REVIEW

Autophagy in neuronal cells: general principles and physiological and pathological functions

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Abstract Autophagy delivers cytoplasmic components and organelles to lysosomes for degradation. This pathway serves to degrade nonfunctional or unnecessary organelles and aggregate-prone and oxidized proteins to produce substrates for energy production and biosynthesis. Macroautophagy delivers large aggregates and whole organelles to lysosomes by first enveloping them into autophagosomes that then fuse with lysosomes. Chaperone-mediated autophagy (CMA) degrades proteins containing the KFERQ-like motif in their amino acid sequence, by transporting them from the cytosol across the lysosomal membrane into the lysosomal lumen. Autophagy is especially important for the survival and homeostasis of postmitotic cells like neurons, because these cells are not able to dilute accumulating detrimental substances and damaged organelles by cell division. Our current knowledge on the autophagic pathways and molecular mechanisms and regulation of autophagy will be summarized in this review. We will describe the physiological functions of macroautophagy and CMA in neuronal cells. Finally, we will summarize the current evidence showing that dysfunction of macroautophagy and/or CMA contributes to neuronal diseases. We will give an overview of our current knowledge on the role of autophagy in aging neurons, and focus on the role of autophagy in four types of neurodegenerative diseases, i.e., amyotrophic lateral sclerosis and frontotemporal dementia, prion diseases, lysosomal storage diseases, and Parkinson's disease.

Keywords Autophagosome · Lysosome · Chaperone-mediated autophagy · Neurogenesis · Aging · Neurodegeneration · FTD/ALS · Prion disease · Lysosomal storage disease · Parkinson's disease

Abbreviations

AAA$^+$-ATPase	ATPase associated with diverse cellular activities
ALS	Amyotrophic lateral sclerosis
AMBRA1	Activating molecule in beclin 1-regulated autophagy
AMPK	AMP-activated protein kinase
ATF6	Activating transcription factor 6
ATG	Autophagy-related
BECN1	Beclin 1
BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3
CHMP2B	Charged multivesicular protein 2B
CMA	Chaperone-mediated autophagy
CNS	Central nervous system
DAP1	Death-associated protein 1
DFCP1	Double FYVE-containing protein 1
DRG	Dorsal root ganglion
ESCRT-III	Endosomal sorting complex required for transport-III
ER	Endoplasmic reticulum
ERK2	Extracellular signal-regulated kinase 2
FIP200	200 kDa FAK family kinase-interacting protein
FTD	Frontotemporal dementia
GFP	Green fluorescent protein
GTPase	Guanosine triphosphatase

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HIF	Hypoxia-inducible factor
HSC70	Heat shock cognate protein of 70 kDa
IRE1	Serine/threonine-protein kinase/endoribonuclease protein
LAMP	Lysosomal associated membrane protein
LC3/MAP1-LC3	Microtubule associated protein 1 light chain 3
LRRK2	Leucine-rich repeat kinase 2
LSD	Lysosomal storage disorder
MEF2D	Myocyte enhancer factor 2D
ML-II	Mucolipidosis type II
NCL	Neuronal ceroid lipofuscinosis
NPC	Niemann–Pick C
mTOR	Mammalian target of rapamycin
mTORC1	mTOR complex 1
MVB	Multivesicular body
OPTN	Optineurin
PD	Parkinson's disease
PERK	Protein kinase-like ER kinase
PI3P	Phosphatidyl inositol 3-phosphate
PI3KC3	Phosphatidyl inositol 3-kinase class 3
PKC	Protein kinase C
POMC	Pro-opiomelanocortin
RAPTOR	Regulatory associated protein of mTOR)
RB1CC1	RB1-inducible coiled-coil 1
REST	Repressor element 1 silencing transcription factor
Rheb	Ras homolog enriched in brain
ROS	Reactive oxygen species
SNARE	Soluble *N*-ethyl-maleimide-sensitive factor attachment protein receptor
SOD	Superoxide dismutase
SQSTM1	Sequestosome 1
TDP-43	Tar-DNA binding protein 43
TFEB	Transcription factor EB
TSC	Tuberous sclerosis complex
UBA	Ubiquitin-binding domain
UBQLN2	Ubiquilin 2
ULK1	Unc-51-like kinase 1
UPR	Unfolded protein response
V-ATPase	Vacuolar ATPase
VCP	Valosin-containing protein
WIPI	WD repeat domain, phosphoinositide interacting

Introduction

Autophagy is a catabolic process in which cytoplasmic material and/or organelles are transported to lysosomes for degradation by lysosomal acid hydrolases [19, 55, 58, 81, 180]. The delivery of cytoplasmic material to lysosomes can occur via three different routes (Fig. 1). In macroautophagy, the cytoplasm and/or organelle is first engulfed by a flat membrane cistern that forms a double-membrane vesicle, called the autophagosome, which then fuses with endosomal and lysosomal vesicles. In a poorly defined process called microautophagy, lysosomes internalize small portions of cytoplasm in a process that resembles endocytosis. In chaperone-mediated autophagy (CMA), cytosolic soluble proteins containing a certain sequence tag (the KFERQ-like motif) in their amino acid sequence are recognized by the A isoform of the lysosomal associated membrane protein 2 (LAMP-2A), and transported through the lysosomal membrane in a process assisted by chaperones [108].

Autophagy is functioning under basal conditions, but during nutrient starvation it is upregulated in order to supply nutrients by degradation of redundant cytoplasmic material. Clearance of aggregate-prone proteins and damaged organelles by macroautophagy is imperative for post-mitotic cells like neurons, which cannot dilute these components by cell division [27]. The role of autophagy in neurons is highlighted by a recent systems biology study revealing the pivotal role of dysregulated autophagy in neurodegenerative dementia [25], and the proposed role of autophagy in the turnover of repressor element 1-silencing transcription factor (REST), a central player in the repression of genes promoting cell death and in the induction of genes mediating stress resistance in neurons [135].

Macroautophagy: formation of the phagophore

Autophagosomes are formed by phagophores (also called isolation membranes), flat membrane cisterns that elongate and curve into double-membraned vesicles. The ATG proteins (autophagy-related proteins) were first discovered using yeast mutants. After the initial discoveries in yeast, most of these autophagy proteins were discovered in mammalian cells; some of them had already been described and named before their role in autophagy was determined. Autophagosome biogenesis is initiated by sequential function of several protein complexes, including ULK1/ULK2 complex, PI3KC3 complex, ATG9 and its binding partners, ATG12-ATG5 complex, and LC3-phosphatidyl ethanolamine complex [58, 81, 180]. These complexes and proteins are summarized in Table 1 and Fig. 2.

Mammalian target of rapamycin complex 1 (mTORC1) is a central regulatory hub in nutrient sensing and metabolism. Inactivation of mTORC1 initiates the activation of ULK1/ULK2 complex and autophagosome formation (see below for details) (Fig. 2). The next protein complex to be activated is the class III phosphatidyl inositol 3-kinase (PI3KC3) complex, for which beclin 1 acts as a scaffold. Beclin 1, encoded by the *BECN1* gene, functions at an early
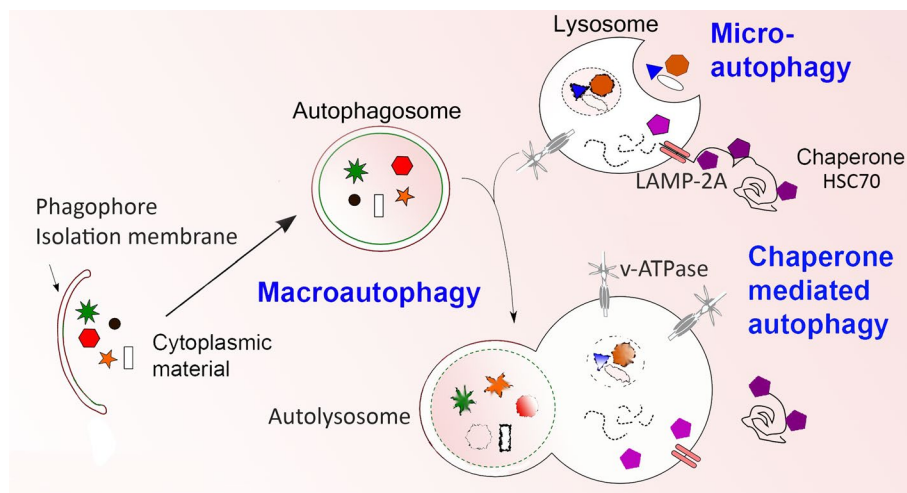
Fig. 1 A schematic representation of the three different forms of autophagy. In macroautophagy, the cytoplasmic cargo is first segregated into autophagosomes. Autophagosomes are formed by phagophores, also called isolation membranes, that expand and capture the cargo inside the forming autophagosome, which then fuses with a lysosome. In microautophagy, lysosomal membrane invaginates and engulfs cytosol. In chaperone-mediated autophagy, protein substrates are unfolded by the cytosolic chaperone HSC70 prior to transport through the lysosomal membrane. The lysosomal associated membrane protein LAMP-2A is thought to function as a receptor and/or channel for CMA. Intralysosomal HSC70 assists the transport across the lysosomal membrane

step during autophagosome formation. Beclin 1 can form a complex with several proteins, but during autophagosome formation it interacts with PI3KC3, its regulatory subunit PIK3R4, activating molecule in beclin 1-regulated autophagy (AMBRA1), and ATG14 that targets the complex to the endoplasmic reticulum (ER) [148]. Localized synthesis of phosphatidyl inositol 3-phosphate (PI3P) in the ER recruits PI3P-binding proteins called double FYVE-containing protein 1 (DFCP1) and WIPI1 or WIPI2 (WD repeat domain, phosphoinositide interacting). This recruitment is connected to the formation of specialized ER subdomains called omegasomes (Fig. 2) [6]. Autophagosome biogenesis occurs inside these omega-shaped subdomains. ATG9 is a membrane spanning protein that is needed for autophagosome formation. ATG9 recycles between the trans-Golgi network and endosomes in an ULK-dependent manner, and has been suggested to assist in the delivery of membrane to phagophores [219, 260]. Recent studies in yeast showed that Atg1 (the yeast equivalent to ULK1) phosphorylates Atg9, and that this phosphorylation is required for autophagosome formation [178]. Another group showed that the amount of Atg9 protein directly correlates with the frequency of autophagosome formation and rate of autophagic degradation [100]. It remains to be shown whether the mammalian ATG9 has similar functions. During phagophore elongation the membrane recruits ATG12 that is covalently conjugated to ATG5. These conjugates form large oligomers by additionally recruiting ATG16L1. The ATG12-ATG5 conjugate determines the site where the protein called microtubule associated protein

1 light chain 3 (MAP1-LC3) is conjugated to phosphatidyl ethanolamine (PE), giving rise to the lipidated form of LC3, called LC3-II [67, 94]. The lipidation is needed for the recruitment of LC3 to the phagophore [104]. LC3 is the most widely used marker of autophagosomes in mammalian cells. Soon after sealing of the autophagosome, ATG12-ATG5/ATG16L1 dissociates from the membrane, while part of LC3-II stays on the inner limiting membrane and is delivered to the lysosome together with the cytoplasmic cargo. Before lipidation, newly synthesized LC3 is cleaved by ATG4, which is also able to delipidate LC3-II after a sealed autophagosome has formed. The conjugation reactions of ATG12 to ATG5 and LC3 to PE are similar to protein ubiquitination. The equivalents to the ubiquitin ligases E1 and E2 are ATG7 and ATG10 for ATG12-ATG5 conjugation, and ATG7 and ATG3 for LC3-PE conjugation [180]. Several recent reviews have been published on phagophore and autophagosome formation [58, 73, 211, 219].

One of the most debated questions in macroautophagy concerns the origin of phagophore and autophagosome membranes [54]. As described above recent results indicate that ER plays a role in phagophore biogenesis. In starved cells, phagophores locate between two cisterns of rough ER [84, 256]. The omegasome marker protein DFCP1 has been localized by immunoelectron microscopy in delicate membrane tubules extending from the ER to the phagophore [235]. Recent studies also suggest that coat protein II (COPII) coated vesicles budding from ER exit sites deliver membrane for phagophores [76, 223]. During starvation, the outer mitochondrial membrane may also participate in

Table 1 Proteins functioning during autophagosome formation

Protein complex	Autophagy protein	Alternative names	Yeast protein	Localization or likely function
ULK complex	ULK1, ULK2		Atg1	Ser/Thr protein kinase, needed for autophagosome initiation, regulated by phosphorylation
ULK complex	ATG13		Atg13	Regulated by phosphorylation
ULK complex	FIP200	RB1CC1	Atg17	Scaffolding protein, phosphorylated by ULK1
ULK complex	ATG101	C12orf44		
PI3KC3 complex	BECN1	Beclin 1	Atg6/Vps30	Scaffolding protein, forms a complex with PI3KC3, PIK3R5, ATG14 and AMBRA1. Needed for autophagosome initiation or formation
PI3KC3 complex	PI3KC3	Phosphoinoside 3-kinase class 3, VPS34	Vps34	Lipid kinase activity needed for autophagosome formation
PI3KC3 complex	PIK3R4	Phosphoinoside 3-kinase regulatory subunit 4, VPS15	Vps15	Regulatory subunit of PI3KC3
PI3KC3 complex	ATG14	ATG14L, Barkor	Atg14	Directs the complex to ER
PI3KC3 complex	AMBRA1			Facilitates autophagosome formation
	ATG9A, ATG9B		Atg9	Transmembrane protein, thought to target membrane to forming phagophores
	WIPI1, WIPI2		Atg18	PI3P-binding proteins, needed for autophagosome formation
	DFCP1	ZFYVE1		Localizes in omegasomes, function not known
ATG12-ATG5 complex	ATG5		Atg5	Conjugated with ATG12, needed for autophagosome formation, present in phagophore membrane
ATG12-ATG5 complex	ATG12		Atg12	Ubiquitin-like protein, conjugated with ATG5, needed for autophagosome formation, present in phagophore membrane
ATG12-ATG5 complex	ATG16L1		Atg16	Needed for autophagosome formation, present in phagophore membrane
ATG8-PE conjugate	LC3	MAP-LC3 LC3-I (unlipidated form), LC3-II or LC3-PE (lipidated form)	Atg8	Ubiquitin-like protein, conjugated with phosphatidyl ethanolamine to from LC3-II, which is needed for autophagosome formation; present in phagophore and autophagosome membranes
ATG8-PE conjugate	GABARAP GABARAPL1		Atg8	Ubiquitin-like protein, conjugated with phosphatidyl ethanolamine, present in phagophore and autophagosome membranes
ATG8-PE conjugate	GAPARAPL2	GATE16	Atg8	Ubiquitin-like protein, conjugated with phosphatidyl ethanolamine, present in phagophore and autophagosome membranes
	ATG4			Cleaves LC3 to prepare it for conjugation to PE, also delipidates LC3-II
	ATG7			E1-like enzyme for conjugation of Atg12 to Atg5 and LC3 to PE
	ATG10			E2-like enzyme for conjugation of Atg12 to Atg5
	ATG3			E2-like enzyme for conjugation of LC3 to PE

Since yeast is a key organism in studies on the molecular mechanisms of autophagy, the names of the corresponding yeast proteins are also given
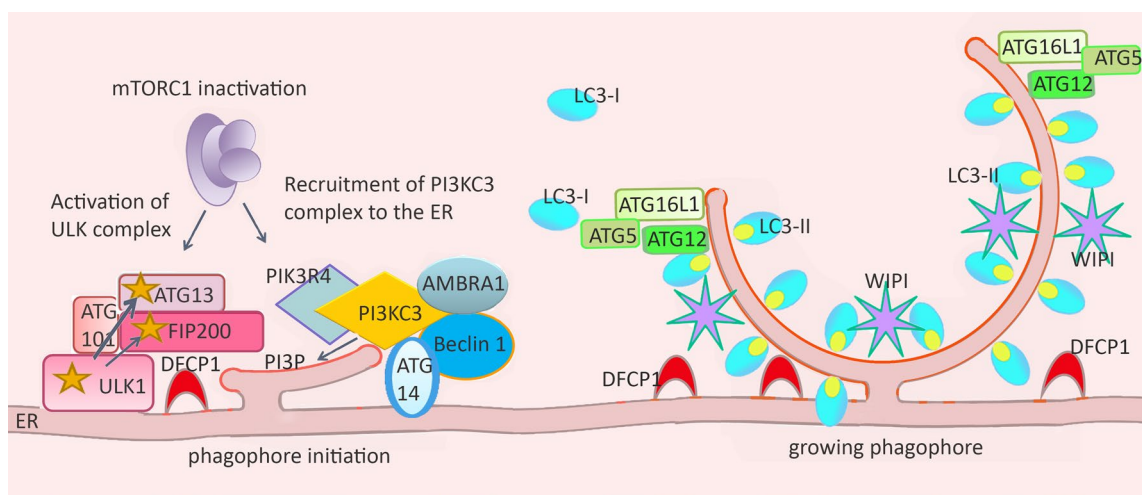
Fig. 2 A schematic representation of phagophore formation in ER-associated, DFCP1-positive omegasome. ULK1/2-ATG13-FIP200-ATG101 (induction) complex is stable regardless of nutrient status. Under nutrient-rich conditions mTORC1 associates with the ULK complex and inactivates it by phosphorylation of ULK1/2 and ATG13. During nutrient starvation autophagy begins when mTORC1 dissociates from the ULK complex, which results in partial dephosphorylation of ULK1/2 and ATG13, and induction of macroautophagy. ULK1/ULK2 becomes activated by phosphorylation of two other serine residues by AMPK, which in turn makes it capable of phosphorylating ATG13 and FIP200 (these phosphorylations are symbolized by *stars*). This initiates the recruitment of PI3KC3 complex to the omegasome where it produces PI3P, which in turn recruits PI3P-binding proteins including WIPIs to the growing phagophore. ATG12-ATG5 complex binds ATG16L1 and associates with the phagophore. The localization ATG12-ATG5 determines the site of LC3 lipidation to LC3-II, which associates with the phagophore membrane. ATG9 is not depicted in this figure since it is currently unclear at which stage it functions. The function of the omegasome marker DFCP1 is also unclear

phagophore biogenesis [80], and a recent paper showed that autophagosomes form in ER-mitochondria contact sites [82]. Further, plasma membrane may contribute to the formation of autophagosome precursors, structures that give rise to phagophores [187]. Exit from the Golgi complex may be necessary for the delivery of membrane during the elongation of phagophores [77, 222, 238]. Recycling endosomes may also contribute membrane to forming autophagosomes [134]. Taken together, it is possible that phagophores have more than one membrane source, possibly depending on the cargo or the signals that initiate autophagy.

After their formation, autophagosomes undergo a maturation process that includes fusion with endosomal and lysosomal vesicles (Fig. 3) [52]. These fusion events are controlled by several RAB and SNARE proteins [16, 31, 120]. Autophagosomes that have fused with endosomes are called amphisomes, and fusions with lysosomes produce autolysosomes. During maturation, the autophagosome limiting membrane acquires lysosomal membrane proteins, for example LAMP-1, LAMP-2 and the vacuolar proton pump V-ATPase, the activity of which acidifies the contents of autophagosomes. Lysosomal hydrolases that degrade the autophagosome cargo are also delivered into autophagosomes via the fusion of lysosomes with autophagosomes. Lysosomal membrane protein transporters then carry the degradation products back to cytoplasm, where the cell can use them for energy production and/or biosynthetic reactions. It is currently unclear what happens after the degradation in autolysosomes is complete, or alternatively, in the case that the degradation is not complete, but different possibilities have been reported. (1) New lysosomes may reform from autolysosomes as budding tubular extensions, which then separate and form new lysosomes [261]. (2) The second possibility is exocytosis of autolysosome contents by fusion of the limiting membrane with the plasma membrane and extrusion of the contents into the extracellular space. These two alternatives are not mutually exclusive. Recent studies suggest that autophagosome exocytosis increases if autophagic degradation capacity is compromised by inhibiting autophagosome–lysosome fusion. This process may depend on the transcription factor EB (TFEB) [51, 122, 150] and/or other transcription factors like TFE3 [143]. Impaired acidification of lysosomes may also increase exocytosis of autolysosome contents [186].

Nutritional and transcriptional control of macroautophagy

Macroautophagy induction during starvation is controlled by a sophisticated system of amino acid sensing, which is coupled to transcription-dependent and -independent activation of autophagosome formation. This system
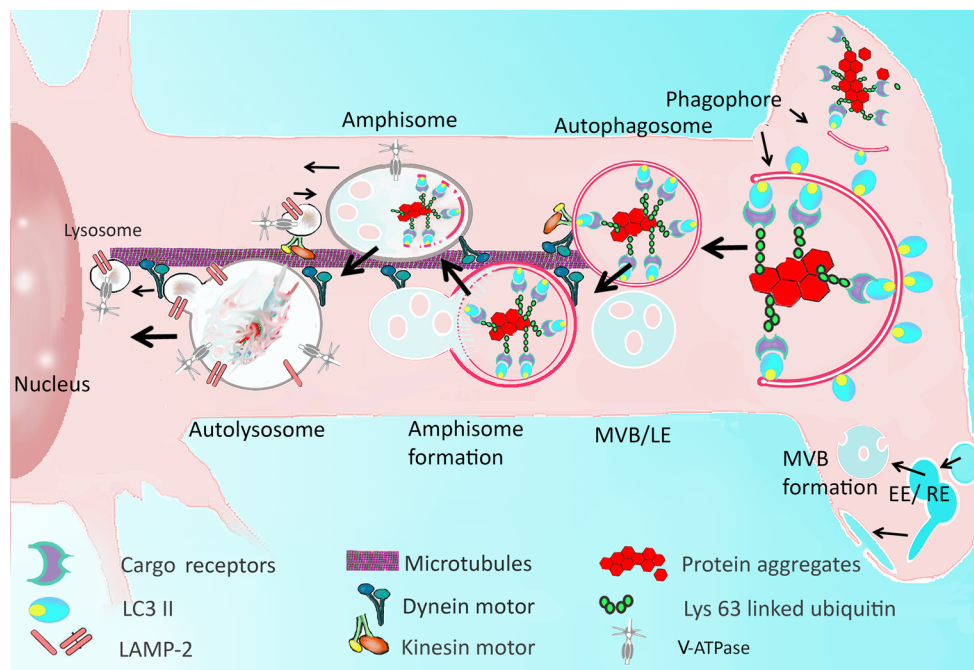
Fig. 3 Autophagosome formation and maturation are spatially controlled in neuronal cells. Autophagosomes form in the neurite tips, where ubiquitinated protein aggregates bind different cargo receptors that also bind the autophagosome protein LC3-II. This directs phagophore growth and leads to engulfment of harmful aggregates to autophagosomes (in the drawing in axon terminals). Autophagosomes as well as endocytosed material in multivesicular bodies (*MVB*) and late endosomes (*LE*) are transported by dynein motors towards the neuron soma. During the transport, autophagosomes fuse with multivesicular bodies and late endosomes forming amphisomes, and these finally fuse with lysosomes to create autolysosomes. Fusion with lysosomes leads to acidification of the cytosolic cargo by proton pump V-ATPase in lysosomal membrane (acidification is illustrated by fading of the *pink* and *blue* color). Lysosomes move bidirectionally by means of kinesin and dynein motors. *RE/EE* recycling endosome/early endosome

allows the cell to respond to starvation by increased allocation of amino acids from proteins among the sequestered autophagic cargo. The central part of the amino acid sensing machinery is the conserved protein complex named *m*ammalian *T*arget *O*f *R*apamycin (mTOR) complex 1 (mTORC1), which is a potent repressor of autophagy in all eukaryotes (Fig. 4) [155]. mTORC1 consists of (1) the Ser/Thr protein kinase mTOR; (2) regulatory associated protein of mTOR (RAPTOR) that acts as a scaffold for the complex; and (3) the additional subunits PRAS40 and mLST8. mTORC1 acts on several intracellular pathways and its direct substrates include the translational regulator 4EBP, ribosomal S6 kinase (S6K1), death-associated protein 1 (DAP1, a negative regulator of autophagy [119]), and AMBRA1, a protein needed for autophagosome formation [164]. The primary autophagy regulating targets of mTORC1 are ULK1 and/or Unc-51-like kinase 2 (ULK2) [103]. This linkage of mTOR and ULK1/ULK2 connects autophagy regulation to the availability of nutrients. The ULK1/ULK2 complex contains ULK1 or ULK2, 200 kDa FAK family kinase-interacting protein/RB1-inducible coiled-coil 1 (FIP200/RB1CC1), ATG13 and C12orf44/ATG101, and functions during autophagy induction. Under starvation conditions, ULK1/ULK2 phosphorylation by mTORC1 at serine 757 is reduced, and consequently, ULK1/ULK2 becomes activated by phosphorylation of two other serine residues by AMP activated protein kinase (AMPK), which makes it in turn capable of phosphorylating ATG13 and FIP200 (Fig. 2). This initiates autophagy by the formation of an active ULK kinase complex. Under nutrient-rich conditions, mTORC1 is active and inhibits autophagy by phosphorylating ATG13 and ULK1/ULK2 subunits and thereby repressing ULK1/ULK2 kinase activity and ultimately autophagy [112]. mTORC1 activity in turn is negatively regulated by the tuberous sclerosis complex (TSC) 1/2. Neurons deficient in TSC2 were recently shown to have an mTORC1-independent mechanism for autophagy regulation [46]. Unlike non-neuronal cells, neurons deficient in TSC2 show increased accumulation of autolysosomes and increased autophagic flux, despite mTORC1-dependent inhibition of ULK1. In the absence of TSC2, ULK1 was activated by AMPK. These findings suggest that, in TSC2 knockdown neurons, AMPK activation is the dominant regulator of autophagy, and that neuronal TSC1/2 complex is required for coordinated regulation of autophagy by AMPK [46].
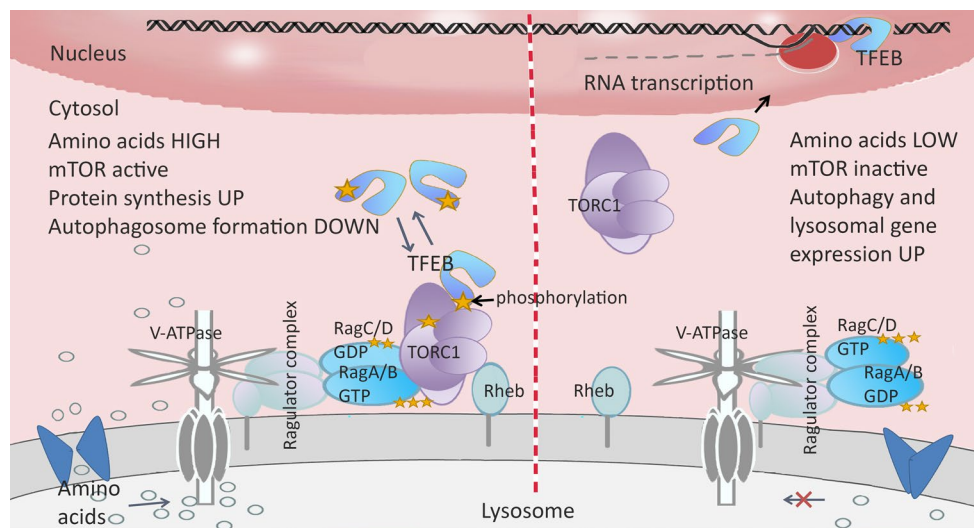
Fig. 4 mTORC1 is the main regulator of starvation-induced autophagy. mTORC1 senses the nutrient status of the cell via different pathways. Amino acid sensing occurs on the lysosomal surface. When the amount of amino acids is high (*left* side of the figure) V-ATPase signals to Ragulator complex, which leads to activation of the small GTPase RagA or B by GDP exchange to GTP. Mammals express four Rag proteins—RagA, RagB, RagC, and RagD—that form heterodimers consisting of RagA or RagB with RagC or RagD. GTP-bound RagA or RagB and mTORC1 are recruited to the lysosomal surface. mTORC1 then interacts with Rheb–GTP, and phospho-rylates its downstream effectors leading to inhibited autophagy and enhanced cell growth. Also TFEB is phosphorylated and remains in the cytosol. When amino acids are missing or low (*right* side of the figure), V-ATPase prevents the guanine nucleotide exchange activity of the Ragulator complex, and mTORC1 remains inactive and resides in the cytosol. Downstream effectors of mTORC1 are not phosphorylated, leading to general translational halt and induction of autophagy (see Fig. 2). Also TFEB is not phosphorylated and thus it translocates to the nucleus, where it activates the transcription of autophagy and lysosomal genes

The precise mechanism for amino acid sensing is not yet resolved, but recent findings indicate that it is initiated by the accumulation of amino acids within the lysosomal lumen. This induces V-ATPase to form a complex with Ragulator, a guanine nucleotide exchange factor, and small heterodimeric Rag (A + C or B + D) guanosine triphosphatases (GTPases) (Fig. 4). Rags are heterodimers consisting of RagA or RagB, which are highly similar to each other, bound to RagC or RagD, respectively. Ragulator and Rag GTPases locate on the lysosomal surface, forming a docking site for mTORC1. mTORC1 then becomes activated to inhibit autophagy (Fig. 4) [270].

In addition to this transcription-independent regulation of autophagy by mTORC1, autophagy is also regulated by a transcription-dependent mechanism [202]. This mechanism depends on TFEB, which is also critical for the coordinated expression of lysosomal genes [202]. In the presence of nutrients, mTORC1 phosphorylates TFEB at serine 211. This causes retention of TFEB in the cytoplasm. In the absence of nutrients, when mTORC1 is inactive, TFEB translocates into the nucleus [192, 209]. After binding to its target sequence in the promotor-region of lysosomal and autophagic genes TFEB induces the expression of these genes. Thus, TFEB directly links the enhanced formation of autophagosomes and the subsequent degradation of autophagocytosed cargo in autolysosomes by increased lysosomal biogenesis (Fig. 4).

mTORC1-dependent and TFEB-induced induction of autophagy are linked, connecting nutrition sensing at the lysosomal membrane by mTORC1 and V-ATPase, and induction of autophagic and lysosomal gene expression by TFEB (Fig. 4). Puertollano and coworkers showed that, upon inhibition of mTORC1, TFEB transiently associates with mTORC1 and then rapidly translocates to the nucleus [142]. Under normal conditions, most TFEB is retained in the cytosol by a phosphorylation-dependent interaction with members of the YWHA (14-3-3) protein family, whereas a small amount associates with lysosomal membranes. TFEB bound to the cytosolic side of the lysosomal membrane becomes phosphorylated by mTORC1 and cycles back to the cytosol [142]. In the presence of nutrients, active Rag GTPases A and B recruit TFEB and mTORC1 to lysosomal membranes [144, 145], where mTORC1 comes in close contact with Ras homolog enriched in brain (Rheb), an essential GTPase for amino acid activation of mTORC1 [133]. Rheb is a target of growth-factor-dependent signaling and thereby further modulates mTORC1 activity [72, 93]. This nutrient sensing at lysosomal membranes allows the cell to coordinate availability of amino acids with the induction of a number of autophagy genes and genes encoding lysosomal hydrolases and membrane proteins.

In addition to amino acid starvation, other cellular stress factors can induce macroautophagy, including hypoxia,

glucose deprivation, endoplasmic reticulum stress and reactive oxygen species (ROS). In experimental neuronal models of hypoxia–ischemia (oxygen and glucose deprivation) autophagy was found to increase dramatically, ultimately leading to cell death [177, 210]. In agreement with these results, a recent study showed that hexokinase, a central enzyme in glucose metabolism, directly activates macroautophagy by inhibition of mTORC1 [191]. Papadakis et al. [177] examined why hippocampal CA1 neurons are more vulnerable to ischemic insults than CA3 neurons and how ischemic preconditioning affects these neurons. Hamartin, the product of *TSC1*, was shown to confer neuroprotection against ischemia both in vitro and in vivo in mice by inducing neuronal macroautophagy in an mTORC1- and presumably Rheb-dependent manner [177]. Another study showed that macroautophagy induction under hypoxic conditions was independent of mTORC1, but at least in part dependent on hypoxia-inducible factor (HIF), a key transcription factor that allows adaptation to and survival in reduced oxygen concentrations. HIF was shown to induce the expression of *Bnip3,* whose gene product (BNIP3, BCL2/adenovirus E1B 19 kDa interacting protein 3) is proposed to induce macroautophagy via beclin 1 (reviewed in [149]).

Another cellular stressor leading to activation of macroautophagy is endoplasmic reticulum stress, induced by unfolded or misfolded proteins [63, 165, 170, 200]. Several different pathways have been implicated, including direct induction of autophagic genes by the unfolded protein response (UPR) sensors. In mammals, serine/threonine-protein kinase/endoribonuclease protein (IRE1), protein kinase-like ER kinase (PERK) and activating transcription factor 6 (ATF6) [170] function as UPR sensors. Furthermore, additional signaling pathways have been described to induce macroautophagy, including negative regulation of the Akt-mTOR pathway by UPR and calcium signaling via Ca^{2+}-dependent protein kinase C theta (PKCθ) activity [200]. Finally, ROS were shown to be a common inducer of autophagy in neurons. Since ROS are highly toxic for cells, macroautophagy clearly plays a neuroprotective role in this regard, by removing ROS-generating organelles including mitochondria and endoplasmic reticulum. However, how precisely ROS induce macroautophagy is poorly understood. In cancer cells both Akt-mTOR pathway and BNIP3-dependent pathways were described to contribute to autophagy induction [24, 265]. Since ROS are highly reactive, direct interaction with autophagy-related proteins are also likely to contribute: the redox-sensitive cysteine protease ATG4 was shown to be directly influenced by ROS. A cysteine residue near the catalytic center becomes oxidized, thereby inhibiting ATG4 activity and the delipidation of LC3-II [204]. Intriguingly, mitophagy, selective macroautophagy of mitochondria, was demonstrated to be mediated by mitochondria-generated ROS and a direct interaction of Rheb and LC3-II [151].

Localization and movement of autophagosomes in neuronal cells

Neuronal cells are morphologically and functionally specialized. In agreement with this specialization, the formation and maturation of autophagosomes are spatially controlled in neurons. Autophagosomes were identified in neurite tips by electron microscopy already in 1973 [23]. Live cell imaging showed that both endocytic and autophagic vesicles move along axons towards the cell soma [90]. Later studies demonstrated that autophagosomes positive for green fluorescent protein (GFP) tagged LC3 move in axons bidirectionally, with net movement towards the cell soma where the majority of lysosomes are located. This movement depends on microtubules and on the motor proteins dynein and kinesin [107, 255]. Dynein mediates the movement towards cell soma, while kinesin assists movement to the opposite direction [138]. A recent study showed that the scaffolding protein JIP1 regulates autophagosome transport in neuronal cells. JIP1 binds LC3 and the dynein activator protein dynactin, and these interactions are needed for autophagosome transport towards the cell soma [66]. Interestingly, JIP1 also binds and activates kinesin-1 in a phosphorylation-dependent manner. Phosphodeficient JIP1 activates transport towards the cell soma, while phosphomimetic JIP1 aberrantly activates transport to the opposite direction. In primary neurons, autophagosomes constitutively form in the tips of neurites and then travel towards the cell soma, undergoing fusion with endosomal and lysosomal vesicles and turning acidic during the transport [66, 138] (Fig. 3). These autophagosomes may contain organelles like mitochondria and/or aggregate-prone proteins as cargo. The small GTPase RAB7 is needed for the maturation of autophagosomes to autolysosomes, most likely for the fusion with lysosomes [78, 97]. In Purkinje neurons, insulin-like growth factor I enhances autophagosome maturation by increasing the interaction of RAB7 with RILP, a RAB7 effector protein that is needed for association of RAB7 with dynein motor proteins [8]. Fusion of autophagosomes with lysosomes initiates the degradation of autophagic cargo. Inhibition of lysosomal proteolysis with drug treatment or due to genetic deficiency of lysosomal enzymes (discussed later) slows down the axonal transport of autolysosomes, endosomes and lysosomes. As a consequence, these organelles accumulate in axonal swellings while other organelles like mitochondria are still able to move normally [127]. Accumulation of autolysosomes in axonal swellings is a typical finding in Alzheimer's disease [168].

Fig. 5 Autophagic cargo receptors implicated in genetic forms of neuronal diseases discussed in this article. The *text* below the drawing indicates the stage of the macroautophagic pathway which is disturbed by each of the disease-causing mutation or neurological disease
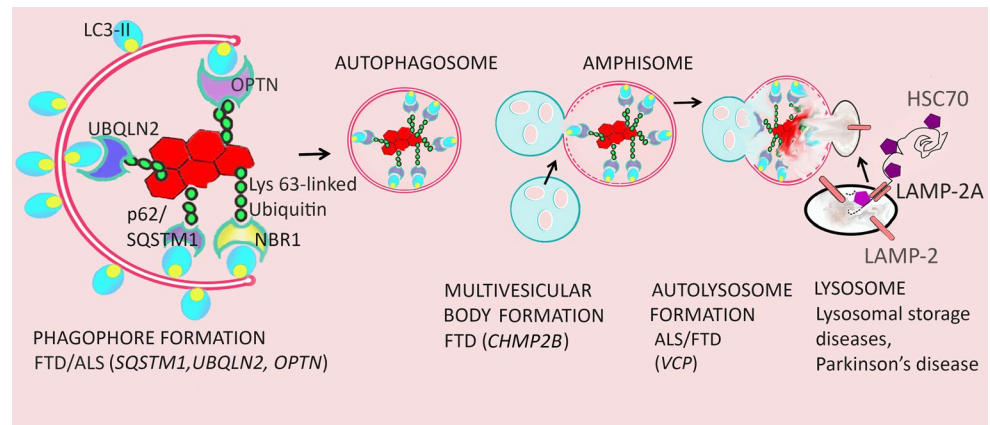
Basal and induced macroautophagy and selective and non-selective macroautophagy in neurons

Basal macroautophagy occurs at low level without a specific stimulus. It is important for cellular homeostasis, because it prevents metabolic and oxidative stress by degrading long-lived proteins, damaged organelles like mitochondria, and aggregated or aggregate-prone proteins. These functions are especially important for postmitotic cells like neurons, which are not able to dilute the harmful constituents by cell division. Stress such as starvation enhances macroautophagy several-fold in isolated tissues and cultured cells [158]. This allows cells to mobilize energy from internal resources and to survive the stress. Induced macroautophagy is inhibited by mTORC1 that senses cellular energy and nutrient levels and regulates cell growth, while basal macroautophagy is less sensitive to mTORC1 activity [4]. Although both basal and induced autophagosomes may form in a similar manner [160], the proteins mediating their fusion events with endosomes and lysosomes may differ. The small GTPase RAB7 is essential for the maturation of starvation-induced, but not basal, autophagosomes [97]. Recent results also show that the histone deacetylase HDAC6 and valosin-containing protein (VCP/p97) are essential for the maturation of basal, but not starvation-induced, autophagosomes [124, 234]. In agreement with these results, proteomic analysis also revealed that basal and induced autophagosomes contain proteomes that only partially overlap [44].

In mammals, the induction of macroautophagy is organ dependent. Fasting causes a more profound induction of macroautophagy in heart and muscle when compared to brain [156]. This was suggested to be due to nutrient supply from other organs to brain during food deprivation. A more recent study, however, detected a significant increase in autophagosomes in cerebral cortical and cerebellar Purkinje cells in nutrient-deprived mice [5]. Interestingly, another recent study showed that physical exercise induces macroautophagy in the cerebral cortex of adult mice [85]. These observations suggest that macroautophagy may participate in mediating the known beneficial effects of calorie restriction and exercise on brain function.

Recent studies support the idea that basal macroautophagy is selective, acting as a quality control mechanism, while induced macroautophagy is less selective, acting mainly to support survival by producing nutrients by degrading randomly segregated cytoplasmic components and organelles [124]. As discussed below, in neuronal cells defective macroautophagy leads to accumulation of protein aggregates and neurodegeneration. Selective macroautophagy of aggregates is called aggrephagy [121]. Dysfunction of mitochondria can cause oxidative stress and is also linked to aging and neurodegeneration [50, 188, 233, 269]. Cells have developed a mechanism to remove damaged mitochondria by selective macroautophagy, called mitophagy [14, 225, 228]. This process is induced by mitochondrial depolarization, which stabilizes the serine/threonine kinase PINK1 on the mitochondrial surface. PINK1 then recruits the E3 ubiquitin ligase parkin/PARK2 [162], which leads to ubiquitination of mitochondrial proteins. Ubiquitination recruits mitophagy adaptor proteins, which in turn recruit autophagy proteins and initiate mitophagy [50]. Loss of the PINK1/PARK2 pathway causes one form of familial Parkinson's disease. Mitochondrial depolarization increases the interaction of PARK2 with AMBRA1, a component of the PI3KC3 complex. AMBRA1 favors the interaction of beclin 1 with PI3KC3 and the production of phosphatidylinositol 3-phosphate by the PI3KC3. This in turns enhances mitophagy [239].

Protein aggregates or damaged organelles are hallmarks of many neurological diseases. They are selectively engulfed by a mechanism involving one or more cargo receptors, including sequestosome 1 (SQSTM1/p62), NBR1, NDP52, optineurin (OPTN), NIX/BNIP3L, and FUNDC1 [113, 121, 196, 205] (Fig. 5). The cargo receptors contain a cargo-binding domain and another

domain that binds LC3 or another member of the ATG8 family (LC3A, LC3B, LC3C, GABARAP, GAPARAPL1, GATE16/GAPARAPL2), which enables the recruitment of autophagic membranes to the cargo. BNIP3L and FUNDC1 are integral membrane proteins of the mitochondrial outer membrane and interact with LC3 in order to mediate hypoxia-induced mitophagy [132, 169]. Additional scaffolding proteins like HDAC6 and ALFY may be needed for the selective macroautophagic segregation of protein aggregates [95, 190, 213]. Similar to the proteasomal system, ubiquitination serves as a general cargo recognition signal for selective macroautophagy, and many of the cargo receptors have a ubiquitin-binding domain. Unlike in proteasomal degradation, selective macroautophagy degrades the cargo together with the ubiquitin tag, as well as the associated cargo receptors and scaffolding proteins. Lysine 63-linked polyubiquitin has been suggested to be the main signal for macroautophagic degradation of protein aggregates and mitochondria [125, 172, 224]. Lysine 63-linked ubiquitination increases the formation of aggresomes, inclusion bodies containing aggregated proteins, as well as the macroautophagic degradation of the ubiquitinated proteins. HDAC6 and the motor protein dynein participate in the aggresome formation by transport of ubiquitinated cargo, i.e., proteins or mitochondria, along microtubules towards their minus ends (in neurons towards the cell soma) [69, 95, 125, 171, 172]. HDAC6 regulates microtubule dynamics and microtubule-mediated transport by deacetylating tubulin [41]. During proteasome inhibition, HDAC6 promotes the localization of the E3 ubiquitin ligase TRIM50 to protein aggregates, which promotes their transport to aggresomes [69]. HDAC6 also controls the fusion of autophagosomes with lysosomes by recruiting the machinery that assembles filamentous actin, which in turn stimulates autophagosome–lysosome fusion [124].

Mechanism and regulation of CMA

The cytosolic chaperone heat shock cognate protein 70 (HSC70) binds to the canonical KFERQ-like motif of substrate proteins, mediating their binding to the C-terminal cytosolic tail of the lysosomal membrane protein LAMP-2A. This binding mediates the assembly of monomeric LAMP-2A into a high molecular weight complex, which was suggested to act as a translocation pore [11]. In addition to HSC70 and LAMP-2A, other proteins such as co-chaperones are present in the translocation complex [108]. How the translocation pore accommodates its substrates remains an open question. After translocation of substrate proteins the complex was proposed to dissociate into monomers. Intralysosomal form of HSC70 is also needed for the transport of the CMA substrates across the lysosomal membrane [36].

The selectivity of CMA is mediated by the KFERQ-like motif which binds to cytosolic HSC70. This motif is present in ~30 % of cytosolic proteins. Similar to macroautophagy, also the level of CMA changes according to conditions, allowing adaptation to long-term needs of the cells. The extent of macroautophagy and CMA are tightly linked; a block in macroautophagy has been shown to upregulate CMA and vice versa [109, 147]. The extent of CMA depends on the amount of its receptor LAMP-2A on the lysosomal membrane [47]. The levels of available LAMP-2A can be modulated by changes in its biosynthesis, degradation, localization in the lysosomal membranes, and conformation [11]. In addition, the stability of the translocation complex was shown to be regulated by GTP [12]. Phosphorylation and acetylation where shown to modify amino acid charge of the KFERQ-like motifs, which may modify the efficiency of recognition by HSC70. Partial unfolding of the protein may reveal the KFERQ-like motif in case it is normally concealed in the interior of a protein and this may facilitate CMA-mediated degradation [136, 185].

Similar to macroautophagy, CMA is activated by starvation of nutrients, but the timing of these two autophagy pathways differ. Macroautophagy is activated as the first response to starvation, and CMA is upregulated later, when the activity of macroautophagy decreases. In cultured cells, removal of serum from the culture medium for more than 10 h increases the activity of CMA and reduces the levels of KFERQ-containing proteins [173]. It has been suggested that the switch from macroautophagy to CMA is beneficial since it allows more selective degradation of proteins that are not necessary for cells during nutrient deprivation. CMA may also allow the cells to adjust their proteome and adapt to the changed conditions during long starvation [38].

Physiological functions of autophagy in neuronal cells

A growing number of physiological functions have been described for both macroautophagy and CMA [55, 173]. In postmitotic cells like neurons, autophagy prevents the accumulation of oxidized proteins, harmful aggregates and nonfunctional, potentially harmful organelles like mitochondria. The importance of autophagy for neurons is highlighted by a recent systems biology study that revealed a pivotal role of dysregulated autophagy in neurodegenerative dementia [25].

The role of macroautophagy in neurons has been studied using mice with central nervous system (CNS)-specific, or neuronal cell type-specific, knockout of autophagy proteins (ATG5, ATG7 or FIP200/RB1CC1) that are indispensable for autophagosome formation [83, 117, 128]. These studies report shortened life span and progressive motor and behavioral defects in the mutant mice. Histological examination

of CNS-specific knockout mice revealed neurodegenerative changes in brain that were most prominent in the cerebral cortical region and in cerebellar Purkinje cells. In *Atg5* and *Atg7* mutant mice, an increase in the number of TUNEL-positive cells was detected in the cerebral cortex and within the granular cell layer of the cerebellum [83, 117]. In the cerebellum of *FIP200* mutant mice, neuronal loss, spongiosis and neurite degeneration were observed [128]. Ubiquitin-positive inclusions and protein aggregates were reported in *Atg5* mutant mice in large neurons in the thalamus, midbrain, pons, medulla and dorsal root ganglion, as well as in neurons in the cerebral cortex, hippocampus, striatum and olfactory bulb [83], while in *Atg7* mutant mice these were encountered in the cerebral cortex, cerebellar Purkinje cells, hippocampal pyramidal neurons, thalamus, hypothalamus, amygdala and pontine nuclei [117], and in *FIP200* mutant mice in Purkinje cells and white matter in the cerebellum [128]. Furthermore, inclusion bodies were only observed in neurons, not in glial cells of *Atg5* mutant mice [83]. Notably, the proteasomal activity was not affected in *Atg7* and *FIP200* mutant mice, indicating that ubiquitin-positive aggregates did not accumulate due to impaired proteasome function [117, 128]. In addition, abnormal mitochondria were detected in Purkinje cells of *FIP200* mutant mice, possibly due to defective removal via mitophagy [128]. Taken together, these studies indicate that macroautophagy is necessary for the elimination of aggregate-prone proteins in neurons even in the absence of disease-causing mutations in these cargo proteins, and that loss of macroautophagic activity leads to severe neurodegeneration.

To circumvent the early lethal effects of the classical knockout mice, neuronal cell type-specific knockout mice have also been generated. Knockout of *Atg7* specifically in dopamine neurons of midbrain caused axonal and dendritic dystrophy and loss of dopamine neurons [3, 65]. Inclusions positive for ubiquitin and α-synuclein were detected in the degenerating neurons, similar to the findings in *Atg5* and *Atg7* CNS-specific knockout mice [83, 117]. Purkinje cell-specific conditional *Atg5* or *Atg7* knockout mice were used to show that macroautophagy is essential for the prevention of axonal degeneration and maintenance of homeostasis in axon terminals [118, 167]. Interestingly, the *Atg7* knockout Purkinje cells did not show atrophy of dendrites or dendritic spines. However, the axonal damage was followed by Purkinje cell death. Deletion of *Atg7* in mouse pro-opiomelanocortin (POMC) neurons led to an age-related accumulation of ubiquitin and SQSTM1 positive aggregates in the hypothalamus, and disrupted maturation of POMC-containing axonal projections [33]. Higher body weight, increased adiposity and glucose intolerance were also observed in these mutant mice [33, 184]. The results suggest that macroautophagy in the hypothalamus may be able to control and reduce obesity. Additionally, in Purkinje cells and POMC neurons, macroautophagy may be specifically important for the homeostasis of axons. Knockout mice for *Atg7* in ventral midbrain dopamine neurons were used to demonstrate that macroautophagy plays a role in the regulation of presynaptic transmission. The axonal profiles of the *Atg7* knockout dopamine neurons were one-third larger than the control axonal profiles. The mutant neurons released ~50 % more dopamine after stimulus, and the synaptic recovery was also faster than in the control neurons [89, 231]. Induction of macroautophagy by rapamycin treatment reduced the axon profile size of dopamine neurons by ~30 % in control mice, but had no effect on the *Atg7* knockout axons. Rapamycin treatment also decreased the dopaminergic synaptic vesicle density and inhibited dopamine release by 25 % in control mice, but not in the *Atg7* knockout mice. These findings illustrate that macroautophagy can reduce presynaptic activity in vivo, likely by degrading synaptic vesicles [89, 231].

A recent study showed that macroautophagy negatively regulates axon growth in cortical neurons [10]. Silencing of the autophagy protein ATG7 caused elongation of axons, while activation of macroautophagy by rapamycin treatment suppressed axon growth. The regulation was shown to be mediated via the Rho A-ROCK pathway. Finally, both defective and excessive macroautophagy have been shown to contribute to neurite degeneration in various model systems. These studies are summarized in a recent review [254].

Autophagy can protect neuronal cells during stress or injury

Several studies have shown that macroautophagy can protect neuronal cells under stress conditions. The class III histone deacetylase sirtuin1 (SIRT1) mediates the beneficial metabolic effects of caloric restriction. SIRT1 was shown to protect neurons against the toxicity of mutant huntingtin, while reduction of SIRT1 increased huntingtin toxicity [99]. Another group showed that SIRT1 overexpression prevented prion peptide neurotoxicity by inducing macroautophagy, while preventing autophagy by knockdown of *Atg5* abolished the SIRT1 induced neuroprotection [98]. Further, the macrolide drug FK506 delayed the accumulation of prion protein in brains of mice inoculated with Fukuoka-1 prion [161]. This was accompanied by an increased expression of autophagy proteins in brain tissue. Cell culture experiments revealed that FK506 upregulated macroautophagy and enhanced degradation of prions in autolysosomes. Finally, in Purkinje cells and retinal ganglion cells suffering from axonal dystrophy or traumatic injury, induction of macroautophagy in axons apparently serves as a stress response that protects the cell soma [193, 194, 241, 262].

Macroautophagic segregation and degradation, or autophagy proteins, can have both antiviral and proviral roles, depending on the tissue and virus type [49, 259]. It has been proposed that in postmitotic cells like neurons, macroautophagy, rather than an interferon response, plays the predominant role in controlling viral infection [258]. Several studies support a vital role for macroautophagy in antiviral defense in neurons. Because neurons do not divide and thus cannot replace dead cells, they need non-cytolytic mechanisms to control viral replication. Deficiency in the autophagy gene *Atg5* or autophagic cargo receptor gene *Sqstm1* accelerated Sindbis virus-induced cell death in mouse brain without affecting virus replication [176]. The results suggest that SQSTM1 targets the viral particles for autophagic degradation. Yordy and coworkers studied neurotropic herpes simplex type 1 virus infection in dorsal root ganglion (DRG) neurons. They showed that in neuronal DRG cells—in contrast to mucosal epithelial cells and other renewable cells—little type I interferon was produced, nor was interferon treatment able to block viral replication or to induce interferon-primed cell death. Instead, in DRG neurons macroautophagy was required to restrict viral replication [257]. Viruses have also developed ways to resist the macroautophagic defense system. Herpes simplex virus produces the protein ICP34.5 that binds to the autophagy protein beclin 1 and prevents its function in macroautophagy. Viral mutants that lack this beclin 1 inhibitor were less infective in a mouse intracerebral infection model [175]. Viruses can also exploit the macroautophagy pathway or individual autophagy proteins for their replication [49]. This was first discovered for poliovirus that uses macroautophagy for both replication and nonlytic release [96, 189]. Positive-stranded RNA viruses like poliovirus utilize autophagosome maturation into acidic autolysosomes to promote their replication.

There are also situations where macroautophagy may enhance neuronal degeneration. One example is degeneration as observed in the rat optic nerve. In this case, inhibition of macroautophagy by 3-methyladenine (an inhibitor of PI3KC3) attenuated acute axonal degeneration after a lesion caused by crushing the optic nerve [114]. Another example of possibly harmful macroautophagy is cerebral ischemia and ischemic brain damage. The induction of macroautophagy after cerebral ischemia has been observed in numerous studies, but the role of macroautophagy in this pathological process is under debate: inhibition of macroautophagy has been reported to have both beneficial and harmful effects for neurons [7, 244, 252]. The effect of macroautophagy in neurons may be influenced by various physiological and pathological conditions, such as the age of the patient, affected brain region, severity of the insult, and stage of ischemia. A recent study showed that macroautophagy may have different roles during cerebral ischemia and subsequent reperfusion [267]. During permanent ischemia, inhibition of macroautophagy reduced brain and cell damage. However, during reperfusion, inhibition of macroautophagy increased both cell injury in vitro and brain injury in vivo. The neuroprotection during reperfusion was mediated by increased mitophagy [267].

Increasing evidence suggest that CMA can also protect neurons from stress and injury. CMA is upregulated during oxidative stress, and oxidized proteins are more readily transported into lysosomes than non-oxidized proteins [110]. Transcriptional upregulation of LAMP-2A levels was shown to be the mechanism of the increased CMA. Cells unable to upregulate CMA during oxidative stress accumulate oxidative damage and show increased cell death [147]. It has been shown that LAMP-2A levels and CMA activity decline with age and this may contribute to the accumulation of oxidized proteins that is observed in most tissues in old animals [173, 264].

In addition to macroautophagy, CMA may also contribute to neuronal cell survival after hypoxic stress. A recent study showed that LAMP-2A was upregulated in the ischemic hemisphere after brain ischemia induced by occlusion of the middle cerebral artery. In addition, hypoxia increased LAMP-2A levels and activated CMA in neuronal cell cultures. Activation of CMA by drug treatment reduced hypoxia-mediated cell death, while silencing LAMP-2A increased it [48].

CMA was also shown to act neuroprotectively by regulating the activity of the myocyte enhancer factor 2D (MEF2D), a transcription factor required for neuronal survival [253]. MEF2D was shown to continuously shuttle from the nucleus to the cytoplasm where it interacts with HSC70 and is degraded by CMA. Inhibition of CMA was shown to lead to the accumulation of inactive MEF2D in the cytoplasm and depletion of active MEF2D from the nucleus. It was also shown that wild-type α-synuclein disrupts CMA-mediated degradation of MEF2D, which could explain why both wild-type and mutant α-synuclein can cause Parkinson's disease.

Role of macroautophagy in neuronal development and neurogenesis

Emerging evidence supports a role for macroautophagy in neuronal development and neurodevelopmental disorders [126]. AMBRA1 is a component of the PI3KC3 complex that functions during autophagosome formation (Fig. 2). *Ambra1* is a highly conserved vertebrate-specific gene, and it is highly expressed in the neuroepithelium during early neurogenesis. Functional knockout of AMBRA1 in mice showed that this protein plays a role in the development of the nervous system. Embryos deficient in *Ambra1*

died before birth and showed severe defects in the neural tube, including impaired macroautophagy, accumulation of ubiquitinated proteins, and unbalanced cell proliferation followed by excessive apoptosis [60]. Interestingly, mice heterozygous for *Ambra1* showed an autism-like phenotype that was only detected in female animals [45]. Autism is considered to be associated with defects in neurodevelopment. Vazquez and colleagues [243] studied the role of autophagy proteins in neurogenesis of neural stem cells. The expression of autophagy genes *Atg7*, *Becn1*, *Ambra1* and *Lc3* increased in mouse embryonic olfactory bulb during the initial neuronal differentiation. In addition, macroautophagy was increased during neuronal differentiation in cultured stem cells derived from mouse olfactory bulb, and inhibition of macroautophagy by drugs or deletion of autophagy proteins decreased neurogenesis. The results also suggest that neural stem cells activate macroautophagy in order to fulfill their energy demands that are high due to remodeling of the cell shape and cytoskeleton [243].

Aburto and colleagues [1] investigated the role of macroautophagy in otic neurogenesis in chicken inner ear. They showed that autophagy genes *Becn1*, *Atg5* and *Lc3B* were expressed and autophagic vesicles were present in the otic neurogenic zone. Inhibiting macroautophagy by antisense morpholinos or by drugs caused aberrant morphology, accumulation of apoptotic cells, impaired neurogenesis and poor axonal outgrowth. The results also suggest that macroautophagy provides the energy for the clearance of dying neuroepithelial cells and migration of otic neuronal precursors [1].

Role of autophagy in neuronal aging and neuronal diseases

In this review we will give a short overview of our current knowledge on the role of autophagy in aging neurons, and focus on the role of autophagy in four types of neurodegenerative diseases, i.e., amyotrophic lateral sclerosis and frontotemporal dementia, prion diseases, lysosomal storage diseases, and Parkinson's disease. Other neuronal diseases where autophagy has been shown to play a role are discussed in the other review articles of this cluster.

Autophagy in aging neurons

As stated above, removal of harmful oxidated and aggregate-prone proteins and damaged organelles that produce oxidative damage is particularly important for postmitotic neuronal cells. Removal of damaged mitochondria by mitophagy is vital for cell survival, because damaged mitochondria can produce ROS and induce harmful events including protein carbonylation, lipid peroxidation and

DNA damage. Further, damaged mitochondria can trigger apoptosis by releasing cytochrome *c* [236]. Thus, is not surprising that both macroautophagy and CMA have been shown to delay aging [27, 38]. Promotion of macroautophagy has been shown to extend the lifespan of many model organisms including yeast, worms, flies, and mice [86]. In these studies, macroautophagy has been increased either by genetic manipulations (e.g., overexpression of SIRT1) or by drugs (e.g., rapamycin, resveratrol and spermidine). Further, studies with several model organisms have also shown that single-gene mutations, e.g., in the insulin and mTOR signaling pathways, can extend lifespan, and many of these mutations also induce macroautophagy [74].

The neurological phenotypes of macroautophagy-deficient animals, described above, reflect at least in part the phenotype of normal aging, including accumulation of the aging pigment lipofuscin, aggregates consisting of ubiquitinated substrates and autophagy cargo receptors, and dysfunctional mitochondria. These observations are highly suggestive of a pivotal role for autophagy in the prevention of aging [13, 221]. The rate of macroautophagy decreases with age in several cell types including the brain cells [131], even though differences between various brain regions are described, e.g., for the expression of *Ambra1* in mouse brain [208]. In addition to these transcriptional alterations, decreased lysosome–autophagosome fusion events also contribute to the inefficient clearance of macroautophagic substrates in aging tissues. Extremely reactive hydroxyl radicals are formed during the degradation of iron-containing metalloproteins and ROS-generating mitochondria in lysosomes under acidic and reducing conditions (partly due to the presence of the reducing amino acid cysteine). Hydroxyl radicals generate aldehydes which react with free amino groups within proteins, crosslinking them tightly, which ultimately leads to the formation of non-degradable, insoluble lipofuscin aggregates (reviewed in [21]), which can fill up to 75 % of some brain neurons [232]. These abundant lipofuscin aggregates are likely to contribute to the impairment of the lysosome–autophagosome fusion process and the degradative capacity of lysosomes and autolysosomes.

As stated above, both macroautophagy and CMA have been shown to delay aging [27, 38]. Similar to macroautophagy, CMA levels decrease with aging [35]. In the liver, the decrease in CMA was assigned to decreased levels of the CMA receptor LAMP-2A, due to apparent loss of its stability with aging [111, 195]. Whether this mechanism is also responsible for decreased CMA in neurons remains to be determined. CMA can be involved in neurodegeneration via two alternative mechanisms [38]. The first possibility is that CMA contributes to the degradation of the pathogenic protein, and the age-related or other type of decline

in CMA causes or contributes to disease. The second possibility is that the pathogenic protein blocks CMA, for example by irreversibly binding to the translocation complex. In both scenarios, the decline of CMA leads to the accumulation of all CMA substrates, including physiological and pathological proteins.

Amyotrophic lateral sclerosis and frontotemporal dementia

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are diseases in which a large subset of patients typically accumulate cytosolic aggregates consisting of the Tar-DNA binding protein 43 (TDP-43). TDP-43 is degraded by the ubiquitin proteasome system (UPS) and macroautophagy. In addition, it was also shown to be degraded by CMA, mediated through interaction between HSC70 and ubiquitylated TDP-43 [91].

Pathogenic mutations in several autophagy-related genes were identified in a small subgroup of patients suffering from FTD and ALS, including mutations in cargo receptors like *UBQLN2*, a gene encoding the ubiquitin-like protein ubiquilin 2 (UBQLN2). Like the autophagic cargo receptors described above, this protein directly interacts with LC3 and contains an ubiquitin-binding domain (UBA) that binds polyubiquitinated proteins [198]. *UBQLN2* mutations were found in patients presenting with ALS/FTD symptoms, and mutated ubiquilin 2 co-localized with aggregated proteins in neurons of the hippocampus and the spinal cord [43]. Another cargo receptor protein mutated in rare familial ALS cases is optineurin (OPTN). Sequencing of a cohort of 371 FTD patients failed to detect any mutations, indicating that *OPTN* mutations are only seen in ALS [197]. Similar to UBQLN2, OPTN directly binds to LC3 via an interacting motif and ubiquitin via an independent ubiquitin interacting motif [247, 268]. In addition to their autophagy cargo receptor function, both UBQLN2 and OPTN act in the degradation of substrates via the ubiquitin–proteasome system. The third cargo receptor protein found to be mutated in a subset of ~3 % of FTD and ALS patients is SQSTM1 [56, 199], which preferentially binds Lys63-polyubiquitinated aggregates [17, 18, 207]. It seems to act predominantly in the macroautophagic degradation, since most autophagy gene knockout mouse models show a profound accumulation of ubiquitin and SQSTM1-positive aggregates especially in neurons [33, 118, 128].

Another gene mutated in TDP-43 proteinopathies is *VCP*, which encodes the valosin-containing protein (VCP). VCP is a member of the AAA$^+$-ATPase (ATPase associated with diverse cellular activities) protein super family. Mutations in *VCP* have been documented on both arms of the ALS and FTD disease continuum [75, 101], indicating essential function in neurons. In the proteasomal degradation pathway of misfolded proteins, VCP binds to polyubiquitinated

proteins and extracts them from the membrane of the endoplasmic reticulum in an ATP-dependent manner to facilitate delivery to the proteasome [152]. In addition, mutations in *VCP* lead to an accumulation of nondegradative autophagosomes due to impaired fusion of autophagosomes with lysosomes [102]. As described earlier, VCP was shown to be essential for the fusion of ubiquitin-containing, basal autophagosomes with proteolytically active acidified vesicles [234]. These data suggest important functions for VCP in both macroautophagy and proteasomal degradation. With regard to TDP-43 proteinopathies, another intriguing function of VCP is its role in the degradation of stress granules, which are commonly observed in neurons of patients with neurodegenerative diseases. Stress granules are composed of cytoplasmic mRNA and RNA-binding proteins (such as TDP-43), which become sequestered by macroautophagy. VCP was shown to be essential for this process and it was suggested that it might act in the disassembly and/or targeting of stress granules to macroautophagy [22]. However, the mechanisms that lead to inclusions of TDP-43 in *VCP* mutant neurons need to be determined. Interestingly, *VCP* mutations directly affect mTOR-mediated nutrient sensing. Mutations in *VCP* or inhibition of VCP lead to a failure in mTOR activation upon nutrient stimulation, even though the precise mechanism remains unclear [30]. However, this additional regulatory function implicates a complex interplay between degradative pathways in the cell.

Rare mutations in charged multivesicular protein 2B (CHMP2B) have been described in FTD patients [22, 179, 214] and also in some ALS patients [34]. CHMP2B is part of the endosomal sorting complex required for transport-III (ESCRT-III), which is needed for efficient sorting of ubiquitinated integral membrane proteins into the internal vesicles of multivesicular bodies (MVBs), and the formation of MVBs in general. Moreover, MVBs fuse with autophagosomes and were shown to be essential for the degradation of macroautophagic cargo and the macroautophagic clearance of protein aggregates, including TDP-43 aggregates [59, 123, 206]. These findings suggest that CHMP2B is an essential player in the macroautophagic pathway.

One-fifth of familial ALS cases bear gain-of-function mutations in *SOD1*, leading to accumulation of aggregated superoxide dismutase 1 (SOD1) in the cytosol of motor neurons. In neuronal cells, SOD1 was shown to be degraded by both the proteasomal pathway and macroautophagy [106]. Even though SOD1 apparently does not directly act in the autophagic pathway, autophagic alterations were observed in patients and mouse models with mutated SOD1 [70, 71, 79, 154]. How aggregated SOD1 leads to inhibition of autophagic flux is still unknown, but impairment of vesicular trafficking, heterotypic organelle fusion and lysosomal function might contribute to the pathology of ALS [266]. Of note, induction of macroautophagy by rapamycin

augmented neuronal cell death and decreased the life span of SOD1 mutant mice, suggesting that increasing the uptake of macroautophagic cargo in the context of impaired clearance is harmful [266]. In contrast, a previous study in which lithium was applied to induce macroautophagy in ALS mice and patients, revealed a beneficial effect of this treatment [61]. It is unknown whether this positive effect was due to different signaling routes induced by lithium and rapamycin, or to side effects of lithium unrelated to macroautophagy induction [62]. Interestingly, when trehalose was used to induce macroautophagy in an mTOR-independent manner, the treatment prolonged the life span of mutant SOD1 transgenic mice and attenuated the progression of the disease [28]. The complexity of macroautophagy modulation in the context of ALS is highlighted by a recent study using a transgenic ALS mouse model expressing mutant SOD1 in a beclin 1 heterozygous background. Surprisingly, even though lower beclin 1 levels limited the autophagic flux, these mice had a significantly increased lifespan, suggesting that beclin 1 has a pathogenic role in ALS [163]. Nevertheless, beclin 1 was shown to have a functional role in the macroautophagic clearance of mutant SOD1 in cultured motoneurons. The molecular mechanism underlying the unexpected results with beclin 1 heterozygous ALS mice was suggested to include an abnormal interaction of mutant SOD1 with beclin1, mediated by the macroautophagy inhibitory protein BCL2L1, which may alter macroautophagy stimulation [163].

Similar to ALS, most patients with familial FTD harbor mutations in genes that are apparently unrelated to autophagy, including the frequent mutations in *GRN* (encoding progranulin), *MAPT* (encoding tau) and hexanucleotide repeat expansion in *C9ORF72*. In the case of tau, a proteolytic tau-fragment was shown to interact with HSC70 facilitating its binding to the CMA receptor LAMP-2A. However, in contrast to typical CMA substrates, these forms of tau are not translocated into the lysosomal lumen by CMA. Instead, association of tau to lysosomes through the CMA machinery seems to contribute to the cleavage of this mutant form of tau and the organization of the protein into oligomeric complexes [242]. It remains to be determined whether this association of tau with CMA is relevant in the pathogenesis of FTD.

Rare FTD cases also bear mutations in *TARDBP* gene (encoding TDP-43 and causing frontotemporal lobar degeneration of type FTLD-TDP) and *FUS* (encoding fused in sarcoma protein) [230]. Whether autophagy is impaired in these patients is currently unknown. However, the findings that certain pathogenic TDP-43 species can bind SQSTM1 and become degraded via macroautophagy further strengthen findings that autophagy might play a role in the degradation of the aggregating TDP-43 species [20, 227]. Moreover, in contrast to the SOD1 mouse model

(see above), a mouse model for FTLD-TDP showed beneficial effects of rapamycin treatment on TDP-43 aggregation, neuronal survival and motor dysfunction [240]. These results support a role for dysfunctional macroautophagy in the pathogenesis of TDP-43 proteinopathies.

Transmissible spongiform encephalopathies (prion diseases)

Prion diseases are transmissible neurodegenerative disorders characterized by an accumulation of misfolded pathogenic isoforms (PrP^{Sc}) of normal prion protein (PrP^{C}) [183]. Autophagy has been suggested to mediate the degradation of PrP^{Sc} and/or PrP^{C} at least to some extent [2, 88, 250]. As mentioned above, induction of macroautophagy by SIRT1 overexpression prevented prion peptide neurotoxicity, while prevention of macroautophagy by knockdown of *Atg5* abolished the SIRT1-induced neuroprotection [98]. In a recent study, macroautophagy induction by caffeine was shown to reduce neurotoxicity of human PrP^{Sc} in neuronal cells [157]. Both prion disease mouse models and human patients exhibit increased steady-state numbers of autophagosomes in neurons [130, 203, 212]. Several approaches have been used to test the effect of macroautophagy induction in prion diseases. Imatinib, an autophagy-inducing cancer drug, delayed the disease onset and appearance of PrP^{Sc} in an induced scrapie mouse model [263], and rapamycin and lithium modestly extended survival in scrapie infected mice [87]. In a genetic prion disease model, rapamycin delayed the disease onset and prevented plaque formation [32]. In contrast, trehalose did not change the course of the disease in prion-infected mice [2]. Taken together, these results suggest that macroautophagy is involved in the clearance of prion proteins in neurons, and that induction of macroautophagy is a potential treatment for prion diseases.

Lysosomal storage disorders

Lysosomal storage disorders (LSDs) are caused by deficiencies in lysosomal hydrolases or membrane proteins [181]. LSDs are characterized by the intralysosomal accumulation of different types of substrates and an impairment of endocytic trafficking pathways [9]. Since autophagic cargo is degraded by lysosomal hydrolases, a defect in the autophagic flux is also observed in many LSDs. This is demonstrated by the observations showing that a deficiency of the major lysosomal membrane protein LAMP-2 in mice [226] and man [166] leads to an accumulation of autophagic vacuoles due to an impaired lysosomal/autophagosomal fusion process [53]. After the fusion of lysosomes and autophagosomes, an efficient lysosomal degradation is necessary for successful completion

of the autophagic flux. For example, deficiencies in the major lysosomal proteinases, cathepsin D or cathepsins B and L, in mice cause a massive neuronal accumulation of autophagic structures [57, 115], leading to neuronal ceroid lipofuscinosis (NCL) and severe neurological symptoms. In humans, deficiency of cathepsin D causes type 10 NCL (CLN10) and that of cathepsin F results in CLN13.

The findings in cathepsin-deficient mice, but also in other NCL models (e.g., CLN2, CLN3, and CLN6 deficient mice) underline the major role of macroautophagy in the pathogenesis of many NCL diseases [26, 153, 229]. A block of autophagic flux is a characteristic feature in several other lysosomal storage disorders, due to either defective autophagosome–lysosome fusion or defective lysosomal degradation, or both. As a consequence, polyubiquitinated protein aggregates and dysfunctional mitochondria accumulate in neurons, which is thought to cause cell death [17]. As an example of this model, an impaired basal macroautophagy associated with severe neurodegeneration has been revealed in the brain of mucolipidosis type II (ML-II) mouse model [116]. ML-II (also called I-cell disease) results from defective intracellular targeting of mannose-6 phosphate tagged lysosomal hydrolases due to a deficiency of the *N*-acetylglucosamine-1-phosphotransferase. Autophagic dysfunction is also evident in mucolipidosis type IV patients and mice [40, 246]. Mutations in the lysosomal cation channel mucolipin 1, the protein mutated in mucolipidosis type IV, led to increased autophagosome formation and decreased autophagosome–lysosome fusion in mucolipidosis IV patient fibroblasts. Another study showed that the impaired autophagic clearance in mucolipidosis type IV fibroblasts was caused at least in part by impaired CMA, which was due to reduced level of LAMP-2A in the lysosomal membrane [245]. However, in mucolipin 1-deficient mouse neurons, autophagic dysfunction was assigned to impaired macroautophagic clearance [40]. These at first glance conflicting data may be due to different roles of macroautophagy and CMA in different cell types.

It is not understood how accumulation of lysosomal cargo or mutations in lysosomal membrane proteins cause the defects in the fusion of lysosomes and autophagosomes, but different mechanisms have been proposed. One possibility is that the defects are at least partly due to a secondary accumulation of cholesterol inside endosomes and lysosomes, which is observed in many LSDs [237]. It is possible that in LSDs with neuronal involvement, this secondary accumulation of cholesterol in endosomal and lysosomal contents and/or membranes contributes to the reduced fusion capacity. How this cholesterol imbalance occurs is not understood, but it is possible that an impaired lysosomal cholesterol export contributes to it. Interestingly, Fraldi and colleagues [64] showed that in two severe neurodegenerative LSDs, SNAREs (soluble *N*-ethyl-maleimide-sensitive factor attachment protein receptors) were abnormally localized in the lysosomal membrane, with a preference for the cholesterol-rich membrane domains. It was proposed that this causes an impaired SNARE sorting and recycling, preventing efficient lysosomal fusion. The role of a defective cholesterol homeostasis is also supported by the observations in Niemann–Pick type C (NPC) disease, in which cholesterol accumulates in endosomes and lysosomes due to mutations in NPC1 or NPC2, proteins that function in lysosomal cholesterol export. An early neuronal autophagic vacuolation is observed in NPC disease, which is connected with neurodegeneration [129]. In agreement with this, a recent study with induced pluripotent stem cells showed that NPC1 function is needed for macroautophagy [140].

One possibility to overcome the problems associated with an impaired autophagic flux in LSDs was revealed in studies analyzing glycogen storage disease type II (Pompe disease), an LSD due to lack of α-glucosidase (acid maltase) activity affecting mainly skeletal muscle and heart. It was shown that increasing the expression of TFEB significantly improved the pathological alterations in this specific LSD. TFEB increased lysosome biogenesis and exocytosis of autophagosomes, which both contributed to the removal of the excessive accumulation of autophagic cargo in cells [68, 216]. Increased expression of TFEB can be anticipated to work in a similar beneficial manner in LSDs with CNS involvement. Interestingly, the effect of two drugs, 2-hydroxypropyl-β-cyclodextrin and genistein, used for therapeutic intervention in mucopolysaccharidoses and NPC disease, was recently shown to depend at least in part on the TFEB-mediated modulation of the expression of lysosomal and autophagic genes [159, 215]. Clearly, more experiments are needed to evaluate, whether this approach is also beneficial in neurological LSDs, and whether the increased appearance of storage material in the extracellular space due to autophagosomal exocytosis is well tolerated in neuronal tissues.

Damaged lysosomes, which may occur in LSDs, may also be a substrate for macroautophagy [92, 139], and this autophagy of lysosomes was shown to be important for the recovery of lysosomal degradation capacity. Therefore, it is possible that lysosomal storage causes trouble from both ends, i.e., both by inhibition of lysosome–autophagosome fusion and by reduced autophagic clearance of storage lysosomes.

Parkinson's disease

Increasing evidence shows that lysosomal and autophagosomal function and the development of Parkinson's disease (PD) are closely linked [137]. Cytosolic α-synuclein accumulation and associated neurotoxicity are major

hallmarks of this disease [218], and mutations within the α-synuclein gene are linked to PD [182]. Both CMA and macroautophagy have been linked to PD pathogenesis [38, 141]. α-Synuclein contains the KFERQ-like motif that acts as a zip code for CMA, and CMA has been reported to participate in the degradation of α-synuclein [249]. Mutated α-synuclein was suggested to inhibit the transport of CMA substrates into lysosomes, possibly by blocking the binding sites in the CMA receptor LAMP-2A [146]. This block can lead to abnormal cytoplasmic accumulation of α-synuclein and other CMA substrates [37]. Neurons expressing mutated α-synuclein also show accumulation of autophagosomes, defective dopamine release and higher susceptibility to autophagic cell death [220]. An increase in macroautophagy accompanied α-synuclein accumulations in neuronal cells, and cell death was reduced in mutant α-synuclein expressing cells when ATG5 was silenced, suggesting that macroautophagy contributed to cell death (Fig. 4d in [251]). The upregulated macroautophagy was proposed to be a consequence of the impaired CMA. However, other studies have shown that macroautophagy is beneficial for cells expressing mutant α-synuclein. Induction of macroautophagy by overexpression of beclin 1 and rapamycin treatment could rescue α-synuclein degradation in neuronal cell lines overexpressing α-synuclein [217]. Lentivirus-mediated overexpression of beclin 1 also decreased the PD pathology in α-synuclein transgenic mice. These results suggest that macroautophagy also contributes to degradation of α-synuclein, and that induction of macroautophagy may be a potential treatment for diseases associated with α-synuclein accumulation. In agreement with this proposition, increased expression of wild-type α-synuclein negatively influences the rate of macroautophagy in cell lines and mice. This effect is mediated by impaired activity of RAB1a and mislocalization of the autophagy protein ATG9, which are needed for the formation of autophagosomes [248].

CMA may also be involved in PD caused by mutations in other proteins. Leucine-rich repeat kinase 2 (LRRK2), the mutations of which cause familial PD, was recently shown to be a CMA substrate [174]. Mutant LRRK2 was observed to interfere the function of the CMA translocation complex on the lysosomal membrane. Thus, mutant LRRK2 may inhibit the degradation of CMA substrates including α-synuclein. Similar observations were published for another PD-associated CMA substrate protein, the ubiquitin C-terminal hydrolase L1 [105].

Neurons are heavily dependent on mitochondrial ATP production. Dysfunctional mitochondria have been reported to accumulate in the brains of PD patients, and mitochondrial defects, implicated as reduced bioenergetic capacity, oxidative stress and reduced stress resistance, are observed in several PD models [29]. In agreement with these observations, mutations in mitophagy and mitochondrial quality control genes PARK2, PINK1 and DJ-1 have been associated with autosomal recessive PD [29, 50, 188].

Mutations that lead to impaired lysosomal degradation capacity have also been linked to synucleinopathies. Mutations in the vesicular P type ATPase ATP13A2 are associated with autosomal recessive familial PD. Disease-causing mutations impair lysosomal acidification and proteolytic activity, thereby also decreasing autophagic flux and clearance of α-synuclein aggregates [42]. Mutations within the gene encoding the lysosomal sphingolipid degrading hydrolase β-glucocerebrosidase influence lysosomal protein degradation and clearance of α-synuclein [39, 201] and are one of the genetic risk factors of synucleinopathies including PD and Lewy body disease. Overexpression of β-glucocerebrosidase in the central nervous system of mice expressing mutant α-synuclein decreased the levels of α-synuclein, suggesting that this enzyme may be linked to the clearance of α-synuclein. A recent study showed that parkin/PARK2 and β-glucocerebrosidase interact with each other, and that parkin mediates ubiquitination of mutant β-glucocerebrosidase, which facilitates its degradation in proteasomes. Further, mutant β-glucocerebrosidase competes with other substrates of parkin such as PARIS and ARTS proteins, whose accumulation triggers apoptosis [15].

Conclusions and open questions

The importance of macroautophagy as a central degradative pathway in nutrient homeostasis, clearance of damaged organelles and aggregate-prone substrates in postmitotic cells like neurons is highlighted by mice with neuron-specific autophagy deficiencies. An impaired autophagic function has also been reported in many neurodegenerative diseases. There is also increasing evidence showing that CMA dysfunction contributes to neurodegeneration. However, even though major advances have been made to describe functions and dysfunctions of macroautophagy and CMA in neurodegenerative disorders, we are far from understanding their exact roles in different diseases. In a disease context it is not always clear whether autophagy is beneficial or detrimental in an affected neuron. Thus, it is not always certain whether an induction or inhibition of autophagy would serve as a therapeutic option. The conflicting results in many of the initial studies applying autophagy modulating agents underline this lack of knowledge and call for more detailed and thorough investigation of the roles of autophagy in neurons.

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