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Review Article

Ubiquitin–proteasome pathway and cellular responses to oxidative stress

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ABSTRACT

The ubiquitin–proteasome pathway (UPP) is the primary cytosolic proteolytic machinery for the selective degradation of various forms of damaged proteins. Thus, the UPP is an important protein quality control mechanism. In the canonical UPP, both ubiquitin and the 26S proteasome are involved. Substrate proteins of the canonical UPP are first tagged by multiple ubiquitin molecules and then degraded by the 26S proteasome. However, in noncanonical UPP, proteins can be degraded by the 26S or the 20S proteasome without being ubiquitinated. It is clear that a proteasome is responsible for selective degradation of oxidized proteins, but the extent to which ubiquitination is involved in this process remains a subject of debate. Whereas many publications suggest that the 20S proteasome degrades oxidized proteins independent of ubiquitin, there is also solid evidence indicating that ubiquitin and ubiquitination are involved in degradation of some forms of oxidized proteins. A fully functional UPP is required for cells to cope with oxidative stress and the activity of the UPP is also modulated by cellular redox status. Mild or transient oxidative stress up-regulates the ubiquitination system and proteasome activity in cells and tissues and transiently enhances intracellular proteolysis. Severe or sustained oxidative stress impairs the function of the UPP and decreases intracellular proteolysis. Both the ubiquitin-conjugating enzymes and the proteasome can be inactivated by sustained oxidative stress, especially the 26S proteasome. Differential susceptibilities of the ubiquitin-conjugating enzymes and the 26S proteasome to oxidative damage lead to an accumulation of ubiquitin conjugates in cells in response to mild oxidative stress. Thus, increased levels of ubiquitin conjugates in cells seem to be an indicator of mild oxidative stress.

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Introduction

There are two major intracellular proteolytic pathways in the cells: the lysosomal pathway and the ubiquitin–proteasome pathway (UPP) [1–3]. Whereas the lysosomal pathway plays an important role in degradation of long-lived bulk proteins, particularly membrane-bound proteins, the UPP is the primary cytosolic protein degradation pathway [4–6]. In this article we review roles for the UPP in response to oxidative stress and the effects of oxidative stress on the function of the UPP. In its simplest form, the UPP involves two discrete steps: (1) covalent attachment of multiple ubiquitin molecules to the protein substrate and (2) degradation of the ubiquitin-tagged protein by the 26S proteasome with the release of free and reusable ubiquitin. In some

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cases, ubiquitin is degraded together with the tagged substrates by the proteasome [7].

Ubiquitin is a highly conserved 76-amino-acid polypeptide and its most widely understood function is to tag intracellular proteins for proteasomal degradation. Covalent attachment of ubiquitin to the protein substrate proceeds via a three-step cascade mechanism. Initially, the ubiquitin-activating enzyme, E1, activates the C-terminal glycine residue of ubiquitin via formation of a high-energy thiol ester with an internal E1 cysteine residue. One of dozens of ubiquitin-conjugating enzymes, E2's, transfers the activated ubiquitin, also via an E2–ubiquitin thiol ester intermediate, to the substrate that is specifically bound to a member of the ubiquitin–protein ligase family, E3's. In some cases, an E3–ubiquitin high-energy thiol ester intermediate is formed before the ubiquitin is transferred to the E3-bound substrate. The E3 catalyzes the formation of a peptide/isopeptide bond between a carboxyl group at the C-terminus of ubiquitin and an amine group of the substrate. There are 2 genes in the human genome that encode different isoforms of E1 and each form has a distinct preference for E2's [8–11]. There are at least 37 genes in the human genome that encode distinctive E2's [12]. The number of the genes encoding E3's is over 1000 [13,14]. The diversity of E2's and E3's and the combinatorial possibilities of various E2's and E3's in various cellular contexts provide the molecular basis for the stringent substrate specificity of the UPP.

In most cases, multiple ubiquitins are conjugated to the initial ubiquitin moiety to form polyubiquitin chains. A chain of four or more ubiquitin moieties is often required for substrate recognition by the 26S proteasome complex [15–17]. For most substrates, the first ubiquitin is often linked to the ε-amino group of an internal lysine residue of the target protein. However, for some protein substrates, such as MyoD and $p16^{INK4a}$, the first ubiquitin is fused to the free and exposed N-terminal residue of the substrate to generate a linear peptide bond [18]. In successive reactions, a polyubiquitin chain is synthesized by the progressive transfer of additional activated ubiquitin moieties to an internal lysine residue or the N-terminus of the previously conjugated ubiquitin molecule.

Ubiquitin has seven internal lysine residues. Together with the amine group at the N-terminus there are 8 possible positions for the second ubiquitin to attach. For the third ubiquitin, there are 15 possible positions of attachment. Thus, the structures of polyubiquitin chains in cells can be highly diverse [19–22]. The synthesis of particular polyubiquitin chain linkages seems to be catalyzed by specific E2–E3 combinations [23–29], and different topologies and lengths of the ubiquitin chains have different functional outcomes [30]. For example, ubiquitin chains conjugated through the lysine at position 48 of ubiquitin (Lys 48) lead to proteasomal degradation of the modified substrate, whereas chains linked at Lys 63 are instead implicated in signaling or trafficking events [31–33].

The 26S proteasome is a 2.4-MDa complex composed of two multisubunit complexes: a catalytic core, also called the 20S proteasome, and a regulatory complex, also called the 19S regulatory particle or PA700. The 20S proteasome is a 700-kDa complex composed of two copies of 14 different gene products arranged in four axially stacked heptameric rings (α1–7, β1–7, β1–7, α1–7). The α subunits form the gates of the cylindrical structure of the 20S proteasome and the β subunits carry the catalytic sites that line the central lumen of the proteolytic chamber. Substrates reach this proteolytic chamber via 13-Å pores formed by the α-subunit rings at either end of the cylinder [34]. However, these pores are normally obstructed by the amino termini of the α subunits. The topological arrangement of the 20S proteasome precludes access of native proteins to the catalytic sites and partially contributes to the selectivity of the proteasome [34].

The 19S regulatory particle (PA700) is a multisubunit complex that binds to either or both ends of the 20S cylinder, thereby positioning PA700 as a gatekeeper for substrate entry to the 20S proteasome. PA700 includes six distinct AAA-family ATPases (Rpt1–Rpt6) arranged in a hexameric ring that extends axially from the outer α rings of the 20S proteasome [35]. The ATP-dependent interaction between PA700 and the catalytic core promotes opening of the pores of the 20S proteasome and provides an access portal for substrates to the catalytic core. The ATPase subunits of PA700 also contribute to the unfolding of the protein substrates and delivery of the unfolded protein substrates into the proteolytic chamber through the narrow pores of the 20S proteasome [36–39]. Some of the non-ATPase subunits of PA700 display deubiquitinating activity (also called isopeptidase activity), and others serve as ubiquitin-interaction subunits to recruit polyubiquitinated substrates to the proteasome [40]. The overall process of 26S proteasome-catalyzed proteolysis depends on ATP hydrolysis. The exact energy-consuming steps in proteolysis remain unclear but are probably linked to substrate unfolding, translocation, and deubiquitination [41].

There are many additional proteins that reversibly associate with the proteasome and play a role in its regulatory functions. These proteasome-associated proteins include ubiquitin ligases, deubiquitinating enzymes (enzymes that release ubiquitin moieties from ubiquitin conjugates), and polyubiquitin-chain-binding proteins [42–45].

The modular and dynamic composition of the proteasome and its multiple regulators allows the formation of various isoforms of the proteasome to fulfill a wide array of physiological functions. In cells, the total number of proteasomes as well as the subunit composition of the proteasomes can be altered in response to physiological demands [46,47]. The 20S proteasome exists in two distinct forms that differ in their catalytic subunits. Mammals contain two genes for each of the three catalytic subunits. Two of these genes (β1i and β5i) are encoded in the major histocompatibility locus. Under certain physiological states such as enhanced immune function, β1i and β5i, together with a third gene (β2i), are conditionally expressed and selectively incorporated into newly synthesized proteasomes instead of their constitutive counterparts (β1, β5, and β2, respectively) [34]. This specific class of proteasome, which contains inducible catalytic subunits, is called immunoproteasome and participates in antigen presentation by enhancing production of certain class I peptides [48]. Animals lacking genes for the inducible catalytic subunits cannot produce the antigenic peptides, whereas overexpression of these inducible proteasome subunits enhances antigen presentation.

In addition to PA700, there are several other regulatory proteins or protein complexes, such as PA200 and PA28, that bind directly to the outer α rings of 20S proteasomes [49]. Unlike PA700, these alternative regulators are not ATPases and do not bind polyubiquitin chains, suggesting that they may direct the proteasome in ubiquitin-independent proteolytic functions. Furthermore, two proline-rich proteins, PI31 and Pr39, also bind and inhibit proteasome function via directly blocking 20S proteasome activity or attenuating binding of proteasome activators [50,51]. The dynamic states and diversity of the regulatory complex of the proteasome suggest that the proteasome may function in both ubiquitin-dependent and ubiquitin-independent manners.

Consistent with multiple configurations of the proteasome in cells, proteasome-mediated degradation also occurs in various manners. In the canonical UPP, proteins are degraded by the 26S proteasome in an ATP-dependent and ubiquitin-dependent manner. However, the 20S proteasome also exists in free form and some proteins, including oxidized proteins, are degraded by the 20S proteasome in an ATP-independent and ubiquitin-independent manner [52–58]. In rare instances proteins are degraded by the 26S proteasome in an ATP-dependent but ubiquitination-independent process [39,59–62]. The ubiquitin-independent proteasomal degradation is considered a noncanonical UPP function. The substrate specificity of various forms of the proteasome has been cleverly exploited to determine