

Physiology of Aging

Invited Review: Theories of aging

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Weinert, Brian T. and Paola S. Timiras. *Physiology of Aging.* Invited Review: Theories of aging. *J Appl Physiol* 95: 1706–1716, 2003; 10.1152/japphysiol.00288.2003.—Several factors (the lengthening of the average and, to a lesser extent, of the maximum human life span; the increase in percentage of elderly in the population and in the proportion of the national expenditure utilized by the elderly) have stimulated and continue to expand the study of aging. Recently, the view of aging as an extremely complex multifactorial process has replaced the earlier search for a distinct cause such as a single gene or the decline of a key body system. This minireview keeps in mind the multiplicity of mechanisms regulating aging; examines them at the molecular, cellular, and systemic levels; and explores the possibility of interactions at these three levels. The heterogeneity of the aging phenotype among individuals of the same species and differences in longevity among species underline the contribution of both genetic and environmental factors in shaping the life span. Thus, the presence of several trajectories of the life span, from incidence of disease and disability to absence of pathology and persistence of function, suggest that it is possible to experimentally (e.g., by calorie restriction) prolong functional plasticity and life span. In this minireview, several theories are identified only briefly; a few (evolutionary, gene regulation, cellular senescence, free radical, and neuro-endocrine-immuno theories) are discussed in more detail, at molecular, cellular, and systemic levels.

evolution; gene regulation; cellular senescence; free radical; neuro-endocrine-immunologic regulation

IN RECENT DECADES, THE STUDY of aging has expanded rapidly both in depth and in breadth. This growth has been stimulated by 1) the extraordinary lengthening of the average human life span, worldwide; 2) the less spectacular, but nevertheless significant, lengthening of the maximum life span; 3) the increasing percentage of elderly in the population, especially in some developed countries; and 4) the increased proportion of the national health expenditures utilized by the elderly (96). Biological, epidemiologic, and demographic data have generated a number of theories that attempt to identify a cause or process to explain aging and its inevitable consequence, death. However, in recent years, the search for a single cause of aging, such as a single gene or the decline of a key body system, has been replaced by the view of aging as an extremely complex, multifactorial process (43). Several processes may interact simultaneously and may operate at many levels of functional organization (31). Similarly, different theories of aging are not mutually exclusive and may adequately describe some or all features of the

normal aging process alone or in combination with other theories. The definition of aging itself is open to various interpretations (14, 79). In response to the question “Why do we age?” aging is presented as an ontogenic issue; the process of growing old and/or the sum of all changes, physiological, genetic, molecular, that occur with the passage of time, from fertilization to death. In response to the question “Why do we live as long as we do?” an evolutionary-comparative framework is the preferred address. To the question “Why do we die?” the answer should underline the lack of necessary relation between aging (a definite period of the life span) and death (an event that may occur at all ages). However, because aging is characterized by the declining ability to respond to stress and by increasing homeostatic imbalance and incidence of pathology, death remains the ultimate consequence of aging. Theories formulated to explain aging processes have been grouped into several categories, some of the most widely used being the programmed and error theories of aging. According to the “programmed” theories, aging depends on biological clocks regulating the timetable of the life span through the stages of growth, development, maturity, and old age: this regulation would depend on genes sequentially switching on and

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off signals to the nervous, endocrine, and immune systems responsible for maintenance of homeostasis and for activation of defense responses. The “error” theories identify environmental insults to living organisms that induce progressive damage at various levels (e.g., mitochondrial DNA damage, oxygen radicals accumulation, cross-linking).

In the present review, we have categorized the various theories of aging as evolutionary, molecular, cellular, and systemic. The choice of these categories and the order in which they are presented reflect their affinity to physiological discourse (90). Thus theories of aging may overlap at various levels of organization: alterations with aging of molecular events may lead to cellular alterations, and these, in turn, contribute to organ and systemic failure with evolutionary implications for reproduction and survival. In complex, multicellular organisms, the study of interactions among intrinsic (genetic), extrinsic (environmental), and stochastic (random damage to vital molecules) causes provides a fruitful approach conducive to a comprehensive and realistic understanding of the aging process. In humans, for example, the current longevity is the result of an early (middle of last century) “epidemiologic transition,” referring to the decline in death rates due to acute infectious disease (because of improved hygiene and the discovery of antibiotics) (101). This was followed in the 1970s to 1980s by a second mortality decline at older ages in the reduction of death rates due to cardiovascular disease (101). In several animal species (rodents, monkeys), experimental interventions such as restriction of dietary calories show that it is possible to delay the onset of pathology and to prolong the life span by manipulating molecular (e.g., free

radical reduction), cellular (e.g., mitochondrial protection), and systemic (e.g., endocrine shifts) mechanisms (57). Although these interventions extend beyond the limits of the theories of aging themselves, they will be mentioned here in their support. Some of the principal theories of aging to be discussed here are listed in Table 1: several will be identified only briefly, whereas a few will be discussed in detail. The latter include evolutionary, gene regulation, cellular senescence, free radical, and neuro-endocrine-immuno theories.

EVOLUTIONARY THEORIES

Why do we live as long as we do? Evolutionary theories argue that aging results from a decline in the force of natural selection. Because evolution acts primarily to maximize reproductive fitness in an individual, longevity is a trait to be selected only if it is beneficial for fitness. Life span is, therefore, the result of selective pressures and may have a large degree of plasticity within an individual species, as well as among species. The evolutionary theory was first formulated in the 1940s based on the observation that Huntington’s disease, a dominant lethal mutation, remained in the population even though it should be strongly selected against (34). The late age of onset for Huntington’s disease (30–40 yr) allows a carrier to reproduce before dying, thereby allowing the disease to avoid the force of natural selection. This observation inspired the Mutation Accumulation Theory of aging, which suggests that detrimental, late-acting mutations may accumulate in the population and ultimately lead to pathology and senescence (59). Currently, there is scant experimental evidence for this theory of aging (67).

Table 1. *Classification and brief description of main theories of aging*

Biological Level/Theory	Description
Evolutionary	
Mutation accumulation*	Mutations that affect health at older ages are not selected against.
Disposable soma*	Somatic cells are maintained only to ensure continued reproductive success; after reproduction, soma becomes disposable.
Antagonistic pleiotropy*	Genes beneficial at younger age become deleterious at older ages.
Molecular	
Gene regulation*	Aging is caused by changes in the expression of genes regulating both development and aging.
Codon restriction	Fidelity/accuracy of mRNA translation is impaired due to inability to decode codons in mRNA.
Error catastrophe	Decline in fidelity of gene expression with aging results in increased fraction of abnormal proteins.
Somatic mutation	Molecular damage accumulates, primarily to DNA/genetic material.
Dysdifferentiation	Gradual accumulation of random molecular damage impairs regulation of gene expression.
Cellular	
Cellular senescence-Telomere theory*	Phenotypes of aging are caused by an increase in frequency of senescent cells. Senescence may result from telomere loss (replicative senescence) or cell stress (cellular senescence).
Free radical*	Oxidative metabolism produces highly reactive free radicals that subsequently damage lipids, protein and DNA.
Wear-and-tear	Accumulation of normal injury.
Apoptosis	Programmed cell death from genetic events or genome crisis.
System	
Neuroendocrine*	Alterations in neuroendocrine control of homeostasis results in aging-related physiological changes.
Immunologic*	Decline of immune function with aging results in decreased incidence of infectious diseases but increased incidence of autoimmunity.
Rate-of-living	Assumes a fixed amount of metabolic potential for every living organism (live fast, die young).

*Discussed in the text.

However, the basic concept that aging results from a lack of selection enjoys a wealth of experimental support. Long-lived *Drosophila* strains can be bred by selecting the offspring of older adults, demonstrating that life span can be altered directly by selective pressure (68, 75). Life span is species specific because it is largely a function of survivability and reproductive strategy in a competitive environment. Consequently, organisms that die primarily from predation and environmental hazards will evolve a life span optimized for their own particular environment. This idea was tested in a natural environment by comparing mainland opossums that are subject to predation to a population of opossums living on an island free of predators (4). The Evolutionary Theory predicts that the protected island opossums would have the opportunity to evolve a longer life span, if it were beneficial to fitness. Indeed, island opossums do live longer and age more slowly than their mainland counterparts (4). The observation that organisms can age in a natural environment (51) indicates that although extending life span can be beneficial to fitness, other considerations might necessitate sacrificing longevity for reproductive fitness. This basic idea of the Disposable Soma Theory of aging argues that the somatic organism is effectively maintained only for reproductive success; afterward it is disposable. Inherent in this theory is the idea that somatic maintenance, in other words, longevity, has a cost; the balance of resources invested in longevity vs. reproductive fitness determines the life span.

The concept of an evolutionary tradeoff is essential in both the Disposable Soma Theory and the Antagonistic Pleiotropy Theory. The Disposable Soma Theory explains why we live for a certain period of time but does not postulate the specific cause of aging, whereas the theory of Antagonistic Pleiotropy suggests that some genes may be selected for beneficial effects early in life and yet have unselected deleterious effects with age, thereby contributing directly to senescence. Antagonism between reproduction and longevity is supported by experiments in which limiting reproduction by destroying germ line cells can extend life span in both *Drosophila* and *Caenorhabditis elegans* (1, 83). In humans, the growth and normal function of the prostate gland are promoted by androgens, the male gonadal hormones. In old age these same hormones may contribute to the etiology of prostate cancer, one of the major causes of death in old men. The relationship between longevity and fecundity is not absolute; some long-lived *Drosophila* strains have no loss in reproductive capacity (2), and long-lived three-toed box turtles continue to reproduce for more than 60 yr (64). Animals (such as the box turtle above) that adapt to escape predation might favor the selection of both longevity and fecundity. For example, eusocial insects, such as ants, will cooperate to support a single queen. The queen, protected from the environment and cared for by worker ants, will give rise to hundreds and even thousands of offspring each day and, in some cases, lives for 30 years (40). In contrast, related, noneusocial

insects have average life spans that are measured in months, not years.

MOLECULAR THEORIES

The Gene Regulation Theory of aging proposes that senescence results from changes in gene expression (38). Although it is clear that many genes show changes in expression with age (71, 98, 104), it is unlikely that selection could act on genes that promote senescence directly (42). Rather, life span is influenced by the selection of genes that promote longevity (see above). Recently, DNA microarrays have been used to assay genome-wide transcriptional changes with age in several model organisms (71, 74, 98, 104). Genome-level analysis allows researchers to compile a transcriptional fingerprint of "normal" aging. This data can be compared with interventions that slow or accelerate aging, perhaps enabling the identification of gene expression changes that are relevant to the aging process (71, 99, 104).

One of the most exciting developments in aging research is the identification of an insulin-like signaling pathway that regulates life span in worms, flies, and mice (87). Life span extension results from the activation of a conserved transcription factor in response to a reduction in insulin-like signaling, indicating that gene expression can regulate life span. Understanding how nature delays aging through changes in gene expression should reveal much about the process of aging itself and provide a starting point for developing therapies to slow aging.

Studies of human centenarians and their relatives have identified a significant genetic aspect of the ability to survive to exceptional ages. In one study, the mortality rate of centenarian siblings was shown to be, on average, half the mortality rate of the U.S. year 1900 cohort (69, 70). This sustained life-long reduction in mortality rate is taken to imply that the effect is due to genetic rather than environmental or socioeconomic factors. A recent study supports the idea that exceptional longevity has a genetic component by identifying a locus on chromosome 4 that may contain gene(s) that promote longevity (72). Genetic analysis of human longevity is especially important given that genetic aspects of aging are studied primarily in short-lived model organisms. It will be particularly interesting to see whether the recent advances in understanding genetics of longevity in model organisms are verified in human studies, and vice versa.

CELLULAR THEORIES

Cell senescence/telomere theory. The Cellular Senescence Theory of aging was formulated in 1965 when cell senescence was described as the process that limits the number of cell divisions normal human cells can undergo in culture (36). This "limit in replicative capacity" occurs after a characteristic number of cell divisions and results in terminally arrested cells with altered physiology (11). Cellular senescence can also occur in response to distinct molecular events; in this

discussion, we distinguish between cellular senescence (of all types) from senescence due to cell replication (replicative senescence) and senescence due to other causes [stress-induced senescence (SIS)]. Replicative senescence is a specific type of cellular senescence that ultimately results from loss of telomeres (specialized structures composed of a repeating DNA sequence and located at the ends of each linear chromosome; Ref. 8). With each cell division, a small amount of DNA is necessarily lost at each chromosome end, resulting in ever-shorter telomeres, altered telomere structure, and eventual replicative senescence (8). Activation of the telomerase enzyme will regenerate telomeres, prevent replicative senescence, and immortalize human primary cell cultures (10). SIS occurs in response to a variety of stressors, including but not limited to 1) DNA damage, 2) modifications in heterochromatin structure, and 3) strong mitogenic signals resulting from oncogene expression (11). Specialized immortal cell types such as stem cells, germ cells, and T lymphocytes express telomerase and will either maintain telomere length or delay telomere attrition (17, 102). Additionally, all cancer cells activate telomerase or an alternate pathway of telomere extension to avoid replicative senescence (41, 73).

Initial experiments with cells in culture showed a correlation between replicative potential and donor age, suggesting that cells from older individuals have a more limited capacity for further cell divisions. Similarly, organisms with short life spans have cells that senesce more rapidly than organisms with longer life spans. However, recent experiments have cast considerable doubt on these observations, and further research is required to clarify these divergent data (reviewed in Refs. 8, 78, 103). Cells expressing stress-induced markers found in senescent cells accumulate with age in many tissues (20, 44), although it remains unclear whether this indicates the presence of senescent cells *in vivo*. Several studies suggest that atherosclerosis results from senescent changes in arterial endothelial cells (15, 26, 94). Werner's syndrome is a remarkable progeroid syndrome due to an apparently normal period of development until puberty, followed by early manifestation of many aging-related physiological changes. Notable among these changes is the early onset of atherosclerosis (54); in addition, cells from both Werner's patients and a mouse model for the disease are marked by accelerated senescence in cell culture (47, 55). The altered physiology of senescent cells might contribute to aging and cancer through secondary effects on neighboring cells in tissues (44). For example, senescent endothelial cells upregulate the proinflammatory cytokine interleukin-1 α and EGF-like growth factors (50, 52). Such changes may result in a hazardous local environment in which inflammation and mitogenic stimulation lead to a decline in organ function and increased risk of cancer. Consistent with this idea, the growth of preneoplastic and neoplastic epithelial cells in culture is stimulated by the presence of senescent fibroblasts compared with presenescent fibroblasts (45).

The tumor suppressor protein p53 is a key regulator of cellular checkpoint responses to genome crisis. Among the many functions attributed to p53 are essential roles in activating transient cell cycle arrest, apoptosis, replicative senescence, and SIS in response to radiation-induced DNA damage and replication-induced telomere loss (23). The type of p53-dependent cellular response (cell arrest, apoptosis, or senescence) is often dependent on the particular cell type being examined or the type and severity of stress that the cells are exposed to. Mice mutated for p53 have a dramatically increased incidence of cancer (24), and p53 signaling is altered in ~80% of human cancers (23), indicating that p53-mediated functions are important for tumor suppression. Replicative senescence and/or SIS may have the biological role of preventing cancer by limiting the replicative potential of any given cell. However, if cellular senescence acts to suppress tumor formation, then how do we explain the observation that both cancer and cellular senescence are more prevalent with age? One way in which this apparent contradiction can be resolved is by the evolutionary hypothesis (antagonistic pleiotropy) that cellular senescence was selected to suppress cancer early in life yet has the unselected effect of contributing to age-related pathology and cancer in older, postreproductive individuals (44).

The requirement of telomerase for human cell immortality together with the observation that telomeres shorten with age led to the speculation that telomere length regulates cell replicative life span *in vivo* and contributes to aging. Although difficult to test directly in humans, experiments in rodents have provided little support for this idea. Gene targeting showed that telomerase-deficient mice do not age rapidly; in fact, overt phenotypes are not observed for several generations (9, 49). This showed that telomere shortening cannot account for normal aging in mice; however, similarities between aging and the late-generation telomerase-deficient phenotype might indicate that cellular senescence of some type contributes to aging in mice. The tumor suppressor protein p53 is required for cellular senescence; p53 deficiency suppresses the early aging phenotype of late-generation telomerase-deficient mice (16). These data suggest that p53-dependent processes (including, but not limited to cellular senescence) are responsible for the early aging phenotype in telomerase-deficient mice, an interpretation supported by the recent finding that a hyperactive p53 mutant mouse ages rapidly and has a markedly reduced incidence of spontaneous tumors (92). The essential role of p53 in cellular senescence is underscored by recent reports indicating that p53 is required for maintenance of cellular senescence in some cell types. Treatments that inactivate p53 in senescent cells can trigger reentry into the replication cycle and cell proliferation (5a, 21), although some human senescent cells (those with elevated p16 expression) are refractory to senescence release by p53 inhibition (5a).

Although telomere shortening does not appear to have a significant role in aging mice, there is some

evidence that telomeres may contribute to normal human aging. Dyskeratosis congenita (DKC) is an X-linked disorder marked by skin and bone marrow pathologies largely attributed to the loss of functional stem cells in these tissues (22, 25). The mutation responsible for DKC affects an enzyme involved in the metabolism of the telomerase RNA subunit (hTR) (65). A rare dominant autosomal form of DKC can result from mutation of the hTR gene directly (95), supporting the idea that DKC manifests itself through telomerase dysfunction. Interestingly, patients with the dominant hTR-defective form of DKC have a more severe pathology in later generations (95), mirroring the delayed phenotype observed in telomerase-deficient mice (see above). Although DKC patients develop pathologies that partly resemble normal aging, these phenotypes are limited compared with a more extensive progeroid disorder such as Werner's syndrome and suggest a limited role for telomere shortening in normal human aging. For example, telomere length may restrict the replicative potential of hemopoietic cells, perhaps contributing to the well-documented decline in immune function with age. Patients with DKC are not completely telomerase deficient; depending on the specific type of disease (X-linked or autosomal), telomerase levels may be from two- to fivefold reduced (18). Interestingly, the age of disease onset may be correlated with the degree of telomerase deficiency, with the most deficient individuals developing pathologies at an earlier age. An interesting model suggests that telomere shortening can promote tumorigenesis by enhancing genome instability: telomere-induced genome crisis leads to cell transformation, which is followed by telomerase activation to allow for unlimited cell proliferation (56). Consistently, some DKC patients have an increased incidence of carcinomas, suggesting that telomere shortening may contribute to the development cancer that is more prevalent with age (18).

There is an ongoing debate as to the relative contributions of replicative senescence (due to telomere loss) and SIS (due to damage accumulation and other factors) in the aging process. The validity of conclusions based on the replicative life span of cells in culture has been questioned in several recent reviews (8, 78, 103). In addition, experiments in mice have provided little, if any, support for a role of replicative senescence in aging, although it is not unreasonable to assume that humans and mice may differ as to the ultimate causes of cell senescence in culture (84). Recent results illustrate this point by showing that mouse embryonic fibroblasts (MEFs) enter SIS in response to the elevated oxygen (20%) present in normal tissue culture, a characteristic that distinguishes these cells from human cells (66a). MEFs normally enter cellular senescence after just 10–15 divisions in cell culture and with very long telomeres; this phenomenon was previously considered the replicative life span of these cells. Cells grown in 3% oxygen do not senesce at all, indicating that previous estimates of MEF replicative potential were based on observations of cells that enter SIS owing to oxidative damage in tissue culture. These

data suggest that, in mice, oxidative damage is responsible for cellular senescence in culture and may account for cellular senescence in vivo, an interpretation that lends credence to both free radical and cellular senescence theories of aging. It is worth noting that the question of replicative senescence vs. SIS has a wider implication in terms of theories of aging in general. Replicative senescence can be considered a cause of aging in and of itself, as it is largely attributed to the number of cell divisions as determined by telomere length. On the other hand, SIS is a response to stress, particularly genome crisis and DNA damage. SIS should, therefore, be considered a cellular response to age-related molecular changes that likely acts to exacerbate or accelerate organismal aging. This view of cellular senescence in aging is compatible with the various damage accumulation theories (such as free radical, error catastrophe, and somatic mutation) that may account for the ultimate cause of cellular senescence with aging.

Free radical theory. The Free Radical Theory of aging was first proposed in 1957 (35); it is one of the best-known theories and remains controversial to this day. All organisms live in an environment that contains free radical-containing reactive oxygen species (ROS); mitochondrial respiration, the basis of energy production in all eukaryotes, generates ROS by leaking intermediates from the electron transport chain (29). The universal nature of oxidative free radicals is underscored by the presence of superoxide dismutase (SOD), an enzyme found in all aerobic organisms that scavenges superoxide anions exclusively (29). In addition, cellular oxidative damage is indiscriminate; there is evidence for the oxidative modification of DNA, protein, and lipid molecules (60). The Free Radical Theory supposes that free radical reactivity is inherent in biology and results in cumulative damage and senescence. In fact, elevated levels of both oxidant-damaged DNA and protein are found in aged organisms (6, 86). Although it is clear that oxidative damage accumulates with aging, it is not clear whether this process contributes to aging in all organisms. A more thorough review of the Free Radical Theory may be found in several excellent reviews that focus exclusively on this topic (29, 60).

Some of the strongest evidence in support of the Free Radical Theory comes from life span experiments in flies and worms. The increased life span of transgenic flies expressing SOD (91) indicates that free radical-scavenging enzymes are sufficient to delay aging in *Drosophila*. In addition, flies selected for increased longevity have elevated levels of SOD and increased resistance to oxidative stress (3). Long-lived mutant worms are also resistant to oxidative stress and show an age-dependent increase in SOD and catalase activity (46). Extension of *C. elegans* life span by synthetic small molecules that mimic catalase and/or SOD demonstrates that antioxidant compounds can delay aging in worms (61). Clearly, free radical damage opposes longevity in these small, short-lived organisms; but

what about larger, long-lived organisms such as mammals?

Dietary antioxidants can reduce the accumulation of oxidized molecules in mice, yet they fail to extend life span (60). Rodents with SOD mutations are quite sick and die prematurely, although it is not clear that they actually age rapidly. Ubiquitous overexpression of SOD does not extend life span in rodents, indicating that SOD does not limit longevity (37). Ionizing radiation generates free radicals; surprisingly, chronic radiation exposure actually causes a reproducible increase in rodent life span (13). The longevity-enhancing effect of chronic radiation may be explained if radiation exposure results in stable activation of cellular defenses. Similar stress conditioning can lead a positive compensatory response (hormesis) that protects against oxidative damage and extends life span (29). Calorie restriction is an intervention that prolongs the life span of nearly every organism to which it has been applied (see below). In rodents, calorie restriction reduces generation of ROS from isolated mitochondrial preparations and attenuates the accumulation of oxidative damage (63). Free Radical Theory may provide an attractive explanation for the longevity-promoting effects of calorie restriction (i.e., reducing dietary intake reduces metabolism and ROS production); however, calorie restriction is known to alter the function of many other molecular, cellular, and organ systems (see below). Although it is easy to find correlations between many physiological functions and calorie restriction, it remains difficult to distinguish the ultimate cause of life span extension by this technique from the abundant molecular and cellular changes that accompany it.

The Free Radical Theory is further divided into several hypotheses focusing on the exclusive role of particular organelles and types of damaged molecules in the aging process. One such hypothesis argues that mutations in mitochondrial DNA accelerates free radical damage by introducing altered enzyme components into the electron transport chain. Faulty electron transport results in elevated free radical leakage and ultimately more mitochondrial DNA mutation and exacerbated oxidant production. This "vicious cycle" of mutation and oxidant production eventually leads to cellular catastrophe, organ failure, and senescence (53). Another hypothesis argues that free radicals cause aging when oxidized proteins accumulate in cells. An age-dependent reduction in the ability to degrade oxidatively modified proteins may contribute to the build-up of damaged, dysfunctional molecules in the cell (86). The Somatic Mutation Theory of aging supposes that accumulation of genetic mutations in somatic cells is the specific cause of senescence; free radical damage may be an important source of somatic mutations (6). However, mice have been serially cloned by somatic nuclear transfer for six generations without any sign of premature aging (97), indicating that somatic mutations cannot account for aging in mice and free radicals are not likely to promote senescence in this manner.

SYSTEM-BASED THEORIES OF AGING: NEUROENDOCRINE AND IMMUNE THEORIES

In these theories, the aging process is related to the decline of the organ systems essential for 1) the control and maintenance of other systems within an organism, and 2) the ability of organisms to communicate and adapt to the environment in which they live. In humans, all systems may be considered indispensable for survival. However, the nervous, endocrine, and immune systems play a key role by their ubiquitous actions in coordinating all other systems and in their interactive and defensive responsiveness to external and internal stimuli.

Neuroendocrine theory. This theory proposes that aging is due to changes in neural and endocrine functions that are crucial for 1) coordinating communication and responsiveness of all body systems with the external environment; 2) programming physiological responses to environmental stimuli; and 3) maintaining an optimal functional state for reproduction and survival while responding to environmental demands. These changes, often detrimental in nature, not only selectively affect the neurons and hormones that regulate evolutionarily significant functions such as reproduction, growth, and development, but also affect those that regulate survival through adaptation to stress. Thus the life span, as one of the cyclic body functions regulated by "biological clocks," would undergo a continuum of sequential stages driven by nervous and endocrine signals. Alterations of the biological clock, e.g., decreased responsiveness to the stimuli driving the clock or excessive or insufficient coordination of responses, would disrupt the clock and the corresponding adjustments (27, 28, 88, 89). An important component of this theory is the perception of the hypothalamo-pituitary-adrenal (HPA) axis as the master regulator, the "pacemaker" that signals the onset and termination of each life stage. One of the major functions of the HPA axis is to muster the physiological adjustments necessary for preservation and maintenance of the internal "homeostasis" (steady state) despite the continuing changes in the environment (7, 12). During life span, chronic exposure to severe stress from a multitude of physical, biological, or emotional stimuli may exhaust or weaken the capacity to adapt and lead to the so-called "diseases of adaptation" and death (58, 82). Aging would then result from "a decreasing ability to survive stress," one of the many definitions of aging that suggests a close relationship between stress and longevity.

Integration of responses to environmental stimuli would be carried out by the hypothalamus from information derived in various cerebral structures (primarily the cerebral cortex, limbic lobe, and reticular formation). The hypothalamus itself regulates 1) several important nervous functions (e.g., sympathetic and parasympathetic visceral functions), 2) behaviors (e.g., sexual and eating behaviors, rage, fear), and 3) endocrine functions, such as producing and secreting hypophysiotropic hormones that stimulate or inhibit hor-

mone release from the pituitary (or hypophysis). In response to hypothalamic signals, the pituitary, often referred to as the master endocrine gland, produces and secretes several hormones that act to regulate many important functions of the body. Pituitary regulation occurs by releasing hormones (e.g., growth hormone, oxytocin, vasopressin), or by stimulating a peripheral endocrine gland such as the adrenal cortex, thyroid, or gonads. The adrenal gland is formed of a cortex that surrounds a central core or medulla. Major hormones of the adrenal medulla are the catecholamines epinephrine and norepinephrine, which function as neurotransmitters for the sympathetic division of the autonomic nervous system: these respond rapidly to any external or internal stress through circulatory (increased blood pressure) and metabolic (facilitating carbohydrate and lipid utilization for energy) adjustments (12). With aging, a reduction in sympathetic responsiveness is characterized by 1) a decreased number of catecholamine receptors in peripheral target tissues; 2) a decline of heat shock proteins that increase stress resistance in many animal species, including humans, and 3) a decreased capability of catecholamines to induce these heat shock proteins (93). The hormones of the adrenal cortex are glucocorticoids, for the regulation of lipid, protein, and carbohydrate metabolism; mineralocorticoids, for that of water and electrolytes; and sex hormones. Among the latter is dehydroepiandrosterone, which decreases with aging; dehydroepiandrosterone replacement therapy has been advocated in humans, despite unconvincing results (19). Glucocorticoids, as well as other (ovarian and testicular) steroid hormones, are regulated by positive and negative feedback between the target hormones and their central control by the pituitary and hypothalamus. With aging and in response to continuing and severe stress, not only feedback mechanisms may be impaired, but also glucocorticoids themselves may become toxic to neural cells, thus disrupting feedback control and hormonal cyclicality (80, 81).

The Neuroendocrine Theory has recently been supported by data showing that an "ancestral" insulin pathway controls stress responses and longevity in the nematode *C. elegans* (see also above) (39). Mutations of a number of genes in this pathway confer 1) resistance to environmental stress, including heat shock (93), 2) enhanced resistance to starvation, and 3) extended longevity. Many of these same genes are conserved in humans: the insulin/insulin-like growth factor-I (IGF-I) peptide and *Daf-2* gene are homologs of the human insulin and IGF-I receptor, *unc-64* and *unc-31* are homologous to human synthaxin and catabolite activator proteins that are involved in the release of neurotransmitters at the synapse, *Age-1* is related to a conserved phosphoinositol-3-kinase that responds to insulin receptor activation, and *Daf-16* is the homolog of the human forkhead box, class-O transcription factor (5). In *C. elegans*, a relatively complex organism, these genes constitute a primordial neuroendocrine system in which the insulin/IGF-I peptide integrates information from environmental stress. The resulting inte-

grated responses play an important role in monitoring metabolic and reproductive status to permit appropriate energy adjustments and, ultimately, extend life span (87). Thus it may be assumed that this primitive neuroendocrine system has the capacity not only to coordinate what occurs in each cell and tissue of the body, but also to avoid disorganization (e.g., overexpression leading to toxicity) of stress responses. These landmark studies in nematodes encourage further exploration of hierarchical programming among the multiple factors that regulate longevity.

Neuroendocrine-immune theory. In the hierarchy of multisystem regulation throughout the sequential stages of life, there is a significant role for the interaction and integration of the neuroendocrine and immune systems. Such interaction occurs through 1) neuropeptides and cytokines present in the immune system that mediate both intrimmune communication and communication between the neuroendocrine and immune systems, 2) several hormones from the posterior (vasopressin) and anterior (thyroid-stimulating hormone, prolactin, adrenocorticotrophic hormone, and growth hormone) pituitary that control many important immune functions (cytokine and antibody production, lymphocyte cytotoxicity and proliferation, and macrophage function), and 3) reciprocal action of cytokines on neuroendocrine functions. For example, interleukin-1 activates the HPA by stimulating the secretion of cortico-releasing and adrenocorticotrophic hormones and may also act on the release of other pituitary hormones (thyroid-stimulating hormone, growth hormone, prolactin, luteinizing hormone).

Parallel to neuroendocrine interactions, the immune system has several essential functions. The immune system must control and eliminate foreign organisms and substances in the host body while at the same time recognizing and therefore sparing from destruction the molecules (cells and tissues) from oneself. In most elderly humans, immunosenescence is characterized by a decreased resistance to infectious diseases, a decreased protection against cancer, and an increased failure to recognize self (hence, autoimmune pathology) (31, 33). However, different immune responses are differentially affected with age. In humans, the thymus is one of the most important immune organs: it is involved in the selection and maturation of T cells and production of peptide hormones. The thymus reaches a peak in both size and function during puberty; shortly thereafter, it atrophies and progressively reduces its production of mature T cells and hormones. This sign of early immunosenescence may be interpreted as a tradeoff between the decreasing usefulness of the thymus once the repertoire of T cells has been set up and the cost of maintenance of the organ. (32). Yet other functions, for example the activities of several types of lymphocytes (natural killer and dendritic cells, macrophages) and of the complement system, are well preserved in healthy centenarians (30).

Both the neuroendocrine and immune systems are endowed with a high degree of plasticity, that is, the ability to modify their function according to demand.

Plasticity is most efficient at early ages but also persists at advanced age. Although studies of human aging in the 1960s to 1980s focused on functional decrements with aging involving all organs and systems of the body (85), in the 1980s and 1990s there was a reconceptualization of the aging process that 1) deemphasized the view that aging is exclusively characterized by declines in function and in health, 2) refocused on the substantial heterogeneity among older persons, 3) underscored the existence of positive trajectories of aging (i.e., without disease, disability, and major physiological decline), and 4) highlighted the possible avoidance of many usually aging-related diseases and disabilities (76). Thus three trajectories of aging were delineated, the first characterized by disease and disability, the second, known as "usual aging," characterized by absence of overt pathology but presence of some decline in function, and the last, the so-called "successful aging," with little or no physiological loss and no pathology (76). Mechanisms of successful aging would consist of 1) persistence of normal function and plasticity, 2) compensatory responses to restore normal function (as may be induced by exercise, good nutrition, and better education), 3) interventions to replace deficient function (as represented by replacement therapies), 4) changing of health outcome by modifying risk profiles (as in Ref. 2), 5) prevention of disease, and 6) strengthening of social interactions and support (77). A successful example of this "functional remodeling" may be mediated by neuroendocrine and immune signals (66). For example, insulin sensitivity by peripheral target tissue is decreased in old age but may be improved through caloric restriction (100). Another example is the significant lengthening (by 40% and more) of the life span induced by caloric restriction (57). This experimental intervention acts at various levels of function and involves a number of molecular, cellular, and systemic changes. Only a few aspects of caloric restriction will be discussed below.

Caloric restriction is the most potent and reproducible environmental variable capable of extending the life span in a variety of animals from worms to rats. This simple intervention is achieved by providing a diet containing all the essential nutrients and vitamins but significantly restricted (by 30–70%) in calories. In addition to the severity of the restriction, the degree of life span lengthening depends on several factors: the specific animal species, age at onset of restriction, and others (62). Not only is longevity increased but also metabolic responses (e.g., increased tissue sensitivity to insulin), neuroendocrine and immune responses (e.g., increased defenses against stress, infections, cancer), and collagen responses (e.g., reduction of cross-linking) are significantly enhanced (66). Such functional changes may be associated with changes in gene expression profile. For example, after chronic (28 mo) severe (76%) caloric restriction, the genetic changes that occurred in aging mice fed *ad libitum* (i.e., non-caloric restricted) were significantly (by 84%) attenuated: for those genes characterized by increased expression, 29% were completely prevented and 34%

were diminished (48). Caloric restriction may act to promote longevity through metabolic reprogramming with a transcriptional shift (perhaps triggered by insulin) toward reduced energy metabolism and increased biosynthesis and turnover of proteins. Caloric restriction also markedly influences the expression of pathological phenotypes in rodent species selectively bred as models of human pathology. However, the many benefits of caloric restriction are accompanied by a number of untoward effects that may prevent its applicability in humans and other animals; among these, the most significant are delayed (or stunted) growth and failure of sexual maturation. The molecular mechanisms of caloric restriction remain unresolved; however, this intervention is a useful experimental manipulation of aging in a variety of animal species, a property that fully merits its current widespread use in the study of aging.

CONCLUDING REMARKS

It should be clear from the content of this review that the ultimate causes of aging remain unknown. On the other hand, a great deal of the aging process is understood and may only require the integration of various models and theories to account for normal aging. In our view, the aging process is multifactorial and complex, but not irreducibly so. Many of the pleiotropic changes that occur with aging may result from one or more primary changes that affect many downstream processes. This interconnectivity of the aging process often obfuscates the root cause of aging and limits the ability to draw definitive conclusions from experimental results. Life span extension by calorie restriction is often cited in support of one or another theory of aging. For example, calorie restriction reduces oxidant production from mitochondria (see above), and it also prevents or delays age-related changes in endocrine function (such as estrogen receptor density in the hypothalamus). A free radical theorist may argue that oxidative damage causes aging in a universal manner and that the changes in endocrine function with calorie restriction are secondary to changes in oxidant production in endocrine cells. An endocrinologist might offer the counterargument that, because hormones regulate metabolism, calorie restriction delays aging by acting on the endocrine system directly, and all other physiological changes are secondary to this effect. One of the most important goals in aging research is to determine how a physiological intervention such as calorie restriction signals the body to delay aging. Is it a passive process dependent on metabolic changes that accompany reduced caloric intake, or is the organism actively responding to a caloric reduction to prolong reproductive life span? At present, the answer is not entirely clear. The ability of insulin-like signaling to regulate life span argues for the latter, even though a definitive connection between calorie restriction and insulin-like signaling awaits demonstration. However, the ability to study an active regulatory system that affects life span is an enormous benefit to aging research, because

we can now explore the molecular mechanisms that connect changes in gene expression due to insulin signaling (and perhaps calorie restriction) with its ultimate consequence, the delay of aging.

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