Protein Redox-Regulation Mechanisms in Aging

Ufuk Çakatay

Abstract The perspicuity of the general mechanisms of in vivo protein oxidation was achieved in the 1980s and that of the redox-homeostasis mechanisms of reactive oxygen species (ROS)/antioxidants in the 1990s. Publications in the scientific literature dealing with protein redox-regulation mechanisms in aging have appeared only within the past 10–15 years. As is well known, the group of protein disulfide oxidoreductases, such as thioredoxin (Trx), glutaredoxin, and Trx-dependent oxidoreductases, as well as methionine sulfoxide reductase (Msr), and the mechanisms related to these systems work synergistically to regulate the level of oxidized proteins and to repair mildly oxidatively modified proteins, keeping a balanced redox potential to maintain the function of aging cells. The proteolytic enzyme systems such as proteasome complexes, caspases, and the Lon protease, which are regulated by redox mechanisms, eliminate oxidized proteins. These mechanisms, in turn, affect redox homeostasis of proteins in aging cells. The ubiquitination and sumoylation of proteins are other mechanisms by which selectively oxidized proteins are targeted for degradation and compartmentalization with such specificity believed to be necessary for maintenance of cellular redox homeostasis. However, some of the extensively oxidized proteins of an unrepairable nature can escape degradation pathways and form high-molecular-weight aggregates that accumulate with age. Such oxidized protein aggregates can become cytotoxic and have been associated with a large number of age-related disorders, including Alzheimer's disease, Parkinson's disease, cataractogenesis, and cancer. Considering the variations that have emerged in redox-regulation mechanisms and antioxidant systems related to age-related disorders, it is found that these are of an extremely complex nature. Work communicated to us in the current scientific literature now shows the extent of oxidative protein damage in aged subjects and in age-related disorders. Future research will probably be concerned with understanding the relationship between

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the aforementioned redox-regulatory proteins and age-related disorders. Such scientific progress will bring preventive and therapeutic approaches to control altered redox homeostasis in these disorders.

Keywords Aging · Protein oxidation · Oxidative protein damage · Redox regulation

1 Introduction

Since the completion of the Human Genome Project in 2002 and the recognition that this cannot provide all the answers to the etiology of age-related diseases, attention has turned to assessing changes in the expressed proteins of a given genome. Proteins are highly sensitive to oxidative modifications by reactive oxygen species (ROS) and reactive nitrogen species (RNS). In addition, native proteins can be modified by highly reactive aldehydes and ketones produced during ROS-mediated oxidation of lipids and glycated proteins during the aging process [1]. Most of our understanding about the modification of proteins by ROS comes from the pioneering studies of Garrison [2, 3], Swallow [4], and Schuessler and Schilling [5], who studied the effects of ionizing radiation on the modification of amino acids, peptides, and proteins. The results of these studies demonstrate that the oxidation of proteins by ROS can lead to oxidation of amino-acid-residue side chains, cleavage of peptide bonds, and formation of covalent protein-to-protein cross-linked derivatives. In later years, mechanisms of chemical modification of proteins by ROS/RNS were investigated until the 1990s, notably by E.R. Stadtman, R.L. Levine, R.T. Dean, K.J. Davies, and others, and were demonstrated extensively [6].

On the other hand, a broad community of investigators focused on the role of protein oxidation in the etiology and/or progression of several age-related diseases [7]. As a result of all these studies, it was determined that oxidized protein levels in tissues of subjects having such diseases are higher than those in normal subjects. Publications in the scientific literature dealing with protein redox-regulation mechanisms in aging appeared only within the past 10–15 years. As is well known, the group of protein disulfide oxidoreductases, such as thioredoxin (Trx)/Trx reductase and glutaredoxin (also known as thioltransferases), as well as methionine sulfoxide reductase (Msr), and the antioxidant mechanisms related to these enzymes work synergistically to regulate and repair oxidatively modified proteins, keeping a balanced redox potential to maintain the function of aging cells [8]. The proteolytic enzyme systems such as proteasome complexes, caspases, Lon protease, and the small ubiquitin-like modifier (SUMO), which are regulated by redox signaling mechanisms, that affect redox homeostasis of proteins in aging cells [7, 9–11]. However, some of the extensively oxidized proteins of an unrepairable nature can escape from the degradation pathways and form high-molecular-weight aggregates that accumulate with age. Such oxidized protein aggregates can become

cytotoxic and have been associated with a large number of age-related disorders, including Alzheimer's disease, Parkinson's disease, cataractogenesis, and cancer [12].

Because protein oxidation mechanisms and their markers in aging and age-related disorders have been treated extensively in various reviews and textbooks, including those by the aforementioned leading authors, we have chosen not to deal with the same material in this chapter and concentrate instead on protein redox-regulation mechanisms in the aging cell.

2 Postmitotic Aging and Redox Homeostasis

Aging is a progressive decline in an organism's adaptability and consequent increase in morbidity and mortality. It largely depends on changes occurring in long-lived postmitotic cells (nondividing cells), such as neurons, cardiac myocytes, and retinal pigment epithelial cells [13, 14]. Available data from various studies support the development of mitochondrial failure in old age in fixed postmitotic cells suggesting that mitochondrial failure may be central to the senescent process. It is now generally accepted that aging in such long-lived cells is induced by endogenously formed (primarily from mitochondria) ROS causing irreversible damage (destined mainly to mitochondria) with increased oxidative protein damage and triggering apoptotic cell death [14, 15]. The delicate balance between beneficial/harmful effects of free radicals is a very important aspect of living organism and is achieved by a mechanism called *redox regulation* (Fig. 1). The process of redox regulation protects living organisms from various oxidative stresses and maintains *redox homeostasis* by controlling the redox status in vivo [14, 16].

Postmitotic tissues of high energy demand are at greater risk of being damaged by free radicals, consistent with the notion that signs of oxidative damage usually start to appear at these body sites [17]. Due to the differing efficacy of redox homeostasis mechanisms, postmitotic tissues are generally much more vulnerable to oxidative protein damage than the mitotic cells. Whereas the liver is an organ with high mitotic rate and the major organ of antioxidant enzyme release, the brain, which is of a postmitotic nature, produces more ROS per gram of tissue than any other organ, because of its high lipid content, high oxygen consumption (20% of all oxygen), and relatively poor antioxidant defense [7, 18].

These cells are very rarely (or never) replaced because of the division and differentiation of stem cells, causing the accumulation of biological waste materials (e.g., lipofuscin, irreversibly damaged mitochondria, and aberrant proteins) that gradually replace normal structures, leading to functional decay and cell death [19, 20]. The free radical theory of aging, as already proposed in 1956 by Denham Harman [21], has recently been extended to the oxidative "garbage-catastrophe theory" by Alexei Terman [22] according to which ROS or reactive oxygen intermediates are responsible for the accumulation of oxidatively damaged biomolecules in aging. According to garbage-catastrophe theory, aging may

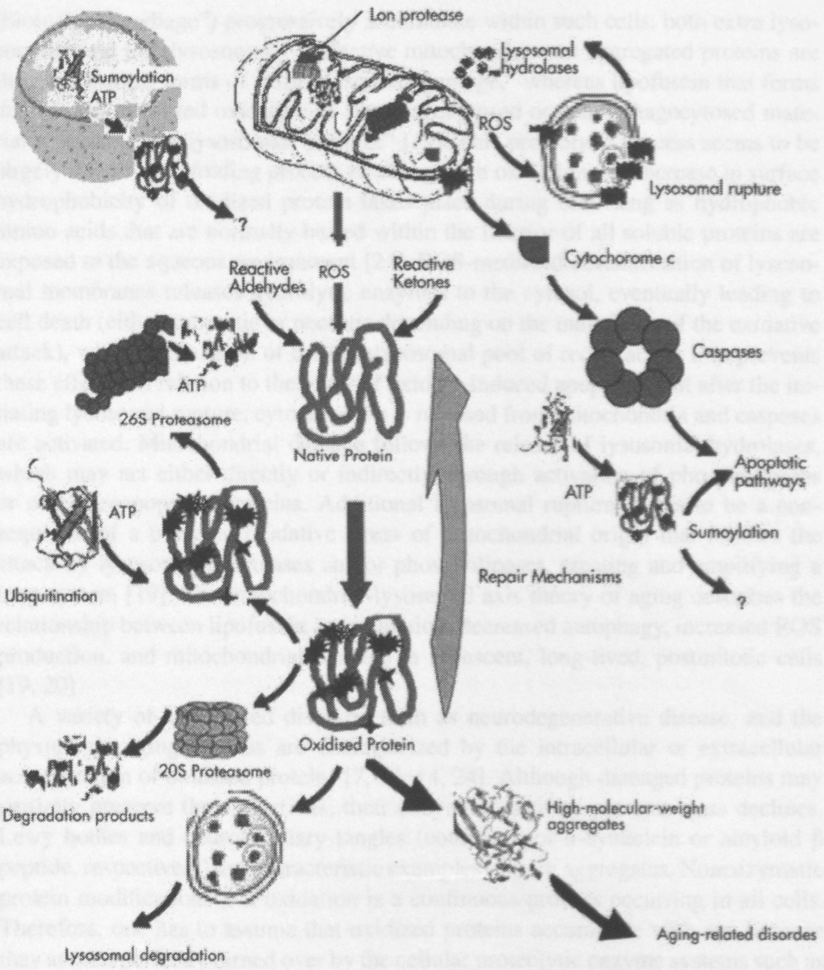

Fig. 1 Proteins can become oxidatively modified by a large number of reactions involving ROS, reactive aldehydes, and ketones. Intracellular degradation pathways and repair systems maintain redox homeostasis of the proteins in aging postmitotic cells. Oxidized proteins are generally destined for proteolysis by the lysosomal system, proteasome, small ubiquitin-like modifier (SUMO), and the Lon protease but can escape degradation and form high-molecular-weight aggregates that accumulate with aging. Depending on the type and degree of the oxidative modification, intracellular elimination of oxidatively modified proteins materializes in the different cellular compartments by either degradation or repair systems including the group of protein disulfide oxidoreductases, such as Trx/Trx reductase, glutaredoxin, and Msr. The accumulation of oxidized proteins is known to be linked to age-related diseases such as Alzheimer's disease, Parkinson's disease, and cancer

derive from imperfect elimination of oxidatively damaged, relatively indigestible cellular material, the accumulation of which further hinders metabolic functions and mainly affects such cells [22–24]. Because of inherently imperfect lysosomal degradation (autophagy) and other self-repair mechanisms, damaged structures

(biological "garbage") progressively accumulate within such cells, both extra lysosomally and intralysosomally. Defective mitochondria and aggregated proteins are the most typical forms of extralysosomal "garbage," whereas lipofuscin that forms due to iron-catalyzed oxidation of autophagocytosed or heterophagocytosed material represents intralysosomal "garbage" [19]. This proteolytic process seems to be largely due to an unfolding process resulting from oxidation. An increase in surface hydrophobicity of oxidized protein takes place during unfolding as hydrophobic amino acids that are normally buried within the interior of all soluble proteins are exposed to the aqueous environment [24]. ROS-mediated destabilization of lysosomal membranes releases hydrolytic enzymes to the cytosol, eventually leading to cell death (either apoptotic or necrotic depending on the magnitude of the oxidative attack), whereas chelation of the intralysosomal pool of redox-active iron prevents these effects. In relation to the onset of oxidant-induced apoptosis, but after the initiating lysosomal rupture, cytochrome c is released from mitochondria and caspases are activated. Mitochondrial damage follows the release of lysosomal hydrolases, which may act either directly or indirectly, through activation of phospholipases or other proapoptotic proteins. Additional lysosomal rupture seems to be a consequence of a transient oxidative stress of mitochondrial origin that follows the attack by lysosomal hydrolases and/or phospholipases, creating and amplifying a loop system [19]. The mitochondrial-lysosomal axis theory of aging describes the relationship between lipofuscin accumulation, decreased autophagy, increased ROS production, and mitochondrial damage in senescent, long-lived, postmitotic cells [19, 20].

A variety of age-related diseases, such as neurodegenerative disease, and the physiologic aging process are characterized by the intracellular or extracellular accumulation of oxidized proteins [7, 13, 14, 24]. Although damaged proteins may partially preserve their functions, their enzymatic activity per unit mass declines. Lewy bodies and neurofibrillary tangles (composed of α-synuclein or amyloid β peptide, respectively) are characteristic examples of such aggregates. Nonenzymatic protein modifications and oxidation is a continuous process occurring in all cells. Therefore, one has to assume that oxidized proteins accumulate with age because they are not perfectly turned over by the cellular proteolytic enzyme systems such as 20S and 26S proteasome complexes in cytoplasm and nucleus, caspases in cytosol, Lon protease in mitochondria, and lysosomal enzymes [7, 9, 11, 19, 20, 24]. The ubiquitination [7, 11, 12, 24] and sumoylation [10, 11] of proteins are other crucial mechanisms by which selectively oxidized proteins are targeted for degradation with such specificity believed to be necessary for maintenance of cellular redox homeostasis in the aging cell.

Although the SUMO system was only discovered 10 years ago, extensive studies in the past few years have demonstrated that sumoylation is a remarkably versatile regulatory mechanism of protein functions involved in the regulation of diverse cellular process. SUMO can either regulate the functional activity of a target protein by direct sumoylation or indirectly regulate a signaling pathway via sumoylation of a key signaling molecule. SUMO proteases may also act as redox sensors and effectors modulating the desumoylation pathway and specific cellular responses to oxidative stress. Recent evidence indicated that equilibrium between

SUMO conjugation–deconjugation under high oxidative stress could be affected by ROS [10].

Other evidence was provided previously by the finding that overexpression of antioxidant enzymes that prevent the generation of excess free radicals, such as superoxide dismutase (SOD) and catalase [25], reduce the levels of oxidized proteins. On the other hand, the oxidation of methionine plays an important role in oxidative protein damage in the aging cell. Depending on the nature of the oxidizing species, methionine may undergo a two-electron oxidation transforming it into methionine sulfoxide or a one-electron oxidation into methionine radical cations. Methionine radical cations will be destined to predominately irreversible reaction channels, which ultimately yield carbon-centered and/or peroxyl radicals. These may become starting points for chain reactions of protein oxidation [26]. Methionine sulfoxide levels may increase as a result of insufficient active Msr and/or the required cofactors as a consequence of pathologies and biological aging. Msr [27], which catalyzes the repair of oxidized methionine in proteins by reducing methionine sulfoxide back to methionine, extends the life span of *Drosophila melanogaster* markedly. 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 CuZn-SOD and double the life span [28]. It is worthwhile to mention that the germ line of egg and sperm has been maintained alive and safe from senescence and oxidative damage for more than a billion years. Because multicellular organisms are able to reproduce with germ-line cells before senescence of their soma, which incapacitates them, there was never any evolutionary impetus to develop biochemical mechanisms of preventing senescence in their postmitotic cells. Notably, both *Drosophila melanogaster* and *Caenorhabditis elegans* are mostly composed of postmitotic cells; the results from these invertebrates are much more supportive of the free radical theory of aging than are results from rat.

Our understanding of the intricate and delicate redox-regulation mechanisms of cellular proteins and antioxidant systems in postmitotic tissues in aging and age-related diseases is much less advanced although many detailed studies have been performed in recent years.

3 Redox-Regulation Pathways and Repair of Proteins

3.1 General Principles

Although excessive oxidative damage in proteins and nucleotides may occur, physiologic amounts of oxidative stress transduce intracellular signals for activation, differentiation, and proliferation. Special pathways exist in aging cells to accompany redox regulation of proteins including redox-regulating enzymes, proteolytic enzyme systems, and tagging systems. Several possibilities may be the cause of an increase in the steady-state level of oxidatively modified proteins [23]. These include [1] an increase in the formation of oxidizing species [2], a decreased

antioxidant capacity to scavenge those species [3], an increased susceptibility of the proteins to become oxidized as a consequence of transcriptional and translational errors, and [4] a decrease in the levels or activities of the proteasome or proteases that selectively degrade oxidized proteins. Moreover, it has been shown that the activity of the redox-regulating enzymes catalyzes the repair of oxidized proteins, and tagging systems affect the steady-state level of oxidatively modified proteins [8, 10, 11, 27]. On the other hand, gender-related hormonal status and other possible regulatory events are other important factors that contribute to the type and extent of oxidative protein damage in various tissue proteins in aged subjects [29–31]. It is still obscure how gender-related factors affect the redox-regulating mechanisms and, in turn, the steady-state level of oxidatively modified proteins.

Reversible oxidation–reduction reactions of sulfur atoms in cysteine and methionine provide a common mechanism for the control of physical and functional properties of cellular proteins. Cys-based redox signaling mechanisms are an essential cellular response on oxidative stress. Oxidation of cysteine residue side-chains in proteins forms disulfides, sulfenic acids, sulfinic acids, and sulfonic acids. Many amino acids can undergo oxidation, and sulfur atoms can be oxidized to a number of different oxidation states; but only Cys and Met undergo reversible oxidation reactions, and these involve only a limited number of oxidation states. The cellular redox measurements largely reflect the cytoplasmic compartment. The restricted movement of biomolecules imposed by both the plasma membrane and intracellular membrane systems creates multiple compartments with different redox status. Based on current data, which are limited for some organelles, the redox status of the secretory pathways and lysosomes appears to be relatively oxidizing, whereas that of the nucleus is relatively reducing [32]. The *i*ntegrated mechanisms for controlling redox homeostasis in different cellular compartments need to be elucidated in future aging studies.

Low-molecular-weight thiols such as reduced glutathione (GSH) and protein thiol (–SH) groups undergo reversible oxidation to form disulfides [32]. Cellular thiol systems are important in the control of redox regulation of proteins, both by protecting the aging cells against oxidative damage and serving in redox signaling mechanisms to sense danger and to repair damage [33]. Studies by a number of research groups show that the redox state of the central tissue antioxidant, GSH, can be measured in plasma and provide a quantitative systemic indicator of oxidative stress. The GSH/GSSG (oxidized glutathione) redox couple in humans and experimental animals tends to oxidize with age [29, 30]. However, the GSH/GSSG redox couple is not in equilibrium with the larger plasma cysteine/cystine (Cys/CySS) pool, and the Cys/CySS redox couple varies with age in a pattern that is distinct from that of the GSH/GSSG redox couple. Furthermore, in vitro studies show that variation in Cys/CySS redox-couple status over the range found in vivo affects signaling pathways that control cell proliferation and oxidant-induced apoptosis. The results point to the conclusion that free radical scavenging antioxidants are of increased importance when thiol/disulfide redox states are oxidized. Because thiol/disulfide redox states, per se, function in redox signaling and control as well as antioxidant protection, GSH/GSSG and Cys/CySS redox states may provide central parameters

to link environmental influences and progression of changes associated with aging [33].

The current knowledge on the oxidized protein repair systems and degradation mechanisms in aging is reviewed herein. The possible interactions between the ubiquitin-proteasome system, the SUMO system, the protein repair mechanisms, and other antioxidative defense strategies are highlighted without going into extensive detail.

3.2 Intracellular Mechanisms

3.2.1 Role of Thiol-Based Repair Systems

Oxidized protein repair systems are limited to the reduction of certain oxidation products of sulfur-containing amino acids. Two principal systems foresee cellular thiol/disulfide redox state: GSH and Trx. These systems are complementary but also have overlapping activities that provide a partial redundancy in their functions. GSH is a low-molecular-weight thiol present at millimolar concentrations in cells and is well suited for functions in detoxification, interorgan cysteine homeostasis, and redox control, whereas Trx is a key molecule for redox regulation and small multifunctional protein (12 kDa) and is present at micromolar concentrations and has a redox–active disulfide/dithiol within the conserved active site sequence: Cys-Gly-Pro-Cys [32, 34, 35]. Trx is induced by a variety of oxidative stress conditions and plays crucial roles as a redox-regulator of intracellular signal transduction and as a radical scavenger. The dithiol motif at its active site is ideally suited for reduction of protein disulfides, sulfoxides, and sulfenic acids, but also is capable of peroxide elimination [32].

Methionine is first oxidized into methionine sulfoxide, which can be further oxidized into methionine sulfone. The oxidation of methionine into methionine sulfoxide is accompanied by a decreased hydrophobicity and flexibility and has been associated with the impairment of protein function. Disulfide bridge and sulfenic acid reduction is achieved by the Trx/Trx reductase system, whereas the glutaredoxin/glutathione/glutathione reductase system can reduce both disulfide bridges and low-molecular-weight mixed disulfides, including glutathione. Trx and glutaredoxin are small ubiquitous proteins belonging to the thiol/disulfide oxidoreductase family, the members of which contain a redox-active disulfide at their active centers. Oxidized Trx, which carries a disulfide bridge, is subsequently reduced in an NADPH-dependent manner by Trx reductase, an enzyme containing selenocysteine and flavin that is present in both the cytosol and mitochondria of mammalian cells. The inactivation of Trx-dependent peroxidases and peroxiredoxins as the result of cysteine-sulfinic acid formation was found to be reversible in mammalian cells [36].

Oxidation of methionine residues leads to the formation of two diastereoisomers, Met-S(O) and Met-R(O), which can be enzymatically reduced back to methionine by the ubiquitous enzymes methionine sulfoxide reductase A (MsrA) and B (MsrB), respectively. MsrA and MsrB are both found in the cytosol, nucleus, and

mitochondria, and endoplasmic reticulum provides an additional location for MsrB izoenzyme. Oxidized MsrA and MsrB are then reduced by the Trx/Trx reductase system in vivo [36, 37]

3.2.2 The Roles of Proteasome, Ubiquitin, and SUMO

Modification of proteins by the covalent attachment of small polypeptides is an important regulatory mechanism. The best-known molecule of this type of modification is ubiquitin: Ubiquitination is critical for targeting proteins to be degraded by proteasome complexes. Proteasome-mediated protein degradation is responsible for a large percentage of bulk protein turnover in the aging cell [7, 24]. The proteasomes are multicatalytic protease complexes that play an important role in the degradation of short-lived, oxidatively damaged proteins, including mutant or misfolded proteins, and are localized in the cytosol, the nucleus, as well as being attached to the endoplasmic reticulum. Generally, the proteasome operates in the following manner: The oxidatively damaged protein is recognized by the protease as it enters the proteasome complex through the narrow opening (α-annulus) in the middle of the outer α-ring and is processed in the catalytic chamber between the two β-rings, and the small polypeptides leave the proteasome through the opening of the second α-ring. Within the catalytic chamber of the proteasome between the two β-rings are located the six active centers that exhibit different catalytic activities [7]. The core proteasome degrades the protein only partially to single amino acids. The resulting oligopeptides from proteasome activity are further hydrolyzed by several intracellular peptidases to single amino acids. Proteins must be deaggregated and unfolded in order to be able to enter the proteasome. Most aggregated proteins, particularly cross-linked aggregates, may no longer "fit" into the proteasome. The cross-linking of these proteins may thus result in restricted entry into the core particle of the proteasome and incomplete degradation [24]. The proteasome also plays a pivotal role in several other cellular activities including cell cycle regulation, antigen presentation, and apoptosis [7]. It has been described that the 20S proteasome is activated under oxidative stress conditions and is able to degrade oxidized proteins in an ATP- and ubiquitin-independent manner [38], different from the 26S proteasome. The latter contains a central, barrel-like core particle (the 20S proteasome) composed of four stacked seven-membered rings, with the subunit stoichiometry $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ [7]. The ubiquitin-dependent 26S proteasome as well as ubiquitin-activating and -conjugating enzymes are very sensitive to direct oxidative inactivation, and cells deficient in ubiquitin-conjugating activity are able to degrade oxidatively damaged proteins at near normal rates. The key role of the 20S proteasome in the clearance of oxidized proteins is supported by data showing that several genes encoding 20S proteasome subunits are upregulated in cells exposed to oxidative stress and also during recovery after oxidative damage [39]. Within the 20S proteasome, subunits β1, β2, and β5 of both adjacent β-rings expose their proteolytically active sites, exhibiting post–glutamyl peptide hidrolyzing, trypsin-like and chymotrypsin-like cleavage specificity, respectively. As the proteasomal system consists of several proteasomal forms and regulatory particles, which themselves

are composed of different subunits with distinct functions, it seems to be likely that regulation of the proteasomal activity is very complex [7]. The knowledge of how and why the 20S and 26S proteasomes are regulated differently under oxidative stress can be of great importance for the development of new therapeutic strategies in age-related disorders. As aging cells have an altered activity of proteasomes in their nuclei and cytosol, the regulation of proteasomal systems is also at least a co-candidate for understanding age-related disease mechanisms. More information about the redox regulation of the proteasomal system within the aging cell is needed to clarify all these questions.

Similar to ubiquitin, members of the SUMO family of proteins are conjugated to lysine residues in target proteins. A short sequence containing the consensus Ψ-K-X-D/E (where Ψ is a large hydrophobic residue, K is the lysine conjugated to SUMO, X is any amino acid, D or E is an acidic residue) is thought to be necessary for this in vitro protein sumoylation to occur, however sumoylation has also been observed in cases where the consensus site is not conserved lysine residues on the target protein. SUMO proteins are relatively low-molecular-weight proteins. Most of them are approximately 100 amino acids in length and 12 kDa molecular weight. In the SUMO conjugation (sumoylation) process, cysteine residues are crucial for the formation of E1-SUMO and E2-SUMO thioester intermediates as well as catalysis of the isopeptide bond (Fig. 2). Isopeptide bond between SUMO and its target protein can be formed using the sumoylation enzyme cascade and can also be cleaved by SUMO-specific proteases, making the sumoylation process reversible [10, 11, 40, 41]. Although SUMO has very little sequence homology with ubiquitin at the amino acid level, it has a nearly identical molecular architecture with respect to ubiquitin. SUMO proteins are covalently attached to and detached from other cellular proteins to modify their biological function. Sumoylation is a posttranslational modification and may affect many cellular processes such as signal transduction, nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to oxidative stress, and progression through the cell cycle and modulation of ubiquitination. The sumoylation process (Fig. 2) is realized by an enzymatic cascade analogous to that involved in ubiquitination. Ubiquitin is conjugated to target proteins in three steps. These steps are catalyzed by enzymes known as E1 (or ubiquitin-activating enzyme), E2 (or ubiquitin-conjugation enzyme), and E3 (or ubiquitin-protein ligase), respectively. The E1 catalyzes the ATP-dependent activation of the ubiquitin C-terminal carboxyl group, resulting in a covalent

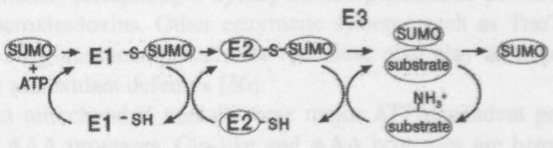

Fig. 2 The formation of the SUMO-substrate (oxidized protein) isopeptide bond. The isopeptide bond between SUMO and its substrate can also be cleaved by SUMO-specific proteases, making the sumoylation process reversible

intermediate in which ubiquitin is linked to an active site Cys residue in the E1 via a high-energy thiolester bond. Activated ubiquitin molecule is then transferred from the E1 to a Cys residue in the E2, yielding ubiquitin-E2 thiolester intermediate. Ubiquitin is then transferred from the E2 to the Lys residue in the target protein with the help of the E3, which is primarily responsible for substrate recognition and regulation of the ubiquitination process. The polyubiquitin chain is finally removed and hydrolyzed into free ubiquitin, which can be reused in a further cycle.

Although little is known about the molecular mechanism of SUMO function in aging cells, it is clear that SUMO does not target proteins for proteasome-dependent proteolysis. In some cases, SUMO appears to act by altering the subcellular localization of the SUMO-modified target protein, whereas in other cases, SUMO prevents ubiquitin-dependent proteolysis of the modified protein.

Xu et al. [10] demonstrate that the SUMO proteases may serve as redox sensor and effector undergoing both reversible and irreversible covalent modification upon exposure to various degrees of oxidative stress. Unlike most of the redox-regulatory switches previously identified, the reversible modification of SUMO proteases is at the intermolecular level [10]. It is anticipated that the seven human SUMO proteases identified to date and its substrate specificities would generate a diverse but specific intracellular redox response. On the other hand, Zhang and colleagues' study for the first time demonstrates that the amount of sumoylated protein increases with age in the spleen, and that in contrast with dietary restriction-mediated effects on the levels of ubiquitinated protein, age-related increases in the sumoylation process are not significantly affected by dietary restriction. Because sumoylation is known to have effects on protein function/localization, their data suggests a new role for sumoylation potentially contributing to altered protein function in the aging spleen [11]. Little is known of the regulation of the SUMO in physiologic aging, but any failure in its function seems to be important in age-related diseases.

3.2.3 The Roles of Mitochondrial Antioxidant Systems and of ATP-Dependent Proteases

As complexes I and III are known to be the major sites of ROS production in the cell, mitochondrial proteins are particularly exposed to oxidative damage. The first line of defense against oxidative injury is composed of a complex network of ROS detoxifying enzymes and nonenzymatic antioxidants. In the mitochondria, the ROS-detoxifying enzymes include manganese superoxide dismutase, catalase, glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, and two isoforms of peroxiredoxins. Other enzymatic systems such as Trx/Trx reductase and glutaredoxin/glutathione/glutathione reductase also play an important role in mitochondrial antioxidant defenses [36].

Mammalian mitochondria contain three major ATP-dependent proteases: Lon, Clp-like, and AAA proteases. Clp-like and AAA proteases are hetero-oligomeric complexes located in the matrix and the inner mitochondrial membrane, respectively. These proteases contribute to the degradation of misfolded and damaged proteins and/or the maintenance of mitochondrial genome stability as a second

line of defense. Mitochondrial proteases contribute to the degradation of misfolded and oxidatively damaged proteins and/or the maintenance of mitochondrial genome stability. Both proteolytic systems appear to exert chaperone activity [36]. The physiologic function of Clp-like protease has still to be determined. Currently, information regarding the redox regulation of each of the ATP-dependent proteases and/or the identities of specific protein substrates is limited.

Irreversibly oxidized proteins are mainly targeted to degradation by mitochondrial matrix proteolytic systems such as the Lon protease. As with numerous mitochondrial proteins, Lon is encoded by the nuclear genome [36, 42]. The *lon* gene encodes a 963-amino-acid protein. The ATP-stimulated Lon protease is believed to play a crucial role in the degradation of oxidized proteins within the mitochondria. Age-related declines in the activity and/or expression of this mitochondrial proteolytic system have been previously reported [7, 9, 36, 42, 43]. Age-related impairment of mitochondrial protein maintenance may therefore contribute to the age-associated build up of oxidized proteins and impairment of mitochondrial redox homeostasis. The Lon protease seems to play a critical role in the removal of oxidized protein. Indeed, aconitase, a Krebs cycle enzyme known to be susceptible to oxidative inactivation, has been shown to be a substrate of the Lon protease when the enzyme is inactivated upon treatment with oxygen radicals. The expression of the Lon protease increased with age in the heart of a rat in comparison with the younger ones, suggesting compensation for loss in specific activity. Although the ATP-stimulated protease activity remained unchanged in the heart's mitochondrial matrix during aging, a decrease was observed in the level of aconitase activity. These results indicate that the Lon protease undergoes age-dependent alterations leading to the accumulation of oxidatively modified substrates of Lon such as aconitase, although the effects are tissue-specific [7, 9, 36, 44].

3.3 Extracellular Mechanisms

Extracellular age-accumulated molecular damage by glycation, oxidation, and cross-linking of long-lived extracellular proteins, mainly collagen and elastin, is a major cause of several important human aging pathologies. Cross-linking increases mechanical stiffness of blood vessels and urinary bladder. Cross-linking impairs the functioning of the kidney, heart, retina, and other tissues and organs. Glycation adducts trigger inflammatory signaling, provoking oxidative damage and cancer [45].

Major differences between cellular and extracellular compartments exist both in terms of the concentrations of thiol/disulfide systems and their relative redox states. Many proteins present on cell surfaces and located in extracellular fluids contain cysteine and methionine residues that are subjected to oxidation. These proteins, which include transporters, receptors, and enzymes, respond to oxidative variations in the extracellular thiol/disulfide redox environment [32]. Changes in the activity of these proteins can alter the ability of organs to function normally in aging.

Perhaps the most frequently recognized difference is that the major low-molecular-weight thiol/disulfide system in cells, GSH/GSSG, is principally in the reduced form, whereas the major low-molecular-weight system in the extracellular compartment, Cys/CySS, is principally in the disulfide form, cystine. On the other hand, recent in vivo data have shown that the redox state of the GSH/GSSG couple in plasma of aged rats varies between genders and may in turn affect the redox state of the postmitotic tissue proteins [30].

4 Altered Redox-Regulation Pathways and Age-Related Disorders

The concept of "redox regulation of proteins" is emerging as an understanding of the novel mechanisms in aging [46–48] and of the pathogenesis of a large number of age-related disorders, including Alzheimer's disease, Parkinson's disease [48–51], cataractogenesis [52, 53], and cancer [54], which were chosen here by this author from among such diseases because the others have not been sufficiently clarified to treat in this instance (Table 1). The decreased efficiency of the autophagic system with age has gained renewed attention. The contents of an increasing number of reports stipulate clues for defective autophagy in the pathogenesis of different age-related diseases. The accumulation of autophagic vesicles and inclusion bodies (protein aggregates or aggresomes) is commonly observed in many protein-conformation disorders and consequent age-related pathologies. In all these disorders, insoluble oligomeric complexes of misfolded or unfolded proteins accumulate in the cytosol as aggregates [55].

Table 1 Protein redox regulation mechanisms in age-related disorders

Diseases	Redox regulation mechanisms
Alzheimer's disease	*Thiol-based repair systems:* Msr and Trx *Ubiquitin-dependent degradation pathway and SUMO*
Parkinson's disease	*Thiol-based repair system:* Msr *Ubiquitin-dependent degradation pathway and SUMO*
Cataracts	*Thiol-based repair systems:* Glutathione, NADPH-dependent Trx system, and thioltransferase *Ubiquitin-dependent degradation pathway*
Diabetes	*Thiol-based repair systems:* Glutathione and Trx system *Ubiquitin-dependent degradation pathway and SUMO*

The neurons of the brain cannot be renewed or replaced, and with time irreversible changes occur. These include the accumulation of defective proteins or protein fragments called peptides, and memory is thereby impaired. Alzheimer's disease is characterized by two hallmark pathologic features: neurofibrillary tangles mainly composed of hyperphosphorylated tau protein aggregates and senile plaques containing deposits of amyloid β peptide (Aβ). These proteins are localized within

both the intracellular and extracellular environment [49]. The accumulation of Aβ in senile plaques is one of the main events in the neuropathology of Alzheimer's disease. Aβ is a peptide derived from the β-amyloid precursor protein (βAPP) by proteolytic cleavage [49]. On the other hand, Parkinson's disease involves a selective loss of dopaminergic neurons from the substantia nigra [56]. The postmortem brains of Parkinson's disease patients are characterized by reduced activity of mitochondrial complex I, an enzyme of the mitochondrial electron transport chain. In turn, this defect may cause a "leakage" of electrons from mitochondria, leading to the accumulation of oxidatively damaged proteins [51, 57]. Dopaminergic neurons also show evidence of impaired proteasomal function (increased oxidative stress and decreased elimination of oxidatively damaged polypeptides). Surviving neurons in the brains of Parkinson's disease patients contain Lewy bodies, which are cytosolic inclusions enriched with aggregated forms of the presynaptic protein α-synuclein. Oxidative stress may play a role in α-synuclein neurotoxicity in two ways: First, oxidative modifications promote the formation of α-synuclein oligomers but not mature fibrils. Second, aggregated forms of α-synuclein may cause an accumulation of ROS, thereby triggering a vicious cycle [51, 58].

Lenses are subject to age-related changes simply because there is no mechanism to replace their protein molecules. The lens is mainly made up of specialized proteins known as crystallins and is constantly subjected to oxidative stress due to environmental radiation and other sources. Cataracts are a common pathologic abnormality of the lens characterized by the loss of lens transparency. They are the leading cause of blindness worldwide and of undue financial burden in the developing countries. The lens has several mechanisms to protect its components from ROS and to maintain its redox state, including enzymatic pathways and high concentrations of ascorbate and GSH [53]. With aging, accumulation of oxidized lens components and decreased efficiency of antioxidant and repair mechanisms (GSH peroxidase, GSH reductase, thioltransferase, and Trx) can contribute to lens opacities or cataracts [52].

4.1 Role of Thiol-Based Repair Systems

Trx plays an essential role in cell function by limiting oxidative stress directly with antioxidant effects and indirectly with protein–protein interactions. Increased experimental evidence demonstrates that, in mammals, cellular redox regulation of many processes is provided by an interaction of the Trx and GSH systems. In fact, Trx and GSH systems are involved in a variety of redox-dependent pathways such as supplying reducing equivalents for ribonucleotide reductase, the first step in DNA biosynthesis, and peptide Msr, an enzyme involved in antioxidant defenses and the regulation of cellular redox state. Together, they form a powerful system controlling signal transduction, protection against oxidative protein damage, as well as regulation of the redox state of the extracellular environment [50].

4.1.1 In Alzheimer's Disease

Previous studies have shown that the pathophysiology of Alzheimer's disease is linked to decreased activity in the peptide Msr. A decline in peptide MsrA activity could reduce antioxidant defenses and increase the oxidation rate of critical neuronal proteins [26, 59]. On the other hand, Trx is a crucial protein for antioxidative defense mechanisms, as well as a redox regulator of the intracellular and extracellular signaling pathways and the transcription factors [60]. Both in vivo and in vitro studies demonstrate that Trx and Trx reductase have protective roles against neurotoxicity mediated by the generation of ROS. The decrease in Trx may contribute to the increased oxidative stress and subsequent neurodegeneration observed in the brains of Alzheimer's disease patients. In contrast with low Trx protein levels, thioredoxin reductase activity was significantly elevated in the amygdala and cerebellum of Alzheimer's disease brain. It is likely that the expression of the Trx cycle enzymes must be tightly regulated to maintain optimal neuronal function and to mount appropriate defenses in response to oxidative stress conditions. Based on the evidence of neuroprotective effects of Trx, upregulation of Trx may be an effective strategy for the prevention and treatment of Alzheimer's disease [50, 60, 61].

4.1.2 In Parkinson's Disease

MsrA is present throughout the brain, including the substantia nigra. Cells with increased or decreased levels of MsrA are relatively vulnerable but resistant to oxidative attacks, respectively. MsrA protects cells from oxidative stress not only by repairing proteins damaged by methionine oxidation but also by engaging the cycle of methionine oxidation and reduction that ultimately results in ROS scavenging. The findings of the recent study by Liu et al. indicate that MsrA protects dopaminergic neurons from the toxic effects of complex I inhibition and synuclein expression [51].

Because soluble α-synuclein lacks Trp and Cys residues, mild oxidation of α-synuclein in vitro with hydrogen peroxide selectively converts all four methionine residues to the corresponding sulfoxides. Both oxidized and nonoxidized α-synucleins have similar unfolded conformations; however, the fibrillation of α-synuclein at physiological pH is completely inhibited by methionine oxidation. Furthermore, the Met-oxidized protein also inhibits fibrillation of unmodified α-synuclein. The degree of inhibition of fibrillation by Met-oxidized α-synuclein is proportional to the number of oxidized methionine residues [62].

4.1.3 In Cataracts

Oxidative protein damage has been observed in cataractous lenses. The lens depends on a balanced redox state for maintaining its transparency. A large percentage of lens proteins are the structural proteins called crystallins, which contain a high level of free thiol (–SH) groups that are in a necessarily reduced state to maintain clarity of the lens [52, 53]. The high content of GSH in the lens plays a vital role as

the first line of defense and is believed to protect –SH groups in structural proteins and enzymes for optimum biological function. The second line of defense for the health of the lens is its intrinsic repair enzymes that constantly dethiolate the protein–thiol mixed disulfide (protein thiolation) or protein–protein disulfides induced by oxidative stress so that lens proteins gain their –SH groups again, thus restoring lens protein and enzyme functions and activities. Protein–thiol mixed disulfides exist in various forms such as protein-S-S-glutathione (PSSG), protein-S-S-cysteine (PSSC), and protein-S-S-γ-glutamylcysteine. Repair enzymes – NADPH-dependent Trx system and thioltransferase can dethiolate protein disulfides and thus are extremely important regulators for redox homeostasis in the lens [52].

4.1.4 In Diabetes

Aging is associated with impaired insulin activity, which may lead to alterations in energy homeostasis and increasing blood glucose levels in the elderly.

Signal transduction mechanisms of mammalian insulin and insulin-like growth factor (IGF-1) have a major role in the control of longevity. Studies of physiologic characteristics and polymorphisms of insulin-related genes in exceptionally long-lived people suggest a role of insulin signaling in the control of human aging [63]. Oka et al. recently reported that Trx binding protein-2/Trx interacting-protein couple is a critical regulator of insulin secretion [64].

The higher glycation rate in diabetic individuals is undoubtedly related to the fact that diabetes greatly resembles accelerated aging. Glycation, thiol oxidation, and aggregation of lens crystalline proteins show parallel changes in streptozotocin-diabetic and aging rats [65]. Disturbances of thiol-related homeostatic mechanisms such as GSH/glutathione disulfide have been observed in both diabetes [66] and aging [31, 67, 68]. On the other hand, it is well known that Trx contributes to the regulation of glucose metabolism and glucose transporter-1 (Glut1) expression [69]. A tangible link between Trx and glucose metabolism is the effect of Trx on mitochondrial membrane potential [70]. In diabetes, glucose toxicity affects different organ systems, including pancreatic islets where it leads to deterioration of beta-cell function during the progression of diabetes via oxidative stress, but the exact molecular mechanisms are not fully understood. Recently, it has been reported that Trx is overexpressed in pancreatic beta-cells and can protect beta cells from destruction in diabetes [71].

4.2 The Roles of Proteasome, Ubiquitin, and SUMO

Oxidatively damaged proteins are first recognized by molecular chaperones, which facilitate protein refolding/repairing process. If the oxidative damage is too extensive for repair or cellular metabolism is unfavorable for protein repair, damaged proteins are targeted for degradation pathways. Three major proteolytic systems are responsible for most of the intracellular protein turnover: the lysosomal system, the

ubiquitin-proteasome system, and the SUMO system. A functional ubiquitin proteasome system is essential for all eukaryotic cells, and any alteration in its components has therefore potential pathologic consequences. Thus, age-related proteasomal dysfunction could be regarded as a factor in these disease processes, which involve the formation of plaques, filaments, and aggregates. Once generated, these protein inclusions have been found to further inhibit proteasome activity and thus amplify the formation of inclusion bodies. Although the exact underlying mechanisms are unclear, an age-related decrease in proteasome activity weakens cellular capacity to remove oxidatively modified proteins and favors the development of age-related diseases [7, 55].

4.2.1 In Alzheimer's Disease

The ubiquitin-dependent degradation pathway plays an important role in the modulation of levels of short-lived regulatory proteins and in the removal of abnormal or damaged neuronal proteins [7, 49, 55]. A growing body of evidence suggests that the ubiquitin-dependent degradation pathway may be altered in brains affected by Alzheimer's disease in several ways. βAPP-soluble isoforms have been shown to be degraded after ubiquitin tagging, and Aβ itself seems to bind to the 20S proteasome and inhibit its chymotrypsin-like activity. On the other hand, β-peptides inhibit the proteolytic activities of the 26S proteasome. When the proteolytic activity of the 26S proteasome is inhibited with lactacystin, there is a marked decrease in Aβ degradation, suggesting that the peptide, in both astrocytes and neurons, could be a possible substrate for this enzymatic complex [49]. On the other hand, Cecarini et al. reported that proteasomes from Alzheimer's disease brain exhibited increases in protein carbonyl groups, 4-hydroxynonenal conjugation, and neuroprostane conjugation [72]. Together, these data confirm that impairment in the function of purified proteasomes occurs in the earliest stages of Alzheimer's disease in the brain and directly support a role for oxidative inactivation contributing to declines in proteasome function in Alzheimer's disease.

The importance of sumoylation in this analogous modification is becoming increasingly apparent in age-related neurodegenerative pathologies [40, 73–77]. Sumoylation is gaining growing interest in aging and age-related disorders in the scientific literature. Many substrates for sumoylation have been identified, and the number is still expanding. Among them, βAPP and the microtubule-associated protein tau-protein are particularly interesting. Sumoylation of tau protein is supposed to be implicated in the pathologic process of Alzheimer's disease in the brain and tauopathies from cell-culture experiments [40, 73, 76]. Sumoylation of protein domains that are exposed to the lumen of the endoplasmic reticulum and other compartments of the secretory pathway had been previously reported by Zhang and Sarge [76]. Their results also provide the first demonstration that the SUMO E2 enzyme is present within the endoplasmic reticulum, indicating how βAPP and other proteins enter this compartment. On the other hand, Takahashi et al. have examined the relationship between hyperphosphorylated tau protein and SUMO protein in the brains of transgenic mice [40]. In this study, authors reported that SUMO-1

immunoreactivity is observed in phosphorylated tau aggregates in βAPP transgenic mice, an Alzheimer's disease model.

4.2.2 In Parkinson's Disease

The brains of Parkinson's disease patients show evidence of impaired proteasomal function, a defect resulting in increased oxidative stress and decreased elimination of oxidatively damaged polypeptides. Dopaminergic neurons of the substantia nigra contain relatively high basal levels of ROS, resulting from dopamine metabolism and auto-oxidation. Therefore, these neurons may be selectively vulnerable to assault that increases oxidative stress in Parkinson's disease brain, including complex I inhibition and proteasome impairment [51, 57].

DJ-1 is a multifunctional protein that plays roles in transcriptional regulation and against oxidative stress, and loss of its function is thought to result in the onset of Parkinson's disease. The condition of patients carrying DJ-1 mutations demonstrate reduced dopamine uptake indistinguishable from that of patients with sporadic Parkinson's disease. Consistent with the role of DJ-1 in transcriptional regulation, several protein interaction studies suggest a potential functional link between DJ-1 and SUMO. DJ-1 interacts with SUMO-1, SUMO-2, and SUMO E3 ligases. However, the functional significance of the link between sumoylation and DJ-1 and the potential relevance to the pathogenesis of Parkinson's disease is still unclear as in other contexts related to sumoylation [73–75, 77].

4.2.3 In Cataracts

Age-related decreasing proteasome content and peptidase activities is associated with the formation of cataracts [78, 79]. Murakami et al. [78] were first to indicate that the lens proteasomes can degrade mildly photo-oxidized lens proteins, but proteins that are extensively damaged are not degraded but may accumulate. In particular, increasing levels of carboxymethylation were observed with age in the proteasome. Viteri et al. concluded that the lower levels of soluble active enzymatic complex present in lenses of the elderly and the posttranslational modifications affecting the proteasome may at least partly explain the decrease in proteasome activity. The concomitant accumulation of carboxymethylated and ubiquitinated proteins occurs with the aging process [79].

4.2.4 In Diabetes

Diabetes-induced oxidative stress can lead to protein misfolding and degradation by the ubiquitin-proteasome system [80]. Exposure to chronic high glucose induces oxidative stress and causes an increase in ubiquitin-protein aggregates in β-cells. Autophagy delivers the ubiquitin-protein aggregates to the lysosome for its degradation [80]. Glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone that is a potent stimulator of insulin release under normoglycemic conditions. However, its insulinotropic effect is reduced or even absent entirely in type

2 diabetic patients. Interference with the binding of GIP to its receptor (GIP-R) results in impairment of insulin secretion and variable degrees of glucose intolerance. GIP-R is continuously degraded when islets are exposed to high glucose for a long period. The results of Zhou et al. suggest that the GIP-R is ubiquitated, resulting in downregulation of the actions of GIP [81].

In contrast with polyubiquitinylation that targets modified proteins for the proteasome degradation pathway, the biological consequences of sumoylation include the increase of protein stability [82]. Aberrant SUMO regulation is a likely cause of a variety of human disease including diabetes [83, 84]. SUMO-4 has recently been cloned in an attempt to identify genes susceptible to human type 1 diabetes mellitus. SUMO-4 expression is primarily restricted in pancreatic islets, immune tissues, and kidneys. Further extensive investigation into these SUMO-4 target proteins is expected to lead to better understanding of the mechanisms underlying the role of SUMO-4 in the pathogenesis of diabetes. Furthermore, proteins that regulate glucose levels in the blood are also regulated by the sumoylation process. Sumoylation promotes the membrane accumulation of GLUT-4, presumably by enhancing the protein stability and facilitating its trafficking [84].

How is the SUMO signaling deregulated in age-related disorders and diabetes? Studies in the years to come will certainly generate exciting answers to many of these questions.

5 Concluding Remarks

It seems clear that most of the effects of ROS in aging cells are related to signaling pathways and redox regulation mechanisms rather than to nonspecific damage of proteins. In fact, the relation of molecular events, such as the control of protein-redox regulation mechanisms, with intracellular signaling pathways are still under extensive investigation. Molecular mechanisms controlling the redox-regulation systems related to proteins and variation in the regulation of these controlling systems can be expected to contribute to the susceptibility of postmitotic tissues to oxidative stress during aging and disease. As the redox regulation mechanisms in aging and age-related disorders become clearer, new therapeutic approaches and prospective solutions are coming into view. A major research and development effort is required to bring forth novel therapies as related to redox regulation of proteins and make these available to the aging population.

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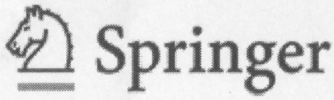

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