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REVIEW

Neurons Specialize in Presynaptic Autophagy: A Perspective to Ameliorate Neurodegeneration

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Abstract

The efficient and prolonged neurotransmission is reliant on the coordinated action of numerous synaptic proteins in the presynaptic compartment that remodels synaptic vesicles for neurotransmitter packaging and facilitates their exocytosis. Once a cycle of neurotransmission is completed, membranes and associated proteins are endocytosed into the cytoplasm for recycling or degradation. Both exocytosis and endocytosis are closely regulated in a timely and spatially constrained manner. Recent research demonstrated the impact of dysfunctional synaptic vesicle retrieval in causing retrograde degeneration of midbrain neurons and has highlighted the importance of such endocytic proteins, including auxilin, synaptojanin1 (SJ1), and endophilin A (EndoA) in neurodegenerative diseases. Additionally, the role of other associated proteins, including leucine-rich repeat kinase 2 (LRRK2), adaptor proteins, and retromer proteins, is being investigated for their roles in regulating synaptic vesicle recycling. Research suggests that the degradation of defective vesicles via presynaptic autophagy, followed by their recycling, not only revitalizes them in the active zone but also contributes to strengthening synaptic plasticity. The presynaptic autophagy rejuvenating terminals and maintaining neuroplasticity is unique in autophagosome formation. It involves several synaptic proteins to support autophagosome construction in tiny compartments and their retrograde trafficking toward the cell bodies. Despite having a comprehensive understanding of ATG proteins in autophagy, we still lack a framework to explain how autophagy is triggered and potentiated in compact presynaptic compartments. Here, we reviewed synaptic proteins' involvement in forming presynaptic autophagosomes and in retrograde trafficking of terminal cargos. The review also discusses the status of endocytic proteins and endocytosis-regulating proteins in neurodegenerative diseases and strategies to combat neurodegeneration.

Keywords Synaptic dysfunction · Autophagy · Endocytic proteins · Presynaptic autophagy · Neurodegenerative diseases · Synaptic proteins

Introduction

Neurons are post-mitotic cells with intricate cellular architecture. Unlike non-polar cells, neuronal morphology is differentiated into compartments—dendrites, cell body (soma), axon, and arborized terminals [1]. The polarized morphology and increased surface area amplify the energy budget required to maintain ion gradients, resting potential, action potential firing, and efficient synaptic transmission [2, 3]; together, increasing the metabolic rate and making them prone to oxidative stress, organelles dysfunction, protein aberrations, and neurodegeneration [3]. Furthermore, the neuronal compartments (soma and axon terminals) differ in morphology, chemical composition, and biochemistry [4]. While the soma of neurons is almost like non-polar cells, the extended axon and terminals are devoid of the nucleus and

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lack major synthetic machinery to synthesize biomolecules [5]; therefore, axon terminals primarily rely on biosynthetic processes occurring in the cell body for the urgent supply of organelles, proteins, and other biomolecules required for neurotransmission. However, during synaptic maintenance and plasticity, presynaptic compartments abruptly adapt autonomy to synthesize local proteins following the distribution of ribosomes, mRNAs, and other synthetic machinery in the compartments [6].

Apart from the reliance on cell body, axon terminals and synaptic boutons are shrunken-cytoplasmic zones with increased bioenergetics due to high mitochondrial density [1], which altogether bows them down in the state of being likely to be affected. Research in many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD), showed that aberrant proteins and organelles accumulate in the axon terminals much earlier than degeneration events [7–9]. It is often impossible for neurons to distribute or eliminate the redundancy due to their post-mitotic nature; mainly, the anomalies are unevenly segregated more in presynaptic terminals than in the neuronal soma [10]. It is probably because of the higher density and significant volume occupancy of terminals and synapses, which uniquely makes them predominantly vulnerable [11, 12], putting them at the degeneration edge. Therefore, presynaptic terminals in neurons deteriorate before their soma degenerates [13–16].

Autophagy is a lysosome-dependent evolutionarily conserved clearance process. Typically, every mammalian cell relies upon autophagy to digest its anomalies. It rejuvenates the cell by catabolizing and recycling several subcellular constituents, including redundant proteins, lipids, and dysfunctional organelles. Also, the increased catabolic process delivers nutrition during starvation by digesting damaged proteins and organelles when no longer required [17]. The process involves the phagophore formation that sequesters ubiquitin-tagged superfluous proteins and organelles and subsequently matures until its open edges fuse to create an enclosed autophagosome vesicle. The mature autophagosome carries the sequestered cytoplasmic debris to the lysosome, which digests and recycles the components back into the cytosol [18]. Neurons depend on efficient clearance mechanisms like autophagy to regularly digest and recycle damaged organelles and aberrant proteins and sustain neuronal health and function [19, 20]. Research showed that in many neurological disorders, including AD, PD, HD, and amyotrophic lateral sclerosis (ALS), defective autophagy leads to proteotoxic and oxidative stress due to augmented protein misfolding and mitochondrial dysfunction, respectively [21–27].

One of the current strategies to combat neurodegeneration in such diseases is to protect deteriorating neurons by enhancing neuronal autophagy to boost the clearance of cellular debris. However, the therapeutic opportunities to treat neurodegenerative diseases by targeting abnormal neuronal autophagy in deteriorating neurons are minimal. One reason for unsatisfying results could be the ignorance of neuronal cytoarchitecture while studying autophagy in neurons. Researchers study neuronal autophagy as they study it in other non-polar cells. But, due to the peerless cytomorphology of the neuron and intra-neuronal difference in the chemical composition of the cell body and axon terminals, they possess compartment-specific autophagy [4, 10]. That is why boosting autophagy in neurons often digests the junks but fails to protect crucial presynaptic terminals in the absence/breakdown of local (presynaptic) clearance. Hoffmann et al. demonstrated that presynaptic boutons possess a robust, highly, and locally regulated distinct autophagy that responds only to local damages and temporally digest misfolded synaptic proteins therein to maintain the synaptic health, integrity, and function [28]. Thus, understanding local quality control mechanisms (presynaptic autophagy), other than general autophagy in neurons, in arborized terminals and presynaptic compartments, is timely, urgent, and needed to ameliorate the early degeneration of synaptic terminals in neurodegenerative diseases.

Endophilin A (EndoA) Remodels Vesicular Structure: The Docking Site for the ATG Proteins

Endophilin A (EndoA) is a presynaptic protein that regulates multiple membrane remodeling through several mechanisms, such as scaffolding, inserting amphipathic α-helices into the lipid bilayer, and protein crowding. By scaffolding and protein crowding mechanisms, the EndoA forcibly changes the conformation of intrinsic proteins and recruits its binding partners, such as dynamin-1 and synaptojanin 1 (SJ1), respectively [29, 30]. Inserting amphipathic α-helices into the lipid bilayer generates wedge force [29]. Structurally, EndoA consists of two major domains: an N-terminal BIN/amphiphysin/Rvs (BAR) domain—for the induction, stabilization, and sensing membrane curvature of the clathrin-coated vesicle (CCV)–and a C-terminal src homology 3 (SH3) domain that facilitates proteins' interaction and recruitment to the membrane curvature site (Fig. 1) [29, 31]. Typically, the BAR domain signals the protein to induce only the internal curvature of the membrane through the scaffolding mechanism. But, on binding with the curved membrane (like the synaptic membrane), the EndoA inserts its amphipathic α-helices (residue 60–87) into the bilipid layer. The protein's BAR domain forms tight contact with the membrane, and its amphipathic α-helices penetrate deeply into the bilipid layer, causing the membrane to shape itself tubular [32]. However, the

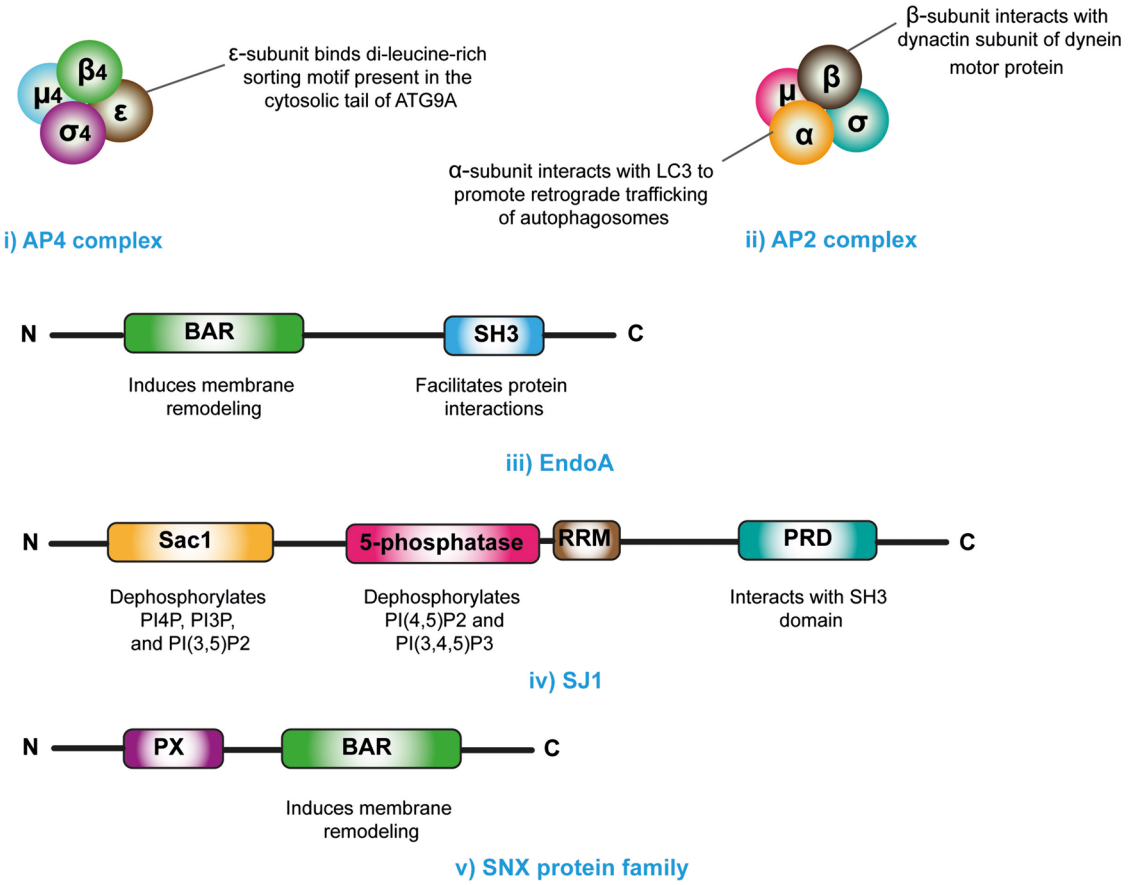

Fig. 1 Structure of AP4, AP2, EndoA, SJ1, and SNX protein family showing roles of their subunits and domains

surface ingression of amphipathic α-helices—where the scaffolding effect is significantly weakened due to the distantly placed BAR domain—remodels the membrane into a vesicle [29, 32–34]. Interestingly, PD-linked leucine-rich repeat kinase 2 (LRRK2) phosphorylates the EndoA at S75, residing in the protein's amphipathic α-helix [29]. The corresponding phosphorylation toggles a conformational switch from the tubular to the vesicular structure [29, 35, 36]. The phosphorylated EndoA associates loosely with the membrane to promote a highly curved membrane compared to the non-phosphorylated form. Research showed that autophagic proteins (ATGs) use the increased membrane curvature as a docking site, and LRRK2-mediated phosphorylated EndoA promotes ATG3 recruitment to the vesicles [36]. The site-specific (S75) mutation in EndoA blocks autophagy in the terminal compartments, which implies the direct role of membrane-bending protein EndoA in presynaptic autophagy, apart from its vital role in endocytosis [36, 37]. Murdoch et al. showed that reduced expression levels of EndoA in mammalian models result in impaired autophagosome formation, decreased autophagy, and aggregation of ubiquitinated and dysfunctional proteins over time, which eventually lead to impaired movement, age-dependent ataxia, and neurodegeneration [38].

Interestingly, Endophilin B1 (EndoB1), an endophilin protein family member, also regulates autophagy in neurons [39]. Like EndoA, EndoB1 has BAR and SH3 domains at N-and C-terminal positions, respectively [40]. The originally discovered Bax-interacting protein, EndoB1, is a versatile protein with involvement in various cellular events, such as apoptosis, autophagy, and mitochondrial health [41]. EndoB1 interacts with the Beclin1 via ultraviolet irradiation resistant–associated gene (UVRAG) [39]. Beclin1 is a nucleation-promoting protein that forms the EndoB1-Beclin1 complex and activates the PI3KC3 to promote autophagy [39]. Contrary to LRRK2-mediated phosphorylation of EndoA, Cdk5 activity phosphorylates EndoB1 at the T145-position in its BAR domain [42]. Research reported significantly decreased EndoB1 protein expression in AD patients, where the cause of pathogenesis is the aggregation of amyloid-β and its reduced clearance in hippocampal neurons [41]. According to Wang et al., the increased load of amyloid-β is secondary to loss of EndoB1-mediated autophagy [41]. Thus, the phosphorylation of both EndoA

and EndoB1 induces autophagy for synaptic health [16, 36, 37], but through a different mechanism.

Synaptojanin 1 Removes PI3P to Promote Autophagosome Biogenesis

SJ1 is a poly-phosphoinositide phosphatase synaptic protein having dual but parallel roles in endocytic recycling and presynaptic autophagy. The protein comprises an N-terminal Sac1 domain to dephosphorylate PI4P and a central 5-phosphatase domain to remove phosphate from PI(4,5)P_2 and PI(3,4,5)P_3. It also consists of RNA recognizing motif (RRM), and an unstructured C-terminal proline-rich domain (PRD); the PRD interacts with SH3 domain–containing endocytic proteins, primarily EndoA, during clathrin uncoating (Fig. 1) [43–45]. The binding of SJ1 to curvature-generating EndoA—near the neck of an endocytic pit—couples to dephosphorylation of synaptic vesicle–enriched PI(4,5)P_2 at 5- and 4-phosphosite by SJ1's 5-phosphatase and the Sac1 domain, respectively. 5-phosphatase domain's preferential hydrolysis of PI(4,5)P_2 at 5-position [44, 46] lowers the affinity of clathrin adaptors from the nascent synaptic vesicular membrane. Thus, the detachment of clathrin adaptors from the synaptic vesicle promotes the downstream proteins, including auxilin, recruitment to disassemble the clathrin lattice. Once the clathrin disperses in the cytosol, the Sac1 domain's 4-phosphatase activity helps to release the auxilin for the next cycle of uncoating [43].

In addition to its activity as a PI4P phosphatase, the Sac1 domain can dephosphorylate PI3P and PI(3,5)P_2, both of which are non-synaptic membrane-enriched phosphoinositide phosphates [44]. The differently phosphorylated phosphoinositide lipids act as molecular tags for translocating specific proteins to the membrane to confer the membrane's identity [47–50] For example, PI(4,5)P_2 is abundantly found on the synaptic membrane and functions as tags to bind clathrin adaptors and other endocytic proteins. Similarly, PI3P and PI(3,5)P_2 are critical phosphoinositide phosphates on the phagophore that recognize ATG18 to promote autophagosome biogenesis [51]. The ATG18 on the phagophore membrane encourages the recruitment of ATG16, a binding partner of the ATG12-ATG5 conjugate [52]. The ATG12-ATG5 conjugate converts LC3 I (un-lipidated form) to LC3 II (lipidated form). At the same time, ATG16 of the ATG12-ATG5-ATG16 complex determines the position of LC3 lipidation [52–54]—the conversion of LC3 I to LC3 II results in membrane elongation and formation of autophagosomes.

Sac1 domain–mediated hydrolysis of PI3P and PI(3,5)P_2 from the membrane removes early-autophagosome markers, including ATG18, thereby promoting the elongation protein, ATG8/LC3, recruitment to the autophagosome [44, 55]. The PD-associated R258Q mutation in the Sac1 domain of SJ1 aggregates PI3P/PI(3,5)P_2-binding protein ATG18 on premature autophagosomes that fail to mature at *Drosophila* synapses and in human neurites [44]. Pan et al. also reported that Parkinsonian mutation (R258Q and R839C) of SJ1 augments autophagosome formation but decreases autophagy [56].

While SJ1 regulates presynaptic autophagy through ATG18, research showed that the active zone constituting cytoskeleton matrix protein Bassoon regulates the process through ATG5. Bassoon consists of an ATG5-binding peptide motif (CC2v1) in its structure to bind and inactivate ATG5's role as an E3-like ligase. The binding of ATG5 with Bassoon disrupts ATG5/12 interaction with ATG16. Thus, it inhibits the catabolic events in the presynaptic compartments [57].

The Adaptor Protein (AP)2 Complex Mediates Retrograde Trafficking of Autophagosomes

The adaptor protein (AP)2 complex is the membrane-derived protein complex that participates in the clathrin-mediated coating. In AD-linked hippocampal neurons, the AP2 complex prevents amyloidogenesis by regulating amyloid precursor protein (APP) processing and amyloid-β formation [58, 59]. The endocytic adaptor interacts with the two crucial proteins of HD, i.e., Huntingtin and Huntingtin-interacting protein 1 (HIP1); however, the physiological role of AP2 binding with these proteins is elusive [60]. The AP2 complex primarily anchors the clathrin to the membrane's PI(4,5)P_2, at 5-phosphosite present on synaptic vesicles [61]. The clathrin-AP2 complex is a crucial center for synaptic proteins' interactions, which regulate clathrin uncoating of clathrin-coated pits [61]. Once the AP2 complex is recruited to the completely formed CCV, the EndoA translocates dynamin-2 to the collar of the CCV to pinch off the CCV from the sites into presynaptic axoplasm [62, 63]. Moreover, the AP2 complex is also responsible for the arborization of dendrites via its upstream kinase. The AP2-associated kinase1 is an upstream kinase that activates the AP2 complex protein to promote dendritic branching [64].

The AP2 is a heterotetramer protein comprising four subunits: α, β, μ, and σ subunits (Fig. 1). The first two subunits participate in the retrograde trafficking of the autophagosomes: the α-subunit binds to LC3 protein on the late-stage autophagosome (marked by LC3 and Rab7). At the same time, the β-subunit anchors the p150Glued subunit of dynactin [65, 66]. The dynactin is a cofactor of a retrograde motor protein dynein [66]. Thus, the LC3-AP2-p150Glued complex is especially crucial in neurons

because the spatially segregated autophagosomes in distal terminals must travel a considerable distance retrograde (Fig. 3) with high precision and rate to accurately reach the soma for ultimate clearance [65]. The AP2 complex also participates in ATG9A (mammalian homolog of ATG9) trafficking; it translocates the protein from the presynaptic membrane to the endosome [40, 67].

The AP3/AP4 Complex Anterograde Transports ATG9 into Presynaptic Sites for Exocytosis

Reports show many other AP complexes that have their role in presynaptic autophagy [11, 68, 69]. In *C. elegans*, one such protein is the AP3 complex that buds off the vesicles from the trans-Golgi network (TGN) and delivers them to the presynaptic compartments. Interestingly, these TGN-derived vesicles released from the juxtanuclear regions are ATG9-positive [11]. In general, ATG proteins are cytosolic proteins, but ATG-9 is the sole evolutionary-conserved transmembrane protein in the family of autophagy proteins and is localized to various subcellular loci. In neurons, the protein is localized to multiple compartments, including TGN-derived vesicles, plasma membranes, early endosomes, recycling endosomes, and phagophores [70]. ATG9 traffics between these sites to create its pool ready to contribute to the autophagosome biogenesis upon autophagy induction [71–73]. The protein works with ATG2 to promote nucleation of the nascent phagophore [74, 75]. The ATG2 plays a vital role in lipid transportation to support membrane elongation [76]. While membrane-bound ATG9 randomly traffics in axoplasm, the AP3 complex binds the cytosolic tail of ATG9 and mediates its anterograde transportation to the plasma membrane of the presynaptic site [11]. The mammalian homolog of AP3 complex is a AP4 complex that drives the ATG9A to the presynaptic membrane [11, 68, 69]. It also interacts with the cytosolic tail of AD-linked amyloid precursor protein (APP) [77]. The AP4 complex is a heterotetrameric adaptor protein that consists of ε, β4, μ4, and σ4 subunits; the ε-subunit binding to ATG9A translocates it to the terminal compartments (Figs. 1 and 2) [68, 69]. In mammalian neurons, AP4-ε KO leads to ATG9A aggregation in TGN and depletion in the peripheral cytoplasm [69]. Intriguingly, the AP2 complex also participates in ATG9 trafficking; it translocates the protein from presynaptic membrane to the endosome (Fig. 2). Both AP2 and AP4 complex bind tyrosine- and di-leucine-enriched sorting motif in the cytosolic tail of ATG9—to traffic the autophagic protein [68].

TBC1D5 and AP2 Complex Together Create an ATG9 Pool for Autophagosome Biogenesis

Popovic and Dikic reported that the deletion of AP2 blocks the presynaptic (intra-compartmental) trafficking of ATG9 to the late endosome under the basal or upon autophagy induction [46, 62]. Under the basal conditions, the deletion enhanced ATG9 localization on the plasma membrane of presynaptic compartments, which infers failed endocytosis of ATG9-positive membrane in the absence of AP2. Upon autophagy induction, the ATG9-positive TGN-derived vesicles accumulate in the juxtanuclear region. Also, the deletion hinders the binding of ATG9 with its intra-compartmental trafficking-carrier TBC1D5 [62]. The TBC1D5 is a crucial Rab-GTPase (RabGAP) protein that interacts with the retromer protein complex to regulate axonal retrograde trafficking [78, 79]. Research showed that TBC1D5 regulates the growth and function of synapses, and their loss leads to aberration in the presynaptic terminals, including abnormal development, change in morphology, synaptic vesicle density, and excessive satellite buttons and branch formation [80].

In a functional neuron, upon autophagy induction, the ATG9-positive TGN-vesicle pool is driven to the presynaptic compartment for exocytosis. At the same time, the plasma membrane–associated ATG9 is endocytosed to the clathrin-AP2-complex positive CCVs [62, 72]. The shedding off clathrin from the vesicle membrane by SJ1 and auxilin provides an opportunity for TBC1D5 to interact with the AP2 bound to the uncoated early endosome [62]. Instantly, the TBC1D5 recruits to the endosomal membrane containing AP2 complex and ATG9; the membrane, along with the proteins, is transferred to the recycling endosome and then to the forming pre-autophagosomal structure to support the supply of membrane, ATG9, and AP2. The ATG9 is an autophagy initiation protein, while the other two proteins, AP2 and TBC1D5, bind to the autophagosome elongation protein LC3. Here, the TBC1D5 interacts with LC3, with its LC3 interacting region 1 (LIR1), and promotes protein internalization and sequesters cytoplasmic materials [62, 81, 82], while the AP2 complex directs retrograde trafficking of the autophagosome (Fig. 3) [59, 62]. Interestingly, TBC1D5 depletion leads to the hang-up of clathrin-mediated endocytosis and missorting of ATG9, suggesting a dual role of TBC1D5 in endocytosis and autophagy [62]. However, it is yet to be determined whether the retrograde trafficking facilitator AP2 complex directly binds to LC3 on the mature autophagosome or indirectly through interacting with the LC3-bound TBC1D5. Also, how the AP2 switches its function—from endocytosis to retrograde trafficking, and vice-versa—in the neuronal terminal is still obscure.

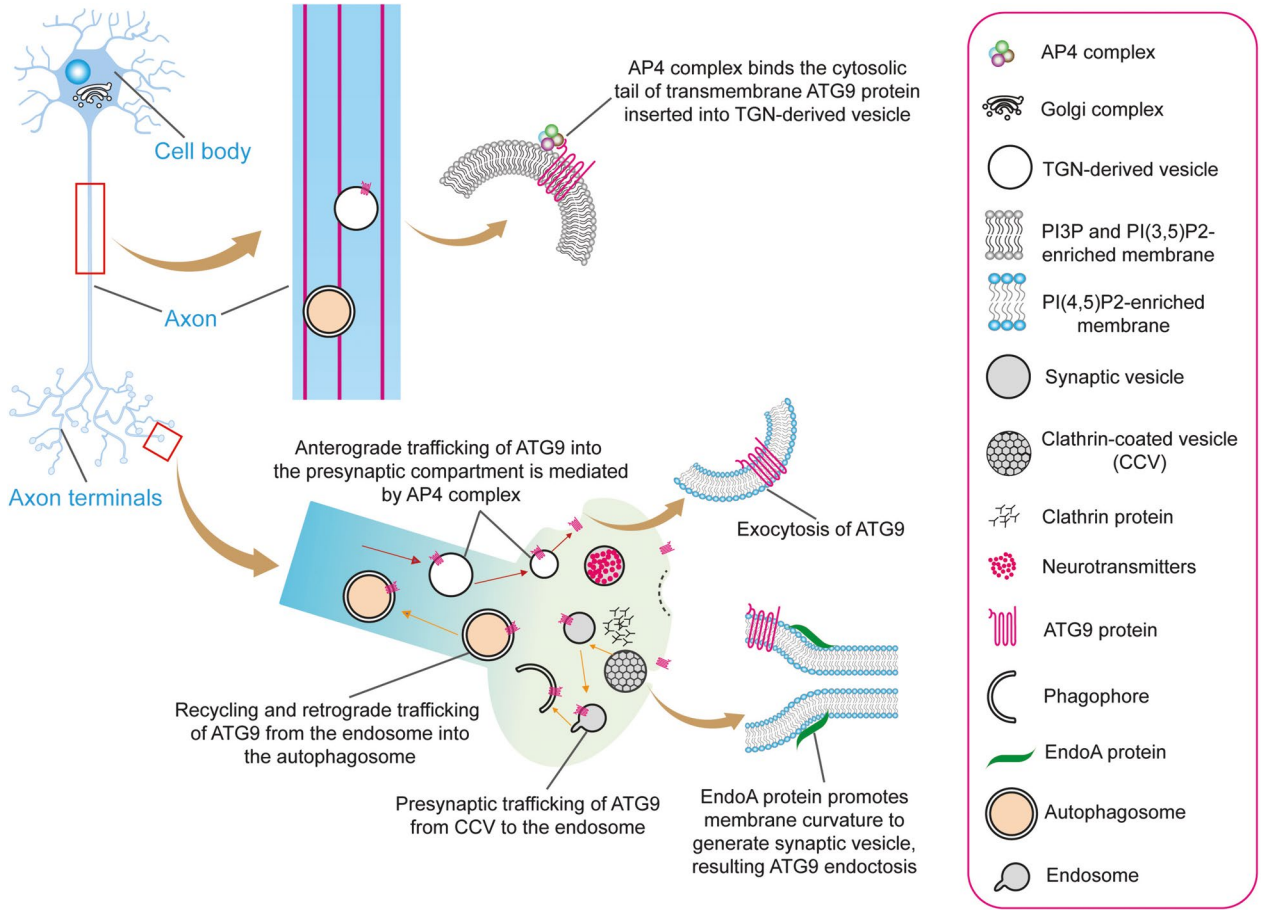

Fig. 2 Anterograde trafficking and delivery of ATG9 to the autophagosome in the presynaptic compartment: The AP4 complex binds the ATG9 protein inserted in the TGN-derived vesicle and anterograde transports it to the presynaptic compartment for exocytosis. The ATG9 protein translocated to the presynaptic plasma membrane is endocytosed into the synaptic vesicle, recycled into the endosome, and then to the phagophore. The phagophore matures into the autophagosome

Role of Retromer Complex and Sorting Nexin (SNX) Proteins

The retromer complex and membrane-associated sorting nexin (SNX) proteins are vital in regulating the sorting of endosomal proteins [83, 84]. Notably, numerous neurodegenerative disorders, including PD and ALS, are related to retromer and SNX protein dysfunction [26, 83, 85–89]. In mammals, two major subcomplexes of the retromer complex are a vacuolar protein sorting–associated protein 26 (VPS26)-VPS29-VPS35 trimeric subcomplex and membrane-associated SNX dimers. VPS26 and VPS29 subunits of the VPS26-VPS29-VPS35 complex bind to a scaffold of the core protein VPS35 at its C- and N-terminal, respectively [90, 91]. The SNX dimers are commonly a collective composition of SNX1, SNX2, SNX5, and SNX6 [92]. While the trimeric assembly of VPS26, VPS29, and VPS35 directly recognizes the cargo and binds to it, the proteins of the SNX family consist of a phox-homology (PX) domain and a C-terminal BAR domain to induce membrane remodeling [93–95]. Members of SNX are reportedly involved in clathrin-dependent endocytosis and in preserving endosome-emanating tubules. SNX proteins are essential for mental health, and their reduced expression is implicated in several neurological disorders, including AD and PD [96]. Most SNX proteins are associated with the membrane's PI3P; therefore, PI3P-enriched early endosomes are a great reservoir of SNXs [97].

A. The retromer complex regulates autophagosome processivity

In neurons, the VPS26-VPS29-VPS35 retromer complex recycles proteins linked to the endolysosomal pathway; it is widely involved in aging, PD, and AD [98]. The VPS29 subunit of the VPS26-VPS29-VPS35 retromer complex interacts with the TBC1D5, a trafficking-carrier protein for ATG9 [62, 81]. As clathrin proteins disperse from the endocytic vesicles, the TBC1D5 leaves the interacting partner VPS29 and translocates to the early endosome to bind the

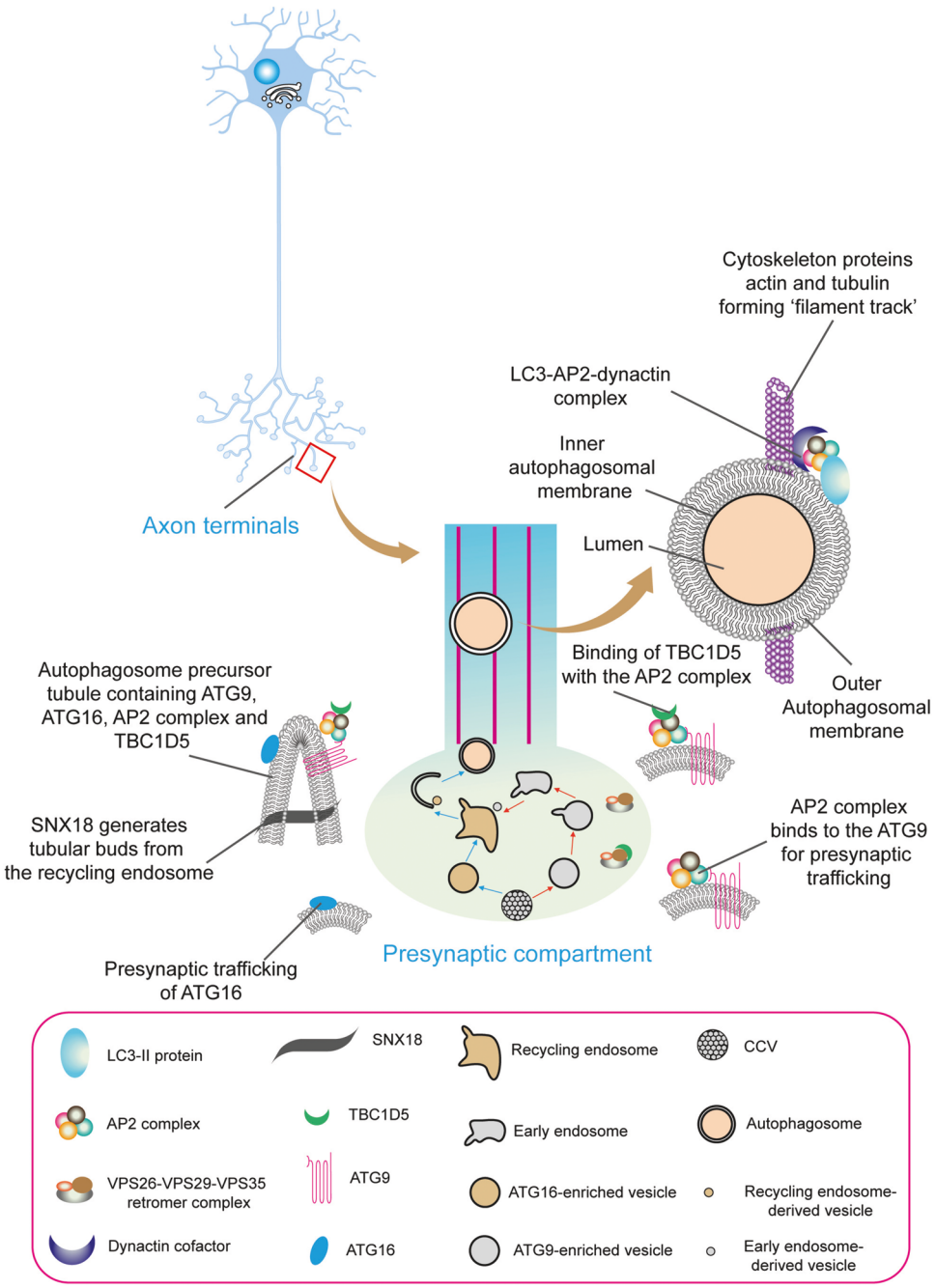

Fig. 3 Formation of the precursor-autophagosome structure and retrograde transport of autophagosome: The plasma membrane–localized ATG9 and ATG16 are endocytosed differently to form a separate pool of vesicles. ATG9 recycles through the conventional route (via early endosome to the recycling endosome), while the ATG16 is directly delivered to the recycling endosome. SNX18 and the dynamin-2 buds off the precursor-autophagosome structure containing ATG9, ATG16, AP2 complex, and TBC1D5. The precursor structure is supplied to the phagophore to form an autophagosome. The LC3-bound AP2 complex protein interacts with the dynactin cofactor of dynein, a retrograde motor protein, to promote retrograde trafficking of the autophagosome

AP2 complex. Further, the two proteins (AP2 complex and TBC1D5) work collectively to facilitate autophagosome formation, maturation, and retrograde transportation. Thus, the retromer subunit VPS29 regulates the switching of the presynaptic autophagy [62]. Moreover, VPS29 plays a pivotal role in brain health in aging [98]. Thereunto VPS29, VPS35, the cargo-binding biggest co-protein of the retromer complex, also participates in presynaptic autophagy by regulating LRRK2 kinase activity, and the PD-linked mutation (D620N) dramatically increases LRRK2-mediated phosphorylation of Rab8, Rab10, and Rab12 [99]. The elevated phosphorylation of Rab proteins by LRRK2 leads to decreased processivity of trafficking autophagosomes [100] and impaired mitophagy in neurons [101]. A study revealed the reduced expression of VPS35 and VPS26 proteins in the entorhinal cortex of AD patients [102].

B. SNX18 and dynamin-2 together generate phagophore's precursor structures

Research on SNX18 showed that the protein generates tubular buds containing ATG9 and ATG16 from the recycling endosome [71]. ATG9 and ATG16 are trafficked from the presynaptic plasma membrane to the phagophore's

precursor structures in endosomes via different routes to form two pools of vesicles (ATG9- or ATG16-positive) that coalescence at recycling endosome [103]. While the delivery of ATG9 to the recycling endosome is via a conventional path through early endosomes [103], the ATG16 traffics through a distinct undeciphered way (Fig. 3). Interestingly, the PX domain of SNX18 binds to the PI3P of the recycling endosome membrane, while BAR domain scaffolds the membrane to induce internal curvature and tubule formation [40, 71]. These ATG9- and ATG16-positive tubules originating from the recycling endosomes are precursor of phagophores/autophagosomes [40, 103]. These precursor autophagosomal tubular structures bud off from the endosome following the interaction of dynamin-2 with the SNX18. The ligation of PRD of dynamin-2 to the SH3 domain of SNX18 mediates their interaction [71]. The ATG9-positive phagophore precursor vesicles, which are budded off from recycling endosomes, are phosphorylated (by ULK1) and redistributed to pre-autophagosomal structure upon autophagy induction [104–106].

Future investigation might show if the SNX18-dynamin-2 protein complex works alone (or the only complex) to bud off the precursor tubule for autophagosome or if some other SH3 domain–containing endocytic proteins are also involved or could compensate for the SNX18 function. This investigation must clarify the story because several SH3 domain–containing membrane remodeling proteins interacting with PRD of dynamin-2 reside in the presynaptic compartments, including EndoA.

LRRK2: A Kinase Switch to Toggle Presynaptic Autophagy

The LRRK2 is a PD-linked multidomain protein that accounts for ~ 1% of sporadic and ~ 5% of genetic cases [107–110]. Piccoli et al. investigated LRRK2's localization in the synaptosomal compartment; it interacts with several synaptic proteins, modulates their trafficking and distribution, and regulates their dynamics [111]. The protein regulates the synaptic vesicle endocytosis and membrane remodeling by phosphorylating the BAR domain, specifically to S75 of the H1 helix in the BAR domain of EndoA protein [29, 36]. The LRRK2-mediated phosphorylation is implicated in switching the EndoA function; the non-phosphorylated EndoA deforms the membrane to tubules, while the phosphorylation induces the protein to create a highly curved membrane. The S75-phosphorylation in the H1 helix prevents the EndoA from penetrating deep into the membrane and associating tightly with it, thus inducing a highly curved membrane, as those found at the growing phagophore's edge [36]. Interestingly, the phosphorylated EndoA-created curved membrane serves as the docking site for an autophagy protein, ATG3 [36], as does the curvature of growing phagophore for the recruitment of autophagic factors, including ATG3, ATG14, and ATG1 [112–116].

The LRRK2 also modulates vesicular trafficking by phosphorylating the Rab proteins. The report shows that increased kinase activity of LRRK2 due to PD-causing G2019S mutation decreases the processivity of trafficking autophagosome, leading to defective axonal transport, acidification, and maturation of autophagosome [100, 109]. Moreover, the mutant LRRK2 protein disrupts the synaptic vesicle endocytosis in PD-linked dopaminergic neurons by abruptly phosphorylating the auxilin in its clathrin-binding domain at S627 [117]. The hyperactivated mutant LRRK2 abnormally recruits the motor adaptor JNK-interacting protein 4 (JIP4) to the autophagosome, promotes binding of JIP4 to LRRK2-phosphorylated Rab proteins, and induces the anterograde protein kinesin to bind with the activated JIP4 on the autophagosome, thereby promoting the anterograde trafficking and reversing the migrating autophagosomes back into the presynaptic compartments [100, 109]. Thus, the G2019S mutation stuck autophagosomes to an unproductive decision between the anterograde and retrograde motors [100]. Moreover, the overexpression of Rab29—one of the several substrates of LRRK2—also induces the hyperactivation of LRRK2 kinase activity and decreases the processivity of autophagosome trafficking [100].

Wauters et al. [101] showed that the LRRK2 kinase activity is essential for the PTEN-induced kinase 1 (PINK1)– and parkin RBR E3 ubiquitin–protein ligase (PRKN)–dependent elimination of depolarized mitochondria through mitophagy. The two usual mutations in LRRK2—G2019S and R1441C—that cause PD led to the aggregation of depolarized mitochondria due to mitophagy failure [101, 118]. PINK-PRKN-mediated clearance of dysfunctional mitochondria requires Rab10 clustering on depolarized mitochondria to recruit and bind the autophagy receptor optineurin (OPTN) on dysfunctional mitochondria to facilitate mitophagy [118]. The mutation-induced hyperactivation of LRRK2 phosphorylation activity enhanced the Rab10 phosphorylation at T73, resulting in decreased interaction of Rab10 and OPTN on dysfunctional mitochondria and impaired mitochondrial clearance [101].

Discussion

The intriguing question is how the presynaptic autophagy is executed in the axon terminals to eliminate aberrant proteins and organelles redundancy. Studies showed that autophagosomes are primarily formed in the presynaptic compartments, where the protein redundancy is relatively higher due to a high metabolic rate [12, 20, 51, 119]. Autophagosome formation in the distantly located presynaptic compartments is a conserved mechanism that rejuvenates axon terminals, enhances their neuroplasticity, and maintains synaptic homeostasis [20, 44, 119–122]. In a highly selective manner, newly developing autophagosomes engulf the ubiquitinated presynaptic junk and travel to the somatodendritic compartment to digest the molecular garbage through lysosomal fusion [123, 124]. During their journey, autophagosomes mature into autolysosomes and digest the redundant synaptic proteins and dysfunctional organelles while reaching the soma [10, 12, 125, 126]. Defective axonal transport significantly induces neurodegeneration in AD, PD, ALS, and HD [127, 128]. The unsolved question is, "Are presynaptic autophagosomes distinct from somal autophagosomes?" Maday and coworkers demonstrated that autophagosomes arising in the axon fiber of hippocampal neurons are morphologically different from those derived in the somatodendritic zone [123, 124]. While autophagosomes formed in the soma and dendritic regions are less developed and mobile, the axonal autophagosomes arriving in the somatodendritic compartment are LAMP1-positive (late endosome/lysosome marker), mature enough, and dynamic in nature [124, 129]. Another research by Hoffmann et al. demonstrated that the terminal compartments possess separate autophagy machinery that gets engaged within minutes following a local insult. They developed a vector system that generated a spatiotemporally controlled ROS-induced damage of local proteins within presynaptic compartments. To their surprise, the insult induces rapid accumulation of LC3-positive vesicles in presynaptic compartments within 5 min, which spread into axons over time (1–2 h) [28]. Thus, autophagosomes formed in presynaptic compartments differ drastically from their counterparts originating in the neuronal cell body or non-polar cells [10, 28, 124, 129, 130]. In the terminal compartments, a few synaptic proteins are parallelly involved in ATG assembly, substrate internalization, membrane distortion/remodeling, and lipid metabolism to support autophagosome formation [29, 38, 44, 131]. At the same time, few proteins escort the autophagosomes retrograde to the cell body [62, 71, 101]. Failure of presynaptic autophagy increases nonfunctional protein accumulation in presynaptic compartments, leading to compromised synaptic transmission. Thus, we reviewed the critical studies and recent findings highlighting the involvement of endocytic/synaptic proteins in the formation and retrograde trafficking of presynaptic autophagosome and examined the altered expression of synaptic proteins in disease onset/progression Table 1.

The present review appeals to study neuronal autophagy differently from that in non-polar cells. Autophagy in neurons might seem the same as in other cells because of the communal site of lysosomal degradation of autophagosomes in the soma. However, the autophagosome formation in neurons is compartmentalized, which makes neuronal autophagy peerless. In axonal/presynaptic autophagosomes, several synaptic proteins support and regulate their formation. However, it will be necessary for researchers to determine in the future whether axonal autophagosomes are molecularly extraordinary and whether they respond to synaptic regulatory signals. Synaptic loss is the earliest hallmark of many neurodegenerative diseases. Therefore, identifying biomarkers of presynaptic autophagy dysfunctions could facilitate early diagnosis that could be extrapolated to monitor disease. Secondly, the involvement of synaptic proteins in autophagosome formation, distribution, and trafficking needs further validation. Future research will validate whether their role in presynaptic autophagy is uniquely obligatory or facultative addition in the classical autophagic process. It will also be compulsory to research the autophagic function of other endocytic and non-endocytic proteins residing in the presynaptic compartments and how autophagy collaborates with other degradation mechanisms to impact neurotransmission. In brief, future investigations on presynaptic autophagy will present significant potential in elucidating the etiology of neurodegenerative disorders and formulating successful treatments to mitigate neurodegeneration. Thus, it is crucial and timely to investigate synaptic autophagy as a field.

Table 1 List of critical synaptic proteins, their role in endocytosis and autophagy, and protein expression levels and mutations in neurodegenerative diseases

Protein	Synaptic role	Autophagic ro`le	Protein expression levels in neurodegenerative diseases	Mutations in neurodegenerative diseases	References
EndoA	Membrane remodeling	It generates vesicular structures and docking sites for ATG protein assembly	EndoA protein level is elevated in the brain of AD patients	-	[36, 37, 132]
EndoB1	Membrane remodeling	Cdk5-mediated phosphorylation promotes neuronal autophagy, but its role in presynaptic autophagy is elusive	Protein expression level is reduced in AD patients	-	[39, 41, 42]
SJ1	Clathrin uncoating	The protein dephosphorylates PI3P and $PI(3,5)P_2$ for the recruitment of autophagosome elongating proteins	SJ1 transcripts and protein expression levels are significantly increased in AD brains	R258Q and R839C mutation in PD	[43, 44, 133–135]
LRRK2	Regulates EndoA-mediated membrane remodeling	Regulates mitophagy and autophagosome trafficking	-	G2019S, R1441G, and R1441C mutation in PD	[36, 37, 100, 109]
AP2	Anchors clathrin to the vesicle membrane	Presynaptic trafficking of ATG9 from the membrane to the endosome	AP2 together with *CALM/PICALM* contribute to clear AD-related Aβ peptide	-	[59, 62, 72, 136]
AP4	-	It facilitates the anterograde transportation of ATG9A to the presynaptic plasma membrane	-	-	[68, 69]
VPS29	It interacts with TBC1D5 and functions as a subunit of the VPS26-VPS29-VPS35 retromer complex	VPS29 regulates the switching of presynaptic autophagy	-	-	[62, 81]
VPS35	It is an essential component of the VPS26-VPS29-VPS35 retromer complex. It also regulates the kinase activity of LRRK2	The protein regulates the LRRK2-mediated processivity of the trafficking autophagosomes	-	D620N mutation in PD	[99, 100]
SNX18	SNX18 Induces internal curvature and tubule formation	It generates ATG-9 and ATG-16-positive tubules in the recycling endosomes	-	-	[71, 92, 103]
Dynamin-2	Buds off the CCVs from the plasma membrane	The protein buds off ATG-9 and ATG-16-associated structures from the recycling endosomes	-	-	[71]
TBC1D5	It is an interacting protein of VPS29 and AP2. It regulates clathrin uncoating and recycling endosome formation	The protein catalyzes the sorting of ATG9 from the early endosome to the recycling endosome presynaptically	-	-	[62, 81]

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Research Involving Human Participants and/or Animals Not applicable.

Competing Interests The authors declare no competing interests.

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