Amino Acids (2003) 25: 397–407
DOI 10.1007/s00726-003-0025-9

Amino Acids
Printed in Austria

Protein oxidation in aging: endoplasmic reticulum as a target

Review Article

D. van der Vlies[1], **J. Woudenberg**[2], and **J. A. Post**[2]

[1] Erasmus MC, Department of Internal Oncology – Josephina Nefkens Instituut, Rotterdam, The Netherlands
[2] Department of Molecular Cell Biology, Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands

Received December 7, 2002
Accepted May 8, 2003
Published online August 28, 2003;

Summary. Oxidatively modified proteins have been shown to correlate with the age of an organism or its tissues. An increase in tissue-susceptibility to experimentally induced protein oxidation not only depends on tissue type and age, but also on the maximum lifespan potential of the species. A general, although tissue dependent, decline in anti-oxidative defenses during aging may very well be responsible for this difference in vulnerability. In addition, the level of protein modifications also depends on the nature and the subcellular localization of the proteins involved. Damage to the endoplasmic reticulum (ER), and its subsequent impaired functionality may be involved in the process of aging. This is suggested by; (1) an upregulation of ER stress-response chaperones, (2) a preferential oxidation of ER-resident proteins and, (3) a disturbance of calcium homeostasis. Therefore, this review will focus on the putative involvement of the oxidized endoplasmic reticulum in the process of aging.

Keywords: Aging – Protein oxidation – Endoplasmic reticulum – Oxidative stress – TyrFluo – Fluorescent probe

1. Introduction

During the last decades, evidence has been obtained that aging is a function of several closely interrelated parameters, such as the metabolic rate, caloric intake, genetics, lifestyle and environmental factors (Schoneich, 1999). But what is aging? Biological aging could be defined as, ''A complex process in which diverse deleterious changes in cells and tissues accumulate with advancing age. These changes cause a loss of cellular function that results in an increased mortality and represents a major risk factor for age-related diseases like cancer, diabetes mellitus, rheumatoid arthritis, neurodegenerative, and cardiovascular diseases.'' Due to its complexity, a very large number of theories have been proposed to explain the process of aging (Merker et al., 2001). For example, the aging process has been attributed to molecular cross-linking (Bjorksten, 1968), changes in immunologic function (Walford, 1974), free radical damage (Harman, 1993), cellular senescence (Hayflick and Moorhead, 1961), telomere shortening (Kruk et al., 1995), mitochondrial DNA damage (reviewed in (Sastre et al., 2002)) and most recently to the Maillard reactions of DNA (Baynes, 2002).

Although no single theory is completely accepted as being the single cause of aging, it is now beyond doubt that reactive oxygen species (ROS) generated *in vivo*, play a role in aging, as already proposed in 1956 (Harman, 1956). This free radical theory of aging has evolved since then (reviewed (Beckman and Ames, 1998)). Due to the high reactivity of several oxygen intermediates, many lipids, proteins and DNA are substrates for oxygen-mediated alterations. Since cellular reactive by-products of normal metabolism also lead to damage, this theory has recently been extended to the oxidative ''garbage catastrophe theory'' where ROS or reactive oxygen intermediates are responsible for the accumulation of age-related cellular damage of biomolecules (Terman, 2001). According to this theory, aging may derive from imperfect clearance of oxidatively damaged, relatively indigestible material, the accumulation of which further hinders cellular catabolic and anabolic functions and mainly affects postmitotic non-proliferating cells. Further support is provided by the finding that overexpression of enzymes that prevent the generation of radicals, like superoxide dismutase (SOD) and catalase (Orr and Sohal, 1994; Parkes et al., 1998), or that reduce oxidized proteins, like methionine

sulfoxide reductase A (MSRA) (Ruan et al., 2002), extend the lifespan of the fruit fly *Drosophila melanogaster* markedly. SOD dismutates superoxide ($O^{2-\cdot}$) to form hydrogen peroxide (H_2O_2), which is subsequently broken down by catalase to water and oxygen. MSRA catalyzes the repair of oxidized methionine in proteins by reducing methionine sulfoxide back to methionine. In agreement with this, mutations in the age-1 gene of *Caenorhabditis elegans* result in an age-specific increase in the activity of catalase and Cu/Zn-SOD and double the life span (Vanfleteren, 1993).

Moreover, it has been shown that, in fibroblasts from individuals with the premature aging diseases progeria or Werner's syndrome, the levels of oxidatively modified proteins are significantly higher than age-matched controls (Oliver et al., 1987). In addition, the basal levels of the primary antioxidant enzymes Mn-SOD, catalase and glutathion peroxidase were found to be decreased in progeria fibroblasts (Yan et al., 1999). These findings also suggest a contribution of ROS-mediated damage to the accelerated aging process characteristic for these diseases.

So, whether or not radicals determine life span, it is becoming increasingly apparent that they play an important role in the (patho)physiology of aging (Stadtman, 2002). After a short overview of the different aging theories and the relation between caloric restriction and protein oxidation, this review will focus on the putative involvement of the endoplasmic reticulum in the process of aging.

2. Age-related protein oxidation

It is now well established that biological aging correlates with the accumulation of chemically modified biomolecules in tissue such as oxidized proteins, lipids, DNA bases, advanced glycation end-products and lipofuscin. Studies on oxidatively modified proteins have revealed an age-related increase in the level of protein carbonyl content (Levine, 2002), oxidized methionine (Wells-Knecht et al., 1997), protein hydrophobicity (Meucci et al., 1991), cross-linked proteins (Squier, 2001) and glycated proteins (Baynes, 2001) as well as the accumulation of catalytically less active enzymes (Friguet et al., 1994; Oliver et al., 1987; Rothstein, 1984; Zhou and Gafni, 1991) that are more susceptible to heat inactivation and to proteolytic degradation (Stadtman, 2001). In order to find out about the role of protein oxidation in the aging process, stable markers of ROS-induced oxidation are required. Several possibilities may be the cause of an increase in the steady-state level of oxidatively modified proteins. These include (1) an increase in the formation of oxidizing species (Schoneich, 1999), (2) a decreased capacity to scavenge those species (Sohal et al., 1990), (3) an increased susceptibility of the proteins to become oxidized as a consequence of transcriptional and translational errors (Dukan et al., 2000), and (iv) a decrease in the levels or activities of the proteasome (Petropoulos et al., 2000) or proteases that selectively degrade oxidized proteins (Giulivi and Davies, 1993).

Although remarks can be put forward about the accuracy of the assays used or the stability of the formed products several protein oxidation markers have been shown to relate with aging. In Table 1 age-related protein oxidation markers are presented of both extracellular and intracellular proteins as well as the tissues they originate from; these modifications are further discussed in the text. One of the best-known markers of age-related protein oxidation is the carbonyl group. The carbonyl content of proteins has been observed to increase with age (Berlett and Stadtman, 1997; Dean et al., 1997; Levine, 2002; Linton et al., 2001). While an increase in carbonyl content was measured in most regions of the mouse brain between the age of 8 to 27 months, age-associated loss of protein sulfhydryls was more uniform across brain regions.

Table 1. Specific protein oxidation markers, in extra- (1–3) and intracellular (4–9) proteins from human tissue, that correlate with aging

Tissue	Marker
1. Skin collagen	N^{ε}-(carboxymethyl)-lysine (CML) N^{ε}-(carboxymethyl)-hydroxylysine (CMHL) pentosidine o-tyrosine methionine sulphoxide (MetSOx)
2. Articular cartilage	CML N^{ε}-(carboxyethyl)-lysine (CEL) pentosidine
3. Lens protein	CML L-3,4-dihydroxyphenylalanine (L-DOPA) o,o′-dityrosine 3-hydroxy valine
4. Brain protein	protein carbonyl content
5. Kidney	protein carbonyl content
6. Liver	MetSOx o,o′-dityrosine
7. Cardiac muscle	o,o′-dityrosine o-tyrosine[1] protein carbonyl content
8. Skeletal muscle	o,o′-dityrosine

[1] The correlation of o-Tyr with aging in cardiac muscle is rather uncertain

Dietary restriction resulted in reversal of age-associated carbonyl and sulfhydryl concentration and was found to retard age-associated decline in learning and coordination problems (Dubey et al., 1996). A comparison of ad libitum fed and dietary restricted mice at 9, 17 and 23 months of age indicated that the protein carbonyl content in the brain, heart and kidney increased with age and was significantly greater in the ad libitum fed group in each organ and at each of the three ages (Sohal et al., 1994). However, the accuracy of the assays used to determine the carbonyl content has been seriously questioned (Ayala and Cutler, 1996), since carbonyls may also be derived from other sources besides oxidation of amino acid residues, such as collagen crosslinking, or glycosylation of proteins (Levine et al., 1994). In addition, nucleic acids also contain carbonyl groups. The main carbonyl products of metal-catalyzed oxidation of proteins *in vitro* have been shown to be glutamic and aminoadipic semialdehydes (Requena et al., 2001).

Other markers may be derived from ROS-induced protein oxidation, but may be susceptible to further reactions. For this reason, a marker like L-DOPA (L-3,4-dihydroxyphenylalanine), a product of tyrosine oxidation, is not an ideal marker for protein oxidation. Protein-bound DOPA may be further oxidized to dopaquinone or directly degraded by proteinases, releasing free DOPA. Subsequently, DOPA may form Michael adducts with free amino acids such as cysteine or may be excreted via the urine as free DOPA or as cysteinyl-DOPA (Davies et al., 1999; Rodgers, 2000). Tyrosine residues may be oxidized by hypochlorite, peroxynitrite or by radicals formed in transition metal ion-catalyzed Fenton and Haber-Weiss reactions (e.g. hydrogen peroxide/Fe^{2+}) (Davies, 1987; Heinecke et al., 1993; Van der Vliet, 1995). The ensuing tyrosyl radicals may form intra- or intermolecular o,o′-dityrosine bonds (Aeschbach, 1976). Levels of o,o′-dityrosine have been shown to increase with age in human lens (Wells-Knecht et al., 1993), and cardiac and skeletal muscle of ad libitum fed mice (Leeuwenburgh et al., 1997). Caloric restriction prevented the increase in o,o′-dityrosine levels in these animals. Moreover, the oxidation-dependent increase of hydrophobicity of rat liver proteins was even correlated with an increase in the levels of methionine sulfoxide (MetSOx) and of o,o′-dityrosine (Chao et al., 1997).

Several other oxidized residues, like hydroperoxides of amino acid side chains are highly unstable. Some oxidation products, like cystine, are inappropriate markers being a natural product as well as a ROS-induced oxidation product, while N-formylkynurenine (an oxidation product of Trp) can be generated enzymatically. MetSOx and disulfides may be enzymatically reduced, resulting in an underestimation of the oxidation levels, whereas other species, like 4-hydroxyproline, coelute with their stereoisomer, making it a poor marker. Besides the accumulation of glycated proteins in lens (see below), extensive hydroxylation of protein-bound amino acid residues has been shown to associate with the development of age related cataract (Fu et al., 1998). The relative abundance of the oxidized amino acids in these lens protein (assessed per parent amino acid) is L-3,4-dihydroxyphenylalanine (DOPA) > o- and m-tyrosine > 3-hydroxyvaline, 5-hydroxyleucine > dityrosine.

In the study of age-related increases in levels of oxidized material, disparities have been observed between intracellular and extracellular proteins. In extracellular proteins, the levels of oxidative markers were found to increase more with age than in intracellular proteins (Linton et al., 2001). This disparity might be explained by a difference in turnover between extracellular (hours-days) and intracellular proteins (minutes-hours). The difference in homeostatic control between extra- and intracellular proteins might also play a role. Upon extracellular radical damage, a lack of availability of antioxidants, reductants and repair mechanisms might explain the higher extracellular levels of oxidative modifications. Furthermore, disparities were observed in levels of oxidized proteins in the organs liver, skeletal muscle and eye lens. These differences may be based on different metabolic rates in these organs. Higher metabolic rates in mice may also account for disparities in rates of ROS production and protein oxidation between mice and rats. In mice, higher levels of oxidized protein have been observed as compared to rats (Linton et al., 2001). In the cardiac muscle of aging mice, an increase in the levels of o-tyrosine has been observed, in contrast with the cardiac muscle of rats (Linton et al., 2001). So the correlation of o-tyrosine with aging in cardiac muscle is rather uncertain. In several studies on the eye lens (Dean et al., 1997; Thomas and Mallis, 2001), increases in protein carbonyls, MetSOx, L-DOPA, o- and m-tyrosine, Val and Leu hydroxides and the oxidation of Cys residues to sulfinic acids and cysteic acid have been reported with aging as well as a loss of Met and Cys. Together with the markers, presented in Table 1, the accumulation of these markers gives growing evidence for the age-related accumulation of oxidized proteins in extracellular tissue. As for intracellular proteins, an age-related accumulation of oxidized proteins still requires further proof, although a decrease in cellular antioxidant defense as well as an increase in intracellular protein oxidation has been reported several times (Berlett and Stadtman, 1997; Stadtman, 1992).

3. Oxidation of long-lived proteins

For the study of aging and age-associated phenomena, long-lived extracellular biomolecules are of fundamental interest. Some of the longest-lived proteins of the body, the crystallins, can be found in the ocular lens. Their low turnover has made crystallins a subject of widespread research activity in determining various posttranslational modifications of aged proteins (Merker et al., 2001). Next to crystallins, extracellular proteins like collagen and elastin have a longer lifetime, making them also suitable for studying age-associated phenomena (Bailey et al., 1998). Perhaps the most important changes in collagen and elastin with age involve the formation of intermolecular cross-links. These cross-links are initially formed (through lysyl oxidase) to provide an optimum function during development and maturation, but can subsequently over-stiffen and compromise the structure and function of the fibers throughout the body when present in excess (Bailey, 2001).

A second process of cross-linking is based on the reaction of proteins with glucose or its metabolites that occurs with age as the turnover of the proteins is reduced to a minimum for that particular tissue (Baynes, 2001). The end-stage products of Maillard reactions in biological systems is the result of rearrangement, dehydration, oxidation and fragmentation reactions of glucose or its adducts to protein and are known as advanced glycation end-products (AGE) (Baynes, 2001). AGEs play an important role in the pathogenesis of angiopathy in diabetic patients and in the aging process. About a dozen different AGEs have been identified of which several are known to accumulate with age, particularly in long-lived proteins such as collagens and crystallins. N^{ε}-(carboxymethyl)lysine (CML in Table 1) is formed on oxidative cleavage of carbohydrate adducts to lysine residues in glycated proteins *in vitro* (Ahmed et al., 1988) and has shown to accumulated in aged human lens (Dunn et al., 1989). Another AGE that has shown to accumulate with aging is pentosidine (Dyer et al., 1993; Verzijl et al., 2000). Pentosidine, named after the reaction of pentoses with proteins, forms crosslinks between arginine, lysine, and a pentose (Sell et al., 1991). In concert with CML and pentosidine, formed during glycoxidation of collagen in aged human skin (Cefalu, 1995), ortho-tyrosine and methionine sulfoxide are formed (Dunn et al., 1991; Dyer et al., 1993; Wells-Knecht et al., 1997). An accelerated rate of this age-dependent chemical modification of collagen was observed in diabetes (Vlassara, 2002), but could not be subscribed to a general increase in oxidative stress in this disease. A marked increase of CML and pentosidine, and N^{ε}-(carboxyethyl)lysine to a lesser extend were also found in articular cartilage collagen, which may very well contribute to the more rigid and fragile nature of cartilage with advancing age (Verzijl et al., 2000).

In view of all the above-mentioned complications only a few side chain oxidation products are appropriate markers for measuring age-related increases in protein oxidation. For measuring these increases, immunologic methods may be used as well as GC/MS and HPLC (Davies et al., 1999). An alternative approach might be the introduction of probes, which will covalently label oxidatively modified proteins. We recently introduced of a probe, denoted as TyrFluo, which only labels oxidized proteins and which has made it possible to detect intracellular and extracellular oxidized proteins (Van der Vlies et al., 2001). The probeconsists of a fluorescein-labelled tyrosine analogue (i.e. tyramine) that upon oxidation by ROS is converted into a tyrosyl radical that can form cross-links with oxidized target proteins. As a result of this coupling reaction these proteins become fluorescently labelled and thus can be visualized by fluorescence microscopy. Further more the covalently modified proteins can be detected on a Western-blot by immunodetection using an anti-fluorescein antibody. The non-membrane permeant TyrFluo has been used to label cell surface proteins. Acetylation of TyrFluo (AcetylTyrFluo) makes the probe membrane-permeable so that intracellular proteins can be labelled. (The nature of the detected proteins will be discussed later)

4. Aging and the endoplasmic reticulum

As referred to above, there are numerous (oxidative) theories of aging. As we will discuss here damage to the endoplasmic reticulum (ER), and its subsequent impaired functionality may be involved in the process of aging. There are several phenomena that suggest its involvement in the aging process, like (1) the upregulation of ER stress response chaperones, the expression of which is decreased upon caloric restriction, (2) the susceptibility towards oxidation of ER resident proteins that are involved protein folding and, (3) impairment of maintaining the calcium homeostasis.

In this perspective it is noteworthy that in age-related neuronal diseases ER dysfunction has been reported (Paschen, 2001). Whereas the ER-associated degradation of misfolded proteins is affected in Parkinson's disease, it is the unfolded protein response that is down-regulated in Alzheimer's disease and the ER calcium homeostasis that is disturbed in ischemia.

4.1. Caloric restriction and stress response proteins

Caloric restriction, the only method known for extending life span, delays most age-related physiologic changes and is the most effective means known for reducing cancer incidence and increasing the mean age of onset of age-related diseases and tumors (Weindruch and Walford, 1982). The process of aging has shown to be associated with specific gene expression profiles, indicative of a marked stress response and lower expression of metabolic and biosynthetic genes of individual organs (Weindruch et al., 2001). When compared at the same chronological age, strains of short-lived mouse contain higher levels of oxidized proteins than the longer-lived strain (Sohal, 1993).

The beneficial age-associated effects of caloric restriction are reflected in, (1) an inhibition of accumulation of oxidatively damaged proteins (Lass et al., 1998; Youngman et al., 1992), (2) a reduction of enzyme activity decline in old rats (Aksenova et al., 1998), (3) a significant decrease of H_2O_2 production of rat liver mitochondria correlating to a significant reduction of oxidative damage to mtDNA (Lopez-Torres et al., 2002), and, (4) alterations of gene expression, which suggests that caloric restriction retards the aging process by causing a metabolic shift toward increased protein turnover and decreased macromolecular damage (Lee et al., 1999; Weindruch et al., 2002).

The glucose regulated proteins (GRP) are a family of stress-induced molecular chaperones (Little et al., 1994). Expression of these proteins is induced in cultured cells by agents that interfere with the normal glycosylation, folding or assembly of proteins in the endoplasmic reticulum thereby increasing the level of misfolded proteins (Lee, 1994). The importance of protein oxidation in biological aging is reflected in the age-dependent upregulation of stress response genes that process damaged or misfolded proteins (Lee et al., 1999). Therefor, the influence of dietary energy restriction on expression of the ER associated chaperone RNA was investigated in mice liver (Dhahbi et al., 1997; Spindler et al., 1990). Although extreme glucose deprivation increases GRP mRNA levels in cultured cell lines, physiologically relevant reductions in blood glucose, as a consequence of long-term energy restriction, had the opposite effect in the liver, *in vivo*. It was suggested that life-prolonging energy restriction might act to reduce misfolded proteins in the endoplasmic reticulum of hepatic cells since the expression of nearly all endoplasmic reticulum chaperones responded rapidly and specifically to dietary energy in mice. A reduction of hepatic expression of the mRNA's of GRP78 (BiP), GRP94 (endoplasmin), GRP170, ERp72, ERp57, calreticulin and calnexin but no change in protein disulfide isomerase (PDI) was observed. PDI is responsible for isomerisation of protein disulfide bonds during or shortly after synthesis to yield proteins with native disulfide bonds (Freedman, 1984). ERp72 and ERp57 are also involved in the rearrangement of both intrachain and interchain disulfide bonds in proteins to form the native structures since they possess the thiol-dependent reductase activity of PDI (Lundstrom-Ljung et al., 1995) and cysteine protease activity (Otsu et al., 1995).

4.2. Oxidation of ER proteins that are involved protein folding

The mitochondrion and the peroxisome as major sites of ROS production in the cell are well protected against oxidative damage by anti-oxidant enzymes. Thus superoxide anion produced in the respiratory chain is converted by Mn-SOD whereas H_2O_2 produced in the peroxisomal β-oxidation is degraded by catalase. Such a defense mechanism has not been described for the ER and may play a role in the recent observation that particularly proteins of the ER are susceptible to oxidation by H_2O_2 (Van der Vlies et al., 2002). Upon incubation of fibroblasts with 10 μM H_2O_2 we identified, by use of the earlier discussed membrane-permeable probe (acetylTyrFluo) (Van der Vlies et al., 2001), proteins of the folding/quality control system in the ER of normal human dermal fibroblasts as the major targets for this oxidative stress. These proteins included BiP/GRP78 (Immunoglobulin heavy chain binding protein or 78 kDa glucose-regulated protein), calnexin, GRP94/endoplasmin, protein disulfide isomerase (PDI), and protein disulfide isomerase ER-60 precursor (also known as ERp60/58 kDa microsomal protein/p58 /GRP58/ERp57) that are all known to reside in the ER lumen. Since H_2O_2 itself is a weak oxidant it must be converted into highly reactive hydroxyl radical ($OH^{\cdot}$) for proteins and TyrFluo to become oxidized. Given the very short lifetime of the $OH^{\cdot}$ ($< 10^{-6}$ sec) we infer that the radical formation most likely occurs in the ER. Here H_2O_2 may be converted into $OH^{\cdot}$ by cytochrome P450 enzymes. It has been shown that cytochrome P450 can operate *in vitro* as a peroxygenase using peroxy compounds as the oxygen donor (Nordbloom et al., 1976). Anari et al. (1995) demonstrated that cytochrome P450 in intact rat hepatocytes could function as a peroxygenase utilizing *tert-butyl*-hydroperoxide. They also showed the involvement of cytochrome P450 in the metabolic bio-activation of cumene

hydroperoxide and suggested the formation of reactive radical metabolites in this reaction (Anari et al., 1996). Another possibility is that H_2O_2 is converted into $OH^{\cdot}$ by the Fenton reaction using transition metal ions available in the ER (Winston et al., 1984).

The ER plays a central role in the synthesis and distribution of many cellular proteins. Before proteins can be transported towards their final destination, disulfide bonds essential for a proper folding have to be formed (Braakman, 1991). A requirement for this oxidative protein folding is a high redox-state (Braakman, 1992), which implies that there must be an optimum ratio of thiol and disulfide (<10). The typical redox-state of the cytosol is too reducing for formation of protein disulfide bonds. In the ER lumen, the relative abundance of the oxidized (GSSG) compared to the reduced (GSH) form of glutathione has led to the proposal that GSSG serves as the oxidizing equivalent during protein folding (Hwang, 1992). The ratio [GSH]/[GSSG] in the ER is 1:1 to 3:1 as compared to 30:1 to 100:1 for the overall cellular ratio.

It is remarkable that all the Tyrfluo labeled proteins identified (i.e. PDI, BiP, calnexin, endoplasmin and PDI precursor ER60/GRP58) reside in the lumen of the ER. So, an explanation that particularly these proteins are highly susceptible to oxidation by H_2O_2 may be found in the peroxygenase activity of cytochrome P450, or an ER associated Fenton reaction in combination with the high redox-state of the ER. Normally, a quality control mechanism ensures that only correctly folded proteins exit the ER. Incorrectly folded proteins are retained and will be degraded. But what if oxidation of the protein folding machinery occurs? May this lead to an improper folding and/or accumulation of proteins meant to be secreted? Will less proteins be secreted and/or be present at the surface of cells? This will not only hamper proper cellular function, but might also result in increased oxidative stress. The latter because impaired secretion and accumulation of proteins in the ER resulted in a large increase in carbonylated proteins in Saccharomyces cerevisiae (Sagt et al., 2002).

The ER quality control system includes a number of chaperones and folding enzymes localized in the lumen or in the membrane of the ER (Ellgaard et al., 1999). It is likely that most, if not all, proteins synthesized in the ER interact with chaperones at some stage of the folding and maturation pathway. The chaperones bind non-mature proteins and are thought to assist folding by preventing irreversible aggregation and misfolding. Exactly how chaperones act in concert to guide immature proteins through the folding pathway, and selectively retain improperly folded proteins, is not fully understood. Although some chaperones in the ER are well studied, including PDI, GRP58, GRP78, GRP94, calnexin, and calreticulin, it is at present impossible to predict with which chaperones a specific protein will interact and to predict the consequences of the interaction.

4.3. Oxidation of calcium regulatory proteins

A nonselective oxidation of many cellular proteins has been suggested during aging since approximately, one-half of intracellular proteins are oxidized in senescent animals (Gafni, 1997). However, in the majority of cases the oxidation of one or two amino acids has a minimal effect on protein function, altering neither the stability nor the function of the protein (Levine et al., 1996). In contrast, some proteins are selectively oxidized at critical sites that regulate their function. The structural and functional consequences associated with the oxidative modification of unique sites of calcium regulatory proteins have been identified (Viner et al., 1999a,b; Yin et al., 2000). Methionines in calmodulin are oxidized to their corresponding methionine sulfoxides. It seems that sarco-/endoplasmic reticulum calcium ATPase pump (SERCA) can be inhibited both by oxidation of its sylfhydryl groups (Scherer and Deamer, 1986) and nitration of specific tyrosines on the ATP-binding site (Viner et al., 1999b). In addition, oxidation by direct attack of hydroxyl radicals on the ATP-binding site (Xu et al., 1997), which could be prevented by antioxidant treatment (Adachi et al., 2002), leads to decreases in ATP-dependent calcium fluxes across membranes and hence decreased ATP-consumption. Subsequent depletion of calcium in the ER inhibits protein-synthesis (Srivastava et al., 1995) and -processing, causing partially folded proteins to accumulate. This activates the transcription of ER chaperone genes, such as GRP78/BiP and GRP94 and calreticulin (Liu et al., 1998) to increases the capacity of intracellular calcium stores and to prevent cellular calcium toxicity. This strongly suggests that oxidative modifications contribute to the age-related decline of function of the SERCA (Pottorf et al., 2000) and the observed increases in intracellular calcium levels (Squier and Bigelow, 2000).

On the other hand, a decreased mitochondrial ATP-production, as a result of lower ATP-consumption by the ER, might result in a decrease of ROS production. By reducing ATP synthesis, reducing equivalents generated in the mitochondrial matrix can be used in the cytosol to enhance antioxidant defense mechanisms and cellular repair processes that minimize the accumulation of oxidized biomolecules (Squier, 2001).

Prolonged retention of misfolded and incompletely folded proteins in the ER leads to their degradation. ER-associated degradation (ERAD) is mainly carried out by the 26*S* proteasome located in the cytosol (Bonifacino and Weissman, 1998). The process occurs in several steps: terminally misfolded or unassembled proteins are recognized by ER chaperones such as calnexin, GRP78/BiP, or by other factors such as specific mannose lectins. They are then retranslocated through the Sec61 channel into the cytosol (Plemper et al., 1997), deglycosylated (in the case of glycoproteins), and polyubiquitinated before proteasomal degradation. How proteins that are destined to be degraded are identified and targeted for retrotranslocation has not been established, but it is likely that the machinery responsible for protein folding again plays a role in the selection and preparation of aberrant products for disposal.

5. Glutathione involvement in aging

Glutathione (GSH) is a tripeptide of glutamate-cysteine-glycine and is involved in a wide variety of biological reactions such as the maintenance of protein thiol groups in the reduced state, removal of hydrogen peroxide and detoxification of xenobiotics. GSH is converted to oxidized glutathione (GSSG) by seleno-dependent glutathione peroxidase (GPx). GSSG is subsequently reduced back to GSH by glutathione reductase (GSSGR) on the expense of NADPH. These two enzymes maintain the cellular balance between GSH and GSSG.

The relative oxidizing environment of the ER compared to the cytosol, as expressed by the ratio of GSH and GSSG, was suggested as being a factor contributing to the preferred oxidation of ER resident proteins. The imbalance of this ratio creates oxidative stress and logically, the lower amount of GSH available would give less protection upon oxidative attack. Until now it is not known whether the ER glutathione balance or concentration changes during aging. More and more evidence is obtained that the systemic GSH concentration decreases with advancing age. In healthy aging human adults low blood glutathione levels are observed (Lang et al., 1992). Erden-Inal et al. (2002) found a positive correlation between age and GPx and a negative with GSSGR resulting in a low ratio GSH/GSSG in human erythrocytes. Studies performed on houseflies showed that H_2O_2 concentration steadily increases with age and that the intracellular redox potential becomes progressively more pro-oxidizing, or less reducing, during the aging process, since the ratios of reduced/oxidized forms of glutathione, NAD and NADP decline with age (Sohal et al., 1987). At the subcellular level both cytosolic and mitochondrial GSH concentration have shown to be lower in old than in adult ad libitum fed rats (Armeni et al., 1998). Life prolonging food restriction did not prevent this decrease, but its extent was attenuated considering the cytosolic GSH. As regards the mitochondrial GSH, its content was higher in adult food restricted animals than in the age-matched ad libitum fed ones.

6. Conclusions

During biological aging, a progressive accumulation of errors at the genetic level might lead to a shift in the balance between protein oxidation and oxidized protein degradation in favor of oxidized protein accumulation and attendant loss of biological function (Berlett and Stadtman, 1997). ROS-induced protein oxidation may lead to protein fragmentation, through the oxidation of the protein backbone, yielding protein carbonyls, and to the oxidation of amino acid side chains. In order to determine the levels of oxidative damage stable oxidation markers are required. As discussed above, in aged extracellular proteins an increase in the levels of these markers has been shown, while this increase is still a matter of debate for intracellular proteins. This relative lack of intracellular accumulation may well reflect the relative efficiency, with which cells can control their intracellular milieu and prevent, or repair, oxidant-mediated events. Another reason for this discrepancy between intracellular and extracellular proteins might be the slow turnover rate of extracellular proteins (Davies, 1999). To date, limited data are available from both human and animal studies, which may be caused by the rapid excretion of oxidized amino acids via urine (Davies, 1999).

Through the oxidation of essential cellular proteins, aging may have deleterious effects on cellular function. The age-dependent upregulation of ER stress response genes that process damaged or misfolded proteins may reflect the importance of protein oxidation for biological aging (Lee et al., 1999) and a common defense mechanism against these effects in diverse species (Verbeke et al., 2001). Upregulation of these genes in aging may imply the presence of accumulated damaged and misfolded proteins in the endoplasmic reticulum caused by improper folding (Fig. 1). Proper folding requires optimal functioning of folding proteins. As a consequence of oxidation of the ER Ca^{2+}-ATPases the required calcium concentration inside the lumen of the ER cannot be established, and will cause an impaired functioning of the calcium-binding chaperones. The cytosolic decline of GSH levels may have its impact in the ER thereby creating a more

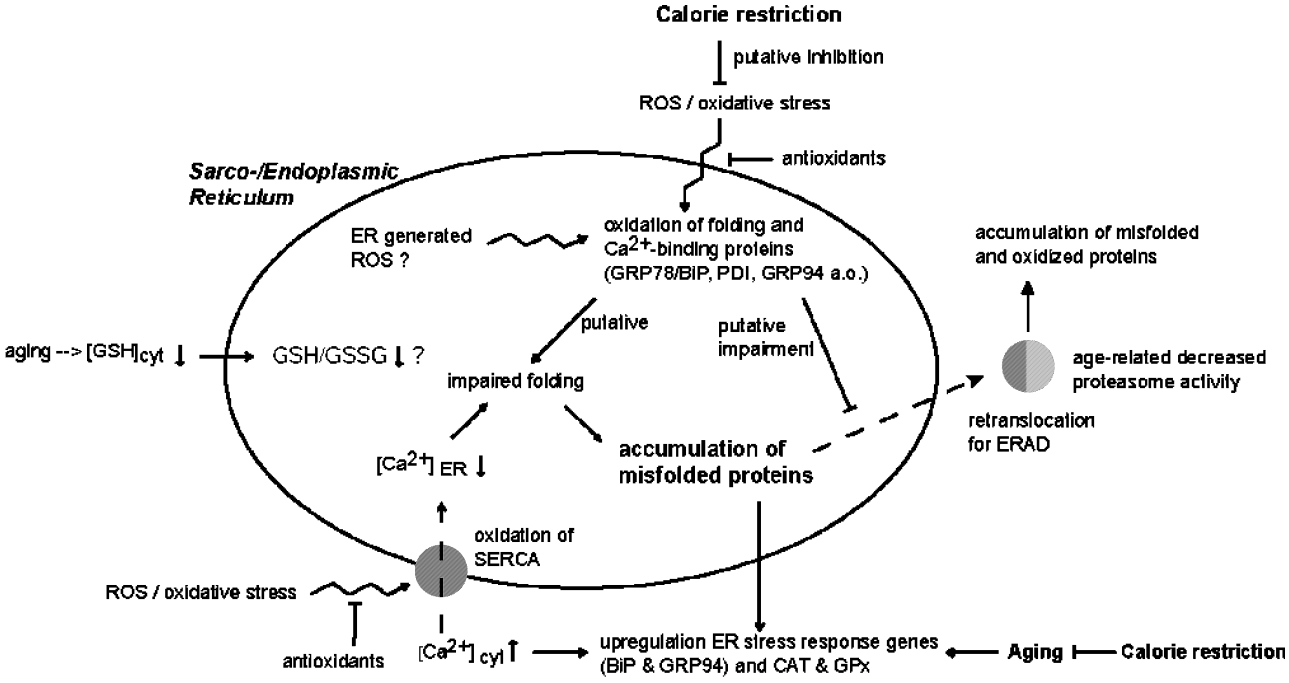

Fig. 1. Proposed mechanism for the contribution of oxidized sarco-/endoplasmic reticulum proteins to the accumulation of damaged and misfolded proteins in aging. *SERCA*, Sarco-/Endoplasmic Reticulum Calcium ATPase; *ERAD*, ER-associated degradation; *CAT*, catalase; *GPx*, glutathione peroxidase

oxidative environment, as expressed by the low ratio of GSH/GSSG. Oxidation of ER resident proteins will cause an even further functional decline and will contribute to an impaired folding as well as a hampered retranslocation of proteins destined for cytosolic degradation. This accompanied by an age-related decrease in the levels or activities of the proteasome (Petropoulos et al., 2000) may contribute to the ''garbage catastrophe theory''. In addition, dietary energy restriction showed a reduction in the expression of these stress response genes. This suggests that life-prolonging energy restriction might act to reduce damaged and misfolded proteins in the endoplasmic reticulum.

References

Adachi T, Matsui R, Xu S, Kirber M, Lazar HL, Sharov VS, Schoneich C, Cohen RA (2002) Antioxidant improves smooth muscle sarco/endoplasmic reticulum Ca2+-ATPase function and lowers tyrosine nitration in hypercholesterolemia and improves nitric oxide-induced relaxation. Circ Res 90: 1114–1121

Aeschbach R, Amadò R, Neukom H (1976) Formation of dityrosine cross-links in proteins by oxidation of tyrosine residues. Bioch Bioph Acta 439: 292–301

Ahmed MU, Dunn JA, Walla MD, Thorpe SR, Baynes JW (1988) Oxidative degradation of glucose adducts to protein. Formation of 3-N(ε)-lysino-lactic acid from model compounds and glycated proteins. J Biol Chem 263: 8816–8821

Aksenova MV, Aksenov MY, Carney JM, Butterfield DA (1998) Protein oxidation and enzyme activity decline in old brown Norway rats are reduced by dietary restriction. Mech Ageing Dev 100: 157–168

Anari MR, Khan S, Liu ZC, O'Brien PJ (1995) Cytochrome P-450 peroxidase/peroxygenase mediated xenobiotic metabolic activation and cytotoxicity in isolated hepatocytes. Chem Res Toxicol 8: 997–1004

Anari MR, Khan S, O'Brien PJ (1996) The involvement of cytochrome P450 peroxidase in the metabolic bioactivation of cumene hydroperoxide by isolated rat hepatocytes. Chem Res Toxicol 9: 923–924

Armeni T, Pieri C, Marra M, Saccucci F, Principato G (1998) Studies on the life prolonging effect of food restriction: glutathione levels and glyoxalase enzymes in rat liver. Mech Ageing Dev 101: 101–110

Ayala A, Cutler RG (1996) The utilization of 5-hydroxyl-2-amino valeric acid as a specific marker of oxidized arginine and proline residues in proteins. Free Radic Biol Med 21: 65–80

Bailey AJ (2001) Molecular mechanisms of ageing in connective tissues. Mech Ageing Dev 122: 735–755

Bailey AJ, Paul RG, Knott L (1998) Mechanisms of maturation and ageing of collagen. Mech Ageing Dev 106: 1–56

Baynes JW (2001) The role of AGEs in aging: causation or correlation. Exp Gerontol 36: 1527–1537

Baynes JW (2002) The Maillard hypothesis on aging: Time to focus on DNA. Ann NY Acad Sci 959: 360–367

Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78: 547–581

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272: 20313–20316

Bjorksten J (1968) The crosslinkage theory of aging. J Am Geriatr Soc 16: 408–427

Bonifacino JS, Weissman AM (1998) Ubiquitin and the control of protein fate in the secretory and endocytic pathways. Annu Rev Cell Dev Biol 14: 19–57

Braakman I, Hoover-Litty H, Wagner KR, Helenius A (1991) Folding of influenza hemagglutinin in the endoplasmic reticulum. J Cell Biol 114: 401–411

Braakman I, Helenius J, Helenius A (1992) Manipulating disulfide bond formation and protein folding in the endoplasmic reticulum. EMBO J 11: 1717–1722

Cefalu WT, Bell-Farrow AD, Wang ZQ, Sonntag WE, Fu MX, Baynes JW, Thorpe SR (1995) Caloric restriction decreases age-dependent accumulation of the glycoxidation products, N(ε)-(carboxymethyl)lysine and pentosidine, in rat skin collagen. J Gerontol A Biol Sci Med Sci 50: B337–B341

Chao C-C, Ma Y-S, Stadtman ER (1997) Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. Proc Natl Acad Sci USA 94: 2969–2974

Davies KJ, Delsignore ME, Lin SW (1987) Protein damage and degradation by oxygen radical. II. Modification of amino acids. J Biol Chem 262: 9902–9907

Davies MJ, Fu S, Wang H, Dean RT (1999) Stable markers of oxidant damage to proteins and their application in the study of human disease. Free Radic Biol Med 27: 1151–1163

Dean RT, Fu S, Stocker R, Davies MJ (1997) Biochemistry and pathology of radical-mediated protein oxidation. Biochem J 324: 1–18

Dhahbi JM, Mote PL, Tillman JB, Walford RL, Spindler SR (1997) Dietary energy tissue-specifically regulates endoplasmic reticulum chaperone gene expression in the liver of mice. J Nutr 127: 1758–1764

Dubey A, Forster MJ, Lal H, Sohal RS (1996) Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. Arch Biochem Biophys 333: 189–197

Dukan S, Farewell A, Ballesteros M, Taddei F, Radman M, Nystrom T (2000) Protein oxidation in response to increased transcriptional or translational errors. Proc Natl Acad Sci USA 97: 5746–5749

Dunn JA, Patrick JS, Thorpe SR, Baynes JW (1989) Oxidation of glycated proteins: Age-dependent accumulation of N(ε)-(carboxymethyl)lysine in lens proteins. Biochemistry 28: 9464–9468

Dunn JA, McCance DR, Thorpe SR, Lyons TJ, Baynes JW (1991) Age-dependent accumulation of N(ε)-(carboxymethyl)lysine and N(ε)-(carboxymethyl)hydroxylysine in human skin collagen. Biochemistry 30: 1205–1210

Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, Baynes JW (1993) Accumulation of Maillard reaction products in skin collagen in diabetes and aging. J Clin Invest 91: 2463–2469

Ellgaard L, Molinari M, Helenius A (1999) Setting the standards: Quality control in the secretory pathway. Science 286: 1882–1888

Erden-Inal M, Sunal E, Kanbak G (2002) Age-related changes in the glutathione redox system. Cell Biochem Funct 20: 61–66

Freedman RB (1984) Native disulphide bond formation in protein biosynthesis: evidence for the role of protein disulphide isomerase. Trends Biochem Sci 9: 438–441

Friguet B, Szweda LI, Stadtman ER (1994) Susceptibility of glucose-6-phosphate dehydrogenase modified by 4-hydroxy-2-nonenal and metal-catalyzed oxidation to proteolysis by the multicatalytic protease. Arch Biochem Biophys 311: 168–173

Fu S, Dean R, Southan M, Truscott R (1998) The hydroxyl radical in lens nuclear cataractogenesis. J Biol Chem 273: 28603–28609

Gafni A (1997) Structural modifications of proteins during aging. J Am Geriatr Soc 45: 871–880

Giulivi C, Davies K (1993) Dityrosine and tyrosine oxidation products are endogenous markers for the selective proteolysis of oxidatively modified red blood cell hemoglobin by (the 19 S) proteasome. J Biol Chem 268: 8752–8759

Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 2: 298–300

Harman D (1993) Free radical involvement in aging. Pathophysiology and therapeutic implications. Drugs Aging 3: 60–80

Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25: 585–621

Heinecke JW, Li W, Daehnke HLd, Goldstein JA (1993) Dityrosine, a specific marker of oxidation, is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages. J Biol Chem 268: 4069–4077

Hwang C, Sinskey AJ, Lodish HF (1992) Oxidized redox state of glutathione in the endoplasmic reticulum. Science 257: 1496–1502

Kruk PA, Rampino NJ, Bohr VA (1995) DNA damage and repair in telomeres: relation to aging. Proc Natl Acad Sci USA 92: 258–262

Lang CA, Naryshkin S, Schneider DL, Mills BJ, Lindeman RD (1992) Low blood glutathione levels in healthy aging adults. J Lab Clin Med 120: 720–725

Lass A, Sohal BH, Weindruch R, Forster MJ, Sohal RS (1998) Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria. Free Radic Biol Med 25: 1089–1097

Lee AS (1994) Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends Biochem Sci 12: 20–23

Lee C-K, Klopp RG, Weindruch R, Prolla TA (1999) Gene expression profile of aging and its retardation by caloric restriction. Science 285: 1390–1393

Leeuwenburgh C, Wagner P, Holloszy JO, Sohal RS, Heinecke JW (1997) Caloric restriction attenuates dityrosine cross-linking of cardiac and skeletal muscle proteins in aging mice. Arch Bioch Bioph 346: 74–80

Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med 32: 790–796

Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233: 346–357

Levine RL, Mosoni L, Berlett BS, Stadtman ER (1996) Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci USA 93: 15036–15040

Linton S, Davies MJ, Dean RT (2001) Protein oxidation and ageing. Exp Gerontol 36: 1503–1518

Little E, Ramakrishnan M, Roy B, Gazit G, Lee AS (1994) The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. Crit Rev Eukar Gene 4: 1–18

Liu H, Miller E, van de Water B, Stevens JL (1998) Endoplasmic reticulum stress proteins block oxidant-induced Ca^{2+} increases and cell death. J Biol Chem 273: 12858–12862

Lopez-Torres M, Gredilla R, Sanz A, Barja G (2002) Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. Free Radic Biol Med 32: 882–889

Lundstrom-Ljung J, Birnbach U, Rupp K, Soling H-D, Holmgren A (1995) Two resident ER-proteins, CaBP1 and CaBP2, with thioredoxin domains, are substrates for thioredoxin reductase: comparison with protein disulfide isomerase. FEBS Lett 357: 305–308

Merker K, Stolzing A, Grune T (2001) Proteolysis, caloric restriction and aging. Mech Ageing Dev 122: 595–615

Meucci E, Mordente A, Martorana GE (1991) Metal-catalyzed oxidation of human serum albumin: conformational and functional changes. Implications in protein aging. J Biol Chem 266: 4692–4699

Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG (1999) The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402: 309–313

Nordbloom GD, White RE, Coon MJ (1976) Studies on hydroperoxide-dependent substrate hydroxylation by purified liver microsomal cytochrome P-450. Arch Biochem Biophys 175: 524–533

Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Stadtman ER (1987) Age-related changes in oxidized proteins. J Biol Chem 262: 5488–5491

Orr WC, Sohal RS (1994) Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster. Science 263: 1128–1130

Otsu M, Urade R, Kito M, Omura F, Kikuchi M (1995) A possible role of ER-60 protease in the degradation of misfolded proteins in the endoplasmic reticulum. J Biol Chem 270: 14958–14961

Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL (1998) Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. Nat Genet 19: 171–174

Paschen W, Frandsen A (2001) Endoplasmic reticulum dysfunction – a common denominator for cell injury in acute and degenerative diseases of the brain? J Neurochem 79: 719–725

Petropoulos I, Conconi M, Wang X, Hoenel B, Brégégère F, Milner Y, Friguet B (2000) Increase of oxidatively modified protein is associated with a decrease of proteasome activity and content in aging epidermal cells. J Gerontol A Biol Sci Med Sci 55B: 220–227

Plemper RK, Bohmler S, Bordallo J, Sommer T, Wolf DH (1997) Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. Nature 388: 891–895

Pottorf WJ, Duckles SP, Buchholz JN (2000) SERCA function declines with age in adrenergic nerves from the superior cervical ganglion. J Auton Pharmacol 20: 281–290

Requena JR, Chao C-C, Levine RL, Stadtman ER (2001) Glutamic and aminoadipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins. Proc Natl Acad Sci USA 98: 69–74

Rodgers KJ, Dean RT (2000) Metabolism of protein-bound DOPA in mammals. Int J Biochem Cell Biol 32: 945–955

Rothstein M (1984) Changes in enzymatic proteins during aging. In: Roy AK, Chatterjee B (eds) Molecular basis of aging, Academic Press, New York, pp 209–232

Ruan H, Tang XD, Chen ML, Joiner MA, Sun G, Brot N, Weissbach H, Heinemann SH, Iverson L, Wu CF, Hoshi T (2002) High-quality life extension by the enzyme peptide methionine sulfoxide reductase. Proc Natl Acad Sci USA 99: 2748–2753

Sagt CMJ, Muller WH, van der Heide L, Boonstra J, Verkleij AJ, Verrips CT (2002) Impaired cutinase secretion in Saccharomyces cerevisiae induces irregular endoplasmic reticulum (ER) membrane proliferatin, oxidative stress and ER-associated degradation. Applied Env Microbiol 68: 2155–2160

Sastre J, Borras C, Garcia-Sala D, Lloret A, Pallardo FV, Vina J (2002) Mitochondrial damage in aging and apoptosis. Ann NY Acad Sci 959: 448–451

Scherer NM, Deamer DW (1986) Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca2 + -ATPase. Arch Biochem Biophys 246: 589–601

Schoneich C (1999) Reactive oxygen species and biological aging: a mechanistic approach. Exp Gerontol 34: 19–34

Sell DR, Nagaraj RH, Grandhee SK, Odetti P, Lapolla A, Fogarty J, Monnier VM (1991) Pentosidine: a molecular marker for the cumulative damage to proteins in diabetes, aging, and uremia. Diabetes Metab Rev 7: 239–251

Sohal RS, Toy PL, Farmer KJ (1987) Age-related changes in the redox status of the housefly, Musca domestica. Arch Gerontol Geriatr 6: 95–100

Sohal RS, Sohal BH, Brunk UT (1990) Relationship between antioxidant defenses and longevity in different mammalian species. Mech Ageing Dev 53: 217–227

Sohal RS, Ku HH, Agarwal S (1993) Biochemical correlates of longevity in two closely related rodent species. Biochem Biophys Res Commun 196: 7–11

Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H (1994) Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. Mech Ageing Dev 74: 121–133

Soti C, Csermely P (2000) Molecular chaperones and the aging process. Biogerontology 1: 225–233

Spindler SR, Crew MD, Mote PL, Grizzle JM, Walford RL (1990) Dietary energy restriction in mice reduces hepatic expression of glucose-regulated protein 78 (BiP) and 94 mRNA. J Nutr 120: 1412–1417

Squier TC (2001) Oxidative stress and protein aggregation during biological aging. Exp Gerontol 36: 1539–1550

Squier TC, Bigelow DJ (2000) Protein oxidation and age-dependent alterations in calcium homeostasis. Frontiers Biosci 5: 504–526

Srivastava SP, Davies MV, Kaufman RJ (1995) Calcium depletion from the endoplasmic reticulum activates the double-stranded RNA-dependent protein kinase (PKR) to inhibit protein synthesis. J Biol Chem 270: 16619–16624

Stadtman ER (2001) Protein oxidation in aging and age-related diseases. Ann NY Acad Sci 928: 22–38

Stadtman ER (2002) Introduction to serial reviews on oxidatively modified proteins in aging and disease. Free Radic Biol Med 32: 789

Stadtman ER, Starke-Reed PE, Oliver CN, Carney JM, Floyd RA (1992) Protein modification in aging. Experientia [Suppl] 62: 64–72

Terman A (2001) Garbage catastrophe theory of aging: imperfect removal of oxidative damage? Redox Rep 6: 15–26

Thomas JA, Mallis RJ (2001) Aging and oxidation of reactive protein sulfhydryls. Exp Gerontol 36: 1519–1526

Van der Vlies D, Wirtz KWA, Pap EHW (2001) Detection of protein oxidation in Rat-1 fibroblasts by fluorescently labeled tyramine. Biochemistry 40: 7783–7788

Van der Vlies D, Pap EH, Post JA, Celis JE, Wirtz KW (2002) Endoplasmic reticulum resident proteins of normal human dermal fibroblasts are the major targets for oxidative stress induced by hydrogen peroxide. Biochem J 366: 825–830 (2002)

Van der Vliet A, Eiserich JP, O'Neill CA, Halliwell B, Cross CE (1995) Tyrosine modification by reactive nitrogen species: a closer look. Arch Biochem Bioph 319: 341–349

Vanfleteren JR (1993) Oxidative stress and ageing in Caenorhabditis elegans. Biochem J 292: 605–608

Verbeke P, Fonager J, Clark BF, Rattan SI (2001) Heat shock response and ageing: mechanisms and applications. Cell Biol Int 25: 845–857

Verzijl N, DeGroot J, Oldehinkel E, Bank RA, Thorpe SR, Baynes JW, Bayliss MT, Bijlsma JWJ, Lafeber FPJG and TeKoppele JM (2000) Age-related accumulation of Maillard reaction products in human articular cartilage collagen. Biochem J 350: 381–387

Viner RI, Ferrington DA, Williams TD, Bigelow DJ, Schoneich C (1999a) Protein modification during biological aging: Selective tyrosine nitration of the SERCA2a isoform of the sarcoplasmic reticulum Ca-ATPase in skeletal muscle. Biochem J 340: 657–669

Viner RI, Williams TD, Schoneich C (1999b) Peroxynitrite modification of protein thiols: Oxidation, nitrosylation, and S-glutathiolation of functionally important cysteine residue(s) in the sarcoplasmic reticulum Ca-ATPase. Biochemistry 38: 12408–12415

Vlassara H, Palace MR (2002) Diabetes and advanced glycation endproducts. J Intern Med 251: 87–101

Walford RL (1974) Immunologic theory of aging: current status. Fed Proc 33: 2020–2027

Weindruch R, Walford RL (1982) Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. Science 215: 1415–1418

Weindruch R, Kayo T, Lee CK, Prolla TA (2001) Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. J Nutr 131: 918S–923S

Weindruch R, Kayo T, Lee C-K, Prolla TA (2002) Gene expression profiling of aging using DNA microarrays. Mech Ageing Dev 123: 177–193

Wells-Knecht MC, Huggins TG, Dyer DG, Thorpe SR, Baynes JW (1993) Oxidized amino acids in lens protein with age. Measurement of o-tyrosine and dityrosine in the aging human lens. J Biol Chem 268: 12348–12352

Wells-Knecht MC, Lyons TJ, McCance DR, Thorpe SR, Baynes JW (1997) Age-dependent increase in ortho-tyrosine and methionine sulf-

oxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes. J Clin Invest 100: 839–846

Winston GW, Feierman DE, Cederbaum AI (1984) The role of iron chelates in hydroxyl radical production by rat liver microsomes, NADPH-cytochrome P-450 reductase and xanthine oxidase. Arch Biochem Biophys 232: 378–390

Xu KY, Zweier JL, Becker LC (1997) Hydroxyl radical inhibits sarcoplasmic reticulum Ca2 + -ATPase function by direct attack on the ATP binding site. Circ Res 80: 76–81

Yan T, Li S, Jiang X, Oberley LW (1999) Altered levels of primary antioxidant enzymes in progeria skin fibroblasts. Biochem Biophys Res Commun 257: 163–167

Yin D, Kuczera K, Squier TC (2000) The sensitivity of carboxyl-terminal methionines in calmodulin isoforms to oxidation by H_2O_2 modulates the ability to activate the plasma membrane Ca-ATPase. Chem Res Toxicol 13: 103–110

Youngman LD, Park JK, Ames BN (1992) Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. Proc Natl Acad Sci USA 89: 9112–9116

Zhou JQ, Gafni A (1991) Exposure of rat muscle phosphoglycerate kinase to a nonenzymatic MFO system generates the old form of the enzyme. J Gerontol 46: B217–B221

Authors' address: Dennis van der Vlies, Erasmus MC, Department of Internal Oncology – Josephina Nefkens Instituut, PO Box 1738, 3000 DR, Rotterdam, The Netherlands,
E-mail: d.vandervlies@erasmucmc.nl