

A high-magnification electron micrograph of a cell, showing a large, dark, textured nucleus on the left and a lighter, more granular cytoplasm on the right. The nucleus contains various internal structures, including what appears to be a nucleolus. The overall image is in grayscale, with the nucleus being the dominant feature.

ADVANCES IN
EXPERIMENTAL
MEDICINE
AND BIOLOGY

Volume 694

Protein Metabolism and Homeostasis in Aging

Edited by
Nektarios Tavernarakis

Protein Metabolism and Homeostasis in Aging

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DEDICATION

To my father

PREFACE

Aging is loosely defined as the accumulation of changes in an organism over time. At the cellular level such changes are distinct and multidimensional: DNA replication ceases, cells stop dividing, they become senescent and eventually die. DNA metabolism and chromosomal maintenance, together with protein metabolism are critical in the aging process. The focus of this book is on the role of protein metabolism and homeostasis in aging. An overview is provided of the current knowledge in the area, including protein synthesis, accuracy and repair, post-translational modifications, degradation and turnover, and how they define and influence aging. The chapters mainly focus on well-characterised factors and pathways, but new areas are also presented, where associations with aging are just being elucidated by current experimental data.

Protein turnover, the balance between protein synthesis and protein degradation are carefully maintained in healthy cells. Chapters 1 and 2 illustrate that aging cells are characterised by alterations in the rate, level and accuracy of protein synthesis compared to young ones, and that mRNA translation, essential for cell growth and survival, is controlled at multiple levels. The theory that growth and somatic maintenance are believed to be antagonistic processes is described in Chapter 3: inhibition of protein synthesis results in decreased rates of growth and development, but also confers an extension of lifespan, as shown for example by the effects of dietary restriction in various model organisms. Quality control mechanisms ensure misfolded or damaged proteins are remodelled or repaired, but when this fails proteins are targeted to go through degradation in order to avoid untimely cell death. The ubiquitin/proteasome system keeps cells clear of abnormal, damaged or denatured proteins (Chapter 4), while autophagy degrades long-lived proteins and small organelles (Chapter 5); compromised activity of both processes has been tightly correlated to aging. Accumulation of damaged or misfolded proteins within cells has been associated with human age-related, neurodegenerative diseases. The paradoxical situation of autophagy up-regulation in models of premature aging is also discussed in Chapter 6.

The insulin/IGF-1, TGF β , TOR and p38/ MAP kinase signalling pathways play a part in regulating protein turnover and have been linked to aging through a number of their components, discussed in Chapter 7. Inhibition of the insulin/IGF and TOR pathways results in lifespan extension in worms, as detailed in Chapter 8, similarly with other longevity pathways, including dietary intake and mitochondrial

function. The role of mitochondria in protein quality control and the influence of reactive oxygen species in aging are presented in detail in Chapter 9. Chapter 10 discusses that different types of stress, intracellular, oncogenic and environmental, such as food and space restrictions, oxidative stress, temperature fluctuations and accumulation of damaged proteins, have been shown to induce premature aging and/or senescence through mechanisms independent of telomere shortening. In Chapter 11 the free radical and oxidative stress theories of aging are portrayed to link such stress factors to the occurrence of aging through the function of mitochondria, the activity of detoxifying enzymes and degradation pathways and their effects on protein turnover, while resistance to stress has been directly associated to lifespan extension and delayed aging in model organisms. Stress is also a major inducer of the sumoylation pathway, a post-translational protein modification that, together with substrate interactions with other ubiquitin-like proteins, show differential activity in aging tissues and has recently been linked to the onset of cellular senescence; these pathways are presented in detail in Chapters 12 and 13. The critical importance of maintaining cell homeostasis is evident in every chapter in this book, but is presented in more detail in Chapter 14, especially focusing on hormone signalling in response to environmental cues. Hormones and cytokines that affect muscle homeostasis during aging are presented in Chapter 15. The main pathways that take part in skeletal muscle atrophy and regeneration are illustrated, followed by a description of current gene and cell therapies to rescue muscle atrophy and wasting. The volume concludes with Chapter 16, where common techniques used in protein metabolism and homeostasis research are presented and critically reviewed.

We would like to thank all authors of this book for their excellent contributions, and hope that the reader will enjoy the chapters and be inspired to further their knowledge in this ever-expanding and exciting field. As the advances of modern technology and medicine have significantly raised life expectancy, it is becoming ever more important to gain a deeper insight and understanding of the mechanisms that influence the aging process. Such knowledge is an essential prerequisite for the development of effective strategies to increase health span and quality of life for the elderly.

*Artemisia M. Andreou, PhD
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March 2010, Heraklion, Greece*

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CHAPTER 1

Synthesis, Modification and Turnover of Proteins during Aging

Suresh I.S. Rattan*

Abstract

Alterations in the rate and extent of protein synthesis, accuracy, post-translational modifications and turnover are among the main molecular characteristics of aging. A decline in the cellular capacity through proteasomal and lysosomal pathways to recognize and preferentially degrade damaged proteins leads to the accumulation of abnormal proteins during aging. The consequent increase in molecular heterogeneity and impaired functioning of proteins is the basis of several age-related pathologies, such as cataracts, sarcopenia and neurodegenerative diseases. Understanding the proteomic spectrum and its functional implications during aging can facilitate developing effective means of intervention, prevention and therapy of aging and age-related diseases.

Introduction

A decline in the rate of total protein synthesis is one of the most commonly observed age-associated biochemical changes in cells, tissues, organs and organisms, including human beings. The implications and consequences of slower rates of protein synthesis are manifold in the context of aging and age-related pathology. Although there is a considerable variability among different tissues and cell types in the extent of decline (varying from 20% to 80%), the fact remains that the bulk protein synthesis slows down during aging.^{1,2} However, it should be pointed out that age-related slowing down of bulk protein synthesis does not mean that the synthesis of each and every protein becomes slower uniformly during aging. Furthermore, even though bulk protein synthesis slows down with age, total protein content of the cell generally increases because of an accumulation of abnormal proteins during aging. Age-related changes in protein synthesis are regulated both at the transcriptional and pretranslational levels in terms of the availability of individual mRNA species for translation,² and at the translational and post-translational levels in terms of alterations in the components of the protein synthetic machinery and the pattern of postsynthetic modifications that determine the activity, specificity and stability of a protein.

The aim of this chapter is to provide an overview of the regulation and misregulation, synthesis, modifications and turnover of proteins, including the role of protein errors and accumulation of abnormal proteins during aging.

Efficiency and Accuracy of Protein Synthesis during Aging

Eukaryotic protein synthesis is a highly complex process, which requires about 200 small and large components to function effectively and accurately in order to translate one mRNA molecule, while using large quantities of cellular energy. There are three major components of the

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translational apparatus: (1) the translational particle, the ribosome; (2) the amino acid transfer system or charging system; and (3) the translational factors.³ The protein-synthesizing apparatus is highly organized and its macromolecular components are not freely diffusible within cells. The rate and accuracy of protein synthesis can be critical for aging. Since, the error frequency of amino acid misincorporation is generally considered to be quite high (10^{-3} to 10^{-4}) as compared with nucleotide misincorporation, the role of protein error feedback in aging has been a widely discussed issue.^{4,5}

So far, no direct estimates of protein error levels in any aging system have been made primarily due to the lack of appropriate methods to determine spontaneous levels of error in a normal situation. However, several indirect estimates of the accuracy of translation in cell-free extracts, using synthetic templates or natural mRNAs have been made. Studies performed on animal tissues, such as chick brain, mouse liver and rat brain, liver and kidney, did not reveal any major age-related differences in the capacity and accuracy of ribosomes to translate poly(U) in cell-free extracts.⁶ However, these attempts to estimate the error frequencies during *in vitro* translation of poly(U) template were inconclusive because the error frequencies encountered in the assays were several times greater than the estimates of natural error frequencies. Another indirect method that has been used to detect misincorporation of amino acids during aging is the method of two-dimensional (2D) gel electrophoresis of proteins, but no age-related increase in amino acid misincorporation affecting the net charge on proteins was observed in histidine-starved human fibroblasts and in nematodes. In contrast to this, using mRNA of CcTMV coat protein for translation by cell extracts prepared from young and old human fibroblasts, a seven-fold increase in cysteine misincorporation during cellular aging has been observed.^{7,8} These studies also showed that an aminoglycoside antibiotic paromomycin (Pm), which is known to reduce ribosomal accuracy during translation *in vivo* and *in vitro*, induces more errors in the translation of CcTMV coat protein mRNA by cell extracts prepared from senescent human fibroblasts than those from young cells. Indirect evidence that indicates the role of protein errors in cellular aging can be drawn from studies on the increase in the sensitivity of human fibroblasts to the life-shortening and aging-inducing effects of Pm and another aminoglycoside antibiotic G418.^{9,10} Further evidence in support of the role of errors comes from experiments which showed that an induction and increase in protein errors can accelerate aging in human cells and bacteria.^{1,2,4,11,12} Similarly, an increase in the accuracy of protein synthesis can slow aging and increase the lifespan in fungi.^{13,14} Although a global "error catastrophe" as a cause of aging due to errors in each and every macromolecule is considered unlikely, it is not ruled out that some kind of errors in various components of protein synthetic machinery, including tRNA charging, may have long-term effects on cellular stability and survival.

Altered Protein Synthesis during Aging

The translational process can be envisaged to proceed in three steps—initiation, elongation and termination, followed by post-translational modifications, including folding, which give the protein a functional tertiary structure. The initiation step is considered to be the main target for the regulation of protein synthesis during cell cycle, growth, development, hormonal response and under stress conditions including heat shock, irradiation and starvation.^{15,16} With respect to aging, however, the rate of initiation appears to remain unaltered. For example, using *in vitro* assays, the conversion of isolated 40S and 60S ribosomal subunits into the 80S initiation complex has been reported to decrease by less than 15% in old *Drosophila*, rat liver and kidney and mouse liver and kidney. On the other hand, since polysomal fraction of the ribosomes decreases during aging, it implies that the activity of an anti-ribosomal-association factor eIF-3 may increase during aging. The activity of eIF-2, which is required for the formation of the ternary complex of Met-tRNA_i, GTP and eIF-2, has been reported to decrease in rat tissues during development and aging. Attenuation of hypusine formation on eIF-5A during senescence of human diploid fibroblasts has been reported.¹⁷ Recently, a novel role of eIF-5A in protein chain elongation has been reported,¹⁸ which may also be important in relation to aging. Similar studies on other eIFs and in other aging systems are yet to be performed and it is necessary that detailed studies on eIFs

are also undertaken in the context of aging and the question of the regulation of protein synthesis at the level of initiation is reinvestigated.

Several studies have been performed on age-related changes in the number of ribosomes, thermal stability, binding to aminoacyl-tRNA, the level of ribosomal proteins and rRNAs, sensitivity to aminoglycoside antibiotics and the fidelity of ribosomes.⁶ Although there is a slight decrease in the number of ribosomes in old animals, this does not appear to be a rate-limiting factor for protein synthesis due to ribosomal abundance in cells. Instead, several studies indicate that the biochemical and biophysical changes in ribosomal characteristics may be more important for translational regulation during aging. For example, the ability of aged ribosomes to translate synthetic poly(U) or natural globin mRNA decreases significantly. A decrease in the translational capacity of ribosomes has also been observed in rodent tissues such as muscle, brain, liver, lens, testis and parotid gland and in various organs of *Drosophila*.^{19,20}

The reasons for the functional changes observed in aging ribosomes are not known at present. Some attempts have been made to study the effect of aging on rRNAs and ribosomal proteins. Although a three-fold increase in the content of rRNA has been reported in late passage senescent human fibroblasts, it is not clear whether the quantity and quality of individual rRNA species undergo alterations during aging and what effect such a change might have on ribosome function. Similarly, although an increase in mRNA levels for ribosomal protein L7 has been reported in aged human fibroblasts and rat preadipocytes, there are no differences in the electrophoretic patterns of the ribosomal proteins in young and old *Drosophila* and mouse liver.⁶

The formation of the 80S initiation complex is followed by the repetitive cyclic event of peptide chain elongation, a series of reactions catalysed by elongation factors (EFs; also abbreviated as eEFs).^{21,22} Various estimates of the elongation rates in eukaryotic cells give a value in the range of 3 to 6 amino acids incorporated per ribosome per second, which is several times slower than the prokaryotic elongation rate of 15 to 18 amino acids incorporated per second.²³ With regard to aging, a slowing-down of the elongation phase of protein synthesis has been suggested to be crucial in bringing about the age-related decline in total protein synthesis. This is because a decline of up to 80% in the rate of protein elongation has been reported by estimating the rate of phenylalanyl-tRNA binding to ribosomes in poly(U)-translating cell-free extracts from old *Drosophila*, nematodes and rodent organs.²⁴ In vivo, a two-fold decrease in the rate of polypeptide chain elongation in old WAG albino rat liver and brain cortex has been reported. Similarly, a decline of 31% in the rate of protein elongation in the livers of male Sprague-Dawley rats has been reported, by measuring the rate of polypeptide chain assembly which was 5.7 amino acids per second in young animals and was 4.5 amino acids per second in old animals.²⁵ However, these estimates of protein elongation rates have been made for "average" size proteins. It will be important to see if there is differential regulation of protein elongation rates for different proteins during aging.

The elongation of polypeptide chain is mediated by 2 elongation factors, eEF-1 and eEF-2 in eukaryotes (a third factor, EF-3, is reported only in yeast), which are highly conserved during evolution.²¹ The activity of eEF-1 declines with age in rat livers and *Drosophila* and the drop parallels the decrease in protein synthesis.^{6,20} This decline in the activity of eEF-1 has been correlated only to EF-1A as no changes were observed in the EF-1beta-gamma-mediated activity. Using more specific cell-free stoichiometric and catalytic assays, a 35-45% decrease in the activity and amounts of active eEF-1A has been reported for serially passaged senescent human fibroblasts, old mouse and rat livers and brains.^{6,20,26}

In the case of eEF-2 that catalyses the translocation of peptidyl-tRNA on the ribosome during the elongation cycle, conflicting data are available regarding the changes during aging. For example, a lack of difference in the rate of translocation has been observed during the translation of poly(U) by cell-free extracts prepared from young and old *Drosophila* and from rodent organs.^{27,28} Similarly, although the proportion of heat-labile eEF-2 increases during aging, the specific activity of eEF-2 purified from old rat and mouse liver remains unchanged.²⁹ In contrast, a decline of more than 60% in the amount of active eEF-2 has been reported during aging of human fibroblasts in culture, measured by determining the content of diphtheria toxin-mediated ADP-ribosylatable

eEF-2 in cell lysates.³⁰ However, using the same assay, no age-related change in the amount of ADP-ribosylatable eEF-2 was detected in rat livers.³¹ Increased fragmentation of eEF-2 due to oxidation has also been reported in old rat livers.³² Acidic variants of eEF-2 in isolated rat heart and cultured cardiomyocytes have also been reported.³³ Further studies are required to determine if there are any qualitative and quantitative changes in eEF-2 at the levels of transcription, translation and post-translational modifications and how such changes are related with the regulation of protein synthesis during aging.

The cycle of peptide chain elongation continues until one of the three stop codons is reached. There is no aa-tRNA complementary to these codons and instead a termination factor or a release factor (RF) binds to the ribosome and induces the hydrolysis of both the aminoacyl linkage and the GTP, releasing the completed polypeptide chain from the ribosome. Studies in aging *Drosophila* and old rat livers and kidneys have shown that the release of ribosome bound N-formylmethionine, a measure of the rate of translation termination, was not affected with age.²⁸ Direct estimates of the activity of the termination factor during aging have not been yet made.

Post-Translational Modifications during Aging

Accurate translation of mRNA, followed by appropriate modifications of the polypeptide chain, is essential for its normal folding, targeting and specificity. A misregulation in any of these steps can have far reaching biological consequences, including its effects on cell growth, division and survival. A large number of post-translational modifications of proteins have been described that determine the activity, stability, specificity, transportability and lifespan of a protein. Several of these modifications are highly specific and regulated involving various enzymatic pathways, but there are several nonenzymatic modifications of proteins which occur stochastically but depend on the ternary structures.^{34,35}

Phosphorylation

Phosphorylation of serine, threonine and tyrosine residues is one of the best studied modifications of proteins. The coordinated activities of protein kinases, which catalyse phosphorylation and of protein phosphatases which catalyse dephosphorylation, regulate several biological processes, including protein synthesis, cell division, signal transduction, cell growth and development.³⁶ Altered pattern of protein phosphorylation may be one of the reasons for age-related alterations in protein function and activity and can be a major cause of the failure of homeodynamics and aging. For example, inhibition of DNA synthesis and the loss of proliferative capacity is the ultimate characteristic of normal diploid cells undergoing aging *in vitro*.³⁷ Although several putative inhibitors of DNA synthesis have been identified in senescent cells, little is known about the mechanisms of action and the regulation of activity of these inhibitors. It is possible that the activity of several of these inhibitors is regulated by phosphorylation. For example, several studies have shown age-related alterations in cell-cycle-regulated gene expression of various genes such as *c-fos*, *c-jun*, *JunB*, *c-myc*, *c-Ha-ras*, *p53*, *cdc2*, *cycA*, *cycB*, *cycD* and retinoblastoma gene *RB1*. Although phosphorylation is involved in regulating the activities of the gene products of almost all these genes, a decrease in phosphorylated cyclin E and Cdk2 and failure to phosphorylate *RB1* gene product p110^{Rb} and *cdc2* product p34^{cdc2} during cellular aging have been reported at present.³⁸ It will be important to find out if there are age-related alterations in the phosphorylation state of other cell cycle related gene products, proteins involved in DNA and RNA synthesis, including various transcription factors.^{39,40}

Various components of the protein synthetic apparatus undergo phosphorylation and dephosphorylation and thus regulate the rates of protein synthesis.²³ For example, phosphorylation of eIF-2 correlates with inhibition of initiation reactions and consequently the inhibition of protein synthesis. Conditions like starvation, heat shock and viral infection, which inhibit the initiation of protein synthesis, induce the phosphorylation of eIF-2 in various cells. Stimuli such as insulin and phorbol esters modulate the phosphorylation of eIF-3, eIF-4B and eIF-4F by activating various protein kinases.²³ Since the activity of eIF-2 has been reported to decrease during aging, it is

possible that the phosphorylation status of eIF-2 also changes during aging. However, no studies are available on age-related changes in the phosphorylation pattern of initiation factors.

At the level of protein elongation, the phosphorylation of elongation factors eEF-1A and eEF-2 appears to be involved in regulating their activities. It will be important to see whether the age-related decline in the activities of elongation factors is accompanied by a parallel change in the extent of phosphorylation of these enzymes. Incidentally, it has been reported that there is an increase in the levels of phosphorylated eEF-1 and eEF-2 during mitosis when minimal protein synthesis occurs. Furthermore, there is indirect evidence that alterations in the phosphorylation and dephosphorylation of eEF-2 due to changes in the activities of eEF-2-specific protein kinase III,⁴¹ and PP2A phosphatase⁴² may affect the rates of protein synthesis during aging in rat livers.

Phosphorylation also occurs in other proteins that participate in the translational process. For example, the regulatory role of phosphorylation of aa-tRNA synthetase in protein synthesis has been suggested.⁴³ However, to what extent the decline in the activity and the accumulation of heat-labile aa-tRNA synthetases reported in studies performed on various organs of aging mice and rats is related to their phosphorylation is not known. Furthermore, since the phosphorylation of the S6 ribosomal protein correlates with the activation of protein synthesis, failure to phosphorylate S6 protein in senescent human fibroblasts in response to serum,⁴⁴ can be one of the reasons for the decline in the rate of protein synthesis observed during aging.

Pathways of intracellular signal transduction depend on sequential phosphorylation and dephosphorylation of a wide variety of proteins. All phosphorylation reactions result from the action of one or more kinases and the ratio between two interconvertible forms of kinases (active and inactive) acts as a control mechanism for many cellular functions. Studies performed on aging cells have not shown any deficiency in the amount, activity or ability of protein kinase-C (PKC) to elicit signalling pathway.⁴⁵ There is also evidence that senescent human fibroblasts retain their ability to phosphorylate proteins in the PKC signal transduction pathway. It appears that the PKCs are largely unaltered in fibroblasts, although the body of information about phosphorylation mechanisms is still very limited.³⁹

Growth factor receptors for EGF, FGF, PDGF, insulin, glucocorticoids and several other hormones also possess protein kinase activity. Therefore, deficiencies in the phosphorylation process of receptors would be a logical explanation for the age-related decline of responsiveness to hormonal action and growth stimulation. However, there is no age-related decline in the autophosphorylation activity of various growth factor receptors.^{46,47} Similarly, most of the PKC-mediated pathways of intracellular signal transduction in response to various mitogens including phorbol esters appear to remain unaltered in senescent fibroblasts.⁴⁶⁻⁴⁸ However, a decline in both serine/threonine- and tyrosine-specific protein kinase signals after activation has been observed in the case of T-lymphocytes in aging mice.⁴⁹ Similarly, alterations in MAPK phosphorylation have been observed in rodents,⁵⁰ while PKC phosphorylation changes are seen in human pathological aging, including Alzheimer's disease and neurodegenerative processes.⁵¹

Oxidation

It is often observed that inactive and abnormal proteins accumulate in old cells and tissues. This increased amount of debris in the cytoplasm can be inhibitory for cell growth and normal metabolism and thus contribute towards failure of homeostasis. One of the reasons for the inactivation of enzymes can be their oxidative modification by oxygen free radicals and by mixed-function oxidation (MFO) systems or metal catalyzed oxidation (MCO) systems. Since some amino acid residues, particularly proline, arginine and lysine, are oxidized to carbonyl derivatives, the amount of carbonyl content of proteins has been used as an estimate of protein oxidation during aging.⁵²⁻⁵⁶

An increase in the levels of oxidatively modified proteins has been reported in old human erythrocytes of higher density and in cultured human fibroblasts from normal old donors and from individuals suffering from progeria and Werner's syndrome.⁵⁷ Similarly, there was a two-fold increase in the protein carbonyl content of brain proteins of retired breeder Mongolian gerbils, which was reversed by treatment with the spin-trapping compound *N-tert*-butyl-phenylnitron.⁵⁸

An age-related increase in the carbonyl content has also been reported in houseflies, fruitflies, nematodes and mouse organs.⁵⁹⁻⁶³

The loss of activity of 6-phosphogluconate dehydrogenase and liver malic enzyme during aging is related to the loss of lysine and histidine residues by oxidation.⁶⁴ Oxidation of a cysteine residue in glyceraldehyde-3-phosphate dehydrogenase may be responsible for its inactivation during aging in rat muscles.⁶⁵ It has also been reported that the concentration of the oxidation products of human lens proteins and skin collagen increases along with the accumulation of oxidative forms of alpha-crystallin in patients with age-related cataract.⁶⁶ However, the content of *ortho*-tyrosine and dityrosine, formed by the oxidation of phenylalanine and tyrosine, respectively, did not increase in the aging human lens.⁶⁷ Structural alterations introduced into proteins by oxidation can lead to aggregation, fragmentation, denaturation and distortion of secondary and tertiary structure, thereby increasing the proteolytic susceptibility of oxidized proteins. Furthermore, toxic products of carbonyl modifications can react with other macromolecules and affect various metabolic processes.

Generally it is thought that there are no systems for the repair of oxidatively damaged proteins and these must be degraded to avoid their accumulation. However, at least one kind of protein repair system has been identified. For example, oxidatively-induced disulphide bridges in cysteine and methionine lead to the formation of sulfenic acid, sulfinic acid and methionine sulphoxide which can accumulate during aging. These damages can be repaired by the methyl-sulfoxide reductase (MSR) system comprising of at least 3 Msr enzymes whose activity is decreased during aging.^{68,69} Senile graying of human hair due to increased oxidative stress is accompanied by a reduction of the activities of MSR system.⁷⁰ In contrast to this, overexpression of one of the Msr genes increases the lifespan of *Drosophila* and improves their stress tolerance to oxidative damage-inducing agents.⁷¹

Glycation

Glycation is one of the most prevalent covalent modifications in which the free amino groups of proteins react with glucose to form a ketoamine called Amadori product. This is followed by a sequence of further reactions and rearrangements producing the so-called advanced glycosylation end products (AGEs).^{72,73} Most commonly, it is the long-lived structural proteins such as lens crystallins, collagen and basement membrane proteins which are more susceptible to glycation. In the case of skin, vimentin has been found to be as the main protein becoming glycated during aging.⁷⁴ The glycated proteins are then more prone to form crosslinks with other proteins, leading to structural and functional alterations.⁵

An increase in the levels of glycated proteins during aging has been observed in a wide variety of systems. For example, there is an increase in the level of glycated lysine residues of rat sciatic nerve, aorta and skin collagen during aging.⁷⁵ There is an increase in the glycation of human collagen and osteocalcin during aging.⁷⁶ The formation and the accumulation of the AGEs are implicated in the physiology and pathology of senescence. It has been observed that pentosidine (cross-linked glycated lysine and arginine), carboxymethyllysine (CML, glycated and oxidated proteins) and pyrroline increase with age in normal and diabetic humans.⁷⁷ By using AGE-specific antibodies, an AGE-modified form of human hemoglobin has been identified, whose levels increase during aging and in patients with diabetes-induced hyperglycemia.⁷⁸

Deamidation, Racemization and Isomerization

Age-related changes in the catalytic activity, heat stability, affinity for substrate and other physical characteristics, such as the conformation of proteins may also be due to the charge change introduced by conversion of a neutral amide group to an acidic group by deamidation. Spontaneous deamidation of asparaginyl and glutaminyl residues of several proteins has been related with the observed accumulation of their inactive and heat labile isoforms during aging.⁶⁵ The sequential deamidation of two asparagine residues of triphosphate isomerase is responsible for the differences of the isoenzymes present in aging cells and tissues, such as bovine eye lens and human skin fibroblasts from old donors and patients with progeria and Werner's syndrome.⁷⁹ Deamidation of glucose-6-phosphate isomerase produces the variant of the enzyme that accumulates in aging bovine lenses.⁸⁰

The interconversion of optical isomers of amino acids, called racemization, has been reported to increase during aging. For example, the concentration of D-aspartate in protein hydrolysates from human teeth, erythrocytes and eye lens increases with age.⁸¹ Racemization of tyrosine has been reported to occur in the aging brunescence human cataract lenses.⁸² The spontaneous prolyl *cis-trans* isomerization in proteins that may cause some of the so-called spontaneous conformational changes has been implicated in the age-related decline in the activity of certain enzymes. However, no definitive examples of enzymes undergoing this kind of post-translational modification during aging are available. It is also not known to what extent the conformational changes associated with old rat muscle phosphoglycerate kinase, enolase and other enzymes are associated with racemization and isomerization.

ADP-Ribosylation

The structure and function of many proteins such as nuclear proteins topoisomerase I, DNA ligase II, endonuclease, histones H1, H2B and H4, DNA polymerases and cytoplasmic proteins adenylyl cyclase and elongation factor eEF-2 is modulated by ADP-ribosylation. ADP-ribosylation of proteins is involved in various cellular processes such as maintenance of chromatin structure, DNA repair, protein synthesis, cell differentiation and cell transformation.^{83,84}

Indirect evidence suggests that poly-ADP-ribosylation of proteins may decrease during aging because the activity of poly(ADP-ribose)polymerase (PARP) decreases in aging human fibroblasts both as a function of donor age and during serial passaging *in vitro*.⁸⁵ Similarly, the direct relationship observed between maximum lifespan of a species and the activity of PARP in mononuclear leukocytes of 13 mammalian species indicates its important role in aging and longevity.^{83,86}

One cytoplasmic protein that can be specifically ribosylated by diphtheria toxin and exotoxin A is the protein elongation factor eEF-2. ADP-ribosylation of the diphthamide (modified histidine 715) residue of eEF-2 results in the complete abolition of its catalytic activity.²¹ There is evidence that increased ADP-ribosylation of eEF-2 is correlated with cellular aging. For example, the amount of eEF-2 that can be ADP-ribosylated in the presence of diphtheria toxin in cell-free extracts decreases significantly during aging of human fibroblasts in culture.^{30,32}

Methylation

Methylation of nitrogens of arginine, lysine and histidine and carboxyls of glutamate and aspartate residues is a widely observed post-translational modification that is involved in many cellular functions. Although most of our present understanding regarding the significance of protein methylation has come from studies on bacterial chemotaxis, muscle contraction, electron transport, processing of pituitary hormones and gene expression, its role in aging is beginning to emerge.⁸⁷

Proteins whose activities are increased by methylation include alcohol dehydrogenase, histones, ribosomal proteins, cytochrome C, eEF-1A, myosin, myelin and rhodopsin. Of these, decreased methylation of histones has been reported in livers and brains of aging rats. On the other hand, there is no difference in the extent of methylation of newly synthesized histones during cellular aging of human fibroblasts in culture. Studies on the levels of methylated histidine, arginine and lysine of myosin isolated from the leg muscles of aging rats, mice and hamsters showed unchanged levels of histidine, decreased levels of arginine and trimethyllysine and increased levels of monomethyllysine.^{6,88}

During the aging of erythrocytes, there is an increase in the number of methyl groups per molecule of band 2.1 (ankyrin) and band 3 protein, which correlates with increased membrane rigidity of erythrocytes during aging.⁸⁹ Similarly, there is a several fold increase in the number of methyl acceptor proteins in the eye lenses from aged humans and persons suffering from cataract.⁹⁰ The number of carboxylmethylatable sites of cerebral membrane-bound proteins also increases in rat brain during aging.⁹¹ At present, age-related changes in the methylation of other proteins such as ribosomal proteins, calmodulin, cytochrome C and myosin have not been studied. It is clear that protein methylation is involved in diverse functions including protein synthesis and turnover and that it should be studied thoroughly in relation to the process of aging.

Proteolytic Processing

Many newly synthesised proteins undergo post-translational proteolytic processing by which certain conformational restraint on the inactive precursor is released and a biologically active protein is generated. Several inactive precursors of enzymes called zymogens, precursors of growth factors, peptide and protein hormones such as insulin, precursors of extracellular matrix and many other secretory proteins including various proteases such as collagenase, undergo proteolytic processing. There are no systematic studies performed on age-related changes in post-translational proteolytic processing of any proteins. However, there is some evidence that alterations in proteolytic processing may be one of the reasons for the appearance or disappearance of certain proteins during aging. For example, the appearance of the "senescent cell antigen" on the surface of a wide variety of aging cells is derived from the proteolysis of band 3 protein.⁹² The exposure of senescent cell-specific epitopes on fibronectin,⁹³ may also be due to altered proteolytic processing. Progressive proteolysis of a 90 kDa protein, Tp-90 terminin, into Tp-60 and Tp-30 terminin in senescent cells and in cells committed to apoptosis has been reported.⁹⁴

Altered proteolytic cleavage of the beta-amyloid precursor protein is well known to play an important role in the pathogenesis of Alzheimer's disease.^{95,96} Increased proteolysis of a conformationally more labile single-chain form of the lysosomal protease cathepsin B has been suggested as a reason for the age-related decline in its activity during aging of human fibroblasts.⁹⁷ Similarly, alterations in the activity of collagenase during aging of human fibroblasts has been suggested to be due to structural and catalytic changes.⁹⁸

Other Modifications

In addition to the types of post-translational modifications mentioned above, there are some other modifications that determine the structure and function of various proteins and may have a role to play during aging. For example, the incorporation of ethanolamine into protein elongation factor eEF-1A may be involved in determining its stability and interaction with intracellular membranes.⁹⁹ Whether this modification has any role in the regulation of the activity of eEF-1A during aging is not known at present. Similarly, the protein initiation factor eIF-5A contains an unusual amino acid, hypusine, which is synthesized post-translationally as a result of a series of enzymatically catalysed alterations of a lysine residue.¹⁰⁰ Since the absence of hypusine in eIF-5A blocks the initiation of protein synthesis, reduced activity of a hypusine synthase enzyme has been reported in senescent human fibroblasts.¹⁷

Detyrosination of microtubules affecting the cytoskeletal organization and many other cellular functions, may also be important during aging. Furthermore, the roles of chaperones in protein folding and conformational organization are yet to be studied in relation to the aging process. There is some evidence that both the pentose-mediated protein crosslinking and transglutaminase-mediated crosslinking of proteins is involved in aging. For example, there is a high correlation between pentosidine protein crosslinks and pigmentation in senescent and cataract affected human lens.¹⁰¹ Similarly, an increase in transglutaminase activity during cellular apoptosis, differentiation and aging of human keratinocytes indicates an important role of this modification in the process of aging.¹⁰²

Protein tyrosine sulfation is another post-translational modification that may have significance in protein alteration during aging because it is involved in determining the biological activity of neuropeptides and the intracellular transportation of a secretory protein.¹⁰³ Similarly, prenylation, the covalent attachment of isoprenoid lipids on cysteine-rich proteins, is involved in the regulation of the activity of some proto-oncogenic ras proteins and the nuclear lamins A and B.¹⁰⁴ These studies have indicated a critical role for prenylation in the regulation of oncogenesis, nuclear structure, signal transduction and cell cycle progression, functions very much related with the causative aspects of aging. There is an age-dependent decrease in the activity of prenyltransferases in the rat liver, which may account for the changes in the synthesis and turnover of mevalonate pathway lipids, including cholesterol, ubiquinone and dolichol.¹⁰⁵