

## REVIEW

## Cellular and Molecular Mechanisms of Aging and Age Related Diseases

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**This review on aging is focused on those cellular and molecular mechanisms which concern age related pathologies. The central question addressed is the relationship between "normal" aging and age-related pathologies such as osteoarthritis, cardiovascular diseases, emphysema, malignant tumors and cognitive decline, dementias. The mechanisms recognized as most important in cell and tissue aging are briefly outlined. Emphasis is laid on the impor-**

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**tance of post-synthetic modifications of the macromolecules of the extracellular matrix and on cell matrix interactions. Loss of intercellular communication and cell-matrix interactions as a result of receptor decay and receptor uncoupling were recently recognized as key events. Unavoidable poly-pathology at advanced age may be the answer to the above question.** (Pathology Oncology Research Vol 6, No 1, 3-9, 2000)

### Introduction

The phenomenon of aging was observed by man in his own species as well as in household animals all through antiquity as witnessed by ancient texts. Although literary and philosophical texts clearly document the interest shown by a number of intellectuals over the ages to this all too general time dependent manifestation of human and animal nature, its scientific study is quite recent. Apparently the first serious experimental approach was that of Fritz Verzar,<sup>66</sup> a Hungarian scientist working in Basel, in the middle of the 20<sup>th</sup> century. About ten years later the cell culture experiments of Hayflick and Moorhead,<sup>21</sup> started the era of cell aging in vitro. Human aging and related pathologies were systematically studied by a Hungarian pathologist, Laszlo Haranghy<sup>20</sup> who published the first monograph (to my best knowledge) on the health status of centenarians.

Since these pioneering experiments of the nineteen-fifties and sixties there has been an exponential explosion of experimental work on aging biology and pathology.

The many thousands of publications which have appeared in this rapidly growing field can be divided according to the level at which the processes of aging were studied (*Table 1*). A number of books are available, addressing some or several aspects of aging (see for references<sup>50,51,52</sup>). The scope of this review will address only one crucial question, still unsolved: is pathology-free aging possible or is aging intrinsically accompanied by age-related diseases? Can we humans age and die of old age or do we have to consider age-associated pathologies as causally, mechanistically related to the aging process. This question arose as a result of the spectacular increase of the life expectancy of men and women in most countries during the last few decades, developed or developing (at the end of this century only some parts of the former soviet Union present a decrease of average life expectancy, mainly for socio-economic reasons). The answer to this question has several important theoretical and practical consequences which will be mentioned in the Discussion.

### *Mechanisms of aging, from the whole organism to its cellular and molecular components*

To discuss the mechanisms underlying aging it would be helpful to have a scientific definition of this process. Curiously there is no generally accepted definition of aging.

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**Table 1. Levels of aging studies**

Populations	→	Epidemiology
Individuals	→	Geriatrics
Organs	→	Pathology
Tissues	→	Cell-matrix interactions
		Cell biology + matrix biology
Cells	→	Cell biology, molecular biology
DNA	→	Genes involved in aging and age-associated pathologies
Macromolecules	→	Biochemistry, physical chemistry modifications of structure – function relationship

For practical (and teaching) purposes we proposed the following definition: aging is the result of the progressive and irreversible decline of the capacity of an organism to adapt to its ever-changing environment. Its multiple causes are all progressive, irreversible and harmful.<sup>50,51,52</sup>

The mechanisms involved are partially intrinsic to the organism, genetic and epigenetic, and partially of external origin: nutrition, radiation, temperature and others (*Table 2*). One of the results of these multiple causes and mechanisms of aging is the observation that all those physiological functions which can be measured with a relatively good precision decline at highly variable speeds (*Table 3*). Some functions decline fast, others quite slowly. The highly simplified representation of this process, as shown on *Table 3*, suggests an increasing imbalance at the level of the whole organism between the slowly and more rapidly declining functions. This process of selective aging (in French: “en pièces détachées”) is similar to what is

**Table 2. Mechanisms and „causes“ involved in aging**

<i>Intrinsic</i>	<i>Extrinsic</i>
Genetic	Nutrition
Epigenetic	Radiation
Strong interactions between genetic and epigenetic factors	Stress
Genes coding for free radical scavenging often increases life expectancy	Caloric restriction increases life expectancy in rodents
Epigenetic mechanisms: Maillard reaction	High sugar intake accelerates epigenetic aging mechanisms
Free radical damage	High saturated lipid intake accelerates vascular ageing
Harmful effect of matrix – degeneration products	
Loss of receptors	

observed with, for instance automobiles where the selective “loss” of only one “function” can stop the whole engine. In order to understand this peacemeal aging we have to examine the underlying mechanisms at the cellular and molecular levels.

### **Cellular aging**

The crucial observation of Hayflick and Moorehead<sup>21</sup> was that cells in culture, kept in standardized conditions, can undergo only a limited number of population doublings. A negative correlation was found between the maximal number of population doublings and the age of the donor of skin fibroblasts (see<sup>35</sup> for a review). This is now attributed to a mechanical process, the shortening of telomeres at every round of cell division because of the stretch occupied by DNA-polymerase on the DNA at the start of its action.<sup>7</sup> Telomerase, the enzyme capable of

**Table 3. Age dependent decline of biological functions (from fastest to slowest decline)**

	<i>Approximate of complete loss</i>
Elasticity of lens capsule	90 years
Glycosaminoglycans in cartilage	95
Vital (respiratory) capacity	103
Distensibility (elasticity) of aorta	105
Proliferation capacity of fibroblasts	110
Loss of memory	125
Collagen content of skin	140
Nervous conduction velocity	# 400

resynthesising the telomeres on each end of chromosomes is repressed in most somatic cells but reexpressed in the large majority of transformed, malignant cells. The transfection of a cDNA coding for the active site of telomerase in some normal cell lines enabled them to accomplish a significantly increased number of population doublings.<sup>6</sup> This is for the moment one of the most active fields of research on the genetic mechanisms of aging and also of carcinogenesis. The loss of telomeres was shown to be more rapid in lymphocytes isolated from the blood of trisomic patients in accord with their more rapid aging.<sup>65</sup> Contrary to general belief, cells at the end of their replicative life span do not die. Their typical morphology, enlarged cell-size, vacuolization, loss of mitochondrial integrity etc. were well characterized.<sup>35</sup> These cells are unable to divide but could be kept for long periods in culture. Such “old” cells, unable to divide were exceptionally observed in vivo, for instance by the team of Russel Ross as smooth muscle cells on the top of proliferative atherosclerotic lesions.<sup>19,43</sup>

Most cells of the organism do not exhaust their division capacity during maximal human life expectancy (about

100 years  $\pm$  20). Besides the keratinocytes of the epidermis and the epithelial cells of the mucosal layers of the gastrointestinal tube or the bone marrow cells, which are in constant mitotic activity, all other cell-types of the organism do not divide regularly after they have reached their mature phenotype (for a review see<sup>52</sup>). Even if they do so, this occurs only exceptionally. Even fibroblasts, maintained in a collagen gel in vitro exhibit a strongly reduced division rate. Post-mitotic cells, such as the neurons, do not divide anymore. Stem cells can be demonstrated in several tissues as striated muscle, bone marrow or even the brain. They keep the possibility of further divisions and differentiation although this does not invalidate the "Hayflick-limit" as discussed above. Only neoplastic transformation enables mitotic cells to escape this limit for reasons quite well understood but beyond the limit of this review. It appears therefore that loss of differentiated cell function more than loss of proliferative capacity is the crucial phenomenon in aging.

#### *Aging of the extracellular matrix*

Postsynthetic molecular aging was first described by Verzar<sup>66</sup> on the rat tail tendon. He measured the mechanical force of thermal retraction of these collagen fibers and found an exponential increase with age (*Figure 4*). This relationship between resistance to heat denaturation and age of collagen fibers was verified from frogs to men. This was attributed by Verzar to an age-dependent increase of cross-linking of collagen. When the natural cross-links were identified<sup>2</sup> it appeared that none of the Schiff base- or aldol-condensation products of lysine or hydroxylysines (the "normal" crosslinks) could account for the Verzar phenomenon. There is now a general agreement to attribute this age-dependent increase of collagen cross-links to the Maillard reaction (for a review see<sup>27</sup>), the progressive non enzymatic glycanation of amino groups on proteins,  $\epsilon$  amines of lysine in particular, followed by the formation of polycyclic, aromatic compounds designated as "advanced glycosylation end products" or AGE. Some of these AGE-products can release free radicals and participate in several epigenetic mechanisms of aging. Improved methodology soon revealed the generality of this non-enzymatic glycanation process. Even short lived macromolecules as for instance fibronectin (half life of the order of 24 hrs) were found to be glycanated in vivo. As this is an organic, bimolecular reaction it could be studied in vitro, with glucose as well as other reducing compounds of biological interest. The speed of this glycanation increases with the concentration of reactants and thus advances much faster in hyperglycemic diabetics. This is one of the mechanisms involved in diabetic micro- and macroangiopathy. Glycanated proteins have altered biological properties. Even

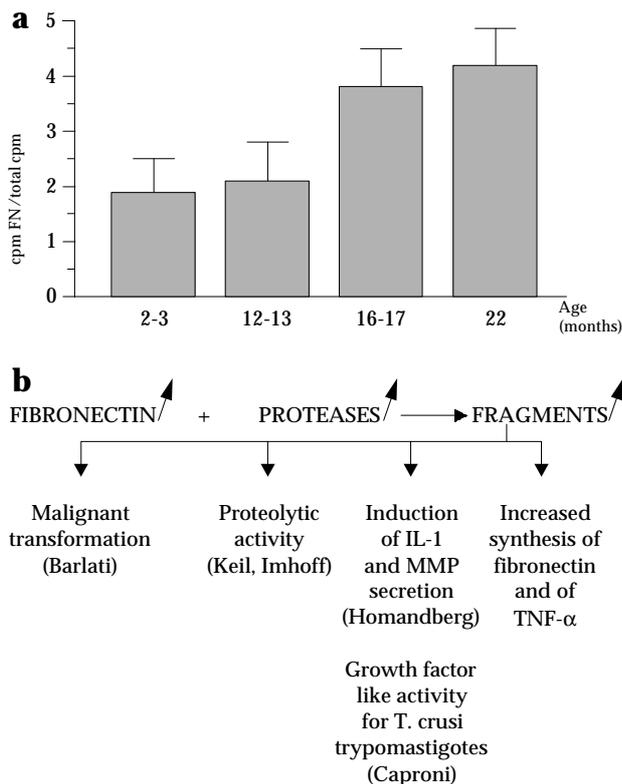
normoglycemic aged persons present advanced glycanation of collagen fibers which become resistant to collagenase, and excluded from the pool of renewable proteins with measurable turnover.

Besides collagen (19 different types were described only in vertebrates), elastin is another fibrous protein of extracellular matrix (ECM). Its post-synthetic aging mechanism was intensively studied in our laboratory over the last decades.<sup>45,46,50</sup> These processes can be summarized as follows : increasing fixation of calcium due to its perfect fit in the  $\beta$ -turns of the elastic fiber,<sup>60</sup> accompanied by lipid deposition. Calcium potentiates cholesterol deposition as shown by in vitro experiments.<sup>28</sup> Calcium and lipid saturated fibers loose their elasticity and are increasingly degraded by elastase-type endopeptidases. Such enzymes were demonstrated in the human vessel wall<sup>44,55</sup> and were shown to be produced in an age-dependent increasing manner by smooth muscle cells and fibroblasts. Atherosclerosis was shown to be an independent increasing factor of vascular elastase production.<sup>24</sup> The result is the production of elastin degradation products, elastin peptides which can be detected and quantified in the circulating blood.<sup>5,15</sup> We shall come back to this process in the section devoted to receptors and aging.

The two other major families of matrix macromolecules, proteoglycans and structural glycoproteins also exhibit age-dependent variations, both at the synthetic and post-synthetic levels. The rate of biosynthesis of matrix macromolecules changes with age. Some decline, others increase. The biosynthesis of hyaluronan, a glycosaminoglycan, was shown to decrease with in vitro aging of human skin fibroblasts.<sup>12</sup> Post synthetic modifications of proteoglycans and glycosaminoglycans consist of both enzymatic and non enzymatic (free radical-mediated) degradation. This process is directly involved in the development of osteoarthritis.

Fibronectin, the most abundant structural glycoprotein is increasingly synthesized by in vivo and in vitro aging cells as was demonstrated by J. Labat-Robert et al.<sup>30,31</sup> As fibronectin is very sensitive to proteolytic degradation, its fragmentation is also increasing with age as well as in some pathologies where its contact with proteases and especially elastases is increased as in cystic fibrosis of the pancreas.<sup>1</sup> *Figure 1* shows schematically the potential harmful effects of fibronectin degradation products. This is a second example of a self-amplifying vicious circle generated by a cascade of epigenetic mechanisms. The first of such examples was the non-enzymatic glycanation, the Verzar phenomenon, discussed above. A third epigenetic aging mechanisms is represented by free radical generation during metabolic processes (for review see<sup>9</sup>). Although scavenging systems are present in cells and tissues, their efficiency is far from 100 % and was shown to decrease with age.

Another important discovery in the field of molecular aging was reported by Gershon and Gershon.<sup>18</sup> These authors described the presence in aged *Cenorhabditis elegans*, a nematode, of enzymatically inactive molecules in a purified sample of citrate lyase. The concomitant determination of enzymatic activity and of enzyme-molecular concentration (by immunological methods) revealed the generality of this phenomenon: aged cells and organisms contain inactive (or less active) enzyme molecules as shown also by their increased rate of heat denaturation (for a review see<sup>36</sup>). The first explanations concentrated on a hypothetical erroneous protein synthesizing machinery in aged cells or organisms. This tendency culminated in Orgel's Error Catastrophe Theory.<sup>37</sup> It was however soon demonstrated that there was no loss of precision of protein synthesis with aging but a slowdown of turnover. This produced longer lived proteins, undergoing post-synthetic modifications, most of them being deleterious for their biological activity. These results produced a disinterest in molecular aging, mainly for psychological reasons. Post-synthetic molecular aging remains however an important mechanism of tissue and organism aging.



**Figure 1.** a – Increase with age of the biosynthesis of fibronectin by mouse skin fibroblasts demonstrated by incorporation of <sup>35</sup>S-labeled methionine in immunoprecipitable fibronectin (Labat-Robert et al., 1981, 1992). b – Harmful effects of fibronectin degradation products. (From Barlati et al. [1986], Keil-Dlouha et al., [1986], Homandberg et al., [1992,1994], Lopez-Armada et al., [1997])

### Cell matrix interactions

Aging at the tissue and organ level was the most intensively studied by classical schools of histopathology. Virchow's descriptions of histopathological alterations concerns a great deal the connective tissues (see the<sup>67</sup>). In Hungary the pioneering work of Huzella<sup>26</sup> contributed a great deal to the realization of the importance of cell-matrix interactions. As a first year medical student I assisted with the projections of his microcinematographic films showing cancer cells climbing on collagen fibers and depositing an excreted material on these fibers.

Connective tissues were however considered for decades as "inert", important only as mechanical protecting devices. Here again pathology played a crucial role in changing this attitude. The degradation of cartilage in osteoarthritis revealed the crucial importance of proteoglycans and of hyaluronan in normal tissue function and in its pathological alterations. This prompted the isolation and characterization of glycosaminoglycans (formerly "acid mucopolysaccharides") later of proteoglycans and revealed the central role of hyaluronan in the formation of multimolecular aggregates of Aggrecan-hyaluronan as essential components is cartilage structure (for a review see<sup>54</sup>). Cells were still considered up to the end of 1970's as producing matrix but functionally independent from it. After the (re)-discovery of fibronectin it became evident that this and other structural glycoproteins mediate cell-matrix interactions. This led to the discovery of integrins, cell receptors mediating cell matrix interactions. Integrins were shown to "recognize" most ECM components except elastin. We therefore decided to try to identify cell receptors capable of mediating cell-elastin interactions. This was accomplished in the mid 1980's by demonstrating the receptor mediated interaction of fibroblasts and vascular smooth muscle cells with micronised, <sup>3</sup>H-labeled elastin fibers.<sup>25,46,54</sup> This matrix receptor was the first to be submitted to pharmacological studies. In collaboration with T. Fülöp, M. P. Jacob, Zs Varga and others we identified the second messengers and the message-transmission pathway of this receptor.<sup>62,64,63,8,48</sup> We took advantage of its expression on mononuclear leukocytes to study its pharmacology, and modifications with aging. *Table 4* shows the physiological and pathological processes associated with the stimulation of this receptor. In collaboration with K. Lapis and J. Timar from the Semmelweis Medical University of Budapest we showed the presence the elastin receptor on metastatic tumor cells and proposed its implication in tumor – matrix interactions and tissue invasion.<sup>59</sup> More recently G. Faury et al.<sup>11</sup> showed the presence of this receptor on vascular endothelial cells. The addition of its agonist, elastin peptides produced an NO-dependent vasorelaxation in noradrenalin-precontracted rat aorta rings. This effect showed a sharp age-dependency: absent

**Table 4. Some functions of the elastin-laminin receptor**

Activity
Chemotactic cell migrations
Adhesion of cells to elastin fibers
Tumor cell-matrix interaction
Chaperon for elastogenesis
Growth factor like activity
Modulation of matrix biosynthesis
Increase of elastase synthesis and release
Release of superoxyde anion
Endothelium and NO' mediated vasodilatation

in newborn aortas, it reached its maximal efficiency in young adults and declined progressively with age.<sup>10</sup> This can be attributed to the chronic overload of the elastin receptor on endothelial cells by circulating elastin peptides. They trigger a sustained release of elastase-type endopeptidases and of free radicals.<sup>51,54</sup> This process will eventually produce more harm than beneficial effects. This probably explains also the age-dependent uncoupling of the elastin receptor. Pertussis toxin, a G-protein inhibitor does not inhibit any more superoxide release in leukocytes from "old" donors as compared to "young" donors. Similar results were reported on the regulation of steroid synthesis by mononuclear leukocytes: elastin peptides produced a dose dependent inhibition of the incorporation of <sup>14</sup>C-acetate in cholesterol. This effect disappeared completely in cells from "old" donors. <sup>38,39,40</sup> In the presence of increasing concentrations of elastin peptides there is first an increase of cell proliferation, but also of the production of a serine-elastase apparently indistinguishable from PMN-elastase. Further increase of elastin peptide concentration leads to an increasing proportion of cells taking up the vital dye, due to a necrotic type of cell-death. At higher elastin peptide concentrations apoptotic cell death became predominant and could be demonstrated by TUNEL, electron microscopy and flow cytometry.<sup>39,40</sup> This is the first demonstration of cell-death produced by the overactivation of a receptor by physiologically occurring agonists. Epidemiological studies showed that the cir-

**Table 5. Age-related diseases and approximate age of onset**

Diseases	Age of onset (appr)
Osteoarthritis	40-50 yr
Atherosclerosis	50-60 yr
Lung emphysema	• 60-70 yr
Malignancies (gastrointestinal, prostate)	60-80 yr
Dementias	70-90 yr

culating elastin peptide concentration is on the average about 10 µg/ml, at least four log units above the Kd of the receptor which is in the nanomolar range.<sup>48</sup>

This or similar mechanisms might be involved in the age-dependent loss or uncoupling of a number of receptors.<sup>54</sup> Loss of receptor function is very probably an important factor in tissue- and organ aging. The loss of β-adrenergic receptors on cardiac muscle cells<sup>32</sup> and of hormone receptors<sup>56</sup> are other examples of this aging mechanism.

### *Aging and age-related pathologies*

*Table 5* shows a list of age related diseases with the indication of their approximate age of onset. Articular pathology, osteoarthritis starts the earliest. Its onset varies according to the different joints. It is currently attributed to the loss of chondrocyte phenotype with aging. Cartilage matrix is composed essentially of collagens II, IX and XI with hyaluronan- aggrecan macromolecular complexes in its interstices. This original matrix is progressively destroyed and replaced by a fibrous matrix. Ensuing inflammation will continue to destroy the original cartilage matrix with the well documented consequences of severe osteoarthritic deformities (for a review see<sup>51</sup>).

But the most important age-related diseases are represented by the cardiovascular diseases. Schematically we prefer to distinguish atheromatosis and arteriosclerosis. Atheromatous plaque formation is observed frequently in young children also. But even normocholesterolemic subjects develop arteriosclerosis, the progressive hardening of elastic vessels.<sup>46,47</sup> As Balo rightly proposed it in the middle of this century<sup>3</sup>, the progressive loss of integrity of elastic fibers plays an important role in the development of arteriosclerosis. It was shown however that hypercholesterolemia accelerates the thickening of the vascular wall and accelerate arteriosclerosis.<sup>16</sup> In most humans both of these processes can be observed, justifying the proposition of Marchand to designate this pathology as athero-arteriosclerosis. In some animals as the rat for instance no lipidic plaques are deposited. The vascular wall shows however the age-dependent modifications of arteriosclerosis: stiffening, increase of wall thickness and of the diameter of the vessel, increase of collagen/elastin ratio (for a review see<sup>47</sup>). One of the most harmful results of this process is its effect on the aging myocardium. It will lose contractile fibers, both through necrosis and apoptosis, « compensated » by an increase of collagen fibers. This process will lead eventually to decompensation, arrhythmias and frequently to heart arrest.

A third age-dependent disease of increasing frequency aggravated by smoking is lung emphysema. The loss of alveolar surface is attributable to elastolytic activity elicited by the inflammatory process entertained by chronic infection of the airways, strongly aggravated by smoking.

Another class of age-related diseases is represented by malignant tumors. The systematic autopsies of all registered patients in the Malmo General Hospital by Ponten showed an age-dependent increase of tumors of the gastrointestinal tract, the prostate and some other organs.<sup>42</sup> A more recent critical assessment of this problem led Macieira-Coelho<sup>34</sup> to exclude from this class of age-dependent malignancies those tumors reaching a plateau with age, followed sometimes by a decrease of frequency. Little is known on the age-dependent factors which might explain these epidemiological observations.

With the recent increase of life expectancy age-related dementias became a predominant problem of aging diseases. Several recent monographies were devoted to this rapidly expanding field of aging research (for a review see<sup>50</sup>). The detailed discussion of their mechanism is beyond the scope of this review. Let us make however one general remark: the age-dependent modifications of cerebral blood vessels appears to play an important role in the non-pathological cognitive decline as well as in age related dementias. Besides the dementias of the Alzheimer type, vascular dementias occupy a comfortable second position as far as frequency and severity are concerned.<sup>50</sup>

### Discussion and Conclusions

We can now come back to the question asked in the Introduction: can we hope to age and die of old age or are age-related pathologies unavoidable? The by now classical work of Alvar Svanborg<sup>57,58</sup> suggested an optimistic outcome. After following three cohorts of 70 year old individuals, 5 years apart each in Gotheborg in Sweden, for decades he found a decreasing rate of several pathologies, occurring later and later in life. These findings prompted optimistic statements and the possibility of dying of "old age". Opposed to these results are those of systematic autopsies of old people showing the presence of several unsuspected pathological alterations. Several of them could have been involved in the death of these patients.

This polypathology of aging contrasts with the above optimistic view suggests a more realistic and more pessimistic opinion. Some or several pathologies accompany aging and lead to final loss of function. Cardiovascular diseases are in particular responsible for the death of old people as shown already by the pioneering autopsies of Haranghy on several centenarians.<sup>20</sup> It appears therefore that the better understanding and treatment of cardiovascular aging and of tumor growth may be the key to an efficient postponement of age related decline and death.<sup>47</sup>

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