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The C-Terminal Domain of *Saccharomyces cerevisiae* DNA Topoisomerase II

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A set of carboxy-terminal deletion mutants of *Saccharomyces cerevisiae* DNA topoisomerase II were constructed for studying the functions of the carboxyl domain in vitro and in vivo. The wild-type yeast enzyme is a homodimer with 1,429 amino acid residues in each of the two polypeptides; truncation of the C terminus to Ile-1220 has little effect on the function of the enzyme in vitro or in vivo, whereas truncations extending beyond Gln-1138 yield completely inactive proteins. Several mutant enzymes with C termini in between these two residues were found to be catalytically active but unable to complement a *top2-4* temperature-sensitive mutation. Immunomicroscopy results suggest that the removal of a nuclear localization signal in the C-terminal domain is likely to contribute to the physiological dysfunction of these proteins; the ability of these mutant proteins to relax supercoiled DNA in vivo shows, however, that at least some of the mutant proteins are present in the nuclei in a catalytically active form. In contrast to the ability of the catalytically active mutant proteins to relax supercoiled intracellular DNA, all mutants that do not complement the temperature-dependent lethality and high frequency of chromosomal nondisjunction of *top2-4* were found to lack decatenation activity in vivo. The plausible roles of the DNA topoisomerase II C-terminal domain, in addition to providing a signal for nuclear localization, are discussed in the light of these results.

The DNA topoisomerase II of the budding yeast *Saccharomyces cerevisiae* is a member of the type II DNA topoisomerase family, a group of evolutionarily and structurally related enzymes that catalyze the ATP-dependent transport of one double-stranded DNA segment through another (for recent reviews, see the articles by Caron and Wang [12], Hsieh [31], Maxwell and Gellert [40], and Wang [61]). In vitro, the DNA transport reaction catalyzed by the yeast enzyme is manifested in interconversions between topological isomers of DNA rings: relaxation of positively and negatively supercoiled DNA, catenation and decatenation, and knot formation and removal. In vivo, the enzyme and its homologs have been shown to unlink multiply intertwined pairs of newly replicated DNA rings (18) and to remove positive and negative supercoils generated by transcription (24). Whereas the supercoiling-relaxation function of the enzyme can be carried out by DNA topoisomerase I, the unlinking of multiply intertwined DNA rings or chromosomal loops without nicks or gaps is a unique function of the type II enzyme; thus, inactivation of the type II enzyme invariably leads to cell inviability (25, 59), which is presumably a consequence of the failure of intertwined pairs of chromosomes to segregate properly during mitosis (18, 29, 60).

In *Schizosaccharomyces pombe* cells lacking DNA topoisomerase II, chromosome condensation is also blocked (58). The involvement of the enzyme in chromosome condensation is supported by in vitro experiments with cell extracts (3, 43) and by results on the time dependence of the cellular localization of the enzyme in *Drosophila* embryos undergoing synchronized cell division (7, 56). The precise role of the enzyme in chromosomal condensation, namely, whether the enzyme enables or facilitates the process through its catalysis of DNA breakage, passage, and rejoining or participates as a stoichiometric component of the nucleoprotein product, is not known.

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The results of several studies have implied a structural role of the enzyme in chromosomal organization. The enzyme has been reported to be distributed within an axial core of isolated metaphase (5, 23) and meiotic (35, 41) chromosomes and has been identified as a major component of the nuclear matrix (4, 21). The interpretation of these observations has been open to question, however, and the physiological significance of the association between eukaryotic DNA topoisomerase II and the nuclear matrix or chromosomal scaffold remains uncertain (see, for example, the discussions in the papers by Hirano and Mitchison [28] and Swedlow et al. [56]).

The uncertainties about the in vivo roles of eukaryotic DNA topoisomerase II other than its unlinking of chromosomal pairs and removal of supercoils led us to examine the function of the carboxy-terminal domain of yeast DNA topoisomerase II. This domain contains approximately 250 amino acid residues and is linked to the remainder of the enzyme via a protease-sensitive region (37). There is a corresponding protease-sensitive region in the bacterial DNA gyrase A-subunit, and the amino-terminal portion of the proteolytic product can combine with the bacterial DNA gyrase B-subunit to give a catalytically active enzyme in vitro (47). Recent studies of C-terminal deletion mutants of *Drosophila* DNA topoisomerase II have led to a similar conclusion, that a C-terminal stretch of 240 amino acids of the *Drosophila* enzyme can be removed without loss of catalytic activity (16). Whereas all type II DNA topoisomerases, including T-even phage DNA topoisomerase, bacterial gyrase, eukaryotic DNA topoisomerase II, and African swine fever virus topoisomerase, are homologous, the viral enzymes lack the carboxy-terminal domain of their bacterial and eukaryotic counterparts. There is also little homology within the carboxy-terminal domains between enzymes from organisms that are evolutionarily widely separated or between two homologs from the same organism (for recent sequence alignments, see the articles by Caron and Wang [12, 13]; see also Fig. 1 in this article). The lack of the C-terminal domain in the viral enzymes and its dispensability for reactions catalyzed by bacterial DNA gyrase and *Drosophila* DNA topoisom-

erase II suggest that the carboxy-terminal domain of bacterial and eukaryotic DNA topoisomerase II is not required for its catalytic activity, which in turn raises the question of whether the domain might have a structural role in vivo.

We report here the construction and genetic and biochemical analysis of a set of mutants of *S. cerevisiae* DNA topoisomerase II with increasing sizes of carboxy-terminal deletions. Our results indicate that removal of up to 210 amino acids from the carboxy terminus of the enzyme has little effect on cell viability or enzymatic activities in vitro; that removal of 230 to 260 amino acids severely compromises cell growth, but the truncated proteins remain catalytically active in vitro; and that removal of more than 290 amino acids from the carboxy terminus leads to complete inactivation of the enzyme. Cell inviability appears to correlate with the loss of DNA decatenation activity of the mutant enzyme in vivo. The identification of catalytically active C-terminal deletions of *Drosophila* DNA topoisomerase II that are physiologically nonfunctional when expressed inside yeast cells has recently been reported by Crenshaw and Hsieh (17).

MATERIALS AND METHODS

Yeast strains and plasmids. Strains CH325 (**a** *his4-539 lys2-801 ura3-52 top2-4*), CH1106 (α *ade2-101 Δleu2 lys2-801 Δtrp1 ura3-52 top2-4*), and CH1117 (**a**/α *ade2-101/ade2-101 his3-Δ200/HIS$^+$ leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 trp1-Δ1/trp1-Δ1 ura3-52/ura3-52 top2-4/top2-4* [*CF TRP1$^+$ SUP11*]) were kindly provided by C. Holm, Harvard University (29, 30). Strain SD119 (**a** *ade2 ura3-52 trp1 leu2 his3 his7 can1 top1-1 top2-1*) was kindly provided by R. Sternglanz (6). Strain BCY123 (**a** *can1 ade2 trp1 ura3-52 his3 leu2-3,112 pep4::his3$^+$ prb1::LEU2$^+$ bar1::HISG$^+$ lys2::PGAL1/10-GAL4$^+$*) was obtained from Neal Lue, Harvard University, and strain DY128 (**a**/α *ade1/ADE$^+$ leu2-3,112/leu2-3,112 arg4-17/ARG4$^+$ lys2/LYS2$^+$ ura3/ura3 trp1/trp1 his3-11,15/his3-11,15 TOP2$^+$/Δtop2::LEU2$^+$* [*cir$^+$*]) was kindly provided by Tao-shih Hsieh, Duke University. The construction of strains JCW10 (α *ade2-101 Δleu2 lys2-801 Δtrp1 ura3-52 Δtop1 top2-4*) and JCW16 (**a** *ura3-52 Δtrp1 leu2 top2-4*) has been described previously (22, 48). Plasmid ARS1-LEU2 was derived from a circularized *Eco*RI *TRP1-ARS1* fragment by replacing the *TRP1* gene with the *LEU2$^+$* marker (48a). Plasmid YEp*TOP2*-PGAL1 contains the *S. cerevisiae URA3$^+$* marker as well as a *TOP2$^+$* gene under the control of the *GAL1* promoter (62). A related plasmid, *Sc*topoII-myc, encodes the first 1,334 amino acids of *S. cerevisiae* DNA topoisomerase II with a decapeptide epitope from human c-Myc protein at its C terminus (37). Plasmid YEp-*topA*-PGPD contains the *Escherichia coli topA* gene under the control of the yeast glyceraldehyde-3-phosphate dehydrogenase gene promoter and a *TRP1$^+$* marker (24). Plasmid pRY131-LEU2, which contains the *LEU2$^+$* marker and the *E. coli* β-galactosidase gene under the control of the yeast *GAL1* promoter, was constructed by inserting an *Xho*I-*Sal*I restriction fragment containing *LEU2$^+$* and deleting a *Stu*I-*Stu*I fragment of pRY131 to destroy the *URA3$^+$* marker (63). Plasmid pBluescript SK− was purchased from Stratagene.

Mutant construction. Deletion mutations *top2*(1–1180) and *top2*(1–1195) were created by using exonuclease III to remove nucleotides from the 3′ ends of *Xho*I-digested plasmid *Sc*topoII-myc, S1 nuclease digestion of the 5′ overhangs, and DNA repair synthesis with the Klenow fragment of *E. coli* DNA polymerase I. This shortened DNA was then ligated to the *Sma*I site of plasmid *Sc*topoII-myc in the presence of an oligonucleotide linker, 5′-CTAGACTAGTCTAG, which contains stop codons in all three frames. After digestion with *Kpn*I and religation, the DNA was used to transform competent *E. coli* cells; individual transformants were examined by restriction mapping and then nucleotide sequencing. A second set of mutations, *top2*(1–1137), *top2*(1–1171), *top2*(1–1208), and *top2*(1–1220), were made by using Bal31 nuclease instead of the exonuclease III-S1 nuclease combination; after the addition of *Sal*I linkers (GGTCGACC) to the shortened pool of DNA, the *Kpn*I-*Sal*I fragment was used to replace the *Kpn*I-*Xho*I fragment in *Sc*topoII-myc. Mutations *top2*(1–1109) and *top2*(1–1166) were created by introducing a termination codon and *Spe*I restriction site by PCR with 5′-GACTAGTACG TATTCTTCAGGACCATTTATAAC and 5′-GACTAGTAC TATCCCACCTCAAAAGCCTT, respectively, as the downstream primer and 5′-ACAATACGTACCTGGTAC as the upstream primer (all oligonucleotides were purchased from Operon). The *Nsi*I-*Spe*I fragment from each reaction was used to replace the *Nsi*I-*Nhe*I fragment of YEp*TOP2*-PGAL1, within which the 3′ end of the *top2* gene lies. Nucleotide sequencing of the entire *Nsi*I-*Spe*I region in the clones was carried out to confirm that the clones contained the intended changes. The coding region of *top2*(1–1334) is identical to the corresponding region in *Sc*topoII-myc described previously (37).

top2(1–1166) was overexpressed by galactose induction, and the Top2(1–1166) protein was purified essentially as described before (62) except that strain BCY123 was used. Extracts of galactose-induced cells expressing several other *GAL1* promoter-linked *top2* mutations were also examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and there was no evidence that the truncated proteins were unstable, as judged from the relative yields of the overexpressed mutant yeast DNA topoisomerase II and of the wild-type enzyme. Highly purified wild-type yeast DNA topoisomerase II was generously provided by James Berger of this laboratory.

Specific activity determination. The DNA strand passage-specific activity was determined as described previously (38), with ^{32}P-labeled pBluescript SK− DNA as the substrate. The labeled DNA was treated with vaccinia virus DNA topoisomerase I (generously provided by Ryo Hanai of our laboratory) in the presence of a predetermined amount of ethidium bromide; removal of the intercalating agent was subsequently carried out to yield a negatively supercoiled DNA substrate with the desired specific linking difference. The topoisomerase-catalyzed change in the linking number of DNA, in a buffer containing 50 mM Tris-HCl (pH 7.6), 120 mM KCl, 6 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 250 μg of bovine serum albumin per ml, and 10 μM ATP, was determined as a function of time from the topoisomer distributions of the radiolabeled DNA samples, as described by Lindsley and Wang (38). The fraction of active enzyme molecules was estimated from the fraction of relaxation of a known concentration of supercoiled DNA under conditions in which the enzyme acts processively (38). The sample of purified wild-type enzyme used in the experiments reported here was estimated to be about 70% active, and Top2(1–1166) was estimated to be nearly 100% active.

Immunofluorescence. The immunofluorescence assay of human c-Myc decapeptide-tagged Top2(1–1334) and Top2(1–1166) protein in strain CH325 was performed as described below with affinity-purified monoclonal antibody MYC1-9E10.2. Cells were grown in 2% raffinose to the logarithmic phase, and galactose was then added to 2% for 3 h at 26°C. Cells were harvested and converted to spheroplasts, and fixation and immunostaining were then carried out by published procedures (46). Nuclei were stained with the DNA dye Hoeschst 33258, and the secondary antibody for the detection

of monoclonal antibody MYC1-9E10.2 was fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin.

In vivo DNA relaxation. In vivo DNA relaxation activity in yeast cells was assayed by taking advantage of the accumulation of positive supercoils when *E. coli* DNA topoisomerase I is expressed in a yeast Δ*top1 top2-4* strain. At a nonpermissive temperature for *top2-4* mutants, 35°C, the *E. coli* enzyme becomes the only active relaxation activity, and positive supercoils accumulate in intracellular plasmids because of the generation of both positively and negatively supercoiled domains by transcription and the preferential removal of negative supercoils by the *E. coli* enzyme (24). The expression of an exogenous topoisomerase that can relax positive supercoils at 35°C would thus prevent the accumulation of positive supercoils in this strain. Cells bearing the plasmid expressing the *E. coli* enzyme and a second plasmid expressing a *top2* C-terminal deletion mutation or the wild-type *TOP2*$^+$ gene or its null control were grown in either 2% dextrose or 2% raffinose until the logarithmic phase at 26°C and then shifted to 35°C for 2 h. Cells were either lysed immediately by the phenol-glass bead method (11) or treated first with a toluene-ethanol-Tris buffer cocktail (32). Spheroplasts were then prepared from the cells for DNA isolation (44). Two-dimensional electrophoresis of the DNA was performed in 0.7% agarose–0.1 M Tris-borate–2 mM EDTA (pH 8.3) with 0.6 μg of chloroquine diphosphate (Sigma) per ml in the first dimension and in the same buffer with 3 μg of chloroquine diphosphate per ml in the second dimension. After electrophoresis, the DNA was transferred to a GeneScreen membrane (DuPont) and probed for the yeast endogenous 2μm plasmid with a ^{32}P-labeled probe prepared from a fragment of the 2μm *FLP* gene by the use of a random-priming kit (Stratagene). Hybridization and washing were performed as described before (14).

In vivo DNA decatenation. JCW16 (*top2-4*) cells containing the ARS1-LEU2 plasmid and various topoisomerase II mutations were grown to the logarithmic phase at 26°C and then treated with 10 μg of α-factor (Sigma) per ml for 3 h at 26°C. Cells were collected by filtration and washed with and then resuspended in fresh medium at 35°C to effect synchronized growth. After various lengths of time, cells were harvested, and DNA was prepared and analyzed by two-dimensional electrophoresis as described above except that the electrophoresis buffers were 0.1 M Tris-borate–2 mM EDTA, pH 8.3, in the first dimension and the same buffer plus ethidium bromide (10 μg/ml) in the second dimension. After electrophoresis, the DNA was transferred to a membrane, and the membrane-bound DNA was subjected to hybridization and washing as described above with a radiolabeled *LEU2* gene probe.

β-Galactosidase assays. Extracts of JCW10 (Δ*top1 top2-4*) cells harboring pRY131-*LEU2*, YEp*topA*-PGPD, and a derivative of YEp*TOP2*-PGAL1 encoding one of the *top2* mutant genes were used in β-galactosidase assays. Cells were grown at 26°C in medium lacking uracil, tryptophan, and leucine and containing 2% raffinose until the logarithmic phase. The cultures were then split into two: one portion was shifted to 35°C, and the other was kept at 26°C. After 1 h, galactose was added to each culture to 2%, and growth of the cultures was continued for 4 h at the respective temperatures. Cells were then harvested for assays of β-galactosidase activity as described before (22, 63).

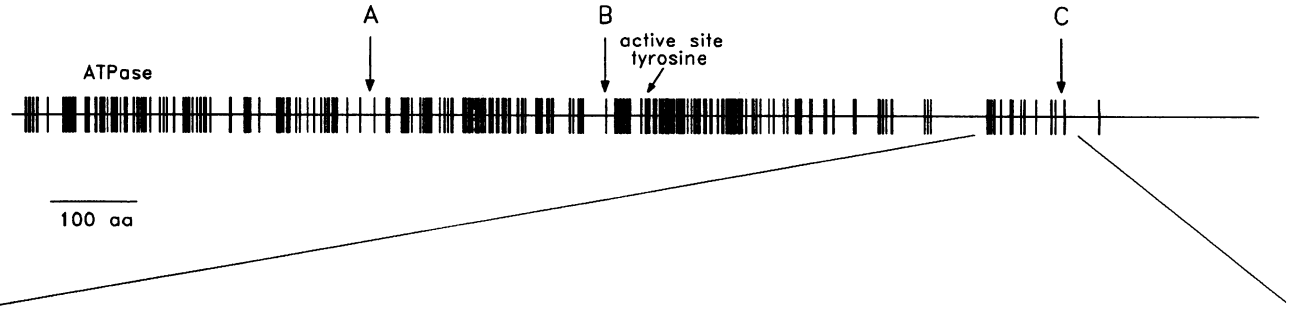
Nondisjunction. Chromosomal nondisjunction frequency was assayed by determining the frequency of loss of a chromosome VII fragment containing the *SUP11* gene in diploid strain CH1117 carrying the *top2-4* as well as the *ade2-101* mutation (30). Cells which contain one copy of the *SUP11* gene form pink colonies, whereas those that have undergone a segregation event to acquire two copies or no copy of *sup11* form white- and red-sectored colonies. Cells were grown at 26°C to the logarithmic phase and then shifted to 35°C for 4 h; appropriate dilutions were plated, and plates were incubated at 26°C for several days. The number of half-sectored white-red colonies and the total number of colonies were scored.

RESULTS

Mutant construction and characterization of the functionality of mutant proteins in vivo and in vitro. The nucleotide sequences of the 3′ termini of the set of *S. cerevisiae* DNA topoisomerase II deletion mutations constructed for the present studies were determined, and the amino acid sequences deduced from them are shown in Fig. 1. The mutations are denoted by their coding regions; *top2*(1–1208), for example, is one in which codons 1209 to 1429 of the wild-type *top2* gene have been deleted. A temperature-sensitive yeast DNA topoisomerase II mutant, CH325 (*top2-4*), was transformed separately with plasmids expressing wild-type DNA topoisomerase II and its C-terminal truncation derivatives from the inducible promoter *PGAL1*. At the restrictive temperature, 35°C, the presence of a plasmid-borne wild-type *TOP2*$^+$ gene complements growth even in medium containing glucose, in which there is only leaky transcription of the gene linked to the glucose-repressible *GAL1* promoter. The presence of plasmid-borne *PGAL1*-linked mutant genes *top2*(1–1208), *top2*(1–1220), and *top2*(1–1334) also complements CH325 (*top2-4*) well under repressed conditions for *PGAL1*. Homologous plasmids carrying *top2*(1–1109), *top2*(1–1137), *top2*(1–1166), *top2*(1–1171), *top2*(1–1180), or *top2*(1–1195), on the other hand, fail to complement the *top2-4* lethality at 35°C in glucose medium.

To investigate whether lethality of these transformant mutations could be overcome by increasing the expression of the mutant proteins, complementation in raffinose medium, in which the *GAL1* promoter is neither repressed nor fully induced and thus expression of the *GAL1* promoter-linked *top2* gene is expected to be higher than in glucose medium, was examined. Cell viability at the restrictive temperature was slightly increased in transformants expressing *top2*(1–1171), *top2*(1–1180), *top2*(1–1195), and *top2*(1–1208). However, only *top2*(1–1208) resulted in growth at the restrictive temperature which resembled that of *TOP2*$^+$ wild-type cells; the other constructs resulted in the formation of much smaller colonies. Quantitative measures of the viability of cells harboring these constructs showed no significant increase in the number of viable cells at the restrictive temperature when the cells were grown in raffinose medium. Transformants expressing *top2*(1–1109), *top2*(1–1137), and *top2*(1–1166) remain inviable at 35°C in raffinose medium. These results are summarized in Table 1. For a number of the deletion mutants, complementation was also tested with strain SD119 (*top2-1*), with results identical to those obtained with CH325 (*top2-4*).

To eliminate the possibility that the presence of the *top2-4* or *top2-1* product might complicate the complementation results, additional experiments were carried out with transformants of a diploid strain, DY128 (**a**/α *ade1/ADE*$^+$ *leu2-3,112/leu2-3,112 arg4-17/ARG4*$^+$ *lys2/LYS2*$^+$ *ura3/ura3 trp1/trp1 his3-11,15/his3-11,15 TOP2*$^+$/Δ*top2::LEU2*$^+$ [*cir*$^+$]), expressing either plasmid-borne C-terminal *top2* truncations or YEp24 as a null control. Transformed cells were allowed to sporulate, and the tetrads were dissected. As expected from the essentiality of the *TOP2* gene, spores from the null control showed predominantly a $2^+:2^0$ viable-nonviable segregation pattern and no $3^+:1^0$ or $4^+:0^0$ segregants. None of the viable spores carried the *LEU2*$^+$

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TCTGATGAAGAAGATGAAGAAAGTTCACACGAAGATACTGAAAATGTTATAAATGGTCCTGAAGAATAG  Top2(1-1109)
S  D  E  E  D  E  E  S  S  H  E  D  T  E  N  V  I  N  G  P  E  E  *

CAAAAGCTGTTGAAACAAAAAGGTCGAGGCATGGAGCAAAAGCTCATTTCTGAAGAGGACTTGAATTAA  Top2(1-1137)
Q  K  L  L  K  Q  K  G  R  G  M  E  Q  K  L  I  S  E  E  D  L  N  *

GAAAACTTGTTAAAACTTTCCGCGAAAGATATATGGAACACTGACTTGAAGGCTTTTGAGGTGGGATAG  Top2(1-1166)
E  N  L  L  K  L  S  A  K  D  I  W  N  T  D  L  K  A  F  E  V  G  *

GTGGGATATCAAGAATTTTTGGGTCGAGGCATGGAGCAAAAGCTCATTTCTGAAGAGGACTTGAATTAA  Top2(1-1171)
V  G  Y  Q  E  F  L  G  R  G  M  E  Q  K  L  I  S  E  E  D  L  N  *

GACTTGAAGGCTTTTGAGGTGGGATATCAAGAATTTTTGCAACGAGATGCAGAAGCTCGCGGTGGGTAA  Top2(1-1180)
D  L  K  A  F  E  V  G  Y  Q  E  F  L  Q  R  D  A  E  A  R  G  G  *

AATAAAGGGAGCAAAACGAAAGGTAAAGGAAAAAGGGTAATAACTGATATAATTAAATTGAAGCTCTAA  Top2(1-1195)
N  K  G  S  K  T  K  G  K  G  K  R  V  I  T  D  I  I  K  L  K  L  *

CTTGTTGACGACGAAGACTACGACCCATCAAAAAAAAAGGTCGAGGCATGGAGCAAAAGCTCATTTCTGA  Top2(1-1208)
L  V  D  D  E  D  Y  D  P  S  K  K  R  S  R  H  G  A  K  A  H  F  *

GACGACGAAGACTACGACCCATCAAAAAAAAACAAGAAAAGTACTGCTAGAAAGGGCAAAAAAATCTAG  Top2(1-1220)
D  D  E  D  Y  D  P  S  K  K  N  K  K  S  T  A  R  K  G  K  K  I  *
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FIG. 1. Diagram of amino acid (aa) sequences of *S. cerevisiae* DNA topoisomerase II and the carboxy-terminal deletion mutants of it constructed in this work. Vertical bars in the top illustration represent positions in the *S. cerevisiae* sequence which are at least 75% conserved in a selected subset of DNA topoisomerase II sequences from various species, including human (α and β variant enzymes), *D. melanogaster*, *Caenorhabditis elegans*, *S. cerevisiae*, *S. pombe*, African swine fever virus, bacteriophage T4, *E. coli* DNA gyrase, and *E. coli* DNA topoisomerase IV (13). Protease-sensitive sites are labeled A, B, and C as in reference 37. The C-terminal amino acid sequences of various deletion constructs in the region between amino acids 1109 and 1220 are shown under the top alignment. Residues in boldface type are those normally present in the wild-type enzyme; underlined residues correspond to the human c-Myc epitope tag; other amino acids not in boldface type were introduced in the construction of these deletions.

marker, which had replaced a segment of the *TOP2*$^+$ gene in the Δ*top2*::*LEU2*$^+$ strain. On dextrose plates, spores derived from transformants harboring plasmids expressing *top2*(1–1137), *top2*(1–1166), *top2*(1–1171), *top2*(1–1195), or *top2*(1–1208) from a *GAL1* promoter showed segregation patterns identical to that of the null control, demonstrating that the expression of these *top2* truncations cannot restore viability to the Δ*top2* cells. Spores of transformants expressing *top2*(1–1220) or wild-type *TOP2*$^+$, on the other hand, showed an abundance of $4^+:0^0$ segregants on dextrose plates. All spores that were *LEU2*$^+$, signifying inheritance of the chromosomal Δ*top2*, were found to carry the plasmid-borne *URA3*$^+$ marker, signifying the presence of the plasmid-borne *top2*. Spores from transformants expressing *top2*(1–1195), *top2*(1–1208), and *top2*(1–1220) were also plated on raffinose plates, and the results were the same as those obtained on dextrose plates. These results are in agreement with those of the genetic complementation experiments with the temperature-sensitive *top2* strains except that *top2*(1–1208) appeared to be ineffective in complementing the Δ*top2* segregants of strain DY128.

In combination, the three sets of complementation experiments with *top2-4*, *top2-1*, and Δ*top2* mutant strains place the effective *TOP2*$^+$ endpoint in the region between codons 1170 and 1220: mutants carrying C-terminal truncation mutations ending before the codon for amino acid 1220 are viable, and those with truncation mutations ending beyond codon 1170 are nonviable. Mutants carrying truncated mutations whose endpoints fall within this region may be viable or nonviable, depending on the growth medium and strain background.

In addition to viability tests, two sets of experiments were carried out to assess the functionality of the mutant enzyme in vivo. In one, the cellular level of β-galactosidase was measured for Δ*top1 top2-4* yeast cells harboring three plasmids: one expresses *E. coli* β-galactosidase from the yeast *GAL1* gene promoter, one constitutively expresses *E. coli* DNA topoisomerase I, and the third expresses a wild-type or C-terminal deletion yeast DNA topoisomerase II. As shown by Gartenberg and Wang (22), in the absence of the third plasmid, β-galactosidase expression is much diminished by inactivation of the enzyme encoded by the *top2-4* gene at 35°C. This reduction has been attributed to the accumulation of positive supercoils in the β-galactosidase gene-carrying plasmid under the experimental conditions, which interfere with transcription. With YEp*TOP2*-PGAL1, expressing wild-type *TOP2*$^+$, as

TABLE 1. Properties of wild-type *S. cerevisiae* DNA topoisomerase II and its C-terminal truncation derivatives in vivo and in vitro

top2 truncation endpoint (codon)	Complementation[a]		Nondisjunction suppression	β-Galactosidase activity ratio, 35°C/26°C	Decatenation		Relaxation	
	2% dextrose	2% raffinose			In vivo	In vitro[b]	In vivo	In vitro[c]
None[d]	−	−	−	0.15	−	−	−	ND[e]
1109	−	−	−	0.14	−	−	−	ND
1137	−	−	ND	ND	ND	−	ND	ND
1166	−	−	−	0.43	−	+	+	0.23
1171	−	±	ND	ND	ND	+	ND	ND
1180	−	±	ND	ND	−	+	ND	ND
1195	−	±	ND	ND	±	+	ND	ND
1208	+	++	ND	ND	ND	+	ND	ND
1220	++	++	ND	ND	+	ND	ND	ND
1334	++	++	ND	ND	ND	+	ND	ND
1429	++	++	+	0.71	++	+	+	0.22

[a] ++, complementation similar to wild-type *TOP2*; +, reduced growth rate; ±, minute colonies.
[b] Decatenation of kinetoplast DNA by crude extracts of cells expressing the Top2 proteins.
[c] Specific activity of the purified protein, in events of DNA passage per dimeric enzyme per second.
[d] Assays done with transformants of YEp24, which does not encode *TOP2*.
[e] ND, not determined.

the third plasmid, the ratio of β-galactosidase activity at 35°C to that at 26°C was found to be 0.74; with YEp24, which lacks *TOP2*$^+$ completely, as the third plasmid, the ratio dropped to 0.15, in agreement with previous results (22). When the wild-type *TOP2* in YEp*TOP2*-PGAL1 was replaced by *top2*(1–1109) or *top2*(1–1166), the ratio was found to be 0.14 and 0.43, respectively. Thus, it appears that the Top2(1–1109) protein is inactive but the Top2(1–1166) protein has at least some DNA relaxation activity inside yeast nuclei, even though its expression is insufficient to restore viability of yeast *top2-4* mutant cells at 35°C.

In the second set of experiments, the effects of the Top2(1–1109) and Top2(1–1166) proteins on chromosome nondisjunction in diploid yeast strain CH1117 (**a**/α *ade2-101/ade2-101 his3-Δ200/HIS*$^+$ *leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 trp1-Δ1/trp1-Δ1 ura3-52/ura3-52 top2-4/top2-4*, plus a chromosome VII fragment bearing *TRP1*$^+$ and *SUP11*) were examined. In this diploid strain, the presence of a single copy of the chromosomal fragment bearing the *SUP11* suppressor permits the scoring of chromosome nondisjunction by the segregation of the *SUP11* gene: cells with one copy of *SUP11* form pink colonies, and cells which have undergone nondisjunction form white- and red-sectored colonies because of the dependence of the degree of suppression of the *ade2-101* mutation on the copy number of *SUP11* (27, 30). In CH1117 cells harboring YEp*TOP2*-PGAL1, the frequencies of half-sectored colony formation were found to be 1.4×10^{-5} for cells grown at 26°C and 1.9×10^{-5} for the same cells subjected to a 4-h incubation at 35°C before plating and incubation again at 26°C. CH1117 cells harboring the control plasmid YEp24, on the other hand, showed a 30-fold increase in the number of sectored colonies in cells with a 4-h exposure to 35°C relative to cells not subjected to the temperature shift; the frequencies with and without incubation at 35°C were found to be 110×10^{-5} and 3.4×10^{-5}, respectively. Similarly, replacing the plasmid-borne *TOP2*$^+$ with *top2*(1–1109) or *top2*(1–1166) greatly increases the number of sectored colonies in cells subjected to incubation at 35°C relative to that in cells grown at a constant temperature of 26°C: the frequencies scored were 640×10^{-5} with the 35°C exposure and 6.8×10^{-5} without it for *top2*(1–1109) and 130×10^{-5} with the exposure and 3.6×10^{-5} without it for *top2*(1–1166).

These results indicate that expression of either *top2*(1–1109) or *top2*(1–1166) has little effect on chromosome disjunction after inactivation of the yeast DNA topoisomerase II encoded by the chromosomal *top2-4* gene, which in turn suggests that neither mutant protein can unlink intertwined pairs of chromosomes during mitosis (29, 30). In the same series of experiments, no increase in the formation of pink-red-sectored colonies resulting from underreplication of the chromosomal fragment bearing *sup11* was observed. Assays of chromosome loss in a *top2-4/top2-4 his4-539/HIS4*$^+$ *LEU2/leu2-Δ1* diploid strain derived by mating strains CH325 and CH1106, in which one chromosome 3 carries a *his4* mutation and the other carries a *leu2* mutation, further demonstrates that expression of a plasmid-borne *top2*(1–1166) mutant gene in *top2-4* mutant cells fails to reduce the high loss rate of 1 to 2% for either marker, whereas expression of a plasmid-borne wild-type *TOP2* gene in the same cells reduces the loss rate to below 0.1%.

Activities of C-terminal truncation mutants of yeast DNA topoisomerase II in vitro. When cells harboring various plasmids carrying *PGAL1*-linked *top2* deletions were exposed to galactose, increases in the amounts of the proteins encoded by the *PGAL1*-linked genes were readily detected in cell extracts by the use of antibodies specific to yeast DNA topoisomerase II (data not shown). Assays of cell extracts for ATP-dependent DNA topoisomerase II activity, which unties knotted phage P4 DNA or unlinks the catenated DNA rings in kinetoplast DNA, showed that proteins encoded by mutant *top2* genes carrying the first 1,166 or more codons of the *TOP2* gene were active and those encoded by *top2*(1–1109) and *top2*(1–1137) were inactive (Table 1).

To test whether there were quantitative differences between active enzymes with truncated C-terminal ends and wild-type yeast DNA topoisomerase II, the Top2(1–1166) protein, which is the smallest of the active mutant proteins studied, was purified. The turnover numbers for DNA passage were then measured for the purified mutant as well as the wild-type enzyme by the procedure of Lindsley and Wang (38). These results are also tabulated in Table 1 together with the complementation results and the results of activity assays of cell extracts. Clearly, purified Top2(1–1166) enzyme can catalyze the ATP-dependent transport of one duplex DNA through another at a rate indistinguishable from that of the wild-type enzyme.

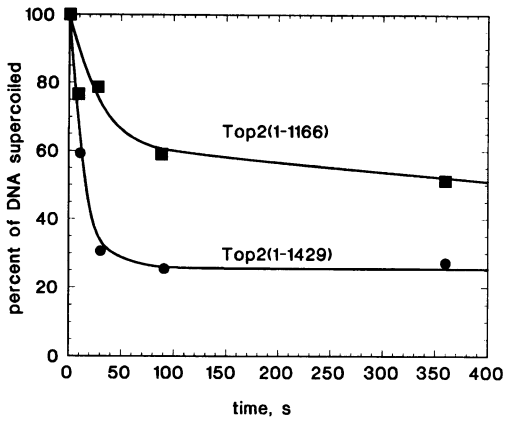

FIG. 2. Processivity of yeast Top2(1–1166) and wild-type DNA topoisomerase II. DNA relaxation experiments were performed at 30°C in a low-salt buffer containing 50 mM Tris-HCl (pH 7.6), 50 mM KCl, 6 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 250 μg of bovine serum albumin per ml, and 1.25 nM ^{32}P-labeled pBluescript SK− DNA and 0.9 nM active enzyme dimers. Reactions were started by the addition of ATP to 10 μM and excess unlabeled pBluescript DNA SK− DNA to 125 nM. Aliquots were withdrawn at various times thereafter and quenched, and DNA topoisomers in each sample were resolved by agarose gel electrophoresis. The amount of supercoiled substrate remaining in each sample is plotted as a function of time after the addition of ATP and excess unlabeled DNA. See text for details.

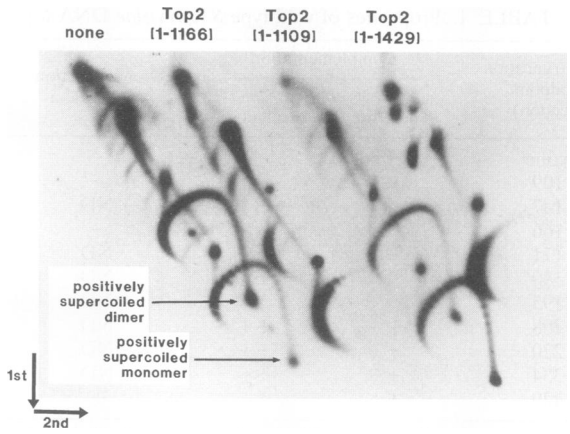

FIG. 3. Relaxation of positively supercoiled 2μm DNA in yeast Δ*top1 top2-4* cells expressing *E. coli* DNA topoisomerase I. DNA was isolated from cells after 2 h at the restrictive temperature of 35°C and resolved by two-dimensional agarose gel electrophoresis with 0.6 μg of chloroquine per ml in the first (vertical) dimension and 3 μg of chloroquine per ml in the second (horizontal) dimension. DNA was transferred from the gel to a nylon membrane and hybridized with ^{32}P-labeled 2μm DNA sequences. The lane labeled none contained DNA from cells bearing YEp24 in addition to the plasmid expressing the *E. coli* enzyme; positive supercoiling of the endogenous 2μm DNA was readily detectable by the appearance of the coalesced spots indicated in the figure. When YEp24 was replaced by YEp*top2*(1–1166)-PGAL1, no accumulation of positive supercoils was detectable. Similar experiments were done with cells harboring YEp*top2*(1–1109)-PGAL1 and YEp*TOP2*-PGAL1 [labeled Top2(1–1429)]. In the particular isolate picked for the experiment shown in the rightmost lane, the 2μm plasmid was present predominantly as dimers.

Whereas the turnover numbers for DNA relaxation reaction are the same for the wild-type and Top2(1–1166) enzymes, the processivity of the mutant enzyme was found to be lower than that of the wild-type enzyme under the assay conditions employed. In the experiment illustrated in Fig. 2, approximately 0.9 nM active wild-type or mutant enzyme was first incubated with 1.25 nM ^{32}P-labeled supercoiled plasmid DNA in assay medium without ATP for 10 min at 30°C. A mixture of ATP and excess unlabeled supercoiled DNA was then added at time zero to give a final ATP concentration of 10 μM and a DNA concentration of about 125 nM. Samples were withdrawn from each reaction mixture and quenched by the addition of sodium dodecyl sulfate and EDTA at various times, and the fraction of labeled DNA that remained supercoiled in each sample was determined after agarose gel electrophoresis to resolve different forms of DNA. In such an experiment, if the enzyme is strictly processive, the reduction of the labeled supercoiled DNA would follow a time course dictated by the turnover number of the enzyme and the distribution of enzyme molecules on the labeled DNA molecules prior to the addition of the unlabeled DNA. If, on the other hand, the enzyme dissociates rapidly from its original substrate, then the labeled DNA would rapidly lose its bound enzyme after the addition of ATP and excess DNA, as rebinding of a dissociated enzyme to a labeled DNA is negligible. In the latter case, nearly 100% of the labeled DNA should remain supercoiled. From the curves shown in Fig. 2, it is readily seen that the Top2(1–1166) mutant enzyme is less processive than the wild-type enzyme. This finding is similar to an earlier observation, that the removal of a C-terminal portion of the A-subunit of *E. coli* DNA gyrase decreases its processivity (47).

Relaxation and decatenation activity of yeast DNA topoisomerase II C-terminal deletion mutants in vivo. As described earlier, intracellular plasmids accumulate positive supercoils in temperature-sensitive yeast Δ*top1 top2-4* cells expressing *E. coli* DNA topoisomerase I after thermal inactivation of the temperature-sensitive *top2-4* mutation-encoded DNA topoisomerase II, and this accumulation should be preventable by the expression of an additional activity capable of relaxing positive supercoils. The results shown in Fig. 3 indicate that the introduction of a multicopy plasmid bearing a *PGAL1*-linked *top2*(1–1166) mutation into strain JCW10 (Δ*top1 top2-4*) cells expressing *E. coli* DNA topoisomerase I prevents the accumulation of positive supercoils in the endogenous 2μm plasmid at 35°C, even when cells are grown in a glucose medium, in which only leaky expression of the *PGAL1*-linked *top2*(1–1166) mutation occurs. This finding provides direct evidence that there is substantial DNA relaxation activity at 35°C in cells expressing *top2*(1–1166), in support of the previous conclusion inferred from the intracellular β-galactoside level measurements. The data shown in Fig. 3 also indicate that expression of *top2*(1–1109) instead of *top2*(1–1166) in the same cells has no effect on the accumulation of positive supercoils at 35°C, confirming the inactivity of the Top2(1–1109) mutant protein.

We have also examined the ability of Top2(1–1109), Top2(1–1166), Top2(1–1180), Top2(1–1195), and Top2(1–1220) mutant proteins and wild-type yeast DNA topoisomerase II to unlink multiply intertwined pairs of plasmid molecules in vivo. The growth of strain JCW16 (*top2-4*) cells harboring an ARS1-LEU2 test plasmid and of YEp*TOP2*-PGAL1 or one of its homologs, expressing a *top2* C-terminal deletion, was arrested by treatment with α-factor, and synchronized growth was started at 35°C after removal of the mating pheromone. As illustrated in Fig. 4, in cells expressing a plasmid-borne wild-type *TOP2*$^+$ gene, most of the ARS1-LEU2 plasmid is in the form of monomeric rings 4 h after release from α-factor arrest; in cells expressing no plasmid-borne *TOP2*$^+$ or plasmid-borne *top2*(1–1166) or *top2*(1–1109) gene, however, a large fraction of the ARS1-LEU2 plasmid migrated on the two-dimensional

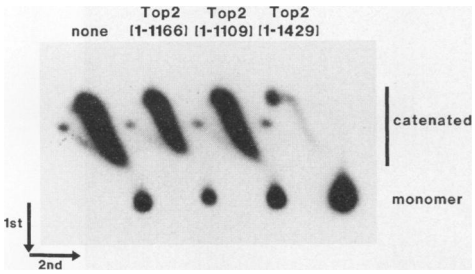

FIG. 4. In vivo decatenation of newly replicated ARS1-LEU2 plasmid DNA. DNA was isolated 4 h after release at 35°C from α-factor-arrested JCW16 (*top2-4*) cells harboring the ARS1-LEU2 test plasmid plus a second plasmid specified above each lane. DNA topoisomers were resolved by two-dimensional gel electrophoresis, with no ethidium bromide in the first (vertical) dimension and 10 μg of ethidium bromide per ml in the second dimension. After transfer of the DNA to a nylon membrane, a ^{32}P-labeled *LEU2*-specific probe was used to detect the test plasmid. Catenated DNA runs as a streak behind monomer DNA. DNA from cells bearing YEp24 (lane labeled none) was mostly in the catenated form because of the lack of DNA topoisomerase II activity at 35°C. In the presence of YEp*TOP2*-PGAL1, expressing wild-type DNA topoisomerase II (rightmost lane), the majority of the ARS1-LEU2 plasmid DNA remained as monomer DNA rings at 35°C.

gel as multiply intertwined catenated dimers (34b, 52). The time dependence of the amount of catenated dimers accumulated after the cells are released from α-factor arrest is shown in Table 2. Under the growth conditions employed, cell doubling time is about 3 h, and the surge in the amounts of catenated dimers in cells lacking a functional DNA topoisomerase II after more than 2 h was anticipated.

Careful examination of the two-dimensional electrophoretic patterns reveals that the tips of the streaks of samples from cells harboring YEp24 and cells harboring YEp*top2*(1–1109)-PGAL1 (Fig. 4) extend downward farther than that of the sample from cells harboring YEp*top2*(1–1166)-PGAL1 (Fig. 4). As the tip of the catenated dimer streak in each DNA sample contains the most highly intertwined catenanes (34b, 52), it appears that cells expressing *top2*(1–1166) can reduce the number of intertwines between a pair of multiply catenated DNA rings but are incapable of achieving their complete separation. Overexpression of *top2*(1–1166) caused by growing the cells in raffinose or galactose medium did not alter the appearance of the streak of catenated dimers, however. Results similar to those obtained with *top2*(1–1166) were found with the *top2*(1–1180) and *top2*(1–1195) mutations; *top2*(1–1220), on the other hand, was found to be close to wild-type *TOP2* in decatenation (data not shown).

TABLE 2. Catenated dimer formation in yeast cells expressing various DNA topoisomerase II mutants[a]

Plasmid	Relative amount of dimeric catenanes (%) at:				
	0 h	0.5 h	1 h	2 h	4 h
YEp24	16	17	13	33	78
YEp*top2*(1–1109)-PGAL1	7	8	9	23	79
YEp*top2*(1–1166)-PGAL1	15	15	25	24	83
YEp*top2*(1–1429)-PGAL1	14	14	14	17	6

[a] Time dependence of catenated dimer formation in yeast strain JCW16 (*top2-4*) harboring test plasmid ARS1-LEU2 and the plasmid indicated. Time zero refers to the time at which cells were released from α-factor arrest and grown at 35°C, a nonpermissive temperature for the chromosomal *top2-4* allele. The relative amounts of dimeric catenanes of the ARS1-LEU2 test plasmid were quantitated from phosphor images of the samples and expressed as a percentage: (amount of dimeric catenanes/amount of dimeric catenanes plus monomeric rings) × 100. YEp24 was used as a negative control.

Effect of C-terminal deletions on cellular localization of *S. cerevisiae* DNA topoisomerase II. The cellular location of yeast DNA topoisomerase II with various portions of the C-terminal domain deleted was assessed qualitatively by immunofluorescence microscopy. In the experiments whose results are shown in Fig. 5, transformants of yeast strain CH325 cells overexpressing Top2(1–1334) or Top2(1–1171), each with a decapeptide immunotag at the carboxyl end of the polypeptide chain, were harvested after induction of the plasmid-borne *PGAL1*-linked *top2* gene in galactose medium, and spheroplasts were prepared from the cells. After fixation, the spheroplasts were processed for immunomicroscopy with a mouse monoclonal antibody specific to the decapeptide tag as the primary antibody and fluorescein-conjugated secondary antibodies for detection by fluorescence microscopy. Whereas Top2(1–1334) is preferentially located in the cell nucleus (Fig. 5B), Top2(1–1171) is abundant in the cytoplasm (Fig. 5A). This difference in the cellular localization of the two proteins was also confirmed by staining with Hoechst dye 33258 to highlight the DNA-containing nuclei (results not shown). For cells overexpressing Top2(1–1334), the nuclei appear to assume irregular shapes, in contrast to the round shape commonly seen in wild-type yeast cells. The reason for this irregular shape is obscure; we note that expression of a high level of wild-type DNA topoisomerase II in a budding yeast is known to be cytotoxic (25, 62).

DISCUSSION

Our dissection of the C-terminal region of DNA topoisomerase II of the budding yeast *S. cerevisiae* has identified several regions demarcated by the positions shown in Fig. 6. Removal of 209 amino acids, up to Ile-1220, has little effect on the function of the enzyme in vivo or in vitro. The dispensability of a large stretch of the C-terminal domain has also been observed for the DNA topoisomerase II of *S. pombe* (50), *Drosophila melanogaster* (16, 17), and mammalian DNA topoisomerase IIα (34a). C-terminal deletions of more than 210 amino acids of the *S. cerevisiae* enzyme show a graded deficiency in complementing temperature-sensitive *top2* mutant strains at the nonpermissive temperatures. Proteins with C-terminal deletions extending to Gly-1166 remain catalytically active in vitro, however; removal of a longer region, to Lys-1137, yields an inactive product. The region between Lys-1137 and Gly-1166 of the *S. cerevisiae* enzyme may contain a part of the polypeptide that is involved in its dimerization to form the active enzyme, as implicated from analysis of the primary sequences of the type II DNA topoisomerases (12); several temperature-sensitive yeast *top2* mutations have also been mapped to this region (57). Inactivation of the enzyme by C-terminal deletion extending to Lys-1137 is consistent with the removal of a peptic segment involved in the dimerization of the two halves of the enzyme, but direct evidence is lacking. It has been suggested previously that there is a dimerization motif closer to the N terminus, in the region between Leu-994 and Leu-1025 of human DNA topoisomerase IIα (64), which corresponds to Ile-971 to Asn-992 of the *S. cerevisiae* enzyme; mutagenesis and biochemical experiments do not support the involvement of this motif in protein dimerization, however (36).

The results summarized above also suggest the presence of a critical stretch of about 40 amino acids between Tyr-1167 and

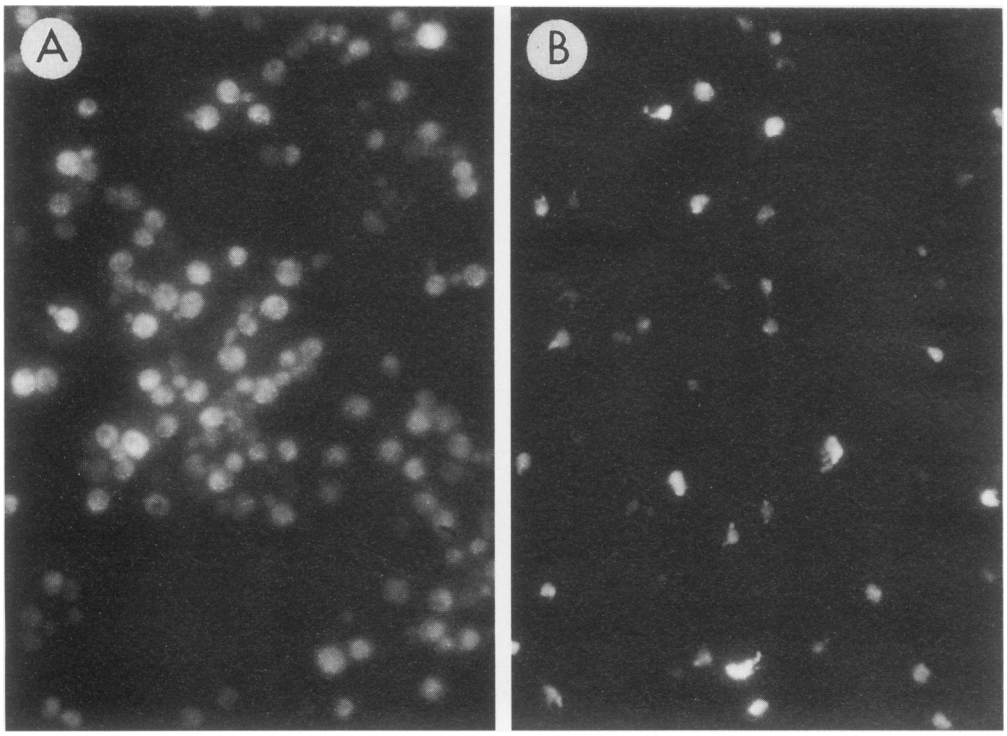

FIG. 5. Immunofluorescence micrographs illustrating *S. cerevisiae* CH325 (*top2-4*) cells overexpressing immunotagged Top2(1–1171) (A) or Top2(1–1334) (B). The magnification factor of the photograph in panel B is 1.5 times higher than that of the one in panel A.

Lys-1208, which, although dispensable for the catalytic functions of the enzyme in vitro, is important for the physiological function of the enzyme in vivo. The region from Tyr-1167 to Lys-1208 of the *S. cerevisiae* enzyme corresponds to the region from Trp-1201 to Thr-1265 of the *S. pombe* enzyme. Previously, studies by Shiozaki and Yanagida (51) have indicated the presence of a nuclear localization signal in this region of the *S. pombe* enzyme, most likely between residues 1227 and 1242. *S. pombe* DNA topoisomerase II terminating at Ala-1198 has partial complementation activity when expressed from a multicopy plasmid, and this partial activity was attributed to the presence of a redundant nuclear localization signal within the N-terminal 75 amino acids of the enzyme, which has no counterpart in the *S. cerevisiae* enzyme; removal of the N-terminal nuclear localization signal from the *S. pombe* Top2(1–1198) mutant protein leads to cell inviability. Sequence analysis of nuclear localization signals has also led Dingwall and Laskey (19) to postulate that the region from Arg-1195 to Gly-1211 of *S. cerevisiae* DNA topoisomerase II constitutes such a signal. In *Drosophila* DNA topoisomerase II, there is a strong nuclear localization signal located within a 60-amino-acid stretch of the C terminus; an additional but weaker signal(s) apparently exists, however, as deletions of the C-terminal 60 amino acids do not abolish the enzyme's ability to genetically complement a temperature-sensitive or null yeast *top2* allele (17).

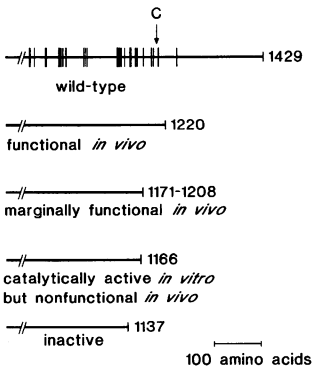

FIG. 6. Summary of catalytic activity and physiological functionality of *S. cerevisiae* DNA topoisomerase II proteins with various portions of the C-terminal domain removed. The top diagram is an enlarged version of the C-terminal region shown in Fig. 1.

Our immunofluorescence results are consonant with the interpretation that nuclear localization is likely to be one contribution of the C-terminal domain to the physiological function of yeast DNA topoisomerase II: whereas the Myc-tagged Top(1–1334) yeast enzyme is found mostly in the nucleus, the similarly tagged Top2(1–1171) protein is abundant in the cytoplasm. On the other hand, our in vivo assays based on inhibition of gene expression by DNA positive supercoiling as well as linking number measurements of intracellular plasmid DNA show that there is at least some Top2(1–1166) protein in the nucleus and that the protein is active in the removal of DNA positive supercoils in vivo. It is difficult to assess, however, whether the nuclear concentration of Top2(1–1166) might be insufficient to sustain growth or whether the less processive nature of the mutant enzyme, as indicated by in vitro assays, may further hinder its nuclear function. The presence of catalytically active Top2(1–1166) protein in the nucleus raises the possibility, however, that the C-terminal domain beyond Gly-1166 may have additional roles. A similar conclusion was reached for *Drosophila* DNA topoisomerase II (17).

A number of studies have implicated phosphorylation in the modulation of eukaryotic DNA topoisomerase II activity. *S.*

cerevisiae DNA topoisomerase II is phosphorylated by a casein kinase II-like protein kinase in vivo, and most of the phosphorylation sites are located in the C-terminal domain, including several sites at which phosphorylation appears to be cell cycle regulated (9, 10). Similar studies with rat, hamster, chicken, and *Drosophila* DNA topoisomerase II (1, 8, 26, 49) indicate that the activity of eukaryotic DNA topoisomerase II is increased by phosphorylation and that phosphorylation is cell cycle dependent. In an analogous case, phosphorylation of a long tail region has recently been shown to occur in the N terminus of human DNA ligase, and this modification appears to correlate directly with increased activity (45); removal of this N-terminal region of DNA ligase yields an enzyme whose activity is no longer modulated by phosphorylation.

Our results indicate that yeast cells expressing *top2* C-terminal deletions, such as *top2*(1–1208), which lack the major cell cycle-dependent phosphorylation sites in the type II DNA topoisomerase are nevertheless phenotypically indistinguishable from cells expressing the wild-type enzyme. Analysis of *S. pombe top2* mutants has also led to the view that phosphorylation of DNA topoisomerase II may not play an essential role in the fission yeast (51). These findings raise some doubt about the physiological importance of the cell cycle dependence of the main phosphorylation sites. They do not necessarily contradict the postulate, however, that the C-terminal domain of the enzyme, together with phosphorylation, may serve a regulatory role; as discussed by Cardenas and Gasser (10), the C-terminal domain in the unphosphorylated enzyme may repress its catalytic activity, and thus either phosphorylation or removal of the C-terminal domain may activate the enzyme.

A third interpretation of the results obtained with *S. cerevisiae* DNA topoisomerase II mutants with C termini falling between Gly-1166 and Lys-1208, which are catalytically active in vitro but functionally inactive or marginally active in vivo, is that this 42-amino-acid stretch is involved in interactions with other cellular entities. Many eukaryotic proteins directly involved in nucleic acid metabolism, including DNA polymerases, RNA polymerases, and helicases, have highly conserved amino acid sequences encoding the active core regions but highly divergent tail regions; the latter regions have often been implicated as sites for chemical modification in the cell or as targets of other cellular factors (15, 20, 42, 54, 55). Strong DNA topoisomerase II cleavage sites have been found in scaffold-associated regions (33, 53), and DNA topoisomerase II has also been identified as a major protein associated with metaphase chromosome scaffolds (41). An association between mammalian DNA topoisomerase II and chromosome scaffold protein Sc2 in sequence-specific DNA-binding protein UB2 has been reported recently (39).

There is strong evidence that the essentiality of DNA topoisomerase II in the yeasts *S. cerevisiae* and *S. pombe* lies in its decatenation activity during the segregation of pairs of intertwined chromosomes at mitosis (29, 60). In the heterologous system studied by Crenshaw and Hsieh (17), a mutant *Drosophila* DNA topoisomerase II in which the C-terminal 240 amino acids of the wild-type enzyme were replaced by a decapeptide encoded by nucleotides introduced during the construction of the plasmid for its expression was found to possess decatenation activity inside yeast nuclei but nevertheless to be incapable of complementing yeast *top2* mutants. In the present study of the homologous system, however, there appears to be a strong correlation between the in vivo functionality and in vivo decatenation activity of the proteins: the *S. cerevisiae* Top2(1–1166), Top2(1–1180), and Top2(1–1195) proteins exhibit little decatenation activity in vivo and are nonfunctional or marginally functional in complementing the temperature-sensitive lethality of the yeast *top2* mutations; Top2(1–1220), on the other hand, has a wild-type level of decatenation activity in vivo and is fully functional in vivo.

It is curious that cells expressing Top2(1–1166) have sufficient supercoil relaxation activity to prevent the accumulation of positive supercoils generated by transcription or other processes involving the translocation of a macromolecular machinery along a DNA, but insufficient decatenation activity to unlink intertwined pairs of newly replicated plasmid molecules. The simplest interpretation of these findings is that a much lower nuclear enzyme level is required for the removal of positive supercoils than for decatenation. It is plausible, however, that whereas the relaxation activity reflects solely the ATP-dependent DNA transport activity of the enzyme, the decatenation activity may in addition involve a process which physically separates the intertwined pairs of plasmids or chromosomes. If the partition process involves the association of a C-terminal domain region of DNA topoisomerase II with other macromolecules, then deletion mutants of the topoisomerase may specifically lack decatenation activity in vivo. The detailed requirements for DNA decatenation in vivo, other than a DNA topoisomerase activity to overcome the topological linkage of the DNA components, are largely unknown. In *E. coli* and *Salmonella typhimurium*, although both DNA gyrase (DNA topoisomerase II) and DNA topoisomerase IV are capable of transporting one duplex DNA segment through another (34), the former appears to be insufficient to fulfill the decatenation function (3).

As discussed above, whereas our results with the DNA topoisomerase II of the budding yeast *S. cerevisiae* as well as the results of others with other eukaryotic type II DNA topoisomerases indicate that the presence of a nuclear localization signal in the C-terminal domain partly explains the functionality of the domain, it is plausible that this domain, especially the *S. cerevisiae* enzyme Gly-1166 to Lys-1208 peptide or its counterparts in its homologs, may serve additional roles in vivo. Genetic and biochemical studies of point mutations in the critical region may offer additional insights on the precise roles of the C-terminal domain of type II DNA topoisomerases.

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