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Short Telomeres: From Dyskeratosis Congenita to Sporadic Aplastic Anemia and Malignancy

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Abstract

Telomeres are DNA-protein structures that form a protective cap on chromosome ends. As such, they prevent the natural ends of linear chromosomes from being subjected to DNA repair activities that would result in telomere fusion, degradation or recombination. Both the DNA and protein components of the telomere are required for this essential function, as insufficient telomeric DNA length, loss of the terminal telomeric DNA structure, or deficiency of key telomere-associated factors may elicit a DNA damage response and result in cellular senescence or apoptosis. In the setting of failed checkpoint mechanisms, such DNA-protein defects can also lead to genomic instability through telomere fusions or recombination. Thus, as shown in both model systems and in humans, defects in telomere biology are implicated in cellular and organismal aging as well as in tumorigenesis. Bone marrow failure and malignancy are two life threatening disease manifestations in the inherited telomere biology disorder, dyskeratosis congenita. Here, we provide an overview of basic telomere structure and maintenance. We outline the telomere biology defects observed in dyskeratosis congenita, focusing on recent discoveries in this field. Lastly, we review the evidence of how telomere biology may impact sporadic aplastic anemia and the risk for various cancers.

Factors that contribute to telomere structure, maintenance and function

Mammalian telomeres are comprised of tandem repeats of a T₂AG₃/C₃TA₂ nucleotide sequence coated with a telomere-dedicated complex known as shelterin.^{1, 2} Along with shelterin are a number of other factors that also contribute significantly to other aspects of chromosome biology.³ This nucleoprotein assembly can adopt a higher order structure known as the t-loop, in which the end of the chromosome terminates in a lasso-like structure (Fig. 1).⁴ The length of the duplex telomeric repeats is in the range of several thousands of base pairs at the time of birth (e.g., 10 kilobase pairs in leukocytes),⁵ although the length varies considerably among normal individuals and cell type.⁶ Additionally, each telomere terminates with a 100 – 200 nucleotide 3' single strand overhang of the G-rich strand (known as the G-overhang), an essential part of the telomere structure.⁷

The length of telomeric DNA is a dynamic sum of shortening and lengthening activities. In contrast to the rest of the genome, semi-conservative DNA replication is incapable of

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faithfully replicating the entire telomere and, consequently, in the absence of a mechanism to restore telomere length, approximately 50 base pairs of telomeric repeats are lost per generation.^{8,9} Telomerase, a specialized reverse transcriptase, can counteract this loss, as well as loss incurred by nucleolytic processing, by catalyzing the addition of telomeric hexanucleotide repeats onto the 3' end of the chromosome.¹⁰ Telomerase minimally consists of the catalytic protein subunit TERT and the integral RNA subunit TERC (also known as hTR), which contains the template for *de novo* telomeric repeat synthesis. TERT and TERC together are sufficient to reconstitute telomerase activity *in vitro*,¹¹ however, *in vivo*, additional factors are required for telomerase assembly, trafficking, and recruitment to telomeres (Fig. 1). One of these factors is dyskerin,¹² which, through its interactions with an H/ACA motif on TERC, facilitates telomerase assembly and TERC stability. Additional factors include NHP2 and NOP10,^{13,14} which function with dyskerin in telomerase biogenesis, and TCAB1,¹⁵ which binds the CAB box sequence on TERC and recruits telomerase to the Cajal body, a step necessary prior to its recruitment to telomeres. Recent studies have also defined a direct role for the shelterin component TPP1 in promoting the recruitment of telomerase from Cajal bodies to telomeres as well as in the catalysis of multiple telomeric repeats onto the DNA end after a single binding event, an activity referred to as repeat addition processivity.¹⁶⁻¹⁸ Lastly, accessory factors that contribute to telomeric DNA structure and length maintenance include such proteins as Apollo, a 5' to 3' exonuclease, and regulator of telomere elongation helicase 1 (RTEL1), an ATP-dependent DNA helicase. Apollo's role is to process the blunt telomeric ends created by semi-conservative leading strand synthesis and restore the terminal G-overhang.^{19,20} RTEL1 contributes at least two critical functions to telomere replication and stability. First, it counteracts telomeric replication fork stalling by unwinding telomeric G quadruplex structures, thus preventing telomere fragility.^{21,22} In addition, Rtel1 prevents massive telomere loss by disassembling t-loops at certain stages of the cell cycle.²²

The major determinant of telomere length regulation is the inherent degree of cellular *TERT* expression, with only a few human cell types expressing levels of telomerase that are sufficient to maintain telomere length or attenuate the rate of telomere shortening.^{23,24} These tissues include germ cells, stem cells, and cells undergoing rapid cellular expansion, such as certain lymphocyte populations. Even when expressed, telomerase levels are just sufficient, such that a reduction in activity levels due to heterozygous loss-of-function mutation of *TERT* or *TERC* can result in failed maintenance of telomere length and telomere dysfunction.²⁵ Similarly, repression of *TERT* expression, which occurs in the majority of human somatic cells,^{23,26,27} results in progressive telomere shortening. Upon reaching a critically short length, a DNA damage checkpoint response is triggered and cellular senescence induced.^{28,29} In contrast, forced *TERT* expression can restore telomerase activity, extend telomeres and convey cellular immortality in a variety of human somatic cells.^{30,31} Correspondingly, *TERT* expression and, consequently, telomerase activity, are upregulated in approximately 90% of primary tumors, thereby removing the telomere barrier to unlimited proliferative potential.²³

Normal telomere function is not conferred by telomere length alone, but rather also requires several telomere-associated factors. Most prominent among these are members of the highly abundant shelterin complex (Fig. 1).² Shelterin is comprised of six subunits: telomeric repeat factors 1 and 2 (TRF1 and TRF2, respectively), which bind to the double-stranded tract of telomeric DNA; repressor/activator protein 1 (RAP1), which is recruited to the telomere via TRF2; TRF1-interacting nuclear protein 2 (TIN2), which binds not only TRF1 and TRF2, but also the TIN2-interacting protein 1 (TPP1) which in turn associates with protection of telomeres 1 (POT1). As POT1 binds the G-overhang and TIN2 can simultaneously bind TRF1, TRF2 and TPP1, TIN2 is thought to bridge the duplex telomere with the G-overhang. The individual shelterin components contribute different functions.³²

Collectively, they (1) inhibit chromosome ends from eliciting a robust DNA damage response, (2) inhibit telomeres from being subjected to unregulated end processing, nonhomologous end joining or homologous recombination, (3) regulate the access and activity of telomerase both positively and negatively, (4) promote replication of duplex telomeric DNA, and (5) promote proper sister telomere cohesion.³

In addition to shelterin, a trimeric complex consisting of conserved telomere maintenance component 1 (CTC1), suppressor of *cdc13* 1 (STN1) and telomeric pathway with STN1 (TEN1), dynamically associates with telomeres and contributes to telomere homeostasis (Fig. 1).^{24, 25} The role of the CTC1/STN1/TEN1 (CST) complex at the telomere is complex, functioning in the replication and processing of telomeres prior to and after telomerase action. The CST complex inhibits telomerase activity *in vitro* and knockdown of CST subunits results in telomerase-dependent telomere elongation *in vivo*.³³ These data have led to the proposal that CST limits the extent to which a telomere-bound telomerase adds telomeric repeats. In addition, CTC1 promotes the re-start of telomere lagging strand synthesis at stalled replication forks through interaction with pol δ primase.^{34, 35} Consistent with these roles in telomere replication, a meta-analysis of 9190 individuals from 6 independent genome wide association studies demonstrated highly significant association of the minor allele of the *CTC1* single nucleotide polymorphism rs3027234 with both short leukocyte telomere length and reduced expression of the *CTC1* gene.³⁶ In the absence of these functions, *CTC1* conditionally null mice exhibit a rapid increase in 3' single stranded telomeric DNA and subsequently catastrophic telomere sequence loss.³⁷ This loss is accompanied by an increase of telomeric circles (t-circles) that are thought to arise when homologous recombination of telomeres is not sufficiently repressed, and are most pronounced in rapidly dividing tissues, suggesting a crucial role for CTC1 in telomere replication. Although initially viable, CTC1 null mice succumb within the first few months of life due to hematopoietic stem cell depletion and bone marrow failure. Of note, several studies point to the impact of CST upon chromosome biology beyond telomeres.^{38, 39} Therefore, ascribing cellular or organismal phenotypes to defects at the telomere versus other chromosomal sites will continue to prove challenging.

Methods for measurement of telomere length

The study of telomeres and their relationship to human disease almost always includes determination of telomere length. Different methods have been used to measure telomere length in human samples (see Aubert et. al⁴⁰ for a comprehensive assessment of the various techniques). Southern blotting is considered by many to be the gold standard.⁴⁰ In this technique, telomeric terminal restriction fragments (TRFs) are separated from heavily restriction-digested genomic DNA by gel electrophoresis and detected by hybridization with a telomeric probe, resulting in a report of mean TRF lengths. More commonly, measurements based on telomere flow fluorescence in situ hybridization (FISH) or telomere quantitative polymerase chain reaction (Q-PCR) are reported. In telomere flow FISH, peripheral blood leukocytes are subjected to flow cytometry following FISH using peptide nucleic acid probes specific for the telomeric repeat sequence.⁴¹ The fluorescence in different leukocyte populations is measured and the absolute telomere length is calculated based on the telomere length of a human reference sample determined by Southern blotting. The Q-PCR or its improved version monochrome multiplex Q-PCR (MMQ-PCR) determines the amount of telomeric DNA relative to a single-copy genomic DNA locus, referred to as the T/S ratio.⁴² This value most often represents an average of telomere lengths in all hematopoietic cells, though specific populations may be isolated for analysis through flow-sorting. Although a more approximate measure, the benefits of the Q-PCR assay are in the small amount of DNA required and the high-throughput design, together

making this a useful technique in larger case-control studies where an absolute measurement is less relevant.

Dyskeratosis congenita, the prototypical disorder of telomere biology

The importance of telomere length maintenance is underscored by the markedly increased risk of bone marrow failure and other diseases observed in individuals harboring shortened telomeres. The impact of short telomeres on human health was first appreciated in the inherited bone marrow failure and cancer predisposition syndrome, dyskeratosis congenita (DC).¹² Clinical manifestations of DC include a variably penetrant but classical disease-defining mucocutaneous triad including oral leukoplakia, abnormal skin pigmentation, and nail dystrophy. Bone marrow failure is a common manifestation of DC, with a greater than 90% penetrance by age 30 years reported among individuals enrolled in DC registries.^{43, 44} Although, it is now appreciated that DC is at the severe end of the spectrum of telomere biology disorders,⁴⁵ the high penetrance of bone marrow failure among those with classical DC underscores the importance of telomere length maintenance to sustained hematopoiesis. Individuals with DC are also at high risk of developing various cancers (discussed further below), pulmonary fibrosis and liver disease, all of which can lead to significant morbidity and decreased life expectancy. Two related disorders thought to be severe variants of DC have been described, Revesz syndrome and Hoyeraal Hreidarsson syndrome.^{46, 47} Cerebral calcifications are observed in both syndromes. Patients with Revesz syndrome additionally manifest bilateral exudative retinopathy, referred to as Coats disease,^{48, 49} whereas the constellation of intrauterine growth retardation, short stature, developmental delay/intellectual disability, progressive immunodeficiency and cerebellar hypoplasia are diagnostic of Hoyeraal Hreidarsson syndrome.^{50–52}

Although DC is a genetically heterogeneous disorder with mutations in nine genes reported to date, a nearly universally molecular feature is excessively short telomeres.⁵³ Each of the DC-associated genes contributes to some aspect of telomere maintenance and function. Among the nine are *TERT*, *TERC*, *DKC1* (encoding dyskerin), *WRAP53* (encoding TCAB1), *NOP10*, and *NHP2* (Fig. 1)^{46, 54–59} Studies with several disease-associated *TERT*, *TERC*, *DKC1*, and *WRAP53* mutations have demonstrated that these are hypomorphic mutations resulting in decreased telomerase activity levels or loss of telomerase recruitment to telomeres.⁶⁰ *NOP10* and *NHP2* mutations, together reported in just a few patients to date, are associated with decreased *TERC* levels, suggesting they lead to telomere shortening through decreased levels of telomerase.^{57, 58}

In contrast to the aforementioned genes, the remaining known DC-associated genes, *TINF2*, *CTC1* and *RTEL1*, do not appear to impact telomere length through direct effects on telomerase.^{46, 61, 62} *TINF2* encodes the shelterin component TIN2. The DC-associated mutations in this gene are autosomal dominant, and comprise a substantial proportion of DC cases, between 10–30% in two large cohorts.^{46, 63} In contrast to individuals carrying heterozygous mutations in *TERT* or *TERC*, *TINF2* mutations most frequently present in early childhood and are often *de novo*, as has been demonstrated in cases where the mutation status of the parents of the affected individual is known.⁶³ Children with deleterious *TINF2* mutation often present with bone marrow failure prior to the development of mucocutaneous features of DC. Thus, the absence of a family history and/or the mucocutaneous features does not preclude the presence of this mutation. To date, 18 unique *TINF2* mutations have been reported in patients with DC, including those with Revesz and Hoyeraal-Hreidarsson syndromes, or isolated aplastic anemia.⁶⁴ Notably, all mutations map to a narrow region encoded in exon 6, which does not encompass known binding sites for either TIN2's shelterin binding partners or SA1, the cohesion subunit through which TIN2 exerts its role in sister telomere cohesion. Instead, this region has been implicated in the

binding of TIN2 to HP1.⁶⁵ Knockdown of TIN2 or HP1 results in a loss of sister telomere cohesion in S phase and cell lines derived from patients with *TINF2* mutations exhibit defective telomere cohesion. Moreover, loss of TIN2's interaction with HP1 impairs telomerase-mediated telomere elongation. These observations have led to a model whereby defective sister telomere cohesion impairs telomerase mediated elongation, resulting in the extreme telomere shortening observed in *TINF2* mutation carriers.^{65, 66} While the existing data are consistent with a loss-of-function mechanism, the failure to identify patients with nonsense or frameshift mutations within the N terminal region upstream of the HP1 binding site of TIN2 suggests that the DC-cluster mutations exert a dominant negative effect. *TIN2*^{-/-} mice are embryonic lethal in both the presence and absence of telomerase, indicating that TIN2 has an essential function that is independent of telomerase-mediated telomere elongation.⁶⁷ Thus, further study is warranted as to the molecular mechanism(s) governing telomere shortening by the DC-cluster mutations.

CTCI was recently implicated in DC. Preceding this discovery, compound missense and truncating mutations in *CTCI* were identified in patients with a rare, autosomal recessive disorder Coats plus, a form of cerebroretinal microangiopathy with calcifications and cysts that includes extra-neurologic manifestations, including osteopenia and gastrointestinal bleeding.^{68, 69} Although the mucocutaneous features and bone marrow failure that are characteristic of DC are less typical of the Coats plus disorder, phenotypic overlap between Coats plus and DC and its more severe variants, including cerebral calcifications and the retinal disease of Revesz syndrome, has been appreciated.⁷⁰ Firmly connecting these disorders was the subsequent identification of *CTCI* mutations in several patients with classical DC.^{61, 71} Notably, some of these patients had no reported retinopathy or brain abnormalities, demonstrating that these are not universal features of patients with germline *CTCI* mutations. Moreover, in striking contrast to patients with DC due to mutations in other genes, patients with biallelic mutations *CTCI* do not consistently have short telomeres.^{61, 71} Discrepant telomere length results have also been observed in patients with Coats plus.^{68, 69} Whether these differences reflect differences in technique for measuring telomere length or differences in biology remains to be determined, and perhaps reflect the differential impact of the *CTCI* mutations on its telomeric versus non-telomeric functions.

Most recently, mutations in *RTEL1* were identified in a small cohort of patients with features of DC, bringing the number of genes clearly implicated in this disorder to nine.⁶² Two of the families included children with Hoyeraal Hreidarsson syndrome. A third proband had classical DC. Both autosomal recessive and autosomal dominant with genetic anticipation inheritance patterns were observed. Whether these cases resulted from loss of RTEL1 function leading to increased telomere fragility, t-loop excision, or both remains to be determined. At the very least, they likely point to yet another molecular mechanism by which telomere mediated disease may occur.

In patients meeting clinical criteria for DC, genetic testing identifies a mutation in one of the known genes in only approximately 50–60% of cases. Solving these genetically uncharacterized cases is of interest research interest. While it is anticipated that mutations in additional genes previously found to be crucial for telomere maintenance or function through basic science research will be identified, the mutated genes may be unexpected or novel. For example, recent work has pointed to TRF1 as a potential etiological factor in telomere mediated bone marrow failure.⁷² However, directed sequencing of *TRF1* and genes encoding the other remaining shelterin components, TRF2, RAP1, TPP1, and POT1, in a cohort of 16 genetically uncharacterized DC or DC-like probands did not reveal any definitive disease-associated variants.^{73, 74} Similarly, although the identification of *CTCI* in several patients with DC and Coats plus has heightened the possibility that *STN1* and *TEN1* mutations might be found, direct sequencing of these genes in cohorts of uncharacterized

DC and Coats plus patients has failed to yield any mutations.^{69, 71} As whole exome sequencing approaches have begun to yield new genes in DC and related disorders,^{62, 68} it is probable that other known telomere-related factors as well as novel factors will be uncovered at a rapid pace.

Finally, in addition to the occurrence of multiple patterns of inheritance (autosomal dominant and recessive, and X linked recessive) and disease anticipation in DC, uncovering the underlying genetics in certain patients may be further complicated by somatic mosaicism. Revertant somatic mosaicism, in which there is spontaneous correction of a pathogenic allele, was recently reported in six individuals from four families with autosomal dominant DC resulting from a small deletion in *TERC*.⁷⁵ Similar to other conditions in which revertant somatic mosaicism has been reported, an outgrowth of the revertant cells was observed. Thus, in cases in which there is a high index of suspicion, sequencing of non-hematopoietic DNA should be highly considered.

Short telomeres and aplastic anemia

Although very short telomeres have been described in association with aplastic anemia within the context of DC, the role of short telomeres in the natural history of acquired aplastic anemia is less well defined. Similarly to what has been observed in DC, these cases of aplastic anemia arise from depletion of functional hematopoietic progenitor and stem cells.⁷⁶⁻⁷⁸ Immunological studies, along with the observation that the majority of patients have at least partial count recovery with intensive immunosuppression, suggest that the hematopoietic progenitor and stem cell depletion is most often immune-mediated.⁷⁹ However, not all patients demonstrate a response to immunosuppression; some lose their response over time, and some experience evolution to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Thus, the role of telomeres in acquired aplastic anemia has been of considerable interest. Although CD34+ hematopoietic stem cells express low levels of telomerase that can be transiently upregulated, telomere length in the hematopoietic stem cell compartment progressively shortens with age and is associated with a decline in proliferative potential *in vitro*.⁸ Therefore, shorter telomere length due to the presence of hypomorphic alleles of telomere biology genes or accelerated telomere shortening secondary to increased hematopoietic stem cell turnover might influence the response to immunosuppression or eventual clonal evolution.

Several studies have begun to address these questions. First, an increased incidence of both known and novel deleterious *TERT* and *TERC* variants (confirmed by telomerase functional analysis) has been noted in two cohorts of largely adult patients with apparently acquired aplastic anemia, none of whom had been diagnosed with an inherited bone marrow failure disorder.^{6, 80} Several of these variants were determined to be functionally significant through *in vitro* studies, although not all mutation carriers had granulocyte or lymphocyte telomere length below the 10th percentile. *TERT* and *TERC* variants identified in patients with apparently acquired aplastic anemia have been considered risk factors for disease development due to the later disease onset observed relative to children with “congenital” aplastic anemia, the absence of other DC clinical features, and the variable penetrance among and within families.^{6, 81, 82} While few reports have noted an inadequate response to immunosuppression in patients with *TERT* or *TERC* mutations, larger cohorts are needed to arrive at definitive conclusions.^{6, 80, 83} The distinction between immune-mediated aplastic anemia and aplastic anemia secondary to rare telomerase variants/mutations is important to establish, as in the case of the former, immunosuppressive therapy is considered the first-line therapy, whereas patients falling into the second category are presumably less likely to respond to immunosuppression.⁸⁴

Correlation of telomere length with severity of disease and treatment outcomes has been explored in cohorts of patients with aplastic anemia. TRF lengths are significantly lower in granulocytes than in the mononuclear cell fraction in patients with apparently acquired aplastic anemia, likely reflecting the shorter lifespan of granulocytes.⁸⁵ It has also been suggested that there is accelerated telomere shortening in both cell populations;⁸⁵ however, this has been inferred by comparing the observed with the expected (mean) TRFs as a function of time from diagnosis as opposed to serial measurements in individual patients. Although initial reports showed a correlation between persistent cytopenias following immunosuppression and short leukocyte telomere length,⁸⁵⁻⁸⁷ a subsequent large, single institution study showed no correlation.⁸⁸ This study was able to demonstrate an association between short telomeres and relapse, clonal evolution, monosomy 7 and complex cytogenetics, as well as mortality.⁸⁸ Furthermore, this shortening was associated with evidence of genomic instability, both preceding and predisposing to malignant transformation.⁸⁹

Telomere shortening has also been observed in Fanconi anemia (FA), one of the inherited bone marrow failure syndromes associated with defects in DNA repair mechanisms. In FA, the mean peripheral blood mononuclear cell (PBMC) TRF length is significantly shorter when compared with controls. Also, the extent of telomere shortening correlates with the degree of bone marrow failure, but not with the presence of clonal abnormalities.^{85, 90} Furthermore, patients with the highest PBMC telomere individual annual shortening rate had the highest risk of developing severe aplastic anemia (relative risk 7.2, 95% confidence interval 4.1-18, $P < 10^{-4}$).⁹¹ Analysis by telomere flow FISH in a small cohort of 11 FA patients yielded somewhat different results, demonstrating telomeres in granulocytes that were less than the 10th percentile for age, but a more normal distribution of telomere lengths in the lymphocyte populations in 10 of the 11 patients studied.⁵³ The relatively shorter telomere length and accelerated shortening in Fanconi anemia cells may be due to the increased recruitment of hematopoietic stem cells as a compensatory mechanism for the increased apoptosis of mature cells, a model supported by a mouse serial bone marrow transplantation model.⁹² However, accelerated telomere shortening was also observed in cultured primary Fanconi anemia fibroblasts in the absence of increased apoptosis or cell division, suggesting the potential contribution of other factors such as increased oxidative damage to telomeric DNA.⁹³ In contrast, the leukocyte relative telomere length in patients with Diamond Blackfan anemia, a congenital pure red cell aplasia, is within the normal distribution, consistent with the isolated impact of this disorder upon cells of the erythroid lineage.⁹⁴

Telomeres and cancer risk

One of the hallmarks of DC is cancer and MDS predisposition. The increased cancer risk is presumed to be related to the excessive telomere shortening characteristic of this patient population, for, as noted above, in the absence of a DNA damage response short telomeres may lead to genomic instability and a potential for malignant transformation.^{95, 96} Nonetheless, this is paradoxical as tumor growth requires some form of telomere maintenance. It is possible that upregulation of *TERT* compensates for reduced telomerase function or telomeres are maintained via a recombination-based, alternative lengthening of telomeres mechanism in tumors that arise in patients with DC.

Analysis of a closely followed National Cancer Institute cohort of 50 DC patients in 31 families revealed an actuarial cancer risk of 40% by age 50 and MDS risk of 3% by age 29.⁹⁷ The odds for any solid tumor were 8-fold over expected and included predominantly squamous cell carcinomas and anorectal and gastrointestinal cancers. The remarkably high 1100-fold odds over expected ratio (O/E) for tongue cancers suggests that screening for DC,

in addition to FA, should be considered when oral cancers, such as cancer of the tongue, present in a non-smoker. Hematologic malignancies are also observed at unexpectedly high frequencies in DC, with a close to 200-fold O/E for AML and a 2500-fold risk for MDS.⁹⁷ As such, DC represents a true cancer predisposition syndrome, and affected individuals should be screened accordingly. It is important to note, however, that cancer risk as determined from DC registries may be overestimated due to a bias toward a more severe case population. Analysis of a group encompassing the full spectrum of telomere biology disorders may one day elucidate the association between cancer risk and specific disease-causing mutations or the severity of telomere shortening.

Within hematopoietic cancer cell populations, associations between telomere length and disease progression, prognosis, and outcomes have been noted (see Jones et al.,⁹⁸ for a comprehensive review of this topic). One example is chronic lymphocytic leukemia (CLL), where shorter telomere length in the malignant cell population was associated with genetic aberrations and evidence of DNA damage at telomeres, specific cytogenetic abnormalities and cell surface markers associated with a poor prognosis, and more rapid disease progression.^{99–101} Given the effects of telomere shortening upon cells in the hematopoietic compartment, and the reported association between DC and both bone marrow failure and AML, the incidence of constitutional *TERT* and *TERC* mutations in cohorts of patients with various hematologic malignancies has also been explored. Variants in the *TERT* gene were shown to be enriched in adults with *de novo* AML when compared with healthy controls.¹⁰² Many of these variants were associated with reduced telomerase activity *in vitro*, suggesting that the cancer may have arisen out of a defect in telomerase's capacity for telomere maintenance in the hematopoietic compartment, resulting in shortened telomeres and coincident genomic instability. Though research in CLL suggests a potential correlation between short telomeres and increased leukemia cell-specific genetic aberrations, in this study no association with cytogenetic complexity in the malignant cell population was noted, aside from a significant association with trisomy 8.¹⁰² One telomerase variant, *TERT* A1062T, although found in the general population as a rare polymorphism, is also significantly enriched in adults with a variety of hematopoietic malignancies including AML, chronic lymphocytic leukemia (CLL), and diffuse large B cell lymphoma.^{102, 103} For many of the variants described in both the aplastic anemia and AML cohorts, introduction of the mutant *TERT* or *TERC* into telomerase-deficient cells demonstrated a reduction in telomerase activity due to haploinsufficiency. However, the significance of the *TERT* A1062T variant remains unclear as its impact upon telomerase activity is negligible,^{104, 105} warranting a more comprehensive evaluation of telomerase function for this variant and others demonstrating normal telomerase activity.

Further supporting a role for telomeres in CLL pathogenesis, however, was the recent identification of recurrent somatic mutations in the gene encoding shelterin subunit Pot1 in 3.5% of cases in a large CLL cohort, placing Pot1 among the most frequently mutated genes in this malignancy.¹⁰⁶ The mutations predominantly mapped to regions of Pot1 required for binding to telomeric DNA and resulted in telomere uncapping and chromosomal aberrations when expressed in cell lines, an affect that corresponded with the higher incidence of telomere-containing chromosomal fusions observed in Pot1 mutant *versus* Pot1 wild type CLL cells. Whether germline mutations in Pot1 may be associated with increased risk of CLL or other malignancies remains to be determined.

Consequent to the variable penetrance of the clinical features of DC, an unknown number of persons with constitutional defects in telomere-related genes may harbor a predisposition toward later manifestations of telomere biology disorders, such as cancer or bone marrow failure. In the 50% of DC cases where no mutation is identified, shortened telomere length may serve as a proxy for a telomere biology defect. In the absence of the spectrum of

clinical criteria suggestive of DC, significantly shortened telomeres, with or without a mutation in a telomere-related gene, have been associated with several related disorders, from cancer to pulmonary fibrosis to hepatic cirrhosis.¹⁰⁷ Numerous case-control studies have examined the risk for cancer associated with telomere length independent of a known associated mutation in a telomere biology gene. Although results from individual studies are somewhat conflicting, when taken together, two meta-analyses including data from 27 publications have demonstrated an overall increased risk for cancer with shortened telomere length.^{108, 109} In addition, the risk for developing *de novo* cancers has been estimated in healthy individuals, within the context of a population-based prospective study. In this cohort of 787 participants, the hazard ratio for developing cancer was 3.11 (95% confidence intervals 1.65–5.84) for the shortest telomere length group, as compared with the longest telomere group, further suggesting that shortened telomeres may be a marker for cancer susceptibility.¹¹⁰ As a result, telomere shortening that naturally occurs with age also has been proposed as a mechanism for the increased cancer risk observed in older populations.¹¹¹ As in disorders of telomere biology characterized by telomere shortening resulting from mutations in telomere-related genes, short telomeres in otherwise healthy individuals appear to also confer an increased risk for the development of malignancy. Thus, discoveries born out of research in relatively rare pediatric diseases have contributed to our understanding of the critical importance of telomere length maintenance in certain cell populations, and the roles that telomeres and telomerase play in the pathophysiology of cancer development.

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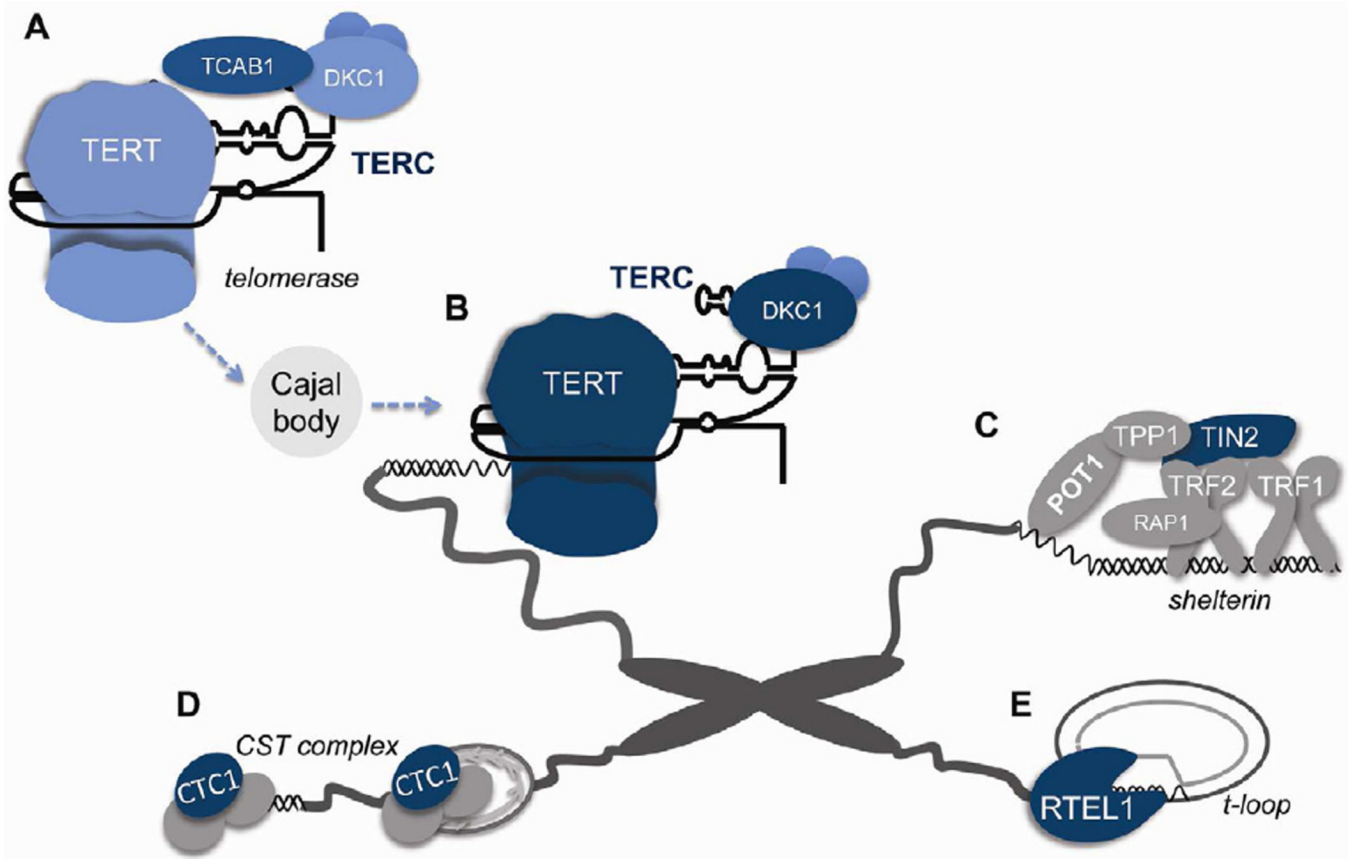


Figure 1.

Factors influencing telomere length maintenance and associated with telomere biology disorders, such as DC. **A.** **TCAB1** associates with telomerase, directing its trafficking through the Cajal body, which is required for its recruitment to telomeres. **B.** **TERT**, **TERC** and **dyskerin (DKC1)** are components of the telomerase holoenzyme. Mutations in these subunits impact telomerase activity and are associated with diseases in the telomere biology disorder spectrum. **C.** Shelterin associates with telomeric DNA, providing protection of the telomeric end and contributing to the regulation of telomerase. Shown here is only shelterin associated with the telomeric end, although it is also bound along the duplex telomeric tract. **TIN2** is the only shelterin subunit that has been found to be mutated in DC or related disorders to date. **D.** The CST complex has been proposed to contribute in telomere maintenance with effects on telomerase recruitment as well as some aspects of DNA replication. Of the CST subunits, only **CTC1** has been implicated in disease thus far. **E.** Mouse studies implicate the **RTEL1** helicase in telomere replication and stability of the telomere t-loops. **RTEL1** is the most recent DC-associated gene.