Understanding and exploiting autophagy signaling in plants

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Autophagy is an essential catabolic pathway and is activated by various endogenous and exogenous stimuli. In particular, autophagy is required to allow sessile organisms such as plants to cope with biotic or abiotic stress conditions. It is thought that these various environmental signaling pathways are somehow integrated with autophagy signaling. However, the molecular mechanisms of plant autophagy signaling are not well understood, leaving a big gap of knowledge as a barrier to being able to manipulate this important pathway to improve plant growth and development. In this review, we discuss possible regulatory mechanisms at the core of plant autophagy signaling.

Introduction

Eukaryotic cells use the catabolic process known as autophagy to degrade nonselectively or selectively dysfunctional or unnecessary cellular components [1,2]. This highly regulated pathway is physiologically essential, ensuring nutrient recycling, and cellular and organismal homeostasis during stress. In plants, mainly two types of autophagy responses have been described: (i) microautophagy, characterized by the tonoplast (vacuolar membrane) invagination and entrapping of the components targeted for degradation and (ii) macroautophagy, characterized by the de novo formation of a double membrane organelle, the autophagosome, engulfing the targeted cytoplasmic components or cargo for degradation. In a plant cell, the outer membrane of the mature autophagosome fuses with the tonoplast, and a single membrane-bound cargo is delivered to a vacuolar lumen and its contents is degraded by the action of vacuolar hydrolases. Both microautophagy and macroautophagy can be bulk or selective. However, the molecular mechanisms of microautophagy, as compared with the more studied macroautophagy (hereafter referred to as autophagy), are not well defined in plants. Other types of autophagy have been described in other eukaryotic systems, for example chaperone-mediated autophagy, endosomal microautophagy and secretory autophagy in animals, or the cytoplasm-to-vacuole pathway in yeast. These specialized autophagy pathways have not been described in the plant cell. However, plant-specific autophagic pathways, as defined by their cargo and possibly the underlying molecular mechanism for the autophagosome formation, do exist. The “sunburn preventing” chlorophagy pathway allows the plant cell to degrade photodamaged (essentially ultraviolet B-induced damage) entire chloroplasts (4–6 μm in length and a mean volume of 20 μm3) [3]. Getting rid of the damaged chloroplasts protects the plant cell from accelerated death due to reactive oxygen species (ROS) accumulation. Autophagy in the plant cell can also target the inactive proteasomal 26S oligomeric complex (up to 2.5 MDa) in a process referred to as proteapathage [4].

Many environmental stimuli including nutrient-limiting conditions, abiotic stress, and pathogen invasion up-regulate autophagy in plants. As sessile organisms, plants have to cope at times with a combination of these stresses. A crucial feature of autophagy is that it is a highly regulated and dynamic process, able to sense intracellular stress within minutes and rapidly mount an appropriate response to cope with the damage [5]. This is possible because a multitude of unrelated cellular pathways converge...
on the autophagy machinery to signal a diversity of conditions. It has been shown in yeast and mammalian cells that the serine-threonine protein kinase ATG1 is a key component of the autophagy machinery integrating these various signals. The sequence of molecular events conducive to autophagosome initiation, entrapment of cytoplasmic material, and eventually fusion of the autophagosome with the lytic vacuole in the plant cell, is under the control of evolutionary conserved specific autophagy complexes (containing autophagy-related or ATG proteins) whose activity is directly or indirectly regulated by stress signaling pathways, including phytohormone pathways [6-10]. The ATG1 complex is thought to be essential in transmitting stress signals to the site where the autophagosome will be formed, and by mediating the activating phosphorylation of downstream autophagy proteins such as the components of the class III vacuolar protein sorting 34 (VPS34) complex. The VPS34 complex catalyzes the formation of the signaling lipid phosphatidylinositol-3-phosphate (PI3P) at the site of autophagosome formation.

In this review, we will discuss the activation and regulation of plant ATG1 and VPS34 complexes, and describe some of the exogenous and endogenous stimuli that modulate autophagy signaling in plants.

Plant ATG1 complex

As a core autophagy complex, the conserved ATG1 complex is a trimeric protein complex composed of a catalytic subunit (ATG1), regulatory subunits (ATG13 and ATG101), and scaffold subunits (FIP200, ATG11, or ATG17). The structure and function of ATG1 complex is not well understood in plants. The *Arabidopsis* genome seems to encode three full-length ATG1 proteins (ATG1a, locus AT3G61960; ATG1b, locus AT3G35960; ATG1c, locus AT2G37840) and a C-terminus-truncated form called ATG1t (locus AT1G49180) [11]. The role of the latter in autophagy is not yet clear. Two functional ATG13 isoforms (ATG13a and ATG13b) and a single ATG101 are also encoded by the *Arabidopsis* genome. A functional bona fide FIP200/ATG17 relative seems to be absent in the plant lineage. Instead, a potential bifunctional protein with domains related to both yeast ATG11 and ATG17 is present in plants, and is required for organelle selective degradation and was dubbed as an ATG11 homolog [12]. It is not yet clear whether all *Arabidopsis* ATG1 complexes contain the ATG11-related protein. However, it has been shown that plant ATG1 and ATG13 are associated with autophagosomes that were not detectable in ATG13-deficient plants [11].

Regulation of the ATG1 complex

The ATG1 kinase in eukaryotes can interact with up to eight other proteins, generating protein complexes with different cellular functions. Although there are examples of autophagy occurring in the absence of mammalian ATG1 homologs unc-51-like kinase 1/2 (ULK1/2) [13], ATG1 kinases are required for efficient stress-induced autophagy under most circumstances in yeast and animal cells. ATG1 interaction with ATG13–FIP200/ATG17–ATG101 stabilizes the kinase, and the clustering and formation of this complex at the preautophagosomal structure (PAS) (also known as the phagophore) is required for the induction of autophagy in response to cellular stress [14-16]. In yeast, the complex containing ATG1–ATG13–ATG11 targets the kinase in the vicinity of the vacuole for cargo recruitment during selective autophagy [17]. ATG13 activates the kinase activity of ATG1, suggesting that the spatiotemporal activation of ATG1 is regulated via its association with ATG13 [18].

The ATG1 complex is also required for basic autophagy. When anabolic processes are preferred by the physiology of the cell, a decrease in catabolism includes the autophagy pathway. The signal transduction kinase TOR (target of rapamycin), in particular mechanistic TOR complex-1 (mTORC1) which positively regulates anabolic processes in mammals, inhibits ATG1 through phosphorylation of Ser-638/758, and its partner ATG13 by phosphorylation at Ser-258 [19,20]. mTORC1 interaction with ATG1 prevents the formation of the core ATG1 complex, resulting in down-regulated ATG1 kinase activity.

The best-characterized stimulus that induces autophagy in eukaryotic cells including plant is nutrient deprivation [19,21]. A high AMP:ATP ratio activates the AMP-activated protein kinase (AMPK) pathway, which stimulates catabolic processes, and in particular, activates autophagy by phosphorylating ATG1 kinase. Plant TOR and AMPK homologs are probable sensors/regulators of the autophagy pathway in plants also (see below). Irrespective of the nature of the initiating stimulus, increasing evidence shows that a series of major stress-response signaling pathways establish a strict cross-talk with autophagy via the ATG1 complex to restore homeostasis.

Downstream targets of the ATG1 kinase

ATG1 kinase can be activated by autophosphorylation. It seems that plant ATG1 kinases can form homodimers in a yeast two-hybrid assay [22]. Autophagosome formation is a tightly choreographed systematic process. The clustering and activation of the ATG1 complex at the phagophore initiate the recruitment of other autophagy core complexes,
and specifically the class III VPS34 complex. In yeast and animal cells, the class III VPS34 complex involved in autophagy contains the catalytic subunit PI3 kinase (PI3K), the regulatory subunits ATG6/Beclin-1 and ATG14, and the scaffold subunit VPS15. Remarkably, ATG14 is absent in plants. Given that ATG14 determines the localization of the VPS34 complex, and is required for both basal and induced autophagy, a structurally unrelated component should exist in plants to fulfill these roles within the VPS34 complex. ATG1 kinase phosphorylates components of the VPS34 kinase including ATG6 (at multiple sites), ATG14, and the catalytic subunit PI3K, resulting in its activation [23-26]. Class III VPS34 complexes devoid of ATG14 appear to be unaffected by ATG1 kinase, suggesting that such complexes are involved in cellular functions other than autophagy. The active autophagic VPS34 complex generates PI3P at the phagophore, and this specific lipid enrichment helps to recruit other core components which bind PI3P, for example ATG2 and ATG18. ATG9, the only membrane protein involved in autophagy, is also a substrate of ATG1 kinase. In contrast with other multicellular organisms, plants express a single, essential PI3K (class III type), which possibly also fulfills the cellular functions ascribed to the various classes of PI3Ks. The plant PI3K can form functional homodimer, in vivo (Batoko, unpublished work) and seems to be involved in plant interactions with microorganisms [27] (see also below). Recent evidence in other systems suggests that the interaction of ATG1 and ATG6/Beclin-1 in a common complex is a key requirement for autophagy induction [28]. AMPK activates autophagy by also phosphorylating ATG6/Beclin-1. The presence of ATG14 within the VPS34 complex promotes the activating phosphorylation of ATG6/Beclin-1 by the autophagy-activating AMPK [29].

The growth of the phagophore and the subsequent cargo selection requires ATG8. The soluble ubiquitin-like ATG8 associates with the growing autophagosomal membranes by conjugation to the membrane lipid phosphatidylethanolamine (PE). ATG8 processing prior to the conjugation reactions and its delipidation are catalyzed by the same ATG4 cysteine protease [30,31]. ATG4 can therefore promote autophagosome biogenesis by processing ATG8, and inactivate autophagy by delipidating ATG8 from autophagosomal membranes. Recent studies in yeast and animal cells have demonstrated that phosphorylation of ATG4 by ATG1 kinase inhibits the activity of the protease, most likely as a regulatory mechanism to prevent delipidation of ATG8 [30,31].

Post-translational deregulation of the ATG1 and VPS34 complexes

Although autophagy is mostly regarded as a positive response for cellular homeostasis, excessive or uncontrolled autophagy can be detrimental and a determinant of cell death. Stopping autophagy responses when needed may be at least as important as initiating these responses, irrespective of the stimulus. In addition to phosphorylation, autophagy initiation is controlled by ubiquitylation of the ATG1 and VPS34 complexes’ defined components. Nondegradative ubiquitylation, degradative ubiquitylation, and reversed deubiquitinases regulate autophagy by modulating protein activity or leading to protein degradation [32]. The VPS34 complex activity is modulated by the ubiquitin–proteasome system by affecting ATG6/Beclin-1 stability in particular [33]. ATG6/Beclin-1 degradation is observed during bacterial infection and the level of ATG14 is also controlled by ubiquitin-dependent degradation. Arabidopsis TRAF (tumor necrosis factor receptor associated factor) proteins, in particular TRAF1a and TRAF1b, translocate to the autophagosome during starvation, and in conjunction with the RING finger E3 ligases SINAT1/2 (seven in absentia homologs 1/2), ubiquitylate ATG6/Beclin-1. Plants lacking both TRAF1a and TRAF1b showed reduced tolerance to nutrient deficiency and increased ROS, resembling the phenotype of autophagy-deficient mutants. These findings suggest that ubiquitylation of ATG6/Beclin-1 also modulates autophagy in plants [34]. Interestingly, it was shown that the ATG1 complex in plants is also an autophagic substrate, suggesting that autophagy can self-regulate its own signaling pathway [11]. This is reminiscent of the proteaphagy regulatory process also described in plants [4].

Contribution of signaling phosphoinositides and the cytoskeleton to the regulation of plant autophagy

Phosphoinositides (PIs) are low-abundance membrane lipids that serve as key regulators of membrane trafficking and that are dynamically regulated by lipid kinases and lipid phosphatases, and control plant development and stress responses [35,36]. As mentioned above, PI3P in particular plays a central role in autophagy, and is required for the membrane recruitment of several PI3P effector proteins containing PI3P-binding domains such as the FYVE (Fab1, YotB, Vacs, and EEA1) domain, the PX (Phox homology) domain, and WD (Trp-Asp)-repeated domains, [37,38]. PI3P is not only critical for the initiation of autophagy, but is also involved in later phases of autophagy [39-42]. In plants, FYVE domain effectors such as FREE1/FYVE1 have been reported to be important for autophagy [39-42]. Some of these plant-specific FYVE domain effectors bind not only to PI3P, but also to
phosphatidylionositol-5-phosphate (PI5P) [43,44]. This is interesting because PI5P has recently been identified as an inducer of noncanonical VPS34-independent autophagy [45,46]. PI5P synthesis is possible via two pathways: either via PIKfyve kinase from PI or via myotubulin phosphatase from phosphatidylinositol-3,5-diphosphate (PI(3,5)P2). Both pathways are possible in plants as PIKfyve kinase and myotubulin phosphatases are present in Arabidopsis [47,48]. Arabidopsis myotubulins AtMTM1 and AtMTM2 control vesicular trafficking between the ER and cis-Golgi [48], and AtMTM1 controls PI5P levels associated with ROS-ABA-mediated stomatal movements [49].

The BAR domain protein SH3P2, which is structurally similar to endophilin A1 and binds PI3P, is involved in autophagosome formation, vacuole trafficking, and cell plate assembly in the plant cell [40-42,50]. Moreover, SH3P2 forms a complex with the plant dynamin DRP1A during cytokinetic cell plate assembly [50]. As dynamins are known to control autophagy and actin cytoskeleton in animal cells [51-53], it is tempting to speculate that dynamins and actin cytoskeleton also regulate plant autophagy. Interestingly, plant DRP1 is known to be involved in endocytic and/or recycling trafficking of plasma membrane lipids and proteins [54].

The role of the actin cytoskeleton in controlling autophagy in animals is well known [55-59]. It was recently shown in plants that a component of the SCAR/WAVE complex, NAP1, colocalizes with ATG8, and NAP1 knockout mutant seedlings cells are defective in autophagy [60]. Also, the recently characterized CFS1 protein, which has FYVE- and actin-binding domains, localizes to ESCRT-I-positive late endosomes, and is required for autophagosome degradation [61]. Interestingly, the NBR1-like autophagy receptor Joka2 [62] colocalizes with both actin filaments and microtubules in plant cells [63].

**Nutrient starvation regulates plant autophagy**

The regulation of nutrient recycling by autophagy was recently reviewed and for substantial details on this topic, the reader is referred to that work [64]. Experiments monitoring autophagy markers in starving plants found an activation of this pathway as measured by an increase in detectable autophagosomes [65,66]. Monitoring the transcriptome, the metabolome and enzyme kinetics of plants defective in autophagy (atg mutants) and grown under nutrient deficiency, as compared to nutrient sufficient conditions, has provided very useful information. Analysis of the atg mutants of Arabidopsis leads to the conclusion that autophagy controls carbon and nitrogen status as well as carbon and nitrogen remobilization from sources during nutrient starvation [67,68]. Etiolated atg seedlings, used as a model for carbon starvation conditions, displayed delayed growth in comparison with wild-type seedlings, and showed modified metabolic and proteomic profiles [69]. These observations are indicative of the importance of autophagy in nutrient remobilization and energy homeostasis in growing plant seedlings. These conclusions from model plant species such as Arabidopsis have been extended to crop plants, showing for example the importance of autophagy in the responses of maize to nitrogen deficiency. Autophagy was reported to be critical for maize productivity during nitrogen starvation [70]. A survey of autophagy-related genes in tobacco (Nicotiana tabacum) reported the up-regulation of many core gene such as NtATG1A, NtATG2, NtATG9, NtATG13, NtATG18, NtVSP15, and NtVSP34 during carbon- and nitrogen-starvation [71]. The significance of autophagy in plant responses to sulfur starvation is much less documented; however, the report on the induction of some autophagy related genes (ATG8 and Joka2/NBR1) in the roots of tobacco plants grown under sulfur-deficient conditions, suggests that sulfur remobilization also might, at least partially, rely on autophagy [62].

**TOR-dependent signaling of nutrient starvation in plant autophagy**

Plant TOR kinase is an integral part of the glucose-signaling network. It reprograms the plant transcriptome via phosphorylation of the transcription factor E2Fa, which activates target genes in the root meristem [72]. It also activates translation and modulates ribosome biogenesis by controlling the phosphorylation status of S6 kinase [73-76], and it plays an important role in the regulation of the primary and secondary plant metabolism [77]. Recent findings highlight the pivotal role of TOR in integrating different environmental signals, such as light, sugar, and phytohormones in the shoot and root meristem [78,79]. As mentioned above, in mammals, TOR negatively regulates autophagy, while autophagy is promoted by AMPK. TOR is a key energy sensor regulating cellular metabolism to maintain energy homeostasis [80]. Apparently, these functions are evolutionary conserved because in plants also, inhibition of TOR resulted in activation of autophagy [81]. Moreover, the elements involved in the TOR-dependent autophagy signaling cascade, namely the ATG1 complex, are also present in plants [11]. Recent data indicated that the SnRK1 complex (the plant ortholog of the mammalian AMPK), with its catalytic subunit KIN10, acts as autophagy activator both upstream [82] and downstream of TOR [83]. Inhibition of the SnRK1 complex blocked the induction of autophagy by abiotic stresses, including salt and oxidative stress, and nitrogen or carbon starvation [82].
Plant hormones and autophagy

The role of plant hormones in autophagy-related signaling is not completely clarified. The stress hormone abscisic acid (ABA) is known to inhibit the activity of plant TOR although the molecular mechanism is not yet clear. The mechanisms of phytohormones–autophagy interplay in general are not well characterized. Changes in the expression of genes related to plant hormone signal transduction pathways are observed during TOR inhibition, indicating that TOR is involved in phytohormone signaling. Dong et al. [76] shows that some genes related to ABA, ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) signal transduction were up-regulated during TOR inhibition, while the genes related to auxin, cytokinin, gibberellin, and brassinosteroid (BR) signal transduction were down-regulated [76]. In accordance, transcriptome analysis of atg mutants revealed that phytohormone signaling pathways are affected. For example, genes involved in SA and ET signaling were up-regulated [68]. Recently published data also demonstrated an interesting link between autophagy and BR signaling. Nolan et al. [84] showed that BES1, a transcription factor and a positive regulator in BR signaling, is selectively degraded during abiotic stress (drought and starvation) via the autophagy pathway. This data corroborate the earlier findings by Zhang et al. [85] who showed a similar autophagy-dependent regulation of BZR1, another transcription factor involved in BR signaling. In contrast, another phytohormone, auxin, which regulates various aspects of plant growth and development, is known to activate plant TOR activity, and was shown to inhibit autophagy during nutrient deficiency and salt and osmotic stress [75]. Auxin seems to have no effect on TOR-independent induced-autophagy resulting from oxidative or ER stress [86]. Mechanistically, it was shown that the auxin-activated small GTPase ROP2 (Rho of Plant 2) interacts physically with TOR resulting in TOR activation [87].

Hydrogen sulfide as a negative regulator of plant autophagy

In mammals, the cellular role of hydrogen sulfide (H₂S) is comparable to the role of nitric oxide [88]. The importance of H₂S homeostasis and its involvement in stress signaling and protection against some environmental cues are recognized also in plants [89]. H₂S can also function as a signaling molecule in autophagy. Analysis of the Arabidopsis des1 mutant impaired in the cytosolic production of H₂S from cysteine led to the conclusion that H₂S acts as an inhibitor of autophagy induced by nutrient deprivation [90]. Its action is independent of ROS and nitrogen starvation [91]. The mechanism of autophagy inhibition by H₂S is poorly understood. However, as speculated by Gotor and colleagues [92], H₂S-mediated signaling in autophagy might be based on the reversible post-translational modification of the enzymes involved in the ubiquitylation process or of other proteins involved in the initiation and completion of the autophagosome. The most probable mode of action of H₂S could be protein S-sulfhydration (also known as persulhydration) at the reactive cysteine residue(s) of the target proteins [93]. Such modification usually increases catalytic activity of the target proteins. Unfortunately, its significance and prevalence have not been sufficiently explored in autophagy-related studies, in contrast with other types of post-translational modifications [5,94].

Emerging role of selective autophagy receptors in maintaining plant homeostasis under stressful conditions

Recent findings have emphasized the emerging role of selective autophagy receptors in regulating plant autophagy under different stress conditions. The commonly shared feature of the candidate receptors is their proven interaction with ATG8 through and ATG8-interacting motif (AIM). Another important domain found in some of these selective autophagy receptors is a ubiquitin-binding domain and ubiquitin-like domain. Examples of such bifunctional proteins are the plant NBR1-related proteins, containing both mammalian p62 and NBR1 characteristics and named AtNBR1 in Arabidopsis [95], or Joka2 in tobacco [62] and potato [96]. Other plant selective autophagy receptors that have been characterized are DSK2 [84], AT1 [97], RPN10 [4] and the heme-binding, ABA-inducible TSPO [98]. Some of their targets are at least partially known. However, links between their involvements in selective autophagy, stress signaling, and plant adaptation to the stressful conditions remain to be deciphered (see Figure 1). How these receptors are integrated in the overall plant autophagy signaling is still not clear. It may be that their molecular target, which should have become a detrimental molecule or organelle for the physiology of the cell, is part of the signaling module.

The role of autophagy in plant–microbe interactions

Pathogens are in direct competition with their hosts to acquire nutrients and if successful at colonizing the plant, they cause starvation [99]. An extensive review of plant–pathogen interactions and autophagy regulation has been published recently [100]. As a starvation-induced survival response, it is not surprising that autophagy is linked to
the immune response against microbes [101]. Although somehow controversial and mechanistically still elusive, several papers have implicated autophagy as a positive regulator of defense against a wide range of plant pathogens [8,102,103]. Interestingly, these responses were lifestyle-dependent. For necrotrophic interactions (i.e. the pathogen needs to kill the host for survival) autophagy was found to be a negative regulator of plant defense [104].

A recent study has shown that autophagic signaling is not only limited to defense responses. Silencing of plant PI3K or plant ATG6/Beclin-1 caused a defect in nodule and mycorrhiza formation in the common bean, suggesting autophagy as an important regulator of mutualistic interactions [27].

Overall, most of these studies are still descriptive and suffer from the pleiotropic phenotypes of the core autophagy mutant plants. Future work focusing on the role of selective autophagy in innate immune responses is likely to yield important insights on this elusive defense mechanism.

In accordance with this, recent studies have shown that NBR1/Joka2-mediated selective autophagy plays a positive role against viral and oomycete pathogens. Hafrán et al have shown that NBR1 targets cauliflower mosaic virus (CaMV) capsid proteins for degradation and reduces viral colonization [105]. Similarly, overexpression of NBR1 enhanced resistance against an oomycete, Phytophthora infestans (the Irish potato famine pathogen) [96]. Both the CaMV and P. infestans have evolved counter-measures to evade the antimicrobial autophagy function. To overcome NBR1-mediated immunity, CaMV induces the formation of inclusion bodies that prevent degradation of capsid proteins, which leads to enhanced viral spreading [105]. On the other hand, P. infestans evolved a specific effector protein, named PexRD54 (an AIM-containing cargo receptor mimic) that binds host ATG8CL protein stronger than
NBR1 and depletes it from host autophagosomes [96,106]. Furthermore, PexRD54 reroutes host ATG8CL-labeled autophagosomes to the pathogen feeding sites, suggesting that the pathogen may have co-opted autophagy for nutrient uptake [107].

In contrast with P. infestans, for the cotton leaf curl Multan virus (CLCuMuV), the expression of ATG8 binding virulence protein βC1 resulted in an "Achilles heel". During infection, βC1 is targeted for autophagy via ATG8 binding which reduces CLCuMuV colonization [108]. Consistently, expression of βC1 mutants that are unable to bind ATG8s (βC1V32A) enhanced viral infection [108]. βC1 up-regulates a calmodulin-like protein (CaM) to suppress antiviral RNA silencing [109]. Recently, it has been shown that CaM suppresses RNA silencing by mediating degradation of SGS3 (an RNA-binding protein that is required for small RNA synthesis) via autophagy [110]. It will be interesting to test if a V32A mutation causes a defect in CaM up-regulation which could explain why the virus has not evolved a βC1V32A variant to evade ATG8 binding.

Together, these findings show that selective autophagy is an important regulator of antimicrobial immunity and spatiotemporal dynamics of host–microbe interactions affect the outcome of autophagy-mediated defense responses. Future studies will elucidate the intricate connections between plant immunity and autophagy. Since autophagy is a membrane trafficking-like pathway, it will be exciting to also see the role of autophagy in microbial accommodation, both in pathogenic and symbiotic interactions.

Concluding remarks
Although plant autophagy has been recognized as an integrated cellular mechanism to cope with a changing environment, the signaling transduction pathway conducive to modulating this degradative process is still poorly understood. Many basic questions remain unanswered, such as the nature of the cargo of some of the less well known cargo receptors, or the receptor of the various selective autophagy pathways described in the plant cell. It will not be surprising that at least some of the putative cargoes are themselves involved in signaling the initiation of autophagosome formation directly in their vicinity. As sessile organisms, it is also likely that plants have evolved, specific signaling pathways to modulate autophagy to fit their lifestyle or particular environment. Understanding these pathways will require full characterization of the proteins involved and the possible integration hubs. Determining the molecular composition of the ATG1 and VPS34 complexes and variant thereof would be a good start.

Summary
- Autophagy is essential for plant growth and development under normal and stressful conditions.
- Environmental cues and phytohormones can modulate autophagy in plants.
- The ATG1 complex can integrate various signaling pathways to regulate plant autophagy.
- The molecular mechanisms of autophagy signaling in plant still await experimental evidence and specifics.

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Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
AIM, ATG8-interacting motif; AMPK, AMP-activated protein kinase; ESCRT, endosomal sorting complexes required for transport; mTORC1, mechanistic TOR complex-1; PE, phosphatidylethanolamine; PI, phosphoinositide; PI3K, PI3 kinase; PI3P;
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