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Biochemistry

Effects of yeast DNA topoisomerase III on telomere structure

(genome stability/genetic recombination/subtelomeric elements/*EST1* gene/*TOP3* gene)

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ABSTRACT **The yeast *TOP3* gene, encoding DNA topoisomerase III, and *EST1* gene, encoding a putative telomerase, are shown to be abutted head-to-head on chromosome XII, with the two initiation codons separated by 258 bp. This arrangement suggests that the two genes might share common upstream regulatory sequences and that their products might be functionally related. A comparison of isogenic pairs of yeast *TOP3*$^+$ and Δ*top3* strains indicates that the $G_{1-3}T$ repetitive sequence tracks in Δ*top3* cells are significantly shortened, by about 150 bp. Cells lacking topoisomerase III also show a much higher sequence fluidity in the subtelomeric regions. In Δ*top3* cells, clusters of two or more copies of tandemly arranged Y′ elements have a high tendency of disappearing due to the loss or dispersion of the elements; similarly, a *URA3* marker embedded in a Y′ element close to the chromosomal tip shows a much higher rate of being lost relative to that in *TOP3*$^+$ cells. These results suggest that yeast DNA topoisomerase III might affect telomere stability, and plausible mechanisms are discussed.**

The budding yeast *Saccharomyces cerevisiae* is known to possess three distinct DNA topoisomerases, each of which belongs to a separate topoisomerase subfamily (for compilation of members of the three subfamilies and their amino acid sequence alignments, see ref. 1). Yeast DNA topoisomerases I and II have been studied extensively. The former closely resembles DNA topoisomerase I of all other eukaryotes and is a member of a subfamily of type I topoisomerases that also includes poxvirus topoisomerase and DNA topoisomerase V of the thermophilic prokaryote *Methanopyrus kandleri* (2). The enzyme relaxes both positive and negative supercoils, and genetic and biochemical evidence suggests that the enzyme is involved in transcription and replication (reviewed in refs. 3 and 4). Yeast DNA topoisomerase II is a type II DNA topoisomerase. Like yeast topoisomerase I, it also relaxes both positive and negative supercoils (reviewed in refs. 5 and 6). Thus, yeast Δ*top1* mutants, lacking DNA topoisomerase I, are viable, as its normal cellular roles can presumably be filled by topoisomerase II (reviewed in refs. 3, 5, and 6). The type II enzyme has the unique ability of unlinking intertwined pairs of newly replicated DNA rings of chromosomes, and in its absence cells cannot survive segregation of the chromosomes during mitosis. The type II enzyme is also involved in chromosome condensation and decondensation (7–10), and the possibility of its involvement in the organization of chromosomes has also been suggested (refs. 11–14; see refs. 10 and 15 for a contrary view).

In contrast to its more extensively studied congeners, yeast DNA topoisomerase III remains largely an enigma. It was initially identified as a member of the DNA topoisomerase subfamily represented by *Escherichia coli* DNA topoisomerases I and III, from a comparison of primary sequences (16). Purification of the protein from yeast cells overexpressing the gene (*TOP3*) encoding yeast topoisomerase III has subsequently confirmed this assignment: the purified protein partially but specifically relaxes highly negative supercoiled DNAs, and biochemical characterization of the yeast enzyme indicates that it is more closely related to *E. coli* topoisomerase III than to *E. coli* topoisomerase I (17). *In vivo*, the enzyme also appears to have a weak activity in the removal of negative supercoils; in cells expressing either topoisomerase I or II, the weak activity of topoisomerase III is masked (17). One might therefore expect that inactivation of a weaker topoisomerase III relaxation activity in the presence of two stronger ones—namely, those of topoisomerases I and II—should have little effect on cell physiology. Surprisingly, null mutations in *TOP3* in a *TOP1*$^+$*TOP2*$^+$ genetic background result in slower growth, hyperrecombination between repetitive sequence elements, and defective sporulation (16).

The lack of a coherent picture relating the known enzymatic activity of yeast topoisomerase III to the phenotypes of cells lacking it has led to the speculation that the *in vivo* role of the protein is probably not directly related to its relaxation of negative supercoils; instead, the enzyme might be involved in the separation of intertwined DNA single strands, perhaps in conjunction with a helicase (6, 18). Interestingly, an extragenic suppressor mutation that suppresses the slow-growth and hyperrecombination phenotypes of *top3* mutants has recently been mapped to a gene (*SGS1*) encoding a protein homologous to the *E. coli* RecQ protein, the primary sequence of which suggests that it might be a helicase (19).

In a search for sequence homologies between yeast topoisomerase III and other proteins, we found that the yeast *TOP3* and *EST1* genes, the latter encoding a putative telomerase (20), might be positioned head-to-head at the same chromosomal location, with 258 bp between the two initiation codons (see below). This finding was unexpected, as *TOP3* and *EST1* were mapped to two different chromosomes, XII and VIII, respectively (16, 20). A divergent pair of genes separated by a short distance of 258 bp would suggest that the two genes might be coregulated, raising the possibility that DNA topoisomerase III might be involved in the maintenance of telomere structure. Here we report evidence in support of these possibilities.

MATERIALS AND METHODS

Yeast Strains and Plasmids. *S. cerevisiae* strains CH1105 (*MATα ade2-101 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52*) and CH1585 (*MAT***a** *leu2-Δ1 trp1-Δ63 ura3-52 his3-200*) were obtained from C. Holm (University of California, San Diego); strain JCW15 was derived from CH1105 by switching its mating type. The Δ*top3* derivatives of these strains were obtained by the one-step gene transplacement method (21), using a segment from the plasmid described below. A 3.3-kb *Eco*RI–*Xba* I fragment of *TOP3* in pUC18 was moved into pBluescript (Stratagene) as an *Eco*RI–*Sal* I fragment. The

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resulting plasmid was digested with *Hpa* I and *Nae* I, which cut respectively at positions 1038 and 1067 of the *TOP3* sequence (16), and an 850-bp *TRP1* marker was inserted between these sites. An *Eco*RI–*Sal* I segment from the *TRP1*-bearing plasmid was then used to transform the various strains to Trp^+ (17).

A plasmid bearing yeast *ARG4* was obtained from N. Kleckner (Harvard University), and plasmids bearing *TOP3* and rDNA were from laboratory stock (17, 22). Probes for the telomere-associated Y′ element and *EST1* gene were derived respectively from YCp50-131Y (23) and from a 5.8-kb *Eco*RI fragment of yeast genomic DNA bearing *TOP3* and part of *EST1* in M13mp18 (17). Introduction of a *URA3* marker into a telomere-associated Y′ element was carried out as described by Louis and Haber (24), by using a *Pvu* I–*Eco*RI fragment of plasmid pEL-2.

Cloning and Sequencing of the *TOP3–EST1* Intergenic Region. A pair of primers, 5′-CAGGAAGCATCTGAACGTGA-3′ and 5′-GAAATCCCTTGAAGTTGATCTGC-3′, whose sequences were based on those of the noncoding strands near the beginning of the *EST1* and *TOP3* open reading frames (16, 20), respectively, were used to sequence the intergenic region on a plasmid clone and to amplify by PCR the intergenic segment in DNA samples isolated from the *Δtop3::TRP1*$^+$ strains used in the present work, JCW53 and JCW153. Chromosomal DNA samples prepared from the two strains were used in the PCRs. The amplified DNAs were individually purified by agarose gel electrophoresis as a 0.5-kb fragment and cloned into the *Sma* I site of pUC18. Nucleotide sequencing of the clones was done by the Sanger dideoxynucleotide chain-termination method.

Other Methods. Genetic manipulations of yeast strains were carried out by established methods (e.g., ref. 25). Blot hybridization of yeast telomeric sequences with poly(dG-dT) was done according to Walmsley *et al.* (26). A nylon membrane blot of yeast chromosomes resolved by orthogonal pulsed-field agarose gel electrophoresis was kindly provided by C. Holm (University of California, San Diego).

RESULTS

Identification of *TOP3* and *EST1* as a Divergent Pair of Genes with 258 bp Between Their Initiation Codons. A search of nucleotide sequences homologous to the published *TOP3* sequence (16) identified a 146-bp segment in the 5′ untranslated region of *EST1* that is nearly identical to a 5′ untranslated region of *TOP3*. Within this stretch there is only a single mismatch between the coding strand of *TOP3* and the noncoding strand of *EST1* in the published sequences. This close match in sequences suggested that *TOP3* and *EST1* might be a pair of divergent genes with overlapping 5′ untranslated regions.

However, *TOP3* has been mapped to chromosome XII (16), and *EST1* to chromosome VIII (20). To check the chromosomal locations of these two genes, blot hybridization of yeast chromosomes resolved by pulsed-field agarose gel electrophoresis was carried out with four radiolabeled probes, two separately prepared from the coding regions of cloned *TOP3* and *EST1* genes, one from an *ARG4* clone known to map to chromosome VIII, and an rDNA segment known to map to chromosome XII. Only the *ARG4* probe hybridized to chromosome VIII, and both *TOP3* and *EST1* hybridized to chromosome XII, where the rDNA cluster resides (data not shown). The assignment of *EST1* to chromosome XII also explains its absence from the recently published sequence of the entire chromosome VIII (27).

To confirm that *TOP3* and *EST1* are indeed a closely linked divergent pair of genes, a pair of primers complementary to the coding strands near the beginning of the *TOP3* and *EST1* coding regions were used to sequence a plasmid clone containing a 5.8-kb *Eco*RI yeast genomic DNA fragment within which *TOP3* and its upstream sequences reside. The sequencing results as well as additional sequencing data on DNA samples amplified from the *Δtop3* strains JCW53 and JCW153 confirmed unequivocally that *TOP3* and *EST1* are abutted head-to-head (data not shown). Where our sequencing data overlapped with the published results, no difference was found between our data and the published sequence of *EST1* (20), and only one discrepancy was found between our data and the published sequence of *TOP3* (16), with C51 in the published sequence being a G in ours. The sequencing results with DNA samples derived from the *Δtop3* strains used in the present work also show that during the construction of these strains, no inadvertent changes were introduced into the upstream region; if such changes had occurred, they could alter the regulation of the *EST1* gene and thus complicate the interpretation of the results to be described below.

Effect of *top3* Null Mutation on Telomere Length. The presence of *TOP3* and *EST1* as a pair of very closely linked genes sharing untranscribed upstream sequences suggested that *TOP3*, like *EST1* (20), might be involved in telomere function. Genomic DNA samples from an isogenic pair of *TOP3*$^+$ *and Δtop3* strains were therefore prepared and digested with *Xho* I restriction endonuclease, which cuts at a highly conserved site in the subtelomeric Y′ element ≈1.3 kb from the chromosomal tips carrying the Y′ elements (23, 24, 26, 28–31). *Xho* I fragments were resolved by agarose gel electrophoresis and blot hybridized with radiolabeled poly[(dG-dT)·(dA-dC)] to detect fragments containing the $G_{1-3}T$ telomeric repetitive sequences (26). The broad band in the *Xho* I digest of the *TOP3*$^+$ strain CH1105 DNA (Fig. 1, lane 1, arrowhead) has an electrophoretic mobility corresponding to DNA fragments of ≈1.3 kb, characteristic of the terminal *Xho* I fragments of many yeast chromosomes (23, 24, 26, 28–30). The broadness of this band is due to heterogeneity in the lengths of the $G_{1-3}T$ tracts at the chromosomal ends (32). Interestingly, the corresponding band in the *Xho* I digest of the *Δtop3* strain JCW53 DNA migrates significantly faster, corresponding to a shortening of the telomeric sequences by 100–150 bp (Fig. 1, lane 2). The *Δtop3* genomic DNA sample used in the experiment shown in Fig. 1 was prepared from cells that had been propagated for 40–50 generations since the disruption of the *TOP3* locus; no further reduction in the sizes of the terminal *Xho* I fragments was apparent upon passage of the cells for an additional 50 generations (results not shown).

High Incidence of Loss of the Telomere-Associated Y′ Element in *Δtop3* Cells. In most *S. cerevisiae* strains there are two classes of telomere-associated middle-repetitive sequences, a highly conserved 6.7-kb Y′ element, which is also present in a form shortened by about 1.5 kb, and a less

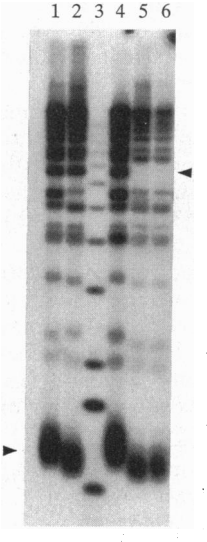

FIG. 1. Agarose gel electrophoresis of *Xho* I digests of DNA from *TOP3*$^+$ yeast strain CH1105 (lanes 1 and 4) and from three individual isolates of its *Δtop3* derivative JCW53 (lanes 2, 5, and 6). Blot hybridization was carried out with ^{32}P-labeled poly(dG-dT) as probe. Lane 3 contained the "1-kb ladder" size markers (BRL) ^{32}P-labeled at their 3′ ends. The position of the ≈1.3-kb band containing the chromosomal tips of DNA from the TOP3$^+$ strain is marked by an arrowhead at left margin, and that of the 6.7-kb Y′ fragment is marked by an arrowhead at right. See text for additional details.

conserved, 0.3- to 3.8-kb X element (23, 31). These elements are embedded in the $G_{1-3}T$ repeats and therefore the presence of adjacent Y′ elements would give larger *Xho* I fragments in addition to the heterogeneous 1.3-kb telomeric band when probed with radiolabeled poly(dG-dT).

The Y′ elements can undergo mitotic and meiotic rearrangements or loss (24, 29, 30, 33–35). In the DNA samples from two of the Δ*top3* isolates, analyzed in lanes 5 and 6 of Fig. 1, which were prepared from individual colonies derived from the same Δ*top3* haploid parent analyzed in lane 2, the 6.7-kb *Xho* I fragment containing the Y′ element is missing.

The loss of the 6.7-kb Y′ fragment in these Δ*top3* isolates was confirmed by additional blot-hybridization experiments using a cloned *Xho* I–*Sal* I Y′ fragment (24) as probe. DNA obtained from the *TOP3*$^+$ parent strain (Fig. 2, lanes 1–3) and the two isolates of its Δ*top3* derivative (lanes 4–6 and 7–9) were digested with *Xho* I, *Hin*dIII, or *Bgl* II and analyzed by agarose gel electrophoresis. The 6.7-kb *Xho* I fragment (band *a*), the 4.6-kb *Hin*dIII fragment (band *b*), and the 4.8-kb *Bgl* II fragment (band *c*) are all derived from two tandemly joined Y′ elements. These bands are prominent in the appropriate digests of DNA from *TOP3*$^+$ cells (lanes 1–3) but are essentially absent in the DNA samples from the two Δ*top3* isolates (lanes 4–6 and 7–9). The 2.1-kb *Hin*dIII fragment (band *d*), which is derived from internal cuts within the 6.7-kb Y′ element, appears to be less abundant in the Δ*top3* samples (lanes 5 and 8) than in the *TOP3*$^+$ sample (lane 2). A similar reduction in the intensity of the 1.7-kb *Bgl* II internal fragment (band *e*), which can form from both the 6.7-kb Y′ element and its shortened derivative, can be seen by comparing the patterns shown in lanes 3, 6, and 9.

Shortening of the $G_{1-3}T$ repeats at the chromosomal tips of Δ*top3* cells can also be seen from Fig. 2 by comparing the *Hin*dIII or *Bgl* II digest of the Δ*top3* samples with the corresponding digest of the *TOP3*$^+$ sample. The telomeric fragments show as a *Hin*dIII band around 2.9 kb (band *f*) or *Bgl* II band around 1.5-kb (band *g*) in the *TOP3*$^+$ sample (lanes 2 and 3, respectively), and these fragments are significantly shortened in the Δ*top3* samples. The telomeric *Xho* I fragments

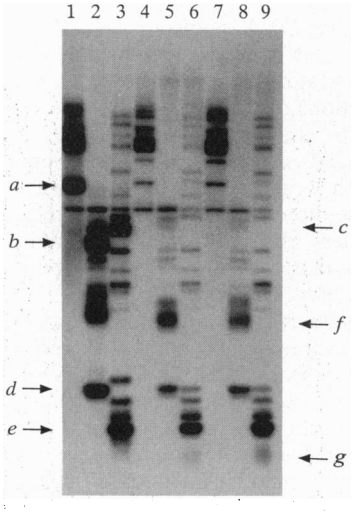

FIG. 2. Agarose gel electrophoresis of three sets of restriction enzyme digests of yeast DNA from strain CH1105 *TOP3*$^+$ cells (lanes 1–3) and from two individual isolates of the isogenic Δ*top3* strain JCW53 (lanes 4–6 and 7–9). In each set of three samples from left to right, digestion was carried out with *Xho* I, *Hin*dIII, and *Bgl* II, respectively. Bands marked by arrows *a*–*g* are explained in the text. Blot hybridization was carried out with ^{32}P-labeled Y′ sequences copied from a gel-purified *Xho* I–*Sal* I Y′ fragment by the use of the Klenow fragment of *E. coli* DNA polymerase I and short primers of random sequences.

are not recognized by the Y′ probe used and are therefore unseen in the Fig. 2 autoradiogram.

From two experiments the frequency of loss of the Y′ 6.7-kb *Xho* I fragment in Δ*top3* cells propagated for about 40 generations is estimated to be ≈5%. In each experiment, a single colony of strain JCW53 (Δ*top3*::*TRP1*$^+$) was picked and colony purified on minimal agar lacking tryptophan, and cells from a single colony were plated on nutrient agar. Eighteen colonies were then randomly picked for DNA preparation and restriction analysis. Among the first set of 18 DNA samples, 2 were found to have lost the 6.7-kb Y′ *Xho* I fragment. Among the second set of 18 samples, 1 had lost the fragment. No loss of the fragment was seen in control experiments with the same number of isolates of the isogenic strain CH1105 (*TOP3*$^+$) (data not shown). To check whether the procedure used in the construction of strain JCW53 from CH1105 might have led to the loss of the 6.7-kb fragment, analysis of DNA samples from 36 colonies in a mock transformation, in which strain CH1105 was transformed from Ura$^-$ to Ura$^+$ with pRY131, was carried out. No loss of the fragment was observed (results not shown).

Effect of Inactivation of Yeast Topoisomerase III on Stability of the Telomeric Region. To test further the plausible involvement of yeast topoisomerase III in telomere function, a *URA3* marker was embedded in a telomeric Y′ element and the frequencies of loss of the marker in *TOP3*$^+$ and Δ*top3* cells were monitored by the procedures of Louis and Haber (29). Because of the increased fluidity of telomeric elements in the Δ*top3* genetic background, as indicated by the experiments described above, a comparison of the rates of Ura$^+$ to Ura$^-$ changes was made through the use of four independent pairs of spores, each consisting of a Ura$^+$ and a Ura$^-$ sibling dissected from the same tetrad. In this experiment, a diploid strain RK102 *TOP3*$^+$/*TOP3*$^+$ was first constructed by mating strain CH1105 (*MAT*α) with CH1585 (*MAT***a**) and selecting for Lys$^+$His$^+$ colonies. The one-step gene transplacement procedure was then applied to yield a *TOP3*$^+$/Δ*top3*::*TRP1*$^+$ strain, RK103. Heterozygosity of the *TRP1*$^+$ product was confirmed by blot hybridization of the *Eco*RI digest of its DNA, using a radiolabeled fragment derived from the *TOP3* gene (data not shown). To integrate a *URA3* marker into a Y′ element of the *TOP3*$^+$/Δ*top3*::*TRP1* diploid, a 3.7-kb *Eco*RI–*Pvu* II fragment of pEL-2 (24), in which a segment containing *SUP11* and *URA3* is flanked by Y′ sequences, was gel purified and used to transform strain RK103 to Ura$^+$. DNA samples prepared from individual Ura$^+$ transformants were digested with *Pst* I, and the digests were analyzed by agarose gel electrophoresis and blot hybridization using Y′ and *URA3* probe in separate experiments. Four individual transformants that yielded restriction fragments signifying the integration of *URA3* into a Y′ element were selected. Each of these four transformants was allowed to sporulate, and *TOP3*$^+$ (Trp$^-$) and *top3*$^-$ (Trp$^+$) pairs of spores, each pair dissected from the same tetrad, were obtained. These pairs were further screened for the presence of the *URA3* marker and their mating types. Four pairs of the selected haploids, each with a Ura$^+$ *TOP3*$^+$ and a Ura$^+$ *top3*$^-$ member of the same mating type, were subjected to Delbrück–Luria fluctuation assays for the determination of the rate of loss of the *URA3* marker during growth under nonselective conditions (Table 1).

Unlike genes immediately adjacent to the telomeric $G_{1-3}T$ repeats, which are subjected to reversible repression (36), *URA3* embedded in a telomeric Y′ element is not repressed (29). The latter finding was confirmed: when the putative *ura3*$^-$ colonies isolated from agar plates containing 5-fluoroorotic acid were restreaked on minimal agar plates lacking uracil, no colonies were formed, indicating that colonies scored on the 5-fluoroorotic acid plates were formed by cells that had lost the *URA3* marker.

It is clear from Table 1 that relative to their isogenic *TOP3*$^+$ siblings, Δ*top3* cells exhibit a much higher rate of loss of the

Table 1. Rates of loss of *URA3* embedded in Y′

Pair	Exp.	Spore	No. of cells*	No. of Ura⁻ cells†	N‡	Rate of loss of marker§
1	1	*TOP3*⁺	4.7×10^9	9.5×10^5	32	6.3×10^{-6}
		Δ*top3*	5.5×10^8	2.2×10^6	26	1.4×10^{-4} (22)
	2	*TOP3*⁺	1.2×10^9	2.3×10^5	30	6.4×10^{-6}
		Δ*top3*	5.6×10^8	1.1×10^6	25	7.8×10^{-5} (12)
2	1	*TOP3*⁺	4.3×10^9	5.0×10^5	32	3.6×10^{-6}
		Δ*top3*	1.0×10^7	1.4×10^6	21	6.7×10^{-3} (1900)
	2	*TOP3*⁺	8.5×10^8	2.0×10^4	30	7.8×10^{-7}
		Δ*top3*	1.2×10^9	4.8×10^6	25	1.6×10^{-4} (205)
3	1	*TOP3*⁺	1.2×10^9	5.0×10^5	31	1.3×10^{-5}
		Δ*top3*	1.3×10^9	4.8×10^6	31	1.2×10^{-4} (9)
	2	*TOP3*⁺	1.6×10^9	5.4×10^5	31	1.1×10^{-5}
		Δ*top3*	4.5×10^8	3.8×10^6	24	3.5×10^{-4} (32)
4	1	*TOP3*⁺	8.8×10^9	2.2×10^7	33	7.6×10^{-5}
		Δ*top3*	1.0×10^7	3.2×10^6	21	1.5×10^{-2} (200)
	2	*TOP3*⁺	1.5×10^9	7.8×10^5	30	1.7×10^{-5}
		Δ*top3*	5.2×10^8	3.2×10^6	24	2.6×10^{-4} (15)

Pairs of *TOP3*⁺ and Δ*top3* spores, each from the same tetrad derived from a sporulating diploid, were used in the comparisons. Eight colonies corresponding to four selected pairs of matched spores were picked and each was resuspended in YEPD medium and serially diluted with the same medium to the limit-dilution end point. Highest dilutions (10 ml each) that showed growth after 48 hr at 26°C in a shaker incubator were used in plating assays of total and Ura⁻ cells (the latter were scored as colonies on agar plates containing 5-fluoroorotic acid; see text for further details).

*Total no. of cells in 10 ml of culture.

†Ura⁻ cells were assayed by plating serial dilutions on 5-fluoroorotic acid plates; streaking of the resulting colonies on uracil-dropout plates indicated that cells in these colonies had lost the *URA3* marker.

‡No. of generations calculated from log(total cells)/log 2.

§(No. of Ura⁻ cells)/(total no. of cells)(N); number in parentheses is the ratio of the rate in Δ*top3* cells to that in the control *TOP3*⁺ cells.

URA3 marker embedded in a Y′ element. Although variation between the two sets of experiments is relatively large, in all cases the rate of loss of *URA3* is higher in Δ*top3* than in *TOP3*⁺ cells, by a factor ranging from 10 to nearly 2000. The broad spread appears to be at least partly due to variability of the position, and perhaps the context as well, of the particular Y′ element within which *URA3* resides. When DNA samples of the four pairs of spores were digested with *Apa* I, which cuts within *URA3* once but does not cut Y′, a pair of fragments 2.5 kb and 10 kb in length, in addition to the 3.5-kb chromosomal *ura3-52* fragment, were detected in spore pairs 2 and 4 by blot hybridization with a *URA3* probe (Fig. 3). The 2.5-kb band was also detectable by hybridization with poly(dG-dT), indicating that in these spores, which show a much higher rate of loss of *URA3* in the Δ*top3* siblings, the *URA3* marker was inserted into a Y′ element immediately adjacent to the terminal $G_{1-3}T$ repeats; thus, cutting with *Apa* I yielded a 2.5-kb terminal fragment that hybridized with either the *URA3* or the poly(dT-dG) probe. For spore pair 3, an *Apa* I fragment of ≈12 kb was recognized by the *URA3* probe in addition to the 3.5-kb fragment, indicating that the URA3 marker had integrated into a more centromerically located Y′ element. In the case of spore pair 1, hybridization with *ura3-52* revealed only the 3.5-kb chromosomal *URA3* fragment within the size range of resolution by conventional agarose gel electrophoresis; thus it appears that the marker had integrated into a Y′ element separated by ≥20 kb from a chromosomal end.

DISCUSSION

Mutations in a number of genes of *S. cerevisiae* have been shown to affect the lengths of $G_{1-3}T$ tracks at telomeres (20, 28, 37, 38). Among these, some are likely to code for components of a telomerase, a ribonucleoprotein which elongates the $G_{1-3}T$-containing strand of telomeres (reviewed in refs. 39–42), and others may affect telomere length through their roles in more general DNA metabolic processes. Genes in the former category may include *TLC1*, which is likely to encode the RNA component of yeast telomerase (38), and *EST1*, mutations in which lead to a progressive shortening of the telomeric $G_{1-3}T$ repeats (20). Genes in the latter category may include *CDC17*, which encodes the catalytic subunit of DNA polymerase I (37), and genes encoding enzymes involved in thymidine biosynthesis (S. Kronmal and T. Petes, quoted in ref. 20).

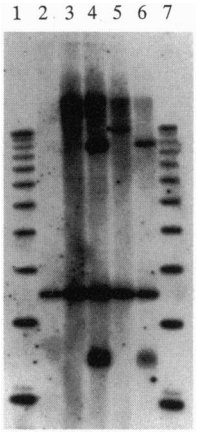

FIG. 3. Mapping of the chromosomal positions of the *URA3* marker embedded in the Y′ elements of four individual isolates. DNA samples were prepared from cells grown from the four individual *TOP3*⁺/Δ*top3*::*TRP1* isolates, each with a *URA3* marker inserted into a subtelomeric Y′ element; the same isolates 1–4 were used to give the spore pairs 1–4 described in the text and Table 1. The DNA samples were separately digested with *Apa* I and 1–4 were loaded in lanes 3–6, respectively. DNA from the parent RK103 *TOP3*⁺/Δ*top3*::*TRP1* cells lacking the *URA3* insert was also prepared, digested with *Apa* I, and run in lane 2 of the same gel as a control. Lanes 1 and 7 contained the "1-kb ladder" (BRL) ^{32}P-labeled at the 3′ ends; the band near the bottom corresponds to the 2036-bp fragment. Blot hybridization was done with a labeled *URA3* probe.

TOP3, encoding DNA topoisomerase III, can now be added to the list of yeast genes known to affect telomere length. Because null mutations in *TOP3* show pleiotropic effects (16), the cellular actions of yeast topoisomerase III are unlikely to be restricted to the telomere regions. It is plausible that topoisomerase III, similar to DNA polymerase I, may affect telomere length through a role or roles in DNA transactions that may not be directly related to telomere growth or shrinkage. Nevertheless, the head-to-head proximity of *TOP3* and *EST1* suggests that the two genes may be coregulated and functionally related.

Yeast topoisomerase III appears to be more closely related to *E. coli* topoisomerase III than to *E. coli* topoisomerase I (17). *E. coli* topoisomerase III can cleave RNA as well as single-stranded DNA through the formation of a covalent protein-nucleic acid complex (43), which suggests a plausible role of the enzyme in a cellular reaction or reactions involving RNA. Yeast topoisomerase III binds strongly to RNA (17), but no covalent protein-RNA intermediate was detected upon incubation of purified enzyme with various RNAs (R.A.K. and J.C.W., unpublished results). It should be of interest to examine interactions between the enzyme and specific RNAs such as the *TCL1* gene transcript (38), and between the enzyme and other proteins such as the *EST1* gene product.

Even though sequences in the telomere region have a high recombination frequency, and subtelomeric repeats are in a dynamic state (20, 24, 29, 30, 34, 40, 44), the very high frequency of loss of the 6.7-kb Y′ *Xho* I fragment in Δ*top3* cells

is striking. The loss of this fragment can be attributed to the disappearance of tandemly juxtaposed 6.7-kb Y′ copies. The 6.7-kb Y′ element and a shortened 5.2-kb form are often present at the chromosomal ends of laboratory strains of *S. cerevisiae*; up to 4 tandem copies of these elements have been found at one end (23, 26, 45). The copy number and distribution of Y′ elements in *S. cerevisiae* show large variations among different strains, however (46). For two strains, YP1 and Y55, that have been examined in detail for their Y′ elements, the former has 26–30 elements but only three chromosomal ends have 2 or more tandem copies of the 6.7-kb Y′ element; the latter has 14–16 Y′ elements, none of which are tandemly arrayed (24). Other than *S. cerevisiae*, few *Saccharomyces* strains possess Y′ elements (47). In the *TOP3*$^+$ parent strains of *S. cerevisiae* used in this work, there is at least one pair of tandemly arrayed 6.7-kb Y′, and most likely more. The disappearance of the 6.7-kb *Xho* I fragment reflects a loss of the tandem copies due to their dispersion to different chromosomal ends or a loss of the component elements, which is likely to occur by recombination between the $G_{1-3}T$ repeats flanking a Y′ element (29).

The high rate of loss or dispersion of more than one Y′ element in tandem in the absence of topoisomerase III is consistent with the high rate of loss of the *URA3*$^+$ marker embedded in a Y′ element of a Δ*top3* strain. Intrachromosomal recombination between the $G_{1-3}T$ repeats flanking a Y′ element and the instability of the excised Y′ ring (35) can account for the loss of Y′ or Y′ bearing a *URA3* marker. When a *URA3* marker is present on a Y′ element farther away from the chromosomal tip, the increment in the rate of its loss due to the deletion of TOP3 is about 10- to 20-fold, which is about the same order of magnitude as the increment seen in the rate of recombination between repeated *delta* sequences when yeast topoisomerase III is inactivated (16).

The stabilization of yeast telomere regions by topoisomerase III extends previous observations that various DNA topoisomerases may serve an important function in genome stabilization in addition to their roles in other vital transactions of DNA (reviewed in refs. 6 and 18; see also refs. 16, 22, and 48). The mechanism(s) by which the topoisomerases stabilize genomes against mitotic recombination is unclear. It has been suggested that a topoisomerase may be required to disrupt recombinational intermediates in which two DNA strands are wound plectonemically (6). Recombination may be normally minimized by dissociating two inadvertently paired DNA strands—through the action of a DNA helicase, for example (49). Other findings suggest that the helicases may indeed be involved in the reduction of recombination (50, 51). It is particularly interesting that the product of the yeast *SGS1* gene, mutations in which suppress the slow-growth and hyperrecombination phenotypes of *top3* null mutations, appears to interact with topoisomerase III and is homologous to a putative helicase encoded by the *E. coli recQ* gene (19). In conjunction with a helicase, DNA topoisomerase may be effective in the separation of plectonemically paired single strands, in aborting either a recombinational joint or a replicative product (18).

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