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Genetics

Identification of yeast mutants with altered telomere structure

(DNA replication/phenotypic lag/simple-sequence DNA)

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ABSTRACT **The chromosomes of the yeast *Saccharomyces cerevisiae* terminate in a tract of simple-sequence DNA [poly($C_{1-3}A$)] that is several hundred base pairs long. We describe the identification of mutant yeast strains that have telomeric tracts that are shorter than normal. A genetic analysis of these strains indicates that these short telomeres are the result of single nuclear recessive mutations and that these mutations can be classified into two different complementation groups. The full expression of the mutant phenotype shows a very long lag (≈150 cell divisions). From our analysis of these mutants as well as other data, we suggest that the duplication of the telomeric poly($C_{1-3}A$) tract involves two processes, semiconservative replication and untemplated terminal addition of nucleotides.**

A number of experiments indicate that the ends (telomeres) of eukaryotic chromosomes represent specific structures with specific functions. For example, linear *Drosophila* chromosomes with telomeric deletions are not observed, although many chromosomes containing sub-telomeric deletions have been isolated (1). Second, in contrast to natural telomeres, chromosomal ends produced by x-rays are unstable, fusing with other broken chromosomes in the cell (2). In addition to these observed properties of telomeres, the telomere is expected to be replicated by a nonstandard mode of DNA synthesis. Watson (3) pointed out that the known properties of DNA polymerase (5′-to-3′ synthesis and a primer requirement) do not explain how the ends of linear chromosomes are replicated. Although the end of one of the daughter DNA molecules could be completely replicated by the action of DNA polymerase, the other daughter DNA molecule, after excision of the primer, would have one DNA strand that was shorter than the original parental strands. Presumably, this loss of sequences would occur at each round of synthesis.

To study the structure and replication of the telomere, we and others have examined the DNA sequences at the ends of chromosomes as well as at the ends of linear eukaryotic plasmids. In all telomeres thus far studied, simple "satellite-like" DNA sequences, with repeats 2–9 base pairs (bp) long, have been observed (4). Among the small repeats that have been characterized are C_4A_2 in *Tetrahymena* (5), C_4A_4 in *Oxytricha* (6), C_3TA_2 in *Trypanosoma* (7, 8), and $C_{1-3}A$ in the yeast *Saccharomyces cerevisiae* (9, 10).

Although most of the telomeric sequences described above are derived either from linear plasmids or from minichromosomes, the yeast DNA sequences are derived from the telomeres of "true" chromosomes. The organization of DNA sequences at the yeast telomere is shown in Fig. 1. The yeast chromosome terminates in a tract of simple-sequence DNA [poly($C_{1-3}A$)] of several hundred base pairs (9, 11, 13). There are two types of repeated sequences containing putative replication origins also associated with yeast telomeres, Y′ and X (14). The Y′ repeat is about 6.7 kilobases (kb) long and is found in one or more copies at most (but not all) of the yeast telomeres; the X repeat is about 2 kb in size and is found at the ends of all yeast chromosomes thus far characterized (10, 14). In this paper, we will refer to those telomeres that contain both X and Y′ repeats as XY′-class telomeres and those that have only an X repeat as X-class telomeres. In addition to the poly($C_{1-3}A$) tract at the end of the chromosome, there are also short poly($C_{1-3}A$) tracts between the X and Y′ repeats and between tandemly arranged Y′ repeats (10, 30).

Several lines of experimental evidence indicate that the yeast telomere is a dynamic structure. First, restriction fragments containing the telomeric sequences are diffuse bands when examined by Southern analysis, indicating size heterogeneity at the telomere (12, 13). Second, telomeres derived from *Tetrahymena* and *Oxytricha*, when cloned in yeast, acquire terminal poly($C_{1-3}A$) sequences through an apparently untemplated process (9, 11, 15). Third, telomeres of cloned linear plasmids recombine with chromosomal telomeres, generating new combinations of telomeric repeats (16). Fourth, laboratory yeast strains with tract lengths of poly($C_{1-3}A$) varying from 350 to 600 bp have been described; this variation is under genetic control (13, 17). Finally, Carson and Hartwell (18) have shown that the yeast cell-cycle mutant *cdc17* has telomeres that are longer than those in wild-type strains.

It is likely that the genetically controlled variation in the structure of the yeast telomere described above is related to the mechanism by which the telomere is duplicated. To analyze this process further, we identified and characterized mutants of yeast that have shorter telomeres than wild-type strains. This analysis and a model of telomere replication based on this study are presented below.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions. The following strains were used in these studies: A364A (*a lys2 tyr1 his7 ade1 ade2 ura1 gal1*), B364B (*α lys2 tyr1 his7 ade1 ade2 ura1 gal1*, isogenic with A364A except at the *MAT* locus), H17C1A1 (*a his7 ura1 cdc17-1*), and H17C1B1 (*α his7 ura1 cdc17-1*). Strains B364B, H17C1A1, and H17C1B1 were provided by L. Hartwell (University of Washington). The Klyce–McLaughlin collection (19) of temperature-sensitive (ts) yeast strains (derived from A364A by nitrosoguanidine mutagenesis) was provided by R. Sternglanz (State University of New York, Stony Brook).

Complementation analysis and tetrad dissection were done using standard procedures (20). Omission media and rich growth medium (YPD) were prepared as described by Sherman *et al.* (20). The extraneous ts mutations in the original

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Abbreviations: bp, base pair(s); kb, kilobase(s); ts, temperature-sensitive.

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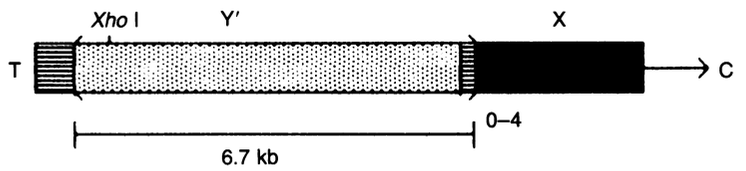

FIG. 1. Structure of the yeast telomere. The organization of sequences at the yeast telomere is summarized (10, 12, 14). The poly($C_{1-3}A$) tracts (striped), the Y' repeats (stippled), and the X repeats (solid bar) are shown in their relative orientation. T and C refer to the telomere and centromere, respectively.

AL-1, AL-4, and AL-5 strains were removed by repeated backcrosses. In each backcross, the spore containing the mutation affecting telomere length was crossed to either A364A or B364B. In all experiments (except for those involving scoring the ts phenotype), cells were grown at 23°C.

In experiments in which strains were subcultured, each strain was streaked for single cells on YPD solid medium and the plates were incubated for 2–3 days to allow colonies to form. The formation of a large colony from a single cell requires about 25 cell divisions.

Isolation and Analysis of Yeast DNA. For the DNA isolations involving the initial mutant screen, yeast cells were grown overnight at 23°C in YPD before they were shifted to 37°C for 6 hr. In all subsequent studies, the yeast cells were grown at 23°C. DNA was isolated by either detergent lysis of spheroplasts (20) or mechanical breakage of cells followed by CsCl centrifugation of the yeast DNA (21); both procedures yielded similar results.

For the analysis of telomere length, DNA was treated with the restriction enzyme *Xho* I (New England Biolabs). The resulting fragments were separated by electrophoresis in a 0.8% agarose gel and transferred to nitrocellulose filters (22). The filters were then hybridized to ^{32}P-labeled poly[d(G-T)·d(C-A)] at 50°C as described (13).

RESULTS

Isolation of Mutant Yeast Strains with Short Telomeric Poly($C_{1-3}A$) Tracts. Since the length of the terminal poly($C_{1-3}A$) tract at the telomere is genetically controlled (13), it should be possible to isolate mutants that have tract lengths that are shorter or longer than wild type. The length of the terminal tract can be conveniently monitored by Southern analysis of yeast DNA samples that have been treated with the restriction enzyme *Xho* I (13). The Y' repeats located near the ends of most yeast chromosomes (Fig. 1) contain a conserved *Xho* I site (14). When genomic DNA is treated with *Xho* I and hybridized to the ^{32}P-labeled copolymer poly[d(G-T)·d(C-A)], the terminal *Xho* I fragments of most yeast chromosomes (the XY' class of telomeres) are represented by a broad diffuse band of hybridization at about 1.3 kb (13). In addition to hybridizing to the poly($C_{1-3}A$) tract of XY' telomeres, the poly[d(G-T)·d(C-A)] hybridizes to three other types of genomic sequences: poly($C_{1-3}A$) tracts at the ends of X-class telomeres, poly($C_{1-3}A$) tracts separating X and Y' sequences, and copolymeric CA tracts not associated with the telomere (10). In previous experiments, we found that different lab strains of yeast often had different sizes for the *Xho* I fragments representing the XY' and X classes of telomeres and that these differences reflected changes in the length of the terminal tract of poly($C_{1-3}A$). We decided, therefore, to attempt to identify mutations affecting tract length by Southern analysis of *Xho* I-treated DNA.

Since mutations affecting the terminal sequences of the chromosome might be lethal, we examined a collection of ts yeast strains for mutants with telomeres of altered size. The collection analyzed was derived from the wild-type haploid strain A364A by Klyce and McLaughlin (19); the strains in the collection grow at 23°C but fail to grow at 37°C. Two hundred strains from this collection were grown in individual cultures at 23°C overnight and then shifted to 37°C for 6 hr. We then isolated DNA from each culture, treated the DNA with *Xho* I, and separated the restriction fragments by gel electrophoresis. The separated fragments were blotted to nitrocellulose and hybridized to labeled poly[d(G-T)·d(C-A)].

Most strains showed hybridization patterns identical to the parental, wild-type strain; however, three strains had telomeric *Xho* I fragments that were substantially smaller than the wild type (Fig. 2, lanes at left). Mutants AL-1 and AL-4 had telomeric fragments that were ≈300 bp shorter than those of the wild-type strain B364B; B364B is an isogenic derivative of A364A that was constructed by a mating-type switch. The mutant strain AL-5 had telomeres that were about 170 bp shorter than wild type. Approximately the same decrease in size was observed for both the XY'-class

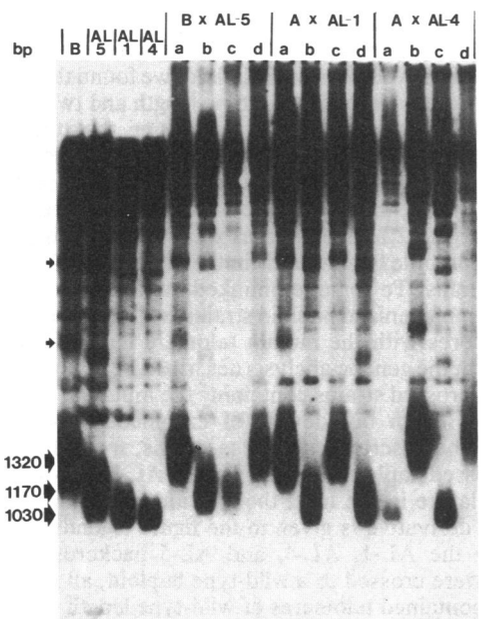

FIG. 2. Identification and meiotic segregation patterns of mutations affecting telomere length. DNA was isolated from a number of mutant and wild-type strains and treated with the enzyme *Xho* I. The resulting fragments were separated by gel electrophoresis, transferred to nitrocellulose, and hybridized to ^{32}P-labeled poly[d(G-T)·d(C-A)]. The four lanes on the left side of the gel contain DNA isolated from strains containing mutations affecting telomere length (AL-1, AL-4, and AL-5) as well as a DNA sample isolated from an isogenic wild-type strain B364B (lane B). The telomeric *Xho* I fragments characteristic of the repeated XY' class of telomeres are indicated by large arrows, and the sizes of these fragments (in bp) are indicated at left. Small arrows indicate the position of single-copy telomeres of the X class. The twelve rightmost lanes of the gel contain DNA derived from one tetrad of each of three different diploid strains. The lanes labeled B × AL-5 contain DNA from four spores (a–d) of a tetrad derived from a diploid formed by crossing B364B with a backcrossed derivative of AL-5 (AJL75-1c). Similarly, the adjacent four lanes represent DNA isolated from spores of a tetrad derived from a cross of A364A and a backcrossed derivative of AL-1 (AJL77-1b); the last four lanes contain DNA isolated from spores of a tetrad derived from a cross of A364A to a backcrossed derivative of AL-4 (AJL80-1c). The loss of one of the X-class telomeres in two of the four spores (lanes b and c) in the AL-1 × A tetrads is the result of the apparent absence of this X-class telomere in one of the A364A strains utilized in backcrossing.

telomeres (large arrows in Fig. 2) and the X-class telomeres (two such telomeres are indicated by small arrows in Fig. 2). We conclude, therefore, that the change in the size of the terminal *Xho* I fragments represents a change in the length of the terminal poly($C_{1-3}A$) tracts rather than an alteration within the Y′ sequence. We calculate that the terminal poly($C_{1-3}A$) tracts in AL-1 and AL-4 average 70 bp long and that the tract length in AL-5 is about 190 bp; the tract lengths in the wild-type strains A364A and B364B are about 360 bp (13). In all strains, there is considerable heterogeneity in the size of the terminal *Xho* I fragments. For all three mutant strains isolated, telomeric fragments of the mutant length were observed at both the restrictive and the permissive temperature. In the experiments described below, therefore, DNA was isolated from cells grown at the permissive temperature.

Genetic Analysis of Mutants Affecting Telomere Length. In our genetic analysis, the mutant strains AL-1, AL-4, and AL-5 (derived from A364A) were crossed individually to a haploid strain (B364B) that was isogenic with A364A except at the mating-type locus. Each of the three diploids produced by these crosses were sporulated and tetrads were dissected. We isolated DNA from each spore culture after ≈150 generations of vegetative subculturing and determined the telomere length by Southern analysis as described above. In one tetrad derived from each diploid, we found that two of the spores had telomeres of wild-type length and two spores had telomeres of mutant length, suggesting that the mutation affecting telomere length was the result of a single nuclear mutation. We observed, however, that in all three of the diploid strains, the phenotype of temperature sensitivity did not segregate 2:2; most spores were ts, indicating the presence of multiple ts mutations in the original AL-1, AL-4, and AL-5 strains. To remove unlinked mutations present in the original mutagenized haploid strains, we did three backcrosses of spores with the mutant telomere length to A364A or B364B. In the genetic analysis described below, we used only the backcrossed strains containing the mutation affecting the telomere length but devoid of extraneous mutations. To simplify the description of these strains, we will refer to the backcrossed haploid strains using the AL-1, AL-4, and AL-5 nomenclature in the text; the specific names of each backcrossed derivative is given in the figure legends.

When the AL-1, AL-4, and AL-5 backcrossed haploid strains were crossed to a wild-type haploid, all three diploid strains contained telomeres of wild-type length (Fig. 3). The mutations in all three strains, therefore, are recessive. When these diploids (heterozygous for the mutations affecting telomere length) were sporulated and the telomeres of the spores examined after extensive subculturing, the mutant phenotype segregated 2:2 in all tetrads derived from each diploid (Fig. 2, lanes at right); a minimum of five complete tetrads were analyzed for each diploid. Thus, the mutations affecting telomere length in AL-1, AL-4, and AL-5 represent changes in single nuclear genes.

While doing the backcrosses between strains containing the mutations affecting telomere length and wild-type strains, we observed that the mutations affecting telomere length in AL-1 and AL-5 were not linked to a ts mutation. We found, however, that the mutation affecting telomere length in AL-4 is tightly linked to a ts mutation, since in 11 of 11 tetrads, the ts phenotype cosegregates with the altered telomere length. To find out whether the mutation affecting telomere length in AL-4 was ts, we analyzed revertants of the ts mutation. DNA was isolated from 30 independent ts^+ colonies spontaneously derived from AL-4, and the telomere tract size was determined. All the revertants maintained the mutant telomeric poly($C_{1-3}A$) tract length. At least three of these revertants were within the gene that was originally ts. It is thus probable that the ts defect and the mutation affecting telomere length

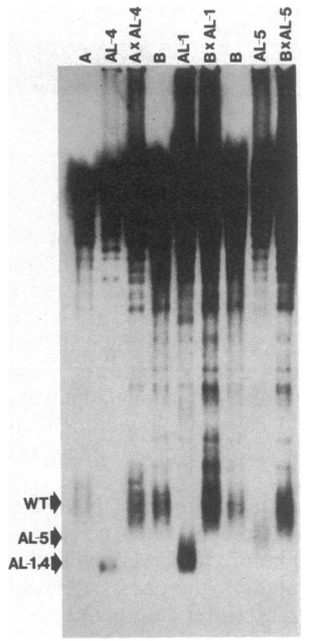

FIG. 3. Mutations affecting telomere length are recessive. Southern analysis (as described in the legend to Fig. 2) using DNA isolated from A364A (A), B364B (B), and backcrossed strains containing the mutations originally found in AL-1, AL-4, and AL-5 [AJL105-2d, AJL 110(H)-1a, and AJL98-1a, respectively] as well as DNA samples derived from diploids constructed by crossing the wild-type and mutant haploids (A × AL-4, B × AL-1, and A × AL-5). Arrows indicate fragments characteristic of wild-type (WT), AL-5, AL-1, and AL-4 telomeres.

represent alterations in closely linked but distinct loci. However, we cannot rule out the possibility that the ts defect and the mutation affecting telomere length are identical, but that a reversion event at a second site within the ts gene is capable of eliminating the ts behavior without restoring the wild-type telomere size.

We did pairwise crosses between strains containing the three different telomere mutations in order to establish the number of complementation groups. When AL-1 and AL-4 were crossed together, the diploid had telomeres of mutant length, indicating that these mutations represent the same complementation group. We name this gene *tel1*; the mutant allele in strain AL-4, *tel1-1*; and the mutant allele in strain AL-1, *tel1-2*. We consider these alleles different because the distribution of telomere lengths in strains containing the *tel1-1* mutation is always narrower than the distribution observed for strains containing the *tel1-2* mutation. When either AL-1 or AL-4 was crossed with AL-5, we found that the diploid had telomeres that were larger than either of the haploid parental strains but were somewhat smaller than wild type. Although this observation suggests that the mutation in AL-5 is in a different complementation group from *tel1*, the result is somewhat ambiguous. To confirm this conclusion, we analyzed telomere length in spores derived from a diploid heterozygous for the mutations found in AL-1 and AL-5. We found that these two mutations segregated independently; in 28 spores (derived from seven complete tetrads) analyzed, we observed 7 spores with wild-type telomeres. Thus, the mutation in AL-5 represents a gene (which we call *tel2*) different from *tel1*. In summary, our search for mutations affecting telomere length has yielded mutations in two different genes, *tel1* and *tel2*; both mutations result in telomeres that are shorter than normal.

Carson and Hartwell (18) found that the ts yeast mutant *cdc17* had longer than normal telomeres. To determine whether *tel1* or *tel2* represent different alleles of *cdc17*, we constructed diploids between strains containing either *tel1-1* or *tel2* and *cdc17*. Since *cdc17* is ts and *tel1-1* is closely linked to a ts mutation, if these genes were allelic almost all the spores derived from a diploid heterozygous for *tel1-1* and *cdc17* should be ts in tetrads derived from such a diploid; however, we frequently found spores that were not ts (10–25% of the spores were wild type). We conclude, therefore, that *cdc17* and *tel1* are not allelic. When tetrads derived from the diploid heterozygous for *tel2* and *cdc17*

were examined by Southern analysis, we found that (in five tetrads examined) five spores had wild-type-length telomeres, five spores had *cdc17*-length telomeres, five spores had *tel2*-length telomeres, and five spores had telomeres intermediate in length between *tel2* and *cdc17*. Since this pattern indicates independent segregation of *tel2* and *cdc17*, we conclude that *tel2* and *cdc17* are not allelic. In summary, three different yeast genes have been identified that affect the structure of the telomere: *cdc17* (ref. 18), *tel1*, and *tel2*.

Phenotypic Lag in Expression of Mutant Telomere Length. As described above, when diploids heterozygous for either the *tel1* or *tel2* mutations were sporulated and DNA was isolated from spore cultures after extensive subculturing, the mutant phenotype segregated 2:2. We found that the extensive subculturing (≈150 generations) was necessary in order to obtain two spores that had telomeres of mutant length. When a similar analysis was done with spore cultures that had undergone only 40 generations of growth, spores containing the *tel* mutations had telomeres that were only slightly shorter than wild type. An example of this phenotypic lag for a haploid strain containing the *tel1-2* mutation is shown in Fig. 4. Similar phenotypic lags were seen with both *tel1-1* and *tel1-2*; strains containing *tel2* appeared to have a lag of shorter duration. Similar phenotypic lags were observed for both X- and XY′-class telomeres. Vegetative subculturing had no effect on telomere length in either the diploid strains from which the spores were derived or in wild-type haploid spore cultures.

Since all mutant strains thus far examined show similar lags in the expression of the mutant phenotype, the lag is not likely to be allele-specific. In addition, Carson and Hartwell (18) showed that yeast strains containing a mutation that increases telomere length (*cdc17*) have a similar phenotypic lag. These lags in expression of the mutant phenotype are too long to be explained by dilution of a wild-type gene product. As described in the *Discussion*, we believe that the lag is a reflection of the mechanism used in duplicating the yeast telomeres.

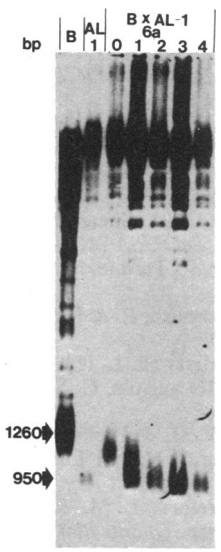

FIG. 4. Phenotypic lag in the expression of mutations affecting telomere length. The wild-type haploid strain B364B (B) was crossed to a haploid strain containing a mutation resulting in short telomeres (AL-1). DNA was isolated from four spores of one tetrad of the resulting diploid, and the telomere length in cultures derived from each spore was examined by Southern analysis. Two of the spores had wild-type-length telomeres and two had telomeres that were slightly shorter than wild type. When the four haploid strains derived from the four spores were vegetatively subcultured, the size of telomeres in two of the strains decreased to the size of telomeres in the original mutant strain AL-1. The figure shows the size of telomeres in the parental haploid strains B364B and AL-1 as well as the size of telomeres in one of the spores [(B × AL-1)-6a] at various stages of subculturing. Lane 0 represents DNA isolated from the spore culture at the earliest time possible (about 40 generations). Each additional subcloning (lanes 1–4) represents about 25 generations. Similar phenotypic lags were observed for backcrossed derivatives of this strain.

DISCUSSION

We have described three yeast strains containing mutations in genes affecting telomere length. These three mutations define two complementation groups, *tel1* and *tel2*. Since all mutants have terminal poly($C_{1-3}A$) tracts that are shorter than normal, the wild-type gene product of these genes is necessary to have telomeric poly($C_{1-3}A$) tracts of normal length. All three mutant alleles are recessive and show a phenotypic lag of many generations.

The existence of mutants that alter the structure of the telomere is relevant to models of telomere replication. As Watson (3) noted, the known properties of DNA polymerase (5′-to-3′ synthesis and a requirement for an RNA primer) predict that one of the two daughter DNA molecules will contain a terminal deletion, resulting from removal of the terminal primer. In addition to explaining how the replication of the telomere circumvents this expected shortening, a model of telomere replication applicable to yeast must also explain the following observations: (*i*) single cloned (12) and chromosomal telomeres (13) are heterogeneous in size, (*ii*) yeast cells have a mechanism to add untemplated poly($C_{1-3}A$) residues to certain linear substrates (9, 11, 15), (*iii*) different lab strains can have different terminal poly($C_{1-3}A$) tract lengths (13, 17), (*iv*) mutants can be isolated that have either longer (18) or shorter telomeres than wild type, and (*v*) mutations affecting telomere length have a long phenotypic lag.

Our data rule out two classes of models. First, any model that involves the perfect semiconservative replication of the telomeric sequences is excluded by the observed heterogeneity in telomeric length. One of the most popular models of this sort is the "hairpin" model (23, 24). In this model, the two complementary DNA strands are covalently linked at the terminus, forming a hairpin. Replication occurs in the 5′ to 3′ direction through the hairpin. The resulting inverted repeat is processed by an endonuclease into two daughter DNA molecules containing hairpins. Although such a mechanism has been demonstrated for certain viral systems (25–27), this model of telomere replication fails to explain the heterogeneity seen for yeast telomeres. In addition, the untemplated addition of poly($C_{1-3}A$) residues to the ends of *Tetrahymena* and *Oxytricha* telomeres is difficult to explain as a consequence of replication of a hairpin. A second type of model that is unlikely is one in which the terminal poly($C_{1-3}A$) tract is synthesized *de novo* every cell cycle from a specific site within the telomere. Such a model is inconsistent with the observation that all mutations that affect telomere length have a long phenotypic lag.

We propose that the terminal poly($C_{1-3}A$) sequences of the yeast chromosome are replicated by a combination of two different mechanisms, semiconservative replication and untemplated DNA synthesis. We suggest that most of the terminal tract is replicated semiconservatively by the known DNA polymerase activities. As mentioned above, we expect that the excision of primers near the end will result in one daughter DNA molecule with a 5′ strand that is shorter than the original template. Since the RNA primers in yeast are short (11 bp; ref. 28), the loss of sequences by this mechanism should be slow. Without a mechanism to compensate for the shortening, however, the terminal tract would eventually be deleted. We suggest, therefore, that this shortening is bal-

anced by an untemplated addition of poly($C_{1-3}A$) residues, probably to the 3′ end of the daughter DNA molecules. This addition of poly($C_{1-3}A$) residues is likely to require a preexisting tract in order to discriminate between specific chromosomal termini and random double-strand breaks. By this model of telomere duplication, the telomeric sequences are maintained by a dynamic equilibrium between processes that shorten and lengthen the terminal tract.

This model accounts for the observations of the behavior of yeast telomeres. In the model, the cellular activity that modifies the *Tetrahymena* and *Oxytricha* ends is the same activity that elongates (by untemplated synthesis) the chromosomal terminal poly($C_{1-3}A$) tracts. The observed heterogeneity of single chromosomal telomeres is an expected feature of the model, since the addition and loss of sequences are likely to be inexact processes. Because the proposed model involves an equilibrium between removal and addition of terminal sequences, one should expect to obtain mutants that affect either elongation or addition. The simplest explanation of the *tel1* and *tel2* mutants is that the mutant gene products are partially defective in the elongation function; this partial defect has no obvious effect on either growth rates or cell viability. The simplest explanation of the effects of *cdc17* is that the mutant gene is partially deficient in a function that shortens the telomere. Finally, the model is also consistent with the observations of long phenotypic lags with *tel1*, *tel2*, and *cdc17*. If most of the poly($C_{1-3}A$) tract is synthesized by semiconservative synthesis and only a small region of the tract is duplicated by untemplated replication, a long lag in the full expression of a mutation affecting untemplated replication is expected.

One complication of our model of telomere replication should be mentioned. It is unlikely that the length of the terminal poly($C_{1-3}A$) tract is maintained by a perfect balance between addition and deletion of a fixed number of base pairs. Such a balance would be extremely unstable, since a mutation that slightly changed either function would lead to constantly increasing or decreasing tract lengths. Since we observe mutations that generate stable tract lengths that are different than wild type, we believe that either the addition or deletion mechanisms must show feedback regulation. For example, feedback regulation may be the result of a tract-specific endonuclease that cleaves long tracts more efficiently than short tracts. If wild-type elongation activity is not limiting, the recessiveness of the *tel* mutations could also be explained.

Although we suggest that the telomere is duplicated by a balance between processes that degrade and extend the terminal poly($C_{1-3}A$) tract, the details of these processes are not understood. Removal of sequences from the telomere may simply be the result of removal of the terminal RNA primer or, alternatively, may also involve a tract-specific endo- or exonuclease, as suggested above. The extension of the tract could be the result of a terminal transferase-like activity (9, 11) or a recombination event between the end of the chromosome and internal poly($C_{1-3}A$) tracts (10). Whatever the details of these mechanisms, it is clear that the duplication of the telomere is not simply a replication process, since the DNA strands are not used solely as templates encoding the complementary strand. The inexact, partially untemplated duplication of the chromosomal telomeres mimics, in some ways, the duplication of cellular organelles rather than the exact replication of other types of DNA sequences.

The fluid nature of the yeast telomeres described above is likely to be a common feature of eukaryotic telomere structure. Bernards *et al.* (29) showed that telomeres of trypanosomes increased in length by about 6 bp per generation when grown in cells. In addition, in many ciliates, simple repeated sequences are added by an untemplated mechanism to the ends of macronuclear DNA fragments (4). The continued genetic and molecular analyses of yeast genes involved in telomere replication, therefore, should have general relevance.

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