



Published in final edited form as:

*Neurobiol Dis.* 2011 July ; 43(1): 4–8. doi:10.1016/j.nbd.2010.11.018.

## Proteasomal dysfunction in aging and Huntington disease

Xiao-Jiang Li and Shihua Li

Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322

### Abstract

Protein degradation plays a central role in many cellular functions. Misfolded and damaged proteins are removed from the cells to avoid toxicity. Eukaryotic cells have two main routes for clearing misfolded or toxic proteins: the ubiquitin-proteasome and autophagy-lysosome pathways. The ubiquitin-proteasome system (UPS) is ubiquitously present in the cytoplasm, nucleus, and various subcellular regions whereas autophagy predominantly functions in the cytoplasm. The activity of the UPS often remains at a high level, whereas basal autophagy constitutively occurs at low levels in cells for the performance of homeostatic functions. Because of the presence of the UPS in the nucleus, the UPS function may be more important for clearing misfolded proteins in the nucleus. Polyglutamine diseases, including Huntington disease (HD), show the age-dependent neurological symptoms and the accumulation of misfolded proteins that are often found in the nucleus. The selective neuropathology in HD is also found to associate with the preferential accumulation of the disease protein huntingtin in neuronal cells. Although it is clear that the UPS is important for clearing mutant huntingtin, it remains unclear whether aging or HD affects the capacity of neuronal UPS to remove toxic and misfolded proteins. In this review, we focus on the relationship between the UPS function and aging as well as Huntington disease. We also discuss findings that suggest that aging is a more important factor that can negatively impact the function of the UPS.

### Keywords

Huntingtin; polyglutamine; ubiquitin; proteasome; autophagy; aging

### Proteasomal function and protein degradation

The ubiquitin-proteasome system (UPS) is critical for cell survival and a variety of cellular functions. Altered UPS function can lead to a wide range of disturbances, such as abnormal animal development and cell degeneration (Ciechanover, 2005; Rubinsztein, 2007; Levine and Kroemer, 2008; Mizushima et al., 2008). The major function of the UPS is to remove degraded or toxic proteins in eukaryotic cells. Compared to the autophagy-lysosome pathway that mainly degrades long-lived proteins and degenerated organelles, the UPS clears most soluble proteins in the cytoplasm and nucleus and plays a key role in degrading short-lived and misfolded proteins.

Two sequential reactions are involved in protein clearance by the UPS: one is a tagging reaction and the other is a subsequent degradation of the tagged proteins in the proteasome

\*Corresponding author: Xiao-Jiang Li, Department of Human Genetics, Emory University School of Medicine, 615 Michael St, Atlanta, GA 30322. Phone 404-727-3290; Fax 404-727-3949; xli2@emory.edu.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(Ciechanover, 2005). The tagging reaction requires ubiquitin, a small (76 residues), heat-stable, and highly conserved protein that is ubiquitously distributed in eukaryotic cells. This tagging reaction leads to the conjugation of proteins with a polyubiquitin chain for their degradation by the proteasome. Three steps are involved in the tagging reaction. First, a ubiquitin monomer is activated by forming an intermolecular thiol ester with the ubiquitin-activating enzyme (E1) in an ATP-requiring reaction. Next, activated ubiquitin is transferred to a Cys residue in the active site of a ubiquitin-conjugating enzyme (E2). Finally, ubiquitin is linked by its C-terminus through an amide isopeptide linkage to the  $\epsilon$ -amino group of a Lys residue in the substrate protein, a step that is catalyzed by a ubiquitin-protein ligase (E3). The E3 ligase confers specificity to the process by selectively binding to a protein target. Activated ubiquitin molecules are then sequentially added to the first ubiquitin proteins, forming a polyubiquitin chain. Proteins tagged with chains of four or more ubiquitins are then recognized by the 26S proteasome for degradation (Chau et al., 1989; Deveraux et al., 1994). After proteasome degradation, ubiquitin monomers are released or actively removed by the ubiquitin carboxyl-terminal hydrolases (Mayer and Wilkinson, 1989).

The 26S proteasome is a multicatalytic protease localized both in the nucleus and the cytoplasm. It is composed of three major subunits: one 20S catalytic core and two 19S regulatory caps. In the inner part of the 20S complex, there are three types of catalytic subunits that execute the corresponding catalytic activities of the proteasome (trypsin-like, chymotrypsin-like, and peptidylglutamyl-peptide hydrolyzing (PGPH) activity) (Layfield et al., 2003). In addition to recognizing the substrates for the 20S proteasome, the 19S regulatory caps facilitate access of the target proteins to the 20S proteasome by unfolding the substrates and opening the catalytic channel (Hershko and Ciechanover, 1998). Upon recognition of the polyubiquitylated substrates, 19S complexes release polyubiquitin chains. Deubiquitylating enzymes (DUBs) then disassemble polyubiquitin chains into ubiquitin monomers that can be reused (Kawakami et al., 1999).

Protein degradation by the UPS is highly regulated and energy dependent (Schrader et al., 2009). This degradation function requires the assembly of the UPS, which is a complicated process because intermediates and subunits for assembly of a eukaryotic proteasome are unstable and heterogeneous. For example, the 19S regulatory particle (19S RP) is the key regulatory component of the 26S proteasome and consist at least of 17 core subunits. Of these core subunits, six subunits are ATPases (Sorokin et al., 2010). To bind the 19S RP to the 20S complex and open the gate, ATP hydrolysis is needed (Ma et al., 1992). ATP is required not only for the assembly of the 26S proteasome (Eytan et al., 1989; Liu et al., 2006) but also for substrate degradation (Hershko et al., 1984). Hydrolysis of ATP is necessary for conformational rearrangements of the proteasomal subunits occurring upon substrate unfolding and probably for its translocation into the 20S proteolytic chamber. The unfolding of the substrate is especially necessary since the dimension of the gate of the 20S is too small to allow a protein with a developed tertiary structure to pass through it. The translocation of the unfolded part of the substrate via the gate into the 20S proteolytic chamber is ATP-dependent (Benaroudj et al., 2003). Upon degradation of the substrate, the ubiquitin attached to the substrate is released, which is also ATP dependent (Yao and Cohen, 2002). In addition, the relative content of the proteasome and its localization in the cell change dynamically and adjust in accordance with the cell requirements and particular stress conditions. The proteasome is constantly assembled and disassembled, and its subunits are targets for a great number of posttranslational modifications including phosphorylation and acetylation (Konstantinova et al., 2008). Because of the highly energy-dependent and regulated nature, the UPS function is vulnerable to various insults and oxidative stress, which can be generated during aging or caused by toxic or misfolded

proteins in diseases. Here, we focus on the influences of aging and Huntington disease, a neurodegenerative disorder caused by protein misfolding, on the UPS.

## Proteasomal function and aging

Aging is a multifactorial process that involves changes at the cellular, tissue, organ, and the whole body levels resulting in decreased functioning, development of diseases, and ultimately death. Oxidative stress is believed to be a very important factor in causing aging and age-related diseases. Oxidative stress is caused by an imbalance between oxidants such as reactive oxygen species (ROS) and antioxidants. ROS are produced from the mitochondrial electron transport chain and many oxidative reactions. In the aging process, mitochondrial function gradually declines with an increase of mutations in mitochondrial DNA (mtDNA) in tissue cells. Some of the aging-associated mtDNA mutations have been shown to result in not only inefficient generation of ATP but also increased production of reactive oxygen species (ROS) such as superoxide anions ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) in the mitochondria of aging tissues. A substantial body of evidence has supported a role for oxidative damage to the mitochondrial respiratory chain and mitochondrial DNA in the determination of mammalian lifespan. In aging mice, the activities of brain mitochondrial enzymes (complexes I and IV and mtNOS) are correlated with neurological performance as well as lifespan and negatively correlated with the mitochondrial content of lipid and protein oxidation products (Boveris and Navarro, 2008).

Although it is clear that oxidative damage increases during aging, the fundamental question regarding whether mitochondrial oxidative stress is causal to the aging process remains unresolved. Despite this, it is generally accepted that aging and eventual death of multicellular organisms is closely related to macromolecular damage by reactive oxygen species generated by mitochondria. Aging appears to particularly affect postmitotic cells, such as neurons, as these cells are rarely or not at all replaced during life. Thus, postmitotic cells may be more vulnerable to aging related increase in oxidative stress and decrease in ATP production (Terman and Kurz, 2010).

The proteasome is responsible for the removal of oxidatively damaged proteins in the cytosol and nucleus. It has been demonstrated that proteasomal degradation increases due to mild oxidation, whereas at higher oxidant levels proteasomal degradation decreases. Moreover, the proteasome itself is affected by oxidative stress to varying degrees. The ATP-stimulated 26S proteasome is sensitive to oxidative stress. Non-degradable protein aggregates and cross-linked proteins are able to bind to the proteasome, which makes the degradation of other misfolded and damaged proteins less efficient. Consequently, inhibition of the proteasome has dramatic effects on cellular aging processes and cell viability (Breusing and Grune 2008; Farout and Friguet, 2006; Ding et al., 2006).

Many reports have shown different degrees of decrease in the activity of the UPS with age in several tissues (Carrard et al., 2002; Ferrington et al., 2005; Keller et al., 2004; Ward, 2002). Of the two major steps for UPS dependent degradation, ubiquitylation and degradation, the former does not seem to be particularly affected by age (Carrard et al., 2002). It is now accepted that the proteolytic ability of the proteasome is modulated in vivo by multiple factors, and that age-dependent modifications in these factors are probably responsible for altered proteasome activity (Carrard et al., 2002; Ferrington et al., 2005). Changes in the oxidation state of the proteasome subunits (oxidation, glycation and conjugation with peroxidized lipid products) increase with age and are likely to result in changes in UPS regulation (Carrard et al., 2002). In addition, oxidized proteins and crosslinked-proteins and lipids can directly inhibit the proteasome (Terman and Brunk, 2004).

Other factors that affect the UPS function with age could be of changes in the expression of proteasomal subunits, the modulatory molecules, and the proteasome substrates. Possible changes with age can also be the reduced ability of the substrates to be ubiquitylated and of the proteasome to recognize the tagged proteins or to remove the polyubiquitin chains prior to degradation.

## Proteasomal function in HD

Huntington's disease (HD) is an autosomal dominant genetic neurological disorder characterized by motor dysfunction, cognitive decline, and psychological dysfunction. HD displays selective neurodegeneration that occurs preferentially in the brain striatum (Gusella et al., 1993; Vonsattel and DiFiglia 1998). The majority of patients with HD show symptoms in midlife and often die 10–15 years after the onset of symptoms. The genetic cause of HD is the expansion of a CAG repeat (>36 CAGs) in exon1 of the HD gene huntingtin, a large-sized protein (348 kDa) that is ubiquitously expressed in various types of cells and that interacts with a number of proteins (Li and Li, 2004). The CAG repeat expansion results in translation of a huntingtin protein with an expanded polyglutamine (polyQ) tract in its N-terminal region. At least nine neurodegenerative disorders, including several types of spinocerebellar ataxia (SCA) and spinobulbar muscle atrophy (SBMA), are caused by the expansion of a polyQ stretch in specific target proteins (Orr and Zoghbi 2007). This expansion of the polyglutamine tract leads to abnormal protein conformations, such as a  $\beta$ -sheet structure (Perutz et al., 1994). As a result, mutant htt with an expanded polyQ tract forms insoluble aggregates or inclusions in the HD brain in an age-dependent manner. Although there is considerable debate over whether aggregates have a toxic versus protective effect, the aggregates in the HD brains apparently result from the accumulation of misfolded proteins with age and are therefore an indicator of the impaired intracellular capacity to clear or remove misfolded proteins.

Identification of the genetic mutation of HD enables the generation of a variety of cellular and animal models for studying HD pathogenesis. Studies of various HD mouse models have provided clear evidence that the extent of protein misfolding and aggregation is correlated with the length of polyQ tracts. For example, smaller N-terminal htt fragments containing an expanded polyQ tract are more prone to misfolding and aggregation. They are also more toxic than full-length mutant htt, which is evident by the fact that cellular models expressing smaller htt fragments show more aggregates than those expressing full-length mutant htt (Hackam et al., 1998; Gutekunst et al., 1999). Comparing different HD animal models demonstrates that mice with more severe neurological phenotypes often have an earlier accumulation of N-terminal mutant htt fragments in their brains, which leads to the formation of prominent aggregates in neuronal nuclei and processes (Wang et al. 2008a). More importantly, HD mouse models expressing small N-terminal mutant htt fragments often show more severe neurological symptoms than those mice transgenic for full-length mutant htt (Heng et al., 2008).

Although the normal function of htt remains to be fully understood, htt is essential for early development, as lack of htt leads to embryonic death of the HD gene knockout mice. Biochemical studies have shown that htt interacts with a number of proteins that are involved in gene transcription, intracellular trafficking, and mitochondrial function. Consistently, a number of studies have shown that mutant htt elicits cytotoxicity via multiple pathogenic pathways, which can be classified as nuclear toxicity that affects gene transcription and cytoplasmic toxicity that increases oxidative stress, affects mitochondrial function, and reduces intracellular trafficking (Borrell-Pagès et al., 2006; Li and Li, 2006). Thus, a key step to prevent htt toxicity is to reduce the expression and accumulation of misfolded and toxic form of mutant htt in cells.

In various cellular and animal models of HD, as well as in the post-mortem brains of HD patients (DiFiglia et al., 1997; Gutekunst et al 1999), nuclear polyQ inclusions are positively labeled by antibodies against ubiquitin. This colocalization led to subsequent studies of whether there is the sequestration of UPS components by polyQ inclusions. There is an indirect assay of UPS function based on the expression of degron-reporter proteins in which a UPS degradation signal is fused to a green fluorescent protein (Bence et al., 2001; Lindsten et al., 2003). The modified protein (such as GFPu) has an extremely short half-life and will accumulate only if the UPS is not working efficiently, providing a sensitive tool to measure the UPS in living cells. Using these assays and specific inhibitors of the proteasome, more polyQ aggregates have been found in cellular models upon pharmacological inhibition of proteasome activity (Bence et al., 2001; Waelter et al., 2001; Jana et al., 2001).

Using peptides containing 10–30 residues of glutamine, Venkatraman and colleagues found that the proteasome could not digest expanded polyglutamine sequence efficiently, but only cut the flanking basic residues (Venkatraman et al., 2004). Their findings raise the important possibility that expanded polyQ tracts or aggregation-prone peptides cannot be digested by the UPS, resulting in an impairment of the function of the UPS. However, by targeting polyQ proteins with N-terminal degradation signal for their digestion by the proteasome in transfected cells, Michalik and Van Broeckhoven found that soluble expanded polyQ proteins do not compromise proteasomal activity. A pulse-chase experiment demonstrated that soluble expanded polyQ proteins are completely and efficiently degraded by the proteasome (Michalik and Van Broeckhoven, 2004). Inhibiting the UPS in transfected PC12 cells that co-express N-terminal htt with a normal (23Q) and expanded (76Q) also showed the same degree in increased accumulation of these htt proteins (Li et al., 2010). Other findings suggest that polyQ protein-mediated impairment of UPS function occurs in the absence of detectable aggregates and of interference of protein aggregates on 26S proteasome function in vitro (Bennett et al., 2005).

Although the above in vitro experiments give us important information about the effect of mutant htt on the UPS, it is crucial to understand the impact of mutant htt on the UPS in vivo. Thus, it is critical to measure proteasomal activity directly in the brain tissues of HD animal models. Several studies that examined UPS activity in the whole cell homogenates of brain tissues did not detect reduced UPS activity in HD mouse brains (Díaz-Hernández et al., 2003; Bett et al., 2006; Wang et al., 2008b). However, quantifying polyubiquitin chains, an endogenous biomarker of UPS function, reveals that Lys 48-linked polyubiquitin chains accumulate early in HD mouse brains (Bennett et al., 2007). On the other hand, it has been found there was no correlation between UPS impairment and polyQ-mediated retinal neuropathology in a mouse model that expresses a fluorescent reporter to reflect UPS function and a mutant polyQ protein (SCA7 protein) (Bowman et al., 2005). Considering that the use of whole cell lysates cannot address whether mutant polyQ proteins affect the UPS in a specific compartment in neurons, Wang et al. generated fluorescent reporters and targeted these reporters to the synapses in the mouse brain to measure synaptic UPS activity and found a decrease in UPS activity in the synapses of HD mice (Wang et al., 2008b). As mutant htt was found to reduce mitochondrial trafficking in neuronal processes and to decrease the amount of mitochondria in the synaptosomal fraction, a low level of ATP in the HD synapses could contribute to the reduced activity of synaptic UPS seen in HD mouse brains (Orr et al., 2008; Wang et al., 2008b). Thus, although mutant htt does not seem to reduce global UPS function in the brain, subcellular localization should be taken into account when investigating potential changes in UPS activity in specific cellular compartments.

Since autophagy is another important system to clear misfolded proteins and degenerated organelles, it is important to know whether mutant htt can affect the function of autophagy. At least three forms of protein degradation via the lysosome have been identified: macroautophagy, microautophagy, and chaperone-mediated autophagy. These forms differ in their physiological functions and in the cargos they deliver to the lysosome. Macroautophagy (often referred to simply as autophagy) is a main pathway for eukaryotic cells to degrade long-lived proteins and organelles. Protein and organelle clearance via autophagy involves the microtubule-associated protein 1 light chain 3, which has two forms, LC3-I and LC3-II. During autophagy activation, LC3-I is processed to produce phosphatidylethanolamine-modified LC3-II that specially associates with the autophagosome membrane. Thus, the conversion of LC3-I to LC3-II is widely used to evaluate the function of autophagy. Western blotting analysis of the brain tissues from N171-82Q mice, which express the first 171 amino acids of htt containing 82Q, and *Hdh* (CAG)<sub>150</sub> knockin mice, which express full-length mutant htt at the endogenous level, did not reveal that mutant htt could significantly affect the conversion of LC3-I to LC3-II. This finding suggests that mutant htt does not impair autophagy function when it is not overexpressed (Li et al., 2010). Furthermore, stereotaxic injection of the UPS or autophagic inhibitors into the striatum of *Hdh*(CAG)<sub>140</sub> knockin mice demonstrated that inhibiting the UPS increased the accumulation of soluble mutant htt and its aggregation to a much greater extent than inhibiting autophagy (Li et al., 2010). These findings suggest that the UPS may be more efficient than autophagy to clear soluble mutant htt in the brain. Because the global activities of the UPS and autophagy in the brain do not seem to be reduced by mutant htt, it is possible that aging-related decrease in the function of UPS and autophagy contributes to the late-onset accumulation of misfolded htt and the related neurological symptoms.

## Conclusion

Misfolded proteins are primarily cleared in cells by two systems: the ubiquitin-proteasome system (UPS) and autophagy. The UPS predominantly degrades short-lived proteins by tagging these substrates with polyubiquitin chains. Autophagy is a cellular degradative pathway for long-lived cytoplasmic proteins, protein complexes, or damaged organelles. There are several facts that are needed to be considered when discussing the roles of these pathways in HD pathogenesis. First, the activity of the UPS often remains at a high level, whereas basal autophagy constitutively occurs at low levels in cells (Levine and Kroemer, 2008). Second, the UPS is ubiquitously presented in various cellular compartments, including the nucleus (Bader et al., 2007), whereas autophagy is restricted to the cytoplasm. Thus, the nuclear UPS may play a more important role in clearing nuclear mutant htt. Since soluble mutant htt may be more toxic than aggregated htt, it is also important to know whether the UPS and autophagy have different capacities to remove soluble mutant htt in the cytoplasm. The findings of our recent studies suggest that the clearance of soluble N-terminal mutant htt is more dependent on the function of the UPS, perhaps because misfolded N-terminal mutant htt fragments are better suited for degradation by the UPS (Li et al., 2010). Autophagy could function as a backup system to clear mutant htt or is more efficient to remove aggregated forms of htt.

In this review, we focus on the effects of aging and HD on the UPS and discussed the complexity of the structure and the highly regulated function of the UPS, which may be targeted by aging or disease-related cellular events. It has been well documented that the UPS function declines with age. Aging related decrease in mitochondrial function and increase in oxidative stress can affect the function of the UPS. Thus, although there is no evidence to show that mutant htt can impair the global UPS function in the brain, it is conceivable that aging related factors can decrease the UPS function. Such reduced function could cause the inefficient clearance of misfolded htt and ultimately leads to the

accumulation of mutant htt and the formation of aggregates. Thus, reducing aging process or improving the cellular capacity to remove misfolded proteins in neuronal cells would ameliorate the neuropathology in HD and other similar neurological disorders. As soluble mutant htt can interact with various proteins and is likely to be more toxic than aggregated htt, preventing the accumulation of soluble mutant htt would be more effective to reduce HD neuropathology. Also, given that mutant htt selectively accumulates in different subcellular regions in affected neurons, the various capacities of different cellular compartments to cope with misfolded htt should also be considered. Understanding the mechanisms underlying the different activities of the UPS during aging and in different subcellular regions could help identify effective therapeutic targets.

### Research Highlights

Huntington disease and other polyglutamine diseases show the age-dependent neurological symptoms and the accumulation of misfolded proteins, which can be cleared by the ubiquitin-proteasome system. This review focuses on the relationship between the ubiquitin-proteasome function and aging as well as Huntington disease. The review suggests that aging is an important factor to negatively impact the function of the ubiquitin-proteasome system, leading to the age-dependent accumulation of misfolded proteins.

### Acknowledgments

We would like to acknowledge the support of several NIH grants (AG109206, NS036232, NS041669 to X.J.L.) and (NS045016 and AG031153 to S.H. L.). We thank Cheryl T. Strauss for her critical reading of the manuscript.

### References

- Bader N, Jung T, Grune T. The proteasome and its role in nuclear protein maintenance. *Exp Gerontol.* 2007; 42 (9):864–870. [PubMed: 17532163]
- Benaroudj N, Zwickl P, Seemuller E, Baumeister W, Goldberg AL. ATP hydrolysis by the proteasome regulatory complex PAN serves multiple functions in protein degradation. *Mol Cell.* 2003; 11 (1): 69–78. [PubMed: 12535522]
- Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science.* 2001; 292 (5521):1552–1555. [PubMed: 11375494]
- Bennett EJ, Bence NF, Jayakumar R, Kopito RR. Global impairment of the ubiquitin-proteasome system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. *Mol Cell.* 2005; 17 (3):351–365. [PubMed: 15694337]
- Bennett EJ, Shaler TA, Woodman B, Ryu KY, Zaitseva TS, Becker CH, et al. Global changes to the ubiquitin system in Huntington's disease. *Nature.* 2007; 448 (7154):704–708. [PubMed: 17687326]
- Bett JS, Goellner GM, Woodman B, Pratt G, Rechsteiner M, Bates GP. Proteasome impairment does not contribute to pathogenesis in R6/2 Huntington's disease mice: exclusion of proteasome activator REGgamma as a therapeutic target. *Hum Mol Genet.* 2006; 15 (1):33–44. [PubMed: 16311253]
- Borrell-Pages M, Zala D, Humbert S, Saudou F. Huntington's disease: from huntingtin function and dysfunction to therapeutic strategies. *Cell Mol Life Sci.* 2006; 63 (22):2642–2660. [PubMed: 17041811]
- Boveris A, Navarro A. Brain mitochondrial dysfunction in aging. *IUBMB Life.* 2008; 60 (5):308–314. [PubMed: 18421773]
- Bowman AB, Yoo SY, Dantuma NP, Zoghbi HY. Neuronal dysfunction in a polyglutamine disease model occurs in the absence of ubiquitin-proteasome system impairment and inversely correlates with the degree of nuclear inclusion formation. *Hum Mol Genet.* 2005; 14 (5):679–691. [PubMed: 15661755]

- Breusing N, Grune T. Regulation of proteasome-mediated protein degradation during oxidative stress and aging. *Biol Chem.* 2008; 389 (3):203–209. [PubMed: 18208355]
- Carrard G, Bulteau AL, Petropoulos I, Friguet B. Impairment of proteasome structure and function in aging. *Int J Biochem Cell Biol.* 2002; 34 (11):1461–1474. [PubMed: 12200039]
- Chau V, Tobias JW, Bachmair A, Marriott D, Ecker DJ, Gonda DK, et al. A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science.* 1989; 243 (4898):1576–1583. [PubMed: 2538923]
- Ciechanover A. Proteolysis: from the lysosome to ubiquitin and the proteasome. *Nat Rev Mol Cell Biol.* 2005; 6 (1):79–87. [PubMed: 15688069]
- Deveraux Q, Ustrell V, Pickart C, Rechsteiner M. A 26 S protease subunit that binds ubiquitin conjugates. *J Biol Chem.* 1994; 269 (10):7059–7061. [PubMed: 8125911]
- Diaz-Hernandez M, Hernandez F, Martin-Aparicio E, Gomez-Ramos P, Moran MA, Castano JG, et al. Neuronal induction of the immunoproteasome in Huntington's disease. *J Neurosci.* 2003; 23 (37): 11653–11661. [PubMed: 14684867]
- DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science.* 1997; 277 (5334): 1990–1993. [PubMed: 9302293]
- Ding Q, Dimayuga E, Keller JN. Proteasome regulation of oxidative stress in aging and age-related diseases of the CNS. *Antioxid Redox Signal.* 2006; 8 (1–2):163–172. [PubMed: 16487050]
- Eytan E, Ganoth D, Armon T, Hershko A. ATP-dependent incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin. *Proc Natl Acad Sci U S A.* 1989; 86 (20): 7751–7755. [PubMed: 2554287]
- Farout L, Friguet B. Proteasome function in aging and oxidative stress: implications in protein maintenance failure. *Antioxid Redox Signal.* 2006; 8 (1–2):205–216. [PubMed: 16487054]
- Ferrington DA, Husom AD, Thompson LV. Altered proteasome structure, function, and oxidation in aged muscle. *Faseb J.* 2005; 19 (6):644–646. [PubMed: 15677694]
- Gusella JF, MacDonald ME, Ambrose CM, Duyao MP. Molecular genetics of Huntington's disease. *Arch Neurol.* 1993; 50 (11):1157–1163. [PubMed: 8215974]
- Gutkunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, et al. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J Neurosci.* 1999; 19 (7):2522–2534. [PubMed: 10087066]
- Hackam AS, Singaraja R, Wellington CL, Metzler M, McCutcheon K, Zhang T, et al. The influence of huntingtin protein size on nuclear localization and cellular toxicity. *J Cell Biol.* 1998; 141 (5): 1097–1105. [PubMed: 9606203]
- Heng MY, Detloff PJ, Albin RL. Rodent genetic models of Huntington disease. *Neurobiol Dis.* 2008; 32 (1):1–9. [PubMed: 18638556]
- Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem.* 1998; 67:425–479. [PubMed: 9759494]
- Hershko A, Leshinsky E, Ganoth D, Heller H. ATP-dependent degradation of ubiquitin-protein conjugates. *Proc Natl Acad Sci U S A.* 1984; 81 (6):1619–1623. [PubMed: 6324208]
- Jana NR, Zemskov EA, Wang G, Nukina N. Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum Mol Genet.* 2001; 10 (10):1049–1059. [PubMed: 11331615]
- Kawakami T, Suzuki T, Baek SH, Chung CH, Kawasaki H, Hirano H, et al. Isolation and characterization of cytosolic and membrane-bound deubiquitinating enzymes from bovine brain. *J Biochem.* 1999; 126 (3):612–623. [PubMed: 10467179]
- Konstantinova IM, Tsimokha AS, Mittenberg AG. Role of proteasomes in cellular regulation. *Int Rev Cell Mol Biol.* 2008; 267:59–124. [PubMed: 18544497]
- Layfield R, Cavey JR, Lowe J. Role of ubiquitin-mediated proteolysis in the pathogenesis of neurodegenerative disorders. *Ageing Res Rev.* 2003; 2 (4):343–356. [PubMed: 14522239]
- Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell.* 2008; 132 (1):27–42. [PubMed: 18191218]

- Li S, Li XJ. Multiple pathways contribute to the pathogenesis of Huntington disease. *Mol Neurodegener.* 2006; 1:19. [PubMed: 17173700]
- Li SH, Li XJ. Huntingtin-protein interactions and the pathogenesis of Huntington's disease. *Trends Genet.* 2004; 20 (3):146–154. [PubMed: 15036808]
- Li X, Wang CE, Huang S, Xu X, Li XJ, Li H, et al. Inhibiting the ubiquitin-proteasome system leads to preferential accumulation of toxic N-terminal mutant huntingtin fragments. *Hum Mol Genet.*
- Liu CW, Li X, Thompson D, Wooding K, Chang TL, Tang Z, et al. ATP binding and ATP hydrolysis play distinct roles in the function of 26S proteasome. *Mol Cell.* 2006; 24 (1):39–50. [PubMed: 17018291]
- Ma CP, Slaughter CA, DeMartino GN. Identification, purification, and characterization of a protein activator (PA28) of the 20 S proteasome (macropain). *J Biol Chem.* 1992; 267 (15):10515–10523. [PubMed: 1587832]
- Mayer AN, Wilkinson KD. Detection, resolution, and nomenclature of multiple ubiquitin carboxyl-terminal esterases from bovine calf thymus. *Biochemistry.* 1989; 28 (1):166–172. [PubMed: 2539853]
- Michalik A, Van Broeckhoven C. Proteasome degrades soluble expanded polyglutamine completely and efficiently. *Neurobiol Dis.* 2004; 16 (1):202–211. [PubMed: 15207277]
- Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature.* 2008; 451 (7182):1069–1075. [PubMed: 18305538]
- Orr AL, Li S, Wang CE, Li H, Wang J, Rong J, et al. N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. *J Neurosci.* 2008; 28 (11):2783–2792. [PubMed: 18337408]
- Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annu Rev Neurosci.* 2007; 30:575–621. [PubMed: 17417937]
- Perutz MF, Johnson T, Suzuki M, Finch JT. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc Natl Acad Sci U S A.* 1994; 91 (12):5355–5358. [PubMed: 8202492]
- Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ. Potential therapeutic applications of autophagy. *Nat Rev Drug Discov.* 2007; 6 (4):304–312. [PubMed: 17396135]
- Schrader EK, Harstad KG, Matouschek A. Targeting proteins for degradation. *Nat Chem Biol.* 2009; 5 (11):815–822. [PubMed: 19841631]
- Sorokin AV, Kim ER, Ovchinnikov LP. Proteasome system of protein degradation and processing. *Biochemistry (Mosc).* 2009; 74 (13):1411–1442. [PubMed: 20210701]
- Terman A, Brunk UT. Myocyte aging and mitochondrial turnover. *Exp Gerontol.* 2004; 39 (5):701–705. [PubMed: 15130664]
- Terman A, Kurz T, Navratil M, Arriaga EA, Brunk UT. Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging. *Antioxid Redox Signal.* 2010; 12 (4):503–535. [PubMed: 19650712]
- Venkatraman P, Wetzel R, Tanaka M, Nukina N, Goldberg AL. Eukaryotic proteasomes cannot digest polyglutamine sequences and release them during degradation of polyglutamine-containing proteins. *Mol Cell.* 2004; 14 (1):95–104. [PubMed: 15068806]
- Vonsattel JP, DiFiglia M. Huntington disease. *J Neuropathol Exp Neurol.* 1998; 57 (5):369–384. [PubMed: 9596408]
- Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, et al. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell.* 2001; 12 (5):1393–1407. [PubMed: 11359930]
- Wang CE, Tydlacka S, Orr AL, Yang SH, Graham RK, Hayden MR, et al. Accumulation of N-terminal mutant huntingtin in mouse and monkey models implicated as a pathogenic mechanism in Huntington's disease. *Hum Mol Genet.* 2008a
- Wang J, Wang CE, Orr A, Tydlacka S, Li SH, Li XJ. Impaired ubiquitin-proteasome system activity in the synapses of Huntington's disease mice. *J Cell Biol.* 2008b; 180 (6):1177–1189. [PubMed: 18362179]

Yao T, Cohen RE. A cryptic protease couples deubiquitination and degradation by the proteasome. *Nature*. 2002; 419 (6905):403–407. [PubMed: 12353037]