Review

Multifunctional Roles of Autophagy Related Genes in Fungi

Aron Osakina , William J. Steinbach , Praveen R. Juvvadi *

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Multifunctional Roles of Autophagy Related Genes in Fungi

Aron Osakina, William J. Steinbach and Praveen R. Juvvadi *

Division of Pediatric Infectious Diseases, Department of Pediatrics, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR 72202, USA

* Correspondence: prjuvvadi@uams.edu

Abstract

Autophagy, also referred to as the "self-eating machinery", is a crucial process where organisms maintain intracellular homeostasis through recycling or degrading non-essential and damaged cellular components, especially during starvation conditions. Autophagy is important in numerous biological functions such as cellular differentiation, aging, nutrient sensing, stress response, tissue homeostasis, immunity, and programmed cell death. To induce autophagy, a double-layered membrane structure called "autophagosome" wraps damaged organelles or proteins and transports them to the vacuole or lysosome for degradation. Autophagy is beneficial to organisms, and it should be optimally regulated because elevated or decreased levels are detrimental for survival. To date more than 40 autophagy-related genes (ATGs) have been identified in the budding yeast *Saccharomyces cerevisiae*, with most having homologs in fungi and higher eukaryotes. Majority of the ATGs in industrial and pathogenic fungal species have been characterized and known to play vital roles in growth, development, and virulence. In this review we provide a comprehensive overview of ATGs in various fungal species and highlight how autophagy is regulated and controls various functions in plant, human, and industrial fungal species.

Keywords: fungi; autophagy; ATGs; autophagosome; mitophagy; golgiphagy; pexophagy; antifungals; TOR; drug resistance

1. Introduction

Autophagy is an evolutionarily conserved process where cytosolic material including organelles and autophagy-related proteins are sequestered by structures called "autophagosomes" and subsequently delivered to vacuoles as autophagic bodies [1]. Autophagy may be broadly classified into non-selective and selective autophagy based on the type of cellular material that is directed for degradation. Non-selective autophagy also called cargo-independent process is induced under starvation conditions to recycle bulk cytosolic materials [2]. On the other hand, selective autophagy also referred to as cargo-dependent autophagy is independent of starvation to selectively degrade and recycle damaged organelles or proteins and maintain intracellular homoeostasis [3]. While non-selective and selective autophagy processes utilize the same mechanism to form autophagosomes, the latter relies on specific autophagic factors that recognize cargos and membrane components.

In the budding yeast, *Saccharomyces cerevisiae*, autophagy has been extensively studied and occurs either non-selectively or selectively with at least 40 autophagy-related genes (ATGs) [4]. Proteins related to autophagy from the plant and the human fungal pathogens, and other industrially useful fungi are summarized in Table 1 and Table 2. Briefly, in selective autophagy process the autophagosome formation occurs via the assembly of a pre-autophagosomal structure (PAS), where organelles such as mitochondria (mitophagy), nuclei (nucleophagy), peroxisomes (pexophagy), are encapsulated by a double membrane envelope derived from the endoplasmic reticulum (ER) (Figure 1). Subsequently, the autophagosomes fuse with the vacuoles and autophagic degradation is mediated by vacuolar hydrolytic enzymes [5]. After the membranes of autophagic bodies are broken

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down by hydrolytic enzymes, degraded products are channeled back to the cytoplasm for reuse in metabolic and biosynthetic pathways [6]. ATG genes that mediate non-selective and selective autophagy are classified into various groups based on their functions in the formation of phagophores and their maturation into autophagosomes [7]. This group includes: the Atg1/ULK complex which comprises Atg1, Atg11, Atg13, Atg17, Atg29, and Atg31, whose function is to scaffold essential sites required for autophagosome initiation at the phagophore. The second group is vital for phagophore expansion, and is a membrane delivery system including Atg2, Atg9, and Atg18. The third group is mainly for vesicle nucleation and is part of the phosphatidylinositol 3-kinase complex (Vps34, Vps15, Vps30/Atg6, and Atg14). Finally, the vesicle expansion group is a ubiquitin-like conjugation system including Atg5, Atg7, Atg10, Atg12, and Atg16.

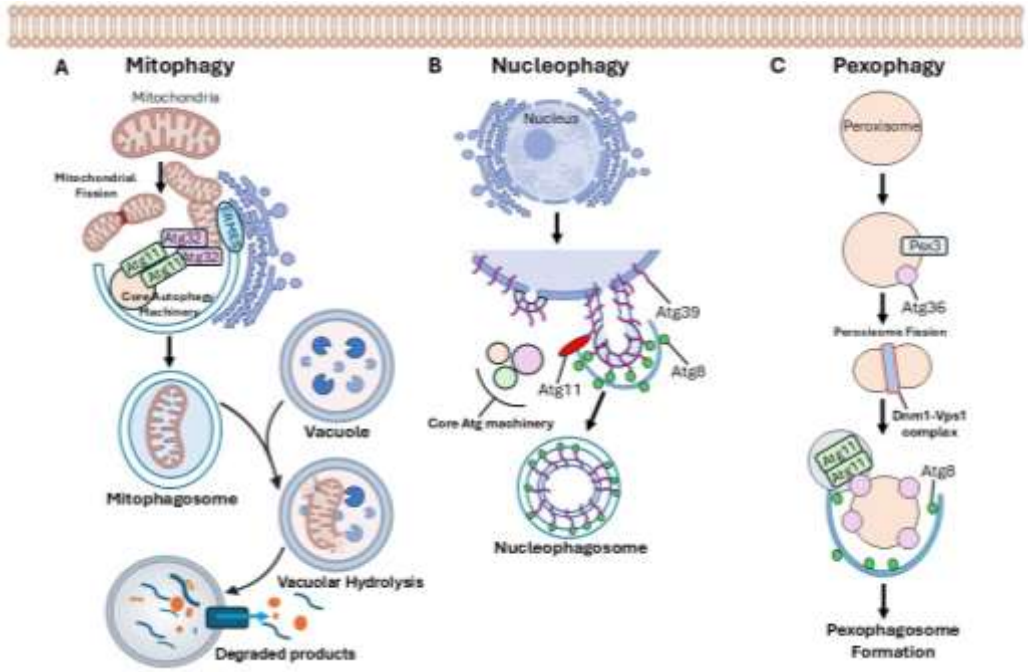

Figure 1. (A) Following mitochondrial fission, the mitophagy transmembrane receptor Atg32 localized on the mitochondrial outer membrane interacts with Atg11. Atg11 tethers the mitochondria to the PAS for selective sequestration of mitochondria. The endoplasmic reticulum-mitochondria encounter structure (ERMES) complex acts as a tether between the ER and mitochondria. Extension of the isolation membrane leads to mitophagosome formation and fusion with the vacuole for degradation by vacuolar hydrolases. The degraded products are released for recycling of cellular material. **(B)** During nucleophagy Atg39 receptor interacts with Atg8 through an Atg8 interacting motif in its N-terminal cytosolic tail and upon nucleophagy induction it binds to Atg8 puncta to form the nucleophagosome destined for degradation of nuclear material. **(C)** In pexophagy process the peroxisomal membrane protein Pex3 acts as a peroxisomal ligand and recruits the Atg36 receptor protein to the peroxisomal membrane. Atg36 binds to Atg11 and recruits peroxisomal fission complexes containing the dynamin-related GTPases Dnm1 and Vps1 to target peroxisomes, thereby facilitating their sequestration by phagophores. Following pexophagosome formation, fusion with the vacuolar membrane releases peroxisomes into the vacuolar lumen for degradation.

1.1. Selective Autophagy Mechanisms in Saccharomyces Cerevisiae

In *S. cerevisiae* the cytoplasm-to-vacuole (Cvt) pathway transports hydrolases, specifically aminopeptidase I (Ape1), from the cytoplasm to the vacuole as part of the selective autophagy mechanism. The Ape1 complex binds to Atg19 receptor to form the Cvt complex, and Atg19 receptor interacts with the adaptor protein Atg11. Atg11 accumulates on PAS by interacting with Atg1 and Atg9. During mitophagy induction the transmembrane mitophagy receptor Atg32 localized on the mitochondrial outer membrane [8–10] interacts with Atg11. Atg11 tethers the mitochondria to the

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PAS for the selective sequestration of the mitochondria by the isolation membrane (Figure 1A). The endoplasmic reticulum-mitochondria encounter structure (ERMES) complex acts as a physical tether between the ER and mitochondria, and aids in expanding the isolation membrane to provide lipid sources from ER and extension of the isolation membrane leading to mitophagosome formation and its eventual fusion with the vacuole for degradation by vacuolar hydrolases.

While the factors participating in the autophagic degradation of nuclear components in other organisms remain largely unknown, *S. cerevisiae* has a unique nucleophagy transmembrane receptor protein, Atg39, localized on the nuclear envelope [11] (Figure 1B). Atg39 interacts with Atg8 through an Atg8-interacting motif in its N-terminal cytosolic tail and upon nucleophagy induction it binds to Atg8 puncta in an Atg8-dependent manner to form the nucleophagosome [12]. While Atg39 is essential for the selective autophagy of proteins within the outer and inner nuclear membrane, nucleolus, and nucleoplasm [11,12], it is not required for the autophagic degradation of nuclear pore components [13–15]. Instead, recent studies have identified nucleoporin Nup159 as an Atg8-binding protein that promotes the autophagic degradation of nuclear pore components [13,14].

The crucial importance of pexophagy in degrading impaired peroxisomes is exemplified by the fact that peroxisomes maintain cellular homeostasis in response to oxidative stress, and play major role in lipid metabolism, including fatty acid β-oxidation [16,17]. In *S. cerevisiae*, the peroxisomal membrane protein Pex3 acts as a peroxisomal ligand and is central to initiating pexophagy by facilitating the recruitment of the Atg36 receptor protein to the peroxisomal membrane (Figure. 1C). Atg36 binds to the adaptor protein Atg11 and recruits peroxisomal fission complexes containing the dynamin-related GTPases Dnm1 and Vps1 to target peroxisomes, thereby facilitating their sequestration by phagophores [18].

In addition to the above established canonical selective autophagic pathways, a Golgi membrane-dependent intracellular proteolytic process called the Golgi membrane-associated degradation (GOMED) pathway has also been reported [19]. Previously, GOMED was classified as alternative autophagy due to its morphological and functional similarities with canonical autophagy. However, recent studies have shown differences between the two processes in terms of the protein machinery involved and the degraded substrates and their biological roles [20].

1.2. Autophagy Genes Regulate Multiple Processes in Plant Pathogenic Fungi

1.2.1. Magnaporthe Oryzae

Magnaporthe oryzae is a hemibiotrophic ascomycetes filamentous fungus that causes blast disease in rice and other cereals [21]. Autophagy in the rice blast fungus *M. oryzae* is well documented and has been shown to control various cellular processes including, fungal growth, conidiation, appressoria formation, and pathogenesis. *M. oryzae* contains a total of 23 autophagy (ATG) genes that are involved in selective and non-selective autophagy and have been identified through genome wide comparison analysis [22]. Loss of function of genes involved in non-selective autophagy such as *MoAtg1*, *MoAtg2*, *MoAtg3*, *MoAtg4*, *MoAtg5*, *MoAtg6*, *MoAtg7*, *MoAtg8*, *MoAtg9*, *MoAtg10*, *MoAtg12*, *MoAtg13*, *MoAtg15*, *MoAtg16*, *MoAtg17*, and *MoAtg18* resulted in almost all of the mutant strains showing defect in host plant penetration and were nonpathogenic with the exception of Δ*MoAtg13* and Δ*MoAtg18* mutant strains that still caused some disease lesions although fewer than the wild-type strains. The loss of virulence in most of these mutant strains was mainly attributed to defects in appressorium function and failure of the mutant strains to colonize the host tissue, indicating a crucial role for autophagy in regulating these processes [22]. In contrast majority of the selective autophagy mutant strains Δ*MoAtg11*, Δ*MoAtg24*, Δ*MoAtg26*, Δ*MoAtg27*, Δ*MoAtg28*, and Δ*MoAtg29* sporulated and caused disease in the host plants [22]. In fact, due to a blockade in the autophagy process in the Δ*MoAtg1* mutant strain, there were fewer lipid droplets present in its conidia which resulted in slower germination rates when compared to the wild-type strain. Also, Δ*MoAtg1* mutant appressorium turgor pressure was lower and as a result the appressorium failed to penetrate and cause disease on rice and barley plants [23]. An *Atg5* deficient Δ*MoAtg5* mutant strain

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exhibited morphogenetic defects including stunted aerial hyphae, shortened lifespan, and impaired disease-causing processes including attenuated conidiation, delayed conidial germination, and defects in the formation of perithecia, appressoria, and penetration peg [24] (Figure 2). Similarly, the deletion of *MoAtg4* drastically impaired aerial hyphae formation, sporulation, delayed conidial germination, perithecia formation, and appressorium formation, reduced appressorium turgor pressure, all resulting in its inability to penetrate the host plant tissue and cause infection [25]. *MoAtg8* was highly expressed at conidiation stage with *MoAtg8* mutant dramatically attenuated in sporulation [26]. Other studies in rice blast disease where deletion of ATG genes impacted fungal conidiation, and pathogenesis include *MoAtg1*, *MoAtg2*, *MoAtg9*, *MoAtg14*, and *MoAtg24* [27]. In addition to ATG genes, the deletion of a RING finger ubiquitin ligase (E3) *MoSnt2* affected autophagy homeostasis resulting in oxidative stress, cell wall integrity defect, impaired conidiation and appressorium development, and cell death ultimately leading to an avirulent phenotype [28].

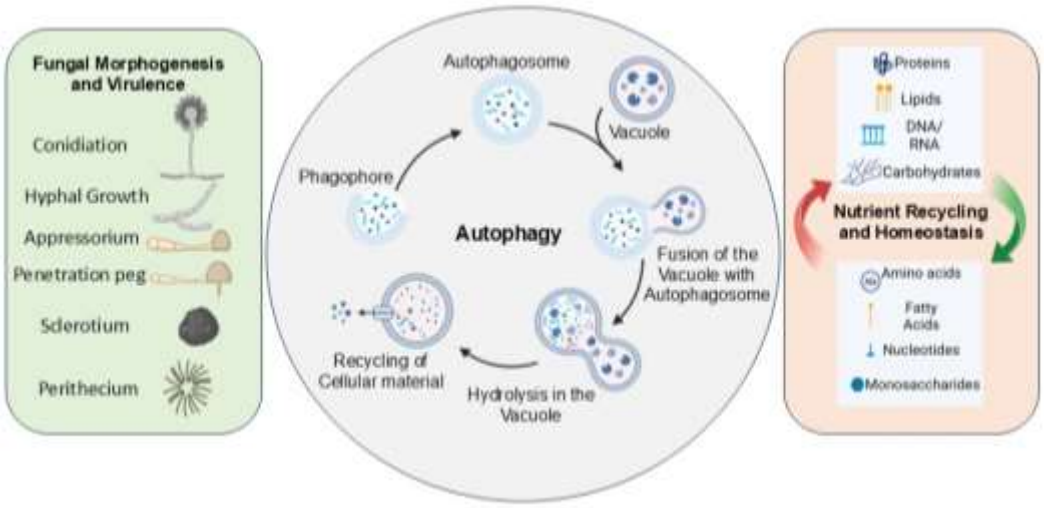

Figure 2. Schematic diagram shows fungal autophagy process and its multi-functional roles in fungal morphogenetic events and nutrient homeostasis.

1.2.2. Colletotrichum spp

Colletotrichum orbiculare (syn. *C. lagenarium)* is a pathogenic fungus that causes disease in cucumber plants [29]. The *CoAtg26* mutant strain formed appressoria, however the appressoria were impaired in host invasion resulting in the loss of virulence [30]. Also, interestingly the autophagic degradation of peroxisomes was significantly delayed in the appressoria of the Δ*CoAtg26* mutant strain indicating the importance Atg26 for pexophagy. Furthermore, Takano et al generated a *CoAtg8* mutant strain and established a role for *CoAtg8* in germination and appressoria development, clearly demonstrating that while CoAtg8-mediated non-selective autophagy is crucial for controlling early stage of infection-related morphogenesis, CoAtg26 mediated pexophagy is important for later stage of host invasion [30].

In *Colletotrichum fructicola,* the pathogen that causes anthracnose disease on *Camellia oleifera* transcriptomic analysis showed that histone acetyltransferase CfGcn5 acts as a repressor of autophagy and mediates the expression of several ATG genes [31]. Loss of function of CfGCN5 resulted in the upregulation of majority of ATG genes. Deletion of *CfAtg8* and *CfAtg9* genes caused defects in mitosis and attenuated appressoria formation, appressoria turgor pressure, and virulence in both the *ΔCfAtg8* and *ΔCfAtg9* mutant strains indicating their importance for autophagy and pathogenicity [31]. An autophagy defective Δ*CfAtg4* mutant strain was severely affected in growth and pathogenesis due to defect in appressoria formation [32]. Introduction of $CfAtg8^{10}$ allele, partially remediated autophagosome formation and autophagic defects in the Δ*CfAtg4* mutant strain subsequently restoring growth and pathogenesis defects.

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In *Colletotrichum scovillei* that causes pepper anthracnose disease *CsAtg8* is critical for asexual development and virulence of the pathogen [33]. Deletion of *CsAtg8* gene resulted in severe defect in sporulation, and the spores were defective in survival, conidial germination, and appressorium formation. Also, the conidia of Δ*CsAtg8* mutant strain showed reduced lipid contents and peroxisomal targeting signal PTS1 selectivity. All these defects reduced anthracnose disease severity upon Δ*CsAtg8* infection on pepper plants.

In *Colletotrichum gloeosporioides* the expression of *CgAtg16* was higher in the conidia, during germination, appressorium formation, at early infection stage, and highly induced during nutrient starvation [34]. Loss of *CgAtg16* significantly reduced autophagosome number, reduced colony growth, decreased sporulation and conidial germination rate, and attenuated appressorium formation ultimately resulting in decreased pathogenicity on rubber tree leaves. These data indicated *CgAtg16* mediated autophagy is crucial for *C. gloeosporioides* growth development and pathogenesis. Lastly, in *Colletotrichum higginsianum* that causes anthracnose in crucifers prohibitins, ChPhb1 and ChPhb2, were shown to interact with ChAtg24 in mitochondria and required for the proper functioning of mitochondria, mitophagy, and pathogenesis [35].

1.2.3. Fusarium Graminearum

In the head blight disease causing fungus *Fusarium graminearum* several ATG genes have been characterized and shown to be crucial for development and pathogenicity. For example, *FgAtg15* gene that encodes a lipolytic enzyme plays an indispensable role in fungal development. Loss of *FgAtg15* resulted in multiple defects in growth rate, conidiation, conidial germination, and aerial hyphae formation [36]. The *ΔFgAtg15* mutant strain was avirulent on wheat and could not degrade stored lipid droplets under nutrient starvation indicating impaired autophagy process in the *ΔFgAtg15* mutant strain [36]. In a genome wide association study 28 ATG genes were identified in *F. graminearum* [37]. Deletion of these genes showed that almost all the mutant strains were impaired in virulence on wheat except for *Atg27* mutant strain indicating the importance of ATG genes for host infection in *F. graminearum*. Further analysis indicated that deletion of *FgAtg1* and *FgAtg5* showed severe defects in autophagy, but these mutant strains could still cause infection on wheat [37]. The remaining strains deleted for other ATG genes were shown to be crucial for conidiation and infection of *F. graminearum*. Loss of Atg8 function in the Δ*FgAtg8* mutant strain resulted in its inability to form autophagic compartments and perithecia structures [38]. The Δ*FgAtg8* mutant strain produced fewer spores and fragile aerial hyphae that collapsed within a few days of culturing. Analysis of the collapsed mycelia of the Δ*FgAtg8* mutant strain indicated the presence of lipid droplets - an indication of nitrogen starvation or failure of the mutant strain to utilize storage lipids. Furthermore, the capacity of the Δ*FgAtg8* to utilize carbon/energy stored in lipid droplets during carbon starvation was compromised. Although the Δ*FgAtg8* mutant strain could still infect the wheat plant, it showed defects in host colonization and reduced growth demonstrating that Atg8-dependent autophagy in *F. graminearum* is crucial for fungal growth and host colonization. A recent study showed that although *F. graminearum Atg27* gene has no effect on vegetative growth and conidiation, its deletion resulted in significant reduction in virulence. Upon starvation induction, the Δ*FgAtg27* mutant strain had fewer autophagosomes present compared to the wild-type and complemented strain [39], indicating that *FgAtg27* is crucial in autophagy through autophagosome formation in *F. graminearum*.

The relevance of autophagy in growth, development, and pathogenesis of *F. graminearum* was also analyzed using the autophagy inhibitor wortmannin and an autophagy activator. This study revealed that nitrogen rich conditions, inhibition and/or activation of autophagy significantly reduced mycelial mass, while both autophagy inhibition and/or activation reduced conidiation [40]. Moreover, the toxin contents in wheat medium upon autophagy activation were elevated revealing increased autophagy. Chai et al showed that inhibition of autophagy and mitophagy processes reduced the virulence of *F. graminearum*, thus providing a new avenue to strategically control plant fungal diseases [40]. Mitophagy selectively eliminates mitochondria an important process of elimination of superfluous proteins or organelles [41].

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1.2.4. Ustilago Maydis

Autophagy in the corn smut fungus *Ustilago maydis* is centered around ATG genes and vacuolar proteases [42,43]. Nadal et al reported that *U. maydis Atg1* and *Atg8* genes are crucial for autophagy induction and autophagosome assembly. Deletion of both *UmAtg1* and *UmAtg8* showed *UmAtg8* as being indispensable for autophagy-dependent processes. The Δ*UmAtg8* mutant strain did not accumulate autophagosomes in the vacuoles during carbon-starvation thus reducing survival rates. Furthermore, the Δ*UmAtg8* mutant strain exhibited defects in the budding of haploid sporidia, with the plant gall induction being abolished. While the loss of *UmAtg1* resulted in a phenotype similar to *UmAtg8* deletion the Δ*UmAtg1* mutant strain was slightly less pathogenic than the wild-type strain although teliospore production remained unaffected [42]. *UmAtg11* was shown to be involved in mitophagy indicating it is also essential for autophagy [44]. However, the Δ*UmAtg11* mutant strain remained unaffected in its ability to cause infection or produce teliospores [45].

Vacuolar protease A and B have also been implicated in the autophagy-dependent response in *U. maydis*. Soberanes-Gutiérrez et al showed the accumulation of autophagosomes within the vacuoles of *U. maydis* wild-type strain when incubated in the presence of the serine protease inhibitor PMSF. Moreover, loss of proteinase B and the protease *Pep4* genes resulted in spherical bodies accumulating in the *ΔUmPep4* mutant strain's vacuoles, but not in the wild-type strain when incubated in carbon starvation medium indicating the mutant was unable to degrade via autophagosome formation a key step in the autophagy process. This study demonstrated the involvement of both proteinase A and B in the degradation of autophagosome in *U. maydis* [43].

1.2.5. Botrytis Cinerea

Botrytis cinerea causes grey mold disease in crops such as grapes and beans [46]. *B. cinerea BcAtg1* was upregulated under nutrient starvation conditions and loss of *BcAtg1* abolished the accumulation of autophagosomes in the vacuoles of nitrogen deficient cells [47]. Vegetative growth, conidiation, sclerotia and appressoria formation, and host tissue penetration were significantly impaired in the *ΔBcAtg1* mutant strain. Furthermore, *ΔBcAtg1* mutant strain was avirulent on different host plants confirming that autophagy is crucial for growth development and pathogenesis of *B. cinerea* [47]. *BcAtg2*, just like *BcAtg1*, is indispensable for growth, development, and virulence of *B. cinerea*. Deletion of *BcAtg2* blocked autophagy leading to attenuated fungal growth, conidiation, and defect in sclerotia production. Moreover, the Δ*BcAtg2* mutant strain failed to form infection structures that infect the host plants [48]. The ubiquitin-like (UBL) protein-activating enzymes BcAtg3 (E2) and BcAtg7 (E1) interact with each other and play important roles in autophagy process in *B. cinerea* [49]. Deletion of *BcAtg3* and *BcAtg7* genes impaired autophagy process with both single deletion mutant strains, Δ*BcAtg3* and *ΔBcAtg7*, being defective in mycelial growth, conidiation, sclerotia formation and pathogenesis [49]. Liu et al showed that *BcAtg6* is important in the regulation of autophagy and its deletion contributed to defects in mycelial growth, conidiation, and sclerotia formation [50]. In another study the loss of *BcAtg4* blocked autophagy and the mutant strain did not produce sclerotia and was significantly reduced in virulence [51]. BcAtg8 directly interacts with BcAtg4. Deletion of *BcAtg8* (Δ*BcAtg8*) blocked autophagy and significantly impaired mycelial growth, conidiation, sclerotia formation, and virulence [52]. In addition, the conidia of the Δ*BcAtg8* mutant strain contained fewer lipid droplets, and expression studies revealed that the basal expression levels of the lipid droplet metabolism-related genes in the mutant were significantly different from those in the wild-type strain.

1.2.6. Aspergillus Flavus

Aspergillus flavus is a saprotrophic and pathogenic fungus that causes postharvest infections in many cereal crops by producing toxic secondary metabolites known as mycotoxins or aflatoxins [53]. While autophagy in *A. flavus* has not been well elucidated, till date only few studies have examined the function of autophagy genes in *A. flavus*. For example, Geng et al 2024 showed that the *A. flavus*

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Atg8 is pivotal for autophagy as deletion of *Atg8* gene resulted in significant reduction in conidiation, spore germination, sclerotia formation and drastically impaired mycotoxin production in the *ΔAtg8* mutant strain compared to the wild-type strain [54]. The *ΔAtg8* mutant strain was defective in autophagy as the hyphae stained with DCFH-DA (Ros detection) emitted significantly brighter green fluorescence relative to the wild-type strain indicating a higher accumulation of reactive oxygen species in the Δ*Atg8* autophagy mutant strain. Apart from ATG related genes, type 2 phosphatases Ptc1 and Ptc2 regulate autophagy in *A. flavus*. Loss of Ptc1 and Ptc2 encoding genes resulted in mutant strains that produced fewer spores both on media and host crops [55]. Also toxin production was severely compromised in the mutant strains with autolysosome formation blocked in the Δ*ptc1*, Δ*ptc2*, and Δ*ptc1/ptc2* mutants when compared to the wild-type strain and complemented strains indicating the importance of Ptc1 and Ptc2 in the autophagy process in *A. flavus*.

1.2.7. Sclerotinia Sclerotiorum

In the necrotrophic fungal pathogen *Sclerotina sclerotiorum* autophagy is implicated in various fungal developmental processes. For instance, *ΔSsAtg1* mutant strain failed to accumulate autophagosomes in starved nitrogen cells, did not form sclerotia, and was impaired in growth under nutrient deficiency conditions. Also, *ΔSsAtg1* appressoria development and host penetration was impaired which subsequently resulted in the *ΔSsAtg1* mutant being avirulent [56]. In another study, six autophagy genes *SsAtg1*, *SsAtg2*, *SsAtg4*, *SsAtg5*, *SsAtg9*, and *SsAtg26* were deleted and phenotypes analyzed [57]. Only *ΔSsAtg26* showed growth defect with the remaining mutants displaying similar growth pattern like the wild-type strain. Several mutants were attenuated in virulence compared with the wild-type strain, however *ΔSsatg1* and *ΔSsatg26* had strongly reduced virulence compared with other mutants. Moreover, the loss of *SsAtg5*, *SsAtg12*, *SsAtg13*, and *SsAtg17* impaired sclerotia development [58]. In another study by Zhang et al, Atg8 was shown to interact with SsNbr1, a homolog of the mammalian autophagy receptor NBR1. This interaction plays a crucial role in the pathogen's growth and ability to cause disease and was required for autophagy with the *Atg8* mutant strain being defective in vegetative growth, sclerotial formation and virulence [59]. These studies indicate autophagy is crucial for *S. sclerotiorum* development and pathogenesis.

Table 1. List of autophagy related genes characterized in plant and industrial fungi.

Gene name	Organism	Growth/Conidiation	Pathogenesis	Reference
MoAtg1	*Magnaporthe oryzae*	Reduced	Reduced	[23]
MoAtg2	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg3	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg4	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg5	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg5	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg7	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg8	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg9	*Magnaporthe oryzae*	Reduced	Abolished	[27]
MoAtg10	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg13	*Magnaporthe oryzae*	No effect	No effect	[27]
MoAtg13	*Magnaporthe oryzae*	Reduced	Abolished	[22]
MoAtg14	*Magnaporthe oryzae*	Reduced	Abolished	[60]
CoAtg8	*Colletotrichum orbiculare*	Not explained	Abolished	[30]
CoAtg26	*Colletotrichum orbiculare*	Not explained	Abolished	[30]
CfAtg4	*Colletotrichum fructicola*	Reduced	Reduced	[32]
CfAtg8	*Colletotrichum fructicola*	Unknown	Abolished	[31]
CfAtg9	*Colletotrichum fructicola*	Unknown	Reduced	[31]
CsAtg8	*Colletotrichum scovillei*	Reduced	Reduced	[33]

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CgAtg16	*Colletotrichum gloeosporioides*	Reduced	Reduced	[34]
ChAtg24	*Colletotrichum higginsianum*	Reduced	Reduced	[35]
FgAtg1	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg2	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg3	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg4	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg5	*Fusarium graminearum*	Reduced	No effect	[37]
FgAtg6	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg7	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg8	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg9	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg10	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg12	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg14	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg15	*Fusarium graminearum*	Reduced	Reduced	[36]
FgAtg16	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg17	*Fusarium graminearum*	No effect	Reduced	[37]
FgAtg18	*Fusarium graminearum*	Reduced	Reduced	[37]
FgAtg27	*Fusarium graminearum*	No effect	No effect	[39]
UmAtg1	*Ustilago maydis*	No effect	Reduced	[42]
UmAtg8	*Ustilago maydis*	No effect	Reduced	[42]
UmAtg11	*Ustilago maydis*	Unknown	No effect	[44,45]
BcAtg1	*Botrytis cinerea*	Reduced	Reduced	[47,61]
BcAtg2	*Botrytis cinerea*	Reduced	Abolished	[48]
BcAtg3	*Botrytis cinerea*	Reduced	Reduced	[49]
BcAtg6	*Botrytis cinerea*	Reduced	Reduced	[62]
BcAtg7	*Botrytis cinerea*	Reduced	Reduced	[49]
BcAtg8	*Botrytis cinerea*	Reduced	Reduced	[49]
AfAtg8	*Aspergillus flavus*	Reduced	Reduced mycotoxin	[63]
AoAtg4	*Aspergillus oryzae*	Abolished	NA	[64]
AoAtg8	*Aspergillus oryzae*	Abolished	NA	[65]
AoAtg11	*Aspergillus oryzae*	Unknown	NA	[64]
AoAtg13	*Aspergillus oryzae*	Reduced	NA	[64]
AoAtg15	*Aspergillus oryzae*	No effect	NA	[66]
AoAtg26	*Aspergillus oryzae*	Reduced	NA	[67]
AnAtg1	*Aspergillus niger*	Reduced	NA	[68]
AnAtg8	*Aspergillus niger*	Reduced	NA	[68]
AnAtg17	*Aspergillus niger*	No effect	NA	[68]
BbAtg1	*Beauveria bassiana*	Reduced	Reduced	[69]
BdAtg1	*Botryosphaeria dothidea*	Reduced	Reduced	[70]
BdAtg3	*Botryosphaeria dothidea*	Reduced	Reduced	[71]
CpAtg4	*Cryphonectria parasitica*	Reduced	Reduced	[72]
FoAtg3	*Fusarium oxysporum*	Reduced	Reduced	[73]
FoAtg8	*Fusarium oxysporum*	Reduced	Reduced	[74]
PsAtg6a	*Phytophthora sojae*	Reduced	Reduced	[75]
PlAtg2	*Peronophythora litchii*	Reduced	Reduced	
SmAtg12	*Sordaria macrospora*	Reduced	Unknown	[76]
SsAtg1	*Sclerotinia sclerotiorum*	Reduced	Reduced	[57]
SsAtg2	*Sclerotinia sclerotiorum*	Unknown	Reduced	[57]
SsAtg4	*Sclerotinia sclerotiorum*	Unknown	Reduced	[57]
SsAtg5	*Sclerotinia sclerotiorum*	Unknown	Reduced	[57,58]

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SsAtg9	*Sclerotinia sclerotiorum*	Unknown	Reduced	[57]
SsAtg12	*Sclerotinia sclerotiorum*	Unknown	Reduced	[58]
SsAtg17	*Sclerotinia sclerotiorum*	Unknown	Reduced	[58]
UvAtg7	*Ustilaginoidea virens*	Reduced	Reduced	[77,78]
UvAtg14	*Ustilaginoidea virens*	Reduced	Reduced	[79]

1.3. Industrially Useful Fungi

1.3.1. Aspergillus Oryzae

Aspergillus oryzae, also known as the koji mold, is an industrially useful fungus utilized to produce enzymes important for fermentation of traditional oriental foods and beverages [80]. Autophagy has been extensively studied in *A. oryzae* and most of the ATG genes characterized have been shown to be pivotal in fungal growth and development. Kikuma et al showed that *AoAtg13*, *AoAtg4*, and *AoAtg15* are involved in sporulation as the Δ*AoAtg13* mutant strain formed fewer conidiophores and conidia while the Δ*AoAtg4* and Δ*AoAtg15* mutant strains completely failed to produce conidia [64]. The *ΔAoAtg1* mutant strain was defective in aerial hyphae development. The *AoAtg13*, *AoAtg4*, and *AoAtg15* genes were all shown to be involved in autophagy, for instance the Δ*AoAtg13* mutant strain showed reduced accumulation of EGFP–AoAtg8 in the vacuoles, PAS, and autophagosomes while only PAS-like structures were present in the Δ*AoAtg4* mutant strain. Also, autophagic bodies were present in the vacuoles of the Δ*AoAtg15* mutant strain. In another study the *AoAtg26* gene that encodes a sterol glucosyltransferase was shown to be essential for sporulation and aerial hyphae formation. The Δ*AoAtg26* mutant strain was defective in the degradation of peroxisomes, mitochondria, and nuclei [67]. Although the deletion of *AoATG11* did not significantly affect growth and sporulation in *A. oryzae*, Tadokoro et al showed that *AoAtg11* is involved in mitophagy and pexophagy [66]. Also, Kikuma et al reported that *AoAtg8* is crucial for aerial hyphae formation, conidiation, and conidial germination [81]. Visualization of autophagy through the expression of DsRed2-AoAtg8 and EGFP-AoAtg8 fusion proteins showed the absence of fluorescence accumulation in the vacuoles of the Δ*AoAtg8* mutant strain under starvation conditions, demonstrating that *AoAtg8* is indispensable for autophagy in *A. oryzae.* Interestingly, the AoAtg8-interacting protein A (AeiA) did not have any effect on colony size, hyphal growth, aerial hyphae formation, conidiation, and sclerotia formation. However, AeiA was shown to be involved in pexophagy by visualizing the expression of EGFP-PTS1 fusion where the number of peroxisomes in the *ΔAeiAGPTS1* strain were notably greater compared to the wild-type strain [82]. Another Rab family GTPase, Ypt7, was shown to be important for nucleophagy. Loss of *AoYpt7* function led to attenuated growth and resulted in complete loss of conidiation. Furthermore, reduced degradation of the histone H2B protein was noted in the Δ*AoYpt7* mutant strain cultured under starvation conditions. Fluorescence and electron microscopic observations revealed the accumulation of autophagosomes and autophagic bodies around the entire nuclei in the Δ*AoYpt7* mutant strain but not in the wild-type strain, indicating that *AoYpt7* is crucial for nucleophagy in *A. oryzae* [83].

1.3.2. Aspergillus Niger

Aspergillus niger is a black-spored saprophytic fungi that secretes various substrate-degrading enzymes and organic acids that are valuable for fermentation industry [84]. Transcriptional activation and induction of ATG genes has been demonstrated in *A. niger* strains that exhibited cryptic growth with empty hyphal compartments under carbon starvation in submerged cultures [68]. Nitsche et al analyzed the phenotypes of *A. niger* strains deleted for *Atg1*, *Atg8*, and *Atg17* and confirmed that *Atg1* and *Atg8* are essential for efficient autophagy. Interestingly, the mutant strains showed differential susceptibility to different kinds of oxidative stressors. Upon carbon starvation in submerged cultures the mitochondrial activity and turnover was blocked in the Δ*Atg1* and Δ*Atg8* mutant strains indicating the importance of autophagy for organelle turnover and protection against cell stress induced due to starvation. The impact of autophagy on ER stress was studied by deletion of genes

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associated with ER-associated degradation along with key autophagy genes *Atg1* and *Atg8* [85]. Interestingly, this study revealed that autophagy does not play any alternative role in the degradation of misfolded proteins due to ER stress. Another important aspect of "acidogenesis" was also studied in the Δ*Atg1* and Δ*Atg8* mutant strains of *A. niger*, where both autophagy and acidogenesis are compromised in these mutant strains [86]. It was demonstrated that an autophagy-like state exists throughout the acidogenic stages of fermentation and a block in autophagy pathway negatively impacts citric acid production. Acidogenic growth thus mimics a nutrient-limited condition and an extended autophagy-like state plays crucial role in *A. niger* physiology.

1.4. Autophagy in Human Fungal Pathogens

1.4.1. Cryptococcus Neoformans

Cryptococcus neoformans causes cryptococcosis in immunosuppressed individuals, especially with compromised immunity due to HIV infection [87]. Several genes related to autophagy have been characterized in *C. neoformans*. For instance, Hu et al reported the crucial role of *Vps34* gene in the autophagy process whereby, *Vps34* null mutant was defective in the formation of autophagic vesicles and exhibited attenuated virulence in a murine model [88]. The *Atg8* RNAi knockdown mutant strain was also reduced in virulence in a murine model [89]. Oliveira et al established that fewer number of autophagic bodies were formed in the *Atg7* mutant in addition to decreased cell viability during nitrogen starvation and reduced pathogenesis in a *Galleria mellonella* infection model [90]. In another study by Ding et al, *Atg1*, *Atg7*, *and Atg8* genes were shown to be critical for the maintenance of amino acid levels during nitrogen starvation, contributed to sensitivity to the proteasome inhibitor bortezomib, and were indispensable for *C. neoformans* virulence in a murine infection [91]. Furthermore, loss of function of the cargo encoding protein aspartyl aminopeptidase-like (Ape4) resulted in the mutant strain that was unable to survive at 37°C and within the macrophages. The Δ*Ape4* mutant strain was defective in expressing phospholipase, an important virulence factor, and also defective in capsule formation. The Δ*Ape4* mutant strain also exhibited higher susceptibility to antifungals and was attenuated in virulence [92].

In addition, Zhao et al generated 22 ATG deletion strains and established that laccase production and growth at 37 °C was significantly reduced in the Δ*Atg6* and Δ*Atg14-03* mutant strains [93]. The Δ*Atg2* and *ΔAtg6* strains were highly sensitive to oxidative stress with almost 50% of the mutant strains exhibiting hypersensitivity to osmotic stress (1.5 M NaCl). Majority of the mutant strains including Δ*Atg5*, Δ*Atg6*, Δ*Atg7*, Δ*Atg8*, Δ*Atg9*, Δ*Atg11*, Δ*Atg12*, Δ*Atg14-03*, Δ*Atg15*, Δ*Atg16*, Δ*Atg20*, and Δ*Atg24* were all attenuated in virulence with Δ*Atg6*, Δ*Atg11*, and Δ*Atg14-03* being completely nonpathogenic. Upon interrogating the exact roles of ATGs in the autophagy process Zhao et al established a lower autophagic flux following the deletion of *Atg1*, *Atg2*, *Atg5*, *Atg7*, *Atg9*, *Atg11*, *Atg12*, *Atg13*, *Atg16*, and *Atg18* genes but the autophagic flux remained unaffected in the *Atg6*Δ and Δ*Atg14* mutant strains confirming that *Atg6* and *Atg14* are dispensable for autophagy in *C. neoformans.* Lastly, it was also reported that autophagy was blocked in the Δ*Atg4* mutant strain because the Δ*Atg4* deletion strain was unable to degrade GFP-Atg8 [94].

1.4.2. Candida Albicans

Candida albicans colonizes the reproductive tract, oral cavity, and gastrointestinal systems. Invasion of C. albicans results in mucosal manifestations such as oral and vaginal thrush [95,96]. The first autophagy study in C. albicans involved the deletion of Atg9 gene, where loss of Atg9 function resulted in a blockade of the autophagic process and cytoplasm-to-vacuole trafficking [97]. The ΔAtg9 mutant strain was highly sensitivity to nitrogen starvation, however, it retained virulence in a murine model infection and remained unaffected in chlamydospore differentiation [97]. Deletion of Atg11 gene resulted in growth defects during nitrogen starvation condition, with the transport and degradation of Atg8 blocked in the ΔAtg11 mutant strain [98]. Cui et al further established impaired autophagy in the ΔAtg11 mutant strain due to suppression of both Csp37 (the indicator of

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mitophagy) and Lap41 (the indicator for cytoplasm-to-vacuole transport). Yu et al reported autophagosome trafficking or fusion with the vacuole was impaired in the Atg1 deletion background as the mutant strain accumulated more than 50% GFP-Atg8 puncta on the vacuolar membrane under normal versus nitrogen starvation conditions, implying a block in autophagy [99].

Recently, Huang et al reported that interaction of Atg1 with Atg9 led to the activation of autophagy [100]. In addition, the loss of *Atg1* and *Atg9* decreased biofilm formation, antifungal resistance, mitochondrial membrane potential, and autophagy. The inhibition of TORC1 through rapamycin treatment increased antifungal resistance in the Δ*Atg1* and Δ*Atg9* mutants and also restored biofilm formation in Δ*Atg1* mutant. Involvement of autophagy in *C. albicans* biofilm formation was also reported by Liu et al where autophagic activities were elevated in matured biofilms but not in premature biofilms [101]. Deletion of *Atg13* and *Atg27* resulted in drastic reduction of autophagosomes, reduced biofilm formation, and antifungal resistance [101]. The *C. albicans* vacuole and mitochondria patch protein Vam6 (vCLAMP) is indispensable for autophagy [102]. The *Vam6* null mutant exhibited growth defects under nitrogen starvation. Mao et al showed impaired degradation of autophagy markers including Atg8, Lap41, and Csp37 in addition to decreased carboxypeptidase Y activity and vacuolar phospholipase Atg15 levels in the Vam6 mutant strain.

1.4.3. Aspergillus Fumigatus

Aspergillus fumigatus is a devastating filamentous fungus that causes invasive aspergillosis in immunocompromised patients [103]. Unlike in other filamentous fungi, only a few ATG genes have been characterized in *A. fumigatus*. The *ΔAfAtg1* mutant strain failed to grow under acute starvation conditions, and the addition of metal ions such as zinc, iron, manganese, magnesium, and copper remediated the growth defect of *ΔAfAtg1* mutant strain confirming the importance of autophagy in fungal metal ion homeostasis. Although sporulation was attenuated in the Δ*AfAtg1* deletion strain, the mutant retained its virulence in a murine infection model [104]. By performing *A. fumigatus* PKA-dependent whole proteome and phosphoproteome analysis, Shwab et al identified 16 autophagy-associated proteins as potential PKA-regulated effectors [105]. Of these 16 autophagy genes identified, *Atg8*, *Atg20* and *Atg24* were characterized. Loss of *Atg20* and *Atg24* significantly reduced growth rate and abolished caspofungin-mediated paradoxical growth. The Δ*Atg20* mutant strain was reduced in virulence with approximately 50% mortality rate occurring in murine infection model [105]. This mortality exhibited by Δ*Atg24* mimics the mortality observed with the Δ*Atg20* mutant strain [106]. These studies demonstrated the role protein kinase A in regulating autophagy and subsequently growth, development, and pathogenesis in *A. fumigatus.*

Table 2. List of autophagy related genes characterized in human pathogenic fungi.

Gene name	Organism	Conidiation	Pathogenesis	Reference
CnAtg8	*Cryptococcus neoformans*	Unknown	Reduced	[89]
CnVps34	*Cryptococcus neoformans*	Unknown	Reduced	[89]
CnAtg1	*Cryptococcus neoformans*	Unknown	Reduced	[91]
CnAtg7	*Cryptococcus neoformans*	Unknown	Reduced	[90,91]
CnAtg8	*Cryptococcus neoformans*	Unknown	Reduced	[91]
CnAtg5	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg6	*Cryptococcus neoformans*	Unknown	Abolished	[93]
CnAtg7	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg8	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg9	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg11	*Cryptococcus neoformans*	Unknown	Abolished	[93]
CnAtg12	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg14-03	*Cryptococcus neoformans*	Unknown	Abolished	[93]
CnAtg15	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg16	*Cryptococcus neoformans*	Unknown	Reduced	[93]

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CnAtg20	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg24	*Cryptococcus neoformans*	Unknown	Reduced	[93]
CnAtg4	*Cryptococcus neoformans*	Unknown	Unknown	[94]
CnApe4	*Cryptococcus neoformans*	Unknown	Reduced	[92]
CaAtg1	*Candida albicans*	Unknown	Unknown	[99]
CaAtg9	*Candida albicans*	Unknown	Normal	[97]
CaAtg11	*Candida albicans*	Reduced	Reduced	[98]
CaAtg13	*Candida albicans*	Unknown	Unknown	[101]
CaAtg27	*Candida albicans*	Unknown	Unknown	[101]
AfAtg1	*Aspergillus fumigatus*	Reduced	Normal	[104]
AfAtg20	*Aspergillus fumigatus*	Reduced	Reduced	[105]
AfAtg24	*Aspergillus fumigatus*	Reduced	Reduced	[106]

1.5. Multifunctional Roles for Autophagy in Fungi

1.5.1. Autophagy in Nutrient Recycling, Homeostasis, Cellular Differentiation, and Degradation

In addition to controlling the morphogenetic and developmental aspects in various fungal species autophagy also plays a critical role in nutrient recycling pathways in several fungal species. For instance, in *M. oryzae* when Δ*MoAtg1* mutant with blocked autophagy was cultured on nutrient-deficient minimal media including, nitrogen and carbon, the mutant was attenuated in growth when compared to the wild-type strain [23]. Also, the Δ*MoAtg8* mutant of *M. oryzae* was significantly reduced in sporulation, which was restored upon the addition of alternative carbon sources, glucose or sucrose, or glucose-6-phosphate [26,107]. The *Aspergillus fumigatus* Δ*AfAtg1* mutant that was deficient in autophagy exhibited limited growth on nutrient starvation medium (i.e., water-agarose), when compared with the wild-type strain and the complemented strains that exhibited normal growth on the starvation medium [104]. However, when the mutant strain was transferred back to rich medium, growth was restored, indicating that growth defect was due to lack of nutrients.

In addition to nutrient recycling metal ion homeostasis impacts autophagy. For instance, depletion of cations such as zinc, manganese, and iron induces autophagy [104]. These studies offer clear evidence on the role of autophagy-dependent nutrient recycling and metal ion homeostasis in fungal growth and development. In the eukaryotes autophagy serves as the key process for protein degradation, where long-lived proteins and whole damaged or obsolete organelles are degraded [108]. Unlike animals, filamentous fungi lack lysosomes, therefore, vacuoles play similar role in degradation process. In *A. oryzae* hyphal vacuolation was reported to rapidly increase in the mycelia of *A. oryzae* under nutrient-starved conditions [109]. In *A. fumigatus* and *M. oryzae* autophagic bodies were present in the vacuoles of their respective wild-type strains upon autophagy induction but absent in the Δ*AfAtg1* and Δ*MoAtg1* mutant strains [23,104]. Lastly, deletion of *MoYpt7*, the Rab GTPase, and *MoMon1*, the guanine nucleotide exchange factor for Ypt7 blocked autophagy and altered vacuole assembly and vacuole fusion in the Δ*MoMon1* and Δ*MoYpt7* deletion strains [110,111]. These studies provide supportive evidence on the role of autophagy in cellular degradation.

Autophagy plays key role in cellular differentiation and mutants blocked in autophagy display various defects in growth and development. In *M. oryzae* autophagy-deficient Δ*MoAtg8* mutant strain was attenuated in sporulation and conidiation being significantly suppressed upon supplementation of exogenous glucose or sucrose, however, appressoria lost the ability to penetrate the plant tissue [26]. Deletion of *MgAtg1*, *MoAtg4*, and *MoAtg5* resulted in reduced conidiation, impaired germination, reduced appressorium turgor pressure with the appressoria of *ΔMoAtg1*, *ΔMoAtg4*, and *ΔMoAtg5* mutant strains losing their ability to penetrate the host plant tissue [23–25]. Loss of *Atg8* function in *C. orbiculare* led to defects in germination and appressoria development [30]. In *B. cinerea* deletion of *BcAtg1* led to defects in growth, conidiation, sclerotial development, appressoria formation, and penetration [47]. Perithecia formation during sexual reproduction is affected by deletion of autophagy related vacuolar protease (PspA) gene in the plant fungal pathogen

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Podospora anserina [112]. Autophagy has been shown to cause and prevent cell death in different fungi. For instance in the plant pathogenic fungus *M. oryzae* deletion of *MoAtg14* blocked autophagy and staining of the Δ*MoAtg14* mutant strain spores with fluorescein diacetate to verify spore viability showed a high percentage of Δ*MoAtg14* mutant cells were still alive compared to the wild-type strain indicating that *MoAtg14* contributes to conidial cell death [60]. In addition, the DNA-binding E3 ubiquitin-protein ligase *Snt2* deletion *M. oryzae* affected autophagy homeostasis and fungal cell death with the *ΔMoSnt1* compromised development of infection structure, conidiation, oxidative stress tolerance, and cell wall integrity. [28]. Also, in *Magnaporthe grisea*, autophagy was shown to be indispensable for spore collapse (cell death) during *in planta* infection [107]. However, in *P. anserina* autophagy is dispensable for cell death, though it tends to be stimulated during cell death by incompatibility [113]. Moreover, autophagy is linked to autolysis which is natural self-degradation process orchestrated by endogenous hydrolases and prolonged autolysis has been shown to result in cell death [114].

1.5.2. Autophagy in Antifungal Drug Resistance and Response Mechanisms

Antifungal drugs specifically target the key components of the fungal cells, the cell wall and the cell membrane, and compromise their integrity and induce cellular damage [115]. Autophagy can be activated as a protective response to drug stress, degrading damaged cellular components, and mitigating oxidative stress, thereby promoting drug tolerance [116,117]. Due to the established roles for autophagy in fungal stress adaptation, it has recently gained significance in antifungal drug resistance processes. Antifungal drug resistance is a complex process that evolves through multitude of factors resulting in fungal adaptation to antifungal drugs exposure [118]. Enhanced activity of drug efflux systems including membrane transport proteins, overexpression of drug resistance-related genes involved in ergosterol and glucan synthesis, the key components of fungal cell membranes and cell walls respectively, and biofilm formation to counteract the stress imposed by antifungals are the major components operating to induce resistance. For instance, previous studies in the autophagy deficient strains of *C. albicans* have shown reduced expression of *Cdr1* and *Mdr1* efflux pump genes indicating the impact of autophagic processes on drug efflux activity [119].

Antifungals induce cellular stress that results in autophagic response. In this regard the azoles (fluconazole, itraconazole), the polyene antibiotic amphotericin B, and the DNA synthesis inhibitor nystatin, have been shown to induce the formation of autophagosomes in *C. albicans* biofilms [120]. Interestingly, Huang et al. recently showed that a combination of antifungals (amphotericin B or 5-fluorocytosine) with aspirin suppressed biofilm formation by activating autophagy and inhibiting TOR signaling [121]. Strategies to target autophagy-related processes/proteins that are fungal-specific are necessary to overcome the challenges of increasing drug resistance observed with current clinical antifungals including the azoles and echinocandins. Previous studies have shown promise in this direction of designing specific inhibitors to autophagy proteins. During autophagy induced by starvation or rapamycin a selective inhibitor, autophinib, has been established to target Vps34 [122]. Another autophagy modulator, berberine, has been shown to synergize with fluconazole treatment and cause growth inhibition of an azole resistant strain of *C. albicans* [123].

1.6. Regulation of Autophagy

1.6.1. Kinase-Phosphatase Modules Regulating Autophagy Machinery

Thus far three kinases including TORC1 (TOR kinase), Atg1, and protein kinase A (PKA), have been identified to phosphorylate a set of autophagy related proteins in *S. cerevisiae*. TOR is a multifunctional Ser/Thr protein kinase regulating cellular growth and development, nutrient acquisition, protein synthesis, and important for nutrient signaling and autophagy. TORC1 phosphorylates *S. cerevisiae* Atg1, Atg13 and Atg29 proteins. During normal growth conditions active TOR phosphorylates Atg13, which in turn modulates Atg1 activity. However, under nitrogen starvation conditions TOR is inactivated resulting in reduced phosphorylation of Atg13 leading to an

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increased affinity of Atg13 for Atg1 and stimulates the formation of Atg1–Atg13 complex required for autophagy induction [124,125]. Atg1 also is a Ser/Thr protein kinase and functions downstream of TOR regulating different steps in autophagosome formation. Atg1 is autophosphorylated [126] and has been shown to phosphorylate other autophagy proteins including Atg4, Atg9, Atg13 and Atg29 proteins. For instance, Atg1-dependent phosphorylation of Vps34 is required for robust autophagy activity in *S. cerevisiae* and Vps34 dependent atg1 phosphorylation was shown to be important for full autophagy activation and cell survival [127]. Similarly, Atg9 was reported to be phosphorylated by Atg1 and this phosphorylation of Atg9 is crucial for regulation of early stages in autophagy through efficient recruitment of Atg8 and Atg18 to the site of autophagosome formation and subsequent expansion of the isolation membrane [128]. Although, Atg1 mediated phosphorylation of Vps34 and Atg9 promotes autophagy process, Sanchez et al showed that phosphorylation of Atg4 by Atg1 blocks autophagy orchestrated by Atg4 [129]. These results demonstrate that Atg1 mediated phosphorylation of autophagy related genes regulates autophagy in a positive and negative manner.

In contrast to *S. cerevisiae*, not much is known about the kinases regulating the phosphorylation of ATG proteins in filamentous fungi. We have earlier reported PKA-dependent phosphorylation of several autophagy related proteins including ATG proteins Atg20 and Atg24, the vacuolar sorting proteins including Vps1 (VpsA), Vps15, Vps17, Vac8, Vtc4 [105]. Vps1 is a dynamin-like GTPase that plays a critical role in the transport of Atg9 containing vesicles to the pre-autophagosomal structure and its role in pexophagy has also been reported[130]. Vps15 is a regulatory protein of Vps34 and is referred to as a pseudokinase as it does not contain the characteristic catalytic domain residues present in protein kinases. Although Vps15 does not phosphorylate Vps34 it forms a complex with it and is required for Vps34 activity in autophagy. Vac8 is a vacuolar armadillo repeat protein shown to be required for efficient induction of nonselective autophagy in *S. cerevisiae*. Localization of Vac8 to the vacuole is required for Atg1 initiation complex recruitment and pre-autophagosomal structure assembly at the vacuoles. Vac8 acts as an anchor at the vacuole and binds to Atg13 which forms an assembly hub for the recruitment of the initiation complex. Interestingly, the deletion of *Vac8* in *S. cerevisiae* did completely abolish autophagy but significantly impacted autophagic activity [131]. Vtc4 belongs to the vacuolar transport chaperone complex of proteins that have recently been found to negatively regulate endocytosis and autophagy [132]. In *M. oryzae*, *MoYck1* which encodes casein kinase, a Ser/Thr protein kinase was shown to negatively regulate autophagy process. Loss of *Moyck1* function affected growth, conidiation, conidial germination, and appressorium formation and penetration. Examination of GFP-MoAtg8 revealed a faster degradation in the Δ*Moyck1* background compared with the wild-type strain. Furthermore, GFP-*MoATG8* showed elevated in the Δ*Moyck1* background indicating MoYck1 negatively controls autophagy [133]. Type PP2C phosphatases, Ptc2 and Ptc3, have also been shown to regulate autophagy in the yeast through interaction and dephosphorylation of Atg1 complex. Loss of function of these phosphatases inhibits starvation-induced macroautophagy and the cytoplasm-to-vacuole targeting pathway via impairing the assembly of the essential autophagy machinery to the phagophore [134]. Similarly, Kondo et al showed that Cdc14 protein phosphatase plays an important role in the induction of autophagy following starvation and the target of rapamycin complex 1 (TORC1) kinase inactivation through rapid dephosphorylated of Atg13 [135]. Also, Cdc14 was necessary for effective induction of ATG8 and ATG13 expression [135]. These studies clearly demonstrate that autophagy is regulated through phosphorylation and dephosphorylation in fungal species.

1.6.2. Other Effectors Involved in the Regulation of Autophagy

Besides phosphorylation, autophagy is also regulated through acetylation. Sin3, a component of the histone deacetylase complex, was shown as a transcriptional repressor of ATGs. Sin3 thus negatively regulates autophagy induction in *M. oryzae* [136]. Loss of function of Sin3 resulted in upregulations of ATGs and promoted autophagy. Moreover, Wu et al established that Sin3 negatively regulated the transcription of Atg1, Atg13, and Atg17 through direct occupancy and histone

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acetylation. During nutrient starvation conditions, the transcription of Sin3 was downregulated, and the reduced occupancy of Sin3 from those ATGs resulted in hyperacetylation and activated their transcription which subsequently promoted autophagy.

In addition, dynamins which are a large superfamily GTPase proteins and function as motor proteins have also shown to be important for autophagy. In *M. oryzae* MoDnm1 was found to localize in the peroxisomes and mitochondria and is essential for vegetative growth, conidiogenesis, and full pathogenicity [137]. MoDnm1 was also shown to interact with the mitochondrial fission protein MoFis1 and the WD adaptor protein MoMdv1. The importance of MoFis1, MoMdv1 and MoDnm1 complex for autophagy was analyzed by monitoring Pex14 (pexophagy marker), Porin (mitophagy marker), and Atg8 (autophagy marker). Fewer stable Pex14 and porin proteins were observed in the Δ*MoDnm1*, Δ*MoFis1*, and Δ*MoMdv1* mutant strains relative to the wild-type strain. Furthermore, the expression of Atg8-GFP to visualize autophagic bodies in the background of these three mutations revealed lesser accumulation of autophagic bodies in the lumen of their vacuoles in comparison to the wild-type confirming their roles in autophagy.

2. Summary and Future Prospects

Research on autophagy in fungi has been extensively undertaken in the past two decades following the identification of ATG genes in the yeast (Ohsumi, 2014). Majority of these studies have clearly elucidated the involvement of autophagy processes in fungal development and pathogenicity. In this review, we have broadly reviewed the roles of various ATG genes in a wide variety of fungal species including plant and human pathogenic fungi, and industrially useful fungi. More importantly majority of the ATG genes have a conserved role in different fungal species and can be exploited for antifungal design. Although most of the studies have only evaluated their involvement in fungal development and pathogenesis, events on how autophagy regulates fungal development and pathogenesis and the molecular mechanism involved remain unclear and calls for further investigation, if autophagy has to be exploited for therapeutic purposes.

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