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Telomeres, Telomerase, Stress, and Aging

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Why should a behavioral scientist study telomeres? Maintenance of telomeres, the natural ends of linear chromosomes, is a fundamental biological mechanism of all eukaryotic cells, from protozoa to humans. Gradual shortening of telomeres after each cell division eventually can lead to loss of cellular division capacity and cell death, and contribute to genomic instability, a characteristic of cancer. Telomeres and telomerase, the enzyme that adds nucleotides to telomere ends, have been linked to human aging and aging-related diseases (Aubert & Lansdorp, 2008). Further, lifestyle and psychological state are increasingly being associated with telomere length and telomerase activity changes. Thus, telomere length and telomerase activity emerge as new biomarkers for cellular aging and may serve as surrogate markers for factors that contribute to aging and aging-related diseases. Therefore, scientists interested in understanding early onset of aging-related diseases, as well as longevity, may want to include this measure of cell aging.

This chapter gives the behavioral scientist a general understanding of the telomere/telomerase maintenance system, from its molecular basis, to clinical observations, to measurement. It addresses such questions as: Why do telomeres shorten? What are the consequences of telomere shortening? How are telomeres and telomerase related to cancer and diseases of aging? And, lastly and most relevant to behavioral scientists, what environmental (nongenetic) factors modulate telomere length? For detailed discussion of each topic, readers are encouraged to read the literature cited in this chapter.

TELOMERE MAINTENANCE AND THE AGING OF CELLS AND ORGANISMS

Telomeres Defined

First named by Hermann Muller in 1938, telomeres are the natural ends of eukaryotic chromosomes. Muller used X-rays to break chromosomes, thus generating mutants of the fruit fly. The mutants recovered often had chromosome fragments that rejoined. However, it appeared that the rejoining did not occur between two natural free chromosomal ends or between one broken end and one natural free end. Barbara McClintock had independently made comparable observations while studying chromosomes in maize. Muller called the natural free ends of chromosomes "telomeres." Telomeres thus behave differently from a broken chromosome end in that they are refractory to the molecular machinery that joins the broken ends.

The first telomeric DNA sequence was determined from the abundant minichromosomes of the ciliated protozoan *Tetrahymena thermophila*. This telomeric DNA consists of approximately 50 tandem repeats of the sequence TTGGGG, with each 3'-OH end of the duplex chromosomal DNA molecule being the G-rich strand (Blackburn et al., 1983; Blackburn & Gall, 1978). Since then, telomeric sequences from many organisms have been determined, including those of many species of yeast, plants, ciliates, birds, and mammals. Human telomeres contain 5 to 10 kilobases of TTAGGG repeats (Moyzis et al., 1989), while the lab strain of mouse *Mus musculus* has telomeres over 40 to 80 kb long of the same sequence as human (Blasco et al., 1997). Interestingly, the fruit fly *Drosophila melanogaster* that Muller used to discover the unusual properties of telomeres lacks the canonical repetitive telomeric sequences characteristic of most eukaryotes. Instead, Drosophila telomeres contain arrays of retrotransposon elements (evolutionally related to retroviruses such as HIV viruses, Pardue & DeBaryshe, 2003). Nevertheless, telomeres in Drosophila are still protected from being recognized as broken ends.

Telomeres are organized into a high-order DNA-protein complex by the binding of multiple telomeric proteins. Evidence for a higher order structure called a T-loop has been found, in which the 3' overhang of the telomeric end is tucked into the double-stranded portion of the telomere sequence to form a loop structure (Figure 65.1). Overall, this higher order structure protects telomeres from being recognized as broken ends. Furthermore, the concerted

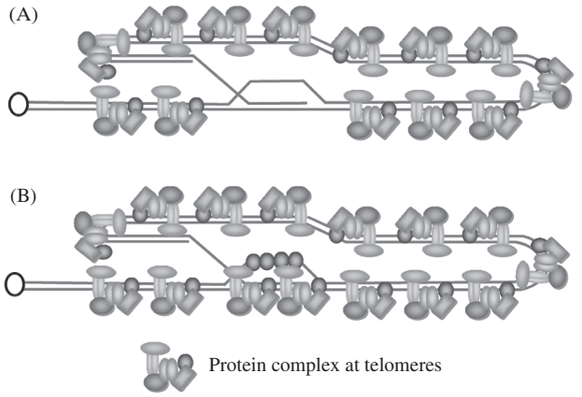

Figure 65.1 Telomeres: A DNA-protein complex at the end of chromosomes, showing the "T-loop" structure.

Note: (A) shows the 3' end of the single stranded region tucked into the double-stranded region to form the "T-loop." (B) shows that the T-loop is bound by a multiprotein complex. From "Structure and Variability of Human Chromosome Ends," by de Lange et al., 1990, *Molecular and Cellular Biology*, 10, pp. 518–527. Adapted with permission.

actions of telomeric proteins and other factors determine the length of telomeres under different conditions.

Why Do Telomeres Shorten? The DNA End Replication Problem and Telomerase

As conventional DNA polymerases need a primer from which nucleotide extension occurs, the removal of the RNA primer at the lagging DNA synthesis strand will result in a 5'-terminal gap after DNA replication (Figure 65.2). Due to this end-replication problem (Watson, 1972), telomeric sequences are lost in each cell division. For immortal single-cell organisms and germ cells, an active cellular mechanism is needed to prevent the loss of telomeres. Since the presentation of the replication problem in the 1970s and early 1980s, many models have been put forward to explain this fundamental phenomenon (for a historical view of this, see Blackburn, 1984). This end-replication problem is solved for eukaryotic chromosomes by the cellular enzyme telomerase, a specialized ribonucleoprotein reverse transcriptase that uses its integral RNA molecule as the template to synthesize telomeric sequences (Greider & Blackburn, 1985, 1987; Figure 65.3).

Telomere Shortening Leads to Cellular Senescence

Hayflick and colleagues first described the limited proliferation capacity of normal human fibroblasts when cultured in vitro (Hayflick & Moorhead, 1961). The term *cellular senescence* refers to this state of irreversible cell cycle arrest. Since it is predicted that gradual shortening of telomeres will lead to eventual cell cycle arrest due to the end replication problem,

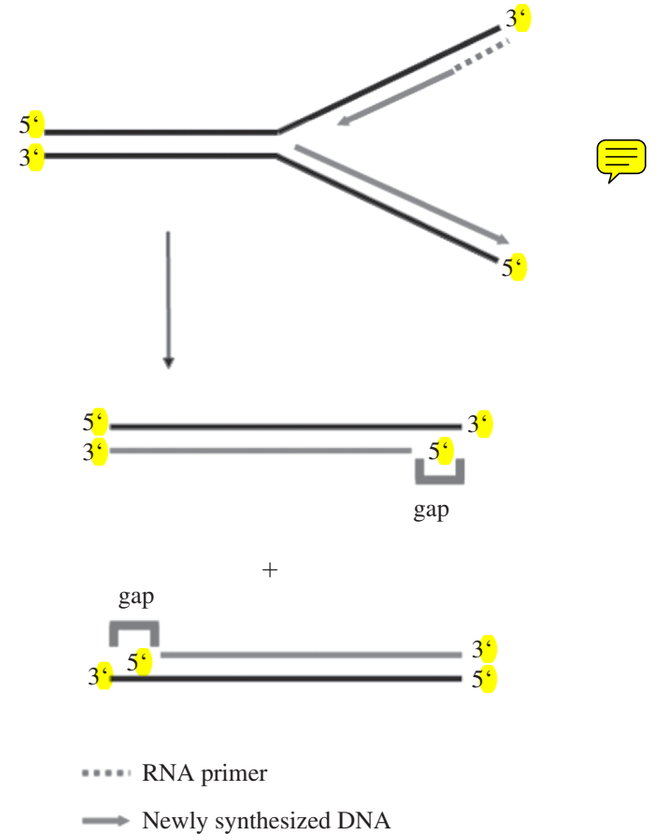

Figure 65.2 The DNA end replication problem.

Figure 65.3 Telomerase: the enzyme that adds nucleotides to and protects telomeric ends.

Telomerase activity was originally discovered in *Tetrahymena*, using extracts from freshly mated cells (Greider & Blackburn, 1985). This developmental stage was carefully chosen because soon after mating, in the somatic nucleus the chromosomes are fragmented, and hundreds of new telomeres are generated de novo, which requires especially high telomerase activity formation. Telomerase activity was subsequently identified in a variety of organisms, including yeast (Cohn & Blackburn, 1995) and human (Morin, 1989). The gene for the RNA component of human telomerase (TER or TERC) was cloned in 1995 (Feng et al., 1995), while the gene for the core protein component (TERT) was cloned in 1997 (Counter, Meyerson, Eaton, & Weinberg, 1997; Lingner et al., 1997; Nakamura et al., 1997). We now know that only the RNA and this protein component are required for minimal enzymatic activity by the telomerase ribonucleoprotein (RNP) enzyme. However, in vivo addition of telomeric DNA onto chromosomal ends requires the collaboration and coordination of dozens of proteins (reviewed in Cong, Wright, & Shay, 2002; Smogorzewska & de Lange, 2004).

the finite doubling capacity of mammalian cells described by Hayflick is proposed to be caused by attrition of telomeric sequence down to a critically short length. Soon after the identification of human telomere sequences, Harley and colleagues reported that telomeres progressively shorten during in vitro culturing of human primary fibroblasts (Harley, Futcher, & Greider, 1990). Furthermore, when fibroblasts directly taken from donors were examined for their telomere length, a loose negative correlation between the age of the donor and their telomere length was reported (Harley et al., 1990). Since then, the correlation between telomere shortening and cellular senescence has been supported by a large body of literature.

Although cellular senescence can be induced by critically shortened telomeres, other cellular signals can induce cellular senescence as well. Current models postulate that two major pathways can lead to cellular senescence (Campisi, 2005). The replicative senescence pathway caused by erosion of telomeres and their dysfunction is dependent on the tumor suppressor p53 (Campisi, 2005). Other cellular stresses, including oxidative stress and overexpression of oncogenes, can lead to stress-induced premature senescence, which is dependent on the p16/pRB pathway (Campisi, 2005). PRB and p16 are tumor suppressor proteins; on activation by cellular stress signals, they cause a chain of reactions that leads to cell-cycle arrest, therefore inhibiting cell proliferation (reviewed in Kim & Sharpless, 2006).

Senescent cells, whether caused by telomere dysfunction or cellular stress, display distinct characteristics. A hallmark of senescent cells is the irreversibility of cell cycle arrest. Senescent cells do not respond to growth stimuli, although they remain metabolically active for long periods. Morphologically, senescent cells are larger than their young counterparts and appear to be flat. They stain positive for the enzyme ß-galactosidase (Senescence-Associated ß-gal, SA-ß-gal) and express p53/p21 (telomere dependent senescence only) and p16 (stress-induced premature senescence). In telomere dependent senescence, DNA damage foci—cytologically visible clusters of proteins involved in DNA damage responses, including histone λ-H_2AX and 53BP1—colocalize with telomeres (d'Adda di Fagagna et al., 2003; Herbig, Ferreira, Condel, Carey, & Sedivy, 2004; Takai, Smogorzewska, & de Lange, 2003). An interesting feature of senescence is that cells are now more resistant to apoptosis; they do not respond to signals that cause apoptosis in normal cells. This may have physiological significance for the mechanism of immunosenescence, the aging of the immune system (discussed later). The gene expression profile of senescent cells is distinctively different from that of young cells. Two cell-cycle inhibitors, p21 and p16, are predominantly expressed in senescent cells. p21 expression is upregulated by the tumor suppressor p53, and p16 is induced by pRB. In addition, senescent fibroblasts express proteins that remodel the extracellular matrix, including metallomatrix proteases and proinflammatory cytokines. These proteins are thought to contribute to carcinogenesis by creating a microenvironment advantageous for cancer cell growth (Campisi & d'Adda di Fagagna, 2007).

It is worth pointing out that for rodents, telomere-dependent replication senescence is not the primary pathway for cellular senescence of in vitro cell cultures; rodent telomeres are long and telomerase expression is generally higher than in the comparable normal human cell types (Chadeneau, Siegel, Harley, Muller, & Bacchetti, 1995; Prowse & Greider, 1995). The observed limited proliferation for mouse cells cultured in vitro is mainly due to insult from high oxidative stress under in vitro culture conditions, which triggers a p53 dependent DNA damage response. Despite this difference, as discussed later in this chapter, telomerase knockout mice, with telomere length similar to that of humans, have proved to be useful in vivo models to examine the potential roles of telomeres and telomerase in human diseases including cancer and other aging-related diseases.

Although the cellular senescence observed for in vitro cultured cells has been proposed to reflect in vivo organismal aging, there has been much controversy about what cellular senescence really means. The relevance of cellular senescence to aging became apparent when markers of cellular senescence were observed in vivo. Telomeres shorten during aging in many tissues that can be renewed throughout life, including peripheral blood, liver, kidney, spleen dermal fibroblasts, and keratinocytes, but not in postmitotic cells (e.g., neurons and cardiomyocytes; Djojosubroto, Choi, Lee, & Rudolph, 2003, and reference therein). This is consistent with the idea that in older organisms, the cells in the self-renewing tissues have gone through more divisions than younger people. Staining of the senescence associated enzyme ß-galactosidase (SA-ß-gal) has been reported in senescent fibroblast and keratinocytes in aging human skin (Dimri et al., 1995) as well as in damaged tissues in various diseases (reviewed in Erusalimsky & Kurz, 2005). Furthermore, expression of the senescence marker protein p16 was also reported (Krishnamurthy et al., 2004). Localization of DNA damage proteins at telomeres was also observed in skin cells of aging baboons and was taken as evidence of replicative senescence in vivo during aging (Herbig et al., 2006). In addition, the link between telomere dysfunction and pathological conditions has now been well established (to be discussed later).

Regulation of Telomerase

Human telomerase activity is highly regulated, both during development and tissue-specifically. Telomerase activity is

expressed in embryonic stem cells and germ lines, but is decreased later in development (Forsyth, Wright, & Shay, 2002; Wright, Piatyszek, Rainey, Byrd, & Shay, 1996). In adults, low levels of telomerase activity were found in stem cells and progenitor cells including hematopoietic stem cells and neuronal, skin, intestinal crypt, mammary epithelial, pancreas, adrenal cortex, kidney, and mesenchymal stem cells (reviewed in E. Hiyama & Hiyama, 2007). Also, very low levels of telomerase activity have been detected in proliferating smooth muscle cells (Cao et al., 2002; Haendeler et al., 2004) and fibroblasts (Masutomi et al., 2003). However, telomerase activity in the adult progenitor cells is not high enough to prevent telomere attrition, as progenitor cells were shown to lose telomeric DNA length as the organism ages. This suggests that modulating telomerase activity in these cells may alter the rates of telomere shortening, thus affecting their proliferation capacity.

The regulation of telomerase activity in lymphocytes has been extensively studied. Telomerase activity is high in early stages of T and B cell development, but decreased at later stages. Only very low activity was detected in mature resting circulating T and B cells from peripheral blood mononuclear cells (K. Hiyama et al., 1995; Weng, Levine, June, & Hodes, 1996). However, telomerase activity is upregulated in T and B cells on stimulation by mitogens or antigens (Broccoli, Young, & de Lange, 1995; K. Hiyama et al., 1995); and this upregulation is required for clonal expansion of T and B cells during an immune response. Upregulated telomerase activity in lymphocytes is still not enough to compensate for telomere loss during proliferation, as telomere shortening was observed during in vitro culturing of activated T and B cells and these cells have a finite life span (Perillo, Walford, Newman, & Effros, 1989; Son, Murray, Yanovski, Hodes, & Weng, 2000). In vivo, memory T cells were found to have shorter telomeres than naive cells, indicating that their telomeres were shortened during clonal expansion. Since a proper adaptive immune response requires extensive and rapid clonal expansion of T and B cells, limited proliferation capacity may lead to compromised immune functions over the long term.

A host of environmental factors regulate telomerase activity (Figure 65.4). Reactive oxidative species (ROS) are reported to decrease telomerase activity in both cancer cells and human umbilical vein endothelial cells (Haendeler, Hoffmann, Brandes, Zeiher, & Dimmeler, 2003; Haendeler, Hoffmann, Rahman, Zeiher, & Dimmeler, 2003; Haendeler et al., 2004). Estrogen upregulates telomerase activity, while progesterone activates telomerase activity transiently, but inhibits activity in longer term experiments. Androgen activates telomerase activity in prostate cancer cells while inhibiting it in normal cells (reviewed in Bayne & Liu, 2005). Many growth hormones

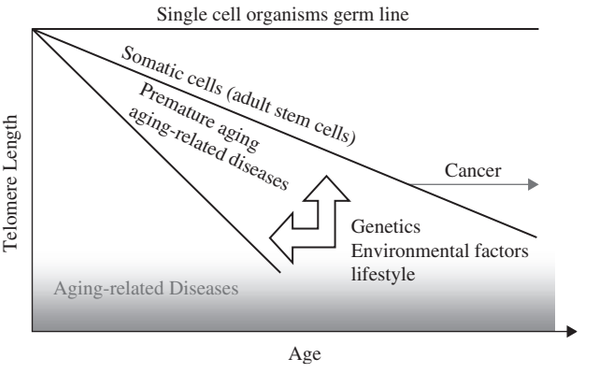

Figure 65.4 Telomere length can be modulated by various factors.

Note: From "The Common Biology of Cancer and Aging," by T. Finkel, M. Serrano, and M. A. Blasco, 2007, *Nature*, 448, pp. 767–774. Adapted with permission.

also play important roles in telomerase activity regulation. Insulin, IGF-1, VEGF and EGF upregulate telomerase activity, while TGF-ß inhibits it (Maida et al., 2002; Torella et al., 2004; Wetterau, Francis, Ma, & Cohen, 2003; Zaccagnini et al., 2005). Many cytokines, including IL-2, IL-6, IL-15, TNF-α and IFN-α, ß, and γ were also reported to regulate telomerase activity (Akiyama et al., 2004; Das, Banik, & Ray, 2007; Kawauchi, Ihjima, & Yamada, 2005; Li, Zhi, Wareski, & Weng, 2005; Xu et al., 2000; Yamagiwa, Meng, & Patel, 2006).

Regulation of telomerase activity is often executed at the level of transcriptional regulation of TERT. Several transcriptional factors have been reported to activate or repress telomerase activity through the hTERT promoter (Flores, Benetti, & Blasco, 2006). Modulations of alternative splicing, posttranslational modification, and subcellular localization and epigenetic modifications have also been reported (Anderson, Hoare, Ashcroft, Bilsland, & Keith, 2006; Flores et al., 2006; Jalink et al., 2007; Liu, Hodes, & Weng, 2001; Saeboe-Larssen, Fossberg, & Gaudernack, 2006).

Telomere Length-Independent Role(s) of Telomerase

There is now evidence suggesting that telomerase may have roles independent of its telomere lengthening function. Constitutive overexpression of hTERT in cancer cells that otherwise maintain their telomere length by telomerase-independent pathways (ALT cells) facilitates malignant transformation, suggesting that this cancer-promoting effect does not rely on extension of telomere length (Stewart et al., 2002). Furthermore, in normal fibroblast cells, further suppression of the already low amount of hTERT in these cells by RNA interference using a shRNA (small hairpin RNA)

impairs the DNA damage response (Masutomi et al., 2003). Telomerase RNA knockdown by shRNAs or ribozymes in malignant cancer cells—rapidly causes a change in gene expression profile (Li et al., 2004; Li & Blackburn, 2005). In postmitotic cells, telomerase may protect against neurotoxicity: PC 12 cells overexpressing hTERT become more resistant to amyloid ß-peptide induced apoptosis (Zhu, Fu, & Mattson, 2000) and to DNA damaging drugs (Lu, Fu, & Mattson, 2001). Along the same lines, TERT mRNA is induced in cortical neurons after ischemic injury in mice, and transgenic mice overexpressing TERT are more resistant to neurotoxicity caused by NMDA (Kang et al., 2004). Although these experiments do not directly address whether it was telomere lengthening or telomerase activity that contributed to these effects, given the short period in which the effects are seen in cultured cells, they suggest that telomerase, as opposed to telomere length change, is the cause mechanistically.

Most convincingly, transgenic mice that overexpress TERT in their skin epithelium have grossly increased proliferation of their hair follicle stem cells. Since this effect is also seen in a telomerase RNA knockout background, this demonstrated that the enzymatic activity (telomere extension function) is not required (Sarin et al., 2005). The mechanisms of telomere length-independent telomerase function(s) remain unknown. A possible pathway is through the maintenance of the very tip of telomeres, that is, the capping function of telomerase. The physical presence of a telomerase complex that includes its RNA component may serve this purpose. An alternative, but not mutually exclusive mechanism is suggested by microarray gene-profiling data, where overexpression of hTERT is shown to upregulate growth-controlling genes (Smith, Coller, & Roberts, 2003). Consistent with this, a recent publication showed that TERT facilitates activation of progenitor cells in the skin and hair follicle by triggering a change in gene expression that significantly overlaps the program controlling natural hair follicle cycling (Choi et al., 2008).

TELOMERE MAINTENANCE AND HUMAN DISEASES

Telomeres and Aging-Related Diseases

The possibility that cellular senescence is associated with organismal aging is consistent with observations that in vivo, senescent cells accumulate with aging (Dimri et al., 1995). Whether replicative senescence caused by telomere erosion is relevant to organismal aging is under debate (Patil, Mian, & Campisi, 2005). However, several lines of evidence strongly suggest a link between telomere dysfunction and aging and aging-related diseases. First, numerous clinical studies link short telomere length in white blood cells (specifically, peripheral blood mononuclear cells, PBMCs) to aging-related disease or preclinical conditions of diseases. A short list of them includes increased mortality from cardiovascular disease and infectious disease (Cawthon, Smith, O'Brien, Sivatchenko, & Kerber, 2003), coronary atherosclerosis (Samani, Boultby, Butler, Thompson, & Goodall, 2001), premature myocardial infarction (Brouilette, Singh, Thompson, Goodall, & Samani, 2003), vascular dementia (von Zglinicki, Pilger, & Sitte, 2000), hypertension with carotid atherosclerosis (Benetos et al., 2004), age-related calcific aortic stenosis (Kurz, 2004), increased pulse pressure (Jeanclos et al., 2000), obesity and smoking (Valdes et al., 2005), Alzheimer's disease (Panossian et al., 2003; Zhang et al., 2003) and insulin-resistance, a preclinical condition for diabetes (Brouilette et al., 2007; Collerton et al., 2007). The main findings of these and several other clinical studies that examined the relationship between telomere length and aging-related diseases are summarized in Table 65.1.

Second, evidence for in vivo cellular senescence was found in affected tissues of cardiovascular patients. Telomere shortening is accelerated in the atherosclerosis-prone areas compared with control areas (Chang & Harley, 1995; Okuda et al., 2000) and telomeres are shorter in diseased coronary arteries than nondiseased age-matched specimens (Ogami et al., 2004). Similarly, putative endothelial cell senescence was observed in human atherosclerosis (Minamino et al., 2002) and vascular smooth muscle cells (VSMCs) with cells in the diseased area containing shorter telomeres than in nondiseased areas in the same patient (Matthews et al., 2006).

Third, in vitro studies with cultured cells have recapitulated some aspects of cellular senescence in vivo regarding cardiovascular diseases. Briefly, stimuli and conditions that affect endothelial and vascular smooth muscle (VSMC) cell function in vitro appear to correlate with telomerase and telomere length maintenance: Oxidative stress reduced telomerase activity and accelerated telomere shortening in EC and VSMC, whereas hypoxia and antioxidants induced telomerase activity and promoted proliferation. Furthermore, overexpression of telomerase in endothelial cells and VSMC extended proliferation life span and improves functional properties (Edo & Andres, 2005).

Fourth, the most compelling evidence for the role of telomeres and telomerase in aging and aging-related diseases came from studies of the genetic disease dyskeratosis congenita (DC) and other related diseases, as discussed in a later section.

TABLE 65.1 Studies linking telomere maintenance to diseases.

Study	Main Findings	Authors
Telomere shortening and prostate cancer	Telomere attrition in the high-grade prostatic, intraepithelial neoplastia and surrounding stroma is predicative of prostate cancer.	Joshua et al. (2007)
Telomere shortening and breast cancer	Short telomeres in PBMCs are associated with breast cancer risk.	Shen et al. (2007)
Telomere shortening and bladder cancer	Short telomeres appear to be associated with increased risks for human bladder, head and neck, lung, and renal cell cancers.	Wu et al. (2003)
Telomere shortening and bladder cancer	Short telomeres in buccal cells associated with bladder cancer risk.	Broberg, Bjork, Paulsson, Hoglund, & Albin (2005)
Telomere dysfunction and renal cancer	Telomere length in lymphocytes is associated with renal cancer.	Shao et al. (2007)
598 participants of the Leiden 85-plus study (average age = 89.8)	Telomere length in leucocytes is not associated with morbidity or mortality in the oldest old.	Martin-Ruiz, Gussekloo, van Heemst, von Zglinicki, & Westendorp (2005)
812 participants (652 twins 73–101 years of age	In twin pairs, the twin with shorter telomere died first. No association between telomere length and survival in this study.	Bischoff et al. (2006)
Lothian Birth Cohort	Telomere length is not associated with age-related physical and cognitive decline or mortality.	Harris et al. (2006)
Scottish Mental Survey (n = 190, born 1921)	Short telomere length is associated with heart disease in old people.	Starr et al. (2007)
183 healthy controls and 620 chronic heart failure patients	Telomere length in PBMCs is shorter in chronic heart failure patients and related to severity of the disease.	van der Harst et al. (2007)
West Scotland Coronary Prevention Study (n = 484)	Short telomere length is associated the risk of coronary heart disease. Statin treatment attenuates the association.	Brouilette et al. (2007)
Newcastle 85+ study	Telomere length is associated with left ventricular function in the oldest old.	Collerton et al. (2007)
Chennai Urban Rural Epidemiology Study (India)	Short telomere length is associated with impaired glucose and diabetic macroangiopathy.	Adaikalakoteswari, Balasubramanyam, Ravikumar, Deepa, & Mohan (2007)
Cardiovascular Health Study	Short telomere length associated with diabetes, diastolic blood pressure, carotid intima-media thickness, and IL-6.	Fitzpatrick et al. (2007)
2509 Caucasian of Askelpios study (n = 35–55 free of overt CVD)	No association between telomere length and cholesterol and blood pressure. Short telomeres are associated with levels of inflammation and oxidative stress markers. Shorter telomere length is associated with unhealthy lifestyle in men.	Bekaert et al. (2007)
Women ages 18–79 (N = 2150)	Telomere length in leukocytes positively correlates with bone mineral density. Shorter telomere length is correlated with osteoporosis.	Valdes et al. (2007)
1086 from TwinsUK Adult Twin Registry	Short telomeres in leucocytes is associated with radiographic hand osteoarthritis.	Zhai et al. (2006)
Caucasian men ages 40–89 from the Framingham Heart Study (N = 327)	Shorter telomere length associated with hypertension, increased insulin resistance, and oxidative stress.	Demissie et al. (2006)
Women ages 18–79 (N = 1517)	Insulin resistance, leptin, and C-reactive protein levels are inversely related to leucocyte telomere length in premenopausal women, but not in postmenopausal women.	Aviv et al. (2006)
Type II diabetes (N = 21) and control (N = 29)	Mean monocyte telomere length in the diabetic group is lower than in control, without significant differences in lymphocyte telomere length.	Sampson, Winterbone, Hughes, Dozio, & Hughes (2006)
Young adults of the Bogalusa Heart Study	Telomere attrition is correlated with insulin resistance and changes in the body mass index.	Gardner et al. (2005)
Women ages 18–76 (N = 1122)	Shorter telomere length is associated with obesity and cigarette smoking.	Valdes et al. (2005)

Hypertensive men (N = 163)	Telomeres are shorter in hypertensive men with carotid artery plaques than hypertensive men without carotid artery plaques	Benetos et al. (2004)
N = 143	Short telomeres associated with high rates of mortality from cardiovascular disease and infection.	Cawthon, Smith, O'Brien, Sivatchenko, & Kerber (2003)
Premature myocardial infarction (N = 203) and control (N = 180)	Telomeres are shorter in leucocytes in premature myocardial infarction than controls.	Brouilette, Singh, Thompson, Goodall, & Samani (2003)
10 patients and 20 controls	Telomere length in white blood cells shorter in patients with severe coronary artery disease.	Samani, Boultby, Butler, Thompson, & Goodall (2001)
49 twin pairs from the Danish Twin Register	Short telomere length in leucocytes correlates with high pulse pressure.	Jeanclos et al. (2000)

Telomerase and Cancer

The finite life span of somatic cells in multicellular organisms has been suggested to be an antitumor mechanism to prevent accumulation of mutations that leads to cancer. However, the secretion of cancer-promoting factors by senescent human cells, as described earlier, argues against this notion. Therefore, it is uncertain whether replicative senescence, caused by critically short telomeres, is a tumor suppression mechanism in humans. Telomerase activity is essential for tumor growth since in over 90% of human cancers, telomerase becomes upregulated during tumorigenesis (Kim et al., 1994), while the rest adopt recombination pathways to maintain their telomere length (ALT for alternative lengthening of telomeres; Bryan, Englezou, Dalla-Pozza, Dunham, & Reddel, 1997). Yet cancer cells often have shorter telomeres than adjacent normal cells (de Lange et al., 1990; Joshua et al., 2007). A commonly invoked model for tumorigenesis, although unproven in humans, is that in normal cells, short telomeres induce replicative senescence, a p53-dependent DNA damage response that results in cell cycle arrest, to serve as antitumor mechanism. Rare events of inactivation of p53 allow cells to bypass this short telomere-induced arrest, known as M1, and continue to grow. Further shortening of telomeres leads to a second cell cycle arrest, M2, also known as crisis. At M2, most cells die from apoptosis, but a few escape by reactivation of telomerase, thereby become cancer cells (Figure 65.5).

Recent clinical studies have also linked short telomeres in PBMCs or, in some cases, buccal cells to greater risk factors for various cancers, including bladder, head and neck, lung, breast and renal cancer (Broberg, Bjork, Paulsson, Hoglund, & Albin, 2005; Shao et al., 2007; Shen et al., 2007; Wu et al., 2003). It is not clear whether the short telomeres in PBMCs and buccal cells reflect genetic predisposition to cancer and/or environmental factors thought to contribute to high cancer risks (e.g., oxidative stress and chronic inflammation).

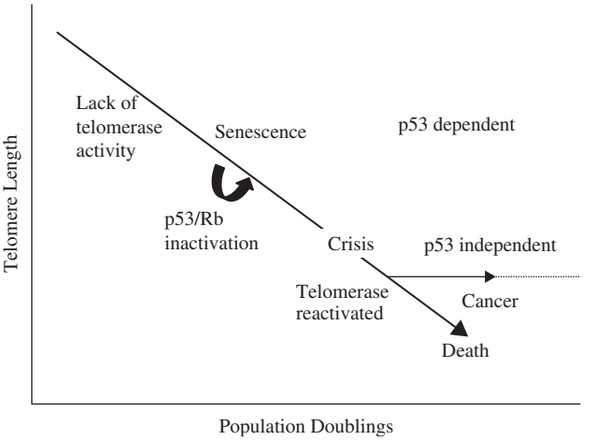

Figure 65.5 One model for telomere maintenance and tumorigenesis.

Telomerase Knockout and TERT Overexpression Mice as Models for Roles of Telomere and Telomerase in Aging-Related Diseases

Studies using telomerase knockout mice have provided essential evidence for the role of telomere and telomerase in health. The first telomerase knockout mouse was created by deleting the RNA component of telomerase mTER (Blasco et al., 1997). As this lab strain of *Mus musculus* normally has over 50 kb-long telomeres (Prowse & Greider, 1995), it was not surprising that the first three generations of TERC-/- did not show any early cytogenetic, morphologic, or physiological phenotypes, despite the expected telomere shortening due to the lack of telomerase activity. However, a notable phenotype is that G1 knockout mice have shorter life span despite their still-long telomeres, indicating that telomerase may have telomere-length independent roles in longevity. Successive breeding of the mTERC-/- mice resulted in phenotypes characterized by deficiencies in tissue renewal. At the sixth generation (G6), TERC-/- mice are

sterile, with males exhibiting testicular atrophy and declined spermatogenesis, while the females have a decreased number of oocytes. In the hematopoietic system, reduced progenitor cell numbers, spleen atrophy, and decreased proliferation of T and B cells on induction by mitogens were reported (reviewed in Blasco, 2005). Thus the phenotypes exhibited in late generation mTERC-/- mouse support the role of telomerase in maintaining telomere length required for cell proliferation for tissue renewal.

The phenotypes of mTERC-/- mouse with regard to cancer seem to be complicated. Chromosomal end-to-end fusions of critically short telomeres, which lead to massive genome instability, were observed in late generation mTERC-/- mice. In addition, gross chromosomal rearrangements, such as nonreciprocal translocations, a common feature of human cancer, were also found. This is consistent with the proposal that for human cancers, critically short telomeres lead to chromosome fusion and breakage—fusion-bridge cycles. Consistent with the role of short telomeres in promoting cancer, these mice have higher incidences of tumors. However, the growth of tumors derived from mTERC-/- mice is decreased compared with wild type, in agreement with the notion that upregulation of telomerase is required for cancer cell growth. Thus telomerase appears to have antagonistic effects on tumorigenesis.

Mice overexpressing the telomerase core protein gene, mTERT, had increased death risk during the first half of their life due to increased tumorigenesis, but had extended life span in the second half of life. These long-lived mice had decreased degenerative lesions in testis, uterus and ovary, and kidney (Gonzalez-Suarez, Flores, & Blasco, 2002; Gonzalez-Suarez, Geserick, Flores, & Blasco, 2005; Gonzalez-Suarez et al., 2001). Notably, kidney dysfunction is a common cause of death in elderly humans.

These findings in telomerase knockout and TERT overexpression mice strengthen the idea that a delicate balance is important to ensure just the right amount of telomerase activity. When the activity is too low, it is not sufficient to allow proliferation of renewable tissues or full protection from cancer, thus contributing to premature aging and aging-related diseases. However, at least in mice, too much telomerase activity predisposes the organism to higher cancer incidence.

Telomerase and Human Genetic Diseases

The strongest evidence suggesting a direct role of telomerase and telomere maintenance in human aging and aging-related diseases came from studies of the rare multisystem disorder *dyskeratosis congenita* (Vulliamy et al., 2006). Classically, dyskeratosis congenita (DC) is characterized by a triad of mucoctaneous symptoms: abnormal skin pigmentation, nail dystrophy, and mucosal leukoplakia. However, a host of other symptoms, including hair graying and loss, pulmonary disease, and predisposition to cancer, were also reported. Patients die of eventual failure of the hematopoietic system (bone marrow failure). Three genetic forms of DC were reported: most DC patients have the X-linked recessive form, caused by mutations in the gene for dyskerin, a protein required for pseudo-uridylation of ribosomal RNA, which is also a component of the mammalian cellular telomerase RNP complex. Of interest here, a more rare genetic type of DC is autosomal-dominant and is caused by mutations in the RNA or protein component of telomerase (TERC or TERT). The apparent autosomal-dominant inheritance mode is due to haploinsufficiency for telomerase activity. Most recently, the genetic cause for one subtype of the autosomal recessive DC is reported to be mutations in another telomerase-associated protein, NOP 10 (Walne et al., 2007).

It has now become clear that the primary molecular basis for the defects in all forms of DC studied so far lies in a deficiency for telomerase activity, which leads to shorter telomeres, especially in the affected tissues. Over a dozen hTER deletion or point mutations have now been reported in DC patients. Using a reconstituted cell-free system that expresses the DC-forms of hTER, or by introducing the DC mutant copy of hTER into a cell line that does not have endogenous hTER, several labs have demonstrated that mutant hTER found in DC patients leads to reduced telomerase activity (Comolli, Smirnov, Xu, Blackburn, & James, 2002; Fu & Collins, 2003; Ly et al., 2005; Marrone, Stevens, Vulliamy, Dokal, & Mason, 2004). Similarly, mutations in the protein subunit of telomerase were also reported in DC patients (Marrone et al., 2007). Retroviral expression of hTER and/or hTERT extended telomere length and rescued DC cells from premature senescence (Westin et al., 2007).

Interestingly, even in the X-linked form of DC caused by dyskerin mutations, telomerase RNA level appears to be the limiting factor for telomere length maintenance (Wong & Collins, 2006), as reintroduction of the wild type hTER and hTERT into cells from DC patients restores the cells to normal rRNA processing and proliferation. DC patients have shorter telomere length than unaffected family members and patients with the severest phenotypes have shorter telomeres than patients with milder symptoms (Vulliamy et al., 2006). Furthermore, autosomal-dominant DC families show an earlier age of onset and more severe disease phenotypes in succeeding generations (Armanios et al., 2005), a phenomenon called disease anticipation. This is consistent with the progressive shortening of telomeres over generations.

Analysis of the immune system of a large family with autosomal dominant DC caused by a TERC mutation

revealed immune abnormalities including severe B lymphopenia and decreased immunoglobulin M (IgM) levels, and T cells that overexpressed senescent cell surface markers. In vitro culturing of the cells from these DC patients showed their lymphocytes had reduced proliferative capacity and increased basal apoptotic rate (Knudson, Kulkarni, Ballas, Bessler, & Goldman, 2005).

Diseases caused by deficient telomerase activity are not limited to family histories of hereditary disorders. Sporadic cases of bone marrow failure syndromes including aplastic anemia (Ly et al., 2005; Xin et al., 2007), melodysplastic syndrome (Field et al., 2006; Ortmann et al., 2006; Yamaguchi, 2006, 2007), and essential thrombocytemia (Ly et al., 2005) were also found to have mutations in hTERC or hTERT. The spectrum of diseases caused by telomerase mutations has now been broadened to include idiopathic pulmonary fibrosis (Armanios et al., 2007).

What Does the Length of Telomeres in Humans Really Reflect?

The length of telomeres is determined by several factors: telomeres that were inherited (genetic), level of telomerase, environmental factors that influence the rate of attrition and telomerase activity, and number of cell divisions (history of division). In the following subsections, we discuss common genetic variations, replicative history, and biochemical environment factors that contribute to person-to-person variation in telomere length and rate of telomere shortening.

Genetic Transmission

A study in 115 twin pairs, 2 to 63 years of age, indicated a 78% heritability for mean telomere length in this age cohort (Slagboom, Droog, & Boomsma, 1994). In 2,050 unselected women aged 18 to 80 years, comprising 1,025 complete dizygotic twin pairs, telomere length was reported to have 36% to 90% heritability (Andrew et al., 2006). In another study of 383 adults including 258 twin pairs, the heritability of telomere length was reported to be 81.9%±11.8% (Vasa-Nicotera et al., 2005). While the preceding studies suggested autosomal genes contribute to telomere length heritability, some reports also suggest X-linked modes of inheritance (between fathers and daughters, between mothers and sons and daughters; and among siblings, but not between father and son). A paternal inheritance (father-son and father-daughter) of telomere length was found in Old Order Amish people (Njajou et al., 2007). However, a study examining telomere length in an elderly population of 686 males including monozygotic (MZ) and dizygotic (DZ) twins reported no evidence of heritable effects, but rather that telomere length was largely associated with shared environmental factors (Huda et al., 2007). While it is still not known why there are discrepancies among findings from different groups, it is likely that it is due to different populations and different ages when the telomeres were measured. It seems plausible that the older the general population, the more environmental impacts might override genetic influences. Further, while there is strong genetic transmission of telomere length, it is not known whether maternal telomere length (e.g., in immune cells) is transmitted to offspring through nongenetic means. In other words, can short telomeres due to environmental exposures in mothers be transmitted through nongenetic or epigenetic means (Epel, in press).

Replication History

Examination of telomere length in various human tissues has suggested that the rate of telomere length attrition roughly reflects the rate of cell turnover in the tissue. A rapid telomere shortening in peripheral blood cells was seen in the first year of life (Frenck, Blackburn, & Shannon, 1998; Rufer et al., 1999), although it is not clear that this reflects higher cell turnover rates. The rate of telomere attrition in adults is estimated to be 31 to 62bp/year (Takubo et al., 2002). Telomeres are shorter in patients with diseases characterized by high cell turnover rates compared with their age-matched peers, including chronic viral infection: HIV, CMV, lupus, and rheumatoid arthritis (Nakajima et al., 2006; Steer et al., 2007). In brain tissues, no evidence for telomere shortening was seen in a cross-sectional study comparing adults of different ages (Allsopp et al., 1995). However, human adrenocortical cells, which divide continuously throughout life, showed a strong age-related decline in telomere length (Yang, Suwa, Wright, Shay, & Hornsby, 2001). The average rate of telomere attrition in PBMCs in women is lower than in men, when measured in cross-sectional studies. It is not clear why, but it has been suggested that estrogen, which is known to upregulate telomerase, may play a role in slowing telomere attrition.

Lifestyle Factors

Lifestyle factors may affect telomere length, such as factors that promote obesity, which is linked to shorter telomeres (Valdes et al., 2005). Cigarette smoking is linked to both shorter telomere length (Valdes et al., 2005) and lower telomerase (Epel et al., 2006). Exercise has been associated with longer leukocyte telomere length (Cherkas et al., 2008). No studies have examined other health behaviors such as alcohol use, although given their relations with insulin and obesity, it is likely that nutrition, dysregulated eating patterns, and overeating would all affect cell aging. Dietary restraint, which is linked to stress, cortisol, and dysregulated eating patterns, is linked to shorter telomere length (Kiefer et al., 2008). It appears that nutrition in early

life may affect telomeres, at least in certain tissues such as kidney (Jennings, Ozanne, & Hales, 2000). Protein restriction while in utero was related to shorter telomeres, whereas protein restriction during early life was related to longer kidney telomeres (Jennings et al., 2000).

Lastly, psychological stress is related to shorter PBMC telomeres. Our group has found this both in young maternal caregivers (Epel et al., 2004) and in elderly dementia caregivers (unpublished data). Others have now replicated this finding in dementia caregivers (Damjanovic et al., 2007), in major depression (Simon et al., 2006), and in stressed mice (Kotrschal, Ilmonen, & Penn, 2007). The mediating pathways are unknown, but likely involve many of the pathways described earlier; both behavioral (such as poor nutrition and fitness, and insufficient sleep) and biochemical pathways are affected by stress. Telomere length is not equivalent to a biological measure of stress, which becomes obvious when we take into account the myriad other factors that modulate telomere length. Telomere length is, however, reflective of stress, as well as of a multitude of other biological, and environmental factors, extending from early in life to throughout the life span.

Possible Mechanisms Relating Telomere Maintenance to Aging-Related Diseases

With the growing body of literature demonstrating the link between telomere dysfunction and aging-related diseases, we might start to ask how mechanistically telomere dysfunction is related to, and contributes to, aging-related diseases. As discussed, telomere shortening is the end result of a multitude of pathways, making attribution of cause and effect difficult. In a notable exception, our group has found evidence that chronicity of psychological stress is quantitatively associated with the degree of telomere shortness, thus implicating chronic psychological stress in a causative role in telomere shortening. Beyond that, the causality of the relationships between telomere shortness and disease and disease risk factors is likely to be challenging to unravel.

In human studies, telomere shortness in leukocytes has been studied extensively. As well as having the practical advantage of being readily obtained, these cells may be closely linked to disease processes. Here we consider three possible, nonmutually exclusive, mechanisms that could link leukocyte aging to risk for aging-related diseases—in particular, cardiovascular diseases:

Mechanism 1: cellular aging in leukocytes simply reflects the same process that is ongoing in cells of other tissues, but does not directly contribute to the aging or disease development of those tissues. Just like cells of the immune system, accumulation of senescent cells limits the proliferation capacity of other tissues that require self-renewal and repair, including tissue stem and progenitor cells. It has been shown in recent years that even in organs that are believed to be postmitotic, progenitor cells are involved in repair after damage or even normal functions. Cardiac stem cells are involved in repair from ischemic injury (Anversa, Kajstura, Leri, & Bolli, 2006; Leri, Kajstura, & Anversa, 2005), and neuronal progenitor cells in the hippocampus may be involved in memory (Kempermann & Gage, 2000).

Mechanism 2: The same detrimental factors (e.g., stress hormones, oxidative stress, proinflammatory cytokines and other risk factors) that cause telomere dysfunction in leukocytes cause damage to cells of other organs through different mechanisms. Therefore, leukocyte telomere dysfunction is not the contributing factor, but rather, it serves as a cellular readout for these damaging factors (telomere dysfunction may be a surrogate marker of other damaging factors that cause aging-related disease). Telomere length thus reflects the cumulative assault the cells receive over the cause of life; particular candidates of interest are oxidative stress and proinflammatory cytokines, as they are known to be associated with CVD. Results from clinical studies are now showing a consensus that PBMC telomere length is linked to the family of biochemical factors reflecting metabolic stress, such as oxidative stress, proinflammatory cytokines, insulin and leptin (Aviv et al., 2006; Bekaert et al., 2007; Demissie et al., 2006; Fitzpatrick et al., 2007). Telomere length in PBMCs may therefore be predictive of morbidity and mortality, in that it reflects cumulative effects, as opposed to the current status.

Mechanism 3: Senescent cells of the immune system secrete proteins detrimental to the surrounding cells. Immunosenescent CD8+CD28- cells are known to produce high levels of proinflammatory cytokines IL-6 and TNF-α. Since local inflammation plays a pivotal role in some aging-related disease including atherosclerosis (Tedgui & Mallat, 2006), the senescent cells of the immune system may contribute to cardiovascular disease through this pathway. In addition, senescent fibroblasts were shown to secrete growth factors that promoted cancer cell growth, providing another potential contributor to the increased risk of cancer with age.

MEASUREMENT OF CELL AGING AND POTENTIAL CONFOUNDING FACTORS

Accurate measurement of telomere length and telomerase activity is crucial, but prone to pitfalls with current

techniques. So for the immediate future, behavioral researchers will need to rely on expertise specifically in these measurements, as well as expert study design to obtain interpretable results.

Telomere Length Measurement

As with any assay, a new lab should test their measure and its reliability against a gold standard lab. Small differences in blood or other tissue collection, reagent solution freshness, DNA quality, and storage conditions have all been found to affect telomere length measurement accuracy. Currently, Southern blot analysis, quantitative-PCR based (Cawthon, 2002) and q-FISH (quantitative fluorescence in situ hybridization (Poon and Lansdorp, 2001) methods are used to measure telomere length. A detailed review of these methods can be found in a recent review (Canela, Klatt, & Blasco, 2007).

Telomerase Detection

The most commonly used method to detect telomerase activity is Telomere Repeat Amplification Protocol (TRAP), developed by Kim and colleagues (Kim et al., 1994; Kim & Wu, 1997). A commercial kit, called TRAPeze, based on the design of the TRAP method, is available from Chemicon, Inc. The TRAP method has been adapted to run on quantitative PCR platforms. Nonetheless, the sensitivity of the method does not reach that of the gel-TRAP method and this lack of sensitivity has to date precluded its use in quantitative measures of telomerase activity in clinical samples of normal human cells such as PBMCs. In the future, further development of this method may render it (or an adaptation) usable for such clinical samples. A detailed review of methods to detect telomerase activity can be found (e.g., Fajkus, 2006).

A second important consideration is that interpretations of telomere length will be affected by subject selection and sample collection. Because measuring cell aging in vivo, in humans, is a new field, we know relatively little about effects of hormones, medications, certain diseases, current infections, and lifestyle factors. Therefore, it is essential either to carefully rule out or to measure these confounding factors. Large studies that have not carefully quantified such health history factors that may leave imprints in telomere length or affect telomerase activity regulation may only epick up the largest effects. For example, the effects of smoking, a large source of oxidative stress, and medications like statins, which are known to alter telomerase, would likely override effects of psychosocial factors, which are typically of smaller magnitude.

SUMMARY AND CONCLUSIONS

The telomere/telomerase maintenance system will be an important focus of behavioral neuroscience research in the coming years. Telomere length and telomerase together appear to be cellular indicators of the potential for viability and self-renewal of cells. We have reviewed the multitude of factors that influence telomere length throughout the life span, including genetics and early nutrition, as well as adulthood obesity, chronic life stress, and biochemical factors. With an in-depth understanding of telomere length and of telomere maintenance by telomerase; their fundamental biological, environmental, and behavioral modifiers; and accurate measurement, behavioral scientists have a valuable role to play in shedding further light on the interwoven environmental, psychological and behavioral modifiers of cell aging.

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