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Review

Ubiquitin-dependent endocytosis, trafficking and turnover of neuronal membrane proteins

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ABSTRACT

Extracellular signaling between cells is often transduced via receptors that reside at the cell membrane. In neurons this receptor-mediated signaling can promote a variety of cellular events such as differentiation, axon outgrowth and guidance, and synaptic development and function. Endocytic membrane trafficking of receptors ensures that the strength and duration of an extracellular signal is properly regulated. The covalent modification of membrane proteins by ubiquitin is a key biological mechanism controlling receptor internalization and endocytic sorting to recycling and degradative pathways in many cell types. In this review we highlight recent findings regarding the ubiquitin-dependent trafficking and turnover of receptors in neurons and the implications for neuronal development and function.

Contents

Introduction

Ubiquitination (also known as ubiquitylation) is the covalent modification of proteins by a highly conserved 76 amino acid protein called ubiquitin. Ubiquitination regulates a myriad of cellular processes, including cell division, differentiation, signal transduction, protein trafficking, and protein quality control (Pickart and Eddins, 2004). Ubiquitination is itself highly regulated and involves a multi-step enzymatic process using three classes of enzymes (E1s, E2s, and E3s) to transfer ubiquitin to a lysine residue on a target protein (Hershko and Ciechanover, 1998). Ubiquitin is first activated by the ubiquitin-activating enzyme (E1) in an ATP-dependent reaction in which the C terminal glycine residue of ubiquitin is attached to the active-site cysteine of an E1 enzyme via a thioester linkage. Specificity of the ubiquitination reaction depends on the later steps of the ubiquitination process. There are dozens of ubiquitin-carrier enzymes (E2s), and several hundred ubiquitin ligases (E3s). Thus, the ubiquitination enzymes form a hierarchical cascade, where the substrate specificity of the overall ubiquitination reaction depends on the specific E2s and E3s that pair up to ubiquitinate the substrate.

Ubiquitinated proteins can be further modified by the attachment of additional ubiquitin molecules to one of seven lysines within ubiquitin to form chains with distinct linkage types (polyubiquitination). Polyubiquitin chains linked through lysine 48 (K48), represent a large fraction of polyubiquitinated proteins found in vivo and are usually

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targeted for degradation by a large energy-dependent multi-subunit protease called the 26S proteasome (Fig. 1a) (Pickart and Eddins, 2004; Xu et al., 2009). However, other forms of ubiquitination exist and are known to regulate protein function in ways distinct from proteasomal degradation. For instance, proteins modified by single (monoubiquitination), short chain and K63-linked polyubiquitinated chains can control the activity, interactions, and subcellular distribution of proteins (Clague and Urbe, 2010).

Similar to phosphorylation, the ubiquitination reaction is reversible. This is accomplished through the action of deubiquitinating enzymes (DUBs). DUBs are ubiquitin proteases that generate free usable ubiquitin from a number of sources including ubiquitin-protein conjugates, ubiquitin-adducts, and ubiquitin precursors (Wilkinson, 1997). Given the emerging roles for DUBs in development, cell growth, oncogenesis, transcription, memory and neurodegenerative disease, they are likely to be as functionally important as their ubiquitin ligase counterparts. Comparatively, however, their regulation, substrate specificity and function are still poorly understood.

The dynamic regulation of protein synthesis and protein degradation is critical for maintaining the stoichiometric balance of the neuronal and synaptic proteome required for proper neuronal function. While protein synthesis received the bulk of attention by researchers in the past, it is now well established that protein degradation plays an equal and important role in neurons. Many recent studies have shown that the ubiquitin proteasome system (UPS) is important for the development, maintenance and remodeling of synapses. Pharmacological inhibition of the proteasome has been shown to impair synaptic plasticity and behavior (Cajigas et al., 2010). In addition, there have been a number of substrates identified whose stability is regulated by the UPS to control neuronal development, presynaptic, and postsynaptic function. Moreover, the stability of many synaptic UPS substrates can be controlled in an activity-dependent fashion (Ehlers, 2003). We refer the reader to several excellent reviews which discuss the overall advancements made in this field regarding the UPS and synaptic development and function (Bingol and Sheng, 2011; Mabb and Ehlers, 2010; Patrick, 2006; Yi and Ehlers, 2007). Furthermore, altered UPS dysfunction is associated with several neurodevelopmental and age-related neurodegenerative disorders (Hegde and Upadhya, 2011).

The covalent modification of neuronal membrane proteins by ubiquitin can also control their internalization and endocytic sorting to

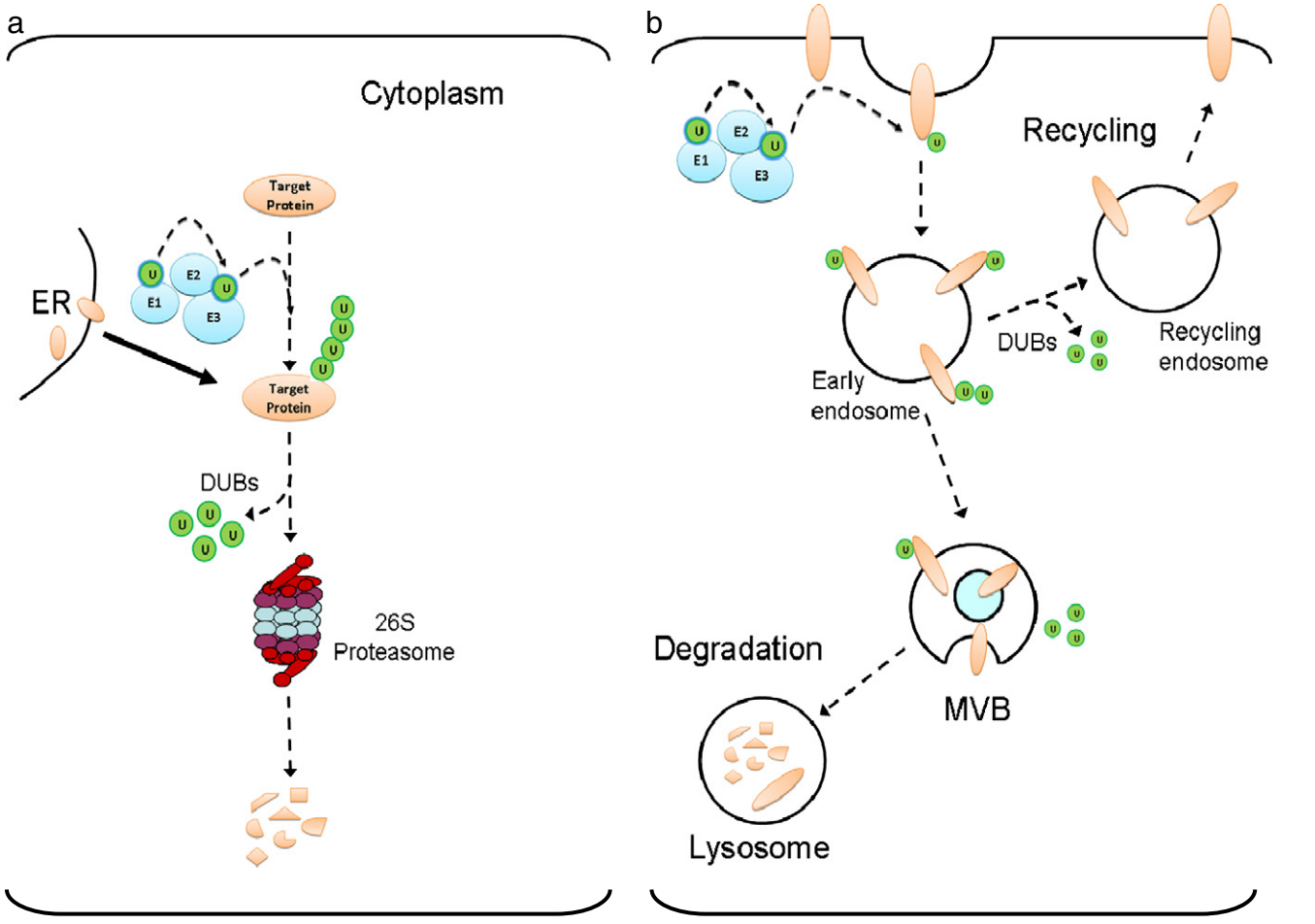

Fig. 1. Major pathways for ubiquitin-dependent protein degradation in eukaryotic cells. Proteasome-mediated degradation of ubiquitinated proteins (a). Ubiquitination is a process whereby target proteins can be marked for degradation by the 26S proteasome. It is a multi-step enzymatic process, using three classes of enzymes (E1s, E2s, and E3s), and involves the sequential transfer of ubiquitin from these enzymes to a lysine residue on the target protein. Ubiquitin is first activated by the ubiquitin-activating enzyme (E1) in an ATP-dependent reaction in which the C terminal glycine residue of ubiquitin binds to the active-site cysteine of an E1 in a thioester linkage. The activated ubiquitin is passed to E2 ubiquitin-carrier enzymes and then to E3 ubiquitin ligases. De-ubiquitinating enzymes (DUBs) reverse the ubiquitination process. DUBs are cysteine proteases that generate free usable ubiquitin from a number of sources including ubiquitin-protein conjugates, ubiquitin-adducts, and ubiquitin precursors. Degradation of ubiquitinated membrane proteins by the lysosome (b). Ubiquitination, usually in the form of single (mono) or short-chain ubiquitin modifications (K63-linkages in many cases) can result in the endocytosis of membrane proteins. In early endosomes, non-ubiquitinated proteins can recycle back to the plasma membrane or be directed to other intracellular compartments. In contrast, ubiquitinated proteins are sorted into multivesicular bodies (MVB) and eventually targeted to the lysosome for degradation. Deubiquitination of cargos plays a critical role in the regulation of intracellular trafficking through the endosomal sorting complexes required for transport (ESCRT) pathway and in some cases ensures recycling of internalized cargos to the plasma membrane.

recycling and lysosomal degradative pathways (Fig. 1b). Since its discovery in yeast, there have been numerous studies of ubiquitin-dependent endocytosis, trafficking, and turnover of membrane proteins in mammalian cells. Several excellent reviews have been published which discuss in detail mechanistic insights of ubiquitin-dependent trafficking in cells (Clague and Urbe, 2010; Acconcia et al., 2009; Hicke and Dunn, 2003). The large majority of these studies have identified substrates and elucidated mechanisms in dividing cells. However, as might be expected, the rapid and dynamic regulation of protein levels at the plasma membrane is also crucial in neurons. Recent findings from *Drosophila*, *Caenorhabditis elegans* and mammals indicate ubiquitin-dependent internalization and endocytic sorting of membrane proteins to be an important mechanism for neuronal development and function. In this review, we specifically discuss recent studies of ubiquitin-dependent trafficking and turnover of membrane proteins in neurons and the implications on neuronal development and function.

Ubiquitin as an endocytosis and sorting signal

Initial indications of the non-proteasomal functions of ubiquitination came from studies in yeast (*Saccharomyces cerevisiae*). Ste6p, the plasma membrane ATP-binding cassette (ABC) transporter required for the export of alpha-factor, was shown to accumulate as ubiquitinated conjugates in endocytosis-defective yeast (Kolling and Hollenberg, 1994). Since then, several membrane proteins have been shown to be regulated by ubiquitination including GPCRs, ion channels, transporters and permeases. In yeast, ubiquitinated membrane proteins traffic through the endocytic pathway to the vacuole (Hicke and Dunn, 2003). In yeast strains lacking ubiquitin conjugation machinery, internalization and degradation of the GPCRs, Ste2p and Ste3p is diminished (Hicke and Riezman, 1996; Roth and Davis, 1996).

Most of our understanding of ubiquitin-dependent internalization and endocytic sorting in mammals comes from studies on receptor tyrosine kinases, and more recently GPCRs. Interestingly, the situation is much more complex in mammals, presumably because of their numerous and divergent signaling pathways. For instance, while it is clear that ubiquitination is involved in the internalization of the growth hormone receptor (GHR), it appears that ubiquitination of the GHR is itself not required (Strous et al., 2004). Furthermore, it appears that the ubiquitination and internalization of receptors can be dependent on the concentration of ligand to recruit them into clathrin-dependent or -- independent pathways (Acconcia et al., 2009).

Ubiquitination, usually in the form of single (mono) or short-chain ubiquitin modifications, can result in the endocytosis of membrane proteins (See Figure 1b). One mechanism by which it does this is through remodeling of the topology of substrate membrane proteins which mediate interactions with other proteins to promote a protein's internalization and endocytic sorting. Ubiquitination has been found to recruit adaptor proteins which then assist clathrin machinery to mediate endocytosis (Hicke and Dunn, 2003). In early endosomes, non-ubiquitinated proteins can recycle back to the plasma membrane or be directed to other intracellular compartments. In contrast, ubiquitinated proteins are sorted into multivesicular bodies (MVB). Sorting in the MVB is mediated by the endosomal sorting complexes required for transport (ESCRTs). There are 4 ESCRT complexes: the first three (ESCRT-0, -I and -II) are engaged early in the sorting process, while ESCRT-III is involved in the delivery of ubiquitinated proteins (cargo) to the MVB and eventually to the lysosome (Williams and Urbe, 2007). Several ubiquitin binding proteins, so-called ubiquitin receptors, are involved in the sorting of ubiquitinated cargo in each ESCRT complex. Deubiquitination of cargos plays a critical role in the regulation of intracellular trafficking through the ESCRT pathway and in some cases ensures recycling of internalized cargos to the plasma membrane (Clague and Urbe, 2006). Several diseases including cancer, retroviral infections, and neurodegeneration are caused by mutations of various components within the ESCRT pathway (Saksena and Emr, 2009).

Ubiquitin-dependent endocytosis and turnover in development

Notch signaling and neuronal cell-fate decisions

A well-recognized function of ubiquitin-dependent endocytosis involves the regulation of Notch signaling in metazoans to mediate a number of cell-fate decisions, such as neurogenesis in flies and vertebrates during development. Notch signaling involves a series of proteolytic cleavage steps of the Notch receptor that ultimately leads to the generation of an intracellular fragment of Notch (Nicd) which enters the nucleus to mediate transcription. This process is highly regulated by binding of the DSL (Delta, Serrate, LAG-2) family of transmembrane ligands to the extracellular domain of the Notch receptor (Bray, 2006). Expression of Notch ligands can be developmentally regulated through transcriptional programs that define which cells activate Notch receptors. However, it is now proposed that ubiquitination of Notch ligands regulates their endocytosis and activation of Notch receptors. This hypothesis, in large part, resulted from the identification of two ubiquitin E3 ligases, Neuralized (Neur) and Mind bomb (Mib), that interact directly with Notch ligands and are required for ligand activity. Loss of Neur in *Drosophila melanogaster* and Neur and Mib1 in *Xenopus laevis* results in a variety of neurogenic phenotypes (Deblandre et al., 2001; Itoh et al., 2003; Lai et al., 2001; Pavlopoulos et al., 2001). In the absence of these ubiquitin ligases, Notch ligands accumulate at the membrane but are inactive (Pavlopoulos et al., 2001; Le Borgne et al., 2005). In addition, the ubiquitin-binding and clathrin adaptor protein epsin and auxilin, which disassembles clathrin-coats, were shown to be required for the activation of Notch ligand, providing further support for a role of ubiquitination and endocytosis in Notch signaling (Overstreet et al., 2004; Wang and Struhl, 2004; Wang and Struhl, 2005). Several models have been proposed for how Notch ligand activity is regulated by ubiquitination (Bray, 2006). Ubiquitin-dependent ligand endocytosis could generate a mechanical force that produces a conformational change in the bound receptor to achieve proper processing and dissociation of Notch signaling proteins (Parks et al., 2000). Alternatively, it has been shown that stabilization and clustering of Notch ligands in the membrane is another way to achieve sufficient and effective levels of Notch signaling (Hicks et al., 2002; Varnum-Finney et al., 2000), however it is not known if ubiquitination of Notch ligands is involved in this regard. Finally, it has been proposed that ubiquitination of Notch ligands promotes internalization into an endocytic compartment, resulting in additional modification and recycling of the ligand into specific membrane domains (Chen and Greenwald, 2004; Emery et al., 2005). It appears that regulation of E3 ubiquitin ligase activity may be a critical mechanism for controlling ligand activation and thus Notch signaling as indicated by the identification of the Bearded (Brd) family of small inhibitory peptides of Neur (Bardin and Schweisguth, 2006; De Renzis et al., 2006). Brd proteins, when overexpressed, can inhibit the endocytosis of Delta by interfering with the interaction of Delta and Neur. Therefore Brd proteins exert spatial and temporal control of Notch–Delta signaling by inhibiting Neur function.

Axon outgrowth and navigation

Developing axons respond rapidly and dynamically as they encounter distinct molecular territories along a defined pathway to correct target destinations (Tessier-Lavigne and Goodman, 1996). Several molecules, such as netrins, semaphorins, ephrins and Slits, have been identified, which provide guidance cues to developing axons navigating long distances to their targets. As axons grow they sometimes change their responsiveness to these extrinsic cues. Growth cones contain protein translation machinery, as well as components of the UPS, and their

chemotropic responses are sensitive to both protein synthesis and proteasome inhibitors (Campbell and Holt, 2001). However, ubiquitin-dependent endocytosis has also been suggested to be involved in the trafficking of key regulatory molecules involved in axon guidance. One of the best characterized choice points for developing axons is the decision to cross the midline of the central nervous system (CNS). In *Drosophila*, *commissureless* (*comm*) controls the axon projection choice between a commissural and ipsilateral pathway. Comm is a short transmembrane protein that regulates the surface levels of the Slit receptor, Roundabout (Robo) (Tear et al., 1996). Prior to crossing the midline, commissural axons are insensitive to the Slit repellent, but once they have crossed, Robo surface levels increase, ensuring that commissural axons do not recross. Two distinct models account for how Comm regulates Robo surface levels. One model involves the downregulation of Robo by Comm at the plasma membrane of growth cones. This involves the recruitment of the E3 ubiquitin ligase, DNedd4, which ubiquitinates Comm and promotes the Comm/Robo complex internalization (Myat et al., 2002). Alternatively, in a model proposed by Keleman et al., ubiquitination of Comm by DNedd4 is not involved. Instead, Comm negatively regulates the delivery of Robo to growth cones by controlling the sorting of Robo into late endosomes and lysosomes and away from vesicles destined for growth cones (Keleman et al., 2002; Keleman et al., 2005). The reasons for these discrepancies are still unclear and perhaps involve specific cell types in the assessment of Comm ubiquitination. Interestingly, however, DNedd4 was recently shown to regulate neuromuscular synaptogenesis in *Drosophila* by similar mechanisms involving Comm ubiquitination and endocytosis (Ing et al., 2007). Regulation of the Slit/Robo pathway by ubiquitination is likely dynamic and reversible. For example, in mammals, it has been found that midline crossing and Slit responsiveness of commissural axons involves Slit-dependent deubiquitination activity. Ubiquitin-specific protease 33 (USP33) was found to directly interact with Robo1, protecting the Robo1 receptor complex from degradation and facilitating recycling of the complex by deubiquitinating Robo in a Slit-dependent manner (Yuasa-Kawada et al., 2009).

Other examples for how ubiquitination is utilized to control the surface level of key regulators of axon outgrowth and guidance have emerged. During development, cell adhesion molecules play an important role in axon growth and navigation. Recently, studies from *Drosophila* showed that the immunoglobulin superfamily cell adhesion molecule (IgSF-CAM) DM-GRASP undergoes ubiquitination. Ubiquitination of DM-GRASP promotes its endocytosis in growth cones and subsequent degradation, thus regulating DM-GRASP surface levels to mediate axon guidance (Thelen et al., 2008). Another IgSF-CAM family member, Fasciclin2 (Fas2), was found to be regulated by the anaphase-promoting complex/cyclosome (APC/C) co-activator fizzy-related/Cdh1 (Fzr/Cdh1) E3 ubiquitin ligase (Silies and Klambt, 2010). Axonal Fas2 directly interacts with glial isoforms of Fas2 in a homophilic manner. Interestingly, APC/$C^{Fzr/Cdh1}$ acts as a novel non-cell autonomous regulator of glial cell migration by controlling surface levels of Fas2 in motor neurons. APC/$C^{Fzr/Cdh1}$ locally down-regulates Fas2 in motor neuron axons and thus establishes a graded distribution of Fas2 important for axonal development and glial migration (Silies and Klambt, 2010).

It is likely that the spatial distribution of ubiquitin ligases and DUBs is an important factor in the regulation of growth cone dynamics and axon guidance. Some studies have indicated that lipid rafts are membrane microdomains that might be used to concentrate signaling molecules in neurons (Simons and Toomre, 2000). Cbl, an E3 ubiquitin ligase that targets receptor tyrosine kinases for ubiquitination and lysosomal degradation, was found to be targeted to lipid raft microdomains in the growth cones of differentiated PC12 cells. Furthermore, overexpression of the RING finger domain of Cbl enhances lamellipodia formation (Haglund et al., 2004). Therefore, the local ubiquitin conjugation and/or de-conjugation in growth cone micro-domains might be a common mechanism to restrict function spatially.

Ubiquitin-dependent trafficking and turnover at synapses

Synaptic plasticity at individual synapses can be accomplished by changes in neurotransmitter release or in neurotransmitter receptor number and function. Longterm potentiation (LTP) and depression (LTD) of synaptic transmission are two of the most compelling and well-studied forms of synaptic plasticity. A prevailing view which has emerged is that these forms of activity-dependent synaptic plasticity ultimately reflect an increase or decrease in the number of receptors at the postsynaptic membrane (Malenka, 2003; Malinow and Malenka, 2002). Therefore a large majority of research in the field has focused on understanding the molecular mechanisms regulating receptor trafficking and surface stability in neurons. Also, it is now understood that the dysfunction of such plasticity pathways at synapses underlie many of the patho-physiological deficits of several age-related and neurodegenerative disorders such as Alzheimer's disease (AD) (Hsieh et al., 2006; Wei et al.). The main class of receptors thought to mediate LTP and LTD in many brain regions are glutamate receptors. In the postsynaptic membrane of glutamatergic synapses there are two types of ionotropic glutamate receptors: the N-methyl-D-aspartate (NMDA) receptor and the alpha-amino-3-hydroxy-5-methyl-4-isoxaolepropionic acid (AMPA) receptor. It is well established that regulation of AMPA receptor trafficking to and from the postsynaptic membrane is a mechanism utilized by neurons for several forms of synaptic plasticity (Shepherd and Huganir, 2007). Many synaptic proteins that regulate the delivery, removal, or stability of AMPA receptors at the synaptic membrane have been found to be modified by ubiquitin. In large part, ubiquitination regulates the degradation and therefore abundance of these proteins at synapses.

Ubiquitin-dependent regulation of glutamate receptors in C. elegans

The first evidence that glutamate receptors are themselves regulated by ubiquitination emerged from studies in *C. elegans*. Burbea et al. demonstrated that GLR-1, the *C. elegans* non-NMDA type glutamate receptor (GluR), is ubiquitinated in vivo, and mutations in GLR-1 which block ubiquitination increase the abundance of the receptor at synapses and alter locomotion behavior in a manner consistent with increased synaptic strength. In contrast, overexpression of ubiquitin decreased the abundance of GLR-1 and GLR-1-containing synapses (Burbea et al., 2002). Furthermore, it was shown that mutations in *unc-11*, which encodes a clathrin adaptor protein AP180, blocked the effects of ubiquitin overexpression, suggesting that the ubiquitin-dependent regulation of GLR-1 receptors at synapses involves a clathrin-dependent endocytic pathway (Burbea et al., 2002). Expression of a mutant form of GLR-1 (GLR-1 (4KR)) that could not be ubiquitinated at C-terminal lysine residues, caused an increased accumulation of these receptors at the plasma membrane and at synapses. Interestingly, co-expression of ubiquitin together with GLR-1 (4KR) did not lead to diminished GLR-1 levels at individual synapses. However, the density of GLR-1(4KR)-containing synapses were decreased to levels similar to GLR-1 (WT). Based on these results, it was hypothesized that ubiquitination and degradation of other synaptic proteins may control the number of GLR-1 containing synapses.

This notion is supported by additional studies in *C. elegans*. For instance, the Lin-23/SCF (Skp1/Cullin/F Box), RPM-1, Cullin 3 and the anaphase-promoting complex (APC) E3 ubiquitin ligases have been identified in genetic screens as regulators of synaptic glutamate receptor abundance by targeting of non-receptor substrates for degradation (Dreier et al., 2005; Juo and Kaplan, 2004; Park et al., 2009; Schaefer and Rongo, 2006). In the case of APC, additional genetic evidence has been found in *Drosophila* at the neuromuscular junction (NMJ). The APC/cyclosome regulates synaptic size by controlling the levels of the downstream effector liprin-α in neurons and also regulates synaptic strength by controlling the concentration of postsynaptic

glutamate receptors in muscles (van Roessel et al., 2004). In addition, post-endocytic sorting to recycling or lysosomal degradation pathways is likely dynamically controlled by GLR-1 ubiquitination. Recently, the deubiquitinating enzyme USP-46 was shown to regulate GLR-1 abundance at synapses by deubiquitinating GLR-1 and thus preventing GLR-1 degradation in lysosomes (Kowalski et al., 2011). As expected due to the decreased GLR-1 levels, the USP-46 mutants display defects in locomotion behavior associated with decreased glutamatergic signaling (Kowalski et al., 2011).

Ubiquitin-dependent regulation of neurotransmitter receptors in mammals

A growing body of literature has shown that ubiquitination may play a similar role in mammalian neurons as it does in other cell-types to mediate the removal of surface receptors from the neuronal synaptic membrane. A new insight emerging from these findings is that ubiquitination and subsequent turnover of synaptic receptors in mammalian neurons is induced by changes in neuronal activity.

A central component of LTP and LTD in the hippocampus is the proper insertion or removal of synaptic AMPARs from the post-synaptic membrane. Initial studies addressing regulation of AMPAR trafficking by the UPS in mammalian neurons showed that AMPAR endocytosis was blocked by proteasome inhibition or expression of chain-elongation defective forms of ubiquitin (Bingol and Schuman, 2004; Patrick et al., 2003). While these papers provide evidence that an intact UPS pathway is needed for AMPAR endocytosis to occur, they do not indicate that AMPARs are themselves modified by ubiquitin. However, a series of recent papers have now shown that AMPARs undergo direct ubiquitination upon various stimuli to regulate their trafficking from the synaptic membrane. The first paper to definitely show this demonstrated that the GluA1 subunit of AMPARs undergoes rapid ubiquitination at C-terminal lysine residues in response to brief application of the agonist AMPA but not NMDA (Schwarz et al., 2010). This ubiquitination was required for the internalization and sorting of internalized AMPARs to the lysosome for degradation, but not necessary for NMDA-induced internalization and receptor recycling back to the plasma membrane. Furthermore, it was found that the E3 ligase neuronal precursor cell-expressed developmentally down-regulated 4-1 (Nedd4-1) directly ubiquitinates GluA1 to mediate its internalization and trafficking. Nedd4-1 is the prototypical protein in a family of HECT-domain containing E3 ligases that have a common architecture (Kumar et al., 1997). The Nedd4 family of E3 ligases is evolutionarily conserved and are involved in a number of diverse cellular processes such as regulation of the trafficking, stability and signaling of membrane proteins in dividing cells and in neurons (Arevalo et al., 2006; Ingham et al., 2004; Sorkina et al., 2006). Ubiquitination of GluA1 was recently confirmed in a separate paper, which also observed that ubiquitination occurs at a specific lysine residue (lysine 868) on the C-terminal tail of GluA1 (Lin et al., 2011).

Separately, Lussier et al. (2011) recently found that the GluA2 AMPA receptor subunit is rapidly ubiquitinated in an activity-dependent fashion (Lussier et al., 2011). From these studies it is apparent that the ubiquitination of AMPARs may be dynamically controlled by neuronal activity. However, it appears that AMPARs may also undergo ubiquitination and proteasomal degradation in response to more long-lasting changes in neuronal activity. Specifically, it was found that chronic application of ephrin-A1 to cortical neurons results in a loss of synaptic and total GluA1 in neurons (Fu et al., 2011). In contrast to lysosomal-dependent degradation discussed above, this study speculates the degradation of GluA1 to involve receptor retrotranslocation, ubiquitination and proteasome-dependent degradation (Fu et al., 2011). Ephrin-A1 application was also shown to induce ubiquitination of GluA1 in neurons, and applying proteasome inhibitors prior to Ephrin-A1 blocked loss of AMPARs in those cells. Furthermore, under these conditions GluA1 is ubiquitinated by the E3 ligase APC and its co-activator Cdh1 via an interaction at its N-terminal domain. Together, these recent papers suggest that AMPA receptor ubiquitination may be controlled by a variety of neuronal signals to regulate synaptic function.

To date, phosphorylation is the major posttranslational modification which occurs on carboxy termini of AMPARs to control their trafficking to and from the postsynaptic membrane (Shepherd and Huganir, 2007). As mentioned above, Schwarz et al. (2010) found that AMPARs are rapidly ubiquitinated and targeted to a lysosomal degradation pathway in response to direct neurotransmitter stimulation (e.g. AMPA), yet they remain unmodified in response to NMDA receptor activation. Interestingly, however, they found that NMDAR activation robustly stimulates AMPAR ubiquitination in neurons pretreated with PKA inhibitors that block GluA1 C-terminal phosphorylation events and prevent their recycling back to the postsynaptic membrane (L.A. Schwarz and G.N. Patrick, *unpublished observations*). It is intriguing to postulate that a dynamic interplay between receptor phosphorylation and ubiquitination exist to regulate AMPAR trafficking and turnover with high specificity in response to differing neuronal signaling cues.

As seen in other model organisms, several synaptic proteins which regulate the delivery, removal, or stability of AMPA receptors at synapses in mammalian neurons have been found to be directly modified by ubiquitin and degraded by the proteasome. In many cases, the degradation of AMPA receptor-interacting proteins is mediated by synaptic activity. For instance, Colledge et al. (2003) found that the PDZ binding domain protein, PSD-95, is rapidly ubiquitinated by the E3 ubiquitin ligase Mdm2 in response to NMDA receptor activation, and mutations in PSD-95 which block its ubiquitination and degradation also block NMDA-induced AMPA receptor internalization. In addition, application of glutamate to cultured neurons promotes the rapid ubiquitin-dependent degradation of glutamate receptor interacting protein 1 (GRIP1), an important AMPA receptor scaffold, which in turns decreases AMPA receptor surface levels (Guo and Wang, 2007). More recently in a very elegant study by Greenberg and colleagues, it was shown that the development of excitatory synapses is regulated by the Angelman's syndrome ubiquitin E3 ligase Ube3A (Greer et al., 2010). Ube3A activity is up-regulated by experience-driven neuronal activity. This in turn regulates synapse development by controlling the degradation of Arc, a synaptic protein that promotes the internalization of the AMPA receptors. Disruption of Ube3A function in neurons leads to an increase in Arc expression and a concomitant decrease in the number of AMPA receptors at excitatory synapses. Beyond the proteins discussed here, there are many other components of the UPS which are regulated in an activity-dependent fashion (Bingol and Schuman, 2006; Cartier et al., 2009; Djakovic et al., 2009). It is likely that further examination of how the UPS is regulated by synaptic activity will yield vital insights into the function of the UPS at synapses.

Similar to AMPARs, several other synaptic receptors have been shown to undergo ubiquitination to regulate synaptic strength. NMDARs have been shown to undergo differential ubiquitination in response to various stimuli. For instance, the N-terminal domain of the NR1 subunit is targeted for ubiquitination and subsequent degradation by the F-box protein Fbx2 when bicuculline is chronically applied to hippocampal neurons (Kato et al., 2005). Separately, the NR2B subunit was shown to be ubiquitinated by the E3 ligase Mind bomb-2 (Mib2). When expressed together with the NR1 and NR2B in heterologous cells, Mib2 decreases the density of NMDA-elicited currents in an ubiquitin-dependent fashion (Jurd et al., 2008). Mib2 was shown to interact with the cytoplasmic domain of NR2B, and ubiquitinate it in a manner dependent on phosphorylation by the tyrosine kinase Fyn (Jurd et al., 2008), indicating that a dynamic relationship between receptor phosphorylation and ubiquitination may exist to control NR2B-containing NMDARs. In addition, kainite receptors have been shown to be modified by ubiquitin. Salinas et al. (2006) found that actinfilin acts as a scaffold for the Cul3 E3 ubiquitin ligase to target GluR6 for degradation. In response to prolonged

nicotine exposure, ubiquitin-dependent proteasomal turnover of the alpha7 nicotinic acetylcholine receptor (nAChR) subunit is decreased (Rezvani et al., 2007). Several other synaptic proteins are stabilized by treatment with nicotine including GluA1, GluN2A, mGluR1, Homer-1A, and PSD-95, suggesting that nicotine negatively regulates UPS function to control synaptic protein composition important for nicotine-dependent synaptic plasticity.

Ubiquitin-dependent regulation of receptor trafficking and turnover has been shown to also be important for inhibitory synaptic transmission. γ-Aminobutyric acid (GABA) receptors, the major receptors in the brain responsible for mediating inhibitory synaptic transmission, are directly ubiquitinated to regulate their trafficking and turnover at synapses. Similar to what was seen with AMPARs, the surface stability of $GABA_A$ receptors was reduced by chronic blockade of neuronal activity (Saliba et al., 2007). In addition, upon 24 h treatment with tetrodotoxin (TTX), the $GABA_A$ β3 receptor subunit became heavily ubiquitinated, and down-regulation of surface $GABA_A$Rs was dependent on proteasome activity. In this case, the loss of surface $GABA_A$Rs is thought to be caused by decreased stability of the receptor in the ER. Further work has described a separate role for ubiquitination of the $GABA_a$ γ2 subunit. Specifically, lysine residues on the γ2 subunit of $GABA_A$ are ubiquitinated to regulate the trafficking of $GABA_A$Rs in the endocytic pathway. Mutation of these ubiquitination sites resulted in a loss of targeting these receptors to the lysosome (Arancibia-Carcamo et al., 2009). In addition, it has been found that the inhibitory glycine receptor (GlyR) is ubiquitinated in the plasma membrane prior to internalization and subsequent endocytic sorting to the lysosome for proteolysis (Buttner et al., 2001). Taken together, ubiquitination may have a crucial role on both excitatory and inhibitory synaptic transmission by controlling postsynaptic receptor numbers.

Conclusions and future considerations

It has become apparent that ubiquitination serves as a major post-translational modification to regulate trafficking and turnover of membrane proteins. Recently, several ubiquitinated synaptic proteins have been identified in developing and mature neurons, suggesting that ubiquitination plays an important role in several aspects of neuronal development and function. Indeed, the trafficking and turnover of membrane proteins involved in neuronal fate specification, axon guidance and synaptic transmission have been shown to be regulated by ubiquitination. Several other pertinent questions lie ahead: What other membrane proteins are directly modified by ubiquitin and how does ubiquitination regulate their function? What other ubiquitin ligases regulate ubiquitin-dependent trafficking of membrane proteins in neurons? There are more than 600 E3 ligases in the mammalian genome, but only a few have known roles in neurons and at synapses. What is the role of deubiquitination to route membrane proteins to recycling or lysosomal degradation pathways? How does neuronal activity control the targeting of membrane proteins and their regulatory protein factors for ubiquitination? Answers to these basic questions will inevitably increase our understanding of how ubiquitination controls the surface levels of neuronal membrane proteins involved in such processes as synaptic development and plasticity. Also, since loss of surface receptors can lead to disruptions in neuronal signaling, an underlying mechanism for many neurological diseases, the answers to these questions may increase our understanding of age-related and neurodegenerative disorders such as AD. In AD, the secretion of the peptide beta amyloid (Aβ), generated from amyloid precursor protein (APP) in neurons, is thought to initiate the pathological defects associated with AD. However, it was recently suggested that decreased synaptic function is due to Aβ's ability to promote AMPAR endocytosis and synaptic depression (Hsieh et al., 2006; Wei et al.; Ting et al., 2007). Direct application of Aβ peptide or over-expression of APP in hippocampal neurons was shown to cause an increase in AMPAR endocytosis and subsequent loss in synaptic strength and spine density. As work emerges relating ubiquitination to AMPAR endocytosis and trafficking (and likely other neurotransmitter receptor), it is worthwhile to consider these recent findings in relationship to such disease paradigms.

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References

Acconcia, F., Sigismund, S., Polo, S., 2009. Ubiquitin in trafficking: the network at work. Exp. Cell Res. 315 (9), 1610–1618.
Arancibia-Carcamo, I.L., et al., 2009. Ubiquitin-dependent lysosomal targeting of GABA(A) receptors regulates neuronal inhibition. Proc. Natl. Acad. Sci. U.S.A. 106 (41), 17552–17557.
Arevalo, J.C., et al., 2006. Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. Neuron 50 (4), 549–559.
Bardin, A.J., Schweisguth, F., 2006. Bearded family members inhibit neuralized-mediated endocytosis and signaling activity of Delta in *Drosophila*. Dev. Cell 10 (2), 245–255.
Bingol, B., Schuman, E.M., 2004. A proteasome-sensitive connection between PSD-95 and GluR1 endocytosis. Neuropharmacology 47 (5), 755–763.
Bingol, B., Schuman, E.M., 2006. Activity-dependent dynamics and sequestration of proteasomes in dendritic spines. Nature 441 (7097), 1144–1148.
Bingol, B., Sheng, M., 2011. Deconstruction for reconstruction: the role of proteolysis in neural plasticity and disease. Neuron 69 (1), 22–32.
Bray, S.J., 2006. Notch signalling: a simple pathway becomes complex. Nat. Rev. Mol. Cell Biol. 7 (9), 678–689.
Burbea, M., et al., 2002. Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. Neuron 35 (1), 107–120.
Buttner, C., et al., 2001. Ubiquitination precedes internalization and proteolytic cleavage of plasma membrane-bound glycine receptors. J. Biol. Chem. 276 (46), 42978–42985.
Cajigas, I.J., Will, T., Schuman, E.M., 2010. Protein homeostasis and synaptic plasticity. Embo J. 29 (16), 2746–2752.
Campbell, D.S., Holt, C.E., 2001. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. Neuron 32 (6), 1013–1026.
Cartier, A.E., et al., 2009. Regulation of synaptic structure by ubiquitin C-terminal hydrolase L1. J. Neurosci. 29 (24), 7857–7868.
Chen, N., Greenwald, I., 2004. The lateral signal for LIN-12/Notch in *C. elegans* vulval development comprises redundant secreted and transmembrane DSL proteins. Dev. Cell 6 (2), 183–192.
Clague, M.J., Urbe, S., 2006. Endocytosis: the DUB version. Trends Cell Biol. 16 (11), 551–559.
Clague, M.J., Urbe, S., 2010. Ubiquitin: same molecule, different degradation pathways. Cell. 143 (5), 682–685.
Colledge, M., et al., 2003. Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. Neuron 40 (3), 595–607.
De Renzis, S., et al., 2006. Dorsal–ventral pattern of Delta trafficking is established by a Snail-Tom-Neuralized pathway. Dev. Cell 10 (2), 257–264.
Deblandre, G.A., Lai, E.C., Kintner, C., 2001. *Xenopus* neuralized is a ubiquitin ligase that interacts with XDelta1 and regulates Notch signaling. Dev. Cell 1 (6), 795–806.
Djakovic, S.N., et al., 2009. Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. J. Biol. Chem. 284 (39), 26655–26665.
Dreier, L., Burbea, M., Kaplan, J.M., 2005. LIN-23-mediated degradation of beta-catenin regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. Neuron 46 (1), 51–64.
Ehlers, M.D., 2003. Activity level controls postsynaptic composition and signaling via the ubiquitin–proteasome system. Nat. Neurosci. 6 (3), 231–242.
Emery, G., et al., 2005. Asymmetric Rab 11 endosomes regulate delta recycling and specify cell fate in the *Drosophila* nervous system. Cell 122 (5), 763–773.
Fu, A.K., et al., 2011. APC(Cdh1) mediates EphA4-dependent downregulation of AMPA receptors in homeostatic plasticity. Nat. Neurosci. 14 (2), 181–189.
Greer, P.L., et al., 2010. The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating arc. Cell. 140 (5), 704–716.
Guo, L., Wang, Y., 2007. Glutamate stimulates glutamate receptor interacting protein 1 degradation by ubiquitin–proteasome system to regulate surface expression of GluR2. Neuroscience 145 (1), 100–109.
Haglund, K., et al., 2004. Recruitment of Pyk2 and Cbl to lipid rafts mediates signals important for actin reorganization in growing neurites. J. Cell Sci. 117 (Pt 12), 2557–2568.
Hegde, A.N., Upadhya, S.C., 2011. Role of ubiquitin–proteasome-mediated proteolysis in nervous system disease. Biochim. Biophys. Acta 1809 (2), 128–140.
Hershko, A., Ciechanover, A., 1998. The ubiquitin system. Annu. Rev. Biochem. 67, 425–479.
Hicke, L., Dunn, R., 2003. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu. Rev. Cell Dev. Biol. 19, 141–172.

Hicke, L., Riezman, H., 1996. Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. Cell 84 (2), 277–287.
Hicks, C., et al., 2002. A secreted Delta1-Fc fusion protein functions both as an activator and inhibitor of Notch1 signaling. J. Neurosci. Res. 68 (6), 655–667.
Hsieh, H., et al., 2006. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron 52 (5), 831–843.
Ing, B., et al., 2007. Regulation of Commissureless by the ubiquitin ligase DNedd4 is required for neuromuscular synaptogenesis in *Drosophila melanogaster*. Mol. Cell. Biol. 27 (2), 481–496.
Ingham, R.J., Gish, G., Pawson, T., 2004. The Nedd4 family of E3 ubiquitin ligases: functional diversity within a common modular architecture. Oncogene 23 (11), 1972–1984.
Itoh, M., et al., 2003. Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta. Dev. Cell 4 (1), 67–82.
Juo, P., Kaplan, J.M., 2004. The anaphase-promoting complex regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. Curr. Biol. 14 (22), 2057–2062.
Jurd, R., et al., 2008. 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. 283 (1), 301–310.
Kato, A., et al., 2005. Activity-dependent NMDA receptor degradation mediated by retrotranslocation and ubiquitination. Proc. Natl. Acad. Sci. U.S.A. 102 (15), 5600–5605.
Keleman, K., et al., 2002. Comm sorts robo to control axon guidance at the *Drosophila* midline. Cell 110 (4), 415–427.
Keleman, K., Ribeiro, C., Dickson, B.J., 2005. Comm function in commissural axon guidance: cell-autonomous sorting of Robo in vivo. Nat. Neurosci. 8 (2), 156–163.
Kolling, R., Hollenberg, C.P., 1994. The ABC-transporter Ste6 accumulates in the plasma membrane in a ubiquitinated form in endocytosis mutants. EMBO J. 13 (14), 3261–3271.
Kowalski, J.R., Dahlberg, C.L., Juo, P., 2011. 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. 31 (4), 1341–1354.
Kumar, S., et al., 1997. cDNA cloning, expression analysis, and mapping of the mouse Nedd4 gene. Genomics 40 (3), 435–443.
Lai, E.C., et al., 2001. *Drosophila* neuralized is a ubiquitin ligase that promotes the internalization and degradation of delta. Dev. Cell 1 (6), 783–794.
Le Borgne, R., et al., 2005. Two distinct E3 ubiquitin ligases have complementary functions in the regulation of delta and serrate signaling in *Drosophila*. PLoS Biol. 3 (4), e96.
Lin, A., et al., 2011. Nedd4-mediated AMPA receptor ubiquitination regulates receptor turnover and trafficking. J Neurochem. doi:10.1111/j.1471-4159.2011.07221.x.
Lussier, M.P., Nasu-Nishimura, Y., Roche, K.W., 2011. Activity-dependent ubiquitination of the AMPA receptor subunit GluA2. J Neurosci. 31 (8), 3077–3081.
Mabb, A.M., Ehlers, M.D., 2010. Ubiquitination in postsynaptic function and plasticity. Annu Rev Cell Dev Biol. 26, 179–210.
Malenka, R.C., 2003. Synaptic plasticity and AMPA receptor trafficking. Ann. N. Y. Acad. Sci. 1003, 1–11.
Malinow, R., Malenka, R.C., 2002. AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. 25, 103–126.
Myat, A., et al., 2002. *Drosophila* Nedd4, a ubiquitin ligase, is recruited by Commissureless to control cell surface levels of the roundabout receptor. Neuron 35 (3), 447–459.
Overstreet, E., Fitch, E., Fischer, J.A., 2004. Fat facets and liquid facets promote Delta endocytosis and Delta signaling in the signaling cells. Development 131 (21), 5355–5366.
Park, E.C., Glodowski, D.R., Rongo, C., 2009. The ubiquitin ligase RPM-1 and the p38 MAPK PMK-3 regulate AMPA receptor trafficking. PLoS One 4 (1), e4284.
Parks, A.L., et al., 2000. Ligand endocytosis drives receptor dissociation and activation in the Notch pathway. Development 127 (7), 1373–1385.
Patrick, G.N., 2006. Synapse formation and plasticity: recent insights from the perspective of the ubiquitin proteasome system. Curr. Opin. Neurobiol. 16 (1), 90–94.
Patrick, G.N., et al., 2003. Ubiquitin-mediated proteasome activity is required for agonist-induced endocytosis of GluRs. Curr. Biol. 13 (23), 2073–2081.
Pavlopoulos, E., et al., 2001. neuralized Encodes a peripheral membrane protein involved in delta signaling and endocytosis. Dev. Cell 1 (6), 807–816.
Pickart, C.M., Eddins, M.J., 2004. Ubiquitin: structures, functions, mechanisms. Biochim. Biophys. Acta 1695 (1–3), 55–72.
Rezvani, K., et al., 2007. Nicotine regulates multiple synaptic proteins by inhibiting proteasomal activity. J. Neurosci. 27 (39), 10508–10519.
Roth, A.F., Davis, N.G., 1996. Ubiquitination of the yeast a-factor receptor. J. Cell Biol. 134 (3), 661–674.
Saksena, S., Emr, S.D., 2009. ESCRTs and human disease. Biochem. Soc. Trans. 37 (Pt 1), 167–172.
Saliba, R.S., et al., 2007. Activity-dependent ubiquitination of GABA(A) receptors regulates their accumulation at synaptic sites. J. Neurosci. 27 (48), 13341–13351.
Salinas, G.D., et al., 2006. Actinfilin is a Cul3 substrate adaptor, linking GluR6 kainate receptor subunits to the ubiquitin–proteasome pathway. J. Biol. Chem. 281 (52), 40164–40173.
Schaefer, H., Rongo, C., 2006. KEL-8 is a substrate receptor for CUL3-dependent ubiquitin ligase that regulates synaptic glutamate receptor turnover. Mol. Biol. Cell 17 (3), 1250–1260.
Schwarz, L.A., Hall, B.J., Patrick, G.N., 2010. Activity-dependent ubiquitination of GluA1 mediates a distinct AMPA receptor endocytosis and sorting pathway. J Neurosci. 30 (49), 16718–16729.
Shepherd, J.D., Huganir, R.L., 2007. The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu. Rev. Cell Dev. Biol. 23, 613–643.
Silies, M., Klambt, C., 2010. APC/C(Fzr/Cdh1)-dependent regulation of cell adhesion controls glial migration in the *Drosophila* PNS. Nat Neurosci. 13 (11), 1357–1364.
Simons, K., Toomre, D., 2000. Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. 1 (1), 31–39.
Sorkina, T., et al., 2006. RNA interference screen reveals an essential role of Nedd4-2 in dopamine transporter ubiquitination and endocytosis. J. Neurosci. 26 (31), 8195–8205.
Strous, G.J., et al., 2004. Ubiquitin system-dependent regulation of growth hormone receptor signal transduction. Curr. Top. Microbiol. Immunol. 286, 81–118.
Tear, G., et al., 1996. commissureless controls growth cone guidance across the CNS midline in *Drosophila* and encodes a novel membrane protein. Neuron 16 (3), 501–514.
Tessier-Lavigne, M., Goodman, C.S., 1996. The molecular biology of axon guidance. Science 274 (5290), 1123–1133.
Thelen, K., et al., 2008. Ubiquitination and endocytosis of cell adhesion molecule DM-GRASP regulate its cell surface presence and affect its role for axon navigation. J. Biol. Chem. 283 (47), 32792–32801.
Ting, J.T., et al., 2007. Amyloid precursor protein overexpression depresses excitatory transmission through both presynaptic and postsynaptic mechanisms. Proc. Natl. Acad. Sci. U.S.A. 104 (1), 353–358.
van Roessel, P., et al., 2004. Independent regulation of synaptic size and activity by the anaphase-promoting complex. Cell 119 (5), 707–718.
Varnum-Finney, B., et al., 2000. Immobilization of Notch ligand, Delta-1, is required for induction of notch signaling. J. Cell Sci. 113 (Pt 23), 4313–4318.
Wang, W., Struhl, G., 2004. *Drosophila* Epsin mediates a select endocytic pathway that DSL ligands must enter to activate Notch. Development 131 (21), 5367–5380.
Wang, W., Struhl, G., 2005. Distinct roles for Mind bomb, Neuralized and Epsin in mediating DSL endocytosis and signaling in *Drosophila*. Development 132 (12), 2883–2894.
Wei, W., et al., 2010. Amyloid beta from axons and dendrites reduces local spine number and plasticity. Nat Neurosci. 13 (2), 190–196.
Wilkinson, K.D., 1997. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. FASEB J. 11 (14), 1245–1256.
Williams, R.L., Urbe, S., 2007. The emerging shape of the ESCRT machinery. Nat. Rev. Mol. Cell Biol. 8 (5), 355–368.
Xu, P., et al., 2009. Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. Cell 137 (1), 133–145.
Yi, J.J., Ehlers, M.D., 2007. Emerging roles for ubiquitin and protein degradation in neuronal function. Pharmacol. Rev. 59 (1), 14–39.
Yuasa-Kawada, J., et al., 2009. Midline crossing and Slit responsiveness of commissural axons require USP33. Nat. Neurosci. 12 (9), 1087–1089.