

Chapter 17

Psychosocial stress and telomere regulation

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17.1 Introduction

“He who is of calm and happy nature will hardly feel the pressure of age, but to him who is of an opposite disposition youth and age are equally a burden,” Plato

Stress is universal; everyone experiences it. But as Plato hinted, perception and disposition are important factors in determining the magnitude of our response, noting that not everyone is equally burdened. More than 2,000 years after Plato, scientists are now seeking to answer, not only how stressful disposition affects our mind, but also how adverse challenges impact health and aging processes by looking at microscopic changes inside the cell. On a cellular level, stress is considered to be a disruption to homeostasis, the relatively stable equilibrium maintained across the multitude of processes within cells. As we get older, continued stress exposure can cause increased damage to individual cells, and eventually induce wear and tear on physiological systems. One factor that is both stress-responsive and has also received growing attention as a marker for cellular aging is telomere length (TL). In fact, the very discovery of telomeres was a result of experiments simulating cellular stress using radiation.

During the 1930s, Herman Muller and Barbara McClintock found they could force breakage and subsequent reformation of new chromosome products by irradiating fruit flies' DNA with X-rays (1, 2), a stressor which can result in DNA damage response and cell death. Interestingly, the products never contained end to end fusions of the original chromosomes, leading Muller to the following hypothesis: “The terminal gene must have a special function, that of sealing the end of the chromosome, so to speak, and that for some reason a chromosome cannot persist indefinitely without having its ends thus sealed. This gene may accordingly be distinguished by a special term, the telomere” (i.e. “end part” from Greek) (3). Subsequent work in the 1950s by Rebecca Gerschman and colleagues showed the same DNA damage induced by X-rays could also be produced through oxidative stress (i.e. the accumulation of highly reactive, oxygen-containing-molecules which are naturally produced by mitochondria during cellular respiration), resulting in decreased survival in mice (4).

This prompted biologists to consider how stress may contribute to aging at the cellular level. Denham Harmon followed Gerschman's work, proposing that cell death is the result of pathways triggered by accumulated oxidative damage to DNA (5). Later in the 1960s Hayflick and Moorhead discovered that cells have an inherent limit to their replicative capacity, the number of times a cell can divide. This threshold was later named the “Hayflick limit.” They theorized that the limited capacity for normal cells to divide is an expression of aging, and that it determines the longevity of the organism (6), building on an idea first theorized in the late nineteenth century by August Weissman (112). In the early 1970s, Olovnikov and Watson implicated telomeres in this process in what they independently formalized as the “end replication

problem,” wherein 12–30 bp of telomeric DNA are lost with each division due to replication inefficiency (7, 8). Further division would be halted when telomeres become critically short, preventing additional loss of critical DNA when cells enter a state of replicative arrest (i.e. senescence). While most cells conform to the Hayflick limit of finite cell divisions, there were multiple reports that certain cells, such as stem cells, seemingly divided indefinitely, somehow circumventing the Hayflick limit. Thus, a protective mechanism must be at work at telomeric ends, at least in some cells.

The first clue came a decade later when Blackburn and Gall, working with the ciliate organism *Tetrahymena*, showed the end regions of chromosomes were not a gene as Muller hypothesized, but rather a 5'-TTGGGG-3' tandem repeats (9) (5'-TTAGGG-3' in humans). Later in the 1980s, Blackburn and Greider discovered a unique protein, telomerase, which adds telomeric repeats to the 3' ends of chromosomes during each cell division, providing a viable solution to the end replication problem (10). As opposed to stem cells, most somatic cells lack sufficient telomerase and as a consequence, telomeres progressively shorten with each cell division.

While no empirical evidence existed to implicate telomere shortening or oxidative damage as the cause of cellular senescence, two seminal studies in the 1990s provided some clarity. In the first, Harley and colleagues confirmed shortening of telomeres with age in human connective tissue cells (11). Once von Zglinicki's work showed exposure to higher levels of oxidative stress increases the rate of telomeric shortening *in vitro*, there was compelling evidence implicating both telomeres and cellular stress in the aging process (12).

Growing body of research over the last 20 years has continued to explore the methods and means of telomere regulation in response to stress, including stress induced by psychosocial factors in humans. The results of these studies are presented in this chapter. We begin with a brief overview of the link between telomeres and health, followed by a state of the science review of the underlying biological mechanisms of telomere regulation. We conclude with some of the open questions in the field and discuss future research directions.

17.2 General impacts of stress on telomeres and health

It is generally accepted that telomere length decreases with chronological age. However, the rate of shortening varies across the lifespan. Although we currently lack within-individual evidence, it is suggested that the greatest loss occurs in the first years of life, corresponding to the rapid growth rates and high production and turnover of cells, followed by a stable plateau into childhood and young adulthood and a gradual decline into old age (13). Beyond this natural erosion of telomeres by age, recent research has shown that the rate of shortening is influenced by physical, psychological, and social conditions, which invites the question; how strong are telomeres as an indicator and predictor of healthspan and lifespan?

Debate still rages on this question, particularly in the realm of gerontology. Based on measurements in peripheral blood leukocytes, some (14), but not all (15), studies have found inverse relationships between TL and mortality, such that shorter telomeres are associated with higher mortality rates. Still others find the association holds, but significantly weakens when controlling for inflammatory markers (16). Possible explanations for these mixed results are varied TL assays and DNA extraction procedures (17, 18), sample sizes, population composition, and statistical adjustments used in the analyses. In a recent population study, including 64,637 individuals, the authors reported significant association between short TL and all-cause mortality (19). Furthermore, the exceptionally long telomeres found in centenarians are associated with above average longevity and increased cognitive function (20). These individuals also display decreased incidence of physically taxing conditions such as obesity, diabetes, and heart disease.

Incidence rates of aging-related health conditions (e.g. cardiovascular disease, diabetes, and stroke) have consistently been shown to be associated with shorter TL, independent of conventional risk factors (21, 22). Further, poor lifestyle behaviors can lead to and intensify negative health outcomes. Smoking is one such example that has been associated with shorter telomeres in cross-sectional studies (23, 24). Another detrimental outcome associated with smoking, and particularly related to telomeres, is cancer. Two large-scale meta-analysis found shorter telomeres to be significantly associated with specific types of cancer (25, 26). It should be noted, however, that findings in this area are mixed. Retrospective studies tend to report shorter telomeres in leukocytes of those with a history or current diagnosis of cancer. By contrast, Mendelian randomization studies report increased cancer risk for those carrying variants associated with longer leukocyte telomere length (113, 114). Taken together, this may imply that longer telomeres predispose one to develop cancer, and that the bout with cancer prompts telomere shortening in turn.

While unhealthy behaviors and exposures can damage telomeres, positive health behaviors, on the other hand, can mitigate telomere loss. For example, endurance training and mindfulness meditation is associated with increased telomerase activity (27, 28, 115), suggesting a protective effect on the rate of telomere erosion. The association between mindfulness and telomerase activity was mediated by changes in feelings of perceived control and neuroticism, highlighting the relationship between psychological and physical well-being. A foundational work in this area was by Epel and colleagues in 2004, showing a relationship between the burden of caregiving and shorter telomeres, which highlighted for the first time the link between psychological stress and TL (29). Follow-up studies provided further support that caregivers of Alzheimer's patients and sisters of women with breast cancer displayed similarly shortened TL (30, 31).

A population which has been a focus of research regarding the effects of stress on telomeres are individuals who were exposed to early-life adversity. This population is of particular importance as early adversity is known to propagate across the lifespan and to powerfully affect health in later life (32, 33). Thus, there is an interest in the role of telomere erosion as a potential mediator of such adverse effects. Beginning with work in 2010 (34), numerous studies have reported the negative impacts of early-life stress on TL in adults (reviewed in 35, 36). In the first study of children, greater exposure to (poor quality) institutional care was associated with shorter TL in middle childhood (37). However, conclusions from these studies have been limited by their cross-sectional and retrospective design. Subsequent longitudinal research involving repeated telomere measurements by Shalev and colleagues provided critical prospective evidence. Children exposed to multiple kinds of violence between age 5 and 10 years showed significantly more telomere erosion over time (i.e. by age 10) than did other children (38).

The fact that telomeres prove sensitive to adversity and predictive of health has resulted in their regard as a "biological clock" for studying accumulated cellular aging throughout the life course. Intriguingly, recent data suggest that this accelerated aging process begins very early in life. One small-sample study linked greater prenatal stress and shorter cord blood TL at birth (39), a finding which was recently replicated in another study using a prospective design over the whole course of gestation (40). This developmental origin of health and disease is known to have long-lasting effects on health and aging processes (41). In a cohort study by Shalev and colleagues, perinatal complications at birth were linked with two aging indicators in midlife, TL and perceived facial aging, independent of family history and social risks present before birth, and of life-course health (42). These associations raise questions of underlying mechanisms of how stress "gets under the skin" at the cellular level. In the sections to follow, we first review basic telomere regulation by intrinsic systems, before discussing stress-related mechanisms and their impact on telomere stability and regulation.

17.3 Telomere length regulation by intrinsic systems

The preceding section highlighted the versatile nature of telomeres as a biomarker for understanding the deleterious impact of various stressors on health and well-being. Given telomeres' association with these processes, extensive research has been conducted to elucidate biological mechanisms responsible for telomere regulation. In order to understand the biological embedding of stress, it is useful to first review the basic biology of the telomere system at the cellular level before turning our attention to regulation of telomeres by stress-related processes. Thus, in the ensuing section we highlight key *intrinsic regulators* of TL, beginning with baseline determinants (Figure 17.1).

17.3.1 Baseline determinants

Despite being composed of DNA bases, telomeres are not like genes. Their length varies between chromosomes and between cells (43), and continually changes within individuals across the lifespan. Even at birth, TL varies significantly across individuals as a result of heritable factors. A recent meta-analysis of 19,713 individuals found TL heritability level of 0.70 based on twins and family members' comparisons (44). A similar heritability level, 64%, was calculated in another recent twin study, with shared environmental experiences accounting for an additional 22% of TL variation (45). Studies are only beginning to examine which specific genetic variations can

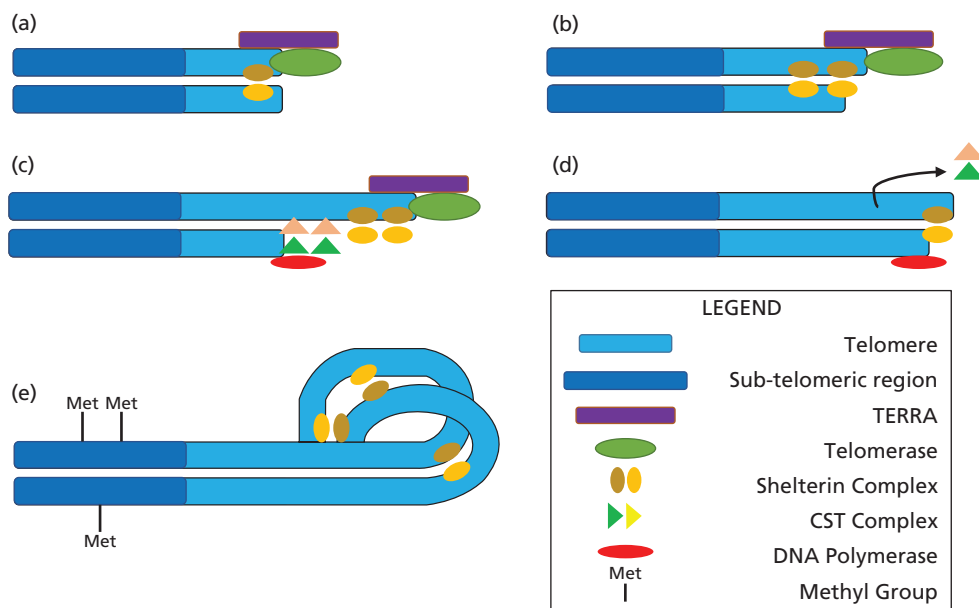


Figure 17.1 Schematic representation of telomere length regulation by intrinsic mechanisms. (A) TERRA assists in recruitment of telomerase to telomere while shelterin stabilizes DNA. (B) Telomerase elongates telomeres through addition of TTAGGG repeats to the leading strand. (C) CST complex competitively excludes shelterin complex by binding to single-stranded DNA and recruits DNA polymerase. (D) DNA polymerase elongates lagging strand of telomeric DNA, leaving a small 3' overhang to which shelterin proteins remain bound. (E) Shelterin proteins induce conformation of a T-loop structure to stabilize telomere ends. DNA is methylated in sub-telomeric regions, preventing further transcription of TERRA.

account for this relatively high heritability level of TL. For example, one study showed that variations in seven autosomal genes are associated with TL and disease (46). Interestingly, three of these genes are directly involved in the formation and activity of telomerase, while another two have been implicated in the function of telomere system proteins. Intriguingly, all seven genes are on different chromosomes, highlighting the complex genomic nature of TL regulation.

Parents influence TL as well, albeit in different ways. Studies reported significant correlation between mother and offspring TL (44, 47), suggesting a strong maternal influence on TL at birth. Paternal influence is more subtle. The strongest evidence has been for a paternal age at conception (PAC) effect, such that increased paternal age at conception is associated with longer telomeres in offspring (44, 48–50). This effect may even be cumulative across generations, as paternal grandfather's age at the time of father's conception is also significantly related to TL, independent of the paternal age effect (51). One potential explanation for this phenomenon is the observation of increased TL in sperm cells of older males (52). Despite genetic influences on TL *before* birth, twin-based studies have provided empirical support for environmental factors as the dominant influence *across the lifespan* (53). These environmental factors are translated to changes in TL through modulation of intrinsic regulation and stress-related processes.

17.3.2 Telomerase and the telomeric repeat containing RNA (TERRA)

As mentioned in section 17.1, telomerase is the master regulator of TL. The telomerase enzyme is a complex protein composed of four subunits (i.e. TERT, TERC, DKC1, and TEP1), which are encoded by four genes located on different chromosomes. All play important roles in telomere maintenance. Although telomerase can protect telomeres from erosion, most somatic cells lack sufficient expression of telomerase. The predominant cells which constitutively express telomerase are stem cells, which need be maintained throughout life to serve as a source for newly differentiated cells, and sperm cells, whose telomeres can actually elongate with age (52). In humans, telomerase adds TTAGGG repeats to the leading strand upon each round of cell division, allowing DNA polymerase a longer consensus sequence to replicate and elongate chromosome ends (54).

For many years the repeat rich telomeric sequence was thought to be a noncoding region similar to other “junk” regions in the genome. Yet, telomeres are transcribed into what is known as the telomeric repeat containing RNA (TERRA), which helps recruit telomerase to the shortest telomeres (55, 56). This ensures that the shortest telomeres in a given cell are prioritized for elongation. Recent work in mouse brain tissue and human lymphocyte cells has elaborated on the importance of TERRA, including a role in the immune response (57). When exported extracellularly, TERRA stimulates the expression of inflammatory cytokines and may cue cell degradation when unable to bind to critically short telomeres in the nucleus (57).

17.3.3 Shelterin and CST complexes

In addition to telomerase and TERRA, telomere intrinsic regulation involves several other proteins, namely the shelterin and CST complexes. Both complexes stabilize and cap the chromosome at various points during the cell division cycle. Specifically, the shelterin complex is a six-protein system (i.e. RAP1, TRF1, TRF2, TIN2, POT1, and TPP1) whose concerted activity promotes accessibility for telomerase and prevents end-to-end fusions of chromosomes (58). To accomplish this, shelterin proteins bind to and stabilize the local area of already replicated double-stranded DNA until telomerase can bind, while additional proteins are bound to the single-stranded DNA overhang to prevent fusion with other chromosomes during cell division (59). As cell division

nears conclusion, shelterin complex proteins are competitively excluded by binding of the three-protein CST complex (i.e. CTC1, STN1, and TEN1), which prevents further elongation by telomerase (60). Additional function of the CST complex is the recruitment of DNA polymerase and ligase for elongation of the lagging strand, complementary to the telomerase-added repeats (61). Once elongation is complete, TERRA interacts with shelterin proteins to induce formation of a stabilizing structure called the T-loop (62). This folded-back structure prevents end-to-end fusions of chromosomes by enzymes responsible for normal repair of double-stranded DNA breaks (63).

17.3.4 Epigenetics

Recent research has shown increased impact of epigenetic regulation on TL. Epigenetic control refers to such processes as methylation and acetylation of DNA and histones, which in turn alters chromatin structure, transcription factor access, and subsequent gene expression. Epigenetic maintenance of TL is a dynamic process involving the regulation of TERRA levels and interactions with other stabilizing proteins in the shelterin and CST complexes (64). Importantly, epigenetic control is one pathway to produce drastically elongated telomeres, most likely through interactions between histones and proteins in the shelterin complex (64). Specifically, increased binding of shelterin proteins TRF1 and TRF2 to histone tails could increase recruitment of telomerase to telomeres (65). While telomere elongation may be perceived as a positive outcome, elongated telomeres cannot effectively form stabilizing T-loop structures, and thus are more prone to breakage or recombination with other chromosome ends. In fact, recombination-mediated telomere elongation (i.e. alternative lengthening of telomeres [ALT]) is a hallmark of certain cancer cells (66). By contrast, DNA methylation in the subtelomeric regions decreases TL, perhaps by inhibiting access to promoter regions to decrease transcription of TERRA (67). Furthermore, loss of methylation occurs in response to critically short telomeres, which may explain the age-associated prevalence of cancer as stem cells are near their Hayflick limit (68).

Future work will continue to elucidate the connection between epigenetics and telomeres. While these intrinsic processes, including variations in specific genes or the mode of parental inheritance can influence TL at birth, substantial influence results from environmental stressors across the lifespan. In the next section, we detail stress-related mechanisms that can further shorten telomeres and impair health.

17.4 Telomere length regulation by stress-related systems

Under normal conditions, the aforementioned intrinsic processes work in concert to maintain and protect telomeres. However, stress, by its very definition, is a disruption to the internal regulation of an organism, and the mechanisms governing TL are no exception. Multiple mechanisms are hypothesized to embed stress biologically in a manner that shortens telomeres. These embedding mechanisms involve complex interactions between stress-related systems and factors which, in turn, can increase the rate of telomere erosion and cellular senescence and promote aging-related diseases (Figure 17.2).

17.4.1 Neuroendocrine systems

Evidence linking shorter TL and increased telomere erosion with psychosocial stressors (35, 69) and internalizing disorders (24, 70, 71) calls attention, when trying to understand the process of telomere regulation, to the physiological stress systems, in particular the sympathetic–adrenal–medulla (SAM) axis of the autonomic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis of the neuroendocrine system. Although the mechanistic link between systemic

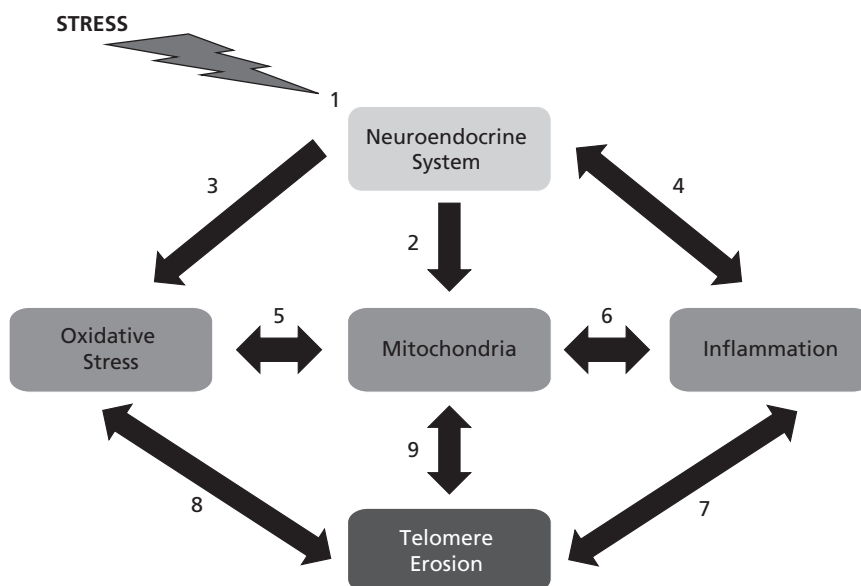


Figure 17.2 Schematic representation of telomere length regulation by stress-related mechanisms. (1) The human stress response initiated via the sympathetic–adrenal–medulla and hypothalamic–pituitary–adrenal axes stimulate the release of catecholamines and glucocorticoids into the periphery. (2) Cortisol release stimulates cellular metabolism through interaction with receptors in mitochondria. (3) Cortisol diminishes antioxidant production, disrupting their balance with free radicals, leading to oxidative stress within cells. (4) Chronic release of cortisol results in over-activation of the inflammatory response. Higher levels of inflammatory cytokines as a result of chronic inflammation also dampen diurnal rhythm of cortisol. (5) Increased cell metabolism increases production of reactive oxygen species (ROS) catalyzed by mitochondria. These can induce oxidative damage to cellular DNA, including mitochondrial DNA. (6) Under severe stress, damaged mitochondria release pro-inflammatory markers. Pro-inflammatory markers, in turn, can suppress mitochondrial function. (7) Inflammatory factors mark cells/tissues for degradation by macrophages. Damaged tissues are replenished by juvenile stem cells, decreasing TL as they divide and differentiate. Critically short telomeres activate senescent pathways, including release of inflammatory markers. (8) Free radicals permeate the nucleus to cause DNA damage, to which telomeres are especially susceptible. If left unrepaired, DNA damage triggers cell death pathways, further increasing stem-cell proliferation and TL erosion. Telomere-induced senescence increase levels of ROS. (9) During times of oxidative stress, TERT, the catalytic subunit of telomerase, localizes within mitochondria which can impair TL elongation. By contrast the age-associated decrease in mitochondrial activity can be rescued by enhancing TERT activity.

dysregulation of the HPA axis and TL is not entirely clear (36), empirical evidence suggests that chronic stress-induced secretion of the glucocorticoid cortisol down-regulates the activity of telomerase in lymphocyte cells, while increasing oxidative stress through mitochondrial dysregulation, which in turn leads to more rapid erosion of telomeres and, eventually, cellular senescence (72–75). Several studies of humans document significant associations between HPA axis indices, including physiological stress reactivity in children, and shorter TL (76–79). Furthermore, dysregulation along three stress pathways, namely the immune–inflammatory pathway, HPA axis, and autonomic nervous system were each associated with shorter TL in an

additive manner (80). To understand further the mechanisms integrating these systems, we need to focus on processes in the periphery, specifically those associated with stress-related cellular metabolism and inflammation.

17.4.2 Inflammation

An important feature of the immune system implicated in the telomere erosion process is inflammation. The inflammatory response increases flow of leukocytes to injured tissues to degrade damaged cells or digest pathogens. Similarly, individually damaged cells release inflammatory cytokines to mark themselves for degradation. As the damaged tissue is removed, stem cells divide and differentiate to give rise to new cells, decreasing TL in the body's whole-cell reserve while simultaneously introducing naïve cells to the system with relatively long telomeres.

Glucocorticoids typically suppress the immune response during stress, redistributing energy to processes more vital to immediate survival. Part of this suppression involves increased release of anti-inflammatory markers, which also down-regulate the overall stress response as part of a negative feedback loop (81). This negative feedback is important to re-establish immune support for an organism's defense. However, when an individual is exposed to chronic stress, the immune system overcompensates, becoming overactive and resistant to normal regulatory measures, which can in turn prompt higher levels of inflammation, increased cell death, and stem cell depletion (82). For example, early-life stress, a "toxic" exposure linked to shorter TL, has been associated with elevated inflammation in adulthood (83). This heightened inflammation occurs in spite of higher than average levels of baseline cortisol, which should suppress inflammatory activity.

Models using human cell lines have also shown decreased telomerase activity in T-cells as an ancillary function of cortisol (73), an effect which likely holds *in vivo* (77). Therefore, chronic inflammation produces a "double-up" effect on TL erosion wherein cells are stimulated to divide as part of the inflammatory response, but have decreased telomerase activity to maintain TL. Moreover, an important feature of senescent cells, apart from growth arrest, is their increased secretion of inflammatory factors, such as interleukins 6 and 8. This feature is known as the senescence-associated secretory phenotype (SASP) (84). Thus, increased senescence rate resulting from increased telomere erosion in turn increases the level of inflammatory markers, making clear that the inflammation–telomere–erosion relation is reciprocal rather than unidirectional. Over time, chronic low-grade inflammation, termed "inflammaging" (116), can damage tissues and accelerate aging.

The inflammation–telomere–erosion process is also influenced by the telomere position effect (TPE) (85). First observed in yeast, and later in human cells in 2001, TPE describes the reversible silencing of genes as a function of TL (86). While DNA damage responses can be triggered by critically short telomeres, TPE does not rely on very short TL. TPE can occur on multiple chromosomes affecting genes near and farther away from telomeres, and importantly, changes as a function of TL (87). Notably, these effects can influence inflammation as the *ISG15* gene, a contributor to the inflammatory response, is within the subset of genes found to be regulated by TPE (87).

17.4.3 Oxidative stress

While mobilization of energy increases an organism's ability to respond to adversity, reactive oxygen species (ROS) generated as a result of cellular metabolism expose the cell to oxidative stress. The G-rich DNA sequences common in telomeres are especially susceptible to oxidative cleavage as demonstrated by *in vitro* experiments showing increased telomere erosion when cells

were exposed to high concentrations of ROS (88, 89). ROS production is also increased in senescent cells, providing more fuel for cellular damage and telomere erosion (90). Adding to this effect, oxidative damage also deters the renewal ability of stem cells (91).

To prevent this damage and potential cell death signaling cascades, cells generate antioxidants, such as glutathione, which reduce ROS in the cytosol, mitochondria, and nucleus to prevent DNA damage (92, 93). However, these defensive processes may be impaired when they are needed most. Specifically, animal models have demonstrated the detrimental effect of stress-induced glucocorticoids on antioxidants' ability to reduce reactive oxygens, which can then further accumulate through interactions between glucocorticoids and mitochondria as discussed more broadly later in the chapter (94). As such, the balance between antioxidants and oxidative stress is an important measure of stress response efficacy. For example, Epel and colleagues (2004) used the ratio of ROS metabolites to the antioxidant vitamin E in their foundational work investigating TL in caregivers (29).

17.4.4 Mitochondria

Mitochondria are other important mediators to consider as these organelles participate in the stress response, in part by sensing levels of glucocorticoids (95). Glucocorticoids permeate the cell membrane, bind to intracellular receptors, and translocate within mitochondria to stimulate ATP production by oxidative phosphorylation. Although ROS are only produced in 1% to 3% of reactions catalyzed in mitochondria, any increase in activity, such as that associated with aging or the stress response, will increase ROS accumulation (96). While this alone can damage telomeres, recent studies provide empirical evidence for a more complex interaction between mitochondria, telomeres, and aging. For example, mitochondrial DNA exhibits a characteristic 5 kb deletion with increased age, and overall mitochondrial respiratory activity is decreased in aging tissues and in individuals with age-related disorders (97–100). Further research has shown higher mitochondrial DNA copy numbers, as well as shorter telomeres, co-occur in individuals with a history of childhood maltreatment (101).

In vitro studies on the oxidative stress response of human fibroblasts have further shown that TERT, the catalytic subunit of telomerase, is excluded from the nucleus and subsequently co-localizes with mitochondria (102). Similarly, the age-associated decrease in mitochondrial activity can be rescued by enhancing TERT activity *in vitro* (103). Taken together, these results imply that TERT may protect mitochondria during stress to allow production of needed ATP, but as a result can decrease the normative telomere elongation. This is in line with a life-history evolutionary perspective of favoring immediate over long-term survival in response to stress. Notwithstanding, protection of mitochondria is nevertheless important for long-term survival. Damaged mitochondria release markers triggering inflammatory and cell death responses similar to those resulting from nuclear DNA damage (74). As a result of their connection to each other and to stress-associated networks, a new theory has emerged purporting a mitochondrial–telomere axis of aging to describe the interconnectedness of the two structures within the aging process (104).

In sum, stress is a complex process with impacts that radiate throughout the human body. While stress functions as an adaptive response to promote short-term survival over gross longevity, under chronic conditions stress can increase stem cell division and differentiation, shorten TL, and eventually promote aging-related disorders such as cancer, diabetes, and cardiovascular disease. Here, we highlighted mechanistic pathways of several key components, including the neuroendocrine response, inflammation, oxidative stress, and mitochondrial control, and their respective impacts on telomere length and erosion.

17.5 Conclusion and future directions

The emergent field of telomere science has opened exciting new opportunities to study stress-related lifelong aging processes. Notably, stress in its various forms is suggested to influence the rate of telomere erosion throughout life and thus, elucidating the underlying mechanisms is of great interest. A better understanding can inform clinical treatments and intervention efforts to reverse the damaging effects of stress on aging processes. Considerable progress in the past two decades has increased our knowledge on the link between TL and health, as well as factors known to influence telomeres. These include intrinsic mechanisms (e.g. heritability, telomerase, TERRA, and epigenetics, to name a few), and biological embedding mechanisms, most likely through complex interaction between the neuroendocrine system, inflammation, oxidative stress, and mitochondrial regulation, although much remains to be illuminated about the mechanisms highlighted here. To put it in historical context, these intrinsic and stress related regulators of telomere length have been discovered in a time frame shorter than that between Muller's and Blackburn's seminal publications. Informed by the literature described herein, research over the next decade will undoubtedly uncover even more knowledge concerning stress, telomeres, and aging.

Future progress will require advancing methodological measurement techniques currently employed by scientists investigating telomeres. The current predominant technique for TL measurement is the quantitative PCR (qPCR) that provides an average length of the telomeric region across all cells in a given sample (105), but suffers from greater measurement error compared with other techniques (106). Other methods such as the “gold standard” Southern blot analysis can measure the distribution of TL within samples (107). Nevertheless, as TL can vary between the *same* chromosome and between *different* chromosomes of the same cell (43), and as telomere-induced senescence may rely on a few (or even single) critically short telomeres (108), future research will benefit from high-resolution and ideally high-throughput techniques. Further, while empirical evidence suggests TL to be correlated across different cell types of the same individual, new knowledge can emerge by measuring TL within specific types of cells and on specific chromosomes (109, 110). For example, how epigenetic processes influence TL regulation may vary across tissues, which inherently express different genes as a function of cell type. Moreover, the observation of “pseudo-lengthening” of telomeres, likely resulting from higher proportions of newly differentiated stem-cells, highlights the need for increased specificity when measuring TL *in vivo* (111). Furthermore, given the diversity of health outcomes associated with early-life adversity, and telomeres' ability to modulate gene expression in a chromosome-specific manner through TPE, future longitudinal studies may reveal associations between specific types of biopsychosocial stressors and chromosome-specific TL erosion. Finally, advancements in nanotechnology for nucleic acid analysis and DNA sequencing techniques will put us closer to finding factors underpinning Plato's old observation of inequality in stress and aging.

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