Review

Autophagy in filamentous fungi

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\begin{abstract}
Autophagy is a ubiquitous, non-selective degradation process in eukaryotic cells that is conserved from yeast to man. Autophagy research has increased significantly in the last ten years, as autophagy has been connected with cancer, neurodegenerative disease and various human developmental processes. Autophagy also appears to play an important role in filamentous fungi, impacting growth, morphology and development. In this review, an autophagy model developed for the yeast \textit{Saccharomyces cerevisiae} is used as an intellectual framework to discuss autophagy in filamentous fungi. Studies imply that, similar to yeast, fungal autophagy is characterized by the presence of autophagosomes and controlled by Tor kinase. In addition, fungal autophagy is apparently involved in protection against cell death and has significant effects on cellular growth and development. However, the only putative autophagy proteins characterized in filamentous fungi are Atg1 and Atg8. We discuss various strategies used to study and monitor fungal autophagy as well as the possible relationship between autophagy, physiology, and morphological development.
\end{abstract}

1. Introduction

Filamentous fungi play important roles in health care, agriculture, food production, and bioprocessing (Adrio and Demain, 2003; Bennett, 1998; Punja, 2001; Sanglard, 2002). In many of these roles, either by default (e.g., in nature) or by design (e.g., industrial fermentations), fungi experience nutrient starvation. Such starvation, in turn, can lead to cellular degradation, autolysis, apoptosis and even cell death. However, fungi also possess a nutrient recycling pathway called autophagy that may prolong cellular survival.

Autophagy is a broad term for catabolic processes involving the lysosomal/vacuolar pathway. Autophagy research has become very popular in the last ten years, so much so that it has been referred to as “the new apoptosis” (Klionsky et al., 2007). Connections have even been discovered linking autophagy to cancer, neurodegenerative diseases and human developmental processes. The term autophagy can describe a number of cellular phenomena (Yorimitsu and Klionsky, 2005) including: macroautophagy (i.e., non-specific engulfment of cytosolic components by double membrane vesicles which subsequently fuse with the vacuole/lysosome where the contents are degraded), microautophagy (i.e., direct invagination of cytosolic material into the vacuole/lysosome), pexophagy (i.e., degradation of targeted peroxisomes in the vacuole/lysosome), and chaperone-mediated autophagy (i.e., degradation of specific cytosolic proteins with assistance from chaperone molecules). However, due to a wealth of information from yeast and mammalian cell studies, current literature often uses the term “autophagy” to refer to macroautophagy, the non-specific form of this cellular recycling pathway. In addition, the few autophagy studies carried out to date using filamentous fungi (Fig. 1) have focused exclusively on macroautophagy. Thus in this review, unless otherwise noted, the term autophagy is limited to macroautophagy.

In filamentous fungi, autophagy appears to be involved in nutrient recycling during starvation, and it has been suggested it may also be involved in normal developmental processes. We believe a review of the current autophagy literature, as related to filamentous fungi, may promote interest in considering the role of autophagy in growth and development as well as its relationship to other cellular processes. Our intent is to provide an overview of autophagy and the current understanding of its role in filamentous fungi. We follow the path taken by other reviewers of early work on higher eukaryotes, using a simple yeast model for macroautophagy as an initial, intellectual framework to guide our discussion. In the context of this model, we discuss the fungal autophagy proteins that have been characterized to date. We also discuss various strategies used to study and monitor autophagy in filamentous fungi, and we suggest directions for future research.

2. Overview of autophagy

2.1. Induction and inhibition of autophagy

As in other organisms, fungal autophagy is typically induced by nutrient (e.g., carbon, nitrogen) starvation (Dementhon et al.,...
to wildtype, suggesting that growth inhibition was directly tied to the mutants inability to recycle growth nutrient through autophagy. The same autophagy mutant strain showed morphological defects that could be reversed when supplemented with ammonium tartate, suggesting the need for high nitrogen content (Richie et al., 2007). Autophagy is also induced by cation depletion, suggesting that autophagy is involved in metal ion (e.g., zinc, manganese, iron) homeostasis and recycling (Richie et al., 2007). These studies provide evidence that autophagy is involved in recycling of nitrogen and of metal ions required to support growth.

2.3. Autophagy in cellular degradation

In general, autophagy serves as a major eukaryotic process for protein degradation, and is the only pathway for degradation of long-lived proteins and whole organelles, particularly those that are damaged or obsolete (Reggiori and Klionsky, 2002). In filamentous fungi, autophagy is typically accompanied by vacuolar enlargement. Vacuoles are degradative organelles, and hyphal vacuolation has been shown to increase rapidly in nutrient-starved Aspergillus oryzae mycelia (Pollack et al., 2008). Autophagic bodies are found inside vacuoles when autophagy is induced, but not in mutant strains defective of autophagy, such as in the A. fumigatus ΔAfg1 strain (Richie et al., 2007). However, vacuolation is not suppressed in P. anserina ΔAfg1, nor ΔAfg8 strains, which suggests that vacuolation itself is not dependent upon autophagy (Pinan-Lucarré et al., 2005).

2.4. Autophagy in cell death

While autophagy has been called type II programmed cell death (PCD) or autophagic cell death (Bursch, 2001), there is evidence that autophagy may play both causative and preventative roles in cell death. For example, in the plant pathogen Magnaporthe grisea, autophagy is required for spore collapse (cell death) during host infection (Veneault-Fourrey et al., 2006). In contrast, autophagy does not appear to be a requirement for cell death in P. anserina, although it is induced during cell death by incompatibility (a model for type II PCD) (Pinan-Lucarré et al., 2005). In fact, autophagy may have a pro-survival function by eliminating “pro-death” signals that move from damaged organelles or compartments to adjacent healthy cells (Pinan-Lucarré et al., 2005).

Autophagy also appears to be connected to autolysis, a natural self-degradation process by endogenous hydrolase activity, which if prolonged leads to cell death (White et al., 2002). Many factors affect fungal autolysis, such as aging, PCD, hyphal differentiation, nutrient limitation, and physical stress; however, the molecular mechanisms of autolysis have not yet been elucidated (White et al., 2002). Autophagy has been shown to precede autolysis (Cebollero and Gonzalez, 2006), and may possibly play a role in the early onset of autolysis.

2.5. Autophagy in cellular differentiation

In a number of species, autophagy is apparently involved in determining cell architecture during differentiation and development (Levine and Klionsky, 2004). Similarly in filamentous fungi, defects in autophagy genes influence morphogenesis and morphology. For example, mutants with deletions of atg1 or atg8 genes consistently show reduced numbers of aerial hyphae, disrupted condiation, and delayed germination; such effects have been shown in M. grisea (Liu et al., 2007), P. anserina (Pinan-Lucarré et al., 2005, 2003), A. oryzae (Kikuma et al., 2006) and A. fumigatus (Richie et al., 2007). Deletion of autophagy genes also inhibits formation of sexual reproductive organs, such as proproterithecia in P. anserina and perithecia in M. grisea (Liu et al., 2007; Pinan-Lucarré et al., 2005).
et al., 2003). These findings strongly suggest that in several species of filamentous fungi a functional autophagy pathway is required for correct cellular differentiation to occur. Autophagy also appears to play a role in differentiation of fungal infectious structures. For example, in the pythopathogen *Colletotrichum lindemuthianum*, a deletion mutant for *clk1* (a homolog of *atg1*) shows defects in leaf cuticle penetration (Dufresne et al., 1998). In another phytopathogen, *M. grisea*, disruption of autophagy by deletion of *Mgatg8* or *Mgatg1* results in loss of ability to infect host plants, likely due to fewer formed appresoria and lower turgor pressure in appresoria (Liu et al., 2007; Veneault-Fourrey et al., 2006). Defects in cellular differentiation are typically recovered when autophagy is restored (e.g., by complementation of the missing gene) or bypassed (e.g., by exogenous nutrient supplementation).

However, autophagy does not appear to always be involved in cellular differentiation. For example, in the filamentous yeast *Candida albicans*, autophagy disruption, in an *Atg9* mutant does not affect hyphal differentiation or formation of chlamydoospores (Palmer et al., 2007). Thus, the role of autophagy in fungal differentiation may, to some degree, be species dependant.

3. Model for fungal autophagy

As the molecular mechanisms of autophagy continue to be elucidated (currently, more than 30 autophagy genes have been identified), increasingly complex models have been proposed (Klionsky et al., 2007; Levine and Klionsky, 2004). However, as only a limited number of autophagy studies have been carried out in filamentous fungi, we put forward a simple autophagy model (Klionsky and Emr, 2000) that consists of four sequential steps (1) induction of autophagy through the target of rapamycin (Tor) kinase, (2) formation of autophagosomes and sequestration of cytoplasm and organelles, (3) docking of autophagosome and fusion with vacuole, and (4) breakdown of autophagic bodies in the vacuole into macromolecules ready for recycling (Fig. 2).

3.1. Induction

Autophagy is typically induced during nutrient starvation conditions or in response to treatment with rapamycin. In both cases, the target of rapamycin (Tor) kinase activity is induced, which leads to activation of the autophagy pathway (Fig. 3). In yeast, genetic studies have shown that the autophagy proteins Atg1, Atg13, and Atg17 must form a complex (“Atg1 complex”) for the induction of autophagy (Kamada et al., 2000). Atg17 has been shown to be specific to the autophagy pathway, and Atg13 is essential for the association of Atg1 and Atg17 (Kabeya et al., 2005). In silico studies have shown that the induction proteins Atg1, Atg13, and Atg17 are fully conserved in filamentous fungi (Meijer et al., 2007; Muthuvijayan and Marten, 2004). Other proteins such as Atg11, Atg20, Atg24, and Vam8 are thought to be involved in both autophagy and the biosynthetic cytoplasm-to-vacuole targeting (Cvt) pathway (Nair and Klionsky, 2005). The Cvt pathway specifically transports two vacuolar hydrolases (Ape1 and Ams1) into the vacuoles (Harding et al., 1995). However, proteins specific to the Cvt pathway are poorly conserved between yeast and filamentous fungi, and are not present in higher eukaryotes (Meijer et al., 2007). Currently, it is unclear whether a functional Cvt pathway exists in filamentous fungi.

3.1.1. Target of rapamycin

Tor is involved in nutrient sensing and regulation of transcription, translation, and protein degradation (Rohde et al., 2001). It is part of a signal transduction pathway conserved from yeast to humans (Fitzgibbon et al., 2005). In filamentous fungi such as *P. anserina, Fusarium fujikoroi* and *A. nidulans*, only one Tor kinase has been identified (Fitzgibbon et al., 2005; Muthuvijayan and Marten, 2004; Pinan-Lucarre et al., 2006; Teichert et al., 2006). This finding is consistent with higher eukaryotes, which generally have a single Tor kinase, but in contrast with yeast which shows at least two multiprotein complexes, TORC1 and TORC2 (De Virgilio and Loewith, 2006).

In *F. fujikoroi*, a rice pathogen, Tor shows conserved domains common to other Tor proteins, including the phosphatidylinositol 3-kinase catalytic domain, the FAT and the FATC domains, FKBP12-rapamycin-binding domain, and a suggested rapamycin-binding domain where a serine residue at position 1973 is located between the FAT and the kinase domains (Teichert et al., 2006). In *P. anserina*, the crucial position for rapamycin binding in PaTOR was identified as a serine residue at position 1895 (Pinan-Lucarre et al., 2006). This serine residue is a known site for missense mutations in yeast and humans, rendering the Tor protein nonfunctional (Chen et al., 1995; Chiu et al., 1994; Helliwell et al., 1994), and thus demonstrating the significant degree of homology between Tor proteins from various species.

Tor can be inhibited by the immunosuppressive macrolide rapamycin by forming a ternary complex between Tor, rapamycin, and the peptidyl-prolyl isomerase FKBP12 (Rohde et al., 2001). Tor inhibition by rapamycin results in upregulation of autophagy-related genes (e.g., homologs of the *Podospora idi7*/*atg8*), but also upregulation of genes involved in transcription, secondary metabolism, and stress response. Tor inhibition by rapamycin also results in the downregulation of genes involved in ribosome biogenesis.
nutrient starvation (Cheong et al., 2005). When active, Tor kinase hyperphosphorylates Atg1 and Atg13 preventing their association. Upon inhibition of Tor kinase by nutrient starvation or rapamycin, Atg1 and Atg13 are dephosphorylated by an unknown phosphatase, allowing them to complex with Atg17 ("Atg1 Complex"), thus inducing autophagy. References to the Cvt pathway have been omitted, as this pathway appears to be absent in a broad spectrum of filamentous fungi (Meijer et al., 2007).

3.1.2. Atg1

Atg1 is a serine/threonine kinase required for autophagy, and overexpression of the Atg1 protein homolog in Drosophila has been shown to induce high levels of autophagy (Scott et al., 2007). The homolog of the yeast ATG1 gene has been identified and characterized in a number of filamentous fungi, including P. anserina, M. grisea, A. fumigatus, and C. lindemuthianum (Dufresne et al., 1998; Liu et al., 2007; Pinan-Lucarré et al., 2005; Richie et al., 2007). Deletion of the ATG1 gene homolog resulted in inhibition of autophagy, as evident by a defect in autophagosome formation and absence of autophagic bodies in the vacuoles in P. anserina and A. fumigatus (Pinan-Lucarré et al., 2005; Richie et al., 2007). These data are consistent with the role of ATG1 in S. cerevisiae in the induction of autophagy (Kamada et al., 2000).

3.1.3. Atg13

In contrast, relatively little is known about the role of the phosphoprotein Atg13 in filamentous fungi. In a recent study, the atg13 gene homolog was deleted in A. nidulans (ΔAnatg13) resulting in a delay in spore germination and reduced growth rate (Pollack et al., unpublished data), which are consistent with morphological defects reported in other autophagy mutants. Moreover, during glucose starvation metabolic activity in the ΔAnatg13 mutant is significantly reduced compared to that of its parent strain, implying a reduction in the mutant’s ability to survive.

3.1.4. Atg17

The third regulatory subunit of the Atg1 complex, Atg17, has yet to be examined in filamentous fungi. Since Atg1–Atg13 interaction is insufficient to induce autophagy in yeast (Kabeya et al., 2005), it is important to elucidate the function of Atg17.

3.1.5. PI3K complex

Another protein complex is required for autophagy in yeast, the phosphotyidylinositol 3-kinase (PI3K) complex, which involves Atg15, Vps34, Atg6, and Atg14 (Klionsky, 2005). All these proteins, except Atg14, are conserved in filamentous fungi, but these gene products have not yet been studied (Meijer et al., 2007). We note that in S. cerevisiae, this complex is not specific to autophagy; but is also involved in the Cvt pathway and a fairly diverse array of signaling and membrane transport events, including Golgi to vacuole transport. Thus, it is likely that autophagy may be impacted by other cellular signaling events.

3.2. Formation of autophagosome

The second step in the autophagy model is the formation of the autophagosome, a double membrane vesicle that sequesters cytoplasmic content and even whole organelles (Abeliovich et al., 2000). The autophagosome is the characteristic morphological feature of autophagy, and the proteins involved are well conserved from yeast to man (Meijer et al., 2007). Autophagosome formation begins with vesicle nucleation where proteins and lipids concentrate to form what is called the pre-autophagosomal structure (PAS). Proteins involved in vesicle nucleation include the PI3K complex, Atg18, Atg20, Atg21, Atg24 (Klionsky, 2005) as well as Atg29 (Kawamata et al., 2005). Next, vesicles expand to form the fully developed autophagosomes; the proteins involved include Atg3, Atg4, Atg5, Atg7, Atg8, Atg10, Atg12, and Atg16 (Fig. 4). The origin of these vesicles was initially thought to be the endoplasmic reticulum (ER), but the current view is that the autophagosome forms in a mainly de novo process (Noda et al., 2002).

3.2.1. Atg8

Atg8 is a ubiquitin-like protein associated with the autophagosome throughout the autophagy process, and is well conserved in most model organisms and higher eukaryotes (Levine and Klionsky, 2004). It is associated with the autophagosome membrane and has been used as a marker for autophagy (Klionsky et al., 2007). Of the proteins associated with the autophagosome, only Atg8 has been studied in filamentous fungi.

Deletion of the atg8 gene in fungi detrimentally inhibits autophagy, and thus affects cellular growth and differentiation. In P. anserina, disruption of the gene idi-7/Paatg8 resulted in decreased hyphal growth density, fewer aerial hyphae, no pigmentation, and no protoperithecia (Pinan-Lucarré et al., 2003). Disruption of Auatg8 in A. oryzae resulted in inhibition of aerial hyphal growth and conidiation, reduced growth on synthetic medium compared to the wild type strain, and defects in autophagy (Kikuma et al., 2006). In M. grisea, disruption of the Mgatg8 gene resulted in the formation of appressoria that could not penetrate the plant surface, such that the mutant was non-pathogenic (Veneault-Fourrey et al., 2006). In the ectomycorrhizal fungus Laccaria bicolor, LB-AU7/ LburG has been identified and observed to be upregulated during interactions with Pinus resinosa (Kim et al., 1999), suggesting that autophagy may be involved in the symbiotic relationship between the two organisms.
3.3. Docking and fusion

In the third step, the autophagosome docks with the surface of a vacuole, and the outer membrane fuses with the vacuolar membrane. The proteins involved in this step are mostly proteins implicated in membrane fusion, such as the SNARE family proteins (Klionsky, 2005). Once inside the vacuolar lumen, the single-membrane vesicle becomes the autophagic body. Studies in mammalian cells have shown that autophagic bodies and fused autophagosomal-lysosome (lysosome is the vacuole equivalent in mammalian cells) are acidic and can be observed with acidic markers (Munafò and Colombo, 2001). To date, this specific autophagy step has not been explored in filamentous fungi.

3.4. Vacuolar breakdown

The last step is the breakdown of the autophagic body in the vacuoles and recycling of cellular macromolecules to support continued tip extension. Atg15 and Atg22, two autophagy proteins identified in yeast, are involved in this process. Atg15 is a putative lipase involved in lysis of autophagic bodies, Cvt bodies and incorporated peroxisomes in the vacuole (Epple et al., 2001). Atg15 is well conserved in yeast and filamentous fungi (Meijer et al., 2007), but only in the latter does it contain a novel yet distinct repeat motif at its C-terminus, preceded by a Ser/Thr rich region (Kiel and van der Klei, 2001). Atg15 is a putative lipase involved in lysis of autophagic bodies, Cvt bodies and incorporated peroxisomes in the vacuole (Epple et al., 2001). Atg15 is well conserved in yeast and filamentous fungi (Meijer et al., 2007), but only in the latter does it contain a novel yet distinct repeat motif at its C-terminus, preceded by a Ser/Thr rich region (Kiel and van der Klei, 2001). Atg15 is a putative lipase involved in lysis of autophagic bodies, Cvt bodies and incorporated peroxisomes in the vacuole (Epple et al., 2001). Atg15 is well conserved in yeast and filamentous fungi (Meijer et al., 2007), but only in the latter does it contain a novel yet distinct repeat motif at its C-terminus, preceded by a Ser/Thr rich region (Kiel and van der Klei, 2001). Atg15 is a putative lipase involved in lysis of autophagic bodies, Cvt bodies and incorporated peroxisomes in the vacuole (Epple et al., 2001). Atg15 is well conserved in yeast and filamentous fungi (Meijer et al., 2007), but only in the latter does it contain a novel yet distinct repeat motif at its C-terminus, preceded by a Ser/Thr rich region (Kiel and van der Klei, 2001). Atg15 is a putative lipase involved in lysis of autophagic bodies, Cvt bodies and incorporated peroxisomes in the vacuole (Epple et al., 2001). Atg15 is well conserved in yeast and filamentous fungi (Meijer et al., 2007), but only in the latter does it contain a novel yet distinct repeat motif at its C-terminus, preceded by a Ser/Thr rich region (Kiel and van der Klei, 2001). Atg15 is a putative lipase involved in lysis of autophagic bodies, Cvt bodies and incorporated peroxisomes in the vacuole (Epple et al., 2001). Atg15 is well conserved in yeast and filamentous fungi (Meijer et al., 2007), but only in the latter does it contain a novel yet distinct repeat motif at its C-terminus, preceded by a Ser/Thr rich region (Kiel and van der Klei, 2001).

4. Methods for evaluation of autophagy

Recently, the autophagy community has agreed on the necessity of standardizing methods for measuring autophagy in different organisms (Klionsky et al., 2008, 2007). Along these lines, there are two general approaches for assessing autophagy: steady state and flux measurements (Klionsky et al., 2008). The former allows monitoring of phagophores and autophagosomes in the cytoplasm at a given time; the latter involves monitoring vacuolar content to indicate completion of the autophagy process. We note, however, that many of the methods that have been developed are either organism or cell condition-dependent, often necessitating multiple assays for accurate evaluation of the extent of autophagy. Here, we focus exclusively on those methods that have been used to study fungal autophagy.

4.1. Construction of autophagy mutants

To study the function of genes involved in the autophagy pathway in filamentous fungi, efficient and versatile methods are needed for the construction of autophagy disruption mutants. Standard cloning procedures using transforming plasmids have been used for making P. anserina ΔPaatg1 and ΔPaatg8 strains (Pinan-Lucarré et al., 2005) as well as the A. oryzae Aoatg8 disruption mutant (Kikuma et al., 2006). The split-marker strategy based on gene deletion in yeast (Catlett et al., 2003) has been used in the disruption of Mgatg8 in M. grisea and Atatg1 in A. fumigatus (Richie et al., 2007; Veneault-Fourrey et al., 2006).

Recent developments in fusion PCR techniques for generating linear fragments for transformation allow more rapid transformation without the need of conventional cloning procedures (Kuwajama et al., 2002; Szweczyk et al., 2006; Yang et al., 2004). In addition, it has been shown that deletion of the gene homolog of the human KU70 and KU80 genes in N. crassa (mus-51 and mus-s2) and A. nidulans (nkuA and nkuB) significantly increases the frequency of correct homologous recombination for gene targeting (Nayak et al., 2006; Ninomiya et al., 2004). These genes control the nonhomologous end joining in DNA repair; thus their deletion increases efficiency of gene replacement by 90%. Together, fusion PCR techniques and deletion of KU70 and KU80 homologs in filamentous fungi will allow more rapid and efficient construction of autophagy mutant strains in filamentous fungi.

4.2. Electron microscopy

Electron microscopy (EM) allows analysis of vacuolar content, in particular, the presence of autophagic bodies in autophagy-induced cultures or the absence of autophagic bodies in autophagy-defective mutants (Liu et al., 2007; Pinan-Lucarré et al., 2003; Veneault-Fourrey et al., 2006). EM has also allowed identification of autophagosomes as double membrane vesicles, which are
characterized by electron density similar to cytosol (Klionsky et al., 2007). However, it has been suggested that the “double membrane” may be an artifact of sample preparation (Klionsky et al., 2007). So it may be more accurate to identify autophagosomes by their delimiting, smooth (i.e., free of transmembrane proteins) membranes and by their content (Klionsky et al., 2007).

4.3. GFP–Atg8 fusion protein

Atg8 is a useful cytological marker for autophagy because of its association with pre-autophagosomal structures (PAS) as well as mature autophagosomes that are characteristic of macroautophagy. Its unique properties allow it to be used in steady state autophagy assays as well as in autophagy flux monitoring. Fusion of GFP to the N-terminus of Atg8 allows visualization of Atg8 localized in the cytoplasm (as punctate dots) and eventually in the vacuole. Upon proteolytic degradation of Atg8 in the vacuole, GFP can continue to fluoresce serving as an indicator of autophagic flux. This method has been successfully used in several fungal species including P. anserina (Pinan-Lucarré et al., 2005), A. oryzae (Kikuma et al., 2006), and A. fumigatus (Kikuma et al., 2006; Richie et al., 2007). Mutants with defects in the autophagy pathway typically do not show an accumulation of GFP in the vacuoles. We note that GFP should be fused at the N-terminus of Atg8, as the C-terminus is susceptible to cleavage by Atg4 prior to entrance into the vacuole. However, a strain of A. nidulans with GFP fused to Atg8 at the C-terminus shows fluorescence in the vacuoles (Harris SD, Semighini C, unpublished results), suggesting cleaved GFP in the cytoplasm may be taken up via the autophagy pathway and thus find its way to the vacuole. A different fluorescent tag, the red fluorescence protein DsRed2, has been used to monitor Atg8 (Kikuma et al., 2006), allowing simultaneous detection when used with proteins tagged with enhanced-GFP (EGFP) (Kikuma et al., 2006; Maruyama et al., 2004).

4.4. Inhibition of autophagic body degradation by vacuolar proteases

Autophagy activity is demonstrated by the presence of autophagic bodies in the vacuoles. Since vacuolar degradation occurs relatively quickly, it is necessary to use a vacuolar protease inhibitor such as PMSF to allow observation of autophagic bodies (Pinan-Lucarré et al., 2003; Takeshige et al., 1992). This method is sometimes performed in conjunction with GFP–Atg8 with fluorescence microscopy and differential interference contrast (DIC) microscopy (Richie et al., 2007; Veneault-Fourrey et al., 2006).

4.5. Probe for acidic compartment

Monoansyl cadaverine (MDC) is an autofluorescent dye that has been used as an indicator of autophagy, as it stains acidic autophagosomes in mammalian cells (Biederbick et al., 1995; Dunn, 1990a,b). The specificity of MDC is derived from ion trapings in acidic compartments and interaction with high levels of lipid characteristic of autophagosomes. MDC also co-localizes with GFP–Atg8 homolog fusion protein in autophagosomes in plants (Conte et al., 2005). Recently, MDC was used in the rice blast fungus M. grisea et al. (2005). Recently, MDC was used in the rice blast fungus

5. Future directions

The study of autophagy in filamentous fungi is still in its infancy. Even though many of the autophagy proteins identified in other species have homologs in filamentous fungi, their functions may not be well conserved. Thus, to better understand the mechanistic details involved in fungal autophagy, it will be important to first identify essential and dispensable proteins, and then to study the functional roles of these proteins.

For example, the particular roles that Atg13 and Atg17 play in the induction of autophagy have yet to be determined. Additionally, most proteins involved in the later steps of autophagy have not yet been studied. Recently, the TOR pathway in yeast was shown to operate in parallel with protein kinase A (PKA) and Sch9 signaling pathways to regulate autophagy. All three pathways appear to be dependent on Atg1 (Yorimitsu et al., 2007), but it has not been reported whether such relationships exist in filamentous fungi.

By using yeast as an intellectual framework, researchers have confirmed that certain characteristics of autophagy are conserved in filamentous fungi. Similar to yeast, autophagy in filamentous fungi is controlled by Tor kinase and characterized by the presence of autophagosomes in the cytoplasm and autophagic bodies in the vacuoles. Autophagy in filamentous fungi can be monitored using the fungal homolog of the GFP–Atg8 fusion protein. Yet filamentous fungi also show similarities to higher eukaryotes, e.g., disruption of autophagy affects cellular differentiation and development.

It is important to note that filamentous fungi differ significantly from yeast in their physiology: the former are considered multicellular organisms, with constant polar growth, that have a different mode of proliferation. As a result, it is likely that these differences may manifest as differences in the role of autophagy in nutrient starvation and/or cellular development. Consequently, it is possible, and perhaps even likely, that there may be genes specific for autophagy in filamentous fungi (and higher eukaryotes) that are not present in yeast. Thus, there is a certain danger in relying exclusively upon the yeast model in identifying autophagy genes. This limitation of the so-called yeast paradigm has previously been discussed in the context of morphogenesis (Harris and Momany, 2004).

Future autophagy research in filamentous fungi will need to tackle such questions as the following: How, exactly, do autophagy and its proteins affect cellular morphology and physiology? How does autophagy affect cell growth, branching, and cell wall properties? What is the relationship between autophagy and other stress responses in filamentous fungi? By answering these questions, we will gain a better functional understanding of autophagy in filamentous fungi.

Acknowledgments

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