

Telomere length, stem cells and aging

Maria A Blasco

Telomere shortening occurs concomitant with organismal aging, and it is accelerated in the context of human diseases associated with mutations in telomerase, such as some cases of dyskeratosis congenita, idiopathic pulmonary fibrosis and aplastic anemia. People with these diseases, as well as *Terc*-deficient mice, show decreased lifespan coincidental with a premature loss of tissue renewal, which suggests that telomerase is rate-limiting for tissue homeostasis and organismal survival. These findings have gained special relevance as they suggest that telomerase activity and telomere length can directly affect the ability of stem cells to regenerate tissues. If this is true, stem cell dysfunction provoked by telomere shortening may be one of the mechanisms responsible for organismal aging in both humans and mice. Here, we will review the current evidence linking telomere shortening to aging and stem cell dysfunction.

Telomeres are specialized chromatin structures at the ends of eukaryotic chromosomes that prevent the chromosome ends from being recognized as a DNA break¹. In vertebrates, telomeres are composed of TTAGGG repeats bound by a protein complex called shelterin (also known as the telosome)^{2,3}. Shelterin components have roles in chromosome protection and in the regulation of telomere length^{2,3}.

Telomere repeats are generated by a cellular reverse transcriptase known as telomerase (telomerase reverse transcriptase, or *Tert*)¹. Telomerase recognizes the 3'-OH at the ends of chromosomes and adds telomere repeats *de novo* by using an associated RNA molecule as a template (telomerase RNA component, or *Terc*)¹. Whereas unicellular eukaryotes have unlimited amounts of telomerase and maintain telomeres at a constant length, most multicellular eukaryotes have limited amounts of telomerase, and telomere shortening occurs coupled to cell division owing to the incapacity of normal DNA polymerases to copy the very ends of chromosomes⁴. Telomere shortening can be further accelerated by the action of nucleases or certain DNA damaging agents. Interestingly, telomere attrition is observed with increasing age in all human tissues in which it has been tested⁵; this reflects the accumulated cell divisions associated with tissue renewal. Indeed, telomere shortening is one of the best-understood mechanisms known to impose a limit on the growth of normal cells in culture, a phenomenon also known as "replicative senescence"⁶.

A number of age-related pathologies and premature aging syndromes are characterized by a faster-than-normal rate of telomere shortening, which suggests that telomere shortening may be a cause of organismal aging. A demonstration for this came from the study of the *Terc*-deficient mouse model. *Terc*-deficient mice show accelerated telomere shortening and a shorter lifespan, which are further aggravated with increasing mouse generations until no more generations can be derived owing to male and female infertility⁷⁻⁹. However, the pathways by which short telomeres provoke aging are still far from being understood. On one hand, telomere shortening may promote aging by inducing apoptosis and cell cycle arrest

in vivo, thus leading to cell loss and tissue dysfunction. On the other hand, telomere shortening may also impair the ability of stem cells to regenerate tissues, thus leading to tissue failure¹⁰. Here, we will review recent data suggesting the importance of telomere length in cancer and aging.

The telomere structure

Vertebrate telomeres end in a 3' overhang of the G-rich strand (G-strand overhang)¹¹, which is generated by the postreplicative processing of the C-rich strand¹², and that is the substrate for telomerase-mediated telomere elongation¹ (Fig. 1a). The G-strand overhang can fold back and invade the double-stranded region of the telomere, thereby generating a looped structure known as the telomere loop or T-loop¹³, which hides the 3' end from telomerase and from DNA repair and degradation activities (Fig. 1a). T-loops may represent a primitive mechanism for telomere protection¹⁴. Furthermore, the fact that they resemble intermediates of homologous recombination suggests that they may be regulated by activities involved in this DNA repair pathway¹⁵.

Telomere repeats are bound by the so-called shelterin multiprotein complex² (Fig. 1a). Shelterin contains factors that bind directly to the G-strand overhang, such as the protection of telomeres 1 (Pot1)-TTP1 heterodimer^{16,17}, and to the double-stranded telomeric region, such as the telomere repeat binding factors TRF1 and TRF2 and their interacting proteins Rap1 (repressor activator protein 1) and Tin2 (TRF1-interacting nuclear protein 2) (Fig. 1a)^{2,3}. TRF1 also recruits to telomeres the TANK1 and TANK2 poly(ADP)-ribosylases (also known as tankyrases)¹⁸. TRF1 and the TRF1-interacting proteins have been proposed to regulate telomere length by controlling the access of telomerase to the telomere¹⁹. TRF2 and Pot1 are also important for telomere length regulation and have additional roles in telomere protection by preventing end-to-end chromosome fusions¹⁹⁻²². The role of TRF2 in telomere protection may be related to their interaction with DNA-damage signaling and repair factors²³. In particular, TRF2 can interact with components of the Mre11 complex²⁴, which is central to both nonhomologous end joining (NHEJ) and homologous recombination. More recently, TRF2 has been shown to interact with the nucleotide excision repair XPF-ERCC1 nuclease²⁵, the Apollo nuclease^{26,27}, and the DNA signaling factor MDC1 (ref. 28). Furthermore, TRF2 has been proposed to bind to ATM and to cancel the ATM-triggered damage

Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre, 3 Melchor Fernandez Almagro, 28019 Madrid, Spain. Correspondence should be addressed to M.A.B. (mblasco@cnio.es).

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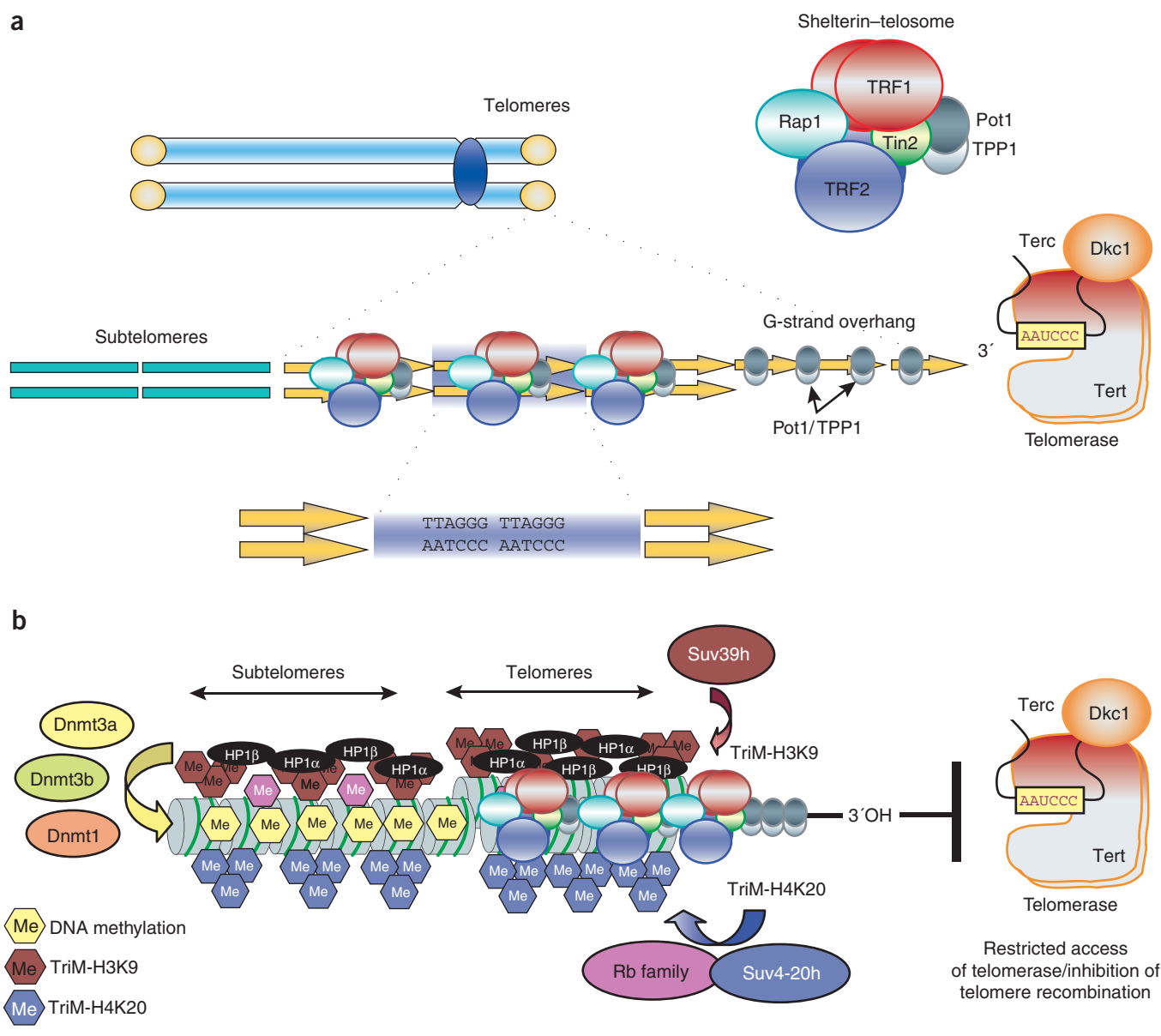


Figure 1 Telomere structure. **(a)** Mammalian telomeres consist of tandem repeats of the TTAGGG sequence that are bound by the shelterin-telosome protein complex. Adjacent to telomeres are the subtelomeric regions, which are also rich in repetitive DNA. **(b)** In addition to shelterin, mammalian telomeres also contain nucleosomes that show histone modifications characteristic of heterochromatin domains. In addition, subtelomeric DNA is heavily methylated. These chromatin modifications at telomeres and subtelomeres have been shown to negatively regulate telomere length and telomere recombination. TriM, trimethyl.

response, which suggests that TRF2 has a role in preventing a DNA damage response at telomeres^{29,30}. A similar role has been proposed for Pot1 based on the fact that Pot1-deficient mice show increased DNA damage signaling at telomeres²². Components of the NHEJ (Ku and DNA-PKcs) and homologous recombination (Rad51D, Rad54, XRCC3) pathways have also been shown to have roles in telomere length regulation and telomere capping^{12,15,31–33}.

In addition to the shelterin complex, both telomeres and subtelomeres are bound by nucleosomes^{34,35} that are enriched in histone modifications characteristic of constitutive heterochromatin domains^{36–38} (Fig. 1b). These histone marks include trimethylation of H3K9 and H4K20 by the histone methyltransferases suppressor of variegation 3-9 homologs (Suv39h1 and h2) and suppressor of variegation 4-20 homologs (Suv4-20h1 and h2), respectively^{36–41}. Furthermore, the

heterochromatin proteins HP1 β , HP1 γ and HP1 α are also recruited to telomeric and subtelomeric domains through their affinity for trimethylated H3K9 residues^{36–38,42,43} (Fig. 1b). These epigenetic marks are characteristic of compacted and transcriptionally silenced heterochromatin domains, such as those found at pericentric heterochromatin^{39–41,43}. In addition, telomeric and subtelomeric regions show low abundance of acetylated H3 (AcH3) and H4 (AcH4)⁴² and hypermethylation of subtelomeric DNA^{37,44}, which further supports that telomeres are silenced chromatin domains. The formation and maintenance of these silenced heterochromatin domains at telomeres has been proposed to act as a negative regulator of telomere elongation (Fig. 1b). In particular, disruption of either histone trimethylation^{36,38} or DNA methylation³⁷ results in abnormally elongated telomeres as well as increased recombination between telomeric sequences (Fig. 1b).

Telomere elongation mechanisms

The main mechanism for telomere elongation in mammals is the enzyme telomerase¹ (Fig. 2a). A recent study with highly purified telomerase extracts has demonstrated that the telomerase enzyme contains two molecules each of the telomerase reverse transcriptase subunit (Tert) and the telomerase-associated RNA molecule (Terc), as well as one molecule of dyskerin⁴⁵, a protein known to stabilize the telomerase complex⁵ (Fig. 2a). The attrition of telomeric DNA that takes place during aging is likely to result from limiting amounts of telomerase activity in the adult organism, which cannot compensate for the progressive telomere shortening that occurs as cells divide during tissue regeneration^{4,5,23}. This progressive telomere loss has been proposed to contribute to organismal aging. In turn, the vast majority of tumors and immortal cell lines have high levels of telomerase, which is thought to sustain their immortal growth by preventing telomere shortening and bypassing senescence and apoptosis²³.

Interestingly, some immortal cell lines and tumors that lack telomerase activity are still able to maintain or elongate their telomeres through activation of mechanisms known as alternative lengthening of telomeres (ALT)^{46,47}. In yeast and mammals, ALT has been shown to involve homologous recombination events between telomeric sequences^{46–48} (Fig. 2b). ALT-positive cells are characterized by heterogeneous telomere lengths, with both very short and very long telomeres being present at the same time, and by the colocalization of telomeres with a specific type of promyelocytic leukemia (PML) body—so-called ALT-associated PML bodies (APBs)^{46–48} (Fig. 2b). ALT mechanisms are also activated in *Terc*-deficient mice in cultured mouse embryonic fibroblasts and embryonic stem cells^{49–51}, and *in vivo* during germinal center formation⁵², which indicates that ALT mechanisms can also be selected in nontumoral settings. However, while ALT can rescue the viability of telomerase-deficient yeast strains⁴⁸, ALT mechanisms cannot rescue the viability of *Terc*-deficient mice, which suggests that ALT mechanisms do not operate to rescue survival of most multicellular organisms.

The fact that ALT is mostly restricted to *Terc*-deficient mice, as well as immortal cell lines and tumors, indicates the existence of mechanisms that actively repress ALT in normal cells. Recent data indicates that components of shelterin complex such as Pot1 and TRF2, or TRF2-interacting proteins such as WRN, can influence telomere recombination^{53–55} and are potential regulators of ALT. Similarly, subtelomeric DNA methylation^{37,42} and histone methylation at telomeres^{42,56} are potent repressors of telomere recombination and ALT activation. However, a causal relationship between ALT activation in tumors and defects in these telomere length regulators is still pending.

Mouse models

The telomerase-deficient mouse model. The *Terc*-deficient mouse model has been instrumental for studying the impact of short telomeres in cancer and aging and is currently considered one of the best models for studying telomere-driven aging. *Terc*-deficient mice were first generated by elimination of the mouse *Terc* gene^{7,57}. The long-term viability of the *Terc*^{-/-} mouse strain is severely compromised, and only a limited number of generations can be derived owing to infertility and the progressive development of pathologies associated with loss of telomeric repeats^{7,8,23,52,58–61} (Table 1 and Fig. 3). These pathologies include male and female infertility, heart failure, and immunosenescence, as well as decreased regeneration of the digestive system, the skin, and the hematopoietic system, among others^{7,8,23,52,58–61} (Table 1). Dysfunctional telomeres in *Terc*-deficient mice are detected as damaged DNA, thereby triggering cell cycle arrest or apoptosis at the cellular level^{60–64} (Fig. 3).

Of notice, the pathologies developed by telomerase-null mice recapitulate human diseases associated with aging that are also characterized

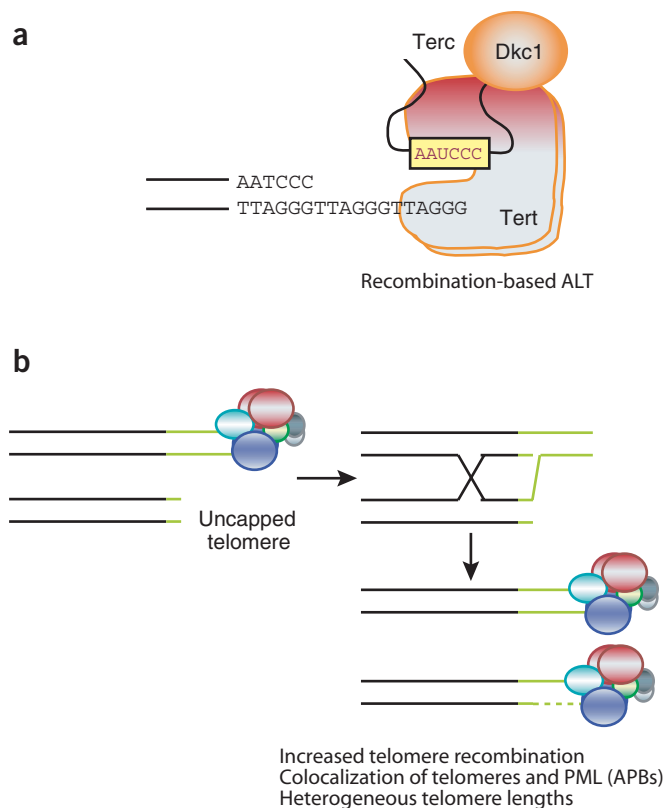


Figure 2 Telomere elongation mechanisms. (a) Telomerase is the main mechanism for telomere length elongation. The telomerase enzyme consists of two molecules of the Tert subunit and two molecules of the associated Terc, which contains the template for the addition of new telomeric repeats. In addition, telomerase contains one molecule of dyskerin, a protein that stabilizes the telomerase complex. Telomerase recognizes the 3' end of the G-rich telomere strand and adds telomeric repeats *de novo*. (b) Telomerase-deficient cells can also maintain telomeres by means of homologous recombination between telomeres, a mechanism known as alternative lengthening of telomeres (ALT).

by defective proliferation and short telomeres (Table 2). By contrast, those age-associated human diseases that are characterized by increased proliferation, such as cancer or atherosclerosis, are not reproduced in the *Terc*-deficient mouse model^{62,65}, which suggests that development of these diseases requires further alterations to bypass the barrier that short telomeres impose on proliferation⁶⁶. In this regard, the *Terc*-deficient mouse model has also been instrumental in dissecting the role of telomeres and telomerase in tumorigenesis (Table 1). In particular, *Terc*-deficient mice with short telomeres are resistant to both induced and spontaneous tumorigenesis⁶². Furthermore, mice that are simultaneously deficient in telomerase and the tumor suppressor proteins p19ARF, p16, p21, APC, ATM, DNA-PKcs, Ku, PARP1 and PMS2 also show reduced tumorigenesis^{67–73} (Table 1). This indicates that short telomeres are potent suppressors of cancer even in tumor-prone genetic backgrounds, most likely because telomere dysfunction induces cellular arrest and apoptosis^{66–73} (Fig. 3). As an exception to this, telomerase deficiency and short telomeres in the context of p53 mutant mice and in the context of TRF2 overexpression lead to a further increase of epithelial tumors characterized by high levels of chromosomal instability, which suggests that mutation of these pathways helps bypass the barrier that short telomeres impose on tumor development^{53,74,75} (Fig. 3).

Interestingly, whereas p53 deficiency rescues degenerative pathologies in *Terc*-deficient mice at the expense of increased tumorigenesis^{74,75} (Fig. 3 and Table 1), p21 abrogation significantly rescues degenerative pathologies and survival of *Terc*-deficient mice without increasing tumorigenesis⁶⁹ (Fig. 3 and Table 1). These different outcomes of p53 and p21 abrogation may be explained by the fact that p21 deficiency rescues proliferative defects but not apoptosis in *Terc*-deficient mice. These results highlight p21 as an important mediator of cell cycle arrest associated with short telomeres. Furthermore, they suggest that cell cycle arrest rather than apoptosis is the main mechanism underlying telomere-driven aging (Fig. 3). In turn, the fact that tumorigenesis is not increased in the context of simultaneous p21 and telomerase deficiencies in spite of increased proliferation pinpoints apoptosis as a major anti-tumoral barrier in response to short telomeres (Fig. 3). More recently, a

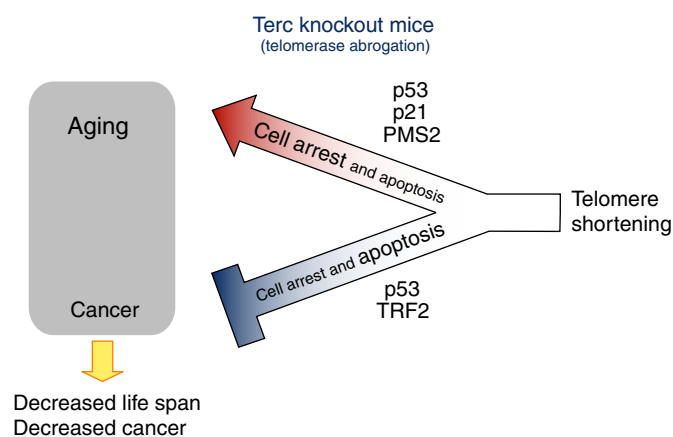
similar role has been described for the mismatch repair protein PMS2, which seems to act in the same pathway as p21 in mediating cell arrest and aging provoked by short telomeres⁷⁰ (Fig. 3 and Table 1).

Mice with altered expression of the shelterin proteins. A putative role for mammalian shelterin components in disease is suggested by the fact that TRF1, TRF2 and Tin2 are upregulated in some human carcinomas^{76–80}. Upregulation of these proteins may have an active role in promoting cancer, especially considering that shelterin components have important roles in regulating telomere length and telomere protection. In particular, TRF2 has been involved in telomere-length regulation¹⁹ and in preventing end-to-end chromosome fusions^{20,21}. Interestingly, mice with increased TRF2 expression in stratified epithelia show dramatically short telomeres and a premature deterioration of the skin, as

Table 1 Mouse models for understanding the role of telomerase and telomere-binding proteins in cancer and aging

	Genotype	Aging phenotype	Cancer phenotype	References
Models of telomerase abrogation	<i>Terc</i> ^{-/-}	Defects in neural tube closure, hypogonadism, infertility, immunosenescence, intestinal atrophy, alopecia, hair graying, heart dysfunction, bone marrow aplasia, kidney dysfunction, hypertension, defective bone marrow, defects in skin, and neural stem cell proliferation	Less cancer	7,8,23,58–61
	<i>Terc</i> ^{-/-} (<i>p16</i> and <i>p19ARF</i>) ^{-/-}	Similar to single <i>Terc</i> ^{-/-} mice	Less cancer	67,72
	<i>Terc</i> ^{-/-} <i>p21</i> ^{-/-}	Delayed aging and rescue of lifespan	Less cancer	69
	<i>Terc</i> ^{-/-} <i>ATM</i> ^{-/-}	Ataxia telangiectasia-like aging pathologies, including neurological defects	Less cancer	73
	<i>Terc</i> ^{-/-} <i>ATM</i> ^{-/-} <i>p53</i> ^{-/-}	Not studied	Chromosomally unstable tumors with genomic alterations similar to those observed in human cancer	75
	<i>Terc</i> ^{-/-} <i>DNA-PKcs</i> ^{-/-}	Accelerated aging	Less cancer	71
	<i>Terc</i> ^{-/-} <i>Ku86</i> ^{-/-}	Accelerated aging	Less cancer	71
	<i>Terc</i> ^{-/-} <i>BLM</i> ^{-/-}	Bloom syndrome-like aging pathologies including bone loss and reduced body weight	Not studied	106
	<i>Terc</i> ^{-/-} <i>WRN</i> ^{-/-}	Werner syndrome-like aging pathologies including bone loss, type II diabetes and cataracts	Not studied	105,106
	<i>Terc</i> ^{-/-} <i>APC</i> ^{min}	Not studied	Less cancer	68
	<i>Terc</i> ^{-/-} <i>p53</i> ^{+/-}	Not studied	More cancer	74
	<i>Terc</i> ^{-/-} <i>ApoE</i> ^{-/-}	Less atherosclerosis	Not studied	65
	<i>Terc</i> ^{-/-} <i>PARP1</i> ^{-/-}	Slightly accelerated aging	Less cancer	71
<i>Terc</i> ^{-/-} <i>PMS2</i> ^{-/-}	Delayed aging and rescue of life span	Less cancer	70	
Models of telomerase overexpression	<i>K5-Tert</i>	Less kidney and germ line dysfunction; slightly extended maximum lifespan	More cancer	113,122,123
	<i>Actin-Tert</i>	Not studied	More cancer	115,124
	<i>Lck-Tert</i>	Not studied	More cancer	125
Models of shelterin abrogation	<i>TRF2</i> ^{-/-}	Embryonic lethal; conditional deletion in liver not essential for liver regeneration	Not studied	21,82
	<i>TRF1</i> ^{-/-}	Embryonic lethal; not studied	Not studied	83
	<i>Pot1a</i> ^{-/-}	Not studied	Not studied	22,54
	<i>Pot1b</i> ^{-/-}	Not studied	Not studied	22
	<i>Tin2</i> ^{-/-}	Embryonic lethal; not studied	Not studied	85
Models of shelterin overexpression	<i>K5-TRF2</i>	Skin hyperpigmentation and premature skin aging	More skin cancer	78
	<i>K5-TRF2 Terc</i> ^{-/-}	Accelerated skin aging	Accelerated cancer	78,53
	<i>K5-TRF2 XPF</i> ^{-/-}	Rescue of some skin aging phenotypes	Not studied	78
	<i>K5-TRF2 K5-Tert</i>	TRF2-induced aging phenotypes not rescued	Not studied	78

APC^{min}, adenomatous polyposis coli min (multiple intestinal neoplasia) allele; *ApoE*, apolipoprotein E; *ATM*, ataxia telangiectasia mutated; *BLM*, Bloom syndrome; *DNA-PKcs*, DNA-dependent protein kinase catalytic subunit; *Ku86*, Ku antigen protein 86; *Lck*, lymphocyte-specific protein tyrosine kinase; *PARP1*, poly(ADP-ribose)polymerase family 1; *Terc*, telomerase RNA component; *Tert*, telomerase reverse transcriptase; *WRN*, Werner syndrome; *PMS2*, postmeiotic segregation increased 2; *TRF1*, telomere repeat factor 1; *TRF2*, telomere repeat factor 2; *Tin2*, TRF1-interacting nuclear factor 2; *Pot1a*, protection of telomeres 1a; *Pot1b*, protection of telomeres 1b; *XPF*, xeroderma pigmentosum group F-complementing protein.



well as increased UV-induced skin carcinogenesis, which recapitulates the skin pathologies associated with the human tumor-prone xeroderma pigmentosum syndrome⁷⁸ (Table 1). Overexpression of telomerase is not able to prevent telomere shortening or the aging pathologies in these mice⁷⁸ (Table 1), which suggests that short telomeres generated by TRF2 overexpression are not susceptible to rescue by telomerase⁷⁸. Short telomeres in TRF2-overexpressing mice are rescued, however, in the absence of the TRF2-interacting XPF nuclease^{25,81} (Table 1). A current model suggests that TRF2 overexpression sequesters most XPF-ERCC1 at telomeres, which results in rapid telomere degradation and induction of skin aging phenotypes; at the same time depletion of XPF-ERCC1 from the rest of the chromatin results in defective nucleotide excision repair (NER), which leads to increased UV-induced DNA damage and skin cancer⁷⁸. Interestingly, the fact that the xeroderma pigmentosum skin phenotypes shown by TRF2 transgenic mice are not observed in mice deficient for different NER components suggest that human xeroderma pigmentosum pathologies are likely the result of a combination of NER deficiency and the presence of critically short telomeres.

Finally, the increased epithelial carcinogenesis shown by TRF2-overexpressing mice is aggravated in the absence of telomerase activity, coincidental with increased chromosomal instability and telomere recombination^{53,78}, which suggests that increased TRF2 expression is actively contributing to tumor development by helping to bypass the cell proliferation barrier imposed by short telomeres by means of inducing ALT pathways, therefore promoting the growth of tumors with a high chromosomal instability (Fig. 3 and Table 1).

Loss-of-function mouse models to study the effects of TRF2 abrogation in the adult organism have been also generated²¹. TRF2 abrogation results in embryonic lethality²¹. However, cells derived from *TRF2*^{-/-} *Trp53*^{-/-} embryos show a dramatic loss of telomere protection and a massive increase in end-to-end chromosome fusions²¹ (Table 1). Strikingly, TRF2 conditional deletion in adult hepatocytes has no effect on viability or on liver regeneration capacity⁸², which suggests tissue specificity in the requirements for TRF2 function. Further studies knocking out TRF2 in additional cell types are required to understand the impact of TRF2 deficiency in organismal and cellular viability. Similarly, the potential effects of TRF2 abrogation on tumorigenesis still need to be determined.

A role for the TRF1 shelterin component in human disease is unknown other than the fact that this protein is upregulated in some human tumors^{76,77,79,80}. Mice deficient in TRF1 show embryonic lethality in the absence of defective telomere length or loss of telomere protection⁸³ (Table 1). Interestingly, a role for TRF1 in mitosis has also been suggested⁸⁴ that may explain mouse lethality. Similarly,

Figure 3 The telomerase knockout mouse as a model for telomere-induced aging. Telomere shortening in the context of *Terc*-deficient mice leads to premature loss of mouse viability and decreased lifespan associated with a number of degenerative pathologies. These pathologies can be rescued in the absence of p53, p21 or PMS2, which indicates that these proteins are important mediators of telomere-induced aging. Importantly, the fact that both p21 and PMS2 abrogation only rescue proliferative defects but not apoptosis triggered by short telomeres indicates that cell arrest rather than apoptosis is responsible for telomere-driven aging. In turn, short telomeres impose a barrier on tumor development that can only be bypassed by abrogation of p53 or by TRF2 overexpression, which indicates that these molecular events are important in mediating cancer driven by short telomeres and chromosomal instability.

mice deficient in TIN2 show embryonic lethality independently of telomere length maintenance and telomerase activity⁸⁵ (Table 1). TIN2 colocalizes to nontelomeric heterochromatin domains through its interaction with HP1 γ (ref. 86), which suggests a putative role for this protein in heterochromatin assembly.

Finally, a role for the Pot1 shelterin component in human disease is suggested by recent findings showing that deletion of the genes encoding Pot1 in mice results in chromosomal instability and increased telomere recombination, which in turn may have an impact on carcinogenesis^{22,54} (Table 1). However, the specific involvement of Pot1 in human disease remains to be demonstrated.

Table 2 Telomere shortening and human disease

	Human disease	Telomere protein affected	Reference
Disease states with short telomeres	Atherosclerosis	Not known	94
	Heart failure	TRF2	91
	Liver cirrhosis	Not known	93
	AIDS	Not known	95
	Ulcerative colitis	Not known	92
Hereditary syndromes with short telomeres	Dyskeratosis congenita	<i>Terc</i> , <i>Tert</i> , dyskerin	98–100, 108
	Aplastic anemia	<i>Terc</i> , <i>Tert</i>	101,102
	Idiopathic pulmonary fibrosis	<i>Terc</i> , <i>Tert</i>	103,104
	Werner syndrome	WRN	105,107
	Bloom syndrome	BLM	107
	Fanconi anemia	<i>FANC</i> genes	23
	Ataxia telangiectasia	ATM	106
	Nijmegen breakage syndrome	Nbs1	23
Ataxia telangiectasia disorder	Mre11	23	
Epidemiological factors that result in accelerated rate of telomere shortening	Age	Not known	89
	Gender	Not known	89
	Smoking	Not known	88
	Obesity	Not known	88
	Perceived stress	Telomerase	87
	Socioeconomic status	Not known	90
Short telomeres are predictive of	Age	Not known	89
	Cardiovascular disease	Not known	96
	Infection	Not known	96
	Cognitive impairment	Not known	89
	Dementia	Not known	97

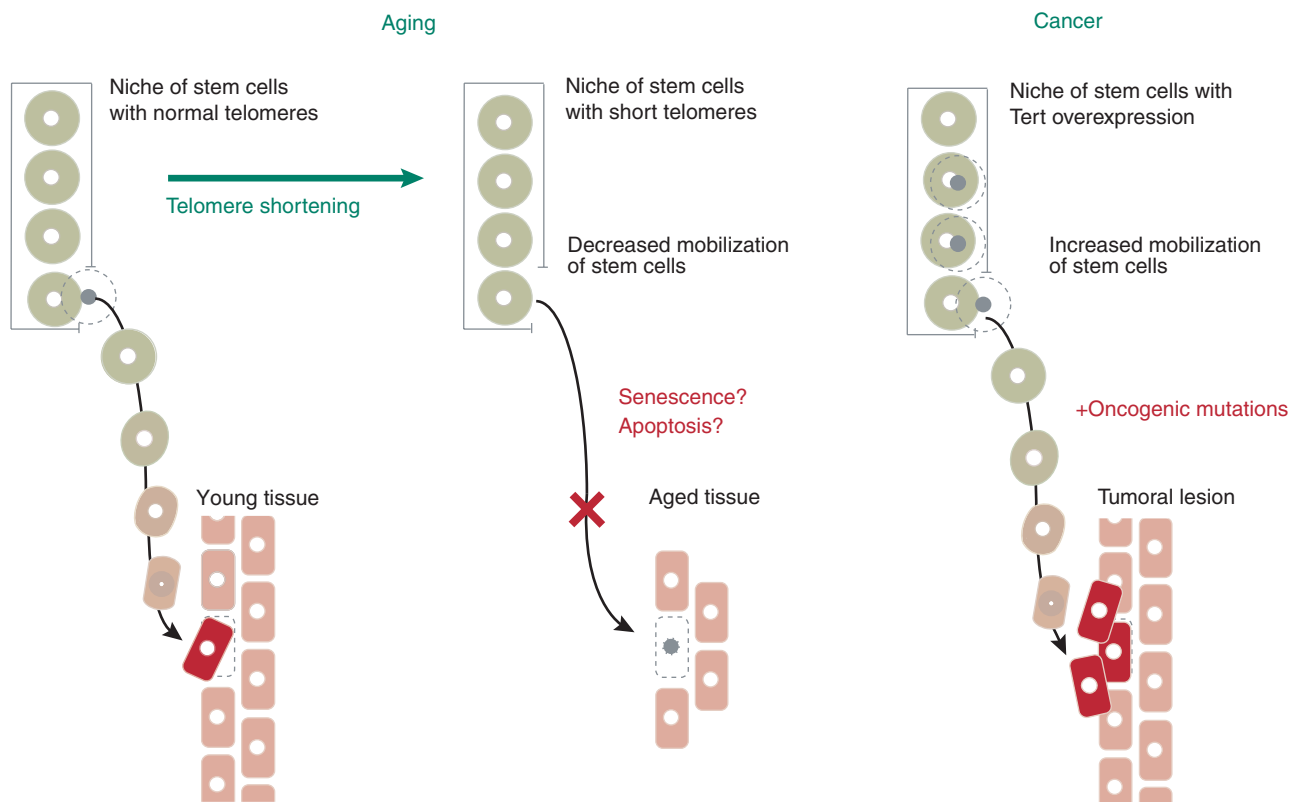


Figure 4 A stem cell theory for the role of telomeres and telomerase in cancer and aging. Telomere shortening leads to a decreased ability of stem cells to leave the stem cell niche and to regenerate tissues. The mechanisms underlying the decreased mobilization of stem cells are still unknown but are likely to involve senescence and apoptosis in response to short telomeres. In turn, telomerase overexpression may lead to increased mobilization of the stem cells out of the niche. This aberrant mobilization of stem cells may contribute to cancer formation in combination with oncogenic mutations.

Telomere shortening in aging pathologies

One of the best-known cell-intrinsic events associated with aging is the progressive shortening of telomeres (Fig. 4). The speed at which telomeres shorten with aging seems to vary between men and women and can be influenced by factors considered to accelerate aging and to be a risk of premature death, such as socioeconomic status, perceived stress, smoking, and obesity, all of which have been proposed to negatively affect telomere length^{87–90} (Table 2). Telomere shortening is also accelerated in various human diseases associated with aging, such as cardiovascular disease and infections, among others^{91–95}. Indeed, a correlation between telomere length and risk of death from heart disease or infections has been reported⁹⁶. Similarly, telomere length has been proposed to be predictive of dementia and cognitive impairment^{89,97}.

In addition, several human syndromes are characterized by mutations in the telomerase genes, which result in accelerated rates of telomere shortening with age (Table 2). These include some cases of dyskeratosis congenita, aplastic anemia and idiopathic pulmonary fibrosis. In particular, people with dyskeratosis congenita carry mutations in components of the telomerase complex that result in decreased telomerase stability and shorter telomeres⁹⁸. These mutations affect either the *TERC* and *TERT* genes (people with the autosomal dominant dyskeratosis congenita variant)^{99,100} or the dyskeratosis congenita 1 gene (*DKC1*; people with the X-linked form of the disease), which encodes a telomerase-interacting protein involved in *Terc* stability and small nucleolar RNA processing⁹⁸. Both mutations result in decreased telomerase activity and shorter telomeres compared with healthy indi-

viduals^{98–100}. Strikingly, people with dyskeratosis congenita develop many of the pathologies shown for the *Terc*-deficient mouse model, such as short stature, hypogonadism and infertility, defects of the skin and the hematopoietic system, bone marrow failure, and premature death. Furthermore, like *Terc*-deficient mice, individuals with dyskeratosis congenita also show increased chromosomal instability with age, which is consistent with a faster rate of telomere loss. Finally, people with dyskeratosis congenita and *Terc*-deficient mice both show disease anticipation in affected progeny, which strongly suggests that short telomeres directly contribute to disease presentation^{9,100}. Of notice, an important difference between people with dyskeratosis congenita and *Terc*-deficient mice is the fact that people with dyskeratosis congenita show an elevated incidence of spontaneous cancer, whereas *Terc*-deficient mice have an increased resistance to cancer, except for those with p53-deficient and TRF2-overexpressing genetic backgrounds^{62,67–75}. Therefore, *Terc*-deficient mice recapitulate closely, but not completely, the human dyskeratosis congenita syndrome pathologies. A reason for this difference may be that, in contrast to *Terc*-null mice, people with dyskeratosis congenita still retain the telomerase genes, which can be upregulated during tumorigenesis.

A number of people diagnosed with aplastic anemia also show mutations in the telomerase *TERC* and *TERT* genes, thus resulting in accelerated telomere shortening and premature death^{101,102} (Table 2). More recently, mutations in the telomerase components have also been found in some cases of idiopathic pulmonary fibrosis, which is an adult-onset lethal disease characterized by lung scarring and subsequent

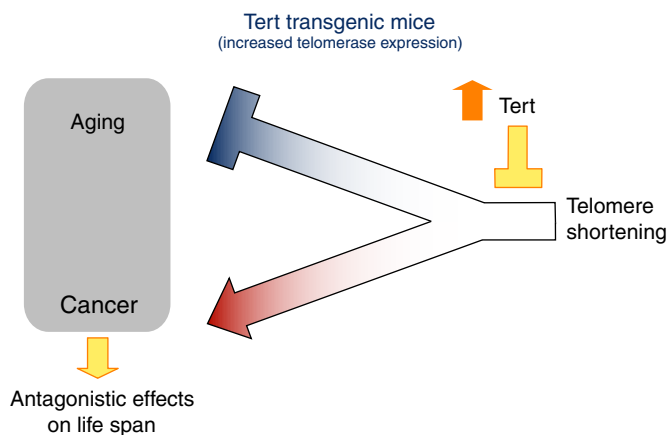


Figure 5 Antagonistic effects of telomerase in cancer and aging. Increased telomerase (*Tert*) expression by means of mouse transgenesis results in decreased aging at the expense of increased tumorigenesis.

respiratory failure. This further expands the number of human diseases characterized by regeneration defects that are associated with telomerase defects^{103,104} (Table 2).

In addition to individuals with dyskeratosis congenita, aplastic anemia or idiopathic pulmonary fibrosis, who have defective telomerase activity and short telomeres, a number of additional human premature aging syndromes are also characterized by an accelerated rate of telomere loss and chromosomal instability (Table 2). Interestingly, these diseases are produced by mutations in DNA repair proteins such as Nbs1 (Nijmegen breakage syndrome), Mre11 (ataxia telangiectasia-like disorder), WRN (Werner syndrome), BLM (Bloom syndrome), ATM (ataxia telangiectasia) and proteins encoded by *FANC* genes (Fanconi anemia) (Table 2), many of which interact with the TRF2 telomere-binding protein²³. The aging pathologies associated with the Werner, Bloom and ATM human syndromes have been modeled in mice only when in combination with telomerase deficiency and short telomeres in the context of the *Terc*-deficient mouse model^{73,105–107} (Table 1), which suggests that short telomeres contribute to the pathobiology of these premature aging diseases in humans.

Telomere shortening and stem cell dysfunction

Cancer and aging, two biological processes in which telomerase activity has been implicated, are increasingly seen as stem cell diseases^{10,23,108}. In particular, cancer may often originate from the transformation of normal stem cells, and aging has been associated with a progressive decline in the number and/or functionality of certain stem cells^{10,108} (Fig. 4).

Although telomerase is expressed at very high levels during embryonic development, its expression is downregulated a few weeks after birth in the majority of adult tissues, with the exception of adult stem cell compartments and cells that undergo rapid expansion, such as lymphocytes or skin keratinocytes¹⁰. The fact that telomerase activity is largely restricted to stem cells suggests that telomerase levels in these cells may be determinant for organism fitness. Evidence for a rate-limiting role of telomerase in human aging and lifespan has come from the study of human diseases associated with mutations in telomerase components. As discussed above, mutations in the telomerase core components *Tert* and *Terc* are present in people that suffer from aplastic anemia, idiopathic pulmonary fibrosis and dyskeratosis congenita (Table 2). These lethal diseases are adult-onset and are characterized by a premature loss of tissue regeneration associated with telomere shortening^{98–104}. In an analo-

gous manner, telomerase deficiency in mice results in decreased median and maximum lifespan already within the first mouse generation, also indicating that telomerase is rate-limiting for aging in mice⁹.

In 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 (HSC)^{109–112}, epidermal stem cells (ESC)^{10,113} and neural stem cells (NSC)¹¹⁴. In particular, HSCs derived from humans and mice lose telomeric DNA with age despite the presence of detectable telomerase activity^{109–112}. 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 *Terc*-deficient mice with short telomeres show a reduced ability to repopulate irradiated mice^{61,111,112}.

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^{10,113,115}. Telomere shortening in the context of *Terc*-deficient mice has been shown to result in decreased functionality of their skin ESC compartment¹¹³. In particular, mobilization (proliferation and migration) of ESCs out of the hair follicle niche upon mitogen-induced proliferation is inhibited in *Terc*^{-/-} mice with critically short telomeres¹¹³ (Fig. 4). This mobilization defect results in lower rates of proliferation in the hair follicle stem cell niche and in the adjacent transient-amplifying compartments, leading to defective hair growth and a stunted hyperplastic response¹¹³.

Interestingly, transgenic mice with constitutive *Tert* overexpression in the epidermis including the ESC compartment (K5-*Tert* mice) present an increased ESC mobilization upon treatment with proliferation stimuli¹¹³ (Fig. 4 and Table 1). This increased ESC mobilization is concomitant with increased keratinocyte proliferation, enhanced hair growth, and augmented skin hyperplasia¹¹³. Similar results regarding ESC activation and hair growth have been reported using a different transgenic mouse in which *Tert* is overexpressed in a conditional manner¹¹⁵ (Table 1). In the latter study, the hair growth-promoting effects of *Tert* were found to be independent of the telomerase RNA component and therefore of telomerase activity, which suggests a noncanonical role for *Tert* other than its known role in telomere synthesis. However, the potential involvement of *Tert* independently of *Terc* in other *in vivo* proliferative responses is still unclear, as it has been recently shown that absence of *Terc* abrogates the enhanced skin tumorigenesis and wound healing responses shown by transgenic mice that constitutively overexpress *Tert* in the skin¹¹⁶. 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^{117,118}.

Besides the skin, other tissues with a high cell turnover such as bone marrow, intestine and testis show atrophies in *Terc*-deficient mice with critically short telomeres^{8,58} (Table 1), which supports 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^{61,113}. In addition, it has been recently shown that short telomeres in the context of the *Terc*-deficient mouse model may also limit the ability of stem cell microenvironments or stroma to sustain the proper functioning of transplanted wild-type stem cells¹¹⁹. All together, these results suggest that telomere shortening with aging is not only an intrinsic factor leading to aberrant stem cell functioning but also may affect the viability of the stem cell environment, thereby further aggravating stem cell dysfunction with aging. This is relevant for designing potential therapeutic strategies based on telomerase reac-

tivation because it indicates that the effects of telomerase and telomere length on stem cell behavior are dependent on both the stem cells and the physiological niche microenvironments.

Telomerase-based therapeutic approaches

The establishment of the role of telomeres and telomerase in human disease has been important for the design of appropriate therapeutic strategies. The fact that diseases associated with human aging and premature aging syndromes are characterized by short telomeres, together with observations from the *Terc*-knockout mouse model demonstrating that short telomeres contribute to these different pathologies, suggests therapeutic potential for strategies based on temporary telomerase reactivation. Therapeutic agents that could be designed to do this would preferentially target those cell types that normally divide to maintain organ homeostasis—such as stem cells, which, although telomerase-proficient, do not have sufficient telomerase activity to maintain telomere length over time. Germ-line reintroduction of telomerase extends inherited short telomeres in mouse models and prevents the occurrence of end-to-end fusions and pathologies associated with short telomeres, such as bone marrow aplasia, atrophy of the intestinal epithelium, hypogonadism and infertility¹²⁰. Similarly, telomerase reactivation could prevent critical telomere shortening and associated pathologies in those premature aging syndromes that are characterized by a faster rate of telomere loss, as well as in age-associated diseases.

By contrast, the fact that the vast majority of human tumors seem to depend on telomerase reactivation to prevent critical telomere loss and to divide indefinitely suggests that telomerase inhibition could be an effective way to abolish tumor growth⁶⁶. An increasing number of therapeutic strategies based on targeting telomerase in cancer have been developed during the last few years, which include pharmacological inhibitors of the enzyme, as well as immunotherapy strategies⁶⁶. Many of these strategies are based on triggering critical shortening of telomeres and loss of cell viability. Interestingly, short telomeres in the context of telomerase deficiency are likely to act synergistically with anticancer therapies based on genotoxic agents, given that telomere dysfunction results in hypersensitivity to DNA damaging agents⁶⁶.

The fact that telomerase deficiency only results in loss of organismal viability when telomeres reach a critically short length is an important point when considering possible secondary effects of these therapies. In particular, this predicts that putative anticancer therapies based on temporary telomerase inhibition will only trigger loss of viability in those cells with short telomeres that depend on telomerase activity. Presumably, these include tumor cells but not healthy tissues, which generally lack telomerase activity and have sufficiently long telomeres to maintain viability during the human lifetime, thus providing a window of opportunity for intervention.

Additional research is needed to establish the role of telomeres and telomerase in the biology of stem cells and of cancer stem cells, which is likely to significantly contribute to the success of therapeutic approaches against cancer and aging. In this regard, generation of new mouse models targeting telomeres and telomerase in adult stem cell compartments is essential to validate telomerase as a good target for stem cell-based therapeutic strategies.

Finally, although there is strong evidence supporting a role for short telomeres in limiting the lifespan of humans and mice, demonstration of whether telomerase re-expression in adult tissues is able to bypass the proliferation barrier imposed by short telomeres and extend the lifespan of organisms is still pending. In this regard, it has been previously demonstrated that Tert overexpression in normal mortal cells is sufficient to immortalize them—which is why Tert has been called the “immortality enzyme”¹²¹. Overexpression of Tert in transgenic mouse models,

however, has been shown to result in increased tumorigenesis^{122–125} (Table 1 and Fig. 5). Nevertheless, those Tert transgenic mice that do not die of tumors seemed to have an extension of the maximum lifespan, which suggests beneficial effects of Tert overexpression on organismal aging¹²⁴. Future studies warrant demonstration of whether telomerase is a longevity gene.

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