# **20** DNA Topoisomerases

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The double-helical nature of DNA and the anchoring of DNA to nuclear structures result in a number of topological problems during replication and transcription, mainly due to DNA-tracking polymerases and helicases. These activities cause the accumulation of positive supercoils ahead of the moving polymerase and negative supercoils behind it (Liu and Wang 1987; Brill and Sternglanz 1988; Giaever and Wang 1988; Wu et al. 1988). (By definition, DNA becomes positively supercoiled when there is a decrease in the number of base pairs per helical turn below 10.3. Likewise, an increase in the number of base pairs per turn above 10.3 results in negatively supercoiled DNA.) The topological imbalance will, if not leveled, ultimately present an impenetrable energy barrier to the tracking protein complexes (Gartenberg and Wang 1992). Enzymes that influence the topological functions of DNA.

In the eukaryotic cell, the topological structure of DNA is modulated by two groups of ubiquitous enzymes known as type I and type II topoisomerases. The enzymes alter the DNA linking number, which is the number of times the two strands are interwound. Type I enzymes (topoisomerase I and the evolutionarily distinct topoisomerase III) interconvert different topological forms of DNA by breaking and rejoining a single strand of the DNA double helix, changing the linking number in steps of one. Type II enzymes (topoisomerase II), however, catalyze topology changes by reversibly breaking both strands of the DNA double helix, resulting in a linking number change of two (Vosberg 1985; Wang 1987).

#### **TYPE I TOPOISOMERASES**

#### **Physical Characteristics**

The first eukaryotic topoisomerase I activity was purified from rat liver cells in 1972 as an untwisting enzyme capable of relaxing negatively supercoiled DNA (Champoux and Dulbecco 1972). Since then, topoisomerase I activities have been purified from a broad range of eukaryotic organisms and tissues (for review, see Vosberg 1985). The enzyme is present as a monomer in solution with a molecular mass of approximately 90–110 kD.

Topoisomerase I coding sequences have been reported for a number of eukaryotic species, including yeasts (Thrash et al. 1985; Uemura et al. 1987a), *Drosophila* (Hsieh et al. 1992), mouse (Koiwai et al. 1993), and human (D'Arpa et al. 1988). Sequence comparisons show that a central region of 400–450 amino acids and a carboxy-terminal region of about 70 amino acids are highly conserved in eukaryotic topoisomerase I. The active site is located in the carboxy-terminal region, and the tyrosine involved in the transient covalent linkage to DNA has been mapped in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* to residues 727 (Lynn et al. 1989) and 771 (Eng et al. 1989), respectively. Alsner et al. (1992) have found that a small amino-terminal region, not required for topoisomerase I activity in vitro, is responsible for nuclear localization, but apart from this, little is known about the functional organization of the enzyme.

Kunze et al. (1990) have characterized the promoter region of the human *TOP1* gene. It holds the characteristics of other housekeeping genes and, accordingly, the enzyme level does not vary significantly during the cell cycle (Heck et al. 1988). Generally, the enzyme is constantly expressed at a level of  $10^5$  to  $10^6$  copies per cell (Champoux and McConaughy 1976; Liu and Miller 1981).

Topoisomerase I is posttranslationally modified and presumably positively regulated by phosphorylation. The kinases involved have been proposed to be protein kinase C (Pommier et al. 1990; Cardellini and Durban 1993) and nuclear casein kinase II (Durban et al. 1985).

## Enzymology of Topoisomerase I

Topoisomerase I relaxes both negatively and positively supercoiled DNA to completion with no requirement for divalent cations or ATP (Table 1 and Fig. 1), and the catalytic cycle can be separated into DNA binding, cleavage, strand passage, religation, and turnover (Fig. 2).

	Subunit					
	structure,	-	Covalent		ATP	Mg <sup>++</sup>
Enzyme	size	Prevalence	linkage	Catalytic activity	requirement	requirement
Topoisomerase I	monomer, 90-110 kD	all eukaryotes examined; some viruses (vaccinia	3′-P	relaxation of negative and positive supercoils:	по	ОП
		Shope fibroma)		decatenation of nicked		
				substrates		
Topoisomerase II	homodimer,	all eukaryotes examined	51-P	relaxation of negative	yes	yes
	160-180 kD/			and positive supercoils;		
	monomer			catenation/decatenation,		
				knotting/unknotting of		
				double-stranded DNA		
Topoisomerase III	monomer,	yeast (S. cerevisiae)	51-P	weak relaxation of negative	no	yes
	~75 kD			supercoils; preference for		
				single-stranded DNA		

Table 1 Characteristics of eukaryotic DNA topoisomerases



Figure 1 Reactions catalyzed by DNA topoisomerases. DNA topoisomerases catalyze the relaxation of positively and negatively supercoiled DNA (A), catenation/decatenation of DNA (B), and knotting/unknotting of DNA (C). For enzyme specificities and other details, see Table 1.

The substrate specificity of topoisomerase I has been widely studied, and it appears that the enzyme recognizes double-stranded DNA, relatively independent of sequence. A highly degenerate consensus sequence has been reported for the four base pairs located immediately 5' to the site of topoisomerase-I-mediated cleavage (Edwards et al. 1982; Been et al. 1984; Jaxel et al. 1991). Despite the apparent promiscuity in bindingsite selection, sites have been described to which topoisomerase I binds with high affinity. Thus, a site initially described in the rDNA of Tetrahymena thermophila is a site of preferential binding and cleavage in vivo and in vitro (Bonven et al. 1985; Andersen et al. 1985; Thomsen et al. 1987), as well as a site mediating preferential catalytic activity (Busk et al. 1987). Several studies suggest that topoisomerase I recognizes gross structural features of the DNA double helix rather than base sequence per se. Particularly, it seems that bent DNA is the substrate of choice. This is reflected in the preferential action on highly supercoiled or otherwise curved DNA (Camilloni et al. 1988, 1989; Caserta et al. 1989,1990) and by the finding that the high-affinity sequence described above exhibits static intrinsic curvature (Krogh et al. 1991).

Footprinting and interference studies of topoisomerase I show protection and interference in the "consensus" region 5' to the nick, but interac-



Figure 2 Steps in the catalytic cycle of eukaryotic DNA topoisomerase I. DNA topoisomerase I binds noncovalently to DNA (I) and cleaves one of the DNA strands, forming a covalent linkage between the newly generated 3'-phosphate and the active-site tyrosine (II). DNA strand passage then takes place, followed by DNA ligation (III). The enzyme can interact with DNA in either a distributive or a processive mode (McConaughy et al. 1981). In the distributive mode (IV), the enzyme dissociates from the DNA after ligation, whereas in the processive mode (V), the enzyme reenters the catalytic cycle without dissociation from the DNA.

tion is also observed 3' to the nick (Stevnsner et al. 1989; Bendixen et al. 1990; Krogh et al. 1991). This additional 3' contact was later confirmed by the use of oligonucleotide substrates (Christiansen et al. 1993; Christiansen and Westergaard 1994), and it has been proposed to operate as a topology sensor (Krogh et al. 1991). The topoisomerase-I-mediated DNA cleavage reaction has traditionally been studied by detergent trapping of cleavage complexes (Vosberg 1985) but has recently been investigated using suicidal DNA substrates; i.e., partially single-stranded DNA or oligonucleotide duplexes (Been and Champoux 1981; Svejstrup et al. 1991; Christiansen et al. 1993). Mechanistically, topoisomerase I acts by directing a nucleophilic attack from the hydroxyl group of an internal tyrosine into a phosphodiester bond in the DNA backbone, resulting in nicking of one strand of the DNA double helix and formation of a covalent linkage between the 3'-phosphate and the tyrosine hydroxyl group.

The mechanism of strand passage has not been described in detail, but two different models have been proposed: (1) the "strand passage model," where the intact strand passes through the nicked strand, requiring local DNA denaturation, and (2) the "swiveling model," where the free 5'-OH end is able to rotate around the intact strand, either freely or in a stepwise fashion. Following topoisomerization, the nick is religated by a nucleophilic attack of the 5'-OH end on the O<sup>4</sup>-phosphotyrosine bond, releasing the enzyme (for reviews, see Maxwell and Gellert 1986; Osheroff 1989b). When oligonucleotide DNA duplexes are used as substrates for topoisomerase I, the generated cleavage complex contains an active enzyme, which is able to ligate the cleaved DNA to a variety of DNA fragments containing a free 5'-OH end. Using this system, it has been found that the enzyme preferentially ligates the cleaved DNA to single-stranded DNA having the ability to base-pair, although religation to substrates with incomplete base-pairing or to DNA with blunt ends has also been described (Christiansen et al. 1993; Christiansen and Westergaard 1993, 1994).

## **TYPE II TOPOISOMERASES**

## **Physical Characteristics**

All type II topoisomerases studied so far are structurally related and show similar physical properties. The gene for topoisomerase II has been cloned from a variety of eukaryotic organisms, including yeasts (Giaever et al. 1986; Uemura et al. 1986), protozoans (Strauss and Wang 1990; Fragoso and Goldenberg 1992; Pasion et al. 1992), *Drosophila* (Wyckoff et al. 1989), mouse (Adachi et al. 1992), rat (Park et al. 1993), and human (Tsai-Pflugfelder et al. 1988; Jenkins et al. 1992; Austin et al. 1993). The enzyme is encoded by a single-copy gene, but it exists in solution as a homodimer (Vosberg 1985) with molecular masses ranging from about 300 kD to 360 kD. Only one topoisomerase II form has been found in lower eukaryotes, whereas two forms, the  $\alpha$  form and the  $\beta$  form, exist in humans and probably in all higher eukaryotes (Drake et al. 1989a). The two isoforms have different DNA-binding/cleavage site preferences and different DNA dissociation rates and also show differences in their susceptibility to drugs (Drake et al. 1989b).

Sequence homology studies have shown that type II topoisomerases from various eukaryotic sources are highly conserved, with the amino acid sequence of the enzymes sharing an overall homology of about 50%. Homology is extensive in the amino-terminal approximately 1200 amino acids, but the carboxy-terminal 200–300 amino acids show no conservation (Wyckoff et al. 1989). The conserved state of the enzymes is further seen from the ability of several eukaryotic topoisomerase II genes to complement a yeast  $top2^{ts}$  mutant or a top2 null mutant (Uemura et al. 1987b; Wyckoff and Hsieh 1988; Adachi et al. 1992).

Eukaryotic topoisomerase II shows significant homology with the bacterial type II topoisomerase enzyme, DNA gyrase. The latter is tetrameric (A<sub>2</sub>B<sub>2</sub>), consisting of two distinct subunits, GyrA and GyrB, where GyrA contains the active site for DNA-cleavage/religation and GyrB holds the ATPase activity (for review, see Reece and Maxwell 1991; Orphanides and Maxwell 1994). Based on the homology of the amino-terminal approximately 650 amino acids in the eukaryotic enzyme to GyrB, this region has been inferred to be responsible for ATP hydrolysis, in agreement with the observation that point mutations in this region abolish ATP binding (Uemura et al. 1987b; Lindsley and Wang 1991). Likewise, the amino-terminal part of GyrA holding the active site is homologous to the region from amino acids approximately 650 to 900 in the eukaryotic enzyme, correlating well with the identification of the active-site tyrosine to Y783 in S. cerevisiae (Worland and Wang 1989). The final carboxy-terminal region from amino acid 1200 has no counterpart in DNA gyrase. The region is rich in highly charged amino acids, suggesting an involvement in DNA binding (Uemura et al. 1986). Nuclear localization signals have been found in the nonconserved region, although the outermost 200-250 amino acids are dispensable for cell viability (Shiozaki and Yanagida 1991, 1992; Crenshaw and Hsieh 1993; Caron et al. 1994).

The promoter region of the gene for human topoisomerase II $\alpha$  shares several features of traditional housekeeping gene promoters, but it con-

tains in addition a regulatory region with a structure similar to that found in proliferation-specific genes (Hochhauser et al. 1992). This is in agreement with observations showing that the amount of topoisomerase IIa varies with respect to the proliferative state of the cell and the position in the cell cycle (Heck and Earnshaw 1986; Fairman and Brutlag 1988; Swedlow et al. 1993). Actively proliferating cells contain about  $10^5$ copies per cell, whereas the enzyme is hardly detectable in guiescent or terminally differentiated cells (Heck and Earnshaw 1986; Fairman and Brutlag 1988; Hsiang et al. 1988). The nuclear level of enzyme raises steadily from the end of mitosis until prophase and drops rapidly during metaphase and anaphase (Swedlow et al. 1993), suggestive of a mitotic involvement of the enzyme. In contrast to the  $\alpha$  form, immunohistochemical studies of topoisomerase IIB expression have shown that this enzyme is mainly expressed in Go cells, and the amount of the enzyme is more or less constant once the cells have entered the cell cycle (Drake et al. 1989a; Woessner et al. 1991; Prosperi et al. 1992).

Topoisomerase II has been shown to be posttranslationally modified by phosphorylation (Cardenas and Gasser 1993), with the major phosphorylation sites located in the extreme carboxy-terminal domain. Phosphorylation modulates the overall catalytic activity of topoisomerase II by stimulating the rate-limiting ATP hydrolysis step and, thus, the catalytic turnover rate of the enzyme (Corbett et al. 1992, 1993). Several lines of evidence have suggested casein kinase II as the kinase responsible for phosphorylation in vivo (Ackerman et al. 1988; Heck et al. 1989; Cardenas et al. 1992; Saijo et al. 1992), but protein kinase C and p34<sup>cdc2</sup> kinase may play a role as well (Rottmann et al. 1987; Cardenas et al. 1992; DeVore et al. 1992). Cell cycle variations exist in the level of topoisomerase II phosphorylation (Heck et al. 1988, 1989; Cardenas et al. 1992; Saijo et al. 1992), with the enzyme being hyperphosphorylated during mitosis. Furthermore, the phosphorylation patterns vary throughout the cell cycle for both topoisomerase II isoforms (Burden and Sullivan 1994; Kimura et al. 1994).

#### Enzymology of Topoisomerase II

Eukaryotic topoisomerase II introduces transient double-stranded breaks into the DNA backbone and changes the linking number of DNA in steps of two, via a double-stranded energy-requiring DNA passage mechanism (Vosberg 1985; Maxwell and Gellert 1986; Osheroff 1989b). The enzyme catalyzes relaxation of negatively as well as positively supercoiled DNA (Osheroff et al. 1983; Schomburg and Grosse 1986) and is able to interconvert different topological forms of double-stranded DNA by catenation/decatenation (Hsieh and Brutlag 1980; Goto and Wang 1982) and knotting/unknotting (see Table 1 and Fig. 1) (Liu et al. 1980; Hsieh 1983).

Topoisomerase II requires the presence of divalent cations (Goto and Wang 1982; Goto et al. 1984; Osheroff 1987; Andersen et al. 1989; Lee et al. 1989) and ATP as an energy source (Shelton et al. 1983; Goto et al. 1984; Osheroff 1986) for all catalytic reactions. The enzyme relaxes supercoiled DNA in a highly processive manner, as the rate of relaxation is considerably faster than the rate of dissociation of the enzyme/DNA complex (Osheroff et al. 1983; Schomburg and Grosse 1986).

A number of mechanistic studies on topoisomerase II have broken the reaction into at least six discrete steps, as shown in Figure 3 (for review, see Osheroff et al. 1991; Orphanides and Maxwell 1994). A current model (Roca and Wang 1992, 1994) suggests that the enzyme acts as a molecular clamp, which is open in the absence of ATP and closed in the presence of ATP or an ATP analog. In the absence of ATP, the enzyme can bind noncovalently to a linear or circular DNA, the gate segment or G-segment (step 1). Another DNA segment, the transported segment or T-segment, can now pass in and out of the clamp as long as the clamp stays in the open form (step 2). Still in the absence of ATP, topoisomerase II can mediate a double-stranded cleavage of the G-segment, which can be religated without any change in the overall DNA topology (step 3). The cleavage/religation process is an equilibrium, which normally is shifted toward religation. However, when ATP is present and binds to the DNA-bound enzyme, a conformational change occurs, whereby the protein clamp closes. If a T-segment has entered the clamp before closure, it will be trapped in the clamp and transported through the transiently cleaved G-segment (step 4). How the final exit of the Tsegment from the complex takes place has been a subject of much debate. A two-gate operation of the enzyme has been proposed to explain how the T-segment leaves the enzyme/DNA complex through a second opening in the enzyme (Roca and Wang 1994). According to this, the second protein gate recloses upon the exit of the T-segment (step 5), and after hydrolysis of ATP the clamp returns to its initial conformation (step 6). The enzyme is then able to enter a new catalytic cycle or dissociate from the DNA.

The steps 1 (noncovalent DNA binding) and 3 (cleavage/religation) have been studied in detail. Noncovalent DNA binding can be analyzed in the absence of divalent cations (Osheroff 1987; Sander et al. 1987). The enzyme binds preferentially to negatively supercoiled DNA (Osher-



Figure 3 Steps in the catalytic cycle of the homodimeric, eukaryotic topoisomerase II enzyme. DNA topoisomerase II binds noncovalently to the G-segment (step 1) and another DNA segment, the T-segment, can pass in and out of the molecular clamp formed by the enzyme (step 2) (Roca and Wang 1992). The enzyme introduces a transient double-stranded cleavage in the G-segment (step 3). In the presence of ATP, the enzyme undergoes a conformational change allowing the T-segment to pass through the break in the G-segment and leave the complex via a second opening in the enzyme (step 4). After reclosure of this opening (step 5), ATP is hydrolyzed, and the enzyme returns to its initial conformation (step 6). The dark and light helices represent the G- and T-segments, respectively.

off and Brutlag 1983; Osheroff 1986), and footprinting has shown that the enzyme protects about 25 bp (Lee et al. 1989; Thomsen et al. 1990).

Subsequent to DNA binding, topoisomerase II will cleave the DNA and enter a cleavage/religation equilibrium if divalent cations are present. The homodimeric enzyme makes a 4-bp staggered cleavage of the DNA duplex (for reviews, see Vosberg 1985; Maxwell and Gellert 1986) and becomes linked to the protruding 5' ends through O<sup>4</sup>-phosphotyrosine bonds (Liu et al. 1983; Sander and Hsieh 1983). The cleavage complex has been studied in detail by treatment of the equilibrium with a strong denaturing agent, which traps the covalently linked enzyme/DNA complex (for reviews, see Vosberg 1985; Maxwell and Gellert 1986; Osheroff 1989b). Identical cleavage complexes can be generated in an active form, when suicidal DNA substrates are used for topoisomerase II, i.e., single-stranded DNA or duplex DNA 5'recessed on one strand (Gale and Osheroff 1990; Andersen et al. 1991). Investigation of cleavage complex formation has shown that eukaryotic topoisomerase II acts at preferred sites and shows some nucleotide sequence specificity in its cleavage reaction (Spitzner and Muller 1988; Andersen et al. 1989; Spitzner et al. 1990), although only rather weak consensus sequences have been obtained (Sander and Hsieh 1985; Spitzner and Muller 1988). The minimum duplex region required for topoisomerase-II-mediated cleavage has been established to be 16 bp located symmetrically around the 4-bp stagger (Lund et al. 1990). DNA cleavage absolutely requires the presence of divalent cations (Sander and Hsieh 1983; Osheroff 1987; Andersen et al. 1991), usually magnesium, but calcium can be substituted (Osheroff and Zechiedrich 1987; Andersen et al. 1989; Zechiedrich et al. 1989). Furthermore, the reaction has no requirement for ATP, although the energy cofactor stimulates cleavage two- to threefold (Sander and Hsieh 1983; Osheroff 1986).

When suicidal DNA substrates are used, the cleavage complex contains a kinetically competent topoisomerase II enzyme able to perform ligation of the cleaved DNA to a free 3'-hydroxyl end on another DNA strand (Gale and Osheroff 1990, 1992; Andersen et al. 1991). Studies of the religation half-reaction per se have shown that the efficiency of the ligation reaction is enhanced if the ligating DNA is able to hybridize to the cleaved DNA (Andersen et al. 1991; Schmidt et al. 1994). Dinucleotides capable of base-pairing are ligated, but the ligation reaction displays a strong preference for double-stranded DNA with a fourbase, single-stranded region (Andersen et al. 1991; Schmidt et al. 1994). Like the cleavage reaction, the religation half-reaction absolutely requires divalent cations, but it takes place in the absence of ATP (Gale and Osheroff 1990; Andersen et al. 1991).

# INHIBITORS OF DNA TOPOISOMERASES

DNA topoisomerases have been identified as the cellular target for a number of clinically relevant antineoplastic drugs (Table 2) (for reviews, see Liu 1989; Osheroff et al. 1991; Pommier 1993; Wang 1994). The drugs interfere with the catalytic cycle of topoisomerase I and II and can be divided into two groups on the basis of their interaction mode. Drugs belonging to the first group (group I) influence the cleavage/religation equilibrium (step 3 in Fig. 3), stabilizing the covalently linked topoisomerase/DNA intermediate by stimulation of the forward cleavage

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Table 2 Inhibitors of DNA to	poisomerases		
Drug classes	Examples	Drug effect	References
		Group I	
Topoisomerase I inhibitors			
camptothecins	camptothecin topotecan CPT-11	complex formation; inhibits catalytic activity	Liu (1989); Hsiang et al. (1985)
Topoisomerase II inhibitors			
epipodophyllotoxins	VM-26 VP-16	complex formation; inhibits religation and catalytic activity	Chen et al. (1984); Osheroff (1989a)
isoflavonoids	genistein	complex formation; inhibits strand passage and ATP hydrolysis	Marcovits et al. (1989)
acridines	m-AMSA	complex formation; inhibits religation, strand passage, and ATP hydrolysis	Robinson and Osheroff (1990); Sørensen et al. (1992); Robinson et al. (1991, 1993)
anthracenediones	mitoxantrone	complex formation	Fox and Smith (1990)
anthracyclines	adriamycin (doxorubicin)	complex formation	Capranico et al. (1990)

ellipticines	9-hydroxy-iso- ellipticine	complex formation	Tewey et al. (1984)
2-nitroimidazoles	Ro 15-0216	complex formation; no inhibition of catalytic activity	Sørensen et al. (1990, 1992)
quinolones	CP-115,953	complex formation; stimulates cleavage; inhibits strand passage and ATP hydrolysis	Robinson et al. (1991, 1993)
		Group II	
anthracenyl peptides	merbarone	inhibits catalytic activity	Drake et al. (1989b)
anthracyclines	aclarubicin	antagonizes complex formation by other drugs; inhibits catalytic activity	Jensen et al. (1990)
bisdioxopiperazines	ICRF-193	antagonizes complex formation by other drugs; inhibits catalytic activity	Tanabe et al. (1991); Roca et al. (1994)
hexasulfated naphthylurea	suramin	antagonizes complex formation by other drugs; inhibits catalytic activity	Bojanowski et al. (1992)

reaction and/or inhibition of the religation half-reaction (Osheroff 1989a; Robinson and Osheroff 1990; Sørensen et al. 1992). Camptothecins, which for the time being are the most potent topoisomerase I inhibitors, belong to this group (Kunimoto et al. 1987; Johnson et al. 1989). Group I also includes the most potent topoisomerase II drugs, which can broadly be categorized into DNA intercalators including amsacrine (mAMSA), ellipticine, and mitoxantrone; and non-intercalators like etoposide (VP16), teniposide (VM26), genistein, and the quinolone, CP115,953 (Liu 1989; Osheroff et al. 1991).

The second group (group II) includes agents that interfere with the catalytic cycle of topoisomerases without trapping the covalent topoisomerase/DNA complex. Members of this group are merbarone (Drake et al. 1989b), aclarubicin (Jensen et al. 1990), ICRF-193 (Tanabe et al. 1991; Roca et al. 1994), and suramin (Bojanowski et al. 1992), all of which target topoisomerase II.

Drugs belonging to both groups have been widely used as tools to probe the reaction mechanisms of DNA topoisomerases. Group I drugs have been used in the mapping of topoisomerase/DNA interaction sites in vitro and in vivo. Drug-trapping of the enzyme covalently linked to cleaved DNA has allowed studies of the DNA sequence specificities of the enzymes, although sites cleaved by topoisomerases are stimulated differently by drug treatment (Kjeldsen et al. 1988).

Studies have shown that topoisomerase inhibitors exert different base preferences, indicating drug interaction with the preferred base (Pommier et al. 1993). This has been further demonstrated by Kreuzer and coworkers, who have shown cross-linking of a photoreactive mAMSA derivative to one of the residues in the base pair 5' to the cleaved phosphodiester bond (Freudenreich and Kreuzer 1994).

Information on the mechanistic activity of group II drugs is scarce. However, ICRF-193 has been found to inhibit topoisomerase II via a novel mechanism (Roca et al. 1994). In the presence of ATP, the drug locks the enzyme in a salt-stable conformation, analogous to the ATPbound closed-clamp form of topoisomerase II. After formation of the closed form, ATP can be removed without opening of the clamp, suggesting that ICRF-193 acts by inhibiting the interconversion between the open and closed form of the enzyme (steps 5 and 6 in Fig. 3).

The final result of cell treatment with most topoisomerase-targeting drugs is cell killing, but little is known about the underlying causes of this event (for review, see Zhang et al. 1990). A major determinant of group I drug cytotoxicity is suggested to be an interconversion of the drug-stimulated, enzyme-mediated cleavages into irreversible doublestranded DNA breaks. This model is supported by the fact that mutations in *RAD52*, which is involved in the repair of double-stranded breaks, increase the cytotoxicity of these drugs considerably (Nitiss and Wang 1988). Some of the likely candidates responsible for the conversion are the replicational and transcriptional machineries through their collision with the drug-induced cleavage complexes (Snapka 1986; Snapka et al. 1988; Hsiang et al. 1989; Bendixen et al. 1990; Thomsen et al. 1990). Cell killing by group II drugs targeting topoisomerase II has been suggested to result from chromosomal breakage and aneuploidy due to progression through mitosis in the absence of a functional topoisomerase II enzyme (Holm et al. 1989; Downes et al. 1991; Wang 1994) (see below).

#### **BIOLOGICAL FUNCTIONS OF DNA TOPOISOMERASES**

# **Topoisomerases as Modulators of Transcription**

In 1987, Liu and Wang proposed that the tracking of an RNA polymerase along a DNA helix would generate a positively supercoiled domain in front of it and a negatively supercoiled domain behind it. Experiments supporting this model have been reported in both prokaryotes (Wu et al. 1988) and eukaryotes (Giaever and Wang 1988). The so-called "twin supercoil domain" model clearly demonstrates a need for topoisomerases in transcription.

Topoisomerase I has been shown to be preferentially associated with genes actively transcribed by RNA polymerase II (Gilmour and Elgin 1987; Stewart and Schutz 1987; Kroeger and Rowe 1989, 1992; Stewart et al. 1990), and RNA elongation has been found to be inhibited by antibodies directed against topoisomerase I (Egyhazi and Durban 1987). Furthermore, a kinetic study of RNA polymerase II and topoisomerase I activities on the *c-fos* proto-oncogene after activation has revealed a close linkage of the two activities (Stewart et al. 1990).

Besides a role of topoisomerase I in transcriptional elongation, a number of recent reports argue that topoisomerase I plays a direct role in the regulation of transcriptional initiation by acting as a repressor of the basal-level transcription (Choder 1991; Merino et al. 1993). A hint toward a specific mechanism for transcriptional repression was given by the finding that topoisomerase I might interact directly with the TATAbinding protein (TBP), thereby competing with the binding of TFIIA and other TBP-binding factors. The repression obtained by this interaction seems independent of topoisomerase I enzymatic activity, because active-site mutants behave similarly (Merino et al. 1993).

An involvement of topoisomerase II in RNA polymerase II transcription has been inferred by the preferential presence of topoisomerase II cleavage sites in DNase-hypersensitive regions 5' or 3' to actively transcribed genes (Reitman and Felsenfeld 1990; Kroeger and Rowe 1992) and by the localization of topoisomerase-II-mediated cleavage sites in transcriptional enhancer regions (Cockerill and Garrard 1986; Gasser and Laemmli 1986).

The transcription of rDNA by RNA polymerase I constitutes around 80% of the total transcription in *S. cerevisiae*, and accumulation of 18S and 25S rRNA is critically dependent on the presence of either topoisomerase I or topoisomerase II (Brill et al. 1987). An abundance of topoisomerase I in nucleoli has been documented by immuno-fluorescence and by SDS cleavage studies (Fleischmann et al. 1984; Muller et al. 1985; Zhang et al. 1988). Furthermore, high-affinity binding sites for topoisomerase I have been reported in the upstream spacer region in the rDNA from *Tetrahymena* and *Dictyostelium* (Gocke et al. 1983; Andersen et al. 1985; Bonven et al. 1985; Ness et al. 1986; Bettler et al. 1988).

In contrast to RNA polymerases I and II, RNA polymerase III is apparently unaffected by topoisomerase activity in vivo, as shown by Brill et al. (1987), who reported that the amount of tRNA produced in an *S. cerevisiae top1-top2<sup>ts</sup>* double mutant was indistinguishable from the amount in wild-type cells.

# DNA Topoisomerases in Replication, Mitosis, and Meiosis

Ongoing replication requires the removal of positive supercoils generated ahead of the replication fork (for review, see Nitiss 1994), and on the basis of studies on the replication of SV40 DNA (Sundin and Varshavsky 1981; Yang et al. 1987; Snapka et al. 1988; Porter and Champoux 1989), it has been proposed that topoisomerase I provides the major swivel activity during replicational elongation. The enzyme is, however, lost from the DNA before replication has finished, due to steric hindrance from the two progressing replication forks. In the absence of swivel activity, continued replication fork movement results in daughter molecule intertwinings, which subsequently are removed by topoisomerase-II-mediated decatenation (Nelson et al. 1986). The model has been supported by data obtained with different yeast mutants. When the replicational elongation process is studied in yeast, *top1* mutants, but not *top2* mutants, show a distinct delay in replicational elongation (Kim and Wang 1989a). Furthermore, topoisomerase II appears essential for cell survival (Goto and Wang 1984), where the onset of inviability coincides with the time of mitosis (Holm et al. 1985). Inviability can, however, be prevented if formation of the mitotic spindle is inhibited by nocodazole, suggesting an involvement of topoisomerase II during chromosome segregation. *Top1* mutants show a dispensability of topoisomerase I activity for yeast survival (Goto and Wang 1985; Thrash et al. 1985), whereas *top1-top2<sup>ts</sup>* double mutants show phenotypes strikingly different from those of the single mutants in that they rapidly stop growth at the nonpermissive temperature, irrespective of the cell cycle stage (Uemura and Yanagida 1984; Goto and Wang 1985; Holm et al. 1985; Brill et al. 1987).

Studies with the yeast mutants have shown that topoisomerase-IImediated decatenation of interwound sister chromatids takes place at mitosis, rather than at the end of replication. This has been proposed to result from a requirement of a directional force for topoisomerase II. During mitosis, a force is given by the pulling of the mitotic spindle, which can direct the untangling activity of the enzyme (Holm et al. 1989; Holm 1994). There seems to be no regulatory checkpoint for the untangling process in yeast. Thus, in the absence of topoisomerase II activity, chromosome segregation still proceeds, resulting in chromosome breakage, nondisjunction, and finally, cell death (Uemura and Yanagida 1986; Uemura et al. 1987b; Holm et al. 1989; Spell and Holm 1994). In mammalian cells, however, strong evidence for a topoisomerase-II-dependent checkpoint in  $G_2$  has recently been described (Downes et al. 1994).

During meiosis I, topoisomerase II is required at the time of chromosome segregation for the resolution of recombined chromosomes (Rose et al. 1990). *Top2ts* yeast mutants induced to sporulate at the nonpermissive temperature complete early meiotic events, including premeiotic DNA synthesis and commitment to meiotic levels of recombination, but arrest in meiosis I prior to spindle formation, before suffering significant DNA damage. Meiotic cells thus have a checkpoint mechanism, in contrast to mitotic cells, preventing an otherwise lethal chromosome segregation (Rose and Holm 1993). In meiosis II, topoisomerase II plays a role similar to that in mitosis (Rose et al. 1990).

## **Topoisomerases in Recombination**

Mutant strains of S. cerevisiae lacking topoisomerase I, II, or III display a dramatic increase in homologous recombination. Christman et al. (1988) reported that strongly increased levels of mitotic recombination occur in top1 and  $top2^{ts}$  single mutants. The effect was only observed when measuring the rate of loss of a marker embedded in the rDNA array. No measurable increase or decrease in recombination was detected when direct repeat recombination was studied elsewhere in the genome (Christman et al. 1988). A strong effect upon recombination has also been observed in yeast cells deficient for topoisomerase III, where a more than 70-fold increase in the frequency of rDNA marker loss is seen (Gangloff et al. 1994). In contrast to the observation with top1 and top2 mutants, top3 mutants have also been found to be hyperrecombinogenic for mitotic recombination events occurring outside the rDNA locus (Wallis et al. 1989; Bailis et al. 1992). It appears that it is the combined action of topoisomerases I, II, and III that stabilizes the genomic rDNA array, performing different, but overlapping, functions. The exact cause for the elevated recombination rates has not been defined, but it has been suggested that highly supercoiled DNA structures in the topoisomerase mutants are processed into recombinogenic lesions by the excision repair machinery (Kim and Wang 1989b; Bailis et al. 1992). A recent finding by Fink and coworkers supports the notion that aberrant DNA structures do arise due to the lack of DNA topoisomerase activity, as they have demonstrated an rDNA-linked chromosome abnormality in S. cerevisiae top1 mutants, but not in top2 mutants (Christman et al. 1993). Whether these structures are a result of recombination or aberrant replication is still unclear. Although topoisomerases act as suppressors of recombination, none of the topoisomerase mutants has so far been shown to have deficiencies in the mechanism of homologous recombination.

Both topoisomerases I and II are able to rearrange DNA fragments in vitro when suicidal DNA substrates are used for cleavage (Been and Champoux 1981; Champoux et al. 1984; Bae et al. 1988; Gale and Osheroff 1990, 1992; Andersen et al. 1991; Christiansen et al. 1993). The covalent complexes generated with these substrates are energetically active and religate intra- and intermolecularly to free hydroxyl ends. Topoisomerases are thus possible candidates as mediators of illegitimate recombination under the assumption that similar aberrant DNA structures might be present in vivo and physically available to the action of topoisomerases. Studies of the junction sequences resulting from illegitimate recombination events have indicated a high occurrence of sites matching the consensus sequence of topoisomerase I, at or near the junctions, both in mammalian cells (Bullock et al. 1985; Konopka 1988; Wang and Rogler 1991) and in yeast (Schiestl et al. 1993). Using a similar approach, it has also been suggested that topoisomerase II promotes illegitimate recombination in vivo (Sperry et al. 1989; Han et al. 1993) and in vitro (Schmidt et al. 1994).

## **CONCLUDING REMARKS**

DNA topoisomerases are ubiquitous enzymes that change the topological state of DNA without altering the overall outline of the DNA double helix (Vosberg 1985; Maxwell and Gellert 1986; Watt and Hickson 1994). In the past decade, the biological importance of these enzymes has been manifested through genetic and biochemical studies that have described roles of the enzymes in transcription, replication, recombination, and chromosome dynamics. Recently, new forms of both type I and type II topoisomerases have been discovered, and it is hoped that the future will elucidate the distinct roles of the different eukaryotic topoisomerase enzymes.

An interesting area in the topoisomerase field is the interaction of topoisomerases with other proteins. The interaction between topoisomerase II and the scaffold protein ScII (Ma et al. 1993), and the newly discovered interaction between topoisomerase III and a DNA helicase (Gangloff et al. 1994), are examples of such interactions. Interacting proteins might be instrumental in unraveling the biological functions of eukaryotic DNA topoisomerases.

## Note Added in Proof

The crystal structures of fragments of topoisomerase I and II have now been determined (Lue et al. 1995; Berger et al. 1996), and together with the biochemical and genetic data, they will contribute significantly to an understanding of the detailed mechanism of action of these enzymes.

## ACKNOWLEDGMENTS

We thank Dr. Kent Christiansen for his critical reading of the manuscript and Lumir Krejcí for assistance with the drawings. The work is supported by the Danish Cancer Society (grants 93-004 and 78-5000), the Danish Centre for Human Genome Research, and the Danish Centre for Respiratory Physiological Adaptation.

#### REFERENCES

- Ackerman, P., C.V. Glover, and N. Osheroff. 1988. Phosphorylation of DNA topoisomerase II *in vivo* and in total homogenates of *Drosophila* Kc cells. The role of casein kinase II. J. Biol. Chem. 263: 12653-12660.
- Adachi, N., M. Miyaike, H. Ikeda, and A. Kikuchi. 1992. Characterization of cDNA encoding the mouse DNA topoisomerase II that can complement the budding yeast top2

mutation. Nucleic Acids Res. 20: 5297-5303.

- Alsner, J., J.Q. Svejstrup, E. Kjeldsen, B.S. Sørensen, and O. Westergaard. 1992. Identification of an N-terminal domain of eukaryotic DNA topoisomerase I dispensable for catalytic activity but essential for *in vivo* function. *J. Biol. Chem.* **267**: 12408–12411.
- Andersen, A.H., E. Gocke, B.J. Bonven, O.F. Nielsen, and O. Westergaard. 1985. Topoisomerase I has a strong binding preference for a conserved hexadecameric sequence in the promoter region of the rRNA gene from *Tetrahymena pyriformis*. Nucleic Acids Res. 13: 1543-1557.
- Andersen, A.H., K. Christiansen, E.L. Zechiedrich, P.S. Jensen, N. Osheroff, and O. Westergaard. 1989. Strand specificity of the topoisomerase II mediated double-stranded DNA cleavage reaction. *Biochemistry* 28: 6237–6244.
- Andersen, A.H., B.S. Sørensen, K. Christiansen, J.Q. Svejstrup, K. Lund, and O. Westergaard. 1991. Studies of the topoisomerase II-mediated cleavage and religation reactions by use of a suicidal double-stranded DNA substrate. J. Biol. Chem. 266: 9203-9210.
- Austin, C.A., J.H. Sng, S. Patel, and L.M. Fisher. 1993. Novel HeLa topoisomerase II is the II beta isoform: Complete coding sequence and homology with other type II topoisomerases. *Biochim. Biophys. Acta* 1172: 283–291.
- Bae, Y.S., I. Kawasaki, H. Ikeda, and L.F. Liu. 1988. Illegitimate recombination mediated by calf thymus DNA topoisomerase II in vitro. Proc. Natl. Acad. Sci. 85: 2076-2080.
- Bailis, A.M., L. Arthur, and R. Rothstein. 1992. Genome rearrangement in *top3* mutants of *Saccharomyces cerevisiae* requires a functional *RAD1* excision repair gene. *Mol. Cell. Biol.* **12**: 4988–4993.
- Been, M.D. and J.J. Champoux. 1981. DNA breakage and closure by rat liver type 1 topoisomerase: Separation of the half-reactions by using a single-stranded DNA substrate. *Proc. Natl. Acad. Sci.* 78: 2883–2887.
- Been, M.D., R.R. Burgess, and J.J. Champoux. 1984. Nucleotide sequence preference at rat liver and wheat germ type I DNA topoisomerase breakage sites in duplex SV40 DNA. *Nucleic Acids Res.* 12: 3097–3114.
- Bendixen, C., B. Thomsen, J. Alsner, and O. Westergaard. 1990. Camptothecin-stabilized topoisomerase I-DNA adducts cause premature termination of transcription. *Biochemistry* 29: 5613–5619.
- Berger, J.M., S.J. Gamblin, S.C. Harrison, and J.C. Wang. 1996. Structure and mechanism of DNA topoisomerase II. *Nature* **379**: 225–232.
- Bettler, B., P.J. Ness, S. Schmidlin, and R.W. Parish. 1988. The upstream limit of nuclease-sensitive chromatin in *Dictyostelium* rRNA genes neighbors a topoisomerase I-like cluster. *J. Mol. Biol.* 204: 549–559.
- Bojanowski, K., S. Lelievre, J. Markovits, J. Couprie, A. Jacquemin-Sablon, and A.K. Larsen. 1992. Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells. *Proc. Natl. Acad. Sci.* **89**: 3025–3029.
- Bonven, B.J., E. Gocke, and O. Westergaard. 1985. A high affinity topoisomerase I binding sequence is clustered at DNase I hypersensitive sites in *Tetrahymena* R-chromatin. *Cell* **41**: 541-551.
- Brill, S.J. and R. Sternglanz. 1988. Transcription-dependent DNA supercoiling in yeast DNA topoisomerase mutants. *Cell* 54: 403–411.
- Brill, S.J., S. DiNardo, K. Voelkel-Meiman, and R. Sternglanz. 1987. Need for DNA topoisomerase activity as a swivel for DNA replication and for transcription of ribo-

somal RNA. Nature 326: 414-416.

- Bullock, P., J.J. Champoux, and M. Botchan. 1985. Association of crossover points with topoisomerase I cleavage sites: A model for nonhomologous recombination. *Science* **230**: 954–958.
- Burden, D.A. and D.M. Sullivan. 1994. Phosphorylation of the  $\alpha$  and  $\beta$ -isoforms of the DNA topoisomerase II is quantitatively different in interphase and mitosis in Chinese hamster ovary cells. *Biochemistry* 33: 14651–14655.
- Busk, H., B. Thomsen, B.J. Bonven, E. Kjeldsen, O.F. Nielsen, and O. Westergaard. 1987. Preferential relaxation of supercoiled DNA containing a hexadecameric recognition sequence for topoisomerase I. *Nature* 327: 638-640.
- Camilloni, G., E. Di-Martino, M. Caserta, and E. Di Mauro. 1988. Eukaryotic DNA topoisomerase I reaction is topology dependent. *Nucleic Acids Res.* 16: 7071-7085.
- Camilloni, G., E. Di-Martino, E. Di Mauro, and M. Caserta. 1989. Regulation of the function of eukaryotic DNA topoisomerase I: Topological conditions for inactivity. *Proc. Natl. Acad. Sci.* 86: 3080-3084.
- Capranico, G., F. Zunino, K. Kohn, and Y. Pommier. 1990. Sequence-selective topoisomerase II inhibition by anthracycline derivatives in SV40 DNA: Relationship with DNA binding affinity and cytotoxicity. *Biochemistry* **29**: 562–569.
- Cardellini, E. and E. Durban. 1993. Phosphorylation of human topoisomerase I by protein kinase C *in vitro* and in phorbol 12-myristate 13-acetate-activated HL-60 promyelo-cytic leukaemia cells. *Biochem. J.* **291**: 303-307.
- Cardenas, M.E. and S.M. Gasser. 1993. Regulation of topoisomerase II by phosphorylation: A role for casein kinase II. J. Cell Sci. 104: 219-225.
- Cardenas, M.E., Q. Dang, C.V. Glover, and S.M. Gasser. 1992. Casein kinase II phosphorylates the eukaryote-specific C-terminal domain of topoisomerase II in vivo. *EMBO J.* 11: 1785–1796.
- Caron, P.R., P. Watt, and J.C. Wang. 1994. The C-terminal domain of *Saccharomyces* cerevisiae DNA topoisomerase II. *Mol. Cell. Biol.* 14: 3197–3207.
- Caserta, M., A. Amadei, G. Camilloni, and E. Di Mauro. 1990. Regulation of the function of eukaryotic DNA topoisomerase I: Analysis of the binding step and of the catalytic constants of topoisomerization as a function of DNA topology. *Biochemistry* 29: 8152–8157.
- Caserta, M., A. Amadei, E. Di Mauro, and G. Camilloni. 1989. In vitro preferential topoisomerization of bent DNA. Nucleic Acids Res. 17: 8463-8474.
- Champoux, J.J. and R. Dulbecco. 1972. An activity from mammalian cells that untwists superhelical DNA-A possible swivel for DNA replication. *Proc. Natl. Acad. Sci.* 69: 143-146.
- Champoux, J.J. and B.L. McConaughy. 1976. Purification and characterization of the DNA untwisting enzyme from rat liver. *Biochemistry* 15: 4638-4642.
- Champoux, J.J., W.K. McCoubrey, and M.D. Been. 1984. DNA structural features that lead to strand breakage by eukaryotic type I topoisomerase. *Cold Spring Harbor Symp. Quant. Biol.* **49:** 435-442.
- Chen, G.L., L. Yang, T.C. Rowe, B.D. Halligan, K.M. Tewey, and L.F. Liu. 1984. Nonintercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem. 259: 13560-13566.
- Choder, M. 1991. A general topoisomerase I-dependent transcriptional repression in the stationary phase in yeast. *Genes Dev.* 5: 2315–2326.

Christiansen, K. and O. Westergaard. 1993. Involvement of eukaryotic topoisomerase I in

illegitimate recombination: Generation of deletions and insertions in DNA repair mechanisms. *Proc. Alfred Benzon Symp.* 35: 361–371.

- Christiansen, K., A.B. Svejstrup, A.H. Andersen, and O. Westergaard. 1993. Eukaryotic topoisomerase I-mediated cleavage requires bipartite DNA interaction. Cleavage of DNA substrates containing strand interruptions implicates a role for topoisomerase I in illegitimate recombination. J. Biol. Chem. 268: 9690-9701.
- Christman, M.F., F.S. Dietrich, and G.R. Fink. 1988. Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. *Cell* 55: 413-425.
- Christman, M.F., F.S. Dietrich, N.A. Levin, B.U. Sadoff, and G.R. Fink. 1993. The rDNA-encoding DNA array has an altered structure in topoisomerase mutants of *Sac-* charomyces cerevisiae. Proc. Natl. Acad. Sci. 90: 7637-7641.
- Cockerill, P.N. and W.T. Garrard. 1986. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* 44: 273–282.
- Corbett, A.H., R.F. DeVore, and N. Osheroff. 1992. Effect of casein kinase II-mediated phosphorylation on the catalytic cycle of topoisomerase II. Regulation of enzyme activity by enhancement of ATP hydrolysis. J. Biol. Chem. 267: 20513-20518.
- Corbett, A.H., A.W. Fernald, and N. Osheroff. 1993. Protein kinase C modulates the catalytic activity of topoisomerase II by enhancing the rate of ATP hydrolysis: Evidence for a common mechanism of regulation by phosphorylation. *Biochemistry* 32: 2090–2097.
- Crenshaw, D.G. and T.-S. Hsieh. 1993. Function of the hydrophilic carboxyl terminus of type II DNA topoisomerase from *Drosophila melanogaster*. J. Biol. Chem. 268: 21335-21345.
- D'Arpa, P., P.S. Machlin, H. Ratrie, N.F. Rothfield, D.W. Cleveland, and W.C. Earnshaw. 1988. cDNA cloning of human DNA topoisomerase I: Catalytic activity of a 67.7-kDa carboxyl-terminal fragment. *Proc. Natl. Acad. Sci.* 85: 2543-2547.
- DeVore, R.F., A.H. Corbett, and N. Osheroff. 1992. Phosphorylation of topoisomerase II by casein kinase II and protein kinase C: Effects on enzyme-mediated DNA cleavage/religation and sensitivity to the antineoplastic drugs etoposide and 4'-(9-acridinylamino)methane- sulfon-m-anisidide. *Cancer Res.* 52: 2156-2161.
- Downes, C.S., A.M. Mullinger, and R.T. Johnson. 1991. Inhibitors of DNA topoisomerase II prevent chromatid separation in mammalian cells but do not prevent exit from mitosis. *Proc. Natl. Acad. Sci.* 88: 8895–8899.
- Downes, C.S., D.J. Clarke, A.M. Mullinger, J.F. Giménez-Abián, A.M. Creighton, and R.T. Johnson. 1994. A topoisomerase II-dependent G2 cycle checkpoint in mammalian cells. *Nature* **372**: 467–470.
- Drake, F.H., G.A. Hofmann, H.F. Bartus, M.R. Mattern, S.T. Crooke, and C.K. Mirabelli. 1989a. Biochemical and pharmacological properties of p170 and p180 forms of topoisomerase II. *Biochemistry* 28: 8154–8160.
- Drake, F.H., G.A. Hofmann, S.-M. Mong, J. O. Bartus, R.P. Hertzberg, R.K. Johnson, M.R. Mattern, and C.K. Mirabelli. 1989b. *In vitro* and intracellular inhibition of topoisomerase II by the antitumor agent merbarone. *Cancer Res.* 49: 2578–2583.
- Durban, E., M. Goodenough, J. Mills, and H. Busch. 1985. Topoisomerase I phosphorylation in vitro and in rapidly growing Novikoff hepatoma cells. *EMBO J.* 4:

2921-2926.

- Edwards, K.A., B.D. Halligan, J.L. Davis, N.L. Nivera, and L.F. Liu. 1982. Recognition sites of eukaryotic DNA topoisomerase I: DNA nucleotide sequencing analysis of topo I cleavage sites on SV40 DNA. *Nucleic Acids Res.* 10: 2565–2576.
- Egyhazi, E. and E. Durban. 1987. Microinjection of anti-topoisomerase I immunoglobulin G into nuclei of *Chironomus tentans* salivary gland cells leads to blockage of transcription elongation. *Mol. Cell. Biol.* 7: 4308-4316.
- Eng, W.K., S.D. Pandit, and R. Sternglanz. 1989. Mapping of the active site tyrosine of eukaryotic DNA topoisomerase I. J. Biol. Chem. 264: 13373-13376.
- Fairman, R. and D.L. Brutlag. 1988. Expression of the *Drosophila* type II topoisomerase is developmentally regulated. *Biochemistry* 27: 560–565.
- Fleischmann, G., G. Pflugfelder, E.K. Steiner, K. Javaherian, G.C. Howard, J.C. Wang, and S.C. Elgin. 1984. Drosophila DNA topoisomerase I is associated with transcriptionally active regions of the genome. Proc. Natl. Acad. Sci. 81: 6958–6962.
- Fox, M.E. and P.J. Smith. 1990. Long-term inhibition of DNA synthesis and the persistence of trapped topoisomerase II complexes in determining the toxicity of the antitumor DNA intercalators mAMSA and mitoxantrone. *Cancer Res.* 50: 5813–5818.
- Fragoso, S.P. and S. Goldenberg. 1992. Cloning and characterization of the gene encoding *Trypanosoma cruzi* DNA topoisomerase II. Mol. Biochem. Parasitol. 55: 127–134.
- Freudenreich, C. and K.N. Kreuzer. 1994. Localization of an aminoacridine antitumor agent in a type II topoisomerase-DNA complex. *Proc. Natl. Acad. Sci.* **91:** 11007–11011.
- Gale, K.C. and N. Osheroff. 1990. Uncoupling the DNA cleavage and religation activities of topoisomerase II with a single-stranded nucleic acid substrate: Evidence for an active enzyme-cleaved DNA intermediate. *Biochemistry* **29**: 9538–9545.
- -----. 1992. Intrinsic intermolecular DNA ligation activity of cukaryotic topoisomerase II. Potential roles in recombination. J. Biol. Chem. 267: 12090-12097.
- Gangloff, S., J.P. McDonald, C. Bendixen, L. Arthur, and R. Rothstein. 1994. The yeast type I topoisomerase *Top3* interacts with *Sgs1*, a DNA helicase homolog: A potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* 14: 8391–8398.
- Gartenberg, M.R. and J.C. Wang. 1992. Positive supercoiling of DNA greatly diminishes mRNA synthesis in yeast. *Proc. Natl. Acad. Sci.* 89: 11461-11465.
- Gasser, S.M. and U.K. Laemmli. 1986. Cohabitation of scaffold binding regions with upstream enhancer elements of three developmentally regulated genes of *D. melanogaster. Cell* **46**: 521–530.
- Giaever, G.N. and J.C. Wang. 1988. Supercoiling of intracellular DNA can occur in eukaryotic cells. *Cell* 55: 849-856.
- Giaever, G.N., R. Lynn, T. Goto, and J.C. Wang. 1986. The complete nucleotide sequence of the structural gene *TOP2* of yeast DNA topoisomerase II. J. Biol. Chem. 261: 12448-12454.
- Gilmour, D.S. and S.C. Elgin. 1987. Localization of specific topoisomerase I interactions within the transcribed region of active heat shock genes by using the inhibitor camptothecin. *Mol. Cell. Biol.* **7**: 141–148.
- Gocke, E., B.J. Bonven, and O. Westergaard. 1983. A site and strand specific nuclease activity with analogies to topoisomerase I frames the rRNA gene of *Tetrahymena*. *Nucleic Acids Res.* 11: 7661–7678.
- Goto, T. and J.C. Wang. 1982. Yeast DNA topoisomerase II. An ATP-dependent type II topoisomerase that catalyzes the catenation, decatenation, unknotting, and relaxation of

double-stranded DNA rings. J. Biol. Chem. 257: 5866-5872.

. 1984. Yeast DNA topoisomerase II is encoded by a single-copy, essential gene. *Cell* **36**: 1073-1080.

. 1985. Cloning of yeast *TOP1*, the gene encoding DNA topoisomerase I, and construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II. *Proc. Natl. Acad. Sci.* **82:** 7178–7182.

- Goto, T., P. Laipis, and J.C. Wang. 1984. The purification and characterization of DNA topoisomerases I and II of the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. 259: 10422-10429.
- Han, Y.-H., M.J.F. Austin, Y. Pommier, and L.F. Povirk. 1993. Small deletion and insertion mutations induced by the topoisomerase II inhibitor teniposide in CHO cells and comparison with sites of drug-stimulated DNA cleavage *in vitro*. J. Mol. Biol. 229: 52– 66.
- Heck, M.M. and W.C. Earnshaw. 1986. Topoisomerase II: A specific marker for cell proliferation. J. Cell Biol. 103: 2569–2581.
- Heck, M.M., W.N. Hittelman, and W.C. Earnshaw. 1988. Differential expression of DNA topoisomerases I and II during the eukaryotic cell cycle. *Proc. Natl. Acad. Sci.* 85: 1086–1090.

\_\_\_\_\_. 1989. In vivo phosphorylation of the 170-kDa form of eukaryotic DNA topoisomerase II. Cell cycle analysis. J. Biol. Chem. 264: 15161-15164.

- Hochhauser, D., C.A. Stanway, A.L. Harris, and I.D. Hickson. 1992. Cloning and characterization of the 5'-flanking region of the human topoisomerase II alpha gene. J. Biol. Chem. 267: 18961–18965.
- Holm, C. 1994. Coming Undone: How to untangle a chromosome. Cell 77: 955-957.
- Holm, C., T. Stearns, and D. Botstein. 1989. DNA topoisomerase II must act at mitosis to prevent nondisjunction and chromosome breakage. *Mol. Cell. Biol.* **9**: 159-168.
- Holm, C., T. Goto, J.C. Wang, and D. Botstein. 1985. DNA topoisomerase II is required at the time of mitosis in yeast. *Cell* **41**: 553–563.
- Hsiang, Y.H., M.G. Lihou, and L.F. Liu. 1989. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.* **49**: 5077–5082.
- Hsiang, Y.H., H.Y. Wu, and L.F. Liu. 1988. Proliferation-dependent regulation of DNA topoisomerase II in cultured human cells. *Cancer Res.* 48: 3230–3235.
- Hsiang, Y.H., R. Hertzberg, S. Hecht, and L.F. Liu. 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem. 260: 14873-14878.
- Hsieh, T.S. 1983. Knotting of the circular duplex DNA by type II DNA topoisomerase from *Drosophila melanogaster*. J. Biol. Chem. 258: 8413-8420.
- Hsieh, T.S. and D. Brutlag. 1980. ATP-dependent DNA topoisomerase from *D. melanogaster* reversibly catenates duplex DNA rings. *Cell* **21**: 115–125.
- Hsieh, T.S., S.D. Brown, P. Huang, and J. Fostel. 1992. Isolation and characterization of a gene encoding DNA topoisomerase I in *Drosophila melanogaster*. *Nucleic Acids Res.* **20:** 6177–6182.
- Jaxel, C., G. Capranico, D. Kerrigan, K.W. Kohn, and Y. Pommier. 1991. Effect of local DNA sequence on topoisomerase I cleavage in the presence or absence of camptothecin. J. Biol. Chem. 266: 20418-20423.
- Jenkins, J.R., P. Ayton, T. Jones, S.L. Davies, D.L. Simmons, A.L. Harris, D. Sheer, and I.D. Hickson. 1992. Isolation of cDNA clones encoding the beta isozyme of human

DNA topoisomerase II and localisation of the gene to chromosome 3p24. *Nucleic Acids Res.* 20: 5587–5592.

- Jensen, P.B., B.S. Sørensen, E.J.F. Demant, M. Sehested, P.S. Jensen, L. Vindeløv, and H.H. Hansen. 1990. Antagonistic effect of aclarubicin on the cytotoxicity of etoposide and 4'-(9-acridinyl-amino)methanesulfon-m-aniside in human small cell lung cancer cells and on topoisomerase II-mediated DNA cleavage. *Cancer Res.* 50: 3311-3316.
- Johnson, R.K., F.L. McCabe, L.F. Faucette, R.P. Hertzberg, W.D. Kinsbury, J.C. Boehm, M.J. Caranfa, and K.G. Holden. 1989. SK&F 10864, a water-soluble analog of camptothecin with broad-spectrum activity in preclinical tumor models. *Proc. Am. Assoc. Cancer Res.* 30: 623-628.
- Kim, R.A. and J.C. Wang. 1989a. Function of DNA topoisomerases as replication swivels in *Saccharomyces cerevisiae*. J. Mol. Biol. 208: 257-267.
- \_\_\_\_\_. 1989b. A subthreshold level of DNA topoisomerases leads to the excision of yeast rDNA as extrachromosomal rings. *Cell* **57**: 975–985.
- Kimura, K., N. Nozaki, M. Saijo, A. Kikuchi, M. Ui, and T. Enomoto. 1994. Identification of the nature of modification that causes the shift of DNA topoisomerase IIβ to apparent higher molecular forms in the M phase. J. Biol. Chem. 269: 24523-24526.
- Kjeldsen, E., S. Mollerup, B. Thomsen, B.J. Bonven, L. Bolund, and O. Westergaard. 1988. Sequence-dependent effect of camptothecin on human topoisomerase I DNA cleavage. J. Mol. Biol. 202: 333-342.
- Koiwai, O., Y. Yasui, Y. Sakai, T. Watanabe, K. Ishii, S. Yanagihara, and T. Andoh. 1993. Cloning of the mouse cDNA encoding DNA topoisomerase I and chromosomal location of the gene. *Gene* **125**: 211–216.
- Konopka, A.K. 1988. Compilation of DNA strand exchange sites for non-homologous recombination in somatic cells. *Nucleic Acids Res.* 16: 1739–1758.
- Kroeger, P.E. and T.C. Rowe. 1989. Interaction of topoisomerase I with the transcribed region of the *Drosophila* HSP 70 heat shock gene. *Nucleic Acids Res.* 17: 8495–8509.
- Krogh, S., U.H. Mortensen, O. Westergaard, and B.J. Bonven. 1991. Eukaryotic topoisomerase I-DNA interaction is stabilized by helix curvature. *Nucleic Acids Res.* 19: 1235-1241.
- Kunimoto, T., K. Nitta, T. Tanaka, N. Uebuara, H. Baba, M. Takeuchi, T. Yokokura, S. Sawada, T. Miyasaka, and M. Mutai. 1987. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res.* 47: 5944–5949.
- Kunze, N., M. Klein, A. Richter, and R. Knippers. 1990. Structural characterization of the human DNA topoisomerase I gene promoter. *Eur. J. Biochem.* **194**: 323–330.
- Lee, M.P., M. Sander, and T.S. Hsieh. 1989. Nuclease protection by *Drosophila* DNA topoisomerase II. Enzyme/DNA contacts at the strong topoisomerase II cleavage sites. *J. Biol. Chem.* 264: 21779–21787.
- Lindsley, J.E. and J.C. Wang. 1991. Proteolysis patterns of epitopically labeled yeast DNA topoisomerase II suggests an allosteric transition in the enzyme induced by ATP binding. *Proc. Natl. Acad. Sci.* 88: 10485–10489.
- Liu, L.F. 1989. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* 58: 351–375.
- Liu, L.F. and K.G. Miller. 1981. Eukaryotic DNA topoisomerases: Two forms of type I DNA topoisomerases from HeLa cell nuclei. *Proc. Natl. Acad. Sci.* **78**: 3487–3491.

- Liu, L.F. and J.C. Wang. 1987. Supercoiling of the DNA template during transcription. *Proc. Natl. Acad. Sci.* 84: 7024-7027.
- Liu, L.F., C.C. Liu, and B.M. Alberts. 1980. Type II DNA topoisomerases: Enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. *Cell* **19**: 697–707.
- Liu, L.F., T.C. Rowe, L. Yang, K.M. Tewey, and G.L. Chen. 1983. Cleavage of DNA by mammalian topoisomerase II. J. Biol. Chem. 258: 15365-15370.
- Lue, N., A. Sharma, A. Mondragón, and J.C. Wang. 1995. A 26 kDa yeast topoisomerase I fragment: Crystallographic structure and mechanism implications. *Structure* **3**: 1315–1322.
- Lund, K., A.H. Andersen, K. Christiansen, J.Q. Svejstrup, and O. Westergaard. 1990. Minimal DNA requirement for topoisomerase II-mediated cleavage in vitro. J. Biol. Chem. 265: 13856-13863.
- Lynn, R.M., M.A. Bjornsti, P.R. Caron, and J.C. Wang. 1989. Peptide sequencing and site-directed mutagenesis identify tyrosine-727 as the active site tyrosine of Saccharomyces cerevisiae DNA topoisomerase I. Proc. Natl. Acad. Sci. 86: 3559–3563.
- Ma, X., N. Saitoh, and P.J. Curtis. 1993. Purification and characterization of a nuclear DNA-binding factor complex containing topoisomerase II and chromosome scaffold protein 2. J. Biol. Chem. 268: 6182–6188.
- Markovits, J., C. Linassier, P. Fossé, J. Couprie, J. Pierre, A. Jacquemin-Sablon, J.-M. Saucier, J.-B. Le Pecq, and A.K. Larsen. 1989. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. *Cancer Res.* 49: 5111–5117.
- Maxwell, A. and M. Gellert. 1986. Mechanistic aspects of DNA topoisomerases. Adv. Protein Chem. 38: 69-107.
- McConaughy, B.L., L.S. Young, and J.J. Champoux. 1981. The effect of salt on the binding of the eukaryotic DNA nicking-closing enzyme to DNA and chromatin. *Biochim. Biophys. Acta* 655: 1–8.
- Merino, A., K.R. Madden, W.S. Lane, J.J. Champoux, and D. Reinberg. 1993. DNA topoisomerase I is involved in both repression and activation of transcription. *Nature* **365**: 227–232.
- Muller, M.T., W.P. Pfund, V.B. Mehta, and D.K. Trask. 1985. Eukaryotic type I topoisomerase is enriched in the nucleolus and catalytically active on ribosomal DNA. *EMBO J.* 4: 1237–1243.
- Nelson, W.G., L.F. Liu, and D.S. Coffey. 1986. Newly replicated DNA is associated with DNA topoisomerase II in cultured rat prostatic adenocarcinoma cells. *Nature* 322: 187–189.
- Ness, P.J., R.W. Parish, and T. Koller. 1986. Mapping of endogenous nuclease-sensitive regions and of putative topoisomerase sites of action along the chromatin of *Dic-tyostelium* ribosomal RNA genes. J. Mol. Biol. 188: 287–300.
- Nitiss, J.L. 1994. Roles of DNA topoisomerases in chromosomal replication and segregation. Adv. Pharmacol. 29A: 103-134.
- Nitiss, J.L. and J.C. Wang. 1988. DNA topoisomerase-targeting antitumor drugs can be studied in yeast. *Proc. Natl. Acad. Sci.* 85: 7501-7505.
- Orphanides, G. and A. Maxwell. 1994. In one gate, out the other. Curr. Biol. 4: 1006-1009.
- Osheroff, N. 1986. Eukaryotic topoisomerase II. Characterization of enzyme turnover. J. Biol. Chem. 261: 9944–9950.
  - -----. 1987. Role of the divalent cation in topoisomerase II mediated reactions.

Biochemistry 26: 6402-6406.

. 1989a. Effect of antineoplastic agents on the DNA cleavage/religation reaction of eukaryotic topoisomerase II: Inhibition of DNA religation by etoposide. *Biochemistry* **28**: 6157-6160.

\_\_\_\_\_. 1989b. Biochemical basis for the interactions of type I and type II topoisomerases with DNA. *Pharmacol. Ther.* **41**: 223-241.

- Osheroff, N. and D.L. Brutlag. 1983. Recognition of supercoiled DNA by *Drosophila* topoisomerase II. In *Mechanism of DNA replication and recombination* (ed. N.R. Cozzarelli), pp. 55–64. A.R. Liss, New York.
- Osheroff, N. and E.L. Zechiedrich. 1987. Calcium-promoted DNA cleavage by eukaryotic topoisomerase II: Trapping the covalent enzyme-DNA complex in an active form. *Biochemistry* **26**: 4303–4309.
- Osheroff, N., E.R. Shelton, and D.L. Brutlag. 1983. DNA topoisomerase II from *Drosophila melanogaster*. Relaxation of supercoiled DNA. J. Biol. Chem. 258: 9536-9543.
- Osheroff, N., E.L. Zechiedrich, and K.C. Gale. 1991. Catalytic function of DNA topoisomerase II. *BioEssays* 13: 269-273.
- Park, S.H., J.H. Yoon, Y.D. Kwon, and S.D. Park. 1993. Nucleotide sequence analysis of the cDNA for rat DNA topoisomerase II. *Biochem. Biophys. Res. Commun.* 193: 787-793.
- Pasion, S.G., J.C. Hines, R. Aebersold, and D.S. Ray. 1992. Molecular cloning and expression of the gene encoding the kinetoplast-associated type II DNA topoisomerase of *Crithidia fasciculata. Mol. Biochem. Parasitol.* 50: 57-67.
- Pommier, Y. 1993. DNA topoisomerase I and II in cancer chemotherapy: Update and perspectives. *Cancer Chemother. Pharmacol.* 32: 103-108.
- Pommier, Y., D. Kerrigan, K.D. Hartman, and R.I. Glazer. 1990. Phosphorylation of mammalian DNA topoisomerase I and activation by protein kinase C. J. Biol. Chem. 265: 9418-9422.
- Pommier, Y., K.W. Kohn, G. Capranico, and C. Jaxel. 1993. Base sequence selectivity of topoisomerase inhibitors suggest a common model for drug action. In *Molecular biology of DNA topoisomerases and its application to chemotherapy* (ed. T. Andoh et al.), pp. 215-227. CRC Press, Boca Raton, Florida.
- Porter, S.E. and J.J. Champoux. 1989. Mapping in vivo topoisomerase I sites on simian virus 40 DNA: Asymmetric distribution of sites on replicating molecules. *Mol. Cell. Biol.* 9: 541–550.
- Prosperi, E., E. Sala, C. Negri, C. Oliani, R. Supino, G.B. Astraldi Ricotti, and G. Bottiroli. 1992. Topoisomerase II alpha and beta in human tumor cells grown *in vitro* and *in vivo. Anticancer Res.* 12: 2093–2099.
- Reece, R.J. and A. Maxwell. 1991. DNA gyrase: Structure and function. Crit. Rev. Biochem. Mol. Biol. 26: 335-375.
- Reitman, M. and G. Felsenfeld. 1990. Developmental regulation of topoisomerase II sites and DNase I-hypersensitive sites in the chicken  $\beta$ -globin locus. *Mol. Cell. Biol.* **10**: 2774–2786.
- Robinson, M.J. and N. Osheroff. 1990. Stabilization of the topoisomerase II-DNA cleavage complex by antineoplastic drugs: Inhibition of enzyme-mediated DNA religation by 4'-(9-acridinylamino)methanesulfon-m-anisidide. *Biochemistry* 29: 2511-2515.
- Robinson, M.J., A.H. Corbett, and N. Osheroff. 1993. Effects of topoisomerase II-

targeted drugs on enzyme-mediated DNA cleavage and ATP hydrolysis: Evidence for distinct drug interaction domains on topoisomerase II. *Biochemistry*. **32**: 3638–3643.

- Robinson, M.J., B.A. Martin, T.D. Gootz, P.R. McGuirk, M. Moynihan, J.A. Sutcliff, and N. Osheroff. 1991. Effects of quinolone derivatives on eukaryotic topoisomerase II: A novel mechanism for enhancement of enzyme-mediated DNA cleavage. J. Biol. Chem. 266: 14585-14592.
- Roca, J. and J.C. Wang. 1992. The capture of a DNA double helix by an ATP-dependent protein clamp: A key step in DNA transport by type II DNA topoisomerases. *Cell* **71**: 833–840.
- \_\_\_\_\_. 1994. DNA transport by a type II topoisomerase: Evidence in favor of a two-gate mechanism. *Cell* 77: 609-616.
- Roca, J., R. Ishida, J.M. Berger, T. Andoh, and J.C. Wang. 1994. Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc. Natl. Acad. Sci.* **91**: 1781–1785.
- Rose, D. and C. Holm. 1993. Meiosis-specific arrest revealed in DNA topoisomerase II mutants. *Mol. Cell. Biol.* 13: 3445–3455.
- Rose, D., W. Thomas, and C. Holm. 1990. Segregation of recombined chromosomes in meiosis I requires DNA topoisomerase II. *Cell* **60**: 1009–1017.
- Rottmann, M., H.C. Schröder, M. Gramzow, K. Renneisen, B. Kurelec, A. Dorn, U. Friese, and W.E.G. Muller. 1987. Specific phosphorylation of proteins in pore complex-laminae from the sponge *Geodia cydonium* by the homologous aggregation factor and phorbol ester. Role of protein kinase C in the phosphorylation of DNA topoisomerase II. *EMBO J.* 6: 3939–3944.
- Saijo, M., M. Ui, and T. Enomoto. 1992. Growth state and cell cycle dependent phosphorylation of DNA topoisomerase II in Swiss 3T3 cells. *Biochemistry* 31: 359-363.
- Sander, M. and T.S. Hsieh. 1983. Double strand DNA cleavage by type II DNA topoisomerase from *Drosophila melanogaster*. J. Biol. Chem. 258: 8421-8428.

- Sander, M., T.S. Hsieh, A. Udvardy, and P. Schedl. 1987. Sequence dependence of *Drosophila* topoisomerase II in plasmid relaxation and DNA binding. J. Mol. Biol. 194: 219-229.
- Schiestl, R.H., M. Dominska, and T.D. Petes. 1993. Transformation of Saccharomyces cerevisiae with nonhomologous DNA: Illegitimate integration of transforming DNA into yeast chromosomes and *in vivo* ligation of transforming DNA to mitochondrial DNA sequences. *Mol. Cell. Biol.* 13: 2697-2705.
- Schmidt, V.K., B.S. Sørensen, H.V. Sørensen, J. Alsner, and O. Westergaard. 1994. Intramolecular and intermolecular DNA ligation mediated by topoisomerase II. J. Mol. Biol. 241: 18-25.
- Schomburg, U. and F. Grosse. 1986. Purification and characterization of DNA topoisomerase II from calf thymus associated with polypeptides of 175 and 150 kDa. *Eur. J. Biochem.* 160: 451-457.
- Shelton, E.R., N. Osheroff, and D.L. Brutlag. 1983. DNA topoisomerase II from Drosophila melanogaster. Purification and physical characterization. J. Biol. Chem. 258: 9530-9535.
- Shiozaki, K. and M. Yanagida. 1991. A functional 125-kDA core polypeptide of fission yeast DNA topoisomerase II. *Mol. Cell. Biol.* 11: 6093–6102.

- Snapka, R.M. 1986. Topoisomerase inhibitors can selectively interfere with different stages of simian virus 40 DNA replication. *Mol. Cell. Biol.* 6: 4221-4227.
- Snapka, R.M., M.A. Powelson, and J.M. Strayer. 1988. Swiveling and decatenation of replicating simian virus 40 genomes *in vivo*. *Mol. Cell. Biol.* 8: 515–521.
- Sørensen, B.S., J. Sinding, A.H. Andersen, J. Alsner, P.B. Jensen, and O. Westergaard. 1992. Mode of action of topoisomerase II targeting agents at a specific DNA sequence: Uncoupling the DNA binding, cleavage and religation events. J. Mol. Biol. 228: 778-786.
- Sørensen, B.S., P.S. Jensen, A.H. Andersen, K. Christiansen, J. Alsner, B. Thomsen, and O. Westergaard. 1990. Stimulation of topoisomerase II mediated DNA cleavage at specific sequence elements by the 2-nitroimidazole Ro 15-0216. *Biochemistry* 29: 9507-9515.
- Spell, R.M. and C. Holm. 1994. Nature and distribution of chromosomal intertwinings in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 14: 1465–1476.
- Sperry, A.O., V.C. Blasquez, and W.T. Garrard. 1989. Dysfunction of chromosomal loop attachment sites: Illegitimate recombination linked to matrix association regions and topoisomerase II. *Proc. Natl. Acad. Sci.* 86: 5497–5501.
- Spitzner, J.R. and M.T. Muller. 1988. A consensus sequence for cleavage by vertebrate DNA topoisomerase II. *Nucleic Acids Res.* 16: 5533-5556.
- Spitzner, J.R., I.K. Chung, and M.T. Muller. 1990. Eukaryotic topoisomerase II preferentially cleaves alternating purine-pyrimidine repeats. *Nucleic Acids Res.* 18: 1–11.
- Stevnsner, T., U.H. Mortensen, O. Westergaard, and B.J. Bonven. 1989. Interactions between eukaryotic DNA topoisomerase I and a specific binding sequence. J. Biol. Chem. 264: 10110-10113.
- Stewart, A.F. and G. Schutz. 1987. Camptothecin-induced in vivo topoisomerase I cleavages in the transcriptionally active tyrosine aminotransferase gene. *Cell* 50: 1109–1117.
- Stewart, A.F., R.E. Herrera, and A. Nordheim. 1990. Rapid induction of *c-fos* transcription reveals quantitative linkage of RNA polymerase II and DNA topoisomerase I enzyme activities. *Cell* **60**: 141–149.
- Strauss, P.R. and J.C. Wang. 1990. The TOP2 gene of Trypanosoma brucei: A single-copy gene that shares extensive homology with other TOP2 genes encoding eukaryotic DNA topoisomerase II. Mol. Biochem. Parasitol. 38: 141-150.
- Sundin, O. and A. Varshavsky. 1981. Arrest of segregation leads to accumulation of highly intertwined catenated dimers: Dissection of the final stages of SV40 DNA replication. *Cell* 25: 659-669.
- Svejstrup, J.Q., K. Christiansen, I.I. Gromova, A.H. Andersen, and O. Westergaard. 1991. New technique for uncoupling the cleavage and religation reactions of eukaryotic topoisomerase I. The mode of action of camptothecin at a specific recognition site. J. Mol. Biol. 222: 669-678.
- Swedlow, J.R., J.W. Sedat, and D.A. Agard. 1993. Multiple chromosomal populations of topoisomerase II detected in vivo by time-lapse, three-dimensional wide-field microscopy. *Cell* **73**: 97–108.
- Tanabe, K., Y. Ikegami, R. Ishida, and T. Andoh. 1991. Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res.* **51**: 4903–4908.
- Tewey, K.M., G.L. Chen, E.M. Nelson, and L.F. Liu. 1984. Intercalative antitumor drugs

interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. J. Biol. Chem. 259: 9182–9187.

- Thomsen, B., C. Bendixen, K. Lund, A.H. Andersen, B.S. Sørensen, and O. Westergaard. 1990. Characterization of the interaction between topoisomerase II and DNA by transcriptional footprinting. J. Mol. Biol. 215: 237-244.
- Thomsen, B., S. Mollerup, B.J. Bonven, R. Frank, H. Blocker, O.F. Nielsen, and O. Westergaard. 1987. Sequence specificity of DNA topoisomerase I in the presence and absence of camptothecin. *EMBO J.* 6: 1817–1823.
- Thrash, C., A.T. Bankier, B.G. Barrell, and R. Sternglanz. 1985. Cloning, characterization, and sequence of the yeast DNA topoisomerase I gene. *Proc. Natl. Acad. Sci.* 82: 4374-4378.
- Tsai-Pflugfelder, M., L.F. Liu, A.A. Liu, K.M. Tewey, J. Whang-Peng, T. Knutsen, K. Huebner, C.M. Croce, and J.C. Wang. 1988. Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21-22. Proc. Natl. Acad. Sci. 85: 7177-7181.
- Uemura, T. and M. Yanagida. 1984. Isolation of type I and II DNA topoisomerase mutants from fission yeast: Single and double mutants show different phenotypes in cell growth and chromatin organization. *EMBO J.* **3**: 1737-1744.
- Uemura, T., K. Morikawa, and M. Yanagida. 1986. The nucleotide sequence of the fission yeast DNA topoisomerase II gene: Structural and functional relationships to other DNA topoisomerases. *EMBO J.* 5: 2355-2361.
- Uemura, T., K. Morino, S. Uzawa, K. Shiozaki, and M. Yanagida. 1987a. Cloning and sequencing of *Schizosaccharomyces pombe* DNA topoisomerase I gene, and effect of gene disruption. *Nucleic Acids Res.* 15: 9727–9739.
- Uemura, T., H. Ohkura, Y. Adachi, K. Morino, K. Shiozaki, and M. Yanagida. 1987b. DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in S. pombe. Cell 50: 917-925.
- Vosberg, H.P. 1985. DNA topoisomerases: Enzymes that control DNA conformation. *Curr. Top. Microbiol. Immunol.* 114: 19-102.
- Wallis, J.W., G. Chrebet, G. Brodsky, M. Rolfe, and R. Rothstein. 1989. A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. *Cell* 58: 409-419.
- Wang, J.C. 1987. Recent studies of DNA topoisomerases. *Biochim. Biophys. Acta* 909: 1-9.
- \_\_\_\_\_, 1994. DNA topoisomerases as targets of therapeutics: An overview. Adv. Pharmacol. 29A: 1-19.
- Wang, H.P. and C.E. Rogler. 1991. Topoisomerase I-mediated integration of hepadnavirus DNA in vitro. J. Virol. 65: 2381–2392.
- Watt, P.M. and I.D. Hickson. 1994. Structure and function of type II DNA topoisomerases. *Biochem. J.* 303: 681-695.
- Woessner, R.D., M.R. Mattern, C.K. Mirabelli, R.K. Johnson, and F.H. Drake. 1991. Proliferation- and cell cycle-dependent differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. *Cell Growth Differ.* 2: 209–214.
- Worland, S.T. and J.C. Wang. 1989. Inducible overexpression, purification, and active site mapping of DNA topoisomerase II from the yeast Saccharomyces cerevisiae. J.

Biol. Chem. 264: 4412-4416.

- Wu, H.Y., S.H. Shyy, J.C. Wang, and L.F. Liu. 1988. Transcription generates positively and negatively supercoiled domains in the template. *Cell* **53**: 433-440.
- Wyckoff, E. and T.-S. Hsieh. 1988. Functional expression of a *Drosophila* gene in yeast: Genetic complementation of DNA topoisomerase II. *Proc. Natl. Acad. Sci.* 85: 6272–6276.
- Wyckoff, E., D. Natalie, J.M. Nolan, M. Lee, and T.S. Hsieh. 1989. Structure of the Drosophila DNA topoisomerase II gene. Nucleotide sequence and homology among topoisomerases II. J. Mol. Biol. 205: 1–13.
- Yang, L., M.S. Wold, J.J. Li, T.J. Kelly, and L.F. Liu. 1987. Roles of DNA topoisomerases in simian virus 40 DNA replication in vitro. *Proc. Natl. Acad. Sci.* 84: 950–954.
- Zechiedrich, E.L., K. Christiansen, A.H. Andersen, O. Westergaard, and N. Osheroff. 1989. Double-stranded DNA cleavage/religation reaction of eukaryotic topoisomerase II: Evidence for a nicked DNA intermediate. *Biochemistry* **28**: 6229–6236.
- Zhang, H., P. D'Arpa, and L.F. Liu. 1990. A model for tumor cell killing by topoisomerase poisons. *Cancer Cells* 2: 23–27.
- Zhang, H., J.C. Wang, and L.F. Liu. 1988. Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. *Proc. Natl. Acad. Sci.* 85: 1060–1064.