



Machinery, regulation and pathophysiological implications of autophagosome maturation

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Abstract | Autophagy is a versatile degradation system for maintaining cellular homeostasis whereby cytosolic materials are sequestered in a double-membrane autophagosome and subsequently delivered to lysosomes, where they are broken down. In multicellular organisms, newly formed autophagosomes undergo a process called ‘maturation’, in which they fuse with vesicles originating from endolysosomal compartments, including early/late endosomes and lysosomes, to form amphisomes, which eventually become degradative autolysosomes. This fusion process requires the concerted actions of multiple regulators of membrane dynamics, including SNAREs, tethering proteins and RAB GTPases, and also transport of autophagosomes and late endosomes/lysosomes towards each other. Multiple mechanisms modulate autophagosome maturation, including post-translational modification of key components, spatial distribution of phosphoinositide lipid species on membranes, RAB protein dynamics, and biogenesis and function of lysosomes. Nutrient status and various stresses integrate into the autophagosome maturation machinery to coordinate the progression of autophagic flux. Impaired autophagosome maturation is linked to the pathogenesis of various human diseases, including neurodegenerative disorders, cancer and myopathies. Furthermore, invading pathogens exploit various strategies to block autophagosome maturation, thus evading destruction and even subverting autophagic vacuoles (autophagosomes, amphisomes and autolysosomes) for survival, growth and/or release. Here, we discuss the recent progress in our understanding of the machinery and regulation of autophagosome maturation, the relevance of these mechanisms to human pathophysiology and how they are harnessed by pathogens for their benefit. We also provide perspectives on targeting autophagosome maturation therapeutically.

Cells in multicellular organisms constantly experience diverse stresses, including protein misfolding, organelle damage, scarcity of nutrients/energy and invasion by pathogens. One mechanism exploited by cells to combat stresses is the lysosome-mediated degradation of intracellular components via autophagy. Autophagy involves the formation of an isolation membrane (or phagophore), which further expands and closes to form the double-membrane autophagosome^{1–3}. The mechanism of autophagosome formation was excellently reviewed recently³. Various cellular materials can be sequestered in autophagosomes, including unselected cytosolic material or selected cargos such as protein aggregates, damaged organelles and pathogens. Following autophagosome closure via membrane abscission, cargos are delivered to the vacuole (yeasts and plants) or to lysosomes (animal cells) (for an overview of the autophagy

process see^{4–8}; BOX 1). After degradation of the autophagic cargo, the digested content in the autolysosomes is released and lysosomes are re-formed to sustain the autophagic flux (BOX 2). A series of proteins encoded by *ATG* (autophagy-related) genes and *EPG* (ectopic PGL granules) genes — identified mainly from genetic screens in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, respectively — act at different membrane remodelling steps for autophagosome formation and maturation^{3,4,9,10}.

In yeast, autophagosomes are formed in the vicinity of and directly fuse with the much larger vacuole, whereas in multicellular organisms, newly formed autophagosomes fuse with different endolysosomal vesicles such as early/late endosomes and lysosomes to form non-degradative, single-membrane structures called ‘amphisomes’, which gradually acquire

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V-ATPase

A proton pump, driven by ATP hydrolysis, which transports protons into the lumen of membrane compartments for their acidification. V-ATPases are multisubunit complexes, containing V1 and V0 sectors.

SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complexes

Complexes consisting of the Qa, Qb, Qc and R SNARE proteins in opposing membranes. The SNARE motifs of these proteins are assembled into a hetero-oligomeric, four-helix bundle to drive membrane fusion.

Tethers

Proteins or protein complexes that link the transported vesicles to target membranes and also promote the assembly of the *trans*-SNARE complex.

Phosphoinositides

Phosphorylated derivatives of phosphatidylinositol that define membrane identity and participate in various signalling and membrane trafficking processes.

RAB proteins

A subgroup of small GTPases that dynamically associate with membrane-bound compartments driven by GTP–GDP cycling to regulate multiple aspects of membrane dynamics, such as vesicle transport, fusion and positioning.

Multivesicular bodies

Endocytic vesicles formed by invagination of the endosomal membrane to form membrane-bound intraluminal vesicles. They subsequently undergo lysosomal degradation or extracellular release.

degradative properties through the accumulation of hydrolases. Maturation also involves autophagosome acidification mediated by V-ATPase, which is required for the activity of degradative enzymes^{9–11}. Fusion of autophagosomes with endosomes/lysosomes requires the concerted actions of cognate SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complexes, tethers, phosphoinositides and RAB proteins¹¹. Bidirectional microtubule-based transport of autophagic vacuoles (a term encompassing autophagosomes, amphisomes and autolysosomes) and late endosomes/lysosomes between the perinuclear space and periphery of cells maximizes the frequency of encounters and fusion efficiency¹².

Recent studies revealed that the machinery mediating autophagosome maturation, such as SNARE complex assembly, tether recruitment and RAB protein dynamics, is tightly controlled and highly responsive to nutrient availability and stress conditions¹¹. Disturbing these processes can cause accumulation of damaged organelles and toxic protein aggregates, and may also hamper endocytic trafficking. Accordingly, impaired autophagosome maturation is associated with the pathogenesis of various human disorders, including neurodegenerative diseases, cancer and myopathies^{11,13}. Furthermore, invading viruses and bacteria also widely target maturation of autophagosomes to block their degradative capability. Viruses and bacteria thereby evade destruction and also accumulate autophagic vacuoles for their survival, replication and release^{14–17}. Notably, unlike impairment of genes essential for autophagosome formation, loss of function of genes acting at autophagosome maturation usually causes a weaker defect and gradual accumulation of autophagic vacuoles. More importantly, in some cases, such defects can be suppressed by promoting the activities of partially redundant factors or mechanisms. For example, facilitating the recruitment of one tethering factor would alleviate the defect caused by depletion of another. This offers opportunities to ameliorate the defects of autophagosome maturation in relevant human diseases.

In this Review, we outline the most recent progress in our understanding of the molecular machinery that drives and regulates autophagosome maturation. We further discuss the causative link between impaired autophagosome maturation and the pathogenesis of neurodegeneration, muscle diseases and cancer. We also consider how viral proteins and bacterial effectors block autophagosome maturation for their own benefits. Finally, we offer insights into how autophagosome maturation can be therapeutically targeted to combat disease.

Machinery for autophagosome fusion

Maturation of autophagosomes requires their fusion with functional endolysosomal compartments such as early endosomes, late endosomes/multivesicular bodies and lysosomes. Accordingly, disturbances in biogenesis of these endolysosomal compartments — such as those caused by the loss of function of subunits of the ESCRT (endosomal sorting complexes required for transport) complex and COPI vesicles — cause accumulation of non-degradative autophagic vacuoles^{18,19}. Fusion of autophagosomes with late endosomes/lysosomes involves the concerted action of RABs, tethers and the SNARE complex. Membrane fusion is driven by the assembly of a *trans*-SNARE complex composed of Qa, Qb, Qc and R SNAREs²⁰. Tethering factors, which are recruited to target membranes and/or fusing vesicles by the active form of small RAB GTPases, phosphoinositides and SNARE proteins, facilitate the initial capture of vesicles and subsequent formation of the *trans*-SNARE complex^{21,22}. Autophagic vacuoles are decorated with lipidated (phosphatidylethanolamine-conjugated) ubiquitin-like autophagy proteins related to yeast Atg8. These proteins, in the LC3 subfamily or the GABARAP subfamily, also help to recruit other factors such as tethers and RAB guanine nucleotide exchange factors (GEFs) for maturation^{23,24}. LC3 is a widely used autophagic marker that labels autophagic structures at different stages of biogenesis, and, together with markers for late endosomes/lysosomes, it accumulates on amphisomes during maturation. Of note, in studies using LC3 to label autophagic structures after closure (which can be differentiated from isolation membranes by co-labelling with markers for other autophagy proteins or use of the HaloTag–LC3 method^{6,25}), double-membrane autophagosomes cannot be distinguished from single-membrane amphisomes and autolysosomes. Hence, LC3-marked structures after closure should be referred to as ‘autophagic vacuoles’ unless the structure has been unambiguously demonstrated.

The role of SNARE complexes. SNARE complexes formed by autophagosomal membrane-localized STX17 (Qa), SNAP29 (Qbc) and late endosomal/lysosomal-localized VAMP8 (Vamp7 in flies and VAMP-7 in worms), or by the autophagosomal YKT6, SNAP29 and late endosomal/lysosomal-localized STX7, function partially redundantly to drive fusion of autophagosomes with late endosomes/lysosomes^{26–28} (FIG. 1a). Targeting of STX17 to forming autophagosomes accompanies autophagosome closure²⁹. The small guanosine triphosphatase IRGM facilitates translocation of STX17 to autophagosomes by interacting with STX17 and ATG8-like proteins on the autophagosomal membrane³⁰. SNAP29 is recruited by interacting with other SNAREs. STX17–SNAP29 complex assembly is promoted by the autophagosome-associated autophagy protein ATG14, which directly interacts with STX17 (REF.³¹) (FIG. 1a). Different SNARE complexes may act at different steps of autophagosome maturation or drive fusion of autophagic vacuoles with different sets of late endosomes/lysosomes^{27,32}. Postfusion SNAREs disassemble and return to their donor compartments to

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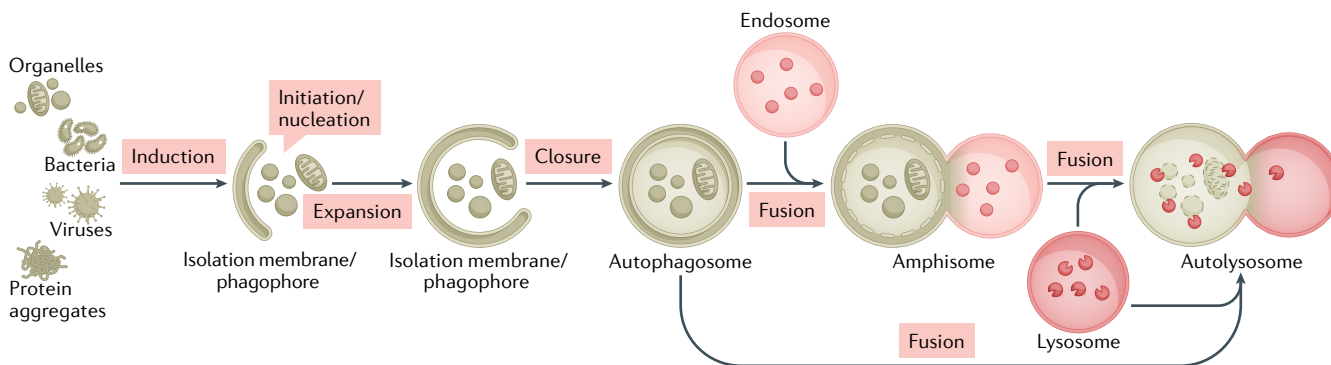
Box 1 | Overview of the autophagy pathway

Autophagy, a lysosome-mediated degradation system, is an evolutionarily conserved pathway for recycling cellular content and for removing aggregated proteins, damaged organelles and invading pathogens (for example, viruses and bacteria) to maintain normal cellular function and homeostasis. Autophagy occurs in a stepwise manner involving sequential membrane remodelling processes (see the figure). Upon induction, autophagy starts with the initiation and nucleation of a double-membrane sac, known as the isolation membrane (or phagophore), in the cytosol^{1–3}. Vesicles carrying the multispansing membrane protein ATG9 have been suggested to serve as one of the membrane sources for isolation membrane initiation and nucleation. The isolation membrane then undergoes expansion to surround the cargos. Multiple membrane sources have been reported to contribute to isolation membrane growth, including the endoplasmic reticulum (ER), lipid droplets, the ER–Golgi intermediate compartment (ERGIC), the plasma membrane, ATG9 vesicles and COPII vesicles^{1–3,10}. Isolation membrane expansion can also be mediated by lipid transfer from the ER and local fatty acid synthesis^{216,217}. Once the autophagosome is fully closed, it fuses with late endosomes/lysosomes to form an amphisome/autolysosome. The sequestered materials are broken down in degradative autolysosomes.

Genetic screens in yeast identified a group of conserved autophagy proteins, named 'Atg (autophagy-related) proteins', which function at different steps of autophagosome formation^{3,10,68}. According to their function, these proteins can be divided into five groups. (1) The Atg1–Atg13–Atg17 kinase complex, whose mammalian counterpart is the ULK1–ATG13–FIP200 complex, is first recruited to the autophagosome formation site and triggers the nucleation of isolation membranes. (2) The phosphatidylinositol 3-phosphate (PtdIns3P) kinase complex, consisting of

Vps34, Atg6 (mammalian beclin 1 homologue) and Atg14, is responsible for local production of PtdIns3P to recruit downstream effectors. (3) The Atg12 conjugation system, comprising the E1 enzyme Atg7 and the E2 enzyme Atg10, catalyses conjugation of ubiquitin-like protein Atg12 with Atg5. The Atg12–Atg5 conjugate further interacts with Atg16. (4) The Atg8 conjugation system, comprising Atg7 and the E2 enzyme Atg3, catalyses conjugation of ubiquitin-like protein Atg8 to phosphatidylethanolamine. The Atg12 and Atg8 conjugation systems function at multiple steps of autophagy, including isolation membrane expansion, isolation membrane closure and cargo recognition in selective autophagy. (5) The Atg2–Atg18 complex and the transmembrane protein Atg9 are possibly involved in tethering the ER with the isolation membrane to transfer glycerophospholipids for isolation membrane growth. By recruiting different effectors, PtdIns3P has multiple functions in autophagy, including initiation and expansion of the isolation membrane and also fusion of autophagosomes with the vacuole¹¹.

Autophagy in multicellular organisms contains steps that are absent in yeast autophagy, including the recruitment of autophagy proteins to the autophagosome formation sites on the ER, the formation of complex ER–isolation membrane contacts during isolation membrane expansion, and autophagosome maturation. Genetic screens in *Caenorhabditis elegans* identified multiple metazoan-specific autophagy proteins, that is, EPG (ectopic PGL granules) proteins. These proteins act at steps unique to autophagy in multicellular organisms. EPG-3 (known as VMP1 in mammals) is required for establishing dynamic ER–isolation membrane contacts¹⁵⁷, while EPG-5 and WDR45/WDR45B mediate autophagosome–lysosome fusion^{25,40}. Thus, ATG and EPG proteins act together to complete the more complex mammalian autophagy process.



maintain intracellular membrane identity and to prepare for new rounds of fusion²⁰. The molecular mechanisms driving SNARE retrieval from autolysosomes have yet to be determined. It has been shown that release of STX17 from autolysosomes coincides with collapse of the autophagosomal inner membrane due to breakdown by lysosomal enzymes²⁹.

The role of tethering factors. Both multisubunit tethering complexes and large individual tether proteins participate in the initial capture of autophagosomes and/or amphisomes and promote fusion efficiency and specificity with early/late endosomes and lysosomes but not with other vesicles such as recycling endosomes or secretory vesicles during autophagosome maturation (FIG. 1b).

The HOPS (homotypic fusion and protein sorting) complex is a prominent tethering complex that promotes assembly of the *trans*-SNARE complex, acts as a GEF for RAB7 and functions as a SNARE chaperone to facilitate fusion of autophagosomes with late endosomes and

lysosomes^{22,33,34}. The HOPS complex can be targeted to both autophagosomes and late endosomes/lysosomes to promote fusion^{33,34}. Targeting of the HOPS complex to late endosomes/lysosomes is mediated by its binding to RAB7 and phosphoinositides such as phosphatidylinositol 3-phosphate (PtdIns3P)³⁵, while multiple mechanisms facilitate its translocation from the cytosol to autophagosomes, including interaction with STX17 (REFS^{33,34}), active RAB7 (REFS^{36,37}), GABARAPs³⁸ or large tether proteins (such as PLEKHM1) described below (FIG. 1b).

EPG5 is an evolutionarily conserved large tether protein for autophagosome maturation^{39,40}. It is a RAB7 effector and translocates from the cytosol to late endosomes/lysosomes by interacting with RAB7 (REF. 40) (FIG. 1b). During autophagosome maturation, EPG5 captures autophagosomes/amphisomes via binding to LC3 and promotes assembly of the STX17–SNAP29–VAMP8 complex⁴⁰. EPG5 depletion causes accumulation of autophagosomes, amphisomes and non-degradative

COPI vesicles

Coat protein I (COPI)-coated vesicles mediating the retrieval of proteins and lipids from the Golgi apparatus to the endoplasmic reticulum and also transport between Golgi apparatus cisternae.

LC3 subfamily

A subfamily of Atg8 homologues containing LC3A, LC3B and LC3C.

GABARAP subfamily

A subfamily of Atg8 homologues containing GABARAP, GABARAPL1 and GABARAPL2.

Guanine nucleotide exchange factors

(GEFs). Factors that catalyse the conversion of the GDP-bound inactive form of small GTPase proteins to their GTP-bound active form.

Recycling endosomes

Endosomal compartments for recycling materials internalized by endocytosis back to the cell surface to maintain the composition of the plasma membrane.

HOPS (homotypic fusion and protein sorting) complex

A multisubunit seahorse-shaped complex consisting of VPS41, VPS39, VPS18, VPS33A, VPS11 and VPS16. It facilitates fusion events involving late endosomes and lysosomes.

SNARE chaperone

A protein chaperone such as Sec1/Munc18 (SM) proteins that binds to individual SNAREs or assembly intermediates. It ensures the fast and accurate assembly of *trans*-SNARE complexes to drive efficient membrane fusion.

autolysosomes⁴¹. The function of EPG5 in autophagosome maturation appears to require its late endosomal/lysosomal localization. The β -propeller proteins WDR45 and WDR45B (mammalian homologues of the yeast PtdIns3P-binding autophagy protein Atg18; also known as WIPI4 and WIPI3, respectively) interact with and mediate late endosomal/lysosomal targeting of EPG5 (REF.²⁵). In *Wdr45-Wdr45b* double-knockout cells, EPG5 is mislocalized on autophagosomes and the EPG5-STX17 interaction is enhanced. However, the assembly of STX17-SNAP29-VAMP8 is reduced, and consequently autophagosome maturation is impaired²⁵.

PLEKHM1, another tethering protein and RAB7 effector, interacts preferentially with GABARAP subfamily members to promote autophagosome maturation^{23,42} (FIG. 1b). PLEKHM1 directly recruits the HOPS complex, ensuring fusion specificity and efficiency^{42,43}. The lysosomal-localized pleckstrin homology (PH) domain-containing TECPR1 selectively binds to LC3C and promotes fusion of LC3C-decorated autophagosomes/amphisomes with phosphatidylinositol 4-phosphate (PtdIns4P)-enriched lysosomes⁴⁴ (FIG. 1b). It has also been shown that TECPR1, upon binding to autophagosomal PtdIns3P, adopts a conformation that associates with the ATG12-ATG5 conjugate, thus facilitating tethering of autophagosomes with lysosomes⁴⁵.

Vesicle transport during maturation

In non-polarized cells, autophagosomes are generated throughout the cytoplasm, while lysosomes locate mainly at perinuclear regions^{12,46}. Autophagosome

maturation requires retrograde transport of autophagic vacuoles and lysosomes driven by the microtubule motor protein dynein, as well as their anterograde transport mediated by kinesin motors^{12,46,47}, which allow the two compartments to meet and fuse.

Machinery mediating transport of autophagic vacuoles and lysosomes. Anterograde transport of lysosomes enables them to fuse with peripheral autophagosomes. Anterograde transport is mediated by the late endosomal/lysosomal-associated multisubunit BORC complex, which recruits the small GTPase ARL8 to promote ARL8-dependent coupling to kinesin motors¹² (FIG. 2). ARL8 directly interacts with kinesin 3, or interacts with its effector SKIP, which in turn couples to kinesin 1 (REF.⁴⁸). Depletion of BORC complex decreases the fusion of peripheral autophagosomes with lysosomes⁴⁹. The RAB7 effector FYCO1 also acts as an adaptor of kinesin 1 to mediate anterograde transport of late endosomes/lysosomes, and of autophagic vacuoles through its binding to LC3 and PtdIns3P⁵⁰ (FIG. 2).

The recruitment of the dynein-dynactin motor complex to late endosomes/lysosomes and autophagic vacuoles for retrograde transport is mediated by RAB7 and its effectors RILP and ORP1L¹² (FIG. 2). RILP interacts with the p150 subunit of the dynein-dynactin motor complex¹², whereas ORP1L promotes the binding of the dynein complex with late endosomal/lysosomal membrane-associated β III spectrin⁵¹. In addition, ORP1L can sense and couple cholesterol levels with transport of lysosomes and autophagic vacuoles (FIG. 2). Under low-cholesterol conditions, ORP1L interacts with the endoplasmic reticulum (ER) protein VAPA to form contacts between the ER and lysosomes/autophagic vacuoles; the dynein motor then disassociates from RAB7-RILP, causing dispersal of these organelles^{43,52}. In Niemann-Pick type C disease and other lysosome storage diseases (LSDs), accumulation of cholesterol in the late endosomal/lysosomal compartments results in their perinuclear clustering and a defect in autophagosome maturation⁵².

Transport of autophagic vacuoles and late endosomes/lysosomes is tightly coordinated with recruitment of tethering factors. Specifically, the HOPS complex can be recruited by ARL8 (REF.⁴⁹) or by the ORP1L-RAB7-RILP complex, which occurs either directly or via PLEKHM1 (REF.⁵³). This allows coupling of anterograde and retrograde transport and fusion events⁵³.

Directional transport of autophagic vacuoles is particularly prominent in polarized neuronal cells, where autophagosomes formed at the distal synaptic termini undergo long-range retrograde transport to the soma^{54,55}. Maturation of autophagosomes is tightly linked with their dynein-driven transport to the soma, which requires coordinated actions of scaffolding proteins, including JNK-interacting protein 1 (JIP1), HTT-associated protein 1 (HAP1) and JIP3 (REFS^{56,57}). These factors bridge autophagic vacuoles with the dynein-dynactin complex, and their actions depend on location and autophagosomal maturity^{56,57}. JIP1 acts in the distal portion of the axon, HAP1 functions in the mid-axon, while JIP3 primarily controls the motility of

Box 2 | Degradation, catabolite export and lysosome re-formation

After autophagosome maturation, the inner membrane disintegrates and is degraded together with sequestered materials in acidic autolysosomes by hydrolases²¹⁸. Specific hydrolases are involved in the degradation of different macromolecules, such as proteins, nucleic acids, lipids and carbohydrates. The glycosylated integral membrane protein Atg15 is a lipase essential for disintegration of inner membranes²¹⁹. In *Caenorhabditis elegans*, the T2 family endoribonuclease RNST-2 degrades ribosomal RNAs that are delivered to lysosomes via autophagy²²⁰. The lysosomal degradation products are exported to the cytosol via lysosomal efflux transporters, such as the lysosomal lysine/arginine transporter LAAT-1 and the sugar transporter Spinster^{221,222}. The exported catabolites provide building blocks (for example, amino acids, nucleotides and monosaccharides) or materials for energy production to maintain cellular homeostasis. For example, autophagy-dependent ribosomal RNA degradation is essential for maintaining nucleotide homeostasis during *C. elegans* development²²⁰. Defective lysosomal degradation or catabolite export from lysosomes leads to impaired lysosomal function and accumulation of undigested materials or digested catabolites.

Upon release of the digestion products, lysosomes are regenerated from autolysosomes, a process known as autophagic lysosome re-formation (ALR), to maintain lysosome homeostasis²²³. ALR involves clathrin-mediated budding of tubular structures from autolysosomes, KIF5B-driven extension of membrane tubules along microtubules, dynamin 2-mediated scission of protolysosomes devoid of lysosomal contents and subsequent maturation of protolysosomes to functional lysosomes²²⁴. During prolonged starvation-induced autophagy, ALR is triggered by mTOR reactivation, which is normally repressed under starvation. This requires degradation of autophagic cargo and release of degraded products, among which are amino acids — the primary activators of mTOR. Defective autophagic degradation or export of the degradation products from the autolysosome, such as in cells derived from patients with lysosome storage diseases, inhibits mTOR reactivation and ALR, resulting in accumulation of enlarged autolysosomes²²³. The activity of the lysosomal channel protein TRPML1 is also involved in autolysosome re-formation²²⁵. Lysosomal regeneration at the end of autophagic flux is critical for maintaining the lysosome pool and thus sustained autophagy.

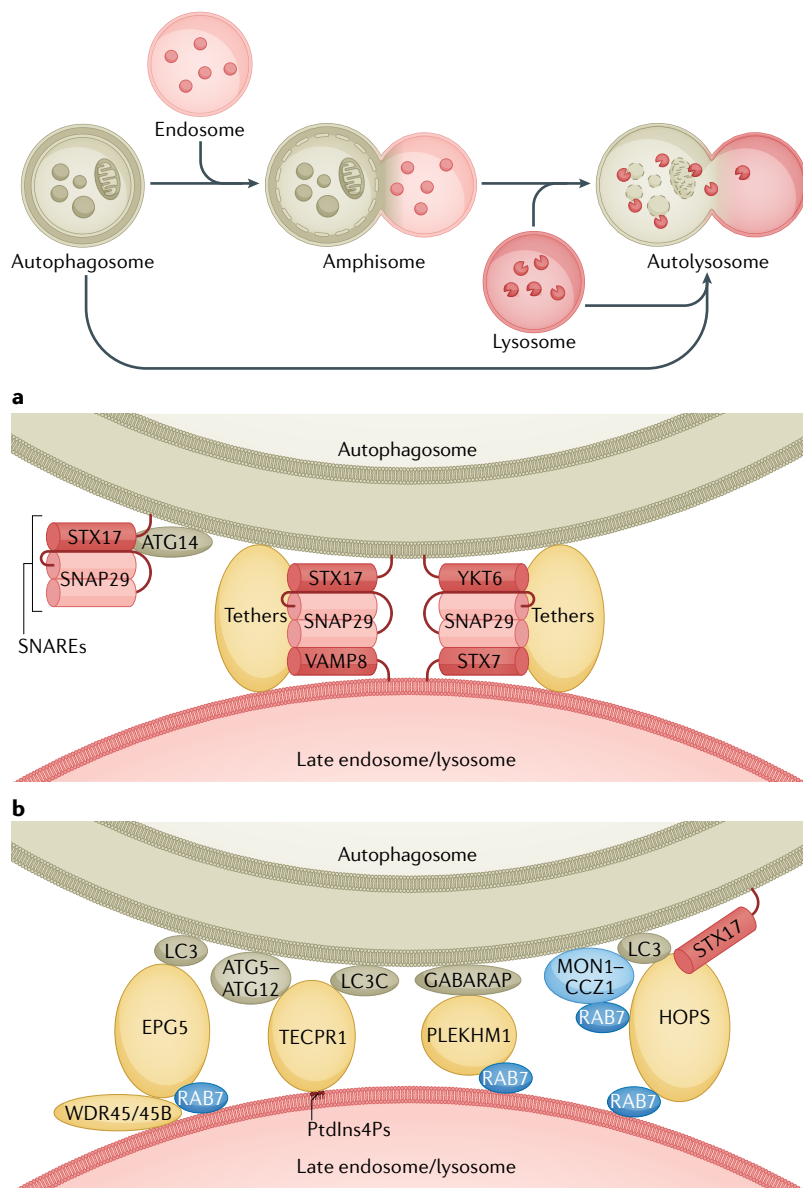


Fig. 1 | SNAREs, tethers and RAB proteins mediate autophagosome maturation. a Fusion between autophagosomes and late endosomes/lysosomes is mediated by two sets of SNARE complexes: the autophagosomal Qa SNARE STX17, the Qbc SNARE SNAP29 and the endolysosomal R SNARE VAMP8 (centre left); and the autophagosomal R SNARE YKT6, SNAP29 and the endolysosomal Qa SNARE STX7 (centre right). The unique hairpin structure formed by the two transmembrane domains of STX17 and the amino-terminal longin domain of YKT6 are involved in their autophagosomal recruitment^{26,27}. The autophagosome-associated autophagy protein ATG14 interacts with STX17 to promote the assembly of the STX17–SNAP29 subcomplex (left). The assembly and function of both complexes are facilitated by tether proteins. **b** Multiple tether proteins are involved in fusion of autophagosomes with late endosomes/lysosomes. EPG5 is targeted to autophagosomes and lysosomes by binding to LC3 and RAB7, respectively. In neural cells, WDR45 and WDR45B facilitate the lysosomal localization of EPG5. TECPR1 interacts with LC3C and ATG5–ATG12 on autophagosomes and phosphatidylinositol 4-phosphate (PtdIns4P) on lysosomes. RAB7 binds to the HOPS complex and PLEKHM1. On the autophagosome side, the HOPS complex binds to STX17 and LC3 proteins, and PLEKHM1 binds to GABARAPs. The active GTP-bound RAB7 associates with the membrane. Localization of RAB7 on autophagic vacuoles is facilitated by the LC3-binding MON1–CCZ1 guanine nucleotide exchange factor complex^{36,37}.

plays a key role here. Nutrient status and other stresses also integrate into the autophagosome maturation machinery to add another level of control of autophagic flux. It is also noteworthy that the machinery governing autophagosome maturation is heterogeneous and demonstrates at least partial redundancy, which may allow flexible regulation of autophagic flux in different cell types.

Trafficking and post-translational modification of SNARE proteins.

SNARE proteins dynamically move between distinct membrane compartments²⁰. Upon starvation, RAB21, an endosomal RAB protein, is activated by its GEF MTMR13, which further promotes the translocation of plasma membrane-localized VAMP8 to late endosomes/lysosomes⁶³ (FIG. 3a). SNARE protein dynamics is required for efficient fusion of the endolysosomal structures with autophagic vacuoles as evidenced by LSDs such as multiple sulfatase deficiency and mucopolysaccharidosis type IIIA, where SNAREs are sequestered in cholesterol-enriched regions of endolysosomal membranes and locked in assembled complexes⁶⁴. Therefore, disassembly of postfusion SNARE complexes and their sorting and recycling back to target membranes is impaired, and, consequently, fusion of lysosomes with endocytic and autophagic vesicles is reduced⁶⁴.

The SNARE activity of STX17 is regulated by acetylation of its SNARE domain, a modification controlled by the histone acetyltransferase CREBBP/CBP and the deacetylase HDAC2 (REF.⁶⁵) (FIG. 3a). Starvation or mTORC1 inhibition inactivates CREBBP, while promoting the deacetylation of STX17. Deacetylation of STX17 facilitates the assembly of the STX17–SNAP29–VAMP8 complex and also enhances its binding to the HOPS

maturer autophagic vacuoles in the proximal portion of the axon⁵⁷.

Regulation of autophagosome maturation

The machinery governing autophagosome maturation is dynamically regulated to allow adaptation of autophagic flux to the needs of the cell and to enable integration of autophagic degradation with external inputs. This involves regulation of the membrane recruitment of the key components mediating fusion events as well as transcriptional regulation, which occurs predominantly via the MiT/TFE family transcription factors TFE3 and TFE3. During autophagosome biogenesis, various stresses and signalling pathways can modulate the initiation of autophagosome formation via multiple mechanisms, such as by regulating the kinase activity, stability and assembly of the Atg1 complex (ULK1 complex in mammals) and the Vps34 PtdIns3P kinase complex^{58–62}. mTORC1, an evolutionarily conserved signalling hub that senses nutrient status and growth factor signals,

β-Propeller proteins

A family of proteins with 4–12 repeats of a β-stranded blade that function as structural scaffolds for ligand binding, enzymatic activity and assembly of multiple protein complexes.

Pleckstrin homology (PH) domain

A protein domain that binds phosphatidylinositol lipids (such as phosphatidylinositol 4,5-bisphosphate) and proteins with high affinity and specificity. It functions in vesicle trafficking, cellular signalling and cytoskeletal remodelling.

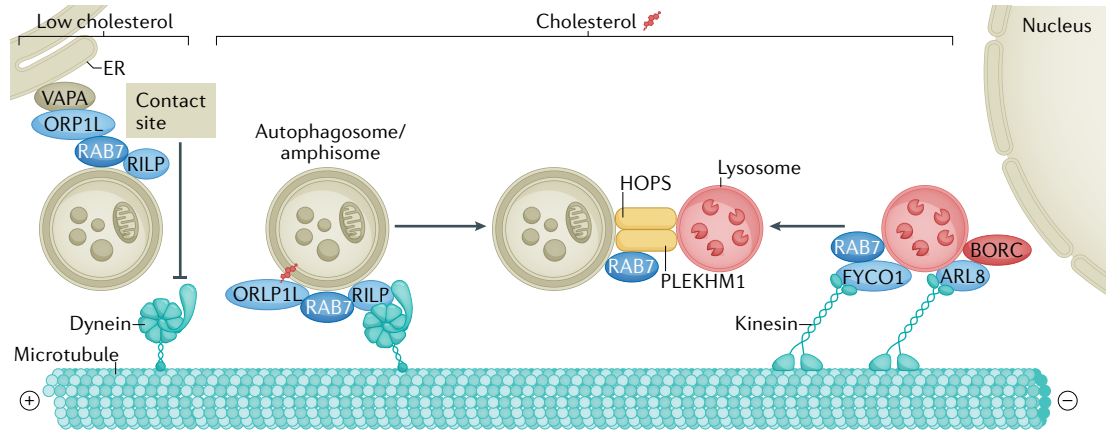


Fig. 2 | Coupling transport and fusion of autophagic vacuoles and late endosomes/lysosomes. Autophagosomes, which form throughout the cytoplasm, and lysosomes, which are localized mainly in the perinuclear region, undergo bidirectional movement on microtubules. The late endosome/lysosome-localized BORC–ARL8 complex and the FYCO1–RAB7 pair recruit kinesin motors for anterograde transport of late endosomes/lysosomes. The RAB7 effectors RILP and ORP1L promote the membrane association of the dynein–dynactin motor machinery to mediate the retrograde transport of autophagic vacuoles (as well as late endosomes/lysosomes (not shown)). When cholesterol levels are low, ORP1L interacts with the endoplasmic reticulum (ER) protein VAPA to form ER–autophagosome membrane contacts, which releases dynein–dynactin and prevents retrograde trafficking, thereby interfering with autophagosome maturation. During transport, the tethering factors HOPS complex and PLEKHM1 are concomitantly recruited to promote fusion processes (see FIG. 1).

Retrograde transport
Intracellular movement from the cell periphery towards the nucleus mediated by the dynein–dynactin complex. Also known as centripetal movement or minus-end transport.

Anterograde transport
Intracellular movement from the nucleus towards the cell periphery mediated by kinesin motors. Also known as centrifugal movement or plus-end transport.

BORC complex
A multisubunit complex that associates peripherally with the lysosomal membrane to regulate lysosomal positioning by recruiting ARL8. It comprises eight subunits: BLOS1, BLOS2, snapin, KXD1, myrlysin, lypersin, diaskedin and MEF2BNB.

ARL8
A small ADP-ribosylation factor-like RAS family GTPase that mediates kinesin-driven lysosome transport, and also regulates lysosome fusion by recruiting the HOPS complex.

Lysosome storage diseases (LSDs). A group of inherited metabolic disorders characterized by abnormal storage of toxic materials. They result from deficiencies of lysosomal enzymes or transporters.

Atg1 complex
The Atg1 complex consists of the protein kinase Atg1 together with Atg13, Atg17, Atg31 and Atg29. Activation of this complex triggers the initiation of autophagy.

Vps34 PtdIns3P kinase complex
A complex, containing the phosphatidylinositol 3-phosphate (PtdIns3P) kinase Vps34 together with Vps15, Atg6 and Atg14, that generates PtdIns3P at the autophagosome formation site to recruit downstream effectors for autophagosome formation.

complex, thus promoting autophagosome–lysosome fusion under stress conditions⁶⁵ (FIG. 3a).

SNAP29 contains two antiparallel helix bundles and is the key SNARE for autophagosome maturation^{26,27}. SNAP29 is post-translationally modified via *O*-GlcNAcylation at multiple serine/threonine residues, a process catalysed by *O*-linked β -*N*-acetylglucosamine (*O*-GlcNAc) transferase (OGT)⁶⁶. *O*-GlcNAcylation of SNAP29 attenuates SNARE complex assembly (FIG. 3a). *OGT* knockdown or expression of *O*-GlcNAcylation-defective SNAP29 facilitates the formation of SNAP29-containing SNARE complexes and promotes autophagic flux⁶⁶. Levels of UDP-GlcNAc, the donor for *O*-GlcNAc addition, are responsive to the availability of glucose, fatty acids, uridine and glutamine⁶⁷. The SNAP29 *O*-GlcNAcylation level is reduced by starvation in mammalian cells and worms⁶⁶, thus integrating nutrient status with autophagosome maturation.

Regulation of HOPS complex recruitment. During autophagosome biogenesis, the VPS34–beclin 1–ATG14 complex (PI3K complex I) generates PtdIns3P on the ER and/or isolation membrane to recruit effectors such as ATG18 for autophagosome initiation^{3,68}. Steady levels of PtdIns3P on autophagic vacuoles also facilitate autophagosome maturation by recruiting the tethering factors HOPS complex (FIG. 3b). The VPS34–beclin 1–UVRAG complex (PI3K complex II), which is known for production of PtdIns3P on endosomes⁶⁹, appears to mediate the generation of PtdIns3P on autophagic vacuoles as well⁷⁰. Pacer (protein associated with UVRAG as autophagy enhancer), which is recruited to autophagic vacuoles by binding STX17 and phosphoinositides, is involved in targeting the VPS34–beclin 1–UVRAG complex⁷¹. Pacer and UVRAG also recruit the HOPS complex^{71,72} (FIG. 3b). Rubicon, a RAB7 effector, which

interacts with beclin 1 and associates with VPS34 complexes, antagonizes UVRAG function and negatively regulates autophagosome maturation^{70,73,74}. The UVRAG–Rubicon interaction is enhanced by mTORC1-mediated phosphorylation of UVRAG⁷⁵, while active GTP-bound RAB7 and Pacer compete with Rubicon to release UVRAG, thus recruiting the HOPS complex and stimulating VPS34 activity under starvation⁷¹ (FIG. 3b). However, the role of UVRAG in autophagosome maturation is debated, as it is dispensable for autophagosome–lysosome fusion in fly fat cells and in certain mammalian cells^{33,34}.

In addition to PtdIns3P, PtdIns4P also participates in multiple steps of the autophagy pathway. During autophagosome biogenesis, PtdIns4P contributes to recruitment of the ULK1 complex subunit ATG13 to the autophagosome initiation site⁷⁶. During maturation, GABARAPs recruit phosphatidylinositol 4-kinase IIa (PI4KIIa) to autophagosomes to generate PtdIns4P, thus facilitating autophagosome–lysosome fusion⁷⁷. Accumulation of PtdIns4P on the endosomes/lysosomes also promotes fusion events. The spatiotemporal distribution of PtdIns4P is regulated by PI4Ks and the PtdIns4P phosphatase SAC1 (REF.⁷⁸). SAC1 is an integral membrane protein that cycles between the ER and Golgi compartments via trafficking mediated by COPI vesicles and COPII vesicles. The ER-localized transmembrane protein SUSR2 (also known as TMEM39A) acts as an adaptor by simultaneously interacting with SAC1 and the COPII coat proteins SEC23 and SEC24 to facilitate ER-to-Golgi apparatus transport of SAC1 (REF.⁷⁹). Retention of SAC1 on the ER in *TMEM39A*-knockdown cells increases the late endosomal/lysosomal PtdIns4P level, which could result from an elevated PtdIns4P level in *trans*-Golgi network-derived anterograde vesicles and/or from an increased level of

O-GlcNAcylation

A reversible post-translational modification in which a single O-linked *N*-acetylglucosamine (O-GlcNAc) moiety is linked to serine/threonine residues of proteins. It is catalysed by O-GlcNAc transferase (OGT) and reversed by O-GlcNAcase (OGA).

COPII vesicles

Vesicles whose formation is driven by COPII coat proteins. COPII vesicles transport proteins from the endoplasmic reticulum to the Golgi apparatus.

GTPase-activating protein (GAP)

A protein that facilitates the GTPase activity of small GTPases resulting in hydrolysis of GTP to GDP and phosphate.

ER stress

A response elicited by accumulation of unfolded proteins in the endoplasmic reticulum (ER). It promotes several intracellular signal transduction pathways, collectively known as the unfolded protein response, to restore ER homeostasis.

Liquid–liquid phase separation

(LLPS). A process triggered by multivalent interactions of constituents, resulting in their concentration in a confined liquid-like compartment that stably coexists with the surrounding liquid milieu.

Mediator complex

A multiprotein complex that interacts with transcription factors and RNA polymerase II and functions as a transcriptional co-activator.

late endosomal/lysosomal PI4KII α . Consequently, the recruitment of the HOPS complex, which binds strongly to PtdIns4P³⁵, is greatly facilitated and subsequently autophagosome maturation is enhanced⁷⁹ (FIG. 3b).

Activity and dynamics of RAB7 proteins. RAB7 activity and dynamics — involving its switch between an active GTP-bound state and an inactive GDP-bound form on autophagic vesicles — are essential for progression of autophagic flux. RAB7 activity is negatively regulated by a GTPase-activating protein (GAP) called ‘Armus’ (also known as TBC1D2A), whose targeting is mediated via binding to LC3 on autophagic vacuoles and PtdIns3P generated by VPS34 on endosomes. Armus finely tunes the nucleotide cycle of RAB7 to promote autolysosome formation and acidification^{80,81} (FIG. 3b). Armus was also demonstrated to be regulated by the nutritional status of the cell via starvation-induced inactivation of the small GTPase RAC1, which competes with Armus for binding to LC3 on autophagic vacuoles⁸¹. Limiting Armus recruitment to target membranes leads to persistence of active RAB7 on autophagic vacuoles and endosomes, which may have multiple inhibitory effects on autophagosome maturation: it reduces the availability of mobile RAB7 required for endosome maturation into late endosomes/lysosomes; it maintains high activity of RAB7 effectors, such as Rubicon, whose aberrant activity may impede autophagosome maturation as described earlier; and it also impedes formation of intraluminal vesicles in multivesicular bodies⁸⁰ — a process that is important for the endocytic pathway and hence the formation of amphisomes as well as for endosome maturation into late endosomes/lysosomes^{11,18,82}. In addition to regulation by Armus, the conversion of endosomal PtdIns4P generated by PI4KII α to phosphatidylinositol 4,5-bisphosphate also inactivates RAB7 and causes its disassociation from late endosomes and from PLEKHM1. This step also facilitates autophagosome maturation⁸³.

Regulation of TFEB/TFE3 activity by cytoplasm-to-nucleus transport. TFEB and TFE3 are transcription factors with key functions in autophagy as they control expression of a network of genes involved in autophagosome formation, autophagosome maturation and lysosomal biogenesis^{84–87}. Their activity is extensively regulated by phosphorylation status, controlled by various kinases and phosphatases, with phosphorylation of TFEB and TFE3 inhibiting their cytoplasm-to-nucleus trafficking^{86,88} (FIG. 3c). This regulation is coupled to nutrient availability via mTORC1. Under nutrient-rich conditions, amino acids promote mTORC1 translocation to the lysosomal surface, where mTORC1 is activated. This lysosome surface-associated mTORC1 phosphorylates TFEB and TFE3, creating binding sites for the scaffold protein 14-3-3 for cytoplasmic retention, thereby preventing nuclear translocation and activity of these factors^{84,89}. In addition to nutrients, various extracellular signals also regulate TFEB/TFE3. For example, it has been shown that glycogen synthase kinase 3 β (GSK3 β) — which is inactivated by protein kinase C (PKC), which responds to several signal transduction

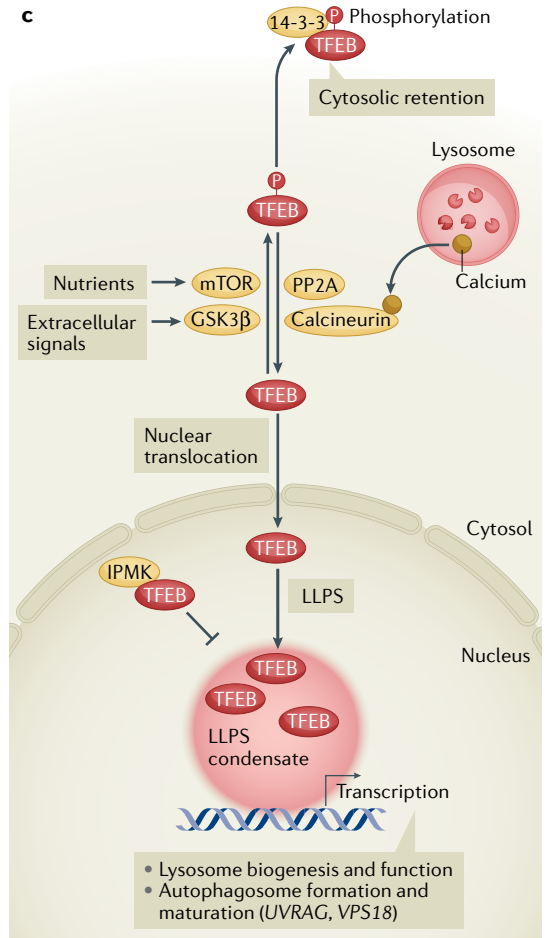
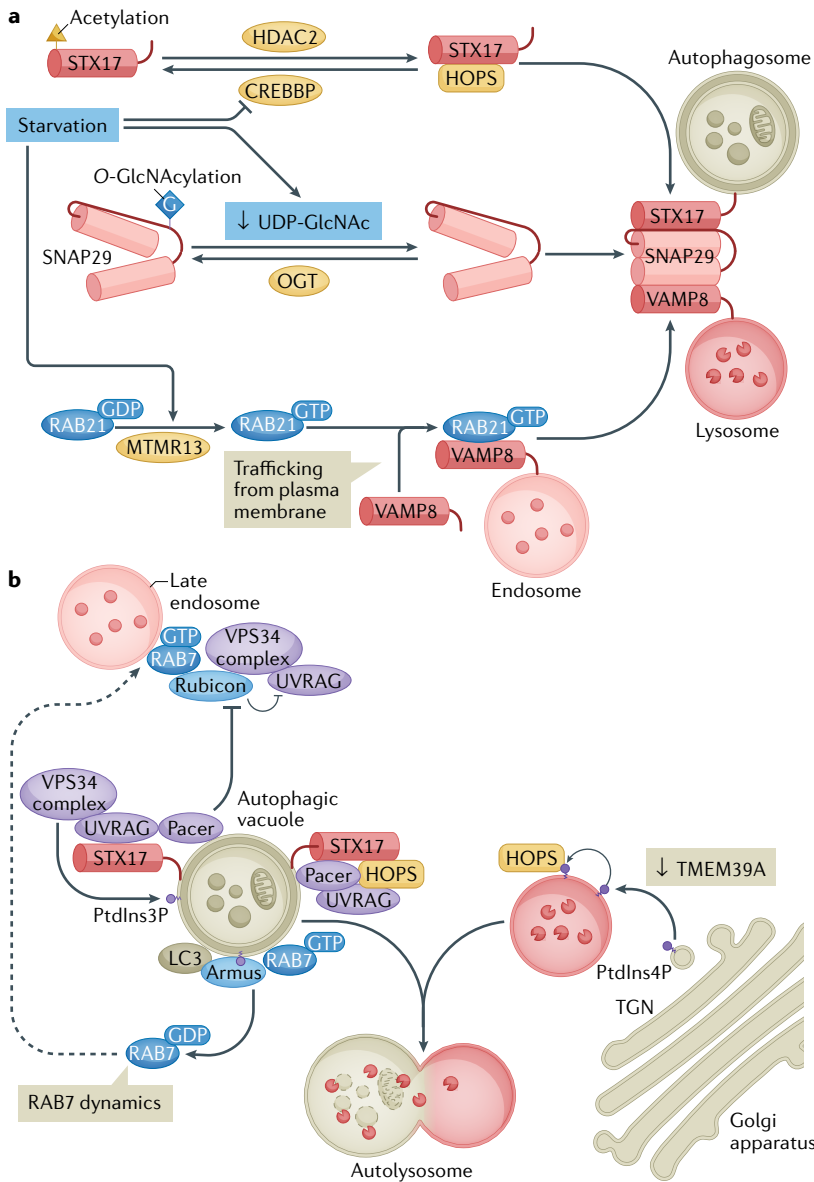
cascades — phosphorylates TFEB to prevent it from translocating into the nucleus⁹⁰. In response to various stresses, TFEB/TFE3 phosphorylation is inhibited and nuclear transport is facilitated⁸⁴. TFEB and TFE3 are also actively dephosphorylated by the ubiquitous protein phosphatase 2A (PP2A) and by calcineurin — a phosphatase activated by starvation-triggered lysosomal calcium release or ER stress^{91,92}.

Regulation of TFEB activity via phase separation.

Protein liquid–liquid phase separation (LLPS) is now an established mechanism for concentrating proteins in confined liquid-like compartments^{93–95}. LLPS also compartmentalizes transcription factors, co-activators, the Mediator complex and RNA polymerase II into condensates for mediating gene expression^{96,97}. It has been recently shown that TFEB undergoes LLPS and that phase-separated TFEB puncta colocalize with the Mediator complex and target mRNAs⁹⁸. The nuclear protein inositol polyphosphate multikinase (IPMK) directly interacts with TFEB and chaperones it to inhibit its LLPS⁹⁸ (FIG. 3c). IPMK knockout increases the formation of TFEB transcriptional condensates without altering TFEB phosphorylation or nuclear transport⁹⁸. Consequently, IPMK knockout facilitates autophagosome maturation and promotes the maturation and degradation capability of lysosomes⁹⁸.

Heterogeneity and redundancy of autophagosome maturation mechanisms. Overall, there is high variability in fusion events leading to mature, degradative autolysosomes. The process involves multiple, probably non-sequential, fusion processes, including fusion of autophagosomes with different endosomal and lysosomal structures, as well as various homotypic and heterotypic fusion events between the different types of autophagic vacuoles themselves^{11,46}. This involves different tethers, which possess differential binding affinity for ATG8 members and thus mediate maturation of distinct populations of autophagosomes decorated with different ATG8-like proteins. Accordingly, different cell types, with distinct organization of endocytic vesicles, may require different tethers for autophagosome maturation. For example, PLEKHM1 depletion causes no autophagy defect in A549 lung adenocarcinoma cells and in *C. elegans*^{40,99}. Loss of function of factors involved in autophagosome maturation may also result in accumulation of autophagic vacuoles at different stages of maturation in different cell types. Depletion of fly *Rab2* causes accumulation of autophagosomes in muscle cells and amphisomes in larval fat cells¹⁰⁰. To acquire degradative potential, autophagic vacuoles must be acidified efficiently, which is accomplished by further rounds of fusion with endosomes/lysosomes. Late endosomes/lysosomes also show heterogeneity, with different surface RAB proteins and distinct resident hydrolytic enzymes. Hence, efficient autophagic degradation requires fusion of autophagic vacuoles with multiple late endosomes/lysosomes^{11,46}, which may require distinct SNAREs and tethers.

Notably, the functions of the multiple SNARE complexes and tethering factors are partially redundant,



which, in some cases, means that impaired autophagosome maturation caused by depletion of one factor can be rescued by promotion of an alternative maturation mechanism. This is exemplified by characterization of suppressors of the autophagy defect associated with loss of EPG-5 activity in *C. elegans*. First, enhanced assembly of the STX17-SNAP29-VAMP8 complex achieved by reduced O-GlcNAcylation of SNAP29 suppresses the autophagy defect in *epg-5*-mutant worms, EPG5-deficient cells, cells depleted of VCP (also known as p97) — which is essential for maturation of ubiquitin cargo-containing autophagosomes (see also the next section) — and *Wdr45-Wdr45b* double-knockout cells^{25,66}. Second, the autophagy defect caused by EPG5 deficiency is also suppressed by enhancement of late endosomal/lysosomal recruitment of the HOPS complex in *TMEM39A*-knockdown cells or by elevation of lysosomal function and biogenesis by promoting TFEB activity via *IPMK* knockout^{79,98}. Finally, defective autophagosome maturation in *epg-5*-mutant worms

was rescued by promotion of RAB-7 dynamics on non-degradative autophagic vacuoles by expression of the GDP-bound form of RAB-7 or by promotion of lysosome biogenesis and function by depletion of the RBG-1-RBG-2 complex — a GEF and GAP complex known for promoting RAB protein dynamics for synaptic transmission and lipid droplet biogenesis. RBG-1-RBG-2 has emerged as a new regulator of RAB-7 activity, which may act by targeting the RAB-7 GEF to lysosomes, or by promoting its activity on lysosomes¹⁰¹. These mechanisms, however, fail to suppress the autophagy defect caused by knockout of genes involved in autophagosome formation^{66,79,98,101}.

Autophagosome maturation is intricately connected with endocytic trafficking pathways that are highly responsive to growth conditions. The heterogeneity and redundancy of the mechanisms governing autophagosome maturation confer flexibility and robustness on dynamic endocytic trafficking to maintain cellular homeostasis.

◀ Fig. 3 | **Multiple mechanisms regulate autophagosome maturation.** **a** | The SNARE domain of STX17 is modified by acetylation, a process controlled by the acetyltransferase CREBBP and the deacetylase HDAC2. Starvation inactivates CREBBP, resulting in deacetylation of STX17. Deacetylated STX17 interacts more strongly with the HOPS complex and SNAP29 and thus promotes autophagosome–lysosome fusion⁶⁵. SNAP29 is O-GlcNAcylated by O-linked β -N-acetylglucosamine (O-GlcNAc) transferase (OGT). This modification attenuates the assembly of SNAP29-containing SNARE complexes. Under starvation conditions that decrease the intracellular UDP-GlcNAc level, or in OGT-knockdown cells, the O-GlcNAcylation of SNAP29 is reduced, which in turn facilitates the assembly of the *trans*-SNARE complex for autophagosome maturation⁶⁶. MTMR13, the guanine nucleotide exchange factor for the endosomal protein RAB21, controls RAB21-dependent trafficking of plasma membrane-localized VAMP8 to late endosomes/lysosomes⁶³. Upon starvation, MTMR13 activates RAB21, which subsequently promotes the translocation of VAMP8 R SNARE to late endosomes/lysosomes to promote fusion of endosomes/lysosomes with autophagic vacuoles (see FIG. 1). **b** | Phosphatidylinositol 3-phosphate (PtdIns3P) on autophagic vacuoles, generated by the UVRAG-c containing VPS34 complex, facilitates autophagosome maturation by recruiting the tethering factors HOPS complex. Rubicon interacts with the UVRAG–VPS34 complex and negatively regulates its function. Pacer is targeted by autophagic vacuole-localized SNARE STX17 and phosphoinositides (PtdIns3P), and it antagonizes Rubicon and recruits the UVRAG–VPS34 complex to autophagic vacuoles. Pacer and UVRAG also recruit the HOPS complex. The RAB7 GTPase-activating protein Armus is targeted to autophagic vacuoles by interacting with LC3 and PtdIns3P, and promotes RAB7 dynamics, whereby it is recycled from the autophagic vacuole membranes. This generates a mobile pool of RAB7 that can be recruited to endosomes to drive their maturation to late endosomes/lysosomes. Depletion of the endoplasmic reticulum-localized transmembrane protein TMEM39A increases phosphatidylinositol 4-phosphate (PtdIns4P) levels on late endosomes/lysosomes (via inhibition of endoplasmic reticulum-to-Golgi apparatus trafficking of the PtdIns4P phosphatase SAC1, not shown), probably by increasing PtdIns4P levels in the *trans*-Golgi network (TGN), which promotes HOPS complex recruitment and enhances autophagosome–lysosome fusion. **c** | The transcription factor TFEB (as well as its homologue TFE3, not shown) activates the expression of genes involved in autophagy (including genes involved in autophagosome trafficking and fusion with lysosomes (UVRAG and VPS18)) and lysosomal biogenesis and function. The nuclear transport of TFEB is regulated by its phosphorylation levels. Various kinases, such as mTORC1 (downstream of nutrients) and glycogen synthase kinase 3 β (GSK3 β) (downstream of protein kinase C (PKC) signalling in response to various extracellular signals), phosphorylate TFEB to prevent its nuclear import, while protein phosphatase 2A (PP2A) and calcineurin (which is activated by calcium release from lysosomes) dephosphorylate TFEB to facilitate its translocation to the nucleus. The activity of TFEB is also controlled by liquid–liquid phase separation (LLPS), whereby TFEB forms condensates that promote gene transcription. The nuclear protein inositol polyphosphate multikinase (IPMK) directly binds to TFEB and inhibits the formation of TFEB condensates.

Autophagosome maturation and disease

Impaired formation of degradative autolysosomes is associated with the pathogenesis of various human diseases, including neurodegenerative disorders, cancer and muscle diseases (myopathies). Defective autophagosome maturation causes accumulation of toxic protein aggregates and damaged organelles that disrupt cellular homeostasis. Non-functional amphisomes, autolysosomes and even hybrid vesicles resulting from promiscuous fusion with other compartments, such as recycling endosomes, may impede endocytic trafficking and recycling, which also contribute to disease pathogenesis.

Dysfunction in neurodegenerative disorders. Autophagy is critical for neuronal function. Its key role in the maintenance of axon homeostasis relies on robust degradation-mediated recycling of synaptic proteins, protein aggregates and damaged mitochondria. Various neurodegenerative diseases have been linked to the dysfunction of the autophagy–endolysosomal system, including Alzheimer disease, Parkinson disease,

Huntington disease, amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD). Notably, in these diseases, autophagosomes are formed but then accumulate as non-degradative autophagic vacuoles^{102,103}. Various mutations have been implicated in these neurodegenerative disorders (FIG. 4). Some of these mutations affect endocytic trafficking at the intersection with autophagosome maturation¹⁰². For example, mutations affecting the p150 subunit of the dynein–dynactin motor cause motor neuron disease¹⁰⁴, while mutations in the ESCRT-III complex subunit CHMP2B cause familial ALS and FTLD¹⁰⁵. Impaired lysosomal function is also related to the pathogenesis of neurodegenerative diseases. When lysosomal acidification is defective, fusion of lysosomes with autophagic vacuoles can still occur, but the resulting autolysosomes are non-degradative¹⁰⁶. Mutant presenilin 1, one of the major causes of familial Alzheimer disease, impairs lysosomal acidification¹⁰⁷. Also, LSDs featuring dysfunctional, non-degradative autolysosomes, such as Gaucher disease, multiple sulfatase deficiency, mucopolysaccharidosis type IIIA and mucopolipidosis type IV, are associated with neurodegeneration^{108,109}. Impaired lysosomal biogenesis is also a feature of neurodegenerative disorders. In this case, disease proteins, such as accumulated α -synuclein in Parkinson disease or androgen receptor with polyglutamine expansion in X-linked spinal and bulbar muscular atrophy sequester TFEB in the cytoplasm, impairing lysosomal function and autophagosome maturation^{110,111}. Lysosomal function can also be impaired by physical damage instigated by disease proteins. Amyloid assemblies of disease-associated proteins, including $A\beta$ and tau in Alzheimer disease, α -synuclein in Parkinson disease and huntingtin (HTT) with a pathological polyglutamine expansion in Huntington disease, can be secreted and transported between cells, and, following endocytosis, can induce endolysosome rupture in recipient cells^{112,113}.

Mutations in genes directly involved in autophagosome maturation can also cause selective damage of certain populations of neurons, resulting in neurodegenerative features (FIG. 4). Mice deficient in *Epg5* show selective loss of motor neurons and display key characteristics of ALS⁴¹. Recessive mutations in human *EPG5* are associated with the multisystem disorder Vici syndrome¹¹⁴. Patients with Vici syndrome exhibit neurodevelopmental and neurodegenerative features that are recapitulated in *Epg5*-knockout mice^{115,116}. Patients with Vici syndrome also display muscle abnormalities (skeletal muscle myopathy and cardiomyopathy)¹¹⁵, which, as discussed later, have been linked to autophagosome maturation defects. In neural cells, WDR45 and WDR45B act redundantly during autophagosome maturation to target EPG5 to late endosomes/lysosomes²⁵. De novo mutations in *WDR45* cause β -propeller protein-associated neurodegeneration, previously known as static encephalopathy of childhood with neurodegeneration in adulthood^{117,118}. *Wdr45*-knockout mice exhibit extensive swollen axons and impaired learning and memory, reminiscent of β -propeller protein-associated neurodegeneration¹¹⁹. A potential causative role of *WDR45B* in intellectual disability recently emerged¹²⁰. *Wdr45b*-knockout mice

α -Synuclein

An aggregation-prone protein that accumulates in Lewy bodies and plays a central role in the pathogenesis of Parkinson disease and other synucleinopathies.

Polyglutamine expansion

An expansion of the CAG-trinucleotide repeat in disease-related genes resulting in polyglutamine expansion disorders such as Huntington disease and spinocerebellar ataxias.

X-linked spinal and bulbar muscular atrophy

An X-linked, recessive lower motor neuron disease caused by a CAG polyglutamine repeat expansion within the first exon of the androgen receptor gene.

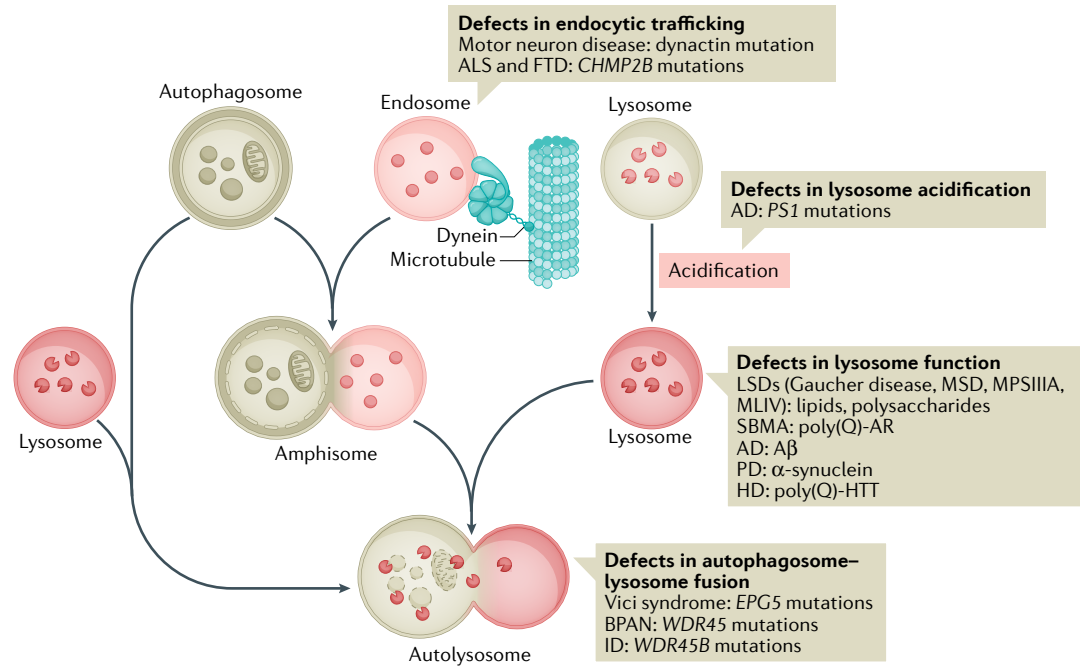


Fig. 4 | Deregulated autophagosome maturation in neurodegenerative diseases. Illustration of endolysosome trafficking and the progression of autophagosomes into autolysosomes. Impairments of several steps in the process have been linked to the pathogenesis of neurodegenerative diseases, and examples of the related diseases are shown. AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; BPAN, β -propeller protein-associated neurodegeneration; FTD, frontotemporal dementia; HD, Huntington disease; ID, intellectual disability; LSD, lysosomal storage disease; MLIV, mucopolipidosis type IV; MPSIIIA, mucopolysaccharidosis type IIIA; MSD, multiple sulfatase deficiency; PD, Parkinson disease; poly(Q)-AR, androgen receptor with polyglutamine expansion; poly(Q)-HTT, huntingtin with polyglutamine expansion; SBMA, spinal and bulbar muscular atrophy.

A β
A mixture of small peptides (37–43 amino acids) derived from sequential secretase-mediated proteolytic processing of amyloid precursor protein (APP). A β aggregates into insoluble neurotoxic plaques in patients with Alzheimer disease.

Tau
A major component of the neurofibrillary tangles found in a number of neurodegenerative diseases, including Alzheimer disease.

Vici syndrome
A multisystem disorder (agenesis of the corpus callosum, progressive microcephaly, cataracts, hypopigmentation, combined immunodeficiency and recurrent infections, and cardiomyopathy) caused by mutations in the autophagy gene *EPG5*.

β -Propeller protein-associated neurodegeneration
A disease caused by *WDR45* mutations. Patients show static psychomotor retardation in early childhood, then develop sudden-onset progressive parkinsonism, dystonia and cognitive impairment in early adulthood.

also display abnormal motor behaviour and cognitive impairment, and pathologically exhibit cerebellar atrophy and accumulation of autophagosomes in swollen axons¹²¹.

Of note, mutations in different genes acting in autophagosome maturation result in distinct neuropathological deficits. This could be because the genes are differentially expressed in different neuronal subpopulations or are required in different types of neural cells or because their mutation results in accumulation of different types of autophagic cargo that may differ in different cells. The differential function of these genes in endocytic trafficking and recycling may also contribute to the distinct pathological defects.

Pathogenesis of muscle diseases. Autophagy is essential for preserving muscle mass and maintaining myofibre integrity¹²². Defects in lysosomal function and their degradative potential have been associated with a group of autophagic vacuolar myopathies, including Pompe disease, Danon disease and X-linked myopathy with excessive autophagy, which all present with accumulation of non-functional autophagic vacuoles¹²³. Pompe disease is caused by deficiency of the lysosomal acid α -glucosidase (GAA), which hydrolyses glycogen to glucose. It is characterized by massive accumulation of autophagic vacuoles, especially in skeletal muscles¹²⁴. Patients with Danon disease, which is linked to deficiency in the lysosomal membrane protein LAMP2, display cardiomyopathy, myopathy and variable mental

retardation¹²⁵. Massive autophagic vacuoles accumulate in cardiomyocytes and skeletal muscle cells of patients with Danon disease and LAMP2-deficient mice^{125,126}. X-linked myopathy with excessive autophagy, a childhood-onset disease with progressive vacuolation and atrophy of skeletal muscle, is caused by compromised lysosomal acidification due to deficiency in the vacuolar ATPase assembly factor VMA21 (REF.¹²⁷).

Muscle cells from patients with inclusion body myopathy, Paget disease of bone and frontotemporal dementia (IBMPFD)-related myopathy accumulate large, ubiquitin-positive rimmed vacuoles, which are non-digested autophagic vacuoles¹²⁸. The causative gene for IBMPFD is *VCP*. *VCP* is primarily known for its roles in extraction of ubiquitylated proteins — which are often misfolded and prone to aggregation — from membranes or protein complexes for degradation or recycling. *VCP* knockdown or expression of IBMPFD mutant *VCP* results in accumulation of autophagic vacuoles containing ubiquitin-positive contents, and *VCP* was found to be essential for autophagosome maturation at late stages (following initial acidification), but the exact mechanisms through which *VCP* promotes further maturation are elusive^{128,129}. *VCP* mutations are also associated with familial ALS¹⁰³.

Deregulation in cancer. Autophagy functions as a tumour suppressor and promoter in a context-dependent manner^{130,131}. Autophagy impedes tumour initiation and early stages of tumour progression by

LAMP2

The receptor for chaperone-mediated autophagy. Mutations in the gene encoding LAMP2 are causatively linked with Danon disease.

EDEMosomes

Endoplasmic reticulum-derived vesicles that tune endoplasmic reticulum-associated degradation activity by targeting regulators of endoplasmic reticulum-associated degradation (such as EDEM1) to lysosomes for degradation.

ER-associated degradation

(ERAD). A process involving extraction of misfolded proteins from the endoplasmic reticulum (ER) for subsequent proteasomal degradation.

Secretion systems

Apparatuses used by bacteria to secrete virulence factors from the bacterial cytosol into host cells or the host cell environment.

acting in quality control processes such as removal of damaged mitochondria and maintenance of genomic stability. By contrast, in advanced tumours or during cancer therapy, autophagy enables tumour cells to survive harsh conditions such as hypoxia and metabolic stresses. In line with this, elevated lysosomal activity resulting from increased activity of TFEB and TFE3 — which may be caused by chromosomal rearrangements, gene amplification or upregulation, and enhanced nuclear transport — is associated with the development or metastasis of various tumours, such as renal cell carcinomas, prostate cancer, pancreatic cancer, non-small-cell lung cancer (NSCLC) and breast cancer^{88,132}. It is important to note that TFE3 and TFEB may also promote cancer progression, at least in part independently of their function in autophagy, by activating signalling pathways implicated in tumorigenesis, such as WNT and TGF β signalling¹³². However, increased expression and nuclear import of TFE3 and TFEB have been demonstrated to maintain a high autophagy level to sustain intracellular amino acid pools in the pathogenesis of pancreatic ductal adenocarcinoma¹³³. Enhanced TFEB activity can also promote lysosome exocytosis, releasing proteolytic enzymes, such as cathepsins, into the cell microenvironment, which fuels extracellular matrix remodelling, thereby stimulating cancer cell invasion and metastasis¹³⁴.

Perturbation of autophagosome maturation machinery may also contribute to the occurrence and development of tumours. Alterations in EPG5 have been suggested to be involved in breast and prostate cancers^{135,136}. *EPG5* expression is significantly lower in NSCLC clinical samples, and *EPG5* knockdown promotes NSCLC cell proliferation and tumorigenesis¹³⁷. *WDR45* is genetically altered in patients with uterine corpus endometrial carcinoma and downregulated in cervical cancer development^{138,139}. However, owing to the heterogeneity and redundancy in the autophagosome maturation machinery highlighted above, changes in individual components are probably not sufficient to drive tumorigenesis and tumorigenic progression, and it is likely that alterations in autophagosome maturation coexist with other oncogenic lesions, further aggravating pathology.

Interplay with pathogen life cycles

Autophagy responds to invading pathogens by capturing them and delivering them to lysosomes for degradation (a process known as xenophagy); this facilitates antigen presentation for activation of innate and adaptive immune responses^{15–17,140}. Pathogens have evolved diverse strategies to inhibit autophagy at the initiation and/or maturation steps to escape destruction. Certain pathogens even block autophagosome maturation and subvert the resulting vesicles for their own benefit.

Harnessing of autophagosomes/amphisomes for viral replication and release. Positive-strand RNA viruses belonging to the family *Picornaviridae*, such as poliovirus, rhinovirus, coxsackievirus B3 and enterovirus D68, utilize double-membrane vesicles (DMVs) as membrane scaffolds for replication and transcription^{141–143}.

DMVs formed in virus-infected cells are smaller than regular autophagosomes. They exhibit hallmarks of autophagosomes/amphisomes such as positivity for LC3 and the late endosomal/lysosomal marker LAMP1, but their further maturation into degradative autolysosomes is blocked^{14,144–146}.

The betacoronaviruses, including mouse hepatitis virus (MHV), Middle East respiratory syndrome coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2, also induce the formation of DMVs for anchoring the viral replication and transcription complexes^{32,147–149}. The viral RNA products are localized in the DMV lumen and transported to the cytosol for translation and virion assembly via double-membrane-spanning molecular pores¹⁵⁰. However, the canonical autophagic machinery, such as the LC3 lipidation system, is not required for DMV formation or coronavirus replication^{151,152}. Instead, the DMVs in MHV-infected cells are related to the ER-derived small vesicles, called ‘EDEMosomes’, that deliver short-lived regulators of ER-associated degradation (ERAD) to late endosomes/lysosomes¹⁵³. Certain autophagy proteins, however, are required for coronavirus infection. LC3 decorates the DMVs and is required for MHV replication¹⁵³. Unlike on autophagic structures, where LC3 is conjugated with phosphatidylethanolamine, non-lipidated LC3 is present on the DMVs in MHV-infected cells¹⁵³. Autophagy proteins involved in the generation of PtdIns3P are required for SARS-CoV-2 infection^{154,155}. The ER-localized transmembrane autophagy proteins EPG3 (also known as VMP1) and TMEM41B are essential for autophagosome formation^{39,156–159}. EPG3 and TMEM41B are also essential for replication of coronaviruses such as SARS-CoV-2 (REFS^{151,160}), but the step at which these proteins act during the viral life cycle has yet to be identified. In betacoronavirus-infected cells, the replicated viruses are transported inside lysosomes and released through the exocytic pathway¹⁶¹. Deacidification of lysosomes by loading with too many viral particles and/or by viral proteins such as ORF3a of SARS-CoV-2 promotes lysosomal exocytosis and thus viral egress from the infected cell^{32,161}. Autophagosomes also can sequestrate and mediate non-lytic extracellular release of poliovirus, coxsackievirus B3 and enterovirus D68 (REFS^{141,145,162}).

Bacterial survival and proliferation in autophagic vacuoles. Bacteria invade host cells via phagocytosis and reside in bacterium-containing vacuoles. If the vacuolar membrane is damaged, bacteria can escape into the cytosol. Autophagy captures bacteria in the cytosol or in damaged vacuoles and delivers them to lysosomes for destruction (via xenophagy)^{16,140}. Bacteria use different secretion systems to deliver effectors or toxins to evade autophagy surveillance and even to exploit autophagic vacuoles for intracellular survival and growth. Bacterial virulence factors can block autophagy by inhibiting the autophagy induction signal, impairing autophagy recognition, or directly attenuating the function of autophagy proteins^{16,140}. For example, the *Legionella pneumophila* effector protein RavZ inhibits host autophagy by functioning as

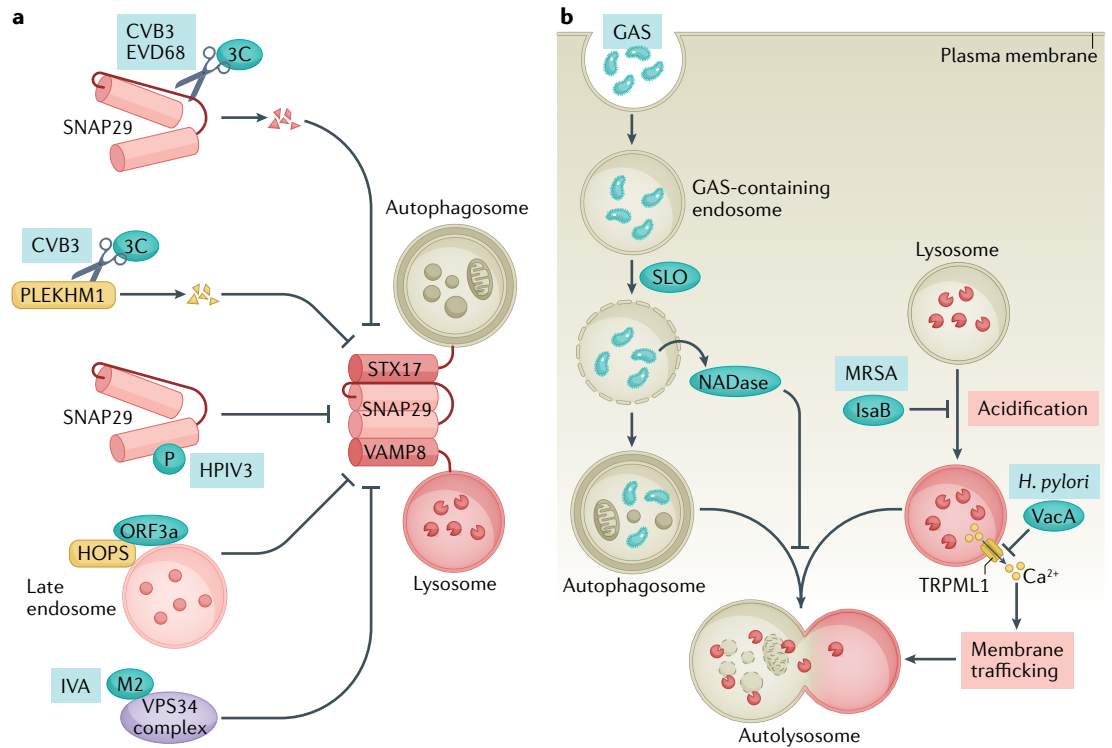


Fig. 5 | Mechanisms used by pathogens to interfere with autophagosome maturation. Autophagy can capture invading pathogens and deliver them to lysosomes for destruction. Pathogens (including viruses and bacteria) therefore use various mechanisms to block autophagosome–lysosome fusion to escape autophagy clearance. Pathogens can also modulate autophagic structures — including induction of accumulation of autophagic vacuoles or autophagy-independent double-membrane vesicles marked by certain autophagosomal proteins and modulation of endolysosomal degradative enzymes and pH — to promote their own survival, replication and/or release (not shown). **a** | Viral proteinase 3C of coxsackievirus B3 (CVB3) and enterovirus D68 (EVD68) cleaves SNAP29 to reduce SNARE assembly^{144,145}. Proteinase 3C of CVB3 also cleaves the tether protein PLEKHM1. Phosphoprotein (P) of human parainfluenza virus type 3 (HPIV3) competes with STX17 for SNAP29 binding¹⁷⁶. ORF3a of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sequesters the HOPS complex on late endosomes, thus impairing SNARE complex assembly. M2 protein of influenza virus A (IVA) dampens the activity of the VPS34 complex to prevent autophagosome maturation. **b** | Streptolysin O (SLO) damages the membrane of group A *Streptococcus* (GAS)-containing endosomes to trigger their engulfment by autophagosomes. Translocation of the co-toxin NAD-glycohydrolase (NADase) into the cytoplasm blocks the fusion of GAS-containing autophagic vacuoles with lysosomes²¹³. The virulence factor IsaB of methicillin-resistant *Staphylococcus aureus* (MRSA) blocks lysosomal acidification to suppress the function of autolysosomes²¹⁴. The virulence factor VacA of *Helicobacter pylori* prevents TRPML1-mediated calcium efflux from endosomes to disrupt endolysosomal trafficking and thus autophagosome maturation²¹⁵.

a cysteine protease that uncouples lipid-conjugated ATG8 proteins¹⁶³. RavZ cleaves ATG8 proteins between the carboxy-terminal glycine and the penultimate aromatic residue, producing ATG8 proteins that cannot be re-conjugated¹⁶³. SopF, the effector of *Salmonella enterica* subsp. *enterica* serovar Typhimurium, impairs initiation of xenophagy by interfering with the binding of the V-ATPase on damaged bacterium-containing vacuoles to ATG16L¹⁶⁴. Maturation of bacterium-containing autophagic vacuoles (autophagosomes or fused vesicles of bacterium-containing vacuoles and autophagosomes) into degradative autolysosomes by deposition of lytic enzymes can also be inhibited. In macrophages, autophagic vacuoles containing the virulent strain of *Mycobacterium tuberculosis* (H37Rv) fail to recruit RAB7 for maturation into autolysosomes^{165,166}. Vacuoles containing *Mycobacterium marinum* and *Yersinia pestis* exhibit features of non-degradative autolysosomes that are devoid of lysosomal enzymes^{167,168}. Some bacteria

(for example, *Serratia marcescens*, *Staphylococcus aureus*, *Anaplasma phagocytophilum* and *Coxiella burnetii*) even exploit autophagic vacuoles as replication niches for intracellular growth and proliferation^{169–174}. Fusion with autophagosomes facilitates the formation of vacuoles where bacteria reside and replicate — by promoting fusion of individual bacterium-containing vacuoles and/or providing a membrane for vacuole expansion — and also supplies nutrients to the pathogen^{171,172,174,175}. In line with this, replication of these pathogens is promoted by autophagy induction and is blocked by autophagy inhibition^{172–174}.

Mechanisms used by pathogens to interfere with autophagosome maturation. Inhibition of the assembly of STX17–SNAP29–VAMP8 is widely used by viruses to prevent the formation of degradative autolysosomes and/or to accumulate autophagosomes/amphisomes for their replication or release (FIG. 5a). Viral proteinase

Tat–beclin 1

A cell-penetrating peptide that induces autophagy. Eighteen amino acids from the evolutionarily conserved domain of beclin 1 are covalently coupled with the HIV-1 Tat protein transduction domain.

Lysosomotropic agents

Substances that are taken up selectively into lysosomes irrespective of their chemical nature or uptake mechanism.

Aptamers

Short, single-stranded DNA or RNA molecules or peptides that non-covalently bind to a target molecule with high affinity and specificity.

Nanobodies

Antibodies consisting of a single monomeric heavy chain variable domain used in pharmaceutical applications such as diagnosis and therapy due to their small size, solubility and stability.

Chloroquine

(CQ). An antimalarial drug also commonly used as a lysosomotropic agent that changes the lysosomal pH.

3C of coxsackievirus B3 and enterovirus D68 mediates cleavage of SNAP29, separating the two SNARE motifs and thus impairing the formation of the SNARE complex^{144,145}. The phosphoprotein (P) of human parainfluenza virus type 3 binds to SNAP29 to inhibit its interaction with STX17 (REF.¹⁷⁶), whereas cells in which hepatitis C virus is replicating exhibit reduced levels of STX17 due to its decreased expression and increased turnover¹⁷⁷. Tethering factors can also be targeted by viral effectors. PLEKHM1 is proteolytically targeted by proteinase 3C of coxsackievirus B3 to separate the HOPS complex-binding and LC3-binding amino terminus from the RAB7-interacting carboxy terminus, thus abolishing its tethering function¹⁴⁵ (FIG. 5a). Cells infected with SARS-CoV-2, or expressing the viral accessory protein ORF3a, sequester components of the HOPS complex on late endosomes³². This prevents functional HOPS complex from interacting with STX17 and consequently inhibits assembly of the STX17–SNAP29–VAMP8 complex³² (FIG. 5a). M2 protein of influenza virus A inhibits autophagosome maturation by interfering with the beclin 1-containing and UVRAG-containing VPS34 complex¹⁷⁸ (FIG. 5a). Bacterial virulence factors also block the maturation and elimination of bacterium-containing autophagic vacuoles, but the mechanisms are less well understood. Examples are shown in FIG. 5b.

Therapeutic interventions

Different steps of the autophagy pathway are potential targets for therapeutic interventions^{179,180}. Inhibitors and activators of autophagosome formation, including VPS34 and ULK1 inhibitors or the activating peptide Tat–beclin 1, are potent modulators of autophagy, but are not yet available for clinical use^{181,182}. By contrast, lysosomotropic agents, which inhibit the activity of lysosomes and block their fusion with autophagic vesicles, have been used in several clinical trials^{183–185}. Modulation of autophagosome maturation through targeting key components of the maturation machinery or through controlling autolysosomal activity by targeting the transcriptional programme of lysosome and autophagosome biogenesis is also a potential therapeutic option to regulate autophagic flux, but such approaches require further optimization if they are to be used in a clinical setting. Inhibiting and stimulating autophagosome maturation are important therapeutic avenues to explore. In many neurodegenerative diseases, autophagosome maturation is blocked^{186,187}, so restoring the autophagy flux would be an important therapeutic option. When inhibition of autophagy is required, as, for example, in cancer cells, it is important to consider the step at which the autophagic pathway must be interrupted. Different outcomes can be observed when autophagy is blocked at autophagosome formation or autophagosome maturation. In line with this, it has been shown that blocking maturation of autophagosomes favours cell death via necroptosis in human cancer cells in response to TNF-related apoptosis-inducing ligand (TRAIL), whereas blocking the formation of autophagosomes triggers TRAIL-dependent apoptosis in the same cells¹⁸⁸.

Targeting the autophagosome maturation machinery.

Targeting the machinery that controls autophagosome maturation (such as ESCRT, SNAREs and the HOPS complex) or targeting post-translational modifications of these proteins would be ideal to either increase or decrease autophagy flux^{189–191}. Inhibitors of O-GlcNAc transferase and those of the opposing enzyme, O-GlcNAcase, can stimulate or inhibit autophagosome maturation, respectively, by modulating the O-GlcNAcylation of SNAP29 (REFS^{66,192,193}) (TABLE 1). As many cellular proteins are O-GlcNAcylated, blocking O-GlcNAc sites on a specific protein would require the development of methods to increase the selectivity of targeting, such as the use of aptamers or nanobodies¹⁹⁴. Another strategy to inhibit autophagosome maturation would be to use the small molecule TCH-165 to specifically activate the proteasomal degradation of SNAP29 and STX17 (REF.¹⁹⁵). Inhibiting Rubicon is a potential approach to promote autophagy; however, pharmacological Rubicon inhibitors have yet to be developed. This strategy must be carefully evaluated because Rubicon is a positive modulator of LC3-associated phagocytosis, a process known to have a protective effect in many inflammatory diseases^{196–198}.

Modulation of the transcription programme of autophagosome maturation.

Modulation of TFEB/TFE3 is an obvious strategy to control autophagy on a transcriptional level. It has been shown that TFEB overexpression has beneficial effects in ameliorating LSDs and obesity by stimulating lipophagy^{199–201}. However, chronic overexpression of TFEB favours the progression of pancreatic tumours and NSCLCs²⁰⁰. Thus, developing small molecules to acutely stimulate TFEB and harness the autophagy–lysosomal pathway would be beneficial in ameliorating diseases in which autophagy has a defensive role²⁰¹. Small molecules can activate TFEB indirectly by modulating its upstream kinases or phosphatases (TABLE 1). For example, TFEB nuclear transport is promoted by rapamycin via inhibiting mTORC1 activity or by compounds isolated from the herb *Euphorbia peplus* Linn via the PKC–GSK3 β cascade⁹⁰.

Blocking autophagosome maturation with lysosomotropic agents.

Chloroquine (CQ), hydroxychloroquine (HCQ) and their derivatives are the only clinically approved drugs that act on autophagosome maturation (TABLE 1). They are used alone or in combination with other drugs, mostly in ongoing oncology trials, in general with the goal of optimizing therapies by blocking autophagy induced by cancer treatments^{183–185}. The new-generation dimeric CQ derivatives Ly05 and DQ661 are active at lower concentrations than CQ and HCQ^{130,183,202}.

CQ and HCQ block autophagy flux by inhibiting the hydrolytic capacity of autolysosomes. They increase the pH in autolysosomal compartments and hence block the activity of acidic proteases and other enzymes²⁰³. Beyond their capacity for H⁺ trapping, monomeric and dimeric CQ derivatives also block lysosomal function by inhibiting the activity of the lysosomal enzyme palmitoyl-protein thioesterase 1 (PPT1), which is involved in stabilizing the lysosomal localization of

Table 1 | Different strategies used to modulate autophagosome maturation

Function on autophagy	Potential therapeutic strategy	Pharmacological mechanism
Autophagy activator	Inhibitors of O-GlcNAc transferase	Stimulating autophagy by suppressing the O-GlcNAcylation of SNAP29
	Small molecules to stimulate TFEB activity	Promoting transcription of the autophagy-lysosome pathway genes and enhancing lysosomal biogenesis
	mTOR or GSK3 β inhibitors	Suppressing TFEB phosphorylation to promote TFEB nuclear translocation
	Small molecules to block Rubicon interaction with the VPS34–UVRAG complex	Stimulating autophagy by activating the VPS34–UVRAG complex
Autophagy inhibitor	Inhibitors of O-GlcNAcase	Inhibiting autophagosome maturation by facilitating the O-GlcNAcylation of SNAP29
	Chloroquine, hydroxychloroquine and their derivatives	Inhibiting autolysosomal hydrolysis
	Inhibitors of V-ATPase	Blocking the transport of protons for lysosomal acidification
	Inhibitors of lysosomal hydrolases	Blocking degradation by lysosomes
	TCH-165	Inhibiting autophagosome fusion with lysosomes by activating proteasomal degradation of STX17 and SNAP29
	PIKFYVE kinase inhibitors	Inhibiting lysosome fission and causing enlarged non-functional lysosomes

GSK3 β , glycogen synthase kinase 3 β ; O-GlcNAc, O-linked β -N-acetylglucosamine.

V-ATPase subunits³⁰². Thus, inhibition of PPT1 results in autophagy inhibition. In addition, lysosomal function and/or fusion with autophagosomes could be targeted by other means of pH regulation (such as via modulation of V-ATPase), inhibition of the cation channel TRPML1, inhibition of lysosomal enzymes or modulation of lysosomal membrane dynamics — fusion and fission — which impact on lysosomal number and function^{179,180,183,204} (TABLE 1).

Of note, lysosomotropic agents target all acidic compartments and also other pathways^{203,205}, and thus in some cases the beneficial effects of lysosomotropic agents can be attributed to mechanisms other than a blockade of autophagy^{130,203}. For example, these drugs inhibit tumour progression, independently of the autophagy blockade, by altering the trafficking of signalling molecules (that is, NOTCH1) in the endocytic pathway²⁰⁶ and by other mechanisms^{184,203}. It is also worth mentioning that the activity of CQ and HCQ observed in vitro may not be the same in vivo due to different parameters. For example, during viral infection, the cell type used in vitro may not reflect the tropism of the virus in vivo and/or the sensitivity to CQ²⁰⁷. Moreover, the acidic environment in tumours can protonate the lysosomotropic agent and greatly reduce its cellular uptake²⁰⁸.

Conclusions and perspectives

Autophagosome maturation is an essential step in the autophagy pathway that ensures the formation of degradative autolysosomes. It adds another layer of complexity and provides an extra node to integrate nutrient status and stresses for regulation of autophagic degradation. The distinct organization and trafficking of the endolysosomal compartment in different cell types

and growth conditions add complexity at the intersection of the autophagy and endocytic pathways. Thus, the *trans*-SNARE complexes and tethering factors act coordinately with context-specific factors to mediate fusion of autophagosomes with endocytic vesicles and lysosomes. Further investigations are needed to elucidate how different signalling pathways and stresses coordinate autophagosome initiation and maturation to ensure efficient progression of autophagic flux and how these processes are adapted in different cell types or pathophysiological contexts.

Autophagosome maturation is widely manipulated by pathogens to escape from destruction and for replication and growth. Pathogens that use autophagic vacuoles for replication can both activate autophagosome initiation and block maturation to achieve their maximal accumulation. Understanding how viral proteins and bacterial virulence factors modulate host autophagy will help us to develop strategies to interfere with the pathogen–host interaction and even to restore autophagy as a defence mechanism. Such strategies are urgently required with the evolution of multidrug-resistant bacteria. Elucidating the underlying mechanisms for autophagosome maturation defects and deregulation of the function of the autophagosome–lysosome system is also key for us to understand the pathogenesis of various human diseases. Targeting autophagosome maturation — via modulation of SNAREs, tethers and their regulators as well as lysosome biogenesis and function — offers an effective strategy for the treatment of these diseases.

Biomarkers and methods that reliably monitor autophagy flux in vivo are needed to examine temporal changes of autophagy activity and to evaluate interventions that target autophagosome maturation.

Positron emission tomography

An imaging technique that visualizes metabolic changes by three-dimensional mapping of administered positron-emitting radiopharmaceuticals.

A combination of assays has been used to measure autophagy flux and to monitor autophagosome maturation²⁵. However, many of these assays are difficult to implement in humans. Several methods have recently been developed to serve as reliable autophagy biomarkers in humans²⁰⁹. Analysis of autophagy flux in isolated peripheral blood mononuclear cells is used to measure autophagy activity in human blood samples^{210,211}. Positron emission tomography can be used with hypoxia tracers to correlate hypoxia and autophagy in tumors and also to gauge the level of specific autophagy substrates in tissues by the use of positron emission tomography ligands that bind to autophagy substrates^{184,209}.

The levels of specific molecules in biological fluids can also be used to determine autophagy flux in tissues. For instance, the blood level of arginase 1 reflects autophagy activity in the liver²¹². Nevertheless, these methods are of low throughput and/or can be applied only to selected cells or tissues. Thus, to screen drugs targeting autophagy, there is an urgent need for reliable, high-throughput clinical biomarkers to measure autophagic activity by the identification of tissue-specific circulating autophagy by-products and the development of flux probes for use in imaging techniques.

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Competing interests

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