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I GENERAL PERSPECTIVES

THEORIES OF AGING

4

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1 GENETIC/ENVIRONMENTAL INTERACTIONS IN AGING

The maximum lifespan potential is a constitutional feature of speciation to polygenic controls and to environmental influences. The enormous genetic heterogeneity that characterizes many species, particularly humans, and the complexity of environmental experiences create quantitative and qualitative variations of the senescent phenotype. Until now, no single theory has accounted for all phenotypes, although many scientists have tried to explain at least some of the major and most frequent aging phenomena. Almost all phenotypes result from an interaction between nature and nurture, and an integrating view of these interactions may help provide a more fundamental understanding of aging. Thus, an analysis of molecular, cellular, and systemic events may reveal a productive path for understanding the biology and pathology of aging. It is with this rationale in mind that the present chapter presents a comprehensive account of the major theories of aging categorized as molecular, cellular, and systemic.

Molecular theories propose that the lifespan of any species is governed by the genes interacting with environmental factors. Genetic information is stored in the genes (nucleotides segment of DNA), is transcribed to RNA, and is subsequently translated into proteins. These proteins, either structural or functional, govern the form and function of organisms. Aging may result from changes in DNA template activity, which regulates the formation of the final cellular products. It is believed that gene expressions are carefully regulated and that the proteins produced by gene activity are involved in multiple interacting processes.

A number of theories propose that changes in cellular proteins and other macromolecules occur as a function of age. These changes occur with the passage of time under the influence of environmental factors (e.g., nutrition and stress). They may be chemical and/or morphologic and involve enzymes, hormones, age pigments, free radicals, membrane permeability, macromolecule crosslinking, and changes in various cell organelles such as lysosomes and mitochondria.

Systemic theories ascribe aging of the entire organism to decrements in the function of a key system, such as the nervous, endocrine, or immune system. Such decrements could be genetically programmed, as are the early developmental phases of the lifespan, or be the consequence of environmental insults. Alterations in the key system will generate changes throughout the entire organism.

The various theories of aging are listed in Table 4-1. Some of these theories are presented here and some are discussed in chapters applicable thereto (Chapters 6, 7, and 11).

2 MOLECULAR THEORIES

These theories begin with the following concepts:

1. All individuals within a species have an almost similar length of life.
2. Individuals from different species have different lifespans.

For example, mayflies live only 1 day, houseflies 30 days, rats 3 years, dogs 12 years, horses 25 years, and humans 100 years.[1] It is presumed that there is some genetic program which determines the maximum lifespan for each species. Another argument for a genetic basis of aging is that the offspring of long-lived parents have a longer lifespan than those born from average-lived parents.[2] The average lifespan for females is generally longer than for males in most developed countries, like the U.S., Sweden, and Japan; this sex difference is also observed in other groups of animals (Chapter 2).[3]

An equally significant contribution to a genetic basis of aging is deduced from the duration of the three phases of the lifespan — developmental, reproductive, and senescent. In most animals, the reproductive phase occupies a very significant period in the lifespan, followed by a postreproductive phase. In mammals, the time taken to reach reproductive maturity is directly correlated with maximum lifespan. Humans and other long-lived mammals take a longer time to reach reproductive maturity than other animals and continue to live even after reproduction has ceased. Conversely, certain lower vertebrates (Pacific salmon, Atlantic eel, and lamprey) and invertebrates (octopus) die soon after their first reproduction, as if reproduction might involve depletion of certain essential factors necessary for maintenance of later life (Chapter 2).

The expression of the genetic program that regulates the lifespan may be altered by various environmental factors. Evidence for genetically programmed lifespan has been reported in a colonial protochordate *Botryllus schlosseri*.[4] Such animals display a parent colony-specific timing of mortality.

Molecular theories of aging discussed here include codon restriction, somatic mutation, error theory, and gene regulation theory, as well as antagonistic pleiotropy, dysdifferentiation, and soma disposal hypotheses (Table 4-2).

2.1 Codon Restriction

All the genetic information stored in DNA directs the structure and function of the organism, although only part of the total DNA information is utilized by the cell at a given time. The information is transferred from DNA to messenger RNA (mRNA) by the process of transcription. The functional mRNA in eukaryotic cells is derived by excision of intervening sequences (introns) and splicing. This mRNA is then translated into protein. *The codon restriction theory of aging is based on*

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TABLE 4-1

Major Theories of Aging

Aging due to external causes	Lifespan indefinite were it not for environmental insults such as	
	Foods/toxins	
	Bacteria/viruses	
	Radiation	
	Pollutants	
Aging due to internal causes	Lifespan genetically determined for a finite period; genetic expression modulated through specific programs leading to	
	Neuroendocrine theory	
	Immunologic theory	
Aging due to cellular and molecular causes	Both internal and external causes may act at one or more cellular levels and/or specific molecules to produce	
	In membranes:	Changes in fluidity, permeability, transport
		Organelle biogenesis and intracellular molecular movements
	In cytoplasm:	Wear and tear
		Free radical accumulation
		Cross-linking
		Lipofuscin
	In nucleus:	Codon restriction
		DNA damage and DNA repair failure
		RNA catastrophe errors
		Mutations
		Gene regulation
		Antagonistic pleiotropy
		Dysdifferentiation
		Disposable soma

the hypothesis that the fidelity or accuracy of translation, which depends on the cell's ability to decode the triple codons (three bases) in mRNA molecules, is impaired with aging.[5] Accurate readings of codons are done by two main biomolecules: transfer RNAs (tRNAs) and aminoacyl-tRNA synthetases. Any changes in these tRNAs and aminoacyl-tRNA synthetases may alter the rate of translation.

There is experimental evidence for quantitative changes in the tRNAs and synthetases during development and aging. Ilan and Patel[6] have reported alterations in $tRNA^{tyr}$, $tRNA^{leu}$, and corresponding synthetases during the developmental period of the insect *Tenebrio molitor*.[7] These quantitative alterations also occur in the isoacceptors of $tRNA^{arg}$ and $tRNA^{tyr}$ during aging of the Free living nematode *Turbatrix aceti*. Support for this theory has come from the findings of Hosbach and Kubli[8] who demonstrated that tRNA isolated from 35-day-old *Drosophila melanogaster* cannot be aminoacylated as efficiently as that of 5-day-old flies. The efficiency of the aminoacylating ability of some synthetases of old flies is only 50% that of the young flies. The fetal rat liver contains six isoacceptors for $tRNA^{tyr}$ compared to the adult which has only three.[9] A lesser aminoacylation has been reported in hepatic parenchymal cells of old rats.[10]

Changes in tRNAs and aminoacyl-tRNA synthetases with aging occur in plant systems as well. Young and old tissues of soybean cotyledons differ from each other in the kinds of completely chargeable tRNA that are present. Moreover, extracts of old tissue are not only deficient in certain aminoacylating abilities but possess factors which inhibit the charging of some tRNAs by extracts of young cotyledons.[11] Gene sequencing of rabbit β-globin reveals a highly restricted use of the synonymous codons for various amino acids, only 39 of the 61 usable codons are employed in the framing of the message.[12] Comparison of the isoaccepting species of $tRNA^{lys}$ from early and late human fibroblasts shows a smaller proportion of these species in senescent cells than in those from early passage cultures.[13]

As a result of differentiation, cells would lose their ability to translate genetic information. Despite much supportive evidence, this theory, based on the view that sequential changes in the tRNAs and aminoacyl-tRNA synthetases during lifespan may lead to the aging of an organism, needs further validation. It is difficult to explain the basic cause(s) for the alterations with aging in these message-reading molecules and the implications of such changes in the aging phenomena.

TABLE 4-2

Molecular Theories of Aging

Codon restriction	Fidelity/accuracy of mRNA message translation is impaired with aging due to cell inability to decode the triple codons (bases) in mRNA molecules
Somatic mutation	Exposure to radiation shortens lifespan due to increased incidence of mutations and loss of functional genes
Error catastrophe	Errors in information transfer due to alterations in RNA polymerase and tRNA synthetase may increase exponentially with age resulting in increased production of abnormal proteins
Gene regulation	Changes in expression of genes regulating both development and aging
Antagonistic pleiotropy	Genes beneficial during development and deleterious at later ages
Dysdifferentiation	Gradual accumulation of random molecular damages impair regulation of gene expression
Disposable soma	Preferential allocation of energy resources for reproductive cells to the detriment of maintenance and survival of somatic cells

2.2 Somatic Mutation

Alteration in the structure of DNA molecules alters the genetic message and results in differences in protein structures which lead to physiologic deficits. This proposed theory was based on the report that rats exposed to limited *irradiation* died at a younger age than nonirradiated controls.[14] These considerations were extended to humans[15,16] and included a higher incidence of neoplasia in irradiated individuals, suggesting that irradiation accelerates the aging process. According to this theory, exposure to radiation damages DNA and subsequently induces mutations which, in turn, lead to progressive loss of genes in postmitotic cells throughout the lifespan. The increased rate of mutations and loss of functional genes decrease the rate of production of functional proteins, and cause cell death at a critical level.

Support for this theory was provided by the observation that increased exposure to X-rays shortens life expectancy and increases chromosomal aberrations in a dosage-dependent way.[17] Older animals have a greater number of chromosomal abnormalities than younger and, in short-lived mice, the rate of development of abnormalities is more rapid than in long-lived ones. These data suggest that natural radiation also affects the aging process. Martin and associates[18] reported a fivefold higher frequency of chromosomal aberrations in primary cultures of kidney from 40-month-old mice compared to young animals. A general consensus has emerged that the frequency of chromosomal aberrations increases greatly with age. In young non-cigarette smoking adults, the frequencies of aneuploidy, breakage, and structural chromosomal rearrangements are six times less than they are in 60-year-old individuals. However, contrasting evidence negates a causative role of somatic mutation in aging.[19]

In some species, such as humans, the sex chromosomes of females are similar (XX) but those of males are different (XY), while in other species the reverse is true. If radiation is a cause of aging, then one might expect a longer life for individuals with identical sex chromosomes. In most species, the females generally live longer than males, irrespective of the chromosomal composition. Another example of the lack of influence of sex chromosomes is the wasp *Habrobracon,* in which males have either two sex chromosomes (diploid) or one (haploid). If both types of males are exposed to X-rays, the haploid males should die earlier than the diploid, but this is not the case: both males have similar lifespans despite the greater resistance of the diploid male to ionizing radiation because of the larger number of repairable chromosomes.[20]

Chemical substances which alter DNA structure have no effect on lifespan.[21] Exposure of human fetal lung fibroblast to colchicine (an alkaloid capable of inducing polyploidy, i.e., higher than normal number of chromosomes), produces 60% tetraploid cells (with four sets of chromosomes) which continue to divide and have growth rates and lifespans similar to diploid cells (two sets of chromosomes).[22] Diploid as well as tetraploid human skin fibroblasts likewise have similar lifespans.[23] The somatic mutation theory is further weakened by the results from studies on the effects of low-dose ionizing radiation on the lifespan of human fibroblasts *in vitro.* Irradiation of early embryonic as well as postnatal cells may shorten, prolong, or have no effect on doubling potential and lifespan.[24,25]

Somatic mutations are no longer regarded as a probable cause of aging because the rate at which they occur in the absence of ionizing radiation is too low to account for overall age changes.[26] Furthermore, this theory does not give a clear picture of the mutation load in different organs and tissues and also of the kinetics of mutation accumulation.[27] Mutation accumulation has been suggested as a consequence rather than a cause of aging.[27]

Most cells have mechanisms for the repair of damaged DNA molecules,[28,29] and there is little evidence that DNA repair mechanisms decline in senescent animals;[30,31] rather, these repair mechanisms appear more effective in long-lived species as compared to short-lived.[28] The species-specific differences in the lifespan of animals might be attributed to the ability of animals to tolerate DNA damage rather than to repair. The presence of multiple copies of the same message coded within the DNA would offer protection to DNA damage.

The number of repetitive genes for the major rRNA is 5 to 10 in bacteria, 100 to 130 in *Drosophila,* and 250 to 600 in vertebrates,[32] suggesting a positive correlation between the number of repetitive genes and the lifespan of the species. Cutler[33] reported that the average redundancy of the transcribing mRNA in the brain was greater in humans than in cows and greater in cows than in mice. The higher the redundancy of the transcribing mRNA the longer is the lifespan. Based on available experimental evidence, radiation does not seem to play a major role in accelerating the aging process or in causing aging.

2.3 Error Theory

The form and function of organisms are determined by specific structural and functional proteins. Certain proteins such as RNA polymerase and tRNA synthetases are involved in the synthesis of other proteins. Medvedev[34] first proposed that *errors in information transfer from DNA to proteins may be responsible for cellular aging.* This concept was extended in a search for errors in transcription and translation processes, which may lead to accumulation of proteins and cause aging.[35,36] It was further argued that production of functional proteins such as enzymes depends not only on the genetic information stored in DNA but also on the protein synthetic machinery. Inaccuracy may occur both in protein and DNA synthesis.[37] The initial error in proteins may be low, but errors may increase exponentially as a function of age and lead to error catastrophe and cell death.

Evidence for the error theory is based on error induced experimentally in fruit flies by feeding them amino acid analogs.[38] Much of the support of the theory came from the work of Holliday and Tarrant[39] who reported an increased accumulation of heat-labile glucose-6-phosphate dehydrogenase in old fibroblasts. This heat-labile enzyme in old fibroblasts also showed an altered substrate specificity suggesting the possibility of errors. Using immunological techniques, Lamb[40] found an age-dependent increase in the proportion of inactive lactate dehydrogenase (LDH) in human fibroblasts, isocitrate lyase in nematodes, and aldolase in mouse liver. Functionally altered enzymes are known to accumulate in various animal tissues with age,[41,42] and the consequent decrease in the functional activity of tissues with age would be due to accumulation of such altered proteins (Chapter 5).

Some experimental evidence is not consistent with the proposal that errors in protein cause aging. Kanungo and Gandhi[43] could not detect any age-related differences in liver malate dehydrogenase (MDH) by immunologic techniques. Kinetic properties (K_m and K_i) and electrophoretic mobilities

of rat liver cytosolic alanine aminotransferase[44] and aspartate aminotransferase[45] do not reveal age-related differences. Studies of cytosolic superoxide dismutase (SOD) from livers, brains, and hearts of rats and mice have not revealed age differences in antigenicity, K_m and K_i, and electrophoretic mobility.[46]

The fidelity of protein synthesis *in vitro* remains unchanged in human diploid skin fibroblasts as a function of age.[47] Although significant quantitative changes occur in aging *Drosophila* mitochondrial proteins, there is no change in the molecular weight or isoelectric point of these proteins.[48] The fidelity of mitochondrial proteins seems to be preserved throughout the lifespan of *Drosophila*. Thus, there is evidence to believe that errors in the fidelity of protein synthetic machinery do not occur with age and, therefore, cannot be responsible for aging. Nevertheless, the findings of altered conformation in some proteins by oxidation may account for physiological impairment during aging of animals (Chapter 6).[48]

2.4 Gene Regulation Theory

According to this theory,[49] *senescence results from changes in the expression of genes after reproductive maturity is reached.* It is based on the presumption that senescence would follow a pattern similar to that of differentiation and growth i.e., a sequential activation and repression of certain genes which are unique to these phases. Sequential activation and repression of genes have been reported for various chains of hemoglobin during the gestational period in humans.[50] Hemoglobin, a tetramer metallo-protein, consists of $\alpha_2\ \varepsilon_2$ chains in the fetus at 1 to 2 months of gestation. In the later phase of gestation the a chain remains the same and the ε chain is replaced by the γ chain. Just before birth, the γ chain is further replaced by the β chain which gives rise to adult hemoglobin $\alpha_2\beta_2$. The synthesis of these chains is governed by different sets of genes which are sequentially activated and repressed during the development of the human fetus.

Another example of gene activation and repression is the differential expression of LDH isoenzymes in mice during embryonic development.[51] The proportion of M4-LDH is significantly lower in heart and skeletal muscle of old compared to young rats (Chapter 5).[52] Studies on rat liver cytosolic alanine aminotransferase (cAAT) have shown that the gene for A subtype is more active in the early period of the lifespan and subsequently repressed, while B subtype gene is activated in old age.[44] The sequential activation and repression of genes would not be restricted to development,[53] but would extend into adulthood and aging.[54,55]

The genes responsible for the synthesis of various enzymes do not appear to undergo any change in their basic sequences during the lifespan. Rather, the observed changes in levels of enzymes may be due to the alterations in the template activity of corresponding genes induced by various extrinsic and intrinsic factors (Chapter 5). For example, the level and inducibility of many enzymes by hormones change in different tissues as a function of age without any possibility of error incorporation into these molecules.[56] Modulating actions may either appear or disappear and/or their levels may change at different phases of the lifespan.[54] The products or by-products of the genes responsible for differentiation and growth, on reaching critical levels, stimulate certain unique genes responsible for the reproductive phase. However, as a result of continued reproduction, certain factors may be depleted and/or they may not be replenished as fast as they disappear. Such factors may be of crucial importance for keeping certain genes expressed or repressed. They may also activate some undesirable genes which may be responsible for a gradual decline in reproductive rate (e. g., number of offsprings) with age. This theory also predicts that should the organism be able to replenish the factors that become depleted due to continued reproduction, the reproductive period and lifespan would be lengthened. It is supported by the data on the lifespan of mammals, particularly the reproductive phase, which has continuously lengthened with the progress of evolution.[57]

The lifespan of a species may be divided into three phases:

1. Developmental
2. Reproductive
3. Senescent

Each phase has a characteristic

1. Duration
2. Rate, i.e., velocity
3. Sequential timetable of events
4. Regulatory mechanisms

Initiation and duration of developmental and reproductive phases depend on a unique set of genes that are sequentially activated and repressed.

Human genetic diseases like *progeria* and *progeroid syndromes* are in agreement with this sequence.[58] Progeria is caused by the mutation of an autosomal gene. In this case, the newborn child appears normal and grows normally up to about 6 years, when signs of aging (e.g., atherosclerosis, accumulation of lipofuscin, graying of hairs) do appear. Fibroblasts taken from a 10-year-old progeria patient do not undergo as many population doublings as those of a normal child of the same age. It appears that some genes responsible for normal development are altered to induce this condition. Perhaps the production of essential factors necessary for development and growth is prevented by this mutation. The reproductive phase is not initiated due to lack of switching on one of the necessary genes during the later phases of development and growth. The lifespan is shortened following expression of the mutated gene (Chapters 3 and 5).

Another example is the sudden death of the female *octopus* which lays eggs only once, broods them, reduces food intake, and dies soon after the hatching of the young ones.[59] Removal of the female's paired optic glands after spawning prevents brooding, and the octopus continues to eat and to grow and its longevity increases. It is apparent that some factors produced by the optic gland are essential for brooding and cessation of feeding followed by senescence and death. Egg laying may deplete these factors, which may in turn cause the optic gland to produce a hormone that causes behavioral change.

A similar phenomenon of semelparous (pertaining to organisms reproducing only once) degeneration and death is observed in *Pacific salmon*[60] and in *marsupial mice*.[61] In all three cases, there is marked evidence for hormone-dependent degeneration and death. Each species has a unique set of genes for development and reproduction. The sequential activation or repression determines the duration of development and the onset of reproduction and is governed by the proper balance of various factors that are essential for maintenance of the reproductive phase. No unique gene would

be responsible for aging; rather, aging would merely be a consequence of the organism's attaining reproductive maturity, irrespective of whether or not it reproduced.

Evidence of semelparous degeneration and death in certain species also provides support for developmentally programmed aging.[1,55] It assumes that aging is controlled in ways similar to those that operate during development. These events are primarily controlled by hormones, which are produced or depleted during and after reproduction. Hormones would play a significant role not only in development but also in regulating the aging of an organism. Developmentally programmed aging,[55] in spite of supportive evidence, needs further testing of genetic approaches to both development and aging in the same organism. Discoveries of homeotic genes[62,63] controlling development of *Drosophila* have provided a way to investigate the role of such related genes in regulating aging of an organism.

Sequential activation and repression of genes for certain senescence marker proteins have been reported.[64] Most striking is the reversible expression of liver senescence marker protein 2 (SMP-2) gene as a function of age. It is expressed maximally during both prepuberty and senescence when liver is insensitive to androgen because of lack of functional androgen receptors, and mRNA expression drops significantly in the postpubertal adult male rat when liver is most responsive to androgen. SMP-2 gene is reported to be an androgen-repressible gene as its high level of expression is maintained in young adult females. These findings are consistent with the concept of hormone-dependent sequential regulation of genes during aging.

Studies of long-lived organisms compared to normal-lived ones may reveal which factors the normal organisms are missing.[65] By selective breeding, longer-lived *Drosophilae* have been generated[65,66] that produce a remarkably active form of SOD, an antioxidant enzyme, indicating that they contain a variant of the normal enzyme encoding gene. An active form of SOD neutralizes superoxide (O_2^-) more effectively leading to a longer lifespan of the flies. Normal fruit flies age more quickly because their free radical defenses are not as effective as those of specifically bred flies.

Another example of genetic clues comes from the search for a gene in the soil nematode *(Caenorhabditis elegans)* that is differentially expressed in the long-lived and normal organism.[67] Mutation of a single gene called age-1 can increase the average lifespan of *C. elegans* by about 70%. These mutant worms also produced enhanced levels of SOD and catalase. Johnson[67] postulated that mutation of age-1 may deplete its protein product, which might have suppressed SOD and catalase gene activity in normal-lived worms.

In a similar approach, Jazwinski[68] identified several genes that prolong the life of brewer's yeast *(Saccharomyces cerevisiae)*. The best studied of these is LAG-1 (longevity assurance gene-1) which is more active in young than in old cells. LAG-1 activity in older cells extends the lifespan of yeast by one third. Strikingly, aged yeast cells harboring the extra-active gene do not become immortal. They simply remain youthful for a longer period of time. The LAG-1 gene product has not yet been functionally characterized. Attempts are being made to isolate such longevity assurance gene(s) from human cells and to correlate their presence with the human lifespan.[65] These examples of longevity-controlling genes reflect a unique feature of gene regulation during aging. Although, the function of such genes remains obscure, the discovery of induced antioxidant enzymes seems to have the potential for affecting longevity in both fruit flies and *C. elegans*.

3 ANTAGONISTIC PLEIOTROPY, DYSDIFFERENTIATION, AND DISPOSABLE SOMA HYPOTHESES

3.1 Antagonistic Pleiotropy

A long-held theory of the cause of senescence is *the declining force of natural selection as a function of age of adult somatic cells*.[69,70] Natural selection that favors genes with early beneficial effects leads to deleterious effects later on. Certain *genes confer survival advantages early in life and cause harmful physiological effects in later stages of lifespan,*[69,71] a phenomenon termed negative or antagonistic pleiotropy. The evolutionary view of aging is supported by mathematics and interesting experimental data. Friedman and Johnson[72,73] have isolated and characterized a mutant allele, age-1, from *C. elegans* which increases maximum lifespan by 60% at 20°C. At the same time, it decreases fertility by 75% in self-fertilizing hermaphrodites.[67] Genes that specify instructions for synthesizing reproductive hormones also serve as examples of antagonistic pleiotropy.[74] Long-term exposure of the estrogen used to enhance fertility increases the risk of breast cancer in aged women. A variety of other cases have been noted. Hypothalamus and pituitary gland control ovarian function and also contribute to aging of the ovary in rodents. At the same time, ovarian signals appear to promote aging of the hypothalamus and pituitary. Age-dependent cytotoxic effect of glucocorticoids on hippocampal and hypothalamic neurons also correlate with antagonistic pleiotropy.[75] However, there is some evidence that is inconsistent with this hypothesis.[76]

3.2 Dysdifferentiation

Gradual accumulation of random molecular damages impairs the normal regulation of gene activity, potentially triggering a cascade of injurious consequences. Cutler[77] has called this process dysdifferentiation. Dysregulation of genes may provide a mechanism that links the antagonistic pleiotropy and disposable soma hypotheses into a unified concept of aging.[78] It is evident that genes are carefully regulated and that the proteins produced by gene activity are involved in multiple, often interacting, processes. Aging may occur when the normal repair and maintenance functions of cells become dysregulated and gradually lead to impaired physiologic functions.

Aberrant expression of genes may play a role in the aging process.[79] Evidence for this came from the discovery of increased levels of globin RNA in the liver and brain of older mice.[80] Loss of epigenetic control has been proposed as a major causal factor for reactivation of genes in aged.[79] DNA methylation at C≡G bases relates to the inactivation of gene expression and has been shown to decrease with age in several systems,[81,82] leading to the reactivation of genes. The extent of demethylation correlates with longevity in two different mouse species.[81] Furthermore, treatment of cultured cells with 5-azacytidine, an inhibitor of methylase activity, results in a decreased doubling potential.[83]

In an attempt to test the universality of the loss of epigenetic control and aberrant gene expression,[79,80] Slagboom and Vijg[84] examined the age-related expression of a number of genes. It appears that the expression of many genes decreases, the

expression of some increases, and others do not change in different systems with advancing age of animals.[84,85] In systematic studies to test the aberrant gene expression phenomena, Sato and his associates[86] measured the steady state level of mRNA for five tissue-specific genes (myelin base protein, atrial natriuretic factor, albumin, κ immunoglobin, and keratin gene expressed in brain, heart, liver, spleen B lymphocytes, and skin, respectively) and found no aberrant expression of these genes with age. Another molecular abnormality with aging is telomere shortening. Telomeres are the tail portion of the chromosomes, which they help to stabilize during cell division. As telomeres shrink and are shed with each cell division, their length gives some indication of the number of divisions undergone and still to occur. In cancer cells, in the presence of the enzyme telomerase, telomeres are not shortened but continue to replace lost sequences; in aging cells, telomerase activity may be reduced, telomeres may be shortened, and cell division may be curtailed.[87] Overall, these studies indicate that aberrant gene products, telomere shortening, or other changes in gene expression are not necessarily a typical feature of the aging process *in vivo*.[86]

TABLE 4-3

Cellular Theories of Aging	
Wear and tear	Intrinsic (e.g., oxidative processes) and extrinsic (e.g., ambient temperature) influence the lifespan
Free radical accumulation	Free radicals formed by oxidative reaction accumulate and damage membrane, cytoplasm, and nucleus (also discussed in Chapter 6)
Age pigments	Accumulation of lipofuscin (fluorescent age pigment) causes several pathophysiological complications and is inversely correlated with aging (also discussed in Chapter 6)
Cross-linking theory	Cross-linkages among molecules develop with aging and alter chemical/physical properties of cell molecules

3.3 Disposable Soma Hypothesis

This hypothesis suggests that aging has evolved as a by-product of optimization of the allocation of energy and resources for the various works performed by the organism. It assumes that *energy resources are better spent for maintenance of reproductive cells, responsible for species survival.* The minimum required for maintenance, repair, and survival of somatic cells is not cost-effective; it is too expensive in terms of energy and the nonreproductive cells of the body are consequently expendable.[88] Aging then would result from a progressive accumulation of somatic defects and damage. Maintenance and repair include the prevention and removal of DNA damage, accuracy in macromolecular synthesis, and degradation of defective proteins. Lifespan of different species depends on the differential level of somatic maintenance and repair. Long-lived species in general have a greater level of maintenance and repair systems compared to short-lived species.

The disposable soma hypothesis *balances the maintenance and repair of somatic cells on one side and the reproduction and fertility on the other.* If more energy is used for maintenance of soma, less will be available for reproduction, and *vice versa*. This hypothesis treats *senescence as a price paid for sexual reproduction.*[89] There exists a direct correlation between the time taken to reach reproductive maturity and the species lifespan (Chapter 2). Experimental animals whose reproductive age is delayed tend to live longer than normal animals.[90]

Semelparous reproductive degeneration and death in certain species also provide support for this hypothesis. Animals with exhaustive reproductive activity seem to expend much more energy than is allocated for this purpose; they are left with little to maintain and repair the somatic cells and hence die soon after their single reproduction. This cost-effectiveness theory also draws support from the free radical theory of aging (Chapter 6).[91] The maintenance and repair of free radical damage to various structural and functional biomolecules play an important role in determining the aging of an organism.[91] Accumulation of age-dependent advanced glycosylation end products (AGEs) occurs because of failure of prevention and repair systems for such damages.[92]

4 CELLULAR THEORIES

These theories relate to changes that occur in structural and functional elements of cells with the passage of time (Table 4-3). They also concern the biomolecules after their synthesis is over, suggesting that these changes impair the effectiveness of molecules as a function of age.

4.1 Wear and Tear

The idea of wear and tear compares living organisms with machines,[16] i.e., with repeated use, parts wear out and become defective, and the machinery finally fails to function. This comparison is not entirely appropriate: organisms have a mechanism by which they can repair their damages, whereas machines do not.

The premise of this theory originates from the observation that the *lifespan of poikilotherms is shortened by increasing the environmental temperature and prolonged by decreasing it;* rates of chemical reactions increased with increasing temperatures, and the reverse is true for low temperatures (Chapter 2). This phenomenon has been reported for fruit flies[93] and rotifers.[94] Even a slower rate of aging has been reported for rat tail tendon collagen, an extracellular protein, at low temperature.[95]

On the other hand, an increase in metabolic rate may shorten the lifespan by accelerating wear and tear. The lifespans of different animal species are inversely proportional to basal metabolic rate.[96] Basal oxygen consumption rates of short-lived animals, such as rats and mice, are much higher than those of long-lived animals, such as elephants and men. However, within the same species, it is difficult to correlate individual differences in lifespan with the metabolic rate.

4.2 Age Pigments

Accumulation of lipofuscin or age pigment is the most prominent age-associated change present in a variety of cell types of many organisms. It is deposited predominantly in nondividing cells such as neurons and cardiac myocytes as a function of age.

Lipofuscin accumulation has been observed in the cortex and hippocampus of man, Rhesus monkey, and rat as one of the common morphological features of aging and has been correlated with the loss of neurons in old age (Chapters 5 and 8). Lipofuscin is also deposited in dividing cells of liver, adrenal cortex, and testes.

Lipofuscin accumulation causes the loss of cytoplasmic mass, mitochondrial number, rough endoplasmic reticulum, and is associated with vacuolization of cytoplasm. Indeed, lipofuscin accumulation may represent a basic feature of cellular aging (Chapters 5, 6, and 8).

TABLE 4-4

System Level Theories of Aging

Neuroendocrine	Control of homeostasis by neural and endocrine signals becomes disorganized with aging; physiologic performance declines while pathologic responses to stress increase in number and severity (also discussed in Chapter 11)
Immunologic	Immune system reduces its defenses against antigens and loses the capacity to recognize self, resulting in increasing incidence of infections and autoimmune diseases (also discussed in Chapter 7)

4.3 Crosslinking Theory

With passage of time, many biological macromolecules develop cross-linkages between identical or different molecules. These linkages alter the physical and chemical properties of the molecules.[97] Major support for this theory was provided by the studies of the extracellular fibrous protein collagen by Verzar.[98] Collagen is synthesized in all cell types, particularly connective tissue cells, and is deposited extracellularly in all tissues. The structural unit of collagen is tropocollagen; units are packed together side by side and stabilized by chemical cross-links between the chains (Chapter 22). The mode of packing creates periodic striations in the structure of collagen fibers. The number of striations in rat tail-tendon collagen and its thermal stability increase with age, while its solubility decreases due to the increased cross-linkages.[99]

Cross-linking agents with charged groups are produced during normal metabolism. Such ionized groups are replaced in early life by normal metabolic processes but accumulate in larger amounts in old age.[99] The groups react irreversibly with macromolecules such as DNA and proteins, inactivating them and thus reducing their functional competence.[98,100]

Increased cross-linking of aged collagen has been correlated with an increased rigidity of the cell membrane, a probable cause of the decreased potassium conductance of the membrane.[101] The higher intracellular potassium would, in turn, increase the intracellular ionic strength and lead to a decreased rate of transcription by chromatin and a decreased rate of protein synthesis.

The free radical hypothesis of aging (Chapter 6) suggests that the important causative agent of aging is the active oxygen species, which produces more damaging effects in compact structures such as cellular membranes than in diluted systems like cytosol.[102] The probability of cross-linking is enhanced in closely packed molecules, making the membranes the most likely targets to be damaged. Cross-linkages are present not only in extracellular collagen but also in intracellular proteins (enzymes) and in nucleic acids (DNA). The decrease in the extractability of chromosomal proteins from chromatin may be attributable to their increased cross-linking with DNA.[103]

Cross-links are produced not only by charged groups but also by some inert molecules such as glucose.[91] Nonenzymatic glycosylation, a chemical attachment of glucose to proteins and nucleic acids, has been implicated in the production of cross-links in these macromolecules.[91] Extensive cross-linking of proteins may contribute to the stiffening and loss of elasticity characteristic of aging tissues.

Even nonenzymatic addition of glucose to nucleic acids may gradually damage DNA. The reaction between glucose and proteins is known as the Maillard or browning reaction. It begins when an aldehyde group (–CHO) of glucose combines with an amino group ($–NH_2$), a Schiff base. This combination is unstable and quickly converts to a substance known as Amadori products. In long-lived proteins, these Amadori products slowly dehydrate and rearrange irreversibly into structures called advanced glycosylation end products (AGEs). Many of these AGEs are also able to cross-link adjacent proteins. It has been suggested that nonenzymatic glycosylation of lens crystallins may contribute to cataract formation in aging individuals. Cross-links generated in proteins and nucleic acids by nonenzymatic glycosylation may contribute to age-related declines in the functioning of cells and tissues.

5 SYSTEM LEVEL THEORIES

Major systemic theory includes the neuroendocrine and immunologic theories (Table 4-4). *For the immunologic theory, the reader is referred to Chapter 7.* Some aspects of the neuroendocrine theory are also discussed in Chapter 11. Because of the interrelation of the immune and neuroendocrine systems, a section including discussion of this interrelation with aging is presented here.

5.1 Neuroendocrine Control Theory

The overall performance of an animal is closely related to the efficacy of a variety of control mechanisms that regulate the interaction between different organs and tissues.[104] *The effectiveness of homeostatic adjustments declines with aging* and leads to consequent failure of adaptive mechanisms, aging, and death.[105] Adaptation to external and/or internal stress depends on *control mechanisms orchestrated by the combined interplay of the nervous and endocrine systems.* The activity of several peripheral endocrine glands, such as thyroid, adrenal, and gonads, is controlled directly by the pituitary gland and indirectly by higher nervous centers, mainly the hypothalamus, which signal the pituitary. For efficient adaptation, nervous and endocrine signals must be synchronized and be responsive to the needs of the many functions they regulate.[106-108] However, with aging, some of the efficiency of the hypothalamo–pituitary interaction is lost or altered, leading to decreased function and increased pathology of most organs and tissue systems.[109,110]

Hormones secreted by the hypothalamus–pituitary–endocrine axis are necessary for the proper functioning of almost every cell in the body. This axis is controlled by a complex mechanism that includes interactions with neurotransmitters of the brain, hormones produced by different endocrine glands, and nutrients from the small intestine and liver. The neuroendocrine theory views aging as part of a lifespan program regulated by neural and hormonal signals. The program unfolds from fertilization through birth, childhood, adulthood, and finally old age and death; command neurons in higher brain centers act as "pacemakers" that regulate the "biological clock" governing development and aging. With the passage of time, aging changes may result from programmed deterioration or cessation of the programming that

regulates homeostasis.[111] In either case, aging would be manifested through a slowing down or imbalance in the activity of the pacemaker neurons with consequent neurotransmitter and hormonal alterations and their repercussion on neural, muscular, and secretory functions. Such functional decrements are exemplified by involution of reproductive organs, loss of fertility, diminished muscular strength, lesser ability to recover from stress, and impairment of cardiovascular and respiratory activity.

5.2 Endocrine–Immunologic Relationship

Hormonal and neural influences on the immune system have long been known; for example, the involutionary action of glucocorticoids on the thymus and lymphatic system has been used for the treatment of allergies and in organ transplants. In addition, the nervous system may regulate some aspects of the immune response. Thus, *neuroendocrine and immunologic theories of aging may converge, or changes in their function may articulate with each other to lead to age-related decline in several bodily functions.*[112]

A neuroendocrine-immunomodulation of thymic aging has been demonstrated in experiments in which tumor (GH3) pituitary adenoma cells, which secrete both growth hormone and prolactin, can reconstitute thymic structure and improve T-cell production and function when implanted in old rats.[113] The possibility of thymic rehabilitation in old age suggests that lymphoid cells in aged animals are not inherently defective, but given the proper stimulus can return to normal function. It will be of immense importance to reactivate the aging thymus, even replacing old T cells with the young ones to make the body's immune system functional for a longer period of time. In addition to cell replacement therapy, one might expect a log of potential of somatic gene therapy in the prevention of immunosenescence.[114,115] These age-related decrements of the body's defense and/or of neuroendocrine systems can be enhanced by using somatic gene therapy, once it becomes fully operative. It can be of great importance to several other age-related disorders such as Parkinson's and Alzheimer's diseases.

5.3 Perspectives on Aging

In spite of tremendous progress in the field of aging research, the mechanism of this process remains elusive. None of the theories proposed explains all the cause(s) for aging; rather, aging seems to be a multifactorial process. Growing evidence suggests that a multitude of parallel and often interacting processes govern the aging of an organism, many of them controlled jointly by a combination of genetic and environmental factors. Although at present it is difficult to advocate a coherent theory of the cause of aging, progress achieved so far and continuing efforts by numerous scientists let us hope that an answer will be forthcoming in the near future.

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