Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein

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The most striking morphological feature of eukaryotic cells is the presence of various membrane-enclosed compartments. These compartments, including organelles and transient transport intermediates, are not static. Rather, dynamic exchange of proteins and membrane is needed to maintain cellular homeostasis. One of the most dramatic examples of membrane mobilization is seen during the process of macroautophagy. Macroautophagy is the primary cellular pathway for degradation of long-lived proteins and organelles. In response to environmental cues, such as starvation or other types of stress, the cell produces a unique membrane structure, the phagophore. The phagophore sequesters cytoplasm as it forms a double-membrane cytosolic vesicle, an autophagosome. Upon completion, the autophagosome fuses with a lysosome or a vacuole in yeast, which delivers hydrolases that break down the inner autophagosome membrane along with its cargo, and the resulting macromolecules are released back into the cytosol for reuse. Autophagy is therefore a recycling process, allowing cells to survive periods of nutrient limitation; however, it has a wider physiological role, participating in development and aging, and also in protection against pathogen invasion, cancer and certain neurodegenerative diseases. In many cases, the role of autophagy is identified through studies of an autophagy-related protein, Atg6/Beclin 1. This protein is part of a lipid kinase complex, and recent studies suggest that it plays a central role in coordinating the cytoprotective function of autophagy and in opposing the cellular death process of apoptosis. Here, we summarize our current knowledge of Atg6/Beclin 1 in different model organisms and its unique function in the cell.

Keywords: lysosome, phosphoinositides, protein targeting, stress, vacuole, yeast

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Introduction

There are various processes that fall under the general name of autophagy, which has the general definition of

any process involving the degradative delivery of a portion of the cytoplasm to the lysosome (or its yeast analog, the vacuole) that does not involve direct transport through the endocytic or vacuolar protein sorting (Vps) pathways [1]. The best characterized of these processes is macroautophagy, which we refer to hereafter as autophagy. Autophagy is highly conserved in eukaryotic cells, ranging from yeast to mammals [2]. It is the major intracellular pathway for degradation and recycling of long-lived proteins and organelles, whereas the ubiquitin/proteasome system degrades primarily short-lived proteins.

One of the most distinctive features of autophagy is its relatively unlimited capacity for degradation, which reflects the unique mechanism of cargo sequestration. During autophagy, a membrane of unknown origin forms

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Abbreviations: activating molecule in Beclin 1-regulated autophagy (Ambra1); autophagy-related (Atg); Bcl-2-homology-3 (BH3); coiled-coil domain (CCD); cytoplasm to vacuole targeting (Cvt); evolutionarily conserved domain (ECD); embryoid bodies (EB); endoplasmic reticulum (ER); hypersensitive response (HR); herpes simplex virus type 1 (HSV-1); Niemann-Pick C (NPC); programmed cell death (PCD); phosphatidylethanolamine (PE); phosphatidylinositol (PtdIns); UV irradiation resistance-associated gene (UVRAG); vacuolar protein sorting (Vps)

a nucleation site that generates a unique organelle, the phagophore (Figure 1). The phagophore expands, probably through vesicular addition, to form a large double-membrane cytosolic vesicle that is termed an autophagosome. Unlike transient transport vesicles such as those that shuttle proteins between the organelles of the secretory pathway, the autophagosome forms *de novo*; it does not bud off intact from a pre-existing organelle, although the membrane that adds to the growing phagophore likely includes vesicles that originate within the secretory pathway. This expansion mechanism allows the autophagosome to sequester entire organelles or large protein aggregates, and even invading pathogens. Following completion of the autophagosome, it fuses with the lysosome or the yeast vacuole. In mammalian cells, autophagy also converges with endocytosis, a form of heterophagy, and the autophagosome may fuse with an endosome to generate an amphisome, which also will ultimately fuse with a lysosome; it is not known whether

Figure 1 A schematic model of macroautophagy in mammalian cells. Autophagy occurs at a low, constitutive basal level, but can be induced by environmental signals including stress (e.g., nutrient depletion) and hormones (e.g., glucagon). The process begins with the formation of a sequestering membrane termed a phagophore. During nonspecific autophagy, bulk cytoplasm (including entire organelles) can be sequestered, whereas specific types of autophagy can target selective cargos. The phagophore expands to form a double-membrane autophagosome. The autophagosome may fuse with an endosome; convergence of endocytosis, a form of heterophagy, and autophagy in the form of a fusion event generates an amphisome. The amphisome or autophagosome acquires hydrolytic enzymes by fusing with a lysosome. The resulting autolysosome breaks down the inner membrane of the autophagosome and its cargo. The macromolecular breakdown products are released back into the cytosol through permeases for reuse.

this process occurs in yeast because the endosome is poorly defined and difficult to detect. In either case, the final fusion event provides access to the diverse hydrolases that reside in the lysosome and yeast vacuole, allowing them to break down the inner membrane of the autophagosome along with its cargo. The resulting macromolecules are then released back into the cytosol for reuse.

Why does the cell maintain a process for eating parts of itself? For proper physiology, the cell must maintain a balance between anabolism and catabolism. Autophagy is a catabolic process that has homeostatic functions, acting in the turnover of various worn out or damaged macromolecules and organelles [3]. Autophagy also has cytoprotective roles during stress conditions, such as nutrient starvation, growth factor depletion [4] and pathogen invasion [5] that might otherwise lead to apoptosis or type I programmed cell death (PCD). For example, damaged mitochondria pose a threat to cell stability due to the generation of excess reactive oxygen species that can cause nuclear DNA damage [6, 7]. The compromised organelle may release cytochrome *c* into the cytosol, which can trigger apoptosis as a barrier against oncogenesis. If the cell can remove the damaged organelle in a timely manner, however, it may avoid the need for apoptosis. Thus, although we are not aware of evidence demonstrating a direct connection between the removal of damaged organelles and the prevention of apoptosis, it seems likely that autophagy can protect the cell against apoptotic death through its action as an organelle quality-control mechanism.

On the other hand, autophagy presents us with a challenging conundrum because it sometimes acts as a killer of cells by promoting autophagic, or type II, PCD with the hallmark of accumulated autophagosomes in dying cells [8]. In this case, extensive autophagy may participate along with, or instead of, apoptosis to ensure the death and removal of cells that cannot be rescued; it is not difficult to imagine that an excessive level of self-digestion would be deleterious. The dual role of autophagy, in cytoprotection and cell death, is one of the most fascinating features of this process, and one that needs to be better understood if we ever hope to harness autophagy for therapeutic use. Indeed, there are many possibilities for modulating autophagy for purposes of human health: autophagy has been implicated in various human diseases, including cancer, cardiomyopathy and the prevention of certain neurodegenerative disorders [9–11].

In the last decade, with the identification of approximately 30 AuTophagy-related (*ATG*) genes in *Saccharomyces cerevisiae* and other fungi [12], the molecular mechanisms of autophagy have gradually been elucidated [2]. As mentioned above, autophagy is conserved across all eukaryotes and homologs of many yeast *ATG* genes have recently been

identified in various eukaryotic systems, and the underlying molecular mechanisms of autophagy are also conserved. Among the *ATG* genes, *ATG6* is relatively unique in its not being “autophagy-specific.” For example, the *S. cerevisiae ATG6/VPS30* gene product is the only protein required for both autophagy and sorting of the vacuole resident hydrolase carboxypeptidase Y through the Vps pathway [13]. Of the Arabidopsis *atg (atatg)* mutant plants examined so far, only those with defects in *AtATG6* are defective in pollen germination [14, 15]. *Beclin 1*, the mammalian homolog of yeast *ATG6*, is a haploinsufficient tumor suppressor gene [16, 17]. Finally, the Beclin 1 protein is also an important convergence point of autophagy and apoptosis; it interacts with anti-apoptotic Bcl-2-like proteins [18], and has recently been found to be a Bcl-2-homology-3 (BH3)-only protein [19, 20].

In the past decade, a substantial amount of research has been carried out on the Atg proteins, and several recent reviews discuss the protein machinery of autophagy [21–24]. However, despite the growing interest in Atg6/Beclin 1 and its central role in autophagic regulation, no review is available on this specific topic. Here, we summarize what we know about Atg6/Beclin 1 from studies in *S. cerevisiae*, plants, *Caenorhabditis elegans*, mice and mammalian cell lines, with an emphasis on its uniqueness compared to other Atg proteins. Finally, the role of Atg6/Beclin 1 in autophagic and apoptotic cell death is discussed.

Atg6/Vps30 participates in autophagy and the Vps pathway

To date, we have been referring to macroautophagy as one process; however, just as there are different types of autophagy, there are also distinct types of macroautophagy. In general, macroautophagy is considered to be nonspecific, but there are also specific types of macroautophagy. For example, excess peroxisomes can be selectively degraded through a specific macroautophagy-like process termed pexophagy [25]. One well-characterized selective type of autophagy is the cytoplasm to vacuole targeting (Cvt) pathway that is used for the delivery of two vacuolar hydrolases, aminopeptidase I and α -mannosidase [26]. The protein machinery of nonspecific autophagy overlaps extensively with the Cvt pathway [27, 28]; however, there are some proteins that are specific for each pathway. All of the proteins that are needed for both the Cvt pathway and autophagy appear to be specifically engaged in autophagy-related pathways, except for Atg6/Vps30 and Atg18 [29]. A brief summary of the functions of the Atg proteins is presented in Table 1.

As noted above, aminopeptidase I and α -mannosidase are delivered to the vacuole through the autophagy-related

Cvt pathway. In contrast, the majority of vacuolar hydrolases (including carboxypeptidase Y) are delivered to their final destination primarily through the Vps pathway [30]. An obvious question that arises is why Atg6/Vps30 is involved in these two separate pathways. One type of complex that is involved in regulating autophagy is a lipid kinase, in particular a phosphatidylinositol (PtdIns) 3-kinase. In mammals, there are two different PtdIns 3-kinases that participate in autophagy regulation: the class I PtdIns 3-kinase generates PtdIns(3,4,5)P₃ and is inhibitory, whereas the class III enzyme generates PtdIns(3)P and is stimulatory [31]. Yeast Atg6/Vps30 is a subunit of two distinct class III PtdIns 3-kinase complexes [32] (Figure 2). Complex I functions in autophagy, whereas complex II is involved in Vps, which explains why Atg6/Vps30 participates in both, otherwise separate, pathways.

The two PtdIns 3-kinase complexes contain three common subunits: the PtdIns 3-kinase enzyme Vps34, the regulatory protein Vps15 and Atg6/Vps30. In addition, each complex has a specific factor, Atg14 for complex I and Vps38 for complex II. Atg14 directs complex I to the phagophore assembly site, also termed the pre-autophagosomal structure (PAS) [33], a perivacuolar site where most of the Atg proteins colocalize at least transiently and autophagosomes are thought to originate [34, 35]. Moreover, Atg14 together with Atg6 are important in mediating the localization of other Atg proteins to the PAS [35, 36]. In contrast, Vps38 is responsible for the endosomal localization of complex II [33]. In *atg14 Δ vps38 Δ* cells, Vps34 and Vps15 still localize to some punctate structures (neither endosomes nor the PAS) and vacuolar membranes, indicating that potential novel PtdIns 3-kinase complexes exist [33]. It is of interest to know what other components, if any, are included in these putative novel complexes and to determine their specific function. Candidate mammalian homologs of Atg14 and Vps38 have recently been identified (N Mizushima and T Yoshimori, personal communication). Considering the conserved roles of class III PtdIns 3-kinases in autophagy and lysosomal or vacuolar protein sorting, it is not surprising that different Beclin 1-containing complexes also exist in mammalian cells.

Beclin 1 is a mammalian tumor suppressor

Bcl-2 is an anti-apoptotic protein [37], and Beclin 1 was first identified as a Bcl-2-interacting protein in a yeast two-hybrid screen [18]. The *beclin 1* gene is monoallelically deleted in up to 75% of ovarian, 50% of breast and 40% of prostate cancers [38]. Decreased expression of Beclin 1 is also observed in other types of cancers including human brain tumors [39] and cervical cell carcinoma [40]. Expression of Beclin 1 in human MCF7 breast carcinoma cells

Table 1 Proteins required for specific and nonspecific autophagy in yeast

Protein	Function
Atg1	A serine/threonine kinase that participates in the induction step and also in the cycling of the Atg9 complex. Atg1 interacts with Atg11, Atg13-Atg17 and Atg29
Atg2	Required for retrograde transport of Atg9 from the PAS to peripheral sites that may mark the membrane donor for sequestering vesicle formation
Atg3	An ubiquitin-conjugating enzyme analog involved in the attachment of phosphatidylethanolamine (PE) to Atg8
Atg4	A cysteine protease that processes the C terminus of Atg8 to expose a glycine residue prior to PE lipidation
Atg5	A protein with two ubiquitin-like domains that is modified by conjugation to Atg12, through an isopeptide bond at an internal lysine of Atg5
Atg6	A component of two PtdIns 3-kinase complexes involved in autophagy and vacuolar protein sorting
Atg7	An ubiquitin activating enzyme homolog required for conjugation of Atg8 to PE and Atg12 to Atg5
Atg8	An ubiquitin-like protein that modifies PE via attachment at the C-terminal glycine. Atg8 is the only protein required for nonspecific autophagy that clearly remains associated with the completed autophagosome
Atg9	A transmembrane protein that is localized at the PAS and at peripheral sites in the cell that may correspond to the donor site(s) that provide membrane for expansion of the phagophore. This protein interacts with Atg23 and Atg27
Atg10	An ubiquitin-conjugating enzyme analog involved in the attachment of Atg12 to Atg5
Atg11	This protein serves as an adaptor or scaffold during specific types of autophagy including the Cvt pathway and pexophagy (selective degradation of peroxisomes). It interacts with Atg1, Atg9, itself, Atg17, Atg19 and Atg20
Atg12	An ubiquitin-like protein that modifies Atg5 through attachment at a C-terminal glycine
Atg13	An Atg1-interacting protein that modulates the kinase activity of Atg1. Atg13 undergoes nutrient-dependent changes in phosphorylation
Atg14	A component of PtdIns 3-kinase complex I that is involved in autophagy; it determines the localization of the complex at the PAS
Atg15	A vacuolar lipase that is needed for the lysis of the inner membrane of the autophagosome after fusion with the vacuole, which releases the inner membrane into the organelle lumen
Atg16	This protein forms a complex with Atg5 that has been conjugated with Atg12. Atg16 self-interacts to form a tetrameric complex
Atg17	An Atg13-interacting protein that is part of the Atg1 complex and modulates the kinase activity of Atg1. This protein is not needed for specific types of autophagy and is not essential for nonspecific autophagy; however, in its absence there are fewer and smaller autophagosomes
Atg18	Interacts with Atg9 and required for retrograde transport of Atg9 from the PAS to peripheral sites. This protein binds PtdIns(3) phosphate and its localization is dependent on the PtdIns 3-kinase complex
Atg19	A receptor for the substrates of the Cvt pathway that interacts with Atg11; this protein is not needed for nonspecific autophagy
Atg20	A PX domain-containing protein that binds PtdIns(3)phosphate and is required for the Cvt pathway, but not nonspecific autophagy. This protein binds Atg11, Atg17 and Atg24, and is therefore part of a putative Atg1 complex
Atg21	A homolog of Atg18 that also binds PtdIns(3)phosphate; this protein is only essential for the Cvt pathway
Atg22	A vacuolar permease that functions in the final step of autophagy, the release of macromolecules (amino acids) generated by vacuolar degradation back into the cytosol
Atg23	A cytosolic protein that displays a localization pattern and cycles similar to, and interacts with, Atg9. This protein is needed for the Cvt pathway and for efficient nonspecific autophagy; an <i>atg23Δ</i> mutant forms a reduced number of autophagosomes
Atg24	A PX domain-containing protein that binds PtdIns(3)phosphate and is required for the Cvt pathway, but not nonspecific autophagy. This protein binds Atg17 and Atg20, and is therefore part of a putative Atg1 complex
Atg25	This protein is not found in <i>S. cerevisiae</i> , but it is required for specific peroxisome degradation in <i>Hansenula polymorpha</i>
Atg26	A GRAM domain-containing protein that binds PtdIns(4)phosphate; this protein is required for specific peroxisome degradation in <i>Pichia pastoris</i> , but not in <i>S. cerevisiae</i>
Atg27	A transmembrane protein that displays a localization pattern and cycles similar to, and interacts with, Atg9. This protein is needed for the Cvt pathway and for efficient nonspecific autophagy; an <i>atg27Δ</i> mutant forms a reduced number of autophagosomes
Atg28	A coiled-coil domain protein required for specific peroxisome degradation in <i>P. pastoris</i>
Atg29	A component of the Atg1 complex that interacts directly with Atg1
Atg30	A protein required for specific peroxisome degradation in <i>P. pastoris</i>
Atg31	A component of the Atg1 complex that interacts directly with Atg17

Figure 2 Two PtdIns 3-kinase complexes function in autophagy and the Vps pathway in yeast. Vps34 is the only PtdIns 3-kinase in yeast, but this enzyme is present in two distinct complexes. Both complexes contain Vps34, Vps15, which is thought to be a regulatory factor, and Atg6/Vps30. Complex I and II additionally include Atg14 and Vps38, respectively, which act as connectors between Atg6/Vps30 and the Vps34-Vps15 core. Complex I functions in autophagy, whereas complex II is required for vacuolar protein sorting. Drawing modified from Kihara *et al.* [32].

promotes autophagy and inhibits *in vitro* clonogenicity and tumorigenesis in nude mice [41]. *Beclin 1* is a haploinsufficient tumor suppressor gene in mice [16, 17], sharing 98% identity with human *beclin 1*. Homozygous *beclin 1*^{-/-} mice die in early embryogenesis, and heterozygous *beclin 1*^{+/-} mice have reduced autophagy levels and increased incidence of spontaneous tumors, which, together with the *in vitro* data, establishes a clear role for autophagy, and Beclin 1, in tumor suppression.

A function for Beclin 1 in tumor suppression is further supported by the identification of additional Beclin 1-interacting proteins. The UV irradiation resistance-associated gene protein, UVRAG, is a positive regulator of the Beclin 1-PtdIns 3-kinase complex; UVRAG promotes autophagy in a manner that is interdependent with Beclin 1 [42]. *UVRAG* is monoallelically deleted in various human cancers, similar to *beclin 1*, suggesting that the UVRAG protein is a tumor suppressor, and expression of UVRAG inhibits the proliferation and tumorigenicity of human colon cancer cells. The activating molecule in Beclin 1-regulated autophagy, Ambra1, is another Beclin 1-interacting protein that positively regulates autophagy and inhibits cell proliferation (see below).

In contrast to *beclin 1*^{-/-} mice, *atg5*^{-/-} and *atg7*^{-/-} mice appear almost normal at birth, but die within 1 day after birth [43, 44]. Furthermore, heterozygous *atg5*^{+/-} mice seem normal during a monitoring period of up to 16 months of age [44], and neither tumorigenesis nor enhanced cell proliferation is detected in Atg7-deficient liver [43]. These studies suggest that Beclin 1 plays a more important role in embryogenesis and tumor suppression than Atg5 and Atg7, and/or the autophagy-independent aspects of Beclin 1 may contribute to these functions. However, it is worth

noting that a truncated calpain-cleaved form of Atg5 induces apoptosis and sensitizes tumor cells to anticancer drug treatment [45].

Human Beclin 1 shares 24.4% identity with Atg6/Vps30 in yeast. It complements the autophagy function, but not the Vps function of *atg6/vps30* mutant yeast [41], and a Vps function for Beclin 1 in mammalian cells has not been established. Maturation of the lysosomal enzyme cathepsin D is normal in MCF7 cells, which have undetectable or very low levels of Beclin 1 [46]. Similarly, knockdown of Beclin 1 in U-251 glioma cells does not affect cathepsin D processing (i.e., lysosomal delivery) and endocytic trafficking [47], suggesting that some other protein may fulfill the Vps role of Atg6/Vps30 in mammalian cells. However, it is still possible that very low levels of Beclin 1 are sufficient for its Vps function, and Beclin 1 might have an unidentified vesicular trafficking function. Endogenous Beclin 1 in HeLa cells localizes predominantly to the *trans*-Golgi network [48], but endogenous Beclin 1 also colocalizes with mitochondria and endoplasmic reticulum (ER) in HT-29 cells [49] in agreement with data from overexpressed protein in COS7 and MCF7 cells [50]. It is possible that Beclin 1 has a slightly different localization in different cell lines, and it has a subpopulation that localizes to mitochondria and ER. In fact, recent studies suggest that ER-localized Beclin 1 plays a role in regulating autophagy [51], although other populations of Beclin 1 may also regulate this pathway.

Sequence and structural studies indicate that Beclin 1 has a BH3-only domain, a central coiled-coil domain (CCD), and an evolutionarily conserved domain (ECD) (Figure 3). The ECD of Beclin 1 is essential for Vps34 binding, autophagy and the tumor suppressor function [46]. BH3 proteins are part of the Bcl-2 family; they are pro-apoptotic damage sensors that play an important role in protecting

Figure 3 Schematic representations of domains of human Beclin 1 and the Bcl-2-Beclin 1-PtdIns 3-kinase-UVRAG multiprotein complex. Beclin 1 has a BH3 domain (amino acids 114-123), a central coiled-coil domain (CCD, amino acids 144-269) and an evolutionarily conserved domain (ECD, amino acids 244-337). Bcl-2 interacts with the BH3 domain of Beclin 1, UVRAG with the CCD, and the class III PtdIns 3-kinase with the ECD and CCD. Beclin 1 functions as a platform or scaffold for the formation of the complex. Drawing modified from Liang *et al.* [42].

against cancer [52]. The BH3-only domain of Beclin 1 can interact with Bcl-2 and Bcl-X_L [19, 20], although one study shows that in U-251 cells endogenous Beclin 1 does not interact with either protein [47]. Both cellular and viral Bcl-2 (vBcl-2), or more specifically ER-targeted Bcl-2, inhibit Beclin 1-dependent autophagy by interfering with the Beclin 1-PtdIns 3-kinase interaction and the Beclin 1-associated PtdIns 3-kinase activity [19, 49]. The interaction between Bcl-2 and Beclin 1 is greatly reduced upon starvation, which suggests that the dissociation of Bcl-2 from Beclin 1 is important for activating autophagy.

UVRAG interacts with the CCD of Beclin 1 through its own central CCD [42]. Unlike the interaction between Bcl-2 and Beclin 1, the UVRAG-Beclin 1 interaction is not affected by starvation. In the multiprotein complex vBcl-2-Beclin 1-PtdIns 3-kinase-UVRAG, Beclin 1 acts as a platform mediating interaction between vBcl-2 and UVRAG, between vBcl-2 and PtdIns 3-kinase, and between UVRAG and PtdIns 3-kinase (Figure 3). UVRAG expression increases the Beclin 1-PtdIns 3-kinase interaction and class III PtdIns 3-kinase activity, which promotes autophagy and suppresses growth and tumorigenesis of HCT116 cells.

Ambra1, a novel activating molecule in Beclin 1-regulated autophagy that is unique to vertebrates, positively regulates autophagy and plays a role in neural tube development and embryogenesis [53]. Ambra1 interacts with Beclin 1 and is in a complex with Beclin 1 and Vps34. Ambra1 overexpression upregulates autophagy and decreases the cell proliferation rate, whereas its downregulation by siRNA decreases rapamycin- and starvation-induced autophagy, impairs interaction between Beclin 1 and Vps34, and increases cell proliferation. Ambra1 null mouse embryos have impaired autophagy and exhibit excessive cell proliferation followed by increased apoptosis. These data support a role of autophagy in neuronal development, in regulating cell proliferation and in cell death.

Beclin 1 levels appear to be one of the critical factors that affect the induction of autophagy. As indicated above, *beclin 1* is haploinsufficient, and various cancer cells show decreased levels of Beclin 1 [39]. The anti-cancer drug tamoxifen may work in part by increasing expression of this protein [54]. Other experiments also support the importance of Beclin 1 levels in autophagy regulation. For example, ceramide is thought to play a role in apoptosis [55, 56]. Recent studies show that ceramide can also induce autophagy, and that it may be involved in the tamoxifen-dependent increase in Beclin 1 levels [57]. Altered levels of Beclin 1 are also seen in situations other than cancer that involve autophagy; high levels of Beclin 1 are associated with neurons at the site of traumatic brain injury [58], whereas inhibition of Beclin 1 expression protects against

cell death due to ischemia/reperfusion [59].

Beclin 1-mediated autophagy and other pathologies

Autophagy also plays a role in diseases other than cancer. For example, expression of Beclin 1 is important for reducing protein aggregates [60], which supports a general protective role of autophagy and Beclin 1 in neurodegeneration [9]. On the other hand, autophagic degradation may contribute to tissue damage in sporadic inclusion body myositis [61, 62]. Similarly, as discussed above, Beclin 1 functions in autophagy as part of a class III PtdIns 3-kinase/Vps34 complex [48]. Amino acid withdrawal stimulates class III PtdIns 3-kinase activity associated with Beclin 1 [63], which further induces autophagy in C₂C₁₂ myotubes. Thus, Beclin 1-dependent autophagy may contribute to the degradation that is associated with certain myopathies.

The dual nature of autophagy in cytoprotection and cell death is also seen in ischemia/reperfusion. Autophagy is induced during ischemia in an AMPK-dependent manner, whereas during reperfusion it is accompanied by upregulation of Beclin 1 [64]. Autophagy plays a protective role during ischemia, whereas cardiac injury associated with reperfusion is attenuated in *beclin 1*^{+/-} mice. Thus, Beclin 1-mediated autophagy during reperfusion can be detrimental in heart cells.

Although some data have implicated autophagy as playing a role in neuronal cell death [65, 66], most evidence indicates that autophagy generally serves a cytoprotective role in neurons. For example, otherwise healthy mice specifically lacking neuronal Atg5 or Atg7 develop symptoms of neurodegeneration [67, 68]. Similarly, autophagy plays a role in preventing Huntington's disease, spinocerebellar ataxia and some forms of familial Parkinson's disease [67, 68]. Even in *C. elegans*, Beclin 1-dependent autophagy plays a role in preventing neurodegeneration associated with the expression of polyQ-containing fragments of huntingtin [69].

Upregulation of autophagy mediated by increased expression of Beclin 1 is observed in a mouse model for Niemann-Pick C (NPC) disease [70], which is characterized by lipid trafficking defects and neurodegeneration due to defects in the NPC1 gene. Similar induction of autophagy and Beclin 1 expression is observed in wild-type fibroblasts treated with U18666A (a compound that causes accumulation of unesterified cholesterol) and in primary fibroblast cells from patients with NPC2 deficiency and Sandhoff disease, two other sphingolipid storage diseases. The implications of these findings are not clear, but they suggest that Beclin 1-dependent autophagy may be induced as a cytoprotective response in some lipid-trafficking diseases. It is also possible, however, that autophagic induction is

an indirect effect of sphingolipid accumulation because sphingolipids regulate autophagy [57].

Beclin 1-mediated autophagy also protects against viral infection. Overexpression of Beclin 1 protects mice against lethal Sindbis virus encephalitis [18]. Autophagy also is involved in the clearance of the herpes simplex virus type 1 (HSV-1) from infected host cells [71]. The HSV-1 neurovirulence protein ICP34.5 inhibits autophagy through its binding to Beclin 1 [72], suggesting that endogenous amounts of Beclin 1 are important in protecting against viral invasion. HSV-1 mutants encoding ICP34.5 proteins that are unable to bind Beclin 1 are neuroattenuated in mice, whereas neurovirulence is restored in *pkr*^{-/-} mice, which are defective in double-stranded RNA-activated protein kinase R, PKR, signaling that normally activates autophagy upon viral infection. Increased viral replication is also observed in *atg6*-silenced plants [73].

Atg6 and the hypersensitive response in plants

Autophagy plays a role in innate and adaptive immunity. For example, autophagy defends cells against invasive bacteria and viruses [71, 74-76], and is involved in major histocompatibility complex II antigen presentation [77, 78]. Similarly, autophagy plays a role in innate immunity in plants. The plant orthologue of yeast Atg6 was first identified in a screen for genes that affect tobacco mosaic virus-induced hypersensitive response PCD (HR PCD) in *Nicotiana tabacum* (Nb) using a virus-induced gene-silencing method [73]. Plants induce the HR at sites of pathogen infection; PCD of infected cells limits the spread of the pathogen. In normal plants, HR PCD is restricted to the infection site and does not spread to neighboring cells, whereas cell death extends to uninfected tissue and distal uninfected leaves in the *NbATG6*-deficient plant indicating that autophagy is involved in negatively regulating PCD. In addition to *NbATG6*, orthologues of *VPS34*, *ATG3* and *ATG7* are also required to restrict HR PCD [73]. Furthermore, in *NbATG*-silenced plants, there is increased accumulation of tobacco mosaic virus at infection sites, suggesting a role for *ATG6* and other *ATG* genes in controlling virus replication.

NbATG6 shares 69% identity with *Arabidopsis ATG6* (*AtATG6*), 36% identity with human *beclin 1* and 24% identity with yeast *ATG6*. No apparent difference is observed between the phenotypes of *NbATG6*-silenced and other *NbATG*-silenced plants, and no obvious developmental defect is noted [73]. In contrast, mutants in *Arabidopsis thaliana ATG6* display defects in pollen germination [14, 15]. Disruption of *AtATG6* by T-DNA insertion results in male sterility, whereas all previously characterized *Atatg* mutants are fertile. These results indicate that autophagy

may not contribute to the pollen germination defect in the *AtATG6*-deficient plants. In contrast to mammalian *beclin 1*, expression of *AtATG6* restores both autophagy and Vps in *atg6/vps30* mutant yeast [14]; vesicular trafficking is involved in pollen tube growth, and hence it is proposed that the Vps function of AtAtg6 is responsible for pollen germination. With regard to NbAtg6, it is possible that a residual level of activity resulting from the gene-silencing system used in that analysis may be sufficient for normal development to occur [73]. It will be interesting to know whether *NbATG6* can complement the Vps defect in *atg6/vps30* mutant yeast, and to determine whether *NbATG6*-deficient, rather than *NbATG6*-silenced, plants are also defective in pollen germination. Nonetheless, all the data point to a general role of autophagy genes, and autophagy, in regulating HR PCD during the plant innate immune response.

BEC-1 and life span extension in *C. elegans*

One of the primary factors that correlate with longevity is caloric restriction [79]. The general idea behind this observation is that the housekeeping function of autophagy that allows the removal of damaged proteins and organelles is critical in maintaining cell and organism viability. Studies with rats demonstrate that autophagy declines with age and that stimulation of *autophagy* in older organisms has beneficial effects [80]. Nonetheless, there are obviously complicating factors in carrying out these types of analyses and drawing definitive conclusions from studies in mammals; however, work from *C. elegans* supports the view that autophagy plays a role in life span extension. The *C. elegans BEC-1* gene shares 28% homology with yeast *ATG6/VPS30* and 31% homology with human *beclin 1* [81]. Expression of *BEC-1* complements the autophagy, but not the Vps defect of the yeast *atg6/vps30* mutant. Knockdown of autophagy genes including *BEC-1* inhibits autophagy and blocks normal dauer formation (a stage of developmental arrest under unfavorable conditions) in *C. elegans* [81], and *bec-1* mutants display a range of developmental defects [82]. *BEC-1* is also required for life span extension in this organism [81, 83]. *BEC-1* interacts with LET-512, the orthologue of the PtdIns 3-kinase Vps34, and is needed for the function of the enzyme; *bec-1* mutants are defective in membrane trafficking [82], suggesting that the *C. elegans* protein may function in two different complexes, one involved in protein sorting and the other in autophagy, similar to yeast Atg6.

Beclin 1 in autophagic cell death and apoptosis

In addition to its role in autophagy, development, tumor

suppression and the clearance of aggregate-prone proteins, Beclin 1 also serves as an important regulator or player in PCD. For example, Beclin 1 is required for autophagic, type II, PCD [84]. As indicated above, mammalian Beclin 1 binds Bcl-2 [18], and similarly, the *C. elegans* Bcl-2 orthologue, CED-9, also binds Beclin 1 [82]. Bcl-2 inhibits Beclin 1-dependent autophagy, and thus autophagic cell death upon starvation [19, 49]. However, overexpression of Bcl-2 or Bcl-X_L in apoptosis-defective *bax*^{-/-} *bak*^{-/-} mouse embryonic fibroblast cells treated with etoposide, an apoptosis-inducing agent, stimulates Beclin 1-dependent autophagic cell death [85]. These findings suggest that Bcl-2 has a different effect on autophagy and autophagic cell death depending on the cell type and stimulus, and are in agreement with the general observation that autophagy can act in both cytoprotective and cell death functions. In addition, the level of Bcl-2 proteins may need to be precisely regulated to ensure proper control over autophagy and apoptosis; however, in physiological conditions it seems that Bcl-2 has an inhibitory effect on autophagy. Additional experiments point to the connection between autophagy and apoptosis. For example, inhibition of autophagy with small interfering RNA to reduce levels of Beclin 1, or other autophagy proteins, leads to apoptotic cell death, and the reduction in autophagy sensitizes cells to apoptotic stimuli, suggesting some type of crosstalk between these pathways [86, 87]. Depletion of Beclin 1 through RNA interference in *C. elegans* also results in elevated apoptosis, and similar effects are seen in homozygous *bec-1*^{-/-} animals [82]. In general, autophagy probably acts initially as a cytoprotective mechanism, and when autophagy is suppressed, cells are more prone to apoptosis. On the other hand, if cell death occurs when apoptosis is blocked, it is likely to involve an autophagic mechanism.

Beclin 1 is not essential for apoptosis, although it has recently been found to have a BH3 domain, which classifies it as a BH3-only protein. The other known members of the BH3-only family (e.g., Bid and Bad) are considered to function in a pro-apoptotic manner, upstream of anti-apoptotic Bcl-2-like proteins [20]. These BH3-only proteins activate Bax-like apoptotic factors (including Bax and Bak), which cause changes in mitochondrial permeability that lead to apoptosis. Along these lines, a synthetic peptide containing the BH3 domain of Beclin 1 induces apoptosis [19]; however, it is important to note that the intact protein, even upon overexpression, does not promote apoptosis. Moreover, in embryonic stem cells derived from *beclin 1*^{-/-} mice, apoptotic cell death induced by UV light or serum deprivation is not compromised [17]. One implication of these findings is that the BH3 domain of Beclin 1 in the native protein is not presented in a similar manner as other BH3-only proteins. Therefore, Beclin 1 could be

a unique member of the BH3-only proteins, the function of which could be regulated by other BH3-only proteins. For example, BH3-only proteins such as Bad may induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X_L, in addition to their pro-apoptotic roles.

As autophagy and apoptosis are interconnected, and the relationship between them may vary depending on the particular context, it is possible that Beclin 1 might have a regulatory role in apoptosis and other related cellular events. For instance, using an *in vitro* mouse model of embryogenesis, a recent study shows that *beclin 1* is not required for apoptotic cell death, but for the generation of signals that allow the phagocytic clearance of apoptotic corpses [88]. As *atg5*^{-/-} embryoid bodies (EB) have the same phenotypes as *beclin 1*^{-/-} EB, the role for clearance of cell corpses is not specific to Beclin 1. It is unexpected that both *atg5*^{-/-} and *beclin 1*^{-/-} EBs fail to cavitate, given that only *beclin 1*^{-/-} mice are embryonic lethal [16, 17, 44]; thus, this *in vitro* study does not fully replicate the *in vivo* situation.

Conclusions and future directions

Atg6/Beclin 1 has roles in many cellular processes, some of which involve autophagy-independent functions. Future studies need to separate Atg6/Beclin 1-specific functions from its general participation in autophagy. The search for Atg14 and Vps38 homologs in mammals will help to establish the different roles, if any, of Beclin 1 in autophagy and lysosomal protein sorting. Additionally, the identification of other Atg6/Beclin 1 interaction partners may provide important information concerning any non-autophagic roles. It is possible that different Beclin 1 complexes exist, and exert their function at different cellular locations or under different stimuli. Generation of knock-in mice expressing mutant Beclin 1 that loses interactions with specific partners is another approach that may help to dissect the function of Beclin 1. Research on Atg6/Beclin 1 will continue to provide insights into the mechanisms of autophagy, the crosstalk between autophagy and apoptosis, and the role of autophagy and Atg6/Beclin 1 in many important cellular processes.

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