Emerging Roles for Ubiquitin and Protein Degradation in Neuronal Function

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Abstract

Alterations in cellular structure and synapse composition are central to proper nervous system function. Recent work has identified the ubiquitin-proteasome system (UPS) as a key regulator of neuronal biology. The UPS is essential for the growth and development of immature neurons and is a critical

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References

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mediator of synaptic adaptability in mature neurons. Furthermore, proteinaceous deposits that accumulate in diverse neurodegenerative disorders are enriched in components of the UPS, suggesting that UPS dysfunction may be pivotal for pathogenesis. Here, we summarize existing knowledge about the role of the UPS in brain function, highlighting recent work delineating its importance in neuronal development, plasticity, and degeneration.

I. Introduction

The idea of protein degradation was first conceptualized in the late 1930s, when Rudolph Schoenheimer used isotope tracers to study the metabolism of biomolecules. By feeding $^{15}$N-labeled amino acids to animals, Schoenheimer demonstrated that although labeled amino acids incorporated into proteins, this signal did not last (Schoenheimer, 1942). In fact, the magnitude of signal loss equaled that of signal gain, a phenomenon that Schoenheimer called “the dynamic steady state” (Schoenheimer, 1942). Despite this early insight, progress in the field of protein turnover remained slow until 1971, when Avram Hershko illustrated the ATP-dependent nature of protein degradation (Hershko and Tomkins, 1971). From this observation, a coherent view of protein degradation began to emerge.

We now know that a highly conserved biochemical pathway exists to discard and renew proteins in the cell. It is remarkably precise and is composed of various regulatory and catalytic proteins that we collectively refer to as the ubiquitin-proteasome system (UPS). The UPS is highly complex and participates in a variety of cellular functions. Initial efforts to characterize the UPS focused on its role in the cell cycle, where it temporally regulates the abundance of proteins involved in cell cycle checkpoint control (Matsui et al., 1979; Goldknopf et al., 1980; Pipkin et al., 1982). More recent work has turned to the role of the UPS in neuronal function, the topic of this review.

Neurons are large, highly complex cells that occupy a niche at the extremes of cellular specialization. They are highly polarized at many levels, with the broadest distinction between the axon and dendrites. Befitting this polarized existence, the UPS seems to be instrumental in properly creating, maintaining, and disassembling protein subdomains within the neuron. This aspect of UPS function is prevalent throughout the life of a neuron, and improper UPS activity is implicated in several neurodegenerative disorders. Here, we summarize existing knowledge about the basic components of the UPS and discuss recent studies that have defined its importance in neuronal development, synaptic function, and neuropathology.

A. Components of the Ubiquitin-Proteasome System

1. Ubiquitin, E1, and E2. There are five general steps associated with UPS-dependent protein degradation: ubiquitin activation, ubiquitin conjugation, ubiquitin ligation, protein degradation, and ubiquitin recycling (Fig. 1). Ubiquitin, named for its nearly universal biological presence, is an ~8-kDa protein originally identified as a thymus hormone in 1975 (Goldstein et al., 1975). It was rediscovered in 1978 as ATP-dependent proteolysis factor-1 (APF-1) by Avram Hershko, Aaron Ciechanover, and colleagues, who observed that APF-1 was required for and covalently linked to proteins targeted for proteolysis (Ciechanover et al., 1978, 1980). Subsequent crystallization of the ubiquitin molecule in 1980 confirmed that APF-1 was indeed ubiquitin (Hershko et al., 1980; Wilkinson et al., 1980).

At the onset of the proteolytic pathway, ubiquitin is biochemically primed by the E1 ubiquitin-activating enzyme in an ATP-dependent manner. In mammals, two isoforms of the E1 enzyme are expressed (E1a and E1b) through alternative translational sites encoded on a single gene. During ubiquitin activation, the E1 enzyme binds an Mg$^{2+}$-ATP molecule and ubiquitin, allowing the formation of a transient ubiquitin-adenylate species. This unstable intermediate serves as the donor of ubiquitin to the catalytic cysteine of E1 through an intramolecular transesterification reaction (Haas and Rose, 1982; Hershko et al., 1983), resulting in a thiol-ester bond between the catalytic cysteine and the terminal glycine (G76) of ubiquitin. When fully occupied, the E1 enzyme sustains two ubiquitin molecules, one in its active center and one as a thiol-ester conjugate.

Ubiquitin molecules activated by the E1 enzyme are accepted by a class of E2 ubiquitin-conjugating enzymes, which form another thiol-ester bond with G76 of ubiquitin molecules activated by the E1 enzyme are accepted by a class of E2 ubiquitin-conjugating enzymes, which form another thiol-ester bond with G76 of ubiquitination in neurons. 15
uitin. In mammals, the family of E2 enzymes consists of >30 genes whose protein products are characterized by a conserved ~150-amino acid ubiquitin-conjugating (UBC) domain and a conserved catalytic cysteine residue (Pickart, 2001; von Arnim, 2001). E2 enzymes can form complexes with E3 ubiquitin ligase enzymes to create necessary topological surfaces for substrate specificity (Huang et al., 1999). Indeed, the domain architectures of E2 enzymes are varied, and phenotypic analyses in the budding yeast, *Saccharomyces cerevisiae*, have indicated that most E2 enzymes have discrete physiological substrates (Johnston and Madura, 2004).

2. E3 Ubiquitin Ligases. E3 ubiquitin ligases accept ubiquitin molecules from E2 enzymes and catalyze their addition to lysine residues of target substrates. Two major classes of E3 enzymes exist, distinguished by conserved homologous to E6-AP carboxyl terminus (HECT) or really interesting new gene (RING) domains. The identification of HECT domain ubiquitin ligases ensued from studies of tumors arising from infection by the human papilloma virus. During infection, the ubiquitin ligase E6-AP, also known as UBE3A, complexes with the E6 viral protein, causing the degradation of the p53 tumor suppressor (Scheffner et al., 1994). Sequence alignments revealed that the ~350 amino acids at the C terminus of E6-AP, including the critical catalytic cysteine, are conserved in several seemingly unrelated proteins (Huibregtse et al., 1995).

Hundreds of genes comprise the family of RING domain E3 enzymes, making it the largest family of ubiquitin ligases. They are characterized by a common 40- to 100-amino acid RING domain consisting of eight conserved cysteine and histidine residues that coordinate two zinc ions in a cross-braced manner, CX$_2$CX$_{9-39}$CX$_{1-3}$HX$_{2-3}$HX$_2$CX$_{4-8}$CX$_4$C (Borden and Freemont, 1996; Freemont, 2000; Jackson et al., 2000; Joazeiro and Weissman, 2000). Ubiquitin ligase activity of RING proteins was first identified in Rbx/Roc1/Hrt1, which is the catalytic component of the multisubunit Skp1/Cul1/F-box ubiquitin ligase (Kamura et al., 1999; Ohta et al., 1999; Seol et al., 1999; Tan et al., 1999). Subsequent studies identified ubiquitin ligase activity in several other RING proteins unrelated to Rbx/Roc1/Hrt1, and sequence analyses revealed that most non-HECT domain E3 enzymes possess a RING domain (Joazeiro et al., 1999; Levkowitz et al., 1999; Lorick et al., 1999; Fang et al., 2000).

The RING class of E3 enzymes is further subdivided into the plant homeobox domain/leukemia-associated protein and U-box families. Both are small families characterized by small modifications in their RING domains. The plant homeobox domain/leukemia-associated protein domain E3 ligases are zinc-binding variants of RING domain enzymes (Boname and Stevenson, 2001; Coscosy et al., 2001; Lu et al., 2002; Fang et al., 2003; Mansouri et al., 2003). They have a histidine to
cysteine substitution in the fourth zinc-coordinating position and possess a common tryptophan residue immediately before the seventh zinc-binding residue (Capili et al., 2001). In contrast, U-box domain E3 enzymes do not have conserved zinc coordinating residues but structurally resemble RING proteins (Aravind and Koonin, 2000; Cyr et al., 2002). Interestingly, ubiquitin fusion degradation 2, the first identified U-box protein in yeast, is also classified as an E4 enzyme (Koegl et al., 1999). Although not well characterized, E4 enzymes are a novel class of proteins that coordinate ubiquitin chain elongation with E3 ligases.

3. The 26S Proteasome. The 26S proteasome was first hypothesized in 1980 and later purified by Rechsteiner and colleagues in 1986 (Hershko et al., 1980; Hough et al., 1986). The proteasome comprises two stable, multimeric complexes, the catalytic 20S proteasome and the 19S regulatory complex (Baumeister et al., 1998; Walz et al., 1998). The 20S component is composed of 14 proteins, seven \( \alpha \) and seven \( \beta \) subunits that are represented twice to form two \( \alpha \) and two \( \beta \) heptameric rings (Fig. 1). The rings are stacked to form a cylinder with two central \( \beta \) rings bordered on each side by an \( \alpha \) ring (Baumeister et al., 1998).

The 19S component is constructed by a base of eight subunits and a cap of eight to nine subunits. A ring of six AAA family ATPases reside at the base of the 19S component, which help gate the lumen of the proteasome and regulate the energy-dependent unfolding of proteins (Glickman et al., 1998; Braun et al., 1999; Kohler et al., 2001; Navon and Goldberg, 2001). Intriguingly, individual mutations within the six ATPase subunits exhibit distinct phenotypes in yeast (Rubin et al., 1998), suggesting that a specific ATPase or a combination of ATPases acts on discrete substrates.

4. Deubiquitinating Enzymes. Ubiquitination is highly dynamic and exists as a balance between deubiquitinating and deubiquitinating processes. Whereas substrates with ubiquitin chains greater than four molecules are targeted for proteasomal degradation, attachment of single ubiquitin moieties can regulate protein trafficking, alter protein function, and serve as docking sites for ubiquitin-binding proteins (Deveraux et al., 1994; Hicke, 2001). Two classes of deubiquitinating enzymes (DUBs) exist that cleave the G76 bond of ubiquitin. These are the ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (USPs) (Wilkinson, 2000). UCH enzymes feature a 230-amino acid catalytic domain and are thought to process ubiquitin precursor molecules into their mature forms and interact with monoubiquitinated proteins (Wilkinson, 2000; Osaka et al., 2003). USP enzymes are characterized by a conserved 350-amino acid core catalytic domain, and they are thought to act primarily to disassemble polyubiquitin chains (Swaminathan et al., 1999). Constituents of both families of enzymes have been implicated in neuopathologies, which are discussed in later sections.

5. Ubiquitin-Proteasome System Adaptor Proteins. Several regulatory or adaptor proteins associated with the UPS contain protein domains that bind or mimic ubiquitin. Such domains include ubiquitin-like domains (UBLs), ubiquitin-associated domains (UBAs), ubiquitin-interacting motifs (UIMs), ubiquitin E2-variant domains (UEVs), and CUE domains. The architecture of UPS adaptor proteins is highly suggestive of the general function of these domains. For example, the yeast DNA repair protein Rad23 contains two UBA domains and an N-terminal UBL domain (Raasi and Pickart, 2003). This arrangement suggests a model in which Rad23 binds multirubiquitinated proteins through its UBA domains and docks at the proteasome through its UBL domain. Indeed, Rpn1, which resides in the regulatory 19S component of the proteasome, binds the UBL domain of Rad23 (Elsasser et al., 2002). Several proteins associated with the endocytic pathway possess UIM domains, which assemble in a monoubiquitination-dependent manner. These include, Eps15, Eps15R, epsins, and Hrs, which are discussed in later sections (Klapsiz et al., 2002; Polo et al., 2002; Raiborg et al., 2002; Shih et al., 2002). Similarly, the ubiquitin-binding CUE domain has also been linked to endocytosis through studies of yeast endocytic protein Vps9, whereas the UEV domain of tumor-susceptibility gene 101 (Tsg101) has been shown to be important in endosomal sorting (Katzmann et al., 2001; Shih et al., 2003).

B. Endoplasmic Reticulum-Associated Degradation

The endoplasmic reticulum (ER) is the main entry portal for proteins destined for the secretory pathway (Horton and Ehlers, 2004; Lee et al., 2004). During translation, proteins are inserted into the lumen of the ER where they undergo various modifications such as N-linked glycosylation and disulfide bond formation. These modifications, along with the actions of various chaperone proteins, regulate proper protein folding. However, protein maturation in the ER is imperfect, often producing defective polypeptides which must be cleared from the ER. This removal is facilitated by the UPS in a process known as endoplasmic reticulum-associated degradation (ERAD).

Since the identification of the yeast integral membrane E2 enzyme, UBC6, many components of the UPS have been identified for their role in ERAD (Chen et al., 1993; Sommer and Jentsch, 1993). These include E3 ubiquitin ligases, Hrd1/Der3 and Doa10, and the soluble E2 enzyme Ubc7 (Chen et al., 1993; Bays et al., 2001; Swanson et al., 2001). Hrd1/Der3 is a membrane-spanning RING domain E3 ligase arranged with its catalytic domain on the cytoplasmic face of the ER (Bays et al., 2001). It helps form the retrotranslocation pore complex by associating with the Sec61 translocon and UBC6 (Plemper et al., 1999). Other components of the UPS also are found at the retrotranslocation pore. These include Cue1, which recruits Ubc7 to the pore complex, and the
CDC48/p97/Vcp ATPase (Vcp), which interacts with the Der1/Derlin-1 protein (Knop et al., 1996; Biederer et al., 1997; Lilley and Ploegh, 2004; Ye et al., 2004). Vcp is a member of the AAA family of ATPases that biochemically copurifies with the proteasome. It is thought to coordinate substrate recruitment and E4-dependent ubiquitin chain assembly and to act as a protein chaperone (Richly et al., 2005). Furthermore, Vcp is an important component of protein retrotranslocation for ERAD (Ye et al., 2003). Taken together, the molecular arrangement of the retrotranslocation pore complex suggests a model in which proteins targeted for ERAD are localized to the pore, retrotranslocated, unfolded with the assistance of the Vcp ATPase, and degraded by the proteasome upon removal from the ER.

C. Monoubiquitination and the Endocytic Pathway

Cells form an extensive and complex network of endosomes that coordinate vesicular transport between membranous structures such as the Golgi, plasma membrane, and lysosomes. Many UPS genes mediate this pathway, participating primarily in the endocytosis of surface transmembrane proteins and the sorting of internalized cargo (Joazeiro et al., 1999; Springael et al., 1999; Cadavid et al., 2000; Klapisz et al., 2002; Polo et al., 2002; Raiborg et al., 2002; Shih et al., 2002; McCullough et al., 2004). In many cases, ubiquitin alone is sufficient to trigger receptor internalization. For example, studies of the yeast Ste6p G protein-coupled α-factor pheromone receptor have shown that an in-frame ubiquitin fusion is sufficient to trigger receptor internalization (Hicke and Riezman, 1996; Terrell et al., 1998). This holds true for a variety of membrane proteins, including ion channels, G protein-coupled receptors, and receptor tyrosine kinases (Mori et al., 1995; Staub et al., 1997; Shenoy et al., 2001; Hicke and Dunn, 2003).

During lysosomal degradation, cargo destined for degradation is sorted at the endosome by trans-acting factors that segregate them into specialized vesicles that invaginate into the lumen of the endosome (Gorden et al., 1978; Haigler et al., 1979). The specialized endosome compartment that retains these vesicles is called the multivesicular body. Immunocytochemical studies have shown that early endosomes exhibit discrete, ubiquitin-positive subdomains that colocalize with clathrin and the UIM domain protein hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) (Raiborg et al., 2002). The formation of this microdomain precedes the sorting of vesicles to late endosomes and subsequent lysosomal degradation. In the case of transferrin receptors, which undergo constitutive endocytic recycling, internalized receptors do not colocalize with Hrs and recycle to the cell surface. However, ubiquitin-fused transferrin receptors localize to Hrs-containing microdomains and are sorted to the degradative pathway (Raiborg et al., 2002). Such studies have given us a general model in which ubiquitination of receptors, in addition to triggering endocytosis, may be used to sort appropriate receptors for degradation or recycling (Fig. 2).

Hrs with its yeast homolog Vps27 is one of several ubiquitin-interacting proteins associated with endosomal sorting. These proteins are found in several subcomplexes, including Hrs-STAM (yeast Vps27/Hse1), and the endosomal sorting complexes required for transport (ESCRT) I, II, and III (Katzmann et al., 2001; Babst et al., 2002a,b; Bilodeau et al., 2002). Whereas Hrs and STAM both bind ubiquitinated cargo through their UIM domains (Bilodeau et al., 2002), the ESCRT-I complex recognizes ubiquitin through the UEV domain of the Tsg101 (yeast Vps23) subunit (Katzmann et al., 2001). Interestingly, the Eap45 (yeast Vps36) subunit of the ESCRT-II complex can also bind ubiquitin. However, this interaction occurs through an unconventional binding of ubiquitin to its Np 14 zinc finger (Meyer et al., 2002).

Mechanisms similar to those described above may regulate neuronal AMPA receptors, the glutamate-gated ion channels that are the primary determinants of synaptic strength at excitatory synapses. In particular, AMPA receptors undergo activity-dependent endocytic sorting (Ehlers, 2000). During elevated activity, AMPA receptors are sorted for the recycling pathway, whereas activity suppression causes receptors to undergo lysosomal degradation (Ehlers, 2000). As ubiquitinated forms of mammalian AMPA receptors have not been observed, the precise mechanisms mediating its internalization and sorting remain to be determined. We discuss this phenomenon and other UPS-dependent neuronal processes in greater detail in the following sections.

II. The Ubiquitin-Proteasome System in Neuronal Function

Interest in the role of the ubiquitin-proteasome system in the brain began in large part with observations that ubiquitin is a component of proteinaceous deposits in neurodegenerative disorders (Mori et al., 1987; Lennox et al., 1988; Lowe et al., 1988a,b). These include neurofibrillary tangles of Alzheimer’s disease, Lewy bodies of Parkinson’s disease, Pick bodies of Pick’s disease, and various other massed filamentous accumulations immunopositive for ubiquitin. Although initially noted in neuronal dysfunction and degeneration, more recent studies have revealed diverse roles for the UPS in neuronal growth and development, synaptic function and plasticity, and neuronal survival. Collectively, this work has greatly expanded the neurobiological scope of the UPS (Tables 1 and 2).

A. Neuronal Development

1. Axon Growth, Steering, and Pruning. An intriguing observation regarding multimeric ubiquitin ligase complexes has been the presence of the anaphase-pro-
moting complex (APC) in postmitotic mammalian neurons (Gieffers et al., 1999). Classically thought to mediate cell cycle progression, components of the APC, including the Cdh1 coactivator, are expressed abundantly in neurons. The enzyme complex is predominantly localized to the nucleus and immunopurifies from brain extracts as a catalytically active ubiquitin ligase (Gieffers et al., 1999). In developing neurons, the APC seems to be a negative regulator of axon growth. Neuronal morphogenesis studies have shown that reducing Cdh1 levels in dissociated cerebellar granule neurons through RNA interference (RNAi) increases the rate of axon growth by 2.5-fold, but does not affect the rate of dendritic growth (Konishi et al., 2004). This effect is emulated when dominant-negative forms of the catalytic APC11 subunit (Leverson et al., 2000; Tang et al., 2001) or the APC inhibitor Emi1 are expressed (Reimann et al., 2001a,b). Little is known about the mechanisms involved in APC regulation of axon growth. One possibility is that the APC regulates the surface presentation of receptors that detect extracellular guidance cues, thus accelerating the rate of axon growth. Consistent with this idea, Cdh1-knockdown neurons readily grow over myelin substrate in culture and exhibit random growth patterns when plated on cerebellar slices. In vivo, the parallel fibers of cerebellar granule neurons defasciate and deviate from anatomically normal projections when they are transfected with Cdh1-specific RNAi (Konishi et al., 2004).

Several other genes involved in UPS-dependent axon growth and targeting have been identified through forward genetics studies in model organisms. One example is LIN-23, a member of the F-box family of proteins, which function as substrate-distinguishing components of SCF ubiquitin ligase complexes (Skowyra et al., 1997). In Caenorhabditis elegans, worms that lack SCF LIN-23 show overactive embryonic cell proliferation (Kipreos et al., 2000), whereas worms containing a point mutation (proline 610 to serine) in the C terminus of LIN-23 are not compromised in cell division but specifically have defects in axon growth (Mehta et al., 2004). These include ectopic outgrowth of motor neuron axons and sprouting defects in sensory neurons and interneurons (Mehta et al., 2004). Based on the phenotypes of LIN-23-null and point mutation animals, LIN-23 function is probably required throughout nervous system develop-
ment, and it presumably targets different substrates at discrete phases of development. Intriguingly, LIN-23 is homologous to the mammalian β-TrCP gene, which is well characterized for its role in the Wnt signaling pathway and also functions in mature worms to regulate glutamate receptor clustering at the synapse (Dreier et al., 2005).

**TABLE 2**

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<th>UPS molecules implicated in neuronal function</th>
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<tr>
<td><strong>Protein</strong></td>
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<tr>
<td>PKA regulatory subunit</td>
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<td>LIM kinase 1</td>
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<td>DLK/Wallenda</td>
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<tr>
<td>Anaplastic lymphoma kinase</td>
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<td>UNC-13</td>
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<td>Epsin 1</td>
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<td>Shank</td>
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<td>Liprin-α</td>
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<td>SPAR</td>
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<td>β-Catenin/BAR-1</td>
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<td>GLR-1</td>
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This postsynaptic role of LIN-23 is discussed in detail below.

In *Drosophila*, the *bendless* mutant has been characterized to have anatomical malformations that manifest at *Drosophila* giant fiber axons, which fail to form synapses with motor neurons innervating the ipsilateral tergotrochanteral muscle. This results in animals with a compromised jumping response (Thomas and Wyman, 1984). The *bendless* locus encodes an uncharacterized E2 enzyme, but the physiological context of this genetic phenomenon remains to be identified (Muralidhar and Thomas, 1993; Oh et al., 1994). *Nonstop* flies are characterized by aberrations in the axonal projections of photoreceptor neurons (R cells). In wild-type flies, the axons of R cells 1 to 6 (R1–R6) terminate at the first optic ganglion, the lamina, which lies directly adjacent to the retina. In contrast, R7 and R8 project through the lamina and terminate at two distinct layers of the second optic ganglion, the medulla (Meinertzhagen and Hanson, 1993). *Nonstop* is disrupted at the locus of a novel USP enzyme (Poeck et al., 2001) and their R1 to R6 cells abnormally project into the medulla (Martin et al., 1995). However, the *nonstop* effect seems to come from glial cells where Nonstop expression participates in the generation of chemokine gradients (Poeck et al., 2001), further illustrating the sweeping effects of the UPS in neural development.

Regardless of the complex nature of UPS expression in the brain, it is clear that axonal growth cones are primed for enhanced UPS signaling. Isolated growth cones of *Xenopus* retinal ganglion neurons are enriched in ubiquitin, proteasomes, and E1 enzymes, and growth cone responsivity to extracellular guidance cues is lost during pharmacological inhibition of the proteasome (Campbell and Holt, 2001). Furthermore, the formation of new growth cones in transected axons is compromised during pharmacological proteasome inhibition (Verma et al., 2005), suggesting that initiation of axon growth may be dependent on the presence of functional proteasomal machinery.

In support of this idea, the RING domain ubiquitin ligase Rnf6 has been shown to target LIM kinase 1 in neurons (Tursun et al., 2005). LIM kinase 1 is a Rho GTPase effector that alters cellular morphology by phosphorylating the actin depolymerizing factor cofilin (Arber et al., 1998; Yang et al., 1998). Phosphorylation of cofilin prevents its association with actin and increases filamentous actin in the cell. Rnf6 localizes to growth cones, and RNAi knockdown of Rnf6 or overexpression of Rnf6 lacking its RING domain increases axon growth, whereas wild-type Rnf6 overexpression suppresses axon growth (Tursun et al., 2005). Moreover, Rnf6 coimmunoprecipitates with LIM kinase 1 and polyubiquitinates LIM kinase 1 in vitro, and proteasomal inhibition of hippocampal neurons leads to increased levels of LIM kinase 1 (Tursun et al., 2005). As it is well known that localized actin dynamics contributes to axon formation and growth (Bradke and Dotti, 1999), localized proteosomal activity may mediate cytoskeletal changes that are necessary to induce polarized extension of neurites.

During development, axonal growth occurs with excessive branching that is accompanied by the formation of exuberant synaptic contacts. During later phases of development, extraneous synapses are eliminated and axon branches are pruned to actualize mature neural circuitry (O’Leary and Koester, 1993). Recognition of this phenomenon dates back to 1911 when Santiago Ramon y Cajal published his observations of the pruning of climbing fibers during the development of the cerebellum (Ramon y Cajal, 1911). Other well-studied systems for axon pruning include nervous system rearrangements during *Drosophila* metamorphosis (Truman, 1990) and the dissolution of motor neuron connections in the vertebrate neuromuscular junction (NMJ) (Sanes and Lichtman, 1999). Studies of the *Drosophila* mushroom body (MB) during metamorphosis have generated a tractable map detailing axon growth and loss in distinct neurons during development (Lee et al., 1999) (Fig. 3). In the MB, a single neuroblast sequentially generates three types of neurons: γ, α′/β′, and α/β. The γ neuron,
which is born in the early larval stage, initially projects an axon that bifurcates to form the dorsal and medial branches. In the early pupal stage, both dorsal and medial branches are pruned, and in the late pupae, the medial branch re-elongates to establish adult-specific axon projections (Lee et al., 1999). Intriguingly, α/β neurons, which have axon projection patterns that are identical to those of γ neurons in the larvae, do not undergo developmental pruning (Lee et al., 1999). This fact underscores the importance of cell specificity during developmental modification of the nervous system.

Several genetic observations have implicated an important role for the UPS in axon pruning. Overexpression of the yeast ubiquitin protease UBP2 in MB γ neurons prevents axon pruning, and this effect is reproduced by loss of function of the Drosophila ubiquitin-activating enzyme Uba1 and loss of function of 19S proteasomal subunits Mov34 and Rpn6 (Watts et al., 2003) (Fig. 3). In all of these cases, both axon branches fail to prune in late pupae, and the adult flies retain the dorsal projections of their MB γ neurons. Interestingly, not all mutations of UPS components, including specific E2 and E3 enzymes, show this defect (Watts et al., 2003). Furthermore, mutations in clathrin or dynamin have no obvious pruning deformities, suggesting that proteasomal degradation, rather than monoubiquitination and endocytosis, is responsible for axon pruning (Watts et al., 2003).

In the future, it will be important to elucidate how appropriate components of the UPS are expressed in specific neurons and how these components are properly positioned in the cell. It is tempting to speculate that collaborative signaling between external factors and internal signaling mechanisms could define spatial and temporal parameters for the proper differentiation and growth of axons. However, links between external growth factors and their receptors and downstream UPS signaling are lacking. UPS genes have also been implicated in several neurodegenerative disorders characterized by axon loss, suggesting that UPS activity may be important in axon maintenance in addition to growth. These models are presented in more detail below and emphasize the importance of ongoing UPS activity throughout the life of a neuron.

2. Synapse Formation and Elimination. Presynaptic terminals of axons directly appose highly animated, actin-based structures called dendritic spines at most excitatory synapses in the brain. At the tips of these spines resides the postsynaptic density (PSD), a dense complex of several hundred proteins that anchors and organizes receptors, adhesion molecules, and enzymes involved in synapse formation, maintenance, and signaling (Kennedy, 2000; Kim and Sheng, 2004) (Fig. 4). An initial link between the UPS and the synapse was the isolation of the highwire (hiw) Drosophila mutant, a mutant compromised in the function of a putative RING domain E3 ubiquitin ligase (Wan et al., 2000; Wu et al., 2005). Disruption of the highwire locus, particularly loss of the highwire RING domain, causes both morphological and signaling defects at neuromuscular synapses (Wu et al., 2005). Loss of function highwire mutations result in exuberant bouton formation and impaired synaptic transmission due to reductions in both quantal size and content (Wan et al., 2000; Wu et al., 2005). Intriguingly, hiw mutants phenocopy the gain of function of fat facets, a USP class of deubiquitinating enzyme, suggesting that a proper balance of ubiquitination and deubiquitination events is critical for synapticogenesis (Huang et al., 1995; Cadavid et al., 2000; DiAntonio et al., 2001).

Highwire, its C. elegans homolog RPM-1, and the mammalian homologs Phr1 and Pam comprise the conserved Pam/Highwire/RPM-1 class of proteins, all of which directly synapse development (Guo et al., 1998; Schaefer et al., 2000; Wan et al., 2000; Zhen et al., 2000; Burgess et al., 2004). Similarly, the signaling components of the highwire pathway also seem to be conserved. For example, a genetic screen in Drosophila for suppressors of the highwire synaptic overgrowth phenotype identified mutations in wallenda, a mitogen-activated protein kinase kinase kinase of the p38 pathway (Collins et al., 2006), an observation that was also made in a suppressor screen for C. elegans RPM-1 mutants (Nakata et al., 2005). In addition, the T10H9.2 receptor tyrosine kinase, a homolog of the mammalian brain-specific anaplastic lymphoma kinase (Iwashara et al., 1997), is also regulated by RPM-1 and the F-box protein, FSN-1, in worms (Liao et al., 2004; Nakata et al., 2005). Anaplastic lymphoma kinase has also been studied for its ability to induce neuronal differentiation (Souttou et al., 2001) and promote neurite outgrowth (Motegi et al., 2004).

The phenotypic effects of RPM-1 or FSN-1 loss of function are varied, exhibiting differing degrees of under- and overdeveloped synapses. Whereas some presynaptic varicosities acquire several presynaptic densities within a single cluster of synaptic vesicles, others fail to differentiate or contain few vesicles (Schaefer et al., 2000; Zhen et al., 2000). RPM-1 functions in a SCF, or cullin-RING, ubiquitin ligase complex in which components such as FSN-1 can be substituted to alter enzyme specificity (Cardozo and Pagano, 2004; Ang and Harper, 2005). Because of the large diversity of interchangeable subunits, there are probably hundreds of distinct cullin-RING ubiquitin ligases (Petroski and Deshaies, 2005). The assorted effects of RPM-1/FSN-1 loss of function may reflect the requirement of synapse-specific SCF substrates or SCF complexes for proper synapticogenesis.

In addition to its role in axon outgrowth, the APC also modulates synaptic activity in mature neurons. At neuromuscular synapses of Drosophila, the APC localizes to presynaptic terminals where it mediates the differentiation of presynaptic boutons and the formation of functional synapses (van Roessel et al., 2004). Drosophila lines carrying a truncated form of the cullin domain APC
component protein APC2, designated APCmr3 (Reed and Orr-Weaver, 1997), survive until the larval or early pupal stages and are characterized by expanded neuro-muscular synapses (van Roessel et al., 2004). This augmentation in synaptic size results from a 2-fold increase in the number of presynaptic boutons per synapse without an increase in the size of individual boutons (van Roessel et al., 2004). These malformations correspond to enhanced spontaneous and evoked excitatory junctional currents and postsynaptically increased clustering of glutamate receptors. The effect of APC on postsynaptic glutamate receptor clustering is also seen in C. elegans (Juo and Kaplan, 2004) and is discussed further in later sections.

Through database searches, liprin-α was identified as a putative neuronal substrate for APC based on its multiple conserved APC destruction box motifs of RXX-LXXXN (Glotzer et al., 1991; King et al., 1996; van Roessel et al., 2004). Liprin-α and its C. elegans ortholog SYD2 were previously shown to affect NMJ bouton numbers in flies (Kaufmann et al., 2002) and affect synaptic size and function in C. elegans (Zhen and Jin, 1999). Furthermore, liprin-α binds the multi-PDZ domain protein glutamate receptor-interacting protein and clusters...
AMPAR receptors at mammalian excitatory synapses (Wyszynski et al., 2002). Accordingly, APC\textsuperscript{mr3} flies show a 33% elevation in levels of liprin-\(\alpha\) in presynaptic boutons and simultaneous loss of liprin-\(\alpha\) function rescues the phenotype in APC\textsuperscript{mr3} animals (van Roessel et al., 2004). Furthermore, immunoprecipitation experiments show that liprin-\(\alpha\) is ubiquitinated, presumably as a monoubiquitin conjugate in vivo (van Roessel et al., 2004). On the basis of all of these findings, the APC controls synapse development by limiting the action of liprin-\(\alpha\) during synapse development.

In addition to synapse formation, and perhaps more intuitively, synapse loss and elimination is regulated by the UPS. Spine-associated Rap GTPase-activating protein (SPAR) undergoes proteasomal degradation after phosphorylation by serum-inducible kinase (SNK), also known as polo-like kinase 2 (Plk2) (Pak and Sheng, 2003). SPAR is an actin-binding positive regulator of spine growth that localizes to the PSD through its interaction with postsynaptic density protein-95 (PSD-95) (Pak et al., 2001). Whereas SPAR overexpression in cultured hippocampal neurons creates enlarged spines with increased PSD-95 content (Pak et al., 2001), SNK/Plk2 overexpression with subsequent phosphorylation of SPAR eliminates spines and increases dendritic filopodia (Pak and Sheng, 2003). As SNK expression is induced by elevated neuronal activity (Kauselmann et al., 1999), SNK and SPAR are thought to participate in homeostatic plasticity or “synaptic scaling,” by dampening synaptic efficacy during chronic increases in neuronal activity. However, the corresponding UPS enzymes associated with SPAR degradation remain to be identified.

**B. Presynaptic Function**

Neurotransmitter release during synaptic signaling occurs when depolarization induces Ca\(^{2+}\) entry into the presynaptic terminal via voltage-gated Ca\(^{2+}\) channels. This influx of Ca\(^{2+}\) allows the fusion of neurotransmitter-containing vesicles with the presynaptic membrane, resulting in the release of neurotransmitter into the synapse (Fig. 4). In biochemically isolated synaptosomes, De Camilli and colleagues showed that acute depolarization in the presence of Ca\(^{2+}\) causes a rapid and global decrease in ubiquitin-immunopositive protein conjugates (Chen et al., 2003). This decrease is sensitive to Ca\(^{2+}\) chelating agents and to FK-506 and cyclosporin A, both pharmacological inhibitors of the Ca\(^{2+}\)-dependent phosphatase calcineurin (Chen et al., 2003). However, proteasome inhibitors only show partial inhibitory effects, suggesting that the loss of ubiquitin immunoreactivity during depolarization results from deubiquitinating events rather than from protein degradation. Indeed, RNAi targeting of FAM, a mammalian homolog of fat facets, causes the UIM protein epsin-1 to remain in its monoubiquitinated form upon depolarization, presumably affecting presynaptic vesicle dynamics by altering epsin-1 binding to components of the endocytic machinery (Chen et al., 2003).

Deubiquitinating activity may also contribute to neuronal function by regulating cellular levels of free monomeric ubiquitin. This is true for the Ataxia mouse, which is characterized by severe tremors and premature death (D’Amato and Hicks, 1965; Wilson et al., 2002). Ataxia mice are deficient in the USP enzyme Usp14, a mammalian homolog of the yeast deubiquitinating enzyme Ubp6 (Wilson et al., 2002). Usp14/Ubp6 physically associates with the proteasome and is thought to recycle ubiquitin molecules by removing them from proteins targeted for degradation (Borodovsky et al., 2001; Leggett et al., 2002). Indeed, monomeric ubiquitin levels in several different tissues of Ataxia mice are decreased by ~35% compared with those in wild-type mice (Anderson et al., 2005), an effect that can be rescued, at least in the brain, by the neuronal expression of a full-length Usp14 transgene (Crimmins et al., 2006). The electrophysiological characteristics of Ataxia mice suggest that Usp14 defects compromise presynaptic function. For example, Ataxia mice display decreased frequency but increased amplitudes of miniature end-plate potentials at neuromuscular synapses and defects in hippocampal short-term plasticity but unaffected long-term plasticity (Wilson et al., 2002).

In addition to rapid ubiquitination and deubiquitination, protein degradation has profound effects on presynaptic function, probably implicating longer lasting changes. For example, experiments with FM dyes have demonstrated that proteasomal inhibition in mammalian hippocampal neurons increases dye uptake in the presynaptic terminal by 76%, whereas the rate or magnitude of dye release is unaffected (Willeumier et al., 2006). This enhancement is independent of protein synthesis, is lost when action potentials are blocked by tetrodotoxin, and is increased when neuronal activity is elevated by picrotoxin (Willeumier et al., 2006). Although the molecular mechanisms of this phenomenon are unclear, these observations suggest that proteasomal degradation at the presynaptic terminal may negatively regulate neurotransmitter release during times of intense synaptic activity.

At the Drosophila NMJ, a presynaptic substrate of the proteasome has been identified. Pharmacological inhibition of the proteasome or genetic perturbation of proteasome components at presynaptic boutons causes the accumulation of Drosophila UNC-13 (DUNC-13), an important molecule for synaptic vesicle priming (Brose et al., 1995; Speese et al., 2003). Electrophysiological data from the Drosophila NMJ support this observation. Whereas acute pharmacological proteasome inhibition increases evoked synaptic current amplitudes by ~50%, spontaneous miniature current amplitudes and frequencies are not affected (Speese et al., 2003). Presumably, proteasome inhibition in the presynaptic terminal changes local stoichiometries of proteasome substrates,
such as UNC-13, thereby altering the efficiency or kinetics in various stages of synaptic vesicle priming, release, or recycling. Interestingly, pharmacological inhibition of protein translation by anisomycin had no effect on synaptic currents, demonstrating the specific involvement of protein degradation (Speese et al., 2003).

Taken together, these studies illustrate the versatile nature of the UPS in regulating presynaptic function. These include rapid mono- and deubiquitination events as well as enduring changes in synaptic protein stoichiometries that lead to changes in the strength of the synapse. Other UPS components characterized in the presynapse await further study. For example, staring, a novel RING domain E3 ligase, is expressed throughout the brain and in conjunction with the brain-specific E2 Ubch8 and polyubiquitinates the SNARE protein syntaxin 1 in vitro (Chin et al., 2002). Siah 1A and Siah 2, which are vertebrate forms of *seven in absentia* (Sina), have been found to target synaptophysin for degradation (Wheeler et al., 2002). The precise roles of these ubiquitin ligases in neuronal biology remain to be determined.

**C. Postsynaptic Plasticity**

Activity-driven changes in synaptic strength such as long-term potentiation (LTP) and long-term depression (LTD) are considered to be a cellular basis for learning and memory (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Malenka and Bear, 2004). Both LTP and LTD are Ca$^{2+}$-dependent and require the respective activation of kinase (Malenka et al., 1986; Hu et al., 1987) and phosphatase (Mulkey et al., 1993; Mulkey et al., 1994) pathways, the balance of which is thought to determine whether synapses are strengthened or weakened, respectively. After activation of downstream signaling cascades, lasting alteration of synaptic efficacy requires enduring changes in synapse structure and composition. Such lasting changes have been traditionally thought to arise from biosynthetic mechanisms, including gene transcription and local protein translation (Steward and Schuman, 2001; Kelleher et al., 2004a,b).

More recently, a role for ubiquitin-dependent protein degradation in long-lasting synaptic modification has emerged (Stanton and Sarvey, 1984; Ehlers, 2003a; Steward and Schuman, 2003).

1. **The Ubiquitin-Proteasome System in Long-Term Potentiation.** Initial observations for the involvement of the UPS in synaptic plasticity were made in behavioral conditioning studies in *Aplysia*, in which long-term facilitation (LTF) was shown to be mediated by the UPS (Boyle et al., 1984; Montarolo et al., 1986; Dale et al., 1988). During LTF, cAMP-dependent protein kinase (PKA) activity endures even after cAMP levels return to baseline concentrations (Hegde et al., 1993). In the absence of cAMP, PKA exists as a holoenzyme composed of two catalytic (C) subunits whose activity is suppressed by the presence of a dimeric regulatory (R) subunit. When cAMP levels rise, four cAMP molecules bind to the R subunit dimer, releasing the constitutively active C subunits until cAMP levels return to baseline, allowing reformation of the inactive holoenzyme.

2. **Ubiquitin-Proteasome System-Dependent Remodeling of the Postsynaptic Density.** Although the PSD is a biochemically enduring structure, its molecular framework is highly dynamic and responsive to changes in synaptic activity (Okabe et al., 1999; Marrs et al., 2001; Toni et al., 2001; Ebihara et al., 2003; Ehlers, 2003a).
For example, time-lapse microscopy has revealed continuous turnover of PSD-95 clusters at excitatory synapses (Okabe et al., 1999), and biochemical pulse-chase experiments have shown the turnover rate of PSD proteins to be on the order of hours (Ehlers, 2003a). It is now clear that these dynamic molecular changes are mediated in part by the UPS.

It has long been established that biochemically isolated PSD fractions are rich in ubiquitin-immunopositive conjugates (Chapman et al., 1992). More recent studies have demonstrated that changing levels of neuronal activity cause reciprocal alterations in the protein composition of the PSD in a UPS-dependent manner (Fig. 5) (Ehlers, 2003a). Whereas activity blockade diminishes the magnitude of ubiquitin-conjugated PSD proteins by 50%, increased spontaneous activity enhances ubiquitin conjugation by 2-fold, indicating a robust increase in the rate of synaptic protein turnover during elevated activity (Ehlers, 2003a). These changes are long-lasting and reversible and occur as coregulated sets of protein ensembles, in which groups of individual synaptic components change in abundance with similar magnitudes and kinetics (Ehlers, 2003a).

Despite the extensive effects of UPS activity at the PSD, surprisingly few PSD molecules have been shown to be ubiquitinated in neurons. Among these are the multivalent scaffolding proteins Shank, GKAP, AKAP79/150, and PSD-95, all of which are capable of arraying several proteins through multiple protein-interacting motifs (Colledge et al., 2003; Ehlers, 2003a). In addition, SPAR, a postsynaptic actin regulatory protein, undergoes activity and phosphorylation-dependent degradation (Pak and Sheng, 2003). Thus, the targeting of a few “master organizing” molecules by the UPS probably alters stoichiometries of distinct protein subsets by disengaging critical proteins from the PSD.

These local changes in synapse composition seem to be facilitated by activity-dependent alterations in the distribution of proteasomes in neurons (Bingol and Schuman, 2006). Whereas proteasomes visualized with a green fluorescent protein-fused Rpt1 component of the 19S subunit or the α4 component of the 20S subunit are diffuse in dendrites at resting potential, depolarization by KCl causes a rapid redistribution of proteasomes into spines (~90% fluorescence increase in 20 min). This spine enrichment is sensitive to NMDA receptor inhibition by AP5, and fluorescence photobleaching experiments suggest that spines increasingly retain an immobile pool of proteasomes in response to activity (Bingol and Schuman, 2006). Furthermore, elevated neuronal activity increases the detergent-insoluble, actin-associated fraction of neuronal proteasomes (Bingol and Schuman, 2006). Taken together, these experiments suggest a model in which neuronal activity and NMDA receptor activation influence proteasome localization, probably through the local reorganization of the actin cytoskeleton. Interestingly, as many synaptic organizing molecules targeted by the UPS directly bind actin or associate with actin-binding molecules (Colledge et al., 2003; Ehlers, 2003a; Pak and Sheng, 2003), this further illustrates the intricate dynamics between neuronal activity and cellular architecture.

**Fig. 5.** PSD proteins are coregulated by the UPS in an activity-dependent manner. Changes in PSD protein levels are observed during activity elevation by bicuculline treatment in the absence (A) and presence (B) of the proteasomal inhibitor MG-132. Prolonged activity in cultured cortical neurons induces coregulated changes in the proteomic composition of the PSD (A), whereas these coordinated changes are lost during proteasomal inhibition by MG-132 (B). 1, PKCγ; 2, CaMKIIβ; 3, CaMKIIα; 4, myosin Va; 5, Homer; 6, PSD-95; 7, mGluR1α; 8, NR2A; 9, PKA RIIβ; 10, PKA catalytic subunit; 11, PP1; 12, spinophilin/neurabin II; 13, AKAP79/150; 14, GKAP/SAPAP; 15, Shank; 16, SAP102; 17, NR2B; 18, NR1. Graphs adapted by permission from Macmillan Publishers Ltd.: Ehlers (2003a) Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat Neurosci* 6:231–242. Copyright 2003.
D. Ubiquitin and Postsynaptic Receptor Trafficking

Tractable genetic models have been instrumental in shaping current paradigms for receptor trafficking in the postsynapse. In particular, many findings regarding UPS-dependent glutamate receptor trafficking have come from forward genetics studies in *C. elegans*. Such experiments have identified UPS adaptor proteins and ubiquitin ligases involved in the presentation and endocytosis of surface receptors. Intriguingly, whereas glutamate receptors in *C. elegans* undergo direct ubiquitination (Burbea et al., 2002), ubiquitination of mammalian glutamate receptors has not been observed. Here, we highlight key experiments on glutamate receptor trafficking in both *C. elegans* and mammals and discuss possible mechanistic differences between the two systems. Furthermore, we extend our discussion to UPS-dependent effects in the trafficking of GABA and acetylcholine receptors in mammals.

1. Glutamate Receptor Regulation in Caenorhabditis elegans. The ubiquitination of GLR-1 glutamate receptors at *C. elegans* synapses induces receptor removal from the postsynaptic membrane through a mechanism requiring the clathrin adaptor AP180 (Burbea et al., 2002). Interestingly, loss of function mutants in the multiscubunit APC ubiquitin ligase complex exhibit locomotion defects that correspond to increases in synapse size and elevated postsynaptic levels of GLR-1 receptors (Juo and Kaplan, 2004). Remarkably, this finding is similar to observations made at the neuromuscular synapse of *Drosophila* APC mutants, which also have enlarged synapses, increased postsynaptic glutamate receptor clustering, and defects in synaptic transmission (van Roessel et al., 2004). Although in *C. elegans*, GLR-1 is not a direct substrate of the APC, the aberrant synaptic phenotypes of these animals can be suppressed by the introduction of a loss of function allele of the unc-11/AP180 clathrin adaptor, suggesting that APC activity is linked to the endocytic pathway (Juo and Kaplan, 2004). The localization of the APC in the synapse remains unclear. However, in *Drosophila*, presynaptic neuronal APC controls synapse size via the downstream effector liprin-α (van Roessel et al., 2004), a protein known to organize the presynaptic active zone and regulate neurotransmitter release.

More recently, worms lacking the neuronal BTB-Kelch protein KEL-8 have been identified for their effect on increased glutamate receptor clustering (Schaefer and Rongo, 2006). KEL-8 is localized to synapses and, similar to APC animals, the KEL-8 phenotype is rescued by a simultaneous loss of function of unc-11/AP180 (Schaefer and Rongo, 2006). As KEL-8 biochemically purifies with the cullin protein CUL-3, the authors concluded that GLR-1 degradation is mediated by CUL-3-containing SCF complexes (Xu et al., 2003; Schaefer and Rongo, 2006). Based on these results, it will be interesting to determine whether GLR-1 ubiquitination and degradation can be achieved by various ligases, depending on physiological state or activity of the synapse.

In addition to axon outgrowth (Mehta et al., 2004), genetic manipulations in *C. elegans* have identified LIN-23 as a negative regulator of synaptic GLR-1 levels in the ventral nerve cord (Dreier et al., 2005). LIN-23 shares sequence similarity to the mammalian F-box protein, β-TrCP, which is a key component of Wnt signaling. During Wnt signaling, β-catenin binds to the TCF/LeF transcription factors and promotes the expression of Wnt target genes (Behrens et al., 1996; Huber et al., 1996). In the absence of Wnt, β-catenin associates with a destruction complex consisting of axin, adenomatous polyposis coli, and glycogen synthase kinase-3β. In the destruction complex, β-catenin is phosphorylated at consensus sites by glycogen synthase kinase-3β and is then recognized by β-TrCP and targeted for proteasomal degradation (Peifer et al., 1994; Yost et al., 1996; Latres et al., 1999; Winston et al., 1999). Consistent with the known role of β-TrCP in mammalian cells, SCF<sup>LIN-23</sup> also targets the *C. elegans* β-catenin homolog BAR-1 for degradation (Dreier et al., 2005). Like APC mutants, the aberrant effects of LIN-23 on GLR-1 clustering could be rescued by a simultaneous loss of function of unc-11/AP180, suggesting that SCF<sup>LIN-23</sup> is also involved in GLR-1 endocytosis. Furthermore, concurrent mutations in the TCF/LeF homolog POP-1 partially rescued the receptor-clustering phenotype of LIN-23-null animals, suggesting that GLR-1 is a target gene of Wnt signaling (Dreier et al., 2005). Thus, SCF<sup>LIN-23</sup> negatively regulates surface GLR-1 levels directly through the endocytic pathway and indirectly through receptor transcription. As little is known about the synaptic effects of Wnt signaling in mammalian neurons, these intriguing findings await further study.

2. Glutamate Receptor Regulation in Mammals. At mammalian excitatory synapses, a principal determinant of synaptic strength is the number of surface AMPA receptors, which, like classic models of receptor dynamics, are internalized and sorted in an activity-dependent manner. For example, in primary hippocampal cultures, AMPA receptors have been shown to undergo endocytosis with a time constant of ~14 min in the presence of the sodium channel blocker tetrodotoxin, which inhibits spontaneous neural activity. When inhibitory inputs are blocked with picrotoxin, AMPA receptor endocytosis is accelerated by ~3-fold (Ehlers, 2000). AMPA receptor internalization can be triggered directly by ligand binding and indirectly by NMDA or insulin receptor activation, and its surface levels are reduced during LTD (Carroll et al., 1999; Luscher et al., 1999; Beattie et al., 2000; Ehlers, 2000; Lin et al., 2000; Bredt and Nicoll, 2003). Although direct and indirect stimuli can initiate AMPA receptor internalization, the intracellular course of the receptor depends on the nature of the endocytic switch. Indeed, stimulation of AMPA receptors alone causes internalized receptors to be sorted for deg-
radation, whereas NMDA receptor activation alone leads to AMPA receptor recycling (Ehlers, 2000).

As many dynamic changes in surface AMPA receptor levels are calcium (Ca\textsuperscript{2+})-dependent, there has been great interest in identifying the molecules that couple Ca\textsuperscript{2+} influx to AMPA receptor endocytosis. Several Ca\textsuperscript{2+} sensors have been proposed in this process, including the Ca\textsuperscript{2+}-binding phosphatase calcineurin (PP2B) (Breatte et al., 2000; Ehlers, 2000), the clathrin-associated complex of AP-2 and hippocalcin (Palmer et al., 2005), and the Ca\textsuperscript{2+}-binding PDZ adaptor protein interacting with protein kinase C 1 (PICK1) (Hanley and Henley, 2005). Although these observations point to a model in which phosphorylation/dephosphorylation events mediate AMPA receptor dynamics at the plasma membrane, activity-dependent endocytosis of AMPA receptors has also been shown to be sensitive to proteasomal inhibition (Colledge et al., 2003; Patrick et al., 2003). This finding suggests that the UPS, at least in some instances, can mediate AMPA receptor endocytosis.

Unlike in C. elegans in which glutamate receptors are directly ubiquitinated (Burbea et al., 2002), ubiquitination of mammalian AMPA receptors has not been observed. Rather, AMPA receptor endocytosis is thought to occur indirectly through ubiquitination of the PSD-95 scaffolding protein by the ubiquitin ligase Mdm2 (Colledge et al., 2003). NMDA-dependent PSD-95 ubiquitination in hippocampal neurons is dependent on an N-terminal PEST motif and is inhibited in the absence of Ca\textsuperscript{2+} or in the presence of the calcineurin inhibitor ascomycin (Colledge et al., 2003). These observations suggest that Mdm2 and calcineurin function in the same signaling axis, with calcineurin operating upstream of Mdm2. In support of a role for protein degradation in AMPA receptor endocytosis, other studies have shown that overexpression of ubiquitin mutated at lysine 48 (K48R), which occludes chain formation but allows monoubiquitination, prevents AMPA-induced receptor internalization (Patrick et al., 2003). As many studies have demonstrated that synaptic PSD-95 levels can influence the content of AMPA receptors at the synapse (El-Husseini et al., 2000; El-Husseini Ael et al., 2002; Stein et al., 2003; Ehrlich and Malinow, 2004), PSD-95 degradation is an attractive model to explain AMPA receptor endocytosis. However, PSD-95 ubiquitination is not detectable in all conditions (Ehlers, 2003a; Bingol and Schuman, 2004). This result may reflect subtle differences in experimental conditions and suggests that PSD-95 ubiquitination may be tightly regulated by the cell. In future studies, it will be important to identify the contributions of both phosphorylation/dephosphorylation and ubiquitination pathways during AMPA receptor endocytosis and to elucidate the physiological contexts in which they occur individually or synchronously. In addition to AMPA receptors, the UPS also mediates degradation of NMDA-type glutamate receptors. An SCF complex composed of the F-box protein Fbx2 mediates activity-dependent ubiquitination and degradation of NMDA receptor NR1 subunits (Kato et al., 2005). The Fbx2 protein was previously characterized for its role in glycoprotein recognition during ERAD (Yoshida et al., 2002), and, similarly, it recognizes high-mannose glycans in the N-terminal extracellular domain of NR1 (Kato et al., 2005). In neurons, Fbx2 localizes to dendritic spines, and the expression of a dominant-negative form of Fbx2 enhances surface NMDA receptor expression and increases NMDA currents (Kato et al., 2005). The degradative capacity of SCF\textsuperscript{Fbx2} for NR1 is activity-dependent, suggesting the involvement of ERAD in homeostatic activity-dependent control of NMDA receptor expression.

3. Trafficking of GABA and Acetylcholine Receptors.

Whereas glutamate receptors primarily determine the strength of excitatory synapses, fast inhibitory transmission in the brain is mediated by GABA receptors. At a general level, GABA receptors are distinguished structurally as they can be either metabotropic (GABA_B) or ionotropic (GABA_A and GABA_C) receptors. Ionotropic receptors can be further distinguished on the basis of their discrete subunit composition, which endows them with different pharmacological properties (Cutting et al., 1991; Polenzani et al., 1991; Shimada et al., 1992). In mammalian brains, GABA_A is the most abundant receptor subtype and acts to hyperpolarize the cell through the influx of chloride ions. GABA_A receptors are pentameric hetero-oligomers that assemble from a diverse pool of six subunits with multiple members: α1 to α6, β1 to β3, γ1 to γ3, δ, ε, and θ. On the basis of studies with recombinant receptors, it is hypothesized that GABA_A receptors assemble with a subunit ratio of 2α:2β:1γ (Chang et al., 1996; Tretter et al., 1997; Farrar et al., 1999) with δ, ε, and θ substituting for γ to increase receptor subtype diversity (Shivers et al., 1989; Saxena and Macdonald, 1994; Davies et al., 1997; Whiting et al., 1997; Bonnert et al., 1999).

Insight into the regulation of GABA_A receptor trafficking was gained when it was discovered that certain α and β subunits (α1–3, α6, and β1–3) interact with protein linking IAP to cytoplasmic 1 (Plic-1), an adaptor protein containing an N-terminal UBL domain and a C-terminal UBA domain (Bedford et al., 2001). In neurons, Plic-1 colocalizes with GABA_A receptors by immunofluorescence imaging, and electron microscopy also showed Plic-1 molecules in close proximity to GABA_A receptors (Bedford et al., 2001). When the interaction between Plic-1 and GABA receptors is disrupted by a peptide inhibitor, GABA-activated currents in hippocampal slices and surface GABA_A receptor levels both diminish by ~20%, suggesting that Plic-1 positively regulates receptor presentation at the plasma membrane (Bedford et al., 2001). Intriguingly, the kinetics of
GABA\(\text{A}\) receptor endocytosis is not affected by its interaction with Plic-1, but rather Plic-1 seems to affect receptor turnover. GABA\(\text{A}\) subunit stability is sensitive to proteasomal inhibition by lactacystin, and pulse-chase experiments with recombinant \(\beta3\) subunits show a 30\% increase in receptor stability when coexpressed with Plic-1 (Bedford et al., 2001). As the UBA domain of Plic-1 is essential for binding to GABA\(\text{A}\) receptors, these results suggest a model in which Plic-1 presumably binds monoubiquitinated GABA\(\text{A}\) receptors and prevents its degradation. However, further study is required to elucidate the physiological context in which this interaction occurs, the relevant points of regulation, and the exact molecular detail of this intriguing mechanism of regulation.

Many multisubunit ion channels and receptors contain ER retention signals and export motifs to assure that only correctly assembled molecules escape the ER and enter the secretory pathway. This mechanism has been well studied in the context of potassium channels (Zerangue et al., 1999), AMPA receptors (Greger et al., 2002; Grunwald and Kaplan, 2003), kainate receptors (Ren et al., 2003b; Jaskolski et al., 2004), and NMDA receptors (Standley et al., 2000; Scott et al., 2001; Mu et al., 2003). Recent evidence has highlighted a role for the UPS in regulating forward trafficking of receptors from the ER. Green and colleagues observed that cultured myotubes constitutively expressing AChRs dramatically increase their levels of surface AChRs in the presence of proteasome inhibitors (Christianson and Green, 2004). Intriguingly, pulse-chase experiments indicate that this effect is independent of increased transcription of AChRs and is probably not caused by a decreased capacity for ERAD. Indeed, the stoichiometric ratios of surface receptors during proteasomal inhibition are consistent with properly assembled AChRs, supporting the view that regulatory elements for amending malformed receptors are not lost. Altogether, these findings suggest a model in which proteasomal degradation during ERAD limits the surface delivery of AChRs early in the secretory pathway, providing an important checkpoint for the forward trafficking of synaptic receptors. Future work examining such a process in neurons may provide clues into the nature of sorting and proper targeting of postsynaptic receptors.

III. The Ubiquitin-Proteasome System in Neurological Disease

Many neurodegenerative diseases are characterized by dense deposits immunoreactive for ubiquitin and UPS-associated proteins (Lowe et al., 1988a, 1990; Shimura et al., 1999). However, the significance of these ubiquitin-rich aggregates in the etiology of neurological disorders remains highly controversial. Whether protein aggregation is a coping response to the accumulation of malfunctioning proteins causing neuronal dysfunction (Arrasate et al., 2004) or a correlative diagnostic by-product of an ongoing disorder remains unclear. However, as mutations in UPS genes are linked to inherited neurodegenerative disorders (e.g., familial Parkinson’s disease), UPS dysfunction almost certainly contributes to diverse neuropathological conditions.

A. Ubiquitin-Proteasome System-Linked Animal Models for Neurodegeneration

Above we considered roles for ubiquitin-dependent mechanisms in axonal outgrowth and targeting during development. In addition to normal physiological axon guidance and pruning, compartmentalized loss of axons occurs in a range of neurological insults. Normally, injured or transected axon segments remain viable for a period of hours and then undergo rapid nonapoptotic degeneration along the entire length of the axon distal to the axon injury (Finn et al., 2000). This type of axon loss, termed Wallerian degeneration (Waller, 1850), is associated with trauma, toxicity, and ischemia (Coleman and Perry, 2002; Raff et al., 2002; Ehlers, 2003b). Historically and quite reasonably, Wallerian degeneration has been thought to result from biomaterial deprivation upon separation of the axon from the cell body. However, a more nuanced view of the molecular basis for axon degeneration has come from Wallerian degeneration slow (Wld\(^{S}\)) mutant mice, which have axons that resist degeneration and remain viable for weeks after transaction (Lunn et al., 1989; Glass et al., 1993).

Wld\(^{S}\) mice possess a gene translocation that results in the overexpression of a chimeric protein consisting of the noncatalytic N terminus of the E4 enzyme ubiquitin fusion degradation 2 and the complete sequence of nicotinamide mononucleotide adenyllyltransferase 1 (Mack et al., 2001). In wild-type dorsal root ganglion neurons, inhibition of the proteasome and Ca\(^{2+}\)-dependent calpain proteases but not of caspases or serine proteases delays degradation of transected axons in a manner reminiscent of that in Wld\(^{S}\) mice (Zhai et al., 2003). The overexpression of the deubiquitinating enzyme, UBP2, mimics this effect, further implying that reduced polyubiquitination and associated proteasome degradation is responsible for the Wld\(^{S}\) phenotype (Zhai et al., 2003). Intriguingly, whereas both axon pruning and Wallerian degeneration are thought to involve the UPS (Watts et al., 2003; Zhai et al., 2003), Wld\(^{S}\) overexpression has no effect on developmental axon pruning in mice or flies even though it protects the same axons at the same developmental stage against injury-induced degeneration (Hooper et al., 2006). Thus, different UPS pathways probably regulate axon degeneration through mechanisms that are highly dependent on the physiological context.

However, a more recent study showed that delayed axon degeneration in transected dorsal root ganglion
neurons only occurs during overexpression of nicotinamide mononucleotide adenyltransferase 1 and not when the E4 portion of the WldS chimera is overexpressed (Araki et al., 2004), suggesting a primarily protective role for NAD biosynthesis in delaying axonal degeneration. More work is clearly needed to distinguish the mechanistic differences and perhaps interplay between the UPS and NAD biosynthesis pathways in axon loss.

Another example of a ubiquitin-dependent mechanism in axonal loss comes from the gracile axonal dystrophy (Gad) mutant mouse, which is characterized by early developmental sensory ataxia with progressive motor ataxia (Saigoh et al., 1999). During this process, axons of the spinal gracile tract, which carry mechanosensory information from the lower extremities, exhibit a “dying back” degeneration, with the formation of spheroid bodies at nerve terminals accompanied by the accumulation of amyloid β-protein and ubiquitin-rich protein deposits (Ichihara et al., 1995). Gad mice exhibit a deletion in the UCH-L1 locus (Saigoh et al., 1999), nullifying the activity of the deubiquitinating enzyme previously discussed for its role in Aplysia LTF (Chain et al., 1999). In addition to gad, the UCH-L1 gene is also implicated in familial forms of PD (Leroy et al., 1998). It is interesting to note that UCH-L1 is estimated to comprise up to 2% of total brain protein composition (Leroy et al., 1998), and, thus, much remains to be discovered about the role of this intriguingly abundant protein in neuronal function.

Another age-dependent mouse model for neurodegeneration is the spontaneous mahogonoid mutant mouse, in which certain mahogonoid alleles cause age-dependent spongiform neurodegeneration, hypomyelination, and tremor (Phan et al., 2002; He et al., 2003). Mahoganoid mice are deficient in the activity of mahogunin, a RING domain E3 ligase previously identified for its role in the melanocortin-1 receptor, and the transmembrane precursor of the secreted protein Attractin (Atrn) (Lu et al., 1991; La Spada et al., 1991). The most prevalent disorders in this group are the polyglutamine (polyQ) repeat diseases, which result from an expansion of CAG repeats ranging up to ≥80 codons in pathological conditions (Tarlac and Storey, 2003). PolyQ disorders progress slowly, show autosomal dominant patterns of inheritance, and increase in severity with successive generational expansion of polyQ repeats. Represented in the family of polyQ disorders are Huntington’s disease, dentatorubral-pallidoluysian atrophy, spinobulbar muscular atrophy (Kennedy’s disease), and various spinocerebellar ataxias. Typically, polyQ disorders result in the deposition of ubiquitin-positive protein inclusions in the brain, suggesting that these diseases may arise from defective protein degradation or toxic stress from aggregated, unfolded proteins (Gatchel and Zoghbi, 2005).

Perhaps the most direct link between UPS dysfunction and polyglutamine disorders is spinocerebellar ataxia type 3, also known as Machado-Joseph disease. Machado-Joseph disease results from a polyQ expansion in the C terminus of the ataxin-3 gene, a ubiquitin-specific protease or USP (Kawaguchi et al., 1994; Burnett et al., 2003). The ataxin-3 protein has three UIM motifs thought to be important for binding ubiquitinated conjugates (Burnett et al., 2003) and the UBL domain of Rad23 (Doss-Pepe et al., 2003). Furthermore, ataxin-3 biochemically copurifies with catalytically active proteasomes, suggesting that it may function to link ubiquitinated proteins to the proteasome.

Recent work has suggested a role for ataxin-3 in ERAD through its interaction with Vcp, an ATPase of the retrotranslocation complex that is instrumental in extracting misfolded proteins from the ER (Ye et al., 2003; Wang et al., 2006; Zhong and Pittman, 2006). Vcp copurifies with ataxin-3, and whereas the overexpression of both wild-type and catalytically inactive forms of ataxin-3 causes an accumulation of the ERAD sub- strates T cell receptor-α and CD3δ (Wang et al., 2006; Zhong and Pittman, 2006), ataxin-3 knockdown by RNAi decreases ERAD substrate levels (Zhong and Pittman, 2006). Taken together, these experiments strongly suggest that ataxin-3 is a negative regulator of ERAD-dependent protein quality control (Wang et al., 2006; Zhong and Pittman, 2006).

However, the role of ataxin-3 polyQ expansion in neuronal pathogenesis remains unclear. Based on the multimodular structure of ataxin-3, elongated polyQ sequences may alter the way ataxin-3 interacts with other components of the UPS pathway. Indeed, polyQ expansions in ataxin-3 increase its association with Vcp, presumably preventing efficient removal of proteins from the ER during ERAD-dependent protein degradation.

B. Spinocerebellar Ataxia

Trinucleotide repeat disorders are a family of neurological disorders grouped by the expansion of repetitive three-nucleotide repeats in a particular gene (Fu et al., 1991; La Spada et al., 1991). The most prevalent disorders in this group are the polyglutamine (polyQ) repeat diseases, which result from an expansion of CAG repeats ranging up to ≥80 codons in pathological conditions (Tarlac and Storey, 2003). PolyQ disorders progress slowly, show autosomal dominant patterns of inheritance, and increase in severity with successive generational expansion of polyQ repeats. Represented in the family of polyQ disorders are Huntington’s disease, dentatorubral-pallidoluysian atrophy, spinobulbar muscular atrophy (Kennedy’s disease), and various spinocerebellar ataxias. Typically, polyQ disorders result in the deposition of ubiquitin-positive protein inclusions in the brain, suggesting that these diseases may arise from defective protein degradation or toxic stress from aggregated, unfolded proteins (Gatchel and Zoghbi, 2005).

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Ubiquitination in Neurons

C. Parkinson’s Disease

PD is a movement disorder that is clinically associated with a gradual degeneration of dopaminergic neurons in the substantia nigra. It affects approximately 1% of the population older than age 65 (Bossy-Wetzel et al., 2004) with >95% of reported cases of PD being sporadic in origin with no clear catalyst for pathological onset (Bossy-Wetzel et al., 2004). Significant advances elucidating the molecular basis of PD have come with the identification of genes responsible for rare, inherited forms of the disorder. The identification of a genetic basis of PD has given tractability to an otherwise mysterious disease. Nine genetic loci (PARK1–9) have been linked to inherited forms of PD and six of these elements have been identified, including α-synuclein (PARK1) (Polymeropoulos et al., 1997), parkin (PARK2) (Kitada et al., 1998), UCH-L1 (PARK5) (Leroy et al., 1998), phosphatase and tensin homolog deleted on chromosome 10-induced kinase 1 (PARK6) (Valente et al., 2004), DJ-1 (PARK7) (Bonifati et al., 2003), and leucinerich repeat kinase 2 (PARK8) (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). In keeping with the scope of this review, we continue our discussion on the role of the UPS genes UCH-L1 and parkin in the etiology of PD.

1. Ubiquitin C-Terminal Hydrolase-L1 in Parkinson’s Disease. Inheritable forms of PD caused by mutations in the UCH-L1 locus were first identified through studies of a German family exhibiting autosomal dominant patterns of PD inheritance (Leroy et al., 1998). The disease-linked mutation that occurs is an Ile83Met substitution in the peptidase domain of UCH-L1, thought to drastically reduce its deubiquitinating activity (Leroy et al., 1998). As previously discussed, mice deficient in UCH-L1 expression (gad mice) are characterized by progressive ataxia and the degeneration of axons along the gracile tract (Saigoh et al., 1999). However, these animals do not exhibit the characteristic death of neurons in the substantia nigra associated with PD.

At a mechanistic level, the enzymatic activity of UCH-L1 has remained mysterious. Whereas wild-type UCH-L1 has been proposed to undergo homodimerization and gain ubiquitin ligase activity, Ile83Met mutants, which are associated with familial PD, lose this specialized function (Liu et al., 2002). Although this observation is intriguing, recent developments point to a more classic role of UCH-L1 in neuronal function. Whereas monoubiquitin pools in the brains of gad mice are decreased by 20 to 30%, monomeric ubiquitin content is increased in the brains of mice overexpressing UCH-L1 (Osaka et al., 2003). These differences in free ubiquitin probably reflect changes at the protein level, as mRNA pools of ubiquitin are unchanged between wild-type and gad mice (Osaka et al., 2003). From these observations, it is plausible to think that UCH-L1 acts analogously to Usp14 to maintain the cellular pool of free ubiquitin molecules and the importance of ubiquitin availability in maintaining neuronal homeostasis is further underscored.

2. Parkin. Parkin is a RING E3 ubiquitin ligase. It has a C-terminal PDZ-binding domain (Fallon et al., 2002), a specially modified C-terminal in-between RING domain flanked by two RING motifs, and an N-terminal UBL domain that binds Rpn10 of the proteasome and is thought to aid the shuttling of ubiquitinated proteins for degradation (Sakata et al., 2003). Genetic studies have shown that diverse mutations in the parkin gene produce PD phenotypes inherited as autosomal recessive traits with detectable symptoms manifesting before age 30 (Dauer and Przedborski, 2003). Individuals carrying disease-linked forms of parkin exhibit the characteristic loss of dopaminergic neurons from the nigrostriatal pathway with corresponding increases in body tremors and deficits in motor control. However, these patients lack the accumulation of Lewy bodies, suggesting that Lewy body deposits alone are not sufficient or necessary for onset of PD and that parkin ubiquitin ligase activity may be responsible for the conjugation of ubiquitin to Lewy body components. Indeed, the ubiquitinated forms of several putative parkin substrates, including α-synuclein (Shimura et al., 2001), synphilin (Chung et al., 2001), and parkin itself (Choi et al., 2000; Zhang et al., 2000), have been shown to be abundant components of Lewy body accumulations (Leroy et al., 1998; Bossy-Wetzel et al., 2004). Other putative substrates targeted for ubiquitination by parkin have been identified, including synaptotagmin XI (Huynh et al., 2003), CD-Crel-1 (Zhang et al., 2000), the Pael receptor (Yang et al., 2003), cyclin E (Staropoli et al., 2003), α and β tubulin (Ren et al., 2003a), the aminoacyl-tRNA synthetase cofactor p38/JTV-1 (Ko et al., 2005), and the UIM protein Eps15 (Fallon et al., 2006).

Parkin expression in the nervous system is widespread with localization to both presynaptic and postsynaptic structures, including synaptic vesicles and dendritic vesicles as well as glial cell bodies (Mouatt-Prigent et al., 2004), suggesting diverse roles for parkin, including a potential role at synapses. Indeed, parkin forms a biochemical complex with NMDA receptors and PSD-95 core components of the postsynaptic density at glutamatergic synapses (Fallon et al., 2002). Consistent with a role in synaptic transmission, mice lacking parkin show subtle behavioral deficits as well as altered excitability in the hippocampus and striatum (Goldberg et al., 2003; Itier et al., 2003; Von Coelln et al., 2004). In
disease-associated alleles of parkin, many mutations cluster around the proposed catalytic region, probably compromising the enzymatic capacity of the protein (Vila and Przedborski, 2004). Yet, mutations associated with autosomal recessive juvenile parkinsonism are found throughout the parkin gene. Such dispersed mutations throughout a multidomain protein, together with the fact that different disease-linked mutations have widely varying effects on the E3 ligase activity, localization, and substrate binding of parkin (Sriram et al., 2005; Matsuda et al., 2006), suggest more complex effects of such mutations than simple loss of function. In other words, although PD-associated symptoms and pathology typically follow recessive inheritance, biochemical and cellular functions of parkin may be selectively disrupted or even enhanced by disease-linked mutations, suggesting that caution is warranted in interpreting the cellular effects of parkin mutations as simply being due to loss of protein function. Supporting this notion, recent genetic studies argue against a simple recessive model of disease transmission (West and Maidment, 2004).

Further complicating our understanding of the function of parkin in normal physiology or in PD, very few proposed parkin substrates are altered in abundance in parkin-null mice, and these animals show no signs of dopaminergic neurodegeneration (Goldberg et al., 2003; Greene et al., 2003; Pesah et al., 2004; Ko et al., 2005). In addition, none of the identified substrates for parkin are particularly enriched in dopaminergic neurons, raising further questions regarding the link between parkin function and disease phenotypes (Vila and Przedborski, 2004). One recent important clue in this regard is the fact that dopamine itself covalently modifies parkin and inactivates its ubiquitin ligase activity (LaVoie et al., 2005), suggesting a mechanism for the progressive and selective loss of parkin activity in dopaminergic neurons during sporadic Parkinson disease.

Although many questions remain unresolved, parkin remains a promising candidate for future PD investigation. Common themes, particularly centered on the role of parkin in neuroprotection, are beginning to emerge. Cyclin E, a parkin substrate and a positive regulator in neuronal apoptosis (Staropoli et al., 2003), is elevated in the midbrains of patients with parkin-linked PD. Cultured neurons overexpressing wild-type parkin show a reduction in cyclin E levels and are correspondingly resistant to excitotoxic stimuli (Staropoli et al., 2003). Ectopic expression of parkin also reduces α-synuclein and Pael receptor-mediated neuronal toxicity (Imai et al., 2001; Petruelli et al., 2002; Yang et al., 2003) and augments the clearance of certain polyQ repeat proteins (Yang et al., 2003). Given that microtubule disruption is the first observable event of axon degradation (Zhai et al., 2003; Ehlers, 2004), parkin may also contribute to the structural maintenance of axons where it localizes along microtubules and mediates tubulin turnover (Ren et al., 2003a). Furthermore, whereas Drosophila models lack dopaminergic deficits, they curiously show an age-dependent degeneration in their flight muscles (Greene et al., 2003).

Recent studies have shown that parkin, through its UBL domain, binds Eps15, a UIM-containing adaptor protein involved in epidermal growth factor (EGF) receptor internalization and trafficking (Confalonieri et al., 2000; Fallon et al., 2006). In the presence of EGF, Eps15 is monoubiquitinated in a parkin-dependent manner, presumably decreasing its affinity for activated, and thus ubiquitinated, surface EGF receptors (Mori et al., 1995; Fallon et al., 2006). Indeed, parkin knockdown by RNAi dramatically increases the rate of internalization of labeled EGF ligand, suggesting that parkin increases the pool of activated receptors at the cell surface by preventing Eps15-dependent endocytosis (Fallon et al., 2006). Moreover, parkin overexpression increases the level of phosphorylated Akt, a downstream component of EGF signaling, and synaptosomes prepared from brains of parkin knockout mice have an attenuated phosphorylated Akt response when stimulated with EGF (Fallon et al., 2006). Given that EGF receptors are known to protect dopamine neurons and are altered in the striatum of patients with PD (Iwakura et al., 2005) and that Akt mediates neuronal survival (Dudek et al., 1997; Kauffmann-Zeh et al., 1997; Philippot et al., 1997; Eves et al., 1998; Kulik and Weber, 1998), the neuroprotective effect of parkin may arise from its ability to increase the cellular response to EGF through an Akt-dependent pathway.

Certain inheritable forms of parkin mutations are responsible for late-onset PD, mimicking the timeline for sporadic forms of PD (West and Maidment, 2004). Biochemically, the biochemical characteristics of parkin are conducive to age-dependent dysfunction. The cysteine-rich RING domains of parkin are susceptible to oxidative damage such that cumulative environmental oxidative stress may contribute to parkin dysfunction. In addition, parkin activity is acutely enhanced when parkin is nitrosylated by nitric oxide, leading to an increase in autoubiquitination and subsequent inhibition of its activity (Chung et al., 2004; Yao et al., 2004). These findings, together with the observation that parkin undergoes complex protein phosphorylation (Yamamoto et al., 2005), point to a high degree of cellular regulation that further argues for important as yet unidentified physiological roles for parkin.

D. Neurodevelopmental Disorders: Angelman Syndrome

Angelman syndrome (AS) was first identified in 1965 by Harry Angelman, an English pediatrician who reported similar clinical features among three children with severe learning disabilities, ataxia, limited speech, inappropriate laughter, epileptic seizures with a characteristic electroencephalogram appearance, and dysmor-
phic facial features (Angelman, 1965). It is now broadly accepted that genomic anomalies underlie AS. Approximately 70% of cases of AS are thought to arise by characteristic breaks in maternal chromosome 15q11-q13, which causes a deletion of 4 megabases of DNA (Knoll et al., 1989). Within this deleted region are several genes, which causes a deletion of 4 megabases of DNA (Knoll et al., 1994; Kishino et al., 1997; Matsuura et al., 1997).

UBE3A expression in the brain is complex and undergoes a highly stereotypical pattern of imprinting in the brain. For example, whereas the maternal copy of UBE3A is expressed highly in hippocampal neurons and Purkinje cells of the cerebellum, the paternal copy in these regions is expressed in very low amounts or not at all (Albrecht et al., 1997; Rougeulle et al., 1997; Vu and Hoffman, 1997). In heterozygous mice lacking maternal copies of UBE3A (m−/p+), decreased levels of UBE3A mRNA is evident in these imprinted areas (Jiang et al., 1998). Furthermore, these animals have defects in hippocampal LTP, context-dependent learning, mild motor dysfunction, and susceptibility to audiogenically induced seizures (Jiang et al., 1998).

Interestingly, the phenotypic characteristics of AS overlap with those of Rett syndrome (RTT), another neurodevelopmental disorder caused by mutations in the X-linked methyl-CpG-binding protein 2 (Tate et al., 1996), a methyltransferase-dependent transcriptional repressor (Fusks et al., 2003). Both AS and RTT are associated with severe cognitive deficits, microcephaly, ataxia, seizures, and stereotypic hand movements (Scheffner et al., 1993). Although both disorders share similar disease manifestations that are apparent postnatally, the timeline of their observable pathophysiology is intriguingly different. Children born with RTT have an initial 12- to 18-month period of normal development followed by intellectual regression (Wilson et al., 2003), whereas patients with AS have no observable window of normal development (Clayton-Smith, 1993).

Despite these initial clues, the molecular details of UBE3A function have been difficult to determine. In addition to its link to CaMKII (Weeber et al., 2003), genetic studies of GABRB3-deficient mice have also implicated GABRB3 in AS (DeLorey et al., 1998). These mice phenotypically mimic UBE3A maternal-null mice by exhibiting seizures, poor performance in memory-dependent tasks, and defects in motor abilities (DeLorey et al., 1998). Given that UPS components are critical for development, and maturation of GABAergic neurons is important for postnatal critical period plasticity in mice (Hensch et al., 1998; Petrucci et al., 2002), it is tempting to speculate that UBE3A may be involved in the development of inhibitory circuits necessary for proper neural maturation. However, more study is needed to understand the developmental context and regulation of UBE3A expression and the significance of its anatomical localization. In addition to providing further insight into the role of the UPS in neuronal function, such studies are likely to provide valuable information regarding the complex nature of neural development.

IV. Conclusions and Perspectives

Many basic questions about the biology of the UPS in the neuron remain. Among the large number of protein components that comprise the UPS, very few have been studied in any detail yet in neurons. We also possess limited knowledge about UPS regulation in the brain. How does a guidance factor or neuronal activity induce or limit the activity of UPS enzymes? How are UPS components positioned at various parts of a large, polarized neuron? How are specific components of the PSD accessed and targeted for ubiquitination while its structural integrity is maintained?

The importance of protein turnover at synapses has raised new intriguing questions about the persistence of neural circuits and their stable properties. A fundamental tenet of learning-related synaptic plasticity is that information storage is represented to a large degree by the composition of proteins at synapses. In the face of robust, ongoing protein turnover, how does the neuron faithfully sustain a molecular history? In other words, how do memories remain when the molecules that physically represent them are continually degraded? Although many challenging questions remain, the multiple roles of the UPS in neuronal development, signaling, and plasticity promises to be a fertile area for future study and may hold the key to linking normal physiology to neuronal dysfunction and degeneration in the many neurological disorders involving aberrant protein degradation by the UPS.

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Correction to “Emerging Roles for Ubiquitin and Protein Degradation in Neuronal Function”


First, in Tables 1 and 2 on page 20, the spelling of “Hedge” in the Hedge et al. (1993) citations should be Hegde.

Second, the year in the Table 1 citation and in the citation at the end of the first paragraph under “The Ubiquitin-Proteasome System in Long-Term Potentiation” should be changed from 1993 to 1997. The complete citation for this reference is hereby listed below:


The authors regret these errors and apologize for any confusion they may have caused.