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Chromosome and Ageing

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Abstract: Aging is the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age. These time-related changes are attributed to the aging process. Aging is mainly due to the cumulative results of free radical oxidation of the protein, lipid and nucleic acid of our body by reactive oxygen species. Genetic instability could be one of the basic reasons for senescence. An important aspect of aging that needs some comment is change in genome structure. Several studies have established the role of DNA alterations in the aging process. At the cellular level, an important recent finding has been that the lifespan of cells in the human body are determined by strings of DNA (genetic material) called telomeres, which are located at the ends of the chromosomes. Each time a cell divides, the telomere becomes shorter; the senescence and death of the cell is triggered when the telomere is reduced to a certain critical length. Telomerase, an enzyme that can intervene in this process, is being closely studied in relation to cancer as well as aging. The 2009 Nobel Prize in Physiology or Medicine is awarded to Drs. Elizabeth H Blackburn, Jack W Szostak and Carol W Greider for their discovery of how chromosomes are protected by telomeres and the enzyme telomerase. They solved a longstanding fundamental problem in biology; how can the ends of chromosomes be maintained and spared from erosion or rearrangement during repeated cellular divisions? These discoveries have significant medical implications for many fields, including cancer, ageing, stem cell maintenance and hereditary disease syndromes.

Key words: Chromosome · Ageing · Telomere and Senescence

INTRODUCTION

Aging is a process that occurs in all members of a species. Typically conceived as characterizing a postreproductive period of life, this process in fact affects organisms of any age. It is the rate and accumulation of aging effects, which distinguishes young and old individuals. Aging in humans appears to result from a combination of genetically programmed phenomena and harmful environmental factors, both exogenous and endogenous. These factors primarily act at the cellular level, rather than at the level of the organism [1, 2]. Cellular aging, termed ‘‘replicative senescence,’’ involves irreversible inhibition of DNA replication and occurs in all eukaryotic cells. It is determined by telomere length, thus limiting cellular proliferation and growth after a certain number of cell divisions occurs (‘‘Hayflick limit’’). The ability of nondividing cells to maintain their metabolic activity leads to the accumulation of senescent cells in the organism [1,3-16]. Because of the increase in the number of DNA lesions that accumulate during multiple repetitions of DNA replication throughout the proliferative life span of a cell, the accumulation of senescent cells in elderly persons might contribute to the aging of tissue and organism [2,17].

The lifespans of different species vary widely and this could be due to genetic control. Long life means long exposure to mutagenic attacks, while aging is accompanied by changes in DNA. Genetic instability could be one of the basic reasons for senescence. Age is accompanied by accumulation of diseases and disorders. An important aspect of aging that needs some comment is change in genome structure. Several studies have established the role of DNA alterations in the aging process. Aging is a universal process which results from interaction of genomes and the environment. The organism is permanently affected by both environmental factors and intra- and intercellular adverse effects (free radicals, radiation, toxins, microorganisms, etc.). During its lifespan, owing to stability of the genetic basis, the organism resists these factors, maintaining homeostasis necessary for normal functioning and survival. However,

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with time, these mechanisms for maintenance of normal conditions decay and change occur in information (mutation), chromosomal structure (structural aberrations), information count (polyploidia and aneuploidia) and methylation intensity and genetic expression. The basic question to be answered is whether these changes are the cause or the effect of aging. In humans, some of the genetic variations for rates of aging encode DNA repair enzymes.

Desire for Long Life or Immortal Life: Ageing as a phenomenon and the possibilities of postponing, reversing or abolishing it, have fascinated humans for a very long time as both mythology and history testify [18]. In the past couple of centuries, scientists proposed a number of ageing theories, but none of them was completely satisfactory. Current theories regarding proximal causes of ageing can be classified into two major categories: theories invoking a program directing ageing and stochastic theories. Programmed ageing theories assume the existence of a purposeful genetic program that determines the ageing process. In contrast, stochastic or error-based theories suggest that ageing is driven by random, accidental events that cause mutations and/or deregulation of cellular processes and finally lead to cellular dysfunction and senescence [19-21]. There are several suggestions that ageing can take place at the cellular level:

- Cellular ageing appears to be related to and perhaps caused by, nuclear DNA and mitochondrial DNA damage and diminished DNA repair. Senescent cells share some molecular features: altered gene expression, disturbance of cell cycle duration, telomere shortening, genetic instability, deregulation of apoptosis [2]. Therefore, many of the processes that occur in most of somatic cells as a consequence of DNA replication (accumulation of DNA errors or mutations that outstrip repair processes, telomere shortening) drive replicative senescence in human cells [22].
- Cell division occurs during life in many tissues either as a part of normal tissue function or in response to tissue damage. But, after a limited number of cell divisions they reach a quiescent state termed senescence and become postmitotic (with few exceptions: spermatogonia, hematopoietic stem cells, tumour cells). The accumulation of cells at the end of their replicative lifespan in the elderly might contribute to tissue ageing [2, 17].
- Dysfunction in many organs observed at an old age, is due to a loss or an altered function of cells. This includes the loss of neurons in the brain, loss of melanin production in hair follicle melanocytes, over and underexpression of collagenase, inhibitors in senescent human skin [2]. The general process of cellular senescence is termed replicative senescence, in contrast to the process of ageing of the organism. Replicative senescence limits the proliferation of normal human cells, irreversibly arrests growth and affects cell function. Replicative senescence is controlled by multiple dominant-acting genes [2].

The ageing process consists of structural alterations and functional declines in body systems with a consequent impairment of homeostasis with increased vulnerability to age-related diseases, ultimately leading to death [23]. The effects of ageing in individuals appear to be a combination of genetically programmed processes [24] and genetic alterations induced by exogenous and endogenous factors [25]. The increase of cellular components damaged by highly reactive free radicals [26-29] and associated with decreased DNA repair capability [30, 31] play a central role in genetic instability, [32] a common marker of cancer and age-associated degenerative diseases [33].

There is an age-related increase in DNA damage and in the mutation rate [34, 35] and variations in different genetic end-points - such as DNA adducts, DNA breaks or alkali-labile sites, [36, 37], mutations at different genetic loci [38-40] and chromosomal damage [1, 41]. The presence of chromosomal abnormalities was the first genetic change to be specifically associated with age [42 -44].

Changes in chromosomal structure or function are strongly associated with ageing, although scientific results cannot determine whether these changes are part of the cause or a consequence of ageing. Chromosomal breakage and loss is a common event induced by carcinogens and reactive oxygen metabolites; [45] furthermore, cancer cells also exhibit a high rate of spontaneous chromosomal breakage and loss [46]. The evidence that chromosomal aberration rate is positively correlated with cancer risk has been further consolidated by epidemiological data from the Italian and the Nordic cohort studies, which showed that overall cancer rates are increased approximately twofold in those with the highest tertile of chromosomal damage relative to those in the lowest tertile [47, 48]. The extent to which diet, genetic background and exposure to environmental genotoxins

contributes to the observed rate of chromosomal damage remains unclear and an understanding of the potential contribution of each of these factors is crucial in evaluating the scope for intervention. The main assumption of intervention strategies is that chromosomal damage rate is the underlying cause of aging [2] and cancer [47, 48] and that a minimization of chromosomal damage rate should result in a reduced risk for cancer and a restraint of the aging process.

In proliferating cells, most of the DNA damage capable to generate chromosomal aberrations is repaired during the G2 phase. It has been postulated that the G2 repair mechanism involves two cooperative pathways: [17] control of mitotic delay (G2 arrest) associated with DNA damage level and [49] DNA repair pathways for removing DNA lesions requiring ATP and NADH. Since chromosomal aberrations arise as a result of misrepaired or unrepaired lesions, the inhibition of G2 repairs should result in an increased number of chromosomal aberrations [50]. Repair-deficient cells may accumulate more DNA damage, resulting in an increased number of chromosomal aberrations with age [1]. DNA breaks are considered to be critical primary lesions in the formation of chromosomal aberrations. They may be induced by exogenous agents but also occur spontaneously during cell cycle [51]. Analysis of peripheral blood lymphocytes provides a possibility to assess structural and numerical chromosome damage *in vivo*. Lymphocytes are generated from hematopoietic stem cells in primary lymphoid organs, such as thymus and adult bone marrow. A portion of mature peripheral lymphocytes are long-lived and can persist in the blood circulation for several years in the quiescent non-proliferative G0 phase until they are activated by a specific antigen. Some of the cytogenetic changes observed in cultured and uncultured lymphocytes are considered to represent aberrations generated *in vivo*. The chromosomal aberrations in peripheral blood lymphocytes should be the best indicator in the detection of senescence. In “normal” somatic cells, the frequency of spontaneously occurring chromosomal aberrations is rather low, with incidence of 0.6% in human lymphocytes [17, 49, 52]. Skin fibroblasts can also be used to assess structural and numerical age-related chromosome damage *in vivo*.

Cellular Senescence: In 1961, Leonard Hayflick at Wistar Institute in Philadelphia observed cell division. After about 70 divisions, its ability to divide gradually slows down and ultimately stops. Hypothesized that each cell had an internal clock. Observed cells from twenty-year old divide more than cells from fifty-year old.

How the Chromosomes Are Protected Against Degradation: Aging is the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age. These time-related changes are attributed to the aging process. The nature of the aging process has been the subject of considerable speculation. Our cells age so we age. Shortening of telomeres leads to inability to divide and produce new cells. Once the telomeres at the end of our cells have shortened too much, new cells are no longer made and eventually the amount of strong cells decreases and the organism as a whole ages.

Telomere and Telomerase: A telomere is a region of repetitive DNA at the end of a chromosome, which protects the end of the chromosome from deterioration. Russian theorist Alexei Olovnikov was the first to recognize [53] the problem of how chromosomes could replicate right to the tip, as such was impossible with replication in a 5' to 3' direction. To solve this and to accommodate Leonard Hayflick's idea of limited somatic cell division, Olovnikov [53] suggested that DNA sequences would be lost in every replicative phase until they reached a critical level, at which point cell division would stop. During cell division, enzymes that duplicate the chromosome and its DNA cannot continue their duplication all the way to the end of the chromosome. If cells divided without telomeres, they would lose the ends of their chromosomes and the necessary information they contain. James Watson [54] named this phenomenon the "end replication problem". The telomeres are disposable buffers blocking the ends of the chromosomes and are consumed during cell division and replenished by an enzyme, the telomerase reverse transcriptase. They have been likened to the aglets (tips) on the ends of shoelaces that keep them from fraying. In 1975-1977, Blackburn, working as a postdoctoral fellow at Yale University with Joseph Gall, discovered the unusual nature of telomeres, with their simple repeated DNA sequences composing chromosome ends. Their work was published in 1978. The telomere shortening mechanism normally limits cells to a fixed number of divisions and animal studies suggest that this is responsible for aging on the cellular level and sets a limit on life spans. Telomeres protect a cell's chromosomes from fusing with each other or rearranging-abnormalities which can lead to cancer-and so cells are normally destroyed when their telomeres are consumed. Most cancers are the result of "immortal" cells which have ways of evading this programmed destruction. Telomeres

are repetitive DNA sequences located at the termini of linear chromosomes of most eukaryotic organisms and a few prokaryotes. Telomeres compensate for incomplete semi-conservative DNA replication at chromosomal ends. The protection against homologous recombination (HR) and non-homologous end joining (NHEJ) constitutes the essential "capping" role of telomeres that distinguishes them from DNA double-strand breaks (DSBs) [55]. In most multicellular eukaryotic organisms, telomerase is active only in germ cells, stem cells and certain white blood cells. There are theories that the steady shortening of telomeres with each replication in somatic (body) cells may have a role in senescence and in the prevention of cancer. This is because the telomeres act as a sort of time-delay "fuse", eventually running out after a certain number of cell divisions and resulting in the eventual loss of vital genetic information from the cell's chromosome with future divisions. Telomere length varies greatly between species, from approximately 300 to 600 base pairs in yeast [56] to many kilobases in humans and usually is composed of arrays of guanine-rich, six-to-eight base-pair-long repeats. Eukaryotic telomeres normally terminate with 3' single-stranded-DNA overhang which is essential for telomere maintenance and capping. Multiple proteins binding single- and double-stranded telomere DNA have been identified [57- 61]. These function in both telomere maintenance and capping. Telomeres form large loop structures called telomere loops, or T-loops. Here, the single-stranded DNA curls around in a long circle stabilized by telomere-binding proteins. At the very end of the T-loop, the single-stranded telomere DNA is held onto a region of double-stranded DNA by the telomere strand disrupting the double-helical DNA and base pairing to one of the two strands. This triple-stranded structure is called a displacement loop or D-loop. Telomere shortening in humans can induce replicative senescence which blocks cell division. This mechanism appears to prevent genomic instability and development of cancer in human aged cells by limiting the number of cell divisions. Malignant cells which bypass this arrest become immortalized by telomere extension mostly due to the activation of telomerase, the reverse transcriptase enzyme responsible for synthesis of telomeres. However, 5-10% of human cancers activate the Alternative Lengthening of Telomeres (ALT) pathway which relies on recombination-mediated elongation. In 2009,. Nobel Prize in physiology or medicine is awarded to three scientists who have solved a major problem in biology: how the chromosomes can be copied in a complete way during cell divisions and how they are protected against degradation. The Nobel Laureates have shown that the solution is to be found in the ends of the chromosomes- the telomeres- and in an enzyme that forms them- telomerase. The long, thread-like DNA molecules that carry our genes are packed into chromosomes, the telomeres being the caps on their ends. Elizabeth Blackburn and Jack Szostak [62] discovered that a unique DNA sequence in the telomeres protects the chromosomes from degradation. Carol Greider and Elizabeth Blackburn [63] identified telomerase, the enzyme that makes telomere DNA. These discoveries explained how the ends of the chromosomes are protected by the telomeres and that they are built by telomerase.

Cell Aging: If the telomeres are shortened, cell age. Conversely, if telomerase activity is high, telomere length is maintained and cellular senescence is delayed. This is the case in cancer cells, which can be considered to have eternal life. Certain inherited diseases, in contrast, are characterized by a defective telomerase, resulting in damaged cells. The award of the Nobel Prize recognizes the discovery of a fundamental mechanism in the cell, a discovery that has stimulated the development of new therapeutic strategies. The chromosomes contain our genome in their DNA molecules. As early as the 1930s, Hermann Muller [64] (Nobel Prize 1946) and Barbara McClintock [65] (Nobel Prize 1983) had observed that the structures at the ends of the chromosomes, the so called telomeres seemed to prevent the chromosomes from attaching to each other. They suspected that the telomeres could have a protective role, but how they operate remained an enigma.

When scientist began to understand how genes are copied, in the 1950s, another problem presented itself. When a cell is about to divide, the DNA molecules, which contain the four bases that form the genetic code, are copied, base by base, by DNA polymerase enzymes. However, for one of the two DNA strands, a problem exists in that the very end of the strand cannot be copied. Therefore, the chromosomes should be shortened every time a cell divides- but in fact that is not usually the case. These discoveries had a major impact within the scientific community. Many scientists speculated that telomere shortening could be the reason for ageing, not only in the individual cells but in the organism as a whole. But the ageing process has turned out to be complex and it is now thought to depend on several different factors, the telomere being one of them. Research in this area remains intense. Most normal cells do not divide frequently, therefore their chromosomes are not at risk of shortening and they do not require high telomerase activity.

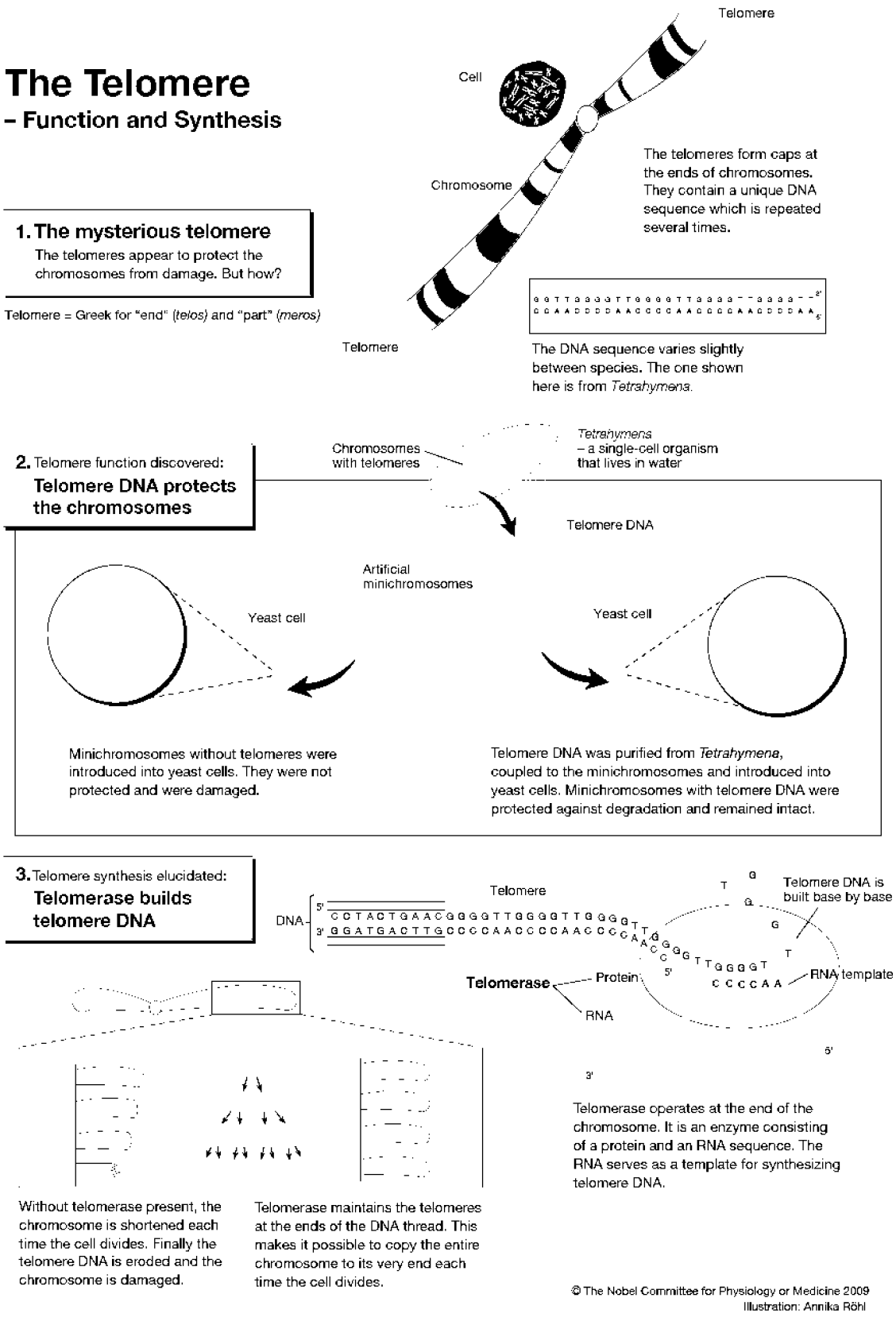
The Telomere
– Function and Synthesis
1. The mysterious telomere
The telomeres appear to protect the chromosomes from damage. But how?
Telomere = Greek for "end" (telos) and "part" (meros)
Telomere
Cell
Chromosome
Telomere
The telomeres form caps at the ends of chromosomes. They contain a unique DNA sequence which is repeated several times.
The DNA sequence varies slightly between species. The one shown here is from Tetrahymena.
2. Telomere function discovered:
Telomere DNA protects the chromosomes
Chromosomes with telomeres
Tetrahymena
– a single-cell organism that lives in water
Telomere DNA
Artificial minichromosomes
Yeast cell
Yeast cell
Minichromosomes without telomeres were introduced into yeast cells. They were not protected and were damaged.
Telomere DNA was purified from Tetrahymena, coupled to the minichromosomes and introduced into yeast cells. Minichromosomes with telomere DNA were protected against degradation and remained intact.
3. Telomere synthesis elucidated:
Telomerase builds telomere DNA
DNA
Telomere
Telomere DNA is built base by base
Telomerase
Protein
RNA
RNA template
Without telomerase present, the chromosome is shortened each time the cell divides. Finally the telomere DNA is eroded and the chromosome is damaged.
Telomerase maintains the telomeres at the ends of the DNA thread. This makes it possible to copy the entire chromosome to its very end each time the cell divides.
Telomerase operates at the end of the chromosome. It is an enzyme consisting of a protein and an RNA sequence. The RNA serves as a template for synthesizing telomere DNA.
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Illustration: Annika Röhl

In contrast, cancer cells have the ability to divide infinitely and yet preserve their telomerase's. How do they escape cellular senescence? One explanation became apparent with the finding that cancer cells often have increased telomerase activity. It was therefore proposed that cancer might be treated by eradicating telomerase. Several studies are underway in this area, including clinical trials evaluating vaccines directed against cells with elevated telomerase activity.

Some inherited diseases are now known to be caused by telomerase defects, including certain forms of congenital aplastic anemia, in which insufficient cell divisions in the stem cells of the bone marrow lead to severe anemia. Certain inherited diseases of skin and the lungs are also caused by telomerase defects.

The discoveries by Black burn, Greider and Szostak [62] have added a new dimension to our understanding of the cell and shed light on disease mechanisms. Besides they stimulated the development of potential new therapies. If one asks the right question: 'What are the main causes of aging?' then one does not have to restrict the quest to search the impossible. Because of the real complexity of an organism, even an answer regarding only one cell type could substantially further contribute to the understanding of the nature of aging. However, one has to be cautious when interpreting the data yielded during model experiments since a lot of the data of animal or cell culture models are not valid in the in vivo human system. It is quite possible that the human body is not perfect, i.e. although avoiding the external or internal harmful effects the information level of the human system is not developed enough to live forever. By providing an optimal state for the living system the maximum life-span of today's people could be substantially lengthened but without changing the present information level of the human body only 'healthy' aging can be expected.

CONCLUSION

Large-scale studies are logistically and financially demanding. However, the reason why some humans live to extreme ages are largely unknown and only a few genetic and environmental factors have been identified. Understanding the genetic basis for longevity is an extraordinarily difficult task, but it has the potential to provide insights into central mechanisms of ageing and disease, which are ultimately hoped to provide targets for the prevention and treatment of late-life disabilities and diseases. The discoveries have also led to the development of new therapeutic strategies for cancer treatment based on the targeting of telomerase activity or expression that are now undergoing clinical testing. The discoveries concerning telomere function and maintenance that are recognized have solved one of the fundamental problems in biology and opened a whole new research field.

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