Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7178–7182, November 1985
Biochemistry

Cloning of yeast *TOP1*, the gene encoding DNA topoisomerase I, and construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II

(*Saccharomyces cerevisiae*/DNA topoisomerase mutants)

TADAATSU GOTO AND JAMES C. WANG

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

Communicated by Mark Ptashne, June 19, 1985

ABSTRACT **Rabbit antibodies specific to yeast DNA topoisomerase I were used in immunological screening of a *Saccharomyces cerevisiae* genomic DNA library in *Escherichia coli*. One of the clones identified by its expression of antigenic determinants of the yeast enzyme is shown to contain the coding sequence of the enzyme: no active DNA topoisomerase I is detectable in cell extracts when insertion or deletion mutations are introduced into a 2-kilobase-pair (kb) region of the sequence in a haploid yeast genome. Blot hybridizations show that there is a single copy of the cloned sequence per haploid and that the sequence is transcribed to give a 2.7-kb poly(A)$^+$ message. Mutants in which 1.7 kb of the sequence is deleted are viable. Temperature-shift experiments using synchronously grown cells of a Δ*top1 top2* temperature-sensitive (ts) double mutant and its isogenic *top2* ts strain show that, whereas mitotic blocks can prevent killing of the *top2* ts mutant at a nonpermissive temperature, the same treatments are ineffective in preventing cell death of the Δ*top1 top2* ts double mutant. These experiments suggest that in yeast DNA topoisomerase I serves a role auxiliary to DNA topoisomerase II.**

In recent years, DNA topoisomerases have emerged as an important class of enzymes with multiple cellular functions (for reviews, see refs. 1–3). Although biochemical and enzymological studies of both eukaryotic and prokaryotic enzymes have been carried out extensively in the past decade and a half, functional studies of the enzymes in eukaryotes have been hampered by the paucity of genetic information. Intensive searches of genes encoding the eukaryotic topoisomerases have been carried out recently, particularly in organisms such as yeast that are easily amenable to genetic manipulations.

Yeast, like other eukaryotic organisms, is known to have a type I and a type II topoisomerase (4–7). Both enzymes have been purified to near homogeneity and are shown to be enzymologically rather similar to their counterparts in other eukaryotes (5, 7). We have used the purifed enzymes to raise rabbit antibodies, and these specific antibody preparations were used to screen yeast genomic libraries cloned in phage λgt11 (8). Both structural genes *TOP1* and *TOP2* encoding yeast DNA topoisomerase I and II, respectively, have been identified. Genetic analysis using the cloned *TOP2* gene has shown that the single-copy *TOP2* gene is essential for yeast viability (9, 10) and that the lethality caused by inactivation of DNA topoisomerase II occurs at the time of mitosis (10). DiNardo *et al.* (11) and Uemura and Yanagida (12) have reached similar conclusions in their analysis of mutants in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *TOP1* gene appears nonessential, however, from analysis of mutants lacking DNA topoisomerase I activity in cell extracts (12, 13). In this communication, we report our initial studies of the yeast *TOP1* gene.

MATERIALS AND METHODS

Materials. Yeast strains used in this study are CH335 (**a** *his4-539 lys2-801 ura3-52 TOP2*) and a *top2* temperature-sensitive (ts) mutant, CH325 (**a** *his4-539 lys2-801 ura3-52 top2-4*), derived from a commom parent (10). Restriction enzymes and other enzymes used in DNA cloning were purchased from New England Biolabs; α factor and nocodazole were from Sigma.

DNA Manipulations and Transformations. Restriction enzyme digestion and other manipulations of DNA and bacterial transformations were performed by standard methods (14). Yeast cells were transformed after treatment with lithium acetate (15). Transformants were selected on plates lacking uracil as described (16).

Cloning of the Gene *TOP1* Encoding Yeast DNA Topoisomerase I. Purification of DNA topoisomerase I from a protease-deficient strain 20B-12 (17) and commercially grown bakers' yeast (Federal Yeast) has been reported (7). Rabbit antibody preparation and gene cloning with the λgt11 vector (8) were carried out as described for yeast DNA topoisomerase II (9). Briefly, rabbit antibodies were prepared by the procedures of Vaitukaitis (18) with DNA topoisomerase I purified from strain 20B-12. About 5×10^5 plaques of a yeast genomic library in λgt11 were screened. Twelve plaques gave positive signals with various degrees of intensity. These phage were purified and screened further with a different preparation of antibodies elicited with the enzyme purified from commercial bakers' yeast. A clone λTOP1ex3 that gave a strong signal in the first round of screening again gave a strong signal in the second screening and was selected for further studies.

Preparation of Yeast Extracts for Activity Assays. Yeast cells were grown in 10 ml of 1% yeast extract/2% Bactopeptone/2% glucose (YPD) medium to about 5×10^7 cells per ml and were harvested by centrifugation. After being washed once with distilled water, cells were suspended in 0.8 ml of TEG-0.3 buffer (50 mM Tris·HCl, pH 7.4/1 mM Na_3EDTA/10% glycerol/1 mM phenylmethylsulfonyl fluoride/1 mM 2-mercaptoethanol/0.3 M KCl). Glass beads (0.7 g) were added, and cells were lysed by vigorous agitation (Vortex mixer) for 1 min. Cell debris and unlysed cells were removed by centrifugation. One microliter of the supernatant was used for DNA topoisomerase I assays, which were performed under the standard assay conditions (7) at 30°C for 30 min. Samples were electrophoresed in 0.7% agarose gels.

Genomic "Walk" by Plasmid Integration and Rescue. To acquire additional yeast genomic sequences bordering the cloned yeast DNA segment in λTOPlex3, a plasmid was first

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: kb, kilobase pair(s); ts, temperature sensitive.

constructed in which the *Eco*RI–*Sal* I segment in the tetracycline-resistance region of pBR322 was replaced by the *Eco*RI–*Sal* I fragment containing the bulk of the cloned yeast sequence in λTOP1ex3. The plasmid was cut at its single *Sph* I site within the yeast sequence (see Fig. 1*a* for the locations of the various restriction sites), and a 1.2-kilobase pair (kb) *URA3* fragment with *Bgl* II ends (9) was inserted after the ligation of *Bgl* II "linkers" to the repaired *Sph* I ends. DNA of the new plasmid was cut with *Mlu* I and used to transform CH335 *ura3* to uracil prototrophy. Integration of the plasmid into the yeast chromosome by homologous recombination at the *Mlu* I site generated one complete and one incomplete copy of *TOP1* separated by the plasmid sequences. The total DNA was prepared from a pool of Ura^+ transformants and digested with *Bgl* II. Among the fragments generated, one is expected to contain most of the original plasmid sequences and additional yeast chromosomal sequences contiguous to the *TOP1* sequence. This fragment was rescued by transforming *E. coli* with the *Bgl* II-digested DNA after ligation and selecting for ampicillin-resistant colonies. The principle of acquisition of additional genomic sequences bordering a cloned segment by plasmid integration and rescue has been described (19, 20).

Other Techniques. Temperature-shift experiments were performed as described (10) with modifications as described in the text. YPD liquid medium was used for routine yeast growth as well as for the temperature-shift experiments. For solid medium, 2% Bacto-agar was added.

RESULTS

A Clone λTOP1ex3 Contains the Structural *TOP1* Encoding Yeast DNA Topoisomerase I. Clone λTOP1ex3 produced antigenic determinants in *E. coli* that were recognized by rabbit antibodies elicited by yeast DNA topoisomerase I purified from a commercial bakers' yeast or by the same enzyme purified from laboratory-grown cells of strain 20B-12. This clone contains a 3.6-kb insert of yeast sequence flanked by two *Eco*RI sites. A restriction map of this fragment is shown in Fig. 1*a*.

To confirm further that λTOP1ex3 contains the structural gene encoding yeast DNA topoisomerase I, we constructed insertion mutants within the cloned yeast sequence and examined DNA topoisomerase I activity in cell extracts of these mutants.

The 3.6-kb insert between the *Eco*RI and *Sal* I sites was first recloned between these sites in pBR322, and five plasmids were constructed by inserting a 1.2-kb fragment containing *URA3* into the *Hin*dIII, *Nco* I, *Mlu* I, *Cla* I, or *Sph* I site. For each plasmid, the *Eco*RI–*Sal* I fragment containing the yeast sequences was purified by gel electrophoresis and used to transform a haploid strain, CH335 (10), to uracil prototrophy. As described by Orr-Weaver *et al.* (21) and by Rothstein (22), this transformation procedure leads to preferential integration of the fragment by homologous recombination into the yeast chromosome at the locus specified by the ends of the linear fragment—in this case within or near the putative *TOP1* gene. Transformants were selected and purified by two more cycles of plating. Integration of the *URA3* fragment at the expected loci were verified by Southern blotting (data not shown), which also showed that there is a single copy of the cloned sequence per haploid yeast genome.

Extracts of these mutant cells were prepared as described, and DNA topoisomerase I activity was assayed in the standard assay medium as described (7). Some of the results are shown in Fig. 2.

Insertion of the *URA3* fragment at the *Nco* I site had no effect on the level of topoisomerase I activity in cell extracts (compare the patterns of lanes 2 and 8 in Fig. 2). Insertion into the *Mlu* I site, which is 200 base pairs away from the *Nco* I site, however, reduced the enzyme level to <10% (Fig. 2, lanes 6 and 7). Insertion of the *URA3* fragment at the *Sph* I site (Fig. 2, lane 3) and the *Cla* I site (Fig. 2, lane 4) led to complete disappearance of the DNA topoisomerase I activity

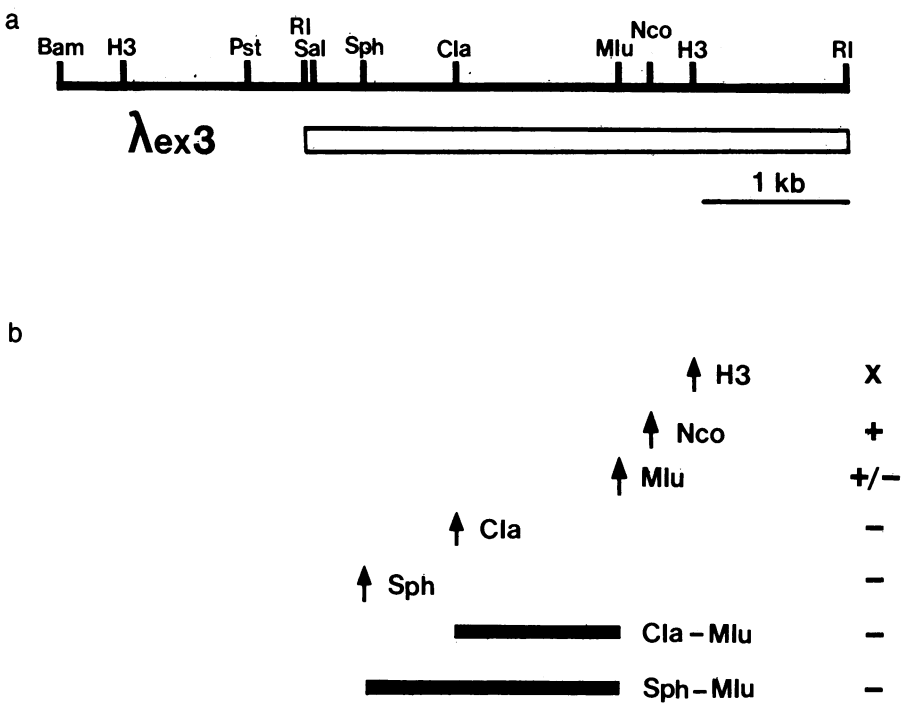

FIG. 1. (*a*) Restriction endonuclease map of the *TOP1* region. The box indicates the region present in λTOP1ex3. Bam, *Bam*HI; H3, *Hin*dIII; RI, *Eco*RI. An additional *Sph* I site near the *Pst* I site is not shown. (*b*) Construction of *top1* mutants. The *URA3* insertion points relative to the map in *a* are indicated by vertical arrows and the regions replaced by *URA3* in the deletion mutagenesis by bars. The presence (+) or absence (−) of DNA topoisomerase I activity in each mutant is indicated to the right. The symbol +/− indicates a reduced level of activity. See the text for the meaning of symbol X.

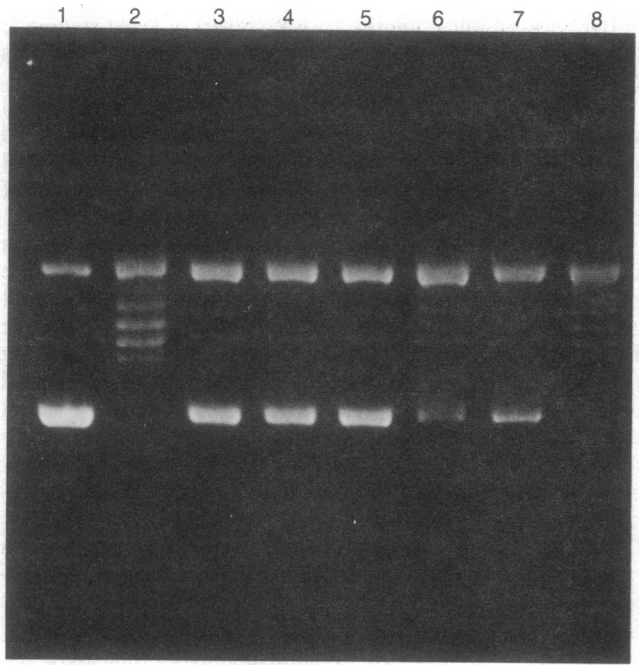

FIG. 2. DNA topoisomerase I activity in wild-type and mutant extracts. Extracts were prepared as described, and DNA topoisomerase I assays were performed under our standard conditions as described (7). Lanes: 1, control without extract; 2, extract from wild type (CH335); 3–8, mutant extracts resulting from *URA3* insertion at *Sph* I (lane 3), *Cla* I (lane 4), *Mlu* I (lanes 6 and 7), and *Nco* I (lane 8) sites and *URA3* replacement of *Cla* I–*Mlu* I (lane 5). The two *Mlu* I mutants (lanes 6 and 7) are independent isolates.

in the extracts. Thus, these results, in combination with the immunological results described above, confirm that the λTOP1ex3 fragment contains the coding sequences of *TOP1*.

***TOP1* Gene Is Nonessential for Yeast Viability.** Thrash *et al.* (13) have shown that a *S. cerevisiae* mutant (*mak1*) with no detectable level of topoisomerase I activity in the extract is viable. Similarly, Uemura and Yanagida (12) observed that *Schizosaccharomyces pombe* apparently lacking DNA topoisomerase I activity is viable. The results of our gene disruption experiments are entirely consistent with their observations and show that yeast cells with their *TOP1* gene disrupted at widely separated loci are all viable. These results do not prove, however, that *TOP1* is entirely dispensable. Although unlikely, these mutants might still retain low levels of DNA topoisomerase I activity not easily detectable in the crude lysates but sufficient to sustain growth. To rule out such a possibility, we extended the gene disruption analysis to include large deletions: one deletion is a 1.2-kb *Mlu* I–*Cla* I deletion, and another is a 1.7-kb *Mlu* I–*Sph* I deletion. The deleted fragments were replaced by a *URA3* fragment in the pBR322-based clone, and the altered *TOP1* fragments were isolated and used to transform yeast as described above. The extracts of these transformants showed no detectable DNA topoisomerase I activity (see Fig. 2, lane 5, for the *Mlu* I–*Cla* I deletion; the *Mlu* I–*Sph* I deletion gave similar results). A blot analysis of yeast poly(A)$^+$ RNA showed that the full-length *TOP1* message was approximately 2700 bases long (data not shown). Therefore, the loss of topoisomerase I activity in these mutants in which as much as 1.7 kb of the coding sequence was deleted most likely reflects the complete inactivation of the gene. These results strongly support the conclusion that the single-copy *TOP1* gene is dispensable in yeast.

Results of the insertion and deletion experiments are summarized in Fig. 1*b*. The arrows indicate the positions of insertions, and the bars indicate the region replaced by the *URA3* fragment; the presence or absence of yeast DNA topoisomerase I activity in cell extracts is indicated in the column to the right. The meaning of the symbol X for the insertion at the *Hin*dIII site will be explained below.

The Possible Presence of an Essential Gene Bordering *TOP1*. In the construction of insertion mutants, we also constructed a plasmid with the *URA3* marker inserted at the *Hin*dIII site (see Fig. 1). Transformation efficiency with the *Eco*RI–*Sal* I fragment containing the insert was lower than those with other fragments described above by 2–3 orders of magnitude. This observation suggests that there might be a single-copy essential gene in this region and that the disruption of this gene by integration of *URA3* at the *Hin*dIII site is lethal to yeast. Screening of yeast cDNA library with this region of DNA as the hybridization probe led to the isolation of a number of clones. The poly(A) end of these clones is located between the *Hin*dIII and *Nco* I sites shown in Fig. 1*a*, suggesting that the transcription unit of this putative essential gene includes the *Hin*dIII site and terminates before the *Nco* I site.

Isolation of Additional Yeast DNA Sequences in the *TOP1* Region by the Insertion and Rescuing of a *TOP1*-Containing Plasmid. The analysis thus far has indicated that one end of *TOP1* lies near the *Mlu* I site and that the *Sph* I and *Cla* I sites are within *TOP1*. Thus, the λTOP1ex3 fragment contains about 2 kb of *TOP1* (Fig. 1), which is shorter than the gene size estimated from both the size of the mRNA (2.7 kb) and the molecular mass of purified yeast DNA topoisomerase I (90,000 daltons; ref. 7). Therefore, we recovered a genomic fragment containing additional sequences adjacent to the λTOP1ex3 insert by using a plasmid rescue method as described (see also ref. 19). The rescued plasmid contained about 4.2 kb of additional yeast sequences beyond the *Sal* I site of λTOP1ex3.

Physiological Studies of Δ*top1* and Δ*top1 top2* ts Double Mutant. In addition to the Δ*top1* deletion mutants described earlier, we constructed a Δ*top1 top2* ts double mutant by replacing the *Sph* I–*Mlu* I 1.7-kb segment within the gene with *URA3* in the *top2* ts strain CH325 (*top2–4*) (10) using the one-sep gene replacement technique. Several independent isolates were found to exhibit similar growth characteristics. Genomic mapping by blot hybridization confirmed the replacement of the *TOP1* sequence by *URA3* in these strains (result not shown). One of these mutants, TG205 (Δ*top1 top2–4*), was selected for further analysis.

TG205 (Δ*top1 top2–4*) grew slowly at a permissive temperature of 26°C. The cell doubling time was about 4 hr, as opposed to 2 hr for the *TOP1*$^+$ *TOP2*$^+$ strain CH335 or its isogenic *top2* ts and Δ*top1* single mutants. We examined cultures of the strains first grown asynchronously to logarithmic phase at 26°C and then shifted to 35°C, a nonpermissive temperature for *top2–4*. Counting of viable cells by plating (at 26°C) aliquots sampled at various times showed that, whereas the wild-type strain and its Δ*top1* derivative continued to grow after the temperature shift, a rapid decrease in the number of viable cells was observed for *top2* ts and Δ*top1 top2* ts mutants (data not shown). The rate of cell death for the double mutant was slower than that of *top2* single mutant by a factor of about 2, reaching 10% survival level in 4 hr as opposed to 2 hr for the single mutant. Examination of cells by optical microscopy revealed that, after one doubling time (2 hr) at the nonpermissive temperature, the *top2* ts cells showed a mixture of two morphologies: unbudded cells and cells with large buds (10, 11). In contrast, the double mutants after one doubling time (4 hr) exhibit a heterogeneous mixture of shapes. The latter observation is consistent with that made by Uemura and Yanagida (12) with *top1 top2* double mutants of *S. pombe*.

To clarify further the apparent differences between *top2* single and *top1 top2* double mutants, we repeated the

temperature-shift experiments for synchronously grown cells in the same manner as carried out for *top2* ts mutants by Holm *et al.* (10). Cultures of the mutants and their wild-type control were first grown at 26°C to the exponential phase. Growth was then arrested by treatment with α factor for 3 hr (*top2* mutant) or 6 hr (*top1 top2* double mutant). In one experiment, the cultures were washed free of the mating factor, resuspended in fresh medium with or without a microtubule-destabilizing drug nocodazole (10, 23, 24), and incubated at 35°C. In a second experiment, α factor was added back to the washed and resuspended cells prior to the temperature-shift. Aliquots were removed from these cultures at different times and plated after appropriate dilutions; the plates were incubated at 26°C to determine the number of viable cells. The results of these experiments are summarized in Table 1.

At 26°C, the control cultures without nocodazole grew as expected; the concentration of viable cells doubled in ≈2 hr for CH325 (*top2-4*) and ≈4 hr for TG205 (Δ*top1 top2-4*). The addition of nocodazole to 20 μg/ml inhibited the growth of yeast (23, 24), and the concentration of viable cells of TG205 (Δ*top1 top2-4*) remained constant during incubation with the drug at the permissive temperature.

Upon shifting the temperature to 35°C, cell death in the absence of the mitotic inhibitor occurred with either the *top2* ts strain or the Δ*top1 top2* ts double mutant. The slower killing rate observed for the double mutant (16% survival after 4 hr at 35°C compared with 15% survival after 2 hr at 35°C for the single mutant) is similar to that observed for asynchronous cultures described earlier. More importantly, whereas the addition of nocodazole prevented cell killing in the case with the *top2* ts strain (ref. 10; Table 1), it did not block cell killing in the case with the Δ*top1 top2* ts strain; comparable rates of loss of viability were seen for the double mutants with and without nocodazole upon shifting to the nonpermissive temperature. Similarly, whereas the presence of α factor after the temperature shift blocked completely the killing of *top2* ts cells, it did not rescue the double mutant.

Microscopic observations of cells made during these experiments revealed that the Δ*top1 top2* ts mutants at the nonpermissive temperature retained the morphology at the time of temperature shift—i.e., the morphology characteristic of α factor-arrested cells. Therefore, the lack of a uniform morphology observed in the temperature-shift experiment with an asynchronous culture of the Δ*top1 top2* ts mutant (see above) suggests that the growth arrest and subsequent cell killing of the double mutant at the nonpermissive temperature occurs irrespective of the stage in the cell cycle.

DISCUSSION

We cloned the structural gene *TOP1* encoding yeast DNA topoisomerase I by immunological screening of a yeast genomic library in an *E. coli* expression vector. Blot hybridizations using the cloned sequence as a probe show that *TOP1* is a single-copy gene and is transcribed into a 2.7-kb message. Four strains in which a *URA3* marker is inserted at widely separated points spanning a 2-kb region of the *TOP1* gene are shown to lack or to have a much reduced level of DNA topoisomerase I in cell extracts. The absence of the enzyme in cell lysates is also shown for two deletion mutants in which as much as 1.7-kb of the *TOP1* coding sequence is deleted. All deletion and insertion mutants are viable, however. Thus, our results confirm the earlier suggestions based on studies of chemically induced mutants that yeast DNA topoisomerase I is nonessential (11, 12). Very recently, Thrash *et al.* (25) independently have cloned the yeast *TOP1* gene by methods that differ from the one used by us and have shown that a *TOP1* deletion mutant is viable. They have sequenced a 3.8-kb fragment containing *TOP1*. The boundaries of the gene mapped in the present work by insertional mutagenesis and blot hybridizations are in complete agreement with predictions based on the presence of a 2.7-kb open reading frame in their sequence.

Whereas mitotic blocks can prevent the killing of yeast *top2* ts cells at a nonpermissive temperature (10), such blocks are ineffective in preventing cell death in Δ*top1 top2* ts mutants. These experiments suggest that DNA topoisomerase I normally has an auxiliary role *in vivo*; in a *top1* mutant, such a role is presumably carried out by DNA topoisomerase II, which is also essential in resolving the intertwined progeny DNA molecules at mitosis (10–12, 26). A similar suggestion was made earlier based on morphological differences of *top1 top2* ts and *top2* ts mutants at nonpermissive temperatures (12). Some of the plausible roles of DNA topoisomerase I consistent with the physiological studies and known biochemical properties of the topoisomerases include relieving the topological constraints in the untwining of parental strands during replication and in transcription and chromatin organization (reviewed in refs. 1–3, and 27).

Table 1. Viability of TG205 (Δ*top1 top2-4*) and CH325 (*top2-4*) after incubation at the restrictive temperature

Incubation		Viability after incubation			
		CH325		TG205	
°C	Treatment	1 hr	2 hr	2 hr	4 hr
26	Control	1.3	2.0	0.92	1.75
	Nocodazole	ND		0.86	0.91
35	Control	0.83	0.15	0.44	0.16
	α factor	1.2	1.1	0.70	0.43
	Nocodazole	0.86	0.93	0.48	0.20

*Numbers refer to the ratio of the number of viable cells at each time point to the number of viable cells at the time of release from α-factor. Control cultures received neither nocodazole nor α factor. ND, not determined (see ref. 10).

We thank Connie Holm for advice and Rolf Sternglanz for sending a manuscript on the cloning of yeast *TOP1* gene prior to publication. This work was supported by grants from the American Cancer Society and the U.S. Public Health Service.

1. Gellert, M. (1981) *Annu. Rev. Biochem.* **50**, 879–910.
2. Wang, J. C. (1985) *Annu. Rev. Biochem.* **54**, 665–697.
3. Vosberg, H.-P. (1985) *Curr. Top. Microbiol. Immunol.* **114**, 19–102.
4. Durnford, J. M. & Champoux, J. J. (1978) *J. Biol. Chem.* **253**, 1086–1089.
5. Gotb, T. & Wang, J. C. (1982) *J. Biol. Chem.* **257**, 5866–5872.
6. Badaracco, G., Plevani, P. L., Ruyechan, W. T. & Chang, L. M. S. (1983) *J. Biol. Chem.* **258**, 2022–2026.
7. Goto, T., Laipis, P. & Wang, J. C. (1984) *J. Biol. Chem.* **16**, 10422–10429.
8. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
9. Goto, T. & Wang, J. C. (1984) *Cell* **36**, 1073–1080.
10. Holm, C., Goto, T., Wang, J. C. & Botstein, D. (1985) *Cell* **41**, 553–563.
11. DiNardo, S., Voelkel, K. A. & Sternglanz, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 857–872.
12. Uemura, T. & Yanagida, M. (1984) *EMBO J.* **3**, 1737–1744.
13. Thrash, C., Voelkel, K., DiNardo, S. & Sternglanz, R. (1984) *J. Biol. Chem.* **259**, 1375–1377.
14. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 202–203.
15. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
16. Sherman, F., Fink, G. & Hicks, J. (1983) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Rev. Ed.
17. Zubenko, G. S., Park, F. J. & Jones, E. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 510–514.

18. Vaitukaitis, J. L. (1981) *Methods Enzymol.* **73,** 46–52.
19. Roeder, G. S. & Fink, G. R. (1980) *Cell* **21,** 239–249.
20. Botstein, D. & Davis, R. W. (1982) in *The Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 607–636.
21. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6354–6358.
22. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–211.
23. Kilmartin, J. V. (1981) *Biochemistry* **20,** 3629–3633.
24. Jacobs, C. W., Adams, A. E. M. & Pringle, J. R. (1984) *J. Cell Biol.* **99,** 43a.
25. Thrash, C., Bankier, A. T., Barrell, B. G. & Sternglanz, R. (1985) *Proc. Natl. Acad. Sci. USA*, **82,** 4374–4378.
26. Sundin, O. & Varshavsky, A. (1980) *Cell* **21,** 103–114.
27. Liu, L. F. (1983) *Crit. Rev. Biochem.* **15,** 1–24.