Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7501–7505, October 1988
Biochemistry

DNA topoisomerase-targeting antitumor drugs can be studied in yeast

(camptothecin/4′-(9-acridinylamino)-methanesulfon-*m*-anisidide/permeability mutants/recombination/DNA damage-inducible genes)

JOHN NITISS AND JAMES C. WANG

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

Contributed by James C. Wang, July 1, 1988

ABSTRACT **The antitumor drugs camptothecin and an anilinoacridine, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (mAMSA), which act on DNA topoisomerase I and II, respectively, are shown to inhibit the growth of *Saccharomyces cerevisiae* mutants selected for their permeability to other inhibitors. In addition to growth inhibition, these drugs induce high levels of homologous recombination and induce the expression of a DNA damage-inducible gene *DIN3*. Cytotoxicity of the drugs is more pronounced in strains that also carry a *rad52* mutation. An analog of mAMSA, 4′-(9-acridinylamino)-methanesulfon-*o*-anisidide (oAMSA), which is ineffective as an inhibitor of DNA topoisomerase II in mammalian cells, is also ineffective in eliciting physiological responses in these yeast strains. The physiological effects of camptothecin, but not those of mAMSA, disappear if the *TOP1* gene encoding DNA topoisomerase I is disrupted. This shows that DNA topoisomerase I is the sole target of camptothecin cytotoxicity and illustrates that a nonessential enzyme can nevertheless be the target for a cytotoxic drug.**

A variety of antitumor chemotherapeutics have been shown recently to target the DNA topoisomerases (for review, see refs. 1–5). Their pharmacological effects correlate well with their effectiveness in trapping a class of complexes between DNA and DNA topoisomerases, termed the cleavable complexes, which are characterized by their conversion to DNA–protein covalent complexes upon their exposure to a protein denaturant (for review, see refs. 6–8).

The chain of events leading from the trapping of a cleavable complex to cell death is unclear; it is also uncertain whether the DNA topoisomerases are the sole targets of the drugs. In strains of *Escherichia coli* lacking the *lon* protease, the cytotoxicity of antibiotics of the 4-quinolone family, which enhance the formation of a cleavable complex between DNA and DNA gyrase (bacterial DNA topoisomerase II), appear to be related to the induction of the SOS repair pathway by these drugs (5, 9). It is tempting to extrapolate the results obtained in bacterial systems to eukaryotes, given the similarities between bacterial gyrase and eukaryotic DNA topoisomerase II and between the actions of the drugs on their respective targets (6–8). No suitable eukaryotic genetic system was available, however, for studies of the actions of these drugs.

The budding yeast *Saccharomyces cerevisiae* appeared to be a natural choice for the genetic dissection of the actions of the topoisomerase-targeting drugs. Purified yeast DNA topoisomerases I and II are very similar to their mammalian counterparts. Although intact cells of ordinary laboratory strains of the yeast are refractory to the drugs, our previously unpublished results showed that spheroplasts or cells treated with LiCl according to the procedures commonly used for uptake of DNA are sensitive to treatment with camptothecin (50 μg/ml), a plant alkaloid that acts on DNA topoisomerase I (10–12) or 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (amsacrine; mAMSA) (100 μg/ml), an acridine derivative that acts on DNA topoisomerase II (1–5). Significantly, the loss of viability of spheroplasts treated with 4′-(9-acridinylamino)-methane-sulfon-*o*-anisidide (oAMSA) (100 μg/ml), the *ortho* analog of mAMSA, which is ineffective as an antitumor agent, was found to be much less. Thus the insensitivity of yeast to the topoisomerase-targeting drugs appeared to be due to a permeability barrier at the cell wall.

Yeast strains have been described previously with pleiotropic drug sensitivity due to enhanced drug permeability (13–16). We report in this communication that by the use of suitable yeast permeability mutants, sensitivity to DNA topoisomerase-targeting drugs can be readily seen. In such mutants, both camptothecin and mAMSA, but not oAMSA, induce high levels of homologous recombination and stimulate the expression of a gene *DIN3*, known to be induced by DNA-alkylating agents and other DNA-damaging agents, including UV light (17, 18). We show also that all phenotypic changes caused by treatment of these mutants with camptothecin disappear when the mutants were made defective in the expression of DNA topoisomerase I, thus demonstrating that DNA topoisomerase is the sole cellular target of this drug.

MATERIALS AND METHODS

Materials. Yeast strains used in this work are listed in Table 1. Strain construction was according to standard methods (19). Various media used in the growth of cells have been described (20); YPDAP and YPDAH are YPDA containing 100 mM potassium phosphate, pH 7.5, and Hepes buffer, pH 7.3, respectively. The lactone form of camptothecin appears to be unstable in yeast media at acidic pH. Camptothecin, the lactone form unless indicated otherwise, was purchased from Sigma; mAMSA, oAMSA, and the sodium salt of the acid form of camptothecin were provided by L. F. Liu (Johns Hopkins University, Baltimore, MD). Sodium camptothecin was dissolved in water at 2 mg/ml, and the other drugs were dissolved in dimethyl sulfoxide at 5–10 mg/ml. All other reagents were purchased from commercial sources.

Yeast Transformation and Construction of Gene Disruptions. Yeast transformation was performed by the lithium acetate method (21). A plasmid-borne *LEU2* disruption of yeast topoisomerase I identical to that of Thrash *et al.* (22) was constructed, and the disruption was used to replace the wild-type *TOP1* gene by the method of Rothstein (23). Similarly, *rad52* derivatives of CG378 and CG379 strains were constructed using a *LEU2* disruption of the *RAD52* gene (24).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mAMSA, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (amsacrine); oAMSA, 4′-(9-acridinylamino)-methanesulfon-*o*-anisidide.

Table 1. Yeast strains

Strain	Genotype
CG378	a *can1 leu2-3,112 trp1-289 ura3-52*
CG379	α *ade5 leu2-3,112 trp1-289 ura3-52 his7-2*
A364a	a *ade1 ade2 ura1 his7 lys2 tyr1 gal1*
FL599	a *ise1*
CG378::rad52	As CG378 but *rad52::LEU2*
CG379::rad52	As CG379 but *rad52::LEU2*
CG379D1	As CG379 but with *DIN1*::β-galactosidase fusion
CG379D3	As CG379 but with *DIN3*::β-galactosidase fusion
JN284	a *leu2 his7 ise1*
JN362	a *leu2 his7 ise1*
JN360	As JN284 but *top1-6*
JN361	As JN362 but *top1-6*
JN384	α *ise1 his7 ade2-1 leu2 ura3 can1 top1-1*
JN385	a *ise1 his7 ade2-1 trp1 ura3 can1 top1-1*
JN386	a *ise1 his7 ade2-1 leu2 trp1 ura3 can1 top1-1*
JN384T1	As JN384 but with YCp50::*TOP1*
JN385T1	As JN385 but with YCp50::*TOP1*
JN386T1	As JN386 but with YCp50::*TOP1*
JN62-3	α *ise1 leu2 trp1 top1-1*
JN62-22	a *ise1 his3 top1-1*
JN387	JN386 × JN62-3
JN388	JN386T1 × JN62-3
JN60-5	*ise1 rad52::LEU2 his7 ura3*
JN57-10	*ise1 RAD52 his7 ade2*
JN127	*a ade1-2 ISE2 ura1 his7-n lys2 tyr1*
JN120	JN127 × CG379D1
JN173	JN127 × CG379D3
JN84	*leu2 ISE2 ade1 DIN3::β-galactosidase*
JN85	*leu2 ISE2 ADE1 DIN3::β-galactosidase*
JN84t1	As JN84 but *top1-6*
JN85t1	As JN85 but *top1-6*

Strains CG378 and CG379 were from C. Giroux (National Institute of Environmental Health Science, Research Triangle Park, NC). FL599 was from E. Nestemann (Environmental Health Center, Ottawa), and A363a was provided by the Yeast Genetic Stock Center (Berkeley, CA). All other strains were constructed in this laboratory.

Isolation and Scoring of Drug-Sensitive Strains. The *ISE2* mutation was obtained by ethyl methanesulfonate mutagenesis of A364a strain to 5% survival. Mutagenized cells were grown in YPDA medium to allow expression of the induced mutations and then plated on YPDA. They were replica plated to YPDA plates with cycloheximide (0.5–5.0 μg/ml). Colonies that grew on YPDA, but not on cycloheximide plates, were tested in liquid medium for sensitivity to aphidicolin (100 μg/ml) (25). A total of 9 of 20,000 colonies screened were reproducibly sensitive to cycloheximide (0.5–2.0 μg/ml), of which one (ISE2; see *Results*) was found sensitive to aphidicolin. Subsequent scoring of *ISE2* was done by plating on YPDA plates containing cycloheximide (2.0 μg/ml) and verified in liquid YPDA containing aphidicolin (100 μg/ml). The *ise1* mutation was scored on 1.5% agar plates containing YPDA plus crystal violet at 0.5 μg/ml or cycloheximide at 0.5–2.0 μg/ml.

Drug Treatments. Cells were inoculated in YPDA, YPDAP, or YPDAH and grown overnight with vigorous shaking at 30°C. After the cells were counted in a hemacytometer and diluted to 1–2 × 10^6 cells per ml, drugs were added. For drugs dissolved in dimethyl sulfoxide, equal volumes of the solvent were added to control cultures.

Detection of Recombination. For detection of recombination at *ade1* or *ade2*, cells were spread on YPDA plates and incubated for 3–4 days at 30°C. The total number of colonies and the number of colonies that were red or had at least one-quarter red sectors were scored. The sectored colonies might be due to residual drug inside the cells at plating. It has also been shown that treatment of cells with DNA-damaging agents can result in mutation or lethality 2–3 generations posttreatment (26). To score gene conversion at *HIS7*, cells were plated either directly or after a 10-fold dilution on synthetic medium lacking histidine. In all cases diploids freshly constructed from their haploid parents were used to ensure a low frequency of spontaneous recombination.

Detection of Mutation. Induction of mutation in the *CAN1* gene by drug treatments was measured by counting the number of colonies on agar plates of synthetic medium lacking arginine but containing canavanine sulfate (50 μg/ml). Mutation induction was also measured from the formation of white colonies in strains carrying *ade2-1* mutation. White colonies mainly arise by suppression of the mutation or by a forward mutation in genes the actions of which precede that of *ADE2* in the adenine biosynthetic pathway (27).

Assay of *DIN* Gene Fusions. Strains carrying a *DIN1* or a *DIN3* fused to the coding sequences of β-galactosidase at the respective chromosomal location of *DIN1* or *DIN3* were constructed as described by Ruby *et al.* (17). Strains carrying these integrated fusions (CG379D1 and CG379D3) were crossed with permeable strains to generate diploids that are heterozygous for the *DIN* gene fusion. Yeast *DIN* genes fused to β-galactosidase were assayed as described (17); reagent blanks were assays with extracts of drug-treated cells that do not carry the *DIN* fusion but are otherwise isogenic.

RESULTS

Permeability Mutants of *S. cerevisiae* Are Sensitive to Topoisomerase-Targeting Antitumor Drugs. We have identified yeast strains that are sensitive to the antitopoisomerase drugs camptothecin and mAMSA. Fig. 1*A* illustrates the effects of mAMSA and oAMSA on the growth of strain JN120. This diploid strain was constructed by mating strain CG379, which is refractory to the drugs, with strain JN127, which is an aphidicolin-sensitive derivative of strain A364a and was found to be sensitive to both mAMSA and camptothecin, but not to oAMSA (data not shown; see Table 1 for a list of all strains). As shown in the figure, sensitivity of JN120 to mAMSA is apparent at drug concentrations as low as 20 μg/ml, whereas oAMSA has little effect at 100 μg/ml. The same strain also retains the sensitivity trait of JN127 to camptothecin (data not shown). Because the drug-sensitivity trait in JN127 strain is retained in JN120 strain and is therefore dominant, we tentatively designated the gene for this trait *ISE2*, and its inhibitor-resistant counterpart *ise2*.

Fig. 1*B* illustrates the effect of camptothecin on the growth of strain FL599, which carries an inhibitor-sensitive recessive mutation *ise1* (15). The mutation *ise1* was initially identified as one that confers recessive sensitivity to crystal violet (0.5 μg/ml) or cycloheximide (0.5 μg/ml). As shown in the figure, the strain is also highly sensitive to camptothecin; inhibition of cell growth is noticeable at a drug concentration as low as 5 μg/ml, and killing of cells is seen at a dose of 50 μg/ml (Fig. 1*B*) or higher (data not shown). In contrast to strain JN120 (*ISE2*), which is sensitive to both camptothecin and mAMSA, strain FL599 (*ise1*) is insensitive to mAMSA at doses to 100 μg/ml.

DNA Topoisomerase-Targeting Antitumor Drugs Induce High Levels of Recombination in Drug-Sensitive Mutants. Treatment of drug-sensitive mutants with mAMSA and camptothecin causes an increase in the level of recombination. We examined crossing over induced by the drugs by the use of the red/white colony test for homozygosis at *ade1* or *ade2* (ref 27; see also *Materials and Methods*). As shown in Table 2, treatment of JN173 (*ise2/ISE2*; *ade1-2/ADE1*) cells with mAMSA or camptothecin resulted in a large increase in red and red-sectored colonies; a large increase was also seen upon treatment of an (*ade2/ADE2*; *ise1/ise1*) diploid strain

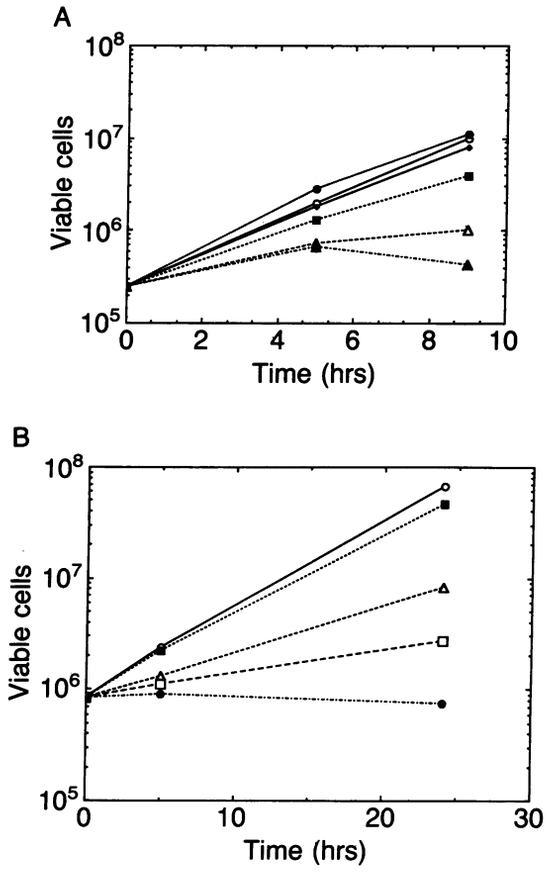

FIG. 1. Dose–response of yeast cells to topoisomerase-targeting drugs. Logarithmically growing cultures of JN120 or FL599 were diluted to about 1 × 10^6 in YPDA medium and then split into separate cultures. The appropriate volume of drug was added with an equal volume of dimethyl sulfoxide in the control culture. Cells were plated on YPDA plates after appropriate dilutions at the times indicated. (*A*) JN120 strain treated with 0 (○), 10 (◆), 20 (■), 50 (△), and 100 (▲) μg of mAMSA per ml and 100 (●) μg of oAMSA per ml. (*B*) FL599 strain treated with 0 (○), 5 (■), 10 (△), 20 (□), and 50 (●) μg of camptothecin per ml in YPDAH medium.

JN388 with camptothecin. Treatment of JN173 with oAMSA (100 μg/ml), on the other hand, showed essentially no effect on the number of red or red-sectored colonies.

Because formation of red or red-sectored colonies by homozygosis in diploid strains can arise by chromosome loss as well as by recombination, we also examined induction of gene conversion by the drugs. As shown in Table 2, treatment of strain JN173 (*ise2/ISE2*; *his7-2/his7-n*) with mAMSA or camptothecin increases greatly the frequency of His^+ colonies. There is considerable spread in data obtained in different experiments with mAMSA; typically a 5- to 20-fold increase was seen upon incubation of cells with 50 μg of the drug per ml for 24 hr. When cells of this strain were incubated with oAMSA (100 μg/ml) for 24 hr, the increase of His^+ colonies over the no-drug control was not > ≈2-fold and was not significantly different from the no-drug control. We also examined the dependence of the number of His^+ colonies on mAMSA concentration. Upon incubation of the cells with the drug for 24 hr, the number of His^+ colonies per 10^6 viable cells was measured to be 4.1, 4.0, 7.3, 14, and 25, respectively, at 5, 10, 20, 50, and 100 μg of the drug per ml. When JN173 cells were incubated with sodium camptothecin (50 μg/ml) for 24 hr, a 50-fold increase in His^+ colonies was seen. For both mAMSA and sodium camptothecin, treatment with low doses of each drug resulted in an increase in the absolute number of recombinants as well as an increase in the frequency of convertants (data not shown).

The effect of camptothecin on mutation frequencies is minor. Treatment of JN127 (*CAN1*) with camptothecin (50 μg/ml) for 8 hr, for example, resulted in a 4-fold increase in the number of canavanine-resistant colonies due to mutations in the *CAN1* gene encoding arginine permease. Similar treatment of the haploid strain JN384T1 (*ade2*), which forms red colonies on YPDA plates, increased 5-fold the number of white colonies due to mutations that suppress *ade2* or mutations in one of the genes preceding *ADE2* in the adenine biosynthetic pathway (27). Thus the high level of red colonies and histidine prototrophs produced by camptothecin are produced mainly by recombination. Nonetheless, both mAMSA and camptothecin appear to act as weak mutagens.

A DNA Damage-Inducible Gene *DIN3* Is Induced by Camptothecin or mAMSA in Yeast Strains Sensitive to the Drug. Several DNA damage-inducible genes have been identified by Ruby and Szostak (18) by joining the promoters of these genes, termed the *DIN* genes, to the coding sequences of β-galactosidase. We have tested two such *DIN–β-gal* fusions, *DIN1* and *DIN3*. Fig. 2 shows that in the strain JN173, either camptothecin or mAMSA induces *DIN3*. The maximal level of β-galactosidase induction by camptothecin or mAMSA in JN173 compares with that induced by treatment with 0.01% methyl methanesulfonate. The analog of mAMSA, oAMSA, has little effect on the expression of β-galactosidase. When the same experiments were done with strain JN120, which is isogenic with JN173 except that it carries a *DIN–β-gal* rather than a *DIN3–β-gal* fusion, no increase in the β-galactosidase level was ever seen.

Table 2. Induction of recombination by antitopoisomerase drugs

Drug	Incubation time, hr	Reds (*ADE1/ade1*), %			Reds (*ADE2/ade2*), %	His^+, × 10^6			
		I	II	III	I	I	II	III	IV
None	24	0.036	0.014	0.18	0.064	1.1	2.3	1.9	3.3
mAMSA (100 μg/ml)	4	0.11							
mAMSA (100 μg/ml)	8	0.58							
mAMSA (100 μg/ml)	24	0.70				6.6	31	25	25
oAMSA (50 μg/ml)	24								2.0
oAMSA (100 μg/ml)	24							3.5	3.3
Sodium camptothecin (50 μg/ml)	4	0.95							57
	9	1.6			0.35				96
	24	2.7	2.7	2.3	0.60	79	86		88

JN173 cells were treated as described with mAMSA, oAMSA, or sodium camptothecin. Homozygosis of *ade2* in camptothecin-treated cells was done with strain JN388. Roman numerals refer to separate experiments with independently constructed diploids. Reciprocal exchange was scored by examining YPDA plates for red or red-sectored colonies. The reds include all colonies with either one-half or one-fourth red sectors and do not include colonies with smaller sectors. Gene conversion in *his7* was scored by plating on agar plates containing a synthetic medium lacking histidine; the frequency of His^+ was obtained by comparing viable titers on "histidine-dropout" plates and YPDA plates.

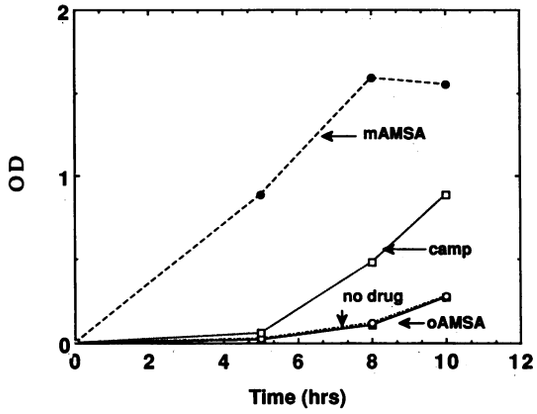

FIG. 2. Induction of *DIN3* fusions by mAMSA and camptothecin (camp). Cells were from a logarithmically growing culture of JN173 and diluted to about 1–2 × 10^6 cells per ml. Drug was added at time 0, with 1-ml samples removed at the indicated times for β-galactosidase assays. The results are shown as OD_{420} as a function of treatment time with the drug. Drug concentrations were 50 μg of camptothecin per ml, 100 μg of mAMSA per ml, and 100 μg of oAMSA per ml.

Sensitivity of Permeable Yeast Cells to Camptothecin Is Much Enhanced by the Introduction of *rad52* Mutation. Because camptothecin is a potent inducer of recombination, it appeared likely that genes involved in repair and recombination might be important in the cellular response to the drug. As illustrated in Fig. 3, the strain JN60-5, which is a congenic *rad52* derivative of strain JN57-10 (*ise1 RAD52*) is much more sensitive to the drug.

DNA Topoisomerase I Is the Sole Cellular Target of Camptothecin. When mutations inactivating DNA topoisomerase I were introduced into strains that are sensitive to camptothecin, the cells became resistant to the drug. In one experiment, strains JN284 and JN362 (*ise1 leu2 TOP1*) were transformed to Leu2$^+$ *top1* by the one-step gene replacement method (23), and five transformants of each were grown and tested for sensitivity to camptothecin. It was found that 85 ± 6% and 89 ± 9% of the *top1* cells survived incubation with camptothecin (50 μg/ml) for 8 and 24 hr, respectively; for the *TOP1*$^+$ control samples the corresponding values are 42% and 17 ± 7%. In a second series of experiments, three *top1-1* mutants JN384, JN385, and JN386 were separately incubated with camptothecin (50 μg/ml). After 6 and 24 hr, the average percentages of surviving cells were 107 ± 15 and 91 ± 15, respectively. For the *TOP1*$^+$ controls, which are identical to the respective mutants except that in each control a copy of the *TOP1* gene on a single-copy plasmid YCp50 (28) had been introduced, the corresponding values were 9 and 17 ± 4%.

Inactivation of DNA topoisomerase I in a camptothecin-sensitive strain not only suppresses the drug sensitivity, it also eliminates all other phenotypes of the drug-treated cells described earlier. When a diploid strain constructed by mating JN85 *ADE1 top1* and JN84 *ade1 top1* was tested for *ADE1* homozygosis upon treatment with sodium camptothecin (50 μg/ml) for 24 hr, 0.07% of the cells were scored as red colonies; this value is not significantly different from that of untreated control cells, which gave 0.1% red colonies. In contrast, when the *TOP1*$^+$ strain JN85 (*ADE1 TOP1*) × JN84 (*ade1 TOP1*) was used, 1.4% of the drug-treated cells gave red colonies compared with 0.03% red colonies for the untreated cells.

A similar series of experiments were done for the effects of incubation with camptothecin (50 μg/ml) on homozygosis at *ADE2*, using the strains JN386 × JN62-3 (*ADE2 top1/ade2-1 top1*) and its isogenic YCp50::*TOP1*$^+$ transformant JN386T1. For the *TOP1*$^+$ cells, 0.35% gave red colonies upon incubation with the drug for 8 hr; the percentage of red colonies increased to 0.6 when the incubation time was lengthened to 24 hr. The corresponding values when incubation was in the absence of the drug were 0.06% and <0.03%. For the *top1*$^-$ cells, 0.07% and 0.05% of the cells gave red colonies upon incubation with the drug for 8 and 24 hr, respectively, which are not significantly higher than the corresponding values for control cells incubated in the absence of the drug, 0.07% and <0.05%.

The effect of inactivation of DNA topoisomerase I on the induction of *DIN3* by camptothecin is shown in Fig. 4. When strain JN85 (*leu2 DIN3–β-gal ISE2 TOP1*) was converted to top1::LEU2, induction of β-galactosidase by the drug is no longer seen. *DIN3* remains inducible by treatment of the *top1*$^-$ strain with 0.01% methyl methanesulfonate, however, as shown in the figure. Taken together, the results in this section show conclusively that DNA topoisomerase I is the only significant cellular target of camptothecin.

DISCUSSION

We show that mutants of the yeast *S. cerevisiae* can be readily selected and used in the study of the mechanisms of action of topoisomerase-targeting antitumor agents. The

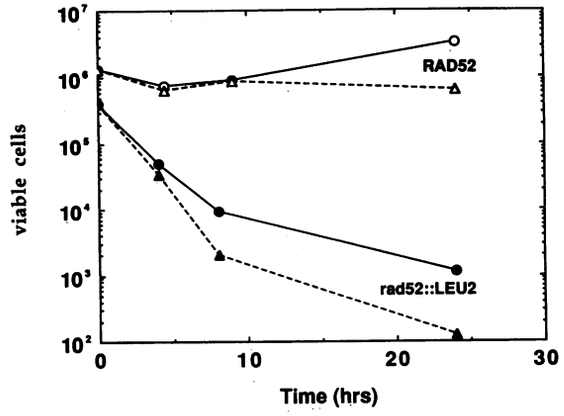

FIG. 3. JN60-5 (*rad52*) or JN57-10 (Rad$^+$) cells were treated with 50 μg of sodium camptothecin per ml in YPDA (solid lines) or 50 μg of camptothecin per ml in YPDAH (dashed lines). Samples were plated on YPDA plates at the times indicated after appropriate dilution.

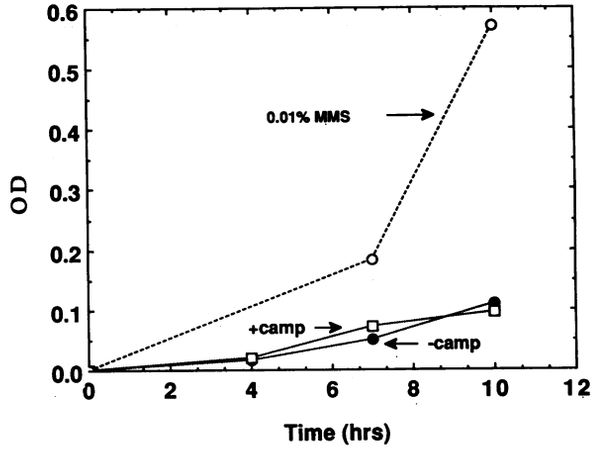

FIG. 4. *DIN3* induction in *top1* cells. JN85t1 cells carrying the *DIN3*::β-galactosidase fusion with an interruption in the *TOP1* gene were assayed for *DIN3* induction as in Fig. 2. Drug concentrations were 50 μg of camptothecin (camp) per ml or 0.01% methyl methanesulfonate (MMS).

advantage of using a genetically tractable model in the dissection of physiologically complex phenomena has been well-recognized. Biochemical studies with mammalian DNA topoisomerase I (10, 11) and the finding that the enzyme purified from a clonal line of human lymphoblastic leukemia cells is resistant to camptothecin (12, 29) have provided strong evidence that DNA topoisomerase I is a cellular target of the alkaloid. It is difficult to show in a mammalian system, however, that the enzyme is the only target of the drug. With the yeast system, experiments with the appropriate mutants show clearly that this is the case. Our results with the yeast mutants demonstrate further a unique aspect of the DNA topoisomerases as targets of therapeutic agents: a nonessential topoisomerase such as yeast DNA topoisomerase I can be the sole target of a cytotoxic drug. In this connection, it is noteworthy that bacterial and viral topoisomerases, whether they are essential or not, could serve as targets of antibiotics and antiviral agents (31–33).

Our results lend further support to the notion that in eukaryotes DNA topoisomerase II is the target of mAMSA. Because of the indispensability of DNA topoisomerase II, whether the enzyme is the sole target of a drug cannot be readily tested by the use of null mutants. The inference is, therefore, based on the differences in the physiological effects of mAMSA and its analog oAMSA. The isolation of drug-resistant *top2* mutants should provide an unequivocal conclusion.

It is interesting that in yeast mutants sensitive to camptothecin and mAMSA, the two drugs elicit similar physiological responses. These similarities could be attributed to the parallel roles of DNA topoisomerases I and II (30) or to the common denominator of the drugs in their trapping of the cleavable complexes. Studies of the topoisomerase-targeting drugs also illustrate clearly that the DNA topoisomerases are an important link between the actions of a diverse spectrum of compounds and the genetic stability of a cell. In mammalian systems, it has been shown that both camptothecin and DNA topoisomerase II-targeting antitumor drugs induce chromosomal aberrations (1–5). The findings discussed in the *Results* section demonstrate that yeast should be a valuable system for the dissection of the mechanisms of processes leading to genetic instability in eukaryotes.

We thank Drs. Craig Giroux, Earl Nestemann, Stephanie Ruby, David Schild, and R. Contoupolou for strains and plasmids. We are grateful to Dr. Leroy Liu for providing some of the drugs used in this work and for communicating results to us prior to publication. This work was supported by grants from the U.S. Public Health Service (GM24544), the American Cancer Society (MV255), and by a postdoctoral fellowship to J.N. from the American Cancer Society.

1. Ross, W. E. (1985) *Biochem. Pharmacol.* **34,** 4191–4195.
2. Chen, G. L. & Liu, L. F. (1986) *Annu. Rep. Med. Chem.* **21,** 257–262.
3. Zwelling, L. A., Silverman, L. & Estey, E. (1986) *Int. J. Radiat. Oncol. Biol. Phys.* **12,** 1041–1047.
4. Potmesil, M. & Ross, W. E. eds. (1987) *Natl. Cancer Inst. Monogr.* **4,** 1–133.
5. Drlica, K. & Franco, R. J. (1988) *Biochemistry* **27,** 2253–2259.
6. Wang, J. C. (1985) *Annu. Rev. Biochem.* **54,** 665–697.
7. Vosberg, H.-P. (1985) *Curr. Top. Microbiol. Immunol.* **114,** 19–102.
8. Wang, J. C. (1987) *Biochim. Biophys. Acta* **909,** 1–9.
9. Drlica, K. (1984) *Microbiol. Rev.* **48,** 273–289.
10. Hsiang, Y.-H., Hertzberg, R., Hecht, S. & Liu, L. R. (1985) *J. Biol. Chem.* **260,** 14873–14878.
11. Hsiang, Y.-H. & Liu, L. R. (1988) *Cancer Res.* **48,** 1722–1726.
12. Andoh, T., Ishii, K., Suzuki, Y., Ikegami, Y., Kusunoki, Y., Takemoto, Y. & Okada, K. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5565–5569.
13. Morita, T. & Yanagihara, Y. (1985) *Chem. Pharm. Bull.* **33,** 1576–1582.
14. Gause, G. F., Laiko, A. V. & Selesneva, T. I. (1976) *Cancer Treatment Rep.* **60,** 637–638.
15. Winsor, B., Potter, A. A., Karst, F., Nestmann, E. R. & LaCroute, F. (1987) *Envir. Mutat.* **9,** 114.
16. Sarsenova, S. Z., Shigaeva, M. K. & Pavlov, Y. I. (1987) *Genetika* **22,** 842–849.
17. Ruby, S., Szostak, J. & Murray, A. W. (1983) *Methods Enzymol.* **101,** 252–269.
18. Ruby, S. & Szostak, J. (1985) *Mol. Cell. Biol.* **5,** 75–84.
19. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Resnick, M. A., Nitiss, J. L., Edwards, C. & Malone, R. (1986) *Genetics* **113,** 531–550.
21. Itoh, H., Fukada, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
22. Thrash, C., Bankier, A. T., Barrell, B. G. & Sternglanz, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4374–4378.
23. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–211.
24. Schild, D., Konforti, B., Perez, C., Gish, W. & Mortimer, R. K. (1983) *Curr. Genet.* **7,** 85–92.
25. Sugino, A., Kojo, H., Greenberg, B., Brown, P. O. & Kim, K. C. (1981) in *The Initiation of DNA Replication*, ed. Ray, D. S. & Fox, C. F. (Academic, New York), pp. 529–553.
26. Wintersberger, U. & Karwan, A. (1987) *Mol. Gen. Genet.* **207,** 320–327.
27. Roman, H. (1956) *Cold Spring Harbor Symp. Quant. Biol.* **21,** 179–183.
28. Rose, M. D., Novick, P., Thomas, J. H., Botstein, D. & Fink, G. R. (1988) *Gene* **60,** 237–243.
29. Kjeldsen, E., Bonven, B., Andoh, T., Ishii, K., Okada, K., Bolund, L. & Westergaard, O. (1988) *J. Biol. Chem.* **263,** 3912–3916.
30. Yanagida, M. & Wang, J. C. (1987) in *Nucleic Acids and Molecular Biology*, eds. Eckstein, M. & Lilley, D. M. J. (Springer, Berlin), Vol. 1, pp. 196–209.
31. Kreuzer, K. N. & Cozzarelli, N. R. (1979) *J. Bacteriol.* **140,** 424–435.
32. Rowe, T. C., Tewey, K. M. & Liu, L. F. (1984) *J. Biol. Chem.* **259,** 9177–9181.
33. Wang, J. C. (1987) *Natl. Cancer Inst. Monogr.* **4,** 3–6.