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Protein Carbonyl and the Methionine Sulfoxide Reductase System

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Abstract

The formation and accumulation of protein-carbonyl by reactive oxygen species may serve as a marker of oxidative stress, aging, and age-related diseases. Enzymatic reversal of the protein-carbonyl modification has not yet been detected. However, an enzymatic reversal of protein-methionine sulfoxide modification exists and is mediated by the methionine sulfoxide reductase (Msr) system. Methionine sulfoxide modifications to proteins may precede the formation of protein-carbonyl adducts because of consequent structural changes that increase the vulnerability of amino acid residues to carbonylation. Supportive evidence for this possibility arises from the elevated protein-carbonyl accumulations observed in organisms, such as yeast and mice, lacking the methionine sulfoxide reductase A (MsrA) enzyme. In addition, advanced age or enhanced oxidative-stress conditions foster the accumulations of protein-carbonyls. This review discusses the possible involvement of methionine sulfoxide formation in the occurrence of protein-carbonyl adducts and their relevance to the aging process and neurodegenerative diseases. *Antioxid. Redox Signal.* 12, 405–415.

Introduction

Reactive oxygen species (ROS), including $O_2^{\bullet -}$, H_2O_2, •OH, and NO•, play an important role in various biologic processes such as signal transduction, aging, immune response, and age-associated diseases (9, 64, 72, 80). Among several biochemical interactions, ROS can react with side chains of the following protein residues: lysine, arginine, proline, threonine, and glutamic acid, which in turn can cause the formation of carbonyl adducts (3, 72, 77). Additionally, aldehydes, such as 4-hydroxy-2-nonenal or malondialdehyde (produced during lipid peroxidation), can be incorporated into proteins by reacting with lysine or cysteine (Cys) residues to form carbonyl derivatives (21, 81). Carbonyl groups also can be formed in proteins by glycation and glycoxidation reactions (7, 33). Consequently, protein-carbonyl groups can be used as markers for ROS-induced protein oxidation.

Aging and Protein-Carbonyl Content

An accumulation of oxidized proteins with age is considered to be one of the characteristics of the aging process. For example, the protein-carbonyl content of human cultured dermal fibroblasts from normal individuals (55), human lens (24), and human brain tissue (68) has been shown to increase as a function of age (Fig. 1) (50). In addition, high levels of carbonylated proteins have been shown to inactivate the proteasome, thereby inhibiting protein degradation, which in turn increases their accumulation (76). The levels of cellular ROS are reflected by the protein-carbonyl content, which has been reported for various human tissues (Fig. 1). The protein-carbonyl content appears to follow an autocatalytic function with an initial lag phase (Fig. 1). This kinetic behavior could be derived from the fact that the antioxidant system was fully functional at a young age and the generated ROS-modified proteins were efficiently degraded. Thus, little or no change is found in the level of protein-carbonyls during the first 45 years, as indicated by the lag phase shown in Fig. 1. However, at age of 45 years or older, the antioxidant and degradation systems become progressively inactivated because of the failure to overcome the constant influx of ROS. As a result, the accumulation of carbonylated proteins accelerates, indicating the age when cells of the individual become increasingly more susceptible to ROS-mediated damage. Moreover, the fact that generation of ROS is likely to increase as a function of age (9, 71) provides further support for the observed phenomenon (Fig. 1) (50). An increase of carbonyl adducts to specific proteins with age was shown in various animal models for aging and age-associated diseases. Accordingly, protein carbonylation was observed in rat bronchoalveolar lavage (82), physically exercised rats (6), aging yeast (31), and aging nematodes (30).

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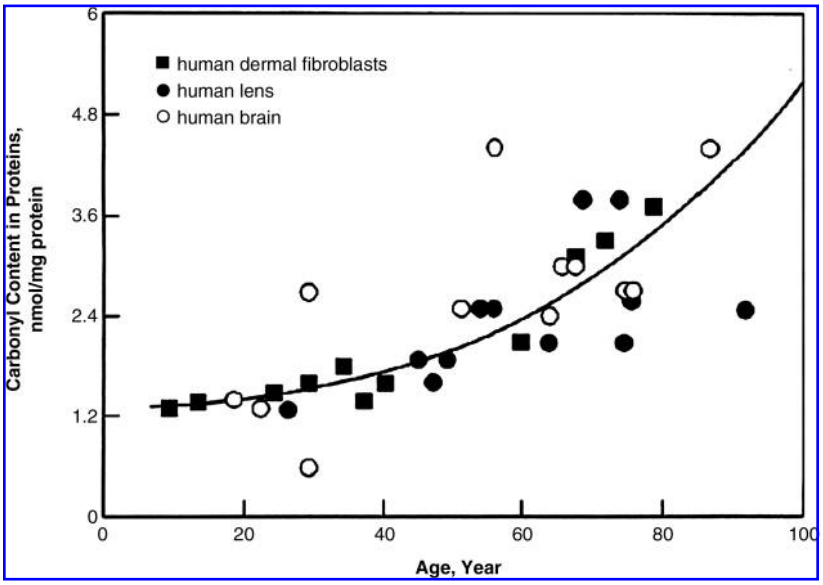

FIG. 1. Age-dependent accumulation of protein carbonyl content. The carbonyl content determined in human cultured dermal fibroblasts from normal individuals (55), in human eye lens cortex (24), and in the occipital lobe of human brain tissue (68) are plotted as a function of age, as previously described [the figure is reproduced from Moskovitz *et al*. (50)].

Protein-Carbonyl and the Methionine Sulfoxide Reductase System

Enzymatic reduction of methionine sulfoxide (MetO) is mediated by the methionine sulfoxide reductase (Msr) system, comprising MsrA and MsrB, which reduce *S*-MetO and *R*-MetO, respectively (42). A compromised Msr system can cause an increase in MetO cellular content in proteins, especially when the cells are exposed to conditions of oxidative stress. Consequently, the structural conformation of MetO-proteins may change (54), while promoting carbonylation in otherwise hidden amino acid residues. For example, a mouse lacking the *MsrA* gene ($MsrA^{-}/^{-}$) was more vulnerable to hyperoxia (100% oxygen), exhibiting enhanced accumulations of protein-carbonyl in kidneys, liver, lungs, and brain (Fig. 2) (44). The MsrB1 enzyme is a selenoprotein that parallels the MsrA distribution in tissues and patterns of expression (5, 48). Moreover, in the absence of MsrA, MsrB1 transcription and expression are downregulated (49).

Selenium is required for the synthesis of selenocysteines that in turn are incorporated into selenoproteins for their activities. Several antioxidant enzymes are also selenoproteins (like thioredoxine reductase, gluthathione peroxidase, and MsrB1), and therefore, their function and expression are compromised when selenium is restricted during synthesis. Accordingly, it was predicted that reducing selenium intake through diet will cause elevation of oxidized proteins, especially in $MsrA^{-}/^{-}$ mice compared with control mice. An increase in Met oxidation was most apparent after the administration of a selenium-deficient diet (SD) to mice through the F_2 generation (43). The

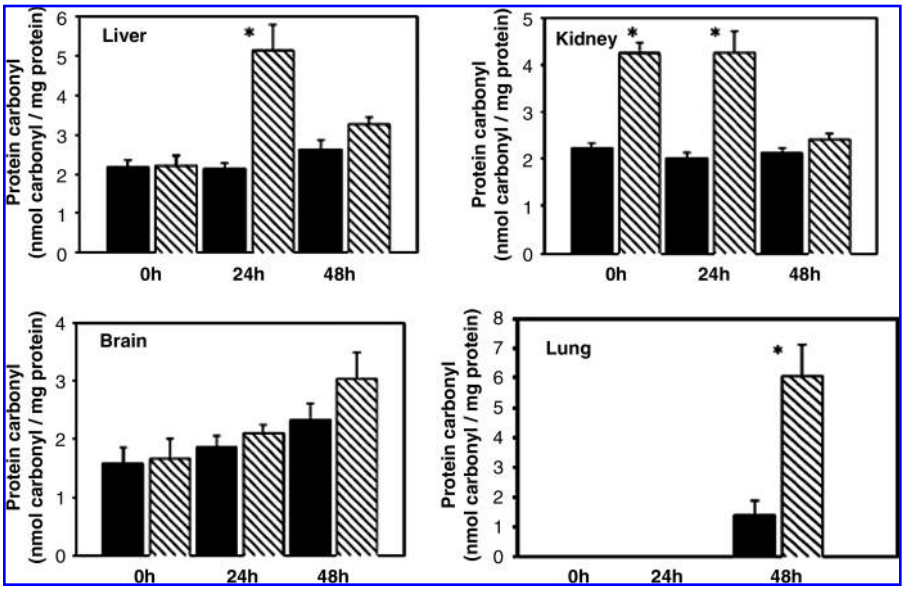

FIG. 2. Protein-carbonyl levels in various tissues from WT and $MsrA^{-}/^{-}$ mice. Protein-carbonyl levels were determined in various tissues from WT and $MsrA^{-}/^{-}$ mice as function of time under hyperoxia (100% oxygen). Time points (h): 0, 24, and 48 h. Each time point represents five animals. *Black bars*, WT; *hatched bars*, $MsrA^{-}/^{-}$. The figure is reproduced from Moskovitz *et al*. (44). *Significant difference between WT and $MsrA^{-}/^{-}$ mice ($p < 0.05$ by *t* test).

biggest effect on protein-MetO accumulation was observed when shifting from a selenium-adequate (SA) diet to an SD diet (43). In the F_2 generation, enhanced protein-MetO accumulations were shown in the *MsrA*$^{-/-}$ mice relative to wild-type (WT) mice in all organs ($\sim\times1.5$) and especially in lungs ($\times2.0$) (Fig. 3). In contrast, no significant differences in the levels of protein-MetO between the two mouse strains were detected for the SD diet through the F_1 generation (except in cerebellum), whereas the SA diet through F_1 and F_2 generations showed no detectable protein-MetO accumulations in both strains (43). Similarly, elevated levels of protein-carbonyl were detected in all *MsrA*$^{-/-}$ mice relative to the WT mouse tissues at the F_2 generation of the SD diet and were most pronounced in brain cerebellum ($\times2.0$), lungs ($\times2.0$), and brain cerebrum ($\times1.5$) (Fig. 4). No significant differences in the levels of protein-carbonyl between the two mouse strains were detected with the SD diet through the F_1 generation. With the SA diet through the F_1 and F_2 generations, no protein-carbonyl accumulations were detected in both mouse strains (43). The lack of detectable levels of protein-carbonyl after the SA diet is mainly due to the fact that the mice tested were too young to accumulate significant amounts of protein-carbonyl (40 days post-weanling). The *MsrA*-knockout effect on protein-carbonylation was shown mostly in two tissues (brain and lungs) and only through the second generation of the SD diet (Fig. 4). These results clearly demonstrate that enhanced posttranslational modification to proteins occurring after the prolonged SD diet is predominantly expressed in brain and lungs of the *MsrA*$^{-/-}$ mouse. Correlative analysis between the MsrB activities and the organ-specific protein MetO and carbonyl accumulations in the *MsrA*$^{-/-}$ and WT mice imply a differential protection by MsrB and MsrA

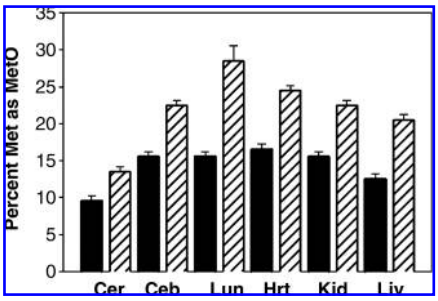

FIG. 3. Methionine sulfoxide accumulations in various tissues from WT and *MsrA*$^{-/-}$ mice after an SD diet. After 40 days of SD at the F_2 generation, protein-MetO content was measured in various tissues of the mice. Cellular protein extracts were treated with and without CNBr, and their relative MetO levels were assayed by using amino acid analysis techniques, as previously described (43). Each value represents an averaged percentage of MetO accumulation ($n=5$). The differences between the protein-MetO levels in the *MsrA*$^{-/-}$ mice and the WT values were all significant ($p<0.05$). Additional data for the appropriate controls are described in Moskovitz (43) (F_1-generation data concerning the SA and SD diets; and F_2-generation data concerning data of SA diet in both mouse strains). The figure is a new presentation of previously published results (43). *Black bars*, WT mice; *hatched bars*, *MsrA*$^{-/-}$ mice; Cer, brain cerebrum; Ceb, brain cerebellum; Lun, lungs; Hrt, heart; Kid, kidneys; Liv, liver.

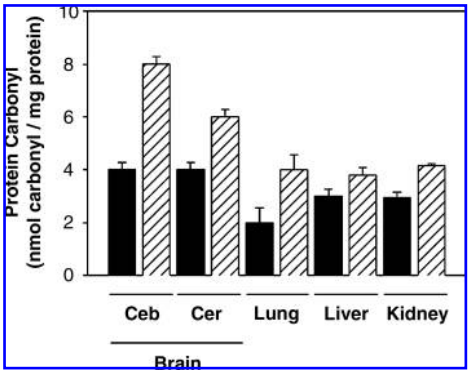

FIG. 4. Protein-carbonyl accumulations in various tissues from WT and *MsrA*$^{-/-}$ mice after the SD diet. After 40 days of SD at the F_2 generation, protein-carbonyl content was measured in various tissues of the mice. Cellular protein levels were determined as previously described (43). Each value represents an averaged percentage protein-carbonyl accumulation ($n=5$). The differences between the protein-carbonyl levels in the *MsrA*$^{-/-}$ mice and the respective WT values were all significant ($p<0.05$, by t test). Additional data for the appropriate controls are described in Moskovitz (43) [F_1-generation data concerning selenium-adequate diet (SA) and SD diet; and F_2-generation data concerning the SA diet in both mouse strains]. The figure is a new presentation of previously published results (43). *Black bars*, WT mice; *hatched bars*, *MsrA*$^{-/-}$ mice; Cer, brain cerebrum; Ceb, brain cerebellum.

from oxidative damage. MsrB1 had a higher contribution in protecting the brain cerebellum from protein-carbonyl accumulation through the F_2 generation of the SD diet (a 50% decrease in MsrB activity compared with $\times2.0$ increase of protein-carbonyl level in *MsrA*$^{-/-}$ relative to WT mice (43). In addition, MsrA has a special protective role in preventing protein-MetO and carbonyl accumulations in lungs and brain of *MsrA*$^{-/-}$ mice through the F_2 generation of the SD diet [as *MsrA*$^{-/-}$ mice exhibited only a 10% decrease in MsrB activity and no MsrA activity compared with elevation of $\times1.5$ to 2.0 of protein-MetO and carbonyl levels (Figs. 3 and 4) (43)].

The effect of MsrA ablation on the protection of cells from oxidative stress–mediated accumulation of protein-carbonyl adducts is demonstrated in the nonreplicative senescence of yeast cells (52). Nonreplicative senescence in yeast is evaluated by isolating old mother cells through the yeast cell generations. The WT yeast cell does not show many signs of aging up to eight cell divisions/generations (41). Accordingly, five- and 21-generation-old cells were isolated, representing young and old mother cells, respectively. Previously, it was shown that *msrA*-null mutant yeast cells (MT) had a shorter life span because of oxidative stress–mediated damage (45, 46). Moreover, an MsrA-overexpressed strain was more resistant to oxidative stress, with a slightly better survival rate than in its respective WT strain (46). These observations suggest that the MT strain is more prone to oxidative damage to proteins, resulting in a shorter life span.

To investigate the possibility that this mutant strain also accumulates nonreversible posttranslational modifications with age, its protein-carbonyl levels were monitored in five- and 21-generation-old cells relative to its control WT strain.

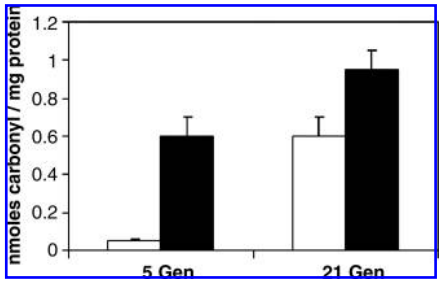

FIG. 5. Age-dependent accumulations of protein-carbonyls in MT and WT control yeast strains. Young yeast cells (five-generation-old cells) and old yeast cells (21-generation-old cells) were isolated by elutriation, as previously described (52). 5 Gen, 5-generation-old cells; 21 Gen, 21-generation-old cells. *White bars*, WT yeast cells; *black bars*, MT yeast cells. Each bar represents an average value ($n = 3$). The differences between WT and MT values were significant ($p < 0.001$, by t test). This figure was reproduced from a previous publication by Oien and Moskovitz (52).

As shown in Fig. 5, the levels of protein-carbonyls in the MT strain were dramatically higher than those in its WT control strain at the age of five generations (a representative generation for young cells). The 21-generation-old cells (a representative age for old cells) of the WT strain accumulated significant amounts of protein-carbonyl, relative to the five-generation-old cells (Fig. 5). However, the protein-carbonyl levels were still significantly lower than those observed with the MT strain, respectively (Fig. 5).

To support the notion that the observed protein-carbonyl accumulations may be a consequence of enhanced oxidative stress, the protein-carbonyl accumulations in both yeast strains were monitored after exposure of the cells to H_2O_2. As expected, the MT strain accumulated 2.6-fold more protein-carbonyls relative to the WT strain under these conditions (Fig. 6).

Accumulations of protein-carbonyl in human tissues (50) and in yeast mother cells (1) were shown to be among the characteristics of aging (50). Thus, it is suggested that the MT strain is aging at a faster rate compared with the WT strain and accumulates higher protein-carbonyl levels as a consequence of oxidative-stress conditions (Figs. 5 and 6). Additionally, lower antioxidant defense is associated with the progression of the aging process.

Among the functions of the Msr system is to serve as an antioxidant *via* its MetO-dependent reducing activities (38). Moreover, the MsrA activity was shown to decline in various mammalian tissues with age or oxidative stress (44, 73). Therefore, it was important to monitor the Msr activity as a function of age in both the MT and control WT yeast strains.

As shown in Fig. 7, both the WT and the MT strains demonstrated a significant loss of total Msr activity at the 21- *versus* five-generation-old cells. The observed decline in Msr activity with age in both examined yeast strains is similar to the activity reduction shown in most aged mammalian tissues (73). The level of the total Msr activity in the MT strain represents MsrB activity only, and its further decline in 21-generation-old cells may contribute to the additional protein-carbonyl accumulation at that age (Fig. 5) (52). However, lack of MsrA activity in the MT strain seems to make a major contribution to its protein-carbonyl accumulation in the five-generation-old cells (Fig. 5).

In summary, these data provide supportive evidence for the importance of the Msr activities in preventing age-associated damage to proteins. Irreversible posttranslation modification to proteins may result in their malfunction, aggregation, and thereby cause cellular damage leading to senescence and apoptosis. Therefore, the removal of faulty proteins that accumulate as a function of age or oxidative-stress conditions is essential for cell survival. When the reversal of the damaging posttranslational modifications to a protein structure fails, we

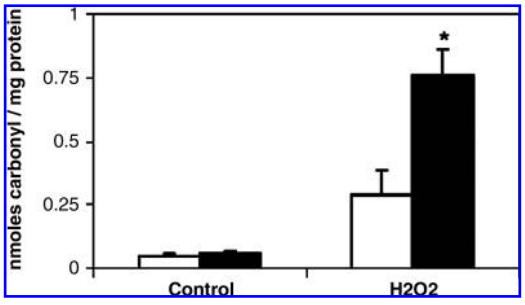

FIG. 6. Oxidative stress–dependent accumulations of protein-carbonyls in MT and WT control yeast strains. Late logarithmic yeast cells were inoculated into liquid YPD media in the presence or absence of 2 mM H_2O_2 at 30°C. When cell density reached the early log phase ($A_{600} = 1.0$), the cells were centrifuged, washed 3 times with buffer, and disrupted. After centrifugation, the resulting supernatants of the cell extracts were used for protein-carbonyl determination, as previously described (52). Control and H_2O_2, non-treated and H_2O_2-treated cells, respectively. The differences between WT and MT values in the presence of H_2O_2 were significant ($^*p < 0.001$ by t test). This figure was reproduced from a previous publication by Oien and Moskovitz (52).

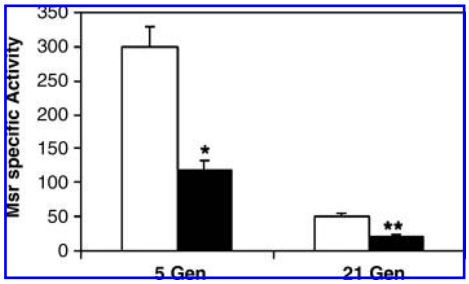

FIG. 7. Total Msr activity in MT and WT control yeast strains. Young cells (five-generation-old cells) and old cells (21-generations-old cells) were analyzed for total Msr activity by using dabsyl-MetO as a substrate and DTT as a reduction power, as previously described (52). Msr specific activity is calculated as pmoles dabsyl-methionine formed per milligram protein per minute. *White bars*, WT yeast cells; *black bars*, MT yeast cells. Each bar represents an average value ($n = 3$). The differences between WT and MT values were significant ($^*p < 0.001$; $^{**}p < 0.005$ by t test). This figure was reproduced from a previous publication by Oien and Moskovitz (52).

must degrade the faulty proteins with the proteasome system. Lower proteasome activity has been implicated as one of the features associated with the aging process in mammals (40) and in yeast (16). Accordingly, it is expected that lack of or compromised Msr activities (mainly of MsrA) may cause faster accumulation of protein-carbonyls in yeast, which in turn may inhibit the 20S proteasome activity. Supportive evidence for the present observations comes from the recent studies revealing marked decreases in the activity of both the proteasomal system and the lysosomal proteases during the senescence of nondividing fibroblasts. Furthermore, this decline in proteolytic capacity was accompanied by an increased accumulation of oxidized proteins (27).

Future research will focus on determining the identity of the oxidized proteins in the MT yeast strain and how their accumulation may affect the 20S proteasome activity.

Protein-Carbonyl and Methionine Sulfoxide in Neurodegenerative Diseases

Methionine oxidation denatures proteins and converts the hydrophobic properties of Met into hydrophilic-causing structural alterations (74). Two major proteins that are involved in the toxicity associated with Alzheimer's disease (AD) and Parkinson's disease (PD) are β-amyloid (Aβ) and α-synuclein, respectively. Both proteins have been shown to lose their fibrillation rate because of their Met oxidation (59, 83), and in the case with MetO-α-synuclein, to refibrillate in the presence of metal ions and ROS, *in vitro* (87). Moreover, it seems that oxidation of Met35 in Aβ contributes significantly to its toxicity *in vitro* (13, 66) and *in vivo*, as is demonstrated by the fact that MetO-Aβ adducts were found in postmortem senile plaques (20). The current hypothesis is that the occurrence of neurodegenerative diseases is linked to the cumulative damage to proteins (including protein-carbonyls) that causes them to unfold and aggregate; thereby interfering with normal neuronal functions and survival under oxidative-stress conditions. The latter effect could be mediated by MetO-proteins that their accumulation may provide signals to either apoptotic events or survival salvation pathways, depending on the function and the level of the targeted proteins.

Alzheimer's disease

Loss of MsrA activity in brains of AD patients correlated with elevated levels of protein-carbonyl, in comparison with normal brains (23). These decreases of Msr activities reached statistical significance in the superior and middle temporal gyri (SMT), inferior parietal lobule (IPL), and hippocampus, regions that are associated with histopathologic changes in AD (23). In parallel to this observation, an increase in brain protein-carbonyl levels was observed and reached statistical significance in the SMT and hippocampus regions of the AD brain (23). Transcription analysis of *MsrA* mRNA levels in the AD brain demonstrated that the decrease of the enzyme activity may be a reflection of a general decline of transcription in AD (23). In addition, a greater loss in total Msr activity was monitored in SMT as compared with the corresponding MsrA mRNA level; suggesting that the enzyme may undergo an oxidative posttranslational modification that affects its activity (23). Alternatively, it is possible that if a decrease in MsrB transcription has occurred as well; that would account for the further observed loss in the total Msr activities. Because the presence of MsrB genes was not known at the time of this study, it is most likely that a decrease in MsrB transcription has contributed to the greater decline in Msr activities as compared with the relative decline in *MsrA* mRNA levels.

Moskovitz *et al*. (47) demonstrated that MsrA immunoreactivity is more pronounced in neurons than in glia, and that some populations of neurons express higher levels than others. They also suggested that MsrA may contribute to the resistance of neurons to oxidative damage. Severe neuron degeneration was found in the hippocampus, SMT, and IPL, regions where MsrA was significantly diminished (23). Moreover, the $MsrA^{-}/^{-}$ mouse exhibited enhanced neurodegeneration, aggregated Aβ accumulation, and elevated tau phosphorylation in the hippocampus region as compared with control mice (58). Thus, it is possible that a decrease in MsrA activity in AD is related to neuron degeneration in regions that are sensitive to an increase of ROS damage to proteins, lipids, and nucleic acids in the brain (8, 39, 69). It is important to note that several other studies have shown elevated protein-carbonyls in brains with AD (28, 68, 69). Oxidative damage to glutamine synthetase (GS) is associated with an increase in carbonylation of protein residues and a loss of enzymatic activity (56, 75). The GS activity is markedly diminished in the hippocampus and neocortex in AD (28, 68). Levine *et al*. (35) demonstrated that oxidation of at least six methionine residues in GS was required before susceptibility to proteolysis occurred *in vitro*. Accordingly, it is possible that under the presence of oxidative-stress conditions in AD, a compromised MsrA function could lead to GS oxidation and consequently to loss of its activity.

Another enzyme that is sensitive to oxidation is creatine kinase. Studies have demonstrated a decline of creatine kinase in the frontal and temporal lobes in AD (2, 12).

Elevated levels of carbonylation to specific proteins in AD brain were identified by using 2D-gel electrophoresis coupled with Western blot analyses for protein-carbonyl and mass spectrometry (14). From theses experiments, it was determined that the following proteins (creatine kinase, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1) were significantly more oxidized in AD than in control brain. In tissues that consume ATP rapidly, like the brain, phosphocreatine (the product of creatine kinase action) serves as an energy reservoir for the rapid regeneration of ATP. For efficient degradation of faulty proteins, both ATP and recycling of ubiquitin by the ubiquitin carboxyl-terminal L-1 (UCH-L1) are needed. Thus, carbonylation of proteins that are involved in degradation may interfere with normal neuronal cellular functions.

A follow-up of this study reported three additional targets of protein oxidation in AD brain: dihydropyrimidinase-related protein-2 (DRP-2), α-enolase, and the heat-shock cognate 71 (HSC-71) (15). The DRP-2 protein is involved in axonal growth and guidance, suggesting impairment in neural network formation in AD when the protein is oxidized. The cytosolic enzyme α-enolase is involved in the glycolytic pathway and may be a consequence of pathologic events of AD. Although oxidation of HSC-71 was increased in AD brain, it was not significant. Nevertheless, oxidative damage leading to even lower levels of dysfunctional heat-shock proteins

may prompt cellular events causing neurodegeneration. These results demonstrate that the process of ROS-mediated protein modification is an important step in fostering AD and suggest that protein oxidation plays a significant role in processes contributing to AD-associated neurodegeneration (15).

As mentioned earlier, one of the characteristic features of the *MsrA*$^{-/-}$ mouse is its hypersensitivity to oxidative stress. This hypersensitivity causes accumulation of oxidized proteins (44) and the presence of markers associated with neurodegenerative diseases, including AD (58). Support for the compromised *MsrA*$^{-/-}$ brain antioxidant capability comes from the recent proteomics and genomics analyses of the *MsrA*$^{-/-}$ brain (53). The acquired data suggest that the genes participating in cellular redox regulation [dehydrogenase/reductase (SDR family) member 8, carbonic anhydrase 8, peroxiredoxin, superoxide dismutase 3, and succinate dehydrogenase complex, subunit A] are upregulated in the *MsrA*$^{-/-}$ mouse as a response to enhanced oxidative stress (53). It is hypothesized that this response is an attempt of the *MsrA*$^{-/-}$ brain cells to reduce the level of the oxidative modification to proteins, which may be increased in the absence of MsrA. In contrast, the levels of the heat-shock protein 1A and ataxia/telangiectasia mutated homologue (ATM) are reduced in the *MsrA*$^{-/-}$ brain, thus reducing the cellular protection against ROS-mediated damage (53). The altered gene expression in the *MsrA*$^{-/-}$ brain may be mediated by oxidation of specific proteins that play an important role in signaling processes and synthesis of key proteins in various pathways. It is still not yet known how multiple processes are simultaneously affected by Met oxidation or the mechanisms controlling the net expressed cellular effect in a specific pathway. Obviously, more-detailed research is required the better to elucidate the role of Met oxidation of specific proteins *in vivo*. Additionally, a clearer understanding of the regulating function of the Msr system may allow future intervention in controlling its expression and function under stages of neurodegenerative diseases, including AD.

Parkinson's disease

As stated earlier, oxidative stress seems to play a role in the development and progression of neurodegenerative diseases, including PD. In a comparison with AD, only limited literature is available for the description and identification PD-associated carbonylated brain proteins. For example, mutations in two enzymes of the ubiquitin system, parkin and UCH-L1, have been identified as causative genetic defects for certain familial forms of PD (11). Although UCH-L1 has been implicated in a rare familial form of PD (26), little is currently known about the role of UCH-L1 in the sporadic forms of PD. With a proteomics approach, UCH-L1 was shown to be significantly oxidized in both PD and AD brains by the addition of carbonyls (18). A significant eight- and 10-fold increase in the specific oxidation level of full-length UCH-L1 was observed in idiopathic AD and PD brains, respectively, compared with age-matched controls (18). Thus, this provides the first evidence linking oxidative modification of UCH-L1 to sporadic PD. With a proteomics analyses, five Met residues were found to be oxidized (Met-1, Met-6, Met-12, Met-124, and Met-179) in full-length of UCH-L1 (isoform 1) in AD and PD brains. These Met residues may play a role in altering the structure and function of UCH-L1 once they are oxidized. It is speculated that if the oxidized Met residues are not adequately reduced by the Msr system, it may cause further oxidation, manifested by the observed carbonyl adducts.

Recently it was shown that familial PD-associated mutation of I93M in UCH-L1 and the carbonylated UCH-L1 shared aberrant structural properties. Compared with wild-type UCH-L1, they exhibit increased insolubility and elevated interactions with multiple proteins (32). Also, interacting of the mutated or carbonylated UCH-L1 with tubulin modulates the polymerization of tubulin (32). These observations may explain the toxicity associated with the mutant form of UCH-L1, found mostly in familial PD. Perhaps the carbonylated form of the enzyme contributes to its toxicity as well and may also constitute one of the causes of sporadic PD (32).

One of the hallmarks of PD is the formation of protein aggregates that develop inside nerve cells, denoted Lewy bodies (19, 37). These inclusion bodies are composed of aggregated proteins and consist mainly of α-synuclein fibrils (4). The α-synuclein protein is a presynaptic protein of unknown function, and the involvement of this protein in PD is still not clear. However, point mutations in α-synuclein (Ala30Pro or Ala53Thr) cause rare, autosomal dominant forms of PD (34, 62). Overexpression of α-synuclein is associated with the risk of developing PD, whereas the cause of PD is yet to be established (78). For example, triplication of a nonmutated form of α-synuclein was found to be associated with the appearance of symptoms of PD (67). Under normal conditions, α-synuclein is hypothesized to be folded in a nontoxic form and to regulate various cellular functions, especially in dopaminergic neurons (86). When α-synuclein is improperly expressed or modified, various studies have shown that it may cause alterations in mitochondrial and proteasomal function, protein aggregation, and accumulation of ROS (36, 65, 70, 84).

Yeast cells have been used to study events related to α-synuclein toxicity. This toxicity was shown to be pronounced mostly in stationary-phase growth, mediated by increased cellular oxidative stress and decreased proteasomal function (17, 60, 61, 85). The monomeric α-synuclein forms fibrils at a relatively slow rate and is not commonly found in healthy cells. Thus, modifications that alter the physical properties of the α-synuclein may be involved in protein oligomerization. Furthermore, PD does not occur in the younger population, suggesting that an age-associated factor contributes to these abnormal modifications. A widely accepted hypothesis is that this factor is an increased level of oxidative stress that is associated with PD, aging, and other neurodegenerative diseases. Indeed, oxidative stress and increased cellular α-synuclein levels promote its aggregation (25, 29). For example, H_2O_2-mediated oxidation can cause posttranslational modifications to proteins, commonly on the sulfur-containing residues Met and Cys. The α-synuclein protein does not have Cys residues but contains four Met residues that have been shown to be readily oxidized *in vitro*. Thus, under conditions of oxidative stress, MetO-α-synuclein can form fibrils when triggered by certain metals (*e.g.*, Zn^{2+}, Al^{3+}) *in vitro* (87). Furthermore, cellular conditions such as higher α-synuclein protein concentrations (22) and a saline environment (51) could increase the rate of α-synuclein fibril formation. One hypothesis to explain the late onset of PD (and other neurodegenerative diseases) is that disease occurs in

aging neurons when the capacity of the quality-control system to cope with accumulating misfolded proteins is exceeded (10, 79). Accordingly, it was shown to be possible to mimic this situation and to exceed the quality-control system of yeast, by increasing α-synuclein expression (57).

To investigate the possible link between protein carbonylation, a compromised Msr system, and α-synuclein in the development of PD, we present here new relevant data. Accordingly, to elucidate the effect of α-synuclein and its mutants on the accumulation of protein-carbonyl under conditions favoring Met oxidation, the proteins were expressed in both a WT stain and in *msrA*-null (MT) yeast strains [yeast strains: the MT (*Mata ΔmsrA::URA3 his6 leu2*) strain and its parent WT strain were obtained from previous studies by Moskovitz *et al*. (45)].

Overexpressing human α-synuclein in yeast

The human α-synuclein protein and its mutants were overexpressed in MT and WT yeast strains. The expression vector pYES6 is designed for inducible expression of recombinant proteins in *Saccharomyces cerevisiae*. This vector harbors the yeast GAL1 promoter for high-level inducible protein expression in yeast by galactose and repression by glucose. In addition, it has a C-terminal peptide encoding the V5 epitope and polyhistidine (6His) tag for detection and purification of the recombinant fusion protein (in this case, α-synuclein). Additionally, the vector contains the ampicillin and blasticidin resistance genes for selection of bacteria and yeast colonies harboring the plasmid, respectively. Three forms of the human α-synuclein cDNA [wild-type (α-synuclein) and naturally occurring mutants: Ala53Thr (SynA53T) and Ala30Pro (SynA30P)] were inserted into the pYES6 vector, keeping the sequence in frame with the 6His peptide. For that purpose, suitable 5′ forward and 3′ reverse complement primers (harboring *Hin*dIII and *Xba*I restriction sites, respectively) were designed and used with each of the three α-synuclein cDNAs (kindly provided by Dr. Mikiei Tanaka, NIH, NHLBI) in a routine PCR procedure. Then the resulting PCR products were subcloned into the suitable complementary restriction sites on the pYES6 and transformed into bacterial cells (DH5α). Each of the desired plasmids was isolated from bacterial cells, selectively grown on media containing ampicillin, and then transformed into WT and MT yeast strains. Vector-transformed cells served as controls for the α-synuclein–expressed yeast cells. After growth selection on media containing blasticidin, all the transformed yeast strains (WT and MT containing either the vector only, vector containing α-synuclein, or vector containing either SynA53T or SynA30P) were grown in the presence of induction media containing 2% galactose and 1% raffinose for up to 24 h. At various times, equal amounts of cells were taken from each culture [as determined by optical density (OD_{600nm})] and extracted with glass beads and a bead-beater homogenizer apparatus (BioSpec Products, Bartlesville, OK). Finally, the α-synuclein proteins were purified from the corresponding extracts by using of ProBond Nickel-Chelating Resin by following the commonly applied purification procedure (Invitrogen). Equal volumes of purified α-synuclein proteins were subjected to SDS–gel electrophoresis and detected with Western blotting by using antibodies specific to the 6His tag.

Protein carbonyl determination

Disrupted yeast cell protein extracts were monitored for their protein-carbonyl content with the use of the Western blot–based carbonyl-assay kit manufactured by Cayman Chemical (Ann Arbor, MI). In brief, both yeast strains with the sham vector (control) and the three types of α-synuclein (α-synuclein, SynA30P, SynA53T) were grown in the protein-expression induction media for 2 h. Next, cells were precipitated and disrupted by glass beads in the presence of phosphate-buffered saline and a protease inhibitors cocktail (Roche). After high-speed centrifugation, equal amounts of the soluble protein extracts were reacted with 2,4-dinitrophenylhydrazine (DNPH), subjected to SDS–gel electrophoresis, and detected with Western blotting by using antibodies specific to DNP. In addition, quantification of the relative amounts of the carbonylated proteins in the corresponding extracts was performed by monitoring the absorbance of the DNPH-protein derivatives at 370 nm.

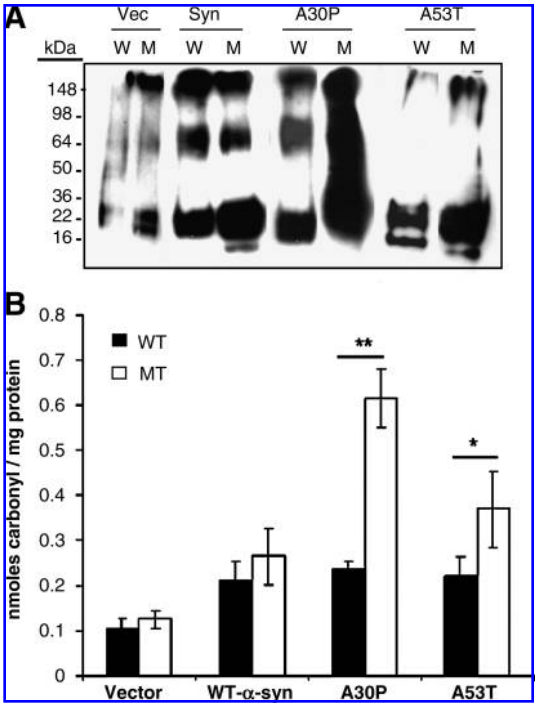

FIG. 8. Accumulation of carbonylated proteins in MT and WT yeast cells expressing α-synuclein and control vector. Both yeast strains with the sham vector control (Vec) and the three types of α-synuclein (Syn, A30P, A53T) were grown in the protein expression–induction medium for 2 h. Then cells were precipitated and disrupted with glass beads in the presence of buffer and a protease-inhibitor cocktail. After high-speed centrifugation, equal amounts of the soluble protein extracts were reacted with DNPH and subjected both to (**A**) SDS-gel electrophoresis and detection by Western blotting by using antibodies specific to DNP (W, wild-type yeast strain; M, *msrA*-null MT yeast strain); and to (**B**) readings by spectrophotometer at $OD_{370\,nm}$. *Black bars,* WT yeast cells; *white bars,* MT yeast cells. Vector, Syn, A30P, and A53T represent sham vector, nonmutated α-synuclein, A30P α-synuclein, and A53T α-synuclein–harboring cells, respectively. Error bars represent standard error ($n = 3$). *$p < 0.05$; **$p < 0.001$ by t test.

Previously we showed that after oxidative stress or nonreplicative senescence, MT cells accumulate increased levels of protein carbonyl (52). Moreover, accumulations of carbonylated proteins have been shown to cause inhibition of degradation with age (76). Together, it suggests that further protein oxidation prompted by Met oxidation may lead to the accumulation of carbonylated proteins, especially in the α-synuclein–expressing MT strain. As shown in Fig. 8, compared with vector-transformed yeast (control), a significant increase in the levels carbonylated proteins was observed when α-synuclein was expressed, especially in the MT strain expressing SynA30P. The latter massive accumulation of carbonylated proteins in the SynA30P expressed in MT correlates with the prominent inhibition of SynA30P degradation into shorter peptides (unpublished data). It is thus suggested that enhanced protein–carbonyl accumulations in the MT strain may be due to an increased oxidation prompted by MetO (that may cause conformational changes to proteins, leading to further irreversible oxidation), along with α-synuclein inhibition of degradation (especially by the SynA30P type).

Supportive evidence on the effect of SynA30P expression on protein carbonylation comes from a proteomics analysis of transgenic mice overexpressing SynA30P (63). In this study, it was shown that more than twofold selective increases in specific carbonyl levels of three proteins occurred in brains of SynA30P mice: carbonic anhydrase 2, α-enolase, and lactate dehydrogenase 2. Furthermore, activity analyses of these oxidized proteins showed decreased functions in these enzymes as well. Accordingly, the authors suggested that proteins associated with impaired energy metabolism and mitochondria are particularly prone to oxidative stress associated with A30P-mutant α-synuclein (63). It is hypothesized that a cross between a transgenic mouse overexpressing SynA30P and the *MsrA*$^{-/-}$ mouse will exacerbate the increase in protein-carbonyl levels, both in these selected proteins and in other proteins yet to be identified.

Conclusions

The consequence of events leading from methionine oxidation to the formation and accumulation of protein-carbonyl is yet to be determined. So far, it is hypothesized that oxidation of specific methionine residues may cause structural changes to a protein, unfold it, and foster carbonylation to otherwise hidden amino acid residues. The resulting carbonylated protein can then be resistant to degradation, accumulate, and interfere with normal cellular processes. Other explanations of the suggested association between methionine oxidation and protein carbonylation may be possible. For example, oxidation of critical methionine residues in antioxidant enzymes or enzymes involved in metabolizing lipid- or sugar-derived carbonylating electrophiles may increase the levels of protein carbonylation. Additionally, inhibition of enzymes involved in degrading carbonylated proteins may enhance the accumulation of cellular protein-carbonyls. The accumulation of protein-carbonyl adducts may be related to the occurrence of aging and age-associated diseases. Among most relevant diseases are neurodegenerative diseases, including AD and PD. Accordingly, it is hoped that adequate reversal of methionine oxidation by the methionine sulfoxide reductase system may prevent further irreversible protein oxidation (including carbonylation) and thus protect against the development of disease.

References

1. Aguilaniu H, Gustafsson L, Rigoulet M, and Nystrom T. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299: 1751–1753, 2003.
2. Aksenov MY, Aksenova MV, Payne RM, Smith CD, Markesbery WR, and Carney JM. The expression of creatine kinase isoenzymes in neocortex of patients with neurodegenerative disorders: Alzheimer's and Pick's disease. *Exp Neurol* 146: 458–465, 1997.
3. Amici A, Levine RL, Tsai L, and Stadtman ER. Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed oxidation reactions. *J Biol Chem* 264: 3341–3346, 1989.
4. Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, and Iwatsubo T. Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am J Pathol* 152: 879–884, 1998.
5. Bar-Noy S and Moskovitz J. Mouse methionine sulfoxide reductase B: effect of selenocysteine incorporation on its activity and expression of the seleno-containing enzyme in bacterial and mammalian cells. *Biochem Biophys Res Commun* 297: 956–961, 2002.
6. Bar-Shai M, Carmeli E, Ljubuncic P, and Reznick AZ. Exercise and immobilization in aging animals: the involvement of oxidative stress and NF-kappaB activation. *Free Radic Biol Med* 44: 202–214, 2008.
7. Baynes JW and Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 48: 1–9, 1999.
8. Beal MF. Energy, oxidative damage, and Alzheimer's disease: clues to the underlying puzzle. *Neurobiol Aging* 15(suppl 2): S171–S174, 1994.
9. Beckman KB and Ames BN. The free radical theory of aging matures. *Physiol Rev* 78: 547–581, 1998.
10. Berke SJ and Paulson HL. Protein aggregation and the ubiquitin proteasome pathway: gaining the upper hand on neurodegeneration. *Curr Opin Genet Dev* 13: 253–261, 2003.
11. Bigelow DJ and Squier TC. Redox modulation of cellular signaling and metabolism through reversible oxidation of methionine sensors in calcium regulatory proteins. *Biochim Biophys Acta* 1703: 121–134, 2005.
12. Burbaeva, G. [Physiologically active brain proteins as possible markers of mental diseases]. *Vestn Ross Akad Med Nauk* 7: 51–54, 1992.
13. Butterfield DA and Boyd-Kimball D. The critical role of methionine 35 in Alzheimer's amyloid beta-peptide (1–42)-induced oxidative stress and neurotoxicity. *Biochim Biophys Acta* 1703: 149–156, 2005.
14. Castegna A, Aksenov M, Aksenova M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery WR, and Butterfield DA. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain, Part I: creatine kinase BB, glutamine synthase, and ubiquitin carboxy-terminal hydrolase L-1. *Free Radic Biol Med* 33: 562–571, 2002.
15. Castegna A, Aksenov M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery WR, and Butterfield DA. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain, Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *J Neurochem* 82: 1524–1532, 2002.

16. Chen Q, Ding Q, and Keller JN. The stationary phase model of aging in yeast for the study of oxidative stress and age-related neurodegeneration. *Biogerontology* 6: 1–13, 2005.
17. Chen Q, Thorpe J, Ding Q, El-Amouri IS, and Keller JN. Proteasome synthesis and assembly are required for survival during stationary phase. *Free Radic Biol Med* 37: 859–868, 2004.
18. Choi J, Levey AI, Weintraub ST, Rees HD, Gearing M, Chin LS, and Li L. Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. *J Biol Chem* 279: 13256–13264, 2004.
19. Dawson TM and Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 302: 819–822, 2003.
20. Dong J, Atwood CS, Anderson VE, Siedlak SL, Smith MA, Perry G, and Carey PR. Metal binding and oxidation of amyloid-beta within isolated senile plaque cores: Raman microscopic evidence. *Biochemistry* 42: 2768–2773, 2003.
21. Esterbauer H, Schaur RJ, and Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 11: 81–128, 1991.
22. Fink AL. The aggregation and fibrillation of alpha-synuclein. *Acc Chem Res* 39: 628–634, 2006.
23. Gabbita SP, Aksenov MY, Lovell MA, and Markesbery WR. Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. *J Neurochem* 73: 1660–1666, 1999.
24. Garland D, Russell P, and Zigler JS Jr. The oxidative modification of lens proteins. *Basic Life Sci* 49: 347–352, 1988.
25. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, and Lee VM. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science* 290: 985–989, 2000.
26. Giorgi FS, Bandettini di Poggio A, Battaglia G, Pellegrini A, Murri L, Ruggieri S, Paparelli A, and Fornai F. A short overview on the role of alpha-synuclein and proteasome in experimental models of Parkinson's disease. *J Neural Transm Suppl* 70: 105–109, 2006.
27. Grune T, Merker K, Jung T, Sitte N, and Davies KJ. Protein oxidation and degradation during postmitotic senescence. *Free Radic Biol Med* 39: 1208–1215, 2005.
28. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM, Lovell M, Markesbery WR, and Butterfield A. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J Neurochem* 65: 2146–2156, 1995.
29. Hsu LJ, Sagara Y, Arroyo A, Rockenstein E, Sisk A, Mallory M, Wong J, Takenouchi T, Hashimoto M, and Masliah E. alpha-synuclein promotes mitochondrial deficit and oxidative stress. *Am J Pathol* 157: 401–410, 2000.
30. Ishii N. Oxidative stress and aging in *Caenorhabditis elegans*. *Free Radic Res* 33: 857–864, 2000.
31. Jakubowski W, Bilinski T, and Bartosz G. Oxidative stress during aging of stationary cultures of the yeast *Saccharomyces cerevisiae*. *Free Radic Biol Med* 28: 659–664, 2000.
32. Kabuta T, Setsuie R, Mitsui T, Kinugawa A, Sakurai M, Aoki S, Uchida K, and Wada K. Aberrant molecular properties shared by familial Parkinson's disease-associated mutant UCH-L1 and carbonyl-modified UCH-L1. *Hum Mol Genet* 17: 1482–1496, 2008.
33. Kristal BS and Yu BP. An emerging hypothesis: synergistic induction of aging by free radicals and Maillard reactions. *J Gerontol* 47: B107–B114, 1992.
34. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, and Riess O. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* 18: 106–108, 1998.
35. Levine RL, Mosoni L, Berlett BS, and Stadtman ER. Methionine residues as endogenous antioxidants in proteins. *Proc Natl Acad Sci U S A* 93: 15036–15040, 1996.
36. Lindersson E, Beedholm R, Hojrup P, Moos T, Gai W, Hendil KB, and Jensen PH. Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J Biol Chem* 279: 12924–12934, 2004.
37. Lucking CB and Brice A. Alpha-synuclein and Parkinson's disease. *Cell Mol Life Sci* 57: 1894–1908, 2000.
38. Luo S and Levine RL. Methionine in proteins defends against oxidative stress. *FASEB J* 23: 464–472, 2009.
39. Markesbery WR and Carney JM. Oxidative alterations in Alzheimer's disease. *Brain Pathol* 9: 133–146, 1999.
40. Martinez-Vicente M, Sovak G, and Cuervo AM. Protein degradation and aging. *Exp Gerontol* 40: 622–633, 2005.
41. McMurray MA and Gottschling DE. An age-induced switch to a hyper-recombinational state. *Science* 301: 1908–1911, 2003.
42. Moskovitz, J. Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. *Biochim Biophys Acta* 1703: 213–219, 2005.
43. Moskovitz J. Prolonged selenium-deficient diet in MsrA knockout mice causes enhanced oxidative modification to proteins and affects the levels of antioxidant enzymes in a tissue-specific manner. *Free Radic Res* 41: 162–171, 2007.
44. Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, and Stadtman ER. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci U S A* 98: 12920–12925, 2001.
45. Moskovitz J, Berlett BS, Poston JM, and Stadtman ER. The yeast peptide-methionine sulfoxide reductase functions as an antioxidant in vivo. *Proc Natl Acad Sci U S A* 94: 9585–9589, 1997.
46. Moskovitz J, Flescher E, Berlett BS, Azare J, Poston JM, and Stadtman ER. Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyces cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proc Natl Acad Sci U S A* 95: 14071–14075, 1998.
47. Moskovitz J, Jenkins NA, Gilbert DJ, Copeland NG, Jursky F, Weissbach H, and Brot N. Chromosomal localization of the mammalian peptide-methionine sulfoxide reductase gene and its differential expression in various tissues. *Proc Natl Acad Sci U S A* 93: 3205–3208, 1996.
48. Moskovitz J, Singh VK, Requena J, Wilkinson BJ, Jayaswal RK, and Stadtman ER. Purification and characterization of methionine sulfoxide reductases from mouse and *Staphylococcus aureus* and their substrate stereospecificity. *Biochem Biophys Res Commun* 290: 62–65, 2002.
49. Moskovitz J and Stadtman ER. Selenium-deficient diet enhances protein oxidation and affects methionine sulfoxide reductase (MsrB) protein level in certain mouse tissues. *Proc Natl Acad Sci U S A* 100: 7486–7490, 2003.
50. Moskovitz J, Yim MB, and Chock PB. Free radicals and disease. *Arch Biochem Biophys* 397: 354–359, 2002.
51. Munishkina LA, Henriques J, Uversky VN, and Fink AL. Role of protein-water interactions and electrostatics in alpha-synuclein fibril formation. *Biochemistry* 43: 3289–3300, 2004.
52. Oien D and Moskovitz J. Protein-carbonyl accumulation in the non-replicative senescence of the methionine sulfoxide

reductase A (msrA) knockout yeast strain. *Amino Acids* 32: 603–606, 2007.
53. Oien D, Wang X, and Moskovitz J. Genomic and sulfoxide reductase a knockout mouse. *Curr Proteomics* 5: 96–103, 2008.
54. Oien DB and Moskovitz J. Substrates of the methionine sulfoxide reductase system and their physiological relevance. *Curr Top Dev Biol* 80: 93–133, 2008.
55. Oliver CN, Ahn BW, Moerman EJ, Goldstein S, and Stadtman ER. Age-related changes in oxidized proteins. *J Biol Chem* 262: 5488–5491, 1987.
56. Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM, and Floyd RA. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. *Proc Natl Acad Sci U S A* 87: 5144–5147, 1990.
57. Outeiro TF and Lindquist S. Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science* 302: 1772–1775, 2003.
58. Pal R, Oien DB, Ersen FY, and Moskovitz J. Elevated levels of brain pathologies associated with neurodegenerative diseases in the methionine sulfoxide reductase A knockout mouse. *Exp Brain Res* 180: 765–774, 2007.
59. Palmblad M, Westlind-Danielsson A, and Bergquist J. Oxidation of methionine 35 attenuates formation of amyloid beta-peptide 1–40 oligomers. *J Biol Chem* 277: 19506–19510, 2002.
60. Perrone GG, Grant CM, and Dawes IW. Genetic and environmental factors influencing glutathione homeostasis in *Saccharomyces cerevisiae*. *Mol Biol Cell* 16: 218–230, 2005.
61. Pocsi I, Prade RA, and Penninckx MJ. Glutathione, altruistic metabolite in fungi. *Adv Microb Physiol* 49: 1–76, 2004.
62. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, and Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276: 2045–2047, 1997.
63. Poon HF, Frasier M, Shreve N, Calabrese V, Wolozin B, and Butterfield DA. Mitochondrial associated metabolic proteins are selectively oxidized in A30P alpha-synuclein transgenic mice: a model of familial Parkinson's disease. *Neurobiol Dis* 18: 492–498, 2005.
64. Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med* 31: 53–59, 1999.
65. Sawada H, Kohno R, Kihara T, Izumi Y, Sakka N, Ibi M, Nakanishi M, Nakamizo T, Yamakawa K, Shibasaki H, Yamamoto N, Akaike A, Inden M, Kitamura Y, Taniguchi T, and Shimohama S. Proteasome mediates dopaminergic neuronal degeneration, and its inhibition causes alpha-synuclein inclusions. *J Biol Chem* 279: 10710–10719, 2004.
66. Schoneich C. Methionine oxidation by reactive oxygen species: reaction mechanisms and relevance to Alzheimer's disease. *Biochim Biophys Acta* 1703: 111–119, 2005.
67. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, and Gwinn-Hardy K. alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302: 841, 2003.
68. Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, and Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc Natl Acad Sci U S A* 88: 10540–10543, 1991.
69. Smith MA, Rudnicka-Nawrot M, Richey PL, Praprotnik D, Mulvihill P, Miller CA, Sayre LM, and Perry G. Carbonyl-related posttranslational modification of neurofilament protein in the neurofibrillary pathology of Alzheimer's disease. *J Neurochem* 64: 2660–2666, 1995.
70. Snyder H, Mensah K, Hsu C, Hashimoto M, Surgucheva IG, Festoff B, Surguchov A, Masliah E, Matouschek A, and Wolozin B. beta-Synuclein reduces proteasomal inhibition by alpha-synuclein but not gamma-synuclein. *J Biol Chem* 280: 7562–7569, 2005.
71. Sohal RS, Agarwal S, and Sohal BH. Oxidative stress and aging in the Mongolian gerbil (*Meriones unguiculatus*). *Mech Ageing Dev* 81: 15–25, 1995.
72. Stadtman ER and Berlett BS. Reactive oxygen-mediated protein oxidation in aging and disease. *Chem Res Toxicol* 10: 485–494, 1997.
73. Stadtman ER, Moskovitz J, Berlett BS, and Levine RL. Cyclic oxidation and reduction of protein methionine residues is an important antioxidant mechanism. *Mol Cell Biochem* 234–235: 3–9, 2002.
74. Stadtman ER, Moskovitz J, and Levine RL. Oxidation of methionine residues of proteins: biological consequences. *Antioxid Redox Signal* 5: 577–582, 2003.
75. Stadtman ER, Starke-Reed PE, Oliver CN, Carney JM, and Floyd RA. Protein modification in aging. *EXS* 62: 64–72, 1992.
76. Starke-Reed PE and Oliver CN. Protein oxidation and proteolysis during aging and oxidative stress. *Arch Biochem Biophys* 275: 559–567, 1989.
77. Taborsky G. Oxidative modification of proteins in the presence of ferrous ion and air: effect of ionic constituents of the reaction medium on the nature of the oxidation products. *Biochemistry* 12: 1341–1348, 1973.
78. Takeda A, Mallory M, Sundsmo M, Honer W, Hansen L, and Masliah E. Abnormal accumulation of NACP/alpha-synuclein in neurodegenerative disorders. *Am J Pathol* 152: 367–372, 1998.
79. Taylor JP, Hardy J, and Fischbeck KH. Toxic proteins in neurodegenerative disease. *Science* 296: 1991–1995, 2002.
80. Thannickal VJ and Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279: L1005–L1028, 2000.
81. Uchida K and Stadtman ER. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase: a possible involvement of intra- and intermolecular cross-linking reaction. *J Biol Chem* 268: 6388–6393, 1993.
82. Umstead TM, Freeman WM, Chinchilli VM, and Phelps DS. Age-related changes in the expression and oxidation of bronchoalveolar lavage proteins in the rat. *Am J Physiol Lung Cell Mol Physiol* 296: L14–L29, 2009.
83. Uversky VN, Yamin G, Souillac PO, Goers J, Glaser CB, and Fink AL. Methionine oxidation inhibits fibrillation of human alpha-synuclein in vitro. *FEBS Lett* 517: 239–244, 2002.
84. Vekrellis K, Rideout HJ, and Stefanis L. Neurobiology of alpha-synuclein. *Mol Neurobiol* 30: 1–21, 2004.
85. Willingham S, Outeiro TF, DeVit MJ, Lindquist SL, and Muchowski PJ. Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. *Science* 302: 1769–1772, 2003.
86. Witt SN and Flower TR. alpha-Synuclein, oxidative stress and apoptosis from the perspective of a yeast model of Parkinson's disease. *FEMS Yeast Res* 6: 1107–1116, 2006.

87. Yamin G, Glaser CB, Uversky VN, and Fink AL. Certain metals trigger fibrillation of methionine-oxidized alpha-synuclein. *J Biol Chem* 278: 27630–27635, 2003.

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Abbreviations Used

Cys = cysteine
Met = methionine
MetO = methionine sulfoxide
Msr = methionine sulfoxide reductase
msrA null = *msrA*-KO
PD = Parkinson's disease
ROS = reactive oxygen species
SynA30P = α-synuclein mutated at position 30
SynA53T = α-synuclein mutated at position 53
WT = wild type

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2. Jos H. M. Schippers, Hung M. Nguyen, Dandan Lu, Romy Schmidt, Bernd Mueller-Roeber. 2012. ROS homeostasis during development: an evolutionary conserved strategy. *Cellular and Molecular Life Sciences* **69**:19, 3245-3257. [CrossRef]
3. Jee In Kim , Seung Hee Choi , Kyong-Jin Jung , Eujin Lee , Hwa-Young Kim , Kwon Moo Park . Protective Role of Methionine Sulfoxide Reductase A Against Ischemia/Reperfusion Injury in Mouse Kidney and Its Involvement in the Regulation of Trans-Sulfuration Pathway. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Jackob Moskovitz, Assaf Malik, Alvaro Hernandez, Mark Band, Aaron Avivi. 2012. Methionine sulfoxide reductases and methionine sulfoxide in the subterranean mole rat (Spalax): Characterization of expression under various oxygen conditions. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **161**:4, 406-414. [CrossRef]
5. Wen Rui Zheng, Zhi Ling Guo, Zhi Chong Chen, Qi Yang, Tao Huang. 2012. S=O homolytic bond dissociation enthalpies in sulfoxides. *Research on Chemical Intermediates* . [CrossRef]
6. Rocio Gomez-Pastor, Roberto Perez-Torrado, Elisa Cabiscol, Joaquim Ros, Emilia Matallana. 2012. Engineered Trx2p industrial yeast strain protects glycolysis and fermentation proteins from oxidative carbonylation during biomass propagation. *Microbial Cell Factories* **11**:1, 4. [CrossRef]
7. Dan Shao, Shin-ichi Oka, Christopher D. Brady, Judith Haendeler, Philip Eaton, Junichi Sadoshima. 2011. Redox modification of cell signaling in the cardiovascular system. *Journal of Molecular and Cellular Cardiology* . [CrossRef]
8. Geun-Hee Kwak, Seung-Hee Choi, Hwa-Young Kim. 2010. Dimethyl sulfoxide elevates hydrogen peroxide-mediated cell death in Saccharomyces cerevisiae by inhibiting the antioxidant function of methionine sulfoxide reductase A. *BMB Reports* **43**:9, 622-628. [CrossRef]
9. Takeshi Ishii, Taiki Mori, Tatsuya Ichikawa, Maiko Kaku, Koji Kusaka, Yoshinori Uekusa, Mitsugu Akagawa, Yoshiyuki Aihara, Takumi Furuta, Toshiyuki Wakimoto. 2010. Structural characteristics of green tea catechins for formation of protein carbonyl in human serum albumin. *Bioorganic & Medicinal Chemistry* **18**:14, 4892-4896. [CrossRef]
10. Jee Eun Oh, Md. Aktar Hossain, Jun_Hyeok Kim, Seung Hee Chu, Eun Hye Lee, Kwang Yeon Hwang, Hana Noh, Suk-Whan Hong, Hojoung Lee. 2010. The ectopic expression of methionine sulfoxide reductase 4 results in enhanced growth performance in arabidopsis. *Plant Science* **178**:3, 265-270. [CrossRef]
11. Yuichiro J. Suzuki , Marina Carini , D. Allan Butterfield . 2010. Protein Carbonylation. *Antioxidants & Redox Signaling* **12**:3, 323-325. [Citation] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Ulrika Törnvall. 2010. Pinpointing oxidative modifications in proteins—recent advances in analytical methods. *Analytical Methods* **2**:11, 1638. [CrossRef]