

The ubiquitin proteasome system in neuropathology

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Received: 6 May 2009 / Revised: 10 June 2009 / Accepted: 11 June 2009 / Published online: 14 July 2009
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Abstract The ubiquitin proteasome system (UPS) orchestrates the turnover of innumerable cellular proteins. In the process of ubiquitination the small protein ubiquitin is attached to a target protein by a peptide bond. The ubiquitinated target protein is subsequently shuttled to a protease complex known as the 26S proteasome and subjected to degradative proteolysis. The UPS facilitates the turnover of proteins in several settings. It targets oxidized, mutant or misfolded proteins for general proteolytic destruction, and allows for the tightly controlled and specific destruction of proteins involved in development and differentiation, cell cycle progression, circadian rhythms, apoptosis, and other biological processes. In neuropathology, alteration of the UPS, or mutations in UPS target proteins may result in signaling abnormalities leading to the initiation or progression of tumors such as astrocytomas, hemangioblastomas, craniopharyngiomas, pituitary adenomas, and medulloblastomas. Dysregulation of the UPS may also contribute to tumor progression by perturbation of DNA replication and mitotic control mechanisms, leading to genomic instability. In neurodegenerative diseases caused by the expression of mutant proteins, the cellular accumulation of these proteins may overload the UPS, indirectly contributing to the disease process, e.g., sporadic Parkinsonism and prion diseases. In other cases, mutation of UPS components may directly cause pathological accumulation of proteins, e.g., autosomal recessive Parkinsonism and spinocerebellar ataxias. Defects or dysfunction

of the UPS may also underlie cognitive disorders such as Angelman syndrome, Rett syndrome and autism, and muscle and nerve diseases, e.g., inclusion body myopathy and giant axon neuropathy. This paper describes the basic biochemical mechanisms comprising the UPS and reviews both its theoretical and proven involvement in neuropathological diseases. The potential for the UPS as a target of pharmacological therapy is also discussed.

Abbreviations

A1Up	Ataxin-1 interacting ubiquitin-like protein
APC	Protein mutated in adenomatous polyposis coli syndrome
APC/C	Anaphase promoting complex/cyclosome multimeric E3 ubiquitin ligase
BMP	Bone morphogenic protein, an extracellular patterning signal in CNS development
Cbl	Casitas B-lineage lymphoma, a small family of RING-type E3 ubiquitin ligases
CDK	Cyclin-dependent kinase
CHIP	Carboxy terminus of Hsc70-interacting protein, a U-box E3 ubiquitin ligase
DUB	Deubiquitination enzyme
E1	Ubiquitin activating enzyme
E2	Ubiquitin conjugating enzyme
E3	Ubiquitin ligase
EGFR	Epidermal growth factor receptor
FTLD-U	Frontotemporal lobar degeneration with ubiquitin-positive inclusions
G76	Terminal glycine residue of ubiquitin
GSK3	Glycogen synthase kinase 3
HDM2	Human double minute 2, human MDM2 homolog, an E3 ubiquitin ligase for p53

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HECTD2	HECT domain containing 2, a HECT-type E3 ubiquitin ligase	UBE3A	E6AP, a HECT-type E3 ubiquitin ligase
HIF-1 α	Hypoxia-inducible factor 1, alpha subunit	UBL	Ubiquitin-like domain
HSP70	Heat shock protein 70, a chaperone protein and ATPase	UCH-L1	Ubiquitin carboxy-terminal hydrolase L1, a deubiquitination enzyme (DUB), and E3 ubiquitin ligase when dimerized
HSP90	Heat shock protein 90, a chaperone protein and ATPase	UPS	Ubiquitin proteasome system
IBM	Inclusion body myopathy	USP7	Ubiquitin specific protease 7, a deubiquitinating enzyme (DUB), its targets include ubiquitinated forms of p53, histones and ataxin-1
IBMPFD	Hereditary inclusion body myopathy with Paget disease of bone and frontotemporal dementia	VEGF	Vascular endothelial growth factor
K48	Lysine 48 of ubiquitin	VHL	von Hippel–Lindau disease
K63	Lysine 63 of ubiquitin		
LRRK2	Leucine-rich repeat kinase-2		
MAFbx	Atrogin-1, a muscle-specific F-box ubiquitin ligase adaptor protein		
MAP8	Microtubule-associated protein 8		
MAP1B	Microtubule-associated protein 1B		
MDM2	Mouse double minute 2, an E3 ubiquitin ligase for p53		
NII	Neuronal intranuclear inclusion		
NCI	Neuronal cytoplasmic inclusion		
NEDD4	Neural precursor cell expressed developmentally down-regulated 4, an E3 ubiquitin ligase		
NEDD8	Neural precursor cell expressed developmentally down-regulated 8, a small ubiquitin-like posttranslational modifier protein		
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate		
PMP22	Peripheral myelin protein 22		
PTTG-1	Pituitary tumor transforming gene-1, securin, an APC/C E3 ubiquitin ligase substrate		
pVHL	von Hippel–Lindau protein, an E3 ubiquitin ligase substrate adaptor protein		
SCF	Skp/cullin/F-box E3 ubiquitin ligase complex		
Shh	A cholesterol-dependent extracellular patterning signal in CNS development		
STK15	Serine-threonine kinase 15, aurora A kinase, an APC/C E3 ubiquitin ligase substrate		
RASSF1A	Ras association (RalGDS/AF-6) domain family member 1		
siRNAs	Small interfering RNAs (ribonucleic acid oligomers)		
SUMO	Small ubiquitin-like posttranslational modifier protein		
TDP-43	TAR DNA binding protein 43		
TRAF6	Tumor necrosis factor receptor associated factor 6, a RING-type E3 ligase		
TSG101	Tumor susceptibility gene 101		
Ubc	Ubiquitin conjugating enzyme (E2)		
UBA	Ubiquitin-associated domain		

Introduction

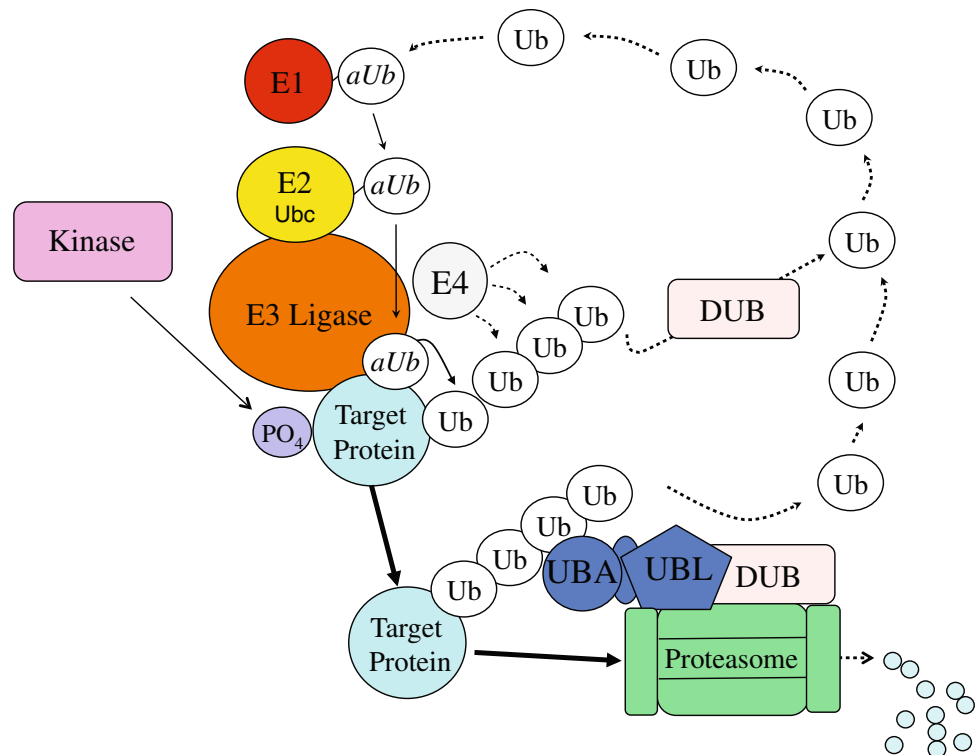
The ubiquitin proteasomal system (UPS) provides both a precise and a general means for the cell to dispose of biologically non-useful proteins, including mutant, misfolded, damaged, terminally modified or over-accumulated proteins [44]. The ability of UPS to cause rapid substrate-specific proteolysis also allows it to function as a molecular switch in many signaling pathways, quickly turning off the function of a target protein.

Ubiquitin is a small 8.5 kD protein composed of 76 amino acids. In the process of *ubiquitination* (also known as *ubiquitylation*), ubiquitin is covalently attached to lysine residues within target proteins via a peptide bond [44]. In many cases, additional ubiquitin monomers are added to the first ubiquitin to form polyubiquitin chains consisting of four to seven ubiquitin monomers. These polyubiquitinated proteins are then shuttled to a multimeric protease complex known as the 26S proteasome, present in both the nucleus and cytoplasm of mammalian cells. At the proteasome, ubiquitin monomers are released from the target protein and recycled for future use. The target protein is then degraded by proteasomal protease activity.

Ubiquitination is a multistep process

Aaron Ciechanover, Avram Hershko and Irwin Rose shared the 2004 Nobel Prize in Chemistry for elucidating the multistep process of ubiquitin-mediated protein degradation [44]. Ubiquitin is first activated by an E1 ubiquitin-activating enzyme (Fig. 1). This activation is ATP-dependent and results in transient adenylation of ubiquitin. AMP is then released and a thioester bond is formed between the C-terminal carboxyl group of ubiquitin and a sulfhydryl group on an E1 cysteine residue [41]. Ubiquitin is then transferred to a cysteine residue of an E2 ubiquitin conjugating enzyme (Ubc). The ubiquitin-bound E2 Ubc then

Fig. 1 Overview of the ubiquitin proteasome system. E1 ubiquitin activating enzymes transfer activated ubiquitin (*aUb*) to E2 ubiquitin conjugating enzymes that associate with E3 ubiquitin ligases. E3 ubiquitin ligases catalyze the formation of an isopeptide bond between ubiquitin (*Ub*) and the target protein. Substrate recognition often requires prior phosphorylation (PO_4) of the target protein. E4 enzymes promote the formation of polyubiquitin chains. Receptor proteins containing UBL and UBA domains transfer the polyubiquitinated target protein to the proteasome for cleavage in to free amino acids and small peptides. Deubiquitinating enzymes (*DUBs*) promote recycling of ubiquitin monomers for reactivation by E1



associates with an E3 ubiquitin ligase capable of recognizing a specific target protein. The Ubc facilitates transfer of activated ubiquitin to the E3 ligase–target protein complex. The E3 ligase catalyzes the formation of an isopeptide bond between a target protein lysine residue and the C-terminal glycine of ubiquitin (Gly76). In rare cases, the Ubc processes both E2 ubiquitin conjugating and E3 ubiquitin ligase activity.

A more recently described E4 activity has been attributed to at least two protein factors that facilitate conjugation of additional ubiquitin monomers to form the polyubiquitin chain, usually through linkages of lysine 48 [64]. Chains formed by linkages involving other lysine residues in ubiquitin, particularly lysine 63, also occur, as do chains with mixed lysine 48 and lysine 63 linkages. The type of linkage, 48, 63, or mixed, affects the fate of the ubiquitinated protein. K48-linked polyubiquitinated proteins are generally degraded in the proteasome and K63-linked polyubiquitinated proteins are ultimately degraded in the lysosome [62, 123].

Another class of UPS proteins sometimes known as *shuttle* or *ubiquitin receptor proteins* act as carriers to deliver polyubiquitinated proteins to the 26S proteasome [8, 55]. The 26S proteasome is composed of a 20S core subunit capped by two 19S regulatory subunits, one at either end of the cylindrically shaped core to form a barrel-like structure [44]. The core subunit itself is composed of

four rings. Ubiquitin-associated (UBA) domains of the ubiquitin receptor protein bind the polyubiquitin chains of target proteins and ubiquitin-like (UBL) domains of the receptor protein bind the 19S regulatory subunits of the proteasome [8, 55]. Like E1-mediated ubiquitin activation, transfer of the polyubiquitinated protein to the proteasome is an ATP-dependent process.

Proteolytic degradation of the target protein takes place within the rings of the core subunit following removal of the ubiquitin monomers by deubiquitination enzymes (DUBs) and unfolding of the substrate protein [44]. The outer rings of the core act as gates to the core and each is composed of seven α -subunits. The inner two rings contain the protease activity and are each composed of seven β -subunits.

Proteins with UBA domains also bind monoubiquitinated proteins and are involved in initiating endocytosis [62, 97]. These proteins may also protect monoubiquitinated proteins from polyubiquitination or deubiquitination. DUBs are thought to also act to deubiquitinate ubiquitinated proteins to prevent their targeting to the proteasome. Ubiquitin receptor proteins effectively bind tetraubiquitin chains and may also function to protect polyubiquitinated proteins from unnecessary polyubiquitin chain expansion or deubiquitination prior to reaching the proteasome [55]. Thus, the process of ubiquitination appears to be highly regulated at various levels of the UPS.

Monoubiquitination, sumoylation and neddylation

Nuclear and cytosolic proteins generally undergo polyubiquitination and degradation by the 26S proteasome, as do some membrane proteins [129]. Membrane proteins may also be monoubiquitinated, triggering their cytoplasmic internalization and degradation in either the endosome–lysosome system or the proteasome [97]. Cbl is a plasma membrane-associated E3 ligase that along with an E2 Ubc, either monoubiquitinates or polyubiquitinates several membrane proteins [74].

More recently, a family of ubiquitin-like modifiers known as *small ubiquitin-like modifier* (SUMO) 1-4 has been described. These modifiers are small proteins similar in size to ubiquitin. Through a process analogous to monoubiquitination termed *sumoylation*, SUMO monomers are conjugated to target proteins. In some cases monoubiquitination or sumoylation may act to protect proteins from ubiquitin-dependent degradation [59] and in other cases they appear to trigger polyubiquitination [112]. Sumoylation and monoubiquitination at one or two lysines within a target protein may also alter the subcellular location of the protein [98]. Monoubiquitination of membrane proteins may trigger their cytoplasmic internalization [97]. Monoubiquitination of the p53 tumor suppressor causes it to be transported from the nucleus to the cytoplasm and

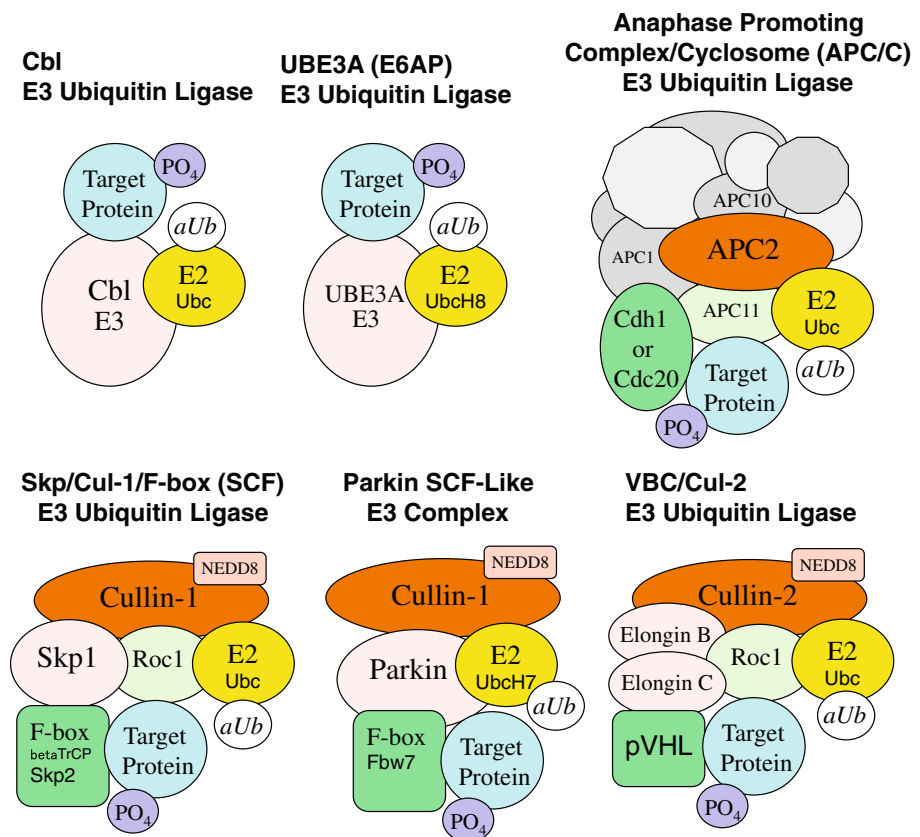
transport may be further enhanced by sumoylation of p53 [71].

An additional small ubiquitin-like posttranslational modifier is known as *neural precursor cell expressed, developmentally down-regulated 8* (NEDD8) [22]. The functions of *neddylation* are less understood; however, neddylation of cullin proteins, essential subunits of certain E3 ubiquitin ligase complexes, appears to be required for normal ligase function (Fig. 2).

Ubiquitination is substrate specific

E1 activity is nonspecific with regard to the protein targets of the UPS and only two isoforms of this enzyme exist in the cell (E1a and E1b) [18]. By comparison about 50 different E2s are known [10]. E3 ligases are the most variable and abundant with over 500 putative E3s identified so far. A given E2 can often associate with multiple different E3s providing for a large variety of functional E2/E3 activities and a relatively high degree of target substrate specificity. However, this specificity is not absolute. Many E3 proteins are able to ubiquitinate several, usually biochemically related, substrate target proteins. These related proteins often share a common small amino acid sequence that acts as a relatively substrate-specific E3 binding domain. Many

Fig. 2 Cbl family E3 ligases and UBE3A E3 ligase are single proteins that associate with an E2 Ubc and the target substrate. The SCF and APC/C E3 ubiquitin ligases are multimeric complexes. The APC/C has only two known adapter subunits, Cdc20 and Cdh1 (not to be confused with E-cadherin, which is sometimes also referred to as Cdh1). The SCF can associate with several different substrate adapter proteins known as F-box proteins



target proteins are thought to be ubiquitinated by only one E3, while others, the tumor suppressor p53 for example, can be ubiquitinated by multiple E3s [71, 116, 141].

Most of the E3 ligases are classified as to whether they contain *homologous to E6AP COOH-terminus* (HECT) or *really interesting new gene* (RING) domains. A third smaller class of E3 ligases is referred to as U-box containing ligases. Both HECT and RING types may possess E3 ligase function as single proteins, for example the HECT-type ligase UBE3A and the RING-type ligase Cbl (Fig. 2). RING-type E3s, however often associate with scaffolding and other auxiliary proteins to form multimeric functional complexes. These complexes can associate with different regulatory subunits, which act as substrate-specific adaptor proteins providing for even greater substrate specificity. Examples include the *Skp/cullin/F-box* (SCF) and *anaphase promoting complex/cyclosome* (APC/C) E3 ubiquitin ligases that exhibit specificity for different substrates depending on the adaptor protein bound to the complex (Fig. 2). SCF complexes containing isoforms of the F-box protein β -TrCP can ubiquitinate several proteins, including the DNA transcriptional regulator β -catenin and the cell cycle progression regulator *Emi1* [40, 43]. In some instances, substrate proteins must be phosphorylated first at specific sites before they can be recognized by the E3 ubiquitin ligase. This may provide an additional level of control as to when and where a target protein is ubiquitinated. For instance, SCF substrates generally must be phosphorylated first on the serine residues (S) of the consensus amino acid sequence motif DSGXXS before the F-box regulatory subunit can bind the substrate [43, 65]. Different substrates may be phosphorylated by different kinases to provide another layer of control, and the activity of the E3 ligase itself is often regulated by phosphorylation [105]. Lastly, the subcellular compartmentalization of the substrate and E3 ligase may add additional substrate specificity. Examples of specific subcellular locations include the cell membrane, nuclear and cytoplasmic compartments, chromatin, centrosome, mitotic spindle, and neuronal synapse. Thus, with regard to control of cell signaling and many biochemical processes, ubiquitination of specific substrates is a tightly controlled process that prevents the random destruction of proteins.

The UPS facilitates diverse biological functions

In addition to its general wastebasket-like function, ubiquitination is also a highly specific way of controlling protein expression in signal transduction systems, in cell–cell communication during development, and at the neural synapse [113, 147]. Other diverse functions controlled by the UPS include regulation of gene transcription via monoubiquitination and deubiquitination of histones,

driving circadian clocks, the auxin-mediated response to light in plants, and the regulation of cell cycle progression and apoptosis [26, 113, 131]. The UPS is also important for the functioning of the immune system and is required for MHC class I antigen presentation [39, 83, 144].

The UPS in neural development

Notch is an integral membrane protein responsive to extracellular signals. Notch has been implicated in neural stem cell proliferation, self-renewal and differentiation [73]. It has also been implicated in plasticity of adult brain hippocampus. It is cleaved by γ -secretase activity, which allows its signal transduction component to translocate to the nucleus and activate transcription. γ -Secretase is derived from the presenilin complex, the constituents of which are all UPS substrates [82]. Notch protein stability is also negatively regulated by the SCF ubiquitin ligase.

The SCF ubiquitin ligase substrate β -catenin is a transcription factor that also regulates proliferation of neural precursor cells. An amazing study by Chenn and Walsh [13] showed that transgenic mice expressing a stabilized form of β -catenin in neural precursor cells develop enlarged cerebral cortices with complex gyral patterns resembling those of primates.

Bone morphogenic proteins (BMPs) and Shh proteins are secreted proteins involved in dorsal–ventral induction in neurogenesis and other developmental and signaling processes [37]. The downstream effector proteins of BMP and Shh, Smad and Gli proteins, respectively, are both transcription factors regulated by ubiquitin-dependent proteolysis, as are BMP cell membrane surface receptors [99]. Furthermore, both Gli proteins and Smad protein complexes induce transcription of E3 ligase proteins. Additionally, the APC/C E3 ligase is involved in the regulation of axonal growth and patterning in the developing cerebellum [66].

UPS pathology in neurodegenerative disease

Alzheimer disease

Dysregulation of the UPS appears to be both a cause and result of neurodegenerative disease processes. In Alzheimer disease, hyperphosphorylation of the microtubule-associated protein tau results in the accumulation of paired helical tau filament involved in the formation of neurofibrillary tangles and neuritic plaques. These abnormal tau aggregates are immunoreactive for anti-ubiquitin antibodies suggesting that the cell is attempting to dispose of these abnormal proteins. Kinases implicated in tau phosphorylation include

those involved in phosphorylation of substrates to allow recognition by ubiquitin ligases, namely *casein kinase 1* and *glycogen synthase kinase 3* (GSK3) which phosphorylate SCF- β -TrCP E3 ligase substrates [24, 56]. The UPS is also involved in early stages of autophagy, and neuronal granulovacuolar degeneration structures are ubiquitin positive [15, 62]. Relative dysfunction or inhibitory overloading of the UPS may contribute to the abnormal accumulation of phosphorylated and ubiquitinated tau. Interestingly, a frameshift mutant of ubiquitin, UBB+1, found in some sporadic and hereditary Alzheimer disease and polyglutamine repeat disease patients, inhibits the UPS and enhances toxic protein aggregation in a yeast model system [30, 124]. Indeed anti-ubiquitin antibodies are good, albeit nonspecific, markers for many of the abnormal cellular inclusions and neuropil deposits found in Alzheimer disease, corticobasal degeneration, progressive supranuclear palsy, Picks disease, other frontotemporal dementias, multiple system atrophy, Parkinson disease, amyotrophic lateral sclerosis and others [15] (Fig. 3).

The relative importance of tau neuronal pathology and β -amyloid deposition in extracellular plaques in Alzheimer disease is a perennial topic of debate. β -amyloid is derived from *amyloid precursor protein* (APP), a neuronal transmembrane protein that appears to function as a cell membrane receptor and is also involved in synaptic vesicle recycling [12, 14].

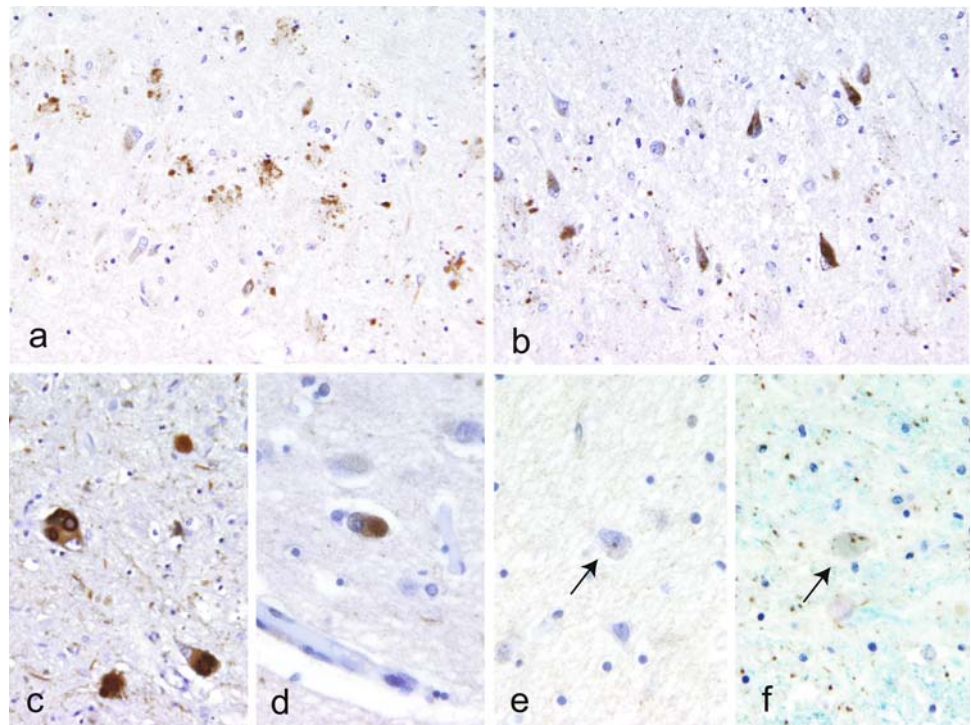
APP binding protein-1 (APP-BP1) binds APP intracellularly at the membrane [14]. APP-B1 also associates with

UBA3 and together they act as an E1-like activating enzyme for the process of neddylation. The APP-B1/UBA3 complex and the NEDD8 conjugating enzyme Ubc12 appear to be necessary for neddylation of cullin proteins and normal cell cycle progression from S-phase to mitosis in Chinese hamster ovary cells [12]. Overexpression of APP or APP-BP1 in cultured primary rat neurons causes DNA synthesis and apoptosis by a mechanism dependent upon the presence of UBA3, Ubc12, and caspase-6 activity.

Similar to Notch, APP is subjected to intramembrane proteolysis via a presenilin-dependent γ -secretase activity. This results in release of β -amyloid, p3 and the APP *intracellular cleavage domain fragment* (AICD). AICD can translocate to the nucleus and result in apoptosis of cells in culture. It is postulated that APP-B1-mediated activation of neddylation, and presumably cullin-based ubiquitin ligases, signals postmitotic neurons to re-enter the cell cycle triggering their apoptosis similar to AICD [12]. Overexpression of APP increased APP-BP1 levels in cell membrane lipid rafts in cultured neurons and increased APP-B1 is found in lipid rafts in the hippocampus of Alzheimer disease patients [11]. These findings suggest that in addition to the role of β -amyloid deposition in apparently disrupting brain circuitry, cellular signaling aberrations involving APP-B1 and AICD resulting in neuronal toxicity and death may be additional mechanisms of APP-mediated Alzheimer neurodegeneration [61].

p62 is a polyubiquitin binding protein processing UBA- and UBL-like domains. It may thus act as a receptor/shuttle

Fig. 3 Anti-ubiquitin immunostaining in neurodegenerative disease. Ubiquitin antibodies highlight Alzheimer disease pathology including hippocampal plaques (a) and neurofibrillary tangles (b). Brain stem (c) and cortical Lewy bodies (d) are also ubiquitin positive. Ubiquitin-positive inclusions are found in the nucleus of cortical neurons in Huntington disease (e) and in the cytoplasm of anterior horn neurons in motor neuron disease (f). Original magnifications are $\times 200$ (a, b) and $\times 400$ (c–f)



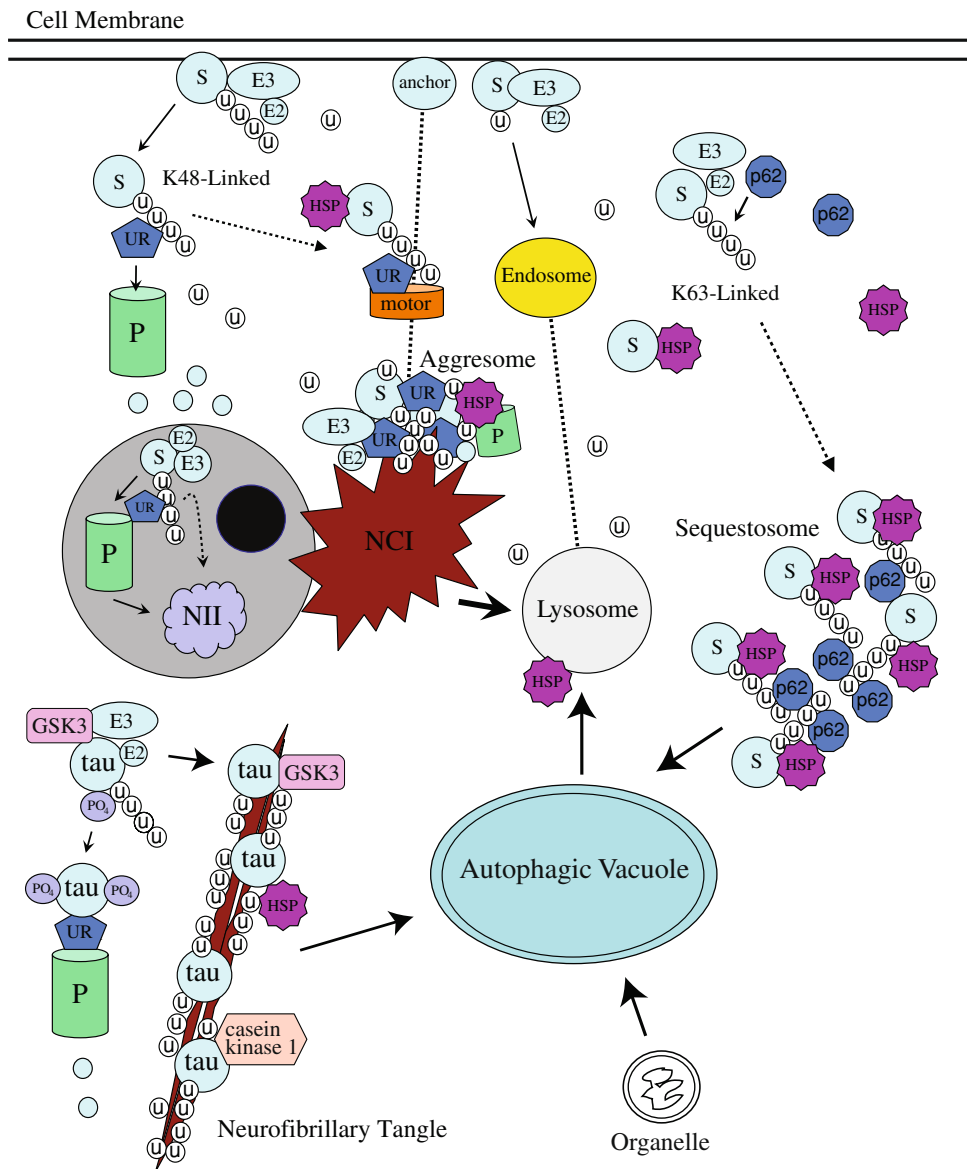


Fig. 4 Simplified model of the UPS, endosome–lysosome system and autophagy cellular pathways. Cell membrane proteins that are UPS substrates (S) are generally monoubiquitinated and degraded in the endosome-lysosome system, or polyubiquitinated and delivered to the 26S proteasome by ubiquitin receptor proteins (UR). Nuclear proteins are degraded in the proteasome within the nucleus or translocated to the cytosol where they can be degraded by various routes. Polyubiquitinated nuclear proteins may be deposited into neuronal intranuclear inclusions (NII) in disease states. Polyubiquitinated cytosolic proteins and UPS machinery proteins such as E2s, E3s and proteasomal subunits may be transported along microtubules (dotted lines) to be

protein capable of transferring polyubiquitinated proteins such as polyubiquitinated tau to the 26S proteasome. Indeed p62 can be found associated with tau in neurofibrillary tangles. p62 appears to bind K63-linked polyubiquitin chains and promote the clearance of K63-linked polyubiquitinated proteins by autophagy [123]. It also may serve as a scaffold protein to promote K63-linked polyubiquitination

deposited into aggresomes, which may form neuronal cytoplasmic inclusions (NCI). Similar to other SCF E3 ligase substrates, tau is probably first phosphorylated by casein kinase 1, which facilitates further phosphorylation by GSK3. Hyperphosphorylated tau is deposited into neurofibrillary tangles, which like NCIs may be cleared by autophagy. K63-linked polyubiquitinated chains bind p62 and form complexes known as sequestosomes. Aggregation of insoluble misfolded proteins is prevented by heat shock proteins (HSP), which also promote the fusion of sequestosomes to autophagic vacuoles and ultimately the lysosome

of membrane proteins by the RING-type E3 ligase *tumor necrosis factor receptor associated factor 6* (TRAF6). p62 also associates with itself and other proteins in a cytoplasmic complex known as the sequestosome and may thus be involved in delivery of proteins to the lysosome (Fig. 4).

A recent study of p62 knock-out mice revealed that adult animals developed age-dependent hyperphosphorylated tau,

neurofibrillary tangles and neurodegeneration and a behavioral and cognitive phenotype mimicking Alzheimer disease [104]. Biochemical fractionation revealed K63-linked polyubiquitinated tau aggregates. p62 therefore binds with K63-linked polyubiquitinated tau to prevent its aggregation so that it can be sequestered and delivered to the lysosome. In support of this pathway, tau binds HSP90, which may facilitate tau phosphorylation [126], but is also important in the binding of proteins to the lysosomal membrane. Interference with this degradative pathway may produce Alzheimer disease or Alzheimer-like dementia.

Frontotemporal lobar degeneration (FTLD) includes tau immunopositive and tau immunonegative forms. FTLD with ubiquitin-immunoreactive lesions (FTLD-U) is a major type of tau negative FTLD, which can be further subdivided into TAR DNA binding protein 43 (TDP-43) immunopositive and immunonegative types [46, 53, 101, 108]. TDP-43 negative FTLD-U subtypes demonstrate either both nuclear and cytoplasmic ubiquitin-positive neuronal inclusions or cytoplasmic inclusions only. Cytoplasmic, but not nuclear inclusions are also p62 positive [46]. Thus, p62 is linked to both cytoplasmic ubiquitination and autophagy in neurons, but may not be involved in proteasome-mediated degradation of proteins. The latter may be less important compared to autophagy in Alzheimer disease and frontotemporal dementias.

Parkinson disease

Familial juvenile onset Parkinson's disease is caused by a defect in an SCF E3 ubiquitin ligase component known as *parkin* (PARK2) (Fig. 2). Substrates of parkin-mediated ubiquitination include a minor glycosylated form of α -synuclein (α -sp22), the α -synuclein binding protein synphilin-1, the *parkin-associated endothelin receptor-like receptor* (Pael-R) and cyclin E [120]. Along with parkin, both α -sp22 and Pael-R are found in the Lewy body cytoplasmic inclusions characteristic of most forms of Parkinson disease. Overexpression of Pael-R results in the rapid loss of dopaminergic striatonigral neurons in rats with no observed effect on GABAergic neurons of the globus pallidus [25]. Since Pael-R protein would tend to accumulate in the presence of mutated parkin, this supports the possible importance of Pael-R in the pathogenesis of autosomal recessive juvenile Parkinsonism.

Parkin also ubiquitinates the ataxin-2 protein which is disrupted by expansion of its polyglutamine repeat in spinocerebellar ataxia type 2 [50]. Like the APC/C E3 ligase, parkin is believed to function as a tumor suppressor protein [33, 77].

Genes mutated in other forms of autosomal recessive Parkinson disease include *phosphatase* and *tensin homo-* *logous deleted on chromosome ten* (PTEN)-induced putative

kinase 1 (PINK1, also known as PARK6) and DJ-1 (PARK7). A recent study has shown that PINK1 and DJ-1 form a complex with parkin and promote parkin's ubiquitin ligase activity [142]. Another study showed that parkin directly interacts with PINK1 and blocks ubiquitin-dependent proteolysis of PINK1 [117]. These studies implicate dysfunction of parkin E3 ligase activity as a common etiologic mechanism for recessive forms of inherited Parkinson disease, all of which may be possibly caused by over accumulation of Pael-R. PINK1/parkin also appears to be important in mitochondrial function, which is deficient in Parkinson disease.

Rare autosomal dominant forms of Parkinson disease in German and Japanese kindred have been linked to polymorphisms in the DUB enzyme known as *ubiquitin carboxy-terminal hydrolase L1* (UCH-L1) [38, 115]. UCH-L1 is an abundant protein in neurons and can also act as an E3 ubiquitin ligase when dimerized. In addition to α -synuclein and synphilin-1, Lewy bodies found in most forms of Parkinson disease contain UCH-L1 and mice lacking UCH-L1 show neurodegeneration [115]. Furthermore, similar to parkin, overexpression of UCH-L1 leads to aggresome formation in response to proteasomal inhibition [2].

Aggresomes are organized cellular structures in which nonfunctional aggregated proteins are shuttled along microtubules by dynein-dependent transport [2, 67] (Fig. 4). Lewy bodies are thought to form by growth or coalescence of perinuclear aggresomes. Neurons in general may be more susceptible to mutations in UPS components such as E3 ligases and DUBs because they have lower overall cellular UPS activity capacity compared to glia [127].

It has been suggested that ubiquitination and sequestration of proteins such as α -synuclein and synphilin-1 in aggresomes and large cytoplasmic inclusions is a mechanism used by the cell to protect itself from the toxicity of these proteins [80]. This is plausible considering evidence that small oligomers of prions can overload the proteasome and are toxic to the cell [68]. This may also explain why Parkinson disease occurs in the autosomal recessive form that harbors defective parkin E3 ligase despite that dopaminergic nigral neurons do not develop Lewy bodies in these patients.

Yet, there is conflicting evidence for the pathogenesis of α -synuclein aggregation. α -Synuclein is mono- and di-ubiquitinated by the E3 ubiquitin ligase SIAH-1. This reportedly promotes α -synuclein aggregation and enhances its neural toxicity [75, 109]. Ubiquitination of synphilin-1 by SIAH-1 also promotes its aggregation into inclusions [28, 80]. Interestingly, SIAH-1-mediated α -synuclein ubiquitination was inhibited by the Parkinson disease-associated A30P α -synuclein mutation supporting a role for toxicity of the unaggregated form of mutant α -synuclein in inhibiting UPS function.

Leucine-rich repeat kinase 2 (LRRK2) (PARK8) is mutated in about 2–5% of sporadic and autosomal dominant Parkinson disease, respectively [34, 149]. LRRK2 is ubiquitinated by the E3 ubiquitin ligase *carboxy terminus of Hsc70-interacting protein* (CHIP), which associates with HSP70 [63]. CHIP and parkin both can bind HSP70 and ubiquitinate polyglutamine repeat expanded proteins such as ataxin-3 [95]. LRRK2 is found associated with α -synuclein in Lewy bodies and in glial cytoplasmic inclusions in multisystem atrophy [49]. LRRK2 has been shown to co-immunoprecipitate with parkin consistent with it also being a parkin substrate. One study showed that LRRK2 aggregates in LRRK2-transfected cells were increased by parkin coexpression, which increased ubiquitination of aggregated LRRK2 [142]. LRRK2 also associates with microtubules and induces protein aggregation at the cell membrane, synapses, and membrane-bound organelles suggesting that it has a physiological role in autophagy. Indeed cytoplasmic neuronal inclusions resulting from proteasomal inhibition can be cleared by autophagy in model systems.

Prion diseases

Spongiform encephalopathies such as sporadic and variant Creutzfeldt–Jakob disease are neurodegenerative conditions caused by mutant hereditary or transmissible prion proteins. The unique property of prions is their ability to induce abnormal conformational changes in normal prion proteins. In some forms of prion disease, e.g., Kuru, abnormal prion proteins form ubiquitin immunopositive amyloid plaques. As misfolded mutant proteins pathological prions are “born” UPS substrates. Although disease-associated prions are deposited into perinuclear cytoplasmic aggregates in prion infected cultured neurons and infected mouse brains, evidence suggests that prion-mediated neuronal toxicity is due to inhibition of proteasome proteolytic activity by prion oligomers [68, 69].

Additional evidence linking prion pathology to disruption of the UPS has come from mouse models. Mutation of the RING-type ubiquitin ligase *mahogunin* results in a spongiform encephalopathy in mice histologically mimicking human prion disease [58]. Mahogunin monoubiquitinates *tumor susceptibility gene 101* (TSG101), a protein involved in endosomal sorting. Mahogunin knockdown by siRNAs interferes with endosome to lysosome trafficking of EGFR receptors in cultured cells. Study of mice strains exhibiting variable incubation times for prion diseases identified an E3 ligase known as *HECT domain containing 2* (HECTD2) as a quantitative trait gene associated with decreased disease incubation periods. Furthermore, analysis of human prion disease patient samples identified specific *HECTD2* haplotypes associated with variant CJD and Kuru, further implicating a role for HECTD2 ubiquitin ligase in prion

disease [84]. The above studies link both ubiquitin ligase or proteasome dysfunction and endosome–lysosome dysfunction to the pathogenesis of prion diseases.

Motor neuron disease

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive fatal motor neuron disease that occurs in sporadic and inherited forms. Approximately 20% of the familial cases harbor mutations in *superoxide dismutase-1*. In ALS, spinal motor neurons demonstrate ubiquitin immunopositive cytoplasmic inclusions (Fig. 3). Two recent studies have revealed that these inclusions are also immunopositive for TDP-43 in all cases of ALS not associated with superoxide dismutase-1 mutations [87]. This finding is accompanied by decreased normal expression of TDP-43 in the nucleus. TDP-43 is a DNA binding protein involved in the pathogenesis of HIV infection and has no known functional role in the UPS. Its presence in both FTLD and ALS inclusions strongly indicates a common link in the neurodegenerative process in these disorders which is at least in part mediated by the UPS [60, 103]. Furthermore, the amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam (ALS/PDC) shows histopathological overlap with all major types of neurodegeneration further illustrating the central role of the UPS in the pathophysiology of neurodegeneration [91, 122].

ALS2 is mutated in a juvenile autosomal recessive form of ALS and codes for an endosomal membrane associated protein involved in endosomal membrane fusion and endosomal trafficking along microtubules. Mutant *ALS2* is unstable and is rapidly degraded by the proteasome [143]. Thus, like other neurodegenerative conditions, motor neuron disease has been linked to dysfunction of both the ubiquitin proteasomal and endosome/lysosome systems.

Polyglutamine repeat diseases

Huntington disease

Huntington disease is characterized by expression of toxic polyglutamine repeat-expanded mutated forms of the protein *huntingtin* and its aggregation in intranuclear and cytoplasmic inclusions. These polyglutamine repeat expanded proteins are degraded by the UPS, components of which are also found within the inclusions, e.g., ubiquitin, proteasomal subunits, chaperones and autophagy proteins [30, 51] (Fig. 3). Decreased UPS function appears to be associated with huntingtin neuronal toxicity. Although the mechanism for this is not known [30, 140], neuronal toxicity may, at least in part, be due to synaptic dysfunction [133]. It is possible that as postulated for other mutant UPS substrates, such as

prions, misfolded mutant huntingtin protein effectively inhibits the proteasome machinery allowing toxicity from over-accumulated mutated huntingtin or other proteins.

Lysine 48-linked polyubiquitin is increased in the brains of mouse Huntington models and Huntington disease patients further suggesting proteasome dysfunction [4]. In cell culture, induction of neuronal death by mutated huntingtin depends on the amount of mutant protein and the length of the polyglutamine expansion. Neuron death can occur in the absence of inclusions, which are associated with increased neuronal survival consistent with toxicity from unaggregated mutant huntingtin [3]. Interestingly, p53 binds a p53-responsive element in the huntingtin gene activating its transcription [29]. This potentially links huntingtin expression to p53-mediated stress responses and implicates control of p53 stability as a possible effector of the expression of mutant huntingtin.

Spinocerebellar ataxias

The spinocerebellar ataxias (SCA) are characterized by progressive cerebellar ataxia, often accompanied by other long tract and variable ophthalmic signs [5, 74, 107]. Most are autosomal dominant disorders and many are mediated by pathological expansion of polyglutamine repeats within functional proteins. Spinocerebellar ataxia type 1 (SCA1) results from polyglutamine repeat expansion of the protein encoded by the gene *ataxin-1*. Ataxin-1 protein is a substrate of the E3 ubiquitin ligase UBE3A and mutant ataxin-1 is resistant to ubiquitin-dependent proteolysis. An ataxin-1 interacting protein known as *ataxin-1 interacting ubiquitin-like protein* (A1Up) possesses N-terminal UBL and C-terminal UBA domains [107]. It also contains heat shock protein chaperonin-binding motifs. Heat shock chaperones, e.g., HSP70 and HSP90, and chaperonin proteins prevent protein aggregation, especially of misfolded proteins and are involved in the ubiquitin-dependent degradation of misfolded and otherwise damaged proteins. The UBL is necessary for A1Up's association with the 19S proteasome and this association is inhibited by misfolded mutant polyglutamine repeat ataxin-1 [107]. Thus, disruption of the delivery of ubiquitinated ataxin-1 to the proteasome may account for ubiquitin immunopositive nuclear inclusions observed in Purkinje, pontine and inferior olive neurons in SCA1. The interaction of ataxin-1 with the deubiquitinating enzyme USP7 is also weakened by ataxin-1 mutation. This may additionally contribute to abnormal accumulation of ubiquitinated mutant ataxin-1 [47].

As previously noted, the SCA2 gene product ataxin-2 is ubiquitinated by parkin [50]. Ataxin-2 binds membrane-associated proteins and transmembrane cell surface receptors. A recent study has shown that ataxin-2 can also associate with endophilin, a protein involved in synaptic

vesicle endocytosis, and the Cbl E3 ubiquitin ligase involved in membrane receptor internalization [102]. Ataxin-2 thus appears to be involved in endocytic cycling of membrane proteins.

The polyglutamine expanded mutant protein of SCA3 (Machado–Joseph disease) contains an ubiquitin-interacting motif [5]. Ataxin-3 has been shown to preferentially bind K63-linked polyubiquitin chains and act as a deubiquitinating enzyme (DUB) [125]. Ubiquitination of ataxin-3 itself may enhance its deubiquitinating activity.

SCA7 is also a polyglutamine repeat disorder. Ataxin-7 was found to associate with the R85 splice variant of the *Cbl-associated protein* (CAP), which contains a nuclear localization signal domain and is therefore translocated to the nucleus [74]. Mutant ataxin-7 interferes with the nuclear translocation of the transcription factor NF- κ B resulting in decreased BCL-X(L) expression and induction of apoptosis in cultured cerebellar neurons [132]. Both translocation of NF- κ B to the nucleus and the nuclear activity of NF- κ B are dependent on the ubiquitin-mediated destruction of *inhibitors of κ B* (I κ Bs). More recently ataxin-7 mutants have been shown to inhibit gene transcription and proteasome activity [121, 132]. Inhibition of NF- κ B-mediated gene transcription by mutant, polyglutamine repeat expanded ataxin-7 can therefore be explained by its apparent ability to inhibit ubiquitin-dependent proteolysis. Ataxin-7 mutants may selectively interfere with the UPS-mediated degradation of proteins bound for the nucleus.

The above-mentioned SCA types all involve mutation of UPS component proteins (Table 1). Thus, this group of diseases associated with similar clinical manifestations appears to be linked by unique disruptions of the UPS, particularly with regard to turnover of membrane and/or synaptic proteins in SCA2 and nuclear proteins in SCA1 and SCA7. Mutant ataxin-2 may also inhibit parkin possibly contributing to the SCA2 disease process. SCA3 appears to differ in that the role of ataxin-3 in the deubiquitination of K63-linked ubiquitin chains suggests dysregulation of autophagy.

Angelman, Prader–Willi, Rett and Autism

Angelman syndrome is characterized by microcephaly, severe developmental delay, seizures, ataxia, a stereotypical hand flapping, and a happy excitable demeanor. It is associated with deletions of chromosomal segment 15q11–13 and is caused by loss of function of the ubiquitin ligase alternately known as UBE3A or E6AP. UBE3A is the same E3 ligase that mediates the human papillomavirus E6 protein-induced ubiquitination of the p53 tumor suppressor protein. Loss or mutation of the maternally inherited UBE3A allele is sufficient to cause Angelman disease [111]. Differences in

Table 1 UPS components and substrates in neuropathological diseases

Disorder	Gene product and function
Parkinson disease	
Autosomal dominant (early onset)	α -Synuclein (SNCA) (PARK1), aggregates in Lewy bodies
Autosomal dominant (late onset)	Leucine-rich repeat kinase 2 (LRRK2) (PARK8), a CHIP ubiquitin ligase substrate, contains a Roc domain as found in SCF ligases
Autosomal dominant (late onset)	Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) (PARK5), a DUB, acts as an E3 ligase when dimerized, polymorphisms linked to rare forms of familial disease
Autosomal recessive (juvenile onset)	Parkin (PARK2), a subunit of a SCF E3 ubiquitin ligase
Autosomal recessive (early onset)	PINK1 (PARK6), promotes parkin ubiquitin ligase activity
Autosomal recessive (early onset)	DJ-1 (PARK7) chaperone, promotes parkin ubiquitin ligase activity
Spinocerebellar ataxias	
SCA1	Ataxin-1, a UBE3A E3 ligase, mutation blocks its ubiquitination and association with the ubiquitin receptor A1Up and the DUB enzyme USP7
SCA2	Ataxin-2, associates with c-Cbl E3 ligase and is involved in membrane protein endocytosis, and is a parkin E3 ligase substrate
SCA3	Ataxin-3, a deubiquitinating enzyme (DUB)
Prion diseases	
Autosomal recessive ALS	Prions may block normal function of the proteasome, HECT2D E3 ubiquitin ligase haplotypes are associated with vCJD and Kuru
Autosomal recessive ALS	ALS2, an endosomal membrane associated protein involved in endosome membrane fusion and trafficking, mutation decreases ALS2 protein stability
Angelman syndrome	Loss or mutation of UBE3A E3 ligase at Angelman/Prader–Willi locus
Rett syndrome	Decreased UBE3A E3 ligase due to MECP2 mutations
Autism	Copy number alterations of <i>UBE3A</i> and other UPS genes
Giant axon neuropathy	Mutation of gigaxonin, an E3 ubiquitin ligase
IBMPFD	Mutation of valosin-containing protein (VCP), involved in ubiquitin-mediated processing of membrane and cytosolic proteins.
Sporadic IBM	VCP and ubiquitin are found in inclusion bodies.
von Hippel–Lindau disease	pVHL, substrate-binding subunit of ubiquitin ligase targeting HIF1- α
Medulloblastoma	Overexpression of several signaling pathway genes that are ubiquitinated by the SCF: c-myc, β -catenin, Gli, stabilizing mutations of Gli and β -catenin prevent their ubiquitin-dependent proteolysis
Adamantinomatous craniopharyngioma	Stabilizing β -catenin mutations preventing its ubiquitin-dependent proteolysis
Gliomas	Misregulation and mutation of cell cycle control proteins regulated by the UPS: CDKs, CDK inhibitors, p53, altered expression of APC/C E3 ubiquitin ligase regulators Emi1 and RASSF1A

expression patterns of paternally and maternally inherited UBE3A alleles are due to differential gene methylation (imprinting). Unlike paternally derived UBE3A, maternal UBE3A is active in the cerebellum and hippocampus. It is needed to maintain CA1 synaptic plasticity, which is lost in *UBE3A*^{-/-} mice and is necessary for normal experience-based maturation of the visual cortex [146]. Known substrates of UBE3A have not been linked to the pathogenesis of Angelman syndrome. The disease can be prevented, however, in an Angelman mouse model by mutation of the inhibitory phosphorylation site of the alpha subunit of *calcium-calmodulin-dependent protein kinase II* (α -CaMKII), suggesting Angelman syndrome may result from increased inhibitory phosphorylation of α -CaMKII [27]. The important target of UBE3A in Angelman disease may thus be a direct or indirect regulator of α -CaMKII phosphorylation.

Since α -CaMKII is also important in maintaining hippocampal plasticity, this is a highly plausible hypothesis [27].

Prader–Willi syndrome, characterized by hyperphagia, cognitive impairment and hypogonadism, results from loss of genetic material from the same chromosomal region as in Angelman, but differs in that the paternally derived segment is lost. UBE3A has not been associated with Prader–Willi in which multiple different genes have been implicated. A common misconception is that Angelman and Prader–Willi are the same disease manifesting differently in the two sexes. They are of course genetically distinct disorders and both can occur in either boys or girls.

Rett syndrome shows similar clinical manifestations to Angelman including microcephaly, cognitive impairment, hypotonia, seizures, ataxia and abnormal hand movements; however, it occurs nearly exclusively in girls. Misdiagnoses

between these two conditions in girls are therefore not uncommon. Unlike Angelman, Rett patient behavior is characterized by frequent loss of speech and other previously attained skills, and poor socialization similar to autism. Most cases are due to sporadic mutations of *methyl-CpG-binding protein-2* (MECP2) on the X chromosome. Male fetuses with the mutation are usually nonviable or die before 2 years of age. Surviving males are 46, XXY. MECP2 binds methylated DNA and acts as a transcriptional repressor and its mutation can lead to disruption of methylation imprinting of the UBE3A gene [88]. Decreased UBE3A has indeed been observed in the brain tissue of MECP2-deficient mice and Rett syndrome patients.

Further evidence of the global importance of UBE3A, other ubiquitin ligases and related UPS proteins in cognitive function has come in a very recent publication identifying these proteins along with neuronal adhesion proteins as exhibiting copy number variation in a cohort of over 800 patients suffering from autism spectrum disorders [36]. UBE3A dysfunction appears to produce a large spectrum of pediatric cognitive functional levels depending on the mechanism and severity of UBE3A loss, i.e., autism to Rett to Angelman syndromes.

The UPS in diseases of the PNS and skeletal muscle

The UPS appears to be involved in the initiation of Wallerian degeneration and a side effect of proteasomal inhibitors is peripheral neuropathy [148]. Altered ubiquitin-dependent degradation of Schwann cell proteins may contribute to the pathogenesis of certain neuropathies [32]. In the Trembler J mouse model of Charcot–Marie–Tooth disease type 1A demyelinating neuropathy the turnover rate of nascent *peripheral myelin protein 22* (PMP22) is decreased. These animals show proteasome impairment correlating with increased levels of polyubiquitinated PMP22 in nerves [32].

Giant axon neuropathy is a severe autosomal recessive motor and sensory disorder presenting in infants and children that is associated with disruption of neurofilaments [145]. It is caused by the mutation of an ubiquitin ligase substrate adapter known as *gigaxonin*, which targets the light chain of *microtubule-associated protein 1B* (MAP1B), MAP8 and the tubulin folding chaperone *tubulin folding cofactor B* (TFCB) [1]. MAP1B over accumulation is toxic to neurons although the mechanism of neurofilament disruption is not known [16].

A significant rare untoward effect of statin-type cholesterol lowering drugs such as atorvastatin is a necrotizing myopathy often accompanied by an inflammatory response, so called statin-induced myopathy. Selective vulnerability of type IIb muscle fibers occurs in statin-induced myopathy [135]; however, the precise pathophysiologic mechanisms of the condition remain poorly understood. Mitochondrial dysfunction has been implicated

because statins inhibit both cholesterol and ubiquinone synthesis. The latter can potentially interfere with mitochondrial oxidative phosphorylation.

Increased turnover of muscle proteins by the UPS may be important in the activation of events that lead to statin-induced myopathy. The muscle-specific F-box protein *MAFbx* (atrogin-1) is part of a SCF E3 ligase induced in muscle atrophy. Hanai et al. [42] found that MAFbx is also induced by lovastatin and that knockdown of MAFbx prevented lovastatin-induced muscle damage in zebrafish embryos. SCF-MAFbx targets the muscle transcription factor MyoD [110]. MyoD is important in both muscle development and maintenance of the differentiated phenotype of type IIb fibers. MAFbx gene silencing by RNA interference inhibits MyoD proteolysis and expression of a stabilized K133R MyoD mutant not recognized by SCF-MAFbx prevents atrophy of mouse skeletal muscle fibers in model systems [72]. These findings support the hypothesis that increased ubiquitination of skeletal muscle proteins, particularly MyoD, may be an important final common pathway in toxic myopathies caused by statins, and perhaps other drugs.

Overloading or other perturbations of ubiquitin-dependent proteolysis are probably involved in inclusion myopathies, such as inclusion body myositis, the myofiber inclusions of which are strongly ubiquitin immunopositive. IBM degenerating fibers contain hyperphosphorylated tau and casein kinase 1 α , a kinase that phosphorylates tau and other SCF E3 ligase substrates [56].

Hereditary inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD) is a rare autosomal dominant disease associated with mutations in the gene encoding *valosin-containing protein* (VCP) [134]. VCP is a chaperone protein involved in the ubiquitin-dependent degradation of membrane and cytoplasmic proteins and in translocation of misfolded proteins from the endoplasmic reticulum into the cytoplasm for proteasomal degradation [96, 137]. As a chaperone, VCP prevents aggregation of UPS substrates prior to their presentation to the proteasome [118, 138]. Frontotemporal dementia is further linked to IBM by the finding of TDP-43 immunopositive sarcoplasmic inclusions in both IBMPFD and sporadic IBM muscle [134]. Additionally, VCP is also found in inclusions of both IBMPFD and sporadic IBM.

The UPS in CNS tumors

von Hippel–Lindau disease

Perhaps the most direct example of misregulation of the UPS in the etiology of neurological tumors is in the vascular tumors of the hereditary cancer syndrome von Hippel–Lindau disease (VHL) [54]. In VHL, CNS and

retinal hemangioblastomas grow in response to overexpression of *vascular endothelial growth factor* (VEGF). VEGF transcription is driven by *hypoxia-inducible factor-1alpha* (HIF-1 α). pVHL protein is the substrate recognition component of the ubiquitin ligase that ubiquitinates HIF-1 α [17] (Fig. 2). The mutant form of pVHL in VHDL is unable to ubiquitinate HIF-1 α leading to its over accumulation. This in turn results in increased VEGF transcription, promoting the formation of vascular tumors. VHDL patients also often develop pheochromocytoma, pancreatic carcinoma and clear cell renal cell carcinoma, and pVHL is mutated in most sporadic clear cell renal cell cancers. Thus, the von Hippel–Lindau ubiquitin ligase complex acts as a tumor suppressor by indirectly regulating levels of the growth-promoting signal VEGF.

p53 and PTEN

The tumor suppressors p53 and PTEN are commonly mutated in human neoplasms including glial tumors, particularly high-grade astrocytomas and secondary glioblastomas. The p53 protein has many functions within the cell [7, 19–21, 70]. Its most well-described functions are in the triggering of the G1/S checkpoint and induction of apoptosis. p53 activation of INK4a gene results in transcription of the cyclin-dependent kinase inhibitor p16 which interferes with the ability of these kinases to phosphorylate and inactivate the pRb tumor suppressor. p53 also induces transcription of the BAX family of proteins responsible for inducing apoptosis via disruption of the mitochondrial membrane, and may initiate apoptosis by direct interaction with mitochondria in the cytosol. Loss of p53 function may thus lead to uncontrolled cellular proliferation by allowing unregulated cell cycling through the G/S checkpoint. It may also contribute to survival and proliferation of genetically damaged cells by removing the signal to initiate apoptosis in such cells. Thus, p53 loss is thought to be a key mechanism in tumorigenesis.

A persistent misconception is that positive nuclear p53 immunohistochemical reactivity indicates p53 mutation. p53 transcription is induced by several forms of cellular stress and nuclear p53 reactivity can be detected in both benign and malignant cells with wild-type p53. However, some p53 mutants are resistant to ubiquitin-dependent degradation rendering them inherently more stable than wild-type p53 protein. Such mutants tend to accumulate, enabling their detection by immunohistochemical staining.

Human double minute 2 (HDM2) (homologous to MDM2 in mice) is transcriptionally induced by p53, and is an E3 ubiquitin ligase targeting p53. p14^{ARF} (homologous to the mouse p19^{ARF}) is encoded by the INK4a locus induced by p53. p14^{ARF} binds HDM2 and prevents its association with p53. Loss of p14^{ARF} therefore leads to uninhibited HDM2 mediated p53-ubiquitination and functional loss of p53.

The AKT (protein kinase B) pathway is activated by the phosphorylation of AKT kinases by phosphoinositide-dependent kinase (PDK). Among their many functions AKT serine–threonine kinases inhibit apoptosis by phosphorylation of the pro-apoptotic BCL-2/BAX family protein BAD, and thus promote tumor cell survival. PTEN is a lipid phosphatase that inhibits the AKT pathway by dephosphorylating *phosphatidylinositol (3,4,5)-trisphosphate* (PIP3), the phosphate donor used by PDK. PTEN loss in tumors thus leads to upregulation of the AKT activating pathway.

Research has also uncovered a link between PTEN and p53 destruction. PTEN inhibits HDM2-mediated p53 destruction (or translocation) in the cell nucleus. Specifically, through inhibition of the AKT pathway, PTEN inhibits the translocation of HDM2 from the cytoplasm to the nucleus [90]. PTEN loss therefore leads to increased ubiquitination of nuclear p53. The resultant decreased nuclear p53 protects tumor cells from DNA damaging chemotherapy [76, 90]. PTEN is ubiquitinated by the pro-oncogenic HECT-type E3 ligase Nedd4-1. Thus, in addition to p53 stabilizing mutations, loss of the UPS regulatory and functional components p14^{ARF}, HDM2 and PTEN may lead to p53 misregulation in tumors.

SCF ubiquitin ligase targets and medulloblastoma

Medulloblastoma is the most common pediatric brain tumor and is thought to arise from cerebellar neural progenitor cells. β -catenin protein binds TCF and LEF transcription factors resulting in transcription of several genes, and has been shown to regulate neural progenitor cell proliferation. Transgenic mice overexpressing β -catenin in the CNS developed enlarged brains because a greater number of their periventricular neural progenitor cells re-entered the cell cycle [13]. Up-regulation of the β -catenin signaling pathway occurs in many human neoplasms. It has been most widely recognized in colon tumors, particularly in familial adenomatous polyposis coli syndrome, but has also been implicated in medulloblastomas. The *adenomatous polyposis coli* (APC) protein is an essential participant in the ubiquitination of β -catenin. Like other SCF target proteins, β -catenin must be phosphorylated before it can be recognized by the SCF. APC is essential for this to occur. Together with the protein axin, APC is thought to act as a scaffold necessary for the binding and phosphorylation of β -catenin by GSK3. APC mutations thus prevent phosphorylation and subsequent ubiquitin-dependent degradation of β -catenin. Mutated β -catenin resistant to phosphorylation by GSK3 is therefore abnormally stabilized. Such mutations are found in some sporadic colon cancers, some medulloblastomas and adamantinomatous craniopharyngiomas [9, 114]. Interestingly, c-Myc, Notch and Gli (a downstream

effector of Shh) have also been found to be mutated or dysregulated in medulloblastomas, and like β -catenin, all are involved in brain development and are regulated by SCF-mediated ubiquitin-dependent proteolysis. This suggests that SCF controlled signaling pathways are particularly important in controlling the fate of cerebellar stem cells. Patch, a negative regulator of Shh signaling, is also mutated in medulloblastoma, but appears to be ubiquitinated by the Nedd4 E3 ligase [23, 85].

Anaphase promoting complex/cyclosome pathway

The anaphase promoting complex/cyclosome (APC/C) (not to be confused with the fore mentioned APC protein) is an E3 ubiquitin ligase that ubiquitinates several important cell cycle regulatory proteins. The APC/C is associated with two different activator subunits that impart general substrate specificity, Cdh1 and Cdc20 (Fig. 2). The APC/C regulates the ubiquitin-dependent degradation of several proteins that drive the cell cycle including cyclins A and B. The APC/C substrates geminin, survivin, polo and aurora kinases are overexpressed in ependymomas and high-grade gliomas [35, 77]. Securin or *pituitary tumor transforming gene-1* (PTTG-1) is an APC/C target frequently overexpressed in pituitary adenomas and is a key component of the mitotic spindle checkpoint, which controls the initiation of chromosome segregation at the metaphase plate of dividing cells. Securin inhibits an enzyme that initiates chromosome segregation known as separase. Upon formation of a mature metaphase mitotic spindle the APC/C ubiquitinates securin. This results in release of separase

inhibition and segregation of chromosomes to daughter cells. Radiation or microtubule destabilizing drugs such as paclitaxel can interfere with the checkpoint and arrest cell division. Overexpression or knockdown of levels of APC/C substrate proteins leads to genomic instability and cellular neoplastic transformation [77, 79]. The APC/C also acts as a tumor suppressor by regulating the levels of these cell cycle proteins.

Emi1 protein is a pseudosubstrate inhibitor of the APC/C and is overexpressed in ependymomas and high-grade astrocytomas [48, 77, 93, 105]. Emi1 regulates APC/C E3 ligase activity during S-phase and early mitosis [48, 130]. The protein product of the *RASSF1A* gene takes over the negative regulation of APC/C activity following the SCF-mediated ubiquitin-dependent degradation of Emi1 in early mitosis [119]. The *RASSF1A* gene is silenced by promoter methylation in a large percentage of glial tumors and medulloblastomas [45, 86, 92]. Thus, dysregulation of the APC/C pathway may be a common feature of glial tumors.

An interesting form of biochemical crosstalk occurs between the SCF and APC/C ubiquitin ligases. p27 is a cyclin-dependent kinase inhibitor whose ubiquitin-dependent degradation serves as an important trigger to allow both the G1/S and G2/M cell cycle transitions. Ubiquitination of p27 is accomplished by the SCF E3 ligase complexed with the F-box protein *Skp2*. *Skp2* is in turn ubiquitinated by the APC/C. Tumors overexpressing *Skp2*, e.g., because of dysregulated Emi1 over-inhibiting the APC/C, tend to have defective regulation of the G1/S or G2/M transitions due to heightened targeting of p27 by SCF^{Skp2} [78] (Fig. 5).

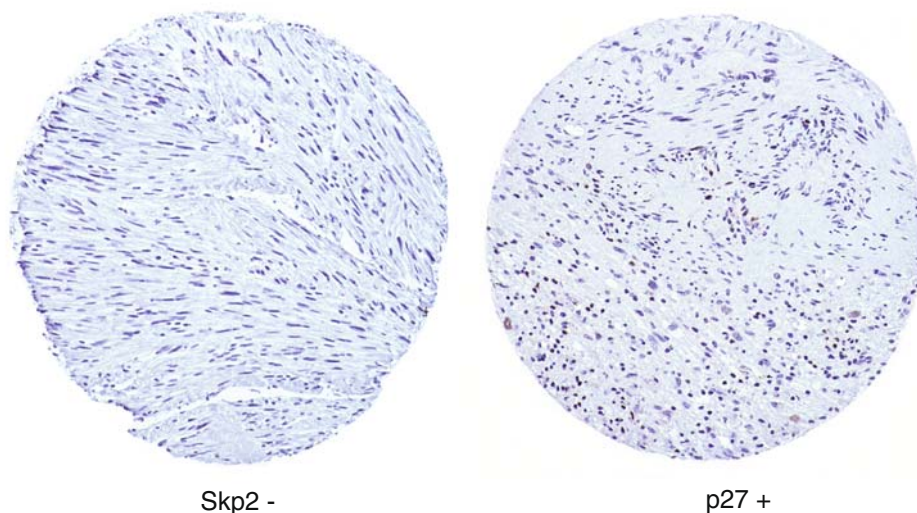


Fig. 5 Cross-talk between the SCF and APC/C regulates cell cycle progression. The SCF E3 ubiquitin ligase F-box adapter protein *Skp2* is expressed at levels undetectable by immunohistochemistry in benign schwannomas while its target the cyclin-dependent kinase inhibitor p27 is expressed at detectable levels. *Skp2* is a substrate

target of the APC/C-Cdh1 E3 ligase, the activity of which may be decreased in many malignant tumors. The opposite pattern of p27 and *Skp2* expression may therefore be found in malignant neoplasms [77, 78]. Immunostained tissue microarray cores are shown ($\times 100$)

The UPS as a target of therapy

Several proteasome inhibitors have undergone clinical trials as anti-cancer agents. The proteasome inhibitor bortezomib (PS-341) has been approved for the treatment of multiple myeloma and mantle cell lymphoma [106]. Bortezomib is thought to have many pharmacological effects including inducing aggresome formation; however, its antineoplastic action may be attributable to induction of apoptosis. This may define a mechanistic pathway of drug action because aggresome formation can itself lead to apoptosis.

A pharmacologic inhibitor of the HDM2 E3 ubiquitin ligase targeting p53, known as MI-63, showed antiproliferative activity and induction of apoptosis in mantle cell lymphoma cells, and was synergistic with rapamycin and bortezomib in vitro [52]. SCF and APC/C E3 ligases are intriguing additional possible pharmacological targets. Small molecule inhibitors of the APC/C substrates aurora A and aurora B kinases have shown antineoplastic activity in animal tumor models and several are currently in clinical trials [89, 128]. Some of these anti-UPS drugs or novel agents targeting other components of the UPS may eventually prove efficacious against CNS neoplasms. For instance, aurora A (STK15) expression was found to be an independent predictor of survival in medulloblastoma [100].

In situations where UPS components are mutated, as in autosomal recessive forms of Parkinson disease and Angelman disease, gene therapy to replace the specific lost E3 ligase activity may be possible in the future. New therapies for disease in which mutant misfolded proteins are produced such as polyglutamine repeat disorders may be particularly challenging. Anti-aggresome therapy for all neurodegenerative diseases involving abnormal protein accumulation has also been postulated. One general form of such therapy would be potentiation of the UPS, which has been proposed to treat neurodegenerative diseases. Finkbeiner and Mitra [30] warn that such treatment would have to target components specific to UPS-mediated degradation of specific substrates, or perhaps groups of substrates to prevent numerous potential off target effects. An interesting study showed that the anti-acidosis drug benzamil enhanced UPS activity, and that blocking the expression of an isoform of the drug's target the *acid-sensing ion channel* also enhanced UPS activity and decreased mutant huntingtin aggregation in the brains of a mouse model of Huntington disease [140]. Other potential drug targets might be components of the UPS relatively specific to neurons such as synaptic endocytosis factors, ubiquitin-like proteins, E2 Ubc's or E3 ligases. Small changes in the activity of such factors might selectively affect neurons because of their lower overall UPS capacity compared to glia [127]. One possible candidate target is

UBE3A. Mutation or loss of function of UBE3A and or its substrates is found in Angelman syndrome, Rett syndrome, autism and SCA1 (Table 1). Overexpression of UBE3A protected against the toxicity of polyglutamine repeat expanded proteins in models of Huntington disease and spinocerebellar ataxia [94]. Thus, UBE3A potentiating therapy may be a means to treat multiple polyglutamate repeat diseases as well as Angelman and Rett syndromes and autisms. A possible approach would be to screen for a compound that inhibits the phosphorylation and/or subsequent ubiquitination of UBE3A. In the absence of small molecule potentiators or gene therapy, perhaps intrathecal delivery of UBE3A might be transiently effective. Since viral E6 can activate UBE3A to target p53, this suggests an intriguing possibility of rationally designed viral-mediated therapy to target other UBE3A substrates.

A different approach might have to be taken for tauopathies, ALS and Parkinsonian conditions which show some commonalities in their UPS pathology. Pharmacologic strategies have been proposed for Parkinson disease based on the effects of the LRRK2 mutation. Reduction in LRRK2 results in decreased neuronal toxicity. Such a reduction could be achieved by antagonizing the protective role of HSP90 in blocking LRRK2 ubiquitination by CHIP E3 ligase [63]. Since many neurodegenerative cytoplasmic inclusions can be cleared by autophagy, upregulation of autophagy has been proposed as a general treatment for Parkinson diseases, polyglutamine repeat disorders, and tauopathies [136]. γ -Secretase inhibitors are in clinical trial for Alzheimer disease and effectively lower serum β -amyloid [31, 139]. Because the same γ -secretase that acts on APP also acts on Notch, γ -secretase inhibition results in some off target effects, namely alterations in lymphopoiesis and intestinal epithelial cell differentiation [139].

Summary

Research into the possible and established roles of components of the UPS in human diseases, particularly in neurological disorders, is appearing in the literature in an explosive manner. Common themes of UPS disruption are emerging in related disorders such as among the autosomal recessive forms of Parkinson disease, the spinocerebellar ataxias, and the cognitive disorders Angelman disease, Rett syndrome and autism.

This review covers only basic UPS components and provides simplified overviews of specific UPS-related diseases. New levels of biochemical, genetic and epigenetic regulation of the UPS, as well as new interconnections between UPS regulated signaling pathways are continually being discovered. The UPS and its substrates are thus topics of growing complexity. Yet there is little doubt that the UPS

and its components will come to represent key factors in our future understanding, diagnosis and perhaps even treatment of neuropathological diseases. The discovery of disease producing defects in the UPS will likely lead to routine molecular subtyping of neurodegenerative, myopathic, neuropathic and neoplastic diseases. Some of these defects might be detectable by simple immunohistochemical methods, e.g., when a protein is absent, overexpressed, or sequestered in aggresomes. In some instances the neuropathologist may be responsible for providing proper neuropathological context and tissue samples to the molecular pathologist, e.g., for gene mutational or methylation status analysis. Familiarity with the basic components of the UPS and knowledge of UPS defects in neurological diseases will definitely be of future importance in neuropathology.

Acknowledgments The author thanks Kathy Roszka for expert performance of anti-ubiquitin immunohistochemical stains, and Nandita Mani and Theresa Hobbs for assistance with formatting the manuscript.

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