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Review article

Axonal autophagy: Mini-review for autophagy in the CNS

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ABSTRACT

Neurons are long-lived and highly polarized cells that depend on autophagy to maintain cellular homeostasis. The robust, constitutive biogenesis of autophagosomes in the distal axon occurs via a conserved pathway that is required to maintain functional synapses and prevent axon degeneration. Autophagosomes are formed de novo at the axon terminal in a stepwise assembly process, engulfing mitochondrial fragments, aggregated proteins, and bulk cytosol in what appears to be a nonselective uptake mechanism. Following formation, autophagosomes fuse with late endosomes/lysosomes and then are rapidly and efficiently transported along the axon toward the soma, driven by the microtubule motor cytoplasmic dynein. Motile autophagosomes mature to autolysosomes in transit by fusing with additional late endosomes/lysosomes, arriving at the soma as fully competent degradative organelles. Misregulation of neuronal autophagy leads to axonal degeneration and synaptic destabilization, and has been implicated in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, and ALS.

1. Autophagy is a conserved degradative pathway required for neuronal homeostasis

Macroautophagy is a critical degradative pathway for eukaryotic cells, important for the clearance of aggregated proteins and dysfunctional organelles. This pathway, referred to here as autophagy, is an essential homeostatic mechanism for neurons. Loss of core components of the autophagy machinery, such as Atg5 or Atg7, leads to cell-autonomous neurodegeneration indicating the importance of the pathway in the maintenance of neuronal function [12,23].

Neurons are post-mitotic and highly polarized cells with distinct compartments that differ in the organization of their endomembrane systems. The soma is the primary site of both biosynthetic and degradative pathways, and thus ribosome-bound rough endoplasmic reticulum (ER) and Golgi are enriched in this compartment, as are degradative organelles such as lysosomes. The axon is an elongated process that extends from the soma to synaptic sites up to 1 m away in humans, which are the primary sites for signal transmission to other cells. Axons are separated from the soma by a selectivity filter at or near the axon initial segment that regulates the trafficking of cargos into this compartment. Mature axons are enriched in some organelles, including ER and endosomes, but there is a dearth of other organelles such as Golgi. Axonal late endosomes/lysosomes are abundant, but appear to be less acidified and have a lower complement of degradative enzymes than those found in the soma [10]. These differences result in distinct dynamics of organelle trafficking that are particularly relevant to the autophagy pathway.

Recent work has established a model for constitutive, non-selective autophagy in the axon, a process that exhibits remarkable spatial specificity. Here, we discuss our current understanding of the spatiotemporal dynamics of axonal autophagy and how this pathway may contribute to the maintenance of neuronal homeostasis. There is evidence that this pathway contributes to the turnover of mitochondria [30,57], synaptic vesicles [38], and aggregated proteins [41,57], as discussed below. Similarly, inhibition of this pathway leads to synaptic destabilization and neurodegeneration [12,23,41,43].

In many cell types autophagy is a significant part of a cellular response to stress, upregulated in response to nutrient deprivation, mitochondrial damage or proteotoxic stress. It is less clear how effectively neurons are able to upregulate axonal autophagy to protect from these stressors [29,30,33]. Potentially, the limited ability of neurons to upregulate autophagy in an efficient stress response may contribute to the vulnerability of the axon to the accumulation of aggregated proteins or dysfunctional organelles seen in diseases such as Alzheimers, Parkinsons, and Huntingtons, and ALS. However, there is intriguing evidence that axonal autophagy can be tuned by synaptic activity and/or integrity [15,38,51]. Defining the underlying mechanisms linking axonal autophagy to synaptic state is currently a very active area of research.

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2. Constitutive autophagy in neurons displays spatiotemporal specificity

The development of live cell microscopy allowed the direct visualization of organelle trafficking along the axon, first with phase contrast approaches, and later using vital dyes or organelle-specific tags visualized with fluorescence microscopy. Landmark work from Peter Hollenbeck characterized endosomal motility in the axons of cultured embryonic peripheral neurons and found evidence for the retrograde motility, meaning movement from the axon terminal back to the soma, of large acidified organelles [16]. Both the size, ~1 μm, and the multilamellar structure of the axonal organelles as seen in EM images suggested that they were part of a degradative pathway and most likely autophagosomes or autophagolysosomes.

Direct observation of the dynamics of axonal autophagosomes in dorsal root ganglion (DRG) neurons cultured from a transgenic mouse expressing the autophagosomal marker LC3 fused to GFP [33] provided new insights into the pathway [30]. Large acidic organelles, positive for GFP-LC3, were observed to form constitutively at the axon tip and then undergo rapid retrograde transport toward the cell soma. Similar findings from the Nixon lab using primary mouse embryonic cortical neurons [26] and the Sheng lab, using DRG neurons from rats [4], support the robust nature of the observations.

The pathway leading to generation of an autophagosome has been established through several decades of work in yeast and mammalian cells, primarily focused on stress-induced autophagy (reviewed in [17,59]) (Fig. 1A). Induction and nucleation phases involve the activation and recruitment of two key protein complexes, the ULK1 complex and the phosphatidylinositol 3-kinase complex. This leads to the generation of a localized PI(3)P signal and the formation of an omegasome at the surface of the ER [17,59]. The recruitment of the PI(3)P effector protein WIPI2B in turn recruits downstream components including the E3-like complex ATG12-ATG5–ATG16L1 [59]. The resulting double-membrane phagophore elongates to become the fully-formed autophagosome, while LC3B is lipidated and incorporated into the delimiting membranes

Dual-label live cell imaging of neurons expressing GFP-LC3B in conjunction with other fluorescently-labeled autophagy components has revealed the kinetics of the stepwise assembly of axonal autophagosomes formed under constitutive conditions [28]. De novo biogenesis events are consistently observed at the axon terminal, using upstream markers such as ATG13, an adaptor protein in the ULK1 kinase complex [54]. ATG13 puncta form on or near the ER in the distal axon [28]. Gradually, over the course of several minutes, ATG13 puncta become LC3B-positive. As the intensity of the GFP-LC3B fluorescence increases, the ATG13 signal declines, indicating dissociation of the kinase complex from the elongating phagophore. In contrast, GFP-LC3B fluorescence stably persists, indicating the continued stable association of lipidated LC3B with the mature autophagosome.

The elongation phase of autophagosome biogenesis involves the recruitment of multi-subunit complexes that coordinate the continued growth of the initial phagophore membrane and the lipidation of LC3. ATG5 is an essential part of the conjugation machinery [12], thus recruitment of ATG5 serves as a marker for the elongating phagophore membrane. Dual-label live cell imaging of ATG5 and LC3B results in time courses with kinetics very similar to those observed for ATG13 and LC3B [28], with the initial formation of an ATG5-positive punctum followed by the stable recruitment of GFP-LC3B.

Overall, the ordered recruitment pathway for autophagosome assembly observed in neurons resembles the dynamics observed in non-neuronal cells [19,24]. However, imaging the distinct phases of autophagosome biogenesis has provided novel insights into the pathway. First, axonal autophagosomes form constitutively in cortical [26], DRG [4,30], and hippocampal neurons [28] cultured under growth-promoting conditions, rather than the nutrient-limiting conditions used to induce autophagy in other cell types. Further, the rate of autophagosome formation remains steady in hippocampal neurons over weeks in culture [28]. Thus, autophagosome formation is a baseline homeostatic mechanism, and not strictly a growth-dependent or stress-related process.

Perhaps the most surprising aspect of autophagosome biogenesis in primary neurons is the pronounced temporal and spatial specificity of the process. Kinetic analysis of the dynamics of autophagosome formation demonstrates a stepwise assembly process that follows a stereotypical time course. Each biogenesis event occurs within minutes, with components recruited with predictable kinetics, indicating high temporal specificity. Still to be determined is the nature of the molecular timer that leads to the steady production of new autophagosomes, at a rate of ~7–20 events/hr [28,30] in mammalian neurons in vitro and ~2 events/hr in vivo in C. elegans [48].

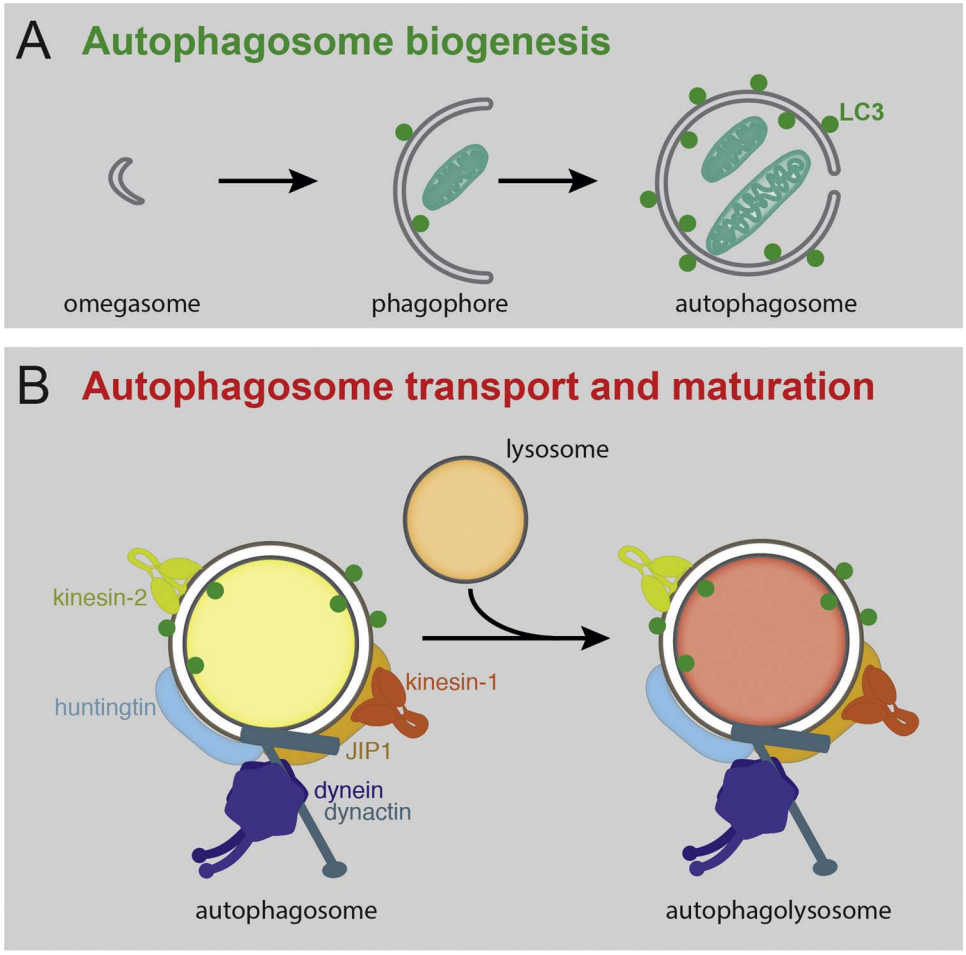
The spatial specificity of autophagosome biogenesis is also remarkable, as the formation of new autophagosomes occurs predominantly at the axon tip. De novo formation of new autophagosomes along the length of the axon is very rare under baseline, constitutive conditions [28]. Given that the axonal volume can far exceed that of the axon terminal or even the soma, this suggests that the molecular determinants leading to autophagosome formation are highly enriched distally at the axon tip. The limiting factor or factors specifying this spatial specificity remain to be identified, but likely involve the targeted delivery of components via kinesins [48].

Given the relatively constricted region in which autophagosomes form in primary neurons, there are limited sources possible for donor membranes. Golgi and mitochondria have both been proposed as sources for the lipids used to assemble the double-membrane autophagosome [52], but Golgi elements are excluded from the axon under normal conditions and it is unclear if there are sufficient mitochondria distally in the axon to serve as a significant source of donor membrane. Plasma membrane has also been suggested as a source [40] but live cell imaging with plasma membrane markers does not support this possibility in neurons [28]. Instead, recent tomographic EM [58] has shown that there is extensive ER along the axon. Thus it is not surprising that live cell imaging data implicate the ER as a primary source for donor membrane. The formation of the omegasome in the distal axon is coincident with the ER, and newly formed autophagosomes include ER markers [28]. During assembly, membrane is trafficked to the elongating phagophore in ATG9-positive carrier vesicles. The kinesin motor KIF1A is required to supply the axon with newly synthesized ATG9 [48], but it remains to be determined if local sources such as recycling endosomes or the ER contribute additional lipid content to ATG9 vesicles in order to build the final organelle in the distal axon.

3. Localized biogenesis in axons in vivo

While studies in primary neurons demonstrate robust autophagosome biogenesis at the axon terminal [4,28], is this an accurate model for autophagosome biogenesis in neurons in vivo? Multiple studies in model organisms including C. elegans and Drosophila support this interpretation.

Live imaging studies in C. elegans demonstrate compartmentalized regulation of autophagy in vivo, with autophagosome biogenesis observed in vivo at synaptic sites during development and in adult animals [48]. Biogenesis of new autophagosomes occurs at a low but consistent rate of 1.7 events/hr at synaptic sites in AIY neurons. Mutations disrupting late stages of the autophagy pathway block formation of new autophagosomes, suggesting that there is a negative feedback loop involved in the regulation of biogenesis in vivo. One of the key limiting factors in distal biogenesis is the delivery of ATG9, which is driven outward from the soma by the anterograde-directed microtubule motor KIF1A, known as UNC-104 in C. elegans. Further, a functional link was demonstrated between axonal autophagy and synapse development, as mutations in multiple genes in the autophagy pathway induced defects in synaptic and cytoskeletal organization [48].



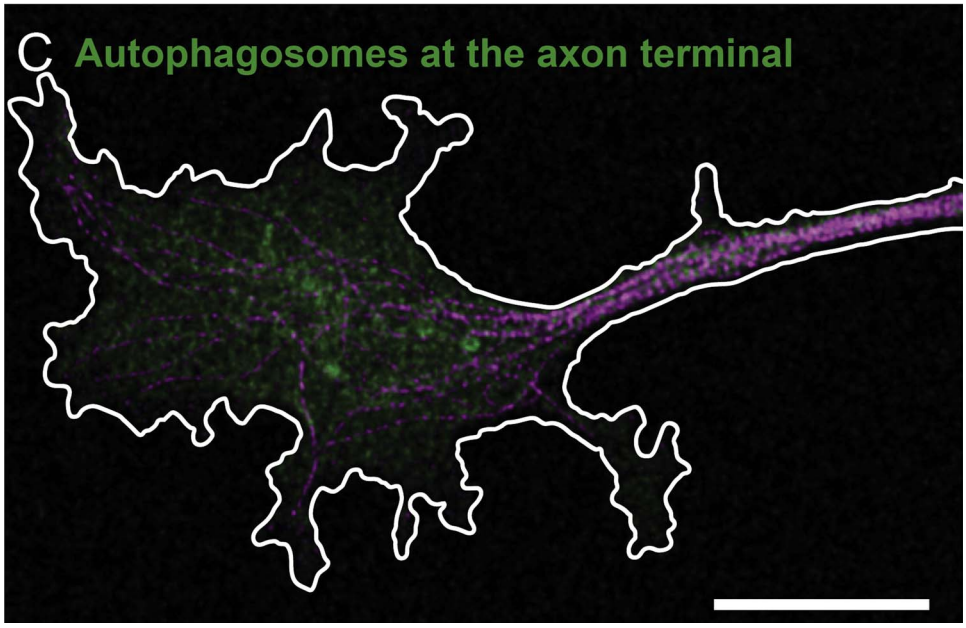


Fig. 1. *Autophagosomes are formed constitutively in the distal axon and are actively trafficked toward the soma by the microtubule motors cytoplasmic dynein and kinesin.* **A.** Autophagosome biogenesis at the axon terminal is a stepwise pathway, starting from an omegasome on the ER, that grows to form a double-membrane phagophore that engulfs cargos including aggregated proteins and mitochondrial fragments, finally sealing to form a spherical compartment marked by LC3B that sequesters its cargos from the cytosol for subsequent degradation. **B.** Once formed at the axon terminal, autophagosomes bind to microtubules via associated dynein and kinesin motors. As autophagosomes move along the axon, they fuse with late endosomes/lysosomes and gradually acidify to form a fully competent degradative organelle, termed the autophagolysosome. **C.** Super-resolution micrograph of a DRG neuron (2DIV) showing the interaction of GFP-LC3B-labeled autophagosomes with microtubules at the axon terminal. GFP-LC3B was stained with an anti-GFP-488 nanobody (Chromtek) and microtubules were stained with an antibody to tyrosinated tubulin (Millipore). Scale bar, 5 μm.

In Drosophila, autophagosomes also form distally at the synapse, as observed at the neuromuscular junctions (NMJs) of motor neurons [36,47]. Either starvation or neuronal stimulation increased the rate of formation of autophagosomes at NMJs [47]. Further, newly formed autophagosomes were found at synaptic termini in Drosophila motor neurons, but only rarely in cell bodies, again supporting the hypothesis that autophagosome biogenesis is compartmentalized in neurons, with formation of new autophagosomes occurring preferentially in the distal axon.

4. Autophagosome trafficking along the axon

Once formed, autophagosomes bind to microtubules within the axon terminal in an interaction mediated by microtubule motor

proteins (Fig. 1B,C). Nascent autophagosomes then undergo a stereotypical pattern of bidirectional movement along microtubules. Processive runs are observed both toward and away from the soma [30]. Biochemical characterization of isolated autophagosomes from mouse brain suggests the basis for this bidirectional motility. Anterograde-directed kinesin-1 and kinesin-2 motors and retrograde-directed dynein motors co-localize and co-purify with neuronal autophagosomes [30]. Multiple mechanisms have been proposed to explain the recruitment of dynein motors to autophagosomes, including the GTPase Rab7 and its effector RILP [21,55] and snapin, a component of the BLOC-1 complex [4], but the basis for kinesin recruitment is not yet known. However, these observations fit a developing paradigm in which multiple types of motors are simultaneously bound to an organelle undergoing transport along a microtubule [11,13]. The unregulated, opposing activities of dynein and kinesin motors operating in a tug-of-war are likely to account for the initial bidirectional movement of newly formed autophagosomes near the axon tip.

Following an initial period of bidirectional motility, axonal autophagosomes then undergo a dramatic switch to unidirectional, highly processive retrograde motility toward the soma. Both in vitro [30], and in vivo in C. elegans [48] and Drosophila [36], > 80% of autophagosomes along the axon move in the retrograde direction at rates of ~0.5 um/sec [30]. Of note, this highly unidirectional and processive motility differs from that of most other axonal organelles characterized to date including lysosomes, which are the other major degradative organelle in axon. Lysosomes exhibit bidirectional motility albeit with an overall retrograde bias; paused lysosomes are also commonly observed [35].

Cytoplasmic dynein is the major minus end-directed organelle motor in high eukaryotes, and many lines of evidence indicate that dynein drives the robust retrograde motility of autophagosomes along the axon. Dynein was first implicated in autophagosome motility by Rubinsztein and colleagues. Either chemical inhibition of dynein in PC12 cells or genetic disruption of dynein function in Drosophila or mouse impaired the clearance of aggregation-prone proteins by autophagy [41]. These observations were further supported by studies in nonneuronal cells [20] where autophagosomes move more bidirectionally than is seen in neurons.

The pronounced switch from bidirectional to highly processive, unidirectional retrograde motility seen for autophagosomes moving along the axon requires the coordinated regulation of opposing motors, a process that often involves scaffolding proteins [7]. Scaffolding proteins bind directly to kinesin and dynein motors and specifically activate or inhibit motor function. In the case of autophagosomes, two key scaffolding proteins involved in motor regulation are JIP1 and Huntingtin [6,57]. JIP1 is a scaffolding protein that binds directly to both the motor and regulatory subunits (heavy and light chains, respectively) of kinesin-1 [8]. JIP1 also binds directly to p150^{Glued}, a subunit of the retrograde motor complex composed of cytoplasmic dynein and its activator dynactin [8]. Live imaging studies demonstrate that JIP1 does not associate with newly formed autophagosomes, including those that are moving bidirectionally along microtubules in the distal axon. Instead, JIP1 loads onto autophagosomes as they exit the axon terminal and enter the axon shaft. The binding of JIP1 induces the switch from bidirectional to unidirectional motility, caused by the JIP1-dependent inhibition of kinesin-1 motors. Of note, phospho-JIP1 activates kinesin, but dephosphorylation of JIP1 inhibits kinesin-1 by favoring the autoinhibited, folded conformation of the motor [6,8].

Huntingtin is another scaffolding protein recruited to autophagosomes, in association with the interacting protein HAP1 [57]. Huntingtin (Htt) is best known for its identification as the causal gene of Huntington's disease, a lethal form of neurodegeneration, following pathogenic expansion of a tract of polyglutamines in the N-terminus of the protein. Htt is a multi-functional scaffolding protein, with roles in both the nucleus and the cytoplasm. Cytosolic Htt is often vesicle-associated and depletion of Htt affects the motility or localization of organelles including endosomes and lysosomes [3]. Htt binds directly to kinesin-1 and a subunit of the cytoplasmic dynein motor. Further, the Htt binding partner HAP1 interacts with both kinesin and the dynactin subunit p150^{Glued}. Knockdown of either Htt or HAP1 in primary neurons significantly inhibited the retrograde motility of autophagosomes along the axon [57].

Recent work has used genetic approaches to further our understanding of the upstream regulatory pathways involved. Specifically, the STRIPAK complex contributes to the regulation of autophagosome motility via its component phosphatase PP2A [36]. Depletion of the core scaffold protein of the STRIPAK complex, CKA, led to the distal accumulation of autophagosomes in the terminal boutons of Drosophila motor neurons and a reduced number of motile autophagosomes along the axon [36]. The reduced axonal transport of autophagosomes in CKA-depleted neurons resembles the transport deficit when dynein is depleted, and GFP-labeled CKA co-precipitated with dynein [36]. Further, STIRPAK component Strip binds to the p150^{Glued} subunit of dynactin [44], a required activator of dynein-driven organelle motility. Of particular interest, both the STRIPAK complex and JIP1 are recruited to autophagosomes through LIR motifs that mediate direct interactions with the lipidated LC3 found on the limiting membranes of the organelles [36,6].

The CKA subunit of STRIPAK is known to bind to the protein phosphatase PP2A [9]. Either disruption of this interaction, or depletion of PP2A was sufficient to induce the accumulation of autophagosomes in nerve terminals [36], indicating a key role for this phosphatase in the regulation of autophagosome transport. The direct target or targets of PP2A remains to be identified, but might include either the dynein motor itself, or the scaffolding protein JIP1, as the dephosphorylated form of JIP1 (at S421) favors the retrograde transport of autophagosomes [6].

It remains to be determined whether there is a single common pathway regulating the motility of axonal autophagosomes involving JIP1, STRIPAK, and Htt, or instead, if there are different classes of organelles that are differentially regulated by these scaffolding proteins and regulatory complexes. However, at present there are no data indicating that there are distinct types of motile axonal autophagosomes translocating from the axon tip to the soma. Nor is there evidence for differential pathways for transport or maturation. Thus, a single consolidated, multi-component mechanism may regulate the motility of axonal autophagosomes. In contrast to this consolidated regulatory hypothesis for motile autophagosomes, there is evidence for a distinct pathway leading to the local formation and local degradation of immotile autophagosomes that are formed along the axon rather than at the distal tip [2]; this pathway is discussed in more detail below.

5. Autophagosome transport is tightly linked to maturation

Once autophagosomes initiate highly processive retrograde transport toward the soma, they tend to transit the length of the axon [29]. Based on live imaging experiments with pH-sensitive constructs, Maday et al. [30] proposed a model in which autophagosomes mature to fully degradative autolyosomes as they move along the axon. The dual color fluorescent reporter mCherry-EGFP-LC3 [39] was observed to mark autophagosomes newly formed at the axon tip with both red and green fluorescence. As autophagosomes neared the soma, the EGFP signal became preferentially quenched by the increasing acidification of the lumen. Experiments with the pH-sensitive dye LysoTracker Red confirmed this model, as distal autophagosomes were not labeled with Lyso Tracker Red while autophagolysosomes in the proximal axon and the soma were strongly positive. In vivo as well, there is evidence indicating that autophagic vesicles become acidified as they traffic toward the soma. Imaging of autophagosome dynamics in larval motor neurons expressing a mCherry-GFP-LC3 reporter in which the GFP signal becomes quenched as autophagosomes acidify to become autophagolysosomes [39] demonstrates that in vivo, autophagosomes mature in transit from the distal axon toward the soma [36].

This acidification is induced by the fusion of a nascent autophagosome with a late endosome or lysosome. There is clear evidence for an initial fusion event prior to the exit of a newly formed autophagosome from the axon tip, as Maday et al. [30] showed that upon initial formation, autophagosomes are LAMP1-negative, but in contrast, axonal autophagosomes are LAMP1-positive. This initial fusion event with a distal late endosome may be required to recruit dynein motors to the autophagosome, and thus for robust retrograde motility along the axon [4]. Syntaxin17 forms a SNARE complex proposed to mediate this late endosome-autophagosome fusion [18,50]; consistent with this hypothesis, depletion of syntaxin17 inhibits autophagosome motility [4].

Multiple studies have shown that inhibition of motility blocks maturation of autophagosomes. For example, replacement of JIP1 with a mutant form that cannot bind LC3 [6] is sufficient to cause a defect in acidification of axonal autophagosomes. Similarly, depletion of Htt blocks autophagosome maturation and leads to a failure in degradation of luminal contents [57]. So why is retrograde motility required for autophagosome maturation to a fully functional degradative compartment? It has been proposed that the dynein-driven motility of autophagosomes mediates efficient encounters with lysosomes [22]. This may be of particular importance in neurons, as it is possible that a single fusion event is not sufficient to fully acidify the lumen of axonal autophagosomes, or to deliver sufficient degradative enzymes to efficiently breakdown engulfed cargo. In support of this hypothesis is the observation that axonal lysosomes are deficient in luminal proteases as compared to somal lysosomes [10]. Thus, active transport along the axon may lead to multiple encounters with axonal lysosomes, and thus may facilitate multiple fusion events along the axon that together lead to effective maturation to form a fully functional autophagolysosome. Alternatively, active transport may function to drive autophagosomes toward more proteolysis-competent lysosomes enriched in the soma relative to the axon. However, a detailed analysis of axonal autophagosomes suggests that under normal circumstances there is significant cargo degradation within autophagosomes in motion along the axon, and that cargo accumulation is only observed under conditions in which transport is inhibited [57]. Further observations of fusion events during the maturation of axonal autophagosomes are required to more directly address this question.

6. Cargo engulfment: selective vs. nonselective uptake

Autophagosomes sequester and degrade engulfed cargos, including protein aggregates and dysfunctional organelles. Both selective and nonselective pathways have been described. Selective autophagy is induced in response to the ubiquitation of protein aggregates or organelle-associated proteins. The best characterized pathway involves the recognition and clearance of damaged mitochondria by the PINK1 kinase and the E3-ubiquitin ligase parkin [37]. Activation of this pathway in response to mitochondrial damage, localized ROS production or membrane depolarization leads to the ubiquitination of outer membrane proteins on the surface of the targeted mitochondria. Receptor proteins including optineurin OPTN), NDP52, and TAX1BP1 are recruited via their UBAN (ubiquitin-binding) motifs; the kinase TBK1 is co-recruited along with optineurin [14,25,34,42,56]. These receptors also have LIR motifs, which bind to LC3 and facilitate the formation of an autophagosome engulfing the damaged mitochondria [37].

p62 has also been implicated in the selective autophagy of ubiquitinated substrates [1], but is not required for mitophagy [25,56]. Similar to mitophagy receptor OTPN, p62 can bind to both ubiquitinated substrates and LC3, which may facilitate autophagosome formation. In vivo, p62 is frequently associated with ubiquitinated protein aggregates in neurons (for example, see [32,43]). However, depletion of p62 did not alter aggregate formation in a mouse model of neurodegenerative disease [43], suggesting that p62 is dispensable for axonal autophagy, potentially due to redundancy with other receptors.

In contrast to selective, receptor-mediated autophagy, cargo can also be taken up nonselectively in bulk autophagy. This pathway has been explored most thoroughly in starvation-induced autophagy in nonneuronal cells. Nonselective autophagy does not require localized protein ubiquitination such as that mediated by the E3 ubiquitin ligase Parkin, nor is there evidence for the involvement of specific receptors nor any preferential uptake of cargos. Somewhat surprisingly, the engulfment of cargos by newly forming autophagosomes at the axon tip appears to be primarily a nonselective process. There is no evidence to date for the involvement of the PINK1-parkin pathway or ubiquitin-binding receptors such as optineurin in the engulfment of cargos in autophagosomes newly formed at the axon terminal. Instead, live cell studies suggest a ‘big gulp’ approach, in which the developing phagophore engulfs local cytosol, which may include mitochondrial fragments or aggregated proteins such as the disease-associated proteins $SOD1^{G93A}$ or Htt with an expanded polyglutamine tract [30,57].

Together, these observations suggest that constitutive autophagy in neurons is nonselective in regard to cargo capture and degradation. This in turn suggests that the major role for autophagy at the synapse is to facilitate protein turnover. Most proteins are synthesized in the soma and may not arrive at distal synapses until hours or days later in neurons with extended axons [53]. Constitutive autophagy may provide a mechanism to facilitate the turnover of aging proteins at the synapse. Alternatively, there may be a specific mechanism for cargo loading into axonal autophagosomes that has not yet been identified. Possibilities include Htt itself, shown to function as a scaffold for selective autophagy in MEFs and neuroblastoma cells [31]. However, this mechanism has not yet been demonstrated to function in axonal autophagy, as Htt depletion had no apparent effect on the generation or cargo-loading of autophagosomes in the axons of primary neurons [57].

7. Induction of axonal autophagy by cellular stress?

There is clear evidence that autophagy is important in neuronal development [46,48]; reviewed elsewhere in this volume. Autophagy is also required for maintenance of homeostasis in neurons. Knockout of core autophagy genes is sufficient to induce degeneration in Purkinje neurons [12,23] and synaptic deficits in motor neurons [43].

Autophagy is also well known as a conserved stress response pathway in many cell types. For example, autophagy can be induced in many cell types by nutrient deprivation, via an mTOR-dependent pathway [45]. It is not clear how effectively neurons can upregulate autophagy in response to cellular stressors including nutrient deprivation and changes in mTOR activity. Neither starvation nor mTOR inhibition was sufficient to upregulate autophagy in hippocampal neurons in vitro [29]; while in mice nutrient deprivation upregulated autophagy in many tissues but not the brain [33]. In contrast, in Drosophila, starvation significantly increased the number of Atg8-positive puncta observed at NMJs [47].

Other forms of stress may also induce neuronal autophagy. For example, Schwarz and colleagues [2] demonstrated the engulfment of mitochondria by the local formation of an autophagosome along the axon, in a pathway that is both PINK1- and Parkin-dependent and is induced in response to localized formation of ROS. These autophagosomes form locally and are degraded locally, remaining immotile, and thus can be readily distinguished from the autophagosomes that form constitutively at the axon tip in vitro and at synaptic sites in vivo. A stress-dependent, but Parkin-independent pathway for the removal of mitochondria from axons has also been proposed [27]. While these alternate pathways have been described in vitro, in vivo studies to date suggest that the major site for mitochondrial quality control is the soma [5,49].

8. Conclusions

The robust, constitutive formation of autophagosomes at synaptic sites in the distal axon is a conserved mechanism that likely contributes

to the maintenance of synaptic homeostasis. Newly forming autophagosomes engulf soluble and aggregated proteins, as well as mitochondrial fragments, in what appears to be a nonselective pathway. Once an autophagosome is formed distally, it rapidly fuses with a late endosome and then undergoes rapid and efficient retrograde transport along the axon, driven by the microtubule motor cytoplasmic dynein. Motile autophagosomes mature to autolysosomes in transit, arriving at the soma as fully competent degradative organelles. While the broad outlines of this pathway are now understood, many questions remain: *What signals regulate the generation of constitutive autophagosomes? Is cargo engulfment completely nonselective? Does this pathway contribute to the maintenance of synaptic homeostasis? Is the pathway upregulated in response to neuronal activity? Can the pathway be upregulated in response to cellular stressors? Is axonal autophagy deleteriously affected by aging or neurodegenerative disease?* The answers to these and other questions are likely to further expand our understanding of the homeostatic mechanisms that maintain neuronal health over decades, and whether deficits in these mechanisms contribute to neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and ALS.

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