How Long Should Telomeres Be?

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What began as a study of the "end-replication problem" took on a new dimension as it became clear that telomeres are a "molecular clock" of replication in human somatic cells. Here we review the biology of telomeres in vitro and in vivo, in mice and humans. We suggest that, in humans, telomeres are involved in the biology of aging and pathobiology of disorders of aging, including cancer and cardiovascular disease. We also propose that the underlying dynamics of telomere biology is in line with broad principles of evolutionary theories.

Introduction

Humans are now living well beyond their reproductive years. The emergence of a host of diseases of aging is, unfortunately, a likely consequence of this extended longevity. Cancer, cardiovascular, skeletal, and neurodegenerative disorders, for example, increase in frequency and severity with age and are the main causes of illness and death in developed nations. The question therefore arises as to whether age-related diseases are intrinsic and inevitable to the aging process or are preventable biological entities that have become increasingly more frequent only because modern humans are living longer than ever before. If age-related diseases are preventable, can they be addressed in the same manner as early-onset diseases? A related question concerns the roles of genetic determinants versus environmental and stochastic factors in these age-related diseases and their impact on human longevity. Ultimately, answers to these questions will emerge as we better understand the role played by biological age in pacing human morbidity and longevity.

While the units of chronological age are based on the calender and are extrinsic and constant, the units of biological age are intrinsic and variable and are subject to genetic-environmental interactions. Moreover, the phases of biological age are probably epigenetic in that they are expressed not in days or years but in a sequential order of changes that mark organismal growth, development, and senescence. An excellent series of review articles [1,2••,3••,4,5] and a commentary [6] appearing in a recent issue of *Nature* has comprehensively addressed the biology of aging from many perspectives. Here we briefly touch on some of the issues discussed in these papers, while focusing in greater depth on one of the biomarkers and contributing factors in human aging—the telomeres.

Evolutionary Perspective of Aging

If genes figure in human longevity or in the development of diseases of aging, the expression of longevity genes must be regarded as the outcome of evolution by natural selection—a process occurring at various levels of biological hierarchy and driven by three fundamental factors: variation, heritability, and survival advantage. Natural selection exerts its primal force through successive cycles of reproduction that sustain variation in polymorphisms within genes and their allelic combinations. However, it does not favor longevity much beyond the reproductive period in multicellular organisms characterized by a distinct germline and a soma [7,8]. Thus, both extrinsic factors (*eg*, predation, starvation, climate changes) and intrinsic biological processes converge on an ultimate common outcome: death of the soma. In different species, this can range from rapid, largely programmed demise after a reproductive burst, through slowly increasing morbidity reflecting an underlying gradual loss of homeostasis, to even negligible senescence, in which death of the soma is completely random or extrinsically determined [9,10••]. Humans and other mammals belong mainly to the category in which both genes and chance events in the environment influence morbidity and longevity in the postreproductive lifespan [10••].

A model explaining how variant genes influence longevity was offered by Williams [8], who proposed the theory of "antagonistic pleiotropy." According to this model, the expression of certain genes provides survival and reproductive advantages in early life but becomes a disadvantage late in the postreproductive period. Variation in longevity and aging within species may thus be attributed to the nature and timing of expression of such pleiotropic genetic factors. Kirkwood *et al.* [11–13] have broadened this concept by articulating the "disposable soma theory," the essence of which is that investment in the soma during the reproductive period occurs at the expense of longevity. In other words, the balance between metabolic energy needed

to maintain and repair the soma and the energy devoted to reproduction accounts for different lifespans among species and, perhaps, variations in longevity within members of the same species. This distinction between the soma and nonsoma (*ie*, the germlines) is critical to the subject of this communication, that is, the potential role of telomeres in the biology of human aging.

Telomeres, Telomerase, Cell Mortality, and Immortality

Telomeres are essential genetic elements that cap linear chromosome ends, protecting them from DNA damage repair pathways that operate to heal broken chromosomes [14]. They are typically composed of repetitive DNA sequences and specialized telomeric DNA binding proteins that assemble into a unique structure that masks the ends of the chromosome, thus distinguishing chromosomal ends from ends that are created by internal chromosome breaks. Such internal breaks occur frequently from DNA processing during replication, transcription, and repair, or result from physical or chemical insults, such as oxidative damage and ionizing radiation.

Because of the "end-replication problem" [15–17], telomeres cannot be fully synthesized by the DNA replication fork complex. To prevent gradual telomere loss with each cell division, most eukaryotes, including humans, have a special DNA polymerase called telomerase [18,19] that compensates for the end-replication problem (Fig. 1). Telomerase is a ribonucleoprotein complex consisting at its core of a catalytic protein component and a functional RNA. The protein component is a unique reverse transcriptase that synthesizes de novo the single-stranded telomeric DNA sequence onto the 3'-end of the chromosome, using a portion of the integral RNA component as the template. In humans, these components are called hTERT (human telomerase reverse transcriptase) [20] and hTR (human telomerase RNA) [21].

Telomerase is usually repressed in normal human somatic cells. As a result, telomeres are gradually lost with cell division, both in culture and in vivo, and telomere loss is accelerated in certain diseases involving chronic stress and an increased cell turnover rate [22–31]. In contrast, telomerase is active and telomeric length is maintained in the germline lineage and in cancer cells (*ie*, in immortal cells) [32–35]. As one might expect, human somatic stem cells in the blood, liver, and skin, which presumably have a considerable but still finite replicative capacity, have a low or transiently inducible expression of telomerase [27,33,36–41]. Therefore, telomere loss in the presence of low or nondetectable telomerase may account for the finite replicative capacity of normal human cells, while constitutive telomerase activation in reproductive cells and cancer may account for the immortality of the germline and tumor cells [42].

Proof that telomerase is necessary for cell immortality in tumor cells and is sufficient for immortalization in normal cells came soon after the essential components of telomerase were cloned [21,43••,44–47]. Repression of *hTERT* gene expression is the reason most normal human cells are telomerase negative, and *hTERT* gene transduction, driven by a constitutive promoter element, is sufficient for immortalization of multiple different primary human cell types. In contrast, repression, inactivation, or inhibition of function of either hTERT or hTR in human tumor cells can "re-mortalize" these cells. Telomeres are thus a "molecular clock" in that their attrition causes replicative aging in human cells in culture.

Telomere Dynamics, Aging, and Disease in the Mouse and Their Implications to Human Disease

The correlative links between abnormal telomerase activation, telomere maintenance, and cancer progression in vivo are as compelling as the correlative link between telomere loss and aging of human cells in vitro. The relationships between telomerase and cancer are further supported by the demonstration in cultured cells of the causality between telomerase activity and cell immortality. In principle, telomeric attrition in normal cells and telomerase activation in malignant cells could be targeted for therapeutic interventions that activate telomerase in degenerative diseases and inhibit telomerase in cancer. Such interventions would also definitively establish the role played by telomere dynamics in human disease. This type of applied research, examining the safety and effectiveness of telomerase therapies in preparation for human clinical trials, is ongoing. In the meantime, the telomerase knockout mouse model has provided added insight into the role of telomere attrition in diseases of proliferative tissues, including cancer.

Telomere biology in the common laboratory species of mice is very different from that seen in humans. In *Mus musculus*, for instance, telomeres are five to 10 times longer than those in humans, and telomerase is not stringently repressed in most somatic tissues during development. In addition, mouse chromosomes are acrocentric, such that telomeric fusions at the short arms can generate pseudo-metacentric chromosomes without undergoing fusion-bridge-breakage cycles [48–50]. As a consequence, telomeres are not significantly eroded during aging or disease in mice, and telomerase activation, although still associated with murine cancers, is not necessary for long-term survival of murine tumor cells, even after considerable telomere loss. Thus, the first generations of telomerase knockout mice had essentially no phenotype other than gradual shortening of telomeric length upon successive breeding of the homozygous knockout mice [51].

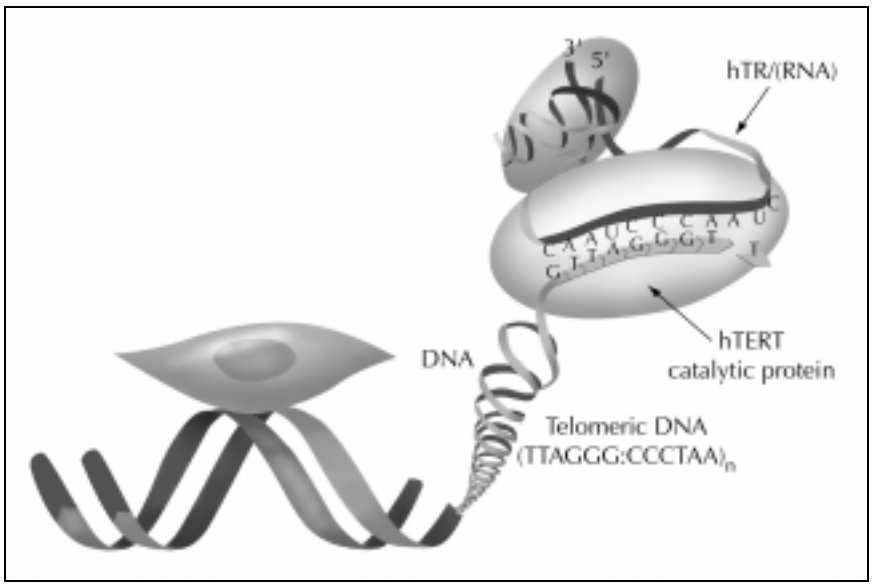

Figure 1. Schematic of telomerase and telomere synthesis.

The early knockout experiments in mice demonstrated that telomerase was not necessary for normal growth and development and that apparently normal tumor initiation and development occurred in its absence. This should have been expected, given the extremely long telomeres in wild-type and early generation telomerase null *Mus musculus.* However, late-generation telomerase knockout mice, with telomere lengths similar to those seen in humans, told a different story [3••,52–57,58••,59]. In these mice, telomere length did reach the threshold limit at which loss of chromosomal integrity signaled cell death. Highly proliferative tissues, such as the immune system, gut, skin, and gonads, in late-generation telomerase knockout mice were the first to exhibit pathology. In addition, pathology could also be induced by chronic environmental or genetic stress in tissues with regenerative capacity, such as the liver. As expected, the accelerated morbidity in these mice was associated with a significantly reduced lifespan.

From the perspective of human disease, studies in knockout mice have generated two very important findings. First, the pathology of diseases induced by chronic stress in these mice more closely matched the pathology in human disease than did that in wild-type mice. Second, in the case of stress-induced liver cirrhosis, telomerase gene therapy in the knockout model effectively prevented the onset of pathology [58••]. Thus, the late-generation telomerase knockout mouse is an important model for degenerative, age-related diseases and telomerase activation therapy in humans.

The cancer phenotype of telomerase knockout mice is harder to interpret in the context of human tumorigenesis, given the inherent differences in chromosome structure (acrocentric vs metacentric) and the stringency of DNA repair and checkpoint mechanisms that control cell proliferation after DNA damage [50]. There are also differences between different laboratories or different cancer models with regard to the effects of telomerase deficiency on tumorigenesis [59,60]. Nevertheless, in the telomerase null mice with short telomeres, growth control mutations can generate a profile of epithelial cancers that much more closely matches those of humans than do the typical sarcomas and hematopoietic tumors of wild-type mice [3••].

Reactive Oxygen Species and Their Role in Telomere Biology

*Reactive oxygen species (*ROS) have been at the center of theories of biological aging. The recent findings that ROS may accelerate the rate of telomeric attrition in cultured cells suggest a potential role for ROS in human aging through their effect on telomere dynamics. Generated by multiple biological processes, ROS are essential for organismal vitality in that they defend against pathogens, transduce signals, and regulate genes, including the response to vascular injury [61••,62–64]. At the same time, ROS promote degenerative senescence. The endogenous targets of ROS are diverse, including nucleic acids, proteins, and lipids. Organisms use an array of antioxidant defenses that include enzymatic scavenging of free radicals [61••,62] and mechanisms that maintain protein thiols and the reduced forms of antioxidant molecules, such as glutathione [65]. In the final analysis, the cumulative oxidative damage in tissues hinges on the balance between the generation of ROS and countermeasures to neutralize their deleterious effects. Accordingly, the rate of tissue accumulation of oxidative end products is negatively correlated, while DNA repair capacity of ROS-evoked damage is positively correlated with maximal lifespan potential among species [2••,61••,62]. Collectively, these findings support the “free radical theory of aging” and the “rate of living theory” [66–68], attributing central roles in aging to ROS and metabolic rate.

Traditionally, the involvement of ROS in aging has been ascribed to oxidative stress-mediated structural damage that promotes age-related diseases. For instance, oxidative mutagenesis causes cancer [69], lipid peroxidation promotes atherosclerosis [70], and oxidized proteins are involved in neurodegenerative disorders [71,72]. However, mild hyperoxia and treatment with hydrogen peroxide or with organic hyperoxides result in activation of the p53 or p16/pRb and p21 pathways in concert with premature senescence of cultured cells [73,74]. These conditions cause considerable single-strand telomeric damage associated with rapid telomeric attrition that cannot be explained by the size of the telomeric overhangs. Single-strand degradation and accelerated telomeric attrition are also observed in cultured cells maintained for long periods in a confluent state [75]. After being released from confluence, these cells attain replicative senescence at a lower cumulative population doubling than control cells. These and other studies indicate that telomeres are considerably more sensitive to ROS-induced single-strand degradation than other chromosomal regions and that ROS accelerate telomeric attrition [76••], which, in principle, may affect the aging process and age-related diseases.

Cardiovascular Diseases: Links to Telomere Biology and the Potential Role of Reactive Oxygen Species and Sex

Most cardiovascular diseases in adult humans are disorders of aging. This is certainly true with regard to atherosclerosis and essential hypertension, which are the most prevalent aging disorders in developed nations. The connection between atherosclerosis and ROS is well established [70]. Evidence suggesting that ROS are a determinant in hypertension pathobiology has been documented only recently. Increased activity of ROS is found in rats with genetic hypertension and experimentally induced hypertension [77–82]. In addition, oxidative stress induced by depletion of glutathione, a major intracellular antioxidant, causes severe hypertension in rats [83]. Production of plasma peroxidase is greater in patients with essential hypertension and in normotensive persons with familial history of essential hypertension than in normotensive persons without familial history of the disorder [84,85]. Less plasma hydrogen peroxide is produced in women than in age-matched men [85]. Women may also differ from men in activities of antioxidant enzymes, such as circulating superoxide dismutase, catalase, and glutathione peroxidase [86]. These sex-related differences might be attributed to the capacity of estrogen to reduce oxidative stress [87,88].

Bearing the marks of ROS and estrogen, links between vascular parameters and telomere dynamics have been identified in humans in vitro and in vivo. As is seen with most other replicative somatic tissues, telomere length in the human vascular endothelium inversely relates to donor age [23,89]. Homocysteine—a major factor in the pathobiology of atherosclerosis—accelerates telomere attrition in human vascular endothelial cells in culture by mechanisms largely mediated via ROS [90].

Among blood pressure parameters, pulse pressure is the most reliable index of cardiovascular risks [91,92]. Pulse pressure is primarily determined by the "stiffness," (*ie*, less distensibility) of central arteries, a characteristic reflecting the biological aging of these vessels. Pulse pressure progressively increases with age and is lower in premenopausal women than in men of the same age. During the postmenopausal period, women's pulse pressure catches up with that of men, so that by age 70 years pulse pressure is about the same in both sexes [92]. In rats, age-dependent increase in arterial stiffness is attenuated by estrogen replacement [93]. Given the observed relationships between pulse pressure, age, and sex, it is interesting that after adjustment for age, pulse pressure correlates inversely with telomere length and is modified by sex [94,95]. In addition, telomere length in patients with vascular dementia is shorter than that in controls [96], suggesting the involvement of telomere dynamics in the biological aging of not only central arteries but also smaller, peripheral arteries.

These intriguing associations between telomeres and measures of vascular biology are largely controlled by genetic factors, since telomere length is highly heritable [94,97], and by sex. Telomere length is the same in newborn boys and girls [Okuda K, Aviv A, Unpublished data], but is longer in adult women than men [94,95]. This finding suggests that telomere attrition is slower in women than men. This may be due to the fact that women produce fewer ROS and that an estrogen response element exists in the catalytic subunit of telomerase so that estrogen can stimulate telomerase [98]. Estrogen and ROS might therefore explain not only differences between men and women in pulse pressure and cardiovascular risks, but also the sex-related differences in telomere length.

Conclusions

Chronological age is an abstraction that presupposes the notion that "one size fits all." Nature rarely, if ever, operates in this fashion, as demonstrated by the heterogeneity that underscores the diversity of biological processes. Telomere biology is no exception to this rule. The telomeric clock is differently set by genetic determinants and unwinds at different paces during different life phases. In addition, telomeric attrition is subject to a host of cellular and extracellular modifiers, including estrogen and ROS, which are centrally involved in signal transduction, cellular energetics, cardiovascular diseases, cancer, and apparently the aging process itself.

Telomere biology may play an important role in disease and longevity of humans, but not in wild-type mice. Humans have roughly 1000 times as many cells and live roughly 50 times as long as mice. Thus, after adjustment for cell number and maximal life expectancy, growth control

and tumor suppressor mechanisms in humans should be 10,000 to 100,000 times as efficient as those in mice. Short telomeres and stringently controlled telomerase expression dictating programmed replicative senescence may be an important component of this difference between mice and man. Somatic cell mortality has long been considered a tumor suppressor mechanism because loss of growth control in cancer would rapidly exhaust the proliferative potential of cells [99]. The deleterious effects of cell senescence following critical telomere loss in normal cells in older humans would then be a classic example of antagonistic pleiotropy: a trait with strong beneficial effects early in life, but detrimental effects late in life. The age-related effect of telomere loss in normal cells would be most apparent in highly proliferative tissues or in anatomical sites of chronic stress, where it could be manifested by degenerative changes, including cardiovascular disease, or even cancer. Different outcomes will depend on genetic endowment, cell types, and whether cell cycle arrest or genomic instability and further loss of growth control predominate.

Telomerase repression in somatic cells but not reproductive cells may also be viewed as an example of the disposable soma theory. Energy to maintain telomeres must be spent in the germline to ensure we are "born young," but the telomerase pathway is turned off in somatic cells as long as telomeres are long enough to allow survival through the peak reproductive years.

The following questions must, therefore, be more fully answered to allow us to understand the role of telomeres in human biogerentology: Is telomere length simply a record that keeps track of our individual passage through life, or is it a determinant of our longevity and susceptibility to age-related disease? How is telomerase regulated in the germline? How is it repressed in normal somatic tissues and abnormally activated in cancer? What is the telomere signal of aging cells? Is it triggered in an all-or-none fashion at a fixed critical length, or does telomere attrition affect biological functions through phenotypic changes in replicating cells even before this putative threshold is reached? The jury may be out for some time on these questions as we continue to explore and ponder the biological structure and function of telomeres. The answers will no doubt have considerable clinical ramifications. From the evolutionary standpoint, the antagonistic pleiotropy and disposable soma theories suggest that, in humans, telomerase regulation and telomere dynamics are fundamentally important to the partition of germline from the soma, the prevention of cancer early in life, and the onset of diseases of aging, including cardiovascular diseases and cancer. The answer to the question "How long should telomeres be?" is still uncertain, but the prospect for treatment of degenerative diseases, including cancer, through therapeutic manipulation of telomerase activity looks promising.

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