CONTRIBUTIONS to SCIENCE, **7** (2): 101–107 (2011)
Institut d'Estudis Catalans, Barcelona
DOI: 10.2436/20.7010.01.114 ISSN: 1575-6343
www.cat-science.cat

Telomeres, the beginning(s) of the end. On the Nobel Prize in Physiology or Medicine awarded to Elizabeth H. Blackburn, Carol W. Greider, and Jack W. Szostak*

Laura Tusell

Cellular Biology Unit, Department of Cellular Biology, Physiology and Immunology, Faculty of Biological Sciences, Autonomous University of Barcelona, Bellaterra

Resum. El Premi Nobel de Fisiologia o Medicina 2009 ha guardonat Elizabeth H. Blackburn, Jack W. Szostak i Carol W. Greider pels seus treballs en el camp dels telòmers i la telomerasa. Els telòmers són formats per seqüències curtes de DNA repetides en tàndem, a les quals s'associen diverses proteïnes. Aquestes estructures nucleoproteiques es troben en els extrems dels cromosomes i són vitals per a mantenir la integritat del genoma, ja que eviten que els cromosomes es fusionin els uns amb els altres. A més de la funció essencial de protecció, els telòmers també tenen un paper fonamental en la replicació completa dels extrems cromosòmics. La longitud telomèrica es manté gràcies a l'enzim telomerasa, un complex constituït per RNA i proteïna que és actiu en cèl·lules germinals i embrionàries, però inactiu en la major part de cèl·lules somàtiques en humans. L'absència de telomerasa en cèl·lules proliferants provoca que els telòmers s'escurcin amb les divisions cel·lulars successives. Quan els telòmers s'escurcen per sota d'una longitud crítica, les cèl·lules aturen la proliferació i entren en una fase de senescència, estat irreversible d'aturada permanent, o bé activen mecanismes de mort cel·lular programada. Malgrat això, algunes cèl·lules poden ser capaces de continuar proliferant, fet que produeix la desprotecció dels extrems cromosòmics. La inestabilitat cromosòmica massiva associada a aquest estat podria ser el mecanisme pel qual alguna cèl·lula adquireix determinades alteracions genètiques necessàries per a la transformació maligna.

Paraules clau: telòmers · telomerasa · *Tetrahymena thermophila* · *Saccharomyces cerevisiae* · progèria · neoplàsia · carcinogènesis

Abstract. The 2009 Nobel Prize for Physiology and Medicine was awarded to Elizabeth H. Blackburn, Jack W. Szostak, and Carol W. Greider for their work on telomeres and telomerase. Telomeres are formed by short DNA sequences repeated in tandem and associated with different proteins. These structures, which are found at the ends of chromosomes, are crucial for maintaining genomic stability by preventing chromosomes from joining with one another. In addition to this essential protective function, telomeres play a vital role in complete DNA end replication. Telomere length is maintained by the enzyme telomerase—a complex made up of RNA and proteins that in humans is active is germinal and embryonic cells but inactive in somatic cells. The absence of telomerase in proliferating cells leads their telomeres to shorten with successive cell divisions. Eventually, the telomeres reach a critically short length that in turn causes massive chromosome instability. This may be the mechanism through which a cell acquires the genetic alternations needed to become malignant. Normally, however, below this critical length, the cells stop dividing and either enter a phase of senescence or an irreversible state of arrest, or mechanisms are activated for programmed cell death. However, some cells that also carry alterations in cell cycle checkpoint proteins continue replicating, giving rise to uncapped chromosome ends and thus to a malignant potential.

Keywords: telomeres · telomerase · *Tetrahymena thermophila* · *Saccharomyces cerevisiae* · progeria · neoplasia · carcinogenesis

---

* Based on the lecture given by the author at the Institute for Catalan Studies, Barcelona, on 14 December 2009, and at the Octubre Centre of Contemporary Culture, Valencia, on 20 January 2010 for the Nobel Prize Cycle.

Correspondence: L. Tusell, Departament de Biologia Cel·lular, de Fisiologia i d'Immunologia, Àrea de Biologia Cel·lular, Edifici C, Despatx C2/050, Campus de la Universitat Autònoma de Barcelona, E-08193 Bellaterra, Catalonia, EU. Tel. +34-935811498. Fax +34-935812295. E-mail: laura.tusell@uab.cat

Fig. 1. From left to right, Elizabeth H. Blackburn**,** Carol W. Greider, and Jack W. Szostak. © The Nobel Foundation. Photos: Ulla Montan.

The 2009 Nobel Prize for Physiology and Medicine was awarded to Elizabeth H. Blackburn, Jack W. Szostak, and Carol W. Greider for their work on telomeres and telomerase (Fig. 1). In 1938, Herman Müller noted the existence of a specialized structure at the natural ends of chromosomes as he was analyzing the chromosomal anomalies induced by X-rays in the fly *Drosophila*. In this study, Müller found that the natural ends of the chromosomes were never involved in the chromosomal reorganizations that appeared, such as inversions, fusions, and translocations [16]. Based on these observations, Müller deduced that the ends of chromosomes must have a specialized structure that protected them from fusion phenomena. Around the same time, Barbara McClintock reached similar conclusions after analyzing the behavior of dicentric chromosomes in the corn plant *Zea mays*. During mitosis, the displacement of two centromeres from a single chromatid towards opposite poles leads to the formation of a structure known as an anaphase bridge. Finally, the tension triggered causes the bridge to break, and the chromosome ends generated tend to fuse once again, leading to a breakage-fusion-bridge (BFB) cycle. McClintock determined that the natural ends of the chromosomes never participated in the formation of these fusions [15]. Therefore, both observations evidenced the existence of structural and functional properties on the natural ends of chromosomes that were unlike the double-strand breaks of DNA and protected the structures from chrmosomal fusion, degradation, and recombination.

The structure of telomere

However, telomeres were ignored until the 1970s, when Elisabeth Blackburn joined Joseph G. Gall's research group at Yale University, New Haven, to sequence the ends of linear minichromosomes in the protozoan *Tetrahymena thermophila*. In 1978, Blackburn and Gall published their findings that the telomeres of the protozoa were made up of G-rich DNA stretches repeated in tandem [2]. Since then, the telomeres of numerous species have been described, and it has become clear that telomere sequences are highly conserved in the different organisms studied. Telomere DNA in eukaryotic organisms consists of a double-strand region with a short sequence rich in G-C tandem repeats and a 3′ single-strand G-rich overhang. In vertebrates, these repeats consist of of six nucleotides $(TTAGGG)_n$

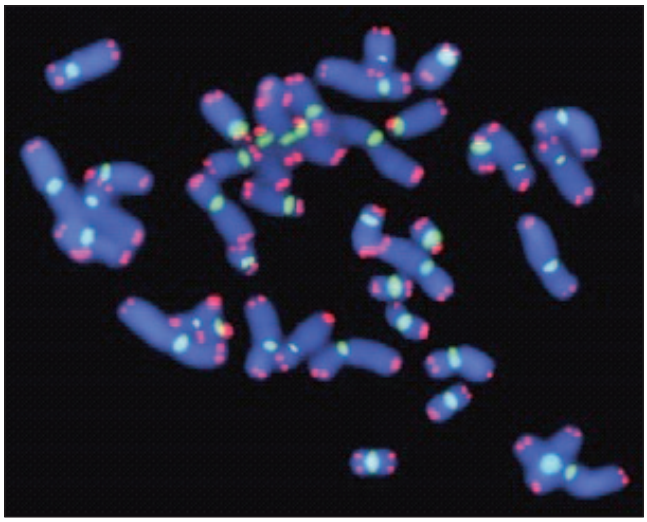

Fig. 2. Human chromosomes (blue) in metaphase hybridized with a centromere probe (green) and a telomere probe (red).

but the length of double-strand telomere DNA and of the 3′ single-strand overhang differ among different species. In *Homo sapiens*, telomere length fluctuates between 5 and 15 kb, while in *Mus musculus* it is 40–60 kb (Fig. 2).

Initially, the telomere was seen as a linear structure; this traditional view was abandoned in 1999, after studies by Griffith and collaborators [11], who showed in vitro that incubation of a telomeric DNA sequence with the telomere protein TRF2 (TTAGGG Repeat Factor 2) resulted in a change in its structure from linear to loop-shaped. This loop structure, which the authors named the t-loop, was produced through folding of the 3′ single-strand overhang over the double helix of telomere repetitions located further back, thus protecting the ends of the chromosomes. Since then numerous telomere-associated proteins have been described. The shelterin protein complex is made up of six proteins (TRF1, TRF2, POT1, TIN2, TPP1, and Rap1) that maintain telomeres during all phases of the cell cycle and serve no other function anywhere else inside the nucleus [18]. These proteins are extremely important in forming and preserving a sealed structure on the chromosome ends, in inhibiting DNA repair processes, and in regulating telomere length. Moreover, there is also a group of less abundant proteins in the telomere that contribute to protecting the chromosome ends, are only temporarily associated with the telomeres, and are known to carry out non-telomere functions both inside and outside the nucleus. Many of these proteins are involved in DNA-damage signaling, DNA repair and replication, and chromatin structure.

The earliest studies

While Elisabeth Blackburn was studying the ends of *Tetrahymena* chromosomes, Jack Szostak was studying gene recombination in the yeast *Saccharomyces cerevisiae* at the Sidney Farber Cancer Institute in Boston, where he had his own laboratory. In his studies, he observed that when circular plasmids were introduced inside yeast cells, instead of integrating

inside the yeast chromosomes they basically remained extrachromosomal, leading to a low recombination frequency of the two genomes. However, if the plasmids were made linear by cutting them with a restriction enzyme, the frequency of recombination phenomena increased and the plasmids that did not integrate into the yeast genome become degraded.

Blackburn and Szostak met in 1980 at the Gordon Research Conference on Nucleic Acids, and decided then and there to embark upon a collaborative project with the goal of ascertaining whether the telomeres of *Tetrahymena* could stabilize the ends of linearized plasmids. After introducing these linear plasmids capped with *Tetrahymena* telomeres $(TTGGGG)_n$ into yeast cells, they found that the linear shape remained, without recombining or degrading [22]. These experiments paved the way for artificial chromosomes and enabled the researchers to determine that telomeres operated in a variety of phylogenetic kingdoms. Subsequent studies led to the identification of the specific sequence of yeast telomeres. However, the most curious observation of all was that *Tetrahymena* telomeres placed inside yeast cells elongate and become more heterogeneous. An exhaustive study of these plasmids by Shampay and collaborators [20] decisively revealed the mechanism underlying the elongation of the chromosomes ends. When linearized plasmids stabilized with the *Tetrahymena* telomeres (TTGGGG) were introduced into yeast, additional telomere repetitions took place. This addition was easy to detect since the new repetitions had the specific sequence of yeast telomeres (TG_{1-3}). Two different models were proposed to explain this phenomenon. The first invoked the presence of a terminal transferase capable of adding new telomere repetitions, and the second a gene recombination process.

Maintenance of telomere length

In 1984, Carol Greider joined the Blackburn laboratory and began to search for the cellular enzyme capable of adding telomere repetitions to the ends of chromosomes. Her studies consisted of incubating synthetic oligonucleotides of different sequences with protein extracts from *Tetrahymena*. On Christmas Eve 1984, these experiments finally revealed the existence of a specific enzyme responsible for maintaining telomere length. Only the oligonucleotides that had the *Tetrahymena* or the yeast telomere sequence were elongated; there was no new addition of telomeric sequences at the ends of DNA fragments with other sequence compositions [8]. These results confirmed the existence of an enzyme capable of elongating the ends of chromosomes, which was named telomerase. Later, the same researchers determined that it was not a conventional enzyme but a ribonucleoprotein [9]. Specifically, telomerase consists of two main subunits, an RNA component (TERC) containing a sequence complementary to the telomeric overhang, and a protein component with reverse transcriptase activity (TERT), as well as numerous additional proteins. Telomerase promotes telomere elongation by copying the nucleotides of the template RNA at the overhanging 3' end of the chromosome, a function carried out by TERT, and afterwards the cell's conventional replication machinery copies the nucleotides at the 5' end [10].

These discoveries were crucial in understanding the events occurring at the ends of chromosomes. Studies subsequent to the discovery of telomerase revealed the presence of mutant yeast cells whose telomere length shortened with cell divisions, called *EST1* (*even shorter telomeres*) [14]. These yeast cells showed an increased rate of chromosomal loss with successive generations; additionally, associated with progressive shortening of the telomeres was the gradual appearance of a senescent phenotype. Similar results were obtained by the Blackburn group in *Tetrahymena* when mutations were generated in the telomerase RNA template, suggesting that telomeres are important to genome stability and to cell viability [24].

In fact, back in 1973 Alexei Olovnikov had postulated that, given the characteristics of DNA replication, DNA polymerase was incapable of copying the 3' ends of linear DNA molecules, which led to shortening of the telomere in each cell division cycle. DNA polymerase can only extend primers from their free 3' OH end, synthesizing DNA in the 5' to 3' direction. Given that DNA is made up of two anti-parallel strands, one of them, the leading strand, is synthesized continuously in the same direction as the replication fork proceeds, and the other, the lagging strand, discontinuously in the opposite direction through the extension of RNA primers (Okazaki fragments) by the action of DNA polymerase. The RNA primers are replaced by DNA as the Okazaki fragments grow, but elimination of the primer on the 5' end of the chromosome results in the shortening of the newly synthesized strand. Today we know that in addition to the phenomenon of the replication terminus, there is also active deterioration of the chromosome ends to generate an overhang long enough to form the t-loop structure. In the absence of a compensatory mechanism, this results in a loss of 50–200 bp in the telomere track after each round of replication.

Therefore, when there is no telomerase activity in the cells, the chromosomes shorten their telomeres, as is the case in vitro or during the aging process of an organism. In humans, chromosomal shortening occurs in the majority of somatic cells, since the enzyme telomerase is inactive; by contrast, in germinal line cells, stem cells, proliferating somatic cells, and tumor cells the enzyme is active, compensating for the loss of nucleotides during DNA replication.

Telomeres and the potential of cell proliferation

For many years it was thought that the cells of pluricellular organisms had the capacity to proliferate indefinitely. However, this view changed radically in 1965, when Hayflick observed that human fibroblasts in culture underwent a finite number of divisions before entering a non-proliferative state. This state, known as replicative senescence or the Hayflick limit, is characterized by a permanent cellular arrest in the G1 phase of the cell cycle, which enables cells to remain metabolically active and viable for long periods of time. These observations gave rise to the hypothesis of the existence of a biological clock that counts the number of

divisions a cell is able to undergo, and is likewise capable of arresting cell growth after a certain number of divisions.

It was not until the 1990s that replicative senescence was associated with telomeres, after Harley, Futcher, and Greider [12] demonstrated telomere shortening after each round of cell division that in human fibroblasts, as predicted in the DNA replication models proposed by Olovnikov [17] and Watson [23]. Therefore, it was confirmed that when telomeres shorten to a critical length, cells stop proliferating and become senescent. Expression of the constitutive form human TERT (hTERT) in pre-senescent fibroblasts, lymphocytes, epithelial cells, and other cell types, by contrast, can extend the proliferative capacity of the cells, thus confirming the role of telomeres in the replicative senescence of cells [4]. We should point out that senescence can also be induced by factors other than telomeres, such as viral infection or oxidative stress.

Today we know that the true cause of the entry into senescence is not the reaching of a critical telomere length but the loss of the t-loop. This vulnerability of the telomere occurs following the loss of repetitions of telomeric DNA or of proteins associated with the t-loop. Loss of the telomere's structure eliminates the ability of the normal cellular repair machinery to distinguish between natural and broken chromosome ends. Indeed, alterations in telomere structure following the overexpression of a negative dominant allele of TRF2 lead to the activation of ATM and ATR, two proteins that signal specifically damaged DNA, which in turn activate cellular repair enzymes. This sequence of events is supported by the observation that proteins recognizing and/or repairing DNA damage accumulate in the vicinity of the excessively short telomeres of senescent human fibroblasts [5]. These findings suggest that short or dysfunctional telomeres activate the same type of response as occurs when DNA is damaged directly, and that senescence involves mechanisms similar to those operating in response to DNA damage.

Therefore, the successive erosion of telomeres in each cell division cycle leads to the loss of the t-loop and consequently the capacity of the telomeres to protect the chromosome ends. This eventually culminates in the loss of genetic material and an increase in chromosomal instability, unless a cellular response to the damaged DNA occurs, such as cell cycle arrest or programmed cell death, also known as apoptosis. For this reason, when telomeres reach a critical length after many rounds of divisions, the cells permanently arrest in the G1 phase of the cell cycle in response to several signals: the actions of DNA damage repair proteins [21], activation of the cell cycle control proteins Rb and p53, and the expression of the Cdk protein inhibitors $p16^{INK4a}$ and p21. For the majority of cells, senescence represents an irreversible state of permanent arrest, but they are still both metabolically active and viable.

The aging organism

Senescent cells are known to accumulate in the tissues of elderly individuals. Thus, while definitive evidence is lacking, it appears that the accumulation of viable, non-proliferating cells compromises tissue functionality and contributes to the different pathologies associated with aging. Indeed, the role of telomere homeostasis and cell senescence in the aging organism is backed by studies relating donor age and shortened telomeres in highly proliferating tissues, such as lymphocytes, muscle cells, endothelial cells, lenticular epithelial cells, and adrenocortical cells, and the lower in-vitro proliferative capacity of cells from patients with premature aging syndromes (progeria), including Werner syndrome, Bloom syndrome, Down syndrome, and Hutchinson-Gilford progeria syndrome. Studies of those patients have provided the most convincing data relating human aging and telomere dysfunction. Surprisingly, the cells of these individuals show alterations in telomere metabolism and telomeres that are much shorter than those of cells from control populations. Many of the progeria syndromes are caused by mutations in the genes encoding TERT or TERC or in those encoding proteins that interact with telomeres. Congenital dyskeratosis was the first human illness whose pathogenesis could be attributed to mutations in telomerase components. These patients suffer from alopecia (hair loss), premature gray hair, tooth loss, osteoporosis, ungual dystrophies, defective skin pigmentation, and deterioration of the immune system. These symptoms are similar to what occurs in the normal aging process in humans and are also seen in mice deficient in telomerase.

The generation of *mTerc*$^{-/-}$ mice, deficient in telomerase RNA [3], enabled researchers to demonstrate the impact exerted by a given telomere length on both the cellular level and the organism as a whole. The mutant mice exhibit a gradual reduction in the length of their telomeres during and over successive rounds of cell division, as is the case in the somatic cells of aging humans. Early generations of these animals do not show an obviously pathological phenotype, most likely because their cells still have sufficiently long telomeres. However, in later generations there are defects in highly proliferative tissues, such as the hematopoietic system, reproductive organs, and the intestinal epithelium, in addition to signs of premature aging. These observations imply that a minimum telomere length is needed to maintain the functional integrity of tissues and that the protection and maintenance of telomere length are important factors in controlling cell life-span; however, when discussing the aging process, we must also bear in mind that it is a highly complex process that is influenced not only by telomere length but also by many other regulatory factors.

Telomeres, chromosomal instability, and carcinogenesis

The loss of telomere function in *mTerc*$^{-/-}$ mice is also associated with the formation of chromosomal fusions and the generation of aneuploidy. Telomeres that have become dysfunctional because of excessive shortening are recognized by the same cell mechanisms that respond to DNA damage but are incorrectly repaired by these mechanisms, leading to chromosomes with two centromeres. These dicentric chromosomes, as McClintock postulated, can give rise to an endless chain of BFB cycles, generating new chromosomal anomalies with each cell

division. Thus, over time, cells with a high proliferative capacity will accumulate extensive chromosomal instability, thus activating p53, which triggers programmed cell death. In fact, the dysfunction of highly proliferative tissues in *mTerc*$^{-/-}$ mice is associated with high rates of apoptosis.

In contrast to the relationship between excessive telomere shortening and senescence or apoptosis, excessive telomerase activity is related to malignancy. In fact, 85% of human cancers show telomerase activity. Given this fascinating connection, telomerase-deficient mice would be a good model to determine the relationship between telomere length, telomerase, and cancer. In fact, experiments in later generation (G4–G6) *mTerc*$^{-/-}$ mice have shown that the absence of telomerase suppresses tumor progression. This tumor-suppressing phenotype of excessively short telomeres is related to the activation of p53. In *mTerc*$^{-/-}$ mice in advanced generations, there is a decline in tumor formation due to the loss of cell viability—coupled with a rise in p53-mediated apoptosis—which is associated with telomere dysfunction. Likewise, studies of mice deficient in both telomerase and other cancer-related proteins, such as APC^{MIN}, INK4a/Arf$^{-/-}$, ATM$^{-/-}$, and Alb-uPA$^{-/-}$, also detected a reduction in carcinogenesis in these animals.

Specifically, experiments conducted with double-mutant in *mTerc*$^{-/-}$/*Apc*^{MIN} mice enabled researchers to associate telomere dysfunction with incipient stages of neoplasia. Even though the initial number of neoplastic lesions was higher in *mTerc*$^{-/-}$/*Apc*^{MIN} mice than in *Apc*^{MIN} mice, the growth and progression of adenomas was significantly suppressed in the double mutants. These studies showed that an initial telomere dysfunction is necessary to promote chromosomal instability capable of generating a preneoplastic lesion; however, later, to allow for tumor growth, telomerase must be reactivated in order to stabilize the genome [19]. If this is not the case, then the chromosomal instability is unsustainable and most of the cells die through apoptosis. These results are consistent with very low or nonexistent telomerase activity in the majority of incipient neoplasias in humans, while the progression to a state of malignancy is associated with high telomerase activity.

Important insights into an association between telomere dysfunction and human epithelial carcinogenesis have come from studies in which *mTerc*$^{-/-}$ mice were crossed with mice deficient in p53 [1]. Telomere erosion triggers a damage signal in p53-dependent DNA; thus, in the presence of functional p53, short telomeres promote apoptosis or the G1-phase arrest of cells. In later generations of *mTerc*$^{-/-}$/*p53*$^{-/-}$ mice, the loss of p53 recapitulates many of the symptoms caused by telomere dysfunction, such as the apoptosis of germinal cells, even though they have short telomeres. The recovery of these cells with short telomeres and a higher proliferative activity results in greater genomic instability, the appearance of tumors at early ages, and a decline in animal survival. However, the most important observation in this study was the appearance of a small proportion of carcinomas, which had not been found in *mTerc*$^{-/-}$/*p53*$^{+/+}$ mice. These observations suggest that the premature death of *mTerc*$^{-/-}$/*p53*$^{+/+}$ mice from lymphoid or mesenchymal cancers masked the impact of telomere dysfunction on epithelial carcinogenesis. In p53 haploinsufficient mice, *mTerc*$^{-/-}$/*p53*$^{+/-}$, there was an increased incidence of tumors and a radical change in their spectrum, evidenced by a shift from lymphoproliferative to carcinomas in old vs. young mice. Moreover, the murine carcinomas showed complex structural chromosomal anomalies similar to those found in human carcinomas. These results along with the fact that human neoplastic cells display short telomeres and complex chromosomal anomalies led to speculations that telomere dysfunction is a risk factor that contributes to tumorigenesis. Accordingly, a model was proposed in which telomere dysfunction coupled with deficiencies in cell cycle checkpoints is responsible for the appearance of carcinomas in the adult human population [6].

Model of human epithelial carcinogenesis

The majority of cancers in the adult human population are epithelial in origin. Indeed, epithelial tumors account for 83.6% of adult neoplasias and the incidence rises with age. As discussed above, most carcinomas show chromosomal instability, manifested as complex chromosomal anomalies. According to the epithelial carcinogenesis model, chromosomal instability in proliferative epithelial cells depends on the telomere shortening that occurs as cells divide, coupled with deficiencies in cell cycle checkpoints.

Epithelial cells proliferate throughout the human lifespan to regenerate the epithelium. However, these cells have no telomerase activity, such that their telomeres become shorter as the individual ages. When the telomeres reach a critically short length, mechanisms are activated to limit cell division, such that cell cycle progression is halted (replicative senescence). If these mechanisms are damaged, the cells may continue to proliferate, with even further shortening of their telomeres. This leads to a loss in telomere structure and thus of the cells' ability to distinguish the natural chromosome ends from double-strand breaks in DNA. Consequently, the cellular repair machinery is activated, and the unprotected chromosome ends are thereby fused. These telomere-telomere fusions give rise to dicentric chromosomes with two centromeres and thus potentially to a genomic instability in successive cell divisions through the advent of BFB cycles. This massive chromosomal instability ends in a phase of cell crisis, characterized by the presence of very short telomeres, telomere fusions, anaphase bridges, and a high rate of apoptosis. Even though most cells in this crisis stage will die, a very low percentage survive and become immortalized through the activation of mechanisms capable of maintaining telomere length, such as telomerase reactivation.

Chromosome stabilization through telomerase or the ALT (alternative lengthening of telomeres) mechanism reduces chromosomal instability at the cost of providing the tumor with unlimited proliferative capacity. For all of these reasons, despite the barriers to cell proliferation posed by the crisis stage and replicative senescence, the massive chromosomal instability associated with crisis might be the mechanism through which some cells acquire the genetic alterations needed to become malignant [21].

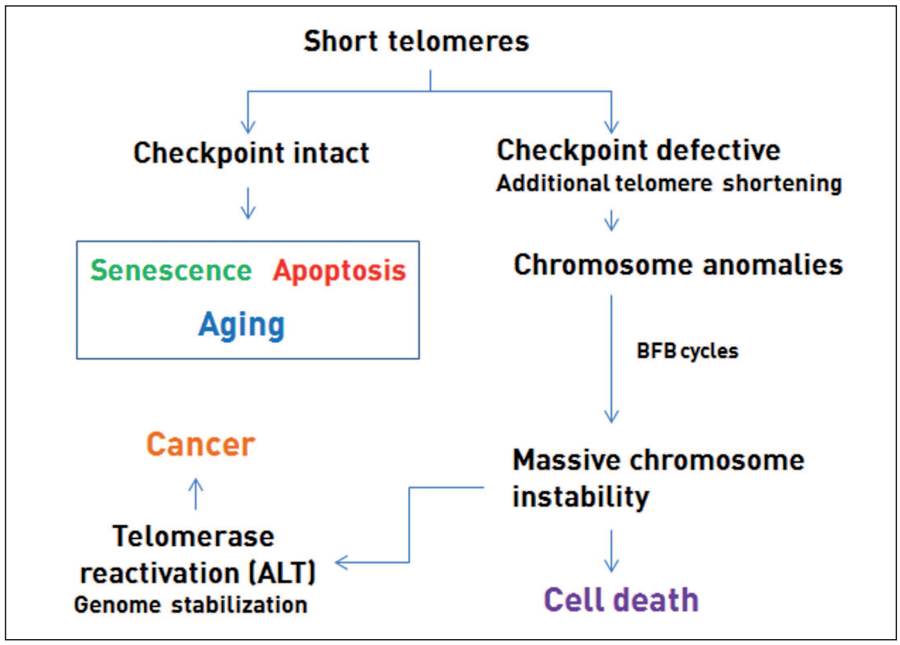

Fig. 3. The relationship between telomeres and telomerase and aging and cancer depends on an environment that is, respectively, proficient or deficient in cell cycle checkpoints.

Therefore, telomere shortening and the activation of telomerase can act as either tumor suppressors or oncogenes, depending on when they take place and their genetic context. On the one hand, the loss in telomere repetitions in an intact cellular environment serves as a tumor-suppressing mechanism, limits cell proliferation, and causes senescence or apoptosis. However, the loss in telomere structure in an environment deficient in genes that act as cell cycle checkpoints leads to telomere instability due to the absence of telomerase and fosters the onset of malignant transformation (Fig. 3).

Pro- and anti-telomerase treatments

Given the close relationship between telomerase, aging, and cancer, a number of studies have recently been conducted to ascertain whether therapies directed at the overexpression or inhibition of telomerase have any effect on the regeneration of tissues damaged during aging or can prevent/inhibit tumor growth. The stimulation or reactivation of telomerase would be expected to prevent aging in addition to providing a therapeutic strategy for telomere phenotype syndromes. However, it is still not clear whether an increase in telomerase activity is sufficient to prevent symptoms of aging. An improvement in tissue generation has been shown in genetically modified K5-Tert mice, which overexpress telomere activity in basal keratinocytes and stem cells of stratified epithelia [7]. The increase in telomere activity reduced both the rate of atrophy in the testicles, uterus, and ovaries of these mice, and renal dysfunctions in elderly mice. Moreover, the life expectancy of the genetically modified mice was longer than that of the control group. By contrast, when K5-Tert mice were treated with chemical carcinogens they had a higher mortality from cancer at advanced ages, suggesting the cooperation of other genetic anomalies over the lifetime of the mice, with an increase in telomerase playing a role in the genesis of neoplasia. These studies therefore suggest certain potential risks in gene therapies involving telomerase overexpression. At the same time, evidence that dysfunctional telomeres can activate both senescence and apoptosis, thus blocking tumorigenesis in different tissues, points to potential therapeutic applications of molecules with anti-telomerase capacity. Currently, several compounds have been shown to act on telomerase: those that directly inhibit the enzyme or target the proteins in the shelterin complex, and vaccines that stimulate the immune system to attack cells expressing the telomerase antigen.

Numerous in vitro studies have demonstrated that the treatment of different tumor cell lines with telomerase inhibitors leads to a shortening of the telomere and subsequently triggers inhibition of the cells' proliferative potential. Furthermore, the treatment of tumor cells in immunocompromised mice inhibits tumor growth. Similarly, the administration of telomerase inhibitors to mice xenotransplanted with breast cancer cells was effective in preventing lung metastasis. These results with human cells in vitro and murine models in vivo show that such treatments can be highly effective in lowering tumor growth. Today, numerous anti-telomerase treatments are currently being tested in clinical trials (phase I, II, and III). Given the prevalence of high telomerase activity and/or the presence of short telomeres in multiple types of tumors compared to normal tissue, targeting telomerase would seem to be a reasonable approach to the treatment of a wide range of cancers.

Brief biography of the Nobel Prize winners

Elizabeth H. Blackburn (Hobart, Tasmania, Australia, 1948) was born into a family of doctors. She moved from Tasmania to Melbourne (Victoria) in 1957, earning her Bachelor's (1970) and her Master's (1972) degrees from the University of Melbourne and her doctorate (1975) from Cambridge University (UK). After working as a postdoctoral fellow at Yale (1975–1977), she went to the University of California at Berkeley in 1978 and has

worked at the University of California at San Francisco since 1990. Although she is primarily known for her studies on telomeres and the role of telomerase, her name was also catapulted into the newspaper headlines in February 2004 when she was removed from the President's Council on Bioethics, presumably due to disagreements with George W. Bush's administration over the issue of research into totipotential cells derived from human embryos.

Carol W. Greider (Davis, USA, 1961) graduated with a Bachelor's degree in Biology from the University of California at Santa Barbara in 1983. In 1984, she began her doctoral studies with Blackburn at Berkeley, in the field of telomere protection. Using the model ciliated protozoa *Tetrahymena*, Greider and Blackburn identified the activity of the enzyme telomerase, directing the controlled regeneration of chromosome ends. After the publication of an important article [8] in the journal *Cell* (1985), she finished her thesis in 1987. After completing her postdoctoral fellowships, Greider joined the Cold Spring Harbor Laboratory (Long Island, New York), with a joint appointment in 1997 at Johns Hopkins University. Her laboratory has primarily pursued avenues of research derived from her doctoral dissertation.

Jack W. Szostak (London, UK, 1952) graduated with a Bachelor's degree in Biology from McGill University in 1971. He earned his doctorate at Cornell University and then permanently moved to Harvard Medical School. His laboratory is part of the Howard Hughes Medical Institute. His career has been linked to the field of genetic recombination and to studies on the processes that govern eukaryotic chromosomes, such as telomere dynamics (shortening and elongation, protection, and destruction). These studies helped him to design the first yeast artificial chromosomes (YACs), which have become fundamental tools in the manipulation of long DNA fragments, used in the comprehensive human genome mapping project completed during last decade. More recently, Szostak, has pursued studies on artificial chromosomes with the intention of generating 'artificial cells,' a process that could provide information on how the first cells emerged from the first organic compounds on primeval Earth.

References

1. Artandi SE, Chang S, Lee SL, Alson S, Gottlieb GJ, Chin L, DePinho RA (2000) Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature 406:641-645
2. Blackburn EH, Gall JG (1978) A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. J Mol Biol 120:33-53
3. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW (1977) Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91:25-34
4. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279:349-352
5. D'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP (2003) A DNA damage checkpoint response in telomere-initiated senescence. Nature 426:194-198
6. DePinho RA (2000) The age of cancer. Nature 408:248-254
7. González-Suárez E, Geserick C, Flores JM, Blasco MA (2005) Antagonistic effects of telomerase on cancer and aging in K5-mTert transgenic mice. Oncogene 24:2256-2270
8. Greider CW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell 43:405-413
9. Greider CW, Blackburn EH (1987) The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with tFwo kinds of primer specificity. Cell 51:887-898
10. Greider CW, Blackburn EH (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature 337:331-337
11. Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, de Lange T (1999) Mammalian telomeres end in a large duplex loop. Cell 97:503-514
12. Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during aging of human fibroblasts. Nature 345:458-460
13. Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. ExpCell Res 37:614-636
14. Lundblad V, Szostak JW (1989) A mutant with a defect in telomere elongation leads to senescence in yeast. Cell 57:633-643
15. McClintock B (1941) The stability of broken ends of chromosomes in *Zea mays*. Genetics 26:234-282
16. Müller HJ (1938) The remaking of chromosomes. Collecting Net 13:181-198
17. Olovnikov AM (1973) 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
18. Palm W, de Lange T (2008) How shelterin protects mammalian telomeres. Annu Rev Genet 42:301-334
19. Rudolph KL, Millard M, Bosenberg MW, DePinho RA (2001) Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. Nat Genet 28:155-159
20. Shampay J, Szostak JW, Blackburn EH (1984) DNA sequences of telomeres maintained in yeast. Nature 310:154-157
21. Stewart SA, Weinberg RA (2006) Telomeres: Cancer to human aging. Annu Rev Cell Dev Biol 22:531-557
22. Szostak JW, Blackburn EH (1982) Cloning yeast telomeres on linear plasmid vectors. Cell 29(1):245-255
23. Watson JD (1972) Origin of concatemeric T7 DNA. Nat New Biol 239:197-201
24. Yu GL, Bradley JD, Attardi LD, Blackburn EH (1990) In vivo alteration of telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. Nature 344:126-132