Autophagy: New emerging functions and concepts

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Abstract

Autophagy is crucial to maintain cell, tissue and organism homeostasis through degradation. This review will emphasize the relevance of its regulation by describing the recent advances in our understanding of the complex post-translational modulation of the Atg (AuTophaGy-related) proteins and the emerging notion of the cell type-specific regulation of autophagy. New data have also revealed unexpected roles of this pathway and that the *ATG* genes carry out autophagy-unrelated functions thus projecting the autophagy research field beyond the boundaries drawn by the simplistic view of a process exclusively dedicated to the turnover of cellular components. This piece is also discussing these new discoveries to highlight the novel frontiers lying ahead.

Introduction

The word autophagy, from the Greek *self-eating*, refers to those catabolic processes through which the cell turns over its own constituents[1]. While the proteasome is also involved in cellular degradation, autophagy only refers to those pathways that lead to the elimination of cytoplasmic components by delivering them into mammalian lysosomes or plant and yeast vacuoles. Autophagy is often labeled as degradative, however, it is more accurate to describe it as a recycling pathway to underscore its important contribution to the cell physiology. In fact, metabolites generated in the lysosome/vacuole are reused either as source of energy or building blocks for the synthesis of new macromolecules. To date, three major types of autophagy have been described: Macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) (Figure 1). This perspective will focus on macroautophagy, hereafter referred to as autophagy.

Autophagy has been shown to participate in the adaptation to starvation, cell differentiation and development, degradation of aberrant structures, turnover of superfluous or damaged organelles, tumor suppression, innate and adaptive immunity, lifespan extension and cell death[2-4]. Deciphering the role of Atg proteins in autophagy was a milestone in the understanding of the importance of this process[1,3]. In addition, the post-translational modifications of the Atg proteins documented so far offer a great plasticity to the system for the integrated transduction of a multitude of stimuli into the autophagy machinery. The recent period has also witnessed new roles for autophagic structures beyond the fusion with lysosomal compartment and a plethora of non-autophagic functions for the Atg proteins.

The mechanism and regulation of autophagy

Autophagy is characterized by the formation of double-membrane vesicles called autophagosomes, which sequester the cytoplasmic structures targeted to destruction (Figure 1). Upon autophagy induction, the Atg proteins (Table 1) assemble at a specialized site that has been named the phagophore assembly site or the pre-autophagosomal structure (PAS)[5]. There, they first mediate the biogenesis of the phagophore by probably participating together with specific SNAREs in the orchestrated fusion of possibly Golgi-, endosome- and plasma membrane-derived membranes[6]. The PAS have been shown to emerge from omegasomes, regions of the mammalian endoplasmic reticulum (ER) enriched in the phosphatidylinositol-3-phosphate (PtdIns3P)-binding protein DFCP1[7]. Contact points between omegasomes and phagophores have led to the hypothesis that lipids are supplied to the nascent autophagosomes by direct transfer from the ER[8,9], but other organelles such as mitochondria has also been implicated in this process[10]. Then complete autophagosomes fuse with the lysosomes (or vacuoles) to expose their content to the hydrolases present in these degradative organelles.

As for all the degradative pathways, regulation is key to specifically turn on a potentially destructive process only for the limited time that it is required. Consequently, eukaryotic cells have several signaling molecules and cascades to modulate autophagy in response to numerous cellular and environmental cues[11,12]. The best-characterized regulator of autophagy is mTOR in the complex 1 (mTORC1). This kinase inhibits the activity of the Atg1/ULK1 complex through direct phosphorylation. The activity of mTORC1 is stimulated by a variety of anabolic inputs, which include the energy and nutrient status of the cell as well as the presence of amino acids and growth factors. Conversely, mTORC1 is inhibited when amino

acids are scarce, growth factor signaling is reduced and/or ATP concentrations fall, and this results in a de-repression of autophagy[12].

Selective type of autophagy

Autophagy has been considered for long time a non-selective process for bulk degradation of either long-lived proteins or cytoplasmic components during nutrient deprivation. Recent evidences, however, have revealed the existence of numerous types of selective autophagy[13]. Under specific conditions, autophagosomes can thus exclusively sequester and degrade mitochondria, peroxisomes, ER, endosomes/lysosomes, lipid droplets, secretory granules, cytoplasmic aggregates, ribosomes, invading pathogens…(Figure 2A). Proteins complexes in signalling cascades as the inflammasome are also regulated through selective autophagy[14]. The small size of these latter factors, however, appears to not always require the stimulation of autophagy but rather the induction of their targeting to the autophagosomes formed by basal autophagy.

The selective types of autophagy rely on specific cargo-recognizing autophagy receptors that assure the cargo sequestration into autophagosomes. Autophagy receptors are proteins being able to interact directly with both the structure that has to be specifically eliminated by autophagy and the pool of the Atg8/LC3 protein family members present in the internal surface of the growing autophagosomes[13,15]. This latter interaction is in most of the cases mediated through a specific amino acid sequence present in the autophagy receptors and commonly referred to as the LC3-interacting region (LIR) or the Atg8-interacting (AIM) motif[15,16].

The study of the biosynthetic transport route present in yeast *Saccharomyces cerevisiae* and called the cytosol-to-vacuole targeting (Cvt) pathway[17], has been pivotal in understanding

the selective types of autophagy in other eukaryotic cells as well. This pathway mediates the delivery into the vacuole lumen of three hydrolases that are all part of a large oligomeric structure. The recruitment of this cargo into autophagosomes depends on Atg11 and the autophagy receptor Atg19 (Atg11 and Atg19 are not required for starvation-induced autophagy). The distribution of Atg19 on the surface of the cargo and its interaction with Atg8 through a LIR motif allow the hermetic formation of a double-membrane vesicle around the targeted structure. Atg32 and Atg36 are yeast autophagy receptors for mitochondria and peroxisomes, respectively, that are on the surface of these organelles and appears to operate in the same way as Atg19[18-20].

One other emerging theme in high eukaryotes is that numerous structures targeted for destruction by autophagy are ubiquitinated and a series of autophagy receptors with an ubiquitin-binding domain and a LIR motif such as p62/SQSTM1, NBR1, NDP52 and optineurin, promote their engulfment into autophagosomes[15,21]. Ubiquitination thus appears to be the key regulatory element during selective types of autophagy highlighting the central stage that the E3 ligases and/or their eventual adaptors and regulators must have in these processes. Despite this relevance, only a handful of these molecules have been identified. Those include the Pink1 kinase, the Parkin ligase and the mitochondrial outer membrane protein FUNDC, which play an important role during mitophagy[22-24], and the SMURF1 ligase and the STING adaptor, which participates in the clearance of pathogens[25,26].

Post-translational modifications in the regulation of autophagy

Originally the mTOR-dependent phosphorylation of the Atg1 complex and the formation of the Atg12—Atg5 conjugate as well as the lipidation of the C-terminus of Atg8/LC3 were considered to be the only post-translational modifications in autophagy. Phosphorylation, ubiquitination and

acetylation are now known to have a broad spectrum of substrates in between the Atg machinery[27].

Yeast protein kinase A (PKA) phosphorylates Atg13 in the presence of nutrients in preventing the association of this protein with the PAS[28]. In mammalian cells, ULK1 is directly phosphorylated by the AMP-activated protein kinase (AMPK) in response to energy restriction[29,30]. AMPK triggers autophagy by both positively regulating the ULK complex and inhibiting mTOR[11]. In addition, this kinase stimulates autophagy in response to glucose starvation by phosphorylating Beclin 1 in the phosphatidylinositol 3-kinase (PI3K) complex I[31]. The PI3K complex I is a major target of phoshorylation to regulate autophagy. For example, the phosphorylation of Beclin 1 by the death-associated protein kinase (DAPK) or phosphorylation of Bcl-2 by the c-Jun N-terminal kinase (JNK) triggers the dissociation of the Beclin 1-Bcl-2 complex in response to various stimuli, allowing Beclin 1 to associate and stimulate PtdIns3P synthesis by the PI3K complex I[32,33]. More recently, it has been shown that Beclin 1 phopshorylation by Akt/PKB inhibits autophagy by favoring the interaction of Beclin 1 with 14-3-3 and the vimentin intermediate filament protein 1[34].

Downstream of the ULK and the PI3K complexes, phosphorylation can regulate the activity of LC3-II. When phosphorylated by PKA or protein kinase C (PKC), LC3 becomes inoperative in autophagosome formation[28,35]. How exactly phosphorylation inhibits LC3 function remains to be elucidated. The PKA site is highly conserved in the human, mouse and rat isoforms of LC3, but is not present in yeast Atg8[28]. Phosphorylation is also able to increase the affinity of the autophagy receptors for substrates and LC3 during selective type of autophagy. Phosphorylation of p62 by the casein kinase 2 favors its interaction with ubiquitinated proteins[36], whereas phosphorylation of optineurin by the TANK-binding kinase 1 enhances its affinity for

LC3 promoting the elimination of cytosolic *Salmonella*[37].

While ubiquitination plays an important role in the selection of the cargo targeted for destruction during selective types of autophagy, there are so far no works reporting this post-translational modification to occur on Atg proteins. Ubiquitination-like reactions, in contrast, are central during autophagosome biogenesis through the generation of the Atg12—Atg5 and the Atg8/LC3—PE conjugates. Recently the formation of an Atg12—Atg3 conjugate though the action of Atg7 and the autocatalytic activity of Atg3 have been described in mammalian cells[38]. This conjugate plays a role in cell death pathways and in the control of mitochondrial expansion.

Following an initial study showing that some of the Atg proteins are acetylated[39], the analysis of the acetylome has revealed that this modification is also playing a key role in the autophagy regulation. Indeed Atg5, Atg7, LC3 and Atg12 are acetylated by the p300 acetyltransferase when cells are maintained in nutrient-rich media and deacetylated by Sirt1 in response to starvation, an event necessary to induce autophagy[40,41]. More recently, the acetylation of Atg3 by Esa1 in yeast and by the Esa1 ortholog TIP60 in mammals under autophagy-inducing conditions has been shown to promote the interaction between Atg3 and Atg8/LC3, which is an important step in the Atg8/LC3 lipidation[42]. TIP60 also acetylates ULK1 in a glycogen synthase kinase-dependent manner in response to growth factor deprivation[43]. Thus, ULK1 is activated by either phosphorylation or acetylation in response to amino acid/glucose and growth factor deprivation, respectively[44].

Autophagy and Atg proteins in membrane transport and secretion

For a long period the Cvt pathway has been the only transport pathway depending on Atg proteins not associated with lysosomal/vacuolar degradation. Recent studies have demonstrated

that the Atg machinery can also be used to release cargo into the extracellular medium sheding some light into the mechanism of at least few types of unconventional protein secretion[45-47]. A mechanism of this type appears to be involved in the exportation of the pro-inflammatory cytokine IL-1 and IL-18 in mammalian cells[47]. This process depends on Atg5, the inflammasome, the peripheral Golgi protein GRASP55 and the small GTPase Rab8a. This autophagy-based unconventional secretion mechanism can probably be extended to modulators of the immune response such as HMGB1[47]. The molecular bases for the formation and the morphology of the autophagy-related organelles involved in secretion remains to be identified. In yeast the putative carriers are formed from a hitherto-unknown compartment for unconventional protein secretion (CUPS)[48]. Like the PAS, CUPS also contains PtdIns3P as well as Atg8 and Atg9. Although these two Atg proteins are required for the generation of the PAS, they appear to be unnecessary for CUPS formation. This mode of secretion could explain the non-lytic release of viruses that subverts the autophagy machinery for egression[49] or the extracellular expel of cellular material by autophagosomes at the late stages of reticulocytes maturation into erythrocytes[50].

In oncogene-induced senescent cells, a specialized compartment known as the TOR-autophagy special coupling compartment (TASCC) stimulates the extracellular release of a specific subset of proteins through the conventional secretory pathway[51]. TASCC, which are juxtaposed to the Golgi apparatus, are principally associated with lysosomes. Lysosomes generate an amino acid efflux from their lumen into the cytoplasm via the degradation of proteins. Importantly, mTOR located at the lysosomal surface is activated by amino acid and positively regulates protein synthesis and cell growth[51,52]. Autophagosomes, which supply lysosomes with proteins, accumulate adjacently the TASCC. This could represent an example of

a coupling between degradation and synthesis/secretion of proteins, which could be key in coordinating the cell metabolism. The discovery of the TASCC has also revealed on the importance of the spatial segregation of autophagosomes and lysosomes for the tight regulation of autophagy. TASCC-like structures have also been observed in non-senescent cells suggesting that this regulatory mechanism for protein secretion may be widely used[51]. Autophagy and Atg proteins also modulate secretion in several specialized tissues including the middle ear, osteoclasts, mast cells, Paneth cells and pancreatic β-cells[53]. LC3-II is involved in numerous of these processes. It is interesting to observe that independently of autophagosome formation, LC3-II mediates the fusion with vesicular carriers containing the protease cathepsin K with the ruffled border of osteoclasts, which is an important step in bone resorption[54]. This situation mirrors the recruitment of LC3-II onto phagosomes to enhance their fusion with lysosomes in phagocytic cells[55]. The role of LC3-II and other members of the Atg8/LC3 protein family in these processes probably relies on the capacities of these factors in mediating tethering/fusion[56,57] and/or binding the cytoskeleton[58].

Autophagy as a regulator of tissue microenvironment metabolism

The classical stimulus to induce autophagy is starvation. Under these conditions, the degradation of intracellular components generates metabolites that are essential to maintain cell viability (Figure 2A). Those can be used to fuel mitochondrial respiration and ATP production, as methyl-pyruvate is able to revert the consequences caused by an impairment of autophagy-dependent ATP production[59,60]. In yeast, amino acids generated by autophagy can be used to sustain new protein synthesis and to maintain mitochondrial functions under nutrient deprivation[61]. Amino acids generated in the liver by autophagy are used for gluconeogenesis to maintain systemic

glycemia under starvation (Figure 2A)[62]. Moreover the selective degradation of lipid droplets in the liver by autophagy, i.e. lipophagy, is an important mechanism to produce free fatty acids from triglycerides beside the activity of hepatic triglyceride lipases[63].

The role autophagy in cancer metabolism is complex and it has been the subject of intense investigation. Autophagy protects cancer cells from metabolic stress (decreased nutrient availability and hypoxic conditions) by reducing oxidative stress and maintaining genomic stability[64]. It is now clear that cancer cells reprogram their metabolism to support their rapid proliferation and growth. In addition to increase nutrient uptake, cancer cells perform aerobic glycolysis (the Warburg effect) by oxidizing glucose into lactate to produce ATP[65]. Although this pathway produces less ATP molecules than glycolysis, it provides cells with intermediary metabolites for anabolic reactions that allow sustaining cell growth[65]. This metabolic switch includes the expression of specific isoforms of glycolytic enzymes but also enzymes metabolizing amino acids and lipids, which display distinct enzymatic activities and substrate preferences[66]. In this context, the activation of the hypoxia-responsive factor HIF-1 regulates the expression of many glycolytic enzymes and induces mitophagy[67]. A recent metabolomic study has demonstrated that cells can maintain minimal levels of mitochondrial respiration to produce ATP even under hypoxia (1% oxygen), although they are lower than in normoxic conditions. These cells moreover display both elevated autophagy activity and increased levels of intermediates resulting from protein and lipid catabolism[68]. Importantly, autophagy inhibition under these conditions leads to a reduction of intracellular ATP levels and an induction of cell death. Thus degradation products resulting from autophagy are able to fuel the tricarboxylic acid cycle for ATP synthesis to maintain cell viability even under low oxygen[68]. This cyto-protective role of autophagy under hypoxic conditions may be modulated through microRNA-dependent

ATG7 expression in hepatic tumor cell lines and *in vivo* xenographs[69]. Although observations in other cell types support this notion[70], is still unknown whether the autophagy-dependent degradation of mitochondria is another mechanism responsible for the metabolic reprogramming. Indeed overexpression of the RCAN1-1L protein induces mitophagy and a shift of neuronal cells from oxidative to glycolytic metabolism[70].

Metabolic coupling, a phenomenon in which two different cell types differentially coordinate their metabolism, has been observed in several tissues including tumors and brain tissue (Figure 2B,C), and it has been associated to autophagy[71]. Tumors are composed by several cell types and a new theory postulates that the metabolic coupling between them is essential for tumor development. In particular, fibroblasts are the cells performing aerobic glycolysis inside tumors and display an increased expression of glycolytic enzymes, elevated HIF-1 activity and augmented autophagy and mitophagy[71]. Metabolites resulting from elevated autophagy and glycolytic activities such as lactate, ketone bodies and amino acids, are released into the tumor microenvironment and sequestered by cancer cells to fuel the oxidative phosphorylation necessary to sustain tumor growth[71]. The autophagy stimulation in fibroblasts results in increased senescence and boosts both the production of ketone bodies and mitochondrial metabolism in adjacent cancer cells, which in turn strongly promotes metastasis[72]. In contrast autophagy upregulation in the cancer cells inhibits tumor growth indicating that the effects of autophagy are tissue-specific in the context of a tumor microenvironment[72]. Other metabolites such as the ammonia generated from glutaminolysis by cancer cells stimulates autophagy in neighbor cells[73]. A similar scenario has also been observed in the brain where astrocytes and neurons also exchange metabolites to support their cellular functions (Figure 2C). A recent report shows that the impairment of lysosomal functions and autophagy affects specific cell types such as

astrocytes making them unable to provide support and protection to neighbor neurons, resulting in cortical neurodegeneration *in vivo*[74]. All together, these data indicate that autophagy in specific cell types could be key in regulating the survival and growth of the surrounding tissue (Figure 2C).

ATG genes beyond autophagy: Their role in other cellular processes

The proteomic analysis of Atg protein interactome suggested that they can function in cellular pathways independently of their role in autophagy[75]. This emerging topic has been recently reviewed[76]and some of the non-autophagic functions of *ATG* genes are illustrated in Figure 3. As discussed above, some modules involved in autophagosome formation such as the two conjugation systems (ATG5—ATG12 and LC3—PE) and the PI3K complex I are recruited to the phagosomal membrane to promote the fusion between phagosomes and lysosomes during phagocytosis triggered by the engagement of Toll-like receptors[55]. These conjugation systems are also required for the generation of the osteoclast ruffled border, a key structure for bone resorption[54]. The ATG5—ATG12-ATG16L1 complex, independently of the LC3 conjugation system can modulate the innate viral immune response. ATG5—ATG12 suppresses the type I interferon (IFN) production by direct association with the retinoic acid-inducible gene I (RIG-I) and IFNβ promoter stimulator (IPS-1)[77]. This complex is also required for type II IFN-mediated host defense against norovirus by inhibiting the formation of its membranous replication complex[78]. Similarly, ATG5 is required for the clearance of the parasite *Toxoplasma gondii* in macrophages[79]. Complexes acting upstream of the conjugation systems such as ULK1 complex and PI3K complex I are necessary for the intracellular cycle of the bacterium *Brucella abortus* to subvert clearance by contribution to form a vacuole containing the bacteria and promote

infection[80]. In addition to the conventional cassette, a new conjugate ATG12—ATG3 has been shown to regulate mitochondria homeostasis and cell death without affecting the formation of autophagosomes in response to starvation[38]. Both LC3 and Atg12 can also function independently from their conventional conjugation to PE and ATG5, respectively. LC3-I is involved in the formation of carriers derived from the ER and called EDEMosomes[81], a pathway hijacked by coronaviruses to form structures needed for the viral genome transcription and replication[82]. ATG12 is a positive mediator of mitochondrial apoptosis by inactivating members of the prosurvival Bcl-2 protein family. The activity of ATG12 is independent of ATG5 or ATG3, and requires a BH3-like motif in ATG12[83]. Another mechanism by which Atg proteins act in an autophagy-independent manner depends on limited proteolysis. For example, ATG5 cleavage by calpain generates a pro-apoptotic fragment that interferes with the anti-apoptotic activity of Bcl-xL[84]. Moreover, Beclin 1 cleavage by caspase 3 generates two fragments without autophagy-inducing capacity[85, 86]. The C-terminal fragment resulting from this processing localizes to mitochondria and sensitizes cells to apoptosis[86]. Thus similarly to proteins involved in apoptosis, which have function beyond apoptosis[87], Atg components and other proteins involved in autophagy such as AMBRA1, Vps34 and p62 not discussed here, are engaged in non-autophagic functions. This notion is crucial and has to be taken into account when we experimentally explore the role of autophagy *in vivo* on the basis of the ablation of a single *ATG* gene.

Conclusion and perspective

Despite the progresses made in our understanding of autophagy, numerous key aspects of this catabolic pathway remain enigmatic and wait to be elucidated. Among them we can for example

mention the regulation of the basal autophagy, which operates under normal growing conditions. The modulation of this process engages actin filaments and the histone deacetylases HDAC6, which in contrast are dispensable for the autophagosome maturation under starvation conditions[88].

The coordination of Atg protein recruitment possibly from different membrane origins to the PAS as well as their hierarchical assembly at this specialized site are aspects of the autophagosome biogenesis that needs to be carefully considered in the future. Although a hierarchical recruitment of the Atg proteins has been proposed for yeast on a genetic basis[89], their effective temporal association with the PAS remains to be investigated. The examinations of the selective sequestration of mitochondria or *Salmonella* by autophagosomes have indicate that there could be a different hierarchy than the one postulated, where groups or cluster of Atg proteins could independently associate to form the PAS[90,91]. Similarly, the different forms of non-canonical autophagy described to date, where only a subset of Atg proteins are used to form an autophagosome, or the autophagy-independent functions of some of the Atg proteins, can help us to better understand the functional importance of these factors engaged during the generation of autophagosomes[92]. On this line, it will be important to understand why metazoans possess more than one gene coding for Atg proteins, which apparently have an identical functions. For example, the human genome contains 6 homologues of the single yeast Atg8 protein. The role of these counterparts in autophagy and beyond remains to be elucidated. Interestingly some of the members of this protein family, i.e. GABARAPL1 and GABARAPL2, are involved in the autophagosome closure[57] whereas LC3C also acts as a specific receptor during selective antibacterial autophagy[93].

The importance of autophagy in many aspects of the cell and organism physiology is now recognized. Not surprisingly a defect or a dysregulation of this process is intimately associated with the onset of numerous human diseases. The better knowledge of the molecular bases of autophagy but also its control by physiological regulators, from cytokines and hormones to dietary restriction or physical exercise, can provide simple and non-invasive ways to modulate this pathway for preventive or therapeutic interventions to fight diseases in the future[11].

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Figure legends

Figure 1. The different types of autophagy. Macroautophagy is characterized by the sequestration into a double-membrane vesicle called autophagosome of the structures targeted to destruction. Complete autophagosme first fuse with endosomes before finally exposing their content to the hydrolytic interior of lysosomes. The resulting metabolites are transported in the cytoplasm and used either for the synthesis of new macromolecules or as a source of energy. During the CMA, proteins carrying the pentapeptide KFERQ-like sequence are recognized by the Hsc70 chaperone, which then associates with the integral lysosome membrane protein LAMP-2A triggering its oligomerization. This event leads the translocation of the bound protein into the lysosome interior through a process that requires the Hsc70 in this organelle lumen. Microautophagy entails the recruitment of the targeted components in proximity of the lysosomal membrane, which subsequently invaginates and pinches off.

Figure 2. Relationship between autophagy and the main metabolic pathways. (A) The catabolic products of the intracellular structures that are targeted by autophagosomes such as amino acids, lipids and sugars are used for anabolic reactions to generate new proteins, glycans, oligonucleotides and membranes to sustain cell functions. Amino acids can also be used to maintain their systemic levels and for de novo synthesis of glycogen (gluconeogenesis) in the liver. Lipids and amino acids can enter the tricarboxylic acid (TCA) cycle and oxidative phosphorylation to generate energy in the form of ATP. Sugars can also be metabolized to generate ATP through glycolysis and to maintain systemic glucose levels. (B,C) Metabolic compartmentalization between different cell types. Inside tumors hypoxia and oxidative stress

trigger autophagy and mitophagy in the stromal fibroblasts. This situation induces a metabolic switch towards aerobic glycolysis (Warburg effect) leading to the generation of lactate and other metabolites that are liberated into the intracellular space and reabsorbed by tumor cells. A more oxidative metabolism in these cells generates oxidative stress and ammonia (from glutaminolysis), which signal back to fibroblasts to further stimulate autophagy. In brain tissue astrocytes produce lactate from glucose through glycolysis and glutamine via autophagy. These metabolites are taken up by neurons and oxidized to generate ATP. Moreover, the neurotransmiter glutamate released by the neurons can be retransformed into glutamine by the astrocytes.

Figure 3. *ATG* genes in autophagy-independent pathways. Upper draw. Schematic diagram illustrating the non-autophagic cellular processes that rely on one or more *ATG* genes, but not others. Bottom table. Detailed list with references of the *ATG* genes that have been implicated in non-autophagic pathways.

Table 1. The key proteins mediating the biogenesis of an autophagosome

Protein	Yeast	High Eukaryotes	Function
Atg1/ULK's	+	+	Protein kinase involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg2	+	+	Protein interacting with Atg18/WIPI4 possibly involved in the PAS/phagophore biogenesis
Atg3	+	+	E2-like enzyme for the ubiquitin-like conjugation system that catalyzes Atg8/LC3's lipidation involved in the phagophore expansion
Atg4	+	+	Cysteine protease processing and delipidate Atg8/LC3, thus involved in the phagophore expansion
Atg5	+	+	Substrate onto which Atg12 is covalently linked generating the Atg12—Atg5 conjugate involved in the phagophore expansion
Atg6/Beclin 1	+	+	Component of various PI3K complexes, one of which involved in induction of autophagy and the PAS/phagophore biogenesis
Atg7	+	+	E1-like enzyme for the two ubiquitin-like conjugation systems and thus involved in the phagophore expansion
Atg8/LC3's	+	+	Ubiquitin-like protein involved in the phagophore expansion
Atg9	+	+	Transmembrane protein involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg10	+	+	E2-like enzyme for the ubiquitin-like conjugation system that mediates the formation of the Atg12—Atg5 conjugate involved in the phagophore expansion
Atg12	+	+	Ubiquitin-like protein involved in the phagophore expansion
Atg13	+	+	Binding partner and regulator of Atg1/ULK's, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg14	+	+	Component of the PI3K complex I involved in induction of autophagy and possibly the PAS/phagophore biogenesis
Atg16	+	+	Associates with Atg12—Atg5 to form a large complex, which acts as an E3 ligase to direct LC3 lipidation on autophagosomal membranes and thus involved in the phagophore expansion
Atg17/FIB200	+	+	Binding partner and regulator of Atg1/ULK's, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg18/WIPI's	+	+	PtsIns3P-binding proteins possibly involved in the PAS/phagophore biogenesis
Atg23	+	-	Binding partner and regulator of Atg9, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg27	+	-	Binding partner and regulator of Atg9, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis

Atg29	+	-	Binding partner and regulator of Atg1, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg31	+	-	Binding partner and regulator of Atg1, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Atg101	-	+	Binding partner and regulator of ULK's, thus involved in the induction of autophagy and possibly in the PAS/phagophore biogenesis
Ambra1	-	+	Regulator of the PI3K and Atg1/ULK complexes, and thus involved in the induction of autophagy
DFCP1	-	+	PtdIns3P-binding proteins concentrating at the omegasome, possibly involved in the induction of autophagy
VMP1	-	+	Transmembrane protein regulating autophagy induction
Vps15/p150	+	+	Kinase regulating Vps34/hVps34 activity; component of various PI3K complexes, one of which involved in the induction of autophagy and the PAS/phagophore biogenesis
Vps34/ PtdIns3PKC3	+	+	PtdIns 3-kinase; component of various PI3K complexes, one of which involved in the induction of autophagy and the PAS/phagophore biogenesis