

The Biology of Aging

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Abstract

In humans, aging is inexorable. The progressive decrease in physiological capacity and the reduced ability to respond to environmental stresses lead to increased susceptibility and vulnerability to disease. Consequently, mortality due to all causes increases exponentially with aging. Attempts at understanding the causes of aging are limited by the complexity of the problem. Aging changes are manifest from the molecular to the organismic level; environmental factors affect experimental observations; secondary effects complicate elucidation of primary mechanisms; and precisely defined, easily measurable “biomarkers” are lacking. No one unifying theory may exist, since the mechanisms of aging could be quite distinct in different organisms, tissues, and cells. Evolutionary pressures have selected for successful reproduction, making it likely that the post-reproductive physiology of an organism (i.e., aging) is an epigenetic and pleiotropic manifestation of the optimization for early fitness. Indeed, antagonistic pleiotropy, wherein genes that enhance early survival and function but are disadvantageous later in life, may play an overriding role in aging. Theories of aging can be divided into two general categories: stochastic and developmental-genetic. These are not mutually exclusive, particularly when considering the free radical/mitochondrial DNA theory of aging. Increasing evidence suggests that cellular senescence and organismic aging are antagonistically pleiotropic manifestations of evolutionary pressures to prevent malignant transformation. In other words, aging may be the price we pay to avoid cancer. The beneficial paradox may be that the maximum lifespan potential of humans may have been achieved, in part, due to our ability to grow old.

Key Words: Aging, senescence, cellular, lifespan, mortality, antagonistic pleiotropy, cancer.

“Every day you get older — that’s a law.”
— *Butch Cassidy and the Sundance Kid* (1)

“There is no such thing as a free lunch.”
— **Anonymous** (2)

“Aging seems to be the only available way to live a long life.”
— **Daniel Francois Esprit Auber** (3)

DISCUSSIONS OF AGING invariably begin by establishing satisfactory definitions for the term “aging” and the related word “senescence.” Although the term “aging” is commonly used to refer to post-maturational processes that lead to

diminished homeostasis and increased organismic vulnerability, the more correct term for this is “senescence.” “Aging” can refer to any time-related process. In this paper, however, “senescence” and “aging” will be used interchangeably. “Normal” aging involves inexorable and universal physiological changes, whereas “usual” aging includes age-related diseases. For example, menopause and the decline in renal function represent aspects of normal aging. In contrast, coronary artery disease is an example of usual aging and is not found in all older persons. This approach to aging can utilize a conceptual framework that identifies intrinsic (developmental-genetic) versus extrinsic (stochastic) causes. However, accumulating evidence increasingly stresses the importance of both. Indeed, the altered homeostasis in older organisms is likely the result of a genetic program that determines the response to exogenous influences and thereby increases the predisposition to illness and death.

Lifespan and Life Expectancy

The average/median lifespan (also known as life expectancy) is represented by the age at

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which 50% of a given population survive, and maximum lifespan potential (MLSP) represents the longest-lived member(s) of the population or species. The average lifespan of humans has increased dramatically over time, yet the MLSP has remained approximately constant and is usually stated to be 90–100 years (Fig. 1) (4). For 99% of our existence as a species, the average life expectancy for humans was very short compared to the present. Due to disease and accidents, people living 50,000 years ago rarely lived beyond the age of 40. Throughout most of recorded human history, socioeconomic status and nutritional status have been strongly associated with life expectancy and, along with disease, resulted in significant variations in the lifespan of individuals. By 1900, improved sanitation helped to raise the average life expectancy at birth in the United States to 57 years, but infectious disease was still a major killer. In the latter half of the twentieth century, better diet, health care, and reduced infant mortality had resulted in an average life expectancy in the United States of about 80 years as of 1980 (5). The increase in the average life expectancy has resulted in a compression of morbidity (a squaring of the mortality curve) toward the end of the lifespan (Fig. 1). The unavoidable presence of trauma and accidents prevents 100% survivability. Of note, the longest-lived human for whom documentation exists was Jeanne Calment, who died in France at the age of 122, in August 1997. The longest-lived

male was Christian Mortensen, who died in San Francisco at the age of 115, in 1998. As causes of early mortality have been eliminated as a result of public health measures and improved medical care, more individuals have approached the maximum lifespan. Between 1960 and 1994, the population of those aged 85 and older increased by 274%. During this interval, the less elderly population doubled while the entire U.S. population increased by 45% (6).

MLSP appears to be species specific, implying a significant genetic component to the rate of aging. For example, humans have an MLSP 25–30-fold higher than mice. Some biodemographic estimates predict that elimination of most of the major killers such as cancer, cardiovascular disease, and diabetes would add no more than 10 years to the average life expectancy, but would not affect MLSP (7, 8). This implies an upper limit to the MLSP. Some models suggest that genes operate to raise or lower the relative risk of death, by making cancer, coronary disease, or Alzheimer's disease more likely, rather than by fixing the lifespan. One mathematical model predicts that if participants in the Framingham Heart Study had been able to maintain the levels of 11 different risk factors to be similar to those of a typical 30 year old, the men and women would have survived to an average age of 99.9 and 97.0 years, respectively (7).

There are three known regimens that can extend lifespan. The first two involve lowering ambient temperature and reducing activity, and are effective in poikilotherms (cold-blooded species). A decrease of 10°C or the elimination of a housefly's capacity to fly extends the maximum lifespan approximately 250% (9). Both of these manipulations decrease the metabolic rate and are accompanied by decreases in free radical generation and oxidative damage to protein and DNA. Dietary restriction without malnutrition can increase both the average and maximum lifespans of mice and rats by more than 50% (10, 11). Although calories are severely restricted (up to 40%), essential nutrients such as vitamins and minerals are maintained at levels equivalent to those found in *ad libitum* diets. The diet-restricted animals also exhibit a delay in the onset of physiological and pathological changes associated with aging (12). These include hormone and lipid levels, female reproduction, immune function, nephropathy, cardiomyopathy, osteodystrophy, and malignancies. Size, weight, fat percentage and some organ weights are markedly less in calorically

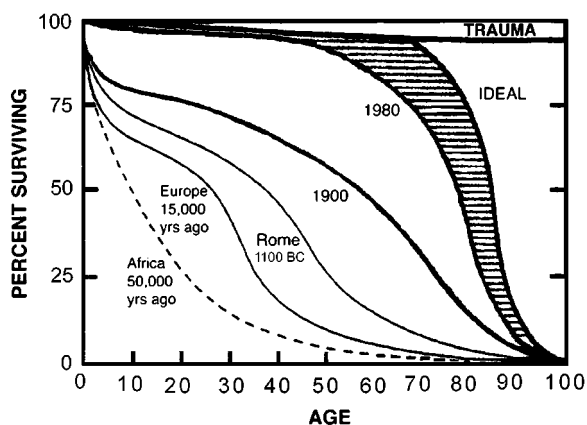


Fig. 1. Percent survival curve for humans at different times in history with varying environments, nutrition, and medical care. The 50% survival values have improved, but maximum lifespan potential has remained the same. Adapted with permission from: Cutler RG. Evolutionary perspective of human longevity. In: Hazzard WR, Andres R, Bierman EL, et al., editors. Principles of geriatric medicine and gerontology. New York: McGraw-Hill; 1990. p. 16 (reference 4).

restricted animals (13). The specific metabolic rate, the amount of oxygen consumed per gram of tissue, decreased in rats subjected to caloric restriction (14, 15). However in one study, long-term food restriction did not alter the metabolic rate (16). This finding suggests that the specific metabolic rate may not be a critical determinant of longevity. To date, the effect of dietary restriction on lifespan has been convincingly demonstrated only in rodents. Caloric restriction in rhesus monkeys leads to reductions in body temperature and energy expenditure, consistent with changes seen in rodent studies in which aging is retarded by dietary restriction (17, 18). Calorie restriction also increases high-density lipoprotein (19) and retards the post-maturational decline in serum dehydroepiandrosterone sulfate in the rhesus monkeys (20).

Characteristics of Aging

There is evidence supporting at least 5 common characteristics of aging in mammals (Table 1):

1. **Increased mortality with age after maturation.** In the early nineteenth century, Gompertz first described the exponential increase in mortality with aging due to various causes, a phenomenon that still pertains today (21). In 1995, the death rate for all causes for people in the U.S. between the ages of 25–44 was 189.5/100,000 and for those aged 65 and over was 5,069.0/100,000: a >25-fold increase (22). Indeed, the pattern of age-related survival is similar across species, including invertebrates and single-cell organisms (Fig. 2) (23).
2. **Changes in biochemical composition in tissues with age.** There are notable age-related decreases in lean body mass and total bone mass in humans (24, 25). Although the amount of subcutaneous fat is either un-

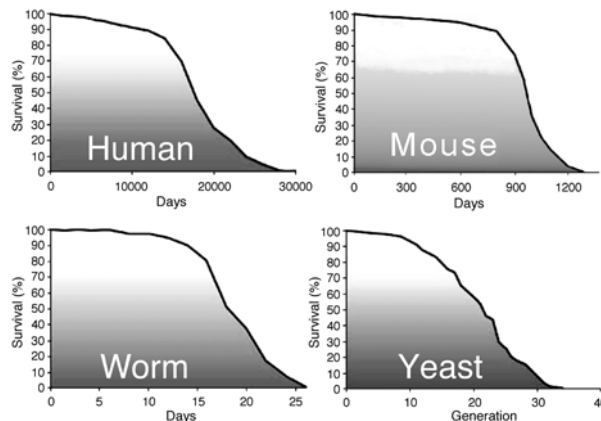


Fig. 2. Viability curves from different model organisms have a similar characteristic shape. Representative mortality data are shown for *Homo sapiens*, *Mus musculus*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae*. Reproduced with permission from reference 23.

changed or decreases, total fat remains the same (24). Consequently, the percentage of adipose tissue increases with age. At the cellular level, many markers of aging have been described in various tissues from different organisms (26). Two of the first to be described were increases in lipofuscin (age pigment) (27) and increased cross-linking in extracellular matrix molecules such as collagen (28, 29). Additional examples include age-related changes in both the rates of transcription of specific genes and the rate of protein synthesis and numerous age-related alterations in post-translational protein modifications, such as glycation and oxidation (30, 31).

3. **Progressive decrease in physiological capacity with age.** Many physiologic changes have been documented in both cross-sectional and longitudinal studies. Examples include declines in glomerular filtration rate, maximal heart rate, and vital capacity (32). These decreases occur linearly from about the age of 30; however, the rate of physiological decline is quite heterogeneous from organ to organ and individual to individual (33, 34).
4. **Reduced ability to respond adaptively to environmental stimuli with age.** A fundamental feature of senescence is the diminished ability to maintain homeostasis (35). This is manifest not primarily by changes in resting or basal parameters, but in the altered response to an external stimulus such

TABLE 1
Characteristics of Aging

1. Increased mortality with age after maturation.
2. Changes in biochemical composition in tissues with age.
3. Progressive decrease in physiological capacity with age.
4. Reduced ability to respond adaptively to environmental stimuli with age.
5. Increased susceptibility and vulnerability to disease.

as exercise or fasting. The loss of “reserve” can result in blunted maximum responses as well as in delays in reaching peak levels and in returning to basal levels. For example, the induction of hepatic tyrosine aminotransferase activity by fasting is both attenuated and delayed in old rodents (35).

5. **Increased susceptibility and vulnerability to disease.** The incidence and mortality rates for many diseases increase with age and parallel the exponential increase in mortality with age (36). For the five leading causes of death for people over 65, the relative increase in death rates compared to the rates for people age 25–44 are: heart disease 92-fold, cancer 43-fold, stroke greater than 100-fold, chronic lung disease greater than 100-fold, and pneumonia and influenza 89-fold (22). The basis for these dramatic rises in mortality is incompletely understood, but presumably involves changes in the function of many types of cells, which lead to tissue/organ dysfunction and systemic illness. Interestingly, a retrospective study of centenarians demonstrated that they live 90–95% of their lives in very good health and with a high level of functional independence (37). The centenarians do suffer a 30–50% annual mortality at the end of their lives, but this represents a marked compression of morbidity toward the end of life and is close to the idealized survival curve in Fig. 1.

Mechanisms / Causes of Aging

Evolutionary

In an effort to adequately explain the phenotype of aged organisms, many theories about the cause(s) of aging have been proposed. However, what is supposedly “known” about the fundamental molecular mechanisms involved in aging remains controversial and largely unproven. A major reason for this is the obvious complexity of the problem. Aging changes are manifest from the molecular to the organismic levels; environmental factors affect experimental observations; secondary effects complicate elucidation of primary mechanisms; and precisely defined, easily measurable “biomarkers” are lacking. No one unifying theory may be valid, since the mechanisms of aging could be quite distinct in different organisms, tissues, and cells.

A general framework for a plausible theory of aging begins with understanding the evolutionary basis of senescence. Evolutionary pressures select for a minimum successful life: this includes the ability to reach reproductive age, procreate, and then care for offspring until they are weaned (so that they, in turn, will achieve reproductive age and continue the cycle) (38, 39). Within this context, it is likely that the post-reproductive/parental physiology of an organism is an epigenetic and pleiotropic manifestation of the optimization for early fitness. Kirkwood proposes that three categories of genes may be involved in senescence (40): (a) those that regulate somatic maintenance and repair, (b) negatively pleiotropic genes that enhance early survival but are disadvantageous later in life (antagonistic pleiotropy), and (c) harmful late-acting mutations upon which little evolutionary selection is exerted. The presence of these genes may represent a spectrum from general to species specific (Fig. 3). Genes involved in cell maintenance and repair are likely to be present in all (or most) organisms, since such essential processes are similar across species. Late-acting mutations are probably species specific, because they are likely to be individualistic and random. Non-maintenance pleiotropic genes could be universally found within a population or species, but may not be shared between species. An example of antagonistic pleiotropy would be the high expression of testosterone in a male gorilla, which could lead to increased aggression and strength that would allow the male to become dominant and mate more frequently, but may eventually lead to a shortened lifespan due to increased atherosclerosis. Recent studies at the molecular genetic level have suggested that cellular senescence may be antagonistically pleiotropic because it prevents tumorigenesis, but also contributes to organismic aging (see below).

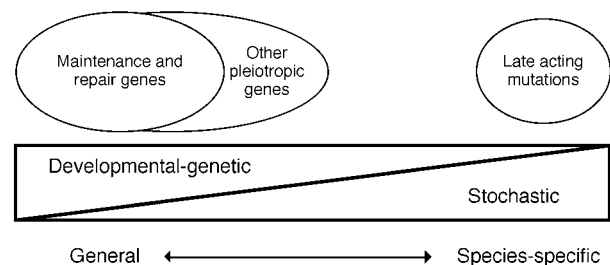


Fig. 3. Genes / events regulating longevity and senescence. Adapted with permission from: Kirkwood TB. Human senescence. *Bioessays* 1996; 18:1009–1016 (reference 40).

Historically, theories of aging have been divided into two general categories: stochastic and developmental-genetic (Table 2). The term “developmental-genetic” implies a more active genetic control of senescence than likely exists (see above). In addition, as described below, these categories are not mutually exclusive, particularly when considering the free radical/mitochondrial DNA theory of aging. Indeed, there is probably a spectrum from birth to senescence that reflects a decreasing influence of active genetic influences and an increasing effect of stochastic events (Fig. 3). This would parallel the shift in importance from general to species-specific genes.

Stochastic Theories

Somatic Mutation and DNA Repair

Stochastic theories propose that aging is caused by random damage to vital molecules. The damage eventually accumulates to a level sufficient to result in the physiological decline associated with aging. The most prominent example is the somatic mutation theory of aging, which states that genetic damage from background radiation produces mutations that lead to functional failure and, ultimately, death (41, 42). Exposure to ionizing radiation does shorten lifespan (43). However, analysis of survival curves of radiation-treated rodent populations reveals an increase in the initial mortality rate without an effect on the subsequent rate of aging (44). The lifespan shortening is probably due to increased cancer and glomerulosclerosis rather than accelerated aging *per se* (45).

The DNA repair theory is a more specific example of the somatic mutation theory. The ability to repair ultraviolet-radiation-induced DNA damage in cell cultures derived from species with a variety of different lifespans cor-

relates directly with the MLSP (46). Unfortunately, there is not enough experimental support to conclude that these differences between species are a causative factor in aging. The cumulative evidence indicates that overall DNA repair capacity does not appear to change with age, although the site-specific repair of select regions of DNA appears to be important in several types of terminally differentiated cells (47). Future studies will need to focus upon repair rates of specific genes rather than indirect general measurements.

Error-Catastrophe

The error-catastrophe theory proposes that random errors in synthesis eventually occur in proteins that synthesize DNA or other “template” molecules (48). Generally, errors occurring in proteins are lost by natural turnover and simply replaced with error-free molecules. Error-containing molecules which are involved in the protein-synthesizing machinery, however, would introduce errors into the molecules that they produce. This could result in an amplification such that the subsequent rapid accumulation of error-containing molecules would result in an “error-catastrophe” that would be incompatible with normal function and life. However, although there are numerous reports of altered proteins in aging, no direct evidence of age-dependent protein mis-synthesis has yet been reported. The altered proteins that do occur in aging cells and tissues are, instead, due to post-translational modifications such as oxidation and glycation (49, 50). The increases in altered proteins appear to be due to decreased clearance in older cells (51).

Protein Modification

In addition to age-related changes in the steady-state levels of proteins, qualitative alterations leading to changes in function occur. Aging is accompanied by decreased specific activity in many enzymes, altered heat stability, and increased carbonyl content of proteins (50). These changes can be caused by direct oxidation of amino acid residues, metal-catalyzed oxidation, modification by lipid oxidation products, and glycation. Kohn (29) and Bjorksten (28) hypothesized that the accumulation of post-translationally altered proteins could impair cellular, and ultimately, organ function. Although collagen undergoes increased cross-linking with age (52), such alterations can lead to improved function at some sites and to impaired function at others (53). The nonenzy-

TABLE 2
Theories of Aging

Stochastic
Somatic Mutation and DNA Repair
Error-Catastrophe
Protein Modification
Free Radical (Oxidative Stress) / Mitochondrial DNA
Developmental-Genetic
Longevity Genes
Accelerated Aging Syndromes
Neuroendocrine
Immunologic
Cellular Senescence
Cell Death

matic reaction of carbohydrates with amino groups of proteins (glycation) can give rise to advanced glycosylation end-products (AGEs) (50). These AGEs increase with aging and are implicated in diabetes, eye disorders, and amyloid accumulation. Many extracellular matrix proteins exhibit increased cross-linking with age. Proper organ function depends upon a normal extracellular matrix for processes such as diffusion of essential molecules. In addition, the extracellular matrix plays an important role in the regulation of gene expression. The cross-linking of macromolecules such as collagen, elastin, osteocalcin, and the eye lens protein crystallin (which may be responsible for cataract formation in both the diabetic and aged lens) could alter both of these processes. These covalent protein-protein interactions probably play a role in the increased stiffness of vascular walls with aging. Protein carboxyl methyltransferase (PCMT) assists in the repair of spontaneously arising atypical protein isoaspartyl residues (54). Overexpression of PCMT at 29°C in flies extended lifespan, suggesting that under certain environmental conditions, protein repair could be important in longevity.

Free Radical (Oxidative Stress) / Mitochondrial DNA

Another potential cause of cross-linking — free radicals — forms the basis for a theory that has elements of both the stochastic and developmental-genetic classes. Harman initially proposed that most aging changes are due to molecular damage caused by free radicals (55, 56), which are atoms or molecules that contain an unpaired electron and are therefore highly reactive. Aerobic metabolism generates the superoxide radical ($O_2^{\bullet-}$), which is metabolized by superoxide dismutases to form hydrogen peroxide (H_2O_2) and oxygen (57). Hydrogen peroxide can go on to form the extremely reactive hydroxyl radical (OH^{\bullet}). These oxygen-derived species can react with macromolecules in a self-perpetuating manner; they create free radicals out of subsequently attacked molecules, which in turn create free radicals out of other molecules, thereby amplifying the effect of the initial free radical attack (9). Reactive oxygen species appear to play a role in regulating differential gene expression, cell replication, differentiation, and apoptotic cell death (in part by acting as secondary messengers in signal transduction pathways) (58, 59). Production of free radicals in the heart, kidney, and liver of a group of mammals was found to be inversely

proportional to the maximum lifespan, although the activities of individual anti-oxidative enzymes were not consistently related to maximum lifespan (60). Overexpression of either superoxide dismutase or catalase alone in transgenic flies does not extend lifespan (61), but some transgenic flies with increased expression of both Cu, Zn-superoxide dismutase and catalase, which act in tandem to remove $O_2^{\bullet-}$ and H_2O_2 , respectively, exhibit up to a one-third extension of average and maximum lifespan (61). In addition, there was increased resistance to oxidative damage and an increase in the metabolic potential (total amount of oxygen consumed during adult life per unit body weight).

The mitochondrial DNA / oxidative stress hypothesis represents a synthesis of several theories and therefore comprises elements of both stochastic and developmental-genetic mechanisms of aging (see below). It is proposed that reactive oxygen species contribute significantly to the somatic accumulation of mitochondrial DNA mutations, leading to the gradual loss of bioenergetic capacity and eventually resulting in aging and cell death (Fig. 4) (62–64). Ozawa has dubbed this the “redox mechanism of mitochondrial aging” (65). Mitochondrial DNA (mtDNA) undergoes a progressive age-related increase in oxygen free radical damage in skeletal muscle (66–68), the diaphragm (69, 70), cardiac muscle (71–74), and the brain (75, 76). This exponential increase in damage correlates with the increase in both point and deletion somatic mtDNA mutations seen with age. Interestingly, extrapolation of the curve to the point where 100% of cardiac mtDNA exhibits deletion mutations produces an age of 129 (65). A deleterious positive feedback results, wherein mtDNA damage leads to defective mitochondrial respiration, which in turn enhances oxygen free radical formation, leading to additional mtDNA damage. Mitochondrial DNA is maternally transmitted, continues to replicate throughout the lifespan of an organism in both proliferating and post-mitotic (non-proliferating) cells, and is subject to a much higher mutation rate than nuclear DNA. This is due, in large part, to inefficient repair mechanisms and its proximity to the mitochondrial membrane

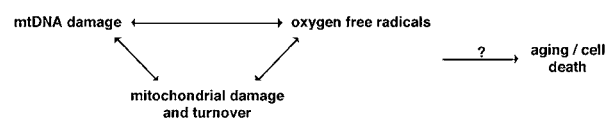


Fig. 4. Mitochondrial DNA and free radical interaction.

where reactive-oxygen species are generated. Defects in mitochondrial respiration with age are found not only in normal tissues (77), but also in people with diseases that are increasingly manifest with age, such as Parkinson's disease (78, 79), Alzheimer's disease (80, 81), Huntington's chorea (82), and other movement disorders (83). Diseases for which mtDNA mutations have been found include Alzheimer's (84, 85) and Parkinson's diseases (75, 85–88), and a large number of skeletal and cardiac myopathies (69, 89–93). Apoptosis has also been associated with mtDNA fragmentation (94). Is the phenotype of aging in tissues actually due to mtDNA mutation? Specific mutations, while increasing with age, seldom account for more than several percent of the total mtDNA. However, some studies suggest that the total percentage of mtDNA affected by mutations is much greater, as much as 85%, and increases with age (65). In addition, caloric restriction in mice retards the age-associated accumulation of mtDNA mutations (95). Inherited mitochondrial DNA variants are associated with aging and longevity (96). The J haplogroup was found in a significantly greater percentage of male centenarians in northern Italy than in younger subjects. Interestingly, this same mitochondrial haplotype is overrepresented in a number of complex diseases (97), raising the possibility of an antagonistically pleiotropic gene or genes that exert deleterious effects in younger individuals, but lead to better health at later ages (successful aging). To complicate matters further, mitochondrial DNA polymorphisms are present in different frequencies in various aged populations from Italy, Ireland, and Japan (98). Ongoing studies in Utah, utilizing extensive genealogical records of Mormons, are testing the hypothesis that longevity is maternally determined, given the inheritance of only maternal mtDNA.

Agents that bypass blocks in the respiratory chain, such as coenzyme Q10, tocopherol, nicotinamide, and ascorbic acid, would be predicted to ameliorate some of the effects of mitochondrial disease and aging. Withdrawal of coenzyme Q from the diet of nematodes extends their lifespan by approximately 60% (99). Caloric restriction, which can extend lifespan, reduces oxidative damage in primates (100). There are epidemiological studies that suggest roles for dietary antioxidants in the reduction of vascular dementia, cardiovascular disease, and cancer in humans (101). However, results to date in treatment of patients with myopathies have been variably or only anecdotally success-

ful (65). This suggests that a complex interaction exists between pro-oxidant and antioxidant forces in the cell, and that regulation of the balance between the two may be the critical determinant in mitochondrial, and subsequently, cellular and tissue integrity during aging.

Developmental-Genetic Theories

Developmental-genetic theories consider the process of aging to be part of the genetically programmed and controlled continuum of development and maturation. Although this is an attractive notion, the diverse expression of aging effects is in sharp contrast to the tightly controlled and very precise processes of development. Also, evolution selects for the optimization of reproduction; the effects of genes expressed in later life probably do not play a large role in the evolution of a species. This class of theories is supported by the observation that the maximum lifespan is highly species specific. As noted above, the maximum lifespan for humans is 30 times that of mice. In addition, studies comparing the longevity of monozygotic and dizygotic twins and non-twin siblings have shown a remarkable similarity between monozygotic twins that is not seen in the other two groups.

Longevity Genes

There is ample evidence in many species that MLSP is under genetic control, though the degree of heritability is likely to be less than 35% (102). Despite this apparently low figure, genetic mutations can significantly modify senescence. In yeast a number of genes affect both the average and maximum lifespan (103). The products of these genes act in diverse ways, including modulating stress response, sensing nutritional status, increasing metabolic capacity, and silencing genes that promote aging. In the nematode (*Caenorhabditis elegans*), mutants with increased lifespan have revealed various genes that appear to play a relevant role (104): *age-1* alters aging rate; *daf-2* and *daf-23* activate a delay in development; *spe-26* reduces fertility; and *clk-1* alters the biological clock. These genes alter stress resistance (particularly in response to ultraviolet light), development, signal transduction, and metabolic activity. The recently isolated *daf-2* gene appears to encode an insulin-receptor family member (105). Mutations in *daf-2* can double the lifespan, but require the *daf-16* gene (106). A mutation in the *daf-16* gene suppresses the UV resistance and

increases longevity of the other gene mutants, suggesting that it acts at a critical point downstream of the other genes (104). The *daf-16* gene is a member of the hepatocyte nuclear factor-3/forkhead family of transcriptional regulators, involved in a variety of signal transduction pathways, including insulin signaling (107). A notable connection between single gene effects upon aging in yeast and higher eukaryotes was revealed by the finding that overexpression of the *SIR2* gene and its homolog extend lifespan in yeast and nematodes, respectively (23). Despite acting via different mechanisms, *SIR2* and its homolog may both exert their effects by linking the regulation of metabolic rate to aging.

A line of *Drosophila melanogaster* has been identified that exhibits an approximately 35% increase in average lifespan and enhanced resistance to various forms of stress, including starvation, high temperature, and dietary paraquat, a free-radical generator (108). The mutation responsible, dubbed "Methuselah," appears to reside within a single gene that is homologous to GTP-binding transmembrane domain receptors. Another single gene mutation leads to an almost doubling of the average adult *Drosophila* lifespan without a decline in fertility or physical activity (109). This gene, named "Indy" (for "I'm not dead yet"), is homologous to a mammalian sodium dicarboxylate cotransporter, which is a membrane protein that transports Krebs cycle intermediates. The investigators speculate that the mutation in the Indy gene may create a metabolic state that mimics caloric restriction. Previous studies have demonstrated that one group of long-lived flies is more resistant to oxidative stress (110), whereas another group exhibits resistance to starvation and desiccation (111).

Genetic analysis of longevity in mammals has not been as revealing. However, immune loci in mice and humans have been implicated in long-lived subjects (103) (see below). In addition, in the gene encoding the signaling molecule p66(shc), there is a mutation which significantly enhances the resistance to oxidative stress and increases the mean lifespan of mice by 30% (112). The Snell dwarf mouse contains a single gene mutation that alters pituitary development and prevents the production of growth hormone, thyrotropin, and prolactin (113). The dwarf mouse also exhibits an extended lifespan of 25–50%, but is much smaller than normal mice. In contrast, the mice with the mutant p66 develop normally and are not significantly smaller than wild type mice.

As noted above, a number of mitochondrial DNA polymorphisms are associated with longevity. In addition, the epsilon 4 allele of apolipoprotein E (ApoE), which is associated with increased coronary disease and Alzheimer's disease, is inversely correlated with longevity (114). In contrast, the epsilon 2 allele of ApoE and an angiotensin-converting enzyme (ACE) allele are found more frequently in French centenarians (114). Interestingly, the ApoE2 allele is associated with type III and IV hyperlipidemia, and the ACE allele predisposes to coronary disease. These findings further suggest that genes can exert pleiotropic age-dependent effects upon longevity. Perls et al. note that support for a genetic contribution to human longevity is further provided by data demonstrating that siblings and parents of centenarians live longer (115). Linkage analysis implicates the presence of a gene or genes on chromosome 4 that are associated with exceptional longevity (115). These authors report that a high percentage of centenarians have had children while in their 40s (well before assisted reproduction). They therefore postulate that an evolutionary force to prolong the period of child bearing would lead to the selection of longevity-enabling genes. Collectively, these studies also raise the question as to whether some genes affect susceptibility to disease rather than alter intrinsic aging.

In contrast to studies that uncover alterations in the expression of single genes during aging, Weindruch and Prolla and their colleagues have begun investigating the broad spectrum of changes in gene expression that occur during aging and calorie restriction in mice and in monkeys (116–119). A common theme is that aging induces a differential gene expression pattern in muscle and brain consistent with inflammatory and oxidative stress, and reduced expression of metabolic and biosynthetic genes. In muscle and brain from mice, caloric restriction either completely or partially prevented the age-related changes in gene expression. Interestingly, caloric restriction did not ameliorate the age-induced alteration in the program of gene expression seen in muscle from aging monkeys. So even though the age-related changes in gene expression may be similar across species, the response to caloric restriction may not be similar.

Accelerated Aging Syndromes

Although no genetic disease exists that is an exact phenocopy of normal aging, several

human genetic diseases, including Hutchinson-Gilford syndrome (the “classic” early-onset progeria seen in children), Werner’s syndrome (“adult” progeria), and Down’s syndrome (trisomy 21), display some features of accelerated aging (120).

Werner’s syndrome (WS) is an autosomally recessive inherited disease (121). Patients prematurely develop arteriosclerosis, glucose intolerance, osteoporosis, early graying, loss of hair, skin atrophy, and menopause. However, they do not typically suffer from Alzheimer’s disease or hypertension. WS patients have an increased incidence of sarcomatous tumors and develop cataracts on the posterior surface of the lens, not in the nucleus, as is usually seen in older people. In addition, they develop laryngeal atrophy and ulcerations on the arm and legs. Most patients die before the age of 50. The gene responsible for WS has been localized to chromosome 8 (122) and appears to be a helicase (123), an enzyme involved in unwinding DNA. DNA helicases play a role in DNA replication and repair. Cells from WS patients display chromosomal instability, elevated rates of gene mutation, and nonhomologous recombination. However, there is no obvious defect in DNA repair mechanisms, as evidenced by a resistance to ultraviolet exposure or other DNA damaging agents, similar to normal cells.

Hutchinson-Gilford syndrome is an extremely rare, autosomal recessive disease in which aging characteristics begin to develop within several years of birth (121). These include wrinkled skin, stooped posture, and growth retardation. These patients suffer from advanced atherosclerosis, and usually die from myocardial infarction, by the age of 30. However, unlike WS patients, they do not typically suffer from cataracts, glucose intolerance, or skin ulcers.

People with Down’s syndrome have trisomy or a translocation involving chromosome 21 (120, 121). They suffer from the early onset of vascular disease, glucose intolerance, hair loss, and degenerative bone and joint disease, as well as increased incidence of cancer. Their lifespan is apparently 50–70 years (not as short as previously believed, since earlier mortality may have represented neglect of these individuals). Dementia occurs earlier and more often in patients with Down’s syndrome than in the general population. Patients develop neuropathological changes similar to the changes seen in dementia of Alzheimer’s type, including amyloid deposition and neurofibrillary tangles. This

may be related to the presence of the β -amyloid gene on chromosome 21.

Kuro-o recently reviewed a number of mouse models which have been developed that exhibit many of the aging phenotypes seen in humans (124) (Table 3). Of these, the *klotho* mouse suffers from a defect in a single gene that codes for a membrane protein; it exhibits a plethora of marked age-related phenotypes that are also seen in humans. These include reduced lifespan, decreased activity, premature thymic involution, skin atrophy, arteriosclerosis, osteoporosis, emphysema, and lipodystrophy. There are a number of strains of senescence-accelerated mice (SAM) that exhibit variable aging phenotypes consistent with multigenic effects. Targeted disruption of genes responsible for premature aging syndromes in humans results in incomplete or absent age-related phenotypes. Despite the fact that none of the mouse models displays all of the phenotypes associated with human aging, they are likely to be valuable tools in identifying some of the molecular mechanisms of aging.

Neuroendocrine Theory

The neuroendocrine theory proposes that functional decrements in neurons and their associated hormones are central to the aging process (125). An important version of this theory holds that the hypothalamic-pituitary-adrenal (HPA) axis is the master regulator of aging in an organism. Because the neuroendocrine system regulates early development, growth, puberty, control of the reproductive system, metabolism, and many other aspects of normal physiology, functional changes in this system could exert effects throughout the organism. The decline in female reproductive capacity is an obvious neuroendocrine age-related change. Mounting evidence suggests that both the ovary and the brain play key roles in menopause (rather than the previously held view of ovarian exhaustion) (126). The neuroendocrine theory of aging is supported by experiments that show that hypophysectomy, followed by the replacement of known hormones, maintains (and may extend) lifespan in rodents (127). In addition, reductions in brain dopaminergic neurotransmission are more prominent in a shorter-lived rat strain (128). Levodopa, a dopaminergic drug, can prolong the mean lifespan in mice (129). Treatment of rats with deprenyl facilitates the activity of the nigrostriatal dopaminergic neurons and protects these neurons from their age-related decay (130), and de-

TABLE 3
Mouse Models for Human Aging

Model	Similarities to human aging	Lifespan and onset of aging	Differences from human aging	Genetics
<i>Kl^{-/-}</i>	Shortened lifespan, infertility, growth retardation, decreased spontaneous activity, premature thymic involution, ectopic calcification, skin atrophy, arteriosclerosis, osteoporosis, pulmonary emphysema, lipodystrophy	Average lifespan: ~9 weeks Onset of aging: ~4 weeks	Phenotypes not observed: neoplasms, cataracts, diabetes mellitus, brain atrophy	Loss of function mutations in the <i>klotho</i> gene (a single-pass membrane protein with homology to beta-glucosidase)
<i>Sam</i>	Amyloidosis, neoplasms, hyperinflation of the lung, hearing impairment, osteoporosis, defects in learning and memory, cataracts, brain atrophy	Average lifespan: ~10 months Onset of aging: < 8 months	Aging phenotypes are distributed among various SAMP strains	Not determined
<i>WRN^{-/-}</i>	Premature loss of proliferative capacity in fibroblasts, sensitivity to topoisomerase inhibitors	Not reported	Mutant mice are apparently normal	Targeted disruption of the Werner syndrome gene (RecQ-like DNA helicase)
<i>CSA^{-/-} CSB^{-/-}</i>	Growth retardation, neurological defects	Almost normal lifespan	Other age-related phenotypes are not observed/examined	Targeted disruption of the Cockayne's syndrome group A (<i>CSA</i>) or group B (<i>CSB</i>) gene (DNA helicase)
<i>Atm^{-/-}</i>	Growth retardation, neurological dysfunction, infertility, malignant thymic lymphoma, sensitivity to X-ray irradiation	Develop thymic lymphoma before 4.5 months of age	Other age-related phenotypes are not observed or not examined	Targeted disruption of the <i>ATM</i> gene (a nuclear protein with PI-3-kinase-like domain)
<i>mTR^{-/-}</i>	Infertility, graying of the hair, alopecia, skin lesions, impaired stress response, impaired proliferation of hematopoietic cells, neoplasms, delayed wound healing	Average lifespan: 18 months in the 6th generation	Aging phenotypes appear only in late-generation telomerase-deficient mice with extremely shortened telomeres. Phenotypes not observed: arteriosclerosis, osteoporosis, cataracts, diabetes mellitus, etc.	Targeted disruption of the gene for the telomerase RNA component
Over-expression of growth hormone	Scoliosis, weight loss, decline or reproductive capacity, insulin resistance, astrogliosis in the brain, glomerulonephritis, glomerulosclerosis, reduced replicative potential of fibroblasts	Lifespan: <50% of controls Onset of symptoms: 6–8 months of age	Growth hormone levels decline with age in humans and in experimental animals	Overexpression of rat, bovine, ovine or human growth hormone under the control of the metallothionein or phosphoenolpyruvate carboxykinase promoters

prenyl increases both the average and maximum lifespan (131, 132). Many human studies demonstrate gradually decreasing levels of peripheral hormones accompanied by normal levels of trophic hormones (125). This suggests either increased response to the peripheral hormones by the HPA axis or inappropriately low expression of the stimulating hormone. However, many organisms with aging phenotypes similar to those of higher vertebrates lack complex neuroendocrine systems. The changes that occur in the neuroendocrine system may be due to fundamental age-related changes in all cells and may therefore be secondary manifestations of the aging phenotype.

Immunologic Theory

The immunologic theory of aging is based upon two main observations: (a) the functional capacity of the immune system declines with age, as evidenced by a decreased response of T cells to mitogens and reduced resistance to infectious disease; and (b) autoimmune phenomena increase with age, such as an increase in serum autoantibodies (133). There is a shift toward increasing proportions of memory T cells, accompanied by enhanced expression of the multidrug-resistance p-glycoprotein (134). Humoral (B-cell mediated) immunity also declines with age, as evidenced by decreased antibody production and a disproportionate loss in the ability to make high affinity IgG and IgA (immunoglobulin G and A) antibodies. In addition, differences in the MLSP of different strains of mice have been related to specific alleles in the major histocompatibility gene complex (135). The genes in this region also contribute to the regulation of mixed-function oxidases (P-450 system), DNA repair, and free-radical-scavenging enzymes. Caruso et al. suggest that mouse and human histocompatibility genes may be associated with longevity via different mechanisms, in mice via susceptibility to lymphomas and in humans via infectious disease susceptibility (136). There is also evidence that cytokine gene polymorphisms may interact with histocompatibility genes to influence longevity (136). Although the immune system obviously plays a central role in health maintenance and survival, similar criticism can be directed at the immunologic theory as has been directed at the neuroendocrine theory. Complex immune systems are not present in organisms that share aspects of aging with higher organisms. In addition, the inability to distinguish between fundamental changes occurring in many types of cells

and tissues, not just those of the immune system, and the secondary effects mediated by the aging-altered immune system, make interpretation of this theory difficult. Proposed mechanistic studies of the immune theory include producing transgenic mice that carry the histocompatibility complex from a longer-lived rodent species, to determine effects on disease incidence and lifespan.

Cellular Senescence

The complexity of studying aging in organisms has led to the use of well-defined cell culture systems as models for cellular aging or senescence. Hayflick and Moorhead (137) pioneered the model of replicative senescence and identified normal human diploid fibroblasts in culture as a model for aging. They observed an initial period of rapid and vigorous proliferation, invariably followed by a decline in growth rate and proliferative activity, finally leading to a cessation of proliferation. This model proposed that aging is a cellular as well as an organismic phenomenon, and that the loss of functional capacity of the individual reflected the summation of the loss of critical functional capacities of individual cells. It is important to note that populations of senescent cells do not necessarily die, and that they can be maintained in culture for years in a post-mitotic (non-proliferating) state, with regular changes of culture medium (138–140). The loss of proliferative capacity of human cells in culture is intrinsic to the cells and not dependent upon environmental or culture conditions (137). In addition, senescence is inevitable unless the cells undergo transformation and acquire a constellation of abnormal characteristics such as multiple chromosomal abnormalities, genetic mutations, and changes in morphology and growth rate. The number of times the cells divide is also more important in determining proliferative lifespan than the actual time the cells spend in culture (141). Cells continuously passaged in culture until the end of their proliferative lifespan achieve approximately the same number of population doublings (PDLs) as cells that are held in a stationary phase for an extended period (months) and then recultured until senescence. The cells therefore seem to possess an intrinsic mechanism that “counts” the number of divisions and not the time that passes.

In addition to studies on fibroblasts, limited *in vitro* lifespan has been reported for glial cells (142), keratinocytes (143), vascular smooth muscle cells (144), lens cells (145), endothelial

cells (146), and lymphocytes (147). *In vivo*, serial transplants of normal somatic tissues, such as skin and breast, from old donor mice to young genetically identical recipients show a decline in proliferative activity and eventual failure of the graft (148). Similarly, skin from old donors retained an increased susceptibility to carcinogens whether transplanted to young or old recipients (149). Do changes in cells in culture parallel changes in cells from aging organisms? The replicative lifespan of fibroblasts in culture is inversely related to the maximum lifespan of several diverse vertebrate species (150). Studies suggest that the replicative lifespan of cells in culture is inversely related to the age of the donor in both humans and rodents (151–153). This *in vivo-in vitro* relationship also holds for several different cell types, including hepatocytes (154), keratinocytes (155), and arterial smooth muscle cells (144). However, in these cross-sectional studies, there is a great deal of variability, and the correlation coefficient, though statistically significant, is low. Cells cultured from healthy individuals do not appear to exhibit a consistent age-related proliferative capacity (156). Cells from people with Werner's syndrome do senesce more rapidly in culture than age-matched controls; however, a consistently similar relationship does not hold for cells from people with Hutchinson-Gilford syndrome (121). Thus, under some circumstances, the proliferative characteristics of cells during aging *in vivo* are maintained in culture. Unfortunately, convincing evidence that senescent cells accumulate with age *in vivo* is lacking to date. A potential biomarker for aging, β -galactosidase, has been described; it initially seemed to distinguish between senescent cells and either pre-senescent or quiescent cells (157). However, subsequent data indicate that *in situ* expression of β -galactosidase exists in confluent, quiescent, pre-senescent cells and is not necessarily specific for senescence (e.g., possibly lysosomal damage rather than senescence *per se*) (158). Rubin proposes that the *in vitro* limit on replication is an artifact that reflects the cells' traumatic response to establishment *in vitro* and that their subsequent maintenance in a foreign environment is starkly different from their *in vivo* milieu (159). He suggests that a decline in the rate of cellular proliferation more accurately correlates with aging in animals.

A major approach to studying the regulation of cessation of replication in senescent cells has been to examine pathways, at various levels, which likely play significant roles in regulating

cell proliferation and adaptive responses. Senescent cells are often less responsive to mitogens but can exhibit variable changes in growth-factor and growth-factor receptor expression compared to young cells (160). Senescent-related alterations in signal transduction pathways and nuclear transcription factors have also been documented. These alterations indicate that senescent cells exist in a growth state that is quite distinct from that of young cells and hint at the complex alteration in cellular physiology during senescence.

The phenomenon of telomere shortening with aging represents a potential "clock" or counting mechanism for senescent cells (161). Telomeres are structures at the end of chromosomes that prevent degradation and fusion with other chromosome ends (162). The average length of the terminal restriction fragment of chromosomes decreases with both *in vitro* and *in vivo* aging of fibroblasts and peripheral blood lymphocytes (161, 163–167). Indeed, telomere length in lymphocytes progressively declines as a function of donor age from newborn to great-grandparents in their eighties (168). Immortalized and transformed cells and germline cells express telomerase, which prevents shortening of the telomeres (169, 170). However, some immortal cells exist without detectable telomerase (171), and stem cells and some normal somatic cells which express telomerase, continue to experience telomeric shortening (172–174). These data suggest that the length of the telomeres *per se*, rather than the degree of telomerase activity, is the more important factor in cellular senescence. A recent study further demonstrates that the shortest telomere, not the average telomere length, determines cell viability and chromosomal stability (175). Experimental nonenzymatic elongation of telomeres extends the lifespan of cells (176). Furthermore, reactivation of telomerase, via the introduction of the telomerase reverse transcriptase unit into normal human cells, increases telomere length and extends the lifespan of both retinal epithelial cells and foreskin fibroblasts (177). Cells that had exceeded their normal lifespan by 20 population doublings exhibited normal karyotype and morphology similar to their younger counterparts. Shortened telomeres also led to a form of premature aging *in vivo* (178). Sixth generation telomerase-deficient mice with markedly shortened telomeres exhibited decreased weight and fecundity, graying and alopecia, increased ulcers and cancer, and shortened lifespan.

Products of the retinoblastoma (Rb) and p53 tumor-suppressor genes have also been implicated in replicative senescence (179, 180). Although similar levels of p53 are expressed in young and old cells *in vitro*, both DNA binding and transcriptional activity are increased in senescent cells (181). The Rb gene product is not phosphorylated in senescent cells (182). Simian virus 40 large T antigen, which is bound by the p53 and Rb gene products, can facilitate escape from senescence (183). T-antigen deletion mutants that lack either Rb- or p53-binding domains are unable to mediate escape from senescence (184). Furthermore, treatment with antisense oligonucleotides to the Rb and p53 tumor-suppressor genes can extend the *in vitro* lifespan of human fibroblasts (185). The p21 (186–188) and p16 (189–191) inhibitors of cyclin kinases (and therefore cell cycle progression) are overexpressed in senescent cells. The p21 protein appears to act by forming complexes with members of the family of E2F transcription factors in senescent cells (Rb/CDK2 [cyclin-dependent kinase 2]/cyclin E or with the Rb-related p107/CDK2/cyclin D), down-regulating transcriptional activity and thereby inhibiting progression through the cell cycle (186). Targeted disruption of the p21 gene delays the onset of senescence in fibroblasts derived from human lung (192). However, adrenocortical cells express high levels of p21 throughout their *in vitro* lifespan up to and including senescence (193). Skin fibroblasts from patients with Li-Fraumeni syndrome are heterozygous for p53. In culture, these cells lose the remaining p53 allele and are subsequently unable to express p21, but still undergo *in vitro* aging (194), suggesting that p53 and p21 are not required for senescence. In senescent cells, p16 complexes to and inhibits both the cyclin-dependent kinase 4 (CDK4) and CDK6 cell cycle kinases (189). The *ras* oncogene product can induce senescence that is accompanied by accumulation of p53 and p16 (195). This occurs only in nonimmortalized cells and may reflect a homeostatic response of the cell to a transforming stimulus. Induction of expression of p16 by demethylation-dependent pathways or of p21 by demethylation-independent pathways can induce senescence in immortal fibroblasts that do not express p53 (196). Of those genes whose expression is required for G1/S cell cycle progression, senescent fibroblasts express no CDK2 and cyclin A, and reduced amounts of the G1 cyclins, C, D1, and E, compared to young cells (197). The expression of early G1

markers, but not late G1 markers, indicates that senescent cells may be blocked at a point in late G1.

The p53 gene plays an important role in a slew of critical cellular processes in addition to senescence, including cell cycle control, apoptosis, DNA repair, and transcription (198). Cells from telomerase-deficient mice (see above) exhibited high levels of p53 (199). In addition, deletion of p53 in the mice initially mitigated the effects of the telomerase deficiency, but ultimately contributed to greater malignant transformation. The p53 and p21 stress response is diminished in the p66shc null mice (also see above) that exhibit an increased lifespan (200). p53 has also been implicated in the effects exerted upon aging by radiation, oxidative stress, and the SIR2 gene (201). Recently p53 has been thrust to center stage in helping to increase our knowledge of the underlying mechanism(s) responsible for *in vivo* aging. Since p53-deficient mice die early due to marked increases in tumors, they do not represent a practical model to study aging. Therefore, Tyner et al. capitalized upon the serendipitous generation of a mouse that expresses a mutant p53 that enhances wild-type p53 activity (202). Not surprisingly, the mice are resistant to tumor development. However, the unexpected and fascinating finding is that the mice with augmented p53 activity also age prematurely and exhibit osteoporosis, generalized organ atrophy, diminished wound healing as well as stress tolerance, lymphoid atrophy, and reduced body weight. Consequently, it appears that increasing p53 activity reduces the incidence of cancer, but concurrently increases the aging rate (Fig. 5). A fine equilibrium between the antineoplastic and pro-aging characteristics of p53 may lead to the optimal lifespan for an organism; too little p53 results in death from can-

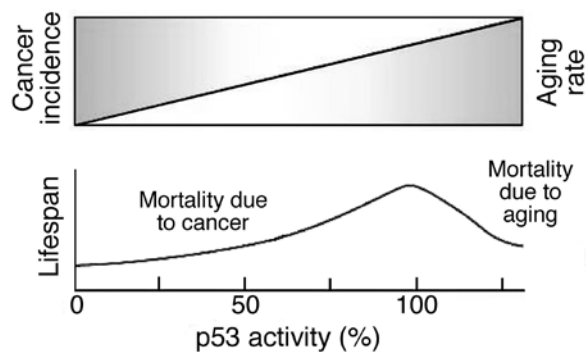


Fig. 5. Balancing cancer and aging. Adapted with permission from: Ferbeyre G, Lowe SW. The price of tumour suppression? *Nature* 2002; 415:26–27 (reference 201).

cer, whereas too much p53 leads to death by accelerated aging. Since p21 can be induced by p53-dependent mechanisms, it is possible that p21 is partly responsible for some of the observed aging phenotypes in these mice (203).

These observations emphasize the presence of complex and incompletely understood, overlapping networks regulating cell cycle progression and proliferation. Depending upon the balance of positive and negative influences, cell proliferation can continue or senescence may ensue. These data are consistent with the theory that cellular senescence evolved as a mechanism of tumor suppression and that aging is an antagonistically pleiotropic manifestation of evolutionary pressures to prevent malignant transformation, perhaps in large part via the actions of p53. The unavoidable conclusion is that cellular senescence is good for the organism; aging may be the price we pay to avoid, for the most part, cancer. However, even this concept has been qualified by the demonstration that senescent cells can foster the growth of premalignant and malignant epithelial cells in culture and the tumorigenesis of these cells in mice (204). The stimulation was due to both soluble and insoluble factors secreted by senescent cells. While these findings seem at odds with the beneficial effects of cellular senescence, they are themselves another example of antagonistic pleiotropy wherein a process that has evolved to protect against cancer may in fact predispose to cancer later in life.

Cell Death

There are two distinct patterns of cell death: necrosis and apoptosis. Massive cell injury, often accompanied by inflammation, can lead to necrosis. Necrosis is essentially accidental and entails clumping of chromatin into ill-defined masses, swelling of organelles, and ultimately membrane and cell disintegration (205). In contrast, apoptosis is an active, gene-directed "suicide" in response to external or internal stimuli, usually in the absence of significant external injury (205). In most circumstances, apoptosis is important, thereby permitting the organism to maintain homeostasis. Apoptosis initially involves compaction and segregation of chromatin adjacent to the nuclear membrane and condensation of the cytoplasm. This rapidly progresses to nuclear/cellular pedunculation and fragmentation. The membrane-bound apoptotic bodies are then phagocytosed by adjacent cells. Although "programmed cell death" and "apoptosis" are often used interchangeably,

they are not actually synonymous terms. Lockshin and Zakeri (203) stress that programmed cell death is a developmental event, whereas apoptosis is a mode of cell death. Programmed cell death often involves increases of lysosomal enzyme and rarely exhibits the laddering of DNA seen in apoptosis. It is likely that programmed cell death is a type of apoptotic (controlled) cell death. Understanding the genetic basis of apoptosis initially depended upon work conducted in the nematode *C. elegans*, which has been a useful model system because the developmental fate of every cell has been determined (206); of the 1,090 cells formed, 131 eventually die. Three genes (*ced-3*, *ced-4*, and *ced-9*) play an important role in cell death in the nematode (207, 208). *Ced-3* is required for apoptosis in *C. elegans*; mammalian homologs include cysteine proteinases (ICE, CPP32, and ICH-1) (209). *Ced-9* blocks apoptosis; mammalian homologs include bcl-2 and bcl-X_L (208). Bcl-2 was originally identified as an oncogene because of its overexpression in a form of B-cell lymphoma. Additional mammalian homologs of bcl-2, which promote apoptosis, include bax, bad, and bak (210). Bcl gene family members can form homodimers or heterodimers, permitting a fine degree of control of cell survival. Heterodimerization with either bcl-2 or bcl-X_L prevents cell death.

Research is ongoing to elucidate the possible role of apoptosis in aging and diseases associated with aging. If cells are unable to repair DNA damage, apoptosis may ensue, followed by replacement via division of another cell. Senescent fibroblasts in culture are resistant to apoptotic signals, being unable to downregulate bcl-2 expression (211). This raises the possibility that damaged senescent cells may accumulate with increased organismal age, potentially compromising tissue function. Induction of apoptosis in the livers of old rats by a genotoxic agent is significantly reduced, compared to the induction of apoptosis in the livers of young rats (212). Caloric restriction in rodents upregulates apoptosis in the liver via the removal of preneoplastic cells (213, 214). Warner et al. suggest that this may counteract the diminished apoptosis in aging and explain life extension induced by caloric restriction (215). Apoptosis plays a critical role in the immune system, where as many as 95% of T lymphocytes undergo cell death (presumably because they recognize self-antigens) (216). Lymphocyte apoptosis is mediated by a cell-surface receptor, Fas. Mice lacking Fas exhibit increased autoimmune

disease and are short lived. Caloric restriction in such mice increases T-lymphocyte apoptosis and extends lifespan (217). Fas expression decreases in older mice, and transgenic overexpression maintains Fas-induced apoptosis (218). Cell death is a characteristic in a number of neurodegenerative diseases common in aging (219). Specific neuronal loss is seen in Alzheimer's disease (hippocampus and cortex), Parkinson's disease (substantia nigra), Huntington's disease (striatum), and amyotrophic lateral sclerosis (motor neurons). β -amyloid protein is cytotoxic to cultured neuronal cells, which then undergo apoptosis.

Conclusions

Despite the near-universal phenomenon of aging in living organisms, there is an extraordinarily varied phenotype that accompanies aging in specific individuals. Furthermore, it appears that evolutionary pressures have led to the development of a remarkable homeostatic complexity to the underlying mechanisms that cause us to grow old. The three quotations at the beginning of this article aptly represent these processes. Butch Cassidy recognized the inexorable forces that cause us to age. The concept of antagonistic pleiotropy is reflected in the other two insights. It seems that we clearly pay a price to maintain a high level of reproductive fitness — there is no free lunch. However, the ironic, yet ultimately satisfying, paradox may be that the only way that we can actually live as long as we do is, in fact, to grow old.

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