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Size Threshold for *Saccharomyces cerevisiae* Chromosomes: Generation of Telocentric Chromosomes from an Unstable Minichromosome

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A 9-kilobase pair *CEN4* linear minichromosome constructed in vitro transformed *Saccharomyces cerevisiae* with high frequency but duplicated or segregated inefficiently in most cells. Stable transformants were only produced by events which fundamentally altered the structure of the minichromosome: elimination of telomeres, alteration of the centromere, or an increase of fivefold or greater in its size. Half of the stable transformants arose via homologous recombination between an intact chromosome IV and the *CEN4* minichromosome. This event generated a new chromosome from each arm of chromosome IV. The other "arm" of each new chromosome was identical to one "arm" of the unstable minichromosome. Unlike natural yeast chromosomes, these new chromosomes were telocentric: their centromeres were either 3.9 or 5.4 kilobases from one end of the chromosome. The mitotic stability of the telocentric chromosome derived from the right arm of chromosome IV was determined by a visual assay and found to be comparable to that of natural yeast chromosomes. Both new chromosomes duplicated, paired, and segregated properly in meiosis. Moreover, their structure, as deduced from mobilities in orthogonal field gels, did not change with continued mitotic growth or after passage through meiosis, indicating that they did not give rise to isochromosomes or suffer large deletions or additions. Thus, in *S. cerevisiae* the close spacing of centromeres and telomeres on a DNA molecule of chromosomal size does not markedly alter the efficiency with which it is maintained. Taken together these data suggest that there is a size threshold below which stable propagation of linear chromosomes is no longer possible.

The yeast *Saccharomyces cerevisiae* contains 16 linear, metacentric chromosomes which range from about 300 to over 2,000 kilobases (kb) in size (3, 14). These chromosomes are duplicated and segregated with high fidelity. During mitosis, a chromosome is lost once in every 10^4 to 10^5 divisions (8–10, 24), whereas in meiosis the rate of chromosome loss is about 2×10^{-4} per viable spore (21).

Three structures are known to be required for efficient maintenance and inheritance of yeast linear chromosomes: origins of DNA replication, centromeres (*CEN*s), and telomeres. Small (10 to 15 kb) artificial chromosomes containing these three structures, as well as a selectable gene, have been constructed in vitro and introduced into *S. cerevisiae* by transformation (4, 15). Although these linear minichromosomes transform *S. cerevisiae* efficiently and are maintained essentially unaltered through mitosis and meiosis, they are markedly less stable than either circular *CEN* plasmids or authentic yeast chromosomes. During mitosis, they are lost at rates of about 10% per division (4, 15). Small artificial chromosomes differ from authentic yeast chromosomes in four major ways: (i) they are about 20 times smaller than even the smallest yeast chromosome, (ii) they are telocentric, (iii) they bear surrogate telomeres derived from ciliate DNAs, and (iv) they lack most of the sequences normally found on a yeast chromosome.

In this report we describe the construction of a 9-kb linear chromosome which transforms *S. cerevisiae* efficiently but is maintained only transiently in transformed cells. Stable transformants were produced by elimination of telomeres, alteration of the centromere, or by an increase in the size of the minichromosome. These data lead to the conclusion that the maintenance of linear chromosomes is exquisitely sensitive to size. There appears to be a critical length below which stable propagation of *CEN4* linear chromosomes is no longer possible. The most common mechanism for rescue of this minichromosome involved the formation of telocentric chromosomes from chromosome IV. This event generated two new chromosomes, each of which had one "arm" identical to an "arm" of the minichromosome used in the transformation. We demonstrate here that the telocentric chromosome formed from the right arm of chromosome IV has an overall size greater than that of all but a few yeast chromosomes. Moreover, this chromosome contains all sequences that normally reside on the right arm of chromosome IV which might influence chromosome stability. However, the distance between the centromere and one of the physical ends of this chromosome is less than 4 kb, and one of its telomeres is derived from ciliate DNA. Nonetheless, the mitotic stability of this telocentric chromosome approaches that of an authentic yeast chromosome. Moreover, the telocentric chromosomes formed from both arms of chromosome IV duplicate, pair, and segregate properly in meiosis.

MATERIALS AND METHODS

The *S. cerevisiae* strains used in this study were 3482-16-1 (*MATa met2 his3Δ-1 leu2-3,112 trp1-284 ura3-52*; from L. Hartwell), RDα5 (*MATα, ade2-101 lys2-901 ura3-52 Δtrp1-901*; from P. Hieter), and FH18 (*MATa ade2-101 lys2 ade8-18 Δtrp1-901*; this study). The linear plasmid YLp1f (Fig. 1A) was constructed as follows. A 1.45-kb *Eco*RI fragment containing *TRP1* and *ARS1* (isolated from YRp7 [23]) was ligated to a 3.6-kb *Eco*RI-*Bam*HI fragment containing *CEN4*

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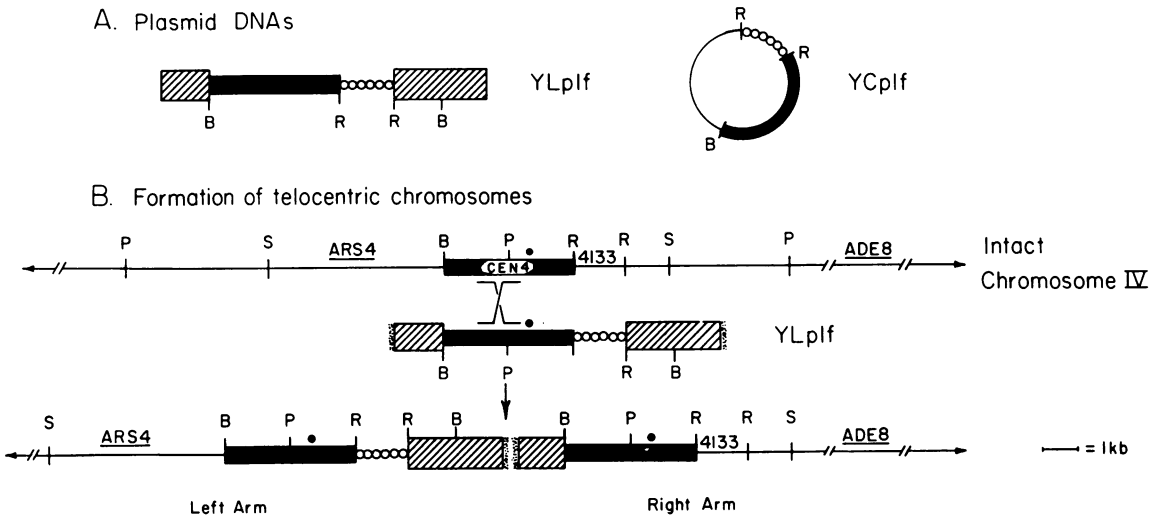

FIG. 1. Structure of DNAs. (A) Plasmid DNAs. YLplf (9 kb) was constructed in vitro by first ligating the 1.45-kb *Eco*RI (R) fragment carrying *TRP1ARS1* (OOO) derived from yeast chromosome IV to the 3.6-kb *Eco*RI-*Bam*HI (B) fragment carrying yeast *CEN4* (■). This 5.0-kb DNA was gel purified and then ligated to a mixture of the 2.6-kb *Eco*RI and 1.3-kb *Bam*HI terminal restriction fragments (▨) derived from *T. thermophila* C3V rDNA. YCplf (9 kb) contains the 1.45-kb *TRP1 ARS1* fragment and the 3.6-kb *Eco*RI-*Bam*HI fragment inserted into pBR322 DNA. (B) Formation of telocentric chromosomes by recombination between intact chromosome IV and YLplf. *ARS4* and 4133 represent segments of DNA from the left and right arms, respectively, of chromosome IV which were used as hybridization probes. *ADE8*, a locus on the right arm of chromosome IV, was used as a genetic marker. There are no *Sal*I (S) sites in YLplf. Abbreviations and symbols are the same as those used in panel A with the addition of *Pvu*II (P). The filled circle marks the position of the functional centromere as deduced from sequencing data (13a). The stippling indicates the DNA added to the *Tetrahymena* termini during propagation in *S. cerevisiae*. Only some of the *Eco*RI sites within the *Sal*I fragment containing *CEN4* on chromosome IV are indicated.

(isolated from YCp19 [22]). The *TRP1 ARS1 CEN4* DNA was gel purified and then ligated to gel-purified *Eco*RI and *Bam*HI terminal restriction fragments from *Tetrahymena* ribosomal DNA (rDNA) prepared from *Tetrahymena thermophila* as described by Wild and Gall (28). After ligation, YLplf was purified by agarose gel electrophoresis and introduced into *S. cerevisiae* by spheroplast transformation. The circular plasmid YCplf (Fig. 1A) was constructed by inserting the 1.45-kb *TRP1 ARS1* fragment and the 3.6-kb *CEN4* fragment into *Eco*RI-*Bam*HI-digested pBR322. Transformed cells were maintained under selection by growth in Y complete medium minus tryptophan (29). For mitotic stabilities, cells were grown to stationary phase at 30°C in YEPD medium (29), sonicated briefly, and spread onto YEPD plates. Under these conditions red and white cells had essentially equivalent rates of growth (data not shown). To ensure that sectored colonies were products of single colonies, individual colonies were marked about 30 h after plating. Colonies were scored for color after about 1 week. Meiotic behavior of telocentric chromosomes was determined as described by Dani and Zakian (4).

For most gels, DNA was isolated from *S. cerevisiae* by modifications (16) of the method of Davis et al. (6). For orthogonal field alteration gel electrophoresis (OFAGE), DNAs were prepared by modifications (3) of the gel insert method of Schwartz and Cantor (20), and gels were run essentially as described by Carle and Olson (2). The following fragments were gel purified and used as hybridization probes: a 1.45-kb *Eco*RI fragment containing *TRP1 ARS1* (isolated from YRp7 [23]), a 1.05-kb *Hin*dIII fragment from near the end of *Tetrahymena* rDNA (isolated from pRP7 [12]), a 1.7-kb *Eco*RI-*Hin*dIII fragment containing *ARS4* (isolated from YRp16-Sc4152 [22]), a 1.4-kb *Eco*RI fragment called 4133 derived from the right arm of yeast chromosome IV (isolated from YIp5-Sc4133 [22]), and a 1.6-kb *Xho*I fragment containing yeast *CEN4* (isolated from YCp19 [22]).

RESULTS

A linear minichromosome transforms *S. cerevisiae* with high efficiency but is not maintained in most cells. The linear minichromosome YLplf (Fig. 1A) was constructed in vitro, purified by agarose gel electrophoresis, and introduced by spheroplast transformation into two strains: 3482-16-1, a strain with a point mutation in *TRP1*, and RDα5, a strain in which the entire 1.45-kb *TRP1 ARS1* locus is deleted. Both the linear (YLplf) and circular (YCplf) DNAs transformed 3482-16-1 efficiently (1×10^5 to 2×10^5 transformants per μg). In contrast, although the circular plasmid produced a large number of transformants in RDα5 (4×10^4/μg), only eight Trp^+ colonies (~160/μg) were recovered in the *TRP1 ARS1* deletion strain after transformation with YLplf (hereafter these transformants will be referred to as RDL1 through RDL8.)

Individual transformants were grown to about 10^8 cells in medium lacking tryptophan. DNAs were prepared from RDL1 through RDL8 and from 20 of the 3482-16-1 colonies produced by transformation with YLplf and examined by agarose gel electrophoresis (Fig. 2). No plasmid-specific sequences were detected in the 3482-16-1 transformants (data not shown, but see, for example, Fig. 3A, lane 6), although the cells were phenotypically Trp^+. In contrast, colonies produced in either strain by transformation with YCplf contained DNA of the size and structure expected for this circular plasmid (data not shown). Likewise, DNAs from RDL1 through RDL8 hybridized both to an *ARS1* (Fig. 2) and a *Tetrahymena* rDNA-specific probe (Fig. 3A). However, the pattern of hybridization was different in different transformants, and in all cases it was different from that expected for a cell simply carrying YLplf.

We interpret these results as follows. YLp1f transforms *S. cerevisiae* with high efficiency (~10^5 transformants per μg in 3482-16-1). However, YLp1f is replicated or segregated so inefficiently that a cell carrying the minichromosome must undergo other changes to produce a stable Trp^+ colony. In 3482-16-1, a stable Trp^+ colony can arise by gene conversion at *trp1*. The unstable minichromosome is subsequently lost by dilution from these cells. Because gene conversion cannot occur in *TRP1*-deleted RDα5 cells, most of the transformants in this strain were abortive. From consideration of the number of YLp1f transformants and after correction for strain differences in transformation frequency with the circular DNA, we estimate that roughly 0.3% of the RDα5 cells that received YLp1f went on to produce a stable Trp^+ colony. The stable transformants in RDα5 were produced as a result of alterations in either YLp1f itself or in chromosomal sequences (or both).

Structure of YLp1f in RDα5 cells. Four of the eight transformants were derived by recombination between intact chromosome IV and YLp1f DNA and are discussed in detail below. The other four transformants each appear to have arisen by different mechanisms and are described only briefly here. RDL1 contained a 6.1-kb circle produced by deletion of a portion of the terminal sequences on YLp1f followed by ligation (Fig. 2, lane 1). When first examined, RDL2 cells contained a 13.3-kb linear minichromosome formed by the terminal addition of yeast DNA to YLp1f (Fig. 2, lane 2). However, continued growth resulted in loss of the linear minichromosome and its replacement by a small circular DNA. RDL6 contained a linear plasmid of 10.3-kb, the behavior of which was most consistent with its carrying an altered *CEN4* (Fig. 2, lane 6). RDL8 contained a linear minichromosome of 50 kb (its size was deduced from mobility in OFAGE gels; Fig. 2, lane 8). The properties and structure of these four transformants will be described in detail elsewhere. However, these data indicate that YLp1f can be rescued by deletion or alteration of either its telomeres (RDL1 and RDL2) or its centromere (RDL6) or by the acquisition of yeast sequences in such a way as to increase both centromere-telomere spacing and overall size (RDL8). These data, as well as those reported below, suggest that telomeres and a centromere cannot both function on a DNA molecule as small as YLp1f.

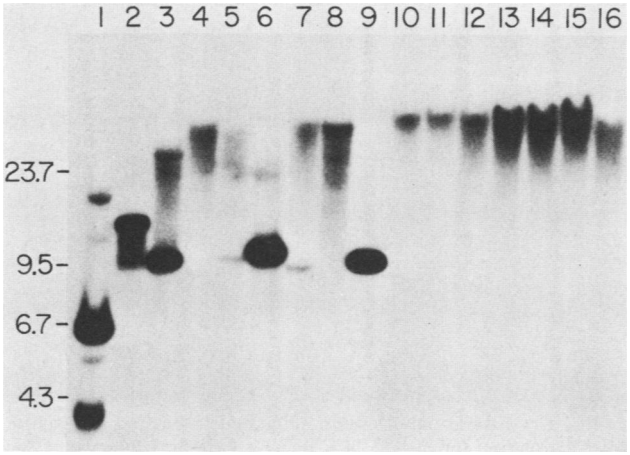

FIG. 2. Structure of DNAs in YLp1f transformants. DNAs were prepared from each of the transformants recovered in the *TRP1 ARS1* deletion strain RDα5 (lanes 1 through 8) and from seven transformants from strain 3482-16-1 (lanes 10 through 16). Undigested DNAs were subjected to electrophoresis in 0.4% agarose, transferred to nitrocellulose, and hybridized to a *TRP1 ARS1* probe. The chromosomal copy of these sequences was deleted in strain RDα5 but not in 3482-16-1. Lane 9 contains linearized YCp1f. The same pattern of hybridization was seen in a duplicate blot hybridized to a probe specific for *Tetrahymena* rDNA, except that with this probe there was no hybridization in lanes 9 through 16. Sizes in this and subsequent gels are in kilobase pairs.

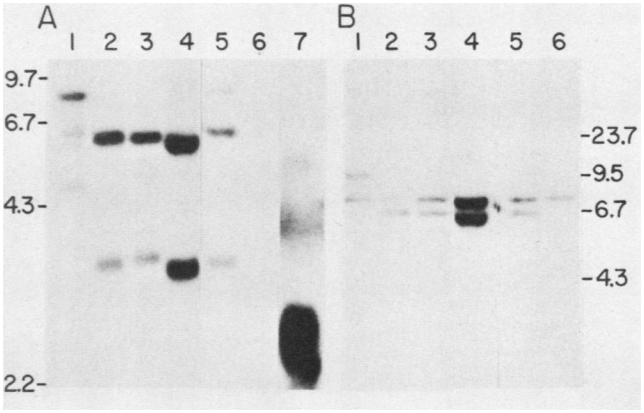

FIG. 3. *Pvu*II digestion of telocentric chromosomes. DNAs were digested with *Pvu*II, subjected to electrophoresis in duplicate 0.7% agarose gels, transferred to nitrocellulose, and hybridized either to a probe specific for *Tetrahymena* rDNA (A) or to yeast *CEN4* DNA (B). The lanes contain DNA from the following: lanes 1, RDL2 (cells contain a 13-kb linear plasmid); lanes 2, RDL3; lanes 3, RDL4; lanes 4, RDL5; lanes 5, RDL7; lanes 6, a Trp^+ 3482-16-1 strain produced by transformation with YLp1f; and lane 7, *Eco*RI-digested *Tetrahymena* rDNA. The size of the smaller *Pvu*II fragment varied among the different transformants (panel A, lanes 2 through 5) because of the addition of different amounts of yeast terminal sequences (26).

Structure of telocentric chromosomes. An unexpected mechanism for the rescue of YLp1f was displayed in four of the eight transformants (RDL3, RDL4, RDL5 and RDL7). In these cells, YLp1f was stabilized via homologous recombination with chromosome IV such that telocentric derivatives were produced from both arms of the original chromosome (Fig. 1B). This conclusion is based on three lines of evidence.

First, when undigested DNA from each of these transformants was hybridized either to an *ARS1* probe (Fig. 2) or to a *Tetrahymena* terminal-specific probe (data not shown), hybridization was detected at the position of chromosomal sequences, even though neither of these probes normally hybridizes to RDα5 DNA. When DNA was first prepared from RDL3, RDL5, and RDL7, hybridization of both probes was also detected to a linear plasmid of 9.0 kb (Fig. 2, lanes 3, 5, and 7). The structure of this plasmid is most easily explained as representing YLp1f to which 200 to 500 base pairs of yeast DNA has been added at each end. In these first DNA preparations, a *Sal*I restriction fragment diagnostic of intact chromosome IV was also detected in RDL3, RDL5, and RDL7 (described in more detail below). Therefore, RDL3, RDL5, and RDL7 cells initially contained a linear plasmid and a chromosome-sized derivative, both of which bear sequences introduced by transformation. The fate of YLp1f and intact chromosome IV in these strains is described below.

Second, the presence of telocentric chromosomes was deduced from the patterns of hybridization of chromosome

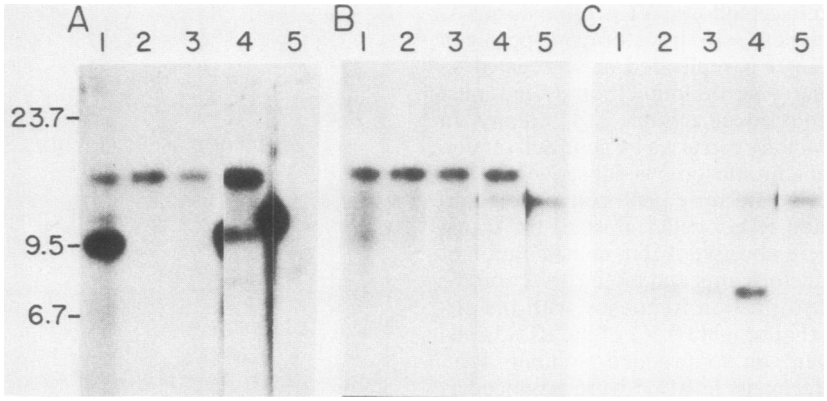

FIG. 4. *Sal*I digestion of telocentric chromosomes. DNAs were digested with *Sal*I, subjected to electrophoresis in 0.4% agarose, transferred to nitrocellulose, and hybridized sequentially to *TRP1ARS1* (A), *ARS4* (B), and 4133 (C). The lanes contain DNA from the following: lanes 1, RDL3; lanes 2, RDL4; lanes 3, RDL5; lanes 4, RLD7; and lanes 5, RDα5. *ARS1* and *ARS4* hybridized to the same 12.6-kb band in DNA from cells with telocentric chromosome IVs (lanes 1 through 4). In DNA from cells containing intact chromosome IV (lanes 4 and 5), *ARS4* and 4133 hybridized to the same 11.3-kb band.

IV sequences to restriction enzyme-digested DNAs. When DNAs from RDL3, RDL4, RDL5, and RDL7 were digested with restriction enzymes with recognition sites within YLp1f DNA (Fig. 1A), such as *Pvu*II (Fig. 3), *Eco*RI, or *Bam*HI (data not shown), the hybridizing bands were those expected from a cell containing YLp1f (even in RDL4, in which YLp1f was not visible in undigested DNA). However, when these DNAs were digested with *Sal*I, an enzyme with no recognition site in YLp1f, new bands hybridizing to chromosome IV or YLp1f sequences were detected (Fig. 4). For example, *ARS1* (a sequence deleted in RDα5) hybridized to a 12.6-kb *Sal*I fragment in RDL3, RDL4, RDL5, and RDL7 (Fig. 4A, lanes 1 through 4; hybridization was also detected at about 9.0 kb in RDL3, RDL5, and RDL7, as was expected from the presence of intact YLp1f in these cells). If the 12.6-kb *Sal*I fragment were derived from a telocentric form of the left arm of chromosome IV, it should also hybridize to a probe containing *ARS4* (Fig. 1B). Indeed, in the four transformants with telocentric forms of chromosome IV, the same band hybridized to both *ARS1* and *ARS4* (Fig. 4B, lanes 1 through 4). In contrast, in the four other transformants (data not shown) and in untransformed RDα5 cells (Fig. 4B, lane 5), *ARS4* hybridized to a *Sal*I fragment of 11.3 kb. Finally, these same DNAs were probed with a sequence called 4133 derived from the right arm of chromosome IV (Fig. 4C). When DNA from RDα5 cells was digested with *Sal*I, an 11.3-kb fragment spanning *CEN4* and containing both *ARS4* and 4133 was produced (Fig. 1B and Fig. 4C, lane 5). However, in cells containing telocentric forms of chromosome IV, 4133 hybridized to a fragment of 7.9 kb (Fig. 4C, lanes 1 through 4), which is the size expected if this fragment is carried on a telocentric chromosome derived from the right arm of chromosome IV (Fig. 1B).

The third line of evidence for the presence of telocentric forms of chromosome IV comes from the mobility of these chromosomes in gel systems capable of resolving intact yeast chromosomes (20) (Fig. 5). When yeast DNA is subjected to OFAGE, it can be resolved into as many as 13 discrete bands (Fig. 5A). These bands can be identified as individual yeast chromosomes by their hybridization to probes specific for different chromosomes (3). Genetic linkage studies predict that chromosome IV should be the largest of the yeast chromosomes and indicate that the right arm should be about twice as big as its left arm (14). Indeed, the band with the slowest mobility in these gels corresponds to intact chromosome IV (3, 20) (Fig. 5B through D).

When DNA from RDα5 cells was displayed on an OFAGE gel, 13 bands were resolved. The slowest migrating band

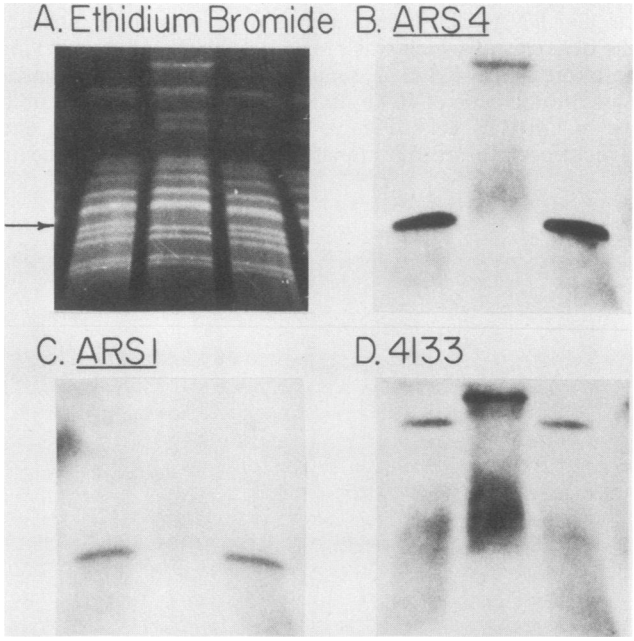

FIG. 5. OFAGE. DNAs were subjected to electrophoresis in a 1.5% agarose gel for 21 h at 14°C using a switching interval of 80 s. After electrophoresis, the gel was stained with ethidium bromide (A). The DNAs were transferred to nitrocellulose, and the blot was probed successively with *ARS4* (B), *ARS1* (C), and 4133 (D). The DNAs were prepared from the following strains: RDL5 (lanes 1), RDα5 (lanes 2), and RDL4 (lanes 3). The arrow in panel A indicates the position of the telocentric left arm of chromosome IV. The ethidium bromide staining band at the position of intact chromosome IV in RDL5 (lanes 1) and RDL4 (lanes 3) is not due to the presence of intact chromosome IV in these strains because it does not hybridize to chromosome IV sequences (panels B and D). This band does hybridize to nick-translated rDNA (data not shown) and thus represents the position of chromosome XII in this gel.

hybridized to probes specific for both the right (4133) and left (*ARS4*) arms of chromosome IV but not to *ARS1*, a locus normally found on chromosome IV but deleted in this strain (Fig. 5, lane 2). In DNAs prepared from two of the four strains with telocentric forms of chromosome IV, an additional band which migrated as the fifth smallest chromosome was visible in the ethidium bromide-stained profile of the gel (Fig. 5A, lanes 1 and 3). This band corresponds to a size of 510 kb, as deduced from its position relative to that of the six smallest yeast chromosomes by using the sizes for these chromosomes determined by Carle and Olson (3). This measurement is in good agreement with that predicted for the left arm of chromosome IV by genetic data (~450 kb [14]). Moreover, this band hybridized to both *ARS1* and *ARS4* (Fig. 5A and B), which is the pattern expected for the smaller of the two chromosomes produced by recombination between YLp1f and intact chromosome IV (Fig. 1B). Finally, in strains containing telocentric forms of chromosome IV, fragment 4133, a sequence derived from the right arm of chromosome IV, hybridized to a band with a mobility greater than that of intact chromosome IV (Fig. 5D). Although there are no size markers for this molecular weight range, the right arm migrates to the same band as that produced by comigration of chromosomes VII and XV (3), that is, to a position corresponding to a size considerably larger than that of the left arm of chromosome IV (as well as most other yeast chromosomes) but smaller than intact chromosome IV.

Formation of telocentric chromosomes. From the initial DNA preparations, three of the four transformants (RDL3, RDL5, and RDL7) carried YLp1f as well as telocentric forms of chromosome IV (Fig. 2, lanes 3, 5, and 7). Indeed, in RDL3, the majority of the hybridization to *ARS1* and *Tetrahymena* rDNA probes was to YLp1f-sized, rather than chromosome-sized, DNA. To explore the generation and maintenance of telocentric chromosomes over time, RDL3, RDL4, RDL5, and RDL7 cells were established as log-phase cultures in media lacking tryptophan and then maintained in log phase in media both with and without tryptophan for about 70 generations. DNAs were prepared from these cultures at intervals of 10 generations, digested with *Sal*I, and subjected to agarose gel electrophoresis. Hybridization with *CEN4* was used to monitor the presence of the different forms of chromosome IV (Fig. 6A). At 10 generations and throughout the course of this experiment, only telocentric forms of chromosome IV were detected in RDL4 and RDL5 (data not shown, but note that there is no intact chromosome IV detected in either of these strains by OFAGE gels; Fig. 5). In RDL3 and RDL7, intact chromosome IV and YLp1f, as well as telocentric forms of chromosome IV, were present after 10 generations; but by the end of the experiment, the majority of *CEN4* hybridization was to fragments derived from the telocentric chromosomes. The data for RDL3, grown with tryptophan, are presented in Fig. 6A because it provides the most dramatic example of this change. Densitometric scannings of the autoradiogram in Fig. 6A were used to determine relative intensities of the bands. From a comparison of the amount of hybridization to the *Sal*I fragment derived from intact chromosome IV to that in the fragments derived from telocentric chromosome IVs, it appears that at early times <50% of the RDL3 cells contained telocentric chromosome IVs (Fig. 6A, lanes 1 and 2). Because at early times there was 20 times more hybridization to YLp1f than to the *Sal*I fragment derived from intact chromosome IV (Fig. 6A, lane1), those cells carrying YLp1f were likely to have multiple copies of the plasmid. After 31 generations, about 70% had telocentric chromosome IVs (Fig. 6A, lane 3). After 53 generations, there was little YLp1f and no intact chromosome IV detectable (Fig. 6A, lane 5). After 60 generations, YLp1f was no longer detectable: all *CEN4* hybridization was to telocentric chromosome IVs (Fig. 6A, lane 6).

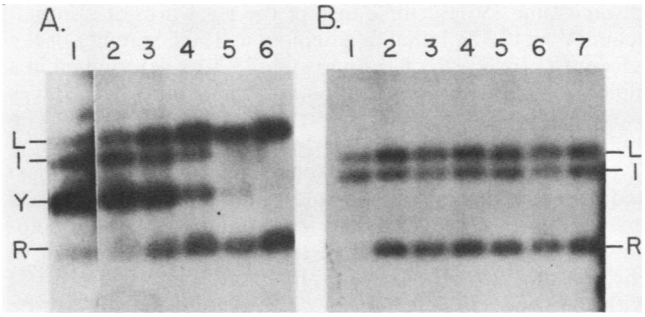

FIG. 6. Mitotic stability of telocentric chromosomes. (A) RDL3 was grown in media containing tryptophan for 9 (lane 1), 19 (lane 2), 31 (lane 3), 41 (lane 4), 54 (lane 5), and 62 (lane 6) generations. DNAs were digested with *Sal*I, subjected to electrophoresis in 0.4% agarose, transferred to nitrocellulose, and hybridized to nick-translated *CEN4* DNA. *Sal*I fragments hybridizing to *CEN4* were derived from the telocentric chromosome formed from the left arm of chromosome IV (L; 12.6 kb), intact chromosome IV (I; 11.3 kb), YLp1f (Y; 9 kb), and the telocentric chromosome formed from the right arm of chromosome IV (R; 7.9 kb). (B) RDL4 was mated to FH18. The diploid was grown to stationary phase in media containing tryptophan and transferred to plates containing low amounts of adenine. DNAs were prepared from red-white half-sectored colonies and treated as described above for panel A. The lanes contain DNA from the white portions of seven different half-sectored colonies. Only one of these sectors (lane 1) was due to loss of the telocentric chromosome derived from the right arm of chromosome IV. Abbreviations are the same as described for panel A.

The appearance of telocentric chromosomes and the concomitant loss of YLp1f and intact chromosome IV support the hypothesis that telocentric chromosomes are generated by recombination between YLp1f and chromosome IV. These data also indicate that telocentric chromosomes, once formed, are reasonably stable. Even without selection for *TRP1*, they were maintained in RDL4 and RDL5 for over 70 generations without detectable reformation of intact chromosome IV. Moreover, in a mixed population of cells, some of which contained intact chromosome IV and some of which contained telocentric chromosomes, those with telocentric chromosomes quickly predominated. For example, in only 12 generations, the percentage of RDL3 cells with intact chromosome IV dropped from 50 to 30 (~4% loss per generation; Fig. 6A, lanes 2 and 3). Because telocentric chromosomes are no more stable than intact chromosome IV (see below), cells containing the intact chromosome must grow slowly compared with cells with telocentric chromosomes. Slow growth of these cells might be due to the fact that they contain many copies of YLp1f which might, in turn, cause them to be limited for centromere or telomere-binding proteins, spindle microtubules, etc.

Mitotic stability of telocentric chromosomes. Loss of the right arm of chromosome IV was monitored by a color assay which takes advantage of the fact that strains blocked late in the purine biosynthetic pathway (e.g., *ade2*) are red, whereas cells blocked earlier in the pathway (e.g., *ade8*) are white (19). Because *ade8* is epistatic to *ade2*, *ade8 ade2* cells are white. The *ADE8* gene is located on the right arm of

chromosome IV. Because loss of the right arm of chromosome IV would be lethal in a haploid cell, the stability of the telocentric right arm of chromosome IV was determined in a diploid. A red diploid (genotype *ade2/ade2 ade8/ADE8*) was produced by mating RDL4 to the *ade8* strain FH18. The diploid was grown to stationary phase in medium containing tryptophan (i.e., no selection for telocentric chromosomes) and plated on plates containing low amounts of adenine; and the number of red, white, and red-white half-sectored colonies was determined. Loss of the telocentric right arm of chromosome IV produces an aneuploid white cell (2n-1/2) with the genotype *ade2/ade2 ade8*. White cells can also be produced by events other than chromosome loss, for example, by conversion of *ADE8* to *ade8* or by formation of a respiratory-deficient (petite) cell. However, the percentage of red-white half-sectored colonies in which the cells in the white sector have lost the 7.9-kb *Sal*I fragment detected by hybridization to *CEN4* (Fig. 1B and 6A) can provide a measure of the rate at which the telocentric right arm of chromosome IV is lost.

In one experiment, a total of 17,544 cells were plated: of these, 16,676 produced red colonies, 868 produced white colonies, and 17 produced a red-white half-sectored colony. DNA was prepared from both halves of each half-sectored colony, digested with *Sal*I, and analyzed by Southern hybridization (Fig. 6B). One of the seventeen white half-sectors was produced by loss of the telocentric right arm of chromosome IV (Fig. 6B, lane 1). Meiotic analysis of cells derived from this half-sector was consistent with its having contained predominately (or solely) 2n-1/2 aneuploid cells. Genetic analysis indicated that the other white sectors were due either to homozygosity at *ade8* (seven sectors) or to the petite phenotype (nine sectors). White sectors were formed in the control diploid (produced by mating RDα5 with FH18) at comparable rates.

The loss rate of the telocentric chromosome would be underestimated if the 2n-1/2 aneuploid cell in which the loss occurred grew slowly compared with the diploid cell from which it was derived. In this case, losses occurring at the first division after plating would not have been scored as half-sectors because the white portion of the colony would have been small compared with the overall size of the colony. To address this potential problem, DNAs were also prepared from the white portions of all colonies with a white sector that was equal in size to about one-quarter of the colony ($n = 20$) and from the white portions of 230 colonies with even smaller white sectors. Care was taken to test all sectors in which the white segment appeared to grow poorly compared with red portions of the colony. None of these white sectors was due to chromosome loss.

In another experiment, there were seven half-sectored colonies of 6,682 red cells, but none of the white sectors was due to chromosome loss. The combined data from the two experiments indicate that the telocentric chromosome formed from the right arm of chromosome IV was lost at a rate of about 4×10^{-5} per division.

Behavior of telocentric chromosomes in meiosis. A chromosome which behaves properly in meiosis should exhibit the following properties: duplication during premeiotic S phase, synapsis with and segregation from its homolog during meiosis I, and segregation from its sister chromatid at meiosis II. The diploid strain created by mating RDL4 with FH18 (see mitotic stability study above) was sporulated and tetrads were dissected. If the telocentric derivatives of chromosome IV duplicate, pair, and segregate with fidelity during meiosis, most of the spores from tetrads containing four spores should be viable. Moreover, both *TRP1* and restriction fragments diagnostic for the telocentric chromosomes should segregate 2+:2− at the first meiotic division.

Four-spore tetrads ($n = 56$) were dissected; of these, 70% yielded four viable spores, a number comparable to the 71% ($n = 59$) obtained when tetrads were dissected from the control diploid (produced by mating RDα5 with FH18). In all of these tetrads, *TRP1* and *URA3* segregated 2+:2−. DNA was prepared from each spore from two of the tetrads with four viable spores (Fig. 7). In these tetrads, the two Trp^+ spores contained restriction fragments characteristic of the telocentric chromosomes derived from both the right and left arms of chromosome IV, whereas the Trp^- spores carried the *CEN4* restriction fragment characteristic of intact chromosome IV. Furthermore, in 85% of the tetrads with four viable spores, *TRP1* segregated at the same division as *URA3*, a centromere-linked marker on chromosome V which exhibits first division segregation in 84% of tetrads (14). Finally, in four spore tetrads with only two or three viable spores, there was no preferential loss of telocentric chromosomes. Assuming 2+:2− segregation of *TRP1*, spores which failed to germinate were as likely to be Trp^+ (45%) as Trp^- (55%). Taken together, these data suggest that telocentric derivatives of chromosome IV can duplicate, pair, and segregate properly in meiosis.

DNA was prepared from cells that were derived from a spore that received the telocentric chromosomes and analyzed in an OFAGE gel (data not shown). No changes in the sizes of the telocentric chromosomes occurred as a result of passage through meiosis.

DISCUSSION

Size requirement for linear chromosomes. We constructed a 9-kb linear minichromosome, YLp1f, containing *CEN4* and *TRP1* which transforms *S. cerevisiae* cells efficiently. However, in the absence of the potential for gene conversion at *TRP1*, only about 0.3% of the transformants produced by YLp1f gave rise to a stable Trp^+ colony, and none of these carried only the original minichromosome. Stable transformants in the *TRP1* deletion strain were only detected in cells in which the minichromosome was fundamentally altered. Stabilization occurred by modification of centromere function

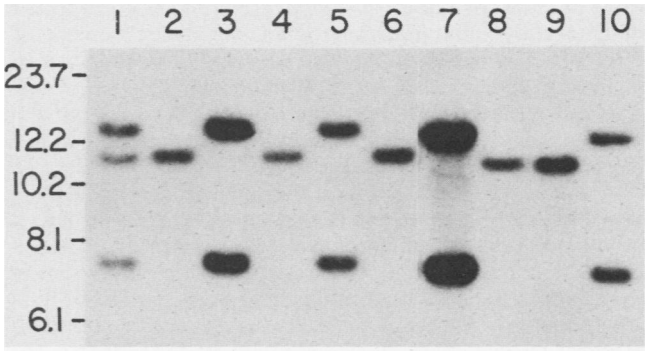

FIG. 7. Segregation of telocentric chromosomes in meiosis. The diploid produced by mating RDL4 with FH18 was sporulated and dissected. DNAs prepared from individual spores were treated as described in the legend to Fig. 6A. The lanes contain DNA from the diploid prior to sporulation (lane 1), each of four spores from one tetrad (lanes 2 through 5), a control diploid strain produced by mating RDα5 with FH18 (lane 6), and each of four spores from a second tetrad (lanes 7 through 10).

(RDL6) or elimination of telomeres (RDL1 and RDL2) or by the acquisition of additional yeast sequences (RDL3, RDL4, RDL5, RDL7, RDL8). The transformation properties of YLp1f are in contrast to those displayed by other plasmid DNAs. For example, circular plasmids without an *ARS* transform via homologous recombination with chromosomal sequences, but they do so at a low frequency (1 to 20 transformants per μg [23]). Linear and circular *ARS* plasmids transform with high frequency and are propagated essentially unaltered as extrachromosomal DNAs (4, 23, 25). The fact that YLp1f, unlike plasmids without an *ARS*, produced many transformants in 3482-16-1 suggests that the minichromosome must be propagated, albeit poorly, in most cells that receive it. However, the efficiency with which it is propagated is not sufficient in itself to produce a stable Trp^+ colony.

Studies with the telocentric chromosomes derived from YLp1f indicate that each "arm" of the minichromosome can function efficiently when it is part of a large DNA molecule. Moreover, linear *CEN4* minichromosomes that are somewhat larger (13 and 15 kb) but in other respects similar to YLp1f also transform efficiently and are stably maintained, as are *CEN3* minichromosomes of about 10 kb. (Although, these linear minichromosomes are markedly less stable than their circular counterparts [4, 15]). These data suggest that a *CEN4* minichromosome must be of a critical minimal size for both centromeres and telomeres to function. This size limitation does not apply to other *ARS* plasmids: an acentric circular plasmid of 1.45 kb (29), a *CEN11* circular minichromosome of 3 kb (1), and a 3-kb acentric linear plasmid (7) can all transform and propagate efficiently in *S. cerevisiae*. At least two of these DNAs are either as stable (1) or more stable (29) than the larger plasmids from which they were derived.

Other studies also demonstrate that size affects chromosome stability. Up to about 100 kb, the stability of *CEN3* and *CEN4* linear and circular artificial chromosomes improves with increasing size (11, 15). Size also influences the stability of natural yeast chromosomes, as demonstrated by the effects of deletions on the stability of chromosome III (R. T. Surosky, C. S. Newlon, and B.-K. Tye, unpublished data). Down to 70 kb, even large deletions on chromosome III produce relatively small decreases in stability. However, for chromosomes from 70 down to 29 kb, small deletions have large deleterious effects (Surosky et al., unpublished data). The data reported here indicate that, in addition to an effect of size on stability, there is also a sharp size cutoff below which stable propagation of linear (but not circular) chromosomes is no longer possible. We believe that very small linear chromosomes are most likely defective in segregation (not replication) because a subset of RDL3 cells accumulated many copies of YLp1f (Fig. 6A).

Formation and stability of telocentric chromosomes. The most frequent event to give rise to a stable Trp^+ transformant in the *TRP1* deletion strain was formation of telocentric chromosomes from both arms of chromosome IV (Fig. 1B). These telocentric chromosomes arose by homologous recombination between the *CEN4* minichromosome and the *CEN4* region of chromosome IV(Fig. 6A). One "arm" on both of these telocentric chromosomes was identical to one "arm" of YLp1f, a minichromosome which cannot itself be stably propagated (Fig. 1B). The presence of telocentric chromosomes was deduced from the pattern of hybridization of chromosome IV and minichromosome sequences to restriction enzyme-digested DNAs (Fig. 2, 3, and 4). Their presence was confirmed by the behavior of DNA from these transformants in OFAGE gels which can separate intact yeast chromosomes (Fig. 5). In these gels, the chromosome derived from the left arm of chromosome IV migrated as the fifth smallest yeast chromosome with a length of about 510 kb. The chromosome derived from the right arm of chromosome IV, although smaller than intact chromosome IV, was still larger than all but four authentic yeast chromosomes. Both chromosomes were telocentric. The functional centromere was only 3.9 kb from one of the physical ends of the chromosome derived from the right arm of chromosome IV and 5.4 kb from one end of the chromosome bearing its left arm (deduced from the structure of YLp1f and the position of the centromere consensus sequence within the *CEN4* fragment [13a]).

Unlike these telocentric derivatives of chromosome IV, all natural yeast chromosomes are metacentric (14). Indeed, it is a matter of controversy as to whether or not true telocentric chromosomes exist in natural karyotypes (13, 27). When telocentric chromosomes arise by, for example, misdivision of the centromere of a metacentric chromosome (5), they are often unstable. One of the best documented examples of telocentric instability was described in maize. A telocentric chromosome generated from one arm of a normally metacentric chromosome was deficient in pairing in meiosis I, generated isochromosomes at meiosis, and was frequently lost or reduced in size during mitosis (17, 18). In contrast, the size and structure of the telocentric chromosomes derived from yeast chromosome IV did not change during mitosis or meiosis. Analysis of DNAs by OFAGE gels rules out formation of isochromosomes from either telocentric chromosome, as well as the deletion (or addition) of as little as 50 to 100 kb from the chromosome derived from the left arm of chromosome IV (Fig. 5). Moreover, results of a visual assay used to measure the mitotic stability of the telocentric chromosome derived from the right arm of chromosome IV indicate that its rate of loss (4×10^{-5} per division) was comparable to that of natural yeast chromosomes. For example, loss of chromosome VII, the size of which in OFAGE gels is indistinguishable from that of the right arm of chromosome IV (Fig. 6), occurs at 0.5×10^{-5} to 1.4×10^{-5} per division (8, 9); chromosome III, one of the smallest yeast chromosomes, is lost at 0.4×10^{-4} to 1.8×10^{-4} per division (24). We conclude that the telocentric chromosome IV is, at worst, 10 times less stable (9) and, possibly, as stable (24) as natural metacentric yeast chromosomes. This result is consistent with results of another recent study. A chromosome generated by internal deletion of 100 kb from the left arm of chromosome III (to produce a chromosome in which the centromere is 12 kb from the end of the molecule) is at worst four times less stable than intact chromosome III (24).

Although loss rates were not determined, the telocentric chromosomes were also propagated with reasonable stability through meiosis. In most or all of the 56 tetrads that were analyzed, the telocentric chromosomes derived from the right and left arms of chromosome IV paired with and separated from intact chromosome IV at meiosis I. A priori, faithful segregation of the telocentric chromosomes might be an unexpected result. However, because each telocentric chromosome is likely to undergo multiple exchanges with intact chromosome IV, recombination is probably sufficient to ensure that both telocentric chromosomes disjoin properly from their homolog (intact chromosome IV). Although telocentric chromosomes can segregate reasonably well in meiosis (compared, for example, with artificial minichromosomes [4, 15]), they do not necessarily do so with the

same high efficiency displayed by authentic yeast chromosomes (2×10^{-4} losses per viable spore [21]).

Are the new chromosomes derived from chromosome IV truly telocentric; that is, do they possess terminal centromeres? Clearly, chromosomes with "arms" of either 4 or 5 kb would be labeled telocentric by conventional cytological criteria (13). The absence of any known genes within the 4 kb between *CEN4* and the chromosome end for the chromosome carrying the right arm of chromosome IV also argues for its being telocentric. Nonetheless, it is possible that there is a minimum size required for formation of a stable chromosome arm. To address this question, most of the 4 kb separating *CEN4* from the end of the chromosome can be eliminated.

In conclusion, these data demonstrate that both "arms" of YLp1f are able to function efficiently on DNA molecules of chromosomal size. Therefore, the close spacing of centromeres and telomeres does not markedly reduce the efficiency with which yeast chromosomes are duplicated or segregated. Moreover, because one "arm" of these telocentric chromosomes lacks all but a few kilobase pairs of yeast DNA, chromosome stability must not require other as yet unidentified *cis*-acting sequences on both arms. Also, termini derived from ciliate DNAs and capped by 200 to 500 base pairs of yeast terminal sequences must function about as efficiently as yeast telomeres in *S. cerevisiae*. Taken together these data suggest that it is size alone that is responsible for the inefficient propagation of very small linear chromosomes.

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