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FORUM REVIEW ARTICLE

The Role of Macroautophagy in Development of Filamentous Fungi

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Abstract

Autophagy (macroautophagy) is a bulk degradative pathway by which cytoplasmic components are delivered to the vacuole for recycling. This process is conserved from yeast to human, where it is implicated in cancer and neurodegenerative diseases. During the last decade, many *ATG* genes involved in autophagy have been identified, initially in *Saccharomyces cerevisiae*. This review summarizes the knowledge on the molecular mechanisms of autophagy using yeast as model system. Although many of the core components involved in autophagy are conserved from yeast to human, there are, nevertheless, significant differences between these organisms, for example, during autophagy initiation. Autophagy also plays an essential role in filamentous fungi especially during differentiation. Remarkably, in these species autophagy may reflect features of both yeast and mammals. This is exemplified by the finding that filamentous fungi lack the *S. cerevisiae* clade–specific Atg31 protein, but contain Atg101, which is absent in this clade. A reappraisal of genome data further suggests that, similar to yeast and mammals, filamentous fungi probably also contain two distinct phosphatidylinositol 3-kinase complexes. This review also summarizes the state of knowledge on the role of autophagy in filamentous fungi during differentiation, such as pathogenic development, programmed cell death during heteroincompatibility, and spore formation. *Antioxid. Redox Signal.* 14, 2271–2287.

Introduction

Autophagy is a broad term covering the processes by which organisms recycle their intracellular components through the vacuole/lysosome, which are essential for cellular survival under conditions that the nutrient supply becomes limiting (143). The importance of autophagy for mammals is underscored by the notion that this process can be been linked to cancer and neurodegenerative diseases [for recent reviews, see (11, 35)]. The term autophagy actually describes multiple cellular phenomena. The best studied of these is nonselective macroautophagy, which involves random uptake of portions of the cytoplasm (cytosol and organelles) into the vacuole/lysosome for recycling (Fig. 1) (127). In many eukaryotes, macroautophagy is not only a survival mechanism, but the recycled building blocks are also utilized during differentiation, for example, in spore formation in some yeast species (80). Macroautophagy is defined by the formation of a double-membrane structure that engulfs a portion of cytoplasm, resulting in the formation of a vesicular structure termed an autophagosome. Subsequently, the outer membrane layer of the autophagosome will fuse with the vacuolar membrane and a single membrane structure, commonly referred to as an autophagic body, enters the lumen of the vacuole. There, this structure is lysed and its contents recycled by vacuolar hydrolases to replenish the nutrient depletion or to stimulate cellular differentiation. Nonselective incorporation of cytoplasmic components by the vacuole can also take place *via* microautophagy, a process that is defined by the direct uptake of a portion of the cytoplasm by vacuolar invagination (70). Similar to macroautophagy, microautophagy is induced by starvation. However, there is no evidence that the specialized macroautophagy machinery (see below) is required for this process. Nevertheless, macroautophagy is a prerequisite to sustain microautophagy. Presumably, this reflects an indirect connection *via* the membrane supply, because microautophagy leads to reduction of the vacuolar membrane surface, which must be compensated for by the fusion of autophagosomes with the vacuole. Since microautophagy has not received much attention in filamentous fungi, we will not consider it further here.

In addition to macro- and microautophagy, multiple autophagy-related processes have been identified that utilize the macroautophagy machinery for selective uptake of proteins, small aggregates, and organelles into the vacuole/lysosome. Most of these processes have been first identied in

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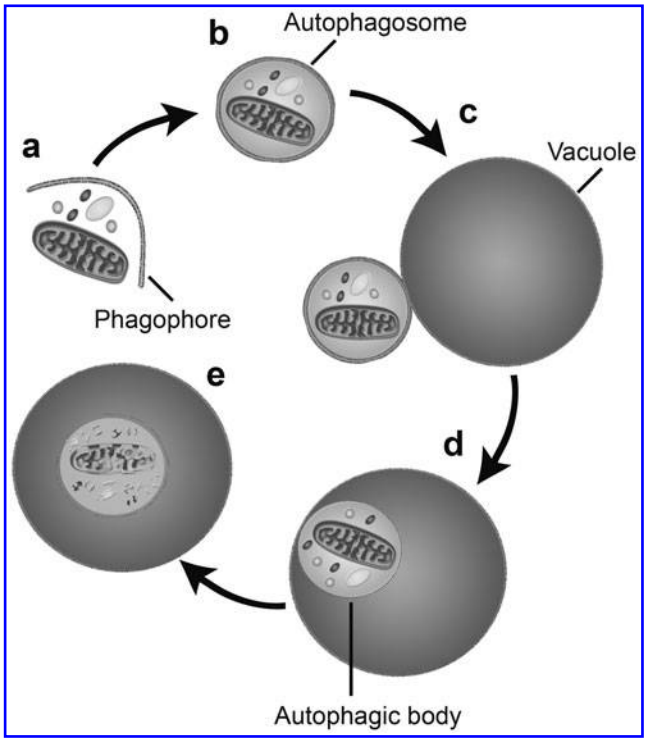

FIG. 1. Schematic model of macroautophagy. (a) Cytoplasmic components (cytosol and organelles) are sequestered by an expanding membrane structure, the phagophore. **(b)** Upon completion, this event results in formation of a double-membrane-bound vesicle, the autophagosome. **(c, d)** The cargo of the autophagosome enters the vacuolar lumen in a single-membrane structure, called the autophagic body, as a result of fusion of the outer membrane layer of the autophagosome with the vacuolar membrane. **(e)** Subsequently, the autophagic body and its contents are degraded *via* vacuolar hydrolases. Building blocks like amino acids, fatty acids, *etc.*, generated in this step can be recycled to the cytosol.

the yeast *Saccharomyces cerevisiae*. In many instances a specific receptor-like protein is involved that links the protein/aggregate/organelle to be transported into the lumen of the vacuole/lysosome to the macroautophagy machinery [for review see (66)]. The best studied selective process is the constitutive cytoplasm to vacuole targeting (Cvt) pathway that sorts certain resident hydrolases to the vacuole (130). Analogous to macroautophagy, Cvt cargo becomes incorporated in a double-membrane structure (the Cvt vesicle) that fuses with the vacuolar membrane to allow release of a single membrane bound structure (the Cvt body) into the vacuole lumen. Upon its lysis, the incorporated hydrolases become activated (118). Remarkably, so far, this pathway has only been observed in *S. cerevisiae* and *Pichia pastoris* (29, 60), although putative Cvt cargo proteins have been identified in other yeast species as well as in filamentous fungi (82).

Another highly selective autophagy-related process is pexophagy, the degradation of redundant peroxisomes, a process that has been mainly studied in methylotrophic yeast species [reviewed in (28, 108)]. Similar to autophagy, this process can be divided into macro- and micropexophagy depending on the formation of a double (or multi-) membrane-layered structure (the pexophagosome) in the cytosol, or the direct uptake of (clusters of) peroxisomes *via* invagination by the vacuole.

Recent data indicate that, when required, also other cellular components can be selectively degraded by processes analogous to macro- and microautophagy. This includes selective degradation of mitochondria [mitophagy, reviewed in (51)], the endoplasmic reticulum (ER) [reticulo- or ER-phagy (7)], ribosomes [ribophagy (65)], and portions of the nucleus [piecemeal microautophagy of the nucleus (107)]. In addition to this, in mammalian cells degradation of aggregate-prone proteins (aggrephagy) occurs *via* a highly selective pathway that utilizes the macroautophagy machinery [reviewed in (63)].

Filamentous fungi are omnipresent and play important roles in human society, including healthcare, agriculture, food production, and bioprocessing. In nature, but also during industrial fermentations, filamentous fungi continuously experience nutrient starvation, which can lead to cellular degradation and even cell death. Under these conditions, autophagy may prolong cellular survival, or allow differentiation. As a result, studies on autophagy in filamentous fungi usually concern the general recycling of cellular constituents by macroautophagy. Little is known about the occurrence of selective forms of autophagy in these organisms. So far, only a few reports of pexophagy in filamentous fungi have appeared (2, 4). Remarkably, it was observed that most of the proteins specific for the Cvt, pexophagy, mitophagy, ER-phagy, ribophagy, piecemeal microautophagy of the nucleus and aggrephagy pathways are apparently not present in filamentous fungi [cf. (82)]. Therefore, in this review we will mainly discuss macroautophagy. First, we will describe the process of macroautophagy at the molecular level using the yeast *S. cerevisiae* as model organism. After that we will summarize the knowledge on the role of macroautophagy in developmental processes in filamentous fungi. Note that in the following, for practical purposes, we will only use the term autophagy, when we refer to macroautophagy.

Proteins Involved in Autophagy

Genes involved in autophagy have been designated *ATG* genes (62), which were first identified in yeast species. However, orthologs for many Atg proteins have been found encoded by the genomes of other eukaryotes, including filamentous fungi allowing to study their molecular characteristics (54, 56, 82, 87). These *in silico* studies showed that the core autophagy machinery is highly conserved. In the following we will discuss the role of Atg proteins in autophagy, with emphasis on *S. cerevisiae*.

Atg proteins required for the initial stages of autophagy

In most organisms, including filamentous fungi, autophagy is typically induced by nutrient starvation or by treatment with the macrolide rapamycin. In baker's yeast, cell growth is regulated by the protein kinase Tor in response to nutrient availability and cellular stress (113). During nutrient-rich conditions, a complex containing Tor (yeast Tor complex 1 [TORC1]) inhibits autophagy. Upon nutient limitation—or rapamycin addition—the kinase activity of Tor is inhibited, which leads to activation of the autophagy pathway. It is believed that TORC1 and possibly members of the type 2A protein phosphatase family (10) regulate the

phosphorylation state of the so-called Atg1 complex. In baker's yeast, genetic studies have shown that the Ser/Thr protein kinase Atg1 and the phosphoprotein Atg13 form the core of a complex essential for autophagy. Under nutrient-rich conditions, yeast TORC1 directly phosphorylates Atg13 at multiple Ser residues, resulting in its hyperphosphorylation (50), which blocks autophagy. Nutrient limitation will result in a significant decrease in the phosphorylation state of Atg13, thereby enhancing its affinity for Atg1. The resulting lowly phosphorylated Atg1/Atg13 complex can then associate with a constitutively present ternary complex consisting of Atg17, Atg29, and the phosphoprotein Atg31 (49, 52). It was demonstrated that this association is markedly enhanced in response to nutrient starvation. The Atg17/Atg29/Atg31 complex is thought to act as a scaffold essential for preautophagosomal structure (PAS) organization. Thus, the phosphorylation state of the Atg1 complex represents an important switch to either prevent or induce autophagy. Moreover, the kinase activity of Atg1 has been shown to be essential for autophagy (48) and appears to increase during starvation (81). However, the identity of the protein target of the kinase remains unresolved.

Atg17, Atg29, and Atg31 are not the sole components to bind to the Atg1 complex. A multitude of other proteins exclusively required for selective autophagy pathways either directly or indirectly associate with the complex. These include the coiled-coil protein Atg11, the Cvt cargo receptor Atg19, the sorting nexins Atg20, and Atg24 as well as the vacuolar membrane protein Vac8. Of these, Atg11 might perform a function as a scaffold protein linking the various selective pathways to Atg1 (10).

In silico studies have shown that Tor (TorA in filamentous fungi) is fully conserved from yeast to human (56, 82, 87). So far, only limited studies have been performed on *torA* in *Aspergillus nidulans* (31), *Fusarium fujikoroi* (129), and *Podospora anserina* (100). As might be expected, deletion of *torA* is lethal. Remarkably, in *F. fujikuroi*, inhibition of TorA by rapamycin not only affected autophagy and development, but also expression of secondary metabolite genes.

The core proteins of the Atg1 complex, Atg1 and Atg13 as well as Atg17 and Atg29, are conserved in filamentous fungi (82). Atg1 has been characterized in *P. anserina* (99), *Magnaporthe grisea* (syn *M. oryzae*) (75), and *Aspergillus fumigatus* (106). Data obtained with *atg1* deletions in these organisms were fully consistent with the role of Atg1 in autophagy in baker's yeast. Remarkably, deletion of *M. oryzae atg13* showed little defect on autophagy-requiring developmental changes [see below; (21, 54)]. Nevertheless, the *A. nidulans atg13* mutant appeared to have a reduced metabolic activity under glucose repression, which might imply a reduction in its ability to survive [unpublished data in (103)]. So far, Atg17 has received little attention in filamentous fungi, whereas Atg31 can only be identified in yeast species of the *S. cerevisiae* clade.

In mammalian cells, the Atg1 complex has a slightly different composition [recently reviewed in (85)]. Although orthologs of the core proteins Atg1 (designated uncoordinated 51-like kinase-1 [ULK-1]) and Atg13 (mAtg13) are present and function similarly as in *S. cerevisiae*, Atg17, Atg29, and Atg31 are not present. Recently, novel binding partners of mAtg13 were identified. These include FIP200 (200 kDa focal adhesion kinase family-interacting protein), a coiled-coil protein essential for autophagy (39), which is not conserved in fungi. Remarkably, the C-terminal portion of mammalian FIP200 shows similarity to the C-terminus of fungal Atg11 proteins, but the significance of this finding is unknown. A second mAtg13 binding protein was identified as Atg101, which is also essential for autophagy (44, 83). We found that this protein is conserved in many yeast species and filamentous fungi, but is fully absent in the *S. cerevisiae* clade (Fig. 2). Thus, it would seem that filamentous fungi contain an Atg1 complex that has features of both baker's yeast and mammals.

The phosphatidylinositol 3-kinase complex

Autophagy requires the formation of a double-layered membrane structure (the phagophore), which can sequester cytoplasmic components. This process starts with the association of proteins and lipids at the so-called preautophagosomal structure or phagophore assembly site [PAS (59, 126)]. A protein complex that controls these early stages of autophagy is the class III phosphatidylinositol 3-kinase (PI3-K) complex, which functions at the PAS. In yeast, this complex is present in the cell in two forms, one essential for autophagy—complex I—and the other essential for the transport of proteins from the Golgi to the vacuole (vacuolar protein sorting [Vps])—complex II (57). Both complexes have as core the Ser/Thr protein kinase Vps15 and the PI3-K Vps34 and also contain Atg6 (also known as Vps30). It is thought that the membrane specificity of each complex is determined by the fourth protein that is unique to each complex, that is, Atg14 in complex I and Vps38 in complex II, both coiled-coil proteins. During autophagy the activity of the type I PI3-K complex allows recruitment of PI3-phosphate binding proteins to the PAS, that is, the WD-40 protein Atg18 (in complex with the peripheral membrane protein Atg2), the sorting nexin Atg24, and the integral membrane protein Atg27 (89, 91, 123, 138). Other Atg proteins with PI3-phosphate binding capabilities, the WD-40 protein Atg21 and the sorting nexin Atg20 are apparently exclusively involved in selective autophagy pathways.

All proteins of the core PI3-K complex are conserved from yeast to human. Similarly, of the PI3-phosphate binding proteins Atg18/Atg2, Atg24 and Atg27 are fully conserved, whereas those involved in selective autophagy pathways (Atg20 and Atg21) are only present in yeast species (82). Initially, it was thought that Atg14 and Vps38 were only present in yeast species. However, recently a protein with extremely low similarity to yeast Atg14 was identified in human (HsAtg14/Barkor), whereas a human homolog of Vps38 turned out to be the earlier identified ultraviolet radiation resistance-associated gene (UVRAG) protein, which had surprisingly also been implicated in autophagy (47, 124). Indeed, it could be demonstrated that these mammalian proteins were in two distinct complexes together with mVps15, mVps34, and Beclin-1 (the mammalian Atg6/Vps30 ortholog). We have performed a reanalysis of protein databases at the NCBI by position-specific iterated and pattern hit-initiated basic local alignment search tool using both yeast and human Atg14

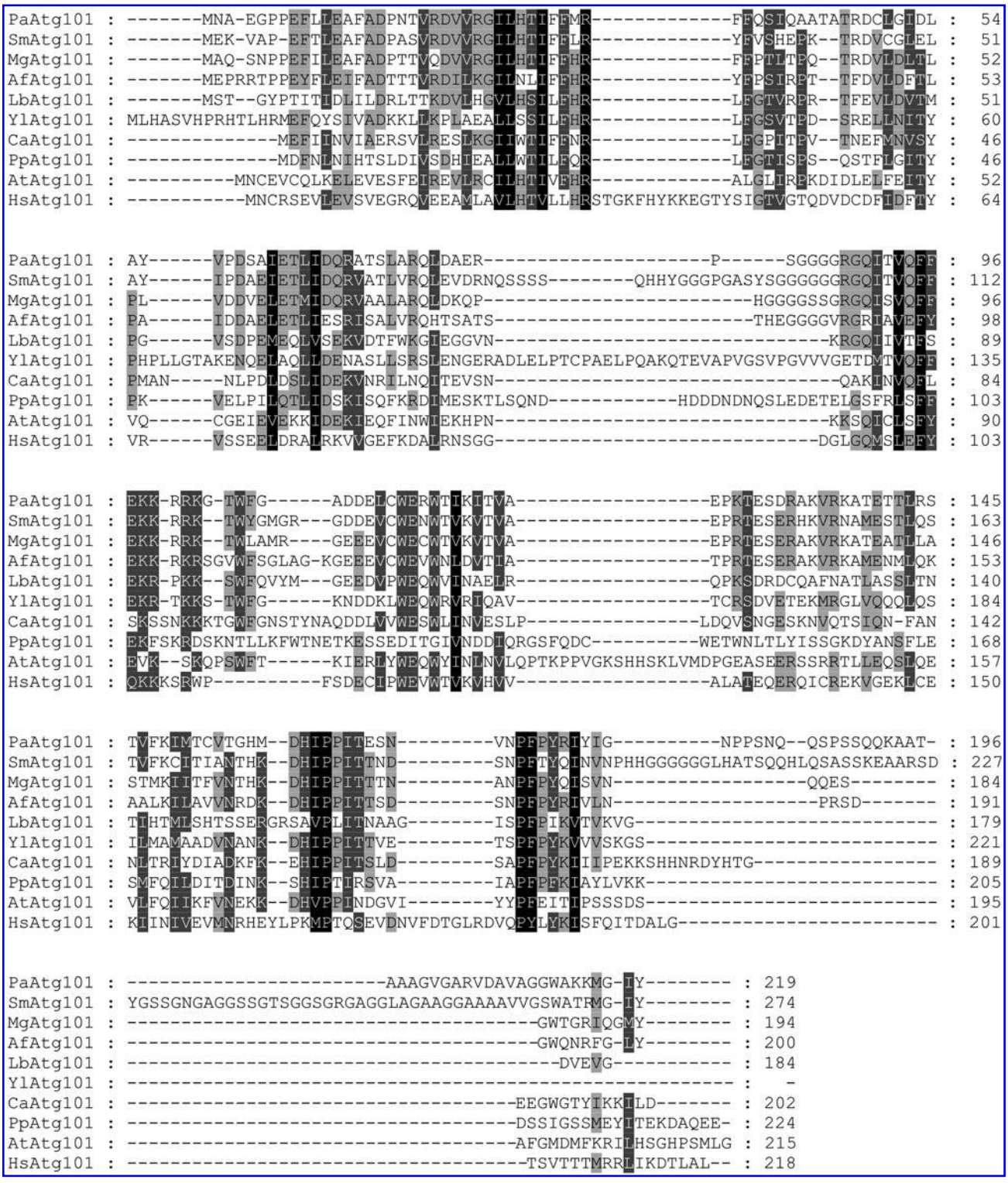

FIG. 2. Alignment of primary sequences of Atg101 proteins. Protein sequences were aligned using ClustalX (131). The one-letter code is shown. Residues that are similar in all proteins are shaded black. Similar residues in at least eight of the proteins are shaded dark gray, while those that are similar in at least six of the proteins are shaded light gray. The following protein sequences were used: Yeast species: *Candida albicans* (Ca; accession number XP_716380, required correction), *Pichia pastoris* (Pp; XP_002492728), and *Yarrowia lipolytica* (Yl; XP_500594). *Filamentous fungi*: *Aspergillus fumigatus* (Af; XP_754002, required correction), *Magnaporthe grisea* (Mg; XP_369908, required correction), *Podospora anserina* (Pa; XP_001908170), and *Sordaria macrospora* (Sm, CBI57327). Basidiomycete: *Laccaria bicolor* (Lb; XP_001878027). Higher eukaryotes: *Arabidopsis thaliana* (At; NP_569042) and *Homo sapiens* (Hs; NP_068753). All proteins belong to the DUF1649 family of conserved proteins.

sequences as queries and identified two conserved coiled-coil proteins encoded by the genomes of filamentous fungi with very weak similarity to both Atg14 and UVRAG. One of these proteins contains a conserved Cys-rich motif at its N-terminus that is also present in yeast and human Atg14, but not in Vps38/UVRAG (Fig. 3). The other protein showed higher similarity to mammalian UVRAG proteins, implying that it might represent the fungal ortholog of Vps38. This implies that also filamentous fungi may contain two distinct PI3-K complexes.

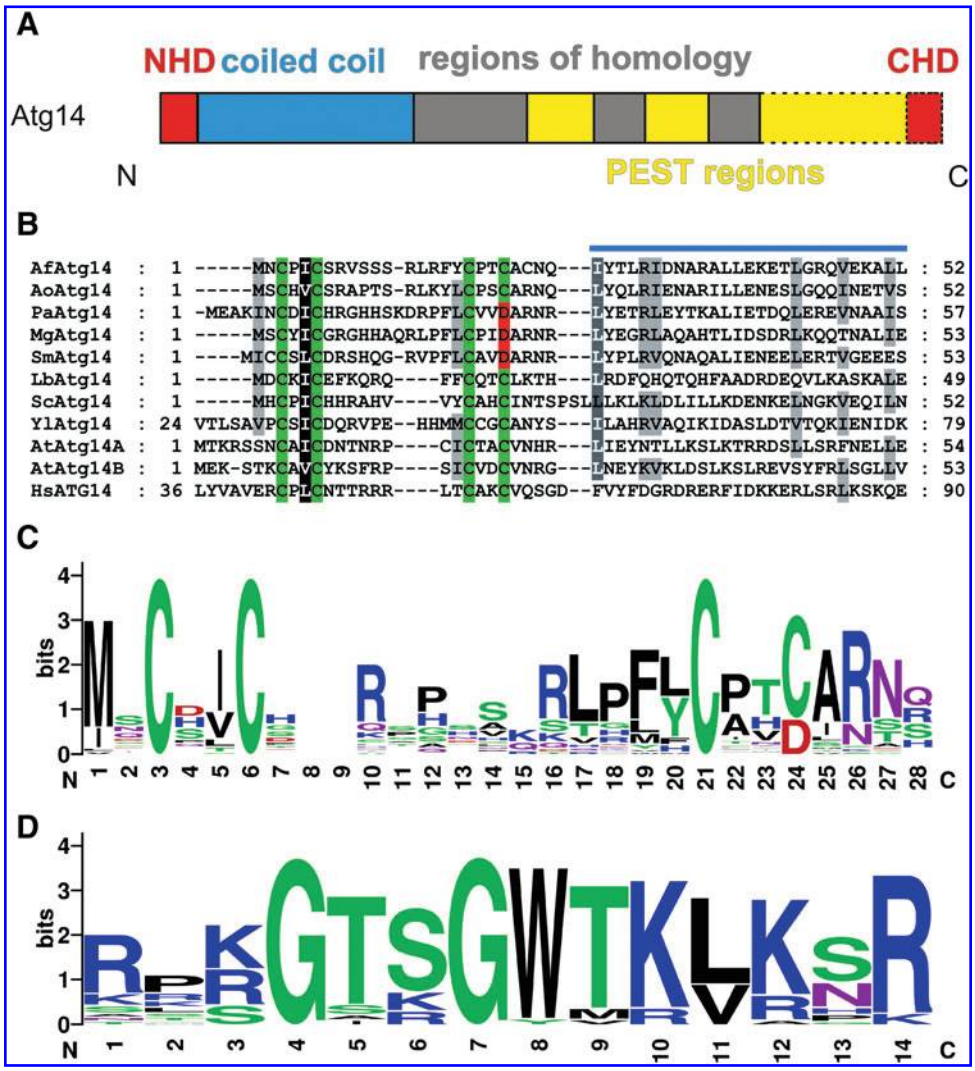

FIG. 3. Atg14 is conserved in filamentous fungi. (A) Schematic representation of Atg14 proteins in eukaryotes. The scheme is based on an analysis of 44 Atg14 orthologs from species ranging from yeast to human, with emphasis on fungi. An N-terminal homology domain (NHD) is indicated in *red*. A conserved coiled-coil region is indicated in *blue*. Other conserved regions are indicated in *gray*. Weakly conserved regions containing putative PEST sequences are indicated in *yellow*. The dotted line at the C-terminus indicates that this portion of the protein is only present in filamentous ascomycetes. This also includes the highly conserved C-terminal homology domain (CHD) that is indicated in *red*. **(B, C)** The N-terminus of Atg14 contains a conserved cysteine-rich motif. **(B)** Alignment of the N-terminus of 11 characteristic Atg14 proteins from species ranging from yeast to human. The conserved Cys residues are indicated in *green*, and conserved Asp residues in *red*. The *blue* bar above the sequences indicates the start of the conserved coiled-coil region. The *Arabidopsis thaliana* genome appeared to encode two Atg14 proteins, which have been designated Atg14A and Atg14B. This feature has been observed before in this organism (82). Af, *Aspergillus fumigatus*; Ao, *Aspergillus oryzae*; Pa, *Podospora anserina*; Mg, *Magnaporthe grisea*; Sm, *Sordaria macrospora*; Lb, *Laccaria bicolor*; Sc, *Saccharomyces cerevisiae*; Yl, *Yarrowia lipolytica*; At, *Arabidopsis thaliana*; Hs, *Homo sapiens.* **(C)** Weblogo graphic showing the distribution and amino acid composition of the N-terminal Homology Domain **(**see **A** and **B)**. The logo is based on 39 fungal Atg14 protein sequences. Residues with similar properties are indicated in the same color. Polar amino acids are *green* (G, S, T, Y, C) or *purple* (Q, N), basic amino acids *blue* (K, R, H), acidic amino acids *red* (D, E), and hydrophobic amino acids are *black* (A, V, L, I, P, W, F, M). **(D)** Atg14 in filamentous ascomycetes contains a highly conserved C-terminal homology domain. Weblogo graphic showing the distribution and amino acid composition of the C-terminal Homology Domain **(**CHD; see **A)**. The logo is based on 27 Atg14 sequences from filamentous ascomycetes. Remarkably, the *M. grisea* Atg14 sequence lacks this region. For color code see **(C)**. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Formation of the autophagosome

The second step of vesicle formation involves expansion of the PAS into the phagophore, which finally closes to become a fully developed autophagosome. The origin of the membrane of these structures has long been under debate. Recently, more substantial evidence was presented pointing toward a role for the ER in this process at least in mammalian cells (40, 142). Phagophore formation involves two sets of proteins that each participate in two ubiquitin conjugation-like reactions (92). First, the protease Atg4 processes the ubiquitin-like protein Atg8, resulting in a free C-terminal Gly residue that is covalently attached to phosphatidylethanolamine (PE) at the PAS. This Atg8-PE conjugation step requires the activities of Atg7 (an E1-like enzyme) and Atg3 (an E2-like enzyme). Moreover, Atg8-PE formation at the PAS also depends on a protein complex formed by a second conjugation reaction that might function analogous to an E3 enzyme (38). This second protein complex involves another ubiquitin-like protein, Atg12, that becomes conjugated *via* its C-terminal Gly residue to Atg5, a reaction also catalyzed by the E1-like enzyme Atg7. For this step Atg3 is not required, rather Atg10 functions as the E2-like enzyme. Finally, the coiled-coil protein Atg16 becomes noncovalently attached to the Atg5-Atg12 conjugate, resulting in formation of an Atg5-Atg12/Atg16 complex. Baker's yeast Atg16 can dimerize through the coiled-coil motif present at its C-terminus [see (34) and the references therein]. Formation of Atg8-PE and dimerization of the Atg12-Atg5 conjugates mediated by Atg16 are essential for autophagosome formation (69). Membrane expansion of the phagophore also requires the integral membrane protein Atg9. This protein appears to cycle in vesicles between the growing phagophore and other ill-defined membranous structures [possibly Golgi vesicles; reviewed in (135)]. Two other proteins with similar behaviour are the peripheral membrane protein Atg23 and the integral membrane protein Atg27, but these proteins are only required for efficient autophagy—but are essential for the Cvt pathway (73).

The proteins involved in autophagosome formation (Atg3, Atg4, Atg5, Atg7, Atg8, Atg9, Atg10, Atg12, and Atg16) are fully conserved from yeast to human (82). Of the proteins involved in the efficiency of autophagy, Atg27 is conserved in filamentous fungi, whereas Atg23 is not. Atg8 has been used as a marker for autophagy in many organisms, including filamentous fungi, since it is present on the growing phagophore as well as the completed autophagosome (54, 99, 102). As might be expected, deletion of *atg8* in filamentous fungi inhibits autophagy. This has been observed for *P. anserina* (102) and *Aspergillus oryzae* (58). Further, the *Laccaria bicolor atg8* and *Sordaria macrospora atg7* genes were shown to functionally substitute for their yeast orthologs during autophagy (61, 90). Similarly, deletion of *atg4*, *atg5*, and *atg9* has been demonstrated to affect autophagy in *M. oryzae* (21, 54, 74, 78). *Mo*Atg4 was shown to interact with *Mo*Atg8 and to cleave the carboxy terminus from this protein. Molecular studies on the other fungal proteins involved in autophagosome formation are still lacking. Nevertheless, it can be expected that these proteins function similarly as their yeast (and mammalian) counterparts.

Recycling of autophagosome components

As indicated above, the cycling membrane protein Atg9 is actively sorted to and retrieved from the growing autophagosome. An early report indicated that this cycling might require the activity of Atg1, Atg2, and Atg18 (105). Recent data indicate that localization of Atg9 to the PAS requires both Atg23 and Atg27, which seem to form a cycling complex with Atg9, despite the fact that these proteins are not essential for autophagy (140). Upon autophagy induction, the localization of Atg9 at the PAS depends on a physical interaction with Atg17, which acts as a scaffold for PAS organization (116). This process also requires the protein kinase Atg1. Additionally, for efficient transport of Atg9 to the PAS also the conserved oligomeric Golgi complex is required (141), whose function is to maintain Golgi structure and is thought to tether vesicles to acceptor membranes. Remarkably, the conserved oligomeric Golgi complex also interacts with Atg17, thus providing a direct link with the Atg1 complex.

During completion of the autophagosome, many Atg proteins are released from the double-membrane structure to prevent them from being degraded in the vacuole together with its contents. This includes Atg8 and the Atg5-Atg12/Atg16 complex that are on the outside of the membranous structure. Release of the covalently attached Atg8 protein depends on its cleavage from PE by the protease Atg4. Nevertheless, a significant portion of Atg8 remains trapped inside the vesicle and will be degraded in the vacuole together with the other contents of the autophagosome.

Trafficking of Atg9 has also been studied in *M. oryzae* (21). In this study, the location of a green fluorescent protein (GFP)-Atg9 fusion protein was significantly altered in *atg1*, *atg2*, and *atg18* mutants, suggesting that *Mo*Atg1, *Mo*Atg2, and *Mo*Atg18 are required for *Mo*Atg9 trafficking. In contrast, deletion of *Moatg13* had no effect on GFP-Atg9 location. However, this latter result should be treated with care, since—unlike the other *atg* mutants—the *Moatg13* mutant was hardly disturbed in autophagy-related developmental changes.

Vacuolar degradation and recycling

After completion of the autophagosome, docking and subsequent fusion of its outer membrane with the vacuolar membrane occurs. Since this step utilizes canonical membrane fusion components, for example SNAREs (soluble NSF [N-ethylmaleimide-sensitive fusion protein] attachment protein receptors), *etc.*, these proteins have not received an Atg nomenclature and will not be discussed further [for review, see (95)].

Fusion results in incorporation of a single membrane-bound autophagic body in the vacuolar lumen, where its membrane will be lysed and its contents degraded. Subsequently, the breakdown products are either stored in the vacuole or can be released again into the cytosol. The baker's yeast vacuole contains many hydrolases (*e.g.*, the proteases PrA, PrB, carboxypeptidase S, and carboxypeptidase Y) that assist in the turn-over of autophagosome contents, but so far only two vacuolar proteins have received an Atg nomenclature. The integral membrane protein Atg15 is a lipase presumably involved in lysis of autophagic bodies and related structures resulting from selective autophagy. Atg15 is transported *via* the secretory pathway to the late Golgi and is thought to enter the vacuole lumen *via* the multivesicular body pathway (24, 25). Remarkably, a recent report has implicated *S. cerevisiae* Atg15 as the only autophagy protein involved in life span extension during caloric restriction (128). This might suggest that Atg15 is also involved in lysis of in-

ternalized membrane material not originating from autophagy-related pathways, but from other pathways (*e.g.*, from the endosome). This is confirmed by the observation that also mutants lacking Vam7 or SNAREs had a significantly reduced life span during caloric restriction. Atg22 is an integral vacuolar membrane protein with similarity to permeases of the major facilitator superfamily (125, 139). The function of this putative transporter is far from resolved, but preliminary data imply that it may function in export of regenerated material from the vacuole to the cytosol. Remarkably, in baker's yeast, Atg22 is only required for nonselective macroautophagy, but not for the selective Cvt pathway.

Atg15 is conserved in yeast species and filamentous fungi (82), but so far, this protein has received little attention. In the pathogenic fungus *Trichophyton rubrum*, transcript levels of the *atg15* gene were upregulated when cells were treated with the antifungal agent ketoconazole (145). However, the significance of this finding is unclear. In contrast to most yeast species, filamentous fungi contain multiple Atg22 paralogs, but molecular details as to the location and function of these proteins are lacking (56, 82). Regarding vacuolar proteases, in *P. anserina*, the *idi-6/pspA* gene encodes a serine protease, which is presumed to be the functional counterpart of *S. cerevisiae* PrB involved in the degradation of autophagic bodies. As expected, a *PapspA* mutant was affected in autophagy (102).

Autophagy in Filamentous Fungi

The degradation and recycling of cellular components *via* autophagy plays a vital role in the life-style of filamentous fungi. In most of the species studied so far, autophagy is indispensable for large-scale cellular remodeling during sexual and asexual reproduction, conidial germination, and survival under nutrient depletion conditions. Autophagy is also essential for effective plant infection by *Magnaporthe* and *Colletotrichum* species. Moreover, autophagic degradation of cell components was also observed during cell death by incompatibility in *P. anserina*, suggesting a role for autophagy in this intriguing phenomenon (103).

A remarkable feature of filamentous fungi is their ability to indefinitely grow in a polarized fashion, which facilitates the population of significant areas of substrate. The mycelium of an ascomycetous fungus is composed of interconnected hyphae that are divided by perforated septa into multinucleated compartments, which allows bulk flow of cytosol (and organelles) throughout the hyphae. Moreover, cells are differentiated along the hyphae. Thus, hyphae can be divided into actively growing regions (apical cells), nongrowing metabolically active regions (sub-apical cells), and the oldest region, which is comprised of degenerating, highly vacuolated cells.

In contrast to yeast species, which enter a nondividing resting state in response to starvation, filamentous fungi are able to sustain growth of hyphal apical cells under these conditions. This phenomenon promotes expansion of the colony into areas with new substrates. It was suggested that degradation of cellular components *via* autophagy in mature hyphal compartments allows recycling of building blocks and ensures continuous growth of apical compartments (119). Indeed, inhibition of autophagy *via* disruption of the *atg1* gene in *A. fumigatus* resulted in inhibition of colony growth under starvation conditions, highlighting the physiological importance of autophagy in filamentous fungi in this adaptive response to starvation (106). Metal ions such as magnesium, zinc, manganese, and copper seem to be the major limiting factors of *A. fumigatus* growth under starvation conditions, since supplementation of media with these compounds restored the growth of the autophagy-deficient strain. Conversely, depletion of cations is a sufficient stimulus to induce autophagy in wild-type *A. fumigatus* (106).

The role of autophagy in pathogenicity and pathogen–host interactions

Autophagy is required for generation of appressorium turgor and pathogenesis of *M. oryzae*. Rice blast is the most serious disease of cultivated rice, which is known to occur in >85 countries world-wide (121). It is caused by the ascomycete fungus *Magnaporthe oryzae* [previously known as a *M. grisea* (14)], which infects all aerial parts of the plant, including leaves, stems, and nodes. Since rice is a staple of diet for >3 billion people world-wide (121), the rice blast disease is of great societal and economic significance. Annual losses caused by *M. oryzae* range between 10% and 30% of the rice yields (121).

M. oryzae attacks aerial parts of rice plants *via* formation of an infection structure called appressorium (Fig. 4), which generates the enormous turgor pressure (up to 8 MPa) required for rupture of the plant cuticle (45). The fungus initiates the infection when its three-celled conidium is attached to the leaf surface *via* adhesives, which are released by the apical compartment in the spore (37). Spore germination produces a single, polarized germ tube, which subsequently evolves into the dome-shaped appressorium (137). The cell wall of this infection structure is chitin-rich and contains a distinct layer of melanin on the inner side of the wall. Some studies indicate that melanin may provide an effective means of preventing solute efflux, allowing the appressorium to accumulate glycerol (16, 133), thereby generating high turgor pressure (53). The produced turgor provides a physical force, allowing the formation of a narrow penetration peg that perforates the host cuticle and invades the plant tissue (45).

During the early stages of invasion, *M. oryzae* proliferates and grows within the plant tissue in a symptomless manner. During this phase, specialized fungal feeding structures employed to derive nutrients from living plant cells are formed. Moreover, the hyphae move to adjacent epidermal cells *via* plasmodesmata, highlighting the biotrophic nature of the ultimate stages of rice blast disease. Currently, it is still unknown how rice blast fungus is able to avoid or suppress the plant defense mechanisms. At later stages, the infection become necrotrophic (22).

Appressorium development by *M. oryzae* is tightly coupled to cell cycle regulation. Once the three-celled fungal spore senses the hydrophobic, hard surface of the rice leaf, the mitogen-activated protein kinase signaling (pathogenicity MAP kinase-1) pathway is induced [reviewed in (137)] and a single nucleus migrates into the developing germ tube, where it undergoes mitosis. Subsequently, one of the daughter nuclei resulting from the mitotic event migrates into the nascent appressorium, whereas the other nucleus returns to the conidium. One of the hallmarks of appressorium formation is the extensive degradation of conidial constituents *via* autophagy-related processes that precedes the collapse of the conidium. Inhibition of mitosis by culturing a

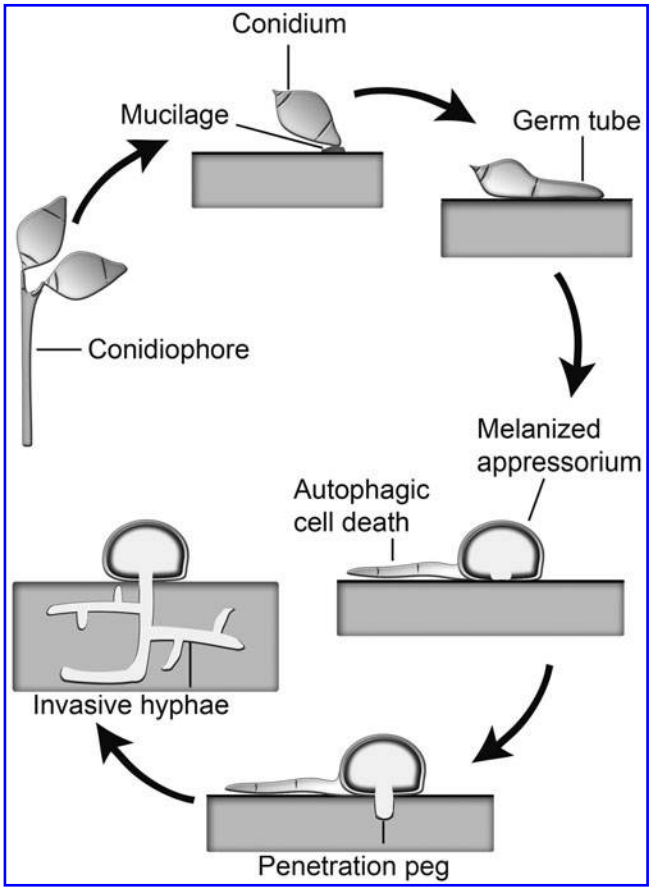

FIG. 4. The infection cycle of *Magnaporthe oryzae*. The infection cycle of *M. oryzae* begins when the three-celled conidium adheres to the host surface using mucilage stored at the spore tip. Subsequently, the conidium germinates producing a narrow germ tube from which the appressorium evolves. Maturation of the appressorium is accompanied by the collapse and autophagy-dependent death of the conidium. At this stage the appressorium becomes melanized and an enormous turgor is generated, which provides a physical force allowing formation of a narrow penetration peg at the base that punctures the host cuticle and invades the plant tissue.

temperature-sensitive *M. oryzae nimA* mutant at nonpermissive temperatures also prevented degeneration of the conidial nuclei and the collapse of the conidium (132). The exact molecular mechanism linking surface perception to induction of the pathogenicity MAP kinase-1 cascade and infection-associated autophagy is unknown and remains to be elucidated.

M. oryzae autophagy-deficient mutants develop appressoria that do not have the ability to penetrate the plant cuticle and infect the plant tissue (54, 75, 78, 132). Also, the deterioration and further collapse of the conidium is arrested in these *atg* mutants, suggesting that autophagic cell death of the conidium is required for the initiation of rice blast disease (132). The loss of pathogenecity of *Moatg* mutants is probably caused by a lowered appressorium turgor (75, 78). Normally, hydrostatic turgor is generated in these structures by accumulation of high concentrations of glycerol (16). Due to the absence of available external nutrients, this phenomenon is solely dependent on turnover of endogenous storage compounds, such as mannitol, glycogen, lipids, and trehalose (32). Lipid bodies are mobilized and degraded in appressorial vacuoles (136), thus liberating glycerol from the fatty acid moieties. Moreover, β-oxidation of fatty acids, released during lipolysis, plays an essential role in appressorium formation, since it provides acetyl-coenzyme A (104, 134), which is a precursor of melanin and polyketides. In addition to this, autophagy is also involved in the control of the storage compound stocks during conidiation. Conidia of *Moatg* mutants are characterized by the presence of significantly reduced amounts of storage materials such as lipid droplets required for generation of turgor in the appressorium (75, 78). As a consequence, impairment of autophagy, necessary for recycling endogenous nutrient sources, results in a disability to accumulate the high concentrations of compatible solutes with concomitant loss of pathogenicity.

Genome-wide functional analysis in *M. oryzae* has revealed that the molecular machinery involved in nonselective bulk autophagy is essential for appressorium functioning. In contrast, the *atg* genes thought to be involved exclusively in selective forms of autophagy (*i.e.*, Mo*atg11*, Mo*atg24*, Mo*atg26*, Mo*atg27*, Mo*atg28*, and Mo*atg29*) are superfluous for appressorium-mediated plant infection (54). However, this may not be a fully conserved feature. Recently, it was suggested that pexophagy might be required for host invasion by the cucumber anthracnose fungus *Colletotrichum orbiculare* (4). Deletion of the fungal *atg26* ortholog, which encodes a sterol glucosyltransferase that enhances pexophagy in the methylotrophic yeast *P. pastoris* (93), specifically affected host invasion, although formation of appressoria was preserved. The *Coatg8* mutant, defective in all autophagy-related pathways, does not form normal appressoria (4), suggesting distinct physiological functions for autophagy in the phytopathogenicity of this filamentous fungus. Although species from the *Colletotrichum* genus are plant pathogens closely related to *M. oryzae* that share similar lifestyle and infection strategies, it seems that these fungi employ different mechanisms of appressorium development and function (88).

Distinct roles of autophagy in pathogenesis of opportunistic human fungi. Fungal pathogen adaptation to the host environment requires dynamic changes involving large-scale alternations in protein expression and remodeling of the cellular architecture (33, 117, 122). These energetically costly changes in cell physiology might be facilitated *via* autophagy. Indeed, it was demonstrated that the opportunistic human fungal pathogen, *Cryptococcus neoformans*, requires autophagy during infection (46). This basidiomycete yeast is predominantly an intracellular pathogen residing within host macrophages, which provides a means to propagate from the primary sites of infection within the lungs to the central nervous system (12, 30, 79). On the other hand, this cellular niche is a source of nutrient stress for the fungal pathogen, and genes encoding proteins involved in autophagy-related processes are upregulated during residence of *C. neoformans* in macrophages (27). Strong evidence showing the significance of autophagy for *C. neoformans* during infection was provided by studies of a mutant lacking PI3-K (*Cn*Vps34) activity and RNAi suppression of *Cnatg8* (46).

A. fumigatus is a saprophyte growing on dead matter in the environment, which can cause life-threatening infections in

populations of immunologically suppressed individuals. Depending on the immune status of the host, *A. fumigatus* may provoke allergic bronchopulmonary aspergillosis, noninvasive aspergillomas, and invasive aspergillosis, of which the latter is the most devastating of *Aspergillus*-related diseases (72). Individuals with hematological malignancies such as leukemia, allogeneic bone marrow transplants, genetic immunodeficiency such as chronic granulomatous disease, or individuals infected with HIV are most at risk of invasive aspergillosis (20). Several lines of evidence suggest that in the host environment *A. fumigatus* is also subjected to nutrient stress (41, 42, 67, 115). Although autophagy is essential for the virulence of *C. neoformans*, this process appears to be dispensable for the pathogenicity of *A. fumigatus*. An *atg1* deletion strain of *A. fumigatus* was hampered in starvation-associated processes, including conidiation and starvation-induced foraging of hyphal tips, but remained fully virulent in a neutropenic mouse model of invasive aspergillosis (106).

The varying demands of autophagy may represent a unique interaction of each fungal pathogen with the host as well as a different physiology of growth. In contrast to *C. neoformans*, *A. fumigatus* occupies predominantly the extracellular environment in an immunocompromised host. The primary ecological niche of *A. fumigatus* is dead and decaying matter, which requires the ability to secrete hydrolyzing enzymes to acquire nutrients (17). This capability may also be beneficial for the survival of *A. fumigatus* in mammalian tissue without a need to induce autophagy. Moreover, *C. neoformans* spreads through host tissue in its yeast obligatory form with limited directional growth. Therefore, during the colonization of mammalian tissue this fungal pathogen may be more prone to localized nutrient exhaustion, where autophagy may be required for survival. In contrast, the filamentous fungus *A. fumigatus* can take advantage of its polarized growth mechanism, which might easier facilitate nutrient acquisition in the human host.

Autophagy is also dispensable for virulence of the yeast *Candida albicans*, another human fungal pathogen. Disruption of the Ca*atg9* gene resulted in impairment of autophagy, but this mutant remained fully virulent in a mouse intravenous model of disseminated candidiasis (96). In contrast to *C. neoformans*, that propagates exclusively as a yeast in host tissue, *C. albicans* transit between yeast and hyphal forms. Surprisingly, the autophagy-deficient *C. albicans* strain had no defect in yeast-hypha differentiation (96). Thus, similar to *A. fumigatus*, this fungal pathogen may take advantage of polarized growth during infection. The differences in requirement of autophagy in the virulence of these fungal pathogens reflect the uniqueness of lifestyle and adaption strategies to their host niches.

The role of autophagy in programmed cell death during the incompatibility reaction

The formation of a complex interconnected network of hyphae is a remarkable feature of the growth of filamentous fungi and relies on extension of apical cells, branching, and hyphal fusion. The growing filamentous fungal colony faces in its natural environment many other microorganisms, including bacteria and fungal colonies of the same and different species. When the colony is confronted with another species of filamentous fungi, it generates high amounts of hydrogen peroxide that may cause the death of the contestant hyphae (120). Once the fungal colony encounters isolates of the same species, a spontaneous, vegetative cell fusion may occur. If these strains bear a genetic difference at specific loci (termed the *het* or *vic* loci), a cell death reaction is triggered in the fusion cell. This phenomenon is known as vegetative, somatic, or heterokaryon incompatibility (36, 111).

Vegetative incompatibility is widespread among filamentous fungi and has been mainly investigated in *Cryphonectria parasita*, *Neurospora crassa*, and *P. anserina* (77). The biological significance of this phenomenon is still under debate. Heterokaryon incompatibility may be a manifestation of a nonself recognition system in filamentous fungi that preserves the biological integrity of these organisms, preventing chimera formation. Moreover, vegetative incompatibility may limit the horizontal transfer of deleterious genetic elements like mycoviruses, senescence plasmids, or transposons (3, 9). Thus, this phenomenon may be considered as a defense mechanism, which prevents propagation of infectious elements and utilization by aggressive genotypes. An alternative hypothesis refers to somatic incompatibility as a consequence of divergent evolution of strains (6). In this perspective *het* genes simply represent genes with accumulated polymorphism in wild-type populations, leading to the presence of allelic variants. The simultaneous appearance of these variants in the same cytoplasm may be harmful for the cell and trigger a cell death reaction.

The heterokaryon incompatibility results from the interaction of different proteins encoded by incompatible *het* genes. Heterocomplex formation may occur between Het proteins encoded by a single locus (allelic system) or two *het* genes from distinct loci (nonallelic system). So far, 10 different *het* loci have been described. Heteroallelism at one of these specific loci is sufficient to provoke cell death. Surprisingly, *het* genes are different among fungal species and encode diverse proteins such as transcription factors, enzymes, and signaling proteins. In many cases the proteins encoded by the *het* genes display other cellular roles in addition to their function in heterokaryon incompatibility (111).

P. anserina displays eight *het* systems and four of them have been characterized (*het*-c, *het*-d, *het*-e, and *het*-s). They represent the nonallelic *het*-c/*het*-d, *het*-c/*het*-e (26, 109, 110) and allelic *het*-s/*het*-S (15) incompatibility systems. In this filamentous fungus, self-incompatible strains—homokaryotic strains bearing incompatible *het* genes in their nuclei—have been successfully employed as a tool to characterize the mechanism of vegetative incompatibility (71). Once the vegetative incompatibility is induced *via* transfer to the restrictive temperature, fungal cells rapidly undergo morphological changes, including vacuolization, increased septation, accumulation of lipid droplets, and abnormal deposition of cell wall material, which together precede cell death (18). At the molecular level, during cell death by incompatibility, an induction of specific *idi* genes (induced during incompatibility) was observed. So far, six of the *idi* genes have been characterized. The *idi*-6 gene was shown to encode a vacuolar protease, PspA, which is required for autophagy-dependent degradation of cellular constituents (97). Additionally, The *idi-7* gene encoded the *P. anserina* Atg8 ortholog. The upregulation of both *idi-6*/*pspA* and

idi-7/atg8 during vegetative incompatibility suggest that autophagy is induced under these conditions (102). Indeed, cytological evidence of autophagy induction was obtained, including accumulation of double membrane-bound autophagosomes and the presence of autophagic bodies inside the vacuolar lumen. The strong activation of autophagy was also illustrated using a *Pa*Atg8.GFP fusion protein, which relocalized from the cytoplasm to the autophagosome membrane during the development of the incompatibility reaction (102). As might be expected, the *idi* genes were also induced under nitrogen starvation and during treatment with rapamycin, a specific inhibitor of the Tor kinase. Moreover, treatment of cells with rapamycin appeared to mimic the incompatibility reaction in *P. anserina* (18).

The presence of autophagic structures in dying cells during vegetative incompatibility indicates that this cell death mechanism displays the features of programmed cell death type II (nonapoptotic). Despite the fact that autophagy was for the first time observed in dying insect cells in the 1960s (112), the relationship between autophagy and cell death is still unclear and controversial (64, 68). However, many studies indicate that induction of large-scale autophagy in dying cells is a pro-survival response and not the cause of cell death. On the other hand, there are also indications that under some circumstances autophagy can facilitate cell death. In *Drosophila melanogaster* autophagic cell death is responsible for clearance of the salivary gland during development (8). Additionally, induction of autophagy in *M. oryzae* conidia leads to conidial cell death, which is required for plant infection (132). The appearance of autophagic structures in dying cells during vegetative incompatibility raises the question what the role is of autophagy in this cell death reaction. *P. anserina* autophagy-deficient mutants lacking Atg1 or Atg8 showed accelerated cell death during the incompatibility reaction. This implies that the strong induction of autophagy observed under these conditions is a survival response (99). It has been suggested that autophagy is induced to restrict programmed cell death by limitation of cytotoxic signals disseminating to adjacent hyphal compartments (99, 101). This assumption was based on the role of autophagy during the hypersensitive response in plants (76). However, recent data indicate that the pro-survival role of autophagy during the plant innate immune response is far from resolved (43). Clearly, more detailed studies are required to confirm the pro-survival role of autophagy in the incompatibility reaction in *P. anserina*.

The role of autophagy in cellular differentiation

The vegetative life cycle of filamentous fungi is initiated by germination of a spore. This process is followed by formation of tubular hyphae that grow in a polar manner by extension of apical cells, coupled with branching of sub-apical hyphal compartments. Extensive growth of filamentous fungi results in formation of the highly complicated network of cytoplasmically connected cells, called the mycelium. At a certain stage of vegetative growth, fungal hyphae undergo drastic, energy-consuming changes that are required for spore production. This phenomenon guarantees reproduction and the survival of the species. Because of the energy-dependent nature of sporulation, the formation of spores is tightly regulated by both endogenous and environmental factors. Depending on the origin of development, the fungal spore can have an asexual character, arising from mitotical divisions (conidiospore) or can be produced during sexual meiotic processes (ascospore). Some species of filamentous fungi reproduce exclusively asexually or sexually.

The effective induction of the sporulation process in filamentous fungi requires appropriate environmental conditions; for example, many filamentous fungi sporulate upon exposure to air. The molecular mechanism of water–air interface induction of sporulation is unknown. However, it was suggested that the intracellular concentration of reactive oxygen species, which increases upon exposure of mycelium to air, could be one of the crucial elements of sporulation induction (23, 84, 98, 144). Initiation of sporulation in filamentous fungi is often light dependent, for example, strongly dependent on blue light in the basidiomycete *Coprinus cinereus* (55) and on red light in *A. nidulans* (86). One of the major factors regulating the process of sporulation in filamentous fungi is the nutritional status of the cells (1). Depletion of nutrients (carbon or nitrogen) is a well-known external factor that induces formation of spores. This nutritional dependence of sporulation suggests a possible involvement of autophagy-related processes in cellular remodeling preceding spore formation. Even favorable environmental conditions are not sufficient for induction of sporulation if the fungus is not competent for development (5). This suggests that the process of sporulation is tightly controlled on the genetic level, although simultaneously tightly regulated by environmental cues.

The significance of autophagy in asexual sporulation. The significance of autophagy for the efficiency of sporulation was demonstrated for species from the *Aspergillus* and *Magnaporthe* genera. Asexual development begins in *Aspergilli* with nondifferentiated hyphae that form an initial structure called the foot (Fig. 5). This structure is the basis for the development of a stalk that vigorously grows and of which the apical part subsequently swells, forming a vesicle-like structure. In the next step a number of cells called metulae are formed from the swollen tip of the hyphal stalk. Each metulae cell is then the source of two to three conidiophore-producing cells, called the phialides. Conidiophores generated by the phialides are organized in long chains that upon environmental conditions can separate and effectively spread.

It was demonstrated that autophagy is induced during conidiation and conidiophore development in *A. oryzae*. This observation implied that autophagy is involved in the sporulation process, which was confirmed by disruption of *Aoatg8*. The autophagy-deficient strain was strongly affected in formation of aerial hyphae and conidiation (58). The importance of autophagy during asexual development was also shown for *A. fumigatus*. An *Afatg1*-deficient mutant strain had a hampered ability of starvation-induced sporulation. It was demonstrated that inhibition of conidiation was associated with the appearance of attenuated conidiophores with abnormal phialides. Both the number of produced conidia and the typical morphology of conidiophores could be restored by supplementation of medium with a nitrogen source (106). These data suggest that starvation-associated sporulation relies on autophagy to provide sufficient nitrogen source for

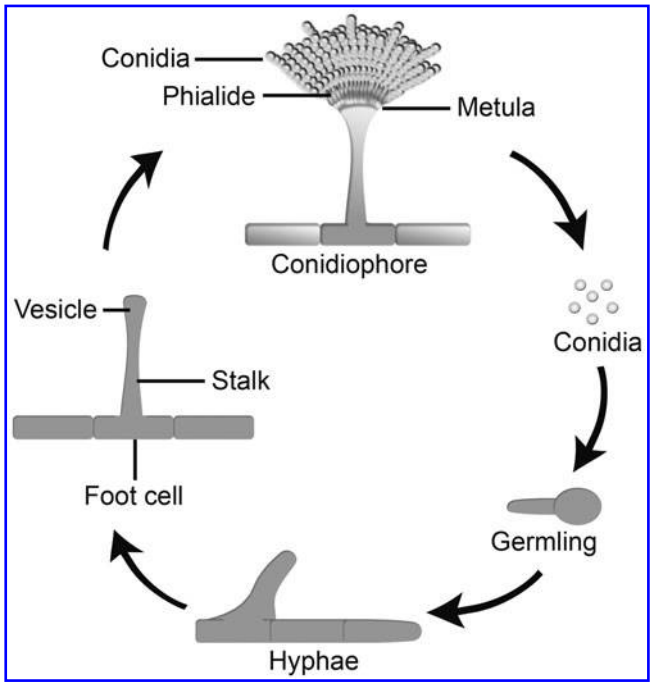

FIG. 5. Asexual sporulation in *Aspergillus* species. For description see text.

conidiophore development. Autophagy may also be critical for the storage of the nitrogen source inside conidia during their development since an exogenous source of nitrogen is not required for germination of conidia of filamentous fungi (94, 114). Moreover, it was demonstrated for *A. oryzae* that autophagy is actually induced during conidial germination. The *Aoatg8* mutant was significantly delayed in conidial germination under nitrogen starvation, suggesting that autophagy may function during the early stages of conidial germination in *A. oryzae*.

A similar function of autophagy during asexual spore development was demonstrated for *M. oryzae*. In this phytopathogenic fungus asexual development begins when the aerial hyphae produce a short stalk from which a proconidium is produced by budding of a single cell. After maturation of the primary budded cell the septa are formed, resulting in production of a three-celled conidium (cf. Fig. 4). It was demonstrated that both the *atg8* gene and autophagy are significantly induced during asexual spore development in *M. oryzae* (19). Moreover, inhibition of autophagy by disruption of *Moatg1*, *Moatg5*, or *Moatg8* affected formation of aerial hyphae and conidiation in this fungus (19, 75, 78). Remarkably, the negative effect of autophagy impairment on asexual reproduction in *M. oryzae* could be significantly restored upon supplementation with an alternative carbon source (glucose or sucrose) or glucose-6-phosphate. Additionally, deletion of *gph1*, encoding a glycogen phosphorylase that catalyzes glycogen breakdown in the cytosol, in the *Moatg8* background had a similar positive effect on conidiation, but this observation is not well understood (19).

Together, these data suggest that asexual sporulation in filamentous fungi induced by sensing the nutritional state of cells relies on autophagy. Under starvation conditions autophagy can directly participate in cell remodeling and provide sufficient amounts of building blocks for conidiophore development and subsequent conidia formation.

Significance of autophagy in sexual reproduction. The importance of autophagy in sexual reproduction was first described for the fungus *P. anserina*, which exclusively produces sexual ascospores as a resistant form (Fig. 6). The sexual cycle of *P. anserina* is induced by starvation and light exposition and begins with differentiation of vegetative hyphae into protoperithecia and microconidia, the female and male sexual reproductive structures, respectively. Fusion of a microconidium and the apical receptive element of a protoperithecium (called the trichogyne) of opposite mating types results in fertilization of the female reproductive structure. This event initiates the development of the fruiting body in which ascospores are ultimately formed [reviewed in (13)].

The impact of autophagy on sexual reproduction in *P. anserina* was studied *via* disruption of selected autophagy-related genes. Impairment of autophagy in this filamentous fungus caused by deletion of *Paidi-7/Paatg8* or *Paatg1* hampered the ability to generate aerial hyphae and to produce pigment. Moreover, it was demonstrated that these autophagy-deficient strains fail to differentiate protoperithecia and are female sterile (99, 102). Interestingly, *P. anserina* autophagy-deficient strains are still male fertile (102). Disruption of the *Paidi-6/pspA* gene, encoding the ortholog of the *S. cerevisiae* vacuolar protease PrB, displayed a less severe phenotype. The *Paidi-6/pspA* mutant strain produced very few protoperithecia, which after fertilization produced fruiting bodies without asci, suggesting that fertilization occurs but further development of fruiting bodies is blocked. The mutant strain also displayed other phenotypic features of autophagy-deficient strains, including impairment of development of aerial hyphae and pigmentation (102).

Together, these data highlight the essential function of autophagy during sexual differentiation in *P. anserina*. The entry into the sexual cycle occurs when mycelium in the stationary phase is subjected to nutrient exhaustion. Therefore, sexual differentiation in *P. anserina*, which requires massive remodeling of the cellular architecture, must rely on endogenous amino acids pools. Since recycling of cellular building blocks under starvation conditions is mediated *via* autophagy, the requirement of this catabolic process for development of female reproductive organs and ascospores seems to be straightforward.

The importance of autophagy for sexual differentiation was also demonstrated for *M. oryzae* (75, 78). Sexual reproduction in *M. oryzae* occurs when two hyphae of opposite mating type meet and fuse forming a perithecium in which ascospores develop. Exhaustion of nitrogen source may promote this phenomenon. It was demonstrated that autophagy is involved in formation of the perithecium. *M. oryzae* autophagy-deficient strains were found to produce fewer perithecia with fertile ascospores. Moreover, sexual differentiation was significantly delayed in these *atg* mutants (78).

Conclusions and Perspectives

During two decades, molecular studies of autophagy have focussed on *S. cerevisiae* as the major model species. This has been pivotal in identifying most of the *ATG* genes involved in

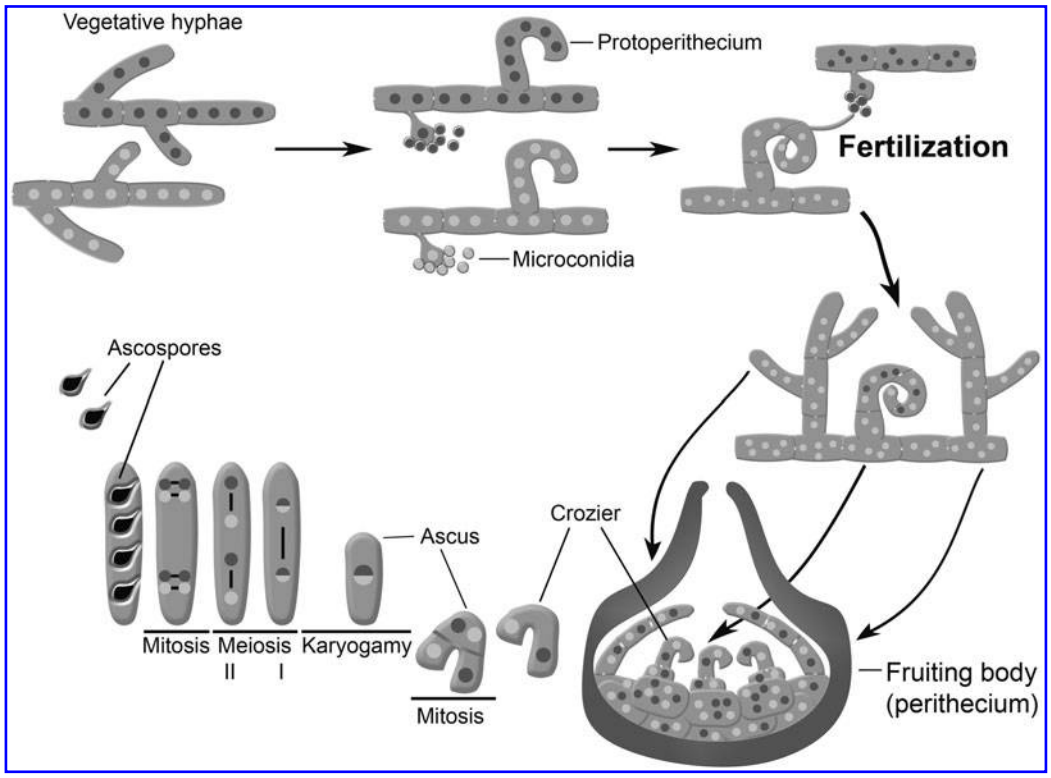

FIG. 6. Sexual reproduction of *Podospora anserina*. Sexual reproduction in *P. anserina* is initiated when the male (microconidia) and female (protoperithecia) sexual reproductive structures differentiate from vegetative hyphae under starvation and light exposure. Fusion of the microconidium with the apical receptive element of a protoperithecium of an opposite mating type results in fertilization of the female reproductive structure and initiation of fruiting body development. After fertilization, the fusion of the male and female nuclei is postponed. Each nucleus divides in plurinucleate cells. Subsequently, pairs of parental nuclei of opposite mating type migrate into specialized structures, termed ascogenous hyphae, from which the so-called crozier cells are formed. In the crozier, nuclei undergo mitosis, which results—after septum formation—in the generation of two monokaryotic basal and lateral cells and one dikaryotic cell that can differentiate into an ascus mother cell in which karyogamy takes place. Subsequently, the diploid nucleus undergoes meiosis followed by postmeiotic mitosis whereupon finally the formation of ascospores occurs.

macroautophagy and the many related, selective processes. The last years, studies on the molecular details of autophagy in mammalian cells have demonstrated that many of the ideas developed in baker's yeast may also be valid in higher eukaryotes. However, also significant differences have been observed, especially during the initial stages of autophagy (*i.e.*, the regulation of the Tor, PI3-K and Atg1 complexes). *In silico* analyses of the putative Atg orthologs in filamentous fungi have demonstrated that autophagy in these species may exhibit features from both yeast and mammals (*e.g.*, lack of Atg31 and presence of Atg101). This implies that for a comprehensive understanding of the functioning of autophagy in filamentous fungi, detailed molecular studies such as those performed in baker's yeast and human should be undertaken. This is all the more important considering the importance of filamentous fungi in medicine, science, agriculture, and industry. Detailed knowledge of autophagy in filamentous fungi may thus not only help in a better understanding of the evolution of this recycling pathway, but will also provide a more rational basis for the design of improved industrial strains [producing secondary metabolites, secreted enzymes, *etc*. (146)] and antifungal drugs.

Already from a limited number of studies conducted on a few species of filamentous fungi, it has become clear that autophagy is essential not only for survival under nutrient-limiting conditions, but also for many developmental processes. Most of these developmental processes are triggered by these nutrient-limiting conditions, suggesting that autophagy is really an indispensible part of the fungal life-style. Thus, studying the details of the autophagy–development relationship should remain a priority. What so far has not been addressed is a possible housekeeping role for autophagy, that is, the continuous removal of damaged proteins and organelles, thereby affording protection against oxidative stress. It remains a possibility that part of the observed impact of autophagy deficiency on developmental processes in filamentous fungi is the result of an increased oxidative stress, accumulation of damaged proteins and organelles, *etc*. Recently, we observed that in hyphae of a *Penicillium chrysogenum* autophagy-deficient mutant, reactive oxygen species levels were significantly increased compared with wild-type hyphae (M. Bartoszewska *et al*., unpublished data). Thus, even at nutrient excess conditions, autophagy may play an essential function in filamentous fungi.

Autophagy studies in filamentous fungi have so far concentrated on macroautophagy, the random uptake of cytoplasmic components by the vacuole. Nevertheless, in

baker's yeast and mammalian cells, also highly selective forms of autophagy have been uncovered, which require specific proteins for cargo recognition to assist in attachment to the macroautophagy machinery. These receptor proteins are usually very weakly conserved, and appear to be absent in filamentous fungi. Remarkably, the Atg11 protein that in yeast species is required for selective autophagy-related processes (*e.g.*, Cvt pathway, pexophagy, and mitophagy) is conserved in all fungi, implying that some sort of selective autophagy may be present in these species. Thus, the identification of Atg11-interacting proteins may be a promising venue to shed more light on this hitherto unexplored facet of autophagy in filamentous fungi.

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Abbreviations Used

Cvt = cytoplasm to vacuole targeting
ER = endoplasmic reticulum
GFP = green fluorescent protein
PAS = preautophagosomal structure/phagophore assembly site
PE = phosphatidylethanolamine
PI3-K = phosphatidylinositol 3-kinase
PrA = proteinase A
PrB = proteinase B
Tor = target of rapamycin
TORC1 = yeast Tor complex 1
UVRAG = ultraviolet radiation resistance-associated gene
Vps = vacuolar protein sorting

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