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An overview of macroautophagy in yeast

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Abstract

Macroautophagy is an evolutionarily conserved dynamic pathway that functions primarily in a degradative manner. A basal level of macroautophagy occurs constitutively, but this process can be further induced in response to various types of stress including starvation, hypoxia and hormonal stimuli. The general principle behind macroautophagy is that cytoplasmic contents can be sequestered within a transient double-membrane organelle, an autophagosome, which subsequently fuses with a lysosome or vacuole (in mammals, or yeast and plants, respectively), allowing degradation of the cargo followed by recycling of the resulting macromolecules. Through this basic mechanism, macroautophagy has a critical role in cellular homeostasis; however, either insufficient or excessive macroautophagy can seriously compromise cell physiology, and thus it needs to be properly regulated. In fact, a wide range of diseases is associated with dysregulation of macroautophagy. There has been substantial progress in understanding the regulation and molecular mechanisms of macroautophagy in different organisms; however, many questions concerning some most fundamental aspects of macroautophagy still remain unresolved. In this review, we summarize current knowledge about macroautophagy mainly in yeast, including the mechanism of autophagosome biogenesis, the function of the core macroautophagic machinery, the regulation of macroautophagy, and the process of cargo recognition in selective macroautophagy, with the goal of providing insights into some of the key unanswered questions in this field.

Graphical Abstract

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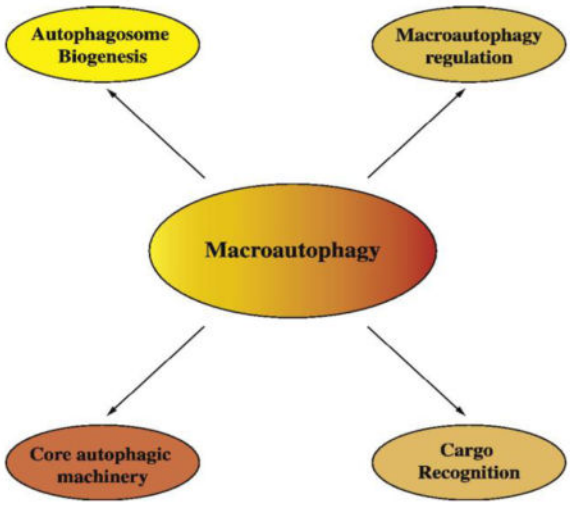

Keywords

autophagosome biogenesis; autophagy; cargo recognition; regulation; stress

1. Introduction

Autophagy, a term from the Greek words “auto” (self) and “phagy” (to eat), is a highly regulated cellular degradation and recycling process, conserved from yeast to higher eukaryotes [1]. Initially in yeast, the limited number of genes that have products primarily involved in macroautophagy (and also in selective microautophagy) were unified under the name autophagy-related (*ATG*) [2]. Mammalian cells carry out at least one additional type of autophagy termed chaperone-mediated autophagy (CMA) [3]. Whereas the proteasome is considered to be responsible for the degradation of most short-lived proteins, long-lived proteins, large protein complexes and organelles are degraded by autophagy. Both microautophagy and macroautophagy have nonselective and selective modes, but information regarding nonselective microautophagy is rather limited [4]. The details about selective autophagy will be discussed in the last part of this review. One key definition of autophagy is that it involves the delivery and typically the degradation of cytoplasmic cargo within the lysosome/vacuole [1, 5]; however, the mechanisms of cytoplasmic sequestration and delivery can be quite distinct. During microautophagy in mammals and yeast, the lysosome/vacuole membrane invaginates or protrudes to capture the cargo that will subsequently be degraded [6, 7]. CMA, as its name suggests, relies on chaperones to recognize individual cargo proteins with a specific KFERQ motif, which are then unfolded and directly translocated across the lysosomal membrane [8–10]. In contrast, during macroautophagy the cargo is initially sequestered in a compartment that is separate from the lysosome/vacuole; the initial sequestration process involves a transient membrane structure, the phagophore [3]. Although precise details regarding phagophore nucleation and formation are not known, this compartment likely expands sequentially around cargo, providing tremendous flexibility with regard to capacity. Upon completion, the phagophore membranes scission to generate the double-membrane autophagosome; this double-

membrane vesicle subsequently fuses with lysosome/vacuole [11]. Among these three types of autophagy the most comprehensively studied is macroautophagy, and accordingly we mainly focus on this process in our review; therefore we use the term autophagy to refer to macroautophagy hereafter.

The morphological hallmark of autophagy is the formation of the phagophore, and its subsequent expansion and maturation into an autophagosome, which is a large cytoplasmic double-membrane vesicle [12]. Once completed, the outer membrane of the autophagosome fuses with the lysosome/vacuole; the inner membrane as well as the cargo are thereby exposed to the lumenal contents of this organelle and subsequently degraded by resident hydrolases. One interesting question concerns regulating fusion of the phagophore with the lysosome/vacuole. Unlike single-membrane vesicles that bud off from organelles of the secretory pathway already containing their cargo, the phagophore does not completely surround its cargo until the final step of autophagosome formation. Therefore, premature fusion of the phagophore with the lysosome/vacuole will not result in delivery of the cargo to the organelle lumen, indicating that the timing of the fusion event must be regulated. In mammals, fusion between the autophagosome and lysosome (which may involve an intermediate fusion between an autophaghosome and an endosome to generate another transient structure, the amphisome) leads to the formation of the autolysosome. However, in yeast, the inner autophagosome vesicle is released into the vacuole lumen as an autophagic body due to the much larger size of this compartment relative to the lysosome [13–15].

The process of autophagy can be divided into the following stages: induction, nucleation of the phagophore, expansion of the phagophore, completion of the autophagosome, docking and fusion with the vacuole, and the final steps of degradation and efflux of the breakdown products (Figure 1). In yeast, the induction of autophagy begins at a single perivacuolar site that is proximal to the vacuole, called the phagophore assembly site (PAS), a step regulated by the Atg1 complex (including at least Atg1, Atg13 and the Atg17-Atg31-Atg29 ternary subcomplex) [16, 17]. Then the Atg14-containing class III phosphatidylinositol 3-kinase (PtdIns3K) complex I (consisting of Vps34, Vps30/Atg6, Vps15, Atg14 and Atg38) is recruited to the PAS in the nucleation stage [18]. From induction to nucleation, the current model is that the PAS would gradually generate a primary double-membrane sequestering compartment, the phagophore [19].

Next, in the expansion and completion stage, the phagophore first begins to expand. There may be different mechanisms that operate in the expansion phase depending on whether the process is selective or nonselective. Key components that participate at this stage of the process include two conjugation systems involving ubiquitin-like (Ubl) proteins [20]. These two systems utilize the Ubl proteins Atg12 (along with the components Atg5, Atg7, Atg10, and Atg16) and Atg8 (and Atg3, Atg4, and Atg7) [21, 22]. One model for the expansion stage also involves cycling of Atg9; this transmembrane protein may cycle between the PAS and peripheral structures, carrying or directing the delivery of membrane, although there are currently no direct data supporting this hypothesis [23]. Following the expansion of the phagophore, the double-membrane autophagosome will ultimately be completed, fully surrounding the cargo. Although the mechanism and regulation of the final closure step have not been well characterized, completion of the autophagosome is likely to involve membrane

scission [24]. Based on other processes requiring vesicular membrane sealing, such as the multivesicular body pathway, it seems likely that the endosomal sorting complex required for transport (ESCRT) components play a role [25, 26]. Along these lines, the *Arabidopsis chmp1* mutant, defective for a component of ESCRT-III, displays a phenotype that is consistent with a partial block in phagophore closure [27]. Once the autophagosome is complete, it will deliver its cargo to vacuole in yeast by fusing with the vacuolar membrane, and the timing of this docking and fusion process is key to ensure degradation and has therefore to be finely regulated [28].

Finally, the autophagic body (the inner autophagosome vesicle) is degraded with the help of the lipase Atg15, and the cargo is typically degraded by various hydrolases present in the vacuole [29]. The final step of the process consists of the release of the breakdown products into the cytoplasm (Figure 1). This last step is not well characterized, although the vacuole contains hydrolases for all of the major macromolecules, essentially nothing is known about the recycling of nucleic acid, carbohydrate or lipid breakdown products.

Generally speaking, autophagy works primarily as a cytoprotective mechanism. Under normal conditions, autophagy occurs constitutively at a basal state. When the cell is exposed to stress stimuli (e.g. nutrient or energy starvation in yeast), autophagy is massively induced thus promoting the turnover of cytoplasmic materials required for cell survival, or removing superfluous or damaged organelles [5]. Too little or too much degradation from uncontrolled autophagy is harmful, and autophagy dysfunction is associated with various diseases, such as cancer, aging, and neurodegeneration [30]. Although a tremendous amount of research has improved our understanding of the different aspects of autophagy over the past two decades, there are still many questions that remain to be answered. In this review, we provide and discuss an overview of our current knowledge about autophagy, especially the morphology of this mechanism, as well as the molecular actors participating in its machinery and regulation, together with a discussion of several currently unresolved questions.

2. Autophagsome biogenesis

The size of the autophagosome can vary substantially depending on the organism undergoing autophagy, or the specific cargo being recognized. In yeast, typical autophagosomes range from 0.3 to 0.9 μm, whereas in mammals the average size is larger, being from 0.5 to 1.5 μm [31, 32]. Although autophagosome biogenesis has been one of the most highly studied aspects of the process, it is also the most complicated, and the underlying mechanism remains largely unclear. There are many unresolved issues concerning the formation of the phagophore and autophagosome including the precise nature of the PAS, the origin/source(s) of the lipid/membrane used for its expansion as well as the role of SNAREs (soluble NSF attachment protein receptors) and other fusion machinery [33, 34].

Most of the Atg proteins, including Atg8, associate at least transiently with the PAS and, when tagged with a fluorophore, can be visualized as a small perivacuolar punctum in the cytoplasm, in addition to having a diffuse cytosolic distribution; this punctum, the PAS, will

literally develop into an autophagosome [35]. The vast majority of the Atg proteins, however, do not remain associated with the completed autophagosome, or at least not at easily detectable levels. The primary exceptions in this regard are the receptor proteins that participate in selective types of autophagy, and Atg8 (which also has a role in determining specificity through cargo recognition). This feature of Atg8 makes it a convenient marker to localize the PAS and monitor autophagy progression [35]. Nonetheless, the PAS has not been well characterized, either biochemically or morphologically.

As noted above, autophagy is constitutive and operates at a basal level even in growing conditions. In addition, the cytoplasm-to-vacuole targeting (Cvt) pathway, which is a biosynthetic mode of vacuolar transport that uses most of the autophagy machinery, also occurs during vegetative growth. Thus, the cell maintains a PAS under either condition. Most of the Atg proteins are peripheral membrane proteins, and their assembly at the PAS occurs in a hierarchical manner [36]. Under growing conditions in yeast, the first component to localize at the PAS appears to be the scaffold protein Atg11, which subsequently recruits the Atg1 kinase complex; following autophagy induction, the Atg17-Atg31-Atg29 subcomplex replaces the function of Atg11 in the starvation-specific PAS [37, 38]. Atg9 is also recruited to the PAS at a similar, early stage, of autophagy [23]. Subsequently, the class III PtdIns3K complex and the Atg12-Atg5-Atg16 conjugation complex followed by the Atg8-phosphatidylethanolamine (PE) conjugation system are recruited [22]. The PAS is therefore a transient structure at which the localization of Atg proteins is dynamic but follows a distinct temporal order of assembly. Following autophagosome completion, the majority of the Atg proteins will temporarily disassemble from the autophagosome and the PAS, until they are reassembled for a new round of biogenesis.

The autophagosome is described as being formed *de novo*, meaning that the sequestering membrane is likely not generated directly from a single pre-existing organelle. The detailed mechanism of phagophore nucleation and expansion is still not known and the models and membrane source(s) participating in this event are highly debated [34]. In one model, following nucleation, the phagophore expands by membrane addition. Various studies have implicated the endoplasmic reticulum (ER), Golgi complex, plasma membrane and mitochondria as contributing to the nucleation and/or expansion of the phagophore in yeast (Figure 2). The ER was perhaps the first organelle proposed to be the source of the autophagosome membrane [39], and subsequent studies show that autophagy is negatively affected in strains having mutations that interfere with the early stages of the secretory pathway including ER-to-Golgi transport [40, 41]. It is not surprising that the ER, considering its crucial function in synthesizing phospholipids, would play a significant role in a process that is so highly dependent on membrane. Along these lines, it should be noted that there are at least two distinct models for phagophore expansion. One model, as described above, involves vesicular addition of lipid, but an alternate model in mammalian cells involves the lateral movement of lipid from the ER into a unique structure termed the omegasome, or direct transfer of lipids from compartments in close proximity [42, 43]. Whether one or both of these mechanisms operate in all cells, or whether this depends on the type of organism or stress condition is currently not known. Here, we will focus on the vesicular expansion model.

Autophagosome formation is further linked to the ER via endoplasmic reticulum exit sites (ERES), corresponding to a specialized ER subdomain, which can function at the very early stage of the process [44]. Genetic studies indicate that the Golgi apparatus is another potential membrane donor. For example, post-Golgi Sec proteins, including Sec2 and Sec4, are involved in autophagy, and under stress conditions they may direct membrane flow into autophagosome biogenesis rather than to the cell surface [45]. The conserved oligomeric Golgi (COG) complex, which functions as a tether, is also required in autophagosome formation, and phagophore expansion is severely impaired when Golgi transport functions are blocked [46, 47]. Another study also revealed the role of other PtdIns kinases in autophagosome formation: the PtdIns 4-kinase Pik1 is involved in Atg9 transport from the Golgi in both nonselective and selective autophagy [48]. Moreover, because Atg9 is the only transmembrane protein of the core autophagy machinery required for autophagosome formation, this protein is often monitored as an indicator of the membrane source in yeast [23]. As with most membrane proteins, Atg9 enters the ER, and subsequently transits to the Golgi apparatus; this observation strongly suggests a role for post-Golgi membranes in autophagosome formation [49]. Atg9 is also concentrated in a, presumably post-Golgi, compartment in close proximity with the mitochondria. This site has been termed the tubule-vesicular cluster, Atg9 reservoirs, or Atg9 peripheral structures [50]. In yeast, one study has revealed that plasma membrane-derived double-membrane vesicles are likely autophagosome-related structures, suggesting that the plasma membrane might also contribute membrane for phagophore expansion [51]. Regulated by the actin capping protein CapZ and phosphatidylinositol-3-phosphate (PtdIns3P), actin may polymerize in the forming autophagosome in response to starvation, which could play a role in forming and maintaining functional autophagosomes in mammalian cells [52]. In addition to the above-mentioned possibilities (i.e., the ER, Golgi, and plasma membrane), the ER-Golgi intermediated compartment (ERGIC) and recycling endosomes are also potential resources for the autophagosome membrane in mammals [53–55]. The autophagosome may also form at the ER-mitochondria contact sites, connecting these two organelles and possibly providing new insight into autophagosome biogenesis [56]; it is possible that the peri-mitochondrial site for Atg9 localization in yeast corresponds to similar functional sites, and there is an interesting finding showing that ER-mitochondria-contact sites are required for mitophagy, a selective type of autophagy [57].

The topic of the membrane source(s) for autophagosome biogenesis is still controversial, and provides many interesting questions, such as if, or how, these different membrane sources of varying composition can function together, whether they have specific contributions under different stimuli, and similarly whether they contribute to different forms of autophagy.

In the vesicular expansion model of autophagosome formation, SNARE proteins likely participate in regulating vesicle fusion [58]. SNAREs also have the potential to function at multiple stages of autophagy in yeast, such as PAS/phagophore assembly, phagophore expansion, and the final fusion step between the completed autophagosome and the vacuole [59]. Sso2 and Sec9, two SNAREs required for exocytosis in yeast, are important for autophagosome formation [60, 61]. These proteins participate in the generation of the tubular network that contains Atg9; in their absence, Atg9 is located in post-Golgi vesicles

that are smaller than those involved in secretion. Other SNAREs including Tlg2, Sec22, and Ykt6 are also involved in this process [62–64]. How SNARE proteins act in the formation of the autophagosome is unclear: They may contribute to the biogenesis of Atg9 peripheral structures, or modulate the fusion of Atg9-containing vesicles with phagophores. SNAREs would presumably also be key elements to integrate membrane from different sources; however, it is not known how the cell would handle the complex issue of maintaining the distinct identity of separate organelles that might contribute membrane to a common destination. After the completion of autophagosome formation, fusion with the vacuole requires certain SNAREs, such as Vam3, the GTP-binding protein Ypt7, the ortholog of the NSF protein Sec18, and the SNAP25 homolog Vam7 [65–68]. In mammalian cells, SNAREs are similarly involved in autophagosome maturation. For example, the homotypic fusion of ATG16L1 precursors, which may participate in formation of the autophagosome, requires the SNARE protein VAMP7 [69]. In addition, the SNARE STX5 can indirectly regulate autophagy by influencing lysosomal biogenesis [70].

Upon completion of the autophagosome, the outer membrane fuses with the lysosome/vacuole; in yeast, this fusion releases the autophagosome inner membrane and its enclosed cargo into the vacuolar lumen—the resulting single-membrane intralumenal vesicle is referred to as an autophagic body. One interesting aspect regarding SNAREs concerns the regulation of this event; that is, how do cells prevent an abortive fusion event between the phagophore and the degradative organelle? In mammalian cells STX17 is recruited to completed autophagosomes, and subsequently acts in the fusion process [71]. In contrast, the interaction between Atg17 and Vam7 may regulate the timing of this step in yeast [72].

Although critical for the autophagy pathway, many aspects of autophagosome biogenesis are still unclear and further studies will be necessary to reach a better understanding of this mechanism. Beyond the morphological features of autophagy, the identification and characterization of the autophagy-related proteins has provided substantial insights into the molecular machinery behind autophagy, which we discuss further below.

3. Structure, function and modification of the core autophagy machinery

Genetic screens for autophagy-defective mutants in yeast and other fungi have at present identified 41 autophagy-related (*ATG)* genes that play a primary role in autophagy; approximately half of these genes have a clear homolog in higher eukaryotes [3, 73]. During the past several years, substantial progress has been achieved in studying components of the core machinery in autophagy. Approximately 16 of the Atg proteins are essential for autophagosome formation, and they are shared by both nonselective and selective autophagy [1]. These proteins participate in different stages of autophagy and interact to form specific functional complexes. In recent years, an increasing amount of crystallographic data of the autophagy-related proteins has been reported, which is providing valuable information to elucidate the mechanism of the autophagic machinery [74]. Furthermore, there is a growing awareness of the importance of intrinsically disordered regions in terms of regulating protein function and protein-protein interaction including those within Atg proteins [75, 76]. Here, we discuss some representative components of the core autophagy machinery at both

structural and molecular levels, connecting the structure with the molecular mechanism of the autophagy core machinery.

3.1. Atg1-Atg13-Atg17-Atg31-Atg29 kinase complex

Atg1 was one of the first identified autophagy-specific proteins, and it remains the only identified protein kinase of the autophagic machinery [77]. The Atg1 kinase complex that functions during nonselective autophagy is comprised of three main components: the Ser/Thr kinase Atg1, Atg13, and the Atg17-Atg31-Atg29 scaffolding subcomplex [17] (Figure 3). The early-acting Atg1 kinase complex is essential for autophagy initiation, but also acts later in the process notably by regulating the movement of Atg9 [23] and controlling the timing of autophagosome fusion with the vacuole [72].

Based on a predicted helical structure, Atg1 may sense membrane curvature, and the C-terminal domain of Atg1, predicted to bind vesicles, contains the binding site for Atg13 [78]. Only limited crystallographic data are available for Atg1; substantially more structural information is available for the other components of this complex. Atg13 is the bridge linking Atg1 and the other subunits in the complex. The C-terminal domain of Atg13 is intrinsically disordered and contains binding sites for Atg1 and Atg17 [79]. These interactions are important for autophagy initiation in which the Atg1 complex functions in part as a scaffold for the PAS [80]. Crystallization of the Atg13 N terminus shows that it contains a HORMA domain [81, 82]. Due to its potential ability to bind to PtdIns3P, the Atg13 HORMA domain is crucial for recruitment of the Atg14-containing PtdIns3K [81, 82]. The Atg13 HORMA domain can also bind to Atg9, thereby facilitating its recruitment to the PAS during autophagosome formation [83].

The three other components, Atg17, Atg31 and Atg29, form a constitutive complex, where Atg31 partly bridges the interaction between Atg17 and Atg29 [84]. The Atg17 monomer has a crescent shape with a curved arc, which reveals a possibility for Atg17 to bind to a curved membrane (e.g., vesicles that arrive at the PAS carrying membrane for phagophore expansion) [79, 84]. In the cell, the Atg17-Atg31-Atg29 ternary subcomplex is present as a dimer, and the two crescents of Atg17 are assembled as to form an “S” shape [38, 79]. Atg31 binds to Atg17 via an α-helix in the C-terminal region, but interacts with Atg29 via a β-sandwich at the N terminus; based on chemical-crosslinking coupled with mass spectrometry, Atg17 and Atg29 also appear to directly interact [85]. Atg17 may also interact with the Atg1-Atg13 complex via Atg11, a scaffold protein that binds to several autophagy-related proteins including Atg1, and Atg29. The C-terminal domain of Atg29 controls its interaction with Atg11 [86], as well as activity of the Atg17-Atg31-Atg29 complex [38]. Even though our understanding of the Atg1 kinase complex has greatly improved, there are still many questions about its structure and function that remain unresolved. For example, it is not clear whether there is a holo-complex that includes components involved in selective and nonselective autophagy (e.g., Atg11 and Atg17-Atg31-Atg29 appear to function primarily in the selective and nonselective modes, respectively), and the mechanism by which this complex regulates phagophore expansion is entirely speculative at this time.

3.2. Atg9 complex

Atg9 is the only transmembrane protein in the core autophagy machinery. Along with its putative cycling system (including Atg23, Atg27, Atg2 and Atg18), Atg9 is proposed to have a key role in expanding the phagophore during formation of the autophagosome [23, 87, 88]. Part of the Atg9 population is located at the PAS, but this protein is also present at the ER, Golgi complex, and peripheral structures proximal to the mitochondrial reticulum. As noted above, one model of phagophore expansion suggests that Atg9 may transit in a cyclical manner between the PAS and the peripheral structures [23, 87, 89]; Atg9 is not detected in the autophagosome membrane, indicating that it may be retrieved (or possibly degraded) during the formation process or after fusion of the autophagosome with the vacuole [88]. Atg9 self-interacts, and the multimerization of Atg9 facilitates its trafficking to the PAS for phagophore formation [90]. Atg9 contains 6 predicted transmembrane domains, and large soluble domains at the N and C termini would be exposed in the cytosol [91]; however, presumably due to its nature as a transmembrane protein, detailed structural information is still unavailable. Atg23 and Atg27 (as well as the scaffold protein Atg11 [92]) participate Atg9 trafficking to the PAS, but no structural data are available [93–95]. A newly characterized protein, Atg41, interacts with Atg9, sharing a similar localization profile, and also appears to participate in regulating Atg9 movement to the PAS [96].

Retrograde movement of Atg9 (i.e., from the PAS back to the peripheral structures) requires two complexes, Atg1-Atg13 and Atg2-Atg18 [23, 80]. The Atg14-containing class III PtdIns3K complex is also crucial for the retrograde pathway, at least in part, because Atg18 binds PtdIns3P [97]. Additionally, the localization of Atg9 at PAS is also dependent on Atg17, further linking Atg9 with the Atg1 kinase complex (Figure 4). The Atg2-Atg18 complex binds the edge of the phagophore and plays a role in controlling Atg9 movement back to the peripheral structures [80]. The localization and recruitment of Atg2 and Atg18 not only depend on each other, but also on Atg1, Atg13 and Atg9 [23]. Atg18 belongs to the “PROPPINs” family, defined by seven-bladed β-propeller fold structures and the conserved phenylalanine-arginine-arginine-glycine (FRRG) motif within this structure [98]. Two Atg18 paralogs, Atg21 and Hsv2, have also been identified, and structural studies provide some clues about Atg18 function [98, 99]. Although Atg18 can bind both PtdIns3P and PtdIns(3,5)P_2, only PtdIns3P is required for the appropriate targeting of the Atg2-Atg18 complex to the PAS [98, 100, 101]. The PtdIns3P binding sites of Atg18 are on propeller blades 5 and 6, and the membrane anchor from the hydrophobic loop in blade 6 also promotes binding to PtdIns3P [97, 100]; further phosphorylation on this loop inhibits membrane-binding activity. The β1-β2 loop and β2-β3 loop in blade 2 of Atg18 are crucial for PAS targeting and its interaction with Atg2 [97, 102]. Further details regarding the interaction between Atg18 and Atg2 still await further studies including Atg2 crystallization and information about their interdependent localization to the PAS.

3.3. Atg8–PE and Atg12–Atg5-Atg16 conjugation systems

The two Ubl systems that mediate conjugation of Atg8 and Atg12 (Figure 5) are key parts of the core autophagy machinery and contribute to phagophore expansion [103]. Although Atg8 and Atg12 are not ubiquitin homologs, they share structural similarity with ubiquitin folds based on their crystal structures [21]. The proteins in these two systems are the best

structurally characterized components in autophagy [104]. Atg12 is C-terminally conjugated to an internal Lys of Atg5, via an enzymatic pathway involving Atg7 (E1-like enzyme) for Atg12 activation and Atg10 (E2-like enzyme) for Atg12-Atg5 conjugation [105, 106]. The function of Atg10 depends on its E2 core consisting of four β sheets and two α-helices, and also on three additional β sheets and two accessory α-helices [107, 108]. Atg5 has two Ubl domains; an N-terminal UblA and a C-terminal UblB, which are connected by a helix-rich domain. UblB is responsible for the interaction with Atg10 [107]. After Atg12-Atg5 conjugation, a small coiled-coil protein, Atg16, binds to Atg5 noncovalently, and the coil-coiled domain of Atg16 further mediates homo-oligomerization [109, 110]. Whereas Atg12 is conjugated to another protein, Atg8 is conjugated to the lipid PE, which allows membrane association. Atg8 is initially synthesized with a C-terminal extension, which is removed by the Atg4 cysteine protease to expose a C-terminal Gly [103]. Next, Atg7 is required for activating the modified Atg8, and the protein is then transferred to Atg3 (an E2 enzyme) that attaches the exposed C-terminal Gly to PE [21, 103]. The Atg12-Atg5-Atg16 complex may work as an E3 enzyme that can facilitate Atg8-PE conjugation, but Atg8-PE can still be generated, albeit with a reduced efficiency, when this complex is absent [111, 112]. Atg8-PE is initially present on both sides of the phagophore; the protein on the concave surface plays a role in cargo recognition, but the function of the protein on the convex side of the membrane is less clear, although it may be involved in determining curvature [113]. Atg8-PE on the outer surface of the autophagosomes is subsequently deconjugated by a second Atg4-dependent cleavage [114]; at present, it is not known how Atg4 activity is regulated to prevent premature deconjugation. Although the specific role of Atg12-Atg5-Atg16 is still not clear, Atg12 negatively regulates a membrane-binding domain on Atg5; blocking this membrane-binding ability inhibits autophagy [115]. The membrane recruitment of Atg3 and Atg7 also depends on the Atg12-Atg5-Atg16 complex that is associated with the lipid bilayer.

4. Regulation of autophagy

Because autophagy plays an important physiological role, autophagic dysfunction is associated with many types of pathology; thereby the magnitude of the process must be tightly regulated to ensure appropriate levels. Accordingly, considerable research has focused on understanding the complex network of regulatory pathways that control autophagy. For example, there is tremendous potential in being able to manipulate autophagy for therapeutic purposes in the treatment of disease [30]. Here, we discuss certain aspects of autophagy regulation from the epigenetic to the post-translational level (Table 1).

4.1. Epigenetic regulation

Different epigenetic modifications including methylation and acetylation/deacetylation of histone H3 and H4 participate in the control of autophagy [116]. For example, spermidine, a natural polyamine that induces autophagy, can cause histone H3 deacetylation by repressing histone acetyltransferases, resulting in a global hypoacetylation in yeast [117]. Based on a genomic study, TOR (a component of a protein complex that acts as a master nutrient sensor in regulating protein synthesis, thereby acting as a primary negative regulator of autophagy) signaling was found to positively modulate the acetylation on histone H3 Lys56 (H3K56ac).

Accordingly, the level of H3K56ac is lower following the addition of rapamycin, a TOR inhibitor [118], and an alanine substitution at this position makes yeast cells more sensitive to rapamycin. The knockout of the sirtuin deacetylases Hst3 and Hst4 can restore the reduced level of H3K56ac caused by TOR inhibition, but the precise role of H3K56 deacetylation in autophagy is still under further investigation [118]. Upon autophagy induction, a decrease of H4K16ac is also observed, which occurs through downregulation of the histone acetyltransferase Sas2 (mammalian KAT8/hMOF) [119]. The joint reduction in the levels of both H4K16ac and trimethylated H3K4 (H3K4me3) is extended when autophagy is stimulated [119]. Collectively, different histone modifications likely play a role in controlling *ATG* gene expression, but current epigenetic studies in this field have been relatively limited.

4.2. Transcriptional regulation

It has been previously demonstrated that Atg protein synthesis is not absolutely required for autophagosome formation, but is important to generate normal-sized autophagosomes [120]. However, the dramatic increase in *ATG8* mRNA transcripts under starvation conditions corresponds to a significant rise of the Atg8 protein level, whereas yeast *atg8*Δ strains form abnormally small autophagosomes, suggesting that transcriptional control—and in particular that of *ATG8*—is important in modulating autophagy [35, 120, 121]. So far, there are several transcriptional regulators that have been identified in autophagy, which can regulate different *ATG* genes.

4.2.1 Ume6 and *ATG8*—Ume6, a yeast DNA-binding protein, is involved in transcriptional repression dependent on Sin3 (a co-repressor) and Rpd3 (a histone deacetylase), both of which can negatively regulate a variety of yeast genes [122–124]. The deletion of *UME6*, *SIN3* and/or *RPD3* results in a substantial increase in *ATG8* mRNA (and subsequently Atg8 protein) in growing conditions (when *ATG8* transcription is otherwise repressed), and also leads to a more rapid autophagic response when cells are shifted to autophagy-inducing conditions [125]. In addition, Ume6-deficient cells form larger autophagosomes than wild-type cells. The URS1 sequence, which corresponds to a consensus binding site for Ume6, is present within the *ATG8* promoter. Therefore, this negative regulator binds the promoter in growing conditions, negatively regulating autophagy at the transcriptional level, while upon autophagy-inducing conditions phosphorylation of Ume6 releases its repression of *ATG8* transcription [125]. This event is also regulated by the kinase Rim15, a positive regulator of autophagy that can integrate signals from TOR, and cAMP-activated protein kinase A (PKA), a second messenger-dependent enzyme that regulates various signaling pathways [126, 127].

4.2.2 Rph1 and *ATG7*—Similar to Ume6, Rph1 is also a DNA-binding protein that regulates autophagy through the control of *ATG* gene expression [128, 129]. Under nutrient-replete conditions, Rph1 inhibits the transcription of several *ATG* genes by binding to, at least, the *ATG7* promoter, and the Rph1-dependent regulation of *ATG7* expression plays a role in modulating autophagy activity [130]. Thus, Rph1 represents another example of how yeast cells maintain autophagy at a low basal level, and the inhibitory phosphorylation of Rph1 is also dependent on Rim15 upon starvation. Moreover, the regulation of autophagy by

Rph1 is conserved; a similar pathway has been confirmed in mammals involving KDM4A, a homolog of Rph1 [130].

4.2.3 Pho23 and *ATG9*—Working together with the Rpd3 histone deacetylase, Pho23 modulates gene expression in yeast [131]. It is yet another factor that negatively regulates mRNA and protein levels of several *ATG*/Atg genes/proteins, including Atg1, Atg7, Atg8, Atg9 and Atg14 [132]. Pho23 has a major impact on *ATG9* expression, and the level of Atg9 correlates with the number of autophagosomes. After autophagy is induced, *pho23*Δ cells would generate more normal-sized autophagosomes, compared to the wild type [132]. Therefore, Pho23 could be an inportant transcriptional repressor of autophagy that targets the frequency of autophagosome formation by negatively regulating *ATG9* level.

4.2.4 Other transcriptional regulators—In addition to Ume6, Rph1 and Pho23, a recent large-scale analysis of *ATG* gene expression has identified several new positive and negative transcription regulators [133]. Gln3 and Gat1 are nitrogen catabolite repression-sensitive GATA-type transcription factors, which can upregulate gene expression upon nutrient starvation conditions [134] [135]. After starvation, the expression of *ATG7*, *ATG8*, *ATG9*, *ATG29* and *ATG32* decreased in cells lacking Gln3 or Gat1, but double deletion of these two factors did not exert an additive effect on *ATG* expression [133]. Moreover, TOR can phosphorylate Gln3 and Ure2, resulting in retention of Gln3 in the cytosol, thereby negatively regulating autophagy [136]. Spt10 represses *ATG8* and *ATG9* expression, and Fyv5 has a negative effect on the expression of *ATG1*, *ATG8*, *ATG9* and *ATG14* [133]. Other DNA-binding proteins have also been identified as positive regulators of *ATG* gene expression. For example, Gcn4, which directly binds to the *ATG1* promoter, is required for its upregulation after nitrogen starvation, while the overexpression of Sfl1 may increase the transcription of several *ATG* genes, especially *ATG8* [133, 137].

In contrast to yeast, there are comparatively more transcription factors of autophagy identified in mammals, such as members from the FOXO (forkhead box O) family, TFEB (transcription factor EB), the E2F family, NFKB and TP53 [138]. FOXO proteins can shuttle between the nucleus and cytoplasm, depending on their phosphorylation status. Upon starvation, the phosphorylation of FOXO is blocked thus allowing positive regulation of autophagy [139]. The homolog of FOXO in yeast, Fhl1, can also directly target the promoters of *ATG13*, *ATG27* and *ATG31* based on ChIP-on-chip studies [140] [141]. Both TEFB and E2F family members are regarded as activators of autophagy. However, it is interesting to find that components such as NFKB and TP53 play a dual role (either as activators or repressors) in autophagy regulation, acting in a transcriptional-dependent or -independent manner [138, 142, 143]. In light of these recent findings about transcriptional regulation of autophagy, we can presume that transcription factors act as important regulators, and they may work together to establish a complicated transcriptional network. However, the contribution of transcriptional regulation of *ATG* genes in yeast and mammals to overall autophagy activity is still poorly understood, and will require a more comprehensive understanding before these regulatory processes can be manipulated for therapeutic purposes.

4.3. Post-transcriptional regulation

A relatively recent investigation has revealed a role of the post-transcriptional regulation on *ATG* transcripts in two fungal species and in human cells. Dhh1/Vad1/DDX6, homologous members of the RNA helicase RCK family in *S. cerevisiae*, *C. neoformans* and mammals, respectively, can negatively regulate autophagy, along with the decapping enzyme Dcp2/DCP2 [144]. Dhh1/Vad1/DDX6 binds the 5′ untranslated region of certain transcripts and recruits Dcp2, and the enzyme is phosphorylated and activated by TOR, resulting in the decapping, further degrading specific transcripts, which maintains autophagy at a basal level [145]. However, a major unresolved question is how this small subset of transcripts is selectively targeted under growing conditions (i.e., when the vast majority of transcripts are actively translated).

4.4. Post-translational modification

With regard to autophagy, there are three major types of post-translational modifications (PTMs), namely phosphorylation, ubiquitination and acetylation [75, 146, 147]. These modifications may act to modulate the function of Atg proteins or other regulatory factors. One or more types of PTMs may occur on a single protein at one or more sites and/or in a temporally-dependent manner in response to different types of stress [148]. Here, we briefly discuss these three types of PTMs as they concern the autophagy core machinery.

4.4.1. Phosphorylation—Phosphorylation is the best-characterized PTM on Atg proteins, and many studies have identified kinases involved in autophagy regulation. Conversely, the complementary phosphatases have been less well characterized. In yeast, one example of the role of phosphorylation-dependent regulation in autophagy is seen with the Atg1 kinase complex [149, 150]. The Atg1 kinase complex (Atg1, Atg13, Atg17-Atg31-Atg29) acts early in the induction step of autophagy and is negatively regulated by the TOR kinase [151]. Under nutrient-rich conditions, TOR-dependent phosphorylation of Atg1 and Atg13 results in reduced Atg1 kinase activity [152, 153]. Upon nitrogen starvation or treatment with rapamycin, TOR is suppressed, leading to dephosphorylation of Atg13 and Atg1 as well as autophagy induction [154]. PKA also directly phosphorylates these two proteins, which, in particular, affects the localization of Atg13 to the PAS [155]. Either the inactivation of PKA alone or the mutation to alanine of the residues on Atg13 can lead to autophagy induction. Furthermore, Atg13 dephosphorylation facilitates its interaction with Atg1 or Atg17, and alters its conformation to activate the Atg1 kinase complex [149] [151, 156]. However, autophosphorylation in the Atg1 kinase-activation loop is required for maintaining Atg1 activity after TOR inhibition [157]. To date, there is still some controversy regarding whether the kinase activity of Atg1 has a more significant role during growing conditions (e.g., for the Cvt pathway) or under starvation conditions [158, 159]. Further phosphorylation events include modifications of Atg29 and Atg31, which are necessary to generate an active Atg17-Atg31-Atg29 complex upon starvation [38, 160]. Some studies in yeast suggest that Atg9 phosphorylation directly by the Atg1 kinase is required in early steps of autophagy/phagophore elongation, and phosphorylated Atg9 then recruits Atg8 and Atg18 to the PAS [161, 162]. Furthermore, phosphorylation of Atg19 and Atg36 by Hrr25, and phosphorylation of Atg32 on Ser114 by casein kinase 2 are involved in selective types

of autophagy [163, 164]. For example, Atg32 phosphorylation mediates the interaction of Atg32-Atg11 during mitophagy [163].

As mentioned above, class III PtdIns3K complex I (Vps34, Vps30/Atg6, Vps15, Atg14 and Atg38) plays a role in the nucleation stage of autophagy. The Ser/Thr protein kinase Vps15 modulates Vps34 phosphorylation, which is important for forming the lipid kinase complex [165]. Additionally, Vps34 can phosphorylate phosphatidylinositol to generate PtdIns3P, which plays a crucial role in autophagosome formation [166]. Indeed, many Atg proteins, including Atg18, Atg20, Atg21 and Atg24 bind to PtdIns3P and are thus recruited to the PAS [101, 167, 168]. In yeast, the PtdIns3P phosphatase Ymr1 is localized at the PAS during an early stage of autophagy, and the Ymr1-dependent dephosphorylation of PtdIns3P is essential for Atg protein disassembly during autophagosome maturation, thus revealing the positive role of Ymr1 in autophagy [169]. In contrast, other PtdIns3P phosphatases may negatively control autophagy by lowering the level of this phosphoinositide, as seen in *C. elegans* or mammalian cells [170, 171]. These data imply that phosphorylation/dephosphorylation of PtdIns/PtdIns3P can either positively or negatively regulate autophagy, depending on the organism and stage of the process.

LC3, the homolog of Atg8, provides a good example of phosphoregulation in mammalian autophagy [172]. A direct phosphorylation site for PKA at Ser12 on LC3 has been confirmed, whereas rapamycin and pathological inducers of autophagy result in the dephosphorylation of endogenous LC3. The status of LC3 phosphorylation affects its interactions with phospholipids and other functions [173].

4.4.2. Ubiquitination—Ubiquitination can serve multiple functions in the cell including protein targeting and degradation. For example, after proteins are tagged by the small regulatory protein ubiquitin (Ub), in particular on Lys 48 and 63, they can be recognized by the 26S proteasome for degradation. The complete process of ubiquitination has three steps including activation, conjugation and ligation, performed by E1 (a Ub activating enzyme), E2 (a Ub conjugating enzyme), and E3 (a Ub ligase) enzymes, respectively [174]. Under some conditions, an E4 (a Ub chain elongation factor) enzyme is also required. The two Ubl systems (Atg8-related and Atg12-related) are the best examples of ubiquitination-like conjugation in yeast. In mammalian cells, an E3 ubiquitin protein ligase TRAF6 can modify both BECN1 (the Vps30/Atg6 ortholog in mammals) and ULK1 (the Atg1 ortholog) [175, 176]. The ubiquitination of BECN1 is required for autophagy induction, while ubiquinated ULK1 is stabilized and can auto-activate. Another E3 ligase, RNF5, promotes the degradation of Atg4 by ubiquitination, leading to suppression of autophagy [177].

4.4.3. Acetylation—Relative to studies on other forms of PTMs that participate in controlling autophagy, there has been much less work on the implication of acetylation in this process [178]. In yeast, Esa1, the catalytic subunit of NuA4, is a histone acetyltransferase participating in autophagy regulation [179]. Upon starvation, acetylation of Atg3 increases in an Esa1-dependent manner. Lys19, Lys48 and Lys183 of Atg3 are acetylated, among which Lys19 and Lys48 modifications are critical for autophagy. The repression of this acetylation blocks Atg8-Atg3 interaction and Atg8 lipidation. The function the Rpd3 deacetylase counters the effect of Esa1: the deletion of RPD3 results in increased

acetylation of Atg3 and upregulation of autophagy. The mammalian ortholog of Esa1, KAT5, can also directly acetylate ULK1, suggesting that acetylation of Atg/ATG proteins may be important in regulating autophagy in all eukaryotes [179, 180].

5. Cargo recognition in selective autophagy

As mentioned above, two types of autophagy can be distinguished based on the type of cargo that is engulfed by the autophagosomes. In nonselective autophagy, the degradation of random portions of cytoplasm is critical for cell survival upon nutrient and energy deprivation. However, autophagy can also be highly specific, and selective autophagy, along with basal constitutive autophagy, functions more in cell maintenance and homeostasis, as well as in the cellular remodeling that accompanies the shift to a preferred carbon source [19]. During selective autophagy (Figure 6), the phagophore membrane is closely apposed to the cargo, preventing the sequestration of bulk cytoplasm, and finally transports the sequestered contents to the vacuole [181]. Due to the *de novo* mechanism of phagophore formation, a wide range of cargo, including entire organelles, can be selectively degraded through autophagy. These include mitochondria (mitophagy), peroxisomes (pexophagy), protein aggregates (aggrephagy), lipid droplets (lipophagy), and intracellular pathogens (xenophagy); accordingly each process involves specific components (such as receptors) and the core machinery [182]. Moreover, in contrast to the normal pattern of autophagy, selective autophagy can also work as a biosynthetic mechanism. In the Cvt pathway, precursor aminopeptidase I (prApe1) is the primary cargo protein. This precursor is synthesized in the cytosol, and assembles into dodecamers, which subsequently form a larger oligomer (termed the Ape1 complex). It is this complex that binds the Cvt pathway receptor Atg19 [183], and the scaffold Atg11, which is selectively sequestered by the phagophore. The binding of Atg11 to Atg19 occurs after Atg19 interacts with prApe1, and the WXXL motif on the C terminus of Atg19 allows for its binding to Atg8 [184]. Upon completion, the double-membrane Cvt vesicle (analogous to an autophagosome in nonselective autophagy) fuses with the vacuole; as with nonselective autophagy this results in the release of the inner vesicle into the vacuole lumen generating the Cvt body (equivalent to the autophagic body). After the single-membrane vesicle is broken down, the cargo is exposed to the resident vacuolar hydrolases, but in this case the contents of the Cvt body are not degraded; prApe1 is proteolytically matured by removal of a propeptide, and it now functions as another vacuolar protease [185]. Atg19 also binds to Ape4 and Ams1, two additional resident hydrolases that are delivered to the vacuole via the Cvt pathway, whereas Atg34 can replace the role of Atg19 in binding to Ams1 under starvation conditions [186–188].

The Cvt pathway provides a general model for selective autophagy, involving interactions among the cargo (prApe1), a ligand (the prApe1 propeptide), a receptor (Atg19) and a scaffold (Atg11), which link the cargo with the autophagy machinery via binding to Atg8. Similarly, during mitophagy, the Atg32 receptor initially binds to Atg11 and subsequently to Atg8, although in this case there is not a clear ligand [189, 190]. Atg33, a transmembrane protein on the mitochondrial outer membrane, is also required by mitophagy that is induced in the post-log phase [191]. In pexophagy, Atg36 functions as the pexophagy receptor, also interacting with Atg11 and Atg8 [192].

6. Concluding Remarks

Autophagy, generally speaking, is a highly regulated, complicated process key to most intracellular degradation events. There has been tremendous research advancing our understanding about autophagy in recent years, and studies of autophagy in yeast allowed researchers to address several questions concerning different aspects of this complex process. However, some fundamental questions still remain to be settled. The detailed mechanism of autophagosome biogenesis is not known. For example, the membrane donors for autophagosome biogenesis are highly debated, and it is unknown whether different membrane sources work together/separately or contribute to different forms of autophagy. Critical mediators of the fusion process, SNAREs, may act in autophagosome formation and in the later steps of the process by regulating autophagosome/lysosome fusion, but the exact mechanisms are still unclear. Although it is obvious that the magnitude of autophagy should be finely regulated by modulating the levels and the activity of autophagy-related proteins, the contribution of the regulation of these proteins to overall autophagy activity is poorly understood, which awaits further studies on the complicated network of regulatory pathways. Understanding the regulation of this pathway is particularly important for the prospect of potential therapeutic modulation of autophagy in the treatment of some major human diseases. Detailed information about the core autophagic machinery would also be garnered from further information on the basic structure of the Atg proteins (both static and dynamic), although structure-related data are still missing for many fundamental autophagy-related proteins including Atg1 and Atg9. The final step of autophagy, and its inherent function in the turnover of cytoplasmic molecules, relies on the release of breakdown products from the vacuole back to the cytosol, yet, this recycling step is still mostly uncharacterized. Therefore, further studies on these different mysterious aspects will bring critical information for a better and comprehensive understanding of autophagy.

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Abbreviations

Ape1	aminopeptidase I
Atg	autophagy-related
CMA	chaperone-mediated autophagy
Cvt	cytoplasm-to-vacuole targeting
ER	endoplasmic reticulum
FOXO	forkhead box O
PAS	phagophore assembly site
PE	phosphatidylethanolamine
PtdIns3K	class III phosphatidylinositol 3-kinase

PKA	cAMP-activated protein kinase A
prApe1	precursor Ape1
PtdIns3P	phosphatidylinositol-3-phosphate
PTM	post-translational modification
TOR	target of rapamycin
Ubl	ubiquitin-like

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Highlights

1. Among the different types of autophagy, macroautophagy is the best characterized.
2. The biogenesis of the phagophore or autophagosome is a key step in the process.
3. Tight regulation of macroautophagy is important for normal cellular function.
4. Autophagy-related proteins that form the autophagosome constitute the core machinery.
5. Macroautophagy can be nonselective or selective for specific cargos.

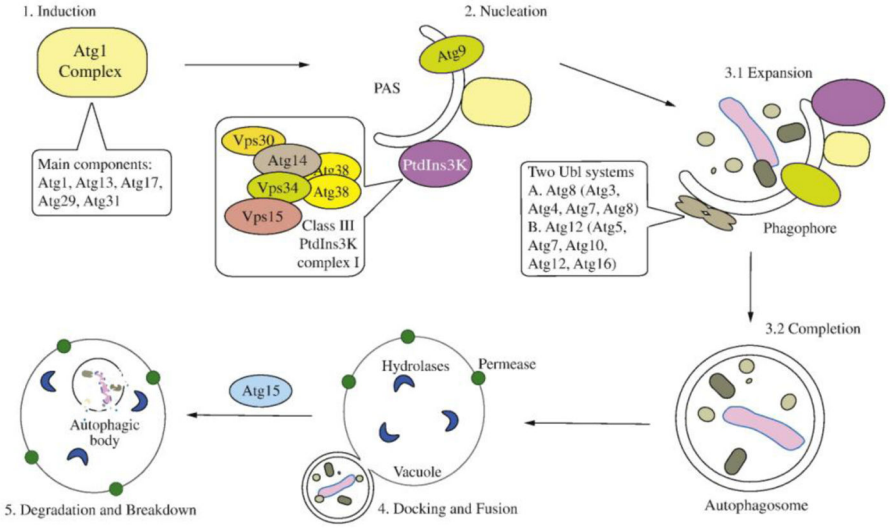


Figure 1. A model of the different stages of autophagy in yeast

In yeast, macroautophagy can be generally divided into the following stages: induction, nucleation, expansion and completion, docking and fusion, and finally degradation and efflux. The Atg proteins form most of the primary machinery that function in each of these stages.

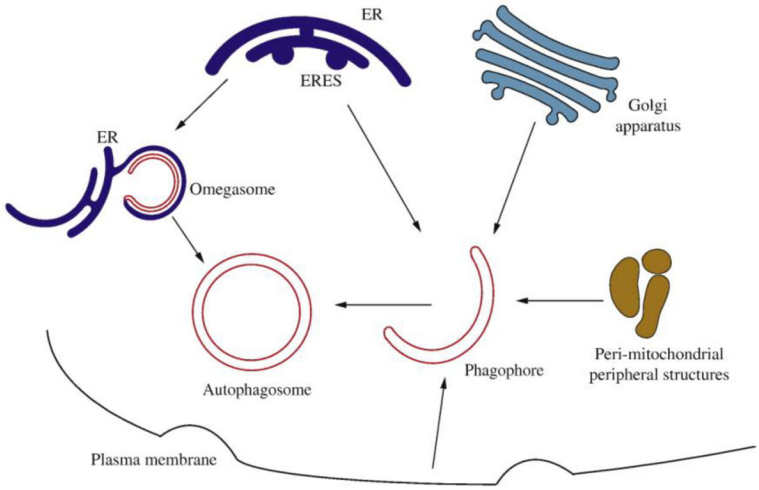


Figure 2. Different membrane sources of autophagosome formation

The formation of the phagophore and the double-membrane autophagosome are the distinguishing morphological hallmark of macroautophagy in yeast. Several membrane donors including the plasma membrane, ER, ER exist site (ERES), mitochondria, and the Golgi apparatus may contribute to the biogenesis of the phagophore and autophagosome in yeast. In mammalian cells an additional structure, the omegasome, forms directly from the ER membrane, but the relationship between the omegasome and phagophore is not clear.

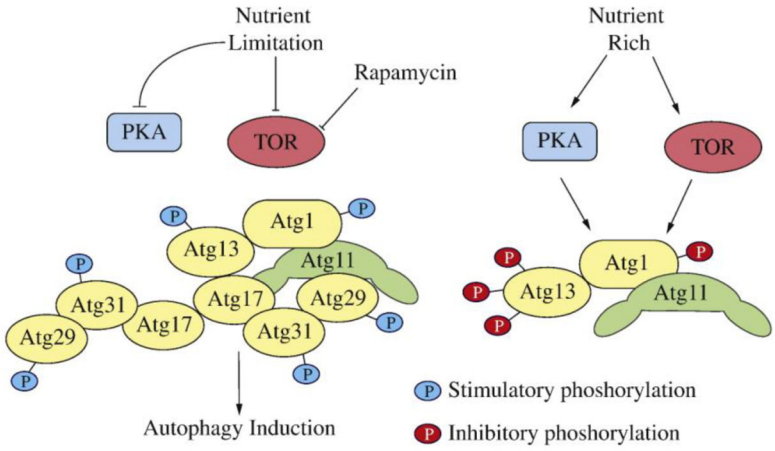


Figure 3. The interactome of the Atg1 kinase complex

Upon autophagy induction, the Atg1 complex is mainly composed of three components: Atg1, Atg13 and the Atg17-Atg31-Atg29 subcomplex. Atg13 can bind to Atg1 and the Atg17-Atg31-Atg29 subcomplex directly, whereas there is no known binding site on Atg1 for the subcomplex. The scaffold protein Atg11, which plays a critical role under growing conditions and for selective autophagy, interacts the with Atg1 kinase complex by binding to Atg1, Atg17 and Atg29.

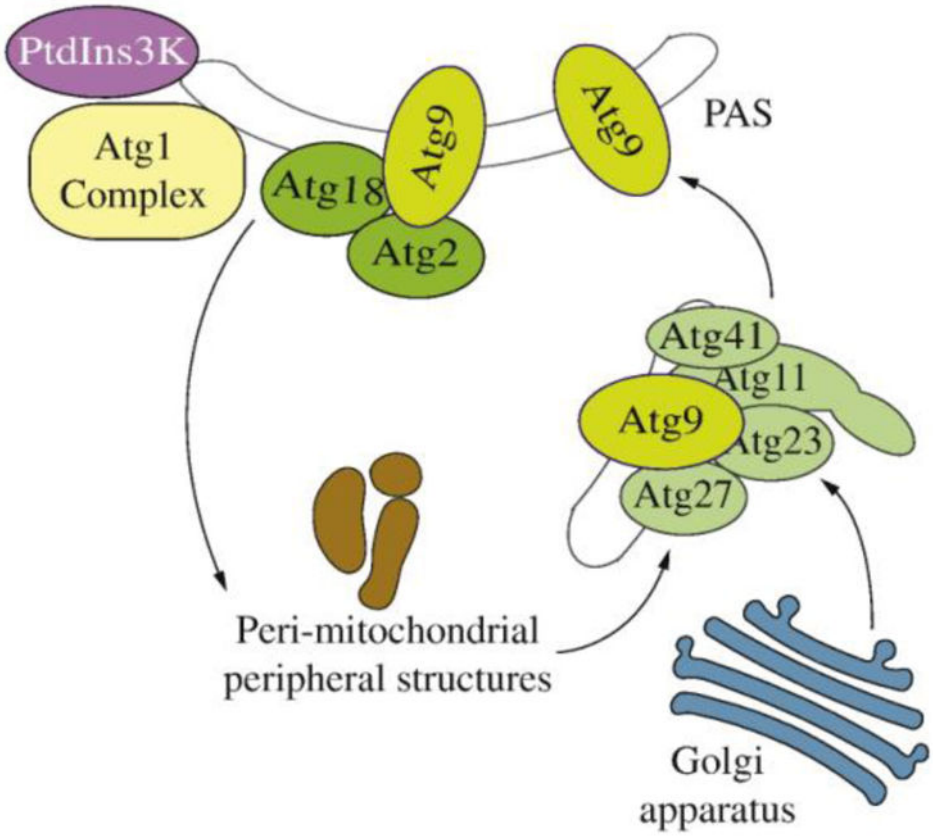


Figure 4. The Atg9 complex

The transmembrane protein Atg9 can reside at the PAS and peripheral structures proximal to mitochondria. With the help of Atg11, Atg23, Atg27 and Atg41, Atg9 can travel from the peripheral structures to the PAS, playing a role in orchestrating membrane delivery. Retrograde movement relies on the Atg2-Atg18, Atg1-Atg13 and Atg14-containing PtdIns3K complexes.

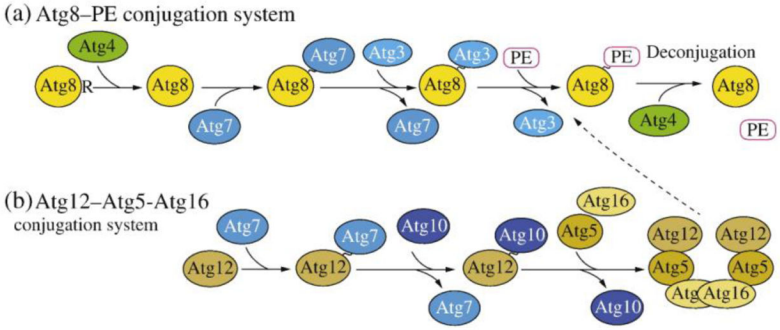
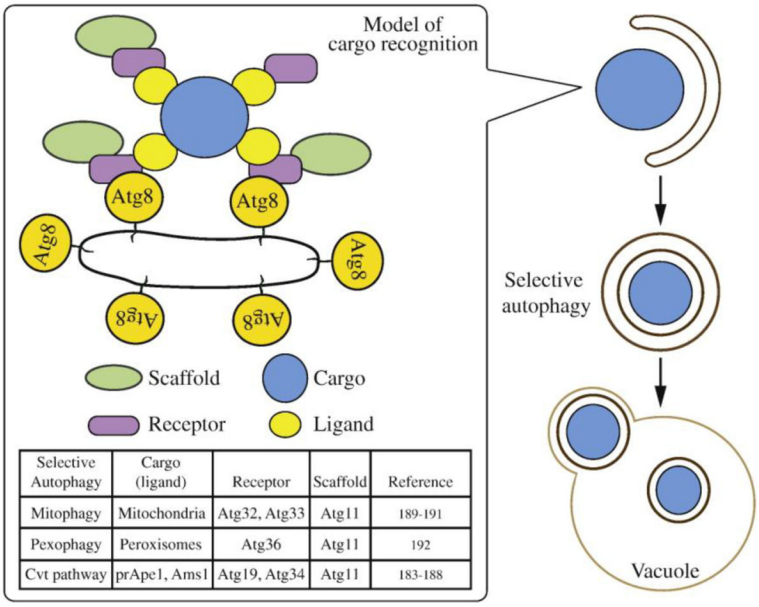


Figure 5. Two ubiquitin-like conjugation systems

The two ubiquitin-like proteins Atg8 and Atg12 are used to generate the conjugation products Atg8-PE and Atg12-Atg5-Atg16, respectively. These two Ubl conjugation systems use the same E1-like enzyme Atg7, but different E2-like enzymes, Atg3 for Atg8 and Atg10 for Atg12. Furthermore, the Atg12-Atg5-Atg16 complex can work as an E3-like enzyme for Atg8-PE formation.



Selective Autophagy	Cargo (ligand)	Receptor	Scaffold	Reference
Mitophagy	Mitochondria	Atg32, Atg33	Atg11	189-191
Pexophagy	Peroxisomes	Atg36	Atg11	192
Cvt pathway	prApe1, Ams1	Atg19, Atg34	Atg11	183-188

Figure 6. A general model of selective autophagy in yeast

For different kinds of selective autophagy in yeast there is a commonly used model to allow cargo recognition. This schematic depicts three kinds of selective autophagy: Cvt pathway, mitophagy, and pexophagy. Detailed information is provided in the corresponding table.

Table 1

The regulation of autophagy in yeast.

Regulation Types		Regulatory factors	Autophagy/autophagy-related targets	Effects on autophagy (+, positive; −, negative)	Refs
Epigenetic regulation	**Acetylation**	Histone acetyltransferase	Histone H3	−	117
		TOR	Histone H3 Lys56	−	118
		KAT8/hMOF	Histone H4 Lys16	−	119
	Deacetylation	Hst3, Hst4	Histone H3 Lys56	+	118
	Methylation	Unclear	Histone H3 Lys4 trimethylation	+	119
Transcriptional regulation		Ume6	*ATG8*	−	125
		Rph1	*ATG7*	−	130
		Pho23	*ATG9*	−	132
		Gln3	*ATG7, 8, 9, 29, 32*	+	133
		Gat1	*ATG7, 8, 9, 29, 32*	+	133
		Spt10	*ATG8, 9*	−	133
		Fyv5	*ATG1, 8, 9, 14* (potential)	−	133
		Gcn4	*ATG1*	+	133
		Sfl1	*ATG8*	+	133
		Fhl1	*ATG13, 27, 31* (potential)	Unknown	140, 141
Post-transcriptional regulation		Dhh1-Dcp2	*ATG7, ATG8, ATG29* and others	−	145
Post-translational regulation	**Phosphorylation**	TOR	Atg1 complex, Atg13	−	151–153
		PKA	Atg1 complex, Atg13	−	155
		Atg1	Atg9	+	161, 162
		Hrr25	Atg19, Atg32, Atg36	+	163, 164
		Vps15	Vps34	+	165
		Vps34	PtdIns3P	+	165
	Dephosphorylation	Ymr1	PtdIns3P	+	169
	Autophosphorylation		Atg1	+	157
			Atg29	+	38
			Atg31	+	160
	Ubiquitination	No clear model in yeast			

Regulation Types		Regulatory factors	Autophagy/autophagy-related targets	Effects on autophagy (+, positive; −, negative)	Refs
	Acetylation	Esa1	Atg3	+	179
	Deacetylation	Rpd3	Atg3	−	179