ISSN 2249-4073
Current Trends in Biotechnology and Chemical Research

Received : Feb. 13, 2011; Accepted : Feb. 26, 2011

Telomerase and Telomeres in Aging and Cancer

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Abstract:

The structure and integrity of telomeres are essential for genome stability. Telomere deregulation can lead to cell death, cell senescence or abnormal cell proliferation. The maintenance of telomere repeats in most eukaryotic organisms require telomerase, which consists of a reverse transcriptase (RT) and an RNA template that dictates the synthesis of the G-rich strand of telomere repeats. Structurally, telomerase reverse transcriptases (TERT) contain unique and variable N- and C- terminal extensions that flank a central RT- like domain. The level of telomerase activity is important in determining telomerase length in aging cells and tissues. Here, evidence on the importance of telomerase activity is reviewed with respect to aging rates, as well as the health and life span of individuals.

Keywords: Telomere, Telomerase, Reverse transcriptase, Aging, Cancer

Introduction

Telomeres are simple repeat elements located at each chromosome end of eukaryotic cells [1]. The main function of telomeres is to cap the ends of chromosomes thus preventing DNA open-end which can lead to activation of DNA-damage responses, chromosomal fusions, and chromosomal instability [2-4]. Due to the 'end-replication problem' of DNA polymerase, telomeres shorten during each cell division by 50−100 base pairs (bp). Telomere length in human is 5−10 kb and the proliferative capacity of primary human cells in tissue culture is limited to 50−70 population doublings [5], before telomeres reach a critical length no longer ensuring telomere function. At this stage, cellular senescence, characterized by permanent growth arrest, is induced [6-8]. Telomerase is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes [9]. Activity of telomerase is required to prevent telomere shortening and thus to ensure cell and organism survival. Telomerase can synthesize telomeres *de novo* and consists of 2 essential components, an RNA component serving as a template for telomere sequence synthesis and a reverse transcriptase [10-14]. The telomerase holoenzyme often contains additional proteins that are not required for catalysis *per se*. These include the usual reverse transcriptase and RNA core components (Est2 and TLC1 respectively) and two accessory factors Est1 and Est3. Although Est1 and Est3 are not required for *in vitro* telomerase activity, mutation in these genes lead to progressive telomere shortening, the so called ever shorter telomeres (est) phenotype [15]. In humans, telomerase expression is regulated very restrictively. It is only active during embryogenesis, whereas its postnatal activity is suppressed in most somatic tissues but remains active in a subset of cells, such as germ cells [16], stem cells, progenitor cells [17] and activated lymphocytes [18-21]. Postnatal suppression of telomerase expression in most somatic tissues is thought to represent a potent anti-tumor barrier not allowing the immortal growth of transformed cells [22]. A flaw of telomerase suppression is the limited growth of primary human cells which might affect the regenerative capacity of organs and tissues during aging and chronic diseases.

Regulation of telomerase

The molecular mechanism for regulation of telomerase in humans is not well known and remains to be elucidated. Several lines of evidence suggest that the existence of repressors rather than the absence of activators might control the tight regulation of telomerase reverse transcriptase (TERT) expression in humans (known as hTERT) [23]. Therefore, it seems certain that identification and characterization of these will provide

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additional information on the molecular mechanism of telomerase regulation possibly pointing to new therapeutic targets for the treatment of regenerative disorders during aging [24]. Exceptionally, limited telomerase activities is detected in some normal human cells such as germ cells, hematopoietic stem cells, intestinal crypt cells and basal layer cells of the epidermis in human skin. Since all of these cells have highly regenerative capacities, the maintenance of telomerase activity in these cell compartments seems to contribute to preservation of regenerative potentials by slowing telomere shortening rates.

Contrary to most normal tissues, over 80% of human cancers show expression and activity of telomerase (Fig.1). Critically short telomeres due to successive cell divisions cause replicative senescence or apoptotic cell death [25-27]. For unlimited proliferation, telomerase activation might be a critical step for normal cells to be transformed to cancer cells. However, activated telomerase itself does not appear to work as an oncogenic factor but rather serves only as a promoting factor in overcoming the barrier of critical telomere shortening caused by continuous proliferation in already transformed cells. Among all components of the telomerase complex, expression level of TERT, the catalytic subunit, is most closely matched to the level of telomerase activity [28-30] but not in all cases [31]. Recent evidence also points that RecQ helicases also contribute to the proper functioning and maintenance of telomeres [32].

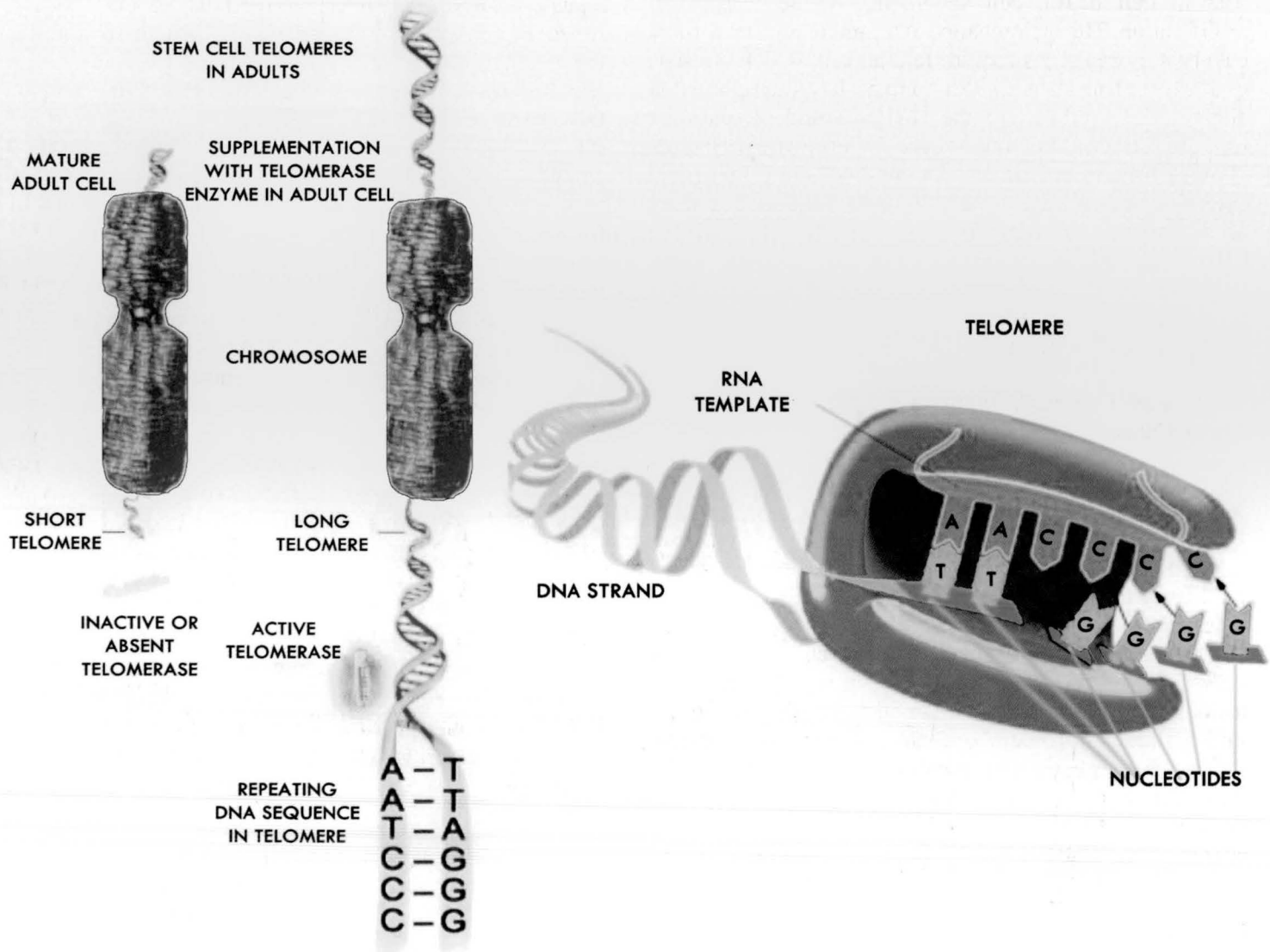

Fig. 1 Chromosome, Telomere and Telomerase

Telomere shortening

Aging is a multifactorial process that has been adjusted by nature to a wide spectrum of life spans, even in closely related species, therefore suggesting that aging is a flexible trait susceptible to the influence of a number of molecular pathways [33-35]. One such process is the progressive attrition of telomeres that occurs in association with organismal aging in humans [36] and in other mammals, such as mice [37]. Telomere length is now considered to be a biomarker of age [38]. Telomeres are specialized structures at the ends of chromosomes that have a role in protecting the chromosome ends from DNA repair and degrading activities. Mammalian telomeres consist of TTAGGG repeats bound by a multiprotein complex known as shelterin. A minimum length of TTAGGG repeats and the integrity of the shelterin complex are necessary for telomere protection [39, 40]. Telomerase is capable of compensating telomere attrition through *de novo* addition of TTAGGG repeats onto the chromosome ends by using an associated RNA component as template (TERC, telomerase RNA component) [41]. Telomerase is expressed in most adult stem cell compartments; however, this is not sufficient to maintain telomere length, as evidenced by the fact that telomere shortening occurs with age in most human and mouse tissues [2, 36, 37]. Furthermore, some diseases characterized by premature loss of tissue renewal and premature death, such as dyskeratosis congenita, anemia, and idiopathic pulmonary fibrosis, are linked to germline mutations in TERT and TERC genes, which result in decreased telomerase activity and accelerated telomere shortening [42-46]. A role for telomerase in tissue renewal and organismal life span is also supported by telomerase-deficient null mice. Longevity is progressively shortened upon successive intercrossing of telomerase-deficient mice [47], an effect already noticeable at the first generation of TERC null mice where both the median and maximum life span are reduced [48]. Finally, telomerase overexpression is sufficient to extend the life span of most human cells in culture [49]. Together, the evidence strongly suggests that telomerase activity and telomere length are rate limiting for mammalian life span and supports a model in which short telomeres actively contribute to aging by limiting tissue renewal.

Telomere shortening during aging occurs in a variety of human tissues and organs including dermal fibroblasts [50], mucosal keratinocytes [51], peripheral blood cells [52, 53], gastrointestinal epithelial cells [54], adrenocortical cells [55], renal cortex [56], liver [57, 58] and spleen. Most of these tissues and organs are mitotically active indicating an outcome of cell divisions on telomere shortening during aging. This finding is also supported by the observation that telomere length is stable in mitotically inactive organs such as brain and myocardium [59]. However, some of the tissues affected by telomere shortening during aging, like liver and renal cortex, show very little mitotic activity indicating that there must be factors other than cell division modulating the attrition of telomeres during aging. In line with this hypothesis, the kinetics of telomere shortening during

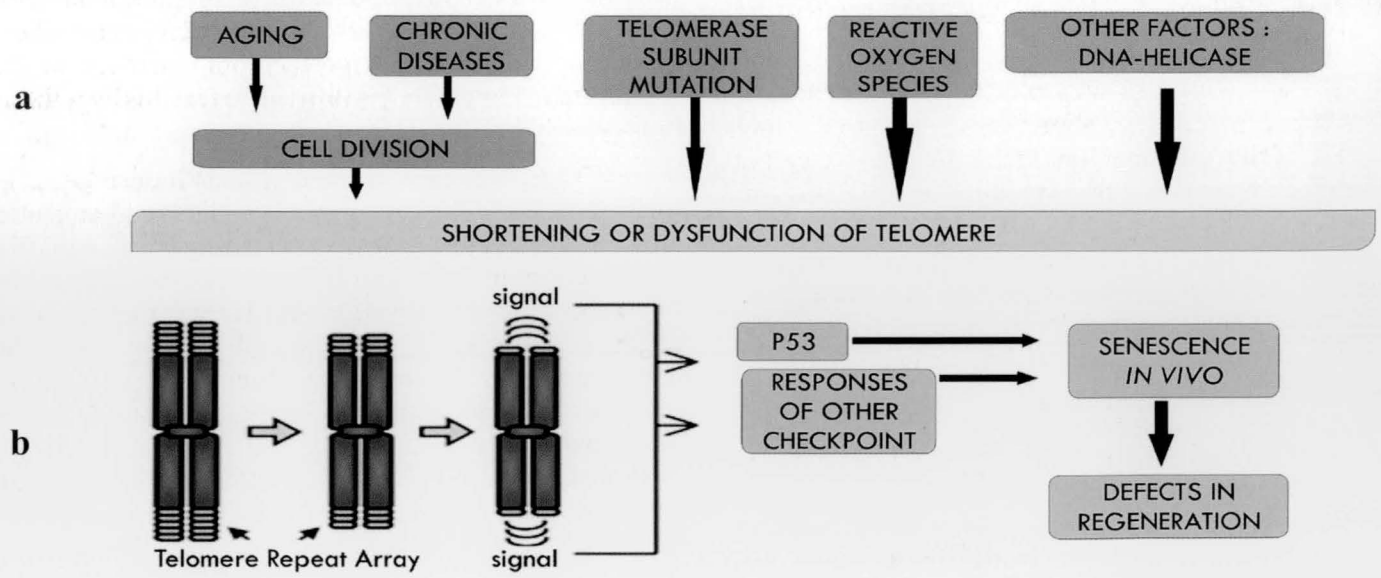

Fig. 2 Telomere shortening

a Most possible causes of telomere shortening or dysfunction. **b** Loss of telomere repeats in cells activates senescence program which result in senescence and regenerative defects

aging is not linear. Telomere shortening is accelerated in peripheral blood cells in young infants, going plateau in older children, and slowly decreased in adults [60, 61]. Another explanation for telomere shortening in mitotically-inactive organs during aging is, that telomere shortening might predominantly affect a proliferative sub-population of cells within an organ (e.g. endothelial cells, lymphocytes, connective tissue) without affecting differentiated organ-specific cell types (e.g. hepatocytes in liver or glomeruli cells of the kidney) [58, 62]. Therefore, a cell-type specific measuring of telomere length is required in future studies to understand the role of telomere shortening in aging tissues and organs.

Different mechanisms have been suggested as the cause of accelerated telomere shortening in chronic diseases (Fig. 2). These include (i) Elevated rates of cell turnover; (ii) Inhibition of telomerase activity by mutation of telomerase subunits; and (iii) Telomere shortening by increased levels of intracellular reactive oxygen species.

Together, there is growing evidence that telomere shortening and senescence might affect the regenerative capacity of organs and tissues during aging and chronic disease. It is important to test this hypothesis by analyzing the effect of telomere shortening and telomere stabilization in animal models. Over the recent years the essential components of mouse telomerase, mTERC and mTERT, have been identified and cloned. Further, knockout and transgenic mouse models have been developed facilitating study on the effect of telomere shortening *in vivo* [63, 64]

Telomere biology and telomerase activity as determinants of aging

When compared with closely related primates, humans have relatively short telomeres [65], and telomerase activity is very low in most cells, except for some types of stem cells, the germ line, and some somatic cells such as T- lymphocytes [66]. If telomere exhaustion were a major cause of aging one would expect humans to be relatively susceptible to this process and mice to be resistant; obviously the much longer life span of humans would suggest that differences in telomere biology is not a major determinant of life span among mammals. However, this simple argument leaves open two related questions: first, are differences in telomere biology important determinants of aging and life span among individuals within a species?; and second, even if telomerase and longevity are not positively correlated, is it possible that they could be negatively correlated: could high telomerase activity be a factor causing shorter life span?

The question of whether differences in telomere biology are important determinants of aging and life span among individuals within a species is only meaningful in species such as humans that have limited telomerase activity. Nevertheless, it is possible to address the question of the consequences of shortened telomeres in tissues by engineering mice to lack telomerase activity. Mice with defects in the TERC gene undergo generation-dependent telomere shortening. In later generation of telomerase-deficient mice, various organs exhibit impaired functions, demonstrating that sufficiently short telomeres do have an adverse impact on tissue function [67]. However, experiments in mice cannot answer the question of whether telomeres ever reach a "critical" length, i.e. a length that impairs proliferation (or conceivably some other cellular property), in any tissue in humans during a normal life span. There is little evidence that commonly observed changes in older individuals, such as anemia and impaired wound healing, result from impaired cellular proliferation, which would be the anticipated consequence of shortened telomeres. Despite the lack of clear evidence for impaired proliferation in aging there is, in fact, good evidence for progressive telomere shortening in many human cell types, including peripheral white blood cells, smooth muscle cells, endothelial cells, lens epithelial cells, muscle satellite cells, and adrenocortical cells, among others [68]. One example is of particular interest: proliferative capacity is closely related to telomere length in endothelial cells. Telomere lengths in endothelial cells decreased as a function of donor age, with a greater decline being observed in cells isolated from the iliac artery in comparison to cells from the thoracic artery [69]. The greater decline in telomere length was observed in the cells that had likely undergone more proliferation *in vivo*, because they resided in a part of the vascular system where blood flow might cause most chronic damage to the endothelium. However, it is difficult to test this hypothesis directly.

Thus, telomere shortening does indeed occur in the human body during aging. The question, as stated above, is whether this telomere shortening is a determinant of differences in aging and life span among individuals. Two aspects to this question are: (i) whether telomere length, as measured in specific cell populations in the body, correlates with longevity or disease; and (ii) whether telomere shortening in any cell population causes functional impairment of that cell population. At the present time the only cell populations that have been subjected to the required depth of analysis are peripheral white blood cells and some white blood cell subsets.

Several observational studies have attempted to gain insight into the question of whether age related telomere shortening in human peripheral white blood cells is associated by way of health and disease status. One study concluded that "in and of itself, SES [socioeconomic

status] appears to have an impact on WBC [white blood cell] telomere dynamics" [70]. Another study of mothers of chronically ill children concluded that "psychological stress is associated with indicators of accelerated cellular aging [including] telomere length" [71]. Both of those studies suggest an influence of perceived psychological status on telomere length. Of course, psychological stress does not necessarily cause stress at the cellular/molecular level. One plausible link is the endocrine system [72]. Possibly, the explanation for the differences in telomere length in individuals of differing psychological status lies in the actions of hormones such as glucocorticoids on cell death and cell proliferation in the hematopoietic system.

Conclusion and future perspective

Telomere shortening resulting from the absence of telomerase activity may be a factor in determining some age-related properties of organs in humans. Reactivation of telomerase removes a barrier to the continued growth of developing cancers; lack of telomerase activity provides a tumor suppressor function. There are at least three major questions that need to be answered. First, we need to know what telomere length in human tissues is associated with functional impairment, of specific organs, tissues or cell populations; second, because of the great heterogeneity in telomere lengths between cells and between different telomeres within cells, we need to know if there could be impairment of individual cells, even if there is no deficit in the cell population as a whole; and third, we do not know if telomere length in white blood cells, or T lymphocytes, correlates with telomere length in other tissues. Gaining access to appropriate tissue samples to test this is problematic. Is there a specific cell population in the body in which telomere length directly determines differences in health, disease or the actual rate of aging among individual humans? This is possible, but we have no evidence to support the existence of such a population of cells. Activation of telomerase on one hand could prevent telomere shortening, chromosomal instability and cancer initiation, but on the other hand could allow cancer progression of nascent, transformed cells in the organism. Extensive research in the years to come should yield more convincing evidence on these issues.

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