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Using yeast to study resistance to topoisomerase II-targeting drugs

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Abstract. We have developed a system utilizing the yeast *Saccharomyces cerevisiae* to probe the mechanism of action of anti-topoisomerase II drugs. This system has enabled us to dissect the mechanism of action of these agents. By inducing the overexpression of yeast topoisomerase II or by reducing the level of activity using temperature-sensitive mutations in topoisomerase II, we have demonstrated that conversion of topoisomerase II to a cellular poison plays a critical role in cell killing. We have also constructed other mutations in the yeast *TOP2* gene that are resistant to etoposide and amsacrine and determined the DNA sequences for several of the drug-resistant alleles. The mutations that confer drug resistance map to several regions of the *TOP2* gene. A mutation of particular interest changes Ser741 to Trp. This mutation results in hypersensitivity to etoposide but does not alter sensitivity to other agents such as mAMSA. We suggest that this mutation defines a site on the *TOP2* protein that is involved in drug:protein interactions.

Key words: Yeast – Topoisomerase – Resistance

Introduction

Anti-topoisomerase antitumor agents exert their cytotoxic effects by stabilizing an intermediate of the topoisomerase enzyme reaction [1, 5, 8, 22]. It has been proposed that this intermediate acts as a type of DNA damage and, therefore, that cells are killed by anti-topoisomerase agents, since the enzyme is converted into a "poison," rather than by the lack of an essential enzyme activity [20, 22].

We have developed a system utilizing the yeast *Saccharomyces cerevisiae* to probe the mechanism of action of these drugs [24, 28]. There are several advantages in using yeast to study anti-topoisomerase drugs. The well-defined genetics available in yeast, along with the ability to carry out targeted gene disruption and gene replacement, makes it possible to assess the effects of single elements on drug sensitivity. The major shortcoming to the use of yeast has been the relative insensitivity of yeast to a variety of chemical agents. The lack of sensitivity of wild-type yeast cells has frequently been attributed to a lack of permeability of the yeast cell wall or cell membrane. Recently, several mutations have been described that enhance the sensitivity of yeast cells to a variety of drugs, including the anti-topoisomerase I agent camptothecin and the anti-topoisomerase II agents mAMSA, etoposide, and doxorubicin [24, 25]. These mutations appear to act on the cell membrane [11, 14] and may produce drug sensitivity due to alterations in the function of endogenous yeast drug transporters because of an altered membrane environment.

Yeast has been of great value in demonstrating the specificity of agents that target topoisomerases. For example, we and other investigators have demonstrated that camptothecin is specific for topoisomerase I by showing that yeast *top1* mutants are completely resistant to the cytotoxic action of the drug [10, 24]. These experiments also demonstrated that conversion of an enzyme into a cellular poison could be the sole determinant of cell killing. Since the *TOP1* gene is not essential in yeast [13, 32], inhibition of enzyme activity by itself cannot lead to cell killing.

The ability to decipher the targets of cell killing using yeast also makes this organism extremely attractive as a screening system to discover new anti-topoisomerase inhibitors. Strains that carry mutations in topoisomerase genes can demonstrate that an agent acts specifically against a particular topoisomerase. The ease of growing yeast cells readily allows testing of a large number of compounds. Although yeast topoisomerase genes are very homologous to their mammalian counterparts, the differ-

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Abbreviations: mAMSA, 4-(9-acridinylamino)-methanesulfon-*m*-anisidide; CHO, Chinese hamster ovary; MDR, multi-drug resistance

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ences between the yeast and mammalian proteins may result in some differences in drug sensitivity. For example, we have found that yeast topoisomerase II shows considerably less sensitivity to mAMSA than mammalian topoisomerase II [17]. This potential shortcoming can be avoided since both human topoisomerase I [3] and human topoisomerase IIα ([35]; Nitiss et al., submitted for publication) have been functionally expressed in yeast. Hence, the drug sensitivity of the human enzymes can be readily assessed in yeast.

As is the case for all other anti-cancer agents, resistance to anti-topoisomerase II drugs frequently develops. Beck and colleagues [2] first demonstrated that some mammalian cell lines develop a resistance that is specific for topoisomerase II-targeting agents. This type of resistance, termed atMDR (atypical multi-drug resistance) was characterized by pleiotropic resistance to agents believed to target topoisomerase II, a lack of cross-resistance to drugs that act against a different target (e.g., vinca alkaloids), and a lack of expression of the multidrug transporter *MDR1*. It was hypothesized that atMDR arose from alterations in the topoisomerase II protein or its expression (reviewed in [1]). Subsequently, it was demonstrated that several of these cell lines carried mutations in the structural gene for topoisomerase II [4, 15, 21]. It was tempting to speculate that the observed mutations were the cause of drug resistance, but since mutations in topoisomerase II are expected to lead to recessive drug resistance, it is quite difficult to demonstrate that the identified mutation can lead to drug resistance. As described below, we have used yeast to demonstrate that the mutation identified by Bugg et al. [4] indeed results in a drug resistant topoisomerase.

In the sections below, experiments using yeast to address critical issues for drugs directed against topoisomerase II are described. Two issues are discussed in detail:

1. Do specific agents kill cells by their action on topoisomerase II, and, in particular, is cell killing due to the stabilization of cleavage by the drug?
2. What mutational changes in *TOP2* can alter sensitivity to anti-topoisomerase II agents, and, especially, are mutations identified in human cell lines that are resistant to anti-topoisomerase II agents contributing to the observed drug resistance?

Experimental tests of the mechanisms of cell killing by putative anti-topoisomerase II agents: effects of levels of topoisomerase activity on in vivo drug sensitivity and determination of drug targets

Overexpression of TOP2 confers drug hypersensitivity

A key prediction of the poison hypothesis is that overexpression of topoisomerase II should lead to an increase in drug sensitivity. We constructed a plasmid that leads to an approximately 10- to 30-fold overexpression of yeast *TOP2* and compared the sensitivity of cells bearing the overexpressing plasmid with that of cells that carry the same vector without the *TOP2* gene. We demonstrated that overexpression of topoisomerase II leads to enhanced sensitivity to topoisomerase II-targeting agents [26]. These experiments demonstrated that poisoning of topoisomerase II was an important mechanism of cell killing but did not rule out the possibility that inhibition of enzyme activity in vivo was also important. Unlike *TOP1*, *TOP2* is essential for viability [6, 16]; hence, inhibition of enzyme activity might also represent an important determinant of cell killing.

Reduced expression of topoisomerase II leads to drug resistance

Most previously identified temperature-sensitive alleles of yeast topoisomerase II show reduced sensitivity to anti-topoisomerase II drugs. The reduced sensitivity could be due either to a decrease in enzyme activity at the permissive temperature or to intrinsic drug resistance due to an alteration in their ability to be affected by topoisomerase-targeting drugs. The *top2-1* allele was of particular interest, since strains with this allele showed relatively high sensitivity to mAMSA and etoposide at the permissive temperature (25° C). It has been demonstrated that yeast strains carrying *top2-1* have nearly wild-type topoisomerase II activity at room temperature and substanti-ally reduced activity at 30° C [6]. Cells bearing *top2-1* are capable of growing at 30° C, although the growth at this temperature is rather slow. If etoposide and mAMSA kill cells because they convert topoisomerase II into a poison, then reducing the enzyme activity should be sufficient to reduce the ability of the drugs to convert the enzyme into a poison. If, on the other hand, the inhibition of enzyme activity by the drugs is important for cell killing, the strains should be quite drug sensitive at 30° C.

We tested sensitivity to mAMSA and etoposide at 30° C in *top2-1* strains and demonstrated that cells became very resistant to etoposide and mAMSA at 30° C [27]. We tested the sensitivity of the *top2-1* cells to camptothecin, an agent that inhibits topoisomerase I, and found that *top2-1* cells become hypersensitive to camptothecin at 30° . This result showed that the drug resistance observed at 30° C was specific for drugs that target topoisomerase II and excluded the possibility that the cells became resistant to cytotoxic drugs because of their slow growth at the semi-permissive temperature. The results obtained with the *top2-1* mutation, taken together with the overexpression experiments described above, strongly supports the model suggesting that these drugs act by converting topoisomerase II into a "poison."

It is worthwhile to emphasize that the reduction in topoisomerase II activity to the level found in *top2-1* cells at 30° C results in hypersensitivity to camptothecin. Similarly, we have found that Δ*top1* mutants are hypersensitive to etoposide and mAMSA (see below). The enhanced sensitivity in both cases may be due to the ability of yeast topoisomerases to substitute for each other in most processes where a topoisomerase is essential [13]. The enhanced dependence of a cell on a single topoisomerase might accentuate the lethal effects when cleavage is stabilized by an anti-topoisomerase agent. These results suggest that appropriate combinations of drugs that target topoisomerase I and topoisomerase II may be used to prevent the development of drug resistance due to reduced expression of to-

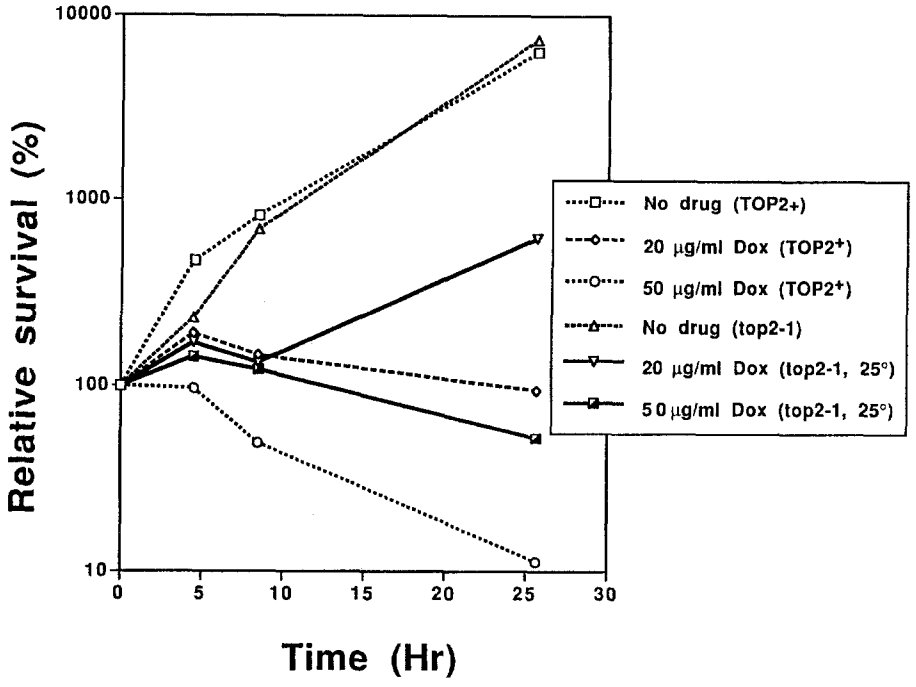

Fig. 1. Sensitivity of *TOP2*+ and *top2-1* strains to doxorubicin. The sensitivity of strains JN394 and JN394t2-1 [27] to doxorubicin (*Dox*) was determined. Doxorubicin was added at the concentration indicated, and cells were incubated in growth medium [24]. Aliquots were removed at various times, and cells were plated to determine viable counts

poisomerases. In yeast, we have found that combinations of mAMSA and camptothecin do result in synergistic cell killing [25]. However, it should be noted that Kaufman [19] has found that combinations of camptothecin and anti-topoisomerase II agents are antagonistic. The mechanism for this antagonism is unclear.

Besides testing the poison hypothesis, the *top2-1* strain can be used to demonstrate whether an agent kills cells by stabilizing cleavage by topoisomerase II. First, it is necessary to show that a drug stabilizes cleavage in vitro using purified topoisomerase II. Then, the drug is tested in *top2-1* strains at 25° C to verify that the drug can be taken up by the strain. The drug sensitivity is also tested in the same strain at 30° C. A drug that kills cells by poisoning topoisomerase II will not be very cytotoxic at 30° C, whereas a drug that has other important targets or a drug that kills cells by completely inhibiting topoisomerase II activity will exhibit cytotoxicity at this temperature.

Sensitivity of cells with the top2-1 mutation to other agents

We have used the *top2-1* strains to analyze several other agents that act against eukaryotic topoisomerase II in vitro. The fluoroquinolone CP-115, 953 has substanti-al activity against eukaryotic topoisomerase II [12, 31]. Using the *top2-1* strains, we have demonstrated that this agent kills cells by poisoning topoisomerase II [9].

We have also used the *top2-1* strain to examine the in vivo targets of the drug doxorubicin. JN394 cells (*TOP2*+) are killed by 50 µg doxorubicin/ml. Cells carrying *top2-1* that are grown at 25° C are somewhat resistant to doxorubicin (Fig. 1); however, little additional resistance is observed when the cells are grown at 30° C (data not shown). This result suggests that although topoisomerase II is a significant target for doxorubicin, other targets are also important for cell killing.

Using other drug resistant mutants

The *top2-1* mutation has been useful for demonstrating that cell killing results from stabilization of cleavage rather than from inhibiting enzyme activity. A more generally useful mutation for identifying new anti-topoisomerase II agents would be one that is highly resistant to all anti-topoisomerase II agents. The mutation that is closest to this ideal is the *top2-5* mutation. Like *top2-1*, *top2-5* is a temperature-sensitive lethal allele that has nearly wild-type topoisomerase II activity in vitro at the permissive temperature [17]. Unlike *top2-1*, the *top2-5* allele confers high levels of drug resistance at the permissive temperature [17]. An example of the resistance conferred by *top2-5* to mitoxantrone is shown in Fig. 2. The strain shown in Fig. 2A carries the *top2-5* allele, the *ise1* mutation to enhance drug permeability, and the DNA-repair mutation *rad52* to increase the killing effects of anti-topoisomerase agents. Even 100 µg mitoxantrone/ml is insufficient to kill cells carrying the *top2-5* allele. By contrast, 10 µg mitoxantrone/ml is sufficient to produce cell killing in the isogenic *TOP2*+ strain (Fig. 2B).

The *top2-5* allele confers only limited resistance to doxorubicin. The *top2-5* strain is killed by 10 µg doxorubicin/ml, with higher drug concentrations leading to more cell killing (Fig. 3A). The sensitivity of the isogenic *TOP2*+ strain to doxorubicin is somewhat greater than that of the *top2-5* strain, with significant cell killing occurring at 5 µg doxorubicin/ml (Fig. 3B). Thus, the doxorubicin resistance of *top2-5* strains is only about 2–3 times greater than that of wild-type *TOP2* strains. This low level of resistance conferred by *top2-5* probably reflects the importance of targets besides topoisomerase II for cell killing by doxorubicin. The importance of other targets is also supported by our observation that 20 µg doxorubicin/ml is cytotoxic in *ise1 RAD52*+ strains, suggesting that non-DNA targets are important at these higher concentrations (unpublished data).

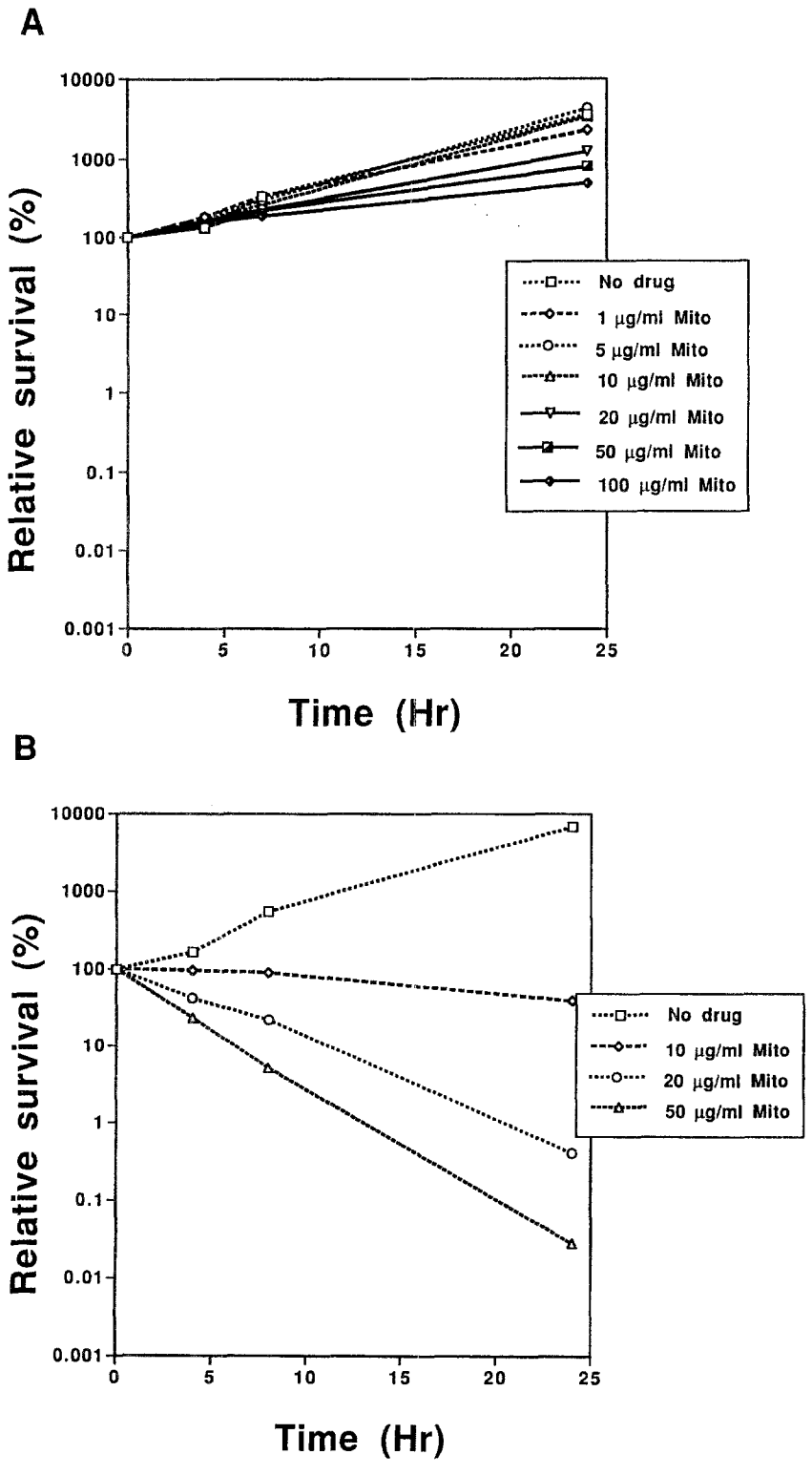

Fig. 2 A, B. Sensitivity of *TOP2*+ and *top2-5* strains to mitoxantrone. The sensitivity of JN271r52t2-5 (relevant genotype: *isel rad52::LEU2 top2-5*) and the isogenic JN271r52 (relevant genotype: *isel rad52::LEU2 TOP2*+) to mitoxantrone (*Mito*) was determined. The drug-sensitivity determinations were carried out at 25° C, the permissive temperature for *top2-5*, using the drug concentrations indicated. **A** Sensitivity of the *top2-5* strain. **B** sensitivity of the *TOP2*+ strain

Since the *top2-5* allele is resistant to many different classes of anti-topoisomerase II agents, including mAMSA, mitoxantrone, CP-115, 953, and etoposide, it is likely to be useful in strains used for characterizing new agents for their ability to stabilize cleavage by topoisomerase II. A comparison of wild-type and *top2-5* strains is probably sufficient to demonstrate whether a particular agent is specific for topoisomerase II.

Effects of top1 mutations on sensitivity to anti-topoisomerase II drugs

Screening for anti-topoisomerase II agents can also be accomplished using Δ*top1* mutants. As described above, anti-topoisomerase II drugs increase the drug sensitivity of Δ*top1* mutants. A similar increase in the sensitivity of *top1* mutants to anti-topoisomerase II agents has been reported

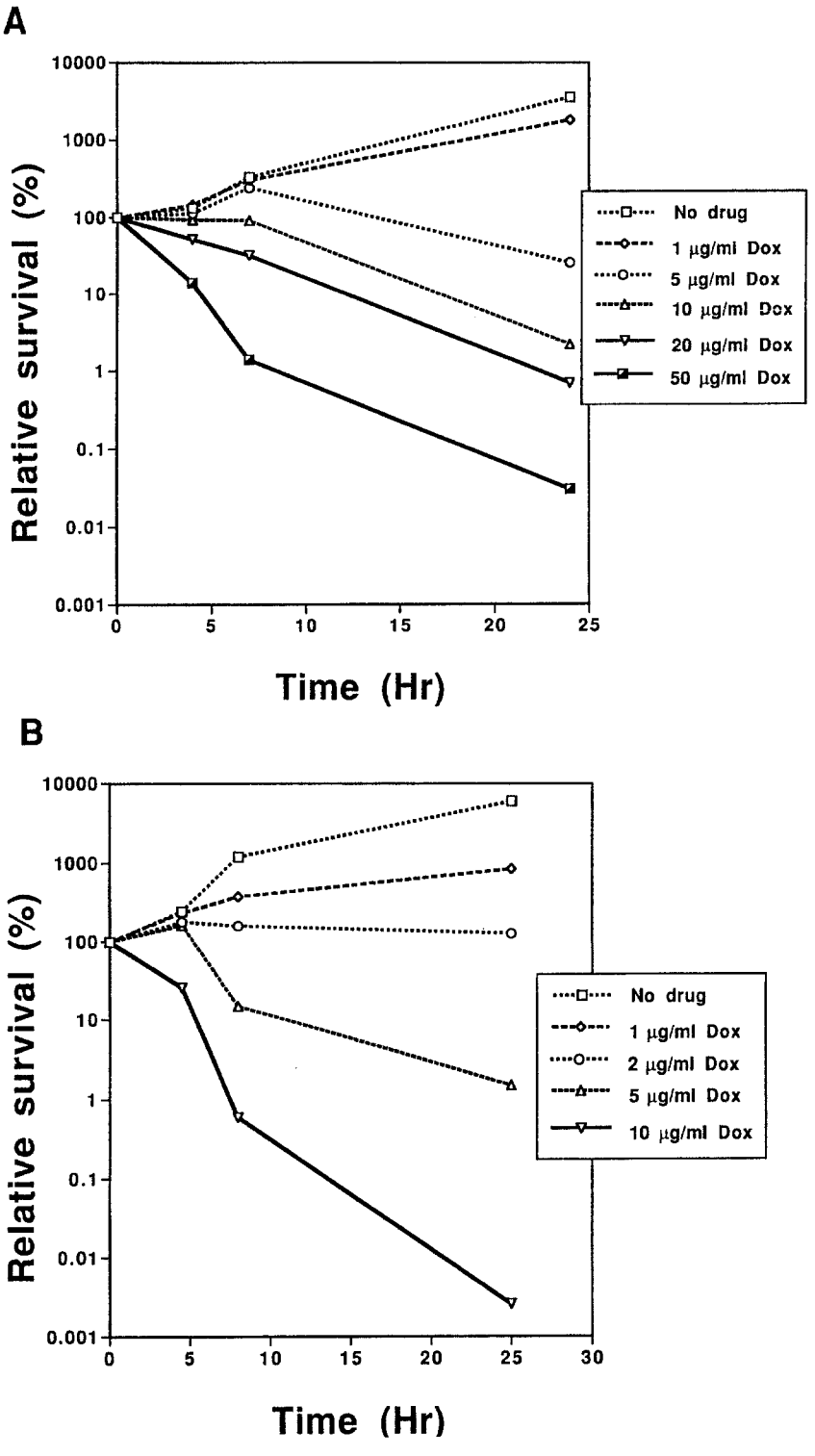

Fig. 3 A, B. Sensitivity of *TOP2*+ and *top2-5* strains to doxorubicin. The sensitivity of JN271r52t2-5 (relevant genotype: *isel rad52::LEU2 top2-5*) and the isogenic JN271r52 (relevant genotype: *isel rad52::LEU2 TOP2*+) to doxorubicin (*Dox*) was determined. The drug-sensitivity determinations were carried out at 25° C, the permissive temperature for *top2-5*, using the drug concentrations indicated. **A** Sensitivity of the *top2-5* strain. **B** Sensitivity of the *TOP2*+ strain.

by Eng et al. [10]. For example, the minimal lethal concentration of mitoxantrone in JN394 is 50–100 µg/ml (note that the experiment shown in Fig. 2 involves a strain that carries a different permeability mutation, *isel*, which confers greater sensitivity to mitoxantrone). JN394t1 is isogenic to JN394 except that it also carries a deletion of the *top1* gene. The deletion of the *top1* gene reduces the minimal lethal concentration to 10 µg/ml, a 5- to 10-fold difference. The sensitivity to mAMSA is similarly increased, with the minimal lethal concentration being 5 µg/ml in JN394 cells; the minimal lethal concentration is reduced to 0.5 µg/ml in JN394t1 cells. Interestingly, *top1* mutations result in only a small effect on sensitivity to the fluoroquinolone CP-115, 953 [31]. The presence of a Δ*top1* mutation increases the minimal lethal concentration of CP-115, 953 by a factor of less than 2. It is noteworthy that whereas etoposide, mitoxantrone, and mAMSA inhibit religation of the strand breaks introduced by topoisomerase

II, CP-115, 953 does not inhibit religation. Instead, the fluoroquinolone acts by increasing the rate of cleavage by the enzyme [30, 31]. How the difference in the mechanism of stabilizing cleavage by fluoroquinolones may affect drug sensitivity in a *top1* strain is not clear.

Mutations in topoisomerase II from cells selected for drug resistance

Mutations identified in mammalian cells

Several mutations have been identified in topoisomerase IIα from mammalian cell lines selected for resistance to topoisomerase-targeting agents. Many of the identified mutations cluster in two regions. The region that contains most of the identified changes is in a region of topoisomerase II that is homologous to *gyrB* [1, 15, 33]. Another group of mutations are located close to the active-site tyrosine of topoisomerase II. It is noteworthy that all of the mutations that have been identified map to topoisomerase IIα; no mutation has been found in the β isozyme [7].

One of the major difficulties in studying these drug resistant mutations has been that most mutations should be recessive to a drug-sensitive topoisomerase. Hence, it is very difficult to demonstrate that the identified mutation actually plays a role in drug resistance. It would be desirable to demonstrate directly that a particular mutation can result in a drug resistant topoisomerase. This can be accomplished by introducing the mutation into a topoisomerase II gene, inducing the overexpression of and purifying the mutant protein, and demonstrating in vitro that the topoisomerase II protein is drug resistant. However, it has proved to be rather difficult to induce the overexpression eukaryotic topoisomerase II. The overexpression of mammalian topoisomerase II has only recently been described, and that of a mutant topoisomerase II has not yet been reported.

Yeast can be used to overcome the difficulty of demonstrating whether a specific mutation in topoisomerase II can lead to drug resistance. Most of the mutations that have been described thus far occur in regions of the topoisomerase II protein that are conserved between yeast and human cells. We have reconstructed the Arg449-to-Gln mutation described by Bugg et al. [4] in yeast topoisomerase II to demonstrate that it encodes a drug resistant topoisomerase II. In yeast, the equivalent amino acid is Lys 439. Mutating Lys439 to either Gln or Glu results in an enzyme that is resistant to mAMSA and etoposide. In addition, we have shown that the Lys-to-Glu mutation can lead to semidominant drug resistance (Nitiss et al., submitted for publication). This result is in agreement with the original characterization of the mutant cell line [4]. This mutation may be an intriguing exception to the hypothesis that mutations in the structural gene of topoisomerase II always lead to recessive drug resistance.

Mutations identified in yeast

The *top2-5* mutation is described in detail above. The DNA sequence of the mutation has been determined, and three tightly clustered changes have been identified [17]. The mutational changes identified in the *top2-5* allele are summarized in Table 1. The *top2-5* mutation defines a new domain for effects on drug sensitivity, since no mutation in prokaryotic or eukaryotic cells that alters drug sensitivity has previously been described in that region. The region around *top2-5* is well conserved among eukaryotic but not prokaryotic topoisomerases. Since prokaryotic enzymes are relatively insensitive to etoposide, mAMSA, and mitoxantrone, it is tempting to speculate that the *top2-5* domain plays an important role in drug interactions and that the lack of this domain leads to the relative resistance of prokaryotic enzymes to these drugs.

Table 1. Yeast mutations with alterations in sensitivity to topoisomerase II agents

Mutation	Amino acid change	Phenotype
top2-5	Arg884 to Pro Arg886 to Ile Met887 to Ile	Resistant to mAMSA, etoposide, and mitoxantrone
top2-101	Gly738 to Ser	Resistant to etoposide, CP-115, 953; sensitive to mAMSA
top2-102	Arg1195 to Lys	Resistant to mAMSA, etoposide
top2-103[a]	Pro824 to Ser	Resistant to mAMSA, etoposide
top2-A4	His507 to Tyr His521 to Tyr	Resistant to mAMSA, etoposide
top2Ser741Trp	Ser741 to Trp	Hypersensitive to etoposide
TOP2Lys439Gln[b]	Lys 439 to Gln	Resistant to mAMSA, etoposide

[a] A separate mutation was detected in this allele. In vitro mutagenesis demonstrated that the other mutation made no contribution to drug resistance

[b] This mutation is in the equivalent position of yeast topoisomerase II as the mutation described by Bugg et al. [4]. It was constructed by oligonucleotide-directed mutagenesis

We have constructed a large number of new mutants in yeast topoisomerase II that lead to altered sensitivity to anti-topoisomerase agents (Nitiss et al., submitted for publication). The region near the *top2-5* mutation is the site for another drug resistant mutation. This mutation, *top2-103*, confers resistance to both etoposide and mAMSA. We identified a mutation in that allele that results in a change at amino acid 824 from proline to serine. We then reconstructed the mutation by oligonucleotide-directed mutagenesis and demonstrated that the change at position 824 is the cause of the resistance to etoposide and mAMSA. This result suggests that the region defined by *top2-103* and *top2-5* represents an important part of the protein for interaction with anti-topoisomerase agents.

Serine 83 of the *gyrA* subunit of prokaryotic topoisomerase II has been demonstrated to interact with fluoroquinolones, which are agents that stabilize cleavage of the prokaryotic enzyme [23, 29]. A mutation has been identified close to the homologous position in yeast topoisomerase II. Interestingly, this mutation confers resistance to fluoroquinolones and etoposide but does not confer resistance to mAMSA. DNA-sequence analysis demonstrated that the *top2-101* allele carries a mutation that converts Gly738 to Asp. We then determined whether the homo-

logue of Ser83 of *gyrA* (Ser741 in yeast) might be important in interacting with etoposide or other agents that target the eukaryotic enzyme. We mutated Ser741 of yeast topoisomerase II (which is homologous to Ser 83 of *gyrA*) to alanine, leucine, or tryptophan. These changes have previously been identified in *gyrA* from *Escherichia coli* strains resistant to fluoroquinolones [29]. Whereas the first two changes resulted in modest changes in sensitivity to anti-topoisomerase agents in yeast, mutation of Ser741 to tryptophan resulted in an enzyme that confers *hypersensitivity* to etoposide (Hsiung and Nitiss, unpublished data). This result strongly suggests that this region is also of critical importance in interacting with anti-topoisomerase agents. We are presently verifying the hypersensitivity of the Ser-to-Trp mutation by inducing the overexpression of and purifying the mutant enzyme and determining its interactions with etoposide.

Yeast mutations near the mammalian mutations in the *gyrB* domain have also been identified. One example, the *top2-A4* mutation, consists of two amino acid changes; His507 and His521 are both changed to Tyr. Reconstruction of the mutations indicated that neither mutation alone could lead to drug resistance; however, when both mutations were included, the resultant topoisomerase II gene conferred high levels of resistance to both mAMSA and etoposide.

In addition to the mutations that we have identified in domains that are known to play a significant role in the topoisomerase II reaction, we have also identified other mutations that can confer drug resistance whose effects on the protein are less clear. For example, the *top2-102* mutation localizes to the carboxy terminal region of yeast topoisomerase II. No specific function has been ascribed to this part of the protein; nonetheless, *top2-102* mutants are highly resistant to etoposide and mAMSA.

The results of the mutation studies suggest that several regions of the topoisomerase II protein are important for the action of drugs that stabilize cleavage by the enzyme. The observation that Ser741 can be mutated to produce an enzyme that is hypersensitive to etoposide strongly suggests that this region of the protein interacts with the drug. Whether the other regions defined by mutations that can confer drug resistance also interact directly with the drugs awaits further biochemical characterization of the mutant proteins.

Discussion: toward biochemical models of drug action on topoisomerase II

Drugs that stabilize cleavage by topoisomerase II are important in the treatment of a variety of tumors. Given their clinical importance, it is critical to understand in detail how cell killing is accomplished by these agents. The effects of anti-topoisomerase II agents in vitro suggest that the drugs may kill cells by converting the enzyme into a type of DNA-damaging agent. The experimental systems described herein have enabled us to substanti-ate this model. By reducing the activity of DNA topoisomerases in vivo, we have demonstrated that the poisoning of topoisomerase II by drugs that stabilize cleavage is the most important aspect of cell killing. Since there are likely to be many ways of reducing topoisomerase II activity in vivo, a diminution of topoisomerase II activity is likely to represent an important mechanism of acquired resistance to anti-topoisomerase II agents.

It is unclear whether mutations in the structural genes for DNA topoisomerases also represent an important mechanism of acquired drug resistance. However, the study of mutations that alter sensitivity to topoisomerase II-targeting drugs is likely to provide an important clue to how these drugs act on the enzyme. Thus, the mechanism of action of drugs that stabilize cleavage by topoisomerase II represents a challenging problem for protein biochemists and cancer pharmacologists. Although a comprehensive model has not yet been put forward, it is useful to summarize some of the results that any model of drug action must explain. The considerations listed below are certainly not inclusive.

There are a diverse range of chemical agents that can interfere with DNA topoisomerase II. It is rather hard to imagine how so many different compounds can interact directly with the enzyme. Given that most (but probably not all) topoisomerase II poisons interact with DNA, it is tempting to speculate that DNA rather than topoisomerase II is the critical target for anti-topoisomerase drugs [22]. However, since agents such as etoposide interact weakly with DNA, interactions with the enzyme, perhaps as part of a drug:DNA:protein ternary complex, are likely to be important in some cases. Since many potent intercalating agents do not stabilize cleavage by topoisomerase II, intercalation cannot be sufficient for drug stabilized cleavage by topoisomerase II.

Mutations that alter drug sensitivity define (at least) three critical domains: a part of the adenosine triphosphatase (ATPase) homology domain (similar to the sites in *gyrB* that lead to fluoroquinolone resistance [36]), the Ser83/active-site tyrosine domain (similar to the *gyrA* resistance domain) and the domain defined by *top2-103* and *top2-5* (3 to tyrosine, the unique eukaryotic enzyme domain). In addition, there are other mutations that do not fall into these categories, e.g., the *top2-102* allele, which is near the 3 end of the *TOP2* coding region. Hopefully, biochemistry studies on these mutant proteins will help to elucidate the biochemical changes that can lead to drug resistance.

The diversity of agents that can stabilize cleavage by topoisomerase II suggests that these agents will be of importance for some time to come. The discovery of new topoisomerases in *E. coli* [18], yeast [34], and human cells [7] suggests that other new topoisomerase targets may await drug discovery efforts.

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