Treball Final de Grau

TELOMERES AND THE AGING PROCESS

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ACKNOWLEDGEMENTS

I have been interested in *Telomeres* since my Biology teacher in High School told us about them. Now, after some years, I have had the opportunity to research and learn a bit more, so I would like to thank the Department of Biochemistry and Molecular Biology for accepting my proposal on this issue. In particular, I am really grateful to my tutor for her guidance during the project. Without her support it would not have been so bearable.

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ABSTRACT

Telomeres are a cornerstone when it comes to protecting coding DNA in chromosomes. They consist of tandem repeats of a sequence of 6 nucleotides: $(TTAGGG)_n$ for humans. Their protective function prevents the ends of linear chromosomes from being recognized as damaged DNA and activate repair processes. However, telomeres slightly shorten every cell cycle due to the inability of DNA polymerase to replicate linear DNA completely. This fact limits the lifespan of cells.

Some types of cells such as germ cells cannot afford this loss and have telomerase activity, the enzyme responsible to lengthen telomeres by adding telomeric sequences to one of the strands of DNA.

Telomeres are involved in aging process as well as in some age-related diseases such as diabetes or cancer. Apart from hereditary factors, there are also a great number of non-genetic external factors that can aggravate telomere shortening. Thus, the probability of suffering illness or even premature death is increased.

This project reviews the structure and function of telomeres, as well as the action mechanism of telomerase, to better understand how they take part in certain diseases afterwards.

RESUM

Els telòmers són una peça clau quan es tracta de protegir l'ADN codificant dels cromosomes. Estan formats per repeticions en tàndem d'una seqüència de 6 nucleòtids: $(TTAGGG)_n$ en el cas dels humans. La seva funció protectora evita que els acabaments dels cromosomes lineals siguin reconeguts com a ADN danyat i s'activin processos de reparació. No obstant, en cada cicle cel·lular els telòmers s'escurcen una mica degut a la impossibilitat de l'ADN polimerasa per replicar completament ADN lineal. D'aquesta manera queda limitada l'esperança de vida cel·lular.

Alguns tipus de cèl·lules com les germinals no poden permetre's aquesta pèrdua i tenen actiu l'enzim telomerasa, encarregat d'allargar els telòmers afegint seqüències telomèriques en una de les dues cadenes de l'ADN.

Els telòmers estan involucrats en el procés d'envelliment, així com amb l'aparició de diverses malalties relacionades amb l'envelliment, com ara la diabetis o el càncer. A part dels factors hereditaris, també hi ha un seguit de factors externs no genètics que poden agreujar la pèrdua de telòmers, augmentant així la probabilitat de sofrir malalties o fins i tot una mort prematura.

En aquest treball es revisa l'estructura i funció dels telòmers, així com el mecanisme d'acció de la telomerasa per, posteriorment, entendre millor com estan involucrats en certes malalties.

RESUMEN

Los telómeros son una pieza clave cuando se trata de proteger el ADN codificante de los cromosomas. Están formados por repeticiones en tándem de una secuencia de 6 nucleótidos: $(TTAGGG)_n$ en el caso de los humanos. Su función protectora evita que las puntas de los cromosomas lineales sean reconocidas como ADN dañado y se activen procesos de reparación. No obstante, en cada ciclo celular, los telómeros se acotan un poco debido a la imposibilidad de la ADN polimerasa para replicar completamente ADN lineal. De esta forma queda limitada la esperanza de vida celular.

Algunos tipos de células como las germinales no pueden permitirse esta pérdida y tienen la enzima telomerasa activa, encargada de alargar los telómeros añadiendo secuencias teloméricas en una de las dos cadenas del ADN.

Los telómeros están involucrados en el proceso de envejecimiento, así como con la aparición de diversas enfermedades relacionadas con el envejecimiento, como la diabetes o el cáncer. Además de los factores hereditarios, también hay una serie de factores externos no genéticos que pueden agravar la pérdida de telómeros,

aumentando así la probabilidad de sufrir enfermedades o incluso una muerte prematura.

En este trabajo se revisa la estructura y función de los telómeros, así como el mecanismo de acción de la telomerasa para, posteriormente, entender mejor cómo están involucrados en ciertas enfermedades.

INTEGRATION OF THE DIFFERENT FIELDS

A great part of this project deals with the structure of chromosomes' ends, the DNA sequence and telomerase enzyme as well as the functioning of it, so the main scope of this project is *Biochemistry and Molecular Biology*.

The other two scopes related to this project in a more minority way are *Physiology and Physiopathology* and *Public Health*. The first one is referred to the age-related diseases where some illnesses are briefly described, while the second one is mentioned at the end, when lifestyle factors are explained to have a negative effect on telomere loss. These non-genetic factors can be reduced or even avoided if people are aware of them, and it is under Public Health's power to make society be concerned.

1. INTRODUCTION

Telomeres were first noticed by Herman Müller in 1938 while analysing chromosomal anomalies in the fly *Drosophila* induced by X-rays. He discovered that natural ends of chromosomes were never involved in reorganizations, fusions or translocations (1). Three years later, in 1941, Barbara McClintock was analysing the chromosomes in the plant *Zea mays* and figured out that during mitosis, a structure called anaphase bridge was formed. The tension between the two centromeres caused the breakage of the bridge, leading the ends of the chromosomes to fusion. McClintock found that the natural ends of chromosomes never cooperated with these fusions (2). So, in conclusion, both scientists deduced that the natural ends of chromosomes must have a special structure that prevent them from participating in such processes.

It was not until the 1970s when telomeres became of interest again with Elisabeth Blackburn and Joseph G. Gall's research group at Yale University. They sequenced the ends of linear mini-chromosomes in the protozoan *Tetrahymena thermophile* and, in 1978, they published the results showing that telomeres were made up of G-rich DNA repeated sequences $(TTGGGG)_n$ (3). From then on, the telomeres of many different species have been described and apparently, these sequences are greatly conserved.

A telomere is a section of DNA located at the end of a linear chromosome. They are a cornerstone when it comes to protection as they confer stability to chromosomes, prevent coding DNA from degradation, end-to-end fusions or fraying usually observed in damaged DNA, either by X-rays or physical fracture (4).

A human repetitive DNA library was constructed from randomly sheared and reassociated DNA and it was then screened with ^{32}P-labeled human repetitive DNA (5). Two of the clones used contained copies of tandem arrays of the hexadeoxynucleotide sequence $(TTAGGG)_n$. Unlike other tandem repetitive patterns which are distributed in different chromosomes, this sequence was present on each human chromosome regardless of chromosome length. The estimated quantity of $(TTAGGG)_n$ sequences in the human genome was 3,000 – 12,000 base pairs per chromosome.

1.1. Telomeres' structure

Like most DNA of the chromosomes, telomeres are constituted by a DNA double-strand of this short sequence rich in G-C deoxynucleotides and a 3' single-strand overhang, the one that contains guanines.

At the beginning, it was thought that telomeres were linear structures, but some investigations demonstrated that it formed a loop due to two telomeric repeat-binding factors, TRF1 and TRF2 (6). These studies showed how, when inhibiting TRF2, the DNA damage checkpoint pathway was activated. Thus, a possible way to form this structure was supposed (Figure 1). It was proposed that TRF1 created a loop-back structure – the T-loop (telomere's loop) – and TRF2 kept it tightly joined by the insertion of the 3' overhang into the double-strand DNA, which led to a triple strand called D-loop or displacement loop (7,8). This was the origin of the capping function of telomeres and how they protect themselves from being recognised as damaged DNA.

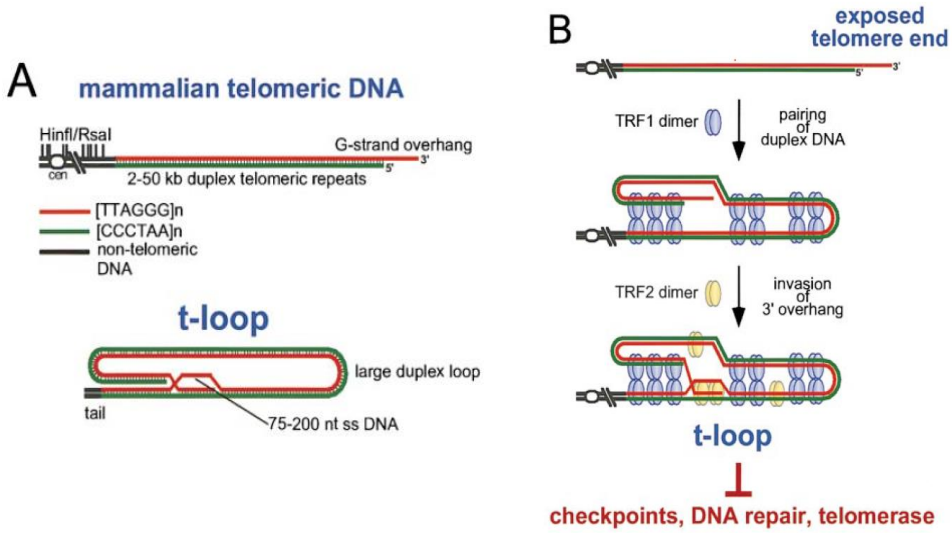

Figure 1: *Proposed structure, formation and function of T loops. Figure adapted from ref.* (6).

(A) The DNA structure at the ends of mammalian chromosomes and a description of the proposed configuration of t loops.
(B) Speculative scheme depicting a possible mode of t loop formation based on the in vitro biochemical activities of TRF1 and TRF2. T loops are proposed to mask telomere termini from cellular activities that can act on DNA ends.

Nowadays, more telomere-associated proteins are known. In human being, the protective complex or shelterin protein complex is composed of six proteins: TRF1, TRF2,

RAP1, TIN2, TPP1 and POT1 (9). They interact with each other to stabilize the loop structure (Figure 2).

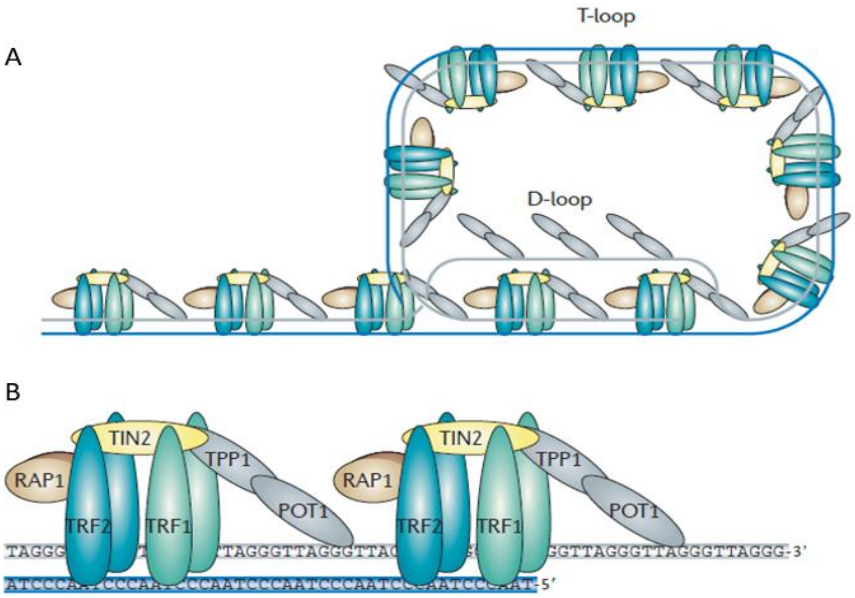

***Figure 2**: Structure of telomeres and the shelterin complex. Figure adapted from ref.* (16).

(A) Model of the telomere's structure with the T-loop and the D-loop.
(B) Representation of the different proteins forming the shelterin complex.

TRF1, TRF2 attach specifically straight to telomeric DNA duplex and POT1 binds only to the 3' strand, either it is double-stranded or the displaced overhang forming the D-loop (9–12). TIN2 acts as a bridge between TRF1 and TRF2 and TPP1 binds both POT1 and TIN2. The amino terminus of TPP1 incorporates a domain related to the recruitment of telomerase (13). RAP1 is associated with TRF2 and even though it is not essential for the capping function, recently studies showed that it impedes telomere recombination and delicacy (14,15).

The protective complex not only avoids the activation of DNA repair processes but it is also involved in the regulation of the enzyme responsible for the extension of telomeres (16).

TRF 1 and **2**: telomeric repeat binding factor 1 and 2		**POT1**: protection of telomeres 1
TIN2: TRF-1 interacting protein 2	**TPP1**	**RAP1**: repressor/activator protein 1

1.2. The end-replication problem

Every time a cell divides, it first replicates the genome with a DNA polymerase but the properties of this enzyme make it impossible to entirely copy the linear DNA molecules out to the extreme by the normal process (17,18). The requisite for a primer to start the synthesis and the unidirectional growth of the new strand cause the known as “end-replication problem”. DNA polymerase can only synthesize DNA in the 5’ to 3’ direction and it also needs an RNA primer with a free 3’-OH group.

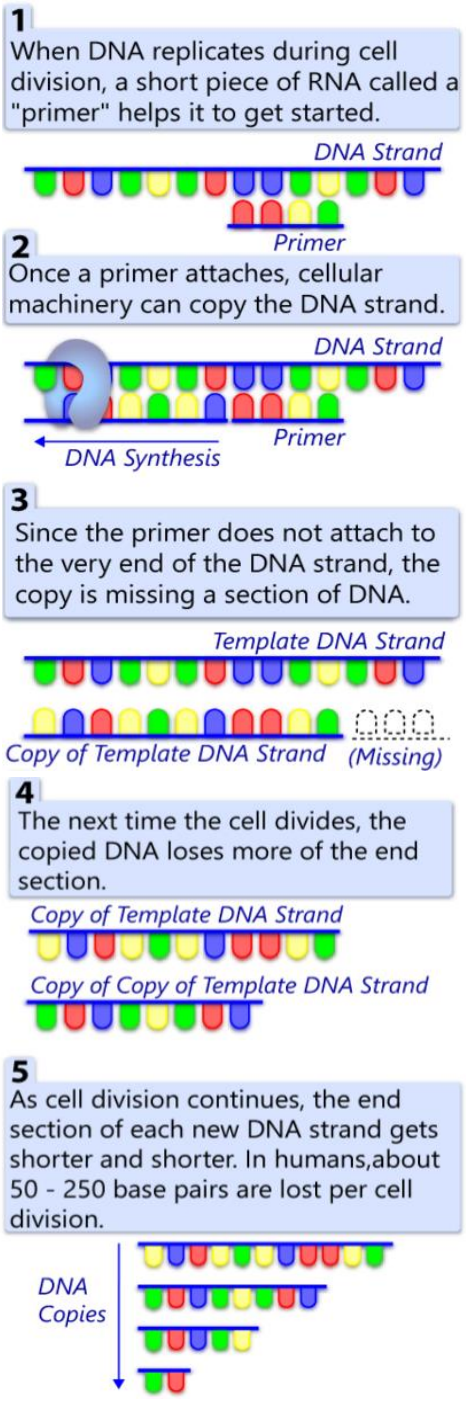

Figure 3: *Explanation of the end-replication problem. Figure adapted from ref.* (87).

Since DNA is formed with two anti-parallel strands, when a bubble of replication is created, the leading strand is synthesized without interruption in the same direction as the replication fork moves forward. However, the lagging strand is synthesized in the opposite direction discontinuously using more RNA primers (Okazaki fragments) (Figure 4). These primers are then eliminated and replaced by DNA, but the removal of the 5’-end primer will imply a loss of a small part of the telomere, as DNA polymerase will not have any 3’-OH group to start from. Furthermore, if the overhang is not long enough, there is an extra deterioration of the telomeres to ensure the t-loop can be formed. As a result, telomeres shorten between 50 and 200 base pairs after each replication cycle (Figure 3), but this is exactly why they are designed for, so that proliferation can take place without losing important genetic information or functions. Considering that, telomeres delimit the number of times a cell can divide.

This problem must be solved at least in germinal cells in order to transfer the complete genome through generations. It was seen that sperm has longer telomeres than somatic cells (19). Diverse organisms have acquired different methods to avoid DNA loss in their chromosome ends. Although most mammalians use a specific enzyme called telomerase, some human cells with telomerase inactivated can also maintain or extend their telomeres by alternative lengthening of telomeres (ALT), which consists in copying DNA sequences from one telomere to another (20). This mechanism would imply homologue recombination.

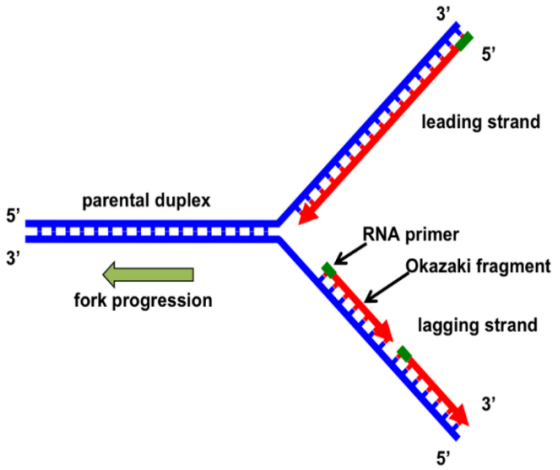

***Figure 4**: The replication fork. Figure from ref.* (88).

1.3. Telomerase, the solution for the end-replication problem

1.3.1. Structure of telomerase

Telomerase was first discovered by Carol Greider and Elizabeth Blackburn in the ciliate *Tetrahymena thermophile* in 1985 (21), some years after Blackburn had sequenced its telomeres. This enzyme is a ribonucleoprotein (RNP), that is an RNA-dependent DNA polymerase which adds telomeric DNA sequences onto chromosome ends. Even though *Tetrahymena*'s telomerase is monomeric, the human telomerase is dimeric and has two main components: an RNA template for the synthesis called hTR or hTERC (22) and a catalytic protein with reverse transcriptase activity known as hTERT (23–25). There are also some associated proteins: dyskerin, NHP2, NOP10, Pontin / Reptin, GAR1 and TCAB1 (Figure 5).

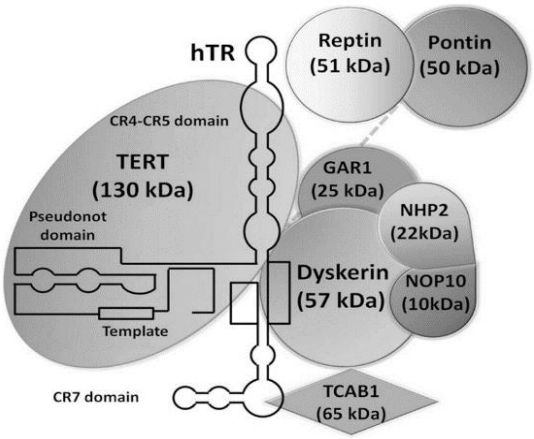

***Figure 5**. Schematic representation of telomerase and its associated proteins. Figure from ref.* (89).

The heterotrimeric complex formed by dyskerin, NHP2 and NOP10 is essential for the stability of hTR *in vivo* (26). The association of this complex and GAR1 to hTR make the enzyme functional. Reptin and Pontin are two ATPases also needed to stabilize dyskerin and hTR *in vivo*. They interact with TERT, regulating it during the S phase of the cell cycle. The protein TCAB1 seems to regulate the subcellular location of telomerase (27).

RNA template

The phylogenetic comparative analysis of vertebrate TR showed three conserved domains: the CR4/CR5 domain, the pseudoknot/template core domain and a box H/ACA domain, each one with different functions (28) (Figure 6).

The box H/ACA domain is necessary for TR stability, nuclear location, processing and telomerase activity *in* vivo. The pseudoknot domain is also needed for telomerase to work properly, as well as the CR4/CR5 domain although this is not fundamental for TR stability (29). These two last referred regions interact independently with hTERT.

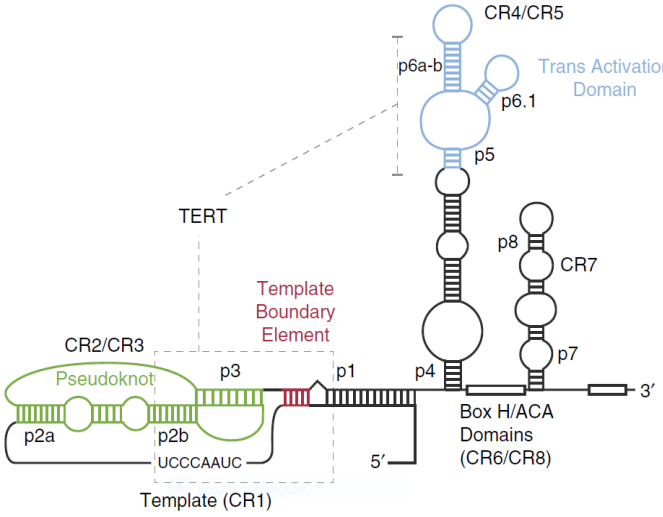

***Figure 6**. Proposed secondary structure of human telomerase RNA (hTR) subunits. The interaction of hTERT with hTR domains is indicated in grey dotted lines. Figure adapted from ref.* (90).

Catalytic subunit hTERT

hTERT is a quite large protein with four main elements: the telomerase essential N-terminal (TEN) domain, the telomerase RNA-binding (TRB) domain, the reverse transcriptase (RT) domain and the C-terminal extension (CTE) (30) (Figure 7).

The TEN domain contains the DAT (dissociates activities of telomerase) region, where mutations in this area stop telomere lengthening *in vivo* but do not affect the catalytic activity *in vitro* (31,32). TEN is therefore a key point to assemble telomerase to telomeres, as it has affinity for single-stranded telomeric DNA.

The TRB domain has some conserved RNA binding sequences one of which is required to place hTR inside the active site of hTERT, specifically the telomerase-specific T motif with the CR4/5 region of hTR.

The RT domain is considered the catalytic heart of the enzyme and it includes some conserved reverse transcriptase motifs (33).

The CTE domain, similarly to TEN domain, is indispensable for *in vivo* lengthening of telomeres but it is not a requisite for *in vitro* activity of telomerase (34).

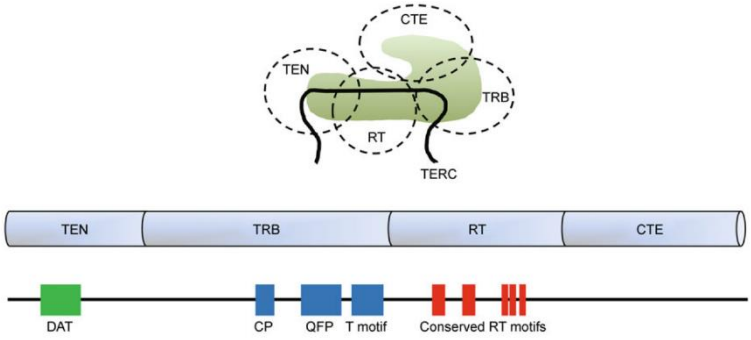

***Figure 7**. Structure of human telomerase reverse transcriptase (hTERT), the four key domains and some of their regions. Figure adapted from ref.* (30).

1.3.2. Elongation of telomeres by telomerase

Telomeres are known to exist in two different configurations. The “closed” state hides the 3’ overhang forming the T- and D- loops, preventing it from telomerase activity. The “open” conformation is the linear structure of telomeres, allowing the interaction with telomerase (35).

Since human telomerase is dimeric, it is able to extend two telomere ends in parallel, in order that sister chromatids can maintain the same telomere length (36).

Being in the open state, the lengthening of telomeres is accomplished within three steps (37,38):

- 1- Substrate recognition and binding: telomerase attaches to the terminal end of the telomere and the nucleotides from the 3' overhang are positioned in hTERT, aligning and hybridizing with the RNA template of hTR.
- 2- Elongation: a telomeric DNA repeat is synthesized by reverse transcriptase action, adding nucleotides complementary to the RNA template, so the overhang becomes longer.
- 3- Translocation or dissociation: telomerase is translocated to restart again the cycle, and when finished, it finally dissociates.

Lastly, the 5' end is completed by the conventional replication method with DNA polymerase.

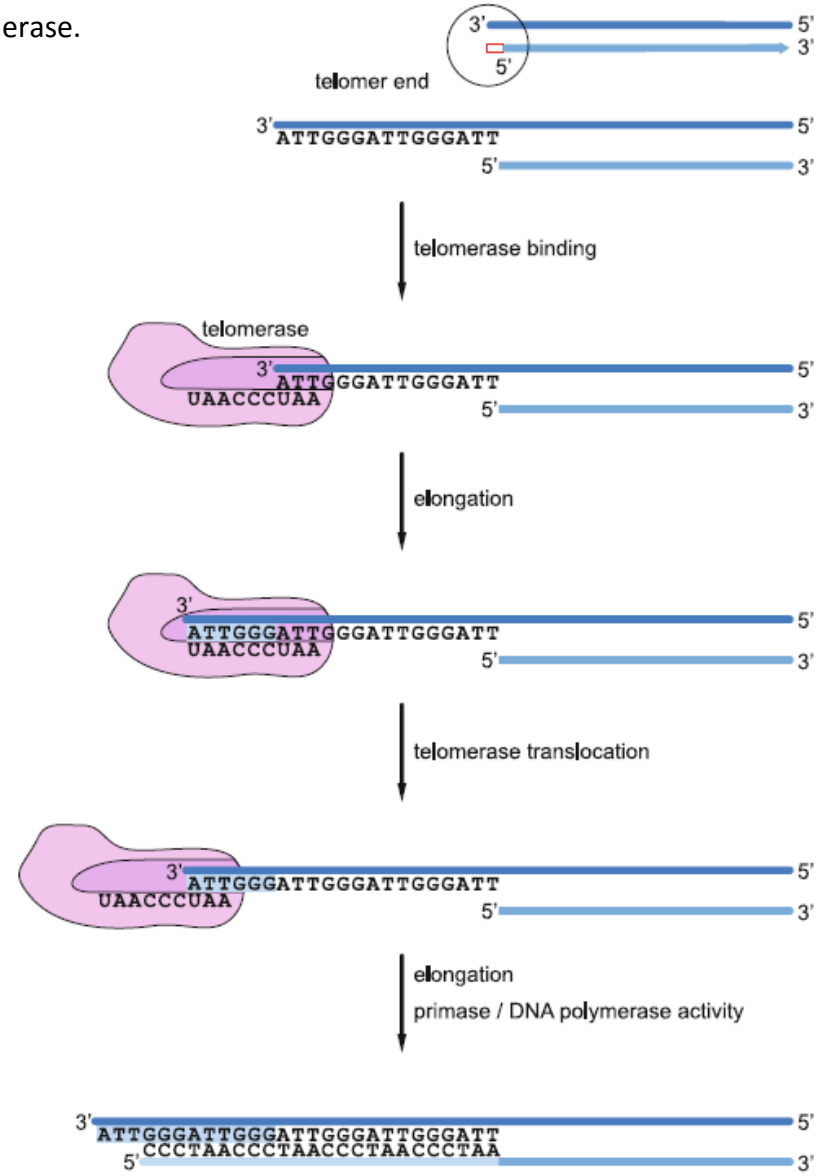

***Figure 8**. Telomerase at work solving the DNA end replication problem. The enzyme telomerase consists of a protein and a short stretch of RNA that is complementary to the sequence at the overhanging 3' end of telomeres. The telomerase activity is able to synthesize DNA from the RNA template whereupon the telomerase moves on, a step that is repeated several times. Then, the missing stretch can be filled in 5' → 3' direction. Figure from ref.* (91).

Telomerase is a highly regulated enzyme in normal human cells. During embryonic differentiation it is repressed in most somatic cells except from some tissues, such as activated lymphocytes, gametes and stem cells (39,40).

When normal mammalian somatic cells are cultured *in vitro*, they proliferate a limited number of times. The maximum possible number of divisions is known as the Hayflick limit (41). At that point, very short telomeres provoke a permanent growth arrest called replicative senescence or mortality stage 1 (M1) (42–44). If any cell cycle checkpoint gene as p53 is inactivated, cells can escape from senescence and continue dividing. If this occurs, telomeres are shortened even more and the cell reaches a second proliferative block or mortality stage 2 (M2) (45–47) where telomeres become dysfunctional and there is massive cell death. Very rare cells that evade M2 are able to activate telomerase leading to cellular immortalization (Figure 9).

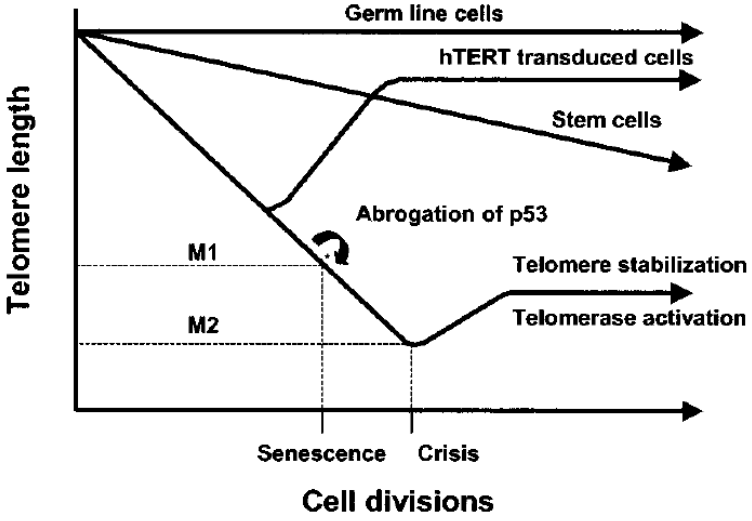

***Figure 9**. Two-step hypothesis of cellular senescence and immortalization. Unlike germ cells, in which telomere length is maintained by telomerase, most human somatic cells have lower levels of telomerase or are telomerase negative and experience telomere shortening with each cell division. Pluripotent stem cells are telomerase positive but do not maintain full telomere length. Telomere length shortens in stem cells at rates slower than that of telomerase-negative somatic cells. Critically shortened telomeres may signal cells to enter senescence at the Hayflick limit, or M1. This proliferative checkpoint can be overcome by inactivation of pRB/p16 or p53. Such cells continue to suffer telomere erosion and ultimately enter crisis, or M2, characterized by widespread cell death. Rare surviving cells acquire unlimited proliferative potential and stabilization of telomere length, almost universally by activation of telomerase. When cells are cultured in adequate conditions, ectopic expression of hTERT allows cells to bypass proliferation barriers and become immortal. Figure from ref.* (92).

2. AIMS

After research on telomeres and telomerase came to light to most science-unaware people by winning a Nobel prize, it caught my attention and became of my interest with the aim of learning a little bit more.

The objectives of this project are the followings:

- To review telomeres' structure and their functions.
- To analyse the structure of telomerase, the enzyme responsible for maintaining telomeres' length, and how it works.
- To study how telomeres and telomerase are involved in aging and age-related diseases.

3. MATERIALS AND METHODS

The methodology of this work has been based in a thorough bibliographic search and the subsequent synthesis of the articles found.

The first general idea of telomeres and telomerase was obtained from Biochemistry books from the library of the university, but then, deeply investigation was done through data bases on internet.

The main source of information has been **PubMed**, through the website www.ncbi.nlm.nih.gov/pubmed. Both full articles and reviews have been used, either searching general words as "structure of telomeres" or concrete papers using advanced research. I have used publications from a wide variety of journals such as *Cell* or *Science.*

4. RESULTS

4.1. Telomere loss with aging

The aging process is generally known by external visible evidences in humans such as skin wrinkling and spotting or hair greying. However, cellular aging is implicated behind these signs and it is related to telomere shortening throughout humans' life. Because of the end-replication problem and lack of telomerase activity in most human cells, telomere loss with aging is unavoidable. A review of telomere shortening in different human tissues was carried out (Table 1) (48).

Organs and tissues	Yearly reduction rate (bp/year)	Sample number	Age range (years)	Reported year and author
1. Peripheral blood	33	47	20–85†	1990 Hastie *et al.*
2. Epidermal cells	19.8	21	0–92	1991 Lindsey *et al.*
3. Fibroblasts	15	43	0–93	1992 Allsopp *et al.*
4. Peripheral lymphocytes	41	140	0–107	1993 Vaziri *et al.*
5. Peripheral lymphocytes (twins)	31	123	2–95	1994 Slagboom *et al.*
6. Endothelium	47–147	13	3.5–102	1995 Chang *et al.*
7. Large and small intestine	42	53	0–89	1996 Hiyama *et al.*
8. Esophageal mucosa	60	177	0–102	1999 Takubo *et al.*‡
9. Endothelium	28	51	1 m–80	1999 Okuda *et al.*
10. Arterial mediastinum	25	51	1 m–80	1999 Okuda *et al.*
11. Liver	55	94	0–101	2000 Takubo *et al.*‡
12. Colonic mucosa	59	129	0–97	2000 Nakamura *et al.*‡
13. Gastric mucosa	47	38	0–99	2000 Furugori *et al.*‡
14. $CD4^+$T Cells	35	121	0–94	2000 Son *et al.*
15. $CD8^+$T Cells	26	121	0–94	2000 Son *et al.*
16. $CD19^+$B Cells	19	121	0–94	2000 Son *et al.*
17. Renal cortex	29	24	0–88	2000 Melk *et al.*
18. Renal medulla	9 & 13	20	0–88	2000 Melk *et al.*
19. Liver	120	23	17–81	2000 Aikata *et al.*
20. Renal cortex	46	137	0–101	2002 Takubo *et al.*‡
21. Liver	60	191	0–104	2002 Takubo *et al.*‡
22. Cerebral cortex	NSD	137	0–104	2002 Takubo *et al.*‡
23. Cardiac muscle	NSD	168	0–104	2002 Takubo *et al.*‡
24. Spleen	29	30	0–102	2002 Takubo *et al.*‡
25. Thyroid	90	44	0–98	2002 Kammori *et al.*‡
26. Parathyroid gland	94	19	0–83	2002 Kammori *et al.*‡
27. Epidermal tissue	36	52	0–101	2002 Nakamura *et al.*‡
28. Lingual mucosa	30	48	0–101	2002 Nakamura *et al.*‡
29. Dental pulp	72†	100	16–70	2003 Takasaki *et al.*
30. Pancreas	36	69	0–100	2006 Ishii *et al.*‡
31. Epidermal tissue	9	100		2006 Sugimoto *et al.*
32. Dermal tissue	11	60		2006 Sugimoto *et al.*
33. Cerebral cortex	NSD	72	0–100	2007 Nakamura *et al.*‡
34. Cerebral white matter	NSD	72	0–100	2007 Nakamura *et al.*‡

†Approximate number; ‡Our Study. bp, base pair; m, month old; NSD, no significant difference.

***Table 1**. Yearly reduction rates of telomere length in human tissues.*

Mean telomere length of neonates for cerebral cortex and liver were 13.1 ± 1.1 and 13.7 ± 2.2 kbp respectively (49) and those for subjects less than 10 years old (neonates included) were 13.1 ± 1.8 and 13.6 ± 2.3 kbp, which do not almost vary. The respective values for centenarians were 13.1 ± 2.3 and 8.7 ± 1.4 kbp. The mean telomere length for centenarians in these and other tissues were not shorter than 6 kbp, so in that point was considered to be the mortality stage 1 (48).

All this data was obtained based on one unique tissue in several people but, when different tissues from one individual are observed, "it was suggested that when longer telomeres are shown in any particular organ in a given individual, the other organs will also have longer telomeres" (48).

4.2. Telomeres and age-related diseases

Apart from working as a biological clock, telomere shortening is also related to several age-related diseases to which elder people are more sensitive.

4.2.1. Cancer

When telomeres are not long enough, chromosomal instability is induced leading to cancer initiation (50). In addition, cancer cells show a high telomerase activity (51) despite normal somatic cells do not have this enzyme active. Approximately 85% - 90% of human cancers have detectable telomerase activity (39). The molecular mechanism for this activation is still unclear, but it is due to the necessity of telomere stabilization for tumour progression (52). Henceforth, some investigators came up with studies inhibiting telomerase activity in those kind of cancer, and the results showed cell death and tumour growth inhibition (53–56).

Trying to extend lifespan in mice, telomerase was activated by constitutive over-expression of TERT in different tissues. The result was a lifespan up to 10% longer compared to wild-type mice (57). On one hand, this increased life expectancy showed a low rate of age-related diseases but on the other hand, both induced and spontaneous

tumours were also induced in higher incidence, what caused mortality in the first year of life (57).

4.2.2. Cardiovascular diseases

Atherosclerosis and heart failure are aging-related diseases and frequently can cause the death. The areas of arterial wall with higher haemodynamic stress are more susceptible to atherosclerosis. This stress is believed to result in a more rapid cell turnover and shorter telomeres (58).

People with short telomeres are more probable to develop hypertension even being healthy, and once they are hypertensive, with shorter telomeres are more vulnerable to suffer from atherosclerosis (59).

4.2.3. Diabetes

Type 2 diabetes is significantly associated with short telomeres and it could be ascribed in part to the oxidative stress that suffer these patients (60,61). A study revealed that subjects with atherosclerosis and type 2 diabetes have shorter telomeres than those with only diabetes (62).

Type 2 diabetes is characterised by peripheral insulin resistance and β-cell dysfunction. It was seen that young adult mice with low telomerase activity expressed impaired glucose tolerance, which means that short telomeres can be responsible for dysfunctional replicative capacity of pancreatic β-cells (63).

In addition, short telomeres can predict all the causes of mortality in the patients with type 1 diabetes (64).

4.2.4. Immune system diseases

The immune system needs a great telomere maintenance as it is a very dynamic cellular system. The rapid expansion of clonal T- and B-cell populations is the key point to be a competent immune system, and short telomeres can cause defective immune responses in old people (65).

Telomere loss is considered one of the greatest factors affecting morbidity and mortality (66). The most harmful effect on the elderly is the decline in T-cell action despite both the innate and adaptive immune responses are debilitated in old people (67).

Moreover, people with short telomeres are eight times more likely to die of infectious diseases than people with long telomeres (68).

4.2.5. Dyskeratosis congenital

Some human diseases related to short telomeres are caused by genetic causes such as mutations in the DNA repair system or defective telomeres, and dyskeratosis congenital (DC) is one of them (69). If the mutations are in the RNA component of telomerase, it is autosomal dominant DC, while mutations in the gene encoding dyskerin protein are caused by X-linked DC (70).

Patients with DC show premature aging signs such as grey hair, alopecia, tooth loss, defective skin pigmentation, osteoporosis and deterioration of the immune system.

4.3. Impact of lifestyle factors on telomeres and aging

It is well known to everyone that some lifestyle factors may negatively affect human health, and accelerated telomere loss is one of these consequences.

4.3.1. Smoking

Smoking accelerates telomere shortening and moreover, it seems to be a relation between the number of smoked cigarettes and the speed of telomere loss, meaning that telomere shortening and smoking are dose-dependent (71).

A study was carried out in white blood cells of women and the results showed that the average rate of telomeric DNA loss was “25.7 – 27.7 base pairs” per year but, if a pack of cigarettes is smoked daily, 5 base pairs are additionally lost (72). As a consequence, the erosion of telomeres by daily smoking of one pack of cigarettes for a 40-year period is comparable to 7.4 years of life (72).

Some other authors have proposed that telomere length may predict the rate of aging by using it as a biomarker of the oxidative damage caused by smoking, although this oxidative stress can be prevented by antioxidant therapy (73).

4.3.2. Obesity

Since the waist circumference and BMI are related to high levels of reactive oxygen species in plasma and urine, obesity correlates with an increase of oxidative stress (74). In case of lean women, their telomeres are significantly longer than those in obese women of the same age group (72).

Given that accelerated telomere loss in obese people equate to 8.8 years of life, it seems that obesity has a worse impact on telomere length than smoking (75).

4.3.3. Environment

The exposure to harmful agents of the environment may affect telomeres. Telomere length in leukocytes was evaluated by some researchers both from traffic police officers and office workers (76). The pollution was measured by levels of benzene and toluene. In each age group, telomeres were shorter for traffic police officers than for office workers. Consistently, telomeres measured in lymphocytes of coke-oven workers were significantly shorter than the control subjects, as exposure to polycyclic aromatic hydrocarbons can damage DNA and cause genetic instability (77). In this case, telomere shortening did not correlate with subjects' age but with the number of years the workers had been exposed to damaging agents.

4.3.4. Stress

Glucocorticoid hormones are released by the adrenal gland because of stress, which reduce the levels of antioxidant molecules (78) and accelerates the loss of telomeric sequences (79). Women exposed to stress in their daily life showed higher oxidative pressure, less telomerase activity and shorter telomeres in peripheral blood mononuclear cells in comparison to women in the control group (80). More importantly, the difference in telomere length in these two groups was comparable to 10 years of

life. Therefore, women with elevated stress levels had more risk to suffer early age-related diseases.

The effect of stress on telomeres can also be seen in new-borns as their telomere length is shorter depending on the stress levels that the mother experienced during her pregnancy (81).

4.3.5. Diet

A study carried out in a group of women showed that dietary intake of polyunsaturated fatty acids is negatively associated with telomere length, while a dietary intake of fibre correlates positively (82). An increase in longevity has also been seen with a reduction in protein intake, as a reduction in the protein content by 40% in rats causes a 15% increase in their lifespans (83,84).

As outlined above, oxidative stress leads to telomere shortening, so the dietary intake of antioxidants will reduce the rate of telomere loss. This was demonstrated in a study where a group of subjects took antioxidant omega-3 fatty acids, and their rate of telomere shortening was lower compared to the control group (85).

Moreover, not only what we eat can affect telomere length but also the quantity of food we consume. Dietary restriction in animals has shown to reduce oxidative burden, DNA damage and reduce growth rate (83). Animals were kept in a biologically younger state and their lifespan was increased by up to 66% (83).

4.3.6. Exercise

Physical activity, together with a healthy diet, is recommended to have a better health as it reduces fat and fastens elimination of waste products. It turns into a reduced oxidative stress and preventing deterioration of telomeres.

Exercise was shown to be associated with high telomerase activity and repression of some apoptosis proteins such as p53 and p16 in mice (86). Furthermore, leukocytes from athletes had more telomerase activity in comparison to non-athletes (86).

5. CONCLUSIONS AND DISCUSSION

Telomeres are a small but very important part of DNA as they are responsible for keeping the integrity of the genome of all living organisms. In case of mammals, telomerase is essential to solve the end-replication problem and to maintain telomere length for specie reproduction. Specifically, in humans, telomeres and telomerase have complex structures and this enzyme is well regulated and controlled.

Both cell and physical aging that can be observed on the surface are caused by telomere shortening, and this also limits individual lifespan.

Different genetic mutations in genes associated with telomeres and telomerase can cause unavoidable illnesses such as dyskeratosis congenital. Howbeit, cells have mechanisms to prevent DNA from damage despite not having genetic problem, such as senescence or apoptosis, but when telomeres are not long enough we are still more prone to suffer diseases as diabetes and cardiovascular diseases, among others. Moreover, when a cell escapes from mortality stage 2 (M2), telomerase can be activated and the cell becomes immortal, possibly leading to cancer.

Various anticancer therapies targeting telomerase are being studied, and also other therapies where telomerase activity is stimulated, although the latter has a high risk of developing undesirable tumours. Much research has to be done yet, but it is clear that this a field with great interest and possibilities for therapy.

However, since there are many external factors that can accelerate telomere loss, it is in everybody's hands to have a healthy lifestyle. Exercising, eating healthy and less quantity, not smoking and reducing stress can contribute to reduce telomere shortening and thus avoid premature aging or age-related diseases.

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