Neuron
Review

Deconstruction for Reconstruction: The Role of Proteolysis in Neural Plasticity and Disease

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The brain changes in response to experience and altered environment. This neural plasticity is largely mediated by morphological and functional modification of synapses, a process that depends on both synthesis and degradation of proteins. It is now clear that regulated proteolysis plays a critical role in the remodeling of synapses, learning and memory, and neurodevelopment. Here, we highlight the mechanisms and functions of proteolysis in synaptic plasticity and discuss its alteration in disease states.

Introduction

Proper protein turnover is critical for maintaining cellular homeostasis and the quality of the cellular proteome. Although essentially all proteins undergo degradation, the process of protein turnover is tightly controlled at multiple levels. Eukaryotic cells have evolved elaborate machineries for targeted protein degradation in which the proteolytic active sites are buried inside a protein chamber (proteasomes) or compartmentalized by a membrane (lysosomes). A diverse array of regulatory proteins controls the access of substrate proteins to these degradative compartments, endowing temporal, spatial, and substrate specificity to the proteolytic pathways.

Regulated proteolysis is crucial for the health and function of neurons and for remodeling of synapses during synaptic plasticity, the process by which synaptic connections are modified in response to past experience and activity. Long-term synaptic plasticity entails not only functional changes in synaptic strength but also structural changes in the shape and size of synapses as well as the physical connectivity of networks. Such modification of synapses depends on coordinated protein synthesis and protein degradation events targeting a variety of molecules in pre- and postsynaptic compartments (Bingol and Schuman, 2005; Yi and Ehlers, 2007). Generally, it is the long-term plasticity (hour to days), rather than short-term plasticity lasting for minutes to an hour, that requires synaptic remodeling through protein synthesis and protein degradation (Tai and Schuman, 2008).

Protein Degradation in Neurons and Synaptic Plasticity

The majority of short-lived proteins in cells are degraded by the ubiquitin-proteasome system (UPS). Substrate proteins covalently tagged by a polyubiquitin chain are targeted to a proteolytic organelle—the 26S proteasome—for degradation. The other main degradation system is the lysosome, which contains multiple proteases and accounts for ~20% of protein turnover in cells (Ciechanover, 2006). Lysosomes mainly degrade organelles and membrane proteins. Cytoplasmic proteins can also be degraded through autophagy, a process in which organelles and bulk cytoplasm are enveloped in double membranes and then delivered to lysosomes (Wong and Cuervo, 2010). Defects in any of these proteolytic pathways are associated with a growing list of human diseases. In particular, many neurodegenerative disorders such as Alzheimer disease (AD) and Parkinson disease (PD) show accumulation of toxic protein aggregates in neurons and evidence of defective protein clearance.

Early evidence of a link between protein degradation and synaptic plasticity and learning came from *Aplysia,* where repeated stimulation of sensory neurons by serotonin induces a form of synaptic plasticity termed long-term-facilitation (LTF), thought to underlie the desensitization of the gill withdrawal reflex (Hegde et al., 1993). LTF depends on persistent activation of protein kinase A (PKA), which is mediated by proteasomal degradation of the PKA regulatory subunit—a negative regulator of the kinase (Chain et al., 1999; Hegde et al., 1993). Activated PKA induces transcription of many genes, one of which is *ApUCH* (*UCH-L1* in mammals)—a deubiquitinating enzyme that recycles free ubiquitin and facilitates degradation of proteasome substrates, including the PKA regulatory subunit (Hegde et al., 1997). ApUCH provides an important positive feedback mechanism to maintain PKA activity, because without ApUCH function, LTF is impaired (Hegde et al., 1997). Thus, in LTF, protein degradation enhances synaptic strength by removing a repressor of a signaling pathway.

The UPS is also critical for learning and memory in vertebrates. In rodents, bilateral injection of proteasome inhibitor lactacystin into the CA1 region of the hippocampus blocks long-term memory formation in a one-trial inhibitory avoidance task (Lopez-Salon et al., 2001). Similarly, extinction of fear memory and consolidation and reconsolidation of spatial memory depend on proteasome activity (Artinian et al., 2008; Lee et al., 2008). Consistent with the need for UPS-mediated degradation, levels of ubiquitinated synaptic proteins increase in the hippocampus following one-trial inhibitory avoidance task (Lopez-Salon et al., 2001) and retrieval of fear memory (Lee et al., 2008).

Synaptic plasticity in mammals requires proteasome function. Long-term depression (LTD) in hippocampus, a well-studied model of synaptic weakening associated with synapse shrinkage, partially depends on proteasome activity (Colledge

et al., 2003; Hou et al., 2006). Perhaps less intuitively, proteasome function is also crucial for the strengthening of synapses. Early and late phases of long-term potentiation (LTP) in CA1 region of the hippocampus are impaired by the proteasome inhibitor MG132 (Karpova et al., 2006). In another study using a more specific inhibitor of the proteasome (lactacystin), early-phase LTP was enhanced but late-phase LTP was blocked (Dong et al., 2008). Interestingly, concomitant inhibition of protein synthesis and degradation did not alter LTP, suggesting an interplay between these opposing processes in this form of plasticity (Fonseca et al., 2006). Taken together, these studies indicate that the UPS is essential to carry out the synaptic modifications associated with plasticity and learning and memory in diverse organisms.

Components of the Ubiquitin-Proteasome System

Substrate proteins destined to be degraded by the 26S proteasome are first ubiquitinated via a series of enzymatic reactions involving ubiquitin-activating (E1), conjugation (E2), and ligase (E3) enzymes (Ciechanover, 2006). E2 enzymes are characterized by a conserved ubiquitin-conjugating (UBC) domain and a catalytic cysteine residue. E2 enzymes, in conjunction with E3 ubiquitin ligases, form substrate binding surfaces to carry out ubiquitination. Two major classes of E3 enzymes are RING domain E3s and HECT domain-containing E3 enzymes. Most HECT-type E3s, and some RING-type ligases such as parkin, function as monomers. Other E3s exist as multiprotein complexes with modular subunits that include a core scaffold protein that interacts with a RING domain E3 and an adaptor protein that binds and recruits the substrate to be ubiquitinated. A well-studied example is the SCF complex composed of Skp1 linker, Cullin scaffold, and one of a variety of F-Box proteins (e.g., β-TRCP) that recruits the substrates to the RING domain E3 (Nagy and Dikic, 2010). There are two E1, ~50 E2, and ~500 E3 enzymes in the human genome; thus the substrate specificity of ubiquitination is mainly determined by different combinations of E2–E3 complexes (Ciechanover, 2006).

E3 enzymes can add a single ubiquitin molecule to the acceptor lysine residue of the substrate (monoubiquitination) or they can add ubiquitin monomers sequentially to form a polyubiquitin chain (Nagy and Dikic, 2010). Monoubiquitination does not signal for proteasomal degradation but rather seems to regulate protein trafficking and other processes. The outcome of polyubiquitination depends on which lysine residue of the seven present in ubiquitin is utilized for constructing the chain. Lysine-48 (K48)-linked polyubiquitin chains target proteins for proteasomal degradation, whereas K63 chains are used for nonproteasomal functions such as protein kinase activation, regulation of protein-protein interactions, and control of receptor endocytosis (Nagy and Dikic, 2010). By utilizing different lysine residues, the ubiquitination system can generate diverse polyubiquitin structures and varied signaling outcomes, which are still not fully understood in neurons or other cell types.

Once a substrate is ubiquitinated by K48 chains, it is conveyed to the 26S proteasome by E3s themselves, substrate-shuttling factors, or binding to resident polyubiquitin receptors on the proteasome (Glickman and Raveh, 2005). Both in neurons and non-neuronal cells, proteasome activity and subcellular localization can be dynamically modulated through posttranslational modifications and regulated interactions with accessory proteins, such as CaMKIIα (Bingol and Schuman, 2006; Bingol et al., 2010; Djakovic et al., 2009; Glickman and Raveh, 2005). There is also evidence for different proteasome-interacting proteins in brain versus other tissues and even between synaptic versus cytosolic compartments within neurons, suggesting proteasome heterogeneity across cell types and subcellular compartments (Tai et al., 2010).

Protein ubiquitination is a dynamic and reversible process owing to the action of deubiquitinating enzymes (DUBs; ~100 in the human genome) (Komander et al., 2009). DUBs can both facilitate and antagonize ubiquitin-mediated signaling and protein degradation. They promote ubiquitination in general by providing free ubiquitin through cleavage of ubiquitin monomers from polyubiquitin chains. On the other hand, DUBs counteract the function of E3 ligases and stabilize proteins by removing ubiquitin from substrates before they can be destroyed by the proteasome. DUBs can also remove monoubiquitin and other types of polyubiquitin linkages (such as K63-polyubiquitin) to terminate proteasome-independent ubiquitin signaling (Komander et al., 2009).

UPS and Synaptic Function

To date, several ubiquitin conjugation and removal enzymes have been described that regulate synaptic function (see Tables 1 and 2 and Figure 1). Below, we summarize these molecules with a focus on mammalian systems. An excellent review covers degradation systems in invertebrates (Hegde, 2010).

One of the first E3 ligases implicated in synaptic plasticity and postsynaptic function was E6-AP (also known as UBE3A), a HECT domain-containing E3 ligase (Jiang et al., 1998). E6-AP is encoded by a maternally-imprinted gene, *Ube3A*, inactivating mutations of which lead to a neurodevelopmental disorder called Angelman syndrome (AS) (Kishino et al., 1997; Matsuura et al., 1997). Loss of UBE3A function in a mouse model of AS impairs LTP and contextual learning (Jiang et al., 1998). CaMKIIα—a major enzyme required for plasticity and learning and memory—is decreased in abundance and activity in postsynaptic densities (PSDs) of UBE3A mice, perhaps explaining the plasticity and learning deficits (Weeber et al., 2003). Remarkably, these molecular and behavioral defects in UBE3A mice are completely rescued by introducing mutations in the phosphorylation sites of CaMKIIα that negatively regulate its activity and synaptic abundance (T305/T306) (van Woerden et al., 2007). The mechanism of CaMKIIα regulation by UBE3A remains unclear.

Recent studies showed that UBE3A directly ubiquitinates Arc, an activity-induced protein that promotes the internalization of the AMPA-type glutamate receptors (AMPARs) (Greer et al., 2010), thus providing another example of degradation of a negative regulator of synaptic strength. Disruption of UBE3A function stabilizes Arc protein and reduces the number of AMPARs at excitatory synapses. Because AMPARs play a central role in excitatory synaptic transmission and plasticity, deregulation of Arc and surface AMPARs offers a plausible mechanism for the deficits observed in AS.

Homeostatic synaptic plasticity operates over a time scale of hours to days to maintain synaptic strength within a dynamic

Table 1. Examples of Substrates and Functions of Ubiquitin Ligases and DUBs in Mammalian Neurons

	Function	Substrate	Reference
Ubiquitin Ligases			
UBE3A (E6-AP)	Regulation of glutamate receptor trafficking and CaMKII abundance in PSD; Loss of function in Angelman syndrome	Arc	Greer et al., 2010; Jiang et al., 1998; Weeber et al., 2003
TRIM3	Activity-dependent postsynaptic remodeling	GKAP	Hung et al., 2010
APC	Negative regulator of axon growth	Liprin-α	Konishi et al., 2004
β-TRCP	Regulation of dendritic spine morphology	SPAR	Ang et al., 2008
Rnf6	Axonal growth - local regulation of actin dynamics in growth cones	LIM kinase 1	Tursun et al., 2005
Nedd4	Dendrite and axon branching	PTEN, Rap2	Drinjakovic et al., 2010; Kawabe et al., 2010
Mdm2	Glutamate receptor trafficking	PSD-95	Colledge et al., 2003
Fbx2	Homeostatic control of NMDARs	NR1	Kato et al., 2005
Parkin	Neuroprotection; Clearance of damaged mitochondria through mitophagy	Synphilin, α-synuclein, synaptotagmin XI, the Pael receptor, cyclin E, tubulin, p38/JTV-1, Eps15, VDAC, mitofusin	Reviewed in Hegde, 2010
Phr-1	Neuronal polarity maintenance		Lewcock et al., 2007; Bloom et al., 2007
DUBs			
UCH-L1	Maintenance of free ubiquitin pool; Turnover of postsynaptic scaffolds		Cartier et al., 2009; Saigoh et al., 1999
USP14	Maintenance of free ubiquitin pool		Chen et al., 2009

range in the face of changing activity levels. This form of plasticity also depends on UPS-mediated degradation. Chronic increases or decreases in neuronal activity induce proteasome-dependent reciprocal changes in the abundance of numerous proteins in the PSD (Ehlers, 2003). However, only a few proteins were found to be directly ubiquitinated in the PSD, suggesting that UPS may target specific "master organizers" of the PSD to regulate a larger set of associated postsynaptic proteins. Indeed, Shank1 and GKAP are highly ubiquitinated and activity-regulated core scaffold proteins of the PSD, organizing cytoskeletal/signaling complexes and maintaining synaptic morphology (Ehlers, 2003; Sheng and Kim, 2000). Recently a RING domain ubiquitin ligase, TRIM3, was identified as a specific E3 ligase for GKAP in hippocampal neurons (Hung et al., 2010). TRIM3 mediates activity-induced ubiquitination and downregulation of GKAP and causes concomitant decreases in Shank1 abundance and synaptic size (Hung et al., 2010). Because degradation of GKAP and Shank occurs during memory consolidation and reconsolidation (Lee et al., 2008), it would be interesting to know how plasticity and memory is affected in animals without TRIM3.

Table 2. Examples of Ubiquitinated Neuronal Proteins Where the Ubiquitin Ligase Is Not Identified

Substrate	Function	Reference
Shank	Postsynaptic scaffold	Ehlers, 2003
GABA-A Receptor β3	Inhibitory neurotransmitter receptor	Saliba et al., 2007
Glycine Receptor	Inhibitory neurotransmitter receptor	Büttner et al., 2001
Synaptophysin	Neurotransmitter release	Wheeler et al., 2002
Syntaxin-1	Neurotransmitter release	Chin et al., 2002
Liprin-α	Presynaptic and postsynaptic scaffold	Hoogenraad et al., 2007
MOV10	Translation repressor	Banerjee et al., 2009
GRIP1	Postsynaptic scaffold	Guo and Wang, 2007
AKAP79/150	Postsynaptic scaffold	Ehlers, 2003

How does neuronal activity control turnover of postsynaptic proteins? Ubiquitination and phosphorylation are often linked (Hunter, 2007). Ubiquitination is frequently preceded by phosphorylation of a specific motif on the substrate (called a degron), which then recruits the ubiquitination machinery. In neurons, synaptic activity could induce phosphorylation of these degrons and prime substrates for UPS degradation, as exemplified by the turnover of a postsynaptic spine-associated Rap GTPase-activating protein (SPAR) (Ang et al., 2008). Following neuronal stimulation, SPAR gets phosphorylated by an activity-induced protein kinase, Polo-like kinase 2 (Plk2) (Pak and Sheng, 2003), which creates a phospho-degron that mediates the physical interaction of SPAR with β-TRCP, an F-box component of a SCF E3 complex (Ang et al., 2008). Functionally, SPAR degradation mediated by Plk2 and the UPS is necessary for homeostatic dampening of synaptic strength following prolonged elevation of activity (Seeburg et al., 2008). SPAR degradation is another example of proteolysis of a negative regulator of signaling, in this case leading to enhanced Rap activity and synapse weakening.

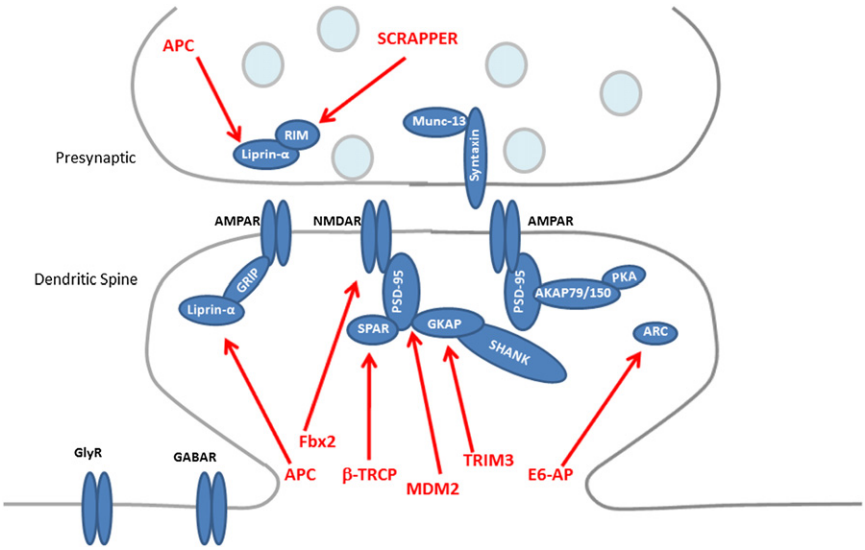

Figure 1. Examples of Presynaptic and Postsynaptic Proteins Targeted by the UPS and, Where Known, the Ubiquitin Ligases Responsible for their Ubiquitination
Presynaptic and postsynaptic proteins are in blue and ubiquitin ligases are in red.

Because synaptic strength is largely determined by the number of postsynaptic AMPARs, mechanisms that target AMPARs or AMPAR trafficking are of great interest. AMPARs undergo endocytosis in response to direct agonist binding or activation of N-methyl-D-aspartic acid receptors (NMDARs), and both processes require proteasome activity (Colledge et al., 2003; Patrick et al., 2003). Although AMPAR homologs in invertebrates were reported to be ubiquitinated and regulated by UPS, it is not clear whether mammalian AMPARs are directly ubiquitinated (Bingol and Schuman, 2004; Burbea et al., 2002; Colledge et al., 2003; Patrick et al., 2003).

The UPS also regulates presynaptic function. In cultured hippocampal neurons, proteasome inhibition for 2 hr increases the size of the recycling vesicle pool by ~75% without changing the release probability, suggesting that proteasomal degradation controls synaptic vesicle cycling (Willeumier et al., 2006). What are the targets of proteasome in mammalian presynaptic terminals? In hippocampal acute slices, proteasome inhibitors increase the frequency of miniature excitatory postsynaptic currents (mEPSC), an effect that depends on SCRAPPER, an F-box protein localized to presynaptic membranes (Yao et al., 2007). SCRAPPER mediates the ubiquitination and degradation of the presynaptic vesicle priming factor, RIM1. In slices prepared from SCRAPPER knockout mice, RIM1 escapes proteasome degradation, and its accumulation is sufficient to occlude enhancement of mEPSCs by proteasome inhibitors. Thus, proteasome activity seems to limit vesicle release by degrading RIM1 ubiquitinated by SCRAPPER (Yao et al., 2007). However, another study found that in cultured hippocampal neurons, proteasome inhibition increases mEPSC frequency without causing a buildup of RIM1 (Rinetti and Schweizer, 2010). Because RIM1 degradation is induced by elevated activity in neuronal cultures (Jiang et al., 2010), it is possible that under basal culture conditions RIM1 stays stable and proteasome inhibition affects presynaptic function via RIM1-independent mechanisms.

A number of E2s and DUBs are reported to be essential for synaptic function and development. A mutation in fly E2 enzyme *bendless* leads to impaired jump response due to aberrant synaptic connectivity between the giant fiber neuron and its muscle target (Muralidhar and Thomas, 1993). Synaptic defects in ataxia mutant mice (characterized by hind limb paralysis, resting tremor, and postnatal lethality) result from mutations in a DUB, Usp14. *Usp14* encodes for a proteasome-associated ubiquitin protease that may function in disassembling polyubiquitin chains, thereby providing free ubiquitin for the UPS (Wilson et al., 2002). UCH-L1, a homolog of *Aplysia* UCH, promotes proteasomal degradation and turnover of postsynaptic scaffolds such as PSD-95 by generating free ubiquitin (Cartier et al., 2009). UCH-L1 may also be associated with neurodegenerative disorders (see below).

Regulation of UPS by Neuronal Activity

How does neuronal activity influence UPS function? Fluorescence-based degradation reporters indicate that proteasome activity rises in response to LTP-inducing stimuli in hippocampal slices and following NMDAR activation in dissociated neuron cultures (Bingol and Schuman, 2006; Djakovic et al., 2009; Karpova et al., 2006). Chronic elevation of neuronal activity increases levels of ubiquitinated proteins in the PSD; decreased neuronal activity has the opposite effect (Ehlers, 2003). CaMKIIα, a postsynaptic kinase activated by calcium entry through NMDARs, phosphorylates and enhances proteolytic activity of the proteasome, linking synaptic excitation to local stimulation of the UPS (Bingol et al., 2010; Djakovic et al., 2009).

The subcellular location of proteasomes in neurons is also regulated by activity (Bingol and Schuman, 2006; Bingol et al., 2010; Shen et al., 2007). Proteasomes rapidly redistribute from dendritic shaft to dendritic spines in response to activation of NMDARs; this redistribution is mediated by binding of proteasomes to activated CaMKIIα (Bingol et al., 2010). Because CaMKIIα translocates to the PSD of stimulated synapses and is itself critical for potentiation of synaptic strength, proteasome recruitment by CaMKIIα provides a mechanism for localizing the effects of proteasome degradation specifically to activated synapses undergoing plasticity. A cocaine-induced protein, NAC1, may also be involved in recruitment of proteasomes to dendrites and dendritic spines (Shen et al., 2007). In addition to subcellular localization, the biochemical composition of proteasomes appears to be dynamic and subject to control by neuronal activity (Tai et al., 2010).

Localized Proteolysis in Neuronal Development and Plasticity

Hebbian forms of plasticity are believed to result from modifications localized to a specific subset of synapses. Thus, synapses must utilize the products of protein synthesis and confine the effects of proteolysis in a synapse-specific manner. Indeed, ribosomes and proteasomes are present at or near postsynaptic sites where they could act locally to make or break down proteins (Bingol and Schuman, 2006; Bourne and Harris, 2008; Sutton and Schuman, 2006). Local protein degradation by UPS operates in growth cones to guide the navigation of axons (Campbell and Holt, 2001; Verma et al., 2005). In support of compartment-specific functions of the UPS, blocking proteasome activity in *Aplysia* throughout the neuron blocks potentiation, whereas proteasome inhibition specifically around synapses has the opposite effect on plasticity (Chain et al., 1999; Hegde, 2004; Zhao et al., 2003). In addition to protein degradation, local protein synthesis is central for plasticity (Cajigas et al., 2010). Interestingly, protein synthesis can be activated through degradation of a negative regulator of translation, the RISC complex, releasing translationally suppressed synaptic mRNAs for local protein synthesis (Ashraf et al., 2006; Banerjee et al., 2009).

Local proteolysis is important during neurodevelopmental processes, such as dendrite pruning. During larval metamorphosis, *Drosophila* sensory neuron dendrite pruning requires UPS components E1, an E2 called ubcD1, and the proteasome, as well as caspase activity (Kuo et al., 2005, 2006). Interestingly, ubcD1 downregulates an E3 ubiquitin ligase, DIAP-1, and in turn DIAP-1 targets a proapoptotic caspase (Dronc) required for dendritic pruning. Caspase activity reporters indicate that Dronc caspase activity is confined to degenerating dendrites of pruning neurons, consistent with the idea that local degradation of DIAP-1 stabilizes Dronc in dendrites destined for destruction (Kuo et al., 2006; Williams et al., 2006). Importantly, these studies not only identify E2/E3 enzymes essential for dendritic pruning but also provide a mechanistic link between the UPS and caspases in a nonapoptotic context. Extending the theme of UPS and caspase involvement in remodeling of neuronal processes, UPS and caspases also appear to function in a spatially-restricted manner during pruning of fly axons and degeneration of mammalian axons (Nikolaev et al., 2009; Watts et al., 2003).

A nonapoptotic requirement for caspase-mediated proteolysis was also shown for synaptic plasticity (Li et al., 2010). Specifically, LTD and AMPAR internalization require activation of caspase-3 via the mitochondrial pathway of apoptosis. Chemically induced LTD was associated with transient and modest activation of caspase-3 in dendrites, but not cell death, implying that caspase-3 activity can be localized to or near synaptic sites without culminating in neuronal apoptosis (Li et al., 2010). Unlike the UPS or lysosomes, caspases probably act by sequence-specific cleavage—rather than degradation—of a select set of target proteins. The molecular mechanism by which caspase effects are restricted to specific neuronal compartments (perhaps even specific synapses) is an important unanswered question.

Local breakdown of proteins—and selective pruning of synapses—can additionally be achieved by spatiotemporal control of E3 ligase assembly. In *Caenorhabditis elegans*, localized inhibition of the assembly of an SCF complex through binding of core protein SKR-1 to a synaptic adhesion molecule, SYG-1, spares synapses from elimination (Ding et al., 2007). It is unknown whether synapse elimination in mammals also relies on local regulation of E3 ubiquitin ligase.

The morphological sculpting of certain synapses is regulated by an evolutionarily conserved RING domain E3 ligase Phr1 (also known as Highwire in *Drosophila*, and RPM-1 in *C. elegans*) (Schaefer et al., 2000; Wan et al., 2000; Zhen et al., 2000). In mammals, Phr1 functions to sculpt motor nerve terminals and is essential for formation of major CNS axon tracts (Bloom et al., 2007). Interestingly, in mice, Phr1 is localized to the axonal shaft and excluded from growth cones, where the protein kinase DLK is restricted (Lewcock et al., 2007). In the absence of Phr1, DLK aberrantly distributes to axons, leading to altered microtubule dynamics and axon-pathfinding deficits. Based on the reciprocal localization of DLK and Phr1, DLK was proposed as a Phr1 substrate, similar to the scenario in invertebrates (Collins et al., 2006; Lewcock et al., 2007; Nakata et al., 2005). However, no increase in DLK was detected in the central nervous system of Phr1 mutant mice and DLK is not required for Phr1 loss-of-function phenotypes (Bloom et al., 2007). In fish, Phr1 localizes to growth cones and regulates pathfinding independent of DLK (Hendricks and Jesuthasan, 2009). Collectively, these studies demonstrate that despite possible cell-type or species-specific differences in the regulation of Phr1, this ubiquitin ligase regulates microtubule remodeling during development and is crucial for axon navigation.

HECT domain Nedd4 is another ubiquitin ligase acting in axons; it promotes branching by targeting PTEN, a PIP3 phosphatase that negatively regulates axonal branching (Drinjakovic et al., 2010). Remarkably, Nedd4 also enhances the branching of dendrites by monoubiquitinating GTPase Rap2 and inhibiting its function (Kawabe et al., 2010). Thus an E3 ligase can target different substrates in different subcellular compartments to carry out similar cell biological functions.

Other Proteolytic Pathways Important for Synaptic Plasticity and Function

Neurons also utilize the lysosome system to degrade organelles and synaptic proteins. For example, following endocytosis, AMPARs either recycle back to the membrane or are sorted into lysosomes, depending on their subunit composition and whether AMPARs themselves or NMDARs were activated (Ehlers, 2000; Lee et al., 2004).

Organelles and cytoplasmic proteins can also be targeted to lysosomal degradation through autophagy (“self-eating”). The canonical autophagy pathway involves sequestration of substrates into double-membrane structures called autophagosomes (APs) and delivering APs to lysosomes for degradation (Wong and Cuervo, 2010). Autophagy can nonselectively degrade bulk cytoplasm and organelles (macroautophagy) or may involve chaperones that mediate selective fusion of substrates with lysosomes (chaperone-mediated autophagy). Autophagy could contribute to remodeling of synapses and neurites in neurons. In *C. elegans*, endocytosed GABA-A receptors, but not acetylcholine receptors, are targeted to

autophagosomes (Rowland et al., 2006). Aberrant membrane structures accumulate in axons of autophagy-deficient mice (Komatsu et al., 2007). In flies, autophagy promotes synapse growth by downregulating Highwire (Shen and Ganetzky, 2009). As discussed below, failure to degrade proteins and organelles due to defects in autophagy may be one of the pathogenic mechanisms associated with neurodegenerative diseases.

The growth and retraction of neuronal processes and the making and breaking of neuronal contacts not only involves remodeling of intracellular structures but also the brain extracellular matrix (ECM). ECM components have profound influences on neuronal signaling, adhesion, and motility and are subject to regulated proteolysis during plasticity (Dityatev, 2010). Generally, the mature ECM environment seems inhibitory for structural plasticity. Chondrotin sulfate proteoglycans appear to be one of the inhibitory components in ECM because their degradation by chondroitinase-ABC can reactivate ocular dominance plasticity (Pizzorusso et al., 2002). Supporting an essential role of ECM remodeling in structural plasticity, the matrix metalloprotease (MMP)-9 is required for spine enlargement that accompanies LTP (Wang et al., 2008). Furthermore, pharmacological or genetic inhibition of MMP-9 impairs LTP and prevents spatial learning (Bozdagi et al., 2007; Meighan et al., 2006), whereas addition of recombinant-active MMP-9 is sufficient to potentiate synapses and occlude further LTP (Bozdagi et al., 2007; Nagy et al., 2006).

Proteolysis and Neurodegenerative Diseases

The aggregation and deposition of misfolded proteins is a hallmark of neurodegenerative diseases and may reflect the failure of cellular protein clearance mechanisms. These pathological protein aggregates include plaques and tangles in AD, Lewy bodies in PD, polyglutamine inclusion bodies in Huntington disease, and TDP-43 inclusions in amyotrophic lateral sclerosis (ALS). Whether these aggregates are the primary cause of the neurodegeneration or secondary by-products remains controversial.

Since intracellular inclusions associated with neurodegenerative diseases are rich in ubiquitinated proteins, it was suggested that these diseases are associated with impaired proteasome function in neurons (Ross and Poirier, 2004). Autophagy also plays a role in the degradation of protein aggregates and in maintaining proteostasis (Wong and Cuervo, 2010). Disruption in mice of key autophagy genes, such as *Atg7* or *Atg5*, causes neurodegeneration and ubiquitin-rich inclusions (Hara et al., 2006; Komatsu et al., 2006). The neurodegeneration is associated with accumulation of aberrant organelles and stacks of cisternal membranes in the dystrophic axons of autophagy-deficient neurons (Komatsu et al., 2007). Consistent with a protective function of autophagy, pharmacological enhancement of autophagy can rescue neurons from the toxicity associated with aggregated misfolded proteins or proteasome inhibition (Pan et al., 2008; Pandey et al., 2007; Tsvetkov et al., 2010).

Neurodegenerative diseases—which involve death of neurons, degeneration of axons, loss of synapses, and impairment of synaptic plasticity—may be a pathological manifestation of cellular processes that are used normally in development, such as apoptosis, neurite pruning, and synapse elimination. In this context, it is interesting that molecular players in physiological plasticity and pathological neurodegeneration are often shared, such as the involvement of proteolytic caspase-3 in LTD and neuronal cell death (Li et al., 2010). In this section, we will focus on how proteolytic pathways are dysregulated in AD and PD.

Alzheimer Disease

AD is characterized by protein deposits composed of Aβ peptide (plaques) and hyperphosphorylated tau (tangles), both of which probably contribute to synaptic dysfunction and neuronal death (Ross and Poirier, 2004). In AD brains, ubiquitin immunoreactivity accumulates in intracellular aggregates suggesting UPS dysfunction (Chu et al., 2000). Reduced proteasome activity is reported in brain regions affected by AD, such as the hippocampus (Keck et al., 2003; Keller et al., 2000). Similarly, primary neurons isolated from APP transgenic mice show decreased proteasome activity (Almeida et al., 2006). Interestingly, transduction of UCH-L1, a DUB that promotes proteasomal degradation, reverses behavioral deficits in AD model mice (Gong et al., 2006; Smith et al., 2009), consistent with an impairment of UPS in AD. Overexpression of an anomalous form of ubiquitin found in some AD patients (UBB^{+1}; generated by a non-DNA-encoded dinucleotide deletion in ubiquitin transcripts) impairs proteasomal degradation and induces neuronal death (Lam et al., 2000; Tan et al., 2007).

Defective proteasomal degradation of hyperphosphorylated tau may contribute to the buildup of tangles. Tau interacts with CHIP, an E3 ubiquitin ligase required for degradation of soluble phosphorylated tau (Dickey et al., 2006; Shimura et al., 2004). In AD, the mechanism of stabilization and accumulation of hyperphosphorylated tau may involve inhibition of tau interaction with CHIP (Dickey et al., 2006). In addition to phosphorylation, tau is also acetylated; acetylation impairs the proteosomal degradation and enhances the accumulation of tau (Min et al., 2010).

Impairment of autophagy is also implicated in AD. Aβ may directly impair lysosomal degradation of autophagic cargo (Ling et al., 2009). Presenilin-1 mutations that cause early-onset AD result in defective lysosomal acidification and autophagy, which might contribute to accumulation of toxic proteins and neurodegeneration (Lee et al., 2010a). Degenerating neurites in AD contain large numbers of intermediate structures of autophagy (autophagic vacuoles), implying deficient autophagic clearance (Boland et al., 2008). These intermediate structures may act as sources of pathogenic Aβ peptide since they harbor the amyloid precursor protein (APP) along with the proteases that cleave APP to produce Aβ (Yu et al., 2005). Furthermore, in a mouse model of AD, genetic reduction of Beclin-1, a component of the autophagy pathway, promotes formation of plaques and neurodegeneration (Pickford et al., 2008). In culture, knockdown of Beclin-1 leads to accumulation of APP and increased secretion of Aβ (Jaeger et al., 2010). Antagonizing IGF-1 receptor signaling (a negative regulator of autophagy) in AD mice ameliorates cognitive defects and neuronal loss (Cohen et al., 2009).

Parkinson Disease

Although more than 95% of PD cases are sporadic, hereditary and sporadic forms of PD share common pathologies that could be linked to UPS dysfunction (Vila and Przedborski, 2004).

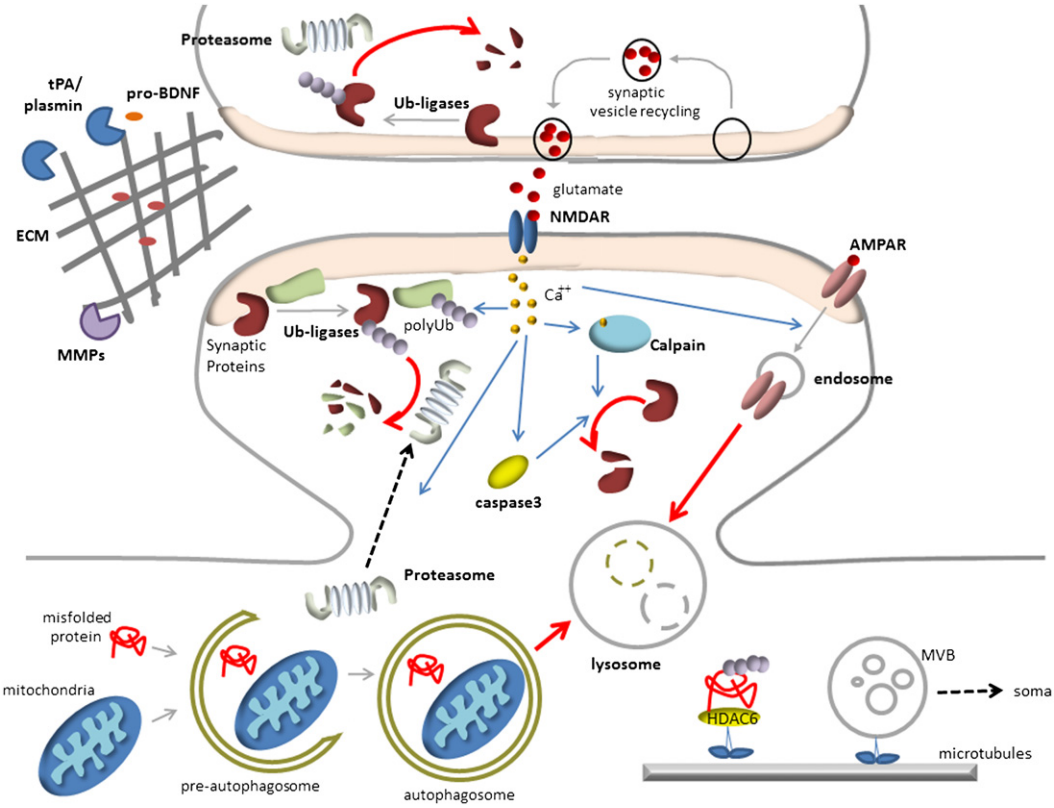

Figure 2. Schematic Diagram of the Degradation of Neuronal Proteins in Synaptic Plasticity

A representation of various proteolytic pathways that regulate synaptic function and plasticity (see text for details). Red arrows indicate proteolytic events and black dashed arrows indicate translocation of the indicated structures. Blue arrows indicate events downstream of glutamate receptor activation. Synaptic activity and stimulation of NMDARs triggers ubiquitination of synaptic proteins, recruitment of proteasomes to spines, internalization of AMPARs, and activation of caspases and calpains (Wu and Lynch, 2006). Misfolded proteins and defective mitochondria are cleared through the autophagy pathway. Internalized surface proteins such as AMPARs are degraded in lysosomes or via multivesicular bodies (MVBs). MVBs and misfolded proteins can be transported to the cell soma by dynein-mediated retrograde transport, involving HDAC6 (Pandey et al., 2007). Extracellular proteases such as tPA, plasminogen, and MMPs proteolyse ECM components and remodel ECM during plasticity.

Selective inactivation of 26S proteasomes in substantia nigra dopaminergic neurons in a conditional knockout mouse model results in neurodegeneration and ubiquitin-positive aggregates resembling Lewy bodies (Bedford et al., 2008). Pathogenic forms of α-synuclein, a principal constituent of Lewy bodies, can directly bind to proteasomes and inhibit their activity (Lindersson et al., 2004; Snyder et al., 2003). More significantly, loss-of-function mutations in parkin—an E3 ligase with two RING domains—underlie a recessively inherited early onset form of PD (Kitada et al., 1998). Interestingly, PD patients with parkin mutations lack Lewy bodies, suggesting that parkin may be required for formation and ubiquitination of these protein aggregates. Parkin could confer neuroprotection by promoting prosurvival signaling through PI(3)K-Akt pathway (Fallon et al., 2006), targeting cyclin E (a prodeath factor in neurons) (Staropoli et al., 2003), and promoting clearance of protein aggregates and unfolded proteins (Dauer and Przedborski, 2003). Recent studies point compellingly to a role for parkin (and another gene for familial PD—the protein kinase PINK1) in the clearance of damaged mitochondria through autophagy (mitophagy) (Narendra et al., 2008). PINK1 becomes stabilized on damaged mitochondria and recruits parkin (Narendra et al., 2010). Parkin ubiquitinates proteins on damaged mitochondria through K63 and K27 linkages with subsequent recruitment of p62, an adaptor protein that links ubiquitinated mitochondria to the mitophagy machinery (Geisler et al., 2010). Without quality control by parkin, defective mitochondria accumulate, leading to neuronal dyshomeostasis and cell death (Geisler et al., 2010; Kawajiri et al., 2010; Lee et al., 2010b; Matsuda et al., 2010; Michiorri et al., 2010; Narendra et al., 2008, 2010; Vives-Bauza and Przedborski, 2010).

PD may also be associated with a general defect in lysosomal degradation. In PD postmortem brains, there is a reduction in lysosomes and an accumulation of autophagosomes (Dehay et al., 2010). α-synuclein is a substrate of chaperone-mediated autophagy (Vogiatzi et al., 2008) and PD-linked mutants or dopamine-modified forms of α-synuclein act as lysosomal uptake blockers, impairing its own degradation and that of other lysosome substrates (Cuervo et al., 2004; Martinez-Vicente et al., 2008).

Future Directions

This review highlights the diversity and importance of proteolytic pathways in synapse development, synaptic plasticity, and the maintenance of neuronal health (Figure 2). The *destruction* of

proteins—which can result in either loss- or gain-of-signaling pathway functions—has generally received less attention than the *production* of proteins in the control of neural plasticity. In most cases of proteolytic control, the details of the regulation (when and how proteolysis is activated) and the molecular mechanisms (for instance, which particular substrates are important and which specific E3s are responsible) have yet to unfold. Of special interest is how protein degradation events are confined to specific compartments such as synapses, dendrite branches, and axon growth cones. It would not be surprising if different proteolytic pathways regulate each other to achieve spatial and temporal specificity of protein turnover. Another major question is how the destruction of existing proteins is coordinated with the synthesis and delivery of new proteins to achieve remodeling of neurons and their connections.

Recent studies suggest a protective role of autophagy in neurons, but we know very little about the physiological roles of this process in mature neurons and whether it is involved in plasticity to remodel neurites and synapses. How autophagy interacts functionally with the UPS and other proteolytic systems in neurons is also unclear. Understanding the roles and mechanisms of regulated protein turnover in the health and plasticity of neurons promises to bring valuable insights into the pathogenesis of common neurodegenerative diseases.

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