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SYNTHESIS, MODIFICATIONS, AND TURNOVER OF PROTEINS DURING AGING

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Abstract—Slowing down of bulk protein synthesis is one of the most commonly observed biochemical changes during aging. The implications and consequences of slower rates of protein synthesis are manifold, including a decrease in the availability of enzymes for the maintenance, repair, and normal metabolic functioning of the cell, an inefficient removal of inactive, abnormal, and damaged macromolecules in the cell, the inefficiency of the intracellular and intercellular signalling pathways, and a decrease in the production and secretion of hormones, antibodies, neurotransmitters, and the components of the extracellular matrix. Age-related changes in the activity, specificity, and stability of a large number of proteins have been reported. However, the molecular mechanisms responsible for such alterations are still poorly understood. Studies on various components of the protein synthetic machinery have revealed a decline in the efficiency and accuracy of ribosomes, an increase in the levels of rRNA and tRNA, and a decrease in the amounts and activities of elongation factors. Because posttranslational modifications of proteins determine their activity and stability, alterations in the extent and level of various modifications such as phosphorylation, methylation, ADP-ribosylation, oxidation, glycation, and conformational changes during aging are being studied. Changes in the regulation of protein synthesis, posttranslational modifications, and protein turnover are crucial determinants of age-related decline in the maintenance, repair, and survival of the organism.

Key Words: translation, posttranslational modifications, protein degradation, accuracy, initiation factors, elongation factors, aging

INTRODUCTION

ALTHOUGH THE genomic instructions of life are written in the language of nucleic acids, the life is actually "lived" in the language of proteins. Protein synthesis is crucial for the survival of a living system, and any disturbance at this level can cause large imbalances and deficiencies. A decline in the rate of total protein synthesis is one of the most common age-associated biochemical changes that has been observed in a wide variety of 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 (for review, see Ward and Richardson, 1991; Rattan, 1992; Van Remmen *et al.*, 1995). Furthermore, it has been shown that the conditions, such as calorie restriction, that increase the lifespan and retard the aging process in many organisms, also slow down the age-related decline in protein synthesis (Van Remmen *et al.*, 1995). 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. 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, and at the translational and posttranslational 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.

SPEED AND ACCURACY OF PROTEIN SYNTHESIS

Eukaryotic protein synthesis is a highly complex process that requires about 200 small and large components to function effectively and accurately to translate one mRNA molecule while using large quantities of cellular energy. The rate and accuracy of protein synthesis (as also of DNA and RNA synthesis) have been presumably gone through natural selection and evolved to optimal levels according to the overall life history of an organism. Because 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.

Accuracy of protein synthesis

At present, 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 errors in a normal situation. Studies on the accuracy of protein synthesis during aging that have been performed on animal tissues did not reveal any major age-related differences in the capacity and accuracy of ribosomes to translate poly(U) in cell-free extracts (Filion and Laughrea, 1985; Laughrea and Latulippe, 1988). However, these attempts to estimate the error frequencies during translation in vitro 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 (for a detailed discussion of this, see Holliday, 1995).

The accuracy of mouse liver ribosomes did not change with age in cell-free assays measuring the incorporation of radioactive lysine during the translation of trout protamine mRNA, which does not have codons for lysine (Mori *et al.*, 1983). However, using mRNA of CcTMV coat protein for translation by cell extracts prepared from young and old human fibroblasts, a sevenfold increase in cysteine misincorporation during cellular aging has been observed (Luce and Bunn, 1989). Furthermore, 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 (Luce and Bunn, 1989). Further indirect evidence indicating the role of protein errors in aging comes 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 (Holliday and Rattan, 1984; Buchanan *et al.*, 1987). Similarly, increased longevity of high-fidelity mutants in *Podospora anserina* indicate the role of protein errors in lifespan (Silar and Picard, 1994).

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, by which at least one kind of error, that is, the misincorporation of a charged amino acid for an uncharged one (or vice versa) can be demonstrated because of "stuttering" of the protein spot on 2D gels. Using this method, no age-related increase in amino acid misincorporation affecting the net charge on proteins was observed in histidine-starved human fibroblasts and in *Caenorhabditis elegans* (Harley *et al.*, 1980; Johnson and McCaffrey, 1985).

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 (Kowald and Kirkwood, 1993). Better methods are still required for measuring the basal levels of translational errors in young and old cells, tissues and organisms.

Initiation of protein synthesis

The translation of an mRNA begins with the formation of a so-called initiation complex between the ribosome and the initiator codon. It is an intricate process, which consumes energy and involves at least seven initiation factors (eIFs) consisting of 24 different subunits, two subunits of ribosomes, and an initiating tRNA called methionyl (Met)-$tRNA_i$. The whole process of the formation of the 80S initiation complex takes about two to three seconds in cell-free assays and is thought to occur much faster in vivo (Merrick, 1992).

Although 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 with respect to aging, the rate of initiation appears to remain unaltered. 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, because polysomal fraction of the ribosomes decreases during aging, it implies that the activity of an antiribosomal-association factor eIF-3 may increase during aging (Rattan, 1992). 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 (Calés *et al.*, 1986). A decline in the amount and activity of GDP/GTP exchange factor eIF-2b has been reported in the brains and livers of 10-month-old Sprague–Dawley rats as compared with one- and four-month-old animals (Kimball *et al.*, 1992). Similar studies on other eIFs and in other aging systems are yet to be performed.

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 have also been reported. 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 a ribosomal abundance in the cell. 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 (Nokazawa *et al.*, 1984; Sojar and Rothstein, 1986). 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* (Webster, 1985).

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. An extensive loss of rRNA gene activity in several tissues of the aging beagles, mice, rats, and senescent human lymphocytes and fibroblasts has been reported (for review, see Medvedev, 1986). Although a threefold increase in the content of rRNA has been reported in late-passage senescent human fibroblasts, it is not clear if the quantity and quality of individual rRNA species undergo alterations during aging. Similarly, although an increase in the levels of mRNA for ribosomal protein L7 has been reported in aged human fibroblasts (Seshadri and Campisi, 1990), and in rat preadipocytes (Kirkland *et al.*, 1993), there are no differences in the electrophoretic patterns of the ribosomal proteins in young and old *Drosophila* and mouse liver (Webster, 1985).

Elongation of protein synthesis

The formation of the 80S initiation complex is followed by the repetitive cyclic event of peptide chain elongation, which is a series of reactions catalyzed by elongation factors (EFs; also abbreviated as eEFs). Various estimates of the elongation rates in eukaryotic cells give a value in the range of three to six 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 (Merrick, 1992). With respect to aging, a slowing down of the elongation phase of protein synthesis has been suggested to be crucial. 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 (Webster, 1985, 1986; Richardson and Semsei, 1987). In vivo, a twofold decrease in the rate of polypeptide chain elongation in old WAG albino rat liver and brain cortex has been reported (Khasigov and Nikolaev, 1987). 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 two-year-old animals (Merry and Holehan, 1991). 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 two elongation factors, EF-1 and EF-2, in eukaryotes (a third factor, EF-3, is reported only in yeast), which are highly conserved during evolution (Riis *et al.*, 1990a). Depending on the relative amounts of EF-1α and EF-1$\beta\gamma$, EF-1 is found in differently aggregated heavy or light forms that might reflect the overall protein synthetic activity of the tissue. There is a high abundance of EF-1α (between 3 and 10% of the soluble protein), and there are multiple copies or isoforms of the gene that undergo cell type and/or developmental stage-specific expression reported in yeast, fungi, brine-shrimp, *Drosophila,* toad, and mammalian cells and tissues (Knudsen *et al.*, 1993). EF-1α has several other functions in addition to its requirement in protein synthesis; for example, it binds to cytoskeletal elements; it is associated with endoplasmic reticulum; it is part of the valyl–tRNA–synthetase complex; it is associated with mitotic apparatus; it is involved in maintaining the accuracy of protein synthesis and protein degradation; it binds calmodulin in protozoan parasites; it induces rapid fragmentation of cytoplasmic microtubule arrays in fibroblasts; and its overexpression increases the susceptibility of mammalian cells to transformation (for review, see Rattan, 1995).

The activity of EF-1 declines with age in rat livers and *Drosophila*, and the drop parallels the decrease in protein synthesis. This decline in the activity of EF-1 has been correlated only to EF-1α, as no changes were observed in the EF-1βγ-mediated activity. Using more specific cell-free stoichiometric and catalytic assays, a 35–45% decrease in the activity and amounts of active EF-1α has been reported for senescent human fibroblasts, old mouse and rat livers and brains (for review, see Rattan, 1991, 1992, 1995). The germ line insertion of an extra copy of EF-1α gene under the regulation of a heat-shock promoter resulted in a better survival of transgenic *Drosophila* at high temperature (Shepherd *et al.*, 1989). This relative increase in the lifespan, however, was not accompanied by any increase in the levels of mRNA, amount, and activity of EF-1α (Shikama *et al.*, 1994). Similarly, no increased expression of EF-1α genes was observed in *Drosophila* with extended longevity phenotype in a long-lived strain (Dudas and Arking, 1994). However, the increased longevity of EF-1α high-fidelity mutants of a fungus *Podospora anserina* suggest that the life prolonging effects of EF-1α may be due to its role in maintaining the fidelity of protein synthesis (Silar and Picard, 1994). Future studies on other aging systems will clarify the role of EF-1α in the regulation of both protein synthesis and longevity.

In the case of EF-2 that catalyses the translocation of peptidyl-tRNA on the ribosome during the elongation cycle, conflicting data are available regarding the changes during aging. 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 (Webster, 1985, 1986). Similarly, although the proportion of heat-labile EF-2 increases during aging, the specific activity of EF-2 purified from old rat and mouse liver remains unchanged (Takahashi *et al.*, 1985a). In contrast, a decline of more than 60% in the amount of active EF-2 has been reported during aging of human fibroblasts in culture, measured by determining the content of diphtheria toxin-mediated ADP-ribosylatable EF-2 in cell lysates (Riis *et al.*, 1990b). Further studies are required to determine if there are any qualitative and quantitative changes in EF-2 at the levels of transcription, translation, and posttranslational modifications, and how such changes are related with the regulation of protein synthesis during aging.

Other major components of protein synthetic machinery are tRNAs and aminoacyl-tRNA sythetases (aaRS). Levels of tRNAs and aaRS have been considered to be rate limiting for protein synthesis. According to the so-called codon restriction theory of aging (Strehler *et al.*, 1971), a random loss of various isoaccepting tRNAs will progressively restrict the readability of codons, resulting in the inefficiency and inaccuracy of protein synthesis. There is some evidence that a shift in the pattern of isoaccepting tRNAs occurs during development and aging in some plants, nematodes, insects, and rat liver and skeletal muscle (Vinayak, 1987). Similarly, a 30- to 60-fold increase in the amount of UAG suppressor tRNA has been reported in the brain, spleen, and liver of old mice, and has been related to increased expression of Moloney murine leukemia virus (MO-MuLV) in fibroblasts (Schröder *et al.*, 1992). Other characteristics of tRNAs that have been studied during aging include the rate of synthesis, total levels, aminoacylation capacity, and nucleoside composition. The aminoacylation capacity of different tRNAs varies to different extents during aging, and the reasons for such variability are not known. The fidelity of aminoacylation did not differ significantly in cell-free extracts prepared from young and old rat livers. There is no generalized pattern that emerges from these studies, and the reported changes vary significantly among different species (Rattan, 1992, 1995).

In the case of aaRS, an increase or decrease in the specific activities of almost all of them has been reported in various organs of aging mice without any apparent correlation with tissue/cell

type and its protein synthetic activity. An increase in the proportions of the heat-labile fraction of several of these enzymes has been reported in the liver, kidney, and brain of old rats (Takahashi *et al.*, 1985b). However, no universal pattern can be seen for the changes in the activities of various synthetases in different organs and in different animals. Although an age-related decrease in the efficiency of aaRS can be crucial in determining the rate and accuracy of protein synthesis, direct evidence in this respect is lacking at present. Studies on aging *Drosophila* and old rat livers and kidneys have shown that the release of ribosome bound N-formylmethionine, a measure of the rate of termination, was not affected with age (Webster, 1985, 1986). Direct estimates of the activity of the termination factor during aging have not been yet made.

POSTSYNTHETIC MODIFICATIONS DURING AGING

Age-related changes in the functioning of proteins can be due to both inefficient protein synthesis and an altered pattern of posttranslational modifications. A large number of posttranslational modifications of proteins have been described that determine the activity, stability, specificity, transportability, and lifespan of a protein. A brief discussion of some of the major protein modifications follows.

Oxidation

Accumulation of inactive and abnormal proteins during aging is a widely observed phenomenon. 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 (Stadtman, 1992). Because 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. Increased levels of oxidatively modified proteins have 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. There was a twofold increase in the protein carbonyl content of the brain proteins of retired breeder Mongolian gerbils, which was reversed by treatment with a spin-trapping compound *N-tert*-butyl-a-phenylnitrone (PBN; Carney *et al.*, 1991). An age-related increase in the carbonyl content has also been reported for houseflies (Sohal *et al.*, 1993a), mouse organs (Sohal *et al.*, 1993b), and *Drosophila* (Orr and Sohal, 1994).

The concentration of the oxidation products of human lens proteins and skin collagen increases along with the accumulation of oxidative forms of α-crystallin in patients with age-related cataract (Stadtman, 1992). 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 (Wells-Knecht *et al.*, 1993).

Structural alterations introduced into proteins by oxidation can lead to the aggregation, fragmentation, denaturation, and distortion of secondary and tertiary structure, thereby increasing the proteolytic susceptibility of oxidized proteins. Thus, the accumulation of abnormal proteins during aging may be due to an impairment of the protein degradation processes and/or defective protection from oxidative damage.

Glycation

Glycation is one of the most prevalent covalent modifications in which the free amino groups of proteins react with glucose forming a ketoamine called Amadori product. This is followed by a sequence of further reactions and rearrangements producing the so-called advanced glycosy-

lation end products (AGEs; Lis and Sharon, 1993). It is the long-lived structural proteins such as lens crystallins, collagen, and basement membrane proteins, which are more susceptible to glycation. The glycated proteins are then more prone to form crosslinks with other proteins, leading to structural and functional alterations.

There is an increase in the level of glycated lysine residues of rat sciatic nerve, aorta, and skin collagen during aging (Oimomi *et al.*, 1988). Similarly, there is an increase in the glycation of human collagen and osteocalcin during aging (Miksík and Deyl, 1991). The formation and the accumulation of the AGEs are implicated in the physiology and pathology of senescence. Pentosidine (crosslinked glycated lysine and arginine) and carboxylmethyllysine (glycated and oxidated proteins) increase with age in humans. Pyrroline, another AGE protein, has been shown to increase in diabetics (Lee and Cerami, 1992). 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 (Makita *et al.*, 1992).

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 (Gafni, 1990). 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 (Gracy *et al.*, 1985). Deamidation of glucose-6-phosphate isomerase produces the variant of the enzyme that accumulates in aging bovine lenses (Cini and Gracy, 1986).

The interconversion of optical isoforms of amino acids, called racemization, has been reported to increase during aging. The concentration of D-aspartate in protein hydrolysates from human teeth, erythrocytes, and eye lens increases with age (Brunauer and Clarke, 1986). Racemization of tyrosine occurs in the aging brunescent human cataract lenses (Luthra *et al.*, 1994). 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 posttranslational 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.

Phosphorylation

Phosphorylation of serine, threonine, and tyrosine residues is one of the best-studied modifications of proteins. The coordinated activities of protein kinases, which catalyze phosphorylation, and protein phosphatases, which catalyze dephosphorylation, regulate several biological processes, including protein synthesis, cell division, signal transduction, cell growth, development, and aging.

Inhibition of DNA synthesis and the loss of proliferative capacity is the ultimate characteristic of normal diploid cells undergoing aging in vitro. 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. 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 (Dulic' *et al.*, 1993), and failure to phosphorylate *RB1* gene product $p110^{Rb}$ (Stein *et al.*, 1990), and *cdc2* product $p34^{cdc2}$ (Richter *et al.*, 1991) during cellular aging have been reported. It will be important to find out if there are age-related alterations in the phosphorylation state of other cell cycle related gene products, and proteins involved in DNA and RNA synthesis, including various transcription factors.

Various components of the protein synthetic apparatus undergo phosphorylation and dephosphorylation and, thus, regulate the rates of protein synthesis (Merrick, 1992). 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. At the level of protein elongation, the phosphorylation of elongation factors EF-1α and EF-2 appears to be involved in regulating their activities (Ryazanov *et al.*, 1991). Because it has been reported that the activity and amounts of active EF-1α and EF-2 decrease significantly during aging, it will be interesting to see whether this decline is accompanied by a parallel change in the extent of phosphorylation of these enzymes. There is indirect evidence that alterations in the phosphorylation and dephosphorylation of EF-2 due to changes in the activities of EF-2–specific protein kinase III (Riis *et al.*, 1993), and PP2A phosphatase (Riis *et al.*, 1995) may affect the rates of protein synthesis during aging.

Phosphorylation also occurs in other proteins that participate in the translational process. 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.

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 a single or multiple kinases and the ratio between two interconvertible, active and inactive, forms of kinases 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 PKC to elicit signalling pathway (Blumenthal *et al.*, 1993). Senescent human fibroblasts retain their ability to phosphorylate proteins in the PKC signal transduction pathway (Shigeoka and Yang, 1990). It appears that the PKCs are largely unaltered in aging fibroblasts, although the body of information about phosphorylation mechanisms is very limited still.

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 (De Tata *et al.*, 1993). 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 (De Tata *et al.*, 1993; Derventzi *et al.*, 1993). 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 (Miller, 1994). Thus, phosphorylation of a wide variety of proteins has significant influence in biological processes and it will be extremely useful to undertake detailed studies on this posttranslational modification of various proteins in relation to the process of aging.

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 α and β, and cytoplasmic proteins adenyl cyclase and elongation factor EF-2 are modulated by ADP-ribosylation (Riis *et al.*, 1990a). 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.

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 (Dell'Orco and Anderson, 1991). Furthermore, 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 (Grube and Bürkle, 1992).

One cytoplasmic protein that can be specifically ribosylated by diphtheria toxin and exotoxin A is the protein elongation factor EF-2. ADP-ribosylation of the diphthamide (modified histidine 715) residue of EF-2 results in the complete abolition of its catalytic activity (Riis *et al.*, 1990a). There is evidence that increased ADP-ribosylation of EF-2 is correlated with cellular aging (Riis *et al.*, 1990b). Many more studies are required to establish the role of ADP-ribosylation on the activity of various proteins during aging.

Methylation

Methylation of nitrogens of arginine, lysine, and histidine, and carboxyls of glutamate and aspartate residues is a widely observed posttranslational 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.

Proteins whose activities are increased by methylation include alcohol dehydrogenase, histones, ribosomal proteins, cytochrome C, elongation factor EF-1α, myosin, myelin, and rhodopsin. Of these, decreased methylation of histones in livers and brains of aging rats has been reported. 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 (for details, see Rattan *et al.*, 1992).

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 (Mays-Hoopes, 1985). 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 (McFadden and Clarke, 1986). The number of carboxylmethylatable sites of cerebral membrane-bound proteins also increases in rat brain during aging (Sellinger *et al.*, 1988). 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

There are no systematic studies performed on age-related changes in posttranslational 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 considered to be derived from the proteolysis of band 3 protein (Kay, 1990). The exposure of senescent cell-specific epitopes on fibronectin (Porter *et al.*, 1992), 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 (Hébert *et al.*, 1994). Proteolytic cleavage of the β-amyloid precursor protein is well known to play an important role in the pathogenesis of Alzheimer's disease (Selkoe, 1992). 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 (DiPaolo *et al.*, 1992). Similarly, alterations in the activity of collagenase during aging of human fibroblasts has been suggested to be due to structural and catalytic changes (Baur *et al.*, 1985; Sottile *et al.*, 1989).

Other modifications

In addition to the types of posttranslational 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 EF-1α may be involved in determining its stability and interaction with intracellular membranes (Riis *et al.*, 1990a). The protein initiation factor eIF-5A contains an unusual amino acid, hypusine, which is synthesized posttranslationally as a result of a series of enzymatically catalyzed alterations of a lysine residue (Park *et al.*, 1993). Because the absence of hypusine in eIF-5A blocks the initiation of protein synthesis, it will be interesting to investigate changes in this modification during aging when total protein synthesis slows down.

Protein tyrosine sulfation is another posttranslational 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. 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. 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. Recent evidence shows 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 cholestrol, ubiquinone, and dolichol (Thelin *et al.*, 1994).

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. According to the crosslinking theory of aging, the progressive linking together of large vital molecules, especially the proteins, results in the loss of cellular functions (Bjorksten and Tenhu, 1990). 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 (Nagaraj *et al.*, 1991). Similarly, an increase in transglutaminase activity during cellular apoptosis, differentiation, and aging of human keratinocytes (Saunders *et al.*, 1993) indicates an important role of this modification in the process of aging.

In conclusion, it is clear that an efficient and accurate translational machinery and the posttranslational modifications of proteins constitute the fundamental biochemical processes for cellular functioning and survival. Alterations at the level of protein synthesis and their postsynthetic modifications can have global detrimental effects on the maintenance and survival of cells, tissues, organs, and organisms leading to aging and death.

PROTEIN TURNOVER

Efficient macromolecular turnover is integral to the normal functioning and survival of a biological system. Protein degradation during aging is a relatively little researched topic as compared with RNA and protein synthesis. Although there are several hundred-fold variations in the rates of degradation of individual proteins, it is generally believed that protein turnover slows down during aging (for a recent comprehensive review, see Van Remmen *et al.*, 1995). The physiological consequences of decreased protein turnover include the accumulation of altered and abnormal proteins in the cell, an altered pattern of posttranslational modifications due to increased dwell time, and a disruption of the organisation of the cytoskeleton and extracellular matrix.

Age-related decline in protein turnover is generally due to a decrease in the proteolytic activity of various lysosomal and cytoplasmic proteases. It is only recently that the molecular details of various pathways of protein degradation, such as the proteasome-mediated, ubiquitin-mediated, and the lysosome-mediated pathways, have begun to be studied in relation to aging. For example, ubiquitin marking of proteins for degradation and ubiquitin-mediated proteolysis did not decline in aging human fibroblasts, and no change in the levels of ubiquitin mRNA and ubiquitin pools was detected (Pan *et al.*, 1993).

Other reasons for age-related changes in the activities of various proteases leading to a decrease in the rate of protein turnover include slower transcription, reduced rates of synthesis and altered pattern of postsynthetic modifications, as discussed above. There is evidence that certain inhibitors of various proteases, such as tissue inhibitor of metalloproteinases (TIMP; Wick *et al.*, 1994), and trypsin inhibitor (Hearn *et al.*, 1994) had increased levels of expression and activities during aging of human fibroblasts. This will also lead to a decrease in the activities of proteases leading to decreased protein degradation during aging.

Finally, the synthesis, modifications, and turnover of proteins are interdependent processes that practically set a limit on the efficiency of genetic information transfer from coded molecules to functional molecules. Aging as the failure of maintenance can be fully understood by studying various enzymatic and other protein mechanisms and networks of repair and maintenance, which progressively become inefficient during aging. Analyzing the synthesis of proteins, their modifications that determine their activity, stability, and specificity, and turnover of inactive and altered proteins is central in this regard.

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