

# Neurodegenerative Diseases and Autophagy

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## AUTOPHAGY CELL BIOLOGY

### Key Autophagy Machinery

Autophagy (macroautophagy) is a degradation process that delivers cytoplasmic materials to lysosomes. By doing so, autophagy sustains cellular renovation and homeostasis by recycling molecular building blocks (such as amino acids or fatty acids) for anabolic processes. The first morphologically recognizable autophagic precursor is a flat, double-membraned, sac-like structure (called a phagophore), whose edges elongate and fuse while engulfing a portion of the cytoplasm. The resulting structure is a spherical double-membrane organelle, called the autophagosome. The formation of autophagosomes requires several steps (nucleation, elongation, and closure) governed by conserved proteins termed ATGs (Autophagy-related proteins) (Mizushima, Yoshimori, & Ohsumi, 2011). Autophagy initiation and autophagosome formation require multiple interactions between different individual proteins and protein complexes. For simplicity, these are referred to by their abbreviated names in the following sections and are described in full in Table 11.1.

During autophagosome formation, the ATG8 ubiquitin-like family proteins are conjugated to the lipid phosphatidylethanolamine (PE) in autophagosomal membranes. Mammalian cells have six ATG8 orthologues; the MAP1-LC3 (LC3) and GABARAP subfamilies. Lipidated ATG8 proteins have been used to distinguish autophagosomes from other

cellular membranes (Itakura & Mizushima, 2010). Measuring the LC3 lipidation, scoring the number of LC3 vesicles, and detecting the degradation of long-lived proteins or damaged organelles are the mainstay methods used for monitoring autophagy (Itakura & Mizushima, 2010). However, this requires careful interpretation since immune receptors engaged by phagocytosed cargoes can also enable LC3 recruitment to single-membrane phagosomes in a process called LC3-associated phagocytosis (Sanjuan et al., 2007).

LC3/GABARAP lipidation requires a protease and two ubiquitin-like conjugation systems (Ichimura et al., 2000; Mizushima et al., 1998) as illustrated in Fig. 11.1. The first reaction involves the conjugation of the proteins ATG12 to ATG5 in a reaction requiring the enzymatic activities of ATG7 and ATG10. The ATG5-ATG12 conjugate forms a complex with ATG16L1. The cysteine protease ATG4 cleaves the C-terminus of LC3 exposing a glycine residue (LC3-I), which is activated by the ATG7 enzyme, initiating events for the second conjugation reaction. In this second reaction, the ATG12-ATG5-ATG16L1 complex, through interaction with the ATG3, acts as the E3-like ligase that determines the site of LC3 lipidation and assists the transfer of LC3-I to PE to form LC3-II (Ichimura et al., 2000). ATG8/LC3 proteins may assist in the expansion and closure of autophagosomal membranes (Nakatogawa, Ichimura, & Ohsumi, 2007) as well as in autophagosome-lysosome fusion and inner autophagosomal membrane degradation (Nguyen et al., 2016; Tsuboyama et al., 2016).

**TABLE 11.1** List of Abbreviations of Proteins, Complexes and Cellular Structures Involved in Autophagy

Abbreviation	Full Name	Function of Protein or Complex in Autophagy
AKT	Protein kinase B	Serine/threonine kinase
AMBRA-1	Activating molecule in Beclin-1-regulated autophagy	Part of VPS34 complex
AMPK	AMP-activated protein kinase	Protein kinase complex
APP	Amyloid precursor protein	Membrane protein cleaved by secretases to form A $\beta$ peptide
ATGs	AuTophagy-related proteins	
ATG3	AuTophagy-related protein 3	E2-ligase-like enzymatic activity
ATG4	AuTophagy-related protein 4	Cysteine protease
ATG7	AuTophagy-related protein 7	E1-ligase-like enzymatic activity
ATG9	AuTophagy-related protein 9	Organization of the preautophagosomal structure/phagophore assembly site
ATG10	AuTophagy-related protein 10	E2-ligase-like enzymatic activity
ATG12	AuTophagy-related protein 12	Ubiquitin-like protein
ATG14	AuTophagy-related protein 14	Part of VPS34 complex; regulates localization of the complex
ATG16L1 complex	Complex comprising ATG5, ATG12, ATG16L1	E3-ligase-like enzymatic activity
Beclin-1	Homologue of BEC-1 ( <i>C. elegans</i> ) and ATG6 (yeast)	Part of VPS34 complex
COPI	Coatmer protein I	Coat protein complex required for ER-Golgi transport and early endosome formation
CREB	cAMP response element-binding protein	Transcription factor
DFCP1	Double FYVE-containing protein 1	Interact with phospholipids
E2F1	E2F transcription factor 1	Transcription factor
ER	Endoplasmic reticulum	
ERES	ER-exit sites	
ESCRT	Endosomal sorting complexes required for transport	Membrane remodeling
FAM134	Family with sequence similarity 134	Selective autophagy cargo receptor
FIP200	Focal adhesion kinase family interacting protein of 200 kDa	Part of ULK1 complex
FOXO	Forkhead box	Transcription factor
FXR	Farnesoid X receptor	Nuclear receptor
FYVE	Zinc-finger domain	Binds and inserts into PI3P membranes

(Continued)

TABLE 11.1 (Continued)

Abbreviation	Full Name	Function of Protein or Complex in Autophagy
GABARAP	$\gamma$ -Aminobutyric acid receptor-associated protein	ATG8 homologue
LYNUS	Lysosome nutrient-sensing	
MAM	Mitochondria-ER-associated membranes	
MAP1-LC3 or LC3	Microtubule associated proteins 1A/1B light chain 3	ATG8 homologue
mTOR	Mechanistic target of rapamycin	Protein kinase
mTORC1	mTOR complex 1; complex comprising mTOR, RAPTOR, G $\beta$ L	Kinase complex
NBR1	Neighbor Of BRCA1 gene 1	Selective autophagy cargo receptor
NCOA4	Nuclear receptor coactivator 4	Selective autophagy cargo receptor
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells	Transcription factor (protein complex)
NDP52/ CALCOCO2	Nuclear domain 10 Protein 52/calcium binding and coiled-coil domain 2	Selective autophagy cargo receptor
OPTN	Optineurin	Selective autophagy cargo receptor
P53/TP53	Tumor protein 53	Transcription factor (tumor suppressor)
p62/SQSTM1	Ubiquitin-binding protein P62/sequestosome1	Selective autophagy cargo receptor
PARKIN	Parkinson's disease protein 2 (PARK2)	E3-Ubiquitin Protein Ligase
PE	Phosphatidylethanolamine	Phospholipids found in membranes
PI3P	Phosphatidylinositol 3-phosphate	Phospholipids found in membranes
PINK1	PTEN-induced putative kinase 1 (PARK6)	mitochondrial serine/threonine-protein kinase
PPAR $\alpha$	Peroxisome proliferation factor-activated receptor $\alpha$	Transcription factor
RAPTOR	Regulatory-associated protein of mTOR	Part of mTOR Complex 1
RE	Recycling endosome	
RPN10	26S proteasome regulatory subunit RPN10	Proteasome component
SNARE	SNAP (soluble NSF attachment protein) Receptor	Mediate vesicle fusion
SNX18	Sorting nexin 18	Membrane remodeling
TAX1BP1	Tax1 (human T-cell leukemia virus type I) binding protein 1	Selective autophagy cargo receptor
TBC1D14	TBC1 domain family member 14	Membrane remodeling
TFEB	Transcription factor EB	Transcription factor

(Continued)

TABLE 11.1 (Continued)

Abbreviation	Full Name	Function of Protein or Complex in Autophagy
TIP60	60 kDa Tat-interactive protein (K-acetyltransferase 5, KAT5)	Acetyl transferase
TOLLIP	Toll interacting protein	Selective autophagy cargo receptor
TRAF6	TNF receptor-associated factor 6	E3-ubiquitin protein ligase
TRIM	Tripartite motif	Pathogen recognition
UBD	Ubiquitin-binding domain	Recognize and bind to ubiquitin
ULK1 and ULK2	Mammalian homologs of the <i>C. elegans</i> uncoordinated 51 kinase	serine/threonine kinase
ULK1/2-complex	Complex comprising ULK1/2, ATG13, ATG101, and FIP200	Regulation of autophagosome biogenesis
VAMPs	Vesicle-associated membrane proteins	Mediate vesicle fusion
VPS	Vacuolar protein sorting-associated protein	Vesicular transport
VPS34 complex	Complex comprising Beclin-1–ATG14–VPS15–VPS34	Class III phosphoinositide 3-kinase (PI3K)
VTI1B	Vesicle transport through interaction with t-SNAREs homolog 1B	Mediate vesicle fusion
WASH	WASP and Scar homologue	Vesicle trafficking
WDR45	WD repeat domain phosphoinositide-interacting protein 45	Interacts with PI <sub>3</sub>
WIPIs	WD-repeat protein interacting with phosphoinositides (homologues of ATG18)	Interact with phospholipids
ZKSCAN3	Zinc-finger protein with KRAB and SCAN domains 3	Transcription factor

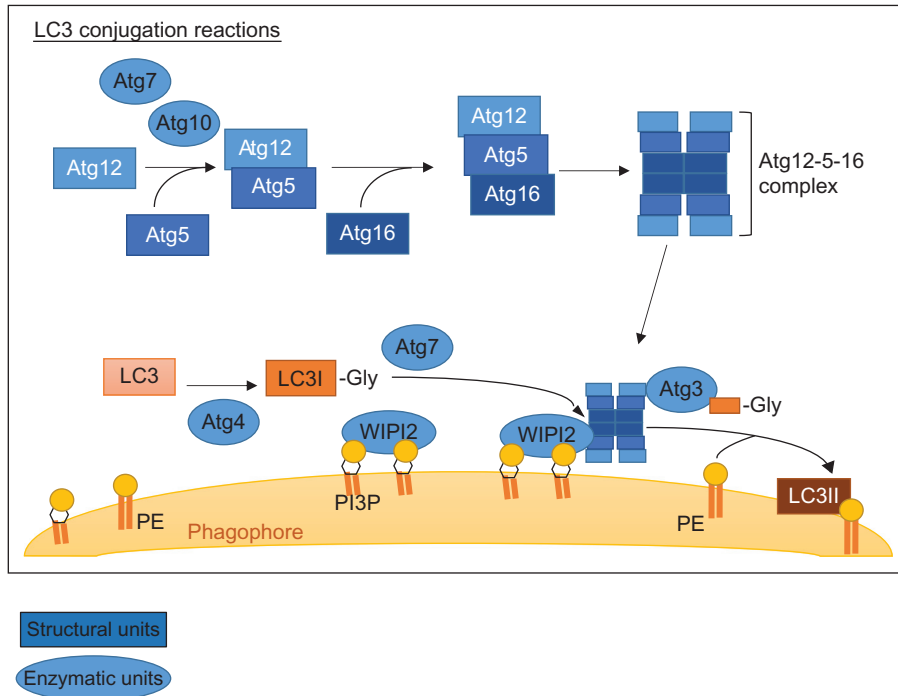
Autophagy initiation and autophagosome formation requires multiple interactions between different individual proteins and protein complexes. For simplicity, these are referred to by their abbreviated names in the text. These proteins and complexes have many diverse cellular functions other than those involved in autophagy; only their main role in autophagy is described here.

### ***ATGs are Organized in Signaling Modules Upstream of LC3 Conjugation***

The ULK1/2-complex is one of the most upstream signaling units in autophagosome formation. This complex includes the ULK1/2 homologues, ATG13, ATG101, and FIP200. AMP-activated protein kinase (AMPK) phosphorylates ATG13 and FIP200 (components of the ULK1/2-complex) and AMBRA-1 and Beclin-1 (components of the VPS34 complex), thereby targeting these two complexes to the

preautophagosomal membrane (see Fig. 11.2) (Di Bartolomeo et al., 2010; Egan et al., 2015; Itakura & Mizushima, 2010; Jung et al., 2009; Park, Jung et al., 2016; Russell et al., 2013).

The generation of the lipid phosphatidylinositol 3-phosphate (PI3P) by the VPS34 complex at the phagophore initiation site aids the recruitment of PI3P-binding ATGs, such as DFCP1 and ATG18/WIPIs family proteins (Proikas-Cezanne, Takacs, Donnes, & Kohlbacher, 2015). WIPI2 is crucial for the localization of ATG16L1 complex



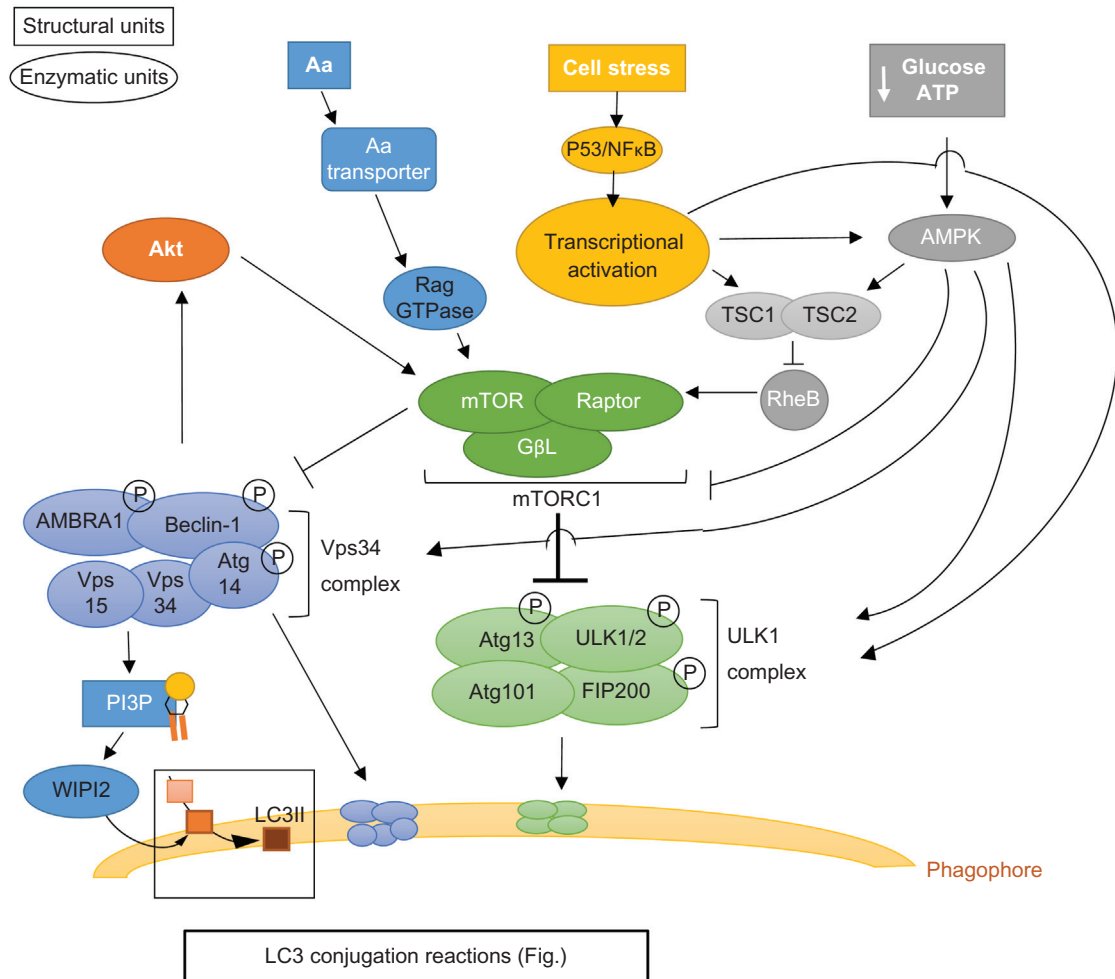
**FIGURE 11.1** Conjugation steps involved in the regulation of autophagy. Two conjugation systems are required for phagophore/autophagosome membrane elongation resulting in the formation of the ATG12-5-16 complex and LC3-II. Structural components are represented as squares, and enzymatic components are represented as circles. Initial conjugation of ATG12 and ATG5 is mediated by the enzymatic activity of ATG7 and ATG10. ATG5 interacts with ATG16, generating the ATG12-5-16 conjugate, which then homo-oligomerizes to form a tetrameric structure called ATG12-5-16 complex. LC3 is cleaved by ATG4 exposing a glycine residue and generating LC3-I, which is activated by ATG7. The ATG12-5-16 complex through ATG3 interaction covalently conjugates LC3-I to PE to form LC3-II. WIPI 2 modulates the localization of the ATG12-5-16 complex through PI3P, facilitating the interaction of LC3-I with PE and its lipidation in the autophagosomal membrane. *PE*, phosphatidylethanolamine; *PI3P*, phosphatidylinositol 3-phosphate.

and dictates where the LC3 lipidation occurs (Dooley et al., 2014). Together with ULK1, WIPI2 may influence the localization of both mATG9 and ATG2A/B. mATG9, the only transmembrane protein among the core ATGs, is considered one of the suppliers of lipid bilayers to the initiation membrane during elongation (Orsi et al., 2012; Papinski et al., 2014), while ATG2A/B regulates autophagosome closure through fission/scission-type events (Knorr, Lipowsky, & Dimova, 2015; Velikkakath, Nishimura, Oita, Ishihara, & Mizushima, 2012).

### Autophagosome Membrane Trafficking Events

The membrane source for autophagosome formation remains a key open question in the field. Membranes from different organelles are believed to contribute to autophagosome formation by meeting in a particular subcellular compartment representing the autophagosome platform or “isolation membrane.” The isolation membrane is a compartment in proximity to mitochondria-endoplasmic reticulum

## Upstream signalling in autophagosome formation



**FIGURE 11.2** Initiation complexes controlling the initiation of autophagy. Many different cellular signals such as level of nutrients, ATP, or cellular stress control autophagy by modulating mTORC1 activity. The activation of the Akt and the presence of amino acids detected by Rag GTPases induce mTORC1 which in turns inhibits both Vps34 and ULK1/2 complexes and hence inhibiting autophagy. Conversely, deprivation of glucose or ATP promotes autophagy by activating AMPK, which directly phosphorylates and induces VPS34 and ULK1/2 complexes. Moreover, AMPK inactivates mTORC1 either directly or through TSC1/TSC2, removing inhibition from ULK1/2-complex. Cell stress, modulated by factors like p53 or NFκB, transcriptionally activates proautophagy genes such as TSC1/TSC2 and ULK1/2 and, therefore, inducing autophagy. The formation of the phagophore as a preautophagosomal structure requires the serial recruitment of ULK1/2-complex first and VPS34 complex after, which generates PI3P, crucial for autophagosome formation. *mTORC1*, mTOR complex; *AMPK*, AMP-activated protein kinase; *PI3P*, phosphatidylinositol 3-phosphate.

(ER)-associated membranes (MAMs) WIPI2 (Axe et al., 2008; Proikas-Cezanne et al., 2009) and is labeled by ATG14, DFCP1, and membrane and surrounding organelles might



contribute to the completion of the autophagosome (Biazik, Yla-Anttila, Vihinen, Jokitalo, & Eskelinen, 2015). Different sources have been proposed as donor membranes including the ER, MAM, ER-exit sites, the ER-Golgi intermediate compartment, recycling endosomes (REs), Golgi, and plasma-membrane (Axe et al., 2008; Biazik et al., 2015; Ge, Melville, Zhang, & Schekman, 2013; Ge, Zhang, & Schekman, 2014; Graef, Friedman, Graham, Babu, & Nunnari, 2013; Itakura & Mizushima, 2010; Karanasios et al., 2016; Knaevelsrud et al., 2013; Longatti et al., 2012; Park, Jung et al., 2016; Puri, Renna, Bento, Moreau, & Rubinsztein, 2013; Ravikumar, Moreau, Jahreiss, Puri, & Rubinsztein, 2010; Shibutani & Yoshimori, 2014; Tan et al., 2013; Yla-Anttila, Vihinen, Jokitalo, & Eskelinen, 2009).

The endocytic compartment is believed to play a primary role in autophagosome formation. The ATG16L1 complex and mATG9 travel on independent clathrin-coated vesicles, and these vesicles fuse in the REs in a VAMP3-dependent manner. The trafficking of these proteins from plasma-membrane to RE trafficking and subsequent vesicle fusion are essential for autophagy (Moreau, Ravikumar, Renna, Puri, & Rubinsztein, 2011; Puri et al., 2013; Ravikumar et al., 2010). The contribution of REs to autophagosome formation is supported by studies showing that autophagic proteins (e.g., ULK1 and LC3) localize on the RE and that the overexpression of RE-resident proteins (e.g., TBC1D14 and SNX18) interfere with the trafficking of mATG9 and ATG16L1 (Knaevelsrud et al., 2013; Lamb et al., 2016; Longatti et al., 2012).

### Key Signaling Pathways

Low cellular energy and nutrient states signal to the autophagy pathway by posttranslationally modifying autophagy-initiating complexes or by regulating the transcription of core

autophagy genes. The energy-sensing AMPK and the growth factor-regulated and nutrient-sensing kinase mammalian target of rapamycin (mTOR) oppositely regulate the ULK1/2 and VPS34 complexes (Kim et al., 2013; Kim, Kundu, Viollet, & Guan, 2011; Yuan, Russell, & Guan, 2013), and thereby autophagy, through a series of phosphorylation events.

AMPK is activated in response to low nutrients (glucose) and low energy (ATP) (Kim et al., 2013). AMPK allosteric activation by AMP binding and phosphorylation of a conserved threonine residue (Thr172) promotes autophagy by directly activating ULK1 through phosphorylation of Ser 317 and Ser 777 (under glucose starvation) (Kim et al., 2011) or Ser 555 (under nutrient starvation and mitophagy) (Yuan et al., 2013). AMPK activates the proautophagy VPS34 complex by phosphorylating Beclin-1 at Ser91/Ser94 (Orsi et al., 2012).

The mTOR complex 1 (mTORC1) is activated by nutrients (amino acids sensed by the Rag GTPases) and growth factors (signaling by receptor tyrosine kinases and the PI3K/Akt pathway) (Laplante & Sabatini, 2012). Under nutrient sufficiency, active mTORC1 inhibits autophagy by binding the ULK1/2-complex, (via raptor-ULK1/2 association) and phosphorylating ATG13 and ULK1 at Ser 757 (i.e., a different site from that phosphorylated by AMPK). This suppresses ULK1/2 kinase activity and prevents the ULK1-AMPK interaction (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009; Kim et al., 2011). Under nutrient starvation, mTORC1 dissociates from the ULK1/2-complex, resulting in ULK1 activation, autophosphorylation, and phosphorylation of ATG13 and FIP200, leading to the autophagosome formation (Ganley et al., 2009; Jung et al., 2009; Kim et al., 2011). mTORC1 also inhibits the phosphoinositide 3-kinase activity of the proautophagy VPS34 complex by phosphorylating ATG14 (Velikkakath et al., 2012). Additional posttranslational modifications that increase ULK1 activity after different inducing stimuli



include ubiquitination, through the E3-ligase TRAF6 (Nazio et al., 2013), and acetylation, by the acetyltransferase TIP60 (Lin et al., 2012).

A plethora of transcription factors integrate a wide range of cellular stimuli to induce core autophagy-related genes expression (Fullgrabe, Klionsky, & Joseph, 2014). Among the autophagy-associated transcription factors, the transcription factor EB (TFEB) (Sardiello et al., 2009; Settembre et al., 2011) has a prominent role, as its overexpression alone is sufficient to induce autophagy and to ameliorate the phenotype of neurodegenerative diseases (Decressac et al., 2013; Tsunemi et al., 2012) and lysosomal storage disorders (LSDs) in vivo (Medina et al., 2011; Spampinato et al., 2013). TFEB connects the lysosome nutrient-sensing machinery to the transactivation of autophagy-related genes (Settembre et al., 2012). Under fed conditions, TFEB is phosphorylated by mTORC1, which leads to its retention in the cytosol. When autophagy is induced, through the inhibition of mTORC1, TFEB becomes dephosphorylated and translocates to the nucleus (Roczniak-Ferguson et al., 2012).

Other well established transcriptional regulators of autophagy include the FOXO family, p53, E2F1, and NF- $\kappa$ B (Hayashi-Nishino et al., 2009). More recently, the farnesoid X receptor (FXR)/peroxisome proliferation factor-activated receptor  $\alpha$  (PPAR $\alpha$ )/cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) axis was discovered as a regulator of a plethora of core autophagy-related genes. Under fed conditions, the nuclear receptor FXR acts as a transcriptional repressor, while autophagy induction by starvation leads to the activation of CREB and PPAR $\alpha$  (Lee et al., 2014; Seok et al., 2014).

## Selective Autophagy

Stress-induced autophagy is thought to be nonselective, leading to bulk degradation of

cytoplasm. However, autophagy contributes to intracellular homeostasis in fed conditions by selectively degrading long-lived proteins or damaged organelles, recognized by specific autophagy receptors (Shaid, Brandts, Serve, & Dikic, 2013; Svenning & Johansen, 2013).

Selective autophagic pathways are generally named after the cargo destined for degradation and include aggregatephagy (protein aggregates), mitophagy (mitochondria), xenophagy (pathogens) (Deretic, Saitoh, & Akira, 2013; Melser, Lavie, & Benard, 2015; Randow & Youle, 2014; Rogov, Dotsch, Johansen, & Kirkin, 2014; Sorbara & Girardin, 2015), ER-phagy (ER) (Khaminets et al., 2015; Mochida et al., 2015), ferritinophagy (ferritin) (Dowdle et al., 2014; Mancias, Wang, Gygi, Harper, & Kimmelman, 2014), pexophagy (peroxisomes) (Kim, Hailey, Mullen, & Lippincott-Schwartz, 2008), ribophagy (ribosomes) (Kraft, Deplazes, Sohrmann, & Peter, 2008), and lipophagy (lipid droplets) (Singh et al., 2009). Autophagy receptors are generally considered as either ubiquitin-dependent or ubiquitin-independent (Khaminets, Behl, & Dikic, 2016).

Aberrantly folded or unused proteins are ubiquitinated, aggregated, and sequestered by proteins containing an ubiquitin-binding domain (i.e., p62/SQSTM1, NBR1, OPTN, TAX1BP1, NDP52/CALCOCO2, TOLLIP, and RPN10) that deliver them to lysosomes via autophagy (Bjorkoy et al., 2005; Kirkin, Lamark, Johansen, & Dikic, 2009; Lu, Psakhye, & Jentsch, 2014; Marshall, Li, Gemperline, Book, & Vierstra, 2015; Newman et al., 2012; Pankiv et al., 2007; Thurston, Ryzhakov, Bloor, von Muhlinen, & Randow, 2009; Wild et al., 2011).

Mitophagy is responsible for disposal of dysfunctional mitochondria. Multiple signals trigger mitophagy, including hypoxia and erythroid differentiation. PTEN-induced putative kinase 1 (PINK1) and PARKIN, proteins encoded by two genes that are mutated in autosomal recessive Parkinson's disease (PD), enable forms of mitophagy. This pathway is activated by nonhypoxic mitochondrial

damage and linked to neurodegenerative diseases, such as PD and amyotrophic lateral sclerosis (ALS) (Hamacher-Brady & Brady, 2015; Liu et al., 2012; Melser et al., 2015; Pickrell et al., 2015; Sandoval et al., 2008). In response to mitochondrial damage, PINK1 is stabilized on the mitochondrial outer membrane and phosphorylates both cytoplasmic PARKIN and ubiquitin on mitochondria (Cunningham et al., 2015; Kane et al., 2014; Kazlauskaitė et al., 2014; Kondapalli et al., 2012; Koyano et al., 2014; Ordureau et al., 2014). p62, OPTN, TAX1BP1, and NDP52 work as ubiquitylated mitochondrial protein receptors (Heo, Ordureau, Paulo, Rinehart, & Harper, 2015; Lazarou et al., 2015; Wong & Holzbaur, 2014).

Cytosolic bacteria can also be ubiquitylated and degraded by autophagy as a part of the innate immune response. Several E3 ligases attach ubiquitin chains to intracellular pathogens, for example, PARKIN on *Mycobacterium tuberculosis* (Manzanillo et al., 2013) and Lrsm1 on *Salmonella enterica* (Huett et al., 2012). Furthermore, *Salmonella*-containing endosomes can undergo ubiquitination, which recruits the autophagic machinery and ultimately incorporates them into autophagosomes (Fujita et al., 2013). Similarly, different members of the tripartite motif protein family have been linked to xenophagy (Kimura et al., 2015; Mandell et al., 2014).

A growing class of ubiquitin-independent selective autophagy pathways have been described (Khaminets et al., 2016), such as NCOA4-mediated ferritinophagy (Dowdle et al., 2014; Mancias et al., 2014) and FAM134-dependent ER-phagy (Khaminets et al., 2015), that appear specialized for degradation of one substrate cargo.

## Lysosomes

Whilst the signaling pathways and machinery for the generation and trafficking of

autophagosomes are important, arguably the key organelle in this process is the lysosome, since it plays a crucial role in maintaining the balance of cellular metabolism and growth by continuously mediating anabolic and catabolic processes. First described by Christian de Duve, the lysosome is a cellular organelle made of a single-lipid bilayer membrane and an acidic lumen (de Duve, 2005), which contains a complex machinery of hydrolases that are responsible for the catabolism of a vast range of substrates (Luzio, Pryor, & Bright, 2007; Saftig & Klumperman, 2009; Schroder, Wrocklage, Hasilik, & Saftig, 2010). The majority of extracellular substrates are transported to the lysosome via the endocytic pathway, and in particular through the fusion of the lysosome with late endosomes (Conner & Schmid, 2003; Huotari & Helenius, 2011), whereas intracellular substrates reach the lysosome via fusion of autophagosomes with lysosomes along the autophagic pathway. The delivery of engulfed cytosolic components is tightly coordinated so that only fully formed autophagosomes fuse with the endocytic system and deliver their contents to the lysosome. In mammalian cells, SNAREs, including VAMP7, VAMP8, and VTI1B, mediate the lysosomal fusion of autophagosomes (Fader, Sanchez, Mestre, & Colombo, 2009; Furuta, Fujita, Noda, Yoshimori, & Amano, 2010). SNAREs are membrane-anchored proteins localized on opposing membrane compartments that can interact with each other to form a highly energetically favorable complex. In order to drive membrane fusion, SNAREs must form a *trans*-SNARE complex consisting of one R-SNARE on the donor membrane and three Q-SNAREs on the acceptor membrane (Jahn & Scheller, 2006). As these SNAREs are common to other intracellular trafficking pathways, it can be difficult to assess their specific roles in endocytosis and membrane trafficking versus autophagy. The Q-SNAREs syntaxin-7, syntaxin-8, and VTI1B, along with the R-SNAREs VAMP7 and VAMP8, have

been linked to the fusion of late endosomes and lysosomes and thus play indirect roles in autophagosome maturation (Pryor et al., 2004). However, these SNAREs also function in autophagosomal fusion. Indeed, VAMP8 and VTI1B, but not VAMP7, syntaxin-7 or syntaxin-8, were shown to be involved in autophagosomal fusion during clearance of intracellular bacteria (Furuta et al., 2010).

### Autophagy in Neuronal Physiology

In the mouse, ubiquitous deletion of core autophagy genes results in neonatal death (Komatsu et al., 2005; Kuma et al., 2004; Sou et al., 2008); however, conditional rescue of autophagy in the nervous system can rescue this lethality (Yoshii et al., 2016). The essential role of autophagy in maintaining normal neuronal physiology has been elucidated through disruption of core autophagy genes using conditional knock-out approaches. Neuronal depletion of ATG5, ATG7, FIP200, or WIPI4 results in progressive neurodegenerative phenotypes, an accumulation of ubiquitinated and often p62/SQSTM1-positive protein inclusions, cell death, and reduced survival of the mice (Hara et al., 2006; Komatsu et al., 2006; Komatsu, Waguri, Koike et al., 2007; Liang, Wang, Peng, Gan, & Guan, 2010; Zhao et al., 2015)[2-6]. However, there are differences in the nature of the behavioral and pathological changes observed depending on the autophagy gene and cell type targeted.

Many of the original nervous system models generated did not disrupt autophagy exclusively in neurons. Depending on the promoter used, such as Nestin-Cre, nonneuronal support cells and neuronal stem cells were also targeted. Hence, there has been continued focus on more precise cellular autophagy disruption to delineate its role within specific nervous system cell types. This includes targeting subsets of neuronal cell types (Chen et al., 2013; Kaushik et al., 2011; Komatsu et al., 2006; Komatsu, Wang et al., 2007; Nishiyama, Miura,

Mizushima, Watanabe, & Yuzaki, 2007; Zhou, Doggett, Sene, Apte, & Ferguson, 2015) as well as nonneuronal (e.g., Schwann) cells (Gomez-Sanchez et al., 2015; Jang et al., 2016; Jang et al., 2015). Studies disrupting core autophagy genes in neural progenitor cells also provide an emerging area of focus, with autophagy disruption resulting in reduced neural stem cell survival and neuronal maturation (Lu et al., 2014; Wang, Liang, Bian, Zhu, & Guan, 2013; Wu et al., 2016; Xi et al., 2016; Yazdankhah, Farioli-Vecchioli, Tonchev, Stoykova, & Cecconi, 2014).

In addition to knock-out studies, enhanced tools are being developed to image and monitor autophagy in vivo in normal and disease conditions, for example, GFP-LC3 reporter mice and more recently GFP-RFP-LC3 mice (Castillo, Valenzuela et al., 2013; Mizushima, Yamamoto, Matsui, Yoshimori, & Ohsumi, 2004; Pavel et al., 2016). Furthermore, mouse models have been generated to study mitophagy in vivo (McWilliams et al., 2016; Sun et al., 2015) in specific cell types as well as under varied genetic conditions such as *Atg5* or *Atg7* disruption. These tools will enable measurement of how mitophagy is modulated in broad genetic and pharmacological conditions. They may be particularly useful for PD, where there is extensive support for a central role for autophagy/mitophagy disruption, mitochondrial damage, and an established importance of the PINK1–PARKIN pathway, as reviewed (Pickrell et al., 2015).

## AUTOPHAGY IN NEURODEGENERATIVE DISEASES

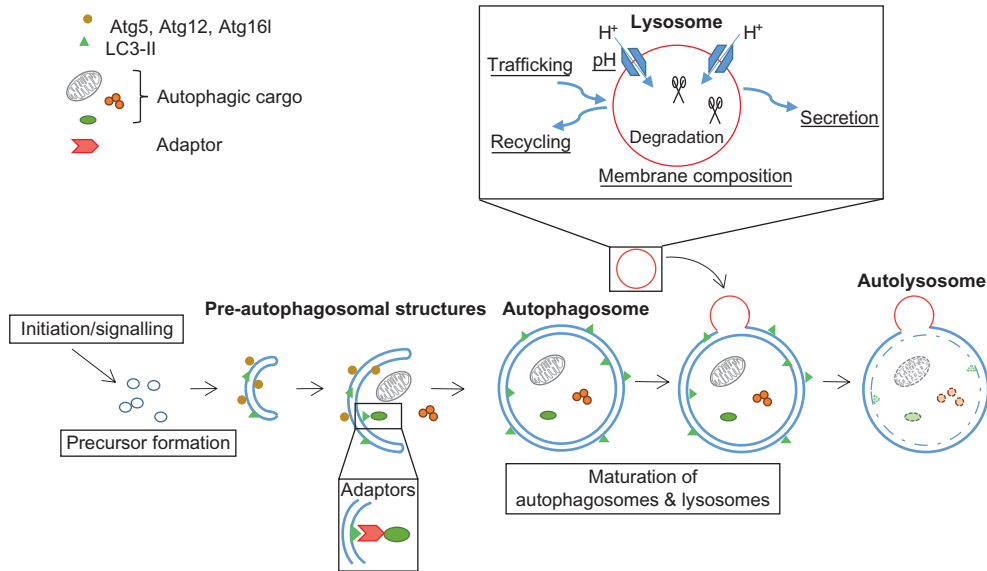
Although the most common neurodegenerative diseases are largely sporadic, mutations that give rise to rare familial forms and genes identified in GWAS studies have highlighted how perturbation of autophagic processes contribute to these diseases. Below, we discuss the evidence for perturbed autophagy in the pathogenesis of various neurodegenerative diseases and the genetic factors that have been

identified which may affect the efficiency of autophagic processes in affected patients (summarized in Fig. 11.3).

## Alzheimer's Disease

Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by the accumulation of intraneuronal tau tangles and extracellular amyloid-beta ( $A\beta$ ) deposits

called plaques. Whereas abundant  $A\beta$  deposits are specific to AD, tau inclusions are also characteristic of other neurodegenerative diseases named tauopathies (Goedert & Spillantini, 2006).  $A\beta$  peptides are cleavage products of the amyloid precursor protein (APP). In sporadic cases, the accumulation of plaques appears to result from impaired  $A\beta$  clearance, in contrast with the overproduction of  $A\beta$  species in the rare dominantly inherited forms of AD



Initiation & signalling	Precursor formation	Adaptor proteins	Maturation	Autolysosome formation	Lysosome function	Trafficking	Secretion	Mitophagy
C9ORF72	WIPI4	P62	EPG5	VPS11	SPG11	Tau	$A\beta$	Parkin
Atg5	PICALM	optineurin	PICALM		GBA	VPS35	SYT11	PINK1
SPG15	VPS35	Htt	SigR1		TFEB	LRRK2	TFEB	SPG59
	$\alpha$ -syn		CHMPB2		ATP13A2	ALS2		
	malin		SPG49		PS-1	CHMPB2		
	Laforin				Tau	DYNC1H1		
					CCT5	Dynactin		
					SNX14	Dynamin2		
					NPC1			

**FIGURE 11.3** Schematic diagram of autophagosome formation and degradation. Genes that are known to play a role in specific steps of the pathway and for which there are known clinical mutations are presented in the table below.

(e.g., APP mutations) (Mawuenyega et al., 2010; Potter et al., 2013). Downregulation of autophagy can enhance AD pathogenesis in model systems and similarly upregulation of the autophagic system has been shown to reduce A $\beta$  levels in a number of models (Boland et al., 2008; Ravikumar, Sarkar, & Rubinsztein, 2008; Spilman et al., 2010; Tian, Bustos, Flajolet, & Greengard, 2011; Vingtdeux et al., 2011).

Neurons from AD patients show an abnormal accumulation of autolysosomes/lysosomes (Nixon et al., 2005; Yu et al., 2005), and autophagic failure by impaired autophagosome formation or defective lysosomal clearance appear to contribute to the increase of A $\beta$ /tau deposits and the development of AD features in fibroblasts and brains from patients and mouse models (Li, Zhang, & Le, 2010; Nixon & Yang, 2011; Zhang, Chen, Huang, & Le, 2013). Wild-type APP has been found in autophagosomes, suggesting that autophagy might also contribute to A $\beta$  formation (Boland et al., 2008; Yu et al., 2005). Mutations in Presenilin-1 (*PS-1*) change the way APP protein is processed into A $\beta$  and are one of the main causes of familial AD (Citron et al., 1997). However, PS-1 is also necessary for the v-ATPase-dependent acidification of the lysosome, and PS-1 mutations are associated with elevated lysosomal pH that would be expected to affect its catabolic activity (Coffey, Beckel, Laties, & Mitchell, 2014; Lee et al., 2010; Wolfe et al., 2013). The lysosomal endopeptidase Cathepsin D (CatD) has also been implicated in the clearance of A $\beta$  and tau peptides through the autophagy-lysosomal system. CatD has been found to colocalize with senile plaques of AD patients (Cataldo, Hamilton, & Nixon, 1994; Cataldo & Nixon, 1990; Khurana et al., 2010) and is involved in the processing APP and apolipoprotein E, both important factors in AD pathogenesis (Vidoni, Follo, Savino, Melone, & Isidoro, 2016; Zhou, Scott, Shelton, & Crutcher, 2006).

While the role of autophagy in degrading A $\beta$  has been extensively studied in

model systems (reviewed in Zare-Shahabadi, Masliah, Johnson, & Rezaei, 2015), autophagy-dependent secretion of A $\beta$  into the extracellular space has been also reported in APP transgenic mice in which ATG7 is knocked out, suggesting that autophagy might regulate plaque formation (Nilsson et al., 2013). Indeed, the autophagic markers LC3, ATG5, and ATG12 have been associated with A $\beta$  plaques and tau tangles in human brains (Ma, Huang, Chen, & Halliday, 2010), and both APP and A $\beta$  peptides can be found within autophagosomes in AD mouse models (Lunemann et al., 2007).

The clathrin adapter protein PICALM (Phosphatidylinositol Binding Clathrin Assembly Protein) has been shown to interact with LC3 and target APP into autophagosomes (Tian, Chang, Fan, Flajolet, & Greengard, 2013). Polymorphisms in the *PICALM* gene are associated with increased risk of AD. PICALM is a key component of clathrin-mediated endocytosis, and loss of function of this protein inhibits autophagy at both the levels of autophagosome formation and degradation and affects tau clearance in model systems (Moreau et al., 2014). Recently, it has been also reported that levels of this protein are decreased in AD brains (Ando et al., 2013, 2016).

Depletion or downregulation of Beclin-1, an autophagy initiator, promotes accumulation and deposition of A $\beta$ , leading to marked neurodegeneration in both cell culture and mouse models (Jaeger et al., 2010; Pickford et al., 2008). Reduced levels of Beclin-1 have been reported in the brains of AD patients (Pickford et al., 2008; Small et al., 2005), and these have been correlated with caspase 3 activation, which in turn cleaves Beclin-1 to a fragment that becomes localized to plaque regions and blood vessels in AD brains (Rohn et al., 2011).

## Tauopathies

Besides AD, the accumulation of tau protein into intracellular tangles is the main feature



of many other neuronal disorders termed tauopathies, including progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), or frontotemporal dementias (FTDs) (Lee, Goedert, & Trojanowski, 2001). Degradation of soluble tau depends on both the ubiquitin-proteasome system and autophagy (Chesser, Pritchard, & Johnson, 2013), whereas oligomers and aggregates are mainly degraded by autophagy (Boland et al., 2008; Lee, Lee, & Rubinsztein, 2013). Hyperphosphorylated tau colocalizes with the autophagosomal marker LC3 and the autophagy cargo receptor p62/SQSTM1 in tauopathy patients with CBD or PSP (Piras, Collin, Gruninger, Graff, & Ronnback, 2016). Some pathogenic tau mutations, like P301L or A152T, impair proteasome activity leading to the accumulation of ubiquitinated proteins and small peptides in animal models (Myeku et al., 2016) that can be ameliorated by inducing autophagy (Lopez et al., 2017). Autophagy induction ameliorates the pathological consequences of aberrant tau in diverse experimental systems including primary neurons, *Drosophila*, zebrafish, and mice (Berger et al., 2006; Caccamo et al., 2013; Kruger, Wang, Kumar, & Mandelkow, 2012; Lopez et al., 2017; Moreau et al., 2014; Schaeffer et al., 2012).

Tau protein binds to and stabilizes microtubules, which are the basic components involved in axonal vesicle transport (Bernhardt & Matus, 1984; Goedert, Wischik, Crowther, Walker, & Klug, 1988; Millecamps & Julien, 2013). Mutations and/or hyperphosphorylation of tau have been reported to impair the dynein–dynactin complex, leading to disruption of axonal transport and increasing the number of autophagosomes in FTDs and AD (Butzlaff et al., 2015; Kimura, Noda, & Yoshimori, 2008; Lacovich et al., 2017; Majid et al., 2014). Small tau fibrils can interact with lysosomal membranes in vitro (Wang et al., 2009) and disrupt their permeability, resulting in lysosomal damage in a mouse model of AD

(Piras et al., 2016) and in AD patients (Perez et al., 2015). Moreover, increased levels of lysosomal proteins LAMP1 and CatD have been recently reported in CBD and PSP patients (Piras et al., 2016).

## Parkinson's Disease

PD results in the progressive loss of dopaminergic neurons in the substantia nigra pars compacta and is associated with the presence of Lewy bodies, alpha-synuclein ( $\alpha$ -syn)-positive intracellular inclusions. Increased  $\alpha$ -syn levels, as a result of multiplication of the *SNCA* gene encoding it, is sufficient to cause PD. Overexpression of  $\alpha$ -syn in cells and mice impairs autophagy and results in mislocalization of mATG9 (Winslow et al., 2010). Accumulation of  $\alpha$ -syn has also been described for PD-associated mutations of VPS35 (vacuolar protein sorting-associated protein 35). VPS35 is a component of the retromer complex that recruits actin nucleation-promoting WASP and Scar homologue complex to endosomes. The D620N mutation in VPS35 causes autosomal-dominant PD and, in transfected cells, has been shown to impair autophagy, mislocalize mATG9 (Zavodszky et al., 2014), and affect the trafficking of the lysosomal protein LAMP2A, leading to  $\alpha$ -syn accumulation (Tang et al., 2015).

Heterozygous mutation of *GBA1* is the most common known genetic risk factor for PD. *GBA1* encodes glucocerebrosidase (GCase), a lysosomal enzyme that cleaves the  $\beta$ -glucosyl linkage of glucosylceramide (GlcCer). Deficiency in GCase activity leads to accumulation of its substrate in the lysosome and compromised lysosomal activity. Loss of GCase activity, with resultant increased GlcCer levels, leads to an increase in  $\alpha$ -syn levels in cultured neurons, mouse, and human brain. Increased  $\alpha$ -syn in turn inhibits lysosomal maturation and GCase activity, resulting in additional GlcCer

accumulation and further  $\alpha$ -syn accumulation (Mazzulli et al., 2011). Even in sporadic PD without *GBA1* mutations, decreased lysosomal GCase activity was directly related to reduced lysosomal chaperone-mediated autophagy and increased  $\alpha$ -syn in the early stages of PD (Murphy et al., 2014). Treatment of iPSC-derived dopaminergic (DA) neurons from PD patients with a modulator of GCase enhanced GCase activity and increased clearance of  $\alpha$ -syn and reversed lysosomal dysfunction (Aflaki, Borger et al., 2016; Mazzulli et al., 2016). In the Thy1-SNCA mouse model of PD, where mice express mutant human A53T  $\alpha$ -syn, ectopic expression of GCase in the striatum led to a decrease in the levels of  $\alpha$ -syn and delayed the progression of synucleinopathy (Rockenstein et al., 2016). In addition to inhibition of the autophagy-lysosomal pathway, GBA mutations activate the unfolded protein response and lead to ER stress (in fibroblasts and iPSC-derived DA neurons from PD patients), which can be reversed by small molecule chaperones in cells and *Drosophila*, by improving GCase trafficking to the lysosomes (Fernandes et al., 2016; Sanchez-Martinez et al., 2016). Homozygous GBA mutation causes Gaucher disease, an LSD, and chaperone treatment of Gaucher patient macrophages induces autophagy, restoring autophagosome maturation, and the fusion of lysosomes with autophagosomes (Aflaki, Moaven et al., 2016). The mechanism by which GCase deficiency leads to reduced autophagy and accumulation of  $\alpha$ -syn has been proposed to be as a result of altered lysosomal recycling, via the process of autophagy-lysosome reformation, resulting in the accumulation of defective lysosomes (Magalhaes et al., 2016).

Mutation in *ATP13A2*, which encodes a lysosomal P5-type ATPase that facilitates cation transport, results in autosomal recessive early-onset PD. Depletion of *ATP13A2*, through modulation of synaptotagmin 11 levels, impairs lysosomal function and the degradation of lysosomal substrates, resulting in

accumulation of  $\alpha$ -syn (Bento, Ashkenazi, Jimenez-Sanchez, & Rubinsztein, 2016; Dehay et al., 2012). Although *Atp13a2*-deficient mice do not exhibit degeneration of DA neurons or significant accumulation of  $\alpha$ -syn, subunit c of mitochondrial ATP synthase accumulated in abnormal lysosomes as a result of lysosomal dysfunction and suggests that clearance of damaged mitochondria may be impaired (Sato et al., 2016). Additionally, trafficking to the lysosome of CatD, which is known to degrade  $\alpha$ -syn, was decreased (Kett & Dauer, 2016). Studies of patient-derived cells show  $Zn^{2+}$  dyshomeostasis, mitochondrial dysfunction, and glycolytic dysfunction as a result of loss of *ATP13A2* (Park, Koentjoro, Davis, & Sue, 2016).

Familial early-onset forms of PD are also caused by recessive mutations in *PARK2/PARKIN* (Kitada et al., 1998), encoding an E3-ubiquitin ligase, and *PINK1* (Valente et al., 2004), encoding a serine-threonine kinase. As described above, both proteins control mitophagy. Both *PINK1* and *PARKIN* patient-derived mDA neurons showed accumulation of  $\alpha$ -syn, increased susceptibility to mitochondrial toxins, mitochondrial dysfunction, and increased intracellular DA levels (Chung et al., 2016).

Mutations in the gene encoding leucine-rich repeat kinase 2 are responsible for the majority of inherited forms of PD as well contributing to some cases of sporadic PD. Whilst the exact function of the wild-type protein is not fully understood, loss-of-function experimental models suggest an important role in intracellular vesicle trafficking (reviewed in Roosen & Cookson, 2016). However, clinical mutations are spread throughout this multidomain protein, and it is unclear how these individual mutations may affect protein function. For example, the kinetics of autophagosome formation and autophagosome-lysosome fusion has been shown to be disrupted in G2019S iPSC-derived human neurons,



resulting in delayed mitophagy (Hsieh et al., 2016), and increased  $\alpha$ -syn levels were found in iPSC-derived DA neurons from PD patients with the G2019S mutation, but not the R1441G mutation. However, in the latter example, impaired canonical NF- $\kappa$ B signaling was proposed to be the mechanism, rather than autophagy (Lopez de Maturana et al., 2016). In G2019S knock-in mice, with abnormally elevated excitatory synaptic activity and altered postsynaptic morphology (Matikainen-Ankney et al., 2016), no changes in  $\alpha$ -syn were observed, but increased levels of LC3-II were reported (Yue et al., 2015). In contrast, R1441G knock-in mice showed no difference in  $\alpha$ -syn, LC3B, or Beclin-1 expression, only perturbed DA homeostasis (Liu, Lu et al., 2014).

### Polyglutamine Disorders

Nine polyglutamine (polyQ) diseases are caused by a mutation in the polyQ domain in different proteins that result in the expansion of the polyQ tract. Examples include mutant huntingtin in Huntington's disease (HD), mutant ataxin-3 in spinocerebellar ataxia type 3 (SCA3), other mutant proteins in other spinocerebellar ataxias, and mutant androgen receptor in spinal and bulbar muscular atrophy (SBMA) (Gatchel & Zoghbi, 2005). Earlier age of disease onset is often correlated with increasing length of the polyQ mutation (Andrew et al., 1993). The expansion of the polyQ domain (usually more than 35 glutamines) results in accumulation of the mutant proteins in oligomeric forms and aggregates in neurons that are found in distinct regions in the brain (e.g., striatum, cerebral cortex, and cerebellum) (Andrew et al., 1993; Gatchel & Zoghbi, 2005; Rubinsztein, 2006). While neuronal toxicity is linked with aggregate formation, toxicity is also observed in neurons without aggregates (Arrasate, Mitra, Schweitzer, Segal, & Finkbeiner, 2004).

Studies have described autophagy perturbation in several polyQ diseases, often by mechanisms that alter upstream signals

required for autophagy induction. For example, the Ras homolog enriched in striatum (Rhes) binds the autophagy initiation protein, Beclin-1, and thus prevents the autophagy inhibitory interaction of bcl-2 with Beclin-1 (Mealer, Murray, Shahani, Subramaniam, & Snyder, 2014). Mutant huntingtin interacts with Rhes and reduces the beneficial effect of Rhes on Beclin-1 activation and autophagy (Mealer et al., 2014). Wild-type huntingtin serves as a scaffold or adaptor for selective autophagy that is induced by cellular stresses (Rui et al., 2015); therefore, mutant huntingtin might also decrease the efficient recruitment of autophagic cargo (Martinez-Vicente et al., 2010). Furthermore, sequestration of Beclin-1 into polyQ aggregates is seen in models of HD, SCA3, and SCA7 (Alves et al., 2014; Nascimento-Ferreira et al., 2011; Shibata et al., 2006) and might impair Beclin-1-autophagic activity. Indeed, reduced Beclin-1 levels have been observed in fibroblasts derived from SCA3 patients (Onofre et al., 2016). Interestingly, while Beclin-1 sequestration into aggregates is likely to inhibit autophagy, the formation of aggregates can also help neurons to cope with polyQ toxicity (Ravikumar et al., 2004). This is evident in studies observing the sequestration of mTOR into aggregates in HD and SCA7 mouse models (Alves et al., 2014; Ravikumar et al., 2004), which is likely to provide signals that induce autophagy to some extent. However, the net effects of autophagy-inducing versus -inhibitory signals are difficult to discern at present and may vary at different stages of disease. Since some autophagy perturbations are influenced by the soluble fraction of the mutant protein and some by its aggregated forms, it also seems that the ratio between the two fractions can affect the outcome of autophagy in these diseases.

Recent data suggest that wild-type ataxin-3 is a positive regulator of autophagy by acting as a deubiquitinase for the core autophagy protein Beclin-1, thereby protecting it from proteasome-mediated degradation. The ataxin-3-Beclin-1

interaction is enabled by the normal polyQ stretch in ataxin-3. When this tract is enlarged by the SCA3 mutation, the ataxin-3-Beclin-1 interaction is strengthened, but the deubiquitinase activity is decreased. This may result in a dominant-negative effect which lowers Beclin-1 levels and impairs autophagy in SCA3 (Ashkenazi et al., 2017). Interestingly, with other polyQ expansion diseases (e.g., HD), at least in cell lines and an animal model, there appears to be competition *in trans* of the disease-causing polyQ tract in the soluble protein with the interaction of ataxin-3 with Beclin-1. This results in a modest impairment of starvation-induced autophagy in these model systems, which may contribute to these diseases (Ashkenazi et al., 2017).

Another autophagy-associated protein that is regulated by mutant ataxin-3 is the E3-ubiquitin ligase PARKIN (Durcan et al., 2011). PARKIN recruits damaged mitochondria for degradation by autophagy (Narendra, Tanaka, Suen, & Youle, 2008) and reduced levels of parkin in the brain of a transgenic mouse model of SCA3 might be linked to disease pathogenesis (Durcan et al., 2011). Some mutant polyQ proteins also perturb transcriptional events that are important for autophagy induction. For example, Sirtuin-1 deacetylates several genes necessary for autophagy induction (Huang et al., 2015; Lee et al., 2008), and lower levels of Sirtuin-1 are observed in SCA3 mouse model (Cunha-Santos et al., 2016). Finally, mutant androgen receptor, which causes SBMA, directly interacts with TFEB, a transcription factor that coordinately regulates expression of genes involved in lysosomal biogenesis and key autophagy genes (Cortes et al., 2014; Settembre et al., 2011). As a consequence of this interaction, TFEB transactivation is abrogated and autophagy is impaired (Cortes et al., 2014).

## Amyotrophic Lateral Sclerosis

ALS is predominantly sporadic, although a growing number of genes have been identified in familial forms. ALS-associated

mutations in *TDP-43* (TAR DNA-binding protein 43), *SOD1* (superoxide dismutase 1), *FUS* (fused in sarcoma/translocated in sarcoma), and *C9ORF72* (Farg et al., 2014; Fecto & Siddique, 2011; Watabe et al., 2014) result in protein misfolding and the accumulation of aggregates. These intracellular aggregates correlate with an accumulation of autophagosomes and decreased proteasome activity in neurons of the spinal cord and brains in ALS patients (Chen, Zhang, Song, & Le, 2012; Cheroni et al., 2009). Growing evidence correlates defects in the autophagy system with the pathogenesis of ALS. The accumulation of the ALS-related protein FUS has been positively correlated with impaired autophagic flux (Watabe et al., 2014). The list of novel mutations in the Rab5 activator ALS2/Alsin that are associated with motor disorders is expanding (Daud et al., 2016; Siddiqi et al., 2014), and loss of ALS2 has been associated with impaired endosomal trafficking, decreased lysosome protein degradation and neurodegeneration in mouse models (Gautam et al., 2016; Hadano et al., 2010).

Recognition of misfolded ubiquitinated proteins by autophagy receptors enables selective autophagic sequestration of ubiquitinated substrates. This mechanism is relevant to many different proteins that are mutated in ALS. Mutations in the autophagy cargo receptor p62/SQSTM1 have been associated with disrupted degradation of mutant SOD1 and TDP-43 due to defective recognition of LC3-II in cell and mouse models and also in patients with ALS (Gal et al., 2009; Goode et al., 2016; Mizuno et al., 2006; Ramesh Babu et al., 2008; Teyssou et al., 2013). Similarly, clinical mutations in the LC3-binding region of p62/SQSTM1, such as L341V, also lead to the impairment of its recruitment into autophagosomes (Goode et al., 2016). Clinical mutations in the receptor ubiquilin-2 also promote abnormal protein accumulation (Williams et al., 2012; Zhang, Yang, Warraich, & Blair, 2014) in ubiquitin-positive inclusions together with

mutant p62/SQSTM1 (Deng et al., 2011; Williams et al., 2012; Zhang, Yang et al., 2014). Similar results were seen for OPTN, another autophagy receptor, where familial ALS-related mutations compromise autophagosome maturation, interaction with p62/SQSTM1, and protein clearance (Maruyama et al., 2010; Shen, Li, Chen, Chern, & Tu, 2015). Most of ALS-associated mutations in OPTN are located in its myosin VI-binding domain and alter autophagosome trafficking (Shen et al., 2015; Sundaramoorthy et al., 2015; Tumbarello et al., 2012). The recent identification of ALS-associated mutations in TANK-binding kinase 1 (Cirulli et al., 2015; Freischmidt et al., 2015) identifies a link between two other familial ALS-associated proteins, since this kinase phosphorylates OPTN (Moore & Holzbaur, 2016) and p62/SQSTM1 (Pilli et al., 2012). Various proteins required for endocytic trafficking have also been implicated in ALS and FTD. Alterations in *C9ORF72* gene are the most common cause of ALS and FTD and are linked by common pathological features (DeJesus-Hernandez et al., 2011; Winklhofer, Tatzelt, & Haass, 2008). The wild-type protein is involved in the regulation of endocytic transport and colocalizes with the autophagic proteins Rab7 and Rab11 in human motor neurons (Farg et al., 2014). *C9ORF72* can interact with the autophagy receptors p62/SQSTM1 and OPTN via Rab8 and Rab39, and affects autophagosome formation (Sellier et al., 2016). In addition, *C9ORF72* mediates the translocation of ULK1, a kinase controlling autophagosome formation, to the phagophore via Rab1a (Webster et al., 2016). It has been recently reported that loss of *C9ORF72* induces autophagy via mTOR and TFEB signaling (Ugolino et al., 2016). In addition to the coding sequence mutations in multiple genes associated with ALS/FTD, epigenetic mechanisms could play a key role in initiating ALS and FTD, especially for sporadic cases (Belzil, Katzman, & Petrucelli, 2016). For example, hypermethylation of the *C9ORF72*

promoter may have a protective function against repeat length expansion that is associated with pathology (Liu, Russ et al., 2014).

Mutations in CHMP2B [charged multivesicular body (MVB) protein 2B] and the sigma nonopioid intracellular receptor 1 (SIGMAR1) have been associated with both FTD and ALS (Al-Saif, Al-Mohanna, & Bohlega, 2011; Krasniak & Ahmad, 2016; Luty et al., 2010), and both proteins play a role in vesicle trafficking. CHMP2B is essential for autophagosome–endosome fusion and endolysosomal trafficking via endosomal sorting complexes required for transport (ESCRT), and defective function of this protein impairs autophagosome degradation (Filimonenko et al., 2010; Krasniak & Ahmad, 2016; West, Lu, Marie, Gao, & Sweeney, 2015). Knockdown of SIGMAR1 impairs vesicle trafficking from the ER to the Golgi leading to reduced fusion of autophagosomes with lysosomes and consequently reduced degradation of autophagy substrates (Vollrath et al., 2014).

## Hereditary Spastic Paraplegias

Hereditary spastic paraplegia (HSP) describes a heterogeneous group of inherited neurodegenerative disorders pathologically characterized by length-dependent axonal degeneration of corticospinal tracts resulting in progressive spasticity and weakness in the lower limbs. The type of HSP is designated by the loci it is associated with—to date, 50 spastic paraplegia genes (SPGs) and more than 70 distinct loci (SPG1-72) have been identified (Fink, 2013).

*SPG11*, encoding spatacsin, is the most commonly mutated gene in autosomal recessive HSPs. *Spg11*<sup>-/-</sup> mice have compromised autophagic lysosome reformation in neurons and a dramatic reduction in the number of lysosomes in the Purkinje cells (Varga et al., 2015), which result in impaired

autophagosome clearance. Similarly, loss or mutation of *SPG15* (encoding spastizin) causes defects in lysosomal biogenesis and autophagosome maturation (Chang, Lee, & Blackstone, 2014; Renvoise et al., 2014). Spastizin also interacts with the Beclin-1–UVRAG–Rubicon multiprotein complex required for autophagosome maturation (Vantaggiato et al., 2013). Thus, the loss of spastizin in fibroblasts of HSP15 patients or neuronal cells leads to compromised autophagy flux due to the accumulation of immature autophagosomes (Vantaggiato et al., 2013). Additionally, depletion of the ESCRT components, such as VPS37A (encoded by *SPG53*), is also known to reduce the autophagy flux (Ganley, Wong, Gammoh, & Jiang, 2011; Rusten & Stenmark, 2009; Sahu et al., 2011).

To date, genes mutated in other SPGs have not been shown to be directly involved in regulating the overall autophagy flux or autophagosome maturation, being required only for specific types of autophagy. For example, the deubiquitinating enzyme USP8 (*SPG59*) is directly involved in regulating PARKIN deubiquitination, which is required for its efficient recruitment to damaged mitochondria. Consequently, loss of USP8 results in compromised parkin-mediated mitophagy (Durcan et al., 2014).

A rare form of HSP is caused by recessive mutations in *SPG49*, encoding TECPR2 (tectonin  $\beta$ -propeller containing protein 2). TECPR2 is an ATG8-binding protein that cooperates with lipidated LC3C to efficiently regulate the ER-exit sites and ER export required for the formation of early autophagosome structures (Stadel et al., 2015). Loss of TECPR2 results in reduced levels and lipidation of LC3, TECPR2 being a positive regulator of autophagy (Oz-Levi et al., 2012). Patient fibroblasts with TECPR2 mutations show compromised ER-exit and reduced autophagosome biogenesis (Stadel et al., 2015).

A very rare form of HSP (characterized by mutilating sensory neuropathy) was identified in four patients from a consanguineous

Moroccan family, being caused by a loss-of-function mutation in *CCT5* (encoding the epsilon subunit of the cytosolic chaperonin CCT/TRiC) (Bouhouche, Benomar, Bouslam, Chkili, & Yahyaoui, 2006). Recently, it has been shown that CCT5 depletion in primary mouse cortical neurons and *Drosophila* causes accumulation of immature autophagosomes and undegraded autophagic cargo, such as p62 and other aggregate-prone proteins (Pavel et al., 2016). This block in autophagy flux caused by CCT5 depletion is mainly due to reduced lysosomal functioning through compromised actin cytoskeleton dynamics required for trafficking of lysosomal enzymes and V-ATPase into the lysosomes (Pavel et al., 2016).

## Lafora Disease

Lafora disease is an autosomal recessive disorder characterized by neurodegeneration and abnormal accumulation of Lafora bodies (LBs), which are polyglucosan bodies comprising a long, hyperphosphorylated, and insoluble form of glycogen. Lafora disease is caused by mutations in the genes encoding either laforin (Minassian et al., 1998), a dual specificity phosphatase that dephosphorylates complex carbohydrates, or malin (Chan et al., 2003), an E3-ubiquitin ligase. Both proteins are involved in the regulation of glycogen biosynthesis. Several studies have revealed an important role for autophagy in the modulation of Lafora disease. Wild-type laforin can induce autophagy (Aguado et al., 2010), and both laforin and malin knock-out mice show impaired autophagy (Aguado et al., 2010; Criado et al., 2012), with an mTOR-dependent mechanism in laforin knock-out mice.

As several reports demonstrated that reducing glycogen synthesis resulted in prevention of formation of LBs and neurodegeneration, it was unclear whether alteration of autophagy was a cause or a consequence of neurodegeneration. Duran, Gruart, Garcia-

Rocha, Delgado-Garcia, and Guinovart (2014) used an elegant in vivo approach to shed light on this matter, demonstrating that autophagy was not upregulated in malin knock-out mice unable to synthesize glycogen in the brain. These results suggest that the autophagy defect seen upon malin knockout is a consequence of glycogen accumulation (Duran et al., 2014).

### Dynein and Dynamin Mutations

As described here, many of the common neurodegenerative diseases are characterized by dysfunctional vesicle trafficking. Cytoplasmic dynein is the molecular motor that drives the retrograde transport of cargoes in cells, acting in concert with its activator dynactin. In the context of autophagy, the dynein complex enables retrograde trafficking of autophagosomes to the part of the cell where lysosomes are clustered, enabling autophagosome–lysosome fusion. Mutations in dynein heavy chain (*DYNC1H1*) cause a wide range of neuromuscular degenerative diseases, including spinal muscular atrophy and axonal Charcot–Marie–Tooth disease (Harms et al., 2012; Weedon et al., 2011). Inhibition of dynein activity results in decreased autophagosome–lysosome fusion and interferes with clearance of aggregate-prone proteins in cells, *Drosophila*, and mice (Ravikumar et al., 2005). Similar results were obtained in human glioma cells, where chemical inhibition of dynein led to an accumulation of autophagosomes indicative of impaired autophagosome–lysosome fusion (Yamamoto, Suzuki, & Himeno, 2010). Missense mutations in a dynactin gene (*DCTN1*), encoding the p150<sup>Glued</sup> subunit of dynactin, have been reported in familial and sporadic ALS (Puls et al., 2003). Mutations in *DCTN1* have been shown to cause Perry syndrome, a progressive neurodegenerative disorder that can present Parkinsonism and psychiatric changes (Farrer

et al., 2009), suggesting a potential role for autophagic dysfunction in this disease.

Dynamin 2 mutations cause dominant centronuclear myopathy (Bitoun et al., 2005) or Charcot–Marie–Tooth disease (Zuchner et al., 2005). Dynamin 2 is a GTPase required for endocytosis and is responsible for the pinching off of nascent vesicles from intracellular membranes. The *Drosophila* orthologue of dynamin 2, *Shi*, was shown to have a direct role in autophagy, as it is required for lysosomal/autolysosomal acidification (Fang et al., 2016). Similar results are reported in knock-in mice expressing the most frequent human dynamin 2 mutation. Embryonic fibroblasts from homozygous knock-in mice showed an accumulation of immature autophagosomes, probably due to a defect in acidification (Durieux et al., 2012).

### Diseases Resulting from Mutations in Core Autophagy Genes

Recent advances in next-generation sequencing have led to the identification of a growing number of autophagy-related genes showing point mutations in neurodegenerative disease patients. The majority of mutations linked to late-onset neurodegenerative disease are found in genes that are not part of the core autophagy machinery but could rather be termed “autophagy accessory” genes. However, recently, some rare congenital neurodegenerative diseases have been linked to mutations in genes that play a central role in the autophagic process (Menzies, Fleming, & Rubinsztein, 2015).

The first link between a core autophagy-related gene was established in 2012 when de novo mutations in WD-repeat domain 45 (*WDR45*) were associated to beta-propeller protein-associated neurodegeneration (BPAN), also referred to as static encephalopathy of childhood with neurodegeneration in adulthood or neurodegeneration with brain iron accumulation-5 (Haack et al., 2012; Saitou et al.,



2013). BPAN patients have global developmental delay in early childhood and progressive dystonia, Parkinsonism, and dementia as young adults. *WDR45* encodes WIPI4, one of the four mammalian homologs of the yeast Atg18 protein (Lu et al., 2011). WIPI4 acts as an autophagy-specific PI3P-binding effector and is essential for autophagosome formation (Lu et al., 2011). The BPAN-associated mutations in *WDR45* result in lower WIPI4 protein levels, due to instability of the protein and therefore accumulation of early autophagosomal membranes and reduced autophagic flux (Saito et al., 2013). Noteworthy, a central nervous system (CNS)-specific knock-out of *Wdr45* in a mouse model replicated several aspects of BPAN, including poor motor coordination, impaired learning and memory, as well as autophagy defects (Zhao et al., 2015).

While the link between Vici syndrome and ectopic P-granules autophagy protein 5 (EPG5) was discovered several years ago (Cullup et al., 2013), the molecular function of EPG5 in autophagy has only been characterized recently (Wang et al., 2016). Vici syndrome is a rare autosomal recessive congenital multisystem disorder characterized by agenesis of the corpus callosum, bilateral cataracts, cutaneous hypopigmentation, progressive cardiomyopathy, and variable immunodeficiency. The affected individuals show psychomotor retardation and hypotonia (del Campo et al., 1999; Dionisi Vici et al., 1988). Most patients with Vici syndrome have truncating, splice site, or missense mutations in the *EPG5* gene with no clear mutational hotspot (Byrne et al., 2016). EPG5 acts as a Rab7 effector that mediates the fusion of autophagosomes with late endosomes/lysosomes. Various tissues from Vici patients as well as *Epg5* knock-out mice display accumulation of nondegradative autophagic vacuoles (Wang et al., 2016; Zhao, Zhao, Sun, & Zhang, 2013).

Recently, a mutation in *ATG5* was identified in two siblings with congenital ataxia with

developmental delay and mental retardation. This E122D mutation results in defects of the conjugation of ATG5 to ATG12 and therefore decreased autophagy. Introduction of the corresponding mutation in a yeast system resulted in a 30%–50% reduction of induced autophagy. A corresponding *Drosophila* model replicated the ataxia phenotype, indicating a causal link of the *ATG5* mutation to the ataxia phenotype (Kim et al., 2016).

## Lysosomal Disorders

The lysosome has a central role in cellular catabolism and in intracellular trafficking by being at the crossroad between endocytic and autophagic pathways. Given this, disruption of this pathway at multiple points leads to impaired lysosomal function and hence defective autophagosome turnover. This can result not only from the accumulation of undegraded autophagic components in the lysosome but also accumulation of autophagosomes that may fail to fuse with the lysosome. For example, disruption of early endosomal function by depletion of COPI leads to the accumulation of autophagic structures that fail to reach the lysosome (Razi, Chan, & Tooze, 2009). Likewise, loss of function of the ESCRT complex that is required for generation of intraluminal vesicles in later endosomal structures known as MVBs not only blocks endocytic degradation but also leads to the accumulation of autophagosomes (Rusten & Stenmark, 2009). Thus, abnormal autophagic flux can be consequence of primary endocytic and lysosomal defects.

LSDs 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 (Poupetova et al., 2010; Wraith, 2002). Most LSDs 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 due to impaired autophagosome–lysosome fusion (Ballabio & Gieselmann, 2009; Platt, Boland, & van der Spoel, 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. This process appears to be relevant to LSDs, since LSD-associated membrane cholesterol abnormalities have been shown to lead to lysosomal accumulation of several substrates, lysosomal dysfunction, and impairment of endocytic membrane trafficking (Fraldi et al., 2010). 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).

In Krabbe disease, a defect of  $\beta$ -galactocerebrosidase causes the accumulation of the glycosphingolipid psychosine, which in turn alters the lipid composition of cellular membranes (Hawkins-Salsbury et al., 2013). In Niemann–Pick type A disease, where mutations in the gene encoding acid sphingomyelinase cause accumulation of sphingomyelin, defects in mATG9 trafficking and autophagosome closure have also been observed (Corcelle-Termeau et al., 2016). Niemann–Pick type C disease is a sphingolipid storage disorder that results from inherited deficiencies in intracellular lipid-trafficking proteins and is characterized by an abnormal intracellular accumulation of cholesterol and glycosphingolipids (Lloyd-Evans & Platt, 2010). Similarly, GM1 gangliosidosis and infantile neuronal ceroid lipofuscinoses have been shown to be associated with the occurrence of chronic ER stress (Sano et al., 2009; Wei et al., 2008). Sphingomyelin storage also leads to lysosomal

membrane permeabilization, thereby liberating cathepsins into the cytosol (Serrano-Puebla & Boya, 2015).

Defects in posttranslational modifications or impaired trafficking of lysosomal enzymes or defective acidification can also result in lysosomal dysfunction (Colacurcio & Nixon, 2016; Hirst et al., 2015; Kyttala, Yliannala, Schu, Jalanko, & Luzio, 2005; Morimoto et al., 1989; Tiede et al., 2005). One example is represented by multiple sulfatase deficiencies, in which a failed posttranslational modification of sulfatases by an ER-resident enzyme abrogates the function of many lysosomal hydrolases (Dierks et al., 2009). Furthermore, 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, Furuta, & Nishino, 2015; Yu et al., 2010). For example, Niemann–Pick disease type C, caused by loss of NPC1 function, leads to impaired  $\text{Ca}^{2+}$  homeostasis and incorrect cholesterol trafficking with accumulation of nonesterified cholesterol and glycosphingolipids in late endosomes and lysosomes, disrupting their fusion (Lloyd-Evans et al., 2008; Lloyd-Evans & Platt, 2010; Pacheco & Lieberman, 2008). More recently, mutations in *SNX14*, encoding a sorting nexin phosphoinositol-binding protein localized on late endosome and lysosomal membranes 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 of the autophagosome–lysosome fusion for lysosomal disease pathology is further underlined by the discovery of a homozygous missense mutation in *VPS11* in patients with a rare form of leukoencephalopathy (Zhang et al., 2016). *VPS11* is a member of the homotypic fusion and protein sorting and class C core vacuole/endosome tethering complexes, and mutations lead to



impaired autophagy. In zebrafish with a null mutation of *vps11*, reduction in CNS myelination and extensive neuronal death were observed in the hindbrain and midbrain (Zhang et al., 2016).

Finally, several studies have linked autophagy to the regulated secretion of the contents of secretory granules or lysosomes in specialized cells or tissues (Cadwell et al., 2008; DeSelm et al., 2011; Michaud et al., 2011; Ushio et al., 2011). Examples of such events at the plasma-membrane include secretion of lysozyme from Paneth cells, which is implicated in Crohn's disease (Cadwell et al., 2008), secretion of ATP under certain conditions (Michaud et al., 2011), secretion of cathepsin K by osteoclasts during bone resorption (DeSelm et al., 2011), and the secretion of cytotoxic proteins stored in the lytic granules of activated cytotoxic T lymphocytes (CTLs), which is a key event in killing target cells (Ushio et al., 2011). Most likely, the fusion events of autophagic vesicles with the plasma-membrane (autophagosome exocytosis) are mediated by SNAREs, in a manner similar to lysosome and lysosome-related organelles, such as lytic granules and melanosomes. Indeed, this has been demonstrated for the secretion of lytic granules: patients with familial hemophagocytic lymphohistiocytosis type 4 who have mutations in the SNARE protein syntaxin-11 have impaired degranulation of CTLs (Bryceson et al., 2007; Hong, 2005). Moreover, recent findings suggest a role for the SNARE proteins VAMP8 and VTI1B (SNAREs that regulate autophagosome–lysosome fusion) in the exocytosis of toxic proteins stored in the lytic granules of CTLs (Dressel, Elsner, Novota, Kanwar, & Fischer von Mollard, 2010). During lysosomal exocytosis, a  $\text{Ca}^{2+}$ -regulated process (mediated by the activation of the lysosomal  $\text{Ca}^{2+}$  channel MCOLN1) enables lysosomes to dock to the cell surface and fuse with the plasma-membrane, emptying their contents outside the cell. This process has an important role in

secretion and plasma-membrane repair. Interestingly, lysosomal exocytosis is transcriptionally regulated by TFEB, a master regulator that coordinates lysosomal biogenesis and autophagy (Sardiello et al., 2009; Settembre et al., 2011), which promotes cellular clearance in models of lysosomal storage diseases (Medina et al., 2011). Therefore, it is tempting to speculate that cells may use this mechanism to coordinate autophagosome–lysosome degradative activity and autophagy-mediated secretory functions in response to specific stimuli, suggesting that promoting lysosomal exocytosis may represent an alternative strategy to treat disorders due to intracellular storage, such as LSDs.

## AUTOPHAGY UPREGULATION

While autophagy is essential for nervous system function, data also supports it decreasing with age across diverse organisms. A range of approaches have been used to assess the potential benefits of constitutive autophagy upregulation in normal health and aging as well as neurodegenerative disease contexts, and these approaches support a clear association of upregulation with beneficial effects. Approaches within invertebrate model organisms show that induction of autophagy with spermidine extends lifespan in *Drosophila* and *Caenorhabditis elegans* (Eisenberg et al., 2009). Depletion of acetyl-coenzyme A through knocking down its synthetase specifically in the *Drosophila* brain also results in prolonged lifespan with enhanced autophagy-mediated protein clearance, which is likely the result of decreasing inhibitory acetylation of autophagy regulators (Eisenberg et al., 2014). Autophagy is induced and lifespan extended by caloric restriction in *C. elegans* (Hansen et al., 2008) as well as by pan-neuronal overexpression of Atg8a or AMPK in *Drosophila* (Simonsen et al., 2008; Ulgherait, Rana, Rera, Graniel, & Walker, 2014).

When assessing the mechanisms of how autophagy upregulation enhances survival, studies in *C. elegans* support the benefit acting through diverse pathways, which include clearance and turnover of mitochondria as well as ER homeostasis and mitotic alterations (Ghavidel et al., 2015; Palikaras, Lionaki, & Tavernarakis, 2015).

In mice, constitutive upregulation of autophagy by ubiquitous overexpression of ATG5 results in extended lifespan and reduced aging phenotypes. These include enhanced motor performance, leanness and, at a primary cellular level, enhanced resistance to oxidative damage (Pyo et al., 2013). Likewise, deletion of the polyQ tract from mouse huntingtin increases autophagy and results in lifespan extension (Zheng et al., 2010).

In cells, autophagy upregulation enhances the clearance of toxic aggregate-prone proteins, such as mutant huntingtin, alpha-synuclein, and tau (Berger et al., 2006; Ravikumar, Duden, & Rubinsztein, 2002; Webb, Ravikumar, Atkins, Skepper, & Rubinsztein, 2003). The process of autophagic engulfment enables removal of oligomeric species, which are inaccessible to the proteasome. While the protective role of autophagy has been widely replicated in animal models of various diseases caused by intracytoplasmic aggregate-prone proteins (Menzies, Fleming et al., 2015), most studies in vivo have used chemical tools that likely have autophagy-independent effects. However, overexpression of Atg5 in zebrafish induces autophagy and ameliorates tau toxicity (Lopez et al., 2017). Likewise, genetic inhibition of calpain protects against tau and huntingtin toxicity in vivo in an autophagy-dependent manner (Menzies, Garcia-Arencibia et al., 2015).

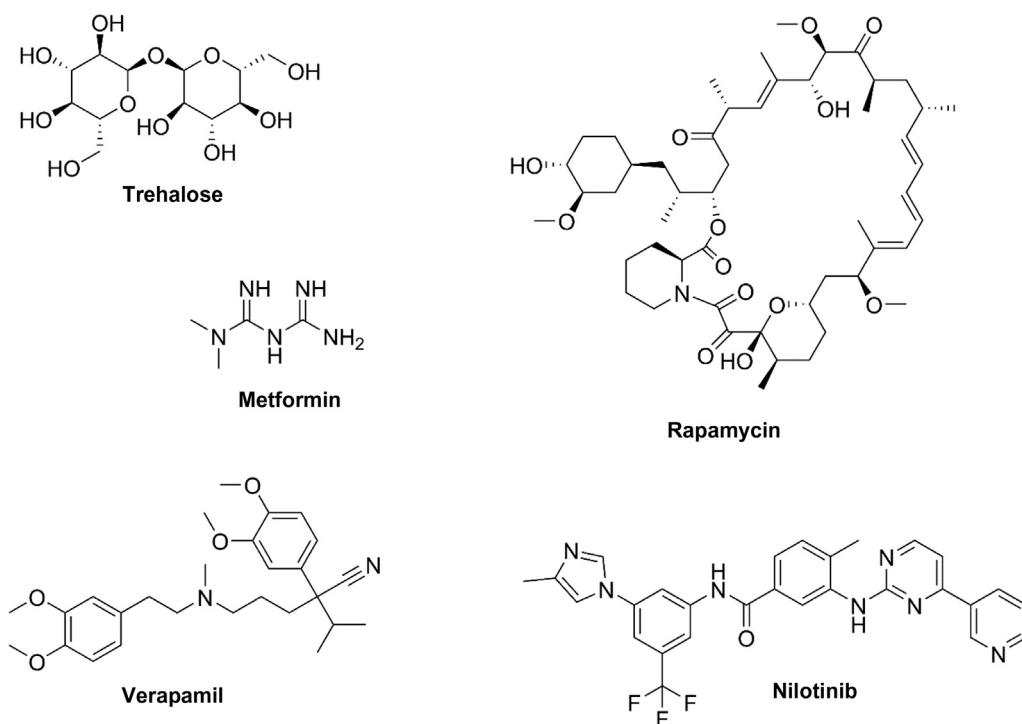
Since upregulation of autophagy has beneficial effects, a number of pharmacological autophagy stimulators have been identified and tested in preclinical models of neurodegenerative diseases (Harris & Rubinsztein, 2011; Levine, Packer, & Codogno, 2015; Sarkar & Rubinsztein, 2008). The most extensively

employed preclinical pharmacological tools to probe autophagy modulation in neurodegeneration are trehalose and rapamycin.

## Trehalose

Trehalose is a stable disaccharide assembled from two molecules of D-glucose (Fig. 11.4). Many organisms, notably fungi, insects, and other invertebrates, biosynthesize trehalose (Richards et al., 2002). Humans, like other mammals, do not synthesize trehalose, but significant quantities may be ingested as part of a normal diet, and some can be absorbed (Kamiya, Hirata, Matsumoto, Arai, & Yoshizane, 2004; Murray, Coupland, Smith, Ansell, & Long, 2000; Richards et al., 2002).

Trehalose has been shown to have activity in a variety of cellular and animal models of neurodegeneration (Emanuele, 2014). Trehalose treatment leads to decreased huntingtin aggregation and reduced toxicity in vitro as well as reduced aggregation and increased lifespan in the R6/2 transgenic mouse model of HD (Tanaka et al., 2004). Trehalose has also been shown to reduce toxicity and formation of mutant PABPN1 aggregates in a cellular model of oculopharyngeal muscular dystrophy (OPMD) and reduced aggregates and cell death and improved disease-associated muscle weakness in a mouse model of this disease (Davies, Sarkar, & Rubinsztein, 2006). More recently, trehalose has also been shown to decrease the processing of both APP and of C-terminal fragments derived from it, to reduce the secretion of A $\beta$  (Tien, Karaca, Tamboli, & Walter, 2016), and to rescue impaired cognition and decrease A $\beta$  deposition in the APP/PS-1 mouse model (Du, Liang, Xu, Sun, & Wang, 2013). Other studies have shown effects in cell and mouse models of tauopathy (Kruger et al., 2012; Schaeffer et al., 2012), tauopathy with Parkin deletion (Rodriguez-Navarro et al., 2010), SCA17 (Chen et al., 2015), and ALS (Castillo, Nassif et al.,



**FIGURE 11.4** The chemical structures of selected autophagy enhancers with demonstrated *in vivo* efficacy in models of neurodegeneration.

2013; Li et al., 2015; Zhang, Chen et al., 2014). Mixed data has been reported in cell and animal models of Lewy body disease (Tanji et al., 2015) and prion disease (Aguib et al., 2009).

Mechanistically, trehalose has been identified as an mTOR-independent enhancer of autophagy (Sarkar, Davies, Huang, Tunnacliffe, & Rubinsztein, 2007) signaling via AMPK. Recently, DeBosch has proposed that trehalose enhances autophagy through inhibition of SLC2A (GLUT) transporters (DeBosch et al., 2016).  $IC_{50}$  values against GLUT transporters were reported as between 17.3 and 126 mM; these values seem inconsistent with the early reports of effects in cell assays at submillimolar concentrations. A further study by this group reports that GLUT8 functions as a trehalose-transporter responsible for trehalose entry into mammalian cells (Mayer et al., 2016).

An intravenous formulation of trehalose, known as Cabaletta, has been developed by Bioblast Pharma for the treatment of PolyA and PolyQ diseases. Cabaletta is in Phase III for OPMD and Phase II for SCA3. Preliminary results from a 24-week open-label Phase II trial have shown that Cabaletta improves dysphagia and muscle function in OPMD (Argov, Gliko-Kabir, Brais, Caraco, & Megiddo, 2016).

### Rapamycin

Rapamycin, a macrolide antifungal and immunosuppressant, was first discovered and characterized as an autophagy activator over 20 years ago (Blommaart, Luiken, Blommaart, van Woerkom, & Meijer, 1995; Heitman, Movva, & Hall, 1991; Noda & Ohsumi, 1998). The

mechanism of action of rapamycin involves binding to FKBP12, and the resulting complex interacts with mTORC1, inhibiting its kinase activity through an allosteric mechanism (Benjamin, Colombi, Moroni, & Hall, 2011; Heitman et al., 1991). Functionally, mTOR forms two complexes termed mTORC1 and mTORC2, with rapamycin's inhibitory action on mTOR showing selectivity toward the first (Schreiber et al., 2015). A significant effort has been devoted toward the evolution of rapamycin analogs (called "rapalogues") as well as toward the targeted development of more potent, ATP-competitive inhibitors of mTOR, such as Torin (Benjamin et al., 2011), these latter in particular as anticancer agents. However, Torin-type inhibitors inhibit both mTORC1 and mTORC2 complexes and display poor selectivity typically associated with ATP-competitive kinase inhibitors, rendering them less suitable for chronic indications.

Rapalogues (including rapamycin) have been employed to probe the therapeutic benefit of autophagy activation in a number of animal models of neurodegeneration. In the triple-transgenic AD mouse model, rapamycin as well as mTOR genetic reduction was shown to rescue cognitive deficits and decrease A $\beta$  and tau pathology through autophagy stimulation (Caccamo, De Pinto, Messina, Branca, & Oddo, 2014; Caccamo, Majumder, Richardson, Strong, & Oddo, 2010). Prevention of AD-like cognitive deficits and lowered levels of amyloid peptide by rapamycin were reported in another AD mouse model, the PDAPP mouse (Spilman et al., 2010). In a model of prion disease, Cortes et al. reported that rapamycin delayed disease onset, increased survival, and ameliorated disease severity (Cortes, Qin, Cook, Solanki, & Mastrianni, 2012). In the P301S TAU mouse model, temsirolimus (a rapalogue) was shown to reduce tau hyperphosphorylation and improve spatial cognitive behavior (Jiang et al., 2014). Similar effects were shown for rapamycin in the same tau mouse model (Ozcelik

et al., 2013). Temsirolimus ameliorated motor performance in a transgenic mouse model of spinocerebellar ataxia type 3, inducing autophagy and reducing the number of aggregates and protein levels of mutant ataxin-3 (Menziés et al., 2010), and ameliorated behavioral performance and decreased aggregate formation in the HD-N171-N82Q mouse model of HD (Ravikumar et al., 2004). In a PD mouse model (Parkin Q311X mice), rapamycin decreased dopaminergic neuronal loss, reduced synuclein levels, improved motor function, and induced autophagic markers (Siddiqui et al., 2015).

While studies in many models of neurodegenerative diseases have converged on the therapeutic efficacy of rapalogues, investigations in ALS models have provided contradictory results. In some studies, detrimental effects were reported (Zhang et al., 2011) but beneficial effects were subsequently observed after uncoupling of rapamycin's immunomodulatory function (Staats et al., 2013). The possibility that the immunomodulatory role of rapalogues may contribute to the phenotypic amelioration (or indeed worsen the phenotype) observed in different animal models of neurodegeneration remains a concern. However, the rationale that autophagy activation is therapeutically relevant in neurodegenerative diseases is strongly supported by the amelioration observed with autophagy activators with different mechanisms of action.

### Repurposing of FDA-Approved Drugs as Autophagy Upregulators

There have been many reports of using repurposed FDA-approved drugs for the treatment of neurodegenerative disorders via upregulation of autophagy (Fleming, Noda, Yoshimori, & Rubinsztein, 2011; Levine et al., 2015; Rubinsztein, Bento, & Deretic, 2015). Selected examples are presented here.

Metformin is a first-line antidiabetes drug that has been demonstrated to upregulate

autophagy in several preclinical animal models of neurodegenerative diseases but with mixed results on the outcome of disease progression. This FDA-approved drug is CNS-penetrant (Labuzek et al., 2010) and is an activator of AMPK. Treatment of transgenic mice expressing a mutant huntingtin with CAG repeat length of 136–151 (HD R6/2) with metformin significantly increased brain AMPK phosphorylation levels (Ma et al., 2007). Whilst decreased hind limb clasping and increased lifespan was observed in males, no effects were observed in female mice. In vivo electrophysiology demonstrated a significant increase in survival of motor units in the SOD1<sup>G93A</sup> mouse model of ALS, although beneficial effects were seen in different muscles of male and female mice (Kaneb, Sharp, Rahmani-Kondori, & Wells, 2011). This effect was observed with treated with 2 mg/mL metformin in drinking water; however, doses of 0.5, 2, and 5 mg/mL metformin did not significantly alter neurological scores and did not increase survival or delay disease onset in male mice. Indeed, metformin dose dependently worsened survival rates for females, possibly owing to inhibition of estrogen production (Kaneb et al., 2011). In transgenic mice overexpressing human A $\beta$ PP 695 (Tg6799), metformin stimulated autophagosome accumulation and promoted the generation of A $\beta$  through increased  $\beta$ - and  $\gamma$ -secretase activity (Son et al., 2016). The results of this study suggest that metformin heightens the progression of AD by increasing A $\beta$  synthesis within autophagosomes.

The Bcr-Abl tyrosine kinase inhibitor nilotinib is approved for the treatment of chronic myelogenous leukemia. The compound is known to be CNS-penetrant but has a low concentration in cerebrospinal fluid (CSF) (Reinwald et al., 2014). Nilotinib has been shown to induce autophagy in vivo (Yu et al., 2013) and increase amyloid clearance in an A $\beta$  transgenic mouse model (Lonskaya, Hebron, Desforges, Schachter, & Moussa, 2014;

Lonskaya, Hebron, Selby, Turner, & Moussa, 2015).

A number of compounds that act on the cAMP/IP<sub>3</sub> pathway have shown benefits in animal models of neurodegeneration, including verapamil, clonidine, rilmenidine, and minoxidil (Rose et al., 2010; Williams et al., 2008). Verapamil is a calcium channel blocker prescribed for the treatment of hypertension, angina pectoris, and cardiac arrhythmia and is known to penetrate the CNS (Narang, Blumhardt, Doran, & Pickar, 1988). In a *Drosophila* model of HD, verapamil slowed the progression of the HD phenotype, and in a zebrafish transgenic model of HD where expression is driven in the rod photoreceptors, verapamil reduced the number of aggregates compared to untreated fish, leading to a reduction of photoreceptor degeneration (Williams et al., 2008).

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## Further Reading

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