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Disruptions in axonal lysosome transport and its contribution to neurological disease

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Abstract

Lysosomes are central to the maintenance of protein and organelle homeostasis in cells. Optimal lysosome function is particularly critical for neurons which are long-lived, non-dividing and highly polarized with specialized compartments such as axons and dendrites with distinct architecture, cargo, and turnover requirements. In recent years, there has been a growing appreciation for the role played by axonal lysosome transport in regulating neuronal development, its maintenance and functioning. Perturbations to optimal axonal lysosome abundance leading to either strong accumulations or dearth of lysosomes are both linked to altered neuronal health and functioning. In this review we highlight how two critical regulators of axonal lysosome transport and abundance, the small GTPase Arl8 and the adaptor protein JIP3, aid in maintaining axonal lysosome homeostasis and how alterations to their levels and activity could contribute to neurodevelopmental and neurodegenerative diseases.

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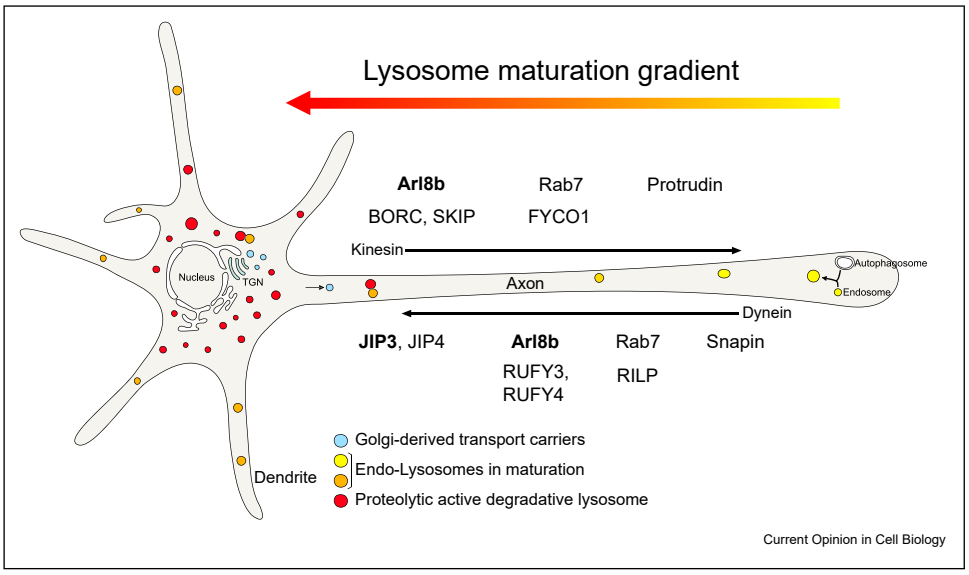
Introduction

Lysosomes, the terminal degradative compartments for endocytic, phagocytic and autophagic pathways, are essential for normal protein and organelle turnover in all cells, and thus necessary for the cell's health and survival. Optimal lysosome function is particularly critical for neurons as these are long-lived, morphologically complex, and post-mitotic in nature, and thus without the ability to dilute toxic aggregates in their cytoplasm by cell division. In fact, neurodegenerative diseases are often characterized by the aggregation of large populations of proteins that would normally be catabolized by two major proteostasis pathways; the autophagic-lysosomal pathway and ubiquitin proteasome system [1]. The contribution of protein aggregates and their propagation and involvement of ubiquitin-proteasomal system in neurodegenerative diseases has been reviewed extensively elsewhere [1,2]. Lysosome dysfunction is associated with several neurodegenerative diseases whose pathologies include accumulation of protein aggregates, such as Alzheimer's disease (AD) and Parkinson's disease [3]. In addition to their role in maintaining protein and organelle homeostasis, lysosomes play an important role as hubs for several signaling pathways including nutrient sensing [3]. There is growing evidence over the last several years that the movement and positioning of lysosomes within a cell is intimately tied to its functioning and cell physiology [4]. This is particularly relevant for neurons where the nature of cargo and cellular demands in the soma, dendritic and axonal compartments are distinct [5]. Evidence from several elegant studies suggests that mechanisms involved in the transport and consumption of cargo in lysosomes are also likely to be differentially regulated in these compartments [5–7]. Here, we review the evidence linking perturbed axonal lysosome transport and abundance to neurological diseases. We highlight the involvement of two key regulators of axonal lysosome transport, the small GTPase Arl8 and the adaptor protein JIP3/MAPK8IP3 to these diseases and discuss potential mechanisms by which their altered activity could contribute to disease pathology.

Axonal lysosomes: their origin, composition and transport

Studies in rat and mouse brain tissue as well as cultured neurons indicate that axons contain relatively fewer lysosomes than neuronal cell bodies or dendrites [6–10]. Several lines of evidence suggest that this might arise primarily from a predominantly retrograde transport and clearance of progressively maturing endolysosomes in axons to the neuronal cell body [11–14]; Figure 1. This retrograde movement plays a critical role in bringing back signaling components, autophagic as well as endocytosed material intended for turnover [11–14]. Given this progressive maturation, axons are largely devoid of fully mature, degradative lysosomes which are highly concentrated in the soma and proximal dendrites [6,7,15,16]. The origin, transport mechanisms of these

Figure 1



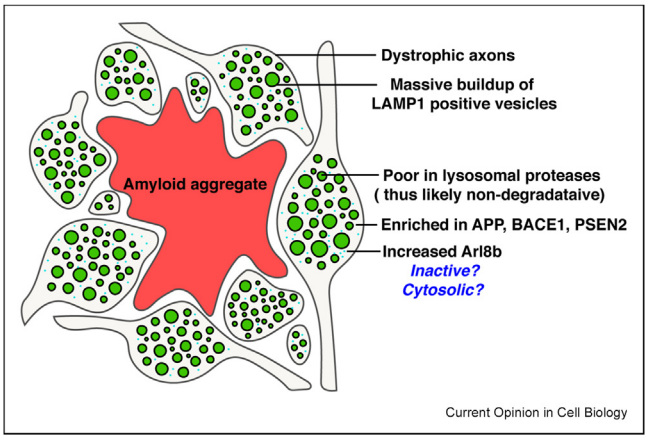
Schematic summarizing axonal lysosome transport and maturation. Organelles arising from fusion of autophagosomes with endo-lysosomes undergo progressive maturation along with their retrograde movement towards the soma. Small GTPases, downstream effectors as well as adaptor proteins are involved in the coordinated dynein-mediated retrograde transport of these organelles. Golgi-derived transport carriers carrying lysosomal proteins move anterogradely to deliver cargo to retrogradely moving organelles as they get closer to the soma.

organelles and their interaction with the closely linked autophagic pathway has been reviewed extensively [17,18]. Ultrastructural studies performed on mouse saphenous nerves where axonal transport was acutely interrupted by local cooling revealed that the cargo that primarily built up distal to the blockade (and thus representing retrograde transport blockade) were multivesicular and multi-lamellar organelles that resemble late endosomes and lysosomes [14]. In contrast, the predominant cargo that accumulated proximal to the site of blockade (and thus representing the anterogradely moving cargo) consisted of clear, smaller vesicles, 50–80 nm in size, some of which were continuous with axonal smooth endoplasmic reticulum [14]. Live imaging studies carried out in primary cultures of hippocampal, DRG neurons over the last few decades as well as in iPSC-derived neurons more recently, examining distinct components of lysosomes and fluorescently tagged autophagy proteins have also corroborated that distally forming endo-lysosomes progressively mature and acidify on their retrograde movement back to the soma [11–13,19]. Likewise, the autophagosomes formed primarily in the distal axons in several neuronal sub-types, fuse with these endo-lysosomal organelles and thus depend on and move processively with the endolysosomal organelles in a retrograde direction [11,13]; Figure 1. While the predominant population of axonal lysosomes are not enriched in proteases and thus not fully mature/degradative and move retrogradely towards the soma, a study carried out in cultured DRG, and cortical neurons has revealed that a small pool of potentially degradative lysosomes (containing fluorescent reporters that bind active cathepsins) move in an anterograde direction towards distal axons [20]. Interestingly, these appear to enrich in the distal tips of these DIV 7 neurons [20], raising the possibility that these anterogradely moving lysosomal populations may be more prevalent during neurodevelopment. Indeed, kinesin-mediated anterograde axonal lysosome transport in rat hippocampal cultured neurons was found to be essential for normal growth cone dynamics strengthening a potential role for these anterogradely moving vesicles in neurodevelopment. However, these vesicles were found to be less acidic, suggesting these may not be degradative [21].

Axonal lysosome transport abnormalities in Alzheimer's disease

Studies in both human AD brain tissue and mouse models of AD have revealed the massive accumulation of lysosomes in swollen axons near extracellular amyloid aggregates, forming part of the neuritic plaque [22]; Figure 2. These organelles were observed to be multilamellar and multivesicular in nature by electron microscopy studies [22], reminiscent of the axonal organelles that build up due to retrograde transport blockade [14]. In further support that these organelles may arise due to a failure of optimal retrograde axonal lysosome transport near the amyloid plaques, these organelles were found to be relatively deficient in multiple lysosomal proteases [9], consistent with the

Figure 2



Axonal lysosome pathology in Alzheimer's Disease. Schematic showing the massive accumulation of LAMP1-positive, protease-poor lysosomal precursors that build up in dystrophic or swollen axons surrounding amyloid plaques. These organelles are enriched in Amyloid Precursor Protein (APP) as well as BACE1and PSEN2 (components of APP processing machinery). Lysosomal GTPase Arl8 has been shown to be highly enriched around plaques, though its membrane association and activity have not been elucidated yet. Increasing axonal lysosome abundance exacerbates amyloid plaque pathology.

composition of less mature organelles normally observed moving retrogradely along axon rather than protease-enriched organelles coming in from the soma. The stalled transport in turn would affect lysosome maturation and protein turnover, leading to more prolonged encounters between APP and BACE1, both of which have been observed to accumulate in these axonal dystrophies [9,23,24]. Indeed, loss of JIP3, a regulator of retrograde axonal lysosome transport, from both murine and human neurons, led to a similar focal accumulation of axonal lysosomes and also increased production of Aβ peptides in the neurons, suggesting that stalled axonal organelles are sites of APP processing and Aβ production [15,19]. Better understanding of mechanisms that control normal axonal lysosome transport as well as efforts to enhance this process or prevent axonal lysosome accumulation could open new therapeutic avenues for AD. Interestingly, a compound identified in a screen for autophagy modulators was observed to enhance net retrograde lysosome movement in human iPSC-derived neurons and interact with LAMP1 [25]. It will be interesting to evaluate the ability of such a compound in clearing axonal lysosome accumulations and modulating Aβ production. Lysosomal phospholipase, PLD3 is also enriched in dystrophic axons around plaques in human AD and mouse models of the same [26,27]. Of functional relevance, AAV-mediated expression of PLD3 in 5xFAD mice led to an increase in size of plaque-associated axonal dystrophies and of individual LAMP1 vesicles inside the swollen axons, which affected axonal conduction but not amyloid plaque size [28]. This effect of PLD3 expression was only observed in lysosomes at the plaques and not in adjacent neuronal soma, suggesting that the extracellular Aβ deposits play a role in PLD3-mediated changes in axonal lysosomes [28]. Further strengthening the connection of lysosomes with amyloid plaques in AD, a study that used laser-capture microdissection of plaques from a small set of EOAD human brain sections in combination with label-free LC–MS, identified endolysosomal proteins including the lysosomal small GTPase Arl8 to be enriched in the plaques [29]. The Arl8 enrichment in axonal dystrophies was subsequently validated in the same study using immunohistochemistry in human AD brain sections [29].

Role of Arl8 in regulating axonal lysosome transport and its involvement in neurological diseases

Studies from almost two decades ago localized the small GTPase Arl8 to lysosomes [30,31] and demonstrated a role for this GTPase in regulating spatial distribution of these organelles [30]. Arl8 has since been demonstrated to play a critical role in endolysosomal maturation as well as multiple fusion events at the lysosome, both homotypic and heterotypic ones with late endosomes and autophagosomes [3,32]. Since then, the mechanism by which Arl8, its effector SKIP and the octameric complex BORC regulate anterograde kinesin-mediated lysosome transport in non-neuronal cells has been extensively worked out [3,4,32]. Studies in mammalian hippocampal neuronal cultures revealed that Arl8, BORC and SKIP work in conjunction with Kinesin 1 to promote anterograde lysosome transport into axons but not dendrites, and perturbation to this process by removal of BORC function led to reduced growth cone dynamics [21]. It is possible that some of these anterogradely moving LAMP1 vesicles are Golgi-derived biosynthetic intermediates/transport carriers. These intermediates would potentially deliver lysosomal proteins to the retrogradely moving and maturing lysosome as similar anterogradely moving LAMP1-positive organelles were positive for TGN38 in a different study [33]. Thus, the anterogradely moving LAMP1 compartments may be a heterogenous population that include some degradative lysosomes as well as TGN-derived carriers of lysosomal proteins that fuse with incoming retrogradely moving vesicles [33]. Consistent with a role for axonal lysosomes in neurodevelopment, compound heterozygous variants and homozygous variants in *BORCS8* were identified in five children with severe early onset-neurodegeneration characterized by severe intellectual disability, global developmental delay, thin corpus callosum, among other features [34] (See also Table 1). Heterologous expression of the mutant BORCS8 resulted in both reduced assembly of the BORC and lysosome movement to the periphery, consistent with them being loss of function

Table 1

Disease-linked mutations in genes encoding lysosome transport regulators.

Gene/Protein	Mutations	Function	Disease	Clinical presentation	Reference
BORCS8/BORCS8	S29P N26Wfs*51 T66P S42P	Anterograde axonal Lysosome transport	Early-infantile neurodegenerative disorder	Severe to profound intellectual disability, Dysmorphic features, hypotonia, Muscle weakness and atrophy, Microcephaly, Thin corpus callosum	[34]
MAPK8IP3/JIP3	G22Afs*3 E27* Y37* G400R L444P R525Q R578C H994Q R1146C D1237N	Retrograde axonal lysosome transport	NEDBA (Neurodevelopmental disorder with or without brain anomalies)	Intellectual disability, Facial dysmorphism. Muscular hypotonia, Cerebellar atrophy, Thin corpus callosum, white matter volume loss	[45,46]
				Severe muscle hypotonia with micrognathia and clenched hands, G-tube feeding	[63]
TMEM106b/TMEM106b	Common variants at 7p21 containing *TMEM106b* (GWAS)	Lysosome transport (in axons and dendrites)	Frontotemporal lobar degeneration (FTLD)	Atrophy of the frontal temporal lobes, Dementia	[62]

alleles, and reiterating the importance of lysosome movement for normal development and functioning of the CNS [34]. A recent study revealed that loss of BORC led to selective depletion of axonal mRNAs of ribosomal and mitochondrial proteins and was also accompanied by mitochondrial defects [35]. Pathway analysis of the depleted mRNAs suggested a potential connection between BORC deficiency and common neurodegenerative disorders [35]. While the role of Arl8 in regulating kinesin-mediated anterograde transport has been well documented [3], its involvement in dynein-mediated retrograde lysosome transport through two effector proteins RUFY3, RUFY4 (RUN and FYVE domain-containing protein) has only recently been elucidated [36,37]. Both studies found that RUFY3 and RUFY4 interact with GTP-bound Arl8 and dynein-dynactin to effect perinuclear lysosome clustering. Consistent with a potential role in regulating axonal lysosome transport, exogenously expressed RUFY3 and RUFY4 colocalize with Arl8 and LAMP1 vesicles in axons of rat hippocampal neurons [36]. Additionally, one study also demonstrated that RUFY3 mediates this retrograde movement through recruitment of JIP4–dynein–dynatcin complex [37]. Thus, much like the GTPase Rab7 [3,17], Arl8 also associates with distinct effectors/adaptors to mediate both anterograde and retrograde transport. The mechanisms and conditions including cell types that may skew Arl8-interaction with one effector over the other need to be further studied. As described previously, Arl8, was found to be enriched in dystrophic axons at AD amyloid plaques [29]. A second study that focused on identifying proteins changing in both human AD brains and in mouse hippocampal lysates in an Aβ-correlated fashion also homed in on Arl8 [38]. They found that Arl8 levels were increased in the hippocampus of 8-month-old 5xFAD animals compared to their littermate controls and localized Arl8 enrichment to axonal dystrophies around Aβ aggregates. Interestingly, the authors of this study propose that the Arl8 accumulation may be a compensatory response to lysosome accumulation potentially to even facilitate lysosomal exocytosis [38]. Given the newly elucidated role of Arl8 in regulating retrograde lysosome transport [36,37], and the lack of clarity as to whether the Arl8 is localized on the surface of lysosomes building up at the plaques, it is also possible that inactivity of Arl8 and/or RUFY3/4 contribute to this pathology (Figure 1). Further studies examining lysosomal recruitment of Arl8 as well as that of its effectors at amyloid plaques, should shed more insight on this.

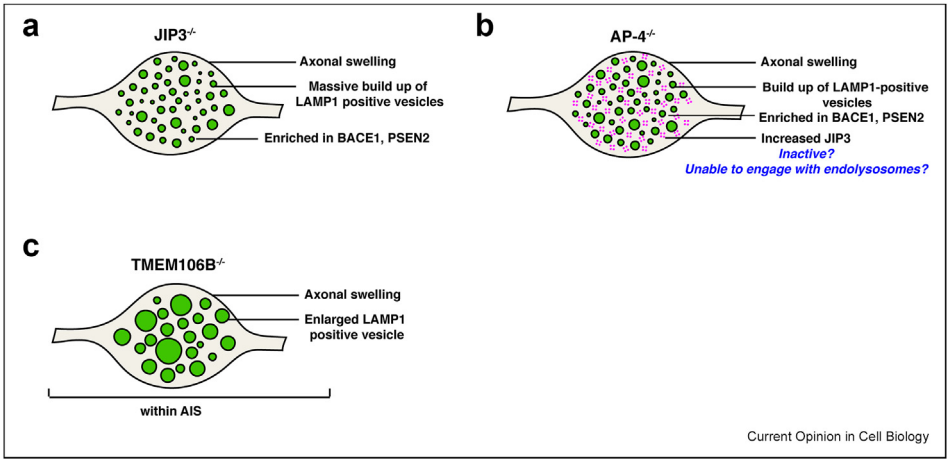
Involvement of JIP3/MAPK8IP3 in axonal lysosome transport and neurological disorders

A role for JIP3 in regulating axonal lysosome transport was first indicated from studies in *Caenorhabditis elegans* and *Danio rerio* models where loss of functional UNC16 and zebrafish JIP3 respectively, led to aberrant axonal

lysosome buildup [39,40]. JIP3/MAPK8IP3 was also shown to be a critical regulator of axonal lysosome transport and abundance in mammalian neurons, with loss of JIP3 in primary mouse cortical neurons and human iPSC-derived neurons leading to large, focal accumulations of lysosomes within axonal swellings [15,19]; Figure 3a. These accumulating lysosomes were deficient in lysosomal proteases and revealed through ultrastructural studies [15] to be electron-dense, multivesicular and multilamellar organelles, highly reminiscent of the organelles that built up on blockade of retrograde transport in the study from Tsukita and Ishikawa [14], as well as those observed around amyloid plaques in AD [9,22]. These results supported a major role for JIP3 in the retrograde movement of these lysosomes out of axons. Consistent with the organelles lacking strong proteolytic activity, the axonal lysosomes in JIP3 KO showed accumulation of lysosomally degraded cargo, BACE1 [15], a critical secretase involved in the amyloidogenic processing of APP. Indeed, both primary mouse neurons and human iPSC-derived neurons lacking JIP3 were observed to have higher levels of intraneuronal Aβ42 [15,19], suggesting that increased axonal lysosome accumulation is a pro-amyloidogenic event. In further support of this model, haploinsufficiency of JIP3 in an Alzheimer's disease mouse strongly enhanced amyloid plaque burden, plaque size as well Aβ42 production [15]. Of relevance to human disease, a study that carried out WES on a well-defined cohort of 60 Austrian Early-onset-dementia patients proposed *MAPK8IP3* as a potential candidate gene linked to dementia [41]. The authors cross-checked their rare gene variant list from the WES data with a curated neurodegeneration candidate gene list and identified two variants in *JIP3* amongst their cohort [41].

Interestingly, loss of Adaptor protein (AP-4) complex-4 in human iPSC-derived neurons causes a similar buildup of axonal lysosomes [42]; Figure 3b. The AP-4 complex acts by sorting of transmembrane cargo at the Trans-Golgi Network (TGN) [43]. Unlike with JIP3, the molecular mechanism by which the AP-4 complex regulates axonal lysosome distribution is not fully understood. Based on its role in cargo sorting, it is possible that AP-4 regulates the sorting of a lysosomal protein that is involved in its retrograde transport. Intriguingly, the axonal swellings arising from AP-4 loss were also enriched in JIP3 [42]. Given the role of JIP3 in mobilizing lysosomes out of axons, it is possible that the JIP3 enriched here is unable to efficiently engage with the lysosomes and effect their retrograde transport. Future studies on AP-4 interactors in neurons may thus reveal new regulators of the lysosome retrograde transport machinery. Interestingly, despite the difference in terms of JIP3 enrichment at these axonal swellings, studies in AP-4ε KO mouse brains indicate that these lysosome-filled axonal dystrophies are also enriched in APP-processing machinery, BACE1 and PSEN2 [44]. It remains to be determined if they contribute to amyloid production. Recently, multiple heterozygous *de novo* variants in *MAPK8IP3* linked to intellectual disability along with variable brain anomalies have been identified [45,46]. Overexpression of human JIP3 carrying the R578C or the R1146C mutations in zebrafish embryos led to axonal varicosities in the posterior lateral line

Figure 3



Axonal lysosome accumulation under different conditions. **(a–c)** Schematic showing axonal lysosome build up in JIP3 KO (a), AP-4 loss of function (b) and TMEM106B KO (c) neurons. APP processing machinery is enriched in axonal organelles building up in both JIP3 KO (a) and AP-4 loss of function (b) neurons, while surprisingly, there are higher levels of JIP3 in axonal swellings of AP-4 depleted neurons. It remains to be determined if this JIP3 increase is a compensatory response and if so, is JIP3 unable to associate with the accumulating lysosomes in this condition. The organelles accumulating in the axonal swellings of TMEM106B KO neurons are enlarged, less acidic and low in protease content (c), suggesting they may also accumulate the lysosomal cargo BACE1. These axonal swellings are present in the Axon Initial Segment (AIS) in TMEM106B KO neurons unlike the other two conditions.

nerve, suggestive of deleterious effect on developing axons [45]. It remains to be determined if the missense mutations are indeed loss of function mutations and cause similar axonal lysosome buildup as observed in the JIP3 KO neurons. A recent study utilized in vitro motility assays as well as cryo-electron microscopy studies to reveal JIP3 to be an autoinhibited, activating adaptor for dynein [47]. Interestingly, mutations in both dynein heavy chain [48] and dynactin [49] have been linked to motor neuron diseases such as dominant spinal muscular atrophy with lower extremity predominance (SMA-LED) and axonal Charcot-Marie-Tooth (CMT) disease. Additionally, *de novo* mutations close to and in the motor domain of dynein heavy chain have been identified in patients with major intellectual disability [50]. *In vitro* and animal model studies have revealed that these mutations are associated with reduced retrograde axonal transport. The effects of these mutations and the insights these provide into the role of dynein in the nervous system have been reviewed in depth elsewhere [51]. It will be interesting to determine if any of the *de novo* JIP3 mutations affect its ability to activate dynein.

While JIP3 is now an established activator of dynein [52,53], JIP3 also binds kinesin [54,55]. Expression of a mutant form of JIP3 abrogating its interaction with DLIC led to endo-lysosomal intermediates building up in neurite tips instead of in focal axonal swellings, suggesting some bidirectional transport of JIP3-bound endo-lysosomes [54]. One of the human mutations is predicted to disrupt JIP3 binding to KLC [46] raising the possibility of distinct lysosomal pathologies with the different *JIP3* mutations.

While a role for JIP3 in regulating axonal lysosome movement is now well-established, phenotypes arising from loss of UNC16 (JIP3 ortholog) in *C. elegans* are more complex, and include large accumulations of Golgi and endosomes in the axon in addition to the lysosome buildup [40]. This led the authors to propose an organelle gate-keeper function for UNC16/JIP3 that controls their entry into axons. UNC16 was also shown to negatively regulate actin and microtubule dynamics in *C. elegans* regenerating neurons [56]. Loss of JIP3 in human neurons is also associated with disruption to the axonal periodic scaffold of actin and spectrin, as well as local microtubule disruption [57]. Given these complex phenotypes arising from perturbations to JIP3, it is possible that alterations to these processes could also contribute to JIP3-linked neurodevelopmental disorder.

Thus, interestingly, alterations to both Arl8 (via BORC) and JIP3 function are linked to neurological disorders characterized by developmental delay, intellectual disability as well as other shared brain anomalies including thin corpus callosum (see Table 1). Likewise, changes in their levels are linked to axonal lysosome buildup around plaques and increased amyloidogenesis [15,38]. Given the structural similarity of JIP3 and JIP4 [58], and the newly described interaction of Arl8 with the JIP4—dynein complex [37], it is possible that an interplay between JIP3 and Arl8 and their different interactors may function to maintain axonal lysosome homeostasis. In further support of a role for normal axonal lysosome homeostasis in maintaining neuronal health, axonal dystrophies filled with autophagic vacuoles were observed in npc1−/− mice [59]. Intriguingly, restoring Arl8 and Kinesin-1 mediated anterograde lysosome transport rescued this pathology in *npc1*−/− neurons, suggesting that either degradative lysosomes and/or LAMP1 vesicles carrying key material for restoring clearance and maturation of AV can restore axonal homeostasis under this pathological condition [59]. In addition to the strong connection between JIP3, Arl8/BORC to neurological disease, TMEM106B, a membrane protein implicated in regulating lysosome transport [60,61], is linked to frontotemporal lobar dementia and Parkinson's disease [62]. Interestingly, loss of TMEM106B in mice led to swellings at the Axon Initial Segment (AIS) that contained enlarged LAMP1-vesicles (Figure 3c) that are poor in proteases and less acidic suggesting they are immature lysosomes [60]. Whether TMEM106B acts in the same pathway or interacts in any way with JIP3 and Arl8 remains to be determined. Further studies (see Table 2) into the mechanisms of action of these proteins in regulating axonal lysosome transport will be vital from both a basic cell biology perspective and in understanding disease mechanisms.

Table 2

Outstanding questions and future directions for the field.

1. Identification and characterization of the distinct endo-lysosomal and autophagic intermediates along the retrograde axonal lysosome transport pathway.
2. What are the different populations of anterogradely moving lysosome-related vesicles and how and where do they intersect with retrogradely moving endo-lysosomal organelles?
3. How are lysosomes recognized by distinct adaptors and how are they coordinated to effect processive movement along axons?
4. How do the *de novo JIP3* mutations alter axonal lysosome transport?
5. What are the differences and similarities in the composition of the endo-lysosomal organelles that accumulate upon loss of JIP3, AP-4 complex and in Alzheimer's disease neurons? Do these differences contribute to selective vulnerability of the neuronal populations in the disease states?

Author contributions

JMP and SG conceptualized and wrote the review.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This is a review that includes work from other scientists whose data we do not have access to

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* of special interest
** of outstanding interest

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