To further characterize the mechanistic basis for cellular resistance/hypersensitivity to anticancer drugs, a yeast genetic system was used to select a mutant type II topoisomerase that conferred cellular resistance to CP-115,953, amsacrine, etoposide, and ellipticine. The mutant enzyme contained a single point mutation that converted Gly437 to Ser (top2G437S). Purified top2G437S displayed wild-type enzymatic activity in the absence of drugs but exhibited two properties that were not predicted by the cellular resistance phenotype. First, in the absence of ATP, it was hypersensitive to all of the drugs examined and hypersensitivity correlated with increased drug affinity. Second, in the presence of ATP, top2G437S lost its hypersensitivity and displayed wild-type drug sensitivity. Since the resistance of yeast harboring top2G437S could not be explained by alterations in enzyme-drug interactions, physiological levels of topoisomerase II were determined. The Gly437 → Ser mutation reduced the stability of topoisomerase II and decreased the cellular concentration of the enzyme. These findings suggest that the physiological drug resistance phenotype conferred by top2G437S results primarily from its decreased stability. This study highlights the need to analyze both the biochemistry and the physiology of topoisomerase II mutants with altered drug sensitivity in order to define the mechanistic bridge that links enzyme function to cellular phenotype.

Topoisomerase II is the primary cellular target for some of the most effective drugs currently used for the chemotherapeutic treatment of human cancers (1–9). These agents act in an unusual fashion. Rather than killing cells by robbing them of the essential activities of topoisomerase II, anticancer drugs “poison” the enzyme (10) and convert it to a potent cellular toxin by increasing levels of covalent topoisomerase II-cleaved DNA complexes that are normal but transient intermediates in the catalytic cycle of the enzyme (1–8, 11–13). This action leads to the generation of permanent double-stranded breaks in the genetic material of treated cells and ultimately triggers cell death pathways (2–8, 14–23).

There is a high degree of variability in the response of different cells to topoisomerase II-targeted drugs (24–26). Despite the impact of drug resistance and hypersensitivity on cancer chemotherapy, relatively few studies have shed light on the mechanistic basis that underlies this physiological variability. In some cases, changes in the cellular response to topoisomerase II poisons appear to result from alterations in the activity or drug sensitivity of the enzyme. All things being equal, increased expression of topoisomerase II tends to produce drug hypersensitivity (27–29). In contrast, decreases in the catalytic activity of the enzyme or in the level of nuclear topoisomerase II (either by reduced protein expression/stability or by improper localization) leads to drug resistance (1, 2, 30–43). Finally, mutations in topoisomerase II have been identified that confer drug resistance or hypersensitivity, depending on how they alter interactions between the enzyme and anticancer agents (36, 44–56).

To characterize more fully the mechanistic basis for cellular resistance/hypersensitivity to topoisomerase II poisons, a yeast genetic system was utilized to select mutant type II enzymes that confer resistance to anticancer agents in vivo (28, 49). The present study describes the characterization of one such yeast topoisomerase II in which Gly437 was mutated to Ser (top2G437S). Although expression of this mutant enzyme conferred cellular resistance to anticancer drugs, purified top2G437S was hypersensitive to these agents in the absence of ATP. The enzyme lost its hypersensitivity to anticancer agents and displayed wild-type sensitivity in the presence of ATP, suggesting a conformational change in the region of residue 437 upon binding of the high energy cofactor. Further analysis indicated that in vivo resistance was caused by decreased levels of active enzyme, while in vitro hypersensitivity reflected (at least in part) an increased affinity for drugs. Thus, top2G437S appears to have distinct physiological and biochemical mechanisms for altered drug sensitivity.

**EXPERIMENTAL PROCEDURES**

Materials—Negatively supercoiled bacterial plasmid pBR322 DNA was prepared as described previously (57). CP-115,953 was provided by Drs. T. D. Gootz and P. R. McGuirk (Pfizer Central Research); amsacrine was obtained from Bristol-Myers Squibb, and etoposide and ellipticine were obtained from Sigma. All drugs were prepared as 20 μM solutions in 100% Me2SO and stored at 4 °C. Tris and ethidium bromide were obtained from Sigma; SDS was from Merck; proteinase K was from U. S. Biochemical Corp.; ATP was from Amersham Pharmacia Biotech; and restriction endonucleases and T4 DNA ligase were from New England Biolabs. All other chemicals were analytical reagent grade.

Yeast Strains and Plasmids—Saccharomyces cerevisiae strains employed were as described in Elsea et al. (54). Yeast cells typically were grown in rich medium (YPDA) or (to select for plasmids carrying URA3
Topoisomerase II (top2G437S) with Altered Drug Sensitivity

as a marker) in synthetic complete medium lacking uracil (SC-URA) and transformed as described previously (58). The plasmid carrying the topoisomerase II (TOP2) gene was YcpDED1TOP2 (28). Wild-type and mutant type II topoisomerases were purified using the inducible overexpression plasmid YepGAL1TOP2 DNA.

In Vitro Transfection and Mutant Selection—Mutations were introduced into the yeast TOP2 gene by exposure to 0.1 M hydroxyurea in vitro (60). Following this treatment, YcpDED1TOP2 was transformed into Escherichia coli strain XLI-1 Blue. Plasmid DNA was purified by the method of Sambrook et al. (57). The mutagenized pool of YcpDED1TOP2 was transformed into yeast strain JN39412-4, and transformants were selected for growth on synthetic media lacking 115,953, as described previously by Nitiss and co-workers (49). Single colonies were selected, and cytotoxicity assays were performed as described below.

Yeast Cytotoxicity Assays—The sensitivity of yeast strain JN39412-4 carrying wild-type or mutagenized YcpDED1TOP2 to CP-115,953, amsacrine, etoposide, or ellipticine was determined as described previously (61). Cells were incubated in SC-URA selection media with drug (0–200 μM) for 24 h, and initial phenotypes were established by following the absorbance of cultures at 600 nm. Transformants with phenotypes of interest were plated in duplicate on YPDA medium solidified with 1.5% Bacto-agar and cultured for 3–4 days at 34 °C. Drug sensitivity was quantitated by counting the number of surviving colonies.

Results Carrying Topoisomerase II Mutations—Plasmids carrying the mutant alleles were recovered as described (62). In summary, yeast cells were lysed, and total nucleic acids were extracted with phenol/CHCl3 and precipitated with ethanol. RNA was degraded by RNase A, and the remaining DNA was precipitated with ethanol and transformed into E. coli strain XLI-1 Blue. Plasmid DNA was purified using Qiagen plasmid kits (Qiagen).

Construction of Plasmids for Sequencing and Overexpression—Plasmids were constructed according the procedure of Elsea et al. (54). Briefly, mutagenized YcpDED1TOP2 was digested with restriction endonucleases KpnI and AvrII. DNA fragments were separated by electrophoresis, and the 2.2-kb fragment (containing the coding sequence for amino acids 317–1045 in yeast topoisomerase II) was gel-purified. In addition, the wild-type plasmids YcpDED1TOP2 and YepGAL1TOP2 were digested with KpnI and AvrII, and the large fragment containing vector sequences was gel-purified. The mutagenized 2.2-kb fragment was then ligated into YcpDED1TOP2 and YepGAL1TOP2, replacing the wild-type fragments, and subsequently transformed into E. coli strain XLI-1 Blue. YcpDED1TOP2 DNA was used for sequence analysis (63) and transformation of the yeast strain JN39412-4 for cytotoxicity assays. YepGAL1TOP2 DNA was used to transform the yeast strain JEL1 for overexpression and purification of topoisomerase II.

Yeast Topoisomerase II Induction, Overexpression, and Purification—Yeast topoisomerase II was overexpressed in yeast strain JEL1 (transformed with YepGAL1TOP2) by the addition of galactose in glucose-free media (59). Wild-type and mutant enzymes were purified to >95% homogeneity (as determined by visualization on silver-stained gel) by a modified procedure (54, 64) of Worland and Wang (59). Although the mutant enzyme appeared to be stable for at least 2 weeks in liquid nitrogen, it lost activity within a few days following thawing and storage at −20 °C. Therefore, enzymes were aliquoted and stored at −80 °C and utilized for assays within 2 days of their transfer from −80 to −20 °C.

Topoisomerase II-mediated DNA Relaxation—DNA relaxation was carried out as described by Osheroff et al. (65). Reaction mixtures contained 0.1–3 nm topoisomerase II, 5 nm supercoiled pBR322, and 0.5 mM ATP in a total volume of 20 μl of assay buffer (10 mM Tris-HCl (pH 7.9), 175 mM KCl, 0.1 mM EDTA, 5 mM MgCl2, and 2.5% glycerol). DNA relaxation was at 20 °C for 15 min and stopped by the addition of 3 μl of 0.77% SDS and 77 mM EDTA. Samples were subjected to electrophoresis in 1% agarose gels in TAE (100 mM Tris borate (pH 8.3), 2 mM EDTA). Gels were stained with 1 μg/ml ethidium bromide, visualized by UV light, and photographed through Kodak 23A and 12 filters with a Polaroid type 665 positive/negative film. Levels of DNA relaxation were quantitated by scanning negatively supercoiled plasmid bands in photographic transparencies with an E-C scanning densitometer in conjunction with Hoefer GS-370 Data System software. The intensity of bands in the negative was proportional to the amount of DNA present. The requirement of wild-type and mutant topoisomerase II for ATP was determined using relaxation assays in which the concentration of ATP was varied from 0 to 0.5 mM.

Topoisomerase II-mediated DNA Cleavage—DNA cleavage assays were carried out as described by Osheroff and Zechiedrich (66). Reaction mixtures contained 150 nm topoisomerase II and 5 nm negatively supercoiled pBR322 DNA in a total volume of 20 μl of cleavage buffer (10 mM Tris-HCl (pH 7.9), 100 mM NaCl, 0.1 mM EDTA, 5 mM MgCl2, 2.5% glycerol, 10% Me2SO) and 0–200 μM drug. For dose-response studies, drug concentrations ranged from 1 μM to 2 mM. Samples were incubated at 28 °C for 6 min and cleavage products were trapped by the addition of 2 μl of 5% SDS. To this mixture was added 1.5 μl of 250 mM EDTA and 2 μl of 0.8 mg/ml Proteinase K, followed by incubation at 45 °C for 30 min. Samples were subjected to electrophoresis in 1% agarose gels in TAE (40 mM Tris acetate (pH 8.3), 2 mM EDTA) containing 1 μg/ml ethidium bromide. When DNA cleavage was carried out in the presence of ATP (1 mM), reaction mixtures contained 40 nm topoisomerase II and 10 nm negatively supercooled pBR322 DNA. Samples were incubated at 28 °C for 3 min, and cleavage products were trapped and analyzed as above.

Topoisomerase II-mediated DNA Religation—DNA cleavage/religation equilibria were established as in the preceding section using 150 nm topoisomerase II, 5 nm negatively supercoiled pBR322 DNA, and 100 μM drug in a total volume of 200 μl of cleavage buffer. After 6 min at 28 °C, religation was induced by the addition of 500 mM NaCl at room temperature. At time points up to 75 s, 20-μl samples were removed and assayed for DNA relaxation as described above.

Results

Thermal Stability of Topoisomerase II—Purified topoisomerase II (1 mg/ml) was diluted 1:250 and incubated at 34 °C. At time points up to 30 min, 1-μl aliquots of topoisomerase II were removed and added to assay buffer that contained 5 nm negatively supercooled pBR322 DNA and 1 mM ATP (total volume of 26 μl). DNA relaxation assays were carried out as above at 28 °C for 6 min. Reaction products were analyzed as described above.

Steady-state Cellular Concentration of top2G437S—The S. cerevisiae strain JEL1 was transformed with YcpDED1TOP2, carrying either the wild-type TOP2 or the mutant top2G437S allele behind the DED1 promoter. Cells were grown to confluency in SC-URA media at room temperature (25 °C). Whole cell homogenates were prepared by adding glass beads to culture aliquots and bead-beating for 2–3 1-min pulses. All homogenates were diluted to 10 mg/ml, and serial dilutions of homogenates were subjected to electrophoresis in a 7.5% homogeneous media PhastGel (PhastSystem, Amersham Pharmacia Biotech). Proteins were transferred to Amersham Pharmacia Biotech Hybond-P paper using the PhastSystem, and immunoblots were prepared using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech). The primary antibody for yeast topoisomerase II was from Topogen; the secondary antibody was supplied in the ECL kit.

RESULTS

Selection of a Mutant Yeast Type II Topoisomerase in Which Gly377 Is Converted to Ser—A yeast genetic system was used to further analyze the mechanistic basis for cellular resistance/hypersensitivity to topoisomerase II poisons (28, 49). In this system, the chromosomal copy of the TOP2 gene was replaced by a temperature-sensitive allele (top2-4) and the resulting strain, JN39412-4, contained a plasmid-based collection of expressed mutated TOP2 genes. Cells were selected for resistance to the quinolone CP-115,953 at the non-permissive temperature (34 °C), to ensure that viability was determined by the phenotype of the expressed mutant type II enzymes. This initial procedure established a library highly enriched for type II topoisomerases that confer altered physiological sensitivity to anticancer drugs (54).

Further screening of the above library identified a yeast colony that exhibited high resistance to CP-115,953, amsacrine, etoposide, and ellipticine. A 2.2-kb fragment of its plasmid was deleted to 2 μl of 5% SDS to release the corresponding fragment in a wild-type construct, and cells containing the chimeric gene were screened for drug resistance (Fig. 1). The phenotype of the chimera was identical to that of the original isolate, indicating that drug resistance resulted from a mutation(s) within the coding region of the TOP2 gene.

To identify the nature of the resistance-conferring muta-

1 The abbreviation used is: kb, kilobase pair.
Topoisomerase II (top2G437S) with Altered Drug Sensitivity

First, as determined by its ability to relax negatively supercoiled plasmid DNA, the Gly → Ser mutation had no effect on the overall catalytic activity of top2G437S. Indeed, over a wide range of enzyme concentrations, rates of DNA relaxation catalyzed by top2G437S were indistinguishable from those of the wild-type enzyme (Fig. 2).

Second, since the mutation in top2G437S is contained within the GyrB (ATPase) domain of topoisomerase II (4), the requirement of the mutant enzyme for ATP was determined by analyzing its ability to relax DNA over a 50-fold range of ATP concentrations. It is notable that two previously described mutants in this region of human topoisomerase II (CEM/VMM-1–5, ArgG450 → Gln (46, 47); HL60/AMSA, ArgK487 → Lys (44)) display either an altered affinity for ATP or an altered response to anticancer drugs in the presence of the nucleoside triphosphate. (The positions of these mutated residues correspond to residues 439 and 476 in the sequence of S. cerevisiae topoisomerase II (4)). Contrary to the mutant human enzymes, top2G437S displayed a requirement for ATP that was similar to that of wild-type topoisomerase II (Fig. 3).

Third, since drug resistance in vivo could result from a decreased level of basal DNA scission activity, the ability of top2G437S to cleave plasmid DNA in the absence of anticancer drugs was compared with its parental enzyme. In both the absence (Fig. 4) and presence (not shown) of ATP, the basal DNA cleavage activity of top2G437S appeared to be similar to that of wild-type yeast topoisomerase II.

Taken together, the above data indicate that the native activity of topoisomerase II is not significantly altered by the substitution of Ser for Gly at position 437.

Hypersensitivity of Purified top2G437S to Anticancer Drugs in the Absence of ATP—The cytotoxic potential of topoisomerase II poisons correlates with their ability to stimulate enzyme-mediated DNA scission (1–8). Therefore, DNA cleavage assays were utilized to characterize the sensitivity of purified top2G437S to the anticancer drugs used in the original cytotoxicity screens. In the first set of experiments, the effects of drugs on the DNA scission event that precedes enzyme-catalyzed DNA strand passage were assessed. This was accomplished by monitoring DNA cleavage in the absence of ATP.

In the absence of a high energy cofactor, top2G437S did not display the expected resistance phenotype against CP-115,953, amsacrine, etoposide, or ellipticine. Indeed, the mutant enzyme was hypersensitive to all of these drugs (Fig. 5). Additional experiments were carried out to explore the basis for this hypersensitivity. CP-115,953 and amsacrine were utilized for these studies because they produced the greatest differential in sensitivity between the wild-type and mutant enzymes.

As seen in Fig. 6, drug hypersensitivity was not due to a fundamental change in the mechanistic basis for drug action. Rates of DNA religation in the presence of CP-115,953 or amsacrine were identical for wild-type topoisomerase II and top2G437S (panels A and B). Furthermore, there did not appear to be a change in the rate-determining step of DNA scis-
sion, since the pre-steady-state profiles for drug-stimulated cleavage (normalized so that maximal cleavage levels for both enzymes were set to 100%) were the same for both type II enzymes (panels C and D).

Dose-response studies for CP-115,953 and amsacrine were carried out over a 3 log concentration range to further define their interactions with top2G437S (Fig. 7). Maximal DNA cleavage levels were attained at ≤1 mM drug in all cases; higher concentrations were inhibitory. As determined from the drug concentration required to stimulate one-half maximal DNA scission, the potency of both drugs against top2G437S was 2–3-fold higher than that for wild-type topoisomerase II. Thus, it appears that the conversion of Gly437 to a Ser yields an enzyme that has a higher kinetic affinity for these topoisomerase II poisons. In addition, the mutant displayed an efficacy (determined by the maximal level of cleavage induced) for amsacrine that was ~2-fold greater than wild type. This suggests that once complexed with the mutant enzyme, amsacrine stimulates higher levels of DNA scission than normally induced in the wild-type topoisomerase II-DNA-drug complex.

Sensitivity of Purified top2G437S to Anticancer Drugs in the Presence of ATP—Topoisomerase II requires ATP binding in order to form a “closed protein clamp” on the double helix and promote the catalytic DNA strand passage reaction (2, 11, 12, 65, 68, 69). Furthermore, the enzyme establishes two distinct DNA cleavage/religation equilibria during its catalytic cycle (one prior to and one following the double-stranded DNA passage event), and drugs have been shown to affect both (2, 11, 12, 54, 70–72). Since the pre-strand passage DNA cleavage activity of top2G437S was hypersensitive to anticancer agents,
the sensitivity of the enzyme to drugs was also examined in the presence of ATP.

Remarkably, the drug hypersensitivity observed in the absence of a nucleoside triphosphate was lost in the presence of ATP (Fig. 8). In fact, the sensitivity of top2G437S toward CP-115,953, amsacrine, etoposide, and ellipticine was similar to that displayed by wild-type topoisomerase II. The drug concentrations utilized for this study represented those that produced the highest levels of scission without generating multiple DNA breaks per plasmid. As seen in the inset, a wild-type DNA cleavage stimulation also was observed at sub-saturating concentrations of amsacrine (the drug that produced the highest level of hypersensitivity in the absence of ATP). Thus, top2G437S appears to be the first reported mutant type II topoisomerase that displays an ATP-dependent drug phenotype.

**Basis for the in Vivo Drug Resistance Conferred by top2G437S**—Although the drug hypersensitivity described in the absence of ATP is lost in the presence of this nucleoside triphosphate, the wild-type profile observed with ATP in *vitro* is insufficient to explain the resistant phenotype conferred to yeast cells carrying top2G437S. Therefore, it is unlikely that drug resistance in *vivo* results from altered interactions between the mutant enzyme and topoisomerase II poisons.

Decreased levels of topoisomerase II activity have been linked to cellular drug resistance (1, 2, 30, 31, 33, 34, 36, 39, 43, 52). As described above, the catalytic activity of purified top2G437S was indistinguishable from that of the wild-type enzyme. However, since the *in vitro* activity assays were carried out at 28 °C and the mutant selection, screening, and cytotoxicity protocols were performed at 34 °C (necessitated by the requirement for a temperature-sensitive chromosomal *TOP2* allele), it is possible that the Gly → Ser mutation at position 437 generated an enzyme that was temperature-sensitive in nature. Therefore, the thermal stability of top2G437S was characterized at 34 °C.

In this experiment, the mutant and wild-type enzymes were incubated at 34 °C in the absence of ATP and DNA. At various times, ATP and negatively supercoiled plasmid were added, and DNA relaxation was monitored at 28 °C. As seen in Fig. 9, the thermal stability of top2G437S at 34 °C was markedly decreased compared with that of wild-type topoisomerase II. While the *t*½ of the wild-type enzyme was estimated to be 60 min, that of the mutant enzyme was ~2.5 min. A similar *t*½ was observed when top2G437S was incubated at 34 °C in the presence of ATP (not shown). Thus, under the temperature conditions utilized for characterization of physiological drug sensitivity, the cellular activity of plasmid-encoded top2G437S would be expected to be significantly lower than that of the wild-type control enzyme.

The decreased thermal stability of top2G437S raises the possibility that the Gly → Ser mutation may reduce the general stability of this enzyme. This is supported by the finding that 1) top2G437S displayed a lifetime at ~20 °C that was considerably shorter (on the order of days, not shown) than that of the wild-type enzyme; and 2) the yield of mutant enzyme was ~2-fold lower than that of wild-type topoisomerase II when purified from an equivalent weight of yeast cells. Since a destabilizing mutation might decrease cellular concentrations of top2G437S even at the permissive temperature, physiological levels of the mutant polypeptide were determined at 25 °C. As characterized by immunoblot analysis, the steady-state concentration of plasmid-encoded top2G437S polypeptide was ~10% that of wild-type topoisomerase II expressed from the same
plasmid system (Fig. 10). Two conclusions can be drawn from the above experiments. First, beyond its effects on drug-enzyme interactions, the Gly → Ser mutation at position 437 leads to temperature-sensitivity and an apparent destabilization of topoisomerase II. This is despite the fact that the mutation does not affect the catalytic activity of the enzyme at the permissive temperature. Second, the basis for the drug resistance of yeast cells harboring top2G437S appears to be related to the instability of the mutant enzyme (and the consequent loss of active enzyme from the cell), rather than its altered interaction with topoisomerase II poisons.

**DISCUSSION**

Alterations in the sensitivity of topoisomerase II to anticancer agents can profoundly affect the efficacy of chemotherapeutic regimens (4, 36, 44–55). In an effort to further our understanding of the mechanistic basis for altered drug sensitivity, a mutant yeast type II enzyme was selected that conferred cellular resistance to several structurally distinct topoisomerase II poisons. The resistance conferring mutation was identified as a single base change that converted Gly → Ser at amino acid position 437 (top2G437S). This point mutation did not affect the intrinsic ability of the purified enzyme to relax or cleave its DNA substrate (at 28 °C) in the absence of anticancer drugs. top2G437S exhibited two properties that were not predicted by the cellular resistance phenotype. First, in the absence of ATP, the enzyme was hypersensitive to all of the topoisomerase II poisons examined. This hypersensitivity correlated, at least for amascarin and CP-115,593, with an increased affinity for drug. Second, in the presence of ATP, top2G437S lost its drug hypersensitivity and displayed wild-type sensitivity toward the same topoisomerase II poisons.

Gly⁴³⁷, which is conserved in a number of eukaryotic species (4), is located in the GyrB domain of topoisomerase II in a tight loop that connects β sheet B’β1 to a helix B’α1 in the crystal structure of the yeast enzyme (73). The residue is located 23 Å from the hydroxyl group of the active site Tyr (residue 783), diagonally across the cleft that is proposed to bind the DNA cleavage helix (73, 74). Given the distance between the mutated residue and the point of DNA cleavage, it is unlikely that either Gly or Ser at position 437 makes intimate contact with a bound drug molecule. However, the fact that position 437 lies along the proposed path of the DNA cleavage helix strongly suggests that alterations at this residue could have ramifications for drug action (73). Finally, Gly⁴³⁷ is located in the region of the GyrB domain that is proposed to undergo a substantial conformational change upon ATP binding and the subsequent closing of the N-terminal protein gate (73–76). It is this structural rearrangement that may mask the effects of the Gly → Ser mutation and ultimately restore wild-type drug sensitivity to top2G437S in the presence of ATP.

Clearly, the resistance phenotype of yeast cells harboring top2G437S cannot be explained by alterations in enzyme-drug interactions. However, since the “wild-type” and “mutant” yeast strains are isogenic except for the replacement of TOP2 with top2G437S, it is reasonable to assume that the Gly → Ser mutation in topoisomerase II is the root cause of the cellular phenotype. As determined by *in vitro* and *in vivo* experiments, the substitution of Ser for Gly 437 reduces the stability of topoisomerase II and decreases the cellular concentration of the enzyme. Therefore, it is proposed that the physiological drug resistance phenotype associated with top2G437S results primarily from its decreased stability.

Topoisomerase II is the primary cytotoxic target for a number of widely prescribed anticancer drugs (1–9, 56). Understanding the mechanistic basis for resistance/hypersensitivity to these agents is critical to the continued development of successful chemotherapeutic regimens. This study highlights the need to analyze both the biochemistry and the physiology of topoisomerase II mutants with altered drug sensitivity in order to define the mechanistic bridge that links enzyme function to cellular phenotype. Assumptions made on the basis of either one alone may lead to erroneous conclusions and obscure the actual mechanism that underlies drug resistance or hypersensitivity.

**Acknowledgments**—We are grateful to Dr. Sarah H. Elsea for preliminary work on this project; to Drs. Paul S. Kingma and D. Andrew Burden for helpful discussions; and to Dr. Paul S. Kingma and John M. Fortune for critical reading of the manuscript.

**REFERENCES**

1. Pommier, Y. (1993) Cancer Chemother. Pharmacol. 32, 103–108
2. Corbett, A. H., and Osheroff, N. (1993) Chem. Res. Toxicol. 6, 585–597
3. Chen, A. Y., and Liu, L. F. (1994) Annu. Rev. Pharmacol. Toxicol. 34, 191–218
4. Liu, L. (ed) (1994) Adv. Pharmacol. 29.
5. Froehlich-Ammon, S. J., and Osheroff, N. (1995) J. Biol. Chem. 270, 21249–21342
6. Pommier, Y., Fonse, M. R., and Goldwasser, P. (1996) in Cancer Chemotherapy and Biotherapy: Principles and Practice (Chabner, B. A., and Longo, D. L., eds) 2nd Ed., pp. 435–461, Lippincott-Raven Publishers, Philadelphia
7. Nittos, J. L., and Beck, W. T. (1996) Eur. J. Cancer 32a, 598–599
8. Petroskov, Y. M. (1997) in Cancer (Cozzarelli, N. R., and Wang, J. C., eds) (Teicher, B. A., ed) Vol. 1, pp. 153–174, Humana Press Inc., Totowa, NJ
9. Wang, H.-K., Morris-Natsche, S. L., and Lee, K.-H. (1997) Med. Res. Rev. 17, 367–425
10. Kreuzer, K. N., and Cozzarelli, N. R. (1979) J. Bacteriol. 140, 424–435
11. Osheroff, N., Zechiedrich, E. L., and Gale, K. C. (1991) Biochim. J. 303, 681–695
12. Watt, P. M., and Hickson, I. D. (1994) Biochim. J. 303, 681–695
13. Wang, J. C. (1996) Annu. Rev. Biochem. 65, 635–692
14. Ferguson, L. R., and Baguley, B. C. (1994) Environ. Mol. Mutagen. 24, 254–261
15. Berger, J. M., and Wang, J. C. (1996) Curr. Opin. Struct. Biol. 6, 84–90
16. Nitiss, J. L., Rose, A., Sykes, K. C., Harris, J., and Zhou, J. (1996) Ann. N. Y. Acad. Sci. 803, 52–43
17. Walker, P. R., Smith, C., Youdale, T., Leblanc, J., Whitfield, J. F., and Srikorska, M. (1991) Cancer Res. 51, 1078–1085
18. Roy, C., Brown, D. L., Littie, J. E., Valentine, B. K., Walker, P. R., Srikorska, M., Leblanc, J., and Chaly, N. (1992) Exp. Cell Res. 200, 416–424
19. Smith, P. J., Rackstraw, C., and Cotter, F. (1994) Ann. Hematol. 69, 87–91
20.Bitke, M., Rusan, D., Atten, W. L., Div, C., Heer, S., and Zalianis, E. (1993) Mol. Pharmacol. 46, 605–611
21. Negri, C., Bernardi, R., Donzelli, M., and Scorsavi, A. L. (1995) Biochimie 77, 893–896
22. Halicka, H. D., Seiter, K., Feldman, E. J., Traganos, F., Mittelman, A., Ahmed, T., and Darzynkiewicz, Z. (1997) Apoptosis 2, 25–39
23. Solomon, E., Bertrand, E., and Pommier, Y. (1994) Leuk. Lymphoma 15, 21–32
24. Potmesil, M., and Silber, R. (1990) in DNA Topology and Its Biological Effects (Cozzarelli, N. R., and Wang, J. C., eds) pp. 391–407, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Potmesil, M., and Kohn, K. W. (eds) (1991) DNA Topoisomerases in Cancer, Oxford University Press, New York
26. Sinha, B. K. (1995) Drugs 48, 11–19
27. Davies, S. M., Davies, S. L., Hall, A. G., and Hickson, I. D. (1990) Mutat. Res.
Topoisomerase II (top2G437S) with Altered Drug Sensitivity

28. Nitiss, J. L., Liu, Y. X., Harbury, P., Jannatipour, M., Wasserman, R., and Wang, J. C. (1992) Cancer Res. 52, 4467–4472
29. McPherson, J. P., Defo, A. M., Jones, N. R., Brown, G. A., Deuchars, K. L., and Goldenberg, G. J. (1997) Anticancer Res. 17, 4243–4252
30. Sullivan, D. M., Latham, M. D., and Ross, W. E. (1987) Cancer Res. 47, 3973–3979
31. Elsea, S. H., Osheroff, N., and Nitiss, J. L. (1992) J. Biol. Chem. 267, 13150–13153
32. Kim, R., Hirabayashi, N., Nishiyama, M., Jinushi, K., Toge, T., and Okada, K. (1992) Anticancer Res. 12, 241–245
33. Alton, P. A., and Harris, A. L. (1993) Br. J. Haematol. 85, 241–245
34. Nitiss, J. L., Liu, Y. X., and Hsiung, Y. (1993) Cancer Res. 53, 89–93
35. Mirski, S. E., Evans, C. D., Almquist, K. C., Slovak, M. L., and Cole, S. P. (1994) Cancer Res. 54, 13150–13153
36. D’Arpa, P. (1994) Adv. Pharmacol.
37. Evans, C. D., Mirski, S. E., Danks, M. K., and Cole, S. P. (1994) Cancer Res. 54, 18586–18592
38. Feldhoff, P. W., Mirski, S. E., Cole, S. P., and Sullivan, D. M. (1994) Cancer Res. 54, 756–762
39. Harrison, D. J. (1995) J. Pathol. 175, 7–12
40. Hasegawa, S., Abe, T., Naito, S., Kotoh, S., Kumazawa, J., Hipfner, D. R., Hasegawa, S., Abe, T., Naito, S., Kotoh, S., Kumazawa, J., Hipfner, D. R., and Goldenberg, G. J. (1997) Anticancer Res. 17, 111–118
41. Wessel, I., Jensen, P. B., Falck, J., Mirski, S. E., and Cole, S. P. (1997) J. Biol. Chem.
42. Malonne, H., and Atassi, G. (1997) Biochemistry 36, 2943–2951
43. Son, Y. S., Suh, J. M., Ahn, S. H., Kim, J. C., Yi, J. Y., Hur, K. C., Hong, W. S., and Goldenberg, G. J. (1997) Mol. Pharmacol. 51, Nitiss, J. L., Vilalta, P. M., Wu, H., and McMahon, J. (1994) Mol. Pharmacol. 46, 773–777
45. Sullivan, D. M., Latham, M. D., Rowe, T. C., and Ross, W. E. (1989) Cancer Res. 49, 4467–4472
46. Bugg, B. Y., Danks, M. K., Beck, W. T., and Suttle, D. P. (1991) J. Biol. Chem.
47. Jannatipour, M., Liu, Y. X., and Nitiss, J. L. (1993) J. Biol. Chem.
48. Chan, V. T., Ng, S. W., Eder, J. P., and Schnipper, L. E. (1993) Anti-Cancer Drugs 4, 3973–3979
49. Harrison, D. J. (1995) J. Pathol.
50. Nitiss, J. L. (1994) Cancer Chemother. Pharmacol. 34, S6–S13
51. Nitiss, J. L., Vilalta, P. M., Wu, H., and McMahon, J. (1994) Mol. Pharmacol. 46, 773–777
52. Pommier, Y., Leuteurte, F., Fesen, M. R., Fujimori, A., Bertrand, R., Solary, E., Kohlhagen, G., and Kohn, K. W. (1994) Cancer Invest. 12, 530–542
53. Wasserman, R. A., and Wang, J. C. (1994) Cancer Res. 54, 1785–1800
54. Elsea, S. H., Hsiung, Y., Nitiss, J. L., and Osheroff, N. (1995) J. Biol. Chem. 270, 1913–1920
55. Hsiung, Y., Elsea, S. H., Osheroff, N., and Nitiss, J. L. (1995) J. Biol. Chem. 270, 29359–29364
56. Patel, S., Sprung, A. U., Keller, B. A., Heaton, V. J., and Fisher, L. M. (1997) Mol. Pharmacol. 52, 658–666
57. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. 9.31–9.57, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
58. Schiestl, R. H., and Gietz, R. D. (1989) Curr. Genet. 16, 339–346
59. Worland, S. T., and Wang, J. C. (1989) J. Biol. Chem. 264, 4412–4416
60. Tartof, K. D., and Hobbs, C. A. (1987) Focus 9, 2–12
61. Nitiss, J., Wang, J. C. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7501–7505
62. Strathern, J. N., and Higgins, D. R. (1991) Methods Enzymol. 194, 319–329
63. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
64. Burden, D. A., Kingma, P. S., Froelich-Ammon, S. J., Bjornati, M. A., Patchan, M. W., Thompson, R. B., and Osheroff, N. (1996) J. Biol. Chem. 271, 29238–29244
65. Osheroff, N., Shelton, E. R., and Brutlag, D. L. (1983) J. Biol. Chem. 258, 9536–9543
66. Osheroff, N., and Zechiedrich, E. L. (1987) Biochemistry 26, 4303–4309
67. Danks, M. K., Schmidt, C. A., Deeneka, D. A., and Beck, W. T. (1989) Cancer Commun. 1, 101–109
68. Osheroff, N. (1986) J. Biol. Chem. 261, 9944–9950
69. Roca, J., and Wang, J. C. (1992) Cell 71, 833–840
70. Osheroff, N. (1989) Biochemistry 28, 6157–6160
71. Robinson, M. J., and Osheroff, N. (1989) Biochemistry 28, 5680–5687
72. Robinson, M. J., and Osheroff, N. (1991) Biochemistry 30, 1807–1813
73. Berger, J. M., Gamblin, S. J., Harrison, S. C., and Wang, J. C. (1996) Nature 380, 225–232
74. Li, W., and Wang, J. C. (1997) J. Biol. Chem. 272, 31190–31195
75. Lindley, D. J., and Wang, J. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10485–10489
76. Cabrall, J. H. M., Jackson, A. P., Smith, C. V., Shikotra, N., Maxwell, A., and Liddington, R. C. (1997) Nature 388, 903–906