

Mechanism of synaptic protein turnover and its regulation by neuronal activity

Tolga Soykan^{1*}, Volker Haucke^{1,2#} and Marijn Kuijpers^{1#},

Affiliations

¹Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Robert-Rössle-Straße 10, 13125 Berlin, Germany

²Freie Universität Berlin, Faculty of Biology, Chemistry, Pharmacy, 14195 Berlin, Germany

*co-first authors

#Correspondence: e-mail: haucke@fmp-berlin.de; kuijpers@fmp-berlin.de; phone: +49-30-947 93-101; fax: +49-30-947 93-109

Highlights

- Synaptic protein turnover is regulated by neuronal activity and other factors
- Multiple systems cooperate to control synaptic proteostasis
- Neuronal autophagy impacts on synaptic function
- Lysosomes may degrade synaptic proteins locally or in somata
- Ubiquitin serves as a common degradation signal for UPS and lysosomes

Abstract

Neurons are long-lived cells with a complex architecture, in which synapses may be located far away from the cell body and are subject to plastic changes, thereby posing special challenges to the systems that maintain and dynamically regulate the synaptic proteome. These mechanisms include neuronal autophagy and the endolysosome pathway as well as the ubiquitin/ proteasome system, which cooperate in the constitutive and regulated turnover of pre- and postsynaptic proteins. Here we summarize the pathways involved in synaptic protein degradation and the mechanisms underlying their regulation, for example by neuronal activity, with an emphasis on the presynaptic compartment and outline perspectives for future research.

Introduction

Protein components (i.e. amino acids) need to be recycled to produce new materials. In addition, proteins can be misfolded, damaged as a consequence of oxidative stress, disease or aging, or be dispensable for function at a given time or place. Protein turnover is therefore essential for maintaining healthy and functional cells. This is of particular importance for neurons, e.g. long-lived cells with a highly polarized architecture and complex morphology that present special challenges with respect to the machineries that spatiotemporally regulate protein turnover, as synapses can be located far away from the cell body where the majority of degradative processes take place [1-3]. To maintain and plastically regulate neurotransmission, a process that involves presynaptic

neurotransmitter release by synaptic vesicle (SV) fusion and recognition of neurotransmitter molecules by postsynaptic receptors and their associated factors, synaptic proteins have to be turned over [4].

Actively releasing SVs have a finite lifetime, and aging vesicles get inactivated and eventually degraded [5]. As protein degradation is essential for the remodeling of synapses and for synaptic plasticity [6], synaptic protein turnover is expected to be regulated by synaptic activity. Indeed, experiments in neuronal cultures show that the turnover of presynaptic vesicle proteins is facilitated by increased neuronal activity. Conversely, synaptic protein synthesis and degradation decrease in response to suppression of activity [5-7]. Moreover, experience-dependent synaptic plasticity in mice accelerates the turnover of the majority of synaptic proteins [7].

In this review, we will summarize three pathways involved in synaptic protein degradation, i.e. autophagy, the endolysosomal pathway and the proteasome system. We discuss the mechanisms underlying their regulation, e.g. by neuronal activity and mTOR with emphasis on the presynaptic and axonal compartments.

Neuronal protein turnover *in vitro* and *in vivo*

Recent methodological advances, for example combining stable isotope labeling of proteins with mass spectrometry, have enabled high-throughput analysis of protein lifetimes. Such studies show that the rate of protein turnover depends on protein function, protein complex architecture, and subcellular localization, as well as on the neuronal cell type and environment [7-9]. As protein turnover appears to be essential for the regulation of neuronal activity and for synapse remodeling [10], neuronal protein half-lives have been intensively investigated in the past years. High-throughput *in vitro* studies in cortical or hippocampal rat cultures have shown that the half-lives of most proteins, including synaptic proteins range from 2 to 5 days [11]. Similar studies in the mouse brain using metabolic labeling *in vivo* have revealed much longer (on average 9 days) half-lives of neuronal proteins [12], i.e. far longer than those observed in other organs such as liver or blood. These studies show that neuronal proteins are longer-lived *in vivo* (2-4 times) than in cultured neurons *in vitro*, but overall the measured half-lives correlate well with each other [4,6]. Recent developments have also enabled the direct visualization of the degradation of specific synaptic proteins. Fluorescent reporters such as fluorescent timers (FTs) or the drug-controllable TimeSTAMPs [13,14] allow tracking of newly synthesized copies of tagged proteins or, by removing an inhibitor, the visualization of their degradative turnover. Another approach uses amino acid labeling/ puromycylation via proximity ligation to visualize degradation of endogenous neuronal proteins [15], revealing degradation rates that overall appear to be comparable to the rates found by metabolic labeling strategies. Collectively, these approaches have yielded important insights into the dynamics and time course of synaptic protein turnover. However, we still lack a clear understanding how neurons determine which of the individual synaptic proteins are targeted for degradation via a specific route and how these routes cooperate to ensure long-term maintenance of the synaptic proteome.

Neuronal autophagy

Recent work in many systems including neurons have established crucial roles for autophagy in the regulation of protein turnover and for the maintenance of neuronal health

[16,17]. Although often used synonymously, there are at least three distinct types of autophagy, i.e., microautophagy, chaperone-mediated autophagy and macroautophagy. While morphologically and mechanistically distinct, all three culminate in the delivery of cargo to the lysosome. Microautophagy is defined by the uptake of material into the lysosomal lumen via membrane invaginations. A variation of this process termed endosomal microautophagy has been shown to operate in *D. melanogaster*. This process, which appears to be repressed by mechanistic target of rapamycin (mTOR) signaling [18], mediates the turnover of soluble synaptic proteins that harbor a recognition motif for the membrane-deforming chaperone Hsc70-4 by engulfment into intraluminal vesicles of late endosomes (also termed multivesicular bodies, MVBs). Endosomal microautophagy thereby regulates the efficiency of neurotransmission via rejuvenation of parts of the synaptic protein pool [19]. Endosomal microautophagy is distinct from chaperone-mediated autophagy, which involves the direct translocation of substrate proteins across the lysosomal membrane. Finally, macroautophagy (hereafter simply referred to as autophagy), the best studied form of autophagy, involves the formation of cytoplasmic double-membrane organelles termed autophagosomes that sequester defective, superfluous proteins and organelles [16,17]. The evolutionarily conserved autophagy-related (ATG) genes tightly regulate this process. Once formed, autophagosomes undergo further maturation steps (i.e. by fusion with late endosomes) before delivering their content to lysosomes for degradation [1].

Lysosomes are the degradative endpoint shared by both autophagy and the endosomal pathway. Akin to other neuronal features, the degradative system in neurons displays a high level of spatial compartmentalization (Figure 1). While degradative compartments have been observed at synaptic terminals [20], degradative lysosomes are enriched in neuronal somata [21,22] and receive cargo from both dendrites and axons [22]. Although autophagosome biogenesis can occur in the soma and in dendrites, under basal conditions most autophagosomes form in distal axons, near or within presynaptic compartments, and then traffic retrogradely to fuse with mature lysosomes in the soma [22]. Recent data show that neuronal autophagy contributes to a variety of pre- and postsynaptic functions, such as the induction of postsynaptic long-term depression (LTD) [23], dendritic spine pruning [24], synapse development [25], and the control of neurotransmitter release at excitatory glutamatergic and dopaminergic synapses [26,27]. Combined electrophysiological, quantitative proteomic, and cell biological analysis in conditional ATG5 knockout mice showed that loss of neuronal autophagy causes the selective accumulation of tubular endoplasmic reticulum (ER) in axons, resulting in increased excitatory neurotransmission as a consequence of elevated calcium release from ER stores via ryanodine receptors [27] (Figure 2A). Surprisingly, the half-lives of key synaptic proteins were unaltered in these ATG5-deficient neurons. Elevated neurotransmitter release was also observed in dopaminergic neurons from α -synuclein-expressing conditional ATG7 knockout mice resulting in improved movement in spite of the observed profound Parkinson disease pathology [26]. These data argue that neuronal autophagy may play important roles in regulated synaptic protein and organelle turnover with important physiological and pathophysiological consequences.

Despite the progress in understanding the mechanisms of autophagy, we still have limited knowledge on the external cues that control the initiation and maintenance of neuronal autophagy. Unlike in other cell-types, starvation has little effect in inducing

autophagy in the brain and in neurons, suggesting alternative external signals [22]. The autophagy pathway shares some key components with the machineries for neurotransmission and SV recycling, thereby linking synaptic activity to autophagic degradation. These include functions of the membrane remodelling protein endophilin and its effector synaptojanin in autophagosome formation [3,28] and of the scaffolding protein Bassoon in limiting presynaptic autophagy by interacting with the autophagy-associated E3-like ligase ATG5 and crosstalk with parkin [29]. Interestingly, recent studies have also implicated Bassoon in the regulation of proteasomal degradation [30,31], a pathway further discussed below (Figure 2B). A further autoregulatory circuit between autophagy and ubiquitination has recently been identified in the *C. elegans* nervous system [32].

While these studies do not directly link neuronal activity and general autophagy, other studies indicate that autophagy can be regulated by neuronal activity, possibly in a compartment-specific fashion. While some studies show that autophagy is inhibited during NMDA receptor-mediated LTD to prevent the endocytic recycling of AMPA-type glutamate receptors [23], other data suggest that LTD stimuli trigger local autophagosome biogenesis in post-synaptic dendrites to mediate degradation of synaptic cargo (i.e. AMPA receptors and/ or associated cytoskeletal proteins) [33,34]. On a global scale NMDA application has been reported to increase the number of autophagosomes [34], e.g. via PI 3-kinase-mTOR pathway inhibition. Consistently, neuronal stimulation has been shown to induce autophagy in a variety of models including *D. melanogaster* and *C. elegans* [1,3]. Conversely, postsynaptic G protein-coupled metabotropic glutamate receptor 1 (mGluR1) may serve a “moonlighting” function in repressing constitutive neuronal autophagy by interacting with FEZ1, an adaptor protein implicated in neuronal autophagy [35]. Clearly, more work is needed to fully explore the relationship between local or global regulation of autophagy, the turnover of specific autophagic substrates, and neuronal activity.

Endolysosomal turnover of presynaptic proteins

In addition to autophagy, the turnover of synaptic proteins and maintenance of synaptic transmission depend on the endolysosomal pathway. While little is known about the role of the endolysosomal system in the turnover of postsynaptic proteins, previous data in cultured neurons suggest that presynaptic vesicle (SV) proteins are subject to quality-control [31,36] via mechanisms involving ubiquitination and sorting into late endosomes via the ESCRT machinery. The ESCRT system comprises a series of protein complexes that catalyze the inward budding and fission of vesicles from the limiting membrane of endosomes. These intraluminal vesicles contain the cargo for lysosomal degradation [37].

How presynaptic protein turnover via endolysosomal sorting is linked to neuronal activity remains poorly understood. ESCRT-mediated turnover of SV proteins has been suggested to be controlled in part by endosomal Rab35 [38] and the Rab GTPase activating protein Skywalker/TBC1D24 [36,38], i.e. proteins whose function maybe regulated by neuronal activity. Growing evidence suggests that the components of the ESCRT machinery, such as Hrs and STAM1, are recruited to synaptic terminals via the anterograde kinesin motor protein KIF13A in an activity-dependent manner [39], providing

a possible explanation for the observation that neuronal activity facilitates the turnover of select SV proteins [38].

Another unsolved question regarding the mechanism of presynaptic protein turnover via the endolysosomal system pertains to the site(s) of synaptic protein degradation. Conceivably, this could either occur by local degradation in synaptically localized degradative organelles, e.g. mature degradative lysosomes, or via retrograde transport of synaptic cargos contained in retrogradely trafficked endosomes that fuse with lysosomes in neuronal somata. In this context it is important to bear in mind that the frequently used lysosome marker LAMP1 is not only found in mature lysosomes, but also in a variety of different organelles including autolysosomes and late endosomes that lack degradative enzymes and often are non- or only mildly acidic. Live imaging studies paired with luminal pH measurements have shown that LAMP1-labelled axonal endocytic organelles become more acidic as they approach the soma. Once axonal endosomes reach neuronal somata, they fuse with active hydrolase-containing organelles (i.e. *bona fide* lysosomes) to degrade axonal and synaptic cargos [21]. Although a recent study indicates that LAMP1-positive, active cathepsin containing organelles can traffic anterogradely into axons [40], it thus appears that the majority of axonal and presynaptic proteins are degraded in neuronal somata following delivery via retrogradely targeted endosomes (Figure 1). Similar mechanisms may apply to the turnover of postsynaptic proteins such as AMPA receptors that undergo endosomal sorting (but also autophagy, see above) and lysosomal turnover during LTD [41] and the cell adhesion receptor neuroligin 2, which uses a retromer-based sorting mechanism to control its levels and, thereby inhibitory synapse number [42].

Control of synaptic proteostasis by the ubiquitin-proteasome system and mTOR

In addition to autophagy and the endolysosomal system, which are mostly responsible for the turnover of membrane proteins and large protein assemblies including entire organelles, most cytoplasmic proteins including axoplasmic and dendritic proteins are degraded by the Ubiquitin-Proteasome System (UPS) [43]. Although protein breakdown via these two pathways takes place in distinct subcellular locations and utilizes different degradative enzymes, the UPS and lysosomal degradation systems share some similarities and functionally cooperate to maintain synaptic protein homeostasis [43]. A central feature of both pathways is the ability to selectively target substrates by site-specific mono-, multi, or poly-ubiquitination.

Proteins labeled with ubiquitin chains of four or more molecules are targeted to the 26S proteasome, a cytoplasmic barrel shaped protein machine that recognizes, unfolds and degrades polyubiquitinated proteins. Pharmacological inhibition of proteasome-dependent degradation causes defects in long-term potentiation (LTP) and LTP-related behaviors [10]. Multiple lines of evidence link the activity of the UPS pathway to synaptic activity and function. For example, neuronal activity can cause UPS-dependent alterations in the composition and turnover of postsynaptic scaffold proteins and receptors, such as AMPA-type glutamate receptors and PSD-95 [44,45]. A recent study also showed that molecular motor KIF17 is degraded locally in dendrites by the UPS in an activity-dependent manner [46]. Using an *in vivo* proteasome-activity reporter, Bingol and Schuman revealed that neuronal activation reduces the exit rate of proteasomes in spines of rat hippocampal neurons, resulting in increased proteasomal degradation [47].

Among the many synaptic clients for UPS-mediated degradation are the presynaptic active zone scaffold proteins Liprin- α [48] and Bassoon [49], which in turn represses proteasome activity [30], and the postsynaptic plasticity regulator Arc [50] among others. That said, a proteomic study based on prolonged pharmacological inhibition of the proteasome in mouse hippocampal cultures did not report significant differences in the half-lives of most neuronal and synaptic proteins [11], suggesting that the UPS, autophagy, and the endolysosomal system may partially compensate for each other with respect to the regulation of neuronal and synaptic protein turnover, consistent with recent studies in non-neuronal cells [51].

In addition to proteasomal activity, protein ubiquitination, which can target substrate proteins for degradation via either the proteasome or the autophagy/endolysosomal pathway [43], is critical for synaptic function and has been shown to be regulated, at least in part, by neuronal activity and/ or synaptic function. For example, depolarization-dependent Ca^{2+} influx into synaptosomes produces a global and rapid decrease in presynaptic protein ubiquitination [52]. The ubiquitin E3-ligases SIAH1 and Parkin, which ubiquitinate synaptic vesicle proteins, are repressed by the presynaptic scaffold protein Bassoon [29,31], thereby providing a possible mechanism for the activity-dependent control of ubiquitination and SV protein turnover at the presynapse (Figure 2B). The Angelman syndrome-linked ubiquitin ligase E3A (Ube3a) plays a crucial role in synaptic plasticity and its expression is dynamically regulated by neuronal activity [53]. The epilepsy-associated Nedd4 family of E3 ligases have been implicated in the ubiquitination and subsequent proteasomal and/ or lysosomal degradation of a wide variety of neuronal membrane proteins such as voltage-gated sodium channels, calcium channels, and ionotropic as well as metabotropic glutamate receptors [45,54]. Finally, transgenic mice that chronically overexpress ubiquitin display reduced AMPA receptor (i.e. GluA1) expression and reduced synaptic plasticity [55], which may conceivably be explained by its elevated turnover. In spite of these evidences in favor of a crucial role for ubiquitin-triggered protein turnover at synapses, one has to bear in mind that ubiquitination can also alter protein function and dynamics independent of degradation.

Another mechanism that can link neuronal activity to degradation via the UPS and/ or the autophagy/endolysosomal pathway is mechanistic target of rapamycin (mTOR) signaling. mTOR is a serine/threonine kinase that regulates cell metabolism in response to extracellular stimuli, such as nutrients and growth factors. mTOR repression during nutrient-deficient conditions suppresses protein synthesis and induces autophagy. In neurons, mTOR is present at lysosomes and at synaptic regions, where it regulates synaptic strength and modulates both pre-and postsynaptic neurotransmission [56]. While neuronal autophagy does not appear to be triggered by nutrient starvation, mTOR can be engaged by other stimuli, including growth factors such as brain-derived neurotrophic factor (BDNF) [57], ischemia [58] and neuronal activity [34]. In addition, mTOR has been implicated in controlling UPS-mediated degradation [59], although the specific consequences of mTOR inhibition/ activation on UPS activity are still under debate.

In conclusion, how neuronal activity, or other extracellular cues, regulate ubiquitination and degradation at synapses, and how this is linked to either UPS or lysosomal degradation remains poorly understood and represents an important field for further study.

Conclusion and perspectives

While we have gained substantial new insights in recent years into the mechanisms and machineries involved in synaptic protein turnover, many fundamental questions remain unsolved. For example, we still do not understand how pre- and postsynaptic proteins are tagged for degradation and how an aged defective synaptic protein is distinguished molecularly from its young functional counterpart. We also do not know whether the components of synaptic organelles such as SVs are degraded *in toto* or whether individual constituent proteins are turned over and replaced by newly synthesized ones as vesicles undergo exo-/ endocytic cycling. Moreover, the relative contributions and modes of regulation of the various pathways involved in synaptic protein turnover remain incompletely understood, both with respect to physiology but also in pathophysiological conditions and during aging. Recent evidence suggests that renewal and/ or readjustment of the synaptic proteome is crucial for the maintenance of synaptic function and for learning. For example, studies in *D. melanogaster* suggest that accumulation of key factors for neurotransmitter release, e.g. active zone proteins such as Brp, during the wake phase must be counteracted by their partial removal during sleep, possibly via sleep-induced degradation, to reset information processing and storage and to support learning [60]. Similar mechanisms may operate in the mammalian brain [61,62] and likely are of relevance for ageing and the prevention of aging-induced memory decline, for example by boosting autophagy [63,64]. The development of novel imaging and proteomic techniques paired with genetic and acute manipulations of the machineries involved in synaptic protein turnover will help to shed light on some of these exciting questions and their implications for the healthy and the diseased brain.

Conflict of interest: none

Acknowledgements

We thank Dr. Barth van Rossum (FMP, Berlin) for help with the artwork. Our own research was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) [HA 2686/13-1 to V.H.], the Neurocore Cluster of Excellence (DFG-Exc-257), the German Ministry of Science (BMBF; SMARTAGE, to V.H.), and a postdoctoral fellowship of the European Union (to M.K.).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Hill SE, Colon-Ramos DA: **The Journey of the Synaptic Autophagosome: A Cell Biological Perspective.** *Neuron* 2020, **105**:961-973.
2. Katsumata K, Nishiyama J, Inoue T, Mizushima N, Takeda J, Yuzaki M: **Dynein- and activity-dependent retrograde transport of autophagosomes in neuronal axons.** *Autophagy* 2010, **6**:378-385.

3. Soukup SF, Kuenen S, Vanhauwaert R, Manetsberger J, Hernandez-Diaz S, Swerts J, Schoovaerts N, Vilain S, Gounko NV, Vints K, et al.: **A LRRK2-Dependent EndophilinA Phosphoswitch Is Critical for Macroautophagy at Presynaptic Terminals.** *Neuron* 2016, **92**:829-844.
4. Cohen LD, Ziv NE: **Neuronal and synaptic protein lifetimes.** *Curr Opin Neurobiol* 2019, **57**:9-16.
5. Truckenbrodt S, Viplav A, Jahne S, Vogts A, Denker A, Wildhagen H, Fornasiero EF, Rizzoli SO: **Newly produced synaptic vesicle proteins are preferentially used in synaptic transmission.** *EMBO J* 2018, **37**.
- *6. Dorrbaum AR, Alvarez-Castelao B, Nassim-Assir B, Langer JD, Schuman EM: **Proteome dynamics during homeostatic scaling in cultured neurons.** *Elife* 2020, **9**.

In this study, dynamic SILAC in combination with MS is used to quantify changes in protein synthesis and degradation in primary hippocampal neuron during synaptic up- or downscaling. Most synaptic proteins were regulated by scaling by a decrease in synthesis or degradation.

7. Heo S, Diering GH, Na CH, Nirujogi RS, Bachman JL, Pandey A, Haganir RL: **Identification of long-lived synaptic proteins by proteomic analysis of synaptosome protein turnover.** *Proc Natl Acad Sci U S A* 2018, **115**:E3827-E3836.
8. Mathieson T, Franken H, Kosinski J, Kurzawa N, Zinn N, Sweetman G, Poeckel D, Ratnu VS, Schramm M, Becher I, et al.: **Systematic analysis of protein turnover in primary cells.** *Nat Commun* 2018, **9**:689.
9. Dorrbaum AR, Kochen L, Langer JD, Schuman EM: **Local and global influences on protein turnover in neurons and glia.** *Elife* 2018, **7**.
10. Fonseca R, Vabulas RM, Hartl FU, Bonhoeffer T, Nagerl UV: **A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP.** *Neuron* 2006, **52**:239-245.
11. Hakim V, Cohen LD, Zuchman R, Ziv T, Ziv NE: **The effects of proteasomal inhibition on synaptic proteostasis.** *EMBO J* 2016, **35**:2238-2262.
- **12. Fornasiero EF, Mandad S, Wildhagen H, Alevra M, Rammner B, Keihani S, Opazo F, Urban I, Ischebeck T, Sakib MS, et al.: **Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions.** *Nat Commun* 2018, **9**:4230.

While earlier studies have measured lifetimes of neuronal proteins *in vitro*, the authors of this study developed an approach to precisely determine lifetimes of mouse brain proteins *in vivo*. This revealed for instance that presynaptic proteins are shorter living in cultured cells than *in vivo*.

13. Lin MZ, Glenn JS, Tsien RY: **A drug-controllable tag for visualizing newly synthesized proteins in cells and whole animals.** *Proc Natl Acad Sci U S A* 2008, **105**:7744-7749.
14. Subach FV, Subach OM, Gundorov IS, Morozova KS, Piatkevich KD, Cuervo AM, Verkhusha VV: **Monomeric fluorescent timers that change color from blue to red report on cellular trafficking.** *Nat Chem Biol* 2009, **5**:118-126.
15. Shahar OD, Schuman EM: **Large-scale cell-type-specific imaging of protein synthesis in a vertebrate brain.** *Elife* 2020, **9**.
16. Fleming A, Rubinsztein DC: **Autophagy in Neuronal Development and Plasticity.** *Trends Neurosci* 2020, **43**:767-779.
17. Lieberman OJ, Sulzer D: **The Synaptic Autophagy Cycle.** *J Mol Biol* 2020, **432**:2589-2604.

18. Khan A, Paro S, McGurk L, Sambrani N, Hogg MC, Brindle J, Pennetta G, Keegan LP, O'Connell MA: **Membrane and synaptic defects leading to neurodegeneration in Adar mutant Drosophila are rescued by increased autophagy.** *Bmc Biology* 2020, **18**.
19. Uytterhoeven V, Lauwers E, Maes I, Miskiewicz K, Melo MN, Swerts J, Kuenen S, Wittocx R, Corthout N, Marrink SJ, et al.: **Hsc70-4 Deforms Membranes to Promote Synaptic Protein Turnover by Endosomal Microautophagy.** *Neuron* 2015, **88**:735-748.
20. Jin EJ, Kiral FR, Ozel MN, Burchardt LS, Osterland M, Epstein D, Wolfenberg H, Prohaska S, Hiesinger PR: **Live Observation of Two Parallel Membrane Degradation Pathways at Axon Terminals.** *Current Biology* 2018, **28**:1027-+.
21. Cheng XT, Xie YX, Zhou B, Huang N, Farfel-Becker T, Sheng ZH: **Characterization of LAMP1-labeled nondegradative lysosomal and endocytic compartments in neurons.** *J Cell Biol* 2018, **217**:3127-3139.
22. Boecker CA, Holzbaur ELF: **Vesicular degradation pathways in neurons: at the crossroads of autophagy and endo-lysosomal degradation.** *Current Opinion in Neurobiology* 2019, **57**:94-101.
23. Shen H, Zhu H, Panja D, Gu Q, Li Z: **Autophagy controls the induction and developmental decline of NMDAR-LTD through endocytic recycling.** *Nat Commun* 2020, **11**:2979.
24. Pascual M, Lopez-Hidalgo R, Montagud-Romero S, Urena-Peralta JR, Rodriguez-Arias M, Guerri C: **Role of mTOR-regulated autophagy in spine pruning defects and memory impairments induced by binge-like ethanol treatment in adolescent mice.** *Brain Pathol* 2020.
25. Shen W, Ganetzky B: **Autophagy promotes synapse development in Drosophila.** *J Cell Biol* 2009, **187**:71-79.
- *26. Hunn BHM, Vingill S, Threlfell S, Alegre-Abarrategui J, Magdelyns M, Deltheil T, Bengoa-Vergniory N, Oliver PL, Cioroch M, Doig NM, et al.: **Impairment of Macroautophagy in Dopamine Neurons Has Opposing Effects on Parkinsonian Pathology and Behavior.** *Cell Rep* 2019, **29**:920-931 e927.

This study shows that mice with a conditional deletion of Atg7 in dopamine neurons have progressive neuron loss. Paradoxically, impaired autophagy also increased dopamine neurotransmission leading to improved movement in elderly mice, indicating that impairing autophagy in Parkinson's disease patients could improve motor performance

- **27. Kuijpers M, Kochlamazashvili G, Stumpf A, Puchkov D, Swaminathan A, Lucht MT, Krause E, Maritzen T, Schmitz D, Haucke V: **Neuronal Autophagy Regulates Presynaptic Neurotransmission by Controlling the Axonal Endoplasmic Reticulum.** *Neuron* 2020.

This article shows that, in the absence of proteotoxic challenges, the axonal tubular ER is a major substrate for neuronal autophagy, this in contrast to synaptic proteins.

28. Vanhauwaert R, Kuenen S, Masius R, Bademosi A, Manetsberger J, Schoovaerts N, Bounti L, Gontcharenko S, Swerts J, Vilain S, et al.: **The SAC1 domain in synaptotagmin is required for autophagosome maturation at presynaptic terminals.** *EMBO J* 2017, **36**:1392-1411.
- **29. Hoffmann-Conaway S, Brockmann MM, Schneider K, Annamneedi A, Rahman KA, Bruns C, Textoris-Taube K, Trimbuch T, Smalla KH, Rosenmund C, et al.: **Parkin contributes to synaptic vesicle autophagy in Bassoon-deficient mice.** *Elife* 2020, **9**.

Together with [30] and [31], this study shows that AZ proteins Bassoon and Piccolo play important roles in maintaining synapse integrity by regulating protein ubiquitination and degradation. This study in particular provides evidence that loss of Bassoon leads to elevated levels of ubiquitinated synaptic proteins and suggests that SVs are degraded *in toto*.

30. Montenegro-Venegas C, Fienko S, Anni D, Pina-Fernandez E, Frischknecht R, Fejtova A: **Bassoon inhibits proteasome activity via interaction with PSMB4.** *Cellular and Molecular Life Sciences* 2020.
31. Waites CL, Leal-Ortiz SA, Okerlund N, Dalke H, Fejtova A, Altmann WD, Gundelfinger ED, Garner CC: **Bassoon and Piccolo maintain synapse integrity by regulating protein ubiquitination and degradation.** *EMBO J* 2013, **32**:954-969.
32. Crawley O, Opperman KJ, Desbois M, Adrados I, Borgen MA, Giles AC, Duckett DR, Grill B: **Autophagy is inhibited by ubiquitin ligase activity in the nervous system.** *Nature Communications* 2019, **10**.
33. Kallergi E, Daskalaki A-D, Ioannou E, Kolaxi A, Plataki M, Haberkant P, Stein F, Savitski MM, Sidiropoulou K, Dalezios Y, et al.: **Long-term synaptic depression triggers local biogenesis of autophagic vesicles in dendrites and requires autophagic degradation.** *bioRxiv* 2020:2020.2003.2012.983965.
34. Shehata M, Matsumura H, Okubo-Suzuki R, Ohkawa N, 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**:10413-10422.
35. Donoso M, Speranza L, Kalinowska M, Castillo C, De Sanctis C, Francesconi A: **The G protein-Coupled Metabotropic Glutamate Receptor 1 controls neuronal macroautophagy.** *bioRxiv* 2020:2020.2011.2002.365783.
36. Uytterhoeven V, Kuenen S, Kaspruwicz J, Miskiewicz K, Verstreken P: **Loss of skywalker reveals synaptic endosomes as sorting stations for synaptic vesicle proteins.** *Cell* 2011, **145**:117-132.
37. Vietri M, Radulovic M, Stenmark H: **The many functions of ESCRTs.** *Nat Rev Mol Cell Biol* 2020, **21**:25-42.
38. Sheehan P, Zhu M, Beskow A, Vollmer C, Waites CL: **Activity-Dependent Degradation of Synaptic Vesicle Proteins Requires Rab35 and the ESCRT Pathway.** *J Neurosci* 2016, **36**:8668-8686.
39. Birdsall V, Imoto Y, Watanabe S, Waites CL: **KIF13A mediates the activity-dependent transport of ESCRT-0 proteins in axons.** *bioRxiv* 2020:2020.2004.2016.044818.
- *40. Farfel-Becker T, Roney JC, Cheng XT, Li S, Cuddy SR, Sheng ZH: **Neuronal Soma-Derived Degradative Lysosomes Are Continuously Delivered to Distal Axons to Maintain Local Degradation Capacity.** *Cell Rep* 2019, **28**:51-64 e54.

This article describes colocalization between LC3-positive structures and reporters of catalytically active lysosomal enzymes in distal axons of DIV14 mouse cortical neurons, indicating that local synaptic degradation can occur.

41. Parkinson GT, Hanley JG: **Mechanisms of AMPA Receptor Endosomal Sorting.** *Front Mol Neurosci* 2018, **11**:440.
42. Binda CS, Nakamura Y, Henley JM, Wilkinson KA: **Sorting nexin 27 rescues neuroligin 2 from lysosomal degradation to control inhibitory synapse number.** *Biochemical Journal* 2019, **476**:293-306.
43. Pohl C, Dikic I: **Cellular quality control by the ubiquitin-proteasome system and autophagy.** *Science* 2019, **366**:818-822.
44. Ehlers MD: **Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system.** *Nat Neurosci* 2003, **6**:231-242.

45. Lin AW, Man HY: **Ubiquitination of neurotransmitter receptors and postsynaptic scaffolding proteins.** *Neural Plast* 2013, **2013**:432057.
46. Iwata S, Morikawa M, Takei Y, Hirokawa N: **An activity-dependent local transport regulation via degradation and synthesis of KIF17 underlying cognitive flexibility.** *Sci Adv* 2020, **6**.
47. Bingol B, Schuman EM: **Activity-dependent dynamics and sequestration of proteasomes in dendritic spines.** *Nature* 2006, **441**:1144-1148.
48. Lazarevic V, Schone C, Heine M, Gundelfinger ED, Fejtova A: **Extensive Remodeling of the Presynaptic Cytomatrix upon Homeostatic Adaptation to Network Activity Silencing.** *Journal of Neuroscience* 2011, **31**:10189-10200.
49. Schattling B, Engler JB, Volkmann C, Rothhammer N, Woo MS, Petersen M, Winkler I, Kaufmann M, Rosenkranz SC, Fejtova A, et al.: **Bassoon proteinopathy drives neurodegeneration in multiple sclerosis.** *Nat Neurosci* 2019, **22**:887-896.
50. Wall MJ, Collins DR, Chery SL, Allen ZD, Pastuzyn ED, George AJ, Nikolova VD, Moy SS, Philpot BD, Shepherd JD, et al.: **The Temporal Dynamics of Arc Expression Regulate Cognitive Flexibility.** *Neuron* 2018, **98**:1124-1132 e1127.
51. Wang D, Xu Q, Yuan Q, Jia M, Niu H, Liu X, Zhang J, Young CY, Yuan H: **Proteasome inhibition boosts autophagic degradation of ubiquitinated-AGR2 and enhances the antitumor efficiency of bevacizumab.** *Oncogene* 2019, **38**:3458-3474.
52. Chen H, Polo S, Di Fiore PP, De Camilli PV: **Rapid Ca²⁺-dependent decrease of protein ubiquitination at synapses.** *Proceedings of the National Academy of Sciences of the United States of America* 2003, **100**:14908-14913.
53. Yang X: **Towards an understanding of Angelman syndrome in mice studies.** *J Neurosci Res* 2020, **98**:1162-1173.
54. Lee S, Park S, Lee H, Han S, Song JM, Han D, Suh YH: **Nedd4 E3 ligase and beta-arrestins regulate ubiquitination, trafficking, and stability of the mGlu7 receptor.** *Elife* 2019, **8**.
55. Vaden JH, Tian T, Golf S, McLean JW, Wilson JA, Wilson SM: **Chronic over-expression of ubiquitin impairs learning, reduces synaptic plasticity, and enhances GRIA receptor turnover in mice.** *J Neurochem* 2019, **148**:386-399.
- *56. McCabe MP, Cullen ER, Barrows CM, Shore AN, Tooke KI, Laprade KA, Stafford JM, Weston MC: **Genetic inactivation of mTORC1 or mTORC2 in neurons reveals distinct functions in glutamatergic synaptic transmission.** *Elife* 2020, **9**.

In this study, mTORC1 or mTORC2 was inactivated in cultured hippocampal neurons by Raptor or Rictor knockout, respectively. This revealed that, while mTORC1 acts mainly post-synaptically, mTORC2 is involved in presynaptic glutamate release.

57. Takei N, Inamura N, Kawamura M, Namba H, Hara K, Yonezawa K, Nawa H: **Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites.** *J Neurosci* 2004, **24**:9760-9769.
58. Zhang X, Wei M, Fan J, Yan W, Zha X, Song H, Wan R, Yin Y, Wang W: **Ischemia-induced upregulation of autophagy preludes dysfunctional lysosomal storage and associated synaptic impairments in neurons.** *Autophagy* 2020:1-24.
59. Zhang Y, Nicholatos J, Dreier JR, Ricoult SJ, Widenmaier SB, Hotamisligil GS, Kwiatkowski DJ, Manning BD: **Coordinated regulation of protein synthesis and degradation by mTORC1.** *Nature* 2014, **513**:440-443.
60. Huang S, Piao C, Beuschel CB, Gotz T, Sigrist SJ: **Presynaptic Active Zone Plasticity Encodes Sleep Need in Drosophila.** *Curr Biol* 2020, **30**:1077-1091 e1075.

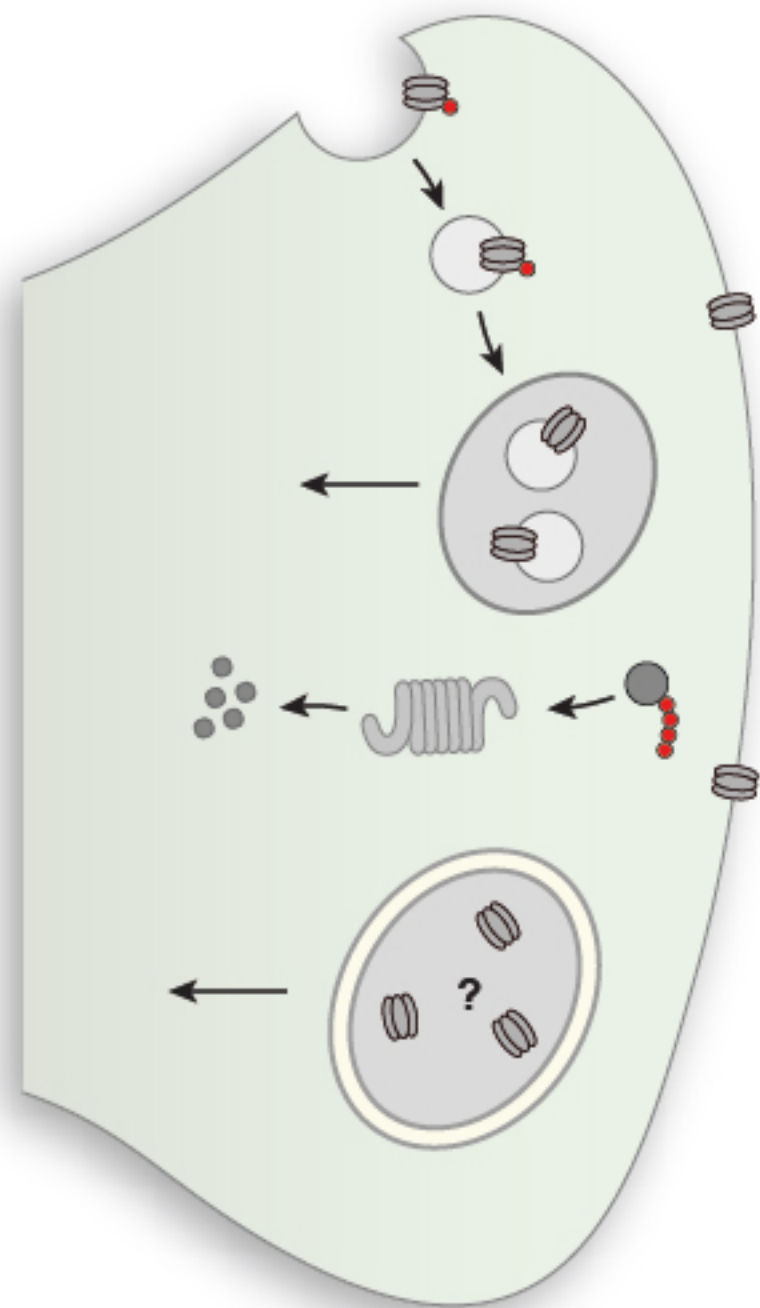
61. Cirelli C, Tononi G: **Effects of sleep and waking on the synaptic ultrastructure.** *Philos Trans R Soc Lond B Biol Sci* 2020, **375**:20190235.
62. Huang S, Sigrist SJ: **Presynaptic and postsynaptic long-term plasticity in sleep homeostasis.** *Curr Opin Neurobiol* 2020, **69**:1-10.
63. Maglione M, Kochlamazashvili G, Eisenberg T, Racz B, Michael E, Toppe D, Stumpf A, Wirth A, Zeug A, Muller FE, et al.: **Spermidine protects from age-related synaptic alterations at hippocampal mossy fiber-CA3 synapses.** *Scientific Reports* 2019, **9**.
64. Wirth M, Schwarz C, Benson G, Horn N, Buchert R, Lange C, Kobe T, Hetzer S, Maglione M, Michael E, et al.: **Effects of spermidine supplementation on cognition and biomarkers in older adults with subjective cognitive decline (SmartAge) study protocol for a randomized controlled trial.** *Alzheimers Research & Therapy* 2019, **11**.

Legends to figures

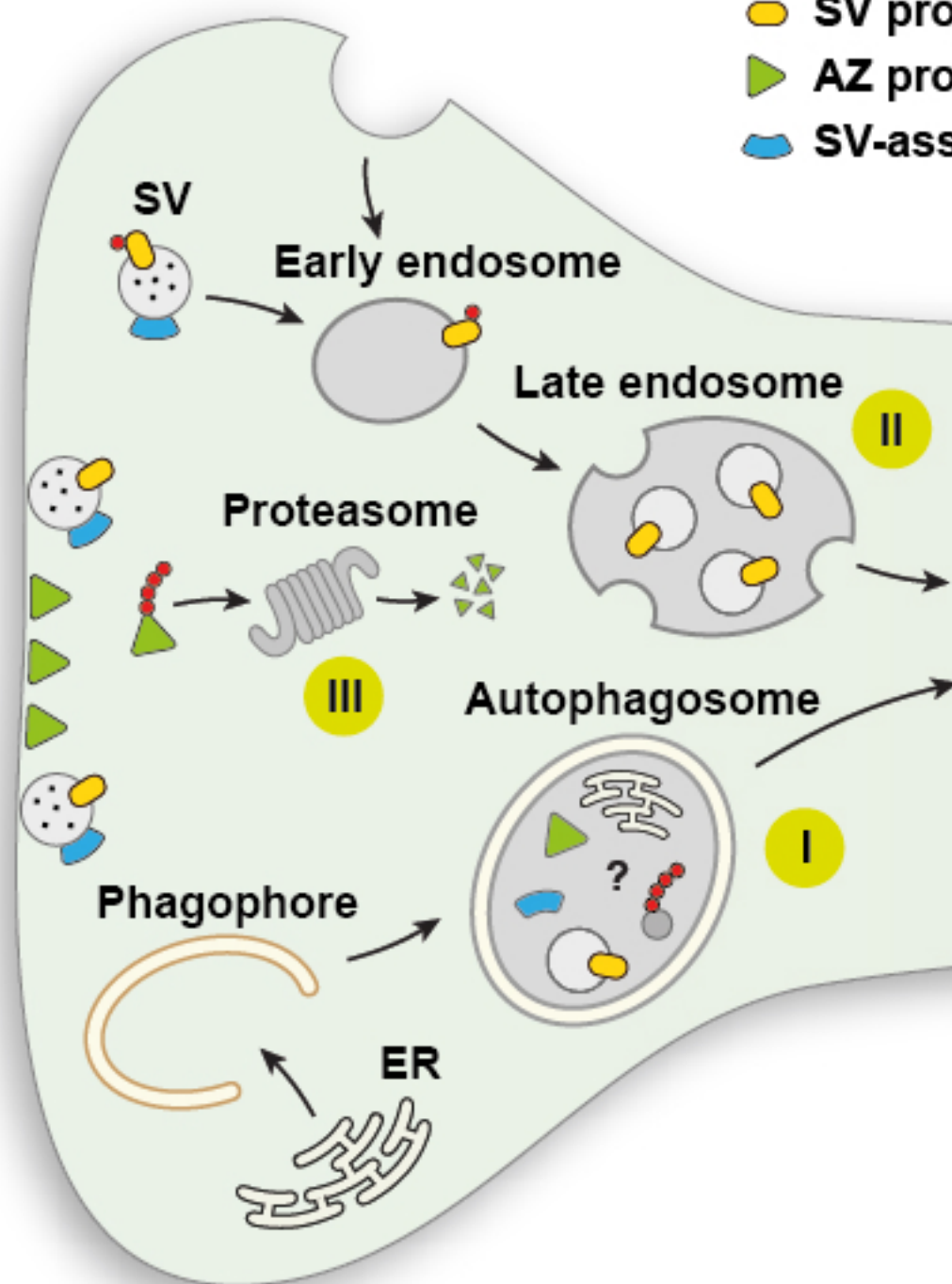
Figure 1: Degradative pathways for synaptic proteins. The synaptic proteome can be recycled via three alternative routes: (I) Neuronal autophagy: Synaptic proteins or organelles destined for degradation by the autophagic route are sequestered by the phagophore, which then closes to form an autophagosome. Autophagosomes travel retrogradely along the axon either as a separate entity, or after fusing with late endosomes to form amphisomes, gradually lower their intralumenal pH, and eventually fuse with somatic lysosomes to undergo lysosomal degradation. (II) The endolysosomal pathway: Individual SV proteins or entire SVs are targeted to early endosomes where a quality-control mechanism operates to sort aged or damaged proteins marked by protein ubiquitination for delivery to multivesicular bodies (MVBs)/ late endosomes. These are then retrogradely transported along the axon to eventually fuse with somatic degradative lysosomes for bulk degradation of their contents. (III) The Ubiquitin- Proteasome system (UPS): Some synaptic proteins, e.g. active zone components (e.g. Liprin- α , Bassoon) are poly-ubiquitinated and degraded locally by synaptic proteasomes.

Figure 2: Roles and regulation of autophagy at the synaptic terminal. Two key mouse models have highlighted important functions and modes of regulation for protein turnover at presynaptic nerve terminals. **(A)** The axonal endoplasmic reticulum (ER) membrane and associated proteins are a major substrate of steady-state neuronal autophagy. Autophagy-deficient Atg5 KO neurons accumulate tubular ER in axons and synaptic terminals. This causes elevated calcium release from ryanodine receptors (RyR) in the ER, eventually causing enhanced presynaptic neurotransmitter release, implying an important role for autophagy in regulating presynaptic neurotransmission. **(B)** A core component of the presynaptic active zone, Bassoon, maintains synaptic integrity by locally repressing proteasomal degradation and autophagic degradation. Bassoon limits proteasome assembly by binding to the PSMB4 subunit of the 26S proteasome. Furthermore, Bassoon represses autophagy by sequestering Atg5 to restrict phagophore formation. By binding and inhibiting the E3 ubiquitin ligase SIAH1, Bassoon also downregulates Parkin-mediated protein ubiquitination, thereby negatively regulating autophagy-mediated clearance of SV proteins. In Bassoon KO neurons, UPS and autophagy are hyperactive, resulting in the downregulation of synaptic activity due to increased synaptic protein degradation and a reduced SV pool size.

Postsynapse

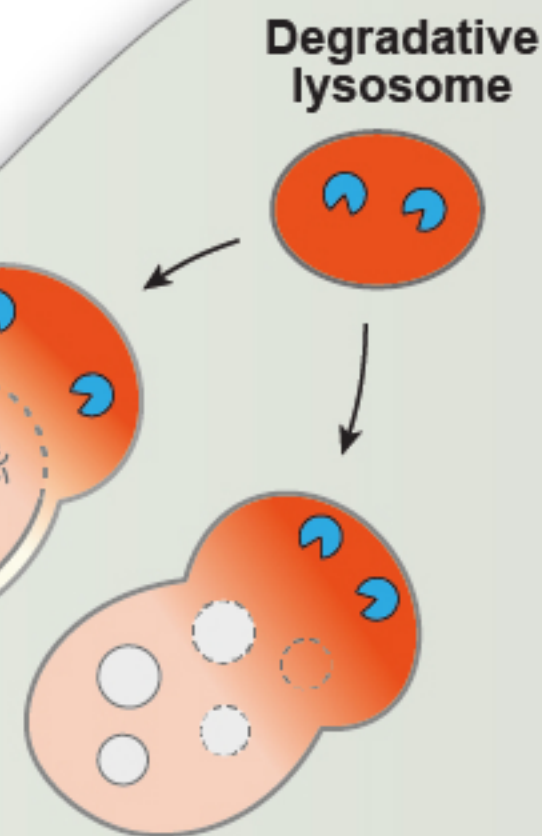


Presynapse



- Ubiquitin
- SV protein
- AZ protein
- SV-associated protein

Soma



Axon

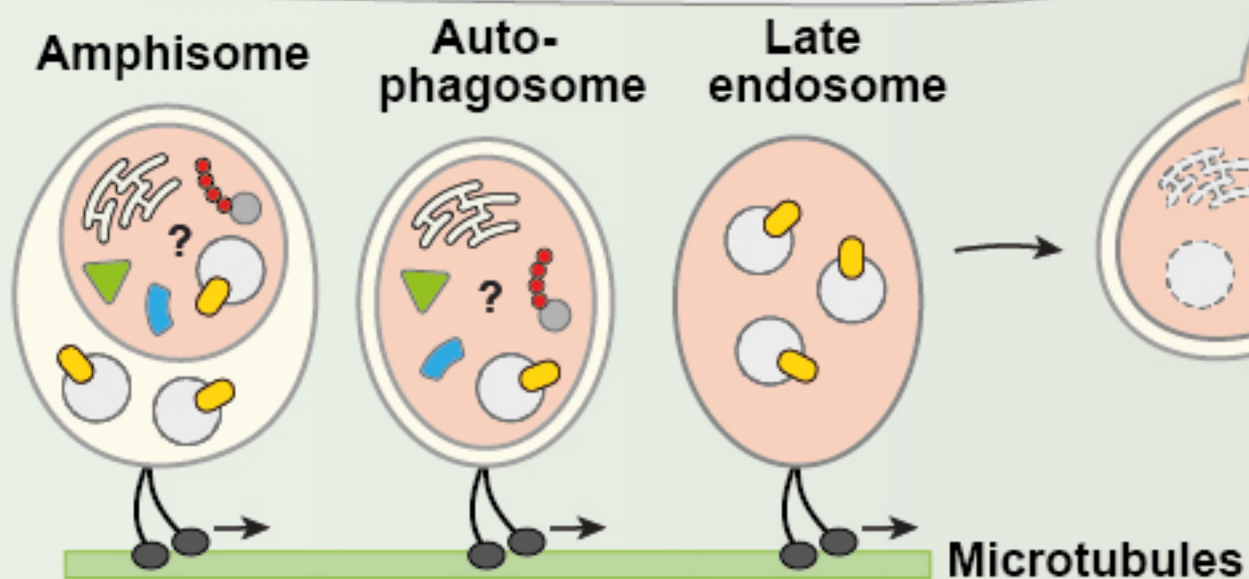


Figure 1

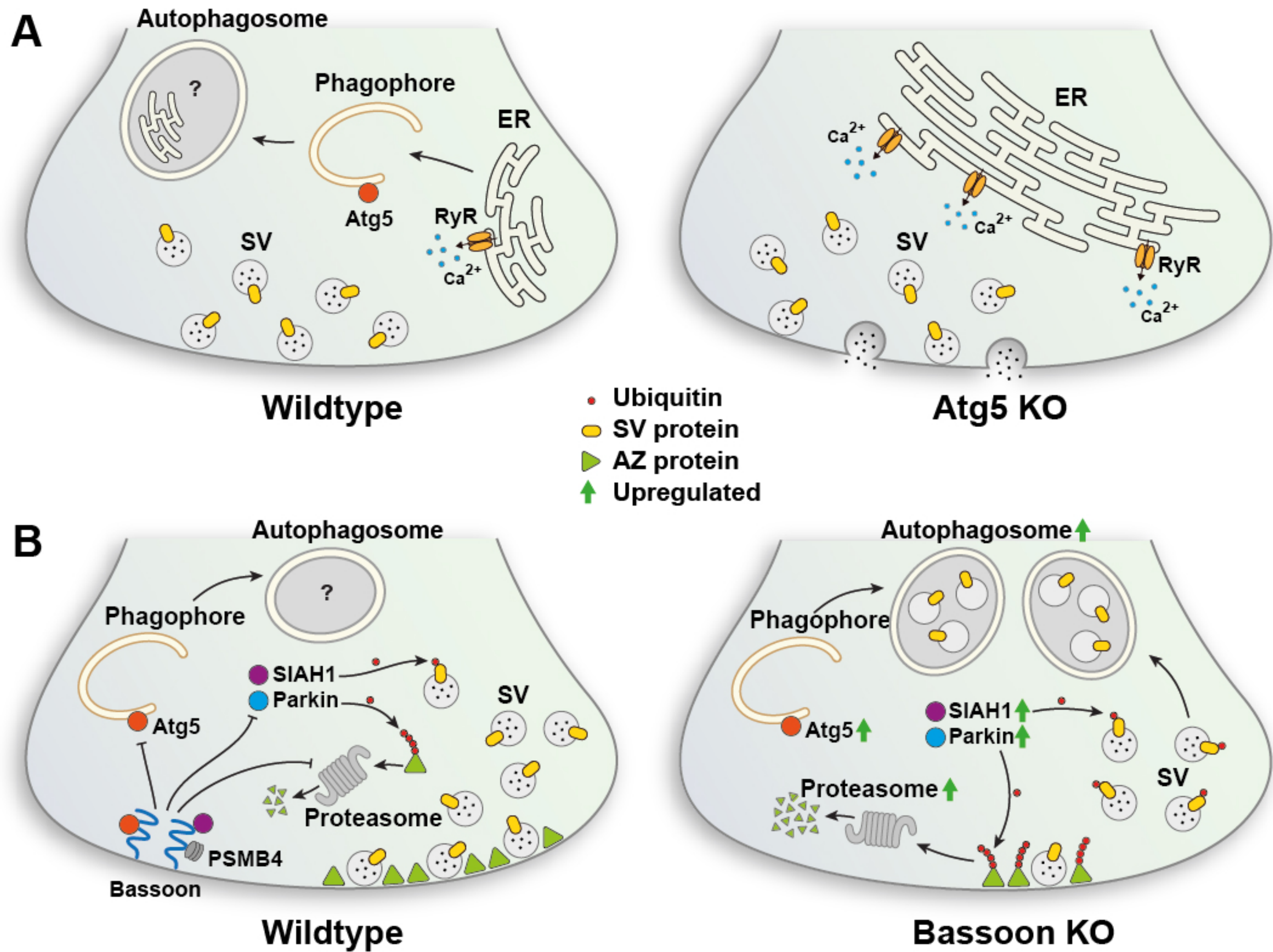


Figure 2