Functional Characterization of Yeast Telomerase RNA Dimerization*

Clay L. Gipson, Zhong-Tao Xin, Shamika C. Danzy, Tristram G. Parslow, and Hinh Ly

From the Department of Pathology and Laboratory Medicine, Experimental Pathology Division, Emory University School of Medicine, Atlanta, Georgia 30322

Telomerase is the cellular RNA-dependent DNA polymerase (i.e. reverse transcriptase) that uses an integral RNA template to synthesize telomeric DNA repeats at the ends of linear chromosomes. Human telomerase RNA (hTERT) is thought to function as a dimeric complex consisting of two RNAs that interact with each other physically as well as genetically. We show here for the first time that the yeast Saccharomyces cerevisiae telomerase RNA TLC1 likewise forms dimers in vitro. TLC1 dimerization depends on a unique 6-base self-complementary sequence, which closely mimics palindromic sequences that mediate functional dimerization of HIV-1 and other retroviral genomes. We found that dissimilar but comparably located TLC1 palindromes from other S. cerevisiae yeasts can functionally substitute for that of S. cerevisiae. Yeast cells expressing dimerization-defective TLC1 alleles have shorter telomeres than those with wild-type TLC1. This study, therefore, highlights dimerization as a functionally conserved feature of the RNA templates utilized by reverse transcriptases of both viral and cellular origins.

Telomerase is the cellular reverse transcriptase (RT)2 that is responsible for adding telomeric DNA repeats onto the ends of linear chromosomes (1). Found in all eukaryotes, it is a multi-molecular complex of which the minimal components are a catalytic RT protein (TERT) and an integral RNA template. TERT proteins from diverse mammalian, protozoan, and yeast species appear to be related to one another evolutionarily as well as to RTs from retroviruses and cellular retroelements (2–4). Telomerase RNAs, by contrast, differ widely in length as a dimeric complex consisting of two RNAs that interact with each other and to retroviral genomes, if any, are unclear. Certain properties that are shared among groups of retroviral or telomerase RNAs may indicate either a common evolutionary origin or the convergent evolution of features involved in RT function. Widely divergent telomerase RNAs, for example, share certain core secondary structures that are critical for telomerase activity but have no obvious counterparts in retroviral RNAs (5, 6). The single-stranded RNA genomes of retroviruses, on the other hand, share a tendency to form stable RNA homodimers that are found in all known retroviral particles and appear to be the natural substrates for viral RTs (reviewed in Ref. 7). The assembly of these viral genomic dimers is generally initiated at a specific palindrome (i.e. a self-complementary base sequence), unique to each virus, that dimerizes readily both in vitro and within cells, and mutations in these palindromes that disrupt genomic dimer formation can markedly impair viral replication and infectivity (7). RNAs derived from at least some cellular retroelements undergo similar dimerization (4). Unexpectedly, studies from our laboratory and others have recently shown that the human telomerase RNA also dimerizes readily and that this capacity to dimerize correlates with telomerase catalytic activity in cells or cell-free extracts (8, 9). This raises the possibility that RNA dimerization plays a fundamental role in telomerase biology.

In this study, we show that the telomerase RNA of Saccharomyces cerevisiae also forms dimers in vitro, although it otherwise bears little resemblance to its human counterpart. Yeast cells harboring a dimerization-defective allele of TLC1 exhibit a progressive telomere shortening effect over successive generations. Collectively, these results provide evidence that RNA template dimerization is a widely conserved property of reverse transcription in both cellular and retroviral contexts.

EXPERIMENTAL PROCEDURES

Yeast Strain—Yeast strain yEHB1002 (MATα ade2::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 tlc1:: TRP1pR5316TLC1) was constructed by disruption of the TLC1 gene with TRP1 (10) (a generous gift of Dr. Elizabeth Blackburn at University of California, San Francisco). The strain carries a CEN/ARS, URA3 plasmid containing the wild-type TLC1 with its endogenous promoter and terminator sequence.

In Vitro TLC1 RNA Dimerization Assay—RNAs were transcribed directly from PCR products containing either the full-length wild-type TLC1 sequence or various mutated or truncated versions of this gene using the MEGAscript kit as suggested by the manufacturer (Ambion, Austin, TX). TLC1 RNA was first denatured in a total of 8 μl of diethylpyrocarbonate water at 95 °C for 3 min and then immediately snap-cooled on ice for an additional 3 min. Two μl of 5 × dimerization buffer (250 mM NaCl, 123 mM Tris (pH 7.0), 50 mM MgCl2) was added to each sample. The samples were either incubated on ice (0 °C) or at 37 °C for 120 min and then separated by nondenaturing gel

* This study was supported by funds from the Leukemia & Lymphoma Society of America and the American Cancer Society (to H. L.) and by National Institutes of Health Grant AI-40316 (to T. G. P.).

1 To whom correspondence should be addressed: Dept. of Pathology & Laboratory Medicine, Emory University School of Medicine, 615 Michael St., Whitehead Bldg., Rm. 105L, Atlanta, GA 30322. Tel.: 404-712-2841; Fax: 404-727-8538; E-mail: hly@emory.edu.

2 The abbreviations used are: RT, reverse transcriptase; nt, nucleotide(s); EMSA, electrophoretic mobility shift assay; HIV, human immunodeficiency virus.
Yeast Telomerase RNA Dimerization

**A.**

| FL (1300 nts) | 01 | 453 | 884 | 1301 |
|---------------|----|-----|-----|------|
| 5' (450 nts)  |    |     |     |      |
| ED (430 nts)  |    |     |     |      |
| 3' (416 nts)  |    |     |     |      |

**B.**

**C.**

| WT (FL) 5' | 4400 | 2370 | 1350 | L1 |
|------------|------|------|------|----|
| WT (3')    |      |      |      |    |
| 42G (FL) 5'| 4400 | 2370 | 1350 | L2 |
| 42C (3')   |      |      |      |    |

**D.**

*Figure 1.* Sequences located within the 3' region of TLC1 are necessary and sufficient to mediate RNA dimerize in vitro. **A**, schematic representation of the full-length TLC1 RNA (FL; 1300 nts), 5' RNA fragment (450 nts), essential domain (ED) fragment (430 nts), and 3' RNA fragment (416 nts). **B**, in vitro dimersizations of the full-length and truncated TLC1 RNAs. The percentage of RNA dimerization (% dimers) is a ratio of the amount of dimeric RNA to the sum of both monomeric and dimeric RNA species in each lane. Lanes 1 and 4, 0.24–9.5-kb RNA size ladder (Invitrogen) (D, dimers; M, monomers). **C**, EMSA showing efficient formations of the homodimers (single asterisks) and heterodimers (double asterisks) between RNA fragments of the wild-type (WT) sequence (lanes 1–6 and 18–19) or between RNA fragments containing either the 42C or the 42G mutation (lanes 7–16 and 20 and 21). Although only a small fraction of the heterodimers can be observed between the 42C- and 42G-containing RNA sequences at 2 h of incubation (lane 15), heterodimers form more readily after 4 h (lane 16) (double asterisk). The percentage of RNA dimerization is a ratio of the amount of dimeric RNA (either homodimer or heterodimer) to the sum of both monomeric and dimeric RNA species in each lane. L1, 0.16–1.77-kb RNA ladder (Invitrogen); L2, 0.24–9.5-kb RNA ladder (Invitrogen).

Electrophoresis in 90 mM Tris borate, 0.1 mM MgCl₂. The percentage of RNA dimerization (% dimers) was calculated by dividing the intensity of the dimeric RNA band, as judged by NIH Image software, to the sum of both monomeric and dimeric RNA species in each lane.

Southern Blot Analysis of Telomeres—Various TLC1 mutants created on the pRS13TLC1 plasmid were transformed into yeast strain yEBH1002 using the method described previously (5). In some instances, two separate pRS313TLC1 plasmids carrying either the 42C or 42G mutant were co-transformed into yEBH1002 cells. Transformants were selected on agar plates that contained −Ura−His medium to retain both wild-type and mutant plasmids. Individual colonies were then streaked on plates that contain 5-flouroorotic acid-His to select against the wild-type copy of TLC1. Colonies were subsequently streaked on −His plate. Genomic DNA was isolated from cells at each streak, restriction digested with XhoI, separated on 0.8% agarose/Tris borate-EDTA, and transferred onto Nytran membrane (Schleicher & Schuell BioScience). The membrane was hybridized with a γ-32P-end-labeled probe, (TGTGGG)₄, and visualized using a phosphorimaging technique (GE Healthcare).

Northern Blot Analysis of TLC1—Total cellular RNAs (15 μg) isolated from yeast cells (yeast RNA purification system, Puregene, Minneapolis, MN) that carried either the wild-type copy or the different mutant versions of TLC1 and 1 ng of the in vitro transcribed 1,300-nt TLC1 RNA were treated with glyoxal prior to loading into a 1% agarose gel. After separation, the RNAs were transferred onto the Hybond membrane (Schleicher & Schuell Bioscience), UV-cross-linked, and hybridized to a TLC1 probe that was labeled by random priming (Amersham Biosciences) with [γ-32P]dCTP. RNA markers (0.16–1.77 and 0.24–9.5 kb) purchased from Invitrogen were also similarly treated but were visualized by a methylene blue staining method (11). The radiolabeled TLC1 RNAs on the membrane were exposed onto a phosphorimaging screen and analyzed. The membrane was stripped and rehybridized to a randomly primed probe corresponding to the yeast Tubulin-1 gene (a kind gift from Dr. Anita Corbett, Emory University) and visualized via a phosphorimaging technique (GE Healthcare).

TLC1 Mutagenesis and Sequencing—Palindromic sequences located within the 3' region of TLC1 were mutated by PCR screen and analyzed. The membrane was stripped and rehybridized to a randomly primed probe corresponding to the yeast Tubulin-1 gene (a kind gift from Dr. Anita Corbett, Emory University) and visualized via a phosphorimaging technique (GE Healthcare).
mutagenesis (QuikChange mutagenesis kit, Stratagene, La Jolla, CA) using pRS313 as the DNA template and primer pairs that contained the desired nucleotide substitutions or deletions. Similar strategy was used to replace the palindromic sequence of the S. cerevisiae with those of the other yeast strains shown in Fig. 4 (boxed sequences). Potential mutants, selected and sequenced in both directions to verify the desired changes, were used as templates for RNA transcription and for transformation into yeast cells. Plasmids containing various TLC1 sequences were isolated from yeast cells using the plasmid DNA purification system (Puregene, Minneapolis, MN) and sequenced. In the instance in which two plasmids expressing the dimerization complementation mutations were extracted from a single yeast cell, the mixture of the plasmids was extracted and transformed (by electroporation) into Escherichia coli. Plasmid DNAs were prepared using the Qiagen Miniprep kit (Qiagen) and submitted for sequencing to verify the identity of each of the dimerization mutant and that no inadvertent recombination had occurred between the two constructs.

RESULTS
Numerous reports demonstrate that synthetic transcripts representing specific portions of human telomerase RNA or of retroviral genomes dimerize spontaneously in vitro under near physiologic conditions (8, 9, 12–16). In this study, we evaluated a synthetic 1300-nt S. cerevisiae TLC1 RNA under these standard conditions using an electrophoretic mobility shift assay (EMSA) and found that it readily formed dimer-sized complexes when warmed to 37 °C (Fig. 1B, lanes 2 and 3). Systematic truncation further revealed that a 416-nt TLC1 fragment comprising nts 885–1301 alone formed dimers at even higher efficiency than the full-length molecule (20–60% versus 15–20%), whereas isolated sequences from farther upstream did not form dimers efficiently (compare Fig. 1B, lanes 7 and 8 with lanes 5, 6, 9, and 10). When this 416-nt 3′ fragment and full-length TLC1 were combined in equal amounts (Fig. 1C, lanes 1–6, 18, and 19), we observed an RNA species migrating at the position predicted for a heterodimer of these two RNAs (double asterisks). Indeed, such heterodimeric RNA species appeared reproducibly to form more readily than would be expected for homodimers of the individual RNA species (Fig. 1C, single asterisks), perhaps because of reduced conformational variability or reduced steric effects on the truncated RNA. These findings confirm that the slow migrating complexes observed on EMSA are indeed dimers, and they suggest that specific sequences within the 3′ region of TLC1 are necessary and sufficient for dimer formation in vitro.

The signals that mediate dimerization of retroviral genomic RNAs typically include a sequence palindrome, 6 or more bases in length, that is critical for the initial interaction between RNA strands. We identified five such palindromes in the 3′ TLC1 region and created a series of mutants of this truncated RNA each of which introduced two or three nucleotide substitutions into a given palindrome (Fig. 2A). As shown in Fig. 2B, most of these mutations had no discernible effect on in vitro dimer formation as compared with the wild-type RNA species, with the exception of mutants 42C and 42G, which replaced the native palindrome CGCGCG at positions 1204–1209 with the alternative, non-palindromic sequences CCCCCG and CGGGGG, respectively (Fig. 1C, base substitutions are underlined). Each of the two latter mutations reduced dimerization to nearly undetectable levels (Figs. 1C, lanes 7–13, and 2B, lanes 5 and 6). Further evidence of a unique role for this palindrome was obtained by progressively truncating the 3′ end of the RNA (Fig. 2C). As shown in Fig. 2D, we found that RNAs that lacked up to 88 nt but retained this palindrome dimerized relatively efficiently (lanes 5–10), but that further deletions or base substitutions that eliminated the palindrome abolished dimer formation (lanes 1–4, 11, and 12).

The significance of palindromes in dimer initiation reflects their ability to mediate homologous base-pairing interactions between RNA strands. The 42C and 42G mutations replace the native palindrome at positions 1204–1209 with sequences that
Yeast Telomerase RNA Dimerization

A.

B.

C.

FIGURE 3. Yeast cells expressing different versions of the TLC1 dimerization-defective RNA grow more slowly and possess shorter telomeres than those that express the dimerization competent TLC1 molecules. Yeast cells harboring either the wild-type TLC1 RNA, dimerization-defective mutants 42G and 42C (either alone or together) or a dimerization-competent palindromic mutant (Mut 43), those that carry either the wild-type TLC1 or Mut 43, that carry only the wild-type TLC1 allele or the dimerization-defective control mutant 43 grew at roughly comparable rates. In contrast, cells harboring either of the dimerization-defective mutants (42C or 42G) alone grew somewhat more slowly and were less efficiently propagated. It is important to note, however, that neither mutant entered replicative senescence, even by the fifth passage, highlighting the plasticity of yeast cells in cultured conditions. Compound heterozygous cells that contained both the 42C and 42G alleles appeared to grow better than those with either mutant alone (Fig. 3A). Similar results were obtained when similar strains of yeast cells were grown in liquid cultures prior to being serially diluted and spotted onto plates for growth (Fig. 3A, lanes 1–4). These results suggest that the 42C and 42G mutants can at least partially complement each other functionally in trans, presumably because of their ability to form RNA heterodimers.

Further evidence of a possible functional role for TLC1 dimerization was obtained by examining telomere lengths of these same yeast strains by Southern blot analysis (Fig. 3B). Over successive passages, telomere length decreased progressively in vitro.

are nonpalindromic but are complementary to each other and so might be predicted to support heterodimer formation. We tested this possibility by combining a full-length TCL1 that contained the 42G mutation with an equimolar amount of a 416-nt fragment containing 42C. As shown in Fig. 1C, neither of these mutant RNAs formed appreciable homodimers when incubated alone for either 2 h (lanes 7, 8, 10–13, 20, and 21) or up to 4 h (data not shown), but small amounts of an apparent heterodimer reproducibly formed when the two RNA species were mixed (lanes 15 and 16). Formation of this mutant heterodimer, however, was slow and inefficient in comparison with homodimerization of wild-type TCL1 (Fig. 1C, compare lane 6 with lanes 15 and 16); this is consistent with evidence from retroviral systems that only a small subset of complementary sequences can support dimerization in vitro or in vivo (13, 17, 18). Taken together, these results indicate that the palindrome at positions 1204–1209 is required for efficient TCL1 dimer formation in vitro by virtue of its ability to form base pairs between strands.

We next asked whether TLC1 dimerization plays a role in the life cycle of yeast cells. To do this, we created artificial expression cassettes encoding wild-type or mutant forms of the full-length TLC1 sequence and introduced these in place of the endogenous TLC1 gene in S. cerevisiae (as outlined under “Experimental Procedures”). Individual transformed yeast clones with the desired genotypes, verified by DNA sequencing, were selected and characterized further by continuous cultivation (i.e. by streaking the same population of cells on agar plates for five successive passages). As shown in Fig. 3A, yeast strains that carried only the wild-type TLC1 allele or the dimerization-competent control mutant 43 grew at roughly comparable rates. In contrast, cells harboring either of the dimerization-defective mutants (42C or 42G) alone grew somewhat more slowly and were less efficiently propagated. It is important to note, however, that neither mutant entered replicative senescence, even by the fifth passage, highlighting the plasticity of yeast cells in cultured conditions. Compound heterozygous cells that contained both the 42C and 42G alleles appeared to grow better than those with either mutant alone (Fig. 3A). Similar results were obtained when similar strains of yeast cells were grown in liquid cultures prior to being serially diluted and spotted onto plates for growth (Fig. 3A, lanes 1–4). These results suggest that the 42C and 42G mutants can at least partially complement each other functionally in trans, presumably because of their ability to form RNA heterodimers.
wild type and each of the dimerization-defective mutants, 42C and 42G, generated stable RNA products that had lengths comparable with the in vitro transcribed 1300-nt TLC1 RNA (lanes 4 and 5).

Finally, we searched for functional counterparts of the dimerization-directed palindrome in TLC1 sequences from Saccharomyces cariocanus and Saccharomyces kudriavzevii, two other yeast species that belong to the senso stricto group and in which TLC1 RNAs exhibit 77 and 65% sequence identity overall, respectively, to that of S. cerevisiae (23). When these sequences are optimally aligned, the regions in question are markedly divergent among the three species but are either palindromic (in the case of S. kudriavzevii) or imperfectly palindromic (in the case of the S. cariocanus) to a degree that would still allow base pairing to occur (Fig. 4A, boxed). As full-length clones of the S. cariocanus and S. kudriavzevii TLC1 genes were not available to us, we were unable to evaluate the dimerization properties of the native RNAs; instead, we asked whether the palindromic sequences from those genes could functionally replace residues 1204–1209 in the context of S. cerevisiae TLC1. Fig. 4B demonstrates that dimerization of the S. cerevisiae RNA was completely abolished when the native palindrome was deleted but could be restored to essentially full efficiency by inserting either the S. cariocanus or the S. kudriavzevii palindrome in its place. Thus, despite wide sequence divergence over the evolutionary history of these species, local palindromic character has been conserved that is capable of supporting RNA dimerization in vitro and may be required for optimal telomerase function. It is noteworthy that the palindromes of S. cariocanus and the S. kudriavzevii species are longer than that of S. cerevisiae, possibly reflecting the fact that the former palindromes are AU-rich.

**DISCUSSION**

RNA dimerization has been shown to be an important process required for the normal functions of a number of viral, bacterial, and eukaryotic RNAs (7–10, 26–28). We and others have previously demonstrated that human telomerase RNA dimerizes readily under conditions that are routinely used to study retroviral genomic RNA dimerization in vitro (8, 9, 12, 29, 30). This is consistent with reports that the minimal functional telomerase complexes isolated from human or yeast cells each may contain two copies of the template RNA (9, 10, 28). The ability of telomerase catalytic proteins to dimerize independently of specific RNA has also been demonstrated (31–34). Although telomerases from the ciliate protozoans Tetrahymena thermophila and Euplotes aediculatus have been purified as monomeric complexes (35, 36), active telomerases isolated from another ciliate, Euplotes crassus, from yeast, and from human cells are all multimolecular complexes (31, 33, 34, 37–40).

We now find that the TLC1 RNA from S. cerevisiae likewise dimerizes readily in vitro and that dimerization of this yeast RNA depends on a specific 6-base palindrome located near the 3’ end of the molecule. The observation that, despite their dissimilar sequences, human and yeast telomerase RNAs each form homodimers suggests an evolutionarily conserved role for dimerization in telomerase function, perhaps related to the

---

**Yeast Telomerase RNA Dimerization**

![Image](https://via.placeholder.com/150)

**FIGURE 4.** Palindromic sequences of other senso stricto yeast species located near positions 1204–1209 can substitute functionally for that of the S. cerevisiae in RNA dimerization. A, partial sequence alignment of the TLC1 sequences of the S. cerevisiae (S. cere), S. cariocanus (S. cario), and S. kudriavzevii (S. kudr) species. The boxed nucleotides indicate the potential dimer-associated palindromic sequences. Shaded nucleotides indicate perfect conservations between the sequences. The available lengths of the TLC1 RNAs of the S. cerevisiae (1300 bases), S. cariocanus (2165 bases), and S. kudriavzevii (2228 bases) are also indicated based on the available data in GenBank. B, EMSA shows defective dimerization of the TLC1 molecule that lacks the palindrome (lanes 3 and 4) as compared with efficient levels of dimer formation of RNAs with either the native the S. cerevisiae palindrome (lanes 1 and 2) or those of other yeast species (lanes 5–8). D, dimer, M, monomer.
Yeast Telomerase RNA Dimerization

reportedly dimeric structure of the telomerase holoenzyme complexes found in both organisms or to the abilities of certain mutant telomerase RNAs from each organism to either activate or inhibit functions of the wild-type RNA in trans (9, 10, 28).

The mechanisms of dimerization in the human and yeast RNAs, moreover, remain to be fully elucidated and may not be fully equivalent. For example, whereas we and other have mapped the cis-acting determinant of hTERC dimerization to the potential pseudoknot structure near the center of that RNA (9, 12, 30, 41, 42), other reports have mapped the signal to a palindromic loop sequence near the 3′ end, (43, 44), a location more closely analogous to those in TLC1. Of note, Zappulla et al. (25) recently reconstituted yeast telomerase enzyme activity in vivo using a highly truncated form of TLC1 that lacks the dimer-initiating palindrome we have identified here; the resulting cells were viable but produced short, stable telomeres and showed reduced fitness, phenotypes that closely mimic those we observed with our own dimerization-defective mutants.

It is also striking to note the close parallels between the dimerization pathways of retroviral and telomerase RT enzymes, particularly in their common use of palindromic RNA sequences to initiate template dimer formation. Among retroviruses, the active palindromes vary widely according to types or subtypes of viruses, but all are believed to function in a similar fashion to initiate interstrand quaternary interactions via Watson-Crick base pairing. Remarkably, the active palindrome we mapped in S. cerevisiae TLC1 (CGCGGC) mirrors that conserved stem-loop structure in the HIV-1 genome, which has no obvious counterpart in TLC1. When the dimer-initiating palindromic sequence in the Rous sarcoma retrovirus genome was intentionally randomized via mutagenesis, serial passaging of the resulting pool of mutant viruses in cell culture led to the emergence of highly “fit” progeny that had reverted to either the wild-type sequence or closely related palindromes (45). A recent study has further illustrated the importance of viral RNA dimer formation by showing that the observed low frequency of genetic recombination between HIV-1 subtype B viruses, which circulate predominantly in Western countries, and the subtype C strains, which are widespread in Africa and Asia, may be attributable to lack of full complementarity between their dimer-initiating palindromes (46). These studies emphasize the fundamental role of such palindromes in mediating genomic RNA dimerization and promoting subsequent events in the retroviral life cycle (46).

The role for template dimerization in telomerase function remains less clear, however. It has been hypothesized that dimeric telomerase promotes its enzymatic processivity by mediating its switching at the growing 3′ telomeric ends between the two RNA templates following each round of template copying (9). Evidence to support this template-switching model comes from findings of interdigitated telomeric repeats of the wild-type and mutated sequences generated in cells of either yeast or Tetrahymena co-expressing both the wild-type and mutated RNA template domains (10, 28, 47). Although biochemical purification of telomerase in human cells carrying a wild-type and a mutated telomerase RNA (hTERC) template has demonstrated template interdependency (8), recent evidence has suggested that processive synthesis by an obligatory dimeric human telomerase does not necessarily require template switching (48). Although we have shown that TLC1 dimerization-defective mutants lead to a telomere shortening effect in cells, it is still unclear whether this is a direct result of failure of the enzyme to synthesize telomeres. It is conceivable that these dimerization mutants may disrupt other processes of the telomere maintenance pathway that can lead to shortened telomeres without directly affecting telomerase enzymatic activity. We are currently in the process of investigating these issues in details.

In summary, we have shown here for the first time that the telomerase TLC1 RNA from the yeast S. cerevisiae spontaneously forms dimers in vitro and that dimerization likely contributes to optimal telomere maintenance in cells. Our findings, moreover, highlight palindromic-mediated template dimerization as a convergent trait shared by cellular and viral reverse transcriptases of widely disparate phylogenetic origins.

Acknowledgments—We thank Arif Hussain for excellent technical assistance and colleagues in Dr. Elizabeth Blackburn’s group at University of California, San Francisco (Dr. Jue Lin and Tetsuya “Tet” Matsaguchi) for providing helpful suggestions, yeast strains, plasmids, and protocols.

REFERENCES

1. Blackburn, E. H. (2001) Cell 106, 661–673
2. Lingner, J., Hughes, T. R., Shevchenko, A., Mann, M., Lundblad, V., and Cech, T. R. (1997) Science 276, 561–567
3. Peng, Y., Mian, I. S., and Lue, N. F. (2004) Mol. Cell 7, 1201–1211
4. Feng, X. X., Moore, S. P., Garfinkel, D. J., and Resin, A. (2000) J. Biol. 74, 10819–10821
5. Lin, I., Ly, H., Hussain, A., Abraham, M., Pearl, S., Tzfati, Y., Parslow, T. G., and Blackburn, E. H. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 14713–14718
6. Chen, J. L., and Greider, C. W. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 14683–14684
7. Paillart, J. C., Shehu-Xhilaga, M., Marquet, R., and Mak, J. (2004) Nat Rev Microbiol. 2, 461–472
8. Wenz, C., Enenkel, B., Amacker, M., Kellerer, C., Damnn, K., and Lingner, J. (2001) EMBO J. 20, 3526–3534
9. Ly, H., Xu, L., Rivera, M. A., Parslow, T. G., and Blackburn, E. H. (2003) Genes Dev. 17, 1078–1083
10. Prescott, I., and Blackburn, E. H. (1997) Genes Dev. 11, 528–540
11. Sambrook, J., and Russell, D. W. (2001) Molecular Cloning: A Laboratory Manual, 3rd Ed., Vol. I, pp. 7.36–7.39, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
12. Moriarty, T. J., Marie-Egyptienne, D. T., and Autexier, C. (2004) Mol. Cell. Biol. 24, 3720–3733
13. Clever, J. L., Wong, M. L., and Parslow, T. G. (1996) J. Biol. 70, 5902–5908
14. Laugrea, M., and Jette, L. (1997) Biochemistry 36, 13464–13474
15. Marquet, R., Paillart, J. C., Skripkin, E., Ehresmann, C., and Ehresmann, B. (1994) Nucleic Acids Res. 22, 145–151
16. Paillart, J. C., Marquet, R., Skripkin, E., Ehresmann, C., and Ehresmann, B. (1996) Biochimie (Paris) 78, 639–653
17. Laugrea, M., Jette, L., Mak, J., Kleinman, L., Liang, C., and Wainberg, M. A. (1997) J. Biol. 71, 3397–3406
18. Laugrea, M., and Jette, L. (1997) Biochemistry 36, 9501–9508
19. Changon, C., Cech, T. R., and Zaug, A. J. (1997) RNA 3, 1337–1351
20. Chappell, A. S., and Lundblad, V. (2004) Mol. Cell. Biol. 24, 7720–7736
21. Seto, A. G., Zaug, A. J., Sobel, S. G., Wolin, S. L., and Cech, T. R. (1999) Nature 401, 177–180

18862 JOURNAL OF BIOLOGICAL CHEMISTRY
22. Singer, M. S., and Gottschling, D. E. (1994) Science 266, 404–409
23. Dandjinou, A. T., Levesque, N., Larose, S., Lucier, J. F., Abou Elela, S., and Wellingler, R. J. (2004) Curr. Biol. 14, 1148–1158
24. Bossoy, D., Peng, Y., Mian, I. S., and Lue, N. F. (2003) J. Biol. Chem. 278, 3882–3890
25. Zappulla, D. C., Goodrich, K., and Cech, T. R. (2005) Nat. Struct. Mol. Biol. 12, 1072–1077
26. Wagner, C., Ehresmann, C., Ehresmann, B., and Brunel, C. (2004) J. Biol. Chem. 279, 4560–4569
27. Ferrandon, D., Koch, I., Westhof, E., and Nusslein-Volhard, C. (1997) EMBO J. 16, 1751–1758
28. Presco, J., and Blackburn, E. H. (1997) Genes Dev. 11, 2790–2800
29. Theimer, C. A., Blois, C. A., and Feigon, J. (2005) Mol. Cell 17, 671–682
30. Keppler, B. R., and Jarstfer, M. B. (2004) Biochemistry 43, 334–343
31. Beattie, T. L., Zhou, W., Robinson, M. O., and Harrington, L. (2001) Mol. Cell. Biol. 21, 6151–6160
32. Moriarty, T. J., Huard, S., Dupuis, S., and Autexier, C. (2002) Mol. Cell. Biol. 22, 1253–1265
33. Wang, L., Dean, S. R., and Shippen, D. E. (2002) Nucleic Acids Res. 30, 4032–4039
34. Arai, K., Masutomi, K., Khurts, S., Kaneko, S., Kobayashi, K., and Murakami, S. (2002) J. Biol. Chem. 277, 8538–8544
35. Bryan, T. M., Goodrich, K. J., and Cech, T. R. (2003) Mol. Biol. Cell 14, 4794–4804
36. Lingner, J., and Cech, T. R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10712–10717
37. Bachand, F., Triki, I., and Autexier, C. (2001) Nucleic Acids Res. 29, 3385–3393
38. Nakamura, T. M., Morin, G. B., Chapman, K. B., Weinrich, S. L., Andrews, W. H., Lingner, J., Harley, C. B., and Cech, T. R. (1997) Science 277, 955–959
39. Schnapp, G., Rodi, H. P., Rettig, W. J., Schnapp, A., and Damm, K. (1998) Nucleic Acids Res. 26, 3311–3313
40. Cohen, S. B., Graham, M. E., Lovrecz, G. O., Bache, N., Robinson, P. J., and Reddel, R. R. (2007) Science 315, 1850–1853
41. Comolli, L. R., Smirnov, I., Xu, L., Blackburn, E. H., and James, T. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 16998–17003
42. Chen, J. L., Blasco, M. A., and Greider, C. W. (2000) Cell 100, 503–514
43. Ren, X., Gavory, G., Li, H., Ying, L., Klenerman, D., and Balasubramanian, S. (2003) Nucleic Acids Res. 31, 6509–6515
44. Marie-Egyptienne, D. T., Cerone, M. A., Londono-Vallejo, J. A., and Autexier, C. (2005) Nucleic Acids Res. 33, 5446–5457
45. Doria-Rose, N. A., and Vogt, V. M. (1998) J. Virol. 72, 8073–8082
46. Chin, M. P., Rhodes, T. D., Chen, J., Fu, W., and Hu, W. S. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 9002–9007
47. Yu, G. L., Bradley, J. D., Attardi, L. D., and Blackburn, E. H. (1990) Nature 344, 126–132
48. Rivera, M. A., and Blackburn, E. H. (2004) J. Biol. Chem. 279, 53770–53781