Chapter 1
Cellular versus Organismal Aging

Andrew Dillin and Jan Karlseder(✉)

Abstract Aging is an extremely complex process, affecting individual cells and organisms as a whole. Here we discuss the impact of telomeres, the natural chromosome ends, on cellular and organismal aging, and how telomere maintenance influences genome stability and tumorigenesis. Since telomeres represent only a fraction of the complexity of the aging process, we discuss how genome integrity and DNA damage response pathways affect postmitotic aging, and which genetic pathways promote survival and elongated life, namely the IGF-1 signaling pathway, dietary restriction, and mitochondrial transport chain. At this point the researchers in the field are intensely studying individual aspects of aging, but it remains a unique challenge to integrate all aspects, with the goal of understanding longevity.

1 Cellular Aging: The Hayflick Limit

The name Leonard Hayflick immediately comes to mind when cellular aging is discussed. In the early 1960s, Hayflick was interested in the division potential of human diploid cells, and performed a number of key experiments at The Wistar Institute in Philadelphia. In two subsequent publications in 1961 and 1965 he described the isolation and growth capacities of primary cells originating from human tissue (Hayflick 1965, Hayflick and Moorhead 1961). He noted that cells are readily recovered from human tissue slices, and can rather easily be subcultivated at a split ratio of 1:2 twice a week. This maintenance schedule can be continued for months, until, after approximately 50 population doublings, the cultures fail to display the numerous mitotic figures characteristic of healthy growth, and cease to divide. Once the cultures arrested, Hayflick found it impossible to recover cell growth, an observation that still holds up today. Based on these results, he suggested

Jan Karlseder
The Salk Institute for Biological Studies, 10010 North Torrey Pines Rd., La Jolla, California, United States.
e-mail: karlseder@salk.edu

K.L. Rudolph (ed.), *Telomeres and Telomerase in Ageing, Disease, and Cancer*.

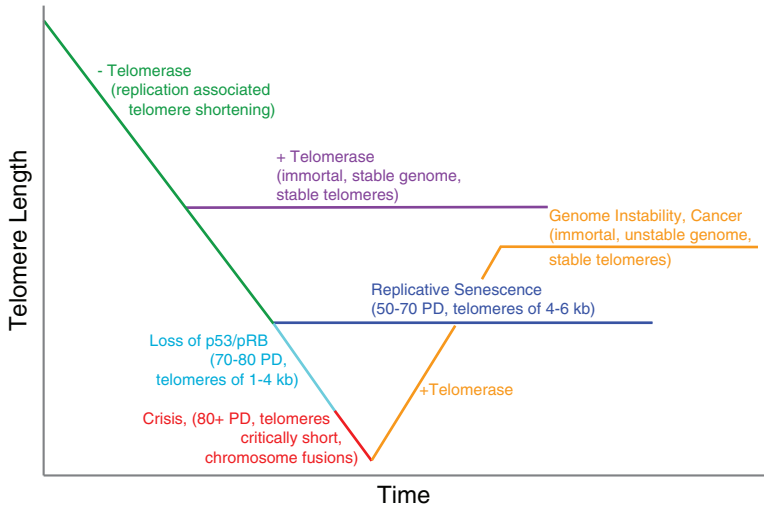

Fig. 1.1 Schematic of telomere dynamics during aging. Telomeres in telomerase-negative cells shorten over time (green line). When telomerase is introduced into such cells, telomere length is stabilized and the cells become immortal without accumulating chromosomal aberrations (purple line). When telomeres become short, cells arrest in senescence (blue line). Suppression of DNA damage pathways allows further telomere shortening (turquoise line), leading to dysfunctional and critically short telomeres, genome fragmentation and crisis (red line). Upregulation of telomerase allows telomere elongation, escape from crisis, and establishment of immortal clones with unstable genomes (orange line) (*See Color Plate*)

that the growth characteristics of such cultures could be divided into three phases. Phase 1, or the early growth phase, is when the culture establishes itself. During Phase 2 the cells grow exponentially, maintain a diploid chromosome set, but eventually stop growing. Phase 3 is termed the degeneration phase, during which tetraploid cells arise occasionally, and nuclei take on various unusual appearances (Hayflick and Moorhead 1961). In more detailed experiments Hayflick demonstrated that primary human cell strains enter Phase 3 after 50 +/−10 passages, no matter when and how long they have been frozen in between passages (Hayflick 1965). Consequently his experiments demonstrate that such cells have a finite lifetime in vitro, and that a cell-intern counting mechanism exists that monitors the accumulative number of population doublings (Fig. 1.1).

2 Cellular Aging: The End Replication Problem

James D. Watson, while investigating the replication pattern of linear molecules of T7 DNA, noted that leading strand replication in the 5′ to 3′ direction should proceed smoothly to the end of its template. However, lagging strand synthesis is unable to copy the parental strand in its entirety (Watson 1972). Lagging strand synthesis is initiated by RNA primers, which are extended to Okazaki fragments

that are ligated together after removal of the initiating RNA. This mechanism does not allow for fill-in synthesis of the gap left by the most distal RNA primer, inevitably leaving the daughter strand shorter than the parental strand. The inability to fully replicate the template, leading to terminal sequence loss during each replication cycle, is termed the end replication problem.

Following an independent approach, Alexey Olovnikov at the Russian Academy of Sciences published "A Theory of Marginotomy" (Olovnikov 1973). His hypothesis holds that the limited doubling potential of primary somatic cells can be explained by terminal sequence loss of the daughter DNA strand. To buffer the progressive erosion, Olovnikov proposed that so-called *telogenes* – vital genes without any coding information – are located at opposite ends of the linear chromosome. Once they are lost due to the end replication problem, the coding chromosomal DNA is no longer protected, replication ceases, and the cells enter what Hayflick called Phase 3 (Hayflick and Moorhead 1961).

Now, of course, we know that the ends of chromosomes do not consist of the hypothesized telogenes, but are specialized structures, called telomeres, consisting of G-rich DNA repeats and proteins that bind to these repeats. Olovnikov was partially correct in suggesting that they fulfill a buffer function, and Watson had precisely predicted the reason for terminal sequence loss. However, at the time it was unclear that telomeres and replication-associated telomere loss represent the counting mechanism that monitors and limits the proliferative potential of primary cells as first discovered by Hayflick. At this point in time we can define the Hayflick limit as a function of initial telomere length and rate of terminal sequence loss per cell division, rendering the telomere the genetic clock that measures cellular replicative aging (Fig. 1.1).

3 Telomeres and Telomerase: A Brief Introduction

Telomeres are the specialized structures at the tips of linear chromosomes. They prevent the natural ends from being recognized as damaged DNA, and consequently protect them from enzymatic modification, such as nucleolytic resection and fusion. Telomeres are nucleoprotein complexes consisting of G-rich repeats of DNA, bound by sets of polypeptides that interact with the repeats and each other. The very terminus of telomeres is not blunted, but consists of a single-stranded 3′ protrusion of the G-rich strand, called a G-tail or G-overhang. These overhangs have been observed in yeast, humans, mice, ciliates, plants, and trypanosomes, demonstrating that they are evolutionary conserved and an essential feature of telomeres (Dionne and Wellinger 1996, Hemann and Greider 1999, Jacob et al. 2001, Makarov et al. 1997, Munoz-Jordan et al. 2001, Riha et al. 2000, Wellinger et al. 1996, Wright et al. 1997). Studies in human and mouse cells led to a telomeric loop-model, which suggests that the G-rich telomeric single-stranded overhang can loop back and invade homologous double-stranded telomeric tracts, resulting in a large lasso-like structure, termed telomeric loop, or t-loop (Griffith et al. 1999). This structure provides an attractive mechanism by which chromosome ends can be distinguished from broken DNA ends, and subsequently, in addition to humans and

mice, t-loops were observed in trypanosomes, ciliates, plants and in yeast species with artificially long telomeres (de Lange 2004).

Mammalian telomeres are associated with the shelterin complex, an assembly of interdependent telomeric core proteins, consisting of TRF1, TRF2, Tin2, Rap1, TPP1, and POT1 (de Lange 2005). It is becoming increasingly clear that the proteins in this complex display multiple interactions, and disruption of individual members leads to disintegration of the complex and telomere dysfunction. In many model organisms loss of telomere function has multiple consequences, such as loss of the telomeric G-rich overhang, resection of the C-rich strand, increased levels of recombination at chromosome ends, altered gene expression patterns, fusion of chromosomes, genome instability, growth arrest and premature senescence, or cell death. In addition to shelterin, mammalian telomeres interact with a plethora of other factors that can influence chromosome end integrity and dynamics, such as Tankyrase 1 and 2, PARP, the MRN complex, the RecQ helicases WRN and BLM, Ku70, Ku86, DNAPK, ATM, ERCC1, XPF and RAD51D (de Lange 2005), pointing to the repair and recombination machineries as important contributors to telomere function.

To counteract replication-associated telomere shortening, a specialized reverse transcriptase complex evolved, which was named terminal telomere transferase at the time of its discovery in the ciliate *Tetrahymena thermophila* (Greider and Blackburn 1985), and is now known simply as telomerase. Telomerase is capable of adding G-rich telomeric repeats to the very ends of chromosomes using its own RNA, named TERC, as a template (Greider and Blackburn 1987, Greider and Blackburn 1989). The identification of the catalytic telomerase subunits in *Saccharomyces cerevisiae* (Lendvay et al. 1996), the ciliate *Euplotes aediculatus* (Lingner and Cech 1996), and humans (Bodnar et al. 1998, Meyerson et al. 1997) suggests a common reverse transcriptase–based mechanism for telomere length stabilization in most organisms with linear genomes (Lingner et al. 1997). Telomerase is active in the germ line and during early development, ensuring that replicative telomere shortening is suppressed, and telomere length is kept constant. However, in most somatic human cells telomerase is not expressed, consequently subjecting chromosome ends in such cells to shortening every time the cell divides (Harley et al. 1990). Progressive telomere shortening ultimately leads to the generation of telomeres that are too short to fulfill their protective and regulatory properties at chromosome ends. Such dysfunctional telomeres are detected by the cell-internal DNA damage machinery, and the cell responds either with death, or by entering a terminally differentiated state, termed replicative senescence (de Lange 2002; see also Allsopp, this volume). Senescent cells are metabolically active, but most likely irreversibly arrested (Fig. 1.1).

As a result, telomere shortening limits the natural replicative life span of somatic human cells, representing a powerful tumor suppressive mechanism (de Lange and Jacks 1999). The finding that all cancer cells maintain a constant telomere length, mostly via activation of telomerase (Kim et al. 1994), points to telomerase-based

telomere length stabilization as a fundamental requirement for immortality (see Rudolph, this volume; Blasco, this volume, Fig. 1.1).

4 Telomere Shortening and Cellular Aging

Telomeres were brought to the forefront of the cellular aging discussion when Olovnikov's hypothesis turned into experimental fact in the model organism *S. cerevisiae*. At the time, it had been suggested that due to the inability of conventional polymerases to fully replicate the 3′ terminus of the G-rich strand, a sequence-specific enzyme would elongate telomeres. Therefore, a mutation screen was designed to identify enzymatic activities responsible for telomere replication via the isolation of mutants defective for the telomere elongation reaction (Lundblad and Szostak 1989). The authors made several predictions, which all turned out to be correct:

- The nature of the screen would allow the identification of enzymatic functions without having to make assumptions about the exact enzymatic activities.
- The identified mutations should not lead to the cells' immediate demise, but rather to lethality or a senescence phenotype over time.
- The *S. cerevisiae* telomeric tracts would become shorter with each generation, and eventually become unable to protect the chromosome ends, resulting in the accumulation of chromosomal abnormalities (Lundblad and Szostak 1989).

The genetic screen, based on the linearization and retention of a circular plasmid carrying telomeric repeats, led to the discovery of a new gene, EST1, which stands for Ever Shorter Telomeres 1. Exactly as predicted by the investigators, a strain mutant for EST1 (the strain est1-1) displays a gradual senescence phenotype, associated with a progressive decrease in telomere length. After 25 generations est1-telomeres are 100 bp shorter than wild type telomeres, and continue to lose terminal sequence for many more generations. Strikingly, the gradual telomere loss is accompanied by a decrease in growth rate and an increase in the frequency of cell death. Finally, after approximately 100 generations the est1-1 strains cease to divide.

The authors had also predicted that mutants in the telomere maintenance pathway should accumulate chromosomal aberrations. Analysis of chromosome loss phenotypes in a strain lacking EST1 demonstrates that mutants do not suffer from chromosome loss in early generations, but as the cells approach senescence, chromosomes are lost at a high frequency, validating the authors' prediction. This landmark publication ends with a statement that finally links Hayflick's observations with telomere biology: " …a loss of telomeric DNA during each somatic cell division may play a role in determining the number of cell divisions possible in a given lineage."

EST1, a protein that binds single-stranded telomeric DNA, turned out to be a subunit of the telomerase complex and to play an essential role in mediating access of the catalytic subunit of telomerase to telomeres (Evans and Lundblad 1999,

http://www.springer.com/978-3-540-73708-7

Telomeres and Telomerase in Aging, Disease, and Cancer
Molecular Mechanisms of Adult Stem Cell Ageing
Rudolph, K.L. (Ed.)
2008, XIV, 330 p. 64 illus., 25 illus. in color., Hardcover
ISBN: 978-3-540-73708-7