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Review

Regulation of proteasomes in prion disease

Ting Zhu, Sher Hayat Khan, Deming Zhao, and Lifeng Yang*

State Key Laboratories for Agrobiotechnology, Key Lab of Animal Epidemiology and Zoonosis, Ministry of Agriculture, National Animal Transmissible Spongiform Encephalopathy Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China
*Correspondence address. Tel/Fax: +86-10-62732987; E-mail: yanglf@cau.edu.cn

The hallmark of prion disease is the accumulation of misfolded protein PrP^{Sc}, which is toxic to neuronal cells. The proteasome system is responsible for the rapid, precise, and timely degradation of proteins and plays an important role in cellular protein quality control. Increasing evidence indicates impaired activity of proteasomes in prion diseases. Accumulated PrP^{Sc} can directly or indirectly affect proteasome activity. Misfolded protein may influence the assembly and activity of 19S regulatory particle, or post-translational modification of 20S proteasome, which may adversely affect the protein degradation activity of proteasomes. In this review, we summarized the recent findings concerning the possible regulation of proteasomes in prion and other neurodegenerative diseases. The proteasome system may enhance its degradation activity by changing its structure, and this activity can also be increased by related chaperones when neuronal cells are subject to stress. When the proteasome system is inhibited, degradation of protein aggregates via autophagy may increase as a compensatory system. It is possible that a balance exists between the proteasome and autophagy *in vivo*; when one is impaired, the activity of the other may increase to maintain homeostasis. However, more studies are needed to elucidate the relationship between the proteasome system and autophagy.

Keywords proteasome; autophagy; prion disease; neurodegenerative disease

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Introduction

Prion diseases are a group of neurodegenerative diseases in animals and humans that have common pathological hallmarks, including spongiform vacuolation, neuronal loss, and astrocytic and microglial proliferation. These diseases have a long delay in the onset of symptoms, hindering early detection. An underlying feature associated with the development of prion diseases is the presence of a misfolded protein PrP^{Sc}, the isoform of endogenous cellular prion protein (PrP^{C}) in brain and lymphoid tissues. When aggregated, protease-resistant PrP^{Sc} seeds are believed to act as a template to promote the conversion of normal PrP^{C} to the pathological state, PrP^{Sc}, which is rich in β-sheets and resistant to degradation [1,2].

The proteasome system is a large multicatalytic, cytoplasmic, and nuclear protease complex that has many forms. All forms contain the core 20S proteasome, which is a hollow, barrel-shaped structure consisting of α- and β-subunits arranged in four rings. Many regulators, such as PA700(19S), PA28αβ(11S), nuclear PA28γ, or PA200, bind to 20S proteasome to form different proteasomes, which function in protein degradation, cell cycle control, DNA repair, chromosome stability, transcriptional activation, signal transduction, antigen presentation, and so on. The ubiquitin proteasome system (UPS) consists of the 19S regulator and 20S proteasome and acts in cellular quality control by degrading misfolded, unassembled, or damaged proteins that could otherwise potentially form aggregates [3]. Accumulation of ubiquitin conjugates or inclusion bodies involved in disease-characteristic proteins have been detected in various neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's diseases (HD), spinal and bulbar muscular atrophy, and prion diseases [4]. The UPS may be associated with these diseases. Lindquist and collaborators [5] have shown that the UPS is a potentially important player in prion pathogenesis. Inhibiting UPS with a proteasome inhibitor leads to PrP^{Sc}-like isoforms in cells. The authors explained that PrP^{Sc} is derived from misfolded PrP^{C} that is retrogradely transported to the cytosol for degradation by proteasomes. The proteasome inhibitor prevents this process, and accumulated PrP^{c} is converted into a PrP^{Sc}-like isoform. This conversion continues even after removal of the proteasome inhibitor from the medium, finally leading to cell death and transmission to neighboring cells [5]. UPS substrates, such as IκB, p27, and p53 accumulate in prion-infected mouse brains, indicating an impairment of the UPS. Aggregated β-sheet-rich

PrP and PrP^{Sc} can inhibit the proteolytic activities of the 26S and 20S proteasomes; even low concentrations of aggregated PrP can inhibit peptide hydrolysis by the 20S proteasome [6]. UPS activity is not only directly decreased by PrP^{Sc}, but also indirectly modulated via other mechanisms in prion disease.

Here, we reviewed the regulation of proteasomes in prion disease and the relationship between the proteasome system and autophagy in prion diseases and other neurodegenerative diseases.

Prion Diseases

Prion diseases are a family of fatal neurodegenerative disorders that affect humans and animals and share the same pathological characteristics: spongiform vacuolation, severe neuronal loss, and astrocytic and microglial proliferation. In humans, normal PrP^{C} is a highly conserved, host-encoded cell surface glycosylphosphatidyl inositol-linked glycoprotein containing 253 amino acids, which are cleaved to produce a mature PrP^{C} of 208 amino acids after translocation into endoplasmic reticulum (ER) and then to Golgi for trimming and modification of the sugar moieties prior to transport to the cell membrane. Accumulating evidence suggests that PrP^{C} has neuroprotective functions and that loss of PrP^{C} could impair cellular responses to oxidative stress [7–10]. However, loss of PrP^{C} is unlikely to be the cause of prion-mediated pathology, as knockout of PrP^{C} did not result in neurodegeneration [11,12]. The 'protein-only' hypothesis was proposed decades ago to explain the surprising transmission mechanism of prion diseases. Now, it is widely accepted that the abnormal isoform of PrP^{Sc} is the exclusive infectious agent of prion diseases. PrP^{Sc} acts as a template that promotes the conversion of PrP^{C} to PrP^{Sc}. The difference between these isoforms lies purely in the monomer conformation and its state of aggregation. Although they share the same amino acid sequence, PrP^{C} is soluble, monomeric, and rich in α-helical structure, whereas PrP^{Sc} is characterized by increased β-sheet structure, detergent insolubility, and partial resistance to proteolysis [13–17].

Considerable evidence demonstrated that PrP^{Sc} can be generated *in vitro* in the absence of genetic material. PrP^{Sc} is able to self-propagate by stimulating the conversion of PrP^{C} to PrP^{Sc} [18–21]. Despite extensive studies, the precise mechanism for PrP^{Sc} replication is still unclear. It is highly likely that prion replication requires exposure to tiny quantities of PrP^{Sc} to trigger the autocatalytic conversion of host PrP^{C} to PrP^{Sc} [22], as breaking large PrP^{Sc} aggregates to many smaller seeding-competent polymers resulted in an exponential accumulation of PrP^{Sc}, a seeding–nucleation mechanism [23,24]. This seeding–nucleation process has been reproduced *in vitro*, and when inoculated into animals, it led to prion disease characteristics [19–21].

The Proteasome System

The proteasome system is a large multicatalytic, cytoplasmic, and nuclear protease complex. It is responsible for the majority of non-lysosomal protein degradation within eukaryotic cells and necessary for cell viability [25–28]. There are many forms of the proteasome, but all forms contain the core 20S proteasome which is a hollow, barrel-shaped structure consisting of α- and β-subunits arranged in four rings, with each ring comprising seven subunits. The two outer rings are α-rings and the N-termini of seven α-subunits form a 'gate' that regulates substrate entry. Every inner β-ring contains three active subunits (β1, β2, and β5) facing a sequestered proteolytic chamber [29–32]. Usually, the 20S proteasome exists in a latent form in cells, because the 'gate' is almost closed, preventing penetration of substrates into the β-ring where they would be hydrolyzed. The latent proteasome is activated by multiple regulators, such as PA700(19S), PA28αβ(11S), REGγ(PA28γ), and PA200, which attach to the end of the 20S proteasome. Furthermore, a special form of the core proteasome is synthesized by substituting the proteolytically catalytic β1, β2, and β5 subunits with β1i, β2i, and β5i subunits, respectively, to form the 'immunoproteasome', which is linked to the 11S regulator (**Fig. 1**). Both the immunoproteasome and 11S are induced by interferon-γ and involved in antigen processing [33,34].

The UPS

The UPS functions in cellular quality control by degrading misfolded, unassembled, or damaged proteins that could otherwise form potentially toxic aggregates [35]. Degradation of proteins via the UPS pathway involves two successive steps: conjugation of multiple moieties of ubiquitin and degradation of the tagged protein by the 26S proteasome [36]. Ubiquitin is an 8.5 kDa protein composed of 76 amino acids, and conjugation of ubiquitin occurs through a series of enzyme-mediated reactions. Initially, ubiquitin-activating enzyme (E1) activates the C-terminal Gly of ubiquitin that is then transferred to ubiquitin-conjugating enzymes (E2), with many different ubiquitin protein ligases (E3). Ubiquitin is continuously ligated to lysine residues of protein substrates, and these intricate processes ensure selective protein conjugates that are subsequently recognized and degraded by the 26S proteasome. Finally, ubiquitinated protein is hydrolyzed into short peptide fragments and amino acids, while polyubiquitin chains are released from targeted proteins and disassembled to monomeric ubiquitin molecules that can be recycled [37,38].

The 26S proteasome is composed of two subcomplexes, a core 20S proteasome and a 19S regulatory particle. Electron microscopy analysis showed that each 20S proteasome is capped by one or two 19S particles [39]. The 19S regulatory

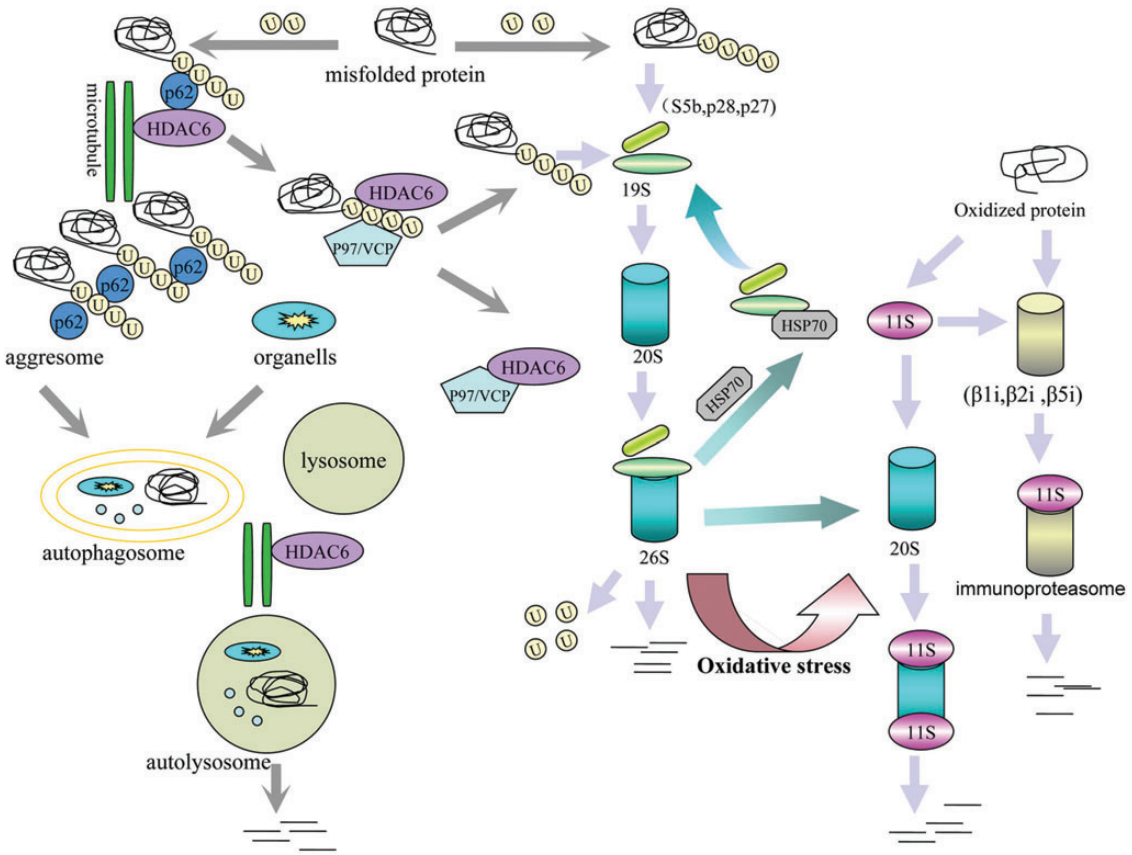

Figure 1. The proteasome system and autophagy degradation system in neurodegenerative disease Misfolded proteins were ubiquitinated and then degraded by the 26S proteasome. S5b/PSMD5 is one of the chaperones involved in the assembly of the 19S regulator, and abnormal expression of S5b/PSMD5 can reduce proteasome activity. When cells are subject to oxidative stress, related chaperones, such as HSP70, bind to the 19S regulator, which will release more free 20S proteasome. The 11S regulator can bind to the 20S proteasome and immunoproteasome to accelerate degradation of oxidized proteins. When the ubiquitinated proteasome is impaired and cannot efficiently degrade ubiqutinated proteins, HDAC6 and P62 will be associated with ubiquitinated proteins and will transfer them along microtubules to microtubule-organizing center (MTOC), from which microtubules emanate throughout the cell cycle, forming aggresome. These aggresomes are then degraded by an enhanced autophagy degrading system, and in addition to promoting the formation of aggresomes, HDAC6 is essential for fusion of an autophagosome and a lysosome. p97/VCP is an AAA-ATPase chaperone that can dissociate HDAC6-ployubiqutin-protein complexes and then transfer polyubiquitin protein to the 19S regulator. Therefore, p97/VCP functions in preventing aggregate formation and accelerating protein degradation by proteasomes.

particle is composed of 19 different canonical subunits and can be divided into two subcomplexes, lid and base, which play important roles in processing ubiquitinated substrates. These roles include binding, deubiquitinating and unfolding ubiquitinated proteins, and translocating them into the 20S proteasome for degradation [40]. The lid consists of 10 non-ATPase subunits (Rpn3, Rpn5–9, Rpn11, Rpn12, and Rpn15). Among them, Rpn11 is a deubiquitinating enzyme (DUB), which deubiquitinates substrates prior to their degradation. The base contains six distinct ATPase subunits (Rpt1–6) and three non-ATPase subunits (Rpn1, Rpn2, Rpn13). Connection between lid and base are stabilized by Rpn10. The six ATPase (Rpt1–6) subunits form a heterohexameric ring and constitute the molecular motor for the proteasome. When 19S ATPase binds a nucleotide, the conserved C-terminal HbYX motifs dock into the pockets between the adjacent α-subunits of 20S, functioning as a 'key in a lock' to destabilize the closed conformation and open the gate of the 20S proteasome. Rpn10 and Rpn13 are ubiquitin (Ub) receptors and can recognize polyubiquitinated substrates [41–44].

Assembly of the 19S regulator involves many chaperones (p28, S5b, and p27), which individually associate with specific pairs of ATPase subunits to form three different models: the p28-Rpt3-Rpt6-Rpn14 complex, the S5b-Rpt1-Rpt2-Rpn1 complex, and the p27-Rpt5-Rpt4 complex. The chaperones regulate the associations between these complexes and dissociations before 26S proteasome formation. The chaperones may accelerate the assembly of the 26S proteasome, although they are not necessary in the conventional process, but are probably required when more efficient proteasomes are needed [45].

Proteasomes in Prion Disease

UPS impairment and its role in prion diseases

Studies suggested that functional impairment in the UPS may be important in prion diseases [6,29]. A null mutation of Mahogunin, a putative E3 ubiquitin-protein ligase, resulted in a transmitted form of spongiform neurodegenerative disease in mice that histologically mimics prion disease [46,47], whereas another E3 ubiquitin ligase, Hectd2, is linked to prion disease incubation time in mouse and is associated with sporadic and variant Creutzfeldt–Jakob disease (CJD) and kuru in humans [48]. Ubiquitination of total brain proteins increased significantly with the development of clinical symptoms in mouse brain infected with ME7 scrapie train, which is associated with failure of the proteasome system. While ubiquitinated PrP is only detected at the terminal stage of prion disease, it is a late event phenomenon and occurs after the formation of protease-resistant PrP^{Sc} [8]. N2a-M cells treated with cyclosporin A produce protease-resistant, 'prion-like' PrP aggresomes as well as disease-linked proline mutants when proteasomes were inhibited [49]. In addition, three mutant prion proteins (PrP V203I, PrP E211Q, and PrP Q212P) associated with familial prion disease accumulated in the cytosol in response to proteasomal inhibition and eventually assembled into aggresomes [50]. Prion-infected neuronal cells became apoptotic after mild proteasome impairment and formed large aggresomes that contain PrP^{Sc}, heat shock chaperone 70, ubiquitin, proteasome subunits, and vimentin [51].

Although many studies have already shown that the UPS is impaired in neurodegenerative disease, the precise mechanism for such impairment is not clear. A significant decrease in proteasome activity was observed in the hippocampus and parahippocampal gyrus, superior and middle temporal gyri, and inferior parietal lobule of AD patients. Post-translational modification may be responsible for the loss of proteasome activity in the AD brain [52]. Surprisingly, Kristiansen *et al*. [53] found that PrP^{Sc} could directly inhibit the catalytic core β-subunits of the 26S proteasome, and recombinant aggregated β-sheet-rich protein had a similar effect. Moreover, denaturation of β-PrP (or PrP^{Sc}) and pre-incubation with an anti-oligomer antibody abolished their inhibitory effect on β-subunit activity. Aggregated β-sheet-rich PrP binding directly to the 20S proteasome inhibits substrate entry and impairs UPS function, because aggregated β-sheet-rich PrP can bind to, but not inhibit, open-gated α3ΔN particles, or 20S-PA26 complexes in which the gate is buried and inaccessible [54,55]. These findings indicated that β-sheet-rich PrP may bind to the outer lateral surface of the 20S particle and act indirectly and allosterically to stabilize the closed station of the 20S proteasome [6,56]. This may be only one of the mechanisms by which the UPS is impaired in prion disease.

The 19S regulator particle is the key component of the 26S proteasome. Except for opening the 20S gate, it is also responsible for recognizing, unfolding, and translocating ubiquitinated substrate into the 20S proteasome. ATP is necessary for the assembly by 19S, as well as for substrate unfolding and translocation into the 20S proteolytic chamber when cells are subject to stress. Low ATP availability may influence the function of the 19S, and therefore, regulating the function of the 19S particle may influence the activity of the 26S proteasome [57]. Shim *et al*. [58] reported that nuclear factor (NF)-κB activation acts as an inhibitor of the 26S proteasome. Tumor necrosis factor-α, interleukin-1β, and lipopolysaccharide each can increase S5b/PSMD5 protein expression by activating NF-κB pathways. This enhances the interaction between S5b/PSMD5 and S7/PSMC2, interferes with the assembly of the 19S particle, and eventually decreases the activity of the 26S proteasome. These processes provide insight into proteasome inhibition in neurodegenerative diseases.

Proteasome regulation in oxidative stress

Oxidative stress is induced by reactive oxygen species (ROS) or free radicals and has been implicated in aging and in the pathogenesis of several neurodegenerative disorders [59]. Several studies have shown that prion proteins impair the cellular response to oxidative stress, and prion-infected hypothalamic neuronal GT1 cells were more susceptible to imposed oxidative stress and showed increased lipid peroxidation and signs of apoptosis [9]. Exposure of neuronal cells to the $PrP_{106-126}$ fragment, the likely etiological agent for a series of encephalopathies and a peptide that mimics the neurotoxicity of PrP^{Sc}, leads to a 70% decrease in the intracellular concentration of glutathione and BCL-2 expression [60]. Exposure of neuronal cell cultures to $PrP_{106-126}$ can also reduce glutathione reductase activity and increase the sensitivity of the cells to H_2O_2 [61].

The proteasome system plays a vital role in the cellular response to oxidative stress, because ROS can target and damage many proteins that then become cytotoxic, and also plays an important role in the selective recognition and degradation of oxidized proteins [62–64]. Previous studies showed that E1 ubiquitin-activating enzymes, E2 ubiquitin-conjugating enzymes, and the 26S proteasome are highly sensitive to oxidative stress, whereas the 20S proteasome is stable and important in the degradation of oxidized proteins [65,66]. Oxidative stress can induce the dissociation of the 20S core particle from 19S regulators, which may involve many regulators and inhibit the 26S proteasome activities. This process finally causes the accumulation of ubiquitinated proteins. However, this process is also beneficial for adapting to oxidative stress, because dissociation of the 26S proteasome can release additional free 20S proteasome that is

required for the degradation of oxidized proteins. Meanwhile, several chaperones such as proteasome-interacting protein Ecm29 can associate with the 19S proteasome, which induces the disassembly of the 26S proteasome and prevents its re-assembly during oxidative stress [67]. Grune *et al*. [68] showed the regulation of cellular proteasomes at different timepoints during oxidative stress. In the first hour, HSP70 chaperone binds to and preserves 19S regulators, which mediates the dissociation of the 26S proteasome, generating extra-free 20S proteasomes to degrade oxidized proteins as an initial response to oxidative stress. Then, HSP70 is released from 19S regulators, and the 26S proteasome is reconstituted from 1 to 3 h. Finally, the transcription/translation of the 20S proteasome, PA28, and immunoproteasome is increased to facilitate the degradation of more oxidized proteins (**Fig. 1**) [68].

In summary, the regulation of the proteasome may be very important for cells' adaptation to oxidative stress in neurodegenerative diseases. Initial survival during oxidative stress may require immediate dissociation of the 26S proteasome into free 20S proteasome to degrade oxidized proteins, and subsequent adaption may depend on the synthesis of the 20S proteasome. Meanwhile, the 26S proteasome is reconstituted and ATP-stimulated proteolysis is restored [68]. However, the exact mechanism remains unclear and needs to be demonstrated in neurodegenerative diseases.

Relationship between the UPS and Autophagy in Neurodegenerative Diseases

Autophagy in prion diseases and other neurodegenerative diseases

Autophagy, especially macroautophagy, is a highly conserved process that involves rearrangement of subcellular membranes to sequester cytoplasm and organelles for delivery to lysosome-forming autophagosomes/autophagic vacuoles (AVs) for degradation and recycling [69]. Autophagy is a fundamental bulk degradation process for organelles and cytoplasmic proteins. Beyond the significant function of basal autophagy in the health of organisms, including humans, the importance of autophagy is even more evident in nervous system diseases, including AD, PD, HD, and prion diseases [70,71]. Increased numbers of AVs are a feature of many neurological diseases. Giant AVs have been detected in neurons of mice with CJD and scrapie-infected hamsters, and the ultrastructural features of autophagy in these two experimental models are similar. Moreover, multivesicular bodies and AVs have been observed in prion-infected cultured neurons and in various forms of human prion diseases [72–74]. Xu *et al*. [75] suggested that microtubule-associated protein 1 light chain 3 (LC3) is converted from a cytolic form to an autophagosome membrane-bound form. Moreover, autophagy substrate sequestosome 1 (SQSTM1) and polyubiquitinated proteins are down-regulated in the brain of scrapie-infected hamsters and in human genetic prion diseases, indicating that the autophagy system is enhanced, likely via the mammalian target of rapamycin pathway.

However, loss of autophagy in the central nervous system (CNS) causes neurodegeneration in mice. Autophagy-related 7 (*Atg7*) gene knockout in the CNS of mice leads to behavioral defects, massive neuronal loss in the cerebral and cerebellar cortices, and accumulation of polyubiquitinated proteins in neurons as inclusion bodies [76], indicating that autophagy is essential for neuron survival. The Atg8a protein level is decreased in the aging CNS, and *Atg8a* mutant flies have a reduced lifespan and neurodegenerative phenotype, including ubiquitinated protein accumulation and increased sensitivity to oxidative stress. In contrast, an enhanced level of Atg8a expression extends the average adult lifespan and promotes resistance to oxidative stress, preventing the accumulation of protein aggregates [77], and up-regulation of autophagy may be beneficial for the healthy CNS under stressful conditions that involve oxidant exposure, protein misfolding, and aging. In addition, a series of studies suggested that neurodegenerative disease-causing proteins are frequently degraded by autophagy. The results from immunoelectron microscopy showed that disease-related proteins are delivered to AVs in cultured cells, showing that autophagy contributes to the degradation of multiple disease-related proteins [78]. PrP^{Sc} does not co-localize with autophagosomes in the brain of scrapie-infected hamsters, whereas PrP^{Sc} does co-localize with autophagosomes in a prion-infected cell line after treatment with Bafilomycin A [75]. In prion-infected mice, imatinib treatment during the early phase of peripheral infection delays both neuroinvasion of PrP^{Sc} and the onset of clinical disease by activating lysosomal degradation of PrP^{Sc} [79]. Moreover, studies have also shown that both lithium and trehalose can enhance PrP^{Sc} clearance in prion-infected cells via the induction of autophagy, whereas the amount of PrP^{Sc} increases in cells treated with autophagy inhibitors [71,80–82].

Autophagy as a compensatory mechanism for proteasome impairment

Autophagy and the UPS are two major cellular protein degradation systems that were previously believed to be distinct, although increasing evidence suggests that they are functionally interrelated [83]. In particular, they share certain substrates and regulators, and several observations have indicated that autophagy may provide an alternate, compensatory route of degradation when the UPS is impaired. Most misfolded soluble proteins are preferentially degraded by the proteasome, but insoluble aggregates are difficult for the proteasome to degrade and even impair its function. Therefore, aggregates are mainly degraded via autophagy, and this is true for large aggregates in cultured cells [57]. One study showed that when aggregation-prone misfolded proteins overwhelm the UPS in cultured cells, single

aggregates were tagged by ubiquitin and then translocated to the microtubule-organizing center to form aggresomes, which were eventually degraded by autophagy (**Fig. 1**) [84]. In addition to the formation of aggresomes, impairment of the UPS has been found to induce autophagy. Ding *et al*. [83] demonstrated that autophagy is activated when the proteasome is inhibited, and this is important for controlling ER stress and preventing cell death in cancer cells. In contrast, autophagy inhibition leads to increased levels of soluble UPS clients and compromised UPS activity [85]. However, Atg5- or Atg7-deficient mice exhibited massive neuronal loss and polyubiquitinated proteins as inclusion bodies. Moreover, these mice showed no deficiencies in UPS function, suggesting that some ubiquitin-tagged proteins may normally be degraded by autophagy (termed 'selective autophagy') [76,86]. Inactivation of the ubiquitin-activating enzyme E1 leads to a defect in autolysosomal degradation and to an absence of ubiquitin-positive proteins within lysosomes [87]. Therefore, more work is needed to elaborate the relationship between autophagy and the UPS. A previous report showed that over-expression of amyloid precursor protein leads to neurodegeneration, increased oxidative stress, inhibition of proteasome activity, and impairment of the autophagic flux, and cells attempt to enhance autophagy in a histone deacetylase-6 (HDAC6)-dependent manner to compensate for this altered scenario [88,89]. Although the exact relationship between selective autophagy and the UPS in neurodegenerative diseases is not well known, abnormal UPS activity in some neurodegenerative diseases may remind us to investigate whether autophagy acts as a compensatory degrading system in response to decreased UPS activity.

Regulators involved in the links between autophagy and the UPS

Although increasing evidence has already demonstrated the coordination between the UPS and autophagy, the exact processes involved are poorly understood. Recently, several regulators have been reported to play important roles in mediating this crosstalk [78].

HDAC6 is a class II HDAC and a microtubule-associated deacetylase. In addition to histone deacetylation, it has been shown to deacetylase multiple non-histone proteins including α-tubulin, Hsp90, cortactin, peroxiredoxin, and Ku70. Different from other HDAC family proteins, HDAC6 also contains a ZnF-UBP domain at its C-terminus, which has a higher affinity for ubiquitinated proteins than other ubiquitin-binding proteins, even Rpn10 [90]. HDAC6 mediates the transport of ubiquitinated proteins along microtubule tracks to aggresomes for eventual degradation by autophagy, and HDAC6 also plays an essential regulatory role in the fusion of autophagosomes and lysosomes (**Fig. 1**) [91]. Autophagy acts as a compensatory degradation system when the UPS is impaired in *Drosophila melanogaster*, and HDAC6 is an essential link in this interaction. Moreover, over-expression of HDAC6 can rescue the degeneration associated with UPS dysfunction *in vivo* in an autophagy-dependent manner [88]. Therefore, HDAC6 may be an efficient medicinal target for the treatment of AD.

p97/VCP is an AAA-ATPase chaperone that has 'segregase' activity and is involved in disassembling various complexes, including HDAC6-polyubiquitin complexes. In the presence of ATP, p97 can dissociate HDAC6-ployubiqutin complexes and transfer the polyubiquitin to Rpn10, which indicates that p97/VCP prevents the formation of aggregates and accelerates protein degradation by proteasomes (**Fig. 1**). Hence, a finely tuned equilibrium in HDAC6 and p97/VCP concentration determines the fate of polyubiquitinated proteins [90].

P62/SQSTM1 is another regulator that links autophagy with the UPS, and it harbors a ubiquitinated-associated (UBA) domain at its C-terminal. This UBA domain can combine with ubiquitinated proteins, p62 and NBR1 (neighbor of BRCA1 gene 1), co-aggregate with ubiquitinated misfolded proteins, and mediate their degradation by selective autophagy [92]. p62 is present in all types of ubiquitinated intracellular protein inclusions found in neurodegenerative diseases, and p62-null mice fail to form ubiquitin-positive protein aggregates in response to misfolded protein stress and exhibit age-related neurodegeneration [78,93]. Researchers have shown that p62 has broad functions in stress situations, such as proteasome impairment, oxidative stress, and increased misfolded protein burden. p62 forms transient, stress-induced 'p62 bodies' containing ubiquitinated, misfolded proteins with NBR1 [94–96]. Kaniuk *et al*. [97] and Lamark and Johansen [98] demonstrated that oxidative stress-induced p62 bodies in pancreatic cells of diabetic rats could only be cleared by autophagy.

Recently, more and more researchers are studying the relationship between autophagy and the UPS, and it has become apparent that the two degradation systems are coordinated. However, the mechanisms and molecular players that regulate the crosstalk between these systems still need to be elucidated, especially in neurodegenerative diseases.

Conclusion

A shared characteristic of many neurodegenerative disorders is the accumulation of misfolded proteins that cannot be removed from neurons and eventually form aggregates. For example, AD is the most common neurodegenerative disease, and hyperphosphorylation of tau protein (p-tau) leads to the formation of paired helical filament/neurofibrillary tangles which accumulate in cells. In contrast, amyloid-β peptide forms oligomers and senile plaques which are deposited outside the cells [91]. PD is the second

most common progressive neurodegenerative disorder, and its hallmark is the Lewy bodies which contain ubiquitin-positive protein aggregates and is accumulated in the cytoplasm [99]. In prion disease, normal PrP^{C} transforms into the poorly soluble PrP^{Sc}, which leads to the accumulation of PrP^{Sc} aggregates. These accumulated proteins are toxic and eventually induce dysfunction and death of neuronal cells.

The proteasome system is a rapid, precise, and timely processing system for many cellular proteins, and it is present in all types of cells in the CNS. Proteasomes degrade multiple substrates including misfolded and damaged proteins and induce the catabolism of oxidized low-density lipoprotein, which is important for cell differentiation, cell cycle procession, circadian rhythms, apoptosis, inflammation, and other biological process. The proteasome is overwhelmed in neurodegenerative diseases, and it can be impaired by directly inhibiting the entrance of substrate to the 20S proteasome or down-regulating specific components. In addition, proteasome activity can be decreased indirectly by other chaperones that participate in the assembly of proteasome. Although the exact mechanism underlying the regulation of proteasome activity remains poorly understood, aberrant proteasome activity is directly associated with the pathogenesis of prion diseases. Therefore, the regulation and normal functioning of the proteasome system is critical for neuronal cells.

Increasing evidence shows that inhibiting proteasome activity can trigger autophagy, which may be the complementary pathway for impairment of proteasomes. However, when an activated autophagic system reaches saturation, it may be impaired, indicating that autophagy has gone awry in neurodegenerative diseases, including AD, PD, HD, ALS, and others [100]. Consequently, more work is needed to elucidate whether a balance exists between the proteasome and autophagy *in vivo*. In such a balance, when one is impaired, the activity of the other pathway is increased to some extent in order to maintain homeostasis. A better understanding of the relationship between the proteasome and autophagy will be beneficial in the treatment of neurodegenerative diseases, in which the proteasome is impaired.

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References

1. Harris DA and True HL. New insights into prion structure and toxicity. Neuron 2006, 50: 353–357.
2. Hu PP and Huang CZ. Prion protein: structural features and related toxicity. Acta Biochim Biophys Sin 2013, 45: 435–441.
3. Hershko A and Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998, 67: 425–479.
4. Ciechanover A and Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. Neuron 2003, 40: 427–446.
5. Ma J, Wollmann R and Lindquist S. Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol. Science 2002, 298: 1781–1785.
6. Deriziotis P, Andre R, Smith DM, Goold R, Kinghorn KJ, Kristiansen M and Nathan JA, *et al*. Misfolded PrP impairs the UPS by interaction with the 20S proteasome and inhibition of substrate entry. EMBO J 2011, 30: 3065–3077.
7. Mastrianni JA. Prion diseases. Clin Neurosci Res 2004, 3: 469–480.
8. Kang SC, Brown DR, Whiteman M, Li R, Pan T, Perry G and Wisniewski T, *et al*. Prion protein is ubiquitinated after developing protease resistance in the brains of scrapie-infected mice. J Pathol 2004, 203: 603–608.
9. Milhavet O, McMahon HE, Rachidi W, Nishida N, Katamine S, Mange A and Arlotto M, *et al*. Prion infection impairs the cellular response to oxidative stress. Proc Natl Acad Sci USA 2000, 97: 13937–13942.
10. Williams A, Lucassen PJ, Ritchie D and Bruce M. PrP deposition, microglial activation, and neuronal apoptosis in murine scrapie. Exp Neurol 1997, 144: 433–438.
11. Mallucci GR, Ratte S, Asante EA, Linehan J, Gowland I, Jefferys JG and Collinge J. Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. EMBO J 2002, 21: 202–210.
12. Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M and Weissmann C. Mice devoid of PrP are resistant to scrapie. Cell 1993, 73: 1339–1347.
13. Griffith JS. Self-replication and scrapie. Nature 1967, 215: 1043–1044.
14. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982, 216: 136–144.
15. Prusiner SB. Prions. Proc Natl Acad Sci USA 1998, 95: 13363–13383.
16. Zhou Z and Xiao G. Conformational conversion of prion protein in prion diseases. Acta Biochim Biophys Sin 2013, 45: 465–476.
17. Yuan Z, Zhao D and Yang L. Decipher the mechanisms of rabbit's low susceptibility to prion infection. Acta Biochim Biophys Sin 2013, 45: 899–903.
18. Legname G, Baskakov IV, Nguyen HO, Riesner D, Cohen FE, DeArmond SJ and Prusiner SB. Synthetic mammalian prions. Science 2004, 305: 673–676.
19. Castilla J, Saa P, Hetz C and Soto C. *In vitro* generation of infectious scrapie prions. Cell 2005, 121: 195–206.
20. Deleault NR, Harris BT, Rees JR and Supattapone S. Formation of native prions from minimal components *in vitro*. Proc Natl Acad Sci USA 2007, 104: 9741–9746.
21. Wang F, Wang X, Yuan CG and Ma J. Generating a prion with bacterially expressed recombinant prion protein. Science 2010, 327: 1132–1135.
22. Lansbury PT, Jr and Caughey B. The chemistry of scrapie infection: implications of the 'ice 9' metaphor. Chem Biol 1995, 2: 1–5.
23. Saborio GP, Permanne B and Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 2001, 411: 810–813.
24. Soto C. Transmissible proteins: expanding the prion heresy. Cell 2012, 149: 968–977.
25. Peters JM, Franke WW and Kleinschmidt JA. Distinct 19S and 20S subcomplexes of the 26S proteasome and their distribution in the nucleus and the cytoplasm. J Biol Chem 1994, 269: 7709–7718.
26. Lodish HBA, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky SL and Darnell J. Molecular Cell Biology (5th end). New York: WH Freeman and CO, 2004: 66–72.
27. Baumeister W, Walz J, Zuhl F and Seemuller E. The proteasome: paradigm of a self-compartmentalizing protease. Cell 1998, 92: 367–380.

28. Fruh K, Gossen M, Wang K, Bujard H, Peterson PA and Yang Y. Displacement of housekeeping proteasome subunits by MHC-encoded LMPs: a newly discovered mechanism for modulating the multicatalytic proteinase complex. EMBO J 1994, 13: 3236–3244.
29. Deriziotis P and Tabrizi SJ. Prions and the proteasome. Biochim Biophys Acta 2008, 1782: 713–722.
30. Jung T and Grune T. Structure of the proteasome. Prog Mol Biol Transl Sci 2012, 109: 1–39.
31. Coux O, Tanaka K and Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem 1996, 65: 801–847.
32. Voges D, Zwickl P and Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 1999, 68: 1015–1068.
33. Pickering AM, Koop AL, Teoh CY, Ermak G, Grune T and Davies KJ. The immunoproteasome, the 20S proteasome and the PA28alphabeta proteasome regulator are oxidative-stress-adaptive proteolytic complexes. Biochem J 2010, 432: 585–594.
34. Murata S, Udono H, Tanahashi N, Hamada N, Watanabe K, Adachi K and Yamano T, *et al*. Immunoproteasome assembly and antigen presentation in mice lacking both PA28alpha and PA28beta. EMBO J 2001, 20: 5898–5907.
35. Bence NF, Sampat RM and Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. Science 2001, 292: 1552–1555.
36. Ciechanover A, Orian A and Schwartz AL. Ubiquitin-mediated proteolysis: biological regulation via destruction. Bioessays 2000, 22: 442–451.
37. Pickart CM. Ubiquitin in chains. Trends Biochem Sci 2000, 25: 544–548.
38. McNaught KS, Olanow CW, Halliwell B, Isacson O and Jenner P. Failure of the ubiquitin-proteasome system in Parkinson's disease. Nat Rev Neurosci 2001, 2: 589–594.
39. Glickman MH, Rubin DM, Coux O, Wefes I, Pfeifer G, Cjeka Z and Baumeister W, *et al*. A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. Cell 1998, 94: 615–623.
40. Liu CW and Jacobson AD. Functions of the 19S complex in proteasomal degradation. Trends Biochem Sci 2013, 38: 103–110.
41. Lander GC, Estrin E, Matyskiela ME, Bashore C, Nogales E and Martin A. Complete subunit architecture of the proteasome regulatory particle. Nature 2012, 482: 186–191.
42. Smith DM, Chang SC, Park S, Finley D, Cheng Y and Goldberg AL. Docking of the proteasomal ATPases' carboxyl termini in the 20S proteasome's alpha ring opens the gate for substrate entry. Mol Cell 2007, 27: 731–744.
43. Rabl J, Smith DM, Yu Y, Chang SC, Goldberg AL and Cheng Y. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. Mol Cell 2008, 30: 360–368.
44. Smith DM, Kafri G, Cheng Y, Ng D, Walz T and Goldberg AL. ATP binding to PAN or the 26S ATPases causes association with the 20S proteasome, gate opening, and translocation of unfolded proteins. Mol Cell 2005, 20: 687–698.
45. Kaneko T, Hamazaki J, Iemura S, Sasaki K, Furuyama K, Natsume T and Tanaka K, *et al*. Assembly pathway of the Mammalian proteasome base subcomplex is mediated by multiple specific chaperones. Cell 2009, 137: 914–925.
46. Kim BY, Olzmann JA, Barsh GS, Chin LS and Li L. Spongiform neurodegeneration-associated E3 ligase Mahogunin ubiquitylates TSG101 and regulates endosomal trafficking. Mol Biol Cell 2007, 18: 1129–1142.
47. Lehman NL. The ubiquitin proteasome system in neuropathology. Acta Neuropathol 2009, 118: 329–347.
48. Lloyd SE, Maytham EG, Pota H, Grizenkova J, Molou E, Uphill J and Hummerich H, *et al*. HECTD2 is associated with susceptibility to mouse and human prion disease. PLoS Genet 2009, 5: e1000383.
49. Cohen E and Taraboulos A. Scrapie-like prion protein accumulates in aggresomes of cyclosporin A-treated cells. EMBO J 2003, 22: 404–417.
50. Mishra RS, Bose S, Gu Y, Li R and Singh N. Aggresome formation by mutant prion proteins: the unfolding role of proteasomes in familial prion disorders. J Alzheimers Dis 2003, 5: 15–23.
51. Kristiansen M, Messenger MJ, Klohn PC, Brandner S, Wadsworth JD, Collinge J and Tabrizi SJ. Disease-related prion protein forms aggresomes in neuronal cells leading to caspase activation and apoptosis. J Biol Chem 2005, 280: 38851–38861.
52. Jeffrey N, Keller KB and William R. Impaired proteasome function in Alzheimer's disease. J Neurochem 2000, 75: 436–439.
53. Kristiansen M, Deriziotis P, Dimcheff DE, Jackson GS, Ovaa H, Naumann H and Clarke AR, *et al*. Disease-associated prion protein oligomers inhibit the 26S proteasome. Mol Cell 2007, 26: 175–188.
54. Forster A, Masters E, Whitby F, Robinson H and Hill C. The 1.9 Å structure of a proteasome-11S activator complex and implications for proteasome-PAN/PA700 interactions. Mol Cell 2005, 18: 589–599.
55. Whitby FG, Masters EI, Kramer L, Knowlton JR, Yao Y, Wang CC and Hill CP. Structural basis for the activation of 20S proteasomes by 11S regulators. Nature 2000, 408: 115–120.
56. Andre R and Tabrizi SJ. Misfolded PrP and a novel mechanism of proteasome inhibition. Prion 2012, 6: 32–36.
57. Lilienbaum A. Relationship between the proteasomal system and autophagy. Int J Biochem Mol Biol 2013, 4: 1–26.
58. Shim SM, Lee WJ, Kim Y, Chang JW, Song S and Jung YK. Role of S5b/PSMD5 in proteasome inhibition caused by TNF-alpha/NFkappaB in higher eukaryotes. Cell Rep 2012, 2: 603–615.
59. Kim JI, Choi SI, Kim NH, Jin JK, Choi EK, Carp RI and Kim YS. Oxidative stress and neurodegeneration in prion diseases. Ann N Y Acad Sci 2001, 928: 182–186.
60. Perovic S, Schroder HC, Pergande G, Ushijima H and Muller WE. Effect of flupirtine on Bcl-2 and glutathione level in neuronal cells treated *in vitro* with the prion protein fragment (PrP106–126). Exp Neurol 1997, 147: 518–524.
61. White AR, Collins SJ, Maher F, Jobling MF, Stewart LR, Thyer JM and Beyreuther K, *et al*. Prion protein-deficient neurons reveal lower glutathione reductase activity and increased susceptibility to hydrogen peroxide toxicity. Am J Pathol 1999, 155: 1723–1730.
62. Jung T and Grune T. The proteasome and its role in the degradation of oxidized proteins. IUBMB Life 2008, 60: 743–752.
63. Davies KJ. Degradation of oxidized proteins by the 20S proteasome. Biochimie 2001, 83: 301–310.
64. Breusing N and Grune T. Regulation of proteasome-mediated protein degradation during oxidative stress and aging. Biol Chem 2008, 389: 203–209.
65. Reinheckel T, Sitte N, Ullrich O, Kuckelkorn U, Davies KJ and Grune T. Comparative resistance of the 20S and 26S proteasome to oxidative stress. Biochem J 1998, 335: 637–642.
66. Shang F and Taylor A. Oxidative stress and recovery from oxidative stress are associated with altered ubiquitin conjugating and proteolytic activities in bovine lens epithelial cells. Biochem J 1995, 307: 297–303.
67. Wang X, Yen J, Kaiser P and Huang L. Regulation of the 26S proteasome complex during oxidative stress. Sci Signal 2010, 3: 1–9.
68. Grune T, Catalgol B, Licht A, Ermak G, Pickering AM, Ngo JK and Davies KJ. HSP70 mediates dissociation and reassociation of the 26S proteasome during adaptation to oxidative stress. Free Radic Biol Med 2011, 51: 1355–1364.
69. Klionsky DJ. Autophagy as a regulated pathway of cellular degradation. Science 2000, 290: 1717–1721.
70. Rubinsztein DC, DiFiglia M, Heintz N, Nixon RA, Qin ZH, Ravikumar B and Stefanis L, *et al*. Autophagy and its possible roles in nervous system diseases, damage and repair. Autophagy 2005, 1: 11–22.
71. Heiseke A, Aguib Y and Schatzl HM. Autophagy, prion infection and their mutual interactions. Curr Issues Mol Biol 2010, 12: 87–97.

72. Boellaard JW, Kao M, Schlote W and Diringer H. Neuronal autophagy in experimental scrapie. Acta Neuropathol 1991, 82: 225–228.
73. Boellaard JW, Schlote W and Tateishi J. Neuronal autophagy in experimental Creutzfeldt–Jakob's disease. Acta Neuropathol 1989, 78: 410–418.
74. Schatzl HM LL, Holtzman DM, Tatzelt J, DeArmond SJ, Weiner RI, Mobley WC and Mobley WC, *et al*. A hypothalamic neuronal cell line persistently infected with scrapie prions exhibits apoptosis. J Virol 1997, 71: 8821–8831.
75. Xu Y, Tian C, Wang SB, Xie WL, Guo Y, Zhang J and Shi Q, *et al*. Activation of the macroautophagic system in scrapie-infected experimental animals and human genetic prion diseases. Autophagy 2012, 8: 1604–1620.
76. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I and Ueno T, *et al*. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 2006, 441: 880–884.
77. Simonsen A, Cumming RC, Brech A, Isakson P, Schubert DR and Finley KD. Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. Autophagy 2008, 4: 176–184.
78. Nedelsky NB, Todd PK and Taylor JP. Autophagy and the ubiquitin-proteasome system: collaborators in neuroprotection. Biochim Biophys Acta 2008, 1782: 691–699.
79. Yun SW, Ertmer A, Flechsig E, Gilch S, Riederer P, Gerlach M and Schätzl HM, *et al*. The tyrosine kinase inhibitor imatinib mesylate delays prion neuroinvasion by inhibiting prion propagation in the periphery. J Neurovirol 2007, 13: 328–337.
80. Heiseke A, Aguib Y, Riemer C, Baier M and Schatzl HM. Lithium induces clearance of protease resistant prion protein in prion-infected cells by induction of autophagy. J Neurochem 2009, 109: 25–34.
81. Aguib Y, Heiseke A, Gilch S, Riemer C, Baier M, Schatzl HM and Ertmer A. Autophagy induction by trehalose counteracts cellular prion infection. Autophagy 2009, 5: 361–369.
82. Yao H, Zhao D, Khan SH and Yang L. Role of autophagy in prion protein-induced neurodegenerative diseases. Acta Biochim Biophys Sin 2013, 45: 494–502.
83. Ding WX, Ni HM, Gao W, Yoshimori T, Stolz DB, Ron D and Yin XM. Linking of autophagy to ubiquitin-proteasome system is important for the regulation of endoplasmic reticulum stress and cell viability. Am J Pathol 2007, 171: 513–524.
84. Johnston JA, Ward CL and Kopito RR. Aggresomes: a cellular response to misfolded proteins. J Cell Biol 1998, 143: 1883–1898.
85. Korolchuk VI, Mansilla A, Menzies FM and Rubinsztein DC. Autophagy inhibition compromises degradation of ubiquitin-proteasome pathway substrates. Mol Cell 2009, 33: 517–527.
86. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R and Yokoyama M, *et al*. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 2006, 441: 885–889.
87. Lenk SE, Dunn WA, Jr, Trausch JS, Ciechanover A and Schwartz AL. Ubiquitin-activating enzyme, E1, is associated with maturation of autophagic vacuoles. J Cell Biol 1992, 118: 301–308.
88. Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB and Schwartz SL, *et al*. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 2007, 447: 859–863.
89. Cecarini V, Bonfili L, Cuccioloni M, Mozzicafreddo M, Rossi G, Buizza L and Uberti D, *et al*. Crosstalk between the ubiquitin-proteasome system and autophagy in a human cellular model of Alzheimer's disease. Biochim Biophys Acta 2012, 1822: 1741–1751.
90. Boyault C, Gilquin B, Zhang Y, Rybin V, Garman E, Meyer-Klaucke W and Matthias P, *et al*. HDAC6-p97/VCP controlled polyubiquitin chain turnover. EMBO J 2006, 25: 3357–3366.
91. Zhang L, Sheng S and Qin C. The role of HDAC6 in Alzheimer's disease. J Alzheimers Dis 2013, 33: 283–295.
92. Kirkin V, Lamark T, Sou YS, Bjorkoy G, Nunn JL, Bruun JA and Shvets E, *et al*. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell 2009, 33: 505–516.
93. Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T and Mizushima N, *et al*. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 2007, 131: 1149–1163.
94. Kuusisto E, Suuronen T and Salminen A. Ubiquitin-binding protein p62 expression is induced during apoptosis and proteasomal inhibition in neuronal cells. Biochem Biophys Res Commun 2001, 280: 223–228.
95. Nagaoka U, Kim K, Jana NR, Doi H, Maruyama M, Mitsui K and Oyama F, *et al*. Increased expression of p62 in expanded polyglutamine-expressing cells and its association with polyglutamine inclusions. J Neurochem 2004, 91: 57–68.
96. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H and Øvervatn A, *et al*. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 2007, 282: 24131–24145.
97. Kaniuk NA, Kiraly M, Bates H, Vranic M, Volchuk A and Brumell JH. Ubiquitinated-protein aggregates form in pancreatic beta-cells during diabetes-induced oxidative stress and are regulated by autophagy. Diabetes 2007, 56: 930–939.
98. Lamark T and Johansen T. Autophagy: links with the proteasome. Curr Opin Cell Biol 2010, 22: 192–198.
99. Lee JY, Nagano Y, Taylor JP, Lim KL and Yao TP. Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. J Cell Biol 2010, 189: 671–679.
100. Wong E and Cuervo AM. Autophagy gone awry in neurodegenerative diseases. Nat Neurosci 2010, 13: 805–811.