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Autophagy in Neurons

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Abstract

Autophagy is the major cellular pathway to degrade dysfunctional organelles and protein aggregates. Autophagy is particularly important in neurons, which are terminally differentiated cells that must last the lifetime of the organism. There are both constitutive and stress-induced pathways for autophagy in neurons, which catalyze the turnover of aged or damaged mitochondria, endoplasmic reticulum, other cellular organelles, and aggregated proteins. These pathways are required in neurodevelopment as well as in the maintenance of neuronal homeostasis. Here we review the core components of the pathway for autophagosome biogenesis, as well as the cell biology of bulk and selective autophagy in neurons. Finally, we discuss the role of autophagy in neuronal development, homeostasis, and aging and the links between deficits in autophagy and neurodegeneration.

Contents

INTRODUCTION: DEGRADATIVE PATHWAYS IN THE NEURON

Neurons are postmitotic and long-lived cells. Both neurodevelopment and the long-term maintenance of neuronal health require effective removal of aggregated proteins and aged or defective organelles. Genetic and cellular studies indicate that defects in protein and organelle turnover closely correlate with cellular stress, degeneration, and death.

Multiple degradative pathways, such as the degradation of misfolded proteins via the ubiquitin-proteasome pathway, are at work in neurons (Bingol & Sheng 2011). Protein degradation also occurs via the endolysosomal trafficking pathway (Lie & Nixon 2018, Winckler et al. 2018), as well as by chaperone-mediated autophagy and endosomal microautophagy (Tekirdag & Cuervo 2018). Recently identified pathways may also contribute to the clearance of misfolded or aggregated proteins in neurons, such as the MAGIC (mitochondria as guardian in cytosol) pathway, which involves the import of misfolded proteins into mitochondria (Ruan et al. 2017).

Macroautophagy, reviewed here, is the major pathway to clear larger targets for degradation, such as protein aggregates and dysfunctional organelles. The molecular players in this pathway have been well defined from decades of elegant work in yeast (Ohsumi 2014). Many of the proteins initially identified in yeast screens have clear homologs required for autophagy in mammalian cells and function in an analogous manner, giving us an overall understanding of the canonical pathway. However, we are just beginning to understand how this pathway is specialized for the turnover of specific cargos in selective autophagy and how it has been adapted to fit the distinct needs of terminally differentiated and highly polarized cells such as neurons (**Figure 1**).

Here we focus on the dynamics of autophagy in neurons, where issues of cellular compartmentalization, long-distance trafficking, activity-dependent plasticity, and high levels of metabolic stress have led to cell type–specific adaptation of the autophagy pathway. For example, neuronal axons can extend up to 1 m from the soma, the principal site of protein biosynthesis and degradation. How proteins that take hours to days to arrive at cellular destinations such as synapses are

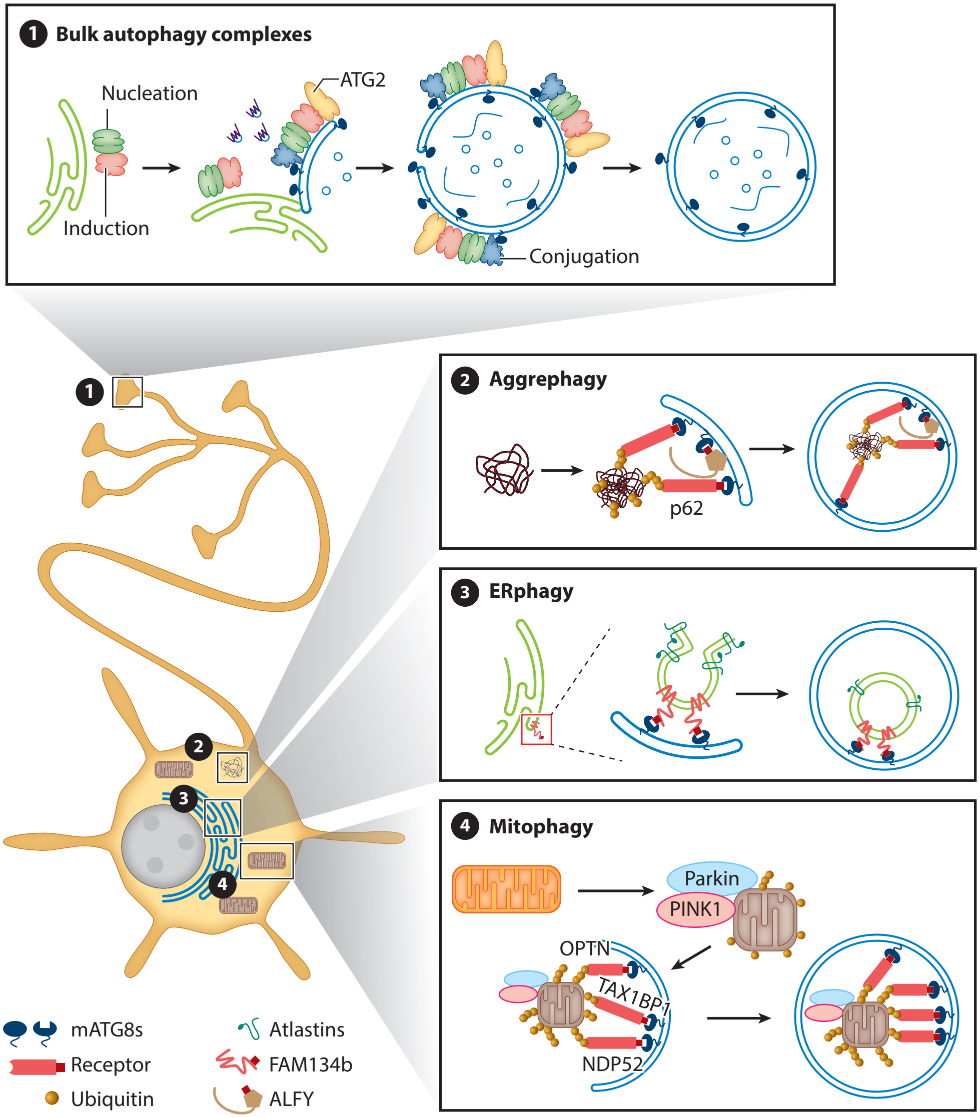

Figure 1

The spatial organization of autophagy in neurons. A cartoon neuron is depicted with the different forms of autophagy in spatially distinct neuronal compartments: ❶ nonselective autophagy in the distal axon and ❷ aggrephagy, ❸ ERphagy, and ❹ mitophagy in the soma. For the types of selective autophagy illustrated in panels ❶–❹, relevant LC3-interacting regions are depicted by dark red boxes on the autophagy receptors. In aggrephagy ❷, WDR81 may perform a function similar to that of ALFY. In ERphagy ❸, RTN3 acts in a similar manner as FAM134b.

effectively turned over is a topic of great interest. Another question is how autophagy contributes to synaptic plasticity, which is required for learning and memory. Significant interest is focused on the role of autophagy in neuronal homeostasis. Neurons often operate under conditions of high metabolic demand, induced by repetitive stimulation, for example. Such conditions can lead to oxidative stress and organelle damage, so damaged organelles such as mitochondria must be subject to vigilant quality control mechanisms, including targeted removal by selective autophagy. While many questions remain, the importance of autophagy in neurons is clear, as the neuron-specific ablation of autophagy leads to neurodegeneration (Hara et al. 2006, Komatsu et al. 2006).

CANONICAL PATHWAY FOR MACROAUTOPHAGY

Bulk and selective macroautophagy (hereafter autophagy) pathways share most of the molecular machinery required to execute these related processes. Autophagy begins with autophagosome biogenesis. In nonneuronal cells, autophagosome biogenesis is activated by cellular stressors such as starvation via the suppression of mTOR (mammalian target of rapamycin 1) kinase activity. In turn, suppression of mTOR removes repression of the induction complex to activate autophagosome biogenesis. Neurons are more dependent on constitutive autophagy than are other cell types and do not exhibit a robust starvation-induced upregulation of autophagy (Maday & Holzbaur 2016, Mizushima et al. 2004), so the specific role of mTOR in the regulation of autophagy in neurons requires further study.

The induction complex is composed of the kinase ULK1 (UNC-51-like kinase 1), along with ATG101, ATG13, and FIP200/RB1CC1 (FAK family kinase–interacting protein of 200 KDa/RB1 inducible coiled-coil 1). When activated, ULK1 phosphorylates other autophagy pathway components, including Beclin1 (BECN1) and ATG9 (Feng et al. 2014, Kamada et al. 2000, Russell et al. 2013, Zhou et al. 2017). Beclin1 is a component of the nucleation complex, along with ATG14, lipid kinase VPS34/PI3KIII, and VPS15/PIK3R4 (Itakura et al. 2008, Matsunaga et al. 2009, Sun et al. 2008, Zhong et al. 2009). The nucleation complex generates PI3P (phosphatidylinositol 3-phosphate), an important component of the autophagosome membrane during biogenesis (Kihara et al. 2001, Obara et al. 2006). Several components of the nucleation complex also function in cellular processes other than autophagy (Liang et al. 2008, Lindmo & Stenmark 2006, Matsunaga et al. 2009, Yan et al. 2009, Zhong et al. 2009). In contrast, ATG14 is specifically required for the proper localization of the nucleation complex to the forming autophagosome (Matsunaga et al. 2010); ATG14 also recruits other autophagosome biogenesis components to the initial autophagosome membrane, termed the isolation membrane in mammals.

Elongation of the autophagosome membrane is mediated by the aptly named elongation complex, composed of two ubiquitin-like conjugation complexes (Ichimura et al. 2000, Mizushima et al. 1998). In the first complex, E1-like ATG7 and E2-like ATG10 conjugate ATG12 to ATG5. In the second complex, ATG7 is again the E1-like activating enzyme, and ATG3 is the E2-like conjugating enzyme. The ATG12-ATG5 conjugate, along with ATG16L1, acts as the E3-like enzyme to conjugate mATG8s to phosphatidylethanolamine (PE) (Mizushima et al. 2001, Suzuki et al. 2001). While yeast has one Atg8 that is conjugated to PE, there are five or six Atg8 homologs in mammals (mATG8s): LC3A (microtubule associated protein 1 light chain 3 alpha), LC3B, GABARAP (GABA type A receptor-associated protein), GABARAPL1, GABARAPL2, and LC3C; LC3C is expressed in humans but likely not in mice (Liu et al. 2017). All mATG8s must be processed before conjugation to PE; ATG4 cleaves the C-terminal amino acids to expose glycine. ATG4 also acts at the end of autophagy to cleave mATG8s from the cytosolic face of the completed autophagosome (Kauffman et al. 2018, Tanida et al. 2004).

ATG9 is a six-pass transmembrane protein and the only transmembrane protein in the core machinery for autophagy (Lang et al. 2000, Noda et al. 2000, Young et al. 2006). Several accessory transmembrane proteins—such as VMP1 and TMEM41B, which primarily localize to the endoplasmic reticulum (ER)—have recently been discovered (Moretti et al. 2018, Morita et al. 2018). ATG9 is thought to shuttle between the membrane source and the growing autophagosome, possibly bringing lipids to the autophagosome (Longatti et al. 2012, Orsi et al. 2012, Popovic & Dikic 2014, Takahashi et al. 2011, Young et al. 2006). ATG9 interacts with ATG2

(Gómez-Sánchez et al. 2018, Velikkakath et al. 2012, Wang et al. 2001), a large protein with regions of homology to VPS13. VPS13 is a lipid transporter between the ER and other membranes, raising the possibility that ATG2 also functions as a lipid transporter that supplies lipids to the growing autophagosome membrane (Kumar et al. 2018). Yeast Atg2 also interacts with Atg18 (Obara et al. 2008, Watanabe et al. 2012), which has four homologs in mammals: WIPI1–WIPI4 (where WIPI denotes WD-repeat protein interacting with phosphoinositides). WIPI1 and WIPI2 are thought to act early in autophagosome biogenesis, linking the nucleation and elongation steps (Dooley et al. 2014, Lu et al. 2011). As WIPIs interact with membranes (Polson et al. 2010) and yeast Atg18 can oligomerize (Gopaldass et al. 2017, Scacioc et al. 2017), it is intriguing to hypothesize that WIPI assembly may bend the membrane of the growing autophagosome.

Since autophagy requires de novo membrane formation and elongation around cargo in the cytoplasm, the source of the lipids that make up the autophagosome membrane has been a pressing question. Virtually every possible membrane source, including the plasma membrane (Hollenbeck 1993, Ravikumar et al. 2010), mitochondria (Hailey et al. 2010), the Golgi complex (van der Vaart et al. 2010), the ER (Hamasaki et al. 2013, Hayashi-Nishino et al. 2009, Ylä-Anttila et al. 2009), and recycling endosomes (Puri et al. 2018), has been implicated in autophagy at some point. However, the ER has been repeatedly identified as crucial for autophagosome formation, both as a source of donor membrane and as a platform for initial biogenesis of the organelle at the omegasome. The importance of the ER has been confirmed in primary hippocampal neurons, in which autophagosomes colocalized with the ER marker Sec61β, but not with plasma membrane or mitochondrial markers (Maday & Holzbaur 2014). In neurons in vivo, ATG9 appears to colocalize and be transported with synaptic vesicles (Stavoe et al. 2016), but there is currently no evidence suggesting that synaptic vesicles directly donate lipids to autophagosomes.

In bulk, or nonselective, autophagy, cytoplasmic contents are nonspecifically engulfed within the isolation membrane during autophagosome formation. In contrast, during selective autophagy, specific cargos are recognized, and sometimes clustered, by receptors that directly link the cargo to the forming autophagosome membrane via mATG8s, discussed in more detail below. Once the contents of the autophagosome are engulfed, the isolation membrane must close by fusing with itself, yielding a double-layer membrane surrounding the contents. Around the time at which the autophagosome membrane fuses with itself, the biogenesis machinery dissociates from the fully formed autophagosome, with the exception of the mATG8s, which remain tightly associated with the limiting membrane (Mizushima et al. 2003). The protein machinery necessary for membrane fusion at the extremities of the isolation membrane is not yet known. However, the mATG8s have been implicated in this step (Tsuboyama et al. 2016).

After autophagosome closure, PI3P is removed from the outer membrane by PI3P phosphatases (Cebollero et al. 2012), and mATG8s are removed from the outer membrane by ATG4 (Kauffman et al. 2018, Nair et al. 2012, Yu et al. 2012), possibly destabilizing the association of other autophagy components with the cytosolic face of the autophagosome membrane. Autophagosomes then fuse with late endosomes or lysosomes. Following fusion to form an autophagolysosome, the internal pH decreases, activating lysosomal enzymes that digest the engulfed cargos for eventual recycling of components.

SELECTIVE AUTOPHAGY

Selective autophagy uses the same core components of the nonselective macroautophagy pathway but generates specialized autophagosomes that selectively engulf specific cargos (**Figure 1**). The specificity is mediated by receptors that connect the selected cargo to the autophagy machinery via the mATG8 proteins, stimulating autophagosome formation around the specified cargo to be degraded. p62/SQSTM1 (sequestome 1) was the first autophagy receptor to be identified (Bjørkøy et al. 2005, Pankiv et al. 2007); p62 interacts with ubiquitin chains through its UBAN (ubiquitin binding in ABIN and NEMO) domain and with mATG8s via its LIR (LC3-interacting region), also known as AIM (Atg8 family–interacting motif) (Pankiv et al. 2007). Other receptors involved in selective autophagy such as OPTN (Optineurin) are also characterized by the presence of both ubiquitin-binding and LIR motifs. These receptors can be either selective for a single cargo or promiscuous, implicated in the removal of multiple types of cargo such as damaged mitochondria or aggregated proteins. Furthermore, there is evidence of functional redundancy, as some receptors appear capable of substituting for other receptors within a specific type of selective autophagy (Rogov et al. 2014, Lazarou et al. 2015).

The best understood example of selective autophagy within the context of the neuron is mitophagy, the clearance of defective mitochondria. However, there is growing interest in parallel pathways mediating the selective turnover of the ER by ERphagy, protein aggregates by aggrephagy, RNA granules by granulophagy, lysosomes by lysophagy, peroxisomes by pexophagy, etc. Only a few of these pathways have been examined in detail in neurons, so here we focus on progress in the understanding of mitophagy, ERphagy, and aggrephagy.

Mitophagy

Interest in the mechanisms driving mitophagy was sparked by the discovery that two genes causal for familial forms of Parkinson's disease (PD), those encoding PINK1 (PTEN-induced kinase 1) and Parkin, are part of a conserved mitophagy pathway. Work has shown that PINK1 is a mitochondrially targeted kinase involved in the recruitment of the E3 ubiquitin ligase Parkin to impaired mitochondria (Narendra et al. 2008, 2010a). PINK1 is usually turned over very rapidly at the outer mitochondrial membrane (OMM), leading to a low steady-state level. Conditions such as mitochondrial depolarization or a block in the mitochondrial import machinery lead to the accumulation of PINK1 on the OMM (Narendra et al. 2010a). This accumulation allows PINK1 to phosphorylate Parkin, leading to Parkin recruitment and activation (Kondapalli et al. 2012, Shiba-Fukushima et al. 2012). PINK1 also phosphorylates ubiquitin, which further enhances Parkin activity (Kane et al. 2014). This feedforward mechanism leads to the rapid ubiquitination of OMM proteins by Parkin (Narendra et al. 2010b) that serve as a binding platform for ubiquitin-binding mitophagy receptors.

Mitophagy can be rapidly induced by either global or focal mitochondrial damage. Either mitochondrial uncouplers such as CCCP or the localized production of reactive oxygen species (ROS) can effectively induce mitophagy in cells expressing Parkin (Wong & Holzbaur 2014a). The ubiquitin-binding domains of mitophagy receptors such as OPTN, NBR1 (neighbor of BRCA1 gene 1), NDP52/CALCOCO2 (nuclear dot protein 52 kDa/calcium binding and coiled-coil 2), and TAX1BP1 (Tax1-binding protein 1) mediate their recruitment to damaged, ubiquitinated mitochondria (Lazarou et al. 2015, Moore & Holzbaur 2016). These receptors then bind LC3 via their LIR motifs to enhance the formation of LC3-positive autophagosomes that surround and engulf damaged mitochondria. Knockout studies in nonneuronal cells indicate that these receptors are partially redundant. Depletion of a single receptor such as OPTN may induce a delay in mitochondrial engulfment (Moore & Holzbaur 2016) but does not block mitophagy completely (Lazarou et al. 2015), indicating some redundancy in the system. One reason why there may be multiple mitophagy receptors is to allow for differential regulation. For example, TBK1 (Tank-binding kinase 1) activates and binds to OPTN (Richter et al. 2016, Wild et al. 2011); OPTN is not recruited to damaged mitochondria when TBK1 activity is inhibited, whereas NDP52 is not affected by TBK1 inhibition (Moore & Holzbaur 2016). Of note, p62 has both a UBAN domain and an LIR motif but does not promote autophagosome formation around damaged mitochondria; rather, p62 appears to cluster damaged mitochondria (Lazarou et al. 2015, Narendra et al. 2010b, Wong & Holzbaur 2014a).

The observation that OPTN and TBK1 are involved in mitophagy downstream from PINK1 and Parkin is particularly interesting because mutations in both OPTN and TBK1 are causative for rare forms of familial amyotrophic lateral sclerosis (ALS) (Cirulli et al. 2015, Freischmidt et al. 2015, Maruyama et al. 2010). Thus, proteins involved in mitophagy are implicated in two major neurodegenerative diseases, both PD and ALS, making it particularly important to understand how this pathway contributes to neuronal homeostasis and neurodegeneration.

In mice, knockout of either PINK1 or Parkin is not lethal. Knockout mice exhibit relatively subtle phenotypes, including altered synaptic excitability and decreased dopamine release, which do not clearly model human PD (Goldberg et al. 2003, Kitada et al. 2007, Perez & Palmiter 2005). Analysis of mitochondrial turnover in PINK1 knockout mice suggests that there are additional, PINK1-independent pathways leading to mitophagy in neurons under basal conditions (McWilliams et al. 2018); such pathways remain to be more fully characterized. For example, mitochondrial ubiquitin ligase 1 (MUL1, or Mulan) has been proposed to function in a pathway parallel to Parkin in the clearance of damaged mitochondria (Yun et al. 2014). One possibility is that MUL1 or another ubiquitin ligase pathway may compensate for loss of PINK1/Parkin activity in some contexts such as neurodevelopment, but not in others such as aging dopaminergic neurons. The importance of more fully understanding mitochondrial quality control is emphasized by the observation that both PINK1 and Parkin knockout mice are susceptible to stress, which in turn leads to inflammation (Sliter et al. 2018). Further work exploring the links between mitophagy and inflammation, and the possible contribution of this cross talk to neurodegenerative disease in humans, is required.

ERphagy

The ER forms an extensive and dynamic network of sheets, tubules, and cisternae that extends throughout the cell. Three-dimensional reconstructions of the ER in mouse brain show the dramatic extension of ER tubules throughout the soma, dendrites, and axons of neurons in vivo (Wu et al. 2017). The ER must be remodeled and renewed in neurons, especially under conditions of stress. One mechanism for turnover is ERphagy (also referred to as reticulophagy), the selective removal of ER segments by autophagy (Grumati et al. 2018).

Recent progress has identified multiple ER-associated proteins with LIR motifs that mediate binding to LC3 family proteins, including the ERphagy receptor FAM134B/RETREG1 (reticulophagy regulator 1), reticulon 3L (RTN3), and CCPG1 (cell cycle progression 1) (Fumagalli et al. 2016, Grumati et al. 2018, Khaminets et al. 2015, Smith et al. 2018). FAM134B and RTN3 are proposed to function in the basal remodeling of ER membranes by autophagy, while CCPG1 is induced by ER stress (Grumati et al. 2018, Smith et al. 2018). As resident ER transmembrane proteins, Atlastins are proposed to remodel the ER to sequester FAM134B-marked membrane for delivery to the autophagosome (Liang et al. 2018). Future studies are likely to further implicate the dynamics of ER remodeling and ERphagy in the maintenance of neuronal homeostasis and to continue to link defects in this pathway to neurodegenerative disease.

Aggrephagy

Misfolded or damaged proteins can be cleared by the ubiquitin-proteasome system, but once misfolded proteins aggregate, these accumulations are cleared by autophagy. The selective removal of aggregated proteins is termed aggrephagy and is mediated in neurons by several receptors that may work either independently or in combination.

The best characterized autophagy receptor known to function in aggrephagy is p62/SQSTM1 (Bjørkøy et al. 2005, Komatsu et al. 2007a, Pankiv et al. 2007). Like other receptors involved in selective autophagy, p62 is recruited to cargo via its ubiquitin-binding domain and recruits autophagy machinery through its LIR motif. In vitro studies indicate that p62 is sufficient to induce the clustering of ubiquitinated proteins, known as cargo nucleation, into aggregates that can be targeted by autophagy (Zaffagnini et al. 2018). In cells, p62 also induces cargo clustering (Komatsu et al. 2007a) but interacts with other proteins to facilitate aggrephagy. Supporting the importance of p62 in neurons, mutations in the gene encoding p62/SQSTM1 are implicated in ALS/FTD (frontotemporal dementia) and ataxia (Fecto et al. 2011, Haack et al. 2016).

ALFY [autophagy-linked FYVE protein, also known as WDFY3 (WD repeat and FYVE domain–containing protein)] is a large scaffolding molecule that binds to p62; the mATG8 proteins LC3C and the GABARAPs; and ATG5, a component of the core autophagy machinery (Lystad et al. 2014). In vivo studies support a role for ALFY in aggrephagy, as the *Drosophila* mutant blue cheese demonstrates accumulation of ubiquitin-positive inclusions, neurodegeneration, and shortened life span (Finley et al. 2003). In mice, deletion of the gene encoding Alfy did not lead to baseline defects in autophagy, but an exogenous aggregation-prone substrate accumulated more rapidly in Alfy knockout mouse embryonic fibroblasts (Dragich et al. 2016).

Another similar protein that can function along with p62 in aggrephagy is WDR81 (WD repeat domain 81), a BEACH (beige and Chediak-Higashi) and WD40 repeat protein. WDR81 can bind directly to LC3, interacts with p62, and has been proposed to facilitate the ability of p62 to bind to aggregated and ubiquitinated proteins (Liu et al. 2017). In cells, depletion of WDR81 leads to accumulation of both p62 and ubiquitinated proteins. p62 appears to sit at a crucial juncture between the ubiquitin proteasome and selective autophagy by detecting the buildup of ubiquitinated substrates (Danieli & Martens 2018), suggesting a key role in the regulation of protein turnover.

SPATIAL ORGANIZATION OF AUTOPHAGY IN NEURONS

Compartmentalization of Neurons

Neurons are highly specialized cells that are polarized, long lived, very metabolically active, and postmitotic. Elucidated by Santiago Ramón y Cajal by using Golgi stain in the nineteenth century, neuronal structure is generally divided into three compartments: the soma, the axon, and the dendrites. Axons can grow from a few micrometers up to many feet to reach their targets, while dendrites are typically much shorter but can form elaborate, highly branched networks. Neurons perform the tasks of processing and transmitting information, placing high metabolic demands on these cells. Furthermore, these demands often occur at distal sites in axons and dendrites, far removed from the cell body. Thus, the most vulnerable regions of neurons bear the brunt of the stress.

In addition, neurons are postmitotic cells, with no ability to dilute damaged or accumulated proteins and organelles by cell division, a strategy commonly employed by mitotic cells. Furthermore, neurons typically terminally differentiate very early during development, usually during embryogenesis, and must survive the lifetime of the organism. While there is some ability to regenerate axons and replace neurons, depending on species, age, neuronal type, and other factors, most organisms do not appear capable of replacing every neuron throughout the organism's lifetime. Therefore, neurons need to robustly manage the stresses placed on remote compartments throughout the lifetime of the organism by removing aggregated and/or damaged proteins and organelles. Autophagy can, and does, perform this function in each neuronal compartment.

Axonal Autophagy

Landmark work in cultured embryonic peripheral neurons determined that large (∼1-µm) acidified vesicles were transported retrogradely in axons toward the soma, suggesting that autophagosomes formed in the distal axon (Hollenbeck 1993). This work was consistent with electron microscopy studies of primary rat sympathetic motor neurons identifying autophagic vesicles in distal growth cones (Bunge 1973). Subsequently, several labs used a variety of neuronal culture paradigms, including mouse and rat dorsal root ganglion (DRG) neurons and mouse embryonic cortical and hippocampal neurons expressing the autophagosome marker GFP-LC3 (Mizushima et al. 2004), to observe autophagosome biogenesis and dynamics in axons. These studies consistently observed large, LC3-positive organelles forming constitutively at the axonal tip. These autophagosomes were then rapidly transported back to the cell body (Cheng et al. 2015, Lee et al. 2011, Maday & Holzbaur 2014, Maday et al. 2012) (**Figure 1**).

Furthermore, these findings have been corroborated in vivo in model organisms. In *Caenorhabditis elegans*, autophagosome biogenesis was observed at synaptic sites in the distal axon of interneuron AIY at a consistent rate (Stavoe et al. 2016). In *Drosophila*, autophagosome biogenesis was observed at the neuromuscular junction (NMJ) in the distal axon of motor neurons (Neisch et al. 2017, Soukup et al. 2016). While autophagosome biogenesis was observed consistently at the NMJ, newly formed autophagosomes were only rarely detected in the soma (Soukup et al. 2016). At the *Drosophila* NMJ presynapse, autophagy is dependent on LRRK2, a protein implicated in PD, and EndophilinA, which recruits Atg3 to membranes (Soukup et al. 2016, Vanhauwaert et al. 2017). Similarly, in the zebrafish photoreceptor, synaptojanin, a lipid phosphatase and EndophilinA interactor, is necessary for autophagy (George et al. 2016).

The stepwise autophagosome biogenesis pathway elucidated in yeast and mammalian cell culture (Itakura & Mizushima 2010, Koyama-Honda et al. 2013) appears to be consistent with the pathway for biogenesis in the distal axon (Maday & Holzbaur 2014). One significant difference is that axonal autophagosomes form constitutively under fed conditions (Cheng et al. 2015; Lee et al. 2011; Maday & Holzbaur 2014, 2016; Maday et al. 2012; Stavoe et al. 2016), in contrast to the induction of autophagy under nutrient-limiting conditions observed in many nonneuronal cell types.

One intriguing aspect of axonal autophagy is the temporal regularity of biogenesis in the distal axon. While the rates of autophagosome biogenesis vary with species and neuronal type, rates of formation are highly consistent across individual neurons within a type, even from different individual animals (Maday & Holzbaur 2014, Maday et al. 2012, Stavoe et al. 2016). Another striking aspect of this pathway is its spatial specificity, with the majority of autophagosome biogenesis events, as labeled with (*a*) either ATG13 or ATG5 and (*b*) LC3B, occurring in the distal axon. Autophagosome formation is only rarely observed in the dendrites or soma in primary mouse adult DRG or embryonic hippocampal neurons under resting conditions (Maday & Holzbaur 2014).

Active zone proteins Bassoon and Piccolo may contribute to modulation of presynaptic autophagy. In primary hippocampal embryonic rat neurons, knockdown of Bassoon caused an accumulation of LC3-positive structures at presynaptic boutons. Bassoon negatively regulated autophagy by interacting, sequestering, and preventing ATG5 from participating in autophagosome biogenesis (Okerlund et al. 2017). Engulfed cargos include mitochondrial fragments as well as cytosolic proteins and protein aggregates, indicating that uptake is via nonselective autophagy (Maday et al. 2012, Wong & Holzbaur 2014b).

Once formed, axonal autophagosomes undergo bidirectional movement along microtubules in the distal axon; such movement is driven by kinesin and dynein motors that localize to neuronal autophagosomes (Maday et al. 2012). Dynein is recruited to autophagosomes by RAB7 and RILP

(Cheng et al. 2015, Jordens et al. 2001, Wijdeven et al. 2016). However, the mechanisms regulating kinesin recruitment have yet to be identified. After an initial phase of bidirectional movement, autophagosomes transition to highly processive retrograde transport toward the cell body; this transport is driven by dynein and is regulated by the scaffolding proteins JIP1 and huntingtin in concert with HAP1 (huntingtin-associated protein 1) (Fu et al. 2014, Wong & Holzbaur 2014b). This robust retrograde trafficking of autophagosomes seen in neurons in culture has been confirmed in vivo in *C. elegans* (Hill et al. 2019, Stavoe et al. 2016) and *Drosophila* (Neisch et al. 2017).

After autophagosomes form and as they transit through the axon toward the soma, they encounter and fuse with late endosomes and lysosomes, leading to increasingly acidic and eventually degradation-competent autolysosomes (Maday et al. 2012). While there are lysosomes present in the axon, they lack the full complement of degradative enzymes found in somal lysosomes (Cheng et al. 2018, Gowrishankar et al. 2015). As an autophagosome is transported retrogradely along the axon, it likely fuses with additional, and more degradatively competent, lysosomes. In support of this possibility, blocking the retrograde trafficking of autophagosomes is sufficient to block their acidification and their degradation of engulfed cargos (Fu et al. 2014, Wong & Holzbaur 2014b).

Dendritic Autophagy

The GABARAP subfamily of mATG8s, as its name suggests, was initially identified to interact with $GABA_A$ receptors by yeast-two-hybrid screening (Wang et al. 1999). GABARAP was then subsequently found to be important for $GABA_A$ receptor intracellular trafficking (Leil 2004), but the cellular mechanism of GABARAP regulation of $GABA_A$ receptor trafficking remains unclear. In PC12 cells, primary hippocampal neurons, and *Xenopus* oocytes, GABARAP lipidation was required for increased surface expression of $GABA_A$ receptors (Chen et al. 2007). In contrast, in *C. elegans*, $GABA_A$ receptors colocalized with autophagosome markers, and autophagy reduced $GABA_A$ receptor surface expression in noninnervated muscle. In contrast, acetylcholine (ACh) receptor subunits did not colocalize with autophagosome markers, and disruption of autophagy did not increase ACh currents. These data indicate that autophagy selectively regulates surface expression of $GABA_A$ receptors, suggesting that autophagy could modulate neuronal excitation and inhibition (Rowland 2006).

Autophagy has also been implicated in AMPA receptor degradation in primary rat embryonic hippocampal neurons. Upon stimulation with low-dose NMDA, dendritic spines displayed an increase in GFP-LC3 puncta. When treated with bafilomycin A to inhibit lysosomal acidification, LC3-positive puncta were also detectable in the dendritic shaft with or without stimulation by NMDA (Shehata et al. 2012).

Autophagy can also modulate dendritic branching. In *Drosophila*, knockdown of autophagy genes reduced dendritic arbor growth and terminal branching in multidendritic sensory neurons in vivo (Clark et al. 2018). Surprisingly, overexpression of Atg1 also decreased dendritic arbor growth and terminal branching in the same neurons. These results suggest that constitutive autophagy must be tightly regulated during dendrite outgrowth and branching. Autophagy may regulate dendritic branching by facilitating organelle or membrane turnover during branch retraction and extension (Clark et al. 2018). Additionally, autophagy may modulate dendritic branching by decreasing levels of Highwire (Shen & Ganetzky 2009), which promotes dendritic arborization. Thus, the tight regulation of autophagy may be required in dendrites to balance dendritic outgrowth and branching (Clark et al. 2018).

Additionally, autophagy can regulate dendritic degeneration. Specifically deleting Atg7 in dopaminergic neurons by using the tyrosine hydroxylase (TH) promoter in conditional knockout mice resulted in dopaminergic dendrites with many large swellings and dendritic dystrophy in TH^+ neurons. These swellings contained ubiquitinated filamentous inclusions that were also observed in cell bodies (Friedman et al. 2012). When Atg7 was specifically deleted from forebrain excitatory neurons in vivo, dendritic spines failed to undergo pruning, leading to social behavioral defects. Similar results were observed in primary hippocampal neurons subjected to Atg7 shRNA, with fewer dendritic spines being eliminated relative to control neurons. These results indicate that basal autophagy is necessary for postnatal spine pruning. To promote synaptic pruning, autophagy may sequester postsynaptic neurotransmitter receptors, or autophagy may eliminate signals that block spine elimination (Tang et al. 2014).

Somal Autophagy

Little is known about bulk autophagy in the neuronal cell body. In mouse primary DRG neurons, autophagosome biogenesis primarily occurs in the distal axon, with very few autophagosome formation events, as marked by ATG13 or ATG5, observed in the soma (Maday & Holzbaur 2014). In mouse primary hippocampal neurons, basal autophagosome biogenesis was observed in the cell body, but autophagosomes generated in the soma were characteristically distinct from axonally generated autophagosomes; soma-derived autophagosomes were less dynamic and mature (Maday & Holzbaur 2016).

There is some debate about whether neuronal mitophagy occurs in the axon or in the soma. In vitro experiments with primary neurons have shown that mitochondrial fragments are engulfed within autophagosomes formed constitutively in the distal axon; however, there is limited evidence that the engulfment of these fragments involves the machinery for selective mitophagy such as PINK1, Parkin, and OPTN. Studies looking more specifically at the mitophagy pathway provide conflicting views. One group reported localized mitophagy along the axons of hippocampal neurons upon induction of cellular stress via mitochondrial depolarization or ROS generation (Ashrafi et al. 2014). In contrast, mitochondrial depolarization in cortical neurons resulted in Parkin-labeled mitochondria accumulation in somatodendritic regions that were subsequently degraded locally by the autophagosomal-lysosomal pathway. Conversely, GFP-LC3 was rarely observed to be associated with axonal mitochondria in these experiments (Cai et al. 2012).

In vivo studies provide more convincing evidence that mitophagy occurs predominately in the cell body (**Figure 1**). Detailed characterization of neurons from PINK1 or Parkin mutant flies indicates that loss of either protein decreases the flux of mitochondria along the axon but does not lead to the accumulation of senescent mitochondria either in the axons of motor neurons or at NMJs. Instead, there is a loss of integrity of somal mitochondria, indicating that active turnover of mitochondria by PINK1/Parkin-dependent mitophagy may be part of a mitochondrial quality control mechanism that is specific to the soma (Devireddy et al. 2015, Sung et al. 2016). Furthermore, initial observations from a mouse model expressing a mitophagy reporter (mito-QC) also support a soma-specific degradation pathway (McWilliams et al. 2016). This pathway may become more important in the maintenance of neuronal function through aging (Cornelissen et al. 2018).

An intriguing possibility is that neurons deploy alternative mechanisms to maintain mitochondrial health in the distal axon. Compared to mitochondria in cell lines, neuronal mitochondria are more resistant to initiation of mitophagy. Independently of Parkin, DRP1 (dynamin-related protein 1), and autophagy, mitochondrial fragments from stressed mitochondria are transported out of the axon in primary rodent neurons. Additionally, the mitochondrial anchoring protein syntaphilin is released from axonal mitochondria upon stress, allowing for their retrograde transport out of the axon (Lin et al. 2017). Thus, damaged axonal mitochondria may undergo retrograde transport to be degraded by mitophagy in the soma.

TEMPORAL ORGANIZATION OF AUTOPHAGY IN NEURONS

Autophagy During Neurodevelopment

Development of a functional nervous system requires neuronal differentiation, neurite outgrowth, neurite guidance, and formation of synaptic connections. Nonneuronal support cells such as astrocytes, microglia, and oligodendrocytes are also required for proper nervous system function and maintenance. All these cells and processes must be coordinated so that each neuron connects with the appropriate partners in the correct order. Autophagy is an intriguing candidate for modulating these neurodevelopmental steps.

Autophagy can act during neurodevelopment in neuronal precursors. Conditionally knocking out mTOR in GABAergic precursors increased autophagy in those cells and suppressed their proliferation, leading to a reduction in cortical interneurons (Ka et al. 2017). Thus, autophagy can modulate the first step in neuronal development, differentiation, or generation.

Autophagy also regulates axon outgrowth. In primary embryonic cortical neurons, depletion of Atg7 led to an increase in neurite length. Conversely, activation of autophagy with rapamycin treatment resulted in a decrease in neurite length (Ban et al. 2013). In SH-SY5Y cells, an in vitro cell line used to model neurons, induction of autophagy by tri-*ortho*-cresyl phosphate also inhibited neurite outgrowth (Chen et al. 2013). In the converse experiment in chick embryonic DRG neurons, when axon outgrowth was blocked with cytochalasin E, retrograde transport of axonal autophagosomes increased threefold (Hollenbeck & Bray 1987). These observations were confirmed in vivo in *C. elegans*; mutants for autophagy components displayed longer axons in the nociceptive sensory neuron PVD. Of note, however, many of the neuronal types examined did not display axon outgrowth phenotypes in the autophagy mutants. Thus, autophagy may regulate different stages of neurodevelopment in different neurons or may not affect the neurodevelopment of certain neurons at all (Stavoe et al. 2016). These subtle differences between neuron types may become apparent only in the context of intact nervous systems in vivo, in which neurons receive and respond to numerous cellular cues.

Selective autophagy has also been implicated in axon outgrowth. In mice, deletion of the gene encoding Alfy leads to pronounced defects in axon outgrowth due to a failure to respond correctly to axon guidance cues (Dragich et al. 2016).

Finally, autophagy can also modulate synaptic formation. In a *C. elegans* interneuron, autophagy is required for synaptic vesicle clustering and active zone formation early during synaptogenesis. Autophagy component mutants displayed defects in active zone assembly and synaptic vesicle clustering in early larval stages, indicating that the observed defects were the result of improper synaptic formation instead of degeneration (Stavoe et al. 2016). Furthermore, mutations in selective autophagy genes did not phenocopy the bulk autophagy mutants (Stavoe et al. 2016), consistent with data indicating that nonselective autophagy occurs predominately in the axon. Similar results were observed in *Drosophila*. In *atg1* (ULK1 in mammals) mutant animals, the NMJ displayed reductions in both the total NMJ area and the number of synapses. As in worms, these defects in autophagy mutants were due to incorrect synaptic formation, not precocious degeneration or synaptic retraction (Wairkar et al. 2009). Similar NMJ undergrowth was observed in *atg2* and *atg18* (WIPIs in mammals) mutants. Additionally, overexpression of Atg1 increased NMJ synaptic bouton number in an autophagy-dependent manner (Shen & Ganetzky 2009). Taken together, these studies indicate that autophagy is an important regulator during neurodevelopment; autophagy negatively regulates axon outgrowth but positively regulates synaptic development. It is compelling to hypothesize that, at least for en passant synapses, these two processes may be related, with restrained axon outgrowth allowing for the proper deposition of synapses during neurodevelopment.

Autophagy in Neuronal Homeostasis

Autophagy regulates cellular homeostasis in nonneuronal cells, especially in response to cellular stressors. Autophagy also maintains neuronal homeostasis. Much of the evidence to support this derives from studies reducing one or more autophagy genes in neurons and observing protein accumulation or neurodegeneration. Conditional knockout of Atg5 in mouse neural precursor cells led to partial loss of Purkinje cells and cerebral cortical pyramidal cells and axonal swelling in numerous brain regions, including the cerebral cortex, hippocampus, and nucleus gracilis. Neurons in a variety of brain regions, such as the cerebral cortex, hippocampus, striatum, and DRG neurons, also exhibited accumulations of ubiquitin-positive inclusion bodies (Hara et al. 2006). Similarly, knockdown of Atg5 in M17 human neuroblastoma cells resulted in increased aggregations of α-synuclein oligomers (Yu et al. 2009).

Conditional knockout of Atg7 in mouse neural precursors by using the Nestin promoter resulted in neuronal death and accumulation of ubiquitin-positive structures and inclusion bodies in neurons in a variety of brain regions, including the cerebral cortex, Purkinje cells, and hippocampal pyramidal neurons (Komatsu et al. 2006). Likewise, conditionally knocking out Atg7 in Purkinje cells by using the Pcp2 promoter in mice did not appear to affect dendrites but did lead to neuronal and axonal dystrophy, degeneration of axon terminals, and eventual neuronal death (Komatsu et al. 2007b). Additionally, conditional knockout of Atg7 in dopaminergic neurons using the DAT, EN, or TH promoters produced neuron loss, loss of striatal dopamine, accumulation of ubiquitin- and p62-containing inclusions, and accumulation of α-synuclein in vivo (Ahmed et al. 2012, Sato et al. 2018).

Neuron-specific knockout of FIP200 in mice by using the Nestin promoter triggered neuronal loss of Purkinje cells and granular cells, spongiosis in the cerebellar white matter, axonal and dendritic degeneration in Purkinje cells, axonal swelling, and accumulation of ubiquitin-positive aggregates (Liang et al. 2010). In complementary experiments, knockdown of FIP200 in Neuro-2a neuroblastoma cells led to neurite atrophy and apoptosis (Chano et al. 2007).

In transgenic mice overexpressing amyloid precursor protein and heterozygous for a Beclin1 deletion, synapses and dendrites degenerated in the neocortex and hippocampus, and layer II neurons of the entorhinal cortex were lost (Pickford et al. 2008). Analogously, knockdown of Beclin1 in M17 human neuroblastoma cells resulted in increased aggregations of α-synuclein oligomers (Yu et al. 2009). Furthermore, lentiviral expression of Beclin1 in the temporal cortex and hippocampus of α-synuclein transgenic mice reduced intraneuronal α-synuclein accumulation (Spencer et al. 2009). These experiments implicate Beclin1 in neuronal homeostasis, especially in the context of Alzheimer's disease (AD) and PD.

FIP200, Beclin1, ATG5, and ATG7 act early in autophagosome biogenesis; deletion of any of these genes results in failure to form autophagosomes. Thus, all of the studies discussed so far examined the effects of loss of autophagosome formation on neurons. However, inhibiting the later stages of autophagy would lead to the initiation of autophagy but would disrupt the complete sequestration and degradation of cargo. Knockout of Epg5, which acts downstream from known Atg genes (Lu et al. 2011, Tian et al. 2010), resulted in p62 aggregations and ubiquitin-positive inclusions in brain and spinal cord extracts, a reduction of pyramidal and motor neurons, axonal degeneration, and the accumulation of TDP43 (TAR DNA-binding protein 43) aggregates in neurons (Zhao et al. 2013).

Selective autophagy has also been implicated in neuronal homeostasis. Genetic studies indicate that FAM134B is required to maintain neuronal homeostasis, as mutations cause a sensory and autonomic neuropathy known as HSAN-II (Kurth et al. 2009). Similarly, $Fam134b^{-/-}$ mice exhibit sensory neuropathy and an age-dependent loss of sensory axons, with impaired ER turnover apparent at the cellular level (Khaminets et al. 2015). Furthermore, a loss-of-function mutation in the gene encoding the Atlastin ATL1, an ER-associated protein that functions downstream from FAM134B (Liang et al. 2018), causes SPG3A, a hereditary spastic paraplegia (Namekawa et al. 2007).

Aggrephagy also plays a role in neuronal homeostasis. Conditional knockout of the aggrephagy receptor WDR81 by using the Nestin promoter led to the accumulation of p62 foci in cortical and striatal neurons (Liu et al. 2017). In humans, a missense mutation in WDR81 is associated with the rare neurodevelopmental condition known as CAMRQ (cerebellar ataxia, mental retardation, and disequilibrium syndrome) (Gulsuner et al. 2011). Similarly, mice carrying a homozygous missense mutation in WDR81 exhibited Purkinje cell and photoreceptor cell death (Traka et al. 2013). These observations support a key role for WDR81 and other p62-interacting proteins (for example, see Haidar et al. 2019) in the maintenance of neuronal homeostasis, likely due to its role in aggrephagy within neurons. Together, these studies indicate the importance of the autophagy pathway in neuronal homeostasis, as loss of a variety of autophagosome biogenesis components results in aggregated proteins, neurite degeneration, and neuron loss.

Autophagy in Neuronal Activity, Plasticity, and Memory

Given the established role of autophagy in presynaptic compartments, an obvious experimental path was to determine the relationship between autophagy and synaptic activity. Upon NMDA stimulation of mouse cerebral microexplant cultures, the number of axonal GFP-LC3-positive puncta increased 2.5-fold. Although the number of autophagosomes increased in the axon, NMDA treatment did not appear to affect retrograde transport of autophagosomes (Katsumata et al. 2010). Similarly, in rat primary hippocampal neurons, stimulation with KCl or NMDA induced axonal autophagy (Shehata et al. 2012, Wang et al. 2015). Stimulation with KCl or chemical long-term potentiation also induced a higher LC3-II–to–LC3-I ratio, a common measure of autophagic flux, in primary embryonic rat hippocampal neurons (Glatigny et al. 2019).

Furthermore, inactivation of ATG7 selectively in dopamine neurons in mice enhanced evoked dopamine secretion and accelerated recovery. Similarly, autophagy induction by rapamycin treatment decreased synaptic vesicles in dopaminergic neurons in wild-type mice, but not in ATG7-defective mice. Thus, these data suggest that autophagy restricts synaptic transmission in dopamine neurons (Hernandez et al. 2012).

Autophagy also influences memory. Mice that had hippocampal injections of AAV with *Fip200*, *Beclin1*, or *Atg12* shRNA displayed defects in hippocampus-associated memory performances, including novel object recognition and context-elicited freezing. Inducing autophagy in the hippocampus during training, but not 12 h after training, enhanced performance in both memory paradigms. Conversely, acute pharmacological inhibition of autophagy during training significantly decreased memory performances in both tests. Consistent with these findings, inhibiting autophagy in primary rat hippocampal neurons or in vivo in the hippocampal dentate gyrus prevented an activity-dependent increase in dendritic spines (Glatigny et al. 2019).

Autophagy in Aging

Whether protein levels of autophagy pathway components change with age is debated. In mouse hippocampus, VPS34, Beclin1, and ATG5 protein levels decreased between 3 and 16 months of age. The LC3-II–to–LC3-I ratio also decreased with age (Glatigny et al. 2019). Similarly, in human brain, mRNA levels of Atg5, Atg7, Beclin1, and GABARAPL2 decreased with age; however, correlated protein level decreases were not assessed (Lipinski et al. 2010, Shibata et al. 2006). In *Drosophila* heads, dAtg8, dAtg2, and dAtg18 mRNA decreased with age (Simonsen et al. 2008).

Conversely, transcriptome and proteome analysis in rat brain did not detect significant changes in autophagy pathway components between 6 and 24 months (Ori et al. 2015).

Autophagosome biogenesis rates could change with age in neurons, despite levels of autophagy proteins remaining constant with age. Posttranslational modifications such as phosphorylation play important roles in the autophagy pathway. Altered levels of protein modification could alter the kinetics of autophagosome biogenesis independently of expression levels. Subcellular localization, described above as being especially important in neurons, could also affect rates of autophagosome biogenesis with age. In *C. elegans*, autophagic activity generally decreases with age. However, age appears to affect neuronal autophagy more variably than in other tissues (Chang et al. 2017).

Modulating autophagy in neurons can impact longevity and life span. In *Drosophila*, Atg8a mutants exhibited decreased life spans and increased ubiquitinated aggregates in neurons. Conversely, overexpression of dAtg8 in the CNS by using the APPL-Gal4 driver prevented accumulation of protein aggregates and dramatically extended life span. However, driving dAtg8 overexpression panneuronally by using earlier-expressing ELAV-Gal4 did not extend life span, suggesting that the age at which autophagy is ectopically induced is important (Simonsen et al. 2008). In *C. elegans*, inhibiting components of the initiation and nucleation complexes in neurons after reproduction ended extended life span, while inhibiting initiation or nucleation in prereproductive animals decreased life span. These data suggest that preventing the accumulation of partially formed autophagosomes is beneficial in postreproductive animals (Wilhelm et al. 2017).

AUTOPHAGY IN DISEASE

The major neurodegenerative diseases associated with aging, including AD, PD, Huntington's disease (HD), ALS, and FTD, are characterized by the accumulation of aggregated proteins and damaged mitochondria (Chu 2018). Observations from both experimental models and postmortem analysis of human tissues suggest that dysfunction of autophagy may be a common contributor to the pathogenic process across these diseases (recently reviewed by Boland et al. 2018, Chu 2018). This hypothesis is further supported by the observation that mutations in the genes encoding many of the proteins involved in autophagy—including PINK1, Parkin, OPTN, TBK1, ATL1, SQSTM1, and WDR81, as discussed above, as well as ATG5, AP4, HTT, WIPI4, and DYNC1H1—are sufficient to cause neurodevelopmental or neurodegenerative disease (reviewed in Zhu et al. 2019).

However, there is no current consensus on what step or steps might be disrupted in the major neurodegenerative diseases such as AD, PD, and ALS. Studies to date have demonstrated aging or disease-associated deficits at multiple points in the pathway, including autophagosome biogenesis (De Pace et al. 2018, Rui et al. 2015, Stavoe et al. 2019), cargo loading (Martinez-Vicente et al. 2010, Rudnick et al. 2017), intracellular transport (Nicolas et al. 2018, Wong & Holzbaur 2014b), and autophagosome-lysosome fusion or acidification (Lie & Nixon 2018, Nixon et al. 2005), across disease models. Deficits in intersecting pathways such as the ubiquitin-proteasome pathway and lysosomal degradation via chaperone-mediated autophagy are also likely to contribute to neurodegenerative disease (Scrivo et al. 2018). Furthermore, it remains to be determined whether defects in neuronal autophagy are central or whether deficits in autophagy or related degradative pathways in support cells such as glia also contribute to age-related neurodegeneration.

It will be essential to fill in these gaps to develop effective therapeutic strategies. For example, nonselective axonal autophagy is differently regulated than the selective removal of damaged mitochondria from the soma (Cornelissen et al. 2018, Devireddy et al. 2015, McWilliams et al. 2016, Sung et al. 2016). It is also unclear whether autophagy in neurons responds to the same activators, such as starvation or inhibition of mTOR, that induce autophagy in other tissues (Maday & Holzbaur 2016). However, the growing prevalence of neurodegeneration in our aging populations means that we must actively screen for therapeutic approaches now. Boland et al. (2018) provide an extensive discussion of pharmacotherapies that have been tested or are currently being considered for the modulation of intracellular degradation pathways in the context of neurodegeneration.

FUTURE DIRECTIONS

Genetic, cellular, and in vivo studies highlight the key role for autophagy in maintaining cellular homeostasis in the neuron and the importance of autophagy in neurodevelopment and neuronal function. While there has been considerable progress in the past few years, key questions remain. First, while the pathway for canonical autophagy is well understood, we need to define the molecular components and cellular dynamics involved in more specific pathways, such as selective autophagy. For example, how is ER health maintained along the extended axon? Is the ER membrane turned over continually, or only when damage is sensed? With regard to mitophagy, we now better understand the outlines of PINK1/Parkin-mediated mitochondrial clearance, but there is good evidence for the participation of other parallel pathways in maintaining mitochondrial quality control, and these need to be explored in more detail.

Next, we are only beginning to understand the effects of neuronal activity on autophagy and how autophagy may contribute to neuronal plasticity such as learning and memory. Does neuronal autophagy change during aging? Does neuronal autophagy affect our ability to learn or maintain memories, or does it have a more direct effect on neuronal homeostasis? If so, how might a decline in autophagy contribute to neurodegeneration? Defects in autophagy have been implicated in all the major neurodegenerative diseases, including ALS, AD, PD, and HD, so further research on the underlying mechanisms is clearly necessary.

And finally, can autophagy and other degradative pathways in neurons be modulated therapeutically? There is evidence that neuronal autophagy is not regulated by the same pathways that regulate stress-induced autophagy in other tissues, so we need to identify the regulatory pathways involved and determine how they can be manipulated in vivo. The availability of small-molecule effectors will allow us to determine whether autophagy can be tuned to make neurons more resilient to the cellular stressors of aging, environmental toxins, and genetic risk factors, offering some hope for the future.

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