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Cargo Recognition and Degradation by Selective Autophagy

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Abstract

Macroautophagy, initially described as a non-selective nutrient recycling process, is essential for the removal of multiple cellular components. In the past three decades, selective autophagy has been characterized as a highly regulated and specific degradation pathway for removal of unwanted cytosolic components and damaged and/or superfluous organelles. Here, we discuss different types of selective autophagy, emphasizing the role of ligand receptors and scaffold proteins in providing cargo specificity, and highlight unanswered questions in the field.

Keywords

mitophagy; receptor; stress; vacuole; yeast

Selective autophagy overview

Autophagy is a highly conserved pathway in eukaryotes involving the cellular recycling of multiple cytoplasmic components during standard physiological conditions and in response to different types of stress including starvation. Macroautophagy (hereafter autophagy) can be either non-selective or selective and involves the sequestration of cytoplasm within double-membrane vesicles termed autophagosomes. Upon maturation, autophagosomes fuse with the vacuole (in yeast and plants) or endosomes and lysosomes (in metazoans) leading to degradation of their cargo by resident hydrolases[1, 2]. Cargo degradation produces molecular building blocks such as amino acids, which are subsequently recycled back into the cytoplasm for reuse[1, 3]. Whereas non-selective autophagy, a cellular response to nutrient deprivation, typically involves random uptake of cytoplasm into phagophores, the precursors to autophagosomes, selective autophagy is responsible for specifically removing certain components such as protein aggregates and damaged or superfluous organelles[4]. Different studies have reported the selective autophagic degradation of several organelles including mitochondria[5], peroxisomes[6], lysosomes[7], endoplasmic reticulum (ER) and the nucleus[8] under various conditions. Furthermore, autophagy selectively degrades aggregation-prone misfolded proteins and protein microaggregates implicated in the pathology of various neurodegenerative diseases[9]. In this review, we address the principal mechanisms of

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selective autophagy in yeast and mammals, with an emphasis on mitophagy, which is to date the best described type of selective autophagy.

Cytoplasm-to-vacuole targeting (Cvt) pathway

The Cvt pathway is a biosynthetic autophagy-related process specific to yeast, in which vacuolar enzymes are transported from the cytoplasm into the vacuole utilizing the autophagic machinery. Among the enzymes that utilize the Cvt pathway are Ape1, Ape4 and Ams1[10]. Ape1 is first synthesized in the cytoplasm as an inactive proenzyme (prApe1). Following oligomerization, prApe1 is selectively recognized by the non-core autophagy-related (Atg) protein Atg19 that functions as a receptor for Ams1, prApe1 and Ape4[11, 12]. Once the prApe1-Atg19 or Cvt complex is formed, Atg19 binds to the scaffold protein Atg11, which in turn recruits the Cvt complex to the perivacuolar location termed the phagophore-assembly site (PAS) where autophagosomes and Cvt vesicles are formed in yeasts[13, 14]; interaction of Atg19 with Atg11 is facilitated by Hrr25-dependent phosphorylation of the receptor[15]. After reaching the PAS, Atg19 interacts with the ubiquitin-like protein Atg8[13]. During autophagy and the Cvt pathway, Atg8 is covalently conjugated through its C terminus to phosphatidylethanolamine (PE); thus, Atg8−PE is present on both the inner and outer membrane of forming autophagosomes[16] (Fig. 1a). Atg8 has been implicated in phagophore expansion and autophagosome size regulation[17]. Thus, Atg19 binding to Atg8 tethers the Cvt complex to the Atg8−PE-conjugated sequestering vesicles. Once fully matured, Cvt vesicles fuse with the vacuole and deliver prApe1, which is then processed into its active form by resident hydrolases.

Using the Cvt pathway as a model for selective autophagy we can propose that although the core autophagy machinery directs phagophore membrane expansion and vesicle formation, cargo selectivity is achieved by a ligand receptor and a scaffold protein, roles taken by Atg19 and Atg11, respectively, in the Cvt pathway. Atg19 has a paralog, Atg34 (also phosphorylated by Hrr25), which functions as an Ams1 receptor during nitrogen starvation[18]. Other types of selective autophagy in yeast, such as mitophagy[19] and pexophagy[20, 21], also rely on Atg11 as a scaffold for cargo delivery to the PAS. However, a counterpart to Atg11 has yet to be discovered in mammals. Similarly, most types of selective autophagy require the binding of the cargo receptor to the core autophagy machinery. In the Cvt pathway, this process is illustrated by Atg19 binding to Atg8 through a specific WXXL motif found on the Atg19 C terminus, similar to that seen in SQSTM1/p62[22, 23]. This interaction is evolutionarily conserved as several proteins in yeasts and more complex eukaryotes contain Atg8-interacting motifs (AIM) or LC3-interacting regions (LIRs), respectively. The AIM or LIR provide selective binding to yeast Atg8 or one of the members of the LC3/GABARAP family of Atg8 mammalian homologs[24]. Recently, a specific type of LIR called GABARAP-interacting motif (GIM) has been proposed, showing enhanced specificity to GABARAP versus LC3 family members[25]. Multiple examples of scaffold and receptor proteins will be showcased as we discuss different types of selective autophagy (Table 1).

Aggrephagy

The selective degradation of protein aggregates by autophagy is known as aggrephagy. Multiple aggregation-prone proteins such as amyloid-β[26], HTT (huntingtin)[27] and SNCA/α-synuclein[28] are autophagy substrates. In yeast, Cue5 is a cargo receptor for the clearance of aggregation-prone poly-glutamine (polyQ)-containing proteins. Cue5 possesses a ubiquitin-binding CUE domain and an AIM, mediating the interaction between the ubiquitinated cargo and Atg8[29]. Overexpression of TOLLIP, a Cue5 human homolog that also has a CUE domain, leads to the degradation of polyQ protein aggregates in human cell lines[30] (Fig. 1b). Ubiquitination of substrates has been demonstrated as a key mediator in the recognition and degradation of these proteins by selective autophagy[31]. At least three additional mammalian cargo receptors, SQSTM1[16, 23], NBR1[32] and OPTN[33], act as ubiquitin binding proteins that mediate the interaction between ubiquitinated proteins and the core autophagy machinery. All three receptors possess LIRs and ubiquitin-binding domains, thus working as a bridge between the LC3/GABARAP family members and the ubiquitinated substrates[16, 23–29, 31–34].

The nucleocytoplasmic shuttling protein WDFY3/ALFY has been proposed as a scaffold in aggrephagy[35]. While unable to directly interact with ubiquitinated substrates, WDFY3 binds the core autophagy protein ATG5, the cargo receptor SQSTM1[36], GABARAP subfamily members[37], and phosphatidylinositol-3-phosphate[38], a prominent lipid in the regulation of autophagosome membrane formation. WDFY3 depletion hinders the clearance of aggregated polyQ proteins. The latter observation, in conjunction with its high number of interacting partners, suggests that WDFY3 is an important scaffold protein in the SQSTM1-dependent degradation of ubiquitinated aggregates by selective autophagy.

Ubiquitination plays an important role not only in substrate recognition and degradation by the ubiquitin-proteasome system (UPS), but also by selective autophagy, raising a set of questions regarding the hierarchy between these two degradation pathways. It has been proposed that protein aggregates that cannot be degraded by the UPS (e.g., due to size) may be cleared by autophagy[39, 40]. At the same time, the Lys residues used for linkage, as well as the length and the nature of the ubiquitin chains, have been proposed as a mechanism to select which degradation pathway is chosen[39]. However, a recent paper by Lu *et al.* emphasizes the role of receptor oligomerization over the type of ubiquitination in selecting a degradation pathway[41]. This finding agrees with data showing the importance of Cue5 and SQSTM1 oligomerization in their association with the phagophore[39, 42]. Thus, both autophagy and the UPS provide dynamic alternatives to different cellular challenges.

Pexophagy

Pexophagy is the selective removal of peroxisomes. Pexophagy has been mostly studied as a pathway for the removal of superfluous organelles in various fungi[43]. Incubating these fungi in oleic acid or methanol leads to peroxisome proliferation; following a shift to a preferred carbon source such as glucose, the excess peroxisomes are rapidly degraded through pexophagy[43]. Similar to other types of selective autophagy, cargo selectivity is provided by receptor proteins; in yeast this role is taken by PpAtg30 in *Pichia pastoris*[20] and Atg36[44] in

S. cerevisiae. Both Atg36 and PpAtg30 tether peroxisomes targeted for degradation to nascent phagophore membranes by linking Atg8 to peroxisomal membrane proteins, with Atg36 binding Pex3, and PpAtg30 binding both PpPex3 and PpPex14[20, 21]. Phosphorylatable variants of the classical AIMs have been reported for both Atg36 and PpAtg30; however, disruption of these AIMs only delays pexophagy rather than abrogating it[45]. As previously mentioned, Atg11 is required for pexophagy[46]. PpAtg37 is an integral peroxisomal membrane protein specifically required for pexophagy in *P. pastoris*. During pexophagy, PpAtg37 is necessary for phagophore formation, as PpAtg37 null cells fail to recruit PpAtg11 to peroxisomes[47].

In contrast to yeast, no pexophagy-specific cargo receptor has been described in mammals. Rather, mammalian pexophagy relies on the ubiquitination of peroxisomal proteins and their recognition by SQSTM1[31] and NBR1[48]. Initially, it was reported that PEX3 overexpression leads to peroxisome ubiquitination and pexophagy induction[49]. However, PEX3 ubiquitination does not prevent pexophagy and this study did not determine the specific peroxisomal proteins targeted for ubiquitination. Subsequently, two studies identified PEX5 mono-ubiquitination as the cargo signal for peroxisome degradation[50, 51]. PEX5 is a cytosolic protein that shuttles between the peroxisomal membrane and the cytosol in a ubiquitin-dependent manner[52]. The accumulation of mono-ubiquitinated PEX5 in the peroxisomal membrane, which was unable to shuttle back to the cytosol, triggers pexophagy[50]. Furthermore, in response to reactive oxygen species (ROS), PEX5 is phosphorylated and subsequently mono-ubiquitinated, which leads to pexophagy induction in a SQSTM1-dependent manner[51]. A recent study has indicated that the peroxisomal E3-ubiquitin ligase PEX2 is responsible for PEX5 ubiquitination[53]. These data suggest a model in which mammalian pexophagy is dependent on the membrane accumulation of ubiquitinated peroxisomal proteins such as PEX5 that are recognized by the ubiquitin-binding receptors SQSTM1 and NBR1, and which in turn link the target peroxisomes to LC3/GABARAP-bound sequestration membranes (Fig. 1c). However, this simple model fails to answer several questions. From a mechanistic perspective, how does PEX5 ubiquitination at a specific site determine whether the protein shuttles into the peroxisome or is directed to proteasomal degradation? Are there distinct mechanisms involving ROS and amino acid starvation-induced pexophagy? Regarding this last point, other studies have reported that the peroxisomal membrane protein PEX14, which acts as a docking factor for PEX5, can directly interact with LC3-II under starvation conditions, outcompeting PEX5[54]. This opens the possibility of different pathways being involved under different pexophagy-inducing stimuli. Finally, the human Atg37 ortholog ACBD5 has also been reported as an essential pexophagy factor[47]. It will be interesting to determine the role of ACBD5 in pexophagy and its connection to possible undiscovered mammalian pexophagy receptors.

Mitophagy

Mitophagy is a critical quality control process that eliminates damaged and/or superfluous mitochondria through their selective autophagic degradation[55, 56]. Deficiencies in mitophagy have been linked to the development of several pathologies, including neurodegenerative disorders[57] such as Parkinson disease (PD).

Mitochondria have multiple metabolic functions and also influence cell fate by regulating apoptosis. Consequently, mitochondrial damage leads to loss of metabolic homeostasis. Additionally, disruption of oxidative phosphorylation (OxPhos) in damaged mitochondria leads to excessive ROS generation[58]. Mitochondria are high-maintenance organelles, and non-functioning/superfluous mitochondria become an energetic burden. Therefore, the regulation of mitochondrial quality and quantity is of paramount importance. Although mitochondria harbour some internal quality control machinery[59], the major contribution towards maintaining mitochondrial integrity comes from mitophagy, which functions in concert with the UPS to ensure mitochondrial homeostasis[60].

In fungi, mitophagy can be triggered by nitrogen starvation[61–63] or post-log phase growth in a non-fermentable medium. In yeast, selectivity is provided by the outer mitochondrial membrane (OMM) receptor Atg32[62], which links targeted mitochondria to the autophagic machinery[19, 64]. The cytosolic N terminus of Atg32 interacts with Atg11[65]. Ectopic targeting of the Atg32 N terminus to peroxisomes leads to pexophagy, underscoring the function and sufficiency of Atg32 as an autophagy receptor[66]. The C terminus of Atg32 faces the intermembrane space, and its proteolytic processing by Yme1 may be required for efficient mitophagy[56]. The interaction between Atg32 and Atg11 promotes the recruitment of mitochondria to the PAS for sequestration. Atg32 also orchestrates the subsequent expansion of the phagophore around the mitochondria through its interaction with Atg8 via the AIM in its cytosolic domain[55, 66]. However, mutating the Atg32 AIM causes only a partial mitophagy defect, suggesting that the Atg32-Atg8 interaction increases mitophagy efficiency, but remains auxiliary[62, 66, 67].

The expression of Atg32 can be influenced by oxidative stress and nutritional status. In *P. pastoris*, the Ume6-Sin3-Rpd3 complex, positively regulated by TOR, suppresses *ATG32* transcription[61]. During starvation, TOR is inactivated, promoting the synthesis of Atg32 and starvation-induced mitophagy. However, the upregulation of Atg32 expression is not by itself sufficient to induce mitophagy. Atg32 is activated by phosphorylation at residues Ser114 and Ser119 in its cytosolic domain, facilitating its interaction with Atg11[67]. Casein kinase 2 (CK2) has been proposed as the Atg32 Ser114 kinase[68] as CK2 phosphorylates Atg32 *in vitro* but fails to phosphorylate $Atg32^{S114A}$. Similarly, CK2 temperature-sensitive mutants fail to phosphorylate Atg32[68]. However, CK2 is a multitasking kinase and its activation is independent of mitophagy-inducing stimuli[62]. Therefore, other signaling pathways may contribute to the temporal selectivity of CK2-mediated phosphorylation of Atg32. Two mitogen-activated protein kinase (MAPK) pathways have been implicated in mitophagy regulation in yeast[69]. Hog1 is a MAPK in the Ssk1-Pbs2 pathway and Atg32 phosphorylation is suppressed in *hog1*Δ cells. However, Hog1 does not phosphorylate Atg32 *in vitro*, suggesting an indirect regulation[67]. The Slt2 pathway plays a role in mitochondrial recruitment to the PAS[69]. Although further investigation is required to identify the signaling circuit regulating Atg32 phosphorylation, cooperative expression and activation of Atg32 highlights the multiple levels of regulation involved in mitophagy induction.

Because the dimensions of intact mitochondria are larger than that of autophagosomes, sequestration of damaged mitochondria might be facilitated by mitochondrial fission[62, 65]. In *S. cerevisiae*, mitochondrial fission is mediated by several factors including Dnm1 and

Fis1[65]. Deletion of either *DNM1* or *FIS1* significantly suppresses mitophagy[63, 70]. Dnm1 interacts with Atg11, allowing the former to be recruited to mitochondria targeted for degradation[63]. The proteins associated with the ER-mitochondrial encounter structure (ERMES) may play a role in modulating mitochondrial fission during mitophagy[65]. Nevertheless, the exact mechanism of mitophagy-associated mitochondrial fission is unclear, and yet unidentified fission factors may be involved.

Mitophagy in mammals is mechanistically more complex than in yeast and is induced by cellular and developmental cues. In mammalian cells the loss of mitochondrial membrane potential is a potent inducer of mitophagy[5, 71]. However, while the use of chemicals that target the electron transport chain or act as protonophores is a convenient and efficient way to study mitophagy, the acute dissipation of mitochondrial membrane potential precludes the study of subtle regulatory phenomenon[72]. Furthermore, such severe mitochondrial damage might not be representative of the true pathophysiological triggers.

In mammals, mitophagy plays important physiological roles in development and cellular differentiation. Erythrocyte development requires the selective degradation of mitochondria in reticulocytes[73] and embryonic development in some organisms involves selective degradation of paternal mitochondria in the zygote[74]. Hypoxia, which disrupts mitochondrial respiration, is another stimulus that promotes mitophagy in mammalian cells[55].

The PINK1-PRKN/PARK2/parkin pathway is the most extensively characterized mechanism effecting mitochondrial quality control in most mammalian cells. PINK1 is a Ser/Thr kinase with a C-terminal kinase domain and N-terminal mitochondrial targeting sequence[55], and PRKN/PARK2 is an E3-ubiquitin ligase[75]. Loss of mitochondrial integrity is usually accompanied by mitochondrial depolarization. PINK1, which requires the mitochondrial membrane potential for its inner mitochondrial membrane (IMM) import, acts as a depolarization sensor[76]. In healthy mitochondria, PINK1 is imported into the matrix where it is cleaved by proteases and subsequently released back into the cytosol for degradation through the N-end rule pathway[77, 78]. In compromised mitochondria, the loss of membrane potential prevents translocation, and PINK1 is stabilized on the OMM, leading to its activation by autophosphorylation[72, 76, 79]. Active PINK1 phosphorylates several substrates including ubiquitin, MFN1 (mitofusin 1), MFN2 and PRKN/PARK2[76, 80]. Unphosphorylated PRKN/PARK2 is autoinhibited[76, 80]; PINK1-mediated phosphorylation of PRKN/PARK2[76] leads to activation. PINK1 also phosphorylates available ubiquitin attached to OMM proteins at Ser65 generating phospho-ubiquitin[81, 82], which acts as a PRKN/PARK2 substrate[77]. PRKN/PARK2 subsequently links phospho-ubiquitin chains to OMM proteins, which possibly results in a feed-forward amplification loop recruiting more PRKN/PARK2[76]. The phosphorylation of MFN2 by PINK1 might also play a role in PRKN/PARK2 recruitment[83], possibly acting along with phospho-ubiquitin at the OMM. However, the role of MFN2 in PRKN/PARK2 recruitment is controversial[84].

The classic model for mitophagy involves the recognition of polyubiquitinated mitochondria by autophagy receptors SQSTM1 and OPTN which bind LC3[76, 85]. This interaction tethers damaged mitochondria to the expanding phagophore and promotes their subsequent

sequestration within autophagosomes (Fig. 2a). Recent progress in the field suggests a complementary model whereby PINK1-mediated phosphorylation of ubiquitin, independent of PRKN/PARK2 activity, is sufficient to recruit the autophagy receptors CALCOCO2/NDP52 and OPTN and induce low-amplitude mitophagy[86]. In this model CALCOCO2 and OPTN can successfully recruit ULK1 and facilitate mitophagy initiation upstream of LC3 binding[86]. The importance of PRKN/PARK2-mediated ubiquitination is indicated by the fact that overexpression of the mitochondrial deubiquitinase USP30 inhibits mitophagy by promoting deubiquitination of PRKN/PARK2 substrates[87].

Polyubiquitination also acts as a signal that promotes VCP/p97-mediated extraction of OMM proteins and their subsequent proteasomal degradation[88], causing disruption of the OMM[88]. Recent findings suggest that OMM disintegration serves to expose the IMM protein PHB2 (prohibitin 2), which possesses a LIR and functions as a mitophagy receptor[74]. PHB2 promotes mitophagy in a PINK1-PRKN/PARK2-dependent manner, and the selective removal of paternal mitochondria in *C. elegans* embryos requires PHB2 function[74]. The PINK1-PRKN/PARK2-dependent generation of mitochondria-derived vesicles (MDVs)[89, 90] is an alternative pathway to conventional PRKN/PARK2-dependent mitophagy. Limited and localized mitochondrial damage promotes MDV formation to ensure the selective removal of damaged portions of a mitochondrion instead of the entire organelle[91]. It is possible that the PINK1-PRKN/PARK2 pathway switches between MDV formation and mitophagy depending on the extent of mitochondrial damage[55].

PINK1 and PRKN/PARK2 are also involved in regulating the arrest of mitochondrial motility following mitochondrial damage[92]. Mitochondria are transported by the kinesin KIF5 on microtubules. KIF5 binds mitochondria through the adaptor TRAK1-TRAK2 and the OMM protein RHOT1/Miro1[93]. Following mitochondrial damage, RHOT1 is one of the earliest proteins to be degraded via PRKN/PARK2-mediated ubiquitination[94], a process which also requires the interaction of RHOT1 with the LRRK2 kinase[95]. The removal of RHOT1 halts mitochondrial motility and quarantines damaged mitochondria for degradation[95]. In cells that harbour mutations in PINK1, PRKN/PARK2 or LRRK2, RHOT1 degradation is inhibited, leading to continued motility of damaged mitochondria and delayed mitophagy[96].

Not all mammalian cell types express PARK2/PRKN and several mitochondria-localized mitophagy receptors exist in mammalian cells. BNIP3L/Nix is one such mitophagy receptor and is involved in the selective elimination of mitochondria during the differentiation of reticulocytes into erythrocytes[73, 91]. BNIP3L localizes to the OMM and contains a LIR near its cytosolic N-terminus[94], the activity of which may be regulated by phosphorylation[25]. However, mutations in the BNIP3L LIR only lead to a partial loss in mitophagy[96]. Another short motif has recently been reported to be indispensable for BNIP3L function[97]. Although the exact mechanism by which BNIP3L mediates mitophagy remains unknown, reports suggest that BNIP3L may promote mitochondrial depolarization[79] leading to PINK1-PRKN/PARK2 recruitment to mitochondria, and activating mitophagy[79]. BNIP3L might also work in concert with the related protein BNIP3[98], which possesses a LIR[99].

BNIP3L is also involved in hypoxia-induced mitophagy[99], as is the LIR-containing OMM protein FUNDC1[100]. Mutations in the FUNDC1 LIR lead to loss of function[101]. Similar to Atg32, FUNDC1 is regulated by reversible phosphorylation. Under normal conditions, FUNDC1 is phosphorylated by SRC kinase and CK2[102], including the modification of one site in its LIR. Hypoxia promotes the dephosphorylation of these residues involving the phosphatase PGAM5[102]. Hypoxia-induced mitophagy is particularly relevant to the pathobiology of tumors, and elucidating the role of BNIP3L and FUNDC1 in these contexts might be an important step towards therapeutic intervention[103, 104].

In mammals, mitochondrial dynamics are regulated by the fission-promoting GTPase DNM1L/Drp1 and the profusion factors MFN1-MFN2 and OPA1[101, 105]. Mitophagy induction is accompanied by a decrease in mitochondrial fusion and an increase in mitochondrial fission to facilitate degradation of damaged mitochondria[98]. PINK1 activation promotes PRKN/PARK2-mediated degradation of MFN1-MFN2, consistent with the idea of reduced fusion[80]. The mitophagy receptor FUNDC1 is also involved in regulating mitochondrial dynamics during mitophagy. Whereas FUNDC1 binds to and recruits OPA1 to mitochondria under normal conditions, upon mitochondrial damage it preferentially recruits DNM1L, promoting fission[106]. Like ERMES in yeast, mitochondria-associated membranes are sites of ER-mitochondria contact in mammals, and have also been proposed to modulate mitophagy-related mitochondrial fission[107], although the mechanism remains unclear.

Whereas most selective autophagy receptors are proteins, recent evidence suggests that mitophagy may also be orchestrated by lipid receptors[107]. Cardiolipin, a lipid unique to the mitochondria, may act as a mitophagy receptor in mammalian cortical neurons. Rotenone-induced mitochondrial damage causes a dramatic translocation of cardiolipin from the inner to the outer mitochondrial membrane[108], where it interacts with the LC3 N terminus. Inhibition of cardiolipin synthesis or translocation reduces the efficiency of mitophagy in these neurons[108]. Cardiolipin was also recently reported to modulate mitophagy in *S. cerevisiae*[33], and ceramide has also been implicated as a mitophagy receptor in certain cancer cell lines[109].

Reticulophagy

Reticulophagy describes the degradation of the ER by selective autophagy. Perturbation of ER function results in the accumulation of misfolded proteins and ER stress, which in turn triggers the unfolded protein response (UPR) and ER-associated degradation, in order to recover cellular homeostasis[109]. Autophagy is also activated by ER stress[110] as a means to control ER size and to counterbalance the ER expansion after the UPR[111, 112]. Other stimuli such as rapamycin treatment and nutrient starvation also activate reticulophagy[113, 114]. Similar to other selective autophagy pathways, cargo receptors have been described for selective ER degradation. In yeast, starvation-induced reticulophagy depends on Atg39 and Atg40, predicted transmembrane proteins that localize to the perinuclear and cytoplasmic ER, respectively. Consistent with their role as cargo receptors, Atg39 and Atg40 contain AIMs, and interact with both Atg8 and Atg11[114]. In mammals, RETREG1/FAM134B is a reticulophagy cargo receptor protein, as well as an Atg40 functional homolog[113]. Similar to

Atg40, RETREG1 localizes to the cytoplasmic ER and interacts with LC3 and GABARAP family members through its LIR (Fig. 2b). Consistent with the reported role of reticulophagy in controlling ER size, RETREG1 overexpression increases ER fragmentation, whereas silencing of this protein results in ER expansion.

Nucleophagy

Nucleophagy has been described as the partial or bulk degradation of the nucleus by the vacuole/lysosome. Nucleophagy is closely related to reticulophagy, given that Atg39 localizes to, and mediates the degradation of, the perinuclear ER and nuclear envelope in yeast[113]. However, to date no Atg39 functional homolog has been described in mammals and it is still unclear how nucleophagy occurs in more complex eukaryotes. However, some studies have suggested selective autophagic degradation of chromatin[115] and the nuclear lamina[115] could play a role in preventing tumorigenesis.

Other types of nucleophagy termed piecemeal microautophagy of the nucleus (PMN) or micronucleophagy[116], and late nucleophagy[117] have been described in *S. cerevisiae*. During PMN the outer nuclear envelope protein Nvj1 interacts with the vacuolar membrane protein Vac8, forming nuclear-vacuolar junctions that pinch off parts of the nucleus, which are later engulfed and degraded by the vacuole[117] (Fig. 2c). PMN is activated soon after nutrient starvation and depends on the core autophagic machinery[118]. In contrast, late nucleophagy occurs after prolonged starvation and is independent of Nvj1, Vac8 and some but not all core autophagy machinery[117]. Further studies will be required to understand the individual roles of Atg39-induced nucleophagy, PMN and late nucleophagy during nitrogen starvation.

Lysophagy

Lysophagy is the selective degradation of damaged lysosomes by autophagy. Leakage of lysosomal enzymes into the cytosol due to lysosomal membrane rupture leads to lysosomal cell death[117]. Therefore, the removal of damaged lysosomes is necessary to maintain cellular homeostasis. LGALS3 (galectin 3) binds to glycoproteins exposed upon lysosomal membrane damage and colocalizes with LC3, working as a key lysophagy marker[119]. Even though the specific mechanisms behind lysophagy are yet to be discovered, two independent reports have suggested a model in which damaged lysosomes are selectively degraded in a ubiquitin-SQSTM1-LC3-dependent manner[7, 119] (Fig. 3a). Thus, lysosome degradation appears analogous to other types of organelle-selective autophagy such as mitophagy and pexophagy. Still, many questions regarding the specific ubiquitination targets and their regulation remain. specific physiological conditions in which lysophagy is triggered will need to be determined.

Xenophagy

Xenophagy is the collective term used for the selective autophagic degradation of intracellular pathogens including viruses, bacteria and fungi, which constitutes an important part of the immune response[120, 121]. Once again, ubiquitination and cargo receptor binding play an important role in xenophagy. Following *Salmonella typhimurium* infection and

release into the cytosol, bacterial proteins are rapidly ubiquitinated and recognized by the cargo receptors SQSTM1[122], CALCOCO2[123] and OPTN[123]. CALCOCO2 binding to invading bacteria depends on lectin LGALS8 recruitment to damaged bacteria-containing vesicles[124]. All three receptors possess ubiquitin binding domains and LIRs, thus mediating the interaction between the ubiquitinated bacteria and LC3/GABARAP family members for phagophore sequestration[122, 123, 125] (Fig. 3b). Wild *et al.* showed that these three cargo receptors can bind to the same bacterium[125]. However, individual silencing of SQSTM1, CALCOCO2 or OPTN is sufficient to increase *S. typhimurium* replication[125]. This finding suggests that all three cargo receptors have individual roles in xenophagy that cannot be compensated by the other two. Although probably linked to their individual abilities to recruit other autophagy-inducing factors, further studies will be necessary to determine the specific contributions of each cargo receptor. Additionally, finding the specific pathogen proteins that are ubiquitinated will prove indispensable to therapeutically counter the strategies that pathogens have evolved to avoid autophagy.

Lipophagy

Initially discovered in hepatocytes and later in other cell types, lipophagy describes the selective degradation of lipid droplets (LD) by autophagy. *In vivo* and *in vitro* experiments have shown that lipophagy occurs during basal and starvation conditions regulating cellular triglyceride content[126]. Chaperone-mediated autophagy (CMA) has been proposed as a regulator of lipophagy. In this model, CMA would degrade the LD-associated PLIN (perilipin) proteins leading to lipophagy activation[127]. Although specific receptors for lipophagy have not been found, the metabolic implications associated with this process have highlighted important insights into energy utilization and possible therapeutic strategies for high-fat diet-induced pathologies. In *S. cerevisiae* lipid droplets are degraded in a process termed microlipophagy that depends on the core autophagy machinery, but not Atg11[128].

Ferritinophagy

Ferritinophagy involves the degradation of the iron-sequestering protein ferritin[129]. Iron is an essential component of various enzymes and proteins, making it indispensable for several cellular processes. However, free iron promotes ROS generation and is detrimental to the cell[130]. Ferritin, consisting of multiple heavy chain (FTH1) and light chain (FTL) subunits, acts as a sink for iron when cellular iron levels are high. Conversely, when bioavailable iron levels are low, ferritin is mobilized by ferritinophagy to release iron[129].

Ferritinophagy was initially identified in *atg5*$^{-/-}$ MEF cells, which fail to degrade ferritin upon iron depletion[131]. Selectivity during ferritinophagy is mediated by the receptor NCOA4 which specifically binds FTH1 and marks ferritin as an autophagic cargo[132, 133]. The level of NCOA4 is kept low in iron-replete conditions by the iron-dependent interaction between the HECT E3 ligase HERC2 and NCOA4, followed by the ubiquitination and proteasomal degradation of NCOA4[132]. In response to iron depletion NCOA4 is stabilized, allowing ferritin to be selectively degraded. NCOA4 does not contain a conventional LIR motif in contrast to other autophagy receptors[129]. Therefore, how NCOA4 links its cargo to phagophores promises to be an intriguing question for the field.

Glycophagy

Glycophagy refers to the selective autophagy-mediated degradation of glycogen, the storage form of glucose in animal cells, by acid α-glucosidase within the lysosome[134]. Glycophagy is distinct from cytosolic glycogen breakdown via glycogen phosphorylase, and these pathways likely have complementary roles in glycogen catabolism because they preferentially act on slightly different glycogen substrates[135]. The putative receptor for glycophagy is STBD1 (starch binding domain 1), which possesses a CBN20 glycan-binding domain[136] as well as a LIR[135]. STBD1 localizes to glycogen particles and binds GABARAPL1[134] but not LC3B[134]. Current evidence indicates an important role for glycophagy in cardiac and hepatic pathophysiology, and further mechanistic investigation of this process will be crucial for realizing the full scope of this pathway in carbohydrate metabolism.

Conclusion

Whereas selective autophagy occurs in different forms corresponding to various targets, there is a unifying principle: a receptor, which binds the cargo or which may be an integral part of the cargo (for example as observed with Atg32), links the cargo to the autophagy machinery. Recent years have shown tremendous progress in understanding the mechanisms behind each of these selective processes resulting in a wealth of knowledge on how distinct subsets of the cell's autophagic machinery are employed to eliminate different cellular components and organelles. As we have highlighted here, there are still important unanswered questions, including the mechanism of mitophagy under physiological (as opposed to experimental) conditions, the post-translational and structural modifications that occur to temporally control receptor-ligand interactions, and the regulatory pathways that integrate stress and developmental signals to coordinate the mode of selective autophagy with the precise cellular needs. Exploring these queries will further our understanding of selective autophagy and may provide important clues for therapeutic strategies.

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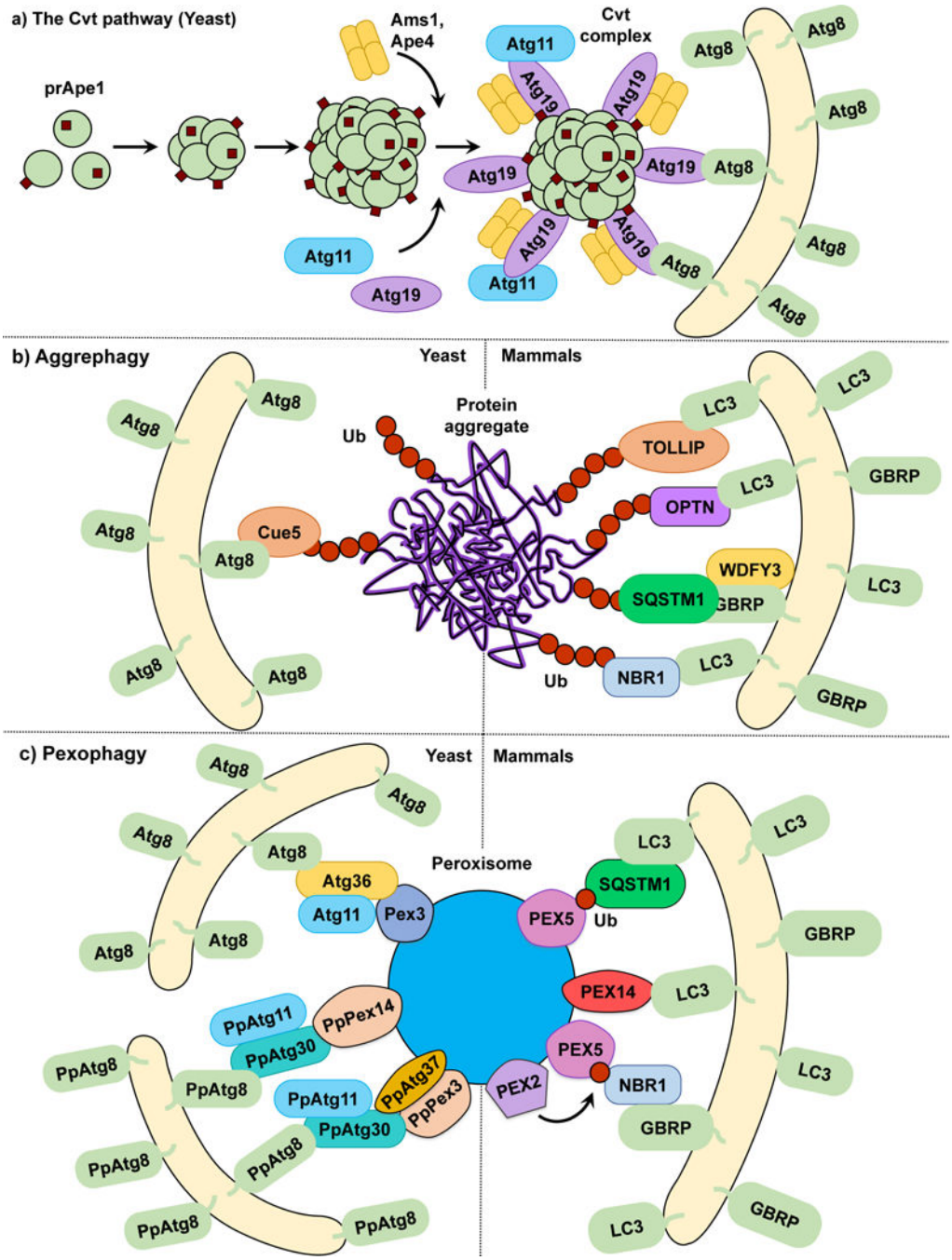

Figure 1.

The Cvt pathway, aggrephagy and pexophagy. (**a**) In the yeast Cvt pathway prApe1, Ape4 and Ams1 are synthesized in the cytoplasm. prApe1 oligomerizes into dodecamers and subsequently higher order structures that are recognized by the receptor Atg19, which in turn binds the scaffold protein Atg11 forming the Cvt Complex. Ams1 and Apr4 also oligomerize and bind Atg19. Atg11 brings the Cvt Complex to the PAS where Atg19 binds Atg8−PE, tethering the Cvt complex to the phagophore. (**b**) In both yeast and mammalian aggrephagy, protein aggregates are ubiquitinated and subsequently recognized by cargo

receptors. In yeast, Cue5 links the ubiquitinated aggregates to Atg8−PE. During mammalian aggrephagy, TOLLIP, SQSTM1, NBR1 and OPTN tether the ubiquitinated aggregates to the phagophore by binding LC3/GABARAP family members. WDFY3 has been described as a scaffold for SQSTM1-dependent degradation. (**c**) In *S. cerevisiae* pexophagy, Atg36 functions as a receptor linking peroxisomes to the phagophore by binding Pex3 and Atg8−PE. In *P. pastoris* pexophagy, PpAtg30 acts as a receptor by linking PpPex3 and PpPex14 to PpAtg8−PE. Atg11 functions as a scaffold for both *S. cerevisiae* and *P. pastoris*. The current model of mammalian pexophagy involves the E3-ubiquitin ligase PEX2-mediated mono-ubiquitination of PEX5, which in turn is recognized by receptors SQSTM1 and NBR1, tethering peroxisomes to the phagophore. PEX14 has also been reported to link peroxisomes to the phagophore by directly binding LC3 family members.

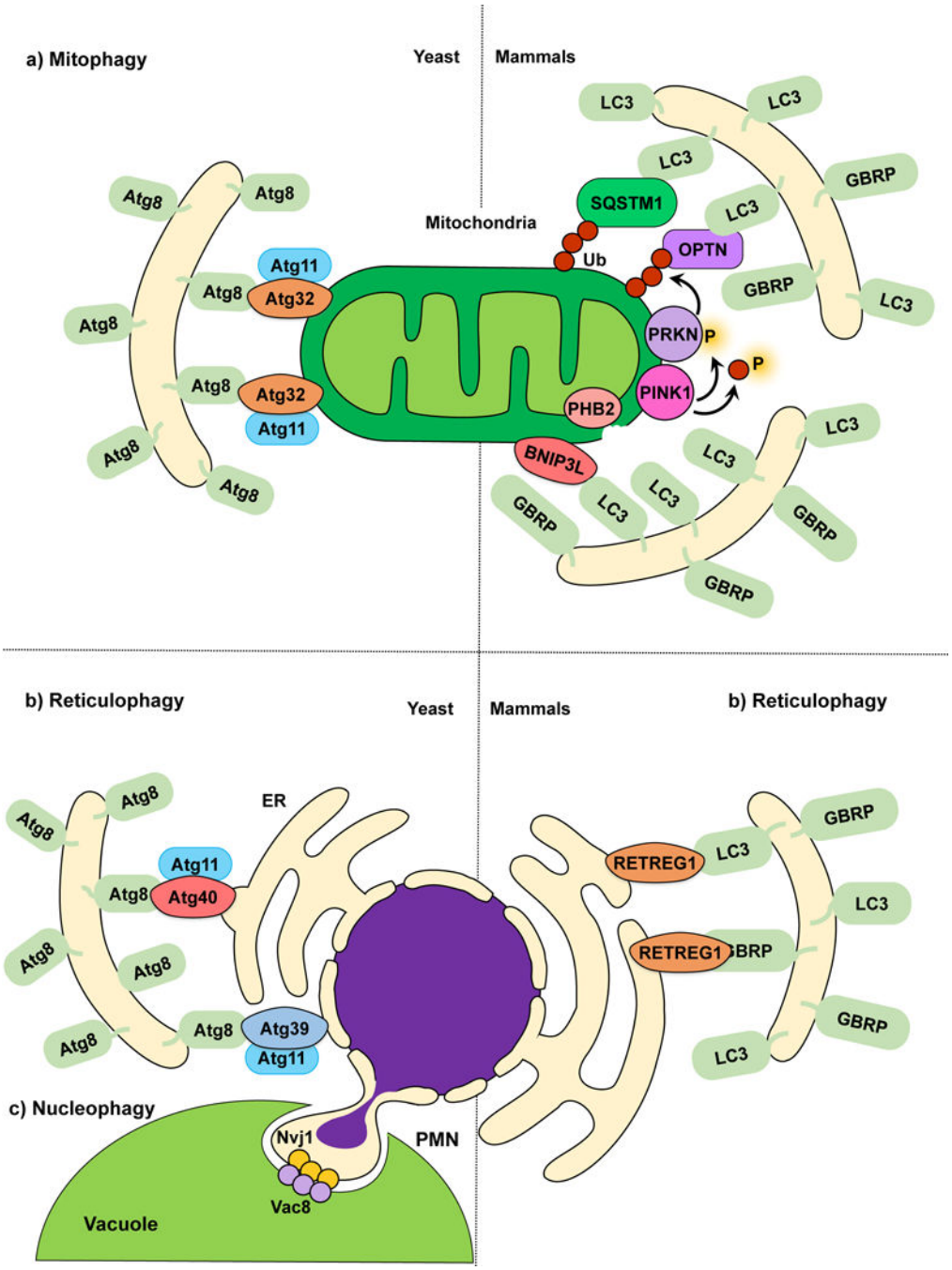

Figure 2.

Mitophagy, reticulophagy and nucleophagy. (**a**) The yeast mitophagy receptor Atg32 links mitochondria to the phagophore by directly binding Atg8−PE; Atg11 functions as a scaffold. Several cargo receptors (not all shown) have been described for mammalian mitophagy. Mitochondria depolarization leads to PINK1 activation and phosphorylation of ubiquitin and PRKN, and OMM disruption exposes PHB2. Receptors link mitochondria targeted for degradation to the phagophore. (**b**) In yeast reticulophagy, Atg39 and Atg40 have been proposed as receptor proteins. Atg39 mediates the degradation of the perinuclear

ER, and Atg40 mediates cytoplasmic ER degradation. Both Atg39 and Atg40 link their respective ER sites to Atg8−PE-conjugated membranes for sequestration. Atg11 has been proposed as a scaffold protein for both Atg39 and Atg40-mediated reticulophagy. During mammalian reticulophagy, RETREG1/FAM134B tethers the cytoplasmic ER to LC3/GABARAP family members for membrane sequestration and degradation. (**c**) Because Atg39 specifically localizes to the perinuclear ER, Atg39-mediated degradation is also considered nucleophagy. During piecemeal microautophagy of the nucleus (PMN), the nuclear envelope protein Nvj1 and vacuolar membrane protein Vac8 form nuclear-vacuolar junctions, which pinch off and engulf part of the nucleus inside the vacuole.

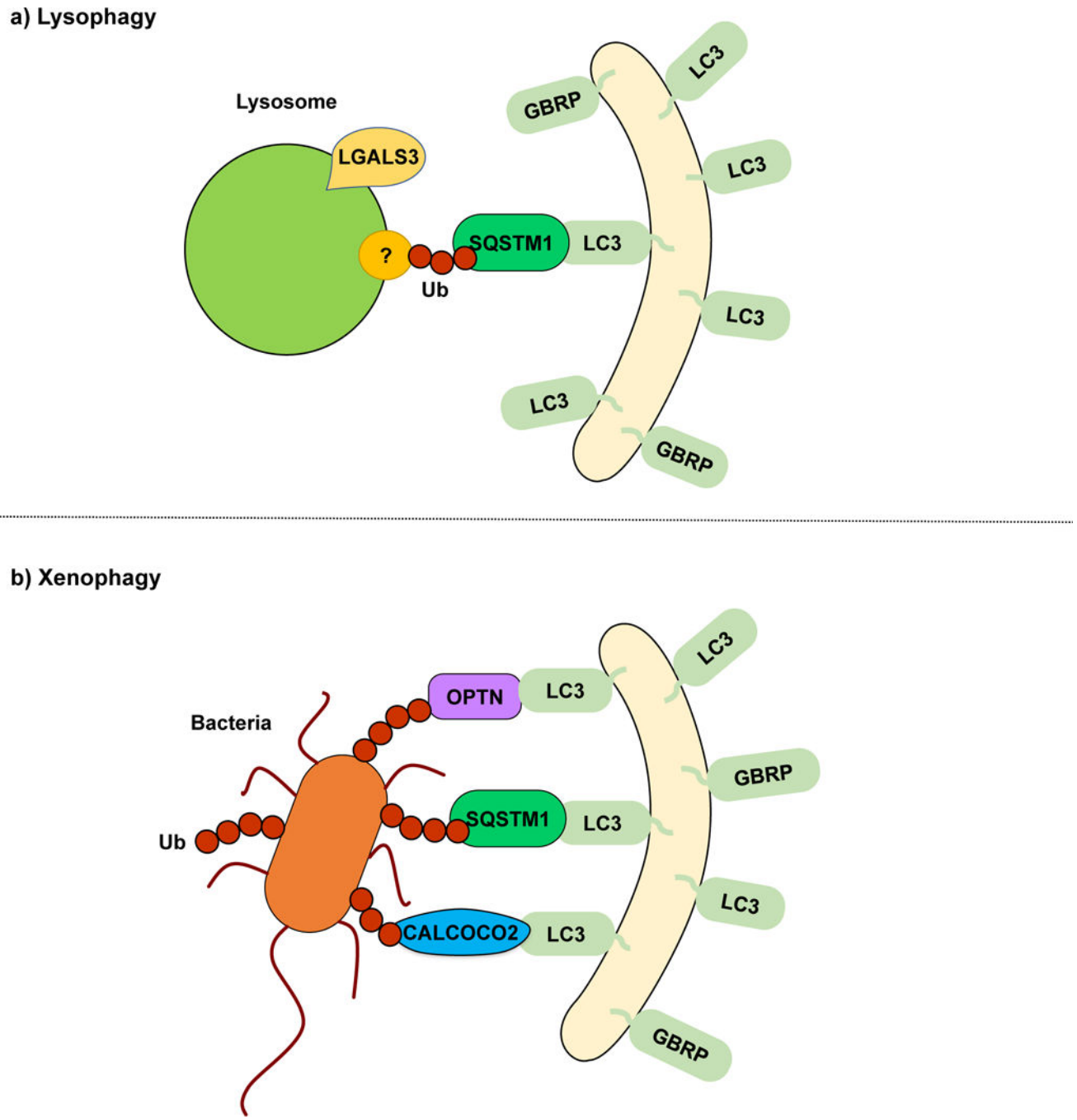

Figure 3.

Lysophagy and xenophagy. (**a**) During lysophagy, unknown lysosomal proteins are ubiquitinated and recognized by SQSTM1, which functions as a receptor, linking the damaged lysosomes with the LC3/GABARAP-conjugated sequestering membranes. LGALS3 binds to exposed lysosomal glycoproteins upon membrane rupture. A specific lysophagy mechanism remains to be elucidated. (**b**) In xenophagy, intracellular pathogens

such as viruses and bacteria are recognized and ubiquitinated. SQSTM1, OPTN, CALCOCO2 and NBR1 have been described as receptor proteins.

Table 1

Selective autophagy ligands, receptor and scaffolds in yeast and mammals.

Process	Organism	Ligand	Receptor	Scaffold
Cvt Pathway	Yeast	prApe1, Ams1	Atg19	Atg11
Aggrephagy	Yeast	Protein aggregates (Ub)	Cue5	–
	Mammals		SQSTM1/p62, NBR1, TOLLIP	WDFY3/ALFY
Pexophagy	Yeast	Peroxisomes (Pex3, PpPex14, PpPex3)	Atg36, PpAtg30	Atg11, PpAtg17
	Mammals	Peroxisomes (Ub)	SQSTM1/p62, NBR1	–
Mitophagy	Yeast	Mitochondria	Atg32	Atg11
	Mammals		SQSTM1/p62, BNIP3L/Nix, OPTN, FUNDC1, PHB2	–
Reticulophagy	Yeast	Endoplasmic reticulum	Atg39, Atg40	Atg11
	Mammals		RETREG1/FAM134B	–
Nucleophagy	Yeast	Nucleus, nuclear Nvj1 portions (PMN)	Atg40, Vac8 (PMN)	–
Lysophagy	Mammals	Lysosomes (Ub)	SQSTM1/p62	–
Xenophagy	Mammals	Bacteria (Ub), viruses	SQSTM1/p62, CALCOCO2/NDP52, OPTN	–
Lipophagy	Mammals	Lipid droplets	–	–
	Yeast		–	–
Ferritinophagy	Mammals	Ferritin	NCOA4	–
Glycophagy	Mammals	Glycogen	STBD1	–