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Telomerase Regulation

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Abstract

The intimate connection between telomerase regulation and human disease is now well established. The molecular basis for telomerase regulation is highly complex and entails multiple layers of control. While the major target of enzyme regulation is the catalytic subunit TERT, the RNA subunit of telomerase is also implicated in telomerase control. In addition, alterations in gene dosage and alternative isoforms of core telomerase components have been described. Finally, telomerase localization, recruitment to the telomere and enzymology at the chromosome terminus are all subject to modulation. In this review we summarize recent advances in understanding fundamental mechanisms of telomerase regulation.

Keywords

telomere; TERT; TR; ribonucleoprotein; repeat addition processivity; TERRA

1. Introduction

The ends of eukaryotic chromosomes are defined by a tract of simple G-rich repeats and associated proteins that constitute the functional unit termed the telomere. The length of the telomeric DNA tract is highly dynamic and subjected to forces that both shorten and extend the repeat array. Telomeres must be long enough to assemble a protective "cap" that can distinguish the terminus from a double-strand break. Dysfunctional telomeres trigger cell cycle arrest, genome instability and in humans, replicative cell senescence and apoptosis [1, 2]. On the other hand, telomeric DNA loss through incomplete DNA replication or nucleolytic processing suppresses tumorigenesis by limiting the proliferative potential of normal somatic cells. At the heart of this balancing act is telomerase, a ribonucleoprotein reverse transcriptase that consists of two core components: a catalytic reverse transcriptase subunit (TERT), and an RNA subunit (TR or TER), which serves as a template for telomeric DNA addition by TERT.

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Telomerase is a highly regulated enzyme and in normal individuals its activity is confined to cells with extended proliferation potential: the germline, embryonic tissues and self-renewing stem cell populations of the hematopoetic system and skin. In other tissues, telomerase is inactivated during gestation, thereby restricting the proliferation program [3]. Mis-regulation of telomerase has dire consequences. As discussed elsewhere in this volume, reactivation of telomerase is associated with approximately 90% of human cancers [4], while insufficient telomerase activity is linked to a litany of stem cell disorders including dyskeratosis congenita, aplastic anemia and idiopathic pulmonary fibrosis [5, 6].

The molecular basis for telomerase regulation is highly complex and entails multiple levels of control. A major determinant of enzyme activity is transcriptional regulation of the catalytic subunit TERT. However, emerging data indicate that TERT is subjected to both post-transcriptional and post-translational control. In addition, transcriptional regulation of TR has also been reported. In some instances the number of genes encoding TERT and TR is expanded, increasing enzyme activity or, over evolutionary time, giving rise to alternative ribonucleoprotein complexes. Finally, telomerase recruitment and enzyme activity at the chromosome terminus are modulated by telomere-associated proteins and by telomeric RNA transcripts. Here we summarize some of the recent advances in understanding telomerase regulation.

2. Transcriptional regulation of TERT

TERT gene expression parallels telomerase activity in many multicellular organisms. For example, in the model plant Arabidopsis, TERT mRNA peaks in flowers and suspension cell culture where telomerase activity is most abundant, but can barely be detected in leaves where telomerase is strongly repressed [4]. Similarly, human TERT is expressed during early development, but with the exception of proliferating cells or renewal tissues, it is absent in most normal somatic cells [7, 8]. Transient transfection of an hTERT promoter-luciferase reporter reveals an expression pattern that mirrors the telomerase activity profile [9]. These and other observations argue that the TERT promoter is a major target of enzyme regulation.

The hTERT promoter has been extensively studied [reviewed in [10, 11]]. A plethora of transcription factor binding sites allow for nuanced hTERT expression (Figure 1). For example, TERT transcription is influenced by Sp1 [11], a general transcription factor that interacts with the TATA binding protein. Notably, TATA boxes are not found in the hTERT promoter, and yet mutations in Sp1 binding sites completely abolish hTERT promoter activity [9, 11]. Telomerase expression is also controlled by oncogenes (i.e. c-Myc) as well as tumor suppressors (i.e. WT1). c-Myc influences telomerase expression by binding the two E-boxes present in the hTERT promoter. Increased expression of c-Myc, which is observed in cancer cells, results in increased telomerase activity [2, 11-13]. Binding of WT1 (Wilm's tumor suppressor) to the hTERT promoter negatively regulates hTERT expression [14]. On the other hand, inactivation of WT1 is associated with telomerase activation during tumorigenesis [15].

Other factors repress hTERT expression. These include MZF-2, a zinc finger transcription factor [16], and member of the E2F family of transcriptional regulators involved in cell-cycle progression [17]. Repression of hTERT transcription may also be accomplished by inhibiting potential activators like c-Myc, via the TGFβ/Smad signaling pathway [18] or BRCA1, a tumor suppressor for hereditary breast and ovarian cancers that is involved in DNA repair [19, 20]. Finally, it has been suggested that p53 may be involved in the negative regulation of hTERT expression, because most cancers are deficient in p53 [21, 22].

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Several lines of evidence indicate that silencing of hTERT is under epigenetic control [23]. The TERT promoter lies within a highly condensed chromatin domain [24] and is associated with hypoacetyled core histones [25, 26]. In addition, both histone and CpG methylation are implicated in hTERT regulation [27, 28], although in the latter case there is controversy as to whether methylation contributes to positive or negative regulation of hTERT expression in cancer cells [29, 30]. CTCF, an insulator that organizes chromatin domains to modulate transcription [31], binds the first exon of hTERT (Figure 1) in cells that repress hTERT expression [32]. Finally, activation of the hTERT promoter is correlated with dramatic changes in the surrounding chromatin environment [23].

3. Post-translational regulation of TERT

Post-translational regulation of telomerase is supported by the observation that TERT mRNA levels do not always correlate with telomerase enzyme activity [33-35]. Moreover, not all cells with active telomerase are capable of maintaining telomere tracts [36]. The biogenesis and assembly of the telomerase RNP represent other avenues of enzyme regulation and are discussed in detail elsewhere in this issue [37]. Figure 2 depicts some of the post-translational modifications of TERT and how they affect protein stability, subcellular localization and ultimately, enzyme activity. A number of studies indicate telomerase activity is modulated by phosphorylation [reviewed in [38]. Putative phosphorylation sites are present in the TERT sequences from mammals [39] and plants [35]. At least two kinases are implicated in hTERT phosphorylation. In response to ionizing radiation, hTERT is phosphorylated by c-Abl leading to a three-fold reduction in telomerase activity [40]. Mice lacking c-Abl display increased telomerase activity and telomere elongation [40]. Thus, c-Abl negatively regulates telomerase activity. In contrast, phosphorylation of hTERT by Akt correlates with increased telomerase activity, presumably resulting from hTERT translocation from the cytoplasm to the nucleus [39, 41].

Ubiquitination may also influence telomerase activity. The MKRN1 ubiquitin ligase (E3) interacts with hTERT in a yeast-two hybrid assay [42]. Over-expression of MKRN1 leads to degradation of TERT, resulting in decreased telomerase activity and shortened telomeres. This finding suggests that telomerase activity is modulated by TERT stability. The half-life of human telomerase activity is approximately 24 hrs [43], while the half-life of TER is extraordinarily long, five days [44]. These observations support the conclusion that hTERT stability contributes to telomerase regulation. A recent study shows that CHIP (C terminus of Hsc70-interacting protein), a co-chaperone with E3 ubiquitin ligase, controls hTERT stability in the cytoplasm [45]. CHIP interaction with hTERT leads to polyubiquitination, blocking hTERT entry into the nucleus and culminating in proteolytic degradation (Figure 2). Intriguingly, the interaction of CHIP with hTERT peaks in G2/M, and is diminished in S phase when telomerase acts on telomeres. Thus, CHIP may regulate telomerase activity during the cell cycle by controlling the intracellular trafficking and consequently the stability of hTERT [45].

The subnuclear localization of telomerase is also dynamically controlled during the cell cycle and contributes to enzyme regulation [46-48]. Emerging data argue that delivery of enzymatically active telomerase to the chromosome terminus first requires the passage of TR through Cajal bodies via a telomerase-specific Cajal body protein termed TCAB1 [49]. Specifically, Cajal bodies are proposed to act as a type of processing center, where TR and possibly associated proteins are modified, in some fashion before the enzyme can become fully competent for telomere elongation [50, 51] (see below).

Negative regulation of telomerase activity can be achieved by sequestration of the enzyme in the nucleolus. Following DNA damage, hTERT transiently moves from the nucleoplasm to

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the nucleolus (Figure 2). This re-localization is hypothesized to reduce the probability of de novo telomere formation at sites of DNA damage [46]. PinX1, an interaction partner for the human shelterin component TRF1, is also proposed to regulate telomerase by sequestration [52] (Figure 2). PinX1 directly binds hTERT [53, 54] and hTR [53] and inhibits telomerase activity in vitro [54]. As in human cells, the interaction of PinX1 with Est2 (TERT) leads to sequestration in the yeast nucleolus [55].

4. Transcriptional and post-transcriptional regulation of TR

Although hTR expression is detected in some tissues where hTERT is not, hTR abundance increases in tumors relative to normal cells [56, 57], arguing that hTR abundance contributes to telomerase regulation. Transcription of hTR is activated by Sp1 and HIF-1 and repressed by Sp3, which integrates cues from the MAPK signaling cascade to silence the hTR promoter [reviewed in [10]. Furthermore, like hTERT, hTR transcription appears to be subjected to epigenetic control as repression of hTR expression is associated with decreased levels of H3 and H4 acetylation [27]. Finally, at least six sites in hTR are subjected to post-transcriptional modification by pseudouridylation [58]. Intriguingly, two of these sites lie in a highly conserved domain essential for telomerase catalytic activity. In vitro telomerase reconstitution assays with model pseudouridylated hTR result in modest alterations in enzyme activity and processivity. Whether hTR modification regulates telomerase activity in vivo is still an open question, although recent findings suggest that this is a distinct possibility (see below).

5. Gene dosage and alternative TERT and TR isoforms

TERT and TR exist as single copy genes in most organisms studied and a null mutation in TERT or TR is ultimately lethal. In mice, both TERT [59] and TR [60, 61] are haploinsufficient for maintaining telomere tracts. Indeed, the etiology underlying a growing list of stem cell diseases is linked to hemizygosity of core telomerase subunits [62]. Conversely, amplification of chromosomal loci encoding TERT or TR is correlated with tumor formation [63-66]. Thus, gene dosage plays a critical role in telomerase regulation.

Alternative splicing of TERT is widespread and has been described for a number of multicellular eukaryotes including a variety of mammals [28, 67, 68], birds [69], fish [70] and plants [71]. Ten different splice variants have been reported for human TERT [28, 72-76]. Three of the major products are depicted in Figure 2. Alternative splicing of hTERT results in both nucleotide deletion and mutation. Several splice variants have been correlated with changes in telomerase activity [77-79]. The most well-studied is hTERTα, which encodes a 183 bp deletion with an accompanying nonsense mutation. Expression of TERTα correlates with decreased telomerase activity, and hence this isoform is proposed to act as dominant negative inhibitor of telomerase activity [74, 75].

Variant isoforms of telomerase subunits have also emerged as a consequence of gene duplication. Three different TERT genes are found the ciliated protozoan *Euplotes crassus* [80]. These genes encode proteins that display 83-87% identity and are differentially expressed during the sexual stage of the life cycle [80]. Expression of the EcTERT-2 gene is limited to macronuclear development, a period when telomeres form de novo on thousands of newly generated mini-chromosomes. In contrast, EcTERT-1 and EcTERT-3 are expressed during vegetative growth when telomerase performs its canonical function of maintaining pre-existing telomere tracts. Remarkably, the *EcTERT-2* gene is destroyed by programmed DNA elimination following new telomere formation, presumably to control promiscuous telomere addition at sites of spontaneous DNA damage [80]. Like most other model organisms, *E. crassus* encodes only a single telomerase RNA. Thus, the *E. crassus* TER assembles with different TERT subunits into alternative RNP complexes to facilitate a

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developmentally programmed switch from de novo telomere formation to telomere maintenance.

A second example of alternative telomerase subunits is found in *Arabidopsis thaliana* [81, 82]. *A. thaliana* encodes two different template RNA components, TER1 and TER2. The two RNAs assemble with the single TERT isoform into alternative telomerase particles. TER1 is a typical telomerase template critical for telomere maintenance [81]. TER2, on the other hand, is a novel negative regulator of enzyme activity [82]. Why an alternative RNP evolved to negatively regulate the plant telomerase is unclear since, unlike mammals, plants do not face the threat of metastatic cancer as a consequence of unbridled telomerase activity. These findings suggest that additional modes of restraining telomerase could be elucidated in mammals where inappropriate expression is much more deleterious.

6. Regulation of telomerase recruitment to the telomere

Once an active telomerase RNP particle is formed, it must engage the chromosome terminus to facilitate the incorporation of telomere repeats. Here we briefly consider how crosstalk between telomerase RNP components and telomere capping proteins influences the recruitment of telomerase to the telomere. Mechanisms to regulate the length of the telomere tract are discussed elsewhere in this volume [83].

The interaction of telomerase with the telomere is best understood in budding yeast. Lundblad and colleagues established that Est1, a non-catalytic telomerase holoenzyme component, physically links the RNP to the telomere through interactions with the telomerase RNA (Tlc1) and Cdc13, a member of the CST telomere capping complex [84]. The telomerase-telomere interaction is regulated during the cell cycle, peaking during S phase [85]. In addition, the interaction of Est1 with Tlc1 is controlled by cell cycle regulated proteolytic degradation [86]. Moreover, a number of studies suggest that Est1-Cdc13 association is controlled via phosphorylation of Cdc13 by Cdk1 (Cdc28) [87] or by Tel1 (ATM) [88-91]. However, recent analyses of Tel1 consensus phosphorylation sites on Cdc13 do not support this model [92]. In conjunction with its role recruiting telomerase to the telomere, Est1 influences the interaction of telomerase with its DNA primer in vitro to stimulate elongation [93-95].

The Ku heterodimer is also implicated in telomerase recruitment. In budding yeast Ku directly interacts with a stem loop in Tlc1 [96]. Cells lacking Ku exhibit defects in Tlc1 nuclear localization and have shorter telomeres with long G-overhangs [97, 98]. The current view is that Ku assists in positioning telomerase at the telomere in G1 to promote telomere synthesis by the enzyme in S phase [99].

The mechanism of telomerase recruitment is less clear in multicellular organisms. Telomerase associates with components of shelterin, the telomere capping complex in human cells [98]. One of these proteins, TPP1, is implicated in telomerase recruitment [100, 101]. TPP1 forms a subcomplex POT1, another shelterin component, simulating interaction of POT1 with the single-strand 3’ overhang on the chromosome end [101]. TPP1 also interacts with the telomerase RNP, binding the TEN domain of TERT [102]. Intriguingly, the TEN domain promotes repeat addition processivity of the core enzyme [103, 104]. Whether interaction of TPP1 with this region telomerase alters enzyme activity is unknown. Furthermore, unraveling precisely how TPP1 influences telomerase function in vivo is hindered by the fact that depletion of TPP1 also dislodges POT1 from telomeres, activating an immediate DNA damage response and cell cycle arrest, not the ever-shorter-telomere phenotype expected for defect in telomerase recruitment [100, 105].

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7. Control of telomerase processivity at the chromosome terminus

Once telomerase engages the single-strand overhang on the telomere, telomere repeat incorporation is facilitated by two enzyme modes: a processive reaction in which multiple telomere repeats are added in a single DNA binding event, and a non-processive or distributive mode in which only one or two repeats are incorporated. The TEN1 domain that promotes repeat addition processivity (RAP) of the core enzyme [103, 104, 106]. Collins and colleagues discovered a new accessory factor, p82, for the Tetrahymena telomerase that strongly stimulates RAP of the catalytic core [107]. In addition, RAP of human telomerase is influenced by the putative human telomerase recruitment factor TPP1 in vitro [108, 109].

Analysis of telomerase dynamics in vivo reveals a striking correlation between RAP and the length of the telomere tract telomerase acts upon. Telomerase is preferentially recruited to shorter telomere tracts [110-112] and in yeast the enzyme does not extend every telomere in every cell cycle [110]. However, telomerase RAP is increased at critically shortened telomeres relative to telomeres in the wild type size range [113]. Thus, modulation of telomerase processivity plays a direct role in establishing telomere length homeostasis.

Recent studies reveal that processivity of human telomerase is highly regulated in cancer cells (Figure 3). Unlike yeast, human telomerase extends every telomere end every cell cycle [114]. However, RAP is altered depending on whether the enzyme is establishing or maintaining telomere length homeostasis [51]. On shortened telomere tracts recovering from telomerase inhibition, telomerase acts in a distributive manner. Multiple telomerase enzymes sequentially engage the same chromosome end to rapidly extend the telomere. In contrast, when telomerase maintaining telomere length homeostasis, RAP is strongly stimulated and only a single telomerase enzyme associates with each telomere. Approximately 10 repeats are added before the enzyme dissociates. This remarkable switch from a distributive to a processive mode for telomere synthesis requires trafficking of hTR through Cajal bodies [51]. Hence, post-transcriptional modification of hTR or an auxillary factor may modulates telomerase processivity in vivo.

In budding yeast, telomerase processivity is negatively regulated by the Pif1 helicase. Pif1 unwinds telomeric DNA from the telomerase RNA template, dislodging telomerase from the chromosome terminus [115]. In cells over-expressing Pif1, the interaction of telomerase with telomeres is reduced leading to telomere shortening. Conversely, Pif1 depletion results in telomere elongation. In conjunction with its action at native chromosome ends, Pif1 also promotes genome stability by ejecting telomerase from non-telomeric DNA substrates [116]. Pif1 is phosphorylated in response to DNA damage, which stimulates its helicase activity, decreasing the opportunity for telomerase to form incorporate telomere repeats at sites of DNA damage [117]. Although mammalian PIF1 physically associates with telomerase [118, 119], it is not required for telomere length regulation [115, 118, 119]. However, it is currently unclear whether human PIF1 regulates telomerase activity at double-strand breaks.

8. Telomerase regulation by TERRA

One of the defining features of telomeres is that they are heterochromatic. Consequently, it was surprising when telomere transcripts termed Telomeric Repeat containing RNA (TERRA) were discovered. TERRA molecules are long non-coding RNAs transcribed by RNA polymerase II from subtelomeric and telomeric DNA. Telomere transcription is reported for a number of eukaryotes including mammals, fish and yeast [120-123]. Arabidopsis appears to be unusual in that it transcribes both strands of the telomere, generating TERRA as well as TERRA antisense transcripts, ARRET [123]. Notably, Arabidopsis TERRA and ARRET are not derived exclusively from telomeres; they are also transcribed from centromere-proximal telomeric sequences.

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TERRA varies in size ranging from 100nt to 9000nt in mammals and ~380nt in yeast [120]. TERRA molecules are capped by 7-methylguanosine (m7G) [122] and at least a subset of them bear a 3' poly(A) tail [121, 122]. Intriguingly, only poly(A) minus TERRA is detected in chromatin [124, 125], suggesting that TERRA may have different functions. TERRA interacts with numerous RNA binding proteins [125, 126]. One of these is hnRNPA1, a single-strand nucleic acid binding protein that recognizes RNA as well as telomeric DNA [127, 128]. A recent study reveals that the interaction of TERRA with hnRNPA1 plays a pivotal role in facilitating the exchange of single-strand binding proteins at the chromosome terminus [127]. Following chromosomal replication, the 3' overhang on the telomere is initially bound by RPA, which is subsequently replaced by hnRPA1. hnRNPA1 is then dislodged from the DNA through its interaction with TERRA, allowing the POT1/TPP1 components of the shelterin complex to bind and thus establish a functional telomere cap.

In addition to this newly discovered role in promoting changes in telomere protein composition, TERRA is also postulated to reinforce the heterochromatic nature of the chromosome terminus [120-122]. Moreover, because TERRA is complementary to the template domain of TR, it has the potential to negatively regulate telomerase. Indeed several studies support this prediction [121, 122, 126]. Human TERRA physically associates with telomerase in nuclear extracts, and as predicted, base pairs with the complementary template region of TR [126]. TERRA may also interact with TERT independently of TR. Further evidence for telomerase regulation by TERRA has been obtained in yeast *rat-1* mutants, where TERRA levels increase as telomeres shorten [121]. It is unknown whether TERRA is released from the telomere to inhibit telomerase in trans, or acts in cis on the chromosome terminus to block telomerase action. The latter model is appealing as long telomeres correlate with increased levels of TERRA. Thus, negative regulation of telomerase by TERRA in cis could provide an elegant feedback mechanism to promote telomere length regulation.

9. Conclusions

Although the initial studies of telomerase regulation focused on transcriptional control of core subunits, it is now apparent that the telomerase RNP is subjected to a highly sophisticated network of regulatory pathways that modulate subunit abundance, intracellular trafficking and the interaction with and activity on the chromosome terminus. The necessity of governing telomerase activity is underscored by the remarkable conservation of factors, both protein and RNA based, that control enzyme behavior. Our understanding of telomerase and its pivotal role in safeguarding the genome will undoubtedly mature as new links between enzyme regulation and fundamental aspects of cellular physiology continue to be revealed.

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Figure 1.
Simplified diagram of regulatory and functional elements associated with the hTERT gene. Rightward arrow depicts start of transcription. Start and stop of translation are indicated. A subset of hTERT promoter elements as well as the binding site for CTCF, a chromatin insulator element, are indicated. Within the TERT coding region, dark blue boxes denote reverse transcriptase motifs, while the light blue box shows the TEN domain. The three major splicing isoforms of TERT (α, β and γ) are shown in pink. Drawing is not to scale. See text for details.

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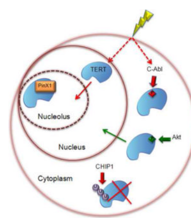

Figure 2.
Summary of post-translational modifications of hTERT and their consequences for telomerase activity. Red arrows indicate negative regulation, green arrows positive regulation. See text for details.

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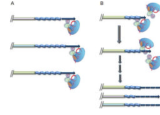

Figure 3.

A model for the regulation of repeat addition processivity (RAP) of telomerase is regulated in human cancer cells. Diagram summarizes results from [51]. A) Telomerase displays high RAP in cells that maintain telomere length. In this setting, a single telomerase enzyme binds each chromosome end, adding multiple telomere repeats (extended blue arrows) before being released from the DNA. B) Telomerase RAP is decreased following artificial telomere shortening. Under these conditions, telomerase is less processive (fragmented blue arrows), adding fewer telomeric repeats before dissociation (low RAP). However, multiple enzymes bind each chromosome end sequentially to rapidly extend telomere tracts and thereby reestablish telomere length homeostasis.