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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.

Keywords

telomerase; cryo-electron microscopy; ribonucleoprotein; TERT; TER

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,

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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 casteneum* (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 Ill 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 pseudouridylase[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(T_{12}TTAGGG)[7,8,10] or d(T_{18}TTAGGG)[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 IncRNA 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

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

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Blackburn EH, Collins K: Telomerase: an RNP enzyme synthesizes DNA. Cold Spring Harb Perspect Biol 2011, 3.
2. Roake CM, Artandi SE: Regulation of human telomerase in homeostasis and disease. Nat Rev Mol Cell Biol 2020, 21:384–397. [PubMed: 32242127]
3. Jiang J, Miracco EJ, Hong K, Eckert B, Chan H, Cash DD, Min B, Zhou ZH, Collins K, Feigon J: The architecture of Tetrahymena telomerase holoenzyme. Nature 2013, 496:187–192. [PubMed: 23552895]
4. Sauerwald A, Sandin S, Cristofari G, Scheres SH, Lingner J, Rhodes D: Structure of active dimeric human telomerase. Nat Struct Mol Biol 2013, 20:454–460. [PubMed: 23474713]
5. Jiang J, Chan H, Cash DD, Miracco EJ, Ogorzalek Loo RR, Upton HE, Cascio D, O'Brien Johnson R, Collins K, Loo JA, et al. : Structure of Tetrahymena telomerase reveals previously unknown subunits, functions, and interactions. Science 2015, 350:aab4070. [PubMed: 26472759]
6. Jiang J, Wang Y, Susac L, Chan H, Basu R, Zhou ZH, Feigon J: Structure of Telomerase with Telomeric DNA. Cell 2018, 173:1179–1190 e1113. [PubMed: 29775593]
7. Nguyen THD, Tam J, Wu RA, Greber BJ, Toso D, Nogales E, Collins K: Cryo-EM structure of substrate-bound human telomerase holoenzyme. Nature 2018,10.1038/s41586-018-0062-x.
8. Ghanim GE, Fountain AJ, van Roon AM, Rangan R, Das R, Collins K, Nguyen THD: Structure of human telomerase holoenzyme with bound telomeric DNA. Nature 2021, 10.1038/

s41586-021-03415-4.** Reports cryo-EM structures of human telomerase holoenzyme at 3.8 Å and 3.4 Å resolution for catalytic core RNP and H/ACA RNP, respectively. Highlights include identification of histone H2A/H2B dimer in the catalytic core RNP, determination of the complete structure of H/ACA RNP, and details of catalytic core including 4 base pairs for template–telomeric DNA duplex.

9. Wan F, Ding Y, Zhang Y, Wu Z, Li S, Yang L, Yan X, Lan P, Li G, Wu J, et al. : Zipper head mechanism of telomere synthesis by human telomerase. Cell Res 2021, 31:1275–1290. [PubMed: 34782750] ** Reports cryo-EM structures of human telomerase holoenzyme at 3.5 Å and 3.9 Å resolution for catalytic core RNP and H/ACA RNP, respectively, and molecular dynamics. For catalytic core RNP proposes that Leu980 in thumb helix limits template–DNA duplex to 3 base pairs and that fingers and TRAP (renamed Fingers B) move in a concerted fashion during nucleotide addition. For H/ACA RNP proposes that TER autoinhibits the pseudoridylase Dyskerin.
10. Liu B, He Y, Wang Y, Song H, Zhou ZH, Feigon J: Structure of active human telomerase with telomere shelterin protein TPP1. Nature 2022, 10.1038/s41586-022-04582-8.** Reports cryo-EM structures of human telomerase holoenzyme with telomeric DNA and with and without shelterin protein TPP1 at 3.5 Å and 3.7 Å resolution for catalytic core RNP. Highlights include characterization of three-way TPP1-TEN–TRAP interface for recruitment and activation, TPP1 binding damps conformational dynamics of TEN–TRAP, detailed interactions in the catalytic cavity including 4 base pair template–DNA duplex, detailed TERT–TER interactions, localization of numerous disease mutations at the TERT–TER interface, and mechanism of telomerase inhibitor BIBR action.
11. Sekne Z, Ghanim GE, van Roon AM, Nguyen THD: Structural basis of human telomerase recruitment by TPP1-POT1. Science 2022, 375:1173–1176. [PubMed: 35201900] ** Reports two cryo-EM structures of human telomerase holoenzyme with telomeric DNA in complex with shelterin proteins TIN1–TPP1–POT1: one focused on catalytic core RNP with TPP1 OB resolved at 3.2 Å resolution and the other one focused on catalytic core RNP with TPP1 OB and POT1 OB1-OB2 resolved at 3.9 Å resolution. Highlights include characterization of three-way TPP1–TEN–TRAP interface, exit path of telomeric DNA, a DNA anchor site specific to vertebrate TEN, and POT1 OB1-OB2 binding site on TEN, indicating both TPP1 and POT1 contribute to recruitment and processivity enhancement.
12. He Y, Wang Y, Liu B, Helmling C, Susac L, Cheng R, Zhou ZH, Feigon J: Structures of telomerase at several steps of telomere repeat synthesis. Nature 2021, 593:454–459. [PubMed: 33981033] ** Reports cryo-EM structures of Tetrahymena telomerase at different steps of telomere repeat synthesis, including 3.3 Å, 4.4 Å, and 3.8 Å resolution structures at the second, fourth, and fifth steps of nucleotide addition. Highlights include direct evidence for template-DNA duplex length and handing at different steps of nucleotide addition, determination of structural basis for template movement during telomeric DNA synthesis, and characterization of three-way p50-TEN–TRAP interface, interactions between p65 La motif and TER, and the exit path of telomeric DNA through Teb1C.
13. Basu R, Eichhorn CD, Cheng R, Peterson RD, Feigon J: Structure of S. pombe telomerase protein Pof8 C-terminal domain is an xRRM conserved among LARP7 proteins. RNA Biol 2020, 10.1080/15476286.2020.1836891:1-12.* Reports the crystal structure of the C-terminal RRM of Pof8, identifying it as an xRRM. As the third example of this motif, refines this class of atypical RRM and highlights the conserved RNA binding residues and other features.
14. Hu X, Kim JK, Yu C, Jun HI, Liu J, Sankaran B, Huang L, Qiao F: Quality-Control Mechanism for Telomerase RNA Folding in the Cell. Cell Rep 2020, 33:108568. [PubMed: 33378677] ** Reports the crystal structure of the C-terminal RRM of Pof8. Establishes that Pof8 interacts with yeast TER pseudoknot and indirectly to polyU tract through binding to LSM complex. Shows that Pof8 only binds correctly folded TER, thus establishing a role in quality control for telomerase assembly.
15. Zhai LT, Rety S, Chen WF, Song ZY, Auguin D, Sun B, Dou SX, Xi XG: Crystal structures of N-terminally truncated telomerase reverse transcriptase from fungidouble dagger. Nucleic Acids Res 2021, 49:4768–4781. [PubMed: 33856462] * Reports the first crystal structures of fungal TERTs, from Candida albicans and Candida tropicalis, with and without CR4/5 but with TEN truncated, revealing two extreme conformations of TERT CTE, and identification of a new U-motif (that includes the CP2/TFLY).

16. Greider CW, Blackburn EH: Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985, 43:405–413. [PubMed: 3907856]
17. Blackburn EH, Gall JG: A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J Mol Biol 1978, 120:33–53. [PubMed: 642006]
18. Myler LR, Kinzig CG, Sasi NK, Zakusilo G, Cai SW, de Lange T: The evolution of metazoan shelterin. Genes Dev 2021, 35:1625–1641. [PubMed: 34764137]
19. Lim CJ, Cech TR: Shaping human telomeres: from shelterin and CST complexes to telomeric chromatin organization. Nat Rev Mol Cell Biol 2021, 10.1038/s41580-021-00328-y.
20. Wang Y, Feigon J: Structural biology of telomerase and its interaction at telomeres. Curr Opin Struct Biol 2017, 47:77–87. [PubMed: 28732250]
21. Prathapam R, Witkin KL, O'Connor CM, Collins K: A telomerase holoenzyme protein enhances telomerase RNA assembly with telomerase reverse transcriptase. Nat Struct Mol Biol 2005, 12:252–257. [PubMed: 15696174]
22. Xie M, Podlevsky JD, Qi X, Bley CJ, Chen JJ: A novel motif in telomerase reverse transcriptase regulates telomere repeat addition rate and processivity. Nucleic Acids Res 2010, 38:1982–1996. [PubMed: 20044353]
23. Lue NF, Lin YC, Mian IS: A conserved telomerase motif within the catalytic domain of telomerase reverse transcriptase is specifically required for repeat addition processivity. Mol Cell Biol 2003, 23:8440–8449. [PubMed: 14612390]
24. Gillis AJ, Schuller AP, Skordalakes E: Structure of the Tribolium castaneum telomerase catalytic subunit TERT. Nature 2008, 455:633–637. [PubMed: 18758444]
25. Podlevsky JD, Chen JJ: Evolutionary perspectives of telomerase RNA structure and function. RNA Biol 2016, 13:720–732. [PubMed: 27359343]
26. Berman AJ, Akiyama BM, Stone MD, Cech TR: The RNA accordion model for template positioning by telomerase RNA during telomeric DNA synthesis. Nat Struct Mol Biol 2011, 18:1371–1375. [PubMed: 22101935]
27. Maraia RJ, Mattijssen S, Cruz-Gallardo I, Conte MR: The La and related RNA-binding proteins (LARPs): structures, functions, and evolving perspectives. Wiley Interdiscip Rev RNA 2017, 8.
28. Singh M, Wang Z, Koo BK, Patel A, Cascio D, Collins K, Feigon J: Structural basis for telomerase RNA recognition and RNP assembly by the holoenzyme La family protein p65. Mol Cell 2012, 47:16–26. [PubMed: 22705372]
29. Stone MD, Mihalusova M, O'Connor CM, Prathapam R, Collins K, Zhuang X: Stepwise protein-mediated RNA folding directs assembly of telomerase ribonucleoprotein. Nature 2007, 446:458–461. [PubMed: 17322903]
30. Akiyama BM, Loper J, Najarro K, Stone MD: The C-terminal domain of Tetrahymena thermophila telomerase holoenzyme protein p65 induces multiple structural changes in telomerase RNA. RNA 2012, 18:653–660. [PubMed: 22315458]
31. Wang Y, Gallagher-Jones M, Susac L, Song H, Feigon J: A structurally conserved human and Tetrahymena telomerase catalytic core. Proceedings of the National Academy of Sciences 2020, 10.1073/pnas.2011684117:202011684.** Multiple sequence alignments and statistical coupling analysis on all identified TERTs show that TEN and TRAP have co-evolved as telomerase specific domains. A model of human TERT–TER catalytic core based on *Tetrahymena telomerase* 4.8 Å cryo-EM structure and NMR structures of human TER domains was bulit into the published ~8 Å human telomerase cryo-EM map by Nguyen *et al.* (2018) including TRAP in previoulsy unassigned density.
32. Wu RA, Upton HE, Vogan JM, Collins K: Telomerase Mechanism of Telomere Synthesis. Annu Rev Biochem 2017, 86:439–460. [PubMed: 28141967]
33. Akiyama BM, Parks JW, Stone MD: The telomerase essential N-terminal domain promotes DNA synthesis by stabilizing short RNA-DNA hybrids. Nucleic Acids Res 2015, 43:5537–5549. [PubMed: 25940626]
34. Chu TW, MacNeil DE, Autexier C: Multiple Mechanisms Contribute to the Cell Growth Defects Imparted by Human Telomerase Insertion in Fingers Domain Mutations Associated with Premature Aging Diseases. J Biol Chem 2016, 291:8374–8386. [PubMed: 26887940]

35. Kim NK, Zhang Q, Zhou J, Theimer CA, Peterson RD, Feigon J: Solution structure and dynamics of the wild-type pseudoknot of human telomerase RNA. J Mol Biol 2008, 384:1249–1261. [PubMed: 18950640]
36. Zhang Q, Kim NK, Peterson RD, Wang Z, Feigon J: Structurally conserved five nucleotide bulge determines the overall topology of the core domain of human telomerase RNA. Proc Natl Acad Sci U S A 2010, 107:18761–18768. [PubMed: 20966348]
37. Kim NK, Zhang Q, Feigon J: Structure and sequence elements of the CR4/5 domain of medaka telomerase RNA important for telomerase function. Nucleic Acids Res 2014, 42:3395–3408. [PubMed: 24335084]
38. Zhang Q, Kim NK, Feigon J: Architecture of human telomerase RNA. Proc Natl Acad Sci U S A 2011, 108:20325–20332. [PubMed: 21844345]
39. Brown AF, Podlevsky JD, Qi X, Chen Y, Xie M, Chen JJ: A self-regulating template in human telomerase. Proc Natl Acad Sci U S A 2014, 111:11311–11316. [PubMed: 24982163]
40. Wu RA, Tam J, Collins K: DNA-binding determinants and cellular thresholds for human telomerase repeat addition processivity. EMBO J 2017, 36:1908–1927. [PubMed: 28495680]
41. Nandakumar J, Bell CF, Weidenfeld I, Zaug AJ, Leinwand LA, Cech TR: The TEL patch of telomere protein TPP1 mediates telomerase recruitment and processivity. Nature 2012, 492:285–289. [PubMed: 23103865]
42. Sexton AN, Youmans DT, Collins K: Specificity requirements for human telomere protein interaction with telomerase holoenzyme. J Biol Chem 2012, 287:34455–34464. [PubMed: 22893708]
43. Zhong FL, Batista LF, Freund A, Pech MF, Venteicher AS, Artandi SE: TPP1 OB-fold domain controls telomere maintenance by recruiting telomerase to chromosome ends. Cell 2012,150:481–494. [PubMed: 22863003]
44. Lei M, Podell ER, Cech TR: Structure of human POT1 bound to telomeric single-stranded DNA provides a model for chromosome end-protection. Nat Struct Mol Biol 2004,11:1223–1229. [PubMed: 15558049]
45. Grill S, Tesmer VM, Nandakumar J: The N Terminus of the OB Domain of Telomere Protein TPP1 Is Critical for Telomerase Action. Cell Rep 2018, 22:1132–1140. [PubMed: 29386102]
46. Schmidt JC, Dalby AB, Cech TR: Identification of human TERT elements necessary for telomerase recruitment to telomeres. Elife 2014, 3.
47. Palka C, Forino NM, Hentschel J, Das R, Stone MD: Folding heterogeneity in the essential human telomerase RNA three-way junction. RNA 2020, 26:1787–1800. [PubMed: 32817241] * Reports in vitro chemical mapping, secondary structural modeling, and single-molecule structural analysis to probe the structure of TER CR4/5 domain in the absence of TERT. In the free RNA, CR4/5 is structurally heterogeneous and the highly conserved P6.1 stem is not formed.
48. Bryan C, Rice C, Hoffman H, Harkisheimer M, Sweeney M, Skordalakes E: Structural Basis of Telomerase Inhibition by the Highly Specific BIBR1532. Structure 2015, 23:1934–1942. [PubMed: 26365799]
49. Podlevsky JD, Bley CJ, Omana RV, Qi X, Chen JJ: The telomerase database. Nucleic Acids Res 2008, 36:D339–343. [PubMed: 18073191]
50. Chen C, Gu P, Wu J, Chen X, Niu S, Sun H, Wu L, Li N, Peng J, Shi S, et al. : Structural insights into POT1-TPP1 interaction and POT1 C-terminal mutations in human cancer. Nat Commun 2017, 8:14929. [PubMed: 28393832]
51. Rice C, Shastrula PK, Kossenkov AV, Hills R, Baird DM, Showe LC, Doukov T, Janicki S, Skordalakes E:Structural and functional analysis of the human POT1-TPP1 telomeric complex. Nat Commun 2017,8:14928. [PubMed: 28393830]
52. Smith EW, Lattmann S, Liu ZB, Ahsan B, Rhodes D: Insights into POT1 structural dynamics revealed bycryo-EM. Pios One 2022, 17.
53. Jurczyluk J, Nouwens AS, Holien JK, Adams TE, Lovrecz GO, Parker MW, Cohen SB, Bryan TM: Direct involvement of the TEN domain at the active site of human telomerase. Nucleic Acids Research 2011, 39:1774–1788. [PubMed: 21051362]
54. Lue NF: A physical and functional constituent of telomerase anchor site. J Biol Chem 2005, 280:26586–26591. [PubMed: 15905172]

55. Jacobs SA, Podell ER, Cech TR: Crystal structure of the essential N-terminal domain of telomerase reverse transcriptase. Nat Struct Mol Biol 2006,13:218–225. [PubMed: 16462747]
56. Zeng Z, Min B, Huang J, Hong K, Yang Y, Collins K, Lei M: Structural basis for Tetrahymena telomerase processivity factor Tebl binding to single-stranded telomeric-repeat DNA. Proc Natl Acad Sci U S A 2011,108:20357–20361. [PubMed: 22143754]
57. Min B, Collins K: Multiple mechanisms for elongation processivity within the reconstituted tetrahymena telomerase holoenzyme. J Biol Chem 2010, 285:16434–16443. [PubMed: 20363756]
58. Schaich MA, Sanford SL, Welfer GA, Johnson SA, Khoang TH, Opresko PL, Freudenthal BD: Mechanisms of nucleotide selection by telomerase. Elife 2020, 9.* Reports new crystal structures of *Tribolium castaneum* TERT-like protein with model template–DNA duplex as pre-nucleotide 'binary', nucleotide bound 'ternary', and product complexes and characterized fidelity and sugar selectivity of single nucleotide insertion using pre-steady state kinetics. Identified a specific tyrosine as a steric gate for discriminating between deoxynucleotides and ribonucleotides, and validated this in human telomerase by activity assays.
59. Choi WS, Weng PJ, Yang W: Flexibility of telomerase in binding the RNA template and DNA telomeric repeat. Proc Natl Acad Sci USA 2022,119.* Presents new Tribolium casteneum TERT-like protein crystal structures and biochemical data to propose an alternative model for separation of template and telomeric DNA strands in the duplex prior to template translocation, involving looping out of the DNA.
60. Yang W, Lee YS: A DNA-hairpin model for repeat-addition processivity in telomere synthesis. Nat Struct Mol Biol 2015, 22:844–847. [PubMed: 26581517]
61. Akiyama BM, Gomez A, Stone MD: A conserved motif in Tetrahymena thermophila telomerase reverse transcriptase is proximal to the RNA template and is essential for boundary definition. J Biol Chem 2013, 288:22141–22149. [PubMed: 23760279]
62. Harkisheimer M, Mason M, Shuvaeva E, Skordalakes E: A motif in the vertebrate telomerase N-terminal linker of TERT contributes to RNA binding and telomerase activity and processivity. Structure 2013, 21:1870–1878. [PubMed: 24055314]
63. Collopy LC, Ware TL, Goncalves T, Kongsstovu SI, Yang Q, Amelina H, Pinder C, Alenazi A, Moiseeva V, Pearson SR, et al. : LARP7 family proteins have conserved function in telomerase assembly. Nature Communications 2018, 9.
64. Mennie AK, Moser BA, Nakamura TM: LARP7-like protein Pof8 regulates telomerase assembly and poly(A)+TERRA expression in fission yeast. Nat Commun 2018, 9:586. [PubMed: 29422503]
65. Paez-Moscoso DJ, Pan L, Sigauke RF, Schroeder MR, Tang W, Baumann P: Pof8 is a La-related protein and a constitutive component of telomerase in fission yeast. Nat Commun 2018, 9:587. [PubMed: 29422664]
66. Yang Y, Liu S, Egloff S, Eichhorn CD, Hadjian T, Zhen J, Kiss T, Zhou ZH, Feigon J: Structural basis of RNA conformational switching in the transcriptional regulator 7SK RNP. Mol Cell 2022, 82:1724–1736 e1727. [PubMed: 35320752]
67. Egloff S, Studniarek C, Kiss T: 7SK small nuclear RNA, a multifunctional transcriptional regulatory RNA with gene-specific features. Transcription 2018, 9:95–101. [PubMed: 28820318]
68. Paez-Moscoso DJ, Ho DV, Pan L, Hildebrand K, Jensen KL, Levy MJ, Florens L, Baumann P: A putative cap binding protein and the methyl phosphate capping enzyme Bin3/MePCE function in telomerase biogenesis. Nat Commun 2022, 13:1067. [PubMed: 35217638] ** Reports the discovery of two proteins, the methyl phosphate capping enzyme (Bin3 or MePCE) and a putative cap binding protein (Thc1), that together with the LARP7 protein Pof8 are components of fission yeast telomerase. MePCE and LARP7 are components of another lncRNP 7SK and also associate with U6 snRNA.
69. Porat J, El Baidouri M, Grigull J, Deragon JM, Bayfield MA: The methyl phosphate capping enzyme Bmc1/Bin3 is a stable component of the fission yeast telomerase holoenzyme. Nature Communications 2022, 13.** Reports the discovery of methyl phosphate capping enzyme (Bin3 or MePCE) as a component of fission yeast telomerase. MePCE and LARP7 are components of another lncRNP, 7SK and also associate with U6 snRNA.
70. Rao T, Lubin JW, Armstrong GS, Tucey TM, Lundblad V, Wuttke DS: Structure of Est3 reveals a bimodal surface with differential roles in telomere replication. Proc Natl Acad Sci U S A 2014, 111:214–218. [PubMed: 24344315]

71. Shepelev NM, Mariasina SS, Mantsyzov AB, Malyavko AN, Efimov SV, Petrova OA, Rodina EV, Zvereva MI, Dontsova OA, Polshakov VI: Insights into the structure and function of Est3 from the Hansenula polymorpha telomerase. Sci Rep 2020, 10:11109. [PubMed: 32632130] * Reports the NMR structure of Est3 from the methylotrophic yeast Hansenula polymorpha, and shows it is structurally homologous to TPP1 and Est3 from fission yeast S. cerevisiae.

72. Williamson JR, Raghuraman MK, Cech TR: Monovalent cation-induced structure of telomeric DNA: the G-quartet model. Cell 1989, 59:871–880. [PubMed: 2590943]

73. Smith FW, Feigon J: Quadruplex structure of Oxytricha telomeric DNA oligonucleotides. Nature 1992, 356:164–168. [PubMed: 1545871]

74. Bryan TM: G-Quadruplexes at Telomeres: Friend or Foe? Molecules 2020, 25.

75. Jansson LI, Hentschel J, Parks JW, Chang TR, Lu C, Baral R, Bagshaw CR, Stone MD: Telomere DNA G-quadruplex folding within actively extending human telomerase. Proc Natl Acad Sci U S A 2019, 10.1073/pnas.1814777116**..**

76. Patrick EM, Slivka JD, Payne B, Comstock MJ, Schmidt JC: Observation of processive telomerase catalysis using high-resolution optical tweezers. Nat Chem Biol 2020, 10.1038/s41589-020-0478-0.** Using single-molecule high-resolution optical tweezers the authors provide evidence that support the hypothesis that G-quadruplexes form during human telomeric repeat synthesis and are involved in anchor site binding and product release.

77. Paudel BP, Moye AL, Abou Assi H, El-Khoury R, Cohen SB, Holien JK, Birrento ML, Samosorn S, Intharapichai K, Tomlinson CG, et al. : A mechanism for the extension and unfolding of parallel telomeric G-quadruplexes by human telomerase at single-molecule resolution. Elife 2020, 9.** Using single-molecule florescence resonance energy transfer (smFRET) microscopy and bulk-phase enzymology the authors propose a mechanism for telomerase resolving parallel G-quadruplexes formed during telomeric repeat synthesis.

78. Moye AL, Porter KC, Cohen SB, Phan T, Zyner KG, Sasaki N, Lovrecz GO, Beck JL, Bryan TM: Telomeric G-quadruplexes are a substrate and site of localization for human telomerase. Nat Commun 2015, 6:7643. [PubMed: 26158869]

79. Chan H, Wang Y, Feigon J: Progress in Human and Tetrahymena Telomerase Structure Determination. Annu Rev Biophys 2017, 46:199–225. [PubMed: 28301767]

80. Premkumar VL, Cranert S, Linger BR, Morin GB, Minium S, Price C: The 3 ‘ Overhangs at Tetrahymena thermophila Telomeres Are Packaged by Four Proteins, Pot1a, Tpt1, Pat1, and Pat2. Eukaryotic Cell 2014, 13:240–245. [PubMed: 24297442]

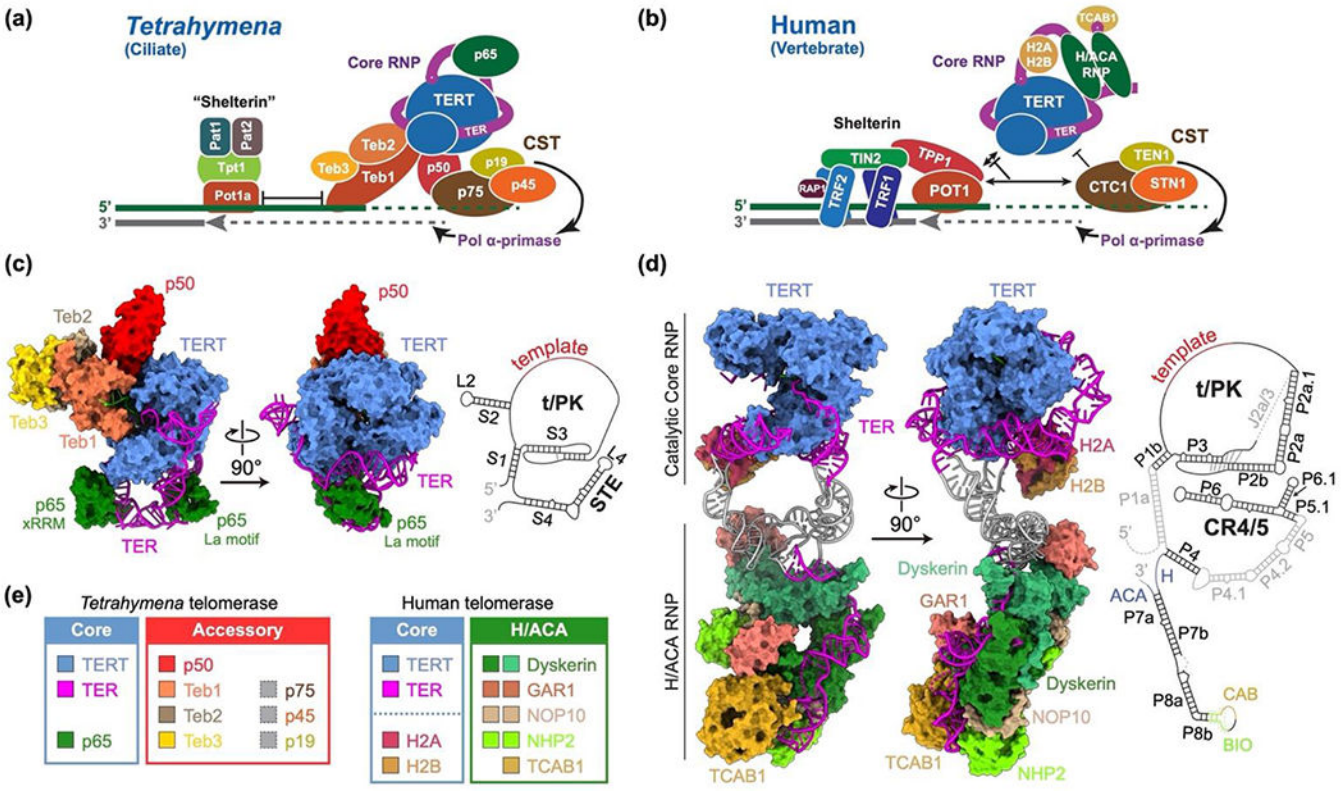

Figure 1. Structures of *Tetrahymena* and human telomerase holoenzymes.

(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.

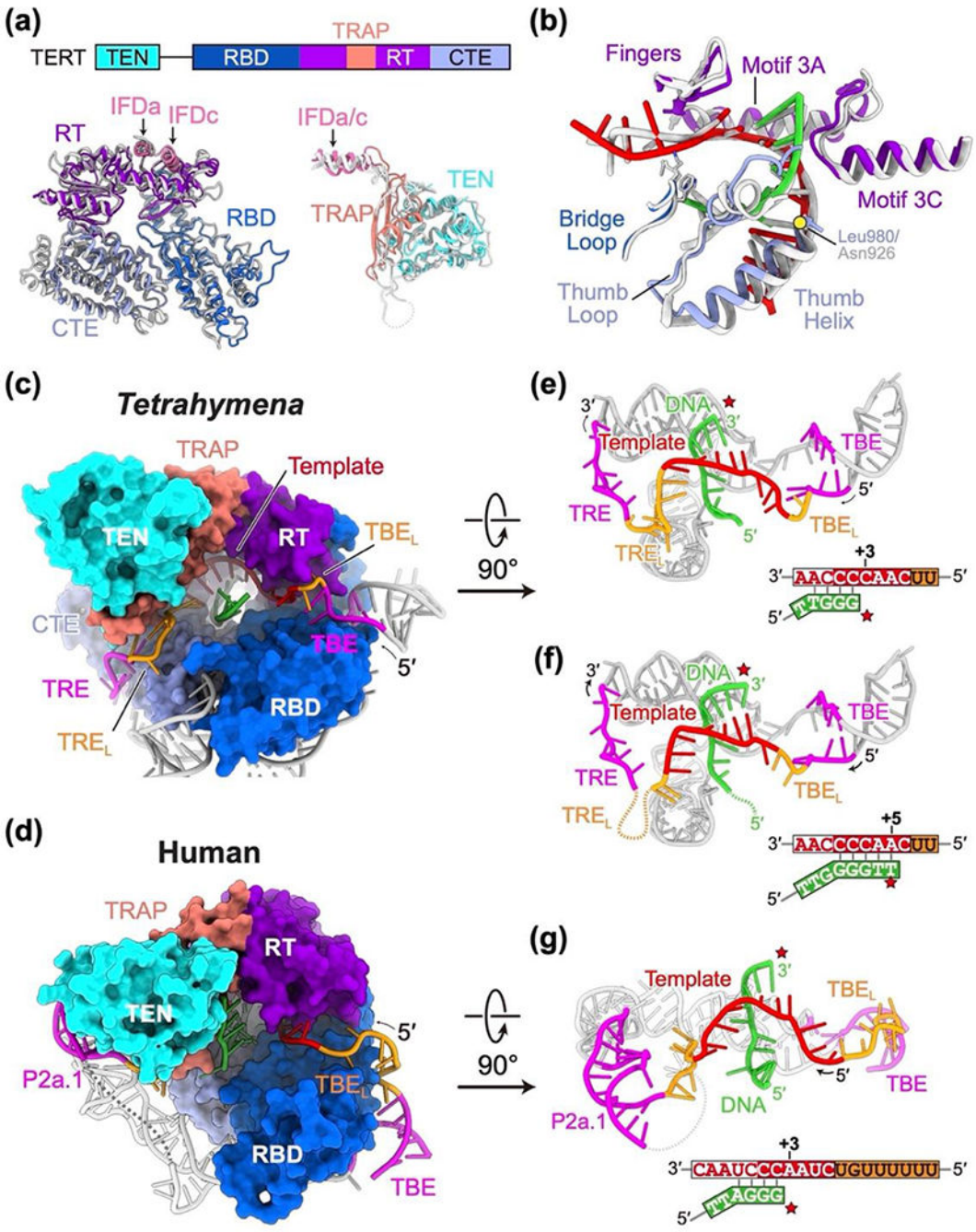

Figure 2. Telomerase TERT–TER catalytic core.

(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.

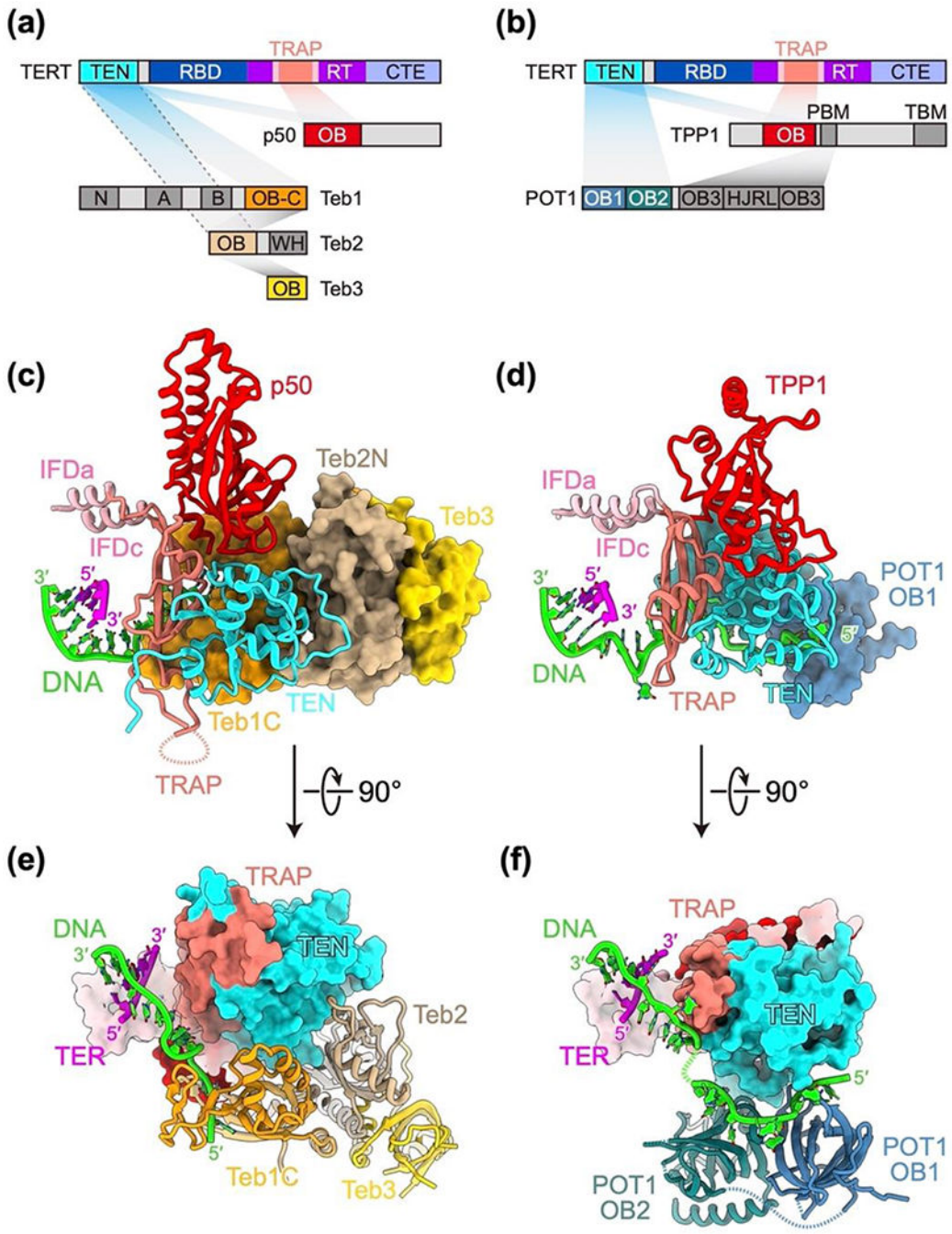

Figure 3. Telomerase activation and recruitment complexes.

(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).

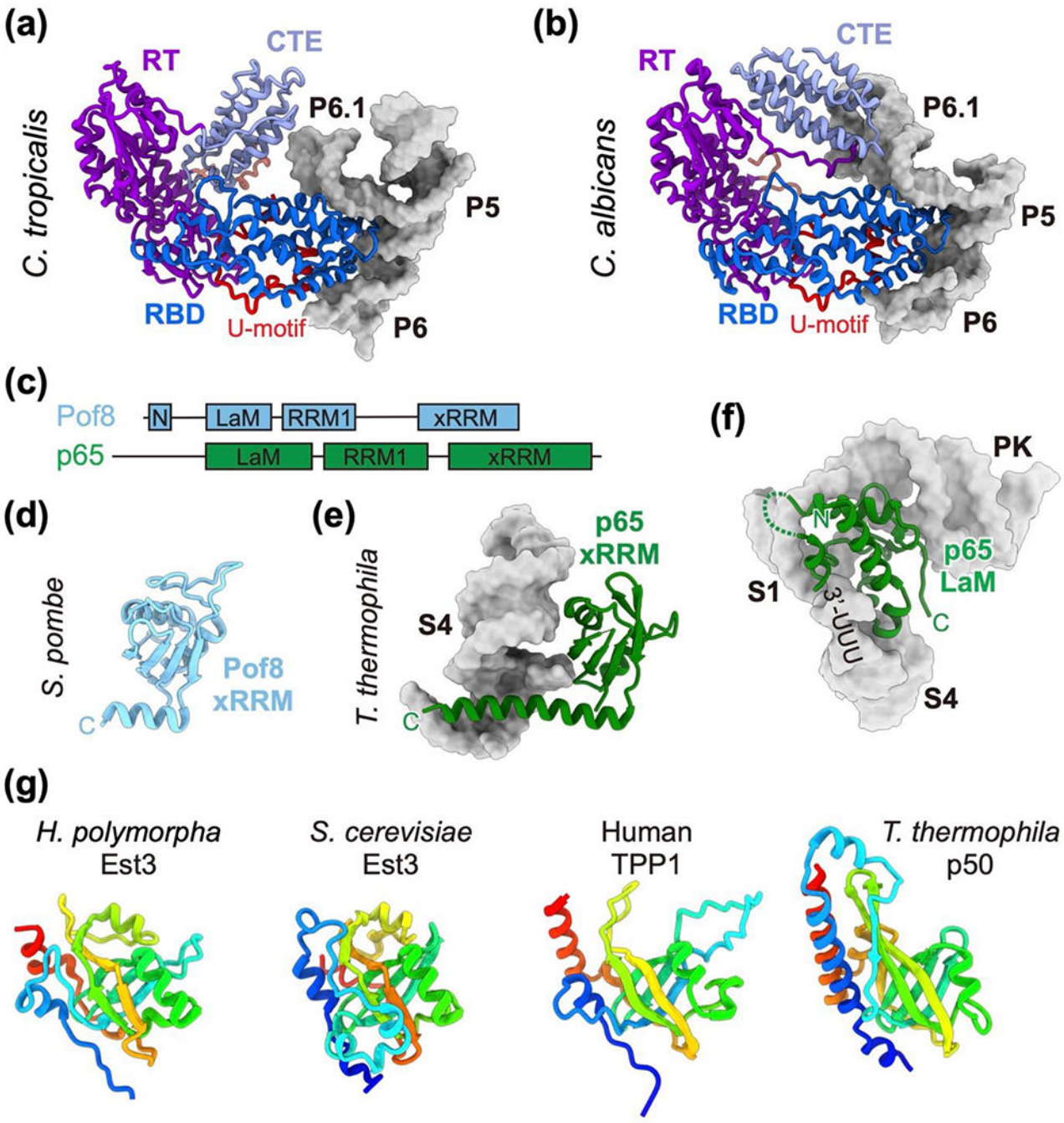

Figure 4. Structural analysis of yeast TERT, Pof8, and Est3.

(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).