

Telomeres and Immunological Diseases of Aging

Nicolas P. Andrews Hiroshi Fujii Jörg J. Goronzy Cornelia M. Weyand

Lowance Center for Human Immunology, Department of Medicine, Emory University School of Medicine, Atlanta, Ga., USA

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Abstract

A defining feature of the eukaryotic genome is the presence of linear chromosomes. This arrangement, however, poses several challenges with regard to chromosomal replication and maintenance. To prevent the loss of coding sequences and to suppress gross chromosomal rearrangements, linear chromosomes are capped by repetitive nucleoprotein structures, called telomeres. Each cell division results in a progressive shortening of telomeres that, below a certain threshold, promotes genome instability, senescence, and apoptosis. Telomeric erosion, maintenance, and repair take center stage in determining cell fate. Cells of the immune system are under enormous proliferative demand, stressing telomeric intactness. Lymphocytes are capable of upregulating telomerase, an enzyme that can elongate telomeric sequences and, thus, prolong cellular lifespan. Therefore, telomere dynamics are critical in preserving immune function and have become a focus for studies of immunosenescence and autoimmunity. In this review, we describe the role of telomeric nucleoproteins in shaping telomere architecture and in suppressing DNA damage responses. We summarize new insights into the regulation of telomerase activity, hereditary

disorders associated with telomere dysfunction, the role of telomere loss in immune aging, and the impact of telomere dysfunction in chronic inflammatory disease.

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Introduction

Over the last decade, the spectrum of immune-mediated diseases has expanded considerably, mostly due to the recognition that syndromes previously viewed as degenerative diseases include a critical component of inflammatory tissue damage [1]. Typical examples are the neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, but also atherosclerosis. In many instances, these inflammatory syndromes affect individuals during the second half of life, with age representing one of the strongest risk factors. Aging also increases the risk to develop classical autoimmunity, as exemplified in rheumatoid arthritis (RA) [2]. Incidence rates for RA are the highest in postmenopausal women, raising the question of how the aging process alters the functioning of the immune system such that tolerance to self antigens is broken and powerful immune effector functions injure the host instead of protecting it from harm.

The immune system is uniquely sensitive to the effects of aging because its cells adaptively respond to immune challenges with massive proliferation and contraction [2]. Immune cells must be able to grow exponentially and die when no longer needed. However, immune protection is intricately linked to the ability to memorize prior antigen encounters, forcing the system to store and maintain information through constant renewal of the existing pool, imposing further proliferative stress. Immune competence in the elderly and immune-mediated diseases of aging must therefore be considerably affected by processes of immune cell regeneration – or lack thereof. More and more information is becoming available on how long immune cells live, how they are turned over, and which factors determine their longevity.

Telomeres have attracted attention because they have emerged as an excellent measure of proliferative history and replicative reserve [3]. While previously considered primarily as protective caps on linear chromosomes, insights into telomere biology are now revealing a much broader role of telomeric structures; regulation of cell fate decisions is now connected to the surveillance and the maintenance of telomeric integrity [4]. Technological advances have made it easier to measure the lengths of telomeric sequences, but much more emphasis has recently been placed on the structural features of the telomere and the protein complex attached to the chromosomal end. Also, the telomere repair enzyme telomerase is finding its place as multifunctional and may direct cellular function far beyond extending telomere length (TL) [5].

In this review, we have summarized current knowledge of telomeric structure, stressing the complexity of the telomeric cap. Telomerase is taking center stage by providing conditions that allow the cell to distinguish telomeric ends from sites of DNA breakage and may play an active role in protecting the telomere from DNA repair activities which, under certain circumstances, are lethal for the cell. Accumulating information about the regulation of the telomerase protein component hTERT has been included. Finally, the connection between preserving DNA integrity, telomere function, and premature aging is delineated with examples of the segmental progeroid syndromes. These genetic disorders are compared with autoimmune diseases in which mechanisms of immune dysfunction are much better understood. Telomeres and their regulation are beginning to provide a conceptual platform to bring together genetic syndromes of nuclear instability, classical autoimmune syndromes, and the expanding spectrum of age-related inflammatory syndromes.

Eukaryotic Chromosomes are Maintained by Telomeres

Somatic cells have a finite lifespan and a limited capacity for proliferation [6]. In a process termed replicative senescence, repetitive *in vitro* stimulation leads to irreversible cell cycle arrest. Cell senescence is thought to limit carcinogenesis by arresting the growth of cells carrying mutations and chromosomal aberrations [3]. Replicative senescence is associated with loss of telomere sequences from the ends of chromosomes. In humans, telomeres consist of 10–20 kb of repetitive DNA hexamers that are lost at a rate of 40–200 bp during each cell cycle [7]. Critically short telomeres become uncapped, recruit components of the DNA damage repair machinery, and trigger replicative senescence or apoptosis [8]. The presence of critically short sentinel telomeres, and not the mean TL, in a cell is thought to initiate this process [9]. To prevent replicative senescence, telomeres are maintained by the reverse transcriptase activity of telomerase. In humans, the catalytic portion of telomerase is encoded by the *hTERT* gene. It associates with an RNA component encoded by *hTERC* that contains a sequence complementary to the G-rich strand of telomeres. In this way, hTERC serves as a template for the addition of 5'-TTAGGG-3' repeats to the G-rich 3'-overhang at the ends of human chromosomes.

Chromosome ends are structurally organized by six telomere-associated proteins (TRF1, TRF2, POT1, TIN2, RAP1, TPP1) that form the shelterin complex [4]. These proteins modulate telomere architecture by binding to the duplex telomere array and the protruding 3' G-rich overhang of telomeres (fig. 1a). *In vitro* studies have demonstrated that telomere ends form a loop, the T loop, that requires the presence of the 3'-overhang [10]. Telomerase has been implicated in 3'-overhang maintenance and, therefore, telomere structure, as attrition of the overhang has been observed in telomerase-negative fibroblasts [11], and telomerase overexpression in human endothelial cells increases overhang length [12]. It has been proposed that the 3'-overhang invades duplex telomeric DNA and base pairs with the C strand to form both the T loop and the D loop, which consists of the displaced G strand (fig. 1b). Shelterin proteins are able to remodel telomere ends *in vitro* and likely have a role in shaping T loops [13]. Tucking in of the 3'-overhang and T-loop formation are thought to prevent recognition of chromosome ends as damaged DNA and limit subsequent activation of cell-death pathways [14]. Loss of the 3'-overhang below a certain threshold may affect T-loop stability and, therefore,

deleteriously expose telomeres to the DNA damage repair machinery. Additionally, by modulating telomere structure, shelterin may control access of telomerase to the 3'-overhang and, thus, regulate telomere repair [14].

DNA damage is recognized by highly conserved proteins that initiate signal transduction cascades to halt cell cycle progression while DNA is repaired. It is now known that telomeric damage is also recognized by these pathways [15]. In the absence of sufficient repair, cells enter senescence or undergo apoptosis. The ataxia telangiectasia-mutated (ATM) kinase recognizes double-strand breaks (DSB), while the ATM and Rad3-related (ATR) kinase recognizes exposed single-stranded DNA. These kinases initiate cell cycle arrest through activation of the cyclin-dependent kinase inhibitor p21 and the tumor suppressor p53. Transfection of dominant negative constructs of shelterin components into human cells leads to telomere loop disruption and either senescence or apoptosis [16]. These studies show that depletion of TRF2 from telomere duplex DNA induces a robust DNA damage response characterized by activation of ATM and accumulation of telomere dysfunction-induced foci containing DNA damage factors such as γ -H2AX and 53BP1. POT1, which binds the telomere 3'-overhang, has been shown to suppress single-stranded DNA responses mediated by ATR [17]. Another protein involved in DNA damage repair, the nonhomologous end-joining protein DNA-dependent protein kinase, has recently been shown to have an essential role in uncapping. Deficiency in this enzyme results in telomere uncapping, recognition of the telomere as a DSB, and telomere end-to-end fusion [18]. Since telomerase has a critical role in maintaining telomere structure [11], it helps to suppress these DNA damage repair responses at telomeres [19].

Regulation of Telomerase Activity

In the process of carcinogenesis, cells escape senescence through the mutation of tumor suppressor genes and survive crisis through telomerase upregulation. Thus, approximately 90% of tumor cells are positive for telomerase activity [20]. A desire to establish a detailed mechanism of carcinogenesis has driven extensive research on the regulation of telomerase expression. Much of this research has focused on transcriptional regulation of *hTERT*, as telomerase activity is primarily limited by expression of the catalytic subunit hTERT, not the RNA component hTR encoded by *hTERC* [21]. The hTERT promoter contains several putative binding sites for tran-

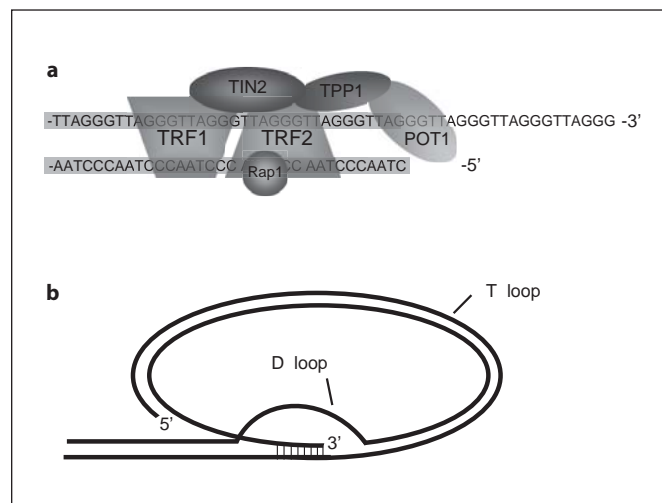


Fig. 1. Organization of telomere ends by the shelterin complex. **a** Schematic of the shelterin complex. The duplex-specific shelterin proteins TRF1 and TRF2 and the single-strand-binding protein POT1 directly recognize telomere sequences via oligonucleotide- or oligosaccharide-binding domains. TPP1 is essential for the recruitment of POT1 to telomeres. TIN2 forms a bridge between these components and is crucial for shelterin stability. Mammalian Rap1, whose function is still unclear, requires TRF2 for telomere localization as it lacks a DNA binding domain. Both TRF2 and TRF1 exhibit in vitro DNA remodeling activity and are thought to promote the formation of telomeric T and D loops in vivo (as in **b**). TRF2 and POT1 have a critical role in suppressing the DNA damage proteins ATM and ATR, respectively. POT1 binds the entire length of the 3'-overhang (not shown here) [4]. **b** Telomeric DNA is organized into T and D loops. The topological arrangement of telomeric ends into T and D loops by shelterin components is thought to prevent the initiation of DNA damage responses at telomeres and to regulate telomere elongation via inhibition of telomerase access to the 3'-overhang.

scription factors (fig. 2) [22]. Activators of *hTERT* transcription include c-Myc, SP1, USF1/2, Ets, HIF-1, and hALP [23–28]. Other transcription factors and several tumor suppressor/oncogene pathways are involved in *hTERT* transcriptional repression, including p53, AP-1, Wilms' tumor 1, Mad1, MZF-2, CTCF, and Smad-3 [29–35]. Transcriptional regulation of *hTERT* is complex with some transcription factors such as HIF-1 α and HIF-2 α having opposing effects in various cell lines [28, 36, 37]. For example, recruitment of histone acetyltransferases to the *hTERT* promoter by HIF-2 α differs between tumor cell lines and may account for cell-type specific telomerase induction or repression by this transcription factor [36]. Telomerase activity is also under hormonal control.

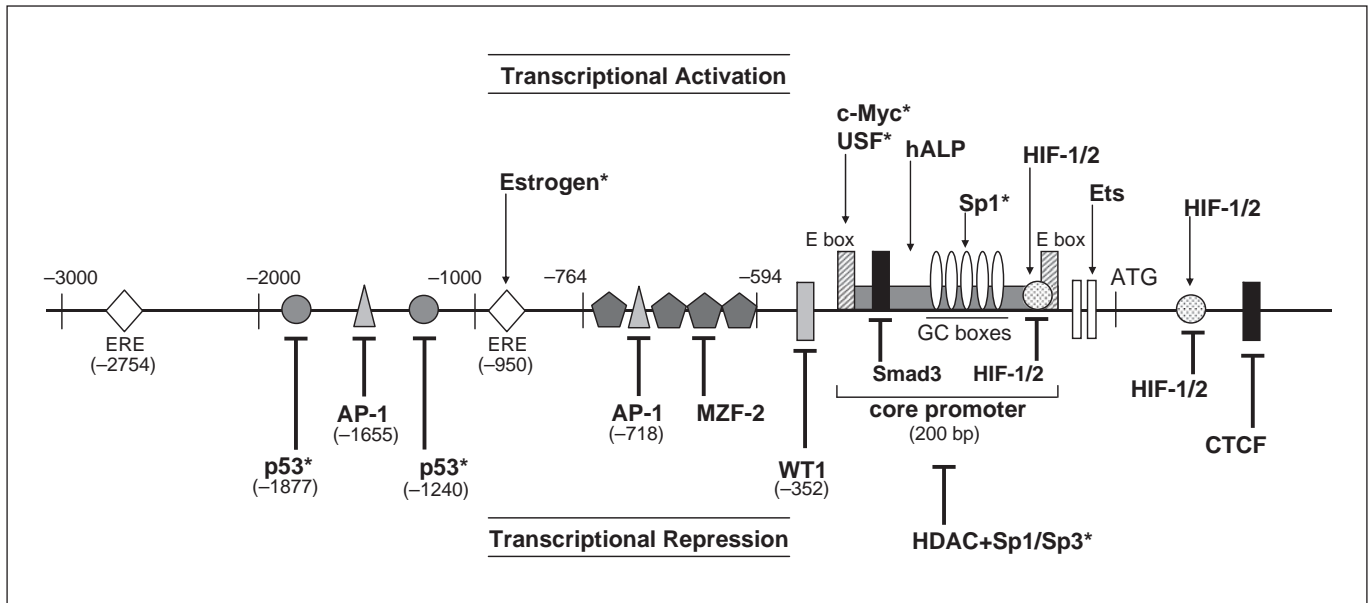


Fig. 2. Schematic of the *hTERT* promoter. The *hTERT* gene is located on the short arm of chromosome 5 at 5p15.33. The binding sites of transcriptional activators and repressors are indicated [148]. The starred transcription factors are known to affect *hTERT* transcription in lymphocytes.

An imperfect palindromic estrogen-responsive element in the *hTERT* promoter binds the estrogen receptor to induce *hTERT* transcription [21]. The ability of estrogen to activate *c-Myc* likely confers an indirect effect on telomerase activity [21]. The hormone cortisol inhibits telomerase activity in CD4 and CD8 T cells, suggesting a mechanism by which stress can negatively affect immune responses [38]. Telomerase is also subject to post-transcriptional regulation. Phosphorylation of *hTERT* by AKT kinase enhances *in vitro* telomerase activity [39]. Further, phosphorylation of *hTERT* protein is required for NF- κ B-mediated translocation of the enzyme from the cytoplasm to the nucleus where it can access chromosome ends [40].

An additional layer of transcriptional regulation is imposed by the methylation status of the CpG island located within the *hTERT* promoter [22]. For most genes, promoter methylation is associated with transcriptional repression [41]. However, several groups have shown that treatment of human cells with the demethylating agent 5'-aza-2'-deoxycytidine results in decreased *hTERT* transcription [42]. A recent study has shown a dual role for methylation in controlling *hTERT* promoter activity. Targeted methylation shows that demethylation of 12 CpG in the core promoter of *hTERT* activates transcription while an absence of methyl groups at exon 1 results in

binding of the 11-zinc finger protein CTCF and dampening of telomerase activity [43]. Analysis of telomerase-positive breast, bladder, and cervical cancers revealed extensive methylation of the CTCF binding site, suggesting that the methylation status of this site is a critical determinant of carcinogenesis. An intriguing implication of these findings is that CTCF-mediated repression of telomerase may have a role in defective immune responses in the elderly, as aging is associated with global hypomethylation [44].

Epigenetic modification of histone proteins with methyl and acetyl groups determines if chromatin is in an open or closed configuration. Chromatin structure thus determines accessibility of promoters to the transcriptional machinery and greatly shapes gene expression. Chromatin remodeling has emerged as a significant mechanism for *hTERT* regulation during cell differentiation. Inhibition of histone deacetylases with the drug trichostatin A relaxes tightly wound heterochromatin and results in *hTERT* transcription in normal somatic cells that developmentally downregulate telomerase activity [45]. Histone modification is linked to differential binding of transcription factors at the *hTERT* promoter [46]. Histone acetylation is associated with *c-Myc* binding and *hTERT* transcription. In contrast, Mad1 binding to the promoter is associated with histone deacetylation

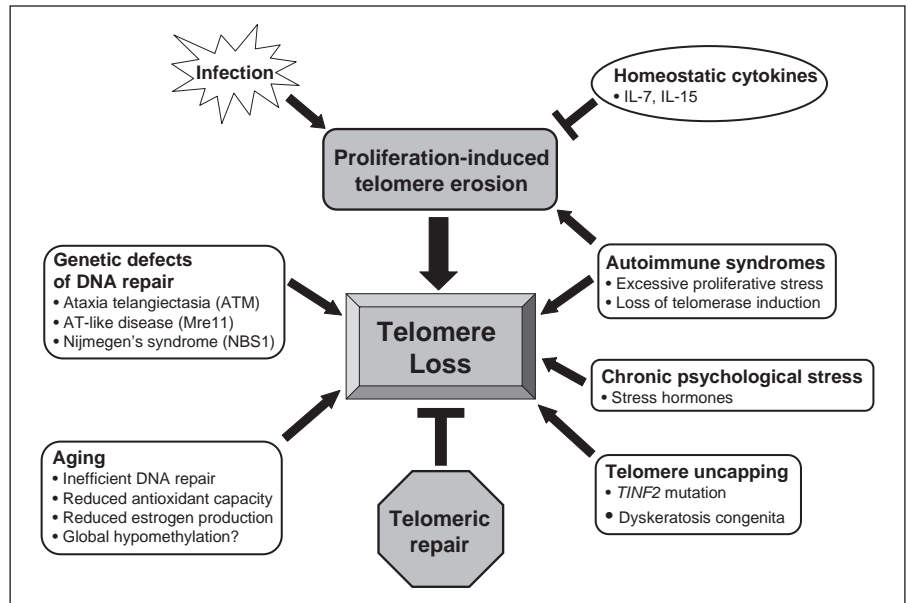


Fig. 3. The multiple pathways affecting telomeric length. Telomere homeostasis is determined by the balance between telomeric repair and factors that promote erosion of telomeric sequences.

and reduced *hTERT* transcripts. The transcription factors SP1/SP3 are thought to directly recruit HDACs to the *hTERT* promoter to effect silencing of telomerase activity in human T cells and fibroblasts [47].

Thus, a confluence of events are necessary for *hTERT* expression and telomerase activity, which include: an open chromatin configuration, demethylation of the *hTERT* core promoter, methylation of the first exon to inhibit CTCF binding, overcoming of negative regulatory factors such as tumor suppressors and inhibitory cytokines/hormones, and NF- κ B-mediated nuclear translocation of phosphorylated *hTERT* protein in complex with *hTERC*-encoded RNA. It is evident that there are many points at which perturbations can lead to loss of telomerase activity.

Genetic Diseases with Premature Telomere Loss

A number of genetic diseases of premature aging are associated with deficient telomere maintenance (fig. 3). Dyskeratosis congenita (DC) is characterized by mucocutaneous features including nail dystrophy and abnormal skin pigmentation, susceptibility to malignancies, bone marrow failure, and other somatic abnormalities [48]. DC is primarily inherited in an X-linked manner, although both autosomal dominant [49] and recessive [50] forms have been described. The X-linked form of DC is caused by mutations in the *DKC1* gene encoding dys-

kerin [51], a protein involved in the processing of ribosomal RNA from precursor transcripts [52]. It is thought that dyskerin, a component of the telomerase complex, processes TERC precursor RNA since telomere erosion in X-linked DC is a direct result of a failure to accumulate mature TERC [53]. Haploinsufficiency for *hTERT* or *hTERC* underlies several diseases that impact highly proliferative cells such as immune and skin cells. Autosomal dominant DC due to *hTERC* mutation [49] is characterized by disease anticipation in carrier families linked to an increased loss of telomere sequences in succeeding generations [54]. Defects in the catalytic subunit of telomerase result in a more diverse set of diseases than mutations in *hTERC* and often display incomplete penetrance [55]. Heterogenous mutations in *hTERT* underlie a small proportion of idiopathic aplastic anemia cases as well as autosomal dominant DC. These diseases are associated with dramatically reduced telomerase activity and poor telomere maintenance [56]. Mutation of *TINF2*, which encodes for the shelterin component TIN2, was recently observed in DC patients [57], suggesting that telomere erosion in these patients is caused by telomere uncapping.

Normal telomere maintenance requires factors that participate in intrachromosomal DNA damage signaling, recombination, and checkpoint regulation [15]. Therefore, deficiencies in the DNA damage repair machinery are also associated with premature telomere loss in a number of diseases. The lethality of DSB is evident

in those individuals who suffer from ATM deficiency [58]. The AT syndrome manifests as chromosome and telomere instability, premature aging, ataxia, severe neurodegeneration, immunodeficiency, and susceptibility to cancer. ATM activation requires recruitment of the MRE11-Rad50-NBS1 (MRN) complex to DNA DSB [59]. The MRN complex directly binds and processes DSB, creating exposed single-stranded DNA. This is an essential step in ATM recruitment to damaged DNA and dissociation of inactive ATM dimers to the active, monomeric form [60]. The importance of MRN in the surveillance of genomic integrity is made evident as hypomorphic mutations of *Nbs1* and *Mre11* genes exhibit many similarities to AT, resulting in Nijmegen breakage syndrome and AT-like disorder, respectively [61, 62]. Immunodeficiency in these syndromes may be explained not only by increased apoptotic death of replicating lymphocytes, but also by the recent finding that the MRN complex participates in the repair of RAG-mediated DSB in lymphocytes during V(D)J recombination [63]. Other examples of genetic diseases associated with premature telomere loss are the Bloom syndrome and Werner syndrome, which are induced by deficiencies in DNA cross-link repair. The group of nuclear instability syndromes includes Fanconi anemia, caused by mutations in one of several Fanconi complementation group proteins that assemble in a common nuclear protein complex in response to genotoxic stress [64]. Lymphocytes are particularly affected in these syndromes, underscoring the importance of telomerase expression and telomere structure in immune cells.

Telomerase Expression and Regulation in Immune Cells

Telomerase is highly expressed in germ line cells and hematopoietic progenitor cells, weakly expressed in somatic cells, and contributes to the immortalization of most tumor cells [3]. Lymphocytes have a unique requirement for clonal expansion that necessitates robust proliferative capacity. To circumvent growth arrest due to replication-associated telomere loss, immune cells maintain their chromosome ends via telomerase induction during entry into the cell cycle. Early studies described very low to absent levels of hTERT protein and telomerase activity in resting human lymphocytes from peripheral blood that were enhanced upon T-cell receptor (TCR) or B-cell receptor cross-linking [65, 66]. A more detailed analysis of lymphocytes from the thymus, tonsils, and blood

showed similar *hTERT* mRNA and protein levels, but tissue-specific enzymatic activities [67]. This disconnect may be explained by a requirement for phosphorylation and nuclear translocation of hTERT for telomerase activity [40].

Telomerase induction in lymphocytes primarily involves NF- κ B activation through upstream PKC θ and phosphoinositide 3-kinase/AKT signaling pathways [68, 69]. AKT has the most crucial role in telomerase activity as it directly activates hTERT via phosphorylation [39]. This process is opposed by dephosphorylation and inactivation of hTERT by protein phosphatase 2A [70]. The effect of PKC θ on telomerase induction appears to be primarily through NF- κ B activation [68]. Further, NF- κ B mediates nuclear transport of hTERT and activates *hTERT* transcription indirectly by promoting SP1 and c-Myc binding to the *hTERT* promoter (fig. 2) [68].

Additionally, cytokines either promote or repress telomerase activity. For example, transforming growth factor beta (TGF- β) transcriptionally activates Smad3, which directly suppresses telomerase activity through interactions with the *hTERT* promoter and c-Myc [35]. Interferon alpha (IFN- α) production in skin may dampen local immune responses by inhibiting telomerase induction in responding memory CD4 T cells [71]. Tumor necrosis factor alpha (TNF- α) facilitates telomerase induction by promoting NF- κ B phosphorylation, which in turn transports hTERT from the cytoplasm to the nucleus where it can access chromosome ends [40]. The cytokines IL-7 and IL-15 maintain naïve and memory T-cell pools by providing survival signals and driving homeostatic proliferation [72]. In vitro stimulation with IL-7 or IL-15 induces proliferation and telomerase activity in CD4 and CD8 T cells that counters telomere loss in mature, but not cord blood, T cells (fig. 3) [73, 74]. These results suggest that the aging of T-cell populations, in terms of TL, is determined by the pattern of cytokines induced by a particular pathogen and is also a result of normal homeostatic maintenance.

Telomere Loss and Aging in Immune Cells

Initial Telomere Length Is Genetically Determined

A study of dizygotic and monozygotic twins suggests that initial TL in human peripheral blood lymphocytes (PBL) at birth is genetically determined [75]. There is additional evidence that TL is paternally inherited, although the effect of inherited TL on an individual's lifespan is not clear [76]. During the first few years of life, 270–1,000

telomeric repeats are lost in PBL each year [77]. During adulthood, the rate of yearly telomere loss in PBL slows to an average of 20–60 bp [78]. At the observed rates of age-associated telomere loss, mean TL in PBL would be reduced from an initial 10 kbp to 3–5 kbp after 80 years of life [79]. However, even mild loss of telomere sequences may significantly impair chromosome protection as it is the shortest telomeres in a cell that trigger damage pathways which lead to senescence [9].

An important question that arises from these observations is what drives age-associated telomere loss. The set point of TL appears to be genetically determined [75], and telomere attrition may be similarly influenced. A recent longitudinal study of leukocyte telomere loss has shown that age-dependent telomere attrition is proportional to baseline TL [80]. The greater rate of telomere loss in African Americans compared to Caucasians, which was also described in this study, was partially explained by the longer initial TL observed in the former group. The underlying mechanism for this proportional telomere loss may involve preferential oxidative damage of long telomeres [81]. Additionally, genetic variation between populations in regulatory elements that influence *hTERT* promoter activity may influence telomere attrition independently of baseline TL. An absence of age-associated telomere loss was observed in a Japanese population with a single nucleotide polymorphism in the *hTERT* promoter [82]. This polymorphism was associated with greater *hTERT* transcription and telomerase activity in PBL. These correlations were not observed in a subsequent study of a Swedish population bearing this single nucleotide polymorphism [83]. These opposing results emphasize the complexity of telomere dynamics and suggest that additional factors that are obscure at this time influence telomere loss associated with aging. For example, differences in age-related global hypomethylation in immune cells [84] may affect expression of factors that directly or indirectly regulate *hTERT* or *hTERC* expression.

Telomere Loss due to External Factors

External factors, including chronic stress, influence telomere loss in human PBL. Chronic psychological stress in caregivers of Alzheimer's patients or chronically ill children is associated with accelerated telomere loss, reduced T-cell proliferation, higher oxidative stress, and decreased telomerase activity in PBL [85]. Increased cortisol levels may explain this association as this stress hormone downregulates *hTERT* transcription and telomerase activity in CD4 and CD8 T cells [38].

An additional non-genetic influence on telomere maintenance is the continual exposure of cells to free radicals. The oxidative stress hypothesis of aging proposes that cumulative free-radical damage is the central cause of aging at the cellular and tissue levels [86]. Telomeric DNA is highly susceptible to oxidative damage because (1) single-stranded breaks induced by oxidative damage are less efficiently repaired in telomeres than in non-telomeric regions [81] and (2) guanine triplets preferentially accumulate 8-oxoG lesions which are targets of the base excision repair pathway [87]. There is substantial evidence that accumulated oxidative stress in fibroblast cell lines results in telomere attrition and cellular senescence [88]. Correlative studies show that telomere loss in PBL is associated with smoking, obesity, cardiovascular disease, and other conditions known to increase oxidative stress [89, 90]. In a cohort of 79-year-old individuals, oxidative gene polymorphisms were associated with variation in TL in PBL and two physical biomarkers of aging: respiratory function and grip strength [91]. These data suggest that oxidative stress genes participate in pathways involved in both telomere shortening and physical aging. Oxidative damage of telomeres may be further exacerbated by inefficiencies in DNA repair that are associated with advanced age [92] and age-related reduction in antioxidant activity [93]. For example, estrogen exhibits antioxidant activity by upregulating mitochondrial manganese superoxide dismutase and glutathione peroxidase which scavenge free radicals [94]. Estrogen replacement therapy in postmenopausal women is associated with longer TL than in untreated individuals [95]. In addition to the positive effect of estrogen on *hTERT* transcription [21], its antioxidant activity may partially account for the observation that in some populations women have longer telomeres than men [96], even though no gender differences in TL are seen in newborns [97]. Thus, there is mounting evidence that telomere loss during an individual's lifespan is much like the susceptibility to cancer. An individual's rate of telomere loss may be genetically set at birth, but may be further modified through lifestyle choices that increase the onslaught of environmental stressors that tax telomere repair mechanisms. However, there is no clear evidence that slowing the rate of age-associated telomere loss will extend an individual's lifespan.

Proliferation-Induced Telomere Loss in Lymphocytes

Lymphocytes are particularly sensitive to the effects of replication-associated telomere loss because they undergo massive proliferation upon encountering cognate an-

tigen. Antigen-experienced memory T cells have shorter telomeres than naïve T cells, suggesting that proliferative stress for these cells is likely a major cause of telomere attrition [78]. Studies of X-linked lymphoproliferative syndrome, a genetic disease resulting in excessive T-cell stimulation [98], provide support for this idea. Young patients exhibit short telomeres similar to normal elderly individuals, indicating that proliferation, not aging per se, drives telomere repeat loss [99]. Excessive T-cell proliferation during chronic viral infection is also associated with telomere loss in PBL [100]. Thus, factors that promote repeated T-cell stimulation, such as persistent antigen and chronic inflammation, appear to drive telomere loss and replicative senescence (fig. 3).

Rates of telomere loss differ between lymphocyte subsets, with age-associated telomere loss occurring at a slower rate in B cells than in T cells [101]. Additionally, there is no difference in TL between naïve and memory B cell populations, and the naïve-to-memory B-cell transition is not associated with telomere loss [102]. This may be due to the unique microenvironment of the germinal center where naïve B-cell differentiation takes place [103]. Telomerase induction is highest in germinal center B cells and may produce a net increase in TL at this stage of differentiation [103]. In contrast, naïve-to-memory CD4 and CD8 T-cell differentiation is associated with TL attrition [78]. This disparity is likely due to differing abilities of memory T and B cells to induce telomerase activity which may involve both transcriptional control of *hTERT* and post-transcriptional modification of the enzyme.

A question that remains unanswered is whether telomere attrition in lymphocytes contributes to adaptive immunity dysfunction in the elderly. There is evidence for a decline of immune memory in the elderly [104]. Akbar et al. [19] have proposed telomere loss as a mechanism that negatively affects T-cell memory. Patients with the premature aging syndrome, DC, inefficiently maintain their telomeres due to genetic defects in telomerase components [48]. DC patients typically succumb to infection before the second decade of life [105], which provides indirect evidence that telomere erosion negatively affects the maintenance of a functional memory T-cell pool in elderly individuals.

T-Lymphocyte Senescence Is Associated with Low Telomerase Activity

Loss of the co-stimulatory molecule CD28 on T cells is considered the best marker of T-lymphocyte aging [106]. CD28 loss can be driven by antigen stimulation

[107], and in vivo accumulation of CD28⁻ T cells likely reflects an individual's continual exposure to previously encountered pathogens and clonal outgrowth of memory T cells specific for persistent antigens. CD28⁻ T cells have features of replicative senescence including oligoclonality and substantially shorter telomeres than CD28⁺ T cells [107]. Elevated levels of senescent CD28⁻ T cells are observed in several inflammatory conditions including atherosclerotic coronary artery disease and autoimmune syndromes including RA and Wegener's granulomatosis [108–110]. CD28⁻ T cells have been proposed to represent a prematurely senescent subset that contribute to inflammatory disease [111], possibly through perforin/granzyme cytotoxicity [112], and IFN- γ secretion [108]. Factors that disturb telomere maintenance, and thus contribute to premature senescence, are likely risk factors for a diverse spectrum of age-related inflammatory diseases.

As T cells are repetitively stimulated, they become refractory to telomerase induction, lose telomere sequences, and enter replicative senescence [113]. Thus, telomerase loss mirrors CD28 loss in settings of chronic T-cell stimulation. The mechanisms responsible for reduced telomerase induction are not completely known. Loss of co-stimulatory molecules that recruit AKT to the T-cell synapse, such as CD28, may explain the progressive loss of telomerase induction in chronically stimulated T cells [113]. There is evidence for reduced AKT phosphorylation in end-differentiated CD8⁺CD28⁻CD27⁻ T cells, which may negatively affect hTERT phosphorylation and nuclear translocation [114]. Transcriptional downregulation of hTERT cannot be discounted as a mechanism since transduction of T cells with hTERT stabilizes TL and substantially extends replicative potential, underscoring the importance of telomerase induction in delaying replicative senescence [115].

Telomere Loss in Autoimmunity

Aberrant TL has been described in a number of autoimmune syndromes (table 1) such as systemic lupus erythematosus [116], systemic sclerosis [117], RA [118], insulin-dependent diabetes mellitus [119], Wegener's granulomatosis [120], atopic dermatitis, and psoriasis [121]. In autoimmune syndromes, increasing evidence is making the general model that autoreactive T cells undergo excessive proliferation, leading to telomeric erosion and loss, less satisfactory. For example, both scleroderma patients and their healthy family members exhibit telomere

Table 1. Telomeric length in chronic inflammatory conditions or factors contributing to chronic inflammatory conditions

Disease/risk factor for oxidative stress	Studied cells	References
Adult-onset Still's disease	PBMC	137*
Atopic dermatitis and psoriasis	PBMC, naïve and memory CD4 ⁺ , CD8 ⁺ , CD28 ^{-/+}	138
Idiopathic pulmonary fibrosis	PBMC	139
Insulin-dependent diabetes mellitus	PBMC	119
Juvenile idiopathic arthritis	CD4 ⁺ CD45RA ⁺ T cells	140
Langerhans cell histiocytosis	Langerhans cells, lymphocytes	141, 142
Limited systemic sclerosis	PBMC	143*
Rheumatoid arthritis	PBMC, CD4 ⁺ and CD8 ⁺ T cells, CD34 ⁺ hematopoietic progenitor cells	118, 144*, 127, 145
Sarcoidosis	PBMC	146
Systemic lupus erythematosus	PBMC, T and B lymphocytes	116, 144*, 147*
Systemic sclerosis	PBMC, lymphocytes	144
Wegener's granulomatosis	PBMC	120
Smoking	PBMC	89
Obesity	PBMC	89
Cardiovascular disease	PBMC	90

The listed inflammatory conditions are associated with reduced telomerase activity and/or telomere erosion. Those references marked with a star (*) describe increased telomerase activity/telomere length.

shortening in PBL [117]. Healthy individuals of the HLA-DR4 haplotype, a major risk factor for RA, have a significant loss of telomere sequences in hematopoietic progenitor cells within the first 20 years of life [118]. Additionally, telomere loss in RA does not correlate with the inflammatory status of the patient, but it is disproportionately associated with the HLA-DR4 haplotype [122]. Thus, it appears that there is a genetic component promoting telomere loss which functions as an underlying risk factor for the development of certain autoimmune diseases.

Chronically stimulated effector/memory CD4 T cells are the primary mediators of synovial inflammation in RA [123]. The memory CD4 T-cell pool in RA patients is characterized by enhanced turnover, outgrowth of clonal populations [124], accumulation of autoreactive, end-differentiated CD28⁻ cells [125], and contraction of the TCR repertoire [126]. These observations suggest a mechanism in which peripheral tolerance breakdown leads to antigen-driven expansion of self-reactive CD4 T cells. However, it is now generally recognized that there is no single antigen that drives T-cell activation and expansion in RA. Aberrations in the naïve CD4 T-cell compartment in RA suggest an underlying non-antigen-associated defect in T-cell homeostasis. In support of this concept, naïve CD4⁺CD45RA⁺ T cells in RA have diminished T-cell repertoire diversity and are prematurely aged as defined by an accelerated loss of telomeric sequences [118]. Recent

work has delineated a molecular mechanism that promotes telomere dysfunction in RA [127]. Naïve CD4 T cells from RA patients are deficient in hTERT induction and telomerase activity upon TCR cross-linking. Stimulated RA naïve CD4 T cells are highly prone to apoptosis, but are rescued by hTERT overexpression. Increased death of stimulated RA T cells due to telomerase insufficiency may place a greater burden on naïve T cells to repopulate the T-cell pool through homeostatic proliferation. This mechanism may be particularly important in maintaining naïve T cell numbers as RA patients have reduced output of TREC⁺ recent thymic emigrants [128]. Further, increased apoptotic sensitivity during priming constrains clonal burst size [127] and may enforce a greater reliance on homeostatic proliferation to maintain the RA memory T-cell pool. Such a disturbance of normal homeostatic mechanisms may provide the impetus for the emergence of senescent, telomere-eroded, CD28⁻ cells that comprise a large fraction of the RA memory T cells [109] and acquire features important in RA pathogenesis [112]. Clonal diversity has emerged as an important mechanism in limiting inappropriate expansion of T cells through interclonal competition for space and growth factors [2]. Inefficient telomere maintenance may promote contraction of the TCR repertoire in RA, leading to a loss of peripheral tolerance.

Elderly individuals often exhibit chronic inflammation characterized by immune system dysregulation with

increased inflammatory cytokine production [129] and redox imbalance due to reduced antioxidant defenses and overproduction of reactive oxygen species [130]. It is now recognized that chronic inflammation is the major risk factor for several age-associated diseases including chronic obstructive pulmonary disease, neurodegeneration, obesity, and vascular disease [1]. Premature telomere erosion in PBL is a common characteristic of these diseases as well as autoimmune syndromes [89, 131]. These findings suggest that telomere loss increases susceptibility to autoimmune disease and may be a predisposing factor for age-related inflammatory disease.

Telomerase overexpression in T cells may serve as a promising therapy for the treatment of autoimmune disease and genetic deficiencies in telomerase components [103]. Several studies have shown that hTERT overexpression in T cells extends their replicative lifespan while maintaining normal cell function [115]. However, two studies have demonstrated chromosomal abnormalities in hTERT-transduced T cells [132, 133]. Further, long-term culture of hTERT-transduced T cells results in accumulation of the cyclin-dependent kinase inhibitors p21 and p16^{Ink4a}, molecules associated with replicative senescence [134]. Thus, further studies are needed before therapeutic telomerase transduction can reach its full potential. An alternative approach is the use of small molecule telomerase activators such as TAT2, which was shown to enhance the immune function of HIV-1-specific CD8 T cells [135]. Given the complications inherent to gene transduction, non-genetic upregulation of telomerase activity may provide an attractive alternative for the treatment of telomere-associated immune deficiencies.

Concluding Remarks

TL is likely paternally inherited and, interestingly, shows a positive correlation with paternal age at the time of the offspring's birth [136]. However, many other factors modulate telomere dynamics, and it is likely that additional polymorphisms that influence telomere maintenance will continue to be described. In the field of telomere biology, a cell's proliferative capacity and replicative reserve were primarily seen as a function of its total TL. However, increasing emphasis has been placed on telomere structure. An important question is how telomere structure affects cell fate decisions, particularly in immune cells which must dramatically upregulate telomerase activity to support their proliferation in response to immune challenge. One aspect of telomere structure that

has received increasing attention is the 3'-overhang. Telomere overhang length dynamics in T cells have not been described and relatively little is known about telomere structure in the context of T-cell responses and autoimmunity. Loss of the 3'-overhang due to insufficient telomerase induction may affect T-loop stability and expose telomeres to DNA damage machinery, triggering apoptosis. Increased telomere damage in RA CD4 T cells due to 3'-overhang exposure may provide an explanation for their increased susceptibility to apoptosis [127]. There is an increasing appreciation of the role of immunosenescence and, by extension, telomere biology in autoimmune diseases. Chemical inhibitors of telomerase to treat cancer have been intensively studied. It is likely that treatments to improve telomere maintenance in autoimmune syndromes will follow the same pharmacological path due to constraints of gene transfer methods. This approach may not prove to be entirely satisfactory as telomerase is now known to have unexpected activities independent of telomere maintenance, including enhancement of cell growth, DNA repair, and protection from apoptosis [5]. Effective pharmacological treatment of telomerase deficiency may necessitate targeting of these extratelomeric functions of telomerase.

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