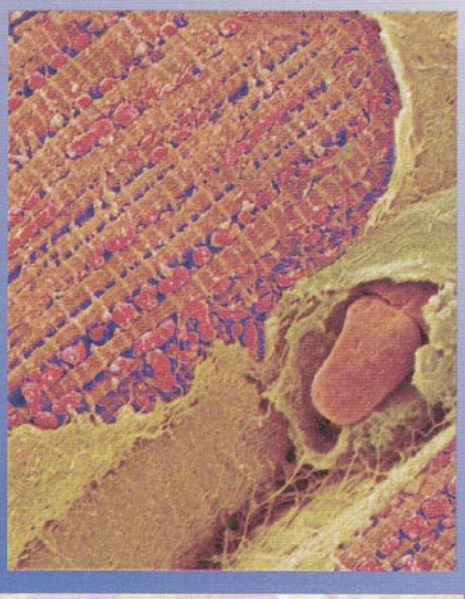

9. Biogerontology – Biochemistry of Aging

Csaba Sőti – Péter Csermely

9.1. Introduction

Authors would have not had the chance to write this chapter in the 17th century. The immature nature of modern natural science would have been only partly to blame; the real reason is that they themselves would have had only a small chance of reaching our current life expectancy (*Fig. 1*). Since then, especially in the 20th century, life expectancy has dramatically increased, which, in turn, led to the birth of *gerontology*, a science investigating the phenomenon and mechanisms of aging. At the same time, civilization and modern Western life created pathological aging and a number of hitherto unknown age-related diseases. Facing this challenge, a novel discipline of medicine, *geriatrics*, was born to deal with many of the diseases and complications related to aging. The science of geriatrics has benefited a great deal from gerontology. The primary aim of medicine today is to effectively treat and prevent age-related civilizational diseases in order to enable healthy and "successful" aging, allowing the elderly to maintain their physical and productive psychosocial capabilities. Molecular and systems biology of aging may provide the key to reach this goal.

This chapter aims to provide an overview of the biological and evolutionary significance of aging, and the phenomenon and mechanisms of aging at the molecular, cellular and organismal level. In addition, some age-related diseases will be presented as examples, with a special focus on the pathobiochemical background and therapeutic approaches. It will also include a summary of our current knowledge about genetic and environmental factors supporting successful and healthy aging.

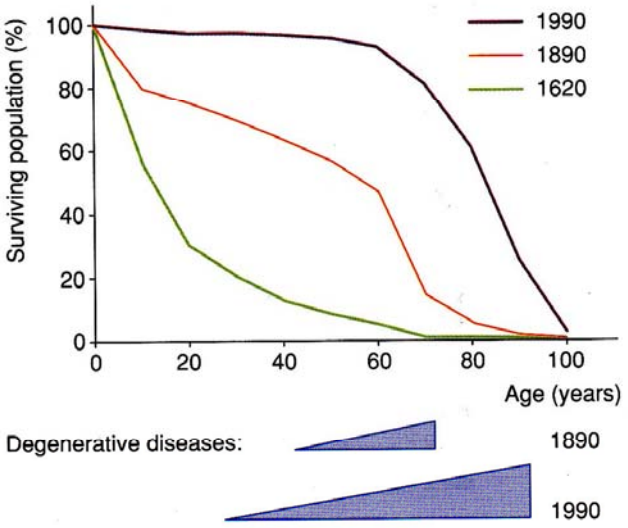

Figure 1 *Lifespan extension during periods of civilization*
In the 1620-s the survival curve was similar to that of natural populations in the wild. Lifespan extension is largely due to the cessation of infant mortality. However, civilized lifestyle is accompanied by an epidemic spread of degenerative (lifestyle) diseases that are not inevitable constituents of healthy aging.

9.2. The Phenomenon of Aging

Definition and Major Characteristics of Aging

Aging is the stochastic and progressive functional decline of the organism, which is accompanied by a decrease in reproduction and an exponential increase of mortality, ultimately leading to death. Organismal aging is not uniform. Our body is an aging "puzzle", possessing different organs and tissues of various biological age. Moreover, growth, development and aging occur in parallel: some body functions are already subject to decline when others have not even emerged (*Table 1*). Aging is not inevitable since stem cells and germinative tissues have an unlimited replication potential: all living organisms are descendent of a cell lineage that has been continuously existing and reproducing for 3 billion years. However, aging and death are universal, involving almost all creatures. Life expectancy (i.e. estimated life-span at the time of birth) is characteristic to the various species and has a great deal of divergence between individuals of the same species. Individual life-span is determined by the intricate interaction between genetic background and environmental factors.

Table 1 *Onset of decay of various functions*

Function	Onset of decay
General decay	60s
Sight	50s
Oocyte maturation	40-50s
Cognitive function	30s
Neuromuscular function	20s
Immunity	10s
Hearing	Year 10
Blood vessel elasticity	After birth
Joint flexibility	After birth
Oocyte production	In utero

Aging Theories

Theories interpreting aging are either rooted in Darwinian natural selection and population genetics (evolutionary theories), or they analyze the changes and causative factors involved in aging (mechanistic theories). The systems/network biology approach of aging integrates premises and experimental data from the cellular to the population level, with the recognition that common causes operate in highly divergent network topology in various cell types, tissues and populations.

Evolutionary Theories

Current evolutionary theories integrate a mass of evidence from evolution and population genetics, including the following:

(1) Individuals in natural populations rarely reach old age.

(2) Natural selection promotes the maintenance and reproduction of genes; thus, physical fitness, i.e. juvenile vitality and fertility, constitute a major evolutionary advantage.

(3) Organismal resources are to be distributed between self-growth, self-maintenance and reproduction. Natural populations are continuously exposed to large and harmful environmental changes; therefore:
- genes specifically inducing or promoting aging could not evolve; thus, aging is not genetically programmed (though there are a variety of genes affecting aging);
- with advancing age, selection pressure declines gradually; thus, genes with harmful consequences in the aged fail to undergo clearance and may accumulate in the population (*mutation accumulation theory*, Medawar 1952);

- furthermore, many genes advantageous for juvenile vitality and fertility are disadvantageous for late self-maintenance and survival (e.g. fast metabolism – more reactive oxygen species, (*antagonistic pleiotropy theory*, Williams, 1957);
- somatic maintenance is worth investing in up to the point where genes are transmitted into healthy progeny; then the body, the "soma", may be disposed. Body repair mechanisms are calibrated to decline and are therefore insufficient to eliminate damage by the time the descendents of the individual are able to start their independent life (*disposable soma theory*, Kirkwood, 1977);
- under harsh environmental conditions (scarcity, stress) survival and self-maintenance are preferred over growth, development and fecundity. Maximal life-span and maximal reproductory success are mutually exclusive; therefore, long-lived individuals are generally more resistant to various stresses and have smaller body-size and reduced fertility.

Based on the above-mentioned, aging is a stochastic process that is not regulated by a specific genetic program. Rather, aging is induced by the accumulation of random errors caused by the exhaustion of repair mechanisms. Aging can also be perceived as a price we pay for early reproduction in the post-reproductive period. However, successful reproduction at the level of the individual does not exclude successful aging and longevity.

According to another theory, aging is a trade-off between individuality and ample genetic variation, which is a consequence of sexual reproduction and recombination. This notion is supported by the role of linear chromosomes in recombination and crossing-over and the role of telomeres in senescence (see below). Moreover, aging does not manifest in those multicellular organisms (like the hydra) in which somatic and germinative cells are unseparated.

Mechanistic Theories

Mechanistic theories try to deduce a (most of the time single) causative factor from the biochemical and biological changes observed during aging. According to the *error catastrophe theory*, translational fidelity is compromised because of ribosomal errors, which in turn give rise to the formation of erroneous proteins, among them translation factors and ribosomal proteins, thus creating a vicious circle. The *somatic mutation theory* states that aging is mainly due to mutations accumulated in the nuclear DNA. The *mitochondrial theory* postulates that mitochondrial DNA mutations impair the electron transport chain and the prevailing energetic incompetence induces aging. According to the *free radical theory*, the single major cause of aging lies in the formation of reactive oxygen species that mostly arise as a by-product of aerobic respiration (see also chapter I. 3.2. (*Oxidative stress* and) below and induce macromolecular damage (The role of mitochondria is outlined in chapter I. 4.3. *Mitochondrial diseases*.)

Network-Theory and Systems Biology of Aging

The network theory (Kirkwood, 1997) postulates that there is no single causative factor and etiology behind aging. Accepting the evolutionary background it emphasizes that (1) pathophysiological changes induced by harmful environmental effects, (2) are opposed by robust repair mechanisms (DNA-repair, antioxidant defense, protein stress response and turnover, immune response, etc.) and (3) the complex and random (stochastic) interaction of the two will determine individual life-span. This complexity can only be comprehended through the use of sophisticated approaches, such as mathematical modeling and comprehensive experimental systems biology.

9.3. Aging at the Cellular and Organismal Level

Aging begins at the molecular level and manifests at each level of hierarchy from individual cells to entire populations. Here we summarize the phenomena and mechanisms of aging observable in both cells and whole organisms.

Cellular Aging – Senescence

Leonard Hayflick (1961) discovered that animal cells in culture are characterized by a finite replication potential. On average, a human fibroblast is able to execute approximately 50–60 divisions (the Hayflick limit) before it irreversibly stops dividing at the G1 phase of the cell cycle, a phenomenon known as *replicative senescence*. In addition to replicative senescence, a variety of harmful agents and conditions can induce an irreversible growth arrest, the so-called *stress-induced premature senescence*, before cells reach the Hayflick limit. Senescent cells live for years and display an intensive metabolism; however, similarly to an individual in the post-reproductive period, they irreversibly lose their replicative potential and are unable to re-enter the cell cycle even in the presence of growth factors, eventually dying in the process. In contrast, tumor cell lines are immortal and can be cultured for an unlimited period of time.

The Senescent Phenotype

Hallmarks of cellular senescence can be identified by light microscopy as well as by functional studies. Major characteristics of the senescent phenotype are highlighted in *Table 2*. In addition to specific morphology, these cells express a neutral lysosomal enzyme. Detection of this enzyme, the so-called senescence-associated β-galacosidase, reflects the increase in lysosomal biogenesis, and this is widely used as an *in vivo* marker of senescence.

Molecular Mechanisms of Senescence

According to the present view, replicative senescence is initiated by the critical shortening of the telomeric region. Telomere erosion is due to the so-called end-replication problem, i.e. the fact that DNA polymerase is unable to replicate the 3' ends of the chromosomal DNA; therefore, during each round of cell replication, 50–200 base pairs are lost from the ends of chromosomes. Thus, telomere length serves as a gauge for replicative age (a so-called mitotic clock). In order to protect the integrity of genetic information, an RNA-dependent DNA-polymerase enzyme, telomerase, extends the chromosome ends by using a non-coding TTAGGG nucleotide sequence, reaching a length of 10–20 kilobases in newborn humans. Initial

Table 2. Characteristics of a senescent cell

Morphology
Bigger size, lower nucleus/cytoplasm ratio
Flat shape, chaotic cell borders
Vacuoles, lipofuscin accumulation
Signs of actin and tubular disorganization
Chromosome aberration, polykaryon
Cellular physiology
Resistance to growth factors and mitogens
Increased sensitivity to stress (oxidative, heat, toxins, etc.)
Decreased respiration, energy output, increased fermentation
Increased free radical production
Biochemistry
Decrease in enzyme function and specificity
Decrease in protein turnover
Accumulation of modified and inactive proteins
Change in membrane lipid composition
Increase in lipid peroxidation
Accumulation of nuclear and mitochondrial DNA mutations
Decreased telomere length
Characteristic gene expression changes

telomere length is determined by the genetic constitution. Telomeres form a loop, known as a T-loop, with a number of telomere-binding proteins. Telomerase activity is high in embryonic and somatic stem cells, as well as in tumors, while its expression is downregulated in somatic tissues. Telomere shortening is proportional to the biological age of the tissue. When the telomere reaches a critically short (2–6 kb) length, it gets uncapped and this is perceived by the cell as DNA damage. The DNA checkpoint response is activated (see chapter I. 8.) via the recruitment and autophosphorylation of the ATM (ataxia teleangiectasia mutated) kinase that, in turn, results in p53 activation and p21 upregulation, which halts the cell cycle at the G1/S restriction point. This is followed by the upregulation of p16 via a secondary, slower mechanism which (in combination with other factors) is responsible for the irreversibility of the process.

Besides replicative senescence, a variety of harmful stimuli can induce an irreversible growth arrest (reactive, *stress-induced or premature senescence*) including:

- DNA damage (ionizing radiation, oxidative stress)
- change in chromatin structure (change in histone acetylation)
- tumor suppressor activation
- transforming growth factor β activation
- hyperintensive growth signal and oncogenic activation (e.g. expression of constitutively active Ras).

Of the above conditions, oxidative stress induces senescence through accelerated telomere shortening, while other genotoxic stress factors, such as ionizing radiation-induced DNA double-strand breaks, also induce the DNA checkpoint response. The remainder of the inducers mediate non-genotoxic stress-induced senescence by activating the other cyclin-dependent kinase inhibitor, p16, that is specific for D-type cyclin complexes. p16 activation results in the hypophosphorylation of the retinoblastoma protein inhibiting the transduction of growth signals into cell division. Indeed, all inducers pose a threat of malignant transformation. It is proposed that cellular senescence serves to guard genetic stability and to prevent/counteract transformation (*Fig. 2*).

Thus, senescence is a side effect of intracellular anti-tumor surveillance, which presents after the reproductive period in the absence of selective pressure for its elimination – a manifestation of antagonistic pleiotropy at the cellular level. Intriguingly, stem cells expressing high level of telomerase show tendencies of malignant transformation; therefore, understanding

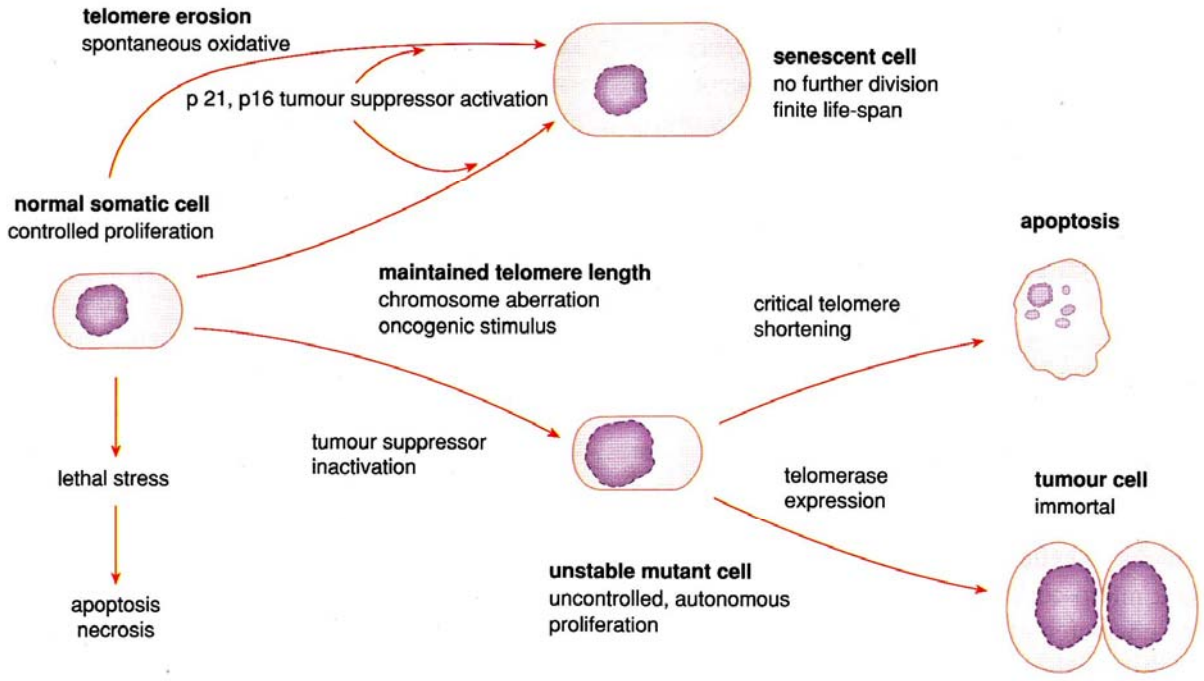

Figure 2. Relationship between cellular senescence and transformation.
A cell may senesce as a consequence of either critical telomere shortening (replicative senescence and oxidative stress) or a threat of oncogenic transformation, by activating the p21 and p16 tumor suppressors, respectively. In the case of tumor suppressor inactivation, critical telomere shortening induces apoptosis; occasionally some cells may undergo a crisis and up-regulate telomerase, resulting in an immortal tumor cell. Note that additional mutations are needed to display the hallmarks of invasive malignant tumors.

Table 3. Aging of replicating and postreplicative cells.
For the sake of simplicity, the following table compares the properties of a middle-aged replicating cell with that of a postreplicative cell

Property	Replicating cell	Postreplicative cell
Telomere length	Decreases	Constant
Number of nuclear mutations	Many, increases	Few, constant
Number of mutant mitochondria	Few	Many, increases
Inclusion bodies (lipofuscine)	Few	Many
Modified proteins	Few	Many, increases
Protein homeostatic buffer capacity	Weakening	Weak
Antioxidant capacity	High	Low

stem cell biology and telomerase activation may be a promising model to intervene into age-related pathologies and the aging of renewing tissues.

Organismal Aging

Cellular senescence may explain the aging of continuously renewing tissues (including the hematopoietic system, immune system, skin and mucous membranes); however, the replicative capacity of human dermal fibroblasts is not exhausted at the time of death. Some monogenically inherited diseases characterized by accelerated aging (premature aging, progerias, progeroid syndromes) are caused by mutations in the replication and repair machinery.

Werner syndrome

Etiology, Pathogenesis

Werner protein is a DNA helicase and exonuclease which has functions in base excision repair, recombination and in telomere maintenance. Loss-of-function mutations of the gene impairs DNA replication (S-phase arrest) and DNA repair. Characteristic biochemical signs are diminished replicative lifespan (less than 20 divisions), increased mutation frequency and hypersensitivity to DNA damaging agents (UV irradiation, alkylating agents).

Clinical Presentation

Werner syndrome presents as the most classical aging phenotype: premature aging, graying hair, hair loss, cataracts, atrophy of the skin, subcutaneous tissue, thymus and muscles. Complications include atherosclerosis, type 2 diabetes mellitus and malignant tumors. Most patients die before the age of 50 due to heart attack or malignancies.

Other segmental progerias are termed as such because they present with many but not all signs and symptoms of aging. These include various mutations affecting the DNA damage response or repair enzymes (Cockayne syndrome, Bloom syndrome, xeroderma pigmentosum, ataxia teleangiectasia), the dominant negative mutant of lamin A involved in the organization of nuclear structure (Hutchinson-Gilford progeria), or chromosomal aberration without known mutation (Down syndrome). Beradinelli-Seip syndrome is caused by mutations impairing the formation of adipose tissue (1-acylglycerol-3-phosphate-O-acyltransferase). It is characterized by a complete lack of fat, severe insulin resistance and diabetes, malignant hypertension, cardiomyopathy and cardiovascular mortality. (The connection between insulin signaling and aging is discussed later.)

The majority of the body is composed of non-dividing, differentiated post-mitotic cells and intercellular (extracellular) matrix connective tissue. Post-mitotic cells include those mostly involved in degenerative diseases, such as neurons and myocytes. There is a great deal of difference between the aging of replicating and post-mitotic cells (*Table 3*).

With respect to the entire body, an increase in macromolecular damage is observable with advancing age, and there is a general dysregulation of various bodily processes. The fidelity and intensity of biochemical pathways is compromised; energy production, transport processes and compartmentalization are impaired; while flexibility and the maximal response of repair mechanisms decline. Cellular disturbances compromise tissue functions (the pancreas is unable to provide the digestive output, fibroblasts are unable to produce connective tissue components), and this impairs the function of the organism.

9.4. Macromolecular Gerontology

An aged cell contains several types of modifications and molecular damage involving all kinds of macromolecular entities. Modifications are mainly induced by reactive oxygen species (ROS, see chapter I. 3.2.), while nitration (peroxynitrite), reduction, alkylation and non-ezymatic glycation also play important roles.

Nucleic Acid Damage – Mutations

Both DNA and RNA damage can be detected during aging. RNA is devoid of protection provided by a nucleosomal structure. However, in order to avoid erroneous protein translation, there exists an RNA repair system, with specialized components such as oxidative demethylase, that specializes in RNA methylation.

DNA telomere damage, strand breaks and base mutations are repaired by classical DNA repair mechanisms. As we have seen, mutation of genes involved in repair induce the accumulation of mutations, increased carcinogenesis and characteristic segmental progerias, thus highlighting the role of DNA mutations in cancer and aging, two sides of the same coin. 8-Oxoguanin formation is the most frequent oxidative base modification that displays an age-dependent increase. 8-Oxoguanin forms perfect H-bonds with adenine, but forms mismatches with its classical partner, cytosine. As a result, the proofreading activity of DNA polymerases integrates a stable mutation, a G-T transversion, into the genome. Indeed, G-T transversion is one of the most prominent mutations in human tumors, offering a plausible example of the connection between aging and increased carcinogenesis.

Protein Aging – Aggregation

A great variety of pathways induce protein damage, considering the 20 amino acids, and the non-protein constituents of proteins (like sugars, lipids, metals, and especially redox-active iron). Both the polypeptide backbone and the amino acid side-chain residues are prone to modification. Sulfur-containing residues are the most sensitive; however, nearly all residues can be oxidized (*Table 4*). Protein carbonyl level is the most reliable aging marker. Nonetheless, its increase with advancing age is quite modest, since a cell from an 80-year old man harbors only twice as many carbonyls as that of a young

Table 4. Oxidative amino acid modifications

Amino acid	Representative derivative
cysteine	cysteine-disulfide, cysteic acid
methionin	methionine-sulfoxide, sulfone
triptophane	hydroxy-derivatives, kynurenine
phenylalanine	hydroxy-derivatives
tyrosine	hydroxy-derivatives, nitrotyrosine, Tyr-O-Tyr
histidine	2-oxohistidine, asparagine, aspartate
arginine	γ-glutamic semialdehyde
lysine	α-aminoadipate semialdehyde
proline	2-pyrrolidone, γ-glutamic semialdehyde
threonine	2-amino-3-ketobutyric acid
glutamate	oxalate, pyruvate

donor. Furthermore, the increase is not specific to aging since similar modifications may be observed in conditions of increased oxidative stress. Carbonyl groups may be formed through the oxidation of the polypeptide backbone, glutamate, lysine, arginine and proline, as well as in reactions with aldehydes (originating from lipid peroxidation and glycoxidation). Protein modifications may lead to the breaking of the backbone, crosslinks, loss of activity, denaturation, and aggregation.

Protein homeostasis is achieved through highly sophisticated mechanisms: damaged side chains may be repaired, misfolded conformation may be stabilized, while irreparable proteins either undergo degradation or are sequestered in inclusion bodies when degradation is inadequate *(Fig. 3)*.

Methionine sulfoxide, a product of oxidation, is reduced back to methionine by the enzyme methionine sulfoxide reductase. Knocking out this enzyme shortens life-span, showing its significance in protein homeostasis. A conformational repair of the whole polypeptide chain is performed by molecular chaperones or heat-shock proteins that are induced by proteotoxic stress. Chaperones recognize hydrophobic patches or the naked backbone of denaturing proteins and stabilize protein structure, prevent incorrect interactions and refold them into their native state. However, oxidatively modified proteins cannot be refolded; therefore, the role of trapped chaperones is confined to preventing aggregation and to directing the proteins for disposal. Protein degradation is achieved by the cytosolic ubiquitin-proteasomal system, as well as by lysosomal autophagy, having these both distinct and overlapping functions in protein turnover. The robustness of both the heat-shock response and protein turnover

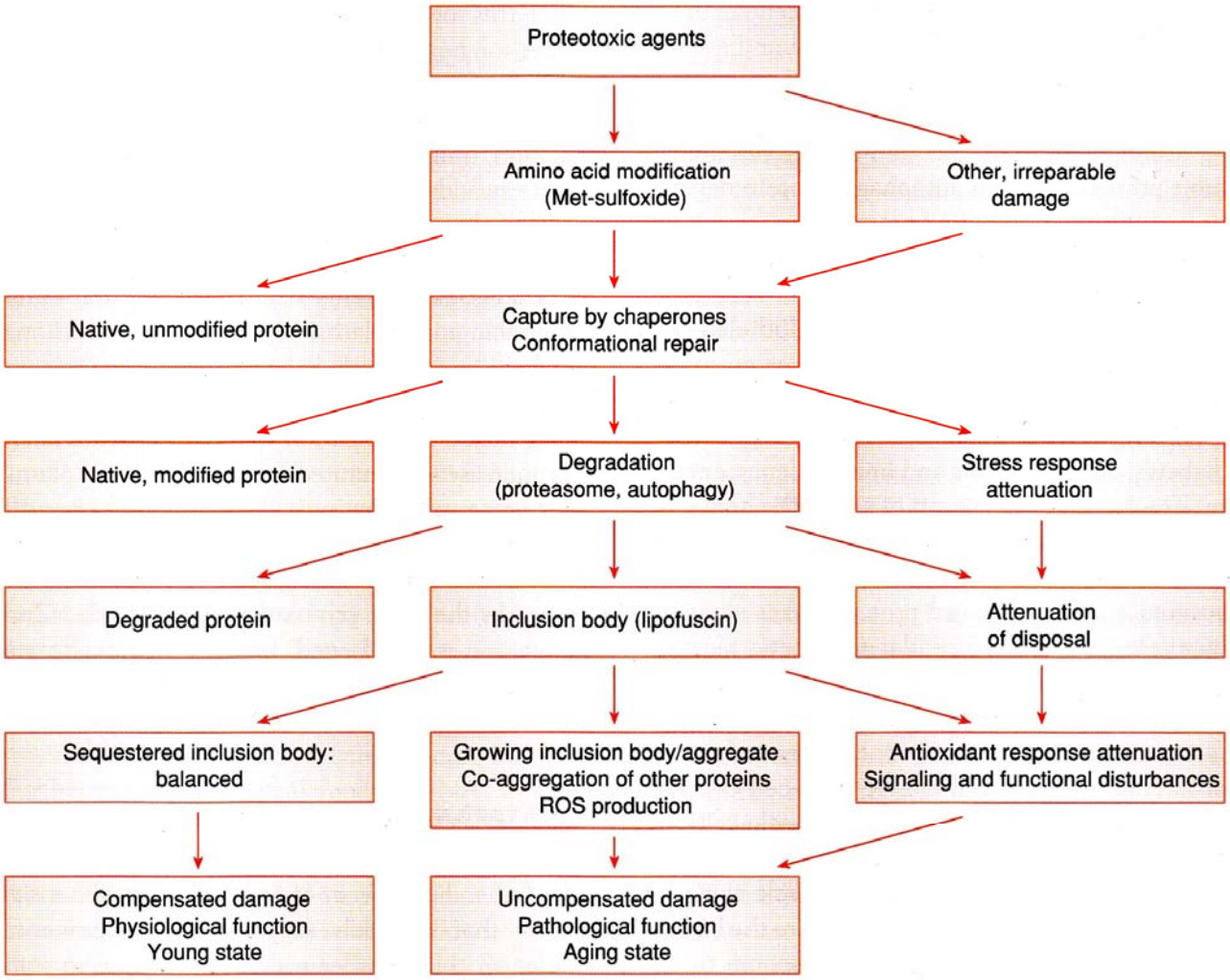

Figure 3. *Protein damage – protein homeostasis and aging.*
The left part of the figure outlines the outcome of successful defense; the center part outlines the complications of defense failure; the right side outlines the overload of defense mechanisms. For the sake of simplicity, interactions with other kinds of macromolecular damage (DNA damage, lipid peroxidation) and with cellular functions (energy production, transport, signaling) have been omitted.

is a major determinant of longevity as rhese shows rapid deterioration during aging.

Proteins that cannot be be degraded form aggregates. These aggregates are separated in special subcellular compartments called inclusion bodies. Aggregates and inclusion bodies are characteristic of a number of degenerative disorders, such as neurological diseases (Alzheimer's and Parkinson's disease) andalcohol-induced liver disease (Mallory body), but they may also occur in a systemic manner (e.g. lipofuscin, a morphological marker of aging postmitotic tissues). Aggregates were previously considered to be the major dangerous element in conformational diseases (pathologies arising from a mutant conformation). Nowadays, they are regarded as the result of a compensatory response against toxic misfolded mono-oligomeric species. Unfortunately, important proteins such as transcription factors and cell structure proteins may be trapped in aggregates and may be depleted; this may result in severe cellular disturbances in signaling, protein expression and motility. additionally, inclusion bodies may also recruit iron and give rise to ROS formation.

Modified proteins also trap chaperones and inhibit proteasomal and autophagic protein degradation, resulting in impaired protein homeostasis and a vicious circle. Glycated proteins are oxidized (glycoxidation) and form *advanced glycation end products* (AGEs). AGEs have receptors on endothelial cells, macrophages and microglia that induce an inflammatory response, contributing to the pro-inflammatory state, a major condition in aging implicated in diabetes, atherosclerosis and immunosenescence.

The longer the life-span of a cell, the higher the contribution of damaged proteins to aging. A special example is neurodegeneration where a lifelong accumulation of damaged proteins takes place and manifests in a picture similar to genetic neurodegenerative diseases (cf. senile dementia and Alzheimer's disease). Another complication of aging is sarcopenia, which is partly due to the exhaustion of satellite cells, special muscle stem cell compartments and the subsequent loss of muscle cells and collagen synthesis accompanied by the rigidification of connective tissue. Accelerated muscle aging can be observed in top-level athletes, where the biological age of a 20-year old subject corresponds to that of a 60-year old fit person, highlighting the importance of moderate exercise.

Aging of the extracellular matrix is determined by its turnover and, in turn, by fibroblast function. Aging fibroblasts divide less and, partly due to HIF1α transcription factor activation, produce increasing amounts of collagen. Connective tissue remodeling is impaired since the expression of matrix metalloproteases is diminished. The net result is the increment of connective tissue and fibers. Fiber proteins are characterized by long half-lives (some of them are as old as we are) and are therefore subject to modifications, crosslinks, and secondary calcification, manifesting in cellulitis, musculoskeletal problems, atherosclerosis, capillary dysfunction and neuropathy. In particular B-crystallin modification leads to the development of cataracts. These processes are much more prominent and malignant in diabetes, suggesting the critical role of metabolism and glycation in (protein) aging.

Membrane Damage – Lipid Peroxidation

Since this topic is summarized in chapter I. 3.2, the discussion here is limited to the major consequences related to aging. Malondialdehyde and 4-hydroxynonenal, two end products of the oxidative chain reaction, transmit the reaction to the watery phase, and form adducts with, and induce damage in, other macromolecules. A special phospholipase repairs oxidized lipids by replacing modified unsaturated fatty acids. Beyond this mechanism and that of hydrophobic antioxidants, like vitamin E, additional mechanisms are likely to exist that preserve membrane integrity.

A major complication of lipid peroxidation is the increase in saturated fatty acids of the plasma membrane, resulting in rigidification. Mitochondrial membrane cardiolipin is significantly decreased, and the antioxidant coenzyme Q10 is gradually displaced by the weak pro-oxidant Q9. Mitochondrial membrane is depolarized, impairing oxidative respiration and sensitizing mitochondria toward apoptotic stimuli.

Oxidative Stress in Aging

The free radical theory of aging is a comprehensive theory that is widely supported by experimental evidence; thus, we deemed it appropriate to summarize it at this point. It has been well documented that (1) reactive oxygen species and the amount of oxidized macromolecular entities increase with increasing age (2) lifespan extending (genetic and environmental) interventions decrease both oxidative stress and oxidative damage; however, the

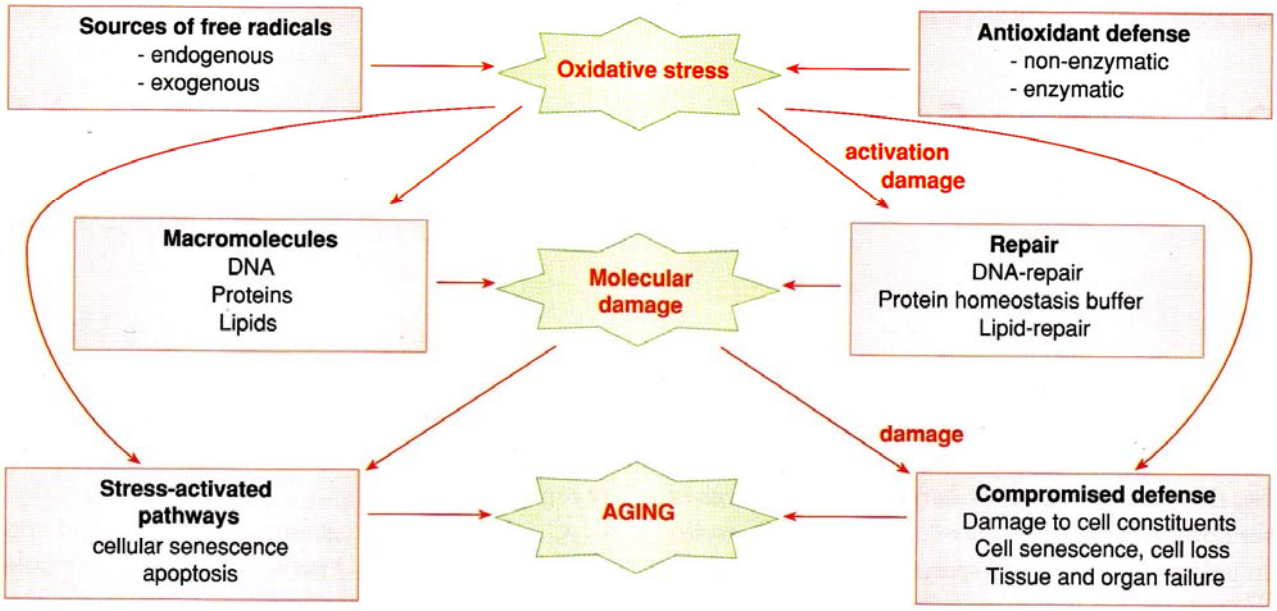

Figure 4. *The free radical theory of aging.*
The rate of aging is determined by the interaction between free radicals and antioxidant defense mechanisms; both are under genetic and environmental control.

most important premise of the theory, i.e. (3) that increasing the oxidative stress increases life-span, while decreasing it decreases life-span, has not be unambiguously demonstrated. Recent observations have challenged the validity of the theory, especially in invertebrate model organisms, and instead of emphasizing damage, has led to the ingreasing appreciation of ros-induced dysregulation in signaling networks. Certainly, free radicals play a central role in aging.

The extent of oxidative stress is an outcome of the interplay between reactive oxygen, nitrogen species and antioxidative capacity (*Fig. 4*). The most prominent endogenous and systemic source of free radicals is mitochondrial respiration (complex I and III). Approximately 0.1 to 1% of respiratory oxygen gives rise to the superoxide anion. Therefore, relative oxygen consumption is considered to be inversely proportional to species-specific life-span. Furthermore, peroxisomal function, NADPH-oxidases, cytochrome P450 enzymes and lipoxygenases contribute to endogenous free radical production, especially in the liver and in the immune system. Exogenous factors include ionizing radiation, UV-light, nutrition, environmental toxins, drugs and microbial inflammatory mediators. The antioxidant defense system is composed of non-enzymatic antioxidants and antioxidative enzymes (see chapter I. 3.2. for details).

The free radical theory fits well with the mitochondrial theory of aging. Metal- and sulfur-containing mitochondrial proteins, such as the components of the respiratory chain or aconitase from the Krebs cycle, are the proteins that are most sensitive to oxidative stress. Moreover, their genes are encoded in the mitochondrial genome, which is placed close to the source of free radicals and has a much less developed nucleosomal structure and set of repair mechanisms than the nuclear genome. Therefore, a vicious circle develops composed of oxidative stress, leading to damaged and mutant mitochondria that, in turn, lead to increasing oxidative stress: all in all a mitochondrial error catastrophe.

During aging there is an increased oxidant load (worsening mitochondrial metabolism, inflammations), while antioxidant defense declines (vitamin deficiencies, enzyme expression and inducibility decreases, and NADPH level decreases). Consequently, oxidative stress increases and creates a wider vicious circle.

9.5. Geronto-Genetics

The first genes affecting life-span were isolated from the 1 mm nematode *Caenorhabditis elegans*, followed by the fruit fly *Drosophila melanogaster*. Loss-of-function mutation of these "gerontogenes" resulted in a two to five-fold life-span extension. Long-lived animals generally display increased stress resistance and decreased fertility, supporting the evolutionary prediction that allocation of limited resources for self-maintenance is only achievable at the expense of reproduction.

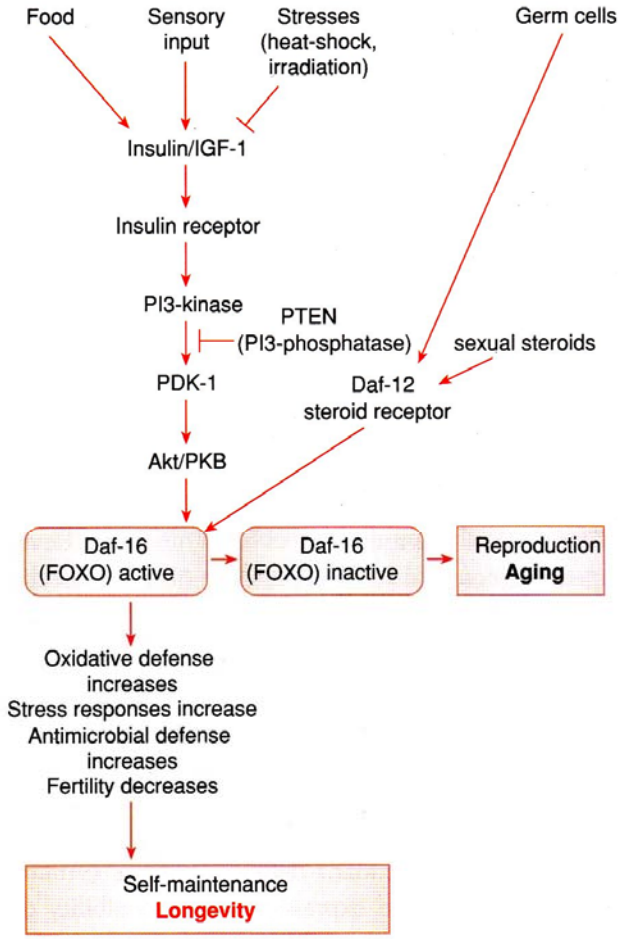

Figure 5. *Role of the insulin-like signaling pathway in aging and longevity in C. elegans.*
Environmental effects regulate the activity of Daf-16 (FOXO) via the insulin-like signaling pathway. Daf-16 controls the allocation of resources between self-maintenance and reproduction. Major elements of the pathway are conserved in humans.

C. *elegans* gerontogenes can be classified into three major groups: (1) genes influencing metabolic flux and free radical production; (2) genes ensuring the coordinated movement of the gut, the lack of which results in diminished caloric consumption; and (3) genes encoding members of the insulin/IGF-1 pathway that signals nutrient abundance (*Fig. 5*). The IGF-1 pathway operates via sensory neuronal stimuli in the worm and regulates the activity of the forkhead transcription factor (FOXO) via phosphorylation by Akt/PKB kinase. This, in turn, inhibits the nuclear translocation and transactivation of FOXO. Mutations in insulin signaling, ablation of germinal cells and sensory neurons activate FOXO, leading to transactivation and an expression change affecting more than 300 genes. Upregulated genes include antioxidant enzymes (superoxide dismutase), metallothioneins, DNA repair enzymes, metabolic enzymes, antimicrobial polypeptides and heat shock proteins. Genes involved in reproduction are generally downregulated. It seems that FOXO controls a switch between a systemic program of self-maintenance and reproduction. Manipulation of individual FOXO target genes results in a slight change in life-span, while the simultaneous ablation of germinative cells and the insulin pathway results in a sixfold increase in life-span. This emphasizes the polygenetic and systemic nature of cellular defense mechanisms, and the intricate connection between stress resistance, metabolism and longevity. The role of insulin signaling in aging has also been recapitulated in *Drosophila* and mice lacking the insulin receptor in adipose tissue.

As a summary, a change in environmental conditions regulates the allocation of resources between self-maintenance/survival and reproduction/aging. This is mediated by the neuroendocrine axis of sensory neurons and utilizes the insulin signaling pathway that determines cellular defense to stress.

9.6. Environment and Aging

Hormesis

Hormesis is a phenomenon in which organisms exposed to certain kinds of mild stress (oxidants, heat stress, heavy metals, etc.) acquire stress tolerance to subsequent exposure. There have been numerous observations about cross-tolerance, i.e. tolerance to additional or multiple stressors, suggesting the involvement of specific and general components of the stress response. Hormesis results in life-span extension in peripheral cells and laboratory *C. elegans* and *Drosophila* strains, and reinforces the link between the genetic control of stress resistance and longevity. Mammalian studies on hormesis are lacking as of yet.

Caloric Restriction

Caloric restriction is the single most robust, universal environmental intervention in mammals that has been shown to extend life-span. There is an inverse relationship between energy restriction and lifespan: while ensuring normal intake of essential nutrients (amino acids, fatty acids and vitamins), a 50% reduction in food consumption consistently extends life-span by as much as 50% percent in various invertebrate and vertebrate species from yeasts to rodents. Three longitudinal studies were initiated in 1987 in rhesus monkeys in the United States. Calorically-restricted monkeys possess excellent health status, smaller body size, and lower body temperature, higher activity and much better glucoregulatory profiles. Furthermore, one of the studies seemed to prove that caloric restriction indeed increases lifespan in primates.

Major effects of caloric restriction are depicted in *Table 5*. Both increased stress resistance and diminished reproduction reinforces the predictions of evolutionary theories and the results of genetic manipulation. The major mechanism of action may be decreased oxidative stress (a more economic and efficient mitochondrial respiration with normal acceptor control in the lack of excess ATP). This is achieved via maintained oxygen consumption and increased physical (food searching) activity. Decreased oxidative stress results in the restoration of the reductive intracellular milieu that favors the quiescent state of major redox-dependent transcription factors such as HIF-1α and NFκB. Another important feature is the downregulation of insulin signaling and the activation of FOXO, which initiates a self-maintenance program.

One of the prime targets of caloric restriction seems to be the silent information regulator sir-

Table 5 Effects of caloric restriction

Fenomenology
Lower blood pressure
Smaller body size and weight
Diminished body fat content, relatively maintained muscle mass
Maintained circadian rhythm of hormones
Increased physical activity
Decreased fertility
Metabolism
Decreased IGF-1, insulin, T3, growth hormone levels
Improved hormone and insulin sensitivity
Decreased fasting plasma glucose and lipid levels
Improved mitochondrial energy production
Decreased free radical production
Function
Improved brain, heart and muscle function
Increased immune system efficiency
Decreased inflammatory response
Increased phase II reactions of drug detoxification
Increased capacity of protein homeostatic buffer
Increased antioxidative capacity
Increased DNA repair
Diminished macromolecular damage
Delayed cellular senescence and tissue loss

tuin gene family member Sir2. It deacetylates and modulates the activity of histones and nucleosomal structure, as well as several key signaling molecules implicated in aging, such as p53, NFκB and PPARγ. Increased gene dosage of Sir2 induces longevity in invertebrate models and recapitulates the effect of caloric restriction, while Sir2 knock-out mice age prematurely. Sir2 is a NAD^+-dependent deacetylase activated by caloric restriction.

Resveratrol, a polyphenolic compound from red wine and a possible mediator of the positive cardiovascular effect known as the "French paradox", is the first natural compound that has been recognized to promote longevity in several organisms, including mice overfed through high caloric intake. Resveratrol acts on a number of targets including Sir2 and induces a lifespan extension without adversely affecting reproduction. Ongoing research targets resveratrol and other plant compounds that show promise of being a tremendous benefit for human health.

Human Aging: Centenarians

Invertebrates are almost completely post-mitotic; their development is under strict genetic control. Vertebrates possess a reserve capacity (stem cells), as well as stronger epigenetic regulation, in addition to a highly organized central nervous system (the brain). The neocortex and the neuroendocrine axes provide much greater plasticity, more instant and more efficient adaptation and consequently a longer life-span.

It seems that genetic factors account for as little as 25% of life-span expectancy, with the rest dependent on the environment and chance. Even genetically fully identical yeast and *C. elegans* strains kept under the same experimental conditions display a 3–10-fold difference in lifespan. Individual life history with stochastic events increases cell-to-cell variation and heterogeneity of the organism with advancing age, and this is especially true in such long-lived species as *Homo sapiens*. Therefore, human life span cannot be predicted.

Interaction of genes and the environment, however, remains an intriguing subject that can be directly investigated in human studies. Centenarians reach an age that is beyond mere statistical chance, and who possess special genetic constitutions and/or a special environmental milieu allowing the prolongation of their lives. International studies of centenarians show that their general health, metabolism, immunity and stress resistance are better than those of the average aged population. They harbor various mild genetic variants occurring in degenerative diseases, suggesting that they induce a mild hormetic stress response and an increased defense capacity resulting in exceptional longevity.

However, recent evidence has challenged both the longevity promoting effects of Sir2 overexpression and its role in caloric restriction in *C. elegans* and *Drosophila*, yet a beneficial effect of Sir2 activation in mammals remains plausible.

9.7. Age-Related Diseases – Diseases of Aging (Degenerative, Civilization Diseases)

This disease group includes age-related pathologies and chronic disorders originating from the disequilibrium between regeneration and tissue (cell) loss, with impaired adaptation. The most important diseases are listed in Table 6 and are discussed in other chapters. Here, we will only discuss the aging of the immune system, that is immunosenescence.

The aging immune system displays a decline in specific and effective immune responses. The number of memory T-cells decreases, accompanied by a clonal expansion of cytotoxic T-cells. This is complemented by a general activation of non-specific immunity (inflamm-aging) characterized by an increased production of pro-inflammatory cytokines (IL-6, IFNγ andTNFα) and a decreased output of anti-inflammatory cytokines (IL-2, IL-3 and IL-10). This leads to recurrent and opportunistic infections (e.g. influenza, herpes zoster, CMV infection, mycoses), increased autoimmunity, a higher frequency of various diseases (e.g. atherosclerosis) and a high incidence of tumors.

Table 6. Age-related pathology and diseases

Immune system
General decline of immunity: infections, tumors
Increased pro-inflammatory response: autoimmunity
Endocrinology
Menopause, andropause
Hypothyroidism
Benign prostate hyperplasia
Musculoskeletal system
Muscle weakness – sarcopenia
Osteoarthritis, joint and ligament rigidity
Osteoporosis
Metabolism
Metabolic X-syndrome, diabetes
Hyperuricemia
Cardiovascular system
Atherosclerosis
Cardiomyopathy
Extracellular matrix, connective tissue
Presbyopy, cataract
Diverticular disease
Incontinence
Chronic obstructive pulmonary disease
Digestive tract
Hypochlorhydria
Malabsorption syndrome
Leaky gut syndrome, dysbiosis
Neural system
Neuropathy
Neurodegeneration (Alzheimer's, Parkinson's, vascular dementia)
Malignant tumors

MEDICAL PATHOBIOCHEMISTRY

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The authors would like to thank the contribution of
Miklós Csala

Medicina Publishing House Co.• Budapest, 2014