Available online at www.sciencedirect.com
SCIENCE DIRECT
Experimental Gerontology 41 (2006) 464–473
Experimental Gerontology
www.elsevier.com/locate/expgero

Accumulation of altered proteins and ageing: Causes and effects☆

Alan R. Hipkiss *

Barts' and the London School of Medicine and Dentistry, William Harvey Research Institute, Centre for Experimental Therapeutics, Charterhouse Square, London EC1M 6BQ, UK

Received 25 November 2005; received in revised form 1 March 2006; accepted 3 March 2006
Available online 18 April 2006

Abstract

Accumulation of altered proteins is the most common molecular symptom of ageing. Altered proteins are also associated with many age-related pathologies. Altered proteins are continuously produced but are normally selectively degraded by cellular proteases; their accumulation during ageing may be explained by either or both increased production or decreased elimination. Sources of altered proteins include erroneous synthesis by cytoplasmic and mitochondrial ribosomes, spontaneous deamidation, isomerization and racemization of unstable amino acids residues, damage inflicted by reactive oxygen and nitrogen species, and glycation and cross-linking by glucose and more reactive metabolites. Glycated proteins may damage mitochondria to increase production of reactive oxygen species, while highly oxidised/cross-linked polypeptides may resist proteolysis, inhibit proteasome function and induce a permanent stress response. Other possible explanations for the age-related changes in the defence systems, enzymatic and non-enzymatic, which normally counter generation of altered proteins are also discussed.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Aberrant polypeptides; Errors; Oxidation; ROS; Glycation; Aldehydes; AGEs; Deamidation; Cross-linking; Proteolysis; Aggregation; Proteasomes; Lysosomes; Mitochondria

1. Introduction

In this short review the possible origins of aberrant protein forms, the methods by which cells deal with them and possible explanations for their age-related accumulation will be discussed. The review will not include a comprehensive account of the origins of protein modification such as attack by oxygen and nitrogen free-radicals and glycating agents, as these processes are frequently discussed elsewhere in the biogerontological literature. Other topics, which seem to the author to be somewhat neglected, will be emphasized however, in order to give a more complete picture of this fundamental aspect of age-related macromolecular dysfunction.

2. Ageing occurs at molecular level

The most common symptom of ageing at the molecular level is the accumulation of altered proteins both within cells and extracellularly (Rosenberger, 1991; Rattan et al., 1992); the alterations may be in either or both polypeptide chain length or amino acid composition. Altered proteins are continuously generated both biosynthetically and post-synthetically (Sitte, 2003; Hipkiss, 2003c), via a variety of processes listed in Table 1. Not unexpectedly, most cells normally possess mechanisms, which either suppress formation of altered proteins, or dispose of them very rapidly, thereby preventing their accumulation. These homeostatic protective processes are listed in Table 2. Explanations of the age-related accumulation of altered proteins therefore involve either an increase in their generation or a decline in the ability to remove them, or both of these.

Altered proteins are also associated with many age-related pathologies such as Alzheimer's disease (AD), Parkinsons' disease (PD), cataractogenesis, atherosclerosis, diabetic secondary complications, etc. (Jay et al., 2006; Grune et al., 2004). Consequently it is thought that these accumulations are possibly causative to much age-related pathology and interventions to either prevent the production of the altered protein forms or facilitate their removal could delay certain age-related diseases. Alternatively, the altered protein structures that accumulate with age may merely be molecular tombstones reflecting an unrecognised deep-seated dysfunction and that preventing their accumulation would be treating

☆ This review is based on a lecture given at a conference on 'Cardiovascular ageing: from molecular biology to clinical perspectives' at Martin-Luther Universität, Halle-Wittenberg, Germany, in September 2005.

* Tel.: +44 1727 867305.
E-mail address: alanandjill@lineone.net

0531-5565/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2006.03.004

Table 1
The origins of altered proteins and effects of the alterations on their structure (ROS, reactive oxygen species; RNS, reactive nitrogen species)

Possible origins of altered proteins	
Source	Type of modification
Biosynthetic	
Mutation in gene	Altered amino acid sequence and/or chain length
Erroneous transcription	Altered amino acid sequence and/or chain length
Erroneous translation	Altered amino acid sequence and/or chain length
Postsynthetic	
Spontaneous	
Amino acid racemization	Altered amino acid orientation
Amino acid isomerization	Alteration to length of side chain and polypeptide backbone composition
Deamidation of amides	Altered amino acid composition
Metabolic	
ROS	Change in amino acid composition and/or chain length, major changes to amino acid structures, introduction of aldehyde and carbonyl groups
RNS	Major changes to amino acid structures
Lipid peroxidation products	Major changes to amino acid structures, introduction of aldehyde and carbonyl groups
Glucose and glycolytic intermediates	Major changes to amino acid structures, introduction of aldehyde and carbonyl groups

symptoms rather than underlying causes. My own prejudice is towards the former case rather than the latter.

3. Origins of altered proteins

3.1. Biosynthetic

Genomic instability is one source of age-related altered protein. DNA is not stable; in fact it possesses a surprisingly high degree of molecular instability (Lindahl, 1993) and is also subject to much molecular insult (Seeberg, 2003). Spontaneous events include loss of bases (depurination and depyrimindation) and deamination of cytosines, guanines and adenines to uracil, hypoxanthine and xanthine, respectively. Collectively these changes occur at a frequency of nearly 600 events per hour at 37 °C in each (human) cell and are potentially mutagenic. Were it not for the presence of the DNA repair apparatus such instability would not be compatible with survival (Seeberg, 2003). Indeed ageing is also accompanied by an increase in somatic mutations quite possibly as a consequence of a decreased efficiency of the DNA repair apparatus. The so-called premature ageing diseases Werner's syndrome and progeria both appear to possess defects in genes involved in genome maintenance and metabolism (Bohr and Opresko, 2003). It may be significant that mitochondrial DNA is repaired less effectively than nuclear DNA and mutations in mitochondrial DNA and mitochondrial dysfunction are frequently associated with ageing and some related pathologies.

Errors also occur during transcription, the RNA polymerase is reported to generate one error in every 10^4–10^5 bases transcribed but translation of messenger RNA is the most error-prone step in protein biosynthesis. In bacteria, at least about three errors occur in every 10,000 codons translated, but the corresponding values in eukaryotic cells (cytoplasmic and mitochondrial ribosomes) are uncertain (Hipkiss, 2003a). If three in every 10,000 codons is representative of the error rate in mammalian tissues, it would imply that about 5% of polypeptide chains 165 amino acids long would possess an error, and a third of all proteins chains with 1000 or more amino acids (e.g. collagen) would be erroneous too. This may explain the very high intra-cellular proteolysis rates of newly synthesized collagen chains produced by fibroblasts, and that pulse-chase experiments show that most cells degrade around 5% of their protein immediately after synthesis.

4. Post-synthetic changes in protein composition

4.1. Amino acid instability

Some amino acid residues spontaneously racemize from the L-configuration to the D-form. D-serine, D-threonine, D-aspartic acid and D-tyrosine have been detected in long-lived proteins such as eye lens proteins and enamel and dentine of teeth (Ritz-Timme and Collins, 2002). These changes may play causative roles in pathology as D-amino acids appear enriched in lenticular cataracts and in the amyloid peptide associated with AD, but the occurrence of these changes may merely reflect the long lived nature of the aberrant protein accumulations.

Table 2
Some of the processes which help protect cells against the formation and accumulation of altered proteins

Name	Function
Chaperone (stress) proteins unfolded protein response	Bind and refold altered proteins, or deliver them to proteolytic apparatus
Proteasomes	Selectively degrade altered proteins
Lysosomes	Degrade altered proteins and organelles
Oxidized protein hydrolase	Selectively degrades oxidized proteins
Neprilysin	Degrades β-amyloid peptide and insulin
Protease Lon	Selectively degrades oxidized and other altered proteins in mitochondria
AAA-proteases	Help maintain protein quality in mitochondria
Anti-oxidant apparatus	Suppress ROS formation
Iron and copper chelators	Suppress ROS formation
Anti-glycating agents	Suppress reactivity of aldehydes and ketones
Anti-cross-linking agents	Suppress reactivity of protein carbonyls
Protein isoaspartate methyl-transferase (PIMT)	Converts protein iso-aspartates to normo-forms
Methionine sulphoxide reductase	Converts protein methionine sulphoxide to methionine

Asparagines and, to a lesser extent, glutamines are prone to spontaneous deamidation where the amide function on the amino acid side chain is replaced by a carboxyl group (Lindner and Helliger, 2001). Following each deamidation event there are four possible products, all of which alter the amino acid composition of the protein to some degree. Deamidation of asparagine residues will produce any one of the following—an L-aspartic acid, D-aspartic acid, L-iso-aspartic acid and D-iso-aspartic acid residue. Not only does the chemical property of the side chain change (amide to carboxyl group) but its orientation in relation to the rest of the polypeptide chain may be altered if the D- or iso-form is generated. Generation of the iso-forms also inserts an extra methylene (CH_2) group in the polypeptide backbone, perhaps perverting intra- or inter-molecular hydrogen bonding by components of the peptide bond, as well as shortening the side chain by one methylene group, which could interfere with protein function and stability. The rates at which particular asparagines residues deamidate is strongly affected by adjacent amino acids, for example, asparagines with glycine, histidine, alanine or serine on their carboxyl sides show a high deamidation incidence, with half-lives as short as one day reported (Robinson, 2002). Deamidation of glutamines proceeds about one hundred-times slower than that of asparagine.

As asparagines deamidation proceeds at a biologically significant rate, there is an enzyme present on most life forms that partially corrects the aberration. This is called protein iso-aspartate methyltransferase (PIMT) whose activity converts the iso-form back to the normo-configuration; it does not, however, convert the carboxyl side chain back to an amide structure (Clarke, 1985). Mice deficient in PIMT show intra-cellular accumulation aberrant polypeptides, especially in their brains, and a decreased lifespan (Kim et al., 1997). Overproduction of PIMT has been shown to extend the lifespan of fruit flies but only when subjected to heat stress (Chavous et al., 2001).

4.2. Modification of proteins by oxygen

Table 3 lists some of the changes that occur in proteins as they age which are thought to be caused by oxygen free-radicals and related reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Much has been written about the potential toxicity of oxygen because of its potential for generation of free-radicals and other reactive species which can then modify proteins [see Stadtman, 1992, 2002; Levine, 2002; Sitte, 2003 and Refs. therein], lipids (Pamplone and Barja, 2003) and DNA (Seeberg, 2003). The most active of the reactive oxygen species is thought to be the hydroxyl radical generated via the reaction of hydrogen peroxide with transition metals such as iron and copper ions. The most common modification to proteins that oxygen free-radicals and other reactive oxygen species (ROS) provoke are carbonyl groups which can arise following oxidative attack on arginine, lysine, threonine and proline residues. Interestingly oxidation of histidine residues generates the unstable amino acids asparagine and aspartic acid. ROS can also modify cysteine residues as well as methionines to generate methionine sulphoxide and its sulphone. Other products of ROS attack on proteins include hydroperoxides and alcohols. Particularly reactive is the hydroxyl radical, which can introduce hydroxyl groups into phenylalanine and tyrosine residues, and cleave the ring structure of tryptophan. Peptide bond cleavage may also occur. Cross-linked species such as dityrosine can arise directly from hydroxyl radical attack, as well as via the reaction of induced carbonyls with any proximal amino group.

Table 3
Some of the products of post-synthetic protein modification mediated by reactive oxygen species and reactive nitrogen species

Changes to amino acid residue side chains induced by ROS and RNS	
Amino acid	Products
Arginine	Glutamic semialdehyde
Cysteine	S-nitrosylated cysteine, cysteine sulphenic, sulphinic and sulphonic acids, cysteine disulphide (cystine)
Glutamic acid	Oxalic acid, pyruvate adducts
Histidine	2-Oxohistidine, aspartic acid, asparagine
Leucine	3-, 4-, and 5-OH-leucine
Lysine	α-Aminoadipic semialdehyde
Methionine	Methionine sulphoxide, methionine sulphone
Phenylalanine	2-, 3-, and 4-OH-phenylalanine, 2,3- dihydroxy-phenylalanine, 3-nitrophenyalanine
Tyrosine	3,4-Dihydroxy-phenylalanine, tyrosine-tyrosine cross-links, 3-nitro-tyrosine
Tryptophan	2-, 4-, 5-, 6-, and 7-OH-tryptophan, formylkynurenine, 3-OH-kynurenine, nitrotryptophan
Proline	Glutamylsemialdehyde, 2-pyrrolidine, 4-, 5-OH- praline, pyroglutamic acid
Threonine	2-Amino-3-ketobutyric acid
Valine	3-Hydroxyvaline

ROS may be generated both within mitochondria and extra-mitochondrially. It is thought that mitochondrial dysfunction generally is a major player in ageing and age-related tissue pathologies. Intra-mitochondrial damage may result from the direct effects of ROS on mitochondrial proteins or derive from their mutagenic effects on organelle DNA. In either case altered protein species may be generated. Modification to the enzyme aconitase appears to be the best characterized mitochondrial protein, which undergoes oxicxative damage during ageing (Yarian et al., 2005) and in exercised muscle (Bota et al., 2002; Radak et al., 2000). Possible consequences of mitochondrial damage are either apoptosis or the age-related accumulation of cells possessing dysfunctional mitochondria.

4.3. Modification by reactive nitrogen species

Reactive nitrogen species (RNS) provide another source of protein damage. Peroxynitrite, formed following the reaction of nitric oxide with the superoxide radical, can modify aromatic amino acids producing nitro-tyrosine, nitro-phenylalanine and nitro-tryptophan (Sitte, 2003).

4.4. Modification by metabolic aldehydes

Non-enzymic protein glycosylation (glycation) is another likely contributor to age-related polypeptide modification

Table 4
Origins of some AGEs (advanced glycation end products) and some ALEs (advanced lipid peroxidation end products)

Modification	AGEs
Cross-linked arginine and lysine	Pentosidine
Lysine and glyoxal	Carboxymethyl-lysine
Lysine and methylglyoxal	Carboxyethyl-lysine
Lysine and 3-deoxyglucosone	Pyrraline
Lysine-lysine cross-linked with glyoxal	GOLD (glyoxal derived lysine dimer)
Lysine-lysine cross-linked with methylglyoxal	MOLD (methylglyoxal lysine dimer)
Lysine-lysine cross-linked with deoxyglucosone	DOLD (deoxyglucosone lysine dimer)
Arginine and glyoxal, methylglyoxal and 3-deoxyglucosone	Hydroimidazolones
Arginine and 3-deoxyglucosone	Argpyrimidine
	ALEs
Lysine and malondialdehyde, 4-hydroxynonenal and acrolein	
Lysine-lysine cross-linked with malondialdehyde	
Histidine and 4-hydroxynonenal and acrolein	
Cysteine and 4-hydroxynonenal and acrolein	

(Baynes and Monnier, 1989; Brownlee, 1995; 2001). Originally called the browning or Maillard reaction, the process whereby reducing sugars generate a brown colouration was first described by food chemists. Many of the details of this process have now been characterized including formation of the initial Schiff's base between glucose and the protein amino group, the subsequent Amadori rearrangement leading to the eventual formation of the complex advanced glycation end-products or AGEs. Table 4 lists some of the AGEs detectable in proteins and their origins. Glucose is the most abundant but least deleterious of the common metabolic sugars in humans; galactose and fructose are between 4 and 7-fold more reactive. Most glycolytic intermediates are also much more reactive than glucose, especially the trioses glyceraldehyde- and dihydroxyacetone-phosphates. The latter two are even more problematic because they can spontaneously generate methylglyoxal (MG), which is a highly reactive glycating agent (Chaplen, 1998; Ahmed et al., 2005). Some MG is also generated metabolically from amino acids and lipids. MG has been implicated in age-related protein, lipid and organelle dysfunction as well as a number of age-related pathologies including Alzheimer's disease and diabetic secondary complications. In fact MG can provoke many of the deleterious changes that accompany normal ageing (formation of protein carbonyls groups and cross-linking, lipid and DNA damage, ROS, mitochondrial damage and apoptosis). It is at least possible that the beneficial effects of dietary restriction and fasting may be explained in terms of suppression of both extracellular glucose and MG levels, compared to the ad libitum-fed condition (Hipkiss, 2006).

4.5. Modification by lipid peroxidation products

Lipids are subject to attack by ROS. Amongst the products are reactive aldehydes such as malondialdehyde, acrolein, glyoxal and hydroxynonenal, all of which can react with protein amino acid side chains especially those of lysine, arginine, histidine (Pamplone and Barja, 2003). Many of the reaction products, called advanced lipid peroxidation end-products or ALEs (see Table 4), possess carbonyl functions which lend themselves to cross-linking to either unmodified polypeptide chains or altered proteins (Baynes and Thorpe, 2000).

5. Ageing and the accumulation of protein carbonyl groups

As stated above, the most common alteration that cytosolic and mitochondrial proteins, (Yan and Sohal, 2003; Bota et al., 2002), undergo during aging is an accumulation of carbonyl groups [see reviews by Levine (2002) and Stadtman (1992, 2002) and Refs. therein]. It is interesting that this accumulation does not occur linearly with age but instead shows a marked tendency to increase in the last third of the life span of many organisms, affecting as much as one protein molecule in every three. Formation of protein carbonyls can occur via the various routes outlined above; attack by oxygen free-radicals and related species (collectively called reactive oxygen species or ROS), attack by lipid peroxidation products following ROS-mediated modification, attack by reducing sugars, their metabolites and other aldehydes and ketones. Clearly such modification can profoundly compromise any protein's function, as well as introduce a potential for intra- and inter-molecular cross-linking which may induce further deleterious effects.

6. Processes which counter the formation of altered proteins

Table 2 lists the various processes, enzymatic and non-enzymatic, which suppress the post-synthetic generation of altered proteins. The most commonly explored are non-enzymatic anti-oxidants especially vitamins C and E, and the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Thomas and Mallis, 2001), all of which contribute to suppressing generation of reactive oxygen species or facilitating their destruction (Sitte and von Zglinicki, 2003). Glutathione is very important for maintenance of reducing conditions within the cell as well as controlling irreversible cysteine oxidation to cysteic acid by forming mixed disulphides (S-thiolation) with target proteins. It is well established that ageing (Hazelton and Lang, 1980) is associated with a decline in cellular glutathione content (Thomas and Mallis, 2001) and a severe decline (about 80%) has been observed in aortic arch of atheroma-prone mice (Biswas et al., 2005). Hence those processes which affect glutathione synthesis and utilization could profoundly affect ageing and related pathologies.

Although protein glycation has been recognised for some time as a potential source of age-related protein damage, less is understood about how cells normally suppress formation of glycated proteins or facilitate their elimination. Indeed much age-related pathology may derive from the glycating activity of

endogenous aldehydes such as methylglyoxal, which may be especially important in the secondary complications of type-2 diabetes (Chaplen, 1998; Berlanga et al., 2005). Possible naturally occurring anti-glycating agents are the dipeptide carnosine, the polyamines spermine and spermidine, and pyridoxamine (Hipkiss, 2005). Many AGEs are often weakly mutagenic (Shibamoto, 1989) but it may be significant that while glucose-modified lysine is mutagenic, glucose-modified carnosine is not (Hipkiss et al., 1995), which supports carnosine's putative protective role in vivo.

7. How do cells normally deal with altered proteins?

Generation of altered protein forms may cause a small loss of function due to the aberration. However the predominant general outcome is a gain of function due to the ability of aberrant and misfolded proteins to aggregate together or interact indiscriminantly with normal polypeptides; indeed misfolding of altered proteins may be the basis of a large number of pathologies (Stefani, 2004) many of which are age-related. One consequence of the accumulation of protein aggregates is the induction of the stress response, possibly in an attempt by the cell to rid itself of the toxic structure. Should the response be insufficient to effect a total clearance of the aberrant structure, then the cell might become permanently stressed. This could be fatal because a second consequence of the stress response is the shut down of the synthesis of regular house-keeping proteins. It is conceivable that under these conditions cell death could result from a failure to replace house-keeping proteins necessary for continued viability.

8. Recognition and refolding

As indicated above, protein biosynthesis is not perfect; furthermore the correctly synthesized polypeptide chain does not always spontaneously form the correct conformation required for biological activity. In fact it is estimated that between 10 and 20% of nascent proteins require assistance from chaperone proteins (especially Hsp70 and Hsp90) to enable them to fold correctly (Verbeke et al., 2001; Proctor et al., 2005). Oxidatively damaged proteins are also recognised by chaperone proteins, and chaperone proteins themselves are subject to oxidative damage. If refolding of the altered proteins is unsuccessful then the chaperone proteins deliver the aberrant chains to the proteasomal system for degradation (discussed below). An indication of the importance of chaperone proteins to cellular function is the fact that they constitute more than 5% of cellular proteins (Soti and Csermely, 2003) and that 10–20% of newly synthesized proteins associate with chaperone proteins (Proctor et al., 2005).

Oxidative and other stress conditions can induce the synthesis of further copies of these stress proteins, principally via the action of heat shock transcription factors (HSF). There appears to be a relationship between the presence of altered proteins and transcription of chaperone proteins due to the fact that HSF is normally bound to the major chaperone proteins Hsp90 and Hsp70, or to itself in an inactive form. But when the Hsp90/HSF complex encounters an aberrant protein, the latter binds to the complex and displaces the HSF, thereby enabling it to bind to heat shock transcription elements in the DNA and stimulate synthesis of more chaperone proteins. The resulting increase in chaperone protein numbers presumably enables the cell to maintain delivery of altered proteins to the proteasomes for destruction and thereby prevent formation of deleterious protein aggregates.

The endoplasmic reticulum (ER) provides another site for control of protein quality. Here, folding occurs co-translationally in three phases; the nascent chain enters ER lumen, folding takes place after release of the completed chain from the ribosome, and finally protein oligomerisation occurs; high concentrations of chaperone and folding proteins are located in the ER lumen to facilitate correct folding and final assembly (Ellgaard and Helenius, 2003). It appears that many of the ER-associated chaperones are similar to those found in the cytoplasm (e.g. Hsp40, Hsp70 and Hsp90) although there are some different ones too (calnexin, calreticulin and thiol-disulphide oxido-reductases). Nevertheless, the outcome of unsuccessful folding is the selective degradation of the aberrant polypeptide via ERAD (ER-associated degradation), which involves the retro-translocation of the unfolded polypeptide to the cytosol for ubiquitination and destruction by the proteasomes. It appears that in some cases the proportion of the newly synthesized protein that fails to satisfy the selection criteria is surprisingly large; for example, 40% of the γ-opioid receptor fails to mature and is degraded (Ellgaard and Helenius, 2003). There is still much to learn about how the quality of those proteins destined for export from the cell is controlled.

Various heat shock or stress proteins protect organisms and cells against ageing in general (Yokoyama et al., 2002; Hsu et al., 2003; Soti and Csermely, 2003; Proctor et al., 2005) and the toxicity of altered proteins in particular (Stefani, 2004) to some degree. Consequently in order to control certain aspects of ageing it will be informative to understand how these protein do their job and how their expression, survival and destruction are regulated.

9. Repair

Unlike DNA, most altered protein forms are not repaired but selectively degraded (see below). Exceptions are PIMT (discussed above) and methionine sulphoxide reductase which reduces methionine sulphoxide to methionine. Loss of the gene for methionine sulphoxide reductase in mice has been shown to decrease life-span by 40% (Moskovitz et al., 2001) whereas over-expression of the gene in fruit flies can increase life-span by 70% ((Moskovitz et al., 2001).

10. Selective proteolysis of altered proteins

There are at least two well-characterised processes present in eukaryotic cells that are responsible for the elimination of altered protein forms, these are the lysosomes and proteasomes

(Ciehanover, 2005; Martinez-Vincente et al., 2005). Additional activities, which may contribute to the elimination of altered proteins include oxidized protein hydrolase (Shimuzu et al., 2004) and mitochondrial proteases such as Lon (Zhu et al., 2002) and paraplegin (Bota and Davies, 2001).

11. Proteasomal elimination of altered proteins

The 20S and 26S proteasomes participate in the selective degradation of altered polypeptide chains (Carrard and Friguet, 2003). Degradation of oxidatively damaged proteins is carried out by the 20S proteasomes without prior ubiquitinization of the substrate polypeptide chains. Other forms of aberrant proteins may be selectively ubiquitinated prior to their ATP-dependent degradation by 26S proteasomes (Ciehanover, 2005). It is thought that the recognition of altered proteins for proteasomal elimination is partly dependent on the exposure of hydrophobic regions of the polypeptide chain and may additionally involve the participation of chaperone proteins such as Hsp70 and Hsp90.

12. Lysosomal activity and ageing

The lysosomes provide another route for intra-cellular proteolysis (Terman and Brunk, 2003). These organelles are also thought responsible for the elimination of dysfunctional mitochondria following macroautophagy (Del Roso et al., 2003). It is interesting that macroautophagy (lysosomal proteolysis) is suppressed by insulin, which may partly explain the beneficial effects of caloric restriction towards age-related dysfunction (Lambert et al., 2004). Indeed recent studies have shown that intermittent feeding which results in prolonged periods of fasting but without any decrease in calorie intake can induce the same beneficial effects as dietary restriction (Mattson and Wan, 2005; Masternak et al., 2005). These observations are consistent with the proposal that lowered circulating insulin levels up-regulate of lysosomal proteolytic activity which may help prevent altered protein accumulation. It has also been suggested that the age-related accumulation of dysfunctional mitochondria is a consequence of compromised lysosomal activity (Brunk and Terman, 2002).

Another possibility whereby glycated proteins might be selectively eliminated involves the so-called amadoriases, fructose-lysine oxidase and fructose-lysine-3-phosphokinase, which are postulated to effect the deglycation of early proteins glycation products (Schiff's bases and Amadori products) (Wu and Monnier, 2003; Gugliucci, 2005; Szwergold, 2005). Such enzymes were initially characterised from fungi (Hirokawa et al., 2003) and evidence suggesting at least one of them, fructosamine-3-kinase, is present of in mammal tissue has been obtained (Szwergold et al., 2005). Their deglycating activity, however, releases an active glycating agent (e.g. 3-deoxyglucosone) which must then be eliminated, possibly by conjugation to an abundant amine such as spermine, glutathione or carnosine, all of which may be present in tissues in millimolar quantities, but which may decline with age. Further, Szwergold (2005) has proposed that an additional transglycating activity, active against the initial Schiff's base, may transfer the sugar moiety to an abundant nucleophile such as glutathione, taurine, anserine or carnosine. Carnosine is elevated in glycolytic muscle, especially after training in humans, and bird muscle tissues possess high concentrations of carnosine and anserine (Abe, 2000), which might help explain the ability of avian species to tolerate levels of glucose (40 mM) considered deleterious in humans. These observations are at least consistent with protective roles, including deglycation, for these dipeptides, which may decline with age (see Hipkiss, 2005; 2006 and Refs. therein). It should be pointed out however that these deglycating enzymes do not appear to be active against protein AGEs.

Many cells possess receptors for AGEs, the ultimate end-products of protein glycation, called RAGEs, but many observations suggest that reaction of AGEs with RAGEs is in fact deleterious as this provokes generation of ROS and an inflammatory response (Schmidt et al., 1994). Perhaps this represents a 'last ditch' attempt to destroy these endogenously-generated toxins.

13. What may cause altered protein to accumulate in old age?

The accumulation of altered proteins, which accompanies ageing may be a consequence of an increase in their production via oxidation and non-enzymic glycosylation and/or a decrease in cellular ability to selectively degrade them. Increased production of ROS may either cause, or result from, mitochondrial dysfunction. Lowered mitochondrial ATP generation may in turn promote a compensating increase in glycolytic activity. Increased detoxification of the consequently raised levels of methylglyoxal may increase utilization of glutathione, which may compromise glutathione peroxidase activity and thereby increase the potential for ROS production and protein damage. Age related declines in intra-cellular concentrations of glutathione, spermine and carnosine may also compromise the ability to prevent formation of deleteriously modified polypeptides or facilitate their detoxification.

There is increasing evidence showing that proteolytic activity declines with age in many cell types. Both cytosolic proteasomal and lysosomal activities have been reported to decline. Much evidence is accumulating which indicates that proteasomal dysfunction may be a cause of the age-related accumulation of altered proteins. (Chondrogianni and Gonos, 2005; Martinez-Vincente et al., 2005; Carrard et al., 2002). Explanations for the lowered activities are mostly related to the inhibitory effects of incompletely degraded and cross-linked peptide species exert on proteasomes (Carrad and Friguet, 2003). Lipid–protein cross-links in the form of lipofuscin (the so-called age pigment) may inhibit lysosomal activity (Szweda et al., 2003; Powell et al., 2005).

Age-related lysosomal dysfunction has been proposed to be a major contributor to ageing and the accumulation of altered proteins (Terman, 2001; Terman and Brunk, 2003). Because lysosomes are important in mitochondrial destruction,

lysosomal dysfunction is likely to contribute to the accumulation of dysfunctional mitochondria.

Dysfunction of the protein ubiquitination system is another possible source of altered protein accumulation (de Pril and Fischer, 2006). For example, the phenomenon of molecular misreading can generate dysfunction ubiquitin, which, if not itself degraded, can adversely affect protein homeostasis and the subsequent elimination of altered polypeptides ((Tsirigotis et al., 2001). Mutant ubiquitin may contribute to the accumulation of the β-amyloid in Alzheimer's disease (Gerez et al., 2004) and increase sensitivity to protein damaging agents generally (Tsirigotis et al., 2001). Ubiquitination may also be necessary for the destruction of mitochondria (Rapoport et al., 1985), so any defect in the ubiquitin-proteasome system (Sullivan et al., 2004) could contribute to the age-related accumulation of dysfunctional mitochondria.

14. Mitochondrial proteolysis

Proteolysis also occurs within mitochondria (Augustin et al., 2005). This is to be expected as these organelles synthesize proteins, presumably some normal and some erroneous, and others may become modified by ROS (Basoah et al., 2005). While the mitochondrial protease Lon (nuclear encoded) has been shown to be important in the elimination of oxidized polypeptides within mitochondria (Bota et al., 2002), other participants in intra-organelle proteolysis, some of which are membrane-associated, also play a role in maintenance mitochondrial integrity (Arnold and Langer, 2002; Bota and Davies, 2002). It is interesting that the levels of Lon appear to be regulated by epidermal growth factor (EGF) (Zhu et al., 2002), which may provide a link between control of cell growth, mitochondrial protein quality control and age-related accumulation of altered proteins (Hipkiss, 2003a, b).

15. A role for nitric oxide in proteolysis of altered proteins?

Nitric oxide is a potential source of altered proteins because of its ability to form peroxynitrite anions as discussed above. Additionally, however, nitric oxide may also facilitate proteolysis of altered proteins by inducing increased synthesis of proteasomal subunits (Kotamraju et al., 2006) via cyclic-GMP and cyclic-AMP-controlled signal cascade, at least in bovine aortic endothelial cells. Interestingly, Stolzing et al. (2006) have recently shown that nitric oxide production is stimulated by glycated proteins in microglial cells. It remains to be seen whether a similar response occurs in other cells.

Nitric oxide has long been recognised as important in cardiovascular health, in particular it may control blood pressure via endothelial vasodilation (Williams et al., 1996). The dipeptide carnosine (β-alanyl-L-histidine), a possible anti-ageing peptide (Hipkiss, 1998), has also been reported to a vasodilatory agent (Ririe et al., 2000; Niijima et al., 2002). Evidence is emerging suggesting that carnosine is involved in nitric oxide metabolism (Tomonaga et al., 2005), that it may be a natural substrate for nitric oxide synthesis rather than arginine (Alaghband-Zadeh et al., 2001) and that it can suppress accumulation of nitrosotyrosine-containing proteins in astrocytes (Calabrese et al., 2005). Despite the complexity of these findings, one is tempted to speculate that the beneficial effects that carnosine towards diabetic complications (Lee et al., 2005) may in part derive from effects exerted via nitric oxide, in addition to the many other protective functions that the dipeptide may possess (Hipkiss, 2005). Carnosine is primarily associated with non-mitotic tissues, especially those of long-lived species, and it is possible that tissue content of the dipeptide may decline with age, although many more studies are required to confirm this (Hipkiss, 2005). Given that ageing is multifactorial, carnosine's apparent pluripotency (Hipkiss, 1998) makes it an attractive lead compound for suppressing accumulation of altered proteins.

16. Could the decline in proteolysis be programmed?

The age-related decline in proteasomal activity, which may be at least partly responsible for the accumulation of altered proteins is thought to result from the inhibitory effects of cross-linked polypeptides produced by oxidation and glycation events. It is assumed that these highly modified polypeptides exert effects directly or indirectly on the proteasomes thereby compromising their proteolytic and other activities. There is, however, another explanation that is not well appreciated but which should also be considered. As stated above protein biosynthesis is not error free. Consequently it is not unreasonable to assume that cells growing rapidly, synthesize more error protein molecules per cell per unit time that those which are growing slowly or not at all. If this is so it follows that rapidly growing cells should possess a higher intrinsic ability to degrade erroneously synthesized proteins than cells which have ceased growing; one might conceivably expect that protein synthetic and proteolytic activities to be closely coordinated. However little evidence has emerged as yet to either support or refute this proposal, at least in animal cells. Nevertheless rapid growth of transformed cells is accompanied by an increase in expression of chaperone proteins, presumably to help combat any deleterious effects of erroneously synthesized or misfolded polypeptide chains. Studies in the bacterium *Escherichia coli,* however, do show that increasing protein biosynthetic rates is accompanied by an increase in constitutive proteolytic activity towards altered proteins; the constitutive degradative activity increased logarithmically (it squared) as growth rates doubled (Rosenberger et al., 1990). Whether a similar relationship pertains in animal tissues is unknown, but the possibility of such leads one to suggest that when the programmed cessation of growth on attaining adulthood occurs, it is accompanied by a decline in protein biosynthesis including that of those degradative proteases responsible for the elimination of altered protein forms. Thus the constitutive proteolytic activity in any cell would be composed of those proteases, which are newly synthesized (in proportion of total protein synthesis) plus any pre-existent proteases, which have not been degraded. One conjectures that as the individual animal progresses from young adult to old adult, even if the rate of synthesis of all proteins including

proteases remains constant in non-mitotic tissues, the level of protease activity would gradually decline as the pre-existing proteases are themselves degraded. The lowered level of constitutive proteolytic activity that may be present in post-mitotic cells of old animals could therefore account for the increased vulnerability to molecular insult which appears to accompany ageing and the consequent accumulation of altered protein forms (Hipkiss, 2003b), especially in the last third of their maximal lifespan.

17. Interaction between altered protein forms

The various forms of altered proteins described above should not be considered independently as one form of protein aberration appears to predispose towards other types of post-synthetic change. For example, erroneous synthesis increases a polypeptide's potential towards oxidative damage, which in turn can raise its tendency for deamidation (Dukan et al., 2000). Additionally, denaturation can increase a protein's glycation potential (Seidler and Yeargans, 2002). Cross-linking of proteins induced by either oxidation or glycation can inhibit proteolytic elimination of oxidized proteins by the proteasomes (Carrard et al., 2002; Chondrogianni and Gonos, 2005) and increase lipofuscin production (Terman, 2001; Szweda et al., 2003; Powell et al., 2005). Proteasome function is also essential for cell cycling and DNA repair. Accumulation of cross-linked proteins in lysosomes can inhibit the elimination of dysfunctional mitochondria (Brunk and Terman, 2002). Indeed even mild proteasome inhibition can affect mitochondrial function including intra-mitochondrial protein biosynthesis (Sullivan et al., 2004). Dysfunctional mitochondria may increase ROS production directly and generate less ATP. Any compensating increase in glycolysis may increase protein glycation by a glycolytic by-product such as methylglyoxal which can itself damage mitochondria, induce further ROS production and as well as interact deleteriously with proteins to produce cross-linked species.

18. Final comments

Although much of this discussion on the causes of age-related accumulation of altered proteins has centred on compromised proteolytic function, it should not be assumed that all age-associated conditions exhibit this phenomenon. Indeed, age-related muscle loss or sarcopenia, which is responsible for much impaired mobility in old age, appears to be caused, at least in part, by raised proteolytic activity in muscle tissues, especially increased substrate ubiquitination and proteasomal function (Husom et al., 2004). Depressed protein biosynthetic activity may also contribute to the net loss of protein during sacropenia. Clearly the situation is complex but the irreversible loss of proteins that accompanies sarcopenia is thought to result from defective regulation of the ubiquitin-proteasome system and an impaired ability to recover from stress (see Attaix et al., 2005 for recent review). It is possible that those hypothetical factors which normally ensure coordination between protein biosynthetic and degradative activities become increasingly disengaged in post-mitotic tissues of old adult animals. In some muscle tissues protein loss predominates as a result of unregulated proteasome-mediated proteolysis producing sarcopenia (Attaix et al., 2005), whereas in other tissues proteasomal activity is insufficient to ensure removal of altered protein species and other pathologies result.

References

Abe, H., 2000. Role of histidine-related compounds as intracellular proton buffer constituents in vertebrate muscle. Biochem. (Moscow) 65, 757–765.

Ahmed, N., Dobler, D., Dean, M., Thornalley, P.J., 2005. Peptide mapping identifies hotspot site of modification in human serum albumin by methylglyoxal involved in ligand binding and esterase activity. J. Biol. Chem. 280, 5724–5732.

Alaghband-Zadeh, J., Mehdizadeh, S., Khan, N.S., O'Farrell, A., Bitensky, L., Chayen, J., 2001. The natural substrate for nitric oxide synthase activity. Cell Biochem. Funct. 19, 277–280.

Arnold, I., Langer, T., 2002. Membrane protein degradation by AAA proteases in mitochondria. Biochim. Biophys. Acta 1592, 89–96.

Attaix, D., Mosoni, L., Dardevet, D., Combaret, L., Mirand, P.P., Grizard, J., 2005. Altered responses in skeletal muscle turnover during aging in anabolic and catabolic periods. Int. J. Biochem. Cell Biol. 37, 1962–1973.

Augustin, S., Nolden, M., Muller, S., Hardt, O., Arnold, I., Langer, T., 2005. Characterization of peptides released from mitochondria. Evidence for constant proteolysis and peptide efflux. J. Biol. Chem. 280, 2691–2699.

Basoah, A., Matthews, P.M., Morten, K.J., 2005. Rapid rates of newly synthesized mitochondrial protein degradation are significant affected by the generation of mitochondrial free radicals. FEBS Lett. 579, 6511–6517.

Baynes, J.W., Monnier, V.M., 1989. The maillard reaction in aging, diabetes and nutrition. Alan R. Liss, Inc., New York.

Baynes, J.W., Thorpe, S.R., 2000. Glycoxidation and lipoperoxidation in atherogenesis. Free Rad. Biol. Med. 28, 1708–1716.

Berlanga, J., Cibrian, D., Guillen, I., Frere, F., Alba, J.S., Lopez-Saura, P., merino, N., Aldama, A., Quintela, A., Triana, M., Montequin, J.F., Ajamieh, H., Urquiza, D., Ahmed, N., Thornalley, P.J., 2005. Methylglyoxal administration induces diabetes-like microvascular changes and perturbs the healing process of cutaneous wounds. Clin. Sci. (Lond.) 109, 83–95.

Biswas, S.K., Newby, D.E., Rahman, I., Megson, I.L., 2005. Depressed glutathione synthesis precedes oxidative stress and atherogenesis in Apo-E(-/-) mice. Biochem. Biophys. Res. Commun. 338, 1368–1373.

Bohr, V.A., Opresko, P.L., 2003. Genomic instability in human premature aging. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 65–77.

Bota, D.A., Davies, K.J.A., 2001. Protein degradation in mitochondria: implications for oxidative stress, aging and disease: a novel etiological classification of mitochondrial proteolytic disorders. Mitochondrion 1, 33–49.

Bota, D.A., Davies, K.J.A., 2002. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. Nat. Cell Biol. 4, 674–680.

Bota, D.A., van Remmen, H., Davies, K.J.A., 2002. Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. FEBS Lett. 532, 103–106.

Brownlee, M., 1995. Advanced protein glycation in diabetes and aging. Ann. Rev. Med. 46, 223–234.

Brownlee, M., 2001. Biochemistry and molecular biology of diabetic complications. Nature 414, 813–820.

Brunk, U.T., Terman, A., 2002. The mitochondrian-lysosomal axis theory of aging. accumulation of damaged mitochondria as a result of imperfect autophagocytosis. Eur. J. Biochem. 269, 1996–2002.

Calabrese, V., Colobrita, C., Guagliano, E., Sapienza, M., Ravagna, A., Cardile, V., Scapagnini, G., Santoro, A.M., Mangiameli, Butterfield, D.A.,

Giuffrida Stella, A.M., Rizzarelli, E., 2005. Protective effects of carnosine during nitrosative stress in astroglial cell cultures. Neurochem. Res. 30, 797–807.

Carrard, G., Friguet, B., 2003. The proteasome in aging. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 213–231.

Carrard, G., Bulteau, A.-L., Petropoulos, I., Friguet, B., 2002. Impairment of proteasome structure and function in aging. Int. J. Biochem. Cell Biol. 34, 1461–1474.

Chaplen, F.W.R., 1998. Incidence and potential implications of the toxic metabolite methylglyoxal in cell culture: a review. Cytotechnology 26, 173–183.

Chavous, D.A., Jackson, F.R., O'Conner, C.M., 2001. Extension of drosophila lifespan by overexpression of a protein repair methyltransferase. Proc. Natl Acad. Sci. USA 98, 14814–14818.

Chondrogianni N., Gonos, E.S. 2005. Proteasome dysfunction in mammalian aging: Steps and factors involved. Exp Gerontol. 40, 931–938.

Ciehanover, A., 2005. Proteolysis: from the lysosome to ubiquitin and the proteasome. Nat. Rev. Mol. Cell Biol. 6, 79–86.

Clarke, S., 1985. Protein carboxyl methyltransferase: two distinct classes of enzymes. Annu. Rev. Biochem. 54, 479–506.

de Pril, R., Fischer, D.F., van Leeiwren, F.W. 2006. Conformation diseases: an umbrella for various disorders with an impaired ubiquitin–proteasome system. Neurobiol. Aging. 27, 515–520.

Del Roso, A., Vittorini, S., Cavallini, G., Donati, A., Gori, Z., Masini, M., Pollera, M., Bergamini, E., 2003. Ageing-related changes in the in vivo function of rat liver autophagy and proteolysis. Exp. Gerontol. 38, 519–527.

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

Ellgaard, L., Helenius, A., 2003. Quality control in the endoplasmic reticulum. Nat. Rev. Mol. Cell Biol. 4, 181–191.

Gerez, L., de Haan, A., Hol, E.M., Fischer, D.F., van Leeuwen, F.W., van Steeg, H., Benne, R., 2004. Molecular misreading: the frequency of dinucleotide deletions in neuronal mRNAs for β-amyloid precursor protein and ubiquitin B. Neurobiol. Aging 26, 145–155.

Grune, T., Jung, T., Merker, K., Davies, K.J.A., 2004. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceriod, and 'aggresomes' during oxidative stress, aging, and disease. Int. J. Biochem. Cell Biol. 36, 2519–2530.

Gugliucci, A., 2005. Alternative antiglycation mechanisms: are spermine and fructosamine-3-kinase part of a carbonyl damage control pathway? Med. Hypoth. 64, 770–777.

Hazelton, G.A., Lang, C.A., 1980. Glutathione content of tissues in the aging mouse. Biochem. J. 188, 25–30.

Hipkiss, A.R., 1998. Carnosine, a protective anti-ageing peptide? Int. J. Biochem. Cell Biol. 30, 863–868.

Hipkiss, A.R., 2003a. Errors, mitochondrial dysfunction and ageing. Biogerontology 4, 397–400.

Hipkiss, A.R., 2003b. Do developmentally-related changes in constitutive proteolysis affect aberrant protein accumulation and generation of the aged phenotype? Mech. Ageing Dev. 124, 575–579.

Hipkiss, A.R., 2003c. Non-oxidative modification of DNA and proteins. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 145–177.

Hipkiss, A.R., 2005. Glycation, ageing and carnosine: are carnivorous diets beneficial? Mech. Ageing Dev. 126, 1034–1039.

Hipkiss, A.R., 2006. Caloric restriction and ageing—is glycolysis the problem? Mech. Ageing Dev. 127, 8–15.

Hipkiss, A.R., Michaelis, J., Syrris, P., 1995. Non-enzymatic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent. FEBS Lett. 371, 81–85.

Hirokawa, K., Gomi, K., Kajiyama, N., 2003. Molecular cloning and expression of novel fructosyl peptide oxidases and their application for the measurement of glycated protein. Biochem. Biophys. Res. Commun. 311, 104–111.

Hsu, A.L., Murphy, C.T., Kenyon, C., 2003. Regulation of aging and ag-related disease by DAF-16 and heat shock factor. Science 300, 1142–1145.

Husom, A.D., Peters, E.A., Kolling, E.A., Fugere, N.A., Thompson, L.V., Ferrington, D.A., 2004. Altered proteasome function and subunit composition in aged muscle. Arch. Biochem. Biophys. 421, 67–76.

Jay, D., Hitomi, H., Griendling, K.K. 2006. Oxidative stress and diabetic cardiovascular complications. Free Rad. Biol. Med. 40, 183–192.

Kim, E., Lowenson, J.D., MacLaren, D.C., Clarke, S., Young, S.G., 1997. Deficiency of a protein-repair enzyme results in the accumulation of altered proteins, retardation of growth and fatal seizures in mice. Proc. Natl Acad. Sci. USA 94, 6132–6137.

Kotamraju, S., Matalon, S., Matsunaga, T., Shang, T., Hickman-Davis, J.M., Kalyanaraman, B. 2006. Upregulation of immunoproteasomes by nitric oxide: potential antioxidative mechanism in endothelail cells. Free Radic. Biol. Med. 40, 1034–1044.

Lambert, A.J., Wang, B., Merry, B.J., 2004. Exogenous insulin can reverse the effects of caloric restriction on mitochondria. Biochem. Biophys. Res. Commun. 316, 1196–1201.

Lee, Y., Hsu, C., Lin, M., Liu, K., Yin, M., 2005. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. Eur. J. Pharmacol. 512, 145–150.

Levine, R.L., 2002. Carbonyl modified proteins in cellular regulation, aging and disease. Free Radic. Biol. Med. 32, 790–796.

Lindahl, T., 1993. Instability and decay of the primary structure of DNA. Nature 363, 709–715.

Lindner, H., Helliger, W., 2001. Age-dependent deamidation of asparagine residues in proteins. Exp. Gerontol. 36, 1551–1563.

Martinez-Vincente, M., Sovak, G., Cuervo, A.M. 2005. Protein degradation and aging. Exp. Gerontol. 40, 622–633.

Masternak, M.M., Al-Regaiey, K.A., Bonkowski, M.S., Panici, J.A., Bartke, A., 2005. Effect of every other day feeding diet on gene expression in normal and in long-lived Ames dwarf mice. Exp. Gerontol. 40, 491–497.

Mattson, M.P., Wan, R., 2005. Beneficial effects of intermittent feeding and caloric restriction on the cardiovascular and cerebrovascular systems. Nutr. Biochem. 16, 129–137.

Moskovitz, J., Bar-Noy, S., Williams, W.M., Requena, J., Berlett, B.S., Stadtman, E.R., 2001. Methionine sulphide reductase (MsrA) is a regulator of anti-oxidant defense and lifespan in animals. Proc. Natl Acad. Sci. 98, 12920–12925.

Niijima, A., Okui, T., Matsumura, Y., Yamano, T., Tsuruoka, N., Kiso, Y., Nagai, K., 2002. Effects of L-carnosine on renal sympathetic nerve activity and DOCA-salt hypertension in rats. Auton. Neurosci. Basic Clin. 97, 99–102.

Pamplone, R., Barja, G., 2003. Aging rate, mitochondrial free-radical production, and constitutive sensitivity to lipid peroxidation: insights from comparative studies. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 47–64.

Powell, S.R., Wang, P., Divald, A., Teichberg, S., Haridas, V., McCloskey, T.W., Davies, K.J.A., Katzeff, H. 2005. Aggregates of oxidized proteins (lipofuscin) induce apoptosis through proteasome inhibition and dysregulation of proapoptotic proteins. Free Radic. Biol. Med. 38, 1093–1101.

Proctor, C.J., Soti, C., Boys, R.J., Gillespie, C.S., Shanley, D.P., Wilkinson, D.J., Kirkwood, T.B.L., 2005. Modelling the actions of chaperones and their role in ageing. Mech. Ageing Dev. 126, 119–131.

Radak, Z., Sasari, M., Nyakas, C., Taylor, A.W., Ohno, H., Nakamoto, H., Goto, S., 2000. Regular training modulates the accumulation of reactive carbonyl derivatives in mitochondrial and cytosolic fractions of rat skeletal muscle. Arch. Biochem. Biophys. 383, 114–118.

Rapoport, S., Dubiel, W., Muller, M., 1985. Proteolysis of mitochondria in reticulocytes during maturation is ubiquitin-dependent and is accompanied by a high rate of ATP hydrolysis. FEBS Lett. 180, 249–252.

Rattan, S.J.H., Derventzi, A., Clark, B.F.C., 1992. Protein synthesis, posttranslational modifications, and aging. Ann. NY Acad. Sci. 663, 48–60.

Ririe, D.G., Roberts, P.R., Shouse, M.N., Zaloga, G.P., 2000. Vasodilatory actions of the dipeptide carnosine. Nutrition 16, 168–172.

Ritz-Timme, S., Collins, M.J., 2002. Racemization of aspartic acid in human proteins. Ageing Res. Rev. 1, 43–59.

Robinson, N.E., 2002. Protein deamidation. Proc. Natl Acad. Sci. USA 99, 5283–5288.

Rosenberger, R.F., 1991. Senescence and the accumulation of abnormal proteins. Mutat. Res. 256, 255–262.

Rosenberger, R.F., Carr, A.J., Hipkiss, A.R., 1990. Regulation of breakdown of canavanyl proteins in Escherichia coli by growth conditions in Lon^+ and Lon^- cells. FEMS Lett. 68, 19–25.

Schmidt, A.M., Hori, O., Brett, J., Yan, S.D., Wautier, J.L., Stern, D., 1994. Cellular receptors for advanced glycation end products. Implications for induction of oxidative stress and cellular dysfunction in pathologies of vascular lesions. Arterioscler. Thromb. 14, 1521–1528.

Seeberg, E., 2003. Oxidative DNA damage and repair—implications for aging. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 11–25.

Seidler, N.W., Yeargans, G.S., 2002. Effects of thermal denaturation on protein glycation. Life Sci. 70, 1789–1799.

Shibamoto, T., 1989. Genotoxicity testing of Maillard reaction products. In: Baynes, J.W., Monnier, V.M. (Eds.), The Maillard Reaction in Aging, Diabetes and Nutrition. A.R.Liss, Inc., New York, pp. 359–376.

Shimuzu, K., Kiuchi, Y., Ando, K., Hayakawa, M., Kikugawa, K., 2004. Coordination of oxidized protein hydrolase and the proteasome in the clearance of cytotoxic proteins. Biochem. Biophys. Res. Commun. 424, 140–146.

Sitte, N., 2003. Oxidative damage to proteins. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 27–45.

Sitte, N., von Zglinicki, T., 2003. Free radical production and anti-oxidant defense: a primer. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 1–10.

Soti, C., Csermely, P., 2003. Aging and molecular chaperones. Exp. Gerontol. 38, 1037–1040.

Stadman, E.R., 2002. Importance of individuality in oxidative stress and aging. Free Radic. Biol. Med. 33, 597–604.

Stadtman, E.R., 1992. Protein oxidation and aging. Science 257, 1220–1224.

Stefani, M., 2004. Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world. Biochim. Biophys. Acta 1739, 5–25.

Stolzing, A., Widmer, R., Jung, T., Voss, P., Grune, T. 2006. Degradation of glycated serum albumin in microglial cells. Free Rad Biol. Med. 40, 1017–1027.

Sullivan, P.G., Dragicevic, N.B., Deng, J.-H., Bai, Y., Dimayuga, E., Ding, Q., Chen, Q., Bruce-Keller, A.J., Keller, J.N., 2004. Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. J. Biol. Chem. 279, 20699–20707.

Szweda, P.A., Camouse, M., Lundberg, K.C., Oberly, T.D., Szweda, L.I., 2003. Aging, lipofuscin formation, and free radical-mediated inhibition of cellular proteolytic systems. Ageing Res. Rev. 2, 383–405.

Szwergold, B.S., 2005. Intrinsic toxicity of glucose, due to non-enzymatic glycation, is controlled in vivo by deglycating systems including: FN3K-mediated deglycation of fructosamines and transglycation of aldomines. Med. Hypoth. 65, 337–348.

Szwergold, B.L., Howell, S.K., Beisswenger, P.J., 2005. Transglycation—a potential new mechanism for deglycation of Schiff's bases. Ann. NY Acad. Sci. 1043, 845–864.

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

Terman, A., Brunk, U.T., 2003. Aging and lysosomal degradation of cellular constituents. In: von Zglinicki, T. (Ed.), Aging at the Molecular Level. Kluwer, The Netherlands, pp. 233–242.

Thomas, J.A., Mallis, R.J., 2001. Aging and oxidation of reactive protein sulfhydryls. Exp. Gerontol. 36, 1519–1526.

Tomonaga, S., Tachibana, T., Takahashi, H., Sato, M., Denbow, D.M., Furuse, M. 2005. Nitric oxide involves in carnosine-induced hyperactivity in chicks. Eur. J. Pharamacol. 524, 84–88.

Tsirigotis, M., Zhang, M., Chiu, R., Wouters, B.G., 2001. Sensivity of mammalian cells expressing mutant ubiquitin to protein-damaging agents. J. Biol. Chem. 276, 46073–46078.

Verbeke, P., Fonager, J., Clark, B.F.V., Rattan, S.I.S., 2001. Heat shock response and ageing: mechanisms and applications. Cell Biol. Int. 25, 845–857.

Williams, S., Cusco, J., Roddy, M., Johnston, M., Creager, M., 1996. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J. Am. Coll. Cardiol. 27, 567–574.

Wu, X., Monnier, V.M., 2003. Enzymatic deglycation of proteins. Arch. Biochem. Biophys. 419, 16–24.

Yan, L.-J., Sohal, R.S., 2003. Prevention of flight activity prolongs the lifespan of the housefly, musca domestica, and attenuates the age-associated damage to specific proteins. Free Radic. Biol. Med. 29, 1143–1150.

Yarian, C.S., Rebrin, I., Sohal, R.S., 2005. Aconitase and ATP synthase are targets of malondialdehyde modification and undergo an age-related decrease in activity in mouse heart mitochondria. Biochem. Biophys. Res. Commun. 330, 151–156.

Yarian, C.S., Toroser, D., Sohal, R.S. 2006. Aconitase is the main functional target of aging in the citric acid cycle of kidney mitochondria in mice. Mech. Ageing Dev. 127, 79–84.

Yokoyama, K., Fukumoto, K., Murakami, T., Harada, S., Hosono, R., Wadha, R., Mitsui, Y., Ohkuma, S., 2002. Extended longevity of Caenorhabtilis elegans by knocking in extra copies of hsp70F, a homolou of mot-2 (mortalin)/mthsp70/Grp75. FEBS Lett. 516, 53–57.

Zhu, Y., Wang, M., Lin, H., Huang, C., Shi, X., Luo, J., 2002. Epidermal growth factor up-regulates the transcription of mouse Lon homologue ATP-dependent protease through extracellular signal-regulated protein kinase- and phosphatidylinositol-3-kinase-dependent pathways. Exp. Cell Res. 280, 97–106.