

Topoisomerase Poisons: Harnessing the Dark Side of Enzyme Mechanism*

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Although the one-dimensional sequence of DNA determines the genetic constitution of an organism, topological relationships within the double helix modulate virtually every physiological function of the genome (1, 2). In the cell, the topology of DNA is orchestrated by enzymes known as topoisomerases (1–7). These ubiquitous enzymes are required for replication, transcription, and recombination and play critical roles in chromosome structure, condensation/decondensation, and segregation (1–9). Over the past decade, interest in topoisomerases has expanded beyond the realm of the basic science laboratory into the clinical arena. These enzymes are now known to be the primary cellular targets for some of the most widely prescribed antibiotics and anticancer drugs used in the treatment of human disease (4, 10–19). This review focuses on the mechanism by which drugs alter the catalytic functions of topoisomerases and convert these essential enzymes into lethal cellular weapons.

Topoisomerase Mechanism

All cells contain two highly conserved classes of topoisomerases that are differentiated on the basis of their mechanistic and physical properties. The type I enzymes, which include bacterial topoisomerases I and III as well as eukaryotic topoisomerases I and III, are monomeric in nature, function without a high energy cofactor, and alter the helical pitch of the genetic material by allowing passage of a single DNA strand through a transient nick they create in the complementary strand of the double helix (1–3, 7, 20–22).

The type II enzymes, which encompass bacterial DNA gyrase and topoisomerase IV as well as eukaryotic topoisomerase II, are multisubunit proteins, require ATP for overall catalytic activity, and modulate topology by passing an intact helix through a transient double-stranded break they create in the DNA backbone (1–4, 6, 21–24). As a result of their double-stranded DNA passage reaction, type II topoisomerases are able to regulate over- and under-winding of the double helix and resolve nucleic acid knots and tangles.

For a number of years, it was believed that cells contained only a single type I and type II topoisomerase. However, it is now known that the mechanistic differences between individual enzymes allow them to fulfill highly specialized functions. For example, the superhelical density of bacterial chromosomes is balanced by the opposing action of topoisomerase I (which removes negative superhelical twists) and DNA gyrase (which actively underwinds DNA) (1, 4, 6). By contrast, topoisomerase IV (which is a potent decatenase) is required to unknot daughter chromosomes following replication (8, 25). In eukaryotes, there may be functional divergence between topoisomerases in lower and higher species. While

yeast (26) and *Drosophila* (27) apparently contain only a single type II topoisomerase, mammals possess two isoforms of the enzyme, α and β (24, 28). Our understanding of these two isoforms, especially β , is still rudimentary.

The Dark Side of Topoisomerases

Despite differences in catalytic mechanism and cellular functions, the critical feature common to all topoisomerases is the DNA strand passage event. However, the ability to pass a single- or double-stranded segment of DNA freely through another comes with a heavy price; it requires enzymes that generate breaks in the genetic material. In an effort to maintain genomic integrity during this cleavage reaction, topoisomerases covalently attach to the newly generated DNA 3' (eukaryotic topoisomerase I) or 5' termini (all other topoisomerases discussed in this review) via phosphotyrosyl bonds (1, 4, 7, 20, 22, 23). Under normal circumstances, these covalent enzyme-DNA cleavage complexes are fleeting catalytic intermediates and are present in low steady-state concentrations. Consequently, they are tolerated by the cell. However, conditions that significantly increase the physiological concentration or lifetime of these breaks unleash a myriad of deleterious side effects, including mutations, insertions, deletions, and chromosomal aberrations (2, 14, 18, 29, 30). Thus, all topoisomerases are fundamentally dualistic in nature. Although they catalyze essential reactions in the cell, they possess an inherent dark side capable of inflicting great harm to the genome of an organism.

Topoisomerase Poisons

Beyond their essential cellular functions, eukaryotic topoisomerases I and II and bacterial DNA gyrase are the targets for a number of clinically important drugs (11, 18). Several examples are shown in Fig. 1. Although agents targeted to eukaryotic topoisomerase I are still in clinical trials, they hold great promise as antineoplastic drugs, especially against cancers for which there are presently no effective treatments (7, 10, 15–19). Many of these agents are structurally related to camptothecin. Recent analogs such as topotecan display greater solubility than the parent compound and enhanced stability of their essential lactone ring in human serum (17, 18, 31).

DNA gyrase is the target for quinolone antibiotics (Fig. 1) (4, 11–13). Early compounds, which were based on the structure of nalidixic acid, were of limited therapeutic value. However, the development of subsequent generations of fluorinated quinolones led to compounds with broad antibacterial activity (11, 13). Ciprofloxacin, a representative fluoroquinolone, is the most efficacious oral antibiotic in clinical use today (11, 13). The role of topoisomerase IV as a target for quinolones has been a subject of debate. However, there is now evidence to suggest that these drugs affect topoisomerase IV, and that this interaction contributes to drug efficacy (32).

In contrast to the limited number of drug classes that act on topoisomerase I or DNA gyrase, topoisomerase II is the target for a number of structurally disparate antineoplastic compounds (14, 17, 18) (Fig. 1). Drugs such as etoposide, doxorubicin, amsacrine, and mitoxantrone are routinely employed for the systemic treatment of human cancers (14, 15, 19). Furthermore, the chemotherapeutic regimens for most curable malignancies either include or are based solely on agents targeted to topoisomerase II. Due to differential sensitivities *in vitro*, it has been assumed that drugs act primarily through the α isoform of the enzyme (28). However, recent studies indicate that topoisomerase II β may also play a role in drug action (33, 34).

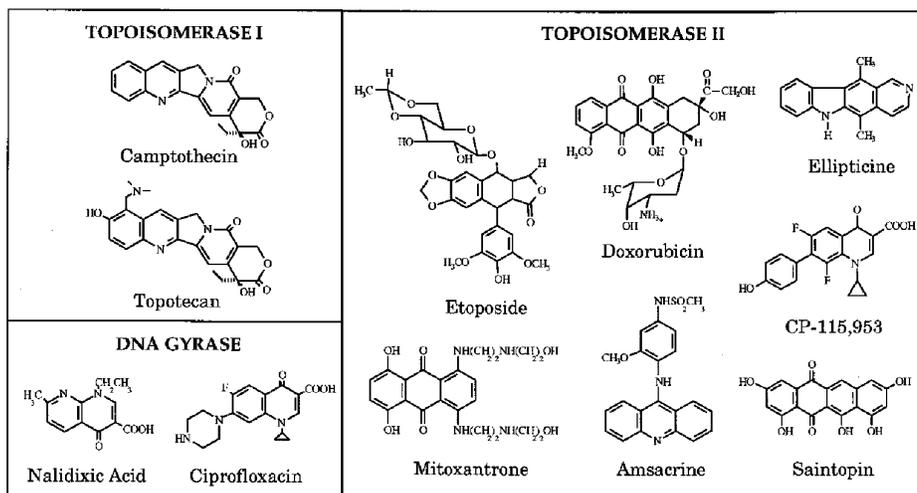
In addition to the clinically relevant topoisomerase II-targeted agents, two compounds that are presently confined to the laboratory, CP-115,953 (35) and saintopin (36), are shown in Fig. 1. CP-115,953, which is a quinolone, displays potent activity against topoisomerase II (greater than etoposide) and DNA gyrase (35). To date, quinolones are the only drug class reported to significantly affect prokaryotic as well as eukaryotic species (13, 35). Saintopin

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FIG. 1. Structures of selected topoisomerase poisons.



is representative of a growing number of drugs that appear to target both eukaryotic topoisomerases I and II (7, 17, 18).

The topoisomerase-targeted drugs discussed above work in an insidious fashion. Unlike most enzyme-targeted agents, the compounds shown in Fig. 1 do not kill cells by blocking topoisomerase catalytic function. Rather they take advantage of the Dr. Jekyll/Mr. Hyde character (37) of topoisomerases (*i.e.* enzymes that perform essential DNA strand passage reactions but cleave the genetic material) and poison these enzymes by increasing the steady-state concentration of their covalent DNA cleavage complexes (4, 12, 14, 15, 17, 18). This action converts topoisomerases into physiological toxins that introduce high levels of transient protein-associated breaks in the genome of treated cells (38) (Fig. 2).

Topoisomerase-DNA cleavage complexes are mutagenic in nature (39). Furthermore, the potential lethality of these drug-induced cleavage complexes rises dramatically when replication machinery or helicases attempt to traverse the covalently bound topoisomerase roadblock in the DNA (40, 41). Such an action disrupts the cleavage complex and converts transient single- or double-strand breaks into permanent double-stranded fractures which are no longer held together by proteinaceous bridges (Fig. 2) (17, 18). Once these untethered breaks are produced in the genetic material, they become targets for recombination and repair pathways. This in turn stimulates sister chromatid exchange, the generation of large insertions and deletions, and the production of chromosomal aberrations and translocations (14, 17, 29, 30). When these permanent DNA breaks are present at sufficient concentration, they trigger a series of events that ultimately culminates in cell death by necrosis or apoptosis (7, 15, 17, 18, 42, 43).

Due to the mechanism of drug action, the higher the physiological concentration of topoisomerases, the more lethal these poisons become (4, 44–48). Levels of topoisomerases I and II are generally elevated in cells that are undergoing rapid proliferation (2, 18, 49). This probably contributes to the increased response of aggressive cancers to topoisomerase-targeted agents (10, 15–19).

Mechanism of Drug Action

Topoisomerase poisons stimulate enzyme-mediated DNA scission by two non-mutually exclusive mechanisms. Drugs can act by impairing the ability of the enzyme to religate cleaved DNA and/or by enhancing the forward rate of DNA cleavage. Thus far, all studies with camptothecin-based drugs indicate that these topoisomerase I poisons act primarily by inhibiting the rate of DNA religation (21, 50–52). In contrast, agents targeted to topoisomerase II have been shown to utilize both mechanisms. While etoposide and amsacrine are potent inhibitors of religation (14, 53), agents such as quinolones and ellipticine have little effect on this enzyme activity and presumably act by enhancing the forward rate of cleavage (14, 35, 54). Comparable studies have not been carried out for DNA gyrase.

Although high concentrations of topoisomerase poisons inhibit other enzymatic activities (*i.e.* ATP hydrolysis and strand passage) (55, 56), they should be differentiated from topoisomerase inhibi-

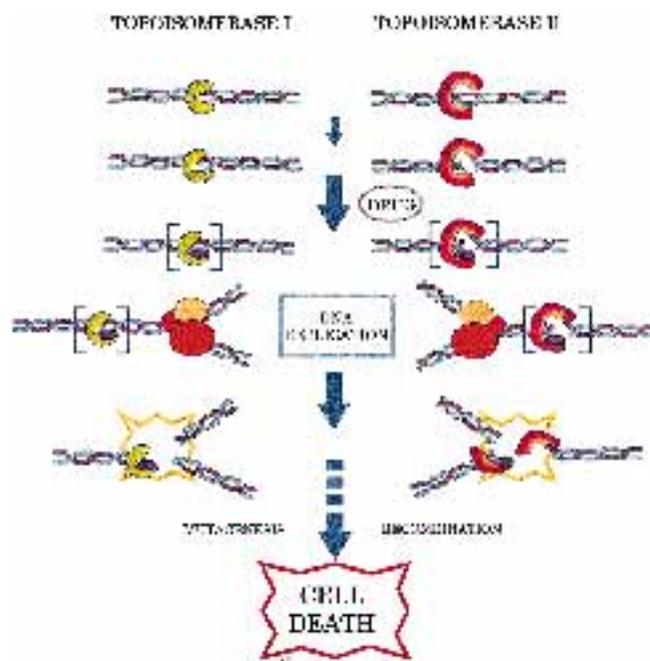


FIG. 2. Mechanism of cytotoxicity by topoisomerase poisons. Topoisomerases bind to DNA in a noncovalent fashion followed by the formation of transient cleavage complexes. In these complexes, topoisomerases I and II create single- and double-stranded breaks, respectively. In the presence of topoisomerase poisons, levels of cleavage complexes (shown in brackets) increase dramatically. Following traversal by replication complexes or helicases, transient topoisomerase-mediated breaks become permanent double-stranded fractures, triggering events that ultimately culminate in cell death. Although this schematic depicts drug-enhanced DNA cleavage by eukaryotic topoisomerases I and II, it is generally applicable to all type I and II enzymes.

tors that act by blocking overall catalytic activity. These include novobiocin which impairs interactions between DNA gyrase and ATP (4), aclarubicin which blocks topoisomerase II-DNA binding (53), and ICRF-193 which blocks topoisomerase II recycling (57). Topoisomerase poisons can be readily distinguished from inhibitors by their cytotoxic criteria (58). Increased levels of topoisomerases render cells hypersensitive to enzyme poisons but resistant to inhibitors (45–48). Conversely, decreased enzyme levels render cells resistant to poisons but hypersensitive to inhibitors (45, 48, 59, 60).

Topoisomerase-Drug-DNA Ternary Complex

The formation of a topoisomerase-drug-DNA complex appears to be critical for nucleic acid cleavage and subsequent cell death. An early indication for the existence of a ternary complex came from kinetic studies on etoposide-topoisomerase II interactions, which showed that the drug had to be present at the time of DNA cleavage

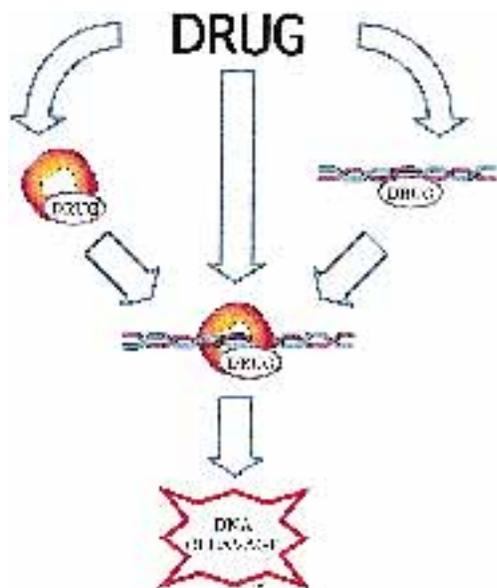


FIG. 3. **Formation of the topoisomerase-drug-DNA ternary complex.** The ternary complex may form by three possible routes. Drug may enter through interactions with its topoisomerase target (left), DNA (right), or the enzyme-DNA complex (center).

to inhibit religation (61). However, despite the clinical relevance of topoisomerase poisons, interactions within this complex are poorly defined.

Several lines of circumstantial evidence place drugs in the ternary complex at the enzyme-DNA interface. The strongest support for this hypothesis comes from studies that used an azido analog of amsacrine (62) or an alkylating analog of camptothecin (63). In both cases, drugs cross-linked to DNA adjacent to the point of cleavage, but only in the presence of their target topoisomerases.

Evidence for drug-DNA interactions in the ternary complex stems from the examination of cleavage site specificity of topoisomerases (7, 14, 64). Numerous studies indicate that site utilization is altered by drugs in a compound-specific manner. Furthermore, saintopin, which targets topoisomerases I and II, enhances cleavage at a similar preferential nucleotide location for both enzymes (65, 66). While these findings suggest drug-DNA interactions contribute to site specificity of the ternary complex, it should be emphasized that DNA binding alone (including intercalation) is insufficient to enhance enzyme-mediated DNA cleavage (67).

Evidence for drug-topoisomerase interactions in the ternary complex is derived from two divergent experimental approaches. First, a number of point mutations that confer drug resistance and/or hypersensitivity have been identified in topoisomerase I, DNA gyrase, and topoisomerase II (4, 7, 14, 58, 68–70). Many of these enzymes still maintain high catalytic activity, suggesting that changes in sensitivity result from altered protein-drug interactions. Second, a recent fluorescence spectroscopy study on topoisomerase II-ellipticine interactions (54) demonstrated that the enzyme dictates the protonation state of the drug in the ternary complex. Moreover, the fluorescence lifetime of bound ellipticine was similar in the binary (enzyme-drug) and ternary complexes, indicating a similar drug environment in both.

Formation of the Ternary Complex

There are three potential routes of ternary complex formation (Fig. 3), and evidence supporting each possibility exists. First, drugs may bind specifically to the topoisomerase-DNA complex and have minimal interactions with either the enzyme or the DNA independently. Strong support for this mechanism comes from binding studies of camptothecin with topoisomerase I (71). In this instance, the agent was found to interact almost exclusively with the enzyme-DNA complex.

Second, drugs may become part of the ternary complex predominantly by interacting with DNA. Support for this mechanism is derived from the fact that many topoisomerase-targeted agents bind to nucleic acids (14, 17, 18). To date, no direct correlation

between cytotoxicity and either the strength or mode of drug-DNA binding has been observed. Thus, the significance of drug-DNA interactions (in the absence of enzyme) remains an open question. However, it is possible that DNA acts as a repository for some topoisomerase poisons and enhances their potency by increasing local drug concentration in the vicinity of these enzymes.

Third, drugs may enter the ternary complex via prior association with their target topoisomerase. Support for this mechanism comes from recent spectroscopy studies on the interaction of intoplicine or ellipticine with topoisomerase II (54, 72). In both cases, drugs complexed with the enzyme in the absence of DNA. Furthermore, the dissociation constant of ellipticine in the drug-enzyme binary complex was an order of magnitude lower than it was in the ternary complex (54).

Drug Resistance/Hypersensitivity

Considerable information regarding drug resistance and hypersensitivity in topoisomerases has been derived from mutagenesis studies. These include analyses of enzymes from clinical isolates as well as laboratory studies that utilized random or site-directed mutagenesis to generate proteins with altered drug sensitivities.

Most studies on topoisomerase I have concentrated on camptothecin-based compounds and have identified a region in the vicinity of the active site tyrosine that appears to be important for drug interaction (7, 65, 66, 69). Other highly conserved regions have also been implicated (7, 50, 69). Cross-resistance to other drug classes has been observed (7, 65, 66), suggesting that topoisomerase I-targeted agents share a common site of action on the enzyme.

Two regions of DNA gyrase have been identified that confer resistance to quinolones. One is found in the *gyrA* subunit (which contains the active site tyrosine) and centers on serine 83 (4, 12, 13). Mutations at this position are prevalent in clinical isolates, confer high levels of drug resistance, and greatly reduce quinolone binding in the ternary complex (4, 12, 13, 73). The second is found in the *gyrB* subunit (which contains the ATP binding site) (4, 12, 13, 74). Although mutations in *gyrB* result in enzymes that display less resistance than do their *gyrA* counterparts, this region contains the first reported mutation conferring drug hypersensitivity (74).

Resistance-conferring mutations have been identified in both the *gyrA* and *gyrB* homology domains of topoisomerase II, but they are scattered over several hundred residues of the enzyme (14, 58, 68, 70). Nonetheless, there appears to be (at least some) evolutionary conservation of drug interaction sites between prokaryotic and eukaryotic type II topoisomerases, since a mutation at the serine residue in yeast topoisomerase II that is homologous to serine 83 in *gyrA* also leads to quinolone resistance (75). Finally, little is known about the mechanism by which mutations in topoisomerase II alter drug sensitivity. However, decreased drug affinity (as derived from potency measurements) has been reported for one resistance mutation (76). Furthermore, increased drug binding affinity as well as decreased rates of DNA religation have been correlated with hypersensitivity (54, 75, 76).

One caveat of topoisomerase mutagenesis studies should be noted; it is impossible to conclude that any amino acid residue identified by this approach interacts directly with drugs. The definitive identification of drug interaction domains is virtually impossible in the absence of detailed structural information. However, based on drug competition studies, it appears that DNA cleavage-enhancing agents share an overlapping site of action, at least on topoisomerase II (56, 77). Therefore, differential cross-resistance patterns observed for specific topoisomerase II mutations (14, 75, 76, 78) probably reflect differences in the microenvironment of bound drug rather than the existence of separate interaction domains.

Summary and Perspectives

Since it was first proposed that cleavage-enhancing drugs acted as topoisomerase poisons (38), numerous advancements have been made in our understanding of the mechanism of topoisomerase-targeted drugs. These inroads have had a tremendous impact on the development of novel topoisomerase poisons and the clinical scheduling of these agents. Considering that drugs have not been identified for all topoisomerases, the full medical potential of these

enzymes remains to be tapped. An important lesson we have learned from the variety of topoisomerase poisons is that generalizations regarding the details of drug mechanism are often precarious. Clearly, more extensive mechanistic studies need to be undertaken in order to fully exploit the dark side of topoisomerases for the treatment of human disease.

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REFERENCES

- Wang, J. C. (1985) *Annu. Rev. Biochem.* **54**, 665–697
- Cozzarelli, N. R., and Wang, J. C. (1990) *DNA Topology and Its Biological Effects*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Osheroff, N. (1989) *Pharmacol. Ther.* **41**, 223–241
- Reece, R. J., and Maxwell, A. (1991) *Crit. Rev. Biochem. Mol. Biol.* **26**, 335–375
- Bjornsti, M.-A. (1991) *Curr. Opin. Struct. Biol.* **1**, 99–103
- Luttinger, A. (1995) *Mol. Microbiol.* **15**, 601–606
- Gupta, M., Fujimori, A., and Pommier, Y. (1995) *Biochim. Biophys. Acta* **1262**, 1–14
- Nitiss, J. L. (1994) *Adv. Pharmacol.* **29A**, 103–134
- Earnshaw, W. C., and Mackay, A. M. (1994) *FASEB J.* **8**, 947–956
- Potmesil, M., and Kohn, K. W. (1991) *DNA Topoisomerases in Cancer*, Oxford University Press, New York
- Hooper, D. C., and Wolfson, J. S. (1991) *N. Engl. J. Med.* **324**, 384–394
- Maxwell, A. (1992) *J. Antimicrob. Chemother.* **30**, 409–414
- Hooper, D. C., and Wolfson, J. S. (eds.) (1993) *Quinolone Antimicrobial Agents*, American Society for Microbiology, Washington, D. C.
- Corbett, A. H., and Osheroff, N. (1993) *Chem. Res. Toxicol.* **6**, 585–597
- Pommier, Y. (1993) *Cancer Chemother. Pharmacol.* **32**, 103–108
- Slichenmyer, W. J., Rowinsky, E. K., Donehower, R. C., and Kaufmann, S. H. (1993) *J. Natl. Cancer Inst.* **85**, 271–287
- Chen, A. Y., and Liu, L. F. (1994) *Annu. Rev. Pharmacol. Toxicol.* **34**, 191–218
- Liu, L. F. (ed) (1994) *Adv. Pharmacol.* **29**
- Sinha, B. K. (1995) *Drugs* **49**, 11–19
- Champoux, J. J. (1994) *Adv. Pharmacol.* **29A**, 71–82
- Andersen, A. H., Svejstrup, J. Q., and Westergaard, O. (1994) *Adv. Pharmacol.* **29A**, 83–101
- Sharma, A., and Mondragon, A. (1995) *Curr. Opin. Struct. Biol.* **5**, 39–47
- Osheroff, N., Zechiedrich, E. L., and Gale, K. C. (1991) *BioEssays* **13**, 269–273
- Watt, P. M., and Hickson, I. D. (1994) *Biochem. J.* **303**, 681–695
- Adams, D. E., Sheekhtman, E. M., Zechiedrich, E. L., Schmid, M. B., and Cozzarelli, N. R. (1992) *Cell* **71**, 277–288
- Goto, T., and Wang, J. C. (1984) *Cell* **36**, 1073–1080
- Wyckoff, E., Natalie, D., Nolan, J. M., Lee, M., and Hsieh, T. (1989) *J. Mol. Biol.* **205**, 1–13
- Drake, F. H., Hofmann, G. A., Bartus, H. F., Mattern, M. R., Crooke, S. T., and Mirabelli, C. K. (1989) *Biochemistry* **28**, 8154–8160
- Anderson, R. D., and Berger, N. A. (1994) *Mutat. Res.* **309**, 109–142
- Ferguson, L. R., and Baguley, B. C. (1994) *Environ. Mol. Mutagen.* **24**, 245–261
- Burke, T. G., and Mi, Z. (1994) *J. Med. Chem.* **37**, 40–46
- Ferrero, L., Cameron, B., Manse, B., Lagneaux, D., Crouzet, J., and Famechon, A. (1994) *Mol. Microbiol.* **13**, 641–653
- Danks, M. K., Qiu, J., Catapano, C. V., Schmidt, C. A., Beck, W. T., and Fernandes, D. J. (1994) *Biochem. Pharmacol.* **48**, 1785–1795
- Brown, G. A., McPherson, J. P., Gu, L., Hedley, D. W., Toso, R., Deuchars, K. L., Freeman, M. H., and Goldenberg, G. J. (1995) *Cancer Res.* **55**, 78–82
- Robinson, M. J., Martin, B. A., Gootz, T. D., McGuirk, P. R., Moynihan, M., Sutcliffe, J. A., and Osheroff, N. (1991) *J. Biol. Chem.* **266**, 14585–14592
- Yamashita, Y., Kawada, S., Fujii, N., and Nakano, H. (1991) *Biochemistry* **30**, 5838–5845
- Stevenson, R. L. (1886) *The Strange Case of Dr. Jekyll and Mr. Hyde*, Longmans, Greene & Co., London
- Kreuzer, K. N., and Cozzarelli, N. R. (1979) *J. Bacteriol.* **140**, 424–435
- Ripley, L. S. (1994) *Mutat. Res.* **312**, 67–78
- Hsiang, Y. H., Lihou, M. G., and Liu, L. F. (1989) *Cancer Res.* **49**, 5077–5082
- Howard, M. T., Neece, S. H., Matson, S. W., and Kreuzer, K. N. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 12031–12035
- Pommier, Y., Leteurtre, F., Fesen, M. R., Fujimori, A., Bertrand, R., Solary, E., Kohlhagen, G., and Kohn, K. W. (1994) *Cancer Invest.* **12**, 530–542
- Beck, W. T., Kim, R., and Chen, M. (1994) *Cancer Chemother. Pharmacol.* **34**, S14–18
- Sullivan, D. M., Latham, M. D., and Ross, W. E. (1987) *Cancer Res.* **47**, 3973–3979
- Bjornsti, M.-A., Benedetti, P., Viglianti, G. A., and Wang, J. C. (1989) *Cancer Res.* **49**, 6318–6323
- Madden, K. R., and Champoux, J. J. (1992) *Cancer Res.* **52**, 525–532
- Nitiss, J. L., Liu, Y. X., Harbury, P., Jannatipour, M., Wasserman, R., and Wang, J. C. (1992) *Cancer Res.* **52**, 4467–4472
- Ishida, R., Hamatake, M., Wasserman, R. A., Nitiss, J. L., Wang, J. C., and Andoh, T. (1995) *Cancer Res.* **55**, 2299–2303
- Heck, M. M., and Earnshaw, W. C. (1986) *J. Cell Biol.* **103**, 2569–2581
- Kjeldsen, E., Bonven, B. J., Andoh, T., Ishii, K., Okada, K., Bolund, L., and Westergaard, O. (1988) *J. Biol. Chem.* **263**, 3912–3916
- Porter, S. E., and Champoux, J. J. (1989) *Nucleic Acids Res.* **17**, 8521–8532
- Tanizawa, A., Kohn, K. W., Kohlhagen, G., Leteurtre, F., and Pommier, Y. (1995) *Biochemistry* **34**, 7200–7206
- Sorensen, B. S., Sinding, J., Andersen, A. H., Alsner, J., Jensen, P. B., and Westergaard, O. (1992) *J. Mol. Biol.* **228**, 778–786
- Froelich-Ammon, S. J., Patchan, M. W., Osheroff, N., and Thompson, R. B. (1995) *J. Biol. Chem.* **270**, 14998–15005
- Robinson, M. J., Corbett, A. H., and Osheroff, N. (1993) *Biochemistry* **32**, 3638–3643
- Corbett, A. H., Hong, D., and Osheroff, N. (1993) *J. Biol. Chem.* **268**, 14394–14398
- Roca, J., Ishida, R., Berger, J. M., Andoh, T., and Wang, J. C. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 1781–1785
- Nitiss, J. L. (1994) *Cancer Chemother. Pharmacol.* **34**, S6–S13
- Nitiss, J., and Wang, J. C. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 7501–7505
- Nitiss, J. L., Liu, Y. X., and Hsiung, Y. (1993) *Cancer Res.* **53**, 89–93
- Osheroff, N. (1989) *Biochemistry* **28**, 6157–6160
- Freudenreich, C. H., and Kreuzer, K. N. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 11007–11011
- Pommier, Y., Kohlhagen, G., Kohn, K. W., Leteurtre, F., Wani, C., and Wall, M. E. (1995) *Proc. Natl. Acad. Sci. U. S. A.*, in press
- Capranico, G., and Zunino, F. (1992) *Eur. J. Cancer* **12**, 2055–2060
- Leteurtre, F., Fujimori, A., Tanizawa, A., Chhabra, A., Mazumder, A., Kohlhagen, G., Nakano, H., and Pommier, Y. (1994) *J. Biol. Chem.* **269**, 28702–28707
- Knab, A. M., Fertala, J., and Bjornsti, M.-A. (1995) *J. Biol. Chem.* **270**, 6141–6148
- Nelson, E. M., Tewey, K. M., and Liu, L. F. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 1361–1365
- Beck, W. T., Danks, M. K., Wolverton, J. S., Kim, R., and Chen, M. (1993) *Adv. Enzyme Regul.* **33**, 113–127
- Bjornsti, M.-A., Knab, A. M., and Benedetti, P. (1994) *Cancer Chemother. Pharmacol.* **34**, S1–S5
- Beck, W. T., Danks, M. K., Wolverton, J. S., Chen, M., Granzen, B., Kim, R., and Suttle, D. P. (1994) *Adv. Pharmacol.* **29B**, 145–170
- Hertzberg, R. P., Caranfa, M. J., and Hecht, S. M. (1989) *Biochemistry* **28**, 4629–4638
- Nabiev, I., Chourpa, I., Riou, J.-F., Nguyen, C. H., Lavelle, F., and Manfait, M. (1994) *Biochemistry* **33**, 9013–9023
- Willmott, C. J., and Maxwell, A. (1993) *Antimicrob. Agents Chemother.* **37**, 126–127
- Yoshida, H., Bogaki, M., Nakamura, M., Yamanaka, L. M., and Nakamura, S. (1991) *Antimicrob. Agents Chemother.* **35**, 1647–1650
- Hsiung, Y., Elsea, S. H., Osheroff, N., and Nitiss, J. L. (1995) *J. Biol. Chem.* **270**, 20359–20364
- Elsea, S. H., Hsiung, Y., Nitiss, J. L., and Osheroff, N. (1995) *J. Biol. Chem.* **270**, 1913–1920
- Osheroff, N., Corbett, A. H., Elsea, S. H., and Westergaard, M. (1994) *Cancer Chemother. Pharmacol.* **34**, S19–S25
- Zwelling, L. A., Mayes, J., Hinds, M., Chan, D., Altschuler, E., Carroll, B., Parker, E., Deisseroth, K., Radcliffe, A., and Seligman, M. (1991) *Biochemistry* **30**, 4048–405