Chapter 21

Telomere Maintenance and Genome Stability

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Chapter Outline

1. INTRODUCTION

As early as the 1930s, it was noted that linear chromosomes possessed special ends that prevented them from being cleaved and fused together [1,2]. These data, along with similar discoveries, provided evidence that the free ends of the chromosomes contributed directly toward genome stability. The free, or natural, chromosome ends were eventually given the name "telomeres" [2]. Later, in the 1960s, Leonard Hayflick discovered that somatic cells could divide only a finite number of times before cell division was halted [3]. In the following decade, Alexei Olovnikov recognized that the ends of chromosomes could not be fully replicated and he suggested that the gradual shortening of chromosome DNA might contribute to the limited replicative capacity of the cell [4]. At around the same time, James Watson was investigating the so-called "end-replication problem" of DNA synthesis [5]. The end-replication problem refers to the inability of DNA polymerases to fully synthesize the ends of DNA on the lagging strand. As a consequence, the ends of chromosomes become shorter with each cell division. It was later demonstrated that, on average, chromosomes in human diploid cells lose between 100 and 150 base pairs per cell division, and most mammalian cells are able to divide about 40–60 times before entering a state of replicative senescence [6,7].

It was not until 1978 that telomere DNA sequence was identified in the ciliate protozoa, *Tetrahymena thermophila* [8]. In this work, it was discovered that the ends of chromosomes contain a repeating DNA sequence (TTGGGG in *T. thermophila*). Furthermore, it was demonstrated that *T. thermophila* telomeric sequence could be recognized in yeast, suggesting that a unique telomere replication process was conserved among distant organisms [9]. In 1985, such a specialized enzyme called "terminal transferase" and eventually named telomerase was identified as being responsible for the

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Genome Stability. http://dx.doi.org/10.1016/B978-0-12-803309-8.00021-5

replication of telomere DNA [10]. The seminal work focusing on the discovery, composition, and maintenance of telomeres and telomerase eventually led to the awarding of the 2009 Nobel Prize in Physiology and Medicine to Elizabeth Blackburn, Jack Szostak, and Carol Greider.

We now know that telomere DNA extends for thousands of base pairs of double-stranded DNA (dsDNA) before ending with a G-rich single-stranded DNA (ssDNA) overhang that serves as the template for telomerase extension. One function of telomeres is to absorb the loss of DNA caused by the end-replication problem and to prevent the loss of genomic information. As such, telomere length in healthy, adult somatic cells is somewhat heterogeneous among individuals and populations; however, telomere lengths tend to gradually become shorter as part of the natural aging process. On average, telomere length in healthy, human adult cells ranges from 5 to 15 kB, with the 3′ overhang extending for an additional 50–200 nucleotides [11,12]. While telomere sequence is conserved among mammals $(TTAGGG)_n$, telomere length varies among species. As an example, mice have extremely long telomeres (~30–150 kb), which has complicated the use of mouse models to explore the processes involved in telomere regulation in humans.

Telomere DNA is bound and protected by a core group of six proteins that is collectively referred to as the shelterin complex [13]. Three shelterin proteins interact specifically with telomere DNA; telomeric repeat-binding factors 1 and 2 (TRF1 and TRF2) bind to the double-stranded region of the telomere and protection of telomere 1 (POT1) binds to the telomere ssDNA. RAP1 (the human ortholog of yeast repressor/activator protein 1) interacts directly with TRF2 to modulate its function. The two remaining shelterin proteins, TIN2 (TERF1-interacting nuclear factor 2) and TPP1 behave as a molecular conduit in the shelterin complex, interacting with TRF2, TRF1, and POT1 to form a direct, protein-mediated link between telomere dsDNA and ssDNA. The removal of individual shelterin proteins induces a complex set of DNA-damage responses, which includes traditional and nontraditional repair mechanisms [14–16]. As such, a primary function of shelterin is to bind telomere DNA and protect it from inadequate recognition of DNA damage–response machinery. However, data are emerging which suggest that the shelterin complex plays a much more versatile role in telomere maintenance and cell-signaling events.

Telomerase is a specialized and unique ribonucleoprotein (RNP) complex that is responsible for maintaining telomere length homeostasis [17]. Telomerase is minimally composed of a catalytic subunit that contains the telomerase reverse transcriptase (TERT) activity and a telomerase RNA component (TR or TERC), which serves as the template for telomeric DNA synthesis [17] (Fig. 21.1). In addition to nucleotide addition, telomerase translocates its RNA template after six nucleotides are synthesized, so that it may be reused as a template for the next set of six nucleotides to be synthesized. This mechanism, referred to as repeat addition processivity, is coordinated by multiple domains within TERT and TR to prevent dissociation from the telomere and to orchestrate realignment of the template RNA with the newly synthesized DNA product.

While TERT is responsible for telomerase catalytic functions, TR provides the RNA template needed to elongate telomeres. In addition to the nucleotides that are responsible for incorporation of the consensus sequence by Watson–Crick base pairing, the TR template contains additional nucleotides that are equally important for initial binding and for proper alignment. Upon recruitment, telomerase binds to the DNA 3′ end-flanking region by complementarity of those nucleotides that are adjacent to the coding template region (Fig. 21.1A). Nucleotides are then reverse transcribed into telomeric ssDNA until the end of the coding template is reached. At this point, telomerase translocates the DNA strand to realign the template region with freshly synthesized telomere DNA to repeat the entire process without primer dissociation.

2. TELOMERE LENGTH AND TELOMERASE REGULATION

In addition to age, telomere length generally correlates with cell function. Cells that exhibit a high proliferative rate (eg, during embryogenesis, in adult germline, and proliferative cells of tissue renewal) express telomerase to maintain longer telomeres and to prevent senescence [7]. In healthy somatic cells, telomerase activity is below detection limits and progressive telomere shortening is observed. Cells that express moderate amounts of telomerase, such as hematopoietic stem cells (HSCs), have the ability to maintain telomere length but not as efficiently as cells that constitutively express telomerase, as is the case in most cancer cells. Generally, cancer cells reactivate and/or upregulate telomerase to maintain telomeres, albeit at reduced lengths. Other evidence suggests a putative mechanism in which telomerase is activated in response to the detection of extremely short telomeres that are at a higher risk for inducing chromosome instability [18]. In these cases, activation of telomerase is sufficient for avoiding cell death mechanisms that would otherwise be initiated. The exact mechanism of how telomere length and telomerase expression is regulated, particularly during cancer progression, remains unclear. Nonetheless, there is a clear connection between telomere length, telomerase activity, and gene stability in a wide range of cell types.

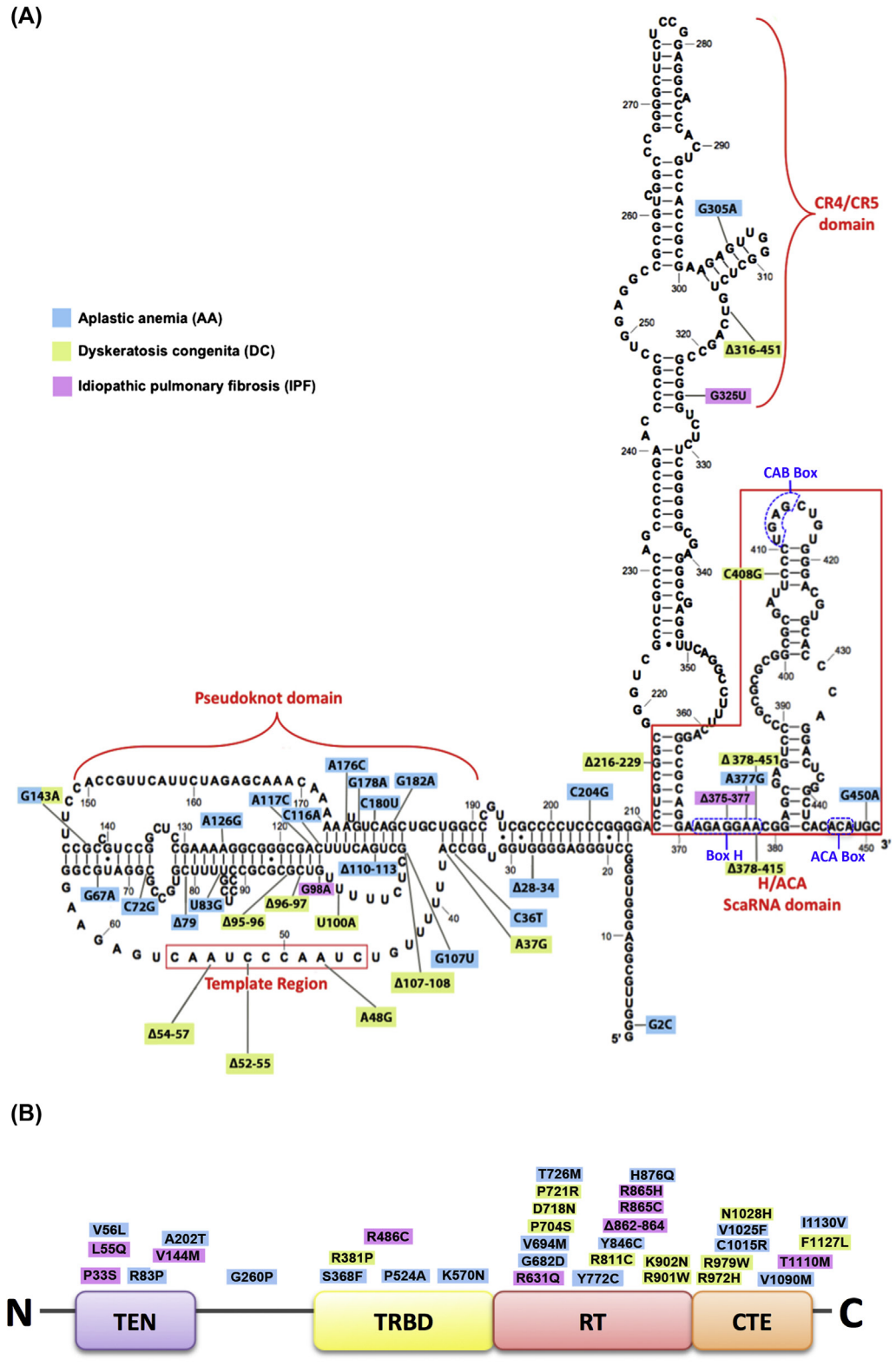

FIGURE 21.1 Telomerase (TERT and TR) domain topology. (A) Schematic representation of telomerase RNA structure. The four primary domains, pseudoknot domain, template region, CR4/CR5 domain, and H/ACA box, are labeled. Mutations associated with telomerase-associated disorders are individually identified and labeled by color code for each disease. (B) Ribbon diagram of TERT organization with N-terminal (TEN), RNA-binding domain (TRBD), reverse transcriptase (RT) domain, and C-terminal domain (CTE) domains labeled. Mutations related to telomerase-associated disorders are identified and labeled in color code for each disease. In both panels, *blue labels* are linked to AA, *green* for DC, and *purple* for IPF.

2.1 Germ Cells and Embryogenesis

In contrast to somatic cells, telomerase activity is detected in adult testes and ovaries. More precisely, the highly proliferative, immature germ cells (oocytes and spermatocytes) display high levels of telomerase activity [19]. Once the germ cells mature and become nonproliferative (eg, sperm and ovum), telomerase activity decreases. As expected, the telomere length in both female and male germline tends to be longer than those in somatic cells. Interestingly, the average telomere length of germ cells in female mice has been reported to decrease with age while maintaining a consistent length, or even increasing, in the germline of male mice [20]. The authors of this study speculate that telomerase activity is associated with fertility, and that telomerase expression and reproductive aging are intertwined. This hypothesis is supported by findings demonstrating that telomerase-negative mice (lacking TR) exhibit enhanced telomere erosion, defective spermatogenesis, decreased proliferation, and an increased incidence of apoptosis.

During embryo development, telomerase activity remains low until the blastocyte stage [21]. At the morula/blastocyte transition, telomeres are elongated significantly in a telomerase-dependent manner. Telomerase levels remains high until total histological differentiation of organs occurs. As an example, telomerase activity in human embryos becomes suppressed at 12 weeks gestation in heart cells when cardiac myoblasts differentiate to myocytes [19]. In mice, by 16 weeks gestation telomerase activity is heterogeneous across tissues, and by birth it diminishes to undetectable levels in brain, liver, skin, lung, muscle, and adrenal tissue [19].

2.2 Stem Cells

As is the case during development, telomerase activity tends to decrease with stem cell differentiation events. For example, when HSCs are stimulated with cytokines to induce differentiation, telomerase expression decreases simultaneously [22]. And although telomerase activity tends to be higher in stem cells than in somatic cells, it is still considerably lower than it is in germ cells, during embryogenesis, or in cancer [23]. One reason that may explain this difference could be due to the quiescent, or very slow growing, nature of some stem cells, which limits the rate of cell division and, thus, the need for telomerase activity. A comparison between HSCs and adult bone-marrow stem cells, and between stem cells from fetal liver and cord blood, revealed that a reduction in telomere length does occur in stem cells [24]. While there is agreement for elevated telomerase activity in HSCs, human mesenchymal stem cells (MSCs) do not have detectable levels of telomerase [25]. Also, MSC telomeres show attrition at a rate that is comparable to somatic cells (30–120 base pairs per cell population doubling) [25]. At least in mice, however, the removal of telomerase adversely affects MSC function and prevents differentiation [26]. Similarly, the proliferation and differentiation of stem cells is linked to telomere length and telomerase activity, further indicating that proper telomere maintenance is an important modulator of stem cell behavior [27]. As with somatic cells, these studies support a correlation between telomere length and telomerase activity in stem cells. Furthermore, they also highlight a critical role for telomerase in maintaining stem cell function, even when its activity is below current levels of detection.

3. ORGANIZATION AND FUNCTION OF TERT AND TR

3.1 TERT Organization

TERT is subdivided into four major domains: the essential N-terminal (TEN) domain, a TERT RNA-binding domain (TRBD), the reverse-transcriptase (RT) domain, and a C-terminal extension (CTE) (Fig. 21.1B) [17,28]. While each domain accounts for individual responsibilities, all four regions are necessary for TERT-orchestrated catalytic functions. The TEN domain facilitates telomerase recruitment and processivity, putatively by acting as a "clamp" to pull the freshly synthesized telomere DNA out of the telomerase active site to assist in translocation of the template, one hexamer at a time [29]. The TRBD domain is highly conserved among species and provides a high affinity-binding platform to facilitate specific interactions with TR that are important for holoenzyme assembly and function [30]. The RT region contains the active, catalytic site of the enzyme that ensures proper alignment of the telomerase RNA template and is responsible for nucleotide addition of the polymerase activity. The RT domain is also highly conserved and shares clear homology, particularly within its active site, with the RT domains of retrotransposons and retroviral RTs [28,31]. Despite sharing little sequence homology, the CTE is structurally analogous to the thumb domain of other RTs [32]. Functionally, the CTE stabilizes the telomerase–DNA complex and is required for telomerase-mediated nucleotide addition and processivity [33].

Although the full-length structure of TERT is yet to be determined, each of its domains have been solved individually or with other domains. The *T. thermophila* telomerase TEN domain represents a single, globular domain with a novel protein fold [34]. In addition to a C-terminal RNA-binding motif, the structure reveals a series of charged and conserved residues

on its surface that comprise a putative DNA-binding groove. The structure of the *T. thermophila* RBD revealed that it contains two conserved (CP and T) motifs within its RNA-binding pocket [35]. The CP and T motifs form a wide hydrophilic pocket that is implicated in binding to the template boundary element of TR. The most complete structure of TERT is for that of the red flour beetle, *Tribolium castaneum* [32,36]. Although the protein lacks a TEN domain, the *T. castaneum* structure reveals how the RBD and RT domains interact to form a large cavity in the center of the protein to accommodate a DNA–RNA, primer–template duplex. The RT domain of TERT is structurally similar to other polymerases and RTs in that it includes a fingers, palm, and thumb topology for gripping nucleic acid. The TERT CTE is localized at the same position as the thumb domain with respect to the palm and finger subdomains of viral RNA and B-family DNA polymerases.

3.2 Telomerase RNA Component

In contrast to TERT, TR from different organisms varies in both sequence and length, ranging from 159 nt in *T. thermophila* and 451 nt in human to 1157 in *Saccharomyces cerevisiae* and 1213 nt in *Schizosaccharomyces pombe* [37]. Even though TR elements are highly divergent, several common motifs have been identified by phylogenetic and mutational studies among different species. These common elements include a template pseudoknot (T-PK) that contains the template used for telomeric DNA synthesis, conserved regions 4 and 5 (CR4/CR5), the vertebrate-specific hairpin-hinge-hairpin-ACA (H/ACA) and conserved region 7 (CR7) motifs (Fig. 21.1A). The T-PK and CR4/CR5 bind to TERT independently, and are the minimal TR elements required for reconstituting telomerase activity in vitro [38]. The H/ACA box, which is a small Cajal body-specific RNA at the 3′ end of vertebrate TR, is critical for telomerase holoenzyme biogenesis and regulation in vivo [39]. The CR7 domain is also located at the 3′ end of vertebrate TR and contains a conserved Cajal body (CAB box) localization element. The CAB box is a conserved feature that is responsible for targeting and localization of small Cajal body-specific RNAs (scaRNAs) to Cajal bodies where posttranscriptional processing occurs [40].

Although the expression of TR and TERT are the minimal requirements for reconstituting telomerase activity in vitro, there are a number of accessory proteins that are important for the composition and/or assembly of the telomerase holoenzyme. Several of these proteins, including dyskerin, NHP2, NOP10, and GAR1 comprise a major class of small nucleolar polypeptides that recognize sequence elements in RNA that include an H/ACA signature. The H/ACA box is a characteristic feature common in two classes of small RNAs—small nucleolar RNAs (snoRNAs) and scaRNAs. This motif generally guides the site-specific pseudouridylation of RNAs in the nucleolus and Cajal bodies, respectively [41]. The dyskerin pseudoduridine synthase, NHP2, NOP10, and GAR1 associate with H/ACA RNAs to guide site-specific pseudoridylation of ribosomal and spliceosomal RNAs, as well as other processes unique to eukaryotes and archaea [42]. In human TR, the H/ACA-motif (Fig. 21.1A) is bound by dyskerin, NHP2, NOP10, and GAR1 to modulate telomerase RNP biogenesis and stability [43]. In spite of lacking any pseudouridylation modifications, TR is classified as a scaRNA, primarily due to the presence and necessity of a CAB box and because of an essential requirement for localization to Cajal bodies for processing. The disruption of H/ACA protein interactions with TR, or abrogated Cajal body localization of TR, is an event that is associated with telomerase dysfunction and impaired telomere extension [39,44]. In addition to the H/ACA-binding proteins, another cofactor called TCAB1 (telomerase Cajal body protein 1; also known as WRAP53) mediates trafficking of telomerase to Cajal bodies for efficient biogenesis and subsequent telomere maintenance [45].

While it has yet to be determined for vertebrate TR biogenesis, the mature 3′-end of TR in fungi is generated by a spliceosome-mediated cleavage event [46,47]. The mature TR molecule in vertebrates contains a 2,2,7-trimethylguanosine cap with no polyA tail [48]. In human cells, mature telomerase exists in equilibrium with unassembled TR and TERT components [49]. Together, these data provide compelling evidence that implicates posttranscriptional processes, biogenesis, and assembly as important regulators in controlling telomerase activity.

4. TELOMERIC DNA STRUCTURE

Telomeric DNA is comprised of repetitive G-rich sequence motifs oriented 5′–3′ toward the chromosome end. The length and sequence of repeats varies among different species. It is represented by 4.5 repeats of (T_4G_4) sequence in the ciliate *Oxytricha nova*, 20–70 repeats of (T_2G_4) sequence in *T. thermophila*, 10–15 kb of (T_2AG_3) repeats in humans, and 20–50 kb in certain mouse and rat species [37]. Meanwhile, the telomeric DNA repeats in *S. cerevisae* is approximately 300 base pairs of a somewhat heterogeneous $(TG)_{1–4}G_{2–3}$ repeating sequence. In all organisms, telomeric DNA is composed mostly of dsDNA followed by a ssDNA overhang that serves as the substrate for telomerase-mediated extension.

Guanine-rich DNA is capable of forming very stable G-quadruplex (GQ) structures [50,51]. GQs are best characterized by the arrangement of planar arrays formed by four guanine bases held together by forming hydrogen-bonded Hoogsteen base-pairing interactions. GQ structures can form both inter- and intramolecularly and the morphology varies depending

on several factors. For example, the orientation of the strands in the GQ can assemble as parallel or antiparallel, or a heterogeneous mix of both, strands. Similarly, the associated metal ion that stabilizes the GQ contributes to GQ topology and strand orientation. Finally, the sequence of nucleotides flanking the GQ structure can influence topology and stability. Multiple GQ topologies assembled using DNA with human telomere sequence have been characterized in molecular detail (Fig. 21.2). The K^+ containing structure of d[AGGG(TTAGGG)$_3$] determined by X-ray crystallography reveals a propeller-like structure with the strands oriented in a parallel fashion [52]. In this arrangement, the guanines are arranged into stacked G-quartets with the K^+ in the center and the TTA loops protruding away like the blades of a propeller. The structure of the same DNA sequence determined by NMR provides a different basket-type GQ conformation [53]. In this topology, all strands reside in an antiparallel orientation. While most of the current knowledge regarding GQ stability and structural polymorphism stems from biophysical experiments using isolated DNA, GQs have also been identified in the telomeres of human cells and in the macronuclei of ciliates and in frog oocytes [54–56].

Telomere loops (T-loop) describe another structure that has been characterized for telomeric DNA. To form a T-loop, the ssDNA overhang is predicted to invade the telomeric duplex DNA to form a lariat configuration [57]. Due to the elevated thermodynamic stability inherent to dsDNA, protein factors such as TRF2 are a requisite for T-loop assembly [57].

5. TELOMERE-INTERACTING PROTEINS

5.1 The Shelterin Complex

Vertebrate telomeres are capped by a multiple-protein complex called shelterin (Fig. 21.3) [13]. Two shelterin components, TRF1 and TRF2, localize specifically at telomeres (Fig. 21.3A). The two proteins are negative regulators of telomere length, as overexpression of either TRF1 or TRF2 leads to gradual telomere attrition in cancer cells [58,59]. TRF1 and TRF2 bind to the telomeric dsDNA as preformed homodimers, which interact through their N-terminal, TRF-homology (TRFH) domains [60]. The structure of the TRFH domain has been determined for both TRF1 and TRF2 and it resembles a twisted horseshoe-like structure with unique interface features to prevent heterodimerization [60]. For example, the amino acid sequence at the TRFH interface differs between TRF1 and TRF2 and the structures implicate these differences in inhibiting TRF1–TRF2 heterodimer formation. The protein–DNA interactions for TRF1 and TRF2 occur exclusively with

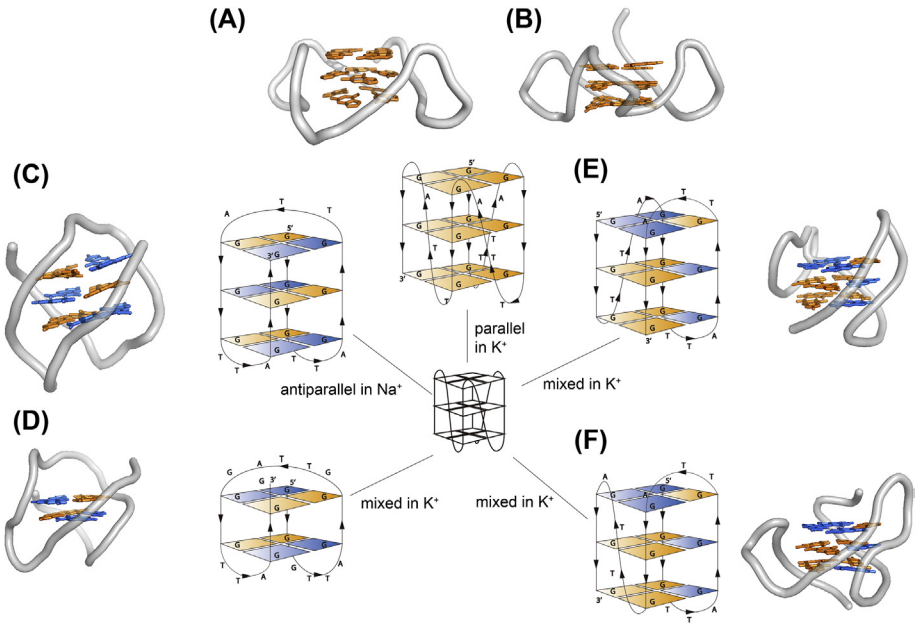

FIGURE 21.2 Three-dimensional and schematic structures of intramolecular G-quadruplexes formed by human telomeric sequences. (A) Parallel G-quadruplex observed for the sequence d[AGGG(TTAGGG)$_3$] in a K^+-containing crystal (PDB ID: 1KF1). (B) Parallel G-quadruplex observed by NMR for the sequence d[TAGGG(TTAGGG)$_3$] in a K^+-containing crowded solution (PDB ID: 2LD8). (C) antiparallel G-quadruplex observed by NMR for the sequence d[AGGG(TTAGGG)$_3$] in Na^+ -containing solution (PDB ID: 143D). (D) Hybrid G-quadruplex with two G-tetrad layers observed by NMR for the sequence d[GGG(TTAGGG)$_3$T] in K^+-containing dilute solution (PDB ID: 2KF8). (E) (3 + 1) form one hybrid G-quadruplex observed by NMR for the sequence d[TAGGG(TTAGGG)$_3$] in K^+-containing dilute solution (PDB ID: 2JSM). (F) (3 + 1) form 2 G-quadruplex observed by NMR for the sequence d[TAGGG(TTAGGG)$_3$TT] in K^+-containing dilute solution (PDB ID: 2JSL); Guanines in an anti-configuration are shown in *blue* and those in a syn-configuration are colored *yellow*.

telomere dsDNA and are orchestrated by conserved Myb domains that reside at the C-terminus of both proteins [61,62]. The tertiary structure of the Myb domain of TRFs is represented by three helices [62–64]. Notably, the third helix recognizes the core TAGGG sequence that resides in the major groove of the duplex, telomere DNA.

TIN2 is retained at the telomere through interactions that stabilize the TRF1 and TRF2 DNA-binding ability [65]. TIN2 comprises a central hub of the shelterin complex that maintains interactions with TRF1, TRF2, and TPP1 (Fig. 21.3B) [66–68]. Mutations in TIN2 that impair its binding with TRF1 or TRF2 destabilize telomeres and induce a DNA-damage response [69]. Meanwhile, interactions that reside between TIN2 and TPP1 are necessary for recruitment of the TPP1–POT1 heterodimer to the telomere to bind and protect the ssDNA overhang [70]. The removal of TIN2 protein in mice abrogates the localization of POT1–TPP1 protein at the telomere and triggers an ATR-mediated DNA-damage response [71]. These data suggest that TRF1 and TRF2 recruit TIN2 to the shelterin complex, which in turn recruits POT1–TPP1 to the telomere. In addition to forming interactions that keep the shelterin complex intact, the TRF1–TIN2 interaction prevents SCF^{Fbx4}-mediated ubiquitination and degradation of the TRF1 protein [72].

RAP1 is the most highly conserved shelterin protein with the least understood role in telomere biology. RAP1 forms a complex specifically with TRF2 to enhance its DNA-binding specificity (Fig. 21.3A) [73]. The RAP1–TRF2 interaction has been shown to protect telomeric DNA from nonhomologous end joining (NHEJ) [74]. The role of RAP1 in NHEJ has been controversial, however, as other data identify a role of RAP1 in suppressing homology-directed repair (HDR) at telomeres and not NHEJ, at least in cell lines devoid of KU70–KU80-signaling proteins [75]. Structurally, the RAP1 C-terminal domain forms a conserved module in proteins across species that guides interactions with TRF2 in humans, and is used to recruit SIR3 proteins to regulate gene silencing in budding yeast [76]. Interestingly, the removal of RAP1 from human cell lines has no affect on the other shelterin components or on telomere length homeostasis [77]. These data suggest that RAP1 may play a more crucial role in regulating transcription as opposed to a direct role in telomere maintenance.

The ssDNA overhang at the 3′ end of mammalian telomeres is bound and protected by POT1 protein (Fig. 21.3C) [78]. POT1 was originally thought to behave exclusively as a negative regulator of telomerase activity, but this interpretation gets complicated when POT1 functions with other shelterin proteins. For example, TPP1 is the binding partner of POT1 and the POT1-TPP1 heterodimer increases telomerase activity on telomere DNA [79,80]. Although TPP1 is not known to interact with telomere DNA directly, it increases the affinity of POT1 for telomere DNA substrates [79,80]. Furthermore,

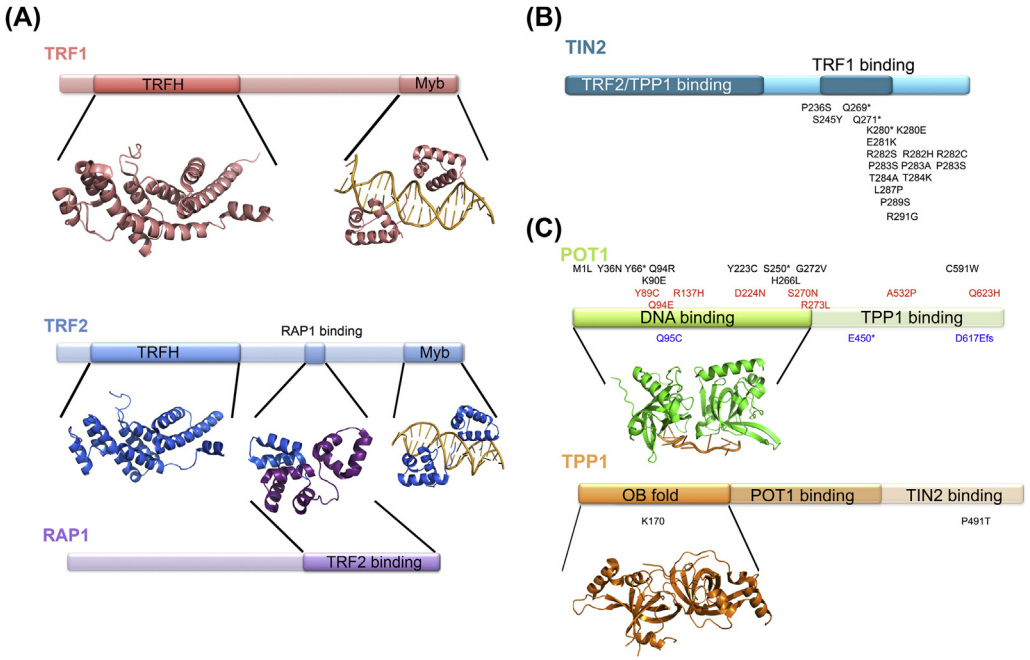

FIGURE 21.3 The structures of shelterin complex components. (A) The top of the panel shows the TRF homology (TRFH) domain (PDB ID: 1H6O) and double-stranded DNA (dsDNA)-bound Myb domain of TRF1; PDB ID: 1W0T). The bottom of the panel displays TRF2 structures, including the TRFH domain (PDB ID: 1H6P), dsDNA-bound Myb domain (PDB ID: 1W0U), and the RAP1–TRF2-interacting domains (PDB ID: 3K6G). (B) Schematic structure of TIN2 with mutations identified related to DC and other telomerase-associated disorders labeled. (C) The top panel shows POT1 organization and includes the structure of the DNA-binding domain bound to telomere DNA (PDB ID: 1XJV). The bottom panel shows TPP1 organization and includes the structure of an internal OB-fold domain (DB ID: 2I46). Mutations identified on POT1 are colored by cancer association: chronic lymphocytic leukemia are colored in black, melanoma in red, and glioma in blue. Mutations identified on TPP1 in Hoyeraal–Hreidarsson syndrome are shown.

TPP1 helps POT1 to discriminate between ssDNA and RNA substrates [81] and plays a central role in the shelterin complex by bridging TIN2, and thus the double-stranded region of the telomere, with POT1 and the ssDNA overhang [70,82,83].

Structurally, the N-terminal domain of POT1 folds into two oligonucleotide/oligosaccharide-binding (OB) domains, which interact intimately with telomeric DNA [84]. In general, the OB fold architecture is a conserved structure comprised of a β-barrel with an α-helix connecting two strands at the end of the barrel. In addition to POT1, a number of nucleic acid–binding proteins, including replication protein A, are represented by OB-fold motifs, indicating a universal role in the direct maintenance of genomic stability [85]. The central domain of TPP1 also represents an OB-fold [79]. However, instead of binding to nucleic acid, the OB-fold of TPP1 is responsible for interactions with telomerase [70,86,87]. Despite poor sequence identity, POT1 and TPP1 are structurally related to the *O. nova* telomere end binding α and β (TEBPα and TEBPβ) heterodimer [79]. TEBPα and TEBPβ are the first identified specific telomeric DNA-binding protein, and the structure of the TEBPα–TEBPβ–DNA complex has been solved by X-ray diffraction [88]. The structure reveals that TEBPα is represented as a series of three OB-fold domains, and TEBPβ is comprised of a single OB-fold motif. The two proteins interact with one another to clamp down on telomere DNA that resides in a groove that is formed between them.

5.2 CST Protein Complex

In addition to shelterin, another multiprotein, telomere-interacting assembly has been described as the CST complex. The CST complex was originally identified in budding yeast to assemble from Cdc13, Stn1, and Ten1 proteins to function in protecting telomeres from DNA degradation [89,90]. In mammalian cells, the homologs of Stn1 and Ten1 have been identified, and a conserved telomere-maintenance component 1 (CTC1) protein, which shares little sequence similarity with Cdc13, associates with STN1 and TEN1 at the telomere [91]. It was originally speculated that the CST complex functions by protecting telomere DNA and downregulating telomerase activity. However, only about 20% of mammalian CST complexes localize to the telomere, suggesting that it is involved in nontelomeric functions as well.

It remains unclear how shelterin and CST coordinate to regulate telomere maintenance. As is the case with shelterin proteins, defects or removal of CST components results in phenotypes that are consistent with telomere dysfunction. For example, the conditional knockdown of *CTC1* impairs efficient replication of telomere DNA [92]. CTC1 and STN1 have been copurified with DNA polymerase α and shown to increase polymerase processivity and assist in the synthesis of RNA–DNA Okazaki fragments [93]. Together, these findings reflect a general role of the CST complex in replication control of telomeres.

6. TELOMERE–TELOMERASE INTERACTIONS AND REGULATION

As mentioned, a primary function of shelterin proteins is to protect telomere DNA from illicit events, such as DNA degradation and end-to-end fusions of different chromosomes. However, the role of shelterin has expanded to include telomere-length maintenance as several of the proteins have been discovered to function in telomerase recruitment and regulation. For example, the POT1–TPP1 heterodimer enhances telomerase activity and processivity [79,80]. The enhancement of telomerase activity can be attributed to a direct protein–protein interaction that allows TPP1 to recruit telomerase to the telomere [70,86,87]. Independent of telomerase recruitment, the POT1–TPP1 heterodimer slows dissociation of telomerase from telomere DNA to assist translocation and enhances telomerase processivity [94]. A number of studies have identified posttranslational modifications to TPP1 that may provide a molecular switch between telomere protection and telomerase recruitment activities [95,96].

Several studies suggest that TIN2 facilitates the localization of POT1-TPP1 to telomeric ssDNA. The removal of TIN2 diminishes the amount of POT1 and TPP1 that localizes at the telomere [71]. Moreover, the depletion of TIN2 but not POT1 results in the failure of TPP1-dependent telomerase recruitment [70]. Together, these data present a scenario in which TRF1 and TRF2 nucleate assembly of the shelterin complex. Indeed, the deletion of both TRF1 and TRF2 in mouse cells results in a complete loss of detection for all six shelterin proteins at the telomere [16].

7. TELOMERE-ASSOCIATED DISEASES

7.1 Telomere Length Homeostasis and Related Diseases

Without question, telomere length homeostasis and the regulation of telomerase are important events to maintain proper genome stability. Short telomeres are associated with several degenerative and age-related disorders. Some examples include dyskeratosis congenita (DC), aplastic anemia (AA), and idiopathic pulmonary fibrosis (IPF). Many of these ailments are

associated with single nucleotide polymorphisms within genes that code for TR or TERT (Fig. 21.1A). Several mutations related to disease, particularly for DC, have been identified in the dyskerin protein, which associates with TR. Still other afflictions, including in a wide range of human cancers, are associated with individual mutations in telomerase or one of the shelterin proteins. A comprehensive list of telomerase-associated mutations associated with human disease is maintained at http://telomerase.asu.edu/diseases.html. Within this communication, we focus on those that are the most prevalent and best understood.

7.1.1 Dyskeratosis Congenita

DC is a heritable, progressive telomerase-associated disorders that is inherited as autosomal dominant, autosomal recessive, or X-linked recessive. The clinical presentation of the disease, which includes hematopoietic deficiency, usually correlates with dysfunctional telomere maintenance. Several mutations in genes involved in telomere and telomerase assembly have been identified to correlate, particularly with X-linked DC, which is the most common form. The vast majority of mutations associated with DC (~40% cases) resides within the *DKC1* gene, which encodes the dyskerin protein [97]. While amino acid changes in DKC1 of DC patients are highly heterogeneous, the most common mutation is a missense change that occurs at position 1058 (C>G) of the gene resulting in an amino acid change at position 353 (A>V) on the protein level [98]. This particular *DKC1* mutation interferes with TR stability and pre-RNP assembly of telomerase [99].

Apart from *DKC1* there is a subset of genes involved in telomere maintenance that have been identified to contain mutations in patients with DC. Like dyskerin, *NHP2* and *NOP10* genes code for proteins that are important for assembly and biogenesis of the telomerase RNP and are associated with mutations in DC patients. DC-related point mutations to *NOP10* or *NHP2* impaired critical interactions between protein products and within dyskerin that are required for TR folding and biogenesis [99]. Mutations in *TCAB1* can abrogate the proper localization of TR to Cajal bodies, thus impairing telomerase holoenzyme assembly. Still other mutations associated with DC have been identified in the two primary components of telomerase, TERT and TR [100].

While these findings tend to agree that mutations linked to DC often result in improper TR assembly with TERT, the molecular pathway to this consequence can vary. For example, some of these mutations result in reduced telomerase catalytic activity, while others impair TR–TERT association, while still others cause loss in fidelity of telomeric repeat synthesis [100]. Regardless of the mechanism, it has become clear that telomerase-associated disorders and telomerase deficiency coincide with one another. It is likely that the telomerase deficiencies and shorter telomeres diminish the proliferative capacity of hematopoietic progenitors.

7.1.2 Aplastic Anemia

AA is another condition characterized by telomerase-associated disorders and associated with mutations in *TR* or *TERT*. Telomere length reduction can be detected in the peripheral blood leukocytes of one-third to one-half of these patients [101,102]. Telomere attrition in AA has been attributed to different mutations on telomerase (TERT and TR), as well as a reduction of shelterin complex proteins POT1 and TIN2 [102]. Like DC, mutations in *TERT* and *TR* found in AA are linked to loss or decrease of telomerase catalytic activity, reduction of telomerase repeat addition processivity, and/or TR instability [103,104]. As in DC, AA molecular mechanism linked to telomerase deficiency is based on haploinsufficiency of telomerase in bone-marrow stem cells that impairs telomere maintenance and proliferation. In fact, many DC patients also develop AA.

7.1.3 Idiopathic Pulmonary Fibrosis

IPF is a chronic lung disease characterized by fibroblast proliferation and excessive accumulation of extracellular matrix. IPF patients typically present with disrupted lung tissue architecture and respiratory difficulties. Although there is not a clear mechanism of the molecular pathway leading to this condition, IPF patients tend to have shorter telomeres in alveolar epithelial cells when compared to healthy individuals [105], suggesting that telomere length is an important feature for the pathophysiology of this disease. Mutations in *TERT* or *TR* are considered risk factors for IPF as they have been observed in 8–15% of familial cases [106]. Mutations in essential telomerase genes have been identified in a small subset of sporadic IPF cases as well [105].

As with DC and AA, mutations in the TERT and TR subunits have been identified in patients with familial IPF and are common genetic risk markers [106,107]. Two of the more characterized mutations in *TERT* (V144M and R865 C/H) (Fig. 21.1B) that are associated with IPF patients have been shown to impair telomerase-mediated telomere extension [107]. While the V144M mutation did not impair telomerase catalytic activity in vitro, it exhibited impaired telomere synthesis in cultured human cells. In contrast, R865H or R865C mutations affected telomerase extension assays in vitro and

impaired telomere synthesis in human cells. The R865 mutation likely affects proper nucleotide binding or incorporation, as that amino acid is within close proximity to the predicted TERT nucleotide-binding pocket.

Also similar to DC and AA, not all patients with IPF have mutations in telomere or telomerase-associated genes. However, a consistency of shorter telomeres among IPF patients suggests that dysfunctional telomere maintenance plays a key role in the molecular pathophysiology of this disease [108]. Mutations that impair telomerase activity or biogenesis commonly result in advanced shortening of telomeres, which limits the replicative potential of progenitor alveolar epithelial cells in IPF cases and can eventually induce apoptosis or senescence. The reduction of alveolar epithelial cell type 2 populations, which are key for the repairing of lung scarring tissue, would make the restructuring of alveolar tissue architecture more difficult in IPF patients with dysfunctional telomeres or telomerase.

7.2 Telomeres and Premature Aging Syndromes

Shortened telomeres are related to an onset of accelerated aging phenotype syndromes such as Werner's syndrome, Bloom's syndrome, and Hutchinson–Gilford progeria syndrome. All are characterized by an increased rate of senescence due to accelerated telomere shortening processes. On average, telomere lengths of the individuals afflicted with these syndromes are significantly shorter when compared to healthy individuals in a similar age group. While telomere shortening is the commonality among patients with advanced aging syndromes, all have different mechanisms leading to this consequence. In the cases of Werner's and Bloom's syndromes, the mechanism involves defects in the DNA helicases WRN and BLM, respectively, which generally increases the rate of transduction of nonfunctional proteins. Both DNA helicases belong to the RecQ family of DNA helicases and are necessary to resolve secondary structure within the telomere DNA (D-loop and GQ) and to unwind dsDNA for replication or DNA damage [109]. Dysfunctional WRN and BLM helicases are associated with incomplete replication of telomeres, recombination between sister chromatids, and inefficient replication of G-rich telomeric strands (WRN) [109,110]. In both cases, the overexpression of telomerase tends to rescue the extreme telomere-shortening phenotype.

As opposed to altered helicase function, Hutchinson–Gilford progeria syndrome is caused by a mutation in the lamin A gene (*LMNA*) at position 1824 (C->T) [111]. Lamin A belongs to a family of proteins that is involved in nuclear assembly, chromatin modifications, and nuclear structure. The C1824T mutation in *LMNA* increases the usage of an internal splicing site that creates a transcript that is missing 150 internal nucleotides. Translation of the transcript results in a protein, called progerin, which is an isoform of LMNA with the deletion of 50 internal amino acids [111]. Telomere shortening and senescent events in normal human fibroblasts activates progerin production and progressive telomere damage increases alternative splicing patterns in multiple genes [112]. In a circuitous cascade of harmful events, some studies have suggested that progerin accumulation leads to telomere deprotection by disrupting heterochromatin structure [112]. Together, these data demonstrate a clear relationship between progerin accumulation and telomere shortening.

7.3 Telomerase Activity in Cancer

While the aforementioned afflictions generally manifest with reduced or aberrant telomerase function, 85–90% of metastatic cancers display increased telomerase activity [113]. The regulation of telomerase activity is presumably governed by TERT expression, as TR and other components of the telomerase complex are constitutively expressed in adult somatic tissues as well as germ cells [114]. Conversely, TERT levels are virtually absent in most adult somatic tissues, but expressed at high levels in most cancer, reproductive, and stem cells where telomerase activity is also elevated [113]. The *TERT* promoter contains several response elements that are recognized by transcription factors, such as c-MYC and SP1 [115]. β-catenin is another protein that regulates gene transcription and interacts with KLF4 at *TERT* promoters to recruit a methyltransferase (Setd1A) to initiate *TERT* transcription [116]. Therefore, the overexpression of c-MYC and WNT/β-catenin that occurs in cancer, may explain the increased levels of TERT in those cells as well. Another explanation can be attributed directly to single-point mutations that have been identified in the *TERT* promoter site in a host of human cancers including skin, central nervous system, bladder, and thyroid [117]. These data indicate that sporadic mutations to regulatory sites might also be responsible for TERT activation in several types of cancer.

Mutations in the *TERT* promoter tend to result in higher *TERT* mRNA levels. One explanation for this correlation can be attributed to an enhanced binding motif that is created for transcription factors such as E-twenty-six (ETS) [117]. While the promoter clearly plays an important role in TERT transcription activation, a number of suppressors that include MAD1, E2F-1, and MZF-2, and PITX1, have been identified to contribute to the regulatory mechanism as well [118–121]. These suppressors exert repressor activity by binding directly to various sites within the *TERT* promoter. In addition, it has been speculated that TERT behaves as a transcriptional modulator of oncogenic genes that are critical for tumor proliferation

[122]. Other mechanisms such as alternative splicing events and epigenetic regulation are likely to be important modulators of TERT regulation as well.

7.4 Shelterin Mutations and Telomere-Related Diseases

Exome sequencing of familial glioma patients has identified inherited mutations in the *POT1* gene that are associated with this type of cancer (p.G95C, p.E450X, and p.D617Efs) [123]. The *POT1* G65C mutation is located within the DNA-binding groove and presumably disrupts interactions with telomere DNA. The *POT1* E450X introduces a premature STOP codon in the translated POT1 protein that would be predicted to lack its TPP1-interacting domain. Similar inherited mutations have been reported in the *POT1* gene of familial melanoma patients [124]. Most of the identified mutations are localized in the POT1 DNA-binding domain, which emphasizes an important relationship between POT1–DNA interactions and the development of familial cancer. Somatic mutations of *POT1* have also been detected in chronic lymphocytic leukemia (CLL) [125]. These studies suggest that mutant POT1 protein fails to localize at the telomere, leaving unprotected telomere ends that could lead to genome instability and tumorigenesis.

Besides POT1, mutations to the genes that code for TIN2 and TPP1 have been identified in patients with telomerase-associated disorders [126–128]. Most of the mutations identified in TIN2 have been identified in patients with DC. Mutations to TIN2 include missense changes as well as nonsense and frameshift mutations to the open reading frame. The corresponding mutations within the synthesized TIN2 protein likely interfere with critical interactions with other shelterin proteins. A 2014 study on Hoyeraal–Hreidarsson syndrome (HH)—a clinically severe variant of DC—revealed *ACD* (codes for TPP1) as a novel DC-related gene [127]. In this study, two mutations (ΔK170 and P491T) were identified at the protein level of TPP1, both of which are highly conserved in mammals. The first mutation is a single amino acid deletion of K170, which is located in a region of TPP1 that is responsible for conducting interactions with telomerase [87]. Interestingly, the ΔK170 mutation of TPP1 has been identified in patients with AA as well [128]. The second mutation is an amino acid substitution (P491T), which is located in the TIN2-interacting domain of TPP1. Together, these data further demonstrate that highly intricate protein–protein and protein–DNA interactions within the shelterin complex contribute to proper genome stability.

8. TELOMERES AS A DNA DAMAGE–PREVENTION SYSTEM

When telomeres become critically short, their capping function is compromised and a range of DNA damage–like responses are induced. In telomerase-deficient yeast cells, short telomeres are recognized as DNA damage and are arrested in G2/M [129]. Markers consistent with DNA-damage response are also triggered in human fibroblasts when telomeres reach a critically short threshold to invoke senescence. These signaling events are remarkably similar to those in cells bearing DNA double-stranded breaks (DSBs) and involve the activation of DNA damage–checkpoint kinases including CHK1 and CHK2. These findings, as well as others, have provided a clear connection between telomere-initiated senescence and innate DNA-damage responses.

In normal telomeres, the shelterin complex collaborates to repress at least six DNA-damage pathways that include ATM and ATR signaling, classical and alternative-nonhomologous end joining (alt-NHEJ), homologous recombination, and resection [16]. Single knockdown studies for each component of the shelterin complex have revealed similar and alternative mechanisms that explain how this protein complex functions to prevent telomeres from appearing as a DNA break in need of repair (Fig. 21.4). The deletion of a POT1 ortholog in mice results in telomere fusions and P53-dependent senescence [130]. POT1 knockdown experiments have provided evidence that it functions, at least in part, to prevent replication protein A (RPA) from binding to telomeric ssDNA, thereby preventing the RPA-induced activation of ATR-dependent DNA-damage responses [14,15,130]. Because of an expanded role in DNA-damage repair and DNA synthesis, RPA exists at a concentration that is much higher in the cell than that of POT1. Both POT1 and RPA display similar binding affinities for telomere DNA, yet physiological levels of POT1 are sufficient to prevent RPA from binding to the telomere. One explanation for this phenomenon can be attributed to a shelterin-related enhancement of POT1 localization and function. Interactions between POT1 and TPP1 with the rest of the shelterin complex effectively localize and concentrate POT1 protein exclusively at the telomere. Furthermore, the inclusion of TPP1 increases the binding affinity of POT1 to telomere DNA nearly 10 times over that of POT1 alone [79]. Highlighting the importance of TPP1 in localizing POT1 to protect ssDNA at the telomere, knockdown experiments revealed that the loss of TPP1 activates ATR-dependent DNA-damage response in a manner similar to that of POT1 removal [131]. Furthermore, TIN2 performs a similar role in recruitment, as its ablation prevents POT1-TPP1 localization at the telomere, thus allowing RPA binding and ATR signaling [71].

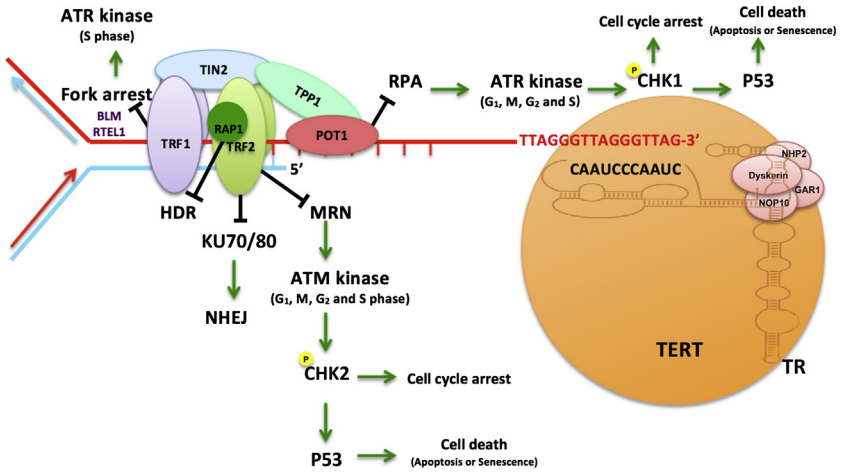

FIGURE 21.4 Shelterin prevents multiple DNA-damage responses at the telomere. The repression mechanism of DNA-damage response by shelterin components are indicated by *blunt-end arrows in black*. The telomerase main components, TR and TERT, are shown in *orange* and telomerase accessory ribonucleotide proteins (dyskerin, GAR1, NHP2, and NOP10) are colored in *pink*. Individual shelterin proteins and their role in preventing various DNA-damage responses are labeled accordingly.

At the double-stranded region of the telomere TRF1, TRF2, and RAP1 also shield telomere DNA from appearing as sites of breaks or damage. Knockdown studies of TRF1 revealed that it is essential for chromosome stability by limiting replicative stress. Mechanistically, TRF1 assists in the proper replication of telomeres by preventing ATR kinase activation and fork stalling [132]. TRF1 may function by coordinated interactions with essential helicases, such as BLM and RTEL1, to facilitate unwinding of GQ structures at the telomere and to avoid fork stalling. Mice deficient for TRF2 are early embryonic lethal. Knockdown of TRF2 in mouse embryonic fibroblast (MEF) cells causes telomere fusions and dsDNA break-like damage activation through MRN (MRE11, RAD50, and NBS1) recruitment and ATM activation [15]. Other studies in cell culture show that the removal of TRF2 allows the KU70–KU80 complex to load onto telomeres to initiate NHEJ DNA repair [133]. Although the TRF2-binding protein, RAP1, is dispensable for ATM activation and NHEJ events, it appears to be critical for repressing HDR at telomeres [75]. Strikingly, these studies demonstrate that HDR events in mouse embryonic fibroblasts lacking RAP1 occur at the telomere even in the absence of a DNA-damage signal. Cumulatively, these knockdown studies reveal a critical function of shelterin proteins in protecting against a range of DNA damage–response mechanisms.

9. CONCLUSIONS AND CLOSING REMARKS

Research relating DNA damage and repair pathways to telomere biology and genome stability will continue to emerge. Cellar phenotypes and molecular pathways have elucidated a role of shelterin and telomerase to function properly to prevent illicit events from occurring. Sequencing data have identified a series of mutations in shelterin proteins and in telomerase that are associated with telomerase-associated disorders and multiple forms of cancer. It will not be surprising to see this list of mutations expand significantly over the next several years. Continued work in telomere biology will also provide further insight into the details of how telomerase gets regulated during development and in cancer.

The basic research focused on telomere biology might lead to improved strategies for treating afflictions related to telomere dysfunction. For example, researchers have explored altering telomere integrity by changing the telomere DNA sequence that is reverse transcribed by telomerase. The overexpression of TR with mutations to its template region inhibits cell growth and induces apoptosis in cancer cells and mouse xenografts [134,135]. In mammals, the template region is 11 nucleotides long (3′-CAAUCCCAAUC-5′) and is localized between positions 46 and 56 of the TR RNA sequence. Studies have shown that different mutations on telomere sequence result in different telomere dysfunction behavior (Table 21.1). Although the usual trend of altering telomere sequence administers a toxic effect, it has been shown that certain sequences can be well tolerated by cells. An example of a nontoxic mutant telomere sequence is mutant TR TSQ1 (3′-CCAACGCCAAC-5′) that codifies telomeric sequence GTTGCG [136]. Studies have shown that TSQ1 mutation can be incorporated into telomeres and the cells are viable for several population doublings. Thus, future insight into what sequence is tolerated and how the telomere mutations coincide with shelterin mutations will provide additional, mechanistic insight into the precise requirements that are necessary for shelterin–telomere DNA interactions and those that result in DNA damage–response induction.

TABLE 21.1 Mutant Telomerase RNA Template

Mutant hTR	RNA Template Sequence	Predicted Sequence	Effect	References
Wild type	3′-CAAUCCCAAUC-5′	TTAGGG	Telomere stability	n/a
hTR-34, MuA, or 47A	3′-CAAACCCAAAC-5′	TTTGGG	Loss of cell viability, cell-cycle deregulation, alteration of nuclear morphology, telomere fusion, DSB-like damages ATM activation, and TRF2 repression	[136–139]
MuC	3′-CAACCCCAACC-5′	TTGGGG	Loss of cell viability, cell-cycle deregulation, alteration of nuclear morphology, and telomere fusion	[138]
MuD	3′-CAAUUCCAAUU-5′	TTAAGG	Loss of cell viability, cell-cycle deregulation, alteration of nuclear morphology, and telomere fusion	[138]
MuE	3′-CACUCCCACUC-5′	TGAGGG	Loss of cell viability, cell-cycle deregulation, alteration of nuclear morphology, and telomere fusion	[138]
AU5	3′-AAUAUAUAUAU-5′	TATATA	Loss of cell viability, DSB-like damages, ATM activation, and TRF2 repression	[134,139]
U11-hTer	3′-UUUUUUUUUUU-5′	AAAAAA	Decreased cell proliferation and cell viability, increased apoptosis, and decreased tumor growth rates	[137]
49A-hTer	3′-CAAUCCCA(AA)AUC-5′	T(TT)TAGGG	Decreased cell proliferation and cell viability, increased apoptosis, and decreased tumor growth rates	[134]
TSQ1	3′-CCAACGCCAAC-5′	GTTGCG	Well-tolerated sequence, do not seem to affect cell survival	[136]

Summary of different mutant TR-template region with their predicted telomeric sequence and genomic consequences. The mutations incorporated in nucleotide bases are underlined.

GLOSSARY

G-quadruplex Guanine-rich DNA capable of forming very stable structures. It is an arrangement of planar arrays formed by four guanine bases held together by forming hydrogen-bonded Hoogsteen base-pairing interactions.

Shelterin complex Group of six proteins (TRF1, TRF2, RAP1, TIN2, TPP1, POT1) that are bound to telomeric DNA. Its main function is to avoid telomere end-to-end fusion and to protect telomeres from being recognized from inappropriate DNA-damage response.

T loop Telomeric DNA structure characterized by the invasion of the ssDNA overhang into the telomeric duplex DNA to form a lariat configuration.

Telomerase Reverse transcriptase that uses its own RNA template to synthesize telomere DNA.

Telomere End cap of linear chromosomes. Its main function is to protect genomic information and control the lifespan of cells.

LIST OF ACRONYMS

AA Aplastic anemia
ACD Adrenocortical dysplasia
alt-NHEJ Alternative-nonhomologous end joining
ATM Ataxia telangiectasia mutated
ATR Ataxia telangiectasia and Rad3 related
BLM Bloom helicase
CAB box Cajal body localization element
CHK1 Checkpoint kinase 1
CHK2 Checkpoint kinase 2
CLL Chronic lymphocytic leukemia
CR4 Conserved region 2
CR5 Conserved region 5

CR7 Conserved region 7
CST Cdc13, Stn1, and Ten1 proteins
CTC1 Conserved telomere-maintenance component 1
CTE C-terminal extension
DC Dyskeratosis congenita
dsDNA Double-stranded DNA
ETS E-twenty-six
GAR1 H/ACA ribonucleoprotein complex subunit 1
GQ G-quadruplex
H/ACA Hairpin-hinge-hairpin-ACA
HDR Homology-directed repair
HSCs Hematopoietic stem cells
IPF Idiopathic pulmonary fibrosis
KLF4 Kruppel-like factor 4
LMNA Lamin A
MEF Mouse embryonic fibroblast
MRN MRE11, RAD50, and NBS1 complex
MSCs Mesenchymal stem cells
NHEJ Nonhomologous end joining
NHP2 H/ACA ribonucleoprotein complex subunit 2
NOP10 H/ACA ribonucleoprotein complex subunit 3
OB Oligonucleotide/oligosaccharide binding
PIP1 POT1-interacting protein 1
POT1 Protection of telomere 1
PTOP POT1- and TIN2-organizing protein
RAP1 Repressor/activator protein 1
RNP Ribonucleoprotein
RPA Replication protein A
RT Reverse transcriptase
RTEL1 Regulator of telomere elongation helicase 1
scaRNA Small Cajal body-specific RNA
SCF Skp, Cullin, F-box-containing complex
SIR3 Silent information regulator 3
snoRNA Small nucleolar RNA
SP1 Specificity protein 1
ssDNA Single-stranded DNA
TCAB1 Telomerase Cajal body protein 1
TEBP Telomere end-binding protein
TEN TERT N-terminal
TEN1 CST complex subunit TEN1
TERT Telomerase reverse transcriptase
TIN2 TERF1-interacting nuclear factor 2
TINT1 TIN2-interacting protein 1
T-loop Telomere loop
T-PK Template pseudoknot
TPP1 TINT1/PTOP/PIP1
TR or TERC Telomerase RNA component
TRBD TERT RNA-binding domain
TRF1 Telomeric repeat-binding factor 1
TRF2 Telomeric repeat-binding factor 2
TRFH TRF-homology domains
TSQ1 Tolerated sequence Q1
WRN Werner helicase

ACKNOWLEDGMENTS

We wish to thank Harry Scott for valuable suggestions and critical reading of this chapter. Research in the Taylor Lab is supported by NIH grants DP2 CA186571 and R21 CA169611, and an American Cancer Society Research Scholar Grant, RSG-13-211-01-DMC. W.H.S. is supported by NIH/NCI supplement DP2 CA186571-01S1.

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