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Review article

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# Autophagy genes in biology and disease

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## Abstract

Macroautophagy and microautophagy are highly conserved eukaryotic cellular processes that degrade cytoplasmic material in lysosomes. Both pathways involve characteristic membrane dynamics regulated by autophagy-related proteins and other molecules, some of which are shared between the two pathways. Over the past few years, the application of new technologies, such as cryo-electron microscopy, coevolution-based structural prediction and in vitro reconstitution, has revealed the functions of individual autophagy gene products, especially in autophagy induction, membrane reorganization and cargo recognition. Concomitantly, mutations in autophagy genes have been linked to human disorders, particularly neurodegenerative diseases, emphasizing the potential pathogenic implications of autophagy defects. Accumulating genome data have also illuminated the evolution of autophagy genes within eukaryotes as well as their transition from possible ancestral elements in prokaryotes.

## Sections



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## Introduction

Autophagy ('self-eating') is a collection of processes by which cellular components such as proteins and organelles are delivered to the lysosome or vacuole for degradation. Two autophagy pathways are well conserved in eukaryotes: macroautophagy and microautophagy (Box 1). In macroautophagy, a cup-shaped membrane structure (known as the phagophore or isolation membrane) emerges near the endoplasmic reticulum (ER), elongates, bends and finally closes via membrane fission to form a double-membraned structure called the autophagosome, capturing part of the cytoplasm inside itself (Fig. 1). The autophagosome then fuses with lysosomes (or the vacuole in yeasts and plants), where its contents are degraded. In microautophagy, the endosomal or lysosomal membrane invaginates inwards to capture part of the cytoplasm directly (Fig. 2).

The macroautophagy pathway was first observed in mammalian cells in the 1960s, and its discovery in *Saccharomyces cerevisiae* in the early 1990s fuelled rapid progress in the field, including the isolation of autophagy-deficient mutants, the identification of autophagy-related (*ATG*) genes and the characterization of related functional protein complexes[1]. Similarly, much of the progress in microautophagy research occurred in *S. cerevisiae* and other yeast species[2].

The most fundamental physiological function common to both macro- and microautophagy is thought to be the supply of nutrients in times of need, such as during starvation or development[2–6]. In addition, autophagy maintains cellular homeostasis by selectively degrading proteins, organelles and foreign entities[2–4]; this process is particularly important for long-living cells such as neurons. Occasionally, autophagy also delivers vacuolar hydrolases to fulfill biosynthetic roles in yeasts[7,8]. Multiple *ATG* genes have also been implicated in the function of non-autophagic pathways, including endocytosis, secretion and extracellular vesicle biogenesis[9]. Owing to these versatile functions, *ATG* genes have attracted attention in various research fields, and the scope of research continues to grow.

In recent years, much progress has been made in understanding the functions of individual *ATG* genes as well as their roles in disease and physiology. The molecular functions of key players have been revealed, and the importance of cargo-driven mechanisms and phase separation have been highlighted. These findings were made possible by the application of new technologies such as cryo-electron microscopy, coevolution-based structural prediction and in vitro reconstitution. In addition to the molecular machinery, another focus of autophagy research is its role in pathophysiology. Genetic analyses of human diseases (for example, neurodegenerative disorders and autoimmune diseases) have identified several *ATG* genes as causative genes or risk factors in the past decade[4,10]. Last, recent genome data have elucidated the evolution of autophagy genes within eukaryotes and possibly from prokaryotes.

In this Review, we summarize recent progress in the molecular and cellular biology of genes involved in macro- and microautophagy, the pathological relevance of these genes and key evolutionary aspects. We do not cover the third type of autophagy, chaperone-mediated autophagy, which does not involve membrane dynamics and is regulated by an entirely different set of proteins[11].

## Genes regulating macroautophagy

### Initiation of macroautophagy

The ULK complex — which is composed of the scaffold protein FIP200 (also known as RB1CC1), ATG13, ATG101 and the serine/threonine kinases ULK1 or ULK2 — is the central regulator in the initiation of macroautophagy (Table 1, Fig. 1a). Its activity is suppressed primarily by mechanistic target of rapamycin complex 1 (mTORC1). Upon starvation, mTORC1 is inactivated, leading to the activation and assembly of the ULK complex in the vicinity of the ER membrane, which recruits downstream ATG proteins to initiate autophagosome formation (Fig. 1a, Initiation). In yeasts, the homologous Atg1 complex forms similar assembled structures known as pre-autophagosomal structures (PASs). The PAS is a higher-order assembly of the Atg1 complexes, in which Atg13 tethers Atg1 (homologous to ULK1/2) and the Atg17–Atg29–Atg31 subcomplex (Atg17 is homologous to part of FIP200)[12,13]. The PAS is a fluid-like condensate resulting from liquid–liquid phase separation, which is driven by the multivalent interactions between Atg1, Atg13 and Atg17 (ref. [13]), and provides an environment that intermolecularly auto-activates the Atg1 kinase[14]. Thus, ULK/Atg1 complex assembly is a key step in initiating macroautophagy.

In addition to starvation-induced assembly, ULK/Atg1 complex assembly is also driven by autophagy cargos (Fig. 1a, Initiation). The soluble autophagy cargo adaptor SQSTM1 (also called p62) forms fluid-like condensates with ubiquitinated proteins and interacts with FIP200 to recruit the ULK complex[15]. (In this Review, 'adaptor' refers to a soluble protein that mediates binding between a cargo and the autophagy machinery, whereas 'receptor' refers to a cargo-resident protein that binds to the autophagy machinery.) During Parkin-dependent mitophagy (see below) and selective degradation of *Salmonella*, NDP52 (also called CALCOCO2) — another soluble autophagy adaptor — localizes to ubiquitinated mitochondria and the cytosol-invading bacteria and recruits the ULK complex via interaction with FIP200 (refs. [16,17]). TAX1BP1 also recruits FIP200 to SQSTM1 condensates[18]. Ubiquitin-independent selective macroautophagy also involves the cargo-driven assembly of the ULK complex: the ER-phagy receptor CCPG1 interacts with the Claw domain of FIP200 to recruit the ULK complex[19,20].

Upon assembly, the ULK complex recruits ATG9 vesicles (Fig. 1a, 'Initiation'), which are thought to be the seeds for autophagosome formation[21]. ATG9 vesicle recruitment is achieved via interactions between the HORMA domains of the ATG13–ATG101 subcomplex and the most C-terminal region of ATG9A[22] (Table 1). During Parkin-dependent mitophagy, ATG9 vesicles are also recruited through binding with the autophagy adaptor OPTN, which is present on ubiquitinated mitochondria[23]. Similarly, in yeast, Atg9 vesicles are recruited via two pathways: one through interaction with the HORMA domain of Atg13 during starvation-induced macroautophagy, and the other through the cargo adaptor Atg11 during selective macroautophagy[24].

### Membrane elongation by lipid transfer

The ULK complex also recruits the class III phosphatidylinositol 3-kinase complex I (PI3KC3–C1), which produces PI(3)P in autophagic membranes (Fig. 1a, 'Membrane elongation'). The PI3KC3–C1 is composed of five subunits: VPS34, VPS15, BECN1 (Vps30 in yeast), ATG14 and NRBF2 (Atg38 in yeast) (Table 1). PI3KC3–C1 binds to membranes via ATG14, BECN1 and VPS34, after which VPS34 generates PI(3)P[25,26].

The PI(3)P effectors include the β-propellers that bind polyphosphoinositides (PROPPIN) family proteins (WIPI proteins in mammals, and Atg18, Atg21 and Hsv2 in yeast), which further recruit ATG2. ATG2 forms a rod-shaped structure that attaches to the ER membrane with its N-terminal tip and the autophagic membrane with its C-terminal tip[27,28] (Fig. 1b). In vitro studies have shown that phospholipids are transferred through a hydrophobic cavity in ATG2, suggesting that this process occurs between the ER and phagophore in vivo[29–31].

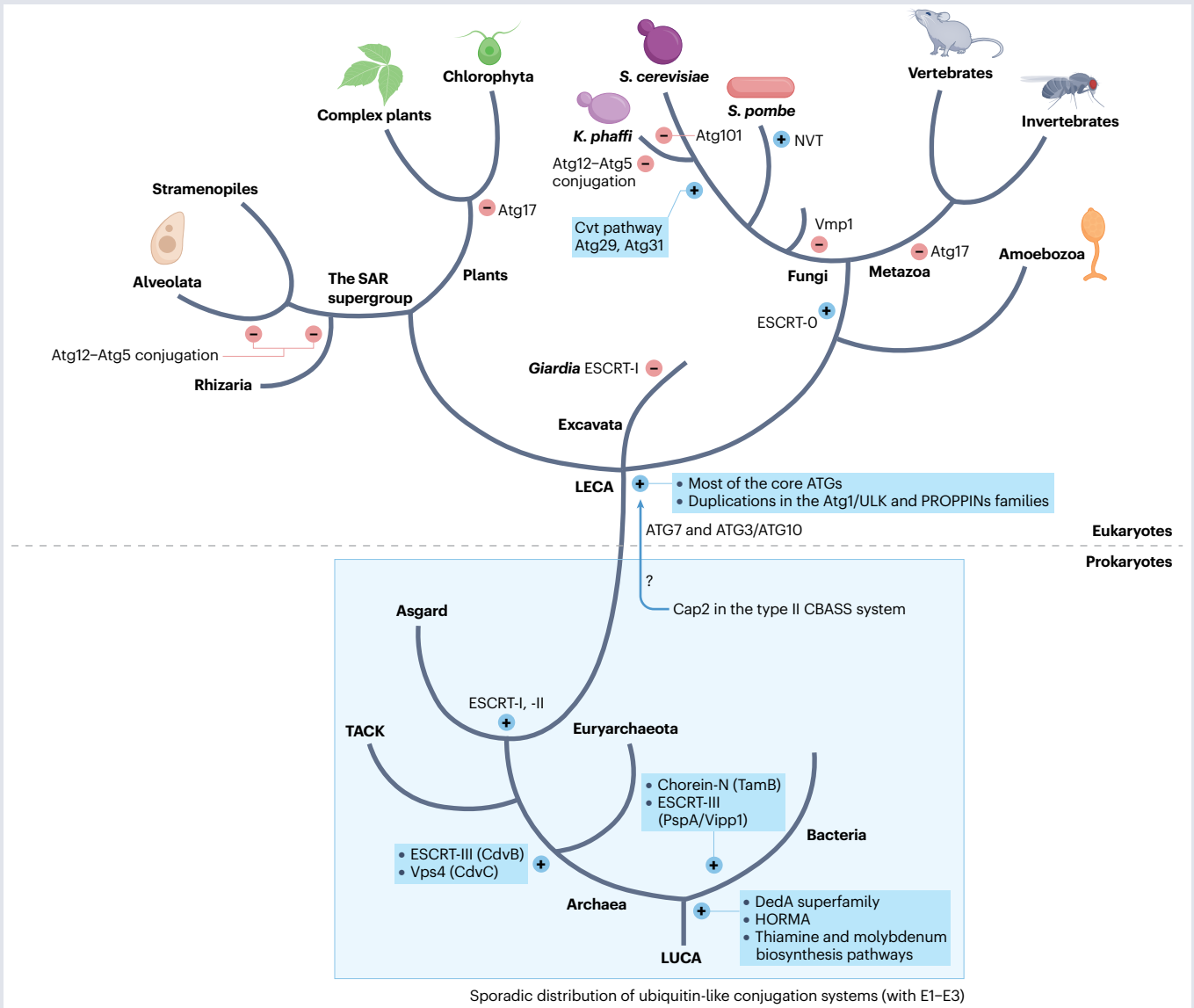
## Box 1

# Evolution of autophagy genes

Bacteria, archaea (collectively the prokaryotes) and eukaryotes constitute the three domains of life (their common ancestor is LUCA, the last universal common ancestor). Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota (TACK), Euryarchaeota and Asgard are major branches in archaea. The autophagy pathway, which requires intracellular membranous compartments, is present in eukaryotes, but absent from prokaryotes. Most, if not all, of the core autophagy-related (ATG) proteins were probably already present in the last eukaryotic common ancestor (LECA; see the figure), with subsequent extensive duplications and losses among eukaryotic lineages contributing to the observed functional diversity[180].

While many of the duplication events occurred in vertebrates and plants, two ATG protein families (the Atg1/ULK and β-propellers that bind polyphosphoinositides family proteins, PROPPINs) had possibly already diversified into different subgroups in or shortly after the LECA, although not all subgroups are involved in autophagy.

In ATG conjugation systems, canonically, ATG12 is covalently conjugated to ATG5, but *Toxoplasma*, *Plasmodium* (both Alveolata, which belongs to the Stramenopiles, Alveolata and Rhizaria (SAR) supergroup) and *Komagataella* (a yeast genus) lost the necessary proteins and/or residues (that is, the E2-like enzyme ATG10 and/or the C-terminal glycine of ATG12) for conjugation and thus rely on




Sporadic distribution of ubiquitin-like conjugation systems (with E1–E3)

(*continued from previous page*)
noncovalent interactions between ATG12 and ATG5 instead[181] (see the figure). The noncovalent form is considered adaptive because it does not require ATP or enzymes. Such covalent-to-noncovalent transitions may have occurred as many as 16 times in eukaryotes[180].

Many breakthroughs in autophagy research, including the first identification of the *ATG* genes, were made in the budding yeast *Saccharomyces cerevisiae*, which is often regarded as the standard model. However, studies in other species have revealed that the autophagy system in *S. cerevisiae* has many unconventional features (see the figure). The Atg1 complex in *S. cerevisiae* lacks ATG101, which forms a complex with and stabilizes ATG13 in other species and contains Atg29 and Atg31 in a complex with Atg17 (ref. [182]). Whether the acquisition of Atg29 and Atg31 and the loss of ATG101 are functionally linked remains unknown. Additionally, *S. cerevisiae* lacks VMP1, the endoplasmic reticulum (ER)-resident downstream (of *hisT*) *Escherichia coli* DNA gene A (DedA) superfamily protein that is required for autophagy in many other species, including metazoa, *Dictyostelium* and possibly green algae. Finally, *S. cerevisiae* has a unique pathway known as the cytoplasm-to-vacuole targeting (Cvt) pathway, which is a biosynthetic pathway that delivers vacuolar hydrolases to the vacuole. *Schizosaccharomyces pombe* has another biosynthetic pathway, termed the Nbr1-mediated vacuolar targeting (NVT) pathway (see details in the main text).

Expansions of gene families are also observed among proteins associated with selective autophagy. NBR1 (Atg19 in *S. cerevisiae*) is broadly distributed in eukaryotes[183], and SQSTM1 could be the result of NBR1 duplication followed by NBR1 domain loss. The OPTN and CALCOCO families are conserved in most metazoan species, and expansions of these families probably occurred in vertebrate lineages.

Even though many ATG proteins are eukaryote-specific, some may have originated in prokaryotes. Indeed, most of the functional complexes in the autophagy pathway contain at least one protein with remote homologues in prokaryotes (for example, the DedA superfamily proteins, including TMEM41B and VMP1, the Hop1, Rev7 and Mad2 (HORMA)-domain-containing proteins, including ATG13 and ATG101, the transmembrane portion of ATG9, the chorein-N domain at the N termini of lipid transfer proteins, including ATG2, and the ubiquitin-like ATG conjugation systems) (see the figure), suggesting that the recruitment of pre-existing genes was important for the evolution of autophagy[180,184].

The endosomal sorting complex required for transport (ESCRT) proteins, which are required for both macro- and microautophagy, also originated in prokaryotes. Although ESCRT-I, -II and -III function sequentially at the site of membrane fission in eukaryotes, the ESCRT-III proteins evolved first, in the form of PspA/Vipp1 and CdvB proteins widely distributed among bacteria and archaea, respectively[185]. By contrast, the ESCRT-I and -II proteins represent later additions and probably originated in the Asgard archaea group[186]. Therefore, the Asgard group, from which eukaryotes have been hypothesized to have emerged, already had a complete ESCRT system (though without ESCRT-0, which only occurs in the Opisthokonta).

However, the mechanism by which the transfer activity is regulated and what drives it remain to be elucidated.

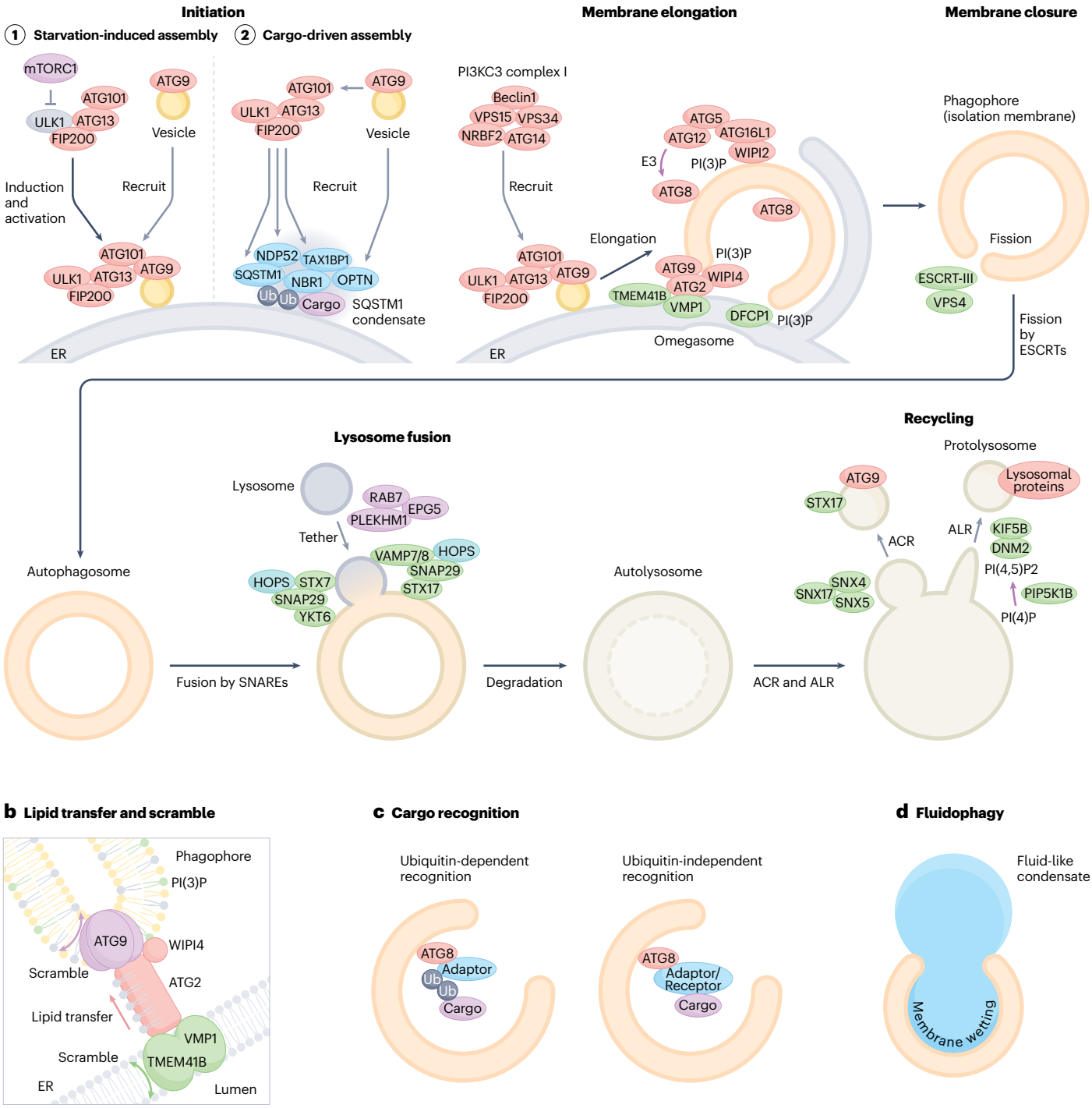
There are four PROPPIN family proteins (WIPI1–4) in mammals. ATG2 is directed to PI(3)P-rich autophagic membranes, probably through the interaction with WIPI3 or WIPI4 (refs. [27,32]). Of the four homologues, WIPI2 functions dominantly, as depletion of only WIPI2 profoundly suppresses autophagy[33], upstream of WIPI3 and WIPI4 (ref. [34]). WIPI2 binds to ATG16L1, a component of the ATG12–ATG5–ATG16L1 complex (Table 1), which has an E3-like activity to promote lipidation of ATG8 proteins (LC3 and GABARAP family proteins in mammals, which are collectively called ATG8 hereafter). ATG8 can further recruit ATG2, as ATG2 has a LC3-interacting region (LIR) (see below)[35]. Thus, WIPI2 also contributes to ATG2 recruitment indirectly.

After ATG2 transfers lipids to the outer leaflet of the autophagic membrane, ATG9 scrambles phospholipids in the membrane (Fig. 1b). ATG9 forms a trimer and translocates phospholipids between the outer and inner leaflets[36–38]. There are also two phospholipid scramblases required for autophagosome formation in the ER membrane: VMP1 and TMEM41B[38–40] (Fig. 1b). Both proteins are multi-spanning membrane proteins with a conserved DedA domain predicted to have two characteristic re-entrant loops[41,42] (Box 1). Because ATG2A interacts with ATG9 as well as VMP1 and TMEM41B[38], VMP1/TMEM41B–ATG2–ATG9 may be considered a lipid transfer unit. These scramblases can equilibrate the imbalance of phospholipid density on each leaflet caused by lipid transfer (Fig. 1b). However, if local lipid synthesis in the outer leaflet of the ER membrane produces pressure for directional lipid transfer, lipid scrambling may weaken that activity. Moreover, VMP1 and TMEM41B are important for the formation of not only autophagosomes but also lipid droplets, lipoproteins and the double-membrane structures required for the replication of several RNA viruses (including SARS-CoV-2)[43]. Therefore, their scrambling activity may be crucial for a fundamental function of the ER, not only in providing lipids to ATG2. Yeast has a TMEM41B-like protein, Tvp38, but it is not required for autophagosome formation[41]. The mechanism by which yeast can form autophagosomes without DedA family proteins has yet to be elucidated.

Two ubiquitin-like conjugation systems, the ATG8 and ATG12 systems, also have key roles in autophagosome formation. In concert with the E1-like enzyme ATG7 and the E2-like enzymes ATG3 and ATG10, ATG8 and ATG12 are conjugated to phosphatidylethanolamine (PE) and ATG5, respectively. The ATG12–ATG5–ATG16(L1) complex has an E3-like activity for ATG8–PE conjugation. Although ATG conjugation systems are essential for autophagosome formation in yeasts, seemingly normal autophagosomes can be generated in mammalian cells even when these systems are disrupted[44–46]. They seem to be more important for fusion with lysosomes or the degradation of the autophagosomal inner membrane[47,48] and, therefore, are still crucial for autophagic flux.

## Cargo recognition

Because autophagosomes engulf part of the cytoplasm (approximately 0.5–1 μm in diameter), the majority of soluble cargos are believed to be incorporated non-selectively. However, autophagosomes can also selectively recognize various cargos, including damaged organelles, intracellular bacteria and certain proteins[49]. Besides its crucial role in membrane dynamics, ATG8 has a central function in cargo recognition in selective macroautophagy (Fig. 1c). ATG8 directly interacts with the LIR motifs (or the Atg8-interacting motif in yeast) in cargos or autophagy adaptors, which




are classified into ubiquitin-dependent and -independent types (Table 2). Ubiquitin-recognizing soluble cargo adaptors include SQSTM1, NBR1, NDP52, OPTN, TAX1BP1 and TOLLIP[49]. The ubiquitin-independent category includes organelle-bound receptors for ER-phagy (for example, CCPG1, TEX264, FAM134B, SEC62, RTN3L and ATL3) and mitophagy (for example, BNIP3, BNIP3L/NIX, FUNDC1, FKBP8 and BCL2L13) as well as LIR-containing soluble proteins such as CALCOCO1 (ref. [49]). Besides cargo recognition, LIRs are used for the recruitment of some core autophagy factors, including FIP200, ULK1 and ATG13 (ref. [50]), as well as the tethering factors PLEKHM1 and EPG5 (refs. [51,52]). As discussed above, selective cargos can also recruit the ULK complex, constituting another layer of cargo recognition (Fig. 1a, 'Initiation').

**Fig. 1 | Membrane dynamics of macroautophagy. a**, (1) At initiation of macroautophagy, the ULK complex assembles near the ER membrane upon starvation and recruits ATG9 vesicles via its interaction with the ATG13–ATG101 subcomplex. (2) Alternatively, cargo adaptors such as p62, NDP52 and TAX1BP1 induce assembly of the ULK complex via interaction with FIP200, whereas ATG9 vesicles are recruited by OPTN. At the membrane-elongation step, the ULK complex recruits the class III phosphatidylinositol 3-kinase complex I (PI3KC3–C1) that produces PI(3)P, which further recruits its effector proteins, DFCP1 to omegasomes and WIPI2 and WIPI4 to phagophores. WIPI4 directs ATG2 to the phagophore membrane, which transfers phospholipids from the ER in concert with ATG9, VMP1 and TMEM41B (see **b**). WIPI2 recruits the ATG12–ATG5–ATG16L1 complex to promote LC3 lipidation on the phagophore membrane. Autophagosomes are closed by the action of the ESCRT machinery. Subsequently, PLEKHM1, EPG5 and RAB7 tether autophagosomes with lysosomes, and the two SNARE complexes, STX17–SNAP29–VAMP7/8 and YKT6–SNAP29–STX7, trigger fusion. After prolonged starvation, lysosomal membrane proteins on autolysosomes are recycled via autophagic lysosome reformation (ALR), whereas autophagosomal membrane proteins are recycled via autophagosomal components recycling (ACR). **b**, The lipid transfer protein ATG2 tethers the ER and phagophore membranes and transfers phospholipids from the ER to the phagophore. ATG9 on the phagophore membrane and VMP1 and TMEM41B on the ER membrane scramble phospholipids. **c**, In selective macroautophagy, cargos are recognized in a ubiquitin (Ub)-dependent manner (through Ub-binding adaptors) or a Ub-independent manner. Cargo adaptors/receptors bind to ATG8 on the autophagic membrane. **d**, In macro-fluidophagy, the phagophore membrane adheres to fluid-like condensates via membrane wetting.

In addition to organelles and individual proteins, fluid-like condensates formed by liquid–liquid phase separation are degraded by macroautophagy entirely or in a piecemeal manner (termed fluidophagy)[53] (Fig. 1d). As shown in the degradation of SQSTM1 condensates, the adherence of fluid-like condensates to ATG8-positive autophagic membranes is promoted by a membrane-wetting effect[53].

## Closure of autophagosomes

Autophagosome closure is mediated by membrane scission of the phagophore membrane into the outer and inner autophagosomal membranes (Fig. 1a). This is topologically identical to membrane scission of the intraluminal vesicle formation in multivesicular bodies and virus budding at the plasma membrane. Indeed, like these two processes, the endosomal sorting complex required for transport (ESCRT) complex has a pivotal role in autophagosome closure[54–56] (Table 2). How the ESCRT complex localizes to the open rim of the phagophore remains unknown in mammals. However, in yeast, Rab5-dependent interactions between Atg17 and Snf7, an ESCRT-III component, may account for the ESCRT recruitment to phagophores[55].

## Autolysosome formation and recycling

After closure, autophagosomes fuse with lysosomes to become autolysosomes. In mammals, fusion is mediated by the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins STX17 and YKT6, which are recruited to autophagosomes when they are closed[57,58] (Table 2), thereby preventing premature fusion between unclosed autophagosomes and lysosomes. STX17 and YKT6 form SNARE bundles with cytosolic SNAP29 and lysosomal VAMP7/VAMP8 and STX7, respectively (Fig. 1a, 'Lysosome fusion'). The relationship and functional differences between these two autophagosomal SNAREs (STX17 and YKT6) are not well understood; thus far, they seem to function redundantly. The role of YKT6 in autophagosome fusion is conserved in yeast[59,60], but STX17 is absent in yeast. Tethering between autophagosomes and lysosomes is mediated by multiple tethering factors, including HOPS, PLEKHM1 and EPG5 (ref. [61]). PLEKHM1 (ref. [51]) and EPG5 (ref. [52]) have a LIR motif and therefore interact with autophagosomal ATG8.

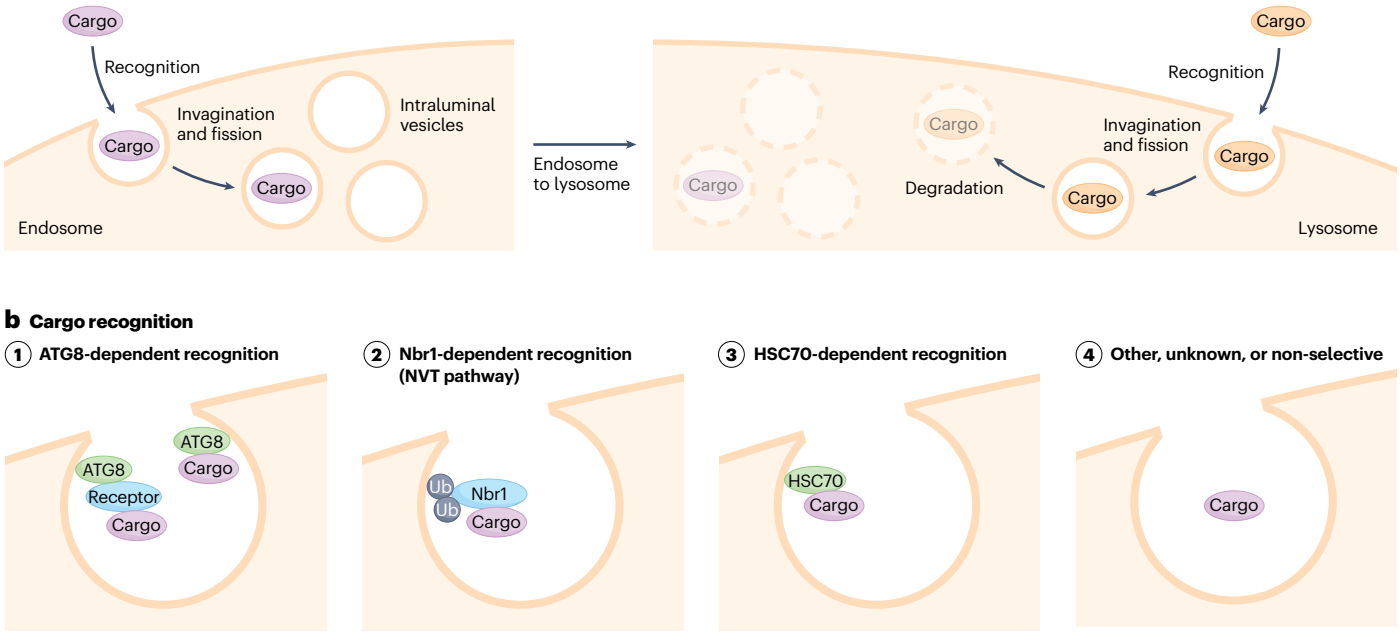
After fusing with lysosomes, the inner autophagosomal membrane is degraded. In yeast, autophagosomes fuse with the large vacuole, releasing autophagic bodies surrounded by the autophagosomal inner membranes. The vacuolar phospholipase Atg15 is responsible for degrading the membranes of autophagic bodies (derived from the autophagosomal inner membrane)[62]. Most organisms, including mammals, do not have Atg15 homologues but instead possess multiple lysosomal phospholipases, which might be redundantly involved in inner membrane degradation. One major remaining question is how degradation is limited to only the inner membrane when both the outer and inner membranes, which are derived from the same phagophore membrane, are exposed to lysosomal enzymes.

After prolonged starvation, autolysosomes deform to generate protolysosomes that then mature into functional lysosomes, in a process called autophagic lysosome reformation (ALR)[63] (Fig. 1a, 'Recycling'). This process recycles lysosomal membrane proteins and is triggered by the reactivation of mTORC1 in response to increased amino acid levels owing to prolonged macroautophagy. Upon induction of ALR, the PI(4)P 5-kinase PIP5K1B produces $PI(4,5)P_2$ on the membrane of autolysosomes, generating $PI(4,5)P_2$-rich microdomains that are further organized by clathrin and adaptor protein 2 (AP2)[64] (Table 2). Lysosomal membrane proteins are captured in these microdomains, which subsequently undergo tubulation driven by the kinesin heavy chain KIF5B[65]. The mechanism by which protolysosomes mature into lysosomes remains to be elucidated.

An additional autolysosome recycling mechanism called autophagosomal components recycling (ACR) was recently reported[66]. This mechanism recycles autophagosome-derived membrane-anchoring proteins such as ATG9 and STX17 via the budding of autolysosomal membranes depending on the SNX4–SNX5–SNX17 recycler complex and the dynein–dynactin complex (Table 2) (Fig. 1a, 'Recycling'). Thus, autophagosomal and lysosomal membrane proteins are recycled by ACR and ALR, respectively. ACR occurs earlier than ALR.

## Non-autophagic functions

Although ATG proteins were originally identified in yeast as factors required for autophagy, it is now apparent that most of them also participate in non-autophagic processes[67] (Table 1). We discuss only a few of these functions here owing to space limitations. Many of the non-autophagic functions of ATG proteins are related to membrane dynamics. BECN1 is a component of not only PI3KC3–C1 but also PI3KC3–C2 (containing UVRAG instead of ATG14), which is involved in the endocytic pathway. The tumour-suppressing activity of BECN1 is at least partially attributable to its non-autophagic function in PI3KC3–C2 (ref. [68]). Another well recognized non-autophagic process is the conjugation of ATG8 to single-membrane compartments in the endocytic pathway rather than double-membrane autophagosomes, which is known as conjugation of ATG8 to endolysosomal single membranes (CASM) or single-membrane ATG8 conjugation (SMAC), including LC3-associated phagocytosis (LAP) and LC3-associated endocytosis (LANDO)[69–71]. LAP is one of the phagocytotic pathways and degrades its contents by fusion




**c**

| Cargos | Location | Organism | Recognition | Induction | ATGs | Fission |
|---|---|---|---|---|---|---|
| Non-selective | Vacuole | Yeast | (4) Non-selective | Diauxic shift, starvation | No | ESCRT |
| ER | Vacuole | Yeast | (4) Unknown | ER stress | No | ESCRT |
| Peroxisome | Vacuole | Yeast | (1) Atg8, Atg30 | Methanol to glucose | Yes | ? |
| Mitochondria | Vacuole | Yeast | (4) Unknown | Respiratory growth | Yes | ? |
| Nucleus | Vacuole | Yeast | (1) Atg8, Atg39 | Nitrogen deprivation | Yes | ? |
| Lipid droplet | Vacuole | Yeast | (4) Unknown | Stationary phase, starvation | Yes | ESCRT |
| Lipid droplet | Vacuole | Yeast | (4) Non-selective | Diauxic shift, ER stress | No | ESCRT |
| ER | Endosome | Mammal | (1) ATG8, SEC62 | ER stress | Yes* | ESCRT |
| Mitochondria | Lysosome | Mammal | (4) Unknown | Oxidative stress | No | ? |
| Nucleus | Lysosome | Mammal | (1) ATG8, cGAS | Genotoxic stress | Yes* | ? |
| Lipid droplet | Lysosome | Mammal | (4) Unknown | Constitutive, starvation | No | ? |
| Autophagy adaptors | Endosome | Mammal | (1) ATG8 | Starvation | Yes* | ESCRT |
| KFERQ-containing proteins | Endosome | Mammal | (3) HSC70 | Constitutive | No | ESCRT |
| KFERQ-containing proteins | Endosome | Fly | (3) HSC70 | Constitutive | Yes | ESCRT |
| STING vesicles | Lysosome | Mammal | (4) TSG101, Ub | DMXAA (STING agonist) | No | ESCRT |
| HNRNPK, SAFB (LDELS cargos) | Endosome | Mammal | (1) ATG8 | Constitutive | Yes* | nSMase2 |
| Chloroplast | Vacuole | Plant | (4) Unknown | Photodamage | Yes | ? |
| Ape2, Ape4, Lap2, Ams1 (NVT cargos) | Endosome | Yeast (Sp) | (2) Nbr1 | Constitutive | No | ESCRT |

**Fig. 2 | Membrane dynamics of microautophagy. a**, Microautophagy involves the invagination of endosomal membranes (left) or lysosomal membranes (right) to incorporate cytoplasmic material. The resulting intraluminal vesicles are degraded inside lysosomes or the vacuole. **b**, Cargos are recognized by (1) ATG8, (2) Nbr1, (3) HSC70 or (4) other proteins. Microautophagy can also uptake cytoplasmic materials non-selectively. **c**, Several types of microautophagy in mammals, yeasts, flies and plants are summarized. The numbers in the 'Recognition' column correspond to those in panel **b**. The 'ATGs' column indicates dependency on autophagy-related (ATG) proteins. Asterisks indicate dependence on only ATG8. Sp, *Schizosaccharomyces pombe*. Ub, ubiquitin.

with lysosomes. LAP does not require the ULK complex but does require the ATG conjugation systems. Notably, the WD40 repeat domain of ATG16L1, which is absent in yeast Atg16 (Table 1), is essential for LAP through binding to V-ATPase, but not for canonical autophagy[72–76]. The PI3KC3–C2 component UVRAG and RUBCN are also required. A recent study reported that during LAP (or CASM), ATG8 is conjugated not only to PE, but also to phosphatidylserine (PS)[77]. It is unknown whether ATG8–PS has a unique function distinct from that of ATG8–PE. Secretory autophagy (autophagy-based unconventional secretion) is another type of non-autophagic process. In secretory autophagy, closed autophagosomes do not fuse with lysosomes, but instead fuse with the plasma membrane, secreting cytosolic proteins lacking conventional leader

**Table 1 | The autophagy-related (ATG) proteins required for autophagosome formation**

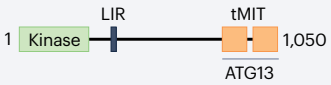
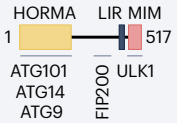
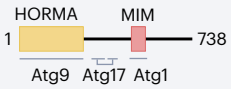
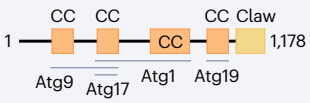
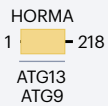
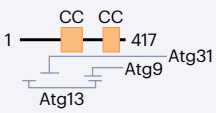
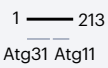
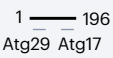
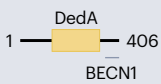
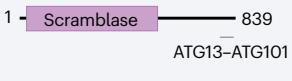
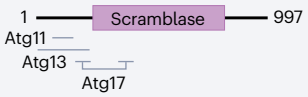
| Domain structure and interacting ATG proteins | | | Autophagic function | Non-autophagic function |
|---|---|---|---|---|
| ULK/Atg1 complex | **ULK1** |  | Serine/threonine kinase that regulates autophagy initiation | Axonal guidance; ER-to-Golgi trafficking; necrotic cell death; type I interferon signalling; regulation of glucose metabolism |
| | **Atg1** |  | | |
| | **ATG13** |  | Tethers ULK/Atg1 and FIP200 (Atg17), and recruits ATG9/Atg9 | Control of viral replication |
| | **Atg13** |  | | |
| | **FIP200** |  | Scaffolding protein in the ULK/Atg1 complex; Atg11 is only required for selective autophagy in the budding yeast | Control of viral replication; maintenance and differentiation of neural progenitor/stem cells; regulation of mitotic spindle alignment; regulation of interferon signalling; secretory autophagy |
| | **Atg11** |  | | |
| | **ATG101** |  | Binds to and stabilizes ATG13 | NR |
| | **Atg17** |  | Scaffolding protein that forms the Atg17–Atg29–Atg31 subcomplex | NR |
| | **Atg29** |  | Component of the Atg17–Atg29–Atg31 subcomplex | NR |
| | **Atg31** |  | Component of the Atg17–Atg29–Atg31 subcomplex | NR |
| DedA superfamily | **TMEM41B** |  | Lipid scramblase on the ER side essential for the autophagosome formation | Viral infection and replication; lipid homeostasis and lipoprotein secretion; regulation of ER–organelle contact sites; regulation of SERCA activity |
| | **VMP1** |  | | |
| ATG9/Atg9 vesicle | **ATG9A** |  | Lipid scramblase essential for the expansion of the autophagosomal membrane | Necrosis; hypoxia response; regulation of innate immunity; Golgi integrity; plasma membrane protection; cell migration; transport of lysosomal hydrolases; coupling of synaptic vesicle cycle with autophagy; lipid mobilization; regulation of tissue homeostasis |
| | **Atg9** |  | | |

**Table 1 (continued) | The autophagy-related (ATG) proteins required for autophagosome formation**

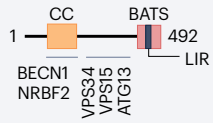
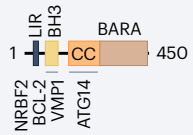
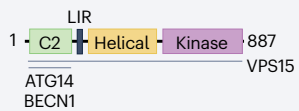
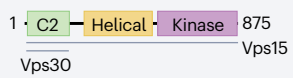
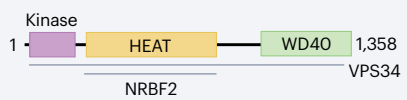
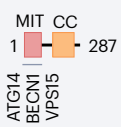
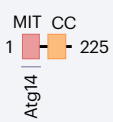
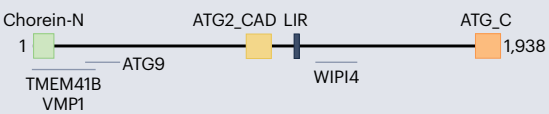
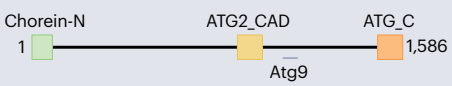
| Domain structure and interacting ATG proteins | | | Autophagic function | Non-autophagic function |
|---|---|---|---|---|
| PI3KC3 complex I | **ATG14** |  | Regulatory subunit of the PI3KC3 complex I, important for membrane binding and localization to the site of autophagosomal formation | Regulated cell death; secretory autophagy |
| | **Atg14** |  | | |
| | **BECN1** |  | Regulatory subunit of the PI3KC3 complex I, important for membrane binding and localization to the site of autophagosomal formation | Forms the PI3KC3 complex II important for endocytic trafficking, vacuolar protein sorting and cytokinesis; LC3-associated phagocytosis; regulated cell death; tumour suppressor; eructophagy |
| | **Vps30** |  | | |
| | **VPS34** |  | Class III PI 3-kinase that produces PI(3)P, essential for the nucleation of autophagosomes and recruitment of downstream factors | Forms PI3KC3 complex II important for endocytic trafficking, vacuolar protein sorting and cytokinesis; LC3-associated phagocytosis |
| | **Vps34** |  | | |
| | **VPS15** |  | Subunit in the PI3KC3 complex I that allosterically regulates VPS34/Vps34 activity | Forms PI3KC3 complex II important for endocytic trafficking, vacuolar protein sorting and cytokinesis; LC3-associated phagocytosis |
| | **Vps15** |  | | |
| | **NRBF2** |  | Accessory subunit in the PI3KC3 complex I that plays a role in assembly and regulation | NR |
| | **Atg38** |  | | |
| ATG2/ Atg2–PROPPINs | **ATG2A** |  | Lipid transfer protein that tethers the ER and the growing phagophore | Regulation of the morphology and distribution of lipid droplets; secretory autophagy |
| | **Atg2** |  | | |

**Table 1 (continued) | The autophagy-related (ATG) proteins required for autophagosome formation**

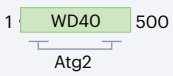
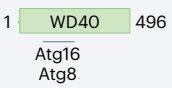
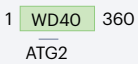
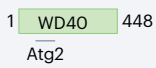
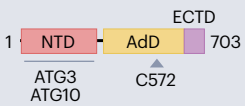
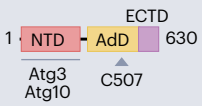
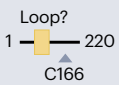
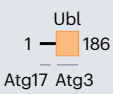
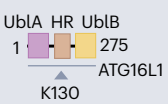
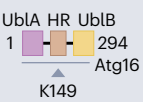
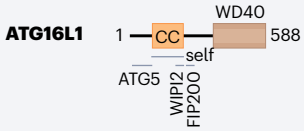
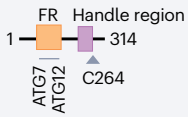
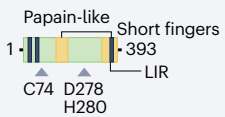
| Domain structure and interacting ATG proteins | | | Autophagic function | Non-autophagic function |
|---|---|---|---|---|
| ATG2/Atg2–PROPPINs (continued) | **WIPI2** |  | PROPPIN family proteins that bind to PI(3)P; WIPI4, Atg18 and Hsv2 form a complex with ATG2/Atg2, while WIPI2 and Atg21 bind to ATG16L1/Atg16 | Regulation of vacuole morphology (Atg18); apicoplast biogenesis (Atg18); membrane fission at endosomes |
| | **Atg18** |  | | |
| | **Atg21** |  | | |
| | **WIPI4** |  | | Endosome-to-Golgi recycling (Hsv2); LKB1–AMPK signalling (WIPI3 and WIPI4) |
| | **Hsv2** |  | | |
| The ATG12/Atg12 and ATG8/Atg8 conjugation systems | **ATG7** |  | E1-like enzyme that activates and forms covalent bonds with the ubiquitin-like proteins ATG12/Atg12 and ATG8/Atg8 | LC3-associated phagocytosis; conventional and unconventional secretion; interferon-mediated immune response; regulated cell death; regulation of cell cycle; TFEB activation; eructophagy |
| | **Atg7** |  | | |
| | **ATG10** |  | E2-like enzyme in the ATG12/Atg12 system | NR (likely functions in LC3-associated phagocytosis) |
| | **Atg10** |  | | |
| | **ATG12** |  | Ubiquitin-like protein in the ATG12/Atg12 system; the ATG12–ATG5–ATG16L1/Atg12–Atg5–Atg16 complex acts as an E3-like enzyme for the ATG8/Atg8 system | LC3-associated phagocytosis; conventional and unconventional secretion; regulated cell death; apicoplast biogenesis |
| | **Atg12** |  | | |
| | **ATG5** |  | Substrate to which ATG12/Atg12 is conjugated | LC3-associated phagocytosis; conventional and unconventional secretion; interferon-mediated immune response; regulated cell death; TFEB activation; eructophagy; lysosomal membrane turnover; apicoplast biogenesis |
| | **Atg5** |  | | |

**Table 1 (continued) | The autophagy-related (ATG) proteins required for autophagosome formation**

| Domain structure and interacting ATG proteins | | Autophagic function | Non-autophagic function |
|---|---|---|---|
| The ATG12/Atg12 and ATG8/Atg8 conjugation systems (continued) | **ATG16L1**   **Atg16**   | Membrane-binding protein that recruits the ATG12–ATG5–ATG16L1/Atg12–Atg5–Atg16 complex to the site of autophagosome formation | LC3-associated phagocytosis; secretion; anti-inflammatory functions; interferon-mediated immune response; TFEB activation; STING-induced LC3 lipidation of single membranes; eructophagy; apicoplast biogenesis |
| | **ATG3**   **Atg3**   | E2-like enzyme in the ATG8/Atg8 system | LC3-associated phagocytosis; multivesicular body distribution; secretion; TFEB activation; interferon-mediated immune response; apicoplast biogenesis |
| | **LC3B**   **Atg8**   | Ubiquitin-like protein that has important roles in autophagosome formation, maturation and cargo selection; the mammalian ATG8 family includes LC3A, -B and -C, GABARAP and GABARAPL1, and -L2 | LC3-associated phagocytosis; conventional and unconventional secretion; interferon-mediated immune response; viral replication and exocytosis; TFEB activation; regulation of vacuole membrane dynamics and function; RNA biology; apicoplast biogenesis |
| | **ATG4B**   **Atg4**   | Cysteine protease that pre-processes and recycles ATG8/Atg8 during autophagy | LC3-associated phagocytosis; secretion; apicoplast biogenesis |

Domain structure and interacting proteins, autophagic and non-autophagic functions are summarized in the left, middle and right columns. The non-ATG proteins VPS34/Vps34 and VPS15/Vps15 are included because they are major components of the PI3KC3 complex I, which is essential for autophagy. Capitalized and non-capitalized protein names refer to human and *Saccharomyces cerevisiae* proteins, respectively. The LC3-interacting region (LIR) motifs (dark blue bars) and the catalytic cysteine and the lysine residue in ATG5 to which ATG12 is conjugated (grey triangles) are shown along with the domain structures. ATG, autophagy-related. AIM, Atg8-family interacting motif. tMIT, tandem microtubule interacting and transport. HORMA, Hop1, Rev7 and Mad2. MIM, MIT-interacting motif. NTD, N-terminal domain. CC, coiled-coil. DedA, downstream (of *hisT*) *E. coli* DNA gene A. BATS, Barkor/ATG14(L) autophagosome-targeting sequence. BARA, β–α repeated, autophagy-specific. BH3, BCL-2 homology 3. PI3KC3, class III phosphatidylinositol 3-kinase. HEAT, Huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1. PI(3)P, phosphatidylinositol 3-phosphate. AdD, adenylation domain. ECTD, extreme C-terminal domain. HR, helix-rich. FR, flexible region. PROPPIN, β-propellers that bind to polyphosphoinositides. NR, not reported. References are available in Supplementary Table S1.

sequences. ATG proteins are required to form autophagosomes in this pathway, and inhibition of autophagosome–lysosome fusion leads to upregulation of secretory autophagy[78]. In addition, autophagy-related genes are involved in viral replication and transmission. Many viruses exploit autophagosome-like vesicles for replication and exocytosis[79]. A genome-wide CRISPR screen identified TMEM41B and VMP1 as host factors for flaviviruses and coronaviruses, including SARS-CoV-2, where they are thought to participate in the formation of specialized replication organelles[43]. Other examples of non-autophagic membrane-related processes that require *ATG* genes include ER-to-Golgi trafficking (ULK1 and ULK2)[80], protection against plasma membrane permeabilization (ATG9A)[81], and TFEB activation during lysosomal damage or lysosomal transient receptor potential mucolipin channel 1 (TRPML1) activation (ATG conjugation systems)[82–84]. In apicomplexan parasites such as *Plasmodium* and *Toxoplasma*, ATG8 (and the ATG conjugation systems) are essential for the biogenesis of apicoplasts[85,86], non-photosynthetic plastids specific to this lineage that support key metabolic functions.

*ATG* genes can also regulate various non-membrane-related processes, such as cell cycle progression and cell death. ATG7 directly interacts with p53, and ATG7-deficient cells show impaired cell cycle arrest and increased apoptosis (both are p53-mediated) upon nutrient starvation[87]. ATG12 promotes mitochondrial apoptosis by binding to and inactivating Bcl-2 family proteins[88]. GABARAPs are required for interferon-γ (IFNγ)-mediated antimicrobial responses through binding to ADP-ribosylation factor 1 (ref. [89]).

## Genes regulating microautophagy

### Membrane dynamics of microautophagy

Microautophagy was first described in mammalian cells as a process involving the invagination of lysosomal membranes that incorporate cytosolic material into lysosomes, followed by membrane fission and degradation[90,91] (Fig. 2a). Because observing microautophagy in small lysosomes is difficult by light microscopy, the underlying molecular mechanisms have been primarily revealed in yeasts and plants, where the vacuole is sufficiently large for optical observation. Like macroautophagy, microautophagy can be both non-selective and selective; the process non-selectively enwraps cytosolic material but also selectively recognizes organelles, such as peroxisomes (micropexophagy), mitochondria (micromitophagy), lipid droplets (microlipophagy), a subdomain of the ER (microER-phagy), a portion of the nucleus (micronucleophagy), and photodamaged chloroplasts (microchlorophagy)[2,92,93]. Microautophagy requires the ESCRT machinery at the membrane fission step[2]. However, its dependency on ATG proteins is complicated and may differ among cargo types and inducing conditions. While ATG proteins seem to be dispensable in general microautophagy and microER-phagy, at least some of the ATG proteins are required for microlipophagy[94–96], micropexophagy[97,98], micromitophagy[99] and micronucleophagy[100,101] in yeast as well as microchlorophagy[102] in plants (Fig. 2b). In mammals, micromitophagy and microlipophagy seem to be independent of ATG proteins[103,104], whereas

**Table 2 | Non-ATG molecules in macroautophagy and microautophagy**

| Functions | Proteins or complexes | |
|---|---|---|
| | Mammal | Yeast[a] |
| **Selective autophagy adaptors/receptors** | | |
| Ubiquitin-binding adaptors | SQSTM1/p62, NBR1, NDP52/CALCOCO2, OPTN, TAX1BP1, TOLLIP | Cue5 |
| ER-phagy | CCPG1, TEX264, FAM134A, FAM134B, FAM134C, SEC62[b], RTN3L, ATL3, CDK5RAP3/C53 | Atg39, Atg40, Epr1 (Sp) |
| Nucleophagy | NR | Atg39 |
| Golgi-phagy | CALCOCO1 | NR |
| Mitophagy | NDP52, OPTN, TAX1BP1, BNIP3, BNIP3L/NIX, FUNDC1, FKBP8, BCL2L13, TRIM5, NLRX1 | Atg32, Atg43 (Sp) |
| Pexophagy | SQSTM1, NBR1, BNIP3L/NIX | Atg30 (Kp), Atg36 |
| Lysophagy | SQSTM1, TAX1BP1 | NA |
| Xenophagy | SQSTM1, NDP52, OPTN, TAX1BP1, TOLLIP | NA |
| Ferritinophagy | TAX1BP1[b], NCOA4[b] | NA |
| Cvt pathway | NA | Atg19, Atg34 |
| Endosomal microautophagy | HSPA8/HSC70, SQSTM1, NBR1, NDP52, TAX1BP1 | Nbr1 |
| **Membrane closure and invagination** | | |
| Autophagosome closure | ESCRT complex, VPS4 | |
| Invagination and/or closure in microautophagy | ESCRT complex, VPS4 | |
| Membrane invagination in LDELS | SMPD3/nSMase2 | NR |
| **Autophagosome–lysosome fusion** | | |
| SNARE pairs | STX17–SNAP29–VAMP7/VAMP8<br>YKT6–SNAP29–STX7 | Ykt6–Vam3–Vti1–Vam7 |
| Tethers | HOPS complex, PLEKHM1, EPG5 | The HOPS complex |
| Rab | RAB7 | Ypt7 |
| **Degradation of the autophagosomal inner membrane** | | |
| Phospholipase | NR | Atg15 |
| **Autolysosome recycling** | | |
| Autophagic lysosome reformation | PIP5K1A, PIP5K1B, clathrin, AP2, KIF5B, DNM2 | NR |
| Autophagosomal components recycling (recycler) | SNX4, SNX5, SNX17 | NR |
| **Other autophagy regulators** | | |
| PI3KC3 complex I/II components | AMBRA1, UVRAG, RUBCN/Rubicon | Vps38 |
| Omegasome protein | ZFYVE1/DFCP1 | NR |

[a]Factors used in *Saccharomyces cerevisiae* are listed unless otherwise noted. [b]Also used for microautophagy. Sp, *Schizosaccharomyces pombe*, Kp, *Komagataella phaffii* (*Pichia pastoris*). LDELS, LC3-dependent extracellular vesicle loading and secretion. NA, not applicable. NR, not reported. References are available in Supplementary Table S2.

micronucleophagy mediated by cGAS requires the ATG8 conjugation system for cargo recognition[105]. Thus, microautophagy can be roughly divided into two types: ATG-independent and ATG-dependent (Fig. 2c). In the latter type, ATG proteins can be involved in the formation of additional membrane structures, the remodelling of vacuolar morphology, and/or the recognition of selective cargos (see below)[2,92,93].

In mammalian cells, multivesicular body formation of endosomes is considered to be a type of microautophagy referred to as endosomal microautophagy[106]. Endosomal microautophagy occurs constitutively and is also induced during early periods of amino acid starvation, leading to the degradation of cytosolic proteins, particularly selective macroautophagy adaptors, such as SQSTM1, NDP52, NBR1, TAX1BP1 and NCOA4 (an adaptor for ferritin)[5] (Fig. 2b). The multivesicular body pathway in yeast is also known to be induced by starvation and contributes to early proteome remodelling during starvation[6]. Starvation-induced endosomal microautophagy in mammals requires ESCRT-III (CHMP4B) and VPS4 but not ESCRT-0, -I or -II (ref. [5]). The necessity of ATG proteins in this pathway is also complicated. The ATG8 conjugation system is required for the degradation of SQSTM1 and NDP52 and partially required for NBR1 and TAX1BP1 but not for NCOA4, whereas FIP200 and VPS34 are not required for any of them[5]. Therefore, ATG proteins should be important for cargo recognition rather than membrane dynamics in this case (Fig. 2c).

Endosomal intraluminal vesicles formed by microautophagy are directed to lysosomes for degradation, but they are also secreted to the extracellular space in mammals. Several RNA-binding proteins, including HNRNPK and SAFB, are incorporated into endosomal intraluminal vesicles by the LC3-dependent endosomal microautophagy-like pathway, referred to as LC3-dependent extracellular vesicle loading and secretion (LDELS)[107]. This process differs from canonical endosomal microautophagy in that it is independent of ESCRTs; however, it is dependent on ceramide produced by neutral sphingomyelinase 2 (nSMase2, also known as SMPD3), which is an alternative pathway of endosomal membrane invagination (Table 2).

### Cargo recognition

For cargo recognition, ATG-dependent microautophagy in yeast utilizes Atg8 and/or Atg11, which interact(s) with organelle-bound selective macroautophagy receptors, including Atg30 in micropexophagy[108] and Atg39 in micronucleophagy[101] (part (1) of Fig. 2b,c). Additionally, in mammals, the ER-phagy receptor SEC62 mediates microER-phagy in an ATG8 binding-dependent manner[109].

By contrast, cargo recognition in ATG-independent microautophagy is not well understood. Specific subdomain formation may be important. For example, in microER-phagy in yeast, the ER membrane forms multilamellar whorls consisting of ribosome-free ER membrane to be subjected to microautophagy[110]. In *Schizosaccharomyces pombe*, microautophagy is used for a biosynthesis pathway for vacuolar enzymes, termed the Nbr1-mediated vacuolar targeting (NVT) pathway (part (2) of Fig. 2b,c), which is functionally similar to the cytoplasm-to-vacuole targeting (Cvt) pathway in *S. cerevisiae* but uses a different route[8,111]. This pathway is independent of ATG proteins but requires Nbr1 to recognize its cargos, such as aminopeptidases (Ape2, Ape4 and Lap2) and α-mannosidase (Ams1). In the NVT pathway, recruitment of Nbr1 to the endosomal membranes is mediated by ubiquitin[8], in sharp contrast to the process of macroautophagy, in which mammalian NBR1 (or its yeast homologue Atg19) is recruited by ATG8.

In mammalian cells, fluid-like ferritin–NCOA4 condensates are subjected to macroautophagy and endosomal microautophagy, both of which require TAX1BP1 for incorporation[112,113]. Because TAX1BP1 interacts with NCOA4, TAX1BP1 can bridge autophagosomal ATG8 and ferritin–NCOA4 condensates in macroautophagy. However, the mechanism by which TAX1BP1 mediates the incorporation of ferritin–NCOA4 condensates to endosomes has yet to be elucidated, because it is largely independent of ATG8 (ref. [5]).

During endosomal microautophagy in mammals, cargo recognition is achieved, in part, by the cytosolic chaperone HSC70/HSPA8 (part (3) of Fig. 2b,c)[106]. HSC70 recognizes the KFERQ-like motif contained in selective cargos and incorporates them into endosomes (the KFERQ-like motif was originally identified as a signal for chaperone-mediated autophagy[11]) (Fig. 2b). In this process, HSC70 binds to PS on the endosomal membrane via its cationic domain and induces inward membrane deformation[106,114]. This KFERQ-dependent endosomal microautophagy is also conserved in *Drosophila*, despite its lack of chaperone-mediated autophagy[115]. Notably, this pathway requires Atg1 and Atg13, but not Atg5, Atg7 or Atg12.

In LDELS, ATG8 is required for recognizing the LIR sequence of RNA-binding proteins such as HNRNPK and SAFB[107]. Membrane invagination occurs even without ATG8 lipidation, but resultant intraluminal vesicles do not contain selective cargos. How ATG8 translocates to endosomes is unknown. A mechanism similar to LAP may be used. Another issue warranting investigation is why only a subset of microautophagy cargos depend on ATG8 to be recognized in both ESCRT-dependent endosomal microautophagy and LDELS.

## Autophagy gene mutations and polymorphisms in human diseases

Given the crucial roles of autophagy in various physiological processes, including stress responses and intracellular clearance, it has been postulated that autophagy is involved in the pathogenesis of human diseases. However, it is difficult to determine which diseases are associated with changes in autophagy owing to a lack of methods with which to measure autophagic activity in humans. Nevertheless, recent genetic studies have identified a number of mutations in autophagy-related genes associated with human diseases, suggesting that autophagy alteration contributes to the development of these diseases. Moreover, studies using acute systemic *Atg7* knockout and *Fip200* knockout mice[116,117] and brain-rescued systemic *Atg5* knockout mice[118] suggest that organs highly susceptible to autophagy deficiency include the nervous system, immune system, liver and intestine. Consistent with these findings, these tissues are often affected in autophagy-gene-related diseases (Tables 3,4). In this section, mutations and polymorphisms of genes involved in general and selective autophagy are discussed. However, it is important to consider that, as emphasized above, most of these autophagy genes also have non-autophagic functions (Table 1). Therefore, the identification of mutations in autophagy genes does not directly implicate a defect in canonical autophagy in the disease phenotype. The involvement of non-autophagic function should always be considered in the interpretation of these mutations.

### Mendelian disorders caused by autophagy gene mutations

Autophagy-related diseases include Mendelian disorders caused by mutations in autophagy genes (Table 3). The most frequently affected tissue seems to be the nervous system. Homozygous mutations in *ATG5* and *ATG7* were found to be associated with human neurological diseases[119,120]. Autophagy is suppressed in these diseases, but only partially, because small amounts of either the ATG12–ATG5 conjugate or LC3-II (the lipidated form) can be detected. Patients with these diseases arising from mutations in *ATG5* and *ATG7* show some overlapping

phenotypes, including ataxia and developmental delay. Patients with *ATG7* mutations also show abnormal cerebellum and corpus callosum structure and facial dysmorphism (it is unknown whether patients with *ATG5* mutations have these abnormalities). SQSTM1 accumulates inpatient-derived cells, confirming reduced autophagic flux[119,120].

Mutations in the PROPPIN family of proteins also cause neurodegenerative diseases, but their phenotypes are somewhat different. A homozygous mutation in *WIPI2* was found in patients with a complex developmental disorder known as intellectual developmental disorder with short stature and variable skeletal anomalies (IDDSSA)[121–124]. The detected Val249Met mutation reduces WIPI2–ATG16L1 binding and autophagic flux[123]. Homozygous nonsense mutations in *WDR45B/WIPI3* cause neurodevelopmental disorder with spastic quadriplegia and brain abnormalities with or without seizures (also called El-Hattab–Alkuraya syndrome)[125–128]. The *WDR45/WIPI4* gene is found on the X chromosome, and its heterozygous mutations in women and hemizygous mutations in men cause β-propeller protein-associated neurodegeneration (BPAN; originally called static encephalopathy of childhood with neurodegeneration in adulthood)[129–131]. This is a biphasic disease that demonstrates infant-onset, non-progressive psychomotor retardation, epilepsy and autism as well as adolescent-onset dystonia, Parkinsonism and dementia. Iron accumulation in the globus pallidus and substantia nigra is one of the hallmarks of this disease, but its relationship with ferritinophagy is unclear because iron accumulation has not been reported in other diseases related to autophagy gene mutations. Given that the deletion of either WIPI3 or WIPI4 suppresses autophagy only mildly compared with deletion of WIPI2, ATG5 or ATG7 (N.M., unpublished results), it is plausible that defects in yet-unknown non-autophagic functions of WIPI3 and WIPI4 may account for the severe phenotype observed in these diseases.

Mutations in genes related to selective autophagy also cause disease (Table 3). The mitophagy-related genes *PARK2/PRKN* (encoding Parkin) and *PARK6/PINK1* are mutated in juvenile-onset familial Parkinson disease[132]. Parkin, a ubiquitin ligase, ubiquitinates various proteins in depolarized mitochondria in a PINK1-dependent manner, recruiting autophagy adaptors such as NDP52 (ref. [16]) and OPTN (Fig. 1a, 'Initiation')[23]. Although Parkin- and PINK1-dependent mitophagy is clearly observed in cell culture, its physiological relevance was initially unclear because *Prkn* or *Pink1* knockout mice show almost normal basal mitophagy levels without an obvious phenotype under normal conditions[133,134]. However, recent studies revealed that aged *Prkn* knockout mice develop locomotor impairments associated with dopaminergic neuronal loss[135]. Intestinal infection could also promote neurodegeneration in *Prkn* knockout mice[136]. Furthermore, Parkin- and PINK1-dependent mitophagy is physiologically important to suppress the release of mitochondrial DNA into the cytosol and subsequent inflammation under stress conditions in vivo[137,138]. By contrast, another report suggests that PINK1-dependent mitophagy in endothelial cells could be pro-inflammatory via the release of mitochondrial formyl peptides[139]. Thus, the pathophysiological role of Parkin- and PINK1-dependent mitophagy may depend on cell type or context. Mutations in autophagy adaptors such as SQSTM1 (ref. [140]) and the ER-phagy receptor FAM134B[141] are also found in childhood-onset neurodegeneration and hereditary sensory and autonomic neuropathy type II, respectively, suggesting that defects in selective autophagy may cause these diseases (Table 3).

Although the diseases listed above are recessive, some exhibit dominant inheritance, which includes amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) (these two are often associated) and Paget disease of bone. Autosomal dominant mutations in the selective autophagy-related genes *SQSTM1, OPTN* and *TBK1* are found in association with these diseases (Table 4). TBK1 phosphorylates and regulates OPTN, but in addition to this canonical role, TBK1 can also directly recruit the PI3KC3–C1 complex in OPTN-dependent mitophagy[142]. In general, many ALS/FTD-related gene mutations, for example, those in *SOD1* and *TARDBP* (encoding TDP-43), are thought to be gain-of-function mutations and show dominant inheritance[143,144]. However, the ALS/FTD-related mutations of OPTN and TBK1 are likely to be loss-of-function mutations[144]. Both autosomal recessive and dominant inheritance patterns have been reported in ALS with OPTN mutations[145]. Thus, haploinsufficiency of OPTN and TBK1 could

**Table 3 | Mendelian diseases associated with autophagy-related-gene mutations**

| Gene | Disease | Inheritance |
|---|---|---|
| **Core ATG** | | |
| *ATG5* | Ataxia with developmental delay | AR |
| *ATG7* | Mild-to-severe intellectual disability, ataxia and tremor | AR |
| *WIPI2* | IDDSSA | AR |
| *WDR45B/WIPI3* | neurodevelopmental disorder with spastic quadriplegia and brain abnormalities with or without seizures (El-Hattab–Alkuraya syndrome) | AR |
| *WDR45/WIPI4* | BPAN | XLD |
| | Rett-like syndrome | XLD |
| *PIK3R4/VPS15* | Cortical atrophy and epilepsy | AR |
| **Tether/fusion/others** | | |
| *EPG5* | Vici syndrome | AR |
| *PLEKHM1* | Osteopetrosis | AD, AR |
| *PLEKHM2* | Recessive dilated cardiomyopathy | AR |
| *TECPR2* | Hereditary spastic paraparesis | AR |
| *RUBCN* | Spinocerebellar ataxia, autosomal recessive 15 | AR |
| **Selective autophagy** | | |
| *SQSTM1* | ALS/FTD | AD |
| | Paget disease of bone | AD |
| | Childhood-onset neurodegeneration with ataxia, dystonia and gaze palsy | AR |
| | Distal myopathy with rimmed vacuole | AD |
| *TBK1* | ALS/FTD, primary open angle glaucoma | AD |
| *OPTN* | ALS | AR, AD |
| | Primary open angle glaucoma | AD |
| *PARK2/PRKN* | Familial Parkinson disease | AR |
| *PARK6/PINK1* | Familial Parkinson disease | AR |
| *FAM134B* | Hereditary sensory and autonomic neuropathy type II | AR |
| *ATL3* | Hereditary sensory neuropathy type IF | AD |

AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; BPAN, beta-propeller protein-associated neurodegeneration; IDDSSA, intellectual developmental disorder with short stature and variable skeletal anomalies; FTD, frontotemporal dementia; XLD, X-linked dominant. Full references are available in Supplementary Table S3.

**Table 4 | Autophagy-related risk factor genes in human diseases**

| Gene | Disease | Method |
|---|---|---|
| **ATG** | | |
| *ULK1* | Crohn's disease | Candidate gene analysis |
| | Tuberculosis | Candidate gene analysis |
| *ATG4C* | Kashin–Beck disease | GWAS |
| | Crohn's disease | Association study with whole-genome and exome sequencing data |
| *ATG5* | SLE, other autoimmune diseases | GWAS |
| | SLE | Candidate gene analysis |
| | Asthma | Candidate gene analysis |
| | Behçet disease | Candidate gene analysis |
| | Cerebral palsy | Candidate gene analysis |
| *ATG7* | Huntington disease | Candidate gene analysis |
| | SLE | Candidate gene analysis |
| | Non-alcoholic fatty liver disease | Association study with whole-exome sequencing data |
| | Familial cholangiocarcinoma | Linkage analysis |
| *MAP1LC3B* | SLE | Candidate gene analysis |
| *ATG9A* | Crohn's disease | Circular chromosome conformation capture-sequencing (4C-seq) |
| *ATG10* | VKH syndrome | Candidate gene analysis |
| *ATG16L1* | Crohn's disease | GWAS |
| | COPD | Candidate gene analysis |
| | Huntington disease | Candidate gene analysis |
| *ATG16L2* | SLE | Association study with replication, Candidate gene analysis |
| | SLE | GWAS |
| | Crohn's disease | GWAS |
| **Selective autophagy-related genes** | | |
| *CALCOCO2/NDP52* | Crohn's disease | GWAS |
| *OPTN* | Paget disease of bone | GWAS |
| *PARK2/PRKN* | Leprosy | Systematic association scan of the chromosomal interval |
| *TOLLIP* | Pulmonary fibrosis | GWAS |

Diseases identified by genome-wide studies or large-scale candidate gene analyses are listed. SLE, systemic lupus erythematosus; VKH syndrome, Vogt–Koyanagi–Harada syndrome; COPD, chronic obstructive pulmonary disease; GWAS, genome-wide association study. References are available in Supplementary Table S4.

contribute to the ALS/FTD spectrum. Although some of the ALS/FTD-associated mutations affect the adaptor function of SQSTM1, OPTN and TBK1 (refs. [146,147]), it remains unknown whether this is the general mechanism. The pathogenic effects of these mutations might be mediated by their non-autophagic roles; for example, the TBK1–OPTN axis is also important for the innate immune and RIPK1-dependent cell death pathways[144]. However, a gain-of-function hypothesis cannot be entirely excluded. Of particular interest is that, like other ALS-related proteins[148], autophagy adaptors such as SQSTM1 (refs. [149–151]) and OPTN[152] form liquid-like biomolecular condensates. Mutations of these genes may exhibit some gain of toxicity.

Although most Mendelian disorders associated with autophagy gene mutations are related to the nervous system, there are some diseases involving other tissues and organs (Table 3). An example is Paget disease of bone, which is characterized by one or multiple focal regions with increased bone remodelling. Of its causative genes, *SQSTM1* is the major one; however, it remains elusive whether its autophagy adaptor function is involved in the pathogenesis of this disease[153,154].

## Genetic risk factors for human diseases

The second category includes diseases whose susceptibility is associated with polymorphism of autophagy-related genes (Table 4). The core autophagy gene first shown to be associated with human disease by a genome-wide association study (GWAS) is *ATG16L1*; the single nucleotide polymorphism resulting in the Thr300Ala (T300A) substitution is a risk factor for Crohn's disease[155,156]; Thr300 is located immediately upstream of the WD40 repeat domain (Table 1). *Atg16L1*$^{T300A}$ knock-in mice exhibit abnormalities in Paneth cells in the intestine[157] and gut microbiota[158]. The WD40 repeat domain in ATG16L1 is essential for LAP but not for canonical autophagy[72]; however, the effect of the T300A substitution on autophagy and LAP is relatively small or undetectable[157,159,160]. Nevertheless, it is possible that autophagy is associated with Crohn's disease because the disorder has also been linked to other autophagy genes (*ULK1*, *ATG9A*, *NDP52* and *ATG4C*)[161–164]. However, the fact that *ATG16L2*, which is considered to be unnecessary for autophagy[165,166], is also associated with Crohn's disease[167] suggests that a yet unknown non-autophagic function shared by ATG16L1 and ATG16L2 may be involved.

Autophagy genes such as *ATG5*, *ATG7* and *MAP1LC3B* have also been identified as susceptibility genes in autoimmune diseases, including systemic lupus erythematosus (SLE)[168] (Table 4). This may reflect the role of autophagy in mitochondrial quality control to suppress the release of SLE-inducible damage-associated molecular patterns from mitochondria[169,170]. In addition to canonical autophagy, these genes are also required for LAP. Because LAP is important for interferon production in response to the incorporation of DNA-containing immune complexes[171], the role of autophagy genes in LAP may be related to their genetic association with autoimmune diseases. Association of ATG16L2 with SLE was also identified, but the role of ATG16L2 in LAP is unclear.

Whole-exome sequencing and subsequent missense variant searches in patients with non-alcoholic fatty liver disease revealed an enrichment of the Phe426Leu and Val471Ala variants of ATG7 (ref. [172]); both are loss-of-function mutations. However, these findings are inconsistent with results obtained in mice showing that a loss of autophagy instead suppresses liver steatosis[173]. Thus, partially reduced autophagic activity in humans may have an impact on the liver different from that caused by the complete loss of autophagy observed in autophagy gene knockout mice.

The relationship between autophagy and cancer has attracted much attention. However, although there are numerous reports suggesting the association of specific tumours with autophagy gene single nucleotide polymorphisms, recurrent or driver mutations of core autophagy genes in human cancers are rather rare[174]. Thus, autophagy may be still functional in most cancers and could even be important. In fact, mouse studies have suggested that, while the deletion of

autophagy genes might promote tumorigenesis, it also affects tumour growth either through cell-autonomous or -nonautonomous mechanisms[175]. Nevertheless, mutations in core *ATG* genes might be associated with familial cancers. For example, a linkage study identified an association of a germline nonsense mutation of *ATG7* (c.2000C>T p.Arg659*) with familial cholangiocarcinoma[176]. In cancer cells, somatic deletion of *ATG7* occurs in the complementary allele, leading to complete inhibition of autophagy. This case suggests that autophagy suppression could also be tumorigenic in humans.

## Conclusions and perspective

A quarter century has passed since the first autophagy gene *ATG1*, named *APG1* initially, was cloned in yeast in 1997 (ref. [177]). During this period, our understanding of the molecular biology underlying autophagy has grown exponentially. In particular, recent structural biological approaches have provided crucial evidence to explain the unique membrane dynamics of autophagy at the molecular level. However, despite the increasing clarity of the functions of individual autophagy gene products, several key cell biological questions remain unanswered. For example, what is the mechanism of unidirectional transport of lipids from the ER to autophagosomes? How is the size of autophagosomes regulated? How is the timing of autophagosome–lysosome fusion regulated? To address these questions, new approaches, including biophysics, theoretical modelling and molecular dynamics simulation, will be useful.

Although we have aimed to summarize the latest knowledge about microautophagy, our efforts may seem incomplete because its mechanisms are still less understood than those of macroautophagy. It is intriguing that some ATG proteins (for example, ATG8) and selective autophagy adaptors (for example, NBR1) are used by both macroautophagy and microautophagy. Whether these molecules exert similar functions in both pathways needs to be elucidated in future studies. In addition, some cargos (for example, ferritin) are selectively degraded by both pathways, but the regulation mechanisms of sorting remain unknown. Further research will reveal a more complete picture of autophagy.

As we mentioned above, one of the apparent bottlenecks in autophagy research is the lack of methods with which to monitor autophagy in humans. It would be ideal if we could estimate autophagic activity by measuring some metabolites (that is, biomarkers) in the blood or urine that are secreted via autophagy-dependent pathways. Alternative techniques may be noninvasive imaging such as fluorescence molecular tomography[178] and positron emission tomography[179].

Finally, although many diseases have been found to be linked to autophagy gene mutations or associated with polymorphisms of autophagy genes, the phenotypes of these diseases are diverse. Thus, it is still difficult to offer a unified explanation of their pathogenesis. This may be due to complementation by homologues, differences in tissue expression and involvement of non-autophagic functions. More investigations will be required to reveal the exact mechanisms by which autophagy gene defects cause a wide range of human diseases.

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## Author contributions

The authors contributed equally to all aspects of the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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