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REVIEW

Olovnikov, Telomeres, and Telomerase. Is It Possible to Prolong a Healthy Life?

Yegor E. Yegorov

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russia
e-mail: yegorov58@gmail.com

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Abstract—The science of telomeres and telomerase has made tremendous progress in recent decades. In this review, we consider it first in a historical context (the Carrel–Hayflick–Olovnikov–Blackburn chain of discoveries) and then review current knowledge on the telomere structure and dynamics in norm and pathology. Central to the review are consequences of the telomere shortening, including telomere position effects, DNA damage signaling, and increased genetic instability. Cell senescence and role of telomere length in its development are discussed separately. Therapeutic aspects and risks of telomere lengthening methods including use of telomerase and other approaches are also discussed.

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Keywords: telomeres, telomerase, aging, carcinogenesis, Olovnikov, telomere crisis, cell senescence, inflammatory aging, genetic instability

HISTORICAL INTRODUCTION

The end regions of chromosomes visible in a light microscope have been termed by cytologists telomeres. These are very large structures covering regions of millions of DNA base pairs. Descriptions of telomeres as special structures that ensure integrity of chromosomes first appeared in the Muller's work performed on the Drosophila chromosomes in 1938 [1], and in the works of McClintock performed on the maize chromosomes in 1938-1941 [2-4]. The essence of these early observations is that a broken chromosome remains unstable until it acquires a new telomere either by recombination or *de novo*. Modern biologists consider the much smaller end regions of chromosomes, thousands of nucleotide pairs long, as telomeres.

Although the idea that the ends of linear chromosome require special stabilization has been recognized by the scientific community, it had not been especially fruitful until it was connected to the cell senescence and cell immortalization.

Back in the 19th century, after numerous proofs of cell theory, it became clear that there are mortal and immortal cells in multicellular organisms, including humans. Immortal cells must exist because living organisms, being the product of long evolution, are still alive. At the same time, our cells die, including via programmed death. Early experiments on the cell cultivation pointed to the potential immortality of cells. Experiments on serial transplantation of tumor cells in peritoneal cavities of rats (end of the XIX century) [5], then the famous experiment of Alexis Carrel, when chicken cells were continuously multiplied for 30 years, created a false belief that all cells are immortal [6].

Retrospectively, we realize that experiments demonstrating cell death in culture are of little informative value. Such result can always be attributed to a technical error. A prime example is the hard-won published paper by Leonard Hayflick, who claimed that "in our hands, cells are not capable of infinite division" [7]. Future Nobel laureate Peyton Rous wrote just one word as a review – "nonsense" [8]. Nevertheless, the "Hayflick limit" turned out to be real, and the term became generally accepted.

The main reason for persistence of the view that cultured cells are immortal was Carrel's reputation. Author of the idea of making organs from the patient cells to avoid rejection (even before the discovery of blood groups), author of the vascular suture (Nobel Prize in 1912), inventor of masses of devices for transplantation and sterile work – he was extremely authoritative. In fact, he developed technology for primary surgical wound care during World War I, saving thousands of lives.

At the same time, he was the man who proposed "a humane and economical way of disposing of inferior human beings in gas chambers" ("should be humanely and economically disposed of in small euthanasic institutions supplied with proper gases"), which was realized already during the Second World War. After 1945 in 20 French cities streets named after Carrel were renamed back. The French disliked very much those who collaborated with the Nazis [9-11].

Limited proliferative capacity of cells found in the Hayflick's experiments had to be explained. After the mechanisms of DNA replication became clear in general terms, a hypothesis on the mechanism of functioning of a cell division counter was suggested. In 1971, Alexey Olovnikov proposed the "principle of marginotomy in template synthesis of polynucleotides", which claims that DNA polymerase is unable to fully replicate a linear template; the replica is always shorter in its initial part [12]. Gradual shortening of DNA (underreplication) limits proliferative potential of the cells and can serve as the basis of the cell division counter in the Hayflick's experiments. In the same work it was postulated that immortal cells should possess an enzyme that completes chromosome ends.

This enzyme, later called telomerase, was first discovered in 1985 in the protozoan cells [13]. At that time, the authors thought that the enzyme they found in infusoria cells was necessary only for replication of the special telomeres of this protozoan. Later they (Nobel laureates Elizabeth Blackburn and Carol Greider) recalled: "We did not know about Olovnikov's ideas until 1988, when Calvin Harley told Greider about them. Intrigued, Greider, Harley, and their colleagues decided to find out if the chromosome shortening in human cells occurs over time." [14].

The very next year, 1989, telomerase was detected in human cells and the length of human telomeres was found to change during development. A year later, telomere shortening during the cell aging was revealed. In 1998, it was proved that telomerase expression induced by gene insertion leads to cell immortalization.

In addition to Olovnikov's visionary articles of 1971 and 1973, several of his other important works should be mentioned. A few months ago, Alexei Matveyevich passed away, and in writing the historical part of the review, I would like to touch on some of his hypotheses, both confirmed and not confirmed.

First, few people remember that Olovnikov suggested that in addition to underreplication, underrepair may also lead to telomere shortening [15, 16]. Indeed, it was subsequently found that telomeres shorten at different rates (per division) depending on the conditions of cell cultivation [17, 18]. Thus, telomere shortening turns not into a simple division count, but into a total index that takes into account various factors, including oxidative stress conditions.

Second, around the millennium boundary, Olovnikov hypothesized existence of perichromosomal particles, which are copies of chromosome segments [19]. It was assumed that transcription of these particles yields some short RNAs controlling many processes related to spatial and temporal regulation of genes and chromatin rearrangement. The terms were introduced: redusomes, chronomers, printomers, fountain RNA, etc. This hypothesis is still waiting for its confirmation. TERRA (TElomeric Repeat-containing RNAs) could be considered as distant analogs of such particles (see below).

It should be noted that scientific events related to the study of cellular immortality have gained wide publicity due to their intensive coverage by the mass media. The author of this article learned about the notorious 50 divisions that human cells are capable of from the popular in USSR weekly review of the foreign press "Za rubezhom (Abroad)". The term "Hayflick limit" has entered the encyclopedias. Unlike the Hayflick and Carrel works, Olovnikov's work was little known, also due to the relative isolation of Soviet science from the world. Only translation of the Olovnikov's paper published in Russian in 1971 into English (2 years later) [20], made it possible to acquaint the world community with it. And it received a well-deserved recognition 15 years later, leading to an explosive growth in the number of papers on the role of telomeres in aging all over the world and, as a result, to the Nobel Prize, but not to A. M. Olovnikov.

TELOMERS

Telomeres are the ends of chromosomes, and they must be packaged so that the repair systems do not confuse them with the double-stranded breaks in DNA. This is achieved through the ability of telomere sequences to fold in a special way and through specialized proteins that protect these "breaks." In humans and all vertebrates, telomeric DNA is represented by the 5'-$(TTAGGG)_n$-3' sequence [21]. At the ends of human telomeres, there are single-stranded 3' regions about 100-150 nucleotides long [22] (Fig. 1).

This single-stranded region is present both in the cells with and without telomerase, so it cannot be explained by telomerase activity. Presence of this free 3'-end is a direct consequence of underreplication of the end, namely, removal of the 5'-end RNA primer on the opposite strand and its inability to be filled by DNA polymerase during replication.

As the structure suggests, this single-stranded site contains repetitive clusters of three guanines (GGG). Calculations show that this sequence easily forms non-canonical structures (triplexes, quadruplexes). G-4 structures (quadruplexes) can be intramolecular, bimolecular, and even tetramolecular, i.e., connecting 4 DNA strands.

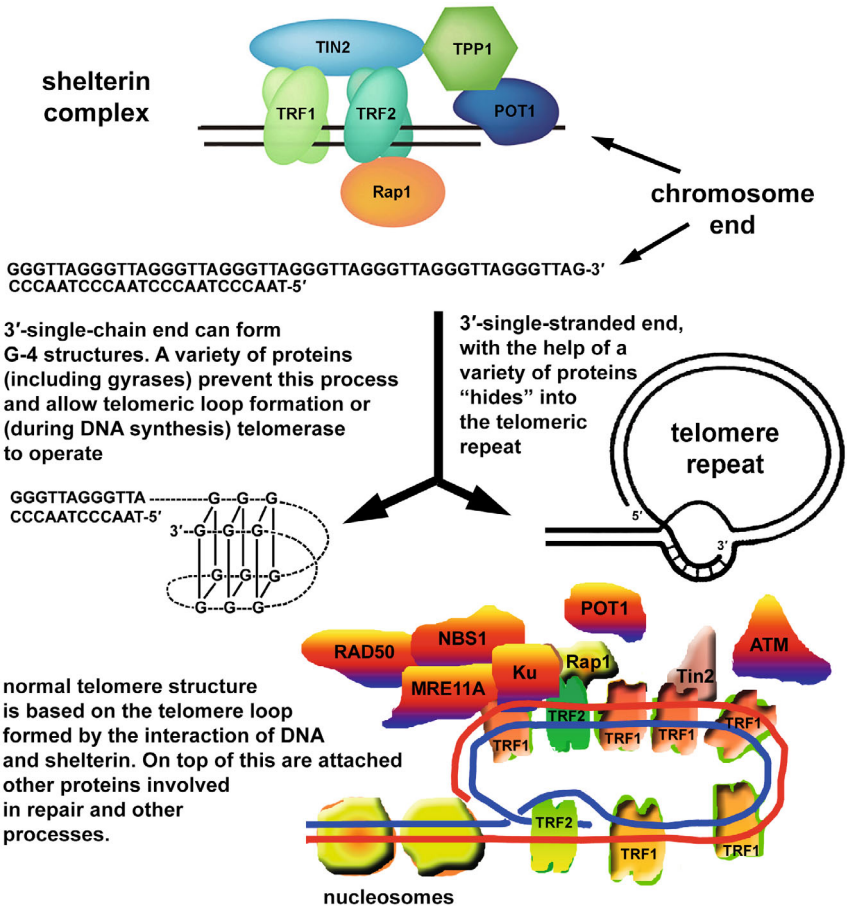

Fig. 1. A simplified scheme of telomere organization.

The strands in them can be parallel and antiparallel. It is believed that at least 12 guanines are required to form a quadruplex [23].

There is an alternative, more widely accepted model of the behavior of the single-stranded 3'-end of DNA. The results of experiments of De Lange and coworkers allowed to propose a telomere model based on the telomeric loop [24]. According to it, the single-stranded end together with the proteins interacts with the double helix of telomeric DNA. Thus, a telomeric loop is formed. Length of this loop correlates with the length of the telomeric repeat measured by independent methods.

Six proteins present in telomeres form a complex called shelterin. Two proteins (TRF1 and TRF2) bind the double-stranded regions of telomeric DNA, two proteins (POT1 and TPP1) bind the single-stranded DNA, and two more proteins (TIN2 and Pap1) have no (at least in humans) DNA-binding sites [25, 26] (Fig. 1).

Despite the fact that the telomeric regions of DNA are devoid of protein-coding sequences, they are transcribed. A long noncoding RNA TERRA (TElomeric Repeat-containing RNA) is formed [27, 28]. TERRA expression is initiated in subtelomeric regions [29]. Thus, it contains subtelomeric sequences at the 5'-end and telomeric UUAGGG repeats at the 3'-end [30].

Various events leading to the changes in TERRA expression are strongly associated with the telomere length [31, 32]. For example, TERRA expression is highly sensitive to stress [33, 34] and depends on epigenetic changes in subtelomeric regions [35]. Interestingly, TERRA is abundantly detected as part of plasma exosomes and is able to modulate the inflammatory response [36]. Also, TERRA expression is altered in Hutchinson–Gilford progeria [37]. Overall, however, a clear picture of TERRA function has not yet emerged. It is obvious that TERRA expression affects numerous heterogeneous cellular responses, including telomere maintenance, chromatin state, stress response, and inflammation induction. Involvement of TERRA in carcinogenesis is being intensively studied. Recent articles [38-42] on this subject can be recommended to the reader.

TELOMERASE

Telomerase is a reverse transcriptase with an integrated RNA template for telomeric repeat synthesis. The enzyme is based on the protein part of human telomerase reverse transcriptase (hTERT) and the RNA component (hTERC). A small part of hTERC contains

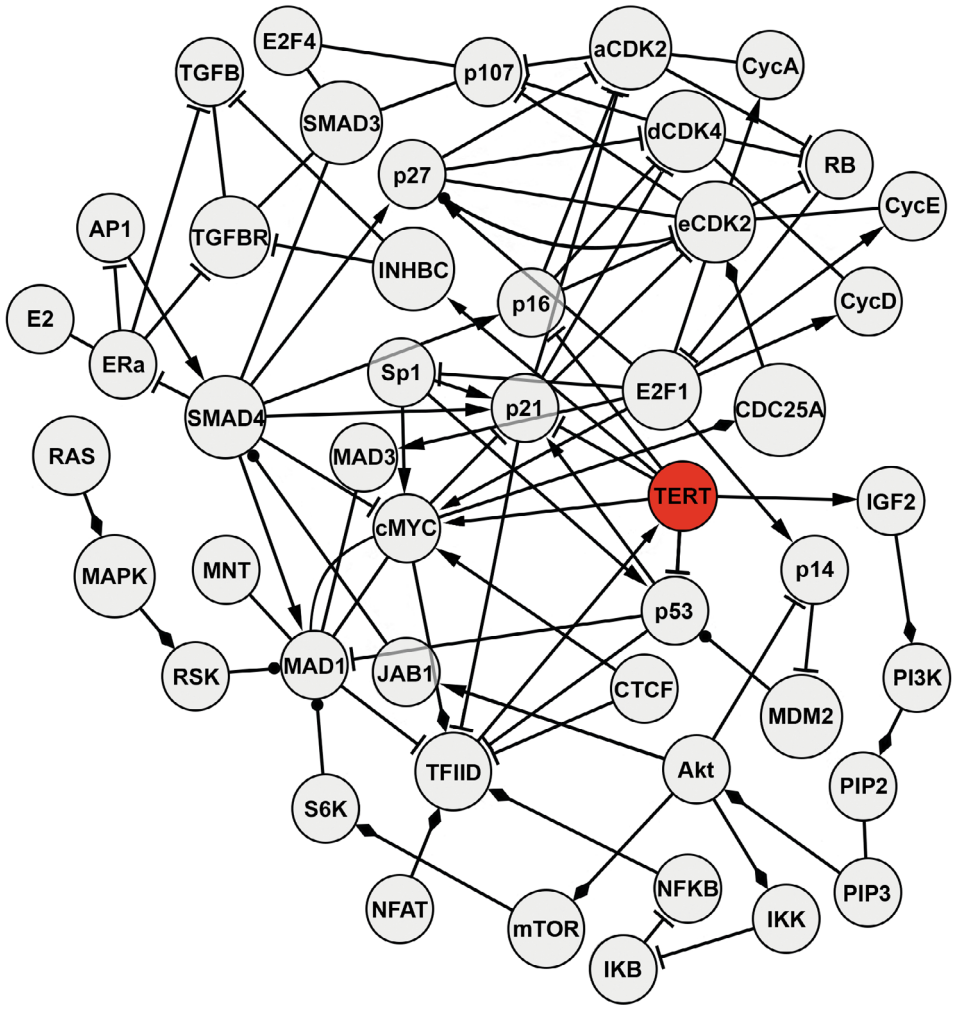

Fig. 2. Partial interactome in hTERT regulation (from [49] with permission).

a template for telomeric DNA synthesis [43]. The enzyme works as a large complex with molecular mass of about 0.5×10^6 Da. The complex includes hTERT, hTERC, dyskerin, TCAB1, as well as temporally associated proteins pontin, reptin, and chaperones HSP90 and TRiC [44].

As predicted in the Olovnikov's hypothesis, telomerase is expressed in germ, stem, and cancer cells. In the latter case, activation of hTERT, which is practically absent in normal somatic cells, is observed. In a relatively small percentage of cases (depending on the tissue origin of cancer), cancer cells activate an alternative mechanism of telomere maintenance (ALT) based on recombination.

The rate-limiting component of telomerase functioning in humans is the hTERT protein. The hTERT gene has a total length of about 37 kb and consists of 16 exons and 15 introns. In humans, 20 hTERT splicing variants have been described [45], some of which strongly influence telomerase activity [46]. Splicing of hTERT changes not only during development, but also during carcinogenesis [47].

Another level of regulating telomerase activity is transcriptional. The hTERT promoter contains many transcription factors binding sites. Among the most studied regulators are c-Myc, estrogen receptor, HIF-1, NF-B, Menin, STAT3/5, MAD1, ETS, Sp1/3, USF, NFX1, etc. [48]. Presence of the sites for numerous activators and repressors suggests a very complex system of gene expression regulation (Fig. 2).

In addition to transcription and splicing, regulation of telomerase activity can occur through posttranslational modifications, including phosphorylation [50]. The ability of telomerase to elongate telomeres also depends on many factors, including localization within the nucleus or in the cytoplasm, and the state of chromatin [51]. Telomerase must undergo a maturation step in Cajal bodies, where TCAB1 is contained [52, 53]. Suppression of activity can be achieved by delaying telomerase in the nucleolus.

Regulation of hTERT is mainly associated with the cell cycle. It involves cyclin/cyclin-dependent kinase (cdk) complexes, which regulate transcription factors that bind to the hTERT promoter. E2F-1 plays a dual

role in the cyclin regulation, acting both as repressor and as activator. PI3K/Akt, NF-kB, and MAP kinase cascades activate hTERT. Phosphorylation of the p107 cyclin/cdk complexes as well as binding of estrogen to its receptor Era relieve the inhibitory effects of the TGF-b cascade. Through the positive feedback, hTERT expression activates the PI3K/Akt cascade, which, in turn, activates the cell cycle via MAD1 and p53 degradation, activates the NF-kB cascade, and blocks the TGF-β cascade.

EXTRACHROMOSOMAL FUNCTIONS OF TELOMERASE

Over time, the data on the action of telomerase began to accumulate, which are difficult to explain only through its effect on chromosomes. For example, enhanced expression of mTERT (murine telomerase) promotes carcinogenesis and wound healing [54]. Enhanced expression of telomerase alters stem cell functions [55]. A surprising result was obtained when the hTERT gene was introduced into the cells of a patient suffering from Niemann-Pick disease, a rare inherited disease characterized by impaired lipid metabolism. Insertion of hTERT normalized the cell phenotype [56]. Telomerase has also been shown to affect activity of the glycolysis genes [57], stimulate transcription of the genes associated with epithelial-mesenchymal transition (vimentin and snail1) [58]. Telomerase was shown to influence the work of NF-kappaB-dependent genes, i.e., participate in the regulation of inflammation [59]. Mutual effects of hTERT on the Wnt cascade and *vice versa* have been described [60].

Under conditions of oxidative stress, hTERT acts as a redox regulator, moving into mitochondria, where it protects mitochondrial DNA and helps maintain the level of anti-oxidative enzymes [61]. It is not entirely clear whether hTERT moves from the nucleus to mitochondria or whether the newly synthesized protein goes directly to mitochondria.

Expression of hTERT is involved in epigenetic regulation by influencing the STAT3 factor, which activates DNA methyltransferase I [62]. In 2009, it was discovered that hTERT protein is able to form complex not only with hTERC, but also with RMRP (RNA component of mitochondrial RNA-processing endoribonuclease) [63]. Such complex has the RNA-dependent RNA polymerase activity, producing long double-stranded RNA. RMRP mutations are associated with cartilage and hair hypoplasia disease in humans [64]. It has been suggested that hTERT may form similar complexes with other RNAs [65]. We have previously shown that even without RNA, the telomerase protein has non-template DNA polymerase activity [66]. Subsequently, these data were confirmed [67].

WHAT HAPPENS WHEN TELOMERES SHORTEN?

The Olovnikov's theory of marginotomy suggested that the trigger for stopping cell divisions and cell death was impairment of the subtelomeric genes critical for cell function. This assumption was not explicitly confirmed, which was one of the points that prompted him to create a new hypothesis of aging. Nevertheless, DNA shortening occurs as a result of end underreplication and this phenomenon has clear physiological consequences. What is the mechanism connecting terminal underreplication of chromosomes and cessation of cell growth? So far, we can clearly see at least three different mechanisms for the effects of telomere shortening on the cells, which do not agree with each other.

Telomere position effect. In 2001, the effect of changing expression of the genes located near the telomere when its length changes was first described [68]. As an explanation, the hypothesis of "heterochromatin stocking" was proposed, which in general can be described as follows: telomeric sequences have a special chromatin packing and special epigenetic changes, and the effect of this packing extends to nearby genes.

Later, a similar effect was described, but already for the genes located at a distance [69, 70]. A similar explanation has been suggested here: telomeres affect expression of the genes located in the space next to them as a result of chromosomal loop formation.

The results of the studies of telomere position effect are still mostly not very convincing, although a number of findings are of interest. For example, the hTERT gene is a subject to the telomere position effect, which opens up a number of hypothetical mechanisms for how the telomere length may be maintained in aging and cancer [71]. At least three mechanisms have been described for regulation of the hTERT expression through formation of chromatin or telomeric loops [72]. Finally, it should be noted that telomere shortening increases expression of the ISG15 gene (interferon stimulated gene 15), which is able to increase inflammation by stimulating IFNγ production [69]. Thus, there is a direct link between aging and inflammation, which provides support to the inflammaging theory.

DNA damage signal emergence. After a critical shortening of telomeres, a DNA damage signal appears. Human cells stop proliferation at an average telomere length of a few thousand nucleotides (i.e., not their complete shortening) and enter a special state, which is called cell senescence in English-language literature.

Probably, there is some minimal telomere length that allows to properly pack the chromosome end so that it differs from the DNA break. As early as 1997, two years before the discovery of telomere loops [24], we suggested a hypothesis about the loop structure of telomeres [73] (Fig. 3).

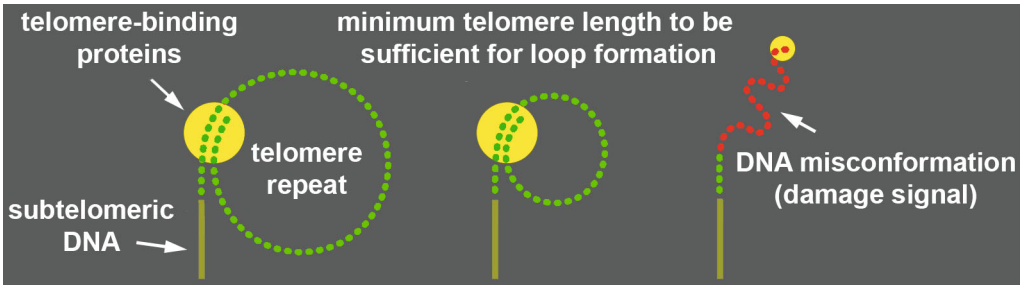

Fig. 3. Telomere looping hypothesis. Telomeric DNA together with telomere-binding proteins can form loops. Due to restriction of free rotation of DNA and interaction with a variety of proteins, DNA in a loop has a tense conformation. In the process of telomeric DNA shortening there is a point when the length of telomeric repeat is not already sufficient for formation of a loop. The telomeric end of DNA acquires a free conformation, which is perceived by the cell as a signal of damage.

There is a great heterogeneity in the telomere length not only between the cells but also within a single cell. Therefore, critical shortening, which causes DNA damage response (DDR), usually concerns only a small part of telomeres. This is sufficient to induce cellular senescence [74, 75].

In most studies on the telomere length, however, data on some average telomere lengths are mostly reported. Such measurements are made using different methods: Southern blot analysis of restriction fragments, PCR, digital PCR, quantitative FISH, FISH cytometry. Occasionally, more sophisticated methods are used that allow to record the lengths of individual, including the shortest telomeres (STELA, TeSLA) [76-78]. Advances in technology already allow sequencing of long sequences, including individual telomeres [79].

Presence of critically shortened telomeres and average telomere length are not strictly related values. It is also worth noting that in the vast majority of gerontological studies telomeres of white blood cells are measured, which also introduces additional ambiguities [80].

There is direct evidence that the cellular senescence arises as a result of DDR signaling from telomeres [81]. At the same time, the signal must be long-lasting, indicating that DNA cannot be repaired [82]. It was proved that a single double-stranded DNA break (if it is not repaired) is sufficient for the induction of senescence [83].

The concept of cellular senescence has changed over the past 60 years, and the generally recognized meaning of this term is still not fully established [84]. There is even an opinion that the term should be replaced [85]. At first, the term was used only for the cells that reached the Hayflick limit, then it was extended to the cells with DNA damage in general [86]. The term 'oncogene-induced senescence' appeared and then others no longer related to DNA. In 2019, leading scientists agreed on what exactly should be considered as a senescence [87], and the following definition emerged. Cellular senescence is a state of the cell caused by stressors and certain physiological processes, characterized by prolonged and generally irreversible cell cycle arrest with secretion changes, macromolecular damage, and altered metabolism. By trying to explain all heterogeneous phenomena, such definition looks too general and does not improve our understanding of the essence of the process.

In simple words, we can define senescence **as an ineffective cell response to any type of stress**. Based on this definition, it becomes clear why no unique (characteristic only for senescence) features have been found so far [88]. It is clear that both complex and simplified definitions can be applied to any cells, including postmitotic and cancer cells.

The difficulty in determining cellular senescence is aggravated by the fact that the senescent phenotype depends on the initial cell type and changes over time; senescence deepens and different mechanisms become involved [89, 90]. Recently, a very interesting phenomenon has been described: increase in activity of the endogenous retrotransposons during senescence [91-93]. Most likely, epigenetic changes are the signal for this [94]. As a result of retroelement activation, in addition to the increase in genetic instability, innate immunity systems are activated and inflammation develops [95, 96].

In the beginning, senescence is an entirely intracellular process. In its development it acquires a specific secretory phenotype (senescence associated secretory phenotype, SASP). The cell begins to affect the life of neighboring cells and further of the whole organism. Mitochondria somehow start to participate in the development of cellular senescence, possibly through redox regulation [97, 98].

In the senescent cells, resistance to apoptosis increases, metabolism shifts towards glycolysis, and production of reactive oxygen species increases. SASP includes DAMPs (damage associated molecular patterns), various proinflammatory cytokines and chemokines that attract immune cells, as well as proteases that alter the extracellular matrix, etc. [99, 100].

Dramatic increase in genetic instability. Telomere fusions and their consequences. The third mechanism of the impact of telomere shortening on the cell fate is the most complex, and leads to sharp increase in the genetic instability. This mechanism is one of the pathways leading to formation of immortal cancer cells.

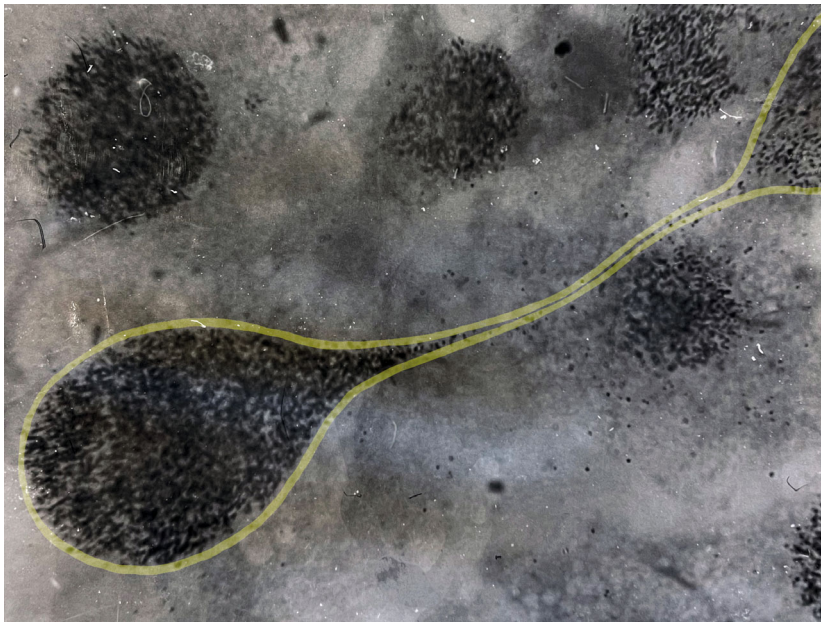

Fig. 4. Chromosome bridge in 3T3 Swiss cells. The cell(s) resemble Siamese twins. Mitosis ended long ago (no chromosomes visible) and the nuclei (cells) started moving away from each other, with the nucleus (and the cells themselves) not separating from each other (the nucleus that did not separate is circled in yellow). DNA of the cells was pre-labelled with ^{3}H-thymidine. Radioautography. Photo by the author.

The mechanism begins to work only in the partially transformed cells, in which, for some reason, the usual mechanism of stopping proliferation upon reaching the Hayflick limit does not work. Such cells, despite the telomere shortening, continue proliferation and can divide dozens of times before reaching the so-called telomere or replicative crisis. About 30 years ago, the existence of two barriers that a normal cell needs to overcome to achieve replicative immortality was theoretically postulated: cellular senescence (M1) and telomere crisis (M2) [101].

Experimentally, the study of telomere crisis is performed on the cells in which p53 or pRb activity is suppressed. For example, in 2023 [102], this was done by introducing genetic constructs encoding human papillomavirus antigens E6 and E7 or the large antigen SV40 into normal fibroblasts. Such cells have an increased proliferative potential, during which telomeres continue to shorten; after that, the cells begin to die *en masse* as a result of catastrophic cell cycle but continue to divide. The outcome of the experiment can be either complete death of all cells or emergence of a replicatively immortal clone, most often with reactivation of the hTERT gene. As recent studies show, cells undergo autophagic death in the process of crisis [103].

If the Hayflick limit is overcome as a result of inactivation of the cell cycle arrest mechanisms in response to DNA damage, the DNA damage repair systems continue to operate. If underreplication continues, DNA damage accumulates, and, at some point, there are options for repairing incorrect DNA ends by means of telomeric fusions.

Either different chromosomes or sister chromatids may undergo fusion. In subsequent mitosis, these dicentrics either mis-segregate or break off. A sequence of events called fusion-bridge-breakage occurs (Fig. 4).

It can be repeated many times. As a result of such seemingly simple processes, a wide variety of mutations can be formed [104]:

1. Aneuploidy, which is lack of a chromosome or acquisition of an extra chromosome.

2. Non-reciprocal translocations resulting from gap-induced replication.

3. Loss of Heterozygosity (LOX) due to terminal deletion. Can become fixed in the genome of cancer cells due to the loss of tumor suppressor genes.

4. General increase in ploidy.

5. During chromatid fusion, local amplification may occur, followed by formation of a homogeneously stained region (HSR) or double minute chromosomes (DM chromosomes).

6. Chromothripsis – dozens of rearrangements within one chromosome segment. It is formed when a chromosome fragment is trapped in the cytoplasm during nuclear envelope rupture and is severely fragmented under the influence of TREX1 exonuclease.

7. Kataegis is editing of the DNA fragments formed during chromothripsis by APOBEC3 deaminase, which converts cytosine residues to uracil. Activity of this enzyme normally limits infection by the DNA and RNA viruses.

In the process of breaking the “bridge”, the DNA in its middle is strongly stretched, it is devoid of nucleosomes [105]. After rupture of the nuclear envelope,

DNA becomes a target of cytoplasmic nucleases and deaminases. In recent years, it has become clear that the components of innate immunity responsible for antiviral defense are involved in these processes. When DNA is present in the cytoplasm, the cGAS-STING pathway is activated, inducing ZBP1 (Z-DNA binding protein 1), which binds to the TERRA transcript, the amount of which increases with telomere dysfunction. The TERRA–ZBP1 complexes oligomerize as filaments on the surface of the outer mitochondrial membrane, where they contribute to formation of MAVS (mitochondrial antiviral signaling complex). An interferon response is initiated [102]. Eventually, the severely damaged DNA with single-strand breaks is incorporated into the nucleus, leading to the phenomena of chromothripsis and kataegis.

The details of the processes of chromosome rearrangement and telomere crisis are too extensive and are not the subject of this review. It should be emphasized, however, that the traces of the above-described events are found with varying frequency in many types of cancer cells. Thus, the impairment of telomere function is involved in carcinogenesis [106-113].

Thus, we see that the telomere shortening (lack of telomerase activity) leads to the appearance of senescent cells, which promotes aging both locally (decreased tissue functionality) and systemically (development of inflammatory aging in the whole organism). The same processes contribute to the growth of genetic instability, which is an important part of carcinogenesis. It has long been observed that despite telomerase activity, cancer cells usually have shortened telomeres [114]. It has been hypothesized that the cancer cells benefit from maintaining short telomeres in order to ensure an increased level of genetic instability.

TELOMERASE, REJUVENATION, AND CANCER

Since telomerase prevents cellular senescence caused by underreplication and enables cell proliferation to maintain normal tissue function, its use for therapeutic purposes for rejuvenation (healthspan) has long been considered. In recent decades, the concept of longevity (lifespan) has been gradually replaced by healthspan as a target for scientists and physicians. The main aspect of telomerase use in medical practice is safety.

On the one hand, telomerase expression does not necessarily lead to cancer-related changes [115, 116] and many normal (non-cancerous) cells have telomerase activity. These include cells of the developing embryo, various stem cells and progenitors, and germline cells in males. At the beginning of the century, Calvin Harley, wishing to emphasize safety of telomerase, wrote an article entitled "Telomerase Is Not an Oncogene" [117].

On the other hand, telomerase activation is the most common feature of cancer cells (about 90%), and, in this regard, purely phenomenologically, we should attribute it to oncogenes [118]. Telomerase activation during carcinogenesis occurs in different ways: these are mutations of the hTERT promoter, genomic rearrangements, and gene amplification. There are positions within the promoter that are most frequently altered [119]. Mutations in the hTERT promoter are the most frequent non-coding mutations in the human cancer cells [120]. In tissues with slow cell self-renewal (tumors of the central nervous system, liver, and melanocytes), mutations in the hTERT promoter are more frequent and appear earlier than in the intestinal and blood tumors [121-123].

Hepatitis B virus (HBV) is able to integrate into the host genome near the hTERT promoter and enhance its expression [124]. Genomic rearrangements that enhance hTERT expression are often observed in neuroblastomas [125, 126]. Amplifications of hTERT have been observed in ovarian tumors and lung adenocarcinomas [127].

In addition to the functional link between telomerase and cancer, namely frequent acquisition of telomerase activity by the cancer cells, various non-canonical telomerase activities unrelated to telomere maintenance but somehow beneficial to cancer cells have been described [128, 129].

It is known that a cancer cell must have a number of changes, one of which is immortalization. In an ageing body there are many cells in which many precancerous changes have already occurred and these cells lack only immortality. Expression of telomerase allows them to pass this stage and turn them into real cancer cells. Consequently, mass acquisition of unregulated telomerase activity by many cells is obviously dangerous.

It is known that cancer cells are characterized by high telomerase activity and tumor malignancy correlates with the level of telomerase activity [130, 131]; the level of activity can vary hundredfold [132]. It could be hypothesized that if telomerase activity is relatively low or erratic, it will only 'heal' dysfunctional, critically shortened telomeres, thereby reducing DDR and SASP and reducing inflammation, but not enabling long-term growth [133]. The protective effect of telomerase under oxidative stress is also likely to be beneficial [61, 134-135].

How can we assess the possible positive health outcomes of telomerase activation? When the telomerase gene was introduced into aging mice using adeno-associated viruses, significant improvement of biomarkers associated with aging was observed [136]. A similar result in mice was achieved by the same authors using the low molecular weight telomerase activator TA-65 instead of AAV9 [137].

TA-65 is a natural product derived from a traditional Chinese medicinal plant (Astragalus). Extracts of this plant have been used for centuries with no reported side effects. Dosage of the drug is easy to control

and it is a fairly weak telomerase activator. In human trials, TA-65 has recently been shown to improve key risk markers for cardiovascular diseases, the leading cause of death in the developed countries. Plasma TNF levels also decreased. The authors conclude that there were changes associated with the reduced inflammation [138]. Reduction of inflammation and normalization of the lymphocyte profile were also shown in another long-term trial of TA-65 conducted on the age-matched patients after a heart attack [139].

Reducing inflammation becomes increasingly important as aging progresses [140, 141]. Therefore, interventions aimed at blocking inflammatory senescence may be justified.

In recent years, the exosome pathway of intercellular communication has attracted special interest. It has been shown that the cells can transfer hTERT transcripts through exosomes, which makes donor cells temporarily telomerase-positive [142, 143].

In addition to the two known ways of telomere maintenance (telomerase-dependent and alternative), a method of direct cell-to-cell telomere transfer during the immune response development has recently been discovered [144]. Upon contact with a T-lymphocyte, the antigen-presenting cell degrades shelterin and cuts off telomeres with participation of the TZAP factor. After that telomeres together with Rad51 (necessary for recombination) are packed into vesicles, which are transferred to the T-lymphocyte via immunological synapse. As a result, the telomeres of the T-lymphocyte are lengthened by on average 3000 base pairs, while those of the presenting cell are shortened. Possible mastering of the direct telomere transfer in medicine would open new opportunities for fighting aging of the immune system, increasing effectiveness of vaccination, and, in future, for the development of new technologies of cellular rejuvenation, in particular, the cells of the vascular wall. Also, the issue of using telomerase for treatment of aging-related pathologies, which has gone out of fashion, requires further study, and we may expect a next, more productive wave of interest in this important enzyme [80, 145].

CONCLUSION

The history of telomerase research, which began with prediction of the enzyme's existence by A. M. Olovnikov in 1971, continues.

The problem of underreplication, as it has been called in the past, is probably not so much a problem, but a mechanism adapted by evolution to control the fate of individual cells in an organism. To date, three ways of maintaining telomere length in humans are known: use of telomerase, alternative, and direct transfer of telomeric DNA. All three methods are utilized by the organism in normal development. Restrictions associated with suppression of telomerase in development are of preventive nature and related to protection against cancer. When these limitations interfere with normal function, the body uses telomerase activation in a limited way (in stem cells and some progenitor cells), or an alternative way to lengthen telomeres (in embryogenesis, prior to implantation). When an ultra-urgent increase in the proliferative potential of lymphocytes is required, there is a method of direct telomere transfer that provides the necessary speed to the immune system functioning.

In the process of organism aging, increase of sterile inflammation plays a growing role. There is a direct link between the state of telomeres and activation of immune antiviral defenses. What was once called a replicometer (telomere shortening as a counter of the number of passed divisions) turned out to be a part of a complex mechanism that largely determines the state of our health and its importance increases with age.

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