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Role of ubiquitin-proteasome-mediated proteolysis in nervous system disease

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Abstract

Proteolysis by the ubiquitin-proteasome pathway (UPP) is now widely recognized as a molecular mechanism controlling myriad normal functions in the nervous system. Also, this pathway is intimately linked to many diseases and disorders of the brain. Among the diseases connected to the UPP are neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases. Perturbation in the UPP is also believed to play a causative role in mental disorders such as Angelman syndrome. The pathology of neurodegenerative diseases is characterized by abnormal deposition of insoluble protein aggregates or inclusion bodies within neurons. The ubiquitinated protein aggregates are believed to result from dysfunction of the UPP or from structural changes in the protein substrates which prevent their recognition and degradation by the UPP. An early effect of abnormal UPP in diseases of the nervous system is likely to be impairment of synaptic function. Here we discuss the UPP and its physiological roles in the nervous system and how alterations in the UPP relate to development of nervous system diseases.

1. Introduction

The link between ubiquitin-proteasome-mediated proteolysis and diseases of the nervous system has come full circle. Originally ubiquitin immunoreactivity was observed by pathologists in diseased brain tissue. No causal link between the presence of ubiquitin and the diseased states was apparent at the time. When the role for ubiquitin in protein degradation was first discovered, it was thought that it targeted only abnormal proteins for degradation. During the 1990s a physiological role for the ubiquitin-proteasome pathway (UPP) was first established in progression of cell cycle. This was soon followed by the discoveries that connected ubiquitin-proteasome-mediated proteolysis to synaptic plasticity. Many studies have established the role of the ubiquitin pathway in various other normal functions of the brain such as development of synaptic connections. With the advancement of our understanding of normal role of the UPP in many parts of the body including the brain, causative links between protein degradation and abnormalities of the brain are now beginning to be elucidated.

In the nervous system, the UPP has been connected to several diseases such as Alzheimer's [1], Parkinson's [2] and Huntington's [3] diseases. A common feature among several chronic neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease

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(PD), motor neuron disease, and prion encephalopathies is abnormal deposition of insoluble protein aggregates or inclusion bodies within neurons. Nuclear insoluble inclusions are also observed in disorders associated with polyglutamine repeats, such as Huntington's disease and spinocerebellar ataxia. Some studies indicate that impairment in the UPP may be linked to disorders such as schizophrenia [4].

A hallmark of neurodegenerative disorders such as AD and PD is the presence of ubiquitin immunoreactivity in the neuronal inclusions such as Lewy bodies and neurofibriallary tangles (NFTs). Ubiquitin immunoreactivity in the intracellular inclusions results from accumulation of ubiquitinated proteins. The accumulation of ubiquitin-protein conjugates in neuropathological lesions was detected first in NFT [5] and later in inclusions observed in a whole range of neurodegenerative diseases [6]. Although different parts of the brain are affected in different neurodegenerative disorders, the presence of ubiquitinated proteins in all the neurodegenerative disorders suggests the possibility that these diseases are associated with an inability of the neuron to degrade specific proteins or accumulated protein aggregates. Impairment of the UPP may contribute to the neurodegenerative process as this proteolytic pathway is known to degrade short-lived regulatory proteins and misfolded or abnormal proteins. The ubiquitinated protein aggregates are likely to result from malfunctioning of the UPP or changes in the protein substrates themselves which renders them resistant to degradation. In addition to a possible role in neurodegeneration, the UPP is known to play role in synaptic function and synaptic plasticity [7,8]. Therefore, malfunctioning of this pathway might also lead to brain disorders without neurodegeneration such as Angelman syndrome which is characterized by mental retardation. In order to comprehend the role of the UPP in abnormalities of the nervous system, it would be helpful to understand its role in the normal nervous system. Therefore, we first describe the UPP and its physiological role in the nervous system, and then discuss the impairment of this pathway in diseases of the nervous system.

2. The ubiquitin-proteasome pathway

The main function of the UPP is to degrade proteins. Proteolysis by the UPP is highly regulated and specific: only proteins that are marked by attachment of ubiquitin are degraded and only at the right time. The tagging of a protein substrate with ubiquitin is regulated by the action of three enzymes: E1, E2, and E3 (So named because of the order of their elution on anion-exchange chromatography [9]). First, ubiquitin is activated by E1. Then, the activated ubiquitin is transferred to one of a family of enzymes termed E2s, which associate with E3 ubiquitin ligases. The E3s ligate the ubiquitin to a lysine (Lys) residue in the protein substrate. A second ubiquitin is covalently attached to the first ubiquitin and thus a polyubiquitin chain is formed. Ubiquitin attachment to other ubiquitins could occur through any of the seven lysine residues (Lys-6, Lys-11, Lys-27, Lys-29, Lys-33, Lys-48 and Lys-63) in the ubiquitin molecules. For marking the substrate for ubiquitin-proteasome-mediated degradation, additional ubiquitins are attached to Lys-11 or Lys-48 in the first ubiquitin. Lys-63-linked polyubiquitin chains are known to have non-proteolytic function such as marking substrates for endocytosis or a role in intracellular signaling such as NFκ-B activation [10]. The functions of polyubiquitin chains with Lys-6, Lys-27, Lys-29 and Lys-33 linkages have not been elucidated [11,12]. The polyubiquitinated substrate with Lys-11 or Lys-48 linkage is recognized by the proteasome and is degraded to small peptides and amino acids [13,14] (Fig. 1).

Before a substrate is recognized by the proteasome and is fated to be degraded, ubiquitination can be reversed by deubiquitinating enzymes (DUBs). The polyubiquitin chains on the substrates degraded by the proteasome are not degraded but disassembled by DUBs that are part of or associated with the proteasome [15] (Fig. 1).

2. 1. Ubiquitin conjugation

The series of enzymatic reactions in the process of attaching a ubiquitin molecule to a substrate is called ubiquitin conjugation or ubiquitination. First reaction in this series is activation of ubiquitin by the enzyme E1. The activation of ubiquitin is ATP dependent. ATP is hydrolyzed to generate AMP, and a high energy thiol-ester ubiquitin-AMP intermediate is formed. The activated ubiquitin is then transferred to an E2, which is also called a ubiquitin carrier. The ubiquitin molecule is then transferred to an E3 which ligates ubiquitin to an ε amino group of a lysine residue in the substrate protein. A second ubiquitin molecule is then attached to an internal lysine reside in the first ubiquitin and thus a polyubiquitin chain grows[13].

2.2. Ubiquitin-conjugating Enzymes: E1, E2, and E3

Among the three classes of ubiquitin-conjugating enzymes, E1 is the least physiologically regulated. E2s are more selective and are believed to interact with specific E3s. Substrate specificity of the ubiquitin conjugation reaction is largely determined by E3s.

Originally, E2 enzymes were believed only to carry the activated ubiquitin and transfer it onto the E3s. Recent studies, however, suggest that at least some E2s can directly conjugate ubiquitin to substrates and are now commonly referred to as ubiquitin conjugating enzymes. E2s are structurally and functionally diverse. Simple eukaryotes like yeast (*Saccharomyces cerevisiae*) have 13 genes potentially encoding E2s. The number of E2s in mammals is estimated to be in the range of 25–30. The diversity of E2 generates some degree of specificity in the ubiquitin-conjugating reaction. E2s transfer ubiquitin to the substrates directly or in case of some ligases through generation of E3~ubiquitin thioester intermediates. The substrate-specificity of ubiquitin ligation is essentially determined by E3s. Sometimes an E3 might prefer a particular E2 by binding to a specific non-catalytic amino or carboxyl terminal extension in an E2. In such cases only specific E2–E3 pairs can ligate ubiquitin to substrates. Since the diversity of E3s is even greater than that of E2s, the combination of E2s and E3s potentially can generate a high degree of specificity [16].

There are two major classes of E3s: HECT (homologous to E6-AP C-terminus) domain E3s and RING (really interesting new gene) finger E3s.

2.2.1. HECT domain E3s—The typical example in this class of E3 is the ubiquitin ligase called E6-AP, which ligates ubiquitin to the tumor suppressor protein p53 in human papilloma virus (HPV) infected cells [17]. E6 protein, which is encoded by oncogenic strains of HPV, associates with a cellular protein called E6-AP (E6-associated protein). The C-terminal region of E6-AP contains the catalytic domain of the ubiquitin ligase. E6-AP ligase can function with either of the E2s called UbcH5 and UbcH7 (Fig. 2). Later studies found that a family of ubiquitin ligases with homology to the catalytic domain of E6-AP exists. These ubiquitin ligases are called HECT domain E3s. In addition to the HECT domain, there is another domain in many E3s called the WW domain. The WW domain-containing E3s also tend to have a C2 domain. The presence of the C2 domain is highly relevant to nervous system function because the C2 domain responds to elevation of intracellular Ca^{2+} and helps in translocation to the plasma membrane. Therefore, presence of this domain in neuronal HECT E3s might be critical in ligating ubiquitin to neurotransmitter receptors or proteins associated with them [7,13].

2.2. 2. RING finger E3s—These E3s are called RING finger E3s because they contain a RING finger domain, which consists of seven cysteine residues and one histidine residue forming a single folded domain binding two zinc ions. During the past few years, numerous ubiquitin ligases were found to contain the RING finger. Unlike the HECT ligases, the

RING finger ligases do not form a thioester intermediate with ubiquitin. Rather, these E3s bind both the substrate and the E2 and mediate the transfer of ubiquitin from the E2 to the substrate. It is now generally believed that the RING finger motif plays a critical role in ubiquitin ligation to substrates or to RING finger proteins themselves. The RING finger category of E3s can be subdivided into single-subunit RING finger E3s and multi-subunit RING finger E3s [14](Fig. 2).

2.2.2.1. Single-subunit RING finger E3s: Single-subunit RING finger E3s contain the RING finger domain and the substrate recognition site in the same protein. One of the well-characterized single-subunit RING finger E3s is Mdm2, which ubiquitinates a postsynaptic density protein PSD-95 in neurons [18]. Another widely studied ligase in this category is parkin which was originally discovered through its association with juvenile recessive parkinsonsism [19].

2.2.2.2. Multi-subunit RING finger E3s: There are two major examples of Multi-subunit RING finger E3s SKP1–cullin–F-box protein (SCF) complex and Anaphase-promoting complex (APC) (Fig. 2).

SCF complex contains at least four proteins: Skp1, Cul1, Roc1/Rbx1/Hrt1, and an F-box protein. The SCF-type ligases have another invariant protein called cullin. The theme appears to be that the cullins provide a scaffold on which the ligase complex is built. Cullins interact with linker proteins such as Skp1 to recruit substrate-interacting proteins such as the F-box proteins. In the center of the SCF complex is the RING finger domain-containing protein Rbx1. The ligases like SCFs are often referred to as cullin-RING ligases [20]. There are at least five different cullins in mammals. There are several F-box proteins as well. A well-characterized substrate of the SCF complex is IkBα [21].

APC also has a subunit with a RING finger domain (APC11) but this ubiquitin ligase is distinct from the SCF ligase in overall subunit combination. For example, instead of one adaptor found in SCF ligases (such as Skp1), APC has multiple subunits that serve as adaptors. Also, unlike SCF ligases, substrate phosphorylation is not an important determinant for specific substrate recognition by the APC ligase. Rather, substrate specificity of APC ligases appears to be modulated by incorporation of ‘specificity factors’ into the ligase complex. APC acts together with an E2 Ubc11 or Ubc X. One of the most studied substrates of APC is mitotic cyclin [22,23].

2.3. The Proteasome

The term proteasome is used to describe two kinds of multi-subunit proteolytic complexes, the 26S and 20S, based on their sedimentation coefficient. The 26S proteasome degrades ubiquitinated protein substrates. The 26S proteasome consists of a 20S catalytic core and two 19S regulatory particles (RP) attached to either end of the cylindrical 20S core.

2.3.1. The catalytic 20S core—In eukaryotes, the 20S core is made up of two outer rings with seven α subunits (α1 to α7) in each ring and two inner rings consisting of seven β subunits (β1 to β7). The catalytic activity of the proteasome is provided by three of the seven β subunits (β1, β2 & β5). The catalytic sites in these β subunits are located at their N-termini which are situated inside the catalytic chamber with an opening 13Å in diameter [24]. Thus only an unfolded substrate can pass through this aperture. The catalytic core of the proteasome is a threonine protease [25]. The 20S proteasome can exist not only as a core of 26S, but also as a separate population that cannot degrade ubiquitinated proteins. However, the 20S proteasome by itself has chymotrypsin-like, trypsin-like, and postglutamyl peptidase activities which cleave after hydrophobic, basic, and acidic residues,

respectively. The peptide-hydrolyzing activity of the 20S proteasome can itself be modulated by an 11S regulatory complex [14].

2.3.2. 19S RC—The 19S RC recognizes the polyubiquitinated substrate, and channels the substrate into the catalytic 20S core of the proteasome. It also has the capacity to regulate the activity of the catalytic core and determine the nature of the degradation process [26]. Usually one 19S RC is attached to either end of the catalytic core. Some of the 19S RC subunits are believed to channel the substrate into the catalytic chamber for degradation by unfolding the substrate. The unfolding activity is believed to be provided by the ATPases that are present in the base of the 19S RP which contains six ATPase subunits Rpt1-Rpt6 (Regulatory particle ATPase 1-6) and four non-ATPase subunits Rpn1, Rpn2, Rpn10 & Rpn13 (Regulatory particle non-ATPases 1, 2, 10 & 13). The other part of the 19S RP is called the 'lid' which comprises only non-ATPase subunits (Rpn3, Rpn5, Rpn6-9, Rpn11, Rpn12, & Rpn15) [14,25]. Among the Rpn subunits, Rpn11 (also called Poh1) and Rpn13 (also called Uch37) are DUBs that are integral part of the 19S RP that assist in deubiquitination of the substrate as it is unfolded and threaded into the catalytic chamber of the 20S core. Another DUB called Usp14 (also known as Ubp6) reversibly associates with the Rpn1 and stimulates substrate degradation through deubiquitination [27,28].

2.4. Deubiquitinating Enzymes

Ubiquitination reaction is reversible before the ubiquitinated protein is committed to degradation by the proteasome. Ubiquitin is removed from substrates by enzymes called DUBs. Based on protein sequence and the molecular size, initially DUBs were classified into two general classes: (1) low molecular weight (20–30K) ubiquitin C-terminal hydrolases (UCHs), and, (2) high molecular weight (around 100 K) ubiquitin-specific proteases (UBPs; also called USPs) [29]. Among the DUBs, UBPs belong to a large family containing diverse genes whereas the UCH family has fewer genes. For example, in yeast *S. cerevisiae*, there are 17 UBPs and 1 UCH. In the human genome, there are 63 genes encoding UBPs and 4 genes that code for UCHs. UCHs and UBPs subserve different functions in the eukaryotic cell. Although the current name for these enzymes, that is, DUBs, emphasizes the removal of ubiquitin from substrates, some DUBs, especially UCHs, function to process linearly linked ubiquitin precursors and generate monoubiquitin. DUBs are important for generating free ubiquitin at various steps of the ubiquitin–proteasome pathway. Ubiquitin is encoded by the tandemly linked polyubiquitin genes UbB and UbC [30,31]. In addition to the polyubiquitin gene, ubiquitin is also encoded by fusion of a ubiquitin-coding region to one of the two ribosomal subunits called L40 and S27. These gene products are thought to be processed by UCHs. Cleavage of isopeptide bond in the ubiquitin chains linked through Lys-48 (and presumably the ubiquitin chains linked through Lys-11) of ubiquitin serves two purposes. One is to recycle ubiquitin after it has been used for marking a substrate for degradation. Another function is to 'edit' the errors made by the ubiquitin-conjugating enzymes and reverse the ubiquitination reaction so that the substrate is no longer degraded. Also, editing function of DUBs probably serves to reverse the monoubiquitin attachment that marks membrane proteins for endocytosis [7,29].

In the post-genome era bioinformatics studies discovered three additional classes of DUBs: the OTU family (genes homologous to ovarian tumor gene), the Josephin/MJD family (genes containing the Josephin domain and the gene homologous to ataxin-3 which is implicated in Machado-Joseph Disease [MJD]), and the JAMM family (the genes containing the JAMM [JAB1/MPN/Mov34 metalloenzyme] domain) [15]. The roles of these classes of DUBs in the nervous system are not well understood.

3. Physiological roles of the UPP in the normal nervous system

3.1. The UPP and synaptic plasticity

Until the early 1990s no physiological or pathological role for the UPP in the nervous system was found. The first discovery of ubiquitin-proteasome-mediated degradation of a physiologically relevant substrate in the nervous system was that of R subunits of cAMP-dependent protein kinase (PKA) [32]. Since then several substrates of the UPP in the nervous system have been identified [4,7,8].

3.1.1. Degradation of the R subunits of PKA—Evidence for a role for the UPP in synaptic plasticity came from the investigation on persistent activation of PKA. Work on the biochemical mechanism of long-term facilitation [33] in *Aplysia* indicated that PKA was persistently activated in the absence of elevated cAMP. How is PKA activated in the absence of sustained increase in cAMP? It was found that the R subunits of PKA were decreased without any change in the catalytic (C) subunit during induction of long-term facilitation. Since there was no change in mRNA for either the R subunit or the C subunit, it was concluded that R subunits were diminished perhaps through proteolysis. What is the mechanism of R subunit degradation? Hegde et al [32] found through a series of biochemical experiments that R subunits were substrates for ubiquitination and proteasome-mediated degradation.

Moreover, a UCH (Ap-uch) that interacts with the proteasome was found to be induced by 5-HT, the neurotransmitter that induces long-term facilitation. Ap-uch was found to be critical for induction of long-term facilitation [34]. The role of UCH in long-term synaptic plasticity and long-term memory have been corroborated by experiments on long-term potentiation and hippocampus-dependent learning in mice [35].

Subsequently Chain et al [36] showed that at sensory-motor neuron synapses, injection of lactacystin, a specific proteasome inhibitor blocked induction of long-term facilitation. Since R subunit inhibit the activity of C subunits of PKA, the results were interpreted to suggest that the UPP operates to remove inhibitory constraints on formation of long-term memory. This has been corroborated by work carried out on the rat hippocampus. Lopez-Salon et al [37] demonstrated that bilateral infusion of lactacystin to the CA1 region of the rat hippocampus caused total retrograde amnesia for a one-trial avoidance learning. They also showed that total ubiquitination increases in the hippocampus 4 h after the training. These results are consistent with the idea that a decrease in some critical inhibitory proteins during long-term memory formation is mediated by the UPP. Proteolysis may play a role in other aspects of memory such as those steps in memory recall and reconsolidation. Using infusion of β-lactone into the CA1 region of the hippocampus Lee et al [38]showed that ubiquitin-proteasome-mediated degradation plays a role in destabilization of retrieved memory as well as extinction of fear memory. Several other studies have provided evidence for the role of the UPP in synaptic plasticity as well (reviewed in [8,39]).

3.1.2. The UPP and transcription: Possible roles in synaptic plasticity—Apart from the roles of the UPP in synaptic plasticity discussed above (see section 3.1.1), the UPP is likely to have a role in controlling transcription that underlies long-term synaptic plasticity. The dependence of long-term synaptic plasticity on gene transcription and new protein synthesis has been intensely studied over the past several years. What has been lacking, however, is the elucidation of the regulation of transcription that occurs in neurons that are modified by learning. For example what molecular processes determine the threshold for transcription? What determines the narrow time window of gene expression observed in many long-term memory paradigms? In many instances, the initial stimuli the neuron receives such as binding of a neurotransmitter to its receptor are the same for short-

term and long-term synaptic plasticity. And yet long-term synaptic plasticity requires induction of gene expression. Long-term synaptic plasticity-producing protocols somehow must initiate a transcription cascade. Thus far the molecular processes that control threshold for transcription have not been elucidated. It is possible that the UPP operates to regulate the threshold for gene induction. Minimally the UPP could play a role in regulating transcription at two steps. One is the relief of transcription repression and the other is control of transcriptional activation. For example, it has been well established that CREB-mediated gene expression is required for long-term memory formation in *Aplysia*, *Drosophila* and mice [40–42]. It is also known that through alternative splicing, CREB gene generates activators and repressors [43]. The repressors are normally present at a higher stoichiometry in the cell than the activators [44,45]. For CREB-mediated gene expression to go forward, the repression has to be relieved. In *Aplysia* neurons, we have found that the repressor CREB1b is degraded by the UPP whereas the activator CREB1a is stable compared to the repressor [46]. The regulation of CREB1b degradation is likely to determine the threshold for induction of CREB-mediated gene expression and hence a threshold for long-term facilitation. With respect to removal of activator by proteolysis, previous work in *Aplysia* has shown that C/EBP, a transcription factor induced during long-term facilitation is degraded by the UPP [47]. The degradation of C/EBP determines a time window during which transcription must take place to induce long-term facilitation. The newly discovered role of the proteasome in transcription might be relevant to synaptic plasticity as well. We have found that proteasome inhibitors block induction of *BDNF* mRNA during hippocampal late phase long-term potentiation (LTP), a mammalian model of long-term synaptic plasticity [48].

There is a growing body of literature on the role of non-proteolytic roles of the proteasome in transcription [49,50]. In addition, ubiquitin-like proteins such as SUMO (small ubiquitin-related modifier) play a critical role in transcription [51,52]. With respect to nervous system function, studies on these other modes of transcriptional regulation have not been studied extensively and therefore are not reviewed here.

3.2. The UPP and developmental synaptic plasticity

Ubiquitin-proteasome-mediated proteolysis also plays a role in establishing and fine-tuning of synaptic connections during development of the nervous system. Synapse formation begins with axon growth towards the dendritic arbors or the cell body of their targets along a correct path. Establishment of mature pattern of synaptic connectivity requires retention of a subset of synapses that are originally formed. Synapse elimination likely requires pruning of axons and dendrites [53]. Consistent with this idea, in cerebellar granule cells axonal growth is inhibited by the multi-subunit ligase Cdh1-APC, which operates in the nucleus [54]. In *Drosophila*, a ubiquitin conjugating enzyme called UbcD1 helps prune dendrites. Also, in *Drosophila*, axon pruning is inhibited by mutations in the ubiquitin activating enzyme E1 or some of the proteasome subunits. Synaptic number in insect neuromuscular junction is controlled by a ubiquitin ligase *highwire* and a DUB called *fat facets* and synaptic size is determined by a subunit of APC (APC2).

4. Roles of the UPP in diseases of the nervous system

The components of the UPP have been linked to several diseases of the nervous system (Table 1). In some instances, mutations in specific genes have been linked to the etiology of the disease while in other cases impairment of UPP might be a late event in pathogenesis [55]. Although the perturbations in ubiquitin-proteasome-mediated proteolysis lead to pleiotropic effects on neurons including cell death or degeneration, one of the early effects is believed to be synaptic malfunction [56]. Many recent reports from animal studies demonstrate a role for ubiquitin-proteasome-mediated degradation of numerous substrate

proteins in synaptic plasticity thus linking UPP to the normal synaptic function [7,57,58]. Although the UPP is linked to several neurodegenerative diseases including AD, PD and HD, impairment in synaptic plasticity and its connection to the UPP is better understood in AD than in other neurodegenerative disease.

4.1. Alzheimer's disease (AD)

AD is a neurodegenerative disorder of the central nervous system (CNS) clinically characterized by progressive loss of memory and other cognitive skills resulting in severe dementia. The condition often begins with mild memory lapses, and then gradually advances to dementia, a progressive deterioration of memory, language and most mental functions. Early during development of AD, neurological examination is normal except for cognitive dysfunction like progressive worsening of memory and other cognitive functions [59–62]. The intellectual decline is accompanied by the progressive extracellular and intracellular accumulation of insoluble fibrous material in the brain in the form of senile plaques and neurofibrillary tangles (NFTs) [63].

AD is the most prevalent neurodegenerative disorder and the most common cause of dementia [64]. Familial AD is a rare autosomal dominant disease with early onset, caused by mutations in the amyloid precursor protein (APP) and presenilins (PSEN1 & PSEN2) genes, both linked to amyloid β peptide (Aβ) metabolism [65]. Aβ is produced from APP by sequential cleavage by β-secretase (β-site APP cleaving enzyme 1; BACE1) and γ-secretase (presenilin complex), and released into the extracellular spaces [66]. Sporadic AD is common heterogeneous disease and caused by a complex interaction of genetic and environmental risk factors [65]. The pathological signs of AD include loss of medium and large pyramidal neurons, the presence of plaques and NFTs, composed of deposits of amyloid filaments and hyperphosphorylated tau respectively, surrounded by altered neurite processes and glia together with a degeneration of the neurons and loss of synapses [67,68]. Tau is a structural protein that is normally associated with microtubules. In abnormal condition like in AD, tau undergoes an abnormal posttranslational modification characterized by hyperphosphorylation. In addition, levels of tau could increase because of translational up-regulation mediated by increased S6 kinase as suggested by studies on postmortem AD brain and SH-SY5Y neuroblastoma cells [69]. Although multiple genetic disturbances are believed to cause AD, a major cause of the disease is buildup of the toxic Aβ peptide [70].

The formation of the neurofibrillary lesions is believed to produce the symptoms of the disease, which result most probably from the degeneration of nerve cells in cerebral cortex and hippocampal formation following synaptic loss. Defective synaptic function and memory loss is seen in early stages of AD and animal models of AD. Cognitive deficits are correlated with the loss of synapses [56]. During the early phase of AD there is 25–35% decrease in the density of synapses, and as the disease progresses loss of synapses is more strongly correlated with the disease than the plaques and tangles [71]. Animal models of AD mimic cognitive impairment seen in human AD patients [72,73].

Recent studies also suggest that AD begins with subtle alterations in synaptic efficacy prior to neuronal degeneration, and diffusible oligomeric assemblies of the Aβ [56] cause this neuronal dysfunction. Although there is eventual loss of synapses in both AD and animal models of AD, deficits in spatial memory and inhibition of LTP (an electrophysiological measure of synaptic strength) precede morphological alterations in the animal models, suggesting earlier biochemical changes in the disease [56,74,75]. Aβ treatment of cultured hippocampal neurons leads to the inactivation of PKA and persistence of its regulatory subunit PKAII alpha suggests that Aβ acts directly on the signaling pathways consisting of cAMP/PKA/cAMP-responsive element binding protein (CREB) required for development

of late phase LTP [76]. Therefore, agents that enhance the PKA pathway have potential for the treatment of AD [76]. In transgenic mice expressing mutant amyloid precursor protein (APP), learning and memory is impaired at 9–10 months of age although no tangles are seen in the brains of these mice [77]. In mice carrying various mutations of APP, both *in vitro* and *in vivo* LTP is impaired much before detectable Aβ deposits are observed [78]. Also, cerebral microinjection of oligomers of Aβ peptide inhibits *in vivo* LTP in rats [79]. Moreover, Aβ dimmers isolated directly from human AD brain inhibit LTP and impair memory [80].

4.1.1. The UPP and AD—Several studies implicate neuronal UPP in the pathogenesis of AD. Abnormal deposition of highly insoluble protein aggregates or inclusion bodies within nerve cells is commonly observed in nervous tissue in association with several chronic neurodegenerative diseases including AD [81]. These inclusions bodies show ubiquitin immunoreactivity in human AD brains along with immunoreactivity to neurofilaments, tau, NEDD8 (Neural Precursor Cell Expressed, Developmentally Downregulated 8), PSEN1 and proteasome subunits [1,82–87]. Recent studies show that Lys-63-linked ubiquitin chains were found in intraneuronal inclusions in the AD brain [88]. The accumulation of ubiquitin-protein conjugates in neuropathological lesions was detected first in NFT isolated from human brain [5,89] and these NFTs best correlate with the degree of dementia [90]. It has been suggested that the UPP might also play a role in mediating the effects of inflammation in the brain and in linking it to formation of NFTs [91]. Aggregated proteins in cell line experiments tend to inhibit the UPP [55,92]. Initiation of aggregation of ubiquitinated proteins might come about by impairment of proteasome function as has been suggested by experiments using a mutation of a proteasome subunit [93].

4.1.2. The UPP and synaptic dysfunction early in AD—Memory impairment in AD is likely to result from synaptic dysfunction such as a defect in ability to change synaptic strength or synaptic plasticity. The findings from basic research on the critical function of Uchl1 (sometimes referred to as Uch-L1) in long-term synaptic plasticity [34] in invertebrates suggested the possibility of using Uchl1 for therapeutic purposes. It was found that treatment with exogenous Uchl1 can reverse the synaptic dysfunction in hippocampal slices from APP/PSEN1 mice (an animal model of AD) or synaptic plasticity impairment in hippocampal slices from normal mice caused by treatment with oligomeric Aβ [35]. Application of exogenous Uchl1 also reversed loss of spine density of hippocampal pyramidal neurons in APP/PSEN1 mice [94]. The essential role of Uchl1 in synaptic plasticity [34] and the application of Uchl1 to treat synaptic dysfunction in AD [35] suggests that perturbations in UPP components may be an important causative factor in AD.

Some other studies have suggested a role for Uchl1 in AD as well. For example, the genetic defect in gracile axonal dystrophy (gad) in mice has been shown to be caused by an in-frame deletion including exons 7 and 8 of *Uchl1*, encoding the ubiquitin carboxyl-terminal hydrolase isozyme Uchl1. The gad mice show ataxia (lack of coordination in muscular movements) characterized by dragging of the hind legs. In the sensory and motor neurons of gad mice accumulation of Aβ and ubiquitin-positive deposition occurs in a manner reminiscent of AD pathology [95,96]. The studies by Osaka et al [97] demonstrated that Uchl1 ensures ubiquitin stability within neurons. Also, UCHL1 protein level is downregulated in idiopathic Parkinson's disease (PD) as well as AD brains [98]. Full-length human UCHL1 appears to be a major target of oxidative damage in AD and PD brains, and the enzyme is extensively modified by carbonyl formation, methionine oxidation, and cysteine oxidation [99]. Also, evidence from studies on Chinese Han population indicates the genetic association of serine to tyrosine mutation (S18Y polymorphism) in the *UCHL1* gene and sporadic AD. This study concluded that Y allele and YY genotype of the S18Y in the *UCHL1* gene may have protective effect against sporadic AD in female subjects [100].

Studies on transgenic mice expressing an aberrant ubiquitin called UBB^{+1} (ubiquitin with 20 extra amino acid residues at its C-terminus, which is implicated in AD) show memory deficits by about 9 months of age even though these mice do not show overt neurological deficits [101]. Since UBB^{+1} impairs proteasome function (see section 4.1.3), memory deficits in UBB^{+1} transgenic mice might be the result of synaptic dysfunction preceding the appearance of memory impairment.

4.1.3. The UPP and proteolytic defects in AD—The ubiquitinated protein aggregates found in AD brains are likely to result from a malfunction or overload of the ubiquitin-proteasome dependent protein degradation pathway or from structural changes in the protein substrates, halting their degradation. Presence of ubiquitinated proteins in all the AD cases [102–104] leads us to speculate that AD is associated with an inability of neurons to degrade specific proteins or accumulated protein aggregates.

How does a defect in UPP in AD come about? An unusual ubiquitin-conjugating enzyme, E2-25K/Hip-2, was found to be an intermediary of Aβ-mediated toxicity and E2-25K/Hip-2 enzymatic activity was required for inhibition of the proteasome in an animal model containing APP mutations [105]. Ubiquitin mutant, UBB^{+1} (a potent inhibitor of proteasome which is observed in AD brains) also functionally interacted with E2-25K/Hip-2 mediated neurotoxicity [105]. Additional studies found that E2-25K/Hip-2 is likely to stabilize Caspase-12 via inhibition of the proteasome (through accumulation of polyubiquitin conjugates and ubiquitinated UBB^{+1}) and thus mediate toxicity caused by Aβ [106]. It has been found that Aβ can bind and inhibit the proteasome and thus block degradation of ubiquitin-conjugated proteins [107]. In *in vitro* experiments, Aβ peptide has been shown to inhibit the proteasome [108]. Intraneuronally accumulated Aβ peptide in APP/PSEN1 mutant neuronal cell culture is also shown to be able to inhibit the proteasome and deubiquitinating enzymes [109]. Ubiquitin immunoreactivity detected in axonal spheroids and dendritic compartments are associated to the elevated intraneuronal Aβ peptide and axonopathy seen in transgenic mouse model of mutant APP/PSEN1 [110,111]. Also, it is thought that other causative factors in AD such as paired helical filaments (PHF) of tau protein impair proteasome function [92]. In the brains of AD patients, proteasome function has been shown to be reduced mostly in the areas critical for long-term memory formation such as hippocampus, parahippocampal gyrus superior and middle temporal gyri and inferior parietal lobule but not in other areas such as the occipital lobe [112]. Another study showed that PHF of tau in brains of AD patients, as well as *in vitro* assembly of PHF using human recombinant tau protein both inhibited proteasome activity [113].

Taking the studies on early cognitive impairment in AD patients and animal models of AD together with the investigations on impairment of the UPP in AD, one could argue that the early synaptic defects result from impairment of proteolysis. There is considerable evidence for a role of UPP in synaptic plasticity [8,39]. Although a secondary role for UPP in AD pathology cannot be ruled out, given the role of UPP in synaptic plasticity and in maintaining the integrity of the synapse, perturbations in these functions might first lead to synaptic dysfunction and eventually loss of synapses.

Presence of ubiquitin and its association with tau in NFT's and senile plaques in AD is common factor [81]. Accumulated ubiquitin is also present in Lewy bodies characteristic to some forms of the disease [85]. In all these cases, the role of tau and other putative target proteins in the pathogenesis is not understood. Also, it is still not clear whether aberration in proteolysis plays a causative role or only a secondary role [114]. Conflicting reports also exist on the E3 that ubiquitinates tau. Two reports claim that ubiquitin ligase, carboxyl terminus of Hsp 70-interacting protein (CHIP) targets tau [115,116] whereas another study argues that ubiquitin ligase TRAF6 is the ligase that conjugates ubiquitin to tau [117]. The

discrepancy in the results perhaps could be resolved if the phosphorylation state of tau and the type of ubiquitin linkage that occurs on tau with each ligase are determined. CHIP has been shown to target hyperphosphorylated tau and TRAF6 ligases causes polyubiquitination via Lys-63-linked ubiquitin. Thus, the two ligases might act on the same substrate under different conditions.

A more direct relationship between the ubiquitin system and pathogenesis of AD was established with the discovery of UBB^{+1} which results from a frameshift mutation in the ubiquitin transcript that elongates the C-terminus of ubiquitin. The UBB^{+1} protein has been observed in the brains of AD patients [118]. UBB^{+1} is an efficient substrate for polyubiquitination, however, causing formation of polyubiquitin chains that cannot be disassembled by deubiquitinating enzymes. Polyubiquitin chains with UBB^{+1} potently inhibit the degradation of a polyubiquitinated substrate by the 26S proteasome [119] and acts as proteasome inhibitor in cells in a dose-dependent manner [120]. This report also suggests that the inhibitory activity of UBB^{+1} may be an important determinant of neurotoxicity and contribute to an environment that favors the accumulation of misfolded proteins [121,122]. In UBB^{+1} transgenic mice protein changes in the brain are remarkably similar to that in the human AD brain [101]. In addition, other genes implicated in AD such as presenilins (PSENs) 1 and 2 which are critical for processing APP are linked to UPP. Both PSEN1 and PSEN2 are targets for the UPP [123]. It is not known whether aberration in the degradation of PSEN1 and PSEN2 plays any role in the pathogenesis of AD.

4.2. Amyotrophic Lateral Sclerosis (ALS)

ALS is a disease of the neurons (motor neurons) that control muscle movement. Degeneration of neurons causes muscle atrophy eventually impairing the movement of people afflicted with the disease. Mutations of *Copper-zinc superoxide dismutase1* (*SOD1*) have been associated with some cases of autosomal dominant familial ALS and some cases of sporadic ALS. Several studies have linked UPP to turnover of SOD1 but the observations have not yielded a clear picture. Some studies reported that mutant SOD1 proteins are degraded more rapidly than wild type SOD1 by the UPP [124,125]. Consistent with this idea, dorfin and NEDL1 ubiquitin ligases were shown to ubiquitinate mutant but not wild type SOD1 [126,127]. Di Noto L et al [128], however, reported that metal free SOD1 is degraded by 20S proteasome *in vitro* without requiring ubiquitination. In their study, monomeric forms of both wild type and mutant SOD1 were susceptible to degradation by the proteasome [128]. Investigations by others reported comparable degradation of both wild type and mutant SOD1 proteins by macroautophagy as well as by the proteasome [129].

Overexpression of the putative SOD1 E3 ligase dorfin can inhibit cell death induced by the mutant SOD1 protein [126]. Gene expression profiling of spinal cords from sporadic ALS patients indicated that genes associated with the UPP (dorfin and ubiquitin-like protein 5), oxidative toxicity, transcription, neuronal differentiation and inflammation might function in the pathogenesis of sporadic ALS [130]. It is possible that the expression of dorfin ligase is increased in an attempt to enhance clearance of the mutant SOD1. Recent studies also reported that heat-shock protein 70 (Hsp-70) or heat-shock cognate Hsc70, and CHIP play a role in proteasomal degradation of mutant SOD1 [131]. In addition, Urushitani et al. [132] found that oxidative damage increases the degree of ubiquitination of mutant SOD1 protein and proteasome activity decreases following one week of expression of a mutant *SOD1* gene in cultured cells. Toxicity of mutant SOD1 protein aggregates is controversial, however. Lee et al. [133] found that aggregates of mutant SOD1 did not cause cell death. Also, others found that motor neurons from wild type animals and motor neurons from transgenic mice with mutant *SOD1* were equally viable [134]. Some studies, however, have demonstrated that proteasome inhibition results in increased cell death in human cells expressing mutant SOD1 protein [135,136]. Recent work has suggested a connection between SOD mutation

and impairment of proteasome. In transgenic mice with SOD gene mutation G93A, expression of proteasome subunits is decreased and the UPP function is impaired in spinal motor neurons [137]. Another molecule TAR-DNA-binding protein-43 (TDP-43), which has been linked to ALS, is a substrate for ubiquitination. It has been suggested that impairment of the UPP might play a role in neurodegeneration caused by TDP-43 dysfunction [138,139]. The role of protein aggregates in familial ALS remains unclear, however. As with other neurodegenerative diseases, the exact connection between SOD1 protein aggregates, the UPP, and disease progression remains to be elucidated.

4.3. Angelman Syndrome (AS)

AS is a neurological disorder with symptoms such as mental retardation, unusually happy demeanor, susceptibility to epileptic seizures, and abnormal gait [140]. Occurrence of AS is estimated to be 1 in 15,000 births. In about 65–75% of AS patients, maternal deletions at chromosome 15q11-q13 are found. Other types of genetic abnormalities such as uniparental disomy and imprinting mutations are also observed in AS, each of which accounts for about 3–5% of the cases. It has been found that the defects occur in a gene called UBE3A [141,142]. Point mutations in UBE3A are found in about 4–6% of the AS cases [143]. UBE3A is a maternally imprinted gene with brain specific expression from the maternal allele [144,145]. UBE3A gene encodes a ubiquitin ligase which had been previously identified as E6-AP (E6-Associated Protein) ubiquitin ligase. E6-AP is the cellular protein that associates with a human papilloma virus protein called E6. E6-AP in association with E6 degrades the tumor suppressor protein p53 [146]. Apart from p53, E6-AP is known to attach ubiquitin to at least three other substrates, RAD23, a human homolog of a yeast DNA repair protein [147]; multi copy maintenance protein 7 (MCM7) which is thought to function in chromosome replication [148]; and E6-AP itself [149].

Mental retardation in human AS patients suggests synaptic malfunction. In support of this idea, mice with deficiency of *Ube3a* maternal allele exhibit impairment of LTP and contextual learning. Previous studies showed an indirect effect of Ube3a on a molecule critical for synaptic plasticity, namely, Calcium/Calmodulin-dependent protein kinase II (CaMKII) has been observed, however. In the hippocampus of AS mouse, an increase in inhibitory autophosphorylation (on Thr305 & Thr306) of CaMKII occurs leading to reduction in kinase activity and dissociation of CaMKII from postsynaptic density [150]. Also, reduction in inhibitory autophosphorylation of CaMKII in AS mice rescues the neurological deficits in AS model mice [151]. So far no explanation as to how mutation in Ube3a causes alteration in CaMKII has been obtained. Another molecule critical for synaptic plasticity Arc has been shown to be a substrate for Ube3a and it has been suggested that Ube3a regulates excitatory synapse development by targeting Arc [152].

4.4. Ataxia

There is a direct link between mutation in a DUB called ubiquitin-specific protease 14 (Usp14) and ataxia. Mice with homozygous recessive mutations Usp14 develop ataxia and severe tremors by 2–3 weeks of age, followed by hindlimb paralysis and death by 6–10 weeks of age [153]. The mutation severely reduces the expression of Usp14 to about 5–10% of the levels found in wild type mice. The function of Usp14 is believed to be recycling of ubiquitin through disassembly of polyubiquitin chains to the monomeric form of ubiquitin. In support of this idea, levels of monomeric ubiquitin are reduced in the brains of Usp14 mutant mice [154]. In contrast to other neurodegenerative disorders such as Parkinson's disease and SCA1 in humans and gad in mice, neither ubiquitin-positive protein aggregates nor neuronal cell loss is detectable in the CNS of these mice. Instead, they have defects in synaptic transmission in both the central and peripheral nervous systems. Neuron-specific expression of Usp14 rescues the growth defects and lethality [155]. The expression of

transgene also rescues motor defects to some extent although some defects in motor coordination remain. Incomplete rescue of motor defects has been attributed to lack of expression of the Usp14 transgene in cerebellar Purkinje cells [155]. Additional evidence gathered by Chen et al [156]showed that Usp14 is critical for maintaining synaptic ubiquitin levels at neuromuscular junctions.

4.5. Huntington's disease (HD)

HD is caused by mutations in a gene called Huntingtin. The disease is caused by abnormal expansion of CAG repeats which encode long stretches of glutamine (polyglutamine). In addition to HD there are about several other known polyglutamine diseases such as spinocerebellar ataxia, and, spinal and bulbar muscular atrophy [81].

HD can also be viewed as a disease of the synapse. In HD patients cognitive deficits appear much before the clinical symptoms of the disease [157–161]. This phenomenon is reproduced in mouse models of HD as well. For example, in transgenic mice carrying exon 1 of human HD mutation, hippocampal plasticity is significantly altered and deficits in spatial learning are observed. Deficits in synaptic plasticity occur before the overt neurological phenotype is observed [162]. Introduction of HD-like CAG repeats into murine huntingtin causes behavioral abnormalities as well [163]. Another study reported abnormalities in synaptic vesicle fusion machinery in HD mutant mice [164]. Even ectopic expression of an N-terminal fragment with 150 glutamine residues in *Aplysia* neurons impaired long-term facilitation without affecting basal synaptic transmission or short-term facilitation [165].

Is the UPP impaired in HD? Altered proteasome function was observed when mutant huntingtin was expressed in Neuro2A cells under the control of an inducible promoter [166]. A convincing demonstration was obtained using expression of huntingtin with a stretch of 103 glutamines in human embryonic kidney (HEK) 293 cells. Expression of mutant huntingtin with 103 glutamine in HEK 293 cells causes aggregate formation, accumulation of ubiquitinated proteins and cell cycle arrest whereas expression of shorter stretch of polyglutamine (25 glutamines) had markedly less effect on all these parameters [167]. Compelling evidence for impairment of the UPP came from a thorough study that used mass spectrometry techniques specifically to identify polyubiquitin chains that are formed through Lys-48 linkage of ubiquitin. This study showed accumulation of polyubiquitin chains in HD. This study revealed that Lys-48 linked ubiquitin chains accumulate in brains of R6/2 transgenic mice (widely used mouse model of HD), a knock-in mouse model (Q150/Q150) of HD, and in brains of HD patients [168].

How do polyglutamine-containing proteins inhibit the proteasome? One explanation is that polyglutamine containing proteins act as direct inhibitors of the proteasome [81]. An alternative explanation is that the UPP is overwhelmed by the load of aggregated or misfolded proteins. Since the nuclear inclusions in HD show ubiquitin immunoreactivity suggest that perhaps ubiquitin conjugation to aggregated protein is taking place and the proteasome is unable to efficiently degrade the ubiquitinated proteins. In support of this idea, proteins inclusions from isolated neurons and brains of conditional HD transgenic mice can be revered if the transgene is turned off. The disappearance of inclusion is proteasome dependent because the proteasome inhibitor lactacystin inhibits the reversal process [169]. Also, when green fluorescent protein containing polyglutamine is expressed in SH-SY5Y cells, basal proteasome activity is not impaired but the ability of the proteasome to respond to stress such as heat shock is dramatically impaired [170].

Based on the results from various studies described above, it is clear that the failure of the UPP ultimately contributes to cell death or degeneration in polyglutamine diseases. This

process is probably progressive. Initial formation of aggregates with proteins with long polyglutamine stretches perhaps impairs proteasome activity which in turn leads to accumulation of more protein aggregates and thus misfolded proteins could build up in the cell. Initially, however, impairment of the proteasome is likely to have effect on synaptic properties of the neuron because as discussed elsewhere in this review, proteasome activity in various subcellular compartments of the neuron is essential for normal synaptic function and plasticity.

4.6. Parkinson's disease

Protein products of four of the genes associated with Parkinson's disease, namely those of *α-synuclein*, *UCH-L1*, *DJ-1* and *parkin* [19,171–174] are linked to the UPP. α-synuclein is part of the Lewy bodies, the intracellular inclusions seen in the brains of Parkinson's disease patients. Lewy bodies contain high amounts of ubiquitinated proteins including ubiquitinated α-synuclein. UCH-L1 is an enzyme of the ubiquitin pathway. DJ-1 is a substrate for conjugation to SUMO-1, a protein related to ubiquitin. SUMOylation of DJ-1 appears to be essential for its function [175]. The amino acid sequence of parkin protein contains a UbL domain at its N-terminus. Also, parkin has been shown to be a ubiquitin ligase. Three of the genes linked to Parkinson's disease have direct synaptic connection. Parkin is present in postsynaptic densities. The *Aplysia* homologue of UCH-L1 is present in presynaptic terminals (Hegde et al., unpublished observations). Synuclein is associated with synaptic vesicles [176]. There is also evidence indicating that PD might be affect synaptic function adversely. In parkin deficient mice, synaptic excitability was reduced in striatal spiny neurons [177]. Also, parkin, which is a ubiquitin ligase [178] has been shown to ubiquitinate synaptotagmin XI [179].

Studies investigating possible connections of parkin to neurodegeneration have found that parkin is a key regulator of the aggresome-autophagy pathway [180–182]. Parkin targets several misfolded proteins to the aggresome-autophagy pathway through Lys-63 linked polyubiquitination. Parkin also promotes ubiquitination and degradation of a polyglutamine-expanded ataxin-3 and reduces its cellular toxicity [183,184]. Ataxin-3 which is implicated in Machado-Joseph Disease (MJD)/spinocerebellar ataxia-3 (SCA3), is a DUB that preferentially cleaves Lys-63 linkages [185]. The DUB activity of ataxin-3 is enhanced by its ubiquitination [186]. Both parkin and ataxin-3 participate in the aggresome formation to remove the misfolded proteins via autophagy [187]. It will be interesting to know the possible role of ubiquitination and deubiquitination activity of these proteins in regulating their own turnover and in the early synaptic failure seen in PD or MJD/SCA3. These studies could also potentially provide an explanation for parkinsonian symptom seen in MJD patients [188].

A link between proteasome dysfunction and neurodegeneration in PD has been provided by an elegant mouse model using targeted conditional depletion of 26S proteasome through inactivation of a 19S proteasome subunit Psmc1 (also called Rpt2/S4). In these mice, 26S proteasome depletion led to neurodegeneration and Lewy-like inclusions. In the brain of these mice intraneuronal inclusions contained ubiquitin and α-synuclein and the inclusions displayed striking similarities to Lewy bodies on the brains of human PD patients [189]. A connection between the proteasome and PD was also provided by a genetic study of German PD patients which found variations in intron 5 of a gene encoding the proteasome subunit S6 ATPase more frequently in early-onset PD patients relative to late-onset PD patients [190].

4.7. Neurodegeneration through autophagy: The ubiquitin connection

Impairment in proteolysis as a possible cause of neurodegeneration seen in many diseases of the nervous system has been previously suggested [3,81]. Several studies during the past few

years point to a possible new connection between the UPP and neurodegeneration through autophagy. While the UPP mediates non-lysosomal protein degradation in the cell, autophagy is the process through which part of the cytoplasm is delivered to the lysosomes to be degraded. Basal autophagy prevents toxic effects of misfolded or abnormal proteins sequestering them inside autophagosomes (the double-membrane vesicle that engulfs portions of the cytoplasm or organelles) that deliver them to the lysosome for degradation [3]. Some of the proteins critical for autophagy are part of a conjugation system similar to that of ubiquitin. For example, Atg12 is activated by Atg7, a E1-like molecule, and transferred to Atg10, an E2-like enzyme, and conjugated to Atg5 through an isopeptide bond. Atg5 appears to be required for vesicle formation and completion which is required for autophagy. Mice lacking Atg7 specifically in the CNS showed neurodegeneration and showed motor deficits [191]. Atg5 null mutation in mice also causes deficits in motor function [192]. In these mice accumulation of ubiquitin immunoreactive cytoplasmic inclusion bodies in neurons is observed. Although the UPP and autophagy were thought to work in parallel, recent investigations suggest a functional link between the two. For example, proteasome inhibition activates autophagy and suppression of autophagy causes polyubiquitinated protein aggregates [193]. Studies on a *Drosophila* model of the neurodegenerative disease spinobulbar muscular atrophy have provided a mechanistic link between the UPP and autophagy. Flies containing a mutation in a proteasome subunit showed neurodegeneration which was rescued by overexpression of histone deacetylase 6 (HDAC6). HDAC6-mediated rescue of neurodegeneration occurred only if autophagy in these flies was intact [194]. Since HDAC6 is known to bind misfolded, polyubiquitinated proteins, it might shuttle these proteins to autophagosomes. Another polyubiquitin binding protein called p62/SQSTM1 might also connect polyubiquitinated proteins to autophagy [195].

4.8. Wallerian Degeneration

Wallerian degeneration is observed at the distal portion of a transected axon following injury. Previous studies have shown that application of proteasome inhibitors slows Wallerian degeneration [196]. Other studies have shown that there are differences between naturally occurring developmental axon degeneration and injury-induced axon degeneration. For example, the WldS protein that protects against Wallerian degeneration has no effect on developmental axon pruning whereas it protects against injury-induced degeneration of the same axons at the same developmental stage. WldS protein is a product of a chimeric gene that is found in laboratory (C57BL) mice which encodes ubiquitination factor E4B (also known as UFD2/E4) fused to nicotinamide mononucleotide adenyltransferase (Nmnat). UFD2/E4 functions in polyubiquitination and Nmnat is an enzyme that facilitates NAD synthesis. In contrast to the function of WldS, the UPP appears to be required for both developmental axon pruning and injury-induced axon degeneration [197].

5. The UPP and nervous system disease: Challenges and major outstanding questions

In spite of the fact that extensive research has been done on the role of the UPP in non-neuronal systems, our understanding regarding the role of the UPP in physiological and pathological functions in the nervous system is in its early stages and many questions remain unanswered and various challenges lie ahead. In terms of identifying the substrates and enzymes of the UPP that play a role in normal neuronal function and in abnormalities of the nervous system there is much more work to be done compared to what has been accomplished so far. Moreover, our knowledge regarding the already identified substrates and the enzymes of the UPP is incomplete. For example, APC appears to have nuclear as well as synaptic roles but it is not clear how this ligase is locally regulated in different

neuronal compartments. Spatial and temporal regulation of the proteasome function in neurons needs to be elucidated as well.

With respect to the diseases and disorders of the nervous system, we need to understand how abnormal proteins and protein aggregates cause synaptic dysfunction and eventual synaptic loss and how the UPP fits into this picture. The overlap of symptoms in certain neurodegenerative diseases can be better understood if we can elucidate how the molecules implicated in the diseases interact with each other. For example, understanding interactions between parkin and ataxin-3 might shed light on parkinsonian symptoms seen in MJD patients. Another major challenge is to develop animal models that faithfully mimic the human disease. If we are able to comprehend the exact role of the UPP in etiology of a given neurodegenerative disease, then we might be able to create animal models of the disease which can be used for testing possible drug targets including the components of the UPP.

6. The elements of the UPP as possible therapeutic targets

Could we envisage targeting enzymes of the UPP or the proteasome as potential therapeutic targets to treat nervous system disease? In principle, it should be possible to use the UPP components as “druggable” targets if a clear causative link between a UPP component and a disease can be established. Among the enzymes that conjugate ubiquitin to substrates, E3s are the best potential therapeutic targets because they mainly determine substrate specificity. Because the substrate binding region imparts specificity to E3s, allosteric modification of this region (or modification of the active site) by small molecules so that specific E3s will have either increased affinity or decreased affinity towards specific substrates is one way of controlling the ubiquitinated substrate accumulation [2]. Selective engineering of the ubiquitin proteasome pathway components to degrade specific accumulated ubiquitinated proteins or protein aggregate and their delivery to specific affected region may also provide an alternative approach to small allosteric molecules. If defective function or deficiency of a UPP component is linked to a nervous system disease such as ataxia gene therapy with administration of specific components of the UPP might be a possibility.

A potential new area for the discovery of drug targets for treating neurodegenerative diseases is the proteasome. Although many inhibitors of the proteasome are available, no effective drugs exist that can stimulate the proteasome. Since abnormal protein aggregation and inhibition of proteasome is a common feature of AD and other neurodegenerative diseases, enhancement of proteasome activity by small molecules might be an efficient way to remove the aggregates that accumulate in the brain [2]. Removal of protein aggregation by the proteasome can be achieved by employing the following strategies: (1) By increasing the activity of the proteasome through increase in assembly of 19S and 20S complex of proteasome; (2) By stimulating the recognition of ubiquitinated proteins in protein aggregate; (3) By overexpressing or modulating the chaperone activity of either chaperonins or ATPase subunits of 19S to unfold the aggregated proteins; and (4) By stimulating the catalytic activity of the 20S core of the proteasome through small molecules [2]. In support of this idea, a study showed that Resveratrol, a naturally occurring polyphenol found in the grapes and red wine is known to lower the Aβ in cell lines by promoting the proteasome-dependent degradation of Aβ suggesting possible use of this compound for therapeutic intervention in AD [198]. A thorough understanding of the role of the UPP in the normal nervous system and elucidation of precise pathological role of ubiquitin-mediated proteolysis in disease states would be essential for future development of therapeutic approaches.

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Fig. 1.

The ubiquitin-proteasome pathway. In this proteolytic pathway, ubiquitin (single ubiquitin molecule is represented by open circles with straight tails) is selectively and covalently liked to the substrate. The enzymatic process of attaching ubiquitin to substrates is called ubiquitination or ubiquitin conjugation and depends on the action of three different classes of enzymes E1, E2 and E3. First ubiquitin is activated by E1 to form a ubiquitin-AMP intermediate. Activated ubiquitin (light orange color-filled circles with straight tails) is passed on to an E2 (ubiquitin carrier enzyme). Some E3s (ubiquitin ligases) such as HECT ligases accept ubiquitin as a thiol intermediate and then ligate it to the substrate. The RING ligases bring the E2 and substrate together and facilitate the transfer of ubiquitin from the E2 to the substrate. To the ubiquitin attached to substrate another ubiquitin is attached and thus through successive linkages of ubiquitin a polyubiquitin chain forms (conjugated ubiquitin is represented by dark orange color-filled circles and a branched pattern of tails). Polyubiquitinated substrates are degraded by a proteolytic complex called the 26S proteasome in an ATP-dependent reaction. Ubiquitin is not degraded but the polyubiquitin chain is disassembled and ubiquitin is recycled by deubiquitinating enzymes (DUBs). Before being committed to be degraded by the proteasome, ubiquitination is reversible. DUBs can disassemble the polyubiquitin chain if a substrate is ubiquitinated erroneously and prevent the degradation of the substrate.

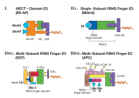

Fig. 2.

Classes/Examples of Ubiquitin Ligases (E3s). (I) HECT-Domain E3. E6-AP ubiquitin ligase in combination with E6 protein and one of the two E2s (UbcH5 or UbcH7) ligates ubiquitin to the p53 tumor suppressor protein. (IIA) Single-subunit RING Finger E3. Mdm2 ligates ubiquitin to PSD 95 with the help of an E2 enzyme. (IIBi) Multi-subunit RING finger E3. SCF ligases contain the substrate recognition site on an F-box protein. Skp1 is an adaptor that joins the F box protein to Cul1. Ring finger domain is on Rbx 1. The E2 is Ubc3. Cul 1 is modified by Nedd 8, a ubiquitin- like protein leading to an increase in the activity of the ligase complex. The substrate is phosphorylated (diamonds) IκBα. (IIBii) Multi-subunit RING finger E3. APC is a more complex example of multi-subunit RING finger E3s and has a subunit composition distinct from that of SCF. Cdc20 protein in APC has the substrate (Cyclin) recognition site. The RING finger domain is on APC11. The E2s Ubc 11 or UbcX can function with the APC ligase. In addition, several adaptor proteins, some labeled (Cdc27, Cdc23, APC 1, Cdc16) and some unlabeled, interact with Cdc20 and APC11. Diamonds on the adaptor subunits indicate phosphorylation. Polyubiquitin chain is shown on the substrates in each panel. In all panels, E2s are light blue, RING finger domains are dark blue and the substrates are purple in color.

Table 1

The ubiquitin-proteasome pathway and diseases of the nervous system

Disease	Component linked to the disease	Reference
Alzhemier's disease	Ubiquitin (abnormal form)	[199]
	Inhibition of proteasome	[113]
Amyotrophic lateral sclerosis	Agrregated superoxide dismutase	[125]
Angelman syndrome	Mutation in E6-AP (UBE3A) ubiquitin ligase	[141,142]
Ataxia	USP14 (DUB)	[153]
Gracile axonal dystrophy	Deletion in the UCH-L1 gene	[200]
Huntington's disease	Abnormal nuclear inclusions of ubiquitinated proteins	[201]
Parkinson's disease	Parkin (E3, ubiquitin ligase) UCH-L1 α-synuclein (substrate)	[19,171,202]
Schizophrenia	Decreased expression of UCHL1 and UBP14	[203,204]
Wallerian degeneration	Possible microtubule fragmentation through the ubiquitin-proteasome pathway	[196]