Published in final edited form as:

Nat Struct Mol Biol. 2015 November ; 22(11): 844–847. doi:10.1038/nsmb.3098.

A DNA Hairpin Model for Repeat Addition Processivity in Telomere Synthesis

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Abstract

We propose a DNA hairpin model for telomere repeat addition processivity. Concomitant with template RNA translocation after each repeat synthesis, the complementary DNA repeat, e.g. AGGGTT, loops out in a non-canonical base-paired hairpin, thus freeing the RNA template for the next round of repeat synthesis. The DNA hairpin is temporarily stabilized by telomerase and the incoming dGTP but becomes realigned for processive telomere synthesis.

Keywords

telomere; telomerase; RAP; primer loopout; translesion synthesis

Introduction

In all eukaryotic cells the G-rich DNA repeats, which form telomeres that cap and protect chromosome ends, are synthesized by the telomerase enzyme¹. Telomerase is a nucleoprotein complex minimally composed of a catalytic protein subunit, known as TERT because of its reverse transcriptase (RT) activity, and telomerase RNA (TR or TER), which contains a template sequence complementary to 1.5 - 2 telomeric repeats for reverse transcription² (Fig. 1). Without telomerase, chromosomes become shorter with every cycle of DNA replication³. The repeat addition processivity (RAP) of telomere DNA synthesis, observed for most organisms except for yeast and some fungi, has been reconstituted in vitro with purified tetrahymena and human telomerases^{4,5} but the mechanism for RNA template translocation and realignment of DNA primer in consecutive cycles of processive DNA repeat synthesis has been unclear.

TERT is highly conserved and contains a catalytic core preceded by a “telomerase essential N-terminal” domain (TEN) and an RNA-binding domain (TRBD) (Fig. 1a). The crystal structures of *Tribolium castaneum* (beetle) TERT protein (which lacks TEN), alone and in complex with RNA-DNA duplex^{6,7}, confirm its homology to reverse transcriptase (RT) and many DNA polymerases in the catalytic core, which is composed of palm, thumb and finger

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The authors declare that there is no competing financial interest.

domains, but give no clue as how TERT is able to reuse an RNA template multiple times. Crystal structures and TR- and telomeric DNA-binding properties of TEN and TRBD have also been reported⁷⁻⁹ It was suggested that the synthesis of DNA repeats requires separation of the RNA template from the DNA product after completion of each repeat addition, followed by RNA translocation and realignment of DNA¹⁰ The interactions between TERT and TR, especially by the TRBD and TEN domains, may facilitate the RNA template movement¹¹.

A telomere RNA template is often flanked by a template boundary element (TBE) on the 5' side, and a template recognition element (TRE), a pseudoknot (PK) structure, and conserved regions (CR) on the 3' side^{2,12} (Fig. 1b). Biochemical analyses indicate that the length, sequence and continuity of nucleotides immediately flanking the RNA template are critical for efficient repeat addition processivity^{13,14} An accordion model supported by FRET experiments has been proposed in which the TBE and TRE extend and contract concertedly to translocate the RNA template back and forth relative to the TERT active site for each repeat synthesis¹³ The FRET changes observed with tetrahymena and human telomerase-telomere complexes suggest a large conformational change between TR and DNA when synthesis reaches the 5' boundary of the RNA template^{13,15} Recent data also suggest that the signal ending each cycle of repeat synthesis and triggering RNA and DNA realignment resides within the RNA template and not in the flanking nucleotides^{14,16}

Telomere sequences

In most eukaryotic organisms except for yeast and some fungi, which have no RAP, telomeric repeats have well defined lengths and sequences (<http://telomerase.asu.edu>)¹⁷. The repeating unit is AGGGTT in all vertebrates¹⁸, AGGGGTT in plants, and GGGGTT in tetrahymena^{12,19}. Because of their G-rich nature, a G-quadruplex is the prevalent secondary structure associated with these telomere DNAs²⁰⁻²² However, experimental observations suggest that telomeric repeats may form other secondary structures. The telomeric G-rich sequences are difficult to replicate and act like a fragile site²⁶, which is likely due to its tendency to form secondary structures. Surprisingly, the secondary structure that impedes DNA polymerase is found to be not the G-quadruplex²⁷

Telomeric repeats are often reported in different permutations, such as GGG(G)(T)TT(A) or GG(G)(T)TT(A)G. However, we note that one particular permutation of the telomere repeats in vertebrates, plants, invertebrates and ciliates, (A)GGG(G)(T)TT has the potential to form a non-canonically base paired hairpin, containing an A:T match, G:T wobble and G:G Hoogsteen pairs (Fig. 2a). Although not standard Watson-Crick A:T and G:C pairs, the G:T wobble and G:G Hoogsteen pairing each maintains two hydrogen bonds. We thus propose that a few consecutive repeats of AGGGTT can form a stable heteroduplex hairpin to block its own replication.

A related hairpin-and-loop structure made of TTTTGGGG has previously been proposed to facilitate RNA template translocation in *Euplotes aediculatus* telomere synthesis²⁵, where TTTT form the 180° hairpin turn. Based on the existing hairpin structures of single-stranded DNA (PDB: 2VJU and 1KR8)^{23,24}, we modeled the 180° turn composed of 2 (vertebrates

and ciliates) or 3 (plants and invertebrates) nucleotides and showed that a single telomeric repeat could form a quasi-stable hairpin (Fig. 2b,c).

Primer loopout in translesion DNA synthesis

As an intrinsic property, DNA polymerases have high affinity for a primer and template pair with a 3'-recessed primer and can capture the primer-strand 3'-OH in the active site. In the presence of a correct incoming dNTP and Mg^{2+} , an even tighter ternary complex is formed, leading to nucleotide incorporation. This is true regardless of whether the polymerase is a low or high fidelity type, and whether an RNA or DNA template is used^{7,28,29} However, DNA polymerases specialized for translesion synthesis, which are often of low fidelity, can bypass DNA lesions that stall normal DNA polymerases. Recently while studying the human translesion DNA polymerase ν (Pol ν)³⁰, we noticed two unusual structural features: (i) a cavity along its interface with the primer strand, and (ii) a flexible thumb domain that can rotate 30°. Exploring these features, we found that 1-2 nucleotides in the primer strand 3 bp upstream from the primer 3'-end can loop out and occupy this cavity. Primer loopout enables translesion synthesis by allowing the DNA polymerase to reuse the upstream template strand when the downstream sequence is unusable or blocked. Primer loopout could lead to product expansion, and has been proposed to be a mechanism for trinucleotide repeat (TNR) expansion in a number of disease conditions³⁰.

TERT

Interestingly, a cavity along the DNA primer-binding surface is also present in TERT. It is formed by the 3-strand β -linker between the palm and thumb domains (Fig. 3a-b)^{6,7} A large rotational movement of the thumb domain similar to that of Pol ν could occur in TERT as well, because the homologous β -linker domain in the heterodimeric HIV-1 RT undergoes conformational changes between p66 and p51 subunits (Fig. 3c)^{29,31}. The β -linker together with the catalytic core (palm, finger and thumb) is superimposable between the p66 subunit of RT and TERT, but the β -linker in the p51 subunit of RT is unfolded and allows the thumb domain of p51 to rotate 80° and move 7 Å^{29,31} (Movie 1). In HIV-1 RT, the movement of the thumb in p66 or p51 is prevented by additional domains in p66 (connector and RNase H), and by close contact between p51 and p66. However, the thumb domain in TERT, which is composed of the CTE (C-terminal extension) at the very C terminus, is free to move. Notably, two distinct conformational states of human telomerase have been observed by electron microscopy³².

Unique to TERT, an extended flexible loop between the β -linker and the thumb domain (known as the thumb loop) makes extensive interactions with the upstream primer strand (Fig. 3)⁷. The exact length of the thumb loop varies between TERT species, but both the thumb loop and β -linker, which includes the signature E motif⁵, are conserved in all TERTs. Therefore, in principle a DNA hairpin loopout can form near the β -linker, and movement of the thumb loop and thumb facilitated by unfolding of the β -linker may provide additional flexibility and space to accommodate the hairpin loopout (Fig. 3).

A DNA hairpin model for repeat addition processivity

With the structural flexibility of TERT and the potential for telomere repeats to form a loopout, we propose a DNA hairpin model to account for repeat addition processivity in telomere synthesis (Fig. 4). In this model, the primer 3'-end remains in the TERT active site when the synthesis reaches the 5'-end of the RNA template, while the upstream RNA-DNA hybrid is held together by 2–3 bps and translocates together towards the TERT active site by one telomere repeat length. During the process, the GT-rich DNA, e.g. AGGGTT in vertebrates, is looped out in a hairpin structure (Fig. 4), while the freed CA-rich RNA template translocates fully and re-forms two base pairs (rU:dA and rC:dG) with the primer 3' end. For translocation and loopout of the DNA hairpin, the thumb domain and thumb loop may re-configure (Fig. 4a, b). The loopout probably interacts with the β -linker and the thumb loop. The translocation of the RNA template is likely supported by the flanking elements (TRE and TBE) and their interactions with the TEN and TRBD domains of TERT^{7,8,11}.

A similar hairpin loopout containing G:T wobble pairs can also form in plant and ciliate telomere repeats^{12,19} (Fig. 2). The quasi-stable DNA hairpin structure can compensate for the energetic cost of melting the RNA-DNA hybrid during RNA template translocation and can also be readily resolved afterwards. Because of base pairings between the RNA and DNA flanking the hairpin loopout, the template and primer remain associated during iterative template translocation, thereby achieving repeat addition processivity. As it has been observed for DNA polymerases that a correct incoming nucleotide stabilizes the polymerase and DNA substrate complex, we find it notable that dGTP, which is the correct incoming nucleotide after template RNA translocation, enhances repeat addition processivity in telomere synthesis.³³ We hypothesize that dGTP binding shifts the equilibrium towards both hairpin formation and its realignment (Fig. 4c,d), which is necessary for the next round of repeat synthesis.

Supporting evidence for the DNA hairpin model

In FRET experiments performed on tetrahymena and human telomerases complexed with telomere DNA, a fluorescence donor and acceptor pair placed on TBE and DNA primer (Fig. 4) produces a mixture of low and high FRET species when the primer reaches the template 5' end and the next incoming nucleotide (ddGTP) is present^{13,15}. The high FRET indicates a reduced distance between the FRET pair. Without the DNA hairpin loopout, the distance between the FRET pair would be the longest at the end of a repeat synthesis cycle and would become even greater when the RNA-DNA hybrid melts and the RNA template translocates (Fig. 1b). In contrast, the DNA hairpin model predicts the highest FRET upon the hairpin loopout formation at the end of one cycle of repeat synthesis (Fig. 4b) and lowest FRET when the DNA primer is realigned relative to the RNA template at the beginning of the next cycle of repeat synthesis (Fig. 4d), which is in full agreement with the FRET observations. The mixed highest and lowest FRET signals at the end of a repeat synthesis in the presence of ddGTP (2', 3'-dideoxy GTP) serving as the incoming nucleotide for the next cycle of repeat addition^{13,15} suggest that the DNA hairpin exists during the transition between synthesis cycles.

In tetrahymena (GGGTTG) and human (GGTTAG) telomere sequences, the first T (underscored) before reaching the end of each repeat synthesis has been shown to be the base specifically recognized by telomerase as the signal to pause synthesis^{14,16}, and its presence in telomere DNA (paired with A48 of hTR) appears to be required for formation of the pausing pattern in human telomere synthesis³⁴. The thymine base may be the signal recognized by TERT to loop out the telomere DNA, which in turn may drive and lead to the RNA template translocation. Based on the TERT crystal structure and mutagenic studies, a conserved telomerase specific motif in the TRBD domain (coincidentally known as the T motif)^{7,35} may directly interact with the T base in the telomere repeat and initiate the DNA hairpin loop formation.

Conclusion

The DNA hairpin model proposed here explains how telomere DNA participates in repeat addition processivity and why the telomeric sequences are highly conserved throughout evolution despite the fragile nature and difficulty posed for replication. This model is fully compatible with the accordion model, which concerns RNA template translocation¹³ (Movie 2). In addition to being consistent with a large number of existing biochemical and biophysical observations, a DNA hairpin structure is the best model to explain the FRET changes during a repeat synthesis cycle^{13,15}. Furthermore, the DNA hairpin model is experimentally testable. The model predicts a specific DNA hairpin structure that is accommodated by telomerase and that TERT undergoes large conformational changes accompanied by folding and unfolding of the β -linker domain in consecutive repeat addition cycles. The model further predicts that TERT serves multiple roles: it interacts with the initiator thymine in telomere DNA, stabilizes the DNA hairpin loop, and interacts with the flanking elements (TBE and TRE) to facilitate RNA template translocation. Protein crosslinking experiments, site-directed mutagenesis, and FRET experiments within TERT and between TERT and TR can be designed to test these predictions. These new experiments will undoubtedly improve our understanding the processivity of repeat addition and telomere synthesis

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank K. Collins for guiding us throughout the project, J.-L. Chen and T. R. Cech for insightful discussion, J. P. Cooper, T. de Lange, C. W. Greider, D. Rhodes and M. D. Stone for encouragement, and D. J. Leahy and M. Gellert for critical reading of the manuscript.

This work is funded by US National Institutes of Health intramural program (DK036146-08, W.Y.).

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Fig. 1. Telomerase.

(a) A diagram of structural domains in a typical TERT. TEN is not present in all TERTs. The small pink oval between palm and thumb represents the β -linker domain, (b) Main components of telomere RNA (TR). The RNA sequence in the template region is shown (human TR as an example). The pseudoknot (PK) and the conserved regions (CRs) are diagrammed disproportionately and smaller than the template region. As observed for tetrahymena, the template boundary element (TBE) and template recognition element (TRE) are included in the diagram for discussion.

Fig. 2. Telomere repeats.

(a) The proposed heteroduplex hairpin formed by telomeric DNA repeats. The vertebrate and plant sequence is used as an example, and similar heteroduplexes can form by all telomeric repeats. In addition to a single duplex, branches of the same heterologous pairings can also form, (b) Representative telomere-repeat sequences in vertebrates, plants and Tetrahymena (ciliates) are shown with the respective RNA templates. In the DNA hairpin model, one telomere DNA repeat (AGGGTT, AGGGTTT or GGGGTT) is looped out, thus freeing the RNA template. In the hairpin, A:T and G:T form normal and wobble pairs, and GG or GGT form the 180° turn. The DNA loopout is flanked by 2-3 bp of RNA-DNA hybrid on its 5' side and 1-2 bp on the 3' side, (c) Structural models of the DNA hairpins are shown in two views. The vertebrate and tetrahymena models are based on the ssDNA transposon structure (PDB: 2VJU)²³ and, the plant model is based on another ssDNA structure (PDB: 1KR8)²⁴ The plant DNA repeat could also adopt a structure similar to that of vertebrates and tetrahymena with the additional nucleotide flipped out²³.

Fig. 3. The structural cavity and flexibility of TERT that can accommodate the DNA hairpin loopout.

(a) The crystal structure of *T. castaneum* telomerase (TERT) complexed with an RNA template and DNA primer (PDB: 3KYL)⁷ is shown. The four structural domains are colored in cyan, blue, red and green. The T motif in TRBD, which may recognize the T base for the DNA hairpin formation, is labeled as "T". The three-stranded β -linker (pink) and the extended thumb loop (purple) between the palm and thumb domains are highlighted. (b) A close-up view of the primer 3'-end with TERT in molecular surface. The two DNA nucleotides visible through the cavity (yellow sticks) mark where the DNA hairpin will form. (c) The β -linker also exists in the p66 subunit of HIV-1 reverse transcriptase, but the same amino acid sequence adopts an extended conformation in the p51 subunit of HIV-1 RT.

The β -linker and the extended thumb loop in TERT may undergo large conformational changes to accommodate the DNA hairpin.

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Fig. 4. A DNA hairpin model for repeat addition processivity.

(a) At the end of one repeat synthesis, recognition of the T base (underscored) leads to a pause. The thumb (drawn as a green box, also known as CTE) may open up. (b) The hybrid of DNA (blue) and the RNA template (orange) is translocated towards the primer 3'-end, which is held in the active site (red star). Because of the cavity in TERT and the flexibility of the thumb, one DNA telomere repeat can be looped out, and the RNA template is fully translocated and freed for the next round of repeat synthesis. (c) The DNA hairpin loopout (in high FRET) is stabilized by TERT and the incoming dGTP. Binding of dGTP also promotes the DNA realignment (in low FRET). The FRET donor (green, D) and acceptor (red, A) are shown according to the published experiment¹⁵ (d) After realignment, DNA synthesis of the next round can take place. The locations of the FRET acceptor on DNA before and after realignment are indicated by faded and dark red, respectively. The thumb opening is hypothesized to facilitate both translocation and hairpin formation, and a closed thumb is required for DNA synthesis as in all DNA polymerases.