Eight

Cell Proliferation in Mammalian Aging

Peter J. Hornsby

I. The Maintenance of Organ Size: Atrophy and Hypertrophy in Aging

Although there are changes in cell proliferation during aging, as reviewed here, the more remarkable fact is that organ and tissue size do not change greatly with aging beyond the point of maturity of the body. This fact is inherent in the life history of most animals, although some continue to grow throughout their life span and have no fixed body size (Finch, 1991). In most species, including all mammals, a fixed body size is achieved at maturity and changes are relatively minor beyond that point. Of course, there are some easily recognized exceptions. Fat deposits change in distribution, and some organs, such as muscle and the dermis of the skin, show some degree of atrophy. Although histological structure may change during aging (see later discussion), most organs and tissues are unchanged in size from maturity to old age. It is easy to overlook the significance of this fact; it is nontrivial and requires explanation, although knowledge of how tissue and organ size are regulated is mostly lacking (Tanner, 1999). Of course for some cell types, such as neurons and muscle fibers, the number of cells is relatively fixed, but even in these cases there is the possibility of replacement from stem cell compartments throughout life (Flax *et al.*, 1998; Carpenter *et al.*, 1999), and other cells in the nervous system and in muscles are not postmitotic. In most tissues there is constant cell turnover throughout life, even though it may be quite slow compared to embryonic growth. In some tissues, such as the skin epidermis, the gut epithelium, and the hematopoietic system, there is relatively rapid cell proliferation occurring throughout the life span. The healthy function of these tissues is dependent on regulated cell proliferation. In these tissues with rapid cell turnover, organ size is determined mainly by the architecture of the tissue: separation of the stem cell compartment from the rest of the tissue and localized loss of cells from another compartment (the surface of the skin, the lining of the gut, or the removal of old red cells from circulation). In other organs, such as the liver and most endocrine organs, total organ size remains constant despite cell proliferation occurring within the tissue,

Handbook of the Biology of Aging, Fifth Edition

and there is not a clear architectural separation of the stem cell compartment. The constancy implies feedback mechanisms that monitor total organ size, presumably by the level of function of the tissue, but these mechanisms are poorly understood.

There are some few exceptions to the general rule of constant organ size. A notorious example is the prostate gland in humans. Beyond the age of about 50 the prostate increases in size, a process termed benign prostatic hyperplasia (Oesterling *et al.*, 1993), which may result from defective apoptosis (Kyprianou *et al.*, 1996). Although this might be viewed as a specific pathological process, i.e., a disease rather than aging, it becomes symptomatic in almost all individuals by the age of about 90, and there is little reason not to consider it part of normal aging, with the extent of the phenomenon and the age of onset of symptoms being quite variable (Oesterling *et al.*, 1993). This example of continued growth highlights by comparison how successful size maintenance is for other organs. Even in the case of the prostate gland, although there must be an excess of proliferation over cell death, the excess is very small or else the gland would expand rapidly like a growing tumor. Therefore, there is only a minimal perturbation in the normal control of organ size, but unfortunately for male human health the control is not perfect.

We need to distinguish these postmaturity changes from changes in tissues that are essentially developmental. This is the case for the involution of the thymus. Because this involution starts well before maturity and is more or less complete before the end of the reproductive period, it should not be considered as an aging process. This is not simply a semantic question, because any process that begins well before reproductive age must be under strong evolutionary selective pressure, whereas those processes that either do not start until after reproductive age or have slight effects before reproductive age may escape the force of natural selection (Lithgow & Kirkwood, 1996). Therefore, mechanisms for pre-maturity and post-maturity changes may be quite different. The changes in the prostate are manifest only well after maturity and have escaped the force of natural selection. A possible example of true involution of a tissue during aging is the decrease in size and function of the zona reticularis of the human adrenal cortex, the zone of the cortex that secretes dehydroepiandrosterone (DHEA) (Hornsby, 1995). It is likely that these cells disappear during aging, whereas the cortisol-secreting zone, the zona fasciculata, is maintained by feedback mechanisms that apparently are lacking for the maintenance of the zona reticularis (Hornsby, 1995).

Questions about changes in cell proliferation in aging must be posed in terms of the overall maintenance of organ size. If there are changes in proliferation, they are compensated for primarily by changes in the rate of cell death. Alternatively, and possibly more likely, changes in cell death rates during aging are compensated for by changes in cell proliferation, so as to maintain constant organ size.

Another consequence of the argument presented previously is that it seems very unlikely that in aging there is simply a cessation of cell proliferation in any tissue that shows some cell turnover over most of the life span. Basic data on cell proliferation rates have come from cell labeling studies *in vivo*. [^{3}H]Thymidine or bromodeoxyuridine (BrdU) is administered to an animal, and the incorporation of these nucleotides into DNA is assessed by autoradiography of tissue sections or by immunohistochemistry. In situations where the administration of precursors is not possible or practical, e.g., for human surgical specimens, direct counting of mitotic figures can be done, but this requires very large numbers of sections.

Surrogate markers for cell proliferation can be useful, such as immunohistochemistry for Ki–67 antigen or proliferating cell nuclear antigen (PCNA). More indirect measures are of enzymes that are required for DNA synthesis or of proteins that increase when cells are stimulated by mitogens, such as c-Myc (Majumdar *et al.*, 1989). Direct measurements of cell proliferation rates confirm that, in tissues with continuously dividing cells, proliferation continues throughout life (reviewed later).

However, raw data on changes in cell proliferation in aging are not necessarily very useful. For each tissue or organ being studied, it is important to understand the specific way in which cell division is regulated, particularly with respect to the stem cell compartment if there are stem cells in the tissue. As emphasized earlier, because most organs maintain a constant size in old age, if there are decreases or increases in the proliferation rate, they must be compensated for by changes in the rate of cell loss by apoptosis or other means.

For each of the tissues and organs where cell proliferation occurs throughout life, the questions that need to be addressed are as follows: (a) whether there are any age-related changes in cell proliferation under normal conditions, and if there are changes, whether these changes result from intrinsic changes in cell proliferation capacity or whether they are secondary to the environment of the cell (exposure of the cell to hormones, cytokines, changes in extracellular matrix, or alterations in the blood supply); (b) whether there are changes that are only apparent when the reserve capacity of the tissue is challenged, e.g., by injury or stresses such as blood loss; and (c) whether changes in cell proliferation have indirect effects, i.e., effects of the presence of cells within the tissue that have lost the ability to divide. The data that are reviewed here show that great progress has been made in answering these questions in some organ systems, but that in others many fundamental questions have yet to be addressed.

II. Principles of Growth Control

A. Cell Proliferation in Culture versus *In Vivo*

There is a very practical reason why the control of tissue and organ size is much less well understood than the basic control of the cell cycle: most of our knowledge of cell cycle regulation comes from experiments initially performed on cells in culture. Studies in knockout mice have complemented the cell culture studies, but it is the rule rather than the exception that basic knowledge of the regulation of cell proliferation has been obtained first in cultured cells and only afterward applied to whole animal systems. Obviously, one must be very careful in applying the knowledge of the regulation of cell proliferation obtained from cell culture to the *in vivo* situation. The problem is that cell culture gives us a rather one-sided view of proliferation control. In culture, proliferation is driven by the availability of mitogens and by the fact that the cells are attached to a surface that is permissive for cell division. Provided the cells do not become too crowded, a parameter that is under the control of the experimenter, proliferation is driven purely by mitogen concentrations. *In vivo*, the situation is much more complex, and we must apply our knowledge of control of the cell cycle to the mechanism by which tissue size is regulated.

B. Role of Angiogenesis

One very important aspect of the regulation of tissue size is the regulation of angiogenesis in tissues. This was first recognized from studies of malignant

tumors that require new angiogenesis to grow beyond a very small size (Hanahan & Folkman, 1996). Angiogenesis also is likely to regulate and limit the growth of normal tissues (Risau, 1997). The sprouting of capillaries, proliferation of endothelial cells, and maturation of new capillaries are tightly linked to proliferation of the other cells in the tissue. This lock-step system is much more suited to fine-tuning of tissue and organ size than simple mitogen availability, as in cell culture. Moreover, the model naturally accommodates normal cell turnover: as cells die, they are replaced by cell division according to the capacity of the vascular system to support a specific mass of cells.

III. Replicative Senescence and Immortalization

A. Human Cells

It has been known since the pioneering experiments of Leonard Hayflick in the 1960s that the limited replicative capacity of human cells in culture is very unlikely to be a experimental artifact, but is a reproducible biological phenomenon (Hayflick, 1980). However, it was not until it was discovered that the limitation of replicative capacity directly correlates with telomere shortening that the notion that it might be a culture artifact was finally laid to rest (Harley *et al.*, 1990; Allsopp *et al.*, 1992). Telomeres shorten in most dividing human somatic cells because of the lack of telomerase activity that is required for telomere maintenance (Greider, 1990; Harley, 1991). The lack of telomerase activity results from the absence of expression of the reverse transcriptase subunit (TERT) of the telomerase ribonucleoprotein complex (Lingner *et al.*, 1997; Meyerson *et al.*, 1997). When cells divide in the absence of telomerase activity, about 40–100 bp of the terminal telomeric repeat DNA are not replicated (Greider, 1990; Harley, 1991). This amount is a constant for various types of human cells, thus providing a kind of mitotic counter (Greider, 1990; Harley, 1991).

After a normal human cell has divided a certain number of times, that number varying with the specific cell type and culture conditions, the telomeres become so short that they trigger a cell cycle checkpoint that puts the cell into a terminally nondividing state. This state commonly has been termed "cellular senescence" or "replicative senescence," the implication of these terms being that it is a form of aging. In this chapter, the phenomenon is termed replicative senescence, but that term is used without the intention of implying that it is necessarily a manifestation of aging at the cellular level. The block to proliferation in replicative senescence is similar to that caused by double-strand breaks in cellular DNA (Harley, 1991), although whether this is actually the mechanism by which short telomeres are recognized is not clear. Further cell division then is blocked by inhibitors of cell proliferation, such as $p21^{SDI1/WAF1/CIP1}$ and $p16^{INK4A}$ (Noda *et al.*, 1994; Smith & Pereira-Smith, 1996). When this checkpoint is abrogated by oncoproteins, such as SV40 T-antigen, this first checkpoint (sometimes termed M1) is bypassed and cells eventually enter a second state, termed crisis or M2 (Holt *et al.*, 1996). In this state, the much shorter telomeres undergo end-to-end fusions resulting in chromosomal breakage—fusion cycles that cause the cells to undergo apoptosis. There is massive loss of cells from the culture, whereas in replicative senescence (M1) cells do not die but enter a permanently nonreplicating state (Smith & Pereira-Smith, 1996). In this state cells are more resistant to apoptosis (Wang, 1995).

The term replicative senescence therefore encompasses two different phenom-

ena: one is the process of telomere shortening, resulting from the lack of expression of TERT, and the second is the process by which telomere shortening shuts off further cell division. It is important to distinguish these two processes because they may have quite different implications for aging *in vivo*.

Most cancer cells that can be grown in culture do not show limits on replication and can divide indefinitely; they are said to be immortalized, and again this term is used here without implying reversal of an aging process. In the absence of genetic changes like the introduction of SV40 T-antigen, most human cells have an unmeasurably low rate of spontaneous immortalization, whereas when SV40 T-antigen is present, rare cells escape from crisis and become immortalized by reexpression of TERT or by other mechanisms (Shay & Wright, 1989; Ray & Kraemer, 1993; Shay *et al.*, 1993; Cheng *et al.*, 1997; Xia *et al.*, 1997; Shammas *et al.*, 1997). The mechanisms by which cancer cells reactivate the expression of TERT or activate alternate mechanisms for avoiding telomere shortening are largely unknown. There appear to be several pathways by which these processes can occur: however, that there are at least four is shown by the fact that immortal human cells may be divided into four complementation groups (Smith & Pereira-Smith, 1996). Within each complementation group, fusion of a cell from one line with a cell from the same or another line produces hybrid cells with indefinite replicative capapcity, whereas fusions of cells from different complementation groups result in hybrid cells that enter the replicative senescent state after a limited number of divisions. The genes involved in these effects have been located to specific chromosomes, and the effects of fusion of two nuclei can be duplicated by the introduction of single chromosomes or chromosome fragments into a cell (Bertram *et al.*, 1999; Cuthbert *et al.*, 1999).

The phenomenon of telomere shortening clearly is the result of the lack of expression of TERT in many normal human somatic cells. At first this was described simply as the absence of telomerase activity in most normal human somatic cells and the presence of telomerase activity in the germ line and in cancer cells. Subsequently, it has become clear that many normal human cells, including many stem cells and other cells that are required to undergo repeated replications, do express TERT, but that expression is tightly regulated by processes not yet well-understood (Belair *et al.*, 1997; Ramakrishnan *et al.*, 1998; Wu *et al.*, 1999; Hodes, 1999). In some cell types, telomerase activity is induced when cells are first isolated from the body and stimulated to divide in culture (Yasumoto *et al.*, 1996; Hsiao *et al.*, 1997; Kunimura *et al.*, 1998). Curiously, however, longer term proliferation is associated with a decline in telomerase activity, sometimes very rapid, so that few long-term cultures of any normal human cells have shown both telomerase activity and telomere maintenance sufficient for immortalization. However, there is at least one and possibly other important exceptions to this generalization. Embryonic stem cells have been isolated and placed in culture at a stage of embryogenesis before TERT expression is down-regulated (Thomson *et al.*, 1998). If they are grown under conditions that prevent differentiation from occurring, telomerase activity is maintained at a level sufficient for indefinite growth. Mesenchymal stem cells show telomerase activity even after extensive growth in culture (Pittenger *et al.*, 1999), but it is not known whether they can grow indefinitely. Additionally, human neural stem cells can be grown for long periods in culture, but their potential for indefinite growth is unknown (Flax *et al.*, 1998; Carpenter *et al.*, 1999).

Proof that the limitation on indefinite cell division in most human cells results from lack of expression of TERT was obtained by showing that forced expression of TERT is sufficient to immortalize normal human fibroblasts and retinal pigmented epithelial cells and is required (although not sufficient by itself) to immortalize keratinocytes and mammary epithelial cells (Bodnar *et al.*, 1998; Kiyono *et al.*, 1998). Immortalization was accompanied by increased or stabilized telomere length, but cells retain a normal karyotype (Jiang *et al.*, 1999; Morales, 1999). Conversely, immortalized cells suffer telomere shortening and the eventual cessation of growth when telomerase is inhibited (Hahn *et al.*, 1999b; Zhang *et al.*, 1999; Shammas *et al.*, 1999).

The second part of the phenomenon of replicative senescence is the state that cells enter when shortening of telomeres has occurred. This state is characterized by high levels of expression of cell cycle inhibitors, principally $p21^{SDI1/WAF1/CIP1}$ and $p16^{INK4A}$ (Noda *et al.*, 1994; Smith & Pereira-Smith, 1996). A useful biochemical marker, although one of unknown biological sigificance, is the high level of β-galactosidase enzymatic activity with a pH optimum of 6.0, termed senescence-associated β-galactosidase (SA-βgal) (Dimri *et al.*, 1995). The same biochemical pathway and molecular markers can occur under circumstances that do not involve telomere shortening; it is possible to drive cells into this state by mechanisms that do not involve cell division at all, such as oxidative stress, radiation, and the ectopic expression of some signal transduction molecules and cyclin-dependent kinase inhibitors (Robles & Adami, 1998; Serrano *et al.*, 1997; Zhu *et al.*, 1998). Interestingly, many of these interventions potentially are oncogenic. There are indications that some human cell types enter replicative senescence by a replication-dependent process that does not involve telomere shortening, but the mechanisms are not well-understood (Wynford-Thomas, 1999).

Patterns of gene expression in replicative senescent cells clearly differ from those of cells in the nonsenescent state. In fibroblasts, the pattern resembles that of fibroblasts in inflammation (Shelton *et al.*, 1999). Of particular significance is the production of proteases that may erode the surrounding extracellular matrix and the production of cytokines that could have effects on neighboring cells (Sottile *et al.*, 1989; West, *et al.*, 1989; Kumar *et al.*, 1992; Millis *et al.*, 1992). Interestingly, other cell types (retinal pigmented epithelial cells and endothelial cells) show different patterns of alteration of gene expression when they reach replicative senescence (Shelton *et al.*, 1999). The data are consistent with the hypothesis that the triggering of the block to DNA synthesis that is characteristic of replicative senescence is accompanied by dysregulation of the expression of various other genes and that the pattern of dysregulation will be cell-type-specific.

Therefore, the replicative senescent state appears to be a universal process that is a reaction of mammalian cells to certain kinds of damage. The process presents a puzzle in terms evolutionary biology. The kinds of damage that cause cells to enter this state are very similar to those kinds of damage that cause other cells to enter apoptosis. From the point of view of the organism and the genome, making cells undergo apoptosis makes sense because the damaged cell and its progeny, carrying potentially damaged copies of the genome, are removed from the body. One may consider cells to be very cheap in terms of the overall economy of the body—millions of cells are born and die every day, and there would seem to be no reason why cells should be preserved via the "replicative senescence" process rather than killed off via apoptosis. It is possible that the process is related to the mechanisms whereby fine

control is exerted over tissue growth and organ size (Martin, 1993), but there is no completely convincing explanation.

B. Important Differences Between Human and Mouse Cells

A recurring theme in this chapter will be to emphasize differences in the changes in proliferation during aging in humans and other primates versus mice and other rodents. There is an emphasis on contrasting human cell properties with those of mouse cells because of the extraordinary value of the experimental ability to make germ-line genetic changes in mice. To summarize the argument made here, although there are changes in cell proliferation observed in mice and rats with age, these changes do not necessarily have the same significance as changes in cell proliferation in humans. Human cells have relatively short telomeres compared to those of mice and rats (although not all rodents have long telomeres) (Blasco *et al.*, 1997; Coviello-McLaughlin & Prowse, 1997). Whereas most somatic human tissues and cells are telomerase-negative, many mouse and rat tissues and cells are telomerase-positive (Prowse & Greider, 1995). It is interesting that both human and rodent cells have a limited replicative capacity in culture. Moreover, some of the biochemical features of the nonreplicating state that the cells enter appear to be common between human and mouse cells. However, the mechanism by which cells enter replicative senescence in culture clearly has been shown to result from telomere shortening in human cells, but this cannot be the case for mouse cells. Mouse cells undergo 10–20 divisions in culture before replicative arrest and arrest without significant telomere shortening. Cells isolated from mice in which the gene for the telomerase RNA component has been inactivated ($TR^{-/-}$ mice), which have shorter telomeres than wild-type cells, also undergo 10–20 doublings in culture before growth arrest (Blasco *et al.*, 1997). Moreover, mouse cells bypass the replicative senescent state and undergo spontaneous immortalization at a very high rate; no specific experimental interventions are required to obtain immortalized cell lines (Wright & Shay, 2000). Immortalization occurs in 100% of cells from $p19^{ARF-/-}$ or $p53^{-/-}$ mice (Harvey *et al.*, 1993; Kamijo *et al.*, 1997). Immortalized mouse cells are almost always aneuploid (Worton & Duff, 1979), but the genetic changes that have taken place to bypass the initial step in replication are not known.

However, the mouse cell "replicative arrest" in culture and human cell "replicative senescence" have some biochemical similarities (Wright & Shay, 2000). The function of p53, although not pRb, is required in mouse cells; the arrest is maintained by high levels of cell cycle inhibitory proteins, and SA-βgal is induced. Mouse cells enter replicative arrest after exposure to the same agents that induce replicative senescence in human cells. A reasonable hypothesis is that replicative arrest in mouse cells is a direct result of the transition from the *in vivo* to the cell culture environment, because of DNA damage or other factors (Wright & Shay, 2000).

Considering the differences between the species, there is a much greater possibility that there could be exhaustion of cellular proliferation in humans than in mice. Consider also that the task of human stem cells is to repopulate and to supply a much larger number of cells over a much longer period than is the case for the mouse. For example, in the hematopoietic system, stem cells in humans have to supply red blood cells for a blood volume that is about 4000 times larger than that of the mouse over a time period that is about 30 times longer than the 3-year maximum life span of the mouse.

Unfortunately, the possibility that human and mouse cellular aging might differ means that data from proliferation during the aging of mice with various genetic changes or experimental manipulations must be evaluated carefully in relation to its potential relevance to aging in humans. The same genetic and other interventions usually are not possible in humans, and available data are restricted to clinical observations and experiments on cells isolated and studied in culture.

Mice with an inactivated telomerase RNA gene ($TR^{-/-}$ mice), whose cells lack telomerase activity, suffer telomere shortening (Blasco *et al.*, 1997). Could the $TR^{-/-}$ mouse provide a more "humanized" rodent model for aging? The data are very interesting, but not conclusive, at the time of writing this chapter (Rudolph *et al.*, 1999; Herrera *et al.*, 1999; Kipling & Faragher, 1999). After three generations, the normally long mouse telomeres have shortened in the $TR^{-/-}$ animals, and these mice present a picture of a "segmental progeroid syndrome," i.e., some aspects of the phenotype resemble accelerated aging. They have premature graying and loss of hair, poor wound healing, increased cancer incidence, gastrointestinal defects, infertility, decreased adipose tissue, and a shortened life span. Clearly, each of these phenotypic effects must be caused by the lack of telomerase activity (i.e., shortening of telomeres) in some cell type or other, but a problem for interpretation is that short telomeres in these animals might not necessarily induce the same cellular state that has been characterized as replicative senescence in human cells. $TR^{-/-}$ mice are viable to the sixth generation. Well before this point, increased numbers of end-to-end chromosome fusions are observed (0 in metaphase in wild type; 0.26 in generation 2; 0.56 in generation 4; and 1.93 in generation 6) (Blasco *et al.*, 1997). Regeneration of the liver is impaired in sixth-generation mice, but cells do not arrest at G1/S, as expected if the short telomeres trigger replicative senescence, but instead hepatocytes have impaired progress through mitosis and show many aberrant mitotic figures (Rudolph *et al.*, 2000). Although there are karyotypic abnormalities in human cells nearing senescence (Chen & Ruddle, 1974), most cells appear to stop dividing with a normal karyotype. Bovine cells also senesce as a result of telomere shortening; the fact that animals can be cloned from the nuclei of bovine cells close to senescence also shows that replicative senescence is not associated with chromosomal abnormalities, at least not those that would prevent the formation of a viable organism (Lanza *et al.*, 2000).

The human genetic disease X-linked dyskeratosis congenita was identified as a disease of impaired telomerase activity and shortened telomeres (Mitchell *et al.*, 1999). The protein product of the gene that is defective in DKC, dyskerin, is required for proper RNA processing, including the RNA of the telomerase ribonucleoprotein complex. In this syndrome, there are proliferative defects in tissues known to have telomerase-positive stem cells (hematopoietic system and skin). DKC patients have very short telomeres in fibroblasts and white blood cells. They usually die of bone marrow failure at a young age. However, the disease is also associated with chromosomal abnormalities and early death from some malignancies. Therefore, there are similarities between telomerase-deficient mice and telomerase-deficient humans. In both, chromosomal aberrations may cause defective proliferation. Whether replicative senescence accounts for some of the pathology in dyskeratosis congenita is unknown, but the evidence could also be interpreted as indicating that shortening of telomeres in human tissues *in vivo* might lead to crisis rather than replicative senescence.

C. Repression of TERT Expression as an Anti-cancer Mechanism

Because TERT appears to be reexpressed in the great majority of human cancers, it has been hypothesized that the process by which TERT is repressed in most somatic cells is an anticancer mechanism. It may contribute to the large difference in susceptibility to cancer (calculated on a per cell basis) between mice and humans. Suppose that mice and humans have the same risk of dying of cancer over their life spans [approximately true at least for some strains of mice (Peto *et al.*, 1985)]. However, a human being is about 3000 times heavier than a 25-g mouse and lives about 30 times as long. Consider also that cells are about the same size in mice as in humans and that cell turnover occurs at about same rate. All of these assumptions may not be entirely correct but this does not substantially affect the basic validity of this argument. It then is evident that human cells are approximately 90,000 times more resistant to tumorigenic conversion per unit of time than are mouse cells. Presumably, as part of the evolution of the life history of the human species, anticancer mechanisms evolved that were not present in short-lived ancestors. In this case, the anticancer process may provide an example of antagonistic pleiotropy, the genetic event (repression of TERT) having beneficial effects early in the life span and possibly negative effects late in the life span (Williams, 1957; Campisi, 1997). The best evidence that TERT repression is indeed an anticancer mechanism in human cells comes from data showing that the well-known oncoproteins Ras and SV40 T-antigen cannot transform a normal human cell into a tumor cell unless they are also expressed together with TERT (Hahn *et al.*, 1999a). Presumably, the reason that TERT can cooperate with other oncogenes is that, during the process by which a normal cell becomes a fully malignant tumor cell, many cell divisions must take place and telomeres would become critically short unless the cell activates telomerase or other mechanisms to prevent telomere shortening. In mice, such anticancer strategies are unnecessary for their life history. Their small size and short life span mean that they are not more likely than humans to die of cancer before being able to reproduce. Thus, there has not been an evolutionary selective pressure to repress TERT expression in this species (and presumably in other similar small, short-lived mammals, although this has not yet been well-studied). Presumably there are similar arguments that can be made in terms of trade-offs between the advantages and disadvantages of long and short telomeres (Wright & Shay, 2000). Evidently, however, an organism that adopts TERT repression as an evolutionary anticancer strategy must also have short telomeres, or TERT repression becomes irrelevant to the suppression of malignant transformation.

D. Replicative Senescence: Summary and Conclusions

Data on the limited replicative capacity of cultured cells isolated from humans and mice of different ages and genotypes must be interpreted carefully in view of the likely different mechanisms by which terminally nondividing states are achieved in human and mouse cells. In humans, there is substantial evidence that telomere shortening occurs *in vivo*, as reviewed later, thus linking *in vivo* aging to the cell culture phenomenon of replicative senescence. When human cells without TERT or with low levels of TERT divide, they suffer telomere shortening, and this occurs whether they are dividing *in vivo* or in culture. However, in the absence of knowing precisely what limits mouse cell growth in culture, it is not possible to state that the process by

which mouse cells exhibit a limited replicative capacity in culture does or does not occur *in vivo*.

IV. Methods for Studying Cell Proliferation in Aging

Experimental transplantation of cells and tissues has been very useful in addressing basic questions of whether there are intrinsic limits on the proliferation of cells *in vivo* and whether those limits are reached during aging. One protocol is to transplant cells or tissues to a recipient animal from which the corresponding endogenous tissue has been removed. For example, this can be done by irradiation in the case of the hematopoietic system or by "clearing" the mammary fat pad in the case of the mammary gland (see later discussion). After the transferred tissue has engrafted and expanded to its maximum size in the host, it is removed and the procedure is repeated with a second recipient animal. The transfer is repeated to successive recipients to test whether there is a point when reconstitution of the recipient fails. Unfortunately, there are a variety of technical obstacles in such transplantation experiments; in stem cell systems, repeated transplantation may fail after some number of passages, not because of an intrinsic limitation on stem cell proliferation but because the handling of the cells during the cell transplantation procedures causes the cells to lose their pluripotent characteristics or because of a failure to transfer a constant number of stem cells each time (see Section VIII on stem cells).

The application of cell transplantation techniques to study human aging has been very limited, but these methods could play an important role. It is important to recognize that cell transplantation can provide both an experimental system for studying fundamental aspects of aging processes as well as potential therapies (Hornsby, 1999). An approach taken in the author's research is to find out how aging affects the ability of human adrenocortical cells to form a functional, vascularized adrenal tissue structure after transplantation into *scid* mice (Hornsby *et al.*, 1998). By transplanting adrenocortical cells into a neutral environment (the young *scid* mouse), we can test how cellular behavior and patterns of gene expression change as a function of population doubling level in culture and as a function of the age of the donor. Cell transplantation experiments can help to elucidate both universal cellular aging processes, the changes that affect all cells in aging, as well as the unique aging processes that occur in specific cell types.

In experimental animals, one can study the effects of interventions that affect life span, such as dietary restriction. Animals under caloric restriction have been reported to show lower cell proliferation rates (Heller *et al.*, 1990; Lok *et al.*, 1990; Hursting *et al.*, 1994; Wolf *et al.*, 1995), but the significance of this observation for the life-prolonging effect is unknown. Genetic effects that affect life span, such as perturbations of growth hormone status, have provided provocative data. Stimulation of growth of the organism as a whole was associated with shortened life span and decreased proliferative potential of isolated cells, whereas genetic defects of growth hormone increase life span (Pendergrass *et al.*, 1993; Brown-Borg *et al.*, 1996).

V. Telomere Shortening and Replicative Senescence *In Vivo*

Human cell proliferation in aging can also be investigated by making primary cultures of cells from donors of different ages and studying their proliferation in culture. In some cases, the complete replicative potential of such cells has been studied, i.e., the cells have been

grown until they reached replicative senescence. In others, colony-forming efficiency has been studied in the primary culture, as a surrogate measure for total replicative capacity (Smith *et al.*, 1978).

Much attention has been paid to the observation originally made in the 1970s that the replicative capacity of human fibroblasts in culture decreases as a function of donor age (Martin *et al.*, 1970). It was well-known even at the time of these initial observations that there was much variation within each decade of age in the maximal and minimal proliferative capacity of the different cell samples. Subsequently, the generality of the observation was challenged by finding that the decrease as a function of donor age applied only to fibroblasts isolated from diabetic and "prediabetic" patients (Goldstein *et al.*, 1978) and was not evident in fibroblasts obtained from non-sun-exposed skin (Gilchrest, 1980; Cristofalo *et al.*, 1998). However, other sets of data have upheld the original observations (Allsopp *et al.*, 1992). Fibroblasts from older donors also showed a higher level of expression of collagenase, which is characteristic of replicative senescent cells (Burke *et al.*, 1994). Additionally, cells from patients with Werner syndrome, a segmental progeroid syndrome, have decreased replicative potential and accelerated telomere shortening (Salk, 1985; Schulz *et al.*, 1996). Fibroblasts are cultured readily, and most cell culture data on the molecular basis for replicative senescence have come from studies on fibroblasts. Unfortunately, fibroblasts as a cell type are not ideal for these kinds of studies. First, the cell population that is isolated is hard to standardize. When fibroblasts are isolated by allowing cells to migrate out from an explant, there is a great deal of selection for which cells form the "starting" culture population (designated as population doubling level zero). Some of the lack of agreement among investigators could result from different isolation techniques, which have provided differing degrees of initial selection. Second, the biology of fibroblasts undoubtedly differs from one organ to another, and even from one part of the skin to another, but these distinctions have not always been considered. Third, most fibroblasts probably are proliferatively quiescent *in vivo* after maturity and undergo very low rates of cell division. Therefore, it would not be surprising if little or no exhaustion of proliferative capacity were observed.

It would be much better to study the effects of donor age on replicative capacity in those cell types where pure populations can be isolated reproducibly from defined sites in the body and where some cell turnover occurs throughout life. In such cell populations there would be at least the potential for exhaustion of proliferative capacity. Such studies become more powerful when there is an ability to correlate the donor age cell culture data with direct *in vivo* data on replicative potential, although obviously this is difficult in humans when such data are from clinical observations rather than from direct experimental intervention.

Studies that have been done on nonfibroblast cell populations have shown much larger decreases in proliferative capacity than was observed as a function of donor age in fibroblasts. In some nonfibroblast cell types, many cells in the population isolated from older donors have very limited or no proliferative capacity. Some examples are age-related decrements in proliferative potential in lens epithelial cells (Tassin *et al.*, 1979; Power *et al.*, 1993), retinal pigmented epithelial cells (Flood *et al.*, 1980), smooth muscle cells (Bierman, 1978; Start *et al.*, 1991; Ruiz-Torres *et al.*, 1999), and osteoblasts (Koshihara *et al.*, 1991; Kassem *et al.*, 1997; D'Ippolito *et al.*, 1999) (Figs. 1 and 2).

Proliferative capacity is closely related to telomere length in endothelial cells

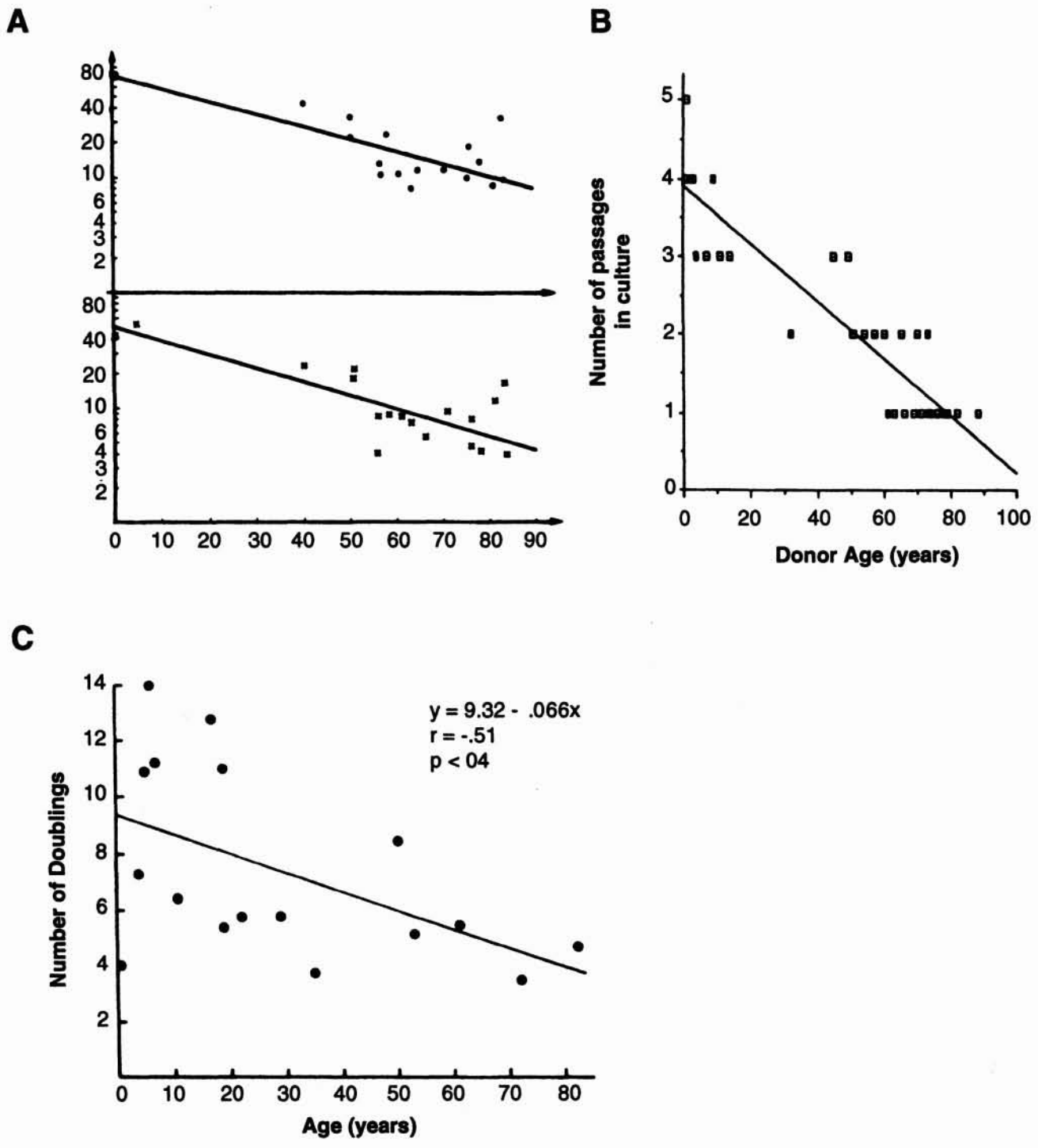


Figure 1. Age-related decline in proliferative potential in human lens epithelial cells and human smooth muscle cells. (A) Labeling index ([^{3}H]thymidine autoradiography) plotted against donor age for primary cultures of lens epithelial cells: top, labeling for 48 hr; bottom, labeling for 24 hr. Reproduced with permission from Tassin *et al.* (1979). (B) Maximum number of passages in culture for lens epithelial cells as a function of donor age. Reproduced with kind permission from Kluwer Academic Publishers from Power *et al.* (1993). (C) Cumulative population doublings for arterial smooth muscle cells as a function of donor age. From Bierman (1978), copyright 1978 by the Society for In Vitro Biology (formerly the Tissue Culture Association). Reproduced with permission of the copyright owner.

(Fig. 2A). Telomere lengths in endothelial cells decreased as a function of donor age, with a greater decline being observed in cells isolated from the iliac artery compared to cells from the thoracic artery (Chang & Harley, 1995) (Fig. 2B). The greater decline in telomere length was observed in the cells that had likely undergone more proliferation *in vivo*, because they resided in a part of the

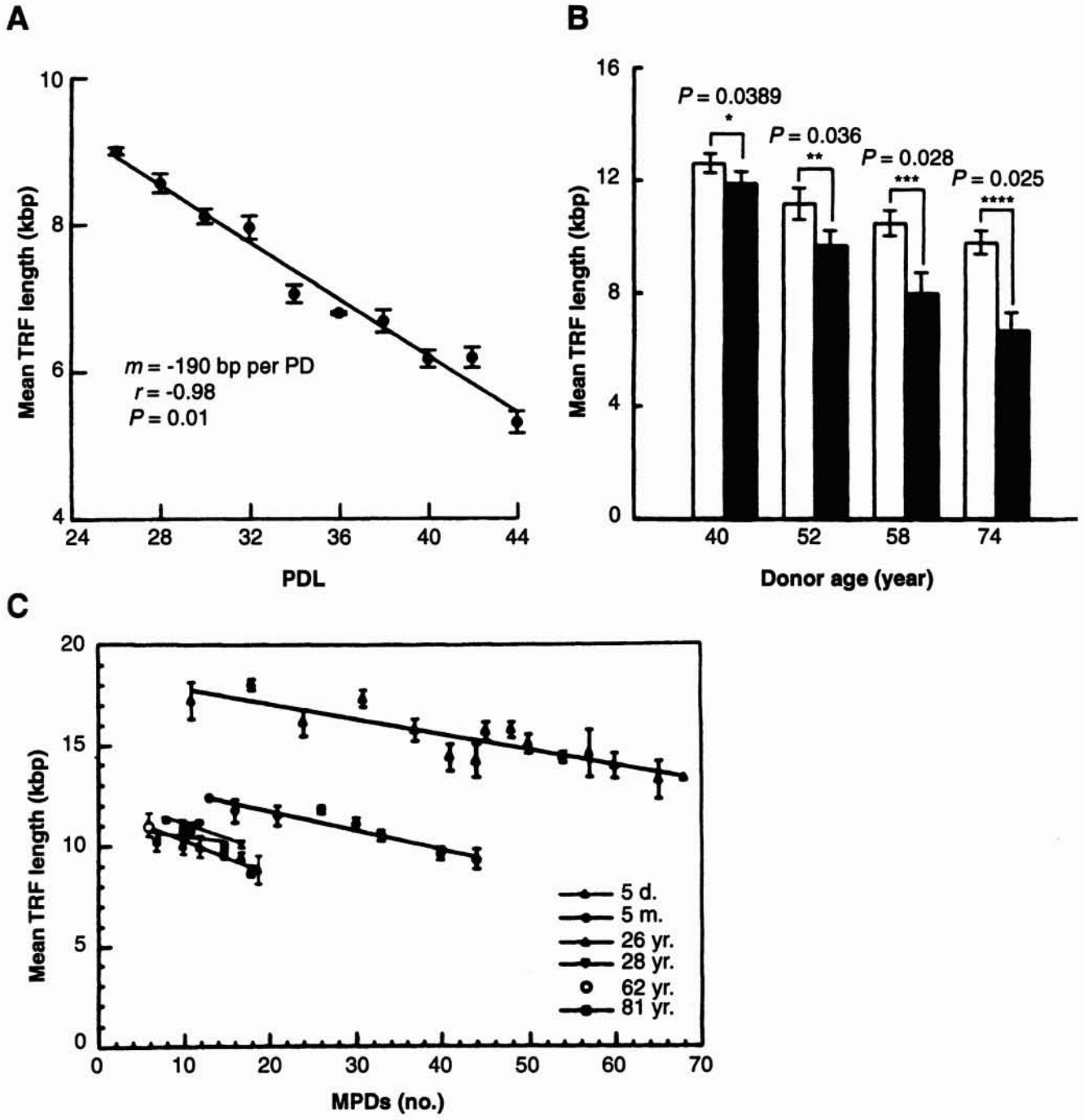


Figure 2. Telomere length and proliferative potential in human endothelial cells and human skeletal muscle satellite cells. (A) Relationship between telomere length (TRF = telomere restriction fragment) in the primary culture and the population doubling level at replicative senescence in umbilical vein endothelial cells (various donors). (B) Decrease in TRF in primary endothelial cells as a function of donor age: open bars, thoracic artery endothelial cells; solid bars, iliac artery endothelial cells. Panels A and B from Chang and Harley (1995), copyright 1995 National Academy of Sciences, USA. (C) Telomere length and population doublings (MPDs) in satellite cells from donors of various ages. Reproduced with permission from Decary *et al.* (1997).

vascular system where blood flow might cause the most chronic damage to the endothelium. Unfortunately, because the data are from human specimens, it is difficult to test this hypothesis directly. Skeletal muscle satellite cells can be isolated from human muscle samples and exhibit a limited replicative potential in culture. They show decreasing proliferative potential as a function of donor age and decreased telomere length (Fig. 2C), but muscle fiber nuclei showed stable

telomere length (Decary *et al.*, 1997). In the author's laboratory, studies on the effects of age on the proliferation of adrenocortical cells from donors show a great decrease in proliferative capacity, and this has also been shown to be associated with short telomeres in cells from older donors (Hornsby *et al.*, 1992; W. Wright & P. Hornsby, unpublished observations).

These examples are consistent with the hypotheses that cell proliferation occurring over the life span of the donor causes telomere shortening and that cell cultures then are initiated with cells that have a lowered remaining proliferative potential because continued cell division in culture shortens telomeres to a point where replicative senescence occcurs.

VI. Diminution of Reserve Capacity

No data show a complete loss of cell proliferative potential in any human organ as a function of age, and it is unlikely that the day-to-day functions of tissues are compromised by changes in replicative potential. However, tissues may suffer a loss of ability to repair damage efficiently or to restore sudden losses of cells during aging. The proliferative reserve capacity of the tissue is impaired, even if the replication capacity that they need for normal maintenance and cell turnover is always adequate. This concept is in agreement with other measurements in aging that show that the gradual decline in organ function, resulting from a large variety of aging processes, has its biggest impact on the loss of the reserve capacity of the organism to react to events that injure the tissue or require its function at a level greater than that normally required. Age-related disease processes may be exacerbated by, or actually caused by, this loss of reserve capacity.

Under conditions where there is a chronic stimulus to divide, there is evidence, albeit limited, that cells can reach replicative senescence under *in vivo* conditions. In patients with chronic ulcers, fibroblasts were observed to have decreased proliferative capacity and increased senescence markers (Raffetto *et al.*, 1999; Agren *et al.*, 1999). These effects were more pronounced in ulcers that had been present for >3 years (Raffetto *et al.*, 1999). In conditions of muscle fiber death and excessive cell turnover in the muscle, such as Duchenne muscular dystrophy, satellite cells show a short replicative potential even when isolated from young donors, and this becomes worse over time (Blau *et al.*, 1983). More examples are found in the hematopoietic system and the liver, as discussed later.

Whether these cells have truly reached the end of their proliferation *in vivo* before isolation is difficult to assess. An unanswered question is whether the short-telomere signal for replicative senescence can be modulated by factors in the environment of the cell. Because a state resembling replicative senescence can be triggered by many manipulations other than telomere shortening, there is the possibility that some factor in the cell culture environment (e.g., some degree of DNA damage by exposure to oxygen and light), which by itself does not cause replicative senescence, can synergize with the short-telomere signal. Thus, although donor age effects clearly indicate the potential for exhaustion of replicative capacity, the imperfect cell culture environment might amplify the effect. On the other hand, telomere shortening can be accelerated in cell culture by DNA damage (Petersen *et al.*, 1998), indicating the possibility that cells isolated from tissues may not always have acquired their short-telomere state by repeated cell division.

VII. Possible Occurrence of Cells with Characteristics of Replicative Senescence in Tissues

Tissue function may be affected by the presence of replicative senescent cells, even if the cell population as a whole retains replicative capacity. Such senescent cells may have been formed by telomere shortening or by one of the numerous other processes that have been shown to place cells in the same state. The presence of cells that have senescence-associated β-galactosidase (SA-βgal) was reported first for human skin (Dimri *et al.*, 1995) and subsequently was shown in the rhesus monkey in retinal pigmented epithelium (Mishima *et al.*, 1999) and in the epidermis (Pendergrass *et al.*, 1999). In all three studies, the number of SA-βgal-positive cells increased as a function of donor age. These intriguing observations require much more study. First, there is an obvious need to study the mechanism by which such cells are formed. Whether SA-βgal positive cells *in vivo* result from telomere shortening is not known. The suspicion that they might not exists for RPE cells, because these cells are mostly postmitotic in adult life (Hjelmeland, 1999). Second, whether such cells *in vivo* actually have the same range of changes in gene expression observed in replicative senescent cells in culture is also unknown. This is important because it has been speculated that these changes may result in a procarcinogenic state in tissues that could aid the growth of premalignant cells and provide a permissive environment for tumor progression (Campisi, 1997; Dunsmuir *et al.*, 1998). At the moment that hypothesis lacks direct experimental confirmation, but deserves full consideration. Many properties of aging tissues might result from the presence of relatively small numbers of replicative senescent cells.

The fact that both human and mouse cells can become SA-βgal-positive by various forms of damage unfortunately leaves us without good *in situ* assays that unambiguously indicate that cells have exhausted their replicative capacity *in vivo* by telomere shortening.

Independent of the question of whether replicative senescent cells affect tissue function, it is clear that telomere shortening does occur in human tissues *in vivo*, potentially putting cells ever closer to replicative senescence. It is important to distinguish the phenomenon of telomere shortening from its significance. The significance is still under debate, but the fact that it does indeed occur should not be considered controversial.

VIII. Stem Cells

An important subject is how aging affects the properties and numbers of stem cells in continuously proliferating tissues. Critical questions for stem cells are the following: (a) is there an age-dependent (i.e., time-dependent) change in the number of stem cells and/or an age-dependent change in their properties; and (b) do stem cells have a finite proliferative potential (or are immortal in terms of cell division) and does repeated cell division change their properties. These two questions must be separated because the most pluripotent stem cells divide only rarely *in vivo* (Morrison, *et al.*, 1997). Most cell division is done by the more committed progenitor cells and by differentiating cells.

In any organ system where cell proliferation is critical to function, such as the hematopoietic system, it would be anticipated that any intrinsic changes in cell proliferation would be compensated via feedback to maintain overall function at the appropriate level. As discussed previously, it might be expected that deficits in aging would become apparent when

the system is stressed by the increased demand for cell proliferation. Various experimental protocols have been devised to search for such deficits. Additionally, experimental systems have been set up to address the questions of whether there are ultimate limits to proliferation and whether such eventual limits may be reached normally. If an organ system does have intrinsic limits on cell proliferation, this should be taken into account in any study of age-related diseases in that organ. It is possible that proliferative limits are reached under at least some set of conditions during aging.

IX. Age-Related Changes in Cell Proliferation

Major organ systems that have been studied for changes in cell proliferation during aging include the hematopoietic system, liver, mammary glands, skin, and gastrointestinal system.

A. Hematopoietic System

When hematopoietic stem cells divide, they regenerate either two new stem cells (equivalent to the parent cell, i.e., a process of self-renewal) or one stem cell and one cell that is more committed to differentiation; the latter gives rise to the various specific cell types of the hematopoietic system. Because methods for characterizing hematopoietic stem cells are complex and have been subject to refinement over time, the nomenclature used by the authors of the various studies discussed has been retained.

1. Mouse

The mouse has been studied extensively as an experimental animal with respect to aging and the hematopoietic system. There are no changes in the ability to maintain normal numbers of red blood cells in old mice except when the animals are stressed by crowding (Williams *et al.*, 1986). Under stressed conditions there were declines in hematocrit, CFU-E (colony-forming units erythroid), BFU-E (burst-forming units erythroid), and CFU-C (colony-forming units culture). On the other hand, measurements of stem cell numbers in mice during aging have shown great variations depending on the strain of animals studied. In the DBA strain, the number of stem cells (cobblestone-area-forming cells, CAFC) decreased markedly between 12 and 20 months of age, but in B6 mice this population increased at a constant rate from late gestation to 20 months (de Haan & Van Zant, 1999). Variable increases in stem cell number were observed in several other strains (Harrison *et al.*, 1989; Morrison *et al.*, 1996; de Haan *et al.*, 1997). In BXD recombinant inbred strains, differences in CAFC numbers from 2 to 20 months ranged from a ~10-fold decrease to a ~10-fold increase. Several genomic loci were found to contribute to these differences (de Haan & Van Zant, 1999).

There is some decline in the repopulating capacity of mouse hematopoietic stem cells with age. In one study, stem cells from old mice were ~25% as efficient as those from young animals in homing and engraftment in the bone marrow of recipients (Morrison *et al.*, 1996). In another study, fetal stem cells had 1.6–3.0 times the functional capacity of young adult stem cells; young adult (3 months) cells had 1.6–2.0 times the functional capacity of old (25–28 months) mice (Chen *et al.*, 1999). It is not clear whether these data indicate true replicative senescence of stem cells or whether this represents an age-dependent shift in the relative populations of pluripotent and more committed cells. There is a decreased replicative capacity in mouse hematopoietic stromal cells (Jiang *et al.*, 1992) and a reduced frequency of stromal

cell precursors in primary cultures of bone marrow cells from old mice (Globerson, 1999). Aging effects on stromal cells may indirectly affect stem cell function.

In any case, the available data show relatively mild effects of aging on overall hematopoietic system function in the mouse, despite the changes in absolute numbers of stem cells. Presumably, homeostatic mechanisms increase the stem cell pool to compensate for age-associated decreases in stem cell function; but the situation in those strains that show decreases in stem cell number in aging is unexplained.

Measurements of stem cell proliferation in mice (long-term self-renewing hematopoietic stem cells, LT-HSC) showed that stem cells divide every 57 days on average (Cheshier *et al.*, 1999). This means that a stem cell would divide about 6 times per year or 18 times over a 3-year mouse life span. Serial cell transplantation experiments have been performed on the hematopoietic system in rodents as a means of attempting to exhaust the proliferative potential of stem cells. Such experiments are important and have had a major impact on the concept of the possibility of age-related exhaustion of cell proliferation. The progeny of a single stem cell can maintain hematopoiesis over the life span of an animal receiving a transplant (Jordan & Lemischka, 1990). However, there are limitations on the interpretation of serial cell transplantation experiments that should be considered carefully. Because of the continuous increase in our knowledge of stem cells, not all experiments in the past have been performed with ideal protocols. The best method would seem to be to isolate highly defined stem cells (at either the most primitive or the more committed stages) and to transplant a constant number of such cells to the recipient (a lethally irradiated or other suitable recipient, such as the anemic mouse mutant *W*; Harrison & Astle, 1991). At the end of the reconstitution process in the recipient, the isolation procedure would be repeated and the transplantation continued in a second recipient, and so on. Unfortunately for aging research, knowledge of how to isolate and handle such stem cells still is not perfect. The methods for isolating and growing highly defined stem cells in culture are still being refined. When they are out of the body, they must not be inadvertently stimulated to enter a more committed state. Even when these technical problems are solved, an unavoidable problem is that the most primitive stem cells may undergo a relatively small number of divisions in each transplant generation, and it is difficult to measure this number. Therefore, the question of whether such stem cells have a finite proliferative capacity or are "immortal" is intrinsically difficult to assess by serial transplantation.

Early serial transplantation experiments in mice suggested a limit to the number of times stem cells could be transferred to successive recipient animals and still successfully reconstitute the hematopoietic system of the recipients, but it was later recognized that this failure likely is technical in nature (Harrison, 1985; Harrison *et al.*, 1988). Subsequent experiments used a strategy of measuring the number of stem cells (long-term reconstituting cells, LTRCs) at each transfer to ensure that similar numbers are transferred. When the number of LTRCs was taken into account, no change in repopulating capacity was observed over four transplant generations (Iscove & Nawa, 1997). It was calculated that there was an expansion of 8400-fold in the stem cell number relative to the original input population. This represents a minimum of ~13 doublings of the stem cells (not taking into account those doublings that produce one stem cell and one committed cell). Thus, it is

clear that a large number of transplant generations would be required before stem cell exhaustion might be anticipated. If the measurement of stem cell proliferation in humans is correct (see later discussion), 13 doublings represent only a small fraction of the total proliferation of a stem cell over the human life span.

An alternative to serial transplantation as a means to study the total replicative potential of stem cells is to repeatedly stimulate stem cell proliferation *in vivo* in a single experimental animal. When such experiments were performed by repeated treatment with hydroxyurea (Ross *et al.*, 1982) or repeated irradiation (Harrison *et al.*, 1984), there was no exhaustion of hematopoietic potential. However, it is not known how many times the stem cells actually divide in these experiments. The problem here is to stimulate the proliferation of the most pluripotent cells rather than more committed successors. Mice with targeted disruption of the $p21^{SDI1/WAF1/CIP1}$ gene exhibit more stem cell cycling and impaired self-renewal of primitive cells in serial transplantation (Cheng *et al.*, 2000). This type of genetic approach to stem cell senescence is very promising. Unfortunately, it is difficult to distinguish whether the genetic intervention (in this case the lack of p21) causes true senescence of stem cells (i.e., causes pluripotent stem cells to divide so many times that they eventually have impaired cell division capability) or in fact changes the probability of the commitment of primitive stem cells to more differentiated cells, resulting in stem cell exhaustion.

Another method for studying the total replicative potential of stem cells is to grow them in culture, followed by the reconstitution of recipient animals after different numbers of cell generations. As in serial cell transplantation experiments, a problem is to keep the cells in their uncommitted state in culture. That such problems essentially are technical is shown by the fact that mouse embryonic stem cells can be grown under special conditions in which differentiation is prevented.

The preceding discussion of the available evidence on hematopoietic stem cells in the mouse suggests that effects of aging on the function of the hematopoietic system are small under normal conditions. The maximum proliferative potential of mouse stem cells is unknown, but evidently it is sufficient to supply the need for cells well past the normal life span. Because of the different characteristics of mouse and human cells with respect to telomerase and telomere regulation emphasized earlier and in view of the fact that human stem cells must supply a much larger population over a much longer time period, it would be unwise to apply these findings uncritically to human biology.

2. *Human*

Most of the data available on hematopoietic stem cells in human aging are from clinical measurements and cell culture data. Strategies that have been valuable for investigating rodent stem cells in aging would need to be modified to study human stem cells. A reconstitution assay is available for human cells in the form of the *scid-hu* mouse, in which mice with the *scid* (severe combined immunodeficiency) mutation that lack functional T- and B-cells are irradiated and rescued with human bone marrow or peripheral blood stem cells (Dao & Nolta, 1999). Mice with the *scid* mutation plus other mutations that enable the xenotransplantation of human cells may also be used (Dao & Nolta, 1999). Studies of donor age effects and replicative potential in serial cell transplantation in this model should be very valuable.

In the absence of disease, the supply of red blood cells to the body in elderly

humans still is efficient. However, anemia is common in older humans (Zauber & Zauber, 1987; Izaks *et al.*, 1999), but is not considered to be a part of normal aging. In most individuals, there is only a slight change in hematocrit as a function of age. There are many causes of anemia (Zauber & Zauber, 1987; Izaks *et al.*, 1999); true age-related changes in cell proliferation, as opposed to disease-related causes, are unlikely. In normal subjects the number of $CD34^+$ bone marrow cells did not change in 70- to 80-year-olds versus 20- to 30-year-olds, although there was a decline in the proliferative response of the cells to G-CSF (granulocyte colony-stimulating factor) (Chatta *et al.*, 1993). In a study of 88-year-old subjects compared to subjects 21–57 years of age, there were no changes in CFU-E or BFU-E, but there was a decrease in CFU-GM (Nilsson-Ehle *et al.*, 1995).

Circulating peripheral blood stem cells ($CD34^+$) and PB-CFU-GM (peripheral blood colony-forming unit granulocyte/macrophage) decreased with age in the range of 20–90 years (Egusa *et al.*, 1998). When peripheral blood $CD34^+$ stem cells are mobilized by the administration of G-CSF, there is a small decline in the number of cells that can be mobilized in donors more than 60 years of age (Anderlini *et al.*, 1997). However, in healthy (nonanemic) donors, the decrease was mild and did not prevent the harvesting of sufficient numbers of these stem cells for transplantation.

These data are in agreement with those in mice, that aging has rather little effect on normal hematopoiesis. However, there is ample evidence, reviewed next, that the reserve capacity of the proliferative potential of the hematopoietic system in humans is much less than in mice. In many pathological conditions the system approaches exhaustion.

There are indications that stem cell number may fall to very low levels in older humans. In many elderly women there is a skewed X-chromosome inactivation pattern in white blood cells, indicating that the number of stem cells giving rise to the peripheral circulating cells has greatly decreased (Champion *et al.*, 1997; Gale *et al.*, 1997). A possible alternate explanation for the observed age-dependent skewing is that there is a selective survival or proliferation advantage for cells expressing a particular allele of a gene on the X-chromosome (Abkowitz *et al.*, 1998).

Telomere length is very valuable in the analysis of cell proliferation in the human hematpoietic system. A major question is whether the level of telomerase activity in human hematopoietic stem cells is sufficient to prevent telomere shortening and thereby to prevent eventual replicative senescence. Cell populations enriched in stem cells have telomerase activity (Yui, *et al.*, 1998).

Telomere shortening is observed in bone marrow cells from adult humans compared to fetal liver and umbilical cord blood cells (Vaziri *et al.*, 1994). A population enriched in stem cells ($CD34^+$ $CD38^-$) also had shorter telomeres in adults (Vaziri *et al.*, 1994). Lymphocytes show a continuous decline in telomere length with age, consistent with a continuous decline in telomere length in stem cells (Hastie *et al.*, 1990; Vaziri *et al.*, 1993). The loss of telomere DNA has been measured by a fluorescence technique in lymphocytes and granulocytes from a large number of human donors in the range of 0–90 years of age. There was a very striking continuous decline in telomere length, which fits a pattern of somewhat greater loss of telomere DNA up to the age of about 1 year followed by a constant linear rate of loss up to the oldest ages studied (Frenck *et al.*, 1998; Rufer *et al.*, 1999) (Fig. 3A). These data all suggest that telomerase in hematopoietic stem cells is insufficient to mantain telomere length. Direct data for this is difficult to obtain because of the fact that

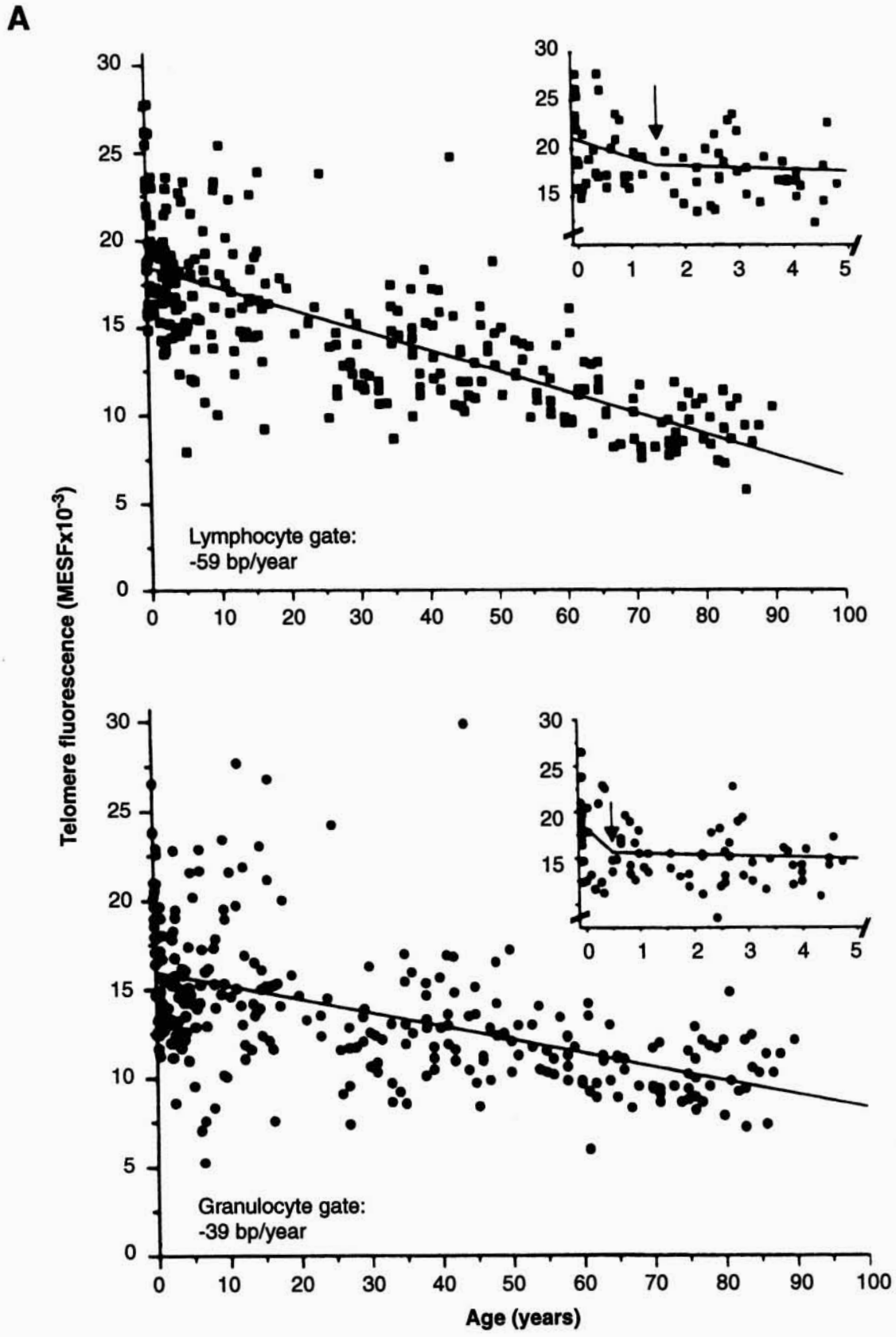
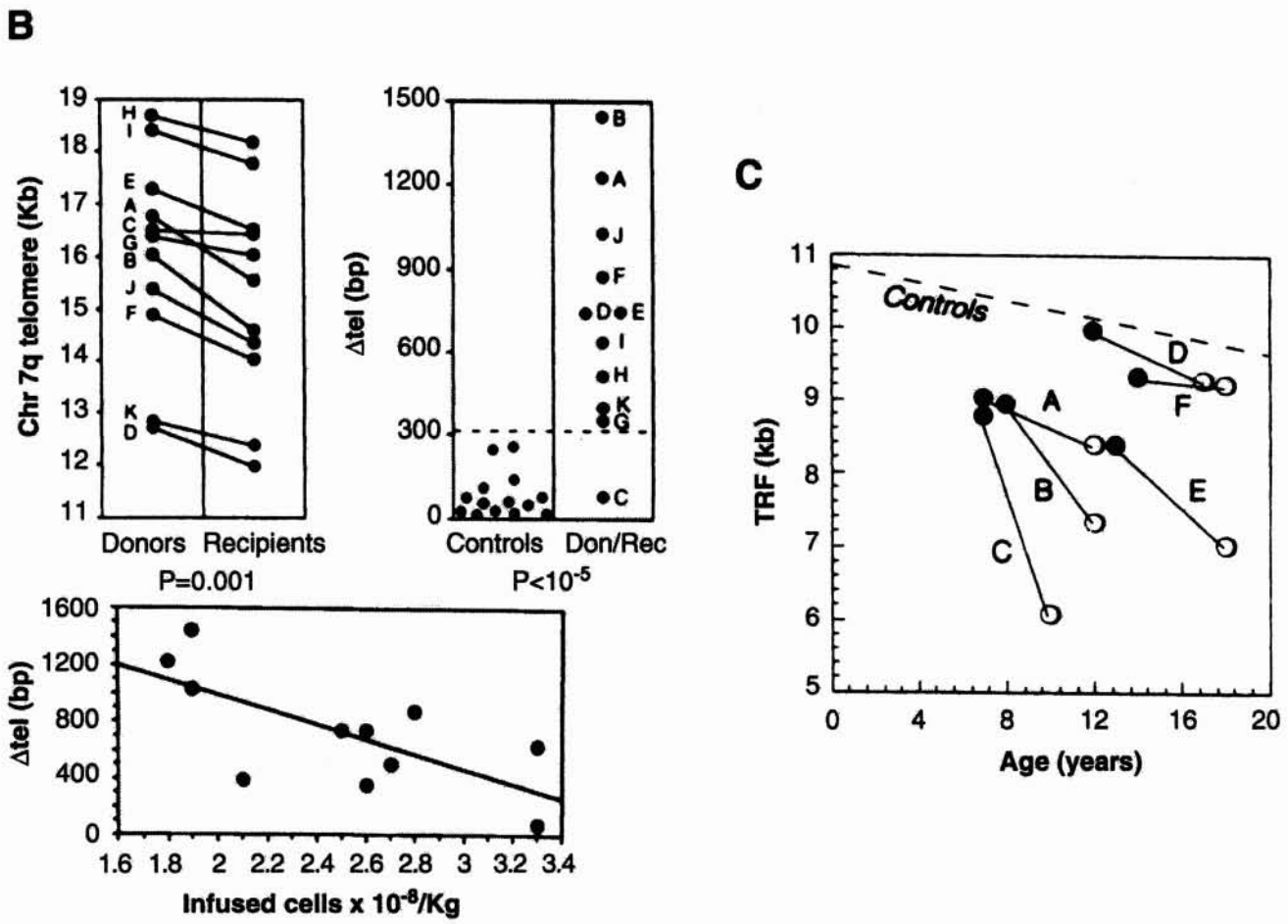


Figure 3. (A) Telomere length decreases as a function of age in lymphocytes and granulocytes. Telomere length was measured by flow FISH (fluorescent *in situ* hybridization). Insets show the results of bisegmented fit analysis. Arrows indicate the optimal intersection of calculated regression lines. Reproduced from Rufer *et al.* (1999) by copyright permission of The Rockefeller Press. (B) Telomere shortening after bone marrow transplantation. DNA was extracted from neutrophils. TRFs were measured using a probe specific for the subtelomeric region of chromosome 7q. Measurements were performed on the donors and on the recipients at a median of 23 months following transplantation. The data also are plotted as a scattergram, the horizontal dotted line indicating the threshold of background in the method used. Additionally, the decrease in telomere length is plotted against the number of infused cells. From Notaro *et al.* (1997),



copyright 1997 National Academy of Sciences, USA. (C) Telomere shortening in Fanconi's anemia. TRFs were measured in DNA extracted from mononuclear cells isolated at the indicated ages. Patients A–C suffered an increase in the severity of their symptoms over the period between the two samples. Reproduced with permission from Leteurtre *et al.* (1999).

it is now understood that telomerase in many cell types is regulated, as discussed earlier. This fact means that very quiescent stem cells that are not stimlated to divide in culture might be capable of telomerase induction, but this cannot be proved until culture conditions are found that cause them to proliferate and retain stem cell properties (Yui *et al.*, 1998; Glimm & Eaves, 1999).

If it is assumed that the level of telomerase activity in stem cells does not prevent telomere loss at all, the data from peripheral white blood cells suggest that human stem cells undergo 15–30 divisions in the first half-year of life, followed by less than one division per year thereafter (Rufer *et al.*, 1999). Such a model requires regulated asymmetric division so that the most primitive stem cells divide far fewer times than more committed descendents (Brummendorf *et al.*, 1998). However, the rate of division could be much higher if telomerase partially, but not completely, prevents telomere shortening in stem cells.

Following the transplantation of bone marrow cells in humans, telomere length in the transplanted cells decreases dramatically, by about 1–2 kb, compared to the length in the donor (Notaro *et al.*, 1997; Wynn *et al.*, 1998; Lee *et al.*, 1999) (Fig. 3B). Thus, during the transplantation procedure, the rate of cell division exceeds the ability of the telomerase activity in the repopulating cells to maintain telomere length. In auto-PBSCT (peripheral blood stem cell transplantation), telomere length decreased by 2.36 kb, which was calculated to be equivalent

to 61.5 years of normal aging (Lee *et al.*, 1999). In contrast, peripheral blood progenitor cells (PBPC) were mobilized with G-CSF in mice and transplanted into lethally irradiated recipients; after one year, PBPC could be remobilized and used to reconstitute the hematopoietic system of a second recipient (Yan *et al.*, 1998). This suggests a much greater capacity for long-term growth of these cells in mice than in humans. The consequence of these observations for aging in human bone marrow recipients is not yet known.

Patients with syndromes of increased replication of hematopoietic cells, such as aplastic anemia, including Fanconi's anemia, show variable increases in the rate of telomere shortening and sometimes are elevated quite dramatically (Ball *et al.*, 1998; Leteurtre *et al.*, 1999) (Fig. 3C). Another situation of increased replication is in patients undergoing cancer chemotherapy. The greater bone marrow toxicity in the elderly suggests a decreased capacity for adapting to the need for increased hematopoietic stem cell function.

It has been pointed out that telomere lengths in differentiated human white blood cells are not as short as might be expected on the basis of the usual concepts of cell turnover in continuously proliferating human tissues (Morris, 1999). This observation suggested the important concept that control of the hierarchy of stem cells in humans and other long-lived species may differ from that in rodents (Morris, 1999). Although this concept deserves thorough experimental investigation, it does not take into account the fact that many stem cell types have some telomerase activity resulting from regulated expression of TERT. This may be sufficient to slow down the rate of telomere shortening in stem cells, making it difficult to calculate the number of cell generations based only on final telomere length.

In summary, direct measures of the proliferative potential of human hematopoietic stem cells await the development of appropriate methods for directly studying this question. The human hematopoietic system begins life with shorter telomeres than in mice, and the level of telomerase does not prevent telomere shortening from occurring in the entire system throughout the life span. The data are consistent with the hypothesis that the stem cell proliferative potential is adequate for the supply of the system over a normal human life span, but has a limited excess capacity that is significant in very old age or under pathological conditions. The limitation of replicative capacity, by the suppression of TERT in most of the system, can be viewed as as an evolutionary trade-off. In a system that is so dependent on cell proliferation for normal function, the opportunity for neoplastic transformation over the life span is very large. Suppression of TERT may prevent many abnormal preneoplastic clones of cells from evolving into lethal cancers, but this evolutionary strategy may leave the system vulnerable to exhaustion in old age.

B. T-Cells

T-cells exhibit a limited proliferative potential in culture, resulting from telomere shortening, like other normal human cell types (Effros & Pawelec, 1997). Senescent T-cells lack expression of CD28 (Effros *et al.*, 1994). In aging, the proportion of $CD28^-$ T-cells increases, and in the elderly such cells may comprise more than 50% of the T-cell subset that controls viral infections (killer, or cytotoxic, T-cells) (Boucher *et al.*, 1998). In HIV infection, more than 65% of cytotoxic T-cells may be $CD28^-$ and have telomere lengths of 5–7 kb, a size consistent with replicative senescence (Effros *et al.*, 1996). Thus, T-cells provide an important and clear example of the occurrence

of replicative senescence *in vivo*. Other aspects of immune senescence are discussed in Chapter 12 of this volume.

C. Liver

There are two cell populations in adult mammalian liver that behave as stem cells. First, the mature differentiated hepatocytes behave functionally as stem cells (Alison, 1998). The liver normally is a mitotically quiescent organ, but it can regenerate efficiently after damage. Liver regeneration can be observed in experimental animals after the removal of two-thirds of the liver. In this experimental protocol, the restoration of the organ normally is dependent on hepatocytes, which clearly are capable of self-renewal like stem cells. Under specific circumstances, however, the liver can be rescued through proliferation of bile-duct-derived cells termed oval cells (Alison, 1998). Oval cells can differentiate into hepatocytes; they appear after parenchymal damage when regeneration by surviving hepatocytes is compromised. Both oval cells and hepatocytes can be transplanted into the liver. Thus, both cell types have stem-cell-like properties, but the available data on cell proliferation in the aging of the liver is for hepatocytes rather than for oval cells.

Following partial hepatectomy, the restoration of liver size occurs through the proliferation of almost all of the hepatocytes, together with biliary epithelial cells, endothelial cells, Kupffer cells and cells of Ito (Michalopoulos & DeFrances, 1997). The restoration of liver size is brought about by coordinated proliferation of these cell types over a 10-day period. In old animals the liver still regenerates after partial hepatectomy, but there are changes in kinetics (Finch, 1991). Regeneration becomes progressively slower as a function of age (Bucher & Glinos, 1950; Bourliere & Molimard, 1957), and the onset of DNA synthesis is delayed (Bucher *et al.*, 1964; Stocker & Heine, 1971; Schapiro *et al.*, 1982). Additionally, although >95% of hepatocytes undergo DNA synthesis in young animals, only about 75% do so in very old animals (Stocker & Heine, 1971).

The age-related changes in regeneration following partial hepatectomy must originate in changes in the microenvironment of the hepatocytes or in the hormonal control of hepatocyte proliferation; they are unlikely to result from any intrinsic changes in the proliferative potential of hepatocytes. Over the life span of the animal, hepatocytes divide relatively infrequently, and the liver can regenerate after at least 12 successive partial hepatectomies (Ingle & Baker, 1957). Serial cell transplantation experiments show a much greater proliferative potential. To perform serial transplantation experiments on hepatocytes, methods have been devised to transplant cells into the livers of recipient animals in which the native hepatocytes have a high rate of cell death, thus providing the transplanted cells with a competitive advantage in colonizing the organ. One system used mice with a plasminogen activator transgene driven by the albumin promoter (Rhim *et al.*, 1994), and another used mice with a lethal type of hereditary tyrosinemia type 1 (Overturf *et al.*, 1997). By using hepatocytes marked with a *lacZ* transgene, it was possible to follow the repopulation of the recipient liver by the transplanted cells. When this was performed through multiple successive recipients, mouse hepatocytes were found to be capable of doubling between 69 and 86 times without a loss of repopulating capacity (Overturf *et al.*, 1997).

Clearly, the vast proliferative potential of rodent hepatocytes means that it would be difficult to reach any limit on proliferative capacity over a normal life span. Comparable data for human hepatocytes are limited. Normal human hepatocytes lack telomerase activity; telomere

shortening has been detected in normal aging and in chronic diseases of the liver, such as hepatitis and cirrhosis, where there is continuous cell turnover (Urabe *et al.*, 1996; Takubo *et al.*, 2000). Indeed, it has been suggested that the cause of cirrhosis of the liver is the ultimate failure of hepatocytes to proliferate, following chronic increased cell death and consequent continuous proliferation of cells, resulting in telomere shortening (Rudolph *et al.*, 2000). Like most cancers, established hepatocellular carcinomas (HCCs) are telomerase-positive. Interestingly, in moderately differentiated HCCs, there is a decrease in telomere length until the HCC reaches about 5 cm in diameter and an increase thereafter, suggesting that the late activation of telomerase allows the continued growth of these cancers (Urabe *et al.*, 1996).

D. Mammary Gland

Experiments on serial transplantation of mouse mammary tissue have been influential in supporting the concept that normal cells can reach a limit of proliferation *in vivo*. In the mouse, mammary tissue can be transplanted into the "cleared" mammary fat pad (a mammary fat pad from which the endogenous epithelium has been removed, surgically or chemically). The tissue grows to fill the fat pad and produces an almost complete mammary gland, with the normal ductal and alveolar structures. Serial transplants of epithelial ductal fragments were made into successive nulliparous hosts until they lost the ability to undergo branching morphogenesis (Daniel & Young, 1971). If fragments from these ducts were then transplanted again, they could not produce branching ducts; however, they could develop secretory lobules in a pregnant host or following the administration of cholera toxin, showing that the cells did not absolutely lose the ability for cell proliferation (Daniel *et al.*, 1984). The rate at which fragments of mammary epithelium reached this stage was greater when transplants were made from the periphery versus the center of previously transplanted tissue (Daniel & Young, 1971). On the other hand, some lines of mouse mammary cells that have undergone spontaneous immortalization in culture, which form ductal outgrowths in the mammary fat pad, can be transplanted indefinitely (Medina & Kittrell, 1993). Taken together, these observations suggest that mammary epithelial cells in the mouse are not capable of indefinite division, unless they undergo genetic changes that permit immortalization. However, in the absence of biochemical data to act as a marker for replicative senescence (such as telomere length or SA-βgal, although neither of these is necessarily useful in this particular case), it is difficult to know whether the limitation of mouse mammary cell proliferation is really a phenomenon of replicative senescence or is a reflection of the loss of pluripotent stem cells during the experimental procedure. This question has been subjected to much more extensive analysis in the hematopoietic system, as discussed earlier, and the lesson from that system is that more definitive interpretation of the data was possible only when certain key features of the cell transplantation were understood—markers for stem cells and knowledge of how many stem cells were transferred to each successive recipient. There is increasing understanding of the biology of mammary epithelial stem cells (Chepko & Smith, 1999), and future experiments on serial transplantation of mammary epithelial cells should be able to take advantage of this progress.

E. Gastrointestinal System

In the aging rat gastrointestinal system, there is hyperproliferation in epithelial cells of the stomach, small intestine, and colon. In the gastric mucosa of the rat,

there is an increase in cell proliferation with aging (Majumdar *et al.*, 1988; 1989; 1990). This is accompanied by a diminished cell proliferation reponse to injury, perhaps as a result of an already maximally stimulated level of cell division that cannot be increased further (Majumdar *et al.*, 1988, 1989, 1990).

Although earlier work suggested that cell proliferation in the small intestine was reduced as a function of age, careful studies later showed that there is an increase in proliferation with aging when care is taken to avoid handling effects on the animals and when animals in ill health are excluded (Atillasoy & Holt, 1993). In the duodenum and jejunum of Fischer 344 rats, the number of villus-absorbing cells was unchanged in 25- to 27-month-old animals compared to 4- to 5-month-old animals (Holt *et al.*, 1984). However, crypt cell numbers were greater in the older animals and the crypt cell production rate increased by about one-third (Holt & Yeh, 1989). In 28- to 30-month-old ICRFa mice there was an increase in the number of clonogenic cells in small intestine crypts compared to 6- to 7-month-old animals (Martin *et al.*, 1998). Aging animals show a dramatic change in the distribution of proliferating cells in crypts. There was an increase in the crypt proliferative zone in all parts of the small intestine under fed, starved, and refed conditions (Holt & Yeh, 1989). Similarly, crypt hyperplasia, an increased rate of proliferation, and a broadened proliferative zone were observed in the colon of 26- to 28-month-old Fischer 344 rats compared to 3- to 4-month-old animals (Holt & Yeh, 1988). In 24- to 26-month-old Fischer 344 rats there was an increase in metaphase chromosome aberrations in jejunal crypts compared to 3- to 7-month-old animals (Ellsworth & Schimke, 1990). In rectal biopsies from elderly human subjects without adenomatous polyps, there is also an increase in proliferating cells in the upper 40% of the crypt (Paganelli *et al.*, 1990; Corazza *et al.*, 1998). This change is a type of proliferative lesion associated in young individuals with many premalignant conditions throughout the gastrointestinal tract, including Barrett's epithelium, chronic gastritis, inflammatory bowel disease, and colonic polyps, and is induced by carcinogens in rodents (Eastwood, 1995).

Food intake normally controls the intestinal cell proliferation rate (Johnson, 1987). Caloric restriction decreases the rate of cell turnover in rodents (Lok *et al.*, 1988; Albanes *et al.*, 1990) and in obese humans (Steinbach *et al.*, 1994). Rectal biopsies from calorically restricted human subjects showed a 39% reduction in the whole-crypt labeling index and a 57% reduction in the upper-crypt labeling index, suggesting a normalization of the abnormal proliferating cell distribution. The changes in distribution of proliferating cells in the aging rat intestine are hypothesized to reflect a failure of the normal control of cell production in response to variations in food intake (Atillasoy & Holt, 1993). In aging, there is a delay in differentiation as the cells move from the crypt to the tip of the villus. Caloric restriction did not alter small intestine villus architecture, but prevented age-associated crypt hyperplasia in the rat (Heller *et al.*, 1990). Additionally, in gnotobiotic rats, which have a much lower intestinal proliferation rate because of the absence of bacteria in the gut, there was no change in proliferation in aging (Ecknauer *et al.*, 1982).

In humans, the lower one-third of the intestinal crypt is telomerase-positive, but interestingly, intestinal cell telomeres are shorter in adults than in children (Hiyama *et al.*, 1996). This suggests that regulation of TERT expression in this system is adequate to provide for continued cell proliferation over the life span, yet not high enough to prevent

developmental changes in telomere length. Moreover, in ulcerative colitis telomeres were shorter than in controls (Kinouchi *et al.*, 1998), suggesting that the telomerase level also does not maintain telomere length under conditions of excessive cell proliferation.

Thus, in the gastrointestinal system, the changes in cell proliferation in aging do not seem to impair the function of the organs. Rather, as in some rodent models of hematopoietic stem cell function, it may be that unknown changes in stem cell function in aging are compensated for by changes in the numbers of clonogenic cells and changes in the distribution of proliferating cells. These changes could predispose the colon to tumorigenesis and might be involved in the increased rate of colon cancer in aging (Eastwood, 1995).

F. Skin

Like the gastrointestinal system, the epidermis of the skin is dependent on continuous proliferation for its function. A population of keratinocytes in the basal layer of human skin has the property of stem cells and is telomerase-positive (Harlebachor & Boukamp, 1996; Yasumoto *et al.*, 1996; Li *et al.*, 1998). The progeny of these stem cells appear to be telomerase-negative. In culture, even though biochemically identified stem cells are present in the starting cell population, the culture becomes telomerase-negative. The cells continue to proliferate and eventually undergo replicative senescence, a process that can be bypassed by TERT in cooperation with oncoproteins that abrogate pRb function (Kiyono *et al.*, 1998). Thus, telomerase activity (i.e., expression of TERT) is closely regulated in keratinocytes, as in other normal human cells. Telomerase activity in stem cells in the basal layer may be required to permit the continued proliferation of cells over the life span.

Although there are well-known changes in human skin structure and function in aging, changes in keratinocyte proliferation in the epidermis are slight. In one study, it was observed that 8% of cells in the epidermis are cycling in the young (20- to 35-year-old donors), as evidenced by immunohistochemistry with the Ki-S3 marker. The value was 5.8% in older donors (>60 years of age), but in biopsies of areas of dry skin (xerosis), the values were slightly higher and were almost identical in young and old skin (Engelke *et al.*, 1997).

Skin wound healing is slower in the elderly (Grove, 1982; Reed *et al.*, 1996). Wound healing is complex, dependending on the interplay of a variety of processes, not only cell division, and there is little evidence for age-related intrinsic changes in cell proliferation (as opposed to changes in the microenvironment of the cells that might change their behavior) that affect wound healing (Ashcroft *et al.*, 1995). The dermis, in contrast to the epidermis, is a proliferatively quiescent tissue, and exhaustion of proliferative capacity would seem unlikely; the need for increased cell proliferation may occur in one specific location in the body only once in a lifetime. This applies to the normal healing of a clean wound, as opposed to chronic conditions like ulcers (see previous discussion). There is a decrease in blood vessel density in the skin in aging, yet hyperemic responses measured by laser–Doppler are more rapid (Kelly *et al.*, 1995). In older animals, there is evidence for delayed angiogenesis in wound healing, but whether this might involve an intrinsic proliferative defect is not clear (Arthur *et al.*, 1998; Reed *et al.*, 1998).

G. Changes in Proliferation Resulting from Endocrine Changes

It is widely assumed that the well-documented changes in the endocrine system

in aging, such as the decline in growth hormone secretion, affect proliferation *in vivo*, but there is little direct evidence for this. Some intriguing data have been obtained on the replicative potential of rat hepatocytes. There is a donor-age-dependent diminution of proliferation in primary culture in response to β-adrenergic hormones; this could be restored by prior transplantation of the animal with thymus from a young animal (Basso *et al.*, 1998). This presumably reflects an endocrine effect of the thymus on the age-related changes in hepatocyte proliferation, but this is unexplained. It was suggested that the life-extending effects of caloric restriction in rodents might be exerted by decreasing insulin concentrations and a consequent reduction in the rate of cell proliferation (Lev-Ran, 1998), but this has yet to be demonstrated directly.

X. Changes in Tissue Structure Resulting from Changes in Proliferation

There are significant changes in histological structure in many organs, which have been suggested to result from focal hyperplasias of various cells (Martin, 1987, 1993). An important concept is that, in these organs, gradual exhaustion of the proliferative potential of some of the cells within the organ increases the proliferation of others within the tissue that have a higher remaining proliferative potential. This is hypothesized to result from feedback control of proliferation—hormonal, paracrine, or some other form of proliferative homeostasis. A range of pathologies observed in aging could result from this process (Martin, 1987, 1993). Of course, these disturbances of tissue structure in turn may result in further changes in cell proliferation.

One example of this is in the aging human adrenal cortex. In aging, the human adrenal cortex becomes somewhat atrophic in comparison with the tissue in the young adult, yet simultaneously accumulates small, presumably clonal, groups of cells termed nodules (Dobbie, 1969; Neville, 1978; Neville & O'Hare, 1982, 1985). Some individuals show minimal detectable histological changes, whereas others have markedly nodular adrenals in old age. With the increased use of CAT and MRI scanning, adrenal nodules became a common incidental finding (Ross & Aron, 1990), and it has become apparent that such nodules represent one end of the spectrum of "normal" aging and are not in themselves of pathological significance. Although nodules are hypothesized to be hyperplasias, they do not necessarily show continued growth; they may remain at the same size over many years. Only very rarely may they undergo neoplastic transformation (Neville & O'Hare, 1982, 1985).

XI. Neoplastic Changes as a Function of Age

The question of the relationship of cancer and aging is complex and has been the subject of much debate (Miller, 1991; Ershler & Longo, 1997). The issue is whether the well-known increases in cancer in aging is the result of fundamental age-related changes in cell properties, rendering them more susceptible to neoplastic transformation, or whether it results simply from the passage of time, enabling cells to accumulate the number of genetic hits that are needed to escape normal growth controls and grow into a malignant tumor. Human fibroblasts or kidney epithelial cells infected with retroviruses encoding TERT, SV40 T-antigen, and oncogenic H-ras become tumorigenic (Hahn *et al.*, 1999a). Therefore, the minimum number of molecular processes in a human cell that must be targeted to

achieve full tumorigenicity is at least three, but perhaps as many as five or six (Hahn *et al.*, 1999a; Weitzman & Yaniv, 1999). The uncertainty arises because proteins like SV40 T-antigen and Ras might affect multiple targets.

The data from the various organ systems reviewed here support a true age-related increase in susceptibility to neoplasia only in the gastrointestinal tract, where the shift in the proliferative zone resembles a preneoplastic lesion known to have the propensity to progress to cancer. However, interpretation of this observation should be cautious, because all individuals show these changes, but most of them will not show a progression to cancer over their entire life span. This lack of progression is made more significant by the fact that, in the colon, the sequence of progression from normal epithelium to cancer is well-described (Lengauer *et al.*, 1998). Additionally, the data on the effects of caloric restriction in the gastrointestinal tract suggest that the proliferative changes might be reversible—if so, they certainly could not be termed intrinsic cellular changes.

It is important to distinguish the intrinsic susceptibility of cells to tumorigenesis from influences on tumorigenesis extrinsic to the cell. For example, tumor angiogenesis is impaired in older host animals (Pili *et al.*, 1994). The more speculative concept that the presence of replicative senescent cells within tissues increases the likelihood of malignant conversion of other cells in the tissue, as discussed earlier, requires a thorough investigation. If this suggestion is correct, it supports the concept that intrinsic cell changes during aging affect tumorigenesis in other cells, but not in the cell undergoing senescent changes. If the age-dependent cancer incidence were the result of intrinsic changes in the cells giving rise to the tumor, we would observe a more ready conversion of replicative senescent cells (or cells close to senescence) to neoplasia. It is difficult to reconcile this concept with the idea that TERT repression, leading to telomere shortening and replicative senescence, is an anticancer mechanism. The absence of *in vivo* evidence that there is an effect of aging on the intrinsic susceptibility of cells to tumorigenic conversion resulted in the famous cynical statement of Richard Peto that "there is no such thing as aging and cancer is not related to it" (Peto *et al.*, 1985).

XII. Conclusions and Summary

Data on rodents show little evidence for intrinsic changes in cell proliferation that might limit the proliferative capacity of tissues in aging, with the important exception of mammary epithelial cells. Apart from that exception, many mouse organs (e.g., hematopoietic system, liver, gut) appear to contain cells that are functionally immortal, although the proof that such cells really have no proliferative limitation will be hard to come by. Under normal conditions, changes in cell proliferation in aging are relatively slight, suggesting that intrinsic changes in proliferative capacity (if any) and changes in the microenvironment of the cells are compensated. For example, in the hematopoietic system, there may be changes in the populations of stem cells, but the overall function of the system is little affected.

As a species, mice and other rodents have a life history in which maximal suppression of cancer timing and incidence is not as important as in long-lived species such as humans. The evolution of anticancer strategies at the cellular level is an essential part of the life history of the human species. Repression of TERT and short telomeres in human cells together form an anticancer mechanism. Telomerase activity is regulated and/or negligible in most cell types, leading to

telomere shortening and replicative senescence. Remarkably, although this process occurs *in vivo*, organ systems seem to be endowed with just enough total replicative capacity for even the extremes of human longevity, if the reserve capacity of the systems is not excessively challenged.

Acknowledgments

Research mentioned in this chapter from the author's laboratory was supported by the National Institute on Aging and the Breast Cancer Research Program of the Army Materiel and Medical Command. I am very grateful to suggestions made by readers of an earlier draft of the text, especially Judith Campisi, George M. Martin, James R. Smith, Rita Effros, and Robert J. S. Reis.

References

Abkowitz, J. L., Taboada, M., Shelton, G. H., Catlin, S. N., Guttorp, P., & Kiklevich, J. V. (1998). An X chromosome gene regulates hematopoietic stem cell kinetics. *Proceedings of the National Academy of Sciences of the USA, 95,* 3862–3866.

Agren, M. S., Steenfos, H. H., Dabelsteen, S., Hansen, J. B., & Dabelsteen, E. (1999). Proliferation and mitogenic response to PDGF-BB of fibroblasts isolated from chronic venous leg ulcers is ulcer-age dependent. *Journal of Investigative Dermatology, 112,* 463–469.

Albanes, D., Salbe, A. D., Levander, O. A., Taylor, P. R., Nixon, D. W., & Winick, M. (1990). The effect of early caloric restriction on colonic cellular growth in rats. *Nutrition and Cancer, 13,* 73–80.

Alison, M. (1998). Liver stem cells: A two compartment system. *Current Opinion in Cell Biology, 10,* 710–715.

Allsopp, R. C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V., Futcher, A. B., Greider, C. W., & Harley, C. B. (1992). Telomere length predicts replicative capacity of human fibroblasts. *Proceedings of the National Academy of Sciences of the USA, 89,* 10114–10118.

Anderlini, P., Przepiorka, D., Lauppe, J., Seong, D., Giralt, S., Champlin, R., & Korbling, M. (1997). Collection of peripheral blood stem cells from normal donors 60 years of age or older. *British Journal of Haematology, 97,* 485–487.

Arthur, W. T., Vernon, R. B., Sage, E. H., & Reed, M. J. (1998). Growth factors reverse the impaired sprouting of microvessels from aged mice. *Microvascular Research, 55,* 260–270.

Ashcroft, G. S., Horan, M. A., & Ferguson, M. W. J. (1995). The effects of ageing on cutaneous wound healing in mammals. *Journal of Anatomy, 187,* 1–26.

Atillasoy, E., & Holt, P. R. (1993). Gastrointestinal proliferation and aging. *Journal of Gerontology, 48,* B43–49.

Ball, S. E., Gibson, F. M., Rizzo, S., Tooze, J. A., Marsh, J. C., & Gordon-Smith, E. C. (1998). Progressive telomere shortening in aplastic anemia. *Blood, 91,* 3582–3592.

Basso, A., Piantanelli, L., Rossolini, G., & Roth, G. S. (1998). Reduced DNA synthesis in primary cultures of hepatocytes from old mice is restored by thymus grafts. *Journal of Gerontology, 53A,* B111–116.

Belair, C. D., Yeager, T. R., Lopez, P. M., & Reznikoff, C. A. (1997). Telomerase activity: a biomarker of cell proliferation, not malignant transformation. *Proceedings of the National Academy of Sciences of the USA, 94,* 13677–13682.

Bertram, M. J., Berube, N. G., Hang-Swanson, X., Ran, Q., Leung, J. K., Bryce, S., Spurgers, K., Bick, R. J., Baldini, A., Ning, Y., Clark, L. J., Parkinson, E. K., Barrett, J. C., Smith, J. R., & Pereira-Smith, O. M. (1999). Identification of a gene that reverses the immortal phenotype of a subset of cells and is a member of a novel family of transcription factor-like genes. *Molecular and Cellular Biology, 19,* 1479–1485.

Bierman, E. L. (1978). The effect of donor age on the in vitro life span of cultured human arterial smooth-muscle cells. *In Vitro, 14,* 951–955.

Blasco, M. A., Lee, H. W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A., & Greider, C. W. (1997). Telomere shortening and tumor formation by mouse