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REVIEW

# Role of plant autophagy in stress response

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## ABSTRACT

**Autophagy is a conserved pathway for the bulk degradation of cytoplasmic components in all eukaryotes. This process plays a critical role in the adaptation of plants to drastic changing environmental stresses such as starvation, oxidative stress, drought, salt, and pathogen invasion. This paper summarizes the current knowledge about the mechanism and roles of plant autophagy in various plant stress responses.**

**KEYWORDS** plant autophagy, stress response, drought and salt stress, pathogen

## INTRODUCTION

Autophagy is an intracellular degradation process that delivers cytoplasmic constituents to the vacuole (yeast and plants) or lysosome (animals) during stress and starvation (Klionsky and Ohsumi, 1999; Klionsky, 2005, 2007). There are two main types of autophagy in plants, namely, microautophagy and macroautophagy. Microautophagy is driven by direct invagination of the tonoplast into the central vacuole. The invagination is then pinched off and the isolated body containing the cytoplasmic materials and membranes is degraded by vacuolar hydrolases. During macroautophagy (hereafter referred to as autophagy), bulk cytoplasmic constituents and organelles are engulfed into a double-membraned vesicle called autophagosome. The outer membrane of the autophagosome fuses with the vacuolar membrane, delivering an inner membrane structure known as the autophagic body into the vacuolar lumen for degradation by resident hydrolases. As a major degradation process, autophagy is extremely important in plant development, starvation, environmental stress, senescence, and immune response. Knowledge on plant autophagy has greatly expanded in the past decade. The mechanism of plant autophagy is first discussed in this review, elaborating on some core autophagy complexes and proteins before summarizing the current knowledge on the role of autophagy in plant stress response.

## AUTOPHAGY MECHANISM IN PLANTS

### Core machinery of autophagy is conserved in plants

The core autophagy (ATG) genes were originally isolated in yeast (*Saccharomyces cerevisiae*). To date, the number of ATG genes has increased to 32 (Barth et al., 2001; Klionsky et al., 2003; Kanki et al., 2009; Nakatogawa et al., 2009; Okamoto et al., 2009). Homologues to the yeast ATG genes have been found in mammals and plants, which indicates the conservation of the core ATG mechanism during evolution. Over 30 *Arabidopsis* ATG genes (*AtATG*) have been identified in the model plant *Arabidopsis thaliana (At)* through genome sequencing. The following genes are present in more than one isoform, displaying functional redundancy: *AtATG1*, *ATG4*, *ATG8*, *ATG12*, *ATG13*, and *ATG18.* Seven ATG genes (*ATG1*, *ATG2*, *ATG3*, *ATG13*, *ATG16*, *VPS15*, and *VPS34*) were identified by sequence similarity and have not been functionally described (Hanaoka et al., 2002; Meijer et al., 2007). Yeast *ATG14*, *ATG17*, *ATG27*, *ATG29*, and *ATG31* have no homologues in *Arabidopsis* (Table 1). Core sequences have relatively high conservation but exhibit low sequence homology compared with their yeast counterparts. Only plant ATG4 and ATG6 complement yeast knockouts in function (Hanaoka et al., 2002; Liu et al., 2005). Two putative *ATG13* genes were found in *Arabidopsis*, but their similarity is restricted to a relatively small region of the protein and it is currently unclear if they are true *ATG13* orthologs (Bassham et al., 2006). Recently, rice and maize ATG genes have also been identified and have been characterized (Su et al., 2006; Chung et al., 2009; Xia et al., 2011).

**Table 1** Autophagy orthologs in yeast and *Arabidopsis*

| Functional group | Yeast autophagy proteins | Homologous autophagy genes in *Arabidopsis thaliana* |
|---|---|---|
| Regulation complex | ATG1 | *At*2g37840, *At*3g53930, *At*3g61960 |
| | ATG13 | *At*3g49590, *At*3g18770 |
| | TOR | Single gene: *At*1g50030 |
| | ATG17 | No homolog |
| | ATG18 | *At*3g62770, *At*4g30510, *At*2g40810, *At*3g56440, *At*5g05150, *At*5g54730, *At*1g03380, *At*1g54710 |
| | ATG20 | *At*5g06140 |
| | ATG27 | No homolog |
| ATG9 complex | ATG9 | Single gene: *At*2g31260 |
| | ATG2 | Single gene: *At*3g19190 |
| | ATG23 | No homolog |
| Phosphoinositide-3-kinase (PI3K) complex | ATG6 | Single gene: *At*3g61710 |
| | ATG14 | No homolog |
| | VPS15 | Single gene: *At*4g29380 |
| | VPS34 | Single gene: *At*1g60490 |
| Ubiquitin-like ATG12 and ATG5 conjugation pathway | ATG5 | Single gene: *At*5g17290 |
| | ATG7 | Single gene: *At*5g45900 |
| | ATG10 | Single gene: *At*3g07525 |
| | ATG12 | *At*1g54210, *At*3g13970 |
| | ATG16 | Single gene: *At*5g50230 |
| Ubiquitin-like ATG8 and PE conjugation pathway | ATG3 | Single gene: *At*5g61500 |
| | ATG4 | *At*2g44140, *At*3g59950 |
| | ATG7 | Single gene: *At*5g45900 |
| | ATG8 | *At*4g21980, *At*4g04620, *At1g62040*, *At*2g05630, *At*2g45170, *At*4g16520, *At*3g60640, *At*3g06420, *At*3g15580 |

## MORPHOLOGY OF AUTOPHAGY AND THE RELATIVE COMPONENTS

Core components of autophagy in yeast, mammals, and plants can be generally divided into three functional groups: (1) ATG9 recycling system including ATG1, ATG2, ATG9, ATG13, ATG18, and ATG27; (2) ATG6/Beclin1 and PI3K/VPS34 nucleation complex including ATG6, ATG14, VPS15, and VPS34; (3) the two ubiquitin-like conjugation systems, ATG8 and ATG12, which are made up of ATG3, ATG4, ATG5, ATG7, ATG8, ATG10, ATG12, and ATG16 (Xie and Klionsky, 2007; Avin-Wittenberg et al., 2011). The identification and function of some core proteins in plants are briefly discussed below in the order of their involvements in the autophagy process: initiation, vesicle nucleation, expansion, and autophagosome formation (Fig. 1).

### Initiation

In yeast, the induction of autophagy by starvation stress or other stress signals is accompanied by the integration of the signaling from the TOR (targets of rapamycin) kinase signaling cascade via the ATG1/ATG13 complex. A single TOR gene exists in *Arabidopsis*, which leads to embryonic lethality during gene knockout. In contrast to yeast and mammals, the vegetative growth of *Arabidopsis* is insensitive to rapamycin (Menand et al., 2002). By reducing the *AtTOR* transcript level through RNA interference (RNAi), Liu et al. found that the expression of some *AtATG* genes are upregulated. Moreover, these plants exhibit constitutive AtATG18a-dependent autophagy, which cannot be inhibited by an NADPH oxidase inhibitor, indicating that *AtTOR* is either downstream of or in a parallel pathway to NADPH

 

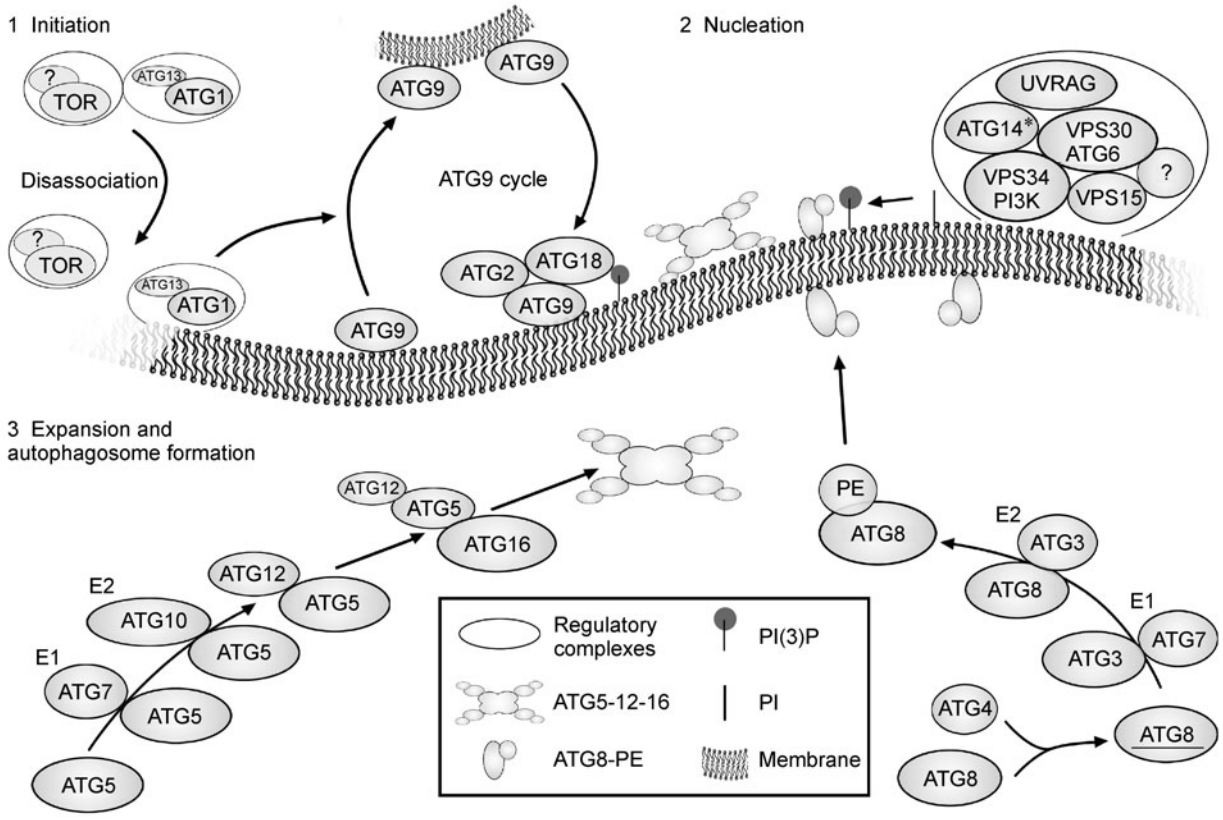


**Figure 1. Schematic of autophagy morphology and core autophagy components in plants.** As the core autophagy players are conserved in plants and yeast, yeast proteins required for the autophagy procedures are used to explain the functions in plants (those with asterisk representing the unidentified ATG proteins in plants). Upon the initiation, the TOR kinase and ATG1 complex disassociates, and the class III PI3K complex is activated. The plant PI3K complex including PI3K, VPS30/ATG6/Beclin1, VPS15 and UVRAG can phosphorylate phosphoinositol (PI) to generate phosphoinositol-3-phosphate (PI3P), and then initiate the nucleation of the autophagosome. ATG9, ATG18 and ATG2 function as membrane recruiters for the vesicle expansion. The membrane elongation and the autophagosome maturation depend on the two-ubiquitin-like conjugation systems. The ATG8 modified by ATG4 is conjugated to phosphatidylethanolamine (PE) by E1-like ATG7 and E2-like ATG3, while ATG5 is conjugated to ATG12 by E1-like ATG7 and E2-like ATG10 and then form a complex with ATG16.

oxidase (Liu and Bassham, 2010). The ATG9 complex is also involved in plant autophagy. The *Arabidopsis* knockout mutant *atg9-1* reportedly has decreased ATG8e levels and a slower accumulation of autophagic bodies in concanamycin A-treated roots, which suggests that the autophagic defect in *atg9-1* plants is leaky compared to the complete null autophagy in yeast *atg9* mutants (Yoshimoto et al., 2004). Obtaining an *ATG1* knockout in *Arabidopsis* is hindered because there are four putative members in *ATG1*. Interestingly, the *ATG13* homologues have been retained in plants but have been lost in animals. Further studies are needed to determine whether ATG13 in plants performs a similar function (Seay et al., 2009).

## Nucleation

In yeast, the regulation of vesicle induction and nucleation involves a class III phosphatidylinositol 3-kinase (PI3K) complex, which has a VPS34 catalytic subunit. A single homologue of VPS34 and VPS15 seems to exist in *Arabidopsis*, which is required for kinase activity (Bassham et al., 2006). However, ATG14, a specific autophagic constituent of the PI3K complex, has not been identified in *Arabidopsis*. ATG6/Beclin1 appears to act as the premier determinant of the level of cellular autophagy that occurs in yeast rather than as a molecular switch (He and Levine, 2010). ATG6/Beclin1 is required for the nucleation of autophagosomal vesicles. ATG6/Beclin1 likely acts as a scaffold for PI3K activity. In plants, knocking down ATG6 gives an autophagy-defective phenotype, indicating its role in plant autophagy (Liu et al., 2005). Homologues of the ATG6 complex core components ATG6, PI3K, VPS15, and UVRAG exist in plants but their interaction is yet to be studied (Hayward and Dinesh-Kumar, 2011).

 

**Autophagosome formation**

The ATG8-PE and ATG 5-12-16 conjugation systems are the two ubiquitin-like conjugation pathways that function during autophagosome membrane expansion and cargo engulfment (Klionsky, 2005; Thompson and Vierstra, 2005). In the ATG8-PE conjugation system, ATG8 is conjugated with phosphatidyl ethanolamine (PE) on both the inner and outer membranes of the phagophore. During the formation of ATG8-PE, ATG4, which functions as a cysteine protease, cleaves the C-terminal of ATG8 and exposes glycine residues. ATG7, the E1-like enzyme, then activates ATG8 and transfers ATG8 to the E2-like enzyme ATG3 and finally forms the ATG8-PE conjugate (Ichimura et al., 2000). In the ATG 5-12-16 conjugation system, ATG12 forms a complex with ATG5 and ATG16. The E1-like enzyme ATG7 activates ATG8 in one complex and ATG12 in another complex. ATG10, an E2-like enzyme present as a single gene in *Arabidopsis*, is required for this complex formation in yeast. The role of AtATG12-AtATG5 conjugates in plant autophagy has been proven through mutant analysis. Phillips et al. showed that *atg10-1*, *atg5-1*, and *atg7-1* plants cannot detect ATG12-ATG5 conjugates because the unconjugated AtATG12 level is higher, which indicates that these mutants have defects in ATG12 targeted E1-like and E2-like enzyme activities (Menzies et al., 2011).

## THE ROLE OF AUTOPHAGY IN THE PLANT STRESS RESPONSE

Autophagy is one of the stress response mechanisms thought to be a central component in the integrated stress response (Kroemer et al., 2010). In plants, such stress can be divided into abiotic and biotic stress. There is a variety of abiotic stress conditions such as, oxidative, nutrient, salt, and drought stresses, as well as biotic stresses, including invasion by various types of microbial pathogens. This study summarizes the recent findings on the role of plant autophagy in stress response.

**Role of autophagy in plant abiotic stress**

Role of autophagy in oxidative stress

Reactive oxygen species (ROS) are toxic products of aerobic metabolism, which are harmful to aerobic life. ROS include reduced or activated oxygen derivatives such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals. Compared with $O_2$, ROS are highly reactive and toxic. ROS accumulation leads to oxidative stress, which may damage all cell components such as proteins, lipids, and DNA. ROS can be removed through antioxidants and anti-oxidative enzymes. ROS-scavenging enzymes include catalase, superoxide dismutase (SOD), monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, peroxiredoxin, ascorbate peroxidase, and glutathione reductase (Noctor and Foyer, 1998). Among these enzymes, SOD converts superoxide ($O_2^-$) into $H_2O_2$, which is further detoxified into $H_2O$ and $O_2$ by catalase or peroxidases (Scherz-Shouval and Elazar, 2011). $O_2^-$ is highly reactive and short-lived, whereas $H_2O_2$ is relatively more stable. $H_2O_2$ and $O_2^-$ have been suggested as regulators of autophagy. In mammal cells, nutrient starvation leads to ROS accumulation, specifically $H_2O_2$ in the mitochondria, thereby inducing autophagy (Scherz-Shouval et al., 2007). $O_2^-$, on the other hand, is induced by prolonged glucose deprivation. Although SOD overexpression neither upregulates nor downregulates the $H_2O_2$ levels, the overexpression of both catalase and SOD inhibits autophagy. Moreover, prolonged exposure to exogenous $H_2O_2$ results in the accumulation of intracellular $O_2^-$ rather than $H_2O_2$, thus, demonstrating the role of $O_2^-$ in inducing autophagy (Chen et al., 2009). ROS generally induce autophagy to reduce oxidative damage in plants. A dramatic accumulation of $H_2O_2$ can be detected in *Arabidopsis atg5* and *atg2* plants (Yoshimoto et al., 2009). In *Arabidopsis* plants, ROS inducer methyl viologen (MV) reportedly induces autophagy. Furthermore, *ATG18* down-regulation leads to the accumulation of oxidized proteins, which subsequently increases sensitivity to oxidative stress (Xiong et al., 2007a, 2007b). In addition, rice *atg10b* mutants are sensitive to treatment with high salt content and ROS inducer MV, resulting in increased amounts of oxidized proteins in MV-treated *atg10b* mutant seedlings (Shin et al., 2009). Clearly, autophagy plays an important role in protecting plant cells from oxidative stresses by degrading oxidized proteins.

Role of autophagy in nutrition starvation

Nutrient starvation includes element deficiency such as carbon and nitrogen deficiency, sucrose starvation, darkness, and so on. Previous studies have proven that sucrose starvation in suspension-cultured cells is an excellent model system for analyzing plant autophagy (Bassham et al., 2006). Autophagy is clearly upregulated during periods of starvation and darkness. The transcripts of some AtATG genes are induced during starvation. *Arabidopsis* autophagy-related genes *AtATG4a*, *AtATG4b*, *AtATG8a-AtATG8i*, *AtATG3*, and *AtATG7* are transiently up-regulated in a coordinated manner at the onset of starvation (Rose et al., 2006). This supports the notion that plant autophagy plays an important role in nutrient recycling, especially under nutrient-starved conditions. Additionally, *Arabidopsis atg*-mutants, such as *atg7-1*, *atg9-1*, *atg4a4b-1*, *atg5-1*, *atg10-1*, are hypersensitive to nutrient-limiting conditions. Recently, chloroplast-derived ribulose-1,5-bisphosphate carboxylase/oxygenase-containing bodies (RCB) and whole chloroplasts have been found mobilized to the vacuole before chloroplast destruction through an ATG gene-dependent process under nutrient-

 

limiting conditions (Ishida et al., 2008). This suggests that plant autophagy plays an important role in nutrient recycling. Autophagy is likely a survival strategy during nutrient stress. Other studies have found that autophagosomes are formed in the cytoplasm shortly after the cells are deprived of nutrients. In contrast to yeast cells, the encircled portion of cytoplasm is usually degraded before the outer membrane of the autophagosome fuses with the tonoplast. Upon fusion, the internal vesicle is released into the vacuole (now called an autophagic body) where the degradation of its membrane and internal remnants is completed. The degradation of the cell components recycles the intracellular substrates, making resources available for the cell to survive (Aubert et al., 1996; Rose et al., 2006). Starvation-induced autophagy in BY-2 cells, as in *Arabidopsis*, is perturbed by bafilomycin A1 and concanamycin A, which are inhibitors of the vacuole-type $H^+$-ATPase, and therefore, prevents vacuolar hydrolase activity. Autophagic bodies cannot be degraded in these cells but can still be expelled into the central vacuole where they accumulate, resembling autophagic bodies in yeast vacuoles (Yoshimoto et al., 2004).

Role of autophagy in salt stress and drought stress

High salt and drought stress are the most common environmental stresses that affect plant growth and development. In contrast to ion stress resulting from high salt, drought stress leads to osmotic stress in plants (Zhu, 2001). These two kinds of stress can create oxidation damage to plant cells, leading to the accumulation of ROS and oxidized proteins (Tsugane et al., 1999). The ability of autophagy to scavenge oxidized proteins and regulate the ROS levels suggests that autophagy may also be involved in salt and drought stresses. Some ATG genes, such as *AtATG8* in *Arabidopsis* and *OsATG10b* in rice, reportedly function in response to salt stress and osmotic stress (Slavikova et al., 2008). The more direct and sufficient proof comes from the research by Bassham et al. who found that high salt and osmotic stresses activate autophagy, concomitant with the upregulation of *AtATG18a* expression. Autophagy-deprived *AtATG18a* RNAi plants show high sensitivity to these two stresses. Considering that ROS may function in the induction of autophagy by high salt and osmotic stresses, they tried to block ROS formation by adding an NADPH oxidase inhibitor. The results show that the NADPH oxidase inhibitors inhibit autophagy induction in plants under high salts stress, but unexpectedly not under osmotic stress; this proves the existence of NADPH oxidase-dependent and-independent pathways for regulating autophagy (Liu et al., 2009).

**Role of autophagy in plant biotic stresses**

In natural environments, plants are continuously attacked by different pathogens. These pathogens can generally be divided in two categories: biotrophs and necrotrophs. Biotrophs are pathogens that derive nutrients from living tissues of plant hosts and often cause minimal damage to the plant. In contrast, necrotrophs depend on dead host tissue for nutrients and reproduction. They often secrete enzymes and toxins to degrade and kill plant cells to make nutrients available. In addition, there are hemibiotrophs that are biotrophic in one stage of the infection and necrotrophic in another stage. During the long co-evolution of plants and pathogens, plants have developed various resistance mechanisms to defeat pathogen ingress. The role of plant autophagy in biotic stress has been a hot topic in the past few years because autophagy reportedly regulates negatively immunity-related cell death and autophagy-defective *Nicotiana benthamiana* plants exhibit spreading cell death once infected by incompatible virus (Liu et al., 2005). This paper discusses recent research in plant autophagy-related immunity.

Role of autophagy in plant responses to the incompatible pathogens

Previous research has revealed that autophagy is a survival strategy in the resistance gene *N*-mediated hypersensitive response (HR) cell death in tobacco plants. The *N* gene confers resistance to tobacco mosaic virus (TMV) and it can be activated by the p50 helicase protein of TMV replicase to induce HR cell death. Autophagy is induced at the onset of TMV infection, which limits TMV-induced cell death to TMV infection sites in wild-type plants. In contrast, the silencing *ATG6/Beclin1* eliminates TMV-induced autophagy and induces spreading cell death. The runaway cell death is not caused by virus movement because the transient expression of TMV-p50 alone induces similar spreading cell death (Liu et al., 2005). Similar phenotypes are observed in another plant model *Arabidopsis* (Patel and Dinesh-Kumar, 2008). The RNAi of *ATG6* in *Arabidopsis* impairs plant autophagy and causes unrestricted plant cell death when HR cell death is triggered by *Pto* DC3000-carrying AvrRpm1. However, Hofius et al. discovered that autophagy may contribute to HR-programmed cell death (PCD) in a pro-death way. The *Arabidopsis* knockout *atg* mutants *atg7* and *atg9* show delayed PCD induced by the avirulent bacterial strain Pto AvrRPS4 or the avirulent isolate Noco2 of the phytopathogenic oomycete *Hyaloperonospora arabidopsidis* (Hofius et al., 2009). Furthermore, the contribution of autophagy to cell death execution in plant *R* gene-mediated resistance is dependent on the type of resistance receptors. CC-NBS-LRR receptors trigger cell death that is partially (RPM1) dependent on or independent (RPS2) of autophagy, whereas TIR-NBS-LRR receptors (RPS4 and RPP1) are strictly dependent on autophagy (Hofius et al., 2009). Yoshimoto et al. proposed an explanation for such apparent controversial observations based on their independent experiment (Yoshimoto et al., 2009). They found the spreading cell death phenotype of the autophagy mutants only in the older leaves of older plants, not

 

in younger plants or younger leaves. The age dependence seems to be caused by age-associated salicylic acid (SA) level difference. In *atg2* and *atg5*, SA accumulates to higher levels. Blocking SA signaling using *npr1* or reducing SA biosynthesis using *sid2* suppresses early senescence, as well as the runaway cell death of *atg5* in their corresponding double mutants. In addition, SA agonist induces autophagy. Thus, they proposed that autophagy negatively regulates cell death by NPR1-dependent SA signaling during senescence and innate immune response (Yoshimoto et al., 2009). Interestingly, despite that autophagy-defective plants accumulate higher level of SA, these plants support increased growth of the incompatible pathogens including virus and bacteria (Liu et al., 2005; Hofius et al., 2009). More bacteria may secrete more effectors that may suppress HR, a form of PCD. This may give another possible explanation for Hofius et al's observation that there is a minor delayed cell death only at the early stage of pathogen infection in *Arabidopsis atg* mutants. Furthermore, plant autophagy may not have a pro-death function during plant immune response (Yoshimoto et al., 2009). However, the possibility that plant autophagy may have a pro-death function during plant immune response cannot be excluded because it has been found to have a pro-death function in tracheary element differentiation (Kwon et al., 2010).

### Role of autophagy in plant responses to the compatible pathogens

The silencing of several autophagy-related genes (*ATG6*, *ATG3*, *ATG7*, and PI3K) results in more TMV accumulation in tobacco plants containing the TMV resistance gene *N* (Liu et al., 2005), which suggests that autophagy has a role in plant immunity to pathogens. *Arabidopsis ATG7* knockout mutants and *ATG6* RNAi plants reportedly support the increased growth of the virulent hemibiotrophic pathogen *Pst* DC3000 (Patel and Dinesh-Kumar, 2008; Hofius et al., 2009). However, these observations obviously contradict the recent report that three *Arabidopsis* mutant lines, *Atatg5*, *atg10*, and *atg18a*, showed an enhanced resistance towards the same virulent pathogen *Pst* DC3000 (Lenz et al., 2011). Lenz et al. reported that these *atg* plants have a higher level of SA and an increased SA-dependent gene expression, indicating that autophagy negatively regulates SA-dependent immunity resistance to the hemibiotrophic pathogen *Pst* DC3000 (Lenz et al., 2011). Similar observation has also been reported in plant response to the infection by pathogen powdery mildew (Wang et al., 2011a, 2011b). *Arabidopsis atg2-2*, *atg5*, *atg7*, *atg10* and *atg18a-2* knockout mutants show enhanced resistance to the biotrophic pathogen powdery mildew and dramatic pathogen-induced cell death, as well as early senescence phenotypes in the absence of pathogens. They found that enhanced resistance to powdery mildew is SA-dependent, but mildew-induced cell death is only partially dependent on SA (Wang et al., 2011a, 2011b). These studies indicate that autophagy may positively contribute to the plant basal resistance to virulent biotrophic pathogens. However, no satisfactory explanation exists for such apparent controversial observations from different laboratories. One possibility is that the role of autophagy in plant basal resistance may differ under different circumstances, such as infection by different types of pathogens.

Autophagy also plays a role in plant responses to necrotrophic pathogens. *Arabidopsis atg* mutants (*atg5*, *atg10*, and *atg18a*) are more susceptible to infections from necrotrophic ascomycetes and *Plectosphaerella cucumerina* (Lenz et al., 2011). Once inoculated with spores of these necrotrophic pathogens, all *atg* mutant plants show leaf chlorosis and spreading necrosis that results in increased lesion sizes, reduced chlorophyll content, and increased disease indices. The *Arabidopsis atg5* mutant and not the control plants show fungal hyphal growth beyond infection foci, which strongly suggests that uncontrolled pathogen-induced necrotic cell death in autophagy-deficient plants facilitates increased necrotrophic pathogen growth. Similar results are reported using two other necrotrophic pathogens to inoculate *Arabidopsis atg* (*atg5*, *atg7*, and *ATG18a*) mutants and *wrky33* mutant (Lai et al., 2011). These *atg* mutants exhibit increased susceptibility to necrotrophic pathogens *Botrytis cinerea* and *Alternaria brassicicola.* *Arabidopsis* WRKY33 is a critical transcription factor that interacts with ATG18a. Furthermore, the increased susceptibility of *atg* mutants is associated with reduced expression of JA-regulated *PFD1.2* gene. These results suggest that autophagy cooperates with WRKY33-meditated and JA-mediated signaling pathways to take part in the plant response to the necrotrophic pathogens (Lai et al., 2011). Altogether, autophagy seems to have a pro-survival role in plant responses to necrotrophic pathogens.

## FUTURE PERSPECTIVES

Several important plant autophagy-related issues need to be addressed. Whether other proteins regulate autophagy in response to specific plant stress, how these proteins regulate autophagy, and what their role is in plant stress response need to be determined. Furthermore, whether autophagy does have a pro-death role during plant stress response, are its pro-death and pro-survival roles in HR PCD mutually exclusive, or how autophagy interacts with other signaling pathways, such as plant hormone and senescence signaling during plant stress response, are still unclear. These questions remain unanswered.

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## ABBREVIATIONS

HR, hypersensitive response; PCD, programmed cell death; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; TMV, tobacco mosaic virus

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