Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity

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Summary

Despite recent progress in the identification of genes that regulate longevity, aging remains a mysterious process. One influential hypothesis is the idea that the potential for cell division and replacement are important factors in aging. In this work, we review and discuss this perspective in the context of interventions in mammals that appear to accelerate or retard aging. Rather than focus on molecular mechanisms, we interpret results from an integrative biology perspective of how gene products affect cellular functions, which in turn impact on tissues and organisms. We review evidence suggesting that mutations that give rise to features resembling premature aging tend to be associated with cellular phenotypes such as increased apoptosis or premature replicative senescence. In contrast, many interventions in mice that extend lifespan and might delay aging, including caloric restriction, tend to either hinder apoptosis or result in smaller animals and thus may be the product of fewer cell divisions. Therefore, it appears plausible that changes in the number of times that cells, and particularly stem cells, divide during an organism's lifespan influence longevity and aging. We discuss possible mechanisms related to this hypothesis and propose experimental paradigms. ***BioEssays*** **30:567–578, 2008. © 2008 Wiley Periodicals, Inc.**

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Abbreviations: Atm, ataxia telangiectasia mutated; Brca1, breast cancer 1; CR, caloric restriction; GH, growth hormone; IGF1, insulin-like growth factor 1.

Introduction

Aging is a major yet mysterious biological process. In humans and other mammals, aging is characterized by a gradual functional decline of virtually every tissue system, increased vulnerability and an increased susceptibility to numerous diseases. Over the years, many hypotheses have been put forward to explain this complex problem.[1,2] These include damage-based hypotheses, such as the free radical and DNA damage hypotheses of aging, and programmed hypotheses, such as those related to endocrine decline. With the advent of modern genetics, researchers have tested these hypotheses, and several genes have been associated with longevity and aging in animal models, including rodents,[3,4] and with premature aging syndromes in man.[5] The fact that mutations in single genes can accelerate or retard multiple components of the aging process is of immense interest to dissect the mechanisms of aging, yet the interpretation of these results is not straightforward.[6–8] Genes that regulate longevity, and that might also determine aging, have been associated with a variety of functions that often span the different hypotheses of aging.[2] It is unsurprising then that research has so far failed to identify the underlying mechanism(s) causing aging, suggesting that new integrative hypotheses may be necessary to elucidate the biological basis of aging.

Cells are the building blocks of life and it is not surprising that they have been associated with aging. One influential hypothesis in the science of aging is the idea that cell division, particularly in the context of replicative senescence, is a primary mechanism of organismal aging.[9–13] In recent years, however, the association between replicative senescence, its associated pathways (e.g. telomere shortening) and aging in vivo has come under attack.[14–16] Rather than discuss specific mechanisms of cellular senescence, though, our goal in this work is to review the association between cell division and organismal aging by primarily focusing on genes reported to influence longevity and aging in vivo. In other words, instead of following a reductionist approach based on molecular mechanisms, one original feature of this review is that we follow an integrative biology

approach to explore how interventions that may modulate the aging process, such as certain genetic manipulations and dietary interventions like caloric restriction, impact on cells, which interact to affect tissues that in turn affect organisms.

The basic hypothesis that we explore in this review is that the amount of divisions cells—and perhaps stem cells in particular—undergo in vivo has an effect on aging differences between individuals of the same species. For instance, one common element in mice with defects in DNA repair that result in premature aging syndromes is at the level of cellular responses to the increased DNA damage, such as an increased vulnerability to apoptosis, which in turn may lead to an imbalance between cell loss and cell replacement.[1,4,6] We review and discuss evidence for this interpretation from what at first may seem disparate molecular pathways shown to impact on longevity and aging in vivo. Lastly, we offer ideas for future research directions on the relationship between cell divisions and aging, including experimental paradigms.

Cell divisions and organismal aging: more than a correlation?

In recent years, a number of studies have shown that it is possible to manipulate longevity, multiple components of the aging process and possibly even the mammalian aging process as a whole by manipulating the expression of one or two genes (Tables 1 and 2). These genes have been related to a variety of functions, including neuroendocrine systems and DNA repair, which in turn can be interpreted as evidence that DNA damage and hormonal alterations are involved in aging.[2,4] Nevertheless, it is possible that many of these genes, albeit related to different molecular functions, impact on aging through common cellular processes, a hypothesis that we explore below.

Mouse models of accelerated or premature aging

Tables 1 and 2 include a selection of major cases of accelerated or premature aging—also called segmental progeroid syndromes—in mice (Table 1) and humans (Table 2) and what is known of their impact on cellular systems. Most of the genes involved are related to DNA repair/metabolism, which can be used to argue that DNA damage plays a causal role in aging.[4] An alternative interpretation, though, is that failure to repair DNA damage and allow transcription to proceed may trigger programmed cell death or prevent cell-cycle progression which in turn impacts on aging.[1,6] Therefore, what follows is a non-exhaustive review of recent results interpreted from a cellular, rather than molecular, perspective.

One key player in DNA repair, cell cycle and apoptosis—among other functions—is the tumor repressor p53. Mice with an activated p53, called $p53^{+/m}$ mice, begin to display signs of premature aging at about 18 months of age. Although their body mass is normal at one year of age, $p53^{+/m}$ mice feature an early loss of body mass presumably due to a decrease in overall cellularity. It has been proposed that the premature aging in $p53^{+/m}$ mice is due to stem cells undergoing premature replicative senescence, which causes a premature exhaustion of their proliferative capacity and in turn a lack of organ homeostasis.[17] This strain exemplifies some key trends observed in many mouse models of accelerated or premature aging. Most notably, they feature cellular alterations, such as increased apoptosis or premature replicative senescence, which may be linked to exhaustion of stem-cell self-renewal and may also help explain the often-observed reduced body weight (Table 1). A few more examples may be helpful.

A somewhat similar phenotype to $p53^{+/m}$ mice is observed in late-generation mice with disrupted Terc (the RNA component of telomerase) and *Atm*, the ataxia telangiectasia mutated gene whose product appears to be related to DNA repair and cell-cycle control. These animals exhibit a slower growth rate and signs of premature aging starting at about 6 months of age accompanied by increased apoptosis, diminished precursor/stem-cell reserves and widespread cellular proliferation defects.[18] Late-generation mice with disrupted Terc and *WRN*, the gene responsible for Werner syndrome (see below), have lower body weights from birth and show premature aging phenotypes only a few weeks after birth. At the cellular level, increased apoptosis and premature replicative senescence have been reported.[19] Whether late-generation mice with disrupted Terc, but wild-type for *WRN* and *Atm*, also display signs of premature aging is debatable,[20] yet it does appear that further disruption of *WRN* and *Atm* emphasizes the premature aging phenotype. Strikingly, disruption of the cell-cycle inhibitor $p21^{WAF1}$ has been recently reported to prolong the lifespan and improve the repopulation capacity and self-renewal of hematopoietic stem cells of late-generation Terc-deficient mice, perhaps by rescuing p21-dependent cell-cycle arrest in stem and progenitor cells.[21]

Brca1, or breast cancer 1, is a cell-cycle checkpoint possibly involved in DNA repair. Most Brca1 hypomorphic mice ($Brca1^{\Delta 11/\Delta 11}$) die at embryonic stages, yet $Brca1^{\Delta 11/\Delta 11}$ mice heterozygous for p53 show increased cellular senescence accompanied by signs of premature aging starting at about 8 months of age. They are also smaller than controls due to slower growth rates.[22] Recently, it has been shown that loss or haploid loss of Chk2, involved in DNA damage response and cell-cycle arrest, also enables $Brca1^{\Delta 11/\Delta 11}$ mice to survive to adulthood and display signs of premature aging.[23] $Brca1^{\Delta 11/\Delta 11}Chk2^{-/-}$ mice outlived $Brca1^{\Delta 11/\Delta 11}p53^{+/-}$ mice and showed signs of premature aging at about 18 months of age. Importantly, the levels of apoptosis were lower in $Brca1^{\Delta 11/\Delta 11}Chk2^{-/-}$ mice than in $Brca1^{\Delta 11/\Delta 11}p53^{+/-}$ mice. $Brca1^{\Delta 11/\Delta 11}Chk2^{-/-}$ mice, though, were smaller than controls and their fibroblasts displayed a premature senescence phenotype. Cao et al.[23] suggested that absence of

Table 1. Selected genetic manipulations in mice that result in putative cases of accelerated or premature aging

Gene name[a]	Common or alternative name	Ageing Phenotype	Growth	Cellular phenotype	Primary reference[b]
ATR		Premature age-related phenotypes when *ATR* is eliminated in adult mice		Loss of proliferating cells	67
Brca1+chk2		Signs of premature aging in mice hypomorphic for Brca1 and double mutant for chk2	Reduced body weight	Premature cellular senescence	23
Brca1+p53		Signs of premature aging in mice hypomorphic for Brca1 and heterozygous for p53	Slower growth rates	Increase in cellular senescence	22
Bub3+Rae1		BUB3/RAE1 haploinsufficient mice show signs of premature aging	Premature loss of body weight	Increase in cellular senescence	26
BubR1	*BUB1B*	Signs of early onset of aging in mice with low levels of BubR1	Growth impaired	Increase in cellular senescence	25
Cdc42GAP	*ARHGAP1*	Signs of premature aging in homozygous knock-outs	Reduced body weight	Premature cellular senescence	98
ERCC1		Signs of premature aging in ERCC1-deficient mice	Growth arrests in the second week	Decreased cellular proliferation and increase in replicative senescence and in apoptotic cells	99
GH	growth hormone, somatotropin	Possible accelerated aging in transgenic mice	Increased postnatal growth		38
p53	*TP53*	Heterozygous mutant mice display signs of accelerated aging	Early loss of body mass at about 18 months	Premature replicative senescence	17
PASG	proliferation associated SNF2-like gene	Putative accelerated aging phenotype due to gene disruption	Growth retardation	Decreased proliferation and premature senescence	24
PolgA	mitochondrial DNA polymerase	Premature aging in mice with a defective PolgA	Slower growth rate from 15–20 weeks	Increased apoptosis	100
SIRT6	sirtuin 6	SIRT6-deficient mice show signs of premature aging	Reduced body size	Impaired proliferation	101
Terc+Atm		Possible premature aging in double mutant mice	Slower growth	Increased apoptosis and cellular proliferation defects	18
Terc+WRN		Possible premature aging in double mutant mice	Lower body weight	Increased apoptosis and premature senescence	19
XPD	xeroderma pigmentosum, group D	Symptoms of premature aging due to homozygous mutation	Growth failure from 12 weeks	Enhanced apoptosis	102

[a]Gene name in accordance with the primary reference. Only the most relevant mouse models were selected.
[b]Only one major reference is featured. For additional references please see the text and consult the GenAge database at http://genomics.senescence.info/genes/.

Table 2. Major segmental progeroid syndromes in humans

Gene name[a]	Common or alternative name	Ageing Phenotype	Growth	Cellular phenotype	Primary reference[b]
CSA	Cockayne Syndrome Type I	Possible premature aging due to recessive mutation	Growth failure early in life	Reduced growth potential	27
WRN	Werner syndrome	Possible accelerated aging due to recessive mutation	Growth failure in teenage years	Slow growth rate and limited proliferation	103
LMNA	Hutchinson-Gilford syndrome	Possible premature aging due to dominant mutation	Growth failure at about age 2	Hyperproliferation and increased apoptosis	104

[a]Gene name in accordance with the primary reference. Only the most important segmental progeroid syndromes were selected.
[b]Only the most relevant reference is featured. For additional references please see the text and consult the GenAge database at http://genomics.senescence.info/genes/.

Chk2 attenuated p53-dependent apoptosis and growth arrest, possibly in stem/progenitor cells and delayed the onset of premature aging.

Premature aging phenotypes in mice are not solely the result of alterations in DNA repair pathways. PASG is a SNF2-like factor that facilitates DNA methylation. It is expressed in rapidly dividing cells and has been linked to cell proliferation. Most PASG-null mice die shortly after birth. In those that survive, signs of premature aging and growth retardation have been reported, though these animals are not known to commonly live more than a month which may make any analysis of aging-related traits difficult. At the cellular level, premature replicative senescence associated with the expression of senescence-associated genes, decreased proliferation—but not increased apoptosis—were also observed.[24]

One final example comes from mitotic checkpoint proteins, which have also been reported to impact on aging. Mice haploinsufficient for BubR1[25] or for Bub3 and Rae1[26] have increased cellular senescence and an early onset of aging-associated phenotypes. *BubR1*$^{H/H}$ mice were growth impaired but not *Bub3*$^{+/-}$*Rae1*$^{+/-}$ mice, though the latter showed a reduced body weight at about two years of age. At the cellular level, fibroblasts from both these strains showed premature senescence and *BubR1*$^{H/H}$ fibroblasts also showed early growth inhibition. Strikingly, though, complete disruption of BubR1, Bub3 or Rae1 results in embryonic lethality.[25,26]

There are many short-lived mouse strains and even those exhibiting signs of premature or accelerated aging do so to different degrees, making it difficult to determine whether they are cases of premature aging or of premature pathology. Nonetheless, one major trend, as pointed out by others,[1,4,6] is for segmental progeroid mouse models to exhibit a reduced body size, often accompanied by stem-cell exhaustion. Importantly, increased apoptosis, premature replicative senescence and/or reduced cellular proliferation also tend to be common hallmarks of animals exhibiting premature signs of aging (Table 1). Therefore, increased cellular loss and/or inefficient cell replacement may, at least in part, mediate accelerated aging phenotypes in mice.

Human segmental progeroid syndromes

Even if segmental progeroid syndromes in man—as in mice—are not exact representations of accelerated aging, because they modulate multiple components of the aging process, they can provide insights into the mechanistic basis of human aging and age-related disease. The same trend described above of premature aging syndromes in mice being associated with defects in mechanisms related to cellular homeostasis is also observed in human segmental progeroid syndromes. Patients with Werner syndrome, Hutchinson-Gilford syndrome and Cockayne syndrome type I, arguably the three main segmental progeroid syndromes caused by single gene mutations, show growth retardation (Table 2). In Cockayne and Hutchinson-Gilford syndromes, growth failure is a typical clinical manifestation in the first years of life and patients usually die in their second decade of life with features resembling premature aging.[5,27,28] Though a slow growth rate is also frequent in children with Werner syndrome, clinical manifestations of the disease typically appear later in life and include the lack of a teenage growth spurt.[5] Patients then go on to display features resembling accelerated aging.

At the cellular level, premature cellular senescence and/or increased apoptosis have been reported in the three major human segmental progeroid syndromes.[28] Fibroblasts from patients with Cockayne syndrome show reduced growth potential,[29] and fibroblasts from patients with Werner's syndrome exhibit slow growth rates, probably due to rapid rates of exit from the cell cycle and into senescence, and a limited proliferative ability.[30] In contrast, fibroblasts from patients with Hutchinson-Gilford syndrome show hyperproliferation and increased apoptosis.[28,31] Indeed, it has been proposed that Hutchinson-Gilford syndrome is the outcome of this increased apoptotic cell death to levels that exhaust the ability of tissues for stem-cell-driven regeneration.[32]

Therefore, one hypothesis is that at least some of the signs of premature aging observed in these pathologies are not due to DNA damage accumulation per se but a result of changes in cellular homeostasis. This is illustrated in the most striking segmental progeroid syndrome—i.e. that which most strongly resembles premature aging—Werner syndrome in which it has been argued that it is the increase in somatic cell senescence that primarily contributes to its phenotype,[7] as further discussed ahead. Although what is considered premature aging remains controversial, other syndromes featuring such signs also tend to be characterized by similar growth defects and cellular alterations, as observed in patients with Bloom syndrome in which apoptosis has been reported to play an important role.[33]

Mammalian models of life extension and delayed aging

Following the above rationale, it is possible that genetic manipulations increasing lifespan in rodents exert their effects, at least partly, at the cellular level. Mutations in *GHR/BP, Pit1* and *Prop1* increase lifespan and may delay aging in mice.[8,34–36] The resulting strains, respectively, Laron, Snell dwarf and Ames dwarf mice, are all considerably smaller than controls with evidence of growth retardation. Although segmental progeroid syndromes are also often associated with stunted growth, the cellular and physiological mechanisms are likely very different from those resulting in life extension. Considering that reduced insulin/insulin-like signaling also extends lifespan in nematodes and fruit flies,[37] the prevailing hypothesis is that mutations in these long-lived mouse strains impact on aging through suppression of growth hormone (GH), insulin and insulin-like growth factor 1 (IGF1) signaling.[2,3] GH and IGF1, though they are involved in multiple processes, are essentially mitogens that have a profound impact on cellular growth and division. Insulin, too, is a mitogenic hormone. Lower levels of these hormones, or resistance to GH, appear to prolong lifespan and maybe delay aging. The possibility that transgenic mice overexpressing GH, which show increased postnatal growth and are 30 to 60% bigger than controls, suffer from accelerated aging is also in line with the idea that cell proliferation and aging are related.[38] At least, overexpression of GH in mice reduces lifespan.[39] Overall, while it would be naïve to assume that changes in body size or growth rates alone explain the extended lifespan observed in these models,[40] a correlation between diminished cellular proliferation and life extension in mouse models appears to exist.

Though it is sometimes unclear whether life extension is related to aging or due to the amelioration of some specific pathology,[8] there are other long-lived mouse strains whose phenotypes may be mediated by cellular mechanisms, such as the *mclk1*$^{+/-}$ mouse. Cells from mclk1-null mice are more resistant to stress and DNA damage and exhibit a slow growth, yet animals die at embryonic stages. In *mclk1*$^{+/-}$ mice, however, hepatocytes that lost mclk1 expression undergo clonal expansion. Interestingly, *mclk1*$^{+/-}$ mice are slightly, 15 to 30%, longer lived.[41] Another long-lived mouse model is the p66^{shc}-null mouse, which has been reported to live 30% longer than controls and whose cells are more resistant to apoptosis. p66^{shc} is a splice variant of *SHC*, involved in signal transduction and transmission of mitogenic signals.[42] Similarly, overexpression of human thioredoxin extends lifespan in transgenic mice by about 35%.[43] Thioredoxin is a redox-active protein implicated in cell growth and apoptosis inhibition.[44] Lastly, it has been recently reported that mice with type 5 adenylyl cyclase knocked out have an about 30% increase in median lifespan and their cells are resistant to stress and apoptosis.[45] Major genes extending lifespan in mice are recapped in Table 3.

In addition to genetic manipulations, one major intervention capable of delaying aging in model organisms is caloric restriction or CR,[46] which can also be interpreted in terms of tissue-level alterations. Succinctly, at least in some tissues, CR leads to a decrease in cellular proliferation.[47] For example, a reduced proliferative capacity of lens epithelial cells has been associated with increased age of mice and is delayed by CR both in vitro and in vivo.[48] In CR animals apoptosis has been reported to be attenuated in some tissues,[49] but maybe not in others.[50] Importantly, CR mammals are considerable smaller than controls, as observed in rodents and rhesus monkeys.[51] In fact, Clive McCay's groundbreaking experiments on CR were based on the hypothesis that stunted growth extends lifespan within species.[52] While this interpretation appears to be overly simplistic,[40,52] it is interesting to note that CR does not appear to extend lifespan in smaller mouse strains,[53] including in wild-derived mice that tend to live longer and be smaller and lighter than laboratory-adapted strains.[54] As argued before,[2,55] and supported by experimental results,[56] CR may exhibit its effects at least partly by suppression of the GH/IGF1 axis. Therefore, it is not surprising that one proposed explanation for CR is that endocrine mechanisms, including lower IGF1 levels, decrease the stimulus for cell replication.[57] In fact, it has been proposed that hormones regulate growth and lifespan in CR and long-lived rodent mutants by exerting their actions on cell-cycle regulation during the course of the lifespan.[58]

In mammals, body size may be affected by many factors (such as fat accumulation) but it is primarily a result of the number of cells in the body. Certainly, numerous factors can affect the final cell number of an organism or organ such as the number of starting cells, rate of cell division and cell death, and the timing of differentiation to terminate cell division. Nonetheless, all other things being equal, a larger number of cell divisions will result in a bigger organism. Intriguingly, a number of results have accumulated in recent years associating, within a given species, small body size with a longer lifespan.[40] Bigger animals tend to die young and vice-versa. This is clear

Table 3. Major genetic interventions that appear to retard aging in mice or at least considerably extend lifespan

Gene name[a]	Common or alternative name	Ageing Phenotype	Growth	Cellular phenotype	Primary reference[b]
AC5	type 5 adenylyl cyclase	Increase in median lifespan by about 30%	Old animals have a reduced body weight	Increased resistance to apoptosis	45
clk-1	*mclk1*	15–30% increase in lifespan in heterozygous mice	Normal		41
GHR/BP	Laron mouse	Increase in lifespan of 40–50% in homozygous knock-outs	Slower growth rate		35
GHRHR	little mouse	Increase in lifespan of about 25% in homozygous knock-outs	Reduced body weight		36
p66^{shc}	*SHC1*	Roughly 30% increase in lifespan in -/- mice	Apparently normal weight	Increased resistance to apoptosis	42
Pit1	Snell dwarf mouse	Lifespan increase of 42% in homozygous mice	Small body size		36
Prop1	Ames dwarf mouse	Homozygous mice show over 50% increases in lifespan	Small body size		34
TRX	thioredoxin	Overexpression increases lifespan by about 35%	Normal weight		43

[a]Gene name in accordance with the primary reference. Only the most relevant genes were selected.
[b]Only one major reference is featured. For additional references please see the text and consult the GenAge database at http://genomics.senescence.info/genes/.

in mice in which growth appears to have a negative effect on lifespan, and body weight is a significant predictor of longevity.[40,52] Moreover, in F_2 hybrids between wild-derived and laboratory-derived stocks, lifespan negatively correlated with body weight.[59] The notion that smaller animals of the same species live longer than larger ones has been consistently observed among mammals and even some non-mammalian species.[40] Smaller breeds of dogs live longer and their fibroblasts show an increased cellular growth potential,[60] and there is anecdotal evidence that smaller breeds of horses also live longer.[40]

In humans, a negative correlation between height and longevity also appears to exist. Findings based on millions of deaths suggest that shorter, smaller bodies have lower death rates[61] and female carriers of a variant GH allele were reported to have a shorter body height and a reduced mortality.[62] These results are even more significant because of the observed negative correlation between height and coronary heart disease.[63] One hypothesis is that, just like people with short stature may tend to have narrower coronary arteries that predispose to heart disease, faster growth may increase the incidence of specific diseases without having an impact on aging as a whole. For instance, smaller animals with lower levels of IGF1 and fewer cells may be expected to be less cancer-prone.[40] Nonetheless, this observation alone does not explain the phenotype of dwarf mice because at least some strains actually appear to age slower.[8,36] Therefore, though the relationship between size and number of cell divisions is not simple, the possibility exists that individuals whose cells divided more during the lifespan have a shorter lifespan not just because of tissue-specific failures but because of alterations in the basic aging process.

Cell divisions and aging: a perspective

Our goal herein is to review how gene products integrate into cells to interpret models of mammalian aging from a cellular perspective. As described above, genetic alterations that appear to regulate longevity and may alter the aging process tend to have an impact on cellular phenotypes, which may help explain the observed organismal phenotypes and suggest an association between cell division in vivo and longevity (Fig. 1). Many, perhaps most, mutations that give rise to signs of premature aging in mice or men appear to increase cell loss by, for instance, increasing apoptosis or by reducing the proliferative capacity of cells and triggering premature cellular senescence (Tables 1 and 2). Mice and patients with such diseases also appear to suffer from growth defects, though it is unclear whether the diminished ability to replace lost cells due to cell death is a causal mechanism. Similarly, loss of tissue mass is also observed during normal aging and some age-related pathologies, even if it is not known whether this is due to an imbalance between lost cells and new cells.[6] Be it as it may, similar mechanisms may ultimately contribute to premature aging phenotypes. In contrast, mutations and interventions that increase lifespan and may delay aging in mice appear to either hinder apoptosis or result in smaller animals that may be the result of fewer cell divisions (Table 3). Smaller individuals of the same species, including humans, also tend to live longer. Therefore, diminished cellular proliferation may contribute to a longer

maintenance of tissue homeostasis and to an extended lifespan (Fig. 1).

All of the above examples highlight the crucial point in this work that molecular functions and pathways might not be the best way to interpret these phenotypes. Just as to appreciate the beauty of a painting one cannot look too closely, perhaps to understand how genes modulate aging and longevity we must look away from the molecular mechanisms and observe the cellular- and tissue-level alterations. This is further supported by several lines of evidence.

In contrast to the aforementioned late-generation mice with disrupted Terc and WRN, WRN-deficient mice, albeit having more tumors, do not show signs of premature aging.[64] Cells from WRN-deficient mice are susceptible to DNA-damaging agents, yet because of differences in telomere metabolism between mice and humans, they do not exhibit premature cellular senescence. In contrast, cells from late-generation mice with disrupted Terc show accelerated replicative senescence. These observations have been suggested as evidence that it is not the molecular functions of WRN that are critical for the observed signs of premature aging but their impact on cellular senescence.[7] In fact, increased genomic instability is observed in some mouse strains displaying signs of premature aging but not in others.[65] Moreover, several other mutations disrupting DNA repair do not cause accelerated aging but only impact on cancer.[2] Interestingly, it has been proposed that differences at the level of damage and DNA-damage response, which in turn determines the outcome of the cell (e.g. whether cells survive or trigger apoptosis), explain whether disruption of DNA-repair pathways have an impact on aging or not.[66] The hypothesis that cellular proliferation per se impacts on aging is supported by the observation that elimination of 80–90% of proliferating cells by disruption of ATR (involved in DNA-damage response and cell-cycle regulation) results in several age-related phenotypes in young adult mice accompanied by a depletion of stem and progenitor cells and exhaustion of tissue renewal and homeostatic capacity.[67]

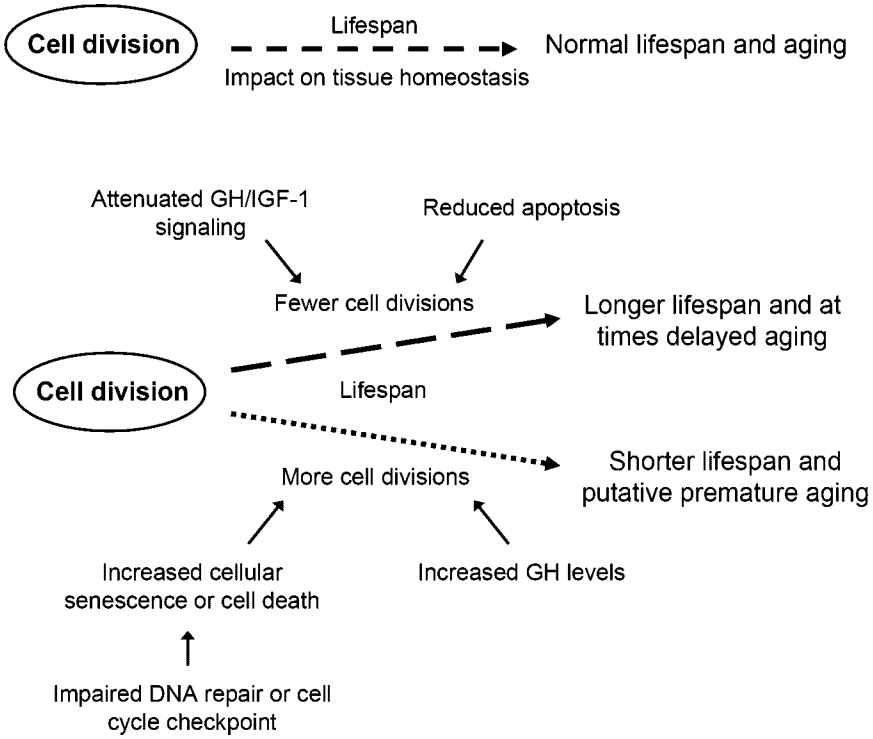

Figure 1. Overview of our cellular perspective of genetic interventions that impact on longevity and aging in mammalian species. Our rationale is based on the hypothesis that cell division throughout the lifespan contributes, at least to some degree, to aging and age-related pathologies. Mutations that result in reduced cell death (e.g. due to reduced apoptosis without interfering with normal physiological functions) or attenuated GH/IGF1 signaling will result in cells undergoing fewer divisions in the same amount of time of the organism's life, possibly contributing to an extended lifespan and, in some cases, a delayed aging process. On the contrary, in mutations that—through whatever molecular mechanism—result in increased cell death or replicative senescence or higher GH levels, the amount of cell divisions for the normal development of organisms must be higher, in the former, to compensate for the cell death or quiescent cells, which as a consequence can contribute to a shorter lifespan with early-aging symptoms.

In light of these observations, it appears plausible that changes in the amount of times cells must divide or in their capacity to divide during the lifespan have an impact on longevity and possibly on aging (Fig. 1). This is in agreement with the hypothesis, inspired by replicative senescence,[9] that as cells divide during the lifespan some form of cellular changes occur that contribute to a gradual disruption of tissue homeostasis.

Unanswered questions and future perspectives

Certainly, not all mutations that modulate apoptosis, cellular senescence and tissue homeostasis in vivo impact on aging. In many cases, alterations of these pathways disrupt normal processes that result in severe deleterious effects and often death at early ages. For instance, mice lacking caspase 9 show reduced apoptosis yet most die perinatally.[68] Probably, an organism's development is based on genetically determined parameters for cell death, replacement and division. Any change in these parameters can potentially be lethal and mask any effects genes involved in these processes may have on aging. Further examples are abundant: *Ku86*$^{-/-}$ mice show signs of premature aging, as they do in a *p53*$^{+/-}$ background despite a higher cancer incidence. In a *p53*$^{-/-}$ background, however, the lifespan is too short (due to cancer induction) to determine whether there is any impact on aging.[69] On the contrary, well-controlled apoptosis can contribute to increased longevity by limiting cancer, as elegantly demonstrated in the cancer resistance and increased survival of mice with ubiquitous expression of the cancer-specific pro-apoptotic domain of the Par-4 tumor-suppressor protein.[70] Therefore, while molecular mechanisms that increase cell loss may contribute to premature aging, the relationship between apoptosis and aging is not simple and the benefits of elevated apoptosis in some contexts might explain why in certain tissues CR appears to increase apoptosis.[50]

The idea that aging has a cellular basis is an old one.[12,13,71–73] This work highlights how cellular phenotypes may at least partly mediate the effects of different genes regulating longevity. Intriguingly, one possible explanation is the presence, at least in some tissues, of a cell division counting mechanism (i.e. a timekeeper) that plays an underlying role in the aging process. Telomere shortening, a mechanism limiting replicative lifespan in vitro, at least in some cell lines, demonstrates how precise genetically determined molecular clocks can limit cell division. In fact, early postnatal growth retardation has been associated with increased longevity and longer telomeres in rats.[74] Nonetheless, a causal relation between replicative senescence/telomere maintenance and human aging is not obvious.[12,15,16]

We speculate that other clocks may exist, even if the essence of these is not clear yet and might depend on localized controls of cell proliferation. For example, one mechanism typically regulating the pace of development is the control of the rate of cell division[75] and thus developmentally regulated mitotic clocks may exist. One mechanism that has been associated with lifespan and development in invertebrates and can impact on cell division, is microRNAs,[76,77] the role of which in mammalian aging (e.g. in the abovementioned long-lived mouse models) merits further investigation. In addition, some evidence suggests an association between mitotic age and methylation,[78] hence it is possible that higher order chromatin and methylation changes can potentially impact on proliferation and differentiation. Lastly, there is increasing evidence that the TOR signaling pathway, involved in nutrient sensing and cell growth, also regulates lifespan, at least in invertebrates,[79] and this an area of great interest for future research in mammals.

Restraints on cell division may not necessarily be due to a counting mechanism. Cell division may contribute to damage or changes, e.g. the accumulation of some protein or molecule, which in turn lead to a gradual loss of cell function and/or renewal, proliferative ability, or to the accumulation of senescent cells that disrupt tissue function and structure. In fact, many plausible mechanisms have been proposed by which cell division can lead to a disease state due to "altered proliferative homeostasis".[80] One appropriate example is $p16^{INK4a}$, a cell-cycle regulator that has been associated with cellular aging in vitro.[13] Recent results from mice suggest that increasing $p16^{INK4a}$ levels limit the self-renewal and regeneration ability in islets, forebrain progenitors and haematopoietic stem cells. Mice deficient in $p16^{INK4a}$ showed a smaller age-related decline in self-renewal potential.[81]

Assuming that a lack of balance between cell loss and cell replacement can contribute to aging, loss of tissue homeostasis could be due to widespread cellular changes or due to changes in the proliferation capacity of specific cell populations, such as stem cells.[82] In other words, the relation between cell divisions and aging can be mediated by a declining ability to recruit cells from stem or progenitor cell niches or by dysfunction in the differentiation pathways.[1] Some premature-aging syndromes in mice have been associated with the depletion of stem-cell stocks which impairs tissue self-renewal.[17,18,23] Moreover, in mice, intrinsic changes appear to occur during aging of hematopoietic stem cells[83] and in muscle satellite cells.[84] Intrinsic age-related changes have also been reported in human mesenchymal stem cells, including increases in p53 expression accompanied by increased apoptosis and reduced proliferation and osteoblast differentiation.[85] Though a definitive causal relation between stem-cell decline (in numbers and/or function) and organismal aging has not yet been established, the idea that aging stem cells contribute to aging in several organ systems remains a powerful one,[82,86] and further research is warranted (see below).

Not only are tissues the product of interactions between different cell types but whole organisms are the result of different interacting tissues. As such, changes in one tissue may be a result of systemic alterations whose underlying mechanism occurs only in another tissue. For example, it has been suggested that the high incidence of cardiovascular disease observed in Werner syndrome patients is not caused by intrinsic changes in the cells of the cardiovascular system but rather by systemic perturbations, for example due to visceral fat accumulation and/or high circulating levels of cytokines.[28] In fact, systemic effects of fat tissue on the levels of hormones, cytokines and complement factors have been suggested to play a role in the benefits of CR,[87] though fat metabolism alone does not explain the life extension observed in long-lived mice. Moreover, massive cell death might overwhelm the immune system and lead to autoimmune conditions and/or inflammatory states associated with age-related pathologies while senescent cells may secrete inflammatory cytokines that disrupt tissue homeostasis even affecting postmitotic cells.[88]

Although we discussed mostly results from mammals, it is remarkable that reducing insulin/IGF1 signaling in nematodes and fruit flies can also extend lifespan.[37] Even though these are mostly postmitotic animals as adults, some results also suggest that cell division may play a role in life-extension phenotypes observed in invertebrates. Succinctly, the insulin receptor and insulin-like peptides appear to regulate cell size and number in fruit flies,[89] as well as stem-cell proliferation.[37] Development in nematodes follows a pattern of cell divisions,[90] yet interestingly the arrested development phase known as "dauer" is long lived and can be triggered by suppression of insulin/IGF1 signaling.[37] Moreover, vertebrate homologs of the DAF-16 protein, often required for life extension in roundworms, downregulate genes promoting cell-cycle progression.[37] The evolutionary conservation of these signals in affecting lifespan and their role in cell proliferation suggest that reduced cell division may be a step in the life extension of different species by the GH–insulin–IGF1 pathway.

Throughout this work, we focused on intraspecies differences. As detailed above, the tendency, at least in mammals, is for individuals of the same species with a small body size to live longer. We hypothesize that certain mouse strains are long lived in part due to molecular and/or genetic alterations that decrease the amount of cellular divisions necessary to produce a viable adult organism. While such hypothesis might partly explain differences in longevity between individuals of the same species, it does not explain differences between species.[40] In fact, it seems to clash with the observation that comparatively small laboratory mice do not commonly live more than 4 years while medium-sized dogs live over 20 and human beings can live over 100 years. This correlation between body size and longevity between species indicates there must be developmentally determined growth and proliferation limits intrinsic to cells that are different between mice, dogs and humans.[13] Identifying the underlying causes of such differences between species is of great interest to understand mammalian aging (see below).

Future experimental paradigms

As detailed above, and reviewed by others,[1,6] the role of cellular mechanisms has been studied in a considerable number of mouse and human models of accelerated or premature aging. There have been surprisingly few such studies, however, in long-lived mouse models. Apart from a small number of studies in CR animals and one study suggesting decreased $p16^{INK4a}$ levels in GHR/BP knock-out mice,[91] the impact of cellular division and the possible role of stem cells have been largely unexplored in long-lived mouse models. We suggest that studying the role of cell division and stem-cell self-renewal in long-lived models is an area of potential interest that merits further attention. For example, even studying cellular senescence biomarkers in long-lived mice, as has been recently done for baboons,[92] would be a potentially important first step, though we acknowledge that a good marker for cellular senescence is lacking. Studying stem-cell renewal, as has been done in normal mice and DNA repair-deficient mice,[83] and the mitotic index of cells in vivo would be additional lines of research.

To test the hypothesis that cell divisions alone contribute to aging in vivo, we require a model where only the amount of cell divisions is altered. Pygmy mice, a result of disruption of the *HMGA2* gene, might be adequate in that respect. They have a small body size as adults even though HMGA2 is not significantly expressed after weaning.[93] Interestingly, one study in mice found a QTL associated with maximum lifespan encompassing the region of the *HMGA2* gene[94] and this gene has been recently associated with height in humans.[95] Moreover, there are no reported alterations in the GH/IGF1 axis in pygmy mice, suggesting that HMGA2 perhaps acts by regulating the cell cycle, maybe through higher-order chromatin modifications. On the contrary, mice with disrupted $p27^{Kip1}$, involved in cell-cycle regulation, exhibit increased cell proliferation and gigantism. Because they have normal GH and IGF1 levels, these mice could be an interesting model to study the impact of increased cell proliferation in aging, though they often develop pituitary tumors.[96] Overall, we think both these strains are potentially adequate choices to test whether cell division alone can have an impact on longevity and aging.

For reasons outlined above, we believe that the relationships between cell division, longevity and aging may be evolutionary conserved to a large extent, yet the correlation between body size and longevity among species indicates major species-specific differences in cellular processes such as apoptosis, proliferation and differentiation[40] For example,

if changes during cell division contribute to aging then large mammals like whales must have evolved different patterns for how these changes occur or different rates of cell division and turnover that offset intrinsic cellular changes. Similarly, small but long-lived animals like birds, bats and naked mole-rats must feature key differences in terms of cell turnover and replication that allow them to live longer and avoid age-related diseases like cancer. Therefore, comparisons between species of parameters like cell growth and proliferation, apoptosis and differentiation (and comparisons of pathways associated with these processes) may yield clues about which and how cellular changes and processes contribute to aging. In the same context, it may be valuable to create transgenic mice with key genes (e.g. apoptotic or cell cycle regulators) replaced by the homologs from long-lived species of similar body size, such as naked mole-rats, to gain insights into cellular mechanisms and genes that could have contributed to the evolution of longevity.

Conclusions

Finding common themes between genes altering aging is important to understand this complex process, particularly now that more and more genes are being associated with aging in animal models. As reviewed above, genes that regulate longevity and appear to modulate aging have a wide range of molecular functions but many tend to have common effects at a cellular level (Fig. 1). This has been particularly well-described in segmental progeroid syndromes in mice and man in which marked cellular alterations have been frequently described, often affecting stem and progenitor cells (Tables 1 and 2). In long-lived mouse strains, even though some results associate cellular processes (e.g. apoptosis) with life extension, the evidence is more modest (Table 3). If cellular processes like proliferation, apoptosis and replicative senescence are causal mechanisms of normal aging then they should be as important in delaying aging as they appear to be in models of accelerated aging. Thus, further cell biology studies in long-lived mice are warranted and, one might argue, essential to assess the value of current cellular models in understanding the mammalian aging process.

Overall, the idea that the amount of divisions that cells undergo in vivo contributes to aging and influences the longevity of individual organisms, perhaps by some sort of cellular clock(s), remains a promising hypothesis. It is not our purpose, however, to claim that differences in cell division explains all observations or are the sole major driving force of aging. For example, results from parabiotic pairings between young and old mice suggest the presence of both systemic and intrinsic factors in aging.[97] Nevertheless, we think the cellular perspective developed herein, bringing together different lines of experimentation, can be helpful to interpret and guide the study of aging at a cellular and organismal level. Finally, we propose experimental paradigms to study the role of cell division in aging, including the use of pygmy mice and $p27^{Kip1}$ knockouts as models of potential interest.

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References

1. Warner HR. 2005. Developing a research agenda in biogerontology: basic mechanisms. Sci Aging Knowledge Environ 2005:pe33.
2. de Magalhaes JP. 2005. Open-minded scepticism: inferring the causal mechanisms of human ageing from genetic perturbations. Ageing Res Rev 4:1–22.
3. Bartke A, Coschigano K, Kopchick J, Chandrashekar V, Mattison J, et al. 2001. Genes that prolong life: relationships of growth hormone and growth to aging and life span. J. Gerontol A Biol Sci Med Sci 56:B340–B349.
4. Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. 2003. Aging and genome maintenance: lessons from the mouse? Science 299:1355–1359.
5. Martin GM, Oshima J. 2000. Lessons from human progeroid syndromes. Nature 408:263–266.
6. Warner HR, Sierra F. 2003. Models of accelerated ageing can be informative about the molecular mechanisms of ageing and/or age-related pathology. Mech Ageing Dev 124:581–587.
7. Kipling D, Davis T, Ostler EL, Faragher RG. 2004. What can progeroid syndromes tell us about human aging? Science 305:1426–1431.
8. de Magalhaes JP, Cabral JA, Magalhaes D. 2005. The influence of genes on the aging process of mice: a statistical assessment of the genetics of aging. Genetics 169:265–274.
9. Hayflick L. 1994. How and Why We Age. New York: Ballantine Books.
10. Campisi J. 1996. Replicative senescence: an old lives' tale? Cell 84: 497–500.
11. Wright WE, Shay JW. 2002. Historical claims and current interpretations of replicative aging. Nat Biotechnol 20:682–688.
12. de Magalhaes JP. 2004. From cells to ageing: a review of models and mechanisms of cellular senescence and their impact on human ageing. Exp Cell Res 300:1–10.
13. Patil CK, Mian IS, Campisi J. 2005. The thorny path linking cellular senescence to organismal aging. Mech Ageing Dev 126:1040–1045.
14. Cristofalo VJ, Allen RG, Pignolo RJ, Martin BG, Beck JC. 1998. Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proc Natl Acad Sci USA 95:10614–10619.
15. Cristofalo VJ, Lorenzini A, Allen RG, Torres C, Tresini M. 2004. Replicative senescence: a critical review. Mech Ageing Dev 125:827–848.
16. de Magalhaes JP, Toussaint O. 2004. Telomeres and telomerase: a modern fountain of youth? Rejuvenation Res 7:126–133.
17. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, et al. 2002. p53 mutant mice that display early ageing-associated phenotypes. Nature 415:45–53.
18. Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, et al. 2003. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. Nature 421:643–648.
19. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, et al. 2004. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nat Genet 36:877–882.
20. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, et al. 1997. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91:25–34.
21. Choudhury AR, Ju Z, Djojosubroto MW, Schienke A, Lechel A, et al. 2007. Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. Nat Genet 39:99–105.
22. Cao L, Li W, Kim S, Brodie SG, Deng CX. 2003. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. Genes Dev 17:201–213.

23. Cao L, Kim S, Xiao C, Wang RH, Coumoul X, et al. 2006. ATM-Chk2-p53 activation prevents tumorigenesis at an expense of organ homeostasis upon Brca1 deficiency. Embo J 25:2167–2177.
24. Sun LQ, Lee DW, Zhang Q, Xiao W, Raabe EH, et al. 2004. Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG. Genes Dev 18:1035–1046.
25. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, et al. 2004. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet 36:744–749.
26. Baker DJ, Jeganathan KB, Malureanu L, Perez-Terzic C, Terzic A, et al. 2006. Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. J. Cell Biol 172:529–540.
27. Henning KA, Li L, Iyer N, McDaniel LD, Reagan MS, et al. 1995. The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. Cell 82:555–564.
28. Cox LS, Faragher RG. 2007. From old organisms to new molecules: integrative biology and therapeutic targets in accelerated human ageing. Cell Mol Life Sci 64:2620–2641.
29. Thompson KV, Holliday R. 1983. Genetic effects on the longevity of cultured human fibroblasts. II. DNA repair deficient syndromes. Gerontology 29:83–88.
30. Faragher RG, Kill IR, Hunter JA, Pope FM, Tannock C, et al. 1993. The gene responsible for Werner syndrome may be a cell division ''counting'' gene. Proc Natl Acad Sci USA 90:12030–12034.
31. Bridger JM, Kill IR. 2004. Aging of Hutchinson-Gilford progeria syndrome fibroblasts is characterised by hyperproliferation and increased apoptosis. Exp Gerontol 39:717–724.
32. Halaschek-Wiener J, Brooks-Wilson A. 2007. Progeria of stem cells: stem cell exhaustion in Hutchinson-Gilford progeria syndrome. J. Gerontol A Biol Sci Med Sci 62:3–8.
33. Davalos AR, Campisi J. 2003. Bloom syndrome cells undergo p53-dependent apoptosis and delayed assembly of BRCA1 and NBS1 repair complexes at stalled replication forks. J. Cell Biol 162:1197–1209.
34. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. 1996. Dwarf mice and the ageing process. Nature 384:33.
35. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. 2000. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 141:2608–2613.
36. Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. 2001. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc Natl Acad Sci USA 98: 6736–6741.
37. Tatar M, Bartke A, Antebi A. 2003. The endocrine regulation of aging by insulin-like signals. Science 299:1346–1351.
38. Bartke A. 2003. Can growth hormone (GH) accelerate aging? Evidence from GH-transgenic mice. Neuroendocrinology 78:210–216.
39. Wolf E, Kahnt E, Ehrlein J, Hermanns W, Brem G, et al. 1993. Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: lessons from transgenic animal models. Mech Ageing Dev 68:71–87.
40. Miller RA, Austad SN. 2006. Growth and aging: Why do big dogs die young?. In: Masoro EJ, Austad SN, editors. Handbook of the Biology of Aging. New York: Academic Press. p 512–533.
41. Liu X, Jiang N, Hughes B, Bigras E, Shoubridge E, et al. 2005. Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice. Genes Dev 19:2424–2434.
42. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, et al. 1999. The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402:309–313.
43. Mitsui A, Hamuro J, Nakamura H, Kondo N, Hirabayashi Y, et al. 2002. Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. Antioxid Redox Signal 4:693–696.
44. Haendeler J, Hoffmann J, Tischler V, Berk BC, Zeiher AM, et al. 2002. Redox regulatory and anti-apoptotic functions of thioredoxin depend on S-nitrosylation at cysteine 69. Nat Cell Biol 4:743–749.
45. Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, et al. 2007. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. Cell 130:247–258.
46. Weindruch R, Walford RL. 1988. The Retardation of Aging and Disease by Dietary Restriction. Springfield: C.C. Thomas.
47. Lok E, Scott FW, Mongeau R, Nera EA, Malcolm S, et al. 1990. Calorie restriction and cellular proliferation in various tissues of the female Swiss Webster mouse. Cancer Lett 51:67–73.
48. Li Y, Yan Q, Wolf NS. 1997. Long-term caloric restriction delays age-related decline in proliferation capacity of murine lens epithelial cells in vitro and in vivo. Invest Ophthalmol Vis Sci 38:100–107.
49. Shelke RR, Leeuwenburgh C. 2003. Lifelong caloric restriction increases expression of apoptosis repressor with a caspase recruitment domain (ARC) in the brain. Faseb J 17:494–496.
50. James SJ, Muskhelishvili L, Gaylor DW, Turturro A, Hart R. 1998. Up-regulation of apoptosis with dietary restriction: implications for carcinogenesis and aging. Environ Health Perspect 106 (Suppl 1):307–312.
51. Roth GS, Ingram DK, Black A, Lane MA. 2000. Effects of reduced energy intake on the biology of aging: the primate model. Eur J Clin Nutr 54 (Suppl 3):S15–S20.
52. Rollo CD. 2002. Growth negatively impacts the life span of mammals. Evol Dev 4:55–61.
53. Forster MJ, Morris P, Sohal RS. 2003. Genotype and age influence the effect of caloric intake on mortality in mice. Faseb J 17:690–692.
54. Harper JM, Leathers CW, Austad SN. 2006. Does caloric restriction extend life in wild mice? Aging Cell 5:441–449.
55. Mobbs CV, Bray GA, Atkinson RL, Bartke A, Finch CE, et al. 2001. Neuroendocrine and pharmacological manipulations to assess how caloric restriction increases life span. J. Gerontol A Biol Sci Med Sci 56 Spec No 1:34–44.
56. Bonkowski MS, Rocha JS, Masternak MM, Al Regaiey KA, Bartke A. 2006. Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. Proc Natl Acad Sci USA 103: 7901–7905.
57. Sonntag WE, Lynch CD, Cefalu WT, Ingram RL, Bennett SA, et al. 1999. Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. J. Gerontol A Biol Sci Med Sci 54:B521–B538.
58. Bowen RL, Atwood CS. 2004. Living and dying for sex. A. theory of aging based on the modulation of cell cycle signaling by reproductive hormones. Gerontology 50:265–290.
59. Harper JM, Durkee SJ, Dysko RC, Austad SN, Miller RA. 2006. Genetic modulation of hormone levels and life span in hybrids between laboratory and wild-derived mice. J. Gerontol A Biol Sci Med Sci 61: 1019–1029.
60. Li Y, Deeb B, Pendergrass W, Wolf N. 1996. Cellular proliferative capacity and life span in small and large dogs. J. Gerontol A Biol Sci Med Sci 51:B403–B408.
61. Samaras TT, Elrick H, Storms LH. 2003. Is height related to longevity? Life Sci 72:1781–1802.
62. van Heemst D, Beekman M, Mooijaart SP, Heijmans BT, Brandt BW, et al. 2005. Reduced insulin/IGF-1 signalling and human longevity. Aging Cell 4:79–85.
63. Silventoinen K, Kaprio J, Koskenvuo M, Lahelma E. 2003. The association between body height and coronary heart disease among Finnish twins and singletons. Int J Epidemiol 32:78–82.
64. Lebel M, Leder P. 1998. A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. Proc Natl Acad Sci USA 95:13097–13102.
65. Dolle ME, Busuttil RA, Garcia AM, Wijnhoven S, van Drunen E, et al. 2006. Increased genomic instability is not a prerequisite for shortened lifespan in DNA repair deficient mice. Mutat Res 596:22–35.
66. Mitchell JR, Hoeijmakers JH, Niedernhofer LJ. 2003. Divide and conquer: nucleotide excision repair battles cancer and ageing. Curr Opin Cell Biol 15:232–240.
67. Ruzankina Y, Pinzon-Guzman C, Asare A, Ong T, Pontano L, et al. 2007. Deletion of the developmentally essential gene ATR in adult mice leads to premature aging phenotypes and stem cell loss. Cell Stem Cell 1:113–126.
68. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, et al. 1998. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 94:325–337.

69. Lim DS, Vogel H, Willerford DM, Sands AT, Platt KA, et al. 2000. Analysis of ku80-mutant mice and cells with deficient levels of p53. Mol Cell Biol 20:3772–3780.
70. Zhao Y, Burikhanov R, Qiu S, Lele SM, Jennings CD, et al. 2007. Cancer resistance in transgenic mice expressing the SAC module of Par-4. Cancer Res 67:9276–9285.
71. Wolf NS, Pendergrass WR. 1999. The relationships of animal age and caloric intake to cellular replication in vivo and in vitro: a review. J. Gerontol A Biol Sci Med Sci 54:B502–B517.
72. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, Wang T, Gregg D, et al. 2003. Aging, progenitor cell exhaustion, and atherosclerosis. Circulation 108:457–463.
73. Chien KR, Karsenty G. 2005. Longevity and lineages: toward the integrative biology of degenerative diseases in heart, muscle, and bone. Cell 120:533–544.
74. Jennings BJ, Ozanne SE, Dorling MW, Hales CN. 1999. Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Lett 448:4–8.
75. Minelli A. 2003. The Development of Animal Form: Ontogeny, Morphology, and Evolution. Cambridge: Cambridge University Press.
76. Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297.
77. Boehm M, Slack F. 2005. A developmental timing microRNA and its target regulate life span in C. elegans. Science 310:1954–1957.
78. Kim JY, Tavare S, Shibata D. 2005. Counting human somatic cell replications: methylation mirrors endometrial stem cell divisions. Proc Natl Acad Sci USA 102:17739–17744.
79. Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, et al. 2004. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr Biol 14:885–890.
80. Martin GM. 2007. The genetics and epigenetics of altered proliferative homeostasis in ageing and cancer. Mech Ageing Dev 128:9–12.
81. Beausejour CM, Campisi J. 2006. Ageing: balancing regeneration and cancer. Nature 443:404–405.
82. Sharpless NE, Depinho RA. 2007. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol 8:703–713.
83. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, et al. 2007. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447:725–729.
84. Conboy IM, Conboy MJ, Smythe GM, Rando TA. 2003. Notch-mediated restoration of regenerative potential to aged muscle. Science 302: 1575–1577.
85. Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, et al. in press. Age-related intrinsic changes in human bone marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell.
86. Van Zant G, Liang Y. 2003. The role of stem cells in aging. Exp Hematol 31:659–672.
87. Barzilai N, Gupta G. 1999. Revisiting the role of fat mass in the life extension induced by caloric restriction. J. Gerontol A Biol Sci Med Sci 54:B89–B96; discussion B97–88.
88. Parrinello S, Coppe JP, Krtolica A, Campisi J. 2005. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J. Cell Sci 118:485–496.
89. Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, et al. 2001. An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr Biol 11:213–221.
90. Koreth J, van den Heuvel S. 2005. Cell-cycle control in Caenorhabditis elegans: how the worm moves from G1 to S. Oncogene 24:2756–2764.
91. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, et al. 2004. Ink4a/Arf expression is a biomarker of aging. J. Clin Invest 114: 1299–1307.
92. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. 2006. Cellular senescence in aging primates. Science 311:1257.
93. Zhou X, Benson KF, Ashar HR, Chada K. 1995. Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGI-C. Nature 376:771–774.
94. Klebanov S, Astle CM, Roderick TH, Flurkey K, Archer JR, et al. 2001. Maximum life spans in mice are extended by wild strain alleles. Exp Biol Med (Maywood) 226:854–859.
95. Weedon MN, Lettre G, Freathy RM, Lindgren CM, Voight BF, et al. 2007. A common variant of HMGA2 is associated with adult and childhood height in the general population. Nat Genet 39:1245–1250.
96. Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, et al. 1996. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell 85:721–732.
97. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, et al. 2005. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 433:760–764.
98. Wang L, Yang L, Debidda M, Witte D, Zheng Y. 2007. Cdc42 GTPase-activating protein deficiency promotes genomic instability and premature aging-like phenotypes. Proc Natl Acad Sci USA 104:1248–1253.
99. Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, et al. 2006. A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature 444:1038–1043.
100. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, et al. 2004. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 429:417–423.
101. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, et al. 2006. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cel. 124:315–329.
102. de Boer J, Andressoo JO, de Wit J, Huijmans J, Beems RB, et al. 2002. Premature aging in mice deficient in DNA repair and transcription. Science 296:1276–1279.
103. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, et al. 1996. Positional cloning of the Werner's syndrome gene. Science 272:258–262.
104. Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, et al. 2003. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423:293–298.