Structure of active dimeric human telomerase

Anselm Sauerwald1,2,5, Sara Sandin1,3,5, Gaël Cristofari2,4, Sjors H W Scheres1, Joachim Lingner2 & Daniela Rhodes4,3

Telomerase contains a large RNA subunit, TER, and a protein catalytic subunit, TERT. Whether telomerase functions as a monomer or dimer has been a matter of debate. Here we report biochemical and labeling data that show that in vivo–assembled human telomerase contains two TERT subunits and binds two telomeric DNA substrates. Notably, catalytic activity requires both TERT active sites to be functional, which demonstrates that human telomerase functions as a dimer. We also present the three-dimensional structure of the active full-length human telomerase dimer, determined by single-particle EM in negative stain. Telomerase has a bilobal architecture with the two monomers linked by a flexible interface. The monomer reconstruction at 23-A resolution and fitting of the atomic structure of the TERT subunit from beetle Tribolium castaneum into the EM density reveals the spatial relationship between RNA and protein subunits, providing insights into telomerase architecture.

A full understanding of telomerase function requires information on the three-dimensional (3D) structure of telomerase. This has been hindered by the difficulty in obtaining sufficient quantities of purified, active and full-length telomerase complex. Therefore, in contrast to other important enzymes, such as the ribosome and RNA and DNA polymerases, knowledge of the telomerase structure has been limited to subdomains of the TER and TERT subunits19–21. Analysis of amino acid sequence and mutational studies have established that the TERT subunit contains three domains: an N-terminal G-overhang–binding (TEN) domain, the RNA-binding domain (TRBD) and a C-terminal domain consisting of the reverse-transcriptase domain (RTD; Fig. 1a)7,20. The most comprehensive structural information on the TERT subunit comes from the crystal structure of the beetle T. castaneum TERT in complex with a short DNA-RNA helix22,23, which confirms a structural conservation with other reverse transcriptases such as HIV reverse transcriptase24 and reveals the relative location of the TRBD with respect to the RTDs within TERT25. This beetle TERT subunit lacks the TEN domain present at the N terminus of most TERTs, including human TERT (hTERT), but the structure of this domain is known from the crystal structure of the isolated TEN domain of the ciliate T. thermophila26.

Although the length of the telomerase RNA subunit is less well conserved than that of the protein subunit (which ranges from 147 nucleotides in protozoa to almost 2,000 nucleotides in budding yeast), phylogenetic and functional studies have revealed that all TERTs contain two conserved structural elements: the catalytically essential pseudoknot–template core domain and a stem-terminus element called CR4-CR5 in vertebrates27, both of which have been shown to interact with TERT28,29. Structural information on the TER subunit is restricted to isolated segments of the RNA, including the functionally

1Medical Research Council Laboratory of Molecular Biology, Cambridge, UK. 2Swiss Institute for Experimental Cancer Research, School of Life Sciences, Frontiers in Genetics National Center of Competence in Research, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland. 3School of Biological Sciences, Nanyang Technological University, Singapore. 4Present address: Institute for Research on Cancer and Aging, Faculty of Medicine, University of Nice–Sophia-Antipolis, Nice, France. 5These authors contributed equally to this work. Correspondence should be addressed to J.L. (joachim.lingner@epfl.ch) or D.R. (DRhodes@ntu.edu.sg).

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Human telomerase is a dimer. Despite the structural information on TER and TERT subdomains, little is known about the overall structure of human telomerase and how the various domains contribute to the architecture of full-length telomerase to form a functional enzyme. Here we present what is, to our knowledge, the first 3D structural analysis of active, full-length human telomerase, by single-particle EM, which revealed a dimeric bilobal structure, as well as experimental evidence that the two TERT subunits in the telomerase dimer cooperate in catalytic activity.

RESULTS

In vivo–assembled human telomerase is a dimer

To obtain sufficient quantities of purified, active human telomerase for structural analyses, we made use of HEK293T ‘super­telomerase’ cells that overexpress hTER and hTERT from transiently transfected plasmid constructs and have 200-fold higher telomerase activity than untransfected HEK293T cells. The purification uses the protein-A tag introduced at the N terminus of hTERT, with subsequent affinity chromatography and fractionation on a sucrose gradient. To facilitate the tracking of telomerase in the sucrose gradient, we incubated telomerase with a 5′-32P-labeled G overhang oligonucleotide substrate (5′-(TTAGGG)2-3′) before the sedimentation. We observed a well-resolved peak that sediments in the same position as the 670-kDa thyroglobulin size marker analyzed in a parallel sucrose gradient (Fig. 1b and Supplementary Fig. 1). We obtained two-dimensional (2D) and 3D structural information by single-particle classification (Fig. 1e) and electron tomography (Fig. 1f), respectively. These analyses revealed that telomerase has an elongated structure, 280 Å in length and 125 Å in width, consisting of two spherical lobes connected by a region of thin density. The size of the telomerase particle is consistent with the estimated molecular weight (670 kDa), and the distribution of density into two lobes of similar size suggests the presence of a homodimeric complex. To establish whether the telomerase complex can bind one or two telomers, we incubated a purified telomerase preparation with a G overhang oligonucleotide 5′-(TTAGGG)2-3′, labeled at its 5′ end with biotin and 5-nm colloidal gold coated with monovalent streptavidin. Colloidal gold can clearly be recognized as 5-nm black dots bound to telomerase dimers, whereas excess monodispersal gold has a thin streptavidin

**Figure 1** Human telomerase is a dimer. (a) Schematic of hTERT domain arrangement showing the essential N-terminal domain involved in DNA binding (TEN), the RNA-binding domain (TRBD), the reverse transcriptase domain (RTD) that contains the catalytic site and the C-terminal extension (CTE). (b) Elution profile of the in vivo–assembled human telomerase in complex with the G overhang oligonucleotide 5′-(TTAGGG)2-3′ fractionated on a sucrose gradient. (c) Telomerase activity profile of a telomerase sample in the absence of a G overhang, fractionated as in b. SG, sucrose gradient. G overhang–bound (b) and unbound (c) telomerase complexes migrate in the same position on the sucrose gradient. (d) Electron micrograph of negatively stained telomerase bound to the G overhang. Top views are indicated by black circle and side views by white circles. (e) Example of reference-free 2D class averages. (f) 3D structure obtained by subtomogram averaging. (g,h) Electron micrographs of telomerase bound by 5-nm colloidal gold coated with streptavidin in the presence (g) and absence (h) of telomerase. Circles denote telomerase dimers in complex with one gold particle (red) or two gold particles (yellow). Arrows indicate colloidal gold particles that are not bound to telomerase. Scale bars, 50 nm (d,g,h) or 5 nm (e,f).
Figure 2  Active human telomerase functions as a dimer. (a) Western blot analysis, using an anti-C-terminal hTERT antibody, of TEV protease–treated input whole-cell lysates (left) and anti-ZZ immunoaffinity-purified and TEV protease–released telomerase complexes (right). Whole-cell lysate of untransfected HEK 293T cells was used as an expression control. Left, the intensities of the TERT subunit bands are consistent with input expression plasmids. Right, the intensities of the bands are consistent with the release of Flag-hTERT homodimers and Myc-hTERT–Flag-hTERT dimers or Myc-hTERT–Flag-hTERT dominant negative (DN) dimers (DN refers to V709A D710I in motif A in TERT). Top, ratios of expression plasmids used in transient transfection. (b) Telospot telomerase activity assay of input whole-cell lysate (left) and immunoaffinity-purified and TEV-released telomerase complexes (right). Telomerase activity in the transfected cells is shown as a multiple of the immunopurification and TEV-released telomerase complexes, showing that catalytic-pocket mutant hTERT (DN) has a dominant negative effect on telomerase activity. We next asked whether the two TERT subunits in a telomerase complex function independently of each other or whether they cooperate for catalytic activity. For this analysis, we generated a catalytically dead active site mutant (V709A D710I)34, ZZ–TEV site–3× Flag–hTERT (DN) (Supplementary Fig. 2a,b). This mutant, or the corresponding wild-type ZZ–TEV site–3× Flag–hTERT (WT), was coexpressed with wild-type 13× Myc–hTERT (WT) at plasmid ratios of 1:1 or 6:1. The western blot analysis (Fig. 2a, lanes 2–5) showed that the wild-type and mutant TERT subunits are expressed in the expected ratios. To test whether TERT subunits function independently or cooperate in telomeric DNA synthesis, we affinity-purified the telomerase complexes by the ZZ tag and released the complexes by TEV cleavage as described above (Fig. 2a, lanes 6–9). Thus, with the ZZ-tagged hTERT mutant, released complexes contained wild-type hTERT only as wild-type–mutant heterodimer but not as wild-type–wild-type homodimers (Fig. 2a, lanes 6 and 8). Notably, quantification of telomerase catalytic activity by both the telospot and direct telomerase activity assays14 (Fig. 2b,c, lanes 6 and 8) revealed that the telomerase complexes containing a mixture of catalytically inactive and catalytically active hTERT are totally catalytically inactive for processivity of both repeat addition and nucleotide addition (Fig. 2c, lanes 6 and 8; quantification in Supplementary Fig. 2c,d, lanes 2–5, 7 and 8). In contrast, the wild-type–wild-type TERT-containing complexes are active, as expected (Fig. 2b,c, lanes 7 and 9). Therefore, the presence of a catalytically inactive TERT subunit in the telomerase complex has a dominant-negative impact on catalytic activity. These results establish not only that the human telomerase is structurally a dimer but also that telomerase activity requires the cooperation between the two TERT subunits in a dimer.

3D reconstruction of the telomerase dimer

For single-particle EM analysis of the telomerase dimer bound to the G-overhang oligonucleotide 5′-(TTAGGG)2-3′, the sucrose gradient fractionation described above (Fig. 1b) was carried out in the presence of 0–0.1% glutaraldehyde (GraFix)35. This gentle cross-linking...
method has previously been demonstrated to improve structural homogeneity, particularly for low-abundance complexes that may otherwise dissociate during the preparation of EM grids. Notably, parallel sucrose gradient fractionations of purified telomerase samples in the presence or absence of glutaraldehyde show the same sedimentation profile (Fig. 1b). This indicates that the cross-linking did not affect the structural composition and that the cross-linked telomerase sample represents the active conformation of the enzyme (Fig. 1c). After the sucrose gradient fractionation, the concentration of the purified telomerase was very low, and this precluded 3D structure determination by cryo-EM. After concentrating the sample (also shown in Supplementary Movie 1), in which the two monomers in the dimer exhibit considerable differences in their relative orientations. (f) Four views of a refined dimer density map after MLF3D classification. The relative rotations are indicated. Double asterisk, V-shaped opening in the top monomer; single asterisk, closed view of the lower monomer. (g) Individual telomerase dimers in complex with biotinylated (TTAGGG)_2 bound to two streptavidin-coated gold particles. The distance between the two gold particles in the dimer is 180–190 Å. (h) Structure of the dimer, showing the locations of the two G overhang–binding sites and the hinge region. Scale bars, 50 nm (a) or 10 nm (b–d,g).

We obtained a representative field view of the raw telomerase particles in negative stain, in which top views and side views of the telomerase dimer can clearly be recognized (Fig. 3a). A data set containing 20,127 telomerase particles was subjected to 2D class averaging by reference-free procedures using ~200 images per class (image-processing procedures are described in Supplementary Note). From this, we obtained a gallery of 2D class averages representing top, tilted and side views (Fig. 3b–d). Two representative class-average side views after rotational alignment displayed a bilobal structure in which the two lobes are connected by a thin region of density (Fig. 3e). One of the two lobes has a V-shaped opening, and the other appears closed (Fig. 3f). Inspection of the rotationally aligned class averages revealed similar monomer densities; however, the relative orientation of monomers in the dimer differed. The difference detected is illustrated by a simple movie composed of these two 2D class averages in sequence (Fig. 3e and Supplementary Movie 1). This analysis suggests that telomerase has a flexible dimer interface that could act as a hinge region.

In addition to 2D classification, we refined the 3D structure of the telomerase dimer by using the low-resolution subtomogram average (Fig. 1f) as a reference model for 3D single-particle refinement (Supplementary Note). Distinct conformations were further classified by 3D maximum-likelihood analysis in Fourier space (MLF3D). After 25 rounds of nonsupervised angular refinement, four reconstructions (Supplementary Fig. 3c–f) were calculated, and the absolute hand and the overall correctness of the dimer structure were assessed by using a modified version of the tilt-pair validation method37 (Supplementary Fig. 4). Different orientations of a representative density map calculated from a subpopulation of 3,659 particles at a resolution of 30 Å were obtained (Fig. 3f). Top views, tilted views and side views of the dimer density agree well with independently obtained reference-free 2D class averages (Fig. 3b–e). These features include a characteristic V-shaped opening in one of the monomers, whereas the other is more closed. From the location of the nanogold-labeled G overhang, we estimate that the distance between the two catalytic pockets in a telomerase dimer is 180–190 Å (Fig. 3g). To test whether the difference in monomer conformation observed was due to subsaturated levels of bound G-overhang oligonucleotide, we repeated the 2D classification (as described above) of a telomerase prepared in absence of DNA. No difference could be detected between class averages of telomerase in the presence (Fig. 3b) or absence of bound DNA (data not shown), which indicates that the conformation of the monomers as well as the flexibility of the dimer interface is independent of G-overhang binding. In summary, the 3D structural determination of the telomerase dimer established that the two monomers in the dimer have different conformations, one open and one closed, and they are linked by a flexible dimer interface (Fig. 3h). Future analyses will be required to establish the role of the structure dynamics of the telomerase in the binding of two telomere substrates and in synthesis of telomeric DNA.

Independent of refined monomers and composite dimers
To circumvent refinement problems arising from the flexibility in the telomerase dimer interface, we processed the two halves of the telomerase dimer independently (Fig. 4). From each class average of the telomerase dimer (excluding top views), we manually measured the center of each monomer and calculated the corresponding centers in the raw image stack. Next, we boxed out subimages (as shown in Fig. 4a), keeping images corresponding to the open V-shaped monomer separate from those of the closed monomer. The 3D refinements were carried out independently for the two monomer types by MLF3D as described above. As expected, the resolution of the monomer structures markedly improved, with measurements of 23 Å for the open monomer and 21 Å for the closed one (Fig. 4b). Although both structures had similar resolutions, the V-shaped open-monomer density had more clearly defined density distribution, as can be explained by...
 Independently refined monomers and composite dimers. (a) Left, boxed-out areas used for the independent refinement of both the open and closed monomer. (b) Four views of the open monomer (top) and closed monomer (bottom), in which the side views are related by a 90° rotation around the horizontal axis. (c) Comparison of three 2D class averages (top) from which three composite dimers were reconstructed (middle) and the corresponding projections (bottom). (d) Surface-rendered refined monomer reconstructions (nontransparent) placed within the EM density of the telomerase dimer (wire frame).

alignment procedures that, in general, work better with pronounced features. The dimer interface was not refined with this procedure, and therefore we removed it from side views shown in Figure 4b.

Next, we reassembled the independently refined 3D monomer reconstructions into composite dimers on the basis of the angles previously measured in the individual 2D class averages. The composite dimer had many structural features in common with the density calculated for the telomerase dimer but is better resolved (Fig. 3f compared to Fig. 4d). We compared three 2D class averages, the corresponding composite 3D reconstruction and projections of composite dimers (Fig. 4c). The close similarity between features in the 3D EM density of composite dimers and the 2D class averages indicates that the open and closed monomers were well-refined.

The open-monomer reconstruction shows several distinct features that allowed the fitting of the atomic structure of the beetle TERT subunit22,23 into the EM density (Fig. 5a–c). We did this by using an automatic six-dimensional search in real space, for which only one solution was obtained. The excellent fit is supported both by the similarity in the overall shape of the protein subunit and by the coincidence of the catalytic-core channel in the crystal structure, with a channel in the EM density (Fig. 5c). These features can also be recognized in the crystal structure of the beetle TERT after filtering to 20-Å resolution (Supplementary Fig. 5). To validate our assignment of the TERT subunit to the correct region of the EM density map, we first tested different antibodies to pinpoint the protein subunit. Although we could detect binding, the EM images were not informative, as the molecular weight of IgG is in the same range as that of the TERT subunit. Instead, we produced and purified telomerase (as described above) in which the TERT subunit contained an N-terminal histidine tag (ZZ–TEV–6× His–5× AAKE–37-Å-long α-helix–3× Flag–hTERT). We identified the location of the histidine tag by using Ni-NTA–coated 5-nm colloidal gold. Visual inspection of particles showed that the majority of the gold particles were located at the periphery of the telomerase dimer (Fig. 5d). To obtain the distribution of the location of the gold particles in the telomerase dimer, we measured the distance from the center of 100 gold particles to the dimer interface. The resulting plot (Fig. 5e) shows a pronounced peak at 110-Å distance, which is in good agreement with the fitted location of the TERT subunit in the EM density of the open monomer (Fig. 5a–c).

DISCUSSION

The determination of the first 3D structure, to our knowledge, of active full-length human telomerase assembled in vivo shows that it has a bilobal architecture and a molecular weight consistent with it being a dimeric complex (Fig. 1). These data, together with our biochemical data (Fig. 2), provide strong evidence that the bilobal structure arises from the presence of two TERT subunits and two TER subunits and can bind two telomeric G-overhang substrates. For the TERT subunit, the crystal structure of the beetle TERT subunit23,24 fits well into the EM map, thus revealing the location of the RNA-binding TRBD and catalytic RTDs in TERT. For in vivo–assembled telomerase, catalytic activity required the cooperation between two TERT subunits in the telomerase dimer. A catalytic-pocket mutation in one of the two TERT subunits was dominant negative (Fig. 2). These observations substantially strengthen our previous conclusions derived from...
studies on an in vitro–assembled telomerase, wherein an RNA-template mutant interfered with wild-type template function when coassembled into telomerase particles. They are also in agreement with molecular-weight estimations by us and others. However, our results are in disagreement with publications in which it has been concluded that human telomerase is monomeric. Our work also underlines the importance of revisiting the conclusion that short telomere disease–associated telomerase mutations are not dominant negative.

Why should telomerase function as a dimer? Our work demonstrates that a human telomerase dimer contains two catalytic pockets, located about 180 Å apart (corresponding to a stretch of ~50 nucleotides), that can associate with two telomeric DNA substrates (Fig. 3). This suggests that telomerase may function by extending two telomeric ends in parallel. Notably, it has been demonstrated recently that the cohesion of replicated telomeres is important for telomere maintenance by telomerase, a function that is defective in dyskeratosis congenita–associated mutations in TIN2 (ref. 40). Therefore, an attractive hypothesis is that telomerase dimers extend aligned sister telomeres in parallel. This mechanism could enable sister telomeres to maintain equal lengths after extension. In contrast, our data showing that the two TERT subunits in a telomerase dimer cooperate in telomerase activity could also have been explained with a mechanism in which telomerase binds and extends a single DNA substrate in a processive manner, as has been suggested from studies using partly purified Euplotes telomerase. The observed flexibility of the interface between the two monomer lobes in the telomerase structure (Supplementary Movie 1) could be functionally important by providing a hinge mechanism for the intramolecular translocation of the DNA substrate from one catalytic pocket to the other, as has been reported for other DNA and RNA polymerases such as the eukaryotic primosome. However, because the mutant–wild-type telomerase heterodimers were inactive even for the first round of telomeric DNA synthesis (Fig. 2c), which according to the translocation model should not require two active sites, we favor the parallel extension model.

The identification of the location of the TERT subunit in the EM density (Fig. 5) provides important insights into the domain organization of the telomerase dimer. First of all, the TERT subunit is located at the periphery of the open monomer, where it is separated from the rest of the complex by the V-shaped opening in the EM density. Because the fitted crystal structure of the beetle TERT subunit corresponds to two-thirds of hTERT, the majority of the EM density that is not occupied by the TERT subunit has to correspond primarily to RNA, that is, the hTER subunit. This interpretation agrees with the observation that the EM map in that region consists of three continuous regions of tubular density with diameters of 20–30 Å (Fig. 5a–c), which is an expected feature of RNA helices (Supplementary Fig. 5a).

This interpretation of the telomerase architecture implies that the dimer interface involves RNA–RNA interactions (Fig. 5f), which is in agreement with previous results. However, we could not dissociate dimeric TERT into monomers by RNase treatment (data not shown), and therefore we cannot exclude the involvement of some telomerase-accessory protein in the formation of the dimer interface. Because the crystal structure of the beetle TERT subunit was solved in complex with a short DNA-RNA helix, its fitting into the EM density also pinpoints the location of the 3′ end of the G overhang in the catalytic pocket of the RTD (Fig. 5f). The consequence of this fitting is that the 5′ end of the G overhang points toward the center of the monomer and a continuous region of density in its vicinity that we have tentatively assigned to the TER subunit (Fig. 5 and Supplementary Fig. 5), which suggests that the DNA-binding TEN domain would be located adjacent to TRBD domain (Fig. 5f), consistent with the TERT domain structure (Fig. 1a).

Future work will aim at improving the resolution of the telomerase structure to reveal the fold of the telomerase RNA and its specific interaction with the various TERT domains as well as how the different conformational states that we observed may relate to different functional states of the telomerase catalytic cycle. This study also provides the starting point for investigating the structural basis of telomerase dimerization and its significance for catalysis and function.

METHODS Methods and any associated references are available in the online version of the paper.

Accession codes. EM maps were deposited in the EMDB, with accession codes EMD-2312, EMD-2311 and EMD-2310.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS A.S. designed and carried out all the biochemical work and contributed to image processing. S.S. collected EM data and solved the structures. G.C. developed the super-telomerase cells. S.H.W.S. designed and contributed to the structure refinement. J.L. and D.R. designed and supervised the project. All authors contributed to the writing of the paper.

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ONLINE METHODS

Telomerase expression and purification. The telomerase complex was overexpressed in HEK293T cells46 transiently transfected with hTERT- and hTER-expressing plasmid DNA (pVan145: pCDNA6–ZZ–TEV–3′–Flag–hTERT (WT); pCDNA6–ZZ–TEV–3′–Flag–hTERT (DN); or pCDNA6–13×Mye–hTERT (WT); and pBS-U1–KTR) as described45, and whole-cell lysates (WCL) were prepared as described46. For telomerase-affinity purification, the WCL was adjusted to a protein concentration of 4 mg/ml with buffer A (20 mM HEPES-KOH, pH 7.9, 2 mM MgCl₂, 300 mM KCl, 10% glycerol (v/v), 1 mM DTT, 1 mM EDTA, 0.1% Triton X-100 (v/v), 1 mM PMSF) and clarified by filtration to produce the input. To 20 ml input, 1 ml buffer A was added and dialyzed extensively with ice-cold buffer A. Telomerase was released from 1 ml IgG Sepharose by overnight incubation with 5 ml 0.05 mg/ml TEV protease (>90% efficient) in buffer A at 4 °C. In a second affinity purification step, the soluble telomerase-containing fraction was applied to a 1-ml HiTrap heparin HP column (GE Healthcare, 17-0406-01) previously equilibrated with buffer A and washed with 10 column volumes of buffer A to remove unbound proteins. Bound proteins were eluted with a gradient of 0.3–1 M KCl in buffer A. Fractions containing telomerase were identified by using a direct telomerase activity assay (Fig. 1c) as described47, and active fractions were pooled, concentrated, and dialyzed against buffer B (20 mM HEPES-KOH, pH 7.9, 2 mM MgCl₂, 150 mM KCl, 10% glycerol (v/v), 1 mM DTT, 1 mM EDTA, and 1 mM PMSF). Telomerase yields were quantified by northern blot analysis using KTR run-off transcripts as standards, and the purity was assessed by SDS-PAGE.

Telomerase sucrose-gradient sedimentation in combination with fixation (GraFix). For EM analysis, the telomerase complex was further purified by using GraFix47. Briefly, solutions of buffer B containing 5% and 30% sucrose were prepared in conical tubes. Glutaraldehyde (0.1%; EM-grade, 25%, Science Services) was added to the high-density solution only. Gradients of (4 ml) for centrifugation in a Beckman SW60 rotor were prepared by using a gradient former (Gradient Master 107, BioComp Instruments). Freshly prepared gradients were kept at 4 °C for 1 h before sample loading. Subsequently 200 µl containing 10–30 pmol of purified telomerase bound to 5 µl reactions containing 1′-[32P]dGTP (3,000 Ci/µmol) and 1 µM of telomeric primer 5′-biotin-(T₆₋₄₅₈₃₋₃′). Reactions were stopped by the addition of 3 mM EDTA and 1% SDS. Biotinylated telomeric primers were recovered with Dynabeads M-280 streptavidin (Invitrogen), following the instructions provided by the manufacturer. Beads were resuspended in 98% formamide containing 10 mM EDTA and 0.005% xylene cyanol, heated to 95 °C for 5 min and analyzed on 12% polyacrylamide-urea sequencing gels.

Western analysis. For western analysis, extract (4 µl) was boiled for 5 min in Laemmli sample buffer and fractionated on 4–15% SDS-PAGE gradient gels. Immunoblots used standard protocols and an affinity-purified rabbit polyclonal antibody against hTERT (1:10,000, R484)48. A secondary IRDye 800CW goat anti-rabbit IgG (Li Cor cat. no. 926-32210) was used (1:7,000). The Li-Cor infrared fluorescence detection of IRDye infrared dye substrate and imaging system was used to detect bound antibodies.

Teloposť assay. Teloposť telomerase activity assays were performed as previously described49. Briefly, telomerase activity reactions were carried out for 45 min at 30 °C in 20-µl reactions containing 1 µl of cell extract, 50 nM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM spermidine, 1 mM MgCl₂, 0.5 mM dATP, 0.5 mM dTTP, 2 mM dGTP, 20 µCi of [α-32P]dATP (3,000 Ci/µmol) and 1 µl of telomeric primer 5′-biotin-(T₆₋₄₅₈₃₋₃′). Reactions were stopped by the addition of 3 µl of 50 mM EDTA and 1% SDS. DNA samples were UV cross-linked to the membrane with the auto–cross-link function of a Stratalinker (Stratagene). Without prior denaturation, membranes were blocked for 1 h at 60 °C with church buffer. After overnight hybridization at 60 °C with a randomly labeled probe derived from a 600-bp TTAGGG repeat-containing DNA fragment, membranes were washed twice for 15 min with 2× SSC buffer at room temperature and twice for 30 min with 2× SSC, 0.1% SDS at 65 °C and exposed to a phosphorimager screen. Spot intensities were quantified by using 2D densitometry and the Aida software (Raytest).

Additional methods. Further methodology can be found in Supplementary Note.