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Review

Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities

Fiona M. Menzies,[1,3,4] Angeleen Fleming,[1,3] Andrea Caricasole,[2,3] Carla F. Bento,[1,3] Stephen P. Andrews,[2,3] Avraham Ashkenazi,[1,3] Jens Füllgrabe,[1,3] Anne Jackson,[1,3] Maria Jimenez Sanchez,[1,3] Cansu Karabiyik,[1,3] Floriana Licitra,[1,3] Ana Lopez Ramirez,[1,3] Mariana Pavel,[1,3] Claudia Puri,[1,3] Maurizio Renna,[1,3] Thomas Ricketts,[1,3] Lars Schlotawa,[1,3] Mariella Vicinanza,[1,3] Hyeran Won,[1,3] Ye Zhu,[1,3] John Skidmore,[2,3] and David C. Rubinsztein[1,5,*]
[1]Department of Medical Genetics, Cambridge Institute for Medical Research, Cambridge Biomedical Campus, Wellcome Trust/MRC Building, Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0XY, UK
[2]Alzheimer's Research UK Cambridge Drug Discovery Institute, University of Cambridge, Cambridge Biomedical Campus, Hills Road, Cambridge CB2 0AH, UK
[3]Co-first author
[4]Present address: Eli Lilly and Company Limited, Erl Wood Manor, Sunninghill Road, Windlesham, Surrey GU20 6PH, UK
[5]Lead Contact
*Correspondence: dcr1000@cam.ac.uk
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Autophagy is a conserved pathway that delivers cytoplasmic contents to the lysosome for degradation. Here we consider its roles in neuronal health and disease. We review evidence from mouse knockout studies demonstrating the normal functions of autophagy as a protective factor against neurodegeneration associated with intracytoplasmic aggregate-prone protein accumulation as well as other roles, including in neuronal stem cell differentiation. We then describe how autophagy may be affected in a range of neurodegenerative diseases. Finally, we describe how autophagy upregulation may be a therapeutic strategy in a wide range of neurodegenerative conditions and consider possible pathways and druggable targets that may be suitable for this objective.

Autophagy Cell Biology

Macroautophagy (henceforth called autophagy) is a major intracytoplasmic protein degradation pathway whereby cytoplasmic contents are delivered, by double-membraned vesicles called autophagosomes, to the lysosome for degradation. It should be differentiated from other pathways that will not be considered in this review, like chaperone-mediated autophagy and microautophagy, where substrates are directly translocated into the lysosome without vesicular transport. The first morphologically characteristic structure in autophagy is the double-membraned, cup-shaped autophagosome precursor, called the phagophore, that engulfs substrates as its edges extend. After the phagophore edges close to form a vesicle, the completed autophagosomes traffic along microtubules to enable autophagosome-lysosome fusion, which leads to the degradation of the autophagic contents (Figure 1). Autophagy is regulated by a series of proteins defined as autophagy-related (ATG) proteins.

Autophagy was initially characterized as a bulk and non-selective degradation pathway induced by nutrient deprivation. However, more recent studies made clear that autophagy also contributes to intracellular homeostasis in non-starved cells by degrading cargo material such as aggregate-prone proteins, including those causing many neurodegenerative conditions (aggrephagy), damaged mitochondria (mitophagy), excess peroxisomes (pexophagy), and invading pathogens (xenophagy) (Stolz et al., 2014). In the classic example, aggregates of aberrantly folded proteins are tagged with ubiquitin chains that are recognized by ubiquitin-binding domain-containing receptors such as Sequestosome 1 (SQSTM1)/p62 (Bjørkøy et al., 2005), neighbor of BRCA1 gene 1 (NBR1) (Kirkin et al., 2009), optineurin (OPTN) (Wild et al., 2011), Tax1 binding protein 1 (TAX1BP1), nuclear dot protein 52 (NDP52)/CALCOCO2 ((Newman et al., 2012), TOLL-interacting protein (TOLLIP) (Lu et al., 2014), and 26S proteasome regulatory subunit (RPN10) (Marshall et al., 2015; Khaminets et al., 2016; Stolz et al., 2014). These receptors also contain LC3-interacting region (LIR) motifs in their sequences that can recognize the key autophagosome-associated protein microtubule-associated protein 1 light chain 3 (LC3). Thus, these receptors serve as a bridge between ubiquitinated cargo and autophagosomes and enhance the incorporation of cargo into autophagosomes for subsequent lysosomal degradation (Bjørkøy et al., 2005; Pankiv et al., 2007).

This concept of selective autophagy has now been extended with the description of precision autophagy. This is defined by the involvement of receptors with the ability to recognize LC3 family member proteins, their specific substrates, and also, importantly, the capacity to act as platforms for the assembly of other core ATG factors (Kimura et al., 2015). To date, receptors identified in this class come from the tripartite motif-containing (TRIM) family, a diverse group of proteins associated with the degradation of targets such as innate immunity signaling molecules (reviewed by Kimura et al., 2016). It seems likely that there are many other molecules that play such roles in autophagy, and identifying them will be key for our understanding of autophagy in non-starved cells.

Key Autophagy Regulators

Autophagy activation in response to the primordial stimuli of nutrient deprivation and/or low cellular energy levels is mediated by signaling pathways that converge on ULK1/2 (mammalian homologs of the *C. elegans* uncoordinated-51 kinase) (Figure 2).

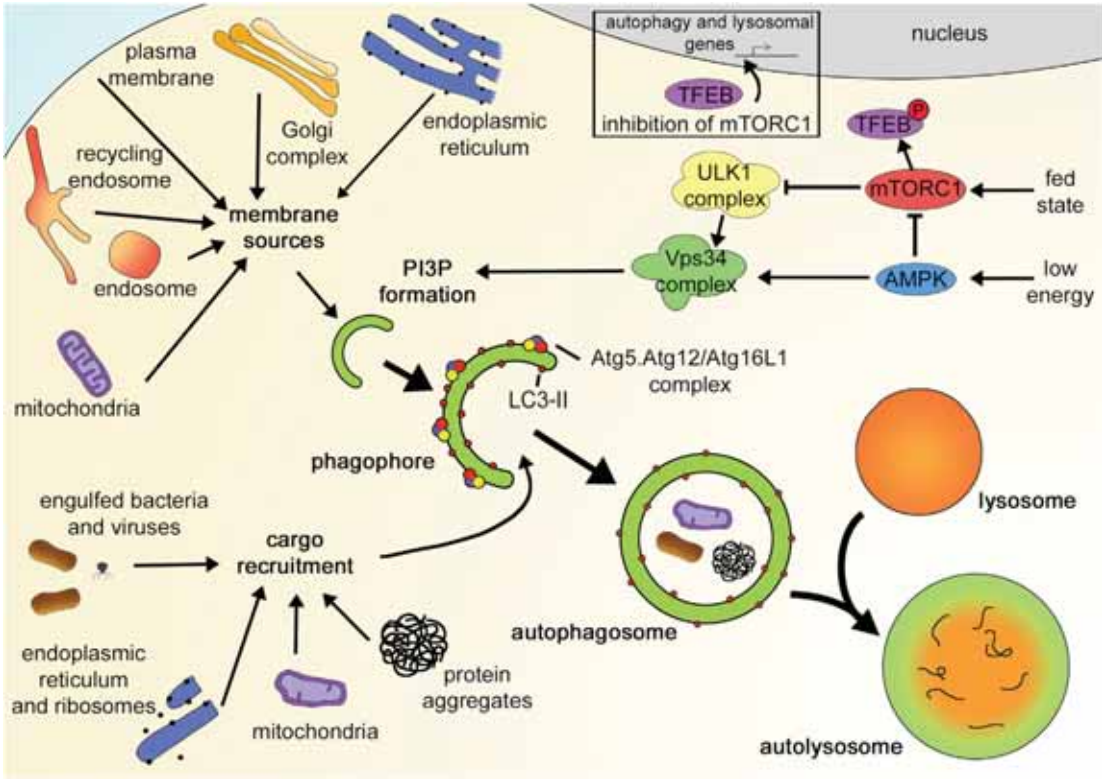

Figure 1. Overview of the Mammalian Autophagy Pathway

Nutrient depletion, growth factor deprivation, and low energy are well established autophagy inducers. These culminate in mTORC1 inhibition and AMPK activation, which, in turn, positively regulate the ULK1 complex through a series of phosphorylation events. Induction of the ULK1 complex subsequently activates the VSP34 complex, which leads to PI3P synthesis in pre-autophagosomal structures. The membranes of these structures appear to have multiple sources, such as the endoplasmic reticulum, Golgi apparatus and *trans*-Golgi network, plasma membrane, endosomal compartment, and mitochondria. PI3P defines the LC3 lipidation sites for autophagosome precursors by assisting in the recruitment of the ATG12-ATG5-ATG16L1 complex. This complex is essential for the conjugation of LC3-I to PE in membranes, which sustains membrane elongation and engulfment of a variety of substrates (e.g., protein aggregates, infectious agents, and damaged mitochondria) that end up being degraded in the lysosome after fusion with autophagosomes. Conditions characterized by mTORC1 inactivation are also accompanied by translocation of TFEB to the nucleus and TFEB activation, leading to the transcription of many autophagy and lysosomal genes, which ensures synthesis of key components of the pathway and efficient autophagy-dependent degradation. AMPK, AMP-dependent protein kinase; mTORC1, mammalian target of rapamycin complex 1; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol 3-phosphate; TFEB, transcription factor EB; ULK, mammalian homologs of the *C. elegans* uncoordinated-51 kinase.

ULK1/2 forms a complex with ATG13, ATG101, and focal adhesion kinase family-interacting protein of 200 kDa (FIP200). Nutrients and growth factor availability and levels of AMP/ATP (which reflect the energetic status of the cell) are sensed by mammalian target of rapamycin complex 1 (mTORC1) and AMP-dependent protein kinase (AMPK), respectively, which, in turn, oppositely regulate the ULK1/2 complex through a series of phosphorylation events. For instance, activation of AMPK by allosteric binding of AMP and phosphorylation of Thr172 promotes autophagy by directly activating ULK1 through phosphorylation of Ser317 and Ser77 under glucose deprivation (Kim et al., 2011) or Ser555 under amino acid starvation and mitophagy (Egan et al., 2011). On the other hand, in medium replete with amino acids (sensed by the Rag-Ragulator complex) and growth factors (that signal by receptor tyrosine kinases and the phosphatidylinositol 3-kinase (PI3K)/AKT pathway), mTORC1 is activated and inhibits autophagy by binding to the ULK1 complex (via Raptor-ULK1 interaction) and by phosphorylating both ATG13 and ULK1 (at Ser 757), thereby suppressing ULK1 kinase activity and preventing the interaction between ULK1 and AMPK (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009; Kim et al., 2011; Laplante and Sabatini, 2012).

Activation of the ULK complex is required for the recruitment of the class III PI3K VPS34 to the phagophore initiation sites where VPS34 generates phosphatidylinositol 3-phosphate (PI3P) while in a complex with VPS15, ATG14, and Beclin 1. The exact functions of PI3P in autophagy are still unclear. However, it appears to aid the recruitment of tryptophan-aspartic acid (WD) repeat domain phosphoinositide-interacting (WIPI) proteins to the phagophore membrane, which, in turn, controls the recruitment of crucial downstream autophagic proteins (e.g., ATG16L1 by WIPI2) that dictate where the phagophores form (Dooley et al., 2014).

The ATG12 and ATG8/LC3 ubiquitin-like conjugation systems are then required for sustaining the expansion of the phagophore. In the first system, ATG12 is conjugated to ATG5 in a reaction that involves ATG7 and ATG10 (E1-like and E2-like enzymes, respectively), and the resulting complex binds non-covalently to

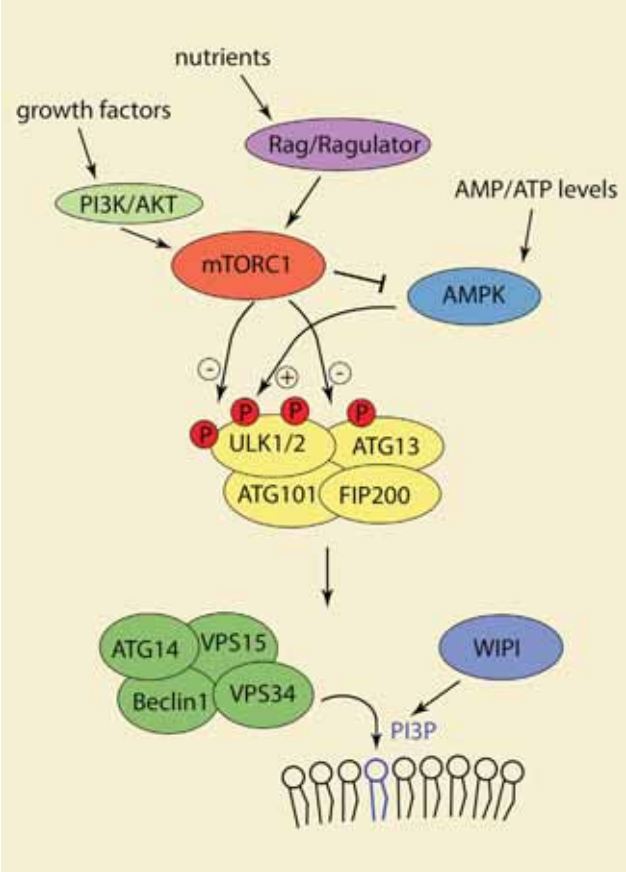

Figure 2. Autophagy Regulation in Response to Nutrients
The key signaling pathway controlling autophagy response to nutrient and energy levels converges on the phosphorylation of the ULK1/2 complex at different sites. Conventionally, AMPK inhibits mTOR. Additionally, as indicated here, mTOR phosphorylation of ULK1 disrupts the AMPK-ULK1 interaction. Phosphorylation of the ULK1/2 complex regulates the recruitment of the VPS34 complex to the phagophore and, hence, the production of PI3P and downstream autophagy effectors via the binding of WIPI proteins.

ATG16L1. ATG12-ATG5-ATG16L1 associates with pre-autophagosomal membranes, enabling their elongation by assisting in the recruitment of LC3. However, before this can happen, LC3 has to be processed by the cysteine protease ATG4, which cleaves the C terminus of LC3, exposing a glycine residue (LC3-I form). This cleavage is crucial for LC3-I conjugation to phosphatidylethanolamine (PE) by a mechanism dependent on ATG7, ATG3, and ATG12-ATG5-ATG16L1 (E1-like, E2-like, and E3-like enzymes, respectively), leading to the formation of LC3-II, which is tightly associated with autophagosomal membranes. This cascade of reactions then sustains extension of the phagophore edges and its closure to form mature autophagosomes (Bento et al., 2016b).

Extension of the phagophore is also assisted by mATG9, the only identified multi-pass transmembrane protein among the core ATG proteins. This protein appears to localize to the *trans*-Golgi network and the endocytic compartment, including early endosomes, late endosomes, and recycling endosomes, and is postulated to aid in the supply of lipid bilayers to the nascent phagophore, enabling its further elongation prior to closure of the fully formed autophagosome (Bento et al., 2016b). This late stage, where the outer and the inner membranes of the pre-autophagosomal structure become separate entities, is poorly understood, but ATG2, in combination with WIPI1, appears to regulate autophagosome closure (Velikkakath et al., 2012), a process likely involving membrane fission/scission-type events akin to the genesis of multi-vesicular bodies by endosomal sorting complexes required for transport (ESCRT)-mediated membrane budding (Knorr et al., 2015).

The source of autophagosome membranes is an area of active investigation. The endoplasmic reticulum (ER), Golgi and *trans*-Golgi network, mitochondria, plasma membrane, and endosomal compartments have all been suggested as possible sources of phagophore membranes (Bento et al., 2016b). The ER emerged as one of the possible sources of membranes for pre-autophagosomes, not only because isolation membranes were observed cradled within a subdomain of the ER and interconnected with it (Axe et al., 2008; Hayashi-Nishino et al., 2009) but also because ATG14- and ATG5-positive isolation membranes were found in close proximity to a subdomain of the ER in contact with mitochondria during starvation (Hamasaki et al., 2013). Post-Golgi tubulo-vesicular compartments undergoing remodelling and homotypic fusion (Guo et al., 2012; Orsi et al., 2012; Young et al., 2006) and the ER-Golgi intermediate compartment (ERGIC) have also been considered as pre-autophagosomal membrane sources (Ge et al., 2013, 2014). The ERGIC was specifically observed to bud LC3 lipidation-active vesicles that may enable autophagosome biogenesis and expansion (Ge et al., 2013, 2014).

The plasma membrane and the endocytic compartments are also suggested as membrane sources for early autophagosomal precursor structures. Clathrin-dependent endocytosis has been implicated in this process by delivering ATG16L1 and mATG9 to recycling endosomes (via different routes and involving VAMP3-dependent membrane fusion events), which leads to the formation of early autophagosomal structures and mature autophagosomes (Moreau et al., 2011; Puri et al., 2013; Ravikumar et al., 2010). Overexpression of the recycling endosome proteins TBC1D14 (a Rab11 effector) or PX-containing SNX18 were shown to induce accumulation of mATG9 and ATG16L1, respectively, along with other autophagic proteins (e.g., ULK1 and LC3) in this compartment (Knævelsrud et al., 2013; Lamb et al., 2016; Longatti et al., 2012), reinforcing the possible role of the recycling endosome as an important phagophore membrane source.

Transcriptional Regulation of Autophagy

In addition to phosphorylation, discussed above, autophagy is also regulated at the transcriptional level. mTORC1, apart from regulating the ULK1/2 complex, also connects the lysosome nutrient-sensing (LYNUS) machinery to the transcriptional regulation of autophagy genes via transcription factor EB (TFEB) (Settembre et al., 2012). In resting cells, phosphorylation of TFEB by active mTORC1 induces TFEB binding to 14-3-3 proteins and, therefore, TFEB retention in the cytosol (Roczniak-Ferguson et al., 2012; Settembre et al., 2011). However, under starvation and consequent mTORC1 inactivation, TFEB is no longer phosphorylated and translocates to the nucleus, where it binds to coordinated lysosomal expression and regulation (CLEAR) consensus sequences in promoters of target genes and induces

their transcription. Among these genes, many are directly related to the lysosome and autophagy (e.g., lysosomal hydrolases, vacuolar-type H+-ATPase (v-ATPase) subunits, and ATG proteins), and, thus, TFEB appears to co-ordinately regulate the expression of many of the key genes required for autophagy/lysosome function (Sardiello et al., 2009; Settembre et al., 2011). Zinc-finger protein with KRAB and SCAN domains 3 (ZKSCAN3) is a transcriptional repressor of autophagy that appears to oppose TFEB. ZKSCAN3 silencing enhances autophagosome and lysosomal biogenesis, and mTORC1 inhibition induces its accumulation in the cytosol (Chauhan et al., 2013). More than 20 other transcription factors have now been linked to transcriptional regulation of autophagy following a wide range of stimuli (Füllgrabe et al., 2014). For instance, microphthalmia-associated transcription factor (MITF) (which belongs to the same family of proteins as TFEB) (Martina et al., 2014), p53 (Kenzelmann Broz and Attardi, 2013) and forkhead box O3 (FOXO3) (Mammucari et al., 2007) have all been shown to *trans*-activate ATG genes.

Autophagy Physiology and the Nervous System

The mammalian nervous system requires autophagy to maintain its normal functions and homeostasis. Because ubiquitous deletion of core autophagy genes results in neonatal and embryonic lethality, multiple nervous system-specific knockout mouse models have been generated to allow analyses of the roles of autophagy in neuronal function. A Nestin-Cre promoter that switches on embryonically in neuronal precursor cells has been used to excise floxed alleles of *Atg5* and *Atg7*. This results in autophagy deficiency in neuronal cells and glia, accompanied by the accumulation of intra-neuronal aggregates (Hara et al., 2006; Komatsu et al., 2006, 2007a). The accumulation of these aggregates in otherwise normal mice suggests that autophagy plays a key role in removing aggregate-prone proteins, supporting earlier studies in cell culture models of Huntington's disease (Ravikumar et al., 2002). Subsequent Nestin-Cre models have been generated targeting *FIP200* and *Wipi4* as well as a double knockout for *Ulk1/2* (Joo et al., 2016; Liang et al., 2010; Orosco et al., 2014). The result in all models is reduced survival and early-onset, progressive neurodegeneration across broad areas of the brain. However, the nature of pathology varies according to the gene targeted; e.g., progressive spongiosis is seen in *FIP200* Nestin-Cre mice but not the *Atg5* and *Atg7* Nestin-Cre models. The variability observed between models will likely be accounted for by the stages at which autophagy is disrupted as well as non-autophagy functions for each target.

To further delineate the function of autophagy in different neuronal types, targeted knockouts of *Atg5* and *Atg7* in Purkinje neurons of the cerebellum (Komatsu et al., 2006; Nishiyama et al., 2007), *Atg7* in agouti-related peptide (AgRP) neurons of the hypothalamus (Kaushik et al., 2011), as well as *Atg5* and *Atg7* in rhodopsin neurons of the retina (Chen et al., 2013; Zhou et al., 2015) have been developed. Deletion of *Atg5* and *Atg7* results in cell-autonomous Purkinje neuron degeneration. The earliest signs of homeostatic disruption in these mice are Purkinje axonal swellings, which are observed prior to progressive dystrophy and degeneration of the axon (Komatsu et al., 2007b; Nishiyama et al., 2007). These findings support a key role of autophagy in axon homeostasis and highlight an early sign of neuronal dysfunction. Studies from cell culture systems have led to the proposal that different neuronal types may have varying capacities to degrade autophagy substrates, such as mutant polyglutamine proteins, and that this correlates with the sensitivity of that cell type to the toxicity of aggregate-prone proteins (Tsvetkov et al., 2013).

In the peripheral nervous system (PNS), a specific role for autophagy in Schwann cell (SC) function has been identified during myelination and re-myelination. In the early postnatal period, when myelination is occurring, autophagy is involved in the maturation of SCs, particularly in the reduction of abaxonal cytoplasm volume. In conditional knockouts where ATG7 is absent in SCs, the abaxonal cytoplasm remains abundant, and, consequently, small fiber hypermyelination is observed (Jang et al., 2015). Conversely, autophagy upregulation via rapamycin treatment during the early post-natal period leads to a greater reduction in abaxonal cytoplasm within the SCs. After nerve injury, myelin debris is cleared by SCs through a newly described form of selective autophagy named myelinophagy (Gomez-Sanchez et al., 2015), and this debris clearance is delayed in *Atg7*-SC conditional knockouts (Jang et al., 2016).

In addition to protecting against neurodegeneration, autophagy also regulates neurogenesis. An important role for autophagy in sustaining the post-natal pool of neural stem cells (NSCs) has recently emerged. In the adult brain, NSCs within the sub-ventricular zone (SVZ) of the lateral ventricle wall and subgranular zone (SGZ) of the dentate gyrus can produce new functional neurons in response to physiological and pathological stimuli (Gage, 2000). Ablation of FIP200 results in a reduced number of NSCs in the SVZ and the dentate gyrus, affecting the self-renewal capacity of NSCs (Wang et al., 2013a). Key autophagy proteins, including AMBRA1 and Beclin 1, are highly expressed in the SVZ of the adult mouse brain, and heterozygosity for *Becn1* results in decreased cell proliferation in vitro (Yazdankhah et al., 2014). Depletion of ATG5 in adult NSCs in the dentate gyrus also leads to reduced survival and neuronal maturation (Xi et al., 2016). In all of these studies, the depletion of the NSC pool could be attributed to apoptosis (Wang et al., 2013a; Xi et al., 2016; Yazdankhah et al., 2014). However, although apoptosis is likely responsible for reduced survival of autophagy-defective NSCs, it is unclear whether NSC differentiation and neuronal maturation are apoptosis-dependent (Xi et al., 2016; Yazdankhah et al., 2014). It will be interesting to examine whether potential beneficial effects of increasing basal autophagy act via changes in NSCs, and this is likely to be an exciting topic for future research. In addition to effects on the stem cell pool, autophagy is required for normal neural progenitor cell proliferation and differentiation, as revealed by studies in ubiquitous ATG16L1 hypomorph mice and knockdown of ATG5 in vivo in embryonic brains (Lv et al., 2014; Wu et al., 2016).

Although impairment of autophagy is deleterious, autophagy upregulation appears to be protective in many normal contexts. Studies in *C. elegans* have shown a clear association between increased longevity and constitutive autophagy, which may be mediated through multiple different but overlapping mechanisms, including nutrient restriction (Hansen et al., 2008), altered mitosis (Ghavidel et al., 2015), and mitochondrial turnover (Palikaras et al., 2015). Similarly, pan-neuronal overexpression of

Atg8a (Simonsen et al., 2008) or AMPK (Ulgherait et al., 2014) in *Drosophila* resulted in an extended lifespan. Overexpression of ATG5 in mice induces autophagy and extends the lifespan and is associated with anti-aging phenotypes including leanness, increased insulin sensitivity, and improved motor function (Pyo et al., 2013). In Huntington's disease (HD), expansion of the polyglutamine tract in the huntingtin (HTT) protein causes disease with a polyglutamine length-dependent severity. In mice where the polyglutamine expansion was removed from the endogenous *Htt*-coding gene (delta-Q mice), an increase in autophagosome biosynthesis was observed, and this was also associated with a significantly longer lifespan. (Zheng et al., 2010). Together, these studies indicate a beneficial effect of enhancing basal autophagy during aging.

Neurodegenerative Diseases in which Autophagy Is Impaired

As outlined above, there is increasing evidence for the physiological importance of autophagy in neuronal health, raising the possibility that autophagy dysfunction may play a role in neurodegenerative diseases. Circumstantially, this is further supported by the major pathological phenotype of most late-onset neurodegenerative diseases, the presence of intraneuronal aggregates of misfolded proteins, which are substrates for autophagic degradation (Menzies et al., 2015a; Ravikumar et al., 2002). In some forms of disease, these aggregate-prone proteins are the result of specific mutations (such as in Huntington's disease). However, in the vast majority of diseases, the underlying reason for the presence of aggregates is unknown.

For the most part, neurodegenerative diseases do not follow simple, monogenic inheritance patterns. However, in all major neurodegenerative diseases, a subset of cases are associated with inherited genetic mutations. These familial forms of disease allow an insight into the potential mechanisms of pathogenesis. Identification of disease-associated genes and investigations into their functions reveal that many affect autophagy. The absolute contribution of autophagy dysfunction to disease progression has yet to be established. Given that many neurodegenerative diseases are late-onset, it is possible that small alterations in the turnover of proteins will have cumulative effects that manifest later in life.

The autophagy pathway is complex, with multiple steps and modes of regulation. This makes identifying potentially minor perturbations in the pathway difficult. Therefore, in some cases, the evidence for autophagy involvement in neurodegenerative disease pathogenesis appears controversial. This evidence is discussed in a disease-specific manner below (Figure 3).

Alzheimer's Disease

The pathological hallmarks of Alzheimer's disease (AD) are intracellular tau tangles and extracellular amyloid β (Aβ) plaques. Aβ is formed from amyloid precursor protein (APP) by two cleavage events. There is a complex interplay between Aβ and autophagy. Aβ may be degraded by autophagy, and upregulation of autophagy has been shown to reduce Aβ levels in a number of systems (Boland et al., 2008; Spilman et al., 2010; Tian et al., 2011; Vingtdeux et al., 2011). However, Aβ may also be generated in autophagosomes, which appear to contain both APP and Presenilin-1 (PS-1), an enzyme involved in the cleavage of APP to Aβ (Boland et al., 2008; Yu et al., 2005). Furthermore, autophagy may play a role in the secretion of Aβ into the extracellular space, where it forms plaques, because deletion of ATG7 in APP transgenic mice results in less Aβ extracellular secretion and plaque formation, contrary to what one may expect if autophagy simply degraded Aβ (Nilsson et al., 2013).

Genetic studies have implicated phosphatidylinositol-binding clathrin assembly protein (*PICALM*) in Alzheimer's disease (Harold et al., 2009; Jun et al., 2010), and changes in the level of this protein have been reported in the brains of patients with Alzheimer's (Ando et al., 2013, 2016). PICALM is a clathrin adaptor protein required for the endocytosis of soluble NSF attachment protein receptors (SNAREs), and loss of this function has been demonstrated to inhibit autophagy at multiple steps, including early autophagosome formation and maturation of autophagosomes (Moreau et al., 2014). Of course, altered trafficking of these SNAREs is also likely to affect other vesicle trafficking pathways, and, therefore, as with all of the disease mutations discussed in this review, it is yet to be established whether or how much PICALM-mediated autophagy perturbation contributes to disease. However, a second mechanism through which PICALM function may affect autophagy has also been proposed. Through its action in a complex with assembly polypeptide 2 (AP2), PICALM may act as an autophagy receptor that is able to interact with LC3 and target APP into autophagosomes (Tian et al., 2013).

Mutations in *PS-1* cause familial AD, and there is a great deal of evidence demonstrating that these mutations change the way the APP protein is processed (for example, Citron et al., 1997). However, other functions of PS-1 may also contribute to disease pathogenesis. PS-1 has been shown to function as an ER chaperone for the V_0a_1 subunit of the lysosomal v-ATPase. Familial AD-associated mutations in PS-1 result in decreased maturation of the lysosomal v-ATPase and, thus, increased lysosomal pH (Coffey et al., 2014; Lee et al., 2010; Wolfe et al., 2013), which would be predicted to reduce autophagosome clearance.

Autophagy may also be affected by reduced levels of Beclin 1 mRNA and protein, which have been reported in AD brains (Pickford et al., 2008; Small et al., 2005). Similarly, Beclin 1 protein levels are thought to be decreased because of caspase 3 cleavage, which is activated in the brains of AD patients (Rohn et al., 2011).

Tauopathies

Tau accumulation into intracellular tangles is one of the hallmark pathologies of AD and is also seen in a group of neuronal disorders termed tauopathies, which include progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and frontotemporal dementias (FTDs) (Lee et al., 2001). Hyperphosphorylated tau co-localizes with LC3-positive vesicles and the autophagy cargo-receptor SQSTM1/p62 in CBD and PSP patients (Piras et al., 2016). Moreover, aberrant tau appears to disrupt axonal vesicle transport by impairing the dynein-dynactin complex, increasing the number of autophagosomes and contributing to tau-induced toxicity in FTDs and AD (Butzlaff et al., 2015; Majid et al., 2014). Tau has also been shown to bind lysosomal membranes and perturb lysosomal permeability in vitro and in a mouse model of AD (Collin et al., 2014; Wang et al., 2009). Defective

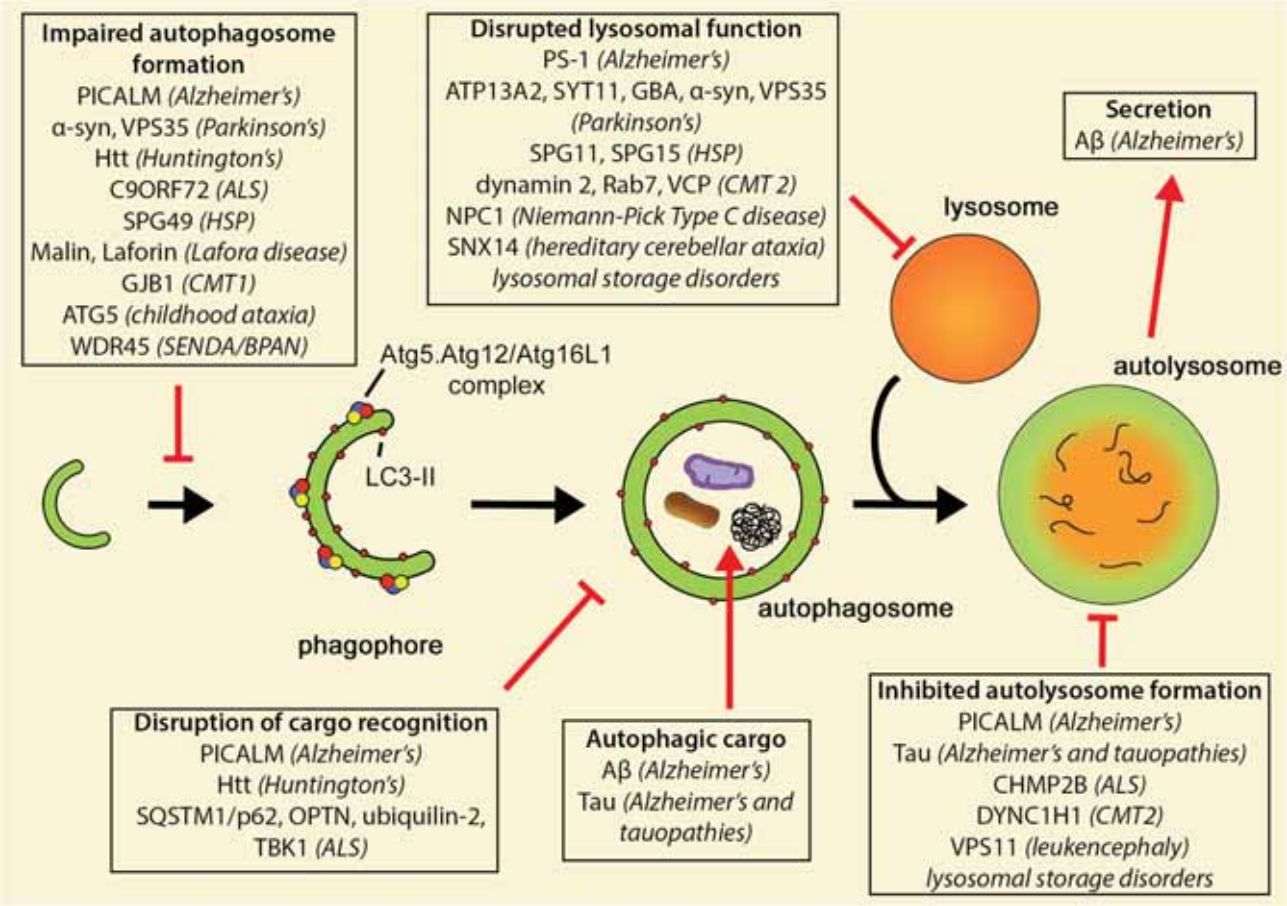

Figure 3. Intersections between Autophagy and Disease-Associated Genes
An increasing number of genes associated with neurodegenerative diseases have now been implicated in autophagy function. These genes act at a number of different steps throughout the autophagic process, from early steps of autophagosome formation through autolysosome formation. Their proposed sites of action are indicated, along with the neurodegenerative disease with which they are associated.

lysosomal membrane integrity was also found in AD patients (Perez et al., 2015), and, more recently, increased levels of the lysosomal components lysosomal-associated membrane protein 1 (LAMP1) and cathepsin D were reported in CBD and PSP patients (Piras et al., 2016).

Parkinson's Disease

Compelling evidence supporting a role for dysfunctional autophagy as a causative factor in neurodegenerative disease comes from the studies of mitophagy in Parkinson's disease (PD). Autosomal recessive forms of early-onset PD are associated with mutations in genes encoding phosphatase and tensin homolog-induced putative kinase 1 (PINK1) (Valente et al., 2004) and the E3 ubiquitin ligase Parkin (Kitada et al., 1998). Elegant studies have demonstrated that PINK1 and Parkin act in the same pathway to promote mitophagy. PINK1 is stabilized on the outer membrane of damaged mitochondria, leading to the recruitment and activation of the E3 ubiquitin ligase Parkin on these mitochondria and, ultimately, their sequestration into autophagosomes (Matsuda et al., 2010; Narendra et al., 2008). This disease-associated mitophagy signaling pathway has been the subject of recent comprehensive reviews (Pickrell and Youle, 2015; Nguyen et al., 2016).

Several models have been generated to elucidate the function of these mitophagy effectors in the nervous system. Although no gross behavioral changes were found in mice where *Parkin* was deleted (Goldberg et al., 2003; Itier et al., 2003; Perez and Palmiter, 2005; Von Coelln et al., 2004), the activity of striatal neuron mitochondria was impaired (Palacino et al., 2004). A deficit in evoked dopamine release response and striatal synaptic plasticity in the striatum was also observed (Kitada et al., 2009). Similarly, deletion of *Pink1* resulted in impaired respiration in striatal mitochondria and increased sensitivity to oxidative stress (Gautier et al., 2008). Furthermore, dopaminergic neurons derived from *Pink1* or *DJ-1* knockout mice also showed defective morphology and reduced activity (Shim et al., 2011). Indeed, loss of endogenous Parkin synergizes with a loss of DNA polymerase γ that results in increased mitochondrial mutations and, in turn, in dopaminergic neuron damage (Pickrell et al., 2015). The use of a recently generated reporter mouse to assess in vivo mitophagy will shed light on how mitophagy flux is

modulated in response to different genetic and pharmacological perturbations (Sun et al., 2015).

PD is characterized by loss of dopaminergic neurons in the substantia nigra and, typically, by the presence of α-synuclein (α-syn) inclusions. These inclusions have been shown to affect autophagy function (Tanik et al., 2013). In cells with α-syn inclusions, although lysosomal function appears normal, autophagosome maturation and fusion with the lysosomes are decreased, resulting in a decrease in protein degradation. It is interesting to note that this alteration in vesicle trafficking does not seem to result from a non-specific physical blockade of the axons by the inclusions but, rather, a specific inhibition of endocytic and autophagic vesicles (Volpicelli-Daley et al., 2014). Independent of the formation of inclusions, increased levels of α-syn (as found in disease) has been shown to impair autophagy because α-syn overexpression in cell and mouse models led to mislocalization of mATG9 (Winslow et al., 2010). Similarly, abnormal trafficking of mATG9 has been shown to be caused by the vacuolar protein sorting-associated protein 35 (VPS35) D620N mutation, which causes an autosomal dominant form of PD. VPS35 is a component of the retromer complex, which recruits the actin nucleation-promoting WASP and Scar homolog (WASH) complex to endosomes. D620N VPS35 prevents this recruitment and, thus, causes mATG9 mislocalization and autophagy impairment (Zavodszky et al., 2014).

The most common genetic risk factor for PD is heterozygosity for mutations in the lysosomal enzyme glucocerebrosidase (GBA). These mutations have been reported in up to 31.3% of Ashkenazi Jewish patients with Parkinson's disease and up to 9.4% in patients of other ethnic origins (Sidransky and Lopez, 2012). Homozygous GBA mutations cause the lysosomal storage disorder Gaucher disease (reviewed in Michel et al., 2016), where loss of GBA leads to the accumulation of its substrate glucosylceramide within lysosomes and, thus, to autophagy impairment because of lysosomal dysfunction. In PD patients without GBA mutations, the levels and activity of the enzyme are decreased in brain areas with increased α-syn levels in early disease stages (Mazzulli et al., 2011; Murphy et al., 2014).

Loss-of-function mutations in the P-type ATPase ATP13A2 (also known as PARK9) cause familial Kufor-Rakeb syndrome, characterized by early-onset Parkinsonism (Ramirez et al., 2006). Lysosomal ATPases are required for the maintenance of lysosomal pH and, therefore, the activity of lysosomal proteases. Mutations in *ATP13A2* impair these processes and lead to an increase in the number of autophagic vesicles, which are unable to fuse with lysosomes (Dehay et al., 2012). Impaired lysosomal degradative capacity in cells with ATP13A2 mutations leads to an accumulation of α-synuclein, which may contribute to the toxicity of ATP13A2 mutations (Usenovic et al., 2012). Recent studies show that ATP13A2 depletion leads to a decrease in the levels of another PD-associated gene, synaptotagmin 11 (SYT11), and that it is this SYT11 decrease that causes lysosomal dysfunction and impaired autophagosome degradation resulting from loss of ATP13A2 activity. Overexpression of SYT11 in ATP13A2 knockdown cells is able to rescue the autophagy defects seen in these cells, demonstrating that they act in the same pathway (Bento et al., 2016a).

Polyglutamine Disorders

Nine neurological disorders are caused by mutant proteins with expanded glutamine repeats (polyQ), including HD, various spinocerebellar ataxias (SCAs), and spinal and bulbar muscular atrophy (SBMA). Autophagy perturbation is observed in several of these diseases. At the transcriptional level, the mutant polyQ androgen receptor (which causes SBMA) interacts with TFEB, a potent autophagy gene inducer, and inhibits TFEB transactivation (Cortes et al., 2014). A transgenic mouse model of SCA3 showed low levels of sirtuin 1 (Cunha-Santos et al., 2016), an enzyme that normally deacetylates several autophagy proteins to promote autophagy (Huang et al., 2015; Lee et al., 2008). Additionally, brains from mutant SCA3 transgenic mice have low levels of Parkin (Durcan et al., 2011) and Beclin 1 (Nascimento-Ferreira et al., 2011). Beclin 1 appears to be sequestered into aggregates in the brains of mouse models of HD and SCA7 (Alves et al., 2014; Shibata et al., 2006).

The most investigated polyQ disease protein is huntingtin, mutated in HD. Mutant huntingtin impairs efficient cargo recognition by autophagosomes (Martinez-Vicente et al., 2010), whereas wild-type huntingtin serves as a scaffold for the recruitment of several autophagy proteins during selective autophagy of targets, such as mitochondria and protein aggregates (Ochaba et al., 2014; Rui et al., 2015). Furthermore, a role for huntingtin in axonal transport of autophagosomes has been suggested by live-cell imaging studies in striatal neurons. Following either loss of huntingtin or expression of the mutant protein, a decrease autophagosome transport and subsequent inhibition of substrate degradation were observed (Wong and Holzbaur, 2014a). Mutant huntingtin also interacts with and inactivates Rhes, a protein selectively expressed in the striatum, the brain region selectively affected in HD. Rhes is required for autophagy because it interacts with Beclin 1 and reduces the inhibitory interaction of Bcl-2 with Beclin 1 (Mealer et al., 2014). Another important autophagy negative regulator, mTOR, is sequestered to aggregates in HD and SCA7 brains (Alves et al., 2014; Ravikumar et al., 2004).

In addition to potential mechanistic roles in autophagy, it has also been proposed that mutant huntingtin may accumulate because of the loss of an acetylation signal that would normally target it for degradation by this pathway (Jeong et al., 2009). Overall, mutant huntingtin is involved in different pathways that modulate autophagy—some toxic and others that may be protective. The overall outcome in terms of autophagy activity may depend on the ratio of soluble to aggregated mutant protein.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is associated with the accumulation of misfolded proteins such as superoxide dismutase 1 (SOD1), TAR DNA-binding protein 43 (TDP-43), fused in sarcoma/translocated in sarcoma (FUS), and/or C9ORF72 (reviewed in Blokhuis et al., 2013). It is most commonly a sporadic disease, but approximately 5%–10% of cases are familial. A range of genes have been associated with the disease, but recently it has become apparent that a number of these genes encode for proteins that act as autophagy receptors, like SQSTM1/p62 (Fecto et al., 2011), OPTN (Maruyama et al., 2010), and Ubiquilin 2 (Williams et al., 2012), which enhance

the incorporation of autophagy substrates into autophagosomes via interactions with LC3, as discussed above (Khaminets et al., 2016). Mutations in SQSTM1/p62 have been associated with disrupted autophagic degradation of mutant SOD1 and TDP-43 (Gal et al., 2009; Teyssou et al., 2013). ALS-causing mutations in the LC3 binding region of SQSTM1/p62 have been shown to impair its recruitment into autophagosomes (Goode et al., 2016). However, mutations have been identified throughout the length of the protein, and it is not clear how these mutations affect SQSTM1/p62 function. Similarly, disease-causing mutations in OPTN decrease autophagy and protein clearance (Shen et al., 2015) and also inhibit the ability of OPTN to recruit LC3 to damaged mitochondria and induce mitophagy (Wong and Holzbaur, 2014b). OPTN also functions to bind LC3 and Myosin VI, which is required for the trafficking of autophagosomes (Tumbarello et al., 2012); the majority of ALS-associated mutations in OPTN are located in the myosin VI-binding domain and show loss of this binding (Shen et al., 2015; Sundaramoorthy et al., 2015). Recently, mutations in TANK-binding kinase 1 (TBK1) have been associated with ALS (Cirulli et al., 2015; Freischmidt et al., 2015); TBK1 has been shown to phosphorylate OPTN to promote efficient mitophagy and SQSTM1/p62 to allow autophagosome maturation (Moore and Holzbaur, 2016; Pilli et al., 2012). Disease-associated mutations in this kinase have been shown to reduce binding of TBK1 to OPTN and, thus, decrease the clearance of dysfunctional mitochondria (Richter et al., 2016).

Alongside autophagy receptor proteins, other ALS-associated genes have been identified as having a role in autophagy. Disruption of the ESCRT machinery through mutations in charged multivesicular body protein 2B (CHMP2B) have been associated with both ALS and frontotemporal dementia, a neurodegenerative disease thought to be on a spectrum with ALS. Disease-associated mutations in CHMP2B impair autolysosome formation and lead to the accumulation of protein aggregates (Filimonenko et al., 2010; Lee et al., 2007).

The most common cause of ALS is a hexanucleotide repeat expansion in the C9ORF72 gene (DeJesus-Hernandez et al., 2011; Renton et al., 2011). These mutations may cause disease through a number of different mechanisms, including toxic gain of function from ATG-independent translation of the RNA repeats and loss of normal protein function (Todd and Petrucelli, 2016). A role has been suggested for C9ORF72 in endocytic vesicle trafficking and autophagy (Farg et al., 2014). Recently, C9ORF72 was found to form a complex with Smith-Magenis syndrome chromosome region, candidate 8 (SMCR8) and WD repeat domain 41 (WDR41) (Sellier et al., 2016; Sullivan et al., 2016). This complex acts as a guanosine diphosphate (GDP)/guanosine triphosphate (GTP) exchange factor (GEF) for the activation of Rab8 and Rab39, which are involved in the formation or maturation of autophagosomes (Pilli et al., 2012; Seto et al., 2013), and interacts with the autophagy receptors SQSTM1/p62 and OPTN (Sellier et al., 2016). Furthermore, C9ORF72 can interact with ULK1, a key kinase in the control of autophagosome formation, and this interaction mediates the translocation of the ULK1 autophagy initiation complex to the phagophore via RAB1a (Webster et al., 2016). Understanding the role of these genes in autophagy may be beginning to provide a link in the pathogenic mechanism between ALS genes; for example, Rab8 has been identified as a modifier of toxicity in a *Drosophila* mutant CHMP2B model (West et al., 2015). Finally, another Rab GEF, Alsin (ALS2), has been associated with ALS (Yang et al., 2001). Alsin is an activator of Rab5 (Topp et al., 2004), which has been shown previously to regulate autophagy (Otomo et al., 2011; Ravikumar et al., 2008).

Hereditary Spastic Paraplegia

Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurodegenerative diseases characterized by length-dependent degeneration of upper motor neuron axons resulting in progressive lower limb spasticity. To date, more than 70 distinct loci (SPG1-72) and 50 spastic paraplegia genes have been identified; the type of HSP is designated by the locus with which it is associated (i.e., SPG1-56) (Fink, 2013). *SPG11*, encoding spatacsin, is the most prevalent gene mutated in autosomal recessive HSP. Spatacsin knockout mice have a reduced number of lysosomes in Purkinje cells and impaired autophagic lysosome reformation in neurons (Varga et al., 2015). SPG11 and SPG15 (encoding spastizin) are both similar in their symptoms and in their cellular mechanisms; loss or mutation of either gene disrupts autophagosome maturation and lysosomal biogenesis (Chang et al., 2014; Renvoisé et al., 2014). Spastizin also interacts with the Beclin 1-UVRAG (UV radiation resistance-associated gene)-Rubicon complex required for autophagosome maturation (Vantaggiato et al., 2013).

A rare form of HSP is caused by mutations in tectonin β propeller-containing protein 2 (TECPR2), encoded by SPG49. The loss of TECPR2 results in a reduction in the levels of LC3 along with a reduction in the lipidation of LC3 (Oz-Levi et al., 2012). TECPR2 contains a LIR, and its interaction with LC3 is required for maintenance of functional ER exit sites (Stadel et al., 2015). In patient fibroblasts with TECPR2 mutations, ER exit is inhibited, and this loss is associated with decreased autophagosome formation.

Lafora Disease

Lafora disease is a progressive neurodegenerative disease that manifests with myoclonus epilepsy caused by loss-of-function mutations in either malin or laforin, which form a complex. A characteristic feature of the disease is the formation of inclusions of insoluble forms of glycogen called polyglucosan bodies (or lafora bodies). Because malin and laforin have been implicated in glycogen metabolism, it was originally assumed that perturbations in this function cause Lafora disease, but there is also clear evidence to support a key role for dysfunctional autophagy in this disease. Knockout mice for either gene show decreased LC3-II levels, and this is thought to result from increased mTOR activity in the case of mutations in laforin (Aguado et al., 2010; Garyali et al., 2014), although the mTOR involvement is more controversial in the case of malin mutations (Criado et al., 2012; Garyali et al., 2014).

Charcot-Marie-Tooth Disease

Charcot-Marie-Tooth (CMT) diseases are hereditary motor and sensory peripheral neuropathies caused by inherited mutations in genes encoding either myelin-related (CMT type 1, CMT1) or axonal (CMT type 2, CMT2) proteins (Bird, 1993a). CMT type 1 X-linked disease, one of the most common neurological disorders, is caused by loss-of-function mutations in the gene *GJB1*

encoding the gap junction protein connexin 32 (Bird, 1993b; Xie et al., 2016). Recent studies reported that connexin 32 depletion reduces autophagosome biogenesis not via its gap junction function but, rather, through directly interacting with autophagy core proteins (VPS34, BECN1, and ATG16L1) to sequester them at the plasma membrane (Bejarano et al., 2014). Upon starvation-induced autophagy, ATG14 localizes with a complex containing connexins and several autophagy proteins, facilitating the recruitment of ATG9 and formation of autophagosomes required for the degradation of connexins (Bejarano et al., 2014). However, these data have not yet been validated in neurons or in the context of CMT1.

The genes associated with the axonal CMT type 2 disease include those encoding the dynein-dynactin motor protein complex, also known to cause distal spinal and bulbar muscular atrophy (Puls et al., 2005; Punetha et al., 2015). Many of these mutations affect trafficking pathways relevant to autophagy. *DYNC1H1* (dynein heavy chain 1) mutations, found in some familial cases of CMT2, directly affect neuronal retrograde axonal transport and phenocopy disease pathogenesis in rodent models (Weedon et al., 2011). Additionally, dynein mutations impair autophagosome-lysosome fusion by reducing the retrograde trafficking of autophagosomes to the microtubule-organizing center where lysosomes are clustered. This causes reduced autophagic clearance and accumulation of the aggregate-prone proteins (Jahreiss et al., 2008; Ravikumar et al., 2005). Other supportive evidence shows that mutations in the p150 subunit of dynactin cause motor neuron degeneration and result in protein aggregation (Levy et al., 2006). Additional genes affected in some cases of CMT2 encode endocytic-regulatory proteins such as dynamin. Both knockin mice bearing the dynamin 2 mutations (Durieux et al., 2012) and *Drosophila* models (*Shi* ortholog loss of function) (Fang et al., 2016) showed accumulation of immature autophagosome structures because of defects in autolysosome acidification rather than affecting the fusion step per se. Mutations in genes encoding proteins involved in endosomal maturation, such as RAB7, lead to impaired autophagic flux (Ganley et al., 2011; Spinosa et al., 2008). A recently characterized CMT2 mutation in valosin-containing protein (*VCP*) leads to a similar phenotype: accumulation of immature autophagosome and impaired autophagic flux (Gonzalez et al., 2014).

Lysosomal Diseases

Lysosomal diseases are rare inherited disorders with variable phenotypes. They represent the most common cause of neurodegeneration in childhood but can also result in neurological impairment in adults (Poupetová et al., 2010; Wraith, 2002). Most lysosomal storage disorders are caused by loss of function of specific lysosomal hydrolases, leading to the accumulation of the substrates of these enzymes and accumulation of general autophagic substrates because of impaired autophagosome-lysosome fusion (Ballabio and Gieselmann, 2009; Platt et al., 2012; Settembre et al., 2008).

There is increasing evidence that changes in membrane lipid composition as a result of lysosomal dysfunction contribute to lysosome fusion defects. In mouse models of mucopolysaccharidosis type III (Sanfilippo syndrome), defects in the breakdown of heparin sulfate cause an altered membrane lipid composition, with SNARE protein redistribution resulting in impaired autophagosome-lysosome fusion and a block in autophagy (Fraldi et al., 2010; Settembre et al., 2008). The accumulation of the glycosphingolipid psychosine in Krabbe disease, caused by a defect of β-galactocerebrosidase, alters membrane lipid composition (Hawkins-Salsbury et al., 2013). In Niemann-Pick type A disease, where mutations in the gene encoding acid sphingomyelinase cause accumulation of sphingomyelin, defects of mATG9 trafficking and autophagosome closure have also been observed (Corcelle-Termeau et al., 2016). Sphingomyelin storage also leads to lysosomal membrane permeabilization, thereby liberating cathepsins into the cytosol (Serrano-Puebla and Boya, 2016).

Apart from mutations in genes encoding lysosomal hydrolases, defects in posttranslational modifications, impaired trafficking of lysosomal enzymes, or defective acidification also result in lysosomal dysfunction (Colacurcio and Nixon, 2016; Hirst et al., 2015; Kyttälä et al., 2005; Morimoto et al., 1989; Tiede et al., 2005). One example is multiple sulfatase deficiency, in which a failed posttranslational modification of sulfatases by an ER-resident enzyme abrogates the function of a whole group of lysosomal hydrolases (Dierks et al., 2009).

Beside this, impaired lysosomal structure, regeneration, fusion, and signaling also contribute to lysosomal malfunction (Blanz et al., 2010; Chang et al., 2014; Cortes et al., 2014; Endo et al., 2015; Yu et al., 2010). For example, Niemann-Pick disease type C, caused by loss of NPC1 function, leads to impaired Ca^{2+} homeostasis and incorrect cholesterol trafficking with accumulation of unesterified cholesterol and glycosphingolipids in lysosomes and late endosomes, disrupting their fusion (Lloyd-Evans et al., 2008; Lloyd-Evans and Platt, 2010; Pacheco and Lieberman, 2008).

Recently, the group of diseases associated with lysosomal dysfunction has expanded. Mutations in SNX14, a sorting nexin phosphoinositol binding protein localized on the late endosome and lysosomal membrane and involved in cargo sorting upon endocytosis, were found in patients with hereditary cerebellar ataxia. Autophagosome clearance was slowed in patient cells, suggesting lysosome-autophagosome dysfunction (Akizu et al., 2015). A specific role for autophagosome-lysosome fusion in lysosomal disease pathology is underlined by the discovery of a missense mutation in VPS11 in patients with a rare form of leukoencephalopathy. VPS11 is a member of the homotypic fusion and protein sorting (HOPS) and class C core vacuole/endosome tethering (CORVET) complexes, and mutations lead to impaired autophagy. A zebrafish VPS11 mutant (*vps11(plt)*) shows a reduction in CNS myelination and extensive neuronal death in the hindbrain and midbrain (Zhang et al., 2016).

Core Autophagy Genes Implicated in Neurodegenerative Disease

As outlined above, mutations in what might be termed ''autophagy accessory'' genes have been identified in a number of diseases, but diseases have also been identified that result from mutations in core autophagy genes. Recently, an E122D *ATG5* mutation was identified in two siblings with childhood ataxia, characterized by lack of coordination and cerebellar hypoplasia (Kim et al., 2016). The E122D mutation weakens binding of ATG5 to ATG12, resulting in decreased autophagosome formation and reduced autophagy flux.

Another autophagy gene involved in disease is WDR45, a gene coding for the protein WIPI4, one of the four WIPI proteins (Proikas-Cezanne et al., 2004). WIPI proteins are key autophagic lipid sensors that facilitate autophagosome maturation by bridging PI3P production and LC3 lipidation in mammalian cells (Lamb et al., 2013). De novo mutations in WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood (SENDA) (Haack et al., 2012; Saitsu et al., 2013), also called β propeller protein-associated neurodegeneration (BPAN). In lymphoblastoid cell lines derived from BPAN patients, mutant WDR45 is less stable and degraded, resulting in decreased protein levels compared with unaffected individuals. These cells showed an impairment in autophagy flux and an accumulation of LC3-positive autophagosome membranes (Saitsu et al., 2013). CNS-specific WDR45 knockout mice exhibit some aspects of the BPAN phenotype: poor motor coordination, impaired learning and memory, and extensive axon swelling with numerous axon spheroids, along with autophagy perturbation in the form of accumulation of SQSTM1/p62 and ubiquitin-positive aggregates (Zhao et al., 2015).

Autophagy Therapeutics for Neurodegenerative Diseases

The neuropathology of protein misfolding diseases is intimately linked with the propensity of specific proteins to misfold and self-associate, giving rise to an array of oligomers and aggregates. Because many of these proteins cause disease primarily via toxic gain-of function mechanisms, one way of combatting disease is to reduce the levels of such proteins (Ciechanover and Kwon, 2015). Furthermore, most aggregate-prone, neurodegenerative disease-associated proteins have been shown to be autophagy substrates, including mutant huntingtin, α-synuclein, and tau (Berger et al., 2006; Ravikumar et al., 2002, 2004; Webb et al., 2003; Rubinsztein et al., 2012). Importantly, induction of autophagy reduces the levels of both the soluble and aggregated species in such models and is associated with beneficial effects. It is likely that the autophagic process is capturing small oligomeric species and not the very large aggregates visible by light microscopy—the reduction of the large aggregates after autophagy induction may reflect a reduced input of the smaller species into the aggregates and the flux of the mutant proteins on and off the aggregates (Berger et al., 2006; Ravikumar et al., 2002, 2004; Webb et al., 2003).

Many small molecules employed to induce autophagy have an impact on cellular effectors with pleiotropic effects, such as mTOR and AMPK (Fleming et al., 2011; Levine et al., 2015). Nevertheless, in many cases, the effects of such autophagy modulators on the levels of the substrates have been shown to be autophagy-dependent (e.g., Williams et al., 2008). Table S1 lists key autophagy enhancing molecules that have been validated in vivo, their mechanism of action, biological targets, and CNS penetration profiles.

Autophagy inducers can be classified into two main groups: those acting via mTOR-dependent or mTOR-independent targets. mTOR inhibitors are either ATP-competitive inhibitors (e.g., Torin1 and related compounds) or non-ATP-competitive inhibitors (e.g., rapamycin and rapalogs) (Kim and Guan, 2015). Because of their inhibition of mTORC1, mTORC2, and, in some cases, PI3K activities, chronic dosing of ATP-competitive inhibitors of mTOR activity in animals presents significant toxicity issues (Kim and Guan, 2015), and, for this reason, they are generally unsuitable for studies in mouse models of neurodegeneration, which typically require chronic dosing. Although mTORC1 inhibits autophagy, mTORC2 is required for autophagy, and the autophagy inhibition resulting from prolonged exposure to such compounds may explain at least some of their toxicity in vivo (Renna et al., 2013). Rapamycin and rapalogs have relatively safer profiles because of their non-ATP competitive mode of action and apparent selectivity for mTORC1 (Wander et al., 2011), allowing everolimus (a rapalog; Lebwohl et al., 2013) to be recently approved by the Food and Drug Administration (FDA) for the treatment of tuberous sclerosis. Rapamycin and its analogs have shown benefits as autophagy inducers in animal models of AD, PD, FTD, HD, and prion protein (PrP) (Cortes et al., 2012; Jiang et al., 2014; Menzies et al., 2010; Ozcelik et al., 2013; Ravikumar et al., 2004; Sarkar et al., 2008; Spilman et al., 2010; Wang et al., 2013b). The therapeutic efficacy of rapamycin in SOD1 ALS mouse models is complex, with some studies reporting detrimental effects (Zhang et al., 2011) and others reporting beneficial effects only after uncoupling of its immunomodulatory function (Staats et al., 2013).

Many mTOR-independent autophagy activators signal via AMPK. Trehalose, a widely studied autophagy inducer in neurodegeneration models (Sarkar et al., 2007), has recently been characterized as acting via AMPK activation (DeBosch et al., 2016). The molecular targets of this disaccharide have been proposed to be GLUT proteins, a family of glucose transporters whose inhibition by trehalose results in AMPK activation (DeBosch et al., 2016). Trehalose dosing in mice has shown therapeutic effects, concomitant with autophagy induction, in an impressive variety of mouse models of neurodegeneration, including AD, PD, FTD, HD, SCA17, PrP, ALS, and oculopharyngeal muscular dystrophy (OPMD) (Aguib et al., 2009; Castillo et al., 2013; Chen et al., 2015; Davies et al., 2006; Du et al., 2013; Li et al., 2015; Rodríguez-Navarro et al., 2010; Schaeffer and Goedert, 2012; Schaeffer et al., 2012; Tanaka et al., 2004; Tanji et al., 2015; Zhang et al., 2014).

Metformin is another AMPK-dependent autophagy inducer that has shown beneficial effects in animal models of neurodegeneration. Metformin has shown efficacy in animal models of AD, HD, and Lafora disease (Berthier et al., 2016; Ma et al., 2007; Son et al., 2016) but showed no efficacy in ALS models (Kaneb et al., 2011). Berberine, which showed efficacy in an HD model (Jiang et al., 2015), may also induce autophagy via AMPK activation (Yu et al., 2014). Methylene blue, a reported anti-aggregant and AMPK activator (Shin et al., 2014), was shown to induce autophagy and ameliorate the disease phenotype in FTD models (Congdon et al., 2012; Hochgräfe et al., 2015; Melis et al., 2015). Nilotinib, a c-Abl kinase inhibitor and AMPK activator (Yu et al., 2013), was shown to induce autophagy and ameliorate the disease phenotype in PD mice (Hebron et al., 2014; Lonskaya et al., 2015).

Finally, a growing number of autophagy inducers are being characterized that may act on other pathways, including compounds acting on the modulation of cyclic AMP (cAMP)/inositol triphosphate (IP_3), such as rilmenidine, clonidine, minoxidil,

and verapamil, which have been shown to ameliorate phenotypes in animal models of HD (Rose et al., 2010; Williams et al., 2008; Table S1).

In addition to the small molecules with in vivo validation reviewed above, there are other molecules that have been shown to be active in cell-based models but not yet in vivo. These are presented, with their mechanism of action and any available evidence for potential CNS penetration, in Table S2.

Non-Small-Molecule Approaches

In addition to small-molecule therapeutics, a number of other strategies for autophagy enhancement have been adopted. Shoji-Kawata et al. (2013) have reported the development of a Tat-Beclin 1 peptide that consists of the HIV-1 Tat protein transduction domain linked to a modified 18 amino acid sequence based on the residues 267–284 of Beclin 1. This region was identified as being required for the autophagy-inducing effects of Beclin 1. The Tat-Beclin 1 peptide was found to increase autophagic flux via the canonical pathway using a range of cell models and to decrease the number of small huntingtin aggregates in HeLa cells. It was also shown that Tat-Beclin 1 increased GFP-LC3 dots in a range of tissues in a transgenic mouse model (Shoji-Kawata et al., 2013).

A number of mouse studies exploring the potential of gene therapy have been reported. For example, delivery of the TFEB gene ameliorates signs of disease in a PD model based on adeno-associated virus (AAV) vector-mediated overexpression of human wild-type α-synuclein in the rat midbrain (Decressac et al., 2013), and, in a transgenic mouse model overexpressing tau, TFEB was able to decrease the levels of pathological tau and improve cognitive performance and synaptic function (Polito et al., 2014). Beneficial effects have also been reported with Beclin 1 overexpression in an α-synuclein model of PD/Lewy body diseases (Spencer et al., 2009) and in a model of Machado-Joseph disease (Nascimento-Ferreira et al., 2011). Parkin overexpression has beneficial effects in an AD model (Khandelwal et al., 2011).

Another possible candidate target for such approaches is calpain. Knockdown of calpain in a Huntington's disease *Drosophila* model (RNAi) results in upregulation of autophagy, reduction in huntingtin aggregate number, and increases cell survival and is also protective against tau toxicity in HD and tauopathy models, respectively (Menzies et al., 2015b). In mice, neuronal overexpression of calpastatin (CAST), an endogenous inhibitor of calpain, increased autophagy and confers protection when crossed to HD mice, improving aggregate burden, behavioral measures of degeneration, and long-term survival (Menzies et al., 2015b). These CAST transgenic mice were compared with wild-type littermates, and no deficits were found by SmithKline Beecham, Harwell, Imperial College, Royal London Hospital phenotype assessment (SHIRPA) behavioral testing or in survival, suggesting that such a strategy may be well tolerated.

Considerations for Autophagy as a Therapeutic Strategy in Neurodegeneration

Taken together, the evidence from pharmacological efficacy mouse studies suggests that autophagy activation has significant therapeutic potential across a wide range of neurodegenerative diseases. Although the majority of studies of autophagy-modulating approaches in neurodegenerative disease models have reported beneficial effects on disease signs, this is not universally true (Hernandez et al., 2012). For example, in the SOD1 G93A mutant model of ALS, treatment with rapamycin decreased the lifespan and increased motor neuron degeneration (Zhang et al., 2011). However, rapamycin is protective in such mice lacking mature lymphocytes, suggesting that the immunosuppressive effects of this drug (unrelated to autophagy) may be deleterious in this disease and may counterbalance the protective effects of autophagy induction (Staats et al., 2013). Indeed, an alternative mTOR-independent autophagy inducer, trehalose, is protective in this SOD1 ALS model (Zhang et al., 2014).

Similarly, the time point in disease at which autophagy is induced may be critical. For example, many studies have demonstrated the protective effect of autophagy upregulation on α-synuclein toxicity (e.g., Decressac et al., 2013; Hebron et al., 2014; Spencer et al., 2009). However, at least in tissue culture, α-synuclein aggregate-containing cells appear to have impaired autophagosome maturation, and autophagy upregulation may actually be toxic under these conditions (Tanik et al., 2013). Likewise, the point of intervention in the autophagy pathway might be an important consideration. For example, where autophagosome clearance or degradation is impaired, upstream induction of autophagy may lead to a largely unproductive accumulation of autophagosomes, possibly leading to toxic consequences; this may be avoided by more directly targeting lysosomal function or using approaches that enhance the entire pathway, including increasing lysosomal capacity (for example, with TFEB, as discussed above). Thus, one should ideally understand autophagy dynamics in diseases one is aiming to treat to best tailor the therapeutic approach to the most suitable diseases. Further developments in autophagy-based therapeutics for neurodegeneration must await the advent of sharper pharmacological tools for autophagy induction, with more selective molecular targets within the autophagic process, and of higher resolution readouts for different steps of the autophagic flux with which autophagy dysfunction in different neurodegenerative diseases can be profiled.

Conclusions

In this review, we have highlighted how autophagy defects at various stages of the pathway may be seen in diverse neurodegenerative conditions and that autophagy upregulation may be a promising therapeutic strategy for some diseases. However, the roles of autophagy in health and disease may be more complex and not simply cell-autonomous. For example, autophagy affects inflammatory and immune processes, which are increasingly being implicated in various neurodegenerative diseases (Rubinsztein et al., 2015). Future studies aiming to understand non-cell-autonomous effects of autophagy in the nervous system and its relevance to neuronal-glial interactions are likely to be informative.

From a therapeutic perspective, we believe that autophagy is a promising target mechanism. In many cases, it enhances the removal of the primary toxic entity causing disease (e.g., mutant tau or mutant huntingtin) and, thus, targets such diseases at their roots. Autophagy upregulation has additional protective effects

by reducing the susceptibility to pro-death insults (Boya et al., 2005; Ravikumar et al., 2006). One benefit of autophagy upregulation as a therapeutic approach is that one need not require constitutive activation of the pathway because a pulsatile strategy (like periodically taking out the rubbish) may be sufficient to have efficacy, especially when employed over a long time period. This would have major benefits from a drug toxicity perspective.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.01.022.

AUTHOR CONTRIBUTIONS

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