Physiol Rev 88: 557–579, 2008;
doi:10.1152/physrev.00026.2007.

Telomeres and Aging

GERALDINE AUBERT AND PETER M. LANSDORP

Terry Fox Laboratory, British Columbia Cancer Agency, and Division of Hematology, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

Aubert G, Lansdorp PM. Telomeres and Aging. *Physiol Rev* 88: 557–579, 2008; doi:10.1152/physrev.00026.2007.—Telomeres play a central role in cell fate and aging by adjusting the cellular response to stress and growth stimulation on the basis of previous cell divisions and DNA damage. At least a few hundred nucleotides of telomere repeats must "cap" each chromosome end to avoid activation of DNA repair pathways. Repair of critically short or "uncapped" telomeres by telomerase or recombination is limited in most somatic cells and apoptosis or cellular senescence is triggered when too many "uncapped" telomeres accumulate. The chance of the latter increases as the average telomere length decreases. The average telomere length is set and maintained in cells of the germline which typically express high levels of telomerase. In somatic cells, telomere length is very heterogeneous but typically declines with age, posing a barrier to tumor growth but also contributing to loss of cells with age. Loss of (stem) cells via telomere attrition provides strong selection for abnormal and malignant cells, a process facilitated by the genome instability and aneuploidy triggered by dysfunctional telomeres. The crucial role of telomeres in cell turnover and aging is highlighted by patients with 50% of normal telomerase levels resulting from a mutation in one of the telomerase genes. Short telomeres in such patients are implicated in a variety of disorders including dyskeratosis congenita, aplastic anemia, pulmonary fibrosis, and cancer. Here the role of telomeres and telomerase in human aging and aging-associated diseases is reviewed.

> In the future attention undoubtedly will be centered on the genome, and with greater appreciation of its significance as a highly sensitive organ of the cell, monitoring genomic activities and correcting common errors, sensing the unusual and unexpected events, and responding to them, often by restructuring the genome.
>
> Barbara McClintock, 1983

I. INTRODUCTION

A. Telomeres From Cytogenetics to Replicative Senescence: Historic Background

That chromosome ends play an important role in ensuring chromosome stability was first proposed in the 1930s by Barbara McClintock working with maize (142) and Hermann Muller working with fruitflies (155). Both investigators proposed that chromosome ends have special structures required for chromosome stability. Muller coined the term *telomere*, from the Greek for "end" (*telos*) and "part" (*meros*). McClintock noted that without these special end structures, chromosomes would fuse and often break upon mitosis, and she observed that the resulting chromosome instability was detrimental to cells. These pioneering studies established that functional "telomeres" are required to protect chromosome ends, to provide chromosome stability, and to ensure faithful segregation of genetic material into daughter cells upon cell

division. These conclusions have stood the test of time, and since this work was published, an enormous amount of data on telomeres and their function have been produced. Some of the most striking contributions are reviewed here. However, despite this progress, it is also clear that many mysteries around telomeres and their function remain. The increasing amount of detail about individual molecules and pathways involved in telomere biology and DNA damage responses has not at all diminished the challenge of understanding how telomeres are integrated and involved in DNA damage responses, cellular fitness, and human aging. While it has become clear that telomeres play a central role in the cellular response to stress and DNA damage, neither the relative importance to other factors nor all the connections between proteins and signaling pathways that directly or indirectly involve telomeres are fully understood. The future of telomere research is bright!

In the early 1960s, Leonard Hayflick observed that human cells placed in tissue culture stop dividing after a limited number of cell divisions by a process now known as replicative senescence (90, 92; reviewed in Ref. 89). He proposed that the cell culture phenomenon could be used as a model to study human aging at a molecular and cellular level. However, the role of replicative senescence in human aging and the relevance of the in vitro studies remained subject to much debate. Cells presumably divide either to balance normal cell loss or in response to injury. Many cells in the human body can divide many more times than needed during a normal lifetime. A mitotic "reserve capacity" was used as an argument against the idea that replicative senescence has any relevance to human aging. However, one would not expect all (stem) cells in the body to have a similar replicative history (or potential), and cells that no longer exist (or can no longer divide) are easily overlooked. It has furthermore been difficult to estimate the actual turnover of the stem cells in tissues such as the intestine and hematopoietic stem cells over a normal lifetime with any degree of accuracy. Estimates range from more than 1,000 times for intestinal epithelial cells in rodents (170) to less than 100 times for hematopoietic stem cells in humans (115). Recent studies of the levels of ^{14}C remaining in tissues from nuclear weapons test during the Cold War have shown that the turnover of blood cells far exceeds that of the cells in the gut (197), and these data seem incompatible with thousands of cell divisions. Uncertainties about actual turnover and the fact that model organisms such as worms and flies clearly "age" without cell renewal being a major factor have been used to question the role of cell turnover and replicative senescence in human aging. However, as will be discussed, the tight association of telomeres to overall cellular fitness does not exclude a role for telomeres even in the aging of tissues that contain mostly long-lived postmitotic cells such as the brain, heart, or kidney. For example, it is possible that damage to telomeric DNA by reactive oxygen species (ROS) produced by either dysfunctional mitochondria (85, 220) or by signaling pathways (e.g., overexpression of oncogenes such as Ras, Refs. 152, 239) contributes or predisposes cells to apoptosis and senescence. Thus DNA damage signals originating from telomeres could be replication independent, and the sensitivity of cells to DNA damage could increase as the overall telomere length declines. More information is needed on the role of telomeres in the cellular response to various types of insults (177).

Why most primary human cells in culture stop dividing after a limited number of divisions remained a puzzle for another decade. The first scientist to link the programmed cessation of cell division observed by Hayflick to replication of telomeres was Alexei Olovnikov (163). He proposed that human somatic cells might not be able to correct the chromosomal shortening that occurs when cells replicate DNA and that the repeated nucleotide sequences at telomeres could act as a buffer to protect downstream genes from such replication losses. He furthermore had the remarkable insight to propose that the length of the repeated sequences could determine the possible number of DNA replication rounds. That the unidirectional nature of DNA replication poses a problem for complete replication of chromosome ends was also recognized by Watson (224). It would take another two decades before the predicted causal link between replicative senescence and telomere shortening was formally established. The first observations connecting telomeres directly to aging were made in 1986 when Cooke and Smith (48) noticed that the average length of telomere repeats capping sex chromosomes in sperm cells was much longer than in adult cells. They considered the possibility that adult cells could be deficient in the enzyme telomerase which had just been discovered in the unicellular organism *Tetrahymena* (79). Several studies in the next few years confirmed a reduction in average telomere length with cell divisions in fibroblasts (49, 83) and with age in somatic cells from the blood and colon (87), but not in the cells of the germ line. These observations supported the conclusion that somatic cells are apparently unable to maintain telomere length. For the first time, the aging of cells could be linked to readily detectable and reproducible changes in genomic DNA. Similar observations were subsequently made with cells from many other human tissues (reviewed in Refs. 91, 98).

The correlation between telomere length and replicative potential became a mechanistic link when it was demonstrated that the replicative potential of primary human fibroblasts can be extended indefinitely by artificially elongating telomeres. The latter was achieved in primary human fibroblasts by overexpression of the telomerase reverse transcriptase (*hTERT*) gene (25, 211). These experiments established that progressive telomere

loss is indeed the major cause of replicative senescence as had been proposed earlier (3, 84).

B. About This Review

Telomeres used to be obscure functional elements at chromosome ends studied by a few eccentric scientists.[1] Telomere research now has become (almost) "mainstream" with many more papers published on the topic (indicated by >5,000 articles in PubMed) than can be reviewed in a comprehensive manner. This represents a serious challenge for a review in which it is attempted to link the telomere field to the even larger fields of (stem) cell biology and human aging (both "stem cells" and "aging" yield over 100,000 hits in PubMed). Furthermore, the ever-increasing amount of data in all areas of science makes a balanced review that ranges from molecules, cell biology, to aging an impossible task. For the interest of readers with diverse backgrounds, we have decided to focus this review as much as possible on original reports as well as recent observations related primarily to human telomere biology. Most basic principles about the role of telomeres in cells were discovered in various model organisms. We apologize to all colleagues whose work is not discussed, and we strongly encourage readers to study the review articles that are cited and the papers cited in those reviews to get a more complete picture of the field.

We start with a discussion of the structure and function of telomeres and the methods that are available to measure telomere length. It is important to note that, despite the increasing realization that telomeres are important in human aging and cancer, the actual amount of information on telomere length in different human cell types of normal individuals in relation to their age is surprisingly modest. Thus the field is relatively "data poor." This is because all available methods to delineate telomere length have limitations and do not measure what is presumably the most important parameter: the few (?) short telomeres that may result in cell death or senescence if elongation cannot be achieved either by recombination or by telomerase. After briefly discussing the reasons why telomere length decreases with cell division and with age, we focus on the assembly and function of the telomerase reverse transcriptase enzyme complex. We then discuss heritable and stochastic variation in telomere length and the age-related decline in telomere length that has been documented in various human tissues. The consequences of inherited telomerase deficiencies and the resulting telomere dysfunction are presented next. Finally, we discuss the prospects of interventions and novel therapies that target telomeres or telomerase.

[1] Telomeric as well as centromeric sequences continue to be underrepresented in most of the genomes that have been "completely" sequenced.

C. Aging and Evolution

Aging can be defined as the progressive functional decline of tissue function that eventually results in mortality. Such functional decline can result from the loss or diminished function of postmitotic cells or from failure to replace such cells by a functional decline in the ability of (stem) cells to sustain replication and cell divisions. Aging is not a disease, and the biology of aging, which varies between individuals, is best understood in the context of evolution. The Disposable Soma model provides a useful framework for such considerations (109). This model proposes that an increase in longevity in mammals is due to a concomitant reduction in the rates of growth and reproduction and an increase in the accuracy of synthesis of macromolecules. The notion that the fidelity of DNA repair is subject to selective forces and not necessarily better than (strictly) needed for a particular cell type, tissue, or species is not easily grasped. Differences in the fidelity of DNA repair pathways between cells of the germ-line and somatic (stem) cells and between comparable somatic cells from small, short-lived animals and large, long-lived species greatly complicate generalizations about the molecular mechanism of aging across different species. Limitations in the use of model organisms to study the role of telomeres in human aging are perhaps best illustrated by the different consequences of telomerase deficiencies in humans and various model organisms. In laboratory mice (*Mus musculus*), Baker's yeast (*Saccharomyces cerevisiae*) (126), mustard plants (*Arabidopsis*) (172), and roundworms (*Caenorhabditis elegans*) (43), complete loss of telomerase is tolerated for at least several generations. In contrast, a modest twofold reduction in telomerase levels in humans (e.g., resulting from haploinsufficiency for one of the telomerase genes) is now known to cause severe clinical symptoms including aplastic anemia, immune deficiencies, and pulmonary fibrosis after one to three generations. The indirect relation between clinical phenotype and mutations in genes that affect telomere length or telomere maintenance has been confusing to many and certainly has greatly complicated genetic linkage analysis. As a result, the involvement of abnormalities in telomeres and telomere biology in human disease is probably underestimated. A major objective of this review is to set the stage for future studies of telomeres and telomerase in relation to (stem) cell turnover, tissue function, and aging.

The progressive loss of telomeric DNA in human somatic (stem) cells is believed to act as a tumor suppressor mechanism that limits clonal proliferation, prevents clonal dominance, and ensures a polyclonal composition of (stem) cells in large, long-lived multicellular organisms. Unfortunately, limits to the clonal expansion of somatic (stem) cells also provide strong selection for cells that can ignore or bypass the "telomere" checkpoint (214),

e.g., because their DNA damage responses are defective. Such cells can continue to grow despite the presence of dysfunctional telomeres. The loss of telomere function in such cells results in chromosome fusions, broken chromosomes, break-fusion bridge cycles, translocations, and aneuploidy. This genetic instability allows selection of cells with abnormal growth characteristics and also facilitates rapid acquisition of genetic alterations that provide further growth advantages (60, 185). Thus, while telomere loss may act as a tumor suppressor mechanism, it also promotes tumor growth by driving selection of cells with defective DNA damage responses (e.g., loss of p53; Ref. 8). The aneuploidy and genomic rearrangements in cells with short telomeres and defective DNA damage responses complicate the analysis of the molecular changes that are most relevant for tumor growth initiation and progression. The fact that loss of telomere function has consequences both for aging and carcinogenesis (199) explains much of the current interest in telomeres. The interconnections between normal and dysfunctional telomeres and intracellular signaling pathways involved in DNA damage responses and DNA repair involving proteins such as ATM, ATR, and p53 (216) support a view of telomeres as pivotal dynamic elements required for genome stability that determine how a cell responds to stress and growth stimulation.

II. TELOMERE STRUCTURE AND FUNCTION: OVERVIEW

A. Telomere Structure and Function

Linear chromosomes pose a general challenge: how to protect the natural ends of chromosomes from breakdown and degradation and avoid recognition and processing as double-strand breaks. There are many different solutions to this problem, ranging from covalently closed hairpin ends in some viruses, bacteria, and phages (111) to specific transposable elements in certain insects (167). However, in organisms as diverse as protozoan, fungi, mammals, and plants, telomeres consists of G-rich repetitive DNA maintained by a specialized reverse transcriptase enzyme called telomerase. A detailed discussion of the structure and function of telomeres and telomerase in model organisms is outside the scope of this review, and the reader is referred elsewhere (102, 173, 206). While many excellent reviews also exist regarding telomeres and telomerase in mammals (see, e.g., Refs. 38, 47, 195), some further discussion is needed here to provide a context for understanding the consequences of telomere shortening and telomerase deficiencies in humans.

1. Telomere binding proteins

The DNA component of telomeres is characterized in all vertebrates by tandem repeats of $(TTAGGG/CCCTAA)_n$ (153). Telomeric DNA typically ends in a single-strand G-rich overhang of between 50 and 300 nucleotides at the 3′ end, which has been proposed to fold back onto duplex telomeric DNA forming a "T-loop" structure (80). The length of the repeats varies between chromosomes and between species. In humans and mice, the length of telomere repeats at individual chromosome ends in individual cells is strikingly variable (15, 116, 241). Human chromosome ends are typically capped with between 0.5 and 15 kilobase (kb) pairs of detectable telomere repeats depending on the type of tissue, the age of the donor, and the replicative history of the cells. Individual ends of human chromosomes show marked variation in telomere length (Fig. 1) and the average length varies between chromosome ends. For example, chromosome 17p typically has shorter telomeres than most other chromosome ends (26, 137). In human nucleated blood cells, the average telomere length shows a highly significant decline with age that is most pronounced for the cells of the immune system (Fig. 2). Telomeres prevent the ends of linear chromosomes from appearing as DNA double-strand (ds) breaks and protect chromosome ends from degradation and fusion. It has been proposed that telomeres can switch between an open state (in principle allowing elongation by telomerase) and a closed state (inaccessible to telomerase) with the likelihood of the open state inversely related to the length of the repeat tract (21). A model of how telomeres and telomerase interact in a telomere length-dependent manner is shown in Figure 3. This model is supported by data in yeast (205). Recent studies in this model organism suggest that the timing of telomere replication is important for elongation by telomerase (18).

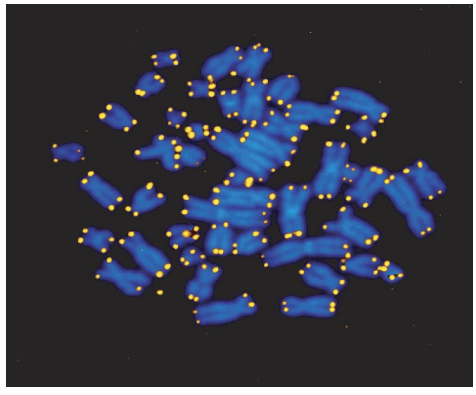

FIG. 1. The length of telomere repeats at individual chromosome ends is highly variable. Telomere repeats in a normal human lymphocyte are visualized using quantitative fluorescence in situ hybridization (Q-FISH) using peptide nucleic acid probes (116). Telomeres are shown in yellow, whereas the DNA of chromosomes, counterstained with DAPI, is shown in blue. Note that the fluorescence on sister chromatid telomeres is typically of equal intensity in line with expectations for quantitative hybridization.

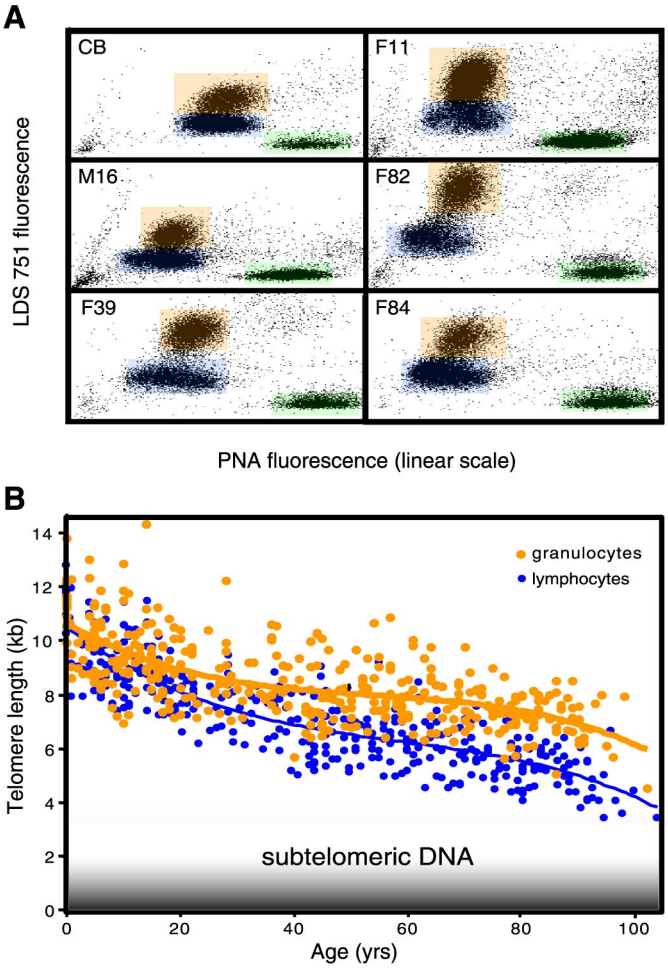

FIG. 2. The telomere length in human granulocytes and lymphocytes from human peripheral blood declines with age. *A*: bivariate flow cytometry analysis of the nucleated blood cells (CB, cord blood; M, male; F, female; number, age) hybridized with fluorescently labeled $(CCCTAA)_3$ peptide nucleic acid (PNA) probe specific for telomere repeats counterstained with LDS751. For details, see Ref. 13. The LDS fluorescence allows discrimination between granulocytes (orange shaded boxes), lymphocytes (blue shaded boxes), and bovine thymocytes (green shaded boxes). Results from separate experiments are shown to illustrate how inclusion of aliquots of the same internal control cells (bovine thymocytes, with known telomere length) in every tube is used to correct for day-to-day variation between experiments to obtain accurate calculations of the median telomere length in selected cell populations. Note that the telomere fluorescence in granulocytes and lymphocytes is similar early in life but that, as the proportion of memory T cells relative to naive T cells increases with age, the telomere fluorescence in lymphocytes becomes increasingly short relative to granulocytes. *B*: results of calculated median telomere fluorescence in lymphocytes (orange dots) and lymphocytes (blue dots) of 400 normal individuals over the entire age range (Baerlocher and Lansdorp, unpublished data). Note that the telomere length is highly variable at any given age and shows a biphasic decline with age. Most likely hematopoietic stem cells proliferate rapidly early in life followed by a marked decrease in turnover in infancy (12). The acceleration in telomere attrition over the age of 60 is as yet unexplained.

A large number of proteins have been found to directly or indirectly associate with telomeric DNA (Table 1). Some of these proteins, such as TRF1, TRF2, TIN2, TPP1, Rap1, and POT1 (59), can be found at telomeres at any time, although the dynamic exchange between telomere-bound and unbound proteins can be high. For example, fluorescence recovery after photobleaching (FRAP) of TRF1 tagged with fluorescent protein takes less than a minute (140). FRAP studies also showed that POT1 and TRF2 bind to telomeric DNA in at least two different modes: one unstable (rapid exchange with unbound proteins) and one more stable mode (slow exchange with unbound proteins). Differences in binding modes presumably reflect differences in structures and protein (abundance) at telomeres to which these proteins bind, for example, single-strand G-rich DNA versus double-strand telomeric repeats (Fig. 3). Other important telomere proteins or protein complexes, such as the telomerase enzyme complex, associate with telomeric DNA only transiently (Fig. 3). Much progress has been made in the last decade regarding the characterization of specific proteins at telomeres and their role in telomere function (59). Many proteins that are known to (transiently) associate with telomeric DNA have roles outside telomeres, and the factors regulating their interactions and traffic are incompletely understood. Most likely, posttranslational protein modifications including phosphorylation, dephosphorylation, poly-ADP ribosylation, and deribosylation, acetylation, ubiquitination, sumoylation, etc., are crucial for the accumulation of specific proteins at telomeres during specific stages of the cell cycle. Modulation in cellular and nuclear protein levels related to protein turnover, gene expression, and other variables that are difficult to measure independently in single cells, greatly complicate the development of an integrated view of telomere function in relation to specific proteins and cell function. Many "telomeric" proteins can be found at cytoplasmic and nontelomeric nuclear sites, and some proteins appear to localize at telomeres for yet unknown reasons. In general, the "cross-talk" between the many proteins involved in telomere function and various cellular signaling pathways is poorly understood. Challenges are differences in the recruitment of specific proteins to telomeric DNA between primary (diploid) cell types and the immortalized cell lines that are typically studied in the laboratory. Such differences complicate generalizations about the function of proteins that have been found to associate with telomeric DNA. A discussion of the individual proteins that are known to bind to telomeric DNA and that are an integral part of telomere function is outside the scope of this review. The reader is referred to excellent recent reviews on these topics (59, 196).

2. *Telomerase*

Telomerase is a specialized reverse transcriptase capable of extending the 3′ end of chromosomes by adding TTAGGG repeats (10, 47). The human core enzyme consists of a reverse transcriptase protein (TERT) of 1,132

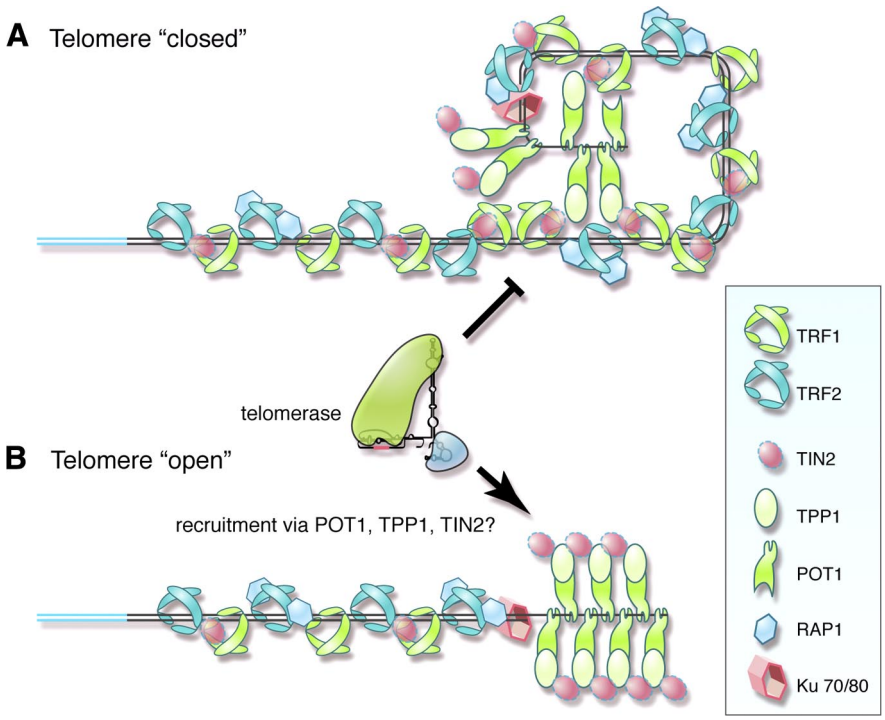

FIG. 3. Telomere function is linked to telomere length via the proteins that interact with double-strand telomere repeats (homodimers of TRF1 and TRF2), proteins that bind to the 3′ single strand G-rich overhang present at the very end of chromosomes (POT1), and proteins that interact with these proteins such as RAP1, TPP1, and TIN2 as well as the Ku70/80 complex present at the junction of single- and double-stranded telomeric DNA. *A*: telomere "closed". TPP1 and POT1 form a complex with telomeric DNA via TIN2 and TRF1/2 if the length of telomere repeat tract is sufficiently long. TRF1 and -2 are known to bend telomeric DNA (5, 19), and telomerase access is proposed to be blocked in a repeat length-dependent manner. *B*: telomere "open." The TPP1/POT1 recruits and stimulates enzymatic activity of telomerase (222) preferentially at short telomeres (205).

amino acids encoded by the *hTERT* gene (86, 147, 158), located on chromosome 5p15.33 and telomerase RNA containing 451 nucleotides (including the CAAUCCCAAUC telomere template) encoded by the telomerase RNA gene *hTERC* (74), located on chromosome 3q21-q28 (see Figs. 3 and 7). The ribonucleoprotein dyskerin (encoded by the *DKC1* gene on the X chromosome) is required for proper folding and stability of telomerase RNA (228) and was recently found to be part of the basic human telomerase enzyme complex (45). Both the reverse transcriptase and telomerase RNA are expressed at very low levels, and haplo-insufficiency for either gene or mutations in *DKC1* can give rise to various clinical manifestations (see sect. IV). Telomerase levels are regulated at multiple levels including transcription, alternative splicing, assembly, subcellular localization, and posttranslational modifications of various components and of the enzyme complex itself. Expression of *TERT* is stimulated by c-Myc and estrogen and suppressed by Rb and p21. Multiple splice forms of *TERT* have been described with some having a dominant negative effect on telomerase activity (46). Many questions about the efficiency of the assembly of fully functional as well as inactive telomerase complexes and the regulation of the subcellular trafficking of such complexes by posttranslational modification also remain largely unexplored. As a result, the relative importance of the such factors that have been proposed to affect the activity of telomerase at telomeres is difficult to discern, and the relative importance of such factors could vary between cell types. Another complicating factor is that the likelihood of a functional interaction between telomerase and repetitive DNA at telomeres is almost certainly also regulated at the level of telomere chromatin, an emerging research topic of much interest.

TABLE 1. *Proteins found at human telomeres*

Proteins	Reference Nos.
TRF1	47, 256
TRF2	22, 30, 138, 254
TIN2	118, 135
Rap1	118, 135
TPP1	108, 239, 248
POT1	18
Transient presence at telomeres	
Rif1	89, 207, 250
ERCC1/XPF	258
Mre11/Rad50/Nbs1	219
WRN helicase	54, 143, 178, 179
BLM helicases	179, 214, 253
DNA-PK	56, 109, 110, 172, 258
PARP-2	60
Tankyrases	44, 71, 114, 208, 209, 254
Rad51D	220
Apollo	133, 226

"Resident" proteins, part of the "Shelterin" complex (62).

3. *Telomeres and DNA damage responses*

When the telomere pioneer Barbara McClintock received the Nobel prize in 1983 for her work on transposable genetic elements in maize, she referred in her acceptance speech to the importance of responses of the genome to challenges (143). She concluded her lecture with:

"In the future attention undoubtedly will be centered on the genome, and with greater appreciation of its significance as a highly sensitive organ of the cell, monitoring genomic activities and correcting common errors, sensing the unusual and unexpected events, and responding to them, often by restructuring the genome. We know about the components of genomes that could be made available for such restructuring. We know nothing, however, about how the cell senses danger and instigates responses to it that often are truly remarkable." While studies in the general areas of DNA repair, DNA damage responses, and apoptosis have all progressed tremendously, it is doubtful whether we are very much closer to an integrated view of the role of the genome in general and telomeres in particular in relation to how cells respond to stress of various kinds. Studies on p53, one of the major components of the response to stress, have highlighted that this protein has a very broad role in normal development and tumor formation, life expectancy, and overall fitness (216). DNA damage signals are known to originate from short telomeres (54, 203) and contribute to p53 activation and the cellular responses to stress. The telomere binding protein TRF2 binds to ataxia telangiectasia mutated (ATM) kinase and can inhibit its function (106), yet DNA damage signals appear to originate from telomeres with each replication cycle (213). It has been proposed that telomeres switch between closed and open states (21) as is illustrated in Figures 3 and 4: perhaps the likelihood of the open state is proportional to the overall telomere length of the repeat tract (214). As telomere length decreases with age, the amount of DNA damage signals originating from short telomeres is expected to increase (Fig. 4). Higher "background" levels of activated p53 could decrease the threshold for activation of senescence or apoptosis in "old" cells, in line with the increased sensitivity to stress and more fragile nature of cells and tissues from the elderly. The role of telomeres in cellular aging relative to other proposed molecular mechanisms of aging including oxidative stress resulting from mitochondrial dysfunction or loss of ribosomal function remains to be precisely

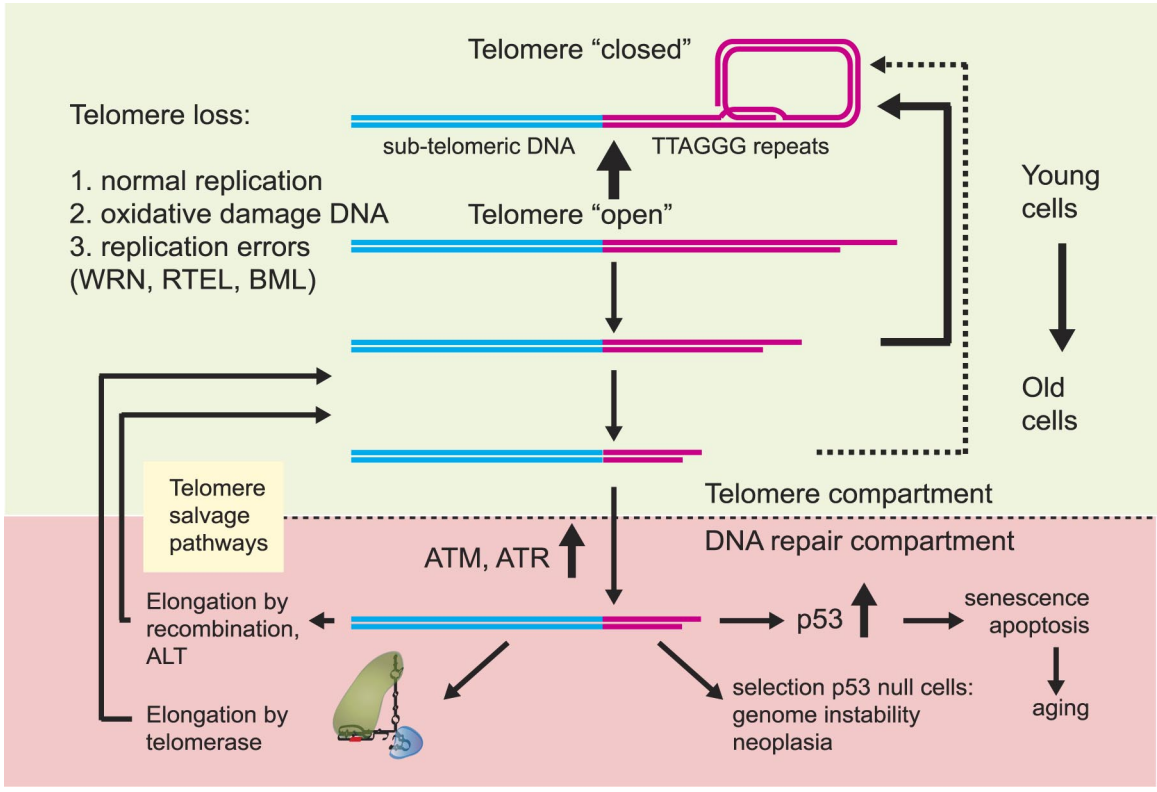

FIG. 4. Diagram of factors affecting the telomere length in primary somatic cells from human tissues. According to the model shown, telomeres in "young" somatic cells have long tracts of telomere repeats that favor folding into a "closed" structure that is invisible to the DNA damage response pathways and telomerase. As the telomere length at individual chromosome ends decreases, the likelihood that telomeres remain "closed" also decreases (see also Fig. 3). At one point telomeres become too short and indistinguishable from broken ends. Such ends will be processed by enzymes in the DNA repair compartment (proposed to occupy a different nuclear domain than long telomeres). Depending on the cell type and the genes that are expressed in the cell, a limited number of short ends can be elongated by limiting levels of telomerase or recombination. However, with continued cell division and telomere loss, eventually too many short ends accumulate for the limited capacity of these "telomere salvage pathways." At this point, defective telomeres will trigger levels of DNA damage signals such as p53 to which cells respond by either apoptosis or senescence. Rare (mutant) cells that do not upregulate functional DNA damage responses (e.g., by loss of functional p53) continue cell divisions in the presence of dysfunctional telomeres causing genome instability via chromosome fusions, chromosome breaks, and repetitive break-fusion bridge cycles.

delineated. The development of an integrated view of the various molecular mechanisms of aging that have been proposed remains as formidable a challenge. However, it has become clear that telomeres are directly responsible for sustained DNA damage signals in senescent cells (54, 203), and DNA damage foci originating from telomeres in senescent cells can readily be detected in vivo (104).

4. Telomere length measurements

All methods to measure telomere length rely on the binding of nucleic acid probes or primers to telomere specific DNA repeat sequences (summarized in Table 2).

Telomere restriction fragment analysis (TRF) was the first methodology devised to estimate the average telomere length of a cell population (3, 83, 84) and is a reference for all methods that have been set up since. It exploits the specificity and redundancy of telomere DNA repeats by digesting genomic DNA with common restriction enzymes that cannot use telomere repeats as a substrate. The DNA fragments produced by digestion are resolved by gel electrophoresis, Southern blotted and probed with a labeled telomere oligonucleotide probe (typically complimentary to 3 or 4 telomere repeats) to reveal a telomere specific smear. The median length of this smear can then be estimated by comparison with known size markers. The method requires a relatively large amount of DNA and has low resolution, but it has the benefits of a simple design and has no requirements for specialized laboratory equipment. However, the relatively large amount of material needed to measure telomere length by TRF has limited its use mostly to cell lines and broad research questions, and the method is of limited use for screening of telomere length in clinical samples.

Quantitative fluorescence in situ hybridization or Q-FISH using image cytometry and metaphase chromosomes (Fig. 1; Refs. 137, 169) as well as in situ hybridization and flow cytometry or flow FISH (Fig. 2; Refs. 13, 180) both use directly fluorescently labeled $(CCCTAA)_3$ peptide nucleic acid (PNA) probes as a high-affinity alternative to DNA oligonucleotide probes that specifically hybridize to denatured telomere DNA repeat arrays. The fluorescent signal can then be detected and measured relative to standards of known telomere length in metaphase spreads with specific software for Q-FISH image analysis (169) for Q-FISH (freely available at www.flintbox.com), or, following hybridization in suspension by flow cytometry (flow FISH, Ref. 13). Q-FISH is the method of choice for high-resolution telomere length measurements at specific chromosome ends (Fig. 1). Q-FISH has also been used to detect ends without detectable repeats ($<$0.5 kb) as well as chromosome fusion events. The technique is well established to study telomere biology in many settings (8, 81). By comparison, flow FISH accurately mea-

TABLE 2. *Summary of telomere length measurements and necessary requirements*

Method	Cell Number Required	Estimate of Telomere Length and Resolution	Resolution	Additional Information	Time, days	Reference Nos.
Telomere restriction fragment analysis (TRF)	1–3 $\times$ 10^6(0.5–10 μg DNA)	Mean length for total cell population	1 kb	NA	2	3, 90
Quantitative-fluorescence in situ hybridization (Q-FISH)	Actively dividing cells for chromosome spread (cell type dependent)	Cell average length and chromosome end-specific distribution	0.3 kb	Antibody staining, alternative probe hybridization strand-specific CO-FISH	3	150, 184
Fluorescence in situ hybridization and flow cytometry (Flow FISH)	0.5–2 $\times$ 10^6 freshly isolated or frozen cells	Cell-specific average length	0.5 kb	Antibody staining, mitosis tracking comparison between subpopulations	2	14, 196
Single telomere length analysis (STELA)	1 to 1 $\times$ 10^5, no viability requirements	Single chromosome end-specific length	0.1 kb	Allele-specific average length and distribution	2	16
Telomere Q-PCR	Blood sample	Relative product ratio to single copy gene	In theory, 76 bp	NA	1	38

NA, not available.

sures the median telomere length in individual cells in suspension (Fig. 2) and can be used to measure the telomere length in distinct cell populations within a single sample by antibody staining (for a very limited set of cell surface markers that are retained after the hybridization procedure; alternatively, specific cell populations can be cell sorted prior to flow FISH). Flow FISH has been adapted for higher throughput by using a semi-automated 96-well format with a robotic microdispenser to reproducibly perform some of the steps in the procedure (13). Flow FISH has become the method of choice for the measurement of telomere length in peripheral blood cells from human samples and has allowed the determination of the normal range of telomere lengths for specific cell subsets (11) that are being used both for research and for clinical investigations (4, 28, 29, 76, 233, 234). Data on telomere length relative to age are particularly useful in the context of the cells of the immune system (discussed in sect. III, illustrated in Fig. 5) as well as in cases of suspected telomerase deficiencies (4) (see Fig. 6). Although both Q-FISH and flow FISH offer the most complete quantitative data related to telomere length currently available, both techniques require specialized equipment, and both methods are time consuming as well as labor intensive. New molecular strategies to assess telomere length have recently been reported: single telomere length analysis (STELA) (15) and Q-PCR (35). Both methods rely on specific PCR amplification of telomere repeats. While PCR products are directly quantified in Q-PCR, STELA requires gel electrophoresis to separate the amplified products that are characterized by Southern blot hybridization. STELA can be used to measure the size distribution of single telomeres using primers for chromosome-specific subtelomeric DNA, while Q-PCR estimates the average telomere length by measuring the ratio of telomere amplicons relative to single-copy gene amplicons. Although the Q-PCR method is very attractive for its shorter timeline, more data are required to establish its precision. STELA offers the most precise measurement of telomere length currently available, can be used with very few and even single cells, and has the advantage that it can detect short outlier telomeres in a sample. However, STELA is very labor intensive, technically

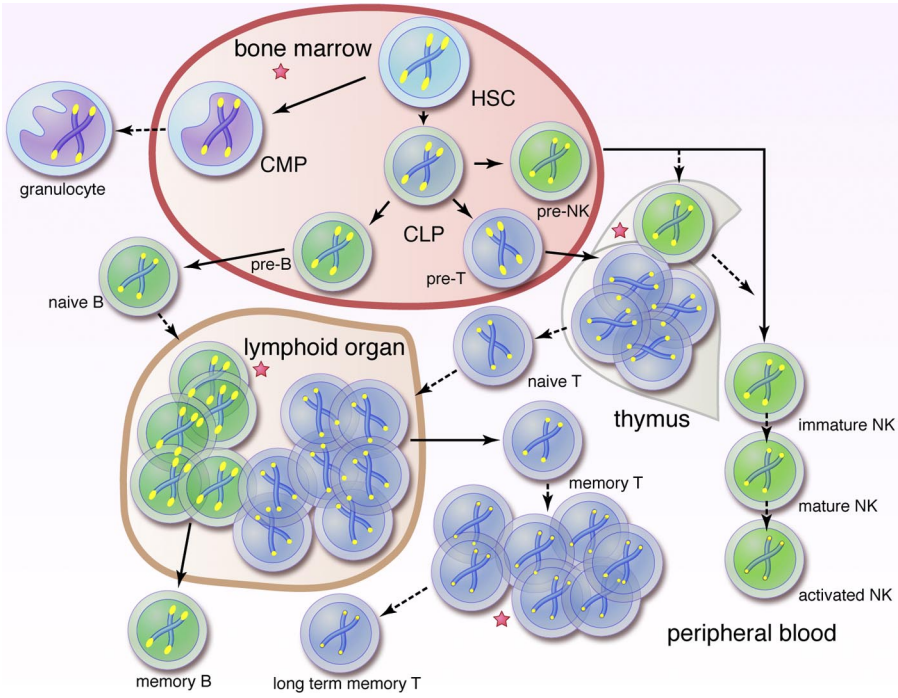

FIG. 5. Schematic diagram of telomere length in the hematopoietic system. The hematopoietic system can be subdivided into a hierarchy of distinct populations. The least differentiated cells are the hematopoietic stem cells (HSC) which have the longest telomere length (212) (bright yellow spots on a "representative" metaphase chromosome: blue). HSCs differentiate and produce committed progenitor cells [common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) are shown], or contribute to the long-term maintenance of hematopoiesis by self-renewal divisions. The telomerase activity (relative amounts indicated by red stars) in stem and progenitor cells is insufficient to maintain telomere length over time. Telomere length also decreases over the course of differentiation and expansion; the telomere length in stem cells remains linked to that in granulocytes (*top left*), while lymphocytes show a more pronounced decline, reflecting a larger number of cell divisions only compensated in part by relatively high telomerase activity in the thymus and secondary lymphoid organs (such as lymph nodes) during clonal expansion. An interesting exception is memory B cells, which in the elderly often have telomeres that are longer than can be found at birth (135, 214). Terminally differentiated cell types (memory or activated cells) display the shortest telomeres, phenotypes associated with reduced replicative potential, reduced function, and senescence. These characteristics accumulate and are accentuated with aging.

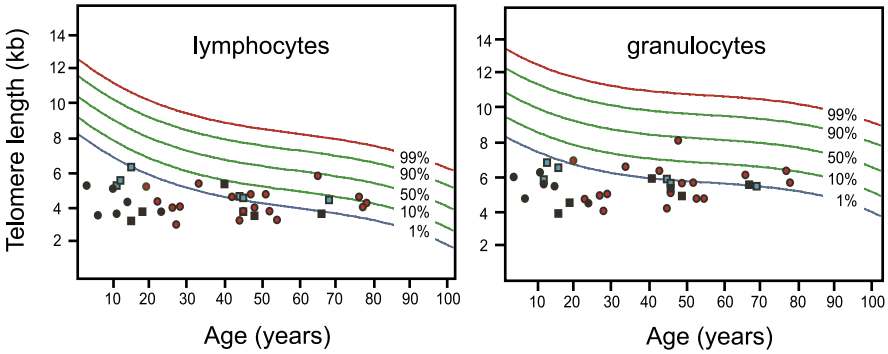

FIG. 6. Telomere length in leukocytes from normal individuals and in individuals with known mutations in telomere genes. The age-related loss of telomere length in lymphocytes (*A*) and granulocytes (*B*) from the peripheral blood of 400 normal individuals measured by flow FISH shown in Fig. 2 was used to establish the distribution of telomere length in the normal population using a best-fit approach (red, green, and blue curves representing expected telomere length for the indicated proportion of normal individuals). Lymphocyte and granulocyte telomere length in patients with a known telomerase gene mutation measured in the context of several studies are shown. Each symbol represents an individual patient diagnosed with clinical symptoms associated with dyskerin (*DKC1*) gene mutation (black circle), *hTERT* mutation (red circle), and *hTERC* mutation (black square). Some individuals have a mutation in one copy of the *hTERC* gene but no clinical symptoms (blue square) (4, 6, 129, 231, 234). The majority of individuals that carry mutations in telomerase genes display critically short telomeres, nearly all of them below the 10th percentile of the normal distribution and a majority of these below the first percentile (typically for both cell subsets shown). Note that individuals with early onset of disease (in the first three decades of life) show the most striking difference between observed and expected telomere length. The observations point to a critical median telomere length threshold below which the proliferative capacity and function of the cells in tissues is failing. Judging from the best-fit curves, such failure would not be expected to occur before the eighth decade of life in normal individuals.

demanding, and is limited to chromosome ends for which specific primers and probe sets can be designed.

B. Telomere Length Variation

One of the most striking features of telomeres revealed by Q-FISH is the heterogeneity in the length of telomere repeats at individual chromosome ends (Fig. 1). Some of this diversity is generated in somatic cells and not in the germline (114), and specific chromosome ends in clonally derived cells can show an almost complete loss of telomere repeats (136). Sporadic telomere losses complicate the relationship between telomere length and cell division history and potential. It is important to realize this uncertainty in the context of aging. Some of the unresolved questions are as follows: How does repeat length variation at individual ends relate to progressive telomere loss? How important are sporadic loss events relative to the more predictable replicative loss events in overall telomere loss with age, replicative senescence, and apoptosis in somatic cells? Does the presence of (very) long telomeres support repair of short and dysfunctional telomeres by recombination in the absence of telomerase? This explanation was proposed to explain the survival of inbred laboratory mice that lack telomerase (113), where a clear phenotype only develops when the average telomere length is less than half of that in wild-type animals (23). If recombination is indeed used to salvage short telomeres in the absence of telomerase, how frequent are exchanges between telomeric DNA in different chromosomes and between sister chromatids in different cells and in different tissues? Of note, some diseases in humans cannot be reproduced in the mouse unless the specific genetic defect is introduced in mice that have short telomeres or as a result of a telomerase deficiency. This is exemplified by mice deficient for the *WRN* gene (40), the *BLM* gene (66), and by the carcinomas that can only be observed in murine models when telomeres are short (9, 73). In humans, mutational inactivation of the *WRN* gene causes Werner syndrome, an autosomal recessive disease characterized by premature aging, elevated genomic instability, and increased cancer incidence. *Wrn*-deficient mice show no clear phenotype and a classical Werner-like premature aging syndrome typified by premature death, hair graying, alopecia, osteoporosis, type II diabetes, cataracts, and nonepithelial cancers is only observed in late-generation mice that are null with respect to both *Wrn* and *Terc*. Recent studies have provided further support for the idea that the WRN protein plays a direct role in telomere replication (51). However, the precise role of the WRN protein in relation to the disease phenotype remains uncertain (14).

1. *Heritable variation in telomere length*

The average telomere length was shown to be a heritable trait in several studies (78, 179, 192). However, heterogeneity in telomere length at individual chromosome ends is also generated in the germline. For example, it was shown in studies of male littermate mice that the length of telomere repeats at the Yp chromosomes (no confusion about parental origin) was very similar for three but strikingly different for one animal in all tissues

analyzed (241). However, most allele-specific relative telomere length is presumably heritable (78), and variation generated in the germline is probably the exception rather than the rule. Q-FISH has been used to study the length of telomere repeats on specific human chromosomes (137). Remarkably, the heterogeneity in telomere length was not resolved by studying specific chromosome ends: any given chromosome end showed a large variation in the number of telomere repeats, although the average length was found to vary significantly between chromosome arms. Interestingly, chromosome 17p was found to have relatively short telomeres in 10 individuals tested (26, 137). This observation suggests that the 17p telomere will be one of the first to become "uncapped" upon progressive telomere shortening with proliferation and age. However, the rate of telomere erosion also seems to vary between chromosome ends. This is illustrated by inactive X chromosomes in female cells that were found to show accelerated telomere loss relative to autosomal chromosomes and the active X chromosome (201). This observation suggests that differences in the average length of specific chromosomes are in part generated during proliferation and with age. The factors involved in such chromosomes-specific differences are poorly understood but could be related to differences in (sub)telomeric DNA or chromatin, repair by telomerase or recombination, timing of replication, or a combination of these factors.

In general, Q-FISH studies have underscored the importance of having normal chromosome ends capped with a minimum number of telomere repeats (probably in the order of 200–300 base pairs) to prevent replicative senescence, loss of cells, genome instability, and end-to-end fusions of chromosomes characterized by lack of telomere repeats as the junction site (81, 221).

2. Telomere length regulation

The heterogeneity in telomere length in chromosomes of normal cells has complicated studies on the role of factors that regulate telomere length. In general, the length of telomere repeats reflects the balance between additions and losses of telomere repeats. Telomerase and recombination can elongate telomeres, but in most somatic cells additions are outbalanced by losses. Apart from the proteins that bind to telomeres (Table 1), telomerase levels, and telomeric chromatin status, there are a multitude of other possible factors that in principle can codetermine whether telomerase functionally interacts with telomeres.

Telomere loss is typically explained as resulting from incomplete DNA replication (the "end replication problem") (163, 224), and the processing of chromosome ends following replication (124, 132) is sometimes mentioned as well. Studies reporting that (oxidative) damage of telomeric DNA could be the major cause of telomere shortening in human cells (215) are less frequently cited, perhaps because these findings complicate notions about telomere loss acting as a simple "mitotic clock." The guanine-rich nature of telomeric DNA makes it particularly vulnerable to oxidative damage (94, 162). Telomeric proteins are known to actively suppress DNA damage responses in yeast (148), and it is possible that certain lesions in telomeric DNA are encountered only during replication, possibly resulting in variable losses of telomere repeats. Recently, two other mechanisms of telomere shortening were proposed: the failure to unwind or correctly process higher order structures of G-rich telomeric DNA (51, 62) and the deletion of T-loops by homologous recombination (223). The factors and pathways involved in the repair of replication forks that are stalled or collapsed at telomeres are not well understood. Repair during S phase could involve either telomerase or homologous recombination pathways, perhaps including proteins such as the Fanconi proteins, BRCA1 and BRCA2. The relative importance of such DNA repair pathways in the repair of genomic and telomeric DNA in different cell types is an important area for further studies. Studies in this general area should be encouraged because a better understanding of the mechanisms of telomere attrition may yield viable strategies to minimize the inevitable loss of telomeric DNA with age. Such strategies could have significant health benefits without the risks of strategies aimed at elongation of telomeres.

III. LOSS OF TELOMERIC DNA WITH AGE: OVERVIEW

Loss of telomeric DNA at the cellular level is well established and was shown to be related to replicative history and life span in somatic cells (see sect. II and Figs. 2 and 4). However, at the level of tissues or of the entire organism, what is the impact of telomere shortening? Does aging cause telomere shortening, or does telomere shortening cause aging (98)? The issue of organismal aging as a consequence of short telomeres was raised as a concern when Dolly, "cloned" by transfer of an adult mammary gland nucleus into an enucleated egg, was shown to have short telomeres (189). In contrast, nuclear transfer experiments using nuclei from senescent bovine fibroblasts yielded offspring with longer than expected telomeres and a "youthful" phenotype (117). Differences in donor nucleus cell type, nuclear transfer methodology, or species could explain these discrepant results (1, 103, 112). However, the "immortal" growth properties of embryonic stem cell lines derived from preimplantation embryos of many species suggest that telomere length can be maintained or telomere loss attenuated in early development. The loss of telomere repeats in human cells with

age varies greatly between cells and tissues, and the amount of information for different tissues is often very limited. It has been proposed that the number of cell divisions in stem cells is <100 divisions over a human lifetime and that this efficiency is achieved by a strict hierarchy at the level of stem cells with the most primitive cells dividing the least and having the longest telomeres (115). A diagram representation of this model is shown in Figure 7.

A. Hematopoietic Stem Cells

Stem and progenitor cells are required to sustain blood cell formation throughout life. Hematopoietic stem cells were first described in the late 1950s with the advent of bone marrow transplantation (208). The procedure involves the transfer of donor bone marrow containing hematopoietic stem cells to restore hematopoiesis following total marrow ablation (achieved by total body irradiation) in recipients. In 1994 it was shown that the telomere length in purified hematopoietic stem cells shows an age-related decline (212). This study also demonstrated (by TRF analysis) that "candidate" stem cells with a $CD34^+CD38^-$ phenotype have longer telomeres than more mature $CD34^+CD38^+$ progenitor cells in adult bone marrow. For this study "candidate" stem cells were purified from 2×10^{10} organ donor bone marrow cells, a scale of purification that is difficult to reproduce for stem cells of other tissues. Subsequent studies showed that $CD34^+CD38^+$ cells express telomerase at high levels compared with the more primitive $CD34^+CD38^-$ cells or the more mature cells (69). In a murine model, telomerase expression was required to slow telomere shortening and allow consecutive serial hematopoietic stem cell transplants (2). Overexpression of telomerase in this model, while stabilizing telomere length, did not prevent senescence or increase the serial transplantability of stem cells. These observations indicate the existence of telomere-independent senescence pathways in hematopoietic cells, which were also postulated to limit the life span of human $CD4^+$ T cells that overexpress telomerase (174). Further investigations of telomere biology in human hematopoietic stem cells are challenging since these cells are not readily available for study. Even the best purification

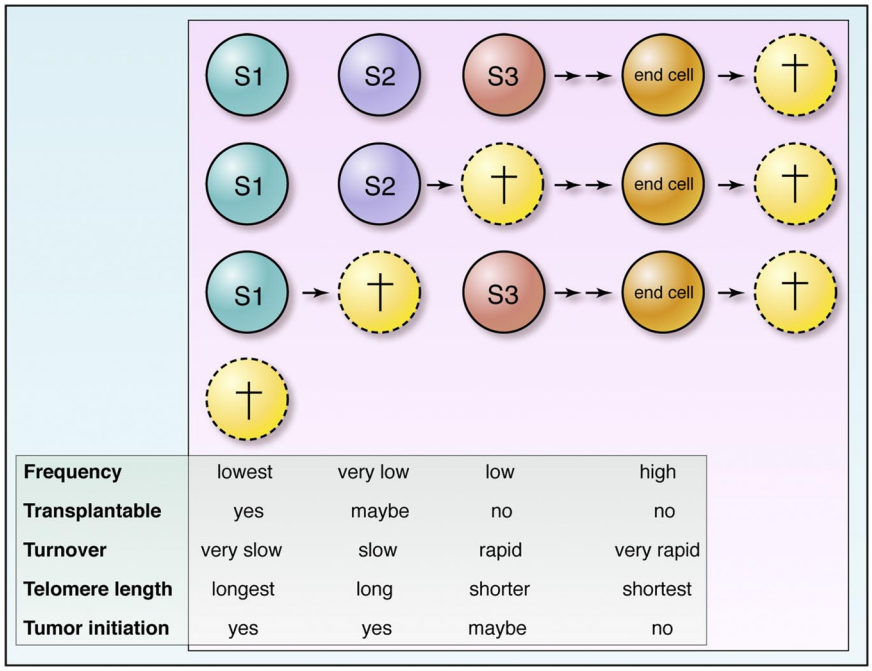

FIG. 7. Organization of adult stem cells into a mitotic hierarchy. On the basis of observations in mice and humans, it was proposed that hematopoietic stem cells divide rapidly during fetal development and infancy to build a reservoir of stem cells that are used economically during adult life by sequential recruitment (115). In this model, the ability of the most primitive stem cells (S1) to sustain hematopoiesis was proposed to be eventually limited by progressive telomere shortening. Note that the three stem cells shown (S1–S3) represent an arbitrary number and that these stem cells are expected to have different properties in terms of requirements for a specific microenvironment, growth factors, gene expression, sensitivity to apoptosis, etc. The number of cell divisions between the most mature stem or progenitor cell (S3 in the model) and end cells is expected to be variable (indicated by 2 arrows). By extrapolation, the number of cell divisions in the stem cell compartment of other tissues with high turnover such as skin and gut could also be kept to a minimum (e.g., <100) by succession of stem and progenitor cells with an increasingly lower turnover rate. Note that according to this model the ability of some stem cells to reconstitute tissues upon transplantation is not shared by all stem cells capable of initiating tumors. Abnormal turnover of more mature stem cells or loss of telomere function, e.g., resulting from inherited telomerase deficiency, could eventually result in very few or no remaining primitive stem cells (S1), resulting in oligoclonal or monoclonal tissue formation and in the selection of abnormal clones.

strategies do not yield pure suspensions of human stem cells, and the number of cells available for telomere length analysis is also typically very limited. The telomere length in peripheral blood leukocytes has been used as a surrogate for measurements directly in hematopoietic stem cells. Assuming that the number of cell divisions separating stem cells from granulocytes is relatively constant, the telomere length in readily available granulocytes has been used to study the proliferation and replicative history of stem cells. The number of divisions separating lymphocytes from stem cells is more variable and increases with age, most likely reflecting a higher turnover of immune cells relative to stem cells (Fig. 2). The telomere length in both granulocytes and lymphocytes at any given age was found to be highly variable, and the overall decline in both cells types with age was found to follow a biphasic curve stabilizing in midlife with a more rapid decline in infancy and in the elderly (see Figs. 2 and 5). The rapid decline in infants was most pronounced in the first years of life (179), and this finding was recently confirmed in a longitudinal study of young primates (baboons), where a steep decline in granulocyte telomere length during the first 50–70 wk (reflecting a high turnover of hematopoietic stem cells) was followed by a marked drop in telomere attrition (12). Rapid proliferation of hematopoietic stem cells is also observed in aplastic anemia (where marrow stem and progenitors are thought to be actively depleted through an autoimmune response) (30) or in the first year following bone marrow transplantation (178).

With new methodologies to assess relative telomere length by Q-PCR, studies were designed to address the impact of telomere length on aging, aging associated pathologies, and mortality. One such study has correlated shorter leukocyte telomere lengths at age 60 with a three times higher risk of heart disease and an eightfold increase in risk of infection-related death (36), thereby associating measured relative cellular aging with disease and life expectancy. In a similar way, chronic stress was shown to correlate with short leukocyte telomere length, a phenomenon attributed to higher levels of oxidative stress at the cellular level (70). More recent studies have linked telomere length in smooth muscle cells with senescence and disease severity in patients with atherosclerosis (141, 150). Leukocyte telomere length was also short in a cohort of similar patients and associated with a higher risk of developing occult cardiovascular disease (71). More data are needed to understand and validate the use of leukocyte telomere length as a biomarker for cardiovascular and other diseases.

B. Cells of the Immune System

Telomerase activity is easily detected in cells of the immune system upon stimulation (Fig. 5). This is thought to occur in a tightly controlled manner to support massive bouts of clonal expansion required for fending off infections as well as maintaining long-term immunological memory (reviewed in Refs. 95, 227). Nevertheless, overall telomere attrition is not prevented in T cells. Similarly to T cells, NK cells showed proliferation and differentiation-mediated telomere length decrease (166). In contrast, germinal center B cells and activated B cells were shown to have longer telomeres than their precursor cells (135, 225; see Fig. 5). The study of these leukocyte populations has benefited from the quantitative measurement of telomere length to help establish the cellular hierarchy for specific immune cell phenotypes (179, 180, 226) and more recently to delineate novel markers. For example, a subset of apoptosis resistant Epstein-Barr virus (EBV) specific T cells with short telomeres that express CD45RA and markers of memory T cells can be distinguished from CD45RA-positive naive T cells with longer telomeres (67). Other studies on telomere length dynamics in immune cells have pointed to specific aspects of "immune aging" in the context of latent or chronic infections. By selecting ex vivo EBV antigen-specific cytotoxic CD8 T cells from blood during acute infectious mononucleosis, it was shown that telomere length was maintained during the acute phase of the infection, but subsequently decreased over time (131, 168). In the context of a recalled memory response to tuberculin, telomere length of helper CD4 T cells was found to be lower at the site of active immune response (skin) compared with similar cell populations found in the peripheral blood of the same vaccinated volunteers (171). These observations suggest that during the initial phases of adaptive immune responses, telomerase activity could maintain the proliferative capacity of responding cells; however, this does not appear to be sufficient to maintain telomere length over time when recalled. Interestingly, late-generation telomerase-deficient (*Terc* knockout) mice have immunosenescent phenotypes in T and B cells that correlate with reduced proliferative capacity (22). It was possible to partially correct these defects and enhance CD8 T cell-mediated specific cytolysis in the context of human immunodeficiency virus (HIV) after overexpression of telomerase in these cells in vitro (56). Taken together, these findings suggest that approaches targeting telomerase could be useful for in vitro expansion of lymphocytes for cell transfer therapies. Culture conditions differ significantly from the biological setting, and telomerase activity was shown to decrease in culture over time. However, inhibition of telomerase by a dominant-negative mutant greatly accentuates telomere loss and limits life span of cell clones (176). Current trials of tumor-specific immunotherapy have demonstrated that the efficacy and maintenance of melanoma tumor infiltrating lymphocytes (TILs) rely on sustained cytokine signaling by interleukin (IL)-2 and correlate with telomere

length (188; see Ref. 119 for a review). Consequently, strategies of controlled hTERT overexpression to achieve telomere length maintenance during culture (144, 180) whilst avoiding genetic instability (174, 175) hold promise and need to be developed further.

C. Cells of Other Tissues

The amount of data on telomere length with age in tissues other than the hematopoietic system is limited. Most data were collected in the context of studies of various diseases, and both age or disease status could affect telomere length (for an example relative to cardiovascular disease, see Ref. 75). More work is needed to better understand the role of telomeres in the normal aging and pathology of various tissues. Loss of telomere function in oocytes has been implicated in meiotic dysfunction in aging women (107), and since lesions in telomeric DNA are poorly recognized and repaired in quiescent cells, it seems possible that damage at telomeric DNA accumulates with age, perhaps posing increasing problems when DNA replication is eventually initiated. Short telomeres are a risk factor in the progression of ulcerative colitis to colon carcinoma (161) and in nonalcoholic fatty liver disease (157), liver cirrhosis (138), and pulmonary fibrosis (6). The impact of telomere shortening is more apparent in rapidly replicating or regenerating tissues where the proliferative pressure is high. This is strikingly illustrated in diseases of accelerated aging involving telomerase deficiencies as well as progeroid syndromes (see sect. IV).

IV. TELOMERE FUNCTION AND HUMAN AGING-RELATED DISEASE

A. Telomere Dysfunction

1. Telomerase deficiencies

Telomerase deficiencies were first implicated (151) in the inherited genetic disorder dyskeratosis congenita (DC) with the discovery of mutations in the dyskerin gene (*DKC1*) associated with the X-linked inheritance form of the disease (93). Mutations in *DKC1* are responsible for symptoms that include pancytopenia, abnormal skin pigmentation, nail dystrophy, leukoplakia, and bone marrow failure or pulmonary fibrosis which ultimately causes death in these patients, with a probability of bone marrow failure by age 20 exceeding 80% (reviewed in Ref. 138). Dyskerin is a nucleolar protein that has been involved in the modification of specific small RNA molecules, specifically ribosomal RNAs and the telomerase template RNA or hTERC. Dyskeratosis can present in a variety of inheritance patterns that include X-linked, autosomal dominant, and autosomal recessive, implying other genetic causes for this disease as well as a possible link with other bone marrow failure syndromes such as constitutional aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), or myelodysplastic syndrome (MDS). AA and MDS reflect ineffective hematopoiesis and are more prevalent in elderly individuals, but most cases are not attributed to telomerase deficiencies despite showing telomere shortening (reviewed in Ref. 16). In view of the studies implicating mutations in *DKC1* with telomerase deficiencies and short telomere lengths (151), the telomerase genes were natural candidates for further investigations of younger patients presenting with DC or bone marrow failure syndromes. *hTERC* was the focus of initial efforts due to its relatively short size providing for relatively easy sequencing compared with *hTERT*, encoded by a fairly large gene. DC patients with hTERC mutations were indeed identified and were found to have reduced telomerase activity down to half of what can be measured in controls (39, 129). This gene-dose effect suggests that levels of telomerase RNA are tightly regulated. Strikingly, telomerase RNA levels also seem to be limiting in mice (88) and yeast (154), indicating that biallelic expression of the telomerase RNA gene is required in a broad range of organisms. In human cell lines, concomitant overexpression of hTERT and hTERC was necessary to substantially increase telomerase activity and elongate telomere length (52). Apart from dyskerin, required for proper folding and stability of telomerase RNA, many other proteins are expected to modulate telomerase RNA levels, and deficiency of such proteins could result in reduced telomerase levels and reduced telomere length. An example of such a protein could be the protein encoded by the *SBDS* gene, which is deficient in the Shwachman-Diamond syndrome (31), although this protein must have other functions as well to explain the disease phenotype that involves specific cell types and tissues. Various mutations in *hTERC* have now been described, and many of these were shown to inhibit telomere elongation in telomerase reconstitution experiments (127, 134, 207). Detection of *hTERC* mutations have been detected in up to 15% of AA or MDS, showing that a clear DC phenotype is not always observed in patients that are telomerase deficient (76, 127). Conversely, *hTERC* mutations were not observed in a subset of patients with short telomeres that were diagnosed with Fanconi anemia (32). Since *hTERC* mutations in marrow failure patient groups remain relatively infrequent, the screening of a large number of samples is required, or additional subset selection such as telomere length can be used to identify novel mutations (58, 128). More recently, substantial efforts have been made to screen for possible *hTERT* mutations, and a number of polymorphisms as well as mutations that cause telomerase deficiencies have been described (182, 219). Both telomerase genes are

linked to the autosomal dominant form of DC, as well as cases of AA, PNH, and MDS (233, 234).

Mutations in *DKC1*, *hTERC*, or *hTERT* all cause defects in telomerase enzymatic activity that result in failure to elongate or maintain telomeres and induce progressive telomere shortening through haploinsufficiency mechanisms, both in patients as they age and in subsequent generations of offspring (summarized in Fig. 8). The latter results in disease anticipation in family trees of patients with telomerase-related genetic defects (218). However, no apparent correlation was found between the type of mutation, the severity, or early onset of symptoms amongst DC patients, particularly in the most severe form of DC: Hoyeraal Hreidarsson disease (HH). Although data are now fairly abundant to make direct causal associations between telomerase deficiencies and DC or constitutive AA, <50% of DC patients have mutations in any of the genes mentioned above. It seems likely that other genes causing DC remain to be discovered. Recent studies have shown that mutations in the genes encoding telomerase components are also implicated in familial idiopathic pulmonary fibrosis (6, 209). These findings support the idea that pathways leading to telomere shortening are involved in the pathogenesis of this disease. Future studies are expected to reveal the involvement of novel genes as well as abnormalities in regulatory sequences, splicing and posttranslational processing, cellular trafficking of the known telomerase genes (see sect. II), as well as the proteins required for functional interaction between telomerase and telomeres in a variety of diseases. Defective telomere maintenance is expected to primarily affect tissues with high cell turnover such as the bone marrow, skin, lung, and gut. At this point, it seems reasonable to assume that the clinical manifestation of the broad class of telomerase deficiencies will depend on inherited telomere length, exposure of specific tissues to pathogens, and/or tissue-specific genetic alterations that result in tissue-specific increases in cell turnover.

2. *Progeroid syndromes*

Patients suffering from progeroid syndromes, or accelerated aging phenotypes, display an array of physical and biological features that vary widely between tissues and diseases and among individuals. Some of the main characteristics for the specific disorders of interest to this review are cited below (for further review of molecules involved and clinical presentation, see Ref. 96). A general dilemma in studies on the role of telomeres in progeroid syndromes (and aging) is that telomere involvement could be direct as well as indirect. For example, the increased cell death resulting from defective DNA repair could result in telomere shortening via increased compensatory (stem) cell turnover or via direct effects on (repair of) telomeric DNA. For many segmental aging disorders, it has proven to be very difficult to distinguish between direct and indirect effects on telomere length. Perhaps phenotypically the most striking segmental aging genetic disorder in humans, Hutchinson-Gilford Progeria syndrome (HGPS), is caused by point mutations in lamin A, a key component of nuclear scaffolding (34, 72). Lamin A deficiency results in absence of hair, craniofacial deformities ("pinched" facial features), emaciated and wrinkled appearance, as well as cardiovascular defects that eventually lead to stroke or heart attack at a very young age. The disease is characterized by specific defects in

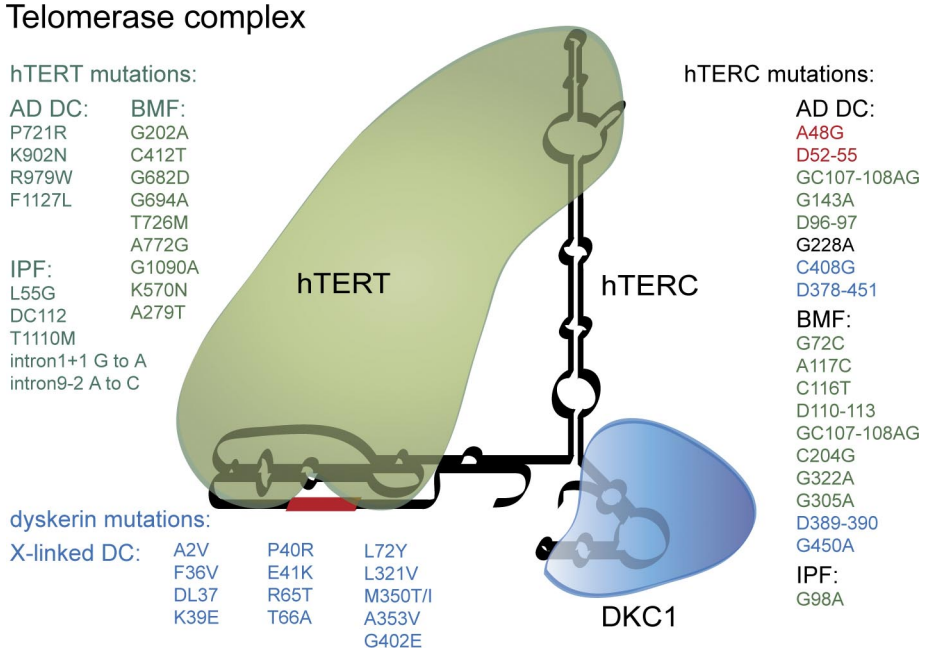

FIG. 8. Defects in human telomerase. The human telomerase complex is minimally composed of two proteins, telomerase reverse transcriptase (hTERT, green) and dyskerin (or DKC1, blue), that both bind specifically to a folded RNA molecule (or *hTERC*, black) containing a telomere repeat anchoring sequence and a template (red box). Known mutations in each component have now been linked to autosomal dominant dyskeratosis congenita (AD DC), bone marrow failure (BMF), and idiopathic pulmonary fibrosis (IPF) (6, 63, 127, 134, 151, 217, 231, 234). The telomerase complex is thought to dimerize, bind to the single-strand G-rich telomere end, and catalyze the addition of new repeats (see also Figs. 3 and 4). The complex translocates along (newly added) telomere tracts for further elongation. Mutations affecting telomerase function lead to failure to assemble a functional complex. In the majority of cases, the level of telomerase activity is reduced by 50%. Such a reduction in telomerase activity compromises telomere length maintenance and increases apoptosis and senescence in proliferating cells (see Fig. 4).

nuclear shape (183). Because expression of (defective) lamin A is limited to certain cell types, some cells and tissues are more affected than others. While there is evidence that DNA damage responses in cells expressing mutant lamin A are abnormal (133), the role of telomeres in this disorders (if any) remains to be clarified. A number of other segmental aging disorders have been more directly linked to telomere (dys)function. Among these, Fanconi anemia (FA) and ataxia telangiectasia (AT) are generally autosomal recessive diseases caused by mutations in, respectively, Fanconi genes (encoding any of 12 Fanconi anemia complementation group proteins) and the ataxia telangiectasia mutated gene (encoding the ATM protein). These proteins are implicated in DNA damage and repair pathways; in addition, ATM is known to phosphorylate FANCD2 (for reviews, see Refs. 64, 118, 190). Both diseases are associated with accelerated telomere shortening (29, 121, 123, 146), and abnormalities in telomere replication or repair are thought to play a role in the pathogenesis, particularly in the progression of the disease to immunodeficiency and bone marrow failure, as well as in the increased predisposition to malignancy in young adults. Other syndromes related to the Fanconi DNA damage response pathway include Nijmegen breakage syndrome (NBS) and Seckel syndrome. Other "progeroid" genes that have been implicated in DNA replication and repair are the family of genes encoding the RecQ DNA helicases. One of the functions of these enzymes is to assist in the resolution and repair of broken or stalled replication forks. Telomeric DNA is known to readily form higher order DNA structures such as G quadruplex structures in vitro (159), and it seems plausible, based on work in *C. elegans* (42), that specialized helicases are required to resolve structures of G-rich DNA arising sporadically during lagging strand DNA synthesis (62). Helicases that could be involved include RecQ protein-like 2 (RecQL2), RecQL3, and RecQL4 with known mutations that give rise to Werner (WRN), Bloom (BLM), and Rothmund Thompson syndromes, respectively. Accelerated telomere shortening is observed in Werner's syndrome (51), and pathology in animal model systems is accentuated in the context of telomerase deficiency (40, 156).

B. Telomeres and Increased Cell Proliferation

In cellular in vitro models, for example, in the case of CD8 positive T cells, hTERT overexpression significantly enhances proliferation and cell survival (55, 149). Similar observations have been made with many different cell types. In vivo findings in animal tumor models showed that *mTERC* was upregulated early in tumorigenesis and that telomerase became activated in late stages of tumor progression (24). These studies led to the examination of what the effects of constitutive expression or overexpression of TERT would be. mTert overexpression was shown to be associated with spontaneous mammary epithelial neoplasia and invasive carcinoma in aged mice (7), while constitutive expression of mTert in thymocytes promotes T-cell lymphoma (33). More recently, work on targeted overexpression in specific tissues showed faster wound healing and increased tumorigenesis in the skin of K5-m*Tert* mice (where m*Terc* is required for the tumor promoting effect) (37). In addition, conditional induction (using a tetracycline-inducible system) in a mouse model showed that mTert causes the proliferation and mobilization of hair follicle stem cells (181). This was visualized in situ as well as through the observation of exacerbated hair growth and faster hair regrowth in a manner independent from telomere synthesis. How TERT protein can also modulate the proliferation of stem cells in the skin even in the absence of telomerase RNA is currently not understood.

C. Cancer

The link between telomere biology and oncogenesis was first proposed when telomerase expression was found to be a hallmark of human cancer: telomerase expression or reexpression and activity can be detected in >90% of tumor samples (reviewed in Ref. 186). Telomerase deficiencies and cancer appear to lie at opposite ends of a spectrum similar to p53: loss of p53 is observed in most tumors and is tumor promoting in mouse models, whereas mice with enhanced p53 responses exhibit increased cancer resistance, a shortened life span, and a number of early aging-associated phenotypes (65, 139). In both models aging appears to be driven in part by a gradual depletion of the functional capacity of stem cells. The link between p53 and telomeres is further illustrated in Li-Fraumeni syndrome (LFS), a cancer predisposition syndrome associated with germ line *TP53* mutations. It was shown that the progressive earlier age of cancer onset (disease anticipation) in LFS is related to a measurable decrease in telomere length, with each generation providing the first rational biological marker for clinical monitoring of LFS patients (202).

Ectopic *hTERT* expression can allow postsenescent cells to proliferate beyond crisis, in a process that could to be independent of catalytic activity (50). Tumorigenesis is often associated with the upregulation of c-Myc that can be induced by retroviral insertion or translocation. c-Myc binding sequences are described within the *hTERT* promoter, and the MYC protein stimulates *hTERT* transcription (229), which may in turn contribute to tumorigenesis or tumor progression. The flip side of continued expression or reexpression of *hTERT* in genetically stable primary cells and in animal models is enhanced longevity and a delay of senescence during in vitro culture (25, 77).

However, sustained (over)expression of telomerase in CD4- or CD8-positive T cells over longer periods in culture was shown to promote genomic instability (174, 184). This may be directly due to hTERT overexpression or may be a consequence of extended proliferation and replication errors that may be exacerbated by culture conditions. In addition, gain of expression of *hTERC* due to the presence of multiple gene copies has also been recently associated with cervical dysplasia and invasive cancer progression (97).

V. INTERVENTIONS TARGETED AT TELOMERES

Interventions targeted at telomeres or telomerase have been the subject of many research projects over the past 10 years, and some applications are now explored in clinical trials.

Strategies to rescue human cells in vitro from senescence or prolong their life span by ectopic telomerase expression were first described about a decade ago (25, 211) and are now routinely used in many laboratories to extend the life span of primary human cells. The approach also has appeal for cell or tissue therapies as available cell numbers are often limiting. Although many of the approaches involving ectopic expression of hTERT are still at the development stage, significant advances have already been made to enhance allografts and engineered tissue for transplantation (110, 237). This type of approach may also benefit specific T-cell immunotherapy protocols where specific T cells recognizing specific antigens (e.g., antigens specific to melanoma cells or HIV) can be purified, expanded in vitro, and infused into patients. The persistence of such tumor-infiltrating lymphocytes and their efficacy was shown to be dependent on telomere length (119, 188). Ectopic expression of *hTERC* and hTERT in fibroblasts from DC patients was shown to rescue the proliferative properties of such cells, suggesting that similar strategies could possibly be useful for the treatment of bone marrow deficiencies linked to mutations in telomerase genes.

Enhancement of telomerase activity and function is not the only attractive application that targeted telomeres: inhibition of telomerase activity remains a very interesting approach for cancer treatment. Most tumors express telomerase, and the possibility to target telomerase has generated a lot of excitement. The original focus was on the discovery of small molecule inhibitors, and moved more recently to siRNA strategies; however, issues with toxicity, half-life, modes of delivery, and the relatively long time delay to achieve telomere-dependent death of cancer cells both in vitro and in vivo remain problematic issues that may impair therapeutic success (for a review on regulation and inhibition of telomerase, see Ref. 145). Despite all these hurdles, one such small molecule targeting the telomerase template unit (GRN163L) is now in clinical trials (61). However, as with many current and improving strategies for cancer therapy, treatment combination approaches are also being tested to enhance antitumor responses, as well as to target the so-called "cancer stem cells" that can give rise to disease relapse after treatment (187). Other approaches in anti-telomerase cancer therapy have aimed at harnessing and enhancing the immune response to telomerase (hTERT) through a vaccination-type approach. TERT protein appears to be expressed at higher levels by some cancer cells (except these that use the ALT pathway), and TERT epitopes can be processed and presented by antigen presenting cells (such as dendritic cells, for example) to antigen-specific T cells that in turn specifically kill target cells. This targeting of hTERT-positive cancer cells is known to actively cause cancer cell death much more rapidly than other strategies mentioned above where progressive telomere shortening has to occur (200). Trials in the context of breast and prostate cancers as well as chronic lymphocytic leukemia are currently underway, stimulating hTERT specific immune responses in combination with conventional chemotherapy

VI. CONCLUSIONS

Accumulated data support the notion that the loss of telomere repeats in (stem) cells and lymphocytes contributes to human aging. This notion is not widely accepted, primarily because the gradual loss of telomere repeats with age in cells of various tissues is not easily measured and because the average telomere length shows a lot of variation between species and between individuals of the same age. However, studies of model organisms as well as patients with telomerase mutations have shown that short telomeres result in dire consequences. It seems plausible that, with age, the proliferation of an increasing number of cells in normal individuals is compromised by progressive telomere loss. This is not necessarily a bad thing, as restrictions in the proliferation of somatic cells pose a barrier for the growth of aspiring tumor cells. Unfortunately, the telomere mechanism that limits the growth of premalignant cells also provides strong selection for cells that no longer respond to the DNA damage signals originating from short telomeres. Such cells are genetically unstable and have greatly increased ability to acquire genetic rearrangements that provide further growth advantages. The intricate involvement of telomeres in both aging and cancer ensures that pathways involving telomeres and telomerase will remain subject to intensive studies for many years to come.

ACKNOWLEDGMENTS

We thank Irma Vulto for assistance in the preparation of Figure 6.

Address for reprint requests and other correspondence: P. M. Lansdorp, Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia V5Z 1L3, Canada (e-mail: plansdor@bccrc.ca).

GRANTS

Work in the laboratory of the author is supported by National Institutes of Health Grant AI-29524, Canadian Institutes of Health Research Grant MOP38075, and the National Cancer Institute of Canada (with support from the Terry Fox Run).

REFERENCES

1. **Alexander B, Coppola G, Perrault SD, Peura TT, Betts DH, King WA.** Telomere length status of somatic cell sheep clones and their offspring. *Mol Reprod Dev* 74: 1525–1537, 2007.
2. **Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL.** Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood* 102: 517–520, 2003.
3. **Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider CW, Harley CB.** Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci USA* 89: 10114–10118, 1992.
4. **Alter BP, Baerlocher GM, Savage SA, Chanock SJ, Weksler BB, Willner JP, Peters JA, Giri N, Lansdorp PM.** Very short telomere length by flow FISH identifies patients with Dyskeratosis Congenita. *Blood* 110: 1439–1447, 2007.
5. **Amiard S, Doudeau M, Pinte S, Poulet A, Lenain C, Faivre-Moskalenko C, Angelov D, Hug N, Vindigni A, Bouvet P, Paoletti J, Gilson E, Giraud-Panis MJ.** A topological mechanism for TRF2-enhanced strand invasion. *Nat Struct Mol Biol* 14: 147–154, 2007.
6. **Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA 3rd, Lansdorp PM, Greider CW, Loyd JE.** Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med* 356: 1317–1326, 2007.
7. **Artandi SE, Alson S, Tietze MK, Sharpless NE, Ye S, Greenberg RA, Castrillon DH, Horner JW, Weiler SR, Carrasco RD, DePinho RA.** Constitutive telomerase expression promotes mammary carcinomas in aging mice. *Proc Natl Acad Sci USA* 99: 8191–8196, 2002.
8. **Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA.** Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* 406: 641–645, 2000.
9. **Artandi SE, DePinho RA.** A critical role for telomeres in suppressing and facilitating carcinogenesis. *Curr Opin Genet Dev* 10: 39–46, 2000.
10. **Autexier C, Lue NF.** The structure and function of telomerase reverse transcriptase. *Annu Rev Biochem* 75: 493–517, 2006.
11. **Baerlocher GM, Lansdorp PM.** Telomere length measurements in leukocyte subsets by automated multicolor flow-FISH. *Cytometry A* 55: 1–6, 2003.
12. **Baerlocher GM, Rice K, Vulto I, Lansdorp PM.** Longitudinal data on telomere length in leukocytes from newborn baboons support a marked drop in stem cell turnover around 1 year of age. *Aging Cell* 6: 121–123, 2007.
13. **Baerlocher GM, Vulto I, de Jong G, Lansdorp PM.** Flow cytometry and FISH to measure the average length of telomeres (flow FISH). *Nat Protocol* 1: 2365–2376, 2006.
14. **Baird DM, Davis T, Rowson J, Jones CJ, Kipling D.** Normal telomere erosion rates at the single cell level in Werner syndrome fibroblast cells. *Hum Mol Genet* 13: 1515–1524, 2004.
15. **Baird DM, Rowson J, Wynford-Thomas D, Kipling D.** Extensive allelic variation and ultrashort telomeres in senescent human cells. *Nat Genet* 33: 203–207, 2003.
16. **Baldwin JG Jr.** Hematopoietic function in the elderly. *Arch Intern Med* 148: 2544–2546, 1988.
17. **Baumann P, Cech TR.** Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* 292: 1171–1175, 2001.
18. **Bianchi A, Shore D.** Early replication of short telomeres in budding yeast. *Cell* 128: 1051–1062, 2007.
19. **Bianchi A, Smith S, Chong L, Elias P, de Lange T.** TRF1 is a dimer and bends telomeric DNA. *EMBO J* 16: 1785–1794, 1997.
20. **Bilaud T, Brun C, Ancelin K, Koering CE, Laroche T, Gilson E.** Telomeric localization of TRF2, a novel human telobox protein. *Nat Genet* 17: 236–239, 1997.
21. **Blackburn EH.** Switching and signaling at the telomere. *Cell* 106: 661–673, 2001.
22. **Blasco MA.** Mouse models to study the role of telomeres in cancer, aging and DNA repair. *Eur J Cancer* 38: 2222–2228, 2002.
23. **Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW.** Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 91: 25–34, 1997.
24. **Blasco MA, Rizen M, Greider CW, Hanahan D.** Differential regulation of telomerase activity and telomerase RNA during multistage tumorigenesis. *Nat Genet* 12: 200–204, 1996.
25. **Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE.** Extension of life-span by introduction of telomerase into normal human cells. *Science* 279: 349–352, 1998.
26. **Britt-Compton B, Rowson J, Locke M, Mackenzie I, Kipling D, Baird DM.** Structural stability and chromosome-specific telomere length is governed by *cis*-acting determinants in humans. *Hum Mol Genet* 15: 725–733, 2006.
27. **Broccoli D, Smogorzewska A, Chong L, de Lange T.** Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat Genet* 17: 231–235, 1997.
28. **Brummendorf TH, Holyoake TL, Rufer N, Barnett MJ, Schulzer M, Eaves CJ, Eaves AC, Lansdorp PM.** Prognostic implications of differences in telomere length between normal and malignant cells from patients with chronic myeloid leukemia measured by flow cytometry. *Blood* 95: 1883–1890, 2000.
29. **Brummendorf TH, Maciejewski JP, Mak J, Young NS, Lansdorp PM.** Telomere length in leukocyte subpopulations of patients with aplastic anemia. *Blood* 97: 895–900, 2001.
30. **Brummendorf TH, Rufer N, Holyoake TL, Maciejewski J, Barnett MJ, Eaves CJ, Eaves AC, Young N, Lansdorp PM.** Telomere length dynamics in normal individuals and in patients with hematopoietic stem cell-associated disorders. *Ann NY Acad Sci* 938: 293–303, 2001.
31. **Calado RT, Graf SA, Wilkerson KL, Kajigaya S, Ancliff PJ, Dror Y, Chanock SJ, Lansdorp PM, Young NS.** Mutations in the SBDS gene in acquired aplastic anemia. *Blood* 110: 1141–1146, 2007.
32. **Calado RT, Pintao MC, Rocha V, Falcao RP, Bitencourt MA, Silva WA Jr, Gluckman E, Pasquini R, Zago MA.** Lack of mutations in the human telomerase RNA component (hTERC) gene in Fanconi's anemia. *Haematologica* 89: 1012–1013, 2004.
33. **Canela A, Martin-Caballero J, Flores JM, Blasco MA.** Constitutive expression of tert in thymocytes leads to increased incidence and dissemination of T-cell lymphoma in Lck-Tert mice. *Mol Cell Biol* 24: 4275–4293, 2004.
34. **Cao H, Hegele RA.** LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). *J Hum Genet* 48: 271–274, 2003.
35. **Cawthon RM.**Telomere measurement by quantitative PCR. *Nucleic Acids Res* 30: e47, 2002.
36. **Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA.** Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361: 393–395, 2003.
37. **Cayuela ML, Flores JM, Blasco MA.** The telomerase RNA component Terc is required for the tumour-promoting effects of Tert overexpression. *EMBO Rep* 6: 268–274, 2005.
38. **Cech TR.** Beginning to understand the end of the chromosome. *Cell* 116: 273–279, 2004.

39. **Cerone MA, Ward RJ, Londono-Vallejo JA, Autexier C.** Telomerase RNA mutated in autosomal dyskeratosis congenita reconstitutes a weakly active telomerase enzyme defective in telomere elongation. *Cell Cycle* 4: 585–589, 2005.
40. **Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, Pathak S, Guarente L, DePinho RA.** Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet* 36: 887–882, 2004.
41. **Chang W, Dynek JN, Smith S.** TRF1 is degraded by ubiquitin-mediated proteolysis after release from telomeres. *Genes Dev* 17: 1328–1333, 2003.
42. **Cheung I, Schertzer M, Rose A, Lansdorp PM.** Disruption of dog-1 in *Caenorhabditis elegans* triggers deletions upstream of guanine-rich DNA. *Nat Genet* 31: 405–409, 2002.
43. **Cheung I, Schertzer M, Rose A, Lansdorp PM.** High incidence of rapid telomere loss in telomerase-deficient *Caenorhabditis elegans*. *Nucleic Acids Res* 34: 96–103, 2006.
44. **Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, de Lange T.** A human telomeric protein. *Science* 270: 1663–1667, 1995.
45. **Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR.** Protein composition of catalytically active human telomerase from immortal cells. *Science* 315: 1850–1853, 2007.
46. **Colgin LM, Wilkinson C, Englezou A, Kilian A, Robinson MO, Reddel RR.** The hTERTalpha splice variant is a dominant negative inhibitor of telomerase activity. *Neoplasia* 2: 426–432, 2000.
47. **Collins K.** The biogenesis and regulation of telomerase holoenzymes. *Nat Rev Mol Cell Biol* 7: 484–494, 2006.
48. **Cooke HJ, Smith BA.** Variability at the telomeres of the human X/Y pseudoautosomal region. *Cold Spring Harb Symp Quant Biol* 51: 213–219, 1986.
49. **Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S.** Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J* 11: 1921–1929, 1992.
50. **Counter CM, Hahn WC, Wei W, Caddle SD, Beijersbergen RL, Lansdorp PM, Sedivy JM, Weinberg RA.** Dissociation among in vitro telomerase activity, telomere maintenance, cellular immortalization. *Proc Natl Acad Sci USA* 95: 14723–14728, 1998.
51. **Crabbe L, Verdun RE, Haggblom CI, Karlseder J.** Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science* 306: 1951–1953, 2004.
52. **Cristofari G, Lingner J.** Fingering the ends: how to make new telomeres. *Cell* 113: 552–554, 2003.
53. **d'Adda di Fagagna F, Hande MP, Tong WM, Roth D, Lansdorp PM, Wang ZQ, Jackson SP.** Effects of DNA nonhomologous end-joining factors on telomere length and chromosomal stability in mammalian cells. *Curr Biol* 11: 1192–1196, 2001.
54. **d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP.** A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426: 194–198, 2003.
55. **Dagarag M, Evazyan T, Rao N, Effros RB.** Genetic manipulation of telomerase in HIV-specific CD8+ T cells: enhanced antiviral functions accompany the increased proliferative potential and telomere length stabilization. *J Immunol* 173: 6303–6311, 2004.
56. **Dagarag M, Ng H, Lubong R, Effros RB, Yang OO.** Differential impairment of lytic and cytokine functions in senescent human immunodeficiency virus type 1-specific cytotoxic T lymphocytes. *J Virol* 77: 3077–3083, 2003.
57. **Dantzer F, Giraud-Panis MJ, Jaco I, Ame JC, Schultz I, Blasco M, Koering CE, Gilson E, Menissier-de Murcia J, de Murcia G, Schreiber V.** Functional interaction between poly(ADP-ribose) polymerase 2 (PARP-2) and TRF2: PARP activity negatively regulates TRF2. *Mol Cell Biol* 24: 1595–1607, 2004.
58. **Danzy S, Su CY, Park S, Li SY, Ferraris AM, Ly H.** Absence of pathogenic mutations of the human telomerase RNA gene (hTERC) in patients with chronic myeloproliferative disorders. *Leukemia* 20: 893–894, 2006.
59. **De Lange T.** Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 19: 2100–2110, 2005.
60. **De Lange T.** Telomere dynamics and genome instability in human cancer. In: *Telomeres*, edited by Blackburn EH, Greider CW. Plainview, NY: Cold Spring Harbor Laboratory, 1995, p. 265–293.
61. **Dikmen ZG, Gellert GC, Jackson S, Gryaznov S, Tressler R, Dogan P, Wright WE, Shay JW.** In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. *Cancer Res* 65: 7866–7873, 2005.
62. **Ding H, Schertzer M, Wu X, Gertsenstein M, Selig S, Kammori M, Pourvali R, Poon S, Vulto I, Chavez E, Tam PP, Nagy A, Lansdorp PM.** Regulation of murine telomere length by Rtel: an essential gene encoding a helicase-like protein. *Cell* 117: 873–886, 2004.
63. **Dokal I.** Dyskeratosis congenita. A disease of premature ageing. *Lancet* 358 *Suppl*: S27, 2001.
64. **Dokal I.**Fanconi's anaemia and related bone marrow failure syndromes. *Br Med Bull* 77–78: 37–53, 2006.
65. **Donehower LA.** Does p53 affect organismal aging? *J Cell Physiol* 192: 23–33, 2002.
66. **Du X, Shen J, Kugan N, Furth EE, Lombard DB, Cheung C, Pak S, Luo G, Pignolo RJ, DePinho RA, Guarente L, Johnson FB.** Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. *Mol Cell Biol* 24: 8437–8446, 2004.
67. **Dunne PJ, Faint JM, Gudgeon NH, Fletcher JM, Plunkett FJ, Soares MV, Hislop AD, Annels NE, Rickinson AB, Salmon M, Akbar AN.** Epstein-Barr virus-specific CD8(+) T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. *Blood* 100: 933–940, 2002.
68. **Dynek JN, Smith S.** Resolution of sister telomere association is required for progression through mitosis. *Science* 304: 97–100, 2004.
69. **Engelhardt M, Kumar R, Albanell J, Pettengell R, Han W, Moore MA.** Telomerase regulation, cell cycle, telomere stability in primitive hematopoietic cells. *Blood* 90: 182–193, 1997.
70. **Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM.** Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci USA* 101: 17312–17315, 2004.
71. **Epel ES, Lin J, Wilhelm FH, Wolkowitz OM, Cawthon R, Adler NE, Dolbier C, Mendes WB, Blackburn EH.** Cell aging in relation to stress arousal and cardiovascular disease risk factors. *Psychoneuroendocrinology* 31: 277–287, 2006.
72. **Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS.** Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423: 293–298, 2003.
73. **Farazi PA, Glickman J, Jiang S, Yu A, Rudolph KL, DePinho RA.** Differential impact of telomere dysfunction on initiation and progression of hepatocellular carcinoma. *Cancer Res* 63: 5021–5027, 2003.
74. **Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J.** The RNA component of human telomerase. *Science* 269: 1236–1241, 1995.
75. **Fitzpatrick AL, Kronmal RA, Gardner JP, Psaty BM, Jenny NS, Tracy RP, Walston J, Kimura M, Aviv A.** Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol* 165: 14–21, 2007.
76. **Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, Read EJ, Lansdorp PM, Young NS.** Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. *Lancet* 362: 1628–1630, 2003.
77. **Gonzalez-Suarez E, Geserick C, Flores JM, Blasco MA.** Antagonistic effects of telomerase on cancer and aging in K5-mTert transgenic mice. *Oncogene* 24: 2256–2270, 2005.
78. **Graakjaer J, Der-Sarkissian H, Schmitz A, Bayer J, Thomas G, Kolvraa S, Londono-Vallejo JA.** Allele-specific relative telomere lengths are inherited. *Hum Genet* 119: 344–350, 2006.
79. **Greider CW, Blackburn EH.** Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43: 405–413, 1985.
80. **Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T.** Mammalian telomeres end in a large duplex loop. *Cell* 97: 503–514, 1999.

81. **Hande MP, Samper E, Lansdorp P, Blasco MA.** Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J Cell Biol* 144: 589–601, 1999.
82. **Hardy CF, Sussel L, Shore D.** A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. *Genes Dev* 6: 801–814, 1992.
83. **Harley CB, Futcher AB, Greider CW.** Telomeres shorten during ageing of human fibroblasts. *Nature* 345: 458–460, 1990.
84. **Harley CB, Vaziri H, Counter CM, Allsopp RC.** The telomere hypothesis of cellular aging. *Exp Gerontol* 27: 375–382, 1992.
85. **Harman D.** Free radicals in aging. *Mol Cell Biochem* 84: 155–161, 1988.
86. **Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Bass MB, Arruda I, Robinson MO.** A mammalian telomerase-associated protein. *Science* 275: 973–977, 1997.
87. **Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC.** Telomere reduction in human colorectal carcinoma and with ageing. *Nature* 346: 866–868, 1990.
88. **Hathcock KS, Hemann MT, Opperman KK, Strong MA, Greider CW, Hodes RJ.** Haploinsufficiency of mTR results in defects in telomere elongation. *Proc Natl Acad Sci USA* 99: 3591–3596, 2002.
89. **Hayflick L.** A brief history of the mortality and immortality of cultured cells. *Keio J Med* 47: 174–182, 1998.
90. **Hayflick L.** The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res* 37: 614–636, 1965.
91. **Hayflick L.** Living forever and dying in the attempt. *Exp Gerontol* 38: 1231–1241, 2003.
92. **Hayflick L, Moorhead PS.** The serial cultivation of human diploid cell strains. *Exp Cell Res* 25: 585–621, 1961.
93. **Heiss NS, Knight SW, Vulliamy TJ, Klauck SM, Wiemann S, Mason PJ, Poustka A, Dokal I.** X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat Genet* 19: 32–38, 1998.
94. **Henle ES, Han Z, Tang N, Rai P, Luo Y, Linn S.** Sequence-specific DNA cleavage by Fe^{2+}-mediated Fenton reactions has possible biological implications. *J Biol Chem* 274: 962–971, 1999.
95. **Hodes RJ, Hathcock KS, Weng NP.** Telomeres in T and B cells. *Nat Rev Immunol* 2: 699–706, 2002.
96. **Hofer AC, Tran RT, Aziz OZ, Wright W, Novelli G, Shay J, Lewis M.** Shared phenotypes among segmental progeroid syndromes suggest underlying pathways of aging. *J Gerontol A Biol Sci Med Sci* 60: 10–20, 2005.
97. **Hopman AH, Theelen W, Hommelberg PP, Kamps MA, Herrington CS, Morrison LE, Speel EJ, Smedts F, Ramaekers FC.** Genomic integration of oncogenic HPV and gain of the human telomerase gene TERC at 3q26 are strongly associated events in the progression of uterine cervical dysplasia to invasive cancer. *J Pathol* 210: 412–419, 2006.
98. **Hornsby PJ.** Short telomeres: cause or consequence of aging? *Aging Cell* 5: 577–578, 2006.
99. **Houghtaling BR, Cuttonaro L, Chang W, Smith S.** A dynamic molecular link between the telomere length regulator TRF1 and the chromosome end protector TRF2. *Curr Biol* 14: 1621–1631, 2004.
100. **Hsu HL, Gilley D, Blackburn EH, Chen DJ.** Ku is associated with the telomere in mammals. *Proc Natl Acad Sci USA* 96: 12454–12458, 1999.
101. **Hsu HL, Gilley D, Galande SA, Hande MP, Allen B, Kim SH, Li GC, Campisi J, Kohwi-Shigematsu T, Chen DJ.** Ku acts in a unique way at the mammalian telomere to prevent end joining. *Genes Dev* 14: 2807–2812, 2000.
102. **Hug N, Lingner J.** Telomere length homeostasis. *Chromosoma* 115: 413–425, 2006.
103. **Jeon HY, Hyun SH, Lee GS, Kim HS, Kim S, Jeong YW, Kang SK, Lee BC, Han JY, Ahn C, Hwang WS.** The analysis of telomere length and telomerase activity in cloned pigs and cows. *Mol Reprod Dev* 71: 315–320, 2005.
104. **Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U.** Accumulation of senescent cells in mitotic tissue of aging primates. *Mech Ageing Dev* 128: 36–44, 2007.
105. **Kaminker PG, Kim SH, Taylor RD, Zebarjadian Y, Funk WD, Morin GB, Yaswen P, Campisi J.** TANK2, a new TRF1-associated poly(ADP-ribose) polymerase, causes rapid induction of cell death upon overexpression. *J Biol Chem* 276: 35891–35899, 2001.
106. **Karlseder J, Hoke K, Mirzoeva OK, Bakkenist C, Kastan MB, Petrini JH, de Lange T.** The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. *PLoS Biol* 2: E240, 2004.
107. **Keefe DL, Marquard K, Liu L.** The telomere theory of reproductive senescence in women. *Curr Opin Obstet Gynecol* 18: 280–285, 2006.
108. **Kim SH, Kaminker P, Campisi J.** TIN2, a new regulator of telomere length in human cells. *Nat Genet* 23: 405–412, 1999.
109. **Kirkwood TB, Holliday R.** The evolution of ageing and longevity. *Proc R Soc Lond B Biol Sci* 205: 531–546, 1979.
110. **Klinger RY, Blum JL, Hearn B, Lebow B, Niklason LE.** Relevance and safety of telomerase for human tissue engineering. *Proc Natl Acad Sci USA* 103: 2500–2505, 2006.
111. **Kobryn K, Chaconas G.** The circle is broken: telomere resolution in linear replicons. *Curr Opin Microbiol* 4: 558–564, 2001.
112. **Kuhholzer-Cabot B, Brem G.** Aging of animals produced by somatic cell nuclear transfer. *Exp Gerontol* 37: 1317–1323, 2002.
113. **Lansdorp PM.** Lessons from mice without telomerase. *J Cell Biol* 139: 309–312, 1997.
114. **Lansdorp PM.** Major cutbacks at chromosome ends. *Trends Biochem Sci* 30: 388–395, 2005.
115. **Lansdorp PM.** Self-renewal of stem cells. *Biol Blood Marrow Transplant* 3: 171–178, 1997.
116. **Lansdorp PM, Verwoerd NP, van de Rijke FM, Dragowska V, Little MT, Dirks RW, Raap AK, Tanke HJ.** Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet* 5: 685–691, 1996.
117. **Lanza RP, Cibelli JB, Blackwell C, Cristofalo VJ, Francis MK, Baerlocher GM, Mak J, Schertzer M, Chavez EA, Sawyer N, Lansdorp PM, West MD.** Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science* 288: 665–669, 2000.
118. **Lavin MF, Kozlov S.** ATM activation and DNA damage response. *Cell Cycle* 6: 931–942, 2007.
119. **Leen AM, Rooney CM, Foster AE.** Improving T cell therapy for cancer. *Annu Rev Immunol* 25: 243–265, 2007.
120. **Lenain C, Bauwens S, Amiard S, Brunori M, Giraud-Panis MJ, Gilson E.** The Apollo 5′ exonuclease functions together with TRF2 to protect telomeres from DNA repair. *Curr Biol* 16: 1303–1310, 2006.
121. **Leteurtre F, Li X, Guardiola P, Le Roux G, Sergere JC, Richard P, Carosella ED, Gluckman E.** Accelerated telomere shortening and telomerase activation in Fanconi's anaemia. *Br J Haematol* 105: 883–893, 1999.
122. **Li B, Oestreich S, de Lange T.** Identification of human Rap1: implications for telomere evolution. *Cell* 101: 471–483, 2000.
123. **Li X, Leteurtre F, Rocha V, Guardiola P, Berger R, Daniel MT, Noguera MH, Maarek O, Roux GL, de la Salmoniere P, Richard P, Gluckman E.** Abnormal telomere metabolism in Fanconi's anaemia correlates with genomic instability and the probability of developing severe aplastic anaemia. *Br J Haematol* 120: 836–845, 2003.
124. **Lingner J, Cooper JP, Cech TR.** Telomerase and DNA end replication: no longer a lagging strand problem? *Science* 269: 1533–1534, 1995.
125. **Liu D, O'Connor MS, Qin J, Songyang Z.** Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins. *J Biol Chem* 279: 51338–51342, 2004.
126. **Lundblad V, Blackburn EH.** RNA-dependent polymerase motifs in EST1: tentative identification of a protein component of an essential yeast telomerase. *Cell* 60: 529–530, 1990.
127. **Ly H, Calado RT, Allard P, Baerlocher GM, Lansdorp PM, Young NS, Parslow TG.** Functional characterization of telomerase RNA variants found in patients with hematologic disorders. *Blood* 105: 2332–2339, 2005.
128. **Ly H, Schertzer M, Jastaniah W, Davis J, Yong SL, Ouyang Q, Blackburn EH, Parslow TG, Lansdorp PM.** Identification and functional characterization of 2 variant alleles of the telomerase RNA template gene (TERC) in a patient with dyskeratosis congenita. *Blood* 106: 1246–1252, 2005.

129. **Ly H, Schertzer M, Jastaniah W, Davis J, Yong SL, Ouyang Q, Blackburn EH, Parslow TG, Lansdorp PM.** Identification and functional characterization of two variant alleles of the telomerase RNA template gene (TERC) in a patient with Dyskeratosis Congenita. *Blood* 106: 1246–1252, 2005.
130. **Machwe A, Xiao L, Orren DK.** TRF2 recruits the Werner syndrome (WRN) exonuclease for processing of telomeric DNA. *Oncogene* 23: 149–156, 2004.
131. **Maini MK, Soares MV, Zilch CF, Akbar AN, Beverley PC.** Virus-induced CD8+ T cell clonal expansion is associated with telomerase up-regulation and telomere length preservation: a mechanism for rescue from replicative senescence. *J Immunol* 162: 4521–4526, 1999.
132. **Makarov VL, Hirose Y, Langmore JP.** Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell* 88: 657–666, 1997.
133. **Manju K, Muralikrishna B, Parnaik VK.** Expression of disease-causing lamin A mutants impairs the formation of DNA repair foci. *J Cell Sci* 119: 2704–2714, 2006.
134. **Marrone A, Stevens D, Vulliamy T, Dokal I, Mason PJ.** Heterozygous telomerase RNA mutations found in dyskeratosis congenita and aplastic anemia reduce telomerase activity via haploinsufficiency. *Blood* 104: 3936–3942, 2004.
135. **Martens UM, Brass V, Sedlacek L, Pantic M, Exner C, Guo Y, Engelhardt M, Lansdorp PM, Waller CF, Lange W.** Telomere maintenance in human B lymphocytes. *Br J Haematol* 119: 810–818, 2002.
136. **Martens UM, Chavez EA, Poon SS, Schmoor C, Lansdorp PM.** Accumulation of short telomeres in human fibroblasts prior to replicative senescence. *Exp Cell Res* 256: 291–299, 2000.
137. **Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK, Lansdorp PM.** Short telomeres on human chromosome 17p. *Nat Genet* 18: 76–80, 1998.
138. **Mason PJ, Wilson DB, Bessler M.** Dyskeratosis congenita–a disease of dysfunctional telomere maintenance. *Curr Mol Med* 5: 159–170, 2005.
139. **Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, Flores JM, Vina J, Blasco MA, Serrano M.** Delayed ageing through damage protection by the Arf/p53 pathway. *Nature* 448: 375–379, 2007.
140. **Mattern KA, Swiggers SJ, Nigg AL, Lowenberg B, Houtsmuller AB, Zijlmans JM.** Dynamics of protein binding to telomeres in living cells: implications for telomere structure and function. *Mol Cell Biol* 24: 5587–5594, 2004.
141. **Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M.** Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. *Circ Res* 99: 156–164, 2006.
142. **McClintock B.** The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proc Natl Acad Sci USA* 25: 405–416, 1939.
143. **McClintock B.** The significance of responses of the genome to challenge. *Science* 226: 792–801, 1984.
144. **Menzel O, Migliaccio M, Goldstein DR, Dahoun S, Delorenzi M, Rufer N.** Mechanisms regulating the proliferative potential of human CD8+ T lymphocytes overexpressing telomerase. *J Immunol* 177: 3657–3668, 2006.
145. **Mergny JL, Riou JF, Mailliet P, Teulade-Fichou MP, Gilson E.** Natural and pharmacological regulation of telomerase. *Nucleic Acids Res* 30: 839–865, 2002.
146. **Metcalfe JA, Parkhill J, Campbell L, Stacey M, Biggs P, Byrd PJ, Taylor AM.** Accelerated telomere shortening in ataxia telangiectasia. *Nat Genet* 13: 350–353, 1996.
147. **Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, Davidoff MJ, Liu Q, Bacchetti S, Haber DA, Weinberg RA.** hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 90: 785–795, 1997.
148. **Michelson RJ, Rosenstein S, Weinert T.** A telomeric repeat sequence adjacent to a DNA double-stranded break produces an anticheckpoint. *Genes Dev* 19: 2546–2559, 2005.
149. **Migliaccio M, Amacker M, Just T, Reichenbach P, Valmori D, Cerottini JC, Romero P, Nabholz M.** Ectopic human telomerase catalytic subunit expression maintains telomere length but is not sufficient for CD8+ T lymphocyte immortalization. *J Immunol* 165: 4978–4984, 2000.
150. **Minamino T, Komuro I.** Vascular cell senescence: contribution to atherosclerosis. *Circ Res* 100: 15–26, 2007.
151. **Mitchell JR, Wood E, Collins K.** A telomerase component is defective in the human disease dyskeratosis congenita. *Nature* 402: 551–555, 1999.
152. **Mooi WJ, Peeper DS.** Oncogene-induced cell senescence–halting on the road to cancer. *N Engl J Med* 355: 1037–1046, 2006.
153. **Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR.** A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* 85: 6622–6626, 1988.
154. **Mozdy AD, Cech TR.** Low abundance of telomerase in yeast: implications for telomerase haploinsufficiency. *RNA* 12: 1721–1737, 2006.
155. **Muller HJ.** The re-making of chromosomes. *Collecting Net, Woods Hole* 13: 181–198, 1938.
156. **Multani AS, Chang S.** WRN at telomeres: implications for aging and cancer. *J Cell Sci* 120: 713–721, 2007.
157. **Nakajima T, Moriguchi M, Katagishi T, Sekoguchi S, Nishikawa T, Takashima H, Kimura H, Minami M, Itoh Y, Kagawa K, Tani Y, Okanoue T.** Premature telomere shortening and impaired regenerative response in hepatocytes of individuals with NAFLD. *Liver Int* 26: 23–31, 2006.
158. **Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, Harley CB, Cech TR.** Telomerase catalytic subunit homologs from fission yeast and human. *Science* 277: 955–959, 1997.
159. **Neidle S, Parkinson GN.** The structure of telomeric DNA. *Curr Opin Struct Biol* 13: 275–283, 2003.
160. **O'Connor MS, Safari A, Liu D, Qin J, Songyang Z.** The human Rap1 protein complex and modulation of telomere length. *J Biol Chem* 279: 28585–28591, 2004.
161. **O'Sullivan JN, Bronner MP, Brentnall TA, Finley JC, Shen WT, Emerson S, Emond MJ, Gollahon KA, Moskovitz AH, Crispin DA, Potter JD, Rabinovitch PS.** Chromosomal instability in ulcerative colitis is related to telomere shortening. *Nat Genet* 32: 280–284, 2002.
162. **Oikawa S, Tada-Oikawa S, Kawanishi S.** Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening. *Biochemistry* 40: 4763–4768, 2001.
163. **Olovnikov AM.** A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol* 41: 181–190, 1973.
164. **Opresko PL, Otterlei M, Graakjaer J, Bruheim P, Dawut L, Kolvraa S, May A, Seidman MM, Bohr VA.** The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2. *Mol Cell* 14: 763–774, 2004.
165. **Opresko PL, von Kobbe C, Laine JP, Harrigan J, Hickson ID, Bohr VA.** Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. *J Biol Chem* 277: 41110–41119, 2002.
166. **Ouyang Q, Baerlocher G, Vulto I, Lansdorp PM.** Telomere length in human natural killer cell subsets. *Ann NY Acad Sci* 1106: 240–252, 2007.
167. **Pardue ML, Rashkova S, Casacuberta E, DeBaryshe PG, George JA, Traverse KL.** Two retrotransposons maintain telomeres in *Drosophila*. *Chromosome Res* 13: 443–453, 2005.
168. **Plunkett FJ, Soares MV, Annels N, Hislop A, Ivory K, Lowdell M, Salmon M, Rickinson A, Akbar AN.** The flow cytometric analysis of telomere length in antigen-specific CD8+ T cells during acute Epstein-Barr virus infection. *Blood* 97: 700–707, 2001.
169. **Poon SS, Lansdorp PM.** Measurements of telomere length on individual chromosomes by image cytometry. *Methods Cell Biol* 64: 69–96, 2001.

170. **Potten CS, Owen G, Booth D.** Intestinal stem cells protect their genome by selective segregation of template DNA strands. *J Cell Sci* 115: 2381–2388, 2002.
171. **Reed JR, Vukmanovic-Stejic M, Fletcher JM, Soares MV, Cook JE, Orteu CH, Jackson SE, Birch KE, Foster GR, Salmon M, Beverley PC, Rustin MH, Akbar AN.** Telomere erosion in memory T cells induced by telomerase inhibition at the site of antigenic challenge in vivo. *J Exp Med* 199: 1433–1443, 2004.
172. **Riha K, McKnight TD, Griffing LR, Shippen DE.** Living with genome instability: plant responses to telomere dysfunction. *Science* 291: 1797–1800, 2001.
173. **Riha K, Shippen DE.** Telomere structure, function and maintenance in *Arabidopsis*. *Chromosome Res* 11: 263–275, 2003.
174. **Roth A, Baerlocher GM, Schertzer M, Chavez E, Duhrsen U, Lansdorp PM.** Telomere loss, senescence, genetic instability in CD4+ T lymphocytes overexpressing hTERT. *Blood* 106: 43–50, 2005.
175. **Roth A, Schneider L, Himmelreich H, Baerlocher GM, Duhrsen U.** Impact of culture conditions on the proliferative life span of human T cells in vitro. *Cytotherapy* 9: 91–98, 2007.
176. **Roth A, Yssel H, Pene J, Chavez EA, Schertzer M, Lansdorp PM, Spits H, Luiten RM.** Telomerase levels control the lifespan of human T lymphocytes. *Blood* 102: 849–857, 2003.
177. **Rubio MA, Davalos AR, Campisi J.** Telomere length mediates the effects of telomerase on the cellular response to genotoxic stress. *Exp Cell Res* 298: 17–27, 2004.
178. **Rufer N, Brummendorf TH, Chapuis B, Helg C, Lansdorp PM, Roosnek E.** Accelerated telomere shortening in hematological lineages is limited to the first year following stem cell transplantation. *Blood* 97: 575–577, 2001.
179. **Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, Schulzer M, Lansdorp PM.** Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *J Exp Med* 190: 157–167, 1999.
180. **Rufer N, Dragowska W, Thornbury G, Roosnek E, Lansdorp PM.** Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. *Nat Biotechnol* 16: 743–747, 1998.
181. **Sarin KY, Cheung P, Gilison D, Lee E, Tennen RI, Wang E, Artandi MK, Oro AE, Artandi SE.** Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* 436: 1048–1052, 2005.
182. **Savage SA, Stewart BJ, Weksler BB, Baerlocher GM, Lansdorp PM, Chanock SJ, Alter BP.** Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure. *Blood Cells Mol Dis* 37: 134–136, 2006.
183. **Scaffidi P, Misteli T.** Lamin A-dependent nuclear defects in human aging. *Science* 312: 1059–1063, 2006.
184. **Schreurs MW, Hermsen MA, Klein Geltink RI, Scholten KB, Brink AA, Kueter EW, Tijssen M, Meijer CJ, Ylstra B, Meijer GA, Hooijberg E.** Genomic stability and functional activity may be lost in telomerase transduced human CD8+ T lymphocytes. *Blood* 106: 2663–2270, 2005.
185. **Sharpless NE, DePinho RA.** Telomeres, stem cells, senescence, cancer. *J Clin Invest* 113: 160–168, 2004.
186. **Shay JW, Roninson IB.** Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene* 23: 2919–2933, 2004.
187. **Shay JW, Wright WE.** Telomerase therapeutics for cancer: challenges and new directions. *Nat Rev Drug Discov* 5: 577–584, 2006.
188. **Shen X, Zhou J, Hathcock KS, Robbins P, Powell DJ Jr, Rosenberg SA, Hodes RJ.** Persistence of tumor infiltrating lymphocytes in adoptive immunotherapy correlates with telomere length. *J Immunother* 30: 123–129, 2007.
189. **Shiels PG, Kind AJ, Campbell KH, Wilmut I, Waddington D, Colman A, Schnieke AE.** Analysis of telomere length in Dolly, a sheep derived by nuclear transfer. *Cloning* 1: 119–125, 1999.
190. **Shiloh Y.** Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu Rev Genet* 31: 635–662, 1997.
191. **Silverman J, Takai H, Buonomo SB, Eisenhaber F, de Lange T.** Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. *Genes Dev* 18: 2108–2119, 2004.
192. **Slagboom PE, Droog S, Boomsma DI.** Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet* 55: 876–882, 1994.
193. **Smith S, de Lange T.** Tankyrase promotes telomere elongation in human cells. *Curr Biol* 10: 1299–1302, 2000.
194. **Smith S, Giriat I, Schmitt A, de Lange T.** Tankyrase, a poly-(ADP-ribose) polymerase at human telomeres. *Science* 282: 1484–1487, 1998.
195. **Smogorzewska A, de Lange T.** Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 73: 177–208, 2004.
196. **Songyang Z, Liu D.** Inside the mammalian telomere interactome: regulation and regulatory activities of telomeres. *Crit Rev Eukaryot Gene Expr* 16: 103–118, 2006.
197. **Spalding KL, Bhardwaj RD, Buchholz BA, Druid H, Frisen J.** Retrospective birth dating of cells in humans. *Cell* 122: 133–143, 2005.
198. **Stavropoulos DJ, Bradshaw PS, Li X, Pasic I, Truong K, Ikura M, Ungrin M, Meyn MS.** The Bloom syndrome helicase BLM interacts with TRF2 in ALT cells and promotes telomeric DNA synthesis. *Hum Mol Genet* 11: 3135–3144, 2002.
199. **Stewart SA, Weinberg RA.** Telomeres: cancer to human aging. *Annu Rev Cell Dev Biol* 2006.
200. **Su Z, Dannull J, Yang BK, Dahm P, Coleman D, Yancey D, Sichi S, Niedzwiecki D, Boczkowski D, Gilboa E, Vieweg J.** Telomerase mRNA-transfected dendritic cells stimulate antigen-specific CD8+ and CD4+ T cell responses in patients with metastatic prostate cancer. *J Immunol* 174: 3798–3807, 2005.
201. **Surralles J, Hande MP, Marcos R, Lansdorp PM.** Accelerated telomere shortening in the human inactive X chromosome. *Am J Hum Genet* 65: 1617–1622, 1999.
202. **Tabori U, Nanda S, Druker H, Lees J, Malkin D.** Younger age of cancer initiation is associated with shorter telomere length in Li-Fraumeni syndrome. *Cancer Res* 67: 1415–1418, 2007.
203. **Takai H, Smogorzewska A, de Lange T.** DNA damage foci at dysfunctional telomeres. *Curr Biol* 13: 1549–1556, 2003.
204. **Tarsounas M, Munoz P, Claas A, Smiraldo PG, Pittman DL, Blasco MA, West SC.** Telomere maintenance requires the RAD51D recombination/repair protein. *Cell* 117: 337–347, 2004.
205. **Teixeira MT, Arneric M, Sperisen P, Lingner J.** Telomere length homeostasis is achieved via a switch between telomerase-extendible and -nonextendible states. *Cell* 117: 323–335, 2004.
206. **Theimer CA, Feigon J.** Structure and function of telomerase RNA. *Curr Opin Struct Biol* 16: 307–318, 2006.
207. **Theimer CA, Finger LD, Feigon J.** YNMG tetraloop formation by a dyskeratosis congenita mutation in human telomerase RNA. *RNA* 9: 1446–1455, 2003.
208. **Thomas ED, Lochte HL Jr, Cannon JH, Sahler OD, Ferrebee JW.** Supralethal whole body irradiation and isologous marrow transplantation in man. *J Clin Invest* 38: 1709–1716, 1959.
209. **Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK.** Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci USA* 104: 7552–7557, 2007.
210. **Van Overbeek M, de Lange T.** Apollo, an Artemis-related nuclease, interacts with TRF2 and protects human telomeres in S phase. *Curr Biol* 16: 1295–1302, 2006.
211. **Vaziri H, Benchimol S.** Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr Biol* 8: 279–282, 1998.
212. **Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM.** Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci USA* 91: 9857–9860, 1994.
213. **Verdun RE, Karlseder J.** The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. *Cell* 127: 709–720, 2006.
214. **Verfaillie CM, Pera MF, Lansdorp PM.** Stem cells: hype and reality. *Hematology* 369–391, 2002.
215. **Von Zglinicki T.** Role of oxidative stress in telomere length regulation and replicative senescence. *Ann NY Acad Sci* 908: 99–110, 2000.

216. **Vousden KH, Lane DP.** p53 in health and disease. *Nat Rev Mol Cell Biol* 8: 275–283, 2007.
217. **Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I.** The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* 413: 432–435, 2001.
218. **Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I.** Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. *Nat Genet* 36: 447–449, 2004.
219. **Vulliamy TJ, Walne A, Baskaradas A, Mason PJ, Marrone A, Dokal I.** Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure. *Blood Cells Mol Dis* 34: 257–263, 2005.
220. **Wallace DC.** A mitochondrial paradigm of metabolic and degenerative diseases, aging, cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 39: 359–407, 2005.
221. **Wan TS, Martens UM, Poon SS, Tsao SW, Chan LC, Lansdorp PM.** Absence or low number of telomere repeats at junctions of dicentric chromosomes. *Genes Chromosomes Cancer* 24: 83–86, 1999.
222. **Wang F, Podell ER, Zaug AJ, Yang Y, Baciu P, Cech TR, Lei M.** The POT1-TPP1 telomere complex is a telomerase processivity factor. *Nature* 445: 506–510, 2007.
223. **Wang RC, Smogorzewska A, de Lange T.** Homologous recombination generates T-loop-sized deletions at human telomeres. *Cell* 119: 355–368, 2004.
224. **Watson JD.** Origin of concatemeric T7 DNA. *Nat New Biol* 239: 197–201, 1972.
225. **Weng NP, Granger L, Hodes RJ.** Telomere lengthening and telomerase activation during human B cell differentiation. *Proc Natl Acad Sci USA* 94: 10827–10832, 1997.
226. **Weng NP, Levine BL, June CH, Hodes RJ.** Human naive and memory T lymphocytes differ in telomeric length and replicative potential. *Proc Natl Acad Sci USA* 92: 11091–11094, 1995.
227. **Weng NP, Palmer LD, Levine BL, Lane HC, June CH, Hodes RJ.** Tales of tails: regulation of telomere length and telomerase activity during lymphocyte development, differentiation, activation, aging. *Immunol Rev* 160: 43–54, 1997.
228. **Wong JM, Collins K.** Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenita. *Genes Dev* 20: 2848–2858, 2006.
229. **Wu KJ, Grandori C, Amacker M, Simon-Vermot N, Polack A, Lingner J, Dalla-Favera R.** Direct activation of TERT transcription by c-MYC. *Nat Genet* 21: 220–224, 1999.
230. **Xin H, Liu D, Wan M, Safari A, Kim H, Sun W, O'Connor MS, Songyang Z.** TPP1 is a homologue of ciliate TEBP-beta and interacts with POT1 to recruit telomerase. *Nature* 445: 559–562, 2007.
231. **Xin ZT, Beauchamp AD, Calado RT, Bradford JW, Regal JA, Shenoy A, Liang Y, Lansdorp PM, Young NS, Ly H.** Functional characterization of natural telomerase mutations found in patients with hematologic disorders. *Blood* 109: 524–532, 2007.
232. **Xu L, Blackburn EH.** Human Rif1 protein binds aberrant telomeres and aligns along anaphase midzone microtubules. *J Cell Biol* 167: 819–830, 2004.
233. **Yamaguchi H, Baerlocher GM, Lansdorp PM, Chanock SJ, Nunez O, Sloand E, Young NS.** Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. *Blood* 102: 916–918, 2003.
234. **Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, Lansdorp PM, Young NS.** Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *N Engl J Med* 352: 1413–1424, 2005.
235. **Yankiwski V, Marciniak RA, Guarente L, Neff NF.** Nuclear structure in normal and Bloom syndrome cells. *Proc Natl Acad Sci USA* 97: 5214–5219, 2000.
236. **Ye JZ, de Lange T.** TIN2 is a tankyrase 1 PARP modulator in the TRF1 telomere length control complex. *Nat Genet* 36: 618–623, 2004.
237. **Young AT, Lakey JR, Murray AG, Mullen JC, Moore RB.** In vitro senescence occurring in normal human endothelial cells can be rescued by ectopic telomerase activity. *Transplant Proc* 35: 2483–2485, 2003.
238. **Zhong Z, Shiue L, Kaplan S, de Lange T.** A mammalian factor that binds telomeric TTAGGG repeats in vitro. *Mol Cell Biol* 12: 4834–4843, 1992.
239. **Zhu J, Woods D, McMahon M, Bishop JM.** Senescence of human fibroblasts induced by oncogenic Raf. *Genes Dev* 12: 2997–3007, 1998.
240. **Zhu XD, Niedernhofer L, Kuster B, Mann M, Hoeijmakers JH, de Lange T.** ERCC1/XPF removes the 3′ overhang from uncapped telomeres and represses formation of telomeric DNA-containing double minute chromosomes. *Mol Cell* 12: 1489–1498, 2003.
241. **Zijlmans JM, Martens UM, Poon SS, Raap AK, Tanke HJ, Ward RK, Lansdorp PM.** Telomeres in the mouse have large inter-chromosomal variations in the number of T2AG3 repeats. *Proc Natl Acad Sci USA* 94: 7423–7428, 1997.

Telomeres and Aging

Geraldine Aubert and Peter M. Lansdorp

Physiol Rev 88:557-579, 2008. doi:10.1152/physrev.00026.2007

Physiological Reviews provides state of the art coverage of timely issues in the physiological and biomedical sciences. It is published quarterly in January, April, July, and October by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2008 by the American Physiological Society. ISSN: 0031-9333, ESSN: 1522-1210. Visit our website at http://www.the-aps.org/.