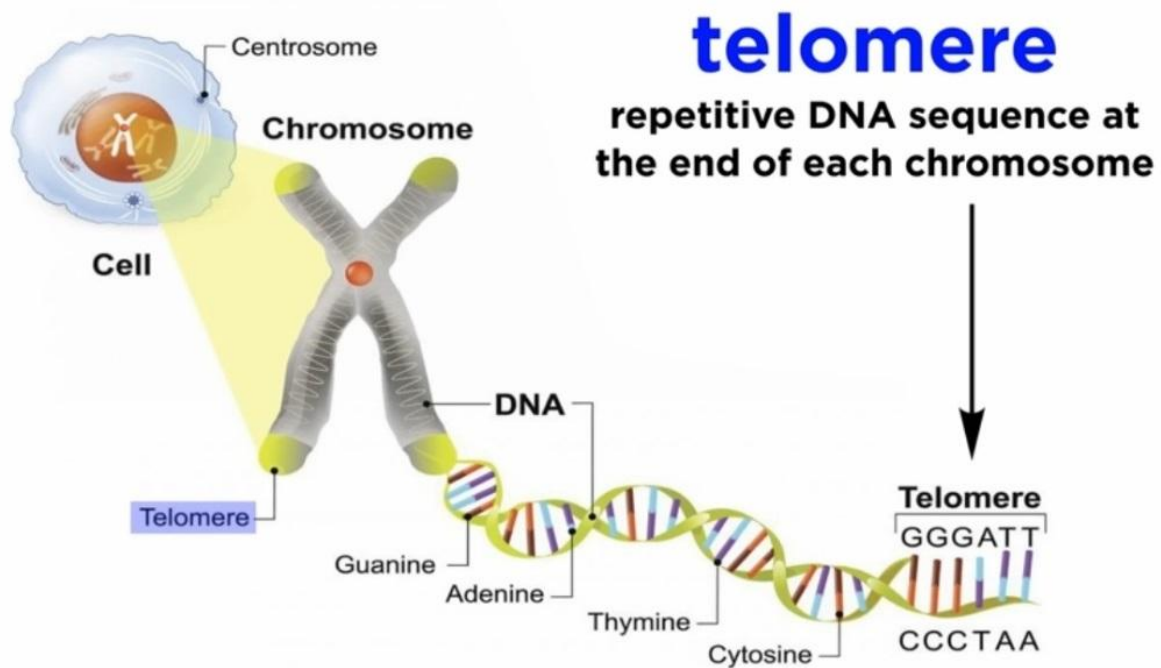


TELOMERES

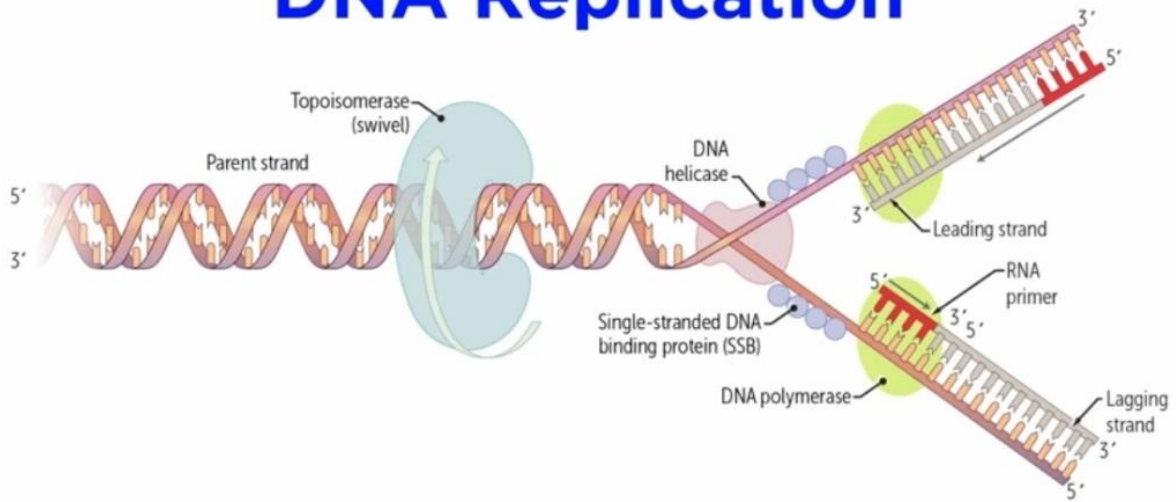


Carol Greider (b. 1961)

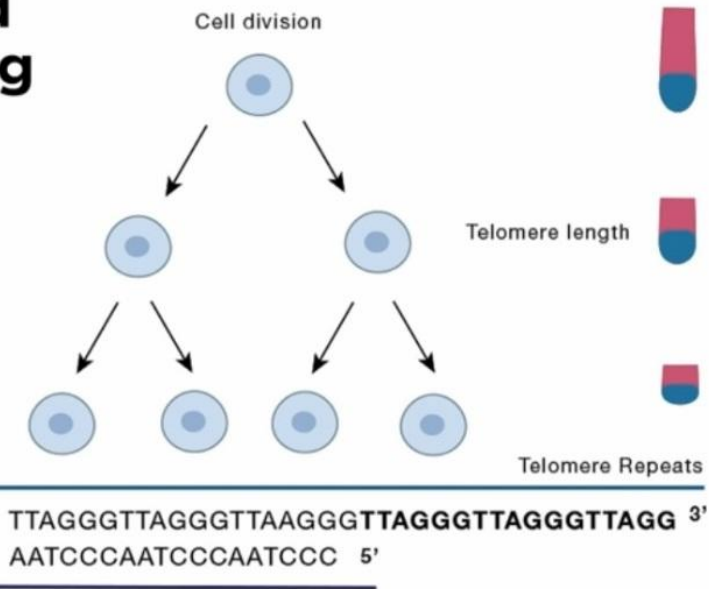


discovered telomerase/pioneered telomere research

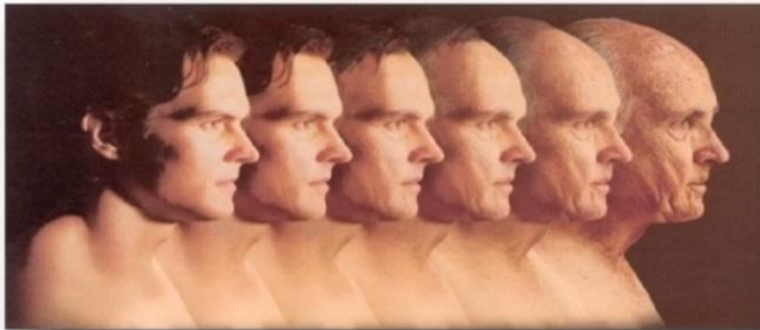
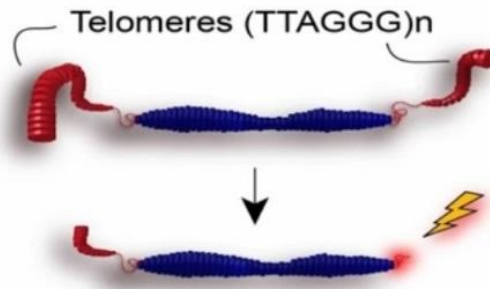
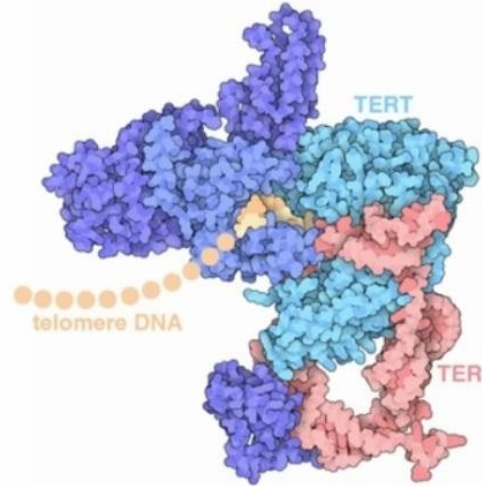
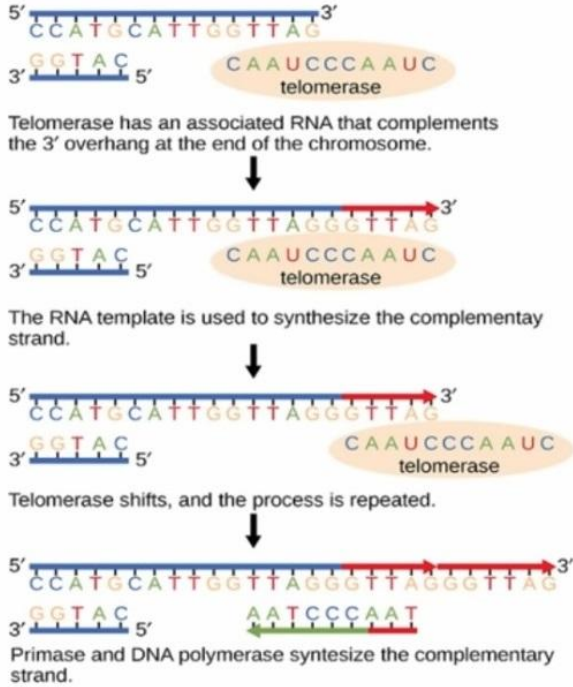
DNA Replication



telomeres can be degraded without losing any genes



telomerase extends telomeres



senescence

Biochemistry, Telomere And Telomerase

Last Update: December 11, 2022.

Introduction

Telomeres are protein structures located at the ends of each eukaryotic DNA chromosomal arm. These chromosomal caps are 1 of the most important structures that preserve the structural integrity of linear DNA during each cycle of replication.[\[1\]](#) Functions of telomeres include protecting the ends of the DNA from binding to one another and to itself, allowing for complete chromosomal replication, and serving as a molecular timer by controlling the lifespan of a eukaryotic cell. Telomeres also prevent the free ends of the chromosome from appearing as DNA double-stranded breaks, which in turn safeguards the ends from accidental DNA repair.[\[2\]](#) Telomeres play a significant role in cellular senescence in humans and have made major contributions to human aging. Pathologically, dysregulated expression of the telomere synthesis mechanism causes cellular immortality, leading to potential oncogenesis and tumorigenesis.[\[3\]](#)

Molecular Level

Telomere

A telomere structure consists of repeats of non-coding nitrogenous bases (5'-TTAGGG-3'). In mammals, telomeres are highly conserved, indicating that this nuclear sequence remains relatively unchanged throughout evolutionary biology.[\[1\]](#) The hexameric segments of DNA are located in tandem with one another. The 3' G-rich end of the chromosome is longer than the 5' C-rich end.[\[4\]\[5\]](#) In humans, the length of the telomere segment is between 5,000 to 15,000 base pairs long.[\[6\]](#) This long stretch of repetitive DNA sequences is characterized by a 3' end single-stranded overhang, which tucks itself into the end of the chromosome, creating a T-loop conformation. Of note, the T-loop biochemical structure is thermodynamically unfavorable. As such, proteins are required to manufacture and maintain the T-loop.[\[4\]](#)

The telomere is associated with 6 proteins that collectively create the Shelterin complex. This complex helps to create the final end cap structure of the chromosome. The associated proteins are described as follows: telomere repeats binding factor 1 (TERF1 or TRF1) regulates the telomere length. Telomere repeat binding factor 2 (TERF2 or TRF2) stabilizes the T-loop. Protection of telomeres 1 (POT1) inhibits DNA damage response at the single-stranded telomere overhang.[\[1\]\[7\]](#) Telomerase recruitment factor (ACD or TPP1) facilitates POT1 binding to single-stranded telomere DNA. TERF1 interacting nuclear factor 2 (TIN2 or TINF2) tethers POT1 and ACD to TERF1 and TERF2. TIN2 is also responsible for stabilizing TERF2 in the telomere. TERF interacting protein 2 (TERF2 or RAP1), in addition to the proteins mentioned above, are all responsible for the regulation of telomere length.[\[8\]\[9\]](#)

Telomerase

The synthesis of a telomere involves a reverse transcriptase telomerase, which functions as an RNA-dependent DNA polymerase. Telomerase is present in germline and stem cells and has enhanced activity in cancer cells. This enzyme is responsible for elongating telomeres by de novo addition of TTAGGG sequences onto 3' chromosome ends to prevent replicative cellular senescence.[10] Telomerase is a ribonucleoprotein structure comprising a functional RNA component and a catalytic reverse transcriptase component. The RNA component houses a template for the synthesis of telomeric DNA. The functional RNA component in humans is called hTERC or hTR [11]. It is encoded by the TERC gene located at the 3q26 region of the chromosome. The reverse transcriptase component is called hTERT and is encoded by the TERT gene located at chromosome 5p13.33.[12] While the telomerase core complex mainly consists of the 2 main components, hTERC and hTERT, essential supportive proteins exist to properly function the entire telomerase structure. Tcab1, Gar1, Nhp2, Reptin, and Pontin are proteins required for telomerase assembly and the proper recruitment of chromosomes.[13][14] Next, the proteins responsible for stabilizing the telomerase structure are TEP1 and dyskerin. Lastly, the additional protein subunits, Es1p, and Es3p, aid in the assembly and maturation of the catalytic complex.[15][16]

Function

The main functions of a telomere are to maintain chromosomal stability and prevent chromosomal degradation. Additionally, telomeres protect the ends of the chromosome from DNA end-joining to one another, damage response to DNA, and accidental DNA recombination.[6] The longer 3' G-rich end overhang that creates the T-loop protects the end of that chromosome from appearing as a double-stranded break in the DNA strand, thus preventing unwanted DNA repair.[17] For these reasons, telomeres and their maintenance are essential to eukaryotic genomic stability and the longevity of cellular information.

Mechanism

DNA replication is facilitated by DNA polymerase. This enzyme can only synthesize DNA in the 5' to 3' direction. DNA replication begins with an RNA primer, which is synthesized by primase. The RNA primer allows the DNA to locate the area of the chromosome where replication begins. The RNA primer anneals to the template DNA to provide a free 3'-OH group where new nucleotides are added. During the synthesis of the leading strand, which runs from the 5' to 3' direction, only 1 primer is needed for synthesis at this location to be continuous. This is due to the addition of new nucleotides in the direction of the replication fork.[18] Simultaneously, the synthesis of the DNA strand occurs in a lagging fashion in the 3' to 5' direction. Multiple RNA primers are necessary for the lagging strand, which is then replaced by DNA nucleotides via DNA polymerase, elongated, and ligated to create the new DNA strand.[19] The challenge arises at the 5' end of the lagging strand, where a stretch of DNA the size of the RNA primer is lost. This "end replication problem" occurs when the final RNA primer is removed after complete replication.[20] DNA polymerase cannot synthesize the end of the lagging strand due to the lack of a 3'-OH group after removing the RNA primer. Thus, due to the inherent properties of DNA polymerase, after each S phase of cell division, telomeres shorten 50-150 base pairs.[21][22]

Telomere replication and maintenance present numerous challenges. Repetitive tandem repeats of DNA predispose DNA polymerase slippage during DNA replication. Frequent slippage of the enzyme may cause insertion or deletion of nucleotide bases and strand mispairing. The next challenge is the G-rich structure of the telomere. A higher number of guanine nucleotides can cause G-quadruplexes to form. Tethered G-rich tetrads are highly stable due to their increased hydrogen bonds. The G-quadruplexes, which require specific helicases for proper disassembly, may induce replication fork stalling if the specialized helicase cannot function.[\[23\]](#) Additionally, the final step of telomere replication involves unwinding the T-loops to facilitate the passage of the replisome. With the considerably large structural make-up of the telomere, inadequate unwinding may cause failure for timely disassembly. Thus, replication machinery cannot copy the end of the chromosome, leading to a significant loss of telomere sequences. To combat the challenges mentioned above, telomeric proteins such as homology-dependent recombination factors, specialized helicases, and nucleases exist to promote smooth replication of the telomere.[\[17\]\[24\]](#)

Pathophysiology

Cellular Senescence

Telomeres play a crucial role in cellular senescence and, thus, biological aging. Cellular senescence refers to the irreversible loss of cellular division capability. The end replication problem, which describes the loss of base pairs during each S phase of cellular synthesis, can expose the ends of the DNA of a somatic cell, activating a process called DNA damage response. The purpose of this phenomenon is to prevent abnormal fusion of exposed chromosomal ends as well as chromosomal instability. The telomeres shorten without telomere elongation, characteristic of most somatic cells. Telomerase can elongate telomere structures; however, with persistent telomeric DNA damage response activation, a senescence-initiating signal can be elicited in addition to DNA damage. Cellular or replicative senescence also initiates when the telomere shortens to below a critical length.[\[25\]\[26\]](#) DNA damage response involves multiple cellular signaling pathways that activate cell cycle checkpoints to prevent the formation of potentially pathophysiologic mutations.[\[27\]](#) In cancer cells, as described later under clinical significance, unlimited self-renewal capacity is acquired through uninhibited telomerase activation.[\[13\]](#)

Clinical Significance

Telomeres and Oxidative Stress

DNA stressors include numerous endogenous and exogenous factors such as mitochondrial dysfunction, cigarette smoking, alcohol consumption, inflammation, a high-fat diet, and other lifestyle and environmental factors.[\[28\]\[29\]](#) Iatrogenically, inducers of cell senescence include chemotherapy and radiation.[\[30\]\[31\]](#) Most importantly, the relationship between these inducers and cellular senescence is the production of reactive oxygen species. Researchers believe that G-rich telomeres are especially susceptible to oxidative stress.[\[32\]](#) Additionally, telomeres have a repressed DNA damage response, leading to inefficient DNA repair if exposed to oxidative damage.[\[6\]](#) Obesity is associated with chronic

inflammation and increased reactive oxygen species levels in adipose tissue. These patients with a higher body mass index are associated with a higher blood volume, leading to greater proliferation of blood cells- all related to the telomere's shortening [29] [33]. It has also been reported that telomere length is inversely correlated with patients who suffer from psychosocial stress and major depressive disorder due to increased oxidative stress and inflammatory factors.[33][34] Notably, it has been reported that those who participate in increased physical activity levels have longer telomeres.[35][36]

Cancer

While the synthesis of telomeres by the reverse transcriptase, telomerase, is absent in most human somatic cells, it is found in greater than 90% of tumorigenic cells and in-vitro immortalized cells.[37] Telomerase gains oncogenic function when its expression is deregulated in human somatic cells.[2][12] hTERT gene amplification, which results from a breakage at DNA sites or abnormal chromosomal fusions, causes a pathologic upregulation of telomerase activity. Cancer cell immortalization through hTERT involvement may also occur by hTERT promoter methylation. Methylation prevents the binding of transcriptional repressors from blocking transcription machinery. TERT promoter mutations have also been implicated in cancer cells, including uroepithelial, bladder, thyroid, cutaneous melanoma, basal cell carcinomas, squamous cell carcinomas, and glioblastoma.[13][38]

Telomerase-targeted Cancer Immunotherapy

Because upregulated telomerase activity is significant in tumor cells, hTERT makes an attractive tumor antigen for telomerase-targeted cancer immunotherapy. Several approaches exist, including oligonucleotide inhibitors, immunotherapeutic approaches, and telomerase-directed gene therapy. Oligonucleotide inhibitors are modified nucleic acids that inhibit telomerase, inducing telomere shortening and forcing cellular senescence and apoptosis. Immunotherapeutic approaches use high-avidity T lymphocytes that are reactive against the catalytic enzyme. Finally, telomerase-directed gene therapy involves the selective killing of tumor cells by targeting telomerase promoters.[39]

Telomeres: History, Health and Hallmarks of Aging

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Abstract

The escalating social and economic burden of an aging world population has placed aging research at center stage. The hallmarks of aging comprise diverse molecular mechanisms and cellular systems that are interrelated and act in concert to drive the aging process. Here, through the lens of telomere biology, we examine how telomere dysfunction may amplify or drive molecular biological processes underlying each hallmark of aging and contribute to the development of age-related diseases such as neurodegeneration and cancer. The intimate link of telomeres to aging hallmarks informs preventive and therapeutic interventions designed to attenuate aging itself and reduce the incidence of age-associated diseases.

DePinho eTOC blurb

Telomere attrition and dysfunction is both a cause and consequence of cellular and molecular aging and age-related disease. Mounting data reveal how telomeres are entwined with other aging hallmarks and may be therapeutic targets for aging and age-related disease.

INTRODUCTION

Over the last century, advances in public health and medicine have fueled a dramatic rise in life expectancy worldwide. On the current trajectory, 2.1 billion individuals will be older than age 60 by 2050 ([United Nations, 2017](#)). This demographic milestone will be accompanied by major increases in age-associated diseases, such as Alzheimer's disease, cardiovascular disease, and cancer ([Melzer et al., 2020](#)), which essentially double in incidence every 5 years after age 60. In the absence of new medical and wellness paradigms, the world will experience an unsustainable burden of chronic disease that already extracts a significant social and economic toll. The link between aging and such diseases has motivated fundamental investigations into the mechanisms of aging and strategies to attenuate its impact.

Aging is a progressive degenerative state accompanied by tissue stem cell depletion, tissue inflammation, matrix alterations, cellular senescence, and metabolic dysfunction ([Lopez-Otin et al., 2013](#)). These cellular and tissue changes reflect underlying molecular aberrations in mitochondria, proteostasis, intercellular communication, nutrient sensing,

epigenetics, and DNA repair which leads to genomic instability and damage including telomere dysfunction ([Lopez-Otin et al., 2013](#)). An ever-greater understanding of the diverse molecular mechanisms of aging points to the telomere as an instigator or amplifier of the molecular circuitry driving the aging process and its associated diseases.

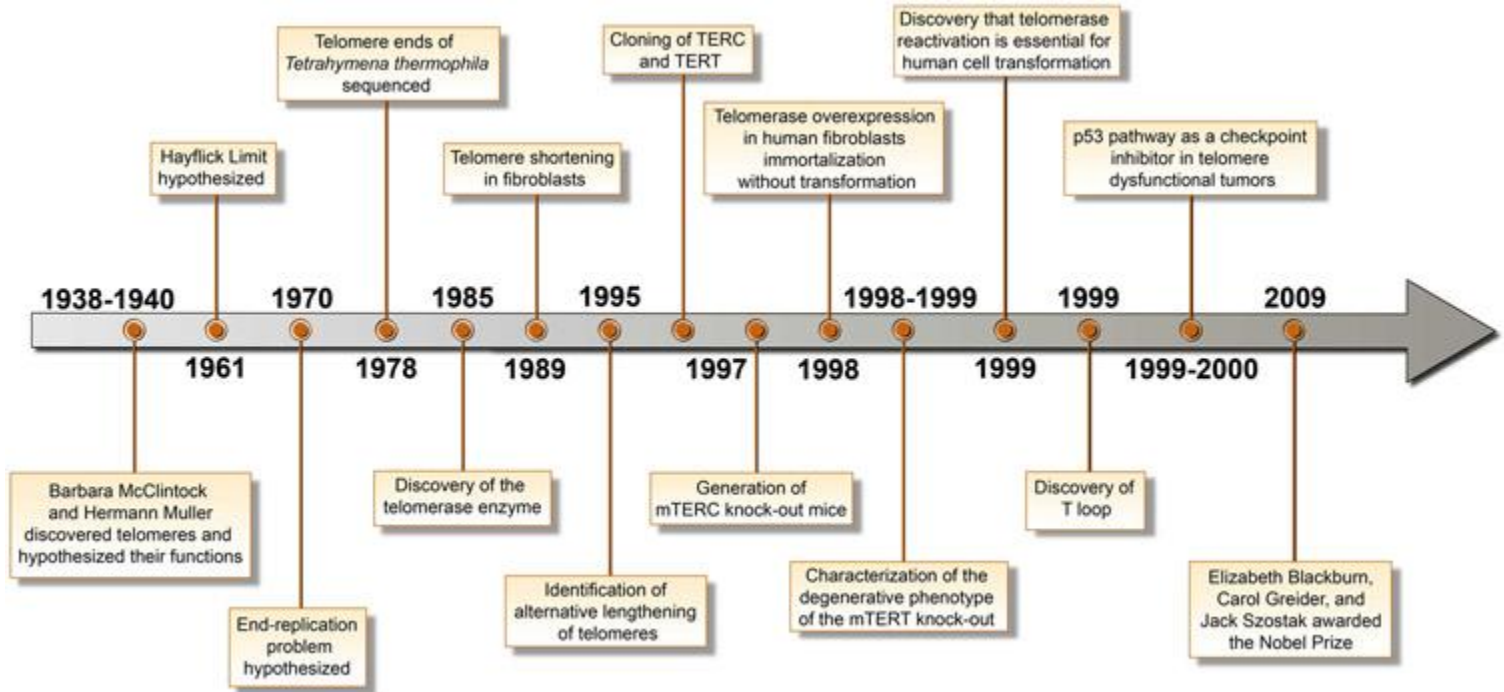
Telomeres are composed of repetitive nucleotide sequences that form a 'cap structure' which functions to maintain the integrity of chromosomes. Human telomere maintenance-associated gene defects are linked to germline and somatic degenerative diseases such as dyskeratosis congenita, idiopathic pulmonary fibrosis, ulcerative colitis and others ([Calado and Young, 2009](#)). Mice engineered with null or inducible telomerase alleles ([Blasco et al., 1997](#); [Jaskelioff et al., 2011](#)) helped researchers to forge a link between telomere dysfunction and aging itself ([Blasco et al., 1997](#); [Lee et al., 1998](#); [Rudolph et al., 1999](#)), progeric syndromes ([Chang et al., 2004](#)), and chronic inflammatory and degenerative conditions ([Chakravarti D., 2020](#); [Rudolph et al., 2000](#)). In mice, reactivation of endogenous telomerase reverses premature aging in mice with telomere dysfunction ([Jaskelioff et al., 2011](#)). Both killifish ([Harel et al., 2015](#)) and zebrafish ([Carneiro et al., 2016](#)) serve as models for telomere biology studies; their telomere length is similar to that of humans, while their telomere dysfunction phenotypes mirror those in murine models.

This review provides an overview of the history and current state of telomere research, highlights mechanistic connections between telomere dysfunction and aging hallmarks, and examines the seemingly pervasive roles of telomeres and telomerase in driving hallmarks of aging, progeria syndromes, and common age-associated diseases such as neurodegeneration and cancer. We describe how the intimate link of telomeres and aging mechanisms informs the development of anti-aging and disease preventive strategies.

HISTORY OF TELOMERES AND TELOMERASE

The concept of telomeres was born in the 1930s, when McClintock and Muller inferred the existence of a unique structure at the ends of chromosomes in *Zea mays* and *Drosophila melanogaster* and hypothesized that it was critical for prevention of chromosome end fusion ([Creighton and McClintock, 1931](#); [Muller, 1938](#)). Muller coined the term 'telomere' from the Greek '*telos*' meaning 'end' and '*meros*' meaning 'part,' hence 'end-part' ([Figure 1](#)). In 1961, Hayflick and Moorhead demonstrated that human fetal cells possessed finite replicative potential of 50 to 60 doublings, subsequently dubbed the 'Hayflick limit' or replicative senescence ([Hayflick, 1965](#); [Hayflick and Moorhead, 1961](#)). In the early 1970s, Olovnikov and Watson introduced the 'end replication problem' by observing the asymmetry in linear DNA replication and predicting that each cell division would incur a loss of chromosomal DNA from the termini of the lagging strand due to removal of the terminal RNA primer, thereby leading to progressive chromosomal shortening ([Olovnikov, 1973](#); [Watson, 1972](#)).

Figure 1.



Timeline of discoveries in the human telomere field.

It was not until 1978 when Blackburn and Gall sequenced the rDNA of the ciliated protozoan *Tetrahymena thermophila*, revealing termini composed of tandem repeats of hexanucleotide sequences of 5'-CCCAA-3' and 3'-TTGGGG-5' on the complementary strand ([Blackburn and Gall, 1978](#)). In 1985, Blackburn and Greider uncovered a novel enzymatic activity capable of adding DNA repeat sequences to chromosome ends and extending telomere length, which is now known as telomerase ([Greider and Blackburn, 1985](#)). In 1989, the RNA component was cloned from *T. thermophila* ([Greider and Blackburn, 1989](#)). In 1990, this journey of discovery culminated in evidence that telomere attrition occurs in parallel with the replicative lifespan of human primary cells in culture, establishing that short telomeres trigger the Hayflick limit ([Harley et al., 1990](#)). The role of telomere attrition and damage in inducing a permanent cell cycle arrest was further demonstrated using human fibroblasts overexpressing a mutant form of TRF2 (TRF2 Δ B Δ M) through activation of DNA damage check-points ([d'Adda di Fagagna et al., 2003](#)).

The next major breakthrough occurred in 1996, with the demonstration that telomerase was a ribonucleoprotein that acted on a 3' overhang ([Lingner et al., 1997](#)). This was followed by the cloning of human telomerase reverse transcriptase (hTERT) ([Harrington et al., 1997](#)) and the telomerase RNA component (hTERC) ([Feng et al., 1995](#)). Consequently, the first TERC knockout mouse was generated in 1997 ([Blasco et al., 1997](#)). In the ensuing years, mice null for TERC and TERT, alone or in combination with

progeria or cancer-relevant alleles, established that telomere dysfunction can drive premature aging, cancer, and various degenerative diseases. These mouse models proved that intact telomeres maintain genome stability, tissue stem cell reserves, organ system homeostasis, and normal lifespan ([Chang et al., 2004](#); [Jaskelioff et al., 2011](#); [Rudolph et al., 1999](#); [Sahin et al., 2011](#); [Wong et al., 2003](#)). In 1998, a capstone study showed that enforced hTERT expression endows unlimited replicative potential to primary human cells including fibroblasts, retinal pigment epithelial cells, and vascular endothelial cells ([Bodnar et al., 1998](#)). Notably, these cell cultures maintained a normal karyotype and showed no malignant properties.

Historically, while telomerase was known to be consistently upregulated in cancer, its role in cellular transformation was documented in 1999 when enforced TERT expression, together with classical oncogenes, promoted full malignant transformation of primary human cells ([Hahn et al., 1999](#)). Contemporaneously, genetic models of cancer established that the status of p53-dependent DNA damage response dictated whether short telomeres promote or suppress cancer *in vivo* ([Chin et al., 1999](#); [Greenberg et al., 1999](#)). In 2000, further analysis of late-generation *mTERC*^{-/-}; *p53*^{+/-} mice ([Artandi et al., 2000](#)) revealed a humanized tumor spectrum of epithelial cancers possessing chromosomal rearrangements and nonreciprocal translocations typical of human cancer genomes. Thus, mice engineered to experience telomere-based crisis in the context of deactivated DNA damage signaling (p53 deficiency) illuminated a major mechanism driving the preponderance of epithelial cancers in aged humans and explained why such cancers develop radically altered cytogenetic profiles.

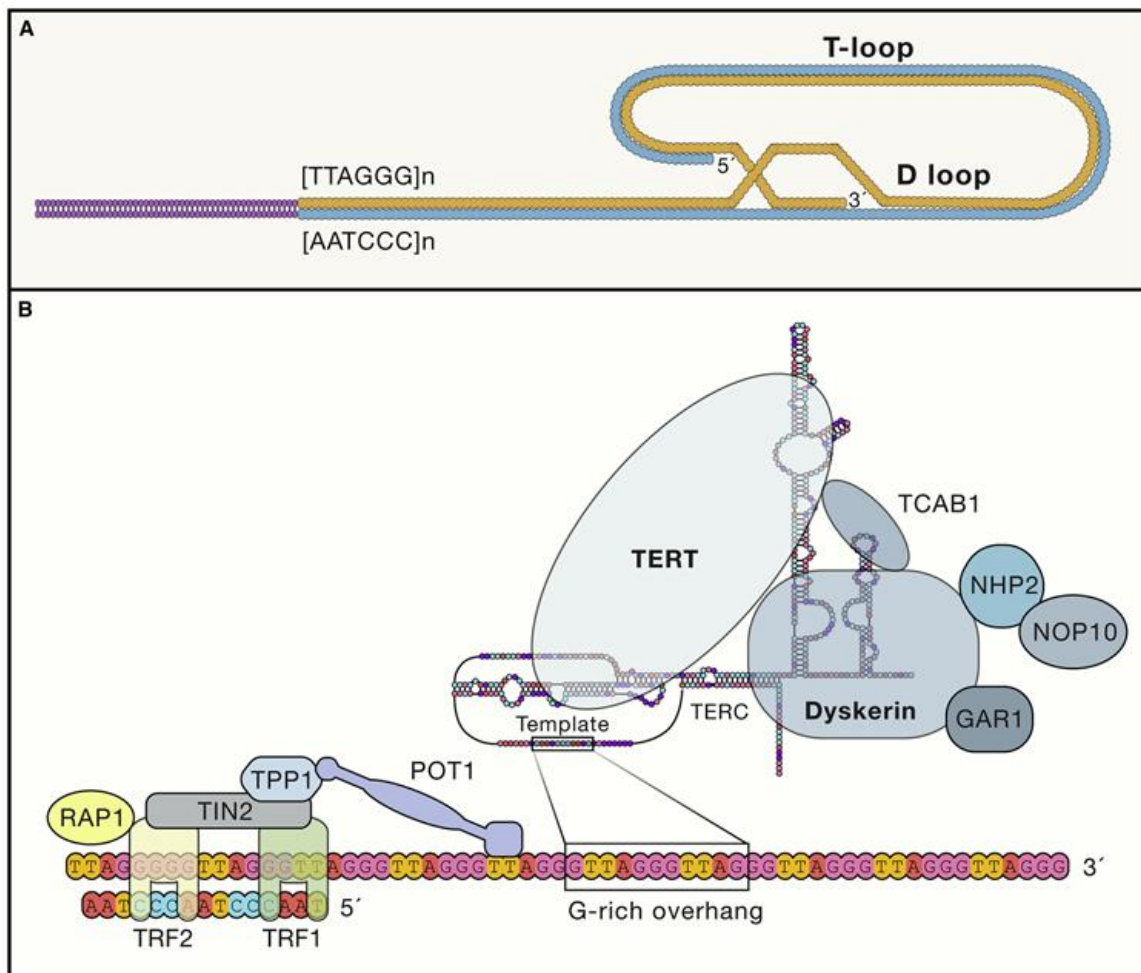
The TERC and TERT knockout mouse models authenticated the role of telomeres in aging and identified a core signaling pathway driving the aging process. First, these models established that telomere dysfunction accelerates signs and symptoms of aging characterized by shortened life expectancy, an aged appearance, declining tissue stem cell reserves, organ atrophy, and diminished capacity to cope with stress, injury, and regenerative demands ([Lee et al., 1998](#); [Rudolph et al., 1999](#)). Second, these models highlighted the essentiality of telomere dysfunction in progeroid syndromes and Parkinson's disease ([Chang et al., 2004](#); [Lee et al., 1998](#); [Rudolph et al., 1999](#); [Wong et al., 2003](#)). Third, unbiased transcriptomic analyses across diverse tissues of the late-generation *TERC*^{-/-} mouse revealed the p53-PGC pathway of aging, integrating three previously separate theories of aging—genotoxic stress (telomere dysfunction), oxidative damage, and mitochondrial decline ([Sahin et al., 2011](#)). Finally, an inducible TERT mouse model demonstrated that reactivation of endogenous telomerase reverses advanced premature aging in mice ([Jaskelioff et al., 2011](#)). Furthermore, adenoviral delivery of telomerase in aged mice improves cardiac function following acute myocardial infarction, enhances muscle coordination and kidney and liver function, reduces insulin resistance and subcutaneous fat depletion, increases bone mineral density, and extends life expectancy without causing an increase in cancer incidence ([Hong and Yun, 2019](#)). Together, this collective body of work across decades and

diverse model systems has defined the molecular biology of telomeres and its role in health and disease.

SHELTERTIN AND THE TELOMERASE COMPLEX

Telomeres maintain chromosomal integrity, which is essential for sustaining health-span and propagation of the species. The evolutionarily conserved function of telomere end protection spans from lower multicellular organisms such as *T. thermophila* to higher-order organisms including *Homo sapiens* (Roake and Artandi, 2020). Structurally, telomeres consist of tandem repeat sequences of TTAGGG measuring from several to tens of kilobases and terminating at the 3' end in a single-stranded 75-to-300-nucleotide overhang enriched in guanine nucleotides (Figure 2B). Seminal work by Griffith and de Lange established that the overhang folds back on itself to form a lariat-like structure named the T loop (Figure 2A) (Griffith et al., 1999).

Figure 2. The vertebrate telomere/telomerase complex.



(A) Structure of the D-loop and T-loop at the telomeric end.

(B) Structure of tetramerase. NOP10, nucleolar protein family A, member 3; NHP2, nucleolar protein family A, member 2; TIN2, TERF1-interacting nuclear factor 2; TPP1, telomere protection protein 1; TRF1, telomeric repeat binding factor 1; TRF2, telomeric repeat binding factor 2, POT1, protection of telomeres 1; RAP1, TERF2-interacting protein; TCAB1, telomerase Cajal body protein 1; GAR1, nucleolar protein family A, member 1.

The telomere is cloaked in specialized proteins dubbed the shelterin complex, a multimer composed of six protein subunits: TRF1, TRF2, TPP1, POT1, TIN2, and RAP1 ([de Lange, 2018](#)). This higher-order structure of telomeres functions to inhibit DNA damage signaling from the telomere ends, prevent DNA repair programs from fusing ends via recombination or classical/alternative non-homologous end joining, and regulate the access and activity of telomerase at the termini. Correspondingly, mutations in the aforementioned components can disrupt the shelterin-telomere complex, causing end fusions and premature senescence ([van Steensel et al., 1998](#)). Similarly, overexpression of *TRF1* or deregulation of *POT1* impairs telomerase binding to telomere ends, causing telomere shortening ([Loayza and De Lange, 2003](#); [Smogorzewska et al., 2000](#)). Conversely, loss of TRF1 also leads to the formation of common fragile sites in telomeric DNA due to its inability to recruit the Bloom (BLM) helicase and therefore BLM's execution of robust double-strand break repair during replication ([Yang et al., 2020](#)).

In normal tissues, telomerase expression is abundant in germ cells ([Lee et al., 1998](#)) and present in undifferentiated stem and progenitor cells of the skin ([Lee et al., 1998](#); [Rudolph et al., 1999](#)), intestine ([Schepers et al., 2011](#)), hematopoietic system ([Colla et al., 2015](#)), hair bulge ([Sarin et al., 2005](#)), and testes ([Rudolph et al., 1999](#)). Differentiated cells such as keratinocytes, fibroblasts, skeletal muscle myocytes, neurons, cardiac myocytes, and spermatids show modest or undetectable TERT levels ([Artandi and DePinho, 2010](#); [Pech et al., 2015](#)).

TERC is related to small Cajal body RNAs (scaRNAs) and small nucleolar RNAs (snoRNAs). While such RNAs are encoded within introns of other genes, *TERC* is a prototypical gene with its own promoter ([Feng et al., 1995](#)). *TERC* RNA is transcribed in a precursor format with a 5' methylguanosine cap and a poly (A) tail. These precursors are de-adenylated by the disease-associated poly (A)-specific ribonuclease (PARN), which promotes *TERC* maturation and accumulation ([Roake and Artandi, 2020](#)). *TERC* contains an H/ACA domain consisting of a three-nucleotide sequence, termed the CAB box, that is essential for binding to telomerase Cajal body protein 1 (TCAB1). TCAB1 is required for both catalytic activity and trafficking of telomerase to Cajal bodies, which aids in trafficking telomerase to telomere ends. Telomerase associates with *TERC* through its PK/T and CR4/5 domains. Multiple proteins are essential for proper

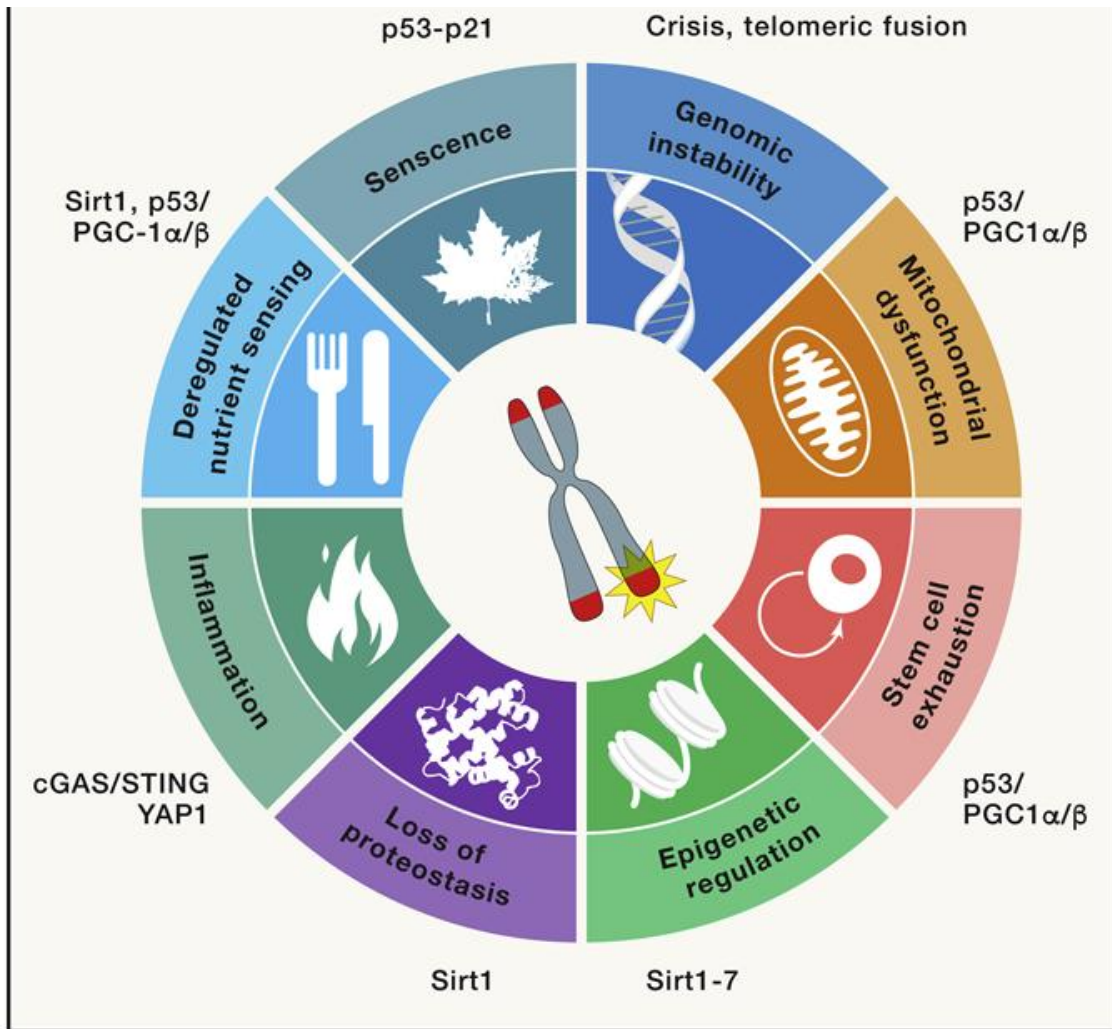
functioning of the holoenzyme, including dyskerin, NHP2, NOP10, and GAR1, which form core components along with other proteins.

In summary, the structure of telomeres, coupled with the highly regulated activity and recruitment of telomerase, ensures proper telomere maintenance in normal cells. At the same time, each of these features is vulnerable to mutations and dysregulation, leading to familial and sporadic diseases. Nevertheless, several questions remain regarding how the telomerase complex senses and is recruited to the shortest telomeres and the specific order in which the different components are assembled. Uncertainty also surrounds how these processes may be differentially regulated between normal and evolving neoplastic cells. Most importantly, a deeper understanding of the regulation of telomerase expression and function is needed to define the contributions of telomerase to normal aging as well as inherited and somatic degenerative disease pathogenesis in humans.

CROSSTALK BETWEEN DYSFUNCTIONAL TELOMERES AND THE HALLMARKS OF CELLULAR AGING

Limiting telomere reserves serve as a roadblock to cellular immortalization, but the loss of telomere function coincides with both age-related decline in fitness and cancer-inducing genome instability. Indeed, telomere dysfunction has been described as one of the nine cellular and molecular hallmarks of aging ([Lopez-Otin et al., 2013](#)). In this section, we detail the role of telomeres in processes of aging with an emphasis of how telomeres interrelate with the other hallmarks of aging, serving to drive or amplify these mechanisms ([Figure 3](#)). Additionally, we summarize the role of genetic model systems in revealing the interconnectedness of telomeres with other mechanisms and pathways driving aging as well as premature aging syndromes. The following subsections outline how telomere dysfunction links to the mechanisms underlying each hallmark of aging.

Figure 3. Relevance of telomere dysfunction to cellular aging hallmarks.



Telomere dysfunction can drive the hallmarks of cellular aging.

Cellular senescence.

Senescent cells accumulate in aging tissues and contribute to aging and age-related diseases via various mechanisms. The following are the two most well-studied ones: the first involves impeding the replicative potential of tissue stem cells (another hallmark of aging: stem cell exhaustion) immune cells (so-called immunosenescence) and stromal cells; the second stems from disrupting organ function through release of pro-inflammatory factors including but not limited to interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF α) ([Coppe et al., 2010](#)) by senescent cells. With respect to the latter, the Hayflick limit triggers senescence and proliferative arrest via activation of the p53-p19^{ARF} and p16^{Ink4a}-Rb signaling pathways ([Shay et al., 1991](#)). Whether and how telomere dysfunction activates the senescence program in low-proliferative stroma tissues during normal aging requires further investigation, although it is tempting to speculate that reactive oxygen species (ROS)-induced damage of telomeres leads to TIF generation and induction of senescence.

Stem cell exhaustion.

Tissue stem cell exhaustion is a cardinal feature of aging. In late generation TERT- or TERC-null mice, progressive telomere erosion leads to p53-dependent apoptotic elimination of tissue stem cells, which contributes to organ atrophy, particularly in highly proliferative tissues with high rates of self-renewal including the skin, intestine, testis, regenerating injured liver, and blood ([Blasco et al., 1997](#); [Chin et al., 1999](#); [Colla et al., 2015](#); [Jaskelioff et al., 2011](#); [Lee et al., 1998](#); [Rudolph et al., 1999](#); [Rudolph et al., 2000](#); [Sahin et al., 2011](#)). Beyond telomere maintenance, TERT may also impact stem cell biology via non-classical functions of TERT involving activation of the WNT pathway, a major regulator of stem cell homeostasis. Specifically, in mouse cells, TERT can physically interact with BRG1 and serve as a co-factor in the β -catenin complex, resulting in upregulation of WNT network genes ([Park et al., 2009](#)).

Genomic instability.

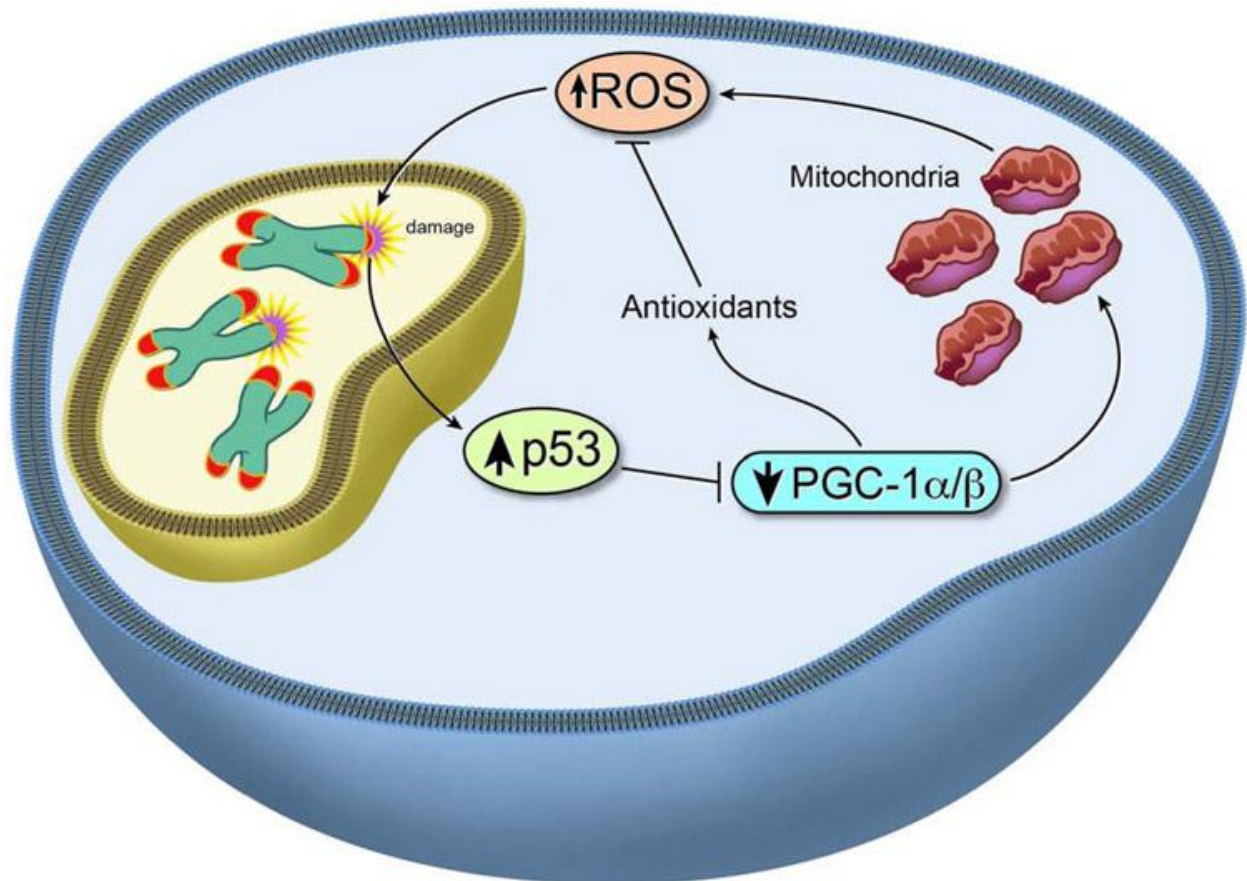
Genomic instability, another hallmark of aging, can lead to stem cell exhaustion and provoke inflammation. Telomere dysfunction can fuel chromosomal instability, as evidenced by cytogenetic analysis of late-generation TERC-null cells and tissues; these analyses showed amplifications, deletions, translocations, and anaphase bridge formation in tumors including colorectal cancers (detailed below) ([Artandi et al., 2000](#); [Blasco et al., 1997](#); [O'Hagan et al., 2002](#); [Rudolph et al., 1999](#); [Rudolph et al., 2001](#)). Mechanistically, eroded or uncapped telomeres undergo end-to-end fusion, forming dicentric chromosomes and resulting in breakage-fusion-bridge (BFB) cycles, aneuploidy and tetraploidization, translocations, amplifications, which create genomic instability through kataegis (localized hypermutations) and chromothripsis (clustered chromosomal rearrangements) during mitosis ([Maciejowski and de Lange, 2017](#)). Moreover, the loss of p53 enables cells to survive these DNA double-strand breakage events to produce aberrant chromosomal imbalances and nonreciprocal translocations that drive cancer initiation (see "[TELOMERES AND TELOMERASE IN AGE-RELATED DISEASES AND CANCER](#)"). These chromosomal abnormalities have been documented in nonmalignant aged stem cell compartments where the mutational burden strongly correlates with increasing age in human tissues, including colonic crypts and the hematopoietic system ([Calado et al., 2012](#); [Hsieh et al., 2013](#)).

Mitochondrial dysfunction.

Telomeres and mitochondria are intimately linked ([Sahin et al., 2011](#)). In aging, mitochondrial function declines, leading to diminished energy (ATP) production as well as increased intracellular ROS. Diminished energy production causes overall frailty, while increased ROS causes cellular damage, including formation of 8-oxoguanine base lesions in DNA (note, telomeres are guanine-rich). Direct evidence of a role for mitochondrial decline in driving processes of aging derives from mice harboring mutations in the mitochondrial polymerase *POLG*. These mutant mice show reduced mitochondrial abundance and abnormal mitochondrial morphology (e.g., fragmented cristae, disrupted external membrane) ([Trifunovic et al., 2004](#)) and exhibit premature aging (e.g., alopecia, kyphosis, reduced body weight, reduced subcutaneous fat, reduced bone mineral density consistent with osteoporosis, anemia, and

cardiomyopathy). Notably, the premature aging phenotypes associated with mitochondrial dysfunction mirror those of telomerase-deficient mice, p53-hyperactivated mice (Tyner et al., 2002), and PGC1 α/β -null mice (Lai et al., 2008). Given the centrality of mitochondria in aging, the overlapping phenotypes of TERC-, PGC1 α/β -, and POLG-null mice enabled us to link telomeres, mitochondria, and oxidative defense mechanisms in driving aging. Indeed, TERC-null mice were documented (Sahin et al., 2011) to have impaired mitochondrial function and diminished oxidative defense. Furthermore, transcriptomic analysis of diverse tissues in the late-generation TERC^{-/-} mouse revealed prominent representation of p53 and PGC1 α/β target genes, enabling us to identify a common pathway integrating three competing theories of aging—accumulating genotoxic stress, declining mitochondrial function, and increasing oxidative damage. Specifically, telomere dysfunction activates p53, which in turn represses PGC1 α and PGC1 β expression (Sahin et al., 2011) (Figure 4). Diminished PGC1 α/β expression, in turn, results in decreased mitochondrial biogenesis and function and reduces expression of genes governing oxidative defense. This signaling circuit of telomere→p53→PGC1 α/β →mitochondria leads to escalating ROS levels and further ROS-mediated 8-hydroxydeoxyguanosine modification of guanosine bases at telomeres (Figure 4). This axis creates a feed-forward loop linking telomere dysfunction, mitochondria, and oxidative stress pathways, resulting in accelerated aging.

Figure 4. Telomere dysfunction drives mitochondrial defects.



Telomere dysfunction compromises mitochondrial function and oxidative defense, increasing ROS and creating a feed-forward loop involving p53/PGC-1 α/β signaling.

Epigenetic dysregulation.

Age-dependent changes in the epigenetic landscape include increased local methylation and decreased global methylation, increased H4K16 acetylation, H3K4 trimethylation, and H4K20 trimethylation, and decreased H3K9 monomethylation and H3K27 trimethylation ([Barnes et al., 2019](#)). Direct telomere and epigenetic interaction is reflected in the regulation of sirtuins, a family of seven (SIRT1-7) NAD⁺ dependent deacetylases regulating life- and health-span ([Amano and Sahin, 2019](#)). In mammals, SIRT1 deacetylates several aging-relevant transcription factors including p53, FOXO, PGC1 α , and NF- κ B, which regulate crucial molecular pathways related to stress resistance, metabolism, and oxidative defense ([Houtkooper et al., 2012](#)). The interplay between telomere dysfunction and SIRT (both SIRT1 and SIRT6) is evidenced by telomere dysfunction-induced p53-mediated repression of all seven sirtuins ([Amano et al., 2019](#)). Moreover, telomere dysfunction-mediated p53 activation represses expression of PGC1 α/β , which in turn regulates expression of mitochondrial sirtuins 3, 4, and 5. In addition, p53 activation results in the transcriptional upregulation of microRNAs (miR-34a-5p, miR-26a-5p, and miR-145-5p) that suppress translation of non-mitochondrial sirtuins 1, 2, 6, and 7 ([Amano et al., 2019](#)). Thus, telomere-regulated epigenetic networks are relevant to aging regulators.

Loss of proteostasis.

Telomere dysfunction and resultant p53-mediated repression of SIRT1 expression is also relevant to the aging hallmark of altered proteostasis. Loss of proteostasis stems from a decline in the activity of the chaperone network that is responsible for proper protein-folding capacity. Mutant mice deficient in the carboxy terminus of HSP70-interacting protein (CHIP), a co-chaperone of the heat-shock family, exhibit accelerated aging phenotypes. In mammalian cells, SIRT1-mediated deacetylation of HSF-1 potentiates transcriptional activation of heat-shock genes such as HSP70. Thus, it is plausible that telomere dysfunction-induced repression of SIRT1 and resultant decreased HSP70 levels impair protein homeostasis and heat shock responses during stress ([Westerheide et al., 2009](#)). Protein folding is essential to maintaining neuronal homeostasis; post-mitotic long-lived neurons are extremely vulnerable to misfolded proteins, as they are unable to reduce misfolded protein load through cell division ([Muchowski and Wacker, 2005](#)). Deregulated protein folding occurs in several neurological diseases of aging, such as Alzheimer's disease and Parkinson's disease, which exhibit misfolded β -amyloid peptide and α -synuclein, respectively. The accumulation leads to gradual neuronal death and cognitive impairment. Recent studies have shown that overexpression of HSP70 is neuroprotective in *Drosophila* and in mouse and rat models of both Alzheimer's disease and Parkinson's disease ([Auluck et al., 2002](#); [Kakimura et al., 2002](#); [Klucken et al., 2004](#)).

Altered nutrient sensing.

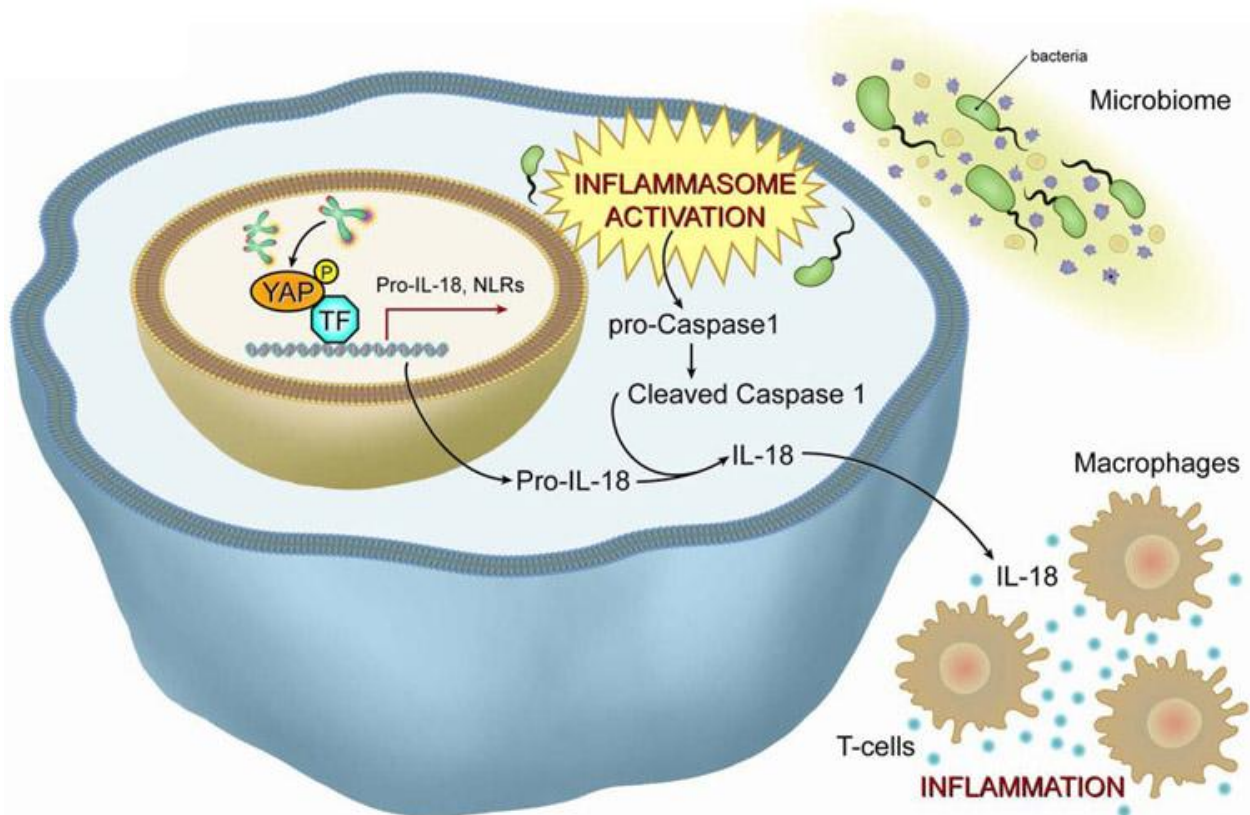
Deregulated nutrient sensing in aging relates to a highly conserved network consisting of IGF-1 and mTOR-S6 signaling pathways and members of the FOXO and AMPK families of proteins. These pathways reciprocally regulate each other to maintain metabolic homeostasis. AMPK is a central sensor of nutrients that modulates mTOR signaling and transcriptionally activates FOXO transcription factors and SIRT1 ([Saxton and Sabatini, 2017](#)). SIRT1 can in turn activate FOXO and PGC1 α , which are essential for mitochondrial biogenesis ([Brunet et al., 2004](#); [Rodgers et al., 2005](#)). Thus, the capacity of telomere dysfunction to impact metabolism stems from its activation of p53 and repression of PGC1 α and SIRT1. Indeed, telomere-dysfunctional mice fail to maintain plasma glucose levels under fasting conditions due to defects in gluconeogenesis regulated by p53-mediated repression of PGC1 α/β and its downstream effectors GLC-6-P and PEPCK ([Sahin et al., 2011](#)). Enforced expression of mTERT or PGC1 α or genetic ablation of p53 elevates expression of PGC1 α/β , GLC-6-P and PEPCK and restores gluconeogenesis ([Sahin et al., 2011](#)). Telomere dysfunction-induced mitochondrial impairment increases the dependency of tissues on glucose metabolism ([Missios et al., 2014](#)). Increases in glycolysis versus mitochondrial oxidative metabolism may also lead to changes in NAD/NADH pools, further impairing sirtuin activity. Additionally, DNA damage signaling and PARP activation, as a consequence of telomere dysfunction, reduce NAD pools and impair sirtuin function. PARP and sirtuins compete for the common pool of NAD⁺, such that inhibition of PARP or genetic ablation leads to increased sirtuin function in brown adipose tissue and muscle, as observed in *PARP1* knockout mice ([Bai et al., 2011](#)).

Inflammation.

Aging processes encompass inflammatory signaling from genomically damaged or senescent cells. Telomere dysfunction can initiate and maintain inflammation on several levels. First, telomere dysfunction provokes cellular senescence, which stimulates production and secretion of interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF α), among other inflammatory factors ([Coppe et al., 2010](#)). Second, telomere dysfunction results in the production of extrachromosomal fragments that can precipitate autophagic cell death via activation of the cGAS/STING pathway ([Nassour et al., 2019](#)). In the context of cancer specifically, a recent study demonstrated, targeting telomeres with a new drug named 6-thio-2'-deoxyguanosine (6-thio-dG) induces generation of TIFs and release of extrachromosomal DNA into the tumor microenvironment of the cancer cells. The uptake of this DNA by dendritic cells triggers the intracellular cGAS/STING pathway and initiates an inflammatory cascade via IRF3-TBK1-mediated upregulation of Type I interferons leading to enhanced cross-priming activity and recruitment of IFN γ producing CD8⁺ T cells ([Mender et al., 2020](#)). Recent studies have forged a direct link between telomere dysfunction and tissue inflammation ([Figure 5](#)) ([Chakravarti D., 2020](#)). Specifically, telomere dysfunction activates ATM/cABL-induced phosphorylation of YAP1, resulting in its nuclear translocation. Nuclear YAP1 upregulates inflammation and inflammasome genes such as *pro-IL18*, *NLRC5*, *NLRP1b*, and *NLRP6*. Together with gut microbiome activation of the inflammasome axis, the resultant activated caspase-1 cleaves pro-IL-18 into its mature IL-18 form, whose secretion recruits and activates T cells and initiates inflammation. The treatment of telomere-dysfunctional

mice with a YAP1 inhibitor, caspase-1 inhibitor, or antibiotics *in vivo*, or the reactivation of telomerase in the intestinal compartment, leads to a reduction in the cleavage of procaspase-1 to caspase-1 and in the production of mature IL-18, consequently reducing tissue inflammation. This study ([Chakravarti D., 2020](#)) established a direct link between telomere dysfunction and inflammation that can partially explain the age-related increase in inflammatory response seen in the aged population. This work also highlights the cooperation between cell-intrinsic telomere dysfunction–driven molecular pathways and the microbiome in driving the inflammatory response.

Figure 5. Telomere dysfunction drives tissue inflammation.



Telomere dysfunction drives tissue inflammation through activation of the ATM/cABL/YAP1 axis and driving secretion of mature IL18 to recruit and potentiate T cells and macrophages.

ATM, Ataxia telangiectasia mutated; cABL, Abelson murine leukemia viral oncogene homolog 1; YAP1, Yes-associated protein1; IL 18, interleukin 18.

In summary, numerous studies link telomere dynamics to pathways and biological processes underlying all hallmarks of aging. Moreover, many of these stress responses create feedback loops that further damage telomeres, amplifying and accelerating degenerative aging phenotypes. This interconnectedness of telomeres virtually all the hallmarks of aging serves to both initiate and escalate the aging process.

TELOMERES AND TELOMERASE IN AGE-RELATED DISEASES AND CANCER

How telomeres contribute to aging and age-related diseases came to light through the study of mice and individuals harboring germline alterations in telomere maintenance genes. Murine and human telomeres show differences in length and regulation. The laboratory mouse, *Mus musculus*, possesses telomeres up to ten times longer than those of humans (30-150 kb in mice versus 10-15 kb in humans) ([Kipling and Cooke, 1990](#)). Moreover, murine somatic cells exhibit high levels of telomerase activity relative to the low activity found in human somatic cells ([Kipling, 1997](#); [Wright et al., 1996](#)). These species differences enabled elucidation of the function of telomeres and telomerase in genome stability, aging, and cancer through the engineering and characterization of mice with shorter, human-like telomere lengths. Specifically, in mice null for TERT or TERC, successive intergenerational crosses progressively shorten telomeres, culminating in telomere dysfunction (end-to-end fusions) by generation 3 (G3) ([Artandi et al., 2000](#); [Blasco et al., 1997](#)). This telomere dysfunction coincides with widespread tissue stem cell depletion, progressive tissue atrophy, germ cell depletion, reduced fecundity, impaired adaptive immunity, decreased memory, delayed wound healing, diminished stress responses, increased hair graying and alopecia, diminished cardiac function, weakened skeletal frame, increased cancer incidence, and overall frailty ([Allsopp et al., 2003](#); [Artandi et al., 2000](#); [Chin et al., 1999](#); [Colla et al., 2015](#); [Jaskelioff et al., 2011](#); [Leri et al., 2003](#); [Pignolo et al., 2008](#); [Rudolph et al., 1999](#); [Sahin et al., 2011](#)).

This section summarizes mounting evidence linking telomere functionality to aging processes such as frailty as well as age-related diseases including cardiovascular diseases (atherosclerosis, vascular dementia, coronary artery disease), metabolic disease (type II diabetes), neurological disease (Parkinson's disease) and cancer.

Telomere dysfunction and telomerase in age-related diseases.

Numerous studies have implicated telomere dysfunction in age-related diseases. First, in high-proliferative tissues such as the skin, gastrointestinal tract, and hematopoietic system, low levels of telomerase in progenitor cell compartments and continual tissue renewal cause progressive telomere attrition over decades. This attrition ultimately triggers DNA damage responses such as cell cycle arrest, apoptosis, impaired differentiation, and/or senescence. Second, low-proliferative tissues such as heart, brain, and liver could experience ROS-induced damage of telomere sequences, causing attrition and uncapping over time. Specifically, murine and human studies show that oxidative stress itself accelerates telomere attrition in vascular endothelium ([Gonzalez-Guardia et al., 2014](#)), skeletal muscle ([Ludlow et al., 2014](#)), cardiomyocytes ([Puente et al., 2014](#)), and innate and adaptive immune cells ([Vaziri et al., 1993](#)) ([Kaneko et al., 1996](#)). Additionally, while telomere shortening *per se* generates telomere dysfunction-induced foci (TIFs), ROS-induced guanine modification also produces TIFs, reflecting

uncapping from shelterin disengagement ([Anderson et al., 2019](#)). This is evident from pioneering studies from the de Lange laboratory demonstrating that perturbations in shelterin components, such as dominant-negative mutant forms of TRF2, can induce TIFs without changes in telomere length ([van Steensel et al., 1998](#)).

The entwined processes of senescence and inflammation may be particularly relevant to the telomere-aging connection. These pleiotropic actions can drive atherosclerosis, type II diabetes, osteoarthritis, and Parkinson's and Alzheimer's diseases. Specifically, a recent study highlighted the role of senescent cells in driving Alzheimer's disease ([Bussian et al., 2018](#)). In this study, mice engineered with mutant *Tau* (*MAPTP301SPS19*) that develop neurofibrillary tangles and Alzheimer's-like symptoms show preservation of cognitive function upon genetic or pharmacological removal of p16^{INK4a}-expressing senescent astrocytes and microglia ([Bussian et al., 2018](#)). The significance of this study lies in the finding that accumulation of senescent cells precedes formation of the neurofibrillary tangles, suggesting that senescent cells may influence initiation of tangle formation. Similarly, removal of senescent cells from the BubR1 progeroid mouse model leads to extended health-span ([Baker et al., 2011](#)). These studies catalyzed development of 'senolytic' agents capable of clearing senescent cells in humans ([Ellison-Hughes, 2020](#)). The senescence-inflammation axis may also be active in patients with end-stage heart failure, cardiac hypertrophy, and coronary artery disease ([Birks et al., 2008](#)).

Telomere dysfunction in inflammatory diseases.

That telomere dysfunction can drive inflammatory diseases in human aging is evident from the study of telomeropathy. Telomeropathy results from germline defects of telomere maintenance genes including *TERT*, *TERC*, *DKC*, *PARN*, *RTEL1*, *TINF2*, and *POT1* as reviewed elsewhere ([Opresko and Shay, 2017](#)). On the cellular level, telomeropathy is characterized by (i) hematopoietic stem cell depletion leading to bone marrow failure, (ii) immunosenescence of lymphocytes and (iii) intestinal stem cell loss resulting in intestinal villous atrophy associated with crypt cell apoptosis, villous blunting, basal plasmacytosis, and intraepithelial lymphocytosis ([Jonassaint et al., 2013](#)). On the tissue level, telomeropathy shows increased incidence of idiopathic pulmonary fibrosis, liver cirrhosis, and kidney diseases ([Armanios and Blackburn, 2012](#)), all of which are related to increased inflammation. Given that high ROS levels are associated with telomere shortening and are postulated as drivers of tissue inflammation, even in the absence of genetic mutations in telomere-maintenance genes, telomere shortening and damage could be instrumental in driving various inflammatory diseases in older individuals without classical germline mutations affecting telomeres ([Wiemann et al., 2002](#)). These conditions could stem from dysfunctional telomeres in a few cells of an affected tissue leading to a local increase in inflammatory cytokines and driving further tissue damage and telomere shortening. Such inflammatory diseases include inflammatory bowel disease ([Risques et al., 2008](#); [Risques et al., 2011](#)), pancreatitis ([van Heek et al., 2002](#)), non-alcoholic fatty liver disease ([Laish et al., 2016](#)), chronic obstructive pulmonary disease ([Birch et al., 2016](#)), and liver cirrhosis as a consequence of chronic liver disease ([Wiemann et al., 2002](#)) among others. In such

conditions, telomere dysfunction may serve to amplify and cooperate with the primary drivers of disease.

Mitochondrial dysfunction and associated increased ROS are linked to aging, and specifically to senescence and inflammation. The maintenance of mitochondrial activity has been a prime therapeutic objective as murine studies have shown beneficial effects of PGC1 α overexpression on various aging-relevant biological processes including metabolism and ROS control ([Dillon et al., 2012](#)). Uncontrolled ROS are also known to impact gluconeogenesis, fatty acid metabolism, and β -oxidation, which can drive age-related metabolic diseases including diabetes, cancer (see “[Telomere dysfunction, telomerase and cancer](#)”), inflammatory conditions, sarcopenia, and neurodegenerative diseases like Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, and amyotrophic lateral sclerosis, as well as overall frailty.

Telomere dysfunction, telomerase and cancer.

Cancer is a disease of aging, with 1 in 2 men and 1 in 3 women in the US receiving a cancer diagnosis by age 80 ([National Cancer Institute](#)). Moreover, epithelial cancers are the predominant cancer types of the aged; these cancers are typically aneuploid and possess many chromosome structural alterations. Curiously, as mice age, the cancer spectrum consists primarily of hematopoietic (lymphoma) and mesenchymal (sarcoma) cancers, and few epithelial cancers ([Brayton et al., 2012](#)). Also, these murine malignancies exhibit minimal chromosome structural aberrations. These cross-species differences enabled elucidation of a key mechanism driving the preponderance of epithelial cancers in aged humans, as well as the basis of genomic instability in these cancers. Specifically, by engineering telomerase-deficient mice possessing shorter human-like telomeres, we demonstrated that ([Artandi et al., 2000](#)) telomere dysfunction, in the setting of p53 deficiency, enables cells in crisis to survive chromosomal breakage events fueling amplification, deletion, and translocation of cancer-relevant genes ([Figure 5](#)). Strikingly, these TERC/p53-null mice not only show increased cancer initiation but also a humanized tumor spectrum possessing complex karyotypes. These mouse genetic experiments, coupled with subsequent genomic evidence of telomere dysfunction in early-stage human cancers ([Ding et al., 2012](#); [Fernandes et al., 2020](#); [Rudolph et al., 2001](#)), established that telomere dysfunction is a major mechanism driving epithelial carcinogenesis in the aged. Stated differently, the absence of this mutational mechanism in the mouse (long telomeres and promiscuous telomerase expression) protects mice from developing epithelial cancers.

These murine models revealed the complex role of telomeres in the evolution of cancer, specifically, how telomere dysfunction enhances tumor initiation while restricting full malignant progression ([Chin et al., 1999](#); [Rudolph et al., 2001](#)). For example, in the APC^{min} TERC-null mouse model, telomere dysfunction initially increases adenoma frequency yet ultimately constrains progression to macroadenomas and increases survival ([Rudolph et al., 2001](#)). This constrained malignant progression relates prominently to the activation of p53 in aspiring cancer cells experiencing telomere

dysfunction. Inactivation of the p53 tumor suppressor is one of the most frequent events in human epithelial cancers ([Brosh and Rotter, 2009](#)) and the status of p53 can dictate whether telomere dysfunction enhances or suppresses cancer development. Specifically, late-generation *TERC*^{-/-} mice null for p53 show an increase in cancer incidence and decreased survival ([Artandi et al., 2000](#); [Chin et al., 1999](#)), whereas late-generation *TERC*^{-/-} mice null for *Ink4a/Arf* show a significant reduction in tumor incidence and increased survival ([Greenberg et al., 1999](#)). Notably, even though *Ink4a/Arf*-null mice lack p19ARF, which signals p53 during oncogenic activation, a functional p53-dependent DNA damage response is retained in these mice (e.g., apoptosis) ([Kamijo et al., 1997](#)). Thus, the role of telomeres in tumor initiation depends on the integrity of p53-mediated DNA damage signaling and associated cellular checkpoint responses.

Recent single-cell DNA sequencing of human cancers has uncovered that many cancers evolve as a consequence of a single punctuated genomic event ([Gao et al., 2016](#); [Navin et al., 2011](#)) where bursts of copy number alterations and mutations dominate all clones. While the mechanisms driving this punctuated evolution or 'episodic instability' are under active investigation, mouse and human evidence clearly implicates telomere-based crisis and subsequent telomerase reactivation as a mechanism shaping cancer genomes and driving epithelial carcinogenesis in the aged. While this episodic instability model is consistent with human genomic data ([Sottoriva et al., 2015](#)) on evolving cancers, we acknowledge that the "Vogelstein mechanism" ([Fearon and Vogelstein, 1990](#))—the stepwise accumulation of driver mutations—may also be at work here, and that the two models are not mutually exclusive

Additional evidence supporting a telomere-cancer link includes age-dependent telomere shortening in colonic mucosa of aged individuals and shorter telomeres in cancers than in adjacent nonmalignant tissues ([Hastie et al., 1990](#)). Additionally, human tumor tissues show elevated TERT and TERC expression compared to matched normal tissues ([Meyerson et al., 1997](#)), and malignant transformation of cultured human primary cells requires enforced TERT expression, indicating that telomere maintenance is essential for full malignant transformation ([Hahn et al., 1999](#)). Finally, cancer-prone mice engineered with an inducible TERT allele first experience telomere-based crisis followed by subsequent telomerase reactivation. This crisis-reactivation sequence generates more aggressive tumors, establishing that crisis enables acquisition of genomic events that endow a more aggressive malignant phenotype ([Ding et al., 2012](#); [Hu et al., 2012](#)). Similarly, transient induction of telomere uncapping in telomerase wildtype mice (by mutant TRF2 protein and disruption of the shelterin complex) increases initiation and progression of hepatocellular carcinoma in carcinogen-treated mice ([Begus-Nahrmann et al., 2012](#)). In contrast, telomere shortening in *TERC*-knockout mice increases tumor initiation but fails to induce tumor progression in a model of carcinogen-induced hepatocellular carcinoma ([Farazi et al., 2003](#)). Thus, multiple lines of evidence substantiate the role of telomere-based crisis and telomerase reactivation in cancer initiation and progression, respectively.

Telomere- and telomerase-dependent mechanisms of carcinogenesis.

As noted, loss of telomere function leads to end-to-end fusion. During mitosis, these dicentric chromosomes generate anaphase bridges with subsequent chromosome breakage leading to the BFB cycle first proposed by Barbara McClintock ([Creighton and McClintock, 1931](#)). The surviving BFB daughter cells accumulate cancer-relevant chromosomal alterations during crisis and ultimately activate telomerase to dampen DNA damage signaling and quell rampant chromosomal instability, enabling full malignant progression. A TERT-inducible model of prostate cancer has experimentally validated this cancer genome evolution concept. In this cancer model ([Ding et al., 2012](#)), critically short telomeres result in increased cancer cell apoptosis and decreased proliferation, producing smaller and less aggressive tumors compared to telomere-proficient controls. In these chromosomally unstable high-grade prostate intraepithelial neoplasias, experimental reactivation of telomerase results in malignant progression including the acquisition of new phenotypes such as bone metastasis. In contrast, telomere intact controls (which did not experience crisis followed by telomerase reactivation) exhibited only local invasion ([Ding et al., 2012](#)). Moreover, genomic analysis of the tumors experiencing crisis showed additional pro-metastasis mutations (e.g., SMAD4 deletion and additional perturbations to the TGF β pathway). Thus, telomere-based crisis drives chromosomal instability to initiate cancer, and subsequent telomerase reactivation enables full malignant progression.

Large-scale sequencing studies show that TERT promoter mutations are the most frequent noncoding mutations in human cancer ([Fredriksson et al., 2014](#); [Icgc Tcga Pan-Cancer Analysis of Whole Genomes Consortium, 2020](#); [Rheinbay et al., 2020](#)); such mutations are highly enriched in cancers originating in tissues with relatively low rates of self-renewal ([Killela et al., 2013](#)). For example, melanoma shows highly recurrent TERT promoter mutations, with an average 4-fold increase in TERT expression ([Horn et al., 2013](#)), and primary glioblastoma shows TERT promoter mutations in over 80% of cases ([Killela et al., 2013](#)). The most common recurrent single-nucleotide mutations in the TERT promoter occur at G228A and G250A, which generate *de novo* ETS consensus binding motifs to recruit GAPB, and potentially other ETS transcription factors, to elevate TERT expression ([Bell et al., 2015](#)). Additional mechanisms driving increased TERT expression in cancer also result from crisis-induced chromosomal alterations ([Artandi et al., 2000](#)), which produce focal amplifications of the TERT locus in hepatocellular carcinoma ([Totoki et al., 2014](#)). Finally, gene therapy studies have shown that CRISPR-mediated correction of the TERT promoter to wildtype sequences significantly prolongs the survival of glioma-bearing mice ([Li et al., 2020](#)).

In addition to the genomic mechanisms driving TERT upregulation, oncogenic signaling pathways can enhance TERT gene transcription. c-MYC can bind Myc binding elements

in the TERT promoter to induce TERT transcription and increase telomerase activity in primary human fibroblasts, although enforced TERT expression alone was an insufficient substitute for c-MYC in promoting transformation ([Greenberg et al., 1999](#); [Wang et al., 1998](#)). Similarly, activated WNT signaling cooperates with KLF4 to activate TERT transcription ([Hoffmeyer et al., 2012](#)). While key developmental and cancer-related pathways can activate TERT, there is also evidence that TERT can in turn activate these pathways in *cis*. Activation of telomerase in TERT-overexpression mouse models showed proliferation of quiescent hair follicle stem cells and kidney podocytes can occur independent of telomere length, TERC function or TERT reverse transcriptase function ([Flores et al., 2005](#); [Sarin et al., 2005](#)). Mechanistic studies revealed that TERT directly binds TCF elements and enhances MYC and WNT transcriptional programs ([Choi et al., 2008](#); [Park et al., 2009](#))—pathways known to govern stem cell homeostasis and drive cancer ([Dang, 2012](#); [Reya and Clevers, 2005](#)). In addition, TERT interacts with the NF- κ B complex and binds IL-6 and TNF α promoter elements, increasing cell proliferation and resistance to cell death ([Ghosh et al., 2012](#)). The increased cell proliferation and resistance to cell death upon ectopic TERT and TERC expression are attenuated through repression of p65. Functionally, G1 TERC-null mice were more resistant to endotoxic shock than their littermate controls, with more than 50% of mice surviving lipopolysaccharide-induced shock compared to 25% of controls, which suggests that telomerase regulates the NF- κ B inflammatory response independently of telomere length. Together, these studies point to the interactions of oncogenic signaling molecules and TERT in the regulation of cancer-relevant circuits. Further investigation is needed to assess the role of these circuits in human cancer.

Mutations in and/or altered expression of shelterin proteins have been detected in cancer, although their precise roles in cancer initiation and progression are not yet determined. In early-stage chronic lymphocytic leukemia, telomere dysfunction is linked to disease initiation and end-to-end fusions ([Chang, 2013](#)). Accordingly, in this disease, there is reduced expression of shelterin proteins including TRF1, RAP1, and POT1 as well as TIN2 and TPP1. POT1 is the most mutated shelterin-related protein in human cancers, with somatic mutations present in 5% of chronic lymphocytic leukemia cases. Somatic mutations in POT1 or RAP1 can occur in familial glioma, familial melanoma, Li-Fraumeni-like syndrome, parathyroid adenoma, cardiac angiosarcomas, mantle cell lymphoma, and hepatocellular carcinoma ([Fernandes et al., 2020](#)). Both TRF1 and TRF2 are upregulated in various tumor types, including cancers of the lung, breast, colon, stomach, and kidney – suggestive of a supportive role for shelterin proteins in human cancer.

Alternative mechanisms of telomere maintenance in cancer.

While telomerase is the most common telomere maintenance mechanism in cancer, cancer cells also utilize a telomerase-independent recombination-mediated mechanism, termed alternative lengthening of telomeres (ALT) ([Henson et al., 2002](#)). ALT occurs in 5% to 15% of human cancers, particularly in osteosarcoma and glioblastoma, and is typically associated with poor prognosis ([Heaphy et al., 2011](#)). ALT lengthens telomeres using DNA homology-directed repair mechanisms in which telomeric DNA templates

are copied from sister chromatids or non-homologous chromosomes. Essential factors for ALT include MRE11, RAD50, NBS1, FEN1, MUS81, and FANCD2 ([Cesare and Reddel, 2010](#)). Interestingly, ALT-positive cells are commonly defective in their ability to sense cytosolic DNA, and studies have demonstrated that extrachromosomal telomere repeats (ECTR) elicit an interferon response through the cGAS-STING cytosolic DNA-sensing pathway ([Chen et al., 2017](#)). Thus, cancer cells employing ALT have additional anti-proliferative barriers to overcome, such as senescence induction and innate immune surveillance due to the constant production of ECTRs. Interestingly, although ALT is an important factor that drives tumorigenesis in the absence of telomerase reactivation, it is less efficient in driving aggressive malignancy and metastasis. Evidence derives from a study where mouse *mTerc*^{-/-} *Ink4a/Arf*^{-/-} fibroblasts which were passaged extensively and showed development of ALT. While ALT-positive cells maintained telomeres, they were unable to form lung metastases; however, upon mTERC transduction, telomerase-positive cancer cells acquired metastatic potential, establishing that ALT-positive and telomerase-positive cancers are not biologically equivalent ([Chang et al., 2003](#)).

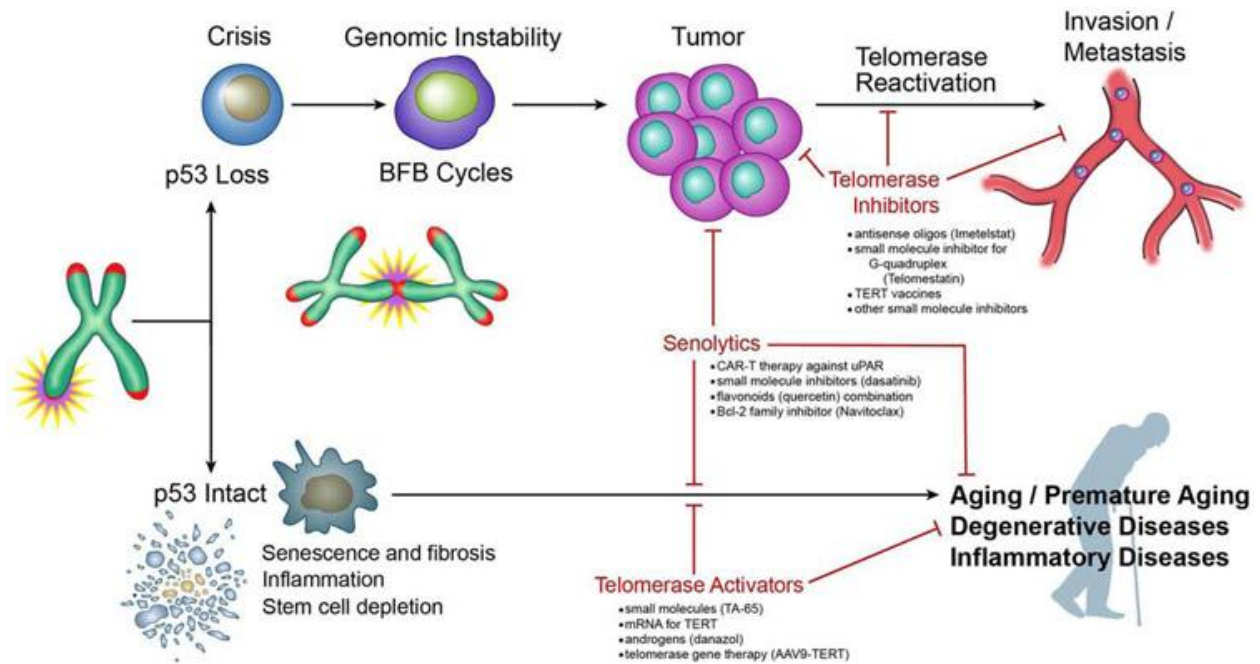
The ALT pathway can also serve as a resistance mechanism for telomerase inhibitor therapies. In a lymphoma-prone ATM-mutant mouse model engineered with a tamoxifen-controlled TERT allele (TERT-ER) ([Hu et al., 2012](#)), extinction of telomerase activity resulted in re-entry of lymphoma cells into telomere-based crisis. While telomerase extinction cured two-thirds of mice, the remaining mice developed recurrent tumors possessing hallmark features of ALT ([Hu et al., 2012](#)). Interestingly, these recurrent tumors acquired amplifications and deletions in genes involved in homologous recombination that are known to play essential roles in ALT, as well as amplification of the PGC1 α locus, which may signify ongoing mitochondrial stress in ALT-positive cells ([Hu et al., 2012](#)). These mechanistic insights inform potential regimens combining telomerase inhibitors with agents targeting homologous recombination or mitochondrial mechanisms. With respect to the latter, ALT-positive cancer cells, which show diminished mitochondrial function and associated high intracellular ROS, are particularly susceptible to agents that target antioxidant proteins.

OUTLOOK FOR TELOMERASE THERAPEUTICS AND FUTURE PERSPECTIVES

The link between telomere dysfunction and the hallmarks of aging, the incidence of age-associated diseases, and the development of inherited and acquired degenerative conditions has catalyzed interest in telomerase restoration therapy as a potential antiaging strategy ([Figure 6](#)). The optimal application of such therapy would likely be in the form of transient telomerase induction to enable restoration of telomere reserves and healing, while avoiding the potential for fueling cancer that might come from constitutive telomerase activation. Indeed, in preclinical mouse models with long telomeres, constitutive TERT expression increased lifespan by 40%, although these mice also harbored extra copies of p53, p16, and ARF ([Tomas-Loba et al., 2008](#)), which enhanced their cancer-resistance. It remains to be determined whether the increased

lifespan and disease-modifying activities of enforced TERT expression relate to TERT's actions on telomeres or to its activation of WNT, which may enhance stem cell reserves.

Figure 6. Telomere/telomerase in aging, cancer and potential therapy.



Telomere shortening leads to senescence, fibrosis, inflammation and stem cell depletion in the presence of a functional p53 checkpoint. These processes lead to aging and other degenerative and inflammatory diseases. Telomerase activators and senolytics can inhibit these processes and inhibit aging and age related diseases. Shortened telomeres also lead to telomeric fusion and cycles of break-fusion-bridges. In the absence of a p53 checkpoint, these events lead to tumorigenesis. Further activation of telomerase leads to the progression to invasion and metastasis. Telomerase inhibitors and senolytics inhibit processes that can thwart tumor progression, invasion and metastasis.

Advancing knowledge of the molecular networks regulated by telomerase and the rejuvenation of prematurely aged mice with telomerase activation ([Jaskeliuff et al., 2011](#)) has fueled interest in the development of agents that activate TERT expression for antiaging therapy. Several small molecules have been identified that appear to activate TERT ([Figure 6](#)), including TA-65 ([Harley et al., 2011](#)) and histone deacetylase inhibitors ([Won et al., 2004](#)), although a clear understanding of their mechanisms of action is lacking. TA-65 (cycloastragenol) is a natural compound isolated from various plant species in the genus of *Astragalus*. Ongoing human trials of TA-65 have reported improved macular function ([Dow and Harley, 2016](#)), reduced high-density lipoprotein levels, and reduced levels of the inflammation markers Creactive protein and TNF α ([Fernandez et al., 2018](#)). In addition, hormonal agents such as danazol (an antiestrogenic and antiprogestogenic) and 5 α -dihydrotestosterone (an androgen), which can increase telomerase levels, are being tested in patients with telomeropathies ([Calado et al., 2009](#); [Townesley et al., 2016](#)). Finally, the recent development of agents

targeting TERC stability may offer new therapeutic options for telomeropathies. Specifically, PAPD5 degrades TERC by 3' oligoadenylation, priming these transcripts for destruction by the RNA exosome. As mentioned earlier, PARN, involved in the de-adenylation and maturation of TERC, is mutated in several diseases including dyskeratosis congenita. A PAPD5 small molecule inhibitor increased telomere length in induced pluripotent stem cells from dyskeratosis congenita patients and was well tolerated in mice over an extended period of time ([Nagpal et al., 2020](#)).

A particularly intriguing application of pulsatile telomerase activation therapy would be the treatment of progeroid diseases such as Werner and Bloom syndromes. This concept emerges from the astonishing lack of degenerative phenotypes in mice null for genes connected to inherited degenerative conditions in humans including (i) ataxia-telangiectasia, which results from mutational inactivation of ATM; (ii) Bloom syndrome, caused by a mutation in BLM of the RecQ helicase family, and (iii) Duchenne muscular dystrophy, which arises from mutation of dystrophin, DMD. Strikingly, when each of these alleles was crossed to the TERC-knockout mouse, the salient phenotypes of these syndromes surfaced as telomeres reached shorter, more human-like lengths (typically at G2-G3) ([Chang et al., 2004](#); [Du et al., 2004](#); [Sacco et al., 2010](#); [Wong et al., 2003](#)). For example, WRN/TERC-null mice recapitulated the hallmark features of Werner syndrome including kyphosis, pathological long bone fractures, alopecia and hair graying, defects in stem cell and immune compartments, senile cataracts, hematopoietic deficiencies such as pancytopenia and unbalanced response to immunogens, and metabolic deficiencies including insulin resistance ([Chang et al., 2004](#)).

As noted, telomere-intact mouse models with mutations in *WRN* or *BLM* genes do not exhibit any degenerative phenotypes, providing a rationale for telomerase activation therapy to delay or reduce the intensity of symptoms and extend life expectancy. Along similar lines, in patients with germline mutations affecting telomere maintenance (e.g., *DKC*), telomerase activation therapy could alleviate progressive symptoms such as anemia, pulmonary fibrosis, and gastrointestinal dysfunction. Another group of diseases with limited treatment options, in which telomerase activation may play a beneficial role, is chronic inflammatory diseases such as liver cirrhosis, pancreatitis, and ulcerative colitis ([Figure 6](#)). Telomere dysfunction at disease onset can drive tissue inflammation, which in turn can accelerate telomere shortening, creating a feed-forward loop that ultimately leads to disease recurrence and even cancer brought about by genomic instability, p53 loss, and telomerase reactivation. Here again, telomerase activation at very early stages of disease prior to entry into telomere-based crisis could prevent disease flares and carcinogenesis. Telomerase activation may also be useful in treating neurodegenerative diseases given the strong improvement in brain health in mice following genetic induction of telomerase ([Ding et al., 2012](#); [Jaskeliuff et al., 2011](#)). In addition to telomerase activation, it may also be possible to ameliorate telomere dysfunction-induced organ degeneration and mortality by impairing the function of checkpoints that mediate the tissue-disruptive effects of dysfunctional telomeres. Along these lines, it was shown that the selective inhibition of p21-dependent cell cycle arrest

([Choudhury et al., 2007](#)) or Puma-mediated apoptosis ([Sperka et al., 2011](#)) could prolong the maintenance of tissue integrity and lifespan in telomere-dysfunctional mice without increasing cancer development. Also, deletion of Exo1 increases tissue maintenance and lifespan in telomere-dysfunctional mice by inhibiting the formation of chromosomal fusions and “BFB “ cycles that drive the induction of DNA damage and the activation of p53-checkpoints in telomere-dysfunctional mice ([Schaetzlein et al., 2007](#)).

Contrary to the potential applications of telomerase activation in antiaging therapy, the increased telomerase activity observed in most cancers has led to the development of antitelomerase therapeutics. Several strategies to target TERT in cancer have been engineered, including antisense oligos, vaccines, and small molecule inhibitors ([Ruden and Puri, 2013](#)) ([Figure 6](#)), but no antitelomerase agents have reached randomized phase III trials. This limited efficacy may be attributable to the time required for telomeres to shorten to a length that can induce tumor shrinkage. In addition, alternative strategies to inhibit telomerase could generate a more meaningful impact in the clinic. To start, cancers with intact p53 would be more suitable for telomerase inhibition owing to their functional checkpoint machinery, which would trigger senescence. This strategy still requires caution, as preclinical animal studies have demonstrated that TERT inhibition can lead to activation of the ALT pathway in lymphoma ([Hu et al., 2012](#)). Thus, combined telomerase and ALT pathway-suppressing drugs ([Flynn et al., 2015](#)) could minimize emergence of resistance. In the subset of cancers that are ALT-positive, targeting relevant immune circuits may also enhance response rates and outcomes. That is, due to the persistent production of cytosolic DNA in ALT-positive cancer cells ([Chen et al., 2017](#)), it is tempting to speculate that these tumors may be more responsive to immunotherapy because cytosolic DNA upregulates interferon signaling via activation of cGAS-STING. In preclinical studies, the nucleoside analog 6-thio-dG, which incorporates into newly synthesized telomeres to cause telomeric DNA damage, can render cancers that are resistant to anti-PD-L1 treatment susceptible to immune checkpoint therapy ([Mender et al., 2020](#)). To summarize, antitelomerase therapy remains a viable anti-cancer strategy based on its potential role in the inhibition of advanced malignancies, although the genotypic and biological context will be important to determine along with the specific combination therapies for synergistic activity.

In closing, the pursuit of the fundamental knowledge of chromosome structure and cell biology has illuminated mechanisms central to many major human diseases and aging itself. The telomere field has been an exemplary model of basic science and multidisciplinary convergence, revealing the role of telomeres and telomerase in the hallmarks of aging and the pathogenesis of cancer. Many knowledge gaps remain, such as elucidating the mechanisms governing telomerase expression and activity, the non-canonical functions of TERT, and the interplay between telomere dysfunction and pathological processes such as inflammatory, fibrotic, and degenerative diseases. Regardless of whether telomere dysfunction initiates the disease or is only a participant, telomeres clearly play an integral pathogenetic role in human disease. Such an elemental role encourages the development and rigorous testing of telomerase activators for the treatment of aging and age-associated diseases as well as the

assessment of efficient telomerase inhibitors for the treatment of advanced cancers. The hallmarks delineated here outline a framework to stimulate further study of the role of telomeres and telomerases, which may help confront the lethal disease ultimately suffered by all—aging.

Acknowledgements

We regret being unable to cite the work of many of our colleagues due to limitations in space. We are grateful to Steven E. Artandi, Karl-Lenhard Rudolph, Sandy Chang, and members of the DePinho lab for helpful suggestions. We would like to thank Denise J. Spring and Amy L. Ninetto for meticulously editing the document as well as Editing Services, Research Medical Library at MD Anderson Cancer Center. We would also like to acknowledge David Aten for his help with the illustrations and the Department of Medical Illustration at MD Anderson Cancer Center. D.C. was supported by the CPRIT Research Training Program (RP140106 & 170067) and by pilot funding through the DDC-NIDDK award P30CA16672. K.A.L. is supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Numbers TL1TR003169 and UL1TR003167. This work was also supported by NIH R01 CA084628 (to R.A.D.).

Footnotes

Declaration of Interests

R.A.D. is co-Founder, Director and Advisor of Tvardi Therapeutics; co-Founder and Advisor of Asyilia Therapeutics; co-Founder and Advisor of Nirogy Therapeutics; and co-Founder and Advisor of Stellanova Therapeutics.

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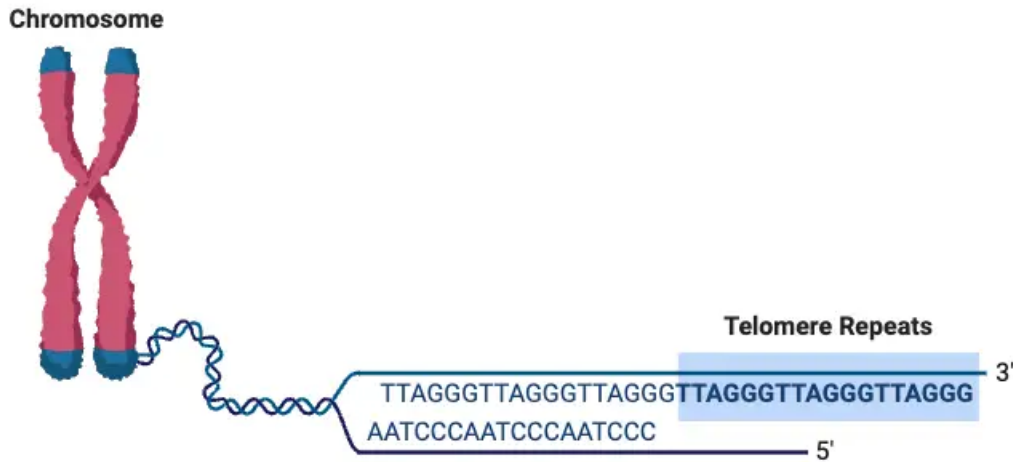
Difference between Telomere and Telomerase

What is a Telomere?

[Telomeres](#) are crucial components found at the ends of chromosomes. They are made up of repetitive nucleotide sequences that protect the genetic material from damage. Each time a [cell](#) divides, the telomeres shorten, playing a key role in cellular aging and health. Here's a breakdown of what telomeres are, how they work, and why they matter.

- **What They Are**
 - Telomeres are repetitive [DNA](#) sequences located at the ends of chromosomes.
 - In humans, the repeated sequence is **5'-TTAGGG-3'**, and it repeats around 3,000 times, which can span up to 15,000 base pairs.
 - They don't code for [proteins](#) but play a crucial role in maintaining [chromosome](#) integrity.
- **Structure**
 - Telomeres are made up of non-coding regions, meaning they don't produce proteins.
 - These sequences are rich in guanine [nucleotides](#), making them critical for their function.
 - At the very end of each telomere is a single-stranded overhang that forms a loop called the **T-loop**, which protects the chromosome from being recognized as damaged.
- **Function**
 - **Protection:** Telomeres act as a shield, preventing the chromosome ends from fraying or sticking together, which could lead to instability.
 - **Limiting [Cell Division](#):** Every time a cell divides, the telomeres shorten slightly. This is a built-in limit to how many times a cell can divide, ensuring that the cell doesn't divide infinitely.
 - **[Telomerase](#):** In certain cells like stem or cancer cells, an enzyme called **telomerase** can extend the telomeres, allowing these cells to bypass the normal aging process and keep dividing indefinitely.
- **Impact on Aging and Disease**
 - Telomere shortening is linked to **aging** and **age-related diseases**. As cells divide, telomeres naturally get shorter, which contributes to the overall aging of [tissues](#).
 - On the flip side, some cells—like cancer cells—often have higher levels of **telomerase activity**, allowing them to keep dividing uncontrollably. This can be a key factor in [tumor](#) growth.

Telomeres are essential in maintaining the stability of our genetic material, but they also play a delicate [balancing](#) act in aging and disease prevention.



Telomere Repeats

What is Telomerase?

Telomerase is an enzyme that helps keep the telomeres—the protective caps at the ends of chromosomes—at a functional length. It does this by adding specific nucleotide sequences to the telomeres, counteracting the natural shortening that occurs during cell division. Here’s how it works and why it matters:



- **What It Is**

- Telomerase is a **ribonucleoprotein**—meaning it consists of both [RNA](#) and [protein](#).
- It includes **telomerase reverse transcriptase (TERT)**, a protein subunit, and an RNA [molecule](#) that provides a template for adding DNA repeats to the telomeres.



- **How It Works**

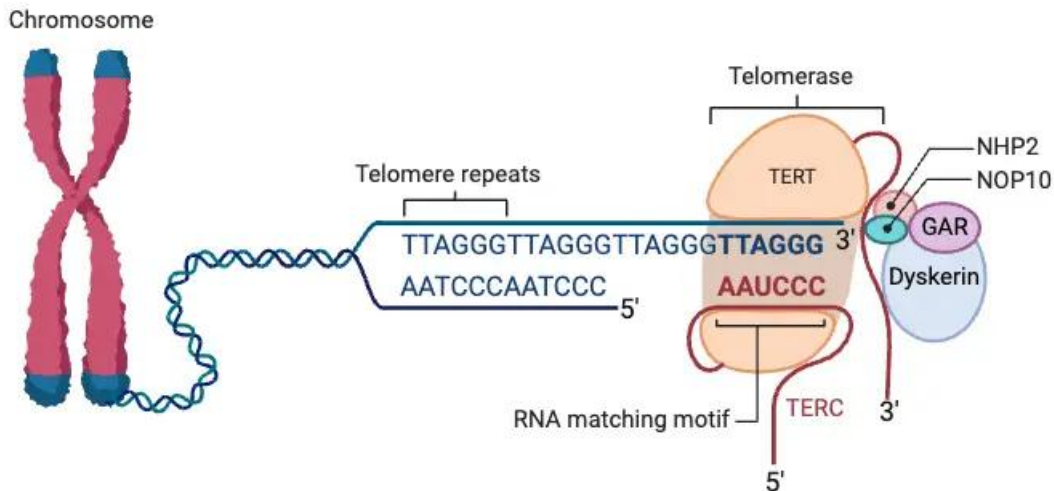
- Telomerase prevents telomere shortening by adding repetitive sequences to the ends of chromosomes.
- Each time a cell divides, a small part of the telomere is lost. Telomerase adds the **TTAGGG** sequence to prevent this loss and keep the telomeres intact.
- It works by binding to the **single-stranded overhang** at the end of a telomere, using its RNA template to extend the telomere length.

- **Key Functions**

- **Length Maintenance:** It adds guanine-rich sequences, which are crucial for preserving the chromosome’s integrity during division.

- **Cellular Longevity:** By extending the telomeres, telomerase ensures that certain cells—like **stem cells** and **germ cells**—can continue dividing without entering **senescence** or **apoptosis** (programmed cell death).
- **Cancer Role:** Many cancer cells have reactivated telomerase, allowing them to bypass normal aging processes and continue dividing uncontrollably.
- **Clinical Implications**
 - **Aging:** As telomeres shorten over time, they are linked to cellular aging and age-related diseases. Telomerase research could provide insights into potential treatments for slowing aging.
 - **Cancer Treatment:** Since cancer cells rely on active telomerase for unrestrained growth, targeting this enzyme is a promising strategy for cancer therapies aimed at limiting tumor growth.

Telomeres and Telomerase



Difference between Telomere and Telomerase

Telomeres and telomerase are both critical components in maintaining cellular stability, but they play distinct roles in the processes of cell division and aging. Here's a breakdown of their differences:

- **What They Are**
 - **Telomeres** are repetitive DNA sequences found at the ends of chromosomes.
 - **Telomerase** is an enzyme made up of both protein and RNA, responsible for extending the length of telomeres.
- **Composition**
 - **Telomeres** consist of non-coding DNA, specifically the repetitive sequence **TTAGGG** in humans.

- **Telomerase** consists of a protein component (**TERT**) and an RNA component (**TERC**), both of which work together to extend telomeres.
- **Function**
 - **Telomeres** protect the ends of chromosomes from degradation and prevent them from fusing with other chromosomes during cell division.
 - **Telomerase** actively adds nucleotide repeats to the telomeres to counteract their shortening during cell division, helping maintain chromosome stability.
- **Location**
 - **Telomeres** are located at the ends of chromosomes in eukaryotic cells.
 - **Telomerase** operates primarily in the [nucleus](#) of cells, particularly active in certain cell types like **germ cells**, **stem cells**, and **cancer cells**.



- **Role in Aging**
 - **Telomeres** shorten each time a cell divides, contributing to **cellular aging** and the eventual entry of cells into **senescence** or **apoptosis**.
 - **Telomerase** helps prevent telomere shortening, allowing some cells, like stem cells, to divide indefinitely and maintain their function.
- **Activity in Cells**
 - **Telomeres** are present in most somatic cells but shorten over time.
 - **Telomerase** is highly active in germ cells, stem cells, and many cancer cells, but it is inactive in most somatic cells.
- **Biological Clock**
 - **Telomeres** act as a **biological clock** that limits the number of times a cell can divide by shortening over time.
 - **Telomerase** resets this clock by adding nucleotide repeats to the telomeres, allowing continued cell division.



- **Mechanism of Action**
 - **Telomeres** function passively as protective structures during [DNA replication](#) and cell division.
 - **Telomerase** functions actively by using its RNA template to extend telomeres, preventing their degradation.
- **Clinical Implications**
 - **Telomere shortening** is linked to aging and **age-related diseases** like cardiovascular disease and cancer.
 - **Telomerase reactivation** is commonly found in cancer cells, enabling them to evade normal cellular aging and continue proliferating uncontrollably.
- **Therapeutic Potential**

- **Telomere shortening** could be targeted for therapies focused on **anti-aging** or age-related diseases.
- **Telomerase inhibition** holds potential as a cancer treatment, aiming to limit tumor growth by preventing the enzyme from extending telomeres.
- **Impact on Cancer**
 - **Shortened telomeres** lead to **chromosomal instability**, often a precursor to cancer progression.
 - **Telomerase** is reactivated in many cancers, enabling tumor cells to bypass senescence and divide endlessly.

Aspect	Telomere	Telomerase
Definition	Repetitive DNA sequences at the ends of chromosomes.	Enzyme that synthesizes telomere DNA sequences.
Composition	Non-coding DNA, specifically the sequence TTAGGG in humans.	Protein (TERT) and RNA (TERC) components.
Function	Protects chromosome ends from degradation and prevents fusion with other chromosomes.	Adds nucleotide repeats to telomeres, maintaining their length during cell division.
Structure	Tandem repeats of DNA forming a protective cap.	Ribonucleoprotein structure, integrating both RNA and protein elements.
Location	Found at the ends of linear chromosomes in eukaryotes.	Found in the nucleus, active in specific cell types.
Role in Aging	Shortens with each cell division, contributing to cellular aging and senescence.	Counteracts telomere shortening, allowing certain cells to divide indefinitely.
Activity in Cells	Present in most somatic cells but shortens over time.	Highly active in germ cells, stem cells, and many cancer cells; inactive in somatic cells.

Aspect	Telomere	Telomerase
Biological Clock	Acts as a biological clock, limiting the number of divisions a cell can undergo.	Resets the biological clock by extending telomeres, allowing continued cell division.
Genetic Stability	Maintains genetic stability by preventing chromosome end degradation or fusion.	Ensures telomeres remain intact and functional, maintaining genetic stability.
Mechanism of Action	Functions passively as a protective structure during DNA replication and division.	Actively adds DNA sequences to telomeres using its RNA template during cell division.
Clinical Implications	Shortening is associated with age-related diseases and cellular senescence.	Reactivation is observed in cancer cells, contributing to uncontrolled growth.
Research Focus	Studied for its role in aging, longevity, and age-related diseases like cancer.	Studied for therapeutic targets in cancer treatment and anti-aging interventions.
Regulation	Length regulated by natural aging and stressors like oxidative stress.	Activity regulated by signaling pathways, gene expression , and environmental cues.
Role in Development	Essential for chromosome function during development; ensures genomic stability.	Critical for stem cells to maintain pluripotency and differentiation ability.
Presence in Organisms	Present in almost all eukaryotic organisms, including plants, animals, and fungi .	Found in specific cell types (germ cells, stem cells), low or absent in most somatic cells.
Telomere Length Variability	Length varies across cell types and species; shorter in somatic cells.	Activity varies across tissues; higher activity correlates with longer telomeres.

Aspect	Telomere	Telomerase
Evolutionary Role	Evolved to protect genetic material from degradation.	Evolved to extend the lifespan of critical cell types for reproduction and tissue maintenance.
Interaction with Other Proteins	Interacts with shelterin complex proteins that protect telomeres.	Interacts with regulatory proteins that modulate its activity and recruitment.
Impact on Cancer Biology	Shortened telomeres lead to chromosomal instability, a hallmark of cancer.	Reactivated telomerase allows cancer cells to evade senescence and proliferate.
Therapeutic Potential	Targeting telomere shortening could lead to therapies for age-related diseases.	Inhibiting telomerase activity offers a potential strategy for cancer treatment.

Telomere Breakthrough: Shown Effective on Identical Twins

And How This Surprising Discovery Could Mean Healthier Aging For YOU.

If you want to stay active and vibrant into your 70s, 80s, and beyond...then you need to hear about this...

The story begins with astronaut Scott Kelly, who blasted off for the International Space Station in 2015. His mission was to spend nearly a year in space.

NASA scientists wanted to study the effects of space on his mind and body over such a long period of time. So they set up a battery of tests. One of them was quite unusual.

You see, Scott Kelly is the only astronaut in history to have *an identical twin brother*. That means he and his brother have the same genetic makeup. So the scientists could compare Scott to his brother and see what effect space flight has on the body.

The scientists compared the brothers on various bio-markers. They also compared the two twins' *telomeres*.

Telomeres, as you may know, are the extra bits of DNA on the end of your chromosomes. They are like the plastic caps on the end of your shoelaces. When the caps on your shoelaces wear down, your shoelaces unravel. Well, telomeres work like that.

Every time your cells divide, your telomeres get a little shorter. Eventually, these telomere “caps” get so short, your cells can no longer make perfect copies of your DNA. So cells go dormant or die. Many scientists think telomere shortening is one of the best markers of your true biological age.

Now, back to Scott Kelly. When Kelly flew off into space, NASA scientists expected his telomeres to shorten. After all, he was going to be under a huge amount of stress:

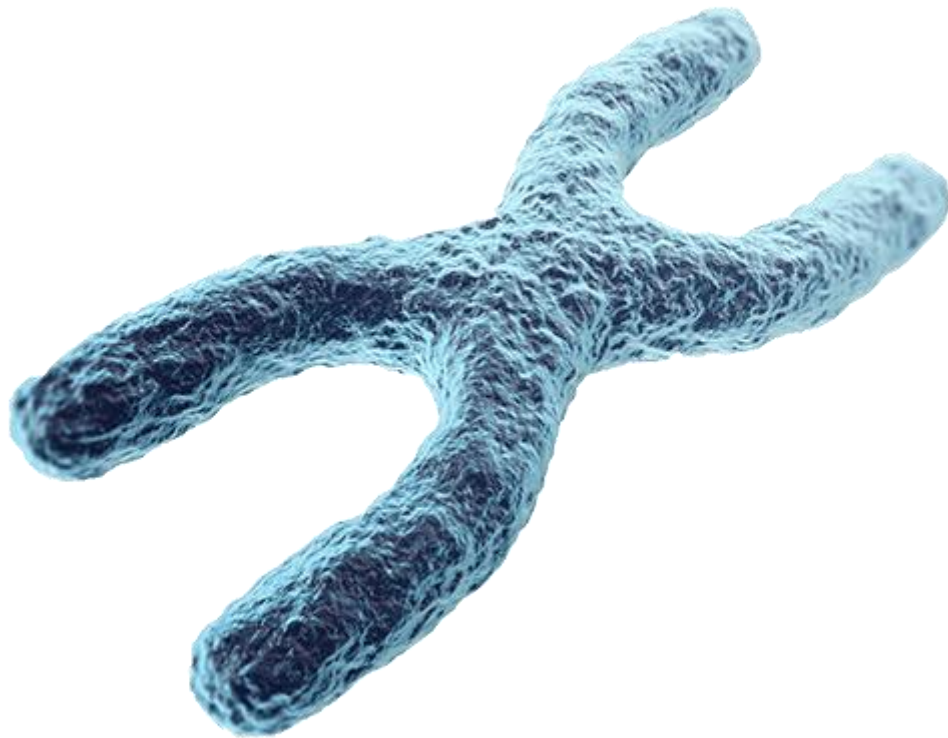
mental, physical, and stress from radiation. Stress shortens your telomeres and ages you.

So when Scott Kelly returned to earth, one year later, scientists expected the worst. When he returned, his body mass had withered. His gut bacteria had completely changed.

But when scientists compared his telomeres to his identical twin brother's, they were shocked at what they saw...

Scott Kelly's telomeres *had actually grown longer!*

That's right, *longer*. How could this happen? I'll tell you how in a second, but first, you need to know...



Why It's So Important To Keep Your Telomeres Long

Your telomeres start shortening even before you are born. They keep shortening throughout your life. By the time you're middle-aged, your telomeres are dramatically shorter. And this is not good news for your health.

It means your cells are becoming less functional and less capable of repairing themselves. Shorter telomeres increase the chance that your cells become old and dormant. Almost like "zombie" cells.

So slowing down the shortening of your telomeres may be helpful in supporting healthy aging at the cellular level.

So How Did Scott Kelly Keep His Telomeres From Shortening?

NASA scientists were dumbfounded when they first saw Scott Kelly's telomeres. But then they realized they had designed an intense diet and exercise program to keep him in tip-top shape during his mission. And this rigorous exercise probably slowed down his telomere shortening.

You see, diet and lifestyle can dramatically influence your telomeres.

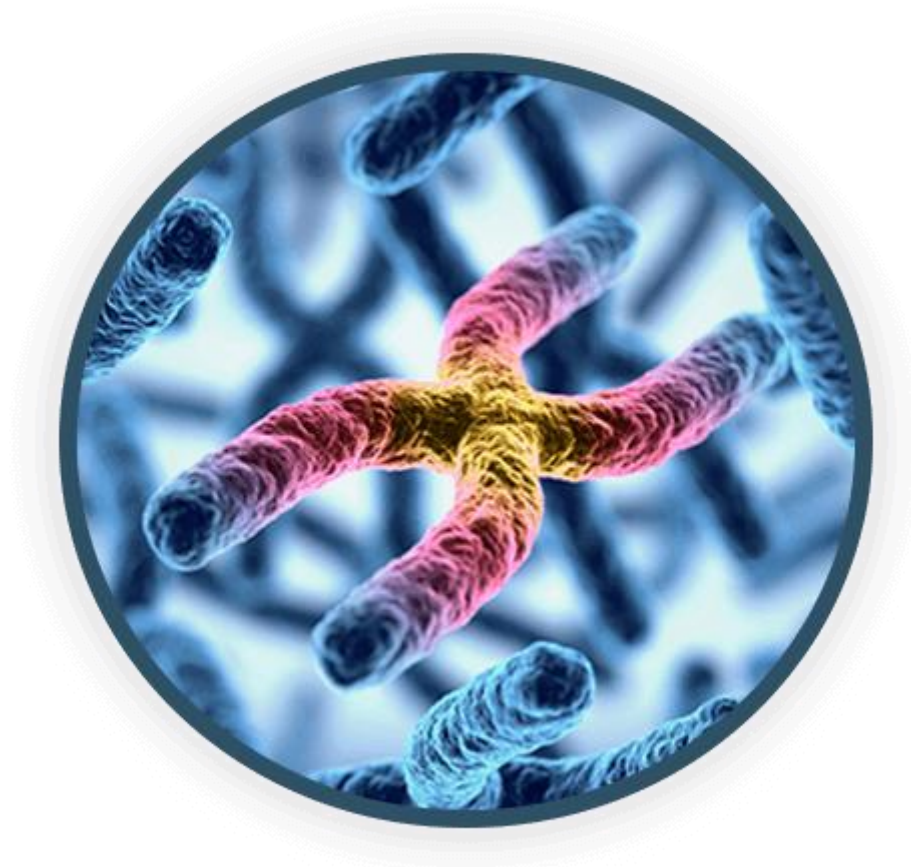
For example, if you exercise regularly, you'll have longer telomeres. Especially after age 40. In fact, adults who are physically active can have a *9-year telomere advantage* over people who don't exercise as much.

You also need to eat well. Get enough sleep. Reduce stress. Stay connected with people socially.

All this is good advice. And as a health-savvy reader, you're probably doing a lot of these things already.

But today, I'm going to show you how to go beyond these obvious choices...to what else you can do to protect your telomeres and support healthy aging...

...without punishing yourself in the gym or changing your diet.



How A Strange Experiment With “Pond Scum” Led To A Breakthrough In Healthy Telomeres

Scientists began studying telomeres back in the 1970s. But it wasn't until they looked at “pond scum” that their research really lit up the science world.

For their experiments, they used a tiny, pond-dwelling microorganism called a **Tetrahymena**. These organisms have over 10,000 pairs of chromosomes—versus just 23 pairs for humans. So they offer a rich source of telomeres to study.

As single-cell organisms, Tetrahymena should die out quickly, as their telomeres shorten. But for some reason, they didn't. So these researchers looked closer. Soon, their persistence paid off. Bingo! They discovered an enzyme hidden deep in the cells of Tetrahymena that seemed to protect telomeres from shortening. They named the enzyme telomerase.

This telomerase enzyme is found in many different types of cells. And in every living organism, including humans.

Telomerase has proteins and genetic bits that basically "rewrite" telomeres to keep them from shortening. It helps replace lost bits of DNA and helps cells repair themselves. It's a key to healthy cell division.

Bottom line:

Telomerase may help slow down the shortening of telomeres!

So you want to do everything you can to pump up your telomerase.

Well I have good news for you today. Scientists have discovered there are all-natural ways to activate your telomerase and support your telomeres. Starting with...

Telomerase Activator #1

The Genetic Miracle Found In An Ancient Chinese Herb

Chinese doctors have used this traditional herb for thousands of years. They consider it a "Qi" tonic—a natural substance that boosts energy in the body.

But Chinese doctors probably never imagined that this herb also contained a powerful genetic secret. Certain molecules in this herb can activate telomerase, the telomere-lengthening enzyme.

It's called **astragalus**. And UCLA scientists found that a chemical inside the plant is powerful enough to slow down and even prevent telomeres from shortening.

In fact, astragalus contains the same active ingredient as the \$25,000-a-year treatment I mentioned before. But in a minute, I'll show you how you can get it without breaking the bank.





Telomerase Activator #2

The Ayurvedic Telomere Protector

This next herb is called the 'King of Medicines' in the ancient Indian system of Ayurveda. It's prized for its many healing powers, including calming inflammation and soothing wounds.

It's called **Terminalia chebula** (also known as 'Haritaki'). But it wasn't until scientists started looking at Terminalia under the microscope, that they discovered its power for healthy aging.

In one study, scientists took human cells and exposed them to harmful UVB light. That's what zaps your skin when you don't protect it from the sun's harmful rays.

Half the cells were treated with Terminalia, half the cells weren't. The result? The telomeres in the cells that weren't protected by Terminalia got shorter—much shorter. In fact, they shortened almost twice as fast.

It turns out, Terminalia contains special flavonoids that protect your telomeres from shortening. Terminalia also increased the longevity of cells by as much as 40% — while protecting telomeres from shrinking.

While this is only preliminary information, it does hold out that it may help with maintaining telomere length as we age.

Telomerase Activator #3

To Keep Stress From Shortening Your Telomeres

It's natural to feel a little stressed from time to time. But being stressed all the time is a big problem for you—and your telomeres.

Constant stress leads to more cortisol—the stress hormone. And too much cortisol can weaken your cells through oxidative damage.

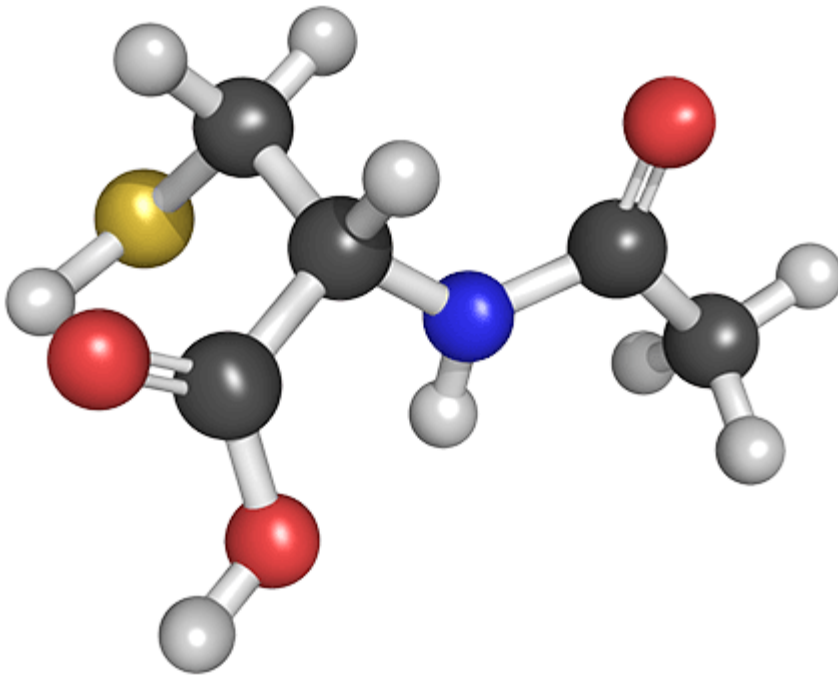
Now, scientists have discovered that too much cortisol also robs you of telomerase. This can speed up the shortening of your telomeres and accelerate aging.

One study even found the damage inflicted by chronic stress was equal to *17 years of faster aging!*

So it's vital you get your stress under control. And one of the best ways is with a herb called **rhodiola**.

Rhodiola really shines at improving your response to stress. That's because it reduces cortisol, the stress hormone. It also stimulates your neurotransmitters—like serotonin and dopamine. So it can improve your energy and mood.





Telomerase Activator #4

Your “Master Antioxidant” For Telomere Health

Another way to slow down telomere shortening is to boost your glutathione levels.

Glutathione, as you may know, is your body’s “master antioxidant.” It helps fight infections and removes toxins from your body.

Now scientists have discovered that having more glutathione can lengthen your telomeres almost as much as high-intensity exercise.

But you can’t take a glutathione supplement, to get more. Because it never gets past your digestive system. So the answer is to take something that helps you make more of it.

One proven way is with **N-acetyl cysteine (NAC)**. NAC not only boosts glutathione, it strengthens your telomeres. In lab tests with animals, NAC boosted telomerase and increased telomere length and may even slow down aspects of aging.

In human studies, NAC kept cells from going “dormant.” And slowed down cellular aging by scavenging free radicals.

Some experts say boosting your glutathione is your “best insurance” against DNA errors. It’s that powerful.

Telomerase Activator #5

The Super Power In Broccoli

You know that eating vegetables like broccoli and cauliflower is good for you. But do you know why these vegetables are so healthy?

One reason is an organic sulfur compound called sulforaphane. Sulphoraphane is a natural detoxifier. It helps your body get rid of many types of free radicals and environmental pollutants. And one of the best sources of sulforaphane comes from... **Broccoli seed extract.**

In scientific studies, broccoli seed extract squashed oxidative stress and supported healthy cholesterol levels.

And now, scientists have discovered that broccoli also protects your telomeres. Broccoli seed extract has been found to significantly boost telomerase—the enzyme that lengthens your telomeres.

So if you don’t get a heaping helping of broccoli every day, do the next best thing: Add broccoli seed extract to your telomere-protecting program.





Telomerase Activator #6

To Fight Free Radical Damage To Your Telomeres

You've heard about free radicals, of course. And know how these rogue molecules can "oxidize" and damage cells in your body.

That's why we try to eat a lot of antioxidant-rich foods like fruits and vegetables. And take antioxidant supplements.

Oxidative stress is nasty business. It attacks your proteins, lipids (fats) and even your DNA. And get this: it goes after your telomeres, too.

As you age, free radicals gum up your ability to produce telomerase. So your telomeres keep shrinking until your cells can no longer divide.

It's time to strike back, with one of the most powerful antioxidants known. Scientists have discovered that a special compound inside **blueberries** can turn things around.

When scientists added these compounds to cells in the lab, it "switched back on" a major group of genes that switch off as you age. It's so powerful, it can revive old, dormant, "sleeping" cells.

Not only that, within hours of treatment, the "sleeping" cells getting blueberry compounds started to divide again. They even developed longer telomeres than the other cells.

"When I saw some of the cells in the culture dish rejuvenating I couldn't believe it," said Dr. Eva Latorre, a researcher on the project. "These old cells were looking like young cells. It was like magic. I repeated the experiments several times and in each case the cells rejuvenated."

That's why you want a concentrated blueberry extract in your corner to keep your telomeres healthy and strong.

Help Support Telomeres For Healthy Aging

I've been on a mission for years to help my patients age gracefully and stay active.

But now I'd like to kick it up a notch. I want to help you go beyond the diet and exercise basics you may already be doing.

Now you can fight aging at the most fundamental level. Deep inside your cells—at the very source of aging—your DNA and telomeres. Your God-given “instructions” for life! That’s why I’ve teamed up with the folks at Advanced Bionutritionalsto create a single supplement with all 6 of these telomere champions. It’s called [Advanced Telomere Support](#). And it has research-level doses of the telomere protectors I’ve mentioned:



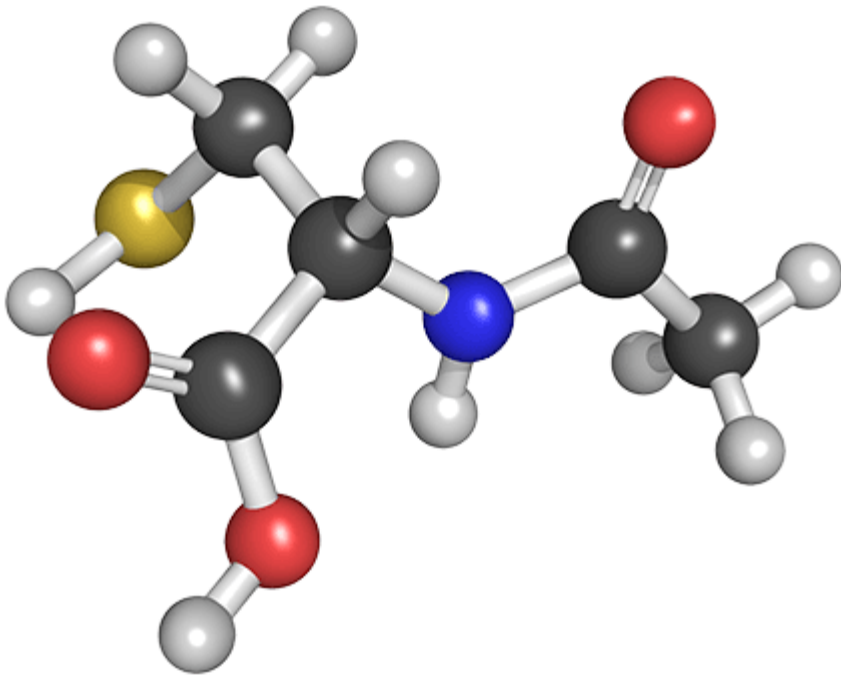
Astragalus root extract: telomerase activator



Terminalia chebula: A potential Ayurvedic telomere protector



Rhodiola rosea: To keep stress from shortening your telomeres



N-acetyl cysteine (NAC): Supports your “master antioxidant”



Broccoli seed extract: With health-supporting sulforaphane



Blueberry extract: To fight free radical damage

All these nutrients were chosen because they support healthy telomeres. They can slow down the rate at which your telomeres shorten...and support healthy aging in every cell of your body.

If you went out and bought these nutrients at your health food store, you could easily spend over \$102.87. But thanks to this private offer, you can get all of them for as little as \$41.50.

Plus, you'll have the peace of mind of knowing you're doing everything you can to support your telomeres' health. To help you stay healthy, active, and independent in your older years!

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9

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Supplement Facts

Serving Size 2 Capsules
Servings Per Container: 30

	%	Daily Value
Astragalus (<i>Astragalus membranaceus</i>) root, dried extract, min 2% 4-hydroxy-3-methoxyisoflavone-7	250 mg	*
TrueBroc®** Broccoli (<i>Brassica oleracea</i> var. italica seeds), dried extract, min 13% glucoraphanin	50 mg	*
Rhodiola (<i>Rhodiola rosea</i>) root, dried extract, min 3% total rosavins, 1% salidroside	100 mg	*
Chebolic Myrobalan (<i>Terminalia chebula</i>) fruit, dried extract, min.45% tannins	50 mg	*
Blueberry (<i>Vaccinium corymbosum</i>) fruit, dried extract, min. 5% anthocyanins	30 mg	*
N-Acetyl-L-Cysteine	200 mg	*
*Daily Value not established.		
Other ingredients: Vegetarian capsule (hydroxypropyl methylcellulose, water), powdered cellulose, silica, calcium stearate, and rice bran extract.		
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Telomere extension turns back aging clock in cultured human cells, study finds

Researchers delivered a modified RNA that encodes a telomere-extending protein to cultured human cells. Cell proliferation capacity was dramatically increased, yielding large numbers of cells for study.

January 22, 2015 - By Krista Conger



Helen Blau

A new procedure can quickly and efficiently increase the length of human telomeres, the protective caps on the ends of chromosomes that are linked to aging and disease, according to scientists at the [Stanford University School of Medicine](#).

Treated cells behave as if they are much younger than untreated cells, multiplying with abandon in the laboratory dish rather than stagnating or dying.

The procedure, which involves the use of a modified type of RNA, will improve the ability of researchers to generate large numbers of cells for study or drug development, the scientists say. Skin cells with telomeres lengthened by the procedure were able to divide up to 40 more times than untreated cells. The research may point to new ways to treat diseases caused by shortened telomeres.

Telomeres are the protective caps on the ends of the strands of DNA called chromosomes, which house our genomes. In young humans, telomeres are about 8,000-10,000 nucleotides long. They shorten with each cell division, however, and when they reach a critical length the cell stops dividing or dies. This internal “clock” makes it difficult to keep most cells growing in a laboratory for more than a few cell doublings.

‘Turning back the internal clock’

“Now we have found a way to lengthen human telomeres by as much as 1,000 nucleotides, turning back the internal clock in these cells by the equivalent of many years of human life,” said [Helen Blau](#), PhD, professor of microbiology and immunology at Stanford and director of the university’s [Baxter Laboratory for Stem Cell Biology](#). “This greatly increases the number of cells available for studies such as drug testing or disease modeling.”

A paper describing the research was published today in the *FASEB Journal*. Blau, who also holds the Donald E. and Delia B. Baxter Professorship, is the senior author. Postdoctoral scholar [John Ramunas](#), PhD, of Stanford shares lead authorship with Eduard Yakubov, PhD, of the Houston Methodist Research Institute.

The researchers used modified messenger RNA to extend the telomeres. RNA carries instructions from genes in the DNA to the cell’s protein-making factories. The RNA used in this experiment contained the coding sequence for TERT, the active component of a naturally occurring enzyme called telomerase. Telomerase is expressed by stem cells, including those that give rise to sperm and egg cells, to ensure that the telomeres of these cells stay in tip-top shape for the next generation. Most other types of cells, however, express very low levels of telomerase.

Transient effect an advantage

The newly developed technique has an important advantage over other potential methods: It’s temporary. The modified RNA is designed to reduce the cell’s immune response to the treatment and allow the TERT-encoding message to stick around a bit longer than an unmodified message would. But it dissipates and is gone within about 48 hours. After that time, the newly lengthened telomeres begin to progressively shorten again with each cell division.

The transient effect is somewhat like tapping the gas pedal in one of a fleet of cars coasting slowly to a stop. The car with the extra surge of energy will go farther than its peers, but it will still come to an eventual halt when its forward momentum is spent. On a biological level, this means the treated cells don’t go on to divide indefinitely, which would make them too dangerous to use as a potential therapy in humans because of the risk of cancer.

This new approach paves the way toward preventing or treating diseases of aging.

The researchers found that as few as three applications of the modified RNA over a period of a few days could significantly increase the length of the telomeres in cultured human muscle and skin cells. A 1,000-nucleotide addition represents a more than 10 percent increase in the length

of the telomeres. These cells divided many more times in the culture dish than did untreated cells: about 28 more times for the skin cells, and about three more times for the muscle cells.

“We were surprised and pleased that modified TERT mRNA worked, because TERT is highly regulated and must bind to another component of telomerase,” said Ramunas. “Previous attempts to deliver mRNA-encoding TERT caused an immune response against telomerase, which could be deleterious. In contrast, our technique is nonimmunogenic. Existing transient methods of extending telomeres act slowly, whereas our method acts over just a few days to reverse telomere shortening that occurs over more than a decade of normal aging. This suggests that a treatment using our method could be brief and infrequent.”

Potential uses for therapy

“This new approach paves the way toward preventing or treating diseases of aging,” said Blau. “There are also highly debilitating genetic diseases associated with telomere shortening that could benefit from such a potential treatment.”

Blau and her colleagues became interested in telomeres when previous work in her lab showed that the muscle stem cells of boys with Duchenne muscular dystrophy had telomeres that were much shorter than those of boys without the disease. This finding not only has implications for understanding how the cells function — or don’t function — in making new muscle, but it also helps explain the limited ability to grow affected cells in the laboratory for study.

The researchers are now testing their new technique in other types of cells.

“This study is a first step toward the development of telomere extension to improve cell therapies and to possibly treat disorders of accelerated aging in humans,” said [John Cooke](#), MD, PhD. Cooke, a co-author of the study, formerly was a professor of cardiovascular medicine at Stanford. He is now chair of cardiovascular sciences at the Houston Methodist Research Institute.

“We’re working to understand more about the differences among cell types, and how we can overcome those differences to allow this approach to be more universally useful,” said Blau, who also is a member of the [Stanford Institute for Stem Cell Biology and Regenerative Medicine](#).

“One day it may be possible to target muscle stem cells in a patient with Duchenne muscular dystrophy, for example, to extend their telomeres. There are also implications for treating conditions of aging, such as diabetes and heart disease. This has really opened the doors to consider all types of potential uses of this therapy.”

Other Stanford co-authors of the paper are postdoctoral scholars Jennifer Brady, PhD, and Moritz Brandt, MD; senior research scientist Stéphane Corbel, PhD; research associate Colin Holbrook; and Juan Santiago, PhD, professor of mechanical engineering.

The work was supported by the [National Institutes of Health](#) (grants R01AR063963, U01HL100397 U01HL099997 and AG044815), Germany's Federal Ministry of Education and Research, [Stanford Bio-X](#) and the Baxter Foundation.

Ramunas, Yakubov, Cooke and Blau are inventors on patents for the use of modified RNA for telomere extension.

Information about Stanford's Department of Microbiology and Immunology, which also supported the work, is available at <http://microimmuno.stanford.edu>.

Mitochondria makeover: unlocking the path to healthy longevity

1. Introduction

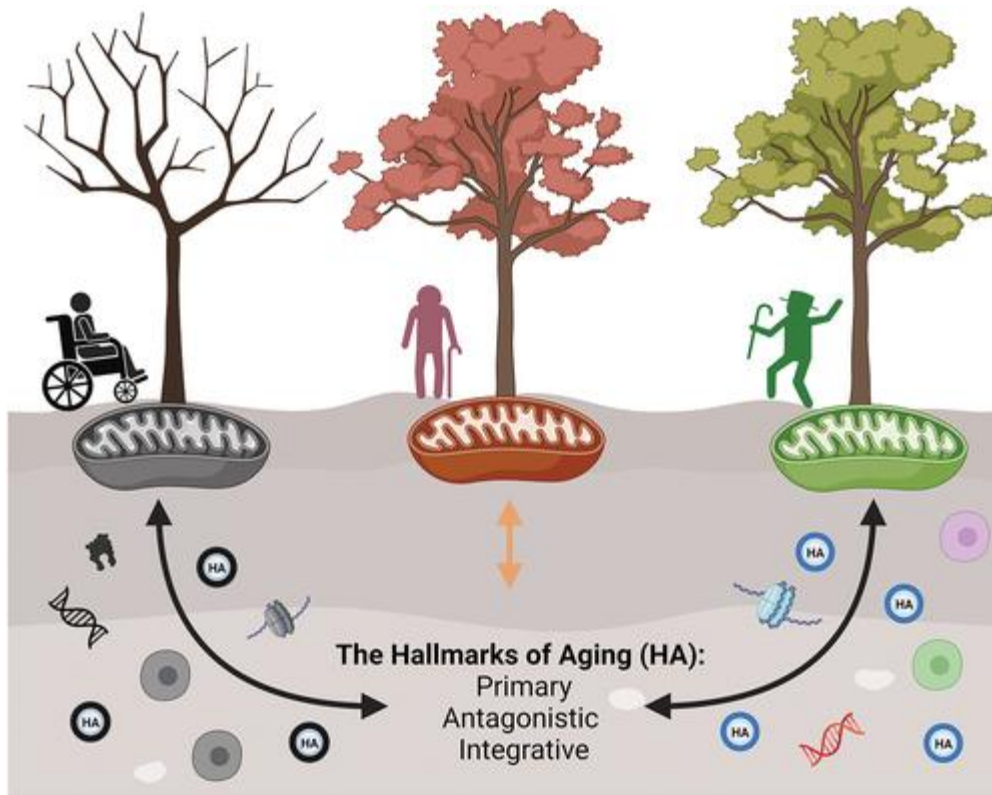
Thanks to biomedical research, significant advances have been made in preventing deaths caused by infectious agents and chronic diseases. Scientific progress has contributed to a remarkable increase in life expectancy, soaring from an estimated 30 years around 1800 to nearly 73 years by 2019 [[Citation1](#)]. This unprecedented global rise in life expectancy has resulted in a noticeable shift in the age distribution toward older demographics. However, even with these advancements, we still experience frailty as we age, accompanied by disabilities that reduce our quality of life. While the prospect of an extended lifespan is generally viewed positively, it is essential to recognize that living longer does not automatically guarantee good health. Therefore, developing interventions aimed at promoting healthy longevity has become imperative.

Achieving healthy longevity and mitigating the progressive loss of physiological integrity is a complex endeavor influenced by various factors that contribute to the development of vulnerability and diseases over time [[Citation2](#)]. In 2013, Lopez-Otin et al. proposed nine hallmarks of aging, sparking the publication of more than 300,000 articles with the aim of dissecting each hallmark to understand the molecular changes and develop interventions to mitigate aging and aging-associated diseases [[Citation3](#)]. Recently 12 hallmarks of aging were described, taking into account age-associated alterations in molecular, cellular, and systemic processes related to the deterioration of biological function over time. These hallmarks include primary (genomic instability, telomere attrition, epigenetic

alterations, loss of proteostasis, and disabled macroautophagy), antagonistic (deregulated nutrient-sensing, cellular senescence, and mitochondrial dysfunction), and integrative (stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis) [Citation3]. Notably among these hallmarks is mitochondria, which are interconnected with all of them, potentially offering a pathway to healthy longevity.

Today, our understanding of mitochondria extends beyond cellular boundaries, as they have been identified as viable entities outside of cells existing in circulation [Citation4-6]. Mitochondria transfer seems to play crucial roles in energy management and facilitating inter-organ metabolic adaptation, particularly in response to nutrient stress and possibly caloric restriction [Citation7]. The phenomenon of natural (natural mitochondrial transfer, NMT) and artificial mitochondrial transfer/transplant (AMT/T) between cells opens up the possibility of modifying the metabolism, mitochondrial DNA (mtDNA), and phenotype of recipient cells, particularly those that could be affected by the aging process [Citation8,Citation9]. This suggests that mitochondria, with their multifaceted roles in intracellular molecular interactions, cellular function, and systemic effects by their transfer between cells, may occupy a central position in the study of the hallmarks of aging and the development of interventions (Figure 1). We focus on uncovering and proposing an untested link between the hallmarks of aging and mitochondrial transfer to promote healthy longevity.

Figure 1. Mitochondria exert an influence on the hallmarks of aging, while the hallmarks, in turn, reciprocally impact mitochondria, shaping the aging process. Trees symbolize the diverse ways we experience aging, and they are intricately linked to mitochondrial function. Mitochondrial function, in turn, is influenced by the hallmarks of aging, which can also be influenced by mitochondria. The hallmarks of aging are categorized into three groups: primary (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, and disabled macroautophagy), antagonistic (deregulated nutrient-sensing), and integrative (cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis). The hallmarks of aging, much like soil, can be differently enriched, leading to changes in mitochondrial function. Created with BioRender.com.



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2. Mitochondrial dysfunction: a central hub linking all other hallmarks of aging

Mitochondria serve as a central hub connecting all other hallmarks of aging and encompassing both their well-understood functions and those crucially remain to be described, such as mitochondrial transfer between cells [Citation10,Citation11]. In light of recent advances in interconnected pathways that may contribute to longevity and reduced age-associated diseases, mitochondria emerge as a key factor of aging decay. Furthermore, it is crucial to delve into how these factors could influence the release and uptake of our 'nomad' mitochondria as they migrate outside the cell and from one cell to another, enriching recipient cells with healthy mitochondria and potentially reducing effects on the aging hallmarks.

Single nucleotide polymorphisms (SNPs) in mtDNA have been associated with healthy longevity in centenarians, providing insights into their key role in the aging process and the importance of genomic stability [Citation12]. In contrast, genomic instability is related to the accumulation of somatic mutations in nuclear and mitochondrial DNA affecting essential genes and transcriptional pathways, leading to cellular dysfunction and compromising our health. Depletion in mtDNA content

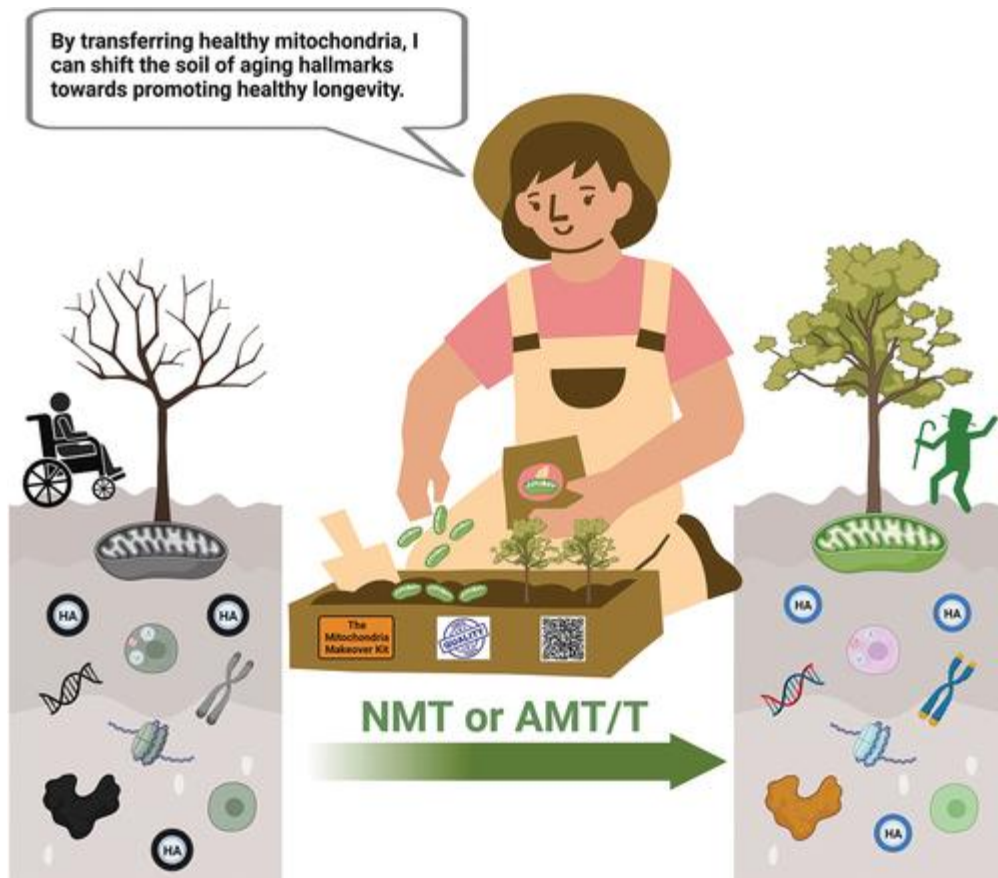
and mitochondrial number is reported during aging [Citation13]. Low mtDNA copy number correlates with frailty and all-cause mortality. Recent research indicates an average loss of four mtDNA copies per decade in humans, linked to age-related physiological changes [Citation14-16]. Additionally, numerous studies affirm the connection between reduced mtDNA content and aging and aging-related diseases [Citation13]. Since the pioneering study conducted by Clark and Shay in 1982, AMT/T has enabled the selective enrichment of a cell's mitochondrial content with a specific type conferring a selective advantage to cells that are less fit or have become unhealthy as a result of the aging process [Citation6,Citation17].

Among the primary hallmarks of aging, the connection between telomere damage and mitochondrial dysfunction is pivotal to understanding cellular decay over time. The relationship between telomere attrition and mitochondrial metabolic malfunction, particularly through the p53-PGC-1 α -NRF-1 axis, remains a subject requiring further research [Citation18]. Interestingly, during the reprogramming of induced pluripotent stem cells (iPSCs), which is associated with potential rejuvenation, telomerase is upregulated, resulting in the lengthening of telomeres to embryonic stem cell (ESC)-like lengths. However, mitochondria that have accumulated mutations during the previous somatic life of the iPSCs exhibit diverse responses, leading to the generation of various mtDNA variants and mitochondrial heterogeneity [Citation19]. Some iPSCs appear to be better suited than others, with specific mtDNA variants improving reprogramming efficiency [Citation20]. The question arises: Is it possible to enhance the reprogramming process using specific AMT/T of mitochondria with mtDNA variants to effectively reset the cellular clock and rejuvenate cells? This intriguing avenue of research holds promise for unlocking new insights into cellular rejuvenation and healthy aging.

The other primary, antagonistic and the integrative hallmarks,—altered intercellular communication, stem cell exhaustion, and chronic inflammation – could also be mediated by NMT and AMT/T of healthy mitochondria. Mesenchymal stem cells (MSCs), along with the hematopoietic niche, are key components of tissue stability and function. Currently, it is unknown how MSCs are affected with time and whether the aging process could change their capacity to transfer mitochondria naturally to other cells. NMT and AMT/T could have strong implications for how cells communicate with each other to maintain their function over time and preserve the health of the immune system (Figure 2). Furthermore, observations indicate that the transfer of mitochondria to immune cells, whether through natural processes or via AMT/T from MSCs, can induce an immunoregulatory profile and potentially reduce chronic inflammation. Indeed, Singh et al. (2018) have shown

that restoring mitochondrial function can mitigate the aging process in mice [Citation13].

Figure 2. Mitochondria, playing critical roles in molecular, cellular, and systemic aspects of aging, have the potential to profoundly impact the hallmarks of aging (HA) through both natural mitochondrial transfer (NMT) and artificial mitochondrial transfer/transplant (AMT/T). Mitochondria play a pivotal role in governing the molecular, cellular, and systemic processes associated with aging, both in accelerating and potentially reversing the aging process for healthy longevity. In addition to their vital intracellular functions, both NMT and AMT/T hold promise as tools for gaining deeper insights into the hallmarks of aging and for the development of therapeutic interventions. Created with BioRender.com.



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It's worth noting that, except for a few exceptions, the spectrum, precise nature and extent of mitochondrial dysfunction concerning the hallmarks of aging remain largely unknown across various cell types and tissues. The factors primarily responsible for the decline in the common core components of the mitochondrial metabolic machinery, leading to dysfunction and subsequent aging in different

tissues, organs, and the entire organism, remain unclear. Intriguingly, not all tissues age in the same manner, adding further complexity to this phenomenon. It could be possible to stimulate NMT between cells in a particular tissue or harness AMT/T with tissue-specific administration of mitochondria. This approach should lead to the utilization of specific mitochondrial transfers in tissues that are particularly affected, offering innovative strategies to rejuvenate and mitigate tissue-specific aging (Figure 2).

Targeting mitochondria and facilitating NMT or AMT/T as a primary approach to extend lifespan and delay aging-associated disease onset necessitates more research. This research should span from basic science to translational and clinical validation. The identification and understanding of core similarities and tissue-specific differences in the nature and scope of mitochondrial dysfunction, along with its connections to the hallmarks of aging, hold the potential to significantly advance our knowledge in the field of healthy longevity (Figure 2).

3. Expert opinion

In the pursuit of achieving healthy longevity, we have discovered that intricate interactions among aging hallmarks converge upon a central player – mitochondria. This dynamic organelle, long recognized for its pivotal role in energy production, management, and metabolism, has now emerged as a linchpin connecting the dots among distinct aging hallmarks. From genomic instability to telomere attrition, from cellular senescence to chronic inflammation, mitochondria stand at the nexus of these age-related processes. Recent breakthroughs in our understanding of NMT and AMT/T have illuminated a path toward restoring mitochondrial function, regenerating cellular vitality, and rejuvenating aging cells, tissues, and organs. These ‘nomad’ mitochondria, migrating between cells, enriching recipient cells with essential functions, and potentially mitigating the effects of other aging hallmarks, present new strategies for extending healthy lifespans (Figure 2). While it is known that the restoration of mitochondrial function can reverse various aging phenotypes [[Citation13](#)], it is currently unknown whether cellular rejuvenation through mitochondrial transfer/transplantation can fully reverse the effects of aging. As research advances and synthetic biology opens up new possibilities, we may harness the power of custom-designed mitochondria to re-energize aging tissues and bolster our health over time. In the ever-evolving landscape of aging research, mitochondria must take center stage, offering the prospect of a healthier, more resilient journey through the passage of time.

Declaration of interest

A Caicedo is the leader of Dragon BioMed. KK Singh is the scientific founder of Yuva Biosciences and serves as Chief Scientific Advisor. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Author contributions

A Caicedo and KK Singh wrote the manuscript, cured, analyze and conveyed the information in the manuscript. A Caicedo and KK Singh proposed natural mitochondrial transfer (NMT) and artificial mitochondrial transfer/transplant (AMT/T) as key mitigators of aging hallmarks. A Caicedo made the figures with comments from KK Singh. A Caicedo and KK Singh conceived this work.

Acknowledgments

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November 13th, 2015

Investigating Mitochondrial Rejuvenation During Cellular Reprogramming and Embryonic Development

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The changes involved in producing [induced pluripotent stem cells](#) from ordinary [somatic cells](#), such as those from a skin sample, are [accompanied by mitochondrial rejuvenation](#), a clearance of [mitochondrial damage associated with aging](#). This also [occurs in the earliest stages of embryonic development](#), turning old parental cells into young child cells. It is not beyond the bounds of the possible to suggest that perhaps just this mitochondrial part of the transformation could be split off and used as the basis for a therapy - though [other approaches to mitochondrial repair](#) are far closer to realization. Also, it may well be that [mitochondria](#) are so vital to cellular function that it is impossible to safely induce such radical changes in adult tissues given the way in which cells are presently structured. As usual, the only way to find out is to dig deeper into what is going on under the hood, as researchers are doing here. The original research release [is in PDF format only](#), unfortunately, but it provides a better explanation than any of the other available resources:

A new study suggests that old mitochondria - the oxygen-consuming metabolic engines in cells - are roadblocks to cellular rejuvenation. By tuning up a gene called [Tcl1](#), which is highly abundant in eggs, researchers were able to suppress old mitochondria to enhance a process known as somatic reprogramming, which turn adult cells into embryonic-like stem cells. Researchers found that Tcl1 does its job by suppressing mitochondrial [polynucleotide phosphorylase \(Pn Pase\)](#), thereby inhibiting mitochondrial growth and metabolism.

Stem cell researchers had known that egg (or [oocyte](#)) [cytoplasm](#) contains some special unknown factors that can reprogramme adult cells into embryonic-like stem cells, either during egg-sperm fertilisation or during artificial cloning procedures. While researchers had invented a technology called induced pluripotent stem cell (iPSC) reprogramming to replace the ethically controversial [oocyte-based reprogramming technique](#), oocyte-based reprogramming was still deemed superior in complete cellular reprogramming efficiency. To address this shortfall, researchers combined oocyte factors with the iPSC reprogramming system. Their bioinformatics-driven screening efforts¹ led to two genes: Tc11 and its cousin [Tc11b1](#). After a deeper investigation, the team found that the Tc11 genes were acting via the mitochondrial [enzyme](#), PnPase. "We were quite surprised, because nobody would have thought that the key to the oocyte's reprogramming powers would be a mitochondrial enzyme. The stem cell field's conventional wisdom suggests that it should have been some other signalling genes instead."

Tc11 is a cytoplasmic protein that binds to the mitochondrial enzyme PnPase. By locking PnPase in the cytoplasm, Tc11 prevents PnPase from entering mitochondria, thereby suppressing its ability to promote mitochondrial growth and metabolism. Thus, an increase in Tc11 suppresses old mitochondria's growth and metabolism in adult cells, to enhance the somatic reprogramming of adult cells into embryonic-like stem cells. These new insights could boost efficacy of the alternative, non-oocyte based iPSC techniques for stem cell banking, organ and tissue regeneration, as well as further our understanding of how cellular metabolism rejuvenates after egg-sperm fertilisation.

Maintaining Telomeres Extends Lifespan in Mice

Overexpression also had a positive impact on tissue repair and regeneration.

Jan 22, 2025

A recent study has found that the overexpression of telomerase reverse transcriptase (TERT), which is a subunit of telomerase, an enzyme essential for telomere maintenance, [leads to lifespan extension in mice without significant side effects](#) [1].

Protecting DNA

[Telomere shortening](#) is a well-known [hallmark of aging](#). Telomeres are protective DNA sequences at the ends of the chromosomes. In most human cells, they become shorter with each division.

Telomerase and TERT have been found to be essential in maintaining telomere length [2]. Since telomeres become shorter with aging, reversing this process and extending telomere length may have the potential to extend longevity and health [3].

Creating genetically modified mice

The authors of a recent study started their research into TERT's impact on lifespan and healthspan by creating mice that express the *TERT* gene. They decided to use a safer, more efficient, and more controllable approach since, as they discuss, such techniques as viruses or exogenous TERT introduction to overexpress it can "potentially lead to unintended effects or immune response," which raises safety concerns.

The researchers genetically modified embryonic stem cells by inserting the *TERT* gene under the control of the human EF1 α promoter. This promoter was selected to ensure stable inheritance and strong TERT expression. They referred to the genetically modified mice as TertKI.

Mating with wild-type Black 6 mice confirmed that the transgene was correctly inherited and didn't have a negative impact on the mice's development, growth, or survival. The

researchers confirmed the transgene to be inherited for at least five generations with no negative impact on litter size.

A comparison of TertKI and wild-type animals didn't show any significant differences in visible features, such as coat color, locomotor activities, or social behaviors, including sniffing, grooming, and play behavior.

However, the researchers noted TERT's impact on postnatal growth and development, as the TertKI group exhibited quicker weight gain from the fifth to twenty-third day postnatal, compared to wild-type mice.

Analysis of organs revealed that organ-to-body weight ratios of the examined organs and organ cellular and tissue morphology didn't differ between genetically modified and wild-type mice. However, analysis of organs during the autopsy revealed five cases of enlarged liver and six cases of enlarged spleen but no evidence of tumor growth.

The researchers ran tests to confirm increased TERT expression, telomerase activity, and telomere length in TertKI mice compared to wild-type mice. The results confirmed their expectations, but expression was at different levels in different organs. The authors suggested that organ-specific regulation of the EF1 α promoter, TERT transcription, and/or the stability of TERT mRNA all played a role in the observed differences.

The researchers also noted that the increase in telomere length and telomerase activity in various organs was not proportional to the increase in the mRNA levels of TERT in a given organ. They suggest that this may be due to tissue-specific gene regulation.

Safety first

The researchers addressed some safety considerations regarding their research, especially since *TERT* gene therapy was previously debated to be either the "natural ally" or the "molecular instigator" of cancer [4]. This debate comes from the observation of telomerase activation in many human cancers.

The researchers did not observe any signs of tumors in the TertKI mice they created. Additionally, they didn't find differences between TertKI and wild-type mice in the levels of the cancer biomarker CA72-4.

However, when the researchers exposed the mice to a mutagen to establish lung cancer, they observed more rapid cancer development in the TertKI mice compared to control animals, suggesting that the overexpression of TERT “can increase the likelihood of carcinogenesis under chronic harmful stimulation.”

Testing whether the genetic modification and TERT overexpression would cause any DNA damage or disturb fetal growth or development revealed no differences between genetically modified and wild-type mice. Blood test results either didn’t show differences or suggested that the genetically modified mice had better health.

Increased lifespan

Lifespan analysis of generations of genetically modified mice revealed an increase in the maximal lifespan of the TertKI mice by 27.48% and a 16.57% increase in median lifespan compared to WT mice.

Previous research suggested that TERT might contribute to lifespan extension through oxidative stress modulation and protection from oxidative damage, which is known to contribute to aging [5]. The researchers measured antioxidant molecules, namely glutathione (GSH) and superoxide dismutase (SOD), in mouse livers, since TERT expression was significantly increased in this organ. Both GSH and SOD were increased in the liver, suggesting improved antioxidant capacity.

However, these results might also suggest an increase in oxidative stress in TertKI mice, resulting in an increase in GSH and SOD levels. Future studies would need to address those possibilities.

Tissue repair and regenerative potential

Significant lifespan extension doesn’t seem to be the only characteristic of TertKI mice. The researchers also observed improved hair growth, faster skin wound healing with reduced infiltration of inflammatory cells, and improved collagen fiber remodeling. In vitro experiments also demonstrated that mouse TertKI skin fibroblasts had more migration ability than wild-type fibroblasts. All of these results suggest improvements in tissue repair and an increase in regenerative capacity.

An assessment of inflammatory factors during wound healing suggested a quick inflammatory response followed by a quick resolution of this inflammation. The researchers suggested that this allows for a rapid response to injury while preventing the adverse effects of an sustained inflammatory state.

The increase in the wound healing capacity of TertKI mice was also supported by the upregulation of growth factor expression and protein levels.

TERT was also found to have benefits when the researchers induced colon inflammation (colitis) in these mice. Their results indicated that their TertKI animals “display less colon deformation, functional disruption, and reduced molecular markers of injury compared to WT animals.”

Limitations

Since this study focused on the common Black 6 strain of mice, more studies are needed to test if these results are strain-specific or can be more generalizable to different strains, animal models, and environments. It is also unclear whether these findings can be applied to future human therapies in the future, especially ones that would start in older age and don't involve TERT overexpression over the entire human lifespan.

Additionally, existing methods of overexpressing genes can be challenging to perform, time- and labor-intensive, expensive, and/or limited to mouse models. The development of easier, human therapy-compatible, and safe methods is essential.

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Is It Possible to Lengthen Telomeres With Diet & Exercise?

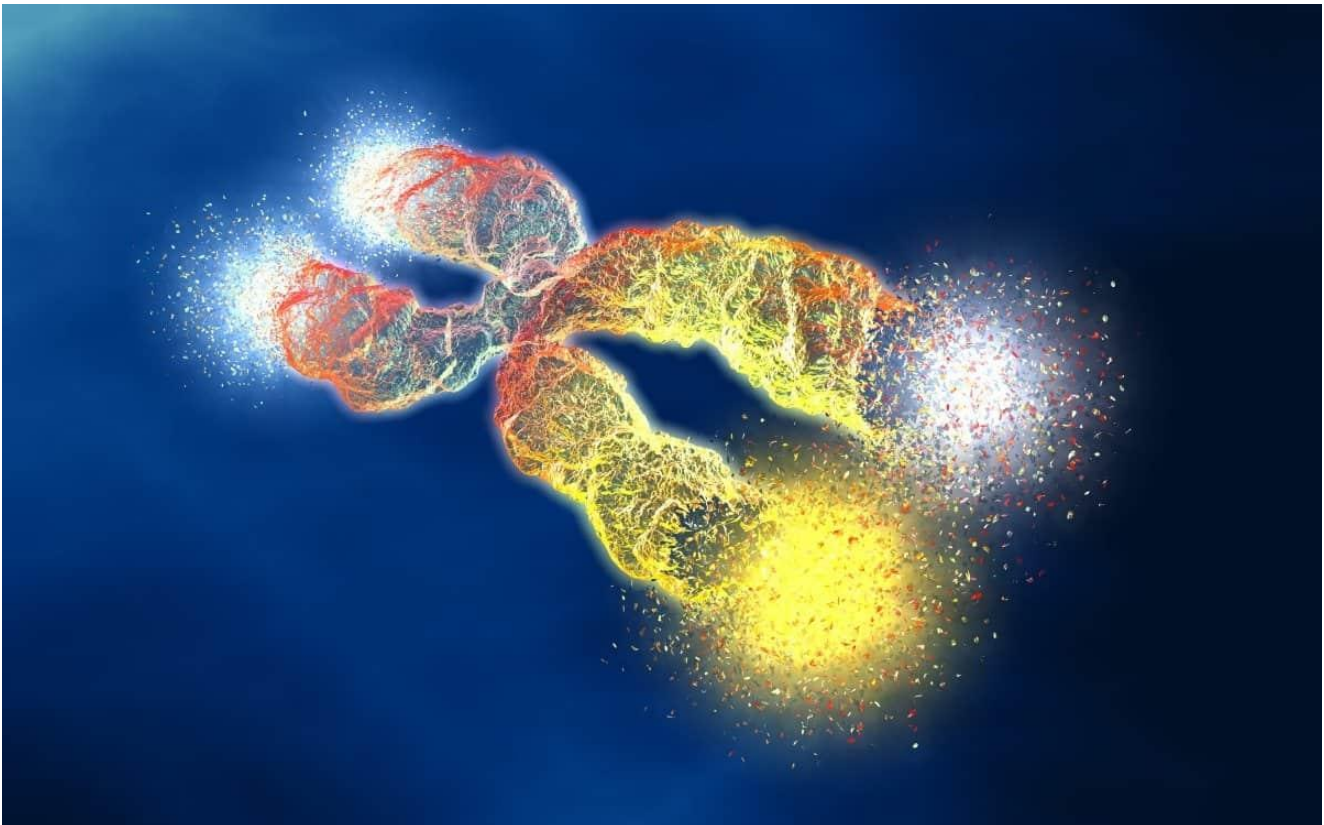


Rusty Moore

Think of a telomere as being similar to a plastic cap on the end of a shoelace that keeps the chromosome from splitting and fraying as it replicates.

As we age and as chromosomes replicate, these caps – the telomeres, become shorter and shorter. The shorter the telomeres get, the more prone we are to age-related diseases like cancer and cardiovascular diseases ([1](#)).

We are really just beginning to understand the significance of telomeres.



In fact, [The 2009 Nobel Prize in Physiology or Medicine](#) was awarded to 3 scientists for their discovery of “*How chromosomes are protected by telomeres and the enzyme telomerase.*”

These scientists made these discoveries in the late '70s, but we have just recently recognized the significance of their work.

Speaking of scientists...

For this article, we contacted a Geneticist who has extensive experience in DNA testing and measuring telomere length.

He was formerly a Population Geneticist for *Ancestry.com* and we have a Q&A section with him at the end of this article.

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Is it possible to lengthen the telomeres and potentially extend life?

There is an enzyme called telomerase that keeps telomeres from shortening too early and can even lengthen these structures (2).

Researchers and science still have a lot to learn here, but the thought is that by increasing telomerase we can extend lifespan.

Is TELOMERASE the key to slowing the aging process?

You can't just take a telomerase supplement.

It's an enzyme produced by the body, so what research is looking into is getting the body to increase telomerase to keep the telomeres from shortening (3).

There are drugs that can increase telomerase activity but unfortunately have been shown to increase the rate of growth of some types of cancer.

This article is going to look at how to lengthen telomeres with diet and exercise.

Let's discuss exercise first.

The trend in fitness today is a heavy focus on [weight training and diet](#) alone to get lean.

Cardio is thought of as unnecessary and even counterproductive.

I have never agreed with this approach to fitness and recommend a combo of weight training, a healthy diet, AND cardio.

Cardio can slow and even reverse cellular aging.

A study published in November 2018 in the *European Heart Journal* (4) compared different types of training on the telomere length and telomerase activity.

This involved 124 people over a 6 month period.

The people were split into 4 groups:

- Endurance Training
- HIIT
- Resistance Training
- Control Group

They were instructed to do three 45-minute sessions of their assigned type of training each week.

The telomere length and telomerase activity were measured in the participants' white blood cells before and after the study.

The lead researcher of the study, Ulrich Laufs, said this:

“Our main finding is that, compared to the start of the study and the control group, in volunteers who did endurance and high intensity training, telomerase activity and telomere length increased, which are both important for cellular aging, regenerative capacity and thus, healthy aging. Interestingly, resistance training did not exert these effects.”

Endurance training (cardio) and HIIT increased the length of telomeres and resistance training did NOT.

Obviously, resistance training is extremely beneficial.

I believe everyone should do some type of resistance training to maintain muscle as they age.

BUT... *Don't skip out on endurance training.*

An interesting thing is that HIIT was also shown to lengthen telomeres.

It doesn't necessarily have to be done with traditional [cardio exercises](#), I have a detailed article on numerous effective HIIT workout approaches here:

[HIIT Workouts | The Definitive Guide to Interval Training](#)

Exercise that increases Nitric Oxide in the blood could POSSIBLY explain why both cardio and HIIT extend telomere length.

In 2000 there was a study (5) showing that Nitric Oxide activates telomerase but in 2007 a study (6) came out with evidence against this.

This seems to be going back and forth...

There's a 2011 study (7) that shows Nitric Oxide positively affecting telomerase activity.

The present work demonstrates that NO affect telomerase activity and cellular replicative capacity in human hematopoietic stem cells. A significant behavior was observed on the telomerase activity and cell proliferation after treatment of cells.

One way to increase Nitric Oxide is to breathe through your nose as much as possible when performing cardio.

I cover this in detail in this article:

[3 Unusual Breathing Techniques to Supercharge Your Workout](#)

Nitric oxide production decreases with age.

So make sure you exercise in a way that keeps your levels as high as possible.

Let's talk about diet.

What can you eat to increase telomere length?

I've been following this and for quite a while studies (8) showed no significance when it came to diet and telomere length.

Some of these studies were comparing specific diets like the *Baltic Sea diet* vs the *Mediterranean Diet*.

Then a study (9) published in April 2018 looked at fiber intake and telomere length.



This study used a sample of 5,674 adults in the U.S.

The U.S. is a good population to study because a large portion of the population has a low fiber intake (and getting lower with the rise in popularity of the KETO and Low Carb diets).

People who eat a high fiber diet have been found to have longer telomeres than those who eat very little fiber.

“A difference of 4.8 to 6.0 years in cell aging was found between those in the lowest compared with the highest quartiles of fiber intake. Overall, the present study highlights the risk of accelerated aging among U.S. women and men who do not consume adequate amounts of dietary fiber.”

This actually may partially explain the results of an earlier study.

In 2014, there was a study (10) showing high fiber intake reduced risk of mortality by 23%.

“By source of fiber, cereal and, to a lesser extent, vegetable fiber were significantly associated with lower total mortality, while fruit fiber showed no association. In conclusion, high dietary fiber intake may reduce the risk of total mortality.”

We have known for a while that a high intake of fiber is associated with a lower risk of chronic disease.



Perhaps fiber's effect on telomere length is one of the reasons for the lower incidence of chronic diseases for those who eat a high fiber diet.

Sometimes we figure out something is healthy before we find out exactly why it is healthy.

Here's a list of foods high in fiber:

- Whole wheat spaghetti
- Whole wheat bread
- Brown Rice
- Oatmeal

- Green peas
- Corn
- Potatoes
- Lentils
- Beans
- Berries

Like I mentioned earlier, the rise in popularity of [low carb and keto diets](#) mean that people are getting less fiber than ever.

Although these diets can result in fat loss, I just don't think they are healthy long term. It's possible to [get lean with a high carb diet](#).

Whatever [diet plan](#) you follow...

Make sure it includes plenty of fiber-rich foods.

How much fiber should you eat each day?

The Dietary Guidelines for Americans (11) recommends 14g of fiber for every 1,000 calories consumed. This comes out to about 21 -25 grams of fiber per day for women and 30 – 38 grams of fiber per day for men.

Only about 10% of Americans hit this recommendation.

I'm not saying the *Dietary Guidelines* are ideal at all, but I think in regards to fiber they have it right.

Here are some other benefits of fiber:

- Reduces cholesterol and blood sugar levels
- Lowers risk of cardiovascular diseases and diabetes
- Increases digestive and bowel health
- Fuels healthy gut bacteria

So the benefits of fiber go beyond extending the lifespan of telomeres.

What about foods that increase Nitric Oxide?



As I discussed earlier an increase in Nitric Oxide is tied to the lengthening of telomeres.

Here are some foods to eat to increase nitric oxide production:

- Leafy Greens
- Beets
- Dark Chocolate
- Citrus Fruit
- Watermelon
- Red Wine
- Rhubarb

Leafy greens like arugula, kale, and spinach top the list.

I would recommend making dishes that combine high fiber foods with these foods shown to boost nitric oxide.

We still have much to learn when it comes to aging, exercise, and diet.

Time to bring in the expert.

Q&A With a Geneticist | DNA, Telomeres, and New Findings



Tim Barclay is the Sr. Editor for the leading DNA testing consumer guide, [Innerbody.com](https://www.innerbody.com). He was also formerly a Population Geneticist for Ancestry.com and earned his Ph.D. in Genetics from Iowa State University.

Question 1: Can you tell how old someone is by telomere length? Is there an average length for each age group?

Answer: It is possible to make an *educated guess* as to how old someone is based on telomere length.

There is an inverse correlation between age and telomere length (measured in TRFs, terminal restriction fragments). By plotting the lengths of thousands of

individuals' telomeres along with their respective ages, we can make educated guesses as to a subject's age based on observed TRF lengths. This plotting method is similar to how we can make an educated guess on how tall a baby will grow up to be based on the heights of the parents.

Question 2: Can I currently have my telomere length tested? I want to find out if I have strong telomeres for a 49-year-old.

Answer: In my opinion, the easiest way to have your telomere length tested is by purchasing a direct-to-consumer test kit from an innovative, Silicon Valley-based company named TeloYears. As far as we know, they are the only company which currently offers such a home test.

On our website, Innerbody.com, we have a detailed [review of TeloYears](#) that a colleague of mine wrote earlier this year. In short, our researchers really thought highly of their tests.

Question 3: What is the biggest benefit someone receives by getting their DNA tested?

Answer: Now I am assuming that you are asking about the biggest benefit associated with taking a telomere DNA test (and not a more general DNA test).

There are many benefits. But if forced to choose one, I would say that if you find out that for whatever reason you have significantly shorter than average telomere lengths (which typically affects 5K – 10K Americans), then you will know early on that you have an increased risk for developing many diseases associated with short telomeres such as pulmonary fibrosis. This information can help you and your doctor make more informed medical decisions.

Question 4: I'm guessing things like obesity, smoking, excessive drinking will all negatively affect telomere length. What should we avoid to ensure our DNA isn't negatively impacted?

Answer: You are correct. There have been many [reputable studies](#) that appear to show a connection between poor lifestyle choices such as smoking, poor diet, etc. and shorter telomere length. However, the science is not at the point where it can explain why this would be the case. Correlation does not necessarily mean causation, so the truth is that we really don't know for sure. I am not a fitness guru, but from a geneticist's perspective, I would say that that you should not be smoking and should maintain a well-balanced diet to keep your body healthy in general. And if keeping your telomere lengths as long as possible is one of the results of these choices then that is icing on the cake.

Question 5: Are there any effective supplements you would recommend that are especially effective when it comes to affecting our DNA in a positive way?

Answer: As a scientist, I only like making assertions that are strongly backed by diligent research. And at this time, there is not a whole lot of evidence, at least that I know of anyway, that would suggest supplements would help. We just don't know currently, so I would not want to make uninformed guesses. I would say that maintaining a healthy diet would surely not hurt. And I would not be surprised if that sometime in the future, a positive correlation between some vitamins or minerals and telomere health was proven.

Question 6: Do you see a day in the near future where we have the ability to drastically slow down the aging process? It feels like we are getting close.

Answer: This is getting very speculative. I don't know if I would say "near" future, but I do personally believe that sometime within the next 20 or 40 years scientists will have figured out a way to slow down the aging process.

Question 7: What excites you most about this area of research?

Answer: The telescope was invented in the early 1600's. The invention sparked an information revolution about what we learned about the universe. Back then, astronomers saw a blurry object and guessed it was a new planet. They were pretty sure that it was a planet, but they didn't know for sure. And just think how we have come since that time, landing a man on the moon, sending a rover to Mars, and seeing distant galaxies billions of light years away.

Well, genetic science today is about where astronomy and the telescope were in the 1700's. But this time, science is moving much, much faster. I can't wait to see what we uncover about our DNA in the next 10 years. There is so much potential.

More About DNA Tests: Tim has coauthored a short guide which compares all the various DNA testing brands: [DNA Health Testing](#)

He covers the best DNA tests for health, nutrition, food sensitivity, ancestry, fitness, telomere health, etc.

Are telomeres really the key to living longer, youthful lives?

Telomeres — the “caps” on the end of chromosomes that protect the DNA from damage — have been associated with greater longevity. In theory, longer telomeres should allow a cell to divide more times and therefore live longer.

However, a new study has suggested that longer telomeres could increase a person’s risk of chronic health conditions. So are longer telomeres key to longevity, or should we be looking to other ways of living longer, healthier lives?

What do we really know about how telomere length relates to aging processes? Image credit: Lucas Ottone/Stocksy.

[Increasing age](#) is the greatest risk factor for many health conditions. However, some people seem to age better than others, enjoying an active, healthy existence long into old age. So how do they do this? Some credit a healthy lifestyle, others luck, and others genetics.

One theory about aging well lies in our chromosomes or, more specifically, our telomeres — protective lengths of repetitive [deoxyribonucleic acid \(DNA\)](#)[Trusted Source](#) and protein found at the end of each chromosome.

Inside every cell in the human body there are 23 pairs of chromosomes. Each chromosome is made up of DNA wound around proteins. That DNA contains [genes](#)[Trusted Source](#) — inherited instructions for all the cell's functions.

Telomeres are found at the terminal region of each chromosome and do not contain genes. Each time a cell divides, the chromosomes replicate and the telomeres shorten. This allows the cell to divide [without losing vital genes](#). Eventually, the telomeres are too short for the cell to divide again and the cell becomes [senescent](#)[Trusted Source](#) or dies.

[Shorter telomeres](#)[Trusted Source](#) have been associated with increased disease incidence and decreased survival times. Senescent cells no longer divide, but remain active and have been [implicated in many diseases of aging](#)[Trusted Source](#), such as [osteoarthritis](#), [atherosclerosis](#), and [cancer](#).

Longer telomeres should mean that cells can divide more often before entering senescence or dying, therefore increasing longevity. [Animal studies](#)[Trusted Source](#) have shown that telomeres shorten faster in short-lived animals than in longer-lived ones.

[A study in mice](#)[Trusted Source](#) bred to have hyper-long telomeres found that they were lean, had low [cholesterol](#) and LDL levels, and improved glucose and [insulin](#) tolerance. They also lived longer and had a lower incidence of cancer than regular mice.

So longer telomeres mean longer, healthier lives. Or do they?

Telomeres and biological age

Telomeres are maintained by the enzyme [telomerase](#)[Trusted Source](#). This adds to the telomeres, preventing them from shortening as rapidly, thereby allowing the cells to live for longer. A good thing, perhaps, until we learn that cancer cells have increased amounts of telomerase, which allows them to continue dividing.

[Sebnem Unluisler](#), genetic engineer and chief longevity officer at the [London Regenerative Institute](#) in the United Kingdom, told *Medical News Today*:

“Studies have demonstrated a correlation between telomere length and biological age. Generally, shorter telomeres are associated with advanced chronological age and increased susceptibility to age-related diseases. Moreover, individuals with certain genetic variations or lifestyle factors that accelerate telomere shortening tend to exhibit a more rapid aging phenotype.”

Telomere length has been likened to a “[biological clock](#)[Trusted Source](#),” with shorter telomeres indicative of greater biological age. Several lifestyle factors have been associated with shorter telomeres. One is a lack of physical activity.

In one study, sedentary women were found to have telomeres that indicated they were biologically 8 years older than women of the same chronological age who exercised more.

Tobacco smoking [increases the risk](#)[Trusted Source](#) of many diseases, and it also accelerates the shortening of telomeres. [A study](#)[Trusted Source](#) found

that telomere shortening was enhanced in the circulating white blood cells of smokers, increasing the rate of biological aging.

Getting [insufficient sleep](#)[Trusted Source](#) can also influence telomere length, shortening telomeres even in childhood, which may lead to impaired health.

All of these are linked to [inflammation](#), which is associated not only with [telomere shortening](#)[Trusted Source](#), but with a number of diseases that are more common in later years.

[Other factors](#) that decrease telomere length are [stress](#), [depression](#), and certain gene mutations, such as that which leads to progeria — a rare condition in which children age extremely rapidly and rarely live past their teenage years.

Maintaining telomere length via diet, exercise

“Recent studies have suggested that telomere length alone may not be a reliable predictor of lifespan or aging. For example, some individuals with shorter telomeres have been found to live longer than those with longer telomeres. Other factors, such as lifestyle, environment, genetics, and stress also play a role in aging and disease.”

– [Dr. Joshua Berkowitz](#), medical director at IV Boost UK

Shorter telomeres may be associated with shorter lifespans and more rapid biological aging, but are longer telomeres therefore associated with longer lifespans and healthier aging? The evidence is not conclusive.

Many lifestyle factors that are associated with better health are also associated with telomere length.

A diet rich in legumes, wholegrain, and fresh fruit and vegetables, such as the [Mediterranean diet](#), is positively associated with telomere length in [several studies](#)[Trusted Source](#). The positive effects of the Mediterranean diet on telomeres may be due to its [antioxidant and anti-inflammatory properties](#)[Trusted Source](#).

Physical activity is advisable for general health, but the evidence for the effect of physical activity on telomere length is not clear-cut — although exercise is thought to be beneficial, the optimal exercise dose is unclear.

One study found that [moderate exercise](#)[Trusted Source](#) helps maintain telomere length, but the benefits decrease with excessive exercise; others found an effect only in people taking [extreme amounts of exercise](#)[Trusted Source](#), such as [ultra-marathon runners](#)[Trusted Source](#).

Other studies have shown that [getting enough sleep](#)[Trusted Source](#), [never having smoked](#)[Trusted Source](#) tobacco, and [avoiding stress](#)[Trusted Source](#) may help preserve telomere length.

Challenging notions about telomeres

“While previous research has suggested that longer telomeres may be associated with longevity, most of this research has been done in cells, and it is not yet clear whether longer telomeres in humans are a cause or a consequence of healthy aging.”

— Sebnem Unluisler

One study [Trusted Source](#) even found that telomeres at both extremes — much longer or shorter than average — were associated with susceptibility to diseases. Short telomeres were linked to organ failure, and long ones to a variety of cancers.

Now, a new study has shown that longer telomeres may not be the key to healthy aging. It suggests, instead, that long telomeres allow cells with age-related mutations to live longer, increasing the likelihood of tumors and other chronic health conditions.

The study, which looked at people with a mutation (*POT1*) that causes longer telomeres, found that, while some showed signs of youthfulness, such as no gray hair in their 70s, those with the mutation had a higher incidence of benign and cancerous tumors, as well as the age-related blood condition [clonal hematopoiesis Trusted Source](#), which increases the risk of several cancers, than those without.

One of the authors, [Dr. Mary Armanios](#), professor of oncology at the Johns Hopkins Kimmel Cancer Center, and professor of genetic medicine, molecular biology and genetics, and pathology at the Johns Hopkins University School of Medicine, suggests an explanation.

According to her, “[c]ells with very long telomeres accumulate mutations and appear to promote tumors and other types of growths that would otherwise be put in check by normal telomere shortening processes.”

Sebnem Unluisler commented that “[t]his study suggests that there may not be a simple relationship between telomere length and aging.”

“While longer telomeres may be associated with increased cancer risk, they may also be associated with decreased risk of other age-related diseases and improved overall health,” she noted.

Are telomere length differences determined at birth?

Telomere length may not depend solely on the influence of external factors either, as evidence increasingly suggests.

A study published in [Science](#) in April 2024 examined telomere lengths in 147 participants using an innovative method called “Telomere Profiling.”

It found that the telomeres “bookending” specific chromosomes were “consistently longer or shorter,” and that these differences in telomere length across different chromosomes occurred in newborn chord blood, too.

This, the study authors explain, suggests that “telomere length is determined at birth and that chromosome end–specific telomere length differences are maintained as telomeres shorten with age.”

The finding contradicts previous notions about telomeres, which held that these “caps” had fairly consistent average lengths that did not vary across all chromosomes.

Senior author [Carol Greider, PhD](#), Distinguished Professor of Molecular, Cell, and Developmental Biology at UC Santa Cruz, qualified the finding as “jaw dropping” in a [press release](#).

The good news, according to the research team behind this study, is that other researchers and clinicians will be able to use their Telomere Profiling tool to help them improve diagnostics and develop new and better drugs.

Taking research on aging further

Nevertheless, telomere length is just one aspect of aging and longevity, and research is investigating many other possible factors.

“The genetic basis of aging is complex, and it is likely that both cellular and whole organism factors contribute to the aging process. While telomeres are one important factor, other genetic and epigenetic factors may also play a role in determining how quickly a person ages.”

– Sebnem Unluisler

Dr. Berkowitz agreed that there are many routes for further study. He suggested that future research might include focus on.

“Identifying genetic and epigenetic factors that contribute to aging and longevity, [...] understanding the role of the microbiome in aging and longevity, and [...] investigating the role of senescent cells in aging and age-related diseases,” he told us.

How to maximize your healthy life years

Although longer telomeres are associated with longevity in cells, the evidence is not conclusive that they are the key to longer, healthier lives. However, many of the lifestyle factors that reduce the risk of disease also result in longer telomeres.

The [National Institutes of Health Trusted Source](#) (NIH) advises the following to promote healthy aging:

- get moving — according to [one study Trusted Source](#), taking around 8,000 steps a day reduced mortality from any cause by 51% compared to taking 4,000 steps.
- eat a healthy diet, such as the Mediterranean diet, with plenty of fresh fruit and vegetable
- maintain a healthy weight — exercise and a healthy diet will help with this
- get a good night's [sleep](#)
- do not smoke, or [stop smoking](#) if you are a smoker
- limit your alcohol intake
- get regular health checks
- look after your [mental health](#) by socializing and managing stress levels.

Dr. Berkowitz echoed this advice: “While genetics play a role in determining lifespan, environmental and lifestyle factors also significantly influence an individual’s health and longevity. By making healthy choices and adopting a healthy lifestyle, individuals can reduce their risk of age-related diseases and improve their chances of living a long and healthy life.”

Longer telomeres may have some influence on your lifespan, but it is a factor you cannot control, and the evidence for their benefit is not conclusive. However, a healthy diet and lifestyle can increase lifespan and [reduce the likelihood of disease Trusted Source](#) even in those with a genetic predisposition.

While research into what is going on in our cells can give us pointers, the tools for healthy aging are largely in our own hands.

Science News

from research organizations

The fountain of youth is ... a T cell?

January 24, 2024

Cold Spring Harbor Laboratory

Date:

Source:

Summary:

Scientists have found a way to reprogram T cells to fight aging. After using them to eliminate specific cells in mice, the scientists discovered they lived healthier lives and didn't develop aging-associated conditions like obesity and diabetes. Just one dose provided young mice with lifelong benefits and rejuvenated older mice.

Share:

FULL STORY

The fountain of youth has eluded explorers for ages. It turns out the magic anti-aging elixir might have been inside us all along.

Cold Spring Harbor Laboratory (CSHL) Assistant Professor Corina Amor Vegas and colleagues have discovered that T cells can be reprogrammed to fight aging, so to speak. Given the right set of genetic modifications, these white blood cells can attack another group of cells known as senescent cells. These cells are thought to be responsible for many of the diseases we grapple with later in life.

Senescent cells are those that stop replicating. As we age, they build up in our bodies, resulting in harmful inflammation. While several drugs currently exist that can eliminate these cells, many must be taken repeatedly over time.

As an alternative, Amor Vegas and colleagues turned to a "living" drug called CAR (chimeric antigen receptor) T cells. They discovered CAR T cells could be manipulated to eliminate senescent cells in mice. As a result, the mice ended up living healthier lives. They had lower body weight, improved metabolism and glucose tolerance, and increased physical activity. All benefits came without any tissue damage or toxicity.

"If we give it to aged mice, they rejuvenate. If we give it to young mice, they age slower. No other therapy right now can do this," says Amor Vegas.

Perhaps the greatest power of CAR T cells is their longevity. The team found that just one dose at a young age can have lifelong effects. That single treatment can protect against conditions that commonly occur later in life, like obesity and diabetes.

"T cells have the ability to develop memory and persist in your body for really long periods, which is very different from a chemical drug," explains Amor Vegas. "With

CAR T cells, you have the potential of getting this one treatment, and then that's it. For chronic pathologies, that's a huge advantage. Think about patients who need treatment multiple times per day versus you get an infusion, and then you're good to go for multiple years."

CAR T cells have been used to treat a variety of blood cancers, receiving FDA approval for this purpose in 2017. But Amor Vegas is one of the first scientists to show that CAR T cells' medical potential goes even further than cancer.

Amor Vegas' lab is now investigating whether CAR T cells let mice live not only healthier but also longer. If so, society will be one mouse step closer to the coveted fountain of youth.

Story Source:

Materials provided by **Cold Spring Harbor Laboratory**. Original written by Luis Sandoval. *Note: Content may be edited for style and length.*

Journal Reference:

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Gut Microbes from Aged Mice Induce Inflammation in Young Mice, Study Finds

June 10, 2024 — When scientists transplanted the gut microbes of aged mice into young 'germ-free' mice -- raised to have no gut microbes of their own -- the recipient mice experienced an increase in inflammation ...

Fecal Transplants Reverse Hallmarks of Aging

May 4, 2022 — In the search for eternal youth, fecal transplants may seem like an unlikely way to reverse the aging process. However, scientists have provided evidence, from research in mice, that transplanting ...

The Bald Truth: Altered Cell Divisions Cause Hair Thinning

Mar. 16, 2021 — Researchers have identified a novel mechanism underlying hair thinning and loss during aging. By studying cell division of hair follicle stem cells in young and aged mice, the researchers found that ...

[A Compound That Slows Bone Loss, and a Resource for Developing Treatments to Slow Aging](#)

Jan. 27, 2021 — A compound that extends lifespan in a tiny nematode worm slows bone loss in aging mice. That surprising result comes from a longitudinal and functional study of 700 aging mice, a project that ...

Vitamin D Rescues Telomere Attrition in Leukocytes

This approach reduced telomere loss in these cells nearly to nothing. *May 23, 2025*

- Vitamin D substantially reduced telomere attrition in leukocytes, a subset of immune cells.
- Improved telomere maintenance may have downstream effects on other aspects of aging.

A sub-study, which was part of the large-scale VITAL trial, determined that vitamin D supplementation **slows telomere attrition in leukocytes almost to a halt**. This could have real-life clinical implications [1].

The chromosome guardians

Attrition of telomeres, repetitive sequences that cap chromosomes, is one of the original hallmarks of aging. Our cells' replication machinery does not copy the last few base pairs at chromosomes' ends. These telomeres act as "surplus" DNA that does not contain valuable information and can be sacrificed. This also means that with each division, telomeres become shorter, which can eventually trigger cellular senescence. Previous research has found that telomere attrition in leukocytes, such as T cells, can predict chronic disease and mortality [2].

The attrition of attrition

A new study co-led by researchers at Mass General Brigham and the Medical College of Georgia has its roots in the VITAL trial, a large, nationwide, randomized, double-blind,

placebo-controlled study that tested daily supplementation with vitamin D₃ (2 000 IU) and/or marine omega-3 fatty acids (1 g fish oil) for primary prevention of cancer and cardiovascular disease. VITAL lasted for five years and enrolled almost 26,000 US females and males aged at least 50 years.

VITAL already yielded some impressive results, showing statistically significant reductions in cancer mortality, the incidence of autoimmune disease, and circulating C-reactive protein levels (a marker of inflammation) in people who took vitamin D.

This new sub-study included slightly more than one thousand VITAL participants who had had their leukocyte telomere length measured at least twice during the study. At baseline, the mean leukocyte telomere length (LTL) was 8,700 base pairs, and the mean LTL loss in the placebo group was 160 base pairs over four years.

Vitamin D abrogated LTL loss almost completely, bringing it down to just about 20 base pairs over four years on average. Omega-3 supplementation did not significantly affect any outcome. While impressive, these results were just barely statistically significant because of the overall slow LTL loss and the limitations of current methods of measuring telomere length. No clinical outcomes were measured.

The association remained robust even after accounting for key demographic, behavioral, and cardiometabolic treatment variables. Those included age, sex, race, body-mass index (BMI), smoking status, hypertension medication use, diabetes, and high cholesterol.

“VITAL is the first large-scale and long-term randomized trial to show that vitamin D supplements protect telomeres and preserve telomere length,” said co-author JoAnn Manson, MD, principal investigator of VITAL and chief of the Division of Preventive Medicine at Brigham and Women’s Hospital. “This is of particular interest because VITAL had also shown the benefits of vitamin D in reducing inflammation and lowering risks of selected chronic diseases of aging, such as advanced cancer and autoimmune disease.”

It’s the shortest telomere that counts

These results require some unpacking. While the average annual LTL loss of 40 base pairs seems minuscule compared to a baseline LTL of 8,700, it might still be clinically significant because of the way cellular senescence works.

It's the single most-eroded telomere in a cell that triggers the DNA damage response and pushes the cell into senescence. Once that "sentinel" telomere falls below a critical length, the cell stops dividing, regardless of how long all the others are [3]. A small shift in the mean LTL of about 40 base pairs per year might be a sign of a steady increase in the fraction of critically short telomeres across the cell population, increasing the probability that any given cell will cross that threshold.

Faster rates of LTL attrition in adults predict higher risks of adverse events even after adjusting for lifestyle and inflammation markers. Those epidemiological associations indicate that even small differences in attrition rate carry real-world health implications.

"Our findings suggest that targeted vitamin D supplementation may be a promising strategy to counter a biological aging process, although further research is warranted," said Haidong Zhu, Ph.D., first author of the report and a molecular geneticist at the Medical College of Georgia, Augusta University.

Why We Age: Telomere Attrition

Jun 5, 2024

Human physiology contains numerous functional redundancies, with multiple mechanisms to address and back up critical processes. This is especially evident in the control of cell division, which is essential for growth, development, wound healing, regeneration, and cancer prevention [1, 2]. Telomere attrition is one of several regulatory mechanisms that ensure that cells do not divide uncontrollably.

However, while telomere attrition helps prevent cancer, it also leads to cellular suicide (apoptosis) and senescence, which reduces the body's ability to function and its regenerative power. This loss of regenerative capacity is a primary aging driver, as senescent cells accumulate and impair the body's ability to repair and renew tissues, and apoptosis depletes the body of functional cells [3]. Consequently, telomere attrition is one of López-Otín's four primary hallmarks of aging [4].

Telomere shortening is especially problematic in cells that divide frequently, including numerous immune, skin, intestine, and hair cells. When telomere erosion reaches the

Hayflick limit, typically after 50 cell divisions, the cell undergoes apoptosis or becomes senescent [5, 6].

Ripple effects on other hallmarks of aging and disease

As telomeres shorten, their protective abilities diminish, leading to increased chromosome end-to-end fusions, loss of genetic information, and genomic instability [7]. Critically short telomeres trigger a persistent DNA damage response, exacerbating genomic instability by activating repair pathways that may introduce mutations or structural chromosome aberrations [8].

Telomere shortening is also associated with changes in the organized combination of DNA and proteins that spool it (chromatin) along with chromosome ends and other genomic regions, leading to altered gene expression patterns and epigenetic drift. This influences the expression of genes involved in maintaining epigenetic marks, resulting in widespread epigenetic changes [9]. The persistent DNA damage response caused by short telomeres can activate stress response pathways, including those that affect protein homeostasis. This can result in the accumulation of misfolded or damaged proteins. Furthermore, senescent cells induced by telomere attrition secrete pro-inflammatory cytokines, proteases, and other factors, known as the senescence-associated secretory phenotype (SASP), which can disrupt proteostasis and promote tissue dysfunction [10].

The cellular senescence induced by telomere attrition also leads to alterations in metabolic pathways, including nutrient-sensing pathways like mTOR, AMPK, and insulin/IGF-1 signaling, which can affect cellular growth and metabolism, contributing to aging. Senescent cells can also contribute to systemic insulin resistance through the SASP, impacting nutrient sensing and metabolic health [11]. Additionally, short telomeres can lead to mitochondrial dysfunction by increasing oxidative stress, further damaging telomeric DNA and other cellular components. Senescent cells induced by telomere attrition often exhibit impaired mitochondrial function, reducing cellular energy production and increasing reactive oxygen species [12].

Telomere attrition is a primary trigger for cellular senescence. As cells reach their replicative limit due to shortened telomeres [5], they enter a state of permanent growth

arrest and adopt the SASP, impacting tissue function and promoting aging. The resulting accumulation of senescent cells contributes to tissue degeneration and dysfunction, promoting the development of age-related diseases [13, 14]. In stem cells, telomere shortening limits their ability to proliferate and maintain tissue homeostasis, leading to stem cell exhaustion [15], which is another hallmark of aging. This reduces tissues' regenerative capacity, as telomere attrition drives stem cells into senescence, further depleting the stem cell pool and impairing tissue repair and regeneration.

The pro-inflammatory environment created by senescent cells can impair normal cell function and communication, contributing to the overall decline in tissue health and function [14]. Telomere attrition, therefore, drives the development of all eight of the other original hallmarks of aging: genomic instability, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. The progressive shortening of telomeres and the resulting cellular senescence create a cascade of detrimental effects that collectively contribute to aging processes and the onset of age-related diseases.

The number of diseases shown to be directly and indirectly driven by telomere attrition is staggering and includes, but is not limited to, cardiovascular disease [16], cancer [17], neurodegenerative diseases [18], diabetes [19], and arthritis [20]. Additionally, several specific diseases are characterized by telomere dysfunction, such as aplastic anemia [21] and dyskeratosis congenita [22].

Thus, scientists from many disciplines are working to understand how telomere maintenance works and how we can augment the body's natural processes to better prevent cancer and promote healthy longevity.

Telomeres, enzyme complexes, and signaling pathways

Telomere attrition occurs as a byproduct of cell division and results from "the end replication problem"; the cell machinery that copies DNA has no place to anchor itself while copying the last few building blocks (nucleotides) of a DNA strand, so it doesn't. This results in a progressive shortening of the chromosome's telomeres each time the cell divides [23].

At each chromosome's end is a long stretch of telomeric repeats. A telomeric repeat is a six-nucleotide sequence. Thousands of these repeats are tacked onto the chromosome before the tip is neatly looped and tucked into it to protect the telomere from damage [24].

The attrition process is complex because the body has mechanisms for extending and shortening telomeres. Telomerase is a critical player in telomere length regulation, as it counters the erosion of telomeres by partially rebuilding them after cell division occurs [25]. However, the rate at which this happens can vary greatly depending on individual genetic predisposition, exposure to oxidative damage, and other cellular stressors. A complex array of biomolecules regulates telomere length as the body simultaneously attempts to protect itself from cancer and optimize tissue regeneration capacity [26].

Two major enzyme complexes and several signaling pathways are crucial in the regulation of telomere length, notably the shelterin complex (TRF1, TRF2, TIN2, TPP1, POT1, and RAP1), the telomerase complex (TERT and TERC), its stabilizing proteins (DKC1, NOP10, NHP2, and GAR), and critical signaling pathways, including the DNA damage response pathways (ATM and ATR), the p53 pathway, the c-Myc pathway, the MAPK/ERK pathway, and the TGF- β pathway. When working correctly, these components and pathways ensure genomic stability, cellular longevity, and proper stress and damage response, forming the telomere maintenance and protection backbone [27].

How the telomere maintenance system works

TERC harbors the RNA template needed to make the DNA sequence (TTAGGG) of the telomeric repeat. TERT binds to TERC to catalyze the synthesis of telomeric repeats [28].

The shelterin complex masks telomeres to prevent them from being treated as DNA damage. Without this feature in place, inappropriate DNA repair mechanisms would be triggered [29].

Shelterin's subcomponents assume several roles. TRF1, TPP1, and POT1 block telomerase from gaining access to the telomere and bind to single-stranded telomeric DNA [30]. TRF2 and RAP1 work together to prevent the activation of the DNA damage response by inhibiting the ATM kinase pathway, which is activated in response to DNA double-strand breaks [31]. TRF2 also tucks the single-stranded telomeric overhang into the body of the

chromosome to add additional protection from the body's DNA damage surveillance mechanisms [32]. TIN2 is the unsung hero that holds the shelterin complex together [33].

Regulation of telomerase in disease and stress

Telomerase regulation is critical for maintaining genomic stability, and its dysregulation is associated with various diseases, including cancer and age-related disorders [34].

TRF1 and TRF2 expression levels can be altered in cancers. Overexpression of TRF2 has frequently been observed in cancer cells, and it facilitates limitless replication by preventing telomere shortening [35]. Under stress conditions, such as oxidative stress, TRF1 and TRF2 can be upregulated to help protect telomeres from damage.

POT1 binds to single-stranded telomeric DNA and regulates telomerase access to the telomere. Mutations in POT1 can lead to dysregulated telomere elongation, contributing to tumorigenesis. During stress, altered binding of POT1 can lead to telomere instability and increased susceptibility to DNA damage [36].

TIN2 is a scaffolding protein stabilizing the shelterin complex. Mutations in TIN2 are linked to telomere syndromes, such as dyskeratosis congenita, which can lead to bone marrow failure and increased cancer risk [37]. TIN2 mutations can impair telomere maintenance under stress, exacerbating telomere shortening and dysfunction [38].

Relevant signaling pathways

ATM and ATR are proteins involved in the cellular response to DNA damage. They respond to double and single-strand DNA breaks, respectively. They are crucial in maintaining genomic stability and orchestrating the DNA damage response (DDR) pathways. TRF2 inhibits ATM signaling at telomeres, preventing inappropriate DNA damage responses [39]. Dysregulation of ATM can lead to genomic instability and cancer [40].

ATR helps maintain telomere integrity under the stress of cell division. POT1 and TRF1 regulate ATR signaling at telomeres [41].

ATM and ATR alike can activate p53, which is a tumor suppressor that can induce cell cycle arrest, apoptosis, or senescence in response to DNA damage [42]. P53 is mutated or

inactivated in many cancers, leading to uncontrolled cell proliferation. Telomere dysfunction can activate p53, linking telomere maintenance to tumor suppression. Under telomere stress, p53 is activated to initiate repair or trigger apoptosis, preventing the propagation of damaged cells [43].

c-Myc is an oncogene that can upregulate telomerase reverse transcriptase (TERT) expression, enhancing telomerase activity. Overexpression of c-Myc is common in cancers, leading to increased telomerase activity and enabling immortalization of cancer cells. Cellular stress can modulate c-Myc levels, influencing telomerase activity and telomere maintenance [44].

Conversely, TGF-beta [45] and P38/MAPK can decrease telomerase expression. P38/MAPK is a crucial regulator of the cellular stress response, which explains why people under stress often have abnormally shortened telomeres [46].

This is not a complete picture of cell signaling as it relates to telomere maintenance. However, it does touch on the primary cell signaling pathways that can influence telomere length.

Recent advances

An avalanche of research culminated in 2024 with publications highlighting the critical functions of telomeres and telomerase in immune cells and the central nervous system, emphasizing their impact on cellular aging and neurodegenerative diseases. Several reviews addressed the limitations of using telomeres as biomarkers for chronological age; others explored the genetic factors influencing telomere length and longevity.

Innovative techniques for precise telomere measurement were established; telomere dynamics on healthspan and space exploration were studied. Several studies were also published to address the associations between telomere length, disease prevalence, and environmental factors such as smoking.

Finally, new insights into the age-dependent relationship between telomere length and aging and the potential of telomerase gene therapy for cardiovascular diseases were investigated. These findings underscore telomeres' complex yet vital role in aging and

disease, opening new avenues for therapeutic interventions. What follows are just a handful of research highlights from late 2023 and 2024.

In August 2023, Savage determined that the baseline length of telomeres is determined by both rare and common genetic variants inherited from parents. The study highlighted that rare genetic variants in genes responsible for maintaining telomeres can cause some individuals to have exceptionally short telomeres for their age (less than the 1st percentile).

These conditions, known as telomere biology disorders, are associated with higher risks of bone marrow failure, myelodysplastic syndrome, acute myeloid leukemia, and squamous cell carcinoma in the head, neck, and anogenital regions. Conversely, rare genetic variants that result in unusually long telomeres are linked to increased risks of other cancers, such as chronic lymphocytic leukemia and sarcoma [47]. This research raises significant questions about the virtues of potential telomere-lengthening therapies.

The findings emphasize that having neither short nor long telomeres is crucial for minimizing cancer risk. The study concludes that maintaining a “just right” length of telomeres is essential for protecting against cancer [47].

In October 2023, Harley and colleagues investigated the role of telomerase reverse transcriptase (TERT) and telomere shortening in the central nervous system (CNS). Using CRISPR/Cas9 to create human induced pluripotent stem cells (hiPSCs) with reduced telomerase function, they studied motor neurons and astrocytes. Their findings showed that telomere shortening induced aging-associated characteristics such as increased cellular senescence, inflammation, and DNA damage. This study highlighted TERT's essential role in neural progenitor cell proliferation and neuronal differentiation, providing a valuable model for studying age-related neurodegenerative diseases [48].

In November 2023, Chebly and colleagues explored aging, focusing on how human immune cells, particularly T-cells, undergo significant changes related to telomeres and telomerase. Their review highlighted the crucial roles of telomeres and telomerase in T-cell differentiation and aging. They noted a strong link between short telomeres, telomerase activation, and various T-cell malignancies. Their comprehensive review of existing literature emphasized the impact of telomere dynamics on health and age-related diseases [49].

In December 2023, Pepke reviewed the role of telomeres as biomarkers for aging and their limitations in predicting chronological age. Despite a correlation between telomere length and age, significant individual variation influenced by genetic and environmental factors limits their predictive reliability. The review emphasized that telomeres should not be used for age estimation, cautioning against misleading efforts in wildlife conservation [50].

In January 2024, Romero-Haro and colleagues investigated the age dependence of the relationship between telomere length and aging in the Japanese quail. They found a robust negative association between telomere length and age in young adults but no such association in older adults. This study suggested that the costs and benefits of telomere maintenance vary throughout life, contributing to the mixed evidence on telomere dynamics and aging [51].

In February 2024, Abdel-Gabbar explored the role of telomeres and telomerase in age-related cardiovascular diseases. Short telomeres activate the DNA damage response (DDR), leading to cell cycle arrest in heart cells. This limited regenerative capacity is linked to conditions like heart failure. The study highlighted the potential of telomerase gene therapy to restore heart cell proliferation and treat cardiovascular diseases [52].

In March of 2024, Torigoe and colleagues examined the FOXO3 gene variant rs2802292 and its relationship with telomere length, telomerase activity, and inflammation. They found that individuals with the longevity-associated G-allele of FOXO3 had longer telomeres and higher telomerase activity. Older female carriers showed a decline in pro-inflammatory IL-6 levels, while older males had increased anti-inflammatory IL-10 levels. This study suggested that FOXO3 promotes longevity through different mechanisms in men and women [53].

In April 2024, Karimian and his team developed telomere profiling, a method of precisely measuring telomere length. They discovered that telomere lengths vary significantly among different chromosome ends and that these lengths are established at birth and maintained as individuals age. Examining telomere lengths in 147 individuals found consistent patterns in specific chromosome ends. Telomere profiling could enhance research and clinical approaches by providing detailed insights into telomere biology and its role in aging and disease [54].

Also in April 2024, Mason and his team discussed the importance of improving human health in light of an aging population. They focused on telomeres, which are protective caps at the ends of chromosomes that shorten with each cell division due to the “end-replication problem.” This shortening is linked to aging and age-related diseases, including reduced fertility, dementia, cardiovascular disease, and cancer. They also discussed the lack of data on how long-duration space missions might affect telomeres and overall health. Insights from the NASA Twins Study have advanced understanding of these effects and could inform strategies for future space missions and human longevity in extreme environments [55].

That is also the month when Liang and his team used a DNA methylation-based telomere length (DNAmTL) estimator to study cancer prevalence and mortality in people with and without HIV. They found that individuals with HIV had shorter DNAmTL, associated with higher cancer prevalence and increased mortality risk. Their findings underscored the impact of HIV infection, physiological frailty, and cancer on telomere length and overall health [56].

In May 2024, Hammami and colleagues studied the impact of cigarette smoke on telomere length and associated gene expression. They found that cigarette smoke extract downregulated telomere-stabilizing genes TRF2 and POT1 while increasing the expression of hTERT, a subunit of telomerase linked to cancer, and ISG15, an inflammatory protein. Their research showed that smokers had higher levels of these markers in lung tissue and blood samples, suggesting that smoking accelerates telomere shortening and contributes to inflammation and cancer development [57].

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