Regulation of human telomerase in homeostasis and disease

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Abstract | Telomerase is a ribonucleoprotein complex, the catalytic core of which includes the telomerase reverse transcriptase (TERT) and the non-coding human telomerase RNA (hTR), which serves as a template for the addition of telomeric repeats to chromosome ends. Telomerase expression is restricted in humans to certain cell types, and telomerase levels are tightly controlled in normal conditions. Increased levels of telomerase are found in the vast majority of human cancers, and we have recently begun to understand the mechanisms by which cancer cells increase telomerase activity. Conversely, germline mutations in telomerase-relevant genes that decrease telomerase function cause a range of genetic disorders, including dyskeratosis congenita, idiopathic pulmonary fibrosis and bone marrow failure. In this Review, we discuss the transcriptional regulation of human TERT, hTR processing, assembly of the telomerase complex, the cellular localization of telomerase and its recruitment to telomeres, and the regulation of telomerase activity. We also discuss the disease relevance of each of these steps of telomerase biogenesis.

Telomeres comprise repetitive sequences at the ends of chromosomes that maintain the integrity of linear chromosomes. These chromosome ends must be distinguished from other types of linear DNA, such as broken DNA ends, which are destined for repair. Telomeric sequences are specifically bound by a set of proteins that together make up the shelterin complex (FIG. 1a). The shelterin complex serves to solve the end-protection problem, by preventing DNA repair responses generated to free DNA ends. These responses include activation of the DNA damage response and repair by non-homologous end-joining, alternative non-homologous end-joining or homologous recombination[1,2]. To serve these functions, the protein subunits of shelterin recruit many other protein cofactors and, in recent years, mass spectrometry-based approaches have expanded the landscape of shelterin-associated proteins[3]. A second critical problem at chromosome ends is telomere shortening, which occurs because the DNA polymerase that synthesizes DNA in the 5′–3′ direction incompletely replicates the lagging strand, thereby causing gradual shortening of telomere sequences over time. This end-replication problem was solved by the evolution of telomerase, which is a ribonucleoprotein (RNP) complex with a catalytic core composed of telomerase reverse transcriptase (TERT) and the non-coding RNA, telomerase RNA component (TERC; herein referred to as human telomerase RNA (hTR)), which serves as a template for the addition of telomeric repeats to chromosome ends.

In single-cell eukaryotes, telomerase is expressed constitutively to enable long-term cell division and viability. In metazoans, and particularly in humans, the expression of telomerase became restricted to certain cell types or cell states. Human telomerase expression is highest in the germline and in progenitor cell compartments, but is limited in many differentiated cell types. This restricted pattern may have evolved in part to reduce the risk of cancer, as nearly all human tumours show increased telomerase expression. Germline mutations in telomerase-pathway genes that decrease telomerase function cause the stem cell failure diseases dyskeratosis congenita, aplastic anaemia and idiopathic pulmonary fibrosis. These genetic findings indicate that cells must maintain an optimum level of telomerase, which is sufficient to support tissue homeostasis but low enough to decrease the risk of cancer. In addition to tight control of enzyme levels, telomerase is regulated at multiple additional steps.

In this Review, we discuss the latest information regarding how the human telomerase complex is regulated in normal progenitor cells and in cancer cells and how these processes are altered in disease. Transcription of the TERT gene is key in the regulation of telomerase expression and dictates which cell types may express the telomerase enzyme. Processing of the 3′ end of hTR is required to make a mature hTR molecule and is subjected to quantitative regulation that dictates steady-state telomerase levels. Telomerase is a large multisubunit RNP, which includes several protein components with distinct roles in telomerase function. Assembly of this complex requires the activity of many additional proteins and movement of the developing complex through

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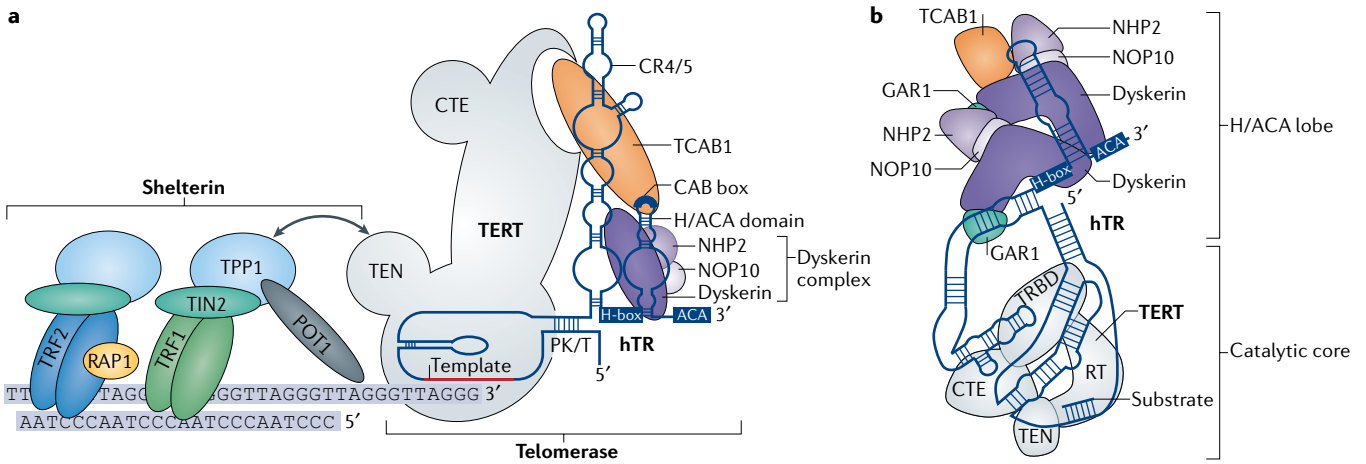

Fig. 1 | **Telomerase interacts with shelterin at telomeres. a** | Telomerase is a ribonucleoprotein complex comprising the scaffolding non-coding human telomerase RNA (hTR), the enzyme telomerase reverse transcriptase (TERT) and associated cofactors. TERT binds to hTR through its telomerase RNA binding domain (not shown) at the pseudoknot/template (PK/T) domain and the conserved region 4/5 (CR4/5) domain of hTR. The dyskerin complex comprising dyskerin, NOP10 and NHP2 binds to the H/ACA domain of hTR. The H/ACA domain includes a single-strand hinge (H-box) followed by a stem-loop and the sequence ACA. The H/ACA domain of hTR also contains a conserved four-nucleotide motif called the CAB box, which binds the protein telomerase Cajal body protein 1 (TCAB1). The template region of hTR binds to the telomeric 3′-end strand. The telomere repeats are bound by the protein complex shelterin. Two double-strand DNA binding proteins, telomeric repeat-binding factor 1 (TRF1) and TRF2, bind directly to telomeric DNA. TRF2 interacts with RAP1 (also known as TRF2-interacting telomeric protein 1), and both TRF1 and TRF2 interact with TRF1-interacting nuclear factor 2 (TIN2), which also binds TPP1. TPP1 additionally binds the single-strand DNA binding protein protection of telomeres protein 1 (POT1) and recruits telomerase to telomeres through the N-terminal domain (TEN) of TERT. **b** | Schematic of telomerase showing the relationship of TERT domains with hTR and telomerase cofactors, adapted from the cryo-electron microscopy structure of human telomerase. Telomerase adopts a flexible, RNA-tethered two-lobed structure. The H/ACA domain of hTR bound by two sets of the dyskerin complex (dyskerin, NHP2, NOP10 and GAR1) and by TCAB1 comprises one lobe. The second lobe contains the catalytic core, where hTR and TERT encircle the telomere substrate. The two lobes are connected by the CR4/5 domain of hTR. CTE, C-terminal extension; RT, reverse transcriptase; TRBD, telomerase RNA binding domain. Adapted from REF.[49], Springer Nature Ltd.

different subcompartments of the nucleus. Recruitment of telomerase to telomeres is controlled by specific protein–protein interactions, and the enzyme is further regulated after telomere engagement. During cancer development, TERT is upregulated through diverse mechanisms to support the enhanced cell proliferation associated with tumorigenesis. Impaired maintenance of telomeres underlies a series of tissue phenotypes characterized by cell depletion and end-stage organ dysfunction. Study of these disease states enables the direct investigation of biochemical defects in telomerase function in human tissues.

Telomerase transcription and maturation

Crucial regulation of telomerase activity occurs through the control of TERT transcription, which dictates TERT levels and determines the cell types in which telomerase is expressed. Regulation of hTR is carried out through a post-transcriptional maturation process of its 3′ end, which sets the levels of mature hTR and, in turn, the levels of the mature telomerase complex (FIG. 2).

Regulation of TERT transcription. Transcription from the *TERT* promoter is highly regulated and is the primary means of controlling telomerase levels in diverse cell types and cellular states. TERT is not expressed in human fibroblasts, which accounts for the progressive shortening of telomeres and replicative senescence encountered by fibroblasts after extensive cell division in culture[4–6]. This 'Hayflick limit' (the finite proliferation potential of primary human cells) is overcome by forced overexpression of TERT or through the introduction of transcription-activating, cancer-associated mutations in the *TERT* promoter[4,7]. Similarly, cultured human keratinocytes or mammary epithelial cells also lack TERT and encounter telomere shortening-based roadblocks to proliferation[8,9]. In contrast to the silenced expression of TERT in these cultured human cell types, TERT is strongly upregulated in human T cells during mitogenic stimulation[10]. This upregulation is presumably important for long-term clonal expansion of lymphocytes during immune responses. Consistent with this notion, telomerase-knockout mouse lymphocytes with short telomeres show impaired proliferative responses when exposed to mitogens[11]. In contrast to the restricted expression of TERT in certain adult human cells, TERT is expressed in many fetal tissues, but its expression becomes limited in early postnatal life[12]. TERT is expressed in human embryonic stem cells, human neural stem cells and human bone marrow progenitors[13–15]. Thus, TERT is expressed in many human stem cell populations, but not in certain cultured cell types.

Analysis of telomerase expression patterns in mouse cells suggested that telomerase is distributed more widely than in human cells. This conclusion is most commonly derived from the observation that telomerase and TERT

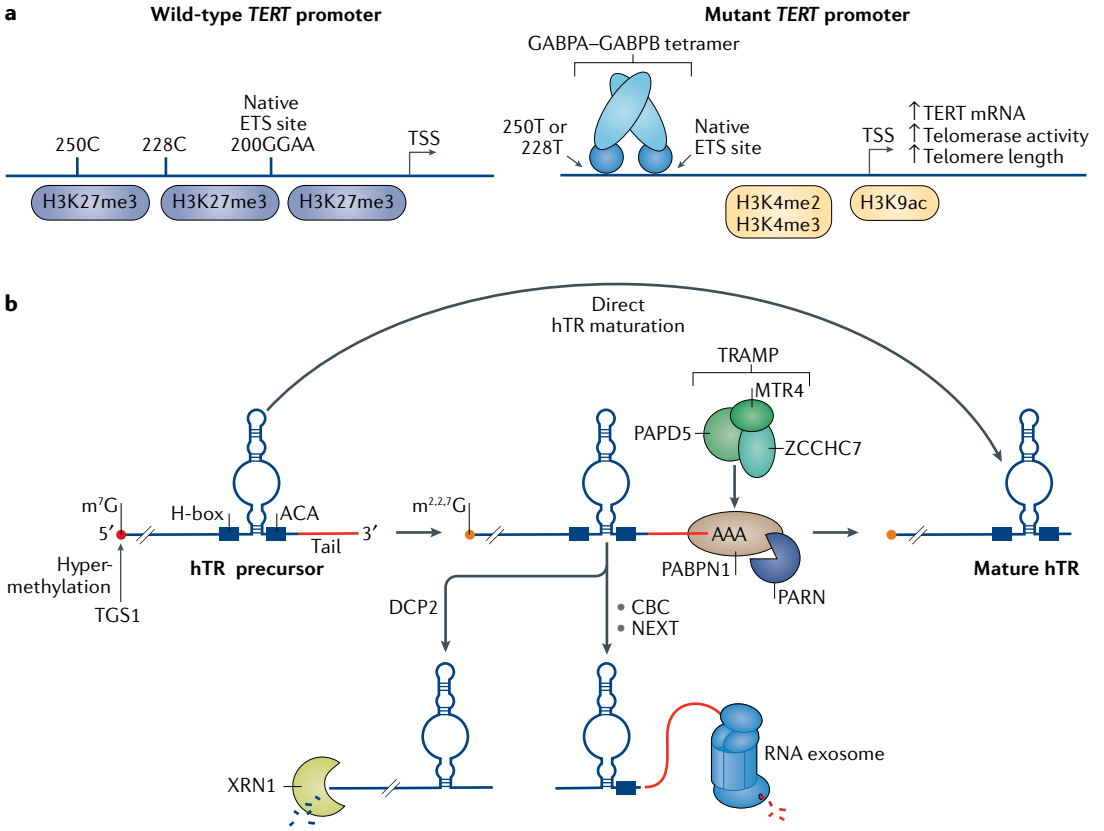

Fig. 2 | **Regulation of the TERT–hTR catalytic core of telomerase. a** | Regulation of telomerase reverse transcriptase (*TERT*) transcription and the role of mutations in the *TERT* promoter. The wild-type promoter of the *TERT* gene is often silenced by the gene-repressive trimethylation of histone H3 Lys27 (H3K27me3) modification. The wild-type promoter contains native binding sites (with the motif GGAA) for the ETS family of transcription factors. In diverse cancer types, C→T transitions occur 124 bp and 146 bp upstream of the translation start codon, which are referred to as C228T and C250T based on their genomic coordinates. These mutations are mutually exclusive and create additional ETS binding sites three to five helical turns (not shown) away from the native ETS sites, which are optimal for recruiting the ETS hetero-tetramer GABPA–GABPB. Mutant *TERT* alleles more commonly associate with RNA polymerase II and are marked by the gene-activating H3K4me2, H3K4me3 and acetylated histone H3 Lys9 (H3K9ac) modifications. Mutant *TERT* alleles cause upregulation of *TERT* transcription and increased telomerase activity. TSS, transcription start site. **b** | Regulation of human telomerase RNA (hTR) biogenesis. hTR is transcribed as a precursor molecule with a 5′ methylguanosine cap (m^7G). The m^7G cap is further methylated by trimethylguanosine synthase (TGS1) into $m^{2,2,7}G$. The majority of hTR transcripts have a short (<10 nucleotides), genomically encoded tail. These precursors are exonucleolytically trimmed to produce mature hTR. hTR precursors can be oligo-adenylated by non-canonical poly(A) RNA polymerase PAPD5, which is a member of the TRAMP complex along with the RNA-exosome helicase MTR4 and the zinc-finger protein ZCCHC7. A subset of these oligo-adenylated hTR intermediates are substrates for degradation by the RNA exosome, to which they are recruited by the nuclear exosomal targeting (NEXT) complex and the nuclear cap-binding complex (CBC). hTR can also undergo decapping by the cytoplasmic decapping enzyme DCP2 and degradation in the 5′–3 direction by 5′–3′ exoribonuclease 1 (XRN1). The remaining oligo-adenylated hTR intermediates are bound by PABPN1 and can be de-adenylated by the disease-associated poly(A) ribonuclease (PARN), which promotes hTR maturation and accumulation. There is also a direct maturation pathway for hTR precursors, which functions in the absence of both PARN and PAPD5; the enzymes involved in this pathway are still unknown. H-box, single-strand hinge in H/ACA domain.

are detectable in mouse fibroblasts[16]. Despite the less restricted expression of TERT in mouse fibroblasts compared with human fibroblasts, the advent of transcription reporters embedded within the *TERT* locus has revealed that the *TERT* promoter is highly regulated in mouse tissues. Generally, telomerase expression is closely associated with germline tissues in organisms from worm to mammals[17–20]. Using a mouse knock-in TERT-transcription reporter, TERT levels were found to be highest in undifferentiated spermatogonia, mitotic stem cells in the adult testis[19,20]. During the differentiation of spermatogonia to committed progenitor cells and subsequent entry into meiosis, telomerase levels diminish in a stepwise fashion. At the spermatid stage, *TERT* promoter activity and TERT expression was undetected[19].

The development of cell lineage tracing from the *TERT* promoter using TERT-CreER mouse strains has enabled the analysis of TERT expression patterns in cell subsets within tissues and the assessment of their functional behaviour as stem cells. Analysis of the small intestine using a transgenic TERT-CreER strain showed that TERT is expressed in mouse intestinal crypts and that these TERT-positive cells serve as intestinal stem cells in renewing the population of differentiated cells of the intestinal villus[21]. A knock-in TERT-CreER strain revealed the presence of a subset population of

TERT-CreER
A mouse strain engineered to induce the expression of the recombinase CreERT2, which enables fluorescence labelling of telomerase-expressing cells in vivo.

Pseudouridylation
Enzymatic alteration of the linkage between the uracil base and the ribose sugar moiety of uridine to create its isomer pseudouridine.

hepatocytes in adult liver with high TERT expression and elevated telomerase activity[22]. These TERT^{high} hepatocytes function as hepatocyte stem cells, repopulating the liver in homeostatic conditions and after injury[22]. Together, these results show that, in renewing tissues in adults, TERT expression is enriched in progenitor cell populations to support their long-term proliferation, but that TERT is downregulated or extinguished in more differentiated progeny cells. Terminally differentiated cells, such as skeletal muscle myocytes, cardiac myocytes, neurons and spermatids, lack telomerase expression even in mice, further supporting a link between proliferative capacity and TERT expression[19,23]. Telomerase enrichment in tissue progenitor cells is crucial for stem cell function, as inactivation of telomerase genes in mice leads to defects in spermatogonial stem cells, gastrointestinal crypt progenitors and haematopoietic stem cells[11,19,24].

Studies of TERT expression patterns in human tissues are limited, but there is evidence that TERT is expressed in stem cell and progenitor cell compartments within tissues, including in the crypts of the gastrointestinal tract, the hair follicle bulb, the hair follicle bulge and the interfollicular epidermis[25–30]. The mechanisms by which TERT is enriched in progenitor cells and silenced during differentiation in both mouse and human remain unknown. A core *TERT*-promoter construct encompassing the transcription start site is active not only in telomerase-expressing human cancer cells but also in telomerase-negative fibroblasts, suggesting that the information controlling cell-type regulation is not encoded in the proximal promoter[31–33] (FIG. 2a). Many studies have dissected the *TERT* promoter and identified *cis*-elements bound by general transcription factors. The elements and factors include two E-boxes with the sequence CACGTG, which are bound by the oncoprotein MYC and its interacting proteins MAX and MAD1, and binding sites for the transcription factors SP1, upstream stimulatory factor 1 (USF1) and USF2, ID2 and ETS2 (REFS[34–37]). Significant advances in understanding the transcriptional regulation of TERT have been recently achieved with the discovery of highly recurrent mutations in the *TERT* promoter in widespread cancer types (FIG. 2a; see below).

Restriction of TERT expression to progenitor cell compartments and downregulation of TERT during differentiation strongly control telomerase activity, but the mechanisms by which this regulation is achieved in either mice or humans remain unknown. Another important open question is why human keratinocytes lack telomerase activity in culture but exhibit telomerase activity in human skin[9,38]. Finally, we lack a fundamental understanding of how TERT is more effectively silenced during differentiation in human tissues compared with differentiation in mouse tissues, or indeed what accounts for the differences in TERT regulation across the many mammalian species that have been studied[39].

hTR synthesis and maturation. The non-coding RNA hTR contains the template for the addition of telomeric repeats[40–42] (FIG. 1a). In vertebrates, hTR belongs to the family of small Cajal body RNAs (scaRNAs) and small nucleolar RNAs (snoRNAs). Both scaRNAs and snoRNAs are encoded in introns and transcribed by RNA polymerase II (Pol II) along with their host genes. By contrast, hTR is transcribed from a dedicated locus with its own promoter[43,44].

Metazoan telomerase RNAs acquired a box H/ACA domain, which includes a hairpin–hinge–hairpin–tail structure with a single-strand hinge (H-box) of the sequence ANANNA and a short tail containing the sequence ACA (FIG. 1a). This RNA domain is shared with a subset of H/ACA-containing snoRNAs and scaRNAs[45,46]. H/ACA-containing snoRNAs and scaRNAs guide the pseudouridylation of ribosomal RNA and of small nuclear RNAs, respectively[47]. A complex comprising dyskerin, NOP10 and NHP2 binds to the H/ACA sequences and executes pseudouridylation on the targets of the RNA guides. The H/ACA sequences are crucial for metazoan telomerase biogenesis, and mutations in the H/ACA domain or in components of the dyskerin complex cause hTR loss and the disease dyskeratosis congenita (see below). Interestingly, although several pseudouridine residues exist in hTR, their relevance to telomerase function remains unclear[48]. Nevertheless, dyskerin is crucial for telomerase function by supporting hTR stability and processing (see below).

The H/ACA domain of hTR contains a three-nucleotide sequence embedded in a terminal stem-loop termed the CAB box, which binds telomerase Cajal body protein 1 (TCAB1; FIG. 1a). TCAB1 is required for both the catalytic activity of telomerase and trafficking of telomerase to Cajal bodies, which are proposed to aid in telomerase RNP assembly and/or in telomerase trafficking to telomeres (see below). Biochemical experiments and a recent cryo-electron microscopy structure revealed that the dyskerin complex and TCAB1 are components of the mature telomerase holoenzyme[49,50].

The mechanism of hTR transcription termination in vertebrates is unknown as, unlike the related snoRNAs, which are embedded in introns, mature hTR is not generated by the spliceosome. Consistent with variations in the length and sequence of telomerase RNAs in evolution, the processing of telomerase RNAs is substantially different between species. However, one commonality is the conservation of post-transcriptional modification of telomerase RNAs from single-cell eukaryotes to mammals. In *Saccharomyces cerevisiae*, transcription termination of the telomerase RNA TLC1 occurs through the Nrd1–Nab3–Sen1 (NNS) pathway[51–53]. Although no homologue of NNS proteins exists in vertebrates, a proposed functional analogue is the Integrator complex, which is responsible for transcription termination of some non-coding RNAs (such as small nuclear RNAs) and mRNAs[54–56]. Following termination, the NNS complex recruits a complex termed the TRAMP complex that oligo-adenylates TLC1 transcripts and thus primes them for degradation by the RNA exosome, which is a multisubunit RNAse[57]. TRAMP homologues in vertebrates include non-canonical poly(A) RNA polymerase PAPD5 (also known as TENT4B), the AIR2 homologue ZCCHC7 and MTR4 (REFS[58,59]) (FIG. 2b). Similar to the yeast complex, vertebrate TRAMP oligo-adenylates precursor telomerase RNA transcripts.

In steady-state conditions, hTR exists as a pool of transcripts, the majority of which end at position 451

and a minority extend past nucleotide 451. These 3′-end extensions are genomically encoded by the hTR locus and, additionally, most of the extended molecules possess short untemplated adenosine tails[60]. It had been unclear whether extended hTR molecules represented precursors for mature hTR or whether they were aberrant transcripts. To address this question, nascent RNA end-sequencing — a technique for enriching RNA precursors and sequencing their 3′ ends — was developed, revealing that hTR is initially transcribed as an extended molecule of approximately 461 nucleotides, and that this precursor is processed to the mature 451-nucleotide RNA[61] (FIG. 2b). Longer hTR precursors, exceeding 1,500 nucleotides in length, have also been detected using PCR with reverse transcription and may represent either very early hTR precursors that are rapidly processed into the short forms or unproductive hTR transcripts that are targeted for destruction by nuclear RNA surveillance[62,63].

hTR precursors are rapidly oligo-adenylated primarily by the poly(A) polymerase PAPD5 (REFS[61,63,64]) (FIG. 2b). The oligo-adenylated hTR intermediates can be deadenylated by poly(A) ribonuclease (PARN), which was initially discovered as a gene mutated in families with short telomeres and pulmonary fibrosis (see below). In the absence of PARN, hTR intermediates initially accumulate and are subsequently degraded, resulting in diminished hTR levels and reduced telomerase activity (see below). PARN has increased activity towards poly(A) substrates and may preferentially remove untemplated A tails from hTR precursors[65,66]. Alternatively, PARN could both remove untemplated A tails and trim the genomic extensions of hTR precursors to produce mature hTR. This idea was suggested on the basis of evidence for PARN in vitro activity towards hTR genomic tail sequences[62]. However, in the absence of both PARN and PAPD5, the extent of oligo-adenylation of hTR precursors is considerably lower, and maturation of hTR transcript proceeds normally[61]. These observations indicate the presence of additional 3′–5′ exonucleases in addition to PARN that are responsible for trimming in vivo the hTR precursor into its mature length directly without the requirement for oligo-adenylation and de-adenylation. One such exonuclease candidate is the Cajal body-localized 3′–5′ RNA exonuclease TOE1, which contributes to the trimming of small nuclear RNAs, snoRNAs and scaRNAs as well as hTR precursor molecules[67–69].

Box 1 | **The telomerase holoenzyme and its accessory factors**

Although combining telomerase reverse transcriptase (TERT) and the telomerase RNA (hTR) in the presence of a cell extract yields telomerase activity, such assembly cannot occur inside a living cell without other components of the telomerase holoenzyme and without many accessory proteins. Distinguishing between the components of the mature holoenzyme and the accessory proteins can be achieved through biochemical and genetic studies. Telomerase holoenzyme components are stoichiometrically associated with TERT and hTR, such that immunodepletion of a holoenzyme component depletes telomerase levels and activity from a human cancer cell extract. By contrast, an accessory protein may be transiently associated with telomerase, and its immunodepletion does not quantitatively deplete telomerase levels or activity. Genetic studies are also crucial for determining whether a telomerase-associated factor is required for telomerase function. Depletion of either a holoenzyme component or an accessory factor may diminish telomerase levels or telomerase activity, or impair telomerase function, depending on the role the protein serves in the pathway.

Oligo-adenylated hTR transcripts that are not processed to mature hTR are degraded by the RNA exosome (FIG. 2b). The poly(A)-binding protein PABPN1 (also known as PABP2) binds oligo-adenylated hTR transcripts, and its loss leads to loss of hTR transcripts[70]. PABPN1-unbound oligo-adenylated hTR intermediates are subject to degradation by the recruitment of the nuclear RNA exosome by the nuclear exosome targeting (NEXT) complex in conjunction with the cap binding complex (CBC)[63,71]. Depletion of exosome components, NEXT or CBC can rescue the phenotype of low hTR levels owing to dyskerin loss or hTR point mutationsin the H/ACA domain, suggesting that the exosome serves also to remove defective or misassembled hTR transcripts[71–73]. Early during transcription, telomerase RNAs acquire the m^7G monomethylguanosine cap, which is hypermethylated to a $m^{2,2,7}G$ trimethylguanosine cap by the enzyme trimethylguanosine synthase (TGS1)[74,75] (FIG. 2b). Hypermethylation by TGS1 suppresses hTR accumulation, and TGS1-negative cell lines exhibit increased telomerase activity and telomere lengthening, indicating that TGS1 negatively regulates telomerase[76]. Cytoplasmic decapping of hTR transcripts by DCP2 (also known as m^7GpppN-mRNA hydrolase) and subsequent degradation by 5′–3′ exoribonuclease 1 (XRN1) also leads to hTR decay[71]. Thus, hTR transcripts undergo a multistep process of maturation that includes cycles of adenylation and de-adenylation, which together control the steady-state levels of hTR. These mechanisms are crucial for the regulation of telomerase levels, and many of these steps are disrupted in disease states.

Assembling the telomerase holoenzyme

Many telomerase-associated proteins have been identified biochemically. These proteins can be divided into two classes: stably associated components of the mature holoenzyme and transiently associated proteins, which typically serve a function in the assembly of the holoenzyme (BOX 1).

The telomerase core. All telomerase enzymes across evolution share a common catalytic core, comprising TERT and telomerase RNA. These core components were originally identified in single-celled ciliated protozoans using biochemical approaches[77,78]. Human TERT and hTR were subsequently cloned based, in part, on sequence similarity[79]. Co-expression of TERT and hTR in vitro is sufficient to reconstitute telomerase activity, but only in the presence of a cell extract that provides additional enzymatic activities that stimulate telomerase assembly[80,81]. The association of TERT and hTR depends on the binding of RNA secondary structures in hTR by TERT. Interactions between TERT and hTR are mediated by specific domains in hTR and TERT. The human TERT protein contains an N-terminal domain (TEN), a telomerase RNA binding domain (TRBD), the catalytic reverse transcriptase (RT) domain and a C-terminal extension (CTE). Human telomerase RNA contains a 5′ pseudoknot/template (PK/T) domain that templates the addition of telomerase repeats, a central domain comprising a three-way junction between stem-loops termed conserved region 4/5 (CR4/5) and the 3′ H/ACA domain (FIG. 1a).

Detailed information on the TERT–hTR interaction is derived from cryo-electron microscopy structures of the telomerase complex, in human and *Tetrahymena thermophila*, and from biochemical analyses[49,82,83] (FIG. 1b). Biochemical experiments showed that TERT binds to the PK/T domain and the CR4/5 domains of hTR[81,84] (FIG. 1a). Although TERT can bind to hTR though the PK/T domain alone, the additional binding with CR4/5 is required for full telomerase activity[84,85]. Structural analysis showed that the TERT domains are arranged in a ring structure characteristic of other reverse transcriptases (FIG. 1b). The assembled catalytic core contains a central canal accommodating the template–substrate RNA–DNA hybrid and providing an exit for the newly synthesized telomere DNA[49,82,83]. Many of the germline mutations that cause dyskeratosis congenita perturb the associations between hTR and hTERT (see below).

Many cofactors are required for the assembly of the telomerase holoenzyme. Although the catalytic core is sufficient for telomerase activity in vitro, the telomerase holoenzyme in human cell extracts comprises a much larger complex of approximately 500 kDa[86,87]. The holoenzyme has been isolated by affinity purification using tagged TERT proteins, antibodies against endogenous TERT proteins or affinity chromatography with a telomere-DNA oligonucleotide substrate[88–90]. Telomerase stability, full activation and recruitment to telomeres require the assembled holoenzyme, which includes two dyskerin complexes that bind to hTR — one at each of the two hairpins in the H/ACA domain. Dyskerin and NOP10 interact with the RNA, and in turn GAR1 (which is not part of the dyskerin complex) and NHP2 interact with dyskerin and NOP10 (REFS[49,91,92]). Dyskerin–NOP10–NHP2 co-localize with the site of H/ACA snoRNA transcription but GAR1 does not, suggesting GAR1 may be a non-core component of telomerase that is assembled at a later stage of maturation. GAR1 is co-localized with the holoenzyme following its recruitment to Cajal bodies[93–96]. TCAB1 is a stably associated component of the holoenzyme and engages telomerase through an interaction with the hTR CAB box. TCAB1 controls the catalytic activity and nuclear trafficking of telomerase (see below).

In addition to these stably associated constituents of the telomerase holoenzyme, several proteins have been identified that associate with telomerase transiently and are required for its biogenesis. NAF1 binds to dyskerin and is required for the stable association of dyskerin with telomerase[96–98]. SHQ1 is another dyskerin-interacting protein that facilitates telomerase assembly[99]. Two AAA^+ ATPases, pontin and reptin, bind to TERT and to dyskerin and promote the assembly of telomerase into a stable complex. Pontin and reptin associate with telomerase in S phase, and their depletion leads to loss of hTR and telomerase activity[88]. TERT binding to hTR is aided by the chaperone heat shock protein 90 (HSP90)[100–102], and telomerase assembly with TCAB1 requires the chaperone TriC, which is dedicated to folding certain classes of protein clients, including WD40 repeat-containing proteins such as TCAB1 (REF.[103]). Thus, although minimal telomerase activity requires only TERT and hTR, telomerase function in vivo requires assembly with stably associated holoenzyme components, such as the dyskerin complex and TCAB1, and with transiently associated proteins, such as pontin, reptin and chaperones like HSP90 and TRiC.

WD40 repeat
A protein domain of 44–60 amino acids, which often mediates protein–protein or protein–RNA interactions.

Telomerase localization

Elongation of telomeres requires telomerase access to telomeric DNA. However, processing of telomerase components and assembly of the complex likely occur at multiple sites within the nucleus, and defects in these processes result in telomerase mislocalization.

Localization to Cajal bodies. Like other scaRNPs, the telomerase complex is localized to Cajal bodies, which are nuclear compartments enriched in factors of splicing and of other RNA-related processes[93,104]. The protein coilin scaffolds Cajal bodies; loss of coilin abrogates Cajal body formation and mislocalizes telomerase[105,106]. Telomerase localization in Cajal bodies is mediated also by TCAB1, which binds to the CAB box of hTR; in the absence of TCAB1–CAB box interaction, telomerase mislocalizes to the nucleolus[50,103,107,108] (FIG. 3).

Single-molecule, live cell imaging showed that telomerase exhibits extended residence time in Cajal bodies[109]. The purpose of telomerase localization in Cajal bodies remains unclear, as telomeres are effectively maintained in coilin-knockout cancer cells despite the disruption of Cajal bodies[106,110]. Furthermore, telomerase does not localize to Cajal bodies in certain mouse cells[111]. Nevertheless, it remains possible that Cajal bodies serve an important function in regulating telomerase in cellular contexts of low-level telomerase expression, or in certain cell types that have not yet been studied. Owing to the technical challenges in studying telomerase, its subnuclear localization has only been studied in a few cell types.

Recruitment to telomeres and association with shelterin. Recruitment of telomerase to telomeres is mediated by an interaction between TERT and the shelterin protein TPP1 (FIG. 1a). TPP1 is connected to the rest of the shelterin complex through TRF1-interacting nuclear factor 2 (TIN2), which also forms contacts with the double-strand DNA-binding proteins telomeric repeat-binding factor 1 (TRF1) and TRF2 (REFS[112,113]). TPP1 also associates with protection of telomeres protein 1 (POT1), which directly binds the single-strand telomere overhang[114]. Loss of TPP1 or TIN2 reduces telomerase recruitment to telomeres[115]. The specific interaction between TERT and TPP1 is mediated by the TEN domain of TERT and a patch of amino acids on the surface of the oligosaccharide/oligonucleotide binding fold domain of TPP1, termed the TEL patch[87,106,115–117] (FIG. 3). The importance of the TEL patch is underscored by the discovery of mutations in this region of TPP1 that cause telomere shortening and severe dyskeratosis congenita[118]. Live cell imaging of single telomerase molecules revealed that telomerase RNPs diffuse through the nucleus and form both transient and long-term interactions with telomeres[109]. Loss of TERT–TPP1 binding by mutation of the TEL patch in human embryonic stem cells causes telomere shortening and loss of cell viability, underlining

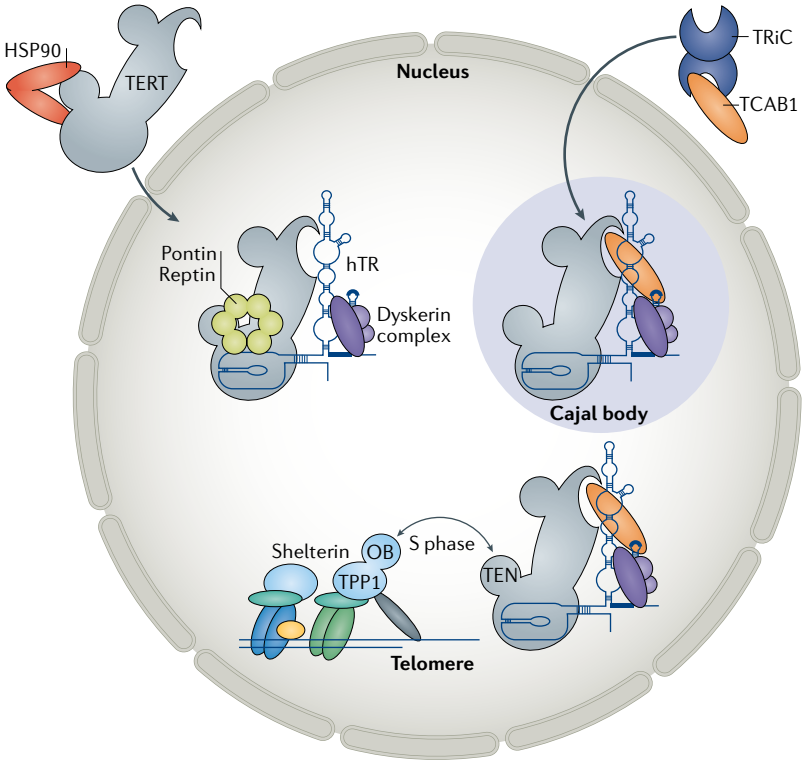

Fig. 3 | **Assembly and trafficking of telomerase within the cell.** Telomerase reverse transcriptase (TERT) is folded with the help of the chaperone heat shock protein 90 (HSP90), whereas human telomerase RNA (hTR) is bound co-transcriptionally by the dyskerin complex, comprising dyskerin, NOP10, NHP2 and the associated factor NAF1. The cofactors pontin and reptin promote assembly of hTR with the dyskerin complex and TERT. Telomerase is localized to Cajal bodies by telomerase Cajal body protein 1 (TCAB1), which is folded by the chaperone TRiC complex. In the absence of TCAB1, telomerase mislocalizes to the nucleolus (not shown) and is not recruited to telomeres. During S phase, the N-terminal domain (TEN) of TERT and the TEL patch (not shown) on the oligosaccharide/oligonucleotide binding (OB) fold domain of TPP1 interact to recruit telomerase to telomeres.

the importance of telomerase recruitment for telomere maintenance[117]. Interestingly, tethering of TERT to TPP1 in this context could not completely rescue telomere shortening, suggesting that the TEL patch of human TPP1 has additional roles in telomerase activation.

TIN2 is also essential for telomere length maintenance[119–122]; in its absence, TPP1 and POT1 cannot localize to the telomere[123]. Owing to this bridging function, TIN2 is required for recruitment of telomerase to telomeres[115]. Reflecting its importance in telomere length maintenance, TIN2 is mutated in individuals with short-telomere syndromes, with mutations clustering in exon 6, near the C-terminal region of the protein[124,125]. Although these mutations do not affect TIN2 localization or telomerase activity, there is some evidence that they impair telomerase recruitment to the telomere in human cells[126,127]. However, a mouse model of the disease-causing TIN2-K280E mutation exhibits telomere shortening through a telomerase-independent mechanism[128]. More studies are needed to precisely clarify how these TIN2 mutations cause telomere shortening.

Telomeric proteins can also inhibit telomerase activity at the telomere. The CST complex, comprising CTC1, STN1 and TEN1, is a conserved trimeric complex that binds the telomeric 3′ overhang[129,130] (FIG. 4). The human CST complex assists DNA replication at telomeres by stimulating DNA polymerase α-primase[131]. Additionally, CST restricts telomere extension by limiting access of telomerase to the telomere and inhibiting telomerase stimulation by TPP1–POT1 (see below)[132]. Thus, the CST complex may facilitate a switch from telomerase-mediated elongation of telomeres to fill-in synthesis of telomeres.

Recruitment of telomerase to telomeres is regulated in a cell cycle-dependent manner. In both budding yeast and fission yeast, interaction of telomerase with telomeres is restricted to S phase[133]. Similarly, in human cells, co-localization of telomerase and telomeres occurs primarily during S phase[134,135] (FIGS 3 and 4). Cell cycle-regulated assembly of the telomerase enzyme could account for some of the restriction of telomerase recruitment to S phase. Along these lines, the association between TERT and pontin varies during the cell cycle and peaks in S phase. Interestingly, assembly of the telomerase complex with TCAB1 varies during the cell cycle[88]. The TCAB1–hTR association is lost in mitotic cells, suggesting that some elements of the telomerase enzyme are assembled in a cell cycle-regulated manner[136]. This observation fits with the previously established importance of TCAB1 in telomerase recruitment to telomeres and suggests that regulation of TCAB1 binding to telomerase accounts for some of the cell cycle-dependent recruitment. Thus, telomerase recruitment to telomeres requires an interaction between TERT and the shelterin protein TPP1 and occurs primarily during S phase; however, the precise mechanisms controlling the timing and regulation of this recruitment remain unknown.

Regulation of telomerase activity

Whether telomerase activity can be regulated by its protein cofactors has been an active area of study. TCAB1 was initially characterized as a telomerase protein required for telomerase trafficking to Cajal bodies and for maintaining telomere length[50,107]. In human and mouse cells deleted of TCAB1 using genome editing, telomerase catalytic activity was impaired. Diminished telomerase activity in these cells was caused by partial unfolding of crucial RNA helices within the hTR CR4/5 domain, which in turn impaired its binding with TERT[110]. These findings indicate that binding of TCAB1 promotes a catalytically active state of telomerase by enhancing RNA folding and encouraging proper association of TERT and hTR (FIG. 4a).

Once recruited to the telomere, telomerase must be further activated to efficiently add telomeric repeats. Regulation of telomerase at the telomere occurs through the activity of the shelterin components. As TPP1–POT1 binds to single-strand DNA through POT1, the initial prediction was that telomerase and TPP1–POT1 would compete for telomere binding, and that TPP1–POT1 would inhibit telomerase activity by sequestering the 3′ overhang. In support of this hypothesis, overexpression of a POT1 mutant incapable of binding single-strand DNA results in rapid telomere lengthening by enhancing telomerase activity at telomeres[137]. In contrast to this finding, addition of recombinant TPP1 and POT1 to

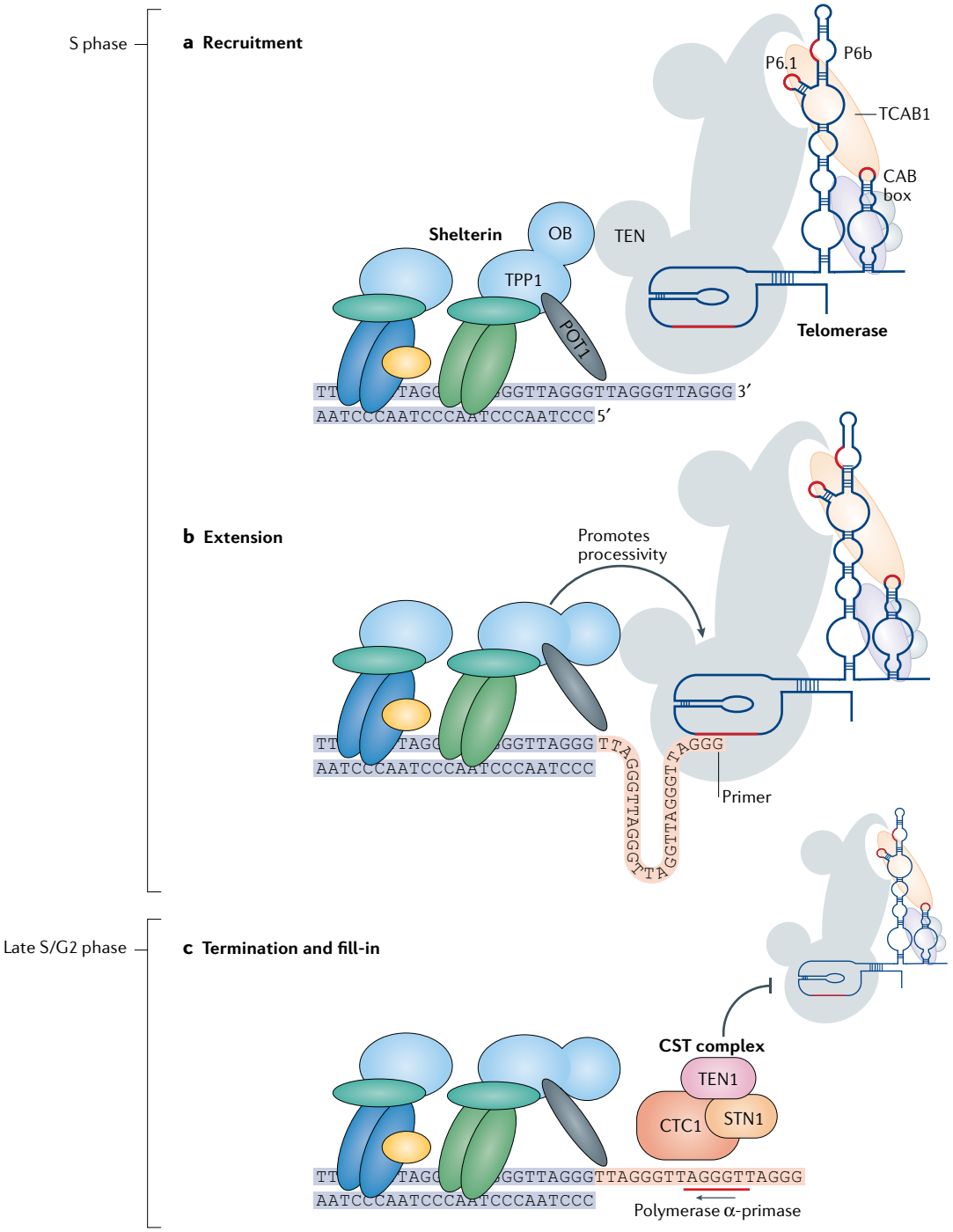

Fig. 4 | **Regulation of telomerase activity at the telomere. a** | Recruitment: telomerase is recruited to the telomere during S phase through contacts between the oligosaccharide/oligonucleotide binding (OB) fold domain of the shelterin complex subunit TPP1 and the N-terminal domain (TEN) of telomerase reverse transcriptase (TERT). Telomerase activity is regulated by telomerase Cajal body protein 1 (TCAB1), which binds to the H/ACA domain of human telomerase RNA (hTR) at a conserved four-nucleotide sequence called the CAB box. TCAB1 binding causes conformational changes in the P6.1 and P6b regions of the hTR conserved region 4/5 (CR4/5) domain, which enhance telomerase activity. TCAB1-interacting sites in hTR are marked in red. **b** | Extension: telomerase extends the G-rich strand (orange) of the telomere. Telomerase processivity — the number of telomere repeats that telomerase can add in one primer-binding event — is enhanced by the shelterin components TPP1 and protection of telomeres protein 1 (POT1). TPP1–POT1 promote telomerase processivity by encouraging the translocation of the telomerase enzyme along the telomere substrate and/or by preventing dissociation of telomerase from the telomeric primer. **c** | Termination and fill-in: during late S phase/G2 phase, the CST complex — comprising CTC1, TEN1 and STN1 — binds the newly synthesized G(-rich) strand of the telomere and prevents telomerase from accessing it. CST also impairs the interaction of telomerase with TPP1–POT1 and promotes the activity of DNA polymerase α-primase, which synthesizes the RNA primer (red line) that is necessary for the fill-in of the telomeric C strand.

telomerase in vitro caused a twofold to threefold increase in telomerase processivity (defined as the number of telomere repeats added following a single telomerase–primer binding event)[138–140]. Further studies showed that TPP1–POT1 improve telomerase processivity in vitro by preventing its dissociation from telomeric DNA or by increasing the efficiency of telomerase translocation after each repeat addition[138] (FIG. 4b). Stimulation of telomerase processivity is mediated by the TEL patch of TPP1, which also functions in telomerase recruitment[116]. In conjunction with recombinant TPP1–POT1, the addition of exogenous TIN2 can also stimulate telomerase processivity in cells[141]. Although in vivo studies of telomerase processivity are lacking, theoretically this activation mechanism of telomerase allows telomerase to synthesize long tracts of telomere DNA in vitro with only a few primer-binding events. Additional investigation is required to better understand this dual role of TPP1–POT1 in enhancing and inhibiting telomerase function.

Telomerase upregulation is nearly universal in human cancers. Upregulation or reactivation of telomerase occurs in more than 90% of all human tumours[142–144]. Although telomerase appears to be expressed in most proliferating progenitor cells, the amount of telomerase is insufficient to maintain telomeres during ageing and in homeostasis. Insufficient levels of telomerase may explain the general observation that telomeres shorten in human tissues during ageing. As discussed above, many types of primary human cells grown in the laboratory completely lack telomerase and show progressive telomere shortening with cell division. After a long lag phase of several months, human fibroblasts cease division and enter a state of replicative senescence. We now understand that replicative senescence is induced by a subset of very short telomeres that lose the ability to protect chromosome ends and are recognized as damaged DNA, which induces the DNA damage response, including activation of the p53 and RB tumour suppressor pathways[145,146]. Inactivation of p53 and RB extends the proliferation of cells with critically short telomeres, but culminates in telomere crisis: a period of genomic instability and cell death. Although telomere crisis impairs cell division, it can also drive malignant transformation by inducing non-reciprocal translocations, aneuploidy and copy number changes[147,148]. The cell death induced during telomere crisis is driven by autophagy[149]. In fibroblasts, overexpression of TERT reconstitutes telomerase, prevents cell senescence and crisis, and enables cell immortality[4]. Upregulation of telomerase in human tumours serves the same function to confer cell immortalization, thereby facilitating tumour invasion and metastasis.

Telomerase is upregulated in cancer through diverse mechanisms, including gene amplification of *TERT* and of the hTR-encoding gene *TERC*. The best understood mechanism by which cancers increase *TERT* transcription is through acquisition of non-coding mutations in the *TERT* promoter (FIG. 2a). *TERT* promoter mutations represent the most common mutations in several tumour types, including glioblastoma, melanoma, bladder cancer and hepatocellular carcinoma[150–154]. Furthermore, they are the most common non-coding mutations in human cancers[155]. *TERT* promoter mutations activate *TERT* transcription, resulting in increased mRNA levels, elevated telomerase activity and increased telomere length in urothelial carcinoma cell lines[156]. The two commonly occurring C→T transition mutations are located in the proximal promoter, 124 bp and 146 bp upstream of the translation start codon. These mutations are referred to as C228T and C250T, based on their hg19 genomic coordinates (chr5, 1,295,228 C>T and chr5, 1,295,25 C>T). The C228T and C250T mutations are heterozygous, are mutually exclusive and create de novo binding sites for ETS family transcription factors (FIG. 2a).

The mutated *TERT* promoter specifically recruits the ETS family transcription factor GABPA with its cofactor GABPB[157]. The two transcription factors form a hetero-tetramer that binds to a motif at the *TERT* promoter as well as to the new motif created by the mutations. In cancer cells harbouring *TERT* promoter mutations, TERT mRNAs specifically derive from the mutated allele[158]. Consistent with this finding, the wild-type allele remains marked by trimethylated histone H3 Lys27 (H3K27me3), which is a gene-repressive histone modification, whereas the mutated allele is marked by the gene-active histone modifications H3K4me2, H3K4me3 and acetylated histone H3 Lys9 (H3K9ac)[158–160] (FIG. 2a). Thus, the mutations in the *TERT* promoter may prevent normal silencing of the promoter. Generation of these *TERT* promoter mutations in telomerase-expressing human embryonic stem cells resulted in a modest increase in TERT mRNA levels. However, upon differentiation into fibroblasts, the mutant cells retained telomerase expression whereas isogenic control cell lines silenced TERT[15]. The *TERT* promoter mutations may also alter the long-range contacts of the *TERT* locus to favour transcription activation[160].

The frequency of TERT upregulation in cancer suggests that it is crucial for tumorigenesis. Based on the restriction of *TERT* promoter mutations to cancers of tissues with typically low rates of self-renewal (such as the central nervous system, the liver and melanocytes) and their absence from cancers of more proliferative tissues (such as the colon and blood), it has been proposed that tissues with low basal telomerase activity require activating telomerase mutations early during tumorigenesis[161]. In favour of this argument, *TERT* promoter mutations occur in early, precursor lesions in cancers, such as hepatocellular carcinoma, bladder cancer, cutaneous melanoma, thyroid carcinoma, squamous cell carcinoma, basal cell carcinoma and oligodendroglioma[153,162–164].

In addition to selecting for *TERT* promoter mutations, cancers upregulate *TERT* transcription by diverse mechanisms. In some cases of hepatocellular carcinoma, the hepatitis B virus (HBV) can integrate its genome in close proximity to the *TERT* promoter, thereby placing viral enhancer elements near the promoter without disrupting coding sequences[165–168]. Chromosomal rearrangements in neuroblastoma position the *TERT* locus near strong enhancer elements, thereby upregulating *TERT* transcription[169,170]. Furthermore, in a set of nearly 7,000 tumour samples, 4% had amplifications of the *TERT* gene, most commonly in ovarian cancer and

lung adenocarcinoma samples[171]. Importantly, *TERT* promoter mutations in glioblastoma and *TERT* rearrangements are each mutually exclusive with mutations in ATRX or DAXX, which are histone chaperones that are commonly inactivated in cancer cells employing a telomerase-independent mechanism of telomere maintenance, termed alternative lengthening of telomeres (ALT). ALT is based on homologous recombination and uses break-induced replication to synthesize tracks of telomere DNA. ALT in cancer cells results in long and heterogeneous telomeres and a lack of telomerase expression[172]. ALT is a common mechanism of telomere maintenance in glioblastoma, neuroendocrine tumours and certain sarcomas[173].

Upregulation of hTR in cancer is less well studied, but some evidence suggests that, like TERT, hTR is transcriptionally activated in human tumours. An increase in copy number of the hTR-encoding gene *TERC* has been detected in multiple cancer types, including in cervix, ovary and lung cancers[174,175]. Finally, although it is estimated that more than 90% of cancers upregulate telomerase activity, most telomerase-positive tumours lack *TERT* promoter mutations. A study of sequencing data from >18,000 tumour and matched normal tissue samples found that, among TERT-expressing tumours, 32% carried *TERT* promoter mutations, gene amplifications or chromosomal rearrangements[171]. These observations suggest that tumours that have detectable telomerase activity but no activating *TERT* mutations must upregulate telomerase by some other mechanism. One possible means for modulating telomerase activity relates to the telomere position effect, an epigenetic means by which telomere shortening can enhance the expression of telomere-proximal genes, such as *TERT*[176,177]. Further work is needed to ascertain whether this is a common mechanism by which *TERT* transcription is increased during carcinogenesis. It also remains to be seen whether cancer cells increase telomerase activity by other mechanisms, such as increasing the assembly, recruitment to telomeres or catalytic activity of telomerase.

Diminished telomerase precipitates tissue-failure diseases. In stark contrast to telomerase upregulation in cancer, germline reduction in telomerase function precipitates a set of tissue-failure diseases characterized by very short telomeres. These diseases, which are known as telomere biology disorders (TBDs), are remarkably heterogeneous in their severity and can manifest at birth, in childhood or even in middle age. The classic telomerase insufficiency disorder was initially defined as dyskeratosis congenita, which is a multiorgan, systemic disease characterized by abnormal skin pigmentation, dystrophic nails, bone marrow failure and predisposition to specific cancers, including squamous cell carcinoma and leukaemia[178]. Based on recent genetic and clinical findings, it is now understood that mutations in telomerase-relevant genes can additionally cause idiopathic pulmonary fibrosis, aplastic anaemia, and liver fibrosis and cirrhosis (TABLE 1). Mutations in every component of the telomerase holoenzyme have been identified in individuals with TBDs and have provided insight into telomerase structure and function.

Heterozygous mutations in the *TERC* (encoding hTR) or *TERT* genes are common in TBDs and cause haploinsufficiency (reduced enzyme levels). Mutations in *TERC* typically reduce telomerase catalysis or decrease hTR levels. Most *TERC* mutations occur within the PK/T domain of hTR, which binds TERT and contains the template sequence for reverse transcription. Many of these mutations impair TERT binding and reduce telomerase function, but preserve hTR expression[85]. Mutations in the central CR4/5 domain of hTR also reduce telomerase catalytic activity, by impairing the hTR–TERT association[84,179]. Mutations in the 3′ H/ACA region of hTR often cause loss of hTR through diminished dyskerin binding[180]. Germline mutations in the *TERT* gene in individuals with TBD are distributed throughout the open reading frame, in contrast to the somatic mutations in the *TERT* promoter in human cancers. Coding mutations in *TERT* commonly lead to reduced catalytic activity, although many mutations preserve catalytic function[181]. These findings suggest that *TERT* mutations may disrupt other aspects of telomerase biology, including protein stability, complex assembly or recruitment to telomeres. In support of this notion, mutations in the TERT TEN domain or TERT C-terminus impair its recruitment to telomeres[106]. Consistent with the importance of telomerase recruitment for telomere maintenance, rare mutations have been identified in TPP1 that specifically disrupt its interaction with TERT and impair recruitment of telomerase to telomeres[118].

The core dyskerin complex (dyskerin–NHP2–NOP10) recognizes that the hTR H/ACA domain is crucial for hTR stability and processing. Mutations in the gene encoding dyskerin reduce hTR levels and cause the X-linked form of dyskeratosis congenita[182], and rare mutations in *NOP10* and in *NHP2* have been identified in autosomal recessive forms of dyskeratosis congenita. Rare mutations have also been found in the gene encoding the dyskerin-associated cofactor NAF1, which lead to diminished hTR levels[183–185]. A common means by which hTR levels are reduced in TBDs is through mutations that inactivate PARN[186]. Such mutations cause autosomal recessive forms of idiopathic pulmonary fibrosis, aplastic anaemia and dyskeratosis congenita and commonly occur in the nuclease or RNA binding domains of PARN. *PARN* mutations slow hTR maturation rates, leading to a relative accumulation of hTR precursors and global reduction in the levels of mature hTR[61]. Rare autosomal recessive mutations in the gene encoding TCAB1 have also been identified in TBDs[107,187]. These mutations reside in the WD40-repeat domain and interfere with TCAB1 protein folding by the TRiC chaperone complex, thereby causing loss of TCAB1 protein[103]. In addition to these mutations directly impairing mutations in genes encoding components of the telomerase holoenzyme, mutations in individuals with TBD are also found in *TIN2*, in *CTC1* and in regulator of telomere elongation helicase 1 (*RTEL1*), which is a DNA helicase involved in the maintenance of telomere integrity[124,125,188,189]. It is striking that most TBD-causing mutations affect hTR, by reducing its levels or function, thereby emphasizing the central role of hTR in telomerase function.

Table 1 | **Telomerase and telomere-associated genes mutated in tissue-failure disorders**

Gene	Product	Normal function	Diseases associated with loss of function	Refs
TERC	hTR	Non-coding RNA template for telomere synthesis	Aplastic anaemia, dyskeratosis congenita, pulmonary fibrosis	190–192
TERT	TERT	Reverse transcriptase that catalyses telomere synthesis	Aplastic anaemia, dyskeratosis congenita, pulmonary fibrosis	192–194
DKC1	Dyskerin	Pseudouridine synthase that controls hTR processing	Dyskeratosis congenita, aplastic anaemia, pulmonary fibrosis	195,196
TINF2	TIN2	Shelterin protein that connects the DNA binding proteins TRF1 and TRF2 with TPP1	Dyskeratosis congenita, aplastic anaemia	125,124
PARN	PARN	RNA exonuclease that aids hTR maturation	Dyskeratosis congenita, pulmonary fibrosis	186
WRAP53	TCAB1	WD40 protein that binds hTR and promotes RNA folding and telomerase localization	Dyskeratosis congenita	107
CTC1	CTC1	Component of the CST complex that promotes telomere DNA replication	Dyskeratosis congenita, Coats plus	188,197
RTEL1	RTEL1	DNA helicase that promotes telomere stability during replication	Dyskeratosis congenita, pulmonary fibrosis	189
Nola2	NHP2	Component of the dyskerin complex	Dyskeratosis congenita	198
Nola3	NOP10	Component of the dyskerin complex	Dyskeratosis congenita	185
Naf1	NAF1	Component of the dyskerin complex	Pulmonary fibrosis	184
ACD	TPP1	Shelterin protein that recruits TERT to telomeres	Dyskeratosis congenita, aplastic anaemia	118,199
ZCCHC8	ZCCHC8	Component of nuclear exosome targeting complex	Pulmonary fibrosis	200

Telomerase and telomere-associated genes that are mutated in telomere biology disorders are listed from the most common to the least common. CST, CTC1–STN1–TEN1; hTR, human telomerase RNA; PARN, poly(A) ribonuclease; RTEL1, regulator of telomere elongation helicase 1; TCAB1, telomerase Cajal body protein 1; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase; TIN2, TRF1-interacting nuclear factor 2; TRF1, telomeric repeat-binding factor 1.

Conclusions and future perspective

Telomerase evolved to provide a simple solution for replicating chromosome ends in single-cell eukaryotes, but the telomerase complex has assumed greater importance in long-lived, complex metazoans, such as humans. The fundamental challenge of supporting robust cell division for tissue renewal while reducing the likelihood of developing cancer accounts for this elevated importance of telomerase in humans. The requirement for facilitating cell renewal while suppressing cancer, combined with the special characteristics of RNPs, explains the complexity of regulation of the telomerase complex. These features account for exquisite transcriptional control of TERT expression, the need to precisely process and stabilize the telomerase RNA, regulation of assembly of the active complex and regulation of recruitment of the enzyme to telomeres. In organisms and tissues, certain cells are licensed to express telomerase, and the amount of telomerase varies with the differentiation state of the cell. The multiple levels of telomerase control are essential as an increase or a decrease in telomerase activity beyond a narrow range is associated with human diseases. Despite important progress, we continue to lack an understanding of the *cis*-regulatory elements and the *trans*-acting factors that activate TERT transcription in normal progenitor cells and that silence TERT during differentiation.

Telomerase RNA was once thought to be unregulated and expressed in a ubiquitous fashion. The study of TBDs has revealed surprising new levels of regulation of hTR, involving the function of several enzymes in processing its 3′ end. These enzymes control the rate of hTR maturation and, in turn, dictate its steady-state levels. The maturation of hTR is dictated by the core dyskerin complex, which binds the hTR H/ACA domain. Thus, the evolution of an H/ACA domain in telomerase RNAs in metazoans served to dictate the mechanism of RNA processing, while regulating the levels and protecting the 3′ end of hTR. Based on these observations, the dyskerin complex can then be considered a telomerase RNA end-processing and stabilizing complex. This feature is visually evident in the cryo-electron microscopy structure of human telomerase, which shows the core dyskerin complex to be distant from the catalytic site of TERT. The hTR-processing enzymes PAPD5 and PARN can modulate the levels of hTR, which represents a potential therapeutic avenue for individuals with TBD. Other potential means of modulating telomerase activity include telomerase assembly, recruitment to telomeres or using catalytic activators and inhibitors.

Telomerase upregulation is nearly universal in diverse cancer types but is now best understood in cancers with activating mutations in the *TERT* promoter. These simple non-coding mutations create a new consensus binding site for the transcription factor GABP, and thus targeting GABP has been suggested as a means for inhibiting TERT in this subset of cancers. However, the majority of human cancers lack *TERT* promoter mutations, and therefore control of *TERT* transcription in cancer continues to remain poorly understood. Advances in this area could improve our understanding of TERT regulation in cancer and provide new avenues for cancer therapy.

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