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Tetrahymena micronuclear sequences that function as telomeres in yeast

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ABSTRACT

We explored the ability of *S. cerevisiae* to utilize heterologous DNA sequences as telomeres by cloning germline (micronuclear) DNA from *Tetrahymena thermophila* on a linear yeast plasmid that selects for telomere function. The only *Tetrahymena* sequences that functioned in this assay were $(C_4A_2)_n$ repeats. Moreover, these repeats did not have to be derived from *Tetrahymena* telomeres, although we show that micronuclear telomeres (like macronuclear telomeres) of *Tetrahymena* terminate in $(C_4A_2)_n$ repeats. Chromosome-internal restriction fragments carrying $(C_4A_2)_n$ repeats also stabilized linear plasmids and were elongated by yeast telomeric repeats. In one case, the C_4A_2 repeat tract was approximately 1.5 kb from the end of the genomic *Tetrahymena* DNA fragment that was cloned, but this 1.5 kb of DNA was missing from the linear plasmid. Thus, yeast can utilize internally located tracts of telomere-like sequences, after the distal DNA is removed. The data provide an example of broken chromo-some healing, and underscore the importance of the telomeric repeat structure for recognition of functional telomeric DNA in vivo.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* has been instrumental in the study of structural elements which confer stability and maintenance to eukaryotic chromosomes (reviewed in 1,2). In particular, the demonstration that yeast will recognize as stable ends the telomeres from two different ciliated protozoa (3,4) has increased our understanding of the requirements of eukaryotic chromosome ends.

Telomeres of the somatic nucleus of the ciliated protozoan *Tetrahymena thermophila* consist of many tandem repeats of the simple sequence d(CCCCAA)·d(GGGGTT) (abbreviated as C_4A_2) which extend out to the very molecular terminus (5,6). This sequence can be used to stabilize the end of a linear plasmid or chromosome in yeast (3,7). It is extended in vivo by the addition of yeast telomeric repeats, $d(C_{1\text{-}3}A)\cdot d(G_{1\text{-}3}T)_n$, (abbreviated as $C_{1\text{-}3}A$) by a mechanism proposed to be analogous to the untemplated addition of nucleotides to DNA by terminal deoxynucleotidyl transferase (8). Such an activity (telomere terminal transferase or telomerase) has been isolated from *Tetrahymena* (9), which in vitro adds *Tetrahymena* $d(TTGGGG)_n$ repeats onto synthetic DNA oligonucleotides that resemble the telomeric sequences of a number of lower eukaryotes. These sequences are not identical, but, like the examples above, are all short, simple repeats which generally segregate the G's and C's to separate strands (reviewed in 10,11). That

yeast, in vivo, and *Tetrahymena* telomerase, in vitro, will recognize heterologous telomeric sequences suggests that these sequences have some structural similarity that distinguishes them from random DNA, a conclusion supported by physical studies (12). In addition to natural chromosomal termini, restriction fragments of bacterial recombinant plasmids terminating in C_4A_2 repeats can also be used as linear plasmid and chromosomal ends (13,14,15,16), while the ends of vaccinia virus (hairpins with no simple-sequence repeats) cannot (17). Thus, it is clear that some feature of the DNA repeat sequence is primarily responsible for telomere recognition and function.

Yeast linear plasmids can be exploited to select for sequences recognizable to yeast as telomeric. In such experiments, recognition is defined as the ability to provide a stable end, to be used as a substrate for the addition of host-specific telomeric repeats. A stability selection scheme was used to clone a yeast chromosomal telomere (3). One end of a linear plasmid carrying two *Tetrahymena* telomeres was removed, and yeast genomic DNA fragments were ligated in its place. Only those plasmid vectors receiving a telomeric fragment were able to transform yeast and be maintained as linear molecules. We used an analogous experiment to determine what segments of DNA from the five micronuclear (germline) chromosomes of *Tetrahymena* would be recognized as telomeric by yeast, as judged by their ability to restore telomere function to a linear plasmid vector.

Micronuclear chromosomal DNA is known to contain many copies of the $(C_4A_2)_n$ sequence, the vast majority of them located at internal positions of the chromosomes (6,18). We demonstrate here that C_4A_2 repeats are also found at the telomeres of micronuclear chromosomes. We show that when random micronuclear DNA fragments were cloned on yeast linear plasmids, C_4A_2 repeats were the only sequences that were able to function as telomeres. Furthermore, C_4A_2 repeat regions that were originally at internal locations of restriction fragments could become functional telomeres upon transformation into yeast.

MATERIALS AND METHODS

Micronuclear and macronuclear DNA from wild-type *Tetrahymena* strain B1979 was prepared by the procedure of Howard and Blackburn (19), except that the filtration of micronuclei was omitted. DNA was usually purified by CsCl density gradient centrifugation prior to treatment with BAL31 nuclease.

Yeast transformations were performed according to Hinnen et al. (20), using as the host *S. cerevisiae* strain LL20 (21). Preparation of whole cell DNA from yeast transformants and DNA enriched for extrachromosomal plasmids have been described (22,8). Selective media were prepared as in ref. 23.

Restriction digestion, gel electrophoresis, radiolabelling, and blot hybridization were carried out under standard conditions or as described (22). Hybridizations were stringent (4XSSC at 65°C). The $C_{1\text{-}3}A$ and LEU2 probes were derived from plasmids pYT103 and prEY3, respectively (8). The C_4A_2 probe used was a 500 bp bacterial plasmid DNA fragment

consisting entirely of C_4A_2 repeats between BamHI linkers (M. Budarf, unpublished). Autoradiograms were scanned with a laser densitometer (LKB) and converted to figures with Thunderscan for the Macintosh.

RESULTS

Telomeric C_4A_2 repeats in the micronucleus

In *Tetrahymena*, C_4A_2 repeats are found at the termini of macronuclear chromosomes, which are derived from a set of the much larger micronuclear chromosomes in a developmental process following sexual conjugation, and at many internal locations in micronuclear DNA. We tested whether or not they were also present at micronuclear telomeres. High molecular weight micronuclear DNA from inbred strain B1979 was treated for various times with the double-stranded exonuclease BAL31, which progressively shortens the ends of linear DNAs, including telomeric ends. DNA was then digested with HindIII, fractionated on an agarose gel, blotted, and probed with C_4A_2 repeats. Results are shown in Fig.1a. Densitometric scans of the first 3 lanes of this autoradiogram are shown in Fig. 1b. A pair of bands migrating at approximately 6.4 and 6.6 kb were shortened by about 250 bp in the first minute of BAL31 treatment, and then disappeared. Note the transient appearance of a broad peak of hybridization at 6.1 kb at one minute of treatment. There were additional, non-telomeric, C_4A_2-hybridizing fragments which initially co-migrated with these bands at 6.4 and 6.6 kb, but which persisted with no change in size after extensive BAL31 digestion. Other autoradiograms (not shown) confirm the interpretation that there are not one but two distinct bands affected by BAL31. The possibility that these BAL31-shortened fragments are due to contaminating macronuclear DNA was eliminated; reconstruction experiments in which different amounts of macronuclear DNA were present showed that the only macronuclear telomeres that would be visible as a discrete class would be those of the highly abundant rDNA (data not shown), and its telomeres have a very small (700 bp) terminal HindIII fragment that would have probably run off the bottom of this gel.

In this experiment, the rate of digestion was approximately 180 bp/min, as measured by the shortening of λ DNA fragments included in the same reaction. Since all of the C_4A_2 hybridization to the BAL31-shortened fragments is gone by 2 minutes of treatment, the C_4A_2 repeats on these micronuclear fragments appear to be confined to less than 400 bp at the very ends of the chromosomes. Length heterogeneity (+/- 50-100 bp) typical of macronuclear telomeres (24), which is responsible for the fuzzy, broad appearance of telomeric restriction fragments, would not be very noticable in telomeric restriction fragments greater than 6 kb in length, such as those seen in Fig. 1.

Cloning strategy

C_4A_2 repeats from the micronuclear genome might stabilize yeast linear plasmids in two different ways. First, a micronuclear telomere terminating in C_4A_2 repeats, when ligated to the vector, should be recognized as a telomere by yeast. Second, a C_4A_2 repeat region in the interior

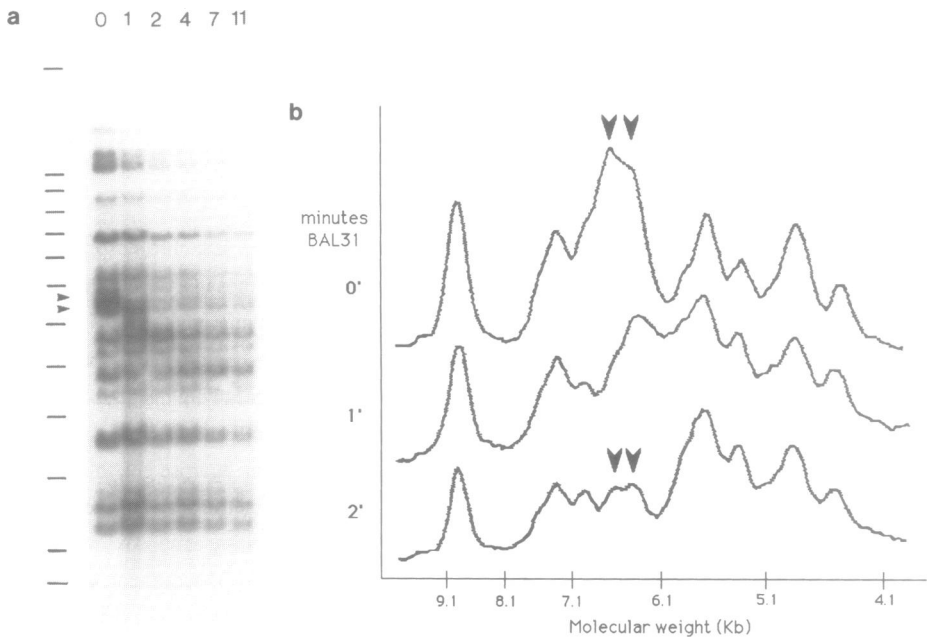


Fig. 1. (a) BAL31 nuclease analysis of micronuclear DNA. Unrestricted DNA was treated with BAL31 (0.4 units per μg DNA) at 30°C for 0, 1, 2, 4, 7, and 11 minutes as indicated above each lane, at which times aliquots of the reaction were stopped by the addition of EGTA. Samples were then restricted with HindIII, run on a 0.6% agarose gel, and blotted to Nytran. The filter was hybridized with a C_4A_2 repeat probe. Molecular weights are indicated by horizontal bars at left: 23, 12.2, 11.2, 10.2, 9.1, 8.1, 7.1, 6.1, 5.1, 4.1, 3.1, 2.0, and 1.6 kb. (b) Portions of lanes 0, 1, and 2 from part (a) were scanned densitometrically and the outputs aligned one below the other. Arrowheads indicate the initial position of the two bands affected by BAL31.

of a restriction fragment could become a telomere, if the DNA distal to the repeats were eliminated. In both cases, the C_4A_2 repeats should become a substrate for the addition of yeast telomeric sequences. This cloning experiment, described below, might also determine if any other internal micronuclear sequences, besides C_4A_2 repeats, could stabilize yeast linear plasmids.

The vector used here was the linear plasmid pSZ219 (3), shown in Fig. 2. The plasmid was digested with BclI and an 8 kb fragment bearing the selectable marker LEU2 and a yeast telomere was gel-purified away from the other telomere, the terminal fragment of the extrachromosomal linear rDNA of *Tetrahymena*. The vector fragment therefore has no C_4A_2 repeats or other *Tetrahymena* sequences. We expected ARS activity to be provided by sequences in the yeast telomere end of this vector, since it is homologous to the ARS-bearing Y' family (25) and contains an ARS consensus sequence (8).

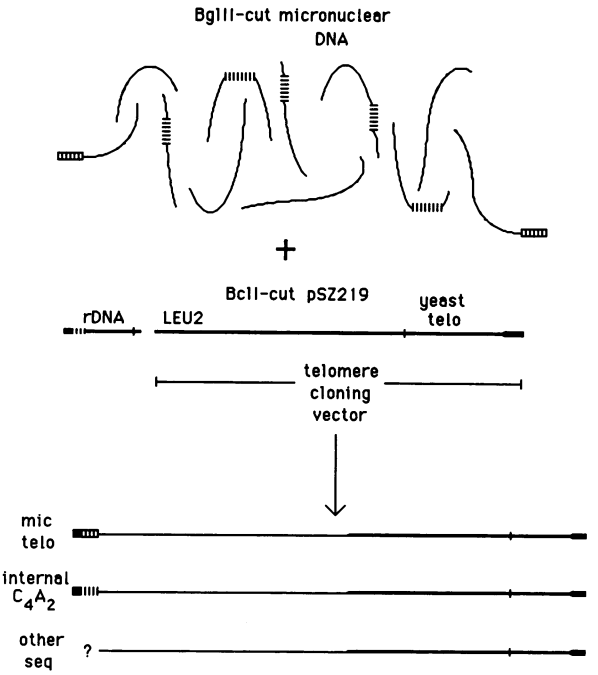


Fig. 2. Cloning strategy. Twelve micrograms of micronuclear chromosomal DNA, containing both internal and telomeric C_4A_2 regions, was digested with BglII and ligated to the right portion (as shown) of BclI-digested pSZ219 (2.5 micrograms). Possible outcomes after transformation into yeast are depicted at the bottom. Thin lines: micronuclear DNA. Thick lines: vector. Striped blocks: C_4A_2 repeats. Solid blocks: yeast telomeric repeats.

Tetrahymena thermophila micronuclear DNA from strain B1979 was digested with BglII (which produces cohesive ends compatible with BclI ends) and ligated to the linear plasmid vector. Approximately equimolar amounts of *Tetrahymena* and vector molecules were used. The mixture was used to transform spheroplasts of yeast strain LL20, and LEU2 prototrophs were selected on defined agar media lacking leucine. This procedure is diagrammed in Fig. 2.

Only C_4A_2 repeats in *Tetrahymena* DNA stabilized linear plasmids in yeast

Whole cell DNA was prepared from small cultures of all 251 transformants obtained and analyzed by agarose gel electrophoresis. The majority (184/251) of transformants contained extrachromosomal linear plasmids that all migrated identically at 16 kb, suggesting that they were composed of 2 copies of the vector ligated together to form a palindrome, with each telomere provided by vector restriction fragments. This was confirmed by further restriction analysis of a sampling of clones from this size class (data not shown). Approximately 35% of these were not perfect palindromes, as indicated by the absence of a BclI site at the center. The ability of a palindromic dimer of our 8 kb vector fragment to be replicated as a multicopy plasmid demonstrates that the yeast telomere segment on pSZ219 contains a functional ARS.

Ten linear plasmid clones (ypT1-10) were obtained which ranged from 12-30 kb in length and hybridized to a $(C_4A_2)_n$ probe. These plasmids are designated C_4A_2 (+) and are discussed in detail below. An additional 18 clones were C_4A_2 (+) but also hybridized to an rDNA-specific probe. Sixteen of these were identical to pSZ219 (10 kb) and were therefore derived from the *Tetrahymena* end of pSZ219; the 2 larger recombinants (~12 kb) were apparently cloned from a small amount of extrachromosomal rDNA contaminating the micronuclear DNA preparation. These two outcomes are distinguishable because the terminal BglII fragment of native *Tetrahymena* rDNA is 2 kb larger than the BclI fragment from pSZ219. Only ten transformants appeared to be gene conversions at the LEU2 locus.

The remaining 29 linear plasmid recombinants were non-C_4A_2-hybridizing, non-palindromic-sized, and were analyzed by digestion with PvuII or BstXI and hybridization with 3 different probes from the vector: LEU2, $(C_{1-3}A)_n$, and Y' (from yeast end of pSZ219, no $C_{1-3}A$). Results were consistent with all of these recombinants being either palindromic dimers of the vector fragment with a central deletion or with otherwise uncharacterized *Tetrahymena* DNA inserted in the center. Thus, the only micronuclear sequence recognized by yeast as a telomere in this experiment is $(C_4A_2)_n$.

Analysis of C_4A_2 -hybridizing linear plasmids

Restriction maps of all 10 C_4A_2 (+) clones were obtained by gel electrophoresis and hybridization to $(C_4A_2)_n$, $(C_{1-3}A)_n$, and LEU2 probes. Hybridization to $(C_4A_2)_n$ was always confined to the smallest, terminal, fuzzy restriction fragment, which in some cases was as small as 400-500 bp, or the average tract length of telomeric repeats in yeast (26), providing further evidence that the C_4A_2 repeats were at the very termini of these recombinant molecules and were thus responsible for telomere function. The C_4A_2 (+) terminal fragments of all 10 clones also hybridized to a $(C_{1-3}A)_n$ probe (which does not cross-hybridize with C_4A_2 repeats), indicating that, like *Oxytricha* and *Tetrahymena* telomeres previously cloned on linear yeast plasmids (27,4,8) these C_4A_2-terminating clones have been lengthened by the addition of yeast $C_{1-3}A$ repeats (data not shown).

In order to determine the origin of the *Tetrahymena* DNA cloned on these linear plasmids, fragments or whole plasmids were used to probe genomic digests of micronuclear and macronuclear DNA. For example, if a micronuclear telomeric fragment were cloned without rearrangement on a linear plasmid, then the probe should hybridize to restriction fragments of the same size as the corresponding fragments on the linear plasmid, minus the short length of $C_{1-3}A$ repeats that has been added. Whenever possible, fragments not containing the terminal C_4A_2 region were used as hybridization probes, since $(C_4A_2)_n$ hybridizes to many bands in micronuclear DNA as well as a large number of broad but characteristic telomeric bands in macronuclear DNA.

Macronuclear telomere clones

Four linear plasmid clones (ypT1 - ypT4) hybridized to restriction fragments from

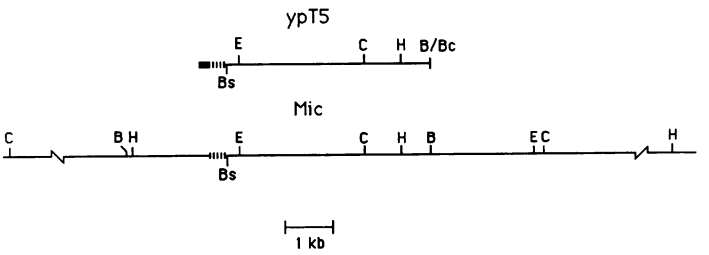


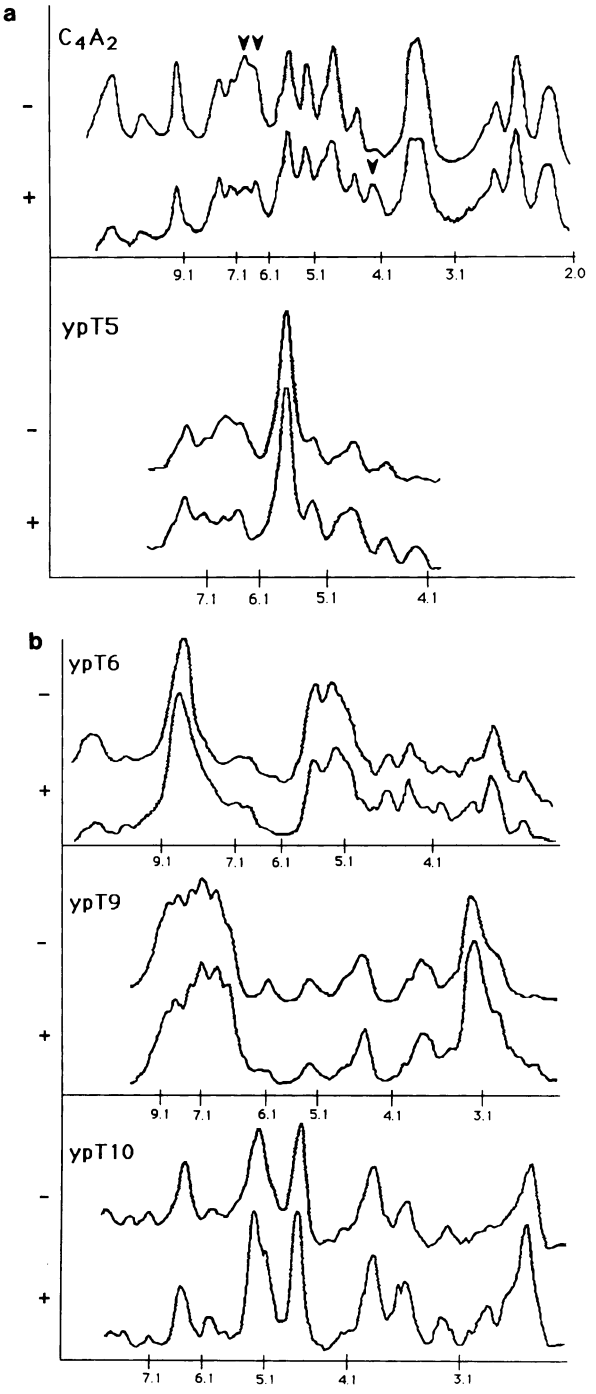
Fig. 3. Structure of ypT5 linear plasmid (excluding vector sequences) and micronuclear DNA counterpart. Restriction enzymes: B, BglII; Bc, BclI; Bs, BstXI; C, ClaI; E, EcoRI; H, HindIII. Striped/solid blocks as in Fig. 2.

macronuclear DNA which were colinear with the restriction maps of these clones, indicating that they were probably derived from the very small amount of macronuclear DNA contaminating the micronuclear preparation. ypT1 has been subcloned into a circular bacterial vector and has been extensively characterized elsewhere (pTtMe4; ref. 28), including the demonstration that ypT1-homologous sequences in macronuclear DNA are progressively shortened by BAL31. Similar results were obtained when fragments from ypT2 and ypT4 were used to probe macronuclear BAL31 blots (data not shown). Each of these macronuclear telomere clones appeared to be unrearranged on the yeast linear plasmid, except for the addition of $C_{1\text{-}3}A$ repeats onto the ends.

Two of the four macronuclear telomere clones - ypT3 and ypT4 - did not hybridize detectably to micronuclear sequences. Further analysis revealed that the micronuclei of strain B1979 had become aneuploid (29). Loss of micronuclear sequences from vegetatively maintained *Tetrahymena* cells has been observed by Allen et al. (30). Therefore, all analyses were done with the same micronuclear DNA as that used in the original cloning experiment, or DNA prepared from the same stock only 3 months later, to avoid confusion caused by further loss of micronuclear DNA sequences homologous to the yeast clones. However, it appears that the strain was already aneuploid when the cloning experiment was performed (data not shown).

Micronuclear-derived linear plasmid clones

The remaining 6 recombinant clones, ypT5-10, hybridized to micronuclear DNA sequences, usually repetitive, that were almost entirely eliminated from macronuclear DNA. Most non-telomeric micronuclear C_4A_2 repeats are found within regions that are eliminated during macronuclear development and are adjacent to a conserved 30 bp sequence which contains a site for the restriction enzyme BstXI (18). Consistent with this observation, all 6 micronuclear-derived clones described here contain a BstXI site adjacent to the terminal C_4A_2 repeats, approximately 400-500 bp from the molecular end of the linear plasmids (see Fig. 3 for an example). Thus it seemed likely that these clones were derived from internally-located C_4A_2 repeats.


a
C_4A_2
-
+
9.1
7.1
6.1
5.1
4.1
3.1
2.0
ypT5
-
+
7.1
6.1
5.1
4.1
b
ypT6
-
+
9.1
7.1
6.1
5.1
4.1
ypT9
-
+
9.1
7.1
6.1
5.1
4.1
3.1
ypT10
-
+
7.1
6.1
5.1
4.1
3.1

An internally-located C_4A_2 repeat region can serve as a telomere in yeast

One linear plasmid clone, ypT5, hybridized to only 1-3 bands in each micronuclear digest and not at all to macronuclear DNA. This simple hybridization pattern allowed us to construct colinear restriction maps of this linear plasmid and the micronuclear DNA from which it was derived (see Fig. 3). The predominantly hybridizing bands in micronuclear genomic DNA were much larger than the corresponding terminal bands from the linear plasmid clone ypT5; e.g., the micronuclear BglII fragment was about 6 kb in length, whereas the distance between the BglII/BclI hybrid site and the C_4A_2 repeat region in ypT5 was only 4.5 kb. In other words, the 1.5 kb of DNA to the left of (distal to) the C_4A_2 repeats in micronuclear DNA is missing from the linear plasmid clone ypT5, leaving the C_4A_2 repeats at the telomere of ypT5. This restriction map strongly suggests that ypT5 is derived from an internally-located C_4A_2 repeat region.

Further evidence that the micronuclear sequences homologous to ypT5 are not telomeric in the micronucleus was provided by a BAL31 nuclease analysis. High molecular weight micronuclear DNA was treated for 3 minutes with 0.4 units BAL31/μg DNA at 30°C. BamHI-digested λ DNA was included in the same reaction as a control; approximately 1kb was removed from each of the λ DNA ends (data not shown). Aliquots of the BAL31-treated DNA were then digested with a restriction enzyme and electrophoresed alongside similarly restricted micronuclear DNA that had not been treated with BAL31. The gel was blotted, and each pair of lanes probed with ypT5 (or other linear plasmids, see below) or with C_4A_2 repeats. The results are shown in Fig. 4. For the C_4A_2 control (Fig. 4a), the pair of HindIII bands which were shortened and removed in the BAL31 experiment in Fig. 1 are again indicated by arrowheads. Note that the relative intensity of these bands was diminished after BAL31 treatment, because the telomeric fragments comigrating with internal fragments had been shortened and their C_4A_2 repeats removed.

However, when this same filter was probed with a fragment of ypT5, the predominant hybridizing species was the expected 5.5 kb HindIII band, which was unchanged in size or intensity after treatment with BAL31 (Fig. 4a). This indicates that the micronuclear DNA sequences homologous to ypT5 (aside from the C_4A_2 repeats) do not lie at a micronuclear telomere, consistent with previous mapping results (Fig. 3). The ypT5 probe was also used on the same BAL31 series filter shown in Fig. 1; the 5.5 kb HindIII band was unchanged during the full course of the digestion (data not shown). Thus it is clear that ypT5 was cloned from an internally-located C_4A_2 repeat region, and that 1.5 kb of DNA between the C_4A_2 repeats and the

Fig. 4. Micronuclear DNA either treated (+) or untreated (-) with BAL31 and subsequently digested with restriction enzymes was run on a gel, blotted to Nytran, and probed with the indicated clone. Autoradiograms were scanned densitometrically and the appropriate lanes aligned, with molecular weights indicated along the X-axes. a) For C_4A_2 and ypT5, digestion was with HindIII; b) for ypT6, EcoRI; for ypT9 and ypT10, BglII.

end of the restriction fragment have been lost, leaving the C_4A_2 repeats at the terminus of the linear plasmid.

In this particular experiment, the ypT5 probe fragment contained a small amount of C_4A_2 repeats, and so all of the C_4A_2-hybridizing bands appeared as well. This pattern appeared identical to the C_4A_2 control filter, providing an internal hybridization control. In addition, in this BAL31 treatment a new C_4A_2 -hybridizing band of about 4 kb appears in the BAL31 treated samples, indicated by an arrowhead in the C_4A_2 control filter (Fig. 4a). The origin of this band is unknown; a possible explanation is that BAL31 pauses at a site approximately 1 kb in from one or more micronuclear telomeres, and an internal C_4A_2 region lies between this pause site and a HindIII site, generating a new, temporarily -C_4A_2 -hybridizing band. Such pause sites during BAL31 digestions have been noted previously (8). Alternative explanations such as gel artifacts or contamination of the sample with plasmid cannot be ruled out.

Each of the remaining micronuclear-derived linear plasmid clones (ypT6 - ypT10) hybridized to many bands in micronuclear DNA. This made it impossible to construct restriction maps of the micronuclear loci that were cloned on the yeast linear plasmids. Therefore, each of these clones was hybridized to the -/+ BAL31 panel described above, to see if any of the many bands were shortened, reduced in intensity, or removed. Representative results are shown in Fig. 4b. For all 5 clones, there appeared to be no significant change in the banding pattern after BAL31 treatment. Furthermore, the region of the gel in which one would expect a telomeric fragment to migrate (given the known size of the homologous fragment on the linear plasmid clone) was often barren of any bands at all. Gross rearrangement of a telomeric fragment could explain this result, but was not observed for any of the macronuclear telomere clones. While we cannot rule out that one of these linear plasmids is derived from a micronuclear telomere, and that the appropriate band is merely hidden by a pattern of hybridization due to some repetitive sequence also present in the clone, we suspect that these other clones are also derived from internally-located C_4A_2 regions in micronuclear DNA.

One micronuclear-derived linear plasmid clone, ypT10, contains an additional $(C_4A_2)_n$-hybridizing region, located at least 3 kb internal to the telomere of this linear plasmid. This region of ypT10 is stable in yeast; that is, no deletion derivatives have been detected in which this internal region of C_4A_2 repeats has now become the telomere of the linear plasmid.

DISCUSSION

We have cloned fragments of DNA from the micronucleus of *Tetrahymena* onto a linear plasmid vector to determine what sequences from micronuclear DNA can be recognized as telomeric in yeast. The only sequences that stabilized these linear plasmids consisted of C_4A_2 repeats, which are found at both telomeric and internal locations in the micronuclear genome. No other sequences from micronuclear DNA were recognized as telomeric in yeast; thus, if other

such sequences exist in the micronuclear genome, they are much more infrequent than C_4A_2 repeats. Cherry and Blackburn (18) have reported that BstX1 sites are found very close to the 3' side of the majority of internally located micronuclear C_4A_2 repeat blocks. The location of BstX1 sites in all 6 of the micronuclear-derived clones described here indicates that the C_4A_2 regions cloned on yeast linear plasmids are in the same orientation as yeast telomeres and ciliate telomeres; that is, the G-rich strand is oriented 5'→3' towards the end of the chromosome or linear plasmid. This is the structure required if a telomerase activity is to elongate the 3' end with G-rich telomeric repeats (9,31). As expected, all of the C_4A_2 telomeres described here have been lengthened by $C_{1\text{-}3}A$ yeast telomeric repeats.

All six of the micronuclear-derived clones obtained in this work apparently originate from internally-located C_4A_2 repeats, which are much more abundant than micronuclear telomeres. A region of C_4A_2 repeats in the middle of a DNA restriction fragment could become a telomere in yeast if the restriction fragment is degraded until the C_4A_2 repeats are at the end of the DNA, at which time they can become a substrate for the addition of yeast telomeric $C_{1\text{-}3}A$ repeats. Thus, the cloned fragment heals at the C_4A_2 repeat region, whereas a cloned fragment not carrying recognizable telomeric sequences would be completely degraded. Murray et al. (32) have analyzed this reaction using plasmid DNAs that carry C_4A_2 repeats at defined positions. Upon transforming these constructs into yeast, as much as several hundred base pairs of non-telomeric DNA could be degraded before healing at or near C_4A_2 repeats occurred. In our experiments, in particular for ypT5, an estimated 1.5 kb of non-telomeric DNA was degraded until the C_4A_2 repeats were reached and could be elongated by yeast telomeric repeats. An alternative mechanism, in which the yeast cell identifies a C_4A_2 repeat region in an internal location, cleaves off the excess DNA, and establishes a telomere, appears less likely, since it would make internally-located telomere-like sequences, such as some $C_{1\text{-}3}A$ repeats (33), highly susceptible to chromosome breakage. The stability of the internally-located C_4A_2 repeats of ypT10 over several years of propagation also argues against this mechanism; if these repeats are in the correct orientation to function as a telomere, shortened derivatives of ypT10 would be generated, and if the repeats are in the opposite orientation, obligate breakage would lead to the loss of the telomere and, consequently, of the plasmid.

Murray et al. (32) have noted that the ability of yeast to add a telomere at sequences that have been exposed after extensive DNA degradation provides one possible mechanism for healing of broken chromosomes (34). Although the function of the internally located $(C_{1\text{-}3}A)_n$ regions of yeast chromosomes is not known, they could act as a second protective telomeric barrier, if loss of distal sequences were to occur. Also, our results show that in any scheme to clone telomeres by function in yeast, one must be mindful that this healing reaction will allow many internal sequences to be cloned. Thus, restriction fragments carrying poly $d(GT)_n$ tracts, which occur at many locations in several eukaryotic genomes, will stabilize yeast linear plasmids, since this simple repetitive sequence will function as a telomere in yeast (32).

The number of C_4A_2 repeat regions at internal positions in the micronuclear genome has been estimated at about 100 (35,36). Therefore, only about 0.6% of the BglII fragments of micronuclear DNA will carry a C_4A_2 tract. Using the number of vector palindromes obtained as a measure of ligation and transformation efficiency, it can be estimated that 600 to 700 micronuclear restriction fragments would have been cloned in the experiment described here, of which only 0.6%, or 4 fragments, should carry C_4A_2 repeats and therefore not be lost. Six clones were obtained in this way; thus cloning of internally-located C_4A_2 tracts was efficient.

Cloning of macronuclear telomeres contaminating the micronuclear DNA preparation was also efficient. Macronuclear DNA is divided into approximately 270 minichromosomes (37), and therefore has 540 telomeres per genome equivalent, excluding the small rDNA molecules. Using either microscopic examination of nuclei or measurement of extrachromosomal rDNA contamination, by hybridization to an rDNA-specific probe, the amount of macronuclear DNA in the micronuclear preparation can be estimated at only 0.5%-2.5% by weight. Thus, compared to the 100 internal micronuclear DNA fragments which carry C_4A_2 repeats (35,36), there are only 2.7-13.5 contaminating macronuclear telomeres (540 per genome times 0.5-2.5%). By comparison, in our experiment we cloned 6 internal micronuclear C_4A_2 regions and 4 macronuclear telomeres, which indicates that recognition of the natural telomere structure, or recognition of C_4A_2 repeats already at a molecular terminus, may be more efficient than healing at an internal location. This same relative efficiency is found when examining the frequency at which we cloned rDNA telomeres. If there are about 225 rDNA telomeres per macronuclear genome (10,000 total copies per 45-ploid macronucleus) one would clone half as many rDNA telomeres as other macronuclear telomeres, and as expected, we obtained 2 such clones.

We have demonstrated that telomeres of micronuclear chromosomes of *Tetrahymena*, like the telomeres of macronuclear minichromosomes, also terminate in C_4A_2 repeats. This is the same arrangement as in another ciliate, *Oxytricha*, in which both the germline and somatic chromosomes terminate in C_4A_4 repeats (38). Thus, the same telomere replication apparatus is very likely to be responsible for maintenance and replication of telomeres in both nuclei of each organism. Unlike *Oxyticha* micronuclear telomeres, however, *Tetrahymena* micronuclear telomeres appear to have a fairly short tract of C_4A_2 repeats, no longer than that of macronuclear telomeres. Also, since we could only detect 2 BAL31-sensitive bands in Fig. 1, we would expect that each of these represents several micronuclear telomeres. If so, then there is a conserved HindIII site either 6.4 or 6.6 kb from all or most micronuclear chromosomal termini. This is reminiscent of the situation in yeast, which has a large Y' repeat associated with many, but not all, chromosomal telomeres, in addition to the actual telomeric $C_{1\text{-}3}A$ repeats (25,39). If our 2 BAL31-sensitive bands do not represent all of the micronuclear telomeres, then most likely we do not detect them, because they were unique bands obscured by the chromosome-internal C_4A_2 repeat bands, or because they were located in large HindIII fragments in a region of the gel where transfer to the filter is inefficient and general degradation of the DNA might obscure the BAL31 digestion profile typical of telomeres.

We have shown here that *S. cerevisiae* efficiently recognizes C_4A_2 repeats from *Tetrahymena* as telomeric, even when such repeats are initially as far as 1.5 kb from molecular termini. This provides a potential pathway for the healing of broken chromosomes. The recognition of C_4A_2 repeats was specific; other sequences from *Tetrahymena* did not stabilize yeast linear plasmids. In addition, we report that micronuclear chromosomes terminate in C_4A_2 repeats. Telomeric repeats thus appear to be indispensable features of functional telomeres in eukaryotes.

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