FEATURE ARTICLE

An Instant Update on Telomeres and Telomerase

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Abstract

The DNA sequence of Tetrahymena *telomeres was the first to be determined among all telomeres by Elizabeth Blackburn and Joseph Gall in 1978. In 1982, Jack Szostak and Blackburn showed that the unique DNA sequence contained in the telomeres served to protect the chromosomes from degradation. In 1985, Carol Greider and Blackburn showed how the telomeres could be elongated by the enzyme telomerase. These discoveries are milestones marking the start of the molecular era of telomere biology. Telomeres occur at the ends of chromosomes, like the plastic sheaths at the ends of shoelaces. The significance of telomere research was recognized by the award of the Nobel Prize in Physiology or Medicine to Blackburn, Szostak, and Greider in 2009.*

Although the award of the Nobel Prize is undoubtedly one of the most exciting events in the history of telomere biology, it by no means signals that we have reached the peak of telomere research. Research on telomeres and telomerase is still moving at a very rapid pace, and new findings about telomere functions and the underlying mechanisms are unveiled daily. This paper reviews the early work as well as recent advances in the field.

Key Words: *telomeres; telomerase; aging; cancer.*

Introduction

Both normal cells and cancer cells can be cultured in the laboratory. However, they behave quite differently. Normal cells can divide only a limited number of times, whereas cancer cells can divide endlessly. There is considerable evidence that cellular aging is determined by cell divisions and that the total cellular lifespan is quantified by the number of cell divisions and not by chronological time. This implies that there is a limit to the number of times a normal cell can divide and that cells possess a built-in counting mechanism for cell divisions. It is now known that the shortening of telomeres of chromosomes is an inherent cellular monitoring mechanism of cellular aging (Armanios, 2013). Chromosomes can be likened to shoelaces, whereas telomeres can be thought of more like the plastic sheaths at the ends of shoelaces because they prevent chromosomes from sticking together, becoming frayed or damaged, and they protect the genetic information on a chromosome. However, the analogy between telomere and plastic sheath is limited by the static nature of plastic sheath. In contrast, telomere is dynamic in nature and undergoes rapid assembly and disassembly.

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Early Work on Telomeres

Interest in telomeres has its origins in the experiments carried out in the 1930s by outstanding geneticists: Barbara McClintock, then at the University of Missouri at Columbia, and Hermann Muller, then at the University of Edinburgh. McClintock worked on the broken ends of chromosomes in maize and concluded that experimentally induced or accidental chromosomal breaks were different from the natural ends of chromosomes (McClintock, 1931). The broken ends of chromosomes were easily degraded and had a certain stickiness that could cause chromosomal fusions. Conversely, the natural ends of chromosomes had no such stickiness. Independently, Muller reached the same conclusion from his work on fruit fly and coined the term "telomeres" from the Greek words *telos* (end) and *meros* (part) for the natural ends of chromosomes (Muller, 1938). Both scientists suggested that telomeres could have a protective role but their mechanism of action remained an enigma then.

Prior to 1960s, normal human cells were thought to be able to divide indefinitely. However, Hayflick and Moorhead (1961) reported that normal human cells could divide only a limited number of times in culture. In other words, these cells have a finite replicative lifespan and undergo cellular or replicative senescence eventually.

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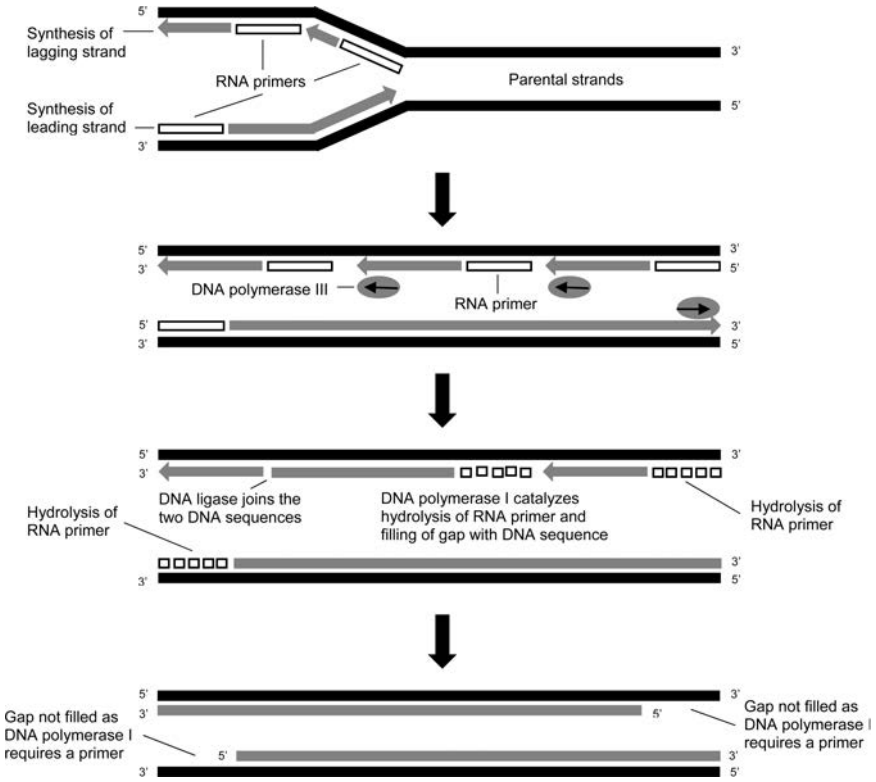

Figure 1. The end-replication problem.

Alexey Olovnikov of the Russian Academy of Sciences and James Watson of Cold Spring Harbor Laboratory also contributed to the early work on telomeres. Since DNA replication can proceed only in the 5′ to 3′ direction and requires RNA primers that are subsequently hydrolyzed, gaps are formed (Figure 1). These gaps are filled using the adjacent Okazaki fragments as primers. However, the terminal gaps at the 5′ ends of the newly synthesized strands cannot be filled due to the lack of such a primer. This is known as the end-replication problem. Both Olovnikov (1971) and Watson (1972) independently recognized this problem and pointed out that the newly synthesized DNA molecules would be left with a 3′ overhang. They suggested that such incomplete DNA replication would lead to shortening of telomeres with each cell division. Olovnikov also proposed that the end of the replicative lifespan might be due to the telomere length decreasing below a critical limit.

○ Structure and Function of Telomeres

The molecular era of telomere research started in the late 1970s when Elizabeth Blackburn was doing her postdoctoral studies with Joseph Gall of Yale University. They were researching the chromosomes of a single-celled ciliated protozoan, *Tetrahymena thermophila*. *Tetrahymena* possesses large amounts of a type of relatively short linear DNA molecules called mini-chromosomes. Blackburn isolated and analyzed the telomeres of these mini-chromosomes and found that they contained a six-nucleotide sequence, TTGGGG, that was repeated many times on one DNA strand, and tandem repeats of the hexa-nucleotide CCCCAA on the other DNA strand (Blackburn & Gall, 1978).

Since 1978, researchers have characterized the telomeres of a wide range of organisms such as micro-organisms, plants, humans, mice, and other vertebrates. For example, human and mouse telomeres consist of repeating sequences of TTAGGG, whereas roundworm telomeres contain repeating sequences of TTAGGC. All the chromosomes in a given organism have the same species-specific telomeric repeat sequence. However, the same telomeric repeat sequence can occur in very diverse organisms. For example, the telomeric repeat sequence found in humans, TTAGGG, is also found in the telomeres of the mold, *Neurospora*, the slime mold, *Physarum*, and the protozoan, *Trypanosoma* (Blackburn, 1991). It has been shown that the number of repeating units in telomeres varies among different organisms of a species and even among different cells in the same organism. However, every species has a characteristic average. For example, the average telomere in *Tetrahymena* has 70 repeats, whereas that in humans has 2000.

The function of telomeres was discovered by Szostak and Blackburn in the early 1980s after their serendipitous meeting at a Gordon Research Conference in 1980. Szostak was then a researcher from Harvard Medical School, and he observed that linearized yeast plasmid DNA molecules were rapidly degraded when introduced into yeast cells. They decided to carry out an experiment that would cross the boundaries between two very different species. They investigated whether telomeres from *Tetrahymena* could protect linearized yeast plasmid DNA molecules from being broken down in yeast. Blackburn purified the telomeres from *Tetrahymena*, while Szostak ligated the telomeres to both ends of linearized yeast plasmid DNA molecules and inserted them into yeast cells. The results were astonishing: the telomeres from *Tetrahymena* protected the linearized yeast plasmid DNA molecules from degradation (Szostak & Blackburn, 1982). As telomeres from *Tetrahymena* protected DNA molecules in an entirely different organism, yeast, Szostak and Blackburn postulated the existence of a previously unknown fundamental biological mechanism that was common to distantly related species. The protective function of telomeres is now known to be highly conserved in the evolutionary chain from *Tetrahymena* to humans.

Research has shown that telomeric DNA is protected by bound proteins (Gomez et al., 2012). Many of these proteins have been characterized with regard to structure and biochemistry. Researchers have proposed various functions for these proteins by looking at the consequences to cells of altering or deleting the proteins. In humans, telomeric DNA is bound by a six-protein complex called shelterin, consisting of TRF1, TRF2, TIN2, TPP1, Rap1, and POT1. The current picture from the results of numerous studies is that the telomere is a highly dynamic structure. The proteins can come on and off the telomere at great speed. Hence, the telomeric structure is constantly being assembled and disassembled.

Besides protecting DNA molecules from degradation, telomeres also protect chromosomal ends from participation in fusion events.

The proteins on telomeres also prevent the cellular DNA repair machinery from mistaking telomeres for broken ends. If the DNA repair machinery is activated, cells will stop dividing and will eventually die.

○ Telomerase

Telomerase is the enzyme that is responsible for the synthesis of telomere DNA. It was discovered by Carol Greider, who was then a graduate student, and her supervisor, Blackburn (Greider & Blackburn, 1985). They purified telomerase and showed that it was a ribonucleoprotein complex. The protein moiety has reverse transcriptase activity and is abbreviated as TERT. The RNA component serves as a template for synthesizing telomere DNA and is abbreviated as TERC. Both the protein and RNA components retain well-recognizable conserved features in even the most distantly related eukaryotes.

The action of telomerase enables DNA polymerase to copy the entire length of the DNA template strand, and thus prevents telomere shortening. Figure 2 shows a model for elongating telomeres in *Tetrahymena* (Zvereva et al., 2010). The CAACCCCAA sequence of the telomerase RNA serves as a template for the extension of the telomere TTGGGG strand. The terminal TTGGGG of the telomere pairs with CCCCAA of the RNA template. This leaves three bases (CAA) that can serve as a template for elongation. Telomerase adds TTG to the 3′ end of telomere. The CAACCCCAA sequence of the RNA template moves six nucleotides down the TTGGGG sequence such that base pairing between the terminal TTG and the telomerase RNA is maintained. The GGGTTG sequence is added to the 3′ end of telomere, and the process repeats itself. Many cycles of the process can occur, resulting in the addition of a few dozen to several hundred repeat sequences. Once telomerase has completed its action, DNA primase synthesizes a RNA primer near the 3′ end of telomere, and DNA polymerase fills the vacant region.

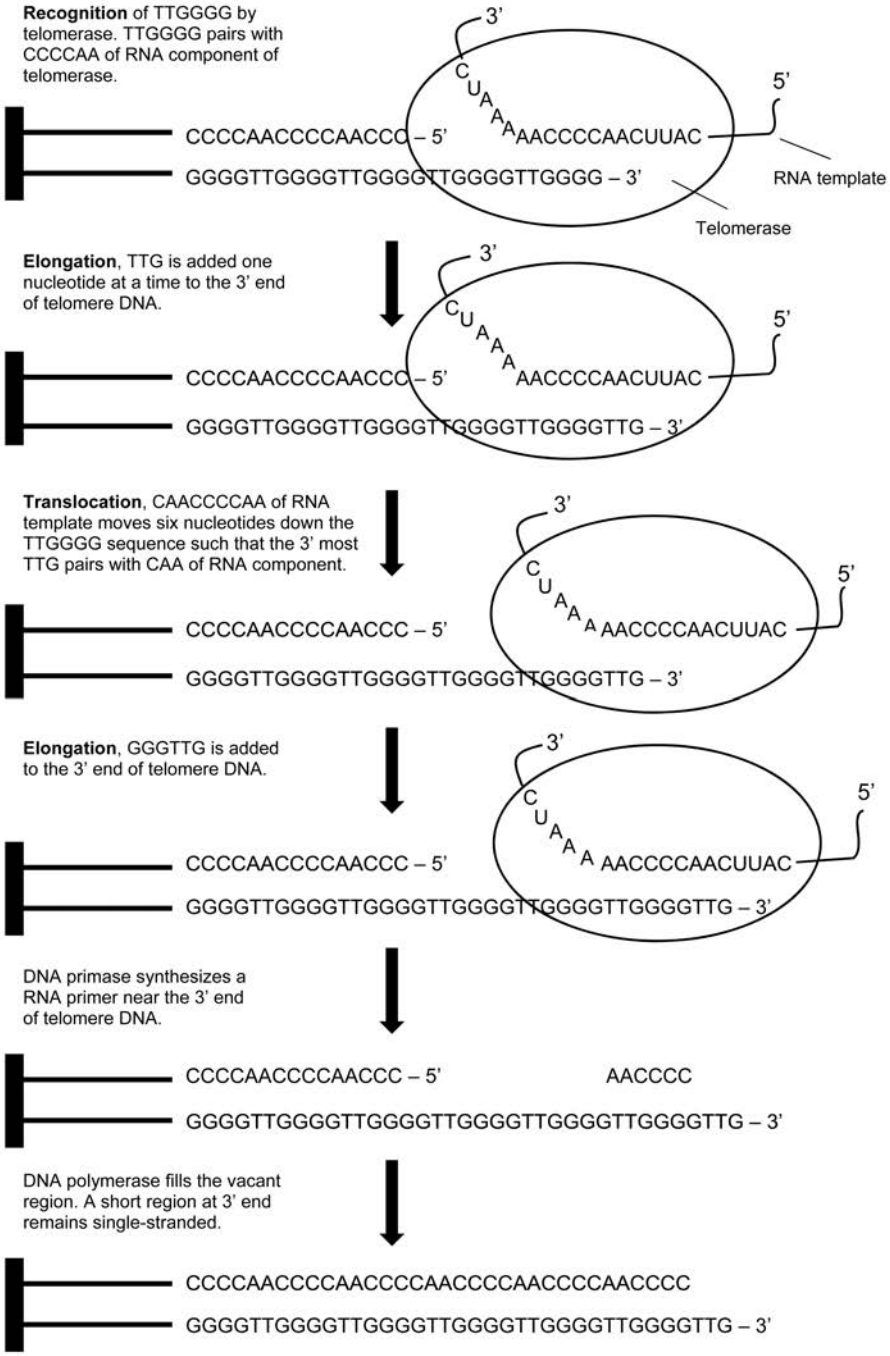

Figure 2. A model for elongating telomeres in *Tetrahymena*.

Telomerase is synthesized in nearly all eukaryotic organisms. The numbers and types of telomerase-expressing cells vary widely among species. In mice, for example, many cells in the adult animal are telomerase-positive. In humans, however, such cells are rare. Telomerase-positive human cells include germ cells, embryonic stem cells, certain adult stem cells, about 90 percent of cancer cells, and a few somatic cells, for example, white blood cells (Artandi & DePinho, 2010). The remaining 10 percent of human cancer cells lack telomerase and maintain telomere length by a mechanism referred to as alternative lengthening of telomeres (Dunham et al., 2000). This explains why germ cells, stem cells, and cancer cells are capable of dividing repeatedly whereas somatic cells cannot.

○ Telomeres, Telomerase, and Aging

Tetrahymena is rich in telomeres and telomerase, and hence can proliferate indefinitely. Such unicellular organisms are immortal in that they can divide endlessly. This immortal trait of unicellular organisms puts them at selective advantage and prevents them from becoming extinct. However, if the telomerase in *Tetrahymena*

is inhibited, the telomeres gradually become shorter and the cells eventually cease to divide. Thus, an immortal organism can be changed to a mortal one by blocking telomerase, thereby showing the importance of telomerase in maintaining telomeres and cell proliferation. This logically leads one to ask, What roles do telomeres and telomerase play in the aging of multicellular organisms such as mice and humans, which are a lot more complex than *Tetrahymena*?

Research on aging has made headway on many fronts. It is now known that aging is a very complicated process and is affected by multiple factors, telomere shortening being one of them. This hypothesis can explain why mice and humans have very different lifespans. Mice are short-lived compared with humans, yet mice have long telomeres, and adult mouse somatic cells often have telomerase activity. Conversely, humans have relatively short telomeres, and telomerase activity is absent in most human somatic cells. Obviously the much shorter lifespan of mice results from factors other than species-specific differences in telomere biology.

It has been established that telomere length decreases with biological age in normal human somatic cells (Armanios, 2013). Cawthon et al. (2003) reported that individuals with shorter telomeres had poorer survival rates. Among people over 60 years of age, individuals with shorter telomeres are three times more likely to die from heart disease and eight times more likely to die from infectious disease as compared with those with longer telomeres. In many studies, telomere shortening has been linked to human aging and diseases of old age (Shammas, 2011). For example, short telomere length in leukocytes is associated with increased incidence of cardiovascular diseases, hypertension, osteoporosis, and type 2 diabetes. In contrast, long telomere length is associated with many years of healthy life.

The relation between telomere, telomerase, and aging is well-illustrated in patients suffering from a genetic disease known as dyskeratosis congenita (Armanios, 2013). Mutations in genes encoding telomerase result in diminished telomerase levels. Accelerated telomere shortening in sufferers of this disorder is associated with premature onset of many age-related diseases and early death. Sufferers are also likely to have early graying of the hair, balding, poor wound healing, gut abnormalities, and infertility.

Would a mouse model that mimics dyskeratosis congenita have similar effects? Not only do aging telomerase-deficient adult mice have extensive tissue damage and organ failure, but reactivating the telomerase results in the striking reversal of a wide range of tissue damage and organ degeneration (Jaskelioff et al., 2011). This is encouraging news, suggesting that premature tissue aging and organ decline associated with decreased telomerase activity may be reversible.

○ Effects of Lifestyle Factors on Telomeres and Aging

Numerous studies have been conducted to investigate the effects of lifestyle factors on telomeres and aging. Factors such as cigarette smoking, obesity, exposure to pollutants, psychological stress, and dietary intake of linoleic acid seem to accelerate telomere shortening. In contrast, dietary intake of fiber, omega-3 fatty acids, and antioxidants, and physical exercise have been shown to slow down telomere shortening.

Cigarette smoking is associated with accelerated telomere shortening (Valdes et al., 2005). A study carried out on 1122 women shows that telomere length of leukocytes decreases steadily at a mean rate of 27 base pairs a year ($p < 0.0001$) and with daily smoking of one pack of cigarettes, an additional five base pairs ($p < 0.0004$) is lost. Thus, telomere erosion caused by smoking one pack of cigarettes a day over a period of 50 years corresponds to a possible loss of 9.3 years of lifespan (p value is not given). However, not all smokers experience earlier mortality; in fact, a small proportion manage to survive to extreme ages. It has been postulated that these smokers are long-lived because they possess a set of 215 single nucleotide polymorphisms in their genomes, which enables them to offset the damage caused by long-term smoking through the activation of somatic maintenance and repair mechanisms (Levine & Crimmins, 2016).

Obesity has also been linked to accelerated telomere shortening. Valdes et al. (2005) reported that the telomeres of white blood cells of obese women are significantly shorter than those of lean women. Another study conducted on both adults and children shows that obese adults have shorter telomeres in their white blood cells than their normal-weight counterparts, but this is not observed in children (Zannolli et al., 2008).

Hoxha et al. (2009) compared the telomere length of leukocytes obtained from traffic police officers exposed to traffic pollution with that of indoor office workers. Exposure to traffic pollution is indicated by the levels of benzene and toluene. The results show that the telomeres of traffic police officers are shorter than those of office workers within each age category. Among the traffic police officers, individuals working at high traffic density have shorter telomeres as compared with those at low traffic density. Similarly, in a study by Pavanello et al. (2010), it is found that the telomeres of lymphocytes of coke-oven workers who are exposed to polycyclic aromatic hydrocarbons are significantly shorter than those who are not exposed, with the length decreasing with number of years of exposure. Of interest to note is that the authors discount the effect of the telomeres decreasing naturally with age and thus contributing to a spurious causal effect in the trials, by having control groups for each age bracket.

Psychological stress increases the rate of telomere erosion and aging. Epel et al. (2004) reported that healthy premenopausal women who are exposed to a high stress level in their daily life have lower telomerase activity and shorter telomeres in their white blood cells as compared with those in the control group. The difference in telomere length between the experimental and control groups of women corresponds to at least ten years of additional aging in women at a high stress level.

Cassidy et al. (2010) studied the association between leukocyte telomere length and various dietary factors in 2284 female participants. It is found that telomere length correlates positively with dietary intake of fiber but correlates negatively with dietary intake of linoleic acid. A study carried out by Farzaneh-Far et al. (2010) shows that a diet rich in omega-3 fatty acids is associated with a decreased rate of telomere erosion in human leukocytes, whereas one lacking in omega-3 fatty acids is linked to an increased rate of telomere shortening. Shen et al. (2009) reported that consuming a diet with lots of antioxidants such as Vitamin C, Vitamin E, and beta-carotene yields longer telomeres in human white blood cells, whereas a diet lacking in such antioxidants is associated with shorter telomeres.

Antioxidants are known to protect telomeres from oxidative damage caused by extrinsic and intrinsic DNA damaging agents.

Werner et al. (2009) reported that leukocytes isolated from endurance athletes show higher telomerase activity and expression of telomere-stabilizing proteins, but reduced telomere shortening, as compared with untrained controls. This result highlights the importance of physical exercise in preserving telomeres and reducing the pace of aging.

○ Telomeres, Telomerase, and Cancer

The most widely accepted hypothesis for tumorigenesis is as follows. When an individual is fully developed, telomerase is produced by germ cells and stem cells but repressed in most somatic cells. When somatic cells divide, telomeres shorten progressively. When the telomeres reach a critical length, the cells enter senescence. If, however, mutations occur in genes such as tumor suppressor genes, the cells can circumvent senescence and continue to replicate. For the vast majority of the cells, telomerase remains repressed and the telomeres become almost totally eroded as the cells continue to proliferate, and the cells eventually die. In a small number of cells, further mutations may result in activation of telomerase; hence the shortened telomeres can be saved or maintained, and the cells become immortal or cancerous. Scientists believe that successive telomere shortening in normal cells and their eventual death may be an important anti-cancer mechanism. In essence, activation of telomerase for the maintenance of telomeres is an important step in tumorigenesis (Artandi & DePinho, 2010). However, this hypothesis does not explain why some advanced tumors lack telomerase and why some somatic cells such as white blood cells produce telomerase.

The presence of telomerase in most cancer cells and its absence in most normal cells have raised hopes that it might be possible to treat cancer by blocking telomerase, using either chemicals to inhibit telomerase activity or vaccines to activate the immune system to mount an immune response against telomerase-expressing cells. Several vaccines for fighting cancers have been tested in clinical trials (Gomez et al., 2012). However, two problems must be solved before blocking telomerase can be efficacious for cancer treatment. The first issue is that about 10 percent of cancer cells have developed alternative pathways to maintain telomeres, and thus their proliferation cannot be stopped by telomerase inhibitors or vaccines. The second issue is the risk of damage to healthy cells in which a high telomerase level is necessary for their functioning, such as germ cells and stem cells. Inhibiting telomerase in such cells can therefore impair fertility, healing of wounds, and the production of blood cells.

○ Conclusion

The discoveries on telomere and telomerase are typical examples of curiosity-driven research. Though the work was originally of interest to only a few scientists within a very narrow field, it was still pursued actively for the sake of generating fundamental knowledge, and with little inkling that it might lead to medical applications in the future. Teachers could share the work done by Elizabeth Blackburn, Jack Szostak, and Carol Greider with students and help them realize that some research projects they may embark on in the future could have implications that they might not have foreseen.

Research on telomeres has made considerable progress since the recognition of telomeres as repetitive DNA at the chromosomal ends in *Tetrahymena*. The lengthening of telomeres by telomerase was originally thought to be simply a means by which *Tetrahymena* maintains or protects its chromosomes, but it turns out to be a fundamental process in eukaryotic cells. The study of this once unknown process may lead to novel strategies for regenerating tissues and battling cancers in humans. Although several challenges stand in the way of realizing this potential, research has resulted in solutions to some of the challenges, thus making the use of telomerase and telomerase inhibitors in fighting degenerative diseases and malignancies a reality in the future.

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