DNA TOPOISOMERASES-ENZYMES THAT CATALYSE THE BREAKING AND REJOINING OF DNA

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I. INTRODUCTION

DNA topoisomerases are a group of enzymes defined by their in vitro reactions that interconvert various topological isomers of DNA. Because of these topological transformation reactions, DNA topoisomerases must interact intimately with DNA by transiently breaking one or both strands of the DNA helix. Furthermore, the topological passing of DNA strands is also an unique feature of the enzyme mechanism. The biological functions of this group of enzymes are diverse and in most cases still unknown. Because of the fundamental importance of these enzymes in various genetic processes, it has recently been an exciting area of molecular genetic research. The rapid development in this field has resulted in many recent review articles.[1-7] I will focus this review only on some selected areas of this field.

DNA topoisomerase activity is monitored in vitro by the use of various forms of circular DNA. I first will summarize certain aspects of these forms before proceeding to the classification of the topoisomerases.

II. TOPOLOGICAL ISOMERS OF DNA RINGS

A. Supercoiled DNA

About 19 years ago, Vinograd and his co-workers discovered that the DNA of the animal virus polyoma is covalently closed.[8] Since then, closed circular DNA has been shown to be the most abundant form of genetic material. All closed circular DNA molecules isolated from cells are negatively superhelical. In other words, they are underwound. The topological and thermodynamic properties of superhelical DNAs have been elegantly studied.[9-15] The basic topological parameter that characterizes a superhelical DNA is the linking number (deisgnated as α or Lk), which specifies the number of times the two DNA strands are intertwined. The linking number of a given closed circular DNA is an integer and is invariant so long as the phosphodiester linkages are not broken. To change the linking number of a closed circular DNA, it is necessary to break and reseal the phosphodiester linkages of DNA. The degree of supercoiling of a given closed circular DNA is best described by the quantity called linking differences. Linking difference ($\Delta\alpha$) is the difference between the linking number of the superhelical DNA (α) and the linking number of the same DNA when it is completely relaxed (α°). The specific linking differnce $\Delta\alpha/\alpha^\circ$ is the ratio of $\Delta\alpha$ and α°. In the earlier literature, the linking difference ($\Delta\alpha = \alpha - \alpha^\circ$) has been referred to as the number of superhelical turns and the specific linking difference has been referred to as the superhelical density. The specific linking difference is about -0.06 to -0.07 for most closed circular DNAs isolated from cells.

A superhelical DNA is in a higher free energy state than its relaxed counterpart because of the bending and twisting of the DNA helix. The free energy of superhelix formation has been estimated and shown to be an important factor in many processes involving the winding and unwinding of the DNA helix.[12-16] Negative superhelical twists destabilize the DNA duplex and thus aid any process that involves the unwinding of the DNA, including strand separation. Negative superhelical twists have been shown to be important in DNA replication, transcription, transposition, and viral integration.[1-7] Two independent methods have been used to measure the superhelical state of DNA inside *Escherichia coli*.[17-21] Both methods indicate that DNA inside *E. coli* is under negative superhelical tension and the actual superhelical density inside the *E. coli* cells is about -0.05. The state of DNA inside a eukaryotic cell, however, seems to be more or less relaxed. Firstly, the superhelical turns of SV40 DNA can be fully explained by the wrapping of DNA around histone octamers.[22,23] Secondly, the rate of binding of trimethylpsoralen to intracellular DNA in *Drosophila* and HeLa cells is characteristic of relaxed DNA.[21] However, transient and/or localized supercoiling in eukaryotic cells has not been ruled out. It is interesting to point out that regions on the chicken globin genes have been shown to be sensitive to single-strand specific nuclease S1 in red cell nuclei and the S1 sensitivity correlates with the switching pattern of globin gene expression in embryonic and adult red cells.[24] Similar regions on the cloned globin genes are also sensitive to S1 nuclease when the genes are under negative superhelical tension.[24] These results suggest that DNA supercoiling in eukaryotic cells may be important developmentally in the determination of gene expression and DNA supercoiling may be limited to genetically active regions of the chromosomes.

B. DNA Catenanes

Double-stranded DNA circles are sometimes found in a topological form that involves interlocking of different DNA circles.[25-29] The most remarkable example of such catenated DNA circles is the DNA network found in the kinetoplasts of trypanosomes.[30] Figure 1A shows an electron micrograph of the DNA network isolated from the kinetoplast of *Crithidia fasciculata*.[30] About 5000 minicircles and 30 maxicircles are topologically interlocked to form such a network. A mechanism has been proposed for the replication of the DNA network.[31] Why DNA circles are catenated in the mitochondria of trypanosomes is still unknown. However, enzymes that can unlink these interlocked DNA circles exist (see discussion of type II DNA topoisomerases below). Figures 1B and 1C show electron micrographs of a kinetoplast DNA network decatenated by such an enzyme. Catenated DNA has also been shown to be the product of DNA replication.[32,33] Complex interlocking between the two daughter DNA molecules of SV40 DNA has been shown to occur immediately prior to the final segregation of the two daughter DNA molecules.[32,33] Possibly, a topoisomerase is responsible for the decatenation reaction. Catenated DNA may also be generated from other genetic processes such as excision of the integrated lambda DNA from *E coli* chromosome.[34]

C. Knotted DNA Rings

It is well known that random cyclization of a linear polymer can result in the formation of a knotted circle. Fritsch and Wasserman found that stereochemically reasonable models of trefoils (the simplest knot) can be made for hydrocarbon rings containing more than 50 carbon atoms.[35] Despite considerable effort, previous attempts to synthesize such a structure have been unsuccessful. The first knotted polymer was reported by Liu et al.[36] The knotted single-stranded DNA circles were formed as a result of treating the circular single-stranded DNAs (fd DNA or ϕX174 DNA) with *E. coli* DNA topoisomerase I. The distribution of the various knotted species actually reaches equilibrium in the presence of the enzyme. Statistical-mechanical treatment of random cyclization of a polymer chain has been devel-

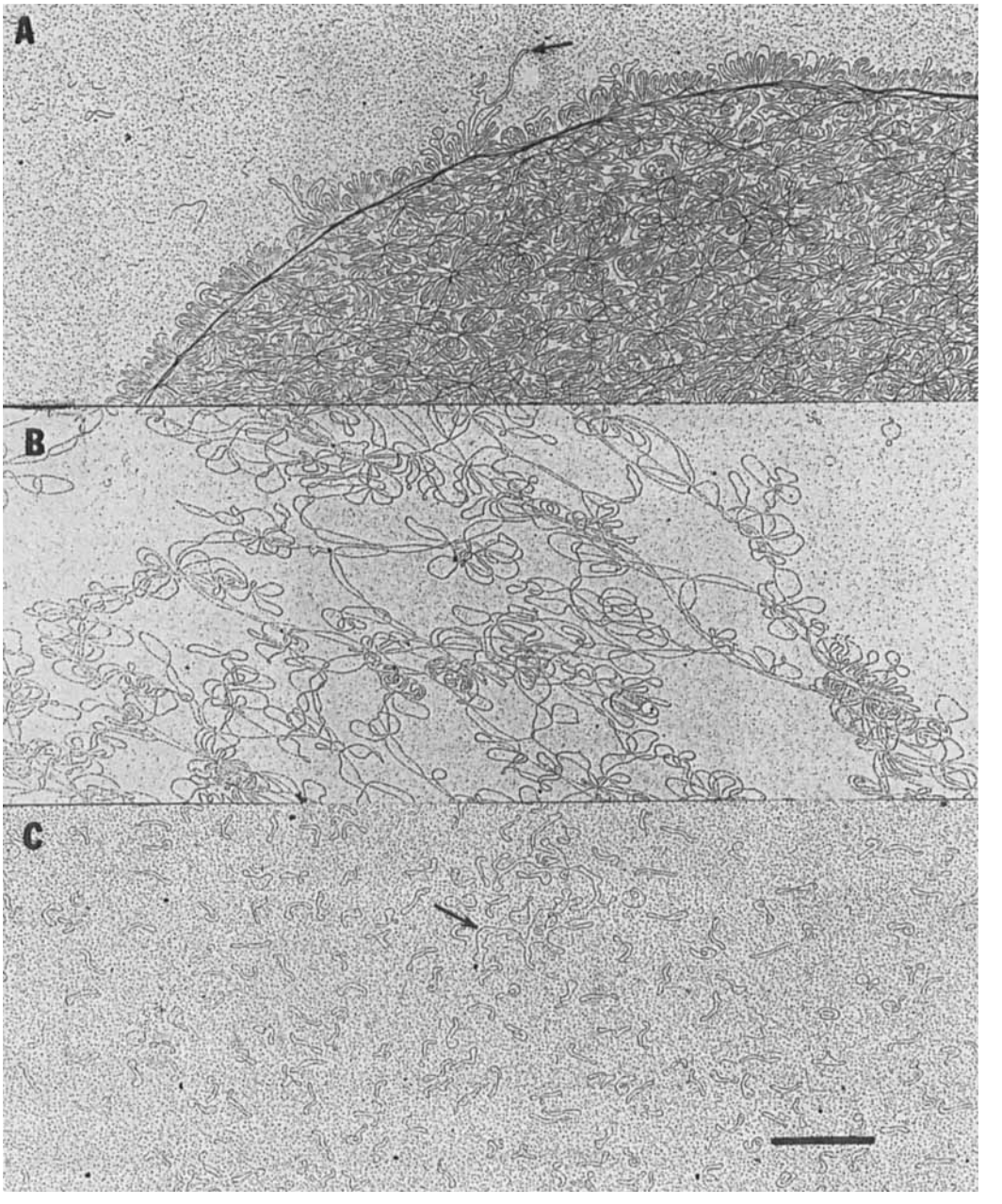

FIGURE 1. Electron micrographs of *C. fasciculata* kinetoplast DNA networks (Form I) treated with T4 DNA topoisomerase. (A) shows the edge of an untreated network (the entire network is about 15 to 20 μm in diameter), (B) shows a network after limited treatment with T4 DNA topoisomerase, and (C) shows the products of a virtually complete digestion. The arrow in (A) indicates a presumed maxicircle "edge-loop" and that in (C) indicates a free maxicircle. The bar represents 1 μm. A sample of each reaction mixture was prepared for electron microscopy using the formamide spreading technique. (From Marini, J. C., Miller, K. G., and Englund, P. T., *J. Biol. Chem.*, 255, 4976, 1980. With permission.)

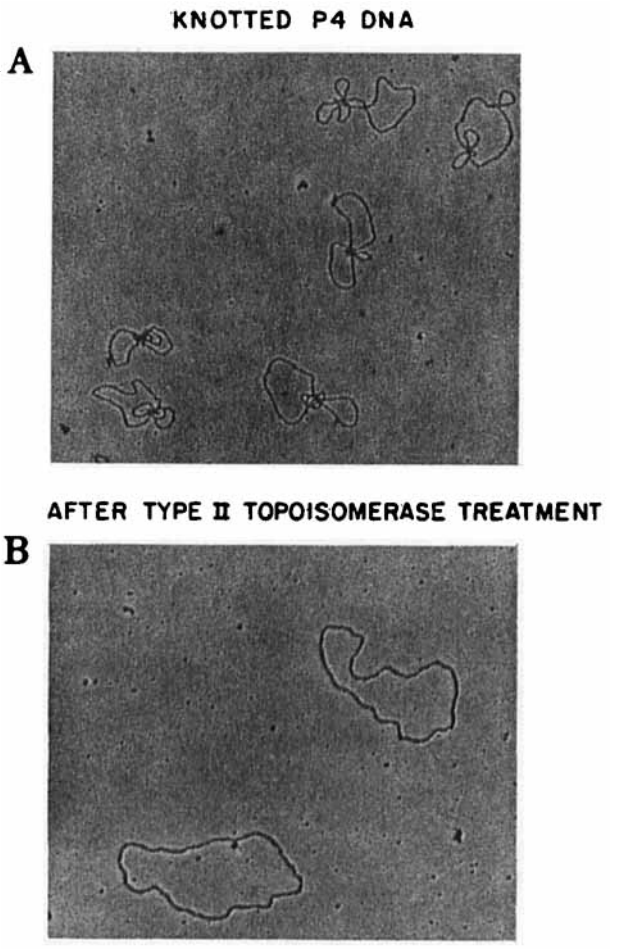

FIGURE 2. Electron micrographs of the P4 head DNA before and after type II DNA topoisomerase treatment. (A): P4 head DNA. (B): P4 head DNA treated with HeLa topoisomerase II. Samples were spread for electron microscopy using the aqueous technique. (From Liu, L. F., Davis, J. L., and Calendar, R., *Nucl. Acids Res.*, 9, 3979, 1981. With permission.)

oped. The probability of knot formation has been shown to be a linear function of the number of DNA statistical segments.[37] For example, random cyclization of λ DNA (MW = 32 × 10^6 daltons, or about 150 Kuhn segments) is predicted to produce 40% knotted molecules. Since most plasmid DNAs are much smaller than λ DNA, random cyclization of a linearized plasmid DNA is not expected to produce any significant amount of knotted DNA. Complex topologically knotted DNA has recently been shown to exist in nature.[38,39] The majority of the DNA prepared from tailless capsids of bacteriophage P2 consists of monomeric rings that have their cohesive ends joined. Electron microscopic and ultracentrifugal studies have indicated that these molecules are topologically knotted.[38] Further studies have also shown that DNA from its satellite phage P4 is also topologically knotted. An electron micrograph of DNA isolated from the bacteriophage P4 capsids is shown in Figure 2. The origin of the topological knots is still unknown. The complexity of the knots, however, must reflect the way the DNA is packaged in the phage head. Whether topological knots exist in other

chromosomes is still unknown. Although small circular DNA such as the plasmid DNA is not expected to be topologically knotted from statistical mechanical considerations, large circular DNA such as the *E. coli* chromosome would be expected to possess complex knots.[37] However, as revealed by the autoradiographic image of a replicating *E. coli* chromosome, it seems to exist as a simple circle without any topological knots.[40] If *E. coli* chromosome is indeed a simple ring, a mechanism must exist to avoid the topological entangling of DNA strands.

III. TYPE I DNA TOPOISOMERASES

DNA topoisomerase activity was first observed by James Wang in 1969.[41] Wang noted that an activity in *E. coli* cell extract was capable of converting highly twisted DNA into a more relaxed form. Further purification of this enzyme activity resulted in a single polypeptide.[42,43] Since the only way to change the topological structure of DNA is to break and rejoin the phosphodiester linkage of DNA, Wang concluded that this enzyme activity must possess both endonuclease activity and ligase activity. This *E. coli* enzyme was first termed ω protein and later named Eco DNA topoisomerase I. A similar enzyme activity from mouse nuclei was reported in 1972 by Champoux and Dulbecco.[44] It is now clear that enzymes that can untwist the superhelical DNA are ubiquitous in nature.[1-7] In 1976, another enzyme activity that can supertwist a relaxed DNA was reported by Gellert and his co-workers.[45] This new enzyme, named DNA gyrase, can transduce the energy from ATP hydrolysis to drive DNA into a highly negatively supertwisted form. Although both *E. coli* ω protein and DNA gyrase must transiently break the phosphodiester linkage of DNA, the mechanisms of these enzymes are fundamentally different. It was later shown that *E. coli* ω protein transiently breaks one strand of DNA while DNA gyrase breaks both DNA strands more or less simultaneously.[1-7] Based on this fundamental difference between the two enzymes, DNA topoisomerases are classified into type I (*E. coli* ω protein) and type II (DNA gyrase) DNA topoisomerases.[5,46] As of now, all DNA topoisomerases can be classified into these two types. In this review, I will discuss only two type I DNA topoisomerases, *E. coli* DNA topoisomerase I and eukaryotic DNA topoisomerase I, because they are the major type I DNA topoisomerases in prokaryotic and eukaryotic cells, respectively. Other type I DNA topoisomerases such as lambda *int* protein,[47,48] vaccinia virus-induced nicking-closing enzyme,[49] and mitochondrial topoisomerases[50] will not be discussed.

A type I DNA topoisomerase is defined by its ability to transiently break one DNA strand and thus permitting the interconversion of various topological isomers of DNA. None of the type I DNA topoisomerases identified so far require energy cofactors for their catalytic reactions. It was first proposed by Wang that the energy required for the rejoining reaction of the phosphodiester linkage may be provided by a covalent enzyme-DNA intermediate which conserves the energy of the phosphodiester bond.[41] This notion was later proven correct. It has now become clear that covalent enzyme-DNA intermediates are characteristic of all known DNA topoisomerases even for the ATP-dependent type II DNA topoisomerases. There has not been a single exception to this interesting proposal. Studies on these covalent enzyme-DNA complexes have been very useful in the elucidation of the molecular mechanisms of DNA topoisomerases. Many type I DNA topoisomerases have been identified since the discovery of *E. coli* ω protein. Although they all share the property of transiently breaking one DNA strand, detailed mechanisms for these type I DNA topoisomerases are different. Such mechanistic differences may be reflected in the diverse functional roles of type I DNA topoisomerases in various genetic processes.

A. *E. Coli* DNA Topoisomerase I (ω Protein)

Purified *E. coli* DNA topoisomerase I is a monomeric protein of about 100,000 daltons. DNA topoisomerases with the same enzymatic properties have also been purified from other

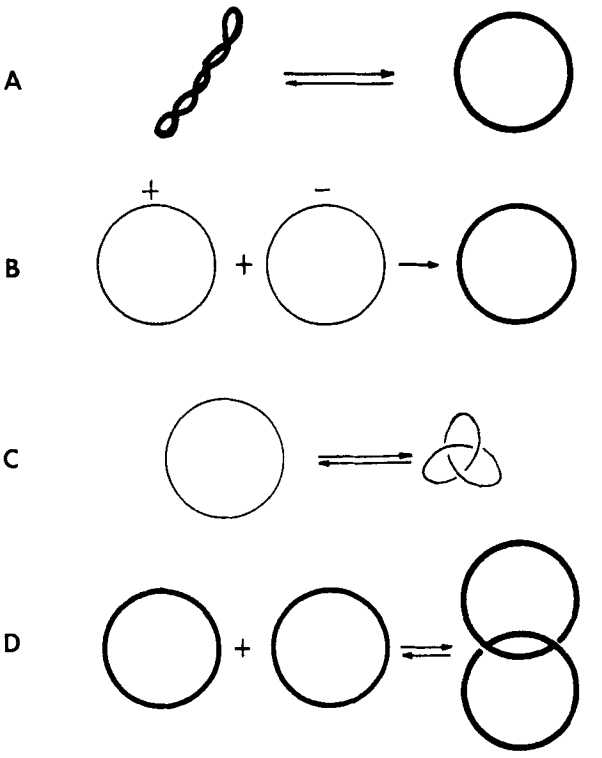

FIGURE 3. Topoisomerization reactions promoted by *E. coli* DNA topoisomerase I. (From Wang, J. C., in *The Enzyme*, Boyer, P., Ed., Academic Press, New York, 1981, 331. With permission.)

prokaryotic organisms.[51-54] Most early experiments were focused on demonstrating the hypothetical covalent enzyme-DNA intermediate and the trapping of the disjoined state of DNA which must exist in the reaction sequence. During these studies, new DNA topoisomerization reactions were discovered which added more clues to the reaction mechanism.

1. DNA Topoisomerization Reactions

As of now, four different types of topological isomerization reactions have been demonstrated and they are shown in Figure 3A.[55] Relaxation of negative twists was the first topological isomerization reaction shown to be catalyzed by *E. coli* DNA topoisomerase I.[41] This reaction requires Mg (II) ion. No energy cofactor is required for this reaction. Positively twisted DNA is not relaxed by *E. coli* DNA topoisomerase I. Relaxation of the negative twists is usually not complete. The residual negative twists are removed at a very slow rate. Under proper conditions, one enzyme molecule can relax many DNA molecules. Single-stranded DNA is a very potent inhibitor of the relaxation reaction. Why *E. coli* DNA topoisomerase I relaxes only negatively twisted DNA and why the reaction is usually not complete will be discussed later.

The second topological reaction (Figure 3B), the interconversion between single-stranded DNA rings with and without topological knots, was discovered in 1976.[36] This reaction obviously requires that the DNA backbone bond be transiently broken, and a segment must cross the broken strand. The final distribution of topological knots is shown to reach an equilibrium state that is dependent on the ionic strength and temperature. Since conditions which favor DNA helix formation also favor knotted ring formation, it was proposed that

the knot formation is closely related to helix formation and DNA topoisomerase serves the role of removing the topological constraints involved. Alternatively, as suggested by the catenation reaction described below, *E. coli* DNA topoisomerase I may pass two single-stranded DNA segments topologically by an enzyme-bridged, DNA strand-passing mechanism.

When complementary rings of PM2 DNA were annealed in the presence of *E. coli* DNA topoisomerase I, the formation of duplex rings was demonstrated.[56] This topological reaction is shown in Figure 3 C. Because the linking number increased from zero (two separated single-stranded rings) to a large positive number (linking number of a nearly relaxed duplex ring), the phosphodiester linkages of DNA must have been transiently broken.

The fourth reaction was discovered rather recently (Figure 3D). *E. coli* DNA topoisomerase I is shown to promote the interlocking of two duplex rings provided that at least one partner is nicked (Figure 3D).[57,58] Although the detailed mechanism is still not worked out, it seems likely that *E. coli* DNA topoisomerase I may bind to the phosphodiester bond opposite to the nick and then mediate an enzyme-bridged, DNA strand-passing reaction similar to the reaction mechanism proposed for type II DNA topoisomerases (see below). Although *E. coli* DNA topoisomerase I promotes four different types of DNA topoisomerization reactions, the mechanism which underlies all these reactions is probably the same.

2. Reaction Mechanism

It was predicted by Wang that *E. coli* DNA topoisomerase I forms a covalently linked enzyme-DNA intermediate.[41] Although direct evidence for this mechanism is still lacking, recent experiments have indicated that covalent topoisomerase-DNA complexes can be trapped under strong protein-denaturing conditions. How such covalent enzyme-DNA complexes are related to the reaction mechanism has not been firmly established.

In the presence of nM concentrations of Mg (II) ion, *E. coli* DNA topoisomerase I exhibits no detectable enzymatic activity but can bind to single-stranded DNA or negatively supercoiled DNA to form a tight complex.[59,60] Such a complex was termed "alkali-cleavable complex" because exposure of this complex to alkaline pH results in DNA strand breakage at the binding site of the DNA topoisomerase and the covalent linking of the protein to the 5′-phosphoryl end of the broken DNA strand. Alkali-cleavable complex also exhibits remarkable stability in high salt solutions in the presence of excess EDTA. In higher Mg(II) ion concentration (mM concentraction range), *E. coli* DNA topoisomerase I breaks and rejoins the DNA efficiently, resulting in the rapid relaxation of the negative superhelical twist and hence the disappearance of the alkali-cleavable complex. On single-stranded DNA, however, the alkali-cleavable complex persists and is unaffected by higher Mg (II) ion concentrations. The simplest interpretation of these results is that *E. coli* DNA topoisomerase I may locally unwind the DNA double helix and form the alkali-cleavable complex with the melted single strands. Furthermore, the formation of the alkali-cleavable complex may be essential for the catalytic event. Such an interpretation is consistent with the observation that *E. coli* DNA topoisomerase I relaxes only negatively superhelical DNA and the residual negative twists are removed at an exceedingly slow rate. Supporting evidence for such a proposal came from the studies of the dependence of alkali-cleavable complex formation on DNA superhelicity and the direct measurement of the unwinding angle of *E. coli* DNA topoisomerase I on DNA. It was shown that the alkali-cleavable complex formation is sharply dependent upon the negative superhelicity of DNA.[1] Direct measurement of the unwinding of *E. coli* DNA topoisomerase I using DNA ligase and nicked circular DNA also indicated that this enzyme unwinds DNA.[1]

The disjoined state of the DNA strand accompanying the formation of the covalent enzyme-DNA complex has been demonstrated only when strong protein denaturants are used. As of now, there has been no evidence that the topoisomerization reactions promoted by *E. coli* DNA topoisomerase I involve a transient "free" swivel (free swivel is used here to describe

the hypothetical situation where the 3′-end of the enzyme-induced transient break can diffuse out of the influence of the enzyme which is bound to the 5′-end of the transient break). It seems likely that the enzyme molecule bridges both ends of the transiently broken DNA single-strands (the 5′-phosphoryl end of the broken DNA strand may be covalently bound by the enzyme and the 3′-OH end may be noncovalently bound by the enzyme) at all times during the topoisomerization reactions, and the topoisomerization reactions proceed by an enzyme-bridged, strand-passing mechanism. This reaction mechanism will be compared with the mechanism proposed for eukaryotic type I DNA topoisomerases later.

The cleavage reaction of topoisomerase I on DNA has been exploited to reveal some of the detailed molecular interactions between topoisomerase and DNA. Firstly, the cleavage sites have been determined by direct DNA sequencing and shown to exhibit little sequence homology among them.[61] Secondly, the covalent protein-DNA linkage has been determined to be a phosphotyrosine diester linkage.[61]

3. Possible Biological Functions of E. coli DNA Topoisomerase I

The ability of *E. coli* DNA topoisomerase I to break and rejoin DNA phosphodiester bonds has led to suggestions that it may be involved in providing a swivel for processes such as DNA replication, transcription, and recombination. *E. coli* deletion mutants totally lacking DNA topoisomerase I gene (*top*A gene) have recently been isolated and shown to grow at a normal rate.[62-65] DNA topoisomerase I is thus not essential for the growth of *E. coli*. However, parallel pathways are well known to exist in vivo. Whether *E. coli* DNA topoisomerase I functions in these genetic processes remains to be elucidated. It is interesting that the *top*A gene is identical to *sup* X gene which was originally identified in *Salmonella typhimurium*.[66,67] It is firmly established that *top*A (*sup*X) mutations affect the transcription of certain operons. This is most likely due to the increased DNA supercoiling in *top*A mutant cells. Consistent with this interpretation, DNA isolated from *top*A mutants showed higher negative superhelical density.[68] Recently, it has been demonstrated that *E. coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes.[69] Furthermore, the increased supercoiling due to topoisomerase I mutations can be corrected by mutations near gyrase genes.[70] It seems possible that the primary role of *E. coli* DNA topoisomerase I may be the modulation of DNA superhelicity. Whether DNA topoisomerase I is directly involved in other genetic processes has yet to be determined.

B. Eukaryotic DNA Topoisomerase I

A DNA topoisomerase similar to *E. coli* DNA topoisomerase I was discovered in 1972 by Champoux and Dulbecco from mouse nuclei.[44] Similar to *E. coli* topoisomerase I, the mouse enzyme relaxes superhelical DNA and the reaction does not require any energy cofactor. Several differences were noted, however. First, the mouse enzyme can relax positively twisted DNA as well as negatively twisted DNA. Second, the relaxation reaction of the mouse enzyme usually goes to completion, and third, the mouse enzyme does not require Mg (II) ion for its catalytic activity.[71-72] All other eukaryotic type I DNA topoisomerases isolated from nuclei share the same properties as the mouse enzyme (see reviews[1-7]). Despite these differences, both *E. coli* DNA topoisomerase I and eukaryotic DNA topoisomerase I are type I DNA topoisomerases.[73] Eukaryotic DNA topoisomerase I is also a monomeric protein of about 100,000 to 130,000 daltsons depending upon the organism.[73-75] However, the native form is often proteolyzed to smaller but active products. In addition to the relaxation reaction, eukaryotic DNA topoisomerase I has also been shown to promote the linking of two complementary DNA rings to a fully relaxed duplex DNA ring,[76] and the catenation of double-stranded DNA circles provided that at least one of the circle is nicked.[58] The catenation reaction is very inefficient and its mechanism may be drastically different from that of *E. coli* DNA topoisomerase I. I will discuss this reaction later.

1. Mechanism

Similar to the bacterial topoisomerase I, eukaryotic DNA topoisomerase I can also be trapped as covalently linked enzyme-DNA complexes.[77-81] However, significant differences exist between the eukaryotic and prokaryotic enzymes:

1. Cleavage of DNA by eukaryotic DNA topoisomerase I occurs efficiently on relaxed DNA or linear DNA.
2. The presence of Mg (II) ion in the millimolar concentration range does not significantly affect the efficiency of the cleavage reaction by eukaryotic DNA topoisomerase I.
3. Eukaryotic DNA topoisomerase I is covalently linked to the 3′-phosphoryl end of the broken DNA strand, whereas *E. coli* DNA topoisomerase I is covalently linked to the 5′-phosphoryl end of the broken DNA strand.
4. Eukaryotic DNA topoisomerase I probably does not bridge both ends of the transient break at all times (see below).

Cleavage of relaxed DNA by eukaryotic DNA topoisomerase I is probably not too surprising because of the fact that the relaxation reaction catalyzed by the eukaryotic DNA topoisomerase I is independent of DNA superhelicity.[72] It is likely that the relaxation of superhelical DNA by eukaryotic DNA topoisomerase I does not involve the local unwinding step as is proposed for the prokaryotic DNA topoisomerase I. Indeed, direct unwinding measurement has indicated that binding of eukaryotic DNA topoisomerase I to DNA does not involve any overall winding or unwinding of the DNA double-helix.[1,2] The role of Mg (II) ion in the relaxation reaction by eukaryotic DNA topoisomerase I is unclear. Although the relaxation of a superhelical DNA by eukaryotic DNA topoisomerase I does not require Mg (II) ion, the presence of mM Mg (II) ion stimulates the reaction 5- to 25-fold depending on the assay conditions.[73] The covalent linkage of eukaryotic DNA topoisomerase I to the 3′-phosphoryl ends of the broken DNA strands has been firmly established. The amino acid residue which is covalently linked to the 3′-phosphoryl end has also been determined to be tyrosine.[82] The chemical basis for the differences between the prokaryotic enzyme (5′-end linkage) and the eukaryotic enzyme (3′-end linkage) is still unknown. The biological significance of this difference is also unknown.

The most intriguing question concerning the mechanism of eukaryotic DNA topoisomerase I is whether it bridges both ends of the break generated by the enzyme at all times during the breakage and rejoining reaction. As discussed earlier, *E. coli* DNA topoisomerase I probably bridges both ends of the transient break at all times during the breakage and rejoining reaction. Several experiments suggest that eukaryotic DNA topoisomerase I does not bridge both ends of the break at all times. When single-stranded DNA is treated with eukaryotic DNA topoisomerase I, spontaneous fragmentation of the single-stranded DNA occurs.[81,83,84] This apparent single-strand specific nuclease activity, however, is quite different from the conventional nucleases because it is stoichiometric rather than catalytic. Although it has not been definitely proven, it seems most likely that each broken DNA fragment is tightly (perhaps covalently) associated with one eukaryotic DNA topoisomerase I molecule at its 3′-phosphoryl end. One possible interpretation for the reaction of topoisomerase I on single-stranded DNA is that eukaryotic DNA topoisomerase I does not bridge both ends of the transient break. It binds tightly to the 3′-phosphoryl end of the break but does not hold on to the 5′-hydroxyl end at all times during the breakage and rejoining reaction. On a duplex DNA, the transiently free 5′-hydroxyl end cannot diffuse away from the active site of the enzyme because of the base pairing to the complementary strand. On a single-stranded DNA, the generation of the transiently free 5′-hydroxyl end results in the complete separation of the two ends of the enzyme-induced break.

If the fragmentation of single-stranded DNA by the treatment of eukaryotic DNA topoisomerase I is indeed due to the simple diffusion of the transiently free 5′-hydroxyl end, one would expect that the reunion reaction to occur at a certain low probability. In other

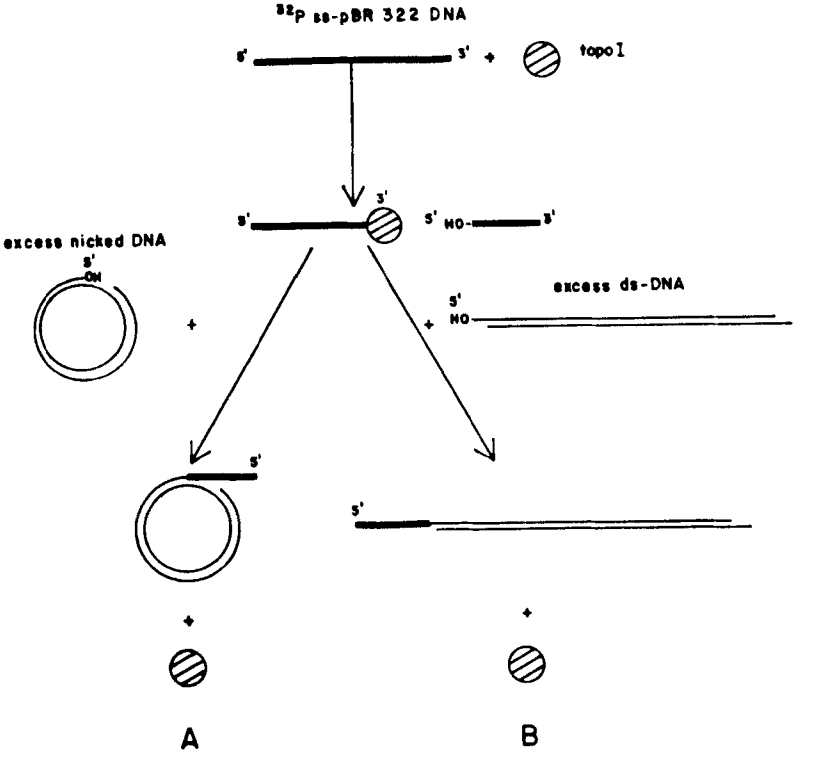

FIGURE 4. Strategy of the strand-transfer experiment. ^{32}P-labeled pBR 322 DNA (labeled by nick translation) was reacted with HeLa topo I to form a protein DNA complex with the active protein linked to the 3′-end of the broken strand. The reaction mixture was then challenged with excess unlabeled heterologous DNA (either linear double-stranded DNA or nicked circular DNA possessing a 5′-OH end). Intermolecular strand-transfer is monitored by the transfer of ^{32}P label to the unlabeled DNA. (From Halligan, B. D., Davis, J. L., Edwards, K. A., and Liu, L. F., *J. Biol. Chem.*, 257, 3995, 1982. With permission.)

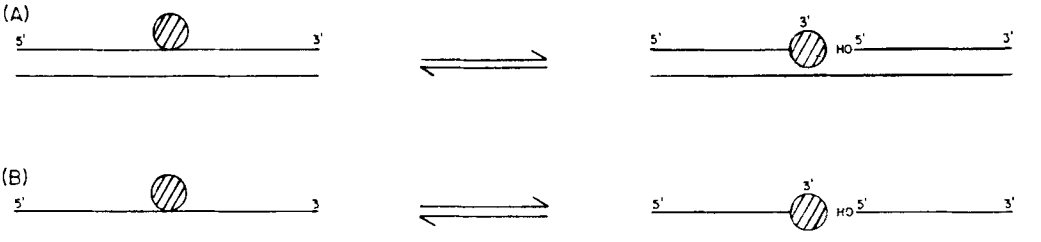

FIGURE 5. The proposed reaction mechanism for eukaryotic DNA topoisomerase I on (A) double-stranded and (B) single-stranded DNA.

words, the enzyme which is linked to the 3′-phosphoryl end of the other half of the broken DNA strand may be an active intermediate and is capable of completing the partial reaction. Several experiments have been performed to support this idea.[83,84] The strategy of one of the experiments is shown in Figure 4. The idea is to trap the hypothetical intermediate with excess DNA possessing 5′-hydroxyl ends. The covalent joining of the ''active intermediate'' to the 5′-hydroxyl end of the added duplex DNA could occur by a process simlar to the completion of the partial reaction. Experimental results clearly show that topoisomerase I linked single-stranded fragments (donors) can covalently join to double-stranded DNA possessing a 5′-hydroxyl group (acceptors). The donor is transferred to the 5′-hydroxyl end of the acceptor, independent of the position of the end (internal nick or ends of linear DNA) or the configuration of the end (flush, 5′-protruding, or 5′-recessed end) of the acceptor.[84] The fact that the ends generated by various restriction enzymes all accepted the donor suggests that the 5′-hydroxyl end of the transient break contains little sequence information at least for the rejoining reaction.[84] Direct DNA sequence analysis of the topoisomerase I cleavage sites on SV40 DNA has suggested that the sequence information for enzyme recognition resides mostly on the 3′-end of the transient break.[85] It seems possible that the DNA sequence at the 5′ end of the transient break plays little role in both the cleavage and the rejoining reactions. Based on these and other results, we have proposed a model (see Figure 5) for the mechanism of eukaryotic DNA topoisomerase I.[84] On single-stranded DNA (Figure 5B), eukaryotic DNA topoisomerase I binds and then cleaves the phosphodiester bond at certain sites leading to the formation of two DNA fragments, one containing the enzyme-DNA complex with the enzyme covalently linked (through the hydroxyl group of a tyrosine) to the 3′-phosphoryl end of the broken DNA strand and the other possessing a free 5′-hydroxyl end. The enzyme-DNA complex thus formed is capable of reversing the reaction by promoting the heterologous joining to the 5′-hydroxyl end of another DNA molecule. Similarly, on double-stranded DNA (Figure 5A), eukaryotic DNA topoisomerase I binds and cleaves only one strand of the DNA duplex. The presence of the complementary DNA strand presumably holds the 5′-hydroxyl end and the covalent protein DNA complex at the 3′-end in close proximity. In our view, the enzyme at the nick in this model does not bridge between the two broken ends at all times. Free rotation of the chemical bond opposite to the nick is allowed to occur. Rejoining of the 5′-hydroxyl end to the enzyme-DNA complex occurs readily because of their close proximity. If this is true, the linking number change for each breakage and rejoining event does not have to be restricted to unity.

Eukaryotic DNA topoisomerase I, similar to *E. coli* DNA topoisomerase I, has recently been shown to promote the interlocking of nicked circular DNA.[58] Similarly, at least one partner of the participating molecules has to be in the nicked circular form.[58] A unified mechanism has been proposed for both eukaryotic DNA topoisomerase I and *E. coli* DNA topoisomerase I.[58] In this model, topoisomerase I binds at the nick and breaks the phosphodiester bond opposite to the nick. By bridging the break at all times, topoisomerase I mediates the passing of another double-stranded DNA segment through this enzyme-bridged break. Such an enzyme-bridged, strand-passing mechanism is analogous to the strand-passing mechanism proposed for the type II DNA topoisomerases. Because the break is bridged by topoisomerase I at all times, the linking number change for each ''breakage and rejoining'' event has to be one. Alternatively, topoisomerase I may linearize the nicked circular DNA by introducing a single-stranded break opposite to the nick. Recyclization of the linearized DNA in the presence of another double-stranded circular DNA may result in the interlocking of two DNA circles. The enzyme-bridged, strand-passing model is likely to be true for *E. coli* topoisomerase I. However, the linearization model seems more likely for the eukaryotic DNA topoisomerase I because of the recently demonstrated strand transfer reactions. More experiments are necessary to clarify these alternative mechanisms.

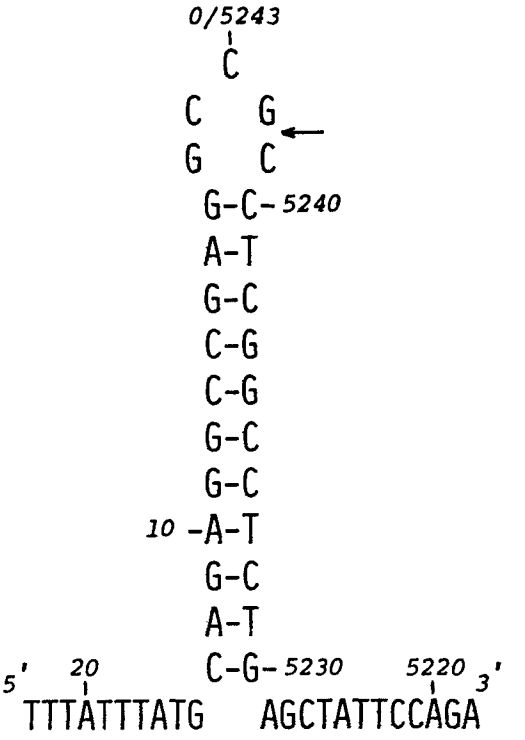

FIGURE 6. Single-strand specific cleavage of a hairpin DNA structure by eukaryotic DNA topoisomerase I. The arrow indicates the site of topoisomerase I cleavate. (From Edwards, K. A., Halligan, B. D., Davis, J. L., Nivera, N., and Liu, L. F., *Nucl. Acids Res.*, 10, 2565, 1982. With permission.)

The sequence specificity of eukaryotic DNA topoisomerase I has been investigated by direct DNA sequencing analyses of the enzyme cleavage sites on SV40 DNA.[85] Both human and calf enzymes cleave SV40 DNA at identical and specific sites. From 827 nucleotides sequenced, 68 cleavage sites were mapped. The majority of the cleavage sites are present on both double- and single-stranded DNA at exactly the same nucleotide positions, suggesting that the DNA sequence is essential for enzyme recognition. By analyzing all the cleavage sequences, certain nucleotides are found to be less favored at the cleavage sites. There is a high probability to exclude G from positions -4, -2, -1, and $+1$, T from position -3, and A from position -1. These five positions (-4 and $+1$ oriented in the 5′ to 3′ direction) around the cleavage sites probably interact with topoisomerase I and thus are essential for enzyme recognition. One topoisomerase cleavage site which shows an atypical cleavage sequence maps in the middle of a palindromic sequence near the origin of SV40 DNA replication (Figure 6). It occurs only on single-stranded SV40 DNA, suggesting that a DNA hairpin can alter the cleavage specificity.

2. Possible Biological Functions

The biological function of eukaryotic DNA topoisomerase I is unknown. Based on its catalytic activity in vitro, eukaryotic DNA topoisomerase I has been implicated in a variety of genetic processes such as DNA replication, RNA transcription, genetic recombination, chromosome condensation and decondensation, and viral encapsidation. Eukaryotic DNA

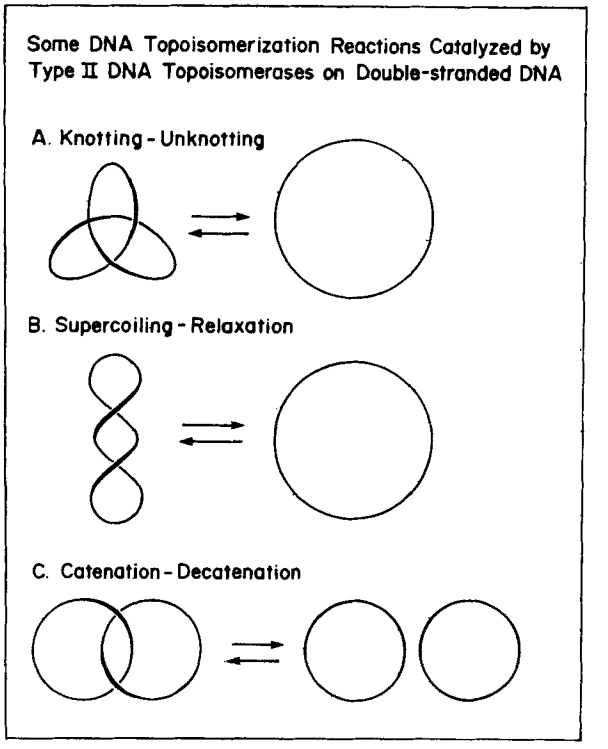

FIGURE 7. Some DNA topoisomerization reactions catalyzed by type II DNA topoisomerase on double-stranded DNA. (From Liu, L. F., in *ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. XIX, Alberts, B. M., Ed., Academic Press, New York, 1980, 817. With permission.)

topoisomerase I has been shown to be tightly associated with chromatin.[86-89] Recently, it has been shown that a fraction of eukaryotic DNA topoisomerase I is tightly associated with active nucleosomes.[90] How eukaryotic DNA topoisomerase I interacts with active nucleosomes remains to be clarified. Several chromosomal proteins (HMG proteins and histone H1) bind tightly to eukaryotic DNA topoisomerase I and stimulate the topoisomerase activity in vitro.[89,91] The intimate interaction of topoisomerase I with chromosomal proteins and nucleosomes may be an essential feature of the topoisomerase function in vivo.

IV. TYPE II DNA TOPOISOMERASE

Type II DNA topoisomerases were discovered rather recently. Type II enzymes are defined by their ability to pass DNA double strands via enzyme-bridged, double-stranded breaks.[5,46] Because of the strand-passing mechanism, type II DNA topoisomerases catalyze a number of novel topological isomerization reactions.[92] Some of these topological reactions are schematically shown in Figure 7. Type II DNA topoisomerases can knot and unknot double-stranded circular DNA, relax and supertwist closed circular DNA, and catenate and decatenate double-stranded DNA circles. Type II DNA topoisomerases can be distinguished from type I DNA topoisomerases by their characteristic changes of the DNA linking number in steps of two. The linking number jump is the result of the crossing of two double-stranded

DNA segments (on the same DNA molecule) via the enzyme-bridged strand-passing mechanism.[10,46,93] Three different type II DNA topoisomerases (*E. coli* DNA gyrase, T4 DNA topoisomerase, and eukaryotic DNA topoisomerase II) will be discussed below. Another type II DNA topoisomerase, *E. coli* DNA topoisomerase II',[94,95] which is closely related to DNA gyrase, will not be discussed in this review.

A. *E. coli* DNA Topoisomerase II (*E. coli* DNA Gyrase)

E. coli DNA gyrase was discovered in 1976 by Martin Gellert and his co-workers during the studies of the integration of bacteriophage lambda in vitro.[45] Many reviews have covered the properties and functions of this enzyme.[3,4,7] I will only discuss briefly about its properties and functions so that comparisons can be made between the eukaryotic type II DNA topoisomerases and prokaryotic type II DNA topoisomerases. Purified DNA gyrase is a tetramer and is composed of two subunits (A_2B_2).[96,100] The gyrase A protein (Mr = 105,000) is the product of *gyr*A gene which is the target of two related antibiotics, nalidixic acid and oxolinic acid. The gyrase B protein (Mr = 95,000) is the product of *gyr* B gene which is the target of several other related antibiotics, coumermycin A_1, novobiocin, and clorobiocin.[17,18,100-107]

1. Topological Isomerization Reactions

DNA gyrase is the only known DNA topoisomerase that introduces negative superhelical turns into a relaxed DNA.[45] Since superhelical DNA is in a higher free energy state than its relaxed form, DNA gyrase uses ATP hydrolysis to drive this energetically unfavorable reaction. In addition to the supercoiling reaction, DNA gyrase has been shown to relax negatively twisted DNA in the absence of ATP.[18,102] More recently, DNA gyrase has been shown to promote a number of type II specific reactions such as catenation/decatenation and knotting/unknotting of duplex DNA.[46,108,109] As is characteristic of a type II DNA topoisomerase, DNA gyrase changes DNA linking number in steps of two, both for the supercoiling reaction and the relaxation reaction.[108,109]

2. Mechanism

a. Wrapping of DNA Around the Gyrase Surface

Three pieces of information have led to the proposal that segments of bound DNA are coiled around the gyrase surface in a unique handedness.

1. DNA gyrase protects a region of 145 bp from digestion by staphylococcal nuclease.[110]
2. Pancreatic DNase I digestion of the gyrase-DNA complex generates a series of DNA fragments with single-strand sizes differing by 10 ± 1 bases.[110] These nuclease digestion results are reminiscent of nuclease digestion on nucleosomes where DNA is wrapped around the histone core.[23]
3. Binding of gyrase to a nicked circular DNA, followed by sealing of the DNA with DNA ligase and deproteinization of the DNA leads to an increase in the linking number of the DNA (i.e., positive supercoiling).[99]

It was proposed that upon binding of gyrase to DNA, some segments of DNA become coiled around the gyrase surface in a unique handedness.[110] From more recent studies, using DNase I footprinting procedure, the regions of gyrase DNA contact have been precisely defined.[111-115] Figure 8 shows the result of the DNase I footprinting around one of the gyrase cleavage sites (see later section on gyrase cleavage reaction).[114] The total length of the protected region from DNase I footprinting is about 140 base pairs, consistent with the staphylococcal nuclease result. The gyrase cleavage site is roughly in the center of this protected region and is strongly protected. Within this 140 base pair region, enhanced DNase

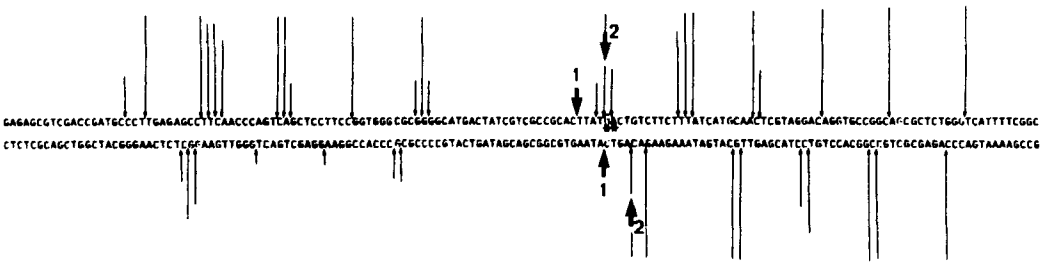

FIGURE 8. Chemical and enzymatic probing of a specific gyrase-DNA complex. The arrows labeled as 1 and 2 show the major and minor gyrase cleavage sites, respectively. The line at the bottom shows the region protected against staphylococcal nuclease. The G and A where methylation by dimethyl sulfate is enhanced are indicated by the * signs. The unlabeled arrows indicate sites where cleavage by pancreatic DNase I is enhanced, the length of the arrows is proportional to the magnitude of the enhancement. (From Wang, J. C., Gumport, R. I., Javaherian, K., Kirkegaard, K., Klevan, K., Kotewicz, M. I., and Tse, Y.-C., in *ICN-UCLA Symposia on Molecular and Cellular Biology.*, Vol. 19, Alberts, B. M., Ed., Academic Press, New York, 1980, 769. With permission.)

I cutting sites still exist at 10 to 11 base pair intervals flanking the gyrase cleavage site. Furthermore, the DNase I cutting sites on complementary strands are staggered by about 2- to 4 base pairs, again reminicent of the action of DNase I on nucleosomal DNA.[23]

b. DNA Cleavage

Similar to the type I DNA topoisomerases, gyrase-DNA complex can also be trapped by protein denaturants so that the disjoined state of the DNA can be revealed.[18,61,102] Oxolinic acid strongly stimulates the reaction for the *E. coli enzyme*. Detailed studies on gyrase cleavage reaction have shown that the double-strand break is staggered by four bases and gyrase A protein is linked to each 5′-protruding end.[116,117] The covalent linkage between gyrase and DNA has been identified to be tyrosyl phosphate.[61]

c. ATP Hydrolysis and Energy Transduction

E. coli DNA gyrase is also a DNA-dependent ATPase.[98,118,119] The hydrolysis product is ADP and inorganic phosphate. Novobiocin and coumermycin strongly inhibit the ATPase activity by competition with ATP binding to DNA gyrase. Negatively superhelical DNA is not as good an effector as relaxed DNA for the ATPase activity, suggesting that the supercoiling reaction and ATP hydrolysis are tightly coupled. It appears that novobiocin and coumermycin interfere with the energy coupling of the supercoiling reaction, while oxolinic acid and nalidixic acid block the breakage and rejoining of DNA. An important clue to the energy transduction mechanism came from studies using the nonhydrolyzable ATP analogue, (β,γ-imido) adenosine triphosphate.[118] In the presence of this ATP analogue, only limited supercoiling is induced. The supercoiling is stoichiometric with the amount of gyrase added. About 1 to 2 negative superhelical turns are introduced per gyrase tetramer added. This result strongly suggests that ATP binding alone is sufficient to drive one step of strand passage in the supercoiling direction.

d. DNA Strand-Passing and a Model for Gyrase Action

Several lines of evidence have suggested that the supercoiling reaction of DNA gyrase involves enzyme-bridged, DNA strand-passing. Firstly, the cleavage reaction of DNA gyrase has revealed that the intermediate of gyrase reaction may involve DNA double-stranded breaks.[18,61,102] Secondly, gyrase has been shown to promote knotting/unknotting and catenation/decatenation reactions of double-stranded circular DNA. Thirdly, gyrase has been shown to change DNA linking number in steps of two, both in the supercoiling reaction

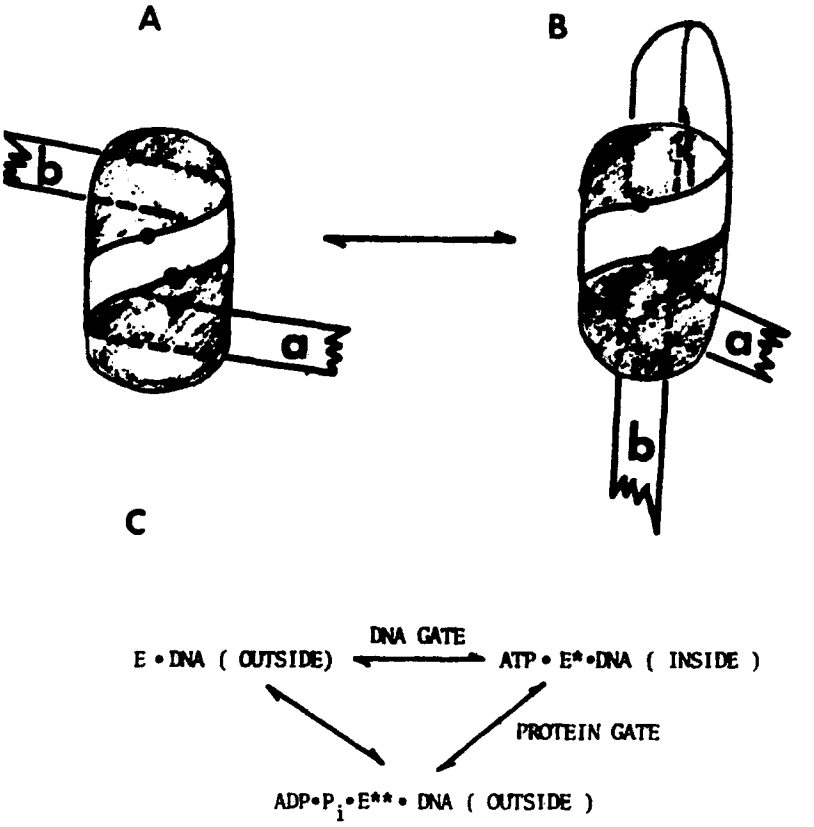

FIGURE 9. The outside (A) and inside (B) states of the DNA-gyrase complex are illustrated diagrammatically, with the DNA represented by a ribbon and the enzyme by the darkened body. The two black dots signify sites where breakage and rejoining of DNA strand occur. Only a segment from a to b of the DNA ring is shown. In C, a scheme of the ATP hydrolysis-coupled DNA supercoiling is illustrated. (From Wang, J. C., Gumport, R. I., Javaherian, K., Kirkegaard, K., Klevan, K., Kotewicz, M. I., and Tse, Y.-C., *ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. 19, Alberts, B. M., Ed., Academic Press, New York, 1980, 769. With permission.)

and in the relaxation reaction. The linking number change experiment requires that the ends of the double-strand break be bridged by the enzyme so that the relative rotation of the two ends cannot occur. Many models have been proposed which are all based on the enzyme-bridged, strand-passing mechanism.[92,93,109,114] One of the models, termed the outside-inside model,[112] is shown in Figure 9. The basic features of this model are as follows:

1. The wrapping of DNA on gyrase is always right-handed during the entire cycle of the strand-passing event.
2. A DNA gate, which is a reversible double-stranded break (with gyrase A subunits covalently linked to each 5′-phosphoryl end), is located somewhere in the middle of the bound DNA.
3. The outside-state and the inside-state are in equilibrium and are affected by ATP binding and DNA superhelicity.
4. The sequence of events is proposed to be as follows: the DNA goes from the outside state (Figure 9A) to the inside state (Figure 9B) by transporting a segment through the DNA gate. ATP binding shifts the equilibrium in favor of the inside state via a

protein conformational change. The hydrolysis of ATP causes a second protein conformational change to close the DNA gate and open a protein gate. The release of the bound ADP, or phophate or both returns the protein to its initial conformation.

All the topological isomerization reactions promoted by DNA gyrase can be explained by this model.

3. Biological Functions

It has been clearly demonstrated that DNA gyrase is responsible for the negative superhelical state of DNA in vivo. Many biological functions of DNA gyrase, such as DNA replication, RNA transcription, DNA transposition, and recombination and repair are probably dependent on such an indirect effect (see reviews[4,7]). A direct role of DNA gyrase in illegitimate recombination has been suggested.[120,121] Whether gyrase has direct roles in other genetic processes has yet to be determined. It is interesting to point out that it has been suggested that catabolite-repressible (CAP-protein dependent) promoters are particularly sensitive to DNA supercoiling.[122] It seems possible that DNA supercoiling may also be an important way of regulating a large number of promoters. Transcription of ribosomal RNA genes of *E. coli* is also stimulated by DNA supercoiling.[123] The significance of such a global regulation of gene expression awaits further studies.

B. T4 DNA Topoisomerase

T4 DNA topoisomerase is a multisubunit enzyme, coded for by T4 genes 39, 52, and 60 (DNA-delay genes).[124,125] Genetic and biochemical studies of mutants in these genes have indicated that T4 DNA topoisomerase is essential for phage T4 DNA replication and is functionally analogous to DNA gyrase. Similar to DNA gyrase, T4 DNA topoisomerase catalyzes ATP-dependent DNA strand-passing reactions (knotting/unknotting, catenation/decatenation, and relaxation).[124,92] T4 DNA topoisomerase also changes the DNA linking number in steps of two.[46] However, T4 DNA topoisomerase is unable to catalyze the supercoiling reaction in vitro.[124] Mechanistic studies have indicated that ATP hydrolysis and DNA strand-passing activities are tightly associated. It is not clear why ATP hydrolysis is necessary for the energetically favorable strand-passing reactions. It seems possible that an unidentified energy-utilizing function is tightly coupled to the DNA strand-passing reaction. It is also possible that T4 DNA topoisomerase is part of a larger functional complex in vivo. Indeed, when the crude extracts prepared from bacteriophage T4 infected *E. coli* cells were sedimented in sucrose density gradients, nearly all the T4 DNA topoisomerase activity was sedimenting at about 30 S.[124] It has been proposed that T4 DNA topoisomerase may function as an origin specific DNA gyrase in vivo, and DNA supercoiling may be localized and/or transient. Localized DNA supercoiling requires the closure of a topological domain. Perhaps, the functional complex of T4 DNA topoisomerase is capable of closing a topological domain. Anchoring of DNA segments on a large structure such as the cell membrane may also restrict the rotation of the DNA helix and thus closes a topological domain. Whether T4 DNA topoisomerase functions as a DNA supercoiling activity in vivo has to await further studies.

C. Eukaryotic DNA Topoisomerase II

The discovery of T4 DNA topoisomerase and the realization of DNA strand-passing reactions had prompted searches for similar enzymes (type II DNA topoisomerases) in eukaryotic cells. Indeed, a type II DNA topoisomerase with enzymatic properties completely analogous to T4 DNA topoisomerase has been detected and in some cases purified from every eukaryotic organism examined.[92,126-129] Eukaryotic DNA topoisomerase II is a rather large protein, the polypeptide detected by SDS gel electrophoresis is about 170,000 daltons. The sedimentation coefficient of eukaryotic DNA topoisomerase II is about 9 to 10 S in a

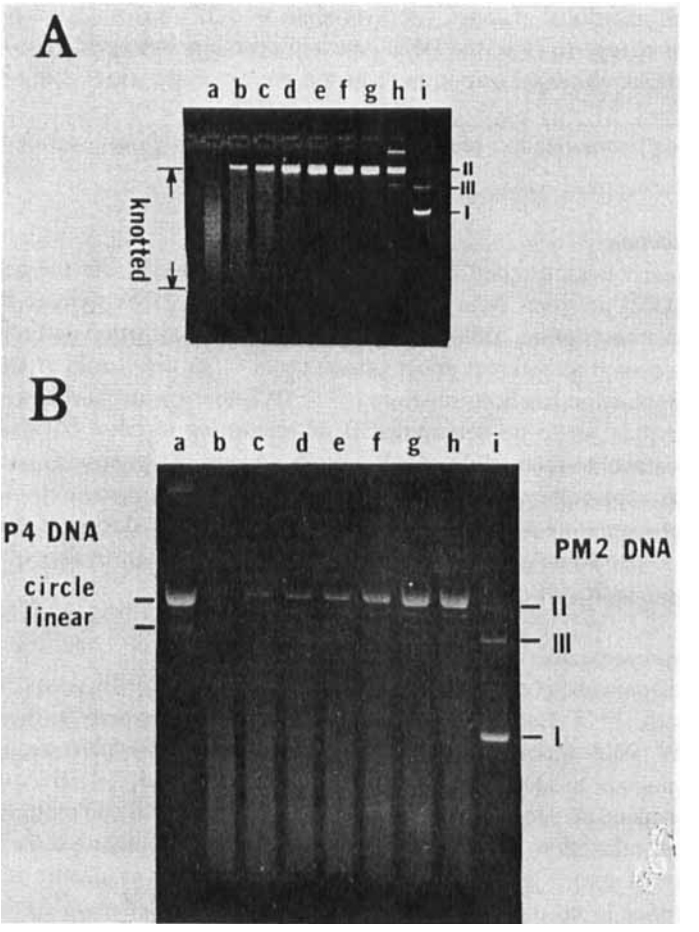

FIGURE 10. Unknotting of P4 head DNA by a type II DNA topoisomerase. (A) P4 head DNA was treated with HeLa topoisomerase II (25 ng/mℓ) at 30°C. 1 m*M* ATP was present in all reactions. 20 $\mu\ell$ aliquots of the reaction mixture were withdrawn at each time point and subjected to gel electrophoresis in a 0.7% agarose gel. (a) 0 min, (b) 1 min, (c) 2 min, (d) 4 min, (e) 8 min, (f) 16 min, (g) 32 min, (h) P4 phage DNA (untreated) and (i) PM2 DNA marker. (B) P4 head DNA was treated with HeLa topoisomerase II (3 ng/mℓ) at 30°C. Aliquots were analyzed as described in (A). (a) P4 phage DNA (untreated), (b) 0 min, (c) 30 seconds (d) 1 min, (e) 2 min, (f) 4 min, (g) 8 min, (h) 10 min, and (i) PM2 DNA marker. A 0.4% gel was used for this analysis. (From Liu, L. F., Davis, J. L., and Calendar, R., *Nucl. Acids Res.*, 9, 3979, 1981. With permission.)

sucrose gradient, suggesting that eukaryotic DNA topoisomerase II may be a multimeric protein. Similar to T4 DNA topoisomerase, eukaryotic DNA topoisomerase II has also been purified as a large protein complex (about 30 S in a glycerol density gradient) which has, in addition to the topoisomerase activity, DNA polymerase α and DNA ligase activities.[130] Whether this large protein complex is the functional form of eukaryotic DNA topoisomerase II or not has not been established. A number of assays have been used to purify eukaryotic DNA topoisomerase II, including ATP-dependent relaxation, catenation and decatenation, and unknotting. Because of the excess topoisomerase I in the cell extracts, relaxation is generally not a suitable assay for topoisomerase II. The presence of DNA condensing agents in the cell extracts also interferes the catenation and decatenation assays. So far the most

satisfactory assay for the eukaryotic DNA topoisomerase II has been the unknotting assay.[39] The knotted DNA can be isolated from the tailless capside of bacteriophage P4 (a satellite phage of P2). Agarose gel electrophoresis is used to monitor the unknotting reaction (Figure 10).[39] The knotted DNA is in a higher free energy state than its unknotted form. The conversion of the knotted P4 DNA to the unknotted form is thus an energetically favorable reaction.

Purified DNA topoisomerase II catalyzes a number of ATP (or dATP)-dependent DNA strand-passing reactions, such as relaxation of superhelical DNA, catenation and decatenation of double-stranded circular DNA, and unknotting of a knotted DNA. DNA topoisomerase II is also a DNA-dependent ATPase which hydrolyzes ATP to ADP and orthophosphate.[126,127] However, unlike DNA gyrase but like T4 DNA topoisomerase, eukaryotic DNA topoisomerase II does not catalyze the negative supercoiling reaction. Why ATP hydrolysis is required for the passive DNA strand-passing reaction is still unknown. Similar to prokaryotic type II DNA topoisomerases, eukaryotic DNA topoisomerase II can induce double-stranded DNA breaks at higher enzyme concentrations. The 5′-ends of the double-stranded breaks are blocked by topoisomerase II and the 3′-ends are free. Similar to T4 DNA topoisomerase, eukaryotic DNA topoisomerase II can also break down single-stranded DNA.[130] Whether these double-strand breaks and single-strand breaks are the result of protein-denaturants treatment or not has not yet been established.

Although all type II DNA topoisomerases catalyze the DNA topoisomerization reactions via the DNA strand-passing mechanism, differences have been noted among the three type II DNA topoisomerases (DNA gyrase, T4 DNA topoisomerase, and eukaryotic DNA topoisomerase II) discussed here. T4 DNA topoisomerase and eukaryotic DNA topoisomerase II do not supercoil DNA in the presence of ATP and do not wind or unwind DNA duplex upon binding,[130] whereas DNA gyrase can supercoil DNA in the presence of ATP and the binding of the enzyme winds the DNA duplex. Whether DNA is wrapped around the enzyme surface of T4 DNA topoisomerase and eukaryotic DNA topoisomerase II is not known. Nuclease protection studies should clarify this point.

Similar to eukaryotic DNA topoisomerase I, eukaryotic DNA topoisomerase II is also tightly associated with isolated cellular SV40 minichromosomes.[130] Furthermore, catenation and decatenation of SV40 minichromosomes has been demonstrated in vitro using purified eukaryotic DNA topoisomerase II.[130] Cleavage of SV40 minichromosomes by eukaryotic DNA topoisomerase II has also been demonstrated.[130] The strand-passing mechanism of eukaryotic DNA topoisomerase II thus may be functional in vivo on chromatin. Many biological functions, such as DNA replication, RNA transcription, sister chromatid exchange, DNA condensation and decondensation, and illegitimate recombination may require a eukaryotic DNA topoisomerase II. It is interesting that eukaryotic DNA topoisomerase II is very sensitive to a number of DNA intercalators such as adriamycin, ellipticine, ethidium bromide, and acridine orange. The inhibitory effect of these antitumor drugs may not be entirely due to their capacity to intercalate DNA, because actinomycin D which intercalates DNA does not inhibit eukaryotic DNA topoisomerase II. In addition, elleipticine and acridine orange stimulate the cleavage reaction of eukaryotic DNA topoisomerase II both on DNA and on chromatin.[130] How these drugs interact with topoisomerases II in vivo is still not known.

V. CONCLUSIONS

The tertiary structure of DNA plays an important role in determining the various genetic activities. The functional roles of DNA topoisomerases which control the tertiary structure of DNA have been partially understood in prokaryotic cells. Very little is known about the biological functions of eukaryotic DNA topoisomerases. The discovery of multiple DNA

topoisomerases in eukaryotic cells will undoubtedly stimulate further research in this area. It is expected that many genetic processes such as DNA replication, RNA transcription, recombination (homologous and illegitimate), chromosome condensation and decondensation, and DNA transposition may all require a DNA topoisomerase activity. Localized and/or transient DNA supercoiling may be an important feature of eukaryotic chromosomes. Localized and/or transient DNA supercoiling may be important in cell differentiation. Global regulation of gene expression by DNA supercoiling may also be important in eukaryotic cells. It is expected that new DNA topoisomerases will be discovered in the future. How DNA topoisomerases manage the DNA structures and cooperate with other nuclear components in the eukaryotic cells will be a major task in the future.

REFERENCES

1. **Champoux, J. J.,** Proteins that affect DNA conformation, *Ann. Rev. Biochem.*, 47, 449, 1978.
2. **Wang, J. C. and Liu, L. F.,** DNA topoisomerases: enzymes that catalyze the breaking and rejoining of DNA backbone bonds, in *Molecular Genetics*, Part 3, Taylor, J. H., Ed., Academic Press, New York, 1979, 65.
3. **Cozzarelli, N. R.,** DNA gyrase and the supercoiling of DNA, *Science*, 207, 953, 1980.
4. **Cozzarelli, N. R.,** DNA topoisomerases, *Cell*, 22, 327, 1980.
5. **Gellert, M.,** DNA topoisomerases, *Ann. Rev. Biochem.*, 50, 879, 1981.
6. **Wang, J. C.,** Type I DNA topoisomerases, in *The Enzyme*, Boyer, P., Ed., Academic Press, New York, 1981, 331.
7. **Gellert, M.,** Type II DNA topoisomerases, in *The Enzyme*, Boyer, P., Ed., Academic Press, New York, 1981, 345.
8. **Weil, R. and Vinograd, J.** The cyclic helix and cyclic coil forms of polyoma viral DNA, *Proc. Natl. Acad. Sci. USA*, 50, 730, 1963.
9. **Fuller, F. B.,** The writhing number of a space curve, *Proc. Natl. Acad. Sci. USA*, 68, 815, 1971.
10. **Fuller, F. B.,** Decomposition of the linking number of a closed ribbon: a problem from molecular biology, *Proc. Natl. Acad. Sci. USA*, 75, 3557, 1978.
11. **Crick, F. H. C.,** Linking numbers and nucleosomes, *Proc. Natl. Acad. Sci. USA*, 73, 2639, 1976.
12. **Bauer, W.and Vinograd, J.,** Interaction of closed circular DNA with intercalative dyes, II. the free energy of superhelix formation in SV40 DNA, *J. Mol. Biol.*, 47, 419, 1970.
13. **Hsieh, T.-S. and Wang, J. C.,** Thermodynamic properties of superhelical DNAs, *Biochemistry*, 14, 527, 1975.
14. **Depew, R.E. and Wang, J. C.** Conformational fluctuations of DNA helix, *Proc. Natl. Acad. Sci. USA*, 72, 4275, 1975.
15. **Pulleyblank, E., Shure, M., Tang, D., Vinograd, J., and Volsberg, H.-P.,** Action of nicking-closing enzyme on supercoiled and nonsupercoiled closed circular DNA: formation of a Boltzmann distribution of topological isomers, *Proc. Natl. Acad. Sci. USA*, 72, 4280, 1975.
16. **Davidson, N.,** Effect of DNA length on the free energy of binding of an unwinding ligand to a superhelical DNA, *J. Mol. Biol.*, 66, 307, 1972.
17. **Gellert, M., O'Dea, M. H., Itoh, T., and Tomizawa, J.,** Novobiocin and coumercin inhibit DNA supercoiling catalyzed by DNA gyrase, *Proc. Natl. Acad. Sci. USA*, 73, 4474, 1976.
18. **Gellert, M., Mizuuchi, K., O'Dea, M. H., Itoh, T., and Tomizawa, J.,** Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity, *Proc. Natl. Acad. Sci. USA*, 74, 4772, 1977.
19. **Drlica, K. and Snyder, M.,** Superhelical *Escherichia coli* DNA: relaxation by coumermycin, *J. Mol. Biol.*, 120, 145, 1978.
20. **Pettijohn, D. E. and Pfenninger, O.,** Supercoils in prokaryotic DNA restrained *in vivo*. *Proc. Natl. Acad. Sci. USA*, 77, 1331, 1980.
21. **Sinden, R. R., Carlson, J. O., and Pettijohn, D. E.,** Torsional tension in the DNA double helix measured with trimethylpsoralen in living *E. coli* cells: analogous measurements in insect and human cells, *Cell*, 21, 773, 1980.
22. **Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M., and Chambon, P.,** Folding of the DNA double helix in chromatin-like structures from SV40, *Proc. Natl. Acad. Sci. USA*, 72, 1843, 1975.
23. **Felsenfeld, G.,** Chromatin, *Nature (London)*, 271, 115, 1978.
24. **Larson, A. and Weintraub, H.** An altered DNA conformation detected by S1 nuclease occurs at specific regions in active chick globin chromatin, *Cell*, 29, 609, 1982.

25. **Sakakibara, Y., Suzuki, K., and Tomizawa, J.,** Formation of catenated molecules by replication of colicin E1 plasmid DNA in cell extracts, *J. Mol. Biol.*, 108, 569, 1976.
26. **Jaenisch, R. and Levine, A. J.,** DNA replication of SV40-infected cells. VII. formation of SV40 catenated and circular dimers, *J. Mol. Biol.*, 73, 199, 1973.
27. **Kupersztoch, Y. M. and Helinski, D. R.,** A catenated DNA molecule as an intermediate in the replication of the resistance transer factor R6K in *Escherichia coli*, *Biochem. Biophys. Res. Commun.*, 54, 1451, 1973.
28. **Novick, R. P., Smith, K., Sheehy, R. J., and Murphy, E.,** A catenated intermediate in plasmid replication, *Biochem. Biophys. Res. Commun.*, 54, 1460, 1973.
29. **Flory, P. J., Jr. and Vinograd, J.,** 5-Bromodeoxyuridine labeling of monomeric and catenated mitochondial DNA in HeLa cells, *J. Mol. Biol.*, 61, 543, 1971.
30. **Marini, J. C., Miller, K. G., and Englund, P. T.,** Decatenation of kinetoplast DNA by topoisomerases, *J. Biol. Chem.*, 255, 4976, 1980.
31. **Englund, P. T.,** Kinetoplast DNA, in *Biochemistry and Physiology of Protozoa*, 2nd ed., Vol. 4, Levandowsky, M. and Hutner, S. H., Eds., Academic Press, New York, in press.
32. **Sundin, O. and Varsharvsky, A.,** Arrest of segregation leads to accumulation of highly interwined catenated dimers: dissection of the final stages of SV40 DNA replication, *Cell*, 25, 659, 1981.
33. **Sundin, O. and Varsharvsky, A.,** Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers, *Cell*, 21, 103, 1980.
34. **Nash, H. A.,** Integration and excision of bacteriophage λ: the mechanism of conservative site specific recombination, *Ann. Rev. Genet.*, 15, 143, 1981.
35. **Fritsch, H. L. and Wasserman, E.,** Chemical topology, *J. Am. Chem. Soc.*, 83, 3789, 1961.
36. **Liu, L. F., Depew, R. E., and Wang, J. C.,** Knotted single-strangded DNA rings: a novel topological isomer of circular single-stranded DNA formed by treatment with Escherichia coli ω protein, *J. Mol. Biol.*, 106, 439, 1976.
37. **Frank-Kamenetskii, J. D., Lukashin, A. V., and Vologodskii, A. V.,** Statistical mechanics and topology of polymer chains, *Nature (London)*, 258, 398, 1975.
38. **Liu, L. F., Perkocha, L., Calendar, R., and Wang, J. C.,** Knotted DNA from bacteriophage capsids, *Proc. Natl. Acad. Sci. USA*, 78, 5498, 1981.
39. **Liu, L. F., Davis, J. L., and Calendar, R.,** Novel topologically knotted DNA from bacteriophage P4 capsids: studies with DNA topoisomerases, *Nucl. Acids Res.*, 9, 3979, 1981.
40. **Cairns, J.,** The bacterial chromosome and its manner of replication as seen by autoradiography, *J. Mol. Biol.*, 6, 208, 1963.
41. **Wang, J. C.,** Interaction between DNA and an *Escherichia coli* protein β, *J. Mol. Biol.*, 43, 263, 1969.
42. **Wang, J. C.,** Interaction between DNA and an *Escherichia coli* protein ω, *J. Mol. Biol.*, 55, 523, 1971.
43. **Wang, J. C.,** Protein ω: a DNA swivelase from *Escherichia coli*?, in *DNA Synthesis in Vitro*, Wells, R. D. and Inman, R. B., Eds., University Park Press, Baltimore, Md., 1973, 163.
44. **Champoux, J. J. and Dulbecco, R.,** An activity from mammalian cells that untwists superhelical DNA-a possible swivel for DNA replication, *Proc. Natl. Acad. Sci. USA*, 69, 143, 1972.
45. **Gellert, M., Mizuuchi, K., O'Dea, M. H., and Nash, H. A.,** An enzyme that introduces superhelical turns into DNA, *Proc. Nal. Acad. Sci. USA*, 73, 3872, 1976.
46. **Liu, L. F., Liu, C. C., and Alberts, B. M.,** Type II DNA topoisomerases: enzyme that can unknot a topologically knotted DNA molecule via a reversible double-strand break, *Cell*, 19, 697, 1980.
47. **Kikuchi, Y. and Nash, H. A.,** Nicking-closing activity associated with bacteriophage *int* gene product, *Proc. Natl. Acad. Sci. USA*, 76, 3760, 1979.
48. **Nash, H. A., Mizuuchi, K., Enquist, L. W., and Weisberg, R. A.,** Strand exchange in λ integrative recombination: genetics, biochemistry and models, *Cold Spring Harbor Symp. Quant. Biol.*, 45, 417, 1980.
49. **Bauer, W. R., Ressner, E. C., Kates, J., and Patzke, J. V.,** A DNA nicking-closing enzyme encapsidated in vaccinia virus: partial purification and properties, *Proc. Natl. Acad. Sci. USA*, 74, 1841, 1977.
50. **Fairfield, F. R., Bauer, W. R., and Simpson, M. V.,** Mitochondria contain a distinct DNA topoisomerase, *J. Biol. Chem.*, 254, 9352, 1979.
51. **Kung, V. and Wang, J. C.,** Purification and characterization of an ω protein from *Micrococcus luteus*, *J. Biol. Chem.*, 252, 5398, 1977.
52. **Burrington, M. G. and Morgan, A. R.,** The purification from *E. coli* of a protein relaxing superhelical DNA, *Can. J. Biochem.*, 54, 301, 1976.
53. **LeBon, J. M., Kado, C. I., Rosenthal, L. J., and Chirikjian, J. G.,** DNA topoisomerase from *agrogacterium tumefacieus:* purification and catalytic properties, *Proc. Natl. Acad. Sci. USA*, 75, 4097, 1978.
54. **Hecht, R. and Thielman, H. W.,** DNA relaxing enzyme from *Micrococcus luteus*, *Nucl. Acids Res.*, 4, 4235, 1977.
55. **Wang, J. C.,** Superhelical DNA, *Trends in Biochemical Sciences*, 219, August, 1980.
56. **Kirkegaard, K. and Wang, J. C.,** *Escherichia coli* DNA topoisomerase I catalyzed linking of single-stranded rings of complementary sequences, *Nucl. Acids Res.*, 5, 3811, 1978.

57. **Tse, Y.-C. and Wang, J. C.,** *E. coli* and *M. luteus* DNA topoisomerase I can catalyze catenation or decatenation of double-stranded DNA rings, *Cell*, 22, 269, 1980.
58. **Brown, P. O. and Cozzarelli, N. R.** Catenation and knotting of duplex DNA by type I topoisomerases: a mechanism parallel with type 2 topoisomerases, *Proc. Natl. Acad. Sci. USA*, 78, 843, 1981.
59. **Depew, R. E., Liu, L. F.,and Wang, J. C.,** Interaction between DNA and *Escherichia coli* Protein ω: formation of a complex between single-stranded DNA and ω protein, *J. Biol Chem.*, 253, 511, 1978.
60. **Liu, L. F. and Wang, J. C.,** Interaction between DNA and *Escherichia coli* DNA topoisomerase I: formation of complexes between the protein and superhelical and nonsuperhelical duplex DNAs, *J. Biol. Chem.*, 254, 11082, 1979.
61. **Tse, Y.-C., Kirkegaard, K., and Wang, J. C.,** Covalent bonds between protein and DNA, *J. Biol. Chem.*, 255, 5560, 1980.
62. **Sternglanz, R., DiNardo, S., Wang, J. C., Nishimura, Y., and Hirota, Y.,** Isolation of an *E. coli* DNA topoisomerase I mutant, in *ICN-UCLA Symposia on Molecular and Cellular Biology*, Vol. 19, Alberts, B., Ed., Academic Press, New York, 1980, 833.
63. **Trucksis, M. and Depew, R. E.,** Identification and localization of a gene which specifies production of *Escherichia coli* DNA topoisomerase I, *Proc. Natl. Acad. Sci. USA*, 78, 2164, 1981.
64. **Sternglanz, R., DiNardo, S., Voelkel, K. A., Nishimura, Y., Hirota, Y., Becherer, K., Zumstein, L., and Wang, J. C.,** Mutations in the gene coding for *Escherichia coli* DNA topoisomerase I affect transcription and transposition, *Proc. Natl. Acad. Sci. USA*, 78, 2747, 1981.
65. **Trucksis, M., Golub, E. I., Zabel, D. J., and Depew, R. E.,** *Escherichia coli* and *Salmonella typhimurium supX* genes specify deoxyribonucleic acid topoisomerase I, *J. Bacteriol.*, 147, 679, 1981.
66. **Dubnau, E. and Margolin, P.,** Suppression of promoter mutations by the pleiotropic *sup*X mutation, *Mol. Gen. Genet.*, 117, 91, 1972.
67. **Dubnau, E., Lenny, A. B.,and Margolin, P.,** Nonsense mutations of the *sup*X locus: further characterization of the *sup*X mutant phenotype, *Mol. Gen. Genet.*, 126, 191, 1973.
68. **Wang, J. C. and Sternglanz, R.,** unpublished data, 1982.
69. **DiNardo, S., Voelkel, K. A., Sternglanz, R., Reynolds, A. E., and Wright, A.,** *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes, *Cell*, 31, 43, 1982.
70. **Pruss, G. J., Manes, S. H., and Drlica, K,.** *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes, *Cell*, 31, 35, 1982.
71. **Keller, W.,** Determination of the number of superhelical turns in simian virus 40 DNA by gel electrophoresis, *Proc. Natl. Acad. Sci. USA*, 72, 4876, 1975.
72. **Vosberg, H.-P., Grossman, L. I., and Vinograd, J.,** Isolation and partial characterization of the relaxation protein from nuclei of cultured mouse and human cells, *Eur. J. Biochem.*, 55, 79, 1975.
73. **Liu, L. F. and Miller, K. G.,** Eukaryotic DNA topoisomerases: two forms of type I DNA topoisomerases from HeLa cell nuclei, *Proc. Natl. Acad. Sci. USA*, 78, 3487, 1981.
74. **Dynan, W. S., Jendrisak, J. J., Hager, D. A., and Burgess, R. R.,** Purification and characterization of wheat germ DNA topoisomerase I (nicking-closing enzyme), *J. Biol. Chem.*, 256, 5860, 1981.
75. **Javaherian, K., Tse, Y.-C., and Vega, J.,** *Drosophila* topoisomerase I: isolation, purification and characterization, *Nucl. Acids Res.*, in press.
76. **Champoux, J. J.,** Renaturation of complementary single-stranded DNA circles: complete rewinding facilitated by the DNA untwisting enzyme, *Proc. Natl. Acad. Sci. USA*, 74, 5328, 1977.
77. **Champoux, J. J.,** Strand breakage by the DNA untwisting enzyme results in covalent attachment of the enzyme to DNA, *Proc. Natl. Acad. Sci. USA*, 74, 3800, 1977.
78. **Keller, W., Muller, U., Eicken, I., Wendel, I., and Zentgraf, H.,** Biochemical and ultrastructural analysis of SV40 chromatin, *Cold Spring Harbor Symp. Quant. Biol.*, 42, 227, 1977.
79. **Champoux, J. J., Young, L. S., and Been, M. D.,** Studies on the regulation and specificity of the DNA-untwisting enzyme, *Cold Spring Harbor Symp. Quant. Biol.*, 43, 53, 1978.
80. **Champoux, J. J.,** Mechanism of the reaction catalyzed by the DNA untwisting enzyme: attachment of the enzyme to the 3′-terminus of the nicked DNA, *J. Mol. Biol.*, 118, 441, 1981.
81. **Prell, B. and Vosberg, H. -P.,** Analysis of covalent complexes formed between calf thymus DNA topoisomerase and single-stranded DNA, *Eur. J. Biochem.*, 108, 389, 1980.
82. **Champoux, J. J.,** DNA is linked to the rat liver DNA nicking-closing enzyme by a phosphodiester bond to tyrosine, *J. Biol. Chem.*, 256, 4825, 1981.
83. **Been, M. D. and Champoux, J. J.,** DNA breakage and closure by rat liver type I topoisomerase: separation of the half-reactions by using a single-stranded DNA substrate, *Proc. Natl. Acad. Sci. USA*, 78, 2883, 1981.
84. **Halligan, B. D., Davis, J. L., Edwards, K. A., and Liu, L. F.,** Intra- and intermolecular strand transfer by eukaryotic DNA topoisomerase I, *J. Biol. Chem.*, 257, 3995, 1982.
85. **Edwards, K. A., Halligan, B. D., Davis, J. L., Nivera, N., and Liu, L. F.,** Recognition sites of eukaryotic DNA topoisomerase I: DNA nucleotide sequencing analysis of topo I cleavage sites on SV40 DNA, *Nucl. Acids Res.*, 10, 2565, 1982.

86. **Sen. A. and Levine, A. J.,** SV40 nucleoprotein complex activity unwinds superhelical turns in SV40 DNA, *Nature (London),* 249, 256, 1974.
87. **Hamlin, C. and Yaniv, N.,** Nicking-closing enzyme is associated with SV40 DNA *in vivo* as a sodium dodecyl sulfate-resistant complex, *Nucl. Acids Res.*, 7, 679, 1979.
88. **Bina, M., Beecher, S., and Blasquez, V.,** Stability and components of mature simian virus 40, *Biochemistry,* 21, 3057, 1982.
89. **Javaherian, K. and Liu, L. F.,** Association of eukaryotic DNA topoisomerase I with nucleosomes and chromosomal proteins, *Nucl. Acids Res.*, in press.
90. **Weisbrod, S. T.,** Properties of active nucleosomes as revealed by HMG 14 and 17 chromotography, *Nucl. Acids Res.*, 10, 2017, 1982.
91. **Rowe, T. C., Rusche, J. R., Brougham, M. J., and Holloman, W. K.,** Purification and properties of a topoisomerase from *Ustilago maydis.*, *J Biol. Chem.*, 256, 10354, 1981.
92. **Liu, L. F.,** DNA strand passing and the functions of type II DNA topoisomerases, in *ICN-UCLA Symposia on Molecular Biology and Cellular Biology,* Vol. XIX, Alberts, B. M., Ed., Academic Press, New York, 1980, 817.
93. **Brown, P. O. and Cozzarelli, N. R.,** A sign inversion mechanism for enzymatic supercoiling of DNA, *Science,* 206, 1081, 1979.
94. **Brown, P. O., Peebles, C. L., and Cozzarelli, N. R.,** A topoisomerase from *Escherichia coli* related to DNA gyrase, *Proc. Natl. Acad. Sci. USA,* 76, 6110, 1979.
95. **Gellert, M., Fisher, L. M., Ohmori, H., and O'Dea, H. H.,** DNA gyrase: purification and catalytic properties of a fragment of gyrase B protein, *Proc. Natl. Acad. Sci. USA,* 76, 6289, 1979.
96. **Higgins, N. P., Peebles, C. L., Sugino, A., and Cozzarelli, N. R.,** Purification of subunits of *E. coli* DNA gyrase and reconstitution of enzymatic activity, *Proc. Natl. Acad. Sci. USA,* 75, 1773, 1978.
97. **Mizuuchi, K., O'Dea, M. H., and Gellert, M.,** DNA gyrase: subunit structure and ATPase activity of the purified enzyme, *Proc. Natl. Acad. Sci. USA,* 75, 5960, 1978.
98. **Liu, L. F. and Wang, J. C.,** *Micrococcus luteus* DNA gyrase: active components and a model for its supercoiling of DNA, *Proc. Natl. Acad. Sci. USA,* 75, 2098, 1978.
99. **Liu, L. F. and Wang, J. C.,** unpublished data, 1978.
100. **Klevan, L. and Wang, J. C.,** A DNA-DNA gyrase complex containing 140 base pairs of DNA and an $\alpha_2\beta_2$ protein core, *Biochemistry,* 19, 5229, 1980.
101. **Sugino, A. and Bott, K. F.** *Bacillus subtilis* deoxyribonucleic acid gyrase, *J. Bacteriol.*, 141, 1331, 1980.
102. **Sugino, A., Peebles, C. L., Kreuzer, K. N., and Cozzarelli, N. R.,** Mechanism of action of nalidixic acid: purification of *E. coli nal* A gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme, *Proc. Natl. Acad. Sci. USA,* 74, 4767, 1977.
103. **Ryan, M. J.,** Coumermycin A_1: a preferential inhibitor of replicative DNA synthesis in *Escherichia coli.* I. *In vivo* characterization, *Biochemistry,* 15, 3769, 1976.
104. **Goss, W. A., Deitz, W. H., and Cook, T. M.,** Mechanism of action of nalidixic acid on *Escherichia coli.* II. Inhibition of deoxyribonucleic acid synthesis, *J. Bacteriol.*, 89, 1068, 1965.
105. **Staudenbauer, W. L.,** Replication of *Escherichia coli* DNA *in vitro.* Inhibition by oxolinic acid, *Eur. J. Biochem.*, 62, 491, 1976.
106. **Hansen, F. G. and von Meyenburg, K.,** Characterization of the *dna* A, *gyr*B, and other genes in the *dna* A region of the *Escherichia coli* chromosome cloned on specialized transducing phages λ tna, *Mol. Gen. Genet.*, 175, 135, 1979.
107. **Fairweather, N. F., Orr, E., and Holland, I. B.,** Inhibition of deoxyribonucleic acid gyrase: effects on nucleic acid synthesis and cell division in *Escherichia coli* K-12, *J. Bacteriol.*, 142, 153, 1980.
108. **Kreuzer, K. N. and Cozzarelli, N. R.,** Formation and resolution of catenanes by DNA gyrase, *Cell,* 20, 245, 1980.
109. **Mizuuchi, K., Fischer, L. M., O'Dea, M. H., and Gellert, M.,** DNA gyrase action involves the introduction of transient double-strand breaks into DNA, *Proc. Natl. Acad. Sci. USA,* 77, 1847, 1980.
110. **Liu, L. F. and Wang, J. C.,** DNA-DNA gyrase complex: the wrapping of the DNA duplex outside the enzyme, *Cell,* 15, 979, 1978.
111. **Kirkegaard, K. and Wang, J. C.,** Mapping the topography of DNA wrapped around gyrase by nucleolytic and chemical probing of complexes of unique DNA sequences, *Cell,* 23, 721, 1981.
112. **Morrison, A. and Cozzarelli, N. R.,** Contacts between DNA gyrase and its binding site on DNA: features of symmetry and asymmetry revealed by protection from nucleases, *Proc. Natl. Acad. Sci. USA,* 78, 1416, 1981.
113. **Fisher, L. M., Mizuuchi, K., O'Dea, M. H., Ohmori, H., and Gellert, M.,** Site-specific interaction of DNA gyrase with DNA, *Proc. Natl. Acad. Sci. USA,* 78, 4165, 1981.
114. **Wang, J. C., Gumport, R. I., Javaherian, K., Kirkegaard, K., Klevan, L., Kotewicz, M. L., and Tse, Y.-C.,** DNA topoisomerases, in Mechanistic Studies of DNA Replication and Genetic Recombination, *ICN-UCLA Symposia on Molecular and Cellular Biology,* Vol. 19, Aberts, B. M., Ed., Academic Press, New York, 1980, 769.

115. **Morrison, A., Brown, P. O., Kreuzer, K. N., Otter, R., Gerrard, S. P., and Cozzarelli, N. R.,** Mechanisms of DNA topoisomerases, in Mechanistic Studies of DNA Replication and Genetic Recombination, *ICN-UCLA Symposia on Molecular and Cellular Biology,* Vol. 19, Alberts, B. M., Ed., Academic Press, New York, 1980, 785.
116. **Gellert, M., Fisher, L. M., Ohmori, H., O'Dea, M. H., and Mizuuchi, K.,** DNA gyrase: site-specific interactions and transient double-strand breakage of DNA, *Cold Spring Harbor Symp. Quant. Biol.,* 45, 391, 1981.
117. **Morrison, A., and Cozzarelli, N. R.** Site specific cleavage of DNA by *Escherichia coli* DNA gyrase, *Cell,* 17, 175, 1979.
118. **Sugino, A., Higgins, N. P., Brown, P. O., Peebles, C. L., and Cozzarelli, N. R.,** Energy coupling in DNA gyrase and the mechanism of action of novobiocin, *Proc. Natl. Acad. Sci. USA,* 75, 4838, 1978.
119. **Sugino, A. and Cozzarelli, N. R.,** The intrinsic ATPase of DNA gyrase, *J. Biol. Chem.,* 255, 6299, 1980.
120. **Ikeda, H., Moriya, K., and Matsumoto, T.,** *In vivo* study of illegitimate recombination: involvement of DNA gyrase, *Cold Spring Harbor Symp. Quant. Biol.,* 45, 399, 1981.
121. **Ikeda, H., Aoki, K., and Naito, A.,** Illegitimate recombination mediated *in vitro* by DNA gyrase of *Escherichia coli:* structure of recombination DNA molecules. *Proc. Natl. Acad. Sci. USA,* 79, 3724, 1982.
122. **Sanzey, B.,** Modulation of gene expression by drugs affecting DNA gyrase, *J. Bacteriol.,* 138, 40, 1979.
123. **Oostra, B. A., Geert, A. B., and Gruber, M.,** Involvement of DNA gyrase in the transcription of ribosomal RNA, *Nucl. Acids Res.,* 8, 4235, 1980.
124. **Liu, L. F., Liu, C. C., and Alberts, B. M.,** T4 DNA topoisomerase: a new ATP-dependent enzyme essential for initiation of T4 bacteriophage DNA replication, *Nature (London),* 281, 456, 1979.
125. **Stetler, G. L., King, G J., and Huang, W. M.,** T4 DNA-delay proteins, required for specific DNA replications, form a complex that has ATP-dependent DNA topoisomerase activity, *Proc. Natl. Acad. Sci. USA,* 76, 3737, 1979.
126. **Hsieh, T. S. and Brutlag, D.,** ATP-dependent DNA topoisomerase from *Drosophila melanogaster* reversibly catenates duplex DNA rings, *Cell,* 21, 115, 1980.
127. **Miller, K. G., Liu, L. F., and England, P. T.,** A homogeneous type II DNA topoisomerase from HeLa cell nuclei, *J. Biol. Chem.,* 256, 9334, 1981.
128. **Baldi, M. I., Benedetti, P., Mattoccia, E., and Tocchini-Valentini,** *In vitro* catenation and decatenation of DNA and a novel eucaryotic ATP-dependent topoisomerase, *Cell,* 20, 461, 1980.
129. **Goto, T. and Wang, J. C.,** Yeast DNA topoisomerase II, an ATP-dependent type II topoisomerase that catalyzes the catanation, decatenation, unknotting, and relaxation of double-stranded DNA rings, *J. Biol. Chem.,* 257, 5866, 1982.
130. **Liu, L. F., Halligan, B. D., Nelson, E. M., and Edwards, K. A.,** unpublished data, 1982.