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# Telomeres, cellular senescence, and aging: past and future

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## Abstract

Over half a century has passed since Alexey Olovnikov's groundbreaking proposal of the end-replication problem in 1971, laying the foundation for our understanding of telomeres and their pivotal role in cellular senescence. This review paper delves into the intricate and multifaceted relationship between cellular senescence, the influence of telomeres in this process, and the far-reaching consequences of telomeres in the context of aging and age-related diseases. Additionally, the paper investigates the various factors that can influence telomere shortening beyond the confines of the end-replication problem and how telomeres can exert their impact on aging, even in the absence of significant shortening. Ultimately, this paper stands as a tribute to the pioneering work of Olovnikov, whose seminal contributions established the solid foundation upon which our ongoing explorations of telomeres and the aging process are based.

### Keywords

Telomeres; Senescence; Aging; Olovnikov

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## Introduction

In 1971, Alexey Olovnikov introduced a theory suggesting that segments of the genomic DNA located at the chromosome ends, known as telomeres, are regularly lost during each round of DNA replication (Olovnikov 1971). In addition, Olovnikov predicted that the preservation of telomeres in germ line and tumor cells could be attributed to the presence of a unique DNA polymerase absent in typical somatic cells, later identified as telomerase. This theory was inspired by a lecture he attended a few years earlier, where he heard about a major discovery by American scientist Leonard Hayflick, of a phenomenon called cellular senescence (Hayflick and Moorhead 1961; Olovnikov 1996).

Although Olovnikov's theory went unnoticed by Western scientists for many years due to language barriers (given that the original paper was published in Russian) and geopolitical divisions, its lasting impact is beyond dispute, as all of its predictions have been experimentally confirmed. Importantly, the exploration of telomeres, cellular senescence, and their implications in the aging process and various diseases has experienced a surge in recent years, offering tangible possibilities for clinical interventions. This stands as a testament of Olovnikov's exceptional foresight.

In this paper, we present a comprehensive overview of the cutting-edge knowledge regarding telomeres, cellular senescence, and their pivotal roles in the aging process and age-related diseases.

## What is cellular senescence?

In the early 1960s, a groundbreaking discovery challenged the prevailing notion that cells grown outside the body environment could be cultivated indefinitely (Carrel 1912). Leonard Hayflick observed that cells could only undergo approximately 50 population doublings before experiencing a halt in their replicative capacity (Hayflick and Moorhead 1961). This phenomenon was termed "cellular senescence," and the associated proliferative limit is commonly referred to as "the Hayflick limit." Since Hayflick's discovery, researchers have dedicated substantial efforts to characterizing cellular senescence. It is now accepted that senescence is a multifaceted phenotype, characterized by marked changes in morphology, gene expression, metabolism, and others which manifest in a time dependent manner (Gorgoulis et al. 2019). Furthermore, it has become evident that senescence is not solely a result of extensive replication but can also be triggered by exposure to various stressors, including DNA damage, oxidative stress, and other factors. Senescent cells also produce and release a collection of factors known as the senescence associated secretory phenotype (SASP) which includes various pro-inflammatory cytokines, chemokines, and extra-cellular degrading proteins (Coppé et al. 2010). The SASP is thought to be a mechanism by which senescent cells communicate with the immune system (likely to orchestrate their own clearance) (Kang et al. 2011).

Indeed, senescent cells are believed to play a number of important roles in vivo. While it was initially believed that they evolved as a mechanism to counteract cancer (Serrano et al. 1997), more recent studies have identified additional roles for senescent cells in

processes like development and tissue repair (Demaria et al. 2014; Muñoz-Espín et al. 2013; Ritschka et al. 2017; Storer et al. 2013). Senescence markers were identified throughout mouse embryonic development and deletion of p21 in mice led to developmental defects, underscoring the significant role of senescence in this process (Jun and Lau 2010; Muñoz-Espín et al. 2013; Storer et al. 2013). Furthermore, senescent cells have been demonstrated to play a role in tissue repair in diverse situations, including liver injury, skin fibrosis, and wound healing (Demaria et al. 2014; Krizhanovsky et al. 2008). Importantly, the presence of senescent cells in these contexts is transient, unlike what is observed in aged tissues, suggesting that temporal control of these cells is an important determinant of how they impact different physiological functions.

Cells bearing senescent markers have been shown to accumulate in many tissues during aging and age-related diseases (Rossiello et al. 2022). The exact reasons for this accumulation remain unclear, but the most likely explanation is that as individuals age, the immune system becomes less effective at clearing senescent cells (Ovadya et al. 2018). These persistent senescent cells generate a chronic SASP, which has been hypothesized to be a driver of age-related tissue dysfunction.

In line with this idea, recent studies have shown that if senescent cells are selectively eliminated from tissues, this can alleviate a multitude of age-related pathologies, suggesting that senescent cells play a causal role during the aging process (Baker et al. 2016; Xu et al. 2015). In fact, the field has expanded in directions likely beyond the wildest dreams of early pioneers like Hayflick or Olovnikov. Cellular senescence has become the focus of extensive drug screening efforts aimed at discovering new drugs capable of targeting these cells (Zhang et al. 2021). Drugs designed to target senescent cells are already undergoing human clinical trials for age-related diseases (Hickson et al. 2019). The National Institutes of Health (NIH) is presently supporting a multi-million-dollar initiative with the goal of mapping senescent cells and their heterogeneity, akin to the genome mapping project (Gurkar et al. 2023; Lee et al. 2022). In the subsequent sections of this review, we will examine the data that reveals potential mechanisms through which telomeres play a role in cellular senescence. In addition, we will delve into the impact of telomeres on the processes of aging and the development of age-related diseases.

## Telomeres and their link to senescence

Telomeres are protective structures present at the ends of eukaryotic linear DNA and serve as a cap on each arm of all chromosomes. These regions serve to maintain genomic stability by protecting eukaryotic genetic material from nucleolytic attack and double-strand break detection (Stroik and Hendrickson 2020). These telomeric regions consist of highly repetitive DNA segments with TTA GGG repeats and comprise two strands, one rich in C nucleotides (the lagging strand) and the other rich in G nucleotides (the leading strand). Together, they form a 3’ overhang, which is believed to bind to the double-stranded telomeric regions upstream, forming a structure known as the telomere-loop (t-loop). The primary function of the t-loop is to secure loose DNA ends, preventing them from being recognized as double-stranded breaks that require repair (Doksani et al. 2013; Griffith et al. 1999). Telomeres interact with a set of six proteins: telomeric repeat binding factor

1 (TRF1), telomeric repeat binding factor 2 (TRF2), TRF2 interacting protein (RAP1), TRF1-interacting nuclear factor 2 (TIN2), adrenocortical dysplasia protein homolog (TPP1) and protection of telomeres 1 (POT1). Together, these proteins create a specialized complex referred to as “Shelterin”, which plays a vital role in stabilizing the t-loop structure (de Lange 2005).

As cells continue to divide, their telomeric region shortens with each replication of DNA due to what is known as the “end-replication problem”, where base pairs are lost from the ends of chromosomes. This issue arises during lagging-strand synthesis when primase synthesizes an RNA primer that can be recognized by DNA polymerase to form an Okazaki fragment. These RNA fragments are later replaced with nucleotides by the enzyme DNA polymerase in all parts of the DNA except for the end of the telomeres which lack an RNA primer. As we previously described in the introduction, this phenomenon was first theorized by Alexei Olovnikov in 1971 which proposed it as an explanation for Hayflick’s observation of senescence (Olovnikov 1971). On the other side of the world, in the year 1972, James D. Watson also recognized that when the DNA polymerase approached the terminus of a linear DNA molecule, a significant challenge in achieving complete replication would arise (Watson 1972). It took nearly two decades before these predictions were experimentally substantiated. In 1990, Harley, Futcher and Greider, observed that as fibroblasts reached replicative senescence, telomeres became progressively shorter (Harley et al. 1990). Later, it was shown that ectopic expression of telomerase—an enzyme that maintains and elongates telomeres- was sufficient to prevent the onset of senescence, demonstrating that telomere shortening is a limiting factor in replicative senescence (Bodnar et al. 1998). The observation that telomere shortening serves as a limiting factor in cell division, along with the capacity of telomerase to counteract this process, both align with predictions made by Olovnikov and Watson (Olovnikov 1971; Watson 1972).

Subsequent studies revealed novel insights into how short telomeres can trigger senescence. It was hypothesized that extensive shortening of telomeres due to cell division results in the loss of shelterin components from telomeres thereby disrupting its t-loop configuration and exposing the chromosome ends (Griffith et al. 1999). This has been further demonstrated by studies where a dominant-negative form of TRF2 was expressed in human cells, which hinders TRF2 accumulation at telomeres and causes uncapping. Consequently, this uncapping triggers the recruitment and activation of DNA damage response (DDR) proteins, such as 53BP1, the Mre11 complex, phosphorylated ATM, H2A.X, and Rad17 (Takai et al. 2003). In line with the hypothesis that extensive loss of telomeric repeats exposes chromosome ends and triggers a DDR, human fibroblasts undergoing replicative senescence were shown to exhibit an accumulation of DDR proteins, including phosphorylated H2A.X, 53BP1, MDC1, and NBS1, at telomeres (d’Adda di Fagagna et al. 2003) (Fig. 1).

One of the primary effector molecules downstream of the DDR is the transcription factor p53 (Lakin and Jackson 1999). P53 induces the expression of the cyclin-dependent kinase inhibitor p21 which in turn promotes cell-cycle arrest (Campisi and d’Adda di Fagagna 2007). Evidence supporting the involvement of p21 in telomere-induced senescence stems from studies conducted on late-generation telomerase-deficient mice (Blasco et al. 1997). These mice exhibit generation-dependent telomere shortening in multiple tissues and an

accelerated onset of aging-related traits. Notably, p21 deletion was found to reinstate the proliferation of hematopoietic stem cells and intestinal progenitor cells in these mice and extend their lifespan (Choudhury et al. 2007).

Another crucial pathway for inducing and maintaining senescence is the p16-pRb pathway, but its involvement in telomere-induced senescence is less clear. For instance, it has been observed that p16 activation can occur independently of telomere dysfunction in human cells (Herbig et al. 2004). Conversely, in Wrn-deficient mice which lack the WRN protein —a member of the RecQ family of DNA helicases important for efficient replication of G-rich telomeric DNA—deletion of p16 enhanced the proliferative capacity of mouse embryonic fibroblasts (Crabbe et al. 2004; Zhang et al. 2012). Additionally, p16 deletion was sufficient to prevent senescence triggered by telomere dysfunction in these cells (Zhang et al. 2012). Activation of p16 in response to telomere damage has also been observed upon TRF2 deletion (Jacobs and de Lange 2004). However, in these cells, p16 deletion did not fully rescue the growth arrest induced by telomere dysfunction. Complete restoration of proliferation was achieved only when both p16 and p53 were simultaneously inhibited, suggesting that p16 may act as a secondary barrier to impede cell cycle progression in response to telomere damage (Jacobs and de Lange 2004).

## Telomere dysfunction can occur in a length-independent manner

Recent research has expanded our understanding of telomeres and their role in cellular aging. Traditionally, telomere shortening has been associated with cellular senescence, but emerging studies have unveiled a more nuanced picture. Studies have brought to light that the DNA damage response (DDR) can be initiated at telomeric regions, regardless of their length (Fig. 1). This phenomenon can also contribute to cellular senescence. In fact, exposure to stressors that have the capacity to induce senescence has been shown to trigger DNA damage signaling at telomeric regions, without telomere shortening (Fumagalli et al. 2012; Hewitt et al. 2012).

These observations have extended to non-dividing cells as they age. In murine cardiomyocytes, which are terminally differentiated postmitotic cells, there is an age-dependent accumulation of telomere-associated DNA damage, regardless of telomere length (Anderson et al. 2019). Similar observations were made in other postmitotic tissues in mice, including adipocytes (Xu et al. 2018), neurons (Fielder et al. 2020), osteoclasts, osteocytes (Farr et al. 2016), and skeletal muscle fibers (Zhang et al. 2022), where age-related telomere damage has been noted. In human melanocytes, which have limited replicative capacity, age-related telomere damage has been observed, accompanied by an increase in the expression of senescence-associated markers. Importantly, this telomere damage occurred without a significant reduction in telomere length (Victorelli et al. 2019). Therefore, while the concept of telomere shortening remains a compelling explanation for cell aging and proliferative exhaustion, it is becoming evident that it alone cannot account for the aging of non-proliferating, quiescent, or terminally differentiated cells. The role of telomere damage, independent of telomere length, is emerging as an essential aspect of cellular aging, especially in postmitotic cell types.

Mechanistically, there are some clues to explain how length-independent telomere dysfunction contributes to cellular senescence. In contrast to the rest of the genome, damage to telomeric regions tends to persist over time. Live-cell imaging of cells undergoing stress-induced senescence have revealed that DNA damage foci associated with telomeres persist for longer than non-telomeric foci (Hewitt et al. 2012). After exposure to sub-lethal levels of irradiation, mouse hippocampal neurons showed persistent DDR signaling in telomeric regions even three months after exposure to genotoxic stress (Fumagalli et al. 2012). Supporting the notion that telomeric damage is repaired differently compared to non-telomeric damage, the inducible expression of an endonuclease (FOKI) fused to TRF1, specifically targeting telomeres, resulted in sustained telomeric damage, ultimately leading to senescence. In contrast, the inducible expression of the endonuclease I-PpoI led to random genomic damage, which was promptly repaired and was insufficient to initiate the process of senescence. This underscores the distinct nature of DNA repair at telomeric regions and the persistence of damage at telomeres in comparison to non-telomeric damage (Anderson et al. 2019). The reason behind this deficiency in DNA repair, is thought to be related to the presence of shelterin components. Shelterin components have evolved mechanisms to prevent end-to-end fusions of chromosomes (Bae and Baumann 2007). These very mechanisms that safeguard against end-to-end fusions also hinder the repair process if damage occurs within telomeric repeats, contributing to long-lasting DDR signaling at chromosome ends (Fumagalli et al. 2012).

## Mechanisms by which stress accelerates telomere dysfunction

While repeated rounds of cell division in the absence of telomerase cause telomere attrition, other factors can influence the rate at which telomeres shorten. Multiple studies have shown that mild oxidative stress can accelerate the rate of telomere shortening, reducing the cells' ability to proliferate, and inducing premature cellular senescence (von Zglinicki 2002) (Fig. 1). Consistent with this idea, studies have shown that manipulating the antioxidant capacity of cells can impact on the rate of loss of telomeric repeats. A study conducted in human fibroblasts demonstrated that reducing ROS (Reactive Oxygen Species) levels, either by overexpressing the antioxidant enzyme superoxide dismutase 3 (SOD3) or by antioxidant treatment, resulted in a slower rate of telomere shortening and extension of the proliferative potential of human fibroblasts (Serra et al. 2003). Moreover, cells with lower antioxidant capacity and shorter replicative lifespans tend to experience faster telomere shortening compared to those with greater antioxidant capacity (Richter and Zglinicki 2007).

In addition, studies in vivo have shown correlations between levels of intracellular ROS and the rate of telomere attrition. For instance, studies suggest that telomeric attrition due to oxidative stress can be linked to reproductive aging in females and a reduction in oocyte development (Igarashi et al. 2016; van der Reest et al. 2021). Oxidative stress has also been implicated as a contributing factor to the demyelination and axonal damage in multiple-sclerosis patients (MS) displaying evidence of oxidative stress and shortened telomeres (Gilgun-Sherki et al. 2004). Accelerated telomere shortening has also been linked to age-related conditions associated with oxidative stress such as osteoporosis, cardiovascular disease, malignancies, and Alzheimer's disease (Cai et al. 2013; Rossiello et al. 2022; Zhu et al. 2016).

The precise source of ROS responsible for causing telomere dysfunction is not yet fully understood. However, there is substantial evidence indicating that ROS originating from mitochondria plays a significant role. When cells are treated with the mitochondria-targeted antioxidant Mito-Q, this results in decreased rate of telomere shortening and extends the replicative lifespan of fibroblasts exposed to hyperoxic conditions (Saretzki et al. 2003). Moreover, mild mitochondrial uncoupling, which reduces mitochondrial superoxide levels, has been demonstrated to prolong the replicative lifespan of fibroblasts and slow down the rate of telomere shortening (Passos et al. 2007). This emphasizes the role of mitochondrial ROS in this process.

Telomeres are believed to be particularly vulnerable to oxidative damage when compared to other regions of the genome. This susceptibility is attributed to the rich presence of guanine triplets in telomeric repeats, which are prone to oxidative damage (Oikawa et al. 2001). The structure and low redox potential of the guanine nucleotide makes it particularly susceptible to reacting with ROS and other guanine nucleotides (Singh et al. 2019). Exposing cells to mild oxidative stress has been shown to result in single-stranded breaks in telomeric regions, which leads to the stalling of the replication fork, incomplete replication, and accelerated telomeric shortening (Petersen et al. 1998; von Zglinicki et al. 2000). It is important to note that the ROS responsible for driving telomere dysfunction do not necessarily need to originate from within the affected cells. In a recent study, it was demonstrated that neutrophils can induce telomere dysfunction in non-immune cells through the generation of ROS. In this study, short-term co-culture of neutrophils with human primary fibroblasts led to an accelerated rate of telomere shortening and induced premature replicative senescence in the fibroblasts. Notably, this process was found to be dependent on the presence of ROS, emphasizing the role of extracellular ROS in driving telomere dysfunction (Lagnado et al. 2021). In agreement with this, SASP factors released by senescent melanocytes were also shown to induce telomere damage in neighboring cells in a ROS-dependent manner (Victorelli et al. 2019).

In mice, ROS is also implicated in causing telomere damage in postmitotic cells. Mouse models with elevated ROS levels ($MnSOD^{+/-}$ and $Catalase^{-/-}$) and mitochondrial dysfunction ($Polg^{mut/mut}$) display early-onset age-related telomere dysfunction in postmitotic cardiomyocytes (Anderson et al. 2019). Given that these cells do not divide, it is unlikely that this damage will be caused by accelerated telomere shortening. Recently, a mechanism was suggested to explain this observation. Acute induction of telomere-specific 8oxoG was shown to cause telomere dysfunction and cellular senescence without significant shortening (Barnes et al. 2022). This study suggested that oxidative lesions at telomeres induced replication-dependent fragile sites at telomeric regions, which triggered premature senescence without causing telomere shortening (Barnes et al. 2022).

In addition to their involvement in triggering senescence through the induction of telomere dysfunction, ROS are believed to act as effectors during the senescence process. This is further supported by the observation that the activation of the DNA damage response (DDR) during senescence leads to the generation of ROS within mitochondria. These mechanisms are partly reliant on factors like p53 and p21 and ATM-dependent activation of mTORC1 (Correia-Melo et al. 2016; Passos et al. 2010). Supporting this idea, deletion of p21 in

late-generation TERC$^{-/-}$ mice (Blasco et al. 1997), which exhibit telomere dysfunction, has been shown to reduce the levels of oxidative damage observed in tissues (Passos et al. 2010). Studies also suggest a positive feedback loop exists between mitochondrial ROS and DNA damage, which promotes the stabilization of senescence through the continual activation of the DNA damage response.

## Telomere-associated DDR foci accumulate during aging and disease

While the relationship between telomere shortening and lifespan is not entirely linear, research has demonstrated that late-generation mice lacking telomerase (Terc or Tert), the enzyme responsible for maintaining telomere length, exhibit remarkably short telomeres and accelerated aging phenotypes and disease (Blasco et al. 1997; Samper et al. 2001). In addition, links between reduced telomere length and increased mortality risk have also been reported in humans. For instance, in one study, it was shown that among individuals over 60 years old, those who had shorter telomeres in blood cells were more likely to succumb to conditions such as heart disease and infectious diseases (Cawthon et al. 2003). Interestingly, a recent study revealed a positive correlation between the telomere length of peripheral blood cells and telomere length observed in various tissues, suggesting that length of telomeres in circulating cells can be used as a proxy for telomere length in other organs (Demanelis et al. 2020). However, it is unlikely that telomere length on its own is the only determinant of lifespan, as some shorter-lived species have longer telomeres than humans (Gomes et al. 2011). Therefore, it has been proposed that the rate at which telomeres shorten throughout an organism's life may play a more important role in determining lifespan. In support of this hypothesis, studies have shown that the rate of telomere shortening positively correlates with life expectancy of different species (Whittemore et al. 2019). However, whether telomere shortening alone can result in critically short telomeres that can trigger a persistent DNA damage response and induce senescence still requires further investigation. Although mice, for example, experience age-dependent telomere shortening, their telomeres at older ages are still considerably longer than those in young humans. Moreover, while telomere shortening can explain aging in actively dividing tissues, it does not explain why quiescent or non-dividing cells also accumulate DDR signals at telomeres during aging.

For these reasons, we suggest that a more reliable indicator of telomere dysfunction is to analyze if DDR proteins accumulate at telomere regions rather than just assessing telomere length. Telomere-associated DDR can be assessed in groups of cells using methods like ChIP-seq (d'Adda di Fagagna et al. 2003) or at the single-cell level using Immuno-FISH or a modified proximity ligation assay (TIF-PLA), which enables the microscopic observation of the co-localization between telomeres and DDR proteins like γH2A.X, 53BP1, and others (Hewitt et al. 2012; Wang et al. 2022). Other methods, such as detecting telomeric non-coding RNA, allow the detection of telomeric DDR both in situ (FISH) or in bulk tissues (qPCR) (Rossiello et al. 2017).

A substantial body of consistent data supports the notion that the co-localization of DNA damage response (DDR) proteins with telomeres increases with age in various tissues and mammalian species. This phenomenon, often referred to as Telomere-Associated DDR Foci (TAF), has been observed in several contexts. TAF have been observed to increase in

multiple tissues in aging mice, including the liver (Hewitt et al. 2012), intestine (Hewitt et al. 2012), lung (Birch et al. 2015), bone (Farr et al. 2016), brain (Fielder et al. 2020), and heart (Anderson et al. 2019). Additionally, TAF have also been found to increase in the skin and brain of aging baboons (Fumagalli et al. 2012; Herbig et al. 2006), and in aged human skin (Victorelli et al. 2019) as well as human skeletal muscle (Zhang et al. 2022). Conditions known to accelerate aging, such as inflammation (Jurk et al. 2014; Lagnado et al. 2021), obesity (Ogrodnik et al. 2017, 2019), mitochondrial dysfunction (Anderson et al. 2019) and impaired autophagy (Cassidy et al. 2020), have also resulted in increased TAF in vivo.

Interventions aimed at targeting senescent cells, such as rapamycin (Correia-Melo et al. 2019, 2016), anti-inflammatory drugs (Fielder et al. 2020; Jurk et al. 2014) and dietary restriction (Ogrodnik et al. 2017) have significantly reduced the frequency of TAF in various tissues. Moreover, senolytic drugs or the genetic removal of $p16^{Ink4a}$-positive cells have been shown to reduce TAF in the aorta (Roos et al. 2016), liver (Ogrodnik et al. 2017), heart (Anderson et al. 2019), brain (Ogrodnik et al. 2019), bone (Chandra et al. 2020), and adipose tissue (Farr et al. 2023; Ogrodnik et al. 2019; Xu et al. 2018) of mice. These findings support the notion that TAF are a reliable marker of cellular senescence.

Furthermore, to substantiate the notion that telomere dysfunction and DNA damage signaling play a role in the aging process, recent studies have revealed that targeted inhibition of DNA damage response (DDR) at dysfunctional telomeres can potentially avert senescence and yield positive effects on aging. Inhibition of DDR at telomeres was achieved through the use of telomeric antisense oligonucleotides (tASOs) (Rossiello et al. 2017). Notably, this approach extended both lifespan and health span in a mouse model of Hutchinson-Gilford progeria syndrome (HGPS) (Aguado et al. 2019). These findings offer intriguing insights into the potential for interventions at the molecular level to mitigate the aging process and its associated consequences.

## Conclusions and future perspectives

More than five decades have passed since Olovnikov's seminal paper, and it has become increasingly evident, supported by a wealth of research, that telomeres play pivotal roles in cellular senescence, aging, and age-related conditions. It is clear that the end-replication problem alone cannot fully account for the presence of senescent cells during aging and in various diseases, given the complexity and heterogeneity in division rates of cells within tissues. As our review indicates, there are likely other contributing factors at play. Despite the intricacies and challenges involved, it is safe to say that Olovnikov's predictions and the collective efforts of subsequent scientists have left a profound impact. This underscores the importance of collaboration between theoretical and experimental scientists to further advance our understanding of complex biological processes. Unfortunately, the contributions of theoreticians, such as Olovnikov and many others, often go underappreciated.

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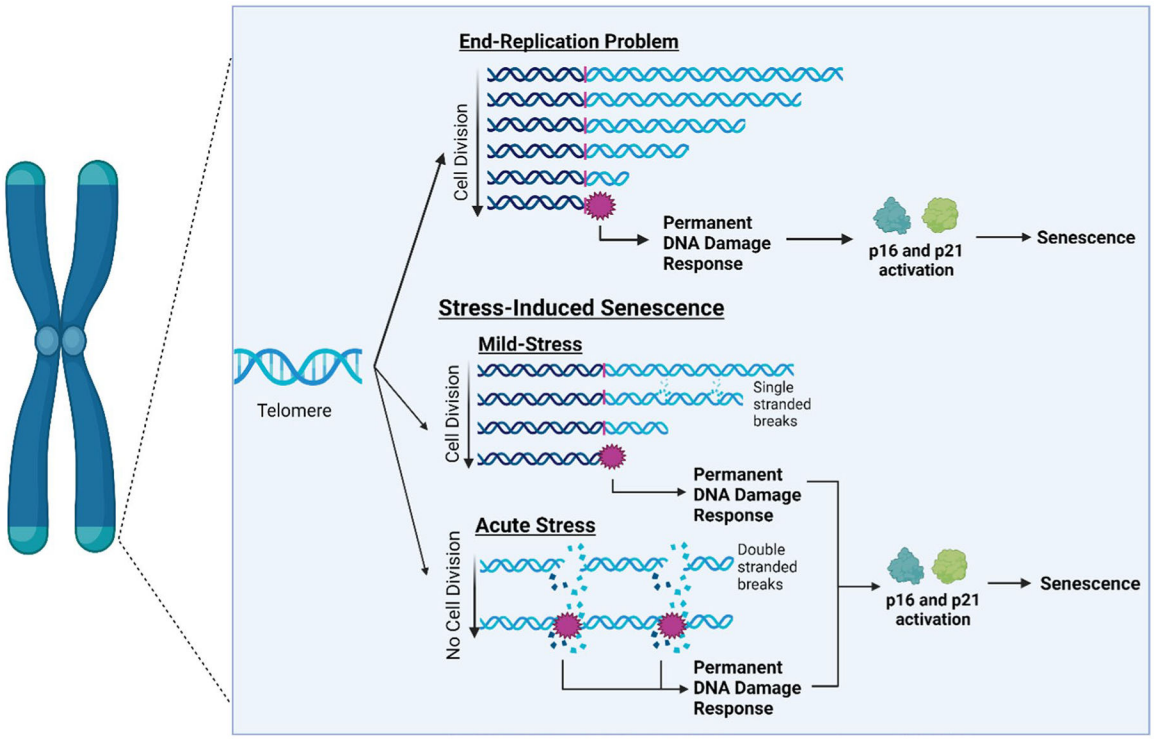


**Fig. 1.**

Dysfunctional telomeres activate a persistent DDR that contributes to cellular senescence. Extensive telomere shortening that occurs as a consequence of repeated rounds of cell division ultimately leads to exposure of chromosome ends and activation of a DNA damage response, contributing to the senescence-associated cell-cycle arrest. However, mild oxidative stress can also induce breaks within telomeric regions, accelerating telomere shortening and senescence. Telomere dysfunction can also occur independently of shortening, whereby damage arising within telomeric repeats triggers persistent DNA damage signaling and senescence induction. Created using BioRender.com