The Atg8 family: multifunctional ubiquitin-like key regulators of autophagy

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Abstract

Autophagy is an evolutionarily-conserved catabolic process initiated by the engulfment of cytosolic components in a crescent-shaped structure, called the phagophore, that expands and fuses to form a closed double-membrane vesicle, the autophagosome. Autophagosomes are subsequently targeted to the lysosome/vacuole with which they fuse to degrade their content. The formation of the autophagosome is carried out by a set of autophagy-related proteins (Atg), highly conserved from yeast to mammals. The Atg8s are Ubl (ubiquitin-like) proteins that play an essential role in autophagosome biogenesis. This family of proteins comprises a single member in yeast and several mammalian homologues grouped into three subfamilies: LC3 (light-chain 3), GABARAP (γ-aminobutyric acid receptor-associated protein) and GATE-16 (Golgi-associated ATPase enhancer of 16 kDa). The Atg8s are synthesized as cytosolic precursors, but can undergo a series of post-translational modifications leading to their tight association with autophagosomal structures following autophagy induction. Owing to this feature, the Atg8 proteins have been widely served as key molecules to monitor autophagosomes and autophagic activity. Studies in both yeast and mammalian systems have demonstrated that Atg8s play a dual role in the autophagosome formation process, coupling between selective incorporation of autophagy cargo and promoting autophagosome membrane expansion and closure. The membrane-remodelling activity of the Atg8 proteins is associated with their capacity to promote tethering and hemifusion of liposomes in vitro.
Introduction

Autophagy is a catabolic process initiated by the sequestration of cytoplasmic proteins and entire organelles within a double membrane sac called the isolation membrane or phagophore. The phagophore expands and is ultimately closed resulting in the formation of the mature autophagosome. The outer membrane of the autophagosome is subsequently fused with the cellular lytic compartment (lysosome in mammals, vacuole in yeast) where the inner membrane together with the sequestered content, termed the autophagic body, is released and degraded by hydrolytic enzymes (Figure 1).

Autophagy was originally implicated in adaptive response of cells to provide nutrients and energy on exposure to stress conditions; however, it has since been connected to diverse physiological and pathological processes including differentiation, development, neurodegeneration, immune function, cancer and aging [1].

Two independent molecular genetic studies in yeast have led to the identification of the autophagy-defective apg and aut mutants [2,3]. A third overlapping group of mutants (the cvt mutants) were subsequently isolated on the basis of defects in the mechanistically related cvt (cytoplasm-to-vacuole-targeting) pathway [4]. The cvt pathway mediates the transport of vacuolar resident enzymes such as the precursor form of API (aminopeptidase-I; proAPI), Ams1 (α-mannosidase) and Ape4 (aspartyl aminopeptidase) from the cytosol to the vacuole under...
nutrient-rich conditions. The nomenclature of the autophagy-related genes was subsequently unified as ATG [5]. Within the past few years, analysis of the ATG gene products has progressed rapidly and many of their mammalian homologues have been identified and characterized.

The Atg8 family of proteins, constituting a single member in yeast and multiple homologues in higher eukaryotes, were shown to be essential regulators of autophagy [6]. These were the first molecules found to be specifically associated with autophagy-related membrane structures. Atg8 proteins are Ubl (ubiquitin-like) proteins, but rather than conjugating to another protein, they are attached to PE (phosphatidylethanolamine) leading to their tight membrane association. Newly synthesized Atg8 is rapidly C-terminally cleaved by a cysteine protease of the Atg4 family. This cleavage exposes a glycine residue which is then covalently conjugated to PE in a reaction catalysed by the E1- and E2-like enzymes, Atg7 and Atg3 respectively.

Atg8 proteins appear to play multiple roles in the autophagy process. They are responsible for specific recruitment of cargo proteins destined for lysosomal degradation and promote autophagosome maturation. This latter role is suggested to be mediated by their interaction with different effectors involved in regulating the basic autophagy machinery. In addition, Atg8s may directly support membrane fusion events driving the expansion and closure of autophagosomes.

The Atg8 family

Although yeast express only one Atg8 protein, the mammalian Atg8 homologues constitute a family of proteins subdivided into three subfamilies on the basis of amino-acid-sequence homology: MAP1LC3 (microtubule-associated protein 1 light-chain 3; hereafter referred to as LC3), GABARAP (γ-aminobutyric acid receptor-associated protein) and GATE-16 (Golgi-associated ATPase enhancer of 16 kDa). The subfamilies in different organisms vary in their gene numbers due to gene duplication and lost events that occurred during evolution. Humans have a single GATE-16 gene, two GABARAP genes [GABARAP and GABARAPL1 (γ-aminobutyric acid receptor-associated protein-like 1)] and four LC3 genes (LC3A, LC3B, LC3B2 and LC3C) [6].

LC3 was the first mammalian Atg8 homologue to be characterized. It was originally identified as the light chain of the microtubule-associated proteins 1A and 1B in rat brain. Its first implication in autophagy was provided by Kabeya et al. [7] who showed that LC3 is post-translationally processed into two forms: LC3-I, located at cytosolic fractions, and PE-conjugated LC3-II, which associates with the autophagosome membrane. The amount of LC3-II was found to be correlated with the extent of autophagosome formation. The other Atg8 homologues, belonging to the GABARAP and GATE-16 subfamily, were initially characterized as intracellular trafficking factors. GATE-16 was found to interact with the Golgi v-SNARE (vesicle-associated N-ethylmaleimide-sensitive factor attachment protein receptors) GOS-28 as well as with NSF (N-ethylmaleimide-sensitive factor) leading to activation of the ATPase activity of the latter. Through these interactions, GATE-16 was shown to modulate intra-Golgi transport by coupling between NSF activity and SNARE activation [8]. In addition, GATE-16 was implicated in post-mitotic Golgi re-assembly [9]. GABARAP was identified as a cytosolic factor regulating the intracellular transport of the γ2 subunit of GABA<sub>A</sub> receptors [10]. It was later shown that, similar to LC3, both GATE-16 and GABARAP are subjected to post-translational processing and exist in two modified forms, I and II [11]. Conversion into the II form correlates with their association with the autophagosome.
Structural studies of several Atg8 family members indicated that Atg8 proteins share a strong structural similarity to ubiquitin [12]. In addition to the ubiquitin core, comprising five β-strands flanked by two α-helices, Atg8 proteins contain two N-terminal α-helices which play a crucial role in the functionality of these proteins, probably by regulating protein–protein interactions.

Despite their structural resemblance, mammalian Atg8s show some differences in amino acid sequence. For example, the first α-helix in LC3 is basic, whereas in GATE-16 and GABARAP it has an acidic nature. The surface of the second α-helix is acidic in LC3, neutral in GATE-16 and basic in GABARAP. Whereas conserved domains among the mammals Atg8s are likely to be responsible for characteristic interactions of these proteins, such as the binding to the conjugation machinery proteins, structural differences between the various Atg8 proteins may confer differential specificity towards target proteins and reflect their diverse functions. Indeed, the mammalian homologues of Atg8 were shown to have diverse interaction profiles and to regulate different steps along the autophagic process. Moreover, some differences in the tissue distribution of Atg8s point to a tissue-specific function for some family members [6]. For simplicity, ‘Atg8’ will hereafter refer to all family members. The specific names will be used to distinguish between yeast Atg8 and the mammalian homologues.

**Atg8 processing**

The function of the Atg8 proteins is associated with their membrane-binding state. Atg8s are found in cells either as a free cytosolic form, designated Atg8-I, or a tightly membrane-bound form termed Atg8-II. Atg8-II is localized both to the inner and the outer membrane of the autophagosome. The recruitment of Atg8 on to the autophagosomal membrane depends on a series of post-translational modifications (Figure 2). Nascent Atg8 is proteolytically cleaved at its C-terminal end by a constitutively expressed cysteine protease Atg4. Mammalian homologues contain four Atg4 homologues (named Atg4A–D or autophagin1–autophagin4), showing sequence similarity to a single Atg4 in yeast [13]. This proteolytic event produces the mature Atg8-I which possesses a C-terminally exposed glycine residue. Atg8-I is then covalently conjugated to PE through an amide bond between the C-terminal glycine residue and the amino group of PE. PE-conjugated Atg8 (Atg8-II) is associated with the autophagosomal membrane. The lipidation reaction is mediated by a ubiquitination-like system involving the sequential action of Atg7 and Atg3, which serve as E1-activating- and E2-conjugating-like enzymes respectively [14]. Finally, Atg4 can attack Atg8 associated with the outer membrane of the autophagosome, deconjugating it from its target lipid. This makes the association of Atg8 with the autophagosomal membrane a reversible process. The liberated Atg8 is probably recycled and participates in a new conjugation reaction, whereas Atg8–PE entrapped inside the autophagosome is degraded following fusion with the lysosome. Both the conjugating and the deconjugating activities of Atg4 are required for normal progression of autophagy. The recruitment of Atg8 to the autophagosome is preceded by a second Ubl system mediating the conjugation between Ubl, Atg12 and Atg5 [14]. Homologous with the ubiquitin system, Atg12 is first activated by the E1-like enzyme, Atg7, followed by its transfer to an E2-like enzyme, Atg10. Finally, the C-terminal glycine residue of Atg12 is covalently conjugated to an internal lysine residue of Atg5 through an isopeptide bond. The Atg12–Atg5 conjugate then associates with Atg16 which can form homo-oligomers through a coiled-coiled domain. This allows Atg16 to cross-link.
multiple Atg12–Atg5 conjugates into a single large protein complex. It has been shown that the localization of the Atg12–Atg5–Atg16 complex dictates the site of Atg8 lipidation and facilitates the lipidation reaction by promoting the transfer of Atg8 from Atg3 to PE thus acting as a ubiquitin ligase (E3)-like enzyme [15].

Recruitment of Atg8 on to the autophagosome may be controlled by additional post-translational modifications. LC3 was found to be subjected to PKA (protein kinase A)-mediated phosphorylation [16]. It was further demonstrated that LC3 was dephosphorylated following autophagy induction in neuronal cells. Membrane association of dephosphorylated LC3 was

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**Figure 2. Processing of Atg8s**

Following their synthesis as C-terminally extended proforms, Atg8 homologues are cleaved by Atg4 cysteine proteases, resulting in the formation of the Atg8-I form harbouring an exposed C-terminal glycine residue (G) (1). The exposed glycine residue forms a thioester bond with a catalytic cysteine residue (C) of the E1-like enzyme Atg7 (2). Activated Atg8 is then transferred to the E2-like enzyme Atg3, also through a thioester bond (3). Finally, the C-terminal glycine residue of Atg8 is conjugated to PE through an amide bond, resulting in the formation of the tightly membrane-associated Atg8-II form (4). Membrane association of Atg8 is reversible as Atg8–PE, which is associated with the outer autophagosome membrane, can be cleaved by Atg4 to release free Atg8-I (5).
enhanced, whereas formation of LC3-positive autophagosomes was inhibited by the expression of a pseudophosphorylated LC3 mutant. Finally, acetylation of Atg8 and other Atg proteins by the acetyltransferase p300 was proposed to negatively regulate their activity [17].

Function of the Atg8 family

Autophagosome biogenesis

Initial observations in yeast showing that autophagosome formation was severely impaired in Atg8-null mutants, implicated Atg8 as a crucial player in the process of autophagosome biogenesis [18]. Subsequent findings using both yeast and mammalian systems have highlighted the importance of the Atg8 proteins in the regulation of autophagy. Expression of Atg8 is induced under starvation in yeast. The amount of lipidated Atg8, localized to autophagosomal structures, is greatly increased following autophagy induction and is correlated with the extent of autophagosome formation. The levels of Atg8 determine the levels of autophagy and control the size of autophagosomes [19].

Analysis of the Atg8 role in mammals was initially hampered owing to the existence of multiple homologues. Overexpression of a catalytically dead Atg4B (Atg4B<sup>C74A</sup>) in mammalian cells was found to inhibit autophagy by sequestration of non-lipidated Atg8 homologues [20]. These cells, overexpressing mutant Atg4B, accumulated unclosed pre-autophagosomal structures, suggesting that lipidation of Atg8 homologues is required for completion of autophagosomes in mammalian cells. Manipulation in the expression of the Atg8 subfamilies in mammals (LC3, GABARAP and GATE-16) by siRNA-mediated knock-down and overexpression approaches revealed that each subfamily contributes to autophagosome biogenesis, but act in different steps along this process. Although the LC3 subfamily mediates the elongation of the phagophore membrane, GABARAP and GATE-16 families may be involved in a downstream step along the maturation process possibly in sealing of the autophagosomes [21].

The first mechanistic insight into the function of Atg8 was provided by Ohsumi and colleagues [22] using an in vitro system to reconstitute Atg8–PE conjugation in the presence of liposomes. They showed that lipidated Atg8 mediated the tethering and hemifusion of liposomes to which it was anchored. The study showed further that Atg8 mutants defective in membrane tethering and hemifusion activities impaired the formation of autophagosomes in yeast. These observations indicated a role of Atg8 in membrane remodelling, driving the growth and maturation of autophagosomal structures. It was subsequently shown that the two Atg8 mammalian homologues, LC3 and GATE-16, promote tethering and membrane fusion. This activity is mediated by positively charged and hydrophobic amino acids in the N-terminal α-helices of LC3 and GATE-16 respectively [23].

In addition to the crucial role of Atg8–PE in autophagy, it has been proposed that Atg4-mediated delipidation of Atg8, namely the release of Atg8 from the autophagosome membrane, is an important step required to facilitate normal autophagy. Prevention of Atg8 delipidation was shown to result in its mislocalization to the vacuolar membrane and to have negative effects on autophagosome biogenesis reflected by a reduction in both the number and size of autophagosomes [24]. A possible explanation to this finding is that Atg8 conjugation to PE occurs not only at pre-autophagosomal structures, but also in other sites within the cell. The release of Atg8–PE from these sites is crucial to supply the growing demand for Atg8 following
autophagy induction. Deconjugation of Atg8–PE appears to be additionally required in later stages, facilitating autophagosome maturation and fusion with the vacuole [25].

**Sorting of autophagy cargo**

Autophagy has long been considered to be a non-selective bulk degradation process. Recent experimental data, however, present mounting evidence for another mode of autophagy responsible for selective delivery of cargo for lysosomal degradation [26]. A wide range of substrates found to be specifically cleared by autophagy include: protein inclusions caused by aggregate-prone or misfolded proteins (aggrephagy), organelles such as peroxisomes (pexophagy), mitochondria (mitophagy) and surplus ER (endoplasmic reticulum) (reticulophagy), bacteria and virus (xenophagy), and ribosomes (ribophagy) [26].

In addition to their role in autophagosome biogenesis, Atg8 proteins appear to be central factors in mediating selective cargo sorting into autophagosomes. This activity is largely achieved by interaction with adaptor proteins, also called autophagy receptors, that link autophagy substrates to autophagosome-associated Atg8s. Mostly, the autophagy receptors are themselves degraded by autophagy [26]. In fact, direct Atg8-mediated cargo sorting was first demonstrated in the autophagy-related cvt pathway in yeast. Atg8 was found to interact with Atg19, a receptor for the cvt cargoes facilitating their vacuolar targeting. The cargo-sorting function of mammalian LC3 was subsequently revealed by studies demonstrating its role in the clearance of ubiquitinated molecules. In addition to its fundamental role in protein degradation by the proteasome system, ubiquitin has emerged as a selective degradation signal for the lysosomal targeting of various types of autophagy substrates such as protein aggregates, membrane-bound organelles and microbes [27]. Clearance of such ubiquitinated substrates depends on a group of UBD (ubiquitin-binding domain)-containing autophagy receptors which simultaneously bind to the Atg8 proteins [26].

The rapidly expanding list of autophagy receptors in mammals include: p62/SQSTM1 and neighbour of Brca1 (Nbr1), which are involved in the autophagic degradation of ubiquitinated protein aggregates; p62/SQSTM1, NDP52 (nuclear dot protein 52 kDa) and optenurin, which link LC3 to intracellular pathogens; Nix, the outer mitochondrial membrane protein Bcl2-related protein, which may be directly involved in the recruitment of the autophagy machinery to damaged mitochondria; and autophagy-linked FYVE protein (ALFY). The interaction of autophagy receptors with Atg8 homologues is mediated by a short linear motif termed LIR (LC3-interacting region), conforming to the consensus W/F/Y-X-X-L/I/V and surrounded by at least one acidic residue.

**Recruitment of autophagy machinery**

LIR-dependent interactions of Atg8 with autophagy core proteins that may serve a regulatory function have also been reported. A direct LIR-mediated binding of Atg8s to the essential autophagy regulator Atg1 (in yeast) and the ULK1 (uncoordinated-51-like kinase 1) complex (in mammals) was found to facilitate autophagosome formation [28,29]. Likewise, Atg8 was found to interact with several members of the TBC (Tre2, Bub2 and Cdc16) domain-containing GAPs (GTPase-activating proteins) of Rab proteins [30]. Rab-type small GTPases are evolutionarily conserved membrane-trafficking proteins, some of which were implicated in regulating tethering and fusion of autophagy-related membranes. Thus, in addition to their
Figure 3. (See facing page for legend)
role in recognition of degradation targets, Atg8s may function as scaffold proteins promoting the assembly of critical autophagy complexes on the surface of autophagosomes.

**LC3 as a tool to monitor the autophagic process**

As Atg8s are specifically associated with premature as well as completed autophagosomes, and since conjugated Atg8 correlates with the number of autophagosomes, these proteins are widely used as specific markers to monitor autophagosomes and autophagic activity. Multiple assays, utilized to detect and quantify Atg8 proteins in yeast and mammals, include Western blotting, fluorescence microscopy and flow cytometry [31,32].

Induction of autophagy is accompanied by an increased conversion of soluble Atg8-I into PE-conjugated membrane-bound Atg8-II. Thus accumulation of Atg8-II should be indicative of autophagy induction. Lipidated Atg8 can be separated from the non-lipidated form owing to its faster migration by electrophoresis to an apparently lower $M_r$ position (albeit it is a larger molecular mass). However, since Atg8-II, associated with the inner autophagosome membrane, is degraded following fusion of the limiting membrane with the lysosome/vacuole, a decrease in Atg8-II levels is often seen under autophagy-inducing conditions. Therefore to reliably measure autophagic activity, Atg8 levels should be determined in the presence or absence of lysosomal degradation inhibitors (Figure 3A). In yeast, the discrimination between the non-lipidated and the lipidated forms of Atg8 can be more complicated due to their nearly identical SDS/PAGE motilities. The more commonly employed assay to follow autophagy in this organism is the GFP–Atg8 processing assay. This assay relies on the use of an ectopically expressed

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**Figure 3. Analysing autophagy using Atg8 proteins**

(A) Upper blot shows HeLa cells that were incubated for 2 h in nutrient-rich or in starvation medium in the presence (+) or absence (−) of the lysosomal inhibitor bafilomycin A1. Cells were then lysed and subjected to Western blot analysis using an antibody directed against LC3. Lower blot shows HeLa cells, stably expressing GFP-LC3B, that were similarly treated. Western blot analysis was done with a GFP-directed antibody. The asterisk indicates a non-related band. Note that both in the absence and in the presence of the lysosomal inhibitor, starvation results in a higher amount of lipidated LC3 (LC3-II) compared with the non-lipidated form (LC3-I), suggesting an increase in autophagosome formation. On lysosomal inhibition, the LC3-II/LC3-I ratio is significantly augmented due to the block in lysosome-mediated consumption of autophagosome-associated LC3. Actin served as a loading control. Notably, the LC3 mobility shift can be quantified and used as a measurement for autophagic activity [32]. (B) Yeast cells expressing GFP–Atg8 were starved of nitrogen for the indicated times and analysed by Western blotting using an anti-GFP antibody (for details see main text). PGK (phosphoglycerate kinase) served as a loading control. (C) Upper panel shows HeLa cells that were treated as in (A) and analysed using direct fluorescent microscopy following staining with DAPI to visualize nuclei. Lower panel shows cells stably expressing GFP-LC3B that were similarly treated, fixed and stained with an LC3-directed primary antibody followed by a rhodamine-conjugated secondary antibody. Nuclei were stained with DAPI. (D) HeLa cells transiently expressing tandem fluorescent LC3 (RFP–GFP–LC3) were treated as in (A) and visualized by fluorescence microscopy. Note the increase in LC3-positive puncta following autophagy induction by starvation. In the absence of lysosomal inhibitor, a higher amount of red puncta (representing autophagosomes already fused with the lysosome) are observed compared with green/yellow puncta (representing the autophagosomal structure before fusion). On addition of the lysosome inhibitor there is a marked increase in the number of LC3-associated autophagosomes which appear as yellow dots due to the block in the degradative activity of the lysosome.
N-terminal GFP-tagged Atg8 (not C-terminally tagged, as the C-terminus of Atg8 is proteolytically processed by Atg4). Following autophagy induction, GFP–Atg8 decorating the inner autophagosome membrane is released into the vacuole lumen. Although Atg8 is rapidly degraded by vacuolar proteases, the relatively stable GFP remains intact leading to the accumulation of free GFP. Thus accumulation of free GFP indicates an increase in the autophagy flux (Figure 3B).

The GFP-tagged Atg8/LC3 can also be used as functional markers for monitoring autophagy through direct fluorescence microscopy in cultured cells and transgenic organisms. Alternatively, endogenous Atg8 proteins can be detected in immunocytochemistry or immunohistochemistry procedures using specific antibodies. By following the endogenous protein, one obviates the need for transfection or generation of a transgenic organism and avoids potential artefacts resulting from overexpression. Atg8-associated autophagic membranes and autophagosome are visualized as bright cytosolically scattered puncta (Figure 3C). It should be noted that accumulation of intracellular Atg8-positive dots may not be indicative of autophagy induction, but rather reflects autophagy inhibition due to blockage in autophagosome consumption (caused by impaired trafficking to lysosomes, compromised autophagosome–lysosome fusion or reduction in lysosomal degradative activity). These possibilities can be distinguished by using lysosomal degradation inhibitors. An increase in the number of Atg8 puncta in the presence of such inhibitors would reflect active autophagy whereas unchanged puncta levels on lysosomal inhibition would be suggestive of autophagy blockage. Another fluorescence approach, capable of analysing both autophagy induction and flux without the necessity for drug treatment, makes use of the tandem monomeric RFP–GFP-tagged Atg8. Whereas the GFP signal is sensitive to the acidic and/or proteolytic conditions of the lysosome lumen, RFP is more stable. Therefore a yellow signal (derived from colocalization of the GFP and then RFP fluorophores) corresponds to autophagosomal structures before their fusion with the lysosome. Autophagosomes, already fused with the lysosome, would appear as red puncta resulting from the RFP, without GFP, signal (Figure 3D). Lastly, FACS (fluorescence-activated cell sorting) has been used for the analysis of autophagy in living mammalian cells. Autophagy induction leads to a lysosomal-activity-dependent decrease in the total cellular signal of fluorescently tagged Atg8. This simple approach allows precise automated analysis of a large number of cells to obtain robust data.

It is important to point out that although the Atg8 family is routinely used to study autophagy, the analysis methods on the basis of these proteins may suffer from various caveats. The amount and the processing of the Atg8s may be tissue- and cell context-dependent. In addition, Atg8s may be involved in non-autophagy cellular processes. Thus it is recommended to include additional tools, such as EM (electron microscopy), and more direct approaches to measure autophagic flux by monitoring autophagic substrate degradation.

Conclusions and future aspects

Since the term ‘autophagy’ was first coined in 1963 by Christian de Duve, the study of this important cellular process has significantly expanded. Autophagy is now recognized as a crucial pathway involved in normal physiological processes as well as in pathological conditions. In addition to its role in maintaining cellular homoeostasis, autophagy is implicated in a growing list of diseases including cancer, metabolic and neurodegenerative disorders, infectious and inflammatory diseases, diabetes and obesity. Therefore understanding the cellular and
molecular mechanisms underlying autophagy is of great interest. Such an improved knowledge would enable identification of new targets for both diagnostic and therapeutic implications.

The Atg8 proteins are key regulators of the autophagy process. Owing to their localization on autophagosomes and intermediate structures, these proteins also serve as reliable markers to monitor autophagy in various biological systems. Although the Atg8 proteins were extensively investigated in the last decade, mechanisms of their action and regulation are not yet fully resolved.

Atg8s were shown to promote proper autophagosome biogenesis. This activity was mechanistically associated with their lipidation-dependent capacity to mediate tethering and fusion of liposomes in vitro. A plausible model to explain how these functions of Atg8s mediate autophagosome maturation is that the growth of the autophagosome depends on the supply of lipids and/or proteins provided by vesicular structures. Atg8s associated with these structures as well as with autophagosomal intermediates may drive their fusion leading to the expansion of autophagosomes. Alternatively, Atg8-mediated membrane fusion may promote the final step of autophagosome sealing. Of note, the fundamental cellular membrane fusion molecules, soluble SNAREs, were proposed recently to mediate membrane fusion events along the autophagy process [33]. Whether Atg8s act independently or assist in these SNARE-dependent fusion events needs further study.

Another open question in the field relates to the regulation of Atg8s processing namely, the lipidation/de-lipidation cycle. On the one hand, association of Atg8s with autophagosomal structures is essential for proper autophagosome biogenesis suggesting that there must be a mechanism that protects lipidated Atg8 and prevents unregulated deconjugation. On the other hand, several reports indicate that Atg4-mediated delipidation of Atg8s is also required for normal autophagy. It is not known at what step of autophagosome biogenesis Atg8 deconjugation occurs (following autophagosome completion or earlier). Atg8 processing is likely to be subjected to complex temporal and spatial regulations and, indeed, regulation of Atg4 was documented in mammalian cells. Atg4 activity was shown to be inhibited by the accumulation of ROS (reactive oxygen species), preventing the release of Atg8 proteins from the autophagosomal membrane during autophagy [34].

The Atg8 family emerge as multifunctional autophagy regulators promoting recruitment of degradation targets as well as supporting autophagosome biogenesis by acting as ‘fusogens’ and by interacting with additional autophagy effectors. However, whether and how these functions of Atg8s are coordinated is not entirely clear. Ho et al. [35] have reported that impaired binding of cargo receptor by Atg8 negatively affects its general autophagy regulation function, suggesting that Atg8 roles in autophagosome formation and cargo sorting are coupled.

Summary

- Autophagy is a catabolic pathway for the delivery of a wide range of substrates including protein aggregates, cellular organelles and invading pathogens to lysosomal degradation through their entrapment in double-membrane structures called autophagosomes.
- Atg8 family members are essential components of the core autophagy machinery.
• The Atg8 family comprises a single member in yeast, whereas in higher organisms it constitutes several homologues subdivided into three subfamilies: LC3, GABARAP and GATE-16.

• Atg8s exist as three forms in cells. They are produced as a C-terminally extended precursor (Atg8) which is rapidly cleaved by the Atg4 cysteine protease to yield the cytosolic Atg8-I form with an exposed C-terminal glycine residue. Following autophagy induction, Atg8-I is converted into the Atg8-II form which tightly associates with autophagosomal membranes through its conjugation to the lipid PE.

• Conversion of Atg8-I into lipated Atg8-II depends on the sequential action of the E1-activating-like enzyme (Atg7), E2-conjugating-like enzyme (Atg3) and the Atg12–Atg5–Atg16 complex acting as the E3-ubiquitin ligase-like enzyme.

• Atg8s can be dissociated from the autophagosome membrane through their Atg4-mediated deconjugation from PE.

• Atg8s were shown to act as membrane modifiers promoting the expansion and closure of maturing autophagosomes. In addition, Atg8s play a central role in selective recruitment of autophagy substrates into autophagosomes thus mediating their lysosomal targeting and degradation.

References
related protein kinase Atg1 interacts with the ubiquitin-like protein Atg8 via the Atg8 family interacting motif to facilitate autophagosome formation. J. Biol. Chem. 287, 28503–28507


