

Telomerase structural biology comes of age

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Abstract

Telomerase is an RNA-protein complex comprising telomerase reverse transcriptase, a non-coding telomerase RNA, and proteins involved in biogenesis, assembly, localization, or recruitment. Telomerase synthesizes the telomeric DNA at the 3'-ends of linear chromosomes. During the past decade, structural studies have defined the architecture of *Tetrahymena* and human telomerase as well as protein and RNA domain structures, but high-resolution details of interactions remained largely elusive. In the past two years, several sub-4 Å cryo-electron microscopy structures of telomerase were published, including *Tetrahymena* telomerase at different steps of telomere repeat addition and human telomerase with telomere shelterin proteins that recruit telomerase to telomeres. These and other recent structural studies have expanded our understanding of telomerase assembly, mechanism, recruitment, and mutations leading to disease.

Introduction

Telomerase is a ribonucleoprotein complex (RNP) essential for maintenance of chromosome ends in most eukaryotes. Telomerase synthesizes multiple copies of short G-rich telomeric repeats (dTTAGGG in humans, dTTGGGG in ciliates) found in telomeres[1]. Telomerase is active in stem cells and most cancer cells, but inactive in somatic cells[2]. Mutations in telomerase holoenzyme components lead to diseases collectively called telomere biology disorders, and telomerase upregulation appears essential for the immortalization of cancer cells[2].

All telomerases contain a unique telomerase reverse transcriptase (TERT) that utilizes a templating region within its component telomerase RNA (TER, TR) to processively synthesize multiple copies of the G-strand repeat[1]. The repetitive use of the same template, which requires template translocation back to the starting point for each round of telomere repeat addition, is unique to telomerase. Additional species-specific proteins associate with TERT and/or TER to form the holoenzyme; these proteins play various essential roles *in vivo* for biogenesis, assembly, localization, or recruitment (Figure 1). The low levels of telomerase in

most cells, variation in TER, and variety of different biogenesis proteins among organisms have made structural studies of telomerase challenging.

Early structural studies focused on protein and TER domain structures using NMR and crystallography. Almost a decade ago, negative stain electron microscopy (EM) structures of telomerase were reported for *Tetrahymena*[3] and human[4]. The first cryo-EM structure of telomerase (~9 Å), for *Tetrahymena*[5], was published in 2015, followed in 2018 by structures of *Tetrahymena*[6] (4.8 Å) and human[7] telomerase (~8 Å) with telomeric DNA bound. In 2021-2022, four publications on cryo-EM structures of human telomerase[8-11] and one on *Tetrahymena* telomerase[12] at resolutions ranging from 3.3 Å to 3.9 Å appeared. These and other structural studies from the past two years, including some yeast telomerase proteins[13-15], are reviewed here.

***Tetrahymena* telomerase structure and mechanism**

Both telomerase and telomere DNA repeats were discovered in the ciliated protozoan *Tetrahymena*[16,17]. Early EM studies[3,5] proved useful in the identification of several constitutively associated accessory proteins (p50, p75, p45, p19, Teb1, Teb2, Teb3), that appeared to be unique to *Tetrahymena*, as orthologs of telomere binding or associated proteins that only transiently associate with human telomerase (TPP1, CTC1, STN1, TEN1, POT1, respectively)[18-20] (Figure 1a,b). *Tetrahymena* telomerase core RNP comprises TERT, TER, and a biogenesis protein p65[21] (Figure 1a,c). TERT contains RNA binding domain (RBD or TRBD), reverse transcriptase domain (RT; palm and fingers), and C-terminal extension (CTE; thumb) that form a TERT ring, and a telomerase essential N-terminal domain (TEN) unique to TERT[20]. TEN, which is connected to RBD by a flexible linker, forms a complex with another TERT-unique region within RT named TRAP (for its physical role in trapping TER as well as a functional role in regulating telomerase repeat addition processivity[6]; previously called IFDb[22]). Two helices, IFDa and IFDc[22,23], also found in some other reverse transcriptases including the *Tribolium castaneum* (flour beetle) TERT-like protein[24], flank TRAP. TER is a rapidly evolving non-coding RNA that includes two regions essential for catalysis: the template

and pseudoknot domain (t/PK) that forms a circle closed by a helix; and a stem-terminus element (STE), called stem-loop 4 (SL4) in *Tetrahymena* and CR4/5 or three-way junction in human[25] (Figure 1c,d). STE interacts with RBD and CTE to stabilize the TERT ring, and t/PK encircles the TERT ring. The template and adjacent single-stranded RNA traverse RBD-RT-CTE on one side of the TERT ring and TEN-TRAP above it completes the catalytic cavity.

In 2021, He *et al.*[12] published structures of *Tetrahymena* telomerase with telomeric DNA at three steps of telomere repeat synthesis, at 3.3, 3.8, 4.4 Å resolution (Figure 2a,c-f). Although all proteins of the holoenzyme are present in these samples, the flexible p75-p45-p19 (*Tetrahymena* CST) was masked out during cryo-EM data processing to improve the resolution of the catalytic core. Together with a structure at another step[6], analysis of the catalytic cores provided new insights into mechanism. Most notably, all four structures showed an RNA template-DNA duplex length of four base pairs (or five, prior to nucleotide translocation out of the active site) (Figure 2e,f). Consequently, the separation of the two strands for the template translocation step should not require much energy; rather, the short duplexes would need to be stabilized in the catalytic cavity during telomere repeat synthesis. The authors proposed that TEN-TRAP (Figure 2a) and a newly identified bridge loop motif in the RBD, along with previously observed motifs, help retain the short template-DNA duplex throughout nucleotide addition and also have an essential role in template translocation (Figure 2b). On the template 3'-side, TER enters a TRAP-TH (thumb helix) channel. Comparison of the telomerase structures revealed fixed anchors and flexible linkers on either side of the template that determine template boundaries and allow template movement through the active site[6,26], respectively (Figure 2c,e,f). The authors presented a model for all the steps of telomere repeat synthesis, including the template translocation step. Structural proof of the latter will require trapping intermediates in that step.

The 3.3 Å resolution structure[12] also revealed unexpected interactions for the telomerase La related group 7 (LARP7) protein p65 with TER (Figure 1c,e; see also Figure 4e,f). LARP7 proteins have a La module (La motif and RRM) that binds the 3' polyU end of RNA polymerase

III transcripts and a C-terminal xRRM that binds a specific site[27]. p65 xRRM bends TER stem 4 (S4) to help position loop 4 (L4) at the interface between TERT RBD and CTE [6,28]. Despite lower resolution for the rest of p65 in the cryo-EM map, the authors succeeded to model the La motif[12]. Surprisingly, it interacts not only with the 3'-UUUU-OH, but also binds the junction between the pseudoknot and stem 1 (S1) and the 5'-end. These multiple interactions help explain the role of p65 as a chaperone for telomerase assembly[21,29,30].

Human telomerase holoenzyme structure

The first cryo-EM structure of human telomerase at ~8 Å resolution[7] established that it has a bilobal structure, comprising a catalytic core RNP of TERT and TER (or hTR) and an H/ACA RNP. The two RNPs are flexibly connected by TER, so their structures were refined separately[7]. (This approach has also been used for the more recent cryo-EM studies[8-11].) Due to the limited resolution, modeling was primarily conducted by rigid-body fitting of the known structures or homology models[7].

The ring-like (TRBD-RT-CTE) structure of a putative TERT from *Tribolium castaneum*[24] has served as a model for telomerase studies since 2008, but it lacks TEN and TRAP (as well as TER). Using multiple sequence alignments and statistical coupling analysis on all identified TERTs, Wang *et al.*[31] found that TEN and TRAP have co-evolved as telomerase-specific domains. Based on this analysis and the essential role of TEN–TRAP in telomerase activity[6,32-34], the authors concluded that the presence of TEN–TRAP is a hallmark of functional TERTs[31]. Integrating this data and the structure of *Tetrahymena* telomerase[6] plus NMR structures of hTR domains[35-38], they built a pseudoatomic model of human telomerase catalytic core[31] into the published ~8 Å cryo-EM map[7] including TRAP which fits in previously unassigned density.

All four papers with cryo-EM structures of human telomerase at sub-4 Å resolution[8-11] have telomeric DNA bound after the second step of nucleotide addition for telomere repeat synthesis (Figure 1d,e). Ghanim *et al.*[8] reported structures of the catalytic core RNP and H/ACA RNP at 3.8 and 3.4 Å resolution, respectively. Several notable findings emerged from

the *de novo* models determined in this study. *First*, a histone H2A–H2B dimer was identified corresponding to an unmodeled density in the previous map[7]. The authors present a convincing case that these histones are telomerase subunits. Histones H2A–H2B pack against CR4/5, which interacts with TRBD and CTE to close the TERT ring. They suggested that histones H2A–H2B may play a role analogous to *Tetrahymena* p65[12] in TER–TERT assembly and stabilization. Whether the histone dimer is a constitutive component remains an open question, as it is sub-stoichiometric among purified telomerase particles[9,10]. *Second*, this is the first model of a complete eukaryotic H/ACA RNP, revealing cross-hairpin interactions between the H and ACA boxes mediated by dyskerin, dyskerin–dyskerin interactions, and details of CAB box recognition by both TCAB1 and NHP2 (Fig. 1d). Significantly, most dyskeratosis congenita mutations in the H/ACA RNP map to interfaces between one dyskerin N-terminal extension and a hydrophobic pocket in the other, suggesting they affect assembly[8]. *Third*, details of TER, telomeric DNA, and TERT interactions in the catalytic cavity are revealed. TERT structure, including the predicted TEN–TRAP[31], and interactions with template and telomeric DNA are mostly similar to those seen in *Tetrahymena* telomerase (Figure 2a-d). Notably, as observed for *Tetrahymena* telomerase at several steps of telomere repeat synthesis[12], there are 4 base pairs between template and telomeric DNA (Figure 2g). This suggests that a short template–DNA duplex may be a universal feature of telomerase mechanism.

Wan *et al.*[9] combined analysis of their 3.5 Å resolution telomerase catalytic core RNP structure with extensive molecular dynamics simulations to propose details of telomerase mechanism. The authors noted an interesting structural similarity between the fingers motif, which regulates step-wise flipping of template bases in opposite the active site in polymerases, and the extended β -sheet between TEN–TRAP. They proposed that the fingers (renamed Fingers-A) and TEN–TRAP (renamed Fingers-B) regulate nucleotide translocation steps through coordinated opening and closing at either end of the duplex. This is partially in line with the conformational dynamics of TEN–TRAP observed in *Tetrahymena* telomerase[12]. The

manuscript focuses on the role of a TERT-specific residue, Leu980, located in the thumb helix, which they call a zipper head. The thumb helix binds in the minor groove of the template–DNA duplex (Figure 2b), where Leu980 is proposed to sterically disrupt the end base pair thereby limiting the duplex length to three base pairs[9]. They proposed that different base pairs would be more or less disrupted by Leu980, and correlated this to a previously observed template sequence-defined pausing signal[39]. The importance of Leu980 to telomerase repeat addition processivity (RAP) has been convincingly demonstrated[9,22,40]; we note however that four base pairs with Watson-Crick geometry are well-defined from the cryo-EM density for the other recent telomerase structures [8,10-12] . For the 3.9 Å resolution structure of H/ACA RNP, one interesting point is that TER binds dyskerin in such a way as to block the pseudouridylation pocket, thus autoinhibiting it from acting as a pseudouridylylase[9].

Structures of human telomerase catalytic core RNP in recruitment complexes

Telomerase recruitment to telomere ends involves shelterin proteins TIN2, TPP1, and POT1[19]. Recruitment is dependent on direct interaction of TPP1 with telomerase[41-43], while POT1 binds the single-stranded telomeric DNA[44] (Figure 1b). Liu *et al.*[10] determined cryo-EM structures of human telomerase with and without TPP1 OB at 3.3–3.7 Å resolution (Figure 2a,b,d,g). Notably, TPP1 binding damps the conformational dynamics of TEN–TRAP, which hinges as a unit above the TERT ring, and stabilizes the TEN–TRAP interface. The consequently improved density allowed accurate modeling of TEN (Figure 2a and 3). The three-way TPP1–TEN–TRAP interface is structurally homologous to *Tetrahymena* p50–TEN–TRAP[12], despite sequence diversity (Figure 3c,d). The interface reveals how regions on TPP1, called the TEL patch[41] and NOB[45], interact with both TRAP and TEN, and explains why the charge-swap TEN K78E, TPP1 E215K rescues RAP stimulation by TPP1–POT1[46].

In addition to defining the structural basis of TPP1 recruitment and activation of telomerase, analysis of the catalytic cavity[10] provided important insights into telomerase mechanism, TER structure, and disease. As noted above, cryo-EM density clearly delineates four base pairs between template and telomeric DNA (Figure 2g and 3d,f). The RBD bridge loop, proposed to

help regulate flipping in of the template and out of the DNA nucleotides at each end of the 4 base pair helix in *Tetrahymena*[12], has similar interactions with the template end but stacks on the second unpaired DNA nucleotide at the other end (Figure 2b). Leu980 proposed by Wan *et al.*[9] to act as a zipper head is at the duplex–single strand junction rather than sterically breaking the last base pair. The 5' template boundary element (TBE) is formed by interactions of TERT RBD with P1b-PK junction (Figure 2d,g), and additional interactions were observed between the template-adjacent TER (TBE_L) and a pocket on the RBD. CR4/5 folds into a L-shape closed by a 3 bp helix (P5.1) that was previously predicted to form during assembly[31,47] (Figure 1d). In the only place where PK and CR4/5 closely approach each other, as previously noted[8,9], a nucleotide from PK and a nucleotide from CR4/5 insert into separate pockets on opposite sides of the CTE. Notably, docking the non-nucleoside telomerase inhibitor BIBR1532 onto a previously identified binding loop on CTE[48] showed it would disrupt these critical TERT–TER interactions. An important conclusion from all the *Tetrahymena*[12] and human telomerase[8-11] structures is that TER t/PK and CR4/5 (SL4 in *Tetrahymena*) form a framework that stabilizes the catalytic cavity that accommodates the short template–DNA duplex and inhibits large-scale conformational changes in the TERT ring during telomere repeat synthesis. Analysis of 185 TERT and 75 TER mutations[49] in the catalytic core RNP linked to telomere biology disorders showed that almost all TER mutations are at or adjacent to TER nucleotides that contact TERT and numerous TERT mutations would also disrupt TERT–TER interactions[10], indicating the importance of the TER scaffold for telomerase activity[9,10].

Sekne *et al.*[11] obtained cryo-EM structures of telomerase recruitment complexes with TIN2–TPP1–POT1 and d(TTAGGG)₅, one where only TPP1 OB is resolved (3.2 Å) and another where TPP1 OB and POT1 OB1–OB2 are resolved (3.9 Å), using chemical cross-linking. The interactions between TPP1 and TEN–TRAP revealed in these structures, as well as the conformational changes of TPP1 and TEN–TRAP upon binding, are almost identical to those in the structure from Liu *et al.*[10] with TPP1 OB alone. Unexpectedly, POT1 OB1–OB2 binds TEN

(Figure 3b,d,f), although dynamically as evidenced by the lower resolution (7-9 Å) in this region of the map. TIN2 and POT1 OB3-HJRL that interacts with TPP1[50,51] are not visible in the cryo-EM maps, consistent with their conformational heterogeneity observed in the cryo-EM structures of TIN2-TPP1-POT1 alone[52]. The other human telomerase structures all used oligoT followed by a single telomeric repeat [d(T₁₂TTAGGG)[7,8,10] or d(T₁₈TTAGGG)[9]] and only the last six nucleotides were resolved in the catalytic core; here, density for two telomeric repeats was visible[11]. The newly resolved DNA density exits CTE along TRAP and TEN, and then apparently turns sharply to traverse POT1 OB2 then OB1 at their interface to TEN (Figure 3f). Thus, this study revealed a long-proposed DNA anchor site on TEN[53-55], for retaining the DNA during template translocation, along a positively charged surface conserved in vertebrates but not ciliates or yeasts. We note that previous studies had also proposed that TERT motifs within the catalytic cavity constitute a DNA anchor site[12,32,40], and we suggest that both anchor sites play essential roles in retaining the telomeric DNA in human telomerase.

Comparison of *Tetrahymena* telomerase holoenzyme to human telomerase recruitment complexes

While the interactions of human TPP1 and *Tetrahymena* p50 with TERT are highly similar, the interactions of human POT1 and *Tetrahymena* Teb1 with TERT are significantly different (Figure 3) [10-12]. Teb1 OB-C binds constitutively to TERT, mostly to TEN (in concert with Teb2 OB) with a few contacts to p50[12] (Figure 3c). The telomeric DNA exits directly from TERT CTE to the C-shaped DNA binding cleft of Teb1 OB-C (Figure 1c and 3e), explaining why it does not traverse TEN or require a secondary anchor site there[12]. The DNA is then presumably handled by Teb1 OB-B and OB-A, which bind telomeric DNA with at least 10-fold higher affinity than Teb1 OB-C *in vitro*[56,57], but no visible density for these domains has been observed by cryo-EM[12] (Figure 3a). In contrast, in human telomerase, POT1 OB3-HJRL (equivalent to Teb1 OB-C) is apparently flexibly tethered to TPP1 and is not visible in the cryo-EM map[11] (Figure 3b). A segment of TPP1 that binds in the C-shaped cleft of POT1 OB3 would apparently occlude DNA binding[50,51]. Instead, the exiting telomeric DNA turns to follow

a path along TEN anchor site to POT1 OB2 and OB1[11] (equivalent to Teb1 OB-B and OB-A) (Figure 3f).

Mechanistic insights from *Tribolium* TERT-like protein

Two mechanistic studies utilized *Tribolium* TERT-like protein as a model system[58,59]. Schaich *et al.*[58] solved crystal structures of *Tribolium* TERT with a model 16 nt RNA–15 nt DNA duplex, with added non-hydrolysable nucleotide analog (pre-catalytic) or nucleotide (post catalytic) to characterize nucleotide insertion steps. They used pre-steady-state kinetics of nucleotide insertion to identify the roles of various active site residues. A steric gate residue (Y256 in *Tribolium* TERT-like) for selectivity against rNTPs, as found in most DNA polymerases, was identified, and the importance of the equivalent residue (Y717) in human telomerase activity was supported by activity assays and the recent cryo-EM structures [8-12]. The second study[59] is a follow-up on a previous proposal[60] that the newly synthesized telomeric DNA forms a hairpin on the template for the translocation step and this step requires large scale opening of the TERT ring. In the present study[59], they propose a revised model where the looped-out DNA is accommodated in a preformed cavity between the RT palm and CTE.

Structural studies of yeast telomerase proteins: TERT, Pof8, and Est3

Due in part to their larger TERs and protein components other than TERT that differ between budding and fission yeast, there has been less progress on yeast telomerase structural biology, and to date no cryo-EM studies have been published. Zhai *et al.*[15] reported crystal structures of fungal TERTs, from the budding yeasts *Candida albicans* and *tropicalis* without and with the three-way junction element (CR4/5 in human) (Figure 4a,b). The protein constructs that crystallized all lack TEN, and TRAP is only partially folded, consistent with the hypothesis that TRAP folding is dependent on assembly of TEN–TRAP[31]. While *Candida albicans* has a TERT ring structure, *Candida tropicalis* has a collapsed TERT ring due to a large change in position of CTE. The authors proposed that CTE rotation between the two observed conformations is important for processivity. Whether the fungal TERT ring is still conformationally flexible when assembled with their large TER t/PK remains to be established.

An unusual ~45 residue U-motif at TRBD N-terminus, which includes and extends the CP2/TFLY[61,62] motif identified in *Tetrahymena*/Human (Figure 4a,b), was described and shown to be important for telomerase activity.

In 2018, three laboratories identified LARP7 protein Pof8 as a constitutive component of fission yeast (*Schizosaccharomyces pombe*)[63-65]. This was somewhat surprising since yeast TER is an RNA polymerase II transcript, although it does have a 3'-polyU end after processing. Two groups subsequently reported structures of Pof8 RRM2, revealed as an xRRM[13,14] first identified in *Tetrahymena* p65 with conserved features for RNA binding[13] (Figure 4c-e). Hu *et al.*[14] additionally addressed Pof8 binding to yeast TER using gel shifts and phosphorothioate footprinting, and found that Pof8 recognizes the pseudoknot fold, which is notable given that *Tetrahymena* p65 La motif also binds the pseudoknot[12] (Figure 4f). Additionally, Pof8 does not bind the 3'-polyU by itself, but rather through interaction with the biogenesis protein complex Lsm2-8. Overall, they demonstrated that Pof8 is a TER folding quality-control factor, and suggest this may be general to LARP7 proteins for RNAs.

In metazoans, Larp7 and methylphosphate capping enzyme (MePCE) constitutively associate with the lncRNA 7SK[66], which is involved in regulating RNA polymerase II transcription through binding and releasing of the kinase P-TEFb[67]. Recently, it was discovered that Bmc1 (also called Bin3)[68,69], the yeast orthologue of MePCE, as well as Thc1, a putative cap binding protein[68], associate with Pof8 as a complex for telomerase assembly, setting the stage for future structural studies of yeast telomerase biogenesis.

S. cerevisiae Est3 is a cell cycle regulated component of telomerase, with structural and functional homology to TPP1[70]. The NMR structure of Est3 from another budding yeast, *Hansenula polymorpha*[71] also has structural homology with human TPP1 and *S. cerevisiae* Est3 (Figure 4g). No interaction was detected between Est3 and TERT TEN domain, but this is perhaps not surprising as the interface of TPP1/p50 with TERT involves both TEN and TRAP[10-12].

Roles for G-quadruplexes in telomerase mechanism

271 It has long been known that telomeric repeat sequences can form G-quadruplexes[72,73], and
272 many studies have investigated whether they are inhibitory, activating, and/or mechanistically
273 important for telomerase activity[74]. Studies using single-molecule FRET and kinetics[75] and
274 single-molecule high-resolution optical tweezers[76] suggest that G-quadruplexes form in the
275 human telomerase catalytic cavity during telomere repeat synthesis and contribute to
276 translocation, product release, and/or DNA anchor site binding[75]. Other single-molecule FRET
277 studies[77,78] show that telomerase is a G-quadruplex resolvase that can both extend and
278 unfold parallel G-quadruplexes in a translocation dependent manner. It will be of interest to see
279 if G-quadruplexes can be visualized in future structures of telomerase actively synthesizing
280 telomeric DNA without or with TPP1 and POT1.

281

282 **Conflict of interest**

283 None declared.

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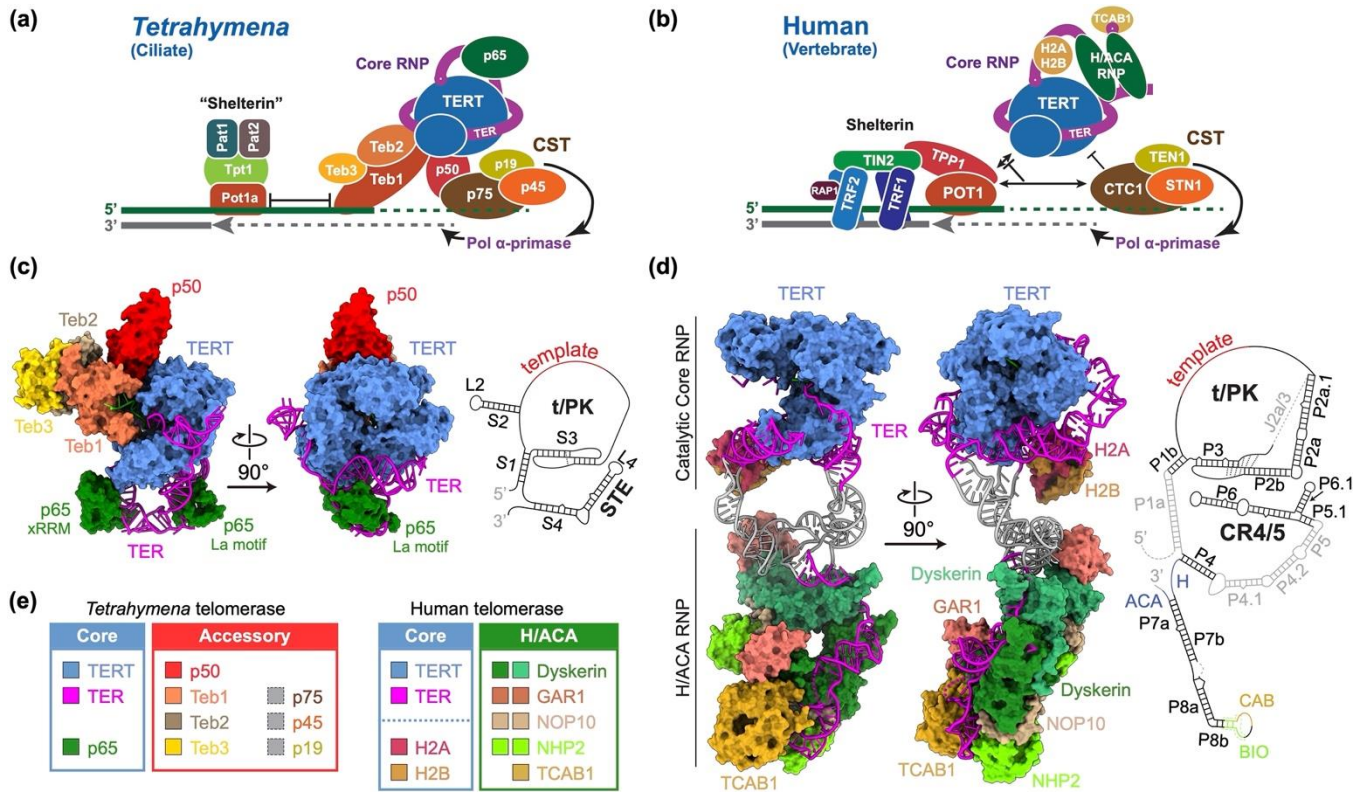


Figure 1. Structures of *Tetrahymena* and human telomerase holoenzymes. (a,b) Schematics of *Tetrahymena* (a) and a human (b) telomerase holoenzymes and their interactions at telomere ends. *Tetrahymena* telomerase catalytic core RNP is TERT, TER, and p65. p50, TEB (Teb1–Teb2–Teb3, a Replication Protein A (RPA) related complex), and p75–p45–p19 (*Tetrahymena* Ctc1–Snt1–Ten1, CST) are constitutively associated with the core RNP[79]. Human telomerase catalytic core RNP is TERT, TER, and possibly histone H2A/H2B dimer[8]. The H/ACA RNP has two each Dyskerin, GAR1, NOP10, and NHP2, plus one TCAB1 protein. Human and *Tetrahymena* CST recruit DNA polymerase α -Primase for synthesis of the C-strand, after G-strand synthesis by telomerase[19]. In human, six proteins collectively called shelterin bind the telomeric DNA[18]; a less well-defined “shelterin” complex of four proteins has been identified in *Tetrahymena*[80]. (c) 3.3 Å resolution cryo-EM structure of *Tetrahymena* telomerase (PDB 7LMA), with the dynamic p75–p45–p19 (CST) complex masked out[12], and schematic of TER. For TEB, only the Teb1C, Teb2N, Teb3 heterotrimer is visible; for p50, only the OB-domain is visible; and for p65, only the La motif and xRRM are well defined in the cryo-EM map. (d) Cryo-EM structure of human telomerase (PDB 7TRC for H/ACA RNP and PDB 7TRD for catalytic core RNP) and schematic of TER. Gray colored regions of TER between the two RNPs are modeled based on low resolution cryo-EM densities[8]. (e) Color chart for the telomerase proteins and RNA.

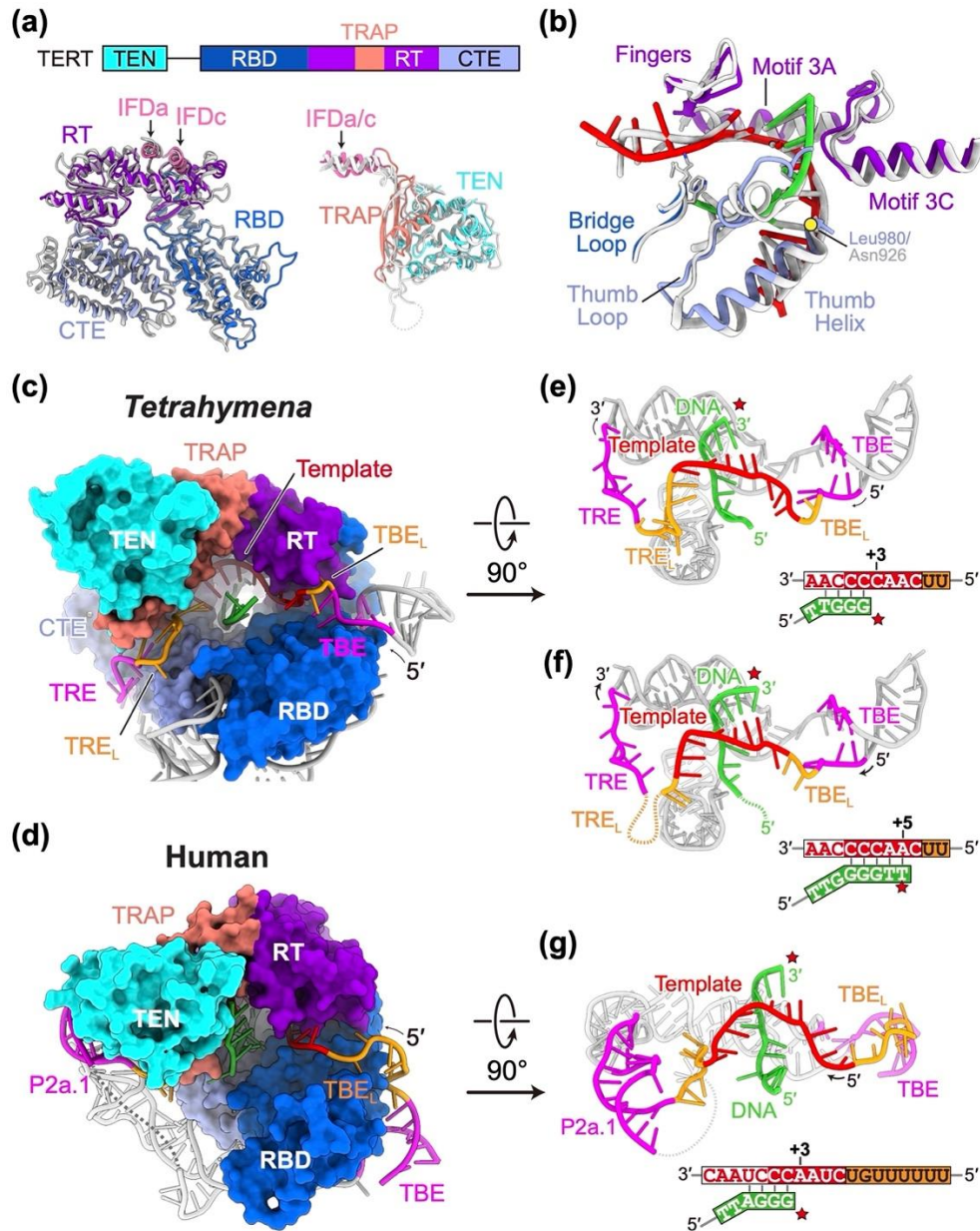


Figure 2. Telomerase TERT-TER catalytic core. (a) Schematic of TERT domains and overlay of human (colored) and *Tetrahymena* (gray) RBD-RT-CTE TERT ring and TEN-TRAP. (b) Overlay of human (colored) and *Tetrahymena* (gray) TERT ring showing some motifs that interact with the template and telomeric DNA. (c,d) Surface renderings of telomerase catalytic cavity of *Tetrahymena* (c) and human (d) with TER and telomeric DNA shown as ribbon-and-sticks. CR4/5 is removed for clarity in (d). Colors for TERT domains are as in (a). (e-g) TER t/PK and telomeric DNA structures in *Tetrahymena* at the second (e) and fifth (f) steps of telomere repeat addition and in human at the second step of telomere repeat addition (g). Template is red, flexible regions of TER next to template are orange, and fixed regions of TER are magenta. TBE is template boundary element. TBE_L is TBE linker. TRE is template recognition element. TRE_L is TRE linker. In (f) the telomeric DNA is still in the active site, while for (e) and (g) the active site is empty. In the accompanying schematics, red star is active site and numbering refers to template position in the active site for telomere repeat synthesis. The alignment nucleotides in the template are red with white background.

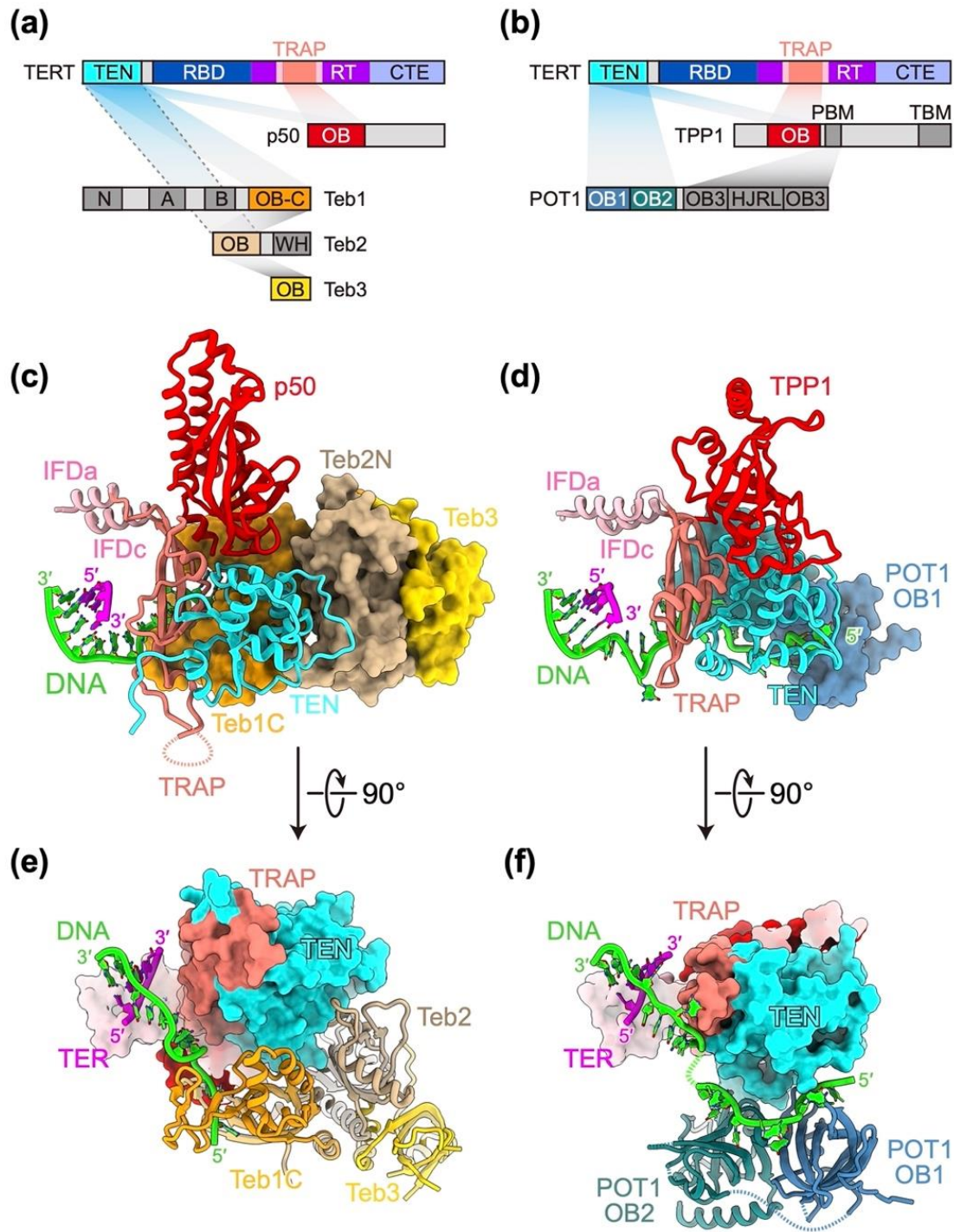


Figure 3. Telomerase activation and recruitment complexes. (a,b) Schematics of domain structures and interactions between (a) *Tetrahymena* TERT, p50, and TEB (PDB 7LMA) and (b) human TERT, TPP1, and POT1 (PDB 7QXB). Domains that are not visible or defined by the cryo-EM map are shown in gray. (c,d) View of the three-way interactions between *Tetrahymena* TERT TEN, TRAP, and p50 (c) and human TERT TEN, TRAP, and TPP1 (d), shown in ribbon. TEB (Teb1, Teb2, Teb3) in *Tetrahymena* (c) and POT1 OB1-OB2 in human (d) are shown as space fill. The short template–telomeric DNA duplex and single-strand exiting telomeric DNA are also shown. (e,f) 90° rotated views of (c,d) showing the path of telomeric DNA in *Tetrahymena* (e) and human (f).

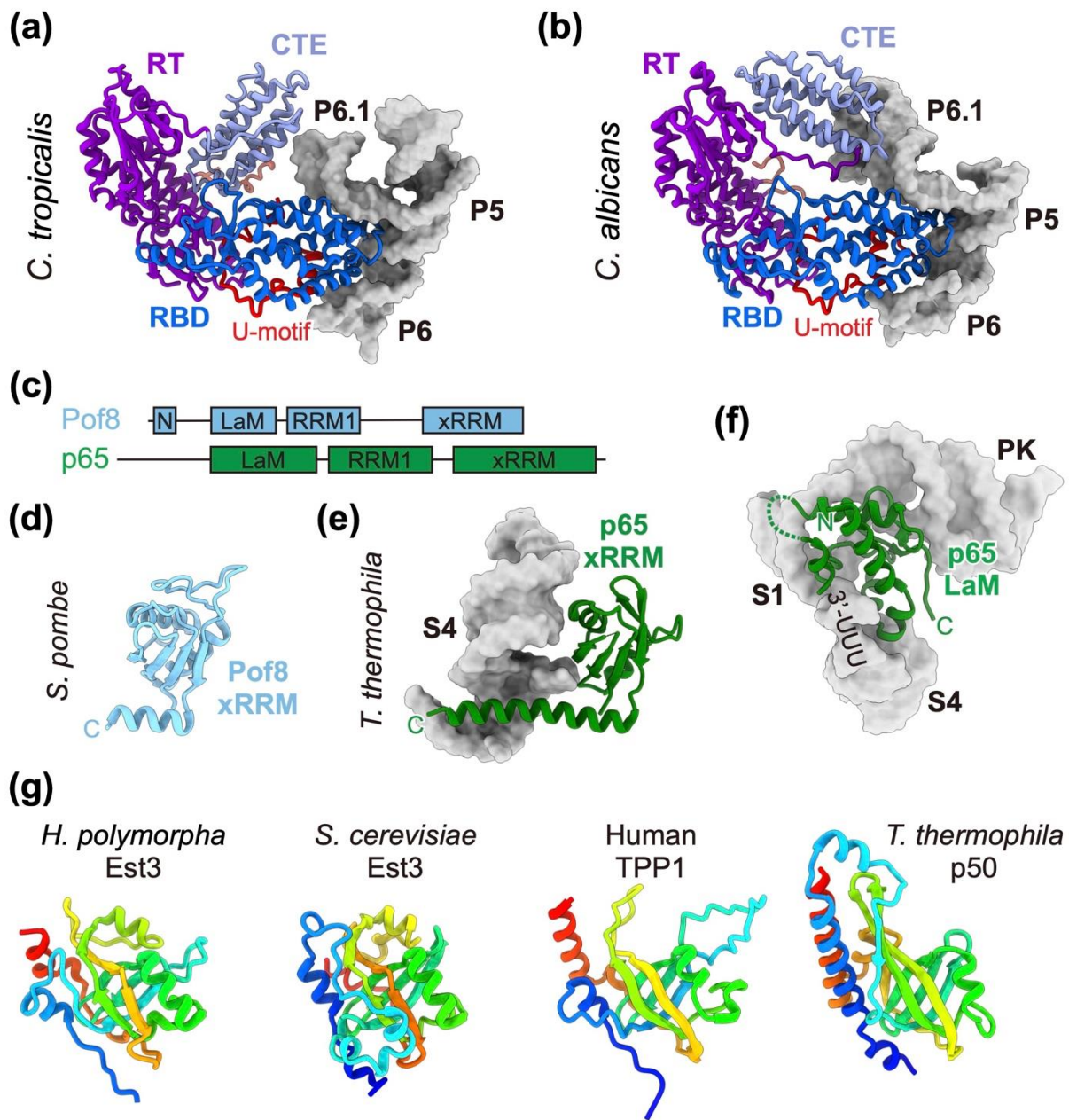


Figure 4. Structural analysis of yeast TERT, Pof8, and Est3. (a) Structure of *C. tropicalis* TERT in complex with TER three-way junction (PDB 6ZDP). (b) Structure of *C. albicans* TERT in complex with TER three-way junction (PDB 6ZDU). Two different conformations of CTE were observed in these two *Candida* TERT structures. The U-motifs are colored in red. (c) Domain architectures of *S. pombe* Pof8 and *T. thermophila* p65. LaM, La motif. (d) Structure of Pof8 xRRM domain (PDB 6TZN and 6U7V). (e) Structure of p65 xRRM domain with TER S4 (PDB 7LMA). (f) Structure of p65 LaM with TER PK, S1, S4 and the 3'-UUU (PDB 7LMA). (g) Structure of *H. polymorpha* Est3 OB domain (PDB 6Q44) and its comparison with the OB domains of *T. thermophila* p50 (PDB 7LMA), human TPP1 (PDB 7TRE), and *S. cerevisiae* Est3 (PDB 2M9V). Structures are rainbow colored from N-terminal (blue) to C-terminal (red).