Mutation in TERT separates processivity from anchor-site function

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Abstract

Telomerase shows repeat-addition processivity (RAP): synthesis of multiple telomeric DNA repeats without primer dissociation. Leu14 mutants in the telomerase essential N-terminal domain of Tetrahymena thermophila telomerase reverse transcriptase retain full activity and anchor-site function but lose RAP, suggesting models for how this domain facilitates DNA translocation.

Telomerase is the complex of RNA and a reverse transcriptase (TERT) that synthesizes telomeric DNA repeats at chromosome ends, thereby contributing to genomic stability and cell immortalization. Although telomerase has only one catalytic center1, it copies the same small stretch of its internal RNA template numerous times before product dissociation2, indicating that translocation must occur to reposition the primer-template pair with respect to the active site. It has long been proposed that translocation is facilitated by an anchor site that binds telomeric DNA upstream of the 3′ end2,3. Biochemical evidence implicated the telomerase essential N-terminal (TEN) domain of TERT as forming at least part of the yeast4, Tetrahymena5,6 and human7 telomerase anchor sites (see also refs. 8,9.) The crystal structure of the TEN domain of Tetrahymena TERT revealed a single-stranded DNA binding groove that contributes to anchor-site function5.

We reconstituted TERT proteins containing mutations on the TEN domain surface (Fig. 1a) with telomerase RNA (TER) and tested extension of a single-stranded telomeric DNA primer in vitro. Several mutations (for example, G101V, Y106A, Q108A, Q168A and G144A) decreased telomerase activity but did not affect RAP, as evidenced by the fraction of the extension products corresponding to addition of multiple repeats (Supplementary Fig. 1a online). The L14A mutation, however, extended the telomeric primer Tel3a by one G, to the end of the template, but failed to synthesize a discernible ladder of higher multimers (Fig. 1b). Yet, L14A retained full activity (defined in Supplementary Methods online): the number of primers extended per unit of time for a constant amount of TERT was 130 ± 20% of that of wild-type telomerase (mean ± s.d., eight measurements). Under a wide range of
DNA primer concentrations (0.1−100 μM), dGTP concentrations (1−500 μM) and temperatures (25−42 °C), wild-type telomerase always showed processivity, whereas L14A gave predominantly the single extension product. Other primers that bind further upstream on the template were extended by L14A with unimpaired nucleotide-addition processivity (NAP) but, again, no RAP (Supplementary Figs. 1b and 2 online).

This RAP defect was not due to the substituted alanine, as various other amino acids, even isoleucine, were just as defective (Fig. 1b). Thus, L14A is a loss-of-function mutation. Because the preceding residue is semi-conserved among diverse TERT proteins, we also constructed the M13A mutant. It showed 50% RAP relative to wild-type telomerase (Fig. 1b; quantification described in Supplementary Methods). The phenotypes of these mutants identify a small patch on the surface of the TEN domain, adjacent to the DNA binding groove (Fig. 1a), that is involved in RAP. Met13 could contribute independently of Leu14, or could function by positioning and orienting Leu14.

Amino acids in other domains of TERT affect RAP10, so we made those mutants in our system to allow side-by-side comparison. We confirmed that these mutations reduce RAP (Supplementary Fig. 3 online), but none had the extreme reduction shown by L14A, again suggesting a key and specific role for Leu14.

A simple explanation for the phenotype of the L14A telomerase would be weakened binding of the DNA primer; rapid dissociation of the product after one round of synthesis would prevent RAP. Three independent tests of this idea caused us to discount this hypothesis.

First, we measured the amount of radiolabeled primer bound to L14A and other TERT mutants relative to wild-type TERT by photo–cross-linking (Fig. 2a). Each TERT was mixed with a truncation mutant missing the entire TEN domain (ΔTEN-TERT) as an internal control; the primer cross-links both in TEN and elsewhere within TERT, so the difference in intensity between the two bands reveals the TEN domain cross-linking. Instead of reduced primer cross-linking, L14A and M13A showed slightly increased cross-linking relative to wild-type TERT (Fig. 2a), which may reflect tighter binding of the primer to L14A or perturbations that bring the photoactive 5-iodo-deoxyuridine residues into better proximity to an aromatic residue in TEN.

To monitor the kinetics of DNA product release and productive binding and extension of a different primer, we conducted a ‘primer challenge experiment’2 (Fig. 2b). L14A and wild-type telomerase had similar half-times of 7.4 ± 0.9 min and 7.3 ± 0.4 min (mean ± s.d. over four experiments), respectively. Finally, we measured similar values of the rate constant for primer Tel3a dissociation, $k_{off} = 0.07 ± 0.01 \text{ min}^{-1}$ and $0.17 ± 0.08 \text{ min}^{-1}$ (mean ± s.d. over four experiments), for L14A and wild-type telomerase, respectively (Supplementary Fig. 4 online). Thus, the L14A mutant stops DNA synthesis after one round not because of premature release of the extended primer but presumably because it fails to undergo translocation.

Despite weak amino acid conservation in the N terminus of the TEN domain, it seemed that human Leu14 or perhaps the neighboring Leu13 might correspond to Tetrahymena Leu14 (Supplementary Fig. 5a online). Human telomerases reconstituted with L14A or L13A.
TERT had markedly decreased activity but retained RAP (Supplementary Fig. 5b). This result persisted with DNA primers representing all six permutations of the human telomeric sequence and was not rescued by a 100-fold increase in primer concentration (representative data in Supplementary Figure 6 online). In contrast, the L13A L14A double mutant was defective in RAP (Supplementary Fig. 5b). Apparently, a leucine at either position 13 or 14 is sufficient for RAP in human telomerase, whereas introduction of a leucine at position 13 did not rescue the Tetrahymena L14A mutant (data not shown). Mutation of amino acid 13 or 14 also resulted in a dramatic loss of activity of human telomerase assembled in living cells, so RAP could not be assessed (Supplementary Fig. 5c). Thus, human and Tetrahymena telomerase TEN domains both contribute to RAP but in somewhat different ways.

In conclusion, Tetrahymena Leu14 mutants provide new evidence for the essential role of the TEN domain in RAP. Because Leu14 mutants show normal activity and NAP, it seems that Leu14 does not merely make the same interaction at every nucleotide-addition step but rather has a function specific to the translocation step. In one model, Leu14 acts as the pawl in a molecular ratchet11 (Supplementary Discussion online). Alternatively, Leu14 may serve as a ‘door latch’ connecting the TEN domain to another domain of telomerase and mediating an intramolecular switch necessary for RAP (Fig. 3 and Supplementary Movie online). Clearly, repeat-addition processivity involves numerous protein, RNA12,13 and DNA elements moving in a stepwise or concerted manner relative to each other. Identification of the role of Leu14 is necessary but not sufficient for a complete understanding of this unique enzymatic property of telomerase.

Supplementary Material

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References

1. Lingner J, et al. Science. 1997; 276:561–567. [PubMed: 9110970]
2. Greider CW. Mol. Cell. Biol. 1991; 11:4572–4580. [PubMed: 1875940]
3. Hammond PW, Lively TN, Cech TR. Mol. Cell. Biol. 1997; 17:296–308. [PubMed: 8972210]
4. Lue NF, Li Z. Nucleic Acids Res. 2007; 35:5213–5222. [PubMed: 17670795]
5. Jacobs SA, Podell ER, Cech TR. Nat. Struct. Mol. Biol. 2006; 13:218–225. [PubMed: 16462747]
6. Romi E, et al. Proc. Natl. Acad. Sci. USA. 2007; 104:8791–8796. [PubMed: 17494734]
7. Moriarty TJ, Marie-Egyptienne DT, Autexier C. Mol. Cell. Biol. 2004; 24:3720–3733. [PubMed: 15082768]
8. Finger SN, Bryan TM. Nucleic Acids Res. 2008; 36:1260–1272. [PubMed: 18174223]
9. Wyatt HD, Lobb DA, Beattie TL. Mol. Cell. Biol. 2007; 27:3226–3240. [PubMed: 17296728]
10. Miller MC, Liu JK, Collins K. EMBO J. 2000; 19:4412–4422. [PubMed: 10944124]
11. Bar-Nahum G, et al. Cell. 2005; 120:183–193. [PubMed: 15680325]
12. Lai CK, Miller MC, Collins K. Mol. Cell. 2003; 11:1673–1683. [PubMed: 12820978]
13. Mason DX, Goneska E, Greider CW. Mol. Cell. Biol. 2003; 23:5606–5613. [PubMed: 12897134]
Figure 1.
Leu14 mutants of *Tetrahymena* TERT have a specific defect in RAP. (a) Surface representation of the crystal structure of the TEN domain of *Tetrahymena* TERT5. Red, DNA binding groove; yellow, amino acids 13 and 14, the main subject of this study; blue, other amino acids mutated for comparison (Supplementary Fig. 1a). (b) Direct telomerase activity assays with recombinant TERT and telomerase RNA, primer Tel3a ((GGGGTT)_3 at 1 μM and 10 μM), ^32^P-dGTP and unlabeled dTTP. Right, diagram showing pairing of primer Tel3a with the RNA template, with added nucleotides in red.
Figure 2.
L14A mutation does not disrupt the anchor site. (a) Photo–cross-linking assay for primer binding. The anchor-site mutant Q168A serves as negative control. Below left, gel showing relative amounts of $^{35}$S-labeled protein in each sample. Below right, bar graph showing calculation of single-stranded DNA cross-linking to the mutant TEN domains relative to the wild-type (WT) TEN domain, normalized to the amount of each protein present, following the method of ref. 5. Error bars represent mean ± s.d. for five independent experiments. CON, control nontelomeric oligonucleotide, IU, 5-iodo-U residue. (b) Primer challenge experiment shows that L14A recycles primers at a rate similar to WT telomerase. The telomerase reaction was initiated with a saturating amount of primer Tel3a; the enzyme was allowed to extend for 2 min and was then challenged with a ten-fold excess of a second ‘challenge’ primer, TTTel3a, which has two additional thymines at its 5' end (Supplementary Methods). 1:10, Tel3a and challenge primer were mixed at a 1:10 ratio at time zero.
Figure 3.
Model. (a) Direction that the template primer must move relative to a single active site. (b) Model in which Leu14 provides a door latch holding the TEN domain close to the remainder of TERT or TER (‘closed state’, I). One round of telomeric DNA synthesis moves the template-primer pair relative to the active site, resulting in a high-energy state (II) that is then relaxed by breaking the interaction of Leu14 with the rest of the telomerase (‘open state’, III). With only a few base pairs between primer and template, melting can occur at any time during the cycle. Repositioning the RNA template and restoring base-pairing in the alignment region of the RNA template occur between states III and IV. Sliding of the DNA through the TEN domain allows Leu14 to re-establish its intramolecular protein-protein interaction (IV); stated in thermodynamic terms, restoring this protein-protein interaction compensates for the energy required for translocation. State IV is essentially identical to state I, and the system is ready for another round of telomeric DNA synthesis. In Leu14 mutants, the door latch would always be open and the system would stall at state III.