Chapter 14
Forms and Functions of Telomerase RNA

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Abstract Telomerase adds single-stranded telomeric DNA repeats to chromosome ends. Unlike other polymerases involved in genome replication, telomerase synthesizes DNA without use of a DNA template. Instead, the enzyme active site copies a template carried within the integral RNA subunit of the telomerase ribonucleoprotein (RNP) complex. In addition to providing a template, telomerase RNA has non-template motifs with critical functions in the catalytic cycle of repeat synthesis. In its complexity of structure and function, telomerase RNA resembles the non-coding RNAs of RNP machines like the ribosome and spliceosome that evolved from catalytic RNAs of the RNA World. However, unlike these RNPs, telomerase evolved its RNP identity after advent of the Protein World. Insights about telomerase have broad significance for understanding non-coding RNA biology as well as chromosome end maintenance and human disease.

14.1 Telomerase Biological Function and Regulation

In all but few eukaryotes, nuclear chromosome ends terminate in a tandem array of simple-sequence repeats. Groups of eukaryotes have different sequences and lengths of telomeric repeat array, such as ~1,000 repeats of T_2AG_3 at an average human cell telomere or ~50 repeats of T_2G_4 at an average telomere in the single-celled model ciliate. The array of telomeric repeats serves at least two conserved functions (Gilson and Geli 2007; Verdun and Karlseder 2007). (1) telomeric repeats and their associated proteins form chromatin structures that protect authentic chromosome termini from fusion or degradation. (2) telomeric repeats provide a mechanism for telomere maintenance that can compensate for incomplete terminus replication by DNA-templated DNA polymerases.

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Eukaryotic chromosome end maintenance relies on new telomeric repeat synthesis by the specialized polymerase telomerase. The telomerase ribonucleoprotein (RNP) complex carries an internal RNA template, complementary to one or more copies of the telomeric repeat. In the presence of a single-stranded DNA oligonucleotide primer in vitro or an appropriate chromosome 3′ end in vivo, telomerase uses its internal template to accomplish de novo synthesis of telomeric DNA repeats (Greider and Blackburn 1989; Yu et al. 1990). Reconstitution of core telomerase enzyme activity in vitro requires template-containing telomerase RNA (TER) and telomerase reverse transcriptase protein (TERT). TERT shares the active site motifs of viral reverse transcriptases (RTs), including the aspartic acids that coordinate catalytic magnesium ions (Autexier and Lue 2006). However, unlike other RT or DNA polymerase polypeptides, the catalytic activity of TERT depends both on the TER template and TER motifs physically separable from the template (Miller and Collins 2002). Non-template RNA motifs play key roles in establishing telomerase's specialized repeat synthesis activity, as described in detail below.

In single-celled eukaryotes, telomerase extends at least a subset of telomeres in each cell cycle. The shortest telomeres preferentially recruit telomerase, giving rise to telomere length homeostasis (Hug and Lingner 2006). In contrast, in cells of multicellular eukaryotes, telomerase activation and telomere maintenance are highly restricted. All human somatic cells express and accumulate TER, but few of these cells express TERT and even fewer show evidence of telomerase-dependent telomere maintenance (Collins and Mitchell 2002; Cong et al. 2002). With ongoing cell proliferation in the absence of telomerase, telomeric repeat tracts become too short to protect chromosome ends from recognition as DNA double-stranded breaks. The presence of critically short telomeres induces cells to cease dividing and instead undergo proliferative senescence or apoptosis (Verdun and Karlseder 2007). The general repression of TERT in somatic cells acts as tumor suppression mechanism (Serrano and Blasco 2007). Cancer cells dramatically up-regulate telomerase to gain extended proliferative capacity (Shay and Wright 2006). Inherited or spontaneous gene mutations that cause as little as 50% deficiency in human TER or TERT cause premature bone marrow failure and a spectrum of other proliferative defects (Wong and Collins 2003; Garcia et al. 2007; Vulliamy and Dokal 2007).

14.2 Telomerase Subunit Composition

Success in the heterologous reconstitution of active telomerase was first achieved using rabbit reticulocyte lysate to express and assemble human TER and TERT (Weinrich et al. 1997). The same approach was successful in reconstitution of the catalytic core of *T. thermophila* telomerase (Collins and Gandhi 1998) and reconstitution of *Saccharomyces cerevisiae* telomerase from a minimized version of *S. cerevisiae* TER and TERT/Est2 (Zappulla et al. 2005). Against these successes are many failures to reconstitute active RNP from the combination of TERT and TER of other species. All telomerase in vitro reconstitution systems require crude eukaryotic cell extract for assembly of TER and TERT, due to the need for folding

and assembly chaperones (Holt et al. 1999; Licht and Collins 1999). How in vitro reconstitution conditions either fulfill or bypass the physiological requirements for active RNP assembly is not yet understood.

The physiologically assembled telomerase RNP complexes with telomeric repeat synthesis activity are designated telomerase holoenzymes. Telomerase holoenzymes harbor numerous proteins other than TERT. Telomerase catalytic activity in a ciliate, vertebrate, other cell extract fractionates by gel filtration with an apparent mass of ~500 kDa or more, with differences depending on the cells and methods used (Collins 1999; Lingner et al. 1997; Schnapp et al. 1998; Aigner et al. 2003; Muñoz and Collins 2004). At the molecular level, telomerase holoenzyme proteins differ between ciliates, vertebrates, and yeasts. Despite this divergence in identity among telomerase-associated proteins, the biological roles of holoenzyme proteins have some similarity. Known roles of telomerase-associated proteins include folding and stabilization of TER, localization of TER and/or TERT, and telomerase activation on telomere substrates (Collins 2006; Zappulla and Cech 2006; Bertuch and Lundblad 2006; Collins 2008; Gallardo et al. 2008). Much remains to be learned about how TER and TERT are folded, assembled, and regulated in function in vivo.

14.3 A Multistep Catalytic Cycle of Repeat Synthesis

Telomerase has an atypically complex catalytic cycle for a nucleic acid polymerase. The overall features of the catalytic cycle can be illustrated using the *T. thermophila* enzyme as an example, because it is intensively studied with regard to enzyme mechanism. The template region of *T. thermophila* TER harbors a 1.5-copy complement of the organisms' telomeric repeat (Fig. 14.1). The 3′ end of a primer can be positioned relative to the template by two mechanisms. If the primer 3′ end is complementary to the template, it can base-pair to create a primer-template hybrid (Fig. 14.1a). Primer alignment by hybridization ensures that any permutation of telomeric repeat at a primer 3′ end is recognized and extended accordingly, to maintain a precise register of telomeric repeats in the product DNA. Alternately, some telomerases including the *T. thermophila* enzyme can position non-telomeric sequence primers at the template "default" position without hybridization. For *T. thermophila* telomerase, the template 3′ cytidine is copied first, resulting in addition of a -G4T2 permutation of the telomeric repeat. This specificity of default template alignment is observed in vitro and also in vivo, when telomerase heals chromosome ends generated by developmentally programmed chromosome fragmentation (Yu and Blackburn 1991; Wang and Blackburn 1997).

Nucleotide addition processivity (NAP) describes the efficiency of copying each successive template position required for synthesis of a telomeric repeat (Fig. 14.1a–c). For the *T. thermophila* enzyme, copying to template 5′ boundary gives product with the 3′ permutation G3T2G-3′ (Fig. 14.1c). This differs from the 3′ telomeric repeat permutation of chromosome ends in vivo, suggesting that cellular factors either modulate telomerase NAP or trim the released product DNA (Jacob

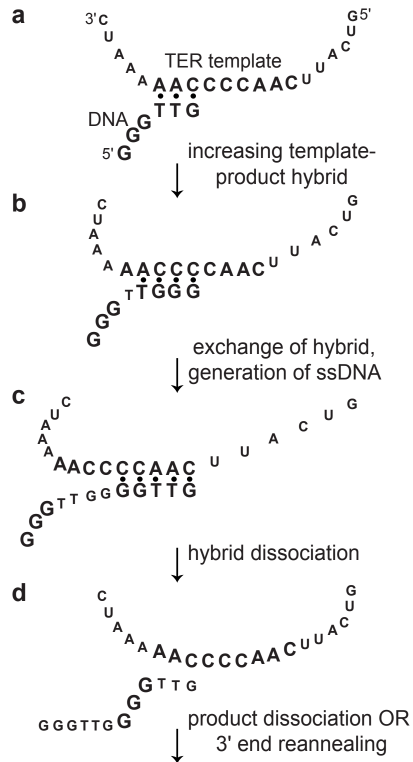

Fig. 14.1 The telomerase catalytic cycle. *T. thermophila* TER template and flanking sequences are depicted over the course of repeat synthesis. Template region nucleotides including the redundant 3′ primer alignment site are shown in bold. Primer and product nucleotides forming the template hybrid are also indicated in bold, along with the putative primer 5′ region bound to TERT

et al. 2003). Notably, primer or product positioning to copy 5′ template positions requires a greater length of template hybrid than to copy 3′ template positions: although 2–3 base-pairs are sufficient to uniquely specify addition of the second G (Fig. 14.1a), at least four base-pairs are required to unambiguously specify addition of the fourth G (Fig. 14.1b). Experiments using sequence-altered templates or chemical modification suggest that the template 3′ end must unpair from product prior to copying the template 5′ end (Fig. 14.1c), limiting the number of base-pairs of template-product hybrid (Wang et al. 1998; Förstemann and Lingner 2005). This feature of the catalytic cycle may serve to equalize the binding affinity of primers with different 3′ permutations or promote product-template dissociation after repeat synthesis (Collins and Greider 1993; Hammond and Cech 1998).

In vitro telomerase activity assays are typically conducted with a large excess of primer. Under these conditions, many telomerases will add multiple repeats to a bound substrate before dissociation. Repeat addition processivity (RAP) describes the efficiency of successive complete repeat additions to each bound primer. RAP requires product to release from pairing with the template and yet remain bound to the enzyme, such that the product 3′ end can be repositioned at the 3′ end of the template (Fig. 14.1d). The *T. thermophila* telomerase holoenzyme assayed in cell extract has over 95% probability of next repeat addition (Greider 1991). In comparison, recombinant

T. thermophila TER + TERT has a maximum ~25% probability of repeat addition and unlike the case for holoenzyme, this relatively limited RAP requires allosteric stimulation by dGTP (Hardy et al. 2001). The extent of telomerase RAP in vivo is unclear (see below). The cellular conditions that determine telomerase RAP in vivo may not be recapitulated in assays of single-stranded primer DNA elongation in vitro.

Telomerase enzymes have additional biochemical activities that are of uncertain biological significance. *T. thermophila* and other telomerases can catalyze endonucleolytic cleavage of bound substrates or products, stimulated by conditions that stall forward DNA synthesis near the template 5′ end (Autexier and Lue 2006). *T. thermophila* and other ciliate telomerase holoenzymes will also readily polymerize a poly-guanosine ladder instead of a ladder of telomeric repeats (Collins 1999). Homopolymer synthesis is not detected using dTTP, perhaps due to the higher Km for the pyrimidine nucleotide.

14.4 Biochemical Differences In Telomerase Holoenzymes

Telomerase activity assays using cell extracts from different organisms have revealed heterogeneity of telomerase catalytic properties, varying around the general theme of template-directed telomeric repeat synthesis described above. A major difference between telomerase holoenzymes of different species is in their relative NAP and RAP. *S. cerevisiae* telomerase predominantly acts with low NAP and low RAP, with product synthesis in vitro limited by slow turnover of the product-template hybrid (Prescott and Blackburn 1997). The degenerate TG1–3 telomeric repeats cloned from telomeres in vivo are produced by frequent copying of a variable, incomplete subset of template positions (Singer and Gottschling 1994; McEachern and Blackburn 1995). In contrast, other yeast telomerases efficiently copy the entire template; some even display substantial RAP (Cohn and Blackburn 1995). Mammalian telomerase holoenzymes differ dramatically in RAP, ranging from high RAP for human telomerase to little or no RAP for telomerase from mice (Morin 1989; Prowse et al. 1993). A synthetic drug that reduces human telomerase RAP in vitro induces telomere shortening in vivo (Pascolo et al. 2002). Likewise, some *T. thermophila* TER variants that reduce holoenzyme RAP in vitro maintain shorter telomeres than wild-type when expressed in vivo (Cunningham and Collins 2005). However, other *T. thermophila* TER variants that reduce RAP maintain telomeres longer than wild-type (Cunningham and Collins 2005). These studies suggest that the balance of telomere length homeostasis depends on specific features of the telomerase catalytic cycle, but the details of this relationship remain to be understood.

More surprising than telomerase holoenzyme differences in NAP and RAP are deviations in the fidelity of primer alignment and template copying. Apparently, for some telomerases, infidelity has a crucial biological role in telomerase function. The variable telomeric repeats of the fission yeast *Schizosaccharomyces pombe* require telomerase to elongate telomere substrates that are 3′-mismatched with the TER template (Leonardi et al. 2008; Webb and Zakian 2008). Plasmodium telomerase generates

mixed telomeric repeats of T2(T/C)AG3 by inserting either dTTP or dCTP across from a particular template guanosine (Chakrabarti et al. 2007). Telomerases from different Paramecium species copy the same A2C4 TER template with different fidelity: *P. caudatum* telomeres are expected repeats of T2G4, but *P. tetraurelia* telomeres are ~75% T2G4 and ~25% T3G3 (McCormick-Graham and Romero 1996). Expression of *P. caudatum* TER in *P. tetraurelia* does not increase fidelity of telomeric repeat synthesis in vivo (McCormick-Graham et al. 1997), suggesting that *P. tetraurelia* TERT or another telomere factor is responsible for reduced fidelity of repeat synthesis.

14.5 TER

The primary sequence of TER is divergent across eukaryotes. Genome sequencing projects have enabled TERT identification in diverse organisms, but identification of TER has been more challenging. Three major phylogenetic groups have been the dominant model systems for molecular studies of TER: ciliates, vertebrates, and budding yeasts. TERs from these organisms are described in greater detail below. Secondary structure models have been derived by comparative sequence analysis. High-resolution structures have been reported for individual motifs within *T. thermophila* or human TER (Theimer and Feigon 2006; Legassie and Jarstfer 2006). Knowledge of global tertiary structure of any TER is still lacking.

14.5.1 Ciliate TERs

Relative abundance of ciliate telomerases has facilitated TER biochemical and molecular characterization. TERs have been cloned from 28 ciliates, ranging in length from 147 to 209 nt (Romero and Blackburn 1991; Lingner et al. 1994; McCormick-Graham and Romero 1996; Ye and Romero 2002). Ciliate TER is an unprocessed primary transcript of RNA Polymerase III with conserved secondary structure (Fig. 14.2a), including a template/pseudoknot region closed by stem I (P1) and a 3′ stem (P4). P2 is absent in some ciliate TERs, and others have an extra stem-loop immediately following the pseudoknot.

Studies of *T. thermophila* telomerase reconstituted in vitro and in vivo have characterized numerous roles for TER motifs in the catalytic cycle (Fig. 14.2a). Substitutions 5′ of the template in the template boundary element (TBE) allow copying of TER positions beyond the normal template 5′ boundary (Autexier and Greider 1995; Lai et al. 2002). The TBE also provides a high-affinity binding site for TERT. TERT contact with the 5′ half of the *T. thermophila* TBE is most critical for high-affinity interaction, while contact with the 3′ half is most critical for template boundary definition; however, all TER substitutions that compromise TBE function reduce TERT binding and vice-versa (Lai et al. 2002). Substitutions 3′ of the template in the template recognition element (TRE) impose a modest defect in NAP and a more

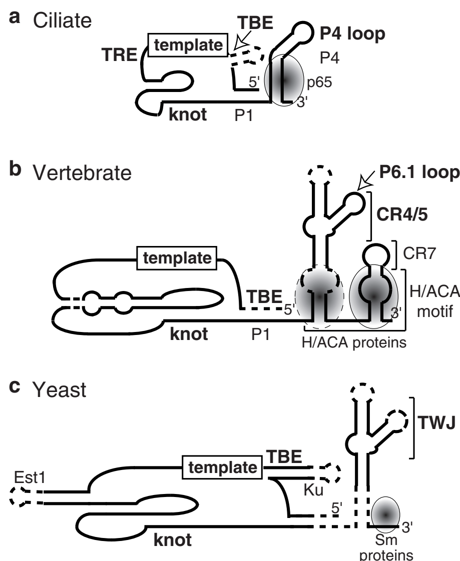

Fig. 14.2 TER secondary structure and functional motifs. TER secondary structures are illustrated with dashed lines representing regions that are variable within the phylogenetic group. Pseudoknots are labeled as knot; other functional motifs are labeled with abbreviations explained in the text: *TWJ* three way junction; *TBE* template boundary element; *TRE* template recognition element. In yeast TER, the three arms extending from the template/pseudoknot core are not drawn to scale; they are truncated in this illustration. TER motifs crucial for biogenesis of a biologically stable RNP are shown with a shaded oval depicting the location of the protein(s) that bind the motif, which are p65, the H/ACA proteins, and the Sm proteins in ciliates, vertebrates, and yeasts, respectively. Whether H/ACA proteins bind to one or both stems of the TER H/ACA motif is not yet established

severe one in RAP (Licht and Collins 1999; Lai et al. 2001; Cunningham and Collins 2005). Substitutions in the P4 loop drastically reduce overall activity (Licht and Collins 1999; Sperger and Cech 2001; O'Connor et al. 2005). Unpairing or deletion of either pseudoknot stem induces a surprisingly modest defect in RAP and RNP stability (Lai et al. 2003; Cunningham and Collins 2005).

The P4 stem and 3′ polyuridine tail are the binding site for a ciliate telomerase holoenzyme protein p65 (Prathapam et al. 2005; O'Connor and Collins 2006). In vivo, p65 is essential for accumulation of TER and TERT (Witkin and Collins 2004). Reconstitution of p65-dependent telomerase RNP assembly in vitro demonstrated that p65 binding to TER induces a stable kink in central P4, which allows subsequent assembly of TERT to generate a unique, conformationally stable p65-TER-TERT ternary complex (Stone et al. 2007). The TER TBE and P4 loop are brought closer upon p65 binding and even closer when TERT joins the p65-TER complex (Stone et al. 2007). These observations are consistent with a model that the TBE/template/TRE region and the P4 loop each bind TERT and perhaps also make a tertiary structure contact, as proposed for the vertebrate TER template region and loop of P6.1 (see below).

An RNA oligonucleotide containing the *T. thermophila* template and 3′-flanking TRE can be positioned in the TERT active site and copied with NAP, if the template-less remainder of the telomerase RNA is supplied as a separate fragment (Miller and Collins 2002). The low NAP and RAP of TER with a P4 loop substitution or deletion can also be fully rescued by addition of the P4 stem-loop as a separate oligonucleotide (Lai et al. 2003; Mason et al. 2003). These and related findings from circular permutation of the TER 5′ and 3′ ends (Miller and Collins 2002) suggest that non-template TER motifs have modular function. Some non-template motifs act in direct physical linkage with the template (for example, the TBE and TRE) while others can function without covalent connection (for example, the P4 loop). Division of labor among TER non-template motifs suggests that these RNAs could have gained their functional complexity in an incremental manner over evolution (see Conclusions below).

14.5.2 Vertebrate TERs

Vertebrate TERs are transcribed by RNA Polymerase II as 3′-extended precursors, which are processed to create mature, functional TER (Feng et al. 1995; Mitchell et al. 1999a). Mature TER also bears a post-transcriptionally added 5′ trimethylguanosine (TMG) cap (Fu and Collins 2006). Phylogenetic sequence comparison of 35 vertebrate TERs ranging from 382 to 559 nt in length revealed elements of shared secondary structure (Chen et al. 2000), including a template/pseudoknot region closed by stem P1 (Fig. 14.2b). The vertebrate TER pseudoknot has much greater sequence conservation than its ciliate counterpart and vastly increased stability of folding, due in part to base-triple interactions formed by one stem and the crossing uridine-rich loop (Theimer et al. 2005). Beyond the template/pseudoknot region, vertebrate TERs possess a 3′ H/ACA (hairpin-Hinge-hairpin-ACA) motif and two Conserved Regions (CRs) of primary sequence, CR4/5 and CR7 (Fig. 14.2b). The 5′ half of the CR7 loop harbors a non-essential CAB box for RNA localization to Cajal bodies (Jády et al. 2004; Fu and Collins 2007; Cristofari et al. 2007).

The vertebrate TER H/ACA motif and a motif on the 3′ side of the CR7 loop termed BIO box are both essential for TER accumulation in vivo (Mitchell et al. 1999a; Martin-Rivera and Blasco 2001; Fu and Collins 2003). The H/ACA motif is shared by a large family of eukaryotic H/ACA-motif RNAs and binds a heterotrimer of dyskerin, NOP10, and NHP2 proteins, each of which is essential for accumulation of mature RNA in vivo and remains associated with mature RNA in the biologically stable RNP (Fu and Collins 2007; Cohen et al. 2007; Collins 2008). Single amino acid substitutions of dyskerin identified to cause the X-linked form of the bone marrow failure syndrome dyskeratosis congenita (DC) reduce the accumulation of TER but not other H/ACA-motif RNAs and impose a telomere maintenance deficiency (Mitchell et al. 1999b; Wong and Collins 2006). Heterozygous TER mutations that either delete a large portion of the H/ACA motif or disrupt BIO box function were identified in patients with autosomal dominant DC (Vulliamy

et al. 2001). As expected, these mutations prevent mutant TER accumulation in vivo (Fu and Collins 2003; Westin et al. 2007).

Vertebrate TER motifs required for telomerase catalytic activity have been investigated by both forward mutagenesis and reverse analysis of disease-linked TER variants identified in patients with DC, aplastic anemia, and other disorders (Garcia et al. 2007; Vulliamy and Dokal 2007). The template/pseudoknot region and CR4/5 are both important for activity reconstituted in vivo or in vitro (Mitchell and Collins 2000; Martin-Rivera and Blasco 2001). Each region alone can bind TERT (Mitchell and Collins 2000; Chen et al. 2002). As expected from the high level of vertebrate TER pseudoknot sequence conservation, pseudoknot stem mutations or mutation of the uridines involved in triple-helix formation inhibit activity assayed in vitro (Fu and Collins 2003; Ly et al. 2003; Chen and Greider 2005). Changes in P6.1 stem pairing, stem length, or loop sequence within the CR4/5 region also inhibit activity (Chen et al. 2002). Short-range cross-links between P6.1 and the template region have been detected using recombinant human TER (Ueda and Roberts 2004). This finding suggests the possibility that in the vertebrate as well as ciliate telomerase RNP, a stem-loop structure distant from the template in primary sequence (vertebrate P6.1, ciliate P4) may be brought in close proximity to the template in the active site of the RNP.

Surprisingly, TERs from mouse and some other rodents lack P1 and instead have only 2 nt 5′ of the template (Chen et al. 2000). These rodent TERs also lack the template 3′ region that is the site of primer hybridization for RAP. This change in TER structure may have evolved in collaboration with a gain in function by a regulatory factor such as a telomere-associated helicase, which could have assumed biological control of template/product dissociation. In vitro reconstitution assays swapping segments between human and mouse TERs have examined the basis for some activity differences between the two species' telomerase enzymes (Chen and Greider 2003). Human TER with a mouse-like 5′ end only 2 nt before the start of the template supports RAP, but lack of P1 abolishes correct specification of the template 5′ boundary. Mouse TERT combined with the human template/pseudoknot has RAP, while human TERT combined with the mouse template/pseudoknot is more inactive than merely lacking NAP or RAP. These and other findings suggest that there has been rapid evolutionary fine-tuning of telomerase activity and regulation among mammals.

14.5.3 *Yeast TERs*

Yeast TERs are transcribed by RNA Polymerase II and, like vertebrate TERs, gain a 5′ TMG cap (Seto et al. 1999; Leonardi et al. 2008; Webb and Zakian 2008). Yeast TERs are also 3′-processed like vertebrate TERs to form the functional mature RNA, but unlike the case of vertebrates, the yeast TER precursor is implicated to be a polyadenylated transcript (Chapon et al. 1997; Leonardi et al. 2008). Mature yeast TERs range in length from 930 to 1,540 nt, larger than all other known TERs except that of Plasmodium (Chakrabarti et al. 2007). Partial secondary structure

has been predicted for budding yeast TERs by phylogenetic sequence comparison and modeling (Chen and Greider 2004; Tzfati et al. 2003). In contrast with ciliate and vertebrate TERs, the yeast TER template/pseudoknot region forms from non-contiguous primary sequence elements (Fig. 14.2c). However, there is strong evidence for a pseudoknot with the same type of triple-helix discovered in human TER (Shefer et al. 2007). As in ciliate and human TERs, the yeast TER template 5′ end is adjacent to a base-paired stem that acts to prevent copying past the template 5′ boundary (Tzfati et al. 2000; Seto et al. 2003).

Three base-paired arms extend from the template/pseudoknot core (Fig. 14.2c). These harbor a binding site for the telomerase holoenzyme protein Est1, a binding site for the chromosome end-binding complex Ku (in Saccharomyces only), or a three-way junction (TWJ) potentially analogous to vertebrate CR4/5 (Peterson et al. 2001; Seto et al. 2002; Chen and Greider 2004; Brown et al. 2007). In vivo reconstitution assays in *S. cerevisiae* indicate that TERT interaction occurs with the central template/pseudoknot region (Livengood et al. 2002; Chappell and Lundblad 2004; Lin et al. 2004). The TWJ arm includes a binding site for Sm proteins near the mature RNA 3′ end. In both *S. cerevisiae* and in *S. pombe*, mutations that disrupt the TER Sm site result in loss of mature RNA (Seto et al. 1999; Leonardi et al. 2008).

Yeast TER motifs important for catalytic activity have been assayed predominantly by in vivo reconstitution, with the exception of a recent rabbit reticulocyte lysate-based reconstitution assay using TERT and a minimized *S. cerevisiae* TER (Zappulla et al. 2005). Due to the large size of yeast TERs, these assays have focused on defining general regions required for function and protein interactions described above. Extensive mutagenesis has been done within the pseudoknot region and TWJ motif to establish more detail about the structures and sequences important for enzyme activity (Chappell and Lundblad 2004; Lin et al. 2004; Shefer et al. 2007; Brown et al. 2007).

14.6 TERT

TERTs have four structural or functional domains (Fig. 14.3): (1) a far-N terminal (TEN) domain, (2) a high-affinity telomerase RNA binding domain (TRBD), (3) reverse transcriptase active site motifs (RT), and (4) a C-terminal extension (TEC). High-resolution structures have been solved for a large portion of the *T. thermophila* TEN domain (Jacobs et al. 2006) and a truncated version of the *T. thermophila* TRBD lacking TER binding activity (Rouda and Skordalakes 2007). How these and the other TERT domains fold relative to each other in the overall active RNP architecture has not been established. *T. thermophila* TERT and TER assemble in vitro and in vivo to form an active RNP with a monomer of each subunit (Witkin and Collins 2004; Cunningham and Collins 2005; Bryan et al. 2003). Human TERT + TER RNPs reconstituted in vitro are said to contain a dimer or higher-order multimer of TER and/or TERT, while reconstitution in vivo produces an active RNP architecture with subunit monomers (Autexier and Lue 2006; Errington et al. 2008).

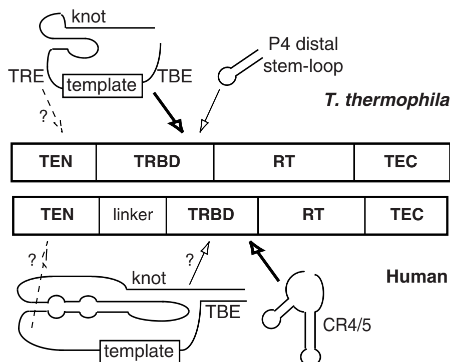

Fig. 14.3 TERT domains and TERT–TER interactions. See text for explanation

Yeast TERs purified from cell extract do not appear to be dimerized (Livengood et al. 2002). However, cooperation between telomerase active sites may occur during telomere elongation (Yu and Blackburn 1991; Prescott and Blackburn 1997).

The TRBD domain of *T. thermophila* or human TERT is sufficient to mediate a specific, high-affinity interaction with TER (Lai et al. 2001). Amino acid substitutions or deletions within the TRBD greatly inhibit TERT assembly with TER in ciliate, vertebrate, and yeast systems (Friedman and Cech 1999; Bryan et al. 2000; Armbruster et al. 2001; Lai et al. 2002; Bosoy et al. 2003). Purified recombinant *T. thermophila* TRBD binds to TER dependent on the TBE motif 5′ of the template, but the TBE alone is not sufficient for high-affinity interaction (O'Connor et al. 2005). In the physiological context of a p65-TER complex, *T. thermophila* TRBD interaction with TER involves both the TBE and the distal stem-loop of P4 (Fig. 14.3). Likewise, the human TRBD can bind both the human TER template/pseudoknot region and CR4/5 (Fig. 14.3). Vertebrate TERT binding to CR4/5 occurs with higher affinity than binding to the template/pseudoknot region (Mitchell and Collins 2000; Chen et al. 2002), and the TRBD is sufficient for this interaction (Mitchell and Collins 2000; Moriarty et al. 2004).

Cross-linking and mutagenesis-based structure/function assays establish the *T. thermophila* TEN domain as a site of DNA interaction in the active RNP (Romi et al. 2007; Jacobs et al. 2006). Similar studies implicate human and *S. cerevisiae* TEN domains in primer binding as well (Lee et al. 2003; Lue 2005). The TEN domain also has RNA binding activity (O'Connor et al. 2005; Jacobs et al. 2006; Moriarty et al. 2004), though the relatively weak TER interaction affinity compared to that mediated by the TRBD has hampered study of its functional significance. High-resolution structure of the *T. thermophila* TEN domain suggests a possible surface for RNA binding apart from the prominent groove for interaction with single-stranded DNA (Jacobs et al. 2006). One tantalizing model for TEN domain function would be to coordinate template positioning in the active site with the positioning of single-stranded product DNA. It will be necessary to develop methods

for monitoring dynamic protein–RNA and protein–DNA interactions across the catalytic cycle in order to understand the complex interplay of protein and nucleic acid associations that drive telomeric repeat synthesis.

14.7 Conclusions: An Evolutionary Perspective on TER Functional Complexity

Studies of structures and functions of TER illuminate many roles for non-coding RNA in the telomerase RNP enzyme, both in the enzyme's complex catalytic cycle and the biological regulation of telomerase function at telomeres. When the predecessor of modern-day telomerase began elongating genome 3′ ends, it may have copied template RNAs only loosely associated with the active site. Specificity would have been improved by anchoring a unique RNA into a stable RNP complex (Fig. 14.4a). The original TER may have harbored only a template and a TERT binding site, but evolution would have subsequently utilized this physical connection to specialize telomerase through changes in TER and TERT. TER-TERT interactions in template-flanking regions would have refined the precision of repeat synthesis (Fig. 14.4b). Additional TER structures could have evolved to improve template use and elongation processivity (Fig. 14.4c). The order of these gains in TER function is purely speculative, but the expanding size and structural complexity

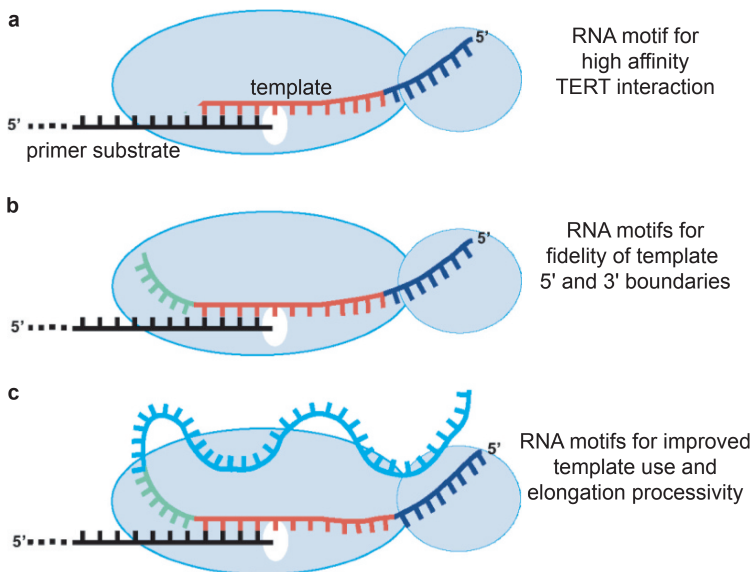

Fig. 14.4 A speculative evolutionary route for gains of TER non-template motif functions. See text for explanation (See figure insert for color reproduction)

of TER may have in turn promoted evolution of a hierarchical RNP assembly pathway with ordered steps of RNA folding and protein–RNA interaction (O'Connor and Collins 2006; Stone et al. 2007). Continuing studies of telomerase RNA form and function will illuminate the mechanism of a fascinating RNP enzyme and also provide insights into the diversity of biological roles of non-coding RNA beyond its ancestral roles as reaction catalyst.

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