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Telomerase regulation and stem cell behaviour

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Telomerase expression is restricted to a few cell types of the adult organism, most notably germ cells and stem/progenitor cells. Telomerase activity in germ cells is sufficient to prevent telomere shortening with age. Stem cells, however, do not have sufficient telomerase to prevent telomere shortening associated with continuous tissue renewal with increasing age. Indeed, telomerase levels in the adult organism are thought to be rate-limiting for longevity. This is supported by rare human syndromes caused by mutations in telomerase components, which are characterized by premature loss of tissue renewal and premature death. More recently, the role of telomerase and telomere length in stem cells is starting to be elucidated.

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Introduction

The ends of chromosomes are formed by a special chromatin structure, known as the telomere, which is essential to protect chromosome-ends from degradation and DNA repair activities [1••,2]. Telomeric chromatin is formed by tandem TTAGGG repeats and associated proteins [1••,2]. Telomere repeats span ~10–15 Kb in humans and 25–40 Kb in mice [3]. The proteins that associate with these repeats include the telomere repeat binding factors TRF1 and TRF2 as well as their interacting factors, which form a large protein complex recently named 'shelterin' [1••]. This complex is proposed to regulate both telomere length and telomere protection [1••]. Importantly, telomeres are also bound by nucleosome arrays, which show histone modifications characteristic of constitutive heterochromatin domains [3,4]. Constitutive heterochromatin is generally found at transcriptionally inactive ('silenced') genomic regions of repetitive DNA, such as pericentric satellite repeats. Similar to pericentric chromatin, telomeres are enriched for binding of the heterochromatin protein 1 (HP1) and contain high levels of trimethylated H3-K9 and H4-K20, two histone modifications carried out by the histone methyltransferases (HMTases) suppressor of variegation 3–9 homolog (Suv39h) and suppressor of variegation 4-20 homolog (Suv4-20h), respectively [5,6••,7••]. The Retinoblastoma proteins are also required for efficient H4-K20 trimethylation at both telomeres and centromeres through direct interaction with the Suv4-20h HMTases [5,7••]. The heterochromatic nature of telomeres, therefore, suggests that chromosome ends are in a compacted and 'silenced' chromatin conformation, which has to be finely regulated in order to properly control telomere length.

Interestingly, during cell division telomeres lose TTAGGG repeats as a result of the incomplete replication of linear chromosomes by conventional DNA polymerases, the so-called 'end-replication problem'. This progressive telomere shortening is proposed to be one of the molecular mechanisms underlying organismal aging, since critically short telomeres trigger chromosome instability and loss of cell viability [3,8]. As an exception, germ cells, certain populations of stem cells, and the vast majority of cancer cells express high levels of telomerase [3]. Telomerase is a reverse transcriptase encoded by the *Tert* (telomerase reverse transcriptase) and *Terc* (telomerase RNA component) genes, which adds telomeric repeats onto the chromosome ends [2,8].

Defective telomerase activity and short telomeres have been implicated in the pathobiology of several age-related diseases and premature aging syndromes [3,8,9]. In contrast, telomerase is abnormally up-regulated in >90% of human tumors, where it is though to sustain tumor growth by maintaining telomeres above a threshold length. In this review, we will discuss recent advances on how telomerase is regulated, as well as on novel roles of telomeres and telomerase in stem cell biology. These new findings have profound implications for how telomerase regulates the balance between aging and cancer.

Telomerase regulation

It is of great interest to understand how telomerase activity is regulated in normal and pathological conditions in order to evaluate its potential as a therapeutic target. A number of different mechanisms have been shown to regulate telomerase activity. Regulation of Tert mRNA expression seems to be the most important and rate-limiting step for telomerase activation [10]. Other mechanisms for telomerase regulation include alternative splicing [11], post-translational Tert modification [12–14] and sub-cellular Tert localization [15]. The human Tert (hTert) promoter regulatory region contains potential

binding sites for a number of positive and negative hTert transcriptional regulators, among which the Myc/Mad binding sites have been extensively studied (Table 1). In particular, hTert is a direct transcriptional target of c-Myc, which up-regulates telomerase expression [16–20], while the Myc antagonist Mad1 suppresses hTert expression [21,22]. In addition, a number of tumor suppressors and oncogenic pathways have been shown to negatively regulate hTert (Table 1). Among them, the Smad-interacting protein 1 (SIP1) mediates TGF-β-induced hTert repression, while Menin directly represses hTert through inhibition of the trans-activation ability of several transcription factors [23]. Other tumor suppressors, such as RAK and BRIT1 [23] as well as p53 and MDM2 [24,25], have been shown to regulate hTert expression. In addition, the transcription factor E2F-1 has been identified as a repressor that down-regulates hTERT promoter activity in human tumor cells. Interestingly, in contrast to its repressive activity in human tumor cells, E2F-1 activates the hTERT promoter in normal human somatic cells [26,27]. Among the hTert promoter activators, estrogen up-regulates telomerase in both mammary and ovarian epithelial cells [28–30]. Similarly, the oncogenic variant of human papillomavirus E6 [31] and the oncogenic constituents of the RAS signaling pathway [32] have been shown to up-regulate telomerase activity. Finally, a number of additional hTert transcriptional activators have been described over recent years, including the transcription factor activator protein 1 (AP-1) [33] and the signal transducer and activator of transcription 3 (STAT3) [34], as well as hTert repressors MZF-2 and the Tax oncogene [35,36].

Besides these different transcriptional regulators, the Tert promoter is also a target of epigenetic modifications, which in turn can modulate promoter activity and hTert expression [37•]. In particular, the hTert promoter contains clusters of CpG dinucleotides [38], which can be methylated by DNA methyltransferases. Indeed, the hTert promoter is hypermethylated in untransformed, differentiated and senescent cells that do not express telomerase [39]. In contrast, some cancer cells show high levels of telomerase activity despite having a densely methylated promoter [40], highlighting the fact that telomerase levels in the cell depend on both genetic and epigenetic factors.

Table 1

Transcription factors shown to regulate hTert gene expression.

Transcription factors	Role	References
AP-1	Repressor	[33]
BRCA-1	Repressor	[20]
Mad 1	Repressor	[22]
Mdm2	Repressor	[24]
Menin	Repressor	[23]
MZF-2	Repressor	[35]
P53	Repressor	[25]
RAK/BRIT1	Repressor	[23]
SIP-1	Repressor	[23]
Tax	Repressor	[36]
TGF-β	Repressor	[25]
Wt-1	Repressor	[68]
E2F-1	Repressor in cancer cells	[26]
E2F-1	Activator in normal cells	[27]
Estrogen	Activator	[29,30]
Sp1	Activator	[19]
STAT3	Activator	[34]
C-Myc	Activator	[16,17]
U2F1/2	Activator	[32]
Survivin	Activator	[69]

Besides the above-described genetic and epigenetic regulators of hTert expression, telomerase-mediated telomere maintenance and elongation are also likely to depend on telomere structure. Telomere structure is regulated both by the telomere-binding proteins and by specific chromatin modifications at telomeres [3,5,6••,7••,41,42]. As mentioned above, telomeres show histone modifications characteristic of heterochromatic and 'silenced' chromatin domains, such as tri-methylation of H3K9 and H4K20 and binding of HP1 [3,5,6••,7••]. Furthermore, loss of these heterochromatic marks at telomeres [5,6••,7••] leads to a less compacted chromatin and to abnormal telomere elongation, suggesting a higher-order control of telomere length by the state of telomeric chromatin. The current view is that the action of telomerase at individual chromosome ends is likely to be controlled by a balance between the molecular interactions that recruit telomerase to telomeres and the negative feedback mechanisms that maintain telomeres within a set size range and that involve changes in telomere structure. In support of this notion, it has been reported that telomerase does not elongate all telomeres at the same time but selectively acts on the shortest telomeres [43••]. In particular, yeast telomerase only elongates a subset of telomeres (40%) within a single cell cycle, showing a strong preference (around six-fold) for the shortest ones [43••]. Similarly, telomerase re-introduction in telomerase-deficient mice with critically short telomeres specifically elongates the shortest telomeres [44,45]. These observations imply that telomere length influences whether the chromatin at telomeres is in a 'closed' or 'open' conformation for telomerase, which in turn depends on both histone modifications and the telomere-binding proteins. In this regard, shelterin, a protein complex formed by six telomere-specific binding factors — TRF1, TRF2, TIN2, Rap1, TPP1 and POT1 — is proposed to modulate the access of telomerase to telomeres [1••,42]. In support of this, decreased TRF1 binding to telomeres by inhibition of TRF1 ADP-ribosylation has been shown to reduce the affinity of telomerase for telomeres and to enhance the efficacy of telomerase inhibitors in human cancer cells [46]. All together, these findings underline the importance and complexity of telomerase regulation in order to achieve a fine balance between the need to maintain telomeres

within a functional length and the need to prevent aberrant telomere elongation.

Telomerase and stem cell behavior

Telomerase is up-regulated in cells that undergo rapid expansion, such as lymphocytes or keratinocytes, and notably in germ cells and in different stem cell compartments, even within tissues with a low cell turnover such as the brain [47]. The fact that telomerase activity is largely restricted to stem cells suggests that telomerase levels in these cells may be determinant for organism fitness. Indeed, mutations in the telomerase core components, Tert and Terc, are present in patients suffering from aplastic anemia and dyskeratosis congenita. Both diseases are characterized by skin abnormalities and bone marrow failure, the latter resulting from defects in maintaining the hematopoietic stem cell compartment [48–50]. Moreover, cancer and aging, two biological processes in which telomerase activity has been implicated, are increasingly seen as stem cell diseases [3,51]. In particular, cancer may often originate from the transformation of normal stem cells, while aging has been associated with a progressive decline in the number and/or functionality of certain stem cells [3].

During the past few years the specific role of telomerase in different stem cell compartments has started to be elucidated, mostly in well-characterized stem cell subtypes such as hematopoietic stem cells (HSCs), epidermal stem cells (ESCs) and neural stem cells (NSCs). In particular, HSCs derived from human and mice lose telomeric DNA with age despite the presence of detectable telomerase activity [52,53]. This progressive telomere shortening is proposed to act as a developmental barrier for HSCs, which may limit hematopoietic regeneration. In support of this notion, HSCs from telomerase-deficient mice with short telomeres show a reduced ability to repopulate irradiated mice [54,55]. Interestingly, stabilization of telomere length in these cells by Tert over-expression throughout the hematopoietic system is not sufficient to extend their transplantation capacity, suggesting that additional telomere-independent barriers limit HSC regeneration capacity [56].

The use of loss-of-function and gain-of-function mouse models for telomerase has also served to establish the role of telomere length and telomerase activity on ESC behavior. Telomere shortening in the context of telomerase-deficient mice has been shown to result in decreased functionality of their skin ESC compartment [57••]. In particular, mobilization (proliferation and migration) of ESCs out of the hair follicle niche upon mitogen-induced proliferation is partially inhibited in mice with a slight reduction in telomere length (G1 $Terc^{-/-}$ mice) and strongly inhibited in mice with critically short telomeres (G3 $Terc^{-/-}$ mice) [57••]. The immediate consequences of such mobilization defects are lower rates of proliferation in the hair follicle stem cell niche and in the adjacent transient-amplifying compartments, resulting in defective hair growth and a stunted hyperplasic response [57••].

Interestingly, transgenic mice with constitutive Tert over-expression in the epidermis including the ESC compartment (K5-*mTert* mice) present increased ESC mobilization upon treatment with proliferation stimuli. This increased ESC mobilization is concomitant with increased keratinocyte proliferation, enhanced hair growth and augmented skin hyperplasia [57••]. Similar results regarding ESC activation and hair growth have been reported using a different transgenic mouse in which Tert is over-expressed in a conditional manner [58••]. Interestingly, in the later study, the hair-growth-promoting effects of Tert were found to be independent of the telomerase RNA component and therefore of telomerase activity, suggesting a non-canonical role for Tert in addition to its known role in telomere synthesis. However, the potential involvement of Tert independent of Terc in other *in vivo* proliferative responses is still unclear, since it has been recently shown that absence of Terc abrogates the enhanced skin tumorigenesis and wound healing responses shown by transgenic mice that constitutively over-express Tert in the skin [59]. These different requirements for Terc in epidermal growth versus hair growth may be explained by the existence of distinct cell populations involved in these processes. Indeed, recent data indicate the existence of distinct stem cells populations within the epidermis, which are separately involved in regenerating either the hair follicles or the epidermis [60–62].

Besides the skin, other tissues with a high cell turnover, such as bone marrow, intestine and testis, show atrophies in telomerase-deficient mice with critically short telomeres [63,64], supporting the notion that telomere length is a determinant for tissue fitness in the wide context of the organism.

Finally, it is important to note that the effects of telomere length and telomerase activity on different stem cell compartments (ESC, HSC and adult NSC) are cell-autonomous, as demonstrated using *in vitro* clonogenicity assays [54,57••,65]. This fact is relevant for designing potential therapeutic strategies based on telomerase reactivation, since it indicates that the effects of telomerase and telomere length on stem cell behavior are intrinsic to the stem cells and do not depend on physiological niche micro-environments.

Terc as an optimal target for telomerase inhibition in cancer

As discussed above, Terc is required for the tumor-promoting effects of transgenic Tert over-expression *in vivo* [59], as well as to maintain the enhanced proliferative response of Tert-transgenic ESCs *in vitro* [57••]. Similarly, it has recently been reported that Terc is needed to

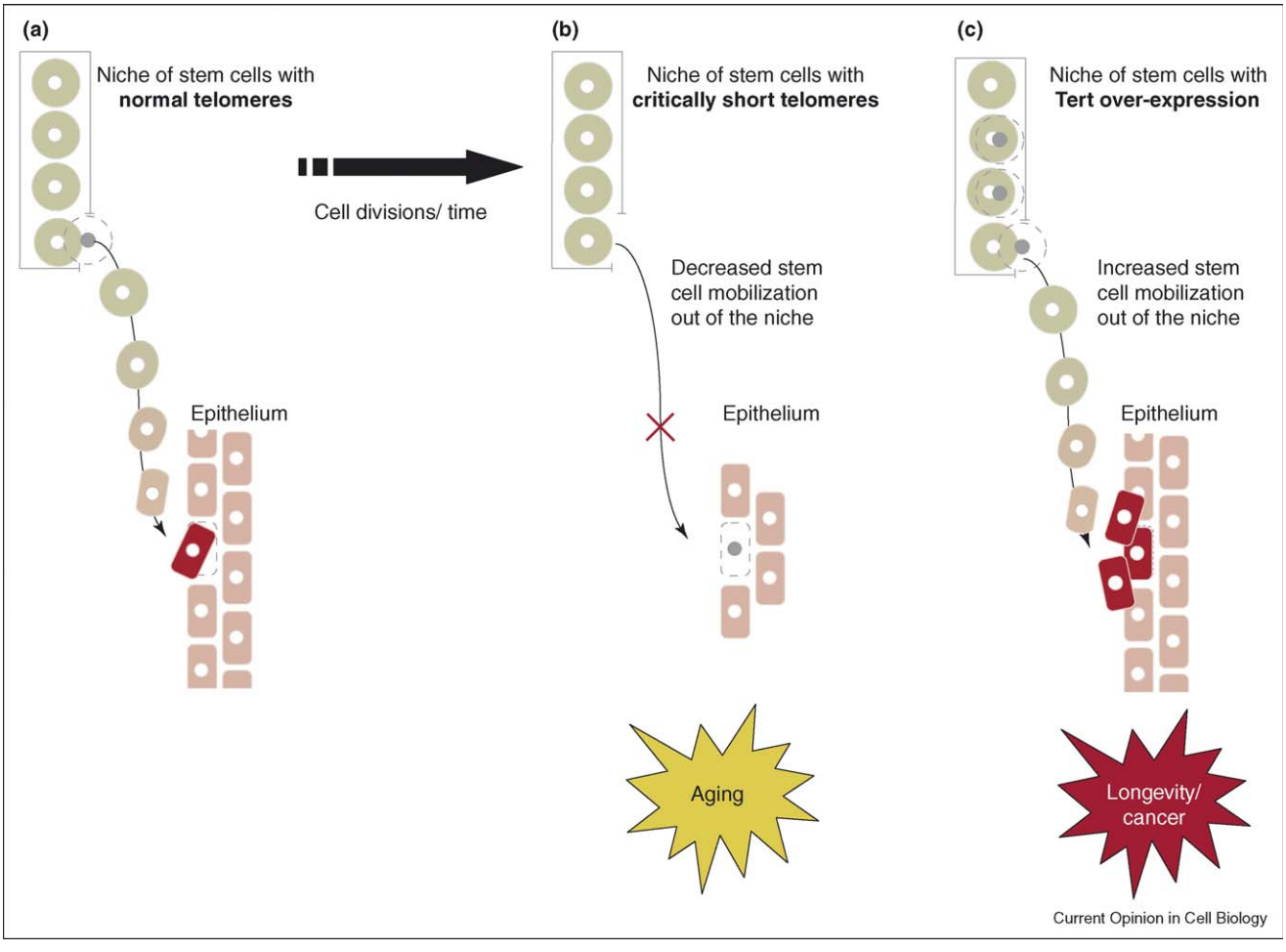
maintain cell growth in different human cancer cell lines that over-express Tert [66,67]. In particular, Terc knockdown rapidly inhibits the growth of human cancer cells in the absence of bulk telomere shortening or telomere uncapping [66,67]. These results uncover novel roles for telomerase independent of telomere maintenance, which require Terc, therefore highlighting Terc as an optimal target for telomerase-mediated therapeutic intervention, even when telomeres are sufficiently long.

Conclusions and perspectives

Despite great progress having been made on how telomere length and telomerase activity are regulated by genetic and epigenetic factors, additional biochemical and genetic studies are required to fully understand these processes during normal development and disease. Further knowledge on how telomerase is regulated should provide new avenues for targeting telomerase in cancer patients as well as in premature aging pathologies associated with short telomeres.

Importantly, the fact that telomerase is specifically expressed in highly proliferative stem/progenitor compartments has opened the possibility that telomerase may be viewed as a 'stem cell' factor. In fact, both telomere length and telomerase levels have profound effects on stem cell behavior. However, the precise role of telomeres and telomerase in specific stem/progenitor

Figure 1



A general model for cancer and aging based on telomeres, telomerase and stem cell (SC) behavior. **(a)** Despite the presence of telomerase in SC compartments, SC telomeres progressively shorten as we advance in life. In consequence, SCs gradually lose their ability to mobilize out of the niche and to regenerate different organs. Decreased SC mobilization also reduces the probability of accumulating abnormal cells in tissues, which provides a mechanism for cancer protection. The ultimate consequence of impaired mobilization, however, will be organ failure due to tissue degeneration **(b)**. **(c)** In contrast, SCs that possess high telomerase activity (high number of functional Tert/Terc complexes) mobilize their SCs more efficiently than normal, which may increase cell numbers in tissues and therefore the risk of tumor formation. On the other hand, under these conditions of higher mobilization tissue fitness would be maintained for longer times, therefore increasing life span. Finally, since all the ESC mobilization effects are detected months before any sign of premature aging or spontaneous tumor formation occurs in telomerase mutant mice, stem cell functionality could be used to predict the fate of individuals.

compartments is still emerging. Novel areas have yet to be explored, such as the different signalling networks that connect telomerase activity and telomere state with stem cell functionality. A careful analysis of telomere biology in stem cells will help us to refine our current model of how we age or suffer from diseases such as cancer (Figure 1).

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- •• of outstanding interest

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