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Genetics

Elaboration of telomeres in yeast: Recognition and modification of termini from *Oxytricha* macronuclear DNA

(chromosome structure/DNA replication)

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ABSTRACT **The termini of macronuclear DNA molecules from the protozoan *Oxytricha fallax* share a common sequence and structure, both of which differ markedly from those deduced for yeast telomeres. Despite these differences, terminal restriction fragments from *O. fallax* macronuclear DNA can support telomere formation in yeast. Two linear plasmids (LYX-1 and LYX-2) constructed by ligating *Bam*HI-digested total *Oxytricha* macronuclear DNA to a yeast vector were analyzed. One end of LYX-1 and both ends of LYX-2 are derived from the *Oxytricha* DNA that encodes rRNA (rDNA) whereas the other end of LYX-1 is from an *Oxytricha* fragment other than rDNA. After propagation in yeast, both ends of LYX-1 and LYX-2 retain the C_4A_4 repeat characteristic of the *O. fallax* terminal sequence. In addition, both ends of both plasmids acquire 300–1000 base pairs of DNA containing the sequence $(C\text{-}A)_n$, a sequence found near the termini of yeast chromosomes. Thus, at least two different *Oxytricha* termini display distinctive properties in yeast cells in that linear plasmids containing them are not degraded nor are they integrated into chromosomal DNA. These *Oxytricha* termini may act directly as telomeres in yeast; alternatively, the *Oxytricha* DNA may serve as a signal that results in the elaboration of a yeast telomere on the ciliate DNA.**

Cytological and genetic studies indicate that telomeres, the physical ends of eukaryotic chromosomes, are essential for the maintenance of linear chromosomes. Telomeres are distinguished from artificially created ends by their stability: they are not degraded nor do they fuse with other DNA ends. Moreover, there must be special structures or mechanisms for replication of DNA termini to avoid the production of 5′ gaps at the ends of newly synthesized DNA strands. Although telomere structure in eukaryotes remains elusive, the terminal regions of achromosomal DNA molecules from a number of lower eukaryotes have been extensively studied. For example, the terminal region of the extrachromosomal DNA that encodes rRNA (rDNA) from the macronucleus of the ciliated protozoan *Tetrahymena thermophila* consists of 20–70 copies of the sequence 5′ C_4A_2 3′ with specific single-base gaps in the repeated sequence and small hairpins at the ends of the molecule (1, 2). A strikingly different structure is found at the termini of most or all DNA molecules in macronuclei of hypotrichous ciliates, such as *Oxytricha fallax* (ref. 3; Fig. 1*A*). Although the repeat sequence at *Oxytricha* termini (5′ C_4A_4 3′) is similar to that at *Tetrahymena* rDNA ends, *Oxytricha* DNA molecules are different in having 16-nucleotide 3′ single-stranded tails at both ends. Moreover, the *Oxytricha* terminal sequence is short (36 bases), exhibits very little length heterogeneity, and lacks internal nicks.

The *Saccharomyces cerevisiae* transformation system provides an assay method for telomere function. For a plasmid to be maintained in linear form in yeast cells, it must carry special structures at both ends of the DNA molecule (5). Terminal restriction fragments from either *Tetrahymena* macronuclear rDNA (5, 8) or from yeast chromosomes (5) can allow maintenance of linear plasmids in yeast. Here we show that natural ends of macronuclear DNA from *O. fallax*, ends that differ in sequence and structure from those of both *Tetrahymena* rDNA and yeast chromosomes, can support telomere formation in yeast.

MATERIALS AND METHODS

Poly[d(A-C)]·poly[d(G-T)] (hereafter called poly[d(C-A)]) was from Boehringer Mannheim. The following yeast strains were used: fH8 (*a, ade2-1, ade8-18, trp1, ura3-52, leu2-1, his3*), 3482-16-1 (*a, met2, his3∇-1, leu2-3, leu2-112, trp1-289, ura3-52*), and 34 $cir^0\rho^0$ (isogenic with 3482-16-1 except that it lacks the endogenous yeast plasmid 2-μm DNA and mtDNA; constructed by B. Veit and K. Keegan). Symbols that follow a slash in the name of a strain refer to the recombinant DNA plasmid contained within it. DNA was isolated from cells as described (9) except that diethyl pyrocarbonate was not used, and DNA was further purified by sequential extraction with phenol/chloroform/isoamyl alcohol and with chloroform. DNA enriched in plasmid sequences was prepared essentially as described (10). A hybridization probe specific for *Oxytricha* termini was made as follows. A 430-base-pair (bp) *Bgl* II/*Pst* I fragment from the cloned *O. fallax* actin molecule pOfACT(1.6) (Fig. 1*B*) was inserted into *Bam*HI/*Pst* I-digested M13mp8 (called 430mp8). This fragment contains $(C_4A_4)_4$ (6). 430mp8 was digested with *Bgl* II and *Apa* I [to remove the poly[d(G-C)] added in cloning of the actin molecule], and a fragment containing 430 bp of the actin molecule (including the C_4A_4 repeats) as well as a portion of M13 was isolated by gel electrophoresis. *Oxytricha* macronuclei were obtained from G. Herrick and DNA was prepared as described (4) except that it was not digested with either RNase or proteinase and was not purified through CsCl. One μg of pSZ213 (ref. 5; Fig. 1*B*) was linearized with *Bgl* II, treated with calf alkaline phosphatase (Sigma), and ligated to 10 μg of *Bam*HI-digested total *Oxytricha* macronuclear DNA (16 hr, 15°C). The ligation mixture was used to transform yeast strain fH8. Two-dimensional agarose gel electrophoresis was carried out as described (11) except that no agarose block was used in first-dimension gels. Hybridization conditions for the C_4A_4 probe and poly[d(C-A)] are described in ref. 12.

RESULTS

Isolation of Linear Plasmids. Total *O. fallax* macronuclear DNA was digested with *Bam*HI, ligated to *Bgl* II-digested

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Abbreviations: rDNA, DNA encoding rRNA; bp, base pair(s); kb, kilobase pair(s).

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pSZ213 (Fig. 1*B*), and then used to transform yeast strain fH8. DNA was isolated from individual Leu$^+$ colonies and analyzed for the presence of plasmid DNA by Southern hybridization using nick-translated pBR322 DNA. Based on their pattern of hybridization, 51 of 71 transformants contained circles (equal to or greater than pSZ213 in size) and 18 of the transformants contained integrated plasmids. Two transformants hybridized to a single discrete band and were, therefore, candidates for linear plasmids. The estimated sizes of these putative linear plasmids, LYX-1 and LYX-2, are 17.5 and 23.4 kb, respectively (Fig. 5*C*).

Two-dimensional agarose gel electrophoresis was carried out to verify the linearity of LYX-1 and LYX-2. DNA was prepared from transformed cells, mixed with *Bam*HI-digested adenovirus 2 DNA, and subjected to electrophoresis using conditions that permit separation of circular and linear DNA molecules (ref. 11; Fig. 2). The positions of the nicked and covalently closed forms of 2-μm DNA, a naturally occurring multiple-copy yeast plasmid, define an arc of circular DNA molecules visible in the ethidium bromide-stained profiles of second-dimension gels (Fig. 2*B*). When nick-translated 2-μm DNA was hybridized to Southern blots of these gels, the dimer (12.6 kb) and trimer (18.9 kb) forms of 2-μm DNA were also seen, indicating that even large circles fall on the circular arc (data not shown). An arc of linear DNA molecules is defined by the positions of both the adenovirus restriction fragments and the majority of yeast DNA (Fig. 2*B*).

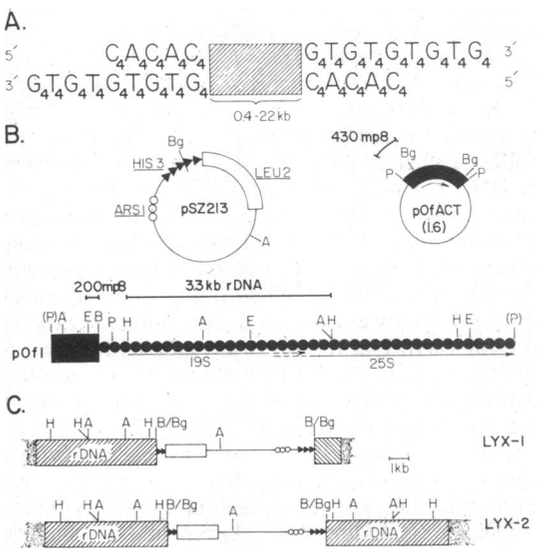

FIG. 1. DNA structures. (*A*) Termini of macronuclear DNA from *O. fallax*. The linear achromosomal DNA molecules that comprise the *O. fallax* macronuclear genome range in size from 0.4 to 22 kb (4) and bear a common repeated sequence at both termini (3). (*B*) Recombinant DNA plasmids. pSZ213 (8.5 kb; from J. Szostak; ref. 5) was used for construction of linear plasmids. pOfACT(1.6) (6 kb) has a 1.6-kb *O. fallax* macronuclear DNA molecule coding for actin inserted by G·C tailing into pBR322 (6). The region of the actin gene subcloned into M13mp8 is indicated. Plasmid pOf1 (7) contains the 7.4-kb macronuclear rDNA molecule from *O. fallax* coding for 19S and 25S rRNAs cloned into the *Pst* I site of pBR322 (only the *Oxytricha* portion is shown). *Pst* I sites in parentheses are present only in the cloned version of the molecule. The 6.6-kb portion of the molecule indicated by solid circles designates the *O. fallax* *Bam*HI fragment cloned onto three of the four ends of LYX-1 and LYX-2. Regions of the cloned rDNA molecule isolated for subcloning and for use as hybridization probes are indicated. (*C*) Linear plasmids. LYX-1 (17.5 kb) and LYX-2 (23.4 kb) were constructed by ligating *Bam*HI-digested total *O. fallax* macronuclear DNA to *Bgl* II-linearized pSZ213. DNA added to the ends of LYX-1 and LYX-2 during propagation in yeast are indicated by stippling. A, *Ava* I; B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; Xm, *Xma* I.

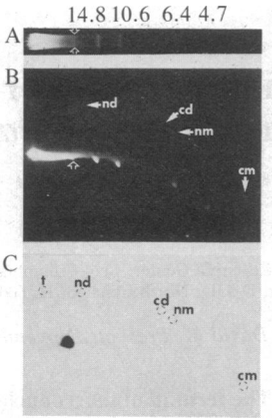

FIG. 2. Two-dimensional agarose gel electrophoresis of LYX-2. (*A*) DNA isolated from 3482-16-1/LYX-2 was subjected to electrophoresis at 1 V/cm in 0.35% agarose containing ethidium bromide at 0.5 μg/ml. Size markers (kb) were restriction fragments of *Bam*HI-digested adenovirus 2 DNA added to the yeast DNA before electrophoresis. The position of LYX-2 is indicated by arrows. (*B*) The lane in *A* was removed from the gel, rotated 90°, and subjected to electrophoresis at 2 V/cm in 1% agarose containing ethidium bromide at 0.5 μg/ml. (*C*) Autoradiogram of hybridization of ^{32}P-labeled pBR322 to the DNA in *B*. Positions of the various forms of 2-μm DNA (covalently closed monomer, cm; nicked monomer, nm; covalently closed dimer, cd; nicked dimer, nd; and trimer, t) were determined by removal of ^{32}P-labeled pBR322 and subsequent hybridization with nick-translated 2-μm DNA.

DNA from cells transformed with LYX-1 or LYX-2 contains a single species that hybridizes to nick-translated pBR322. This species was found on the arc of linear DNA molecules (for example, Fig. 2*C*). In contrast, hybridization to DNA extracted from cells carrying the circular plasmid pSZ213 occurs at positions on the circular arc expected for the covalently closed and nicked circular forms of pSZ213 (data not shown). Thus, LYX-1 and LYX-2 are linear plasmids.

LYX-1 and LYX-2 Contain Sequences Homologous to *Oxytricha* DNA. Hybridization of nick-translated LYX-1 to *Oxytricha* macronuclear DNA was used to identify the *Oxytricha* *Bam*HI fragments that form the ends of the linear plasmid. DNA from cells carrying LYX-1 was fractionated by agarose gel electrophoresis (Fig. 3*A*) and the linear plasmid was isolated by electrophoresis onto DEAE-paper. The plasmid was nick-translated and hybridized to undigested and *Bam*HI-digested *Oxytricha* macronuclear DNA (Fig. 3*B*). Because LYX-1 had been constructed by ligating *Bam*HI-digested macronuclear DNA to linearized pSZ213, we anticipated hybridization to at least two *Oxytricha* *Bam*HI restriction fragments. In undigested macronuclear DNA, nick-translated LYX-1 hybridized to a 7.4-kb band that corresponds in size to the predominant rDNA band in the ethidium bromide-stained profile of the gel. An unidentified band at 2.8 kb also hybridized to the plasmid probe. Hybridization to *Bam*HI-digested macronuclear DNA was detected at a band of 6.6 kb, which corresponds in size to a *Bam*HI fragment containing the 19S and 25S coding regions of *O. fallax* rDNA (ref. 7; Fig. 1*B*). In addition, hybridization was detected to both a 2.8-kb and a 1.5-kb fragment.

Since LYX-1 hybridized to a band corresponding in size to *Oxytricha* rDNA, we asked directly whether rDNA is found on LYX-1 and LYX-2. When the *O. fallax* rDNA plasmid pOf1 was digested with *Hin*dIII, a 3.3-kb fragment was generated that contained all of the 19S and a portion of the 25S rRNA encoding regions (Fig. 1*B*). This fragment was isolated and hybridized to DNAs from cells containing LYX-1 and LYX-2 (Fig. 4). Because of homology between *Oxytricha* and yeast rDNAs, the 3.3-kb fragment was expected to

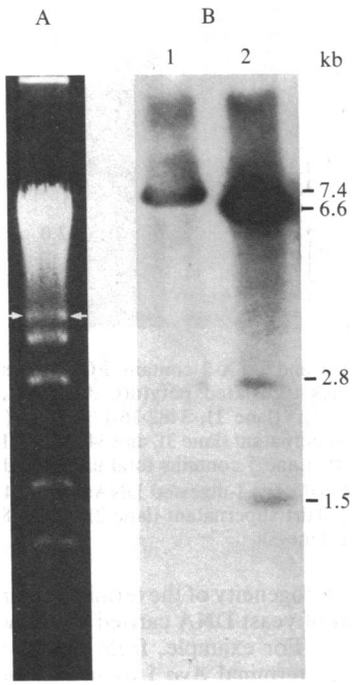

FIG. 3. LYX-1 contains *Oxytricha* DNA. (*A*) Total DNA from 34 $cir^0\rho^0$/LYX-1 was mixed with *Bam*HI-digested adenovirus 2 DNA and fractionated on a 0.7% agarose gel. (*B*) LYX-1 (arrows in *A*) was isolated, nick-translated, and hybridized to Southern blots of undigested (lane 1) and *Bam*HI-digested (lane 2) *Oxytricha* macronuclear DNA in the presence of a 10-fold excess of unlabeled yeast DNA. (pSZ213 DNA does not hybridize to *Oxytricha* DNA.)

cross-hybridize with yeast rDNA. Digestion of yeast rDNA with *Hin*dIII yields three fragments of which only one (6.5 kb) contains sequences for 18S and 25S rRNAs (13). As expected, the 3.3-kb *Oxytricha* rDNA fragment hybridizes to a 6.5-kb band in *Hin*dIII-digested total yeast DNA (Fig. 4*A*). However, hybridization of the 3.3-kb probe to *Hin*dIII-digested total DNA from cells carrying LYX-1 or LYX-2 detected two bands, the 6.5-kb band corresponding to yeast rDNA and a band of 3.3 kb (Fig. 4*A*). The 3.3-kb band comigrated with the hybridizing band from both the *Hin*dIII-digested *O. fallax* rDNA plasmid and *Hin*dIII-digested total *O. fallax* macronuclear DNA (data not shown). Thus, both LYX-1 and LYX-2 contain the 6.6-kb *Bam*HI rDNA fragment.

To determine which end of LYX-1 and LYX-2 contains

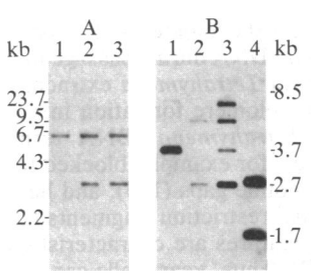

FIG. 4. LYX-1 and LYX-2 contain *Oxytricha* rDNA. Hybridization of the 3.3-kb *Hin*dIII fragment of pOf1 (Fig. 1) was carried out in the presence of a 100-fold excess of unlabeled pBR322 DNA. (*A*) A 0.7% agarose gel containing *Hin*dIII-digested DNAs from 34 $cir^0\rho^0$/pSZ213 (lane 1), 34 $cir^0\rho^0$/LYX-1 (lane 2), and 3482-16-1/LYX-2 (lane 3). (*B*) A 1% agarose gel containing *Ava* I-digested DNA from 3482-16-1/pSZ213 (lane 1), *Ava* I-digested DNA from 34 $cir^0\rho^0$/LYX-1 (lane 2), *Ava* I-digested DNA from 3482-16-1/LYX-2 (lane 3), and *Ava* I/*Bam*HI-digested *Oxytricha* macronuclear DNA (lane 4).

Oxytricha rDNA, DNAs from cells carrying the plasmids were digested with *Ava* I and hybridized to the 3.3-kb *Hin*dIII *Oxytricha* rDNA probe (Fig. 4*B*). The 3.3-kb rDNA probe hybridized to a single band of 3.7 kb in *Ava* I-digested yeast DNA (Fig. 4*B*). Based on the *Ava* I restriction sites in pSZ213 and *Oxytricha* rDNA (Fig. 1*B*) and on the method used to generate LYX-1 and LYX-2, two unique *Ava* I fragments, which contain the *Oxytricha* rDNA-pSZ213 junction site, are possible. A junction fragment of about 5.2 kb is predicted if the *Oxytricha* rDNA fragment is attached to the *LEU2* proximal portion of pSZ213. Alternatively, if this fragment is attached to the *ARS1* proximal portion of pSZ213, the junction fragment should be about 6.7 kb. The common fragments anticipated from digestion of LYX-1 and LYX-2 with *Ava* I after hybridization to the 3.3-kb *Hin*d III probe are a 3.7-kb fragment from yeast rDNA and a 2.7-kb fragment from the internal portion of the 6.6-kb *Oxytricha* rDNA end. In cells carrying LYX-1, hybridization was detected to yeast rDNA, to the internal rDNA band, and to a third band of 5.8 kb. Thus, in LYX-1 the 6.6-kb *Oxytricha* rDNA fragment is attached to the *LEU2* proximal portion of pSZ213. In DNA from cells carrying LYX-2, hybridization was detected at the three bands detected in LYX-1. However, an additional band at 7.2 kb was also detected, suggesting that LYX-2 has *Oxytricha* rDNA on both ends (Fig. 4*B*). To verify this interpretation, the *Oxytricha* probe was removed from the blot and the DNA was rehybridized to a nick-translated fragment containing *ARS1*. Of the four fragments that hybridized to the *Oxytricha* rDNA probe, only the 7.2-kb *Ava* I fragment hybridized to *ARS1* (data not shown). Thus, LYX-2 contains the *Oxytricha* 6.6-kb rDNA fragment on both ends.

We also tested LYX-1 and LYX-2 for homology to the other fragment produced by *Bam*HI digestion of *Oxytricha* rDNA. An M13 probe containing a 200-bp *Eco*RI/*Bam*HI fragment isolated from pOf1 (Fig. 1*B*) did not hybridize to DNA from cells carrying either LYX-1 or LYX-2 but did hybridize to the proper size fragment in total *Oxytricha* DNA (data not shown). Thus, LYX-1 does not contain the small *Bam*HI fragment from *Oxytricha* rDNA at its *ARS1* proximal end.

LYX-1, LYX-2, and Yeast DNA Contain Sequences Homologous to the Ends of *Oxytricha* Macronuclear DNA. Hybridization of LYX-1 to macronuclear DNA identified specific *Bam*HI fragments contained on the plasmid. In addition, hybridization to total macronuclear DNA was observed (Fig. 3). This pattern of hybridization would be expected if LYX-1 retained the C_4A_4 sequence common to the ends of all macronuclear DNA molecules (Fig. 1*A*). A fragment that contains $(C_4A_4)_4$ was isolated, nick-translated, and hybridized to a variety of DNAs. Hybridization was detected to both untransformed yeast DNA and total macronuclear DNA (Fig. 5*A*). *Xho* I digestion of yeast DNA produces a 1.4-kb fragment from the distal-most portion of the chromosome (5). The C_4A_4 probe did not hybridize to the terminal 1.4-kb *Xho* I fragment but did hybridize to larger fragments (Fig. 5*A*). Thus, yeast DNA contains sequences homologous to the end-specific sequence of *O. fallax*. The C_4A_4 probe also hybridized to LYX-1 and LYX-2 but not to pSZ213 nor to LYT-1 (Fig. 5*B* and *C*). LYT-1 is a linear plasmid containing *Tetrahymena* rDNA terminal fragments at each end (8). Hybridization of the C_4A_4 probe to *Ava* I-digested DNA from cells carrying LYX-1 and LYX-2 suggests that both ends of the linear plasmids carry the *Oxytricha* sequence. A 2.7-kb fragment containing the terminus of the rDNA was produced by *Ava* I digestion of the 6.6-kb *Bam*HI *Oxytricha* rDNA (Fig. 1*B*). This fragment should be found on both ends of LYX-2 and on the *LEU2* proximal end of LYX-1. Hirt supernatants from cells carrying LYX-1 and LYX-2 were digested with *Ava* I and hybridized to the C_4A_4 probe (Fig. 5*D*). Both DNAs hybridized to a broad band of about 3.1 kb.

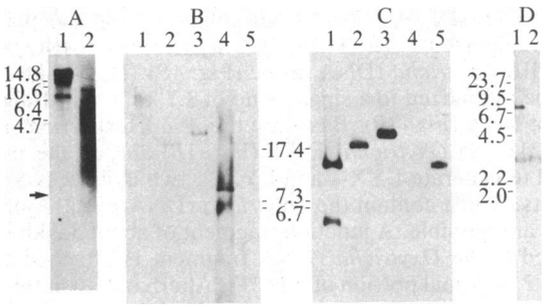

FIG. 5. LYX-1, LYX-2, and yeast DNA hybridize to the *O. fallax* terminal repeat. DNAs in *A*, *B*, and *D* were hybridized with a nick-translated fragment isolated from 430mp8 (Fig. 1*B*). (*A*) A 0.7% agarose gel containing *Xho* I-digested untransformed yeast DNA (lane 1) and undigested *O. fallax* macronuclear DNA (lane 2). The arrow indicates the position at which a poly[d(C-A)] probe hybridized to *Xho* I-digested yeast DNA on a duplicate blot. (*B*) A 0.4% agarose gel containing undigested DNAs: 3482-16-1/pSZ213 (lane 1), 34 $cir^0\rho^0$/ LYX-1 (lane 2), 3482-16-1/LYX-2 (lane 3), *O. fallax* macronuclear DNA (lane 4), and 3482-16-1/LYT-1 (lane 5). Lane 1 contains more yeast DNA than lanes 2, 3, and 5 and therefore hybridization of C_4A_4 to yeast chromosomal DNA is visible at this exposure. (*C*) The C_4A_4 probe was removed from the blot in *B*, and the blot was rehybridized to nick-translated pBR322 to determine the positions of plasmid DNAs. The DNAs in *D* (0.7% agarose gel) were digested with *Ava* I and are Hirt supernatants from 34 $cir^0\rho^0$/ LYX-1 (lane 1) and 3482-16-1/LYX-2 (lane 2). Sizes are indicated in kb.

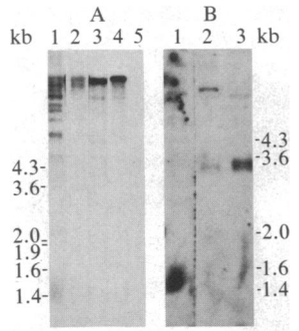

FIG. 6. LYX-1 and LYX-2 contain d(C-A) tracts. DNAs were hybridized to nick-translated poly[d(C-A)]. (*A*) *Xho* I-digested DNAs from 34 $cir^0\rho^0$ (lane 1), 3482-16-1/pSZ213 (lane 2), 34 $cir^0\rho^0$/LYX-1, Hirt supernatant (lane 3), and 3482-16-1/LYX-2, Hirt supernatant (lane 4). Lane 5 contains total undigested *Oxytricha* macronuclear DNA. (*B*) *Ava* I-digested DNAs from 34 $cir^0\rho^0$ (lane 1), 34 $cir^0\rho^0$/LYX-1, Hirt supernatant (lane 2), and 3482-16-1/LYX-2, Hirt supernatant (lane 3).

In DNA from cells carrying LYX-1, hybridization was also detected to a 7.3-kb *Ava* I fragment. Thus, both ends of LYX-1 (and, presumably, LYX-2) carry the *Oxytricha* end sequence. Because the *Oxytricha* end probe does not hybridize to LYT-1, we assume that the C_4A_4 detected on LYX-1 and LYX-2 was retained from *Oxytricha* DNA rather than being added to the plasmids during their propagation in yeast.

Both Ends of LYX-1 and LYX-2 Contain Sequences Homologous to poly[d(C-A)]. Tracts of poly[d(C-A] are found in the terminal 1.4-kb *Xho* I fragments of yeast chromosomes as well as at other locations in the genome (14). *Tetrahymena* rDNA does not hybridize to poly[d(C-A)]; however, linear plasmids containing ends from *Tetrahymena* rDNA hybridize to this probe after their propagation in yeast (14).

Poly[d(C-A)] was nick-translated and hybridized to *Oxytricha* macronuclear DNA and to a variety of yeast DNAs (Fig. 6). No hybridization was detected to *Oxytricha* sequences (Fig. 6*A*) nor to pSZ213 (data not shown). In *Xho* I-digested DNA from untransformed yeast, hybridization was detected to fragments of about 1.4 kb as well as to multiple bands larger than 4 kb (Fig. 6*A*). In *Xho* I digests of DNAs from cells carrying LYX-1 and LYX-2, hybridization occurred at the positions of the intact plasmids (Fig. 6*A*). Since LYX-1 and LYX-2 lack recognition sites for *Xho* I, this result suggests that both plasmids carry tracts of d(C-A). To verify this result, DNA preparations enriched in plasmid sequences were prepared (10). These DNAs were digested with *Ava* I and then hybridized to nick-translated poly[d(C-A)] (Fig. 6*B*). *Ava* I digestion of the *Oxytricha* rDNA produces a terminal fragment of 2.7 kb, a fragment found on both LYX-1 and LYX-2. Hybridization with the d(C-A) probe to *Ava* I-digested DNAs from Hirt extractions detects a broad band with an average size on this gel of 3.3 kb (Fig. 6*B*). No band of this size was detected in *Ava* I-digested DNA from untransformed yeast cells (Fig. 6*B*). The average size of this fragment from measurements from four different gels was 3.2 kb. This result indicates that there is an average of 500 bp of DNA added near the rDNA ends of LYX-1 and LYX-2 and that this DNA contains stretches of poly[d(C-A)]. The size heterogeneity of the terminal fragment suggests that the amount of yeast DNA carried by individual plasmid molecules varies. For example, from the gel with the best resolution of this terminal *Ava* I fragment, we estimate its size as ranging from 3.0 to 3.2 kb (for LYX-1) and from 3.1 to 3.3 kb (for LYX-2). DNA from cells carrying LYX-1 also displays hybridization to a second band (Fig. 6*B*). Although the size of this fragment cannot be estimated accurately from this gel, its average size from determinations in three other gels is 7.3 kb. Since pSZ213 contributes 5.0 kb to this 7.3-kb *Ava* I fragment, there must be less than 2.3 kb of *Oxytricha* DNA on the *ARS1* proximal end of LYX-1. These data suggest that the 1.5-kb fragment (rather than the 2.8-kb fragment) detected by hybridization of LYX-1 to *Oxytricha* DNA (Fig. 3) is the fragment on the *ARS1* proximal end of LYX-1 and that an average of 800 bp of DNA was added to the end of this fragment during propagation in yeast. We assume that the 2.8-kb fragment hybridizes to LYX-1 because it has homology with the 1.5-kb fragment.

DISCUSSION

The ability to propagate linear plasmids in *S. cerevisiae* provides an opportunity to investigate the sequence and structure requirements for telomere function. We report here that termini from *O. fallax* macronuclear DNA enable a yeast plasmid to be propagated as an extrachromosomal linear DNA molecule. The sequence characteristic of *Oxytricha* DNA termini is maintained in yeast; but, in addition, sequences found near the telomeres of yeast chromosomes are added to *Oxytricha* DNA during passage in yeast. Fragments from the termini of *Tetrahymena* extrachromosomal rDNA can also support telomere formation in yeast (5, 8). Yeast telomeres and *Tetrahymena* rDNA termini share many structural features; for example, blocked termini (2, 14), the presence of nucleotide gaps (1, 5), and heterogeneity in the lengths of terminal restriction fragments (1, 5, 14). None of these structural features are characteristic of DNA termini from *O. fallax* (3). Thus, yeast cells can recognize a variety of DNA termini, termini that differ from each other in both structure and sequence. Instead of degrading or integrating linear DNAs with these specialized ends, the yeast cell ultimately transforms them into functioning telomeres.

New telomeres are also created during formation of the ciliate macronucleus. For example, in *Oxytricha*, the macronuclear genome is derived from the chromosomes of the micronucleus by an intricate process involving the fragmentation and selective degradation of polytenized micronuclear chromosomes (see ref. 15). Since C_4A_4 is not detected on the

micronuclear counterpart of a specific macronuclear DNA molecule (16), the terminal repeats must be added to nascent macronuclear DNA after their excision from micronuclear chromosomes. A similar process seems to operate during formation of the macronucleus in *Tetrahymena*: the multiple extrachromosomal copies of macronuclear rDNA are derived from a single copy in the micronucleus that bears only one C_4A_2 repeat (17).

Are rDNA termini the only ones from ciliates capable of providing telomere activity in yeast? From consideration of both the average spacing of *Bam*HI sites and the amount of rDNA in total *Oxytricha* DNA, we estimate that the 6.6-kb rDNA fragment should equal no more than 10% of the *Oxytricha Bam*HI fragments with terminal repeats. However, three of the four *Oxytricha* fragments identified by functional cloning in yeast are 6.6-kb *Bam*HI rDNA fragments (Fig. 4). We have also identified a fourth *Oxytricha* fragment, not derived from the 7.4-kb rDNA molecule that can provide telomere function in yeast (Fig. 3). Thus, at least two, and possibly all, *Oxytricha* terminal fragments can function in yeast. However, it is likely that some property of the 6.6-kb rDNA fragment facilitates its use as a telomere in the yeast cloning system. For example, minor differences in the terminal sequence could explain the preference for rDNA in our cloning experiments: although the 6.6-kb rDNA fragment hybridizes to the C_4A_4 probe, direct sequence analysis of *Oxytricha* rDNA will be necessary to determine whether the length and sequence of its termini are identical to those on other *Oxytricha* DNA molecules. Alternatively, it is possible that the 6.6-kb rDNA fragment contains an *ARS* sequence and that both an *ARS* and the *Oxytricha* terminal repeat are required for telomere function in yeast. This hypothesis is attractive because fragments from both yeast telomeres and *Tetrahymena* rDNA ends function as *ARS* elements in yeast when they are inserted into circular plasmids (18, 19).

It has been argued that the ability of ciliate DNAs to provide telomere function in the phylogenetically distant yeast cell argues for conservation of mechanisms of replication and resolution of eukaryotic telomeres (5). However, if linear plasmids carrying *Oxytricha* and *Tetrahymena* DNAs are capped by yeast telomeres, the replication and resolution of their ends may proceed by mechanisms unique to yeast. An alternative explanation for the behavior of *Tetrahymena* and *Oxytricha* termini in yeast is that, unlike termini produced by restriction enzymes, these natural ends are stable by virtue of being protected from, for example, exonucleolytic degradation. A simple repeated terminal sequence, a terminal *ARS* sequence, or both may be responsible for the stability of natural termini. A linear molecule that persists intact until the time of telomere replication might then acquire a yeast telomere. New telomeres could be formed by transposition of tracts of poly[d(C-A)] found elsewhere in the yeast genome (Fig. 6). A transposition event that depends on sequence homology seems unlikely because of the absence of C_4A_4 repeats in the terminal 1.4-kb region of yeast chromosomes and the fact that no more than 1 kb of yeast DNA is added to the ends of LYX-1 and LYX-2. Alternatively, replication of telomeres could occur at the telomere itself by a process that does not require template DNA, for example by an enzyme like terminal transferase. Although both of these mechanisms for telomere replication may seem baroque, a similar process must be invoked to explain the addition of terminal repeats to *Oxytricha* and *Tetrahymena* DNAs during the formation of macronuclei (16, 17).

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