Oxidative Stress-Mediated Regulation of Proteasome Complexes*

Charity T. Aiken‡, Robyn M. Kaake‡, Xiaorong Wang‡, and Lan Huang‡§

Oxidative stress has been implicated in aging and many human diseases, notably neurodegenerative disorders and various cancers. The reactive oxygen species that are generated by aerobic metabolism and environmental stressors can chemically modify proteins and alter their biological functions. Cells possess protein repair pathways to rescue oxidized proteins and restore their functions. If these repair processes fail, oxidized proteins may become cytotoxic. Cell homeostasis and viability are therefore dependent on the removal of oxidatively damaged proteins. Numerous studies have demonstrated that the proteasome plays a pivotal role in the selective recognition and degradation of oxidized proteins. Despite extensive research, oxidative stress-triggered regulation of proteasome complexes remains poorly defined. Better understanding of molecular mechanisms underlying proteasome function in response to oxidative stress will provide a basis for developing new strategies aimed at improving cell viability and recovery as well as attenuating oxidation-induced cytotoxicity associated with aging and disease. Here we highlight recent advances in the understanding of proteasome structure and function during oxidative stress and describe how cells cope with oxidative stress through proteasome-dependent degradation pathways. ***Molecular & Cellular Proteomics 10: 10.1074/mcp.R110.006924, 1–11, 2011.***

Reactive oxygen species (ROS)[1] are routinely produced as a byproduct of aerobic metabolism and oxidative phosphorylation (1–4). Exposure to various environmental stressors (*e.g.* ionizing and nonionizing radiation, or certain chemical agents) can also result in the production of ROS (5–8). In addition, ROS production and accumulation can be generated during disease pathogenesis (*e.g.* Abeta-mediated production of ROS in Alzheimer's disease (9)), or even the natural aging process (10, 11) (Fig. 1). Unneutralized ROS cause oxidative damage to lipids, proteins, and DNA, thus leading to aberrant molecular activities (12–14). Protein oxidation is particularly detrimental as the resulting conformational changes to protein structures can render damaged proteins inactive or lead to functional abnormalities.

To maintain cell viability and normal homeostasis, aerobic organisms have evolved several defense mechanisms for reducing the deleterious effects of oxidative stress, including the production of antioxidants (*e.g.* glutathione, vitamins A, C, and E, and flavenoids) and enzymatic scavengers of ROS (*e.g.* superoxide dismutases (SOD), catalase, and glutathione peroxide). Cells also possess oxidation-reduction (redox)-dependent protein repair pathways, which are triggered by oxidation of redox proteins (15, 16). Redox signaling pathways activate kinase cascades and gene transcription aimed at rescuing oxidized proteins and restoring their functions (15–18). If cellular defense and repair processes fail, oxidatively damaged proteins can undergo direct chemical fragmentation, or form large aggregates (19, 20). Although the pathogenicity of protein aggregates remains uncertain (21), it is known that unrestricted accumulation of damaged proteins can disrupt important cellular processes, including proteasome-mediated protein degradation (22). Therefore, timely removal of oxidatively damaged proteins is of critical importance to maintain normal cellular homeostasis and viability. Although there is evidence suggesting that chaperone mediated autophagy is activated during oxidative stress response (23), the proteasome represents the major proteolytic machinery for the removal of oxidized and misfolded proteins (19, 24–27). If homeostasis is not restored, cells ultimately undergo apoptotic or necrotic cell death (28, 29).

Oxidative stress has been implicated in aging and many human diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), cataract formation, and human cancers (30–36). In particular, pathological developments in neurodegenerative diseases have been strongly linked to oxidation triggered protein aggregation partly because of elevated ROS levels in the brain (37–39). To prevent cytotoxicity induced by oxidized proteins, normal proteasome-dependent degradation is essential for cells to cope with oxidative stress (25, 40, 41). Proteasomal dysfunction can lead to decreased degradation of misfolded proteins, thus resulting in accumulation of oxidized proteins and subsequent protein aggregation. Protein aggregates can then feedback to further inhibit proteasome activities, generate additional cellular stress, and lead to cytotoxicity and human pathologies. Such phenomena have been implicated in many oxidative stress-associated disorders (42, 43).

From the ‡Departments of Physiology and Biophysics and Developmental and Cell Biology, University of California, Irvine, CA 92697

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[1] The abbreviations used are: ROS, reactive oxygen species; CP, core particle; PIP, proteasome interacting protein; GSH, glutathione.

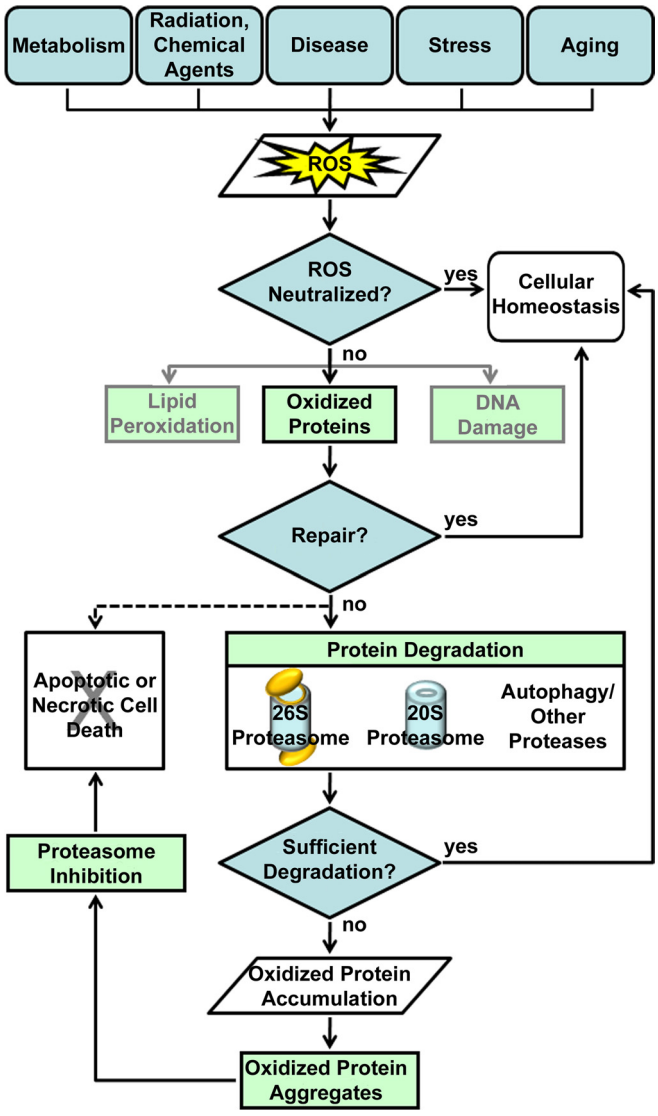

FIG. 1. **Cellular Response to Oxidative Stress.** Shown here is a flow chart detailing the production of reactive oxygen species (ROS) and the subsequent cellular response resulting in either the return to normal cellular homeostasis or apoptotic/necrotic cell death.

Despite the proteasome's critical role in oxidative stress response, our current understanding of how proteolysis of oxidized proteins is regulated and how oxidative stress modulates proteasome structure and function remains limited. Further understanding of how proteasome-dependent degradation pathways are regulated in response to oxidative stress may provide a molecular basis for developing new strategies for curbing oxidative stress and preventing the formation of intracellular protein aggregates during aging and disease. Although other types of cellular stress, such as ubiquitin stress and metal stress, share overlapping components and response pathways as those involved with oxidative stress, the differing overall responses and distinct requirements for signaling and survival indicate these types of stress are not functionally synonymous with oxidative stress (44–47), and are beyond the scope of this review. This review focuses on the recent developments in our understanding of proteasomal regulation during oxidative stress.

Proteasomes and Oxidative Stress

The 26S proteasome is a multicatalytic protease responsible for ubiquitin/ATP dependent protein degradation (48–50). This macromolecular protein complex is composed of the 20S core particle (CP), capped by a 19S regulatory particle (RP, also known as CAP or PA700) on one or both sides (51, 52). The eukaryotic 20S CP is composed of two copies each of 14 subunits, 7α and 7β, which form a conserved barrel-shaped structure with four stacked seven-member rings in the order of $\alpha\beta\beta\alpha$ (48, 53). Three of the β subunits (*i.e.* β1(Y), β2(Z), and β5(X)) are catalytically active and are responsible for the various proteolytic activities of the proteasome (*e.g.* chymotrypsin-like, trypsin-like, and caspase-like activities) (54). Upon Interferon-γ induction, mammalian 20S proteasomes can incorporate three alternative catalytic β subunits, β1i (LMP2), β2i (MECL), and β5i (LMP7), to constitute immunoproteasomes that are best known for generating immunopeptides for MHC class I antigen presentation (55, 56). Although α subunits are catalytically inactive, they are critical for gating the opening of the 20S core particle and for forming associations with regulatory complexes (49, 53).

The 19S regulatory complex is composed of at least 19 different subunits, which are arranged into two subcomplexes, the base and the lid (57, 58). The base complex contains six ATPases (Rpt1–6) plus four non-ATPase subunits (Rpn1, Rpn2, Rpn10, and Rpn13) and directly associates with the 20S core. The lid is found distal to the base and contains nine non-ATPase subunits (Rpn3, Rpn5–9, Rpn11–12, and Rpn15). The 19S particle carries several biochemical functions including recognition of polyubiquitinated substrates, cleavage of polyubiquitin chains to recycle ubiquitin, unfolding of substrates, assisting in opening the 20S core, and subsequent translocation of unfolded substrates into the catalytic chamber (49, 59–61). The activities of the 19S regulatory complex and its assembly with the 20S proteasome have been shown to be strictly ATP-dependent.

In addition to association with 19S regulatory particles, 20S proteasome can bind to alternative activator proteins. Three mammalian 20S activators have been identified to date: PA28$\alpha\beta$, PA28γ, and PA200 (Blm10 in yeast) (58, 62–65). These proteasome activators modulate 20S proteasome structure and generate "active" 20S proteasomes by opening the α ring channel, thereby facilitating the entry of protein substrates for degradation. Because these alternative regulatory proteins lack deubiquitinases and ATPase activity, they promote protein degradation in an ubiquitin/ATP-independent manner.

Although the degradation of oxidatively damaged proteins can occur by both ubiquitin/ATP-dependent (*i.e.* 26S-depen-

dent) and ubiquitin/ATP-independent (*i.e.* 20S-dependent) mechanisms (25, 66), various studies have implied that 20S proteasomes may be more critical for the removal of damaged proteins (19, 24, 26, 67). This may be in part because of the fact that the 20S proteasome is more resistant to oxidative stress than the 26S proteasome as the 20S complex can maintain activity even upon treatment with moderate to high concentrations of H_2O_2, whereas the 26S proteasome is much more vulnerable (68, 69). Recently, it has been shown that 20S proteasomes can degrade oxidized proteins (*e.g.* histones, hemoglobin, superoxide dismutase) *in vitro*, independent of ubiquitin/ATP (19, 24, 26, 70, 71). This phenomenon has been attributed to 20S proteasome recognition of, and interaction with, abnormally exposed hydrophobic patches in oxidatively damaged and unfolded proteins that induce conformational changes in the 20S structure and promote channel opening followed by protein degradation (19, 24, 26). It remains unclear, however, if degradation of oxidatively damaged proteins by the 20S proteasome can occur *in vivo* in a similar manner as was shown *in vitro*.

The combination of associating regulatory complexes, post-translational modifications, proteasome interacting proteins (PIPs), and subunit composition define the structure and activity of a given proteasome entity (50, 58, 72–79). The diverse range of regulatory and activating complexes that modulate 20S core activity supports the idea that the proteasome is a highly dynamic protein complex, capable of adjusting its proteolytic activity depending on the needs of the cell. Accordingly, protein-protein interactions, post-translational modifications, and proteasome gene regulation represent additional levels of regulation for fine tuning the collective proteasome activity upon oxidative stress.

Regulation of the Proteasome by Interacting Proteins

Protein-protein interaction is one of the major mechanisms regulating protein functions. Therefore, characterizing PIPs is important for understanding the regulation of proteasome function. Various approaches have been developed to capture and identify PIPs using genetic and biochemical techniques. Among them, mass spectrometry coupled with affinity purification has evolved as an attractive and powerful tool (80, 81), which has led to the discovery of hundreds of PIPs (46, 75, 76, 82–91). In addition to the proteins that form the proteasome holocomplex, a broad class of PIPs have been identified, including ubiquitin receptors, ubiquitin ligases, deubiquitinases, proteasome activators and inhibitors, chaperones, and other types of modulators (46, 50, 58, 74–76, 82–94). These proteins associate with proteasomes dynamically in response to environmental changes and affect the function and structure of proteasome complexes.

Ecm29-dependent Disassembly of the 26S Proteasome—We recently employed biochemical and quantitative mass spectrometry-based proteomic approaches to monitor the structural dynamics of the 26S proteasome in yeast and mammalian cells in an effort to understand the molecular mechanisms underlying the regulation of 26S proteasomes upon H_2O_2-induced oxidative stress (77). In this study, we determined that acute H_2O_2 stress disrupts the integrity of the 26S proteasome complex and causes the dissociation of the 20S core from the 19S particle in a dose-dependent manner. We also detected H_2O_2-induced loss of 26S proteasome proteolytic activities, likely because of the observed separation of the 19S particle from the 20S core. Additionally, we characterized the dynamic changes of PIPs using stable isotope labeling with amino acid in cell culture (SILAC)-based quantitative mass spectrometry, and identified that one of the yeast PIPs, Ecm29, is substantially recruited to the 19S particle in response to H_2O_2 stress. Biochemical and genetic experiments revealed that the H_2O_2 stress-induced attenuation of yeast 26S proteasome activity is because of Ecm29-dependent disassembly of the 26S proteasome complex, indicating that Ecm29 is a key regulator of 26S proteasome structure in response to H_2O_2 stress. Ecm29-dependent proteasome dissociation has proven important for cell survival, particularly for recovery following oxidative stress. This phenomenon is independent of yeast activator protein 1 (Yap1), a transcription factor critical for oxidative stress response in yeast, and therefore functions as a parallel defense pathway against H_2O_2-induced stress. In addition to the previously established Ecm29 functions (83, 95, 96), our results describe a role for Ecm29 in the response to oxidative stress in yeast, suggesting that Ecm29 may have multiple functionalities in controlling 26S proteasome structure.

H_2O_2 stress-induced disassembly of the 26S proteasome was observed in both yeast and mammalian cells (77), suggesting that this is a conserved mechanism for regulating proteasome activities in an effort to cope with oxidative insults. Several studies have suggested that degradation of oxidized proteins is likely more dependent on 20S than 26S proteasomes (19, 24, 26, 67). Therefore, we suspect that disassembly of 26S proteasomes during oxidative stress serves to increase 20S proteasome abundance, allowing cells to more effectively clear irreparably damaged proteins and mitigate the cytotoxic effects of their accumulation (19, 71, 97). This notion is further supported by studies using mutants defective in 26S proteasome assembly (98), or activities (99), which demonstrated that mutant cells are more resistant to H_2O_2 exposure, and are able to degrade oxidized proteins more effectively than their wild-type controls. Despite its identification as a PIP in mammalian cells, mammalian Ecm29 appears to be functionally distinct from its yeast ortholog (100, 101). Extensive analyses by Gorbea *et al.* revealed that mammalian Ecm29 associates with various molecular motors and endosomal components, and serves as an adaptor protein, recruiting 26S proteasomes to specific cellular compartments such as flotillin-positive endosomes, endoplasmic reticulum (ER), and the centrosome (100, 101). In addition,

studies in HeLa cells demonstrated that human 26S proteasomes remain assembled even following detergent-induced dissociation of Ecm29 (100). Furthermore, the levels and distribution of Ecm29 vary markedly among mouse organs, and can be absent in some tissues (100). These results indicate that Ecm29 is not necessary for the association of the 20S core and the 19S particle. From these studies, it is evident that Ecm29 has some distinct functions in higher eukaryotes that are not present in lower eukaryotic systems. This brings into question whether the reverse is also true. Consequently, the question of whether mammalian Ecm29 is involved in modulating the stability of 26S proteasome assembly in response to oxidative stress, like its yeast ortholog, remains unanswered, and the details regarding the regulator(s) responsible for the observed H_2O_2-triggered dissociation of the 20S core from the 19S particle in mammalian cells (77) are in need of further elucidation.

Usp14-dependent Modulation of Proteasomal Degradation—Human Usp14 is a proteasome-associated deubiquitinating enzyme that disassembles polyubiquitin chains from the end distal to the substrate, thus shortening chains rather than removing them together (84, 102, 103). Usp14 and its yeast ortholog, Ubp6, have been identified as potent inhibitors of proteasomal degradation of selected ubiquitinated substrates *in vitro* and in cells by two different modes of action (47, 84). The decreased degradation of some proteasome substrates is dependent on Usp14 deubiquitinase activity; whereas other substrates are stabilized by a mechanism that is independent of Usp14 deubiquitinase activity (47). Lee *et al.* has recently identified a selective small molecule (IU1) that inhibits the deubiquitinating activity of Usp14 (47). It has been shown that IU1 strongly reduces the accumulation of oxidized proteins by accelerating their degradation in cells exposed to oxidants (*e.g.* menadione, H_2O_2), thus promoting cell survival and enhancing cell resistance to proteotoxic stress. However, the IU1 inhibitor had little to no effect on ubiquitin-independent proteasomal degradation indicating that modulation of proteasomal degradation by Usp14 is mediated by changing the accessibility of ubiquitinated substrates for proteasomal degradation, rather than directly altering proteasome catalytic activity. This represents a very different mechanism from Ecm29-dependent regulation of the 26S proteasome in response to oxidative stress as discussed above (77). Together, these results demonstrate that regulation of proteasomal degradation is a very complex process and multiple mechanisms exist in cells that target various aspects of the degradation process in response to cytotoxic stress. Whether and how these regulatory steps work independently or together require further clarification.

Chaperone-mediated Proteasome Regulation—Given the association of chaperone proteins with unfolded and misfolded proteins, and the contribution of oxidative stress to protein misfolding, it is not surprising that chaperone PIPs contribute to proteasomal regulation in an effort to protect cells from oxidative damage (104–109). For example, it has been shown that neural cells overexpressing the human chaperone protein HDJ-1/Heat shock protein 40 (Hsp40) are more resistant to cytotoxicity associated with both oxidative stressors and general proteasome inhibitors. This suggests that heat shock proteins may confer resistance to oxidative stress by preserving proteasome function and attenuating the toxicity of proteasome inhibition (105). Similarly, Hsp90 and α-crystalline both associate with the proteasome and are important regulators of specific 20S proteasome activities when cells are submitted to oxidative challenge (106–108). Interestingly, under non-stressed conditions Hsp90 and α-crystalline inhibit 20S proteasome activity (108, 110, 111), but upon oxidative stress, these chaperones protect activated 20S proteasomes from oxidative inactivation (106–108). Hsp90 also appears to selectively promote the degradation of oxidized substrates by the 20S proteasome *in vitro* (112). Taken together, these results suggest that molecular chaperones may play a role in regulating proteasome activity in response to oxidative stress by both stabilizing specific proteolytic activities and by aiding the recognition and degradation of oxidized substrates. However, the molecular mechanisms by which chaperone proteins regulate proteasome activity in response to oxidative stress have yet to be determined.

Regulation of the Proteasome by Post-translational Modifications

Protein post-translational modifications can regulate protein functions by changing their structures and physiochemical properties (113, 114), including their biochemical activity, intracellular localization, turnover rate, and protein-protein interactions. Identification and characterization of protein post-translational modifications is therefore important for defining how proteins are regulated in various cellular environments. With the vast and rapid improvements in mass spectrometry-based proteomic approaches (81, 114, 115), various post-translational modifications of proteasome subunits have been reported, including phosphorylation, acetylation, oxidation, and myristoylation (86, 116–123). Most of these modifications were identified from large scale analyses at the proteome level or studies of purified proteasome complexes. Following the identification of proteasomal post-translational modifications, further analyses using genetic and/or biochemical approaches are required to determine the functional and biological significance of each modification. This review will focus on those post-translational modifications that have been linked to proteasome function associated with oxidative stress.

Oxidative Modifications—Oxidative modification refers to a process by which ROS attack proteins, leading to fragmentation of the polypeptide backbone, modification of amino acid side chains, and/or the generation of protein-protein

cross-linkages. Side chain modifications include β-scission of alanine, valine, leucine, and aspartic acid, oxidation of methionine, and carbonylation (124). Intra- and interprotein cross linking can occur through a variety of mechanisms, including the formation of Schiff base cross-linkage (*e.g.* resulting from 4-hydroxy-2-nonenal (HNE) modification), and the formation disulfide bridges between oxidized and reduced thiol groups (124). Recent studies have shown that 19S and 20S proteasome subunits are susceptible to oxidative modifications, including carbonylation, HNE modification, and *S*-glutathionylation (27, 125–128). It has been shown that carbonylation of Rpt3 resulted in impaired Rpt3 ATPase activity and a subsequent decrease in ubiquitin/ATP-dependent proteolysis of the 26S proteasome (126). In addition, carbonylation or HNE modification of the 20S proteasome has been shown to suppress its proteolytic activities (125). These results suggest that oxidative modifications of proteasomes can contribute to the regulation of proteasome functions in response to oxidative stress.

S-glutathiolation is the covalent attachment of glutathione (GSH) to protein thiol groups. There are two mechanisms by which proteins can be *S*-glutathiolated: GSH can react with oxidized thiol groups (*e.g.* Cys-SOH or Cys-S-S-Cys), or oxidized glutathione (GSSG) can react with reduced thiol residues (*e.g.* Cys-SH) (129). GSH is considered to have antioxidant function, by stabilizing oxidized protein thiol groups, preventing further, possibly irreversible thiol oxidation through *S*-glutathiolation, but *S*-glutathiolaton is also known to regulate protein activity (130). Upon H_2O_2-induced oxidative stress in yeast, *S*-glutathiolation of 20S subunits was demonstrated both *in vitro* and *in vivo* (127). Further functional studies determined that treatment of purified 20S proteasomes with GSH lead to the inhibition of chymotrypsin-like and trypsin-like activities (127). In comparison, mammalian proteasomes appear to have a biphasic response to *S*-glutathiolation, as low concentrations of GSH or GSSG increased the chymotrypsin-like activity of purified mammalian proteasomes whereas high levels of GSH or GSSG led to decreased activity (128). Although *S*-glutathiolation of the 20S proteasome generally inhibits proteasome activity, the biphasic response observed for *S*-glutathiolation of mammalian proteasome may be evidence of proteasome *S*-glutathiolation acting as a redox signaling trigger through which proteasome activity is regulated depending on the redox status of mammalian cells.

ADP-Ribosylation—In addition to oxidative modifications, other types of modifications may be involved in altering proteasome activities during oxidative stress. Poly [ADP-ribose] polymerase 1 (PARP1), a nuclear enzyme that transfers ADP-ribose moieties from NAD^+ to glutamic acid, aspartic acid, or lysine residues, is activated in response to oxidative stress (70, 131–133). Interestingly, evidence exists suggesting that nuclear 20S proteasomes can be ADP-ribosylated by PARP1 in human hematopoietic K562 cells, resulting in increased chymotrypsin-like activity of the nuclear 20S proteasome (70). Given the nuclear localization of PARP1 and its role in DNA repair (134), ADP-ribosylation is likely unique to nuclear proteasomes and may function to enhance proteasomal degradation of oxidized nuclear proteins (70, 135).

Phosphorylation—The proteasome is extensively and dynamically phosphorylated, though only a few phosphorylation events have been linked to the regulation of proteasome activity (136–139). One recent study revealed that Rpt5 (19S subunit) can be phosphorylated by human apoptosis signal-regulating kinase 1 (Ask1) (136). Although the specific Rpt5 functional sites have yet to be identified, phosphorylation did result in the inhibition of Rpt5 ATPase activity and in the reduction of 26S proteasome proteolytic activities (136). The impairment of the 26S proteasome activity is not because of changes in the 26S proteasome assembly. It is interesting to note that Ask1 is required for the H_2O_2 stress-induced inhibition of 26S proteasome activity in mouse fibroblasts and that Ask1 is activated by Thioredoxin in response to various stresses including oxidative stress (140–143). Therefore, Ask1-dependent proteasome phosphorylation may act as a regulatory mechanism of proteasome activities during various stress responses.

Apart from Ask1, additional kinases have been found to phosphorylate proteasome subunits including CK2 (formerly casein kinase II), cyclic AMP-dependent kinase (PKA), Ca^{2+}/calmodulin-dependent kinase (CaM-K) II, AMP-activated protein kinase (AMPK), and c-Abl and *abl*-related gene (Arg) tyrosine kinases (137, 139, 144–148). Phosphorylation of proteasome subunits by these kinases appears to be involved in several proteasomal related functions and regulations including proteasome assembly (137, 144–146, 149), and proteolytic activities (139, 147). For example, CK2 phosphorylation of α7 is important for stabilizing the association of the 20S CP to the 19S RP (144, 145, 149). Although α7 phosphorylation, is not required for assembly of the 26S proteasome, it was reported that dephosphorylation of α7 following INFγ treatment correlated with decreased 26S proteasome stability. Several putative PKA target substrates have also been identified from murine cardiac and hepatic tissue (147). In this study it was shown that proteasomal peptidase activities were elevated following *in vitro* phosphorylation of the 20S CP, at multiple sites, by PKA (147). Another recent report demonstrated that CaMKII can directly phosphorylate Rpt6, and that constitutive activation of CaMKII results increased proteasome activity, whereas pharmalogical inhibition of CaMKII decreases the degradation of a GFP reporter protein *in vivo*, suggesting that Rpt6 phosphorylation may regulate proteasome activity (137). Proteasome activity can also be negatively regulated by phosphorylation, as Liu *et al.* conclusively demonstrated that c-Abl and Arg phosphorylation of α4 results in suppressed 20S and 26S proteasome proteolytic activities (139). Although proteasome phosphorylation by these kinases has not been directly linked to oxidative stress, activities of CK2, PKA, CaM-KII, c-Abl, and Arg have been shown to be modulated during oxidative stress (150–157).

Given the biological significance of proteasome phosphorylation by these kinases, we speculate that these phosphorylation events may provide additional means of regulating proteasome activities upon oxidative insult.

Oxidative Stress-Mediated Proteasome Gene Regulation

Oxidative stress-mediated gene regulation is a known component of the defense mechanism for cellular responses to proteotoxic stress (158, 159). In yeast, much of the oxidative stress-driven transcriptional activation is controlled by the redox reactive transcription factor Yap1 (160). Rpn4, the transcriptional activator for proteasome genes, is a Yap1 targeted gene (161–164). Upon oxidative stress, transient Yap1-mediated Rpn4 mRNA up-regulation (163) and Yap1-dependent expression of several yeast proteasome components (165) have been observed, however the biological consequences of these changes were not evaluated. Nevertheless, overexpression of proteasome catalytic subunits β1 or β5 in mammalian cells increased proteasome catalytic activities that correlated with enhanced cell viability and reduced accumulation of oxidized proteins following oxidative stress (166). It has also been shown that overexpression of proteasome assembly protein UMP1 improves cell viability following exposure to various oxidants (167, 168). The increased resistance to oxidative stress by UMP1 overexpression may be because of increased levels of proteasome activity (167, 168) resulting from up-regulation of proteasome β-subunits (168). Together, these studies suggest that increased 20S expression and assembly would enhance a cell's capacity to cope with oxidative stress. Alternatively, disruption of Rpn4-mediated proteasome induction leads to reduced viability in response to oxidative stress (169), demonstrating the critical role of proteasome gene regulation for combating oxidative insult.

In higher eukaryotes, nuclear factor κB (NFκB) and activator protein-1 (AP-1; Yap1 homolog) are the most widely accepted transcriptional regulators of mammalian oxidative stress response, but they are not responsible for activation of proteasome gene transcription (170, 171). Instead, transcription factor 11 (TCF11; long isoform of Nrf1) and NF-E2-related factor 2 (Nrf2) have been shown to promote the expression of several proteasome genes (171, 172), and may act as functional orthologs of yeast Rpn4 (78, 173, 174). Information detailing how these transcription factors are regulated under stress is still unknown and needs to be further investigated. Although the transcriptional control of proteasome expression in the mammalian system appears to be more complex than the yeast system, up-regulation of proteasome expression has also been observed in mammalian cells as an adaptive cellular response to prolonged exposure of oxidative stress (67, 175).

In addition to standard proteasome subunits, mammalian systems, unlike their yeast counterparts, also contain IFN-γ inducible catalytic β subunits that are integral parts of immunoproteasomes. Recently it has been recognized that immunoproteasomes are up-regulated under ROS attack and also contribute to the removal of oxidized proteins in mammalian cells (67, 175–177). Interestingly, it has been suggested that immunoproteasomes are more resistant to oxidative stress than standard proteasomes (67). Cells and mice deficient for immunoproteasome subunits are more susceptible to oxidation-induced cell death because of reduced proteasome activity and accumulation of oxidized proteins (176, 177). It appears that increased immunoproteasome expression not only helps preserve proteasome function, but also makes cells more resistant to oxidative insult (67, 175–177). Whether the same class of transcription factors regulates expression of standard and inducible proteasomal subunits remains to be determined.

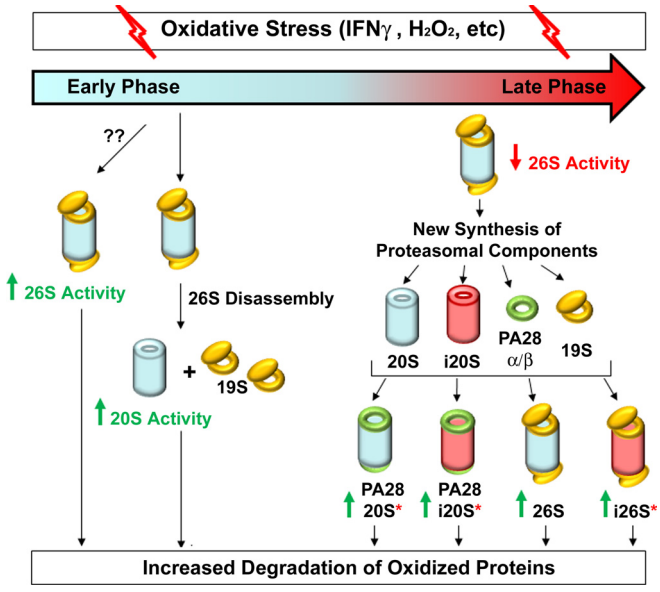

FIG. 2. **Model of oxidative stress-dependent regulation of proteasomes.** In the early phase of cellular response to oxidative insult, various changes occur to modulate 26S and 20S proteasome activity in order to promote the degradation of oxidized proteins, and limit the damage of oxidative stress. Initially, under milder stress conditions, 26S proteasomes are activated by mechanisms still unknown. With persistent oxidative insult, or application of acute oxidative stress, proteasomes disassemble into 20S CPs and 19RPs. In yeast, the PIP Ecm29 is required for this disassembly (77). Following dissociation, free 20S proteasomes are activated and oxidized proteins are degraded independently of ATP and ubiquitin. If cells undergo prolonged exposure to oxidative stress (at least 12 h), cells enter the late phase of cellular response to oxidative stress. Though the exact mechanism is unknown, 26S proteasome inhibition ultimately signals the synthesis of new proteasome components and the formation of functional proteasome degradation units. Of note * 20S, i20S, and i26S proteasomes are more effective than standard 26S proteasomes for degrading oxidized proteins.

Proposed Model of Oxidative Stress-dependent Regulation of the 26S Proteasome

In order to effectively defend the cell against oxidative insults, cells must coordinate repair systems with protea-

some-dependent degradation. Based on recent findings (19, 41, 67, 68, 71, 77, 97, 165, 175, 176, 178, 179), we propose a working model to illustrate how compositional and structural changes of proteasomes modulate their proteolytic activities in response to ROS attack (Fig. 2). In the absence of stress, the 26S proteasome represents the major cellular degradation machinery and carries out ATP-dependent degradation of ubiquitinated substrates. At the onset of oxidative stress, it has been suggested that activities of the 26S proteasome can be initially stimulated by unknown mechanisms for degrading mildly oxidized proteins, thus protecting cells from oxidative damage (175, 178). However, when the oxidative challenge persists, or acute oxidative stress is applied, partial inhibition of 26S activity occurs, leading to an accumulation of ubiquitinated substrates (41, 68, 77). Although inhibition of 26S proteasomes could be caused by oxidation products such as protein aggregates or oxidized lipids (178, 179), it is most likely because of oxidative stress-triggered 26S disassembly as shown recently (41, 77). The dissociation of the 20S core from the 19S particle allows the liberation of 20S complexes and therefore increases cellular capacity for ATP/ubiquitin-independent removal of oxidized proteins. Whether other types of regulatory proteins are required for such 20S-dependent degradation *in vivo* requires further investigation. In yeast cells, 26S proteasome disassembly is regulated by proteasome interacting protein Ecm29, and we hypothesize that a similar type of regulator exists in mammalian cells. Because mammalian cells have more regulatory proteins and proteasomal components, we suspect that the molecular details underlying the regulation of the mammalian 26S proteasome are likely much more complicated than the yeast system. At this stage, 26S proteasome disassembly is reversible (77); once the oxidative stress is removed, the reassembly of the 26S proteasome occurs and the degradation of ubiquitinated substrates can resume, leading to cellular recovery.

During prolonged exposure of oxidative stress (*i.e.* later phase—at least 12 h following stress induction), proteasomal activities are inhibited and *de novo* proteasome synthesis is activated (67, 165, 175, 176). Up-regulation of both standard and inducible proteasomal components leads to the formation of more functional 20S and i20S proteasomes, respectively. The newly produced 20S and i20S complexes can associate with PA28 and/or 19S regulatory complexes respectively to form diverse functional proteasome complexes for ubiquitin/ATP- independent and/or dependent degradation of oxidized proteins (41, 67). It has been suggested that activated 20S, i20S, and i26S proteasomes are all better able to degrade oxidized proteins than the standard 26S proteasome (41, 67), and the production of immunoproteasomes may be of particular importance for mounting a cellular response against oxidative stress (176). Ultimately, the heterogeneous populations of proteasomes act in concert to degrade toxic oxidized proteins and protect cells from oxidative damage.

CONCLUSION

The proteasome is regulated by complex and poorly understood mechanisms. Attempts to clarify proteasome functional dynamics in response to oxidative stress are complicated by the presence of heterogeneous proteasome populations and multiple regulatory pathways. Additionally, cells exhibit diverse, often contrasting, responses to oxidative stress that are dependent on the type, dose, and duration of oxidative insults. Despite well-established knowledge that proteasomes are important for the removal of oxidatively damaged proteins and the more recently proposed model whereby proteasome activities are modulated by elevated ROS levels, many key questions remain unanswered. These include the following: (1) how do the subtypes of proteasome complexes work together to effectively degrade damaged proteins; (2) what are the mechanisms controlling proteasomal activities and how do these adapt to oxidative stress; (3) how is proteolysis of oxidatively damaged proteins regulated; (4) how are 20S proteasomes activated *in vivo* for the degradation of oxidized proteins; (5) what molecular mechanisms link proteasome inhibition and/or activation to oxidative stress-associated human pathologies. Although several recent studies have provided new insights that shed light on some of these questions, we have only just begun to unravel the molecular details underlying oxidative stress-triggered regulation of proteasome complexes. To fully address these questions, systematic analyses using biochemical, genetic and proteomic approaches are required. This will not only allow the understanding of ROS-induced regulation of proteasomes, but also provide potential molecular targets for screening proteasome inhibitors and activators. Given that oxidative stress-induced human diseases are associated with the accumulation of misfolded proteins and the loss of proteasome activities, strategies that enhance endogenous proteasome activity would be beneficial. Recent success of using a Usp14 inhibitor to accelerate proteasomal degradation of oxidized proteins (47) demonstrates the possibility of developing proteasome activating reagents for preventing protein aggregation in aging and/or neurodegenerative disorders.

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§ To whom correspondence should be addressed: Medical Science I, D233, Department of Physiology and Biophysics, Department of Developmental and Cell Biology, University of California, Irvine, Irvine, CA92697-4560. Phone: (949)824-8548; Fax: (949)824-8540; E-mail: lanhuang@uci.edu.

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