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Telomere and Telomerase: brief review of a history initiated by Hermann Müller and Barbara McClintock

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SUMMARY

What is the nature of the biological clock that determines the cells aging? What is the way to explain the genesis of diseases associated in aging? The above are only some of the questions whose answer could be found within the modus operandi frame of the telomere-telomerase pair, which had become in objective of study for a number of science men and women since the pioneers Hermann Müller and Barbara McClintock, until the most recent Jack Szostak, Elizabeth Blackburn and Carol Greider, among others.

Keywords: *Biological locks; Research personnel; History.*

Telómeros y Telomerasa: Breve recuento de una historia iniciada por Hermann Müller y Bárbara McClintock

RESUMEN

¿Cuál es la naturaleza del reloj biológico que determina el envejecimiento de las células? ¿De qué manera se explica la génesis de las enfermedades asociadas con el envejecimiento? Las anteriores son sólo algunas de las preguntas cuya respuesta puede ser hallada en el marco del *modus operandi* de la dupla telómeros-telomerasa, convertida en objeto de estudio para un sinnúmero de hombres y mujeres de ciencia, desde los pioneros Hermann Müller y Bárbara McClintock hasta los más recientes Jack Szostak, Elizabeth Blackburn y Carol Greider, entre otros.

Palabras clave: *Relojes biológicos; Investigadores; Historia.*

In 1938, when the young North American geneticist Hermann J. Müller used to work with flies of the species Drosophila melanogaster, exposed to X rays at the Edinburgh Animal Genetics Institute (United Kingdom), he did not foresee the implications that his findings would have in the molecular biology and genetics in the following 70 years. He had just observed that the ends of the irradiated chromosomes, different from the other genome, did not present alterations such as deletions or inversions, thanks to the presence of a protective cap that himself called «terminal gene» and afterwards «telomere», from the greek terms «telos» (end) and «meros» (part)[1].

Two years after, Barbara McClintock, respected investigator from the University of Missouri (Columbia, USA), who was dedicated to the study of corn genetics (Zea mays), described how the rupture of the chromosomes resulted in adhesion and fusion of their ends, with the consequent formation of dicentric chromosomes. She demonstrated that regardless of this damage, the ends could be restored thanks to the acquisition of new telomere. According to her conclusions, telomeres play a crucial role in the integrity of the chromosomes, since they prevent the appearance of «rupture-fusion-bridge» cycles which are catastrophic for the cellular survival[2].

The term «telomere» coined by Müller had apparently a premonitory character, though everything pointed to an immediate promissory future. The predominant skepticism of that age in the genetic field made that research on the importance of the ends of the chromosomes in replication and integrity of the cell steeply stopped. It was only reinitiated 30 years after, when the mechanisms subjacent to the replication of the deoxyribonucleic acid (DNA) were revealed, fact in which James Watson worked (the same that described the double helicoidal structure of the DNA). He identified the «problem of the terminal replication» consisting on the incapacity of the cells to completely replicate the linear ends of the DNA. Watson postulated that, because of the special characteristics of

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the synthesis of the left behind chain of nucleic acid, which cause that the DNA polymerase can not completely replicate its end 3', the telomere and therefore the chromosomes were shortened. In addition he postulated the existence of a protective mechanism to prevent the chromosomal shortening[3].

By the same age, Alexsei Matveevich Olovnikov, an unknown russian scientist, found the link between the problem of the end replication -named by Watson-, and the cellular senescence at the same time described by Leonard Hayflick. According to Hayflick (1961), senescence corresponded to a status of detention of the proliferation to which the human somatic cells entered with signs of biochemical and morphologic alterations as a result of having exceeded their replicative lifespan[4].

For Olovnikov, the problem of the terminal replication was the cause of the progressive shortening of the telomere the one that as well, acted as an internal clock to determine the number of divisions that the cell could experience throughout its life and therefore to control the process of aging[5,6]. The proposed model demonstrated to have an impressive precision. At the present time, it is not only accepted that the telomere shortening is the main cause of the cellular senescence, but that it is actually the molecular clock that counts the number of cycles that the cell can support[7].

Like James Watson, Aleksei Oloknikov believed that the cell had a strategy to maintain its telomeric length during the normal replication of the DNA. It did not take long time to discover that such strategy had own name. It was telomerase, reverse transcriptase enzyme in whose discovery Elizabeth Blackburn, investigator from Tasmania, played a crucial role.

Blackburn y Joseph Gall, famous biologists of the University of Yale, initiated in 1975 their work with *Tetrahymena thermophila*, ciliated protozoa that unlike other eukaryotic organisms, has in addition of a micronucleus with normal chromosomes, a macronucleus in which fragmented chromosomes are found in multiple small segments of DNA, having the same coding gene for ribosomal RNA.

It was then possible to determine the sequence of the extra-chromosomal DNA in whose ends they appeared multiple repetitions of the hexanucleotid CCCCAA those that also were found in the micronucleus[8]. Nevertheless the finding brought with him doubts: it was perhaps a rare property of an extra chromosomal gene pertaining to a ciliated organism that in addition did not form part of the main eukariota evolutive line? Or it was perhaps an authentic telomeric sequence?

The question was without response until Blackburn solved to be associated with Jack Szostak, molecular and geneticist biologist of the Harvard Medical School, in a certain joint venture that finally lead to the discovery of the telomeric sequences and the enzyme that synthesizes them. The work of Szostak, unfruitful until that moment, consisted of constructing artificial chromosomes that allowed cloning big groups of human genes in a yeast single linear DNA molecule as a vector. The results were discouraging, inasmuch as the chromosomes thus generated were unstable and they did not replicate, due possibly to the telomere deficiency. But when Szostak decided to prove if the repetitive sequences discovered by Blackburn and Gall could act like telomere in their experiment with *Saccharomyces cerevisiae*, the results were forceful: the yeast linear plasmids -assembled from the vector and of the telomeric ends of the *T. thermophila* DNA- were replicated in a stable way. Blackburn and Szostak concluded that if the yeast was able to recognize and use such ends pertaining to a so evolutionary distant organism, this fact constituted reasonable evidence regarding the high evolutionary conservation of the telomeric replication mechanisms. This finding was linked to other not less important: one when by means of maps of restriction and tests of hybridization they analized the ends of *T. thermopila* DNA as well as the yeast fragments that were supposed to act as telomere, and it was found that the telomeric sequences were common for both organisms[9,10]. On the other hand, the replicated plasmids had a greater length, understood if it were considered that the strategy of the yeast cells perhaps consisted of adding new repetitive sequences to the ends of the repetitive sequences of *T. thermophila*. Thus, Blackburn and Szostak suggested that the telomere elongation was due to the activity of an unknown enzyme that synthesized telomere, later called telomerase[11].

Then Blackburn and Szostak had created not only the first known functional test for telomere, but also laid the foundations for the later construction of the first yeast artificial chromosomes, the famous YACs[12], used afterwards in the development of the Human Genome Project, for cloning great human DNA segments, that could be sequenced later[13,14]. Without still counting on a satisfactory explanation to clarify the true reason for

which the replicated plasmids in their work with Szostak had a greater length, Elizabeth Blackburn and her student Carol Greider they later undertook years in the University of California the memorable work that took them to propose the existence of an enzymatic activity to which they did not doubt in calling «telomere terminal transferase», same telomerase. They used extracts of cells of *T. thermophila* and primers constituted per identical sequences to the present ones in the telomere yeast and *T. thermophila* cells. Their results demonstrated the synthesis of novo of tandem TTGGGG repetitions, which were added to the oligonucleotid primers, thanks to the activity of the new enzyme described by them[15].

The results obtained by Blackburn and her collaborators marked a landmark in the investigation of the biology of the telomere, because they explained the apparent contradiction between two verified facts: first, the progressive shortening of the telomere during each cell division and second, its replication, process that happens in independent way to the one of the rest of the chromosomal DNA. While the non-telomeric DNA uses for its replication the DNA polymerase enzyme, the DNA of the telomere uses a RNA template that adds new telomeric repetitions. This forms an integral part of the telomerase molecule and it is in fact the mold on which the copy of the telomere is generated, in a process denominated reversed transcription. In addition to RNA (TR) subunit, telomerase contains a catalytic subunit (TERT). Absent or little expressed in the somatic cells, telomerase is found in embryonic and germinative cells (oogonia and spermatogonia), as well as in most of transformed cells (immortalized cellular lines and cancerous cells), where resists the problem of absence of replication at the telomeric ends[16,17].

At the present time it is accepted that telomere length and telomerase expression varies considerably with age and cellular type, facts that justified their use as biomarkers to evaluate history and replicative potential between tissues and age groups. Thus, in most of these, it has been possible to identify an approximated pattern of telomeric dynamics and telomerase expression. On the matter, it is interesting to highlight that in the genesis of certain pathologies like Alzheimer's disease, coronary disease and cancer, among others[16-22], the difference between biological age (predicted based on the telomeric length) and chronological age plays a key role. On the other hand, the expression of telomerase has been associated through solid evidence with oncogenesis and cellular immortalizations, powerful reasons to propose it for diagnosis and as therapeutic target in the cancer treatment[23].

In normal cells, telomere shortening during cell division acts as a tumor-suppressor mechanism that «forces» to that the cells leave the cellular cycle and enter an irreversible state of senescence[24], where they cease to divide and finally die. However, in the process of malignant transformation, the senescence is also an important risk factor[25,26]: It exists ample evidence that demonstrates that it can be eluded by cell with short telomere that have begun to express telomerase. In this case, «the fugitive» cell acquires a new status, because it is transformed not only into malignant, but in addition in immortal, thanks to the stabilizing action that the enzyme exerts on telomere[27]. But senescence is not only a state of that prevents the cellular growth: it also involves changes in the expression of certain genes and confers resistance to apoptosis[25,28].

This explains why senescent cells can be accumulated in tissues and contributes as much to the aging process, and to the genesis of associated diseases as well. When this happens, there are hyperplasic and pre-malign pathological alterations which favor the theory that cancer development is depending on age, due possibly to a sum of multiple mutations[29,30]. Therefore, senescence exerts a protective effect against cancer to early ages, whereas to greater ages it promotes the typical phenotype of aging[25].

It is not surprising that tumor-suppressor mechanisms like telomeric shortening and therefore senescence, can stimulate cancer development in the delayed stages of life. This apparent paradox is the one that has taken in the last decade to that many men and women of science deeply investigate the telomere-telomerase pair, with the purpose of discovering the until now hidden key of immortality or well to reveal the secret of the malignity that so jealously keeps.

REFERENCES

1. Müller HJ. The remaking of chromosomes. *Collecting Net* 1938; *13:* 181-198.
2. McClintock B. The stability of broken ends of chromosomes in Zea mays. *Genetics* 1941; *26:* 234-282.
3. Watson JD. Origin of concatemeric T7 DNA. *Nat New Biol* 1972; *239:* 197-201.
4. Hayflick L Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961; *25:* 585-621.
5. Olovnikov AM. Principle of marginotomy in template synthesis of polynucleotides. *Dokl Akad Nauk SSSR* 1971; *201:* 1496-1499.
6. Olovnikov AM. A theory of marginotomy. The incomplete

copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol* 1973; *41:* 181-190.
7. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. *Curr Opin Genet Dev* 2001; *11:* 98-103.
8. Yao MC, Blackburn E, Gall J. Tandemly repeated C-C-C-C-A-A hexanucleotide of Tetrahymena rDNA is present elsewhere in the genome and may be related to the alteration of the somatic genome. *J Cell Biol* 1981; *90:* 515-520.
9. Szostak JW, Blackburn EH. Cloning yeast telomeres on linear plasmid vectors. *Cell* 1982; 29: 245-255.
10. Birmingham K. Elizabeth Blackburn. *Nature Med* 2001; *7:* 520.
11. Shampay J, Szostak JW, Blackburn EH. DNA sequences of telomeres maintained in yeast. *Nature* 1984; *310:* 154-157.
12. Brown WRA. Molecular cloning of human telomeres in yeast. *Nature* 1989; *338:* 774-776.
13. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, *et al.* The sequence of the human genome. *Science* 2001; *291:* 1304-1351.
14. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, *et al.* Initial sequencing and analysis of the human genome. *Nature* 2001; *409:* 860-921.
15. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* 1985; *43:* 405-413.
16. Wai LK. Telomeres, telomerase and tumorigenesis. A review. *MedGenMed* 2004; 6: 19.
17. Polychronopoulou S, Koutroumba P. Telomere length and telomerase activity: variations with advancing age and potential role in childhood malignancies. *J Pediatr Hematol Oncol* 2004; *26:* 342-350.
18. Bischoff C, Petersen HC, Graakjaer J, Andersen-Ranberg K, Vaupel JW, Bohr VA, Kølvraa S, *et al.* No association between telomere length and survival among the elderly and oldest old. *Epidemiology* 2006, *17:* 190-194.
19. Jenkins EC, Velinov MT, Ye L, Gu H, Li S, Jenkins Jr.EC, *et al.* Telomere shortening in T lymphocytes of older individuals with Down syndrome and dementia. *Neurobiol Aging* 2006; *27:* 941-945.
20. Tristano A, Chollet ME, Willson ML, Adjounian H, Correa MF, Borges A. Actividad de la telomerasa en leucocitos de sangre periférica de pacientes con hipertensión arterial esencial. *Med Clin (Barc)* 2003; *120:* 365-369.
21. Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol* 2003; *23:* 842-846.
22. Wu X, Amos CI, Zhu Y, Zhao W, Grossman BH, Shay JW, *et al.* Telomere dysfunction: A potential cancer predisposition factor. *J Natl Cancer Inst* 2003; *95:* 1211-1218.
23. Mu J, Wei LX. Telomere and telomerase in oncology. *Cell Res* 2002; *12:* 1-7.
24. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C *et al.* A biomarker that identifies senescent human cells in culture and in aging skin *in vitro*. *Proc Natl Acad Sci USA* 1995; *92:* 9363-9367.
25. Campisi J. Aging, tumor suppression and cancer: High-wire act! *Mech Ageing Dev* 2005; *126:* 51-58.
26. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. *Curr Opin Genet Dev* 2001; *11:* 98-103.
27. Blasco MA. Mammalian telomeres and telomerase: why they matter for cancer and aging. *Eur J Cell Biol* 2003; *82:* 441-446.
28. Krtolica A, Campisi J. Cancer and aging: a model for the cancer promoting effects of the aging stroma. *Int J Biochem Cell Biol* 2002; *34:* 1401-1414.
29. Campisi J. Cancer and ageing: rival demons? *Nature Rev Cancer* 2003; *3:* 339-349.
30. Campisi J. Cellular senescence and apoptosis: how cellular responses might influence ageing phenotypes. *Exp Gerontol* 2003; *38:* 5-11.