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Review

It all comes together at the ends: Telomerase structure, function, and biogenesis

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ABSTRACT

Telomerase is a reverse transcriptase specialized in the addition of telomeric DNA repeats onto the ends of chromosomes. Telomere extension offsets the loss of telomeric repeats from the failure of DNA polymerases to fully replicate linear chromosome ends. Telomerase functions as a ribonucleoprotein, requiring an integral telomerase RNA (TR) component, in addition to the catalytic telomerase reverse transcriptase (TERT). Extensive studies have identified numerous structural and functional features within the TR and TERT essential for activity. A number of accessory proteins have also been identified with various functions in enzyme biogenesis, localization, and regulation. Understanding the molecular mechanism of telomerase function has significance for the development of therapies for telomere-mediated disorders and cancer. Here we review telomerase structural and functional features, and the techniques for assessing telomerase dysfunction.

Contents

The ends of eukaryotic chromosomes are capped by the protective DNA–protein complex known as the telomere [1,2]. Following genome duplication, telomeres shrink due to the incomplete replication of telomere ends by conventional DNA polymerases [3–5]. The progressive loss of telomeric DNA limits the number of cell divisions and threatens genome stability [reviewed in [6]]. Telomerase is a specialized reverse transcriptase (RT), adding telomeric DNA repeats to chromosome ends to offset this persistent loss [7]. So far, mutations in at least six telomerase components have been linked to human telomere-mediated disorders such as dyskeratosis congenita (DC), aplastic anemia (AA), and idiopathic pulmonary fibrosis (IPF) [8–10]. Additionally, the vast majority of cancer cells have telomerase up-regulated to sustain growth [11]. Thus the study of telomerase is important for understanding the basis of many human diseases and the development of potential therapies.

1. Telomerase enzymatic properties

Telomerase is unique among RTs by functioning as a ribonucleoprotein [12–14]. The catalytic core of telomerase is minimally composed of the telomerase reverse transcriptase (TERT, also known as TRT and Est2) and the integral telomerase RNA (TR, also known as TER, TERC, and TLC1). The TERT protein contains the catalytic site for DNA synthesis, and assembles with the TR which provides the template. While dispensable for telomerase activity, a variety of accessory proteins in the holoenzyme play crucial roles in telomerase biogenesis, localization, and regulation [15–18].

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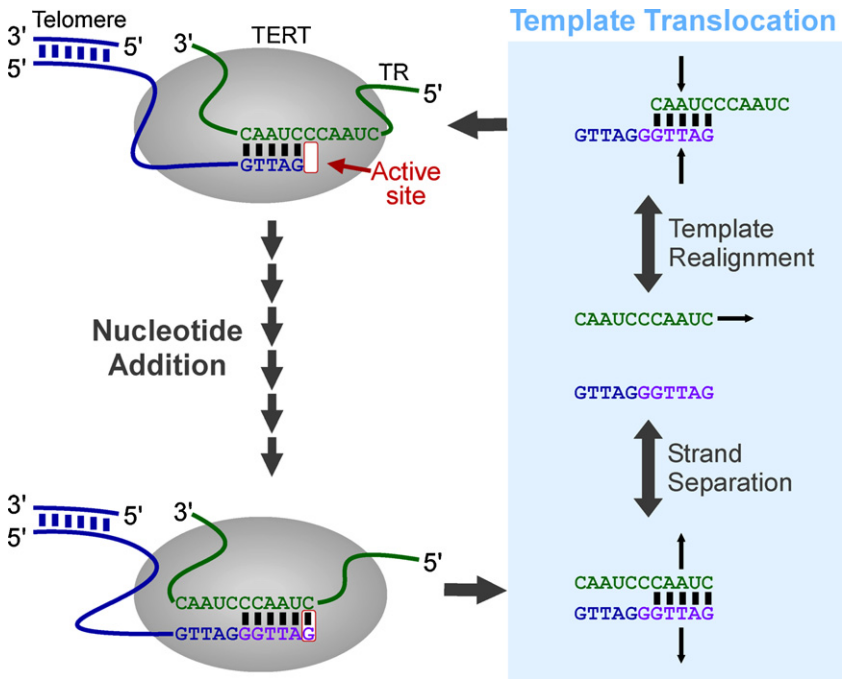

Fig. 1. A model of the human telomerase reaction cycle. The telomerase reaction is divided between nucleotide addition (left) which is common to all polymerases and template translocation (right, pale-blue box), a unique property of telomerase. After assembly of the telomerase catalytic core, composed of TERT (grey) and TR (green), with the DNA primer (blue), six nucleotides (violet) are sequential added in a template-dependent manner (dark-grey arrows). The template is then regenerated though a multi-step process involving separation of the RNA/DNA hybrid, realignment of the RNA template relative to the DNA primer, and the reformation of an RNA/DNA hybrid. Movements of the RNA and DNA strands are denoted by small black arrows.

The telomerase reaction produces a long tract of telomeric DNA repeats from a single short RNA template [7,13]. The telomerase catalytic cycle involves two phases: the synthesis of a single telomere repeat onto the 3′ end of the telomeric DNA primer and the regeneration of the template for the synthesis of additional repeats. The processive synthesis of multiple telomere repeats from the same template to a given primer is unusual for a polymerase and requires a specialized mechanism for template regeneration after each repeat synthesis. The telomerase reaction initiates by base-pairing the 3′ end of the telomeric DNA primer with the 5′ region of the RNA template to form an RNA/DNA hybrid (Fig. 1, top/left). For human telomerase, the active site reverse transcribes six nucleotides 5′-GGTTAG-3′ onto the 3′ end of the DNA primer from the RNA template (Fig. 1, bottom/left). Upon reaching the end of the template, nucleotide addition arrests, awaiting either the regeneration of the template for next repeat synthesis or complete disassociation of the enzyme from the DNA product.

Processive repeat addition requires the translocation of the RNA strand after each repeat synthesis to regenerate the template (Fig. 1). Template translocation is a complex, multi-step process which is currently poorly understood. However, it is known that the RNA template/DNA primer hybrid must first separate, translocate, and re-anneal so that the 5′ portion of the RNA template is no longer base-paired with the DNA primer and available for the next round of nucleotide addition (Fig. 1, top/right). Template translocation has been shown to occur outside the active site [19]. Through this process, a given primer can be extended with numerous telomere repeats before complete disassociation from the telomerase enzyme. Nucleotide addition is believed to proceed quickly within the telomerase reaction, thus template translocation would be the rate-limiting step [20,21].

While nucleotide addition is common to all polymerases, processive repeat addition is unique to telomerase and requires telomerase-specific elements. In the TR, template length affects the template realignment efficiency and thus repeat addition processivity [22]. The TERT protein contains several DNA-binding motifs which enhance primer retention [23–26] and additional motifs for binding the realigned RNA/DNA hybrid during template translocation [19,27–29]. Mutations in these motifs alter repeat addition processivity. Additionally, the complex formed by the telomere DNA-binding protein POT1 (protection of telomeres 1) and TPP1 (TIN2 and POT1-interacting protein 1) has been found to delay primer release [30]. POT1 directly binds to the telomeric DNA primer while TPP1 simultaneously binds to POT1 and TERT. Thus the POT1–TPP1 complex holds the DNA primer in close proximity to telomerase, delaying primer release from the enzyme, and enhancing repeat addition processivity [31]. The structural elements within the TR and TERT which increase processivity are discussed further in the following sections.

2. TR structure and function

The TR is the integral RNA component within the telomerase catalytic core. In addition to providing the template which specifies the telomere repeat sequence, this non-coding RNA contains motifs necessary for the reconstitution of telomerase activity. The TR from all known species contains two conserved, and potentially universal structures: the template/pseudoknot domain [32–34] and the CR4/5domain (also known as the three-way junction or stem-terminus element) [34–37]. These two domains comprise the regions of the TR required for telomerase activity (Fig. 2, top). In fact, these two elements can be excised from the TR and combined in trans with the TERT protein to generate an active telomerase enzyme in vitro [38,39]. The human TR contains a third domain conserved among all known vertebrate TR sequences, the H/ACA domain [32,40,41]. As the name indicates, this domain has homology to small nucleolar (sno) and small Cajal body-specific (sca) RNAs, which contain two stem-loops separated by box H and box

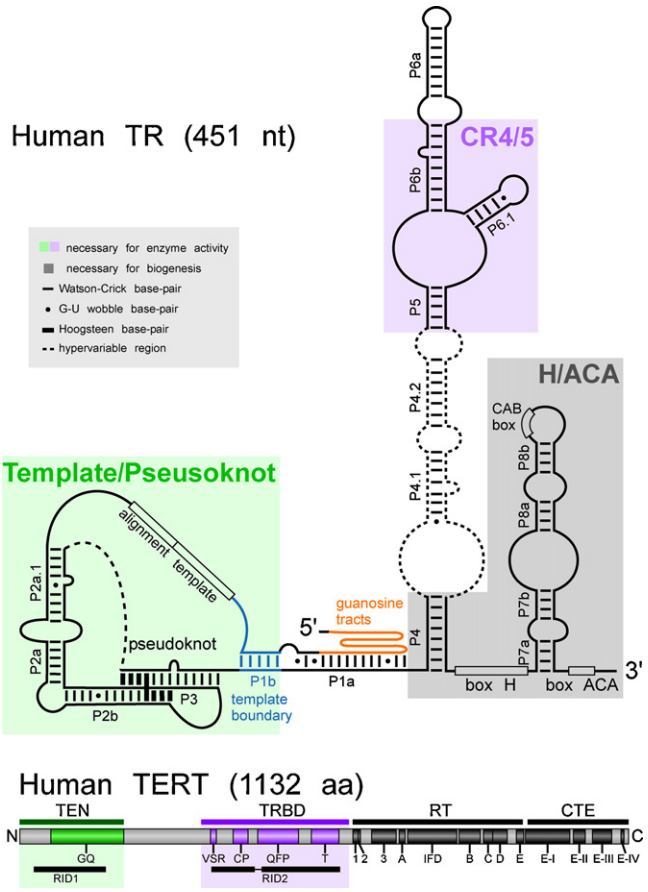

Fig. 2. TR and TERT motifs and domain organization. Top, the TR is composed of three functional domains. The template/pseudoknot (pale-green box) and CR4/5 (pale-violet box) domains bind to TERT and are essential for enzymatic activity. While the H/ACA domain (grey box) is dispensable for activity, it is essential for in vivo biogenesis, accumulation, and RNP assembly. Additionally there is the RHAU RNA helicase binding to the G-quadruplex structure (orange) at the 5′ end and the TBE composed of helix P1b (blue) located upstream of the template. Bottom, the TERT protein is composed of 4 independently folding domains. The TEN domain (green) and TRBD (violet) are shaded in colors corresponds to the template/pseudoknot and CR4/5 domains. Important motifs within each domain are colored similarly to the domain.

ACA moieties [42]. The TR H/ACA domain binds a quartette of proteins, dyskerin, NOP10, NHP2, and GAR1 essential in vivo for telomerase biogenesis and localization [41,43,44].

The secondary structure of the TR has provided insight into telomerase function. The template/pseudoknot domain contains several structural features that position the template in the active site and define the template boundary. The human pseudoknot has been found to contain a triple helix and the pseudoknot from other species is predicted to contain a similar triple helix [45,46]. While the function of the pseudoknot or triple helix is not precisely known, the loss or disruption of this structure drastically reduces telomerase activity [47–49]. Although not located in proximity in the primary sequence, the pseudoknot and template are located adjacent to each other in the secondary structure [32]. A sharp structural kink was found between helices P2a and P2b in the pseudoknot domain, facilitating positioning of the template in the template-pseudoknot core domain [50]. The template sequence can be divided into a 5′ region encoding for telomeric DNA repeats and a 3′ region annealing to the DNA primer after template translocation (Fig. 2, top). The template is flanked on the 5′ end by the template boundary element (TBE) that defines the end of the templating sequence. Within the human TR, helix P1b functions as the template boundary element [51]. Mutations disrupting the P1b helix result in 'read-through', where the non-telomeric sequence immediately upstream from the template is utilized for nucleotide synthesis [51,52]. Recent evidence suggests that the human TR contains a G-quadruplex structure, formed from the guanosine-rich tracts located at the 5′ end (Fig. 2, top). The DEXH box RNA helicase RHAU (also known as DHX36, G4R1) stably associates with the 5′ end of the TR and resolves the G-quadruplex structure, increasing TR accumulation within the cell [53,54]. In summary, the TR template/pseudoknot domain is a remarkably complex structure, containing elements defining the template boundary, binding to the TERT protein, and enhancing enzymatic activity of telomerase.

The other domain required for enzymatic activity is the CR4/5domain, which is distal to the template/pseudoknot domain in both the primary sequence and secondary structure [35,36]. The CR4/5 domain is composed of a three-way junction of helices, known in vertebrates as P5, P6, and P6.1 (Fig. 2, top) [32,37]. Outside of vertebrates, fungi appear to also have a similar structural element [36]. It has been proposed that ciliates have an abridged variant of this structural feature. While not quite a three-way junction, the ciliate TR has important nucleic acid–protein contacts which may be conserved [35]. The detailed molecular basis for how the CR4/5 domain, as well as the pseudoknot, contribute to activity and facilitate catalysis remains poorly understood.

The vertebrate TR has a conserved H/ACA domain located at the 3′ end (Fig. 2, top). The H/ACA domain contains two stem-loops separated by the box H/ACA moieties which function as binding sites for dyskerin, NOP10, NHP2, and GAR1 [40,55,56]. Additionally, in the 3′ stem-loop of the H/ACA domain is the Cajal body localization (CAB box) moiety for binding the telomerase Cajal body protein 1 (TCAB1) [18,40,57]. Mutations in the H/ACA moieties abolishes 3′ end processing and reduces TR accumulation, while mutations in the in the CAB box moiety causes TR accumulation in nucleoli instead of Cajal bodies [18,42,58,59].

Numerous unique mutations within the TR gene have been found to reduce the levels of active telomerase and are linked to a variety of human telomere-mediated disorders [55,60–75]. These mutations frequently exhibit a syndrome complex of AA, IPF, or the full spectrum of DC [reviewed in [76]]. The genetic mechanism for disease within these patients is haloinsufficiency, where a single functional TR allele is insufficient for the accumulation of active telomerase, resulting in telomere shortening [77]. When mapped onto the TR secondary structure (available at http://telomerase.asu.edu), these mutations concentrate within the three functional domains, with the vast majority located in the template/pseudoknot domain [78]. Changes in the TR primary sequence can disrupt RNA base-pairing and local RNA structure, affecting telomerase function in several ways. First, they may affect the assembly of TR and TERT or template positioning which would result in reduced telomerase activity. Additionally, the association of TR with accessory proteins may be affected, resulting in reduced telomerase level in cells. The reduction in telomerase activity or RNA accumulation has been experimentally confirmed for many of these disease-linked mutations [15,59,70,74,75,79–83], thus complementing our understanding of telomerase function in vivo.

3. TERT structure and function

The TERT protein is the catalytic component of the core telomerase enzyme. The protein comprises four conserved structural domains, the telomerase essential N-terminal (TEN) domain, the telomerase RNA binding domain (TRBD), the RT, and C-terminal extension (CTE) (Fig. 2, bottom). The central catalytic RT domain contains seven conserved motifs shared with conventional RTs: motifs 1, 2 and A, B, C, D, and E (Fig. 2, bottom) [84]. The tertiary structure of TERT, like other DNA polymerases resembles and is

described in terms of a right hand [85]. The fingers domain, composed of motifs 1 and 2, is believed to bind incoming nucleotides, while the palm domain, consisting of motifs A–E, forms the catalytic site [86]. Mutational analysis of the essential Asp residues from motifs A and C supports the telomerase enzyme employing acidic metal-coordination by an aspartic acid triad for DNA polymerization, a mechanism common to conventional RTs [87]. Motif E functions as a primer grip, interacting with telomeric DNA [88]. Conserved residues in the RT domain when mutated abolish telomerase enzymatic activity in vitro, these TERT mutants fail to maintain telomere length in vivo, and many of these mutations have been identified in individuals with telomere-mediated disorders [70,77,82,87,89–93]. Recently, a telomerase-specific motif was discovered within the RT domain. This motif 3 is unique to TERT and exclusive to enzymes with high repeat addition processivity [27]. The CTE in TERT has been postulated to share functionality with the HIV RT C-terminus, commonly referred to as the thumb domain. The retroviral thumb domain binds to the RNA template/DNA primer duplex, while the telomerase CTE binds to telomeric DNA, enhancing nucleotide polymerization [85,94–96]. A schematic of the conserved motifs within TERT is shown in Fig. 2.

While the RT and CTE domains are broadly conserved between TERT and conventional RTs, the TEN and TRBD domains are telomerase-specific and unique to the TERT protein (Fig. 2, bottom) [85,97–100]. The TEN domain contains 'anchor' sites which bind single-stranded telomeric DNA [101]. This delays complete disassociation of the DNA product from the enzyme and thus increases repeat addition processivity. Mutational analysis has identified regions within the TEN domain specific for repeat addition processivity which do not affect nucleotide addition [23,24]. The TEN domain also contains RNA interacting domain 1 (RID1), a low-affinity binding site for the TR template/pseudoknot domain [102]. The TRBD contains RNA interacting domain 2 (RID2) which is a high affinity binding site for the TR CR4/5 domain (Fig. 2, bottom) [103]. The protein–RNA interactions through RID1 and RID2 are essential for telomerase assembly and disruptions of these domains abolish activity both in vitro and in vivo [39].

Numerous unique mutations have been identified within the TERT gene which are linked to human telomere-mediated disorders [55,63,64,66,70,74,77,82,93,104–108]. Similar to TR mutations, TERT mutations manifest as a syndrome complex which includes AA, autosomal dominant or recessive DC, and IPF. It is of note that there are cases of compound heterozygous DC from several TERT mutations [65,106,107]. When mapped onto the amino acid sequence, these TERT mutations are located almost exclusively within the conserved functional domains, with the majority concentrated within RT motifs [78]. While mutations which disrupt nucleotide addition are well characterized, only recently have mutations which disrupt repeat addition processivity been discovered [108].

4. Telomerase accessory protein structure and function

In addition to the catalytic TERT protein, the TR binds a variety of additional telomerase accessory proteins. These ancillary proteins are essential for in vivo TR localization and telomerase RNP biogenesis (Fig. 3). As previously discussed, the 3′ end of the vertebrate TR contains two stem-loop structures separated by a box H and box ACA moiety, aptly named the H/ACA domain (Fig. 2, top) [42]. Each of the two stems in the TR H/ACA domain binds a copy of the protein complex formed from dyskerin, NOP10, NHP2, and GAR1proteins (Fig. 3) [17]. This protein complex is important for RNA maturation, 3′ processing, and RNP biogenesis [16,56]. Cajal body localization of TR is dependent on the TCAB1 protein binding to the CAB box (Fig. 2, top and 3) [18].

Dyskerin is the mammalian ortholog of the archaeal H/ACA RNA pseudouridine synthase which contains the catalytic TruB domain and the pseudouridine synthase and archaeosine transglycosylase (PUA) domain involved in RNA modification [109]. NOP10 is a small basic protein with a conserved zinc ribbon domain in the N-terminal region. This protein does not directly bind to the RNA, and instead binds to the dyskerin protein [57,110,111]. NHP2 is another small basic protein, which binds to the RNA [57,110–112]. GAR1 is defined by, and named for, the glycine and arginine rich (GAR) domains which flank the highly conserved central domain [111]. As with NOP10, GAR1 also does not directly bind to the RNA and instead binds to the dyskerin protein. GAR1 is not required for H/ACA snoRNP stability in vivo or snoRNP assembly in vitro [57,110,112,113]. While dyskerin bound H/ACA snoRNAs localize to both nucleoli and Cajal bodies, TCAB1 bound scaRNAs exclusively localize to Cajal bodies. TCAB1 is responsible for TR localization to Cajal bodies since the depletion of TCAB1 alters the localization of the TR to nucleoli [18].

Numerous unique mutations have been identified within the DKC1gene, encoding for dyskerin [55,114–124]. Mutations in a limited number of families have also been reported in NOLA2, encoding for NHP2 [125]; and NOLA3, encoding for NOP10 [73]. These mutations retain wild-type telomerase activity in vitro while reducing the amount of active telomerase within the cell. No reports of mutations within NOLA1, encoding for GAR1, have been linked to telomere-mediated disorders. Mutations within the DKC1 gene are associated with X-linked recessive DC [126], while mutations in the NOLA2 and NOLA3 genes correlate to autosomal recessive DC [73,125]. Dyskerin and other associated proteins are crucial for ribosomal, as well as telomerase biogenesis [55,127]. However, mutations within these genes appear to have no significant negative effect upon ribosome maturation in human cells [40,128]. Since the discovery of TCAB1 and its gene, WRD79, there has been a report of mutations linked to two cases of autosomal recessive DC. These mutations produce defects in TR trafficking, reducing the amount of active enzyme [129].

5. Telomerase RNP biogenesis

The summation of telomerase biogenesis is the localization of an active telomerase enzyme to the telomere where nucleotide addition can then proceed (Fig. 3). While the intricate details of telomerase RNP assembly have yet to be fully elucidated, much progress has been made in uncovering many of the steps necessary for individual component maturation and the assembly of these components into an active ribonucleoprotein enzyme. TERT protein expression follows the canonical mRNA transcription, maturation, and cytoplasmic translation. The TERT protein is then recruited to nucleoli and then Cajal bodies for RNP assembly [130–133]. The accumulation of TERT in the nucleoli reduces the levels of active telomerase, supposedly by sequestering TERT from TR [134,135]. TR begins as a precursor RNA polymerase II transcript capped by trimethyl-guanosine (TMG) [reviewed in [136]]. Binding by RHAU to the 5′ end resolves the G-quadruplex structure while binding dyskerin and other proteins to the 3′ end, trim and internally modify the RNA to produce a mature TR [53,54,137]. The initial binding of dyskerin to the TR, and other H/ACA snoRNA species, relies on the sequential binding of SHQ1 (snoRNA H/ACA family quantitative accumulation 1), followed by NAF1 (nuclear assembly factor 1) to the dyskerin protein. NAR1 is exchanged for GAR1 and SHQ1 is lost prior to the localization of the mature TR to Cajal bodies [138]. TCAB1 binding is thought to then direct the mature TR to Cajal bodies [18]. The TERT protein is then localized near the Cajal body where telomerase RNP assembly then proceeds [133]. The assembled telomerase localizes to the telomere for nucleotide addition

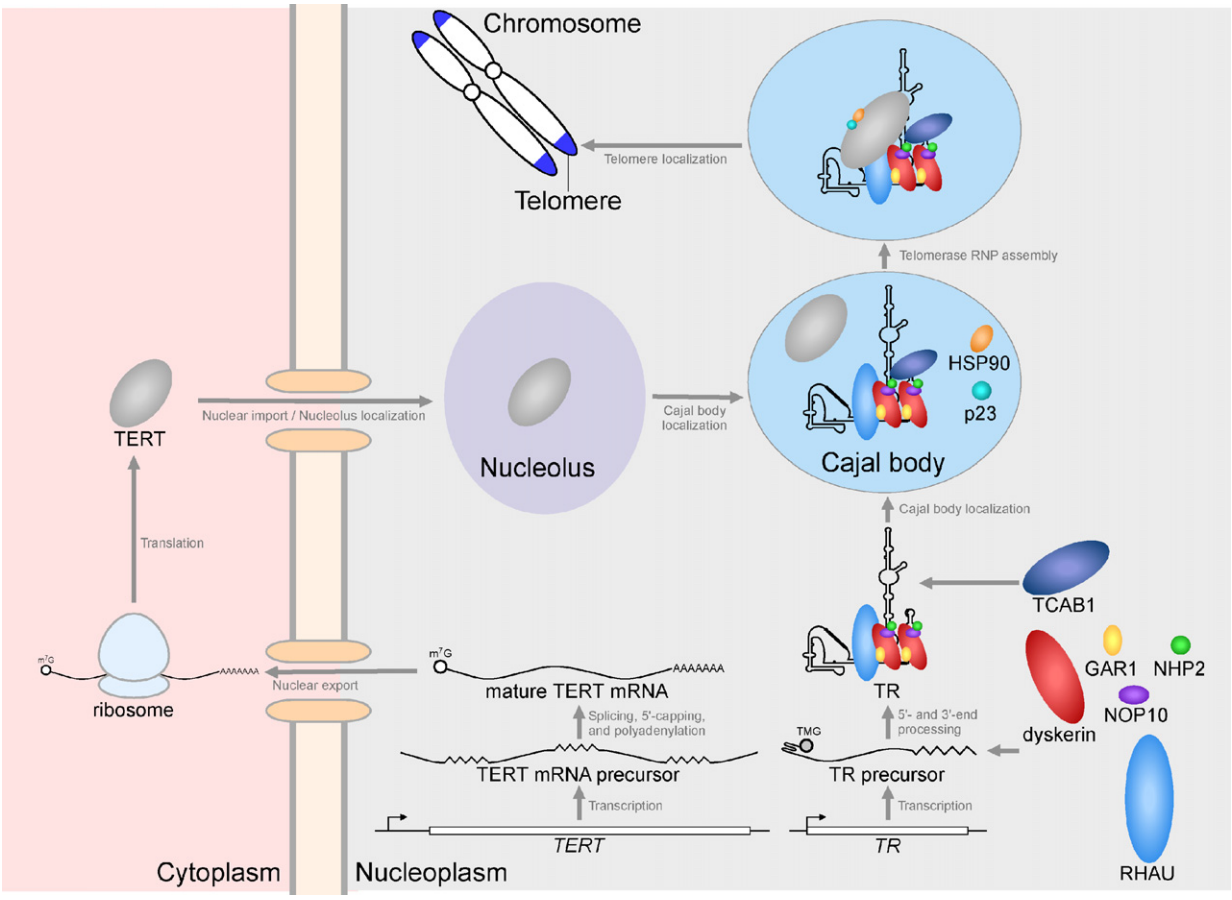

Fig. 3. A model of telomerase RNP biogenesis. Active telomerase localizes to the telomere through a complex pathway. TERT protein expression follows the canonical eukaryotic transcription, RNA maturation, and nuclear export to the cytoplasm (pale-red) for translation. The TERT protein (grey) is then imported back into the nucleus and localizes to the nucleolus (pale-violet) prior to assembly with the TR (black). The TR precursor, synthesized by RNA polymerase II and TMG capped, is bound by two copies of the protein complex formed by dyskerin (red), NOP10 (violet), NHP2 (green), and GAR1 (yellow) for 3′ end processing and internal modifications. The RHAU RNA helicase (light-blue) resolves the G-quadruplex at the 5′ end. TCAB1 (dark-blue) binds to the CAB box for localization of the mature TR to Cajal bodies (pale-blue). TERT localizes to Cajal bodies for assembly with the TR, aided by the chaperone proteins hsp90 (orange) and p23 (cyan). The now fully assembled active telomerase complex localizes to the telomere (blue) for telomeric DNA synthesis.

to proceed (Fig. 3) [139]. The regulation of each component of the telomerase holoenzyme has implications for the accumulation of active telomerase within the cell [reviewed in [140]].

6. Assaying telomerase mutations

In the last two decades, mutagenesis and functional characterization of telomerase has brought about several assays for the assessment of telomerase function both in vitro and in vivo. These assays have been beneficial in establishing the mechanisms by which mutations in telomerase components cause telomere shortening. While telomerase mutations vary by the enzymatic property affected and the clinical presentation of symptoms, thus far, all telomerase mutations reduce the levels of active telomerase within the cell. Telomere-mediated disorders stem from critically short telomeres, which can be measured by a variety of techniques including telomere restriction fragment (TRF), single telomere length analysis (STELA), Q-PCR, Q-FISH, and flow FISH [reviewed in [141]]. The detection of telomere shortening validates the mutation as affecting telomerase function. However, determining the enzymatic property affected and the molecular basis of telomerase dysfunction requires more detailed assays.

Reduced levels of active telomerase are potentially the result of reduced enzyme catalysis. Under such conditions, the amount of the telomerase enzyme is retained at wild-type levels while the specific-activity of the enzyme is decreased. Telomerase activity assays detect the addition of telomeric repeats onto the 3′ end of a given substrate. Fewer telomeric repeats added would indicate diminished levels of telomerase activity. The two frequently employed assays for assessing telomerase enzymatic activity are the telomere repeat amplification protocol (TRAP) and the direct primer-extension activity (direct activity) assay.

The TRAP assay is a PCR-based assessment of telomerase nucleotide addition activity. Telomerase first extends an oligonucleotide by incorporating onto the 3′ end multiple telomeric repeats. The telomerase-extended products are then PCR amplified to generate several fold more product. This PCR amplification step requires the oligonucleotide substrate to include a 5′ non-telomeric sequence specific for PCR amplification [reviewed in [142]]. However, the 3′ ends of the telomerase-extended products are repetitive. This is problematic for primer specificity, since the primer has the potential to anneal to multiple sites, making this assay only semi-quantitative. Thus telomerase-extended product lengths are re-distributed by the PCR application step. These altered telomerase-extended products obscure the number of nucleotides and repeats incorporated by telomerase, concealing the processivity for both nucleotide and repeat additions. While the PCR application of the telomerase-extended products greatly increases the sensitivity of the assay, the processivity cannot be determined [143].

The direct activity assay for detecting and characterizing telomerase activity relies on the extension of an oligonucleotide, typically a telomeric sequence, by the telomerase enzyme in the presence of triphosphate nucleosides, often radiolabeled for detection. The pattern of telomerase-elongated products directly correlates with the number of telomeric repeats added to the oligonucleotide substrate [142,144,145]. Since the telomerase-extended products are directly assayed, the number of incorporated nucleotides is unaltered, in contrast to the PCR-based TRAP assay. Quantitatively measuring the telomerase-extended products reveals not only the nucleotide addition activity of the enzyme, but also the repeat addition processivity [51]. So far, the vast majority of mutations identified from human patients have decreased telomerase activity while retaining wild-type processivity. Recently, hypomorphic TERT mutations that primarily affect repeat addition processivity have been identified and found to cause telomere-mediated disease [108].

The direct activity assay is advantageous in quantifying telomerase nucleotide addition activity and repeat addition processivity. This assay has been established over the past decade to have enough sensitivity to detect activity from telomerase reconstitution both in vitro and in cells [51]. The in vitro reconstitution of telomerase relies on rabbit reticulocyte lysate for in vitro transcription and translation of the TERT protein and in vitro transcription of the TR. Mutations localized to the TR and TERT can be rapidly assessed by this method [70,82,108]. The in vivo reconstituted telomerase holoenzyme contains numerous accessory proteins which have been shown to affect telomerase activity [30] and could potentially mask or amplify TR or TERT defects. Thus in vivo reconstitution of telomerase is more holistic and comprehensive [145,146]. Although the direct activity assay has lower sensitivity than the TRAP assay for detecting telomerase activity, the over-expression of both the TR (under the U3 snRNA promoter) and TERT (under the CMV promoter) in cells generates sufficiently high levels of telomerase activity for detection [15,147]. While this over-expression system produces high levels of TR and TERT, the holoenzyme is composed of numerous addition accessory proteins. These accessory proteins could become limiting and might then alter the enzyme stoichiometry. For example, dyskerin could become limiting, and the TR would be bound with less than two copies of the protein. To compensate, the known accessory proteins could also be over-expressed, however, not all telomerase accessory proteins are known.

While the TRAP and direct activity assays are well-designed for the detection of defects in telomerase processivity and activity, mutations altering the localization of telomerase components or the biogenesis of the telomerase RNA would remain undetected. The loss of elements essential in vivo, but dispensable in vitro, such H/ACA domain motifs, could only be detected by directly measuring the levels of each telomerase component [75]. TCAB1 is necessary for TR localization and biogenesis, but not required for in vitro reconstitution of telomerase nucleotide addition activity and repeat addition processivity. The consequences for these lost functions would have to be detected by other methods, such as FISH [16,129,148]. The failure of the TR to localize and accumulate within Cajal bodies would indicate the TR could no longer bind to TCAB1. However, determining if this is due to a TR or TCAB1 mutation would require further investigation and experimentation. Mutations affecting TR stability require a separate program of study beyond enzymatic function and localization. The stability of telomerase holoenzyme components is essential for the accumulation of sufficient active telomerase. While the reduction of apparent telomerase activity could be the result of enzymatic dysfunction, there is also the possibility that the TR could prematurely degrade. The reduced accumulation of TR would reduce the amount of active telomerase [17,54]. Assaying the relative levels of telomerase components, for TR by northern blot analysis or qRT-PCR, would separate decreased TR accumulation from functional defects within the holoenzyme.

7. Concluding statements

The structure, function, and localization analysis of telomerase components have expanded our understanding of the telomerase holoenzyme and provided extensive insights to the underlying cellular mechanism behind telomere-mediated disorders. The structural features within each component directly relates to their functional role within the enzyme. This close association between the structure and function is further evidenced by naturally derived or experimentally induced mutations. Further understanding of this remarkable enzyme may provide insights into the development of treatments for diseases linked to telomerase dysfunction.

Conflicts of Interest

None.

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