TELOMERES. TELOMERASE

RUXANDRA PETRUT, ION I. BARA

Key words: telomere, telomerase, structure, function, cell proliferation, length regulation, cancer, aging.

Abstract: Telomeres, nucleoprotein structures at the ends of linear eukaryotic chromosomes, are essential for maintaining the integrity of the genome by protecting chromosomes against end-to-end fusion, recombination and degradation. Telomerase, a ribonucleoprotein enzyme that synthesizes telomeric DNA repeats onto chromosomal ends, can compensate for telomere shortening. In germ lines and immortalized cells, telomerase activity is high and telomeres do not shorten.

INTRODUCTION

Telomeres are very important in modern biology. Whether the subject refers to cancer, gene regulation, organism aging, or the cloning of mammals, much seems to depend on what happens at the ends of chromosomes [3]. That is why, telomerase and telomere length have received a lot of recent attention from the biomedical research community [7].

In most organisms that have been studied, the tips called telomeres are changing: they shorten and lengthen repeatedly [8].

During the past 15 years, investigation of this unexpected flux has produced a number of surprising discoveries. In particular, it has led to identification of an extraordinary enzyme named telomerase that acts on telomeres and is thought to be required for the maintenance of many human cancers. This last finding has sparked much speculation than drugs able to inhibit the enzyme might combat a wide array of malignancies. The research also opens the possibility that changes in telomere length over time may sometimes play a role in the aging of human cells [8].

Modern interest in telomeres and telomerase has its roots in experiments carried out in the 1930s by two remarkable geneticists: Barbara McClintock, then at the University of Missouri at Columbia, and Hermann J. Muller, then at the University of Edinburgh. Working separately and with different organisms, both investigators realized that chromosomes bore a special component at their ends that provided stability. Muller coined the term “telomere” from the Greek for “end” (telos) and “part” (meros). McClintock noted that without these end caps, chromosomes stick to one another, undergo structural changes and misbehave in other ways. These activities threaten the survival and faithful replication of chromosomes and, consequently, of the cells housing them. [8]

STRUCTURE OF TELOMERE

Telomeres are extended arrays of tandem repeat sequences of G- and C-rich complementary hexanucleotide strands and their binding proteins. Telomeric DNA possesses a general structure of (T or (A) m (G) n, with the vertebrate sequence being (TTAGGG) [10]. Telomeric repeats are synthesized by the enzyme telomerase [1].

Telomeric DNA is evolutionarily conserved among eukaryotes. For instance, repeats of the sequence 5`-d (TTAGGG)-3` are found in all vertebrates as well as in some fungi and slime mold. Telomeric DNA is oriented 5` to 3` towards the chromosome end. The majority of this strand is base paired to a complementary cytosine-rich strand, while an unpaired tail extends to form in different species, a 12-150 bases single-strand overhang. The guanine-rich strand of the telomeric DNA can form four-stranded DNA structures, which are presumed to be involved in the control of telomere synthesis and telomere function. The length of telomeres in human cells is variable. Indeed, any determination of telomere length is based on a calculation of an average value for all chromosomes [4].

The telomeric DNA is complexed with specific non-nucleosomal telomere-binding proteins (TBPs) in addition to nucleosomes. Two classes of TBPs have been identified: those that bind the double- strand (ds) region of telomeric DNA and those that bind the single stranded (ss) overhangs. The first class (dsDNA-TBP) includes, e.g. Rap1p from *Saccharomyces cerevisiae*, and two related mammalian proteins, TRF1 and TRF2. All

these dsDNA-TBPs are able to affect telomere length. Lack of TRF2 results in loss of the 3` overhang, end-to-end chromosome fusion and activation of p53, which mediates cell cycle arrest and apoptosis. The recent discovery of t-loops suggests a possible mechanism by which TRF2 may affect the single-stranded overhang: TRF2 can remodel linear telomeric DNA into large duplex loops in vitro, whose size is proportional to the telomere length. Binding of TFR1 and SSB protein suggested that t-loops are formed by invasion of the 3` overhang into the duplex telomeric repeat array. The t-loop model for telomere structure proposes a solution to the protection and maintenance of telomeres in mammals. The other class of telomere-binding proteins interacts with the single-stranded 3` overhang, forming very salt-stable complexes which may act as a molecular chaperone for G-quartet formation [6].

TELOMERE METABOLISM

Telomeres are metabolized by progressive shortening as a function of chromosomal DNA replication in normal human cell cultures and with age in vivo. In each round of chromosome replication, for instance, telomeres typically lose ~ 150 base pairs of nucleotide sequence at the 5` end of a DNA molecule, reflecting the inability of conventional DNA polymerases to replicate the extreme ends of telomeres and the effects of a putative 5`-3` exonuclease.

Thus, an increasing cell division number is usually accompanied by declining telomere length; in a given population, the more that cells have undergone cell division, the shorter their telomeres will be. This DNA replication-dependent loss of telomere length has accordingly been proposed to operate as a mitotic clock in order to count the number of cell divisions and to signal cellular senescence. When the number of cell division is high, the telomeres become so short that the cells are then permanently directed to exit the cell division cycle, characteristic of replicative senescence. Thus, degradation of telomeres appears to constitute a signal with which a cell is no longer able to undergo cell division.[10]

Although the signaling pathways whereby telomere shortening induces cellular senescence remain ill defined, it has been proposed that short telomeres may lead to activation of multiple signaling machanisms. One possible mechanism may be that with shortening telomere, transcription of genes nearby is affected. Repression of euchromatic gene transcription by repetitive sequences of condensed heterochromatin is known as position effect variegation. In organisms such as *Saccharomyces cerevisiae*, transcription of genes near telomeres is reversibly repressed, a phenomenon called telomere position effect (TPE). Extensive cutting or elimination of telomeres may, on the other hand, activate gene transcription. Little is known about what genes are regulated by TPE in human chromosomes and whether or not these genes are involved in signaling cellular senescence in response to telomere length reduction. Identification of the telomere shortening-sensitive genes (TSSG) is therefore important for understanding the cellular effects of telomere metabolism. Recent studies in mice have shown that significant shortening of telomeres results in activation of the tumor suppressor p53 expression in association with cell growth arrest and apoptosis. In contrast, elimination of p 53 allows direct oncogene-induced cell immortalization. Thus, p53 may be one of TSSG involved in regulating cell growth arrest and apoptosis. In addition, it is conceivable that telomere shortening may be associated with production and accumulation of molecules regulating permanent exit from the cell cycle into senescence [10].

ROLE IN ANIMAL CLONING

The cloning of livestock from adult nuclei raised initial questions as to the effect of telomere length in the cloned progeny. An earlier analysis of telomeric length from cloned sheep and age-matched controls suggested that the telomeres were shorter in the cloned animals.[5]

However, given the activation of telomerase by blastocyst stage, it was likely that the reprogramming of the adult nucleus might also involve re-expression of telomerase and restoration of telomere length to normal levels. The demonstration that aged fibroblasts were suitable as nuclear donors suggested that during embryogenesis the replicative lifespan was reset irrespective or initial length. This was confirmed by demonstrating that telomere lengths were longer (15-23 kb) in cloned fetuses than in the adult donor cells (14-18 kb), and age-matched controls showed no significant differences. Regardless of nuclear donor age, telomerase activity was present by the blastocyst stage of postclonal embryonic development [5].

TELOMERE-LENGTHENING MECHANISMS

To accomodate telomere shortening, organisms have evolved complex mechanisms to compensate for the loss of telomeres during cell replication under particular circumstances. Three different telomere-lengthening mechanisms have been described. In human cells, de novo synthesis of telomeres by telomerase is the predominant mechanism in telomere lengthening. In *Drosophila melanogaster*, telomeric DNA can be elongated by transposition of specific retrotransposons (HeT-A and TART) to chromosome ends. In yeast, telomere extension can occur by homologous or heterologous chromosomes. Thus, telomere length in cells is regulated not only by the gradual loss of telomeric DNA with each round of cell replication, but also by differential gains of telomeric repeats through various mechanism across different animal species. At a given time in the development of a eukaryotic organism, the length of telomeres is thus a dynamic equilibrium between the rate of the DNA replication-dependent shortening and the degree of a compensatory (or anomalous) lengthening of telomeres through processes such as telomerase activation. Erroneous activation of telomerase has been widely believed to be involved in telomere stabilization or elongation in humans, triggering the continuous divisions and proliferation characteristic of immortal cell [10].

STRUCTURE OF TELOMERASE

In contrast to normal somatic cells, many immortal lines do not exhibit telomere shortening during DNA replication, suggesting that maintenance of these structures is required to escape replicative senescence. Telomere shortening is frequently arrested in immortal and tumor cell lines. Telomere maintenance in these cells is the result of the activity of a ribonucleoprotein complex known as telomerase [11].

Telomerase is a large ribonucleoprotein complex, containing an RNA subunit and several protein components. The RNA moiety is essential for the enzymatic function of telomerase. Human telomerase RNA (hTR) is 445 nucleotides lon with an 11 nucleotide putative template sequence (5`-CUAACCCUAAC-3`) coding for the telomere repeats of (TTAGGG)n. In vitro mutagenesis studies suggest that the region required for minimal function is located between residues 44 and 203, with mutation between residues 170-179, 180-189, or 190-199 inhibiting both templating function and telomerase activity. Besides serving as a template for reverse transcription in telomere DNA synthesis, the RNA subunit is also involved in the enzyme active site, probably with specific nucleotides interacting with structural components of the DNA primer substrate and protein subunits. Interspecies substitutions of telomerase RNA with an identical template base sequence from the ciliate *Glaucoma chattoni* into *Tetrahymena thermophila* cells produces a functional but aberrant telomerase, suggesting that non-template RNA domains play roles in regulating enzyme structure and function via intermolecular interactions within the telomerase ribonucleoprotein complex. Removal or down-regulation of the RNA subunit leads ti inhibition of telomerase, erosion of telomeres, compromise of growth capacity of highly proliferative embryonic stem cells, testicular cells, and hematopoietic cells in the mouse, and death of both cultured HeLa cells and malignant human glioma cells [10].

ELONGATION OF TELOMERE

In the absence of telomerase, telomeric repeats are lost with each cell division, because DNA polymerases cannot replicate the very end of a linear DNA molecule. This is thought to be due to the fact that lagging-strand synthesis proceeds as a series of discrete events (the formation of Okazaki fragments), each requiring an RNA primer. Since there is no DNA beyond the 3` end of the chromosome to which this primer can anneal, DNA polymerases cannot fill in the gap between the final Okazaki fragment and the end of the chromosome (the “end replication problem”). The incompletely replicated telomeres are inheited by daughters cells, and the process repeats itself in subsequent cell divisions, progressively shortening the telomers as cell divisions increase. [9]

The mode of telomerase action in synthesizing and elongating telomeres is incompletely understood. It is believed that the telomerase holoenzyme interacts with the single strand of 3` GT-rich telomeric primer and polymerizes deoxynucleoside triphosphates in the 5` to 3` direction. This reaction appears not to require ATP in yeast, but utilizes the telomerase RNA as a guiding template complementary to the telomeric DNA repeats. Once telomerase binds to telomere primer and positions itself in such a way that the RNA template sequence aligns with telomeric DNA primer, the enzyme transcribes one DNA nucleotide onto the telomere at a time according to the complementary coding sequence of the RNA template. When a full telomeric repeat TTAGGG

is formed, the telomerase may then translocate to the next site with the process cycling to allow telomerase to add iterative telomere DNA repeats.[10]

Both the catalytic activity and processitivity require optimal conditions in temperature, ionic strength, and enzyme-substrate interactions. Use of synthetic TTAGGG oligodeoxynucleotide or reverse transcriptase inhibitors (azidothymidine and carbovir) inhibits telomerase activity and induces cell programmed death [10].

TELOMERE AND TELOMERASE FUNCTIONS

Telomeres, intact or unwounded, function to protect chromosomes ends from recombination, fusion, and degradation by exonucleases and ligases, to regulate mitotic chromosome recognition and separation, and to position and anchor chromosomes with the nuclear machinery to facilitate DNA replication at various stages of the meiotic and mitotic cell cycle [10]. As the functional components of chromosomes, telomeres allow complete replication of the chromosome ends and represent a biological clock that determines lifespan. Telomeres seems to be associated with the nuclear matrix and migth play a role in nuclear architecture [6].

Together, telomeres and telomerase functions combine to form what has been referred to as the " telomere hypothesis of cellular aging and tumorigenesis". This hypothesis states that after a certain number of cell doublings, when a threshold level of telomeric length is reached, a signal is initiated to cease cell division and further progression to S-phase is prevented The hypothesis further suggests that in carcinogenesis, certain cells such as those that have undergone viral transformation, irradiation or mutagenesis will continue to divide, have their telomeres further shortened and eventually die. However, prior to death, the resulting genomic instability causes a small number of these cells to undergo multiple mutations, including what appears to be a key step in the generation of immortalized cells: the regaining of telomerase activity which thereafter serves to maintain telomere length and genomic stability indefinitely.[4]

It should be noted, however, that not all data support a role for telomerase activity in cell senescence and immortalization. It has been reported that significant telomere elongation rather than stabilization follows telomerase activation in immortal cell lines and tumors. It is now known that telomeres can also be elongated by a telomerase-independent mechanism, designated ALT (Alternative Lengthening of Telomeres), which can explain the finding of some immortalized cells without detectable telomerase activity but with long telomeres [4].

REGULATION OF TELOMERASE ACTIVITY

Currently, very little is known about the regulation of telomerase activity in in vertebrates. If telomerase synthesizes telomeric repeats only during S phase, a variety of mechanisms may be involved in the regulation of telomerase during the other phases of the cell cycle. For instance, it is possible that telomerase is expressed and active only during Sphase when telomeres are replicated, is present during all phases of the cell cycle, but is able to add repeats only during S phase, or is continuously synthesizing telomeric DNA throughout the cell cycle. [9]

Reactivation of telomerase is a constant finding in carcinogenesis, rendering the telomerase enzyme is a potential target for the future therapeutic studies. Conceptually, telomerase activity can be down-regulated by several mechanisms, including inactivation of the RNA component of the enzyme, blockage of the telomerase nucleotide binding site, inhibition of phosphorylation, and/or manipulations of telomerase regulatory proteins [4].

DETECTION OF TELOMERASE ACTIVITY

A highly sensitive assay for telomerase activity called the Telomeric Repeat Amplification Protocol (TRAP) have provided a useful tool for detection of telomerase activity in variuos clinical and research studies. The TRAP assay consists of an incubation of detergent extract of tested cells or tissue with an oligonucleotide that serves as a primer for the synthesis of telomeric repeats by telomerase. The telomeric product is then amplified by polymerase chain reaction (PCR) in the presence of the same oligonucleotide as a forward primer, and a specific reverse primer, complementary to the telomeric DNA repeats. The assay is sufficiently sensitive to detect telomerase activity in a single cell. Recently, it has been further improved by the inclusion of an internal standard in the PCR reaction, thus enabling semi-quantitative analysis and elimination of false- negative results due to inhibition of the PCR reaction. A fluorescent assay, as well as in situ TRAP have also been develop for documenting telomerase activity [4].

TELOMERASE AND CELL PROLIFERATION

Although telomerase was proposed to be expressed in tumor and not normal human tissue, evidence has accumulated that telomerase is expressed in a variety of normal tissue and that it is growth-regulated. The discovery of low levels of telomerase activity in normal human blood samples led to the observation that mitogenic stimulation of lymphocytes causes telomerase up-regulation. In fact, in normal mature T cells, the activity is specifically up-regulated on entry into S phase. [7]

In addition to blood, telomerase has been detected in several other normal human cell types. Typically, it is the mitotically active cells in a given tissue that express telomerase. Skin samples have very low levels of telomerase activity. Relativelly high levels of telomerase activity were found in the proliferative basal layer whereas the quiescent dermis was telomerase-negative. Isolated normal epithelial cells and epithelial cell cultures are telomerase-positive, as are cultures of normal human endotheloal cells.[7]

Telomerase activity is also growth-regulated in human tissues in vivo. Three recent reports indicate that activity is present in endometrial tissue and is correlated tightly with proliferation during menstrual cycle. In addition, mitotically active regions of human hair follicles and the proliferative zone of intestinal crypts also have telomerase activity [7].

HUMAN AGING

Aging is a process associated with progressive changes, ultimately leading to death, and the mechanisms involved in aging are still far from well understood Telomeres are shorter in certain human tissues in older people than in younger people, and aging in human diploid fibroblast and hematopoietic cells is accompanied by the progressive loss of telomeres. Short telomeres may activate multiple signaling pathways to induce cell senescence, including activation of the p53 and p21 cell cycle checkpoint pathway. In two diseases of very marked premature aging, Hutchinson-Gilford progeria and Down sindrome, short telomeres have been detected. It is thus possible that an onset of the major aging-related diseases (atherosclerosis, hypertention, Alzheimer`s disease) and debilitating diseases (degenerative joint disese and sensory impairment) may result from early cellular senescence in the relevant tissue.[3]

Short telomeres trigger multiple aging-related processes including cell growth arrest, apoptosis, and/or decreased capacity in response to stresses in highly proliferative organs, demonstrating a critical role for telomere length in genomic stability, cell replicative life span and aging [3].

TELOMERASE AND CANCER

There has been a vast increase in telomerase research over the past several years, with many different pre-cinical approaches being tested for inhibiting telomerase activity as a novel therapeutic modality to treat malignancy. To confirm action trough a telomerase-dependent mechanism, inhibitors, but not chemically-related molecules should reduce telomerase activity, but not initialyy affect cell growth rates; should lead to progressive shortening of telomeres with each cell division; and ultimatelly cause cells to undergo growth arrest/ apoptosis in a time-frame dependent on initial telomere length. Thir approach has been validated using antisense oligonucleotides directed against the telomerase RNA template region, expression of a dominant-negative version of the catalytic protein component (hTERT) to competitively inhibit endogenous telomerase from maintaining telomeres, and by using a small molecule inhibitor of telomerase [2], [12].

CONCLUSIONS

The telomerase enzyme is the object of so much attention today. Many studies have suggested that such an enzyme could resolve the important problems in modern biology: cancer, gene regulation, aging, cellular senescence and of course, the cloning of mammals.

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