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Genetics

Genetic control of chromosome length in yeast

(repeated sequences/telomeres/DNA replication)

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ABSTRACT **The chromosomes of the yeast *Saccharomyces cerevisiae* terminate with sequences that have the form poly(C_{1-3}-A). In this paper, we show that within an individual yeast strain all chromosomes end with tracts of poly(C_{1-3}-A) of similar lengths; however, different strains can have tracts that vary in length by a factor of two. By a genetic analysis, we demonstrate that yeast cells have a mechanism that allows them to change rapidly the length of their chromosomes by altering the length of the poly(C_{1-3}-A) tract.**

Watson (1) pointed out that conventional mechanisms of DNA replication do not allow the complete duplication of a linear DNA molecule, because all known DNA polymerases require a primer and elongate the growing strand exclusively in the 5′ to 3′ direction. To investigate how the ends (telomeres) of eukaryotic chromosomes are replicated, we have analyzed the structure and stability of the telomeres of the yeast *Saccharomyces cerevisiae*.

The yeast chromosomes terminate in the simple DNA sequence poly(C_{1-3}-A) (2). Chan and Tye (3) described two classes of repeated sequences, X and Y′, that are found associated with the terminal poly(C_{1-3}-A) tract. The Y′ sequences are a collection of homogeneous repeats 6.7 kilobases (kb) long found at the telomeres of most but not all of the yeast chromosomes. The sequences of the X family of repeats are heterogeneous in size (0.3–3.75 kb per repeat) and have thus far been present on all yeast telomeres examined. The order of these sequences (from telomere to centromere) is as follows: [poly(C_{1-3}-A)]-$(Y')_{0-4}$ copies-[poly(C_{1-3}-A)]-(X)-single copy chromosomal sequences (3, 4). There are, therefore, two types of yeast telomeres; those that have only an X repeat and a poly(C_{1-3}-A) tract, and those that have one or more Y′ repeats integrated within the poly(C_{1-3}-A) tract associated with the X sequence. In this paper, we call the first class X telomeres and the second class XY′ telomeres.

Although the poly(C_{1-3}-A) tracts at the telomere have only short runs of alternating C and A residues (2, 4), these tracts hybridize strongly to the synthetic copolymer poly(dG-dT·dC-dA) (5). In *Xho* I digests of genomic DNA, poly(G-T) hybridizes to a broad band of ≈1.3 kb as well as to a large number of fragments >5 kb; the broad band at 1.3 kb represents a large class of yeast telomeres (5). By doing this type of analysis with a number of different yeast strains, we demonstrate that the lengths of the telomeres vary between different strains but are relatively constant within a strain. The variation in length is the result of variation in the size of the terminal poly(C_{1-3}-A) tract and is under the control of several yeast genes.

MATERIAL AND METHODS

Yeast Strains and Growth Conditions. The haploid strain A364a was provided by L. Hartwell (University of Washington) and has the genotype *a ade1 ura1 gal1 ade2 tyr1 his7 lys2*. The strain 2262 (provided by C. McLaughlin, University of California, Irvine) has the genotype *α ade1 ura1 gal1 his5 lys11 leu2*. The diploid D4 (RW) was constructed by mating A364a to 2262. The diploid strain MGG2 (genotype, *a/α his3/his3 leu2-3,112/leu2-3,112 SUC2/SUC2*) was constructed by diploidizing a haploid strain and is, therefore, homozygous at all loci except mating type. Conditions for growing and sporulating the yeast strains were standard (6).

Restriction Enzymes, Reagents, and Reaction Conditions. The restriction enzymes *Xho* I and *Hpa* I as well as the exonuclease BAL-31 were purchased from New England Biolabs and were used with the buffers recommended by the manufacturer. The poly(dG-dT·dC-dA) was bought from Boehringer Mannheim. For the BAL-31 digestion experiments, ≈80 μg of yeast DNA was incubated at 32°C with 1.2 units of the BAL-31 enzyme in 1 ml of the recommended reaction buffer (600 mM NaCl/12 mM $CaCl_2$/12 mM $MgCl_2$/20 mM Tris·HCl, pH 8/1 mM EDTA). At various time intervals, 80-μl samples were removed, added to 16 μl of 0.2 M EGTA, then precipitated with 50 μl of 7.5 M ammonium acetate and 150 μl of isopropanol. The precipitate was recovered by centrifugation and washed twice—once with 70% ethanol and once with 95% ethanol. Samples (1 μg) were used in the subsequent Southern analysis.

Yeast DNA Isolation. To isolate large DNA (50 kb), we used the "mini-prep" procedure, which involves lysis of spheroplasts (6). To ensure that the results were unaffected by the specific DNA isolation procedure, we also isolated DNA from A364a, 2262, and D4 (RW) using a procedure in which cells were disrupted by Vortex mixing with glass beads (7).

Southern Analysis. The DNA samples, after treatment with restriction enzymes, were analyzed on either 0.8% or 1% agarose gels containing ethidium bromide. The DNA fragments were transferred either to nitrocellulose (8) or to Gene-Screen (New England Nuclear). The hybridization probe used in the analysis was nick-translated poly(dG-dT·dC-dA) (5). For nitrocellulose filters, the hybridization was done for 16 hr at 55°C in 6× NaCl/Cit (0.9 M NaCl/0.09 M Na citrate) with Denhardt's solution (5). For the Gene-Screen filters, hybridization was done at 52°C in 0.3 M NaCl/60 mM Tris·HCl, pH 8/20 mM EDTA/5% sodium dodecylsulfate/Denhardt's solution (0.2% Ficoll/0.2% polyvinylpyrrolidone/0.2% bovine serum albumin).

RESULTS

Telomeres in Different Yeast Strains Vary in Length. By examining a number of different laboratory yeast strains, we found two haploid strains in which the lengths of the telomeres were reproducibly different. We detected these differences by hybridizing *Xho* I-treated genomic DNA to poly(G-T) (Fig. 1*a*). The broad band of hybridization ≈1.3 kb in size represents the distance between the tip of the chromosome and a conserved *Xho* I site in the XY′ class of telomeres; this

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Abbreviations: kb, kilobase(s); bp, base pair(s).

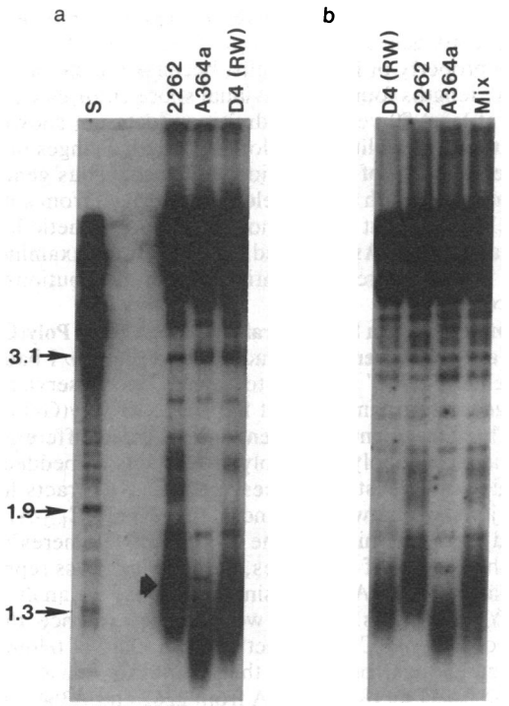

FIG. 1. Telomere sizes in different yeast strains can be different. Genomic DNA was isolated from the haploid yeast strains 2262, A364a, and the diploid D4 (RW) (constructed by mating A364a with 2262). This DNA was treated with the restriction enzyme *Xho* I, and the resulting DNA fragments were separated by agarose gel electrophoresis. The separated fragments were transferred to nitrocellulose (8) and hybridized to ^{32}P-labeled poly(dG-dT·dC-dA) (5). Lane labeled S (in all figures) contains *Eco*RI-treated A364a DNA; these bands of hybridization serve as size standards to allow comparisons of different gels. Sizes (in kb) of some of the labeled bands in lane S are indicated by numbered arrows. Larger arrow in *a* indicates the bank of hybridization in 2262 that is characteristic of the XY′ class of telomeres (4, 5). (*b*) Similar analysis of *Xho* I-treated DNA in which the fragments were separated on a 0.8% agarose gel instead of a 1% gel. Lower gel concentration results in a narrower size distribution of the telomeric fragments.

class accounts for more than half of the ≈32 telomeres of the cell (3). Although both haploid strains shown in Fig. 1 have a broad band at approximately the expected position, the positions of the bands are detectably different. For strain 2262, the fragments within the band are centered on a size of 1.5 kb with a distribution of ±0.25 kb (the ± designation indicates the size distribution of fragments in the band, not a standard deviation of the average fragment size). For A364a, the comparable fragments are centered on a size of 1.26 kb with a distribution of ±0.18 kb.

The observed difference of 250 base pairs (bp) in size between the telomeres of A364a and 2262 could represent a different placement of the *Xho* I site within the Y′ repeat in the two strains or a different average size for the terminal poly(C_{1-3}-A) tract. We showed experimentally that the latter explanation was correct. Large (>50 kb) genomic DNA was isolated from the two strains, treated for various times with the BAL 31 exonuclease, and then digested with the *Xho* I restriction enzyme. The resulting fragments were separated by gel electrophoresis and hybridized to poly(G-T). As shown in Fig. 2, the broad band representing the XY′ telomeres is rapidly digested by BAL-31, confirming its telomeric identification. In control experiments using the yeast gene *lys2* (which is located internally on the chromosome) as a hybridization probe, no degradation is observed, even at the longest time point (data not shown). For both the A364a and 2262 DNA samples, with increasing time of digestion with BAL-31, the *Xho* I band representing the XY′ telomeres becomes steadily smaller until it abruptly disappears. This abrupt disappearance (which occurs between 80 and 100 min for 2262 and between 40 and 50 min for A364a) presumably represents the complete removal of the poly(C_{1-3}-A) tract. Thus, by measuring the difference between the size of the major telomeric fragments before treatment with BAL-31 and the size of the fragments just prior to their disappearance, we calculated tract lengths of 620 ± 250 bp for 2262 and 360 ± 180 bp for A364a. The terminal poly(C_{1-3}-A) tracts in 2262, therefore, are almost twice as long as those of A364a. In addition, the difference in tract lengths is large enough to explain the difference in size of the *Xho* I fragments derived from the XY′ telomeres.

Since A364a and 2262 are of opposite mating types, we

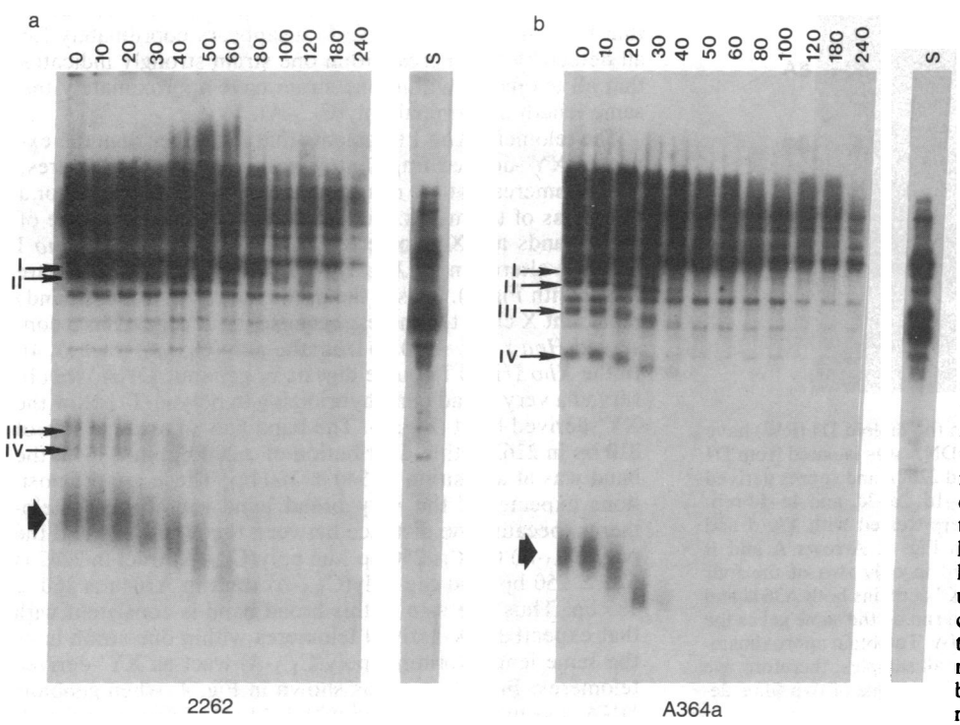

FIG. 2. BAL-31 sensitivity of telomeres of strains 2262 and A364a. Genomic DNA was isolated from haploid strains 2262 (*a*) and A364a (*b*). DNA was treated with the BAL-31 exonuclease for various times and then digested with the enzyme *Xho* I. The *Xho* I fragments from each sample were separated by gel electrophoresis, transferred to nitrocellulose, and hybridized to poly(G-T). Numbers at the top of the lanes show time of incubation (minutes) with BAL-31. Large arrow indicates the *Xho* I band that represents the XY′ class of telomeres. Small arrows next to roman numerals show bands that disappear with the same kinetics as the XY′ class of telomeres.

constructed a diploid strain D4 (RW) by mating (Fig. 1). Although the telomeric *Xho* I fragments of the diploid had sizes that were intermediate between those of the two haploid parents (1.37 ± 0.23 kb), the distribution of fragment sizes was as narrow as that of the individual haploid parents. These results suggest either that there is genetic control of telomere length or that recombination between different telomeres in the diploid results in all telomeres having the same average length. To distinguish between these possibilities, we sporulated the diploid and examined the lengths of the XY′ telomeres in cultures derived from individual spores.

Meiotic Segregation of Genes Affecting Chromosome Length. When the lengths of the XY′ telomeres in spores derived from D4 (RW) were examined (Fig. 3), it was clear that different spores often had telomeres of different lengths. This result, in combination with the observations concerning the sizes of the telomeres in haploid parents and the diploid strain, suggests that there are a number of genes that control telomere length. These genes specify different lengths in A364a and 2262 but are codominant in the diploid. The variability in telomere lengths in different haploid spores, therefore, represents the meiotic segregation of the heterozygous genes. The lengths of the XY′ telomeres within one tetrad do not show simple 2:2 segregation (two spores with 2262-length telomeres to two spores with A364a-length telomeres), indicating that telomere length is not controlled by a single pair of heterozygous alleles.

An alternative, although less likely, explanation of the data is that the lengths of the telomeres are altered in meiosis by a mechanism that is unrelated to meiotic segregation of heterozygous genes. This process would have to be exclusively a property of meiotic cells, because the characteristic telomere lengths found in individual spore cultures were stable for at least 80 vegetative divisions (data not shown). To rule out the possibility that telomere length changes in meiosis independently of segregation of heterozygous genes, we examined the length of XY′ telomeres derived from a diploid strain (MGG 2) that was homozygous at all genetic loci except mating type. As expected, in two tetrads examined, all spores had telomeres of identical length distributions (data not shown).

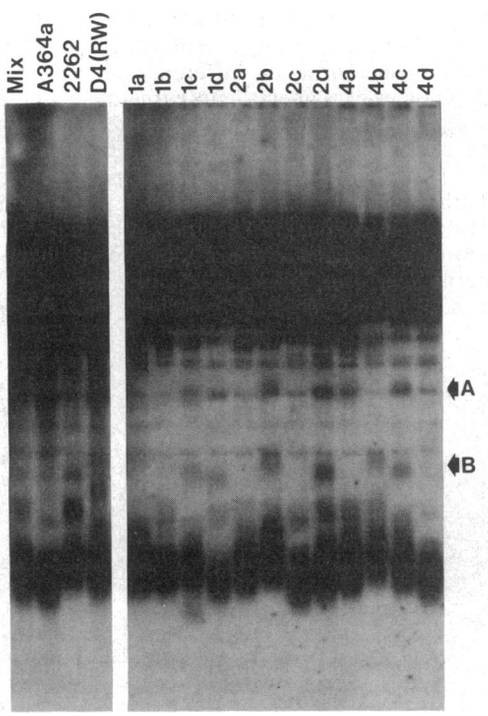

FIG. 3. Different spores derived from the diploid D4 (RW) have telomeres of different lengths. Genomic DNA was isolated from D4 (RW), haploid parental strains (A364a and 2262), and spores derived from D4 (RW). DNA samples labeled 1a–1d, 2a–2d, and 4a–4d represent complete tetrads. All samples were treated with *Xho* I and hybridized to poly(G-T) as described in Fig. 1. Arrows A and B point out telomeres that are represented in only two of the four spores of each tetrad. Lane labeled "MIX" contains both A364a and 2262 DNA. Leftmost four lanes, although run on the same gel as the spore DNA samples, contained more DNA. To obtain approximately the same intensity of hybridization for all samples, therefore, we assembled a composite of complementary portions of two films developed after different exposure times.

Telomeres Within Single Strains of Yeast Have Poly(C_{1-3}-A) Tracts of Similar Lengths. In addition to the *Xho* I band that represents the XY′ class of telomeres, we observed many other genomic fragments that hybridize to poly(G-T) (Figs. 1–3). These fragments represent at least three different types of sequences: copolymeric poly(C-A) tracts embedded within single-copy yeast sequences, poly(C_{1-3}-A) tracts located at the junction between X and Y′, and poly(C_{1-3}-A) tracts located at the terminus of the X class of telomeres (4). Of these three types of sequences, only the last class represents terminal poly(C_{1-3}-A) tracts similar to those we analyzed for the XY′ telomeres. Below we present evidence that the length of the poly(C_{1-3}-A) tract of the X class of telomeres is coordinately controlled with that of the XY′ class.

In the *Xho* I digests of DNA from 2262 and A364a (Figs. 1 and 2), we observed several "fuzzy" bands, representing hybridization of poly(G-T) to DNA fragments larger than the *Xho* I band characteristic of the XY′ telomeres. Since cloned telomeres show some heterogeneity in length (9), the fuzzy appearance of these bands suggested that they might represent non-XY′ telomeres. This possibility was confirmed by the BAL 31 experiments shown in Fig. 2. For both 2262 and A364a, at least four fuzzy bands were digested with BAL 31 at the same rate as the *Xho* I band characteristic of the XY′ telomeres. The telomeric fragments I and II of 2262 appear to be identical in size with the telomeric fragments I and II of A364a. Bands III and IV appear different in the two strains, presumably as a result of polymorphic *Xho* I restriction sites in single-copy DNA centromere proximal to the telomere; the allelic relationships between bands III and IV of A364a and bands III and IV of 2262 are not known. The observation that hybridization to poly(G-T) disappears coordinately for all detectable telomeres within one strain strongly indicates that all telomeres within one strain have approximately the same length of terminal poly(C_{1-3}-A).

The telomeric *Xho* I fragments that are larger than the expected XY′-derived fragments could be either X telomeres, XY′ telomeres that are missing the conserved *Xho* I site, or a new class of telomeres. One argument that at least some of these bands are X telomeres is that their size in an *Xho* I digest is altered in an *Xho* I/*Hpa* I double digest (compare Fig. 2 with Fig. 4). This is the predicted result if these bands represent X class telomeres, because the X repeat has a conserved *Hpa* I site ≈250 bp from the poly(C_{1-3}-A) tract (3, 4). In the *Xho* I/*Hpa* I double digests of genomic DNA, we observed a very broad band hybridizing to poly(G-T) below the XY′-derived band (Fig. 4). The band had an average size of 810 bp in 2262 with a distribution of ±200 bp; in A364a, the band was at a position of 540 ± 100 bp. These are the positions expected if the very broad band represents X telomeres, because the distance between the *Hpa* I site and the poly(C_{1-3}-A) tract is 250 bp, the poly(C_{1-3}-A) tract in 2262 is 620 ± 250 bp, and the poly(C_{1-3}-A) tract in A364a is 360 ± 185 bp. Thus, the size of this broad band is consistent with that expected if X-derived telomeres within one strain have the same length terminal poly(C_{1-3}-A) tract as XY′-derived telomeres. Furthermore, as shown in Fig. 4, when genomic DNA is sequentially treated with BAL-31, *Xho* I, and *Hpa* I,

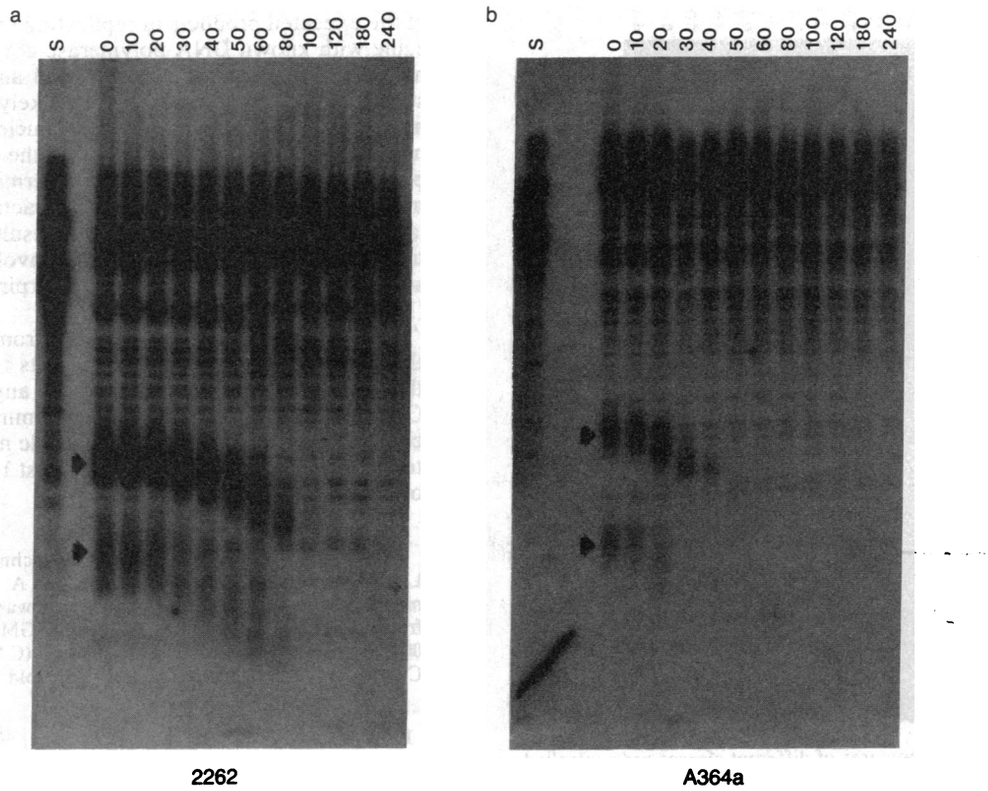

FIG. 4. BAL-31 sensitivity of two classes of telomeres in haploid yeast strains 2262 and A364a. Genomic DNA was isolated from strains 2262 (*a*) and A364a (*b*) and treated for various times (shown in minutes at top of each lane) with the enzyme BAL-31. DNA was then treated with *Xho* I and *Hpa* I, and Southern analysis was done with each sample. As in other experiments, the hybridization probe was poly(G-T). Arrows indicate positions of the XY′ class of telomeres (upper arrows) and the X class of telomeres (lower arrows).

the two classes of telomeres are degraded with the same kinetics.

The conclusion that individual telomeres within one strain are similar in length is supported not only from an analysis of the haploid parental strains A364a and 2262 but also by examination of the meiotic segregants of D4 (RW). In *Xho* I digests of DNA from the meiotic spores, we observed several fuzzy bands segregating 2:2 (Fig. 3). The clearest bands are labeled "A" and "B." Band A is present in spores 1c, 1d, 2b, 2d, 4a, and 4c; band B is present in spores 1c, 1d, 2b, 2d, 4b, and 4c. This 2:2 segregation pattern indicates that each of these bands is represented once in the D4 (RW) diploid genome. Since bands A and B are fuzzy, hybridize to poly-(G-T), and are at approximately the same position as BAL-31-sensitive *Xho* I fragments in A364a and 2262, we believe that A and B represent X class telomeres. Band A is at a position identical to that of band IV of A364a in Fig. 2. Band B is approximately the same size as band III of 2262 (Fig. 2). Thus, bands A and B in Fig. 3 represent single chromosomal telomeres segregating in meiosis.

Four conclusions can be made from the segregation patterns of bands A and B in Fig. 3. First, single chromosomal telomeres vary slightly in size (±50 bp) within one strain: previously, the heterogeneity of the yeast telomere had been shown only for multicopy plasmids containing cloned telomeres (9). Second, single chromosomal telomeres vary in length depending on the genetic background. For example, the B band telomere in spore D4-2b is ≈75 bp larger than the B band telomere in spore 2d. Third, the length of the poly-(C_{1-3}-A) tracts of the A and B band telomeres is controlled coordinately with the length of the XY′ telomeres. Thus, when the major *Xho* I band is large, the single-copy telomeres are large and vice versa. Fourth, the A and B bands do not represent alleles. If A and B represented allelic telomeres, then these bands would segregate apart from one another in all tetrads; none of the tetrads shown in Fig. 3 has this type of segregation.

In the last experiment, we examined the sizes of both the X class and XY′ classes of telomeres in the D4 (RW) spores by hybridizing poly(G-T) to *Xho* I/*Hpa* I-digested spore DNA (Fig. 5). As expected, the sizes of these two classes of telomeres was controlled coordinately. When the XY′ class of telomeres is large, the X class of telomeres (represented by the diffuse band below the XY′ class) is large, and vice versa.

In summary, the length of the yeast chromosomes can be different in different strains. The source of variation is in the length of a simple sequence [poly(C_{1-3}-A)] tract located at the end of each chromosome. Within one strain, the lengths of the tracts on all the chromosomes are approximately the same; there is, however, a small amount of heterogeneity (±50 bp) in the size of the terminal poly(C_{1-3}-A) tract even for a single chromosomal end within one strain. We have found that the size of the tract can be changed fairly rapidly. The sizes of the telomeric fragments from the spores of D4 (RW) are established by the time DNA is isolated for analysis (≈30 generations). The characteristic size of the telomeres is not affected by repeated vegetative subculturing (80 cell generations) or by different methods of DNA isolation.

DISCUSSION

The fluid structure of the yeast telomeres, although surprising, is not without precedent. Bernards *et al.* (10) showed that the telomeres of trypanosomes grow at the rate of ≈10 bp per division, with occasional large deletions. Van der Ploeg *et al.* (11) suggested that the additions occur in increments of the 6-bp sequence C-C-C-T-A-A, since this sequence is repeated many times at the ends of the trypanosome chromosomes (11, 12). In addition, C. Berg and J. Gall

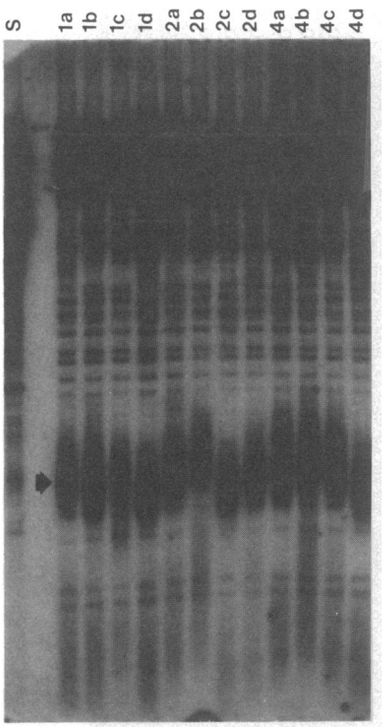

FIG. 5. Sizes of telomeres of different classes are controlled coordinately in spores derived from D4 (RW). Genomic DNA was isolated from spore cultures derived from three complete tetrads of D4 (RW). This DNA was treated with *Xho* I and *Hpa* I and examined by Southern analysis; hybridization probe was poly(G-T). Molecular weight of band representing XY′ telomeres (arrow) is correlated with molecular weight of band representing X class of telomeres. The latter band is a diffuse smear below the XY′ band.

(personal communication) found that ribosomal DNA molecules of *Tetrahymena*, when introduced into *Xenopus* eggs, acquired DNA sequences by terminal addition.

The alteration in length of poly(C_{1-3}-A) tracts of yeast telomeres as demonstrated above is consistent with earlier observations concerning the modification of non-yeast telomeres introduced into the yeast cell by transformation. Both *Tetrahymena* (2, 5) and *Oxytricha* (13) telomeres when cloned in yeast acquire a yeast-like poly(C_{1-3}-A) tract.

We suggest that the cellular mechanism that adds poly(C_{1-3}-A) sequences to the telomeres may be part of the normal replication process. Watson (1) first pointed out that one of the expected products of replication of a linear DNA molecule with known DNA polymerase activities is a daughter molecule with a protruding 3′ strand and an incomplete 5′ strand. Thus, eukaryotic cells are likely to have a requirement for a mechanism that adds nucleotides to the telomeres. We do not yet know whether the terminal addition of poly(C_{1-3}-A) sequences involves a terminal transferase-like mechanism or a recombinational interaction with other cellular poly(C_{1-3}-A) tracts (4, 11). Our results also indicate that models of telomere replication that involve simple semiconservative replication, such as the hairpin model of Bateman (14), are unlikely to be correct.

The analysis of the spores derived from D4 (RW) indicates that more than one yeast gene affects telomere length. Although we have not yet characterized any of these genes, M. Carson and L. Hartwell (personal communication) have recently shown that one of the cell cycle mutants of yeast has telomeres that are elongated by at least 1 kb relative to those of the isogeneic parental strain.

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