Biogerontology (2011) 12:503–515
DOI 10.1007/s10522-011-9346-3

REVIEW ARTICLE

Cell division and aging of the organism

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Received: 22 March 2011 / Accepted: 23 June 2011 / Published online: 6 July 2011

Abstract The capacity to regenerate cell compartments through cell proliferation is an important characteristic of many developed metazoan tissues. Pre- and post-natal development proceeds through the modifications occurring during cell division. Experiments with cultivated cells showed that cell proliferation originates changes in cell functions and coordinations that contribute to aging and senescence. The implications of the finite cell proliferation to aging of the organism is not the accumulation of cells at the end of their life cycle, but rather the drift in cell function created by cell division. Comparative gerontology shows that the regulation of the length of telomeres has no implications for aging. On the other hand there are interspecies differences in regard to the somatic cell division potential that seem to be related with the "plasticity" of the genome and with longevity, which should be viewed independently of the aging phenomenon. Telomeres may play a role in this plasticity through the regulation of chromosome recombination, and via the latter also in development.

Keywords Telomeres · Asymmetric cell division · DNA methylation · Fibroblasts · Immortalization · Longevity

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Rationale for the use of proliferating cells in vitro to investigate aging

Years ago gerontologists thought that proliferating cells were of secondary importance to study aging, it was supposed that terminal differentiated cells were the relevant ones to investigate human senescence. This view led to criticisms in regard to the so called Hayflick limit. However, cell division is crucial for aging of the organism, it concerns inter alia tissue regeneration, wound healing, and the immune response. The fusion of the gametes triggers an accelerated expansion of the number of cells, which decelerates progressively. Cell renewal persists in several compartments all along the human life span. The cells of connective tissue are mitotic cells, the pancreas replaces its cells every 24 h, the cells lining the stomach are renewed every 3 days, white blood cells are renewed every 10 days, and the skin is replaced every 6 weeks (Capra 1997). Approximately 70 g of proliferating cells are discarded in the small intestine every day (Potten et al. 1987). Moreover, it was thought that neurons were completely devoid of renewal potential but in some regions of the central nervous system regeneration could be ascertained. Maintenance of another terminally differentiated cell, striated muscle, depends on mitotic satellite cells.

Some gerontologists rejected the use of cells cultivated in vitro for the study of aging of the organism. They were not aware that cells maintained in vitro reveal many of the properties they display in

the organism. Indeed cell culture in vitro has been used to detect developmental modifications and to understand many human pathological conditions (reviewed in Macieira-Coelho 1988). Hence, if cells can express in vitro the changes occurring during development and disease, there is no reason to believe that they cannot express the changes taking place during aging of the organism. Unfortunately the field was misdirected through wrong experiments mentioned below that led to the concept that the accumulation of non-dividing cells is the hallmark of aging. This originated obvious criticisms from gerontologists using other approaches to study aging. In spite of these misunderstandings a clear picture has finally emerged giving new views of the mechanisms of aging.

The understanding of how cell division contributes to aging was possible thanks to the work of Hayflick and Moorhead (1961) who suggested that the finite division potential of human fibroblasts in vitro is the expression of aging at the cellular level. Hayflick (1965) compared the division potential of human embryonic fibroblasts with those of postnatal origin and found that the latter had a shorter division potential, showing that the proliferation potential declines with development. Several groups compared the long term proliferation of postnatal fibroblasts in vitro with donor age. Most investigators observed an inverse correlation between proliferation an donor age, not only for fibroblasts but also for other cell types (reviewed in Macieira-Coelho 1988). However, at least two groups did not find a correlation (Goldstein et al. 1978; Cristofalo et al. 1998). It should be stressed that this parameter is difficult to ascertain since there are different fibroblast populations differing in growth potential, which is related to the developmental characteristics of each organ and tissue (Macieira-Coelho 2003). Indeed, the paper from Cristofalo's group suggests that the cell populations studied from older donors come from tissues containing a mosaic of cells each having different population doubling potential. These cell populations evolve during proliferation with increasing heterogeneity of the division cycle that becomes longer to complete and whose initiation becomes more random so that the mathematical formula used to calculate the number of divisions is unrealistic; indeed after subcultivation, some cells divide once, others two or three times, and others do not divide but do so after the following subcultivation. In other words, there is a great intercellular variation in the latency of the initiation of the division cycle. The heterogeneity in cell cycling is progressive illustrating the continuous functional changes taking place throughout the proliferation life span (Macieira-Coelho et al. 1966; Macieira-Coelho 1967, 1974; Absher and Absher 1976; Macieira-Coelho and Azzarone 1982). These kinetics of cell proliferation end up with a post-mitotic state with a particular morphology, which has been denominated phase IV (Macieira-Coelho and Taboury 1982) in addition to the phases I, II and III described by Hayflick and Moorhead (1961). There are other parameters that render the detection of a correlation difficult, they concern the physiopathology of the donor; certain conditions decrease and others increase the division potential (Macieira-Coelho 2003).

Two groups tested the correlation between the fibroblast division potential in vitro and the longevity of the respective species. One study did not find a correlation (Stanley et al. 1975) and another found a direct correlation (Röhme 1981). Unfortunately in both studies embryonic cells from some species were compared with postnatal cells from other species. Moreover, life tables for the animals studied in the paper by Stanley et al. do not exist and the longevity of the zoo animals cited is dependent on observations made on very few captured individuals, so we still have to wait for the right evidence.

Two works led to a wrong direction: they concluded that post-mitotic fibroblasts increase exponentially at each cell population doubling (Merz and Ross 1969; Cristofalo and Sharf 1973); the post-mitotic fibroblasts were called senescent cells and considered as the hallmark of cell aging. These two works used a wrong methodology that measured only fast dividers (Macieira-Coelho and Azzarone 1982); the first publication used cloning and in the second, cells were plated at low densities and labelled with high doses of thymidine. The probability of entering the division cycle is not the same for an isolated cell or a cell making contacts with other cells. The cooperation between cells, either through secretion of metabolites and creation of a microenvironment or through contacts, can enhance the commitment to divide. Hence these works measured only the division capacity of the most vigorous cells and led to the belief that the main event was the final non-mitotic state. Other results that deviated the attention from

the essential question concern an experiment that indicated an increase with donor's age of the number of terminal non-mitotic fibroblasts in vivo (Dimri et al. 1995). Several experiments showed that the marker used is not valid (Macieira-Coelho 2010).

In general it is accepted that there is a slow down with age of the proliferation of mitotic cells in the mammalian organism, that this decline influences some aspects of aging of the organism, but obviously one does not age because our cells stop dividing. Several works have shown that the fibroblasts of very old people maintain the capacity to divide. It should be reminded that cells can regenerate to a certain extent in vivo the long term division potential during resting phase (reviewed in Macieira-Coelho 1988).

Several markers known to be present in the terminal postmitotic cells in vitro were not found in cells directly taken from old donors (reviewed in Macieira-Coelho 2010). It was reported that a marker for telomere-associated DNA damage increased exponentially in baboon skin fibroblasts cultivated from animals of different ages; it reached a value of 15–20% in cells grown from very old animals (Herbig et al. 2006). The cellular marker used is associated with the so called senescent cells in cultured human fibroblasts aged in vitro (Herbig et al. 2004). No tests were performed to check the health status of the baboons. This is an important point since in human tissues terminal post-mitotic fibroblasts were found to accumulate only in pathological conditions (reviewed in Macieira-Coelho 2010). The accumulation of terminal post-mitotic cells only in pathological conditions raises the hypothesis that a regular evolution of the mesenchymal fibroblast proliferation life span is necessary for homeostasis to prevail. Hence it was suggested that the accumulation of post-mitotic terminal fibroblasts in pathological processes signals a disturbance of the homeostatic regulation of the differentiation cycle and is part of the pathological picture (Macieira-Coelho 2010). It favors the view that the evolution of the fibroblast compartment is a developmental process (Martin et al. 1974). There is strong evidence showing that fibroblasts play a crucial role in pre- and postnatal development, that their properties evolve with proliferation through the organism's life span, and that these modifications can be studied in vitro. It led to significant contributions concerning the control of the cell division cycle, how cells are modified through division, and how this is responsible for several aspects of the aging syndrome.

Molecular modifications caused by cell divisions

Cytogenetic and epigenetic modifications

The first attempts to elucidate the causes of the decline of the proliferation potential, concerned the chromosomes. A progressive reorganization at the cytogenetic level was described during serial proliferation (Saksela and Moorhead 1963; Chen and Ruddle 1974). Moreover, it was thought that due to semiconservative DNA synthesis cells get the same amount of genetic material during the division cycle. However, it was demonstrated that newly synthesized DNA is distributed asymmetrically between daughter cells (Macieira-Coelho et al. 1982); it results in an accumulation of differences between cells during serial divisions. During the last 3–4 divisions (phase IV) the distribution of DNA becomes chaotic (Macieira-Coelho 1995).

Epigenetic modifications were also thought to be implicated in the limited division potential. The first work performed in this respect examined DNA methylation in diploid human fibroblasts, early and late in their replicative life-span (Reis and Goldstein 1982). Methylation at internal (3′) cytosines increased from 59 to 64% of sites in one cell strain at late passage, remained constant in another, and decreased in four other strains. Three discrete classes of highly repetitive DNA had consistently 70–80% of sites methylated at 3′ cytosines in all clones and in all strains examined both at early and at late passage. Considerable interclonal and intraclonal heterogeneity was observed for methylation in the γ-globin coding region of DNA. It was concluded that the pattern of methylation in endogenous gene regions appears to undergo random drift during replication of diploid fibroblasts. Later Wilson and Jones (1983) observed a decrease during replication in 5-methylcytosine in cultured human, hamster, and mouse fibroblasts. The greatest loss was observed in mouse cells, which survived the least number of divisions. The authors concluded that it remains to be shown if this represents a response to the culture environment or has any significance in vivo. Bork et al. (2010) studied methylation through the long-term culture of

human mesenchymal stromal cells. Overall, methylation patterns were maintained throughout aging but there were significant differences at specific CpG sites, inter alia in homeobox genes and genes involved in cell differentiation. Interestingly the methylation changes were overlapping in aging in vitro and in vivo, supporting the view that senescence in vitro represents a developmental process. It should be reminded that mesenchymal stromal cell are precursors for mesodermal cell lineages such as osteocytes, chondrocytes, and adipocytes. The same group (Koch et al. 2011) analyzed methylation in long-term cultures of human dermal fibroblasts, which can not be induced towards differentiated lineages. In 766 CpG sites they found hypermethylation and in 752 hypomethylation. Age-associated DNA methylation changes in dermal fibroblasts and mesenchymal stromal cells were often regulated in opposite directions. Thus, the second highest hypermethylation upon aging of dermal fibroblasts was found in the CDKN2B (INK4a) locus, which was hypomethylated in mesenchymal stromal cells. Hornsby et al. (1991) followed DNA methylation in the steroid 17 alpha-hydroxylase flanking region of bovine adrenocortical cells senescing in culture. Two CpG sites that are methylated in adrenocortical cells in vivo were observed to undergo rapid demethylation in the cultivated cells in vitro. Two adjacent sites that are methylated in vivo did not demethylate; they are completely nonmethylated in fibroblasts.

More than 50 metabolic, genetic, and structural modifications were described at different hierarchical levels of the organization of DNA and more than 40 genes have been implicated in the final arrest of division (reviewed in Macieira-Coelho 2003).

Telomeres and aging

One modification has particularly drawn the attention of gerontologists, it concerned the shortening of chromosome ends, the telomeres. One can still see claims in the literature that this is one of the main causes of aging. The hybridization of the terminal restriction fragments (TRF) of DNA from serially dividing human fibroblasts with the appropriate probe showed a shortening of the hybridization signal which was interpreted as a shortening of the telomeres as population doublings increase (Harley 1991). The extension of the human fibroblast life span accompanied by an increase in the hybridization signal, after the introduction of telomerase by transfection of the cells with vectors encoding the human telomerase catalytic unit, supported the relationship of causality between telomere shortening and the cell's finite division potential (Bodnar et al. 1998). Since some immortal cell lines express the enzyme telomerase, and develop the capacity to reconstitute telomeres after replication, the link between telomere integrity and replication potential seemed established. Investigators were quick to equate telomere shortening with proliferation and aging. The reasoning was based on the syllogism: the number of potential divisions decreases with aging, telomeres are shortened during the proliferation of fibroblasts, hence aging is a function of telomere shortening. The syllogism, however, is unjustified because the major and minor propositions have not been ascertained in comparative gerontology studies.

The erosion of telomeres through division is not universal. In humans the division potential in vitro of normal keratinocytes (Kang et al. 1998; Rheinwald et al. 2002), cardiomyocytes (Ball and Levine 2005) and astrocytes (Evans et al. 2003) is independent of telomere size. Results obtained with normal in vivo and in vitro lymphocytes vary with the laboratory and the methodology. Some investigators found that telomeric sequences are lost in proliferating lymphocytes (Vaziri et al. 1993), others could not find any alteration of chromosome ends (Luke et al. 1994; Kormann-Bortolotto et al. 1996), while still others observed a decline early during the human life span, a plateau between age 4 and young adulthood, and a gradual attrition later in life (Frenck et al. 1998). This development-related rather than senescence-related modification in telomere structure is compatible with another work showing that human telomeres are modified already during the embryonic stage in different organs (Ulaner et al. 2001). In humans, telomere lengths did not show a clear correlation with tissue renewal times in vivo and are characteristic of each human individual (Takubo et al. 2002); moreover the rate of telomere loss slows throughout the human life span (Unryn et al. 2005). Fibroblasts from patients with Werner's syndrome, which have a shorter life span than those of normal age-matched control donors do not have shorter telomeres than control cells (Schultz et al. 1996). The mean terminal repeat fragments of cells from patients with Werner's

syndrome that had ceased replication were significantly longer than those of the controls. This can be interpreted in terms of the deviation from normal development that characterizes the Werner syndrome, rather than premature aging. Experiments performed with cells from Hutchinson-Gilford progeria syndrome showed that telomere length is related to factors other than replication (Decker et al. 2009). Telomere length was reduced in fibroblasts and was variable; in contrast in hematopoietic cells it was within the normal range. These data raise the possibility that lamin A, which is mutated in this syndrome and is expressed in fibroblasts but not in hematopoietic cells, decreases telomere length. In benign leiomyoma tumors telomere size is unrelated with the proliferation potential (Rogalla et al. 1995). In malignant human tissues results are variable. Nonisotropic Southern hybridization revealed a reduction of telomere repeat arrays in 14 of the 35 tumors analyzed. In other cases, 60% showed either no reduction or an increase in telomeric length (Schmitt et al. 1994).

There are other caveats concerning the relationship between telomere shortening and proliferation. Human fibroblasts maintained in the presence of 3% O_2 instead of the usual concentration of 20%, have an increased proliferation potential but have shorter telomeres (Betts et al. 2008). Radiation-induced senescence-like growth arrest is independent of telomere shortening (Suzuki et al. 2001). No significant (Chen et al. 2001) or only a slight telomere shortening (Magalhaes et al. 2004) was observed in senescence-like induced growth arrest of human fibroblasts after H_2O_2 stress.

Telomere biology seems to vary with the species in a way unrelated with aging and with the respective cells proliferation life span in vitro. In non-human primates such as rhesus monkey, Japanese monkey, crab-eating monkey, chimpanzee, and orangutan, TRF length was more than double that of human somatic tissues (Kakuo et al. 1999). The only exception of human tissues is sperm with a TRF longer than 23 kb, which increases slightly with donor age (Allsop et al. 1992). Other authors reported a somewhat shorter TRF for rhesus monkey, pigmy chimpanzee, and orangutan (Steinert et al. 2002). The European White Rabbit, the Black-tailed Jack Rabbit, the Swamp Rabbit, and the North American Pika have extremely long telomeric arrays and their fibroblasts do not exhibit a limited division potential in vitro (Forsyth et al. 2005). Of these four species, only Pika displays endogenous telomerase activity in culture. Hamster embryonic fibroblasts express telomerase throughout their replicative life span and the average telomere length does not decrease (Carman et al. 1998).

Long telomeres, short cell replicative aging in vitro and short longevity is found in either wild mice or inbred laboratory strains. Restriction fragments of up to 150 kb have been reported in DBA/2, C57Bl/6j and CBA/Ca mice colonies, without any difference detectable between DNA from liver, spleen or testes of the same animal (Kipling and Cook 1990). The size seems largely unchanged through somatic cell division or during the animal's life span. In Mus spretus telomere lengths have approximately the same length as in humans (Coviello-McLaughlin and Prowse 1997). Significant TRF length changes with age were found in brain and spleen, but not in liver, testis and kidney. Some tissues tended to have longer or shorter TRF lengths compared to other tissues. Males had TRF lengths that were on average 0.5–1 kb shorter than females in every tissue examined. The data suggested that telomere lengths may decrease due to factors other than the end replication problem during cell division (Coviello-McLaughlin and Prowse 1997). Chicken telomeres resemble human telomeres in length but the distribution of telomerase activity is present in the germ line as well as in a wide variety of somatic tissues as in mice (Venkatesan and Price 1998). It is well known that chicken fibroblasts have a finite proliferation span and contrary to mice exhibit extremely low rates of spontaneous immortalization. Telomerase activity is greatly down-regulated when chicken cells are cultivated in vitro, which raises the question on the extrapolation of in vitro studies to the in vivo data (Venkatesan and Price 1998). In another phylum, Paramecium caudatum, no shift in telomerase activity, or telomere length was observed at the point of maturation; telomeres elongate successively as cells divide (Takenaka et al. 2001). On the other hand in P. tetraurelia, the length of telomeres is kept constant.

Telomeres and development

Several data suggest that variation of telomere length is related to developmental regulations. This was

proposed by Barbara McClintock in 1943; she demonstrated a gradient of crossing-over in the distal part of telomeric regions and proposed a regulatory role of TRF in the chromosome recombinations necessary for development to proceed. In adult normal mice changes in telomere length are tissue-specific and seem to be developmentally regulated (Prowse and Greider 1995); long telomeres and telomerase activity were detected in the testis about 5–6 weeks after birth, at a time when the testis increase dramatically in size due to the production of the first spermatocytes. Indeed several works favor the role of telomeres in development. A telomere elongation program was demonstrated at the transition from morula to blastocyst in mice and cattle, which restores telomeres in cloned embryos derived from fibroblasts, regardless of the telomere length of donor nuclei (Schaetzlein et al. 2004). This program is telomerase-dependent since it is abrogated in telomerase-deficient mice. The telomere length in immature rat testis containing type A spermatogonia were compared with adult testis containing more differentiated cells (Achi et al. 2000). Mean telomere length in the immature testis was significantly shorter in comparison to adult testis. Pachytene spermatocytes exhibited longer telomeres compared to type A spermatogonia. The results indicated that telomere length increases during development of male germ cells from spermatogonia to spermatozoa and is inversely correlated with the expression of telomerase activity.

Mice without telomerase, obtained with gene knockout techniques, reproduce normally for several generations (Blasco et al. 1997). These telomerase null mice do not present pathophysiological symptoms of aging (Rudolph et al. 1999); sixth generation null mice, however, are infertile and have an increased embryonic lethality due to neural tube closure defects, long-term renewal of hematopoietic stem cells is compromised, and there is delayed reepithelialization of skin wounds. Two independent lines of mice reiteratively cloned to four and six generations showed no signs of premature aging (Wakayama et al. 2000). Moreover, there was no shortening of telomeres, on the contrary telomeres lengthened with each generation. Cloned sheep on the other hand had a decrease in mean TRF as compared with age-matched controls but were healthy without signs of abnormal aging (Shiels et al. 1999). One of the cloned animals had undergone two normal pregnancies and delivered healthy lambs.

Human subtelomeres have been described as hotspots of interchromosomal recombination (Linardopoulou et al. 2005). Alternative lengthening of telomeres is also characterized by high rates of telomeric exchange (Londono-Vallejo et al. 2004). The telomeres of human fibroblast chromosomes were analyzed with the canonical probe that detects all telomeres and with a TelBamm 11 probe that is specific for a subset of human telomeres (Ben 1997). The heterogeneity of fragment size was much greater when the canonical probe was used, relative to that seen using the TelBamm 11 probe. This could be attributable to a greater variability in the location of the restriction enzyme sites rather than to differences in the length of terminal repeats. Ben concluded that variation in terminal repeat length may be related to how much telomeres participate in chromosome rearrangement. Telomere-promoted recombination can lead to degeneration of the telomeric sequence, and subsequent loss of the hybridization ability (Ashley and Ward 1993). Indeed, several examples of apparent terminal deletions are actually subtelomeric translocations (Meltzer et al. 1993). Therefore the exact nature of the modifications occurring during proliferation has yet to be ascertained.

Blackburn (2000) considered that the structure of telomeres is more important than their length, in particular the capping of the chromosome ends by a protein complex and telomerase avoiding non-homologous end-joining, is critical for cell division. The role of telomerase is not only that of adding telomeric DNA repeats to telomere ends; some alleles code for a telomerase that can permit continued proliferation with short telomeres, suggesting that telomerase helps to cap telomeres since recombination in the telomeric regions is repressed. Human telomerase allows cell proliferation without requiring lengthening of telomeres (Zhu et al. 1999). Blackburn also showed the role of the various DNA-binding structural proteins in the regulation of the integrity of chromosome ends. She concluded that one has to look beyond length to understand telomere functionality (Blackburn 2000).

In summary it seems that the regulation of the length of telomeres has no implications for aging. Several experiments suggest that they might have implications for normal development through

recombinational events as originally suggested by McClintock (1943). The understanding of the regulation of normal development at telomeres could help to ascertain if the human fibroblast proliferative life span is indeed a developmental process. For the moment it is difficult to grasp the implications of the interspecies differences in the biology of telomeres.

Telomeres and longevity

On the other hand, the control of chromosome recombination through telomeres could be connected with cell stability and species longevity. The potential for chromosome rearrangements is not identical across species and seems to confer characteristics that have a bearing with patterns of longevity and to other properties of the respective species (Macieira-Coelho 2000).

The long term proliferation potential of animal cells seems related with their ability to carry on genomic rearrangements. Human embryonic fibroblasts when cultivated in vitro go through continuous chromosomal rearrangements without any definite pattern becoming apparent (Chen and Ruddle 1974). The data showed that multiple clones arise continuously and compete between each other without any one overgrowing the others. These cells have a longer division potential than postnatal fibroblasts, which go through more stable type chromosome rearrangements during serial divisions (Harnden et al. 1976; Bourgeois et al. 1981). Moreover, in human fibroblasts close to the end of their proliferative life span, it becomes more difficult to induce sister chromatid exchanges (Schneider and Gilman 1979). This decline in the recombination potential of the genome during development could be related to the decreased number of long telomeres available for recombination (Martens et al. 2000). Moreover, fibroblasts from Werner's syndrome patients, which have a reduced doubling potential when compared with cells from age-matched normal donors, present chromosomal rearrangements (variegated translocation mosaicism), which become fixed and remain predominant during the proliferative life span of the cell population (Salk 1982). These findings suggest that a "rigidity" of the genome is associated with a shorter doubling potential as opposed to a higher "plasticity" which would favor a longer potential.

The mouse genome is particularly prone to chromosomal recombinational events, which could explain why the proliferation of mouse fibroblasts can escape the finite division potential; it may have a bearing with the structure of mouse telomeres. The mouse genome is endowed with the capacity to survive pronounced chromosome reorganizations. Indeed, the chromosomes of mouse fibroblasts cultivated in vitro display a very unusual capacity for recombination, which is expressed by the presence of radial figures (somatic crossing overs) and bridges between chromosomes corresponding to genetic exchanges (Macieira-Coelho and Azzarone 1988).

Comparison between the mouse and the human genomes in respect to the recombination potential shows a higher rate of sister chromatid exchanges in mouse chromosomes. The plasticity of the mouse genome is also revealed by the rapidity with which mouse cells can switch from the diploid to the tetraploid state (Macieira-Coelho and Azzarone 1988). Furthermore, the chromatin lability, which accumulates during the decline of the growth potential, disappears in the case of mouse cells during the chromosomal rearrangements, which occur during the transition to immortalization (Macieira-Coelho 1991). DNA elimination in mouse fibroblasts during the period preceding immortalization could be germane to disappearance of the fragile chromatin sites. Indeed, DNA measurements on cells in interphase after ethidium bromide staining showed that occasionally at early population doubling levels, the DNA content of G2 cells is less than that expected from the G1 content (Macieira-Coelho and Azzarone 1988). This suggests that a significant process of DNA elimination goes on in these cells during the division cycle.

The potential for chromosomal recombinational events expressed by mouse fibroblasts in vitro also has a counterpart in vivo. Although 40 acrocentric chromosomes is the usual diploid number of the mouse species, localized races with 38–22 chromosomes resulting from Robertsonian fusions, have been found in the wild (Capanna 1973). This property of the mouse genome could be responsible for the high probability of mouse cells to immortalize spontaneously and acquire malignant characteristics, for the high susceptibility to viral, chemical and physical carcinogens and to oncogenes, and for the facility with which one can induce tumors in mice.

The instability of the mouse genome, due to yet unknown properties, is favorable for the species to adapt and survive, but reduces the species life span because of the high rate of mutational events.

Very slight differences created by sequence divergence can give properties to the genome that seem critical for cell stability and species survival. Although both the rat and human H-ras genes encode the same aminoacid sequence, they have only 75% identity at the DNA level. Loss of the TGG-triplet that overlaps with codon 12 in the human gene dramatically reduces the number of potential cruciform structures by decreasing the purine content of the purine-reach strand, and the pyrimidine content of the pyrimidine-rich strand. It also introduces an additional CG that, if methylated, would produce a more stable duplex. One would expect the net effect to be that the human codon 12 sequence would be less prone to the formation of paired fold-backs that are a source of recombinations (Smith 1994). This could be germane to the stability of the life span of human cells in contrast to rat cells, which immortalize regularly in vitro. The rat species, like other rodents, also has a short life span but a high adaptability in all environments. However, the characteristics manifested by rodent cells in vitro have been observed with cells obtained from inbred animals; it remains to be demonstrated whether cells from animals in the real feral world manifest the same properties.

In summary, telomeres have a role in the recombination potential of the chromosomes which seems to have implications for normal development, it also seems associated with attributes of cell stability, long term cell proliferation, and species survival.

Consequences for aging of the organism of the changes occurring through cell division

The ascertainment that there is a limit to the proliferation of certain mitotic cell compartments implicates that cells change at each division tending to the limit. The connotation of the finite proliferation potential to aging of the organism is not the accumulation of cells at the end of their life cycle, as has been claimed, but rather the drift in cell function due to the changes through replication well before the terminal stage. A functional drift was reported by Houck et al. (1971) and later reinforced (Hayflick 1977). Many more modifications through cell divisions were later described (reviewed in Macieira-Coelho 1988), which contribute to new regulations and interactions with other cell compartments that are an important component of the permanent evolution taking place in a mammalian organism; it fits into what is known of the physiology of the organism. Hence in order to understand the mechanisms of aging it is more relevant to analyze the events occurring in the proliferating cells rather than the final step as has been done in general.

Most studies have concerned human fibroblasts but other cell systems go through a differentiation cycle and have a limited division potential that declines with aging of the organism, although their share in senescence is played in a different way from that of the mesenchymal fibroblast. That is the case of keratinocytes that proliferate in vivo and in vitro to a terminally differentiated cell (Rheinwald and Green 1977). Keratinocytes cultivated in vitro acquire the characteristics of terminal keratinocytes as in vivo, i.e., they develop detergent-insoluble cytoplasmic filaments, there is increase in size, formation of a cornified cell envelope, and destruction of the cell nucleus (Green 1977). The increase in size is accompanied by the appearance of new proteins such as keratins, involucrin, and other specific proteins (Simon and Green 1984). The role of this cell system is that of tissue renewal for the production of the external cell layer protecting the skin.

Chondrocytes represent another differentiated cell system whose rate of division declines with senescence (Evans and Georgescu 1983). It is not a terminal differentiating system since the cells are already differentiated and their function does not evolve towards a more differentiated state. Their role is that of forming the layer that covers the joints, the modifications occurring through aging contribute to the inadequate function of the joints during senescence.

Lens cells constitute an example of a terminal differentiation that evolves with formation of elongated enucleated fibers with synthesis of specific proteins called crystallins differently distributed in the central epithelium, in the pre-elongation fibers, and in the fiber cells (Simonneau et al. 1983). Alpha-cristallin, the major protein component of the vertebrate lens, plays a critical role in the maintenance of

transparency through its ability to inhibit stress-induced protein aggregation. Lens cells are derived from the ectoderm and like the hair or the nails continue growth throughout life. The proliferation potential of lens cells declines with aging of the organism. Senescence of this cell system is the result of the accumulation of fibers with a progressive loss of focusing power. Later an opacification of the lens can take place with the formation of cataracts due to the accumulation of membrane-bound α-cristallin and of high molecular complexes of α-cristallin with other crystallins. Cataracts can also be the result of deviations from the control of cell division during embryonic development (Muggleton-Harris et al. 1987) or of pathological processes.

Senescence in these different cell types is the expression of the diversity of the mammalian organism. They all have in common of being differentiated cells with a finite division potential, the proliferation declining with senescence, but the role of these cell types on the remodeling of the human body and on the effects of senescence is different. Cell culture has been a critical tool to understand these different ways of cell senescence through division.

With regard to the interactions with other cell compartments the physiology of the brain constitutes a pertinent example. Glial cells are fundamental for the survival of neurons, inter alia through their action on myelination and neuron membrane recycling, creation of the blood brain barrier, and regulation of the neuron's ionic environment. Astrocytes control synapse number, few synapses form in the absence of glial cells and the few synapses that do form are functionally immature (Ullian et al. 2001). Glial cells evolve through the human life span like the fibroblasts, i.e., with longer division cycles (Fedoroff et al. 1990). This evolution reflects the metabolic changes they incur, which alter their multiple interactions with the neuron and are responsible for many aspects of aging of the brain. The restructuring involving the glial cell compartment will influence neuron signal transcription.

A remodeling at the cellular level resulting from a different equilibrium between cell compartments, the decline of one leading to over expression of another, also contributes to the aging syndrome; it renders the organs more prone to pathology and explains to a great extent the functional changes evolving in the mammalian organism through senescence. The following examples illustrate this feature of the evolution of the mammalian organism. With advancing age, a progressive decrease in the density of striated muscle can be observed in the urinary bladder's rhabdosphincter with the concomitant replacement by fat cells and connective tissue (Strasser et al. 1999). A progressive age-dependent decrease of the density of striated muscle cells was observed to the point where only 34.2% of the rhabdosphincter in the elderly women can consist of striated muscle cells. This work shows that the cell remodeling in the sphincter may be the main reason for the high incidence of urinary incontinence with advancing age. The esophagus in aging mice becomes markedly thick with the disappearance of ciliated cells and keratinization of superficial cells (Duan et al. 1993). DNA synthesis is almost confined to the basal layer and declines with age, with an enlargement of the nuclei and nucleoli of the basal cells. In humans the number of parietal cells in the gastric mucosa tend to increase and that of mucous cell is reduced during normal aging (Farinati et al. 1993). Since the mucus protects from acid secretion the findings can explain an increased susceptibility of the gastric mucosa to damage in the elderly.

The permanent restructuring that occurs in bone is the result of the continuous shift that takes place in the balance between the activity of osteoblasts and osteoclasts leading to a functionally deficient architecture. The maturation of pro-osteoblasts into osteoblasts diminishes and the proliferation of osteoblasts declines so that their activity is progressively offset by the activity of osteoclasts (Roholl et al. 1994). Other cells in bones contribute to this process. With age there is a decline in the expression of osteoprotegerin (OPG) secreted by stromal cells, which blocks the osteoclast-stimulatory effect of OPG ligand (Makhluf et al. 2000). The remodeling in the synthesis of long-distance messengers such as hormones, of course, also play a role in the bone structural changes. In the skin the loss of elasticity and increased wrinkling are the result of the rearrangements in the relative proportion of the molecular and cellular constituents. Hydroxylation of type I collagen decreases and the proportion of type IV collagen and fibronectin increase with age in mouse skin (Boyer et al. 1991). In senescent human skin, due to functional changes in fibroblasts, collagen fibers are fragmented, the fibroblasts lose contact

with collagen, and the ground substance increases. This constitutes the most important modification, i.e., the destruction of the relationship between fibroblasts and interstitial matrix breaking the relationship between the fibroblast cytoskeleton, the plasma membrane, fibronectin, and collagen (Pieraggi et al. 1984). The drop in glycosaminoglycan content in old age contributes to this modified relationship (Fleischmajer et al. 1972).

There is a causal relationship between changes in vascular compliance and the evolution of the collagen/elastin ratio, and the proportion of endothelial and smooth muscle cells. The elastic recoil of the aorta is rendered possible through the coordinated extension and retraction of its concentric elastic laminae. During aging, elastin is degraded and the collagen/elastin ratio increases. As a result, the elastic recoil of the vessel wall decreases and the aorta has to increase its volume to compensate for the decreasing elasticity of its wall (Robert et al. 1984). In addition, there is a modification of the genetic program that alters the balance of the mediators of remodeling such as nitric oxide synthase, platelet-derived growth factor, and transforming growth factor $\beta 1$ (Gibbons and Dzau 1994).

The cellularity and ultrastructure of the spleen is profoundly modified (Cheung and Nadakavukaren 1983). The spleen increases in weight due to an increased number of reticular cells and macrophages with enlarged cytoplasm in the white pulp whereas the number of lymphocytes falls. The age-related decline of immune functions proposed by the immune theory has been replaced by a completely different view (Globerson and Effros 2000). Instead of the unidirectional decline in immune responses, immune senescence appears as a shift in lymphocyte populations leading to enhanced as well as diminished functions determined by developmental changes. In contrast to the general age-related decline in T- and B-cell activity, the NK cell-system is highly active in the age group above 80 years (Krishnaj and Blandford 1987). The percentage of CD45RA (virgin) cells among $CD8^+$ T lymphocytes in centenarians was about 50%, only slightly lower than that of young donors (about 55–60%) (Cossarizza et al. 1996).

The cellular and molecular remodeling causes an impaired adaptive response to the environment due to a different synchronization of effectors (e.g., hormones) or regulation of enzymes. The induction for instance of glucokinase, tyrosine aminotransferase and microsomal NADPH:cytochrome *c* reductase in rat liver following treatment with glucose, ACTH, and phenobarbital respectively, are characterized by an age-dependent adaptive latent period whose duration increases progressively from 2 to 24 months of age (Adelman 1972).

These are just a few examples of how the mammalian organism evolves leading to senescence, with the changes occurring through cell proliferation. The capacity to regenerate a large part of its cells allows the elimination of damaged cells and to renew itself but there is a price to pay. As is described above, the investigation of aging of mitotic cells has shown that through divisions cells are modified with the consequent repercussions on cell function and cell interactions; it creates a drift that contributes to aging and senescence. This was the main contribution of the research of aging of proliferating cells for the understanding of the mechanisms of senescence.

References

Absher PM, Absher RG (1976) Clonal variation and aging of diploid fibroblasts. Cinematographic studies of cell pedigrees. Exp Cell Res 103:247–255

Achi MV, Ravindranath N, Dym M (2000) Telomere length in male germ cells is inversely correlated with telomerase activity. Biol Reprod 63:591–598

Adelman RC (1972) Age-dependent control of enzyme adaptation. In: Strehler B (ed) Advances in gerontological research, vol 4. Academic Press, New York, pp 1–23

Allsop RC, Vaziri H, Patterson C, Goldstein S, Younglali EV, Futcher AB, Greider CW, Harley CB (1992) Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci USA 89:10114–10118

Ashley T, Ward DC (1993) A "hot spot" of recombination coincides with an interstitial telomeric sequence in the Armenian hamster. Cytogenet Cell Genet 62:169–176

Ball AJ, Levine F (2005) Telomere-independent cellular senescence in human fetal cardiomyocytes. Aging Cell 4:21

Ben P (1997) Aging chromosome telomeres: parallel studies with terminal repeat and telomere associated DNA probes. Mech Ageing Dev 99:153–166

Betts DH, Perrault SD, King WA (2008) Low oxygen delays fibroblast senescence despite shorter telomeres. Biogerontology 9:19–31

Blackburn EH (2000) Telomere states and cell fates. Nature 408:53–55

Blasco MA, Lee HW, Hande MP, Smaper E, Lansdorp PM, De Pinho RA, Greider CW (1997) Telomere shortening and tumor formation by mouse cells lacking telomerase. Cell 91:25–34

Bodnar AG, Ouelette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichsteiner S, Wright WE (1998) Extension of life span by introduction of telomerase into normal human cells. Science 279:349–352

Bork S, Pfister S, Witt H, Horn P, Korn B, Ho AD, Wagner W (2010) DNA methylation pattern changes upon long-term and aging of human mesenchymal stromal cells. Aging Cell 9:54–63

Bourgeois CA, Raynaud C, Diatloff-Zito C, Macieira-Coelho A (1981) Effect of low dose rate ionizing radiation on the division potential of cells in vitro. VIII. Cytogenetic analysis of human fibroblasts. Mech Ag Dev 17:225–235

Boyer B, Kern P, Fourtanier A, Labat-Robert J (1991) Age-dependent variations of the biosynthesis of fibronectin and fibrous collagens in mouse skin. Exp Gerontol 26:375–383

Capanna E (1973) Cytotaxonomy and vertebrate evolution. Academic Press, London

Capra F (1997) The web of life. Anchor Books, Doubleday, New York

Carman TA, Afshari CA, Barrett JC (1998) Cellular senescence in telomerase-expressing Syrian hamster embryo cells. Exp Cell Res 244:33–42

Chen TH, Ruddle EH (1974) Chromosome changes revealed by the Q-band staining method during cell senescence of WI-38. Proc Soc Exp Biol Med 147:533–536

Chen QM, Prowse KR, Tu V, Purdom S, Linskens HK (2001) Uncoupling the senescent phenotype from telomere shortening in hydrogen peroxide-treated fibroblasts. Exp Cell Res 265:294–303

Cheung HT, Nadakavukaren MJ (1983) Age-dependent changes in the cellularity and ultrastructure of the spleen of Fischer F344 rats. Mech Ageing Dev 22:23–33

Cossarizza A, Ortolani C, Paganelli R, Barbieri D, Monti D, Sansoni P, Fegiolo U, Castellani G, Bersanii F, Londei M, Franceschi C (1996) CD45 isoforms expression on $CD4^+$ and $CD8^+$ T cells throughout life, from newborns to centenarians: implications for T cell memory. Mech Ageing Dev 86:173–195

Coviello-McLaughlin G, Prowse KR (1997) Telomere length regulation during postnatal development and ageing in Mus spretus. Nucleic Acids Res 25:3051–3058

Cristofalo VJ, Sharf BB (1973) Cellular senescence and DNA synthesis. Thymidine incorporation as a measure of population age in human diploid cells. Exp Cell Res 76:419–427

Cristofalo VJ, Allen RG, Pignolo RJ, Martin BJ, Beck JC (1998) Relationship between donor age and the replicative lifespan oh human cells in culture. A reevaluation. Proc Natl Acad Sci USA 95:10614–10619

Decker LM, Chavez E, Vulto I, Lansdorp PM (2009) Telomere length in Hutchinson-Gilford Progeria syndrome. Mech Ag Dev 130:377–383

Dimri G, Lee G, Basile M, Acosta M, Scott G, Roskelly C, Medranos E, Linskens M, Rubellj I, Pereira-Smith OM, Peacocke M, Campisi J (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 92:9363–9367

Duan H, Gao F, Li S, Nagata T (1993) Postnatal development and aging of esophageal epithelium in mouse: a light and electron microscopic radioautographyh study. Cell Mol Biol 39:309–316

Evans CH, Georgescu HJ (1983) Observations on the senescence of cells derived from articular cartilage. Mech Ageing Dev 22:179–191

Evans RJ, Wyllie FS, Wynford-Thomas D, Kipling D, Jones CJ (2003) A p-53-dependent, telomere-independent proliferative life span barrier in human astrocytes consistent with the molecular genetics of glioma development. Cancer Res 63:4854–4861

Farinati F, Formentini S, Della Libera G, Valiante F, Fanton MC, Di Mario F, Vianello F, Pilotto A, Naccarato R (1993) Changes in parietal and mucous cell mass in the gastric mucosa of normal subjects with age. Gerontology 39:146–151

Fedoroff S, Ahmed I, Wang E (1990) The relationship of expression of statin, the nuclear protein of non-proliferating cells, to the differentiation and cell cycle of astroglia in cultures and in situ. J Neurosci Res 26:1–15

Fleischmajer R, Perlish JS, Bashey RI (1972) Human dermal glycosaminoglycans and aging. Biochem Biophys Acta 279:265–275

Forsyth NR, Elder FFB, Shay JW, Wright WE (2005) Lagomorphs (rabbits, pikas and hares) do not use telomere-directed replicative aging in vitro. Mech Ag Dev 126:685–691

Frenck RW Jr, Blackburn EH, Shannon KM (1998) The rate of telomere sequence loss in human leukocytes varies with age. Proc Natl Acad Sci USA 95:5607–5610

Gibbons GH, Dzau VJ (1994) The emerging concept of vascular remodeling. New Engl J Med 330:1431–1438

Globerson A, Effros RB (2000) Ageing of lymphocytes in the aged. Immunol Today 21:515–521

Goldstein S, Moerman EJ, Soeldner JS, Gleason RE, Barnett DM (1978) Chronologic and physiologic age affect replicative lifespan of fibroblasts from diabetic, prediabetic and normal donors. Science 199:781–783

Green H (1977) Terminal differentiation of cultured human epidermal cells. Cell 11:405–416

Harley CB (1991) Telomere loss: mitotic clock or genetic time bomb. Mutat Res 256:271–283

Harnden DG, Benn PA, Oxford JM, Taylor AMR, Webb TP (1976) Cytogenetically marked clones in human fibroblasts cultured from normal subjects. Somatic Cell Genet 2:55–60

Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 37:614–636

Hayflick L (1977) The cellular basis for biological aging. In: Finch CE, Hayflick L (eds) Handbook of the biology of aging. Van Nostrand Reinhold Company, New York, pp 159–186

Hayflick L, Moorhead PS (1961) The serial subcultivation of human diploid cell strains. Exp Cell Res 25:585–621

Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIPI), but not p16(INK4a). Mol Cell 14:420–430

Herbig U, Ferreira M, Condel D, Carey D, Sedivy M (2006) Cellular senescence in aging primates. Science 311: 1257

Hornsby PJ, Yang LQ, Raju SG, Cheng CY (1991) Changes in gene expression and DNA methylation in adrenocortical cells senescing in culture. Mutat Res 256:103–113

Houck JC, Sharma VK, Hayflick L (1971) Functional failures of cultured human diploid fibroblasts after continued population doublings. Proc Soc Exp Biol Med 137:331–333

Kakuo S, Asaoka K, Ide T (1999) Human is a unique species among primates in terms of telomere length. Biochem Biophys Res Commun 263:308–314

Kang MK, Guo W, Park NH (1998) Replicative senescence of normal human oral keratinocytes is associated with the loss of telomerase activity without shortening of telomeres. Cell Growth Differ 9:85–95

Kipling D, Cook HJ (1990) Hypervariable ultra-long telomeres in mice. Nature 347:400–402

Koch CM, Suchek CV, Lin Q, Bork S, Georgens M, Joussen S, Pallua N, Ho AD, Zenke M, Wagner W (2011) Specific age-associated DNA methylation changes in human dermal fibroblasts. PLoS 6:e16679

Kormann-Bortolotto MH, Borsatto B, Smith MAC (1996) Telomere shortening, ageing, and chromosome damage. Mech Ageing Dev 89:45–49

Krishnaj R, Blandford G (1987) Age-associated alterations in human natural killer cells. I. Increased activity as per conventional and kinetic analysis. Clin Immunol Immunopathol 45:268–285

Linardopoulou EV, Williams EM, Fan Y, Friedman C, Young JM, Trask BJ (2005) Human subtelomeres are hotspots of interchromosomal recombination and segmental duplication. Nature 437:94–100

Londono-Vallejo JA, Der-Sarkissian H, Cazes L, Bacchetti S, Reddel RR (2004) Alternative lengthening of telomeres is characterized by high rates of telomeric exchange. Cancer Res 64:2324–2327

Luke S, Birnbaum R, Verma RS (1994) Centromeric and telomeric repeats are stable in nonagenarians as revealed by the double hybridization fluorescent in situ technique. Genet Anal Tech Appl 11:77–80

Macieira-Coelho A (1967) Influence of cell density on growth inhibition of human fibroblasts in vitro. Proc Soc Exp Biol Med 125:548–552

Macieira-Coelho A (1974) Are non-dividing cells present in ageing cell cultures? Nature 248:421–422

Macieira-Coelho A (1988) Biology of normal proliferating cells in vitro. Relevance for in vivo aging. Karger SA, Basel

Macieira-Coelho A (1991) Chromatin reorganization during senescence of proliferating cells. Mutat Res 256:81–104

Macieira-Coelho A (1995) Chaos in DNA partition during the last mitoses of the proliferative life-span of human fibroblasts. FEBS Lett 358:126–128

Macieira-Coelho A (2000) Cell Immortalization. Progr Mol Subcell Biol, vol 24. Springer, Heidelberg

Macieira-Coelho A (2003) Biology of Aging Progr Mol Subcell Biol, vol 30. Springer, Heidelberg

Macieira-Coelho A (2010) Cancers and the concept of cell senescence. Biogerontology 11:211–227

Macieira-Coelho A, Azzarone B (1982) Aging of human fibroblasts is a succession of subtle changes in the cell cycle and has a final short stage with abrupt events. Exp Cell Res 141:325–332

Macieira-Coelho A, Azzarone B (1988) The transition from primary culture to spontaneous immortalization in mouse fibroblast populations. Anticancer Res 8:669–676

Macieira-Coelho A, Taboury F (1982) A re-evaluation of the changes in proliferation in human fibroblasts during aging in vitro. Cell Tissue Kinetics 15:213–224

Macieira-Coelho A, Pontén J, Philipson L (1966) The division cycle and RNA synthesis in diploid human cells at different passage levels in vitro. Exp Cell Res 42:673–684

Macieira-Coelho A, Bengtsson A, Van der Ploeg M (1982) Distribution of DNA between sister cells during serial subcultivaton of human fibroblasts. Histochemistry 75:11–21

Magalhaes JP, Chainiaux F, de Longueville F, Mainfroid V, Migeot V, Marcq L, Remacle J, Salmon M, Toussaint O (2004) Gene expression and regulation in H_2O_2-induced premature senescence of human foreskin fibroblasts expressing or not telomerase. Exp Geront 39:1379–1389

Makhluf HA, Mueller SM, Mizuno S, Glowacki J (2000) Age-related decline in osteoprotegerin expression by human bone marrow cells cultured in three-dimensional collagen sponges. Biochem Biophys Res Commun 268:669–672

Martens UM, Chavez EA, Poon SSS, Schmoor C, Landsorp PM (2000) Accumulation of short telomeres in human fibroblasts prior to replicative senescence. Exp Cell Res 256:291–299

Martin GM, Sprague CA, Norwood TH, Pendergrass WR (1974) Clonal selection, attenuation, and differentiation in an in vitro model of hyperplasia. Am J Path 74:137–154

McClintock B (1943) Maize genetics. Yearbook Carnegie Inst 42:148–152

Meltzer PS, Guan XY, Trent JM (1993) Telomere capture stabilizes chromosome breakage. Nat Genet 4:252–255

Merz GS, Ross JD (1969) Viability of human diploid cells as a function of in vitro age. J Cell Physiol 74:219–223

Muggleton-Harris AL, Hardy K, Higbee N (1987) Rescue of developmental lens abnormalities in chimaeras of non-cataractous and congenital cataractous mice. Development 99:473–480

Pieraggi MT, Julian M, Bouissou H (1984) Fibroblast changes in cutaneous ageing. Virchows Arch [Pathol Anat] 402:275–287

Potten CS, Hendry JH, Moore JV (1987) Estimates of the number of clonogenic cells in crypts of murine small intestine. Virchows Arch B Cell Pathol 66:227–234

Prowse KR, Greider CW (1995) Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proc Natl Acad Sci USA 92:4818–4820

Reis RJS, Goldstein S (1982) Variability of DNA methylation patterns during serial passage of human diploid fibroblasts. Proc Natl Acad Sc USA 79:3949–3953

Rheinwald JG, Green JH (1977) Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature 265:421–424

Rheinwald JG, Hahn WC, Ramsey MR, Wu JY, Guo Z, Tsao H, de Luca M, Catricalà C, O'Toole M (2002) A two-stage, p16INK4A and p53-dependent keratinocyte senescence mechanism that limits replicative potential independent of telomere status. Mol Cell Biol 22:5157–5172

Robert L, Jacob MP, Frances C, Godeau G, Hornebeck W (1984) Interaction between elastin and elastases and its role in the aging of the arterial wall, skin and other connective tissues. A review. Mech Ageing Dev 28:155–166

Rogalla P, Rohen C, Henning Y, Deichert U, Bonk U, Bullerdiek J (1995) Telomere repeat fragment sizes do not

limit the growth potential of uterine leiomyomas. Biochem Biophys Res Commun 211:175–182
Röhme D (1981) 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 USA 78:5009–5013
Roholl PJM, Blauw E, Zurcher C, Dormans JAMA, Theuns HM (1994) Evidence for a diminished maturation of preosteoblasts into osteoblasts during aging in rats: an ultrastructural analysis. J Bone Mineral Res 9:355–366
Rudolph KL, Chang S, Lee H-W, Blasco M, Gottleib GJ, Greider C, DePinho RA (1999) Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell 96:701–712
Saksela E, Moorhead PS (1963) Aneuploidy in the degenerative phase of serial cultivation of human cell studies. Proc Natl Acad Sci USA 50:390–395
Salk D (1982) Werner syndrome: a review of recent research with an analysis of connective tissue metabolism, growth control of cultured cells and chromosome aberrations. Hum Genet 62:1–25
Schaetzlein S, Lucas-Hahn A, Lemme E, Kues WA, Dorsch M, Manns MP, Niemann H, Lenhard-Rudolph K (2004) Telomere length is reset during early mammalian embryogenesis. Proc Natl Acad Sci USA 101:8034–8038
Schmitt H, Blin N, Zankl H, Scherthan H (1994) Telomere length variation in normal and malignant human tissues. Genes Chromos Cancer 11:171–177
Schneider EL, Gilman B (1979) Sister chromatid exchanges in human diploid fibroblasts. Hum Genet 46:57–62
Schultz VP, Zakian VA, Ogburn CE, McKay J, Jarzebowicz AA, Edland SD, Martin GM (1996) Accelerated loss of telomeric repeats may not explain accelerated replicative decline of Werner syndrome cells. Hum Genet 97:750–754
Shiels PG, Kind AJ, Campbell KHS, Waddington D, Wilmut I, Colman A, Schnieke AE (1999) Analysis of telomere length in cloned sheep. Nature 399:316–317
Simon M, Green H (1984) Participation of membrane-associated proteins in the formation of the cross-linked envelope of the keratinocyte. Cell 36:827–834
Simonneau L, Hervé B, Jacquemin E, Courtois Y (1983) State of differentiation of bovine epithelial lens cells in vitro. Exp Cell Res 145:433–446
Smith SS (1994) Species-specific differences in tumorigenesis and senescence. Trends Genet 10:305–306
Stanley JF, Pye D, MacGregor A (1975) Comparison of doubling numbers attained by cultured animal cells with life span of species. Nature 255:158–159
Steinert S, White DM, Zou Y, Shay JW, Wright WE (2002) Telomere biology and cellular aging in nonhuman primate cells. Exp Cell Res 272:146–152
Strasser H, Tiefenthaler M, Steinlechner M, Bartsch G, Konwalinka G (1999) Urinary incontinence in the elderly and age-dependent apoptosis of rhabdosphincter cells. Lancet 354:918–919
Suzuki K, Mori I, Nakayama Y, Miyakoda M, Kodama S, Watanabe M (2001) Radiation-induced senescence-like growth arrest requires TP53 function but not telomere shortening. Radiat Res 155:248–253
Takenaka Y, Matsuura T, Haga N, Mitsui Y (2001) Expression of telomerase reverse trranscriptase and telomere elongation during sexual maturation in Paramecium caudatum. Gene 264:153–161
Takubo K, Izumiyama-Shimomura M, Homa N, Sawabe M, Arai T, Kato M, Oshimura M, Nakamura K-I (2002) Telomere lengths are characteristic in each human individual. Exp Geront 37:523–531
Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR (2001) Tissue-specific alternate splicing of human telomerase reverse transcriptase (HTERT) influences telomere lengths during human development. Int J Cancer 91:644–649
Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001) Control of synapse number by glia. Science 291:657–660
Unryn BM, Cook LS, Riabowoll KT (2005) Paternal age is positively linked to telomere length of children. Aging Cell 97:97
Vaziri H, Schächter F, Uchida I, Wei L, Zhu X, Effros R, Cohen D, Harley CB (1993) Loss of telomeric DNA during aging of normal and trisomic 21 human lymphocytes. Am J Hum Genet 52:661–667
Venkatesan RN, Price C (1998) Telomerase expression in chickens: Constitutive activity in somatic tissues and down-regulation in culture. Proc Natl Acad Sci USA 95:14763–14768
Wakayama T, Shinkai Y, Tamashiro KLK, Niida H, Blanchard DC, Ogura A, Tanemura A, Tachibana M, Perry ACF, Colgan DF, Mombaerts P, Yanagimashi R (2000) Cloning of mice to six generations. Nature 407:318–319
Wilson VL, Jones PA (1983) DNA methylation decreases in aging but not in immortal cells. Science 220:1055–1056
Zhu J, Wang H, Bishop JM, Blackburn EH (1999) Telomerase extends the lifespan of virus-transformed human cells without net telomere lengthening. Proc Natl Acad Sci USA 96:3723–3728