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Stringent sequence requirements for the formation of human telomeres

(artificial chromosome/chromosome breakage/telomerase/telomeric protein/telomere healing)

JOHN P. HANISH, JUDITH L. YANOWITZ, AND TITIA DE LANGE*

The Rockefeller University, New York, NY 10021

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ABSTRACT **In human cells, transfection of telomeric T_2AG_3 repeats induces the formation of functional telomeres at previously interstitial sites. We report that telomere formation has stringent sequence requirements. While $(T_2AG_3)_n$ telomere seeds formed telomeres in ≈70% of the transfected cells, five T_2AG_3-related heterologous telomeric DNAs seeded new telomeres in <5% of the transfectants. Telomere formation did not correlate with the ability of human telomerase to elongate telomeric sequences *in vitro*. Homologous recombination is probably also not involved because a $(T_2AG_3)_n$ telomere seed with nontelomeric DNA at 160-bp intervals formed new telomeres frequently. Instead, the sequence dependence of telomere formation matched the *in vitro* binding requirements for the mammalian T_2AG_3 repeat binding factor (TRF). Human TRF failed to bind ineffective heterologous telomere seeds and had a 4-fold lower affinity for $(T_2AG_5)_2T_2AG_3$ repeats, which seeded telomeres with reduced frequency. The results suggest that telomere seeds interact with TRF and predict that mammalian artificial chromosomes will require wild-type telomeric repeats at, or near, their termini.**

The telomeres of most eukaryotic chromosomes carry a tandem array of short repeats, which are thought to form a protective nucleoprotein complex at chromosome ends (1, 2). The telomeric complex prevents detection of chromosome ends by DNA damage checkpoints and protects the termini from exonucleases and ligases (1–3). In addition, telomeric DNA is important for the replication of linear chromosomes. Chromosome ends are predicted to shorten gradually with cell divisions due to the fixed direction of chromosomal DNA replication and the requirement for a primer. Telomeres can counter this effect by engaging telomerase, a ribonucleoprotein reverse transcriptase that adds telomeric repeat DNA to 3′ telomere termini (reviewed in refs. 4 and 5).

Human telomeres contain several kilobase pairs of the highly conserved sequence $(T_2AG_3)_n$ with the G-rich strand oriented 5′ → 3′ toward the chromosome ends (6–9). These telomeric tracts progressively shorten from ≈10 to ≈1.5 kb during normal development, as well as in primary tumors, and in normal cells undergoing *in vitro* senescence (refs. 9–11; reviewed in refs. 5 and 12). As expected from the attrition of their telomeres, primary human cells lack telomerase activity (11). In contrast, immortal human cells express telomerase and the decline of their telomeres is halted (5, 11–14).

Human telomeres are expected to interact with specific telomeric proteins in addition to telomerase. Telomeric factors and specialized telomeric chromatin have been demonstrated in *Saccharomyces cerevisiae* and several ciliates (reviewed in refs. 1, 2, and 15). Human telomeres display an unusual chromatin structure and are attached to the nuclear matrix; a highly conserved candidate telomeric protein (TRF; T_2AG_3 repeat binding factor) has been identified in mammalian cells (16–18).

The functional significance of telomeric repeats is demonstrated by the formation of new mammalian telomeres after transfection of linear DNAs with a terminal $(T_2AG_3)_n$ stretch (19–21). These telomere seeds become linked to chromosome internal sequences by illegitimate recombination between interstitial DNA and the nontelomeric end of the transfected molecules (refs. 19–21; this report). The resulting fragmented chromosomes apparently become stabilized when the $(T_2AG_3)_n$ DNA is recognized as a telomere seed and heals into a functional telomere. We have examined the cis-acting requirements for telomere formation in human HeLa cells. The results indicate that subtle alterations in the sequence of telomere seeds interfere with their conversion into functional telomeres. The sequences required for telomere formation match the binding site of TRF and implicate this factor in the formation of new telomeres in human cells.

MATERIALS AND METHODS

Construction of Telomere Seeds. Short telomeric arrays were derived from previously described plasmids (18) or formed by self-ligation of synthetic DNAs. Telomere seed plasmids were constructed by tandem ligation of these short (250–800 bp) telomeric repeat segments in pSXneo (see Fig. 1*a* and ref. 18). pSXneo-1.6-T_2AG_3 (Fig. 1*a*) contains two tandem 0.8-kb T_2AG_3 repeat arrays separated by a 23-bp nonrepeat polylinker segment. pSXneo-3.2-T_2AG_3 contains four 0.8-kb T_2AG_3 repeat arrays separated by 23-, 32-, and 23-bp segments. pSXneo-1.6-TAG_3 contains three 530-bp TAG_3 interrupted by 20-bp nonrepeat segments. pSXneo-1.1-T_2G_4 contains two 540-bp T_2G_4 arrays interrupted by a 28-bp nonrepeat sequence. pSXneo-1.2-T_3AG_3 contains three 385-bp T_3AG_3 arrays interrupted by 19-bp nonrepeat segments. Additional heterologous telomere seeds were constructed from synthetic DNAs. For pSXneo-1.0-T_2AG_5, DNA oligonucleotides with the sequence $(T_2AG_5)_3$ and $(TA_2C_5)_3$ were annealed and self-ligated to form a 250-bp T_2AG_5 repeat array. A multimer of this array was subcloned into pSXneo to form a 1.0-kb stretch in which four 250-bp T_2AG_5 segments are linked by 15-, 38-, and 15-bp nonrepeat segments. Similarly, oligonucleotides with the sequence $(T_2AG_2C)_3$ and $(TA_2GC_2)_3$ were annealed and ligated to form a 400-bp array, which was multimerized to a stretch of five 400-bp T_2AG_2C segments interrupted with 15-bp nonrepeat segments in pSXneo-2.0-T_2AG_2C. pSXneo-2.4-$(T_2AG_5)_2$ T_2AG_3 was generated by using the oligonucleotides $(T_2AG_5)_2$ T_2AG_3 and $(TA_2C_5)_2TA_2C_3$, resulting in a 2.4-kb repeat array with six 400-bp $(T_2AG_5)T_2AG_3$ units interrupted with nonre-

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Abbreviations: MAC, mammalian artificial chromosome; TRF, TTAGGG repeat binding factor.

*To whom reprint requests should be addressed at: The Rockefeller University, 1230 York Avenue, New York, NY 10021.

peat segments of 15-bp. pSXneo-1.0-$(T_2AG_3)_{168}N_{14-22}$ contains 1.0 kb to T_2AG_3 repeats formed by the ligation of six 168-bp T_2AG_3 arrays from pTH5 (11) joined by nontelomeric segments of 14 and 22 bp. All cloning steps were performed with *Escherichia coli* HB101 grown logarithmically at 30°C in brain/heart infusion broth. Precursor plasmids with short repeat arrays were sequenced and final telomere seed constructs were verified by partial sequence analysis.

Transfection of HeLa Cells. Plasmids were linearized at sites 4–20 bp 3′ of the G-rich repeat strand and transfected into adherent HeLa cells (HeLa II in ref. 22) with Lipofectin (BRL). For each construct 100–500 $G418^R$ (400 μg/ml) colonies (from transfection of 1 pmol of DNA) were pooled for genomic analysis. Clonal cell lines were derived by limiting dilution or with cloning cylinders.

Quantitation of Telomeric Neomycin Phosphotransferase (neo) Signals. Genomic DNA from pooled colonies was treated with BAL-31 (IBI; 10 units of BAL-31-M/40 μg of DNA) for 6 hr at 30°C. Untreated samples were processed in parallel. *Hin*dIII-digested DNAs (14 μg per sample as measured by Hoechst fluorescence) was analyzed by blotting as described (9). neo signals on fragments larger than 1.2 kb (the size of the neo cassette) were quantitated with a PhosphorImager. The fraction of telomeric *neo* genes was calculated as

$$\%\ \text{telomeric } neo \text{ genes} = \frac{\text{neo signal before BAL-31} - \text{neo signal after BAL-31}}{\text{neo signal before BAL-31}} \times 100.$$

Hybridization with a T_2AG_3 repeat probe (16) demonstrated that BAL-31 had removed 89–93% of the telomeric sequences in each case. The residual signal is due to interstitial T_2AG_3-related sequences. Hybridization with the human *DEK* gene (R. Bose and T.d.L., unpublished data) verified that BAL-31 had not degraded chromosome internal DNA.

Plasmid Rescue. *Xba* I-digested genomic DNA was circularized and plasmids were isolated that contained telomeric *neo* loci and several kilobases of flanking HeLa sequences.

Telomerase Assays. HeLa S100 extract was incubated with oligonucleotide primers (at 0.4 or 2.0 μM) and [α-^{32}P]dGTP for 1 or 2 hr at 30°C and processed as described by Morin (14).

TRF Assays. HeLa TRF was assayed as described (18) with an end-labeled $(T_2AG_3)_{12}$ probe (12merA in ref. 18) in the presence of circular plasmids containing human and heterologous telomeric sequences. Plasmid concentrations were determined by Hoechst fluorescence and verified by ethidium bromide staining of agarose gels.

RESULTS AND DISCUSSION

Frequent Telomere Formation with Telomere Seeds of 0.8–3.2 kb. To determine which features of the $(T_2AG_3)_n$ telomere seeds are required for telomere formation in human cells, we measured the frequency with which different telomeric sequences are recovered at chromosome ends. In this assay, HeLa cells were transfected with linear DNAs containing the *neo* gene proximal to the telomeric sequence (Fig. 1*a*) and stable $G418^R$ cells were isolated. Clonal lines with telomeric *neo* genes were readily identified based on the typical heterogeneous size of telomeric restriction fragments (Fig. 1*b*). Telomere formation was also detected in pools of transfected colonies. In this case, the frequency of telomere formation was determined by quantitating the BAL-31 sensitivity of the *neo* loci (Fig. 1*c* and Table 1). These frequencies correlated well with those obtained from clonal cell lines (Table 1). We found that T_2AG_3 repeat arrays of 0.8–3.2 kb seeded telomeres in up to ≈75% of the stably transformed cell lines (Table 1). The remaining 25% of the transfectants had chromosome

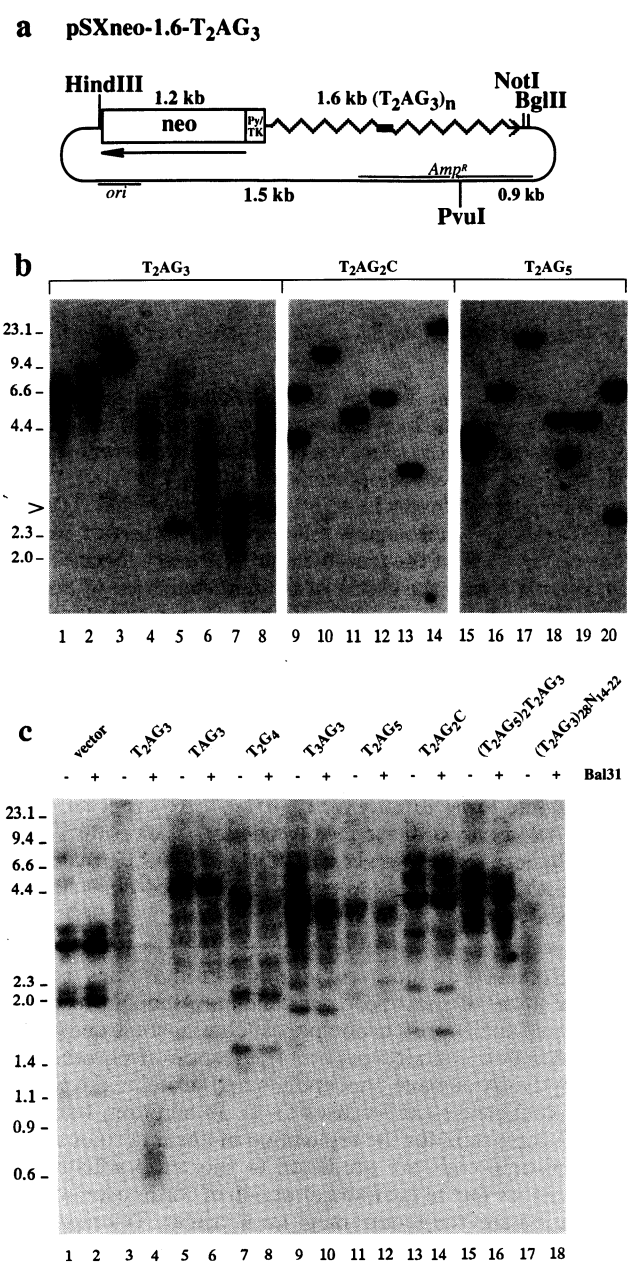

FIG. 1. Telomere formation in HeLa cells. (*a*) Telomere seed plasmid pSXneo-1.6-T_2AG_3. The wavy line represents 1.6 kb of T_2AG_3 repeats with a 23-bp interruption (solid box). 1.6N and 1.6P (Table 1) are generated by cleavage with *Not* I and *Pvu* I, respectively (9 and 900 bp 3′ to the T_2AG_3 repeat strand, respectively). (*b*) Telomere formation with T_2AG_3, T_2AG_2C, and T_2AG_5 telomere seeds. Blots of neo fragments in *Hin*dIII-digested DNA of HeLa clones transfected with pSXneo-1.6-T_2AG_3 (lanes 1–8), pSXneo-2.0-T_2AG_2C (lanes 9–14), or pSXneo-1.0-T_2AG_5 (lanes 15–20). Arrowhead indicates expected position of pSXneo-1.6-T_2AG_3 telomere seeds without healing—i.e., the 2.8-kb *Hin*dIII/*Not* I fragment (see *a*). Molecular sizes (kb) of *Hin*dIII-digested λ DNA marker fragments are indicated. (*c*) BAL-31 sensitivity of *neo* loci in pools of colonies transfected with telomere seeds. DNA from pooled colonies was treated with BAL-31 for 0 hr (odd-numbered lanes) and 6 hr (even-numbered lanes), cleaved with *Hin*dIII, fractionated, blotted, and hybridized with neo. The following telomere seed plasmids were used: lanes 1 and 2, pSXneo vector; lanes 3 and 4, pSXneo-1.6-T_2AG_3; lanes 5 and 6, pSXneo-1.6-TAG_3; lanes 7 and 8, pSXneo-1.1-T_2G_4; lanes 9 and 10, pSXneo-1.2-T_3AG_3; lanes 11 and 12, pSXneo-1.0-T_2AG_5; lanes 13 and 14, pSXneo-2.0-T_2AG_2C; lanes 15 and 16, pSXneo-2.4-$(T_2AG_5)_2T_2AG_3$; lanes 17 and 18, pSXneo-1.0-$(T_2AG_3)_{28}N_{14-22}$.

Table 1. Telomeres formed with human and heterologous telomeric DNAs

	pSXneo telomere seed derivative			Fraction of telomeric *neo* genes		
	Length, kb	Sequence	Configuration	In pooled colonies, %	In clonal lines	Median telomere length, kb
1.	0.8	TTAGGG	Linear	67 ± 5	16/24	4.7 ± 1.5
2.	1.6N	TTAGGG	Linear *Not* I*	69 ± 13	9/12	3.4 ± 2.0
3.	1.6P	TTAGGG	Linear *Pvu* I*	54, 61	7/11	3.0 ± 1.3
4.	1.6C	TTAGGG	Circular	45	13/33	2.7 ± 1.0
5.	3.2	TTAGGG	Linear	69, 59	ND	ND
6.	1.1	TTGGGG	Linear	≤10†	0/22	NA
7.	2.0	TTAGGC	Linear	≤10†	0/20	NA
8.	1.6	-TAGGG	Linear	≤10†	0/19	NA
9.	1.2	TTTAGGG	Linear	≤10†	1/31	7.5‡
10.	1.0	TTAGGGGG	Linear	≤10†	0/33	NA
11.	1.0	$(T_2AG_3)_{28}N_{14-22}$	Linear	55, 56	6/9	2.5 ± 1.2
12.	2.4	$(T_2AG_5)_2T_2AG_3$	Linear	37, ≤10†	3/28	2.6 ± 0.8

See *Materials and Methods* and Fig. 1*a* for constructs. The fraction of telomeric *neo* genes (%) in pooled colonies was deduced from BAL-31 sensitivity. Values in lines 1 and 2 represent means ± SD (%) of three and seven measurements, respectively. Lines 3, 5, 11, and 12 represent two measurements; line 4 represents a single experiment. Fraction of clonal cell lines with heterogeneous *Hin*dIII neo fragments (see Fig. 1*b*) is indicated. Most cell lines (72%) contained one copy of neo; for multiple copies of neo, each was scored independently. Telomeres (median length) are assumed to be 1.2 kb shorter than the *Hin*dIII neo fragments (see Fig. 1). Shown are averages ± SD. ND, not determined; NA, not applicable.
*pSXneo-1.6-T_2AG_3 linearized with *Not* I or *Pvu* I (see Fig. 1*a*).
†Telomere formation frequencies < 10% are not detected.
‡Median length of the single telomere formed with T_3AG_3 repeats.

internal integrations of the transfected DNAs. A similar high frequency of telomere formation was observed in two other human cell lines (HT1080 and a different HeLa subclone; data not shown). The results also demonstrated that T_2AG_3 repeat arrays of 0.8, 1.6, and 3.2 kb were equally effective (Table 1), indicating that the efficacy of telomere seeds in this size range is not strongly influenced by their length.

To verify that the telomere seeds used in these experiments induced the formation of new telomeres at interstitial sites (19–21), several kilobases of HeLa genomic DNA flanking two independently formed new telomeres were isolated by plasmid rescue (see *Materials and Methods*). Both integration sites appeared interstitial in the parental HeLa cells based on the presence of restriction endonuclease sites around the integration site and the BAL-31 resistance of these loci (data not shown). In addition, a 404-bp region immediately adjacent to one of the newly formed telomeres lacked similarities to T_2AG_3 repeats or telomere-associated sequences (GenBank data base, accession no. U02502). These results are consistent with the occurrence of an illegitimate recombination between the nontelomeric end of the telomere seed plasmids and an interstitial site in the HeLa genome.

Effects of Telomere Seed Configuration. We next examined the effect of different DNA configurations on telomere formation. The frequency of telomere formation was not significantly lowered when the segment 3′ of a 1.6-kb telomere seed was increased from 9 to 900 bp (1.6N and 1.6P, respectively; see Table 1). Introduction of this plasmid in circular form (1.6C in Table 1) also resulted in new telomeres in a considerable percentage of the transfected cells (40–45%). In yeast, telomeres can be formed on nonterminal telomere seeds with retention of the distal segment (23, 24). Furthermore, occasional retention of distal nontelomeric DNA has been demonstrated in newly formed HeLa telomeres (21). However, the 20 telomeres formed with 1.6C and 1.6P all lacked a *Bgl* II site positioned 24 bp beyond the telomeric repeats in the transfected construct (data not shown). One possibility is that the telomere seeds are modified during transfection, resulting in linear DNAs with T_2AG_3 at their termini. Regardless, our data indicate that the nontelomeric DNA distal to the telomere seeds does not inhibit telomere formation significantly.

Sequence Dependence of Telomere Formation. The sequence requirements for telomere formation were addressed using heterologous telomeric stretches of TAG_3, T_3AG_3, T_2AG_5, T_2G_4, and T_2AG_2C repeats (reviewed in refs. 1 and 2). For each sequence, a plasmid with a telomere seed of at least 1 kb was linearized close to the 3′ end of the G-rich strand and the ability of these constructs to form additional telomeres was measured in clonal cell lines and pooled colonies (Fig. 1*c* and Table 1). In most of the transfected cells, these heterologous telomeric repeats clearly failed to seed new telomeres, resulting in interstitial *neo* genes as evidenced by discrete neo fragments in clonal cell lines and BAL-31-resistant neo signals in pooled colonies. Telomere formation was not observed in the 20–30 clonal cell lines that were analyzed for each sequence. The only exception was a single clone (of 31 examined) in which the heterologous sequence T_3AG_3 had seeded a new telomere (Table 1). Restriction endonuclease analysis confirmed that this telomere was formed on a T_3AG_3 repeat array (data not shown). These results demonstrate remarkably stringent sequence requirements for human telomere seeds and suggest that the formation of telomeres requires a highly sequence-specific interaction.

Testing for Homologous Recombination. We considered three mechanisms that could explain the stringent sequence requirements for telomere formation. First, the requirement for T_2AG_3 repeats might be due to homologous recombination. The fact that telomere formation is accompanied by chromosome fragmentation indicates that the telomere seeds do not simply integrate into homologous DNA at endogenous telomeres. However, a one-sided homologous recombination with an endogenous telomere could be responsible for elongation of the transfected telomere seeds. Since the telomeres of the HeLa cell line used for these experiments contain 3–6.5 kb of T_2AG_3 repeats (22), homologous recombination could explain the observed addition of several kilobases to the transfected DNAs (Fig. 1*b* and Table 1). Furthermore, the presence of distal nontelomeric DNA in newly formed HeLa telomeres (21) does not rule out a role for homologous recombination in telomere healing, because the retention of

distal nonhomologous sequences is occasionally observed in homologous recombination (25).

To test for the involvement of homologous recombination in telomere healing, we created a 1.0-kb T_2AG_3 repeat array in which a 14- or 22-bp nonrepeat sequence occurs every 168 bp. Similar mismatches have been shown to inhibit homologous recombination 10- to 200-fold in mammalian cells (refs. 26 and 27; T. Jacks and R. Weinberg, personal communication). However, the infrequent interruptions of the T_2AG_3 repeat array did not inhibit telomere formation significantly and the new telomeres were elongated (Fig. 1*c* and Table 1). A more drastic departure from the T_2AG_3 repeat sequence was created by oligomerization of a synthetic $(T_2AG_5)_2T_2AG_3$ DNA, which seeded new telomeres in 3 of the 28 cell lines we examined (Table 1). Telomere formation could also be detected in pooled colonies transfected with the $(T_2AG_5)_2$-T_2AG_3 telomere seed (Fig. 1*c*).

These results suggest that homologous recombination does not contribute to a rate-limiting step in the formation of telomeres. Furthermore, it is unlikely that the addition of ≈1.5 kb to the telomere seed that contained nontelomeric interruptions (Table 1) involved homologous recombination. We do not believe that the abundance of telomeric loci in HeLa cells confounds the interpretation of these experiments because the search for homology is not rate-limiting for homologous recombination in mammalian cells (28). It cannot be excluded, however, that the telomere seeds are healed by some specialized recombination pathway active at telomeres. In yeast, telomere healing can occur by RAD52-independent recombination but, unlike telomere formation in human cells, this pathway can utilize heterologous telomeric DNA (see ref. 29 for review).

Priming of Telomerase by Heterologous Telomeric DNAs. As a second possibility, we considered that telomerase (refs. 11, 13, 14, and 30; reviewed in refs. 4 and 5) could be responsible for the sequence specificity of telomere formation. The involvement of telomerase is consistent with the occasional retention of distal segments in healed telomeres (21) because the enzyme is known to elongate G-rich primers with only 3 nt identity to T_2AG_3 repeats at their 3′ end (14). To determine whether failure to engage telomerase limits the ability of the heterologous telomeric DNA to form new human telomeres, we performed *in vitro* telomerase assays with $(T_2AG_3)_4$ and five similarly sized primers that represent the pertinent heterologous telomeric repeats (Fig. 2). As expected from previous work on the primer specificity of mammalian telomerases (13, 14, 30), each of the heterologous telomeric oligonucleotides primed telomerase elongation *in vitro*. The length of the products and the overall incorporation of labeled guanine varied with the different primers. However, the size distribution and the total yield of telomerase products did not correlate with the capacity of the telomeric sequences to seed new telomeres in HeLa cells. Although it is possible that telomerase has a different sequence specificity *in vivo*, these *in vitro* assays suggest that the sequences of the heterologous telomere seeds *per se* do not prevent telomere elongation by telomerase.

Matching Specificity of Telomere Formation and TRF. A third interaction that could explain the stringent sequence requirements for telomere formation is the binding of a telomeric protein. HeLa cells express the candidate telomeric protein TRF, which binds $(T_2AG_3)_{n\geq 6}$ in linear and circular DNAs (18). To examine the interaction of TRF with the telomeric DNAs used in this study, the factor was allowed to bind to a $(T_2AG_3)_{12}$ probe in the presence of the relevant telomere seed plasmids and the residual TRF complex was quantitated by gel-shift analysis (Fig. 3). Plasmids with T_2AG_3 repeats were the most effective competitors for TRF. Each of the heterologous telomeric repeat arrays were at least 10-fold less active in this competition titration assay

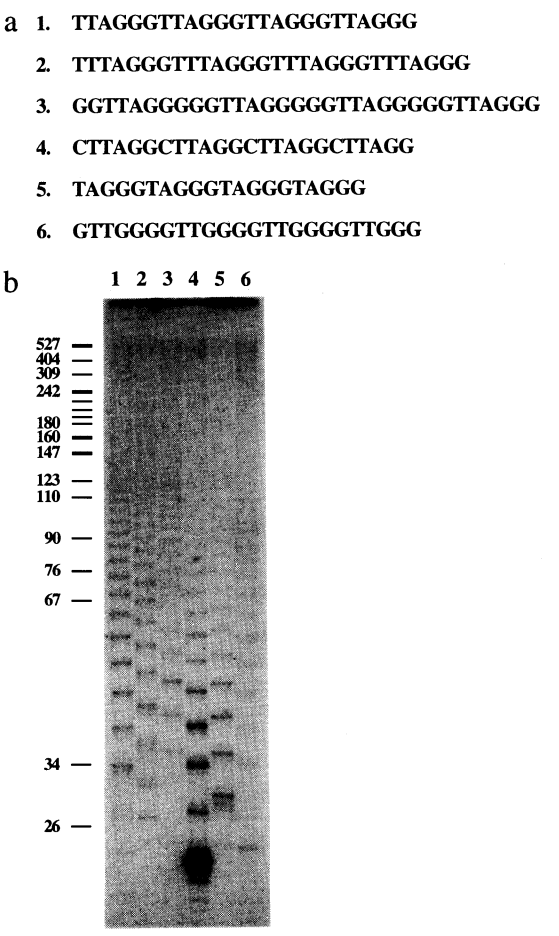

FIG. 2. Telomerase activity with human and heterologous telomeric repeat primers. (*a*) The sequence (5′ to 3′) of six single-stranded DNA oligonucleotides representing human (oligonucleotide 1) and heterologous (oligonucleotides 2–6) telomeric DNAs. (*b*) Telomerase products with the oligonucleotide primers depicted in *a*. See *Materials and Methods* for experimental details. Origin of the shortest strongly labeled product in lane 4 is unknown. Marker fragments are from *Msp* I-digested pBR322 DNA. Numbers on left are bp.

(Fig. 3; ref. 18). An intermediate effect was observed with an artificial array of $(T_2AG_5)_2T_2AG_3$ repeats. Although this mixed sequence competed for TRF, the competitor plasmid had to be present at an ≈4-fold higher concentration to achieve the same competition level as T_2AG_3 repeats (Fig. 3). These data indicate that the sequence specificity of TRF matches the requirements for telomere formation in HeLa cells. The involvement of TRF, which could bind along the length of the telomeric repeat array rather than to its terminus, is in agreement with the observation that the terminal sequence of the transfected DNA is not important (Table 1) and with the occasional retention of distal nontelomeric sequences in healed telomeres (21). Interestingly, in *S. cerevisiae*, efficient healing of short telomere seeds also requires a site for a telomeric DNA binding factor, RAP1 (31, 32). Whether TRF has any other similarities to RAP1 remains to be determined.

Mammalian cells contain nucleic acid binding activities that display some preference for single-stranded telomeric DNA *in vitro*. Several heterogeneous nuclear ribonucleoproteins bind single-stranded T_2AG_3 repeats with considerable specificity even though these proteins prefer the RNA version of this sequence (33–35). Since the exact sequence requirements of these factors is not known, we cannot rule out the involvement of heterogeneous nuclear ribonucleo-

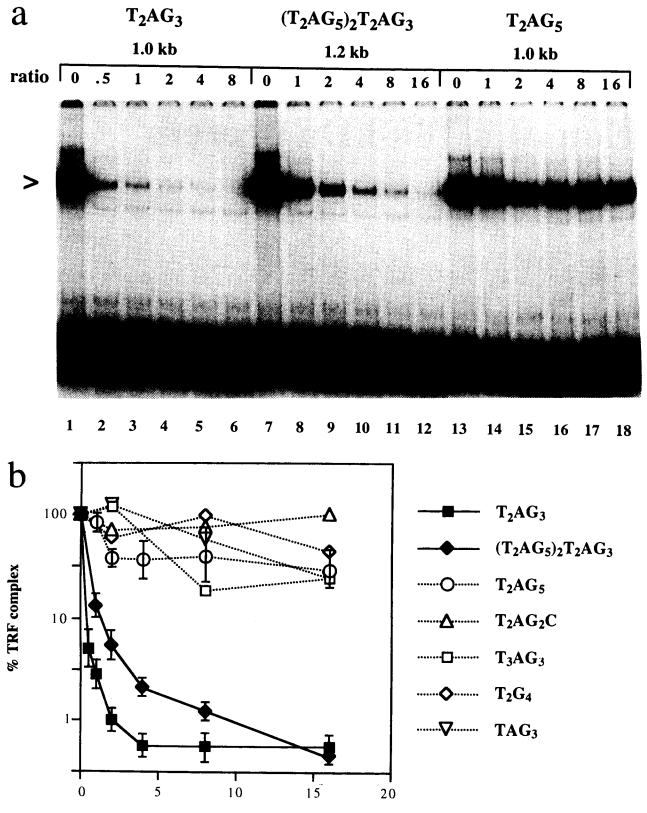

FIG. 3. TRF matches the sequence requirements for telomere formation. (*a*) Competition of telomere seeds for TRF gel shifts complex formation with double-stranded $(T_2AG_3)_{12}$. Competitors: lanes 1–6, pSXneo-1.0-$(T_2AG_3)_{28}N_{14-22}$; lanes 7–12, pSXneo-1.2-$(T_2AG_5)_2T_2AG_3$; lanes 13–18, pSXneo-1.0-T_2AG_5 repeats. Molar excess of the competitor DNAs is indicated above each lane. Arrowhead indicates TRF complex. (*b*) Relative competition of telomeric DNAs for TRF. Competition efficiency of the heterologous telomeric DNAs (open symbols) is similar to the vector (18). pSXneo-1.0-$(T_2AG_3)_{28}N_{14-22}$ competes similarly to pSXneo-0.8-T_2AG_3. Quantitative data were obtained by analysis of gel-shift experiments (see *a*) with a PhosphorImager.

proteins in the formation of human telomeres. Avian cells express a single-stranded DNA binding protein that forms a complex with single-stranded T_2AG_3 repeats (36). However, it is not known whether HeLa cells contain a related activity and the sequence preference of the avian factor does not match the sequence requirements for telomere formation in human cells (36).

Implications for Mammalian Artificial Chromosomes (MACs). The stringent sequence requirements for human telomere seeds predict that heterologous telomeric sequences cannot stabilize the ends of MACs. Therefore, the development of MACs will require vectors that carry T_2AG_3 repeat arrays. Specifically, the use of yeast artificial chromosome precursors for MACs is predicted to require T_2AG_3 repeats at chromosome ends because the yeast telomeric $(TG_{1-3})_n$ tract is unlikely to seed mammalian telomeres. However, it may not be necessary to engineer the T_2AG_3 repeats at a terminal position in MAC precursors, since 1.6 kb of T_2AG_3 repeats seeded telomeres efficiently when positioned 900 bp from the end of the transfected DNA.

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