Review

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The needs of a synapse—How local organelles serve synaptic proteostasis

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Abstract

Synaptic function crucially relies on the constant supply and removal of neuronal membranes. The morphological complexity of neurons poses a significant challenge for neuronal protein transport since the machineries for protein synthesis and degradation are mainly localized in the cell soma. In response to this unique challenge, local micro-secretory systems have evolved that are adapted to the requirements of neuronal membrane protein proteostasis. However, our knowledge of how neuronal proteins are synthesized, trafficked to membranes, and eventually replaced and degraded remains scarce. Here, we review recent insights into membrane trafficking at synaptic sites and into the contribution of local organelles and micro-secretory pathways to synaptic function. We describe the role of endoplasmic reticulum specializations in neurons, Golgi-related organelles, and protein complexes like retromer in the synthesis and trafficking of synaptic transmembrane proteins. We discuss the contribution of autophagy and of proteasome-mediated and endo-lysosomal degradation to presynaptic proteostasis and synaptic function, as well as nondegradative roles of autophagosomes and lysosomes in signaling and synapse remodeling. We conclude that the complexity of neuronal cyto-architecture necessitates long-distance protein transport that combines degradation with signaling functions.

Keywords autophagy; Golgi satellites; lysosomes; secretory trafficking
Subject Categories Membranes & Trafficking; Neuroscience
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See the Glossary for abbreviations used in this article.

Introduction

Neurons are highly polarized cells with a complex dendritic tree and a long axon that can bridge vast distances (Fig 1). Typically, the number of synapses is huge, their molecular makeup extraordinarily complex, and the distance from the cell body, where most protein synthesis and lysosomal degradation occurs, can be enormous. Because neurons are both postmitotic and long-lived, maintaining the integrity of their proteome is of crucial importance. Several hundred different proteins can be found in forebrain synapses (Wilhelm *et al*, 2014; Dieterich & Kreutz, 2016; Koopmans *et al*, 2019) and this complex proteome creates a unique situation with respect to the molecular dynamics of protein exchange. A single pyramidal neuron can harbor up to 17,000 spine synapses (Ballesteros-Yáñez *et al*, 2006) and their axonal terminal fields might establish around 3,000 presynaptic boutons (Ziv, 2018). Along these lines, the soma of a pyramidal neuron contributes only 5% to the entire cell volume, and its share of the membrane is negligible (< 1%) as compared to the rest of the cell (Ishizuka *et al*, 1995). This complexity poses a significant challenge for proteostasis.

In non-neuronal cells, newly-synthesized integral transmembrane (TM) proteins are transported in the soma from the endoplasmic reticulum (ER) to the Golgi apparatus (GA) via the ER-Golgi intermediate compartment (ERGIC), a cluster of tubular membranes ensuring proper quality and cargo folding. Subsequently, the cargo passes through the compartments of the GA, where it undergoes a series of modifications, which include most prominently glycosylation. Following sorting at the GA, the protein is delivered in post-Golgi carriers to the plasma membrane (PM). The degradation of TM proteins can occur either in the endolysosomal system or through macroautophagy. In the endolysosomal system, the protein is first trafficked to early endosomes from which it can be directed to recycling (recycling endosomes (REs)) or late endosomes. Macroautophagy (hereafter called autophagy) is characterized by formation of a double membrane phagophore around the cargo sorted for degradation. Both pathways share a common endpoint—an acidic, degradative organelle filled with active proteases, the lysosome.

Different modes of secretory trafficking in dendrites

The bewildering complexity of neuronal processes and the fact that protein synthesis occurs predominantly in the soma and much less in axons and dendrites, poses a logistic challenge for transport,

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Glossary

Amphisome
A hybrid, double-membrane organelle generated upon fusion of a LE/MVB with a autophagosome. Recently shown to contain active TrkB receptors, be endowed with signaling properties.
Early endosome (EE)
First compartment of the endolysosomal system to receive endocytic cargo, where cargo is sorted for recycling or degradation. Enriched in Rab5.
Endolysosomal system
Ensemble of single-membrane organelles that are dynamically interconnected with the ultimate function of sorting cargo for recycling or delivery into lysosomes for degradation upon fusion.
Endoplasmic reticulum (ER)
A continuous, tubular organelle that expands from the outer nuclear membrane to dendrites providing a protein trafficking route and that plays a role in proper protein folding and lipid synthesis. Smooth ER (SER) is a Ca2+ store whereas rybosomes associated with ER form rough ER (RER), involved in modulating protein synthesis.
ER exit sites (ERES)
ER secretion regions marked by COPII coat specialized in anterograde cargo transfer from ER to Golgi.
ER-Golgi intermediate compartment (ERGIC)
Tubulovesicular membrane clusters, an intermediate point for proteins en route to Golgi, contributing quality control, concentration, and folding of cargo.
Glycosylation
A posttranslational, reversible modification, where polysaccharide chains (glycans) are enzymatically added to protein. The classes of glycans depend on the attachment side, where N-linked glycans are attached to nitrogen (e.g., Asp or Asn) and O-linked glycans are attached to oxygen (e.g., Ser, Thr, Tyr). N-glycosylation is associated with immature proteins.
Golgi apparatus/complex
Organelle composed of cisternae stack involved in maturation (e.g., glycosylation) and packaging of cargo into membrane-bound transport vesicles.
Golgi outposts (GOs)
A Golgi-related organelle containing Golgi matrix located in the apical, proximal dendrite and branch points in the pyramidal neurons.
Golgi satellites (GS)
A small, Golgi-related organelle distributed throughout the dendritic tree.
Late endosome (LE)/multivesicular body (MVB)
Organelles of the endolysosomal system resulting from EE maturation and enriched in Rab7. It contains intraluminal vesicles (ILVs) that gather degradative cargo generated upon the activity of the ESCRT complex. The complex sorts cargo in endosomal membrane subdomains and mediates membrane invagination and scission into ILVs with the ultimate goal of cargo degradation upon fusion with lysosomes.
Lysosomal fusion
Merging of lysosomal membrane with the cell membrane.
Lysosomal exocytosis
An active release of the content of the lysosome (including secretory lysosome) to the extracellular space.
Lysosome
Single-membrane, electron-dense organelle of acidic pH (below 5.0), containing active proteases.
Macroautophagy/autophagosome
A degradative process that engulfs cargo into a double-membrane organelle called autophagosome that transports and delivers it to lysosomes (forming autolysosomes) for degradation upon fusion.
Proteostasis
Dynamic, regulatory processes ensuring balance between anabolic and catabolic mechanisms of the cells and functional protein levels.
Recycling endosome (RE)
Organelle of the endolysosomal system in which recycling cargo is sorted back to the plasma membrane.
Retromer
Complex of proteins crucial for recycling of transmembrane cargo from endosomes to Golgi and for local endosomal insertion of transmembrane proteins.
Secretory lysosome
A type of lysosome, which can undergo fusion, and contains additional, secretory components (e.g., secretory lysosomes in basophils contain serotonin and histamine).
Ubiquitin-proteasome system (UPS)
Intracellular protein degradation system, where cytosolic proteins are sorted to proteasome after attachment to the ubiquitin polypeptide and degraded in specialized protein complex, proteasome.

synthesis, sorting, posttranslational processing, and degradation of TM proteins. Three routes of membrane trafficking have been described in dendrites—lateral diffusion in the PM, vesicular transport, and dwelling in the ER—that account to a varying degree for delivery of cargo to synaptic sites (Fig 2) (Pick & Ziff, 2018; Ribeiro *et al*, 2018; Buonarati *et al*, 2019; Kennedy & Hanus, 2019).

Lateral diffusion following insertion in the PM is the most inefficient mean of delivery of TM proteins. It was calculated that a protein following synthesis and insertion in the soma would need several days to reach distal dendrites (Earnshaw & Bressloff, 2006, 2008) and it is therefore unlikely that a significant number of membrane proteins at a given synapse take this route. Lateral diffusion of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) in and out of the synapse, however, plays a central role in the expression of plasticity (Choquet & Triller, 2013; Groc & Choquet, 2020). Here, the distance of trafficking is limited to a few micrometers.

A well-documented transport route of TM proteins synthesized in the cell body is based on vesicular transport subsequent to processing of these proteins in the canonical secretory pathway. Several studies have shown anterograde vesicular transport of key synaptic TM proteins including AMPAR, N-methyl-D-aspartate receptors (NMDAR), neuroligins, and other synaptic cell adhesion proteins from soma to dendrites (Pick & Ziff, 2018; Ribeiro *et al*, 2018; Buonarati *et al*, 2019; Bourke *et al*, 2021).

At present, however, the contribution of vesicular transport for proteostasis of postsynaptic TM proteins is still a matter of debate. The ER in pyramidal neurons of the hippocampus is continuous between dendrites, a subset of spines and the outer nuclear membrane. The dendrites contain ERGIC, retromer, dendritic mRNA, polyribosomes, and various other organelles and components for secretory trafficking (Fig 1) (Dieterich & Kreutz, 2016; Hanus & Ehlers, 2016; Kennedy & Hanus, 2019). Conclusive evidence was found for the existence of a satellite microsecretory system in dendrites that even allows for local synthesis and processing of synaptic TM proteins (Ye *et al*, 2007; Ramírez & Couve, 2011; Cui-Wang *et al*, 2012).

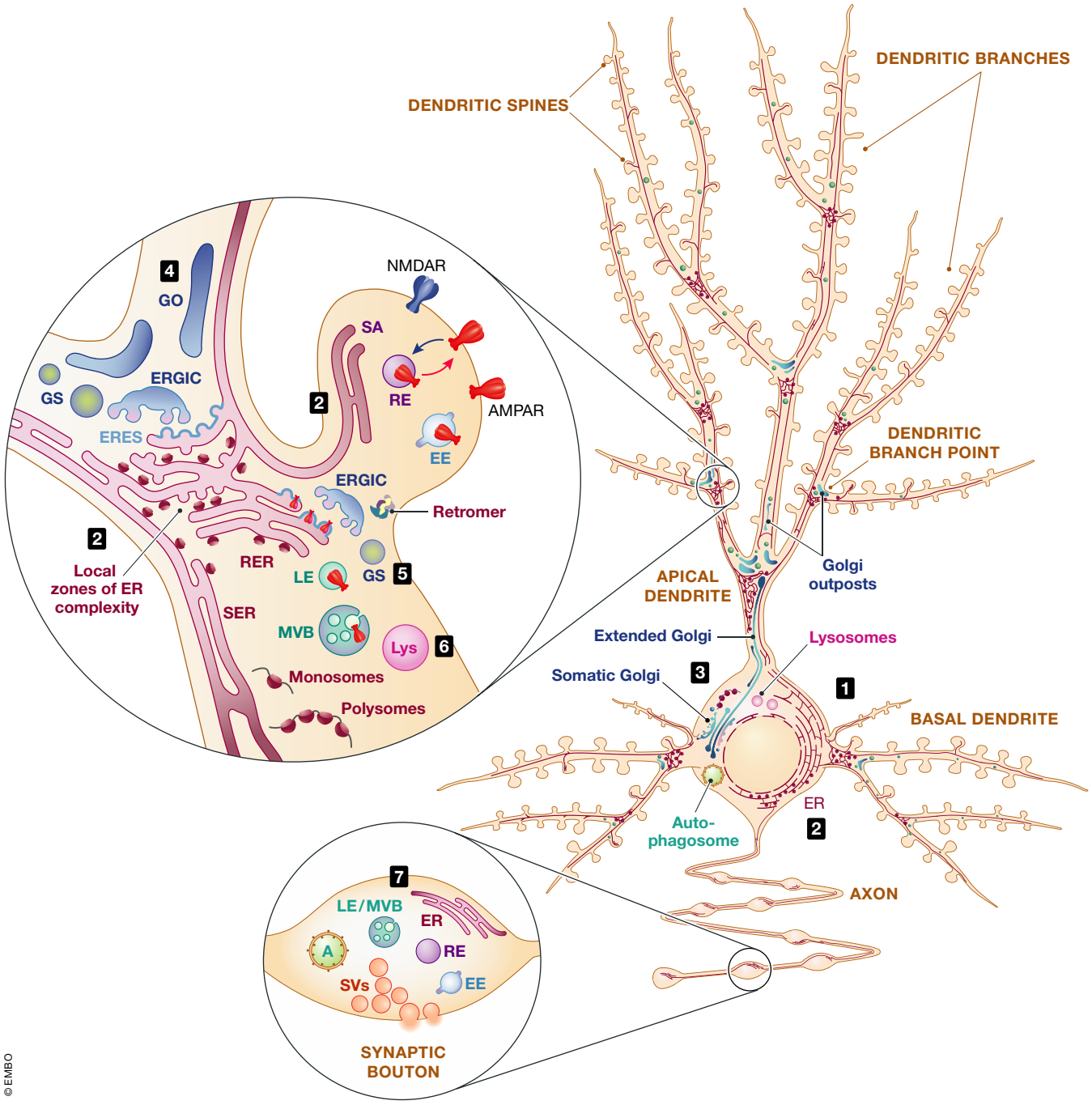

Figure 1. Membrane trafficking in dendrites and axons.

(1) Most protein synthesis and lysosomal degradation occur in the soma and to a much lesser degree in dendrites and axons. (2) The endoplasmic reticulum (ER) in pyramidal neurons of the hippocampus is continuous between the outer nuclear membrane, dendrites, and a subset of dendritic spines. Some of these spines contain a spine apparatus (SA). Local zones of ER complexity compartmentalize ER-exit sites (ERES) preferentially at dendritic branch points and a subset of dendritic spines. Local zones of ER complexity associate prominently with ribosomes. RER—rough endoplasmic reticulum; SER—smooth endoplasmic reticulum. (3) The Golgi apparatus extends in a few neurons into the apical dendrite (Extended Golgi). (4) Golgi Outposts (GO) are mainly localized at dendritic branch points in proximal parts of apical dendrites, whereas Golgi satellites (GS) are part of a local microsecretory system (5) that might allow for processing of synaptic transmembrane proteins in all segments of basal and apical dendrites. Microsecretory systems also include ERES–Golgi interface—ER-Golgi intermediate compartment (ERGIC). (6) Components for secretory trafficking in distal dendrites include recycling endosomes (RE), early endosomes (EE), late endosomes (LE), multivesicular bodies (MVB), lysosomes (Lys), and retromer. (7) Proteostasis of transmembrane proteins at the presynapse involves the ER, RE, EE, LE, MVB, autophagosomes (A), and synaptic vesicles (SV).

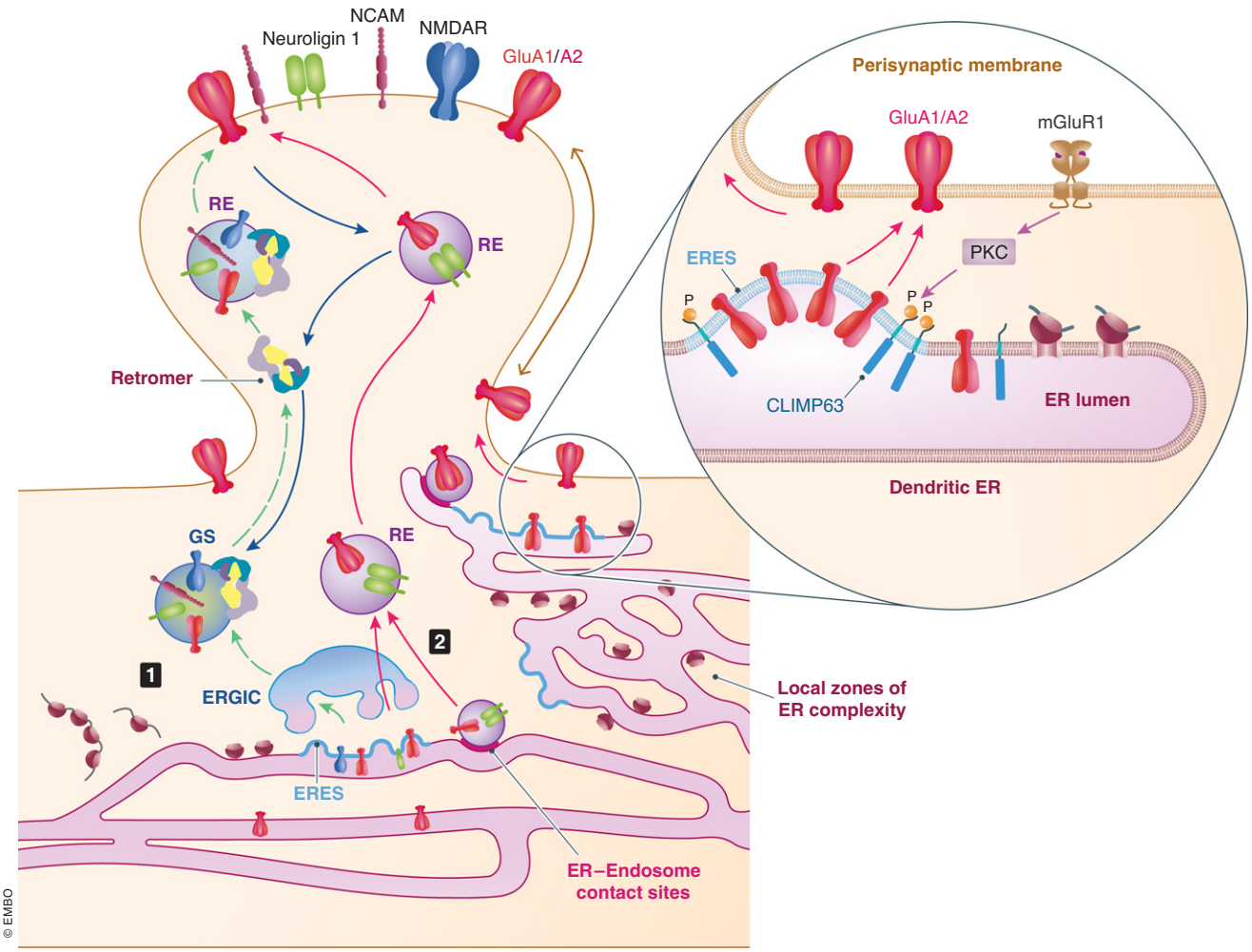

Figure 2. Forward and retrograde secretory trafficking in dendrites.

Forward secretory trafficking of synaptic transmembrane proteins in neuronal dendrites occurs either via cargo exit from the ER-Golgi intermediate compartment (ERGIC) and passes through Golgi satellites (GS, (1) green arrows) or a Golgi-independent trafficking route mediated by REs from ER-endosome contact sites ((2), red arrows). A broader spectrum of synaptic transmembrane proteins including GluA1, GluN1, GluN2B, NCAM, and Neuroligin-1 pass through and recycle back to GS (1) localized in close proximity to ERGIC and retromer (dark blue arrows). Recycling of synaptic transmembrane proteins might enable their local (re)-glycosylation. Local delivery to synaptic sites as such might derive from local zones of increased ER complexity near dendritic branch points and dendritic spines (inset) that confine mobility of membrane cargo and compartmentalize ER export. The spatial range of AMPA receptor mobility is restricted by bidirectional regulation of ER complexity involving type I metabotropic glutamate receptor (mGluRI) signaling through activation of protein kinase C (PKC) and subsequent phosphorylation of the cytosolic part of the ER protein CLIMP63 (inset). Biosynthesis of transmembrane proteins including AMPAR occurs on ER-bound ribosomes that are also prominently localized at zones of increased ER complexity.

Cell-surface trafficking of TM proteins via a route that bypasses the Golgi?

Currently, it is still an open question whether post-ER carrier in dendrites requires a Golgi-related compartment for glycosylation or whether they bypass the Golgi and synaptic TM proteins are inserted without mature glycosylation. In neurons, discontinuous structures resembling Golgi cisternae are present along dendrites, which are known as Golgi outposts (GOs). The term "Golgi outposts" is not well defined and used in the literature (i) for extended Golgi in the primary apical proximal dendrite in a subset of hippocampal pyramidal neurons (Fig 1) and (ii) for Golgi membranes located at branch points of the primary apical dendrite (Fig 1) where this type of GOs is present mainly in a subset of neurons during dendritogenesis (Hanus & Ehlers, 2016; Kennedy & Hanus, 2019). Given the low abundance of GOs in dendrites (Hanus & Schuman, 2013; Hanus & Ehlers, 2016; Hanus *et al*, 2016), new locally synthesized proteins in basal or distal apical dendrites might not undergo all of the processing steps of the canonical secretory pathway and could be functionally different from somatically synthesized ones. Thus, faster and spatially restricted delivery might come at the expense of functional maturity and protein stability (Rosenberg *et al*, 2014).

According to this latter view, following synthesis integral membrane proteins dwell in the ER for variable periods that are typically rate limiting for PM delivery. Membrane proteins, including AMPAR, rapidly diffuse within the continuous network of dendritic ER but are confined by increased ER complexity at dendritic branch points and near dendritic spines (Fig 2). The spatial range of receptor mobility is rapidly restricted by type I metabotropic glutamate receptor (mGluRI) signaling through a mechanism involving protein kinase C (PKC) and the ER protein Cytoskeleton-linking Membrane Protein 63 (CLIMP63) (Fig 2) (Cui-Wang *et al*, 2012). Moreover, local zones of ER complexity compartmentalize ER export and correspond to sites of new dendritic branches. Newly assembled proteins in the ER accumulate at these ER exit sites (ERES) (Fig 2). Thus, local control of ER complexity spatially scales secretory trafficking within elaborate dendritic arbors (Cui-Wang *et al*, 2012).

PM delivery is routed via ERGIC and REs (Bowen *et al*, 2017). This was shown in rat cortical neurons where the AMPAR subunit GluA1 as well as Neuroligin 1 accumulate in RE located in dendrites and spines before reaching the PM (Fig 2). Surprisingly, GluA1 surface delivery occurred even when GA function was disrupted (Bowen *et al*, 2017). Thus, in addition to their canonical role in protein recycling, RE also mediate forward secretory trafficking in neuronal dendrites and spines through a specialized GA-independent trafficking network (Fig 2). Of note, most of this work was based on the analysis of AMPAR and it remains unclear whether the same holds true for other classes of synaptic TM protein trafficking. Thus, whether the complete repertoire of proteins that might be locally translated in response to synaptic signals, dwell in the ER and accumulate in ERES before traffic to the PM is currently unknown.

The glycosylation of synaptic TM proteins

N-linked protein glycosylation in the ER involves the assembly of an oligosaccharide on a lipid carrier and the transfer of the oligosaccharide to selected asparagine residues of polypeptides that have entered the lumen of the ER (Moremen *et al*, 2012). N-linked core glycosylation is then modified by the sequential addition of complex sugars to several classes of membrane proteins as they progress through the ER and the GA. However, it was found that hundreds of neuronal surface membrane proteins are only core-glycosylated (Hanus *et al*, 2016). Thus, surprisingly high levels of glycosylation profiles that are classically associated with immature intracellular proteins are displayed at the surface of neuronal membranes. It was argued that this atypical glycosylation of surface neuronal proteins can be attributed to a bypass of the GA, indicating that the canonical secretory pathway is not only absent in dendrites but also hypofunctional in the soma. Interestingly, core-glycosylation is regulated by synaptic activity, modulates synaptic signaling and accelerates the turnover of GluA2-containing glutamate receptors, revealing a novel mechanism that controls the composition and sensing properties of the neuronal membrane (Hanus *et al*, 2016). The dynamics of nascent membrane proteins in dendritic post-ER compartments were already previously investigated under regimes of low or increased neuronal activity (Hanus *et al*, 2014) where it was shown that increasing synaptic activity restricts the length scales of early secretory trafficking in dendrites. Other studies of GluA2 surface trafficking in hippocampal neurons demonstrate that GluA2 accumulated in the ER is found to reside in puncta associated with internal membranes along the dendrite, and may be targeted directly to the synaptic membrane (Pick & Ziff, 2018). GluA2 exit from the ER depends upon Ca^{2+} release from inositol triphosphate (IP3) and ryanodine receptors (RyR) (Pick & Ziff, 2018). If GluA2 indeed bypasses the Golgi, the insertion of GluA2-containing AMPAR from the ER to the synapse may be direct and rapid. This points to the possibility that synthesis of different subunits of glutamate receptors is spatially coordinated and assembly as well as trafficking is regulated locally. Moreover, the sites of protein synthesis and ER release might be spatially and functionally coordinated. An interesting question is how many spine synapses can profit from such a mechanism or, in other terms, how far will TM proteins traffic from their sites of synthesis before insertion into the PM? In a recent study performed with a zapalog-mediated ER trap, which allows to trigger forward trafficking with subcellular spatial resolution, it was found that TM proteins following exit from the ER appeared at the cell surface in a relatively broad area (Bourke *et al*, 2021). More sophisticated techniques might be necessary to resolve whether synaptic activity is needed to spatially constrain trafficking to dendritic segments or even individual synapses.

However, several lines of evidence contradict the scenario outlined above. In hippocampal neurons, it was reported that GFP-tagged GluA1 exits the ER and traffics to the plasma membrane via the conventional somatic Golgi network rather than the dendritic ER to the plasma membrane involving vesicular transport (Jeyifous *et al*, 2009). Also, post-Golgi vesicles carrying GluA2 and N-Cadherin were reported to undergo soma to dendrite trafficking in a kinesin-dependent manner prior to synapse delivery (Heisler *et al*, 2014). In addition, surface-expressed GluA1 consistently show complex glycosylation (Midorikawa *et al*, 2020). Finally, the notion that the majority of synaptic TM proteins will only show core-glycosylation is hard to reconcile with reports suggesting that several important aspects of AMPAR regulation in a cellular context are regulated by mature N-glycosylation, including ligand-binding affinity (Kawamoto *et al*, 1995; Pasternack *et al*, 2003), surface expression (Kandel *et al*, 2018), oligomerization, and trafficking (Takeuchi *et al*, 2015). In addition, the sensitivity to glycolytic enzymes removing immature high mannose glycosylation from TM proteins is only high for GluA1 and already considerably less for GluA2, whereas the auxiliary subunit of AMPAR TARPγ8 even seems to be devoid of immature N-linked carbohydrates (Bowen *et al*, 2017).

Golgi satellites in dendrites

These contradictory findings show how fragmented our current knowledge is and that it is far from clear how molecular and functional heterogeneity of surface-expressed TM proteins is established and maintained within neuronal dendrites. In our own work, we took advantage of the Golgi-targeting properties of the trans-Golgi network (TGN)-resident neuronal EF-hand calcium sensor protein Calneuron-2 to develop a simple but efficient plasmid-based system called pGolt to study Golgi organelles in neurons (Mikhaylova *et al*, 2009, 2016; Bera *et al*, 2016). With this tool, we found the presence of Golgi-related organelles termed Golgi Satellites (GS) in all dendrites of pyramidal neurons in close proximity to ERGIC and retromer (Fig 2) (Mikhaylova *et al*, 2016). The GS secretory system

is much more widespread in dendrites than previously described GOs and it contains at least part of the cellular glycosylation machinery but, as opposed to GOs, lacks many protein components for sorting and the organization in cis-, medial- and trans- Golgi cisternae. Interestingly, in *Drosophila* neurons, the stack-like organization of Golgi appears to be disrupted and TGN-like Golgi compartments are frequently observed in dendrites (Ori-McKenney *et al*, 2012; Zhou *et al*, 2014). Of note, in *Drosophila* neurons, GOs are reportedly part of the microtubule organizing center (MTOC) and acentrosomal nucleation at GOs appears to play an important role for dendrite growth and maintenance (Ori-McKenney *et al*, 2012). However, this finding could not be replicated in cultured pyramidal neurons where γ-tubulin controls neuronal microtubule polarity independent of GOs (Nguyen *et al*, 2014).

Nonetheless, it has been speculated based on the presence of proteins like TGN38 that Golgi membranes are present in dendrites and even in spines of pyramidal neurons (Gardiol *et al*, 1999). Trafficking of cargo from ER to Golgi in dendrites has been shown for NMDAR (Jeyifous *et al*, 2009), alpha-7 nicotinic acetylcholine receptor (α7 nAChRs) (Alexander *et al*, 2010), and GluK2-containing kainate receptors (Evans *et al*, 2017). Accordingly, a broad spectrum of synaptic TM proteins (including GluA1, GluN1, GluN2B, neural cell adhesion molecule (NCAM), and Neuroligin-1) might pass and even recycle through GS (Mikhaylova *et al*, 2016) (Fig 2). Thus, GS might enable local glycosylation of proteins, which can then be recruited to membranes in spatially confined dendritic segments.

A question that arises in light of the widespread distribution of GS is whether and how they differ from GOs. It appears unlikely that GS is an integral part for modifying, sorting, and packaging of macromolecules for cell secretion like classical GA. We reason that the widespread distribution of GS makes a local insertion of TM proteins passing through this structure via retromer very likely and tightly regulated sorting as well as packaging processes might be dispensable. At present, it is also unknown how their biogenesis is regulated. GOs appear to be generated from the somatic GA (Quassollo *et al*, 2015). GS might either be formed locally in close proximity to ERES in an activity-dependent manner (Govind *et al*, 2021) or bud off from somatic Golgi (Mikhaylova *et al*, 2016). It was reported that nicotine and other excitatory stimuli trigger dispersal of Golgi membranes in soma and dendrites (Govind *et al*, 2021). Distal glycosylation involved prominently mature sialylation and was accompanied by remodeling of the dendritic surface as evidenced by altered lectin binding (Govind *et al*, 2021).

Microsecretory pathways in dendrites

Retromer is a complex of proteins that is crucial in recycling TM receptors from endosomes to the TGN (Burd & Cullen, 2014). In neurons, retromer supports specialized and regulated PM trafficking pathways, including localized recycling of cargo near the dendritic spine (Choy *et al*, 2014; Wu *et al*, 2017). Depletion of retromer affects localization of specific cargo and does not appear to affect bulk trafficking to the PM. β-adrenergic receptors, dopamine transporters, and ionotropic glutamate receptors are sorted for local endosomal membrane insertion via retromer (Choy *et al*, 2014; Temkin *et al*, 2017; Wu *et al*, 2017), and it appears that this sorting has implication for the induction of long-lasting changes in synaptic efficacy (Temkin *et al*, 2017). A long list of potential synaptic retromer cargo that includes apart from β2-adrenergic receptors (Choy *et al*, 2014; Varandas *et al*, 2016; Temkin *et al*, 2017) and AMPA-type glutamate receptors (Zhang *et al*, 2012; Munsie *et al*, 2015; Tian *et al*, 2015; Kadgien *et al*, 2021), glycine receptors (del Pino *et al*, 2011), D1-type dopamine receptors (Wang *et al*, 2016), NMDAR (Clairfeuille *et al*, 2016; Mikhaylova *et al*, 2016; Ma *et al*, 2017; Kadgien *et al*, 2021), neuroligin 1 and 3 (Binda *et al*, 2019), and transporters for biogenic amines including dopamine (Wu *et al*, 2016, 2017) indicates the potential importance of local retromer-mediated trafficking in dendrites. Moreover, the close spatial relationship between retromer and GS suggests that this Golgi-related organelle might also receive retrograde traffic of synaptic receptors (Fig 2) (Mikhaylova *et al*, 2016). In accord with this notion, GluN2B contains NMDAR recycle through retromer and GS (Mikhaylova *et al*, 2016), and it is tempting to speculate that recycling is tightly controlled by synaptic activity.

As already outlined above, an alternative route bypassing the Golgi appears to be established by RE (Fig 2) (Bowen *et al*, 2017) and evidence was provided for a role of retromer also in endocytic trafficking of signaling receptors and in mediating direct endosome-to-plasma membrane traffic (Choy *et al*, 2014; Varandas *et al*, 2016). The ER can modulate endosome dynamics through ER–endosome contact sites (Fig 2), which regulate endosomal forward trafficking, lipid transfer, endosome fission, positioning, or sorting (Raiborg *et al*, 2015a, 2015b). Cargo for forward membrane trafficking can in principle reach the plasma membrane following exit from ER–endosome contact sites without passing through any other organelle, and RE could provide a very fast local means for membrane insertion (Fig 2). Some synaptic membrane proteins exit for PM trafficking from the ERGIC (Hanus *et al*, 2014), and forward trafficking of neurotransmitter receptors has been shown to involve the retromer (Choy *et al*, 2014).

Collectively, these—somewhat controversial—results raise a number of questions. For instance, why are there discrepant reports regarding forward trafficking of AMPAR and potentially recycling of membrane proteins through biosynthetic pathways? Could differences in the age (from day *in vitro* 11–2 months) and source of the studied primary neurons (hippocampal versus cortical pyramidal neurons) account for these discrepant findings in published studies? Or do we need a re-evaluation of current concepts and, critically, novel tools to study these aspects? In addition, the current focus on AMPAR and here in particular GluA1 ignores a large number of molecules including cell adhesion proteins and NMDAR that have a crucial role in synaptic function. Therefore, further studies are needed that address the molecular machinery underlying fast and direct insertion of synaptic membrane proteins in synapses undergoing plasticity. Moreover, previous work was focused on N-glycosylation of membrane proteins but ignored O-glycosylation, which is reversible and occurs in GS (Mikhaylova *et al*, 2016; Evans *et al*, 2017). GS are rather small (in the range of 0.2–1 μm) and it is not clear how their biogenesis is regulated and how they retain their membrane integrity. Is outgoing and incoming traffic coupled to each other? Are the underlying mechanisms of forward trafficking and the assembly and organization of microsecretory systems the same in all neuronal cell types or are there significant differences between brain regions as well as between excitatory and inhibitory neurons? An

important question also relates to the role of retromer in forward and backward trafficking: Is it involved in sorting of cargo for recycling of membrane proteins through biosynthetic pathways? If true, why two pathways exist for local secretory membrane trafficking and how do they compare to the canonical pathway present in the soma? And finally, can synaptic signals induce a switch of the trafficking route for certain proteins?

The capacity of local protein synthesis to replenish the postsynaptic pool of TM proteins

Computational modeling shows that protein synthesis in the cell soma and subsequent long-distance transport to distal dendrites is relatively slow and inefficient when it comes to the need of replenishment of the existing protein pool and the incorporation of plasticity-related protein in an input- and activity-dependent manner (Williams *et al*, 2016). Several forms of synaptic plasticity rely on the stimulus-dependent local translation of proteins. Compelling evidence has shown that *de novo* protein synthesis indeed takes place in axons and dendrites where the machinery for both protein synthesis and degradation are present (Steward & Schuman, 2001; Jung *et al*, 2012) and that this regulate protein availability during synaptic transmission (Hanus & Schuman, 2013; Kim & Jung, 2015). A broader range of different synaptic TM proteins can be synthesized locally in dendrites, including neurotransmitter receptors, ion channels, and cell adhesion molecules (Cajigas *et al*, 2012; Holt *et al*, 2019). Also, excitatory and inhibitory presynaptic terminals contain the machinery necessary for protein synthesis, and numerous transcripts, including TM proteins, were detected (Hafner *et al*, 2019).

What is still unclear is whether global and local synthesis of membrane proteins are functionally segregated and if locally synthesized proteins serve different functions or are endowed with different properties that serve the specific needs of neurotransmission (Jeyifous *et al*, 2009; Hanus & Schuman, 2013). Dendrites, and to lesser extent axons, contain rough ER and ribosomes. Biosynthesis of TM proteins occurs on ER-bound ribosomes and on ribosome-associated vesicles that are only present in dendrites (Carter *et al*, 2020). It is interesting that the partitioning of mRNAs that associate with ER-bound ribosomes occurs rather early after transcription, when ribosomes engaged in the translation of mRNAs encoding signal-sequence-bearing proteins are targeted to the ER (Stephens *et al*, 2008). Thus, dendritically targeted mRNAs encoding TM proteins might have privileged access to this subset of ribosomes if they contain the corresponding signal peptide. Although the number of polyribosomes in dendrites is very limited, recent work has pointed to a significant role of monoribosomes in local protein synthesis (Biever *et al*, 2020). Interestingly, some transcripts exhibited a preference for monoribosomes including transcripts encoding for glutamate receptor subunits and synaptic cell adhesion molecules (Biever *et al*, 2020). Because AMPA-receptors have a very low copy number per synapse (15–20 molecules; Choquet, 2018; Böger *et al*, 2019; Buonarati *et al*, 2019), even minute local changes in protein synthesis can have huge impact. Therefore, one intriguing question is whether locally synthesized membrane proteins use different routes to the synaptic membrane depending upon their synthesis in poly- or monoribosomes.

ER-bound ribosomes are indeed prominently localized at zones of increased ER complexity (Fig 2), which makes tight coupling between protein synthesis and ER exit possible. Since ER-exit sites are in close proximity to ERGIC, the entire network can be spatially coordinated (Spacek & Harris, 1997; Cooney *et al*, 2002; Cui-Wang *et al*, 2012; Wu *et al*, 2017). ERGIC not only participates in the folding and quality control of nascent proteins but is also involved in O- and N-glycosylation, and in the synthesis of glycosaminoglycans and lipids (Krijnse-Locker *et al*, 1995; Jönsson *et al*, 2003; Sannerud *et al*, 2006; Ge *et al*, 2013; Sirkis *et al*, 2017; Saraste & Marie, 2018). Thus, input-specific and activity-dependent membrane insertion with high spatial specificity is conceivable but has not been shown yet with high spatial resolution.

Membrane trafficking and lysosomal protein degradation

Proteostasis of synaptic proteins requires degradation machinery that allows for local control of synaptic protein composition. Two main degradative pathways exist that act as surveillance mechanisms to ensure efficient cargo degradation at synapses by vastly different mechanisms. The ubiquitin-proteasome system (UPS) locally degrades ubiquitin-tagged proteins that are recognized by the proteasomal machinery (see for instance, Hakim *et al*, 2016/ reviewed in Bingol & Schuman, 2005, Tai & Schuman, 2008). The second degradative pathway relies on the delivery of cargo to catalytically active lysosomes (Fig 3). Lysosomes are the common endpoint for endosomes, autophagosomes, and phagosomes, and are therefore specialized in the degradation of vesicular cargo (Luzio *et al*, 2007, 2014; Lawrence & Zoncu, 2019). Their acidic pH (4.5–5.0) provides the environment for activation of around 60 soluble hydrolases (Fig 3) (Luzio *et al*, 2007, 2014; Saftig & Klumperman, 2009; Lawrence & Zoncu, 2019). The lysosomal containment consists of a single membrane coated with a glycosylated intraluminal part of the residing TM proteins. The prevailing lysosomal biogenesis model proposes gradual delivery of lysosomal components to intermediate stages of endocytic or autophagosomal pathways and their progressive maturation upon fusion with lysosomal membrane (Luzio *et al*, 2007, 2014; Saftig & Klumperman, 2009). This leads to the formation of a heterogeneous group of lysosomal vesicles that are commonly identified by staining of marker proteins (the most frequently used lysosomal markers are summarized in Box 1). It is important to note that due to the continuous fusion of vesicles from different origins, the majority of proteins used as markers do not exclusively identify mature degradation-competent lysosomes (Box 1). This has led to some confusion in the literature concerning the presence and function of lysosomes in dendrites and axons. Endo-lysosomal maturation progresses during trafficking to the soma where most lysosomal degradation occurs (Maday *et al*, 2012; Wang *et al*, 2015; Farías *et al*, 2017; Cheng *et al*, 2018; Yap *et al*, 2018; Farfel-Becker *et al*, 2020). Along these lines, electron microscopy (EM) studies revealed a variety of lysosomes of different size and morphology, from electron-dense small vesicles to bigger entities packed with a multilamellar membrane whorl (Luzio *et al*, 2007, 2014; Saftig & Klumperman, 2009).

Compelling evidence was provided for the presence of mature lysosomes in dendrites (White *et al*, 2016; Goo *et al*, 2017; Padamsey *et al*, 2017a, 2017b), and even in dendritic spines (Fig 3)

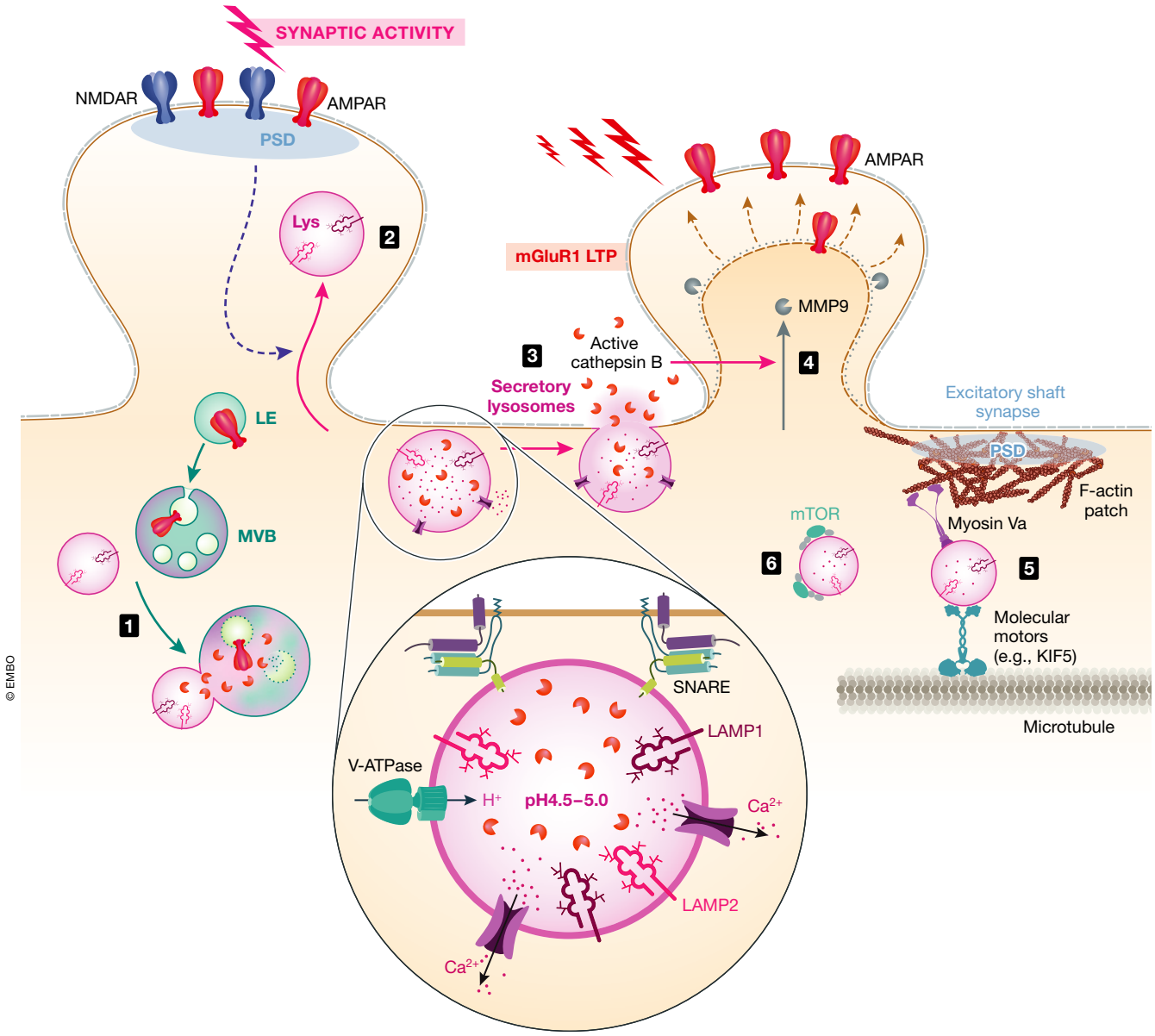

Figure 3. Membrane trafficking and lysosomal protein degradation.

Lysosomes of different sizes and morphology are present in dendrites and even dendritic spines. Lysosomes that specialize in the degradation of vesicular cargo are endpoints for endosomes in dendrites. Fusion of multivesicular bodies (MVB) with the lysosomal membrane is indicated (1). Synaptic activity and in particular activation of synaptic NMDAR (blue dashed arrow) controls trafficking and the distribution of lysosomes in dendrites. Synaptic activity enhances the recruitment of LAMP-positive organelles into dendritic spines in an NMDAR-dependent manner (2). In addition, it results in the immobilization of these organelles at the base of dendritic spines. SNARE-proteins constitute the fusion machinery and association of lysosomes with SNARE complex could prime them for secretion (3). Secretory lysosomes are present in dendrites (3) and involved in the maintenance of structural plasticity. Lysosomes are Ca^{2+} stores with a high intraluminal Ca^{2+} concentration and activity-induced lysosomal Ca^{2+} release precedes the fusion with the plasma membrane in a mGluR1-dependent manner and result in the secretion of active Cathepsin B (CatB) into the extracellular space. In turn, this leads to Matrix metalloproteinase (MMP)9-dependent remodeling of the extracellular matrix and, consequently, results in an enlargement of dendritic spines (indicated with dashed arrows in 4). (5) Transport of dendritic lysosomes along the microtubules is mediated by molecular motors and transport is stalled at F-actin patches. (6) Lysosomes associate with mammalian target of rapamycin (mTOR), a regulator of the local protein synthesis, and serve as cellular metabolic hubs in dendrites integrating nutrient sensing and ensuring balance in lipid and protein synthesis.

(Goo *et al*, 2017). In non-neuronal cells, lysosomes are recruited to the cell periphery in a stimulus-dependent manner (Czibener *et al*, 2006; Samie *et al*, 2013) and lysosomal trafficking in dendrites is tightly controlled by synaptic activity (Goo *et al*, 2017). The distribution of lysosomes is regulated by synaptic AMPAR signaling by yet unknown mechanisms and synaptic activity enhances the recruitment of LAMP1-GFP positive organelles into dendritic spines in a NMDAR-dependent manner (Fig 3, (Goo *et al*, 2017)). These

Box 1

Being an endpoint for endocytosis and autophagy, lysosomes receive membrane from upstream organelles. In addition, their gradual maturation makes it difficult to clearly define experimentally mature lysosomes (Luzio *et al*, 2014). Below, we summarize the most commonly used strategies for the study and identification of lysosomes in neurites (Schröder *et al*, 2010).

- Electron microscopy—lysosomes are identified as a heterogeneous population of globular or tubular shape organelles with an electron-dense lumen and irregular content (e.g., vesicles, membrane sheets).
- Glycyl-L-phenylalanine 2-naphthylamide (GPN)—a dipeptide used to disrupt lysosomal structure. It is assumed that its cleavage by Cathepsin C (CatC) leads to osmotic stress and subsequent disruption of lysosomal structure. This approach is widely used to prove the lysosomal specificity of used probes such as lysotracker (Goo *et al*, 2017; Padamsey *et al*, 2017a; Atakpa *et al*, 2019). However, some reports postulate that GPN also induces increase in lysosomal and cytoplasmic pH and transient release of Ca^{2+} from ER stores leaving lysosomes intact (Atakpa *et al*, 2019; Morgan *et al*, 2020).
- LysoTracker™ DND99—probe that accumulates in acidic compartments, including lysosomes and used to identify these organelles. However, the pKa of i.e. Lysotracker Red is ~7.5 (meaning that > 90% of the dye will be visible at pH = 6.5 (Duvvuri *et al*, 2004)), values that are far away from the optimal pH required by most lysosomal enzymes (~4.5; Sun-Wada *et al*, 2003), and it very likely its labeling refers to nonmature lysosomes and other mildly acidic organelles. Furthermore, it was reported that under certain illumination conditions, the dye is partially converted and acquire additional, green fluorescence, which may pose difficulties during multicolor imaging (Freundt *et al*, 2007).
- Lysosomal-associated membrane proteins—the most abundant are LAMP1, LAMP2, and LAMP3 (also called tetraspanin). Although significantly enriched in lysosomes, they do not localize exclusively to lysosomal membrane and their presence does not suffice for lysosomal identification (Saftig & Klumperman, 2009; Cheng *et al*, 2015; Vukoja *et al*, 2018). Despite their similarity in structure, they seem to play different roles. It is important to note that LAMP2 exists in 3 different splice variants (Eskelinen *et al*, 2005) that take part in distinct functions. For example, LAMP2a is crucial for chaperone-mediated autophagy (Alfaro *et al*, 2018), whereas LAMP2c was implicated in nucleic acid degradation (Fujiwara *et al*, 2013).
- The absence of mannose 6-phosphate receptors (M6PR). M6PR is a key component of the targeting system, by which newly synthesized acidic hydrolases are delivered to immature lysosomes (Luzio *et al*, 2007; Saftig & Klumperman, 2009). The proteolytic activity is achieved only in low pH environment when the M6PR tag dissociates; therefore, catabolically active, mature lysosomes should be M6PR negative (Luzio *et al*, 2014).
- Magic Red™—a Cathepsin B, K, or L substrates that display red fluorescence only upon cleavage by cathepsins, lysosomal enzymes active only in lower pH of acidified organelle.
- Pepstatin A, BODIPY™ conjugate—a Cathepsin D (CatD) inhibitor coupled to green fluorescent probe, labels specifically active Cat D.

results were corroborated by experiments performed in organotypic slices, which revealed immobilization of LAMP1-GFP at the base of dendritic spines upon glutamate uncaging (Goo *et al*, 2017).

Noncanonical roles of lysosomes in dendrites

Apart from a degradative function, dendritic lysosomes may also fulfill a noncanonical exocytotic role (Fig 3). Lysosomal secretion was studied mainly in the context of hematopoietic lineage cells, as well as melanocytes and adipocytes, where specialized subpopulations of secretory lysosomes have been described (Blott & Griffiths, 2002; Morgan *et al*, 2011; Villeneuve *et al*, 2018; Buratta *et al*, 2020). Secretory lysosomes, next to active hydrolases and acidic milieu, contain additional secretory molecules. For example, cytotoxic T cells contain dense cores lysosomes filled with cytolytic proteins, whereas basophils release histamine and serotonin via lysosomal exocytosis (Blott & Griffiths, 2002; Buratta *et al*, 2020). In the brain Ca^{2+}-dependent fusion of lysosomes was reported in astrocytes (Zhang *et al*, 2007; Liu *et al*, 2011; Božić *et al*, 2020) as well as in neurons (Fig 3) (Padamsey *et al*, 2017a, 2017b). Lysosomes are Ca^{2+} stores with a high intraluminal Ca^{2+} concentration (Christensen *et al*, 2002; Patel & Docampo, 2010). Lysosomal Ca^{2+} release precedes fusion (Jaiswal *et al*, 2002; Roy *et al*, 2004; Czibener *et al*, 2006; Luzio *et al*, 2007; Padamsey *et al*, 2017a, 2017b; Foster *et al*, 2018) and is modulated by neuronal activity in dendrites leading to transient steep increases in cytoplasmic Ca^{2+} concentration (Pandey *et al*, 2009; Hui *et al*, 2015; Padamsey *et al*, 2017a, 2017b; Foster *et al*, 2018). It was shown that Ca^{2+} release from acidic stores is essential for mGluR1-dependent LTP, and is upstream to Ca^{2+} release from ER, thus contributing to neuronal depolarization (Foster *et al*, 2018). It is thus likely that this release contributes to membrane fusion and this could provide another level of regulation.

Lysosomal fusion in dendrites results in the secretion of active Cathepsin B (CatB), leading to matrix metallopeptidase 9 (MMP-9)-dependent remodeling of the extracellular matrix (ECM) and in consequence an enlargement of dendritic spines (Fig 3) (Padamsey *et al*, 2017a). Furthermore, the inhibition of neuronal lysosomal fusion significantly reduces dendritic spine density (Padamsey *et al*, 2017b). It is not clear, whether lysosomal fusion has a more defined role for spinogenesis or spine maturation. One exciting possibility is that digestion of the ECM, that confines synapses forming surface compartments, affects the mobility of synaptic TM proteins. Thus, lateral diffusion of AMPAR could be facilitated to increase extrasynaptic receptor diffusion and the exchange of synaptic AMPAR between synapses (Frischknecht *et al*, 2009) but this has not been investigated yet. Along these lines, an interesting question that has not been addressed in detail is whether lysosomal fusion in dendrites happens at discrete positions of the dendritic membrane, whether a specific subpopulation of lysosomes is secretion competent (e.g., tagged with v-SNARE proteins, e.g., Synaptotagmin 7 (Syt7) (Padamsey *et al*, 2017a)) or whether lysosomal fusion does not require membrane specialization. Lysosome positioning has been shown to occur at actin hot spots located in proximity to shaft synapses in dendrites (van Bommel *et al*, 2019) but it is currently unclear whether these hot spots define the region for membrane fusion (Fig 3).

Lysosomal fusion might also have functions that go beyond ECM remodeling. In several cell types including neurons (Padamsey *et al*, 2017b), lysosomal fusion provides additional PM. Lysosomes are found in all neuronal compartments during development and were shown to be crucial for growth cone development (Farías *et al*, 2017; Tran *et al*, 2018; Ibata *et al*, 2019; De Pace *et al*, 2020; Jiang *et al*, 2020). The Ca^{2+}-dependent fusion of mature axonal lysosomes leads to co-release of CatB and the synaptic organizer Cerebellin 1 (Cbln1), a member of the complement component 1q (C1q) family, during granule cell development (Ibata *et al*, 2019). Released Cbln1 is retained by binding to its presynaptic receptor Neurexin and

diffuses laterally along the axonal surface, until binding to the NMDAR subunit GluN2D, which promotes subsequent formation of a synaptic connection (Ibata *et al*, 2019).

Finally, lysosomes are a key cellular metabolic hub, integrating nutrient sensing and ensuring balance in lipid and protein synthesis (Puertollano, 2014; Marat *et al*, 2017; Rabanal-Ruiz & Korolchuk, 2018; Lawrence & Zoncu, 2019). They are central regulators of mechanistic target of rapamycin complex 1 (mTORC1). mTOR, a master controller of protein synthesis, can form an active complex only on lysosomal membranes (Puertollano, 2014; Rabanal-Ruiz & Korolchuk, 2018; Lawrence & Zoncu, 2019; Sanders *et al*, 2019). Some reports indicate a role of lysosomal mTOR signaling in local protein synthesis in dendrites (Takei *et al*, 2004; Wang *et al*, 2011; Miller *et al*, 2014), and it is therefore conceivable that dendritic lysosomes have a dual catabolic and protein synthesis-related function to meet the need of fast-changing demands for synaptic proteostasis. A central question for future work is whether the same organelle is involved in the many facets of outlined above lysosome function in dendrites or whether the dendritic lysosome pool is functionally segregated (Fig 3, Box 1).

Membrane trafficking and presynaptic function

In light of the need to replace and dispose the entire presynaptic proteome, at least a few hundred if not thousand times during the lifetime of a synapse, the remoteness of presynaptic boutons and their intricate structure pose probably the most extreme challenge for membrane trafficking in neurons. However, there is astonishingly little known on mechanisms of protein replacement at presynapses. Gaps in our knowledge concern which degradative pathways are involved and how they contribute to the presynaptic proteome. The specific synaptic function of autophagy, a predominantly axonal degradative pathway in basal conditions (Maday, 2016; Stavoe & Holzbaur, 2019a), is still to a large degree unclear. For this reason, it is important to understand which proteins are eventually sorted for certain degradative mechanisms and how sorting itself is accomplished. This goes along with fundamental questions like which presynaptic sensor mechanisms identify protein "damage," how synaptic activity affects degradation and sorting, and whether there is crosstalk between different degradative pathways, and last but not least how will different modes of protein degradation interconnect with the need for protein replenishment. This long list of unknowns shows how fragmented our current knowledge is and we are just at the beginning to understand the specific contribution of autophagy, proteasome-mediated and endolysosomal degradation to presynaptic proteostasis and how degradative processes impact synaptic signaling and function.

In the following, we will largely focus on the impact of autophagy on synaptic signaling and function and we want to refer to recently published reviews that have covered various aspects of autophagosomal protein degradation (Andres-Alonso *et al*, 2021; Soykan *et al*, 2021). Evidence for a local degradative role of lysosomes in axons is currently scarce (Maday *et al*, 2012; Farfel-Becker *et al*, 2019). As outlined above, continuous membrane exchange hampers the study and characterization of vesicles and organelles that stem from the endolysosomal system and the autophagy pathway, in particular in axons. Available evidence is mainly based on the identification of organelle populations by molecular markers, which comes along with certain shortcomings in the identification of mature lysosomes (see Box 1).

Following internalization, cargo incorporates into endosomes that either undergo homotypic fusion or fuse with an already formed endosome called "early endosome" (EE). The EE constitutes the first major cargo sorting station, where multiprotein complexes localized in specific endosomal subdomains sort cargo for recycling to the plasma membrane or degradation (Fig 4) (Naslavsky & Caplan, 2018). Sorting of cargo in endosomes is typically determined by the presence of ubiquitinated residues, which function as a tag that is recognized by the endosomal sorting complexes required for transport (ESCRT). The ESCRT complex enables the retrieval of cargo and the formation of intraluminal vesicles (ILVs) in the endosomes that are subsequently delivered to lysosomes (Raiborg & Stenmark, 2009). Sorting of cargo takes place not only in early but also in late endosomes (LE)/multivesicular bodies (MVB), organelles that result from the maturation of EE into organelles with a more acidified lumen and new molecular identity (Fig 4). The latter is called "Rab conversion" as it consists of an exchange of endosomal Rab proteins from Rab5, mostly present in EE, to Rab7, abundant in LE (Rink *et al*, 2005; Kiral *et al*, 2018). Acquisition of Rab7 is essential for the long-range transport of LE from dendrites and axons to the soma as well as for the fusion with lysosomes (Guerra & Bucci, 2016). In addition, Rab7 is also required for the retrograde transport of autophagosomes (Fig 4) (Cheng *et al*, 2015).

Autophagy and the endolysosomal system differ in mechanisms of cargo selection although they might take up the same cargo (Andres-Alonso *et al*, 2021). In autophagy, cargo is taken up through a cup-shaped structure called phagophore that expands and ultimately closes generating a double-membrane organelle called autophagosome where cargo is isolated within the inner membrane (Fig 4). Cargo degradation via autophagy comprises not only organelles (i.e., ER, mitochondria, synaptic vesicles (SVs) among others) and protein aggregates but also signaling molecules (Dikic & Elazar, 2018; Pohl & Dikic, 2019). Autophagy receptors can confer cargo selectivity by physically connecting the cargo to the autophagosome by interaction with the lipidated form of microtubule-associated protein 1 light chain 3 (LC3) (Khaminets *et al*, 2016).

The existence of two cellular systems that deliver cargo to lysosomes suggests that both mechanisms serve complementary functions and might be endowed with distinct cargo selectivity. However, little is known with regard to the mechanisms that direct substrates to each pathway. Selectivity for each pathway could be provided by protein adaptors that recognize and sort these ubiquitinated substrates for autophagic or endolysosomal degradation (Bonifacino & Traub, 2003; Mayers *et al*, 2013; Shaid *et al*, 2013). Along these lines, organelles such as the ER (Kuijpers *et al*, 2021), mitochondria (Maday *et al*, 2012; Ashrafi *et al*, 2014), and SVs (Hernandez *et al*, 2012; Binotti *et al*, 2015; Lüningschrör *et al*, 2017; Hoffmann-Conaway *et al*, 2020) as well as scaffold proteins like Liprin-α and Syd-1 (Kiral *et al*, 2020) are engulfed by autophagy at presynapses. However, components of SVs have also been shown to enter the endolysosomal system at boutons (Uytterhoeven *et al*, 2011; Sheehan *et al*, 2016). Moreover, the presence of both degradative pathways *in vivo* within filopodia of neurons in *Drosophila* indicates highly regulated turnover mechanisms within the same compartment (Jin *et al*, 2018).

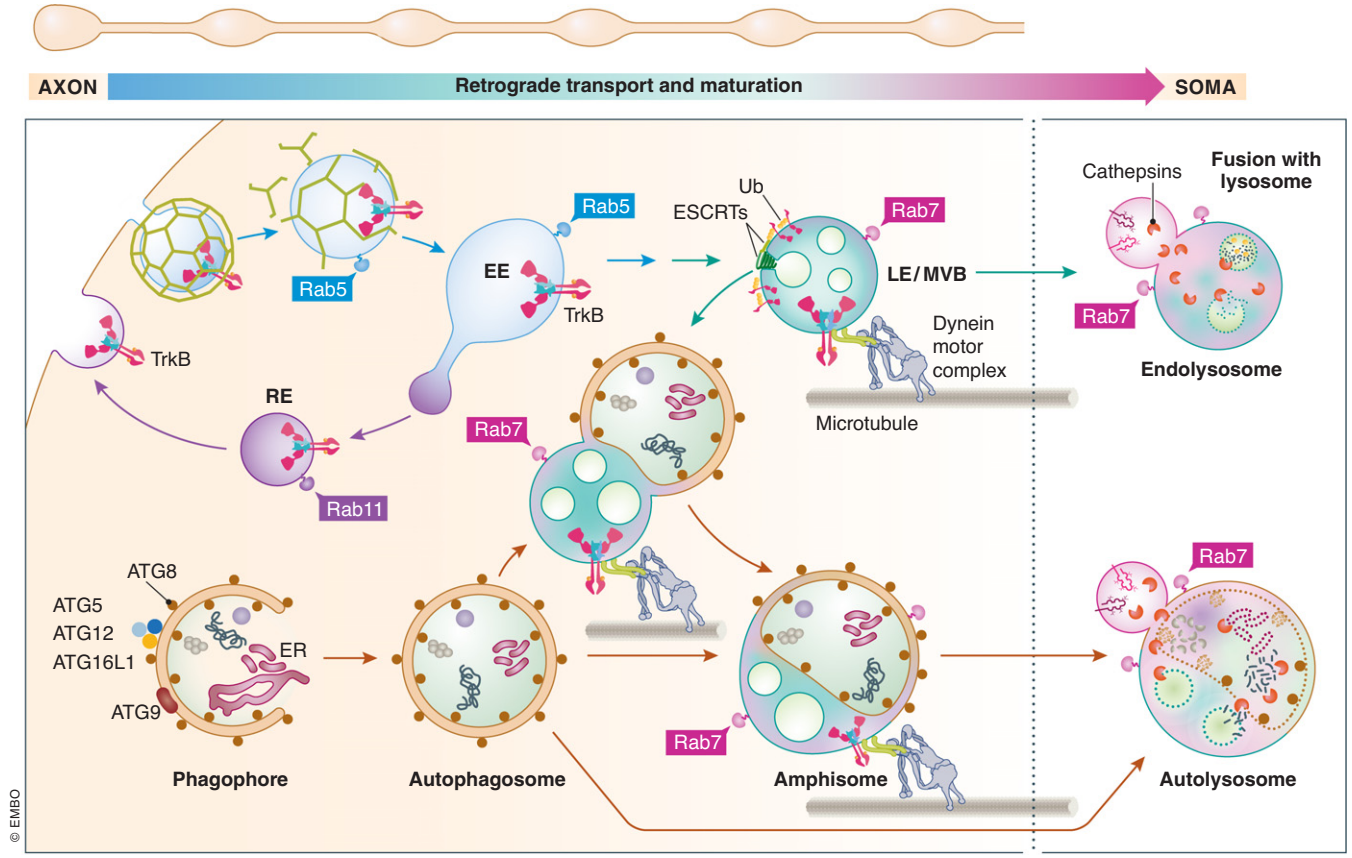

Figure 4. Crosstalk of endo-lysosomal and autophagic degradative pathways in axons.

Following internalization, cargo is incorporated into endosomes. The early endosome (EE) constitutes a major cargo sorting station where multiprotein complexes sort cargo for recycling to the plasma membrane or degradation. Sorting of cargo in endosomes is determined by the presence of ubiquitin residues (Ub) that is recognized by the endosomal sorting complexes required for transport (ESCRT) and enables cargo retrieval. EE mature into organelles with a more acidified lumen and specific molecular identity so-called Rab conversion. Acquisition of Rab7 is essential for the long-range transport of LE/MVB from distal axons to the soma as well as for the fusion with lysosomes. Cargo is then degraded in endolysosomes that arise from the fusion of lysosomes with LE/MVB. Rab7 is also critical for the retrograde transport of autophagosomes. In macroautophagy, at distal axons, cup-shaped phagophore expands engulfing and sequestering cytosolic cargo (i.e., ER, mitochondria, synaptic vesicles (SVs) among others) and after the closer generates a double-membrane organelle called autophagosome. Autophagosomes are retrogradely transported to the soma in a dynein-dependent manner (indicated with the red arrow). Gradient cyan-to-magenta arrow indicates gradual organelle acidification (maturation). Autolysosomes result from fusion of autophagosomes or amphisomes with lysosomes.

Autophagosomes are continuously formed in distal axons

For many years, it was believed that biogenesis of autophagy is strictly compartmentalized. Under basal conditions, autophagosomes form continuously in distal axons (Maday *et al*, 2012; Maday & Holzbaur, 2014; Soukup *et al*, 2016). While they undergo retrograde trafficking to the soma, they become increasingly acidified upon fusion with lysosomes (Fig 4) (Maday *et al*, 2012; Maday & Holzbaur, 2014, 2016). It has been proposed that retrograde transport of damaged organelles and long-lived proteins is the major function of autophagy in axons (Stavoe & Holzbaur, 2019a, 2019b; Andres-Alonso *et al*, 2021; Kuijpers & Haucke, 2021). Along these lines, removal of dysfunctional proteins is associated with improved presynaptic function (Hernandez *et al*, 2012; Truckenbrodt *et al*, 2018) and boosting autophagy in aged neurons rejuvenates synaptic function (Vijayan & Verstreken, 2017; Liang & Sigrist, 2018; Maglione *et al*, 2019). Basal autophagy seems to decline with age (Lipinski *et al*, 2010; Glatigny *et al*, 2019) and expression of key autophagy genes is reduced during aging (Kroemer, 2015; Gupta *et al*, 2016). Conversely, a growing list of studies have demonstrated that upregulation of autophagy not only increases lifespan and neuronal health in a variety of organisms from yeast through nematodes to mice (Pyo *et al*, 2013; Ruckenstuhl *et al*, 2014; Eisenberg *et al*, 2016; Hansen *et al*, 2018; Chen *et al*, 2019) but also ameliorates memory in aged mice (Glatigny *et al*, 2019; Schroeder *et al*, 2021) and fruitflies (Gupta *et al*, 2016). On the contrary, attenuation of neuronal autophagy in *Drosophila* brains is sufficient to mimic age-induced memory decay in younger animals (Bhukel *et al*, 2019).

Accordingly, transgenic mice lacking key autophagy proteins present signs of neurodegeneration such as axonal swelling and accumulation of ubiquitinated proteins at a very early stage (Hara *et al*, 2006; Komatsu *et al*, 2006). Surprisingly, the degenerative phenotypes of mice deficient in expression of essential autophagy genes in the brain are heterogeneous and while inactivation of autophagy

results in cellular death and lethality in some models, in others it does not seem to affect neuronal viability (see Table EV1). These discrepancies likely stem from the different promoters used to drive Cre-expression and that the developmental stage at which autophagy impairment is triggered and the affected cell types might be different. Accordingly, stronger phenotypes are found when autophagy gene inactivation occurs from embryonic stages onward and in several neuronal cell types (see phenotypes induced by EMX1- and Nestin-dependent expression of Cre in Table EV1). Significantly milder phenotypes are observed in mice with conditional alleles when the gene knockout occurred exclusively in neurons at a late stage of brain development (Table EV1). On one hand, this shows the importance of autophagy for neuronal development but on the other hand, this is begging the question whether basal autophagy is essential for presynaptic proteostasis in adulthood. Hence, the study of the specific synaptic role of autophagy in knockout mice might be hampered by functional compensation via the endolysosomal system and more prominent neurodegeneration might only occur with increasing age or when demands for protein degradation are high.

Still the question remains what might be the specific synaptic role of autophagy? Loss of autophagy in dopaminergic terminals from transgenic mice lacking autophagy-related protein 7 (ATG7), an essential protein for autophagosome formation, enhanced evoked dopamine release that was accompanied by a faster presynaptic recovery (Hernandez *et al*, 2012), suggesting that basal autophagy in these terminals controls presynaptic morphology and limits neurotransmitter release. In contrast, impairment of autophagy by knockdown of autophagy-related protein 5 (ATG5) did not have any effect on evoked glutamate release under basal conditions, but evoked release was affected when protein damage was locally induced at boutons by light-activated superoxides (Hoffmann *et al*, 2019). Studies employing conditional mouse lines lacking essential autophagy genes have revealed specific functions of autophagy at boutons that go beyond protein degradation (Negrete-Hurtado *et al*, 2020; Kuijpers *et al*, 2021). Removal of *atg5 and atg16l1*, two proteins of the LC3 lipidation complex, in forebrain neurons induces axonal swelling and accumulation of organelles such as endosomes and mitochondria at terminals, and influences microtubule dynamics with a negative effect on trafficking of axonal cargo (Negrete-Hurtado *et al*, 2020). These effects resulted from blocking lipidation machinery and not autophagy induction suggesting that LC3 lipidation regulates microtubule stability (Negrete-Hurtado *et al*, 2020). Moreover, in the absence of autophagosome formation, ER accumulates specifically at axon terminals but not in dendrites and that this accumulation favors neurotransmitter release by elevating Ca^{2+}-release from intracellular stores via RyR (Kuijpers *et al*, 2021). Thus, basal autophagy in axons mediates to a large extent the turnover of cortical/tubular ER membranes and loss of ER-phagy facilitates excitatory neurotransmission by increasing presynaptic release probability (Kuijpers *et al*, 2021).

The formation of autophagosomes is enhanced by synaptic activity

Autophagy is enhanced at boutons upon increased synaptic activity (Wang *et al*, 2015; Soukup *et al*, 2016) and one might speculate that activity-dependent autophagy has functions that could differ from basal autophagy (Andres-Alonso *et al*, 2021; Kuijpers *et al*, 2021). The mechanisms that couple autophagosome biogenesis locally at synapses to neuronal activity are still unknown but it has been suggested that trafficking of ATG9, which is the only TM protein in the core autophagy pathway, links the SV cycle to autophagy in *C. elegans* (Fig 5) (Yang *et al*, 2022). Interestingly, ATG9 is generated from the TGN and undergoes exo-endocytosis at presynaptic sites in an activity-dependent manner (Yang *et al*, 2022). Thus, ATG9 exo-endocytosis and subsequent endocytosis might be instrumental in autophagosome biogenesis at presynaptic sites based on an intimate link with the activity-dependent synaptic vesicle cycle (Yang *et al*, 2022). The presence of GS has been reported in axons (Cornejo *et al*, 2020), and this raises the intriguing possibility that ATG might traffic from local axonal Golgi membranes to boutons (Fig 5).

In contrast to axons, basal autophagy occurs only at a very low rate in dendrites (Stavoe & Holzbaur, 2019a). It has become apparent in recent years that autophagosome formation in dendrites is prominently induced following induction of long-term depression (LTD) (Fig 5) (Shehata *et al*, 2012; Kallergi *et al*, 2022; Compans *et al*, 2021), raising the possibility that autophagy directly contributes to activity-dependent synaptic changes (see also Nikoletopoulou *et al* (2017)). LC3 positive vesicles contain cargo of postsynaptic origin including AMPAR and the postsynaptic scaffolding protein postsynaptic density protein 95 (PSD-95) (Fig 5) (Kallergi *et al*, 2022; Compans *et al*, 2021). Moreover, NMDAR-dependent LTD induction requires the autophagy machinery to remove PSD-95 from synapses, which leads to an increase in AMPAR surface mobility (Compans *et al*, 2021) and induces transcription-dependent autophagy for synaptic turnover and late-phase LTD (Pan *et al*, 2021). Transcription-dependent autophagy depends upon activity-dependent nuclear import and dephosphorylation of CREB-regulated transcription coactivator 1 (CRTC1) (Pan *et al*, 2021). In sharp contrast, another study claimed that autophagy in dendrites is inhibited upon LTD induction and that this inhibition is essential for the expression of this type of plasticity (Shen *et al*, 2020). At present, it is hard to overlay these discrepant findings into a coherent picture, but they clearly point to a spatially segregated regulation of autophagosome biogenesis and potentially function. Interestingly, synaptic activity reduces the motility of autophagosomes in dendrites (Fig 5) but not in axons of hippocampal primary neurons, whereas neuronal silencing has the opposite effect (Kulkarni *et al*, 2021).

Signaling amphisomes

As outlined above, maturation and transport of autophagosomes requires fusion with endosomal compartments for the acquisition of molecular motors that enable their retrograde transport to the soma (Maday *et al*, 2012; Cheng *et al*, 2015). This fusion generates a hybrid organelle called amphisome that ultimately undergoes fusion with lysosomes. Two studies have shown a role of amphisomes in neuronal signaling (Kononenko *et al*, 2017; Andres-Alonso *et al*, 2019). Brain-derived neurotrophic factor (BDNF) binds to and activates Tropomyosin-related Kinase B (TrkB) receptors localized in the PM. The BDNF/TrkB complex is subsequently endocytosed into signaling-competent compartments termed signaling endosomes that undergo long-range retrograde transport to the soma where

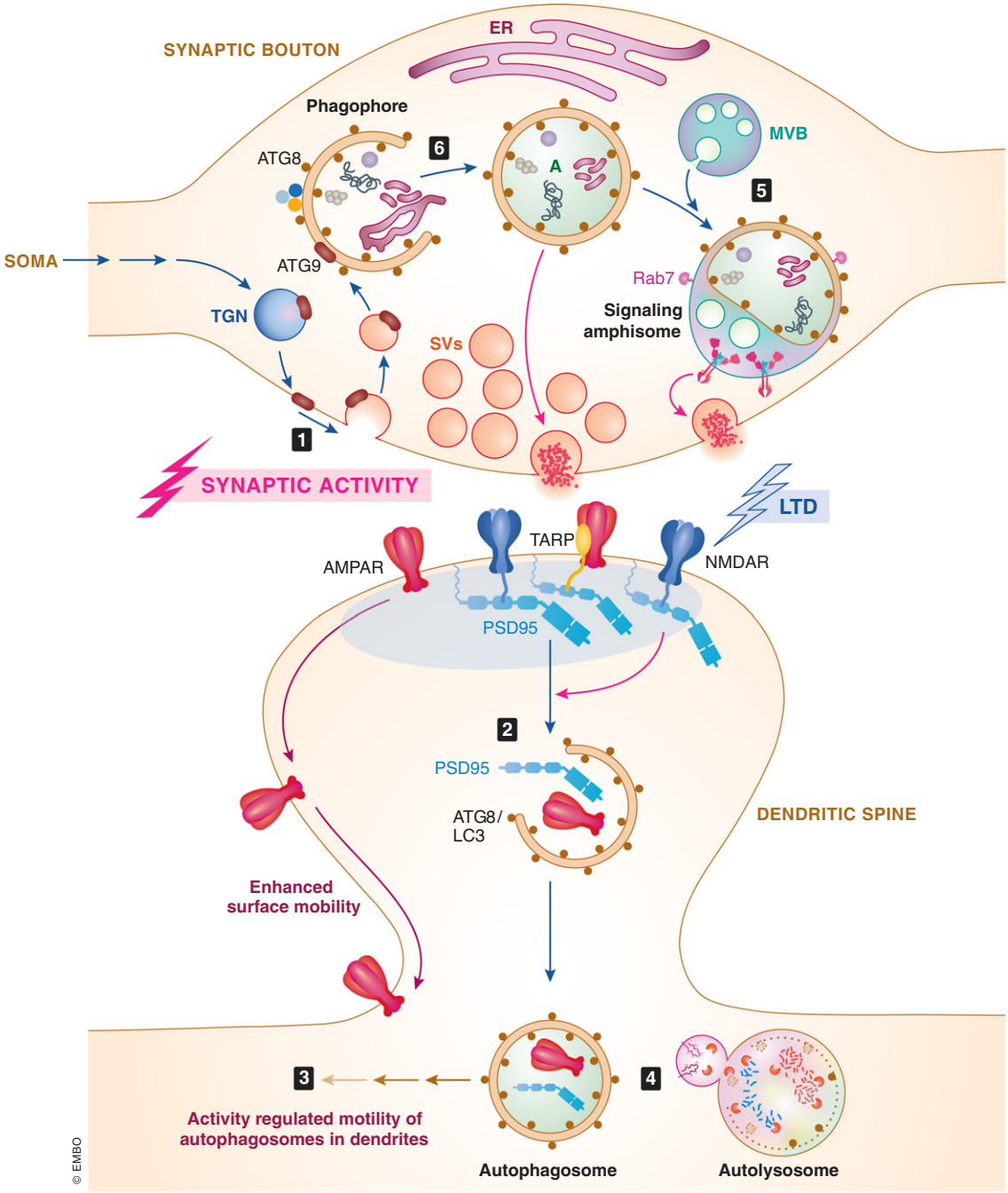

Figure 5. Roles of autophagy in pre- and postsynaptic function.

(1) Autophagosome biogenesis is locally regulated at synapses and increases in response to neuronal activity. ATG9, the only transmembrane protein in the core autophagy pathway, undergoes exo- and endocytosis in an activity-dependent manner and couples autophagosome biogenesis at presynaptic sites with the activity-dependent synaptic vesicle cycle. (2) At the postsynapse, autophagic ATG8/LC3 containing vesicles can be generated in dendrites upon induction of long-term depression (LTD). Collectively, this mechanism contributes to the removal of PSD-95 from synapses, which in turn leads to an increase in AMPAR surface mobility, as well as removal of AMPAR raising the possibility that autophagy pathway is involved in activity-dependent synaptic changes. (3) Synaptic activity reduces the motility of autophagosomes in dendrites and (4) increases their presence in dendrites. (5) Amphisomes, hybrid organelles resulting from the fusion of autophagosomes with endosomal compartments, enable local TrkB signaling at bouton. This promotes neurotransmitter release and might serve to mediate activity-dependent synaptic changes. (6) Neuronal ER-phagy is involved in regulation of pre-synaptic excitatory neurotransmission by controlling the axonal endoplasmic reticulum and calcium release from ER stores.

they regulate gene expression (Huang & Reichardt, 2001). Little is known about the molecular identity of signaling endosomes and how they escape degradation and ensure long-range signaling. Kononenko and colleagues revealed the localization of TrkB receptors in vesicles of the autophagy pathway, whose retrograde transport is mediated by the endocytic adaptor activator protein 2 (AP-2)

and the dynein activator p150^{Glued} (Kononenko *et al*, 2017). The absence of AP-2 rendered these organelles immobile and conditional ablation of AP-2 triggered a significant deficit in neuronal arborization and a decrease in the expression levels of TrkB-target genes *in vivo*, indicating that TrkB transport in autophagic vesicles is essential for the long-range signaling function of the receptor (Kononenko *et al*, 2017). In addition, we have demonstrated that axonal TrkB-amphisomes enable local TrkB signaling at single boutons on their way back to the soma, thereby promoting neurotransmitter release at single terminals (Andres-Alonso *et al*, 2019). This is mediated by the protein signal-induced proliferation-associated 1-like protein 2 (SIPA1L2), which directly interacts with TrkB and provides a link to dynein motors via interaction with the motor adaptor Snapin. Moreover, SIPA1L2 allows the temporal and spatial control of TrkB signaling by regulating the activation of the TrkB-effector Rap1 required for the downstream long-range activation of ERK1/2. Direct interaction of SIPA1L2 with LC3 in amphisomes promotes the RapGAP activity and controls both the trafficking and the signaling properties of the complex. PKA-dependent phosphorylation triggers the stopover of the complex at boutons and decreases SIPA1L2 Rap1 GTPase-activating protein (RapGAP) activity, which locally activates ERK1/2 and ultimately promotes neurotransmitter release (Andres-Alonso *et al*, 2019). In agreement with the involvement of this mechanism in plasticity-related mechanism, animals lacking *sipa1l2* present cognitive deficits and an impairment in presynaptic mossy-fiber long-term potentiation (Andres-Alonso *et al*, 2019). Hence, it can be hypothesized that in the absence of autolysosome formation, amphisomes serve as signaling and sorting platforms while trafficking in a retrograde direction to the cell soma. This could give an answer to questions such as why neurons transport autophagic and endocytic cargos back to the cell body for degradation instead of disposing them locally. How signaling endosomes escape a degradative pathway following endocytosis and how autophagy regulates presynaptic plasticity is not known.

Conclusions and future directions

In the past decade, the relevance of membrane trafficking processes for synaptic function has been appreciated but the role of local organelles for the proteostasis of synaptic proteins is still less than clear. In particular, the complexity of endosomal sorting processes, the highly dynamic exchange of membrane, the limited reliability of marker proteins to identify organelles in neurites, and the potential for compensation by alternative pathways in loss of function studies have hampered progress in our understanding of forward and retrograde membrane trafficking.

Collectively, published studies suggest that the enormous complexity of neuronal cytoarchitecture has led to ways of long-distance protein transport that combines degradative with signaling functions. Nonetheless, several key questions are still unanswered: What are the specific contributions of autophagy, proteasome-mediated, and endolysosomal degradation to synaptic proteostasis? How are synaptic function and synaptic plasticity regulated by autophagy? How is autophagy regulated locally? Why is this regulation different for axons and dendrites? And, finally, how do noncanonical functions of autophagosomes in signaling impact synaptic development, maintenance, and function?

Recent reports point to an intriguing scenario in which lysosomes (Ibata *et al*, 2019) and lysosome-related organelles (Vukoja *et al*, 2018) may fuse to axolemmas in an activity-dependent manner. As outlined above, the axon of mature neurons is largely devoid of catabolically active lysosomes (Maday *et al*, 2012; Cheng *et al*, 2015; Lie *et al*, 2021). Instead, a gradual delivery of lysosomal proteins to retrogradely transported cargo was described (Lie *et al*, 2021). The gradual acidification of axonal organelles appears to require Golgi-related transport carriers (Lie *et al*, 2021) and GS have been found in axons (Cornejo *et al*, 2020). Furthermore, this subset of GS was also characterized by markers associated with exocytic vesicles, which implicates their role in a cargo delivery for yet undefined studies on axonal endocytosis. In light of these findings, axonal GS emerge as a mobile hub for both forward trafficking of axonal proteins and delivery of lysosomal component to retrogradely transported cargo. Furthermore, Cornejo and colleagues speculate that passing of LAMP1 through GS leads to the generation of nonconventional secretory vesicles (Cornejo *et al*, 2020). Along these lines, it is unclear whether and how such vesicles are related to secretory autophagy that was proposed to explain the unconventional secretion of cytosolic proteins (Ponpuak *et al*, 2015). Secretion of aggregation-prone proteins like α-synuclein requires autophagosome formation and secretory autophagy of α-synuclein is reportedly enhanced by inhibiting the fusion of autophagosomes with lysosomes (Ejlerskov *et al*, 2013).

Similarly, key open questions regarding secretory forward trafficking in dendrites will likely be addressed in the very near future. It is tempting to speculate that two segregated pathways of forward trafficking exist, one direct pathway that bypasses the Golgi and the other ERGIC-GS-Retromer pathway, and that a switch between both pathways might occur in response to neuronal activity or the induction of synaptic plasticity. Membrane trafficking could then be mainly routed through the faster direct pathway and following endocytosis and retrograde transport to GS cargo might be glycosylated before membrane insertion possibly via RE. Alternatively, and possibly after removal of sugar residues local re-modelling of glycans on receptors and cell adhesion molecules in confined dendritic segments. Thereby, even subtle changes in the neuronal glycoproteome might affect local neuronal excitability and synaptic properties.

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The authors declare that they have no conflict of interest.

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