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# A functional telomerase RNA swap *in vivo* reveals the importance of nontemplate RNA domains

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**ABSTRACT** **The ribonucleoprotein (RNP) enzyme telomerase is required for replication of eukaryotic chromosomal termini. The RNA moiety of telomerase is essential for enzyme function and provides the template for telomeric DNA synthesis. However, the roles of its nontemplate domains have not been explored. Here we demonstrate that a novel interspecies telomerase RNA swap *in vivo* creates a functional but aberrant telomerase. Telomerase RNA from the ciliate *Glaucoma chattoni* was expressed in *Tetrahymena thermophila* cells. The telomerase RNAs from these two species have almost superimposable secondary structures. The template region base sequence is identical in the two RNAs, but elsewhere their sequences differ by 49%. This hybrid telomerase RNP was enzymatically active but added only short stretches of telomeric repeat tracts *in vivo* and *in vitro*. This new enzyme also had a strong, aberrant DNA cleavage activity *in vitro*. Thus, molecular interactions in the RNP involving nontemplate RNA domains affect specific aspects of telomerase enzyme function, raising the possibility that they may regulate telomerase activity.**

The G-rich telomeric DNA tracts found at most eukaryotic chromosomal termini are added by a specialized component of the cellular DNA replication machinery, the ribonucleoprotein (RNP) reverse transcriptase telomerase. Telomeric DNA has numerous important functions and properties, including allowing completion of DNA replication, mediating chromosome stability (1), and affecting mitotic chromosome separation (2). Therefore, telomere synthesis and maintenance are crucial aspects of stable genomic inheritance in eukaryotes. The RNA moiety of the telomerase RNP is essential for enzymatic function and contains an internal sequence that templates telomeric DNA polymerization (3). Studies of template point mutations also have revealed other critical roles for specific template residues in enzyme action (4, 5). However, little is known about the function of other portions of telomerase RNA.

Telomerase RNAs in ciliated protozoa (3, 6, 7), yeasts (8, 9), and mammals (10, 11) have diverged rapidly in primary sequence. Phylogenetic (12–14) and RNA conformational analyses (15, 16) of the small (≈150–200 nt) ciliate telomerase RNAs have shown that their secondary structures are highly conserved. Comparison of 13 telomerase RNAs from one group of ciliates has shown a strikingly bipartite sequence arrangement; they all contain a 17-nt stretch of identical sequence centered on the template domain, and the primary sequence of the rest of the RNA differs by up to 76% yet preserves similar structural domains (12, 14). To investigate directly how this nontemplate portion of telomerase RNA affects enzymatic function, we tested whether the ≈160-nt telomerase RNAs in the ciliates *Tetrahymena thermophila* and *Glaucoma chattoni* were functionally interchangeable *in vivo*. These two telomerase RNAs share an identical 23-base sequence in and around the template domain. In contrast, the rest of the RNA sequence, which has an almost identical secondary structure (15), is 49% different (Fig. 1*A*).

Here we demonstrate that, in a cross-species telomerase RNA swap, the *Glaucoma* telomerase RNA, despite its ≈50% sequence divergence outside the template region, assembles and functions *in vivo* in *Tetrahymena* cells. The resulting hybrid enzyme, containing *Glaucoma* telomerase RNA and *Tetrahymena* telomerase proteins, has lower activity and polymerizes repeats less processively than the wild-type *Tetrahymena* enzyme. Strikingly, the hybrid telomerase activity also displays an aberrant cleavage activity. This study reveals a new class of telomerase RNA mutants, indicating that telomerase RNA domains other than the template affect enzymatic processes taking place at the polymerization active-site.

## METHODS

**PCR and Transformation.** The chimeric *Gc.TER1* gene was constructed as three consecutively overlapping PCR fragments using methods described previously (4, 5). Sequences of oligonucleotide primers used to synthesize the three fragments are indicated.

*Upstream.* 5′GGGGGGGGGGGGTGATCACTCGAGGGAGCTCATAAAA; 5′GGATCTACCAGGAGGTAAAAGACTTAAAATAATTTCTAC. Coding region: 5′GTAGAAATTATTTTAAGTCTTTTACCTCCTGGTAGATCC; 5′GAATACAAATCGAAATAGATAAAAAAAA CTTGGCATTCCATAAGATAAATAGTG.

*Downstream.* 5′GGAATGCCAAGTTTTTTTTATCTATTTCGATTTGTATTC; 5′GGGGGGGTACCCTCGAGGGAAGCTATTTTTAG.

The 43A template mutation was introduced into the chimeric *Gc.TER1* gene by a similar PCR method using the following primers: 5′CCTGTCATTAAACCCCAAAAATC and 5′GATTTTTGGGGTTTAATGACAGG.

*Gc.TER1* and *Gc.ter1–43A* PCR fragments were cloned into a TA-cloning vector (Invitrogen). Correct sequence of gene inserts was verified (Sequenase, United States Biochemical), and ≈0.5 kb *Xho*I DNA fragments bearing *Gc.TER1* or *Gc.ter1–43A* genes were subcloned into the high copy, episomal vector prD4–1. Transformation of wild-type *T. thermophila* cells (B2086 and CU428) was performed using established electroporation methods. Drug selection (100 μg/ml paromomycin) was applied 16 h after electroporation, and transformants were selected 3–4 days later.

**Northern Blot Analysis.** Pooled transformants (>15–20 single transformant cell lines) were grown in liquid culture at 30°C with shaking (100 rpm) for 1–2 days. Total RNA was prepared from 50-ml cultures. Total RNA (10–20 μg) was

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Abbreviation: RNP, ribonucleoprotein.

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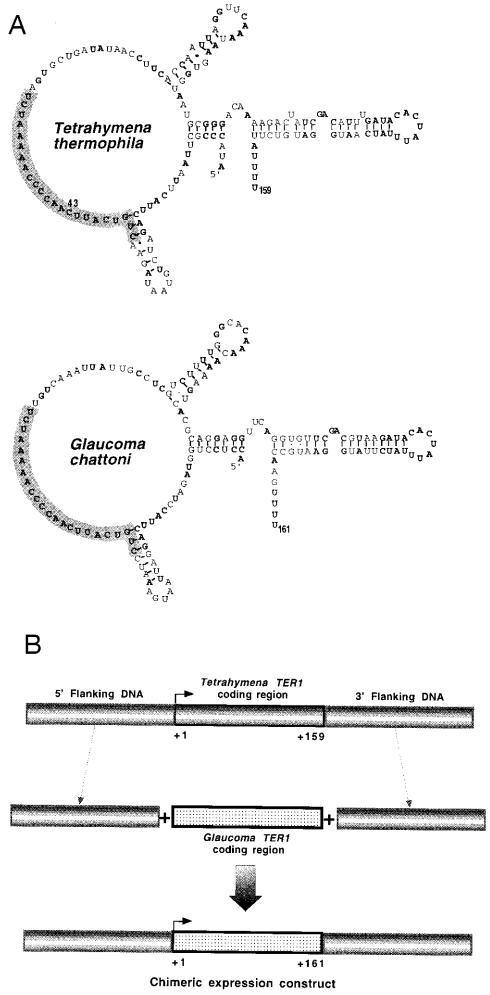


FIG. 1. Conserved similarities between *T. thermophila* and *G. chattoni* telomerase RNAs (*TER1*). (*A*) Schematic summary of the conserved secondary structure between *Tetrahymena* and *Glaucoma TER1* RNAs. Residues in bold represent bases conserved between these two RNAs. Shaded region contains 23 bases of absolute sequence identity centered upon the template region. The templating region contains an RNA sequence specifying the complementary telomeric DNA sequence polymerized at chromosome termini. The position of the C-to-A template base change (43A) is indicated. Despite an overall 35% difference in RNA sequence (49% excluding the template region), both *TER1* RNAs fold into similar conformations. (*B*) Schematic strategy for the heterologous expression of the *G. chattoni* telomerase RNA gene (*Gc.TER1*) in *T. thermophila* cells. A PCR approach was devised to precisely surround the *Glaucoma TER1* coding region with *Tetrahymena TER1* expression signals producing a chimeric *Gc.TER1* gene, which was introduced into wild-type *T. thermphila* cells.

electrophoresed through 5% acrylamide/7 M urea denaturing gels. After transfer to Hybond-$N^+$ membrane (Amersham), blots were probed with random-prime, labeled PCR product for *Tt.TER1* or *Gc.TER1* gene probes. Primers to generate *TER1*-specific probes have been described (15). Hybridization was performed using the SDS/$Na_2HPO_4$ method in 0.5 M phosphate at 25°C overnight. *Tt.TER1*-specific probed blots were washed three times at 25°C for 5 min, once at 55°C for 5 min, and once at 25°C for 5 min in 50 mM phosphate buffer. *Gc.TER1*-specific, probed blots were washed as described above, except once a 60°C, 5-min wash replaced the 55°C wash.

**RNP Gel Analysis.** S100 extract was prepared from *T. thermophila* cells, grown as described above. RNP complexes were analyzed on composite 3.5% polyacrylamide (60:1)/0.6% agarose in 50 mM Tris·acetate (pH 7.5) buffer. Approximately equal amounts of total S100 protein (Bradford, Pierce, IL) were loaded onto gels. RNase sensitivity of RNP complexes was determined by RNase A treatment (50 $\mu$g/ml for 5 min at 25°C) before gel loading. After electrophoresis, gels were denatured in 50% urea and transferred to membrane. Gel blots for Western blot analysis were transferred onto poly(vinylidene difluoride) membrane (Millipore).

**Western Blot Analysis.** Anti-p80 and -p95 antibodies were a generous gift from Kathleen Collins (University of California, Berkeley, CA). Western blot analysis was performed with an enhanced chemiluminescence detection kit (Amersham) according to the manufacturer's instructions.

**Southern Blot Analysis.** Southern blot detection of contiguous $G_4T_3$ and $G_4T_2$ telomeric repeat tracts from *Glaucoma* and *Tetrahymena* telomerase RNA transformant genomic DNAs (*Pst*I-cut) was performed as described (17). The $G_4T_3$ repeat probe used was 5′-TTT(GGGGTTT)$_3$, and the $G_4T_2$ probe was 5′-GTT(GGGGTT)$_3$G. *TER1* gene dosage studies were determined using duplicate Southern blots of *Pst*I-cut genomic DNA prepared from *Tetrahymena* and *Glaucoma TER1* and *ter1–43A* transformants probed with *Tt.TER1* or *Gc.TER1* gene probes. Vector plasmids bearing *Tt.TER1* or *Gc.TER1* genes provided positive controls for probe hybridization.

**Cloning and Screening Transformant Telomeres.** Uncut *Tt.ter1–43A* or *Gc.ter1–43A* transformant genomic DNAs (≈1 $\mu$g) were T4 DNA polymerase-treated before ligation to *Eco*RV-cut Bluescript vector (Stratagene). After *Eco*RI cleavage and religation, approximately one–sixth of both telomere libraries was transformed into *Escherichia coli* (XL1 Blue MRF', Stratagene) and plated. Libraries were screened first with the $G_4T_3$-specific oligonucleotide probe and reprobed with the $G_4T_2$-specific probe. Plasmids containing $G_4T_3$ and $G_4T_2$ repeat tracts provided positive controls (17). Extent of $G_4T_3$ probe hybridization was >10 fold in *Tt.ter1–43A* telomere clones compared with *Gc.ter1–43A* telomere clones, likely resulting from better probe hybridization to longer variant repeat tracts present in the *Tt.ter1–43A* telomeric clones vis-à-vis shorter tracts in *Gc.ter1–43A* clones. $G_4T_3$-positive clones were DNA sequenced using a T7 promoter primer.

**Purification of Telomerase Activity.** Extracts prepared from pooled *T. thermophila* transformant cell lines were purified over heparin–agarose, DEAE–cellulose, and octyl–Sepharose columns as described (4, 5). Labeled dTTP*-only and dGTP* plus dTTP telomerase reactions were performed as described (4) except final primer and nucleotide concentrations were 1 $\mu$M and 5 $\mu$M, respectively. Reactions were performed typically in a 20-$\mu$l volume at 25°C for between 10 and 60 min. In mixing experiments, relative ratios of *Tt.TER1:Tt.ter-43A* extracts were mixed as follows and were subject to a G + T reaction: 1:0.9, 1:0.7, and 1:0.5. The $(T_2G_4)_3$ primer was gel purified to nucleotide resolution and heat denatured before use. Labeled reaction products were separated on denaturing gels and visualized by autoradiography.

## RESULTS

**Heterologous Expression of *Glaucoma* Telomerase RNA.** The *Glaucoma* telomerase RNA gene (*Gc.TER1*) was expressed on a high copy number vector in *Tetrahymena* cells (Fig. 1*B*). Previous work has shown that such an introduced, template-marked *T. thermophila* telomerase RNA can replace the endogenous telomerase RNA in the telomerase RNP (4, 5). A template mutation was introduced into the *Gc.TER1* gene to provide a direct "read-out" of *in vivo* gene activity distinguishable from the endogenous, wild-type *Tetrahymena* telomerase activity. The same mutation was a C-to-A substitution at the 5′-most position in the RNA template (43A mutation; Fig. 1*A*). This mutation in the *T. thermophila* RNA

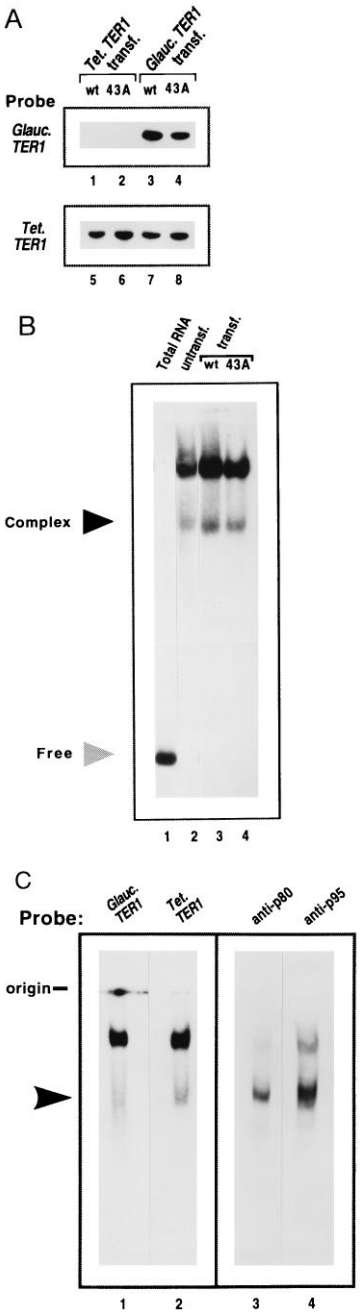


FIG. 2. Heterologous expression and assembly of *Glaucoma* telomerase RNA into an RNP complex. (*A*) Northern blot analysis of *Tetrahymena* transformants expressing the *Gc.TER1* gene *in vivo*. Total RNA from *Tetrahymena* or *Glaucoma TER1* or *ter1–43A* transformants was fractionated on denaturing gels. Blots probed with a *Gc.TER1*-specific gene probe (lanes 1–4) revealed presence of *Gc.TER1* RNA *in vivo* (lanes 3 and 4). Blots were reprobed with a *Tt.TER1*-specific probe (lanes 5–8); endogenous *Tetrahymena* TER1 RNA was observed in *Gc.TER1* and *Gc.ter1–43A* transformants (lanes 7 and 8). (*B*) Assembly of *Tetrahymena* TER1 RNA into a telomerase RNP complex. RNP complexes prepared from untransformed, vegetative *Tetrahymena* cells (lane 2) and *Tt.TER1* or *Tt.ter1–43A* transformants (lanes 3 and 4) were studied by RNP gel analysis; blots were probed with a *Tetrahymena TER1*-specific probe (see *Methods*). Lane 1 contains total RNA (≈10 μg) prepared from *Tt.TER1* transformants. Light shaded arrow indicates free telomerase RNA, and dark arrow indicates lower putative telomerase RNP complex. (*C*) Identity of RNP complexes in *Gc.ter1–43A* transformants. RNP complexes from *Gc.ter1–43A* transformants were analyzed by sequentially probing with a *Gc.TER1*-specific probe (lane 1) and then a *Tt.TER1*-specific probe (lane 2). Lanes 3 and 4 are duplicate loadings subjected to Western blot analysis using telomerase anti-p80 and -p95 antibodies, respectively. Arrow identifies lower RNP complex.

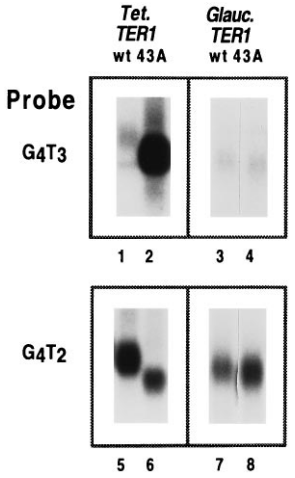


FIG. 3. Southern blot analysis of genomic DNA from *T. thermophila* transformants containing *Tetrahymena* and *Glaucoma TER1* and *ter1–43A* genes. *Pst*I-cut transformant genomic DNAs were separated by 0.7% agarose gel electrophoresis and subjected to Southern blot analysis. Transformant macronuclear rDNA telomeres were analyzed using either a $G_4T_3$- (lanes 1–4) or a $G_4T_2$-specific (lanes 5–8) oligonucleotide probe (17). Lanes 5–8 are duplicate lanes to lanes 1–4. Only part of the Southern blot containing the rDNA telomeres is shown.

(*Tt.ter1–43A*) causes synthesis of $G_4T_3$ variant repeats instead of wild-type $G_4T_2$ repeats *in vitro* and *in vivo* (4). The *Gc.TER1* and *Gc.ter1–43A* constructs, or corresponding *Tt.TER1* and *Tt.ter1-43A* gene constructs as controls (4, 18), were introduced into wild-type *T. thermophila* cells. Southern and Northern blotting analyses of transformants showed that the *Gc.TER1* gene was present at the expected high copy number (data not shown) and was transcribed to produce stable RNA (Fig. 2*A*). The steady-state levels of *Gc.TER1* RNA were similar to those found in the control *Tt.TER1* transformants (Fig. 2*A*, lanes 5 and 6) and were comparable to endogenous *Tt.TER1* RNA levels (Fig. 2*A*, lanes 7 and 8).

**Telomerase RNP Assembly.** Telomerase RNP formation was assessed by nondenaturing gel electrophoretic analysis of S100 fractions prepared from transformed cells or untransformed control cells (Fig. 2 *B*, lanes 2–4, and *C*, lane 2). A *Tt.TER1*-specific probe identified two RNP complexes both sensitive to RNase A pretreatment that were similar in all of these cell lines. In analyses of the *Gc.ter1–43A* S100 extracts, sequential probing for either the *Glaucoma* or the *Tetrahymena TER1* RNA showed that comparable levels of both RNAs were present in the RNP complexes (Fig. 2*C*, lanes 1 and 2). Western blotting analysis of these same RNP complexes, using separate antibodies specific for the *T. thermophila* telomerase 80- and 95-kDa protein components (19), showed that the majority of signal for both telomerase proteins was in the lower RNP complex (Fig. 2*C*, lanes 3 and 4, arrow). These results indicated that both *Tetrahymena* telomerase p80/p95 proteins and *Glaucoma* telomerase RNA were present in the lower RNP complex.

Thus, despite its 49% sequence difference outside the template region, the *Glaucoma* telomerase RNA assembles *Tetrahymena* protein components into a telomerase RNP complex. However, the lower RNP complex detected with the *Gc.TER1* probe ran as a slightly broader band than that visualized with the *Tt.TER1* probe, suggesting that conformational differences exist between the RNP complexes containing the two different RNAs. In Western blotting of the RNP gels, antibody signals from the upper complex diminished with more stringent washing, and other experiments indicated that some of the nucleic acid hybridization signal from this complex came from genomic DNA present in the S100 extract (T. Ware

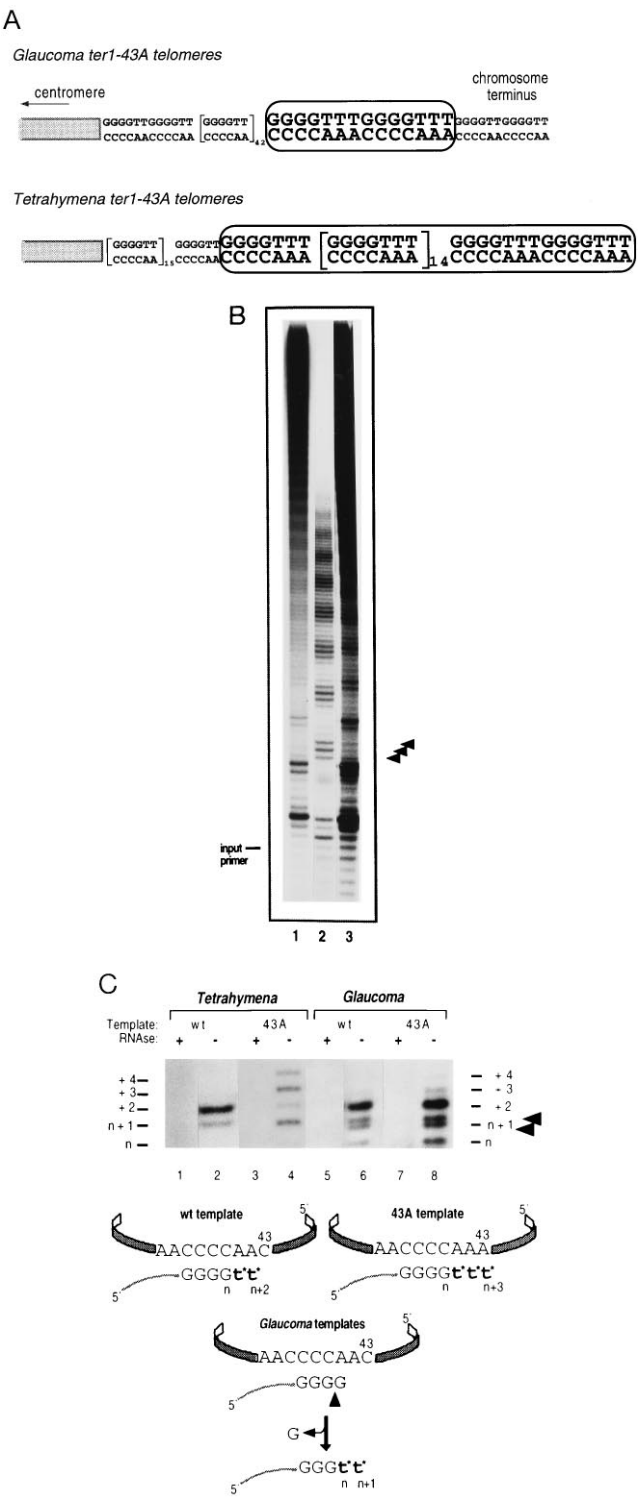


FIG. 4. Incorporation of $G_4T_3$ variant telomeric repeats *in vivo* and *in vitro*. (*A*) DNA sequences of representative $G_4T_3$-containing telomere clones isolated from *Gc.ter1–43A* and *Tt.ter1–43A* transformant telomere libraries. Boxes highlight occurrence of variant $G_4T_3$ repeats. (*B*) *In vitro* telomerase activity from *Tt.TER1* (lane 1), *Tt.ter1–43A* (lane 2), and *Gc.ter1–43A* (lane 3) extracts in presence of labeled dGTP and unlabeled dTTP. Arrows indicate positions of $G_4T_3$ repeat products in *Glaucoma ter1–43A* extract amid the largely wild-type repeat pattern, consistent with only two rounds of 43A-templated elongation synthesis. Size of unlabeled input primer is indicated. (*C*) *In vitro* telomerase activity from transformant extracts in the presence of [$\alpha$-$^{32}$P] dTTP-only. Lanes 1, 3, 5, and 7: telomerase fractions were pretreated with RNase A. Lane 2, extension products from *Tt.TER1*; lane 4, *Tt.ter1–43A* extracts. Lengths of control DNA fragments are indicated. Schematic summaries represent products predicted from wild-type or 43A telomerase RNA template. The lower band in the $n + 1$ doublet (arrowed, lanes 6 and 8) likely represents cleavage of the 3′-terminal dG from primer $d(T_2G_4)_3$ and addition of two radiolabeled dT* bases, producing a $d(T_2G_4)_2TTGGGT^*T^*$ product. This species is expected to migrate slightly faster than the $d(T_2G_4)_2TTGGGGT^*$ product, generated by a single dT* residue addition onto the uncleaved primer.

and E.H.B., unpublished observations). Previous studies of purified telomerase in the ciliate *Euplotes aediculatus* have shown only a single RNP complex (20). It is possible that the upper complex may represent a form of the telomerase holoenzyme, with partially masked protein epitopes associated with genomic DNA.

**Incorporation of Variant Telomeric DNA Repeats *in Vivo*.** To address whether *Gc.ter1–43A* transformants produced enzymatically active telomerase, we first used Southern blotting analysis to detect $G_4T_3$ repeats in the macronuclear telomeres of transformant cells using a $G_4T_3$ repeat-specific probe (17) (Fig. 3). Compared with control *Tt.ter1–43A* transformants, *Gc.ter1–43A* transformants showed only low levels of $G_4T_3$ repeats (Fig. 3). To obtain an estimate of the relative numbers of telomeres containing $G_4T_3$ repeats, partial libraries of cloned telomeres were screened with $G_4T_3$- and $G_4T_2$-specific probes. In a *Gc.ter1–43A* telomere library, three cloned telomeres contained $G_4T_3$ repeats as well as $G_4T_2$ repeats, and 41 contained only $G_4T_2$ repeats. Of the cloned telomeres analyzed from a control *Tt.ter1–43A* library, five clones had $G_4T_3$ repeats, and 40 contained only $G_4T_2$ repeats. These results suggested that the *Gc.ter1–43A* and *Tt.ter1–43A* enzymes synthesized repeats onto comparable numbers of telomeres *in vivo*. However, DNA sequence analysis of two of the $G_4T_3$-containing clones from the *Gc.ter1–43A* library showed that the *Gc.ter1–43A* enzyme had added only two contiguous repeats in both clones (Fig. 4*A*). In contrast, in four cloned telomeres from the *Tt.ter1–43A* library, 16–17 consecutive $G_4T_3$ repeats were found in each clone.

**Hybrid *Glaucoma* Telomerase Enzyme Functions Aberrantly *in Vitro*.** We directly assessed the enzymological properties of the *Gc.TER1* telomerase *in vitro*. Telomerase activity, prepared from *Gc.TER1* and *Gc.ter1–43A* transformants and from *Tt.TER1* or *Tt.ter1–43A* transformant controls, was compared in complete telomerase reactions containing radiolabeled dGTP and unlabeled dTTP (G + T reaction) primed with the telomeric oligonucleotide $d(T_2G_4)_3$. The *Tt.TER1* and *Tt.ter1–43A* extracts produced the described (4) patterns of 6-base $G_4T_2$ or 7-base $G_4T_3$ repeats, respectively (Fig. 4*B*, lanes 1 and 2). In contrast, the *Gc.ter1–43A* transformant activity produced the largely wild-type 6-base repeat pattern of the endogenous *Tetrahymena* telomerase activity (Fig. 4*B*, lane 3). However, there were additional, clearly visible bands corresponding to up to two $G_4T_3$ repeats (arrowheads, Fig. 4*B*). This pattern differed from that of *Tt.ter1–43A* transformants, in which the bands corresponding to addition of three and four $G_4T_3$ repeats were stronger than those from addition of two repeats (Fig. 4*B*, lane 2). In control experiments, such longer $G_4T_3$ repeat products were clearly apparent when low ratios of telomerase activity from *Tt.ter1–43A* transformants were mixed with *Tt.TER1* extracts and assayed (data not shown). Both *Gc.ter1–43A* and *Tt.ter1–43A* RNAs and RNPs were present in comparable amounts in the *Gc.ter1–43A* transformant cells. Hence, the hybrid *Gc.ter1–43A* RNP in *Tetrahymena* cells was not only enzymatically less active but also was less processive than its all-*Tetrahymena* counterpart enzyme.

The *Glaucoma* RNA-substituted telomerase was assayed with radiolabeled dTTP alone (T*-only reaction), using a $d(T_2G_4)_3$ oligonucleotide primer. In control reactions, the *Tt.TER1* (wild-type) telomerase added two dT* residues ($n$ + 2 product, where the primer is $n$ nucleotides long) to the 3′ end

of this $dG_4$-ending primer (Fig. 4*C*, schematic and lane 2), and the *Tt.ter1–43A* enzyme added the predicted three dT* residues (n + 3 product) as has been shown (4) (Fig. 4*C*, schematic and lane 4). As described (4), the *Tt.ter1–43A* telomerase activity "stuttered," incorporating an extra dT* nucleotide in addition to the predicted three-templated dT* bases (Fig. 4*C*, lane 4). The *Gc.ter1–43A* activity also synthesized the predicted $n + 3$, labeled product, plus the analogous $n + 4$ band (Fig. 4*C*, lane 8).

In marked contrast to the *Tt.TER1* or *Tt.ter1–43A* enzyme activities, both the *Gc.TER1* and *Gc.ter1–43A* activities produced a labeled, primer-sized product (*n*) plus a labeled doublet at the $n + 1$ position (Fig. 4*C*, lanes 6 and 8, arrowheads). These products were all sensitive to RNase pretreatment. The primer-sized (*n*), labeled band must contain at least one labeled dT* residue and can be explained by the presence of a telomerase-based cleavage activity, induced by the presence of *Glaucoma* RNA in the telomerase RNP, removing one (or more) dG residues from the 3′ end of the DNA primer and adding one (or more) labeled dT* residues. Because only dTTP was present, the labeled $n + 1$ doublet represents two products of the same length but different base composition. We deduced that the lower band was generated by 3′ cleavage and two dT* additions, and the upper band (which comigrated with the $n + 1$ product in Fig. 4*C*, lane 4; data not shown) was generated from a dT* addition directly to the uncleaved primer (Fig. 4*C*, lanes 6 and 8, arrows and schematic). Such cleavage and 3′ replacement reactions have been reported for wild-type *Tetrahymena* and *Euplotes* telomerases with certain primers but have not been reported for $(T_2G_4)_3$ or comparable primers (21, 22).

## DISCUSSION

**Role of Telomerase RNA Domains in Enzymatic Function *in Vivo*.** In this first reported cross-species telomerase RNA swap, remarkably, a telomerase RNA that was ≈50% divergent outside the template region assembled and functioned *in vivo*. Nevertheless, this new hybrid enzyme had lower activity and polymerized repeats less processively. It also displayed an aberrant cleavage activity. We propose that the compromised polymerization and miscleavage resulted from altered RNA–protein and/or protein–protein interactions in the hybrid telomerase. Specifically, these appear to place the cleavage active site in an altered spatial relationship to the template/primer. The telomerase cleavage activity was reminiscent of nascent transcript cleavages stimulated by transcription elongation factors in eukaryotes and prokaryotes (23–26), proposed to relieve "dead-end" transcription complexes (27, 28). Thus, it is possible that the aberrant cleavage and lowered processivity of the hybrid telomerase may be mechanistically related.

We had suggested a domain model of the telomerase RNA based on function (15), with the template region associated with enzymatic function and other regions of the RNA that might be involved more directly with binding telomerase protein components. This work reveals a new class of telomerase RNA mutants, demonstrating that telomerase RNA domains other than the template domain affect crucial molecular events taking place at the polymerization active site. Thus, our present study indicates that these "domain-associated" functions are not mutually exclusive and in fact likely influence each other.

This work raises the intriguing possibility that telomerase activity can be controlled or regulated by changing the molecular interactions involving its nontemplate RNA domains. At present, it is not known if specific telomerase RNA structural elements affect specific telomerase enzymatic functions. Pleij and colleagues (29) have proposed, for example, that the phylogenetically conserved helix III element (3′ to the template region; Fig. 1*A*) adopts a pseudoknot conformation. We had postulated (15) that the helix III region in the naked telomerase RNA *in vitro* was conformationally flexible, likely undergoing transitions between hairpin and pseudoknot states, while in the telomerase RNP the pseudoknot form might be stabilized by protein interactions. It also has been speculated that this helix-to-pseudoknot RNA structural transition might affect polymerization events at the template (15, 16). Thus, elucidating the RNA determinants of telomerase action may identify RNP domains that interact with naturally occurring telomerase control factors and point to new drug targets in the RNP for control of telomerase activity *in vivo*.

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