Am J Neurodegener Dis 2013;2(1):1-14
www.AJND.us /ISSN:2165-591X/AJND1211003

Review Article

Protein aggregation and degradation mechanisms in neurodegenerative diseases

Mari Takalo, Antero Salminen, Hilkka Soininen, Mikko Hiltunen, Annakaisa Haapasalo

Institute of Clinical Medicine – Neurology, University of Eastern Finland and Department of Neurology, Kuopio University Hospital, Kuopio, Finland

Received November 29, 2012; Accepted January 18, 2013; Epub March 8, 2013; Published March 18, 2013

Abstract: Neurodegenerative diseases are characterized by selective neuronal vulnerability and neurodegeneration in specific brain regions. The pathogenesis of these disorders centrally involves abnormal accumulation and aggregation of specific proteins, which are deposited in intracellular inclusions or extracellular aggregates that are characteristic for each disease. Increasing evidence suggests that genetic mutations or environmental factors can instigate protein misfolding and aggregation in these diseases. Consequently, neurodegenerative diseases are often considered as conformational diseases. This idea is further supported by studies implicating that impairment of the protein quality control (PQC) and clearance systems, such as the ubiquitin-proteasome system and autophagosome-lysosome pathway, may lead to the abnormal accumulation of disease-specific proteins. This suggests that similar pathological mechanisms may underlie the pathogenesis of the different neurodegenerative disorders. Interestingly, several proteins that are known to associate with neurodegenerative diseases have been identified as important regulators of PQC and clearance systems. In this review, we summarize the central features of abnormal protein accumulation in different common neurodegenerative diseases and discuss some aspects of specific disease-associated proteins regulating the PQC and clearance mechanisms, such as ubiquilin-1.

Keywords: Protein quality control, ubiquitin-proteasome system, autophagy, protein misfolding, neurodegenerative diseases, inclusion body, aggresome, IPOD, JUNQ, ubiquilin-1

Introduction

The pathogenesis of different neurodegenerative diseases, such as Alzheimer's (AD), Parkinson's (PD), and Huntington's disease (HD), shares several common features. One of these is the abnormal accumulation and aggregation of disease-specific proteins, which is suggested to lead to neurodegeneration [1, 2]. The accumulated proteins typically form intracellular inclusions or extracellular aggregates in specific brain areas. These are considered specific pathological hallmarks for the diseases. The proteins that accumulate in neurodegenerative diseases are typically misfolded and yield a β-sheet structure that promotes aggregation and fibril formation, suggesting that these diseases are conformational diseases [1, 2]. Genetic mutations or different environmental factors, such as oxidative or metabolic stress, can induce protein misfolding and aggregation, but the exact underlying mechanisms of protein aggregation in different neurodegenerative disorders are still not completely understood.

It is estimated that approximately 30% of newly synthesized proteins are incorrectly folded and degraded [3]. Under normal conditions, the cells are able to efficiently utilize their protein quality control (PQC) system to handle the misfolded proteins and maintain the protein homeostasis. The molecular chaperones involved in the cellular PQC systems, such as heat shock proteins (Hsp), recognize misfolded proteins, assist in their refolding, prevent their aggregation, and help to repair the damaged proteins [4]. The molecular chaperones may also interact with the ubiquitination machinery and target the misfolded proteins to degradation by the ubiquitin-proteasome system (UPS) [5, 6] or the autophagosome-lysosome pathway

(ALP) [7-9]. Accumulating evidence suggests that deficiencies in PQC and clearance mechanisms may lead to the abnormal accumulation of proteins in neurodegenerative diseases. Moreover, the excessive accumulation of misfolded and aggregated proteins may overwhelm the PQC and clearance systems, which leads to further protein accumulation, cellular stress, and finally to neurodegeneration [7, 10, 11]. Interestingly, several proteins that are known to associate with the pathogenesis of specific neurodegenerative diseases have also been identified as central regulators of PQC and protein clearance systems [12]. In this review, we will discuss common aspects of PQC systems and specific proteins, such as ubiquilin-1, regulating the protein levels, accumulation, and targeting in the context of common neurodegenerative diseases.

Protein accumulation and pathological inclusions in neurodegenerative diseases

Abnormal intracellular or extracellular protein accumulation in the affected brain regions is a typical pathological hallmark of neurodegenerative diseases and thought to lead to neurotoxicity, neurodegeneration, and finally clinical manifestation of the disease. The intracellular inclusions detected in the brain of patients with a neurodegenerative disease are often ubiquitin-positive and contain misfolded disease-specific proteins that have acquired an amyloidogenic conformation containing β-sheet structures [1, 2]. This conformational change results in the exposure of the hydrophobic regions of the protein that are normally buried within the protein structure when it is in its natively folded conformation. The exposed hydrophobic structures promote oligomerization and aggregation of the protein [1, 13-15].

In AD, the pathological hallmarks in specific cortical areas of the brain include extracellular amyloid plaques consisting of aggregated β-amyloid peptide, and intracellular neurofibrillary tangles (NFTs) containing aggregated, hyperphosphorylated tau protein [16, 17]. In a subset of patients with frontotemporal dementia (FTD), TAR DNA-binding protein 43 (TDP-43)- or tau-positive inclusions are detected [18]. In PD, the intracellular Lewy bodies containing aggregated α-synuclein [19], and in HD, the intranuclear inclusions of aggregated huntingtin protein containing polyglutamine (polyQ) expansion [20], are typical hallmarks. In amyotrophic lateral sclerosis (ALS), aggregates of superoxide dismutase (SOD) in motor neurons can be detected [21]. It has been suggested that genetic mutations, environmental factors, or different stress conditions induce protein misfolding and aggregation in these diseases [22], implicating that similar pathological mechanisms may underlie their pathogenesis. Recent evidence also indicates that the aggregated proteins may spread from one cell or brain area to another and function as seeds to instigate protein misfolding and aggregation in these previously unaffected cells or areas [23]. This may explain the gradual progression of the disease pathology in the brain over time in the case of many neurodegenerative disorders.

Underlying factors of protein misfolding and aggregation

Several genetic and environmental factors have been suggested to promote protein misfolding and aggregation in different neurodegenerative diseases. These include gene mutations, gene dose, and promoter polymorphisms, which may affect protein levels and conformation. Also, inefficient protein biogenesis, excess unassembled units of oligomeric protein complexes, and inefficient translocation of secretory or mitochondrial protein precursors may result in the accumulation of misfolded proteins [22, 24]. Different conditions, such as metabolic or environmental stress or aging, further increase the production of misfolded proteins and thus challenge the capacity of the PQC system [25]. It is also suggested that during aging, the cells lose their ability to efficiently deal with misfolded proteins as the capacity of the PQC system declines. Reduced activity of UPS and ALP are known to associate with neurodegenerative diseases and aging [26-31]. In neurodegenerative diseases, the deficiencies in the PQC system together with mutations in the disease-associated proteins and inflammation and oxidative stress, which are intimately involved in the pathogenesis of these diseases, further enhance the accumulation and aggregation of proteins and may lead to aberrant protein modifications. These mechanisms together are thought to underlie the excessive accumulation and aggregation of proteins, which cause neuronal dysfunction and neurotoxicity and ulti-

mately lead to widespread neurodegeneration [32].

Many neurodegenerative diseases have a strong genetic component that affects the disease onset and progression. The patients with Down syndrome are a good example of the effects of gene dose on disease pathogenesis. These individuals develop AD-like pathology and typically suffer from the symptoms of AD already early in their life. The trisomy in the chromosome 21 leads to the triplication of the *APP* gene, resulting in increased levels of the APP protein and subsequently augmented early deposition of β-amyloid [33, 34]. Also, PD may be caused by α-synuclein gene locus triplication, in addition to mutations in the α-synuclein gene [35, 36]. Indeed, many neurodegenerative diseases are associated with the inheritance of pathological gene mutations that lead to the disease onset. These forms of the disease are typically rare, but cause an earlier onset of the disease and often lead to a more aggressive disease progression as compared to the more common sporadic cases with a late-onset and slow progression. For example, the P301L mutation in the *MAPT* gene encoding tau protein or mutations in other genes, such as *C9ORF72* or *GRN*, are known to cause FTD [18]. Rare early-onset familial forms of ALS (fALS) are caused by mutations in the *SOD1, TARDBP* (encoding TDP-43)*,* or *FUS* genes, but the pathogenesis can differ between these different types of fALS [37]. Early-onset familial forms of AD are known to result from mutations in genes encoding APP or presenilins (*PSEN*) [38]. A subset of early-onset PD cases has mutations in the genes encoding parkin, DJ-1 or PINK1 [39-41]. On the other hand, apart from gene mutations, polymorphisms in the promoter areas of disease-associated genes may lead to increased transcription or alternative splicing of the gene, resulting in a general increase in protein levels or in the levels of a more aggregation-prone transcript variant [42].

In addition to the genetic factors, the pathogenesis of many neurodegenerative diseases is often associated with inflammation and increased oxidative stress [32]. These conditions may induce alterations in the covalent post-transcriptional protein modifications, which consequently may affect the protein function, interaction, and levels. These modifications include changes in protein oxidation, nitration, phosphorylation, ubiquitination, SUMOylation, and proteolytic cleavage [25]. The levels of oxidized and nitrated proteins are known to increase in the brains of AD patients, implying that inflammation and oxidative stress are central phenomena that associate with the disease process [43-45]. In AD, hyperphosphorylation of the tau protein is known to result in increased tau aggregation and destabilization of the microtubules. Tau aggregates are harmful and they further accumulate as NFTs within neurons, eventually causing neurodegeneration [46]. In addition, missorting of tau from the axons to the somatodendritic compartment in AD brain is an early sign of neurodegeneration and is at least partially caused by oligomeric β-amyloid peptides [47, 48]. Furthermore, increased proteolytic processing of APP by β- and γ-secretases leads to an augmented generation of β-amyloid, and consequently increased deposition of amyloid plaques [49]. The familial causative mutations in *APP* or *PSEN* genes also result in the enhanced cleavage of APP by β- and γ-secretase and subsequently increased β-amyloid production [38, 49, 50], confirming that enhanced amyloidogenic processing of APP is a central mechanism underlying the pathogenesis of AD. Interestingly, many disease-specific inclusions, such as NFTs or Lewy bodies, typically contain proteins that are ubiquitinated [2]. This suggests that the accumulated proteins with abnormal conformation have been detected and tagged by the PQC system, but due to inefficient or impaired clearance, they remain within the cells and are deposited in the inclusions.

Protein quality control systems

In the case of protein misfolding and aggregation in cells, the PQC system uses three main parallel strategies to maintain protein homeostasis (**Figure 1**). The misfolded protein may be refolded to recover the protein's normal conformation. Different molecular chaperones, such as Hsps, play an essential role in protein refolding. Alternatively, if the protein cannot be refolded, it is targeted to the UPS or ALP for degradation. In the case when the misfolded or aggregated proteins cannot be directed for refolding or degradation, they may be sequestered as specific protein inclusions within the

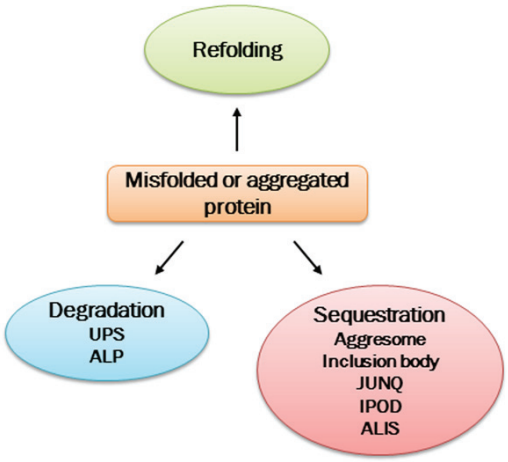

Figure 1. The main parallel strategies to maintain protein homeostasis. The misfolded proteins may be refolded, degraded, or sequestered within cells. All these mechanisms centrally involve the function of different molecular chaperones, such as heat shock proteins (Hsps). Abbreviations: ALIS, aggresome-like inducible structure; ALP, autophagosome-lysosome system; IPOD, insoluble protein deposit; JUNQ, juxtanuclear quality control; UPS, ubiquitin-proteasome system.

cells. Also these steps involve the function of molecular chaperone proteins [9].

The signal for targeting a protein for UPS- or ALP-mediated degradation is polyubiquitination. When the protein is polyubiquitinated, it is covalently tagged with four or more ubiquitin molecules in its lysine residues [51]. Ubiquitin itself has seven lysines (e.g. K48 and K63) and canonical binding of other ubiquitin molecules to these internal lysines then forms the polyubiquitin chain. Ubiquitination of proteins requires the coordinated function of different ubiquitin ligases E1, E2 and E3. Differently linked polyubiquitin chains have been shown to mediate differential targeting of the polyubiquitinated proteins. K48-linked polyubiquitin chains are the classical signal for proteasomal degradation. The ubiquitinated proteins are then recognized and degraded by the 26S proteasome, which is a complex structure comprising two regulatory 19S subunits and the 20S catalytic core subunit [52]. The 26S proteasome is a barrel-shaped structure containing a channel through which the protein travels and is enzymatically degraded on the way. During degradation, the ubiquitin moieties are removed from the proteins by deubiquitinating enzymes and recycled [52]. The K63-linked polyubiquitination, on the other hand, may target the protein for autophagy [53]. In the ALP, the proteins or protein aggregates are engulfed within a double-membrane, which forms the autophagosome. Different autophagy receptors, such as p62/SQSTM1, are essential in the recruitment of K63-ubiquitin-linked proteins to autophagic degradation [54, 55]. The Atg family proteins on the membranes of the autophagosomes are important for the formation of the autophagosomal vesicles. Finally, the mature autophagosomes fuse with lysosomes, which results in the degradation of the contents of the autophagosome [56].

If the protein cannot be refolded and is not targeted for degradation, it may be sequestered to a specific cellular site to generate an intracellular inclusion body, such as an aggresome [57-59]. The presence of the inclusions typically reflects a pathological state and can be used as a disease marker. However, according to current understanding, the formation of the inclusion bodies likely functions as a cytoprotective mechanism rather than a pathogenic one [57]. Active sequestration of misfolded proteins or protein aggregates in intracellular inclusions may reduce the accumulation of potentially toxic protein oligomers and aggregates and prevent abnormal interactions of these with other proteins, cell organelles, or the PQC machinery [9]. Protein refolding, targeting to UPS- or ALP-mediated degradation, or sequestration in inclusions centrally involve the function of different molecular chaperones. Interestingly, a number of these proteins have been associated with the pathogenesis of neurodegenerative diseases.

Protein sequestration in intracellular compartments

Increasing evidence suggests that impairment in the protein clearance systems, such as UPS, ALP or chaperone-mediated autophagy (CMA), takes place in the diseased brain [26, 27, 29-31]. The abnormal protein accumulation may further overwhelm these systems and, as the result, even more proteins start accumulating within the cells. Furthermore, the aggregated proteins typically cannot be degraded by the UPS, shifting the burden in their clearance to the ALP [60]. When the level of protein accumulation and aggregation exceeds the capacity of the UPS or ALP disposal pathways, the misfold-

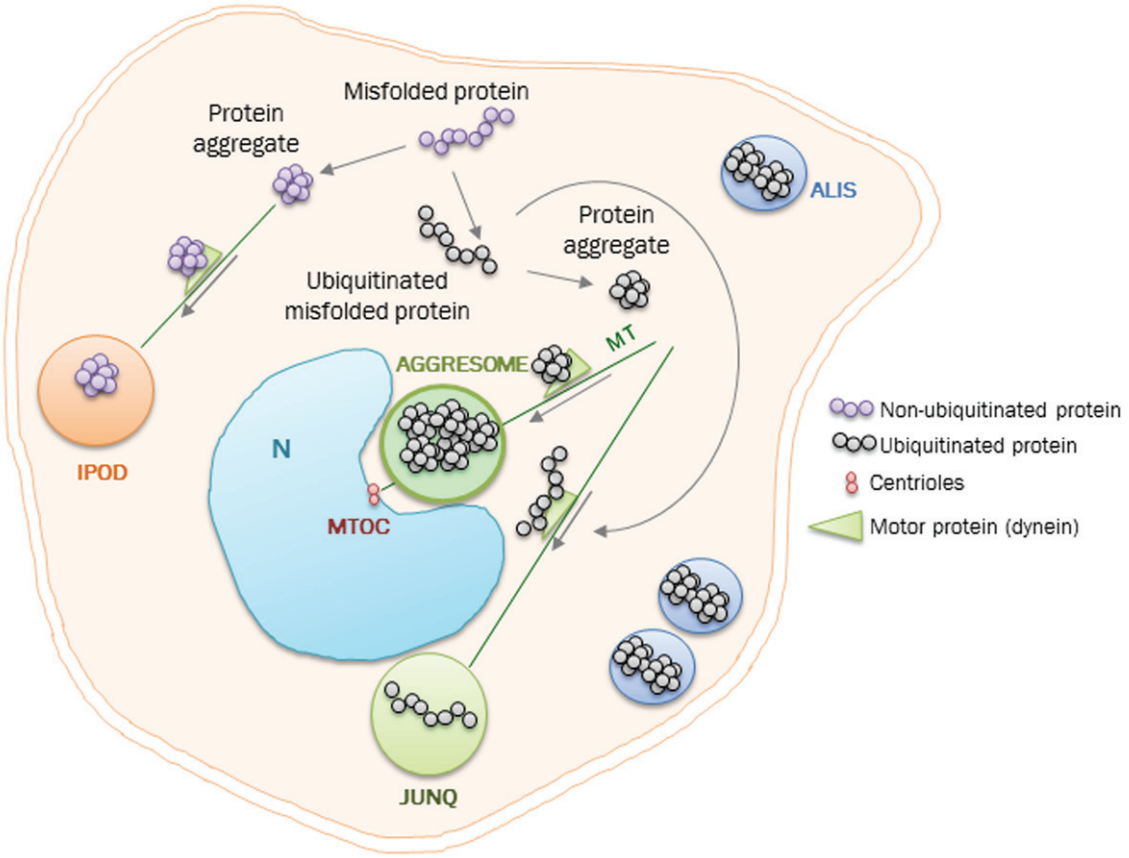

Figure 2. Protein sequestration into different compartments. The misfolded or aggregated proteins may be targeted to different intracellular compartments. After misfolding, most proteins are recognized and ubiquitinated, which directs them to the JUNQ, a region that contains chaperones and 26S proteasomes. JUNQ concentrates soluble misfolded proteins, which may be proteasomally degraded or refolded by the chaperones. The insoluble aggregated proteins which may not be ubiquitinated, such as HD-associated huntingtin or prions, can be targeted to IPOD. It does not contain proteasomes, but colocalizes with autophagy-associated proteins, such as Atg8. Aggregated proteins can also be targeted to the aggresomes, which localize at the MTOC, are surrounded by a vimentin envelope, and cause an indentation of the nucleus. Aggresomes also contain chaperones and components of the ubiquitin-proteasome system (UPS). Targeting to the aggresomes, IPOD, or JUNQ involves active retrograde transport of the cargo by the motor proteins on the microtubules. Immune activation or stress conditions may induce the formation of transient ALIS inclusions. These colocalize with ubiquitin and p62/SQSTM1 and concentrate soluble proteins targeted to clearance by the UPS or autophagy. Abbreviations: ALIS, aggresome-like induced structure; IPOD, insoluble protein deposit; JUNQ, juxtanuclear quality control; MT, microtubule; MTOC, microtubule-organizing center; N, nucleus.

ed or accumulated proteins may be actively compartmentalized as different kinds of inclusions at specific cellular sites to minimize their toxic effects. Depending on their solubility and other properties, the proteins may be targeted to different kinds of compartments or inclusions (**Figure 2**).

Insoluble misfolded proteins are often targeted to juxtanuclear structures termed aggresomes [57-59]. The misfolded proteins or protein aggregates are ubiquitinated and actively transported via the microtubules on retrograde motor proteins to the microtubule-organizing center (MTOC) next to the nucleus. There they typically colocalize with γ-tubulin, an MTOC marker. The aggresome core contains components of the proteasome, Hsps, and mitochondria in addition to the ubiquitinated misfolded proteins. The core is enveloped within a cage formed by vimentin or other filament proteins. The aggresomes appear to be relatively stable structures within cells, but there is increasing evidence that they are eventually cleared from the cells by autophagy [57-59]. Soluble misfolded proteins that are targeted to the UPS for

degradation or alternatively to refolding by cytoplasmic chaperones are typically concentrated to a structure called juxtanuclear quality control (JUNQ), which contains chaperones and proteasomal subunits [61]. Here, the proteins may be refolded or degraded by the proteasome. The insoluble aggregated proteins, such as disease-associated huntingtin or prion proteins, can be directed to a compartment termed insoluble protein deposit (IPOD) [61]. These colocalize with autophagy-associated proteins, suggesting that the IPODs or their constituents may be disposed of by autophagy. Enhanced ubiquitination of the protein at IPOD may redirect it to JUNQ. In addition, immune activation or stress conditions may induce the formation of transient inclusions termed aggresome-like inducible structure (ALIS) that colocalize with ubiquitin and p62/SQSTM1 and concentrate soluble proteins targeted to clearance by the UPS or autophagy [62].

Accumulating evidence implies that sequestration of potentially harmful misfolded or aggregated proteins into specific compartments and formation of intracellular inclusions is a cytoprotective response, which aims to prevent unspecific interactions of the harmful proteins or protein aggregates with other proteins, cell organelles, or components of the PQC system and thus diminish their toxicity [9, 63]. It has been suggested that the soluble misfolded oligomers or aggregates are especially toxic and reduce the capacity of protein folding systems by sequestering chaperones and other factors. This leads to impaired protein homeostasis. In contrast, large insoluble aggregates can be protective and promote cell survival [64]. Therefore, the compartmentalization of the harmful misfolded proteins and protein aggregates may enhance their clearance and prevent them from blocking the UPS or ALP and occupying the cellular chaperones.

Disease-associated proteins involved in protein quality control systems

Several key proteins, which are involved in the function of UPS and ALP at different stages, are known to contain mutations that lead to neurodegenerative disease. Many of these proteins typically contain domains that mediate interaction with polyubiquitinated proteins or the proteasome, such as ubiquitin-like domain (UBL), ubiquitin-associated domain (UBA), or ubiquitin-interacting motifs (UIM) [65, 66]. The presence of such domains in these proteins suggests that they may function as shuttles targeting polyubiquitinated proteins to the UPS or ALP for degradation or to intracellular inclusions. A number of genes, which encode proteins that are involved in the function of the UPS or are degraded by the UPS, are mutated in inherited forms of PD. For example, mutations in the *PARKIN* gene, which encodes the E3 ubiquitin ligase parkin containing an N-terminal UBL-domain, are known to cause autosomal recessive juvenile parkinsonism [41]. Furthermore, wild-type parkin protein is found in Lewy bodies in sporadic PD [67]. UCHL1, a ubiquitin C-terminal hydrolase L1, is a deubiquitinating enzyme that generates free monomeric ubiquitin from polyubiquitin chains, and is also associated with PD [68]. In addition, α-synuclein, parkin, synphilin (polyubiquitinated by parkin), and mutated DJ-1 proteins, all implicated in PD pathogenesis, are substrates for UPS-mediated degradation [69]. Interestingly, the PD-linked mutation in *DJ-1* disrupts the correct folding of DJ-1 protein. As a result, a misfolded, aggregation-prone protein is generated [70].

Ataxin-3 is a deubiquitinating enzyme harboring UIM domains that bind polyubiquitinated proteins. Wild-type ataxin-3 is found in the intranuclear inclusions in spinocerebellar ataxia (SCA) [71]. Furthermore, ataxin-3 gene has been reported to contain mutations, which associate with a specific type of SCA [72].

HDAC6 is a histone deacetylase that links polyubiquitinated proteins to the dynein motor complex for transport along the microtubules. HDAC6 has been shown to localize in aggresomes *in vitro* and the Lewy bodies in PD brain [73, 74]. Furthermore, HDAC6 has been reported to control autophagosome-lysosome fusion, suggesting that it is involved in the regulation of the ALP [75] Interestingly, parkin-mediated K63-linked polyubiquitination targets misfolded DJ-1 to aggresomes via binding to HDAC6 [76], indicating that specific disease-associated proteins may interact with each other and are able to affect each other's functions. This further suggests that defect in the function of one of these proteins may disrupt the function of its interacting partners and thus potentially amplify neurodegeneration.

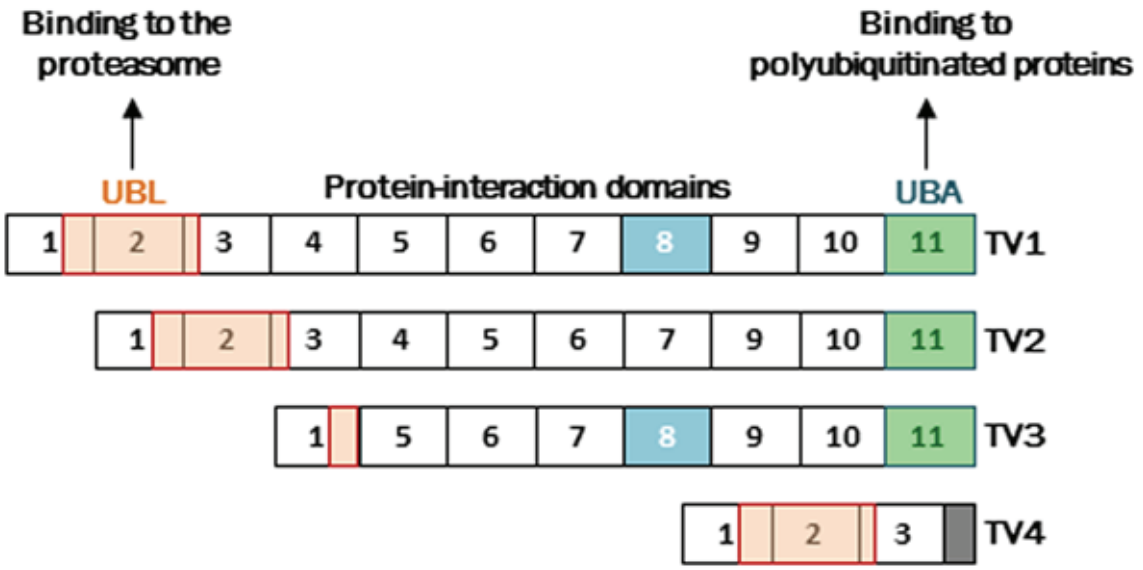

Figure 3. Ubiquilin-1 transcript variants (TV). The full-length ubiquilin-1 TV1 is encoded by 11 exons. Ubiquilin-1 has two signature domains of ubiquitin-like proteins: The N-terminal UBL (ubiquitin-like) domain (orange) and the C-terminal UBA (ubiquitin-associated) domain (green). UBA binds to polyubiquitinated proteins and UBL mediates interaction with the proteasome. The central region consists of conserved asparagine- and proline-rich repeats that mediate ubiquilin-1 interaction with other proteins. TV2 lacks exon 8 (light blue). TV3 lacks exons 2, 3, and 4 and therefore has an incomplete UBL domain. TV4 contains the first 3 exons and a unique short C-terminus (grey) due to a frame shift leading to a 32-amino acid insertion after the exon 3/5 junction.

p62/SQSTM1 is a major cargo receptor for autophagic degradation of ubiquitinated targets. p62/SQSTM1 binds to Atg8 on the autophagosomes and is itself a substrate for ALP-mediated degradation [77, 78]. In addition to many protein-protein interaction domains, it contains an UBA domain in its C-terminus through which it binds polyubiquitinated proteins and targets them for sequestration or degradation [79, 80]. p62/SQSTM1 is found present in neuronal and glial inclusions in AD and inclusions in Pick's disease and in synucleinopathies, e.g. PD, dementia with Lewy bodies and multiple system atrophy [81]. The early accumulation of p62/SQSTM1 in NFTs in AD brain suggests that p62/SQSTM1 may be involved in the formation of NFTs [82]. Also, p62/SQSTM1 colocalizes with TDP-43 and with or without ubiquitin in neuronal and glial inclusions in frontotemporal lobar degeneration (FTLD) and in polyQ-containing inclusions in SCAs [83, 84]. These facts together with the large number of proteins that p62/SQSTM1 interacts with suggests that it is a multifunctional protein that likely regulates a variety of physiological and pathophysiological functions [85]. Another disease-associated protein that is structurally related to p62/SQSTM1 and has also been reported to regulate the levels and targeting of many proteins is ubiquilin-1 (see below).

Ubiquilin-1

Ubiquilin-1 (also known as PLIC-1) is an AD-associated protein, which belongs to the highly conserved group of ubiquitin-like proteins that deliver polyubiquitinated proteins to UPS for degradation [86-88]. We have previously demonstrated an association between sporadic AD and genetic variation of *UBQLN1*, the gene encoding ubiquilin-1 [89] and shown that ubiquilin-1 protein regulates APP processing and β-amyloid production [90, 91], suggesting that ubiquilin-1 may influence the AD pathogenesis at both genetic and mechanistic levels. Interestingly, mutations in a homologous gene, *UBQLN2,* were recently found to cause fALS and ALS/dementia [92], further implying a role for ubiquilin family proteins in neurodegenerative diseases. The full-length ubiquilin-1 is encoded by eleven exons. These give rise to specific domains in the ubiquilin-1 protein, such as the characteristic UBL domain in the N-terminus and the UBA domain in the C-terminus (**Figure 3**) [86, 87, 93]. The central

region of ubiquilin-1 consists of conserved asparagine- and proline-rich repeats that mediate the interaction of ubiquilin-1 with specific domains of other proteins [87]. We have identified four alternatively spliced ubiquilin-1 transcript variants (TVs) in human brain [89, 94] that encode different forms of ubiquilin-1 protein having different domains. In contrast to the full-length ubiquilin-1 TV1, TV2 variant lacks the exon 8, whereas TV3 lacks exons 2, 3 and 4 and therefore has an incomplete UBL domain. The smallest isoform, TV4 has only exons 1, 2 and 3, and thus is devoid of the UBA-domain. TV4 also contains a unique 32-amino acid insertion in its C-terminus (**Figure 3**). It is possible that the different ubiquilin-1 transcript variants have different functions.

Ubiquilin-1 is ubiquitously expressed in different tissues, such as brain, liver, kidney, heart and skeletal muscle. In cells, ubiquilin-1 localizes in the cytoplasm and to a lesser extent, in the nucleus and periphery of the cells [87] Staining of human brain has revealed the presence of ubiquilin-1 in neurons [87]. Ubiquilin-1 interacts specifically with a variety of cytosolic and transmembrane proteins, including γ-aminobutyric acid (GABA) and nicotinic acetylcholine receptors, G-proteins, and CD47, via its functional domains [95]. Moreover, many ubiquilin-1-interacting proteins have been implicated in the pathogenesis of neurodegenerative disorders, suggesting that ubiquilin-1 may regulate many physiological and pathophysiological events.

Ubiquilin-1 and UPS and ALP

Ubiquilin-1 appears to play a versatile role in regulating protein levels and subcellular targeting under different stress conditions that are centrally associated with the pathogenesis of neurodegenerative diseases. Ubiquilin-1 mediates the proteasomal targeting of misfolded or accumulated proteins by binding to their polyubiquitin chains with its UBA domain and by directly interacting with the S5a-domain of the 19S proteasomal subunit through its UBL domain [86-88] (**Figure 3**). These observations suggest that ubiquilin-1 functions as a shuttle protein between the proteins targeted for degradation and the proteasome. Our previous studies suggest that under excessive protein accumulation, specific ubiquilin-1 TVs may promote targeting of the accumulated proteins to both UPS and aggresomes [96, 97] (see below). Furthermore, in the same study, we observed that ubiquilin-1 TVs are present in autophagosomes, suggesting that ubiquilin-1 may regulate the ALP [96]. Other studies have also reported that ubiquilin-1 colocalizes with the autophagosome marker LC3, associates with autophagosomes most probably through its UBA domain, and regulates ALP-mediated degradation of cellular cargo [98, 99]. Additionally, ubiquilin-1 regulates recycling of nutrients and protects cells from apoptosis by enhancing the maturation of autophagosomes during nutrient starvation [98]. In contrast, depletion of ubiquilin-1 has been shown to inhibit autophagosome formation [99]. Ubiquilin-1 has been shown to bind the protein kinase mammalian target of rapamycin (mTOR), which is a major inhibitor of autophagy, but the effects of this interaction on autophagy are currently not known [100]. Ubiquilin-1 itself has been suggested to be a substrate for CMA [99].

Several studies indicate that ubiquilin-1 localizes in the ER. ER is an organelle, which is essential for protein folding and for redirecting undesired proteins to degradation in the UPS or APL pathways [94, 101, 102]. Disturbances in the ER-associated degradation system (ERAD) cause ER-stress and activate the unfolded protein response (UPR) in cells. These mechanisms have been suggested to be involved in the pathogenesis of many neurodegenerative diseases [103]. We and others have shown that ubiquilin-1 levels are up-regulated in cells by ER-stress and that ubiquilin-1 alleviates ER-stress and subsequently increases cell viability [94, 101]. In accordance with this, loss of ubiquilin-1 in *Caenorhabditis elegans* increases the accumulation of misfolded proteins in the ER, activates ER-stress, and shortens the lifespan of the worms [102].

Ubiquilin-1 and accumulation and targeting of disease-associated proteins

Ubiquilin-1 has been shown to colocalize with NFTs in AD brain and Lewy bodies in PD brain [87]. Moreover, ubiquilin-1 interacts with and regulates many proteins involved in the pathogenesis of AD and other neurodegenerative diseases. Ubiquilin-1 was first identified as a presenilin (PS)-interacting protein [87, 91]. PS1 and PS2 are essential catalytic components of the γ-secretase complex, which generates

β-amyloid by proteolytic cleavage of APP. Ubiquilin-1 was shown to specifically increase the accumulation of full-length PS to form ubiquitinated high-molecular-weight (HMW) complexes [87]. Since that, the role of ubiquilin-1 as a PS1- and PS2-stabilizing protein has been confirmed by us and others [96, 104, 105]. Massey *et al*. [104] reported that ubiquilin-1 reduces the degradation of HMW-PS2 and colocalizes with PS2 in the aggresomes under proteasomal inhibition. We have recently shown that specific ubiquilin-1 variants, TV1 and TV3, regulate HMW-PS1 formation and targeting to aggresomes [96]. The increased aggresome formation was not associated with UPS impairment in our study, suggesting that ubiquilin-1 does not globally inhibit proteasomal activity [96]. The potential functional consequences of ubiquilin-1-induced accumulation and aggresomal targeting of PS1 remain to be resolved in future studies.

In addition to AD-related proteins, ubiquilin-1 is implicated in the regulation of other neurodegenerative disease-associated proteins. Heir *et al.* reported that the UBL domain of ubiquilin-1 is required for aggresomal targeting of aggregated proteins containing polyQ expansions [106]. Furthermore, ubiquilin-1 has been shown to regulate the aggregation and suppress the toxicity of polyQ proteins involved in HD in several studies [106-109]. Recently, ubiquilin-1 was reported to colocalize in intracellular inclusions with TDP-43, a protein which is a major component of ubiquitin-positive cytosolic inclusions in patients with ALS and ubiquitin-positive frontotemporal lobular dementia (FTLD-U). In these studies, the UBA domain of ubiquilin-1 was shown to mediate the stability and toxicity of the TDP-43 aggregates [110, 111].

Taken together, the current data show that ubiquilin-1 interacts through its functional domains with a number of proteins and therefore regulates a variety of physiological and pathophysiological functions. Ubiquilin-1 also may play a crucial role in dictating the pathway to which specific proteins are targeted for degradation, especially under different stress conditions. The lack of UBL or UBA domains from specific ubiquilin-1 TVs suggests that different ubiquilin-1 TVs may have differential effects on the regulation of protein degradation pathways. As ubiquilin-1 participates in the aggregation, deposition, and degradation of several abnormally accumulated proteins in neurodegenerative diseases, it might represent a common mechanistic link between distinct neurodegenerative diseases.

Concluding remarks

Mounting evidence suggests that the pathogenesis of different neurodegenerative diseases centrally involves deficits in the PQC systems, which lead to the pathogenic accumulation and aggregation of proteins. The deficits in the PQC together with aging and other factors involved in the pathogenic mechanisms underlying neurodegenerative disorders, such as inflammation and oxidative or metabolic stress and pathogenic disease-associated mutations, play an important role in determining the onset and progression of the disease and finally causing widespread neurodegeneration in specific brain regions. Many of the disease-associated proteins also interact with each other and a number of other binding partners that are involved in important physiological functions, which may further aggravate the pathogenic events during disease pathogenesis. Therefore, characterization of the specific interactions of the disease-associated proteins and identification of factors regulating the PQC systems may help to recognize common molecular mechanisms between different neurodegenerative diseases. This may provide novel opportunities to better understand the disease pathogenesis and subsequently to identify new disease biomarkers and therapeutic targets for an earlier diagnosis and treatment of patients suffering from different neurodegenerative disorders.

Acknowledgments

This study has been supported by the Health Research Council of the Academy of Finland, EVO grant 5772708 of Kuopio University Hospital, Sigrid Juselius Foundation, the strategic funding of the University of Eastern Finland (UEF-Brain), and the Doctoral Program of Molecular Medicine of the University of Eastern Finland.

Declaration of conflicts of interest

The authors do not have any conflicts of interest to declare

Address correspondence to: Dr. Annakaisa Haapasalo, Institute of Clinical Medicine – Neurology, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland. Tel: +358 40 3552768; Fax: +358 17 162048; E-mail: annakaisa.haapasalo@uef.fi

References

[1] Soto C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat Rev Neurosci 2003; 4: 49-60.

[2] Ross CA and Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med 2004; 10 Suppl: S10-7.

[3] Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 2000; 404: 770-774.

[4] Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature 2011; 475: 324-332.

[5] Ciechanover A, Orian A, Schwartz AL. Ubiquitin-mediated proteolysis: biological regulation via destruction. Bioessays 2000; 22: 442-451.

[6] Dikic I, Wakatsuki S, Walters KJ. Ubiquitin-binding domains - from structures to functions. Nat Rev Mol Cell Biol 2009; 10: 659-671.

[7] Kundu M and Thompson CB. Autophagy: basic principles and relevance to disease. Annu Rev Pathol 2008; 3: 427-455.

[8] Ihara Y, Morishima-Kawashima M, Nixon R. The ubiquitin-proteasome system and the autophagic-lysosomal system in Alzheimer disease. Cold Spring Harb Perspect Med 2012; 2: 10.1101/cshperspect.a006361.

[9] Chen B, Retzlaff M, Roos T, Frydman J. Cellular strategies of protein quality control. Cold Spring Harb Perspect Biol 2011; 3: a004374.

[10] Cuervo AM, Wong ES, Martinez-Vicente M. Protein degradation, aggregation, and misfolding. Mov Disord 2010; 25 Suppl 1: S49-54.

[11] Hol EM and Scheper W. Protein quality control in neurodegeneration: walking the tight rope between health and disease. J Mol Neurosci 2008; 34: 23-33.

[12] Mittal S and Ganesh S. Protein quality control mechanisms and neurodegenerative disorders: Checks, balances and deadlocks. Neurosci Res 2010; 68: 159-166.

[13] Chiti F and Dobson CM. Protein misfolding, functional amyloid, and human disease. Annu Rev Biochem 2006; 75: 333-366.

[14] Bolognesi B, Kumita JR, Barros TP, Esbjorner EK, Luheshi LM, Crowther DC, Wilson MR, Dobson CM, Favrin G, Yerbury JJ. ANS binding reveals common features of cytotoxic amyloid species. ACS Chem Biol 2010; 5: 735-740.

[15] Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 2003; 300: 486-489.

[16] Glenner GG and Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 1984; 120: 885-890.

[17] Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem 1986; 261: 6084-6089.

[18] Goedert M, Ghetti B, Spillantini MG. Frontotemporal dementia: implications for understanding Alzheimer disease. Cold Spring Harb Perspect Med 2012; 2: a006254.

[19] Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997; 388: 839-840.

[20] DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997; 277: 1990-1993.

[21] Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, Ohama E, Reaume AG, Scott RW, Cleveland DW. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. Science 1998; 281: 1851-1854.

[22] Voisine C, Pedersen JS, Morimoto RI. Chaperone networks: tipping the balance in protein folding diseases. Neurobiol Dis 2010; 40: 12-20.

[23] Walker LC and Levine H 3rd. Corruption and spread of pathogenic proteins in neurodegenerative diseases. J Biol Chem 2012; 287: 33109-33115.

[24] Balch WE, Morimoto RI, Dillin A, Kelly JW. Adapting proteostasis for disease intervention. Science 2008; 319: 916-919.

[25] Haigis MC and Yankner BA. The aging stress response. Mol Cell 2010; 40: 333-344.

[26] McNaught KS and Jenner P. Proteasomal function is impaired in substantia nigra in Parkinson's disease. Neurosci Lett 2001; 297: 191-194.

[27] Keller JN, Hanni KB, Markesbery WR. Impaired proteasome function in Alzheimer's disease. J Neurochem 2000; 75: 436-439.

[28] Keller JN, Hanni KB, Markesbery WR. Possible involvement of proteasome inhibition in aging: implications for oxidative stress. Mech Ageing Dev 2000; 113: 61-70.

[29] Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA, Nixon RA. Abnormalities of the endosomal-lysosomal system in Alzheimer's disease: relationship to disease pathogenesis. Adv Exp Med Biol 1996; 389: 271-280.

[30] Kegel KB, Kim M, Sapp E, McIntyre C, Castano JG, Aronin N, DiFiglia M. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. J Neurosci 2000; 20: 7268-7278.

[31] Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 1997; 12: 25-31.

[32] Tarawneh R and Galvin JE. Potential future neuroprotective therapies for neurodegenerative disorders and stroke. Clin Geriatr Med 2010; 26: 125-147.

[33] Zigman WB and Lott IT. Alzheimer's disease in Down syndrome: neurobiology and risk. Ment Retard Dev Disabil Res Rev 2007; 13: 237-246.

[34] Zigman WB, Schupf N, Sersen E, Silverman W. Prevalence of dementia in adults with and without Down syndrome. Am J Ment Retard 1996; 100: 403-412.

[35] Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K. alpha-Synuclein locus triplication causes Parkinson's disease. Science 2003; 302: 841.

[36] Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997; 276: 2045-2047.

[37] Liscic RM and Breljak D. Molecular basis of amyotrophic lateral sclerosis. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35: 370-372.

[38] Tanzi RE. The genetics of Alzheimer disease. Cold Spring Harb Perspect Med 2012; 2: 10.1101/cshperspect.a006296.

[39] Dawson TM and Dawson VL. Rare genetic mutations shed light on the pathogenesis of Parkinson disease. J Clin Invest 2003; 111: 145-151.

[40] Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004; 304: 1158-1160.

[41] Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998; 392: 605-608.

[42] Singleton A, Myers A, Hardy J. The law of mass action applied to neurodegenerative disease: a hypothesis concerning the etiology and pathogenesis of complex diseases. Hum Mol Genet 2004; 13 Spec No 1: R123-6.

[43] Smith MA, Richey Harris PL, Sayre LM, Beckman JS, Perry G. Widespread peroxynitrite-mediated damage in Alzheimer's disease. J Neurosci 1997; 17: 2653-2657.

[44] Castegna A, Thongboonkerd V, Klein JB, Lynn B, Markesbery WR, Butterfield DA. Proteomic identification of nitrated proteins in Alzheimer's disease brain. J Neurochem 2003; 85: 1394-1401.

[45] Sultana R, Perluigi M, Butterfield DA. Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurodegeneration from redox proteomics. Antioxid Redox Signal 2006; 8: 2021-2037.

[46] Mandelkow EM and Mandelkow E. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. Cold Spring Harb Perspect Med 2012; 2: a006247.

[47] Braak E, Braak H, Mandelkow EM. A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 1994; 87: 554-567.

[48] Zempel H, Thies E, Mandelkow E, Mandelkow EM. Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci 2010; 30: 11938-11950.

[49] Haass C, Kaether C, Thinakaran G, Sisodia S. Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2012; 2: a006270.

[50] Tanzi RE and Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 2005; 120: 545-555.

[51] Hershko A and Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998; 67: 425-479.

[52] Navon A and Ciechanover A. The 26 S proteasome: from basic mechanisms to drug targeting. J Biol Chem 2009; 284: 33713-33718.

[53] Tan JM, Wong ES, Kirkpatrick DS, Pletnikova O, Ko HS, Tay SP, Ho MW, Troncoso J, Gygi SP, Lee MK, Dawson VL, Dawson TM, Lim KL. Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. Hum Mol Genet 2008; 17: 431-439.

[54] Long J, Gallagher TR, Cavey JR, Sheppard PW, Ralston SH, Layfield R, Searle MS. Ubiquitin recognition by the ubiquitin-associated domain of p62 involves a novel conformational switch. J Biol Chem 2008; 283: 5427-5440.

[55] Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Overvatn A, Bjorkoy G, Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 2007; 282: 24131-24145.

[56] Knaevelsrud H and Simonsen A. Fighting disease by selective autophagy of aggregate-prone proteins. FEBS Lett 2010; 584: 2635-2645.

[57] Kopito RR. Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 2000; 10: 524-530.

[58] Johnston JA, Ward CL, Kopito RR. Aggresomes: a cellular response to misfolded proteins. J Cell Biol 1998; 143: 1883-1898.

[59] Olzmann JA, Li L, Chin LS. Aggresome formation and neurodegenerative diseases: therapeutic implications. Curr Med Chem 2008; 15: 47-60.

[60] Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. Science 2001; 292: 1552-1555.

[61] Kaganovich D, Kopito R, Frydman J. Misfolded proteins partition between two distinct quality control compartments. Nature 2008; 454: 1088-1095.

[62] Liu XD, Ko S, Xu Y, Fattah EA, Xiang Q, Jagannath C, Ishii T, Komatsu M, Eissa NT. Transient aggregation of ubiquitinated proteins is a cytosolic unfolded protein response to inflammation and endoplasmic reticulum stress. J Biol Chem 2012; 287: 19687-19698.

[63] Taylor JP, Tanaka F, Robitschek J, Sandoval CM, Taye A, Markovic-Plese S, Fischbeck KH. Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. Hum Mol Genet 2003; 12: 749-757.

[64] Liu B, Larsson L, Caballero A, Hao X, Oling D, Grantham J, Nystrom T. The polarisome is required for segregation and retrograde transport of protein aggregates. Cell 2010; 140: 257-267.

[65] Grabbe C and Dikic I. Functional roles of ubiquitin-like domain (ULD) and ubiquitin-binding domain (UBD) containing proteins. Chem Rev 2009; 109: 1481-1494.

[66] Su V and Lau AF. Ubiquitin-like and ubiquitin-associated domain proteins: significance in proteasomal degradation. Cell Mol Life Sci 2009; 66: 2819-2833.

[67] Schlossmacher MG, Frosch MP, Gai WP, Medina M, Sharma N, Forno L, Ochiishi T, Shimura H, Sharon R, Hattori N, Langston JW, Mizuno Y, Hyman BT, Selkoe DJ, Kosik KS. Parkin localizes to the Lewy bodies of Parkinson disease and dementia with Lewy bodies. Am J Pathol 2002; 160: 1655-1667.

[68] Maraganore DM, Farrer MJ, Hardy JA, Lincoln SJ, McDonnell SK, Rocca WA. Case-control study of the ubiquitin carboxy-terminal hydrolase L1 gene in Parkinson's disease. Neurology 1999; 53: 1858-1860.

[69] Kruger R, Eberhardt O, Riess O, Schulz JB. Parkinson's disease: one biochemical pathway to fit all genes? Trends Mol Med 2002; 8: 236-240.

[70] Olzmann JA, Brown K, Wilkinson KD, Rees HD, Huai Q, Ke H, Levey AI, Li L, Chin LS. Familial Parkinson's disease-associated L166P mutation disrupts DJ-1 protein folding and function. J Biol Chem 2004; 279: 8506-8515.

[71] Uchihara T, Fujigasaki H, Koyano S, Nakamura A, Yagishita S, Iwabuchi K. Non-expanded polyglutamine proteins in intranuclear inclusions of hereditary ataxias--triple-labeling immunofluorescence study. Acta Neuropathol 2001; 102: 149-152.

[72] Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 1994; 8: 221-228.

[73] Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell 2003; 115: 727-738.

[74] Miki Y, Mori F, Tanji K, Kakita A, Takahashi H, Wakabayashi K. Accumulation of histone deacetylase 6, an aggresome-related protein, is specific to Lewy bodies and glial cytoplasmic inclusions. Neuropathology 2011; 31: 561-568.

[75] Lee JY, Koga H, Kawaguchi Y, Tang W, Wong E, Gao YS, Pandey UB, Kaushik S, Tresse E, Lu J, Taylor JP, Cuervo AM, Yao TP. HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy. EMBO J 2010; 29: 969-980.

[76] Olzmann JA, Li L, Chudaev MV, Chen J, Perez FA, Palmiter RD, Chin LS. Parkin-mediated K63-linked polyubiquitination targets misfolded DJ-1 to aggresomes via binding to HDAC6. J Cell Biol 2007; 178: 1025-1038.

[77] Lamark T, Kirkin V, Dikic I, Johansen T. NBR1 and p62 as cargo receptors for selective autophagy of ubiquitinated targets. Cell Cycle 2009; 8: 1986-1990.
[78] Johansen T and Lamark T. Selective autophagy mediated by autophagic adapter proteins. Autophagy 2011; 7: 279-296.
[79] Shin J. P62 and the sequestosome, a novel mechanism for protein metabolism. Arch Pharm Res 1998; 21: 629-633.
[80] Vadlamudi RK, Joung I, Strominger JL, Shin J. p62, a phosphotyrosine-independent ligand of the SH2 domain of p56lck, belongs to a new class of ubiquitin-binding proteins. J Biol Chem 1996; 271: 20235-20237.
[81] Kuusisto E, Salminen A, Alafuzoff I. Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. Neuroreport 2001; 12: 2085-2090.
[82] Kuusisto E, Salminen A, Alafuzoff I. Early accumulation of p62 in neurofibrillary tangles in Alzheimer's disease: possible role in tangle formation. Neuropathol Appl Neurobiol 2002; 28: 228-237.
[83] Pikkarainen M, Hartikainen P, Alafuzoff I. Neuropathologic features of frontotemporal lobar degeneration with ubiquitin-positive inclusions visualized with ubiquitin-binding protein p62 immunohistochemistry. J Neuropathol Exp Neurol 2008; 67: 280-298.
[84] Pikkarainen M, Hartikainen P, Soininen H, Alafuzoff I. Distribution and pattern of pathology in subjects with familial or sporadic late-onset cerebellar ataxia as assessed by p62/sequestosome immunohistochemistry. Cerebellum 2011; 10: 720-731.
[85] Salminen A, Kaarniranta K, Haapasalo A, Hiltunen M, Soininen H, Alafuzoff I. Emerging role of p62/sequestosome-1 in the pathogenesis of Alzheimer's disease. Prog Neurobiol 2011; 96: 87-95.
[86] Kleijnen MF, Shih AH, Zhou P, Kumar S, Soccio RE, Kedersha NL, Gill G, Howley PM. The hPLIC proteins may provide a link between the ubiquitination machinery and the proteasome. Mol Cell 2000; 6: 409-419.
[87] Mah AL, Perry G, Smith MA, Monteiro MJ. Identification of ubiquilin, a novel presenilin interactor that increases presenilin protein accumulation. J Cell Biol 2000; 151: 847-862.
[88] Ko HS, Uehara T, Tsuruma K, Nomura Y. Ubiquilin interacts with ubiquitylated proteins and proteasome through its ubiquitin-associated and ubiquitin-like domains. FEBS Lett 2004; 566: 110-114.
[89] Bertram L, Hiltunen M, Parkinson M, Ingelsson M, Lange C, Ramasamy K, Mullin K, Menon R, Sampson AJ, Hsiao MY, Elliott KJ, Velicelebi G, Moscarillo T, Hyman BT, Wagner SL, Becker KD, Blacker D, Tanzi RE. Family-based association between Alzheimer's disease and variants in UBQLN1. N Engl J Med 2005; 352: 884-894.
[90] Hiltunen M, Lu A, Thomas AV, Romano DM, Kim M, Jones PB, Xie Z, Kounnas MZ, Wagner SL, Berezovska O, Hyman BT, Tesco G, Bertram L, Tanzi RE. Ubiquilin 1 modulates amyloid precursor protein trafficking and Abeta secretion. J Biol Chem 2006; 281: 32240-32253.
[91] Thomas AV, Herl L, Spoelgen R, Hiltunen M, Jones PB, Tanzi RE, Hyman BT, Berezovska O. Interaction between presenilin 1 and ubiquilin 1 as detected by fluorescence lifetime imaging microscopy and a high-throughput fluorescent plate reader. J Biol Chem 2006; 281: 26400-26407.
[92] Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, Ajroud K, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 2011; 477: 211-215.
[93] Kleijnen MF, Alarcon RM, Howley PM. The ubiquitin-associated domain of hPLIC-2 interacts with the proteasome. Mol Biol Cell 2003; 14: 3868-3875.
[94] Lu A, Hiltunen M, Romano DM, Soininen H, Hyman BT, Bertram L, Tanzi RE. Effects of ubiquilin 1 on the unfolded protein response. J Mol Neurosci 2009; 38: 19-30.
[95] Haapasalo A, Viswanathan J, Bertram L, Soininen H, Tanzi RE, Hiltunen M. Emerging role of Alzheimer's disease-associated ubiquilin-1 in protein aggregation. Biochem Soc Trans 2010; 38: 150-155.
[96] Viswanathan J, Haapasalo A, Bottcher C, Miettinen R, Kurkinen KM, Lu A, Thomas A, Maynard CJ, Romano D, Hyman BT, Berezovska O, Bertram L, Soininen H, Dantuma NP, Tanzi RE, Hiltunen M. Alzheimer's disease-associated ubiquilin-1 regulates presenilin-1 accumulation and aggresome formation. Traffic 2011; 12: 330-348.
[97] Haapasalo A, Viswanathan J, Kurkinen KM, Bertram L, Soininen H, Dantuma NP, Tanzi RE, Hiltunen M. Involvement of ubiquilin-1 transcript variants in protein degradation and accumulation. Commun Integr Biol 2011; 4: 428-432.
[98] N'Diaye EN, Kajihara KK, Hsieh I, Morisaki H, Debnath J, Brown EJ. PLIC proteins or ubiquilins regulate autophagy-dependent cell survival during nutrient starvation. EMBO Rep 2009; 10: 173-179.

[99] Rothenberg C, Srinivasan D, Mah L, Kaushik S, Peterhoff CM, Ugolino J, Fang S, Cuervo AM, Nixon RA, Monteiro MJ. Ubiquilin functions in autophagy and is degraded by chaperone-mediated autophagy. Hum Mol Genet 2010; 19: 3219-3232.

[100] Wu S, Mikhailov A, Kallo-Hosein H, Hara K, Yonezawa K, Avruch J. Characterization of ubiquilin 1, an mTOR-interacting protein. Biochim Biophys Acta 2002; 1542: 41-56.

[101] Ko HS, Uehara T, Nomura Y. Role of ubiquilin associated with protein-disulfide isomerase in the endoplasmic reticulum in stress-induced apoptotic cell death. J Biol Chem 2002; 277: 35386-35392.

[102] Lim PJ, Danner R, Liang J, Doong H, Harman C, Srinivasan D, Rothenberg C, Wang H, Ye Y, Fang S, Monteiro MJ. Ubiquilin and p97/VCP bind erasin, forming a complex involved in ERAD. J Cell Biol 2009; 187: 201-217.

[103] Wang S and Kaufman RJ. The impact of the unfolded protein response on human disease. J Cell Biol 2012; 197: 857-867.

[104] Massey LK, Mah AL, Ford DL, Miller J, Liang J, Doong H, Monteiro MJ. Overexpression of ubiquilin decreases ubiquitination and degradation of presenilin proteins. J Alzheimers Dis 2004; 6: 79-92.

[105] Massey LK, Mah AL, Monteiro MJ. Ubiquilin regulates presenilin endoproteolysis and modulates gamma-secretase components, Pen-2 and nicastrin. Biochem J 2005; 391: 513-525.

[106] Heir R, Ablasou C, Dumontier E, Elliott M, Fagotto-Kaufmann C, Bedford FK. The UBL domain of PLIC-1 regulates aggresome formation. EMBO Rep 2006; 7: 1252-1258.

[107] Doi H, Mitsui K, Kurosawa M, Machida Y, Kuroiwa Y, Nukina N. Identification of ubiquitin-interacting proteins in purified polyglutamine aggregates. FEBS Lett 2004; 571: 171-176.

[108] Wang H, Lim PJ, Yin C, Rieckher M, Vogel BE, Monteiro MJ. Suppression of polyglutamine-induced toxicity in cell and animal models of Huntington's disease by ubiquilin. Hum Mol Genet 2006; 15: 1025-1041.

[109] Wang H and Monteiro MJ. Ubiquilin interacts and enhances the degradation of expanded-polyglutamine proteins. Biochem Biophys Res Commun 2007; 360: 423-427.

[110] Kim SH, Shi Y, Hanson KA, Williams LM, Sakasai R, Bowler MJ, Tibbetts RS. Potentiation of amyotrophic lateral sclerosis (ALS)-associated TDP-43 aggregation by the proteasome-targeting factor, ubiquilin 1. J Biol Chem 2009; 284: 8083-8092.

[111] Hanson KA, Kim SH, Wassarman DA, Tibbetts RS. Ubiquilin modifies TDP-43 toxicity in a Drosophila model of amyotrophic lateral sclerosis (ALS). J Biol Chem 2010; 285: 11068-11072.