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Antioxidants & Redox Signaling
The Ubiquitin-Proteasome System in Neurodegeneration (doi: 10.1089/ars.2013.5802)

FORUM REVIEW ARTICLE

The Ubiquitin-Proteasome System in Neurodegeneration

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Abstract

Significance: Impairment of the ubiquitin-proteasome system (UPS) has been implicated in the pathogenesis of a wide variety of neurodegenerative disorders including Alzheimer's, Parkinson's and Huntington's diseases. The most significant risk factor for the development of these disorders is aging, which is associated with a progressive decline in UPS activity and the accumulation of oxidatively modified proteins. To date, no therapies have been developed which can specifically upregulate this system.

Recent advances: In the neurodegenerative brain, dysfunction of the UPS has been associated with the deposition of ubiquitinated protein aggregates and widespread disruption of the proteostasis network. Recent research has identified further evidence of impairment in substrate ubiquitination and proteasomal degradation which could contribute to the loss of cellular proteostasis in neurodegenerative disease. Novel strategies for activation of the UPS by genetic manipulation and treatment with synthetic compounds have also recently been identified.

Critical issues: Here we discuss the specific roles of the UPS in the healthy central nervous system and establish how dysfunctional components can contribute to neurotoxicity in the context of disease.

Future directions: Knowledge of the UPS components specifically or preferentially involved in neurodegenerative disease will be critical in the development of targeted therapies which aim to limit accumulation of misfolded proteins without gross disturbance of this major proteolytic pathway.

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Introduction

Efficient folding of nascent polypeptides and rapid elimination of misfolded proteins is critical to the maintenance of cellular and organismal health. Under normal conditions, this protein homeostasis (also known as proteostasis) is achieved by an integrated network of molecular chaperones and proteolytic clearance systems, including the ubiquitin-proteasome system (UPS) and autophagy (79). The UPS is a highly conserved and tightly regulated pathway for the coordinated degradation of a wide variety of proteins with half-lives ranging from minutes to several days (70). It is therefore unsurprising that dysfunction of the UPS has been implicated in the pathogenesis of many human pathologies, including cancer, autoimmunity and neurodegeneration. Despite heterogeneous clinical phenotypes, Alzheimer's, Huntington's and Parkinson's diseases are all characterised by the accumulation of misfolded, aggregate-prone proteins and the pathognomonic accumulation of ubiquitinated conjugates in post-mortem brains of affected patients (Table 1). Studies in animal models indicate that early impairment of the UPS and resulting loss of cellular proteostasis could be primary mediators of neurodegeneration, raising the possibility of proteostasis-based therapies to slow disease progression.

Each neurodegenerative disease has a unique profile of protein aggregate composition and distribution. The most common form of dementia, Alzheimer's disease (AD) is characterised by the appearance of two types of protein deposit: extracellular amyloid plaques and intraneuronal neurofibrillary tangles (NFTs). NFTs are composed of aggregates of hyperphosphorylated tau, whilst the principal component of amyloid plaques is a 40- to 42-residue peptide called β-amyloid protein ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$) (161). Parkinson's disease (PD) is classically associated with the selective loss of dopaminergic neurons in the substantia nigra. At the cellular level, PD is characterised by the appearance of eosinophilic cytoplasmic

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inclusions, termed Lewy bodies, which contain aggregated forms of the protein α-synuclein (84). In Huntington's disease (HD), translation of a CAG repeat expansion in exon 1 of the *HTT* gene results in expression of mutant huntingtin (mtHtt) protein with a polyQ expansion in the N-terminal region (157). Increased β-sheet content of mtHtt promotes the age-dependent formation of insoluble protein aggregates in structures known as inclusion bodies (157). Misfolded forms of the Cu^{2+}/Zn^{2+} superoxide dismutase SOD1 have been identified in amyotrophic lateral sclerosis (ALS), the most common cause of adult-onset motor neuron disease (20). The G93A mutant form of SOD1 is the most commonly studied protein in cellular and animal models of ALS, and is thought to mediate pathology through a toxic gain-of-function. Lastly, prion diseases are a collection of rapidly progressive and, in some cases, infectious neurodegenerative disorders characterised by the conformational rearrangement of a normal host-encoded protein, PrP^{C}, into the abnormal, aggregate-prone conformer PrP^{Sc} (153). Whilst PrP^{C} is critical for the templated misfolding of PrP, its depletion is not associated with overt pathology (25). As a result, prion disease pathogenesis is also believed to occur by a toxic gain-of-function of the misfolded conformer.

As the principal route of protein degradation in mammalian cells, the UPS represents a major defence against these misfolded proteins, particularly in post-mitotic neurons which are unable to divide to reduce their burden of damaged proteins. Proteins are marked for proteasomal degradation by covalent conjugation of ubiquitin, a highly conserved 76-residue polypeptide, in a three-step cascade (Fig. 1). Initially, the ubiquitin-activating enzyme, E1, activates ubiquitin by creating a high-energy thiol ester intermediate in an ATP-dependent reaction (80). The activated ubiquitin moiety is subsequently shuttled from E1 to E2, an ubiquitin-conjugating enzyme, creating a second high-energy thiol ester intermediate (80). A third class of enzyme, the ubiquitin E3 ligases, mediate the covalent attachment of

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polyubiquitin chains to an internal lysine residue of specific UPS substrates (70). Similar to other forms of post-translational modification, the process of ubiquitination is reversible under the influence of specific deubiquitinating enzymes (DUBs) (82). Polyubiquitinated proteins are recognised and subsequently degraded by the 26S proteasome. This ATP-dependent proteolytic complex consists of a 20S core particle and one or two 19S regulatory particle(s) (Fig. 2). The barrel-shaped 20S complex is composed of four heptagonal rings: two identical outer α-rings (α_{1-7}) and two identical inner β rings (β_{1-7}). β_1, β_2 and β_5 subunits are responsible for peptide bond cleavage, with preference for acidic (caspase-like activity), basic (trypsin-like activity) and hydrophobic (chymotrypsin-like activity) residues, respectively (55). The N-termini of the α subunits function as a gate, restricting substrate entry to the proteolytic chamber (75). The 19S regulatory particle is important for the recognition, unfolding and translocation of ubiquitinated substrates into the 20S core particle for degradation (11).

In addition to the UPS, protein quality control is also mediated by clearance of misfolded proteins through the autophagic pathway. Autophagy, and more specifically macroautophagy, is responsible for the bulk turnover of redundant cellular constituents, including damaged organelles and protein aggregates. In this highly regulated process, a region of cytosol is isolated in a double membrane-bound structure called an autophagosome, which later fuses with the lysosome to expose sequestered contents to hydrolytic enzymes (140). Extensive cross-talk between the UPS and autophagy has been described, including the compensatory upregulation of autophagy in conditions of UPS impairment (112). Although beyond the scope of this review, defects in autophagy have been reported in the pathology of various disorders including Alzheimer's and Parkinson's diseases, and may be additional source of proteostasis disruption in the neurodegenerative brain (extensively reviewed in (140)).

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In this review, we will evaluate the specific roles of the UPS in the healthy central nervous system (CNS) and discuss how impairment of substrate ubiquitination or proteasomal degradation can lead to loss of cellular proteostasis in the context of neurodegenerative disease. In the absence of effective strategies to upregulate UPS activity, we also highlight possible components of the UPS which may be valid therapeutic targets for the treatment of these debilitating disorders.

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The UPS in the healthy CNS

Despite being highly conserved across species, structurally and functionally distinct subpopulations of the UPS have been identified in different tissues (58, 71, 174). This variation has been attributed to alterations in ubiquitin ligase activity, proteasome subunit composition and tissue-specific proteasome-interacting proteins (58, 71, 174). Following isolation of 26S proteasomes from rodent brain, mass-spectrometry identified 28 interacting proteins, of which only 12 were shared with muscle tissue (174). This heterogeneity is likely to reflect marked variation in the cellular proteomes of different tissues and the varying regulatory factors required for their degradation. In order to maintain cellular proteostasis, it is critical that components of the UPS are adapted to meet the specific physiological demands of the tissue (14). In the CNS, synaptic transmission is a critical process by which neurons receive, process and transmit information. A synapse is composed of two distinct functional units: presynaptic terminals and postsynaptic dendritic spines. In the presynaptic terminal, neurotransmitters are packaged in synaptic vesicles which later dock and fuse with specialised regions of plasma membrane called active zones. Exocytosis of neurotransmitters into the synaptic cleft stimulates cell surface receptors localised on dendritic spines of the post-synaptic neuron, activating downstream signal transduction pathways. The UPS has been implicated in the regulation of neurotransmission at pre-and post-synaptic sites, thus playing a critical role in neuronal signalling.

At the presynaptic terminal, the E3 ligase SCRAPPER was shown to ubiquitinate and promote degradation of Rab3-interacting molecule 1 (RIM1), a scaffold protein which links synaptic vesicles to membrane fusion machinery in the active zone (206) (Fig 3). Consistent with a role in the regulation of vesicle exocytosis, SCRAPPER knockout mice display

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impaired RIM1 ubiquitination and dysregulation of synaptic vesicle release (206). At the postsynaptic terminal, the UPS plays a key role in the dynamic remodelling of dendritic spines in response to changes in neuronal activity (57). These structural rearrangements underlie the process of synaptic plasticity, in which synapses are strengthened or weakened in an activity-dependent manner to facilitate processes such as learning and memory. To compensate for excessive neuronal stimulation, post-synaptic excitability can be dampened by reducing the size of dendritic spines and the number of cell surface receptors (19) (Fig 3). For example, in response to synaptic stimulation, the E3 ligase TRIM3 was shown to ubiquitinate and promote degradation of the postsynaptic scaffold protein GKAP, leading to a reduction in dendritic spine size (85). In a similar activity-dependent mechanism, the E3 ligase $SCF^{\beta\text{-}TRCP}$ was shown to ubiquitinate the Rap GTPase activating protein SPAR, targeting it for degradation (4). As a positive regulator of dendritic spine size, proteasomal clearance of SPAR promotes synaptic shrinkage, tempering postsynaptic activation in conditions of chronically elevated activity (163). Dampening of excitatory synaptic transmission can also be achieved by the internalisation of postsynaptic neurotransmitter receptors (Fig 3). Neuronal activity can induce transcription of the E3 ligase E6-AP which ubiquitinates and controls degradation of Arc, a synaptic protein involved in the internalisation of the AMPA subtype of glutamate receptors (73). Inactivating mutations in the *UBE3A* gene which encodes E6-AP are associated with the neurodevelopmental disorder Angelman syndrome, demonstrating the importance of E3 ligase activity to normal synaptic function (108, 131).

Activity-dependent remodeling of dendritic spines requires increased turnover of postsynaptic proteins, a process which is facilitated by an upregulation of proteasome function (17, 57). In response to activation of neurotransmitter receptors, influx of calcium

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results in autophosphorylation of the postsynaptic protein kinase CaMKIIα (Fig 3), enhancing its association with proteasomes and directing their relocalisation to dendritic spines (18). In addition to the redistribution of proteasomes, CaMKIIα was also shown to enhance proteolytic activity by phosphorylation of the proteasome subunit Rpt6 (57). This localised upregulation of proteasome activity in spines may be important for the structural remodelling of active synapses. Consistent with this hypothesis, recent work by Hamilton and colleagues demonstrated that CaMKIIα -mediated phosphorylation of Rpt6 was critical to the activity-induced outgrowth of new dendritic spines (78).

Taken together, the above studies support a direct role for the UPS in normal brain function, and in particular, learning and memory. Dysfunction of the UPS would therefore be expected to interrupt activity-induced synaptic plasticity. This effect was elegantly demonstrated by Lopez-Salon and colleagues, who studied the effect of proteasome inhibition on long-term memory formation in rats (124). Avoidance training was associated with increased levels of ubiquitinated proteins and 26S proteasome activity in the hippocampus (124). Infusion of the proteasome inhibitor lactacystin in the post-training interval resulted in full retrograde amnesia, demonstrating the critical role of the UPS in memory consolidation (124). In similar experiments, proteasome inhibitors have also been shown to prevent extinction of contextual fear memory (116) and to disrupt the consolidation of spatial memory (6). Efficient turnover of synaptic proteins by the UPS therefore plays a critical role in synaptic plasticity. In the context of neurodegenerative disease, rising levels of misfolded proteins may divert UPS activity away from these critical regulatory functions, leading to impairments in neuronal function.

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The UPS in neurodegenerative disease

Dysfunction of the UPS in neurodegenerative disease can arise from impairments in ubiquitination, substrate delivery to the proteasome or a loss of proteasome activity (Fig. 4), each of which can contribute to progressive disruption of cellular proteostasis.

(1) Impairments in ubiquitination

Ubiquitin

A mutant form of ubiquitin, UBB^{+1}, has been implicated in the pathogenesis of several tauopathies and polyglutamine diseases (117). UBB^{+1} is generated by a process known as molecular misreading, in which a dinucleotide deletion at the level of ubiquitin mRNA results in a 19 amino acid C-terminal extension (118). In the absence of a Gly76 residue, this frameshift mutant is unable to ubiquitinate other proteins, yet is itself efficiently ubiquitinated. Since the resulting polyubiquitinated UBB^{+1} is refractory to disassembly by DUBs, it is thought to compete with other polyubiquitinated substrates for recognition and degradation by the proteasome (114). Transgenic mice with postnatal neuronal expression of UBB^{+1} display a marked reduction in UPS activity, accumulation of ubiquitinated proteins and an early impairment in contextual memory, mirroring the clinical hallmarks of AD (63). A significant proportion of UBB^{+1} neurotoxicity may, however, be independent of an effect on the UPS since studies in primary neurons revealed that UBB^{+1} induces dysregulation of mitochondrial trafficking in neurites, leading to mitochondrial stress and activation of p53 cell death pathways (175). Whilst these effects were effectively reversed by UBB^{+1} silencing, such strategies are likely to have limited therapeutic potential due to the inability to identify clinical cases affected by the accumulation of UBB^{+1}.

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E1/E2 enzymes

Evidence of impairment in the earliest stages of the ubiquitination process has been identified in post-mortem AD brain tissue. One study reported a significant reduction in E1 and E2 enzyme activities in human AD brain tissue, which was associated with impaired formation of high-molecular-weight ubiquitin-protein conjugates (123). It remains unclear whether similar deficits are present in other neurodegenerative diseases.

E3 ligases

Parkin

Mutations in the cytosolic E3 ligase parkin are the most common cause of autosomal recessive monogenic PD (109). In addition to inherited mutations, cysteine residues in the RING domains of parkin are particularly susceptible to oxidation and nitrosylation, which may lead to functional impairment in cases of sporadic PD (41, 205). In response to mitochondrial depolarisation, parkin is normally recruited from the cytosol to direct proteasomal degradation of outer mitochondrial membrane (OMM) proteins, including Mitofusins 1/2, Tom 20/40/70 and Omp25 (31, 176, 210). Depletion of Mitofusins 1 and 2 is thought to prevent mitochondrial fusion, thus segregating dysfunctional depolarised mitochondria from healthy mitochondria. In addition, the degradation of OMM proteins may be an important prerequisite for mitophagy, by exposing inner mitochondrial membrane (IMM) proteins to the cytosol for secondary degeneration. Consistent with these findings, loss of parkin function has been associated with reduced polyubiquitination of OMM proteins, the accumulation of defective mitochondria and increased cell death (190). In addition to its role in substrate ubiquitination, parkin has also been shown to enhance assembly and activity of the 26S proteasome (46, 87, 186). Parkin gene therapy may

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therefore be an attractive strategy to preserve mitochondrial integrity and enhance degradation of misfolded proteins. The potential therapeutic benefit of parkin overexpression may not be restricted to PD as parkin has also been shown to interact with Aβ and was found to be depleted in post-mortem AD brains (156) . Consistent with these observations, overexpression of wild-type parkin effectively depleted levels of $A\beta_{1-42}$ in AD cell and rodent models (27, 156).

CHIP

Misfolded proteins must be refolded by molecular chaperones (e.g. Hsc70/Hsp70 and Hsp90) or targeted for degradation by the UPS to prevent aggregation and cytotoxicity. Carboxy terminus of Hsc70-interacting protein (CHIP) is a 35-kDA member of the RING domain family of E3 ligases, which binds to E2 ubiquitin-conjugating enzymes through a C-terminal U-box domain and to Hsc70/Hsp70 and Hsp90 chaperones through an N-terminal tetratricopeptide repeat domain (42). As suggested by its interacting partners, CHIP plays a key role in the ubiquitin-mediated degradation of unfolded chaperone substrates (42, 151) . One such substrate is LRRK2, a multi-domain protein with kinase and GTPase activities (129). Mutations in LRRK2 are the most common known cause of PD and are associated with the accumulation of α-synuclein in intraneuronal aggregates (213). CHIP-mediated clearance of LRRK2 was shown to rescue SH-SY5Y cells from mutant LRRK2 toxicity (110). CHIP has also been shown to promote the degradation of other disease-associated proteins including oligomeric forms of α-synuclein (179), hyperphosphorylated tau species (56, 151), mutant SOD1 (187) and mtHtt (91, 136). Overexpression of CHIP protected against Aβ-induced accumulation of tau in a mouse model of AD, suggesting that CHIP gene therapy could be a general strategy to enhance clearance of misfolded proteins (142). In addition to directly promoting tau clearance by ubiquitination, CHIP may also facilitate its

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degradation by abrogating the protein folding activity of chaperone Hsp90 (43). This could have important wider implications for proteostasis by preventing the functional loss of chaperones through their pre-occupation with folding of aggregate-prone disease-associated proteins.

E6-AP

The *UBE3A* gene encodes the HECT-domain E3 ligase E6-AP and was initially identified as the sole causative gene underlying the neurodevelopmental disorder Angelman syndrome (108, 131). Recently, a novel role of E6-AP in the context of neurodegeneration has started to emerge. A pronounced depletion in levels of E6-AP was identified in the motor neurons of mutant SOD1 transgenic mice (137) and E6-AP was reported to be a key component of Lewy bodies in postmortem PD brains (139). Recruitment of E6-AP to aggregates may result in a depletion of functional soluble pools, with detrimental consequences for synaptic plasticity. This effect was recently observed in the R6/2 mouse model, where E6-AP recruitment to nuclear huntingtin aggregates was accompanied by decreased levels of AMPA receptors and various pre- and post-synaptic proteins (127). While E6-AP gene therapy has proven effective in ameliorating learning deficits in a mouse model of Angelman syndrome, it remains unclear whether similar strategies would be effective in the context of neurodegenerative disease where no inactivating mutations in *UBE3A* have been identified (49). Elevated levels of E6-AP may still have important neuroprotective effects by maintaining soluble E6-AP pools for the continued regulation of synaptic function.

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Deubiquitinating enzymes (DUBs)

USP9X

In addition to an age-related decline in proteasome activity, recent evidence suggests that the accumulation of aggregate-prone monoubiquitinated α-synuclein in PD brains may be accounted for by a failure of DUB activity. USP9X interacts with and deubiquitinates α-synuclein *in vitro* and was found to be depleted in post-mortem brain tissue of Diffuse Lewy-Body Dementia (DLBD) and PD patients (158). The development of compounds to activate USP9X may prove useful in promoting deubiquitination of monoubiquitinated α-synuclein, reducing aggregate formation and cytotoxicity.

UCH-L1

Initially identified as a DUB, UCH-L1 has a multitude of reported functions, including ubiquitin ligase activity and the stabilisation of mono-ubiquitin. PARK5, a rare autosomal dominant form of PD, is caused by a missense mutation in *UCH-L1* resulting in a I93M substitution (119). UCH-L1^{I93M} has increased affinity for LAMP-2A, which may disrupt CMA-mediated turnover of α-synuclein and promote nigral cell death (99, 208). Proteomic analyses revealed a reduction in wild-type UCH-L1 levels in post-mortem AD and PD brains, and identified that the protein is a major target of oxidative damage (28, 37). These findings have important implications in the context of sporadic disease as carbonyl-modified UCH-L1 shares similar physicochemical properties to UCH-L1^{I93M} (100). The neuroprotective effect of UCH-L1 overexpression was demonstrated in the *APP/PS1* mouse model of AD, where it rescued Aβ-induced inhibition of LTP and ameliorated associative memory deficits (72). Further evidence of a neuroprotective role of UCH-L1 comes from the identification of the *UCH-L1* S18Y polymorphism which has been associated with a significantly lower risk of

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PD (128) and has modest regulatory effects on Huntington's disease age of onset (204). Intrastriatal adenoviral overexpression of *UCH-L1*^{S18Y} was found to protect mouse nigral neurons against the toxic effects of the MPTP (203). Taken together, these findings suggest that therapeutics aimed at enhancing UCH-L1 function may help to maintain ubiquitin homoeostasis and synaptic plasticity in the context of disease.

Ataxin-3

Ataxin-3 (Atx3) is a highly conserved DUB with a structured globular N-terminal domain, termed the Josephin domain, and a flexible C-terminal tail (130). The Josephin domain displays ubiquitin protease activity, while the flexible tail encompasses three ubiquitin-interacting motifs (UIMs) flanking a polyQ region of variable length. Abnormal expansion of the polyQ region to over 53 glutamines is pathological and causes the autosomal dominant neurodegenerative disorder Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3) (125) . In addition to the toxic gain-of-function conventionally attributed to polyQ repeat expansions, one hypothesis suggests that polyQ expansion leads to a loss of Atx3 function, which could have important implications for proteostasis. Expanded Atx3 retains its ability to bind polyubiquitinated substrates but the mutant protein may be less-efficient in substrate binding or proteolysis as it is associated with higher global levels of ubiquitination than the non-expanded form (202). Further work is required to establish specific physiological substrates of Atx3 and to determine how these interactions are affected by expansion of the polyQ region in the context of disease.

USP14

Ubiquitin-specific protease 14 (USP14) is a DUB which resides on the 19S regulatory particle and plays an important role in substrate deubiquitination and proteasomal gate

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opening (148, 149). The *ataxia* (*ax*^{J}) mutation is a spontaneous recessive mutation that results in reduced Usp14 expression in mutant mice, leading to severe growth retardation, resting tremor and hind limb paralysis (201). These neurological deficits could be attributed to impairments in the developmental maturation and function of neuromuscular junctions (16, 34). Compared with wildtype mice, *ax*^{J} mice showed a 30-40% reduction in levels of monomeric ubiquitin, suggesting a critical role for ubiquitin homeostasis in synaptic function (3). Consistent with this hypothesis, transgenic complementation of *ax*^{J} mice with neuronally expressed ubiquitin was sufficient to prevent developmental and functional deficits (33). Whilst disease-associated mutations in *USP14* have not been reported, these findings could yield important insights into neurodegenerative disease, where accumulation of ubiquitinated deposits may lead to functional depletion of ubiquitin pools and associated synaptic dysfunction.

(2) Impairments in substrate delivery to the proteasome

Ubiquilins

The ubiquitin-like protein family, or ubiquilins, are characterised by an N-terminal ubiquitin-like domain and C-terminal ubiquitin-association domain, implicating them in the delivery of polyubiquitinated substrates to the proteasome for degradation (111). Polymorphisms in the *UBQLN1* gene, have been identified as a modest risk-conferring haplotype for the development of AD (13, 102). While studies in other populations have failed to replicate these findings (169), a significant depletion of ubiquilin-1 was reported in late-onset AD brains, regardless of *UBQLN1* genotype (172). The role of ubiquilin-1 in AD pathogenesis may be independent of any effect on substrate delivery to the proteasome since ubiquilin-1

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also plays a critical role in APP maturation and processing by controlling K63-linked polyubiquitination of the APP intracellular domain (8).

Mutations in *UBQLN2*, another member of the ubiquilin family, are associated with rare, dominantly-inherited X-linked forms of ALS and ubiquilin-2 immunoreactive inclusions have also been identified as a common pathological feature in non-*UBQLN2*-linked ALS and ALS/dementia cases (51, 52). Consistent with a role of ubiquilin-2 in ubiquitinated substrate delivery to the proteasome, expression of mutant ubiquilin 2 in SH-SY5Y cells induced UPS dysfunction (52). Interestingly, overexpression of wild-type ubiquilin-2 enhanced clearance of TDP-43 *in vitro,* suggesting a possible role for ubiquilin-2 in TDP-43-associated neurotoxicity (30).

VCP

Valosin-containing protein (VCP) is a type II member of the ATPase associated with diverse cellular activities (AAA+) family of proteins (93). VCP associates with a broad range of polyubiquitinated proteins through its N- terminal domain, facilitating their extraction from large multimeric complexes for degradation by the proteasome (48, 209). VCP mutations have been implicated in sporadic ALS (2), familial ALS (94), Parkinson's disease (171) and the rare hereditary disease Inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD) (196). Depletion or mutation of VCP has been associated with impairments in both the UPS and autophagy (50, 69, 95, 183). A recent study in *Drosophila* identified a critical role of VCP in proteasome-dependent degradation of Mitofusins1 and 2 and demonstrated that VCP mutations lead to a loss of mitochondrial quality control (107). Depletion of cellular ATP levels may contribute to the loss of UPS activity associated with VCP mutations.

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(3) Impairments in proteasome activity

Genetic ablation of neuronal 26S proteasome activity

As the terminal step in UPS-mediated protein degradation, dysfunction of the 26S proteasome has potentially catastrophic consequences for the maintenance of cellular proteostasis. Genetic ablation studies have elegantly demonstrated the importance of 26S catalytic activity to neuronal function and survival. Bedford and colleagues developed conditional genetic mouse models with spatially restricted inactivation of the 19S subunit *Psmc1* (Rpt2) in the forebrain or substantia nigra (10). Since Rpt2 directs gate-opening of the 20S core particle on arrival of ubiquitinated substrates, this system was able to test the specific role of ubiquitin-dependent degradation by the 26S proteasome in targeted brain regions. Depletion of 26S proteasomes in the substantia nigra resulted in Lewy-like body formation, axonal die-back and death of dopaminergic neurons, closely mirroring the neuropathology observed in PD patients. Depletion of 26S proteasome activity in the forebrain also resulted in a progressive neurodegeneration with widespread neuronal loss and marked learning deficits. Recently, a similar ablation study investigated the effects of conditional knockout of *Psmc4* (Rpt3) specifically in motor neurons. Tashiro and colleagues reported that depletion of Rpt3 was associated with progressive motor neuron loss and locomotor dysfunction (178). Surprisingly, the mice also developed neuropathological hallmarks of sporadic ALS, including inclusions immunoreactive for TDP43, FUS, optineurin and ubiquilin-2. These effects were not observed when autophagy was impaired by specific knockout of Atg7 in motor neurons, underlining the central role of the UPS in the maintenance of neuronal proteostasis (178).

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Age-related decline in proteasome activity

The brain is particularly susceptible to oxidative stress and protein misfolding due to a high rate of oxygen consumption and only low to moderate activities of antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase (59). Several studies have identified an age-related decline in proteasome activity which may account for the late onset of most sporadic and inherited neurodegenerative diseases (132). In human keratinocytes, an age-related decline in proteolytic activity was attributed to a decrease in proteasome number (150) and structural alterations in 20S proteasome subunits (26). Similar changes were reported for 26S proteasomes isolated from the lymphocytes of elderly donors (29). Declining proteasome activity can partly be explained by an age-related accumulation of reactive oxygen species (ROS) which result in oxidative modification of proteasome subunits (74). Disruption of 26S proteasome structure may be aggravated by the accumulation of aggregate-prone cross-linked proteins which have been shown to inhibit proteasome activity in conditions of oxidative stress (66).

The importance of preserved UPS activity to longevity was recently demonstrated in a transgenic mouse line with ubiquitous expression of the β_{5t} subunit, normally expressed only in the thymus (181). Since the enzymatically less active β_{5t} subunit is preferentially incorporated into 20S proteasomes in place of the β_5 subunit, these mice display reduced levels of proteasome activity. Compared with wild-type controls, β_{5t} mice have significantly elevated levels of polyubiquitinated and oxidised proteins, as well as a marked reduction in life span (181). Whilst the effect of reduced proteasome activity in the brain was not described, the authors reported an early onset of age-related metabolic disorders such as obesity and hepatic steatosis. In the context of neurodegenerative disease, a reduction in

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proteasome activity with age may shift the balance towards accumulation of mutated or aggregate-prone proteins and thus potentiate the onset of cytotoxicity.

Loss of proteasome activity in neurodegeneration

A reduction in all three proteasome peptidase activities has been reported in the brains of AD patients (106, 123). Similar impairment was also described in the spinal cord of the SOD1 G93A mouse model of familial ALS and sporadic ALS patients (97, 98). Whilst total proteasome number appeared unchanged, a decrease in the expression of specific proteasome subunits, including the β_5 catalytic subunit, was reported (36, 96). A reduction in all three peptidase activities was also found in the substantia nigra of sporadic PD brains, which may be accounted for by a marked reduction in levels of the α subunits which are critical for the structural integrity of the 20S core particle (134, 135). Proteasome dysfunction has since been replicated in transgenic and toxin-induced mouse models of PD, suggesting that these observations are unlikely to be an artifact of end-stage disease, post-mortem delay in tissue processing or limited sample size (32, 64). The mechanisms underlying proteasome impairment in neurodegenerative diseases remain controversial. A disruption in proteolytic activity may represent a primary mechanism of disease in which direct interactions between misfolded proteins and the proteasome impair its function. Alternatively, secondary effects of neurodegeneration, such as impaired ATP production or oxidative damage, may precipitate a decline in proteasomal activity.

Primary dysfunction of the proteasome

Alzheimer's disease

Aβ peptides are generated as a product of two sequential endoproteolytic cleavage events of amyloid precursor protein (APP) by β- and gamma- secretases (164). Growing evidence

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supports a role of soluble Aβ oligomers in AD neurotoxicity, including impairment of learning and memory function (reviewed in (193)). The triple-transgenic mouse model 3xTg-AD expresses three major genes associated with familial AD (APP_{Swe}, $PS1_{M146V}$ and tau_{p301L}) and develops plaque and tangle pathology which recapitulates that observed in AD patients (141). Treatment of 3xTg-AD mice with the proteasome inhibitor epoxomicin was found to accelerate accumulation of Aβ, suggesting the disease-associated protein is the subject of proteasomal degradation (184). Despite being catalytic substrates, misfolded Aβ species have also been shown to impair proteasome activity. A direct interaction between synthetic Aβ oligomers and purified human 20S core particle was sufficient to inhibit all three peptidase activities (184). Consistent with these *in vitro* observations, Aβ immunotherapy effectively reversed the age-dependent decline in proteasome activity in the 3xTg-AD mice (184). The cellular mechanism underlying this effect remains unclear since Aβ is produced in the secretory pathway and must gain cytosolic access in order to interact directly with proteasomes (192).

Tau is an abundant axonal cytosolic protein which associates with and stabilises microtubules. Phosphorylation of tau disrupts its interaction with microtubules and hyperphosphorylated forms of tau have been identified as a key component of paired-helical filaments (PHFs), the building blocks of NFTs commonly associated with AD neuropathology (89). A positive correlation between the number of NFTs and the duration and severity of AD has been reported (5). Tau can be degraded by a wide variety of cellular degradation systems including calpains, lysosomes and proteasomes. As a natively unfolded protein, tau can be degraded by the 20S proteasome without the need for ubiquitin modification (76), however a ubiquitin-dependent pathway has also been described. In cooperation with Hsc70, CHIP facilitates ubiquitin-dependent degradation of abnormal forms

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of tau, including hyperphosphorylated tau (56, 151, 167). These findings suggest that the UPS may play a selective role in the degradation of abnormal forms of tau, rather than normal soluble tau (88). Tau has also been found to interact directly with proteasomes in human AD brains (105). Incubation of isolated proteasomes with PHFs resulted in a marked reduction in proteasome activity (105). Taken together, these findings suggest that the accumulation of tau and Aβ may contribute to the overall reduction in UPS degradative capacity in human AD brains and emphasise that strategies to upregulate proteasome activity may be beneficial in aiding clearance of these misfolded protein species.

Parkinson's disease

Autosomal dominant cases of PD are associated with point mutations (A30P, A53T or E46K) in the *SNCA* gene which encodes α-synuclein (21). By combining *in vivo* pharmacologic and multiphoton imaging strategies, Ebrahimi-Fakhari and colleagues demonstrated that the UPS is the major degradation pathway for the clearance of endogenous and overexpressed levels of α-synuclein in the living mouse brain (61). In contrast, autophagy was only recruited to degrade α-synuclein when levels of the protein were massively increased. *In vitro* studies have provided conflicting accounts on the relative importance of the UPS and autophagy to the degradation of mutant forms of α-synuclein (191, 197). These variable results are likely a result of different α-synuclein expression levels and cell culture conditions, which can have a marked effect on the proteolytic processing of α-synuclein.

Since α-synuclein knockout mice lack an overt phenotype which resembles PD, mutated forms of the protein are thought to be associated with a toxic gain-of-function (1). Stable overexpression of mutant α-synuclein was shown to impair proteasomal function in several mammalian cell lines (60, 62), a transgenic mouse model (32) and a novel zebrafish model of

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Parkinson's disease (152). In PC12 cells, soluble, intermediate size oligomers of mutant α-synuclein were found to co-elute with the 26S proteasome and were associated with a significant inhibition of its catalytic activity (62). Several studies have also reported a direct physical interaction between aggregated forms of α-synuclein and the proteasome (121, 170, 212). Zhang and colleagues demonstrated that α-synuclein protofibrils, but not monomeric or dimeric species, bound purified 26S proteasome and resulted in a marked inhibition of ubiquitin-dependent and -independent proteasomal degradation (212). Due to the large size of the protofibrils relative to the narrow 20S channel pore, this inhibitory effect could result from allosteric inhibition of substrate translocation or sequestration of proteasomal substrates prior to their degradation. Since impairment of proteasome activity can reduce α-synuclein solubility, a positive feedback loop may be established in PD whereby mutant α-synuclein inhibits the proteasome, leading to further accumulation of misfolded protein species and additional suppression of proteasome activity (60).

Amyotrophic lateral sclerosis

More than 150 autosomal dominant mutations have been identified in the gene encoding SOD1, which together account for up to 25% of familial ALS cases (20). Misfolded wild-type SOD-1 has also been identified in sporadic ALS which represents more than 90% of disease cases (22, 65). Misfolded forms of SOD1 are not typically associated with a loss of antioxidant enzyme activity, suggesting that a toxic gain-of-function is likely to be the primary mechanism of pathology (188). Accumulation of $SOD1^{G93A}$ in immortalised motor neurons resulted in marked inhibition of proteasome activity, as measured by the reporter substrate YFPu (45, 160). Using double transgenic $SOD1^{G93A}$ mice which express the proteasome reporter substrate Ub^{G76V}-GFP, Cheroni and colleagues identified UPS dysfunction in the spinal and cranial motor neurons of symptomatic mice (35). The

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appearance of the reporter was attributed to reduced expression of catalytic and non-catalytic proteasome subunits, however these effects were observed after the onset of other pathological hallmarks such as mitochondrial swelling and disrupted axonal transport. The identification of UPS dysfunction as a late-stage effect in ALS is supported by data suggesting that detergent-insoluble mutant SOD1 only becomes ubiquitinated after its aggregation in the spinal cord of $SOD1^{G93A}$ mice (9). As previously described for α-synuclein, inhibition of the proteasome is associated with an exponential increase in levels of insoluble aggregated $SOD1^{G93A}$ (160). Thus, late impairment of the proteasome in ALS may contribute to the severe neurotoxicity which ultimately overwhelms motor neurons at end-stage disease.

Huntington's disease

Prior to its degradation, huntingtin (Htt) is phosphorylated by IKK, activating the protein for ubiquitination and subsequent clearance by the proteasome and lysosome (180). Expansion of the Htt polyQ repeat may reduce the efficiency of this phosphorylation, leading to impaired clearance and accumulation of mtHtt. Early *in vivo* studies in the conditional HD94 (54) and double transgenic R6/2 ubiquitin-reporter mouse models (15, 133) reported that the UPS remained functionally active in HD. These findings appeared to contradict the marked accumulation of polyubiquitin chains in the brains of R6/2 mice and human HD patients, including Lys-48 linked conjugates which have been established as the proximal substrates of proteasomal proteolysis (12). This apparent controversy was elegantly resolved by Ortega and colleagues who crossed inducible HD94 mice with Ub^{G76V}-GFP proteasome reporter mice to show transient dysfunction of the UPS shortly after induction of mtHtt expression, which was reversed on formation of inclusion bodies (145). Prevention of aggregate formation with the drug riluzole blocked this recovery, suggesting that inclusion body

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formation may be an important neuroprotective response against proteotoxic stress. Wang and colleagues have also reported synapse-specific loss of proteasome activity in R6/2 mice by measuring peptidase activity in isolated synaptosomes and by fusing synaptic targeting sequences to the GFPu proteasome reporter (194) .

One possible explanation for observed UPS dysfunction in HD is that aggregate-prone polyQ proteins become trapped in the channel of the 20S core particle, blocking access to other ubiquitinated substrates. This theory was recently dismissed by the observation that ubiquitinated mtHtt, whether aggregated or not, did not clog purified 26S proteasomes (81). Instead, proteasomes may become sequestered into polyQ aggregates, supported by evidence of an increase in proteasome activity in the insoluble cellular fractions of mtHtt-Q150 expressing neuronal cells (92) and the spatial restriction of proteasomes within aggregates in Fluorescence Recovery After Photobleaching (FRAP) experiments (83). Whilst proteasome sequestration may contribute to UPS dysfunction, it is unlikely to play a major role since a large proportion of the neuronal proteasome population remains “free”. Recently, Hipp and colleagues proposed a theory of global proteostasis network dysfunction in which rising concentration of mtHtt causes delayed maturation of other cellular chaperone-clients, promoting their ubiquitination and proteasomal degradation (81). Subsequent competition between increasing numbers of ubiquitinated substrates may result in UPS dysfunction, independent of any impairment in proteasome activity.

Prion diseases

Although the critical pathological event in prion pathogenesis is thought to be the templated conversion of soluble PrP^{C} into insoluble aggregate-prone PrP^{Sc}, the mechanisms underlying neurotoxicity remain unclear. The brains of prion-infected mice have increased levels of

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ubiquitin conjugates, which correlate with a reduction in proteasome catalytic activities (103). Prion infection of neuroblastoma cells and transgenic mice expressing the proteasome reporter substrate Ub^{G76V}-GFP revealed functional impairment of the UPS (113). Incubation of purified 26S proteasomes with semipurified PrP^{Sc} from diseased brains or recombinant β-sheet rich forms of PrP resulted in a marked reduction in all three peptidase activities (113). Similar impairment was not observed when purified 26S proteasomes were incubated with recombinant PrP folded into a predominantly α-helical structure similar to that of PrP^{C}. A direct interaction between the 20S core particle and misfolded PrP isoforms was found to stabilise the closed conformation of the substrate entry channel, inhibiting the translocation of ubiquitinated substrates into the catalytic chamber for degradation (53). These findings contradict a recent study which reported no evidence of UPS impairment in transgenic mice expressing mutant, albeit non-infectious, PrP isoforms associated with inherited prion diseases (154). This apparent discrepancy may be explained by differences in the subcellular trafficking pathways of different PrP isoforms, which play a critical role in determining whether misfolded PrP species gain access to the cytosol to interact with the proteasome.

Secondary dysfunction of the proteasome

As an ATP-dependent process, the efficiency of ubiquitinated substrate degradation by the 26S proteasome is inextricably linked to mitochondrial respiration. MtHtt was shown to interfere with the association of microtubule-based transport proteins with mitochondria, leading to a reduction in mitochondrial trafficking (144). Consistent with these observations, reduced ATP content has been detected in synaptosome fractions prepared from the brains of HD knock-in mice (144, 194). Early aberrations in mitochondrial structure and function have also been reported in the $SOD1^{G93A}$ mouse model of ALS (126). In addition to impaired ATP

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production, dysfunctional mitochondria are major sources of oxidative stress through the production of ROS (185). Treatment of neuroblastoma cells with rotenone, an inhibitor of mitochondrial complex I, resulted in impairment of proteasome activity and a secondary decline in levels of the 20S core particle (40, 166). Taken together, these findings suggest that mitochondrial dysfunction may contribute to UPS impairment in neurodegenerative disease by depleting critical ATP levels and inducing oxidative damage of proteasome subunits.

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Therapeutic strategies to enhance UPS activity

(1) Enhance ubiquitination

Maintenance of ubiquitin reserves

In the human brain, 82% of processed ubiquitin is found as free monomer, which may function as an important reserve for the rapid degradation of misfolded proteins in conditions of cell stress (101). In neurodegenerative disease, the accumulation of ubiquitinated protein aggregates may result in "trapping" of ubiquitin molecules, depleting ubiquitin reserves with detrimental effects on ubiquitin-dependent proteolysis (77). Consistent with this theory, mouse models of ubiquitin depletion are associated with a severe neurodegenerative phenotype (3, 159). Strategies to augment ubiquitin levels may help to protect against progressive proteostasis disruption. Levels of free ubiquitin could be stabilised by overexpression of UCH-L1, which has been shown to bind monoubiquitin with high-affinity and increase ubiquitin half-life in cultured cells and mice (146).

Upregulation of E3 ligase activity

In addition to the stabilisation of ubiquitin reserves, ubiquitin-dependent proteolysis could be enhanced by upregulation of E3 ligase activity. Parkin gene therapy may be an effective strategy to mitigate neuronal toxicity associated with the accumulation of misfolded forms of α-synuclein, particularly in autosomal recessive forms of PD characterised by loss of function mutations in the *PARKIN* gene (138). Injection of a recombinant adeno-associated viral vector carrying parkin cDNA (rAAV1-parkin) successfully reduced accumulation of α-synuclein when co-expressed in the striatum of macaque monkeys (207). The E3 ligase CHIP is another important regulator of ubiquitination and could represent a gene therapy target for the treatment of various neurodegenerative diseases. In a cell culture model of PD,

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overexpression of CHIP reduced levels of α-synuclein by promoting its proteasomal and lysosomal degradation (168). In cell culture models of HD and MJD, overexpression of CHIP increased the proteasomal degradation of polyQ-expanded huntingtin and Atx3, respectively (91, 200). The potential therapeutic benefit of CHIP overexpression *in vivo* was demonstrated in the 3xTg-AD mouse model where injection of a CHIP-expressing lentivirus rescued animals from Aβ-induced tau pathology (142).

Modulation of E3 ligases is an attractive therapeutic approach since the specificity of ligase-substrate interactions can restrict effects to a single cellular pathway, rather than the UPS as a whole. Despite this, traditional methods of gene overexpression by viral delivery of cDNA can be limited by compensatory changes in protein networks or a lack of spatiotemporal control. As a result, small-molecule modulators that modify protein activity at the post-translational level may be preferable alternatives. Proteolysis targeting chimeric molecules, or PROTACs, are small heterobifunctional molecules designed to induce the degradation of specific target proteins by the UPS (162). PROTACs comprise two distinct recognition motifs separated by a linker moiety. One motif recognises the target protein of interest; the other recognises a specific E3 ligase, enhancing ubiquitination and proteasomal degradation of the selected target protein. This technology has proved effective in the selective degradation of hormone receptors in prostate and breast cancer cells, leading to growth arrest in G1 and ultimately apoptosis (155). Similar approaches may enable the selective proteolysis of disease-associated misfolded proteins in neurons; however further development of PROTAC molecules will be required prior to testing in animal models and humans.

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(2) Enhance proteasome activity

As previously discussed, impairment in proteasome activity has been reported in post-mortem brain or spinal cord tissue from patients with AD (106, 123), PD (134, 135) and ALS (97). Faced with rising levels of misfolded proteins, proteasomal insufficiency may become the rate-limiting step in the UPS, leading to a backlog of ubiquitinated proteins. As a result, strategies to enhance substrate ubiquitination may have limited therapeutic potential, particularly in patients with established neurodegenerative disease. An alternative strategy is to induce activity of the 26S proteasome, enhancing clearance of toxic misfolded proteins to ensure that cellular proteostasis is maintained.

20S core particle induction

Despite an abundance of small molecule inhibitors of the proteasome, effective methods of upregulation of the proteasome remain scarce. To date, strategies to enhance proteasome activity have predominantly focussed on the genetic upregulation of specific proteasome subunits, however a small number of compounds have been identified which can stimulate proteolytic activity *in vitro*. Early evidence that proteasome activity could be increased came from genetic manipulation of 20S core particle subunits. Goldberg and colleagues observed that lymphoblasts and HeLa cells transfected with the inducible β_{5i} subunit had increased chymotrypsin- and trypsin-like peptidase activity (68). Transfection of the inducible β_{1i} subunit into the same cell lines resulted in a selective increase in trypsin-like activity. Later work by the same group reported an increase in caspase-like activity following overexpression of the constitutive β_1 subunit in HeLa cells (67). Interestingly, such genetic manipulation of a single proteasome subunit appears to be sufficient to drive changes in the proteasome complex as a whole. Chondrogianni and colleagues reported that stable overexpression of the β_5 subunit in W138/T and HL60 cells resulted in upregulation of the

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other β-type subunits and recruitment of "free" α-type subunits to increase the number of assembled 20S complexes (39). Evidence of co-regulation of proteasome subunit levels was also observed when β_5 was overexpressed in lens epithelial cells and aged human fibroblasts (86, 122). The resulting increase in proteolytic activity enhanced the clearance of oxidised proteins and promoted cell survival. These findings were replicated by overexpression of the POMP proteasome accessory protein in human fibroblast cultures, suggesting that the rate-limiting step in proteasome activity may be 20S core particle assembly, rather than the expression level of individual proteasome subunits (38). Taken together, these studies confirm that activation of the proteasome is feasible by genetic manipulation of 20S proteasome subunits or proteasome accessory proteins.

Stimulation of proteasome activity *in vitro* has also been reported following treatment with various natural compounds, including some fatty acids such as olein, linoleic and linolenic acids, as well as oleuropein isolated from *Olea europaea* leaves (47, 104, 195). These compounds are thought to activate the proteasome through structural changes that promote opening of the 20S gate. Synthetic peptidyl alcohols, nitriles, *p*-nitroanilides and esters were also shown to stimulate proteasome catalytic activity, possibly through interaction with the PA28 activator binding site (199). In most cases, the mechanisms underlying compound-mediated activation of the proteasome remain unclear, limiting their use for research or therapeutic purposes.

19S/11S regulatory particle induction

Consistent with their role in activation of the 20S core particle, upregulation of various subunits of the 19S or 11S regulatory particles has been shown to enhance proteasome activity. PA28, also known as the 11S regulatory particle, can consist of a heteroheptamer of

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$PA28_{\alpha}$ and $PA28_{\beta}$ subunits or a homoheptamer of PA28γ subunits. Ectopic expression of PA28γ was shown to recover proteasome function in HD patient fibroblasts and improved survival in mtHtt-expressing striatal neurons in conditions of excitotoxic stress (165). In *Drosophila*, overexpression of the 19S subunit *Rpn11* delayed the age-related decline in 26S proteasome activity and slowed polyglutamine-induced neurodegeneration (182). Vilchez and colleagues recently reported that overexpression of the 19S regulatory subunit Rpn6 was sufficient to prolong lifespan and confer resistance to proteotoxic stress in *C.elegans* (189). Rpn6 has been shown to interact with α2 and Rpt6 subunits, suggesting an important role in stabilising the otherwise weak interaction between 20S core particle and 19S regulatory particle (147). Consistent with these observations, ectopic expression of Rpn6 was sufficient to increase assembly of 26S proteasomes *in vivo*, with an associated increase in proteasome activity and clearance of polyubiquitinated substrates (189). These findings may have relevance to neurodegenerative disease, since Rpn6-overexpressing worms also displayed enhanced clearance of an aggregated polyQ protein. Taken together, these findings suggest that upregulation of *PSMD11*, the mammalian homologue of Rpn6, may be a potential strategy to enhance misfolded protein clearance in the context of neurodegenerative disease.

In addition to modulation of regulatory subunit expression levels, recent studies have identified an important role of post-translational modifications in the control of proteasome activity. Phosphorylation of Rpt6 by PKA was sufficient to enhance assembly and activity of the 26S proteasome (7, 211). Chronic treatment with CGS, an agonist of the A_{2a} adenosine receptor, rescued proteasome activity and facilitated mtHtt clearance in the striatum of the R6/2 HD mouse model (120). These findings suggest that drugs targeting the A_{2a} receptor and other cAMP-inducing reagents may prove beneficial in the treatment of HD and other neurodegenerative diseases characterised by proteasomal insufficiency.

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Inhibition of polyubiquitin chain-trimming

Recently, the small molecule inhibitor IU1 was shown to accelerate proteasomal degradation of oxidised and misfolded proteins in cultured cells, including the disease-associated proteins Tau, TDP-43 and Atx3 (115). This enhancement of proteasome activity was shown to be independent of any change in proteasome subunit composition and instead relied on inhibition of ubiquitin chain-trimming by the proteasome-associated DUB Usp14. Chain-trimming by Usp14 is thought to occur in a stepwise manner, disassembling the chain from its distal tip, which may suppress proteolytic activity by promoting substrate dissociation from the proteasome prior to degradation. Thus, inhibition of Usp14 by IU1 may act to stabilise ubiquitinated substrates on the proteasome until they are unfolded and translocated into the 20S core particle for proteolytic clearance. It remains unclear whether IU1 provides resistance to proteotoxic stress in neurons and more importantly, whether it has efficacy in animal models of neurodegenerative disease.

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Conclusion

Proteostasis network dysfunction due to impairment of the UPS is likely to have pleiotropic effects in the neurodegenerative brain. Whilst distinct pathogenic mechanisms will operate in different neurodegenerative diseases, many common pathways have been proposed which could contribute to progressive neurotoxicity, synaptic dysfunction and ultimately cell death (Fig. 5). Therapeutic strategies to target these downstream neurotoxic sequelae may have limited efficacy due to their late-onset in the course of disease progression. Thus, early intervention to compensate for the accumulation of misfolded proteins by induction of protein catabolism may be an important defensive, or even therapeutic, strategy against these age-related disorders. At first glance, the UPS appears an unlikely target since ubiquitination and proteasomal degradation are involved in the regulation of most cellular protein networks. However, carefully tailored therapies which target CNS-specific components of the UPS may restrict adverse effects. CNS-specific induction of general UPS components such as the 20S or 19S complexes could be achieved by the use of viral vectors with neuronal tropism. Alternatively, viral delivery of specific E3 ligases (e.g. parkin, CHIP) which target defined disease-associated proteins could represent customised therapies for the treatment of different neurodegenerative conditions. Whilst considerable progress has been made in the development of viral vector-based therapies for clinical use, significant challenges still remain including the regulation of transgene expression levels and methods for widespread anatomical delivery (198). Due to these technical limitations, the development of synthetic or naturally occurring compounds which upregulate the UPS and are capable of crossing the blood-brain barrier will remain a research priority. The success of any future therapies will depend upon the identification of reliable biomarkers to facilitate early diagnosis and allow intervention before global disruption of the cellular proteome becomes established (143).

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Abbreviations used	
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
Atx3	ataxin-3
Aβ	β-amyloid protein
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CHIP	carboxy terminus of Hsc70-interacting protein
CNS	central nervous system
DLBD	diffuse Lewy body disease
ERAD	ER-associated protein degradation
FRAP	fluorescence recovery after photobleaching
FUS	Fused in Sarcoma protein
HD	Huntington's disease
IB	inclusion body
IBMPFD	Inclusion body myopathy with Paget's disease of bone and frontotemporal dementia
IKK	IκB kinase
IMM	inner mitochondrial membrane
LB	Lewy body
LTD	long term depression
LTP	long term potentiation
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mtHtt	mutant huntingtin
NFT	neurofibrillary tangle
OMM	outer mitochondrial membrane
PD	Parkinson's disease
PHF	paired-helical filament
PKA	protein kinase A
POMP	proteasome maturation protein
PROTAC	proteolysis targeting chimeric molecules
PrP	prion protein
PrP^{C}	normal isoform of the prion protein
PrP^{Sc}	disease-associated prion protein
Rpt	regulatory particle triple-A
Rpn	regulatory particle non-ATPase
rAAV	recombinant adeno-associated virus
RIM1	Rab3-interacting molecule 1
ROS	reactive oxygen species
SCA3	Spinocerebellar ataxia type 3
SOD1	superoxide dismutase 1
SPAR	spine-associated Rap GTPase activating protein
TDP-43	TAR-DNA binding protein 43
UIM	ubiquitin-interacting motif
UPS	ubiquitin-proteasome system
VCP	valosin-containing protein

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Tables

Table 1. Neuropathological hallmarks of neurodegenerative diseases

Disease	Neuropathological hallmarks	Affected brain regions	Ubiquitinated inclusions
Alzheimer's disease (AD)	Extracellular Aβ deposits and intracellular NFTs (23, 24)	Transentorhinal, limbic and neocortical regions (23, 24)	+
Parkinson's disease (PD)	Intracytoplasmic LB composed of α-synuclein (84)	Predominantly substantia nigra, with additional LB pathology in brainstem and cortical regions (84)	+
Huntington's disease (HD)	Intranuclear and intracytoplasmic IB composed of mtHtt (157)	Predominantly striatum, with additional atrophy of cerebral cortex and subcortical white matter (157)	+
Amyotrophic lateral sclerosis (ALS)	Intranuclear and intracytoplasmic aggregates of ALS-associated proteins (e.g. SOD1, FUS, TDP-43) (44)	Cortical, bulbar and spinal motor neurons (44)	+
Prion diseases	Intracytoplasmic and extracellular deposition of PrP^{Sc} (177)	Heterogeneous distribution including cortex, cerebellum, striatum, thalamus, hippocampus and brainstem (90)	+

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Figure legends

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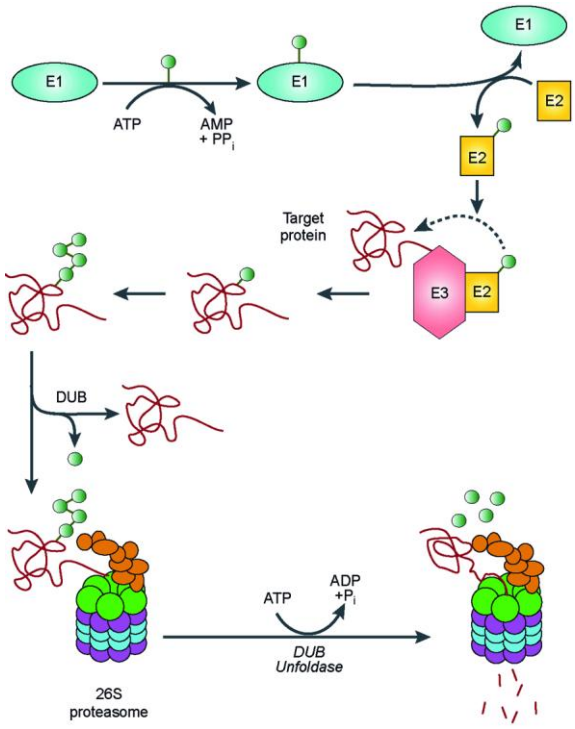

Figure 1. The Ubiquitin-Proteasome System

Ubiquitin is activated by the ubiquitin-activating enzyme E1, generating a high-energy thiol ester intermediate. Ubiquitin is next transferred, still as a high-energy intermediate, to an ubiquitin-conjugating enzyme, E2. From E2, ubiquitin is covalently attached to an internal lysine residue of a target protein that is bound specifically to an E3 ligase. By a similar mechanism, successive addition of ubiquitin moieties to the previously conjugated one generates a polyubiquitin chain. Ubiquitin conjugation is reversible by the action of DUBs. Polyubiquitin chains are recognised by the 26S proteasome as a degradation signal. This enzyme complex unfolds the substrate in an ATP-dependent manner, recycles conjugated ubiquitin moieties by the action of proteasome-associated DUBs and lastly degrades the target protein into short peptides. (To see this illustration in color the reader is referred to the web version of this article at www.libertonline.com/ars).

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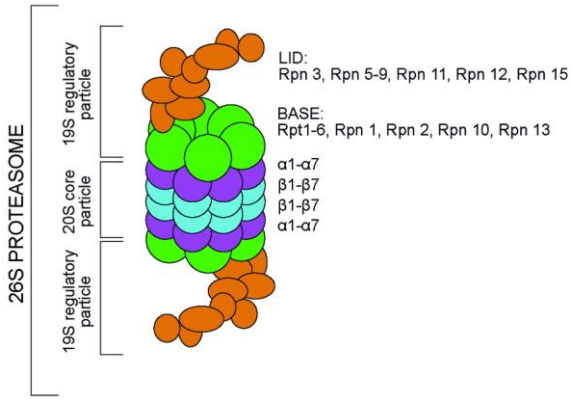

Figure 2. The 26S proteasome

The 26S proteasome consists of the catalytic 20S core particle, comprising four stacked rings (two outer α-rings and two inner β-rings) and one or two 19S regulatory particle(s). The regulatory particle is composed of lid and base subcomplexes, which contain regulatory particle triple-A (Rpt) and regulatory particle non-ATPase (Rpn) subunits. (To see this illustration in color the reader is referred to the web version of this article at www.libertonline.com/ars).

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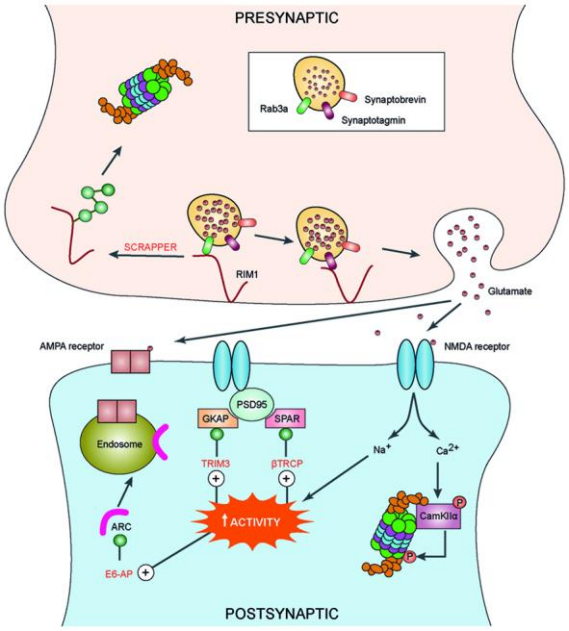

Figure 3. UPS-mediated degradation of proteins in synaptic plasticity

A schematic diagram of the UPS pathways that regulate synaptic plasticity (see text for details). The release of neurotransmitters from presynaptic terminals by synaptic vesicle exocytosis results in stimulation of post-synaptic NMDA and AMPA glutamate receptors. The E3 ligase SCRAPPER mediates the ubiquitination and degradation of the presynaptic vesicle priming factor RIM1, negatively regulating neurotransmitter release. Activation of NMDA receptors triggers ubiquitination of synaptic scaffold proteins and recruitment of proteasomes to dendritic spines by interaction with autophosphorylated CAMKIIα, which also enhances proteolytic activity by phosphorylation of Rpt6. Increased neuronal activity results in internalisation of AMPA receptors by E6-AP mediated degradation of Arc. (To see this illustration in color the reader is referred to the web version of this article at www.libertonline.com/ars).

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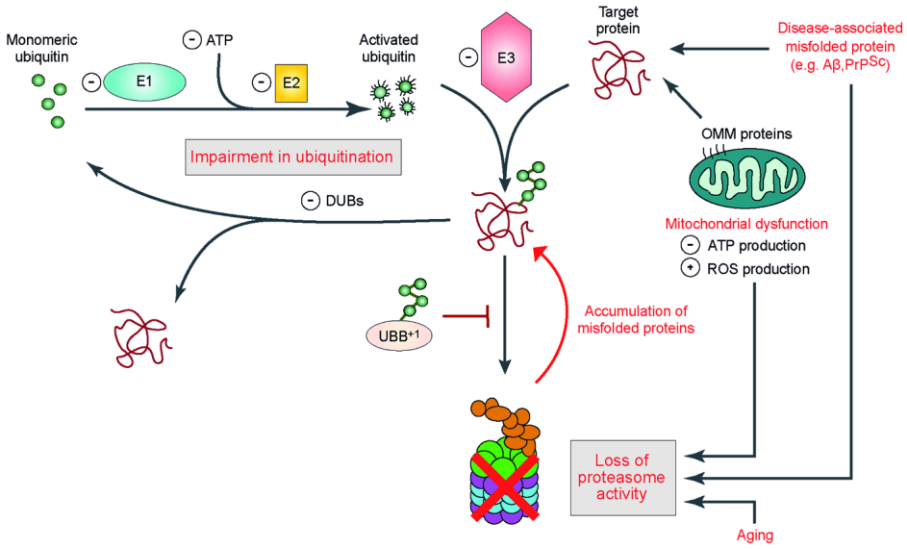

Figure 4. Dysfunction of the UPS in neurodegenerative disease

Dysfunction of the UPS can arise from impairments in ubiquitination due to depletion or reduced activity of E1, E2 or E3 enzymes. In addition, impaired degradation of target proteins can arise from direct inhibition of proteasome activity by disease-associated misfolded proteins, aggravated by an age-related decline in catalytic activity. Secondary impairment of the proteasome can occur in conditions of mitochondrial dysfunction, due to depletion of ATP and production of ROS. Loss of proteasome activity results in the accumulation of misfolded proteins, which can further impair catalytic activity. (To see this illustration in color the reader is referred to the web version of this article at www.libertonline.com/ars).

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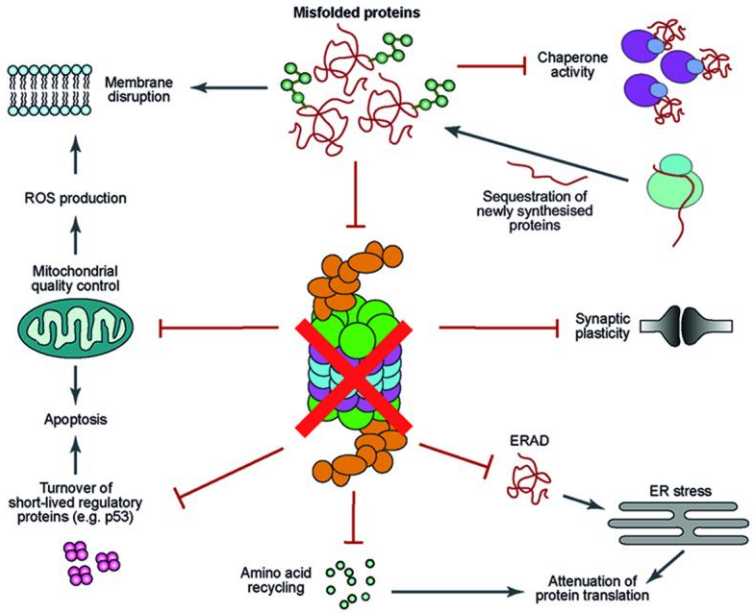

Figure 5. Neurotoxic sequelae of UPS dysfunction

Dysfunction of the UPS is associated with widespread disruption of cellular homeostasis. Due to its critical role in the degradation of synaptic proteins, impairment of the UPS is associated with a loss of synaptic plasticity. Progressive accumulation of misfolded proteins can overwhelm molecular chaperones, leading to the delayed maturation of other chaperone-clients (81). β-sheet rich disease-associated proteins can also sequester newly-synthesised proteins, many of which occupy critical hub positions in cellular protein networks (143). The exposure of interior hydrophobic residues in misfolded proteins can disrupt biological membranes leading to reduced cellular viability. Due to its critical role in the degradation of OMM proteins, impairments of the UPS can lead to a loss of mitochondrial quality control, leading to production of ROS and apoptosis. A pro-apoptotic state can also arise from the accumulation of short-lived regulatory proteins such as p53. Proteasome failure is associated with a depletion of amino acids (173) and impairment of the ER-associated protein degradation (ERAD) pathway, which can ultimately lead to a lethal attenuation of protein translation. (To see this illustration in color the reader is referred to the web version of this article at www.libertonline.com/ars).

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