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The Autophagy Lysosomal Pathway and Neurodegeneration

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The autophagy lysosomal pathway (ALP) is a major mechanism for degrading intracellular macromolecules. The catabolic products can then be used by the cell for energy or as building blocks to make other macromolecules. Since its discovery, a variety of cellular pathways have emerged that target components with varying specificity for lysosomal degradation. Under some circumstances, lysosomes may release their contents into the extracellular space where they may serve signaling or pathogenic functions. The ALP is active in healthy cells, and the level of activity can be regulated by nutrient-sensing and metabolic signaling pathways. The ALP is the primary pathway by which lipids and damaged organelles are degraded and may be the only pathway capable of degrading aggregated proteins. As such, there has been intense interest in understanding the role of the ALP in the accumulation of aggregated misfolded proteins characteristic of many of the major adult-onset neurodegenerative diseases. This review focuses on recent advances in our understanding of the ALP and its potential relationship to the pathogenesis and treatment of neurodegenerative diseases.

HISTORY

The work of Drs. Christian de Duve and Yoshinori Ohsumi led to the discovery of the autophagy lysosomal pathway (ALP), and the contributions of each were recognized with the awards of the Nobel prize in physiology or medicine in 1974 and 2016, respectively. de Duve discovered the membrane-bound acidic compartment called the lysosome with biochemistry approaches (De Duve and Wattiaux 1966). Ohsumi discovered the core machinery responsible for autophagy by identifying the genes in yeast that are necessary for survival in the setting of caloric restriction (Klionsky et al. 2003; Tooze and Dikic 2016).

LYSOSOME—THE HUB OF A PROTEOSTASIS NETWORK

The common way to portray the ALP is as a unidirectional pathway that begins with the de novo formation of double-membrane organelles called autophagophores and culminates in the fusion of autophagosomes and their contents with lysosomes to form autophagolysosomes, in which the contents of the autophagosomes are degraded. However, further study of the ALP has revealed multiple distinct pathways to the lysosome including nonspecific macroautophagy, substrate-specific forms of autophagy (e.g., mitophagy), chaperone-mediated autophagy (CMA), microautophagy, and micropinocy-

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tosis, to name a few (Fig. 1). One common feature of all these pathways is that degradation ultimately occurs in the lysosome. For this reason, it may be easier for the reader to understand the ALP using a hub and spoke model (Nixon et al. 2008; Perera and Zoncu 2016). Here, the hub, the lysosome, will be introduced first and then the different pathways into and out of the lysosome will be described.

Lysosomes are defined as membrane-bound organelles delimited by a single-lipid bilayer and characterized by an acidified milieu (pH ~4.5).

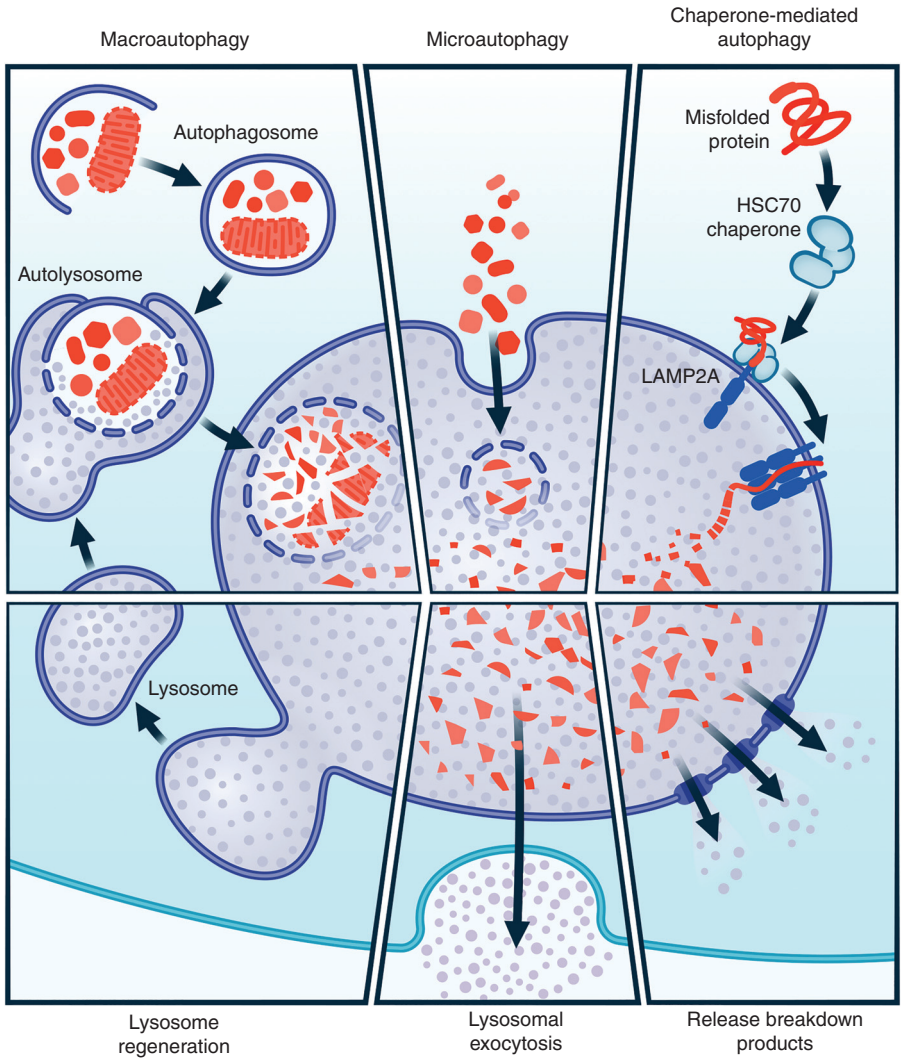

Figure 1. The autophagy lysosomal pathway. The lysosome is the hub of a network of pathways that feed cargo into its lumen for degradation. These include pathways depicted here to deliver intracellular cargo such as macroautophagy, chaperone-mediated autophagy and microautophagy, as well as others that deliver extracellular cargo to the lysosome including endocytosis and micropinocytosis (see text for details). Cargo delivered to the lysosome can undergo degradation into molecular building blocks that can return to the cytoplasm to be catabolized further to supply cellular energy needs or to be reused in the synthesis of new macromolecules. Contents of lysosomes can also be extruded extracellularly by a Ca^{2+}-dependent exocytic process. LAMP2A, Lysosome-associated membrane glycoprotein 2; HSC70, heat shock cognate 71.

The membrane contains a variety of structural and signaling proteins, channels, transporters and trafficking, and fusion machinery. For example, the lysosomal membrane v-type ATPase (v-ATPase) pumps protons across the membrane and is the major mechanism for generating an acidic lumen and a pH gradient. Lysosomes are frequently located in the perinuclear area in the cell soma but undergo trafficking and fusion, mediated by a subset of membrane-associated RAB GTPases and SNARE proteins (Ao et al. 2014; Perera and Zoncu 2016). RAB5 and RAB7 function specifically to tether and dock endolysosomal membranes. LAMP1 makes up ~50% of the lysosomal membrane protein, and it couples lysosomes to transport machinery.

Lysosomes contain approximately 60 soluble hydrolases, which are active at acidic pH, and carry out the degradation of macromolecules transported from the cytoplasm to the lumen of the organelle. Membranes and lipids are degraded within intralysosomal vesicles that contain specialized hydrolases and activator proteins suited to the task. Lipid droplets shuttled to lysosomes are hydrolyzed into free fatty acids and glycerol. Catabolites such as short peptides, amino acids, and other molecules are translocated from the lumen to the cytoplasm or other cellular compartments for the cell to use.

Lysosome biogenesis is coordinated by transcription factors that belong to the microphthalmia-transcription factor E family (MiT), including transcription factor EB (TFEB) and TFE3 (Settembre et al. 2011). They bind to a DNA response element motif known as the coordinated lysosomal expression and regulation (CLEAR) element within genes that encode proteins required for lysosomal biogenesis, autophagy, exocytosis, and endocytosis. In effect, CLEAR-dependent transcription promotes processes that lead to the isolation from the cytoplasm of unwanted cytoplasmic macromolecules, and their permanent removal via degradation and/or exocytosis (Decressac et al. 2013; Polito et al. 2014). TFEB and TFE3 are normally located in the cytoplasm, bound to the scaffold 14-3-3 protein owing to phosphorylation of two serines in the transcription factors by TFEB kinases such as ERK2 or mTORC1. In response to stresses such as starvation or lysosomal dysfunction, the lysosomal nutrient sensing (LYNUS) machinery, which sits on the cytoplasmic side of the lysosomal membrane, senses the nutrient content of the lysosome, and communicates the information to the nucleus (Alers et al. 2012; Inoki et al. 2012a; Lin et al. 2012; Settembre et al. 2013; Shanware et al. 2013). TFEB is dephosphorylated by mTOR, translocates to the nucleus, and drives the transcription of CLEAR-containing genes (Settembre et al. 2013). For example, TFEB induces expression of PPARα and PGC1α, which play a role in lipid oxidation and ketogenesis (Settembre et al. 2011, 2013; Tsunemi et al. 2012), emphasizing the tight integration of these catabolic processes. TFEB was also shown to be the main mediator of PGC1α-induced improvement in a mouse model of Huntington's disease (Tsunemi et al. 2012). Transcription of TFEB itself is controlled via an autoregulatory loop (Settembre et al. 2013).

Although it is well established that the protein composition of lysosomes evolves during biogenesis and maturation, an important unanswered question in the field is whether functionally important subpopulations of mature lysosomes exist within different cell types or even within the same cell. Lysosomes uniformly express LAMP-1, but other lysosome-associated proteins are expressed more variably. For example, LAMP-2A, which is required for CMA, is absent from ~80% of lysosomes at the baseline (Cuervo et al. 1997; Kaushik and Cuervo 2012). It is unclear whether the heterogeneity reflects functionally distinct lysosomes in which production is regulated, or if it is related to stochastic variations in protein sorting or maturation. Lysosomes undergo "reformation" (Yu et al. 2010), suggesting that there is a lot of exchange between different endocytic compartments.

PATHWAYS TO THE LYSOSOME—MACROAUTOPHAGY

Macroautophagy is the major intracellular pathway by which intracellular cargo are delivered to the lysosome (Biswas et al. 2008). The complex biochemistry of the pathway is excellently reviewed elsewhere (Klionsky 2005, 2007; Ya-

mamoto and Yue 2014; Bingol 2018). Induction is governed by the Ulk1 complex, which induces the nucleation of the isolation membrane (IM) or phagophore. Then, the Beclin1-Atg14L-Vps34 lipid kinase complex catalyzes the production of PI3P at the IM, resulting in the recruitment of PI3P-binding proteins such as WIPI-1, -2, and DFCP1 (Ferguson et al. 2009). The sites of autophagy induction and the source of membranes has been controversial (Mari et al. 2011); endoplasmic reticulum (ER) and ER-mitochondrial contacts are likely sources but others have been suggested (Axe et al. 2008; Itoh et al. 2008). The IM expands and envelops the cargo in a process that requires Atg9 and two ubiquitin-like conjugation systems, which trigger the covalent attachment of the lipid phosphatidylethanolamine (PE) to Atg8 or its mammalian homolog, microtubule-associated protein 1 light chain 3 (LC3) (Nakatogawa et al. 2007; Fujita et al. 2008; Sou et al. 2008). Embedded into the autophagosome membrane via the PE moiety, LC3-II serves as an adaptor protein that binds cargo being engulfed by the autophagosome through other adaptor proteins described below (Tan et al. 2008; Tung et al. 2010). The mature autophagosome fuses with lysosomes by a mechanism that likely depends on SNAREs to form autophagolysosomes, exposing cargo to the lysosomal degradation machinery for digestion (Jäger et al. 2004; Stroikin et al. 2004; Cai et al. 2010; Yuzaki 2010; Itoh et al. 2011; Hyttinen et al. 2013; Albanesi et al. 2015; Murrow et al. 2015). Interestingly, although LC3 remains the most specific known marker of autophagosomes, LC3-independent forms of autophagy have been described (Kuma et al. 2007; Szalai et al. 2015; Engedal and Seglen 2016).

Initially, macroautophagy was thought to be nonselective, with phagophores scooping up and engulfing cytoplasm and cargo that happen to be in the neighborhood. Subsequently, a large number of adaptor proteins have been discovered, including p62/SQSTM1 (Sequestosome 1), NBR1, Nix, NDP52, Alfy/WDFY3, and OPTN (optineurin), which play a role in targeting specific cargo for autophagic clearance including protein aggregates, mitochondria, other damaged organelles, etc. (Fortun et al. 2003; Komatsu et al. 2007, 2010; Farré et al. 2008; Geisler et al. 2010; Brady et al. 2011; Matsumoto et al. 2011; Lim et al. 2015). Many of these adaptors contain ubiquitin- and LC3-interacting regions and often undergo oligomerization, which increases the avidity of their binding to cargo. In some cases, binding is regulated by clinically relevant modulators of these adaptor proteins such as TBK1, which phosphorylates OPTN (Wild et al. 2011). Defining the extent of substrate specificity and the associated mechanisms of regulation are a major focus of ongoing research in the field (Anding and Baehrecke 2017).

PATHWAYS TO THE LYSOSOME—MICROAUTOPHAGY, MULTIVESICULAR BODIES, AND PINOCYTOSIS

Another path to lysosomal degradation is microautophagy (Sahu et al. 2011; Shpilka and Elazar 2011) and the closely related endosomal microautophagy (Bingol 2018). These relatively less studied pathways begin with the invagination of the lysosomal membrane. Eventually, the invaginations, which contain contents from the cytoplasm, pinch off to form vesicles in the lysosomal lumen (Shpilka and Elazar 2011). The degree of substrate specificity and relative importance of microautophagy for physiology remains unclear.

PATHWAYS TO THE LYSOSOME—CHAPERONE-MEDIATED AUTOPHAGY

CMA is a pathway to translocate polypeptides with the KFERQ motif directly from the cytoplasm into the lumen of the lysosome (Olson et al. 1991; Kiffin et al. 2004). Translocation is mediated by LAMP-2A and HSP8A, a member of the HSP70 family. Interestingly, the KFERQ motif is found in ∼30% of the proteome and in many proteins implicated in neurodegenerative diseases including α-synuclein (Olson et al. 1991; Cuervo et al. 2004; Martinez-Vicente et al. 2008). Importantly, and unlike macroautophagy, proteins evidently need to be translocated into the lysososme as monomers, so CMA may be less effective than autophagy at degrading aggregated protein. Nevertheless, induction of

CMA has been shown to accelerate the clearance of some disease-causing proteins (Massey et al. 2006; Bauer et al. 2010; Wang et al. 2010b), which presumably reduces their propensity to accumulate and aggregate.

OTHER PATHS TO THE LYSOSOME

Autophagy, microautophagy, and CMA are pathways to convey cytoplasmic contents to the lysosomal lumen for degradation. Additional pathways, including macropinocytosis, phagocytosis, and endocytosis can bring extracellular material to the lysosome for degradation (Binotti et al. 2015). In cancer cells, macropinocytosis may be a critical way to take up macromolecules for metabolic support, but relatively less is known about the role and importance of macropinocytosis in neurons. In the context of neurodegenerative disease, these pathways may be important routes by which extracellular aggregation-prone proteins enter neurons and transmit proteinopathy between neighboring cells. An important future direction will be to better understand mechanisms by which misfolded proteins are taken up by neurons and how they get access to the cytosol to template and propagate misfolded protein conformations.

PATHWAYS FROM THE LYSOSOME

Cargo delivered to the lysosome can be degraded into substituent molecular building blocks, then released into the cytoplasm to be metabolized or reused in the synthesis of new macromolecules. However, lysosomes or autolysosomes can also translocate to the plasma membrane, and fuse and release their contents into the extracellular space (Takenouchi et al. 2009; Settembre et al. 2013). This process of lysosomal exocytosis is believed to begin with activation of the lysosome, causing kinesin-associated lysosomes to be trafficked to the plasma membrane (Perera and Zoncu 2016). Ca^{2+} efflux from the lysosome mediated by the TRPML1 channel is likely critical for triggering SNARE-mediated plasma membrane fusion (Xu and Ren 2015; Perera and Zoncu 2016). TRPML1 gating is modulated by lysosomal pH and by phosphoinositides, such as $PI(3,5)P_2$, $PI(4,5)P_2$, $PI(3,4)P_2$, and $PI(3,4,5)P_2$. Interestingly, lysosomal Ca^{2+} release and exocytosis may be triggered in neurons by back-propagating action potentials, and exocytosed proteolytic enzymes may play a role in synaptic plasticity (Padamsey et al. 2017). Fe^{2+} reportedly accumulates in lysosomes, likely as a consequence of degrading Fe^{2+}-containing cellular proteins, and abnormal neuronal accumulation of Fe^{2+} is a feature of neurodegenerative diseases (Kwan et al. 2012; Wang et al. 2016; van Duijn et al. 2017). Whether that is so because of lysosomal dysfunction is not known. How lysosomal exocytosis is related to other release mechanisms, including exosomes, is also not fully understood (Baixauli et al. 2014; Poehler et al. 2014).

ALP IN THE CONTEXT OF THE PROTEOSTASIS NETWORK

It is important to remember that the ALP does not function in isolation. It exists within an elaborate proteostasis network and signaling system that enables cells to sense and respond to metabolic changes (Crighton et al. 2006; Maiuri et al. 2010; He et al. 2012). Although the ALP plays an important role in the turnover of proteins, other pathways including the unfolded protein response (UPR) and the ubiquitin proteasome system (UPS) are also critical (Webb et al. 2003; Keller et al. 2004; Kabuta et al. 2006; Wang et al. 2009, 2010a; Benbrook and Long 2012; Wang and Mandelkow 2012; Lee et al. 2013), and there is abundant evidence that there is cross talk between these pathways (Bernales et al. 2006; Pandey et al. 2007; Matus et al. 2008; Kirkin et al. 2009; Rouschop et al. 2010; Watanabe et al. 2010; Senft and Ronai 2015). For example, inhibitors of the UPR are capable of inducing autophagy, perhaps because they lead to ER-associated degradation and the extrusion of aggregated protein to the cytoplasm that must be cleared by autophagy (Høyer-Hansen and Jäättelä 2007; Hetz et al. 2009; Suh et al. 2012). Cross talk and redundancy between pathways probably explain how cells and organisms can survive at least some significant impairments in specific components of the proteostasis network.

ALP IN NEURODEGENERATIVE DISEASE—DO NEURONS MANAGE AUTOPHAGY DIFFERENTLY?

The genetics of autophagy were elucidated in yeast, and the majority of autophagy studies involve the use of nonneuronal cells (Kim and Klionsky 2000), raising the question of whether neuronal autophagy is unique (Larsen and Sulzer 2002; Boland and Nixon 2006; Moruno Manchon et al. 2016). Because neurons are postmitotic, they have lost one important mechanism to clear long-lived proteins—cell division and dilution (Eden et al. 2011). Neurons also have an elaborated morphology, with long dendrites and axons that can be a meter long or longer, and therefore must manage the degradation of cargo at distant sites (Shen and Ganetzky 2009; Bowling and Klann 2014; Tang et al. 2014). The cell biology of the autophagy pathways in neurons was initially characterized by Holzbaur and colleagues, who showed that autophagosomes are formed constitutively in axon terminals, and are trafficked to the cell body (Maday and Holzbaur 2014). En route, the protein composition gradually resembles mature lysosomes and the intralumenal pH drops. Eventually, in the soma, they fuse with lysosomes to form autophagolysosomes, and the degradation of their contents ensues.

In addition, neurons in the central nervous system are substantially buffered by astrocytes from large variations in nutrients that can occur elsewhere in the body and that can induce starvation-dependent activation of mTOR-dependent autophagy. Indeed, a number of studies have suggested that mTOR-dependent autophagy may be regulated in neurons differently than in nonneuronal cells (Boland et al. 2008). For example, inhibition of mTOR with rapamycin or sirolimus induces autophagy less effectively in neurons compared with nonneuronal cells (Tsvetkov et al. 2010; Krüger et al. 2012). Rapamycin binds FKB12, a nonobligate component of the mTORC1 complex, but not the mTORC2 complex, and one study has suggested that differences in the TORC complexes in neurons could contribute to the differences in reported efficacy of rapamycin (Roscic et al. 2011). There is also evidence that subtypes of neurons may differ in their dependence on autophagy (Hara et al. 2006; Komatsu et al. 2006; Friedman et al. 2012).

ALP IN NEURODEGENERATIVE DISEASE—WHAT IS THE EVIDENCE?

A common thread cutting across multiple adult-onset neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's, Huntington disease, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia, is the abnormal deposition of misfolded aggregated protein(s) (Friedman et al. 2012). Given the critical role of autophagy in the clearance of aggregated protein, this could indicate that autophagy dysfunction is a common mechanism in neurodegenerative disease.

That autophagy dysfunction may be a major mechanism in neurodegenerative disease is further suggested from the established role of mitochondrial damage and associated deficits in bioenergetics or excessive reactive oxygen species in these conditions (Beal et al. 2006; Lezi and Swerdlow 2012; Ryan et al. 2015; Wang 2017). Because autophagy is the principal pathway by which cells remove catastrophically damaged mitochondria (Youle and Narendra 2011), it stands to reason that impairment in this process may result in abnormal accumulation of deleterious mitochondria (Dagda et al. 2008; Narendra et al. 2008; Ferree et al. 2013; Lemasters 2014; Lazarou et al. 2015; Chen et al. 2017; Jinn et al. 2017).

More broadly, the link between autophagy and aging may be an important consideration (Meléndez et al. 2003; Bergamini et al. 2004; Rubinsztein et al. 2011; Inoue et al. 2012b; Carnio et al. 2014). Aging is the number-one risk factor for neurodegenerative diseases, and is associated with down-regulation of autophagy in the brain (Lipinski et al. 2010). Conversely, induction of autophagy has been shown in model organisms to increase their longevity (Simonsen et al. 2008; Zheng et al. 2010; Pyo et al. 2013). More importantly, autophagy induction is associated with an increase in their health span and quality of life, suggesting that the promotion of

autophagy may confer a more fundamental improvement in quality of the function of biological systems that could bring broad benefits (Cuervo et al. 2005; Levine et al. 2011; Oka et al. 2012; Kang et al. 2015).

Beyond the intuitive appeal of the pathway as a therapeutic target (Levine and Kroemer 2008; Nixon 2013), there is a remarkable genetic association between neurodegenerative disease and autophagy. Mutations in many different genes associated with different steps in autophagy have been linked to AD, Parkinson's, or Huntington's disease, as well as ALS, frontotemporal dementia, and others (Fig. 2; Table 1) (Kegel et al. 2000; Petersén et al. 2001; Skibinski et al. 2005; Ramirez et al. 2006; Cheung and Ip 2009; Ju et al. 2009; Montie et al. 2009; Winslow et al. 2010; Dehay et al. 2012; Lucin et al. 2013; Ochaba et al. 2014; Wong and Holzbaur 2014; Martin et al. 2015; Ciura et al. 2016; Sellier et al. 2016; Sullivan et al. 2016; Manzoni 2017; Fujikake et al. 2018), indicating that mutations and variants in genes associated with autophagy are sufficient to cause neurodegenerative disease in some cases. Indeed, mutations in some genes critical for lysosome function lead to lysosomal storage disorders that cause neurological symptoms in childhood (Nixon et al. 2008). The extent to which a mismatch between the production and autophagic clearance of misfolded proteins exists in the much more common cases of idiopathic or sporadic AD and Parkinson's disease or ALS is not known (Ahmed et al. 2012). However, recent studies in patients with AD and the dynamics of amyloid β turnover suggest the intriguing possibility that genetic forms of AD may cause an overproduction of misfolded proteins, whereas idiopathic forms may be associated with reduced clearance, with all forms of AD associated with a fundamental mismatch between production and clearance, and the same may hold for other common but idiopathic forms of neurodegenerative disease (Mawuenyega et al. 2010).

A number of studies have sought to further determine whether brains of patients with neurodegenerative disease exhibit morphological abnormalities that indicate autophagic malfunction. Although widespread abnormalities have been reported, the nature of the morphological changes appear to be, at least partly, disease-specific (Anglade et al. 1997; Sikorska et al. 2004; Nixon 2007; Boland et al. 2008; Chu et al. 2009; Crews et al. 2010; Lucin et al. 2013; Yamamoto and Yue 2014; Martin et al. 2015; Fujikake et al. 2018). Some of these differences have been highlighted in excellent recent reviews (Nixon 2013; Yamamoto and Yue 2014). That different neurodegenerative diseases exhibit sometimes striking differences in patterns of autophagy-associated cytopathology underscores the importance of defining the underlying disease-associated mechanisms. This knowledge will help guide approaches to the development of effective therapeutics and for stratifying patient populations to do sensitive clinical trials.

Based in part on these lines of reasoning, many groups have found evidence in nonhuman models of neurodegenerative disease that autophagy modulation accelerates clearance of disease-causing proteins (Ravikumar et al. 2002; Sarkar et al. 2007a; Dolan and Johnson 2010; Tsvetkov et al. 2010; Wang et al. 2010a; Roscic et al. 2011; Congdon et al. 2012; Kruger et al. 2012; Barmada et al. 2014; Polito et al. 2014; Marrone et al. 2018) and improves disease phenotypes (Ravikumar et al. 2004; Jia et al. 2007; Sarkar et al. 2007b; Hetz et al. 2009; Montie et al. 2009; Spencer et al. 2009; Bauer et al. 2010; Rodríguez-Navarro et al. 2010; Tsvetkov et al. 2010; Watanabe et al. 2010; Schaeffer et al. 2012; Castillo et al. 2013; Decressac et al. 2013; Barmada et al. 2014; Höllerhage et al. 2014; Polito et al. 2014). It is worth noting that some important limitations apply to many of these studies. The assays available to measure autophagy induction are limited in their sensitivity. In general, these assays depend on making inferences about flux based on snapshots of levels of pathway intermediates, which can be prone to misinterpretation, even failing to distinguish upstream autophagy induction with downstream blockade (Klionsky et al. 2011, 2012, 2016). One assay that avoids this pitfall is the optical pulse labeling approach, which monitors the dynamic clearance of an autophagy substrate fused to a photo-switchable protein in live cells, allowing clearance to be calculated directly (Barmada

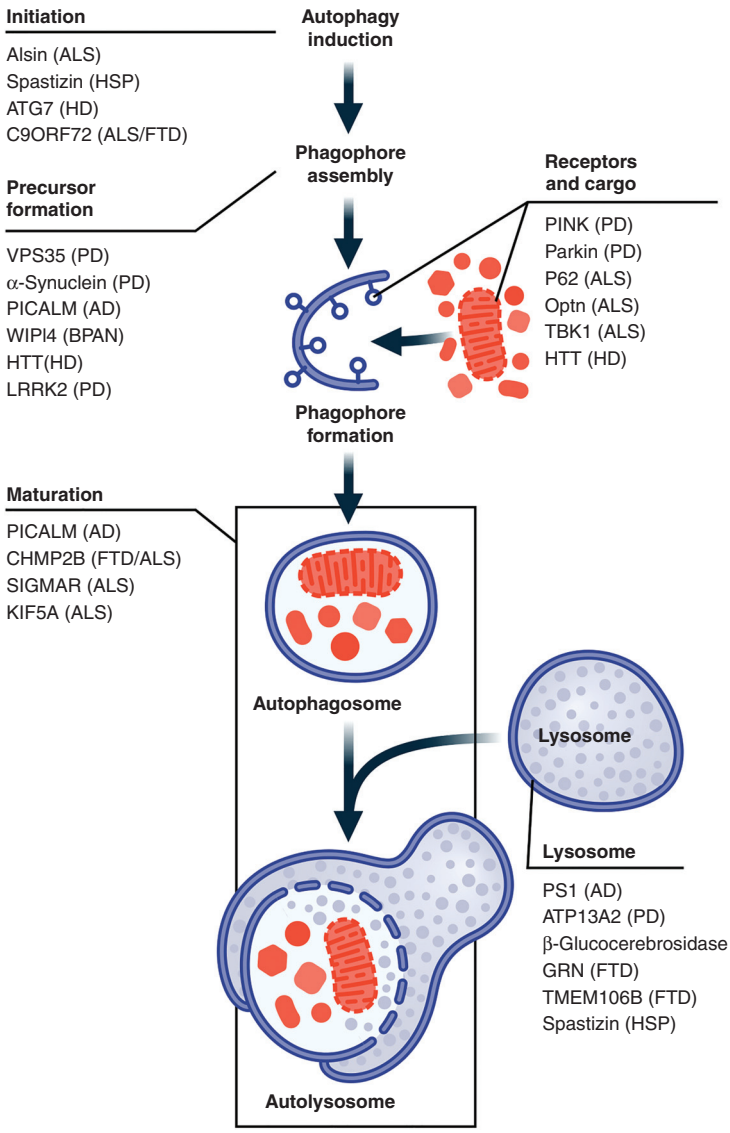

Figure 2. Genetic links between autophagy and neurodegenerative disease. Mutations in many genes that encode proteins that play a role in the autophagy lysosomal pathway (ALP) lead to neurodegenerative disease syndromes in humans, indicating that impairment of the ALP can be sufficient to produce neurodegenerative disease. Some examples are shown in this figure. AD, Alzheimer's disease; ALS/FTD, amyotrophic lateral sclerosis/frontotemporal dementia; BPAN, β-propeller protein-associated neurodegeneration; CMT2, Charcot-Marie-Tooth disease 2; HD, Huntington's disease; HSP, hereditary spastic paraplegia; PD, Parkinson's disease.

et al. 2014). Assays in vivo are even more limited (Mizushima et al. 2004), making it difficult to accurately assess the effects of putative autophagy inducers, and complicating any correlations seen with disease phenotypes. Very few studies that have reported positive effects of autophagy induction on disease-associated phenotypes have done appropriate pharmacokinetic/pharmacodynamic studies. Without a direct demonstration that the intervention is, in fact, inducing autophagy, the exact relationship is difficult to determine.

Table 1. Autophagy lysosomal pathway and Parkinson's disease–associated genes

Genetic locus	Gene	Possible roles in autophagy
PARK1	SNCA (synuclein)	Blocks chaperone-mediated autophagy (CMA); may promote Atg9 mislocalization; vesicle fusion
PARK2	Parkin	Ubiquitin ligase that tags mitochondria for binding of adaptor proteins for mitophagy
PARK6	PINK1	Ubiquitin kinase whose accumulation on mitochondrial membranes is a marker for recruitment of Parkin to mediate mitophagy
PARK 7	DJ-1	Works in parallel with PINK1/Parkin to play a role in mitophagy
PARK8	LRRK2	Kinase for key Rab proteins that may play a role in endosomal/lysosomal trafficking
PARK9	P-type ATPase ATP13A2	Essential for the maintenance of lysosomal pH and autophagosome–lysosome fusion
PARK14	PLA2G6	May play a role mediating mTOR-independent autophagy
PARK15	FBOX7	Mitophagy
PARK17	Vps35 (vacuolar sorting-associated protein 35)	Mutant Vps35 may mislocalize Atg9, leading to impaired autophagy
PARK19	DNAJC6	Endocytosis and endocytic trafficking
PARK20	SYNJ1	Endosomal trafficking and autophagy
PARK21	DNAJC13	Endocytosis
Unassigned	GBA	Lysosomal function, synuclein metabolism

Some studies have reported that interventions designed to modulate autophagy flux have unexpected effects (Zhang et al. 2011). Maniatis and colleagues tested whether genetic inhibition of the autophagy pathway would worsen disease-associated phenotypes in a mouse model of ALS based on mutations in superoxide dismutase 1 (Rudnick et al. 2017). They specifically knocked out Atg7, a gene required for autophagy, in motor neurons early in development in mice expressing a transgene encoding the ALS-associated mutant $SOD1^{G93A}$. Early on, denervation and behavioral deficits were accelerated compared with $SOD1^{G93A}$ mice in which Atg7 was intact. But later, they observed that the loss of Atg7 was associated with less interneuron and astrocyte pathology and an extension of life span.

What could be happening? One possibility is that autophagy may play multiple roles in disease that differ depending on the stage of disease progression. For example, perhaps the initial and somewhat expected worsening of deficits in motor unit function and motor neuron-dependent phenotypes could result from an acceleration in pathogenesis in motor neurons. But Cleveland and colleagues have shown by selectively reducing mutant SOD1 expression in a cell-type specific manner that motor neurons, astrocytes, microglia, and oligodendrocytes each contribute to different extents and in different ways to deficits exhibited by murine models of ALS (Ilieva et al. 2009). The exact nature and mechanisms of the cell-nonautonomous contributions to ALS pathogenesis still need to be worked out. But it is conceivable that if disease begins in motor neurons and if propagation of disease to neighboring nonneuronal cells depends on the release of misfolded proteins by lysosomal exocytosis, then blocking autophagy might actually mitigate the cell-nonautonomous component of disease at the expense of worsening the cell-autonomous component. It is interesting to note that whereas Maniatis and colleagues reported almost no effects of knocking out Atg-7 on baseline motor neuron structure or function (Rudnick et al. 2017), Komatsu found that disruption of Atg-7 and autophagy throughout the brain caused significant neurodegeneration (Komatsu et al. 2006).

But the explanation could be even more complicated. Others have shown that germline mutations that are designed to block specific arms of the protein homeostasis pathway, such as autophagy, are sufficient to induce neurodegeneration (Komatsu et al. 2005, 2006; Hara et al. 2006). Presumably, germline mutations in the autophagy pathway must induce widespread adaptive remodeling of other arms of the proteostasis network to compensate, possibly even in a complicated cell-specific way, which could confound the simple interpretation of the role of autophagy in disease. To what extent does the loss of autophagy at an early stage in development instruct us on the role of autophagy impairment in the aged brain when many neurodegenerative diseases develop? Answers to these questions will be critical for shaping our thinking about pathogenesis and treatment.

ALP AS A POTENTIAL THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISEASE

The evidence suggesting that autophagy is a therapeutic target is intriguing and warrants further investigation. However, while it has been shown that it can be safe to chronically target a major protein homeostasis pathway (e.g., the drug bortezomib, which inhibits the UPS, is a clinically approved treatment for multiple myeloma), there have as yet been no clinical trials of autophagy modulators in humans, and there are multiple caveats to consider.

The mechanisms by which disease-associated genetic mutations in the autophagy pathway cause neurodegeneration is a focus of ongoing research (Nixon 2006), and is critical to the design of therapeutic strategies. Deficits that attenuate the pathway might respond to interventions that stimulate the pathway to restore normal levels of activity (Bose et al. 2011), but that depends on the step in the process that is impaired and the nature of the impairment. For example, mutations in CHMP2B, which plays a role in autophagosome/lysosome fusion (Lu et al. 2013), cause frontotemporal dementia (Skibinski et al. 2005). If disease-causing mutations block that step completely, therapeutically stimulating autophagy might be detrimental, because induced autophagosomes would accumulate without a path to clear them.

Unlike the UPS, autophagy is active basally and can be induced normally by caloric restriction, which can occur during fasting (Alirezaei et al. 2010). Chronic caloric restriction in nonhuman organisms has generally been associated with positive outcomes including increases in longevity and resistance to disease (Bergamini et al. 2007). However, the mechanism by which caloric restriction induces autophagy is thought to be via the inhibition of the mTOR kinase (Balgi et al. 2009; Inoki et al. 2012a). Whether mTOR could be a safe drug target is controversial (Zhang et al. 2011) because mTOR regulates other critical biological pathways in addition to autophagy (Inoki et al. 2012a), such as protein translation (King et al. 2008), and the mTOR inhibitor rapamycin is an immunosuppressant. mTOR inhibitors would be expected to have activity in the periphery, and it has been difficult to develop brain penetrant inhibitors. Partly for these reasons, a focus of the field has been on the development of autophagy inducers that act by mTOR-independent mechanisms (Høyer-Hansen and Jäättelä 2007; Høyer-Hansen et al. 2007; Williams et al. 2008; Lipinski et al. 2010; Decuypere et al. 2011; Bootman et al. 2017).

One additional concern with targeting this pathway has been the potential that systemic induction of autophagy might increase the risk of developing cancer (Shintani and Klionsky 2004; Amaravadi et al. 2007; Kimmelman 2011; Lock et al. 2011; Rosenfeldt et al. 2013; Cui et al. 2014; Rao et al. 2014; Galluzzi et al. 2015; Lévy et al. 2015; Perera et al. 2015; White 2015). Several studies suggest that cancers, particularly as they form tumors that outstrip their blood and nutrient supply, up-regulate the autophagy pathway, possibly in conjunction with macropinocytosis, to enable the cells to endure stress, scavenge nutrients, survive, and proliferate (Qu et al. 2003; Amaravadi and Thompson 2007; Mathew et al. 2007; Guo et al. 2011; Lopez et al. 2011; Yang et al. 2011, 2014, 2016; Amaravadi and Debnath 2014; Karsli-Uzunbas et al. 2014). It is important to note that although autophagy induction might enhance the growth of a preexisting cancer

by these mechanisms, there is also evidence that autophagy can function to suppress the initial development of cancer (Liang et al. 1999; Yue et al. 2003; Takamura et al. 2011; Wu et al. 2012; Guo et al. 2013). Interestingly, Levine and colleagues developed a cell-permeant version of beclin, an autophagy-inducing gene, and showed that chronic administration did not significantly increase the incidence of tumors in mice. In the end, it will be critical to do careful preclinical safety studies for any potential autophagy inducer before first-in-human trials. In addition, the field would benefit tremendously from the development of target-engagement biomarkers for autophagy to guide dosing regimens and to ensure that any clinical trial of a putative autophagy inducer is, in fact, inducing autophagy at the doses tested.

SUMMARY AND CONCLUSIONS

Previously relegated to a narrow role as the cell's trash bin, the lysosome has gained new prominence as an organelle centrally positioned to send and receive critical signals and to regulate cellular metabolism as well as catabolism of macromolecules and other organelles. Autophagy is one of several cellular pathways to deliver intracellular cargo to lysosomes for degradation, a process that depends in many cases on special sets of adaptor proteins that are specific for certain cargoes.

Autophagy may have particular importance for neurodegenerative disease given its demonstrated capacity to metabolize aggregated proteins, damaged organelles linked to neurodegenerative disease such as mitochondria, and its close association with aging, the most important risk factor for neurodegenerative diseases. The myriad mutations in genes with established or apparent roles in autophagy, which result in neurodegenerative disease underscore the conclusion that deficits in autophagy are sufficient to cause neurodegenerative disease and suggest that lysosomal dysfunction may be a common thread that even extends to the more common idiopathic forms of adult-onset neurodegenerative disease.

Nevertheless, it remains unclear whether modulation of autophagy and lysosomal function will be a successful strategy to treat one or more neurodegenerative diseases. Neuropathology studies support the conclusion that dysfunction in the ALP is a common feature of neurodegenerative diseases. However, the ALP is complex and our understanding of the nature of the impairments remains limited in most cases, making it uncertain whether the same type of intervention is likely to work in all diseases and at all stages of those disease. Some of these questions may be difficult to fully address in nonhuman models, given the poor predictive value many of these models have for results in human clinical trials. Frustratingly, knowledge of druggable targets in the pathway remains limited, and the throughput and resolution of current autophagy assays pose challenges for the effective prosecution of potential lead optimization programs. Finally, there is the question of whether and how modulation of the ALP could be achieved safely in humans. Good pharmacodynamic markers for the ALP would be invaluable to enable investigators to test the hypothesis in humans that modulation of the autophagy pathway could be a safe and effective treatment for neurodegenerative diseases.

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REFERENCES

Ahmed I, Liang Y, Schools S, Dawson VL, Dawson TM, Savitt JM. 2012. Development and characterization of a new Parkinson's disease model resulting from impaired autophagy. *J Neurosci* **32:** 16503–16509. doi:10.1523/jneurosci.0209-12.2012

Albanesi J, Wang H, Sun HQ, Levine B, Yin H. 2015. GABARAP-mediated targeting of PI4K2A/PI4KIIα to autophagosomes regulates PtdIns4P-dependent autopha-

gosome-lysosome fusion. *Autophagy* **11:** 2127–2129. doi:10.1080/15548627.2015.1093718

Alers S, Löffler AS, Wesselborg S, Stork B. 2012. Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: Cross talk, shortcuts, and feedbacks. *Mol Cell Biol* **32:** 2–11. doi:10.1128/MCB.06159-11

Alirezaei M, Kemball CC, Flynn CT, Wood MR, Whitton JL, Kiosses WB. 2010. Short-term fasting induces profound neuronal autophagy. *Autophagy* **6:** 702–710. doi:10.4161/auto.6.6.12376

Amaravadi R, Debnath J. 2014. Mouse models address key concerns regarding autophagy inhibition in cancer therapy. *Cancer Discov* **4:** 873–875. doi:10.1158/2159-8290.CD-14-0618

Amaravadi RK, Thompson CB. 2007. The roles of therapy-induced autophagy and necrosis in cancer treatment. *Clin Cancer Res* **13:** 7271–7279. doi:10.1158/1078-0432.CCR-07-1595

Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A, Thompson CB. 2007. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. *J Clin Invest* **117:** 326–336. doi:10.1172/JCI28833

Anding AL, Baehrecke EH. 2017. Cleaning house: Selective autophagy of organelles. *Dev Cell* **41:** 10–22. doi:10.1016/j.devcel.2017.02.016

Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC, Agid Y. 1997. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol Histopathol* **12:** 25–31.

Ao X, Zou L, Wu Y. 2014. Regulation of autophagy by the Rab GTPase network. *Cell Death Differ* **21:** 348–358. doi:10.1038/cdd.2013.187

Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT. 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J Cell Biol* **182:** 685–701. doi:10.1083/jcb.200803137

Baixauli F, López-Otín C, Mittelbrunn M. 2014. Exosomes and autophagy: Coordinated mechanisms for the maintenance of cellular fitness. *Front Immunol* **5:** 403. doi:10.3389/fimmu.2014.00403

Balgi AD, Fonseca BD, Donohue E, Tsang TC, Lajoie P, Proud CG, Nabi IR, Roberge M. 2009. Screen for chemical modulators of autophagy reveals novel therapeutic inhibitors of mTORC1 signaling. *PLoS ONE* **4:** e7124. doi:10.1371/journal.pone.0007124

Barmada SJ, Serio A, Arjun A, Bilican B, Daub A, Ando DM, Tsvetkov A, Pleiss M, Li X, Peisach D, et al. 2014. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat Chem Biol* **10:** 677–685. doi:10.1038/nchembio.1563

Bauer PO, Goswami A, Wong HK, Okuno M, Kurosawa M, Yamada M, Miyazaki H, Matsumoto G, Kino Y, Nagai Y, et al. 2010. Harnessing chaperone-mediated autophagy for the selective degradation of mutant huntingtin protein. *Nat Biotechnol* **28:** 256–263. doi:10.1038/nbt.1608

Beal FM, Bossy-Wetzel E, Finkbeiner S, Fiskum G, Giasson B, Johnson C, Khachaturian ZS, Lee VM, Nicholls D, Reddy H, et al. 2006. Common threads in neurodegenerative disorders of aging. *Alzheimer's Dement* **2:** 322–326. doi:10.1016/j.jalz.2006.08.008

Benbrook DM, Long A. 2012. Integration of autophagy, proteasomal degradation, unfolded protein response and apoptosis. *Exp Oncol* **34:** 286–297.

Bergamini E, Cavallini G, Donati A, Gori Z. 2004. The role of macroautophagy in the ageing process, anti-ageing intervention and age-associated diseases. *Int J Biochem Cell Biol* **36:** 2392–2404. doi:10.1016/j.biocel.2004.05.007

Bergamini E, Cavallini G, Donati A, Gori Z. 2007. The role of autophagy in aging: Its essential part in the anti-aging mechanism of caloric restriction. *Ann NY Acad Sci* **1114:** 69–78. doi:10.1196/annals.1396.020

Bernales S, McDonald KL, Walter P. 2006. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol* **4:** e423. doi:10.1371/journal.pbio.0040423

Bingol B. 2018. Autophagy and lysosomal pathways in nervous system disorders. *Mol Cell Neurosci* **91:** 167–208. doi:10.1016/j.mcn.2018.04.009

Binotti B, Pavlos NJ, Riedel D, Wenzel D, Vorbrüggen G, Schalk AM, Kühnel K, Boyken J, Erck C, Martens H, et al. 2015. The GTPase Rab26 links synaptic vesicles to the autophagy pathway. *eLife* **4:** e05597. doi:10.7554/eLife.05597

Biswas D, Qureshi OS, Lee WY, Croudace JE, Mura M, Lammas DA. 2008. ATP-induced autophagy is associated with rapid killing of intracellular mycobacteria within human monocytes/macrophages. *BMC Immunol* **9:** 1–10. doi:10.1186/1471-2172-9-35

Boland B, Nixon RA. 2006. Neuronal macroautophagy: From development to degeneration. *Mol Aspects Med* **27:** 503–519. doi:10.1016/j.mam.2006.08.009

Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, Nixon RA. 2008. Autophagy induction and autophagosome clearance in neurons: Relationship to autophagic pathology in Alzheimer's disease. *J Neurosci* **28:** 6926–6937. doi:10.1523/jneurosci.0800-08.2008

Bootman MD, Chehab T, Bultynck G, Parys JB, Rietdorf K. 2017. The regulation of autophagy by calcium signals: Do we have a consensus? *Cell Calcium* **70:** 32–46. doi:10.1016/j.ceca.2017.08.005

Bose JK, Huang CC, Shen CKJ. 2011. Regulation of autophagy by neuropathological protein TDP-43. *J Biol Chem* **286:** 44441–44448. doi:10.1074/jbc.M111.237115

Bowling H, Klann E. 2014. Shaping dendritic spines in autism spectrum disorder: mTORC1-dependent macroautophagy. *Neuron* **83:** 994–996. doi:10.1016/j.neuron.2014.08.021

Brady OA, Meng P, Zheng Y, Mao Y, Hu F. 2011. Regulation of TDP-43 aggregation by phosphorylation and p62/SQSTM1. *J Neurochem* **116:** 248–259. doi:10.1111/j.1471-4159.2010.07098.x

Cai Q, Lu L, Tian JH, Zhu YB, Qiao H, Sheng ZH. 2010. Snapin-regulated late endosomal transport is critical for efficient autophagy-lysosomal function in neurons. *Neuron* **68:** 73–86. doi:10.1016/j.neuron.2010.09.022

Carnio S, LoVerso F, Baraibar MA, Longa E, Khan MM, Maffei M, Reischl M, Canepari M, Loefler S, Kern H, et al. 2014. Autophagy impairment in muscle induces neu-

romuscular junction degeneration and precocious aging. *Cell Rep* **8:** 1509–1521. doi:10.1016/j.celrep.2014.07.061

Castillo K, Nassif M, Valenzuela V, Rojas F, Matus S, Mercado G, Court FA, van Zundert B, Hetz C. 2013. Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. *Autophagy* **9:** 1308–1320. doi:10.4161/auto.25188

Chen L, Ma K, Han J, Chen Q, Zhu Y. 2017. Monitoring mitophagy in mammalian cells. *Methods Enzymol* **588:** 187–208. doi:10.1016/bs.mie.2016.10.038

Cheung ZH, Ip NY. 2009. The emerging role of autophagy in Parkinson's disease. *Mol Brain* **2:** 29. doi:10.1186/1756-6606-2-29

Chu CT, Plowey ED, Dagda RK, Hickey RW, Cherra SJ III, Clark RS. 2009. Autophagy in neurite injury and neurodegeneration: In vitro and in vivo models. *Methods Enzymol* **453:** 217–249. doi:10.1016/S0076-6879(08)04011-1

Ciura S, Sellier C, Campanari ML, Charlet-Berguerand N, Kabashi E. 2016. The most prevalent genetic cause of ALS-FTD, C9orf72 synergizes the toxicity of ATXN2 intermediate polyglutamine repeats through the autophagy pathway. *Autophagy* **12:** 1406–1408. doi:10.1080/15548627.2016.1189070

Congdon EE, Wu JW, Myeku N, Figueroa YH, Herman M, Marinec PS, Gestwicki JE, Dickey CA, Yu WH, Duff KE. 2012. Methylthioninium chloride (methylene blue) induces autophagy and attenuates tauopathy in vitro and in vivo. *Autophagy* **8:** 609–622. doi:10.4161/auto.19048

Crews L, Spencer B, Desplats P, Patrick C, Paulino A, Rockenstein E, Hansen L, Adame A, Galasko D, Masliah E. 2010. Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of α-synucleinopathy. *PLoS ONE* **5:** e9313. doi:10.1371/journal.pone.0009313

Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, Gasco M, Garrone O, Crook T, Ryan KM. 2006. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* **126:** 121–134. doi:10.1016/j.cell.2006.05.034

Cuervo AM, Dice JF, Knecht E. 1997. A population of rat liver lysosomes responsible for the selective uptake and degradation of cytosolic proteins. *J Biol Chem* **272:** 5606–5615. doi:10.1074/jbc.272.9.5606

Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. 2004. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. *Science* **305:** 1292–1295. doi:10.1126/science.1101738

Cuervo AM, Bergamini E, Brunk UT, Dröge W, Ffrench M, Terman A. 2005. Autophagy and aging: The importance of maintaining "clean" cells. *Autophagy* **1:** 131–140. doi:10.4161/auto.1.3.2017

Cui J, Hu YF, Feng XM, Tian T, Gui YH, Ma JW, Nan KJ, Zhang HY. 2014. EGFR inhibitors and autophagy in cancer treatment. *Tumour Biol* **35:** 11701–11179. doi:10.1007/s13277-014-2660-z

Dagda R, Zhu J, Kulich S, Chu C. 2008. Mitochondrially localized ERK2 regulates mitophagy and autophagic cell stress: Implications for Parkinson's disease. *Autophagy* **4:** 770–782. doi:10.4161/auto.6458

Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Björklund A. 2013. TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. *Proc Natl Acad Sci* **110:** E1817–E1826. doi:10.1073/pnas.1305623110

Decuypere JP, Bultynck G, Parys JB. 2011. A dual role for Ca^{2+} in autophagy regulation. *Cell Calcium* **50:** 242–250. doi:10.1016/j.ceca.2011.04.001

De Duve C, Wattiaux R. 1966. Functions of lysosomes. *Annu Rev Physiol* **28:** 435–492. doi:10.1146/annurev.ph.28.030166.002251

Dehay B, Martinez-Vicente M, Ramirez A, Perier C, Klein C, Vila M, Bezard E. 2012. Lysosomal dysfunction in Parkinson's disease: ATP13A2 gets into the groove. *Autophagy* **8:** 1389–1391. doi:10.4161/auto.21011

Dolan PJ, Johnson GV. 2010. A caspase cleaved form of tau is preferentially degraded through the autophagy pathway. *J Biol Chem* **285:** 21978–21987. doi:10.1074/jbc.M110.110940

Eden E, Geva-Zatorsky N, Issaeva I, Cohen A, Dekel E, Danon T, Cohen L, Mayo A, Alon U. 2011. Proteome half-life dynamics in living human cells. *Science* **331:** 764–768. doi:10.1126/science.1199784

Engedal N, Seglen PO. 2016. Autophagy of cytoplasmic bulk cargo does not require LC3. *Autophagy* **12:** 439–441. doi:10.1080/15548627.2015.1076606

Farré JC, Manjithaya R, Mathewson RD, Subramani S. 2008. PpAtg30 tags peroxisomes for turnover by selective autophagy. *Dev Cell* **14:** 365–376. doi:10.1016/j.devcel.2007.12.011

Ferguson CJ, Lenk GM, Meisler MH. 2009. Defective autophagy in neurons and astrocytes from mice deficient in $PI(3,5)P_2$. *Hum Mol Genet* **18:** 4868–4878. doi:10.1093/hmg/ddp460

Ferree AW, Trudeau K, Zik E, Benador IY, Twig G, Gottlieb RA, Shirihai OS. 2013. MitoTimer probe reveals the impact of autophagy, fusion, and motility on subcellular distribution of young and old mitochondrial protein and on relative mitochondrial protein age. *Autophagy* **9:** 1887–1896. doi:10.4161/auto.26503

Fortun J, Dunn WA Jr, Joy S, Li J, Notterpek L. 2003. Emerging role for autophagy in the removal of aggresomes in Schwann cells. *J Neurosci* **23:** 10672–10680. doi:10.1523/jneurosci.23-33-10672.2003

Friedman LG, Lachenmayer ML, Wang J, He L, Poulose SM, Komatsu M, Holstein GR, Yue Z. 2012. Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of α-synuclein and LRRK2 in the brain. *J Neurosci* **32:** 7585–7593. doi:10.1523/jneurosci.5809-11.2012

Fujikake N, Shin M, Shimizu S. 2018. Association between autophagy and neurodegenerative diseases. *Front Neurosci* **12:** 255. doi:10.3389/fnins.2018.00255

Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. 2008. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell* **19:** 2092–2100. doi:10.1091/mbc.e07-12-1257

Galluzzi L, Pietrocola F, Bravo-San Pedro JM, Amaravadi RK, Baehrecke EH, Cecconi F, Codogno P, Debnath J, Gewirtz DA, Karantza V, et al. 2015. Autophagy in malignant transformation and cancer progression. *EMBO J* **34:** 856–880. doi:10.15252/embj.201490784

Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. 2010. PINK1/Parkin-mediated mi-

tophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* **12:** 119–131. doi:10.1038/ncb2012

Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kamphorst JJ, Chen G, Lemons JM, Karantza V, et al. 2011. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev* **25:** 460–470. doi:10.1101/gad.2016311

Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, Strohecker AM, Chen G, Price S, Lu W, Teng X, et al. 2013. Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev* **27:** 1447–1461. doi:10.1101/gad.219642.113

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, et al. 2006. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* **441:** 885–889. doi:10.1038/nature04724

He C, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, An Z, Loh J, Fisher J, Sun Q, et al. 2012. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature* **481:** 511–515. doi:10.1038/nature10758

Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, Martinez G, Cuervo AM, Brown RH, Glimcher LH. 2009. XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. *Genes Dev* **23:** 2294–2306. doi:10.1101/gad.1830709

Höllerhage M, Goebel JN, de Andrade A, Hildebrandt T, Dolga A, Culmsee C, Oertel WH, Hengerer B, Höglinger GU. 2014. Trifluoperazine rescues human dopaminergic cells from wild-type α-synuclein-induced toxicity. *Neurobiol Aging* **35:** 1700–1711. doi:10.1016/j.neurobiolaging.2014.01.027

Høyer-Hansen M, Jäättelä M. 2007. Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. *Cell Death Differ* **14:** 1576–1582. doi:10.1038/sj.cdd.4402200

Høyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T, Bianchi K, Fehrenbacher N, Elling F, Rizzuto R, et al. 2007. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2. *Mol Cell* **25:** 193–205. doi:10.1016/j.molcel.2006.12.009

Hyttinen JM, Niittykoski M, Salminen A, Kaarniranta K. 2013. Maturation of autophagosomes and endosomes: A key role for Rab7. *Biochim Biophys Acta* **1833:** 503–510. doi:10.1016/j.bbamcr.2012.11.018

Ilieva H, Polymenidou M, Cleveland DW. 2009. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol* **187:** 761–772. doi:10.1083/jcb.200908164

Inoki K, Kim J, Guan KL. 2012a. AMPK and mTOR in cellular energy homeostasis and drug targets. *Annu Rev Pharmacol Toxicol* **52:** 381–400. doi:10.1146/annurev-pharmtox-010611-134537

Inoue K, Rispoli J, Kaphzan H, Klann E, Chen EI, Kim J, Komatsu M, Abeliovich A. 2012b. Macroautophagy deficiency mediates age-dependent neurodegeneration through a phospho-tau pathway. *Mol Neurodegener* **7:** 48. doi:10.1186/1750-1326-7-48

Itoh T, Fujita N, Kanno E, Yamamoto A, Yoshimori T, Fukuda M. 2008. Golgi-resident small GTPase Rab33B interacts with Atg16L and modulates autophagosome formation. *Mol Biol Cell* **19:** 2916–2925. doi:10.1091/mbc.e07-12-1231

Itoh T, Kanno E, Uemura T, Waguri S, Fukuda M. 2011. OATL1, a novel autophagosome-resident Rab33B-GAP, regulates autophagosomal maturation. *J Cell Biol* **192:** 839–853. doi:10.1083/jcb.201008107

Jäger S, Bucci C, Tanida I, Ueno T, Kominami E, Saftig P, Eskelinen EL. 2004. Role for Rab7 in maturation of late autophagic vacuoles. *J Cell Sci* **117:** 4837–4848. doi:10.1242/jcs.01370

Jia K, Hart AC, Levine B. 2007. Autophagy genes protect against disease caused by polyglutamine expansion proteins in *Caenorhabditis elegans*. *Autophagy* **3:** 21–25. doi:10.4161/auto.3528

Jinn S, Drolet RE, Cramer PE, Wong AH, Toolan DM, Gretzula CA, Voleti B, Vassileva G, Disa J, Tadin-Strapps M, et al. 2017. TMEM175 deficiency impairs lysosomal and mitochondrial function and increases α-synuclein aggregation. *Proc Natl Acad Sci* **114:** 2389–2394. doi:10.1073/pnas.1616332114

Ju JS, Fuentealba RA, Miller SE, Jackson E, Piwnica-Worms D, Baloh RH, Weihl CC. 2009. Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J Cell Biol* **187:** 875–888. doi:10.1083/jcb.200908115

Kabuta T, Suzuki Y, Wada K. 2006. Degradation of amyotrophic lateral sclerosis-linked mutant Cu,Zn-superoxide dismutase proteins by macroautophagy and the proteasome. *J Biol Chem* **281:** 30524–30533. doi:10.1074/jbc.M603337200

Kang C, Xu Q, Martin TD, Li MZ, Demaria M, Aron L, Lu T, Yankner BA, Campisi J, Elledge SJ. 2015. The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. *Science* **349:** aaa5612. doi:10.1126/science.aaa5612

Karsli-Uzunbas G, Guo JY, Price S, Teng X, Laddha SV, Khor S, Kalaany NY, Jacks T, Chan CS, Rabinowitz JD, et al. 2014. Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discov* **4:** 914–927. doi:10.1158/2159-8290.CD-14-0363

Kaushik S, Cuervo AM. 2012. Chaperones in autophagy. *Pharmacol Res* **66:** 484–493. doi:10.1016/j.phrs.2012.10.002

Kegel KB, Kim M, Sapp E, McIntyre C, Castaño JG, Aronin N, DiFiglia M. 2000. Huntingtin expression stimulates endosomal–lysosomal activity, endosome tubulation, and autophagy. *J Neurosci* **20:** 7268–7278. doi:10.1523/jneurosci.20-19-07268.2000

Keller JN, Dimayuga E, Chen Q, Thorpe J, Gee J, Ding Q. 2004. Autophagy, proteasomes, lipofuscin, and oxidative stress in the aging brain. *Int J Biochem Cell Biol* **36:** 2376–2391. doi:10.1016/j.biocel.2004.05.003

Kiffin R, Christian C, Knecht E, Cuervo AM. 2004. Activation of chaperone-mediated autophagy during oxidative stress. *Mol Biol Cell* **15:** 4829–4840. doi:10.1091/mbc.e04-06-0477

Kim J, Klionsky DJ. 2000. Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mamma-

lian cells. *Annu Rev Biochem* **69:** 303–342. doi:10.1146/annurev.biochem.69.1.303

Kimmelman AC. 2011. The dynamic nature of autophagy in cancer. *Genes Dev* **25:** 1999–2010. doi:10.1101/gad.17558811

King MA, Hands S, Hafiz F, Mizushima N, Tolkovsky AM, Wyttenbach A. 2008. Rapamycin inhibits polyglutamine aggregation independently of autophagy by reducing protein synthesis. *Mol Pharmacol* **73:** 1052–1062. doi:10.1124/mol.107.043398

Kirkin V, McEwan DG, Novak I, Dikic I. 2009. A role for ubiquitin in selective autophagy. *Mol Cell* **34:** 259–269. doi:10.1016/j.molcel.2009.04.026

Klionsky DJ. 2005. The molecular machinery of autophagy: Unanswered questions. *J Cell Sci* **118:** 7–18. doi:10.1242/jcs.01620

Klionsky DJ. 2007. Autophagy: From phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* **8:** 931–937. doi:10.1038/nrm2245

Klionsky DJ, Cregg JM, Dunn WA, Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, et al. 2003. A unified nomenclature for yeast autophagy-related genes. *Dev Cell* **5:** 539–545. doi:10.1016/S1534-5807(03)00296-X

Klionsky DJ, Baehrecke EH, Brumell JH, Chu CT, Codogno P, Cuervo AM, Debnath J, Deretic V, Elazar Z, Eskelinen EL, et al. 2011. A comprehensive glossary of autophagy-related molecules and processes (2nd edition). *Autophagy* **7:** 1273–1294. doi:10.4161/auto.7.11.17661

Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, et al. 2012. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* **8:** 445–544. doi:10.4161/auto.19496

Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, Adachi H, Adams CM, Adams PD, Adeli K, et al. 2016. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* **12:** 1–222. doi:10.1080/15548627.2015.1100356.

Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, et al. 2005. Impairment of starvation-induced and constitutive autophagy in *Atg7*-deficient mice. *J Cell Biol* **169:** 425–434. doi:10.1083/jcb.200412022

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, et al. 2006. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* **441:** 880–884. doi:10.1038/nature04723

Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, et al. 2007. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* **131:** 1149–1163. doi:10.1016/j.cell.2007.10.035

Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, Sou YS, Ueno I, Sakamoto A, Tong KI, et al. 2010. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* **12:** 213–223. doi:10.1038/ncb2021

Krüger U, Wang Y, Kumar S, Mandelkow EM. 2012. Autophagic degradation of tau in primary neurons and its enhancement by trehalose. *Neurobiol Aging* **33:** 2291–2305. doi:10.1016/j.neurobiolaging.2011.11.009

Kuma A, Matsui M, Mizushima N. 2007. LC3, an autophagosome marker, can be incorporated into protein aggregates independent of autophagy: Caution in the interpretation of LC3 localization. *Autophagy* **3:** 323–328. doi:10.4161/auto.4012

Kwan JY, Jeong SY, Van Gelderen P, Deng HX, Quezado MM, Danielian LE, Butman JA, Chen L, Bayat E, Russell J, et al. 2012. Iron accumulation in deep cortical layers accounts for MRI signal abnormalities in ALS: Correlating 7 tesla MRI and pathology. *PLoS ONE* **7:** e35241. doi:10.1371/journal.pone.0035241

Larsen KE, Sulzer D. 2002. Autophagy in neurons: A review. *Histol Histopathol* **17:** 897–908.

Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL, Sideris DP, Fogel AI, Youle RJ. 2015. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* **524:** 309–314. doi:10.1038/nature14893

Lee MJ, Lee JH, Rubinsztein DC. 2013. Tau degradation: The ubiquitin-proteasome system versus the autophagy-lysosome system. *Prog Neurobiol* **105:** 49–59. doi:10.1016/j.pneurobio.2013.03.001

Lemasters JJ. 2014. Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (type 3). *Redox Biol* **2:** 749–754. doi:10.1016/j.redox.2014.06.004

Levine B, Kroemer G. 2008. Autophagy in the pathogenesis of disease. *Cell* **132:** 27–42. doi:10.1016/j.cell.2007.12.018

Levine B, Mizushima N, Virgin HW. 2011. Autophagy in immunity and inflammation. *Nature* **469:** 323–335. doi:10.1038/nature09782

Lévy J, Cacheux W, Bara MA, L'Hermitte A, Lepage P, Fraudeau M, Trentesaux C, Lemarchand J, Durand A, Crain AM, et al. 2015. Intestinal inhibition of Atg7 prevents tumour initiation through a microbiome-influenced immune response and suppresses tumour growth. *Nat Cell Biol* **17:** 1062–1073. doi:10.1038/ncb3206

Lezi E, Swerdlow RH. 2012. Mitochondria in neurodegeneration. *Adv Exp Med Biol* **942:** 269–286. doi:10.1007/978-94-007-2869-1_12

Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* **402:** 672–676. doi:10.1038/45257

Lim J, Lachenmayer ML, Wu S, Liu W, Kundu M, Wang R, Komatsu M, Oh YJ, Zhao Y, Yue Z. 2015. Proteotoxic stress induces phosphorylation of p62/SQSTM1 by ULK1 to regulate selective autophagic clearance of protein aggregates. *PLoS Genet* **11:** e1004987. doi:10.1371/journal.pgen.1004987

Lin SY, Li TY, Liu Q, Zhang C, Li X, Chen Y, Zhang SM, Lian G, Liu Q, Ruan K, et al. 2012. GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. *Science* **336:** 477–481. doi:10.1126/science.1217032

Lipinski MM, Hoffman G, Ng A, Zhou W, Py BF, Hsu E, Liu X, Eisenberg J, Liu J, Blenis J, et al. 2010. A genome-wide siRNA screen reveals multiple mTORC1 independent signaling pathways regulating autophagy under normal nutritional conditions. *Dev Cell* **18:** 1041–1052. doi:10.1016/j.devcel.2010.05.005

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, Debnath J. 2011. Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Mol Biol Cell* **22:** 165–178. doi:10.1091/mbc.e10-06-0500

Lopez G, Torres K, Liu J, Hernandez B, Young E, Belousov R, Bolshakov S, Lazar AJ, Slopis JM, McCutcheon IE, et al. 2011. Autophagic survival in resistance to histone deacetylase inhibitors: Novel strategies to treat malignant peripheral nerve sheath tumors. *Cancer Res* **71:** 185–196. doi:10.1158/0008-5472.CAN-10-2799

Lu Y, Zhang Z, Sun D, Sweeney ST, Gao FB. 2013. Syntaxin 13, a genetic modifier of mutant CHMP2B in frontotemporal dementia, is required for autophagosome maturation. *Mol Cell* **52:** 264–271. doi:10.1016/j.molcel.2013.08.041

Lucin KM, O'Brien CE, Bieri G, Czirr E, Mosher KI, Abbey RJ, Mastroeni DF, Rogers J, Spencer B, Masliah E, et al. 2013. Microglial beclin 1 regulates retromer trafficking and phagocytosis and is impaired in Alzheimer's disease. *Neuron* **79:** 873–886. doi:10.1016/j.neuron.2013.06.046

Maday S, Holzbaur EL. 2014. Autophagosome biogenesis in primary neurons follows an ordered and spatially regulated pathway. *Dev Cell* **30:** 71–85. doi:10.1016/j.devcel.2014.06.001

Maiuri MC, Galluzzi L, Morselli E, Kepp O, Malik SA, Kroemer G. 2010. Autophagy regulation by p53. *Curr Opin Cell Biol* **22:** 181–185. doi:10.1016/j.ceb.2009.12.001

Manzoni C. 2017. The LRRK2-macroautophagy axis and its relevance to Parkinson's disease. *Biochem Soc Trans* **45:** 155–162. doi:10.1042/BST20160265

Mari M, Tooze SA, Reggiori F. 2011. The puzzling origin of the autophagosomal membrane. *F1000 Biol Rep* **3:** 25. doi:10.3410/B3-25

Marrone L, Poser I, Casci I, Japtok J, Reinhardt P, Janosch A, Andree C, Lee HO, Moebius C, Koerner E, et al. 2018. Isogenic FUS-eGFP iPSC reporter lines enable quantification of FUS stress granule pathology that is rescued by drugs inducing autophagy. *Stem Cell Rep* **10:** 375–389. doi:10.1016/j.stemcr.2017.12.018

Martin DD, Ladha S, Ehrnhoefer DE, Hayden MR. 2015. Autophagy in Huntington's disease and huntingtin in autophagy. *Trends Neurosci* **38:** 26–35. doi:10.1016/j.tins.2014.09.003

Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, Hodara R, Fredenburg R, Wu DC, Follenzi A, et al. 2008. Dopamine-modified α-synuclein blocks chaperone-mediated autophagy. *J Clin Invest* **118:** 777–788. doi:10.1172/JCI32806

Massey AC, Zhang C, Cuervo AM. 2006. Chaperone-mediated autophagy in aging and disease. *Curr Top Dev Biol* **73:** 205–235. doi:10.1016/S0070-2153(05)73007-6

Mathew R, Karantza-Wadsworth V, White E. 2007. Role of autophagy in cancer. *Nat Rev Cancer* **7:** 961–967. doi:10.1038/nrc2254

Matsumoto G, Wada K, Okuno M, Kurosawa M, Nukina N. 2011. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol Cell* **44:** 279–289. doi:10.1016/j.molcel.2011.07.039

Matus S, Lisbona F, Torres M, León C, Thielen P, Hetz C. 2008. The stress rheostat: An interplay between the unfolded protein response (UPR) and autophagy in neurodegeneration. *Curr Mol Med* **8:** 157–172. doi:10.2174/156652408784221324

Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ. 2010. Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science* **330:** 1774. doi:10.1126/science.1197623

Meléndez A, Tallóczy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. 2003. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science* **301:** 1387–1391. doi:10.1126/science.1087782

Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. 2004. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol Biol Cell* **15:** 1101–1111. doi:10.1091/mbc.e03-09-0704

Montie HL, Cho MS, Holder L, Liu Y, Tsvetkov AS, Finkbeiner S, Merry DE. 2009. Cytoplasmic retention of polyglutamine-expanded androgen receptor ameliorates disease via autophagy in a mouse model of spinal and bulbar muscular atrophy. *Hum Mol Genet* **18:** 1937–1950. doi:10.1093/hmg/ddp115

Moruno Manchon JF, Uzor NE, Finkbeiner S, Tsvetkov AS. 2016. SPHK1/sphingosine kinase 1-mediated autophagy differs between neurons and SH-SY5Y neuroblastoma cells. *Autophagy* **12:** 1418–1424. doi:10.1080/15548627.2016.1183082

Murrow L, Malhotra R, Debnath J. 2015. ATG12-ATG3 interacts with Alix to promote basal autophagic flux and late endosome function. *Nat Cell Biol* **17:** 300–310. doi:10.1038/ncb3112

Nakatogawa H, Ichimura Y, Ohsumi Y. 2007. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* **130:** 165–178. doi:10.1016/j.cell.2007.05.021

Narendra D, Tanaka A, Suen DF, Youle RJ. 2008. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* **183:** 795–803. doi:10.1083/jcb.200809125

Nixon RA. 2006. Autophagy in neurodegenerative disease: Friend, foe or turncoat? *Trends Neurosci* **29:** 528–535. doi:10.1016/j.tins.2006.07.003

Nixon RA. 2007. Autophagy, amyloidogenesis and Alzheimer's disease. *J Cell Sci* **120:** 4081–4091. doi:10.1242/jcs.019265

Nixon RA. 2013. The role of autophagy in neurodegenerative disease. *Nat Med* **19:** 983–997. doi:10.1038/nm.3232

Nixon RA, Yang DS, Lee JH. 2008. Neurodegenerative lysosomal disorders: A continuum from development to late age. *Autophagy* **4:** 590–599. doi:10.4161/auto.6259

Ochaba J, Lukacsovich T, Csikos G, Zheng S, Margulis J, Salazar L, Mao K, Lau AL, Yeung SY, Humbert S, et al. 2014. Potential function for the Huntingtin protein as a scaffold for selective autophagy. *Proc Natl Acad Sci* **111:** 16889–16894. doi:10.1073/pnas.1420103111

Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, et al. 2012. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* **485:** 251–255. doi:10.1038/nature10992

Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Olson TS, Terlecky SR, Dice JF. 1991. Targeting specific proteins for lysosomal proteolysis. *Biomed Biochim Acta* **50:** 393–397.

Padamsey Z, McGuinness L, Bardo SJ, Reinhart M, Tong R, Hedegaard A, Hart ML, Emptage NJ. 2017. Activity-dependent exocytosis of lysosomes regulates the structural plasticity of dendritic spines. *Neuron* **93:** 132–146. doi:10.1016/j.neuron.2016.11.013

Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, et al. 2007. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature* **447:** 859–863. doi:10.1038/nature05853

Perera RM, Zoncu R. 2016. The lysosome as a regulatory hub. *Annu Rev Cell Dev Biol* **32:** 223–253. doi:10.1146/annurev-cellbio-111315-125125

Perera RM, Stoykova S, Nicolay BN, Ross KN, Fitamant J, Boukhali M, Lengrand J, Deshpande V, Selig MK, Ferrone CR, et al. 2015. Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature* **524:** 361–365. doi:10.1038/nature14587

Petersén A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P, Sulzer D. 2001. Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. *Hum Mol Genet* **10:** 1243–1254. doi:10.1093/hmg/10.12.1243

Poehler AM, Xiang W, Spitzer P, May VE, Meixner H, Rockenstein E, Chutna O, Outeiro TF, Winkler J, Masliah E, et al. 2014. Autophagy modulates SNCA/α-synuclein release, thereby generating a hostile microenvironment. *Autophagy* **10:** 2171–2192. doi:10.4161/auto.36436

Polito VA, Li H, Martini-Stoica H, Wang B, Yang L, Xu Y, Swartzlander DB, Palmieri M, di Ronza A, Lee VM, et al. 2014. Selective clearance of aberrant tau proteins and rescue of neurotoxicity by transcription factor EB. *EMBO Mol Med* **6:** 1142–1160. doi:10.15252/emmm.201303671

Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, Jung S, Jung YK. 2013. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat Commun* **4:** 2300. doi:10.1038/ncomms3300

Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, et al. 2003. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* **112:** 1809–1820. doi:10.1172/JCI20039

Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J, et al. 2006. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* **38:** 1184–1191. doi:10.1038/ng1884

Rao S, Tortola L, Perlot T, Wirnsberger G, Novatchkova M, Nitsch R, Sykacek P, Frank L, Schramek D, Komnenovic V, et al. 2014. A dual role for autophagy in a murine model of lung cancer. *Nat Commun* **5:** 3056. doi:10.1038/ncomms4056

Ravikumar B, Duden R, Rubinsztein DC. 2002. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum Mol Genet* **11:** 1107–1117. doi:10.1093/hmg/11.9.1107

Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, et al. 2004. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington's disease. *Nat Genet* **36:** 585–595. doi:10.1038/ng1362

Rodríguez-Navarro JA, Rodríguez L, Casarejos MJ, Solano RM, Gómez A, Perucho J, Cuervo AM, García de Yébenes J, Mena MA. 2010. Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. *Neurobiol Dis* **39:** 423–438. doi:10.1016/j.nbd.2010.05.014

Roscic A, Baldo B, Crochermore C, Mercellin D, Paganetti P. 2011. Induction of autophagy with catalytic mTOR inhibitors reduces huntingtin aggregates in a neuronal cell model. *J Neurochem* **119:** 398–407. doi:10.1111/j.1471-4159.2011.07435.x

Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, MacKay G, Mrowinska A, Au A, Rai TS, Zheng L, Ridgway R, et al. 2013. p53 status determines the role of autophagy in pancreatic tumour development. *Nature* **504:** 296–300. doi:10.1038/nature12865

Rouschop KM, van den Beucken T, Dubois L, Niessen H, Bussink J, Savelkouls K, Keulers T, Mujcic H, Landuyt W, Voncken JW, et al. 2010. The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* **120:** 127–141. doi:10.1172/JCI40027

Rubinsztein DC, Mariño G, Kroemer G. 2011. Autophagy and aging. *Cell* **146:** 682–695. doi:10.1016/j.cell.2011.07.030

Rudnick ND, Griffey CJ, Guarnieri P, Gerbino V, Wang X, Piersaint JA, Tapia JC, Rich MM, Maniatis T. 2017. Distinct roles for motor neuron autophagy early and late in the SOD1(G93A) mouse model of ALS. *Proc Natl Acad Sci* **114:** E8294–E8303. doi:10.1073/pnas.1704294114

Ryan BJ, Hoek S, Fon EA, Wade-Martins R. 2015. Mitochondrial dysfunction and mitophagy in Parkinson's: From familial to sporadic disease. *Trends Biochem Sci* **40:** 200–210. doi:10.1016/j.tibs.2015.02.003

Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, Potolicchio I, Nieves E, Cuervo AM, Santambrogio L. 2011. Microautophagy of cytosolic proteins by late endosomes. *Dev Cell* **20:** 131–139. doi:10.1016/j.devcel.2010.12.003

Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. 2007a. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and α-synuclein. *J Biol Chem* **282:** 5641–5652. doi:10.1074/jbc.M609532200

Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, Webster JA, Lewis TA, O'Kane CJ, Schreiber SL, et al. 2007b. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat Chem Biol* **3:** 331–338. doi:10.1038/nchembio883

Schaeffer V, Lavenir I, Ozcelik S, Tolnay M, Winkler DT, Goedert M. 2012. Stimulation of autophagy reduces neurodegeneration in a mouse model of human tauopathy. *Brain* **135:** 2169–2177. doi:10.1093/brain/aws143

Cold Spring Harbor Perspectives in Biology
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Sellier C, Campanari ML, Julie Corbier C, Gaucherot A, Kolb-Cheynel I, Oulad-Abdelghani M, Ruffenach F, Page A, Ciura S, Kabashi E, et al. 2016. Loss of C9ORF72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. *EMBO J* **35:** 1276–1297. doi:10.15252/embj.201593350

Senft D, Ronai ZA. 2015. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem Sci* **40:** 141–148. doi:10.1016/j.tibs.2015.01.002

Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, Erdin SU, Huynh T, Medina D, Colella P, et al. 2011. TFEB links autophagy to lysosomal biogenesis. *Science* **332:** 1429–1433. doi:10.1126/science.1204592

Settembre C, Fraldi A, Medina DL, Ballabio A. 2013. Signals from the lysosome: A control centre for cellular clearance and energy metabolism. *Nat Rev Mol Cell BIol* **14:** 283–296. doi:10.1038/nrm3565

Shanware NP, Bray K, Abraham RT. 2013. The PI3K, metabolic, and autophagy networks: Interactive partners in cellular health and disease. *Annu Rev Pharmacol Toxicol* **53:** 89–106. doi:10.1146/annurev-pharmtox-010611-134717

Shen W, Ganetzky B. 2009. Autophagy promotes synapse development in *Drosophila*. *J Cell Biol* **187:** 71–79. doi:10.1083/jcb.200907109

Shintani T, Klionsky DJ. 2004. Autophagy in health and disease: A double-edged sword. *Science* **306:** 990–995. doi:10.1126/science.1099993

Shpilka T, Elazar Z. 2011. Shedding light on mammalian microautophagy. *Dev Cell* **20:** 1–2. doi:10.1016/j.devcel.2010.12.010

Sikorska B, Liberski PP, Giraud P, Kopp N, Brown P. 2004. Autophagy is a part of ultrastructural synaptic pathology in Creutzfeldt–Jakob disease: A brain biopsy study. *Int J Biochem Cell Biol* **36:** 2563–2573. doi:10.1016/j.biocel.2004.04.014

Simonsen A, Cumming RC, Brech A, Isakson P, Schubert DR, Finley KD. 2008. Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. *Autophagy* **4:** 176–184. doi:10.4161/auto.5269

Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, et al. 2005. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* **37:** 806–808. doi:10.1038/ng1609

Sou YS, Waguri S, Iwata J, Ueno T, Fujimura T, Hara T, Sawada N, Yamada A, Mizushima N, Uchiyama Y, et al. 2008. The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. *Mol Biol Cell* **19:** 4762–4775. doi:10.1091/mbc.e08-03-0309

Spencer B, Potkar R, Trejo M, Rockenstein E, Patrick C, Gindi R, Adame A, Wyss-Coray T, Masliah E. 2009. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in α-synuclein models of Parkinson's and Lewy body diseases. *J Neurosci* **29:** 13578–13588. doi:10.1523/jneurosci.4390-09.2009

Stroikin Y, Dalen H, Lööf S, Terman A. 2004. Inhibition of autophagy with 3-methyladenine results in impaired turnover of lysosomes and accumulation of lipofuscin-like material. *Eur J Cell Biol* **83:** 583–590. doi:10.1078/0171-9335-00433

Suh DH, Kim MK, Kim HS, Chung HH, Song YS. 2012. Unfolded protein response to autophagy as a promising druggable target for anticancer therapy. *Ann NY Acad Sci* **1271:** 20–32. doi:10.1111/j.1749-6632.2012.06739.x

Sullivan PM, Zhou X, Robins AM, Paushter DH, Kim D, Smolka MB, Hu F. 2016. The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway. *Acta Neuropathol Commun* **4:** 51. doi:10.1186/s40478-016-0324-5

Szalai P, Hagen LK, Sætre F, Luhr M, Sponheim M, Øverbye A, Mills IG, Seglen PO, Engedal N. 2015. Autophagic bulk sequestration of cytosolic cargo is independent of LC3, but requires GABARAPs. *Exp Cell Res* **333:** 21–38. doi:10.1016/j.yexcr.2015.02.003

Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, Eishi Y, Hino O, Tanaka K, Mizushima N. 2011. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev* **25:** 795–800. doi:10.1101/gad.2016211

Takenouchi T, Fujita M, Sugama S, Kitani H, Hashimoto M. 2009. The role of the P2X7 receptor signaling pathway for the release of autolysosomes in microglial cells. *Autophagy* **5:** 723–724. doi:10.4161/auto.5.5.8478

Tan JM, Wong ES, Kirkpatrick DS, Pletnikova O, Ko HS, Tay SP, Ho MW, Troncoso J, Gygi SP, Lee MK, et al. 2008. Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. *Hum Mol Genet* **17:** 431–439. doi:10.1093/hmg/ddm320

Tang G, Gudsnuk K, Kuo SH, Cotrina ML, Rosoklija G, Sosunov A, Sonders MS, Kanter E, Castagna C, Yamamoto A, et al. 2014. Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron* **83:** 1131–1143. doi:10.1016/j.neuron.2014.07.040

Tooze SA, Dikic I. 2016. Autophagy captures the Nobel prize. *Cell* **167:** 1433–1435. doi:10.1016/j.cell.2016.11.023

Tsunemi T, Ashe TD, Morrison BE, Soriano KR, Au J, Roque RA, Lazarowski ER, Damian VA, Masliah E, La Spada AR. 2012. PGC-1α rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. *Sci Transl Med* **4:** 142ra197. doi:10.1126/scitranslmed.3003799

Tsvetkov AS, Miller J, Arrasate M, Wong JS, Pleiss MA, Finkbeiner S. 2010. A small-molecule scaffold induces autophagy in primary neurons and protects against toxicity in a Huntington's disease model. *Proc Natl Acad Sci* **107:** 16982–16987. doi:10.1073/pnas.1004498107

Tung YT, Hsu WM, Lee H, Huang WP, Liao YF. 2010. The evolutionarily conserved interaction between LC3 and p62 selectively mediates autophagy-dependent degradation of mutant huntingtin. *Cell Mol Neurobiol* **30:** 795–806. doi:10.1007/s10571-010-9507-y

van Duijn S, van Duinen SG, Nabuurs RJA, van Buchem MA, van der Weerd L, Natté R. 2017. Cortical iron reflects severity of Alzheimer's disease. *J Alzheimer's Dis* **60:** 1533–1545. doi:10.3233/JAD-161143

Wang X. 2017. Destructive cellular paths underlying familial and sporadic Parkinson's disease converge on mitophagy. *Autophagy* **13:** 1998–1999. doi:10.1080/15548627.2017.1327511

Wang Y, Mandelkow E. 2012. Degradation of tau protein by autophagy and proteasomal pathways. *Biochem Soc Trans* **40:** 644–652. doi:10.1042/BST20120071

Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM, Mandelkow E. 2009. Tau fragmentation, aggregation and clearance: The dual role of lysosomal processing. *Hum Mol Genet* **18:** 4153–4170. doi:10.1093/hmg/ddp367

Wang X, Fan H, Ying Z, Li B, Wang H, Wang G. 2010a. Degradation of TDP-43 and its pathogenic form by autophagy and the ubiquitin-proteasome system. *Neurosci Lett* **469:** 112–116. doi:10.1016/j.neulet.2009.11.055

Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM, Mandelkow E. 2010b. Synergy and antagonism of macroautophagy and chaperone-mediated autophagy in a cell model of pathological tau aggregation. *Autophagy* **6:** 182–183. doi:10.4161/auto.6.1.10815

Wang JY, Zhuang QQ, Zhu l, Shu H, Li T, Chen SF, Huang CP, Zhang X, Zhu JH. 2016. Meta-analysis of brain iron levels of Parkinson's disease patients determined by postmortem and MRI measurements. *Sci Rep* **6:** 36669. doi:10.1038/srep36669

Watanabe T, Nagase K, Chosa M, Tobinai K. 2010. Schwann cell autophagy induced by SAHA, 17-AAG, or clonazepam can reduce bortezomib-induced peripheral neuropathy. *Br J Cancer* **103:** 1580–1587. doi:10.1038/sj.bjc.6605954

Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. 2003. α-Synuclein is degraded by both autophagy and the proteasome. *J Biol Chem* **278:** 25009–25013. doi:10.1074/jbc.M300227200

White E. 2015. The role for autophagy in cancer. *J Clin Invest* **125:** 42–46. doi:10.1172/JCI73941

Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B, Korac J, Waidmann O, Choudhary C, et al. 2011. Phosphorylation of the autophagy receptor optineurin restricts *Salmonella* growth. *Science* **333:** 228–233. doi:10.1126/science.1205405

Williams A, Sarkar S, Cuddon P, Ttofi EK, Saiki S, Siddiqi FH, Jahreiss L, Fleming A, Pask D, Goldsmith P, et al. 2008. Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. *Nat Chem Biol* **4:** 295–305. doi:10.1038/nchembio.79

Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, Lichtenberg M, Menzies FM, Ravikumar B, Imarisio S, et al. 2010. α-Synuclein impairs macroautophagy: Implications for Parkinson's disease. *J Cell Biol* **190:** 1023–1037. doi:10.1083/jcb.201003122

Wong YC, Holzbaur EL. 2014. The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. *J Neurosci* **34:** 1293–1305. doi:10.1523/jneurosci.1870-13.2014

Wu WK, Coffelt SB, Cho CH, Wang XJ, Lee CW, Chan FK, Yu J, Sung JJ. 2012. The autophagic paradox in cancer therapy. *Oncogene* **31:** 939–953. doi:10.1038/onc.2011.295

Xu H, Ren D. 2015. Lysosomal physiology. *Annu Rev Physiol* **77:** 57–80. doi:10.1146/annurev-physiol-021014-071649

Yamamoto A, Yue Z. 2014. Autophagy and its normal and pathogenic states in the brain. *Annu Rev Neurosci* **37:** 55–78. doi:10.1146/annurev-neuro-071013-014149

Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, Dell'Antonio G, et al. 2011. Pancreatic cancers require autophagy for tumor growth. *Genes Dev* **25:** 717–729. doi:10.1101/gad.2016111

Yang A, Rajeshkumar NV, Wang X, Yabuuchi S, Alexander BM, Chu CG, Von Hoff DD, Maitra A, Kimmelman AC. 2014. Autophagy is critical for pancreatic tumor growth and progression in tumors with p53 alterations. *Cancer Discov* **4:** 905–913. doi:10.1158/2159-8290.CD-14-0362

Yang M, Liang C, Swaminathan K, Herrlinger S, Lai F, Shiekhattar R, Chen JF. 2016. A C9ORF72/SMCR8-containing complex regulates ULK1 and plays a dual role in autophagy. *Sci Adv* **2:** e1601167. doi:10.1126/sciadv.1601167

Youle RJ, Narendra DP. 2011. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* **12:** 9–14. doi:10.1038/nrm3028

Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, Peng J, Mi N, Zhao Y, Liu Z, Wan F, et al. 2010. Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature* **465:** 942–946. doi:10.1038/nature09076

Yue Z, Jin S, Yang C, Levine AJ, Heintz N. 2003. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci* **100:** 15077–15082. doi:10.1073/pnas.2436255100

Yuzaki M. 2010. Snapin snaps into the dynein complex for late endosome-lysosome trafficking and autophagy. *Neuron* **68:** 4–6. doi:10.1016/j.neuron.2010.09.036

Zhang X, Li L, Chen S, Yang D, Wang Y, Zhang X, Wang Z, Le W. 2011. Rapamycin treatment augments motor neuron degeneration in $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis. *Autophagy* **7:** 412–425. doi:10.4161/auto.7.4.14541

Zheng S, Clagough EB, Sarkar S, Futter M, Rubinsztein DC, Zeitlin SO. 2010. Deletion of the huntingtin polyglutamine stretch enhances neuronal autophagy and longevity in mice. *PLoS Genet* **6:** e1000838. doi:10.1371/journal.pgen.1000838

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