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REVIEW ARTICLE

The complex roles of autophagy in plant immunity

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Plant immunity is the result of multiple distinct cellular processes cooperating with each other to generate immune responses. Autophagy is a conserved cellular recycling process and has well-established roles in nutrient starvation responses and cellular homeostasis. Recently, the role of autophagy in immunity has become increasingly evident. However, our knowledge about plant autophagy remains limited, and how this fundamental cellular process is involved in plant immunity is still somewhat perplexing. Here, we summarize the current understanding of the positive and negative roles of autophagy in plant immunity and how different microbes exploit this process to their own advantage. The dualistic role of autophagy in plant immunity emphasizes that much remains to be explored in this area.

Keywords: autophagy; ETI; HR-PCD; immunity; pathogen effectors; PTI; reactive oxygen species; salicylic acid

Plants possess a sophisticated immune network to combat pathogen infections [1–4]. Plants pattern triggered immunity (PTI) involves recognition of conserved microbial components, such as fungal chitin or bacterial flagellin, by cell-surface localized pattern recognition receptors (PRRs). This results in a cascade of defence responses including the production of reactive oxygen species (ROS), phytohormones, callose deposition and transcriptional reprogramming of defence-related genes [3]. Consequently, some pathogens have evolved strategies to overcome PTI by secreting effector proteins into host cells [5]. Such effector proteins modulate various cellular and molecular activities to suppress PTI thereby promoting pathogenesis [5]. Not to be outdone, plants can deploy nucleotide-binding leucine-rich repeat (NLR) class of intracellular immune receptors that detect the presence or activity of effectors. This effector-triggered immunity (ETI) results in the activation of immune signalling that culminates in localized programmed cell death, known as the hypersensitive response (HR-PCD). The initiation and control of HR-PCD can be mediated by various signalling molecules, including salicylic acid (SA) and ROS. This localized programmed cell death restricts pathogens from spreading to adjacent cells [1,6]. In general, plants have two classes of NLRs: Toll-interleukin-1 receptor homology (TIR) domain containing NLRs (TNLs) and coiled-coil (CC) domain containing NLRs (CNLs). Despite having distinct triggers and characteristics, the overlap between PTI and ETI is becoming increasingly evident, and the

Abbreviations
AIM, ATG8 interacting motifs; APCB1, Aspartyl Protease Cleaving BAG; ATG, autophagy-related genes or proteins; BAG, Bcl-2-associated athanogene; BAK, BCL2 antagonist/killer; BAX, BCL2-associated X; Bcl-2, B-cell lymphoma 2; BI-1, Bax inhibitor 1; CC, coiled-coil; CNLs, coiled-coil (CC) domain containing NLRs; ETI, effector-triggered immunity; FLS2, FLAGELLIN-SENSING 2; GAPDH, gyceraldehyde-3-phosphate dehydrogenases; H_2O_2, hydrogen peroxide; HR-PCD, hypersensitive response-programmed cell death; MIT, microtubule interaction and transportation; NLR, nucleotide-binding leucine-rich repeat; ORM, orosomucoid; PRRs, pattern recognition receptors; *Pst* DC3000, *Pseudomonas syringae* pv. *tomato* strain DC3000; PTI, pattern triggered immunity; RLK, receptor-like kinase; ROS, reactive oxygen species; SA, salicylic acid; TIR, Toll-interleukin-1 receptor homology; TMV, *Tobacco mosaic virus*; TNLs, Toll-interleukin-1 receptor homology (TIR) domain containing NLRs; UPS, ubiquitin-proteasome system.

cooperation between these two immunity programs is crucial in the perpetual fight against plant diseases [7–10].

Autophagy is a conserved eukaryotic process in which cytoplasmic materials and damaged organelles are recycled or degraded inside a lytic cellular compartment to maintain homeostasis [11,12]. While multiple types of autophagy exist, macroautophagy has been the most extensively explored and is commonly referred to simply as autophagy in the literature (as well as hereinafter). The primary distinguishing characteristic of autophagy is the formation of autophagosomes, which are specialized double-membrane vesicles capable of delivering cytoplasmic components into either the plant vacuole or the animal lysosomes for degradation [11,13]. Selective autophagy occurs when only specific types of organelles or molecules are targeted [14,15]. In plants, more than 40 autophagy-related (*ATG*) genes have been identified, which have distinct yet collaborative roles in mediating autophagy [14,16]. Disruptions of ATGs can not only impair autophagy but also impact other cellular and developmental processes [12,17]. Although the history and mechanisms of autophagy are not the focus of this review (more information can be found in [11–14,16,18]), the process of autophagy includes initiation, nucleation, elongation, completion and ultimately fusion of autophagosomes with the vacuole or lysosome for the delivery and subsequent breakdown or recycling of cargoes.

Autophagy has been extensively researched in animal systems, yet plant autophagy has only begun to be explored. Several studies have demonstrated that plant autophagy is indispensable for proper function of plant immunity (Fig. 1 and Table 1). Moreover, pathogens possess different strategies to target autophagy to compromise the host immunity (Fig. 2). In this review, we explore various dimensions of the relationships between autophagy and plant immunity, including the roles of autophagy in plant defence and strategies that pathogens have evolved to manipulate autophagy, with a focus on bacterial and oomycete pathogens. A more complete review regarding plant viruses and their ways to manipulate autophagy can be found in this issue [19].

Roles of autophagy in PRR-mediated defence

In *Arabidopsis*, autophagy regulates levels of FLAGELLIN-SENSING 2 (FLS2), a PRR receptor kinase that recognizes bacterial flagellin and activates PTI [20] through orosomucoid (ORM) proteins [21] (Fig. 1). ORMs can act as autophagy receptors, allowing FLS2 to be targeted for autophagic degradation. Both ORM RNAi and CRISPR knockout *orm1* and *orm2* plants exhibited over-accumulation of FLS2 and hyperactive PTI after infection with *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst* DC3000). In contrast, overexpression of ORMs resulted in reduced FLS2 accumulation and enhanced susceptibility to *Pst* DC3000. Furthermore, overexpression of ORMs in *atg7-2* and *atg10-1* mutants had no effect on FLS2 accumulation and resulted in resistance to *Pst* DC3000

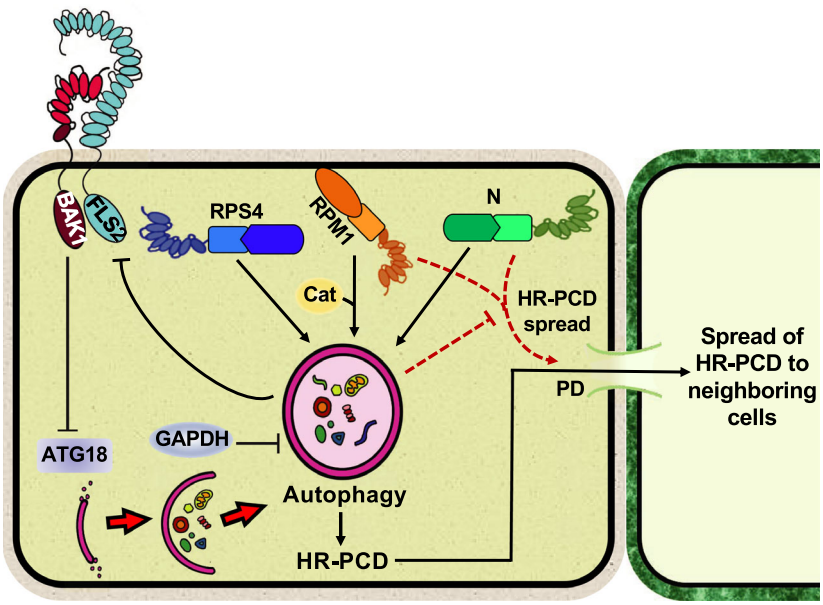

Fig. 1. Functions of autophagy in plant immunity. Autophagy negatively regulates FLS2 PRR levels, whereas BAK1 co-receptor inhibits the function of ATG18, which is required for the formation of the phagophore. Autophagy is required for the initiation of HR-PCD triggered by the RPM1 CNL and the RPS4 TNL. Additionally, autophagy is required for restricting HR-PCD to the infection site triggered by RPM1- or N-mediated immunity (represented by red dashed lines). Catalase (Cat) functions upstream of autophagy in RMP1-mediated HR-PCD. Gyceraldehyde-3-phosphate dehydrogenases (GAPDH) is a negative regulator of autophagy. PD, plasmodesmata.

Table 1. Roles of autophagy (*ATG*) genes in plant defence and susceptibility.

Function in autophagy	Gene	Knockdown or knockout phenotype	References
Nucleation of autophagosomes	*ATG6*	Growth defects Unrestricted HR-PCD during N- and RPM1- mediated resistance Susceptibility to *Pst* DC3000	[26,27]
	VPS34	Unrestricted HR-PCD during N-mediated resistance	[26]
Delivery of lipids for expansion of autophagosomal membrane	*ATG2*	Autoimmune phenotype Increased SA signalling and ROS accumulation Resistance to powdery mildew in *Arabidopsis* and *Pseudomonas syringae* pv. *glycinea* in soybeans	[28,37,46]
	ATG9	Suppression of HR-PCD during RPS4- and RPP1- mediated resistance	[33–35]
	ATG18	Resistance to *Pst* DC3000 Susceptibility to *Botrytis cinerea* and *Alternaria brassicicola*	[23,36]
A part of ATG8 conjugation system mediating lipidation of ATG8 and promoting expansion of autophagosomal membrane	*ATG3*	Unrestricted HR-PCD during N-mediated resistance	[26]
	ATG5	Early senescence Increased SA and ROS accumulation Suppression of HR-PCD in young plants and unrestricted HR-PCD in older plants during RPM1- mediated resistance Susceptibility to *B. cinerea* and *A. brassicicola*	[23,28,33–37]
	ATG7	Unrestricted HR-PCD during *N*-mediated resistance Suppression of HR-PCD in young plants and unrestricted HR-PCD in older plants during *RPM1*- mediated resistance Resistance to powdery mildew Susceptibility to *B. cinerea* and *A. brassicola*	[23,28,33–37]
	ATG10	Increased SA accumulation Resistance to *Pst* DC3000 and powdery mildew Susceptibility to *A. brassicola*	[36,37]

compared with that of wild-type plants [21]. Overall, this study shows the negative role of autophagy on FLS2-mediated PTI by mediating autophagic degradation of FLS2. As ORMs had no effect on other PRR-mediated signalling pathways tested, it will be interesting to learn if autophagy also plays a role in maintaining levels of PRRs levels through other modes of targeting and selective degradation.

BAK1 is a receptor-like kinase (RLK) co-receptor for multiple PRRs, which is crucial for activation of immune signalling [3]. Recently, BAK1 has been shown to negatively regulate ATG18a activities upon *Botrytis cinerea* infection [22] (Fig. 1). ATG18a is essential for host defence against *B. cinerea* likely through its roles in activating autophagy-mediated degradation and expression of the defence-related transcription factor WRKY33 [23]. BAK1 phosphorylated and suppressed ATG18a activity during resistance against *B. cinerea*. Loss-of-function in *BAK1* revealed low levels of phosphorylated ATG18a and strong induction of autophagy, resulting in enhanced resistance to *B. cinerea* [22]. Together, this study has discovered a novel connection between PRR-mediated defence and autophagy, in which the immune system modulates autophagy to keep the pathogen-induced defence responses in check.

Dual role of autophagy in immunity-induced cell death

Autophagy can play a dual role in the plant immune system, contributing to both pro-cell survival and pro-cell death activities (Fig. 1). Evidence suggests that this depends on multiple factors, including types of pathogens, plant age and the defence mechanisms invoked. Generally, recognition of pathogen-encoded effectors [also known as avirulent (Avr) protein] by a host NLR, triggers the ETI response, leading to lesion of cell death at the infection site and containment of pathogens [1,6]. The restriction of HR-PCD to the infection site is necessary to prevent spread of HR-PCD to neighbouring cells and distal tissues.

The tobacco N protein is a TNL that confers resistance to *Tobacco mosaic virus* (TMV) [24]. In *Nicotiana benthamiana* plants expressing the N TNL, TMV infection induces HR-PCD and limits TMV to the infection site [25]. However, silencing of plant ortholog of *ATG6*/*Beclin1* that is required for nucleation of autophagosomes [11,12] in *N*-containing plants resulted in spreading of HR-PCD into surrounding healthy tissue and systemic leaves [26]. Similar results could be seen after silencing other key genes involved with autophagy, such as *ATG3*, *ATG7*, and *VPS34*

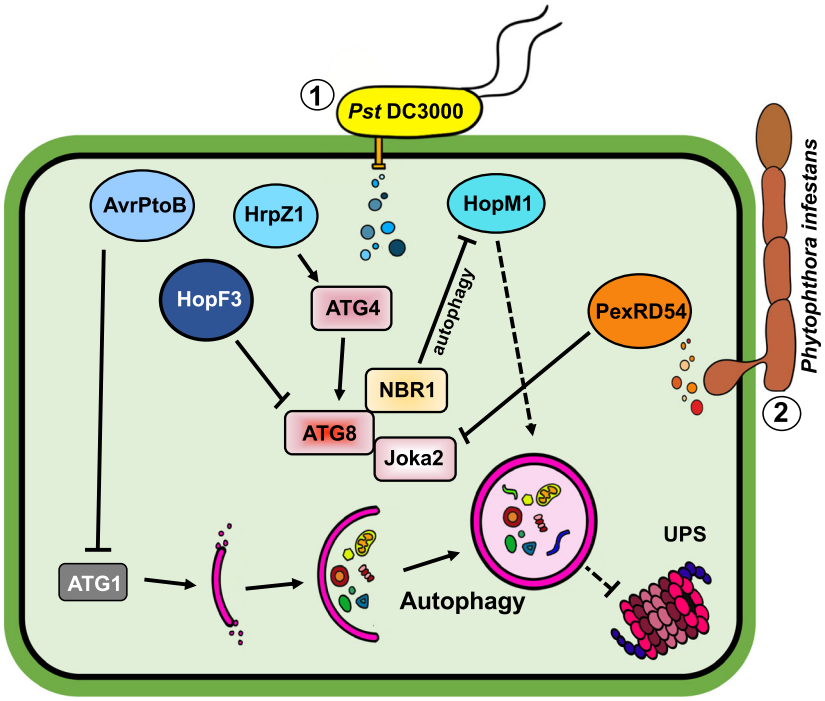

Fig. 2. Manipulations of autophagy by phytopathogens. Phytopathogens employ different effectors to promote pathogenicity in host plants. *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) (1) utilizes a set of effectors to enhance virulence. HopF3 and AvrPtoB inhibit autophagy through their interactions with ATG8 and ATG1, respectively, whereas HrpZ1 activates autophagy by promoting ATG4 activity. HopM1 enhances autophagy to mediate degradation of ubiquitin-proteasome system (UPS) in plants, thus compromising host defence (dotted lines). However, the effects of HopM1 are antagonized by the NBR1-mediated selective autophagy. *Phytophthora infestans* (2) also secretes effectors that disrupt host immunity. PexRD54, for example, specifically and competitively binds to an ATG8 ortholog and prevents ATG8 from interacting with the autophagy cargo receptor Joka2 (NBR1 homolog), which initiates the formation of defence-related autophagosomes during *P. infestans* infection.

[26]. These findings indicate that in autophagy-deficient cells, the pro-death signals that cause HR-PCD are no longer restricted, and therefore support a pro-survival role for immunity-induced autophagy. A similar spreading cell death phenomenon was observed in 4-week-old *Arabidopsis ATG6* RNAi plants when infected with the hemibiotroph *Pst* DC3000 harbouring *AvrRpm1* effector (*Pst-AvrRpm1*) [27]. Additionally, *Arabidopsis atg5-1* knockout plants exhibited unrestricted HR-PCD in response to *Pst-AvrRpm1* infection [28]. These findings overall suggest that immunity-induced autophagy plays an important pro-survival role by eliminating the pro-death signals associated with HR-PCD (Fig. 1 and Table 1).

In mammalian systems, anti-apoptotic B-cell lymphoma 2 (Bcl-2) family members and pro-apoptotic BAX (BCL2-associated X) and BAK (BCL2 antagonist/killer) proteins regulate autophagy and cell death [17]. Plants lack Bcl-2, BAX and BAK homologs but contain the evolutionarily conserved cell death suppressor, Bax inhibitor 1 (BI-1)-like protein [29]. Plant BI-1 interacts with ATG6 and this interaction is required for induction of autophagy during N TNL-mediated resistance to TMV [30]. Silencing of BI-1 resulted in increased accumulation of TMV-GFP and enhanced cell death, indicating that BI-1 is required for induction of autophagy to negatively regulate cell death. Contrary to the cell death suppressing role of BI-1, overexpression of BI-1-induced cell death in plants and BI-1-induced cell death requires autophagy. These findings provide evidence for both the death promoting and inhibiting role of plant BI-1. Although how BI-1 shifts between these functions remains elusive, it is likely that autophagy, which is also modulated by BI-1, plays a pivotal role in this process.

Cytoplasmic gyceraldehyde-3-phosphate dehydrogenases (GAPDH) has also been proven to modulate autophagy in plants [31]. In *N. benthamiana*, GAPDH acts as a suppressor of autophagy and its function could be carried out by its interaction with ATG3 (Fig. 1). Additionally, silencing of GAPDH led to enhanced HR-PCD during N-TMV interaction and also

increased resistance to the virulent *Pst* DC3000 and *P. syringae* pv. *tabaci* [31]. Similarly, *Arabidopsis GAPDH* knockout plants accumulated increased levels of ROS and exhibited constitutive autophagy. The enhanced HR-PCD in response to *Pst-AvrRpt2* and basal resistance against *Pst* DC3000 infection were also observed in the mutant plants [32]. Together, GAPDH can function as a negative regulator of immunity-mediated cell death and basal resistance, which could link to its inhibitory role on plant autophagy.

Plant autophagy can also operate in a pro-cell death manner during some plant-pathogen interactions (Fig. 1). The *Arabidopsis* RPS4 and RPP1 TNLs recognize *Pst* DC3000 harbouring the *AvrRps4* effector (*Pst-AvrRps4*), and the *AvrAtr1* effector of the oomycete *Hyaloperonospora arabidopsidis*, respectively. The *Arabidopsis* RPM1 and RPS2 CNLs recognize *Pst-AvrRpm1* and *Pst-AvrRpt2*, respectively. Successful recognition of these effectors induces HR-PCD; however, HR-PCD becomes inhibited in *atg7-1* and *atg9-1* mutants after infection with *Pst-AvrRps4* and *H. arabidopsidis* race Noco2, as measured by an electrolyte leakage assay [33]. This pro-death function of autophagy may possess a level of specificity, as very little reduction in electrolyte leakage was observed in *atg7-1* and *atg9-1* mutants after infection with *Pst-AvrRpm1* or in *atg5-1* and *atg7-2* mutants after infection with *Pst-AvrRpt2* [33]. However, in a single-cell death assay, HR-PCD induced during *Pst-AvrRpm1* was suppressed in 2-week-old *atg5-1* and *atg18a* mutant plants [34]. Furthermore, catalase, an antioxidant enzyme, seems to function upstream of autophagy in *Pst-AvrRpm1*-induced cell death [35]. Although cell death was compromised in the cases described above, there was no effect on the bacterial growth in *atg2*, *atg5-1*, *atg7-1* or *atg18a* mutants compared with wild-type plants [33–35]. Together, these findings provide support for the role of autophagy in cell death triggered by certain NLRs when young plants are challenged with pathogens.

Dual role of autophagy during disease-associated cell death

The disease-associated cell death generally refers to necrotic cell death that is induced by necrotrophic pathogens such as *B. cinerea* as a result of host susceptibility. In addition to its pro-survival role in immunity-induced cell death, autophagy can play a role in the regulation of disease-associated cell death (Table 1). During infection with virulent *Pst* DC3000, *Arabidopsis ATG6* RNAi lines displayed unrestricted spread of disease-induced cell death [27]. *Arabidopsis atg5-1*, *atg10-1* and *atg18a-1* and *ATG18a* RNAi (*atg18a-2*) plants exhibited spread of disease-associated cell death and enhanced susceptibility upon infection with necrotrophic fungi *Alternaria brassicicola* [36]. Similarly, *Arabidopsis atg5-1*, *atg7-2*, *atg7-3,* and *atg18a-1* and *atg18a*-2 lines showed increased disease-associated cell death and susceptibility to the necrotrophic fungi *B. cinerea* [23]. Together, these studies support a positive role for autophagy in necrotrophic pathogen defence.

In contrast, *atg2-2* plants displayed enhanced disease resistance to the powdery mildew *Golovinomyces cichoracearum*, an obligate biotrophic fungi [37]. Consistent with the enhanced resistance phenotype, *atg2-2* plants had increased expression of defence-related genes, including *PR1*, *PR2* and *PR5*, and increased levels of SA and ROS. Additionally, other *atg* mutants such as *atg5-1*, *atg7-1* and *atg10-1* also acquired enhanced *G. cichoracearum* resistance, similar to that of *atg2-2*. These findings suggest that autophagy additionally plays a negative role in resistance against this obligate biotroph.

Bcl-2-associated athanogene (BAG) family members have been known to play an important role in cell death regulation [38]. In *Arabidopsis*, BAG6 plays a role in disease-associated cell death in response to *B. cineria* infection [38,39]. Wild-type Col-0 *Arabidopsis* plants generally induce symptomatic cell death at the site of *B. cinerea* inoculation. However, in *Arabidopsis bag6* mutants, cell death rapidly spreads beyond the inoculation site and promotes enhanced susceptibility to *B. cineria* [39]. Interestingly, the ability of BAG6 to confer immunity to *B. cineria* requires cleavage of BAG6 by aspartyl protease APCB1 (Aspartyl Protease Cleaving BAG) [39]. Overexpression of a cleavage-resistant mutant of BAG6 in *bag6* mutant failed to rescue resistance against *B. cinerea*. Both infection of plants by *B. cinerea* and expression of cleaved BAG6 can induce autophagy that is crucial for immune activation and autophagic cell death to limit *B. cineria* to the infection site [39]. These studies highlight the functions of BAG6 as a positive regulator of plant immunity through its ability to modulate host autophagy and subsequently pathogen-induced cell death.

The role of autophagy in SA and ROS modulation

Salicylic acid and ROS are pro-defence compounds in plant that are tightly controlled [6]. In plants, SA is crucial defence signalling hormone, and pathogen perception can trigger SA biosynthesis and accumulation. While ROS is induced upon pathogen recognition and is critical for defence signalling, uncontrolled ROS accumulation could have detrimental effects, including

cellular damage. Both SA and ROS have been linked to the formation and regulation of HR-PCD. Autophagy has been shown to negatively regulate SA and ROS accumulation [28]. During infection with *Pst* DC3000, *Arabidopsis atg5-1* plants accumulated threefold higher SA compared with wild-type plants. Consistent with this, expression of the SA-responsive genes *PR1* and *PR2* was elevated in *atg2-1* and *atg5-1* plants. Furthermore, these *atg* mutants accumulated higher levels of hydrogen peroxide (H_2O_2). The spreading cell death phenotype observed in *atg5-1* in response to *Pst-AvrRpm1* was suppressed when SA-related pathways were inactivated in the *atg5-1* plants. These findings indicate that over-accumulated SA and ROS may play a role in pathogen-induced cell death spread in the absence of autophagy [28].

Similar results were found when analysing immunity in *atg2-2* plants in response to powdery mildew *G. cichoracearum* [37]. Besides the enhanced resistance to the fungal pathogen, both *atg2-1* and *atg2-2* displayed autoimmune phenotypes, designated by stunted growth, early senescence and spontaneous cell death. Further analysis revealed that the key defence-related factors such as *PR1*, *PR2*, *PR5* and ROS were upregulated in the *atg2-2* mutants [37], which was consistent with the previous report of *atg2-1* [28]. Although hyperaccumulation of SA in both *atg2-1* and *atg2-2* was not reported in both studies, the upregulation of SA-responsive genes might imply high levels of SA in the mutant plants. Additionally, inactivation of SA signalling in the *atg2-2* background suppressed the autoimmune phenotypes and the powdery mildew resistance based on the analysis of fungal growth [37]. As SA and ROS are essential for the regulation of both senescence and immunity in plants [40], the increase in SA, ROS and *PR* genes likely explain the autoimmune phenotype observed on the *atg2* mutants and also enhanced resistance to pathogen infection. However, many other *atg* mutants are normal and they induce cell death similar to wild-type plants but the cell death spreads. This suggests a role for autophagy in eliminating the SA and ROS signals after host-induced HR-PCD, and the SA-ROS amplification signalling that mediates HR-PCD could be a target of active downregulation by autophagy.

Despite coordination of SA and ROS accumulation in *Arabidopsis atg* mutants during host-microbe interactions, Lenz et al. [36] further explored this relationship across multiple types of pathogens. *Arabidopsis atg5-1*, *atg10-1*, *atg18a-1* and *atg18a-2* were found to be more resistant to *Pst* DC3000 infection compared with wild-type plants. Phytohormone quantification in some of these *atg* mutants revealed that *atg5-1* and *atg10-1* accumulated twofold higher amounts of SA during the infection of *Pst* DC3000 than the wild-type plants, while wild-type levels of PTI responses were still found in these mutants. This report was consistent with a previous study [28], supporting the negative regulatory roles of autophagy on SA-dependent defence response to biotrophic pathogens. However, these mutants responded differently to the necrotrophic pathogen *A. brassicicola*. During infection with *A. brassicicola*, the *atg* mutants used previously for *Pst* DC3000 disease assays exhibited significantly enlarged necrotic lesions without an increase in senescence molecular markers. Surprisingly, ROS accumulation, which is often associated with cell death phenotype, was not altered in the *atg* mutants in comparison with the wild-type plants [36]. The results show that the misregulation of host autophagy leads to the increased vulnerability of these mutants to the necrotrophic pathogen without significant alterations in ROS production. It should be noted that an interplay exists between SA and the phytohormone jasmonic acid (JA), where JA often mediates resistance to necrotrophic pathogens and antagonizes SA-mediated resistance to biotrophic pathogens. However, there were no significant changes in levels of JA and *PDF1.2b* expression, a JA-responsive gene, between the wild-type and *atg* mutants upon *A. brassicicola* infection [36]. Overall, this study emphasizes the dynamic roles of autophagy in modulating two primary defence compounds in plants, SA and ROS, and the regulatory functions of autophagy depend on specific lifestyles of pathogens and their interactions with host plants.

Microbial manipulation of autophagy

Multiple effectors from diverse pathogens appear to target the autophagy pathway and molecular machinery to promote pathogenesis, suggesting a fundamental role for autophagy in the determination of infection outcomes and the pathogen-plant arms race. Here, effectors from bacterial and oomycete pathogens are discussed (Fig. 2).

The success of plant bacterial infection relies on the pathogens ability to subvert host immunity. While multiple bacterial effectors and their targeted biological pathways in plants have been extensively studied, effectors capable of manipulating plant autophagy are only recently being identified. To promote virulence, type 3 effectors from *Pst* DC3000 inhibit the ubiquitin-proteasome system (UPS), which is a major protein degradation pathway in eukaryotes [41] (Fig. 2). However, *Pst* effectors failed to inhibit UPS in *atg5-1* knockout mutant suggesting the importance

of the pathogen-induced autophagy in interfering with plant UPS. Moreover, HopM1 was identified as an effector that activates host autophagy [42]. However, NBR1, an autophagic cargo receptor, antagonized the HopM1-induced water-soaked lesions and bacterial growth [42]. Collectively, *Pst* DC3000 facilitates host autophagy through the HopM1 effector to mediate degradation of UPS, which promotes bacterial growth and infection. Meanwhile, plants combat the effects of HopM1 by NBR1-mediated selective autophagy utilizing target proteins that have yet to be identified.

The HrpZ1 effector of *Pst* DC3000 also activates plant autophagy and supports disease development [43] (Fig. 2). HrpZ1 interacts with multiple *Arabidopsis* ATG8 isoforms both *in vitro* and *in vivo*, insinuating that HrpZ1 might function to manipulate the host autophagy pathway. Further functional analysis revealed that HrpZ1 enhanced autophagy through increasing the activity of ATG4b protease to processes ATG8 at the conserved C-terminal glycine residue, which is a vital step in autophagosome biogenesis [43].

In addition to inducing autophagy, some bacterial effectors have been shown to inhibit autophagy as a strategy to promote bacterial virulence. HopF3 is a *Pseudomonas* effector that interacts directly and selectively with a subset of *Arabidopsis* ATG8s. Unlike HrpZ1, HopF3 attenuates autophagy (Fig. 2). Expression of HopF3 in *Arabidopsis atg5-1* mutant diminished the enhanced *Pst* DC3000 virulence observed in wild-type plants, further suggesting that host autophagy is required for HopF3-mediated virulence [43]. The AvrPtoB effector of *Pst* DC3000 has also been shown to suppress autophagy similarly to HopF3. However, instead of targeting ATG8s, AvrPtoB interacts with ATG1 kinase, a key initiator of the autophagy process (Fig. 2). Strong interaction was detected between AvrPtoB and the microtubule interaction and transportation (MIT) domain of ATG1, and this interaction depended on the previously uncharacterized N-terminal domain of AvrPtoB. Biochemical assay showed that AvrPtoB inhibited phosphorylation of ATG1 which limits autophagy but promotes bacterial virulence [43].

Like bacteria, oomycete pathogens also employ effector proteins to impede host immunity (Fig. 2). The oomycete effector PexRD54 from *Phytophthora infestans* contains two predicted ATG8 interacting motifs (AIM) [44]. One of these AIMs and the host small GTPase Rab8a, a key player in the vesicle trafficking pathway, were necessary for the interaction between PexRD54 and ATG8CL. This interaction allows the effector to be loaded into autophagosomes, eventually perturbing the interaction between ATG8CL and the autophagy cargo receptor Joka2 in tobacco, which is an NBR1 homolog. This ultimately confers defence against *P. infestans* infection [44,45].

Considering the evolutionary arms race between plants and pathogens, it is not surprising that pathogens can manipulate or hijack the autophagy pathway to promote pathogenesis. This microbial manipulation is often executed using microbial effectors. While some effector proteins induce autophagy, others function to suppress it. Additionally, some effectors compete to interact with host autophagy components without altering autophagic flux. Despite differences in modes of function, enhanced pathogen virulence remains a shared goal. Nevertheless, only a few effectors have been shown to specifically interfere with autophagy. Several effectors from pathogens across different kingdoms were found to interact with ATG proteins [43]. However, how they function to modulate autophagy remains unclear. Understanding their functions and how plants counteract these effectors would provide a more complete picture of the interplays between autophagy and plant immunity.

Conclusions and perspectives

Autophagy is a vital recycling pathway responding to stresses, especially nutrient deprivation. Impairment of autophagy causes abnormality in eukaryotic organisms both developmentally and physiologically. There is growing evidence supporting a connection between autophagy and immunity against pathogens in plants. Indeed, many studies have shown that defects in different *ATG* genes affect how plants interact with pathogens both *via* the PTI and the ETI branches of host defence. In terms of PTI and basal resistance, plant lines with either loss-of-function mutations or gene silencing of different *ATG* genes revealed both enhanced and dampened host resistance to different types of virulent pathogens. The alterations of host defence were found to be linked to changes in hallmarks of basal resistance and homeostasis of immune receptors. In relation to ETI, autophagy is required for proper regulation of HR-PCD. Pro-death and pro-survival roles of autophagy in ETI-mediated PCD have been found upon pathogen infection. It should, however, be noted that different types of pathogens and plant *atg* mutant genotypes certainly contribute to the discrepancies observed in both PTI and ETI studies, which may suggest potential roles for individual ATGs in other biological pathways intertwined with autophagy. A plethora of host factors involved in the route from pathogen perception to induction of autophagy and HR-PCD have been established. However, understanding their exact roles and mechanisms of

action within this pathway, as well as whether or not other unknown players exist, will require further research that piece together the puzzle. Despite some seemingly contradictions, pathogens have found ways to promote their virulence by manipulating plant autophagy. Overall, the dualistic role of autophagy in plant immunity emphasizes how intricate this relationship is and how much remains to be explored in this field.

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