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REVIEW

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Evolutionary perspectives of telomerase RNA structure and function

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ABSTRACT

Telomerase is the eukaryotic solution to the 'end-replication problem' of linear chromosomes by synthesising the highly repetitive DNA constituent of telomeres, the nucleoprotein cap that protects chromosome termini. Functioning as a ribonucleoprotein (RNP) enzyme, telomerase is minimally composed of the highly conserved catalytic telomerase reverse transcriptase (TERT) and essential telomerase RNA (TR) component. Beyond merely providing the template for telomeric DNA synthesis, TR is an innate telomerase component and directly facilitates enzymatic function. TR accomplishes this by having evolved structural elements for stable assembly with the TERT protein and the regulation of the telomerase catalytic cycle. Despite its prominence and prevalence, TR has profoundly diverged in length, sequence, and biogenesis pathway among distinct evolutionary lineages. This diversity has generated numerous structural and mechanistic solutions for ensuring proper RNP formation and high fidelity telomeric DNA synthesis. Telomerase provides unique insights into RNA and protein coevolution within RNP enzymes.

Abbreviations: RNP, ribonucleoprotein; TERT, telomerase reverse transcriptase; TR, telomerase RNA

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Introduction

It has been more than a quarter century since the initial discovery of telomerase as the solution to the long standing end-replication problem for linear eukaryotic chromosomes.[1,2] Much progress has been made in the identification of telomerase core components, the catalytic telomerase reverse transcriptase (TERT) and intrinsic template-bearing telomerase RNA (TR), from evolutionary distinct groups of species across eukaryotic lineages.[3] Characterization of these disparate TRs revealed profoundly excessive diversity in length, sequence, and structure. Nonetheless, within this multiformity of divergent structures lies shared specific features necessary and sufficient for TR to function as the essential RNA component of the telomerase ribonucleoprotein (RNP) rather than merely a rudimentary and common template for reverse transcription. The origin of telomerase is seemingly associated with the internalization of an RNA that facilitates telomerase enzymatic function, regulation, and localization within the cell. Recent TR discoveries and structure determination have uncovered the evolutionary connections and implied common ancestors for TR, upheaving and overturning long held assumptions for telomerase evolution. This review will discuss the possible origins of the telomerase RNP core complex, the driving forces behind telomerase evolutionary diversity, and the fundamental features that distinguish and separate TRs from other non-coding RNAs.

Solutions to the end-replication problem

Early in the eukaryotic lineage, linear chromosomes became the universal genetic structure.[4] As opposed to circular chromosomes that are simple to replicate and maintain, linear chromosomes generate uniquely specific problems inherent to the DNA polymerases and require novel solutions. Conventional DNA polymerases fail to fully replicate linear DNA termini by requiring an RNA primer for the initiation and 5′-to-3′ directionality for DNA catalysis. The outcome of these limitations were initially proposed and described as the 'end-replication problem' for lagging strand synthesis.[5,6] With the improved understanding that telomeric DNA has 3′-overhangs, the end-replication problem has been revised and designated for leading strand synthesis.[7] Following chromosome duplication, the blunt ends of DNA produced from leading strand synthesis undergo resection by 5′–3′ exonucleases to recreate the characteristic 3′-overhangs necessary for specific protein binding.[8-10] The progressive loss of the terminal DNA segments after each genome duplication event eventually prevents further cell replication, commonly referred to as the 'Hayflick limit', resulting in cellular senescence.[11,12]

The telomerase enzyme counterbalances the progressive loss of chromosome terminal DNA by adding telomeric DNA repeats onto chromosome ends.[13] Telomerase produces vast arrays of telomeric DNA tracts from its inordinately shorter template located within the TR component.[2,13] This unique property of telomerase stems from the two discrete phases of the telomerase catalytic cycle. The initial phase of this catalytic cycle is the synthesis of a single telomeric DNA repeat directed by the TR template. Upon reaching the template boundary, a template-translocation event regenerates the template, granting additional telomeric DNA repeat synthesis. The reiterative use of the short internal TR template underlies telomerase repeat addition

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processivity. This reiterative catalytic cycle for processive telomeric DNA repeat synthesis by telomerase is extraordinary for a DNA polymerase, requiring a specialized mechanism and telomerase-specific structural elements for the unique template regeneration process following each repeat synthesized.

A myriad of other, less common, resolutions to the end-replication problem have arisen in some eukaryotes, while telomerase is the prevailing solution.[14,15] Repeated insertions of retrotransposons into chromosome terminal regions is employ ed for telomere length maintenance in a small group of insect species that lacks discernable telomerase enzyme components or detectable telomerase activity.[16,17] The lack of telomerase within only a small and closely related group of species, yet retained in neighboring species, indicates that telomerase was recently lost within the dipteran fly insect lineage.[18] Telomerase-independent maintenance has been most extensively studied in *Drosophila*, whose telomeres are composed of tandem arrays of the HeT-A and TART retrotransposons—parasitic genetic elements capable of self-replication through an RNA intermediate.[19] Outside of the *Drosophila* genus, the chromosome ends of dipteran fly species comprise satellite sequences—highly repetitive DNA sequences of 50–800 bp—which are prone to homologous recombination for satellite sequence amplification to extend these terminal sequences.[20-22]

While the overwhelming majority of examined cancer cells rely on telomerase for cellular immortalization, a small subset of specific cancers relies on homologous recombination-mediated pathways for telomere length maintenance. Collectively termed Alternative Lengthening of Telomeres (ALT), the primary mechanism driving ALT is homologous DNA strand invasion and using the invaded strand as template for telomere elongation. Telomeric DNA strand invasion can occur as either (1) self-strand invasion where the DNA forms 't-loops',[23] displacement loops formed at the telomere ends, or as (2) neighboring strand invasion of sister telomeres, resulting in excessive telomere sister exchanges. A hallmark of ALT—for which there is active assay development for clinical testing[24]—is the formation of circular extrachromosomal telomeric DNA fragments that result from these recombination events, termed C-circles. Interestingly, in disparate bacterial families that harbor linear chromosomes as well as extragenomic linear plasmids, DNA ends are maintained by covalently linking the 3′- and 5′-ends of each terminus to form various closed hairpin structures or by capping chromosome ends with covalently bound terminal proteins.[25-28]

The origins of telomerase

The telomerase enzyme arose specifically within and is found ubiquitously throughout the eukaryotic lineage as a successful solution to the end-replication problem.[1,2] Of the two essential components comprising the telomerase RNP core complex, the TERT protein is highly conserved with a central catalytic domain that is homologous to conventional RTs found in both eukaryote and bacteria domains of life.[29-31] Moreover, the presence of the catalytic TERT protein in early branching eukaryotes[32,33] suggests that telomerase originated as an ancient RT that internalized a primitive template-bearing RNA early during eukaryote evolution and later evolved into modern telomerase RNPs with essential and stably associated TR components.

The catalytic domains of TERT and conventional RTs share highly conserved motifs that form the active site for RNA-dependent DNA polymerization.[29,30] Sequence analysis of the conserved RT motifs between TERT and other RTs indicates that TERT is most closely related to non-LTR retrotransposon RTs (Fig. 1).[17,29,34] Telomerase and non-LTR retrotransposons similarly employ target-priming reverse transcription for DNA synthesis. The endonuclease domain of non-LTR retrotransposons permits target-priming and insertion throughout the chromosome interior. In contrast, the lack of an endonuclease domain in the TERT protein restricts telomerase to targeting chromosome termini exclusively. Remarkably, a select group of Penelope-like Elements (PLEs) retrotransposons RTs shares outstanding sequence homology,[35] as well as several key characteristics, with the TERT protein (Fig. 1).[35] These PLEs lack an endonuclease domain and employ target-priming for DNA synthesis targeting chromosome termini.[35] Thus, it has been postulated that an ancient retrotransposon, with properties similar to these termini-proximal PLE RTs, is the ancestor for extant TERT proteins.[17,34]

While an ancient RT with properties reminiscent of PLEs seemingly fulfills a 'missing link' and possible common ancestor for TERT proteins, far less is known of the ancestral RNA

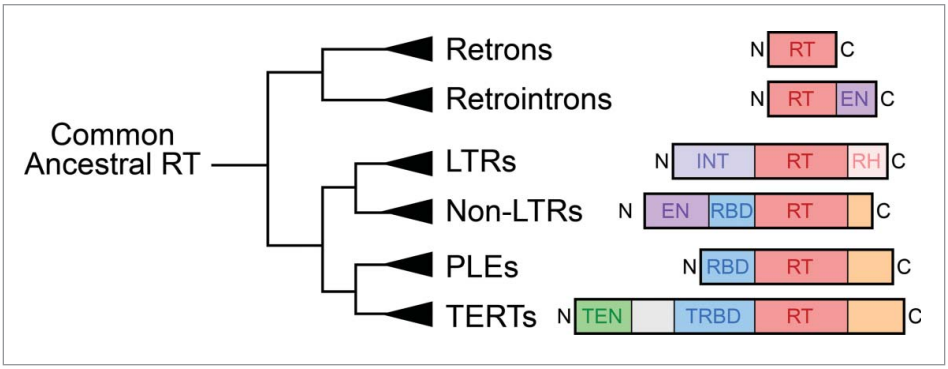

Figure 1. Phylogenetic relationship and structural domains of TERT and conventional RTs. (Left) The TERT protein is closely related to the RTs from PLEs and non-LTR retrotransposons,[147] which similarly employ target-priming reverse transcription. The phylogenetic tree is based on the shared motifs of the RT domain with bacterial retrons and retrointrons as the outgroup for eukaryotic retrotransposons.[148] (Right) Domain organization of retron, retrointron and retrotransposon RTs. The central catalytic RT domain (red) is flanked by variable accessory domains, including endonuclease (EN, violet), integrase (INT, indigo), RNase-H (RH, pink), RNA binding domain (RBD, blue),[37] and a thumb domain (orange). TERT contains a large N-terminal extension compromising of the DNA binding TEN (green) domain and TR binding domain (TRBD, blue).

that gave rise to the integral and exceedingly diverse TRs. TRs from all examined species are highly structured RNAs that form stable RNP complexes with TERT proteins and are indispensable components of functional telomerase enzymes.[36] Given the fact that TERTs and retrotransposon RTs are closely related and share a common ancestor, a possible progenitor for TRs is a retrotransposon RNA intermediate that physically associated with its retrotransposon RT.[4,37,38] However, there are fundamental differences between TRs and retrotransposon RNA intermediates. The TERT protein and TR component are encoded by two separate and distinct genes;[29,30,39-42] the TR transcript is disparate from the TERT protein mRNA.

TERT and TR encoded by two separate genes is noticeably reminiscent of the curious relationship between the separately encoded retrotransposons, long and short interspersed nuclear elements (LINEs and SINEs, respectively). SINEs are long non-coding RNAs that coopted the separately expressed LINE RTs for DNA synthesis.[43] Therefore, it is conceivable that TERT emerged from an ancient RT—similar to the LINE RT—and was coopted by a separately expressed RNA molecule that provided the template sequence for DNA synthesis primed at chromosome termini (Fig. 2A). The evolution of telomerase from this proposed ancient RT and coopting RNA would require several critical events to shape and form a nascent telomere-extending enzyme (Fig. 2). It has recently been proposed that retrotransposon elements are responsible for the very formation of telomeres as arrays of short, highly repetitive DNA segments.[4] This coopting RNA would require a region compatible with early telomere sequences for DNA synthesis by the appropriated RT to function as a nascent telomere maintenance enzyme. Internalization of a progenitor template within the larger RNA would have necessitated a physically defined template boundary (Fig. 2B). RNA appropriation of an RT would have been improved by the development of structural elements that enhanced RNA-protein interactions (Fig. 2C). This stable RNP assembly would promote further integration of this RNA with the RT and, over the course of evolution, greater complexity and dependency of the RT on the RNA would be expected (Fig. 2D). The details of these RNA-protein elements for RNP formation within modern telomerases will be explored further below.

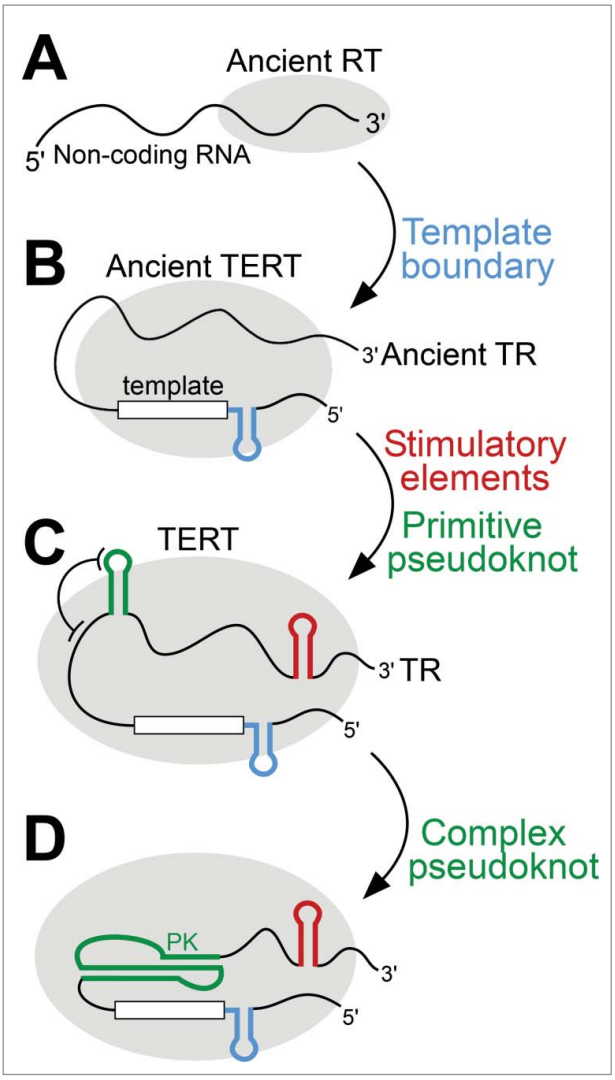

Figure 2. A model for the origin of the telomerase RNP. (A) Telomerase likely originated from an ancient retrotransposon RT that lost its endonuclease domain and associated with a non-coding RNA transcribed from a separate gene. (B) The ancient TR contains a specialized template with the 5′ boundary defined by a TBE (blue). (C) Toward becoming an integral component of the telomerase enzyme, this proto-TR would have evolved a primitive pseudoknot (green) as found in protozoan TRs and a protein-binding structural element (red) for RNP assembly and activity stimulation, which is present in all known modern TRs. (D) TR evolution in fungal and metazoan lineages accompanied the development of a more complex pseudoknot (green).

An RNA template specialized for DNA repeat synthesis

The minimal requirement for a TR progenitor would be to provide the template for *de novo* synthesis of DNA onto chromosome termini for telomeric DNA length homeostasis. In the vast majority of eukaryotes, including vertebrates, echinoderms, filamentous fungi and early diverging flagellates, telomeric DNA contains repeats of the simple hexanucleotide sequence 5′-$(TTAGGG)_n$-3′ (Fig. 3).[3] Having deviated from this canonical sequence, ciliates and plants synthesize telomeric DNA sequences with a single point mutation or insertion, while telomeric DNA from most yeast species is more divergent.[44-46] The predominance of a single sequence—albeit permutated or slightly modified—suggests there was a common ancestral telomerase enzyme that harbored an RNA template for the synthesis of this telomeric DNA repeat sequence.

For the synthesis of telomeric DNA repeats, the RNA template has two distinct segments: the alignment and templating regions (Fig. 3). The alignment region promotes base-pairing with the DNA primer prior to each cycle of DNA synthesis, while the templating region specifies the nucleotide sequence synthesized. Species with the canonical TTAGGG telomeric DNA sequence have TR templates complementary to typically 1.5 to 2 repeats of the telomeric DNA sequence.[39-41,47] For instance, the human TR template is 11 nucleotides in length with a 5 nucleotide alignment region and a 6 nucleotide templating region that encodes for the specific telomeric DNA sequence GGTTAG, a permutation of TTAGGG (Fig. 3).[48] Telomerases from different groups of species synthesize distinct specific telomeric DNA sequences, one of the 6 possible permutations of TTAGGG (Fig. 3). However, within a

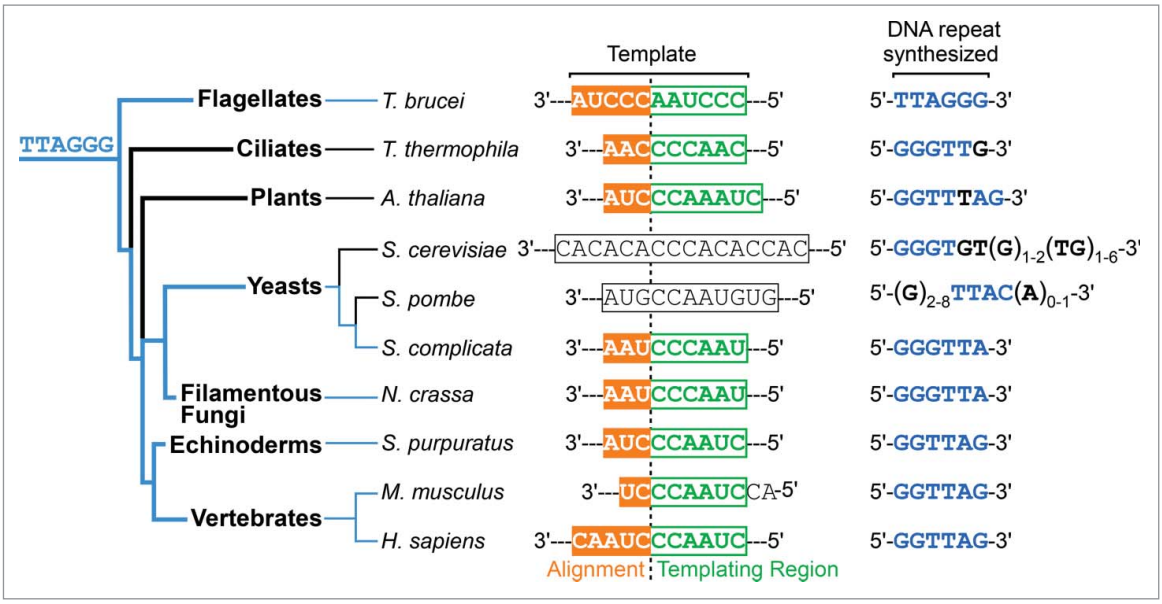

Figure 3. Evolution of telomeric DNA repeat and TR template sequences. (Left) Simplified phylogenetic tree of eukaryotic lineages.[149] Branch length does not reflect evolutionary distance. (Right) The TR template is composed of the 3′ alignment (orange) and 5′ templating (green) regions. The alignment region positions the 3′-end of the target DNA through base-pairing interactions, while the templating region specifies the DNA sequence synthesized. Budding and fission yeast TR templates are degenerate, with the alignment and templating regions poorly defined (open box). The 5′-TTAGGG-3′ (blue) telomeric DNA repeat is evolutionary conserved and found in most groups of eukaryotes including early branching flagellates. Deviations from the putatively ancestral telomeric DNA repeat sequence are denoted (black). Telomerases from different species synthesize different permuted registers of the TTAGGG sequence. Representative species shown include *Trypanosoma brucei, Tetrahymena thermophila, Arabidopsis thaliana, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Saitoella complicata, Neurospora crassa, Strongylocentrotus purpuratus, Mus musculus, and Homo sapiens*.

tract of telomeric DNA, the specific DNA sequence forming an individual repeated unit is indistinguishable.

The alignment region within the TR template is essential for telomerase repeat addition processivity, the reiterative synthesis of telomeric DNA repeats without complete disassociation of telomerase from the DNA strand.[49] The length of the template alignment region directly impacts telomerase repeat addition processivity. Select rodent species have TRs with a shortened alignment region of merely 2 nucleotides and subsequently lower repeat addition processivity (Fig. 3).[50] The low repeat addition processivity of rodent telomerase can be dramatically increased by extending the length of the alignment region.[51] Reciprocally, reducing the length of the human TR alignment region correspondingly reduces human telomerase repeat addition processivity. The small population of yeast TRs harbor considerably larger and more divergent template sequences that is consistent with their more degenerate telomeric DNA repeat sequences.[52] The alignment region of these yeast TRs is less well defined due to the longer and more degenerate telomeric repeats synthesized (Fig. 3), which reduces yeast telomerase repeat addition processivity.[53]

Beyond influencing processive telomeric DNA repeat addition, the specific sequence of the template directly affects telomerase enzymatic activity and even regulates template utilization. Mutations within the template sequence of human TR alter the rate of telomeric DNA repeat synthesis.[54] A single nucleotide within the human TR template was found to serve as a critical signal for pausing continuous nucleotide addition at the template boundary.[55] Loss of this pause signal would introduce stuttering across the template, stalling nucleotide addition at random points and generating heterogeneous telomeric DNA products. This pause signal functions, in part, to define the end of the templating region together with RNA structural elements.

The 5′ boundary of the TR template requires physical definition and enforcement to prevent DNA synthesis into the flanking non-template regions. Reverse transcribing the unintended sequence beyond the template boundary results in the synthesis of non-telomeric DNA sequences onto the chromosome ends, which impedes the binding of telomeric proteins and is deleterious for telomere function.[56] Among all known TRs examined, two distinct types of template boundary elements (TBEs) have been found for 5′ boundary definition: (1) a local template-adjacent helix common to the vast majority of eukaryotes, or (2) a long-range base-paired core-enclosing helix found exclusively within the vertebrate lineage (Fig. 4A).

The template-adjacent helix TBE is conserved across eukaryotic lineages that include ciliates, flagellates, fungi and echinoderms (Fig. 4A). This TBE defines the template boundary by physically restricting the availability of single-stranded RNA to be used as template through either TERT-binding or simple base-pairings that form the helix. In ciliate TRs, the template-adjacent TBE is a short stem-loop structure, termed helix II, with a highly conserved sequence at the base of the stem that serves as the binding site for motif CP2 located within the TR-binding domain (TRBD) of the TERT protein (Fig. 4B).[57-60] TERT binding impedes utilization of template-flanking sequence as template. In the basal eukaryote species, flagellates, the TBE similarly comprises a larger template-adjacent helix that potentially serves as a TERT binding site.[61] The fungal TBE is a stable helical structure located immediately upstream of the template.[41,62,63] Within fission yeast, the TBE helix even partially overlaps with the template, resulting in DNA products with sequence heterogeneity due to the intermittent use of the overlapping residue as template.[64]

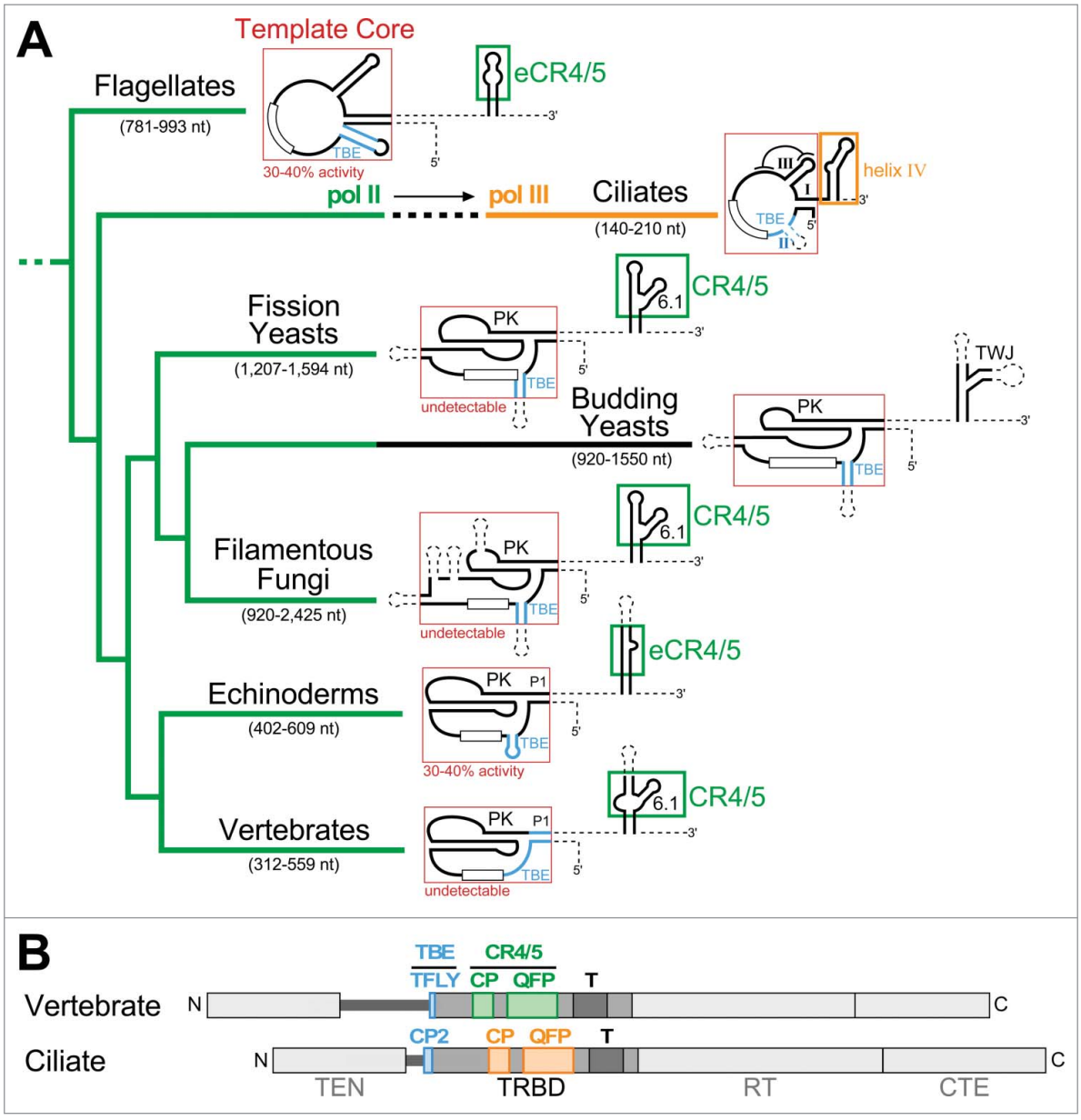

Figure 4. TR domains essential for telomerase enzymatic activity. (A) Within all TRs is the template core domain (red box) with the TBE (blue) and later branching species include an essential pseudoknot structure (PK). The percentage of activity generated by the template core, without the remainder of the RNA is denoted. TR in most species is transcribed by RNA pol II, with a specific and unique transition event to RNA pol III (orange) within the ciliate lineage. The shared CR4/5 (green) among the evolutionarily distant vertebrate and fungal lineages implies a common ancestor with the CR4/5 element.[41] The echinoderm eCR4/5 (green) lacks the P6.1 stem-loop and its presence is less essential for function, while the *S. cerevisiae* TWJ (black) lacks the P6.1 stem-loop and is not required for function, demonstrating more outlier features from the presumed common TR ancestor (green line). Ciliate helix IV has potentially arisen from convergent evolution (orange). (B) RNA binding motifs in the TERT protein. Schematic of the TERT protein from vertebrates and ciliates denoting the 4 structural domains, TEN, TRBD, RT and C-terminal extension (CTE). Within the TRBD, the TFLY and CP2 motifs (blue) bind TBE, while the CP and QFP motifs (green) associate with CR4/5. Association of ciliate helix IV with TRBD motifs (orange) is speculative.

The core-enclosing helix TBE is found throughout vertebrate TRs and is dissimilar from the helix proximal TBE, functioning as a tether that restricts the template-flanking linker region from reaching the TERT active site.[51] The vertebrate TBE mechanism relies on the length of the single-stranded linker spanning the template and the distal core-enclosing helix, termed P1 (Fig. 4). The TFLY motif, identified in the crystal structure of a teleost fish TRBD, has been implicated in binding the P1 helix or potentially the adjacent single-stranded linker.[65] Interestingly, TRs from select rodents lack a physical TBE and instead have the 5′-end of TR merely 2 nucleotide upstream of the template.[66] This lack of sequence upstream of the template evidently functions as the rodent TBE by preventing DNA synthesis beyond the template boundary.[50]

Despite the close relationship to vertebrates, echinoderm TR does not employ the vertebrate-type TBE mechanism. Instead, the echinoderm TBE comprises a template-adjacent stem-loop, termed P1.1, that resembles those found in ciliate, flagellate, and fungal TRs (Fig. 4A).[47] Remarkably, the deletion of the echinoderm P1.1 stem-loop shifts the physical boundary to the distant P1 core-enclosing helix, which is then structurally equivalent to the vertebrate core-enclosing helix TBE. This would suggest that the vertebrate TBE is likely is a functional compensation for the loss of the template-adjacent TBE. The

more prevalent template-adjacent TBE is presumably an early-adopted essential feature of the common ancestral TR, as it is even found in the early branching flagellate TRs (Figs. 2 and 4).

Evolution of the template core domain

The TR template is located within a large enclosed loop, termed the template core domain, that itself exhibits binding affinity to the TERT protein.[67,68] This TERT-binding affinity has been proposed necessary for positioning the template within the active site and seemingly important for facilitating the movement of the RNA template during telomerase catalytic cycles.[69] Curiously, the template can be excised from the template core domain and added back *in trans* with a DNA primer as an RNA/DNA duplex to serve as substrate for DNA synthesis by telomerase,[70] similar to the reaction catalyzed by conventional RTs. The remaining non-template portion of the template core domain, however, remains essential for telomerase enzymatic function. This indicates that the remainder of the template core domain is an essential element of the telomerase RNP and functions beyond merely bringing the template to the TERT active site.

In the vast majority of known TRs, a pseudoknot structure is present and located downstream of the template within the TR secondary structure.[36,71,72] However, the specific size and secondary structure complexity of pseudoknots in the template core domain are highly variable among distinct evolutionary lineages. Metazoan and fungal TR pseudoknots consist of larger, more stable helices and are necessary for telomerase enzymatic activity (Fig. 4A).[40,41,52,73-75] In contrast, ciliate TR pseudoknots are rather primitive (Fig. 4A) and are functionally dispensable for telomerase *in vitro* enzymatic activity, yet apparently necessary for *in vivo* telomerase function.[76,77] Recent functional determination of the trypanosome minimal TR domains found no evidence to support even a primitive pseudoknot in the template core domain (Fig. 4A).[61] The lack of a pseudoknot structure in trypanosome species profoundly alters the paradigm for TR functional requirements and its structural evolution. The lack of a pseudoknot in the basal eukaryote species trypanosome, together with the minimally structured and seemingly dispensable ciliate pseudoknot, suggests that complex and essential pseudoknot structures found in higher eukaryotes was later evolved. Thus, the common ancestor for extant TRs presumably lacked a template adjacent pseudoknot structure.

The disparity in pseudoknot presence is reflected in different TERT binding locations within the TR template core domain. In ciliate TRs, the TBE helix II is a crucial binding site for the TERT protein.[59,78] The base of helix II contains a highly conserved sequence motif that is recognized and bound by the TERT protein.[60] Within metazoan and fungal TRs, the pseudoknot appears to contain a TERT-binding site, as the human TR pseudoknot can be physically disjoined from the template and the core-enclosing helix P1, yet assemble with its TERT protein to reconstitute catalytic activity with an exogenous RNA/DNA duplex substrate.[70] However, specific motifs or residues within the pseudoknot structure responsible for TERT protein interactions have not yet been identified.

Apart from TERT binding, vertebrate and fungal TR pseudoknots are essential for telomerase enzymatic function.[73,75,79] Within human TR, a unique triple helix structure was found at the helical junction of the pseudoknot structure.[80] Similar triple helix structures have also been identified, or predicted, in TR pseudoknots from other species that include fungi.[81,82] Within the triple helix, the 2′-hydroxyl groups of invariant adenosine residues appear to be critical for telomerase activity.[81] Moreover, structural studies of addition helical regions within the human pseudoknot structure revealed a sharp kink within the helix proximal to the template, which is possibly important for template positioning or dynamic movements during the telomerase catalytic cycle.[83,84] However, the precise function of either the essential pseudoknot or triple helix has yet to be elucidated, nor the underlying reason for tolerating the lack of a pseudoknot within basal eukaryote species.

A second TERT-bound TR element for activity stimulation

Telomerase functions as an RNP enzyme, requiring specific and stable interactions between the TERT protein and TR. While the TR template core domain provides weak interaction with TERT,[67,68] a second and stronger TERT binding site is located at a variable distance from the template core domain (Fig. 4A). Within separate evolutionary lineages, this TR template-distal TERT-binding site folds into distinct secondary structures and was identified as the conserved regions 4/5 (CR4/5) domain in vertebrates,[85] 3-way junction (TWJ) in budding yeasts,[86] and helix IV in ciliates.[87] Analogies have been suggested for these lineage-specific distal stem-loop structures,[71] however, evolutionary connections have remained tenuous. While vertebrate CR4/5 and budding yeast TWJ similarly comprise an intersection of 3 helical regions,[86] budding yeast TWJ lacks the vertebrate-specific stem-loop P6.1 that is extremely well-conserved and consists of a 4-bp stem followed by a 5-nucleotide distal loop.[85] In contrast, ciliate helix IV is merely a simple stem-loop structure.[88] The evolutionary origins and connections among these lineage-specific structural domains from ciliates, budding yeasts and vertebrates have remained enigmatic.

Despite their massive size, the recent identification of filamentous fungal TRs were instrumental in reconciling the evolutionary connections among vertebrate and fungal TRs.[41,89] Remarkably, the minimal TR core elements for filamentous fungi and fission yeasts were found to contain structural elements that are highly similar to vertebrate CR4/5 and termed herein fungal CR4/5. Structurally, fungal CR4/5 comprises three highly conserved short helices, which includes the exceptionally critical P6.1 stem-loop (Fig. 4A).[41] Functionally, fungal CR4/5 from filamentous fungal and fission yeast TRs are identical to vertebrate CR4/5, being absolutely essential for the reconstitution of telomerase activity and can be bound by the TERT protein *in trans* as an RNA fragment physically separated from the template core domain.[41] Moreover, vertebrate and fungal CR4/5 require an invariant adenosine residue located between the P6 and P6.1 helices for TERT binding, further supporting structural and functional homology among vertebrate and fungal CR4/5.[90] The presence of CR4/5 in vertebrate and fungal lineages indicates the metazoan-fungal common ancestor likely

harbored a CR4/5 element. This suggests that the TWJ from budding yeast is divergent, having evolved away from the implied fungal common ancestor. Moreover, budding yeasts do not seem to require the TWJ for telomerase function. Budding yeast cells survive and retain cellular proliferative capacity with a miniature *Saccharomyces* TR that lacks the TWJ, demonstrating that the TWJ is dispensable within the cell.[91] However, there have been some conflicting results with yeast *Kluyveromyces* TR. Mutations that disrupt this TWJ have been reported to abolish *in vitro* analyzed telomerase activity.[86] Thus, further analysis in budding yeast is necessary to determine the requirement of the TWJ for telomerase function.

Interestingly, despite their evolutionary close proximity to the vertebrate lineage, echinoderm TRs evidently lack the essential and presumed ancestral CR4/5.[47,92] Instead, there is an internalized helical region within the echinoderm TR central region that is functionally equivalent to the vertebrate and fungal CR4/5, termed equivalent CR4/5 (eCR4/5). While functionally equivalent to CR4/5, the echinoderm eCR4/5 structurally lacks the exceptionally critical P6.1 stem-loop (Fig. 4A). As an apparent consequence, echinoderm telomerase is less dependent on eCR4/5 for catalytic activity, while vertebrate and fungal telomerases are absolutely dependent on CR4/5 for activity (Fig. 4A). The echinoderm TERT and the template core domain are sufficient to generate a basal level of activity at 30–40% of the full activity.[47] The gain-of-function for the template core domain presumably facilitated the structural transition from the essential CR4/5 to the less critical eCR4/5 of echinoderm TR.

The functional requirement for two TR structural domains, the template core and either CR4/5 or eCR4/5, to reconstitute a full-level of telomerase activity *in vitro* is conserved in the early branching flagellates. The recent structural and functional study of the minimal trypanosome TR domains identified an eCR4/5 domain that is located approximately 350 nucleotides downstream of the template core domain and is important for telomerase activity.[61] In accordance with echinoderm eCR4/5, flagellate eCR4/5 lacks the vertebrate and fungal essential P6.1 stem-loop (Fig. 4A). Moreover, the flagellate TERT protein and TR template core domain can reconstitute a basal level of telomerase activity without eCR4/5, similar to echinoderms.[61] The identification of an eCR4/5 element in early diverging eukaryotes suggests that a second TERT-bound TR element for telomerase RNP formation and enzymatic function is a conserved feature from the common ancestor of telomerase.

The distal portion of ciliate TR helix IV has been considered analogous to CR4/5, although it merely reconstitutes weak activity *in trans* with the template core domain.[87] Distinct from CR4/5 and eCR4/5, ciliate helix IV has weak affinity to the TERT protein evidenced by its inability to reconstitute a full-level of activity when separated from the template core domain.[87] The weak binding affinity of helix IV is presumably due to the highly compact structure of ciliate TRs. Moreover, the proper positioning of the helix IV distal loop to the TERT protein requires the ciliate-specific p65 protein to stabilize the kink in helix IV,[93] further reducing the evolutionary selection pressure for strong TERT-binding affinity. The primary driver for the compaction of the ciliate TR was likely the transition of transcription machinery from RNA polymerase II (pol II) to pol III which is specific for the synthesis of small RNA transcripts.[88] This single event would have truncated the TR as pol III transcription terminates at U-rich sites that were likely present in the ancestral ciliate TR gene and would have then eliminated an ancestral CR4/5 or eCR4/5 element. The loss of the presumed ancestral ciliate CR4/5 or eCR4/5 could have been compensated for by a basal-level of telomerase activity generated by the template core domain, with the later emergence of helix IV by convergent evolution. It is possible, while seemingly less likely, that ciliate TR was shifting toward a diminutive RNA prior to the transition to pol III. The latter possibility would suggest that helix IV is a degenerate CR4/5 or eCR4/5 with reduced functionalities from divergent evolution.

The exact mechanism of CR4/5 and eCR4/5 conferring or stimulating telomerase enzymatic activity has remained enigmatic. Nonetheless, there has been considerable progress in determining the structure and binding position of vertebrate CR4/5 on the TERT protein.[90,94] While the vertebrate CR4/5 includes three helices, P5, P6 and P6.1, the P6 helix and helical junction alone are responsible for binding by TERT.[85,94,95] UV-crosslinking mapped the location of CR4/5 binding to the TRBD surface comprising the CP and QFP motifs (Fig. 4B).[94] The molecular basis for CR4/5-TRBD recognition was later provided by a high-resolution crystal structure of the RNA-protein complex.[90] However, the function of the extremely conserved and absolutely essential P6.1 stem-loop could not be discerned. It has been postulated that the CR4/5 P6.1 stem-loop reinforces stable folding of TERT domains, allosterically buttressing the critical active site for telomerase DNA catalysis.[90,94] Analysis of CR4/5 in the context of a larger TERT protein fragment is necessary to examine this possibility and resolve the mystery of CR4/5 function. Further experimentation involving the structurally distinct eCR4/5 is needed to discern whether the eCR4/5 mechanism is similar to CR4/5 or has a separate underlying purpose for telomerase activity stimulation.

Diverse mechanisms for TR biogenesis

While the template core and CR4/5 domains are responsible for providing a defined template and stable RNP assembly, additional structural domains located principally in the 3′ portion of TR are essential for TR biogenesis and appear to be the primary driver of TR diversity (Fig. 5). The 3′ biogenesis domains of TRs from evolutionarily distinct groups of species employ seemingly mutually exclusive RNA metabolism pathways for TR 3′-end processing, stabilization, and nuclear compartment localization. TR biogenesis domains provide binding sites for a plethora of species-specific TR accessory proteins necessary for discrete biogenesis pathways.[40,41,47,52,88,96-100] The cooption of these distinct biogenesis pathways by TR requires the incorporation of appropriate binding sites and structural elements to accommodate the necessary accessory proteins (Fig. 5). Thus, separate biogenesis pathways employed across species is the apparent dominant source for TR overall size, sequence and secondary structure disparities.

Vertebrate TR shares a biogenesis pathway with H/ACA small nucleolar (sno) and small Cajal body (sca) RNAs (Fig. 5). The 3′ portion of vertebrate TRs contain a conserved structural domain, termed the H/ACA, that comprises a tandem array of stem-loops interspersed with the highly conserved box H and ACA sequence motifs.[101,102] In common with H/ACA

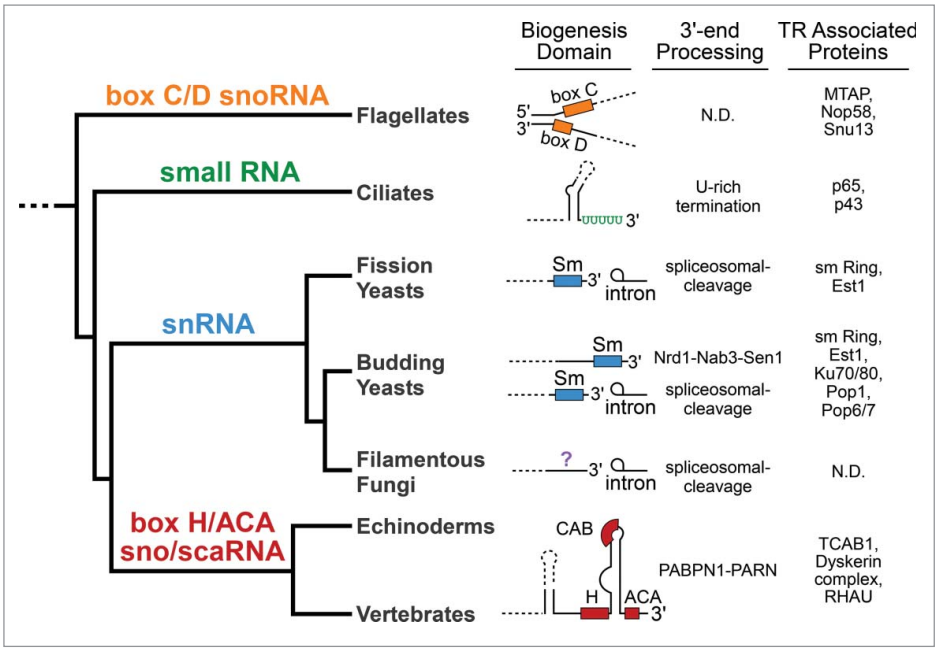

Figure 5. Divergent biogenesis pathways for TR maturation. Four mutually exclusive RNA biogenesis pathways, box C/D snoRNA, pol III transcribed small RNA, snRNA, and box H/ACA sno/scaRNA, are employed for TR biogenesis in separate evolutionary lineages. Schematic of the 3′-end biogenesis domains in TRs with important recognition motifs denoted (colored boxes). The wide array of distinctive 3′-end processing mechanisms is listed. TR associated proteins listed have been determined to directly bind to TR. Mechanisms and accessory proteins that have not been determined (N.D.) as well as telomerase and telomere accessory proteins that do not directly bind to TR are omitted.

snoRNAs, two copies of the dyskerin complex protein tetrad—consisting of dyskerin, NOP10, NHP2, and GAR1—are bound to the vertebrate TR H/ACA domain.[103-108] The 3′-apical loop in the human TR H/ACA domain is bound by telomerase Cajal body protein 1 (TCAB1) for specific localization to Cajal bodies, a nuclear compartment rich in RNA splicing and post-transcriptional modification machineries.[109] Additionally, within this 3′-apical loop is a biogenesis promoting (BIO box) motif.[110,111] At the 5′-end of vertebrate TR is a G-quadruplex structure, which is resolved by the stable association of the RHAU DEAH-box RNA helicase.[112] The nascent vertebrate TR precursor is transcribed by RNA pol II with a poly(A) tail and the 3′-end is processed by the poly(A)-binding protein 1 (PABPN1) and the poly(A)-specific RNase (PARN).[113,114] Echinoderm TRs likely share a highly similar biogenesis pathway with vertebrate TRs, as they contain a homologous H/ACA domain (Fig. 5).[47,92]

While vertebrate and presumably echinoderm TRs have acquired a sno/scaRNA biogenesis pathway,[72] fungal TRs utilize the small nuclear RNA (snRNA) biogenesis pathway for TR maturation.[72,100,115,116] Following the snRNA biogenesis pathway, the 3′-end of budding and fission yeast TR harbor a binding site for the Sm protein heptameric ring for end protection and maturation. However, there is diversity in the TR 3′-end processing mechanism employed by fungal species (Fig. 5). Select budding yeasts that include *Saccharomyces* rely on the Nrd1-Nab3-Sen1 non-coding RNA transcription termination pathway for TR 3′-end processing,[117] while fission yeast, *Candida* budding yeast, and all known filamentous fungal TRs are processed by spliceosomal cleavage.[118-121] Spliceosomal cleavage relies on RNA intron splicing machinery that has been coopted for TR 3′-end cleavage by blocking the second transesterification reaction. Moreover, the vastly larger fungal TRs function as flexible scaffolds for binding and positioning accessory proteins.[91] The fungal-specific helix formed between the template and the pseudoknot in budding and fission yeast TRs is bound by the ever-shorter telomere 1 protein (Est1p), a critical protein for telomere maintenance (Fig. 5).[122-124] In contrast, ever-shorter telomere 3 protein (Est3p) does not directly bind to yeast TR and instead interacts with the TERT protein.[125] The apical stem-loop of the template-adjacent TBE helix from *Saccharomyces* TR harbors a Ku70/80 heterodimer binding site, which plays a role in telomerase recruitment to telomeres (Fig. 5).[126-128] An internal loop adjacent to the Est1p binding site in *Saccharomyces* TR shares a high degree of sequence similarity with the P3 domain of the RNase P and mitochondrial RNase P (MRP) RNA component and was termed the P3-like domain.[125,129] The *Saccharomyces* TR P3-like domain, similar to the P3 domain from the RNase P and MRP RNA component, is bound by the processing of precursor 1 (Pop1) and the Pop6/7 heterodimer proteins for functional telomerase assembly, further demonstrating the phylogenetic diversity of telomerase holoenzyme composition (Fig. 5). The TR biogenesis pathway for the largest fungal group of species, filamentous fungi, has yet to be determined (Fig. 5).

Ciliate TRs follow the biogenesis pathway common for small pol III RNA transcripts (Fig. 5). RNA pol III transcription terminates at a U-rich region, resulting in RNA products with a short 3′-poly(U) tract.[88] Apart from all other known TRs that have the their 3′-ends processed by a variety of mechanisms, the nascent short 3′-poly(U) tail of ciliate TRs is retained and bound by the La proteins, p65 and p43,[130,131] which aid in RNA maturation.[132,133] Binding by the p65 protein induces a distinct bend in the *Tetrahymena* TR helix IV, enhancing interaction between the distal loop of helix IV with the TERT protein, which is critical for telomerase activity.[93,134] While ciliate TRs are the smallest identified, the ciliate telomerase holoenzyme is a large complex with numerous protein components.[130,131,135,136] Comprehensive analysis of

telomerase holoenzyme complexes from other species will permit comparative analysis to determine whether the compaction of the ciliate TR was accompanied by protein component expansion.

Interestingly, there are no similarities in the biogenesis pathways between ciliate and flagellate TRs (Fig. 5). Instead, flagellate TRs share a biogenesis pathway with box C/D snoRNAs,[98,99] which more closely resembles the box H/ACA sno/scaRNA pathway employed by vertebrate TRs.[101,102] As expected from following the box C/D snoRNA biogenesis pathway, flagellate TRs are bound by the box C/D proteins Nop58 and Snu13.[99] While little is known for 5′- or 3′-end processing of mature flagellate TRs, the 5′-end of the nascent RNA pol II TR transcript is *trans* spliced.[97] The *trans* splicing of a separately encoded RNA leader sequence onto the 5′-end of the initial flagellate TR transcript is common for flagellate mRNAs.[137] The spliced leader sequence of flagellate TR is bound by the methyltransferase-associated protein (MTAP), which is related to the vertebrate TCAB1 protein.[99] It will be exciting to discovery whether the flagellate telomerase holoenzyme complex shares any significant similarity with the ciliate telomerase holoenzyme complex, especially for a p65 functional homolog, as *in vitro* trypanosome telomerase activity is not highly processive.[61]

Although TRs are massively divergent in sequence, length, and structural composition, all known TRs encompass two TERT-interacting domain and a biogenesis domain for TR maturation. The two TERT-interacting domains, comprising the template core and CR4/5 or eCR4/5, are well-conserved compared to the biogenesis domains that varies radically across distinct eukaryotic lineages (Fig. 5). The driving force for these separate TR biogenesis pathways would seem to be the inherent volatility of long non-coding RNAs, compared with protein-coding mRNA counterparts, and are strongly affected by the chaos ensuing genome duplications, transpositions, and rearrangements.[138-142] The turmoil following large-scale genomic events would provide ample means for gene fusions with other non-coding RNAs, leading to rapid alterations of TR biogenesis pathways along specific lineages (Fig. 5). In sharp contrast, there is stronger selection pressure on the critical template core and CR4/5 domains to restrain their evolution, evidenced by the higher conservation of these two structural domains (Fig. 4A). TRs from the plant *Arabidopsis thaliana*, and closely related family of species from Brassicaceae, have provided amazing insights into the interplay of genome duplication and telomerase holoenzyme components.[143,144] Duplicated plant TR genes have been reported to lack the unequivocally essential template region, rendering this TR non-function for telomerase activity. Instead, these template-lacking TRs retained TERT association and function for TR regulation as a direct competitor for TERT binding.[145] Determining plant TR secondary structure will be essential for comparative analysis to understand TR evolution and diversity within the plant lineage from the common ancestor of extant TRs. While encompassing the essential element for telomerase RNP formation and enzymatic function, TRs are profoundly flexible in adopting and coopting a multitude of biogenesis pathways while remaining the intrinsic RNA component for telomerase enzymatic function.

Concluding remarks

TR is a complex non-coding RNA with highly organized and specialized structural domains to facilitate telomerase enzymatic function. Initially identified from ciliates, yeasts, and vertebrates, TRs exhibit an unprecedented divergence in length, sequence, and secondary structures across species. The hallmark of TR is its innate ability to adapt, to incorporate distinct and unique mechanisms necessary and sufficient for assembly with the TERT protein, to regulate and impart telomerase enzymatic activity, and assimilate a myriad of disparate biogenesis pathways for RNA maturation. Reconciliation of these disparities has been arduous, stemming from this very lack of conservation among even closely related groups of eukaryotic species. Recent advances in discerning the essential core domains for TRs from major eukaryotic lineages has advantageously revealed the structural and functional consensus from disparate and seemingly unrelated TR elements.[41,71,86,146] There is a less-than-subtle irony that the first identified and extensively studied TRs from the ciliate and yeast lineages appear to be outliers from the implied common ancestral TR structure that is conserved in vertebrates, filamentous fungi, fission yeasts and flagellates. There still remains much work toward elucidating the mechanism by which TR has been capable of incorporating these numerous distinct structural elements, while remaining the functional and intrinsic component of the telomerase enzyme.

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No potential conflicts of interest were disclosed.

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References

1. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985; 43:405-13; PMID:3907856; http://dx.doi.org/10.1016/0092-8674(85)90170-9
2. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 1987; 51:887-98; PMID:3319189; http://dx.doi.org/10.1016/0092-8674(87)90576-9
3. Podlevsky JD, Bley CJ, Omana RV, Qi X, Chen JJ-L. The telomerase database. Nucleic Acids Res 2008; 36:D339-43; PMID:18073191; http://dx.doi.org/10.1093/nar/gkm700
4. de Lange T. A loopy view of telomere evolution. Front Genet 2015; 6:321; PMID:26539211; http://dx.doi.org/10.3389/fgene.2015.00321
5. Watson JD. Origin of concatemeric T7 DNA. Nat New Biol 1972; 239:197-201; PMID:4507727; http://dx.doi.org/10.1038/239197a0
6. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J Theor Biol 1973; 41:181-90; PMID:4754905; http://dx.doi.org/10.1016/0022-5193(73)90198-7
7. Lingner J, Cooper JP, Cech TR. Telomerase and DNA end replication: no longer a lagging strand problem? Science 1995; 269:1533-4; PMID:7545310; http://dx.doi.org/10.1126/science.7545310
8. Makarov VL, Hirose Y, Langmore JP. Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for

telomere shortening. Cell 1997; 88:657-66; PMID:9054505; http://dx.doi.org/10.1016/S0092-8674(00)81908-X
9. Sfeir AJ, Chai W, Shay JW, Wright WE. Telomere-end processing the terminal nucleotides of human chromosomes. Mol Cell 2005; 18:131-8; PMID:15808515; http://dx.doi.org/10.1016/j.molcel.2005.02.035
10. Huffman KE, Levene SD, Tesmer VM, Shay JW, Wright WE. Telomere shortening is proportional to the size of the G-rich telomeric 3′-overhang. J Biol Chem 2000; 275:19719-22; PMID:10787419; http://dx.doi.org/10.1074/jbc.M002843200
11. Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. Exp Cell Res 1965; 37:614-36; PMID:14315085; http://dx.doi.org/10.1016/0014-4827(65)90211-9
12. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. Nature 2003; 426:194-8; PMID:14608368; http://dx.doi.org/10.1038/nature02118
13. Shippen-Lentz D, Blackburn EH. Functional evidence for an RNA template in telomerase. Science 1990; 247:546-52; PMID:1689074; http://dx.doi.org/10.1126/science.1689074
14. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70; PMID:10647931; http://dx.doi.org/10.1016/S0092-8674(00)81683-9
15. Newbold RF. The significance of telomerase activation and cellular immortalization in human cancer. Mutagenesis 2002; 17:539-50; PMID:12435851; http://dx.doi.org/10.1093/mutage/17.6.539
16. Biessmann H, Mason JM. Telomere maintenance without telomerase. Chromosoma 1997; 106:63-9; PMID:9215555; http://dx.doi.org/10.1007/s004120050225
17. Pardue M-LL, Danilevskaya ON, Traverse KL, Lowenhaupt K. Evolutionary links between telomeres and transposable elements. Genetica 1997; 100:73-84; PMID:9440260; http://dx.doi.org/10.1023/A:1018352706024
18. Mason JM, Randall TA, Capkova Frydrychova R. Telomerase lost? Chromosoma 2016; 125:65-73; PMID:26162505; http://dx.doi.org/10.1007/s00412-015-0528-7
19. Biessmann H, Carter SB, Mason JM. Chromosome ends in Drosophila without telomeric DNA sequences. Proc Natl Acad Sci USA 1990; 87:1758-61; PMID:2308935; http://dx.doi.org/10.1073/pnas.87.5.1758
20. Nielsen L, Edström JE. Complex telomere-associated repeat units in members of the genus Chironomus evolve from sequences similar to simple telomeric repeats. Mol Cell Biol 1993; 13:1583-9; PMID:8441399; http://dx.doi.org/10.1128/MCB.13.3.1583
21. Roth CW, Kobeski F, Walter MF, Biessmann H. Chromosome end elongation by recombination in the mosquito Anopheles gambiae. Mol Cell Biol 1997; 17:5176-83; PMID:9271395; http://dx.doi.org/10.1128/MCB.17.9.5176
22. Biessmann H, Zurovcova M, Yao JG, Lozovskaya E, Walter MF. A telomeric satellite in Drosophila virilis and its sibling species. Chromosoma 2000; 109:372-80; PMID:11072792; http://dx.doi.org/10.1007/s004120000094
23. de Lange T. How telomeres solve the end-protection problem. Science 2009; 326:948-52; PMID:19965504; http://dx.doi.org/10.1126/science.1170633
24. Dilley RL, Greenberg RA. ALTernative Telomere Maintenance and Cancer. Trends Cancer 2015; 1:145-56; PMID:26645051; http://dx.doi.org/10.1016/j.trecan.2015.07.007
25. Kirby R. Chromosome diversity and similarity within the Actinomycetales. FEMS Microbiol Lett 2011; 319:1-10; PMID:21320158; http://dx.doi.org/10.1111/j.1574-6968.2011.02242.x
26. Huang CH, Tsai HH, Tsay YG, Chien YN, Wang SL, Cheng MY, Ke CH, Chen CW. The telomere system of the Streptomyces linear plasmid SCP1 represents a novel class. Mol Microbiol 2007; 63:1710-8; PMID:17367390; http://dx.doi.org/10.1111/j.1365-2958.2007.05616.x
27. Suzuki H, Marushima K, Ohnishi Y, Horinouchi S. A novel pair of terminal protein and telomere-associated protein for replication of the linear chromosome of Streptomyces griseus IFO13350. Biosci Biotechnol Biochem 2008; 72:2973-80; PMID:18997415; http://dx.doi.org/10.1271/bbb.80454
28. Huang CH, Lin YS, Yang YL, Huang SW, Chen CW. The telomeres of Streptomyces chromosomes contain conserved palindromic sequences with potential to form complex secondary structures. Mol Microbiol 1998; 28:905-16; PMID:9663678; http://dx.doi.org/10.1046/j.1365-2958.1998.00856.x
29. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science 1997; 276:561-7; PMID:9110970; http://dx.doi.org/10.1126/science.276.5312.561
30. Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR. Telomerase catalytic subunit homologs from fission yeast and human. Science 1997; 277:955-9; PMID:9252327; http://dx.doi.org/10.1126/science.277.5328.955
31. Boeke JD. The unusual phylogenetic distribution of retrotransposons: a hypothesis. Genome Res 2003; 13:1975-83; PMID:12952870; http://dx.doi.org/10.1101/gr.1392003
32. Dreesen O, Li B, Cross GA. Telomere structure and shortening in telomerase-deficient Trypanosoma brucei. Nucleic Acids Res 2005; 33:4536-43; PMID:16091631; http://dx.doi.org/10.1093/nar/gki769
33. Giardini MA, Lira CB, Conte FF, Camillo LR, de Siqueira Neto JL, Ramos CH, Cano MI. The putative telomerase reverse transcriptase component of Leishmania amazonensis: gene cloning and characterization. Parasitol Res 2006; 98:447-54; PMID:16416120; http://dx.doi.org/10.1007/s00436-005-0036-4
34. Eickbush TH. Telomerase and retrotransposons: which came first? Science 1997; 277:911-2; PMID:9281073; http://dx.doi.org/10.1126/science.277.5328.911
35. Gladyshev EA, Arkhipova IR. Telomere-associated endonuclease-deficient Penelope-like retroelements in diverse eukaryotes. Proc Natl Acad Sci USA 2007; 104:9352-7; PMID:17483479; http://dx.doi.org/10.1073/pnas.0702741104
36. Podlevsky JD, Chen JJ-L. It all comes together at the ends: telomerase structure, function, and biogenesis. Mutat Res 2012; 730:3-11; PMID:22093366; http://dx.doi.org/10.1016/j.mrfmmm.2011.11.002
37. Hayashi Y, Kajikawa M, Matsumoto T, Okada N. Mechanism by which a LINE protein recognizes its 3′ tail RNA. Nucleic Acids Res 2014; 42:10605-17; PMID:25143533; http://dx.doi.org/10.1093/nar/gku753
38. Jamburuthugoda VK, Eickbush TH. Identification of RNA binding motifs in the R2 retrotransposon-encoded reverse transcriptase. Nucleic Acids Res 2014; 42:8405-15; PMID:24957604; http://dx.doi.org/10.1093/nar/gku514
39. Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 1989; 337:331-7; PMID:2463488; http://dx.doi.org/10.1038/337331a0
40. Chen J-L, Blasco MA, Greider CW. Secondary structure of vertebrate telomerase RNA. Cell 2000; 100:503-14; PMID:10721988; http://dx.doi.org/10.1016/S0092-8674(00)80687-X
41. Qi X, Li Y, Honda S, Hoffmann S, Marz M, Mosig A, Podlevsky JD, Stadler PF, Selker EU, Chen JJ-L. The common ancestral core of vertebrate and fungal telomerase RNAs. Nucleic Acids Res 2013; 41:450-62; PMID:23093598; http://dx.doi.org/10.1093/nar/gks980
42. Bryan TM, Sperger JM, Chapman KB, Cech TR. Telomerase reverse transcriptase genes identified in Tetrahymena thermophila and Oxytricha trifallax. Proc Natl Acad Sci USA 1998; 95:8479-84; PMID:9671703; http://dx.doi.org/10.1073/pnas.95.15.8479
43. Erwin JA, Marchetto MC, Gage FH. Mobile DNA elements in the generation of diversity and complexity in the brain. Nat Rev Neurosci 2014; 15:497-506; PMID:25005482; http://dx.doi.org/10.1038/nrn3730
44. 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; PMID:642006; http://dx.doi.org/10.1016/0022-2836(78)90294-2
45. Richards EJ, Ausubel FM. Isolation of a higher eukaryotic telomere from Arabidopsis thaliana. Cell 1988; 53:127-36; PMID:3349525; http://dx.doi.org/10.1016/0092-8674(88)90494-1
46. McEachern MJ, Blackburn EH. A conserved sequence motif within the exceptionally diverse telomeric sequences of budding yeasts. Proc Natl Acad Sci USA 1994; 91:3453-7; PMID:8159768; http://dx.doi.org/10.1073/pnas.91.8.3453
47. Podlevsky JD, Li Y, Chen JJ-L. Structure and function of echinoderm telomerase RNA. RNA 2016; 22:204-15; PMID:26598712; http://dx.doi.org/10.1261/rna.053280.115

48. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J, et al. The RNA component of human telomerase. Science 1995; 269:1236-41; PMID:7544491; http://dx.doi.org/10.1126/science.7544491
49. Greider CW. Telomerase is processive. Mol Cell Biol 1991; 11:4572-80; PMID:1875940; http://dx.doi.org/10.1128/MCB.11.9.4572
50. Chen J-L, Greider CW. Determinants in mammalian telomerase RNA that mediate enzyme processivity and cross-species incompatibility. EMBO J 2003; 22:304-14; PMID:12514136; http://dx.doi.org/10.1093/emboj/cdg024
51. Chen J-L, Greider CW. Template boundary definition in mammalian telomerase. Genes Dev 2003; 17:2747-52; PMID:14630939; http://dx.doi.org/10.1101/gad.1140303
52. Dandjinou AT, Lévesque N, Larose S, Lucier J-F, Abou Elela S, Wellinger RJ. A phylogenetically based secondary structure for the yeast telomerase RNA. Curr Biol 2004; 14:1148-58; PMID:15242611; http://dx.doi.org/10.1016/j.cub.2004.05.054
53. Cohn M, Blackburn EH. Telomerase in yeast. Science 1995; 269:396-400; PMID:7618104; http://dx.doi.org/10.1126/science.7618104
54. Drosopoulos WC, Direnzo R, Prasad VR. Human telomerase RNA template sequence is a determinant of telomere repeat extension rate. J Biol Chem 2005; 280:32801-10; PMID:16061476; http://dx.doi.org/10.1074/jbc.M506319200
55. Brown AF, Podlevsky JD, Qi X, Chen Y, Xie M, Chen JJ-L. A self-regulating template in human telomerase. Proc Natl Acad Sci USA 2014; 111:11311-6; PMID:24982163; http://dx.doi.org/10.1073/pnas.1402531111
56. Stohr BA, Xu L, Blackburn EH. The terminal telomeric DNA sequence determines the mechanism of dysfunctional telomere fusion. Mol Cell 2010; 39:307-14; PMID:20670897; http://dx.doi.org/10.1016/j.molcel.2010.06.020
57. Autexier C, Greider CW. Boundary elements of the Tetrahymena telomerase RNA template and alignment domains. Genes Dev 1995; 9:2227-39; PMID:7557377; http://dx.doi.org/10.1101/gad.9.18.2227
58. Lai CK, Miller MC, Collins K. Template boundary definition in Tetrahymena telomerase. Genes Dev 2002; 16:415-20; PMID:11850404; http://dx.doi.org/10.1101/gad.962602
59. 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; 288:22141-9; PMID:23760279; http://dx.doi.org/10.1074/jbc.M113.452425
60. Jansson LI, Akiyama BM, Ooms A, Lu C, Rubin SM, Stone MD. Structural basis of template-boundary definition in Tetrahymena telomerase. Nature Struct Mol Biol 2015; 22:883-8; http://dx.doi.org/10.1038/nsmb.3101
61. Podlevsky JD, Li Y, Chen JJ-L. The functional requirement of two structural domains within telomerase RNA emerged early in eukaryotes. Nucleic Acids Res 2016; http://dx.doi.org/10.1093/nar/gkw605
62. Tzfati Y, Fulton TB, Roy J, Blackburn EH. Template boundary in a yeast telomerase specified by RNA structure. Science 2000; 288:863-7; PMID:10797010; http://dx.doi.org/10.1126/science.288.5467.863
63. Seto AG, Umansky K, Tzfati Y, Zaug AJ, Blackburn EH, Cech TR. A template-proximal RNA paired element contributes to Saccharomyces cerevisiae telomerase activity. RNA 2003; 9:1323-32; PMID:14561882; http://dx.doi.org/10.1261/rna.5570803
64. Box JA, Bunch JT, Zappulla DC, Glynn EF, Baumann P. A flexible template boundary element in the RNA subunit of fission yeast telomerase. J Biol Chem 2008; 283:24224-33; PMID:18574244; http://dx.doi.org/10.1074/jbc.M802043200
65. 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; 21:1870-8; PMID:24055314; http://dx.doi.org/10.1016/j.str.2013.08.013
66. Hinkley CS, Blasco MA, Funk WD, Feng J, Villeponteau B, Greider CW, Herr W. The mouse telomerase RNA 5′-end lies just upstream of the telomerase template sequence. Nucleic Acids Res 1998; 26:532-6; PMID:9421511; http://dx.doi.org/10.1093/nar/26.2.532
67. Moriarty TJ, Marie-Egyptienne DT, Autexier C. Functional organization of repeat addition processivity and DNA synthesis determinants in the human telomerase multimer. Mol Cell Biol 2004; 24:3720-33; PMID:15082768; http://dx.doi.org/10.1128/MCB.24.9.3720-3733.2004
68. Mitchell JR, Collins K. Human telomerase activation requires two independent interactions between telomerase RNA and telomerase reverse transcriptase. Mol Cell 2000; 6:361-71; PMID:10983983; http://dx.doi.org/10.1016/S1097-2765(00)00036-8
69. 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; 18:1371-5; http://dx.doi.org/10.1038/nsmb.2174
70. Qi X, Xie M, Brown AF, Bley CJ, Podlevsky JD, Chen JJ-L. RNA/DNA hybrid binding affinity determines telomerase template-translocation efficiency. EMBO J 2012; 31:150-61; PMID:21989387; http://dx.doi.org/10.1038/emboj.2011.363
71. Blackburn EH, Collins K. Telomerase: an RNP enzyme synthesizes DNA. Cold Spring Harb Perspect Biol 2011; 3:1-9; http://dx.doi.org/10.1101/cshperspect.a003558
72. Egan ED, Collins K. Biogenesis of telomerase ribonucleoproteins. RNA 2012; 18:1747-59; PMID:22875809; http://dx.doi.org/10.1261/rna.034629.112
73. Tzfati Y, Knight Z, Roy J, Blackburn EH. A novel pseudoknot element is essential for the action of a yeast telomerase. Genes Dev 2003; 17:1779-88; PMID:12832393; http://dx.doi.org/10.1101/gad.1099403
74. Cash DD, Cohen-Zontag O, Kim N-K, Shefer K, Brown Y, Ulyanov NB, Tzfati Y, Feigon J. Pyrimidine motif triple helix in the Kluyveromyces lactis telomerase RNA pseudoknot is essential for function *in vivo*. Proc Natl Acad Sci USA 2013; 110:10970-5; PMID:23776224; http://dx.doi.org/10.1073/pnas.1309590110
75. Ly H, Blackburn EH, Parslow TG. Comprehensive structure-function analysis of the core domain of human telomerase RNA. Mol Cell Biol 2003; 23:6849-56; PMID:12972604; http://dx.doi.org/10.1128/MCB.23.19.6849-6856.2003
76. Autexier C, Greider CW. Mutational analysis of the Tetrahymena telomerase RNA: identification of residues affecting telomerase activity *in vitro*. Nucleic Acids Res 1998; 26:787-95; PMID:9443971; http://dx.doi.org/10.1093/nar/26.3.787
77. Gilley D, Blackburn EH. The telomerase RNA pseudoknot is critical for the stable assembly of a catalytically active ribonucleoprotein. Proc Natl Acad Sci USA 1999; 96:6621-5; PMID:10359761; http://dx.doi.org/10.1073/pnas.96.12.6621
78. Lai CK, Mitchell JR, Collins K. RNA binding domain of telomerase reverse transcriptase. Mol Cell Biol 2001; 21:990-1000; PMID:11158287; http://dx.doi.org/10.1128/MCB.21.4.990-1000.2001
79. Chen J-L, Greider CW. Functional analysis of the pseudoknot structure in human telomerase RNA. Proc Natl Acad Sci USA 2005; 102:8080-5; PMID:15849264; http://dx.doi.org/10.1073/pnas.0502259102
80. Theimer CA, Blois CA, Feigon J. Structure of the human telomerase RNA pseudoknot reveals conserved tertiary interactions essential for function. Mol Cell 2005; 17:671-82; PMID:15749017; http://dx.doi.org/10.1016/j.molcel.2005.01.017
81. Qiao F, Cech TR. Triple-helix structure in telomerase RNA contributes to catalysis. Nat Struct Mol Biol 2008; 15:634-40; http://dx.doi.org/10.1038/nsmb.1420
82. Shefer K, Brown Y, Gorkovoy V, Nussbaum T, Ulyanov NB, Tzfati Y. A triple helix within a pseudoknot is a conserved and essential element of telomerase RNA. Mol Cell Biol 2007; 27:2130-43; PMID:17210648; http://dx.doi.org/10.1128/MCB.01826-06
83. Zhang Q, Kim N-K, 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 USA 2010; 107:18761-8; PMID:20966348; http://dx.doi.org/10.1073/pnas.1013269107
84. Zhang Q, Kim N-K, Feigon J. Architecture of human telomerase RNA. Proc Natl Acad Sci USA 2011; 108:20325-32; PMID:21844345; http://dx.doi.org/10.1073/pnas.1100279108
85. Chen J-L, Opperman KK, Greider CW. A critical stem-loop structure in the CR4-CR5 domain of mammalian telomerase RNA. Nucleic Acids Res 2002; 30:592-7; PMID:11788723; http://dx.doi.org/10.1093/nar/30.2.592
86. Brown Y, Abraham M, Pearl S, Kabaha MM, Elboher E, Tzfati Y. A critical three-way junction is conserved in budding yeast and

vertebrate telomerase RNAs. Nucleic Acids Res 2007; 35:6280-9; PMID:17855392; http://dx.doi.org/10.1093/nar/gkm713
87. Mason DX, Goneska E, Greider CW. Stem-loop IV of tetrahymena telomerase RNA stimulates processivity in trans. Mol Cell Biol 2003; 23:5606-13; PMID:12897134; http://dx.doi.org/10.1128/MCB.23.16.5606-5613.2003
88. McCormick-Graham M, Romero DP. Ciliate telomerase RNA structural features. Nucleic Acids Res 1995; 23:1091-7; PMID:7739888; http://dx.doi.org/10.1093/nar/23.7.1091
89. Kuprys PV, Davis SM, Hauer TM, Meltser M, Tzfati Y, Kirk KE. Identification of telomerase RNAs from filamentous fungi reveals conservation with vertebrates and yeasts. PloS One 2013; 8:e58661; PMID:23555591; http://dx.doi.org/10.1371/journal.pone.0058661
90. Huang J, Brown AF, Wu J, Xue J, Bley CJ, Rand DP, Wu L, Zhang R, Chen JJ-L, Lei M. Structural basis for protein-RNA recognition in telomerase. Nature Struct Mol Biol 2014; 21:507-12; http://dx.doi.org/10.1038/nsmb.2819
91. Zappulla DC, Goodrich K, Cech TR. A miniature yeast telomerase RNA functions *in vivo* and reconstitutes activity *in vitro*. Nat Struct Mol Biol 2005; 12:1072-7; http://dx.doi.org/10.1038/nsmb1019
92. Li Y, Podlevsky JD, Marz M, Qi X, Hoffmann S, Stadler PF, Chen JJ-L. Identification of purple sea urchin telomerase RNA using a next-generation sequencing based approach. RNA 2013; 19:852-60; PMID:23584428; http://dx.doi.org/10.1261/rna.039131.113
93. 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; 446:458-61; PMID:17322903; http://dx.doi.org/10.1038/nature05600
94. Bley CJ, Qi X, Rand DP, Borges CR, Nelson RW, Chen JJ-L. RNA-protein binding interface in the telomerase ribonucleoprotein. Proc Natl Acad Sci USA 2011; 108:20333-8; PMID:22123986; http://dx.doi.org/10.1073/pnas.1100270108
95. 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; 42:3395-408; PMID:24335084; http://dx.doi.org/10.1093/nar/gkt1276
96. Xie M, Mosig A, Qi X, Li Y, Stadler PF, Chen JJ-L. Structure and function of the smallest vertebrate telomerase RNA from teleost fish. J Biol Chem 2008; 283:2049-59; PMID:18039659; http://dx.doi.org/10.1074/jbc.M708032200
97. Sandhu R, Sanford S, Basu S, Park M, Pandya UM, Li B, Chakrabarti K. A trans-spliced telomerase RNA dictates telomere synthesis in Trypanosoma brucei. Cell Res 2013; 23:537-51; PMID:23478302; http://dx.doi.org/10.1038/cr.2013.35
98. Vasconcelos EJR, Nunes VS, da Silva MS, Segatto M, Myler PJ, Cano MIN. The putative Leishmania telomerase RNA (LeishTER) undergoes trans-splicing and contains a conserved template sequence. PloS One 2014; 9:e112061; PMID:25391020; http://dx.doi.org/10.1371/journal.pone.0112061
99. Gupta SK, Kolet L, Doniger T, Biswas VK, Unger R, Tzfati Y, Michaeli S. The Trypanosoma brucei telomerase RNA (TER) homologue binds core proteins of the C/D snoRNA family. FEBS Lett 2013; 587:1399-404; PMID:23523918; http://dx.doi.org/10.1016/j.febslet.2013.03.017
100. Leonardi J, Box JA, Bunch JT, Baumann P. TER1, the RNA subunit of fission yeast telomerase. Nat Struct Mol Biol 2008; 15:26-33; http://dx.doi.org/10.1038/nsmb1343
101. Mitchell JR, Cheng J, Collins K. A box H/ACA small nucleolar RNA-like domain at the human telomerase RNA 3′ end. Mol Cell Biol 1999; 19:567-76; PMID:9858580; http://dx.doi.org/10.1128/MCB.19.1.567
102. Jády BE, Bertrand E, Kiss T. Human telomerase RNA and box H/ACA scaRNAs share a common Cajal body-specific localization signal. J Cell Biol 2004; 164:647-52; http://dx.doi.org/10.1083/jcb.200310138
103. Egan ED, Collins K. Specificity and stoichiometry of subunit interactions in the human telomerase holoenzyme assembled *in vivo*. Mol Cell Biol 2010; 30:2775-86; PMID:20351177; http://dx.doi.org/10.1128/MCB.00151-10
104. Girard JP, Caizergues-Ferrer M, Lapeyre B. The SpGAR1 gene of Schizosaccharomyces pombe encodes the functional homologue of the snoRNP protein GAR1 of Saccharomyces cerevisiae. Nucleic Acids Res 1993; 21:2149-55; PMID:8502556; http://dx.doi.org/10.1093/nar/21.9.2149
105. Maiorano D, Brimage LJ, Leroy D, Kearsey SE. Functional conservation and cell cycle localization of the Nhp2 core component of H/ACA snoRNPs in fission and budding yeasts. Exp Cell Res 1999; 252:165-74; PMID:10502409; http://dx.doi.org/10.1006/excr.1999.4607
106. Pogacić V, Dragon F, Filipowicz W. Human H/ACA small nucleolar RNPs and telomerase share evolutionarily conserved proteins NHP2 and NOP10. Mol Cell Biol 2000; 20:9028-40; PMID:11074001; http://dx.doi.org/10.1128/MCB.20.23.9028-9040.2000
107. Cheng X, Roberts RJ. AdoMet-dependent methylation, DNA methyltransferases and base flipping. Nucleic Acids Res 2001; 29:3784-95; PMID:11557810; http://dx.doi.org/10.1093/nar/29.18.3784
108. Hamma T, Reichow SL, Varani G, Ferré-D'Amaré AR. The Cbf5-Nop10 complex is a molecular bracket that organizes box H/ACA RNPs. Nat Struct Mol Biol 2005; 12:1101-7; http://dx.doi.org/10.1038/nsmb1036
109. Venteicher AS, Abreu EB, Meng Z, McCann KE, Terns RM, Veenstra TD, Terns MP, Artandi SE. A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. Science 2009; 323:644-8; PMID:19179534; http://dx.doi.org/10.1126/science.1165357
110. Reichow SL, Hamma T, Ferré-D'Amaré AR, Varani G. The structure and function of small nucleolar ribonucleoproteins. Nucleic Acids Res 2007; 35:1452-64; PMID:17284456; http://dx.doi.org/10.1093/nar/gkl1172
111. Theimer CA, Jády BE, Chim N, Richard P, Breece KE, Kiss T, Feigon J. Structural and functional characterization of human telomerase RNA processing and cajal body localization signals. Mol Cell 2007; 27:869-81; PMID:17889661; http://dx.doi.org/10.1016/j.molcel.2007.07.017
112. Lattmann S, Stadler MB, Vaughn JP, Akman SA, Nagamine Y. The DEAH-box RNA helicase RHAU binds an intramolecular RNA G-quadruplex in TERC and associates with telomerase holoenzyme. Nucleic Acids Res 2011; 39:9390-404; PMID:21846770; http://dx.doi.org/10.1093/nar/gkr630
113. Nguyen D, Grenier St-Sauveur V, Bergeron D, Dupuis-Sandoval F, Scott MS, Bachand F. A Polyadenylation-Dependent 3′ End Maturation Pathway Is Required for the Synthesis of the Human Telomerase RNA. Cell Rep 2015; 13:2244-57; PMID:26628368; http://dx.doi.org/10.1016/j.celrep.2015.11.003
114. Tseng C-K, Wang H-F, Burns AM, Schroeder MR, Gaspari M, Baumann P. Human Telomerase RNA Processing and Quality Control. Cell Rep 2015; 13:2232-43; PMID:26628367; http://dx.doi.org/10.1016/j.celrep.2015.10.075
115. Tang W, Kannan R, Blanchette M, Baumann P. Telomerase RNA biogenesis involves sequential binding by Sm and Lsm complexes. Nature 2012; 484:260-4; PMID:22446625; http://dx.doi.org/10.1038/nature10924
116. Seto AG, Zaug AJ, Sobel SG, Wolin SL, Cech TR. Saccharomyces cerevisiae telomerase is an Sm small nuclear ribonucleoprotein particle. Nature 1999; 401:177-80; PMID:10490028; http://dx.doi.org/10.1038/43694
117. Noël J-F, Larose S, Abou Elela S, Wellinger RJ. Budding yeast telomerase RNA transcription termination is dictated by the Nrd1/Nab3 non-coding RNA termination pathway. Nucleic Acids Res 2012; 40:5625-36; http://dx.doi.org/10.1093/nar/gks200
118. Qi X, Rand DP, Podlevsky JD, Li Y, Mosig A, Stadler PF, Chen JJ-L. Prevalent and distinct spliceosomal 3′-end processing mechanisms for fungal telomerase RNA. Nat Commun 2015; 6:6105; PMID:25598218; http://dx.doi.org/10.1038/ncomms7105
119. Kannan R, Helston RM, Dannebaum RO, Baumann P. Diverse mechanisms for spliceosome-mediated 3′ end processing of telomerase RNA. Nat Commun 2015; 6:6104; PMID:25598145; http://dx.doi.org/10.1038/ncomms7104
120. Box JA, Bunch JT, Tang W, Baumann P. Spliceosomal cleavage generates the 3′ end of telomerase RNA. Nature 2008; 456:910-4; PMID:19052544; http://dx.doi.org/10.1038/nature07584
121. Gunisova S, Elboher E, Nosek J, Gorkovoy V, Brown Y, Lucier JF, Laterreur N, Wellinger RJ, Tzfati Y, Tomaska L. Identification and comparative analysis of telomerase RNAs from Candida species

reveal conservation of functional elements. RNA 2009; 15:546-59; PMID:19223441; http://dx.doi.org/10.1261/rna.1194009
122. Seto AG, Livengood AJ, Tzfati Y, Blackburn EH, Cech TR. A bulged stem tethers Est1p to telomerase RNA in budding yeast. Genes Dev 2002; 16:2800-12; PMID:12414733; http://dx.doi.org/10.1101/gad.1029302
123. Evans SK, Lundblad V. The Est1 subunit of Saccharomyces cerevisiae telomerase makes multiple contributions to telomere length maintenance. Genetics 2002; 162:1101-15; PMID:12454059
124. Beernink HTH, Miller K, Deshpande A, Bucher P, Cooper JP. Telomere maintenance in fission yeast requires an Est1 ortholog. Curr Biol 2003; 13:575-80; PMID:12676088; http://dx.doi.org/10.1016/S0960-9822(03)00169-6
125. Hughes TR, Evans SK, Weilbaecher RG, Lundblad V. The Est3 protein is a subunit of yeast telomerase. Curr Biol 2000; 10:809-12; PMID:10898986; http://dx.doi.org/10.1016/S0960-9822(00)00562-5
126. Stellwagen AE, Haimberger ZW, Veatch JR, Gottschling DE. Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends. Genes Dev 2003; 17:2384-95; PMID:12975323; http://dx.doi.org/10.1101/gad.1125903
127. Fisher TS, Zakian VA. Ku: a multifunctional protein involved in telomere maintenance. DNA Repair 2005; 4:1215-26; PMID:15979949; http://dx.doi.org/10.1016/j.dnarep.2005.04.021
128. Kabaha MM, Zhitomirsky B, Schwartz I, Tzfati Y. The 5′ arm of Kluyveromyces lactis telomerase RNA is critical for telomerase function. Mol Cell Biol 2008; 28:1875-82; PMID:18195041; http://dx.doi.org/10.1128/MCB.01683-07
129. Lemieux B, Laterreur N, Perederina A, Noël J-F, Dubois M-L, Krasilnikov AS, Wellinger RJ. Active Yeast Telomerase Shares Subunits with Ribonucleoproteins RNase P and RNase MRP. Cell 2016; 165:1171-81; PMID:27156450; http://dx.doi.org/10.1016/j.cell.2016.04.018
130. Witkin KL, Collins K. Holoenzyme proteins required for the physiological assembly and activity of telomerase. Genes Dev 2004; 18:1107-18; PMID:15131081; http://dx.doi.org/10.1101/gad.1201704
131. Min B, Collins K. An RPA-related sequence-specific DNA-binding subunit of telomerase holoenzyme is required for elongation processivity and telomere maintenance. Mol Cell 2009; 36:609-19; PMID:19941821; http://dx.doi.org/10.1016/j.molcel.2009.09.041
132. Aigner S, Lingner J, Goodrich KJ, Grosshans CA, Shevchenko A, Mann M, Cech TR. Euplotes telomerase contains an La motif protein produced by apparent translational frameshifting. EMBO J 2000; 19:6230-9; PMID:11080168; http://dx.doi.org/10.1093/emboj/19.22.6230
133. Aigner S, Postberg J, Lipps HJ, Cech TR. The Euplotes La motif protein p43 has properties of a telomerase-specific subunit. Biochemistry 2003; 42:5736-47; PMID:12741831; http://dx.doi.org/10.1021/bi034121y
134. Richards RJ, Wu H, Trantirek L, O'Connor CM, Collins K, Feigon J. Structural study of elements of Tetrahymena telomerase RNA stem-loop IV domain important for function. RNA 2006; 12:1475-85; PMID:16809815; http://dx.doi.org/10.1261/rna.112306
135. Witkin KL, Prathapam R, Collins K. Positive and negative regulation of Tetrahymena telomerase holoenzyme. Mol Cell Biol 2007; 27:2074-83; PMID:17220281; http://dx.doi.org/10.1128/MCB.02105-06
136. 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; PMID:26472759; http://dx.doi.org/10.1126/science.aab4070
137. Günzl A. The pre-mRNA splicing machinery of trypanosomes: complex or simplified? Eukaryot Cell 2010; 9:1159-70; PMID:20581293; http://dx.doi.org/10.1128/EC.00113-10
138. Aversano R, Contaldi F, Ercolano MR, Grosso V, Iorizzo M, Tatino F, Xumerle L, Dal Molin A, Avanzato C, Ferrarini A, et al. The Solanum commersonii Genome Sequence Provides Insights into Adaptation to Stress Conditions and Genome Evolution of Wild Potato Relatives. Plant Cell 2015; 27:954-68; PMID:25873387; http://dx.doi.org/10.1105/tpc.114.135954
139. Glasauer SMK, Neuhauss SCF. Whole-genome duplication in teleost fishes and its evolutionary consequences. Mol Genet Genomics 2014; 289:1045-60; PMID:25092473; http://dx.doi.org/10.1007/s00438-014-0889-2
140. Kapusta A, Feschotte C. Volatile evolution of long noncoding RNA repertoires: mechanisms and biological implications. Trends Genet 2014; 30:439-52; PMID:25218058; http://dx.doi.org/10.1016/j.tig.2014.08.004
141. Freeling M, Woodhouse MR, Subramaniam S, Turco G, Lisch D, Schnable JC. Fractionation mutagenesis and similar consequences of mechanisms removing dispensable or less-expressed DNA in plants. Curr Opin Plant Biol 2012; 15:131-9; PMID:22341793; http://dx.doi.org/10.1016/j.pbi.2012.01.015
142. Brunet FG, Roest Crollius H, Paris M, Aury J-M, Gibert P, Jaillon O, Laudet V, Robinson-Rechavi M. Gene loss and evolutionary rates following whole-genome duplication in teleost fishes. Mol Biol Evol 2006; 23:1808-16; PMID:16809621; http://dx.doi.org/10.1093/molbev/msl049
143. Cifuentes-Rojas C, Kannan K, Tseng L, Shippen DE. Two RNA subunits and POT1a are components of Arabidopsis telomerase. Proc Natl Acad Sci USA 2011; 108:73-8; PMID:21164032; http://dx.doi.org/10.1073/pnas.1013021107
144. Beilstein MA, Brinegar AE, Shippen DE. Evolution of the Arabidopsis telomerase RNA. Front Genet 2012; 3:188; PMID:23015808; http://dx.doi.org/10.3389/fgene.2012.00188
145. Cifuentes-Rojas C, Nelson ADL, Boltz KA, Kannan K, She X, Shippen DE. An alternative telomerase RNA in Arabidopsis modulates enzyme activity in response to DNA damage. Genes Dev 2012; 26:2512-23; PMID:23109676; http://dx.doi.org/10.1101/gad.202960.112
146. Romero DP, Blackburn EH. A conserved secondary structure for telomerase RNA. Cell 1991; 67:343-53; PMID:1840508; http://dx.doi.org/10.1016/0092-8674(91)90186-3
147. Koonin EV, Dolja VV. Virus world as an evolutionary network of viruses and capsidless selfish elements. Microbiol Mol Biol Rev 2014; 78:278-303; PMID:24847023; http://dx.doi.org/10.1128/MMBR.00049-13
148. Arkhipova IR, Pyatkov KI, Meselson M, Evgen'ev MB. Retroelements containing introns in diverse invertebrate taxa. Nat Genet 2003; 33:123-4; PMID:12524543; http://dx.doi.org/10.1038/ng1074
149. Cavalier-Smith T, Chao EE, Snell EA, Berney C, Fiore-Donno AM, Lewis R. Multigene eukaryote phylogeny reveals the likely protozoan ancestors of opisthokonts (animals, fungi, choanozoans) and Amoebozoa. Mol Phylogenet Evol 2014; 81:71-85; PMID:25152275; http://dx.doi.org/10.1016/j.ympev.2014.08.012