

# Cloning, characterization, and sequence of the yeast DNA topoisomerase I gene

(*Saccharomyces cerevisiae*/*mak1* mutants)

CATHERINE THRASH\*, ALAN T. BANKIER†, BART G. BARRELL†, AND ROLF STERNGLANZ\*‡

\*Department of Biochemistry, State University of New York, Stony Brook, NY 11794; and †Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH England

Communicated by Bruce M. Alberts, March 18, 1985

**ABSTRACT** The structural gene for yeast DNA topoisomerase I (*TOP1*) has been cloned from two yeast genomic plasmid banks. Integration of a plasmid carrying the gene into the chromosome and subsequent genetic mapping shows that *TOP1* is identical to the gene previously called *MAK1*. Seven *top1* (*mak1*) mutants including gene disruptions are viable, demonstrating that DNA topoisomerase I is not essential for viability in yeast. A 3787-base-pair DNA fragment including the gene has been sequenced. The protein predicted from the DNA sequence has 769 amino acids and a molecular weight of 90,020.

We have recently identified both DNA topoisomerase I and topoisomerase II mutants in the yeast *Saccharomyces cerevisiae* (1, 2). Our aim was to use the mutants to learn more about the *in vivo* roles of eukaryotic DNA topoisomerases. The topoisomerase II mutant is a temperature-sensitive conditional lethal mutant; its phenotypes demonstrate that topoisomerase II is required for termination of DNA replication and segregation of chromosomes at nuclear division (2). On the other hand, the topoisomerase I mutants that were identified have <1% of the normal level of topoisomerase activity, but they grow normally and have no obvious defects. These mutants were mapped to the *MAK1* gene (1), previously identified as required for the maintenance of a double-stranded linear, killer RNA found in many yeast strains (3). Because of these surprising results we decided to clone the topoisomerase I gene, to determine whether it corresponds to the *MAK1* gene and to study the effects of chromosomal disruptions of the gene. This paper presents those studies as well as the complete nucleotide sequence of the gene.

## MATERIALS AND METHODS

**Escherichia coli Strains and Media.** *E. coli* K-12 strain JA221 *trpE5 leuB6 lacY recA thi hsdR* was used for plasmid propagation. It was grown in L broth (4) with 50  $\mu$ g of ampicillin per ml. Strain JA194 *trpC leuB* grown on M9 medium (4) supplemented with 5  $\mu$ g of tryptophan per ml and 50  $\mu$ g of ampicillin per ml was used to screen for *LEU2* plasmids; the yeast *LEU2* gene complements the *E. coli leuB* mutation. Plasmids were prepared by an alkaline lysis protocol (4).

**Yeast Strains and Media.** The following *S. cerevisiae* strains were used: CT552: *MATa ura3-52 his3 his7 leu2-3,112 trp1 ade2-101 can1*; SD2a: *MATa ade2 ura3 leu2 his3 trp1 can1 top1-1 (mak1-1) top2-1*; SD2c: *MATa ade2 ura3 his3 his7 leu2 can1 top1-1*; CT553: *MATa leu2 lys11 his1 ura3 trp1 pet17*; the diploid CT554 *MATa/α leu2/leu2 trp1/+ lys11/+ pet17/+ his1/+ ade1/+ ura3/+*. All the above strains were constructed in this laboratory using strains described previ-

ously (1, 2). Other yeast strains used include 6287-20B: *MATa kar1-1 leu2* from F. Winston (5); 418D: *ci<sup>0</sup> MATa leu2 his* from J. Broach; and A364A: *MATa adel ade2 ura1 his7, lys2 tyr1 gall* (KIL-o). Three new *top1* alleles created by gene disruptions are described in the text. The *top1-6* allele contains the inserted *LEU2* gene, the *top1-7* allele contains deletion 1447-2295 as well as the *LEU2* insertion, and the *top1-8* allele contains deletion 580-1446 as well as the *LEU2* insertion.

Standard genetic methods and growth media were used (6). Transformation was by the spheroplast method (6). Two yeast genomic libraries were used, one was cloned in YCp50, provided by M. Rose and G. Fink, and the other was cloned in YEp13 (7).

**DNA Topoisomerase I Assays.** A method was developed by S. DiNardo of this laboratory whereby individual yeast colonies could be assayed directly for topoisomerase I activity without growth in liquid medium. A colony was scraped from a plate, resuspended in 30  $\mu$ l of SED (1 M sorbitol/25 mM EDTA/50 mM dithiothreitol), and incubated at 37°C for 30 min. Four microliters of zymolyase 60,000 (2.5 mg/ml) (Miles) was added. Cells were incubated at 30°C for 30 min. Spheroplasts were pelleted in a desktop centrifuge, resuspended in 12  $\mu$ l of yeast lysis buffer (2), and kept on ice for 30 min. After a 10-min centrifugation, 1  $\mu$ l of the supernatant could be assayed in a 10- $\mu$ l reaction mixture as described (1).

Extracts for determining yeast topoisomerase I activity in *E. coli* were prepared by growing 20-ml cultures, pelleting the cells, washing them, and resuspending them in 0.5 ml of yeast lysis buffer. Lysis was by sonication as described (8). One microliter of extract was assayed in a 10- $\mu$ l reaction mixture at 30°C for 1 hr. The same extracts could be assayed for *E. coli* topoisomerase I as described (8).

**Cloning the *TOP1* Gene.** A petite ( $\rho^-$ ) derivative of strain CT552 was isolated and transformed to Ura<sup>+</sup> with the YCp50-derived gene library. The first cloning method involved purifying the transformants and assaying them to find ones that had acquired topoisomerase I activity. To facilitate finding rare transformants with enzymatic activity, they were pooled into groups and assayed by a modification of the method described above for individual colonies. Specifically, 20 colonies were resuspended in 200  $\mu$ l of SED, the suspension was incubated with 30  $\mu$ l of zymolyase, and it was resuspended in 60  $\mu$ l of yeast lysis buffer. Reconstruction experiments showed that if only 1 of the 20 colonies had topoisomerase I activity, it could be detected by this method. When a group of transformants with activity was found, the individual members of the group were reassayed to find the one with activity.

The second cloning method involved screening for transformants that had become Mak1<sup>+</sup> by using a procedure

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Abbreviation: kb, kilobase(s).

‡To whom reprint requests should be addressed.

described previously for introducing the killer RNA into the cells (6). The same Ura<sup>+</sup> transformants generated by the first cloning method were syringed from the top agar of the transformation plates. They were mated with strain 6287-20B on YEPD plates (6) for 60 hr at 30°C. After mating, the cells were diluted into SD medium (6) and spread onto plates containing 0.1% dextrose, 3% glycerol, 0.7% Bacto yeast nitrogen base without amino acids, 20 μg of adenine sulfate per ml, 20 μg of histidine per ml, 30 μg of leucine per ml, and 60 μg of canavanine sulfate per ml. These plates select for transformants of CT552 that have received the cytoplasm of strain 6287-20B and, hence, its killer RNA. After 3 days of incubation at 30°C, colonies were printed onto killer indicator plates (6) containing a lawn of sensitive cells, strain A364A. Colonies that could kill the sensitive cells were considered Mak1<sup>+</sup> candidates; they were assayed for topoisomerase I activity.

The third cloning method involved transforming strain SD2a, a *top1-1 top2-1* temperature-sensitive double mutant, with the YEp13-derived gene library. Leu<sup>+</sup> transformants were selected at 25°C, syringed out of the top agar, and replated on plates without leucine. Rapidly growing colonies were selected and assayed for topoisomerase I.

**DNA Sequencing.** The basic procedure has been described in a recent review article (9). The 3.8-kilobase (kb) *Hind*III fragment from pCT80 was sonicated to generate a library of random fragments. These were size-fractionated, the ends were repaired, and the fragments were subcloned into bacteriophage M13mp8. DNA sequencing by the dideoxynucleotide chain-termination method (10) was carried out using buffer gradient gels and deoxyadenosine 5'-[[α-<sup>35</sup>S]thio]triphosphate (11). The sequence was compiled with the aid of computer programs DBAUTO and DBUTIL (12). On average, each nucleotide was sequenced 6.8 times; both strands were determined for 84% of the sequence.

## RESULTS

**Cloning of the DNA Topoisomerase I Gene.** The gene was cloned by three independent methods. The first method involved transforming a *mak1-1* mutant, which has no detectable topoisomerase I activity (1), with a yeast gene library cloned in the plasmid vector YCp50. Ura<sup>+</sup> transformants were selected and screened by direct enzymatic assay to find ones that had acquired topoisomerase I activity. Transformants were first pooled into groups and assayed. Individual transformants from pools that had activity were then re-assayed. By using this approach, 1140 transformants were screened and three with enzymatic activity were identified.

The second method involved using the same Ura<sup>+</sup> transformants as described above but this time screening them to find ones that had become Mak<sup>+</sup>; i.e., ones that were now able to maintain the killer RNA. The transformants were mated with a *kar1-1* strain (5) in order to introduce the killer RNA, and then screened for the ability to maintain the RNA. Three independent transformants with this property were identified; enzymatic assays showed that all three had acquired topoisomerase I activity.

The third method was designed to complement a phenotype of a *mak1-1 top2-1* temperature-sensitive double mutant. This mutant grows slowly and forms very small colonies at the permissive temperature, 25°C (properties of this mutant will be published elsewhere). A yeast gene library cloned in the vector YEp13 (7) was used to transform the double mutant. Leu<sup>+</sup> transformants were selected and screened to find ones that grew at a normal rate as judged by colony size. Five such transformants were identified and all had acquired topoisomerase I activity.

Plasmid DNA was isolated from all the transformants with topoisomerase I activity, the DNA was passed through *E.*

*coli*, and then used to retransform a yeast *mak1-1* strain to Ura<sup>+</sup> or Leu<sup>+</sup>. In all cases, the yeast transformants regained topoisomerase I activity. Restriction enzyme analysis of the plasmid DNA from these transformants showed that eight different plasmids had been isolated—three from the YCp50 bank and five from the YEp13 bank. All eight plasmids showed common restriction fragments and thus came from the same region of the yeast genome.

To determine whether we had cloned the structural gene for yeast topoisomerase I, as opposed to a regulatory gene, *E. coli* strains containing the plasmids described above were assayed for Mg<sup>2+</sup>-independent topoisomerase activity characteristic of the yeast enzyme. A similar method was used by Goto and Wang to demonstrate that they had cloned the yeast DNA topoisomerase II structural gene (13). Fig. 1 shows that *E. coli* strains carrying these plasmids all show yeast topoisomerase I activity (lanes 1–6), while *E. coli* strains with no plasmid (lane 8) or with a YCp50-derived plasmid containing an unrelated insert (lane 7) do not exhibit such activity. If a strain containing one of the yeast topoisomerase I plasmids is assayed in the presence of Mg<sup>2+</sup>, both the endogenous *E. coli* topoisomerase I and the yeast enzyme are active (lane 10). Under the conditions used in these assays, the two enzymes give different DNA topoisomer distributions and can thus be distinguished (compare lanes 9–11). Some of the plasmids were also used to transform *E. coli* strain DM800, which has a deletion of the *E. coli* topoisomerase I gene (8). These *E. coli* transformants also exhibited yeast DNA topoisomerase I activity (data not shown). The results demonstrate that we have cloned the yeast DNA topoisomerase I structural gene.

**The Structural Gene Maps at *MAK1*, Renamed *TOP1*.** Since one of the methods used to obtain a clone with the DNA topoisomerase I structural gene involved screening for a plasmid that restored the Mak<sup>+</sup> phenotype, it seemed likely that *MAK1* was actually the structural gene for topoisomerase I. To prove this, one of the YEp13-derived plasmids described above, pRSa4-8, was integrated into the chromosome and the site of integration was genetically mapped. Such integrants arise from homologous recombination between yeast sequences on the plasmid and the corresponding sequences on the chromosomes (14), in this case at either *LEU2* or at the topoisomerase I gene. If *MAK1* is the topoisomerase I structural gene, integration of the plasmid at

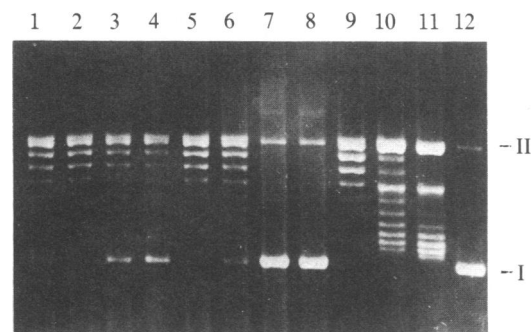


Fig. 1. DNA topoisomerase I assays of *E. coli* strains carrying *TOP1* plasmids. Lanes 1–9, assays carried out in the absence of Mg<sup>2+</sup>, in a buffer specific for yeast topoisomerase (1). Lanes 1–6, *E. coli* transformants carrying the six plasmids identified by cloning methods 1 and 2. Lane 7, an *E. coli* transformant carrying a YCp50-derived plasmid with an unrelated insert that does not restore topoisomerase I activity in yeast. Lane 8, untransformed *E. coli* strain. Lane 9, an assay of partially purified yeast DNA topoisomerase I. Lanes 10 and 11, same extracts as in lanes 1 and 7 but assayed in the presence of Mg<sup>2+</sup> in a buffer designed for *E. coli* topoisomerase I (8). Lane 12, no extract added. I and II show the positions of supercoiled and nicked pBR322 DNA, respectively.

10 20 30 40 50 60 70 80 90 100 110 120 130  
AGCTTGGCGCCCTGAAGACAAAATTCGAAACCGAGTGGAACTGCAAACTCTAGCGCCGACGACGCAATTTTGATATATAGGAGTTTAACTACTAGCCATAAAAATCTAAACAGAAATGGAAACGGACATA

140 150 160 170 180 190 200 210 220 230 240 250 260  
CACATAATATAGCTCATATATATTACATAAATCTTCAATGTCTTGAACACGTAATTTTAGTGTGAGAAACCTTTTCACTCCGGTAAATACCTGCTGTAGTCTTCAAAAAAAAAAAAAAAAAAAGAAA

270 280 290 300 310 320 330 340 350 360 370 380 390  
AAAAACGAGCCATGGAAAGCTCTTTATTTTTTACTTTACGGCTTTTTCCCTTTCTTATATGA TCGATGCACGTAAGAACAACCTGTATTTTTTTGTTTCAACACTAACACGAGCGCAATATCTTTT

400 410 420 430 440 450 460 470 480 490 500 510 520  
TTGTTTTCTCTGTACTCTAATACCTGAGTCTTCTTATAGTATTAACACAGCAAAATAAAAAAATCTAAAGGAGGGCAGAGCTCGAAACTTGAACCGGTAATAAATGACTATTGGCTGATGCTTCC  
M T I A D A S

530 540 550 560 570 580 590 600 610 620 630 640 650  
AAAGTTAATGATGAGTTGCTTCTGATGAGATGACGATGCGCATTATCTCAAACTTTAAAAA AAGAAAGGTGGCTCCATGAACTCTGCCTCTTCAAGACGAAGCGGAACTTATGATAGTGATG  
K V N H E L S S D D D D D V P L S Q T L K K R K V A S H N S A S L Q D E A E P Y D S D

660 670 680 690 700 710 720 730 740 750 760 770 780  
AGGCAATCTCTAAGATTTCCAAGAAAAAGACTAAGAAAAAAGACCGAACCGTCAATCGTGTCAATACCATCGCTCCAGCAAGAAAAAGCGGCACATCAAGGCTAAAAAATCAAGAAAGAAGA  
E A I S K I S K K K K T K K I K T E P V Q S S S L P S P P A K K S A T S K P K K I K K E D

790 800 810 820 830 840 850 860 870 880 890 900 910  
TGGTGATGTAAGGTAACAACTAAAAAGGAAGACAGGAGAACGAAAAAAGAAACGAGAAG AAGAAGAAGAGGAGGACAAGAAAGCGAAGGAGGAGGAGGAAGAATAAATGTTGGGAAAAAGAA  
G D V K V K T T K K E E Q E N E K K K R E E E E E D K K A K E E E E E Y K W W E K E

920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040  
AACGAAATGACACCAATAAATGGGTACACTGAGCATAACGGTGTATATTCCTCCACCATACAGCCCTTACCATCTCACATCAAAATTAATACGATGGGAAGCCAGTAGATTACCTCCGCAAG  
N E D D T I K W V T L K H N G V I F P P P Y Q P L P S H I K L Y Y D G K P V D L P P Q

1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170  
CTGAAGAAGTAGCGGGTTCTTGTGCGCTATTAGAGAGTATGATCCAAAAATCTCTTTTCAAAAGAACTTCTCAATGATTTCTTGCAAGTACTGAAAGAAAGTGGTGGTCCCTCAATGGAAAT  
A E E V A G F F A A L L E S D H A K N P V F Q K N F F N D F L Q V L K E S G G P L N G I

1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300  
TGAGATAAAGGAATTTCTCGTGGATTTCCACAAAATGTTGATTAATCTCCAGTTACAAAAG AACAGAAAAAGCAACTGACTTCCCAAGAAAAAGACAGATTGCTTTGGAAAGAAAAATTCGAG  
E I K E F S R C D F T K M F D Y F Q L Q K E Q K K Q L T S Q E K K Q I R L E R E K F E

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430  
GAAGATTATAAATCTGTGAATTAGATGGCAGAAAGCAAGTAGGAAATTTCAAGGTGAACCTCCCTGATCTATTAGAGGTGCTGGCGCTCACCAAAAAAGCAAGTAAAGAGAAAGTAGAATC  
E D Y K F C E L D G R R E Q V G N F K V E P P D L F R G R G A H P K T G K L K R R V N

1440 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560  
CTGAGGATATCGTTTAAATCTAAGTAAAGACGCCACCGTTCGCGACGCCAGAGGGCACAAG TGGGGTGAATCAGACACGACAATACCGTTCAATGGTTAGCCATGAGGAGAGAAATTTTCAA  
P E D I V L N L S K D A P V P P A P E G H K W G E I R H D N T V Q W L A M W R E N I F N

1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680 1690  
CTCATTCAAATACGTCAGATTGGCAGCGAACTTTCATGAAGGGTCAAAAGTGAATCAAGAAG TTAAGAAGGCGAGACAATTAATAATCTATATCGATGCCATCAGAAAGGATTACACGAGAAATTTG  
S F K Y V R L A A N S S L K G Q S D Y K K F E K A R Q L K S Y I D A I R R D Y T R N L

1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800 1810 1820  
AAAAAGAAATGCTAGAGCGCCAAAAGCCGAGCCATTTATTGATCGATGATTTGCGTTT AAGAGCCGGTGGTGAATAAATCGAAGATGAAGCGGATACTGCTGGTGTGTTGTTGATCGGATG  
K S K V M L E R Q K A V A I Y L I D V F A L R A G G E K S E D E A D T V G C C S L R Y

1830 1840 1850 1860 1870 1880 1890 1900 1910 1920 1930 1940 1950  
AGCATGTTACTTTGAAACCTCCGAATACTGTTATCTTTGATTTCTAGGTAAGGATCTATTAGATTTTATCAAGAGGTAGAAAGTTGACAAACAAGTTTCAAAAAATTGACAATTTTAAAGCGGCC  
E H V T L K P P N T V I F D F L G K D S I R F Y Q E V E V D K Q V F K N L T I F K R P P

1960 1970 1980 1990 2000 2010 2020 2030 2040 2050 2060 2070 2080  
CAACAGCCAGGACATCAAGTGTGATCGTCTAGATCCATCTACTGAACAATACTTACAAAATACATACAGCGGAGTACTGCTAAAGTTTCCGTACATATAAGCTTCCAAAACAAATGGAAGAT  
K Q P G H Q L F D R L D P S I L N K Y L Q N Y M P G L T A K V P R T Y N A S K T M Q D

2090 2100 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200 2210  
CAACTGGATTAATCCAAAATAAGGATCTGTGCGAGAAAAATTTGAAGTACAACGAGCAAAATGAACTGAGCACTGATGATGATCAACCATCAAAAGCACTGTCAGAAAGGGGATGCACAAACACTGG  
Q L D L I P N K G S V A E K I L K Y N A A N R T V A I L C N H Q R T V T K G H A Q T V

2220 2230 2240 2250 2260 2270 2280 2290 2300 2310 2320 2330 2340  
AAAAGCCCAATAAGATACAAGATTTGGAATGGCAAAAGATTCGTTGCAAGAGGGCCATTTTACAAATGGATAAGGATCTTTTAAAGAAAGCCAAAATATTTGGAAGAAATCCAGCATTTGAGCA  
E K A N N R I Q E L E W Q K I R C K R A I L Q L D L K D L L K K E P K Y F E E I D D L T K

2350 2360 2370 2380 2390 2400 2410 2420 2430 2440 2450 2460 2470  
AGAAGTGAAGCCACCATTCCAAAGAGAAATTTGATAGAGAAATTTGAAGAAATATCAGCGAAAAATTTGTTAGGGAGAACGATAAGAGGAAATTTGAAAGGAAGAATTTATGCGCGGAAAGTCAATGAG  
E D E A T I H K R I I D R E I E K Y Q R K F V R E N D K R K F E K E E L L P E S Q L K

2480 2490 2500 2510 2520 2530 2540 2550 2560 2570 2580 2590 2600  
GAATGGTTGGAGAAAGTCGACGAAAAAGAAACAAGAATTCGAAAAGGAATTTGAAAACCGGTGAAGTGAAGTGAATCAAGTTGGAATTCAGTTCGAAAAATAAAGGCACAAAGTAGAGAAATAGAACAGC  
E W L E K V D E K K Q E F E K E L K T G E V E L K S W N S V E K I K A Q V E A K L E Q

2610 2620 2630 2640 2650 2660 2670 2680 2690 2700 2710 2720 2730  
GTATCCAACTAGTTCCAGTTGAAAGATAAAGAGGAAACTCCAGGTTTCTACTGGCACTTCCAAAATCAATTAATAGACCCTAGACTTTCTGTGGTATTGCAAAAAGTATGATGTTCCGAT  
R I Q T S S I Q L K D K E N S Q V S L G T S K I N Y I D P R L S V V F C K K Y D V P I

2740 2750 2760 2770 2780 2790 2800 2810 2820 2830 2840 2850 2860  
TGAAGAATTTTACAAAAACCCTAAGAGAAAAATTCAAATGGGCCATAGAATCCGTAGATGAAAATTTGAGGTTTAAACGCAAGAAAGCAGCATAGGGGAGAAAGCAATACATTCACGCATCAAGTT  
E K I F T K T L R E K F K W A I E S V D E N W R F

2870 2880 2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990  
CGCATTAGGGTTACTAGGAAGATATCTTATACACATATATAGAAAATAATTTAAAACTTTTAAAGAGTATACTGCAGTGAATCATGCCACAGTTACTTTACCCTCCCTATTATTCTTAGTACCTGCAT

3000 3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120  
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3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240 3250  
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3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360 3370 3380  
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3390 3400 3410 3420 3430 3440 3450 3460 3470 3480 3490 3500 3510  
GGATGTGAGATACGTAGTACATCTAGCTTGCTTTATTTACAATAACACATCAGCAGAAAGGACGCTTACGGTGAACAGGAAAGTCTTAACTATAATAGGAAAAAGATTGTCACGATAAATAGT

3520 3530 3540 3550 3560 3570 3580 3590 3600 3610 3620 3630 3640  
AACGACAAATGAGACTGCGAGAAATCTCCATTTGGAAGTGAATACTACTACTTCTTTGTTGCTTACAAGAAATTTCAACTTGTCTCCGCTGCAAAATTTGCCCTCTTATCAAGTAGTACAAGGCAG

3650 3660 3670 3680 3690 3700 3710 3720 3730 3740 3750 3760 3770  
CAGATAGTTCTAGTAAAGGACGCTCTCAGCCAAGCAACACGCTCCTTAGGCAAAAGCTCAGTAACAGGTAAGGATGTTTCGTCAGTCAATATGTCACCTCAAGCACTAAAAATGCCAAAATCACCAC

3780  
GAGCGCTAGTACAAGCT

FIG. 2. DNA sequence of the 3787-base-pair *Hind*III fragment containing the *TOP1* gene. The deduced amino acid sequence is numbered as indicated. The deduced amino acid sequence of DNA topoisomerase I is given in one-letter code below the DNA sequence.

that locus should cause *LEU2* present in the plasmid to become linked to the centromere and to *PET17* (1, 3).

Strain 418-D *leu2* was transformed with pRSa4-8 and a stable *Leu*<sup>+</sup> integrant was isolated. It was crossed with strain CT553 *leu2*, *trp1*, *pet17*. The resultant diploid was sporulated, and tetrads were dissected and analyzed genetically. The *trp1* marker is used to measure centromere linkage. The results showed that *LEU2* had become centromere linked (*LEU2:TRP1*; 7 parental ditypes, 16 nonparental ditypes, 0 tetratypes) and also was linked to *PET17* (*LEU2:PET17*; 13 parental ditypes, 0 nonparental ditypes, 11 tetratypes). These results showed that the plasmid had integrated at or near *MAK1*.

The same haploid integrant was also crossed with a *mak1-1*, *leu2* mutant. Diploids were sporulated, dissected, and analyzed. The results for five tetrads showed that *Leu2*<sup>+</sup> cosegregated with topoisomerase I activity and both markers segregated 2:2 in all cases. The simplest interpretation of these results is that the plasmid integrated at *MAK1*, which we suggest be renamed *TOP1*.

**DNA Sequence of the Topoisomerase I Gene.** Restriction enzyme analysis of the eight different complementing plasmids showed that all of them had a 3.8-kb *HindIII* fragment. This fragment was isolated and cloned into the vector pUC12 to yield plasmid pCT80. This plasmid was used to transform *E. coli* and the transformants were found to have acquired *Mg*<sup>2+</sup>-independent topoisomerase activity characteristic of the yeast enzyme (as in Fig. 1). These results strongly suggested that the *HindIII* fragment contained the *TOP1* gene. The fragment was therefore sequenced using the dideoxynucleotide chain-termination method (9–11).

The 3787-base-pair sequence is shown in Fig. 2. An open reading frame is present, which extends from nucleotide 500 to 2806 and codes for a protein with 769 amino acids and a molecular weight of 90,020. This value agrees well with the published molecular weight of 90,000 for yeast DNA topoisomerase I (15). Results in the next section show that disruptions of this open reading frame eliminate topoisomerase I enzymatic activity in *E. coli* and in yeast. There are two possible transcription start sites at nucleotides 435 and 448. Several possible transcription termination signals are seen beyond the large open reading frame (e.g., at nucleotide 2978) although none agree well with any of the proposed consensus sequences for yeast (16). The sequence TACTAAC, which is found in all yeast introns (17), is not present in this fragment. For these reasons, the amino acid sequence indicated in Fig. 2 is most likely to be the correct sequence for yeast DNA topoisomerase I.

**Topoisomerase I Is Not Essential for Viability.** A major reason for cloning the gene was to determine whether the enzyme is essential for viability in yeast. To this end, null mutations were introduced into the gene. First a 2.2-kb fragment containing the *LEU2* gene was cloned into the *Sal* I site of pCT80 at nucleotide 2486 of the *HindIII* fragment. This insertion within the topoisomerase I coding sequence was sufficient to eliminate yeast topoisomerase I activity in *E. coli* and yeast. Nevertheless, a further gene disruption was performed by deleting an 849-base-pair *Aha* III fragment spanning nucleotides 1447–2295 of the fragment shown in Fig. 2. The resultant plasmid, pCT80ΔTOP, was cut with *HindIII* to generate a 5.1-kb fragment with both a deletion and a *LEU2* insertion in the *TOP1* gene. Linear DNA fragments are recombinogenic in yeast and can be used to replace the homologous sequences on the chromosome (18). In this case, the 5.1-kb fragment was used to transform a diploid yeast strain to *Leu*<sup>+</sup>. Southern blotting with a *TOP1* probe, pCT80, verified that the disrupted *TOP1* gene had replaced the wild-type gene on one of the two homologous chromosomes but not on the other (Fig. 3). To test whether a disruption of the *TOP1* gene is lethal, this diploid was sporulated and

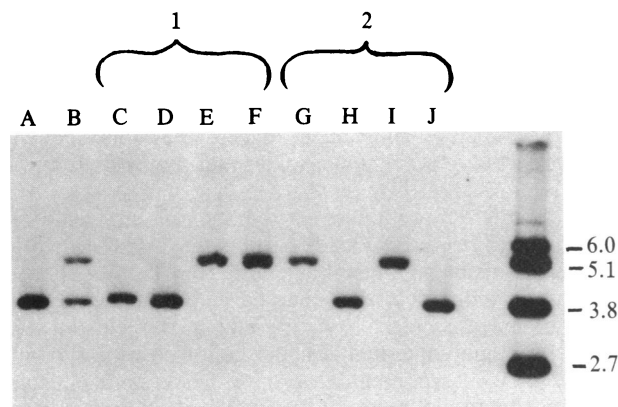


FIG. 3. Southern blotting of strains with a disruption of the *TOP1* gene. Genomic DNA from the following strains was digested with *HindIII*, electrophoresed on a 1.0% agarose gel, blotted to nitrocellulose, and probed with pCT80. Lanes: A, the original diploid CT544; B, CT554 transformed with the 5.1-kb DNA fragment with a deletion and insertion in the *TOP1* gene (see text); C–F, tetrad 1 from the transformed diploid; G–J, tetrad 2 from the transformed diploid. The size of known DNA molecules in kb is shown on the right.

tetrads were dissected. Many fully viable tetrads were found; seven of them were analyzed in detail. As expected, *Leu2*<sup>+</sup> always cosegregated with *Top1*<sup>−</sup> and both markers segregated 2:2. Two complete tetrads were analyzed by Southern blotting. In all cases, the *Leu2*<sup>+</sup> *Top1*<sup>−</sup> haploids had a 5.1-kb *HindIII* fragment diagnostic of the disrupted gene, while the *Leu2*<sup>−</sup> *Top1*<sup>+</sup> haploids all had the wild-type 3.8-kb *HindIII* fragment (Fig. 3). Haploids with the disrupted gene grow at an almost normal rate (the doubling time is ≈30% longer than for wild type) and have no obvious defect in DNA or RNA synthesis. In a separate experiment, we constructed a null mutation with a deletion in the amino-terminal region of the *TOP1* open reading frame (nucleotides 580–1446), as well as the *LEU2* insertion at nucleotide 2486. Haploid yeast strains carrying this mutation also were viable. Thus, we conclude that DNA topoisomerase I is not essential for viability in yeast.

## DISCUSSION

DNA topoisomerase I is present in large amounts in the nuclei of all eukaryotes. Many workers have speculated that it acts as a swivel during DNA replication and possibly during transcription. It is therefore surprising to find that the enzyme is not essential for viability in yeast. A possible explanation is that DNA topoisomerase II can substitute for topoisomerase I. Recently we have examined *top1 top2-1* double mutants and found that they have defects in DNA replication and transcription not seen for either single mutant (unpublished results). These results suggest that topoisomerase action is necessary for DNA replication fork movement and at least some types of transcription, but that either enzyme can perform the required function. A recent paper suggests that the same situation may exist in the fission yeast, *Schizosaccharomyces pombe*. In that case, topoisomerase I mutants also are viable and topoisomerase I and II double mutants have phenotypes exhibited by neither single mutant (19). It remains to be seen whether topoisomerase II can substitute for topoisomerase I in higher eukaryotes.

We have previously shown that *E. coli* DNA topoisomerase I deletion mutants are viable (8), but in that case compensatory mutations, often in the gyrase genes, are necessary for viability (20). It is clear from the ease with which *top1* mutations are introduced into new strains that no such compensatory mutations are necessary for the viability of *S. cerevisiae top1* mutants.

The DNA sequence shown in Fig. 2 has several interesting features. Upstream of the *TOP1* coding sequence beginning at nucleotide 239, there is a stretch of 27 purines, 26 of which are adenine residues. Studies with synthetic polymers and recombinant DNA molecules suggest that this sequence probably will not form nucleosomes and it may therefore be a regulatory signal (21).

The predicted protein is extremely rich in lysine and glutamic acid residues. This is particularly true for a stretch of amino acids beginning at nucleotide 806. Twenty-six of 30 contiguous amino acids are charged, either acidic or basic, and 2 others are polar. Near the end of this cluster are 5 consecutive glutamic acid residues followed by a tyrosine. This sequence is very similar to the *v-src* tyrosine phosphorylation site found in several proteins including polyoma middle T antigen (22). Recently, it has been reported that several topoisomerases can be phosphorylated by tyrosine protein kinases with concomitant loss of activity (23). It would be of interest to learn whether yeast topoisomerase I can be phosphorylated by tyrosine protein kinases and whether this has any functional significance.

We thank D. Treco and M. Bienz for advice, M. Squire for help on the DNA sequencing, and M. Rose and G. Fink for the YCp50-derived gene library. R.S. thanks K. Nasmyth for hospitality during a sabbatical and for critical suggestions about cloning the *TOP1* gene. R.S. was the recipient of a Senior International Fellowship from the Fogarty Center of the National Institutes of Health. C.T. was supported by National Institutes of Health Training Grant T32GM08065. This work was supported by National Institutes of Health Grant GM28220.

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