MOLECULAR AND CELLULAR BIOLOGY, Apr. 1989, p. 1488–1497
0270-7306/89/041488-10$02.00/0

Vol. 9, No. 4

Introduction of Extra Telomeric DNA Sequences into *Saccharomyces cerevisiae* Results in Telomere Elongation

KURT W. RUNGE AND VIRGINIA A. ZAKIAN*

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104

Received 8 November 1988/Accepted 22 December 1988

The termini of *Saccharomyces cerevisiae* chromosomes consist of tracts of $C_{1-3}A$ (one to three cytosine and one adenine residue) sequences of ~450 base pairs in length. To gain insights into *trans*-acting factors at telomeres, high-copy-number linear and circular plasmids containing tracts of $C_{1-3}A$ sequences were introduced into *S. cerevisiae*. We devised a novel system to distinguish by color colonies that maintained the vector at 1 to 5, 20 to 50, and 100 to 400 copies per cell and used it to change the amount of telomeric DNA sequences per cell. An increase in the number of $C_{1-3}A$ sequences caused an increase in the length of telomeric $C_{1-3}A$ repeats that was proportional to plasmid copy number. Our data suggest that telomere growth is inhibited by a limiting factor(s) that specifically recognizes $C_{1-3}A$ sequences and that this factor can be effectively competed for by long tracts of $C_{1-3}A$ sequences at telomeres or on circular plasmids. Telomeres without this factor are exposed to processes that serve to lengthen chromosome ends.

Telomeres are the physical ends of eucaryotic chromosomes. They provide a protective cap from exonucleolytic digestion and, unlike free ends, do not undergo end-to-end fusion. In addition, telomeres provide a solution to the dilemma of how to replicate completely a double-stranded DNA end (4). They also appear to anchor the chromosome to the nuclear membrane in meiosis (6, 32) and in *Drosophila* polytene salivary glands (1). Telomeric DNA sequences are conserved among eucaryotes. For example, ciliates belonging to the genera *Oxytricha* and *Tetrahymena* have C_4A_4 (indicating four cytosine and four adenine residues) and C_4A_2 terminal repeats as their telomeres, respectively, while *Saccharomyces cerevisiae* telomeres carry a more heterogeneous repeat, $C_{1-3}A$ (4). More recently, it has been shown that the plant *Arabidopsis thaliana* has C_3TA_3 at its chromosomal termini (37), and human chromosomes contain the repeated sequence C_3TA_2 at or near their telomeres (33). In addition, C_4A_4 and C_4A_2 sequences can serve as substrates for telomere formation (i.e., $C_{1-3}A$ sequence addition) in *S. cerevisiae* (10, 11, 35, 42). In *S. cerevisiae* the $C_{1-3}A$ repeats are also found at internal chromosomal loci (45).

Several candidates for *trans*-acting factors important for telomere function have been identified biochemically in different systems. *Oxytricha nova* contains two proteins that bind tightly to the termini of its macronuclear DNA (19, 36). Both the specific sequence and structure of the *Oxytricha* termini are required for efficient protein binding. In *Tetrahymena thermophila* an enzymatic activity has been identified that adds G_4T_2 repeats to the 3'-OH of oligonucleotides of telomeric DNA sequences such as G_4T_2, G_4T_4, or $G_{1-3}T$. This addition occurs in the absence of exogenous template (20, 21). A similar activity from *O. nova* adds G_4T_4 repeats to oligonucleotides that end in G_4T_4 (47). A third activity found in crude extracts from *S. cerevisiae* binds to terminal and internal tracts of $C_{1-3}A$ sequences, as monitored by gel retardation assays (3) or nitrocellulose filter binding (5). The properties of these various proteins suggest models of how telomeres might accomplish their functions in the replication and stabilization of chromosome ends. However, it is unclear whether all of these activities are present in any one system or whether any of these activities is actually required for telomere function or replication in vivo.

The very ends of yeast chromosomes consist of tracts of $C_{1-3}A$ sequences, the lengths of which vary both between different yeast strains (46) and between different single colonies of the same strain (38). Mutants that affect the length of the terminal $C_{1-3}A$ repeats have also been isolated (8, 28). Many yeast telomeres also contain the middle repetitive sequences X and Y'. The Y' elements are highly conserved 6.7-kilobase (kb) repeats that can be found at up to four copies per telomere. X elements are less conserved; they are 0.3 to 3.75 kb in size and are positioned centromere proximal to Y' when both elements are present at a single telomere (9). Embedded at the X-Y' and Y'-Y' junctions are internal tracts of $C_{1-3}A$ repeats (Fig. 1). Sequence analysis has revealed that these internal $C_{1-3}A$ tracts range from 50 to 130 base pairs (bp) in length (45), while the terminal tracts of $C_{1-3}A$ vary from 250 to 400 bp in length in wild-type cells (46).

The yeast haploid genome contains 32 telomeres (7). If one assumes that the yeast genome has ~30 X-Y' junctions containing $C_{1-3}A$ sequences of ~100 bp in length and ~450 bp of $C_{1-3}A$ at each chromosomal terminus (as suggested previously [9, 45, 46]), one can estimate that there are about 17 kb of $C_{1-3}A$ sequences in the cell.

In addition to the $C_{1-3}A$-binding protein, *S. cerevisiae* may also contain proteins that specifically interact with chromosomal termini, such as those described in *O. nova* and *T. thermophila*. By introducing more copies of the four types of telomeric DNA sequences (X, Y', internal tracts of $C_{1-3}A$, and terminal tracts of $C_{1-3}A$ [DNA termini]), it might be possible to compete for the binding of telomere-specific proteins. This competition might alter the length of the terminal $C_{1-3}A$ tracts by removing protecting proteins and increasing the accessibility of telomeres to nucleases, recombinases, or terminal transferases. To this end, high-copy-number plasmids bearing each of the four telomeric DNA sequences mentioned above were introduced into *S. cerevisiae*. Only linear plasmids and circular plasmids containing long tracts of $C_{1-3}A$ affected the telomere length. The result was to increase the length of terminal $C_{1-3}A$ tracts on the telomeres of both linear plasmids and chromosomes.

* Corresponding author.

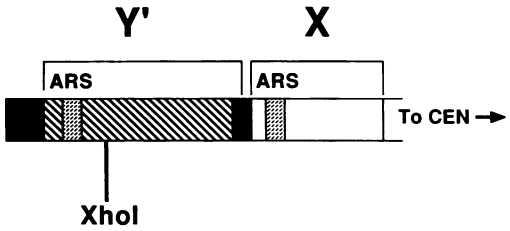

FIG. 1. Structure of the yeast telomere. The termini of the yeast chromosome consisted of $C_{1-3}A$ repeats that varied in different strains from an average of 250 to 620 bp in length (46). Telomeres may also have contained zero to four copies of the highly conserved middle repetitive element Y' and a copy of the less well conserved sequence X (9). Y' elements contain a conserved *Xho*I restriction site which allow analysis of many chromosomal termini at once. Internal tracts of $C_{1-3}A$ sequences are found at the Y'-Y' and Y'-X element junctions.

These data indicate that large numbers of telomeres are not detrimental to the cell, in marked contrast to the case of centromeres, in which ~13 extra copies appear to be toxic (17). These results also suggest a role for a $C_{1-3}A$-binding protein in controlling telomere length.

MATERIALS AND METHODS

Nucleic acids methods. All enzymes were obtained from New England BioLabs, Inc. (Beverly, Mass.). T4 DNA ligase and T4 DNA polymerase were used according to the directions of the manufacturer. Restriction enzyme digests were performed at 37°C in the digestion buffer described by Mirkovitch et al. (31) with the addition of 10 mm dithiothreitol. Digested DNA was analyzed by agarose gel electrophoresis. Southern hybridizations were performed as described by Wahl et al. (44) by using the hybridization solution of Gottschling et al. (18). Yeast genomic DNA samples were prepared either by the method of Davis et al. (12) or by using a glass bead lysis procedure. Cells were grown to the stationary phase in 5 ml of selective medium, spun down, washed with distilled water, suspended in 0.5 ml of 100 mM Tris hydrochloride (pH 8.0)–50 mM EDTA–1.0% sodium dodecyl sulfate, and lysed by vortexing them with glass beads. Twenty-five microliters of 5 M NaCl was added to the disrupted cells. The liquid was recovered and extracted once with TE (10 mm Tris [pH 8.0], 1 mm EDTA)-saturated phenol and once with TE-saturated phenol–chloroform (4:1; vol/vol). The aqueous phase was precipitated with ethanol; and the pellet was washed with 70% ethanol, dried, and suspended in 50 μl of TE. Three microliters of this preparation was digested with restriction enzymes and RNase A (2 μg per digest) and then analyzed by agarose gel electrophoresis and Southern hybridization. Plasmid copy number and the percentage of cells bearing plasmids was determined by using quantitative Southern hybridization as described by Dani et al. (10). In brief, genomic DNA preparations from cells bearing plasmids were digested with restriction enzymes to generate a fragment from a single-copy chromosomal gene (such as *ura3-52* or *LEU2*) and a larger-molecular-weight plasmid band that contained some of the same sequences (such as *URA3* or *leu2-d*). Tenfold serial dilutions were run next to one another on Southern blots and probed with a sequence that was common to the single-copy chromosomal gene and the plasmid fragment. The hybridization intensity of chromosomal and plasmid bands was determined by laser densitometry of several exposures. All other methods that were used have been described by Maniatis et al. (30).

Construction of plasmids. Plasmid pL2 contained the *Eco*RI-*Sal*I fragment of the *LEU2* gene in pBR322 (kindly provided by M. Conrad). The ends of a 1.6-kb *Xho*I fragment containing the *HIS3* gene were rendered flush by treatment with T4 DNA polymerase and cloned into the *Eco*RV site in pL2 (within the *LEU2* gene) to give pL2H3. Cleavage with *Eco*RI and *Sal*I liberated the *LEU2*::*HIS3* fragment.

Plasmids containing $C_{1-3}A$ sequences were derived from plasmid YTCA-1 (kindly provided by D. Gottschling). This plasmid consisted of the 125-bp *Hae*III-*Mnl*I fragment from pYt103 that contained 81 bp of $C_{1-3}A$ sequences derived from a yeast telomere (39) in the *Sma*I site of pUC9 in the orientation *Sal*I-*Bam*HI-*Sma*I/*Hae*III-*Mnl*I/*Sma*I-*Eco*RI. YTCA-1 was converted to YTCA-1X by cutting the plasmid with *Eco*RI, rendering the ends flush with T4 DNA polymerase, and ligating *Xho*I linkers (New England BioLabs) on them. This treatment regenerated the *Eco*RI sites on each side of the *Xho*I linker. Plasmid pC-$C_{1-3}A$ was derived from pC-1/1 (described previously [25]; kindly provided by E. Young) and contained the *Bam*HI-*Xho*I $C_{1-3}A$ fragment from YTCA-1X. The plasmid pC-C_4A_2 was pC-1/1 containing a *Bam*HI-*Sal*I fragment which contained ~300 bp of C_4A_2 derived from pTC5 (kindly provided by A. Murray). The plasmid YEpFAT1 was derived from the *URA3*-bearing plasmid YIp5 (41) and contained the 0.84-kb *Eco*RI-*Hin*dIII fragment of *ARS1* (43), a 1.6-kb *Eco*RI-*Xba*I fragment containing the *Drosophila melanogaster GART* locus (23) and a 3.1-kb *Pvu*II-*Xba*I fragment from pC-$C_{1-3}A$ containing the 2μm origin of replication and the *leu2-d* allele. The last two fragments were inserted into the *Ssp*I and *Eco*RI sites of YIp5, thereby inactivating the Amp^r gene. *Escherichia coli* bearing this plasmid was selected by *leu2-d* complementation of the *leuB6* mutation (see below).

The plasmid YLpFAT1.1 was constructed by linearizing 10 μg of YEpFAT1 by digesting it with *Sal*I and subsequently ligating ~2 μg of the gel-purified *Xho*I-*Pvu*II fragment from YTCA-1X. The ligation mixture was cleaved with *Sal*I, and half of the mixture was used to transform either strain 212 or 213 (see below) by the lithium acetate procedure (26). Transformants were screened for linear plasmids by Southern analysis. All three YLpFAT1.1 transformants that were analyzed contained linear plasmids. The structures of all of these plasmids are shown in Fig. 2.

The plasmid YEpFAT7 contains a *Hin*dIII-*Sma*I fragment containing *URA3*, a *Stu*I-*Xba*I fragment from pC-1/1 containing the *leu2-d* allele and the 2μm origin of replication, and a *Hin*dIII-*Xba*I fragment containing the *D. melanogaster GART* gene in the order *URA3-Sma*I/*Stu*I-*leu2-d-Xba*I-*D. melanogaster GART* in the *Hin*dIII site of pVZ-1 (22). The linear plasmid YLpFAT7.1 was constructed in the same manner as that described above for YLpFAT1.1 by digesting YEpFAT7 with *Sal*I, which cuts in the polylinker of pVZ-1, attaching $C_{1-3}A$ sequences and transforming strain 212. This construction placed the *Sma*I site of the pVZ-1 polylinker adjacent to one of the YLpFAT7.1 telomeres.

The circular plasmids containing long tracts of $C_{1-3}A$ sequences (e.g., YEpFAT7.4 and YEpFAT7.5) were constructed by isolating total genomic DNA from yeast containing YLpFAT7.1 grown on medium without leucine by passing the DNA over an Elutip-d (Schleicher & Schuell, Inc., Keene, N.H.) and converting the telomeres to blunt ends with T4 DNA polymerase. Total DNA was then digested with *Sma*I and treated with 800 U of T4 DNA ligase in 0.5 ml of buffer for 16 h at 15°C. The entire ligation mixture was used to transform either KR36-110 or KR36-60 by the spheroplast method. Transformants were analyzed by

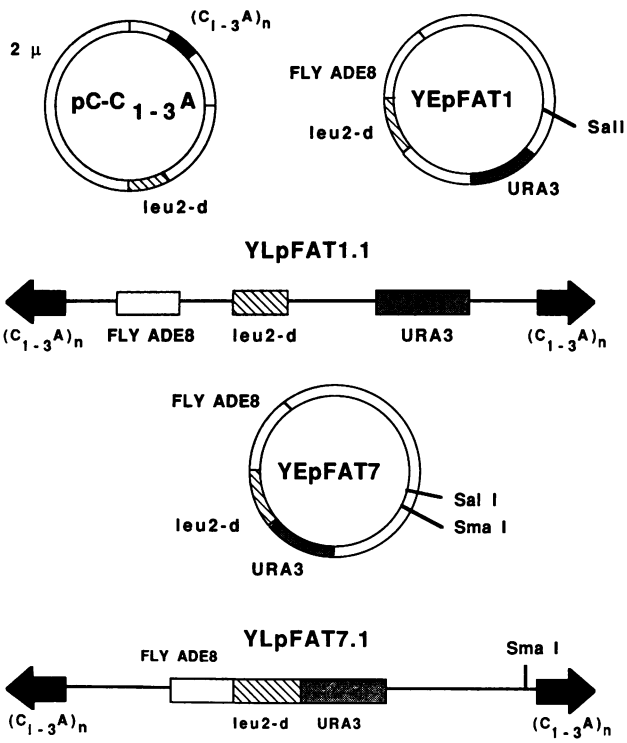

FIG. 2. Plasmids constructed for this study. The construction and relevant sequences on these plasmids are described in the text. YEpFAT1 and YEpFAT7 were linearized at the *Sal*I site, and $C_{1-3}A$ sequences were ligated as described in the text to form YLpFAT1.1 and YLpFAT7.1.

Southern hybridization by using pBR322 or the 81-bp fragment of $C_{1-3}A$ from YTCA-1X as a probe. Transformation into *E. coli* was avoided because of previously published reports that $C_{1-3}A$ sequences are spontaneously deleted when they are propagated in bacteria (39).

Bacterial strains and methods. Bacterial strain *JF1754* (r^- m^- *leuB6 hisB met*) (24) was used as a host for plasmid constructions. For bacterial transformations we used $CaCl_2$-shocked cells, as described by Maniatis et al. (30). When we selected for YEpFAT1 in JF1754, bacteria were allowed to recover for 4 h at 37°C in LB liquid, and then they were washed with M9 medium and plated onto M9 medium-histidine-methionine plates to select for complementation of the *leuB6* mutation by the yeast *leu2-d* allele. LB, LB-ampicillin, and M9 media have been described by Maniatis et al. (30).

Yeast strains and methods. Yeast media have been described by Zakian and Scott (48), except that medium containing low amounts of adenine contained only 10 μg of adenine per ml and 8% glucose (to facilitate the development of a red colony color). Yeast transformation was performed by using the lithium acetate procedure (26) or the spheroplast method described by MacKay (29).

Yeast strains KR36-6 [*MAT***a** *ade2-(1* or *101) ade8-18 ura3-52 trp1-901 his3-100* Can^r] and KR36-11 [*MAT*α *ade2-(1* or *101) ade8-18 ura3-52 trp1-901 his3-100 lys2-801 tyr1* Can^r] were used to construct strains containing an insertion in the *LEU2* gene. Strains KR36-6 and KR36-11 were each transformed with *Eco*RI- and *Sal*I-cleaved pL2H3 (see above), and His^+ transformants were selected. All His^+ transformants tested were Leu^-. Integration at the *LEU2* gene was confirmed by Southern hybridization. Strain KR36-60 was the Leu^- strain derived from KR36-6, and KR36-110 was the analogous strain derived from KR36-11. KR36-60 and KR36-110 cells containing *leu2-d* plasmids (e.g., YEpFAT1 and YLpFAT1.1) were grown on medium that lacked leucine and histidine to select for cells that were Leu^+ because of high plasmid copy number and not as a result of gene conversion of the chromosomal *leu2* gene. Whenever strains KR36-60 and KR36-110 containing a YEpFAT-derived plasmid are reported to be grown on medium without leucine, this medium also lacked histidine.

The karyogamy-deficient yeast strains 213 (*MAT***a** *kar1-1 leu2-3,112 ura3-52 his7*) and 212 (*MAT*α *kar1-1 ade1 ura3-52 leu2-3,112*) were used to transfer YLpFAT1.1 from one yeast strain to another by mating (13, 27). In brief, cells were mated [e.g., 213(YLpFAT1.1) and KR36-110] by mixing two colonies together on a YEPD plate and incubating the plate at 30°C for 6 to 8 h. The mixture of cells was streaked for single colonies onto a YC-uracil plate (48) containing 60 μg of canavanine per ml.

The *rad52* strain XS560-1C-ID2-5 (*MAT***a** *ura3-52* Δ*trp1* Δ*his3 leu2-3,112 RAD52::TRP1*) containing the *TRP1 Bgl*II-*Bam*HI fragment from YRp7 (41) inserted into the *Bgl*II site of *RAD52* (kindly provided by D. Schild) was used to test the effects of the high-copy-number linear plasmid on *S. cerevisiae* that was unable to repair double-stranded breaks.

RESULTS

Experimental design. In order to increase the amounts of yeast telomeric DNA sequences in a cell, yeast strains KR36-110 and KR36-60 were transformed with a variety of plasmids (Fig. 2; see above). X and Y′ sequences were introduced by transformation with the circular plasmids YRp120 and YRp131b, respectively (9). In addition to the X and Y′ sequences, both plasmids contained a *131* class ARS and bore the *LEU2* gene as a selectable marker. Both were probably maintained at 20 to 50 copies per cell, which is similar to the number of other yeast ARS plasmids (for a review, see reference 34). The pC-$C_{1-3}A$ plasmid, which contained a 125-bp DNA fragment with 81 bp of $C_{1-3}A$ sequences and the *leu2-d* allele, allowed the introduction of large numbers of internal $C_{1-3}A$ tracts into cells. The *leu2-d* allele is a promoter-defective gene that is required at 50 to 200 copies per cell, in order to endow the plasmid-bearing cell with a Leu^+ phenotype (2, 14, 16). YLpFAT1.1 is a linear plasmid which contains the *URA3* gene (which can complement *ura3* mutant alleles at one copy per cell), the *leu2-d* allele, and the *D. melanogaster GART* locus. The *D. melanogaster GART* gene encodes the enzyme for the *D. melanogaster* glycin amide phosphoribose transformylase and was isolated by its ability to complement the yeast *ade8-18* mutation (23); however, it does so only poorly. This poor expression of the *D. melanogaster GART* gene allowed us to estimate the plasmid copy number by the color of the colony. Cells bearing the *ade2* and *ade8* mutations gave rise to white colonies on low-adenine medium, while cells bearing just the *ade2* mutation gave rise to red colonies. If *ade2 ade8* cells carried one or two copies of the *D. melanogaster GART* gene (e.g., on a centromere plasmid), they gave rise to white colonies on low-adenine medium. If these yeasts contained 20 to 50 copies of the *D. melanogaster GART* gene per cell (as on a 2μm vector [Table 1] [16]), they gave rise to pink colonies; if they contained 200 to 400 copies per cell, they gave rise to red colonies (Table 1). YLpFAT1.1 was used to introduce different numbers of DNA termini into the cells by growing plasmid-bearing cells on low-adenine me-

TABLE 1. Properties of yeasts bearing high-copy-number plasmids containing $C_{1-3}A$ sequences

Plasmid	Medium without:	% Ura$^+$[a]	% Leu$^+$	Copy no.	Colony color
YLpFAT1.1	Uracil	100	25	30–40	White
	Uracil	100	25	40–50	Pink
	Leucine	100	100	150–200	Red
YEpFAT1	Uracil	100	<1	15–30	White
	Uracil	100	<1	20–50	Pink
	Leucine	100	95	80–300	Red
pC-$C_{1-3}A$	Leucine	NA[b]	100	200–400	NA
YLpFAT7.1	Uracil	60	10	50–90	Pink
	Leucine	100	80	100–400	Red
YEpFAT7	Uracil	90	<1	30–50	Pink
	Leucine	100	90	100–200	Red
YEpFAT7.4	Uracil	90	<1	45–70	Pink
	Leucine	80	90	100–300	Red
YEpFAT7.5	Uracil	80	<1	20–50	Pink
	Leucine	90	95	200–400	Red

[a] The percentage of Ura$^+$ or Leu$^+$ cells is (number of colonies on selective plates/number of colonies on complete plates) × 100. Three plates of 100 to 250 colonies each were counted for each growth condition.
[b] NA, Not applicable.

dium lacking uracil and selecting for white (low-copy-number) or pink (intermediate-copy-number) colonies or by growth on low-adenine medium lacking leucine and histidine and screening for red colonies (see above). YEpFAT1 (the control plasmid) was a circular plasmid with the same sequences as YLpFAT1.1, but it lacked $C_{1-3}A$ tracts. This plasmid was used to control for the effects of vector sequences on the cell.

Yeast telomeres contain various numbers of $C_{1-3}A$ repeats at each terminus. For strain KR36-110, the length of the terminal $C_{1-3}A$ tract was ~450 bp. Thus, when yeast DNA was digested with *Xho*I, an enzyme that had a conserved site within Y′ elements (Fig. 1), and the fragments were analyzed by Southern hybridization (by using $C_{1-3}A$ sequences as a probe), we observed a wide, heterogeneous band with a mean size of 1.3 kb. This band represented the terminal *Xho*I restriction fragments of chromosomes with Y′ telomeres; the size variation can be accounted for by differences in the number of $C_{1-3}A$ repeats at different Y′ termini (46). In addition, several of the higher-molecular-weight $C_{1-3}A$-hybridizing bands corresponded to telomeres bearing only an X sequence or internal restriction fragments containing $C_{1-3}A$ sequences (46) (see Fig. 6). If introduction of a plasmid bearing telomere sequences were to compete for telomere-binding proteins and remove them from chromosomal telomeres, one might expect the position of this wide ~1.3-kb telomere band and the X telomere bands to shift, indicating a change in the average lengths of the terminal $C_{1-3}A$ tracts. For example, removal of proteins which protect the DNA terminus might expose the chromosome end to nucleases and result in a decrease in telomere length. Alternatively, the exposed end might be more susceptible to those processes which cause telomere elongation in the yeast cell, and the length of the telomere fragment would then be expected to increase.

Plasmids bearing X, Y′, or an 81-bp tract of $C_{1-3}A$ sequences do not affect telomere length. The plasmids containing telomere sequences (Fig. 2) were introduced by transformation into yeast strains KR36-110 and KR36-60 (see above). Two individual transformants for each plasmid in both strains were picked and streaked for single colonies five consecutive times on medium requiring the presence of the plasmid, thereby ensuring that each plasmid-bearing strain grew for at least 100 generations in the presence of the excess telomeric DNA sequence. This procedure was followed because mutants that are known to affect telomere length such as *tel1* and *tel2* (28) and *cdc17* (8) all require ~100 generations of growth in order to manifest their phenotypes. In the case of cells containing YLpFAT1.1 or YEpFAT1, cells were streaked onto low-adenine medium lacking either uracil or leucine. On the low-adenine medium lacking uracil, one predominantly white colony and one predominantly pink colony were picked each time. White colonies were always picked from streaks of white colonies, and pink colonies were always picked from streaks of pink colonies. All colonies streaked on low-adenine medium without leucine were red. This protocol allowed the generation of strains that maintained YLpFAT1.1 and YEpFAT1 at low (white colonies), intermediate (pink colonies), and high (red colonies) copy numbers for approximately 100 generations (Table 1). Colonies grew at approximately the same rates on medium without uracil and medium without leucine.

Genomic DNA was digested with *Xho*I in order to examine the length of $C_{1-3}A$ tracts at Y′ telomeres by Southern hybridization. Cells that maintained the control plasmid YEpFAT1 or the plasmid bearing 81 bp of $C_{1-3}A$ (pC-$C_{1-3}A$) on medium without leucine for 100 generations had terminal $C_{1-3}A$ tracts of the same length as the parental cells grown without the plasmid (Fig. 3). Therefore, neither the presence of excess YEpFAT1 plasmid sequences nor the excess internal tracts of $C_{1-3}A$ sequences present on pC-$C_{1-3}A$ affected the telomere length. By using a plasmid copy number of 200 to 400 for pC-$C_{1-3}A$ (Table 1), cells carrying this plasmid had an increase of ~16 to 32 kb in the amount of $C_{1-3}A$ sequences in the cell; this was one to two times more than the total amount of the $C_{1-3}A$ sequences present at the telomeres and at the borders of X and Y′ sequences (approximately 17 kb total; see above) (Table 2). Likewise, increased numbers of X and Y′ sequences did not change the telomere length in the yeast cell (Fig. 3). In addition, 200 to 400 copies of a 300-bp segment of C_4A_2 on pC-C_4A_2 also had no effect on the telomere length (data not shown).

High-copy-number plasmids with long $C_{1-3}A$ tracts cause telomere lengthening. A different result was obtained by increasing the number of DNA termini in the cell. The length of the terminal $C_{1-3}A$ tracts in cells bearing the linear plasmid YLpFAT1.1 increased dramatically as the plasmid copy number increased (Fig. 4). Cells that carried the linear plasmid at a high copy number for ~100 generations had terminal restriction fragments with an average length that was 200 bp longer than those of similar fragments from either the parent cells or the original transformant that was grown for only ~20 generations. This represents a significant increase in length and is comparable to the magnitude of the length decrease seen in *tel1* and *tel2* mutants (28). Moreover, the longest telomeres in the cells with the highest plasmid copy number were up to 500 bp longer than the telomeres of the original transformant (Table 2). A similar difference in the length of telomeres containing X sequences was also seen (see Fig. 6). The termini of the linear plasmid YLpFAT1.1 behaved the same as chromosomal telomeres, gaining an average of 200 bp at each end (Fig. 5). In contrast,

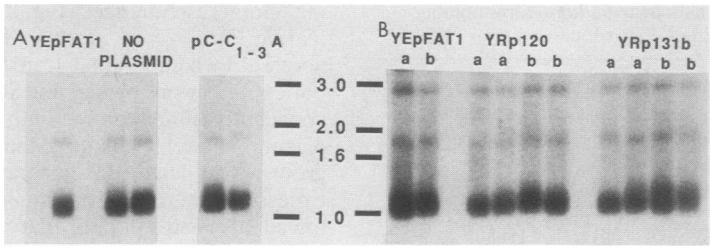

FIG. 3. Introduction of pC-$C_{1-3}A$ and extra Y′ and X elements does not affect the length of terminal $C_{1-3}A$ sequences. KR36-110 cells were transformed with the indicated plasmids and grown for ~100 generations, and individual colonies were used to prepare genomic DNA. Yeast genomic DNA was digested with *Xho*I and examined by Southern hybridization by using the 125-bp $C_{1-3}A$ fragment from YTCA-1X as the probe. The lower part of the gel, which contained the Y′-terminal restriction fragments (telomere fragments, the dark broad band at the bottom of the gel), is shown. Numbers between panels A and B refer to molecular size markers (in kilobases). (A) YEpFAT1 is KR36-110 cells with YEpFAT1 grown for ~100 generations on YC-leucine medium; no plasmid is the parent strain KR36-110; pC-$C_{1-3}A$ is KR36-110 cells bearing pC-$C_{1-3}A$ grown on YC-leucine medium for ~100 generations. (B) YEpFAT1 is the same as that in panel A, except that cells were grown for ~100 generations on YC-uracil medium. YRp120 contains the X sequence and *LEU2* gene, while YRp131b contains the Y′ sequence and the *LEU2* gene (9). a, Two independent transformants of KR36-60; b, two independent KR36-110 transformants. All four transformants were subsequently grown for ~100 generations before genomic DNA was prepared and analyzed.

the strains that maintained the control plasmid YEpFAT1 for ~100 generations had an average telomere length that was essentially unchanged from that of the parent strain or the original transformant grown for only ~20 generations. The length difference seen for one of these isolates (Fig. 4, YEpFAT1 −leu) is consistent with the intrastrain telomere length variation seen in random single colonies (38). This size difference was not observed in any of the other strains containing the control plasmid YEpFAT1 (Fig. 3 and 6 and Table 2). The presence of many copies of the high-copy-number linear plasmid YLpFAT1.1 also caused the telomere length to increase by ~200 bp in the recombination-deficient *rad52* strain XS560-1C-ID2-5 (data not shown).

The elongation of both chromosomal and plasmid telomeres in cells carrying the linear plasmid YLpFAT1.1 was dependent on its presence and copy number. KR36-60 cells were grown for 100 generations on medium without leucine containing either the linear plasmid or the circular control plasmid YEpFAT1. KR36-60 cells bearing the linear plasmid YLpFAT1.1 that were grown for 100 generations also possessed longer telomeres than cells bearing plasmids without $C_{1-3}A$ sequences, and those cells that had maintained the plasmid at the highest copy number (in medium without leucine) had the longest telomeres (data not shown). Cells that had been grown on medium without leucine for ~100 generations were restreaked onto medium without uracil or medium without leucine and grown for approximately an additional 200 generations. Cells were also cured of the plasmid and grown on complete medium for ~200 generations. The telomeres in cells with no plasmid had the same lengths as those in untransformed cells or in cells bearing the circular plasmid control (~450 bp of $C_{1-3}A$), while those grown on medium lacking leucine had the longest telomeres (~650 bp of $C_{1-3}A$) (data not shown). These data indicate that the increase in telomere length (i) requires the continuous presence of the linear plasmid, (ii) is dependent on the

TABLE 2. Amount of $C_{1-3}A$ introduced into *S. cerevisiae*

Plasmid	Medium without:	Length of $C_{1-3}A$ segment (bp)	Copy no./cell	Amt of $C_{1-3}A$ introduced (kb)	Increase in telomere length (bp)[a]	
					Avg	Longest
Endogenous $C_{1-3}A$				[17]	[0]	80
pC-$C_{1-3}A$ (circular)	Leucine	81	200–400	16–32	10	70
YLpFAT1.1 (linear)[b]	Uracil (white)	340	30–40	20–28	40	175
	Uracil (pink)	390	40–50	31–39	100	240
	Leucine (red)	530	150–200	159–212	190	380
YLpFAT7.1 (linear)[b]	Uracil	470	50–90	47–85	40	280
	Leucine	635	100–400	120–500	220	590
YEpFAT7.4 (circular)	Uracil	350	45–70	16–24	190	540
	Leucine	350	100–300	35–135	260	710
YEpFAT7.5 (circular)	Uracil	480	20–50	10–24	210	480
	Leucine	480	200–400	96–190	250	550

[a] The endogenous telomere length is from Fig. 3 (lane a). The control value for YLpFAT1.1 is Fig. 4 (YLpFAT1.1 lane o). The control value for YEpFAT7.4 and YEpFAT7.5 is Fig. 6 YEpFAT7 lane without uracil. The calculation for the amount of endogenous $C_{1-3}A$ is presented in the text. The other data are from Fig. 5 and 7 and Table 1.

[b] For the linear plasmids YLpFAT1.1 .1 and YLpFAT7.1, the $C_{1-3}A$ segment was the average length of the $C_{1-3}A$ tract of each plasmid telomere. The amount of $C_{1-3}A$ introduced for the linear plasmids was twice the product of the segment length and plasmid copy number, as there were two telomeres per plasmid. Values are expressed as the increase in length with respect to a control value on the same Southern blot (as derived in Fig. 7). Values in brackets are the telomere lengths that the cells have before any plasmids are introduced.

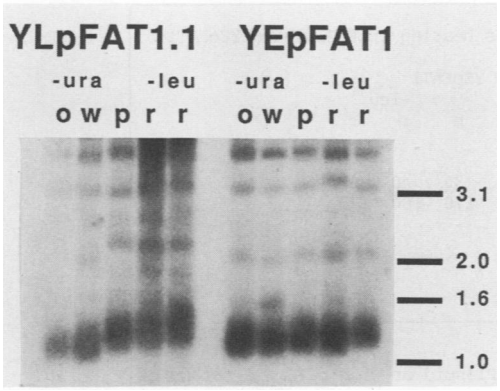

FIG. 4. Introduction of extra DNA termini caused a lengthening of the chromosomal telomeres. Analysis of chromosomal telomeres, which was performed as described in the legend to Fig. 3, was performed on KR36-110 cells containing YLpFAT1.1 and YEpFAT1. Cells were grown as described in the text. −ura refers to YC medium without uracil and low levels of adenine, and −leu refers to YC medium without leucine and low levels of adenine. DNA from the original transformant (o) and white (w), pink (p), and red (r) colonies was analyzed. Red colonies came only from low-adenine medium without leucine. Molecular size standards (in kilobases) are given in the right margin. Plasmid bands were not visible because *Xho*I did not cut in YEpFAT1 or YLpFAT1.1.

copy number of the linear plasmid, and (iii) is stable for at least 200 generations when the linear plasmid is present.

Excess $C_{1-3}A$ tracts cause telomere lengthening. The number of $C_{1-3}A$ sequences introduced into the cell by the circular plasmid with an 81-bp tract of $C_{1-3}A$ sequences, pC-$C_{1-3}A$, or the linear plasmid YLpFAT1.1 is summarized in Table 2. Because the high-copy-number linear plasmid introduced 5 to 10 times more $C_{1-3}A$ repeats than the circular plasmid pC-$C_{1-3}A$ did, we performed additional experiments to determine whether the increase in chromosomal telomere

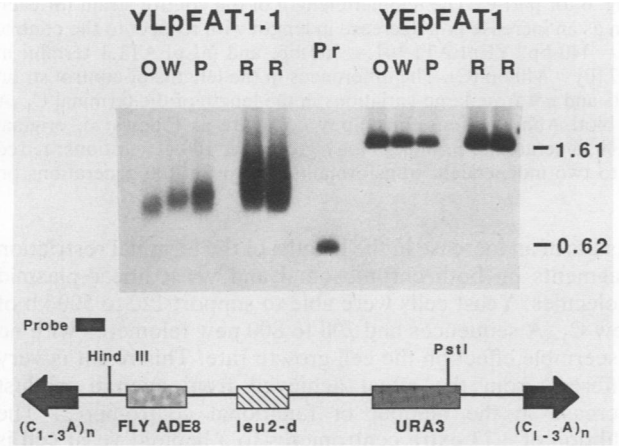

FIG. 5. The length of YLpFAT1.1 linear plasmid telomeres also increased as the number of DNA termini increased. The same genomic DNA analyzed earlier (Fig. 4) was cut with *Hin*dIII and *Pst*I and analyzed by Southern hybridization by using the *Hin*dIII-*Sal*I fragment from YEpFAT1 as a probe (closed box). The lane labeled Pr is of DNA from YEpFAT1 bearing cells from a white colony digested with *Hin*dIII, *Pst*I, and *Sal*I to show the size of plasmid sequences prior to the addition of $C_{1-3}A$. Abbreviations are the same as those described in the legend to Fig. 4, except they are given as capital letters.

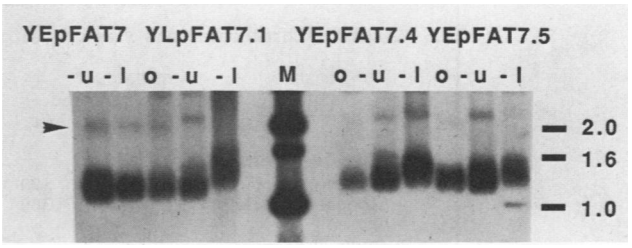

FIG. 6. High-copy-number circular plasmids with long $C_{1-3}A$ inserts also caused telomere lengthening. Genomic DNA from KR36-110 cells bearing YEpFAT7, YLpFAT7.1, YEpFAT7.4, and YEpFAT7.5 was analyzed as described in the legend to Fig. 3. Abbreviations are the same as those described in the legend to Fig. 4. M, Molecular size markers; arrowhead, band derived from an X-telomere that also increases in size.

length was caused by an increase in the number of DNA termini or an increase in the number of $C_{1-3}A$ repeats. A new control vector containing the *URA3*, *leu2-d*, and *D. melanogaster GART* genes and the 2μm origin of replication, YEpFAT7, was constructed and converted into a linear plasmid YLpFAT7.1 (see above). Cells bearing YLpFAT7.1 were grown on medium without leucine and were used as a source of linear plasmid DNA. The telomeres were rendered flush with T4 DNA polymerase, and the DNA was digested with *Sma*I, which specifically removed one plasmid telomere, leaving a blunt end. Cut plasmids were circularized by ligation at a low DNA concentration, and the entire ligation mixture was used to transform yeast strains KR36-110 and KR36-60. Circular plasmids generated in this manner contained one telomere in the YEpFAT7 vector. Because individual telomere lengths varied, plasmids derived by this procedure contained $C_{1-3}A$ segments of different lengths which presumably corresponded to the different lengths of the original telomere (data not shown). Two of these circular plasmids containing $C_{1-3}A$ segments of 350 bp (YEpFAT7.4) and 400 bp (YEpFAT7.5) were analyzed further. Cells bearing YEpFAT7.4, YEpFAT7.5, YEpFAT7 (no $C_{1-3}A$ segment), and YLpFAT7.1 (the high-copy-number linear plasmid) were grown for 100 generations on low-adenine medium without uracil and low adenine medium without leucine. In this case, only the colonies containing the highest plasmid copy numbers for each plasmid were used for each restreaking procedure. Genomic DNA was prepared, and the chromosomal telomere length was analyzed by Southern hybridization (Fig. 6). While the telomere length in cells bearing the control plasmid YEpFAT7 did not change, those containing the high-copy-number linear plasmid YLpFAT7.1 or the high-copy-number circular plasmids containing $C_{1-3}A$ sequences YEpFAT7.4 and YEpFAT7.5 showed increases in telomere length that was related to the plasmid copy number (Fig. 7). The total increase in telomere length caused by YEpFAT7.4 and YEpFAT7.5 was approximately the same as that caused by the high-copy-number linear plasmid YLpFAT7.1 (Table 2). Since these circular $C_{1-3}A$-containing plasmids introduced nearly as many $C_{1-3}A$ repeats as the linear plasmid did (Table 2), these data suggest that it is the increase in the number of $C_{1-3}A$ repeats, and not the increase in the number of DNA termini, that causes telomere lengthening. The other circular plasmids containing 200- to 600-bp tracts of $C_{1-3}A$ were also able to cause telomere lengthening when they were present at a high copy number (data not shown). In addition, the same experiment performed in strain KR36-60 with a circular plasmid containing a 380-bp

A. Length of Chromosomal and Plasmid Telomeres in Cells bearing YLpFAT1.1 and YEpFAT1

PLASMID	YLpFAT1.1					YEpFAT1				
MEDIA	-ura		-leu			-ura		-leu		
COLOR	o	w	p	r	r	o	w	p	r	r
Chromosomal telomeres										
ave.	[0]	40	100	160	190	[0]	-10	30	40	60
longest	125	175	240	315	380	150	110	170	180	175
YLpFAT1.1 termini										
ave.	[0]	40	90	230	230	-	-	-	-	-
longest	65	75	275	530	530					

B. Length of Chromosomal Telomeres in Cells without Plasmid or with pC-$C_{1-3}A$ (81 bp of $C_{1-3}A$)

PLASMID	NONE		pC-$C_{1-3}A$ -leu	
MEDIA	complete			
ISOLATE	a	b	a	b
ave.	[0]	20	0	20
longest	80	130	70	70

C. Length of Chromosomal Telomeres in Cells with Circular Plasmids Containing Long Tracts of $C_{1-3}A$ Segments.

PLASMID	YEpFAT7		YLpFAT7.1			YEpFAT7.4			YEpFAT7.5		
MEDIA	-ura	-leu	o	-ura	-leu	o	-ura	-leu	o	-ura	-leu
ave.	[0]	20	20	40	220	110	190	260	140	210	250
longest	220	180	210	280	590	260	540	710	280	480	550

FIG. 7. Change in telomere length caused by high-copy-number plasmids bearing telomeric DNA sequences. Representative data for the average and longest telomere lengths for KR36-110 as measured from Fig. 3 to 6 are presented. Experiments performed with other yeast strains gave similar results (i.e., as the copy number of YLpFAT1.1 or YLpFAT1.7 was increased, the telomere length increased, while YEpFAT1 or YEpFAT7 had no effect). Telomere lengths were calculated by determining the medium length of the terminal *Xho*I restriction fragment and then subtracting 790 bp (the amount of the Y′ sequence that was present in this fragment) to give the length of the terminal $C_{1-3}A$ tract (39). The longest telomere was determined by measuring the top of the telomere band. Measurements for the longest telomeres were made from different exposures of the same blot, such that the hybridization intensity of the cells grown on medium without leucine was equal to that of control cells that lacked the plasmid. These two values are reported as, respectively, ave. (average) and longest. The measurements reported are derived from Fig. 4 and 5 for part A, Fig. 3 for part B, and Fig. 6 for part C. The telomere length of the control strain for each experiment is defined as 0, and the lengths of the other telomeres are shown as an increase or a decrease in length with respect to the control value. The control values for each part are as follows: YLpFAT1.1 [0] = 440 bp, YEpFAT1 [0] = 500 bp, and YLpFAT1.1 termini in KR36-110 [0] = 440 bp (A); no plasmid control [0] = 510 bp (B); YEpFAT7 [0] = 440 bp (C). The differences in the lengths of control strain telomeres arose from measurement differences from different Southern blots and not from large variations in the length of the terminal $C_{1-3}A$ tract. In all cases, the control strain length was measured on the same blot. Abbreviations for colony color are as follows: o, original transformant grown for only 20 generations; w, white colony grown for 100 generations; p, pink colony grown for 100 generations; r, red colony grown for 100 generations. The a and b headings in part B refer to two independent transformants grown for 100 generations on complete medium or on medium without leucine, respectively.

tract of $C_{1-3}A$ sequences gave equivalent results (data not shown).

Cells bearing either linear plasmid (YLpFAT1.1 or YLpFAT7.1) grown on medium without uracil gave a much higher percentage of Leu^+ cells than did cells bearing circular plasmids without $C_{1-3}A$ sequences (YEpFAT1 and YEpFAT7) or with $C_{1-3}A$ sequences (YEpFAT7.4 and YEpFAT7.5) (Table 1). This large difference in the percentage of Leu^+ cells for cells bearing plasmids with similar copy numbers suggests that linear plasmids are more readily amplified than circular ones. Alternatively, the *leu2-d* allele may be better expressed from a linear plasmid than from a circular one.

DISCUSSION

The experiments reported here demonstrate that the introduction of extra $C_{1-3}A$ sequences into different yeast strains results in an increase in the lengths of the terminal restriction fragments on both chromosomal and yeast linear plasmid molecules. Yeast cells were able to support 120 to 500 kb of new $C_{1-3}A$ sequences and 200 to 800 new telomeres with no discernible effect on the cell growth rate. This result is very different from the effect achieved from even a modest increase in the number of functional centromeres. The addition of ~13 extra centromeres to a haploid yeast cell is toxic because these cells have greatly reduced cell growth rates and show an increased frequency of chromosome loss (17).

The increase in the size of the terminal restriction fragments observed when extra $C_{1-3}A$ sequences are added surely represents elongation of the telomeric $C_{1-3}A$ tracts. Walmsley and Petes (46) have shown that the terminal *Xho*I restriction fragments of different yeast strains vary in size and that this strain variation in size is primarily caused by a

difference in the length of telomeric $C_{1-3}A$ tracts. Analysis of KR36-60 cells bearing YLpFAT1.1 grown on medium without leucine showed that the increase in telomere length was gradual, growing only ~30 bp over ~40 generations instead of the ~130 bp increase seen after ~100 generations (data not shown). This gradual increase in the length of the terminal restriction fragment is consistent with the addition of small amounts of $C_{1-3}A$ repeats over many generations. A similar increase in telomere length caused by the gradual addition of $C_{1-3}A$ sequences to the telomere has also been observed for yeasts bearing the *cdc17* mutation (8). Thus, the elongation of the terminal restriction fragments of the linear plasmids and chromosomal telomeres most likely seems to be caused by terminal $C_{1-3}A$ addition. The observation that the increase is stable after ~200 generations of growth in the presence of the linear plasmid suggests that the chromosomal telomeres reach a new equilibrium length. This new length might be established by different rates of telomere elongation and degradation in cells bearing $C_{1-3}A$-containing plasmids compared with those in cells without plasmids. The amount of telomere elongation is directly proportional to the amount of added $C_{1-3}A$ DNA, with the addition of as little as ~20 kb from the linear plasmid YLpFAT1.1 or the circular plasmids bearing long $C_{1-3}A$ tracts (YEpFAT7.4 or YEpFAT7.5) causing a measurable effect. These data suggest that telomere growth is inhibited by a limiting factor(s). One candidate for the factor(s) being competed for by extra $C_{1-3}A$ sequences is the *RAP1* protein of Shore and Nasmyth (40) or the $C_{1-3}A$-binding activity described by Berman et al. (3) or Buchman et al. (5) in crude yeast extracts. These activities bind specifically in vitro to $C_{1-3}A$ sequences at telomeres, the silent mating type loci, and ribosomal protein gene UAS, the RPG box. Terminal-binding proteins such as those found in *O. nova* (19) or telomerase activities such as those found in *T. thermophila* (20, 21) and *O. nova* (47) are less likely candidates for the limiting factor(s) because circular plasmids are not acted upon by these proteins.

The results summarized in Table 2 indicate that ~20 kb of $C_{1-3}A$ sequences on YEpFAT7.4 and YEpFAT7.5 cause a larger increase in telomere length than do the linear plasmids YLpFAT1.1 or YLpFAT7.1. This difference may reflect a greater affinity of the titrated binding factor for $C_{1-3}A$ sequences that are torsionally constrained. The linear plasmid ends may be free to rotate in the cell, so any superhelicity in the plasmid is lost by rotation of the two strands about one another. The circular plasmid is topologically constrained so that superhelical turns may alter the structure of the $C_{1-3}A$ tracts. Chromosomal telomeres may also be torsionally constrained. Telomeres have been observed to be associated with the nuclear envelope (1, 6, 32). This association may reflect a strong DNA-protein interaction, which in turn may constrain the two DNA strands at the chromosomal terminus and prevent their free rotation about one another. Therefore, all superhelical turns generated by transcription and proteins binding to DNA might not be fully dissipated and the $C_{1-3}A$ duplex may be altered. In this way, the torsionally constrained $C_{1-3}A$ sequences in the circular plasmids YEpFAT7.4 and YEpFAT7.5 may assume a conformation that more closely resembles those of chromosomal telomeres than those of the $C_{1-3}A$ sequences of the linear plasmids. This hypothesis presumes that the linear plasmid telomeres and not all bound to the nuclear envelope.

In contrast to the telomere lengthening caused by many copies of long tracts of $C_{1-3}A$ sequences, the addition of short (81-bp) tracts of $C_{1-3}A$ sequences at a high copy number does not cause telomere lengthening. The total amount of $C_{1-3}A$ introduced on the high-copy-number plasmid bearing the 81-bp $C_{1-3}A$ tract, pC-$C_{1-3}A$, was equivalent to that introduced by the circular plasmids with 350 to 480 bp of $C_{1-3}A$, YEpFAT7.4 and YEpFAT7.5, on medium without uracil (Table 2); but only YEpFAT7.4 and YEpFAT7.5 caused telomeres to lengthen, while pC-$C_{1-3}A$ had no visible effect (compare Fig. 3 and 6). We independently determined that the DNA fragment in pC-$C_{1-3}A$ containing the $C_{1-3}A$ sequences is a substrate for the $C_{1-3}A$-binding activity in a gel retardation assay (K. Runge, unpublished data). In addition, circular and supercoiled plasmids containing $C_{1-3}A$ repeats can effectively compete with linear $C_{1-3}A$ fragments for this binding activity in vitro (3). Therefore, if *RAP1* is the protein being titrated in our experiments, it is surprising that the 81-bp tract of $C_{1-3}A$ sequences on pC-$C_{1-3}A$ does not compete effectively for the $C_{1-3}A$-binding activity in vivo. Our results raise the possibility that whatever the $C_{1-3}A$-binding factor is, it may bind cooperatively, making long tracts of $C_{1-3}A$ sequences a better substrate than short tracts. An alternative explanation is that the factor(s) being titrated out by excess telomeric DNA sequences is not *RAP1* but a protein that recognizes sequences that are present at true telomeres but that are not present in the 81-bp $C_{1-3}A$ fragment in pC-$C_{1-3}A$. Although the 81-bp fragment was derived from a yeast chromosomal telomere, ~400 bp of the most telomere-proximal sequences was deleted during subcloning (39). Therefore, there may be sites for the unknown factor(s) on the linear and circular plasmids YLpFAT1.1 and YLpFAT7.1 and YEpFAT7.4 and YEpFAT7.5 that are not present on pC-$C_{1-3}A$. If this interpretation is correct, the proposed site(s) would not have to be at a chromosomal terminus in order to compete for its binding factor, since the circular plasmids YEpFAT7.4 and YEpFAT7.5 are at least as effective as the linear plasmids YLpFAT1.1 and YLpFAT7.1 in causing telomere lengthening. In either model, the result of titrating this factor(s) would be to leave the chromosomal termini exposed. These telomeres would then be more accessible to lengthening processes such as telomerase or gene conversion. However, if recombination is the process by which telomeres elongate, it must occur in a *RAD52*-independent manner, because telomere lengthening also occurs in a *rad52* strain. The *RAD52* gene is required for those mitotic recombination events that involve healing double-stranded breaks in DNA (for a review, see reference 15). The observation that telomere lengthening is *RAD52* independent is consistent with the fact that elongation occurs either by a telomeraselike mechanism (20, 21, 47) or by recombination between the ends of DNA molecules [A. F. Pluta and V. A. Zakian, Nature (London), in press].

In conclusion, we have shown that yeast cells can support a 6- to 20-fold increase in the number of telomeres and a 2- to 30-fold increase in the amount of $C_{1-3}A$ sequences. The only phenotypic effect of these new $C_{1-3}A$ sequences is that the terminal tracts of telomeres elongate, probably because the high-copy-number plasmids titrate $C_{1-3}A$-binding proteins from the chromosomal termini. A possible candidate for this $C_{1-3}A$-binding protein is the *RAP1* protein. The fact that chromosomal telomeres grow instead of shrink in the presence of excess $C_{1-3}A$ sequences is somewhat surprising, since one might expect the unprotected telomere to be rapidly degraded by nucleases. Our results suggest that the removal of $C_{1-3}A$-binding proteins is not disastrous for telomere integrity, and that those proteins that bind to the telomere may control or limit the normal processes of elongation.

ACKNOWLEDGMENTS

We thank L. Breeden, M.-C. Yao, M. Conrad, R. Wellinger, and D. Gottschling for helpful comments on this study and critical reading of the manuscript.

This work was supported by Public Health Service grant GM26938 from the National Institutes of Health and grant ACS NP-574 from the American Cancer Society (to V.Z.) and by Public Health Service postdoctoral fellowship GM-10472 from the National Institutes of Health (to K.R.).

LITERATURE CITED

1. **Agard, D. A., and J. W. Sedat.** 1983. Three dimensional architecture of a polytene nucleus. Nature (London) **302**:676–681.
2. **Beggs, J. D.** 1978. Transformation of yeast by a replicating hybrid plasmid. Nature (London) **275**:104–108.
3. **Berman, J., C. Y. Tachibana, and B.-K. Tye.** 1986. Identification of a telomere-binding activity from yeast. Proc. Natl. Acad. Sci. USA **83**:3713–3717.
4. **Blackburn, E. H., and J. W. Szotak.** 1984. The molecular structure of centromeres and telomeres. Annu. Rev. Biochem. **53**:163–194.
5. **Buchman, A. R., W. J. Kimmerly, J. Rine, and R. D. Kornberg.** 1988. Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:210–225.
6. **Byers, B., and L. Goetsch.** 1975. Electron microscopic observations on the meiotic karyotype of diploid and tetraploid *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **72**:5056–5060.
7. **Carle, G. F., and M. V. Olson.** 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA **82**:3756–3760.
8. **Carson, M. J., and L. Hartwell.** 1985. *CDC17*: an essential gene that prevents telomere elongation in yeast. Cell **42**:249–257.
9. **Chan, C. S. M., and B.-K. Tye.** 1983. Organization of DNA sequences and replication origins at yeast telomeres. Cell **33**: 563–573.
10. **Dani, G. M., A. F. Pluta, and V. A. Zakian.** 1983. Termini from macronuclear DNA of ciliated protozoans can provide telomere function for yeast plasmids in mitosis and meiosis. UCLA Symp. Mol. Cell. Biol. **10**:553–562.
11. **Dani, G. M., and V. A. Zakian.** 1983. Mitotic and meiotic stability of linear plasmids in yeast. Proc. Natl. Acad. Sci. USA **80**:3406–3410.
12. **Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padgett.** 1980. Rapid DNA isolations for enzymatic and hybridization analysis. Methods Enzymol. **65**: 404–411.
13. **Dutcher, S. K.** 1981. Internuclear transfer of genetic information in *kar1-1/KAR1* heterokaryons in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:245–253.
14. **Erhart, E., and C. P. Hollenberg.** 1983. The presence of a defective *LEU2* gene on 2μ DNA recombinant plasmids of *Saccharomyces cerevisiae* is responsible for curing and high copy number. J. Bacteriol. **156**:625–635.
15. **Friedberg, E. C.** 1988. Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:70–102.
16. **Futcher, A. B., and B. S. Cox.** 1984. Copy number and the stability of 2μm circle-based artificial plasmids of *Saccharomyces cerevisiae*. J. Bacteriol. **157**:283–290.
17. **Futcher, B., and J. Carbon.** 1986. Toxic effects of excess cloned centromeres. Mol. Cell. Biol. **6**:2213–2222.
18. **Gottschling, D. E., T. E. Palen, and T. R. Cech.** 1983. Different nucleosome spacing in transcribed and non-transcribed regions of the ribosomal RNA gene in *Tetrahymena thermophila*. Nucleic Acids Res. **11**:2093–2109.
19. **Gottschling, D. E., and V. A. Zakian.** 1986. Telomere proteins: specific recognition and protection of the natural termini of *Oxytricha* macronuclear DNA. Cell **47**:195–205.
20. **Greider, C. W., and E. H. Blackburn.** 1985. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell **43**:405–413.
21. **Greider, C. W., and E. H. Blackburn.** 1987. The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell **51**:887–898.
22. **Henikoff, S., and M. K. Eghtedarzadeh.** 1985. Conserved arrangement of nested genes at the *Drosophila GART* locus. Genetics **117**:711–725.
23. **Henikoff, S., K. Tatchell, B. D. Hall, and K. A. Nasmyth.** 1981. Isolation of a gene from *Drosophila* by complementation in yeast. Nature (London) **289**:33–37.
24. **Himmelfarb, H. J., E. M. Simpson, and J. D. Friesen.** 1987. Isolation and characterization of temperature-sensitive RNA polymerase II mutants of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:2155–2164.
25. **Irani, M., W. E. Taylor, and E. T. Young.** 1987. Transcription of the *ADH2* gene of *Saccharomyces cerevisiae* is limited by positive factors that bind competitively to its intact promoter region on multicopy plasmids. Mol. Cell. Biol. **7**:1233–1241.
26. **Ito, H., Y. Jukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
27. **Koshland, D., J. C. Kent, and L. H. Hartwell.** 1985. Genetic analysis of the mitotic transmission of minichromosomes. Cell **40**:393–403.
28. **Lustig, A. J., and T. D. Petes.** 1986. Identification of yeast mutants with altered telomere structure. Proc. Natl. Acad. Sci. USA **83**:1398–1402.
29. **MacKay, V. L.** 1983. Cloning of yeast *STE* genes in 2μm vectors. Methods Enzymol. **101**:325–343.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Mirkovitch, J., M.-E. Mirault, and U. K. Laemmli.** 1984. Organization of the higher-order chromatin loop: specific DNA attachment sites on nuclear scaffold. Cell **39**:223–232.
32. **Moses, M. J.** 1968. Synaptinemal complex. Annu. Rev. Genet. **2**:363–412.
33. **Moyzis, R. K., J. M. Buckingham, L. S. Cram, M. Dani, L. L. Deaven, M. D. Jones, J. Jeyne, R. L. Ratliff, and J.-R. Wu.** 1988. A highly conserved repetitive DNA Sequence, $(TTAGGG)_n$, present at the telomeres of human chromosomes. Proc. Natl. Acad. Sci. USA **85**:6622–6626.
34. **Newlon, C. S.** 1988. Yeast chromosome replication and segregation. Microbiol. Rev. **52**:568–601.
35. **Pluta, A. F., G. M. Dani, B. B. Spear, and V. A. Zakian.** 1984. Elaboration of telomeres in yeast: recognition and modification of termini from *Oxytricha* macronuclear DNA. Proc. Natl. Acad. Sci. USA **81**:1475–1479.
36. **Price, C. M., and T. R. Cech.** 1987. Telomeric DNA-protein interactions of *Oxytricha* macronuclear DNA. Gene Dev. **1**: 783–793.
37. **Richards, E. J., and F. M. Ausubel.** 1988. Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell **53**:127–136.
38. **Shampay, J., and E. H. Blackburn.** 1988. Generation of telomere-length heterogeneity in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **85**:534–538.
39. **Shampay, J., J. W. Szostak, and E. H. Blackburn.** 1984. DNA sequences of telomeres maintained in yeast. Nature (London) **310**:154–157.
40. **Shore, D., and K. Nasmyth.** 1988. Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell **51**:721–732.
41. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
42. **Szotak, J. W., and E. H. Blackburn.** 1982. Cloning yeast telomeres on linear plasmid vectors. Cell **29**:245–255.
43. **Tschumper, G., and J. Carbon.** 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10**:157–166.
44. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxy-

methyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.

45. **Walmsley, R. W., C. S. M. Chan, B.-K. Tye, and T. D. Petes.** 1984. Unusual DNA sequences associated with the ends of yeast chromosomes. Nature (London) **310**:157–160.
46. **Walmsley, R. W., and T. D. Petes.** 1985. Genetic control of chromosome length in yeast. Proc. Natl. Acad. Sci. USA **82**:506–510.
47. **Zahler, A. M., and D. M. Prescott.** 1988. Telomere terminal transferase activity in the hypotrichous ciliate Oxytricha nova and a model for replication of the ends of linear DNA molecules. Nucleic Acids Res. **16**:6953–6972.
48. **Zakian, V. A., and J. F. Scott.** 1982. Construction, replication and chromatin structure of TRP1-RI circle, a multicopy synthetic plasmid derived from *Saccharomyces cerevisiae* chromosomal DNA. Mol. Cell. Biol. **2**:221–232.