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Remodeling without Destruction: Nonproteolytic Ubiquitin Chains in Neural Function and Brain Disorders

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Abstract

Ubiquitination is a fundamental posttranslational protein modification that regulates diverse biological processes, including those in the CNS. Several topologically and functionally distinct polyubiquitin chains can be assembled on protein substrates, modifying their fates. The classical and most prevalent polyubiquitin chains are those that tag a substrate to the proteasome for degradation, which has been established as a major mechanism driving neural circuit deconstruction and remodeling. In contrast, proteasome-independent nonproteolytic polyubiquitin chains regulate protein scaffolding, signaling complex formation, and kinase activation, and play essential roles in an array of signal transduction processes. Despite being a cornerstone in immune signaling and abundant in the mammalian brain, these nonproteolytic chains are under-appreciated in neurons and synapses in the brain. Emerging studies have begun to generate exciting insights about some fundamental roles played by these nondegradative chains in neuronal function and plasticity. In addition, their roles in a number of brain diseases are being recognized. In this article, we discuss recent advances on these non-conventional ubiquitin chains in neural development, function, plasticity and related pathologies.

Introduction

Ubiquitination is an essential post-translational protein modification in eukaryotes through which the 76 amino acid protein, ubiquitin is conjugated to a protein substrate [1]. Ubiquitination is completed in sequential enzymatic events through E1 ubiquitin-activating, E2 ubiquitin-conjugating, E3 ubiquitin-ligating enzymes, and sometimes an E4 ligase, and is reversed by a family of deubiquitinases (DUBs) (Fig. 1a). A major form of ubiquitination is polyubiquitination where a chain of ubiquitin molecules linked through the C-terminal glycine of one ubiquitin molecule and an internal lysine of another ubiquitin molecule is conjugated on a lysine residue of a substrate. Given that seven internal lysine residues (K6, K11, K27, K29, K33, K48, and K63) exist in the ubiquitin molecule, seven distinct

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Conflict of Interest Statement

The authors declare no conflict of interest.

polyubiquitin (polyUb) chains can form [2] (Fig. 1b). Another polyUb, the head-to-tail linear chain (M1) linked through the N-terminal methionine of one ubiquitin and the C terminus of a preceding ubiquitin also exists [3]. Various polyUb chains assume distinct topologies and confer differential fates to a substrate. K48-linked chains are the classical signal that targets substrates to the proteasomes for degradation, whereas K63 and M1 chains are nonproteolytic and are involved in signaling activation and transduction [4]. The remaining chains, less prevalent and atypical, have both proteasome-dependent and nonproteolytic functions.

The roles of the ubiquitin proteasome system (UPS) in neural development, function, plasticity, and behavior have been extensively investigated [5–8]. Notably, UPS-dependent protein degradation and turnover represent a major mechanism driving synapse remodeling and plasticity, and are involved in a number of brain diseases [7–10]. Nonproteolytic ubiquitination, however, has not been well studied in neurons. Recent studies begin to reveal involvements of nonproteolytic polyUb chains in neural development, receptor trafficking, pre- and postsynaptic function and remodeling, and synaptic plasticity, representing a new paradigm in synapse biology. The potential roles of nonproteolytic chains in several common brain diseases, including Autism Spectrum Disorders (ASDs), schizophrenia, Parkinson's disease (PD), Alzheimer's Disease (AD) and related dementia, have also been steadily emerging.

In this review, we discuss recent advances on the roles of nonproteolytic polyubiquitination in neural development, functions, and plasticity, as well as several common brain disorders. Although not involved in proteasomal degradation, K63-polyUb chains can target ubiquitinated cargos, including misfolded protein inclusions (aggrephagy) and damaged mitochondria (mitophagy) to the autophagy-lysosome pathway for clearance. This proteasome-independent proteolysis function is outside the scope of our discussion, and interested readers may refer to some excellent recent reviews on this topic [11–14].

Different Faces of Ubiquitin Linkages

Among the eight polyUb linkages (Fig. 1b), K48 chains are the first-identified and best-characterized. These chains adopt a compact conformation and are recognized by the 26S proteasome, where substrates are degraded and the ubiquitin tag is cleaved and recycled, and act as a general degradation signal maintaining cellular protein homeostasis [2, 15]. K63 chains represent the second most abundant polyUb species in the mammalian brain [16]. They adopt a more open and flexible conformation and are not recognized by the 26S proteasome, thus do not signal proteasomal degradation. Instead, they mediate signaling pathways involved in NF- κ B activation, receptor endocytosis, and DNA repair, by stabilizing/activating substrates or acting as a scaffold to assemble signaling complexes [2, 4, 17, 18]. Linear chains are also non-degradable by the proteasome and play important roles in NF- κ B signaling [3, 19]. The remaining linkages are less prevalent and play degradative and/or nondegradative roles in more specialized cellular processes [4, 20]. K6 chains are implicated in DNA damage response (nondegradative) and mitophagy (degradative). K11 chains regulate the proteasomal degradation of proteins involved in cell cycle regulation [21, 22] and endoplasmic reticulum-associated degradation (ERAD) [23], and have a

nonproteolytic signaling role in TNF α activation [24]. K27 chains are more enigmatic, but are implicated in DNA damage, mitophagy, and autophagy as scaffolds to recruit signaling proteins [20]. Both K29 and K33 chains can serve as proteasome degradation signals and are implicated in epigenetic regulation and post-Golgi membrane protein trafficking as nonproteolytic signals [20, 25, 26]. Finally, mixed/branched and unanchored chains also exist and play degradative and nondegradative functions [20, 27].

The various polyUb linkages are assembled by diverse (> 600) E3s in complex with ~40 E2s [2, 28, 29]. E3s contain four families: really interesting new gene (RING), homologous to E6-associated protein C-terminus (HECT), UFD2 homology (U-box), and RING-in-between-RING (RBR) [30, 31] (Fig. 1a). For HECT E3s, ubiquitin is relayed from a bound E2 to a catalytic cysteine residue in the HECT domain of an E3, which then attaches the ubiquitin to a substrate. The chain specificity for most HECT E3s remains to be determined, but one exception is Nedd4 (neural precursor cell expressed developmentally downregulated gene 4) that contains a Ub binding site in the C lobe which may orient the Ub chain and select for K63 chains [32]. In contrast, RING E3s, the most prevalent, serve as adaptors, bringing a ubiquitin-bound E2 and a substrate into close proximity and activating the E2 to transfer the ubiquitin to the substrate. Thus, E2s bound to these RING E3s often determine the polyUb linkage specificity. The RING E3 ligase, TNF receptor-associated factor 6 (TRAF6), for example, catalyzes the formation of K63 chains on substrates together with the K63-specific ubiquitin conjugating enzyme Ubc13 and the Ubc-like protein Uev1A E2 dimers [33]. Some RING E3s function as single subunits (e.g. TRAFs), whereas others function in multisubunit complexes that contain several adaptors for substrate recognition and ubiquitin E3 activity (e.g., Skp1/Cullin/F-Box (SCF)) [34]. U-box E3s, also dubbed E4s, mainly elongate existing polyUb chains [35]. Finally, RBR E3s employ a hybrid mechanism by which the first RING domain acts as a canonical RING ligase while the second RING domain acts like a HECT ligase [36, 37]. Two important RBR E3s are Parkin, involved in Parkinson's disease and mitophagy [13], and LUBAC (linear ubiquitin chain assembly complex), an E3 ligase complex composed of SHARPIN, HOIL-1L, and HOIP that specifically generates linear chains [3]. All ubiquitin enzymes discussed in this review are summarized in Table 1.

More than 100 DUBs, cysteine proteases and metalloproteases that remove or trim PolyUb chains, exist in the human genome [38–40]. Based on their catalytic domains and mechanisms of action, these DUBs are categorized into six superfamilies: Ubiquitin C-terminal hydrolases (UCHs), ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), the Josephin family, the Motif interacting with ubiquitin-containing novel DUB family (MINDYs), and the JAB1/MPN/MOV34 (JAMMs) metalloprotease family [38, 40, 41]. DUB superfamilies exhibit variable levels of linkage specificity. For example, USPs are typically linkage nonspecific [38], but an exception is cylindromatosis (CYLD), which is specific to K63 (and M1 chains which have virtually equivalent structure) due to its unique UBD that contains an extended loop near the catalytic domain selective for K63 chains [42, 43]. In contrast, OTUs are mostly linkage-specific due to their diverse ubiquitin-binding domains (UBDs) [44].

Diverse polyUb chains are “decoded” by a large number of UBD-containing proteins, known as ubiquitin receptors, which translate the ubiquitin code to specific biochemical and cellular outputs [2, 45]. There are more than 20 structurally different UBDs in five subfamilies, α -Helix, Zinc Finger (ZnF), Ubc-like, pleckstrin homology (PH) fold, and other structures [2]. Individual UBDs typically bind ubiquitin with low affinities, and additional interactions between ubiquitinated targets and ubiquitin receptors in a multiprotein complex cooperate to provide sufficient specificity, avidity, and dynamic regulation. The formation of these “signalosomes” through recruitment of binding partners that harbor specific UBDs is a hallmark role of nondegradative polyUb chains.

Major Nonproteolytic Paradigms in Non-neurons

Signalosome assembly and kinase activation.

Roles of nonproteolytic polyUb in scaffolding signaling complexes and activating kinases are best illuminated in the classical NF- κ B signaling pathways in innate and adaptive immunity (Fig. 2a) [18, 46–48]. Activation of Toll-like and cytokine receptors recruits, in a ligand- and receptor-specific manner, adaptor proteins, protein kinases, and a panel of K63- or M1-specific E3 ligases including TRAF6 and LUBAC, promoting their activation. Activated E3s coordinate with specific E2s to assemble K63 and M1 chains on specific substrates or free, unanchored chains. These polyUb chains then serve as scaffolds to simultaneously load the TAK1 (TGF- β activated kinase 1) kinase complex and IKK (Inhibitor-of- κ B kinase) complex through their UBDs, leading to the activation of the IKK complex. Activated IKK complex then recruits a signaling complex consisting of I κ B α (inhibitor of NF- κ B) and NF- κ B subunits p65/p50, leading to phosphorylation and proteasomal degradation of I κ B α by the E3 complex SCF ^{β TrCP} [49]. Liberated NF- κ B then enters the nucleus to regulate expression of diverse target genes important for inflammation and cell survival. The ubiquitin editing enzyme A20, an OTU DUB, and CYLD are potent inhibitors of NF- κ B signaling by cleaving K63 and M1 chains [19, 50].

Endocytosis:

Ubiquitination is a classical signal for surface receptor endocytosis, endosomal trafficking, and sorting [17, 51, 52]. Upon ligand binding, activated receptors are ubiquitinated at the plasma membrane by several K63 E3 ligases recruited to the activated receptors, such as CBL (Cas-Br-M ecotropic retroviral transforming sequence; a RING E3), TRAF6, and the Nedd4 family. AMSH (associated molecule with the SH3 domain of STAM; a JAMM DUB) and USP8/UBPY (ubiquitin-specific protease 8/ubiquitin-specific protease Y) negatively regulate endocytosis by cleaving K63 chains from internalized receptors [53, 54]. Ubiquitinated receptors are internalized through either clathrin-dependent or -independent endocytosis, driven by a set of endocytic adaptors at the plasma membrane, such as EPS15 (epidermal growth factor receptor substrate 15) and epsins, which bind ubiquitin through their UBDs and couple receptors to endocytic vesicles. Once at early endosomes, ubiquitinated cargo is sorted through endosome-associated complexes ESCRT (endosomal sorting complex required for transport)-0, -I, -II, and -III into multivesicular bodies (MVBs) within late endosomes for degradation (Fig. 2b). Importantly, each ESCRT complex contains UBDs in their subunits that facilitate formation of multiprotein complexes necessary for

efficient endosomal sorting. Ubiquitin is removed by ESCRT-III-associated DUBs prior to receptor entry into MVBs. Monoubiquitin and K63-polyUb chains often dominate in endocytosis, however, the latter is believed to be more effective [55].

Maintenance of genome integrity.

Nonproteolytic polyUb plays essential roles in DNA damage response (Fig. 2c). Following DNA double-stranded breaks (DSBs), the E2 Ubc13 and UbcH5c, and RING E3 ligases RNF (RING finger protein) 8 (RNF8) and RNF168 are recruited to sites of damage foci, where they synthesize K27 and K63 chains on Histone H2A/H2AX or other substrates [56–59]. These nonproteolytic chains serve as scaffolds for the receptor-associated protein 80 (RAP80), a ubiquitin receptor, which recruits the BRCA1 (breast cancer type 1 susceptibility protein) E3 ligase repair complex and other crucial mediators to DSB-associated chromatin to promote K6 ubiquitination and initiate DNA repair [56–60].

Neurotrophin Signaling

Neurotrophins (NGF, BDNF, NT4/5, NT-3) regulate neuronal differentiation, survival, and plasticity by signaling through several tyrosine kinase receptors (TrkA, TrkB, and TrkC) and the p75 receptor, a member of the TNF receptor superfamily [61, 62]. The NGF receptor TrkA undergoes K63 polyubiquitination following NGF stimulation in PC12 cells [63], which is mediated by TRAF6 and the E2 UbcH7, and requires the K63-polyUb-binding scaffolding protein p62/sequestosome-1 (SQSTM1) to facilitate the assembly of NGF-induced p75-TrkA-TRAF6-UbcH7 multiprotein complex. This K63 polyUb signal is required for TrkA internalization, downstream signaling, and NGF-induced neurite outgrowth [63]. TrkB and TrkC also contain a consensus site for TRAF6/p62 polyubiquitination, and TrkB is ubiquitinated by TRAF6 following its activation [64].

TRAF6 becomes associated with p75 following stimulation by neurotrophins in transfected HEK293T cells [65]. TRAF6 may act as the E3 ligase for p75, which is polyubiquitinated following NGF stimulation in a mouse hippocampal cell line [66]. Additional signaling intermediates recruited to activated p75 or TRAF6 include p62 [67], IRAK (interleukin 1 receptor-associated kinase) [68], NRIF (neurotrophin receptor interacting factor) [69, 70], and RIP2 (receptor-interacting protein 2) [71]. Among these, NRIF is K63 ubiquitinated by TRAF6 [70]. These cytoplasmic adaptors form complexes following p75 activation and transduce multiple downstream p75-dependent signaling pathways to regulate cell survival or apoptosis in various neuron(-like) cell types [65, 66, 68, 70–72]. In TRAF6 knockout mice, p75-mediated NF- κ B and JNK (c-Jun N-terminal kinase) signaling following NGF or BDNF stimulation were blunted and the p75-induced apoptosis was lost in Schwann cells and sympathetic neurons [73].

Neural Patterning

Sonic Hedgehog (Shh) signaling plays crucial roles in vertebrate neural patterning, mediated by gradients of Ci/Gli family transcription activators (GliA) and repressors (GliR). In a Shh-regulated manner, Glis are modified with degradative K11 or K48 polyUb chains by several multisubunit SCF E3 ligases (among others), which dictates the degradation and

activator/repressor status [74–77]. Recent work indicates that Shh/Gli signaling gradient is also fine-tuned by nonproteolytic ubiquitination during ventral neural tube patterning [78]. Specifically, RNF220, an E3 specifically expressed in the developing ventral neural tube, assembles K63 chains on all Glis, either in their activator or repressor forms, and the K63 chains promote Gli nuclear export and refine their gradients by limiting their nuclear levels. Loss of RNF220 leads to expansion of the GliA and GliR gradients and disruptions of progenitor patterning along the dorsal-ventral axis [78].

Axonal and Dendritic Outgrowth

In developing sensory neurons, neurotrophin signaling promotes axon outgrowth through clathrin-mediated endocytosis and signaling endosomes that are retrogradely transported back to cell bodies to support neuronal survival [79, 80]. Zhou et al. (2007) showed that in addition to this “global” mechanism, neurotrophins also regulate sensory axon elongation and branching via a K63-mediated clathrin-independent TrkA endocytosis locally at the growth cone [81]. In cultured mouse embryonic dorsal root ganglion neurons, NGF stimulates K63 ubiquitination of Ulk1/2 (Unc-51-like kinase 1/2) by TRAF6 and recruitment of Ulk1/2 to the TrkA receptor complex via interaction with p62, routing NGF-bound TrkA to non-clathrin-coated vesicles that attenuates NGF signaling and allows filopodia withdrawal. This study highlights an important role for presynaptic endosomal trafficking in axonal development (Fig. 3). Knocking down Ulk1/2 leads to impaired NGF endocytosis, excessive axon arborization, and severely stunted axon elongation. Mice lacking Ulk1/2 in the CNS showed defects in axonal pathfinding and defasciculation affecting the corpus callosum, anterior commissure, and corticothalamic and thalamocortical axons [82]. However, it should be noted that these loss-of-function experiments did not directly address the role of K63 ubiquitination of Ulk1/2 in these deficits.

Another K63-dependent mechanism regulating neurite outgrowth is mediated by Muscleblind-Like Protein 1 (MBNL1). MBNL1 is a multifunctional protein regulating the transition between differentiation and pluripotency and the pathogenesis of myotonic dystrophy type 1 (DM1), which is associated with dendritic and synaptic transmission deficits at early stages [83]. MBNL1 undergoes K63 ubiquitination, which is required for its localization in the cytoplasm. Cytoplasmic, but not nuclear MBNL1 promotes axon outgrowth and neurite differentiation in hippocampal neurons [84].

Presynaptic Differentiation and Function

Endosomal trafficking at presynaptic terminals plays an important role in synaptic vesicle (SV) cycles during presynaptic development and functions [85–87]. The ubiquitin-binding protein Phospholipase A2 Activating Protein (PLAA) has been shown to act as a ubiquitin adaptor required for post-endocytic sorting of ubiquitinated presynaptic proteins from early endosomes to lysosomes for degradation during SV recycling [88]. Mammalian PLAA specifically recognizes K63-polyUb-modified SV components and targets them for ESCRT-dependent degradation via MVBs. Mice deficient in PLAA show disrupted Purkinje cell migration and dendritic arborization, accumulated K63-ubiquitinated proteins and presynaptic membrane proteins. This accumulation of K63-ubiquitinated proteins is

thought to be a result of impaired trafficking of these proteins to ESCRT-dependent sorting and lysosomal degradation. Mutant NMJs show striking presynaptic swelling/sprouting, drastic loss of reserve pool SVs, and aberrant vesicle recycling during sustained activity. In both human and mouse, hypomorphic mutations in PLAA cause a lethal infantile neurodysfunction syndrome with epilepsy [88]. This study supports the importance of K63-related UBD-containing ubiquitin adaptors and endosomal trafficking in SV homeostasis and presynaptic differentiation (Fig. 3).

In addition to its role in nuclear DNA repair in proliferating cells, RNF8 and associated K63-specific E2 Ubc13 regulate presynaptic differentiation in postmitotic neurons [89]. Knockdown or conditional knockout of RNF8 or Ubc13 in mouse cerebellum granule neurons robustly increases the number of presynaptic boutons and parallel fiber-Purkinje cell synapses as well as functional synaptic transmission. Knockdown of the K48-synthesizing E2 UbcH8, fails to increase the number of parallel fiber presynaptic boutons. Expression of an RNF8 mutant that interacts with Ubc13, but not UbcH8, rescues the number of presynaptic boutons. Cytoplasmic, but not nuclear RNF8 exerts this presynaptic differentiation role. RNF8 interacts with the HECT domain protein HERC2/scaffold protein Neuralized 4 (NEURL4) complex and knocking down either protein mimics the inhibition of RNF8 on synapse formation. These results, though short of directly showing involvement of K63 chains, strongly suggest that RNF8/Ubc13-dependent K63-ubiquitin signaling inhibits synapse formation *in vivo* (Fig. 3). Detailed molecular details of this K63-dependent presynaptic remodeling remain to be determined.

The metabotropic glutamate receptor 7 (mGluR7), a presynaptic G Protein-coupled receptor that inhibits glutamate release especially during sustained and heightened activity [90], is K63 ubiquitinated following agonist stimulation in heterologous cells and cultured neurons [91]. mGluR7 ubiquitination is mediated by Nedd4, which is recruited to activated mGluR7 by the adaptor protein β -arrestin, and leads to endosome-lysosome sorting and degradation of the receptor. This process may play a role in the regulation of presynaptic metaplastic long-term depression (LTD)/long-term potentiation (LTP) at certain synapses, e.g. mossy fiber-CA3 stratum lucidum interneuron (MF-SLIN) synapses [92] (Fig. 4; further discussed below).

Postsynaptic Function and Dendritic Spine Remodeling

Increasing evidence indicates that K63 ubiquitination occurs at mammalian synapses. A mass-spec analysis of rat brain ubiquitome shows that K63-polyUb species are the second most abundant ubiquitin linkage, behind K48 chains [16]. Mouse brain K63-polyUb levels increase during postnatal development and K63-polyUb clusters are abundant in dendritic spines in cultured rat hippocampal neurons [93]. K63-related ubiquitin enzymes, including TRAF3, TRAF6, Ubc13, Uev1A, CYLD, and A20 are present in the mouse brain postsynaptic densities (PSDs) [93–95]. Several signaling components of NF- κ B pathway are also found at synapses [96–98]. These studies suggest that K63-polyUb may play synapse- and neuron-specific roles in addition to their classical functions in host immune surveillance and defense.

Among K63-polyUb machineries localized in the PSD, CYLD emerges as a central player. CYLD transcript is high in fetal brain, and its mutations are associated with familial cylindromatosis, an autosomal dominant genetic predisposition to multiple tumors of the skin appendages [99]. CYLD protein is highly enriched in the rat brain PSD [93, 100]. CYLD in the PSD is endogenously active with K63 deubiquitinase activity at basal conditions maintained by the PSD-associated IKK [100, 101], and is recruited and further activated in a Ca²⁺-dependent manner by CaMKII to the PSD following high K⁺ or NMDA stimulation in cultured hippocampal neurons [102]. Thus, CYLD may participate in activity-dependent regulation of K63 ubiquitination in synapses and synaptic plasticity (Fig. 4; further discussed below). CYLD binds the scaffolding protein and autophagy receptor p62 [103], which is also abundant in the PSD. CYLD also interacts with Shank3, which regulates the abundance of CYLD and total K63-polyUb level in mouse striatal synaptosomes [104]. Proteomic analysis of CYLD interactome has identified more than 103 proteins, many of which are associated with ASD, schizophrenia, and depression. Despite its abundance and function in the PSD, it should be noted that a small amount of CYLD is present in the presynaptic fraction of the rodent brain [93], and a recent study reports that it regulates axonal length in cultured mouse hippocampal neurons [105].

In addition to its abundance and functions at excitatory synapses, CYLD also regulates inhibitory neurons and synapses [106]. In CYLD knockout mice, *in vivo* recording shows that the rhythmic activity in the striatum, where ~95% of neurons are GABAergic, is altered, characterized by shortened spontaneous up-states and increased membrane fluctuations preceding action potential firing. Levels of striatal GABA_A and GABA_B receptors are increased in mutant mice and pharmacological blockade of GABA receptors rescues the electrophysiological phenotypes. This study suggests that CYLD regulates synthesis, turnover, and/or trafficking of GABA receptors.

A bona-fide substrate of K63 ubiquitination at synapses has been identified as PSD-95 [93], a major postsynaptic scaffold with essential roles in PSD organization and synaptic plasticity [107, 108]. PSD-95 is K63-ubiquitinated by TRAF6 together with Ubc13/Uev1A, and deubiquitinated by CYLD (Fig. 3). The K63-polyUb conjugation sites are mapped to several lysine residues in the GK (guanylate kinase) domain, which mediate PSD-95 interactions with its binding partners, including SPAR (spine-associated RapGAP), GKAP (guanylate kinase-associated protein)/SAPAP (SAP90/PSD-95-associated protein), and AKAP79/150 (A-kinase-anchoring protein 79/150). Mutations of these K63-polyUb sites abolish PSD-95-target binding, consistent with the role of K63-polyUb as a signalosome scaffold (Fig. 3). Functionally, mutant PSD-95 lacking K63 ubiquitination cannot target to the synapse and promote synapse formation, maturation, and strength. This study provides the first direct evidence that K63 ubiquitination regulates postsynaptic scaffolding and remodeling in excitatory synapses (Fig. 3).

Recently, the ubiquitin-editing enzyme A20 has been shown to potently inhibit dendritic arborization, spine formation, and synaptic strength in cultured hippocampal neurons [95]. The A20 regulation of dendritic spine remodeling depends on the K63-specific DUB activity of A20 and is mediated by suppression of the (presumably nuclear) NF- κ B signaling

pathway. Thus, K63-linked ubiquitination can regulate postsynaptic remodeling via both local (synapse) and global (nuclear) mechanisms (Fig. 3).

Glutamate Receptor Trafficking and Function

Glutamate receptors mediate the majority of excitatory synaptic transmission and plasticity responsible for cognitive functioning, and ubiquitination plays crucial roles in these processes. Initial studies in *C. elegans* found that the glutamate receptor GLR-1 (about 40% homology to mammalian AMPA-class glutamate receptor (AMPA) subunits [109]) is ubiquitinated, which regulates GLR-1 postsynaptic abundance and synaptic strength [110], and Uev-1 regulates GLR-1 trafficking by controlling its exit from early endosomes post endocytosis [111]. Mammalian AMPARs undergo ubiquitination at specific lysine residues in the C-terminus, regulating receptor trafficking and synaptic strength [112, 113]. All four AMPAR subunits, GluA1-A4, display low levels of ubiquitination under basal conditions, but become rapidly ubiquitinated following short-term AMPAR, but not NMDA receptor (NMDAR) activation that is associated with receptor internalization, endosomal sorting, and lysosomal degradation [114–116]. Ca²⁺ entry via voltage-gated L-type Ca²⁺ channels and CaMKII activation are required for the activity-dependent AMPAR ubiquitination, likely by specific E3s and DUBs recruited/activated to active synapses [113–115, 117]. Importantly, K63-linked ubiquitination represents the primary polyUb linkage for both constitutive and activity-dependent GluA1 and GluA2 ubiquitination [114, 118], consistent with its role in endosomal trafficking. Other glutamate receptors, including NMDA (GluN1, GluN2A, and GluN2B) [119–122], kainate (GluK2) [123, 124], and metabotropic receptor (mGluR1/5) [125], also undergo ubiquitination, but these modifications are associated with proteasomal degradation.

Several ubiquitin enzymes have been identified to regulate AMPAR ubiquitination and trafficking. Nedd4 interacts with GluA1, promotes GluA1 ubiquitination, and is rapidly redistributed to spines in response to AMPAR activation in cultured neurons [115, 126, 127]. Overexpressing Nedd4 increases GluA1 ubiquitination, reduces its surface level, and enhances its internalization and accumulation at lysosomes, and Nedd4 mediates bicuculline-induced homeostatic synaptic downscaling [115, 127, 128]. Nedd4–2/Nedd4L, a closely related but functionally different E3, also ubiquitinates and downregulates GluA1 during synaptic downscaling [129]. RNF167, a plasma membrane-associated but predominately lysosomal RING E3 involved in endosomal trafficking, has been shown to ubiquitinate GluA2 and regulate synaptic AMPARs and activity-dependent AMPAR ubiquitination [117]. Two USP family DUBs can reverse GluA1 ubiquitination and may facilitate AMPAR recycling back to the plasma membrane. USP8/UBPY reduces basal and AMPA-induced AMPAR ubiquitination and positively regulates surface AMPAR levels, and is recruited to synapses following NMDAR activation in a Ca²⁺-dependent manner to regulate homeostatic downscaling [127]. USP46, a DUB that regulates GLR-1 trafficking and levels in *C. elegans* [130], has been reported to cleave K63-polyUb from AMPARs and regulate AMPAR internalization and synaptic strength in cultured rat cortical and hippocampal neurons [118].

Thus, surface AMPARs are ubiquitinated upon activation, internalized, and sorted to late endosomes and lysosomes for degradation or recycled back to the plasma membrane [112, 113, 131, 132] (Fig. 5). However, it remains controversial whether AMPAR ubiquitination occurs before [115, 127, 128, 133] or after [114, 116] endocytosis. AMPARs can also be degraded by the proteasome system [128, 134, 135], especially in response to chronic elevation of synaptic activity [134], or by the autophagy-lysosome pathway during NMDA-induced chemical long-term depression (cLTD) [136]. Finally, although K63 chains are believed to be the primary polyUb chains conjugated to AMPARs [114, 118] and guide AMPAR trafficking, further experiments are needed to elucidate the molecular details underlying each step of intracellular trafficking and sorting processes.

Synaptic Plasticity

In the mossy fiber-CA3 stratum lucidum interneuron synapses, sustained synaptic activity leads to activation of presynaptic mGluR7, which inhibits neurotransmitter release through PLC and PKC-dependent suppression of P/Q Ca²⁺ channels. mGluR7 activation unmasks the ability of these synapses to undergo LTP in response to the same high-frequency stimuli that induce LTD in naive slices [92]. K63 ubiquitination of mGluR7 routes it for lysosomal degradation [91]. These processes may regulate this metaplastic LTD/LTP switch (Fig. 4). Converging evidence also indicates that K63-polyUb mechanisms are regulated by neuronal activity and in turn regulate activity-dependent Hebbian and non-Hebbian synaptic plasticity. Brief exposure to NMDA, a cLTD-inducing stimulus, triggers a rapid and global disassembly of K63-polyUb chains in neuronal cultures and concomitant K63 deubiquitination of PSD-95, which is accompanied by dispersal of PSD-95 from synapses, internalization of AMPARs, and cLTD [93]. Deubiquitination of K63-ubiquitinated PSD-95 by CYLD is required for this cLTD. The activity-triggered, NMDAR-dependent CYLD synaptic translocation and activation [102] may serve as a potential mechanism for deubiquitination of synaptic substrates including PSD-95, and LTD (Fig. 4). Supporting the role of K63-polyUb in synaptic plasticity, Nedd4 heterozygous mice displayed a reduced hippocampal postsynaptic LTP [137], and theta burst stimulation (TBS)-induced LTP is absent in hippocampal CA1 neurons on slices from p62 knockout mice [138]. Finally, K63 ubiquitination, as discussed above, is an important signal for AMPAR trafficking that mediates bicuculline-induced synaptic downscaling, a non-Hebbian form of plasticity (Fig. 5) [115, 127–129].

The involvement of non-proteolytic ubiquitination in behavioral plasticity has also begun to emerge. Conditional knockout of RNF8 or Ubc13 in mouse granule neurons show cerebellar-dependent learning deficits [89]. Conditional knockout of cerebellar Ubc13 also produces disturbances in gait and spontaneous locomotion and exploration [139]. Nedd4 mutant mice show an impaired long-term spatial memory in the Morris water maze [137]. More directly, in the mouse amygdala, memory acquisition has been reported to be associated with a nuclear increase of K48, K63 and M1 chains, whereas memory retrieval induces an increase in these polyUb chains in the synaptic, but not nuclear or cytoplasmic regions [140]. As UPS and synaptic protein degradation underlies destabilization of retrieved fear memory [141], how proteasome-independent polyUb chains regulate memory formation, consolidation, and retrieval remain an important future question.

Emerging Models

Based on above discussions, working models of how nonproteolytic ubiquitination regulates synapse development, function, and plasticity begin to take shape (Fig. 3–5). By regulating protein scaffolding locally in the PSD and globally through NF- κ B signaling in the nucleus, K63-polyUb can regulate dendritic spine remodeling (Fig. 3). By guiding pre- and post-synaptic endosomal trafficking and sorting, K63-polyUb can regulate axonal patterning, SV cycles, terminal differentiation (Fig. 3), LTD (Fig. 4), and homeostatic plasticity (Fig. 5). Through activity-dependent recruitment of ubiquitin enzymes (e.g. CYLD, USP8, Nedd4, and Nedd4L) and synaptic substrate ubiquitination/deubiquitination, K63-polyUb can drive Hebbian (Fig. 4) and non-Hebbian synaptic plasticity (Fig. 5). Molecules with established roles in non-neuron cells (e.g. RNF8) are also repurposed for specific functions at synapses (Fig. 3).

Brain Diseases

In this section, we discuss emerging roles of nonproteolytic ubiquitination in several common neurodevelopmental, neuropsychiatric, and neurodegenerative diseases. Nonproteolytic ubiquitination related genes and their implications in each brain disorder are summarized in Table 2.

Autism Spectrum Disorders.

ASDs are neurodevelopmental disorders characterized by impaired social interaction and communications, as well as repetitive and stereotypic interests and behaviors [142]. Increased dendritic spine density is observed in frontal, temporal, and parietal lobes of some ASD patients [143, 144], although decreased spine density has also been observed in ASD brains and animal models [145–147]. Genome-wide and homozygosity mappings in patients with autism have identified a deletion in the regulatory region of the RNF8 gene [148] and a homozygous missense mutation in the gene HERC2 [149]. RNF8 and HERC2, as discussed above, act to negatively regulate synapse density during development through a K63-dependent mechanism, and disrupting this mechanism results in learning deficits in mice [89].

De novo

heterozygous loss-of-function mutations of Ubiquitin-specific protease 7 (USP7), which can cleave multiple polyUb linkages including short K48 chains and longer K63 chains [150, 151], have been identified in individuals with intellectual disability and ASD [152]. USP7 directly deubiquitinates K63-polyUb chains from WASH (Wiskott-Aldrich syndrome protein (WASP) and SCAR homolog), an actin-nucleating protein essential in the endosomal recycling pathway, to inhibit WASH activity and maintain proper endosomal actin levels. WASH K63 ubiquitination is promoted by the TRIM27 (tripartite motif containing 27)/RNF76 E3 ligase and its regulator melanoma antigen gene L2 (MAGEL2) [152, 153], mutations of which are associated with Prader-Willi syndrome, Schaaf-Yang syndrome, and ASD [154–156]. In addition to buffering WASH K63 ubiquitination, USP7 also deubiquitinates TRIM27 and protects it from auto-ubiquitination and proteasome

degradation. Thus, USP7 appears to act as a “molecular rheostat” to regulate WASH-dependent endosomal actin assembly and protein recycling via both K48 and K63 deubiquitination important in ASD pathogenesis [152].

Schizophrenia.

Schizophrenia is a severe mental illness that affects ~1% of the population worldwide, and is characterized by episodic positive symptoms (delusions, hallucinations, paranoia, and psychosis) and/or persistent negative symptoms (avolition, flat affect, social withdrawal) and cognitive impairments [157]. There is an overall reduction of protein ubiquitination, free ubiquitin, and K48-polyUb, but increased K63-polyUb in the superior temporal gyrus of schizophrenia subjects [158]. Ubiquitin E1 activating enzyme UBA6 and Nedd4 are also decreased, whereas the E2 ubiquitin conjugating enzyme UBE2K is unaltered. Given the important roles of Nedd4 in AMPAR ubiquitination and trafficking, a decreased Nedd4 level may contribute to glutamate hypofunction in schizophrenia. Single nucleotide polymorphisms in the Nedd4 gene have been associated with schizophrenia [159].

Alzheimer’s disease.

AD is the most common neurodegenerative disease, characterized by progressive decline in memory, thinking ability, and cognitive functions. Neuropathological hallmarks of AD include intracellular, filamentous aggregates mainly consisting of hyperphosphorylated Tau (neurofibrillary tangles) and extracellular plaques enriched with Amyloid-beta ($A\beta$; amyloid plaques) [160–162]. $A\beta$, known to have numerous deleterious effects on neurons and synapses [163, 164], is a peptide product generated from the cleavage of the Amyloid Precursor Protein (APP) by the β - (BACE) and γ - (presenilin) secretases after it exits the secretory pathway and reaches the cell surface [165–167]. APP is K63-ubiquitinated and sequestered early in the secretory pathway, primarily within the Golgi apparatus, delaying its maturation and subsequent proteolytic processing by secretases [168]. Ubiquilin-1, a ubiquitin-like molecular chaperone, stimulates this process [168]. Single nucleotide polymorphisms in the Ubiquilin-1 gene have been linked to late-onset AD [169, 170] and Ubiquilin-1 levels are significantly decreased in late-onset AD patient brains [171]. Thus, although mutations in APP and the secretase enzymes often result in familial early-onset AD [164, 166], K63-polyUb may have a role in the pathogenesis of more common sporadic, genetically complex late-onset AD by regulating APP maturation, trafficking, and processing. Finally, presenilins can be K63-ubiquitinated by TRAF6, which does not appear to affect the γ -secretase enzyme activity but increases the levels and Ca^{2+} signaling properties of presenilins in heterologous cells [172]. Presenilin-1 also possesses a ubiquitin-binding CUE (coupling of ubiquitin to ER degradation) domain and can mediate a non-covalent binding to K63 chains [173]. The role of presenilin conjugation or binding by K63-polyUb in AD, if any, is currently unknown.

Frontotemporal Dementia (FTD).

FTD, the second most common cause of dementia after AD and the leading dementia before the age of 65, is caused by degeneration of prefrontal and/or anterior temporal cortex that leads to changes in personality, emotional blunting, disinhibition, and language disability [174–176]. FTD overlaps clinically, pathologically, and genetically with the motor

neuron disease amyotrophic lateral sclerosis (ALS) [177]. TDP-43 (transactive response-DNA binding protein-43) is the major pathologic component of tau-negative and ubiquitin-positive inclusions in ~ 50% of FTD patients [175]. TDP-43 binds noncoding RNAs, introns, and mRNAs and regulates RNA splicing, trafficking, and expression of thousands of genes including Parkin [178, 179]. TDP-43 is predominately nuclear, but in FTD/ALS, is translocated to the cytoplasm where hyperphosphorylated, ubiquitinated, and truncated TDP-43 is accumulated. It is currently unclear how cytosolic TDP-43 accumulation leads to pathogenesis, though it is hypothesized that it is mediated either by a toxic gain of function by aggregated TDP-43, or a loss of function by normal TDP-43 [180–182]. TDP-43 is K63-ubiquitinated by Parkin, which does not seem to alter TDP-43 levels via autophagy process. Rather, Parkin forms a multiprotein complex with cytoplasmic HDAC6 (histone deacetylase 6) and TDP-43 that promotes TDP-43 translocation to and accumulation at the cytoplasm [183]. Furthermore, a mutation in the *CYLD* gene leading to increased K63-deubiquitinase activity of the protein is identified in FTD patients [105]. This mutant, when expressed in primary mouse neurons, results in increased cytoplasmic localization of TDP-43. This, in combination with *CYLD*'s interaction with proteins encoded by several FTD-ALS linked genes including *TBK1* [184, 185], *OPTN* [186, 187] and *SQSTM1* [188, 189], strongly suggest that *CYLD* has a causative role in the pathogenesis of FTD/ALS [105].

Parkinson's disease.

PD, the second most common neurodegenerative disease, is characterized by the loss of dopaminergic neurons in the substantia nigra and the presence of intracellular inclusions termed Lewy bodies (LBs). The major constituent of LBs is misfolded α -synuclein [190], an abundant lipid-binding presynaptic protein often conjugated with K63-polyUb [191]. Nedd4 has been shown to serve as an E3 for α -synuclein ubiquitination and facilitate its targeting to the endosomal-lysosomal pathway for degradation, promoting clearance of inclusions [192, 193]. Independently, unbiased screens in yeast identified Nedd4 and related protein network as druggable targets that ameliorate α -synuclein toxicity in human neurons [194, 195]. The deubiquitinase USP8, also present in LBs, removes K63-polyUb chains from α -synuclein and regulates its clearance and modifies its toxicity in PD [191]. These studies suggest that K63-linked ubiquitination of α -synuclein by Nedd4/USP8 represents an important mechanism in PD pathogenesis.

Concluding Remarks

The functional significance of nonproteolytic ubiquitination in the CNS is steadily emerging, and many fundamental questions need to be answered. Do other atypical polyUb chains have a role in neurons and synapses? How do different polyUb linkages coordinate to regulate activity-dependent assembly and disassembly of the PSD during synapse remodeling and plasticity underlying behavior? What are the precise molecular mechanisms by which K63-polyUb regulates each step of AMPAR trafficking and recycling? What are other synaptic substrates that undergo nonproteolytic ubiquitination and what are their roles? Answering these and other important questions will require the development of new tools for monitoring, manipulating, and characterizing different polyUb chains in the synapse and other specific neuronal compartments.

The brain was initially thought to be immunologically privileged, but it has become abundantly clear that many immune molecules are expressed in neurons and neural-immune interactions profoundly impact synaptic development, function, and plasticity [196–198]. Importantly, abnormal immune activation in the brain, causing abnormal neural circuit rewiring and/or damage to neuronal integrity, is a common pathology across neurodevelopmental and neurodegenerative disorders [199, 200]. Given the essential roles of nonproteolytic ubiquitination in innate and adaptive immune signaling, elucidating how these ubiquitin linkages regulate neural-immune interplays to mediate synapse remodeling in normal and disease states undoubtedly represents a fundamental endeavor.

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References

1. Hershko A and Ciechanover A, The ubiquitin system. *Annu Rev Biochem*, 1998. 67: p. 425–79. [PubMed: 9759494]
2. Husnjak K and Dikic I, Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. *Annu Rev Biochem*, 2012. 81: p. 291–322. [PubMed: 22482907]
3. Ikeda F, Linear ubiquitination signals in adaptive immune responses. *Immunol Rev*, 2015. 266(1): p. 222–36. [PubMed: 26085218]
4. Kulathu Y and Komander D, Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol*, 2012. 13(8): p. 508–23. [PubMed: 22820888]
5. DiAntonio A and Hicke L, Ubiquitin-dependent regulation of the synapse. *Annu Rev Neurosci*, 2004. 27: p. 223–46. [PubMed: 15217332]
6. Tai HC and Schuman EM, Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction. *Nat Rev Neurosci*, 2008. 9(11): p. 826–38. [PubMed: 18931696]
7. Mabb AM and Ehlers MD, Ubiquitination in postsynaptic function and plasticity. *Annu Rev Cell Dev Biol*, 2010. 26: p. 179–210. [PubMed: 20604708]
8. Bingol B and Sheng M, Deconstruction for reconstruction: the role of proteolysis in neural plasticity and disease. *Neuron*, 2011. 69(1): p. 22–32. [PubMed: 21220096]
9. George AJ, Hoffiz YC, Charles AJ, Zhu Y, and Mabb AM, A Comprehensive Atlas of E3 Ubiquitin Ligase Mutations in Neurological Disorders. *Front Genet*, 2018. 9: p. 29. [PubMed: 29491882]
10. Osinalde N, Duarri A, Ramirez J, Barrio R, Perez de Nanclares G, and Mayor U, Impaired proteostasis in rare neurological diseases. *Semin Cell Dev Biol*, 2019. 93: p. 164–177. [PubMed: 30355526]
11. Chin LS, Olzmann JA, and Li L, Parkin-mediated ubiquitin signalling in aggresome formation and autophagy. *Biochem Soc Trans*, 2010. 38(Pt 1): p. 144–9. [PubMed: 20074049]
12. Lim KL and Lim GG, K63-linked ubiquitination and neurodegeneration. *Neurobiol Dis*, 2011. 43(1): p. 9–16. [PubMed: 20696248]
13. Pickrell AM and Youle RJ, The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron*, 2015. 85(2): p. 257–73. [PubMed: 25611507]
14. Khaminets A, Behl C, and Dikic I, Ubiquitin-Dependent And Independent Signals In Selective Autophagy. *Trends Cell Biol*, 2016. 26(1): p. 6–16. [PubMed: 26437584]
15. Finley D, Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem*, 2009. 78: p. 477–513. [PubMed: 19489727]
16. Na CH, Jones DR, Yang Y, Wang X, Xu Y, and Peng J, Synaptic protein ubiquitination in rat brain revealed by antibody-based ubiquitome analysis. *J Proteome Res*, 2012. 11(9): p. 4722–32. [PubMed: 22871113]

17. Mukhopadhyay D and Riezman H, Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science*, 2007. 315(5809): p. 201–5. [PubMed: 17218518]
18. Chen ZJ and Sun LJ, Nonproteolytic functions of ubiquitin in cell signaling. *Mol Cell*, 2009. 33(3): p. 275–86. [PubMed: 19217402]
19. Hrdinka M and Gyrd-Hansen M, The Met1-Linked Ubiquitin Machinery: Emerging Themes of (De)regulation. *Mol Cell*, 2017. 68(2): p. 265–280. [PubMed: 29053955]
20. Swatek KN and Komander D, Ubiquitin modifications. *Cell Res*, 2016. 26(4): p. 399–422. [PubMed: 27012465]
21. Matsumoto ML, Wickliffe KE, Dong KC, Yu C, Bosanac I, Bustos D et al. , K11-linked polyubiquitination in cell cycle control revealed by a K11 linkage-specific antibody. *Mol Cell*, 2010. 39(3): p. 477–84. [PubMed: 20655260]
22. Wickliffe KE, Williamson A, Meyer HJ, Kelly A, and Rape M, K11-linked ubiquitin chains as novel regulators of cell division. *Trends Cell Biol*, 2011. 21(11): p. 656–63. [PubMed: 21978762]
23. Xu P, Duong DM, Seyfried NT, Cheng D, Xie Y, Robert J et al. , Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell*, 2009. 137(1): p. 133–45. [PubMed: 19345192]
24. Dynek JN, Goncharov T, Dueber EC, Fedorova AV, Izrael-Tomasevic A, Phu L et al. , c-IAP1 and UbcH5 promote K11-linked polyubiquitination of RIP1 in TNF signalling. *EMBO J*, 2010. 29(24): p. 4198–209. [PubMed: 21113135]
25. Jin J, Xie X, Xiao Y, Hu H, Zou Q, Cheng X et al. , Epigenetic regulation of the expression of IL12 and IL23 and autoimmune inflammation by the deubiquitinase TRABID. *Nat Immunol*, 2016. 17(3): p. 259–68. [PubMed: 26808229]
26. Yuan WC, Lee YR, Lin SY, Chang LY, Tan YP, Hung CC et al. , K33-Linked Polyubiquitination of Coronin 7 by Cul3-KLHL20 Ubiquitin E3 Ligase Regulates Protein Trafficking. *Mol Cell*, 2014. 54(4): p. 586–600. [PubMed: 24768539]
27. Nakasone MA, Livnat-Levanon N, Glickman MH, Cohen RE, and Fushman D, Mixed-linkage ubiquitin chains send mixed messages. *Structure*, 2013. 21(5): p. 727–40. [PubMed: 23562397]
28. Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A et al. , Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. *PLoS One*, 2008. 3(1): p. e1487. [PubMed: 18213395]
29. Stewart MD, Ritterhoff T, Klevit RE, and Brzovic PS, E2 enzymes: more than just middle men. *Cell Res*, 2016. 26(4): p. 423–40. [PubMed: 27002219]
30. Nagy V and Dikic I, Ubiquitin ligase complexes: from substrate selectivity to conjugational specificity. *Biol Chem*, 2010. 391(2–3): p. 163–9. [PubMed: 20030582]
31. Berndsen CE and Wolberger C, New insights into ubiquitin E3 ligase mechanism. *Nat Struct Mol Biol*, 2014. 21(4): p. 301–7. [PubMed: 24699078]
32. Maspero E, Valentini E, Mari S, Cecatiello V, Soffientini P, Pasqualato S et al. , Structure of a ubiquitin-loaded HECT ligase reveals the molecular basis for catalytic priming. *Nat Struct Mol Biol*, 2013. 20(6): p. 696–701. [PubMed: 23644597]
33. Deng L, Wang C, Spencer E, Yang L, Braun A, You J et al. , Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell*, 2000. 103(2): p. 351–61. [PubMed: 11057907]
34. Deshaies RJ and Joazeiro CA, RING domain E3 ubiquitin ligases. *Annu Rev Biochem*, 2009. 78: p. 399–434. [PubMed: 19489725]
35. Koegl M, Hoppe T, Schlenker S, Ulrich HD, Mayer TU, and Jentsch S, A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell*, 1999. 96(5): p. 635–44. [PubMed: 10089879]
36. Wenzel DM, Lissounov A, Brzovic PS, and Klevit RE, UBC7 reactivity profile reveals parkin and HHARI to be RING/HECT hybrids. *Nature*, 2011. 474(7349): p. 105–8. [PubMed: 21532592]
37. Spratt DE, Walden H, and Shaw GS, RBR E3 ubiquitin ligases: new structures, new insights, new questions. *Biochem J*, 2014. 458(3): p. 421–37. [PubMed: 24576094]
38. Mevissen TET and Komander D, Mechanisms of Deubiquitinase Specificity and Regulation. *Annu Rev Biochem*, 2017. 86: p. 159–192. [PubMed: 28498721]

39. Clague MJ, Barsukov I, Coulson JM, Liu H, Rigden DJ, and Urbe S, Deubiquitylases from genes to organism. *Physiol Rev*, 2013. 93(3): p. 1289–315. [PubMed: 23899565]
40. Reyes-Turcu FE, Ventii KH, and Wilkinson KD, Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem*, 2009. 78: p. 363–97. [PubMed: 19489724]
41. Komander D, Clague MJ, and Urbe S, Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol*, 2009. 10(8): p. 550–63. [PubMed: 19626045]
42. Komander D, Lord CJ, Scheel H, Swift S, Hofmann K, Ashworth A et al. , The structure of the CYLD USP domain explains its specificity for Lys63-linked polyubiquitin and reveals a B box module. *Mol Cell*, 2008. 29(4): p. 451–64. [PubMed: 18313383]
43. Komander D, Reyes-Turcu F, Licchesi JD, Odenwaelder P, Wilkinson KD, and Barford D, Molecular discrimination of structurally equivalent Lys 63-linked and linear polyubiquitin chains. *EMBO Rep*, 2009. 10(5): p. 466–73. [PubMed: 19373254]
44. Mevissen TE, Hospenthal MK, Geurink PP, Elliott PR, Akutsu M, Arnaudo N et al. , OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell*, 2013. 154(1): p. 169–84. [PubMed: 23827681]
45. Komander D and Rape M, The ubiquitin code. *Annu Rev Biochem*, 2012. 81: p. 203–29. [PubMed: 22524316]
46. Haglund K and Dikic I, Ubiquitylation and cell signaling. *EMBO J*, 2005. 24(19): p. 3353–9. [PubMed: 16148945]
47. Chen J and Chen ZJ, Regulation of NF-kappaB by ubiquitination. *Curr Opin Immunol*, 2013. 25(1): p. 4–12. [PubMed: 23312890]
48. Chiang C and Gack MU, Post-translational Control of Intracellular Pathogen Sensing Pathways. *Trends Immunol*, 2017. 38(1): p. 39–52. [PubMed: 27863906]
49. Maniatis T, A ubiquitin ligase complex essential for the NF-kappaB, Wnt/Wingless, and Hedgehog signaling pathways. *Genes Dev*, 1999. 13(5): p. 505–10. [PubMed: 10072378]
50. Ma A and Malynn BA, A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol*, 2012. 12(11): p. 774–85. [PubMed: 23059429]
51. Hislop JN and von Zastrow M, Role of ubiquitination in endocytic trafficking of G-protein-coupled receptors. *Traffic*, 2011. 12(2): p. 137–48. [PubMed: 20854416]
52. Haglund K and Dikic I, The role of ubiquitylation in receptor endocytosis and endosomal sorting. *J Cell Sci*, 2012. 125(Pt 2): p. 265–75. [PubMed: 22357968]
53. McCullough J, Clague MJ, and Urbe S, AMSH is an endosome-associated ubiquitin isopeptidase. *J Cell Biol*, 2004. 166(4): p. 487–92. [PubMed: 15314065]
54. Mizuno E, Iura T, Mukai A, Yoshimori T, Kitamura N, and Komada M, Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. *Mol Biol Cell*, 2005. 16(11): p. 5163–74. [PubMed: 16120644]
55. Piper RC, Dikic I, and Lukacs GL, Ubiquitin-dependent sorting in endocytosis. *Cold Spring Harb Perspect Biol*, 2014. 6(1).
56. Bergink S and Jentsch S, Principles of ubiquitin and SUMO modifications in DNA repair. *Nature*, 2009. 458(7237): p. 461–7. [PubMed: 19325626]
57. Ulrich HD and Walden H, Ubiquitin signalling in DNA replication and repair. *Nat Rev Mol Cell Biol*, 2010. 11(7): p. 479–89. [PubMed: 20551964]
58. Gatti M, Pinato S, Maiolica A, Rocchio F, Prato MG, Aebersold R et al. , RNF168 promotes noncanonical K27 ubiquitination to signal DNA damage. *Cell Rep*, 2015. 10(2): p. 226–38. [PubMed: 25578731]
59. Uckelmann M and Sixma TK, Histone ubiquitination in the DNA damage response. *DNA Repair (Amst)*, 2017. 56: p. 92–101. [PubMed: 28624371]
60. Kalb R, Mallery DL, Larkin C, Huang JT, and Hiom K, BRCA1 is a histone-H2A-specific ubiquitin ligase. *Cell Rep*, 2014. 8(4): p. 999–1005. [PubMed: 25131202]
61. Poo MM, Neurotrophins as synaptic modulators. *Nat Rev Neurosci*, 2001. 2(1): p. 24–32. [PubMed: 11253356]
62. Huang EJ and Reichardt LF, Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem*, 2003. 72: p. 609–42. [PubMed: 12676795]

63. Geetha T, Jiang J, and Wooten MW, Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling. *Mol Cell*, 2005. 20(2): p. 301–12. [PubMed: 16246731]
64. Jadhav T, Geetha T, Jiang J, and Wooten MW, Identification of a consensus site for TRAF6/p62 polyubiquitination. *Biochem Biophys Res Commun*, 2008. 371(3): p. 521–4. [PubMed: 18457658]
65. Khursigara G, Orlinick JR, and Chao MV, Association of the p75 neurotrophin receptor with TRAF6. *J Biol Chem*, 1999. 274(5): p. 2597–600. [PubMed: 9915784]
66. Geetha T, Zheng C, Unroe B, Sycheva M, Kluess H, and Babu JR, Polyubiquitination of the neurotrophin receptor p75 directs neuronal cell survival. *Biochem Biophys Res Commun*, 2012. 421(2): p. 286–90. [PubMed: 22503986]
67. Wooten MW, Seibenhener ML, Mamidipudi V, Diaz-Meco MT, Barker PA, and Moscat J, The atypical protein kinase C-interacting protein p62 is a scaffold for NF-kappaB activation by nerve growth factor. *J Biol Chem*, 2001. 276(11): p. 7709–12. [PubMed: 11244088]
68. Mamidipudi V, Li X, and Wooten MW, Identification of interleukin 1 receptor-associated kinase as a conserved component in the p75-neurotrophin receptor activation of nuclear factor-kappa B. *J Biol Chem*, 2002. 277(31): p. 28010–8. [PubMed: 12034707]
69. Gentry JJ, Rutkoski NJ, Burke TL, and Carter BD, A functional interaction between the p75 neurotrophin receptor interacting factors, TRAF6 and NRIF. *J Biol Chem*, 2004. 279(16): p. 16646–56. [PubMed: 14960584]
70. Geetha T, Kenchappa RS, Wooten MW, and Carter BD, TRAF6-mediated ubiquitination regulates nuclear translocation of NRIF, the p75 receptor interactor. *EMBO J*, 2005. 24(22): p. 3859–68. [PubMed: 16252010]
71. Kisiswa L, Fernandez-Suarez D, Sergaki MC, and Ibanez CF, RIP2 Gates TRAF6 Interaction with Death Receptor p75(NTR) to Regulate Cerebellar Granule Neuron Survival. *Cell Rep*, 2018. 24(4): p. 1013–1024. [PubMed: 30044969]
72. Geetha T and Wooten MW, Association of the atypical protein kinase C-interacting protein p62/ZIP with nerve growth factor receptor TrkA regulates receptor trafficking and Erk5 signaling. *J Biol Chem*, 2003. 278(7): p. 4730–9. [PubMed: 12471037]
73. Yeiser EC, Rutkoski NJ, Naito A, Inoue J, and Carter BD, Neurotrophin signaling through the p75 receptor is deficient in *traf6*^{-/-} mice. *J Neurosci*, 2004. 24(46): p. 10521–9. [PubMed: 15548667]
74. Ou CY, Lin YF, Chen YJ, and Chien CT, Distinct protein degradation mechanisms mediated by Cul1 and Cul3 controlling Ci stability in *Drosophila* eye development. *Genes Dev*, 2002. 16(18): p. 2403–14. [PubMed: 12231629]
75. Zhang Z, Lv X, Yin WC, Zhang X, Feng J, Wu W et al. , Ter94 ATPase complex targets k11-linked ubiquitinated ci to proteasomes for partial degradation. *Dev Cell*, 2013. 25(6): p. 636–44. [PubMed: 23747190]
76. Tempe D, Casas M, Karaz S, Blanchet-Tournier MF, and Concordet JP, Multisite protein kinase A and glycogen synthase kinase 3beta phosphorylation leads to Gli3 ubiquitination by SCFbetaTrCP. *Mol Cell Biol*, 2006. 26(11): p. 4316–26. [PubMed: 16705181]
77. Wang B and Li Y, Evidence for the direct involvement of {beta}TrCP in Gli3 protein processing. *Proc Natl Acad Sci U S A*, 2006. 103(1): p. 33–8. [PubMed: 16371461]
78. Ma P, Song NN, Li Y, Zhang Q, Zhang L, Zhang L et al. , Fine-Tuning of Shh/Gli Signaling Gradient by Non-proteolytic Ubiquitination during Neural Patterning. *Cell Rep*, 2019. 28(2): p. 541–553 e4. [PubMed: 31291587]
79. Ginty DD and Segal RA, Retrograde neurotrophin signaling: Trk-ing along the axon. *Curr Opin Neurobiol*, 2002. 12(3): p. 268–74. [PubMed: 12049932]
80. Howe CL and Mobley WC, Long-distance retrograde neurotrophic signaling. *Curr Opin Neurobiol*, 2005. 15(1): p. 40–8. [PubMed: 15721743]
81. Zhou X, Babu JR, da Silva S, Shu Q, Graef IA, Oliver T et al. , Unc-51-like kinase 1/2-mediated endocytic processes regulate filopodia extension and branching of sensory axons. *Proc Natl Acad Sci U S A*, 2007. 104(14): p. 5842–7. [PubMed: 17389358]
82. Wang B, Iyengar R, Li-Harms X, Joo JH, Wright C, Lavado A et al. , The autophagy-inducing kinases, ULK1 and ULK2, regulate axon guidance in the developing mouse forebrain via a noncanonical pathway. *Autophagy*, 2018. 14(5): p. 796–811. [PubMed: 29099309]

83. Wang PY, Lin YM, Wang LH, Kuo TY, Cheng SJ, and Wang GS, Reduced cytoplasmic MBNL1 is an early event in a brain-specific mouse model of myotonic dystrophy. *Hum Mol Genet*, 2017. 26(12): p. 2247–2257. [PubMed: 28369378]
84. Wang PY, Chang KT, Lin YM, Kuo TY, and Wang GS, Ubiquitination of MBNL1 Is Required for Its Cytoplasmic Localization and Function in Promoting Neurite Outgrowth. *Cell Rep*, 2018. 22(9): p. 2294–2306. [PubMed: 29490267]
85. Melom JE and Littleton JT, Synapse development in health and disease. *Curr Opin Genet Dev*, 2011. 21(3): p. 256–61. [PubMed: 21277192]
86. Morgan JR, Comstra HS, Cohen M, and Faundez V, Presynaptic membrane retrieval and endosome biology: defining molecularly heterogeneous synaptic vesicles. *Cold Spring Harb Perspect Biol*, 2013. 5(10): p. a016915. [PubMed: 24086045]
87. Chanaday NL, Cousin MA, Milosevic I, Watanabe S, and Morgan JR, The Synaptic Vesicle Cycle Revisited: New Insights into the Modes and Mechanisms. *J Neurosci*, 2019. 39(42): p. 8209–8216. [PubMed: 31619489]
88. Hall EA, Nahorski MS, Murray LM, Shaheen R, Perkins E, Dissanayake KN et al. , PLAA Mutations Cause a Lethal Infantile Epileptic Encephalopathy by Disrupting Ubiquitin-Mediated Endolysosomal Degradation of Synaptic Proteins. *Am J Hum Genet*, 2017. 100(5): p. 706–724. [PubMed: 28413018]
89. Valnegri P, Huang J, Yamada T, Yang Y, Mejia LA, Cho HY et al. , RNF8/UBC13 ubiquitin signaling suppresses synapse formation in the mammalian brain. *Nat Commun*, 2017. 8(1): p. 1271. [PubMed: 29097665]
90. Niswender CM and Conn PJ, Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu Rev Pharmacol Toxicol*, 2010. 50: p. 295–322. [PubMed: 20055706]
91. Lee S, Park S, Lee H, Han S, Song JM, Han D et al. , Nedd4 E3 ligase and beta-arrestins regulate ubiquitination, trafficking, and stability of the mGlu7 receptor. *Elife*, 2019. 8.
92. Pelkey KA, Lavezzari G, Racca C, Roche KW, and McBain CJ, mGluR7 is a metaplastic switch controlling bidirectional plasticity of feedforward inhibition. *Neuron*, 2005. 46(1): p. 89–102. [PubMed: 15820696]
93. Ma Q, Ruan H, Peng L, Zhang M, Gack MU, and Yao WD, Proteasome-independent polyubiquitin linkage regulates synapse scaffolding, efficacy, and plasticity. *Proc Natl Acad Sci U S A*, 2017. 114(41): p. E8760–E8769. [PubMed: 28973854]
94. Fernandez E, Collins MO, Uren RT, Kopanitsa MV, Komiyama NH, Croning MD et al. , Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. *Mol Syst Biol*, 2009. 5: p. 269. [PubMed: 19455133]
95. Mei S, Ruan H, Ma Q, and Yao WD, The ubiquitin-editing enzyme A20 regulates synapse remodeling and efficacy. *Brain Res*, 2020. 1727: p. 146569. [PubMed: 31783001]
96. Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, and Baltimore D, NF-kappa B functions in synaptic signaling and behavior. *Nat Neurosci*, 2003. 6(10): p. 1072–8. [PubMed: 12947408]
97. Meffert MK and Baltimore D, Physiological functions for brain NF-kappaB. *Trends Neurosci*, 2005. 28(1): p. 37–43. [PubMed: 15626495]
98. Kaltschmidt B and Kaltschmidt C, NF-kappaB in the nervous system. *Cold Spring Harb Perspect Biol*, 2009. 1(3): p. a001271. [PubMed: 20066105]
99. Bignell GR, Warren W, Seal S, Takahashi M, Rapley E, Barfoot R et al. , Identification of the familial cylindromatosis tumour-suppressor gene. *Nat Genet*, 2000. 25(2): p. 160–5. [PubMed: 10835629]
100. Dosemeci A, Thein S, Yang Y, Reese TS, and Tao-Cheng JH, CYLD, a deubiquitinase specific for lysine63-linked polyubiquitins, accumulates at the postsynaptic density in an activity-dependent manner. *Biochem Biophys Res Commun*, 2013. 430(1): p. 245–9. [PubMed: 23146630]
101. Thein S, Pham A, Bayer KU, Tao-Cheng JH, and Dosemeci A, IKK regulates the deubiquitinase CYLD at the postsynaptic density. *Biochem Biophys Res Commun*, 2014. 450(1): p. 550–4. [PubMed: 24928390]
102. Thein S, Tao-Cheng JH, Li Y, Bayer KU, Reese TS, and Dosemeci A, CaMKII mediates recruitment and activation of the deubiquitinase CYLD at the postsynaptic density. *PLoS One*, 2014. 9(3): p. e91312. [PubMed: 24614225]

103. Wooten MW, Geetha T, Babu JR, Seibenhener ML, Peng J, Cox N et al. , Essential role of sequestosome 1/p62 in regulating accumulation of Lys63-ubiquitinated proteins. *J Biol Chem*, 2008. 283(11): p. 6783–9. [PubMed: 18174161]
104. Jin C, Kim S, Kang H, Yun KN, Lee Y, Zhang Y et al. , Shank3 regulates striatal synaptic abundance of Cyld, a deubiquitinase specific for Lys63-linked polyubiquitin chains. *J Neurochem*, 2019. 150(6): p. 776–786. [PubMed: 31215654]
105. Dobson-Stone C, Hallupp M, Shahheydari H, Ragagnin AMG, Chatterton Z, Carew-Jones F et al. , CYLD is a causative gene for frontotemporal dementia - amyotrophic lateral sclerosis. *Brain*, 2020.
106. Zhang J, Chen M, Li B, Lv B, Jin K, Zheng S et al. , Altered striatal rhythmic activity in cylindromatosis knock-out mice due to enhanced GABAergic inhibition. *Neuropharmacology*, 2016. 110(Pt A): p. 260–267. [PubMed: 27342122]
107. Kim E and Sheng M, PDZ domain proteins of synapses. *Nat Rev Neurosci*, 2004. 5(10): p. 771–81. [PubMed: 15378037]
108. Ting JT, Peca J, and Feng G, Functional consequences of mutations in postsynaptic scaffolding proteins and relevance to psychiatric disorders. *Annu Rev Neurosci*, 2012. 35: p. 49–71. [PubMed: 22540979]
109. Hart AC, Sims S, and Kaplan JM, Synaptic code for sensory modalities revealed by *C. elegans* GLR-1 glutamate receptor. *Nature*, 1995. 378(6552): p. 82–5. [PubMed: 7477294]
110. Burbea M, Dreier L, Dittman JS, Grunwald ME, and Kaplan JM, Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. *Neuron*, 2002. 35(1): p. 107–20. [PubMed: 12123612]
111. Kramer LB, Shim J, Previtiera ML, Isack NR, Lee MC, Firestein BL et al. , UEV-1 is an ubiquitin-conjugating enzyme variant that regulates glutamate trafficking in *C. elegans* neurons. *PLoS One*, 2010. 5(12): p. e14291. [PubMed: 21179194]
112. Goo MS, Scudder SL, and Patrick GN, Ubiquitin-dependent trafficking and turnover of ionotropic glutamate receptors. *Front Mol Neurosci*, 2015. 8: p. 60. [PubMed: 26528125]
113. Widagdo J, Guntupalli S, Jang SE, and Anggono V, Regulation of AMPA Receptor Trafficking by Protein Ubiquitination. *Front Mol Neurosci*, 2017. 10: p. 347. [PubMed: 29123470]
114. Widagdo J, Chai YJ, Ridder MC, Chau YQ, Johnson RC, Sah P et al. , Activity-Dependent Ubiquitination of GluA1 and GluA2 Regulates AMPA Receptor Intracellular Sorting and Degradation. *Cell Rep*, 2015. 10(5): p. 783–795. [PubMed: 25660027]
115. Schwarz LA, Hall BJ, and Patrick GN, Activity-dependent ubiquitination of GluA1 mediates a distinct AMPA receptor endocytosis and sorting pathway. *J Neurosci*, 2010. 30(49): p. 16718–29. [PubMed: 21148011]
116. Lussier MP, Nasu-Nishimura Y, and Roche KW, Activity-dependent ubiquitination of the AMPA receptor subunit GluA2. *J Neurosci*, 2011. 31(8): p. 3077–81. [PubMed: 21414928]
117. Lussier MP, Herring BE, Nasu-Nishimura Y, Neutzner A, Karbowski M, Youle RJ et al. , Ubiquitin ligase RNF167 regulates AMPA receptor-mediated synaptic transmission. *Proc Natl Acad Sci U S A*, 2012. 109(47): p. 19426–31. [PubMed: 23129617]
118. Huo Y, Khatri N, Hou Q, Gilbert J, Wang G, and Man HY, The deubiquitinating enzyme USP46 regulates AMPA receptor ubiquitination and trafficking. *J Neurochem*, 2015. 134(6): p. 1067–80. [PubMed: 26077708]
119. Kato A, Rouach N, Nicoll RA, and Bredt DS, Activity-dependent NMDA receptor degradation mediated by retrotranslocation and ubiquitination. *Proc Natl Acad Sci U S A*, 2005. 102(15): p. 5600–5. [PubMed: 15809437]
120. Yuen EY, Wei J, Liu W, Zhong P, Li X, and Yan Z, Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*, 2012. 73(5): p. 962–77. [PubMed: 22405206]
121. Jurd R, Thornton C, Wang J, Luong K, Phamluong K, Kharazia V et al. , Mind bomb-2 is an E3 ligase that ubiquitinates the N-methyl-D-aspartate receptor NR2B subunit in a phosphorylation-dependent manner. *J Biol Chem*, 2008. 283(1): p. 301–10. [PubMed: 17962190]
122. Gascon S, Garcia-Gallo M, Renart J, and Diaz-Guerra M, Endoplasmic reticulum-associated degradation of the NR1 but not the NR2 subunits of the N-methyl-D-aspartate receptor induced

- by inhibition of the N-glycosylation in cortical neurons. *J Neurosci Res*, 2007. 85(8): p. 1713–23. [PubMed: 17455306]
123. Salinas GD, Blair LA, Needleman LA, Gonzales JD, Chen Y, Li M et al. , Actinfilin is a Cul3 substrate adaptor, linking GluR6 kainate receptor subunits to the ubiquitin-proteasome pathway. *J Biol Chem*, 2006. 281(52): p. 40164–73. [PubMed: 17062563]
124. Helton TD, Otsuka T, Lee MC, Mu Y, and Ehlers MD, Pruning and loss of excitatory synapses by the parkin ubiquitin ligase. *Proc Natl Acad Sci U S A*, 2008. 105(49): p. 19492–7. [PubMed: 19033459]
125. Moriyoshi K, Iijima K, Fujii H, Ito H, Cho Y, and Nakanishi S, Seven in absentia homolog 1A mediates ubiquitination and degradation of group I metabotropic glutamate receptors. *Proc Natl Acad Sci U S A*, 2004. 101(23): p. 8614–9. [PubMed: 15163799]
126. Hou Q, Gilbert J, and Man HY, Homeostatic regulation of AMPA receptor trafficking and degradation by light-controlled single-synaptic activation. *Neuron*, 2011. 72(5): p. 806–18. [PubMed: 22153376]
127. Scudder SL, Goo MS, Cartier AE, Molteni A, Schwarz LA, Wright R et al. , Synaptic strength is bidirectionally controlled by opposing activity-dependent regulation of Nedd4–1 and USP8. *J Neurosci*, 2014. 34(50): p. 16637–49. [PubMed: 25505317]
128. Lin A, Hou Q, Jarzylo L, Amato S, Gilbert J, Shang F et al. , Nedd4-mediated AMPA receptor ubiquitination regulates receptor turnover and trafficking. *J Neurochem*, 2011. 119(1): p. 27–39. [PubMed: 21338354]
129. Jewett KA, Zhu J, and Tsai NP, The tumor suppressor p53 guides GluA1 homeostasis through Nedd4–2 during chronic elevation of neuronal activity. *J Neurochem*, 2015. 135(2): p. 226–33. [PubMed: 26250624]
130. Kowalski JR, Dahlberg CL, and Juo P, The deubiquitinating enzyme USP-46 negatively regulates the degradation of glutamate receptors to control their abundance in the ventral nerve cord of *Caenorhabditis elegans*. *J Neurosci*, 2011. 31(4): p. 1341–54. [PubMed: 21273419]
131. Ehlers MD, Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron*, 2000. 28(2): p. 511–25. [PubMed: 11144360]
132. Lee HK, Barbarosie M, Kameyama K, Bear MF, and Huganir RL, Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature*, 2000. 405(6789): p. 955–9. [PubMed: 10879537]
133. Lin A and Man HY, Endocytic adaptor epidermal growth factor receptor substrate 15 (Eps15) is involved in the trafficking of ubiquitinated alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *J Biol Chem*, 2014. 289(35): p. 24652–64. [PubMed: 25023288]
134. Fu AK, Hung KW, Fu WY, Shen C, Chen Y, Xia J et al. , APC(Cdh1) mediates EphA4-dependent downregulation of AMPA receptors in homeostatic plasticity. *Nat Neurosci*, 2011. 14(2): p. 181–9. [PubMed: 21186356]
135. Zhang D, Hou Q, Wang M, Lin A, Jarzylo L, Navis A et al. , Na,K-ATPase activity regulates AMPA receptor turnover through proteasome-mediated proteolysis. *J Neurosci*, 2009. 29(14): p. 4498–511. [PubMed: 19357275]
136. Shehata M, Matsumura H, Okubo-Suzuki R, Ohkawa N, and Inokuchi K, Neuronal stimulation induces autophagy in hippocampal neurons that is involved in AMPA receptor degradation after chemical long-term depression. *J Neurosci*, 2012. 32(30): p. 10413–22. [PubMed: 22836274]
137. Camera D, Coleman HA, Parkington HC, Jenkins TA, Pow DV, Boase N et al. , Learning, memory and long-term potentiation are altered in Nedd4 heterozygous mice. *Behav Brain Res*, 2016. 303: p. 176–81. [PubMed: 26821291]
138. Jiang J, Parameshwaran K, Seibenhener ML, Kang MG, Suppiramaniam V, Huganir RL et al. , AMPA receptor trafficking and synaptic plasticity require SQSTM1/p62. *Hippocampus*, 2009. 19(4): p. 392–406. [PubMed: 19004011]
139. Wozniak DF, Valnegri P, Dearborn JT, Fowler SC, and Bonni A, Conditional knockout of UBC13 produces disturbances in gait and spontaneous locomotion and exploration in mice. *Sci Rep*, 2019. 9(1): p. 4379. [PubMed: 30867488]

140. Orsi SA, Devulapalli RK, Nelsen JL, McFadden T, Surineni R, and Jarome TJ, Distinct subcellular changes in proteasome activity and linkage-specific protein polyubiquitination in the amygdala during the consolidation and reconsolidation of a fear memory. *Neurobiol Learn Mem*, 2019. 157: p. 1–11. [PubMed: 30458285]
141. Lee SH, Choi JH, Lee N, Lee HR, Kim JI, Yu NK et al. , Synaptic protein degradation underlies destabilization of retrieved fear memory. *Science*, 2008. 319(5867): p. 1253–6. [PubMed: 18258863]
142. American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders (5th ed.)*. 2013.
143. Hutsler JJ and Zhang H, Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res*, 2010. 1309: p. 83–94. [PubMed: 19896929]
144. Tang G, Gudsruk K, Kuo SH, Cotrina ML, Rosoklija G, Sosunov A et al. , Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron*, 2014. 83(5): p. 1131–43. [PubMed: 25155956]
145. Williams RS, Hauser SL, Purpura DP, DeLong GR, and Swisher CN, Autism and mental retardation: neuropathologic studies performed in four retarded persons with autistic behavior. *Arch Neurol*, 1980. 37(12): p. 749–53. [PubMed: 7447762]
146. Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN et al. , Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*, 2011. 472(7344): p. 437–42. [PubMed: 21423165]
147. Martinez-Cerdeno V, Dendrite and spine modifications in autism and related neurodevelopmental disorders in patients and animal models. *Dev Neurobiol*, 2017. 77(4): p. 393–404. [PubMed: 27390186]
148. Morrow EM, Yoo SY, Flavell SW, Kim TK, Lin Y, Hill RS et al. , Identifying autism loci and genes by tracing recent shared ancestry. *Science*, 2008. 321(5886): p. 218–23. [PubMed: 18621663]
149. Puffenberger EG, Jinks RN, Wang H, Xin B, Fiorentini C, Sherman EA et al. , A homozygous missense mutation in HERC2 associated with global developmental delay and autism spectrum disorder. *Hum Mutat*, 2012. 33(12): p. 1639–46. [PubMed: 23065719]
150. Nicholson B and Suresh Kumar KG, The multifaceted roles of USP7: new therapeutic opportunities. *Cell Biochem Biophys*, 2011. 60(1–2): p. 61–8. [PubMed: 21468693]
151. Schaefer JB and Morgan DO, Protein-linked ubiquitin chain structure restricts activity of deubiquitinating enzymes. *J Biol Chem*, 2011. 286(52): p. 45186–96. [PubMed: 22072716]
152. Hao YH, Fountain MD Jr., Fon Tacer K, Xia F, Bi W, Kang SH et al. , USP7 Acts as a Molecular Rheostat to Promote WASH-Dependent Endosomal Protein Recycling and Is Mutated in a Human Neurodevelopmental Disorder. *Mol Cell*, 2015. 59(6): p. 956–69. [PubMed: 26365382]
153. Hao YH, Doyle JM, Ramanathan S, Gomez TS, Jia D, Xu M et al. , Regulation of WASH-dependent actin polymerization and protein trafficking by ubiquitination. *Cell*, 2013. 152(5): p. 1051–64. [PubMed: 23452853]
154. Lee S, Kozlov S, Hernandez L, Chamberlain SJ, Brannan CI, Stewart CL et al. , Expression and imprinting of MAGEL2 suggest a role in Prader-willi syndrome and the homologous murine imprinting phenotype. *Hum Mol Genet*, 2000. 9(12): p. 1813–9. [PubMed: 10915770]
155. Schaaf CP, Gonzalez-Garay ML, Xia F, Potocki L, Gripp KW, Zhang B et al. , Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet*, 2013. 45(11): p. 1405–8. [PubMed: 24076603]
156. Boccaccio I, Glatt-Deeley H, Watrin F, Roeckel N, Lalande M, and Muscatelli F, The human MAGEL2 gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. *Hum Mol Genet*, 1999. 8(13): p. 2497–505. [PubMed: 10556298]
157. Lewis DA and Lieberman JA, Catching up on schizophrenia: natural history and neurobiology. *Neuron*, 2000. 28(2): p. 325–34. [PubMed: 11144342]
158. Rubio MD, Wood K, Haroutunian V, and Meador-Woodruff JH, Dysfunction of the ubiquitin proteasome and ubiquitin-like systems in schizophrenia. *Neuropsychopharmacology*, 2013. 38(10): p. 1910–20. [PubMed: 23571678]

159. Bi X, Cui K, Han C, Sun M, Wang L, Yang L et al. . [Association of NEDD4 gene polymorphisms with schizophrenia and its clinical characteristics in Chinese Han population]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, 2015. 32(3): p. 385–90. [PubMed: 26037357]
160. Glenner GG, Wong CW, Quaranta V, and Eanes ED, The amyloid deposits in Alzheimer's disease: their nature and pathogenesis. *Appl Pathol*, 1984. 2(6): p. 357–69. [PubMed: 6242724]
161. Goedert M, Wischik CM, Crowther RA, Walker JE, and Klug A, Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proc Natl Acad Sci U S A*, 1988. 85(11): p. 4051–5. [PubMed: 3131773]
162. Wischik CM, Novak M, Edwards PC, Klug A, Tichelaar W, and Crowther RA, Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci U S A*, 1988. 85(13): p. 4884–8. [PubMed: 2455299]
163. Mucke L and Selkoe DJ, Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med*, 2012. 2(7): p. a006338. [PubMed: 22762015]
164. Selkoe DJ and Hardy J, The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*, 2016. 8(6): p. 595–608. [PubMed: 27025652]
165. Selkoe DJ, Yamazaki T, Citron M, Podlisny MB, Koo EH, Teplow DB et al. . The role of APP processing and trafficking pathways in the formation of amyloid beta-protein. *Ann N Y Acad Sci*, 1996. 777: p. 57–64. [PubMed: 8624127]
166. Small SA and Gandy S, Sorting through the cell biology of Alzheimer's disease: intracellular pathways to pathogenesis. *Neuron*, 2006. 52(1): p. 15–31. [PubMed: 17015224]
167. Suzuki T, Araki Y, Yamamoto T, and Nakaya T, Trafficking of Alzheimer's disease-related membrane proteins and its participation in disease pathogenesis. *J Biochem*, 2006. 139(6): p. 949–55. [PubMed: 16788045]
168. El Ayadi A, Stieren ES, Barral JM, and Boehning D, Ubiquilin-1 regulates amyloid precursor protein maturation and degradation by stimulating K63-linked polyubiquitination of lysine 688. *Proc Natl Acad Sci U S A*, 2012. 109(33): p. 13416–21. [PubMed: 22847417]
169. Bertram L, Hiltunen M, Parkinson M, Ingelsson M, Lange C, Ramasamy K et al. . Family-based association between Alzheimer's disease and variants in UBQLN1. *N Engl J Med*, 2005. 352(9): p. 884–94. [PubMed: 15745979]
170. Kamboh MI, Minster RL, Feingold E, and DeKosky ST, Genetic association of ubiquilin with Alzheimer's disease and related quantitative measures. *Mol Psychiatry*, 2006. 11(3): p. 273–9. [PubMed: 16302009]
171. Stieren ES, El Ayadi A, Xiao Y, Siller E, Landsverk ML, Oberhauser AF et al. . Ubiquilin-1 is a molecular chaperone for the amyloid precursor protein. *J Biol Chem*, 2011. 286(41): p. 35689–98. [PubMed: 21852239]
172. Yan R, Farrelly S, and McCarthy JV, Presenilins are novel substrates for TRAF6-mediated ubiquitination. *Cell Signal*, 2013. 25(9): p. 1769–79. [PubMed: 23707529]
173. Duggan SP, Yan R, and McCarthy JV, A ubiquitin-binding CUE domain in presenilin-1 enables interaction with K63-linked polyubiquitin chains. *FEBS Lett*, 2015. 589(9): p. 1001–8. [PubMed: 25796185]
174. Neary D, Snowden J, and Mann D, Frontotemporal dementia. *Lancet Neurol*, 2005. 4(11): p. 771–80. [PubMed: 16239184]
175. Goedert M, Ghetti B, and Spillantini MG, Frontotemporal dementia: implications for understanding Alzheimer disease. *Cold Spring Harb Perspect Med*, 2012. 2(2): p. a006254. [PubMed: 22355793]
176. Olney NT, Spina S, and Miller BL, Frontotemporal Dementia. *Neurol Clin*, 2017. 35(2): p. 339–374. [PubMed: 28410663]
177. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W et al. . Amyotrophic lateral sclerosis. *Nat Rev Dis Primers*, 2017. 3: p. 17071. [PubMed: 28980624]
178. Polymenidou M, Lagier-Tourenne C, Hutt KR, Huelga SC, Moran J, Liang TY et al. . Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nat Neurosci*, 2011. 14(4): p. 459–68. [PubMed: 21358643]

179. Tollervey JR, Curk T, Rogelj B, Briese M, Cereda M, Kayikci M et al. , Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nat Neurosci*, 2011. 14(4): p. 452–8. [PubMed: 21358640]
180. Janssens J and Van Broeckhoven C, Pathological mechanisms underlying TDP-43 driven neurodegeneration in FTL-ALS spectrum disorders. *Hum Mol Genet*, 2013. 22(R1): p. R77–87. [PubMed: 23900071]
181. Kim HJ and Taylor JP, Lost in Transportation: Nucleocytoplasmic Transport Defects in ALS and Other Neurodegenerative Diseases. *Neuron*, 2017. 96(2): p. 285–297. [PubMed: 29024655]
182. Gao FB, Almeida S, and Lopez-Gonzalez R, Dysregulated molecular pathways in amyotrophic lateral sclerosis-frontotemporal dementia spectrum disorder. *EMBO J*, 2017. 36(20): p. 2931–2950. [PubMed: 28916614]
183. Hebron ML, Lonskaya I, Sharpe K, Weerasinghe PP, Algarzae NK, Shekoyan AR et al. , Parkin ubiquitinates Tar-DNA binding protein-43 (TDP-43) and promotes its cytosolic accumulation via interaction with histone deacetylase 6 (HDAC6). *J Biol Chem*, 2013. 288(6): p. 4103–15. [PubMed: 23258539]
184. Friedman CS, O'Donnell MA, Legarda-Addison D, Ng A, Cardenas WB, Yount JS et al. , The tumour suppressor CYLD is a negative regulator of RIG-I-mediated antiviral response. *EMBO Rep*, 2008. 9(9): p. 930–6. [PubMed: 18636086]
185. Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Muller K et al. , Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat Neurosci*, 2015. 18(5): p. 631–6. [PubMed: 25803835]
186. Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y et al. , Mutations of optineurin in amyotrophic lateral sclerosis. *Nature*, 2010. 465(7295): p. 223–6. [PubMed: 20428114]
187. Nagabhushana A, Bansal M, and Swarup G, Optineurin is required for CYLD-dependent inhibition of TNF α -induced NF- κ B activation. *PLoS One*, 2011. 6(3): p. e17477. [PubMed: 21408173]
188. Jin W, Chang M, Paul EM, Babu G, Lee AJ, Reiley W et al. , Deubiquitinating enzyme CYLD negatively regulates RANK signaling and osteoclastogenesis in mice. *J Clin Invest*, 2008. 118(5): p. 1858–66. [PubMed: 18382763]
189. Fecto F, Yan J, Vemula SP, Liu E, Yang Y, Chen W et al. , SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch Neurol*, 2011. 68(11): p. 1440–6. [PubMed: 22084127]
190. Goedert M, Spillantini MG, Del Tredici K, and Braak H, 100 years of Lewy pathology. *Nat Rev Neurol*, 2013. 9(1): p. 13–24. [PubMed: 23183883]
191. Alexopoulou Z, Lang J, Perrett RM, Elschami M, Hurry ME, Kim HT et al. , Deubiquitinase Usp8 regulates alpha-synuclein clearance and modifies its toxicity in Lewy body disease. *Proc Natl Acad Sci U S A*, 2016. 113(32): p. E4688–97. [PubMed: 27444016]
192. Tofaris GK, Kim HT, Hourez R, Jung JW, Kim KP, and Goldberg AL, Ubiquitin ligase Nedd4 promotes alpha-synuclein degradation by the endosomal-lysosomal pathway. *Proc Natl Acad Sci U S A*, 2011. 108(41): p. 17004–9. [PubMed: 21953697]
193. Sugeno N, Hasegawa T, Tanaka N, Fukuda M, Wakabayashi K, Oshima R et al. , Lys-63-linked ubiquitination by E3 ubiquitin ligase Nedd4–1 facilitates endosomal sequestration of internalized alpha-synuclein. *J Biol Chem*, 2014. 289(26): p. 18137–51. [PubMed: 24831002]
194. Tardiff DF, Jui NT, Khurana V, Tambe MA, Thompson ML, Chung CY et al. , Yeast reveal a “druggable” Rsp5/Nedd4 network that ameliorates alpha-synuclein toxicity in neurons. *Science*, 2013. 342(6161): p. 979–83. [PubMed: 24158909]
195. Chung CY, Khurana V, Auluck PK, Tardiff DF, Mazzulli JR, Soldner F et al. , Identification and rescue of alpha-synuclein toxicity in Parkinson patient-derived neurons. *Science*, 2013. 342(6161): p. 983–7. [PubMed: 24158904]
196. Boulanger LM and Shatz CJ, Immune signalling in neural development, synaptic plasticity and disease. *Nat Rev Neurosci*, 2004. 5(7): p. 521–31. [PubMed: 15208694]
197. Estes ML and McAllister AK, Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat Rev Neurosci*, 2015. 16(8): p. 469–86. [PubMed: 26189694]

198. Stephan AH, Barres BA, and Stevens B, The complement system: an unexpected role in synaptic pruning during development and disease. *Annu Rev Neurosci*, 2012. 35: p. 369–89. [PubMed: 22715882]
199. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA et al. , Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol*, 2014. 10(11): p. 643–60. [PubMed: 25311587]
200. Hammond TR, Marsh SE, and Stevens B, Immune Signaling in Neurodegeneration. *Immunity*, 2019. 50(4): p. 955–974. [PubMed: 30995509]

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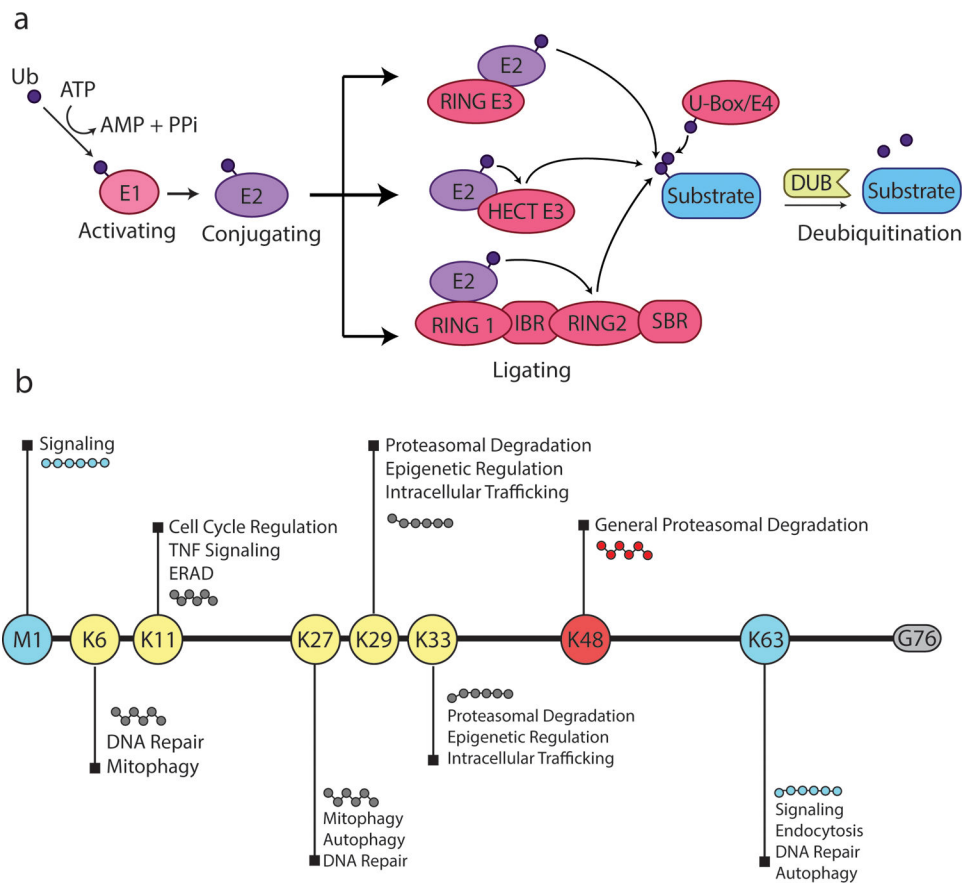


Figure 1. Different faces of polyubiquitin linkages. **a**, Schematic of enzymatic steps involved in substrate ubiquitination and deubiquitination. Ubiquitination by E1, E2, and E3 enzymes, as well as ubiquitin chain extension by U-Box/E4 enzymes and deubiquitination by deubiquitinases (DUB) are shown. Mechanisms for RING, HECT, and RBR E3 ligases are depicted. IBR, in between ring; SBR, substrate binding region. **b**, Eight different chain topologies and their degradative and non-degradative roles in protein fates are shown. TNF, tumor necrosis factor; ERAD, endoplasmic reticulum-associated degradation; M, methionine; K, lysine; G, glycine.

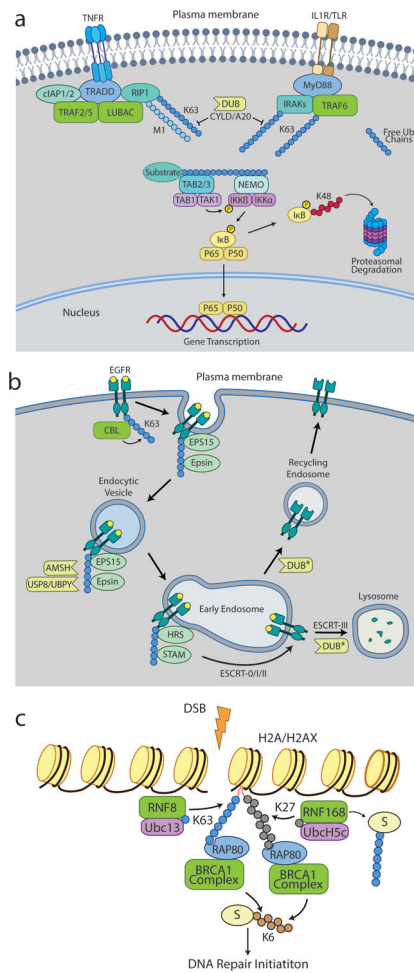


Figure 2.

Roles of nonproteolytic polyUb in signalosome formation and kinase activation. **a**, NF- κ B signaling. Activation of TNF receptor (TNFR), interleukin 1 receptor (IL-1R), or Toll-like receptor (TLR) leads to, in a receptor-dependent manner, recruitment of adaptor proteins (TRADD, MyD88), protein kinases (RIP1, IRAKs), and E3 ubiquitin ligases (TRAF2/5/6, cIAP1/2, and LUBAC) which, together with specific E2s (not shown), catalyze the synthesis of anchored or unanchored polyUb chains of different linkages (K63, M1, and K11). These nonproteolytic chains bind TAB2 subunit of the TAK1 kinase complex, resulting in TAK1 activation, which then phosphorylates IKK β of the IKK complex, leading to IKK activation. Activated IKK phosphorylates I κ B proteins, resulting in their K48-linked ubiquitination and subsequent degradation by the proteasome, freeing the NF- κ B complex to enter the nucleus to regulate gene transcription. CYLD and A20 inhibit NF- κ B signaling by cleaving K63 and M1 chains. **b**, Endocytosis. Following ligand binding of the receptor tyrosine kinases EGFR on the plasma membrane, the E3 CBL conjugates K63 chains onto the activated receptors. DUBs, such as AMSH and USP8/UBPY, cleave K63 chains from internalized receptors. Endocytic adaptors EPS15 and Epsin recognize ubiquitin-bound receptors, targeting them for sorting by the endosome. The ESCRT-0, -I, and -II complexes mediate the sorting process and target the receptors for ESCRT-III dependent lysosomal

degradation, or deubiquitination by DUBs (*, largely unknown) and recycling back to the plasma membrane. **c**, Maintenance of genome integrity. Following DNA double-stranded breaks (DSBs), the E3/E2 complexes RNF8/Ubc13 and RNF168/UbcH5c are recruited to histone H2A/H2AX at sites of DNA damage, where they synthesize K63 and K27 chains on histone or other substrates (S). RAP80 then binds to the polyUb chains, recruiting the BRCA1 E3 ligase complex to sites of the DSB, where it synthesizes K6 chains on substrates to initiate DNA repair process.

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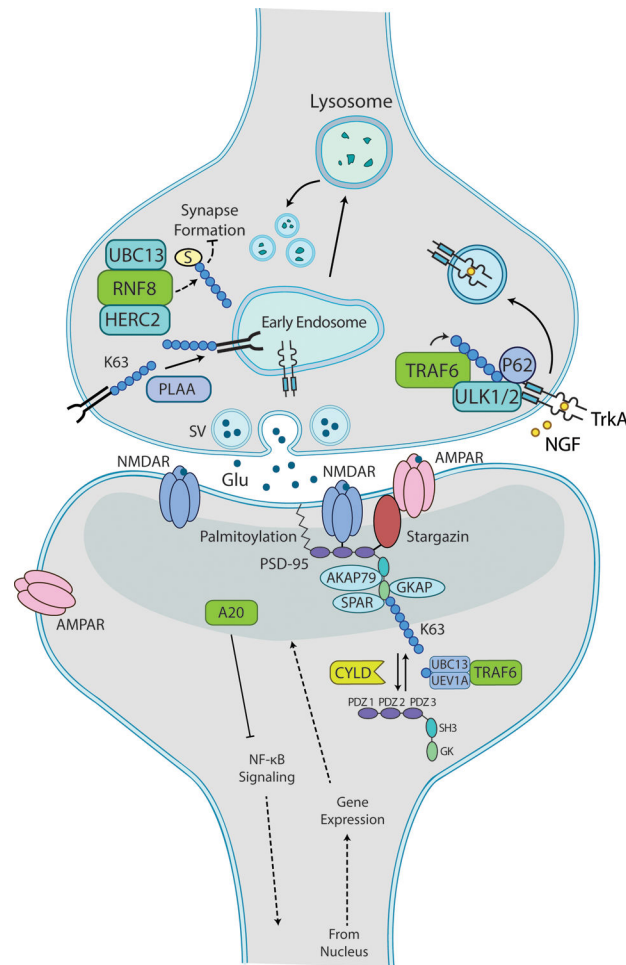


Figure 3. Nonproteolytic ubiquitination in synapse development and remodeling. Presynaptically, the ubiquitin adaptor protein PLAA is required for sorting of ubiquitin-modified membrane proteins into the lumen of MVB/late endosomes. This process plays an important role in synaptic vesicle (SV) recycling, reserve pool size, synaptic transmission, and terminal differentiation. K63 ubiquitination also regulates filopodia extension and branching of sensory axons via an endocytic process. NGF induces K63-polyubiquitination of Ulk1/2 likely by TRAF6, allowing the binding of Ulk1/2 to p62 and the recruitment of Ulk1/2 to active TrkA complex via p62, leading to TrkA internalization, attenuation of NGF signaling, and filopodia withdrawal. RNF8/HERC2 may also regulate presynaptic differentiation in a K63-dependent manner through ubiquitination of unidentified substrates (S). Postsynaptically, A20 inhibits dendritic arborization and spine remodeling through suppression of NF- κ B activity, likely via regulation of gene expression in the nucleus, causing postsynaptic remodeling. TRAF6 and CYLD control local postsynaptic remodeling through K63 polyUb chain conjugation and disassembly on PSD-95. PSD-95 is constitutively K63-ubiquitinated by TRAF6 in conjunction with Ubc13/Uev1A, and deubiquitinated by CYLD. The K63-polyUb conjugation sites are localized primarily in the GK domain, where the assembled K63 chains are essential for PSD-95 interactions with its GK binding partners including SPAR, GKAP/SAPAP, and AKAP79/150. K63-ubiquitinated

PSD-95, but not its un-ubiquitinated form, is targeted to the PSD to promote synapse efficacy (AMPA numbers) and maturation. CYLD removes K63-polyUb from PSD-95, leading to translocation of deubiquitinated PSD-95 away from the PSD, destabilizing the PSD, weakening the synapse, and inhibiting synapse formation and maturation. PDZ, SH3, and GK domains of PSD-95 are indicated. PSD-95 binds NMDARs directly and AMPARs through Stargazin and localizes these receptors at the synapse.

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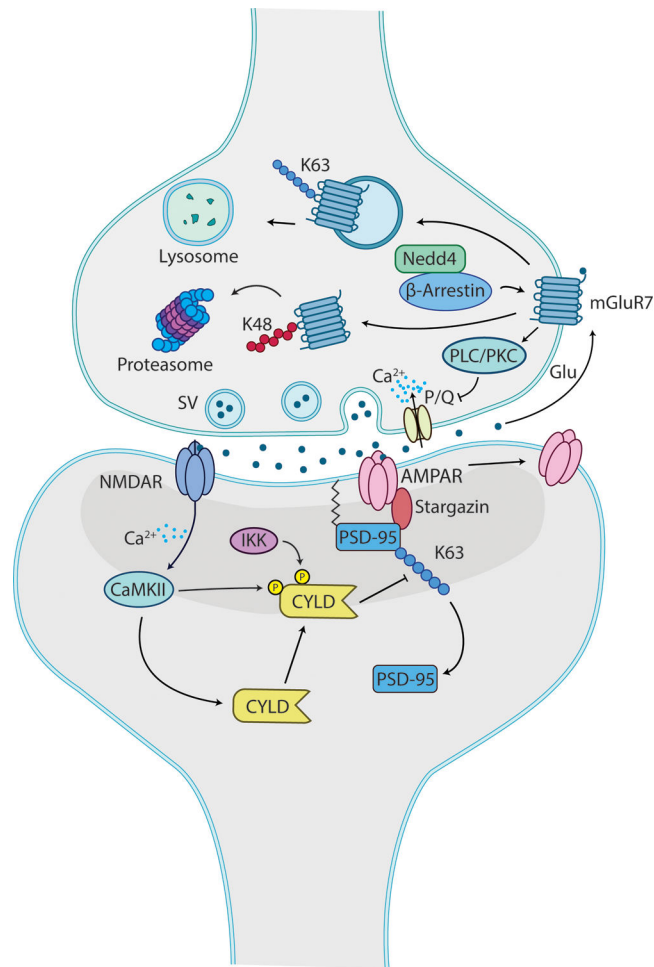


Figure 4. Nonproteolytic ubiquitination regulates Hebbian synaptic plasticity. Presynaptically, sustained synaptic activity leads to activation of presynaptic mGluR7, which inhibits neurotransmitter release through PLC and PKC-dependent suppression of P/Q Ca²⁺ channels. K63 ubiquitination of mGluR7 routes it for lysosomal degradation, whereas K48 ubiquitination targets it for proteasomal degradation. These processes may regulate a metaplastic LTD/LTP switch at MF-SLIN synapses. Postsynaptically, IKK regulates constitutive CYLD activity through phosphorylation under basal conditions. NMDAR-mediated Ca²⁺ influx activates CaMKII, which further activates CYLD through phosphorylation, and/or recruits CYLD to the PSD. CYLD deubiquitinates PSD-95 and causes dispersal of PSD-95, leading to loss of synaptic AMPARs and weakening of synapses.

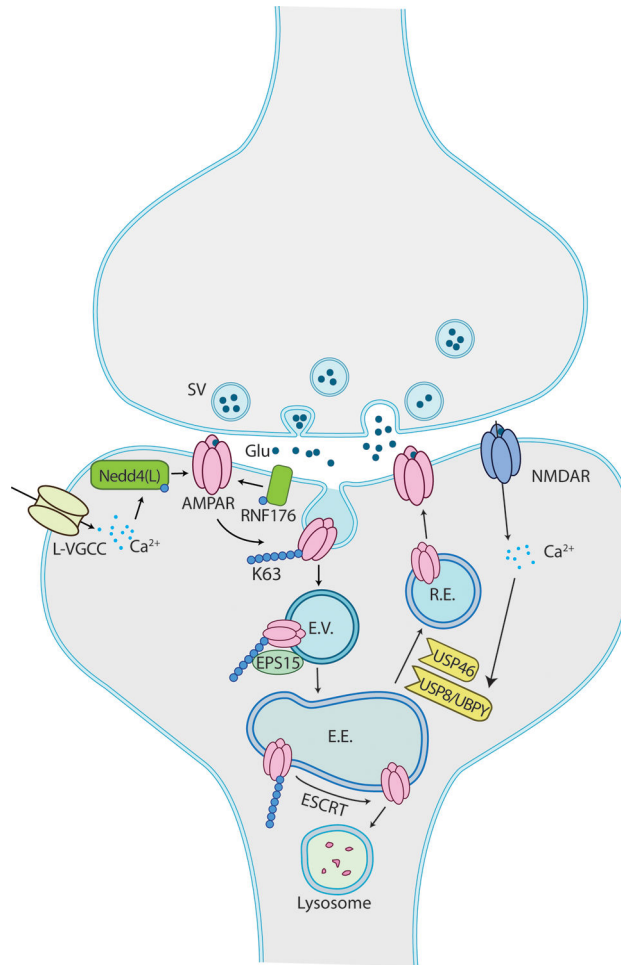


Figure 5. Nonproteolytic ubiquitination regulates homeostatic plasticity. Nedd4 and Nedd4L are activated in response to chronic elevation of synaptic activity (by bicuculline). Nedd4, Nedd4L and RNF176 act as E3s to ubiquitinate AMPARs. Ubiquitination, occurring either before or after receptor endocytosis, regulates endosomal sorting and lysosomal degradation of AMPARs. Deubiquitination by USP8/UBPY, recruited and activated by NMDAR stimulation, and USP46 may promote recycling of AMPARs back to the plasma membrane. L-VGCC, L-type voltage-gated Ca²⁺ channels; E.V., endocytic vesicle; E.E., early endosome; R.E., recycling endosome.

Table 1.

Ubiquitin enzymes and their characteristics discussed in this article.

Ubiquitin enzyme	Enzyme type	Chain specificity	Known substrates	Neuronal function	Refs
UBA6	E1	N/A	ND	Unknown	158
Ubc13	E2	K63	TRAF6, H2A/H2AX	Synapse formation; PSD-95 ubiquitination and scaffolding; locomotor and plasticity behavior	33, 56–58, 93, 139
UbcH5c	E2	K27, K63	H2A	Unknown	58, 59
UbcH7	E2	K63	TrkA	Neurotrophin signaling	63
UbcH8	E2	K48	ND	Unknown	89
UBE2K	E2	K63	ND	Unknown	158
Uev1A	E2	K63	TRAF6	PSD-95 ubiquitination and scaffolding	33, 93
BRCA1	RING E3	K6, others	H2A	Unknown	56–60
CBL	RING E3	K63	Receptor tyrosine kinases, e.g., EGFR	Unknown	51, 52
HERC2	HECT E3	K63	ND	Synapse formation	89
LUBAC	RBR E3 Complex	M1	NEMO, MyD88, IRAKs, TNFR1	Unknown	3, 19
Nedd4	HECT E3	K63	GluA1, mGluR7, α -synuclein	AMPA trafficking; homeostatic plasticity; α -synuclein endosomal trafficking and clearance	91, 115, 126, 127, 192, 193
Nedd4–2 (Nedd4L)	HECT E3	K63	GluA1	AMPA trafficking; homeostatic plasticity	129
Parkin	RBR E3	K63	TDP-43	TDP-43 cytoplasmic translocation and accumulation in FTD/ALS	183
RNF8	RING E3	K63	H2A/H2AX	Synapse formation	56, 57, 89
RNF76/TRIM27	RING E3	K63	WASH	Endosomal actin levels and protein recycling	152, 153
RNF167	RING E3	ND	GluA2	AMPA trafficking, homeostatic plasticity	117
RNF168	RING E3	K63, K27	H2A/H2AX	ND	56–58
RNF220	RING E3	K63	Glis	Neural development	78
SCF	RING E3	K11, K48	Glis	Neural development	74–77
SCF β TrCP	RING E3	K48	I κ B α	NF- κ B signaling	49
TRAF3	RING E3	K63	ND	Present in the PSD with unknown function	94
TRAF6	RING E3	K63	PSD-95, TrkA, TrkB, TrkC, p75, NRIF, Ulk1/2, presenilins	Neurotrophin signaling; synapse remodeling; axonal outgrowth	33, 63–71, 73, 81, 93, 172
A20	OTU DUB, E3	K48, K63	RIP1, TRAF6, NEMO, Ubc13	Synapse remodeling	50, 95
AMSH	JAMM DUB	K63	Receptor tyrosine kinases, e.g., EGFR	Unknown	53
CYLD	USP DUB	K63, M1	PSD-95	Disassembles K63 chains in PSD; regulates PSD scaffolding; cLTD; striatal GABAergic transmission; axonal outgrowth	42–44, 93, 100–102, 104–106
USP7	USP DUB	K48, K63	WASH	Endosomal actin levels and protein recycling	150, 152

Ubiquitin enzyme	Enzyme type	Chain specificity	Known substrates	Neuronal function	Refs
USP8/UBPY	USP DUB	K63	GluA1, α -synuclein, EGFR	AMPA trafficking; homeostatic plasticity; α -synuclein clearance and toxicity	54, 127, 191
USP46	USP DUB	K63	GLR-1, GluA1, GluA2	AMPA trafficking	118, 130

ALS, amyotrophic lateral sclerosis; AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; cLTD, chemical long-term depression; FTD, frontotemporal dementia; GABA, gamma aminobutyric acid; N/A, not applicable; ND, not discussed; PSD, postsynaptic density.

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Table 2.

Nonproteolytic ubiquitination and ubiquitination-related genes and their implications in brain disorders.

Gene (Protein)	Protein and cellular function	Associated brain disorders	Refs
<i>APP</i> (APP)	Subject to Ubiquilin 1-stimulated K63 ubiquitination that delays its maturation through the secretory pathway and subsequent cleavage by secretases	Mutations associated with early-onset familial AD	164, 166, 168
<i>CYLD</i> (CYLD)	DUB that disassembles K63 chains in the PSD, regulates PSD-95 scaffolding, cLTD, striatal GABAergic transmission, and axonal outgrowth; regulated by Shank3 and interacts with >103 synaptic proteins	Mutations associated with FTD/ALS, potentially via regulation of TDP-43 cytoplasmic accumulation; interacts with many proteins associated with ASD, schizophrenia, and depression	93, 100–102, 104–106
<i>HERC2</i> (HERC2)	E3 ligase that interacts with RNF8 and NEURL4 to inhibit synapse formation likely via K63-dependent mechanisms	A homozygous missense mutation linked to ASD	149
<i>MAGEL2</i> (MAGEL2)	Regulator in the TRIM27-MAGEL2 E3 ligase complex that promotes K63 ubiquitination of WASH to regulate endosomal actin assembly and protein recycling	Mutations and dysregulation associated with Prader-Willi syndrome, Schaaf-Yang syndrome, and ASD	152, 154–156
<i>NEDD4</i> (Nedd4)	E3 ligase that regulates AMPAR ubiquitination and trafficking, synaptic plasticity, α -synuclein ubiquitination and endosome-lysosome mediated degradation	Decreased in temporal gyrus of schizophrenia patients; SNPs associated with schizophrenia; regulates clearance and toxicity of α -synuclein in PD	158, 159, 194, 195
<i>PRKN</i> (Parkin)	E3 ligase that promotes K63 ubiquitination of TDP-43 and regulates TDP-43 cytoplasmic translocation and accumulation	Cytoplasmic TDP-43 accumulation is associated with FTD/ALS; mutations and dysfunctions are associated with PD	13, 183
<i>PSEN1</i> (Presenilin-1)	γ -secretase that cleaves APP to form A β ; binds K63 chains and is K63-ubiquitinated by TRAF6 with unknown functional significance	Mutations associated with early-onset familial AD but role of K63 ubiquitination is unknown	165, 166, 172, 173
<i>RNF8</i> (RNF8)	E3 ligase that acts with Ubc13 and interacts with HERC2/NEURL4 to limit synapse formation likely via K63-dependent mechanisms	A deletion in the regulatory region linked to ASD	148
<i>SNCA</i> (α -synuclein)	Major constituent of Lewy bodies that is K63 ubiquitinated by Nedd4	Lewy body formation and toxicity associated with PD	190–195
<i>TARDBP</i> (TDP-43)	Nuclear DNA/RNA-binding protein that regulates expression of thousands of genes; K63 ubiquitination by Parkin promotes its translocation and accumulation in the cytoplasm	Major pathologic component of tau-negative and ubiquitin-positive inclusions in ~ 50% of FTD	105, 176, 178, 179, 183
<i>UBA6</i> (UBA6)	E1 ubiquitin activating enzyme	Decreased in temporal gyrus of schizophrenia patients	158
<i>UBQLN1</i> (Ubiquilin-1)	Ubiquitin-like molecular chaperone that stimulates APP K63 ubiquitination, maturation, and degradation	SNPs associated with late-onset AD; levels are decreased in late-onset AD brains	168–171
<i>USP7</i> (USP7)	DUB that removes K63 chains from WASH to maintain endosomal actin levels and K48 chains from TRIM27/RNF76 to prevent proteasomal degradation to regulate endosomal protein trafficking	Loss of function mutations linked to intellectual disability and ASD	152
<i>USP8</i> (USP8/UBPY)	DUB that removes K63 chains from α -synuclein and regulates its clearance	Regulates α -synuclein toxicity in PD	191

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ASD, autism spectrum disorder; cLTD, chemical long-term depression; DUB, deubiquitinase; FTD, frontotemporal dementia; GABA, gamma-aminobutyric acid; PD, Parkinson's disease; PSD, postsynaptic density; SNP, single nucleotide polymorphism.