Annu. Rev. Pharmacol. Toxicol. 1994. 34:191–218

DNA TOPOISOMERASES: Essential Enzymes and Lethal Targets

Allan Y. Chen and Leroy F. Liu
Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

KEY WORDS: anticancer drug, intercalator, groove binder, DNA damage, drug resistance

INTRODUCTION

In 1971, Wang (1) discovered the first DNA topoisomerase in *Escherichia coli*. The enzyme (*E. coli* DNA topoisomerase I, or ω protein) catalyzed relaxation of negatively supercoiled DNA in the absence of any energy cofactor (1). Wang proposed that this enzyme also catalyzed transient nicking of the DNA double helix and possessed both DNase and ligase activity in one polypeptide (1). The lack of any energy cofactor for the reaction also led Wang to the proposal that the enzyme may form a high-energy covalent bond between itself and the transiently broken DNA phosphodiester bond (1). Both of these predictions have turned out to be correct (2).

Since the discovery of *E. coli* topoisomerase I, investigators have isolated many other DNA topoisomerases from both prokaryotes and eukaryotes. In 1972, Champoux & Dulbecco isolated an enzyme with activity similar to that of *E. coli* topoisomerase I from mouse embryo cells (3). In 1976, Gellert and his colleagues identified an enzyme activity opposing *E. coli* DNA topoisomerase I (4). They demonstrated that this enzyme (*E. coli* DNA topoisomerase II, or gyrase) catalyzed the conversion of relaxed DNA into negatively supercoiled DNA in a reaction requiring ATP hydrolysis (4). These two opposing activities are important for maintaining the superhelical state of the chromosomal DNA during various DNA transactions (5–8). In 1979, Liu et al (9) isolated an enzyme from bacteriophage

0362-1642/94/0415-0191$05.00

T4-infected *E. coli*. Three DNA-delay genes (9, 10) encode this new enzyme (T4 DNA topoisomerase), which is important for T4 DNA replication (9). T4 DNA topoisomerase, unlike either *E. coli* DNA topoisomerase I or *E. coli* DNA gyrase, catalyzes relaxation of both positive and negative supercoils in a reaction requiring ATP hydrolysis (9). Liu et al suggested that this seemingly energy-wasting enzyme might couple ATP hydrolysis to a yet unidentified energy-utilizing function (9).

Interestingly, eukaryotic DNA topoisomerase II has enzymatic activity similar to T4 DNA topoisomerase (11–14). The name "DNA topoisomerases" was introduced in 1979 to describe this ubiquitous class of enzymes that have the ability to manipulate the topology of DNA (15). Clearly, many more DNA topoisomerases are present in cells. For example, topoisomerase III has been identified both in *E. coli* and yeast (16–18); topoisomerase IV (ParC/ParE), which shows sequence homology to gyrase and is involved in chromosome partitioning, has been identified in *E. coli* (19, 20); and topoisomerase V, which shows sequence homology to eukaryotic DNA topoisomerase I, has been identified in the hyperthermophilic methanogen *Methanopyrus kandleri* (21). In addition, researchers have demonstrated that some recombination enzymes such as lambda Int and Tn*3* resolvase have topoisomerase activities (22–27).

Studies on the mechanism of catalysis of T4 DNA topoisomerase have also led to the classification of topoisomerases into two types (28–30). Type I DNA topoisomerases, exemplified by *E. coli* DNA topoisomerase I and eukaryotic DNA topoisomerase I, catalyze DNA relaxation via a transient single-stranded DNA break. Type II DNA topoisomerases, exemplified by *E. coli* DNA gyrase and eukaryotic DNA topoisomerase II, catalyze the topological crossing of two double-stranded DNA segments via a transient DNA double-stranded break (28–30). Characteristically, type I DNA topoisomerases change the linking number of closed circular DNA in steps of one, and type II DNA topoisomerases change in steps of two (28–30).

Scientists realized the importance of topoisomerases as therapeutic targets as soon as *E. coli* DNA gyrase was discovered. Researchers found that gyrase was the target of two different classes of antibiotics, novobiocin and nalidixic acid (30, 31). Nalidixic acid and some of the more recently developed quinolone antibiotics specifically interfere with the breakage-reunion reaction of DNA gyrase by interacting with GyrA (31–34). The impressive potency of quinolone antibiotics may result from the rapid action of these drugs in breaking the chromosomal DNA.

The role of mammalian DNA topoisomerases as molecular targets for anticancer drugs was not recognized until 1984 (35–37). Investigators have since carried out extensive studies of the mechanism of action of topoisomerase-targeting drugs (for reviews, see 38–40). As a result, we have

significantly advanced our understanding of topoisomerase-targeting drugs, but many questions remain unanswered. Recently, researchers have discovered that topoisomerases can also be therapeutic targets for antiparasitic, antifungal, and antiviral drugs (reviewed in 38, 40). The present review focuses on anticancer drugs targeting mammalian DNA topoisomerases. However, the principles underlying these anticancer drugs should be applicable to other topoisomerase-targeting therapeutics.

MAMMALIAN DNA TOPOISOMERASE I: CATALYTIC ACTION AND FUNCTIONS

Mammalian DNA topoisomerase I was initially purified from rat liver as a 67-kDa monomeric protein capable of relaxing both positively and negatively supercoiled DNA even in the presence of ethylenediaminetetraacetic acid (EDTA) (41). By purifying the same type of DNA relaxation activity from HeLa cells, Miller et al have demonstrated that the native enzyme is a 100-kDa monomeric protein, and Mg(II) significantly stimulates the relaxation activity (14). Researchers have sequenced the human DNA topoisomerase I cDNA (42, 43) and mapped the gene to chromosome 20q12–13.2 (43). The cDNA encodes a polypeptide of 765 amino acids, 40% of which are charged amino acids (42).

Investigators have studied the mechanism of catalysis of mammalian topoisomerase I extensively (reviewed in 44) and made many advances, primarily through studies of the partial (cleavage) reaction. At high concentrations of topoisomerase I, a fraction of the topoisomerase I molecules can be trapped covalently on double-stranded DNA (45, 46). Topoisomerase I is covalently linked to the 3′-phosphoryl end of the nick through a tyrosine residue (#723) (46, 47). The cleavage reaction is more efficient at lower ionic strength and in the presence of EDTA, and researchers have deduced a weak consensus sequence at the cleavage site (47–49). Most striking, thymidine is the nucleotide covalently linked to topoisomerase I more than 90% of the time (47, 48). Researchers have also studied the cleavage reaction by using single-stranded DNA substrates (49–52); cleavage on single-stranded DNA can lead to spontaneous cleavage of the strand, and the topoisomerase I–linked DNA strand can be transferred covalently to another single- or double-stranded DNA with 5′-hydroxyl ends (50).

The biological function of topoisomerase I has been studied extensively. Studies in a cell-free SV40 DNA replication system have clearly demonstrated the capacity of topoisomerase I as a swivel during the elongation phase of DNA replication (53). Experiments demonstrating the preferential association of topoisomerase I with the transcribed regions have also suggested the potential role of mammalian DNA topoisomerase I in transcription

(6–8, 54–56). It probably functions as a swivel to release the local supercoils generated during transcription elongation (6–8). The recent finding that topoisomerase I is associated with TBP, the TATA binding protein, is consistent with such a role (57).

TOPOISOMERASE I DRUGS

Camptothecin

Wall et al originally isolated camptothecin from the tree *Camptotheca acuminata* as an antitumor component (58). Camptothecin's broad spectrum of antitumor activity in animal models, especially activity against colon tumors, led to clinical trials in the early 1970s (59, 60). However, because camptothecin lactone had low solubility, researchers used the ring-open form of camptothecin (camptothecin, sodium salt, NSC-100880) in the trials (59, 60). Excessive toxicity resulted in termination of the trials (60). We now know that the ring-open form of camptothecin is inactive against its molecular target, topoisomerase I (61). The residual activity of camptothecin sodium salt could result from the pH-dependent conversion into the lactone form.

The cellular effects of camptothecin have also been studied extensively. Camptothecin inhibits both DNA and RNA synthesis (62–68). RNA synthesis is highly reversible, while DNA synthesis can become irreversible at higher camptothecin concentrations (63, 65). Camptothecin also selectively kills S-phase cells, arrests cells in G_2 phase (69–73), and induces fragmentation of chromosomal DNA as revealed by alkaline sucrose gradient sedimentation (64). Interestingly, removal of camptothecin from the medium rapidly restores the integrity of the chromosomal DNA (64). This unusual phenomenon led to the identification of the receptor for camptothecin (74, 75).

DNA topoisomerase I is now firmly established as the biological receptor for camptothecin (74–77), which explains many of camptothecin's cellular effects. Camptothecin specifically inhibits the rejoining step of the topoisomerase I reaction and, therefore, traps the putative covalent intermediate of the reaction, i.e. the reversible cleavable complex (74). This complex is presumably a topoisomerase I–camptothecin-DNA ternary complex, which when denatured by a strong protein denaturant [e.g. sodium dodecyl sulfate (SDS) and alkali] is converted into a protein-linked single-stranded break (74). Such a model can readily explain reversible fragmentation of chromosomal DNA in alkaline sucrose gradient. Interaction between this reversible cleavable complex and the replication or transcription machinery may be responsible for DNA and RNA synthesis inhibition, respectively.

Figure 1 displays our current understanding of the cytotoxic mechanism

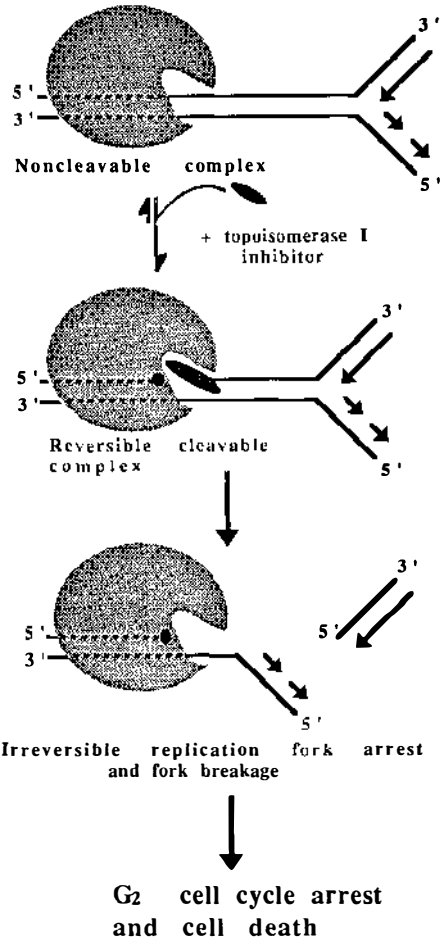


Figure 1 A fork collision model for topoisomerase I drugs. The reversible topoisomerase I–DNA cleavable complex is presumably the covalent intermediate of the topoisomerase I reaction. In a relaxation reaction, the cleavable complex and the noncleavable complex are at equilibrium. Camptothecin perturbs this equilibrium by increasing the concentration of the cleavable complex. In this model, the reversible topoisomerase I–camptothecin-DNA ternary complex is oriented in such a way that the transiently cleaved strand is complementary to the leading strand of DNA synthesis. Topoisomerase I is also represented as an asymmetric enzyme in which the major protein-DNA contact is upstream of the site of cleavage. This polarity-dependent collision triggers cell death and cell-cycle arrest at the G_2 phase.

of camptothecin. This fork collision model is based on studies both in cultured cells and in cell-free extracts (71, 72, 78, 79). Studies in cultured-cell systems have shown that transient and simultaneous inhibition of DNA synthesis abolishes camptothecin cytotoxicity, thus suggesting the involvement of replication in camptothecin cytotoxicity (71–73), and studies in a cell-free SV40 replication extract have provided more details about the role of DNA replication in camptothecin cytotoxicity. As shown in Figure 1, camptothecin stabilizes a reversible topoisomerase I–camptothecin-DNA ternary complex on DNA. In this complex, the tyrosine residue (#723 of the human enzyme) is covalently linked to the 3′-phosphoryl end of the broken DNA strand. The interaction between the replication machinery and the reversible cleavable complex leads to cell death and G_2 arrest of the cell cycle (71–73, 80).

Tsao et al have detected three biochemical events at or near the interphase between the cleavable complex and the replication fork: irreversible arrest of the fork, a double-stranded break at or near the fork, and the conversion of the reversible cleavable complex into an irreversibly cleaved complex (79). Biochemical studies have also suggested a polarity-dependent interaction between the fork and the cleavable complex (79). As shown in Figure 1, the topoisomerase I–mediated transient break is made on the strand that is complementary to the leading strand of DNA synthesis. If the transient break occurs on the strand that is complementary to the lagging strand of DNA synthesis, the collision consequence is not as severe. The reason for this polarity-dependent collision is not clear. One possibility is that the major contact between topoisomerase I and DNA is on the upstream side of the transient break (Figure 1). The collision leads to destabilization of the double helix proximal to the replication fork and melting of the strand that is minimally protected by topoisomerase I.

Not all cellular effects of camptothecin can be explained by the fork collision model. For example, camptothecin increases c-*fos* and c-*jun* mRNA levels (55, 81). However, such an induction is independent of DNA synthesis (E Schneider & LF Liu, unpublished results). Camptothecin also induces cellular differentiation (82–84). Whether or not this cellular effect of camptothecin involves replication has not been tested. More recent studies have also demonstrated that rapid ubiquitination of topoisomerase I occurs in cells treated with camptothecin (P D'Arpa & LF Liu, unpublished results).

One of the most striking observations about camptothecin is its broad spectrum antitumor activity. Several refractory solid tumors including colon tumors have responded to camptothecin treatment in xenograft models (85–88); why camptothecin is effective against these tumors is still uncertain. However, camptothecin and its neutral derivatives overcome MDR1-medi-

ated resistance (89, 90). Camptothecin is also more cytotoxic toward tumorigenic than non-tumorigenic breast cancer cells (87, 88). In this case, the S-phase specificity of camptothecin apparently cannot fully account for its differential effect on these cells. The molecular basis for this tumor specificity is still unknown. More interestingly, topoisomerase I protein levels appear to be higher in colon tumors than in normal mucosa (85). The altered regulation of topoisomerase I in tumor cells has not been investigated. However, topoisomerase I mRNA levels increase substantially upon treatment with the tumor promoter phorbol 12-myristate 13-acetate (PMA) or growth factors (91). Interestingly, as in some oncogenes such as c-*myc* and c-*myb*, the increase of topoisomerase I mRNA levels can be superinduced by treatment with the protein-synthesis inhibitor cycloheximide (91).

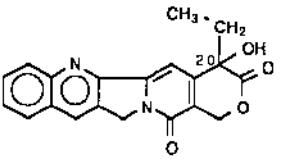
Investigators have synthesized many camptothecin derivatives (92), and some are at various stages of clinical development. Figure 2*A* shows some of the promising camptothecin derivatives. Among these, 9-amino-10,11-methylenedioxy-camptothecin is the most potent, followed by 10,11-methylenedioxy-camptothecin (93). CPT-11, which is a prodrug of SN38, has been successfully developed in Japan (94–96); topotecan and 9-amino-camptothecin are under development in the United States (97).

Other Topoisomerase I Drugs

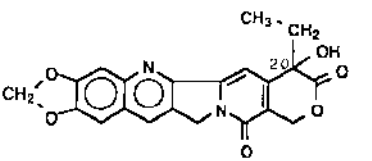
Since the identification of topoisomerase I as the primary molecular target of camptothecin, several new topoisomerase I poisons have been reported (Figure 2*B*). Interestingly, many of them exhibit a DNA minor groove-binding mode (98–104).

DNA minor groove–binding drugs represent a major class of compounds with broad spectrum antimicrobial and antitumor activities (105, 106). Their binding to the minor groove of DNA with A+T specificity, which causes widening of the minor grooves, is well documented (107, 108). Some of these DNA minor-groove binders (e.g. Hoechst 33258 and 33342) are topoisomerase I poisons (101). Similar to camptothecin, these drugs appear to interrupt the breakage-reunion cycle of topoisomerase I by stabilizing a reversible topoisomerase I cleavable complex (101). However, minor groove–binding, drug-induced single-strand DNA breaks in the presence of topoisomerase I are highly site specific (101). Chen et al mapped three of the major Hoechst dyes–induced topoisomerase I–mediated DNA cleavage sites on a 8.4-kb plasmid DNA to highly AT-rich regions and found a common sequence of 5′-T*CATTTTT-3′ (the asterisk marks the site of topoisomerase I cleavage) (101). The nucleotide sequences surrounding these cleavage sites suggest a model in which topoisomerase I interacts mainly

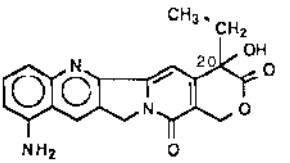
A



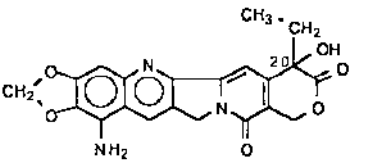
Camptothecin



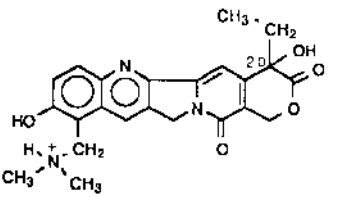
10,11-methylenedioxy-camptothecin



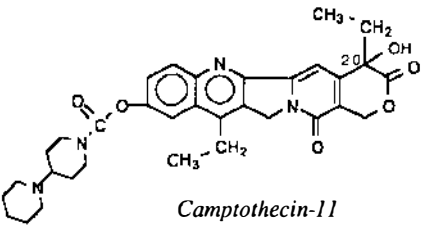
9-Amino-camptothecin



9-Amino-10,11-methylenedioxy-camptothecin



Topotecan



Camptothecin-11

with the sequences 5′ (upstream) of the cleavage site (109). The T-track 3′ (downstream) from the cleavage site is the binding site for these drugs (see Figure 3). One speculation is that drug binding to the minor groove of these sequences near the site of cleavage causes significant bending of the DNA helical axis, which prevents ligation of the two transiently disjoined ends (110).

Although the role of minor groove binding seems important for poisoning topoisomerase I by Hoechst dyes, we still do not know whether minor-groove binding is important for other topoisomerase I drugs. Actinomycin D, which is a classical DNA intercalator, poisons both mammalian DNA topoisomerases I and II (35, 98, 99). While the phenoxazone ring of actinomycin D intercalates into DNA, the pentapeptide rings bind to the minor groove and form hydrogen bonds with the bases (111–113). Researchers have not concluded whether the minor-groove binding of actinomycin D is primarily responsible for its poisoning of topoisomerase I. Nogalamycin, mithramycin, and chromomycin A3 also poison topoisomerase I (101). NMR studies have

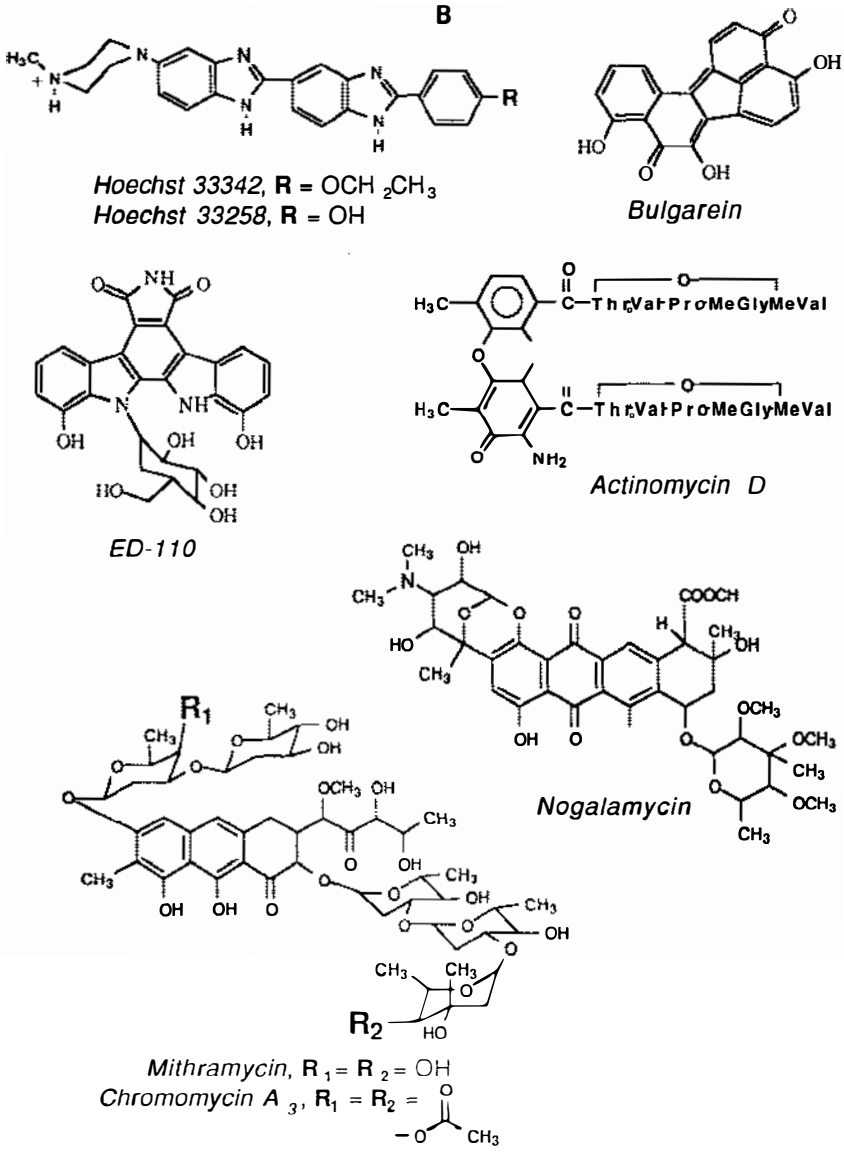


Figure 2 Chemical structures of topoisomerase I drugs. (*A*) Camptothecin derivatives; (*B*) Other topoisomerase I drugs.

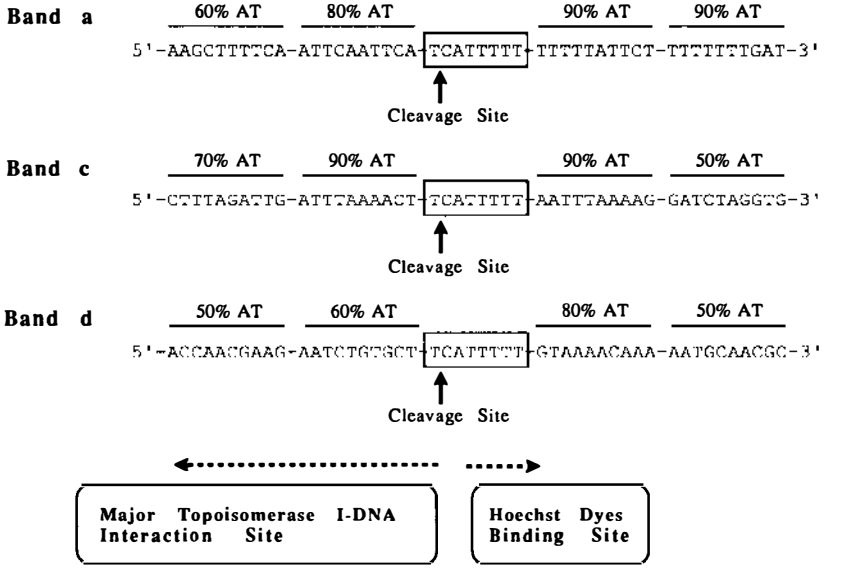


Figure 3 Nucleotide sequences of topoisomerase I-mediated cleavage sites induced by Hoechst 33342 and 33258. Bands *a*, *c*, and *d* represent three major cleavage bands induced by Hoechst 33342 and 33258 in the presence of calf thymus topoisomerase I on linearized YEpG DNA (100).

shown that the sugar moieties of these drugs interact with the minor groove of DNA (114–118). Bulgarein is another mammalian topoisomerase I poison (102). Like netropsin, bulgarein positively winds DNA, which indicates that it interacts with the minor groove (102). Several indolocarbazole derivatives, including ED-110, induce calf thymus topoisomerase I–mediated DNA cleavage (103, 104). Yamashita et al have demonstrated the clear dissociation between the strength of DNA intercalation and the activity in inducing topoisomerase I–mediated DNA cleavage among these indolocarbazole derivatives (103). Because most of these drugs have dual modes of DNA binding (i.e. intercalation and minor groove binding), the precise role of groove binding in poisoning of topoisomerase I needs to be clarified.

MAMMALIAN DNA TOPOISOMERASE II: CATALYTIC ACTION AND FUNCTIONS

Mammalian DNA topoisomerase II has been isolated from calf thymus gland and HeLa cell nuclei (14, 119, 120). The purified calf thymus and HeLa topoisomerase II is a homodimeric protein with a monomer molecular weight of 170 kDa. Like T4 DNA topoisomerase, HeLa topoisomerase II is a type

II DNA topoisomerase that catalyzes the ATP-dependent relaxation, knotting-unknotting, and catenation-decatenation reactions (14, 120). Studies in a cell-free SV40 DNA replication system suggest a role for DNA topoisomerase II in the elongation and termination stages of DNA replication (121). Although topoisomerase I can serve as a functional substitute for DNA topoisomerase II in the elongation stage of DNA replication, the role of topoisomerase II in segregating the multiply interlocked daughter chromosomes is essential and irreplaceable (121–124).

Tsai-Pflugfelder et al have sequenced the human DNA topoisomerase II cDNA and determined its chromosomal location at 17q21–22 (125). This enzyme is homologous to both subunits of DNA gyrase (125, 126). Apparently, during evolution, the two subunits of DNA gyrase fused into a single polypeptide chain.

We still do not fully understand catalysis of ATP-dependent strand-passing by topoisomerase II. Most advances have come from studies of the putative covalent intermediate at high enzyme concentrations (12, 127–129). Figure 4 summarizes a working model for the strand-passing activity of eukaryotic DNA topoisomerase II. Topoisomerase II can break and rejoin the DNA double helix in the absence of ATP by forming an equilibrium mixture of at least two types of complexes—noncleavable and cleavable. The equilibrium strongly favors the noncleavable complex. The cleavable complex is operationally defined by its ready conversion to a protein-linked break upon exposure to a strong protein denaturant such as SDS or alkali (38, 130). The cleaved intermediate can be a single-strand or double-strand break. In both cases, topoisomerase II polypeptide is covalently linked to the 5′-phosphoryl end of the broken DNA strand. In a double-strand break, the 5′ broken-end protrudes precisely four bases (130); the cleavage site has a weak consensus sequence (131, 132); and the role of ATP is unclear. Recent studies have suggested that ATP binding and hydrolysis controls the opening and closing of the protein gate formed by the homodimeric topoisomerase II enzyme (133); protease probing detected these two conformational states of topoisomerase II (134, 135). Apparently, the coupling between opening and closing of the gate with the strand-passing event is not very tight (136). The breakage-reunion site of topoisomerase II can operate more or less independently of ATP binding or hydrolysis. ATP binding triggers closure of the protein gate and therefore locks in the DNA segment(s) that happens to be at the interface of the two protein subunits (133). The presence of two DNA segments at the interface of the two protein subunits presumably results in strand passing. Hydrolysis of ATP reopens the protein gate and releases the DNA segment(s). In this model, all cleavable complexes regardless of the state of the protein gate are at equilibrium with their noncleavable complex counterparts.

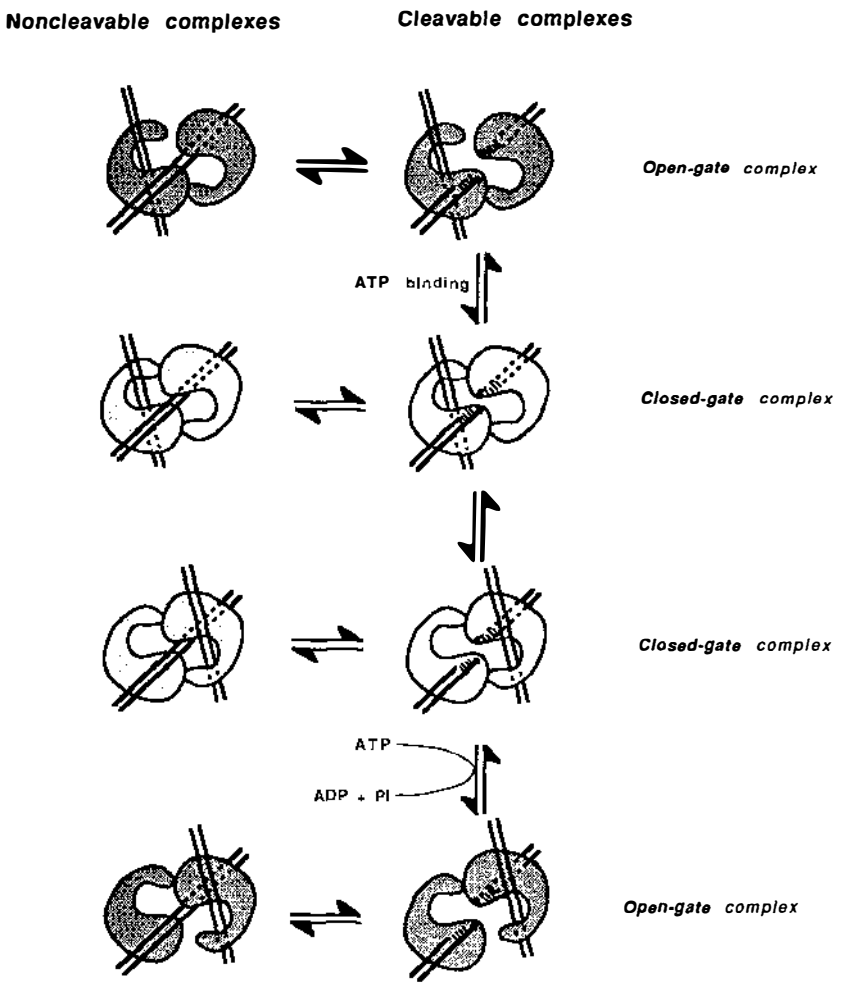


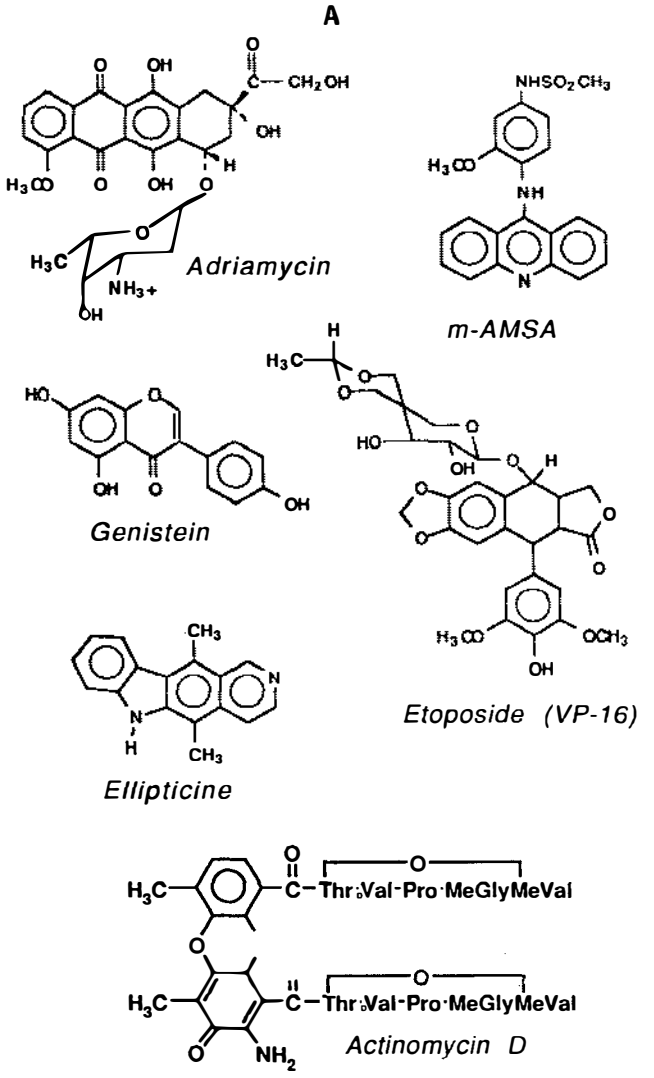
Figure 4 A proposed mechanism of catalysis for mammalian DNA topoisomerasc II. In this model, the homodimeric topoisomerase II has a two-fold rotational symmetry. The enzyme assumes at least two different conformations (see different shades of the enzyme). Depending on the ATP-binding status, the enzyme assumes either the open-gate (no ATP) or the closed-gate (ATP bound) form. The DNA-bound topoisomerase II is at equilibrium with at least two types of complexes, the noncleavable complex and the cleavable complex. The coupling between ATP hydrolysis and DNA binding is not tight. The presence of two DNA segments within the closed-gate complex presumably leads to strand-passing. Hydrolysis of ATP is necessary to reopen the protein gate. Topoisomerase II–targeting drugs can affect either or both of the two conformers of topoisomerase II.

TOPOISOMERASE II DRUGS

We now know that topoisomerase II is the molecular target of many anticancer drugs (see Figure 5 for examples), including some of the most important anticancer drugs such as adriamycin and etoposide (VP-16) (38, 39). As is the case for camptothecin, topoisomerase II drugs characteristically induce reversible protein-linked DNA breaks in cultured cells (137). However, depending on the particular drug, breaks induced by topoisomerase II drugs can be primarily double-strand breaks, as opposed to camptothecin-induced breaks, which are primarily single-strand breaks (38). ICRF193 represents a possible exception (138–140); it is a specific inhibitor of the catalytic activity of topoisomerase II (138, 139).

The way in which various drugs inhibit the mechanisms of topoisomerase II is not fully understood. Presumably, they specifically inhibit the rejoining step in the breakage-rejoining cycle and thereby shift the equilibrium toward the cleavable complex (see Figure 4). However, recent studies have indicated that, depending on the particular drug class, ATP may or may not stimulate topoisomerase II-mediated DNA cleavage (AY Chen & LF Liu, unpublished results). As discussed earlier in this chapter, at least two different conformational states of topoisomerase II can be distinguished by ATP binding (134, 135). The two conformational states may correspond to the open-gate and closed-gate states of topoisomerase II (see Figure 4; the dimeric topoisomerase II molecules are shaded differently to indicate the different conformational states). One speculation is that, depending on the particular drug class, topoisomerase II can be differentially affected in one of the two conformational states. For example, the ATP-stimulated topoisomerase II drugs such as VP-16, VM-26, and adriamycin may preferentially interact with topoisomerase II in the closed-gate conformation. The ATP-independent topoisomerase II drugs such as menadiones may preferentially interact with the open-gate conformation of topoisomerase II (AY Chen & LF Liu, unpublished results).

Many topoisomerase II drugs are known DNA intercalators (e.g. adriamycin, m-AMSA, mitoxantrone, and ellipticine). However, no DNA minor-groove binders (except for the dual mode binders, such as actinomycin D) have yet trapped topoisomerase II cleavable complexes (38, 39). Studies of anthracycline congeners have shown that, for compounds that trap topoisomerase II cleavable complexes, the strength of intercalation (DNA binding) parallels drug potency in trapping the complex, thus suggesting a direct role of drug intercalation (DNA binding) in trapping cleavable complexes (141). A misalignment model, in which the intercalators cause misalignment of the transiently broken DNA ends, has been proposed for intercalative topoisomerase II drugs (142, 143). Studies of the sequence



specificity have also suggested that the intercalators may bind at the enzyme-DNA interface (35, 144–147).

Like many DNA-damaging agents, topoisomerase II drugs induce sister chromatid exchanges and chromosome aberrations (148, 149). Interestingly, topoisomerase II drugs also induce significant levels of recombinant chromosomes (150, 151). More recent studies have strongly suggested that

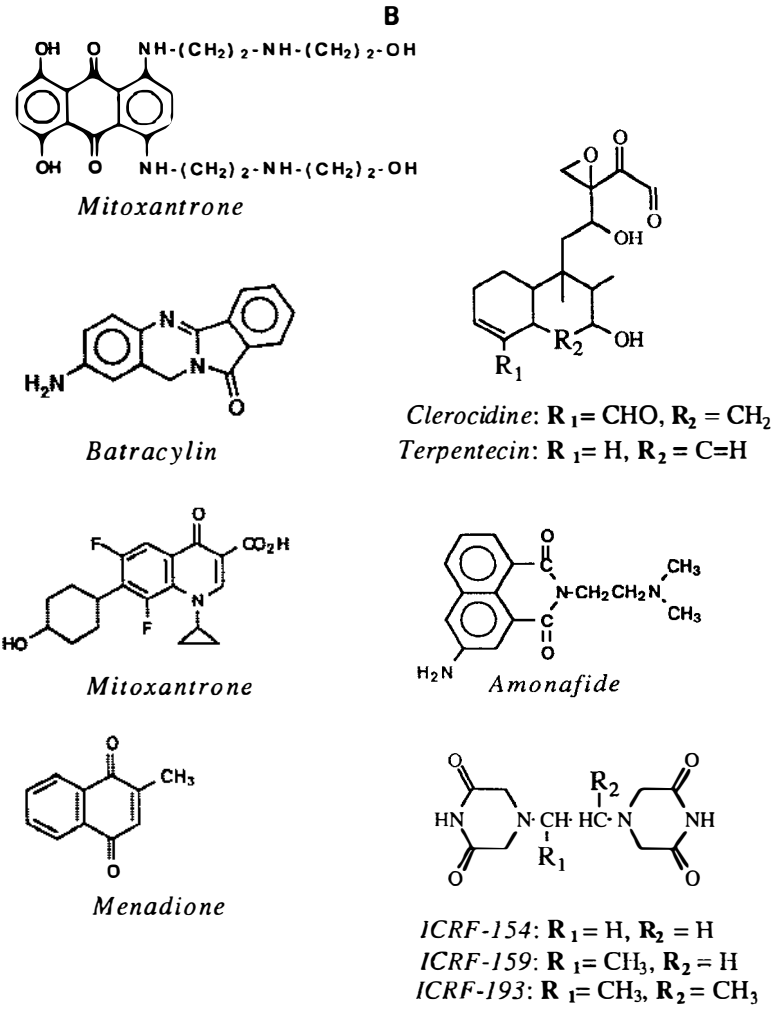


Figure 5 Chemical structures of some topoisomerase II drugs.

topoisomerase II drugs induce high levels of illegitimate recombination (152, 153), as demonstrated by induction of rapid and efficient integration of SV40 DNA (153). The unusual efficiency of topoisomerase II drugs in inducing DNA sequence rearrangements most likely reflects the capacity of topoisomerase II to introduce double-strand DNA breaks.

DRUG-RESISTANCE MECHANISMS

Drug resistance of cancer cells, both intrinsic and acquired, remains one of the major problems in cancer chemotherapy. In theory, drug resistance of cancer cells may arise from alterations at any step in the cell-killing pathway of the particular anticancer drug. Indeed, alterations in cell-cycle progression, drug transport, drug metabolism, drug target, as well as the processing of DNA damage, have all been implicated in cancer cells' development of drug resistance to chemotherapeutic drugs. Particularly, resistance to various topoisomerase I and II inhibitors has been documented in tissue culture cells with respect to MDR1 overexpression, reduced topoisomerase levels, drug-resistant mutant topoisomerase, lengthened cell cycle time, and altered DNA repair functions (see Table 1).

Drug-resistant cell lines, which have been selected with various chemically unrelated hydrophobic anticancer drugs, including adriamycin and vinblastine, overexpress MDR1, a cell membrane–located, energy-dependent drug-effluxing pump (154). These MDR1-overexpressing cells exhibit cross-resistance to most topoisomerase II drugs (reviewed in 155). On the other hand, some topoisomerase I drugs (e.g. camptothecin, 9-amino-campto-

Table 1 Drug resistance mechanisms associated with topoisomerase (topo)-targeting drugs

Drugs[a]	MDR1 overexpression	Reduced topo levels	Drug-resistant mutant topo	Lengthened cell cycle time	Altered DNA repair function
CPTs	–	+	+	+	+
TPT	+	NA[b]	NA	NA	NA
Ho33342	+	NA	NA	NA	NA
ACT-D	+	NA	NA	NA	NA
Epipodophyllotoxins (VM-26/VP-16)	+	+	+	+	+
Anthracyclines (DOX/DAU/MTN)	+	+	+	+	NA
m-AMSA	NA	NA	+	+	+
Ellipticine	+	+	NA	+	NA

[a] CPTs, represent camptothecin, camptothecin-11, 9-amino-camptothecin, and 10,11-methylenedioxy-camptothecin; TPT, topotecan; Ho33342, Hoechst dye 33342; ACT-D, actinomycin D; DOX, doxorubicin; DAU, daunomycin; MTN, mitoxantrone; m-AMSA, 4'(9-acridinylamino)-methane-sulfon-m-anisidide.

[b] NA, not applicable.

thecin, and 10,11-methylenedioxy-camptothecin) overcome MDR1-mediated resistance (89). However, both the positively charged topoisomerase I poisons, topotecan and the newly identified Ho33342, are substrates of MDR1 (89, 100).

Alterations of topoisomerase I and II in cancer cells, by either lowering their expression levels or expressing mutant forms of enzymes, may lead to drug resistance simply because of a reduction in the formation of the cleavable complexes (reviewed in 155). Recently, researchers reported a different method of inducing resistance to topoisomerase II drugs in cultured cells by expressing the dominant negative genetic suppressor elements (GSEs) (156). The expression of GSEs either in the sense form or the antisense form lowers the cellular levels of functional topoisomerase II, thus leading to resistance to topoisomerase II drugs (156). The same approach may be applied to studying other drug resistance mechanisms.

A growing collection of studies has shown that tumor cells resistant to topoisomerase-targeting drugs may contain drug-resistant forms of topoisomerases (76, 157–172). The most well-characterized mutant topoisomerase I is from the CPT-resistant human acute lymphoblastic leukemia cell line (CPT-K5) (76, 159). Among the two mutations found in the cDNA of the topoisomerase I gene from CPT-K5 cells, both are aspartic acid–to-glycine changes; the change at residue 533 is probably responsible for resistance to camptothecin (159, 173). Recent studies on the purified mutant topoisomerase I from CPT-K5 cells have suggested that the alteration in the affinity between the mutant topoisomerase I and DNA is one feature of the mutant enzyme that may be responsible for the mutant enzyme's resistance to camptothecin (174). Studies of the VM-26-resistant form of topoisomerase II, isolated from VM-26-resistant sublines of human leukemia cell line CCRF-CEM, have suggested that the way the mutant topoisomerase II binds ATP can be altered (164). However, whether this mutation is responsible for resistance to VM-26 remains unclear.

Many of the drug-resistant tumor cells selected with topoisomerase inhibitors exhibit lengthened cell cycle time (76, 175–181). Because camptothecin is highly S-phase-specific, lengthened cell cycle time is expected to reduce the relative S-phase cell population and cause resistance. Topoisomerase II drugs at lower concentrations are also S-phase specific (73, 182).

RAD52 is involved in the repair of double-strand DNA breaks in yeast. Studies in *Saccharomyces cerevisiae* have shown that *rad52* mutations can confer hypersensitivity to camptothecin, VP-16, and m-AMSA (77, 183). These results suggest the involvement of a DNA-repair mechanism in cell killing by topoisomerase drugs and that DNA double-strand breaks may be central to this activity. Topoisomerase drugs also have drug resistance mechanisms relating to changes in either the growth or metabolic state of

the cells. Induction of thermotolerant states under glucose-regulated stresses, alterations in the activity of various kinases (129, 184–186), and modulation of the metabolic state of cells (73) significantly affect the cytotoxicity of topoisomerase drugs.

Cloned mammalian DNA topoisomerase IIβ isozyme (187, 188) is sensitive to topoisomerase II poisons (189). Drug resistance to topoisomerase II drugs is associated with alterations in topoisomerase IIβ (190, 191). The differential sensitivity of topoisomerase IIα and IIβ to various topoisomerase II drugs (189) and the differential distribution of topoisomerase IIα and IIβ in different tissues and phases of the cell cycle (192–195) may complicate studies of the cytotoxic mechanisms of topoisomerase II inhibitors. Further studies are necessary to elucidate the role of topoisomerase IIβ in drug resistance.

Human colon tumor xenografts are refractory to most anticancer drugs. However, recent studies have demonstrated that camptothecin analogues exhibit unprecedented antitumor activity against human colon tumor xenografts in nude mice (85). Studies also suggest that the ability of camptothecin to overcome MDR1-mediated resistance is one possible reason for its high activity (89, 90). If this interpretation is correct, the development of topoisomerase II anticancer drugs that can overcome MDR1-mediated resistance may also hold promise for the treatment of some human solid tumors. Interestingly, menadione (vitamin K3) and batracylin derivatives, which were recently identified as topoisomerase II poisons (196, 197), are efficacious in treating MDR1-expressing cancer cells both in cultured-cell and animal-model systems (198, 199; AY Chen & LF Liu, unpublished results). In addition, both menadione and batracylin are equally cytotoxic against the VM-26-resistant CEM/VM-1 cells (AY Chen & LF Liu, unpublished results), which express a mutant form of topoisomerase II (160). Menadione, together with a group of bioreductive alkylating simple quinones, targets mammalian topoisomerase II with a different mode of action that is ATP-independent (AY Chen & LF Liu, unpublished results). These ATP-independent topoisomerase II drugs may interact with both the open-gate and closed-gate cleavable complexes (see Figure 5).

In contrast to the greater than 400-fold resistance to camptothecin, the camptothecin-resistant human lymphoblastic CPT-K5 cell line, which expresses a camptothecin-resistant form of topoisomerase I (76, 159), is minimally cross-resistant to Ho33342 (100). The drug-interaction site on topoisomerase I may, therefore, be different for camptothecin and Ho33342. The sensitivity of Ho33342 to MDR1 and its high potency against camptothecin-resistant cells may suggest a potential use of Ho33342 in cancer chemotherapy (100).

Designing new drugs for various resistant tumors requires fundamental information on various drug-resistance mechanisms. Someday we will be

able to tailor drugs for particular drug-resistant tumors. Some of the parameters that may have to be considered for rational drug design have begun to emerge during recent years. Studies of topoisomerase drugs have contributed significantly to this area.

PERSPECTIVES

Our understanding of chemotherapy of cancer has advanced significantly as a result of studies of various molecular targets for anticancer drugs. The effectiveness of some of these drugs against some tumors has been impressive. However, for the most part, a cure has been limited to a small number of tumors. The discovery of DNA topoisomerases as new therapeutic targets has added a new dimension to the study of anticancer drugs. First, the conversion of an essential enzyme for cell proliferation into a lethal poison is a unique mechanism for killing tumor cells. In this case, drug resistance is often associated with reduced rather than elevated levels of the target enzymes. Second, topoisomerase-mediated DNA damage represents a new type of DNA damage. Because of the specificity of these topoisomerase drugs, DNA damage can be more conveniently studied. Third, these drugs can also be conveniently used to probe the resistance mechanisms of tumor cells. Fourth, these studies may also lead to a better understanding of the mechanism of induced cellular differentiation by anticancer drugs. Finally, one can address the issue of tumor specificity versus proliferation specificity through studies of topoisomerase drugs. We expect that rapid progress in these areas will be made in the coming years.

ACKNOWLEDGMENTS

This work was supported, in part, by the National Institutes of Health grants CA 39662 and CA 40884 (LFL) and the Leukemia Society Special Fellowship (AYC). The authors are indebted to Dr. Peter D'Arpa for his helpful advice in the preparation of this review.

Literature Cited

1. Wang JC. 1971. Interaction between DNA and *Escherichia coli* protein omega. *J. Mol. Biol.* 55:523–33
2. Depew RE, Liu LF, Wang JC. 1978. Interaction between DNA and *E. coli* protein omega: the formation of a complex between single-stranded DNA and omega protein. *J. Biol. Chem.* 253:511–18
3. Champoux JJ, Dulbecco R. 1972. An

activity from mammalian cells that untwists superhelical DNA-a possible swivel for DNA replication. *Proc. Natl. Acad. Sci. USA* 69:143–46
4. Gellert M, Mizuuchi K, O'Dea MH, Nash HA. 1976. An enzyme that introduces superhelical turns into DNA. *Proc. Natl. Acad. Sci. USA* 73:3872–76
5. DiNardo S, Voelkel KA, Sternglanz R, Reynolds AE, Wright A. 1982. *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. *Cell* 31:43–51
6. Liu LF, Wang JC. 1987. Supercoiling of the DNA template during transcription. *Proc. Natl. Acad. Sci. USA* 84: 7024–27
7. Wu H-Y, Shyy SH, Wang JC, Liu LF. 1988. Transcription generates positively and negatively supercoiled domains in the template. *Cell* 53:433–40
8. Tsao Y-P, Wu H-Y, Liu LF. 1989. Transcription-driven supercoiling of DNA: direct biochemical evidence from *in vitro* studies. *Cell* 56:111–18
9. Liu LF, Liu CC, Alberts BM. 1979. T4 DNA topoisomerase. A new ATP-dependent enzyme essential for the initiation of T4 bacteriophage DNA replication. *Nature* 281:456–61
10. Stetler GL, King FJ, Huang WM. 1979. T4 DNA-delay proteins, required for specific DNA replications, from a complex that has ATP-dependent DNA topoisomerase activity. *Proc. Natl. Acad. Sci. USA* 76:3737–41
11. Liu LF. 1980. DNA strand passing and the function of type II DNA topoisomerases. In *Mechanistic Studies of DNA Replication and Genetic Recombination. ICN-UCLA Symp. Mol. Cell. Biol.*, Vol. 19, ed. BM Alberts, 68:817–31. New York: Academic. 1003 pp.
12. Hsieh TS, Brutlag D. 1980. ATP dependent DNA Topoisomerase from *D. melanogaster* reversibly catenates duplex DNA rings. *Cell* 21:115–25
13. Baldi MI, Benedetti P, Mattoccia E, Tocchini-Valentini GP. 1980. In vitro catenation and decatenation of DNA and a novel eukaryotic ATP-dependent Topoisomerase. *Cell* 20:461–67
14. Miller KG, Miller KG, Liu LF, Englund PT. 1981. A homogeneous type II DNA topoisomerase from HeLa cell nuclei. *J. Biol. Chem.* 256:9334–39
15. Wang JC, Liu LF. 1979. DNA topoisomerases. Enzymes that catalyze the concerted breaking and rejoining of DNA bonds. In *Molecular Genetics*, ed. JH Taylor, 3:65–88. New York: Academic
16. Srivenugopal KS, Lockshon D, Morris DR. 1984. *Escherichia coli* DNA topoisomerase III: purification and characterization of a new type I enzyme. *Biochemistry* 23:1899–906
17. Kim RA, Wang JC. 1992. Identification of the yeast TOP3 gene product as a single strand-specific DNA topoisomerase. *J. Biol. Chem.* 267:17178–85
18. Wallis JW, Chrebet G, Brodsky G, Rolfe M, Rothstein R. 1989. A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. *Cell* 58:409–19
19. Kato J, Nishimura Y, Imamura R, Niki H, Hiraga S, Suzuki H. 1990. New topoisomerase essential for chromosome segregation in *E. coli*. *Cell* 63:393–404
20. Kato J, Suzuki H, Ikeda H. 1992. Purification and characterization of DNA topoisomerase IV in *Escherichia coli*. *J. Biol. Chem.* 267:25676–84
21. Slesarev AI, Stetter KO, Lake JA, Gellert M, Krah R, Kozyavkin SA. 1993. DNA topoisomerase V is a relative of eukaryotic topoisomerase I from a hyperthermophilic prokaryote. *Nature* 364:735–37
22. Nash HA. 1981. Integration and excision of bacteriophage: the mechanism of conservative site specific recomination. *Annu. Rev. Genet.* 15: 143–66
23. Weisberg RA, Landy A. 1983. Site-specific recombination in phage lamda. In *Lamda II*, ed. RW Hendrix, JW Roberts, FW Stahl, RA Weisberg, pp. 211–50. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
24. Kitts P, Richet E, Nash HA. 1984. λ Integrative recombination: supercoiling, synapsis, and strand exchange. *Quant. Biol.* 9:735–44
25. Richet E, Abcarian P, Nash HA. 1986. The interaction of recombination proteins with supercoiled DNA: defining the role of supercoiling in Lambda integrative recombination. *Cell* 46: 1011–21
26. Castell SE, Halford SE. 1989. DNA supercoiling determines the activation energy barrier for site specific recombination by TN21 resolvase. *Nucleic Acids Res.* 17:7045–58
27. Droge P, Cozzarelli NR. 1989. Recombination of knotted substrates by Tn3 resolvase. *Proc. Natl. Acad. Sci. USA* 86:6062–66
28. Liu LF, Liu CC, Alberts BM. 1980. Type II DNA topoisomerases. Enzymes that can unknot a topologically knotted

DNA molecule via a reversible double-strand break. *Cell* 19:697–707
29. Cozzarelli NR. 1980. DNA gyrase and the supercoiling of DNA. *Science* 207: 953–60
30. Gellert M. 1981. DNA topoisomerases. *Annu. Rev. Biochem.* 50:879–910
31. Gellert M, Mizuuchi K, O'Dea MH, Itoh T, Tomizawa J. 1977. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. *Proc. Natl. Acad. Sci. USA* 74:4772–76
32. Sugino A, Peebles CL, Kruezer KN, Cozzarelli NR. 1977. 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* 74:4767–71
33. Shen LL, Mitscher LA, Sharma PN, O'Donnell TJ, Chu DWT, et al. 1989. Mechanism of inhibition of DNA gyrase by quinolone antibacterials—A cooperative drug-DNA binding model. *Biochemistry* 28:3886–94
34. Shen LL. 1993. Molecular mechanism of DNA gyrase inhibition by quinolone antibacterial agents. In *Molecular Biology of DNA Topoisomerases and Its Application to Chemotherapy,* ed. T Andoh, H Ikeda, M Oguro, pp. 177–86. Boca Raton, Fla: CRC Press
35. Tewey KM, Rowe TC, Yang L, Halligan BC, Liu LF. 1984. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 226:466–68
36. Nelson EM, Tewey KM, Liu LF. 1984. Mechanism of antitumor drugs. Poisoning of mammalian DNA topoisomerase II on DNA by an antitumor drug m-AMSA. *Proc. Natl. Acad. Sci. USA* 81:1361–65
37. Tewey KM, Chen GL, Nelson EM, Liu LF. 1984. Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. *J. Biol. Chem.* 259: 9182–87
38. Liu LF. 1989. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* 58:351–75
39. Liu LF, D'Arpa P. 1992. Topoisomerase-targeting antitumor drugs: mechanisms of cytotoxicity and resistance. *Important Adv. Oncol.* 1992:79–89
40. Andoh T, Ikeda H, Oguro, M, eds. 1993. *Molecular biology of DNA topoisomerases and its application to chemotherapy,* Boca Raton, Fla: CRC Press. 377 pp.
41. Champoux JJ, Conaughy BL. 1976. Purification and characterzation of the DNA untwisting enzyme from rat liver. *Biochemistry* 15:4638–42
42. D'Arpa P, Machlin PS, Ratrie H III, Rothfield NF, Cleveland DW, Earnshaw WC. 1988. cDNA cloning of human DNA topoisomerase I: catalytic activity of a 67.7-kDa carboxyl-terminal fragment. *Proc. Natl. Acad. Sci. USA* 85:2543–47
43. Juan C-C, Hwang J, Liu AA, Whang-Peng J, Knutsen T, et al. 1988. Human DNA topoisomerase I is encoded by a single-copy gene that maps to chromosome region 20q12–13.2. *Proc. Natl. Acad. Sci. USA* 85:8910–13
44. Champoux JJ. 1990. Mechanistic aspects of type-I topoisomerases. In *DNA Topology and Its Biological Effects,* ed. NR Cozzarelli, JC Wang, pp. 217–42. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
45. Champoux JJ. 1977. Strand breakage by the DNA untwisting enzyme results in covalent attachment of the enzyme to DNA. *Proc. Natl. Acad. Sci. USA* 74:3800–4
46. Champoux JJ. 1978. Mechanism of the reaction catalyzed by the DNA untwisting enzyme: attachment of the enzyme to 3'-terminus of the nicked DNA. *J. Mol. Biol.* 118:441–46
47. Been MD, Burgess RR, Champoux JJ. 1984. Nucleotide sequence preference at rat liver and wheat germ type 1 DNA topoisomerase breakage sites in duplex SV40 DNA. *Nucleic Acids Res.* 12:3097–4014
48. Edwards KA, Halligan BD, Davis JL, Nevera NL, Liu LF. 1982. Recognition sites of eukaryotic DNA topoisomerase I. DNA nucleotide sequencing analyses of topo I cleavage sites on SV40 DNA. *Nucleic Acids Res.* 10:2565–76
49. Champoux JJ, McCoubrey WK Jr, Been MD. 1984. DNA structural features that lead to strand breakage by eukaryotic type-I topoisomerase. *Cold Spring Habor Symp. Quant. Biol.* 49: 435–42
50. Halligan BD, Davis JL, Edwards KA, Liu LF. 1982. Intra- and intermolecular strand transfer by Hela DNA topo isomerase I. *J. Biol. Chem.* 257:3995–4000
51. Been MD, Champoux JJ. 1980. Breakage of single-stranded DNA by rat liver nicking-closing enzyme with the formation of a DNA-enzyme complex. *Nucleic Acids Res.* 8:6129–42
52. Been MD, Champoux JJ. 1981. 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* 78:2883–87

53. Yang L, Wold MS, Li JJ, Kelly TJ, Liu LF. 1987. Roles of DNA topoisomerases in SV40 DNA replication *in vitro*. *Proc. Natl. Acad. Sci. USA* 84:950–54
54. Fleischmann G, Pflugfelder G, Steiner EK, Javaherian K, Howard GC, et al. 1984. *Drosophila* DNA topoisomerase I is associated with transcriptionally active regions of the genome. *Proc. Natl. Acad. Sci. USA* 81:6958–62
55. Stewart AF, Herrera RE, Nordheim A. 1990. Rapid induction of C-fos transcription reveals quantitative linkage of RNA polymerase-II and DNA topoisomerase-I enzyme activities. *Cell* 60:141–49
56. Zhang H, Wang JC, Liu LF. 1988. Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. *Proc. Natl. Acad. Sci. USA* 85:1060–64
57. Merino A, Madden KR, Lane WS, Champoux JJ, Reinberg D. 1993. DNA topoisomerase I is involved in both repression and activation of transcription. *Nature* 365:227–32
58. Wall ME, Wani MC, Cooke CE, Palmer KH, McPhail AT, et al. 1966. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Camptotheca acuminata. *J. Am. Chem. Soc.* 88:3888–90
59. Gottlieb JA, Guarino AM, Call JB, Oliverio VT, Block JB. 1970. Preliminary pharmacological and clinical evaluation of camptothecin sodium (NSC-100880). *Cancer Chemother. Rep.* 54:461–70
60. Muggia FM, Creaven PJ, Hansen HH, Cohen MH, Selawry OS. 1972. Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother. Rep. Part I* 56:515–21
61. Hertzberg RP, Caranfa MJ, Holdern KG, Jakas DR, Gallagher G, et al. 1989. Modification of the hydroxy lactone ring of camptothecin: inhibition of mammalian topoisomerase I and biological activity. *J. Med. Chem.* 32:715–20
62. Bosmann HB. 1970. Camptothecin inhibits macromolecular synthesis in mammalian cells but not in isolated mitochondria or E. coli. *Biochem. Biophy. Res. Commun.* 41:1412–20
63. Horwitz SB, Chang C-K, Grollman AP. 1971. Studies on camptothecin: effects on nucleic acid and protein synthesis. *Mol. Pharmacol.* 7:632–44
64. Horwitz MS, Horwitz SB. 1971. Intracellular degradation of hela and adenovirus type 2 DNA induced by camptothecin. *Biochem. Biophys. Res. Commun.* 45:723–27
65. Kessel D. 1971. Effects of camptothecin on RNA synthesis in leukemia L1210 cells. *Biochim. Biophys. Acta* 246:225–32
66. Kessel D, Bosmann HB, Lohr K. 1972. Camptothecin effects on DNA synthesis in murine leukemia cells. *Biochim. Biophys. Acta* 269:210–16
67. Wu RS, Kumar A, Warner JR. 1971. Ribosome formation is blocked by camptothecin, a reversible inhibitor of RNA synthesis. *Proc. Natl. Acad. Sci. USA* 68:3009–14
68. Abelson HT, Penman S. 1972. Selective interruption of high molecular weight RNA synthesis in HeLa cells by camptothecin. *Nature* 237:144–46
69. Li LH, Fraser TJ, Olin EJ, Bhuyan BK. 1972. Action of camptothecin on mammalian cells in culture. *Cancer Res.* 32:2643–50
70. Horwitz SB, Horwitz MS. 1973. Effects of camptothecin on the breakage and repair of DNA during the cell cycle. *Cancer Res.* 33:2834–36
71. Hsiang Y-H, Lihou MG, Liu LF. 1989. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.* 49:5077–82
72. Covey JM, Jaxel C, Kohn KW, Pommier Y. 1989. Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase-I. *Cancer Res.* 49:5016–22
73. D'Arpa P, Beardmore C, Liu LF. 1990. Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. *Cancer Res.* 50:6919–24
74. Hsiang Y-H, Hertzberg R, Hecht S, Liu LF. 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.* 260:14873–78
75. Hsiang Y-H, Liu LF. 1988. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res.* 48:1722–26
76. Andoh T, Ishii K, Suzuki Y, Ikegami Y, Kusunoki Y, et al. 1987. Characterization of a mammalian mutant with

a camptothecin resistant DNA topoisomerase I. *Proc. Natl. Acad. Sci. USA* 84:5565–69
77. Nitiss J, Wang JC. 1988. DNA topoisomerase-targeting antitumor drugs can be studied in yeast. *Proc. Natl. Acad. Sci. USA* 85:7501–5
78. Zhang H, D'Arpa P, Liu LF. 1990. A model for tumor cell killing by topoisomerase poisons. *Cancer Cells* 2:23–27
79. Tsao Y-P, Russo A, Nyamuswa G, Silber R, Liu LF. 1993. Interaction between replication forks and topoisomerase I-DNA cleavable complexes: studies in a cell-free SV40 DNA replication system. *Cancer Res.* 53:1–8
80. Tsao Y-P, D'Arpa P, Liu LF. 1992. The involvement of active DNA synthesis in camptothecin-induced G2 arrest: altered regulation of p34cdc2/-cyclin B. *Cancer Res.* 52:1823–29
81. Kharbanda S, Rubin E, Gunji H, Hinz H, Giovanella B, et al. 1991. Camptothecin and its derivatives induce expression of the c-jun protooncogene in human myeloid leukemia cells. *Cancer Res.* 51:6636–42
82. McSheehy PM, Gervasoni M, Lampasona V, Erba E, D'Incalci M. 1991. Studies of the differentiation properties of camptothecin in the human leukaemic cells K562. *Eur. J. Cancer* 27: 1406–11
83. Ling YH, Tseng MT, Nelson JA. 1991. Differentiation induction of human promyelocytic leukemia cells by 10-hydroxycamptothecin, a DNA topoisomerase I inhibitor. *Differentiation* 46:135–41
84. Aller P, Rius C, Mata F, Zorrilla A, Cabanas C, et al. 1992. Camptothecin induces differentiation and stimulates the expression of differentiation-related genes in U-937 human promonocytic leukemia cells. *Cancer Res.* 52:1245–51
85. Giovanella BC, Stehlin JS, Wall WE, Wani MC, Nicholas AW, et al. 1989. DNA topoisomerase I-targeted chemotherapy of human colon cancer in Xenografts. *Science* 246:1046–48
86. Pantazis P, Hinz HR, Mendoza JT, Kozielski AJ, Williams LJJ, et al. 1992. Complete inhibition of growth followed by death of human malignant melanoma cells in vitro and regression of human melanoma xenografts in immunodeficient mice induced by camptothecins. *Cancer Res.* 52:3980–87
87. Pantazis P, Early JA, Kozielski AJ, Mendoza JT, Hinz HR, et al. 1993. Regression of human breast carcinoma tumors in immunodeficient mice treated with 9-nitrocamptothecin: differential response of nontumorigenic and tumorigenic human breast cells in vitro. *Cancer Res.* 53:1577–82
88. Pantazis P, Kozielski AJ, Mendoza JT, Early JA, Hinz HR, et al. 1993. Camptothecin derivatives induce regression of human ovarian carcinomas grown in nude mice and distinguish between nontumorigenic and tumorigenic cells in vitro. *Int. J. Cancer* 53:863–71
89. Chen AY, Yu C, Potmesil M, Wall ME, Wani MC, et al. 1991. Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. *Cancer Res.* 51:6039–44
90. Hendricks CB, Rowinsky EK, Grochow LB, Donehower RC, Kaufmann SH. 1992. Effect of P-glycoprotein expression on the accumulation and cytotoxicity of topotecan (SK&F. 104864), a new camptothecin analogue. *Cancer Res.* 52:2268–78
91. Hwong CL, Chen MS, Hwang J. 1989. Phorbol ester transiently increases topoisomerase I mRNA levels in human skin fibrosblasts. *J. Biol. Chem.* 264: 14923–26
92. Wani MC, Nicholas AW, Manikumar G, Wall ME. 1987. Plant antitumor agents. 25. Total synthesis and antileukemic activity of ring A substituted camptothecin analogues. Structure-activity correlations. *J. Med. Chem.* 30: 1774–79
93. Hsiang Y-H, Liu LF, Wall ME, Wani NC, Nicholas AW, et al. 1989. DNA topoisomerase I mediated DNA cleavage and cytotoxicity of camptothecin analogs. *Cancer Res.* 49:4385–89
94. Yokokura T, Furuta T, Sawda S, Sugino E, Mutai M, et al. 1984. Antitumor activity of newly synthesized lactone ring-closed and water-soluble camptothecin derivative in mice. In *Proc. Jpn. Cancer Assoc., 43rd Annu. Meet. Japan,* p. 261
95. Kawato Y, Aonuma M, Hirota Y, Kuga H, Sato K. 1991. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res.* 51:4187–91
96. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, et al. 1992. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J. Clin. Oncol.* 10:16–20
97. Potmesil M, Giovanella BC, Wall ME,

Liu LF, Silber R, et al. 1993. See Ref. 40, pp. 301–11
98. Trask DK, Muller MT. 1988. Stabilization of type I topoisomerase-DNA covalent complexes by actinomycin D. *Proc. Natl. Acad. Sci. USA* 85:1417–21
99. Wassermann K, Markovits J, Jaxel C, Capranico G, Kohn KW, et al. 1990. Effects of morpholinyl doxorubicins, doxorubicin, and actinomycin-D on mammalian DNA topoisomerase-I and topoisomerase-II. *Mol. Pharmacol.* 38: 38–45
100. Chen AY, Yu C, Bodley A, Peng LF, Liu LF. 1993. A new mammalian DNA topoisomerase I poison Hoechst 33342: cytotoxicity and drug resistance in human cell cultures. *Cancer Res.* 53: 1332–37
101. Chen AY, Yu C, Gatto B, Liu LF. 1993. DNA minor groove-binding ligands: a different class of mammalian DNA topoisomerase I inhibitors. *Proc. Natl. Acad. Sci. USA* 90:8131–35
102. Fujii N, Yamashita Y, Saitoh Y, Nakano H. 1993. Induction of mammalian DNA topoisomerase I-mediated DNA cleavage and DNA winding by bulgarein. *J. Biol. Chem.* 268:13160–65
103. Yamashita Y, Fujii N, Murakata C, Ashizawa T, Okabe M, et al. 1992. Induction of mammalian DNA topoisomerase I mediated DNA cleavage by antitumor indolocarbazole derivatives. *Biochemistry* 31:12069–75
104. Yoshinari T, Yamada A, Uemura D, Nomura K, Arakawa H, et al. 1993. Induction of topoisomerase I-mediated DNA cleavage by a new indolocarbazole, ED-110. *Cancer Res.* 53: 490–94
105. Baguley BC. 1982. Nonintercalative DNA-binding antitumor compounds. *Mol. Cell. Biochem.* 43:167–81
106. Zimmer C, Wahnert U. 1986. Nonintercalating DNA-binding ligands: specificity of the interaction and their use as tools in biophysical, biochemical and biological investigations of the genetic material. *Prog. Biophys. Mol. Biol.* 47:31–112
107. Gilbert DE, Feigon J. 1991. Structural analysis of drug-DNA interactions. *Curr. Opin. Struct. Biol.* 1:439–45
108. Neidle S, Pearl LH, Skelly JV. 1987. DNA structure and perturbation by drug binding. *Biochem. J.* 243:1–13
109. Svejstrup JQ, Christiansen K, Andersen AH, Lund K, Westergaard O. 1990. Minimal DNA duplex requirements for topoisomerase-I-mediated cleavage *in-vitro*. *J. Biol. Chem.* 265:12529–35
110. Wu H-M, Crothers DM. 1984. The locus of sequence-directed and protein-induced DNA bending. *Nature* 308: 509–13
111. Jain SC, Sobell HM. 1972. Stereochemistry of actinomycin binding to DNAI Refinement and further structural details of the actinomycin-deoxyguanosine crystal complex. *J. Mol. Biol.* 13:153–90
112. Sobell HM. 1973. The stereochemistry of actinomycin binding to DNA and its implications in molecular biology. *Prog. Nucleic Acids Res. Mol. Biol.* 13:153–90
113. Scott EV, Zon G, Marzilli LG, Wilson WD. 1988. 2D NMR investigation of the binding of the anticancer drug actinomycin D to duplexd dATGC-GCAT: conformational features of the unique 2:1 adduct. *Biochemistry* 27: 7940–51
114. Liaw Y-C, Gao Y-G, Robinson H, Marel GA, Boom JH, et al. 1989. Antitumor drug nogalamycin binds DNA in both grooves simultaneously: molecular structure of nogalamycin-DNA complex. *Biochemistry* 28:9913–18
115. Banville DL, Keniry MA, Kam M, Shafer RH. 1990. NMR studies of the interaction of chromomycin A3 with small DNA duplexes. Binding to GC-containing sequences. *Biochemistry* 29: 6521–34
116. Banville DL, Keniry MA, Shafer RH. 1990. NMR investigation of mithramycin A binding to d(ATGCAT)2: a comparative study with chromomycin A3. *Biochemistry* 29:9294–304
117. Gao X, Patel DJ. 1990. Chromomycin dimer-DNA oligomer complexes. Sequence selectivity and divalent cation specificity. *Biochemistry* 29:10940–56
118. Zhang X, Patel DJ. 1990. Solution structure of the nogalamycin-DNA complex. *Biochemistry* 29:9451–66
119. Liu LF. 1980. DNA strand passing and the function of type 11 DNA topoisomerases. In *Genetic Recombination,* ed. BM Alberts, pp. 817–31. New York: Academic
120. Halligan BD, Edward KA, Liu LF. 1985. Purification and characterization of a type II DNA topoisomerase from bovine calf thymus. *J. Biol. Chem.* 260:2475–82
121. Yang L, Liu LF, Li JJ, Wold MS, Kelly TJ. 1986. The roles of DNA topoisomerase in SV40 DNA replication. *UCLA Symp. Mol. Cell. Biol.* 47:315–26
122. DiNardo S, Voelkel K, Sternglanz R.

1984. DNA topoisomerase II mutant of *saccharomyces cerevisiae:* topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. *Proc. Natl. Acad. Sci. USA* 81:2616–20

123. Uemura T, Ohkura H, Adachi Y, Morino K, Shiozaki K, et al. 1987. DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe. Cell* 50: 917–25
124. Holm C, Stearns T, Botstein D. 1989. DNA topoisomerase II must act at mitosis to prevent nondisjunction and chromosome breakage. *Mol. Cell. Biol.* 9:159–68
125. Tsai-Pflugfelder M, Liu LF, Liu AA, Tewey KM, Whang-Peng J, et al. 1988. Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome 17q21–22. *Proc. Natl. Acad. Sci. USA* 85:7177–81
126. Wang JC. 1987. Recent studies of DNA topoisomerases. *Biochim. Biophys. Acta* 909:1–9
127. Osheroff N, Shelton ER, Brutlag DL. 1983. DNA topoisomerase II from *Drosophila melanogaster. J. Biol. Chem.* 258:9536–43
128. Osheroff N. 1989. Biochemical basis for the interactions of type I and type II topoisomerases with DNA. *Pharmacol. Ther.* 41:223–41
129. Corbett AH, Zechiedrich EL, Osheroff N. 1992. A role for the passage helix in the DNA cleavage reaction of eukaryotic topoisomerase II. A two-site model for enzyme-mediated DNA cleavage. *J. Biol. Chem.* 267:683–86
130. Liu LF, Rowe TC, Yang L, Tewey KM, Chen GL. 1983. Cleavage of DNA by mammalian DNA topoisomerase II. *J. Biol. Chem.* 258: 15365–70
131. Sander M, Hsieh TS. 1983. Double strand DNA cleavage by Type II DNA topoisomerase from *Drosophila melanogaster. J. Biol. Chem.* 258:8421–28
132. Thomsen B, Bendixen C, Lund K, Andersen AH, Sorensen BS, Westergaard O. 1990. Characterization of the interaction between topoisomerase II and DNA by transcriptional footprinting. *J. Mol. Biol.* 215: 237–44
133. Roca J, Wang JC. 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* 71:833–40
134. Lindsley JE, Wang JC. 1991. Proteolysis patterns of epitopically labeled yeast DNA topoisomerase II suggest an allosteric transition in the enzyme induced by ATP binding. *Proc. Natl. Acad. Sci. USA* 88:10485–89
135. Lindsley JE, Wang JC. 1993. Study of allosteric communication between protomers by immunotagging. *Nature* 361:749–50
136. Lindsley JE, Wang JC. 1993. On the coupling between ATP usage and DNA transport by yeast DNA topoisomerase II. *J. Biol. Chem.* 268:8096–8104
137. Hsiang Y-H, Liu LF. 1989. Evidence for the reversibility of cellular DNA lesion induced by mammalian topoisomerase II poisons. *J. Biol. Chem.* 264:9713–15
138. Tanabe K, Ikegami Y, Ishida R, Andoh T. 1991. Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res.* 51:4903–8
139. Ishida R, Miki T, Narita T, Yui R, Sato M, et al. 1991. Inhibition of intracellular topoisomerase II by antitumor bis(2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. *Cancer Res.* 51:4909–16
140. Ishimi Y, Ishida R, Andoh T. 1992. Effect of ICRF-193, a novel DNA topoisomerase II inhibitor, on simian virus 40 DNA and chromosome replication in vitro. *Mol. Cell. Biol.* 12: 4007–14
141. Bodley AL, Liu LF, Israel M, Giuliani FC, Silber R, et al. 1989. DNA topoisomerase II-mediated Interaction of doxocrubicin and daunomycin congeners with DNA. *Cancer Res.* 49: 5969–78
142. D'Arpa P, Liu LF. 1989. Topoisomerase-targcting antitumor drugs. *Biochim. Biophys. Acta* 989: 163–77
143. Liu LF. 1989. Anticancer drugs that convert DNA topoisomerases into DNA damaging agents. In *DNA Topology and Its Biological Effectd,* ed. NR Cozzarelli, JC Wang. pp. 371–89. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
144. Rowe TC, Chen GL, Hsiang Y-H, Liu LF. 1986. DNA damage by antitumor acridines mediated by mammalian DNA topoisomerase II. *Cancer Res.* 46:2021–26
145. Pommier Y, Covey J, Kerrigan D, Mattes W, Markovits J, et al. 1987. Role of DNA intercalation in the inhibition of purified mouse leukemia

(L1210) DNA topoisomerase II by 9-aminoacridines. *Biochem. Pharmacol.* 36:3477–86

146. Capranico G, Zunino F, Kohn KW, Pommier Y. 1990. Sequence-selective topoisomerase II inhibition by anthracycline derivatives in SV40 DNA: relationship with DNA affinity cytotoxicity. *Biochemistry* 29:562–69
147. Pommier Y, Capranico G, Orr A, Kohn KW. 1991. Distribution of topoisomerase II cleavage sites in simian virus 40 DNA and the effects of drugs. *J. Mol. Biol.* 222:909–24
148. West C, Stratford IJ, Barrass N, Smith E. 1981. A comparison of adriamycin and mAMSA *in vitro:* cell lethality and SCE studies. *Br. J. Cancer* 44: 798–809
149. Deaven LL, Oka MS, Tobey RA. 1978. Cell-cycle specific chromosome damage following treatment of cultured Chinese hamster cells with 4′-(9-acridinylampo)methanesulfon-m-anisidide-HCl. *J. Natl. Cancer Inst.* 60:1155–61
150. Bae YS, Chiba M, Ohira M, Ikeda H. 1991. A shuttle vector for analysis of illegitimate recombination in mammalian cells: effects of DNA topoisomerase inhibitors on deletion frequency. *Gene* 101:285–89
151. Charron M, Hancock R. 1991. Chromosome recombination and defective genome segregation induced in Chinese hamster cells by the topoisomerase II inhibitor VM-26. *Chromosoma* 100:97–102
152. Han YH, Austin MJ, Pommier Y, Povirk LF. 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
153. Bodley AL, Huang H-C, Yu C, Liu LF. 1993. Integration of simian virus 40 into cellular DNA occurs at or near topoisomerase II cleavage hot spots induced by VM-26 (teniposide). *Mol. Cell. Biol.* 13:6190–200
154. Gottesman MM, Pastan I. 1993. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* 62:385–427
155. Chen AY, Liu LF. 1993. Mechanisms of resistance to topoisomerase inhibitors. In *Cancer Treatment and Research,* ed. RF Ozols, L Goldstein. Norwell, Mass: Kluwer Academic. In press
156. Gudkov AV, Zelnick CR, Kazarov AR, Thimmapaya R, Suttle DP, et al. 1993. Isolation of genetic suppressor elements, inducing resistance to topoisomerase II-interactive cytotoxic drugs, from human topoisomerase II cDNA. *Proc. Natl. Acad. Sci. USA* 90:3231–35
157. Gupta RS, Gupta R, Eng B, Lock RB, Ross WE, et al. 1988. Camptothecin-resistant mutants of chinese hamster ovary cells containg a resistant form of topoisomerase I. *Cancer Res.* 48:6404–10
158. Kjeldsen E, Bonven BJ, Andoh T, Ishii K, Okada K, et al. 1988. Characterization of a camptothecin-resistant human DNA topoisomerase I. *J. Biol. Chem.* 263:3912–16
159. Tamura H, Kohchi C, Yamada R, Ikeda T, Koiwai O, et al. 1991. Molecular cloning of a cDNA of a camptothecin-resistant human DNA topoisomerase-I and identification of mutation sites. *Nucleic Acids Res.* 19: 69–75
160. Danks MK, Yalowich JC, Beck WT. 1987. Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teniposide (VM-26). *Cancer Res.* 47:1297–301
161. Glisson BS, Sullivan DM, Gupta R, Ross WE. 1987. Mediation of multidrug resistance in a chinese hamster ovary cell line by a mutant type II topoisomerase. *Natl. Cancer Inst. Monogr.* 4:89–93
162. Zwelling LA, Hinds M, Chan D, Mayes J, Sic KL, et al. 1989. Characterization of an amsacrine-resistant line of human leukemia cells- evidence for a drug-resistant form of topoisomerase-II. *J. Biol. Chem.* 264: 16411–20
163. Zwelling LA, Mayes J, Hinds M, Chan D, Altschuler E, et al. 1991. Cross-resistance of an amsacrine-resistant human leukemia line to topoisomerase II reactive DNA intercalating agents. Evidence for two topoisomerase II directed drug actions. *Biochemistry* 30: 4048–55
164. Bugg BY, Danks MK, Beck WT, Suttle DP. 1991. Expression of a mutant DNA topoisomerase II in CCRF-CEM human leukemic cells selected for resistance to teniposide. *Proc. Natl. Acad. Sci. USA* 88:7654–58
165. Kubota N, Kanzawa F, Nishio K, Takeda Y, Ohmori T, et al. 1992. Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. *Biochem. Biophys. Res. Commun.* 188:571–77
166. Hinds M, Deisseroth K, Mayes J, Altschuler E, Jansen R, et al. 1991.

Identification of a point mutation in the topoisomerase II gene from a human leukemia cell line containing an amsacrine-resistant form of topoisomerase II. *Cancer Res.* 51:4729–31

167. Zwelling LA, Mitchell MJ, Satitpunwaycha P, Mayes J, Altschuler E, et al. 1992. Relative activity of structural analogues of amsacrine against human leukemia cell lines containing amsacrine-sensitive or -resistant forms of topoisomerase II: use of computer simulations in new drug development. *Cancer Res.* 52:209–17
168. Lee MS, Wang JC, Beran M. 1992. Two independent amsacrine-resistant human myeloid leukemia cell lines share an identical point mutation in the 170 kDa form of human topoisomerase II. *J. Mol. Biol.* 223:837–43
169. Kamath N, Grabowski D, Ford J, Kerrigan D, Pommier Y, et al. 1992. Overexpression of P-glycoprotein and alterations in topoisomerase II in P388 mouse leukemia cells selected in vivo for resistance to mitoxantrone. *Biochem. Pharmacol.* 44:937–45
170. Danks MK, Warmoth MR, Friche E, Granzen B, Bugg BY, et al. 1993. Single-strand conformational polymorphism analysis of the M(r) 170,000 isozyme of DNA topoisomerase II in human tumor cells. *Cancer Res.* 53: 1373–79
171. Chan VT, Ng SW, Eder JP, Schnipper LE. 1993. Molecular cloning and identification of a point mutation in the topoisomerase II cDNA from an etoposide-resistant Chinese hamster ovary cell line. *J. Biol. Chem.* 268: 2160–65
172. Sullivan DM, Eskildsen LA, Groom KR, Webb CD, Latham MD, et al. 1993. Topoisomerase II activity involved in cleaving DNA into topological domains is altered in a multiple drug-resistant Chinese hamster ovary cell line. *Mol. Pharmacol.* 43:207–16
173. Andoh T, Tamura H, Kohchi C, Yamada R, Ikeda T, et al. 1993. Mechanism of camptothecin resistance in mammalian cells: mutation in topoisomerase I gene and its implication in enzymatic function. See Ref. 40, pp. 229–35
174. Gromova II, Kjeldsen E, Svejstrup JQ, Alsner J, Christiansen K, et al. 1993. Characterization of an altered DNA catalysis of a camptothecin-resistant eukaryotic topoisomerase I. *Nucleic Acids Res.* 21:593–600
175. Tsuruo T, Matsuzaki T, Matsushita M, Saito H, Yokokura T. 1988. Antitumor effect of CPT-11, a new derivative of camptothecin, against pleiotropic drug-resistant tumors in vitro and in vivo. *Cancer Chemother. Pharmacol.* 21:71–74
176. Long BH, Wang L, Lorico A, Wang RC, Brattain MG, et al. 1991. Mechanisms of resistance to etoposide and teniposide in acquired resistant human colon and lung carcinoma cell lines. *Cancer Res.* 51:5275–83
177. Takano H, Kohno K, Ono M, Uchida Y, Kuwano M. 1991. Increased phosphorylation of DNA topoisomerase II in etoposide-resistant mutants of human cancer KB cells. *Cancer Res.* 51:3951–57
178. Odaimi M, Anderson BS, McCredie KB, Beran M. 1986. Drug sensitivity and cross-resistance of the 4′-(9-acridinylamino)methanesulfon-m-anisidide-resistant subline of HL-60 human leukemia. *Cancer Res.* 46:3330–33
179. Harker WG, Slade DL, Dalton WS, Meltzer PS, Trent JM. 1989. Multidrug resistance in mitoxantrone-selected HL-60 leukemia cells in the absence of P-Glycoprotein overexpression. *Cancer Res.* 49:4542–49
180. Chen Y-N, Mickley LA, Schwartz AM, Acton EM, Hwang J, et al. 1990. Characterization of adriamycin-resistant human breast cancer cells which display overexpression of a novel resistance-related membrane protein. *J. Biol. Chem.* 265:10060–80
181. DeJong S, Zijlstra JG, Devries EGE, Mulder NH. 1990. Reduced DNA topoisomerase II activity and drug-induced DNA cleavage activity in an adriamycin-resistant human small cell lung carcinoma cell line. *Cancer Res.* 50:304–9
182. Wilson WR, Whitmore GF. 1981. Cell-cycle-stage specificity of 4′-(9-acridinylamino)methanesulfon-m-anisidide(m-AMSA) and interaction with ionizing radiation in mammalian cell cultures. *Radiat. Res.* 87:121–36
183. Nitiss J, Wang JC. 1991. Yeast as a genetic system in the dissection of the mechanism of cell-killing by topoisomerase-targeting anticance drugs. In *DNA Topoisomerase in Cancer*, ed. P Milan, KW Kohn, pp. 77–90. New York: Oxford Univ. Press
184. Posada J, Vichi P, Tretton TR. 1989. Protein kinase C in adriamycin action and resistance in mouse sarcoma 180 cells. *Cancer Res.* 49:6634–39
185. Fine RL, Ratel J, Chabner BA. 1987. Phorbol esters induce multidrug resis-

tance in human breast cancer cells. *Proc. Natl. Acad. Sci. USA* 85:582–86
186. Corbett AH, Fernald AW, Osheroff N. 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* 32:2090–97
187. Drake FH, Zimmerman JP, McCabe FL, Bartus HF, Per SR, et al. 1987. Purification of topoisomerase II from amsacrine-resistant P388 leukemia cells. *J. Biol. Chem.* 262:16739–47
188. Austin CA, Sng JH, Patel S, Fisher LM. 1993. Novel HeLa topoisomerase II is the II-β isoform: complete coding sequence and homology with other type II topoisomerases. *Biochim. Biophys. Acta* 1172:283–91
189. Drake FH, Hofmann GA, Bartus HF, Mattern MR, Crooke ST, et al. 1989. Biochemical and pharmacological properties of P170 and P180 forms of topoisomerase-II. *Biochemistry* 28: 8154–60
190. Harker WG, Slade DL, Drake FH, Parr RL. 1991. Mitoxantrone resistance in HL-60 leukemia cells: reduced nuclear topoisomerase II catalytic activity and drug-induced DNA cleavage in association with reduced expression of the topoisomerase-II β-isoform. *Biochemistry* 30:9953–61
191. Hochhauser D, Harris AL. 1993. The role of topoisomerase IIα and β in drug resistance. *Cancer Treat Rev.* 19:181–94
192. Woessner RD, Mattern MR, Mirabelli CK, Johnson RK, Drake FH. 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.* 2:209–14
193. Zini N, Martelli AM, Sabatelli P, Santi S, Negri C, et al. 1992. The 180-kDa isoform of topoisomerase II is localized in the nucleolus and belongs to the structural elements of the nucleolar remnant. *Exp. Cell Res.* 200:460–66
194. Negri C, Chiesa R, Cerino A, Bestagno M, Sala C, et al. 1992. Monoclonal antibodies to human DNA topoisomerase I and the two isoforms of DNA topoisomerase II: 170- and 180-kDa isozymes. *Exp. Cell Res.* 200:452–59
195. Negri C, Scovassi AI, Braghetti A, Guano F, Astaldi RG. 1993. DNA topoisomerase II β: stability and distribution in different animal cells in comparison to DNA topoisomerase I and II α. *Exp. Cell Res.* 206:128–33
196. Chen AY, Yu C, Lee W-H, Peng LF, Liu LF. 1992. Menadione (vitamin K3) induces topoisomerase II-mediated DNA cleavage. *Proc. Am. Assoc. Cancer Res.* 33:2588 (Abstr.)
197. Luo Y, Ren YF, Chou TC, Chen AY, Yu C, et al. 1993. A structure-activity relationship study of batracylin analogues. *Pharm. Res.* 10:918–23
198. Nutter LM, Cheng AL, Hung H-L, Hsieh R-K, Ngo EO, et al. 1991. Menadione: spectrum of anticancer activity and effects on nucleotide metabolism in human neoplastic cell lines. *Biochem. Pharmacol.* 41:1283–92
199. Plowman J, Paull KD, Atassi G, Harrison JSD, Dykes DJ, et al. 1988. Preclinical antitumor activity of batracylin (NSC. 320846). *Invest. New Drugs* 6:147–53