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## Autophagy and Mitophagy in ALS

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### Abstract

Amyotrophic lateral sclerosis (ALS) is a debilitating and incurable disease involving the loss of motor neurons and subsequent muscle atrophy. Genetic studies have implicated deficits in autophagy and/or mitophagy in the onset of the disease. Here we review recent progress in our understanding of the pathways for autophagy and mitophagy in neurons, and how these pathways may be affected by mutations in genes including DCTN1, OPTN, TBK1, VCP, and C9ORF72. We also discuss the implications of modulating autophagy in ALS, highlighting both the potential of the approach and the concerns raised by targeting this pathway as a therapeutic strategy in neurodegenerative disease.

### INTRODUCTION

Initially described in the late 19<sup>th</sup> century by Charcot and Joffroy, our scientific understanding of the devastating neurodegenerative disease Amyotrophic lateral sclerosis (ALS) has progressed significantly, particularly through the identification of causative mutations for familial forms of the disease. Genetic studies have implicated key pathways involved in pathogenesis, including autophagy and mitophagy. However, this knowledge has not yet led to the development of effective therapeutic strategies, suggesting that there are still key gaps in our understanding of the underlying mechanisms. This review summarizes progress, investigating neuronal autophagy and mitophagy, and how that knowledge contributes to our understanding of the pathobiology of ALS.

### WHAT IS ALS?

ALS is a debilitating neuromuscular disease characterized by the progressive degeneration of motor neurons in the spinal cord and brain. Degeneration of these motor neurons leads to neuromuscular denervation, atrophy of voluntary skeletal muscles, and ultimately paralysis and death. The worldwide incidence of ALS is estimated at ~5/100,000 (Chio et al., 2013). Onset is usually age-dependent, although rare genetic forms may have a juvenile onset. Global estimates indicate that incidence peaks between the ages of 50 and 75 years (Chia et al., 2018). Disease progression is usually rapid, leading to death within 3–5 years of initial onset. There is currently no cure for this fatal disease, and available therapeutic options show only limited effectiveness.

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Approximately 10% of ALS cases are familial in origin, with the remainder sporadic and of unknown cause. Possible causative factors for sporadic ALS include environmental factors such as infectious disease, chronic neuroinflammation, injury or trauma, and toxin exposure. However, the major shared risk factor across both sporadic and familial forms is aging.

Progress on genetic causes of ALS has been very rapid in recent years, with ~30 different genes now implicated (Chia et al., 2018). Surprisingly, these genes do not fit easily into a single cellular pathway, although some themes do emerge. For example, a number of ALS-associated genes encode RNA-binding proteins, including TDP43 and FUS, suggesting that alterations in ribostasis may contribute to pathogenesis. Alterations in proteostasis, mitochondrial function, cytoskeletal integrity and intracellular trafficking have also been implicated (Taylor et al., 2016).

Strikingly, several of the ALS-associated genes identified to date have been functionally implicated in autophagy and/or mitophagy, specifically the clearance of protein aggregates and/or damaged mitochondria. ALS genes known to function in autophagy include OPTN, TBK1, and SQSTM1. More broadly, proteins encoded by the genes C9ORF72, VCP, CHMP2B, VAPB, ALS2, and DCTN1 have all been implicated in vesicular trafficking and may affect autophagy either directly or indirectly. A central role for autophagy in ALS is well supported by the pathology of the disease, which commonly includes the accumulation of protein aggregates and swollen or dystrophic mitochondria in motor neurons of affected patients (Taylor et al., 2016).

Here we review the two best characterized pathways for autophagy in neurons, axonal autophagy and mitophagy, and discuss the available evidence linking dysfunction in these pathways to the onset and progression of ALS. We also highlight a few of the many questions that remain, such as how mutations that are ubiquitously expressed may lead to the specific loss of motor neurons, and how cell-autonomous and cell-non-autonomous mechanisms may contribute (Box 1). We also discuss the possibilities and limitations of therapeutic interventions affecting autophagy in either neurons or their support cells.

## AUTOPHAGY IN NEURONS

Autophagy is a critical pathway to maintain homeostasis and to respond to cellular stress. The molecular components of the autophagy pathway have been well characterized over several decades of research in model systems, such as yeast. Studies in mammalian cells indicate that the overall pathway is highly conserved. More recently, however, there has been a growing appreciation that autophagy is specifically tuned in highly differentiated cells, with distinct, tissue-specific differences apparent in regulation of autophagy, such as cellular responses to stress. For example, Mizushima *et al.* (2004) used a transgenic mouse model expressing the autophagosome marker GFP-LC3B to demonstrate that upon starvation autophagy is significantly upregulated in tissues such as liver, but not in brain (Mizushima et al., 2004).

Differentiated and highly polarized cells, such as neurons, exhibit spatially segregated pathways for autophagy and mitophagy (Maday and Holzbaur, 2014; Sung et al., 2016).

Direct imaging of autophagy in neurons expressing GFP-labeled Atg8 (LC3B in mammals) has demonstrated a robust, constitutive pathway for autophagosome formation in the distal axon both *in vitro* and *in vivo* (Maday and Holzbaur, 2014; Maday et al., 2012; Neisch et al., 2017). Autophagosomes form in a stepwise manner in the growth cone of neurons in culture and at synaptic sites such as the neuromuscular junction *in vivo* (Neisch et al., 2017; Stavoe et al., 2016). Once formed, distal autophagosomes are rapidly transported toward the soma via the microtubule-based motor cytoplasmic dynein and its activator dynactin (Figure 1; (Maday et al., 2012; Neisch et al., 2017)). Of note, a G59S mutation in the dynactin subunit p150<sup>Glued</sup>, encoded by the DCTN1 gene, has been identified as the cause of the late-onset and slowly progressive motor neuron disease HMN7B (Puls et al., 2003). Thus, mutations in DCTN1 can be considered as a very rare cause of ALS (Chia et al., 2018).

Axonal autophagosomes form constitutively (Maday and Holzbaur, 2014; Maday and Holzbaur, 2016; Maday et al., 2012; Neisch et al., 2017; Stavoe et al., 2016), and cargo-loading appears to be nonselective (Maday et al., 2012). Engulfed cargos found within axonal autophagosomes include mitochondrial fragments and aggregated proteins (Figure 1), and may include synaptic vesicles under some circumstances (Maday et al., 2012; Okerlund et al., 2017; Wong and Holzbaur, 2014b). Both *in vitro* and *in vivo*, autophagosome formation is most prominent in the distal axon (Maday and Holzbaur, 2014; Maday and Holzbaur, 2016; Neisch et al., 2017; Soukup et al., 2016; Stavoe et al., 2016; Vanhauwaert et al., 2017).

During transport toward the soma, autophagosomes fuse with late endosomes and lysosomes to form a more mature, acidified organelle known as an autolysosome. In contrast to newly formed autophagosomes which are primarily found in the distal axon, autolysosomes are found along the length of the axon and accumulated within the soma (Maday and Holzbaur, 2016). The autolysosome contains degradative proteases such as cathepsins and efficiently degrades internalized cargos so that the components can be released and recycled. As effective autophagy depends on fusion of nascent autophagosomes with functional lysosomes to form a proteolytically active compartment, conditions that affect lysosomal integrity, such as mutations in the ALS-linked gene VCP (Papadopoulos et al., 2017) or extracellular deposits of  $\beta$ -amyloid (Gowrishankar et al., 2015) affect both lysosomal and autophagosomal processing in neurons.

## ALS-LINKED GENES IMPLICATED IN AUTOPHAGY

In addition to DCTN1, which encodes an essential component of the microtubule-based motor complex that moves autophagosomes from the axon toward the soma, the protein products of multiple ALS-linked genes have been implicated in the dynamics or regulation of autophagy. These include p62/SQSTM1 (Gal et al., 2009), a ubiquitin-binding protein that associates with both protein aggregates (for example, see (Rudnick et al., 2017)) and damaged mitochondria (Wong and Holzbaur, 2014a). Optineurin/OPTN is another ubiquitin-binding protein that is also implicated in the autophagic clearance of aggregated proteins (Korac et al., 2013) and depolarized or damaged mitochondria (Wong and Holzbaur, 2014a). As noted below, both p62/SQSTM1 and OPTN are recruited to ubiquitinated substrates, and induce their sequestration by an autophagosome via their LC3-binding motifs. TBK1 was

identified as an ALS-linked gene by exome sequencing, and is a kinase known to phosphorylate both p62 and OPTN (Cirulli et al., 2015). While cellular assays have clearly established roles for p62, OPTN, and TBK1 in the autophagic clearance of protein aggregates as well as mitophagy (discussed below), these three proteins have also been implicated in innate immunity pathways, and thus may cause disease by mechanisms unrelated to autophagy/mitophagy. Further work is required to establish the critical pathogenic mechanism or mechanisms involved.

Other ALS-associated genes have also been linked to autophagy, although their specific roles remain to be determined. These include VCP, implicated in the autophagy of stress granules (Buchan et al., 2013) and the maintenance of lysosomal integrity (Papadopoulos et al., 2017), and VAPB, an ER-tethering protein (Dong et al., 2016). Expansion of hexanucleotide repeats within the noncoding sequence of the C9ORF72 gene are the most frequent cause of familial ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Recent data implicate the protein product of the C9ORF72 gene in the regulation of ULK1, and thus in the initiation of autophagy; however, C9ORF72 has also been proposed to function in the regulation of lysosomal fusion or function (Amick et al., 2016; Jung et al., 2017; Yang et al., 2016). However, it remains unclear if decreased C9ORF72 expression contributes to the onset of neurodegeneration, as mouse knockouts do not show a neuron-specific phenotype (O'Rourke et al., 2016); other mechanisms such as RAN translation or the formation of RNA foci may instead drive pathogenesis (Ash et al., 2013; Mori et al., 2013; Zu et al., 2011; Zu et al., 2013)

## AUTOPHAGIC CLEARANCE OF PROTEIN AGGREGATES IN ALS

*In vitro* studies of neurons expressing the ALS-linked mutation SOD1<sup>G93A</sup> demonstrated the uptake of mutant SOD1 into axonal autophagosomes (Maday et al., 2012). Surprisingly, over-expression of the mutant protein did not result in upregulation of autophagosome formation or flux, suggesting that the axonal pathway for autophagosome biogenesis has a limited ability to respond to proteotoxic stress. Similar studies in neurons expressing mutant Huntingtin support this conclusion, as Huntingtin aggregates were readily taken up by axonal autophagosomes, but no increase in biogenesis or flux was induced by the expression of this aggregation-prone protein (Wong and Holzbaur, 2014b). This study demonstrated a detrimental effect of mutant Huntingtin on the ability of autophagosomes to degrade their cargo, suggesting that while autophagosome biogenesis is not effectively upregulated, turnover may be inhibited leading to the accumulation of autophagosomes within neurons exposed to proteotoxic stress.

Rudnick *et al.* (2017) examined autophagy in motor neurons in a transgenic mouse model expressing SOD1<sup>G93A</sup>, and identified the formation of large ubiquitinated protein aggregates positive for p62, the product of the ALS-associated gene SQSTM1. Both large autophagosomes positive for Atg8 homologs, ~3 µm in diameter and termed round-bodies, and skein-like inclusions were observed. The SOD1<sup>G93A</sup> transgenic mouse has a stereotypical time course of disease onset, progression and death, allowing the investigators to compare autophagosome accumulation to disease progression. Prior to frank onset of disease, round bodies were relatively abundant while the skein-like inclusions became more

prevalent with disease progression. Potentially, the accumulation of the skein-like inclusions may reflect a deficit in the ability of the motor neuron to effectively clear protein aggregates via autophagy, due to either an aging-dependent decline in autophagy or the high burden of proteotoxic stress. While the formation of p62-positive aggregates was most prominent in motor neurons early in disease progression, similar structures were observed in interneurons at later time points, indicating that while the observed pathology induced by over-expression of mutant SOD1<sup>G93A</sup> preferentially affected motor neurons, it was not motor neuron-specific. Together, these studies suggest that overexpression of aggregation-prone proteins readily overwhelms the ability of the neuron to effectively clear aggregates by autophagy.

## MITOPHAGY IN NEURONS

Damaged or aged mitochondria are selectively sequestered and eliminated through the autophagic process of mitophagy (Tal et al., 2007). This pathway is important for neuronal homeostasis and is most commonly linked to Parkinson's disease, due to the discovery of two proteins (PINK1 and Parkin) linked to the familial form of the disease (Kitada et al., 1998; Valente et al., 2004; Youle and Narendra, 2011). However, many neurodegenerative diseases overlap on the subcellular level with defects in neuronal mitophagy. In the case of ALS, the accumulation of damaged or dysfunctional mitochondria are thought to be a contributing factor to the disease. Despite our knowledge of this pathway and disease links between PINK-1/Parkin and OPTN/TBK1 to Parkinson's disease and ALS, respectively, it remains unclear as to the significance of this overlap (Cirulli et al., 2015; Freischmidt et al., 2015; Kitada et al., 1998; Maruyama and Kawakami, 2013; Valente et al., 2004).

The mitophagy pathway has been worked out in detail in immortalized cell lines, identifying a highly regulated process that involves the stepwise recruitment of multiple proteins (Lazarou et al., 2015; Moore and Holzbaur, 2016; Wong and Holzbaur, 2014a). Upon damage, PINK1 is stabilized on the mitochondrial surface which leads to the recruitment of an E3 ubiquitin ligase, Parkin (for reviews of PINK1/Parkin mitophagy see (Nguyen et al., 2016; Pickrell and Youle, 2015; Youle and Narendra, 2011)). Phospho-ubiquitination of outer mitochondrial membrane proteins initiates a feed-forward cascade that marks mitochondria for degradation (Kane et al., 2014; Kazlauskaitė et al., 2014; Matsuda et al., 2010; Narendra et al., 2008; Narendra et al., 2010). Specificity for the autophagosome engulfment of damaged mitochondria relies on OPTN, an autophagy receptor, and its kinase TBK1 (Lazarou et al., 2015; Moore and Holzbaur, 2016; Wong and Holzbaur, 2014a). Translocation of OPTN to damaged mitochondria leads to LC3B recruitment and degradation via lysosomal fusion (Figure 1; (Moore and Holzbaur, 2016; Rogov et al., 2014; Wong and Holzbaur, 2014a)). It should be noted that p62 is also recruited to damaged mitochondria, but is independent of OPTN and does not lead to recruitment of LC3B (Wong and Holzbaur, 2014a).

In immortalized cell lines, OPTN translocation was shown to initiate within tens of minutes of mitochondrial damage via a mitochondrial KillerRed (mt-KR) construct, where light-induced activation of mt-KR causes local damage to mitochondria via ROS production (Bulina et al., 2006), or CCCP treatment, an inhibitor of oxidative phosphorylation; robust OPTN recruitment and stabilization was observed in ~40 minutes (Moore and Holzbaur,

2016; Wong and Holzbaur, 2014a). The use of an ALS-associated E478G ubiquitin binding-deficient mutant of OPTN significantly decreased translocation to damaged mitochondria, as the mutant failed to stably associate with the mitochondrial surface (Lazarou et al., 2015; Wong and Holzbaur, 2014a). Moreover, the use of an ALS-linked TBK1 mutant significantly reduced OPTN and LC3B recruitment to damaged mitochondria; this was also observed by silencing TBK1 or using a potent TBK1 inhibitor (Lazarou et al., 2015; Moore and Holzbaur, 2016). Therefore, loss of function of OPTN or TBK1 results in impaired mitophagy and accumulation of damaged mitochondria. Thus, inefficient turnover of mitochondria via ALS-associated mutants could be a contributing factor leading to mitochondrial dysfunction and accumulation, a prevalent feature in the motor neurons of ALS patients.

Surprisingly, substantially less is known about the regulation of mitophagy in neurons. As neurons are highly polarized and require long-range transport, it remains controversial whether distal mitochondria are subject to the same quality control mechanisms as those near the cell body. Since prompt removal of damaged mitochondria is critical for cell viability and the energy demands of neuronal mitochondria are substantial, it raises the question of how/where Pink1/Parkin-mediated mitophagy occur in neurons? Using mt-KR or global damage via antimycin a treatment, Ashrafi *et al.* (2014) showed that damage to axonal mitochondria triggered translocation of the mitophagy machinery within tens of minutes. In neurons lacking PINK1 or Parkin, recruitment was not visualized. It should be noted that despite the implications of PINK1 and Parkin in neurodegenerative disease, knockout mice fail to develop significant neurological defects (Whitworth and Pallanck, 2017). The authors concluded that while autophagosome biogenesis is a constitutive pathway that occurs distally in neurons, autophagosome induced formation around damaged mitochondria is a distinct pathway that can occur anywhere along the axon (Ashrafi et al., 2014).

This idea was recently called into question by studies in *Drosophila* which suggested that the soma is the primary focus of PINK1/Parkin-mediated mitophagy in neurons. Devireddy and colleagues showed that while loss of PINK1 reduced mitochondrial membrane potential, it failed to lead to an accumulation of mitochondria or alter mitochondrial length in axons. However, irregular mitochondrial morphology was observed in the soma (Devireddy et al., 2015). Consistent with this notion, Parkin-deficient flies had normal motility and morphology in the axons of motor neurons, but an abnormal mitochondrial network in the cell body (Sung et al., 2016). The authors argued that in motor neurons *in vivo* mitophagy is a rare event due to an additional quality control step that allows only healthy mitochondria to exit the soma and enter the axon.

Much of the work thus far to study neuronal mitophagy has focused on a PINK1/Parkin-mediated process. The use of chemical uncouplers has shown that mitophagy in neurons is remarkably slow compared to the time-course that was observed in immortalized cell lines. Additionally, neuronal mitochondria are much more resistant to mitophagy initiation than organelles in cell lines, which could be due to the fact that they are terminally differentiated. Thus, it is plausible that neurons have developed additional quality control mechanisms, prior to mitophagy, as preventative measures to decrease the number of stressed or aged

organelles likely to enter the mitophagy pathway (Sugiura et al., 2014). For example, recent work from Lin *et al.* (2017) has provided new evidence to suggest that stressed neuronal mitochondria are removed from the axon via mitochondria-derived cargos and that this pathway is independent of Parkin, Drp1, and autophagy. The authors observed an alteration in mitochondrial membrane potential when synaptophilin (SNPH), a mitochondrial anchoring protein, was overexpressed suggesting that axonal transport of mitochondria is required to maintain integrity and health. Additionally, stressed mitochondria release SNPH containing vesicles as a mechanism to shift from a stationary position to actively transport damaged mitochondria from the axon for degradation (Lin et al., 2017). This finding is in line with a recent report showing mature mitochondria are tethered near presynaptic terminals in cortical neurons (Lewis et al., 2016). Together these results suggest that aged mitochondria are more susceptible to damage due to their immobilization.

It is plausible that these discrepancies in mitophagy location are due to the types of treatments (i.e. global vs. local damage), as well as the severity of the chemical uncouplers (i.e. CCCP or antimycin a) used to induce mitochondrial damage. Additionally, the rates of mitophagy could be heavily influenced by *in vitro* versus *in vivo* model systems (Sung et al., 2016). While Pink1/Parkin-mediated mitophagy in immortalized cell lines appeared to be straightforward, it has become apparent that this mechanism in neurons is complex and work is still needed to fully address where and how this process occurs. For example, the interplay of mitophagy and neuroinflammation is now receiving attention, as neuroinflammation is a hallmark of ALS and increasing evidence suggests that mitophagy and neuroinflammation are linked (Komine and Yamanaka, 2015; Oakes et al., 2017).

## AUTOPHAGY: FRIEND OR FOE FOR THE TREATMENT OF ALS?

Autophagy and mitophagy are considered to be essential homeostatic pathways in neurons, as genetic ablation of key autophagy genes is sufficient to induce the degeneration of neurons (Hara et al., 2006; Komatsu et al., 2006). Further, the identification of mutations in proteins thought to function within the autophagy/mitophagy pathways (SQSTM1, C9ORF72, PINK1, Parkin, OPTN, TBK1) in both Parkinson's disease and ALS further highlights the potential importance of these cellular mechanisms.

In cellular models of ALS, there have been reports that pathology can be rescued by the induction of autophagy. For example, Marrone *et al.* (2018) developed iPSC lines expressing wild type or mutant FUS-eGFP, and observed the recruitment of both proteins to stress granules upon oxidative stress in both iPSCs and an induced mixed population of neuronal cells containing motor neurons. Dynamics of stress granule formation were altered in mutant FUS-eGFP iPSCs and could be rescued by treating the cells with mTOR inhibitors that can induce autophagy (Marrone et al., 2018). Of note, the drug-induced induction of autophagy in iPSC-derived neurons was much less than that observed in the parent iPSC lines, consistent with observations that mTOR inhibition does not effectively upregulate autophagy in neurons (Maday and Holzbaur, 2016).

Surprisingly, however, Rudnick *et al.* (2017) reported that the inhibition of autophagy induced by a targeted knockdown of Atg7 is not sufficient to induce motor neuron cell death

in mice up to 150 days old, although abnormal synaptic structure and function were observed in Atg7 conditional knockout (cKO) mice. Crossing Atg7-cKO mice to mice expressing mutant SOD1 led to some counter-intuitive findings. The deficiency in autophagy induced by depletion of Atg7 led to an earlier disease onset by some but not all measures, as onset of hind limb tremor was observed 22 days earlier but there was no difference in disease-associated weight loss. However, depletion of Atg7 was found to extend lifespan by ~22%, although motor neuron survival was not affected as compared to mice expressing mutant SOD1 in the presence of wildtype levels of Atg7 (Rudnick et al., 2017).

In a parallel study, rilmenidine was administered to induce autophagy in the SOD1<sup>G93A</sup> mouse. While autophagy was upregulated, both motor neuron degeneration and symptom progression were deleteriously affected (Perera et al., 2017). Non-cell autonomous effects of autophagy modulation must also be considered (Fabrizio et al., 2017). For example, Staats *et al.* (2013) found that rapamycin treatment of SOD1<sup>G93A</sup> mice did not increase survival. However, rapamycin has an immunosuppressive effect on lymphocytes. To avoid this complication, SOD1<sup>G93A</sup> mice were crossed to RAG1<sup>-/-</sup> mice to generate animals deficient in mature lymphocytes. In this line, rapamycin treatment induced a mild extension of lifespan (6.5 days). Disease onset was not affected, instead the principal effect was on disease progression (Staats et al., 2013).

Together, results to date raise concern that therapeutic approaches aimed generally at enhancing autophagy may not be uniformly beneficial for patients with ALS. More nuanced strategies may be effective, such as enhancing lysosomal function and thus counteracting the accumulation of autophagosomes or autolysosomes without sufficient degradative ability. These strategies must be built on a better understanding of the cell biology of autophagy in neurons, and how the dynamics of autophagy are affected in both familial and sporadic ALS.

## CONCLUSIONS

Recent progress is beginning to map out how and when autophagy occurs in neurons, and how neurons use this pathway to counter cellular stressors such as proteotoxic stress or damaged mitochondria. Studies at the cellular level are profiting from the recent advances in the genetics of ALS and other, related neurodegenerative diseases. We are beginning to understand how disease-associated genes can be grouped into consensus pathways, allowing an improved focus on disease-causing mechanisms involved in pathogenesis. While current data clearly implicate defects in autophagy and mitophagy in familial ALS, further research is necessary to understand the molecular basis for the defects, as well as the most effective approaches to intervene (Box 1). Hopefully, the progress made in understanding familial cases will then directly inform our understanding of the predominant, sporadic forms of ALS, and lead to the design of more effective therapeutic approaches in future.

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**BOX 1:****Outstanding questions regarding the role of autophagy in ALS**

- ***Is autophagy necessary for motor neurons?*** Data from Rudnick et al. (2017) investigated the effects of Atg7 knockdown on the structure and function of the neuromuscular junction and reported relatively minor deficits? Would more severe deficits be apparent upon longer-term knockdown?
- ***Do mutations in genes required for autophagy and/or mitophagy cause disease through cell-autonomous or cell-non-autonomous pathways?*** For example, is the expression of mutant OPTN or TBK1 in motor neurons sufficient to cause disease, or does disruption glial function also contribute, as has been found for mutant SOD1 (Taylor et al., 2016)?
- ***Are all neuronal mitochondrial subject to the same quality control mechanisms?*** Data from Sung et al. (2016) suggest that mitophagy is rare in vivo, and is limited to the soma. Work is still needed to determine whether mitophagy is spatially constricted or occurs throughout the neuron. In addition, the contributions of other mitochondrial quality control mechanisms in neurons remains unclear.
- ***What are the mechanistic links between deficits in mitophagy and neuroinflammation?*** The genes identified as functioning within the mitophagy pathway downstream of PINK1 and Parkin include OPTN and TBK1, genes which have been previously implicated in inflammatory pathways.
- ***To what extent do defects in autophagy/mitophagy pathways contribute to the pathogenesis of sporadic ALS?*** Genetic studies suggests that the pathogenesis of ALS involves multiple pathways, including proteostasis, ribostasis, mitochondrial health, and neuroinflammation. To what extent these pathways contribute to sporadic ALS remains to be determined.
- ***Will therapeutic approaches that enhance autophagy have a helpful or harmful effect on motor neuron health?*** Data from multiple model systems suggest that activation of autophagy may have differential effects on disease onset and progression.

### HIGHLIGHTS

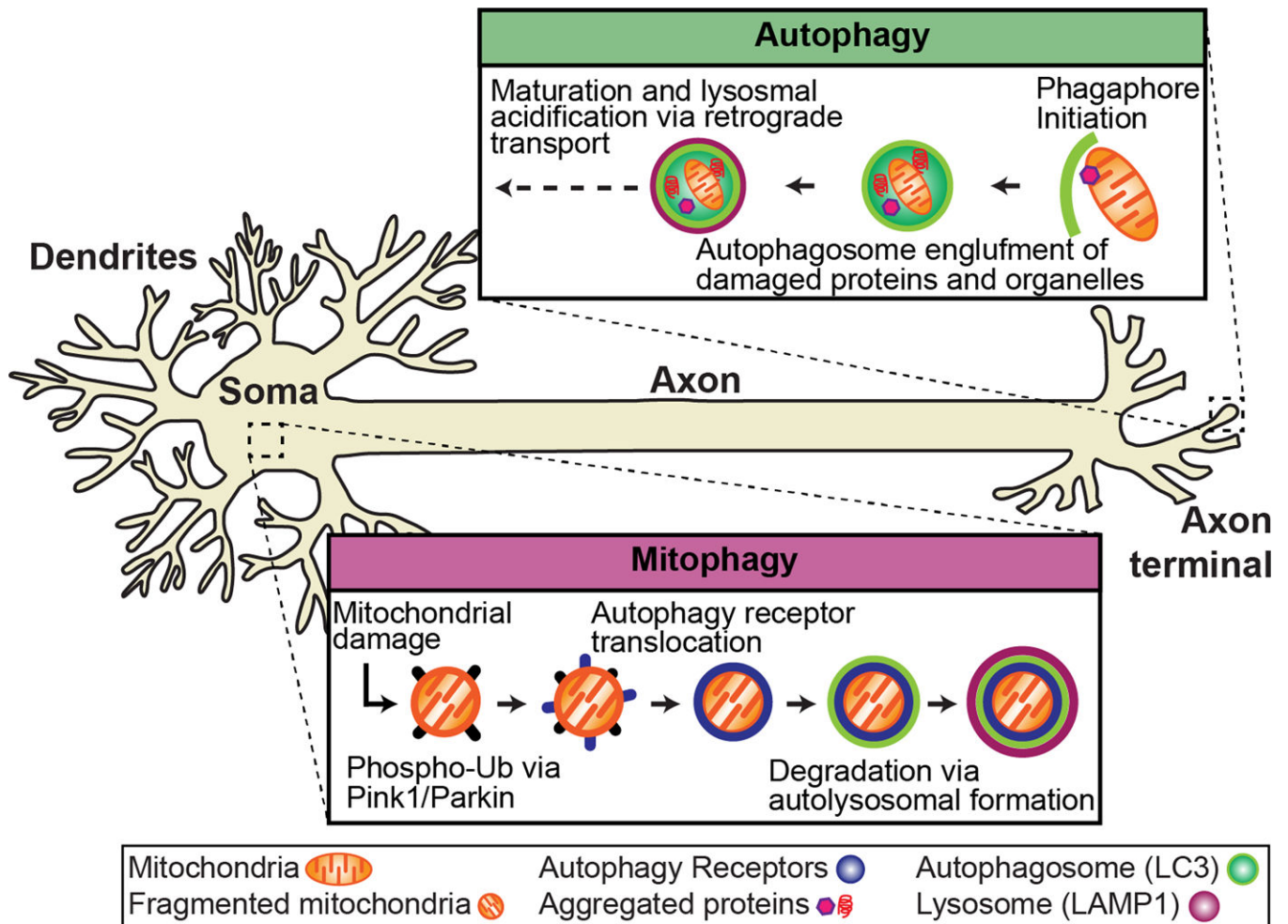
- ALS-associated genes are implicated in autophagy and mitophagy
- Constitutive autophagy and induced mitophagy are distinct pathways in neurons
- Therapeutic targeting of autophagy in ALS has promise but also potential concerns

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**FIGURE 1: Autophagy and mitophagy in the neuron.**

Autophagy and mitophagy are constitutive mechanisms important to in the maintenance of neuronal homeostasis that have been implicated in ALS. Autophagy occurs at the distal tip of the axon, where autophagosomes engulf damaged/aged mitochondria and protein aggregates. Through retrograde transport, the autophagosome matures and fuses with lysosomes to form a mature autolysosome that is degraded in the soma. Evidence suggests that mitophagy is spatially distinct from autophagy and occurs primarily in the soma. Damaged mitochondria are marked for degradation via phospho-ubiquitination of outer mitochondrial membrane proteins via PINK1/Parkin. Autophagy receptors (i.e. OPTN) are recruited to mitochondria and initiate the translocation of the autophagy machinery to degrade damaged mitochondria via lysosomal fusion.