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Molecular basis of telomere dysfunction in human genetic diseases

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Mutations in genes encoding proteins required for telomere structure, replication, repair and length maintenance are associated with several debilitating human genetic disorders. These complex telomere biology disorders (TBDs) give rise to critically short telomeres that affect the homeostasis of multiple organs. Furthermore, genome instability is often a hallmark of telomere syndromes, which are associated with increased cancer risk. Here, we summarize the molecular causes and cellular consequences of disease-causing mutations associated with telomere dysfunction.

In contrast to the circular genomes of prokaryotes, eukaryotic genomes are organized into linear chromosomes, which require mechanisms to protect and maintain the chromosome ends. Eukaryotes have solved this problem with telomeres, complex nucleoprotein structures that protect chromosome ends from promiscuous DNA-repair activities and nucleolytic degradation (reviewed in ref. 1). Telomeres are formed by tandem-repeat sequences (TTAGGG in vertebrates) and terminate in a 3′ single-stranded DNA overhang on the G-rich strand[2,3]. A major challenge to chromosome integrity is the progressive shortening of telomeres with each cell division; this shortening eventually triggers cellular senescence[4]. Three different mechanisms are responsible for progressive telomeric attrition: the 'end-replication problem', processing by exo- or endonucleases and DNA damage localized at telomeric sites. Whereas semiconservative DNA replication occurs bidirectionally (reviewed in ref. 5), DNA polymerases work in a unidirectional fashion and must initiate replication from a primer, thus leaving ~50–200 bp of unreplicated DNA at the 3′ end in each round of DNA replication. This end-replication problem results in progressive loss of telomeric repeats with each round of replication.

Role of telomerase in normal and cancer cells

To counteract progressive telomere shortening with each cell division, telomeric repeats can be extended by telomerase (**Fig. 1**), a reverse transcriptase that uses an RNA moiety (TERC) as a template to extend the 3′ end of the chromosome[6,7]. In human somatic tissues, telomerase is repressed; this repression has been proposed as a mechanism to prevent tumor progression, but it also limits tissue renewal[8,9]. Telomerase expression is retained in certain cell types, such as adult stem cells, mainly in transiently amplifying compartments (reviewed in refs. 10,11), lymphocytes and germ cells. However, its expression is not sufficient to prevent age-associated telomere shortening in stem cells[12]. Strikingly, in 80 to 90% of cancer types, telomerase is reexpressed, thus allowing maintenance of telomere length and enabling cancer-cell immortalization and disease progression[13,14].

Shelterin

Telomere function and integrity are critically dependent on a six-subunit protein complex called shelterin, which specifically associates with telomeric repeats (**Fig. 2**). Shelterin comprises telomere repeat–binding factor (TRF) 1 (TRF1), TRF2, protection of telomeres 1 (POT1), TRF1-interacting nuclear factor 2 (TIN2), repressor activator protein 1 (Rap1) and TINF2-interacting protein 2 (TPP1) (reviewed in ref. 15). The specificity of this protein complex for telomeric DNA is conferred by the ability of TRF1 and TRF2 to recognize duplex TTAGGG repeats and binding of single-stranded TTAGGG repeats by POT1 (ref. 16). Bridging of TRF1 and TRF2 to POT1 is mediated by TIN2 (ref. 17 and reviewed in ref. 16).

TRF1 has been shown to promote telomere replication, which is required to prevent telomeric fragility[18]. TRF2 has an important role in telomeric end protection, which is required to prevent loss of the 3′ overhang and telomere end-to-end fusions[19–21]. Shelterin components also have a key role in suppressing the ATM and ATR checkpoint pathways at telomeres and in inhibiting double-strand break–repair pathways at telomeres, including classical and alternative nonhomologous end joining and homologous recombination.

Telomeres have been shown to adopt a lasso-like structure referred to as a t loop[22], which is believed to form when the 3′ single-stranded overhang invades into duplex TTAGGG repeats. T loops have been proposed as a mechanism that protects chromosome ends from degradation and deleterious DDR (reviewed in ref. 23). T loops must be transiently disassembled for proper telomere replication, in a process requiring the function of the helicase regulator of telomere length 1 (RTEL1)[24]. In the absence of RTEL1, t loops are inappropriately resolved, thus resulting in the loss of telomeres as circles[24]. Additionally, RTEL1 and the BLM helicase are also important in counteracting telomeric G4-DNA structures, thereby facilitating telomere replication and preventing telomeric fragility[18,24,25].

Human telomere syndromes

Telomere dysfunction has been linked to numerous disorders over the years, but how telomere erosion leads to different telomere

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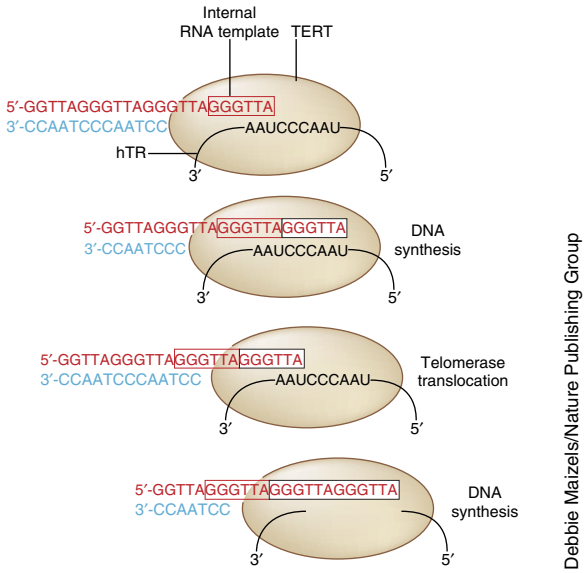

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Figure 1 Role of telomerase in telomere replication. Telomerase is a ribonucleoprotein that contains an intrinsic RNA template (TERC) and the protein catalytic subunit with reverse transcriptase activity (TERT). Telomerase adds single-stranded TTAGGG repeats to the 3′ overhang located at the end of the chromosome and prevents telomere shortening. The integral RNA template in TERC is used by the TERT protein to reverse-transcribe the template into telomeric repeats. Once DNA synthesis has reached the end of the complementary sequence, telomerase translocates, and the telomere DNA extension is repeated. The extension process is completed when DNA polymerase and a primase complex synthesize the complementary strand.

dysfunction syndromes is still unclear. Depending on the mutated gene and inherited telomere length, TBDs present with diverse clinical manifestations and differences in the severity of symptoms. Two of the primary TBDs are dyskeratosis congenita (DC) and Hoyeraal-Hreidarsson (HH) syndrome. In both conditions, the most highly affected tissues and organs are those that undergo frequent cell division.

Dyskeratosis congenita

The majority of mutations that lead to DC either decrease telomerase complex activity (such as that of dyskerin, TERC and telomerase reverse transcriptase (TERT))[26–31] or impair telomerase recruitment (such as mutations in TIN2 (refs. 32–34). This results in defective telomere maintenance, which eventually leads to critically short telomeres, cell-cycle arrest or senescence, and stem-cell exhaustion[35].

People with DC exhibit much shorter telomeres than do unaffected individuals of the same age, but they are born healthy. The first symptoms of ineffective telomere maintenance often appear in the first decade of life and include the mucocutaneous triad of symptoms that are the diagnostic features of DC: abnormal skin pigmentation, nail dystrophy and oral leukoplakia. Other symptoms that are frequently present in DC include pulmonary fibrosis, osteoporosis, developmental delay and liver disease (reviewed in ref. 36). DC is associated with increased morbidity often caused by bone-marrow failure and/or severe immunodeficiency that present in the second decade of life onward. Older people with DC are also prone to cancer, especially to adenocarcinomas (of the stomach, lung, colon and rectum) and carcinomas (of the skin, tongue, colon, pancreas, bronchus, larynx and esophagus) but also to leukemias and lymphomas[37,38]. This may be caused by heightened levels of genetic instability and DNA damage in cells, because critical loss of telomeres can give rise to chromosomal fusions and subsequent breakage-fusion-bridge cycles, thus leading to genetic instability and cellular transformation (reviewed in ref. 39).

There are several modes of DC inheritance (**Table 1**) depending on the underlying mutations. Those types of DC that are caused by autosomal recessive mutations are characterized by genetic anticipation[40].

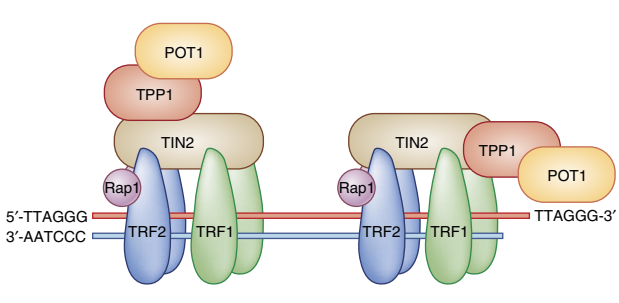

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Figure 2 Shelterin protein complex. Shelterin is a six-subunit complex that specifically associates with telomeric repeats. Shelterin is formed by TRF1, TRF2, POT1, TIN2, Rap1 and TPP1. TRF1 and TRF2 establish the specificity of this protein complex for telomeric DNA through their ability to recognize duplex TTAGGG repeats. POT1 binds to single-stranded TTAGGG repeats.

Individuals carrying a single mutated copy of a DC gene can exhibit diminished telomere maintenance and progressive shortening of telomeres[26,40]. Despite these issues, telomerase activity maintains the telomere length at a level that is sufficient for normal function. As such, carriers of these mutations develop normally and do not present with any overt symptoms of the disease. However, the progressive telomere shortening in the DC carrier can be passed on to subsequent generations and can lead to early onset of the disease in their progeny.

HH syndrome is a rare and extremely severe variant of DC (reviewed in ref. 41), which, like DC, primarily affects highly proliferating tissues. In addition to immunodeficiency and bone-marrow failure, which appear during the first months or years of life, HH syndrome is also characterized by intrauterine growth retardation, microcephaly, cerebellar hypoplasia and developmental delay[42–44]. People with HH syndrome frequently die in their first decade from bone-marrow failure or immunodeficiency. Owing to their short life span, it is currently unclear whether people with HH syndrome are also prone to cancer, because they usually die from other complications of the disease. Although some mutations that are present in DC have also been described in HH syndrome (affecting dyskerin, TERT, TERC and TIN2), recent reports have associated RTEL1 dysfunction with HH syndrome[45–47]. RTEL1 not only acts at telomeres but also acts during DNA replication and functions as an antirecombinase during DNA repair[25,48,49].

Dyskerin and X-linked DC

The most common X-linked form of DC is caused by mutations in dyskerin (DKC1), a protein that is functionally associated with the RNA component TERC[50]. DKC1 is also essential for the function of the H/ACA ribonucleoproteins (RNPs) that modify uracil residues during rRNA processing[50–52]. Numerous DKC1 missense mutations have been identified (**Fig. 3**), and the majority of these are clustered

Table 1 Summary of dyskeratosis congenita inheritance modes

Inheritance mode	Mutated gene
X linked	*DKC1*
Autosomal dominant	*TERC, TERT, TINF2*
Autosomal recessive	*NOP10, NHP2, WRAP53, PARN*
De novo mutations	*TINF2*

into two major groups encompassing amino acids 31–72 and 314–420 (reviewed in ref. 53). The most prevalent mutation in X-linked DC is the missense mutation p.Arg353Val (Human Genome Variation Society nomenclature is used for all mutations herein), which accounts for approximately 40% of all cases. It has been proposed that this amino acid substitution affects pre-RNP assembly with the H/ACA domain of human telomerase RNA fragment (hTR)[54]. Mouse studies have also revealed that p.Arg353Val mutation dramatically decreases the level of mouse telomerase RNA and overall telomerase activity[55]. Moreover, this alteration enhances the interaction of DKC1 with the H/ACA assembly factor SHQ1, which sequesters dyskerin[56]. Reduced availability of DKC1 for RNP assembly has been proposed to decrease telomerase levels, which could account for the short telomeres observed in cells from people with DC[50]. Mice defective for DKC1 are impaired in rRNA pseudouridylation before the onset of DC, thus suggesting that DKC1 initiates DC through the deregulation of ribosomal function[57].

Dyskerin activity is controlled by SUMOylation, and this regulatory mechanism is compromised by disease-causing mutations that alter the DKC1 SUMOylation site at Lys39 (p.Lys39Glu) or by mutations within the highly conserved DKC1 SUMOylation consensus site. These mutations can lead to reduced telomerase activity and telomerase RNA levels[58]. Intriguingly, each amino acid located within the DKC1 hydrophobic-cluster SUMOylation motif is mutated in DC, and deleterious missense mutations of p.Phe36Val, p.Ile38Thr/Met, p.Lys39Glu, p.Pro40Arg, p.Glu41Lys and p.Leu37del have been found in X-linked recessive variants of DC and HH syndrome[27,59,60]. Interestingly, p.Leu37del in DKC1 severely impairs telomerase activity by blocking telomerase assembly and disrupts telomere elongation upon cell reprogramming[61]. Prolonged cultivation of p.Leu37del-mutant induced pluripotent stem cells results in gradual shortening of telomeres and subsequent failure of self-renewal thus raising the possibility that an analogous process may occur in stem cells of individuals with DC.

Autosomal dominant DC: TERT and TERC

Another mechanism leading to short telomeres in people with DC occurs through mutations in the genes encoding the key telomerase components TERT and TERC (hTR). These genes are mutated in approximately 10% of DC cases, and alterations in TERC or TERT are inherited mostly in an autosomal dominant (AD) form; however patients displaying an autosomal recessive mode of inheritance have also been reported[29–31,62]. The central region of TERT comprises reverse transcriptase (RT) active site motifs that are fundamental for catalytic activity[63,64] (**Fig. 3a**). Of note, the p.Arg901Trp and p.Lys902Arg are positioned within the RT domain in a D motif, a TERT region with very high conservation. It has been shown that mutations within this highly conserved domain in

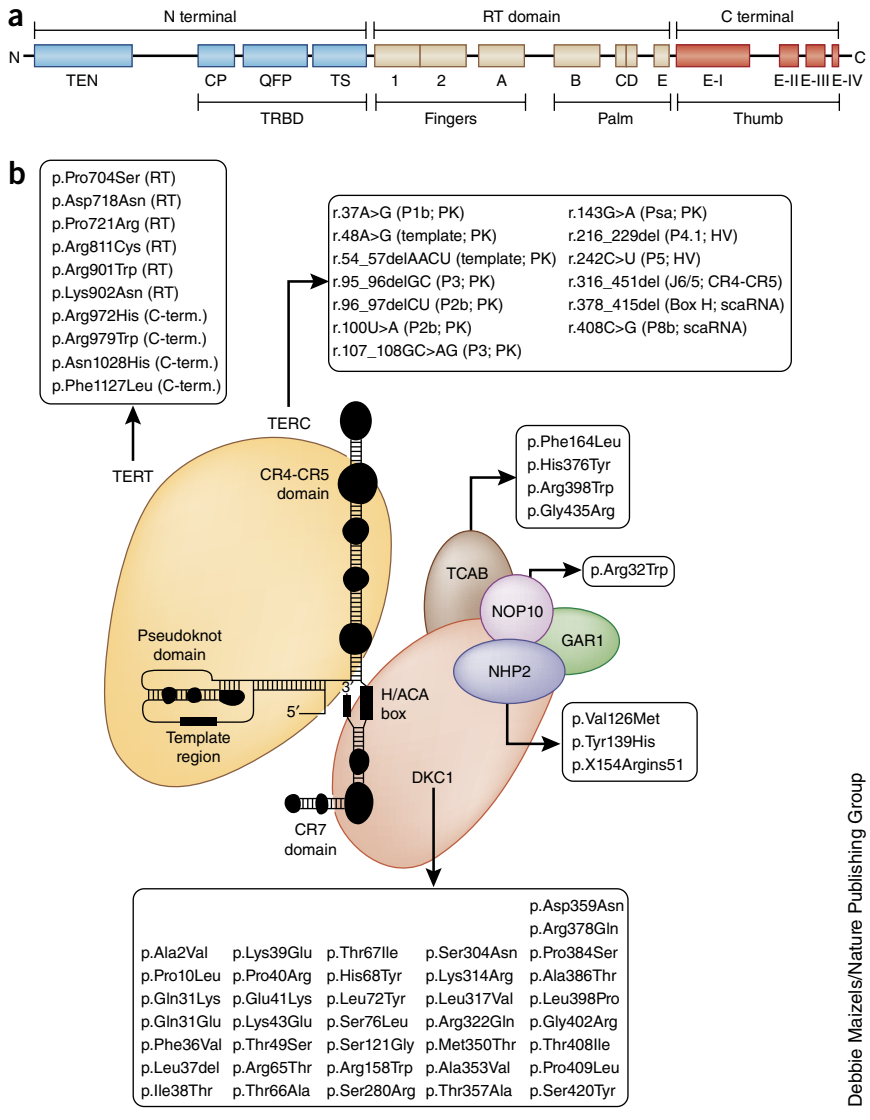

Figure 3 Human telomerase holoenzyme and telomerase accessory proteins. (**a**) Schematic representation of the domains in the TERT protein. Human TERT possesses four evolutionarily conserved domains: the telomerase essential N-terminal (TEN) domain, the TERT RNA-binding domain (TRBD), the reverse transcriptase (RT) domain and the C-terminal domain. CP, QFP and TS are specific conserved domains. (**b**) Structure and mutations found in the human telomerase complex. Depicted are the main components of the human telomerase enzyme complex and telomerase accessory components. Mutations in TERT, TERC, DKC1, NHP2, NOP10 and TCAB1 have been described in people with DC or HH syndromes. Specific domains and/or functional regions affected by the mutations are indicated in parentheses (data based on Telomerase Database, updated 2015; http://telomerase.asu.edu/). C-term, C-terminal domain; P, stem-loop; PK, pseudoknot; HV, hypervariable region; J6/5, internal loop.

HIV-1 RT result in a dramatic decrease in activity and particularly target the selection of the appropriate nucleotide within the catalytic site[65]. Indeed, both mutations detected in human (h) TERT reduce enzyme catalytic activity of the telomerase complex and give rise to short telomeres[26,62]. Moreover, p.Pro704Ser and p.Pro721Arg mutations found in people with Revesz syndrome map to a conserved A region, and the p.Pro868Gly substitution, located in the C motif of the RT domain, also results in diminished telomerase activity *in vitro* and extreme telomere shortening in cells[66–68]. Although, the C terminus of TERT is not highly conserved among members of the TERT family[69], it is important for the function of telomerase[70,71]. It has been suggested that the C terminus of TERT is important for catalytic activity of the enzyme through its C-DAT domain, for cellular immortalization and protein-stability regulation, for nuclear localization through binding to 14-3-3 and for recruitment to chromosome ends through TPP1 interaction[72–75]. Thus, alterations in this region of TERT, such as p.Arg972His, p.Arg979Trp, p.Asn1028His and p.Phe1127Leu identified in people with DC and HH syndrome[76,77], may also affect telomerase-dependent activity distinct from its catalytic function, including telomeric nucleotide addition, processivity and/or protein multimerization. Moreover, some of the patients' mutations (p.Arg979Trp) fall within the 14-3-3–interaction site, located between amino acids 1030 and 1040, flanked upstream by a nuclear export–like sequence between amino acids 974 and 980, whereas others (p.Phe1127Leu) would affect the C-DAT region[74].

Autosomal dominant forms of DC and HH are also caused by mutations in *TERC*, which encodes the RNA component of telomerase (**Fig. 3b**). These *TERC* mutations comprise a range of large and small deletions, insertions and single or multiple amino acid substitutions[40] (**Fig. 3b**). Mutations responsible for the AD variant of DC and aplastic anemia involve base changes in a highly conserved pseudoknot domain of the hTR[78]. The two-base change r.107_108GC>AG in a single copy of *TERC* abolishes telomerase activity by hyperstabilizing the conserved stem-loop P2b structure and blocking pseudoknot formation with P3 (ref. 79). An attractive model suggests that P2b-P3 pairing acts as a telomerase RNA molecular switch, and the 'open-closed' state of the pseudoknot motif controls telomerase activity and interaction with TERT[79]. In addition, the hTR small Cajal body–specific RNA (scaRNA) comprises a conserved Cajal body–localization element (CAB box) in its 3′-terminal hairpin loop, a motif recognized as the CR7 domain (reviewed in ref. 80). NMR studies have revealed that a mutation in the CR7 motif (r.408C>G) disrupts conserved C408-G421 base-pairing and destabilizes the stem structure in the CR7 motif, thus leading to pathological consequences[81].

Ribonucleoproteins and DC: NOP10 and NHP2

NOLA3 is an autosomal recessive DC gene that encodes the NOP10 protein, a part of the H/ACA small nucleolar ribonucleoprotein complex[82], which catalyzes pseudouridylation of rRNA. To date, a single–amino acid substitution (p.Arg34Trp) has been identified in three individuals with reduced telomere length and low TERC levels[83]. The mutation appears in a highly conserved motif of the protein and is anticipated to alter the structure of the NOP10 protein. Intriguingly, the conserved Arg34 residue in NOP10 directly interacts with the P2 region of the RNA component of the H/ACA RNP[84]. Substitution with an aromatic tryptophan may abolish the association of NOP10 with the guide RNA, interrupt correct processing and intranuclear trafficking of TERC, and destabilize the telomerase complex.

Autosomal recessive mutations in *NOLA2*, which encodes NHP2, have also been identified in a cohort of patients with DC[85]. Similarly to NOP10, NHP2 is a component of H/ACA RNP, and two missense mutations, p.Val126Met and p.Tyr139His, alter highly conserved residues of the protein. Interestingly, the mutation p.X154Argins51 gives rise to a replacement of the stop codon with arginine and the addition of 51 amino acids to the C terminus of the protein, and it may affect NHP2's interaction with other proteins and binding to cognate RNA motifs.

Disruption of telomerase trafficking in DC: TCAB1 and TPP1

Defective telomerase trafficking has been proposed to be one of the defects associated with *WRAP53* and *TPP1* mutations linked to DC pathology. Telomerase Cajal body protein 1 (TCAB1), a gene product of *WRAP53*, is a telomerase holoenzyme protein that facilitates trafficking of telomerase RNA to Cajal bodies[86], the nuclear organelles involved in diverse functions such as RNP maturation, telomerase biogenesis and spliceosome formation[87–89] (**Fig. 4**). Sequence analysis of all ten exons in *WRAP53* performed in people with unknown DC etiology revealed new missense mutations (p.Phe164Leu, p.His374Tyr, p.Arg398Trp and p.Gly435Arg) in TCAB1 (ref. 90). Interestingly, these substitutions occurred in proximity to or within WD40 repeats, which associate with the CAB-box motif in TERC[91], a sequence required for telomerase trafficking to Cajal bodies[92,93] (**Fig. 4**). Impaired binding of the evolutionarily conserved WD40 motifs within TERC might underlie an inability of the telomerase RNP to maintain telomeres, owing to mislocalization to the nucleolus. Patient-derived mutations in TCAB1 substantially impair telomerase trafficking and result in a reduction of the telomerase-complex factors (TCAB1, dyskerin and TERC from Cajal bodies), thus leading to severe telomere shortening. Similarly, cells expressing a CAB-box mutant in TERC also exhibit telomere shortening, possibly also because of mislocalization of TERC to the nucleolus[86]. However, telomerase function and telomere lengthening are unaltered in coilin-knockout cells, in which Cajal bodies are absent[94]. These results suggest that Cajal bodies are dispensable in this process, whereas Cajal body–associated factors including TCAB1 are essential in telomere homeostasis. Evidence supporting this notion is further strengthened by the observation that depletion of the chaperonin TRiC, which facilitates TCAB1 folding, causes loss of TCAB1, telomerase and scaRNA mislocalization to the nucleolus as well as telomere-elongation catastrophe[95].

Recent studies have underscored the role of the shelterin component TPP1, a gene product of the adrenocortical dysplasia homolog (*ACD*), in the mechanisms of telomerase recruitment from Cajal bodies to telomeres as well as in stimulating telomerase processivity[75,96,97]. Mutational analysis of two families affected with HH syndrome or aplastic anemia revealed an in-frame single–amino acid deletion (p.Lys170del) in an N-terminal oligonucleotide- and oligosaccharide-binding domain called the TEL patch[98,99]. Although a TPP1 Lys170-deletion mutant (K170Δ) loads correctly onto telomeres, it is unable to recruit and enhance the processivity of telomerase[98,99]. Patients with K170Δ mutation have reduced telomere lengths, thus supporting a vital role of the TEL patch in telomerase function and telomere-length homeostasis. Because the TEL patch is indispensable for telomerase activity, which is fundamental for telomerase-positive neoplasias, alterations in the TEL patch would be predicted to hamper tumor development. The spectrum of cancer susceptibility in DC and HH syndrome has not been fully elucidated. However, tumors arising in people with DC and HH syndrome with TEL-patch alterations might involve cancers with no detectable telomerase activity that maintain their telomeres by an alternative mechanism. Intriguingly, a missense variant (p.Pro491Thr) in the C-terminal TIN2-interacting domain of TPP1 (ref. 98) has been reported in an individual with HH syndrome. Although this mutation does not affect telomerase

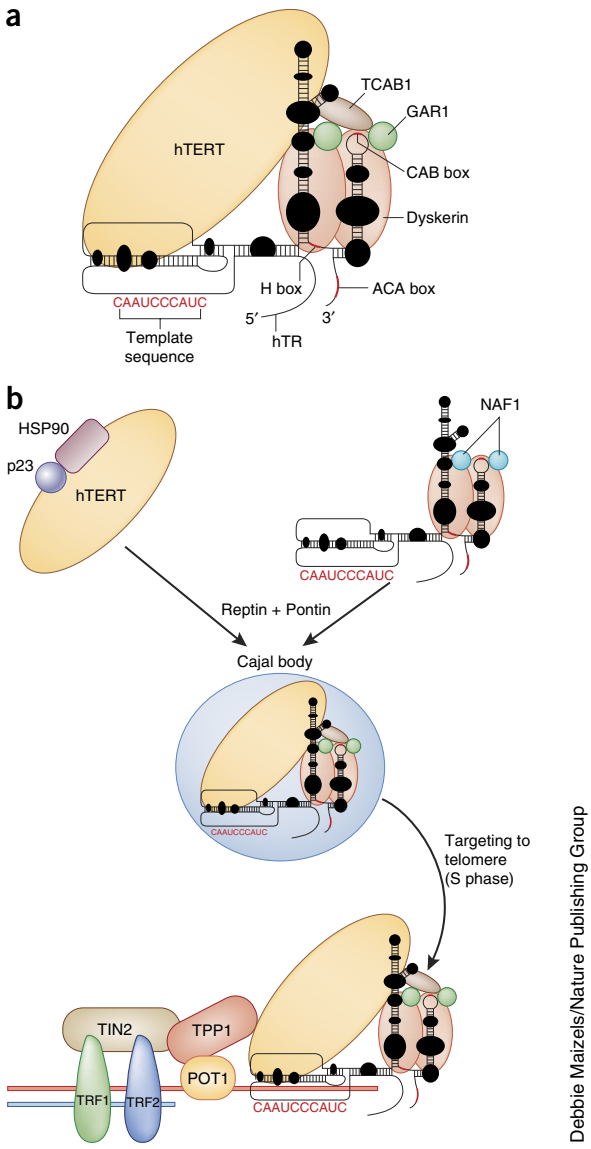

Figure 4 Schematic view of telomerase trafficking. (**a**) Red RNA regions represent binding sites for associated proteins. The CAB box is responsible for TCAB1 binding; the H box and ACA box are responsible for binding of the dyskerin complex (dyskerin, GAR1, NOP10 and NHP2). (**b**) TERT is synthesized in the cytoplasm and is bound by p23 and HSP90 chaperones. hTR is bound by dyskerin, NOP10, NHP2 and NAF1, and NAF1 is subsequently replaced by GAR1. The ATPases Reptin and Pontin help to assemble the telomerase holoenzyme. TCAB1 binding to the CAB box facilitates localization of telomerase complex to Cajal bodies. In S-phase, telomerase is targeted from Cajal bodies to telomeres by binding of hTERT to the TPP1 shelterin subunit.

recruitment, the TIN2-TPP1 interface is essential for POT1 function and the protection of telomeres from the accumulation of single-stranded TTAGGG repeats as well as from activation of the ATR-dependent DNA damage signaling[100].

Shelterin subunit TIN2 is mutated in telomere diseases

Mutations in the *TINF2* gene, which encodes the shelterin component TIN2, have also been implicated in DC. *TINF2* alterations are associated with the heterozygous AD form of DC in approximately 15% of all known cases and are also associated with HH syndrome, Revesz syndrome and aplastic anemia[32,33,101,102]. Although some cases with an autosomal pattern of *TINF2* inheritance have been described, these mutations usually arise *de novo* and result in extremely short telomeres with very severe disease presentation within the first generation[32,33]. Typically, children with *TINF2* mutations are more likely to present with severe bone-marrow failure before developing any symptoms of DC. Interestingly, all 28 unique mutations (Telomerase Database, updated 2015; http://telomerase.asu.edu/) identified to date in the *TINF2* gene localize to a short segment of exon 6a that encodes amino acids 214–298 of the transcribed TIN2 protein. Structural studies have suggested that a peptide of TIN2, $TIN2_{256–276}$ (ref. 103), binds to the homodimerization domain of TRF1, and deletion of the TRF1-binding site of TIN2 leads to reduced TRF1 levels in the shelterin complex[104,105]. Intriguingly, the disease-causing truncation mutant TIN2 p.Gln269X inefficiently binds to TRF1, whereas the TIN2 p.Arg282His mutation has no effect on the TIN2-TRF1 interaction[32,102]. Because patients carrying a TIN2 p.Arg282His mutation display a DC phenotype with extremely short telomeres, it is somewhat unlikely that disruption of binding of TIN2 to TRF1 is the sole driver behind the disease phenotype. Further studies will be required to assess whether mutations in $TIN2_{256–276}$ such as p.Gln271X and p.Gln269X may determine the severity of the disease. These analyses would be valuable, given that chronic TRF1 dysfunction in mice leads to severe telomere shortening over time, increased DNA damage and bone-marrow failure[106], similarly to the phenotype observed in people with TIN2 mutations.

Another intriguing observation is that Pro283 in TIN2, encompassing the PTVML motif, is a binding site for heterochromatin protein 1 gamma (HP1γ), an interacting partner of TIN2 required for establishment and maintenance of proper cohesion of sister telomeres[107]. Disruption of the TIN2–HP1γ complex impairs telomere cohesion in S phase and results in compromised telomere-length maintenance by telomerase. Similarly, human fibroblasts containing mutations resulting in truncated proteins (p.Gln269X and p.Lys280X) or single–amino acid substitutions (p.Pro283His and p.Leu287Pro) exhibit defective TIN2-HP1γ interaction, abnormal telomere cohesion and short telomeres[107,108]. These data strongly support the view that impaired sister-telomere cohesion may affect telomerase function and account for short telomeres in people with *TINF2* mutations. Of note, the PTVMLFP motif in TIN2 is also a potential binding site for the Siah2 E3 ubiquitin ligase, which controls the level of TIN2 in cells[109]. Therefore, ubiquitination of TIN2 may be functionally associated with an allosteric transition of shelterin components leading to dynamic changes in telomere organization. One hypothetical example could involve Siah2-dependent degradation of TIN2 during S phase.

CTC1 and PARN mutations in people with DC or HH syndrome

In addition to shelterin, the CST complex, comprising telomere maintenance component 1 (CTC1), suppressor of cdc13 1 (STN1) and telomeric pathway with STN1 (TEN1), associates with telomeres and plays an important part in telomere homeostasis[110]. The mammalian CST complex functions as a telomerase regulator by coordinating telomerase elongation and fill-in synthesis to complete telomere replication (reviewed in ref. 111). Recently, several mutations in CTC1 have been found to cause DC and other related bone marrow–failure syndromes[112–114]. Systematic analysis of all DC-causing CTC1 mutations has revealed a broad spectrum of molecular defects that negatively affect CST-complex formation (p.Leu1142His and p.1196_1202del), abolish interaction with DNA polymerase α–primase (p.Ala227Val, p.Val259Met, p.Val665Gly and p.Leu1196_Arg1202del) or reduce binding of telomeric single-stranded DNA (p.Val665Gly, p.Cys985del

and p.Arg987Trp)[115]. In addition, single–amino acid substitutions such as p.Ala227Val, p.259M, p.Arg987Trp, p.Leu1142His and the C-terminal–deletion mutant (p.1196_1202del) resulted in mislocalized CTC1 protein in the cytoplasm and impaired telomere association[115].

More recently, mutations in the *PARN* gene, encoding an essential exoribonuclease that controls gene expression through deadenylation, have been identified[116,117]. Mutational analysis by whole-exome sequencing performed on three unrelated families with autosomal recessive DC revealed new homozygous or compound heterozygous missense mutations in the *PARN* gene. Siblings affected with DC exhibited the p.Ala383Val substitution at a conserved residue affecting an α-helix in nuclease domain-2 (ND2) of the PARN enzyme[116]. Similarly, another patient with autosomal recessive DC had a homozygous G-to-T transversion (c.918+1G>T) in intron 13 of the *PARN* gene. This alteration gave rise to two aberrant transcripts causing either an in-frame deletion of residues 281–306 (p.281_306del) in nuclease domain-2 (ND2) or a frameshift and premature termination (p.Gly281ThrfsTer4)[116,118]. The individual with compound heterozygous mutations had a 1-bp duplication (c.863dupA) causing a frameshift and premature termination (p.Asn288LysfsTer23). The same patient had also a 4-bp deletion (c.659+4_659+7delAGTA) in intron 9, resulting in abnormal splicing, the skipping of exon 9 and an in-frame deletion (p.208_220del) in the R3H domain of the PARN exoribonuclease[116,119]. Cells derived from people with DC showed reduced PARN-specific deadenylation activity, an abnormal DNA-damage response, and reduced cell survival after UV treatment. Importantly, PARN deficiency was also associated with decreased expression of *TERC*, *DKC1*, *RTEL1* and *TERF1* transcripts and very short telomeres[116].

RTEL1 and Hoyeraal-Hreidarsson syndrome

RTEL1 is a helicase that was originally identified by mapping of loci that control telomere-length differences between *Mus musculus* and *Mus spretus*[48]. RTEL1 has a critical role in genome stability, because knockout mice are embryonic lethal, and cells derived from these mice exhibit genome instability and telomere dysfunction. Molecular studies have suggested that RTEL1 functions in telomere maintenance by resolving distinct DNA secondary structures, including telomeric G4-DNA structures, which are formed by the telomere repeats, and t loops, which arise when the end of the telomere invades into internal telomeric repeats. The clinical importance of RTEL1's role at telomeres has recently been highlighted with the discovery that it is frequently mutated in HH syndrome[46,47,120–122]. Of the 18 distinct RTEL1 mutations identified in HH syndrome, most are autosomal recessive, and several are autosomal dominant[123]. A recent study has found that 2 of the 18 HH syndrome mutations reside within a C4C4 motif, which is necessary for recruitment of RTEL1 to telomeres via interaction with TRF2. Strikingly, the RTEL1 p.Arg1264His mutation, which resides within the C4C4 domain and specifically disrupts the TRF2-RTEL1 interaction, has a carrier frequency of 1% within the Ashkenazi orthodox Jewish population and 0.45% in the general Ashkenazi Jewish population[124].

Currently, it is unclear how the other HH syndrome mutations in RTEL1 affect the function of RTEL1, although mutations located within the helicase motifs are predicted to affect enzymatic activity. Mutations located within the C terminus (excluding the C4C4) warrant further investigation, because they probably affect new aspects of RTEL1 regulation[125]. Very little is currently known about RTEL1 regulation or how it is dynamically recruited to replication forks and telomeres. In addition, it is not known whether RTEL1 expression or recruitment is regulated by post-translational modifications. Finally, some of the ill-defined HHS mutations reside in uncharacterized regions of the protein, thus making it unclear how they affect RTEL1 function. The study of these uncharacterized mutations is likely to shed light on these unresolved issues.

Concluding remarks

Human TBDs show a broad and complex spectrum of clinical symptoms attributable to critically short telomeres. To date, causative mutations have been described in genes that encode factors involved in telomere maintenance and repair. Currently, however, the only possible interventions for these diseases include bone-marrow, liver or lung transplants, none of which address the underlying cause of the disease. Studies in mouse models of DC have resulted in the generation of relevant disease models that recapitulate the phenotypes of telomere dysfunction observed in DC and HH syndrome. Despite these advances, many features of telomere syndromes remain undefined at the molecular level, and the causative mutations remain unknown. It is hoped that identification of these undetermined mutations will advance understanding of the molecular mechanisms underlying human telomere dysfunction and provide new opportunities for therapeutic intervention.

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