REJUVENATION RESEARCH
Volume 7, Number 2, 2004

Telomeres and Telomerase: A Modern Fountain of Youth?

JOÃO PEDRO DE MAGALHÃES and OLIVIER TOUSSAINT

ABSTRACT

Since ageing is a universal human feature, it is not surprising that, from the Babylonian epic of Gilgamesh to Ponce de Leon seeking the "Fountain of Youth," countless people have dreamed of finding a way to avoid ageing, to no avail. Yet the search continues. In this review, we present one of the latest candidates: the enzyme telomerase, capable of elongating the tips of chromosomes, the telomeres. Research into the causes of cellular ageing established the telomeres as the molecular clock that counts the number of times cells divide and triggers cellular senescence. Herein, we review arguments both in favor and against the use of telomerase as an anti-ageing therapy. The importance of the telomeres in cellular ageing, the low or non-existent levels of telomerase activity in human tissues, and the ability of telomerase to immortalize human cells suggest that telomerase can be used as an anti-ageing therapy. On the other hand, recent experiments in mice have raised doubts whether telomerase affects organismal ageing. Results from human cells expressing telomerase have also suggested telomerase may promote tumorigenesis. We conclude that, though telomerase may be used in regenerative medicine and to treat specific diseases, it is unlikely to become a source of anti-ageing therapies.

INTRODUCTION

AGEING is a universal human feature. It is not surprising then that since the dawn of civilization many have sought to avoid it. From the Babylonian epic of Gilgamesh, to Ponce de Leon seeking the "Fountain of Youth," countless people have dreamed of finding a way to avoid ageing, to no avail. In modern times, despite major advances in technology, there continues to be no proven way of delaying human ageing.[1] Yet the search continues. In this review, we present one of the latest candidates: the enzyme telomerase, capable of elongating the tips of chromosomes, the telomeres. We first introduce the research that led to the suggestion telomeres may be involved in ageing and later discuss whether telomerase may or not be a likely candidate for anti-ageing research.

Research Unit on Cellular Biology, Department of Biology, University of Namur (FUNDP), Namur, Belgium.

CELLULAR AGEING REGULATION BY THE TELOMERES

In 1961, Leonard Hayflick and Paul Moorhead discovered that human cells derived from embryonic tissues can only divide a finite number of times in culture.[2] They noticed that cell cultures stopped dividing after an average of fifty cumulative population doublings (CPDs). This phenomenon is known as Hayflick's limit, phase III phenomenon, or, as it will be called herein, replicative senescence (RS). RS demonstrates how age changes may have a cellular origin and so understanding the mechanisms behind cellular ageing could help explain human ageing.

Hayflick and Moorhead worked with fibroblasts, a cell type found in connective tissue, but RS has been described in other cell types: keratinocytes, endothelial cells, lymphocytes, chondrocytes, etc. In addition, RS occurs in cells derived from either embryonic tissues or from adults of all ages. RS is also witnessed in cells taken from many animals, including mice, chickens, and Galapagos tortoise.[3] In fact, early studies suggested a relation between the number of CPDs cells undergo in culture and the longevity of the species from which the cells were derived. For example, cells from the Galapagos tortoise, which can live over a century, divided about 110 times,[4] while cells from the short-lived mouse divided roughly 15 times.[5,6] In addition, cells taken from patients with progeroid syndromes—diseases resembling accelerated ageing such as Werner's syndrome (WS)—endure far less CPDs than normal cells.[7] Exceptions exist, and certain cell lines never reach RS. As will be detailed ahead, these are said to be "immortal" and include embryonic stem cells and most cell lines derived from tumours, such as HeLa cells.[3]

The difference between "mortal" and "immortal" cells appears to lie in the telomeres: non-coding regions at the tips of chromosomes composed, in vertebrates, of repeated sequences of TTAGGG.[8] Telomeres appear to form duplex loops, called t-loops, that stabilize or cap the telomeres. Initially, it was shown that telomere shortening occurs as cells divide in culture.[9] A complex machinery maintains telomere length and structure, of which a pivotal player is telomerase. Telomerase is a reverse-transcriptase enzyme that elongates the telomeres,[10] thus counteracting the normal telomere erosion. It has two components: an RNA component[11] and a catalytic subunit.[12] Previously, an association between telomere shortening, telomerase, and the immortality of tumour cell lines was already apparent.[13] Yet the definitive breakthrough came when it was shown that expression of the catalytic subunit of human telomerase (hTERT) in both retinal pigment epithelial cells and foreskin fibroblasts avoids RS.[14] Human cells immortalized with hTERT divide vigorously, do not display biomarkers of senescence, and do not show signs of transformation.[15,16] Even expression of hTERT in old cells appears to reverse the loss of function characteristic of senescent cells.[17] It appears that ectopic hTERT expression is sufficient to restore telomerase activity in human cells[18] and that telomere length, not hTERT expression, is the key in avoiding RS.[19]

All known immortal cell lines must stabilize their telomeres.[20] Tumour development, in particular, is dependent on telomere stabilization, normally by telomerase.[21] In contrast, telomerase inhibition can induce senescence in cancer cells.[22] Defects in telomere replication have also been shown to trigger senescence in unicellular organisms such as yeast[23] and the protozoan *Tetrahymena*.[24] Telomere shortening is now considered the main causal mechanism of RS and telomere length appears to be the molecular clock that counts the CPDs cells endure and triggers RS.[25]

TELOMERASE AS AN ANTI-AGEING THERAPY

Most, not all, human somatic tissues have no detectable telomerase activity.[26] For example, in the bone marrow, hematopoietic cells express telomerase. Telomerase activity is higher in primitive progenitor cells and then down-regulated during proliferation and differentiation.[27] Other reports associate, normally low, levels of telomerase activity with human stem cells.[28] On the other hand, human embryonic cells and adult germ cells have been found to express hTERT.[29] Since normal somatic human

tissues have low or no telomerase activity, it is not surprising that telomere shortening has been reported *in vivo*.[30–32]

hTERT expression immortalizes most, though probably not all,[33,34] human cell types.[14] Even so, the principle is that if telomerase can prevent RS, it may also prevent cellular ageing *in vivo* and serve as an anti-ageing therapy by increasing the capacity for renewal. One study found that the telomeric repair efficiency is lower in cells from an old than in cells from a young donor; and a slightly lower efficiency was also reported in WS cells.[35] It has been previously reported that cells from WS patients have a higher rate of telomere shortening.[36] In addition, a recent study found a correlation between telomere length and mortality in people over 60 years of age.[37] As such, telomere dysfunction may play a role in age-related debilitation.

The importance of the telomeres in RS, the low or non-existent levels of telomerase activity in human tissues, and the ability of telomerase to immortalize human cells led to the suggestion that telomerase will be used as an anti-ageing therapy.[38,39]

RELATION BETWEEN REPLICATIVE SENESCENCE AND AGEING

It is known that cells from older donors have a slower proliferative capacity.[3,40] This effect, known as the latent period, appears to occur because fewer cells are in the replication cycle, not because they take longer to divide.[41] Therefore, changes occur with age at a cellular level. In some tissues, such as the immune system, decreased proliferative ability may play a role in age-related degeneration.[42] Even if RS is not a faithful model of changes occurring *in vivo*,[43] if similar mechanisms operate to limit cell function then RS may yield insights into ageing. For instance, a small percentage of senescent cells may interfere with tissue homeostasis and function.[44]

Although it is clear that human ageing has, at least in part, a cellular origin, the connection between ageing and RS is not obvious. At least *post partum*, there is no correlation between the number of CPDs cells can endure and the age of the donor.[45] Studies in centenarians failed to find differences in the CPDs cells from centenarians could endure.[46] In addition, they raised doubts on whether telomere shortening occurs *in vivo* and whether senescence-associated genes *in vitro* are also differentially expressed *in vivo*.[47] In fact, gene expression patterns show differences between *in vitro* senescent cells and cells from old donors.[48] In addition, some types of rat cells have also been claimed as capable of evading RS.[49,50]

The relation between a species' longevity and the CPDs its cells can endure *in vitro* may also be unrelated to ageing. Optimal culture conditions vary from species to species. For instance, O_2 partial pressure can affect cellular proliferation and recent results show that O_2 limits the replicative capacity of murine fibroblasts.[51] These results show that comparisons between different species may be biased due to inter-species differences in O_2 sensitivity; instead of showing maximum cellular proliferate capacity, these results show O_2 sensitivity.[52] In addition, since there is a positive correlation between body size and longevity,[53] perhaps cells taken from long-lived animals endure more CPDs because of the difference in size, not due to the difference in longevity.

TELOMERES AND ORGANISMAL AGEING

Telomerase expression has been found in lobsters and trout, two species in which ageing remains undetected.[54,55] On the other hand, in the frog *Xenopus laevis*, an animal with a slow rate of ageing,[56] not only a great variation in telomere length exists,[57] but telomere length can diminish from parents to offspring, despite telomerase activity in germ cells, with no detectable consequences.[58] Chicken somatic tissues express telomerase,[59] but, overall, our knowledge of telomere biology is limited regarding other species.[60]

No connection exists between mean telomere length and mammalian ageing. Of all studied primates, humans appear to have the shortest telomeres and the longest lifespan.[61] Mice also have long telomeres and feature high telomerase activity in many organs, in contrast to humans.[62] Interestingly, inbred mice have longer

telomeres than wild mice, suggesting telomere length does not affect organismal longevity in mice.[63] Therefore, telomere length and/or telomerase activity do not explain why humans age slower than other primates and mice.

Dyskeratosis congenita is an inherited disease involving skin and bone marrow failure.[64] It is caused by a mutation in the *DKC1* gene. Intriguingly, the protein encoded by *DKC1*, dyskerin, is a component of telomerase. Mutations in the RNA component of telomerase are associated with the autosomal dominant form of dyskeratosis congenita.[65] Families with this form of the disease are more severely affected in later generations, suggesting telomere shortening mechanisms are involved. Features of dyskeratosis congenita include bone marrow failure, which is the most usual cause of death, abnormal skin pigmentation, leukoplakia, and nail dystrophy.[66]

As judged from the phenotype of dyskeratosis congenita and the telomerase knockout mouse (see below), telomeres are crucial in rapidly proliferating tissues but it is unclear whether telomere shortening is involved in human ageing. It is possible, however, that telomere shortening is involved in age-related deterioration. Despite having active telomerase, the telomeres of lymphocytes shorten with age.[67] A decline in telomerase activity was also found in blood mononuclear cells with age.[68] Though mean telomere length at birth does not correlate with longevity in birds, telomere shortening in erythrocytes inversely correlates with bird longevity. Telomere shortening in a variety of tissues also correlates, though to a lesser extent, with mammalian longevity.[69,70] In fact, a correlation between erythrocyte longevity and organismal longevity was previously shown, suggesting a decrease in the number of required cell divisions in long-lived animals.[6] It is, of course, impossible to tell whether increased telomere shortening is a cause rather than a sign of pathology and age-related debilitation.

Mice lacking telomerase were viable up to six generations. Telomeres gradually shortened leading to a number of pathologies, most notably affecting highly proliferative tissues, and cells from animals of generation four displayed aneuploidy and other chromosomal aberrations. Knocking out telomerase in mice through deletion of its RNA component from the germline, while not preventing cancer,[71,72] appears to increase cancer resistance[73,74]; alternative telomere-lengthening mechanisms are likely operating to stabilize the telomeres in these cancer cells. On the other hand, telomerase overexpression in mice promoted cancer development but did not delay ageing or promote longevity.[75,76] Of course mice and humans may feature different mechanisms of ageing, but these results show that, at least in mice, telomerase does not delay ageing.

TELOMERASE ALTERS THE NORMAL CELLULAR FUNCTIONS

Previously, experimental evidence raised questions on whether telomerase could help tumorigenesis.[77,78] Namely, telomerase stabilizes the telomeres which promotes tumorigenesis.[21,22,79] In addition, some reports suggest telomerase favours tumorigenesis by a telomere length-independent mechanism.[80] For example, a recent study found that hTERT expression in HDFs leads to an upregulation of epiregulin, a potent growth factor involved in tumorigenesis.[81] Another recent study found that telomerase modulates the expression of growth-controlling genes to enhance cellular proliferation,[82] and thus hTERT-immortalized cells may not be functionally equivalent to normal cells. In addition, recent results demonstrate that hTERT-immortalized cell cultures accumulate changes as they proliferate, suggesting caution in the use of such cell lines for tissue engineering.[83] Taken together, these results suggest that telomerase activity promotes tumorigenesis and so using hTERT for therapeutic purposes must be approached with great caution.

DISCUSSION

The connection between the telomere signalling pathways and cancer is obvious.[84] In fact, telomerase activation has been associated with skin malignancy as a result of exposure to UV.[85] Telomere shortening is most likely a tu-

mour suppressor mechanism. Telomerase-negative mice are normal up to four generations,[74] and telomerase overexpression does not alter ageing in mice.[76] On the other hand, telomerase dysfunction in humans causes dyskeratosis congenita.[65] It is clear telomere dysfunction is pivotal in RS[86] and telomerase important in cellular proliferation, but there is no evidence that the telomeres are a causal mechanism in mammalian ageing.

As with replicative potential, telomere length *in vivo* is very heterogeneous.[87] Telomere shortening *in vivo* has been reported in skin cells,[31] blood,[68] and colon mucosa.[30] Other studies found weak correlations between donor age and telomere length,[32] while some studies found no correlation.[47,87,88] Moreover, long telomeres have been found in cells from centenarians.[89] Taken as a whole, these results indicate that telomere length varies widely amongst individuals and between different tissues. Although telomere shortening appears to occur in some tissues *in vivo*, there is little evidence linking telomere shortening to ageing. One hypothesis is that increased telomere shortening *in vivo* is associated with age-related pathologies because telomere shortening is a biomarker of DNA damage.[90] If so, then telomere shortening witnessed *in vivo* would be an effect rather than a cause of pathology.

As mentioned before, the relation between RS and organismal ageing is unproven. Cellular immortality just means a cell population can divide indefinitely but it does not mean that the functional capacity and differentiation of cells is preserved. In fact, many non-dividing cells are essential to the organism. Thus, whether telomere shortening plays a role in human ageing is debatable. Not only is it unproven that telomerase can be used as an anti-ageing therapy but some evidence suggests that hTERT transient expression can occur in human cell lines when necessary for regeneration,[91] and there is little evidence to suggest that further hTERT expression is necessary in human tissues.[92,93] Importantly, telomerase may alter the normal cellular functions and promote cancer. One possibility is using a transient telomerase activation in certain diseases—dyskeratosis congenita being the most obvious example—or cell lines with telomerase expression stringently controlled.[94] In regenerative medicine, telomerase expression may be necessary[95] and may be useful to treat a number of pathologies. For instance, cardiac muscle regeneration may be fostered by telomerase expression.[96]

In conclusion, telomerase is a dubious candidate for Fountain of Youth: though it may be used in regenerative medicine or to treat specific diseases (e.g. dyskeratosis congenita or even WS), we think telomerase is unlikely to become a source of anti-ageing therapies.

ACKNOWLEDGMENTS

J.P.M. is funded by the Fundação para a Ciência e a Tecnologia, Portugal, and O.T. is a Research Associate of the Fonds National de la Recherche Scientifique, Belgium. J.P.M. thanks the European Social Fund, III Quadro Comunitário de Apoio. O.T. thanks the European Union, 5th Framework Programme, for the Quality of Life, R&D, Functionage (QLK6-CT-2001-00310) and Protage (QLK6-CT-1999-02193) Projects and the Craft CELLAGE (CRAFT' 1999-71628) Project. O.T. also thanks the Région Wallonne for the Modelage and Arrayage (RW- FSE EPH3310300R0472/215316) Projects.

REFERENCES

1. Olshansky SJ, Hayflick L, Carnes BA. No truth to the fountain of youth. Sci Am 2002;286:92–95.
2. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585–621.
3. Hayflick L. How and Why We Age. New York: Ballantine Books, 1994.
4. Goldstein S. Aging *in vitro*. Growth of cultured cells from the Galapagos tortoise. Exp Cell Res 1974;83: 297–302.
5. Stanley JF, Pye D, MacGregor A. Comparison of doubling numbers attained by cultured animal cells with life span of species. Nature 1975;255:158–159.
6. Rohme D. Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts *in vitro* and erythrocytes *in vivo*. Proc Natl Acad Sci U S A 1981;78:5009–5013.
7. Salk D, Bryant E, Au K, Hoehn H, Martin GM. Systematic growth studies, cocultivation, and cell hybridization studies of Werner syndrome cultured skin fibroblasts. Hum Genet 1981;58:310–316.

8. Meyne J, Ratliff RL, Moyzis RK. Conservation of the human telomere sequence (TTAGGG)n among vertebrates. Proc Natl Acad Sci U S A 1989;86:7049–7053.
9. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458–460.
10. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985;43:405–413.
11. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, Adams RR, Chang E, Allsopp RC, Yu J, et al. The RNA component of human telomerase. Science 1995;269:1236–1241.
12. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science 1997;276:561–567.
13. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 1992;11:1921–1929.
14. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal human cells. Science 1998;279:349–352.
15. Jiang XR, Jimenez G, Chang E, Frolkis M, Kusler B, Sage M, Beeche M, Bodnar AG, Wahl GM, Tlsty TD, Chiu CP. Telomerase expression in human somatic cells does not induce changes associated with a transformed phenotype. Nat Genet 1999;21:111–114.
16. Morales CP, Holt SE, Ouellette M, Kaur KJ, Yan Y, Wilson KS, White MA, Wright WE, Shay JW. Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. Nat Genet 1999;21:115–118.
17. Funk WD, Wang CK, Shelton DN, Harley CB, Pagon GD, Hoeffler WK. Telomerase expression restores dermal integrity to *in vitro*–aged fibroblasts in a reconstituted skin model. Exp Cell Res 2000;258:270–278.
18. Counter CM, Meyerson M, Eaton EN, Ellisen LW, Caddle SD, Haber DA, Weinberg RA. Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase. Oncogene 1998;16:1217–1222.
19. Steinert S, Shay JW, Wright WE. Transient expression of human telomerase extends the life span of normal human fibroblasts. Biochem Biophys Res Commun 2000;273:1095–1098.
20. Colgin LM, Reddel RR. Telomere maintenance mechanisms and cellular immortalization. Curr Opin Genet Dev 1999;9:97–103.
21. Chen HJ, Liang CL, Lu K, Lin JW, Cho CL. Implication of telomerase activity and alternations of telomere length in the histologic characteristics of intracranial meningiomas. Cancer 2000;89:2092–2098.
22. Shammas MA, Simmons CG, Corey DR, Shmookler Reis RJ. Telomerase inhibition by peptide nucleic acids reverses "immortality" of transformed human cells. Oncogene 1999;18:6191–6200.
23. Lundblad V, Szostak JW. A mutant with a defect in telomere elongation leads to senescence in yeast. Cell 1989;57:633–643.
24. Yu GL, Bradley JD, Attardi LD, Blackburn EH. In vivo alteration of telomere sequences and senescence caused by mutated Tetrahymena telomerase RNAs. Nature 1990;344:126–132.
25. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. Curr Opin Genet Dev 2001;11:98–103.
26. Collins K, Mitchell JR. Telomerase in the human organism. Oncogene 2002;21:564–579.
27. Elwood N. Telomere biology of human hematopoietic stem cells. Cancer Control 2004;11:77–85.
28. Sugihara M, Ohshima K, Nakamura H, Suzumiya J, Nakayama Y, Kanda M, Haraoka S, Kikuchi M. Decreased expression of telomerase-associated RNAs in the proliferation of stem cells in comparison with continuous expression in malignant tumors. Int J Oncol 1999;15:1075–1080.
29. Kilian A, Bowtell DD, Abud HE, Hime GR, Venter DJ, Keese PK, Duncan EL, Reddel RR, Jefferson RA. Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types. Hum Mol Genet 1997;6:2011–2019.
30. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. Telomere reduction in human colorectal carcinoma and with ageing. Nature 1990;346:866–868.
31. Lindsey J, McGill NI, Lindsey LA, Green DK, Cooke HJ. *In vivo* loss of telomeric repeats with age in humans. Mutat Res 1991;256:45–48.
32. Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, Greider CW, Harley CB. Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci U S A 1992;89:10114–10118.
33. Di Donna S, Mamchaoui K, Cooper RN, Seigneurin-Venin S, Tremblay J, Butler-Browne GS, Mouly V. Telomerase can extend the proliferative capacity of human myoblasts, but does not lead to their immortalization. Mol Cancer Res 2003;1:643–653.
34. Halvorsen TL, Beattie GM, Lopez AD, Hayek A, Levine F. Accelerated telomere shortening and senescence in human pancreatic islet cells stimulated to divide *in vitro*. J Endocrinol 2000;166:103–109.
35. Kruk PA, Rampino NJ, Bohr VA. DNA damage and repair in telomeres: relation to aging. Proc Natl Acad Sci U S A 1995;92:258–262.
36. Bohr VA, Brosh RM, Jr., von Kobbe C, Opresko P, Karmakar P. Pathways defective in the human premature aging disease Werner syndrome. Biogerontology 2002;3:89–94.
37. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 2003;361:393–395.
38. Fossel M. Reversing Human Aging. New York: William Morrow and Company, 1996.

39. Minton P. The Immortality Enzyme: Aging, Cancer & Heart Disease. New York: Barbara Minto, 2001.
40. Waters H, Walford RL. Latent period for outgrowth of human skin explants as a function of age. J Gerontol 1970;25:381–383.
41. Ponten J, Stein WD, Shall S. A quantitative analysis of the aging of human glial cells in culture. J Cell Physiol 1983;117:342–352.
42. Effros RB. Insights on immunological aging derived from the T lymphocyte cellular senescence model. Exp Gerontol 1996;31:21–27.
43. Gershon H, Gershon D. Paradigms in aging research: a critical review and assessment. Mech Ageing Dev 2000;117:21–28.
44. Shay JW, Wright WE. Hayflick, his limit, and cellular ageing. Nat Rev Mol Cell Biol 2000;1:72–76.
45. Cristofalo VJ, Allen RG, Pignolo RJ, Martin BG, Beck JC. Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proc Natl Acad Sci U S A 1998;95:10614–10619.
46. Tesco G, Vergelli M, Grassilli E, Salomoni P, Bellesia E, Sikora E, Radziszewska E, Barbieri D, Latorraco S, Fagiolo U, Santacaterina S, Amaducci L, Tiozzo R, Franceschi C, Sorbi S. Growth properties and growth factor responsiveness in skin fibroblasts from centenarians. Biochem Biophys Res Commun 1998;244:912–916.
47. Mondello C, Petropoulou C, Monti D, Gonos ES, Franceschi C, Nuzzo F. Telomere length in fibroblasts and blood cells from healthy centenarians. Exp Cell Res 1999;248:234–242.
48. Takeda K, Gosiewska A, Peterkofsky B. Similar, but not identical, modulation of expression of extracellular matrix components during *in vitro* and *in vivo* aging of human skin fibroblasts. J Cell Physiol 1992;153:450–459.
49. Mathon NF, Malcolm DS, Harrisingh MC, Cheng L, Lloyd AC. Lack of replicative senescence in normal rodent glia. Science 2001;291:872–875.
50. Tang DG, Tokumoto YM, Apperly JA, Lloyd AC, Raff MC. Lack of replicative senescence in cultured rat oligodendrocyte precursor cells. Science 2001;291:868–871.
51. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat Cell Biol 2003;5:741–747.
52. Toussaint O, Dumont P, Remacle J, Dierick JF, Pascal T, Frippiat C, Magalhaes JP, Zdanov S, Chainiaux F. Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? Sci. World J 2002;2:230–247.
53. Promislow DE. On size and survival: progress and pitfalls in the allometry of life span. J Gerontol 1993;48:B115–123.
54. Klapper W, Heidorn K, Kuhne K, Parwaresch R, Krupp G. Telomerase activity in "immortal" fish. FEBS Lett 1998;434:409–412.
55. Klapper W, Kuhne K, Singh KK, Heidorn K, Parwaresch R, Krupp G. Longevity of lobsters is linked to ubiquitous telomerase expression. FEBS Lett 1998;439:143–146.
56. Brocas J, Verzar F. The aging of *Xenopus laevis*, a South African frog. Gerontologia 1961;5:228–240.
57. Bassham S, Beam A, Shampay J. Telomere variation in *Xenopus laevis*. Mol Cell Biol 1998;18:269–275.
58. Mantell LL, Greider CW. Telomerase activity in germline and embryonic cells of *Xenopus.* EMBO J 1994;13:3211–3217.
59. Venkatesan RN, Price C. Telomerase expression in chickens: constitutive activity in somatic tissues and down-regulation in culture. Proc Natl Acad Sci U S A 1998;95:14763–14768.
60. Lejnine S, Makarov VL, Langmore JP. Conserved nucleoprotein structure at the ends of vertebrate and invertebrate chromosomes. Proc Natl Acad Sci U S A 1995;92:2393–2397.
61. Kakuo S, Asaoka K, Ide T. Human is a unique species among primates in terms of telomere length. Biochem Biophys Res Commun 1999;263:308–314.
62. Prowse KR, Greider CW. Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci U S A 1995;92:4818–4822.
63. Hemann MT, Greider CW. Wild-derived inbred mouse strains have short telomeres. Nucleic Acids Res 2000;28:4474–4478.
64. Marrone A, Mason PJ. Dyskeratosis congenita. Cell Mol Life Sci 2003;60:507–517.
65. Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. Nature 2001;413:432–435.
66. Knight S, Vulliamy T, Copplestone A, Gluckman E, Mason P, Dokal I. Dyskeratosis Congenita (DC) Registry: identification of new features of DC. Br J Haematol 1998;103:990–996.
67. Pan C, Xue BH, Ellis TM, Peace DJ, Diaz MO. Changes in telomerase activity and telomere length during human T lymphocyte senescence. Exp Cell Res 1997;231:346–353.
68. Iwama H, Ohyashiki K, Ohyashiki JH, Hayashi S, Yahata N, Ando K, Toyama K, Hoshika A, Takasaki M, Mori M, Shay JW. Telomeric length and telomerase activity vary with age in peripheral blood cells obtained from normal individuals. Hum Genet 1998;102:397–402.
69. Haussmann MF, Winkler DW, O'Reilly KM, Huntington CE, Nisbet IC, Vleck CM. Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. Proc R Soc Lond B Biol Sci 2003;270:1387–1392.
70. Vleck CM, Haussmann MF, Vleck D. The natural history of telomeres: tools for aging animals and exploring the aging process. Exp Gerontol 2003;38:791–795.
71. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 1997;91:25–34.
72. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb

GJ, Greider C, DePinho RA. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 1999;96:701–712.
73. Gonzalez-Suarez E, Samper E, Flores JM, Blasco MA. Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. Nat Genet 2000;26:114–117.
74. Rudolph KL, Millard M, Bosenberg MW, DePinho RA. Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. Nat Genet 2001;28:155–159.
75. Gonzalez-Suarez E, Samper E, Ramirez A, Flores JM, Martin-Caballero J, Jorcano JL, Blasco MA. Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. EMBO J 2001;20:2619–2630.
76. Artandi SE, Alson S, Tietze MK, Sharpless NE, Ye S, Greenberg RA, Castrillon DH, Horner JW, Weiler SR, Carrasco RD, DePinho RA. Constitutive telomerase expression promotes mammary carcinomas in aging mice. Proc Natl Acad Sci U S A 2002;99:8191–8196.
77. Vaziri H, Squire JA, Pandita TK, Bradley G, Kuba RM, Zhang H, Gulyas S, Hill RP, Nolan GP, Benchimol S. Analysis of genomic integrity and p53-dependent G1 checkpoint in telomerase-induced extended-life-span human fibroblasts. Mol Cell Biol 1999;19:2373–2379.
78. Wang J, Hannon GJ, Beach DH. Risky immortalization by telomerase. Nature 2000;405:755–756.
79. Hahn WC, Stewart SA, Brooks MW, York SG, Eaton E, Kurachi A, Beijersbergen RL, Knoll JH, Meyerson M, Weinberg RA. Inhibition of telomerase limits the growth of human cancer cells. Nat Med 1999;5:1164–1170.
80. Stewart SA, Hahn WC, O'Connor BF, Banner EN, Lundberg AS, Modha P, Mizuno H, Brooks MW, Fleming M, Zimonjic DB, Popescu NC, Weinberg RA. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. Proc Natl Acad Sci U S A 2002;99:12606–12611.
81. Lindvall C, Hou M, Komurasaki T, Zheng C, Henriksson M, Sedivy JM, Bjorkholm M, Teh BT, Nordenskjold M, Xu D. Molecular characterization of human telomerase reverse transcriptase-immortalized human fibroblasts by gene expression profiling: activation of the epiregulin gene. Cancer Res 2003;63:1743–1747.
82. Smith LL, Coller HA, Roberts JM. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. Nat Cell Biol 2003;5:474–479.
83. Noble JR, Zhong ZH, Neumann AA, Melki JR, Clark SJ, Reddel RR. Alterations in the p16(INK4a) and p53 tumor suppressor genes of hTERT-immortalized human fibroblasts. Oncogene 2004;23:3116–3121.
84. Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. Science 1997;278:1043–1050.
85. Ueda M, Ouhtit A, Bito T, Nakazawa K, Lubbe J, Ichihashi M, Yamasaki H, Nakazawa H. Evidence for UV-associated activation of telomerase in human skin. Cancer Res 1997;57:370–374.
86. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. Nature 2003;426:194–198.
87. Takubo K, Izumiyama-Shimomura N, Honma N, Sawabe M, Arai T, Kato M, Oshimura M, Nakamura KI. Telomere lengths are characteristic in each human individual. Exp Gerontol 2002;37:523–531.
88. Renault V, Thornell LE, Eriksson PO, Butler-Browne G, Mouly V, Thorne LE. Regenerative potential of human skeletal muscle during aging. Aging Cell 2002;1:132–139.
89. Franceschi C, Mondello C, Bonafe M, Valensin S, Sansoni P, Sorbi S. Long telomeres and well preserved proliferative vigor in cells from centenarians: a contribution to longevity? Aging (Milano) 1999;11:69–72.
90. von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002;27:339–344.
91. Osanai M, Tamaki T, Yonekawa M, Kawamura A, Sawada N. Transient increase in telomerase activity of proliferating fibroblasts and endothelial cells in granulation tissue of the human skin. Wound Repair Regen 2002;10:59–66.
92. Rubin H. The disparity between human cell senescence *in vitro* and lifelong replication *in vivo.* Nat Biotechnol 2002;20:675–681.
93. Stephens P, Cook H, Hilton J, Jones CJ, Haughton MF, Wyllie FS, Skinner JW, Harding KG, Kipling D, Thomas DW. An analysis of replicative senescence in dermal fibroblasts derived from chronic leg wounds predicts that telomerase therapy would fail to reverse their disease-specific cellular and proteolytic phenotype. Exp Cell Res 2003;283:22–35.
94. Effros RB. Genetic alterations in the ageing immune system: impact on infection and cancer. Mech Ageing Dev 2003;124:71–77.
95. Thomas M, Yang L, Hornsby PJ. Formation of functional tissue from transplanted adrenocortical cells expressing telomerase reverse transcriptase. Nat Biotechnol 2000;18:39–42.
96. Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, Schneider MD. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. Proc Natl Acad Sci U S A 2001;98:10308–10313.

Address reprint requests to:
João Pedro de Magalhães, Ph.D.
Department of Genetics
Harvard Medical School
77 Avenue Louis Pasteur, Room 238
Boston, MA 02115

E-mail: jpnitya@senescence.info

Received: March 3, 2004
Accepted: April 29, 2004