Reiterative dG addition by *Euplotes crassus* telomerase during extension of non-telomeric DNA

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**ABSTRACT**

Telomerase from the ciliate *Euplotes crassus* incorporates G$_4$T$_4$ telomeric repeats onto both telomeric and non-telomeric single-stranded DNA 3'-ends via reverse transcription of a templating domain in its RNA subunit. Here we describe an unusual mode of template copying that is characteristic of DNA synthesis onto non-telomeric 3'-ends *in vitro*. When dTTP was eliminated from telomerase reactions, telomeric primers or DNA products generated from the telomerase endonuclease were extended by precise copying of the RNA template. In contrast, telomerase catalyzed the addition of up to 13 dG residues onto primers with non-telomeric 3'-ends under the same reaction conditions. Introducing mismatches in the 3'-terminus of telomeric primers that reduced primer complementarity to the RNA template induced reiterative dG incorporation, indicating that the reaction is influenced by Watson–Crick base pair formation between the primer and the RNA template. Unexpectedly, the reiterative dG addition mode was confined to telomerase derived from developing cells that undergo new telomere formation. This reaction was not observed in vegetatively growing cells. We postulate that indiscriminate dG addition by telomerase occurs by reiterative copying of C residues in the telomerase RNA templating domain and reflects lateral instability of the primer–template interaction during *de novo* telomere formation.

**INTRODUCTION**

Telomerase is an enzyme fundamental to the replication and maintenance of telomeres and stabilization of broken chromosome ends. A ribonucleoprotein, telomerase, synthesizes the G-rich strand of telomeric DNA by reverse transcription of a short sequence in its internal RNA (1,2). Telomerase activity has been detected and telomerase RNA components sequenced in a variety of eukaryotic organisms (3). The catalytic subunit of telomerase, containing reverse transcriptase amino acid motifs, has been identified in both budding and fission yeast, the ciliate *Euplotes aediculatus* and in humans (4–7). Proteins associated with the catalytic telomerase protein and telomerase RNA have also been found (8–10).

Ciliated protozoa are attractive model systems for studying telomeric DNA synthesis. During development of a new macronucleus in the sexual stage of the ciliate life cycle, telomerase performs *de novo* telomere addition onto thousands to millions of chromosome fragments (11). During subsequent vegetative growth, telomerase maintains telomeres on all these ends. Telomerase from *Euplotes crassus*, studied herein, contains a 192 nt RNA component with the sequence 5'-CAAACC-CCCCAACCC-3' which templates the synthesis of G$_4$T$_4$ telomeric DNA repeats (12). A second site on the enzyme, termed the anchor site, binds dG residues upstream of a primer 3'-end, allowing the enzyme to maintain contact with the DNA product during successive rounds of primer elongation (13–17).

In addition to extending primers that consist entirely of telomeric repeat sequences, *E.crassus* telomerase from developing macronuclei will add telomeric repeats directly onto non-telomeric DNA *in vitro* in a reaction that mimics developmentally programed *de novo* telomere formation *in vivo* (16). This reaction is observed with primers that bear at least three consecutive dG residues upstream of a non-telomeric 3'-end (16). Upon binding of the dG cluster to the anchor site, the non-telomeric 3'-end of the primer is positioned at a ‘default’ site on the RNA template, so that DNA synthesis always starts with copying of a specific ribonucleotide in the templating domain (16; Fig. 1B). Telomerase from vegetatively growing cells is unable to add telomeric repeats onto non-telomeric DNA 3'-ends *in vitro*, a property that correlates with the telomere maintenance function telomerase performs during this stage of the life cycle (18).

In addition to its reverse transcriptase activity, telomerase from *E.crassus* is associated with a DNA endonuclease (16,19). The cleavage reaction is best observed with oligonucleotides carrying a G$_4$T$_4$ sequence embedded in non-telomeric DNA. Upon hybridization of the G$_4$T$_4$ sequence to the RNA template, telomerase endonucleolytically removes the 3'-terminal non-telomeric DNA from the oligonucleotide, exposing telomeric DNA sequence for elongation by addition of telomeric repeats (16). Thus, *de novo* telomeric DNA synthesis and cleavage-mediated extension are two distinct mechanisms by which non-telomeric DNA 3'-ends can be processed by *E.crassus* telomerase (16).

Here, we describe an unusual mode of telomeric DNA synthesis that telomerase adopts during direct extension of non-telomeric DNA 3'-ends *in vitro*. When dGTP is the only nucleotide substrate present in the reaction, telomerase from...
Figure 1. A model for interaction of *E. crassus* telomerase with telomeric primers and primers that contain a non-telomeric 3′-terminus. (A) A telomeric primer interacts with telomerase at both the anchor and RNA template sites. Potential Watson–Crick base pairs between the primer and the RNA template are indicated. The 3′-end of the primer shown here aligns with ribonucleotides 45–50 in the telomerase RNA. Extension of this primer initiates by copying ribonucleotides 41–44. (B) The G₄T₄-13 primer contains a non-telomeric 3′-terminus which is unable to hybridize on the RNA template. This 3′-terminus is proposed to be positioned at the ‘default’ site (adjacent to ribonucleotide 44, underlined) in the templating region (16). The cluster of dG residues in its 5′-telomeric cassette binds in the anchor site. In the absence of dTTP, telomerase from developing cells ‘slips’ on the RNA template, adding a ladder of up to 13 dG nucleotides onto the 3′-end of the primer. This reaction is not observed with telomeric primers nor is it seen with telomerase from vegetatively growing cells. The reiterative dG addition reaction is reminiscent of template slippage described for other RNA and DNA polymerases (20–22) and may have implications for the mechanism of developmentally programed telomere formation *in vivo*.

**MATERIALS AND METHODS**

**Isolation of *E. crassus* macronuclei and purification of telomerase**

*Euplotes crassus* was cultured with the alga *Dunaliella salina* and mated as described (23). Macronuclei from developing cells (64 h after mating) and vegetatively growing cells were isolated on Percoll–sucrose gradients (18) and resuspended in TMG buffer (30 mM Tris–HCl, pH 7.5, 3 mM MgCl₂, 10% glycerol). Macronuclei were lysed in a French press and lysates were spun in a microfuge for 15 min at 4°C to remove membrane fractions. The supernatant was adjusted to 1 M potassium glutamate (KGlu) and loaded onto a phenyl-Sepharose CL-4B (Sigma) column pre-equilibrated with TMG + 1 M KGlu. The column was washed with 4 vol TMG + 1 M KGlu, 4 vol TMG and 1 vol TMG + 0.5% Triton X-100. Telomerase activity was eluted with 5 vol TMG + 2% Triton X-100. Active telomerase fractions were depleted of Triton X-100 with Extracti-Gel® D Detergent Removing Gel (Pierce) and stored at −80°C until use. Telomerase purified on phenyl-Sepharose had the same properties as telomerase from intact vegetative or developing macronuclei with respect to processivity and ability to extend non-telomeric DNA.

**Oligonucleotide preparation**

DNA oligonucleotides were obtained from Gibco BRL. Oligonucleotide sequences are listed in Table 1. All oligonucleotides were purified on 20% denaturing polyacrylamide gels. To generate molecular weight markers, some oligonucleotides were extended with terminal deoxynucleotidyl transferase in the presence of [³²P]dGTP (16). Products resulting from the addition of one dG residue were excised from a 20% denaturing gel and eluted in TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) before use.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>G₄T₄-13</td>
<td>(G₄T₄)ACTACCCGATCAC</td>
</tr>
<tr>
<td>10-G₄T₄-3</td>
<td>CACTATCGAC(G₄T₄)CAT</td>
</tr>
<tr>
<td>5-G₄T₄-8</td>
<td>CATCA(G₄T₄)GGGATCATCAT</td>
</tr>
<tr>
<td>antisense oligo</td>
<td>TCCGTTTGGGCGTTTGGTTddG</td>
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<tr>
<td>G₄T₄-13-G</td>
<td>(G₄T₄)ACTACCCGATCACG</td>
</tr>
<tr>
<td>G₄T₄-13-GG</td>
<td>(G₄T₄)ACTACCCGATACGGG</td>
</tr>
<tr>
<td>G₄T₄-13-GGG</td>
<td>(G₄T₄)ACTACCCGATACCCGG</td>
</tr>
<tr>
<td>G₄-12-GTTTT</td>
<td>GATCGACTACCCGATTTT</td>
</tr>
<tr>
<td>G₄-13-TTTT</td>
<td>GATCGACTACCCGATTTT</td>
</tr>
<tr>
<td>16-GTTTT</td>
<td>CACTATCGACTACCCGATTTT</td>
</tr>
<tr>
<td>17-TTTT</td>
<td>CACTATCGACTACCCGATTTT</td>
</tr>
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Table 1. DNA primer sequences

Primer sequences run 5′→3′, left to right.

**Telomerase assays**

Telomerase reactions contained 15 µl phenyl-Sepharose purified telomerase extract (10.5 fmol telomerase RNA/ml), 0.4 µM primer, 5 mM MgCl₂, 20 mM EGTA, 50 mM Tris–HCl, pH 8.0, 1 mM spermidine, 1 mM DTT, 0.25 µM [³²P]dGTP (800 Ci/mmol) and 0.1 mM dTTP or 0.5 mM ddTTP where indicated, in
a total volume of 30 μl. Reactions were incubated at 30°C for 1 h, stopped by addition of EDTA, extracted with phenol:chloroform:isoamyl alcohol (50:50:1) and precipitated with ethanol. DNA products were isolated and analyzed on 10% sequencing gels. Gels were exposed overnight unless otherwise stated. Where indicated, DNA products were quantified by phosphorimaging.

RESULTS

Reiterative dG addition by E.crassus telomerase during extension of non-telomeric DNA 3’-termini

To learn more about telomerase interactions with non-telomeric DNA, telomerase was partially purified from developing macro-

To test whether reiterative dG incorporation occurred following endonucleolytic primer cleavage by telomerase, reactions were conducted with 10-G₄T₄₃, an oligonucleotide that contains a telomeric cassette embedded between 10 telomeric repeats in the reactions with [³²P]dGTP and ddTTP (lanes 1, 4 and 7), [³²P]dGTP only (lanes 2, 5 and 8) or [³²P]dGTP and ddTTP (lanes 3 and 6). Primer substrates are indicated. Arrows denote the position of 22 nt DNA products corresponding to addition of a single dG residue onto the 3’-end of each primer. Products 19–22 nt in length in lanes 7 and 8 result from cleavage-initiated primer extension by telomerase. Here and in the following figures, only DNA products resulting from addition of the first few telomeric repeats in the reactions with [³²P]dGTP and ddTTP are shown.

Reiterative dG addition proceeds via reverse transcription of the telomerase RNA template

Reiterative dG addition on non-telomeric 3’-ends could result from a template-independent nucleotide addition mechanism characteristic of terminal transferases. To test this possibility, we examined the sensitivity of the reiterative dG addition reaction to pre-treatment of the extract with RNase A. The primer 5-G₄T₄₃ was used instead of eight nucleotides of non-telomeric DNA, was used in this experiment, because it can be processed by both direct extension and cleavage mechanisms (Fig. 3, lane 1). In the presence of [³²P]dGTP only, 5-G₄T₄₃ was cleaved and the eight non-telomeric 3’ residues were removed. The 13 nt DNA product was then extended by one to four dG residues (Fig. 3, lane 2). In contrast, direct extension of this primer led to formation of DNA products 22–34 nt in length, a result of reiterative dG addition. 

Figure 2. Reiterative dG addition by telomerase from developing E.crassus cells. Telomerase reactions were performed in the presence of [³²P]dGTP and dTTP (lanes 1, 4 and 7), [³²P]dGTP only (lanes 2, 5 and 8) or [³²P]dGTP and ddTTP (lanes 3 and 6). Primer substrates are indicated. Arrows denote the position of 22 nt DNA products corresponding to addition of a single dG residue onto the 3’-end of each primer. Products 19–22 nt in length in lanes 7 and 8 result from cleavage-initiated primer extension by telomerase. Here and in the following figures, only DNA products resulting from addition of the first few telomeric repeats in the reactions with [³²P]dGTP and ddTTP are shown.

Figure 3. Reiterative dG addition proceeds via reverse transcription of the telomerase RNA template

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direct extension by telomerase, but does not block cleavage (16).
Accordingly, the DNA products observed in lanes 4 and 5 in Figure 3 result from cleavage-initiated elongation of the antisense oligonucleotide. The antisense oligonucleotide, but not other oligonucleotides with random DNA sequences, efficiently competed with 5-G4T4-8 for telomerase. No direct extension of 5-G4T4-8 by reiterative dG addition or by the cleavage pathway was observed in the presence of the antisense oligonucleotide (Fig. 3, lane 4, and data not shown). Taken together, these data demonstrate that dG ladder formation occurs by copying the telomerase RNA template and is not a result of a terminal transferase-like activity.

Primer sequence requirements for reiterative dG addition by telomerase

The data presented above indicate that formation of long dG ladders occurs only during direct extension of non-telomeric primer 3'-ends. One obvious distinction of this reaction compared with reactions with standard telomeric primers or products of the telomerase cleavage reaction is the absence of Watson–Crick base pair formation between the primer 3'-terminus and the telomerase RNA template (Fig. 1A). To further investigate the influence of 3'-terminal primer sequences on reiterative dG addition, we altered the primer–template base pairing potential by changing the DNA sequence at the primer 3'-end.

We first tested whether telomerase would perform reiterative dG addition with primers ending in one to three dG residues. Such primers included G4T4-13-G, G4T4-13-GG and G4T4-13-GGG, which ranged in size from 22 to 24 nt. All these primers were elongated by telomerase with similar efficiency in the presence of [32P]dGTP and dTTP (Fig. 4A, lanes 1–3). Increasing the number of dG nucleotides at the primer 3'-end resulted in a decrease in length and abundance of the DNA products formed in the presence of [32P]dGTP only (Fig. 4A, lanes 4–7). The ratios of the amount of radioactive label incorporated into DNA products shown in lanes 5–7 in Figure 4A to the amount of radioactive label incorporated during extension of G4T4-13 (Fig. 4A, lane 4) were 0.67, 0.47 and 0.11 for the primers G4T4-13-G, G4T4-13-GG and G4T4-13-GGG, respectively, as determined by phosphorimaging. The inhibition of dG ladder formation resulted from increasing the number of 3'-terminal dG residues and not the overall increase in primer length. A series of 21 nt primers ending with one to three dG residues also exhibited a decrease in reiterative dG addition as the number of terminal dG residues was increased (data not shown).

Another approach to analyze the relationship between primer–template interactions and reiterative dG addition is to decrease the Watson–Crick base pairing potential in a telomeric primer by introducing nucleotide changes in the primer 3'-terminus. The 3'-end of the telomeric primer G(T4G4)TTA T is predicted to form up to six base pairs with ribonucleotides 45–50 in the telomerase RNA before it will be extended by copying ribonucleotides 41–44 (Fig. 1A). Therefore, we tested whether introduction of single nucleotide mismatches at the 3'-end of this primer, which should destabilize the DNA–RNA hybrid, would induce reiterative dG addition. Primers G(T4G4)TTA T and G(T4G4)ATTT were assayed for extension by telomerase in the presence of [32P]dGTP and dTTP or [32P]dGTP only (Fig. 4B). Although reiterative dG addition was not as efficient as in the case of G4T4-13, both G(T4G4)TTA T and G(T4G4)ATTT were extended by more than four dG residues in the absence of dTTP (Fig. 4B, lanes 2 and 4).

We have previously demonstrated that telomerase eliminates mismatched primer nucleotides when they are positioned opposite ribonucleotides 37–40 in the RNA template (19). Since the four 3'-terminal nucleotides of G(T4G4)TTA T and G(T4G4)ATTT have the potential to hybridize with ribonucleotides 37–40, we expected that some subset of these primers would be cleaved by telomerase to eliminate the mismatch. This proof-reading reaction can be detected when [32P]dTTP is included in the reaction (19; data not shown). However, when [32P]dGTP is the only nucleotide present in the reaction, only DNA products resulting from direct addition of dG residues onto primer 3'-ends can be detected. Hence, the products shown in Figure 4B, lanes 2 and 4, represent direct extension onto primer 3'-ends containing mismatches with the telomerase RNA template and not products from the cleavage-initiated elongation reaction.

As the potential for base pair formation between the primer 3'-end and the telomerase RNA template was decreased, the efficiency of reiterative dG addition increased. The primers G4-12-GTTTT and G4-13-TTTT are predicted to form five and
Figure 4. Effect of primer–template and primer–anchor site interactions on reiterative dG addition. (A) The presence of dG residues in a non-telomeric DNA 3′-end inhibits formation of the dG ladder. G4T4-G4-13-G (lanes 1 and 5), G4T4-G4-13-GG (lanes 2 and 6), G4T4-G4-13-GGG (lanes 3 and 7) and G4T4-13 (lane 4) were assayed in the presence of [32P]dGTP and dTTP (lanes 1–3) or [32P]dGTP only (lanes 4–7). DNA products generated by telomerase in the presence of [32P]dGTP only (lanes 4–7) were quantified by phosphorimaging. Data are presented as ratios between the amount of radioactive label incorporated into G4T4-13-G, G4T4-G4-13-GG and G4T4-G4-13-GGG extension products relative to the amount of label incorporated into products generated with G4T4-13. The average values from three independent experiments are shown. (B) Introduction of mismatches into telomeric primer 3′-ends results in reiterative dG addition by telomerase. Primers G(T4G4)2TTA (lanes 1 and 2) and G(T4G4)2ATTT (lanes 3 and 4) were assayed in the presence of [32P]dGTP and dTTP (lanes 1 and 3) or [32P]dGTP only (lanes 2 and 4). Left and right arrows denote the positions of 22 nt products resulting from incorporation of a single dG residue onto G(T4G4)2TTA and G(T4G4)2ATTT, respectively. (C) Reiterative dG addition is mediated by primer interaction with the anchor site on telomerase. Primers G4-12-GTTTT (lane 1), G4-13-TTTT (lane 2), 16-GTTTT (lane 3) and 17-TTTT (lane 4) were assayed with [32P]dGTP only.

Vegetative telomerase does not perform reiterative dG addition

The primary function of telomerase in the vegetative stage of the *E. crassus* life cycle is to maintain pre-existing tracts of telomeric DNA. Indeed, previous studies indicated that telomerase from vegetatively growing *E. crassus* does not add telomeric repeats onto non-telomeric DNA 3′-ends (18). Therefore, we tested whether telomerase derived from vegetative macronuclei could perform reiterative dG addition. In contrast to telomerase from developing cells, the vegetative telomerase extended G4T4-13 by addition of only a single dG residue, whether dTTP was included in the reaction or not (Fig. 5, lanes 1 and 2). However, like developmental telomerase, vegetative telomerase extended the telomeric primer G(T4G4)2T4 by one to four dG residues, as expected, for precise copying of the telomerase RNA template (Fig. 5, lanes 3 and 4). We failed to detect DNA products resulting from the addition of more than four dG residues in reactions with vegetative telomerase and primers whose 3′-ends are predicted to form four to five base pairs with the telomerase RNA template. For example, vegetative telomerase extended G4T4-13-TTTT and G(T4G4)2ATTT by one to four dG residues in the absence of dTTP (Fig. 5, lanes 6 and 8). Note that the same primers were elongated by more than four dG residues in reactions with developmental telomerase (Fig. 4B and C). Thus, the ability to perform reiterative dG addition is confined to telomerase from developing macronuclei.
DISCUSSION

DNA and RNA polymerases catalyze template-directed elongation of polynucleotides in accordance with Watson–Crick base pairing rules. During template copying, base insertion, deletion and substitution errors can occur as a result of polymerase infidelity. Template slippage is thought to be one of the mechanisms responsible for polymerization errors (20,24–27).

This phenomenon, also known as pseudo-templated polymerization, generally occurs on so-called slippery sequences, marked by short (1–5 nt) repeats. Observed under standard assay conditions in vitro and in vivo, template slippage can also be induced or enhanced by divalent metal substitution (28), template damage (29,30) or by altering specific amino acid residues within polymerase (31).

Although many examples of template slippage have been described, this process is not well understood. Identification of conserved reverse transcriptase motifs in the catalytic subunit of telomerase suggests that telomeric DNA synthesis occurs by a mechanism similar to that of other reverse transcriptases. Since telomerase is a ribonucleoprotein that carries its own template, it provides an unusual opportunity to study many aspects of nucleic acid synthesis.

In this study, we describe a reaction in which multiple dG residues are added onto non-telomeric DNA 3′-ends in vitro. The reaction strictly depends on accessibility of the RNA template. Furthermore, under our reaction conditions the primer is in 104-fold excess over telomerase. Thus, generation of long DNA products is not likely to result from multiple telomerase–primer dissociation and association events. These properties imply that indiscriminate dG addition occurs via reiterative slippage of the primer 3′-end on the RNA template during DNA synthesis.

Telomerase slippage is not observed with primers whose 3′-terminal non-telomeric nucleotides have been eliminated prior to elongation by telomerase. When the three 3′-terminal non-telomeric residues are removed from 10-G4T4-3, one to four dG residues are added as dictated by nt 41–44 in the telomerase RNA. The 3′-ends of telomeric primers and primers cleaved by telomerase are predicted to form a six base pair duplex with nt 45–50 in the telomerase RNA template before copying of nt 41–44 can commence. In contrast, direct extension of non-telomeric primer 3′-ends initiates in the absence of primer hybridization on the RNA template. Reiterative dG addition observed with such primers appears to reflect lateral instability in the primer 3′-terminus–RNA template interaction. Consistent with this idea, we find a strict correlation between the number of potential primer–template base pairs and the efficiency of reiterative dG addition. Relative to primers lacking any 3′-terminal complementarity with the RNA template, primers that contain as few as one to three dG residues on their 3′-ends show decreased dG ladder formation. In contrast, introduction of mismatches in the 3′-portion of telomeric DNA substrate, which is predicted to destabilize primer–template interactions, induces reiterative dG addition.

Slippage often occurs during template copying by other polymerases under conditions where primer–template interactions are relatively unstable. For example, RNA polymerase slippage is observed during transcription initiation, when the length of the RNA–DNA duplex is minimal (20). Nucleotide misincorporation induced by Mn2+ leads to repetitive copying of the template nucleotides by HIV-1 reverse transcriptase (28), an event reminiscent of reiterative dG addition by telomerase when extending telomeric primers containing a 3′-terminal mismatch. Changing the HIV-1 reverse transcriptase amino acid residues responsible for contacting the DNA strand at the second and third nucleotide bases from the 3′-end increases strand slippage (31), demonstrating a critical role for these amino acid residues in enzyme fidelity.

Interestingly, the ability of the primer to contact the telomerase anchor site is crucial for dG ladder formation. Since the anchor site is located some distance away from the RNA template (17; J.Bedneno and D.E.Shippen, unpublished results), the primer–anchor site interaction is not expected to compensate for the destabilizing effect of mismatches between the primer and template. However, this interaction can facilitate primer association with telomerase (15–17).

Our data can be best described by a primer–template realignment model. Weak interaction of the 3′-end of a primer with the telomerase RNA template can result in unpairing and reassociation of the DNA and RNA strands, which would in some cases lead to strand mispairing and re-exposure of C nucleotides for reiterative copying. Interaction of the primer at the anchor site could allow telomerase to remain associated with the primer during transient dissociation of the DNA and RNA strands. A similar model was proposed for RNA polymerase stuttering (32,33).

This model can be extended to telomerases from other organisms. Telomerase slippage is thought to be a primary mechanism for generating irregular repeats in Saccharomyces cerevisiae.
castellii (34). Reiterative dG addition has also been described for telomerase from *Tetrahymena thermophila* (35). Unlike the *E. crassus* telomerase, the *Tetrahymena* enzyme can add multiple dG residues onto telomeric primers when dTTP is omitted from the reaction (35). In addition, the *Tetrahymena* telomerase has an intrinsic ability to extend completely non-telomeric DNA primers, regardless of the stage of the life cycle it is isolated from (36). Wang *et al.* proposed that extension of non-telomeric DNA is mediated by the unique primer specificity of *Tetrahymena* telomerase. The number of primer–template base pairs required for polymerization appears to vary from zero to five, depending on the position of the template base being copied (37).

One obvious structural difference between the *Euplotes* and the *Tetrahymena* telomerases is that the *Tetrahymena* telomerase RNA template, 5′-CAACCCCCAA-3′, is shorter than the *Euplotes* templating region, 5′-CAAAACCCCCAAAAACC-3′ (2,12). A telomeric primer G₂(T₂G₃) is predicted to form only a three base pair duplex with the *Tetrahymena* telomerase RNA before elongation begins (35). Thus, interaction of primer 3′-ends with the *Tetrahymena* telomerase RNA template may be intrinsically less stable than interaction of telomeric primer 3′-ends with the *Euplotes* telomerase RNA template.

Mutations in the *Tetrahymena* and yeast telomerase RNA templates can result in increased telomerase slippage *in vitro* and *in vivo* (38–40). This effect is accompanied by loss of processivity and enzymatic fidelity observed for mutant telomerases *in vitro* and is thought to be a consequence of changing the geometry of the telomerase active site (39–41). *In vitro*, reiterative dG addition by mutant telomerases is not inhibited by addition of ddTTP.

We find no evidence for reiterative dG addition by telomerase derived from vegetatively growing *E. crassus* cells. Vegetative telomerase terminates extension of non-telomeric 3′-ends upon addition of a single dG residue. Moreover, DNA primers containing a few 3′-terminal nucleotides that could base pair with the RNA template, yet still serve as substrates for multiple dG addition in reactions with developmental telomerase, acquire a maximum of four dG residues upon extension by vegetative telomerase. Thus, vegetative telomerase appears to possess a mechanism for preventing primer–template slippage during template copying. This mechanism may increase fidelity of telomeric DNA synthesis during telomere maintenance in vegetatively growing *E. crassus* cells.

Three functional properties by which *E. crassus* telomerases from vegetative cells differs from telomerase in developing cells have been identified. Vegetative telomerase does not extend non-telomeric 3′-ends, it produces a different pattern of DNA products during telomeric repeat synthesis and it does not perform multiple dG addition in the absence of dTTP (18; this study). Interestingly, all these differences appear to be manifested at the level of primer interaction with the telomerase RNA template. It is possible that amino acid modifications and/or factors that change telomerase behavior during development (18) will be found to affect this interaction. Whether the inability of vegetative telomerase to perform reiterative copying of templating nucleotides is directly linked to its inability to add telomeric repeats onto non-telomeric sequences remains to be investigated.

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