Integrated Aging Theory Framework

Core Mechanisms, Testable Predictions, Common Questions and Current Limitations

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Version 1.1 (June, 2026): Revision of Core Mechanisms section. This version refines and expands the mechanistic framework of TRCS, with particular emphasis on strengthening the programmed nature of aging. No changes were made to the testable predictions or overall theoretical structure.

Version 1.2 (July, 2026): Minor revision adding a new testable hypothesis regarding progressive telomere and rDNA array shortening during development and aging, and restructuring the Core Mechanisms section with descriptive subheadings.

Version 2.0 (July, 2026): Major revision reflecting the expanded theoretical architecture. The manuscript has been restructured around the Integrated Aging Theory Framework to clarify the hierarchical relationship between the overall framework and the TRCS model of cellular senescence. The document structure and terminology have been updated accordingly.

Version 2.1 (July, 2026): Minor revision adding a new frequently asked question (Section 3.8) to clarify an additional aspect of the Integrated Aging Theory Framework based on ongoing scientific discussion.

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Overview of the Integrated Aging Theory Framework

The Integrated Aging Theory Framework consists of four interconnected components:

1. The Programmed Nature of Aging
2. Mechanisms of Organismal Aging
3. Mechanisms of Cellular Senescence (TRCS)
4. Causality of Aging Hallmarks

The present living preprint focuses on the mechanistic foundation of this framework, together with its testable predictions, responses to common questions, and current limitations.

1. Core Mechanisms

1.1 Individual Development, Maturation, and Aging Are Fundamentally Programmed Processes.

Over billions of years of evolution, organisms have evolved mechanisms to counteract a wide range of random molecular damage. Furthermore, every living creature follows a relatively rigid timeline governing development, maturation, senescence and death. For this reason, ontogeny and aging are inherently a continuous, end-to-end genetic program, a process in which three major sets of genes are expressed in a programmed sequence along the timeline: the early gene set primarily regulates embryonic development, the middle gene set centers on bodily health and reproduction, and the late gene set is mainly responsible for disrupting normal physiological functions.

1.2 Organismal aging is driven by replicative senescence of adult stem cells within tissues.

Why do organisms age? The tissue cells that make up an organism fall into two categories: terminally differentiated cells and adult stem cells, which account for a very small proportion. Both types of cells are eliminated by the immune system due to cellular senescence, gene mutations, viral infections and other factors, and are

subsequently replenished through self-renewal and differentiation of adult stem cells. However, adult stem cells have a limited number of replication cycles. Therefore, the fundamental cause of organismal aging is the replicative senescence of adult stem cells (Figure 1).

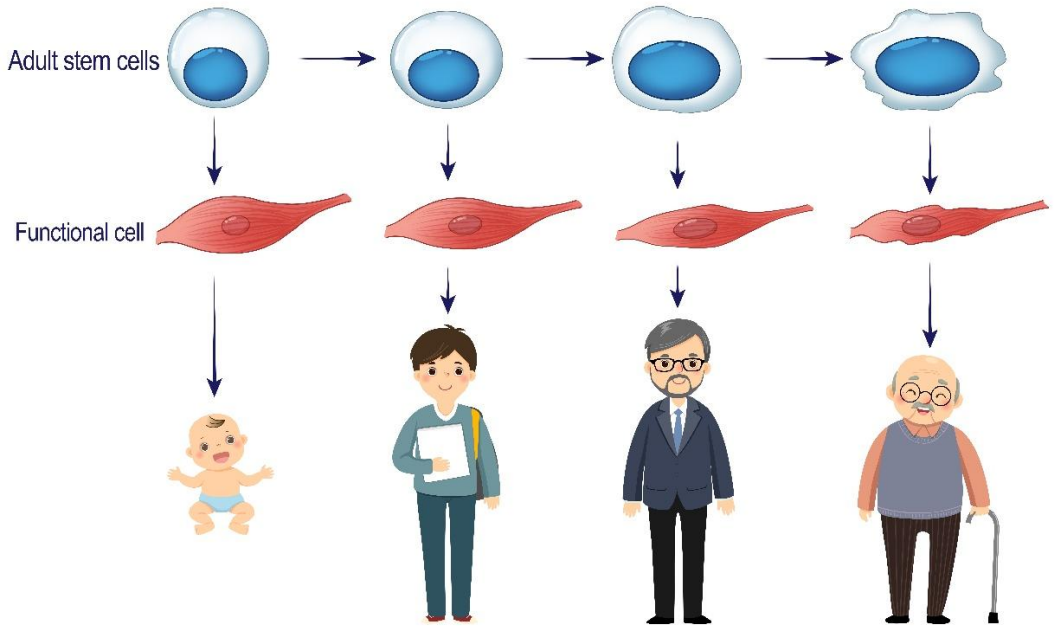


Figure 1. With each division of adult stem cells, daughter cells become progressively more senescent than the previous generation. Functional cells differentiated from senescent adult stem cells are also senescent, contributing to the gradual aging of tissues, organs, physiological systems, and ultimately the whole organism. Reproduced from: Huang, B. Programmed Aging Theory Defeats Damage Accumulation Theory of Aging. Ageing and Longevity Research ***2026****, 2 (1), 2, Figure 4.*

1.3 Cellular senescence is driven by the progressive depletion of cellular countdown elements.

Why do cells age? According to the TRCS model of cellular senescence (Figure 2), the progressive shortening of telomeres and/or rDNA arrays (countdown elements) gives rise to a temporal concentration gradient of the tumor suppressor protein p53. As p53 binds to the promoters and enhancers of numerous genes, the synthesis rates of ATP and proteins decline continuously over time. Meanwhile, the expression of certain genes is selectively upregulated while that of others is selectively downregulated. This cascade drives programmed gene expression, gradually shifting cells from a youthful

state to a senescent state [https://doi.org/10.13276/j.issn.1674-8913.2021.03.003 (in Chinese)].

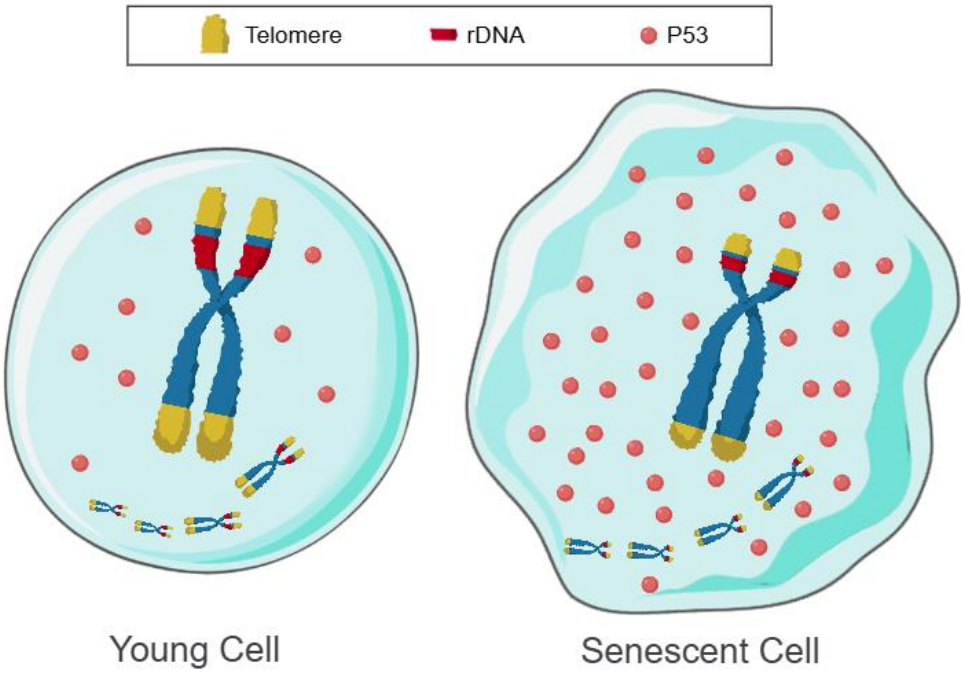


Figure 2. Telomere DNA and ribosomal DNA co-regulation model for cell senescence. Left: Chromosomes with long arrays of telomeres and rDNA: P53 is rapidly degraded, P53 levels are low, and the cell is youthful. Right: Chromosomes with short arrays of telomeres and rDNA: P53 is slowly degraded, P53 levels are high, and the cell is aged. Reproduced from: Huang B, Hu X. Causality of Aging Hallmarks. Aging and Disease. 2026;17(3):1236-1253, Figure 1.

Interpreting the aging mechanism based on the TRCS: In the process of cellular senescence, countdown elements (analogous to sand in an hourglass, Figure 3) function as the upstream triggers of cell aging. p53 acts as the mediator between upstream and downstream pathways. All phenotypes of cellular senescence represent downstream events induced by countdown elements through p53 signaling (Figure 4, Table 1, doi: 10.14336/AD.2025.0541.). A variety of factors that modulate the aging rate affect cellular and organismal aging by regulating the consumption rate of countdown elements.



Figure 3. The sand in the hourglass corresponds to the "countdown elements". Photo by Andrej Lišakov on Unsplash.

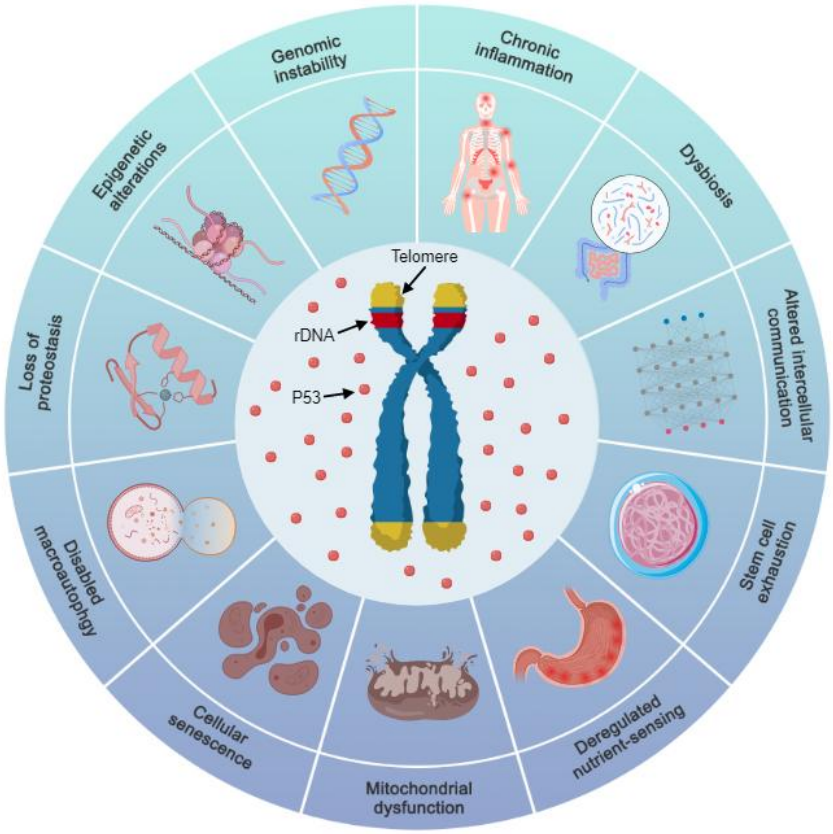


Figure 4. The eleven major hallmarks of aging mediated by telomeres and rDNA through

p53. Reproduced from: Huang B, Hu X. Causality of Aging Hallmarks. Aging and Disease. 2026;17(3):1236-1253, Figure 2.

Table 1. The 11 Hallmarks of Aging Mediated by Telomere and rDNA Array Shortening through P53.

Aging Hallmark	The Hallmarks of Aging Mediated by Telomere and rDNA Array Shortening through P53	References
Genomic instability	P53 mediates the expression of DNMT1 and CENP-A genes, thereby affecting the methylation levels of DNA in heterochromatin regions and the function of centromeres, leading to genomic instability.	Peterson et al. [29], Sikder et al. [54]
Epigenetic alterations	P53 mediates the expression of the DNMT1 gene, affecting epigenetics.	Peterson et al. [29]
Loss of proteostasis	P53 affects the transcription of heat shock protein HSP70 mediated by the SIRT1 gene, thereby affecting proteostasis.	Nemoto et al. [86], Brunquell et al. [87]
Disabled macroautophagy	P53 mediates autophagy in human, mice, and nematode cells.	Tasdemir et al. [88]
Deregulated nutrient-sensing	P53 mediates PGC-1α and plasma glucose levels by affecting SIRT1 expression.	Sahin et al. [95]
Mitochondrial dysfunction	P53 suppresses the expression of PGC-1 α and PGC-1β. Downregulation of PGC-1α/β expression leads to mitochondrial dysfunction and reduced ATP production.	Sahin et al. [95], Hoshino et al. [118]
Cellular senescence	P53 mediates histone acetylation levels and the expression of various transcription factor genes, thereby affecting protein synthesis rates and gene expression profiles.	http://pathcards.genecards.org/, Yang et al. [17]
Stem cell exhaustion	Stem cell exhaustion is characterized by reduced and altered stem cell function, mediated by P53 through decreased protein synthesis rates and altered gene expression profiles.	http://pathcards.genecards.org/, Yang et al. [17]
Altered intercellular communication	Different ages have distinct gene expression profiles, and thus the number and activity of receptors and ligands for various communication molecules change with increasing age.	Schaum et al. [107]
Chronic inflammation	Under the influence of P53, mitochondria and nuclei in senescent cells release mtDNA, dsRNA, and eccDNA into the cytoplasm. The body perceives this as a viral infection, leading to sterile chronic inflammation.	Zhou et al. [59], Liu et al. [60], Newman et al. [108]
Dysbiosis	Changes in bile acid levels mediated by P53 affect the structure of the gut microbiota.	Kim et al. [127], Chen et al. [128]

Table 1. The 11 Hallmarks of Aging Mediated by Telomere and rDNA Arrays Shortening through p53. Reproduced from: Huang B, Hu X. Causality of Aging Hallmarks. Aging and Disease. 2026;17(3):1236-1253, Table 1.

2. Testable Predictions Generated by TRCS

2.1 If the TRCS is valid, the telomeric DNA and rDNA depleted in somatic cells must be replenished in germ cells or early embryonic cells; otherwise, life cannot sustain generational inheritance. Fortunately, existing evidence confirms that telomeric DNA and rDNA lost in somatic cells can be restored in early embryonic cells and germ cells [doi: 10.1038/ncb1664., doi: 10.7554/eLife.32421.].

2.2 Embryonic stem cells (ESCs) are functionally equivalent to germ cells, and induced pluripotent stem cells (iPSCs) are artificially generated ESCs. Accordingly, their telomeres and rDNA arrays undergo substantial elongation. In 2009, Marion et al. first demonstrated that telomeres are markedly elongated during somatic cell

reprogramming into iPSCs [doi: 10.1016/j.stem.2008.12.010.]. Our preliminary experiments data suggest that the rejuvenation of human ESCs (hESCs) and human iPSCs (hiPSCs) arises not from epigenetic reprogramming, but from a pronounced increase in the length of both telomeric DNA arrays and 45S rDNA arrays.

Given that embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) possess the longest telomere and rDNA array lengths, which undergo substantial shortening in somatic cells, I hypothesize that telomere and rDNA arrays sustain progressive shortening throughout embryonic development, postnatal maturation, and aging. We may measure telomere and rDNA array lengths in somatic cells collected from infant, adolescent, and advanced elderly (≥75 years old) populations, then calculate the residual lengths (in kilobases, kb) or copy numbers of telomeres and rDNA arrays across these distinct life stages.

2.3 Mother cells of budding yeast undergo progressive replicative senescence with each cell division, which is caused by the shortening of rDNA arrays [doi:10.1073/pnas.2119593119.], and ultimately die after approximately 20 divisions. In contrast, progeny (bud) cells can elongate rDNA arrays again via meiosis.

2.4 The TRCS proposes that the shortening of telomere and/or rDNA arrays upregulates the level of p53, a master regulator of senescence, and consequently drives cells into a senescent state. Notably, p53 localizes to subtelomeric regions and rDNA loci [doi:10.15252/embj.201490880., doi: 10.1242/jcs.062398.]. Telomere shortening also induces p53 upregulation [doi:10.1093/emboj/cdg013.]. In our preliminary experiments, knockdown of 45S rDNA copy number in primary mouse and human cells resulted in marked upregulation of senescence markers including p53, p21, p16 and SA-β-GAL. Meanwhile, telomere length, cell viability and population doubling capacity were all significantly reduced.

2.5 In rescue experiments aimed at elongating telomere and rDNA arrays in senescent cells, the levels of p53, p21, p16 and SA-β-GAL should be markedly downregulated, accompanied by a significant rise in cell viability and population doubling capacity. In 1998, Bodnar et al. and in 2015, Ramunas et al. transfected cells

with the hTERT gene and hTERT mRNA respectively. Their results showed increased telomere length, reduced expression of senescence markers, rejuvenated cellular phenotype and substantially extended population doubling potential [doi: 10.1126/science.279.5349.349., doi: 10.1096/fj.14-259531.]. This is currently recognized as one of the most effective anti-senescence interventions. Concurrent substantial elongation of both telomeres and rDNA arrays may achieve complete cellular rejuvenation.

2.6 Telomeric DNA and rDNA are multicopy tandem repetitive sequences with inherently poor stability. During transcription or replication, histones are removed, leaving DNA exposed and the double strands unwound. This state renders the DNA vulnerable to various forms of damage and perturbation, ultimately leading to copy number loss [doi: 10.1126/sciadv.1600031., doi:10.1126/science.1147182.]. From first principles, many anti-aging and pro-aging factors may act by inhibiting or stimulating the transcription of telomeres and rDNA. Such regulation slows or accelerates the loss of their copy numbers, thereby modulating lifespan. The supporting evidence is presented as follows:

Caloric restriction (CR) and rapamycin suppress the transcription of 45S rDNA in the nucleolus by inhibiting mTORC1 [doi:10.1038/nrc.2018.3., doi: 10.1093/nar/gkq895]. Spermidine markedly reduces intracellular mTORC1 activity and thereby represses rDNA transcription [https://doi.org/10.1360/TB-2024-0037.]. RPL22 promotes 45S rDNA transcription and induces cellular senescence by disrupting the heterochromatin structure within the nucleolus [doi:10.1093/nar/gkae740.]. Knockout of the 4EBP1 gene in mice activates mTORC1, enhances 45S rDNA transcription and accelerates cardiac senescence [doi:10.1007/s11357-024-01368-w.]. The longevity effect of dietary restriction is well supported: excessive food intake in fruit flies overactivates mTORC1 and accelerates the shortening of 45S rDNA arrays [doi: 10.1371/journal.pgen.1005148.]. Activation of mTORC1 leads to shortening of 45S rDNA arrays in mouse hematopoietic stem cells [doi:10.1371/journal.pgen.1006771.]. IL-11 upregulates the ERK-AMPK-mTORC1

signaling axis, and its expression rises with age in mice. Antibody-mediated inhibition of IL-11 can extend mouse lifespan [doi:10.1038/s41586-024-07701-9.].

SIRTs are a family of anti-aging proteins. SIRT6 binds to telomeres and maintains telomere stability [doi:10.1093/nar/gkw1239.]. SIRT7 localizes to the nucleolus and directly interacts with rDNA, RNA polymerase I (Pol I) and the transcription factor UBF. It facilitates the assembly of the transcription initiation complex and promotes rDNA transcription. Meanwhile, SIRT7 recruits SIRT1, DNMT1 and NoRC to drive heterochromatin formation and preserve rDNA stability. Accordingly, SIRT1, SIRT6 and SIRT7 slow the shortening of telomere and rDNA arrays by maintaining their structural integrity [doi:10.1152/physrev.00045.2021.].

3. Common Questions Regarding the Integrated Aging Theory Framework

3.1 Why do elephants possess far more copies of the TP53 gene than most mammals, yet age much more slowly? Mice have longer initial telomeres than humans, but their lifespan is considerably shorter.

First, a high copy number of the *TP53* gene does not equate to elevated p53 protein levels. For instance, budding yeast cells with 40 and 140 rDNA copies exhibit nearly identical rRNA content [doi: 10.1128/MCB.23.5.1558-1568.2003.].

According to the TRCS, the rate of aging is independent of *TP53* copy number, but correlates with the rate at which p53 concentration forms a temporal gradient. This gradient is in turn determined by the shortening rate of telomeres and/or rDNA arrays. From first principles, the lifespan of a species is linked to the telomere shortening rate rather than initial telomere length.

For example, mice have an initial telomere length of 50 kb, an annual telomere shortening rate of 6420 bp, and a lifespan of 2.5 to 3 years. In humans, the initial telomere length is 15 kb, with an annual shortening rate of 70 bp, and a lifespan ranging from 75 to 85 years.

The correlation between telomere shortening rate and lifespan across species is as follows: African elephants show an annual telomere loss of 109 bp and live

approximately 60 to 70 years; bottlenose dolphins have an annual loss of 766 bp and a lifespan of around 40 years; goats lose 363 bp per year and live 15 years on average; reindeer have an annual shortening of 531 bp, with a lifespan of 10 to 15 years; and Arctic skuas lose 771 bp of telomeric DNA each year, living 10 to 15 years [doi: 10.1073/pnas.1902452116.].

Furthermore, the shortening rate of telomeres and/or rDNA arrays is modulated by a wide range of genetic and environmental factors, which accounts for the substantial lifespan disparities among different species.

3.2 Why cannot we reverse aging and rejuvenate organisms by knocking out TP53 or persistently inhibiting p53?

Telomeres and/or rDNA arrays act as a countdown mechanism that governs the execution of genetic programs and limits the number of cell divisions. If cells are rejuvenated via *TP53* knockout or sustained p53 inhibition, their gene expression profiles will cease to change over time and become locked in an embryonic state. Cells will then undergo uncontrolled proliferation, acquiring characteristics identical to cancer cells, whose gene expression profiles resemble those of embryonic cells. This approach poses severe risks to organisms, as detailed below:

1. Unrestrained cell division will lead to excessive proliferation and tumor formation.
2. Permanent embryonic gene expression will eliminate mature cellular functions, ultimately causing organ failure and organismal death.

Taking the liver as an example, knocking out the *TP53* gene or persistently inhibiting the p53 protein will lock hepatocytes in a proliferative state. The cells will divide uncontrollably and develop into tumors. Meanwhile, their gene expression profiles will be fixed at the embryonic stage, resulting in the loss of all physiological functions of mature liver tissue and eventually liver failure. For instance, during the peak proliferation phase of liver regeneration after partial hepatectomy in mice, hepatocytes express alpha-fetoprotein, a marker specific to fetal liver cells, while bile secretion and clearance capacity decrease significantly [doi:

10.1152/ajpgi.90728.2008.].

Therefore, only by lengthening telomeres and rDNA can p53 levels be reduced. This approach enables cellular rejuvenation without triggering uncontrolled cell division.

3.3 Some reports indicate that the copy number of 45S rDNA does not decline in senescent cells. Does this contradict the TRCS?

Both mouse and human 45S rDNA arrays undergo shortening with advancing age [doi: 10.1038/cr.2017.18]. In fruit flies, the 45S rDNA arrays are reduced by half over their 40-day lifespan [doi: 10.7554/eLife.32421]. Nevertheless, several studies have reported no reduction in 45S rDNA copy number in senescent cells. For example, Geisen et al. performed ddPCR sequencing on more than 160 healthy donors and found that the absolute rDNA copy number remained stable in individuals aged 15 to 71 years [doi:10.1111/acel.14497]. Possible explanations are as follows:

1. Theoretically, there are three patterns by which telomeres and rDNA arrays regulate p53 levels in cells from different species or different tissues of the same species:

① Both telomeres and rDNA arrays undergo shortening;

② Telomeres shorten, while rDNA arrays remain unchanged or even elongate;

③ rDNA arrays shorten, while telomeres stay intact or even elongate.

Accordingly, the copy number of 45S rDNA does not universally decline in senescent cells across various species and cell types. Most human somatic cells experience telomere shortening during aging, yet exceptions exist. For example, human corneal endothelial cells maintain long telomeres throughout life without telomerase activity, and their limited replicative potential is not caused by telomere shortening [PMID: 9501879]. In addition, telomere length does not decrease with age in yeast, nematodes, fruit flies, and *Myotis brandti* (Brandt's bat) — a species weighing merely 7 grams with a maximum lifespan of 40 years [doi: 10.1126/sciadv.aao0926]. In genetically engineered mice with inherently short telomeres, telomeres in tail cells show no shortening and even increase slightly from 2 to 23 months of age [doi:

10.1093/nar/gkaf830]. Newly hatched anacondas have a telomere length of approximately 7 kb, while adult anacondas reach around 28 kb [doi: 10.1371/journal.pone.0007493]. For mouse bone marrow cells, rDNA arrays shorten in one strain but not in another [doi: 10.1128/MCB.00368-20].

2. Chromosomal DNA replication leads to the shortening of telomeres and rDNA arrays. The shortening of telomeres and/or rDNA arrays elevates p53 levels. p53 binds to subtelomeric regions and inhibits telomere shortening [doi: 10.15252/embj.201490880.]. Accordingly, adult stem cells in young individuals generally have low p53 levels and robust proliferative capacity. Their telomeres shorten rapidly at the initial stage. As p53 levels rise gradually, the rate of telomere shortening slows down progressively, and telomere length may even rebound. Meanwhile, rDNA arrays begin to shorten and become the main driver of cellular senescence. For instance, human telomeres shorten rapidly in the first year after birth at a rate of approximately 700 bp per year. The rate then declines year by year to 31 bp per year [doi: 10.1046/j.1365-2141.2000.01970.x.]. Notably, telomere length in human salivary cells increases instead among people aged 80 to 90 years [doi:10.1534/genetics.115.178624.]. Therefore, it is advisable to detect the rDNA copy number in cells from individuals over 75 years old.

The elongation of telomeres or rDNA arrays in senescent cells may result from a compensatory response to the shortening of the other element. In our preliminary work, we knocked down the copy number of 45S rDNA in human T cells and NK cells and observed an increase in telomere length.

In addition, combined measurements of mixed cell populations can obscure the actual changes. Accurate results can only be obtained by testing each cell type separately.

It is worth noting that measuring leukocytes in blood cannot accurately reflect the actual length of telomeres or rDNA arrays in hematopoietic stem cells. Pathogen infection, psychological stress and other factors can stimulate excessive division of leukocytes, thereby causing the shortening of telomeres and rDNA arrays.

3. Accuracy of measurement methods.

3.4 Is cancer cell immortalization attributed to exceptionally long telomeres and rDNA arrays?

No. In fact, the telomeres and rDNA arrays in numerous tumor cells are even shorter than those in healthy senescent cells [PMID: 9305709; doi: 10.1111/j.1600-0676.1996.tb00748.x; doi: 10.1371/journal.pgen.1006994]. Accordingly, the immortalization mechanism of tumor cells is independent of the length of telomeres and rDNA arrays, and is instead associated with TP53 mutations or excessive degradation of p53.

p53 binds to subtelomeric regions to stabilize telomeres and slow the rate of telomere shortening. In p53-deficient cells, telomeric DNA is rapidly lost upon DNA damage [doi: 10.15252/embj.201490880]. This partly explains why tumor cells with high telomerase expression still have shorter telomeres. It also accounts for the gradual decline in the rate of human telomere shortening with advancing age.

3.5 Why have small-molecule telomerase activators failed to extend lifespan in mice?

Telomere elongation represents a valid strategy for anti-aging research. However, the small-molecule telomerase activator TA-65 failed to lengthen telomeres or extend lifespan in mice [doi: 10.1111/j.1474-9726.2011.00700.x]. Similarly, another small-molecule telomerase activator TAC did not increase the average telomere length or prolong the lifespan of mice [doi: 10.1016/j.cell.2024.05.048]. These results have led many researchers to question the telomere theory of cellular senescence.

Telomerase activity in human infants is substantially higher than that in middle-aged and elderly individuals. Nevertheless, telomere shortening reaches approximately 700 base pairs (bp) per year during the first year after birth, and the annual shortening rate gradually drops to 31 bp in later life. Accordingly, the rate of telomere shortening in infancy is more than 20-fold higher than that in middle-aged and aged adults [doi:

10.1046/j.1365-2141.2000.01970.x.]. Patients with Fanconi anemia exhibit a 4.8-fold higher telomerase activity in peripheral blood granulocytes and monocytes compared with healthy controls [doi: 10.1046/j.1365-2141.1999.01445.x]. Their telomere shortening rate is approximately 3.9 times that of healthy individuals (195 bp vs. 50 bp) [doi: 10.1016/j.mad.2007.11.002.]. Hematopoietic stem cells from leukemia patients show 10–50 times greater telomerase activity than normal hematopoietic stem cells, yet their telomeres are substantially shorter [PMID: 10676644]. This suggests that telomerase activation concurrently triggers mechanisms that accelerate telomere shortening, resulting in an elevated shortening rate. In fact, most cancer cells with high telomerase activity possess shorter telomeres than normal cells.

TRF1 and TRF2 are negative regulators of telomerase. A study on lung cancer revealed a positive correlation between the expression of TRF1/TRF2 and telomerase activity [doi: 10.1016/j.lungcan.2007.06.019]. Therefore, small-molecule telomerase activators may simultaneously upregulate TRF1 and TRF2 while activating telomerase. The two effects counteract each other functionally, leaving telomere length unchanged.

From an evolutionary perspective, why is it difficult for small-molecule telomerase activators to effectively lengthen telomeres when they activate telomerase? As early as 1998, I reasoned that if a “countdown elements” were like a fully charged battery, discharging while being recharged with an equal amount of energy, its charge would remain constant forever—and it could no longer function as a clock driving a program [https://doi.org/10.5281/zenodo.20108827 (in Chinese)].

Numerous compounds in plants are capable of activating telomerase. TA-65 is extracted from *Astragalus*, a leguminous plant. Since senescence has been selected for during biological evolution, and telomere shortening triggers cellular senescence, organisms have evolved mechanisms to prevent telomerase activators from driving telomere elongation to outpace telomere shortening.

3.6 Why Telomere Length Fails to Predict or Explain Variations in Human Lifespan

Reasons why telomere measurement cannot accurately assess biological age include:

1. Telomeres act as an upstream regulator of cellular ageing. Given the lengthy signalling cascade from upstream telomere regulation to downstream ageing phenotypes, numerous confounding variables emerge and reduce measurement accuracy.

2. For practical convenience, leukocytes are commonly used for telomere measurement. Pathogen exposure, radiation, mental status and other factors can alter telomerase activity and cell division cycles of leukocytes, leading to substantial variability in telomere length. Since leukocytes differentiate from haematopoietic stem cells, measuring telomere length in haematopoietic stem cells enables more accurate prediction of biological age.

3. The rate of telomere shortening gradually declines with age and eventually reverses. For instance, telomere length increases in humans aged 80 to 90 years [doi:10.1534/genetics.115.178624]. Increased telomere length has also been observed in aged naked mole-rats [doi: 10.7717/peerj.10498]. Accordingly, rDNA arrays begin to shorten at an accelerated pace after humans reach the age of 75, and take over telomeres in regulating cellular senescence. For this reason, telomere length is not an accurate indicator of senescence status.

3.7 Does telomerase therapy induce carcinogenesis?

The prevailing theory holds that telomere shortening restricts the proliferation of cells carrying oncogenic mutations, thereby exerting a tumor-suppressive effect. This has led to a common concern that anti-aging interventions relying on telomerase to elongate telomeres may initiate or promote cancer, which greatly hinders the application of telomerase-based anti-aging strategies. In fact, telomerase is expressed in all adult stem cells within tissues. Hence, although telomerase activity is a hallmark of cancer, it is not a driver of tumorigenesis. The following evidence demonstrates that telomerase does not induce or promote cancer:

1. The immortality of cancer cells is independent of telomere length. Instead, it is

associated with TP53 mutations, or excessive degradation of p53 even when the TP53 gene remains intact.

2. Many tumor cells have shorter telomeres than healthy senescent cells. For example, telomere length ranges from 8 to 13 kb in normal ovarian cells, while it is less than 8 kb in malignant ovarian tumors [PMID: 9305709]. The mean telomere length is 7.8±0.2 kb in normal hepatocytes and 5.2±0.2 kb in hepatocellular carcinoma cells [doi: 10.1111/j.1600-0676.1996.tb00748.x.].

3. Telomerase activity is higher in younger individuals than in the elderly, yet the incidence of cancer is much lower among the young.

4. A single tail vein injection of adeno-associated virus serotype 9 (AAV9) encoding mouse telomerase reverse transcriptase in mice reduced DNA damage, improved glucose tolerance, attenuated cognitive decline, delayed tumor onset, and increased median lifespan by 24% [doi: 10.1002/emmm.201200245.].

5. Mice with ultra-long telomeres exhibit less DNA damage, a lower tumor incidence and a longer lifespan [doi: 10.1038/s41467-019-12664-x].

6. Shorter telomeres correlate with higher DUX4 expression activity. Progressive telomere shortening can increase DUX4 activity by up to 10-fold [doi: 10.1038/nsmb.2571.]. MHC class I (MHC-I) presents intracellular antigens on the cell surface and is expressed in nearly all cell types. DUX4 expression is negatively correlated with MHC-I levels. Accordingly, gradual telomere shortening leads to DUX4 upregulation and concurrent downregulation of MHC-I. Elevated DUX4 impairs antigen presentation by MHC-I [doi: 10.1016/j.devcel.2019.06.011.]. This compromises immune surveillance and clearance of mutated and cancerous cells, resulting in accumulated DNA mutations and a higher cancer incidence in aged tissues.

7. Telomere shortening proceeds at an accelerated rate in cancer patients, making their biological age appear 15 years older than their chronological age. The telomeres in their cells are shorter than normal for their age and keep eroding until approximately four years prior to tumor formation, when the shortening abruptly halts [doi: 10.1016/j.ebiom.2015.04.008.].

8. Shorter telomeres cause T cell immunodeficiency [doi: 10.1016/j.ccell.2023.03.005.], which increases susceptibility to tumors.

9. Among Ashkenazi Jewish centenarians, mutations in hTERT and hTERC are associated with longer telomeres, extended lifespan and protection against age-related diseases [doi: 10.1073/pnas.0906191106.].

10. The incidence of tumors in the group with the shortest telomeres was three times that in the group with the longest telomeres, and the cancer mortality rate in the shortest telomere group was 11 times higher [doi: 10.1001/jama.2010.897.].

However, why do some populations with longer telomeres have a higher risk of developing tumors? For instance, POT1 acts as a negative regulator that maintains telomere stability and modulates telomere length. Carriers of POT1 mutations possess longer telomeres yet face an elevated cancer risk [doi: 10.1056/NEJMoa2300503.]. This has given rise to a common misconception and concern that anti-aging interventions aimed at lengthening telomeres may increase tumor incidence. In fact, the increased cancer susceptibility in individuals with longer telomeres driven by specific gene mutations stems not from telomere length itself, but from other downstream effects of these mutations. In families with familial melanoma, lymphocytes from carriers of POT1 nonsense or frameshift mutations exhibit loss of telomere signals, end-to-end chromosome fusions and chromothripsis-like rearrangements. These findings indicate that genomic instability and immune dysfunction are early events driving tumorigenesis [doi: 10.1038/onc.2016.405.].

3.8 Do telomeres shorten in non-dividing neurons and cardiomyocytes?

It is generally believed that telomeres do not shorten in non-dividing cells, yet this is not actually the case:

1. Autopsy analysis of 530 cases confirmed that cardiac telomeres shorten by approximately 20 base pairs (bp) per year [DOI: 10.1038/srep02401].

2. Oxygen free radicals damage telomeres. It has been found that cells cultured in 40% oxygen quickly stop growing, accompanied by telomere shortening, indicating

that telomere shortening can take place without cell division (von Zglinicki et al., 1995; von Zglinicki, 1998).

3. A meta-analysis of telomere length in patients with Alzheimer's disease (AD), encompassing 860 AD patients and 2022 control subjects, revealed that telomere shortening was significantly more severe in AD patients than in healthy controls. One included study directly measured telomere length in neurons and found that neuronal telomere length was markedly shorter in AD patients relative to healthy controls [doi: 10.1093/gerona/glw053].

4. Current Limitations of the Integrated Aging Theory Framework and Its TRCS Model

To date, comprehensive validation of the TRCS remains insufficient. Further investigations are required to profile changes in rDNA copy number during cellular senescence across diverse species ranging from plants and nematodes to humans, as well as to characterize the reconstruction of telomeres and rDNA following meiosis in germ cells of these organisms.

Large-scale longitudinal measurements and single-cell resolution data on rDNA shortening in humans are still lacking. The interplay between telomere shortening and rDNA compensation has not been fully elucidated, and the mechanisms underlying telomere and rDNA replenishment in germ cells warrant further exploration.

In addition, systematic rescue experiments are needed to fully verify how elongation of telomere and rDNA arrays affects senescence biomarkers and lifespan at both cellular and organismal levels.

We hope the Integrated Aging Theory Framework provides a useful conceptual foundation for future studies on aging biology, cellular senescence, and longevity interventions, and stimulates further experimental investigation of the mechanisms underlying aging and age-related diseases.

Declaration of interests:

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