Autophagy: Pathways for Self-Eating in Plant Cells

Yimo Liu[1] and Diane C. Bassham[1,2]

[1]Department of Genetics, Development, and Cell Biology and Interdepartmental Genetics Program, Iowa State University, Ames, Iowa 50011

[2]Plant Sciences Institute, Iowa State University, Ames, Iowa 50011;
email: bassham@iastate.edu

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Abstract

Plants have developed sophisticated mechanisms to survive when in unfavorable environments. Autophagy is a macromolecule degradation pathway that recycles damaged or unwanted cell materials upon encountering stress conditions or during specific developmental processes. Over the past decade, our molecular and physiological understanding of plant autophagy has greatly increased. Most of the essential machinery required for autophagy seems to be conserved from yeast to plants. Plant autophagy has been shown to function in various stress responses, pathogen defense, and senescence. Some of its potential upstream regulators have also been identified. Here, we describe recent advances in our understanding of autophagy in plants, discuss areas of controversy, and highlight potential future directions in autophagy research.

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INTRODUCTION

Autophagy (meaning "self-eating") is a macromolecule degradation process in which cells recycle cytoplasmic contents when under stress conditions or during developmental transitions. The basic autophagy process is conserved among eukaryotes from yeast to animals and plants (9, 82, 135). Several types of autophagy have been described in many species, including microautophagy (84), macroautophagy (135), chaperone-mediated autophagy (92), and organelle-specific autophagy (103). In plants, microautophagy and macroautophagy have been shown to occur (11). Microautophagy involves the formation of a small intravacuolar vesicle called an autophagic body by invagination of the tonoplast, thus engulfing cytoplasmic components, whereas in macroautophagy, cytoplasmic autophagosomes enclose components to be degraded (11). Here, we focus on the macroautophagy pathway in plants, hereafter referred to as autophagy.

Autophagosome: a double-membrane structure formed upon autophagy induction; it engulfs portions of cytoplasm and delivers them to the vacuole for degradation

The principal characteristic of autophagy is the formation of double-membrane structures called autophagosomes (**Figure 1**). Upon induction of autophagy, an autophagosome forms around the material that is destined for degradation, and the autophagosome delivers this cargo to the vacuole. The outer membrane of the autophagosome fuses with the vacuole membrane, after which vacuolar hydrolases degrade both the cargo and the inner membrane in the vacuole.

Although plant autophagy was discovered several decades ago (80, 81, 120), we have only recently begun to understand its molecular mechanism. Most genes functioning in the autophagy pathway were first identified via mutagenesis studies in yeast. More than 30 autophagy-related genes have been identified in yeast; these genes can be divided into several functional groups (136): the Atg1-Atg13 kinase complex, Atg9 and associated proteins, a phosphatidylinositol 3-kinase (PtdIns3K) complex, and two ubiquitin-like conjugation systems. These studies in yeast have greatly facilitated the identification of homologous genes in plants that are required for autophagy and have provided direction for investigating their molecular functions.

In animals, autophagy is implicated in health and disease processes such as cancer, neurodegeneration, aging, and longevity (136); in plants, it is associated with a variety of stresses, pathogen infection, and senescence (9, 10, 38). Under normal conditions, basal autophagy functions as a housekeeping process to clear damaged or unwanted cytoplasmic contents, whereas under certain stresses, autophagy is upregulated (47, 108, 133). Autophagy-defective plants usually senesce earlier and are more susceptible to stress conditions compared with wild-type (WT) plants (**Table 1**). Several markers have been developed to study autophagy in plants, most commonly green

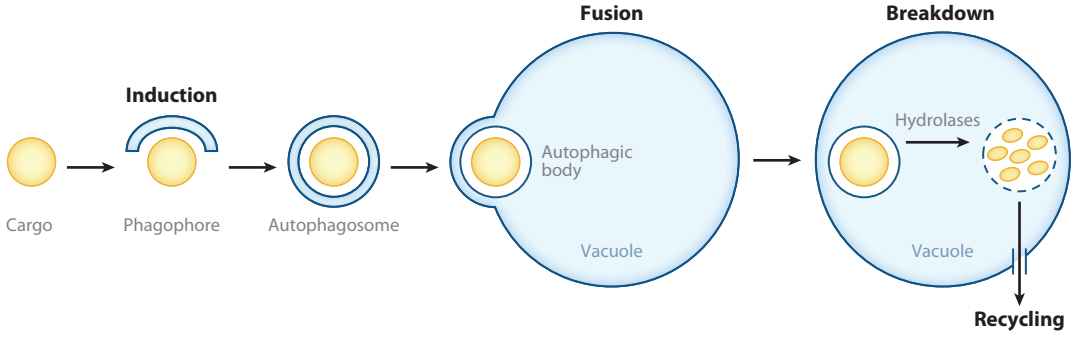

Figure 1

Pathway for autophagy in plant cells. Upon induction of autophagy, a double-membrane structure called an autophagosome forms around a portion of the cytoplasm (cargo). The autophagosome then transports the cargo to the vacuole. During the fusion process, the outer autophagosome membrane fuses with the vacuole membrane, and the remaining single-membrane structure (termed an autophagic body) is delivered inside the vacuole. The autophagic bodies are then broken down by vacuolar hydrolases, and the products are exported from the vacuole to the cytoplasm for reuse.

fluorescent protein (GFP)–ATG8s (19, 117, 142) and monodansylcadaverine (MDC) staining (19). These markers allow the rapid and straightforward detection of autophagy occurrence in plant cells by the specific labeling of autophagosomes. In this review, we summarize recent advances in our understanding of plant autophagy, including the essential machinery, regulation, and physiological roles, and briefly discuss the emerging evidence for selective autophagy.

MACHINERY AND MECHANISMS OF AUTOPHAGY IN PLANTS

Ubiquitin-Like Conjugation Systems

The yeast autophagy pathway requires two ubiquitin-like conjugation systems, which in turn involve two ubiquitin-like proteins, Atg8 and Atg12 (for reviews on mechanistic aspects, see 90, 135). After Atg8 is synthesized, its C-terminus is first cleaved by a cysteine protease, Atg4, to expose a glycine residue. The exposed

Table 1 Common phenotypes of *Arabidopsis* autophagy mutants and their corresponding affected genes

Phenotype(s)	Gene(s) involved	Reference(s)
Accelerated senescence, hypersensitivity to starvation conditions, slower growth	*ATG2*, *ATG4*s, *ATG5*, *ATG6*, *ATG7*, *ATG8*s, *ATG9*, *ATG10*, *ATG12*s, *ATG18a*, *VTI12*	16, 27, 28, 34, 36, 94, 97, 113, 117, 132, 142, 143
Stunted growth, increased anthocyanin production, decreased silique production, abnormal pollen germination	*ATG6*	28, 36, 98
Hypersensitivity to drought and salt stresses	*ATG18a*	74
Hypersensitivity to oxidative stress	*ATG18a*	107, 134
Altered resistance to pathogen infection	*ATG2*, *ATG5*, *ATG6*, *ATG7*, *ATG9*, *ATG10*, *ATG18a*	42, 62, 68, 94, 125, 143

Phosphatidylethanolamine (PE): a lipid component of biological membranes

ATG8-interacting motif (AIM): a WXXL amino acid sequence that can be recognized by ATG8

glycine is bound by an E1-like enzyme, Atg7, and the Atg8 is then transferred to an E2-like enzyme, Atg3. Finally, the Atg8 is conjugated to the membrane lipid phosphatidylethanolamine (PE). This Atg8-PE conjugation is reversible, as the protease Atg4 also cleaves Atg8 from PE; the released Atg8 can thus be recycled. Another ubiquitin-like protein, Atg12, is also activated by Atg7; it is then transferred to an E2-like enzyme, Atg10, and finally conjugated to Atg5. The Atg12-Atg5 conjugate further interacts with a coiled-coil protein, Atg16, to form a tetrameric Atg12-Atg5·Atg16 complex (in which the hyphen indicates a covalent bond and the dot indicates a noncovalent interaction) via Atg16 oligomerization. This complex is also essential for autophagy. Atg8-PE conjugates and Atg12-Atg5·Atg16 complexes reside on the preautophagosomal structure (PAS), and upon induction of autophagy, both of them localize to the expanding phagophore. Atg8-PE conjugates show an equal localization on both the inner and outer autophagosome membrane, whereas the Atg12-Atg5·Atg16 complexes associate mainly with the outer membrane (135).

In *Arabidopsis*, all of the counterparts of the two yeast conjugation systems are also well conserved (**Figure 2**). In contrast to yeast, which has a single *ATG8*, *ATG4*, and *ATG12* gene, *Arabidopsis* contains nine members of the *AtATG8* family (*AtATG8a–AtATG8i*), two members of the *AtATG4* family (*AtATG4a*, *AtATG4b*), and two members of the *AtATG12* family (*AtATG12a*, *AtATG12b*) (27, 34).

Both *AtATG4*s are ubiquitously expressed in plants, and their expression levels are elevated after nitrogen starvation (142). Phenotypic analysis of an *Atatg4a4b* double mutant showed that it displays the phenotypes of early senescence and reduced silique production that are typical of autophagy defects (142). There are contradictory reports on whether the growth of primary roots and lateral roots in the *Atatg4a4b* double mutant are arrested under nitrogen-limited conditions (16, 142). As in yeast, AtATG4s function as proteases to process the C-terminus of AtATG8s, and autophagosomes cannot form in the *Atatg4a4b* double mutant (142). These findings indicate that the *AtATG4*s are essential for plant autophagy and that they function similarly to *ATG4* in yeast.

Under normal growth conditions, all of the nine *AtATG8*s are expressed throughout the plant, although different members show distinct expression patterns (108, 142), implying that each member may have a distinct function during development or under various stress conditions. Several independent studies have demonstrated the existence of the AtATG8 ubiquitin-like conjugation system in *Arabidopsis* (16, 27, 29, 117, 142). The C-terminus of the AtATG8s is cleaved by AtATG4; bound to an E1-like enzyme, AtATG7; transferred to an E2-like enzyme, AtATG3; and finally conjugated to PE (**Figure 2**). ATG8 orthologs have also been identified in *Chlamydomonas* (96), rice (17, 112), and maize (17).

In mammals, ATG8s are divided into two subfamilies according to their protein sequence similarity: the LC3 subfamily (four members) and the GABARAP/GATE-16 subfamily (four members). The LC3 subfamily is involved in elongation of the phagophore membrane, whereas the GABARAP/GATE-16 subfamily is involved in autophagosome maturation (129). It has been suggested that in *Arabidopsis*, AtATG8s bind to microtubules, indicating the possible involvement of the cytoskeleton in plant autophagy (57). In one study, overexpression of a GFP-AtATG8f-HA fusion protein in *Arabidopsis* plants enhanced growth and altered stress responses, and cytokinin-mediated regulation of root architecture and root–shoot communication were affected (109); this suggests that *AtATG8*s may have a physiological role in responding to hormones and abiotic stresses. Recently, several studies showed that in yeast and animals, ATG8/LC3 may be crucial during selective autophagy in recognition of a specific protein motif, the ATG8-interacting motif (AIM) (89). Atg19 and p62 use the same WXXL motif to interact with ATG8/LC3 family members (88). NBR1 (neighbor of *BRCA1* gene), Atg32, and Nix also use a similar motif to bind to the ATG8/LC3 family (89). So far, in plants, two proteins have been found to use an AIM to

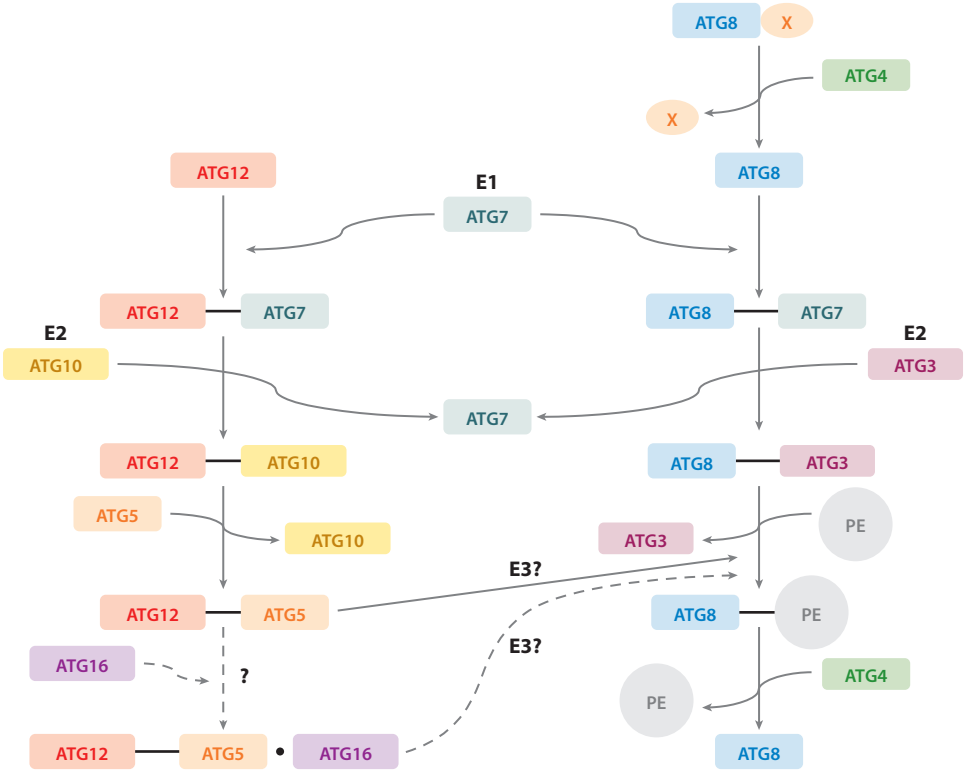

Figure 2

Two ubiquitin-like conjugation systems in *Arabidopsis*. (*Right*) The AtATG8 conjugation system. The C-terminus of the AtATG8 proteins is cleaved by AtATG4; bound to an E1-like enzyme, AtATG7; transferred to an E2-like enzyme, AtATG3; and finally conjugated to phosphatidylethanolamine (PE). The orange Xs represent C-terminal sequences of AtATG8s that are cleaved by AtATG4s (142). There are eight members in the AtATG8 family; AtATG8h and AtATG8i do not possess additional amino acids after glycine and are not thought to be subject to cleavage by AtATG4. (*Left*) The AtATG12 conjugation system. AtATG12 proteins first bind to an E1-like enzyme, AtATG7; are then transferred to an E2-like enzyme, AtATG10; and are finally conjugated to AtATG5. The AtATG12-AtATG5 conjugate may also function as an E3-like enzyme during the AtATG8-PE conjugation. Whether AtATG12-AtATG5 interacts with AtATG16 to form a tetrameric ATG12-ATG5·ATG16 complex (as it does in yeast) and whether the ATG12-ATG5·ATG16 complex is also able to function as an E3-like enzyme are still unknown (*dashed lines*).

interact with ATG8s: AtNBR1 and AtTSPO (tryptophan-rich sensory protein) (116, 122). AtNBR1 is a selective autophagic substrate that binds to AtATG8s via an AIM motif (116). The stress-induced AtTSPO is degraded by the autophagy pathway, and the AIM-like motif is required for this process (122).

In *Arabidopsis*, there are two members of the *AtATG12* gene family: *AtATG12a* and *AtATG12b* (16, 27, 34). These two genes share high amino acid similarities (95%) and have functional overlap; however, *AtATG12b* is more important during basal autophagy, whereas *AtATG12a* is more important during induced autophagy (16). All of the components of the AtATG12 conjugation system have been characterized (16, 27, 97, 117). *Atatg5*, *Atatg7*, *Atatg10*, and *Atatg12a12b* mutants are all autophagy-defective and display the typical phenotypes of early senescence and hypersensitivity to nutrient-limited conditions (16, 27, 97, 115, 117). Just as in yeast, the

AtATG12-AtATG5 conjugate functions in the formation of the AtATG8-PE conjugate (**Figure 2**) (16, 29, 33). In rice, two *ATG10* genes have been identified, *OsATG10a* and *OsATG10b* (107), which play an important role in autophagosome formation and in survival during oxidative stress (107). Maize ZmATG12 has also been identified and found to interact with ZmATG7 (17). These findings suggest that the ATG12 conjugation system is conserved in plants and is essential for the plant autophagy pathway.

ATG9 Cycling System

The source of the lipid membrane during the formation of autophagosomes is a major puzzle. In yeast, the phagophore is thought to be generated at a single perivacuolar site, the PAS (114). An integral membrane protein, Atg9, has been proposed to deliver lipid to the forming autophagosomes (39, 131). Atg9 localizes to the PAS and several non-PAS punctate structures; it is postulated that Atg9 cycles between the PAS and non-PAS structures. In yeast, the Atg9 non-PAS puncta apparently consist of tubulovesicular clusters adjacent to mitochondria, and the autophagosome membrane forms de novo (79). In contrast, mammalian cells may possess multiple PASs, and mAtg9 localizes to a juxtanuclear region corresponding to the *trans*-Golgi network and late endosomes (145). It has been proposed that in animals, the autophagosome membrane derives from several sources, including the endoplasmic reticulum (ER) (37, 139), mitochondria (32), and plasma membrane (100). In plants, however, the membrane origin is not known.

In yeast, the movement of Atg9 from non-PAS puncta to the PAS requires the function of Atg11, Atg23, and Atg27 (131, 135). The movement of Atg9 from the PAS to non-PAS puncta involves the Atg1-Atg13 kinase complex, Atg2, Atg18, and PtdIns3K complex I (131). Defects in any of these components lead to the accumulation of Atg9 at the PAS. Atg2 and Atg18 are peripheral membrane proteins, both of which can interact with Atg9 (131, 135). Atg18 can bind to phosphatidylinositol 3-phosphate [PtdIns(3)P] and phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P_2], and binding to PtdIns(3)P protects Atg8-PE from unregulated cleavage by Atg4 and is required for autophagy (85).

In *Arabidopsis*, although homologs of *ATG1*, *ATG2*, *ATG9*, *ATG13*, and *ATG18* have been found (6), of these only the *ATG2*, *ATG9*, and *ATG18* homologs have been characterized in detail. *Arabidopsis* has a single *AtATG9* gene and a single *AtATG2* gene, which are expressed ubiquitously throughout the plant (34, 125, 143). Both *Atatg9* and *Atatg2* knockout mutants display typical autophagy-defective phenotypes during senescence and stress conditions (34, 125, 143). There are eight members in the *AtATG18* gene family (*AtATG18a–AtATG18h*) (34, 132); each member has a different expression pattern, and only one (*AtATG18a*) shows an increased transcript level in starvation conditions and during senescence (132). *AtATG18a* expression is also upregulated and is required for autophagy during oxidative, salt, and osmotic stresses (74, 133, 134). RNA interference (RNAi)–*AtATG18a* plants are autophagy-defective and display a typical autophagy phenotype, and are more sensitive to various stress conditions (**Figure 3**) (74, 132–134). The identification of these counterparts to yeast genes in *Arabidopsis* may suggest conserved roles. However, their subcellular localizations and specific functions during autophagosome biogenesis remain to be determined.

PtdIns3K Complex

In yeast, a PtdIns3K complex is required for autophagy and localizes to the PAS (135). It includes a class III PtdIns3K, Vps34; a serine/threonine kinase, Vps15; Vps30/Atg6; and Atg14. Vps15 is required for the membrane association of Vps34. Atg14 is thought to connect Vps34 and Vps30/Atg6 (131, 135), whereas the function of Vps30/Atg6 is not clear. The PtdIns3K complex has been postulated to recruit PtdIns(3)P binding proteins, including Atg18, to the PAS (135).

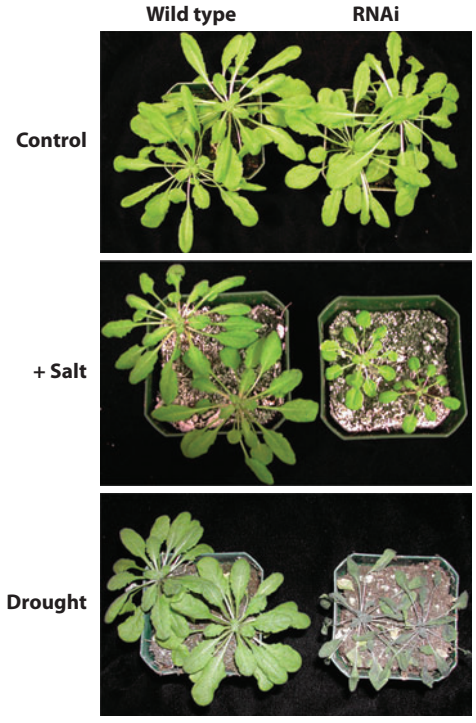

Figure 3

RNA interference (RNAi)–*AtATG18a* plants are hypersensitive to salt and drought stresses. Wild-type and RNAi-*AtATG18a* plants were grown in short-day conditions with regular watering every 2 days for 3 weeks, followed by 0.16-M salt or drought treatments for 5 weeks. In control conditions, little difference was observed between wild-type and RNAi-*AtATG18a* plants. However, under salt and drought stresses, the RNAi-*AtATG18a* plants showed decreased growth and survival. Figure adapted from Reference 74.

In *Arabidopsis*, only one PtdIns3K has been identified, AtVPS34 (130), which is an essential protein (130). It has been reported to have diverse physiological functions in plants (53, 64, 69), and genetic transmission analysis showed that *AtVPS34* is essential for pollen development and vacuole reorganization (65). *ATG6* is also a single gene in *Arabidopsis*, and it is expressed ubiquitously throughout the plant (28, 98). AtATG6 colocalizes with AtATG8s to autophagosomes (28, 36). Several independent reports have shown that *AtATG6* gene function is essential for pollen germination, although this phenotype may be unrelated to its role in autophagy (28, 36, 98). Plants with disrupted *AtATG6* have increased anthocyanin production, short roots, early leaf senescence, dwarfism, fewer flowers, and low fertility (36, 94, 98). Also, *AtATG6* antisense plants fail to limit the pathogen-associated cell death response (94). In tobacco (*Nicotiana benthamiana*), *NbATG6* is required for pathogen-induced autophagy and regulation of programmed cell death (PCD) (73). There is one *VPS15* homolog in the *Arabidopsis* genome (6), but its function has not been studied in detail; *ATG14* appears to be missing. Moreover, how these genes are coordinated to regulate autophagy in plants is still unclear.

Vti12 SNARE

After the double-membrane autophagosome forms, it is delivered to the vacuole for further degradation. This process requires the fusion of the autophagosome membrane with the vacuole membrane. Several components of the SNARE (soluble *N*-ethylmaleimide-sensitive-factor attachment protein receptor) machinery are required for the fusion process and autophagosome membrane expansion in yeast (86, 135). Several homologs of the VTI1 vesicle SNARE are present in *Arabidopsis* (105), and one of these homologs, VTI12, is thought to function during autophagy (113). A *vti12* mutant is more sensitive to starvation conditions and shows an accelerated senescence phenotype, suggesting a role in plant autophagy (113). The partner target SNAREs of VTI12 that may also function in autophagy are not known.

FUNCTIONS OF AUTOPHAGY DURING ABIOTIC STRESS

The first and most common abiotic stress shown to induce autophagy was nutrient deprivation (27, 34, 97, 117, 132). Autophagy-defective plants display accelerated starvation-induced chlorosis, most likely because autophagy is required for nutrient remobilization during the starvation response. In recent

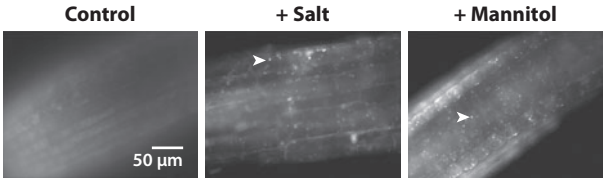

Figure 4

Autophagy is induced under salt and osmotic stresses. A fluorescence microscope was used to visualize autophagy induction in 7-day-old green fluorescent protein (GFP)–AtATG8e transgenic *Arabidopsis* roots. After treatment with 0.16-M NaCl or 0.35-M mannitol, numerous GFP-AtATG8e-labeled autophagosomes appeared, whereas few were present in control conditions. Arrows indicate GFP-labeled autophagosomes. Figure adapted from Reference 74.

years, autophagy has been shown to be a rather general response to a variety of abiotic stresses (9). When oxidative stress is introduced using H_2O_2 or methyl viologen, autophagy is quickly induced. The autophagy-defective RNAi-*AtATG18a* plants are more sensitive to methyl viologen treatment and accumulate higher levels of oxidized proteins (133, 134); similar phenomena have also been observed in a rice *Osatg10* mutant (107). These results suggest a role for autophagy in degrading oxidized proteins in plant cells. Autophagy is also required for plant tolerance to drought and salt stresses (**Figures 3** and **4**) (74), implying that autophagy plays a role in removing damaged proteins or organelles during these stresses. AtTSPO, which is an abscisic acid–induced protein, has been shown to be degraded by the autophagy pathway, indicating the involvement of autophagy in responses to abscisic acid in plants (122).

FUNCTIONS OF AUTOPHAGY DURING DEVELOPMENT

Although autophagy has been investigated most extensively during stress conditions, plants maintain a basal level of housekeeping autophagy even under favorable growth conditions (47, 108, 138). This basal autophagy may function to eliminate damaged proteins and organelles, which are continually generated under normal growth conditions (11). Almost all of the autophagy-defective mutants are able to complete their life cycles but have an early-senescence phenotype even under nutrient-rich conditions (27, 34, 94, 97, 98, 117, 132, 142), which suggests that autophagy has some function under these conditions. Because autophagy recycles cytoplasmic materials, it is understandable that it functions during senescence and germination, the two large-scale nutrient remobilization processes in the plant life cycle. About 80% of leaf nitrogen is contained in chloroplasts (78); during senescence, plants recycle nutrients from senescing leaf chloroplasts to the newly forming organs such as developing seeds (31). Small vesicles containing chloroplast stromal components [RuBisCO-containing bodies (RCBs); for more discussion, see Evidence for Selective Autophagy, below] have been shown to be degraded by the autophagy pathway (48, 49, 123, 124). In *Arabidopsis*, chloroplasts normally decrease in both size and number during senescence, which does not occur in an autophagy mutant (124). This raises an interesting question regarding the relationship between autophagy and chloroplast breakdown during leaf senescence. If autophagy is involved in chloroplast degradation, delayed senescence might be predicted if autophagic degradation is impaired. However, autophagy-defective mutants unexpectedly show early-senescence phenotypes, with accelerated loss of chlorophyll and chloroplast proteins. This indicates that an *ATG* gene–independent mechanism exists that is at least partially responsible for chloroplast recycling (11, 71)—for example, the action of chloroplast-localized proteases. One possibility is that autophagy is normally activated early in the senescence pathway to begin the protein degradation process, leaving the photosynthetic machinery intact to continue photosynthesis. Additional catabolic pathways, including chloroplast-localized pathways for chlorophyll degradation, would be activated later in the senescence process (43). It is therefore hypothesized that when autophagy is blocked, the autophagy-independent pathways are activated prematurely, leading to early breakdown of chloroplast components and premature senescence.

RuBisCO-containing body (RCB): a small vesicle containing only stromal components that pinches off from the chloroplast during senescence

To ensure seed viability, plants synthesize large amounts of seed storage proteins and deposit them into protein storage vacuoles (PSVs) during seed development. Upon seed germination, these proteins are degraded to support the growth of newly forming organs (46). In wheat, during seed development, the prolamin storage proteins are synthesized in the ER and form ER protein bodies; the direct transport of prolamins from ER to PSVs involves a pathway that, under electron microscopy, resembles autophagy (70). In *Vigna mungo*, breakdown of starch granules during seed germination is also associated with the autophagy pathway (119). In *Arabidopsis*, several *ATG* genes are upregulated during seed maturation and desiccation (5), but no obvious defect in seed formation or germination has been observed in the *ATG* mutants under normal growth conditions. During salt stress, the germination of RNAi-*AtATG18a* seeds lags behind that of WT seeds (74), implying that autophagy may be involved in salt tolerance during seed germination. In maize aleurone cells, the prolamin storage protein zein was recently found to be delivered from ER to PSVs in atypical prevacuolar compartments. These zein-containing compartments have multilayered membranes and engulfed cytoplasmic materials, thus morphologically resembling autophagosomes (104). However, they are neither surrounded by a double membrane nor decorated by the Atg8 protein, suggesting that there is an atypical autophagy pathway to deliver storage proteins from ER to PSVs that is independent of *ATG8* (104).

It has been previously suggested that autophagy plays a role in vacuole biogenesis (11, 80). However, *ATG* mutants do not have vacuole defects in either nutrient-limited or nutrient-rich conditions. Research in tobacco (BY-2) miniprotoplasts (protoplasts lacking the large central vacuole) demonstrated that the autophagy pathway involved in vacuole biogenesis is mechanistically distinct from the stress-induced and basal autophagy observed throughout the plant growth cycle (137). In miniprotoplasts, after researchers used cysteine protease inhibitors to inhibit vacuolar protein degradation, cytoplasmic contents were detected in the newly generated vacuole, indicating the occurrence of autophagy during this process. However, inhibitors typically used to block stress-induced and basal autophagy did not affect the autophagy in miniprotoplast vacuole formation, suggesting that this is an atypical autophagy with a mechanism different than that of stress-induced and basal autophagy (137). In animals, alternative autophagy pathways that use only part of the canonical autophagy machinery to form functional autophagosomes have been described, including Atg5/Atg7-independent autophagy (87) and Beclin-1-independent autophagy (106). However, whether such noncanonical autophagy pathways exist in plants is still unknown.

FUNCTIONS OF AUTOPHAGY DURING PROGRAMMED CELL DEATH

In animals, PCD can be morphologically divided into three types: apoptosis, autophagic cell death, and necrosis (59). However, there is not an absolute distinction between the different forms of cell death, as several examples display mixed features (59). To further complicate analysis, in many cases that are defined as autophagic cell death, the studies show only that PCD occurred with concomitant activation of autophagy, rather than that the PCD process was carried out by autophagy (59). In plant cells, the typical animal apoptosis process does not seem to occur, because the presence of a cell wall prevents the dead cell from being engulfed by adjacent cells. Plant PCD seems to share some features with both apoptosis and autophagy in animals (for a more comprehensive review on plant PCD, see 63, 121). For example, the cell takes up organelles into the vacuole, the organelles are degraded, the vacuolar size increases, and eventually the vacuole lyses; these are all characteristics of autophagic cell death. However, the cell also undergoes chromatin condensation, nuclear fragmentation, and DNA laddering, which are characteristics

Hypersensitive response (HR): a mechanism used by plants to limit the spread of pathogen infection

Salicylic acid (SA): a plant hormone implicated in immune responses and senescence

of apoptotic cell death. In mammalian PCD, a group of cysteine proteases called caspases are important regulators of apoptosis (21). True caspase homologs have not been identified in plants (101), although several groups have found caspase-like activities. In barley, VEIDase was found to have a caspase-like activity; it is localized to autophagosomes, linking the caspase activity to autophagic PCD (12). In *Arabidopsis*, type I metacaspases were also shown to control cell death (18). Collectively, known features of PCD in plants indicate that classification is not as clear as it is in animals. Plant PCD seems to have conserved functions but also unique characteristics. Research on plant PCD has focused mainly on two categories: PCD during normal development and PCD during the hypersensitive response (HR) triggered by pathogen infection. Evidence implies that autophagy plays critical roles in both processes.

Functions of Autophagy During Developmental Programmed Cell Death

There are many well-known examples of PCD during various developmental stages (121), including xylem and phloem formation, senescence, shoot elimination, leaf shape formation, and pollen germination and tube growth. Morphological studies have shown that most of these processes involve the gradual disappearance of organelles and eventually the collapse of the tonoplast and plasma membrane, and it has been suggested that autophagy is responsible for the cell death (121). In wheat, accelerated plant development caused by long-day growth conditions can trigger developmentally generated sugar starvation, which in turn initiates autophagic cell death of florets and leads to decreased fertile floret numbers (30). The involvement of autophagy was also demonstrated by the analysis of PCD during xylem fiber maturation in *Populus*. Not only does this type of PCD morphologically resemble autophagic cell death, but several autophagy-related genes are upregulated (20).

Recently, one study (61) showed that autophagy also functions in xylem tracheary element (TE) differentiation in *Arabidopsis*. In this study, both protoxylem and metaxylem cell numbers decreased significantly in an *Arabidopsis atg5* mutant compared with WT plants, suggesting that ATG5-dependent autophagy is involved in xylem development. LysoTracker Green staining showed that autophagy is induced during xylem differentiation. In addition, the study showed that a small GTP-binding protein, RabG3b, which was previously identified as a salicylic acid (SA)–responsive protein, is a positive regulator of autophagy during TE differentiation. These results demonstrate that RabG3b functions as a component of autophagy and regulates TE differentiation by activating the PCD process.

Functions of Autophagy During Pathogen Infection

It has been suggested that autophagy can serve both a "prosurvival" and a "prodeath" role upon pathogen infection, depending on the type of pathogen, the type of immune factor involved, and the age of the plant (38, 42, 67, 68, 140, 144). Phytopathogens can be divided into two types based on their lifestyles: necrotrophic and biotrophic. Necrotrophic pathogens release toxins and lytic enzymes to kill the host cell, whereas biotrophic pathogens depend on their host cell to survive (67). In *Arabidopsis*, infection with the necrotrophic fungal pathogen *Botrytis cinerea* induces autophagy in both the infected and the surrounding areas (62). Compared with WT plants, autophagy-defective mutants are hypersensitive to *Botrytis cinerea* and *Alternaria brassicicola*: They have more dead cells, the degradation of certain proteins accelerates (62), and they develop spreading necrosis (68). Interestingly, the transcription factor WRKY33, which is important for plant resistance to necrotrophic pathogens, interacts with the autophagy protein ATG18a in the nucleus (62). These results clearly show that autophagy functions in a prosurvival role during necrotrophic pathogen infection.

The role of autophagy upon biotrophic pathogen infection is more complicated. When tobacco (*Nicotiana benthamiana*) plants are infected with tobacco mosaic virus, autophagy is induced in both the infected and the uninfected area (73). *BECLIN1/ATG6/VPS30*-silenced plants are capable of initiating HR-PCD; however, the PCD is unrestricted and spreads to healthy uninfected tissue and distal leaves (73). Similar results were later obtained in *Arabidopsis ATG6* RNAi plants and *atg5* knockout mutants (94, 143). After infection with an avirulent bacterial pathogen, *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (*avrRPM1*), the HR-PCD escapes from the infected area and spreads to adjacent healthy tissues (94, 143). These results suggest that autophagy functions to restrict the HR-PCD and prevent runaway cell death, thus serving a prosurvival role. However, another study has reported that cell death is suppressed and resistance is increased in *atg7* and *atg9* mutants infected with the avirulent bacterial strain *Pst* DC3000 (*AvrRps4*) (42). Similarly, the *atg2* mutant displays enhanced powdery mildew resistance (125). Additional *Arabidopsis* autophagy-defective plants were recently tested for their immunity response to different types of pathogens (68); compared with WT plants, the *atg*-defective plants all showed enhanced resistance to the virulent bacterial strain *Pst* DC3000 or to the avirulent *Hyaloperonospora arabidopsidis*, possibly owing to an elevated SA level in the *atg* mutant (discussed below) (42, 68). These results indicate that autophagy serves a prodeath role upon biotrophic pathogen infection. It has been suggested that the contradictory results during biotrophic pathogen infection are caused by the age differences in plants used in the experiments, which is related to SA signaling (see below). The spreading of the PCD was observed only in older leaves of older plants (7–8 weeks old), whereas no difference was observed in younger leaves or younger plants (4–5 weeks old) (38, 62, 143, 144).

The difference in autophagic response to necrotrophic and biotrophic pathogens may be due to the engagement of different plant hormones. The defense response to necrotrophic pathogens depends on jasmonic acid (JA) and ethylene signals, whereas the defense response to biotrophic pathogens involves the SA signal (52, 58, 111). Because the *atg* mutants display altered immune response to both types of pathogens, several research groups have analyzed hormone levels and the expression of hormone-regulated genes during pathogen infection in both WT and autophagy-defective plants (62, 68, 143). Compared with uninfected WT plants, uninfected *atg5*, *atg7*, and *atg18a* mutants had significantly higher basal JA levels and higher transcription levels of a JA-regulated defense gene, *PDF1.2* (62, 143). Upon infection with the necrotrophic fungus *Botrytis*, the *PDF1.2* transcription level was greatly reduced in the *atg* mutants compared with WT plants, despite the elevated basal level. This result suggests that autophagy negatively regulates JA-mediated *PDF1.2* gene expression in healthy plants but positively regulates its level in infected plants (62).

SA was also elevated in uninfected *atg* mutants (62, 68, 143). When *atg* mutants were crossed with several SA-signaling defective mutants, both the early-senescence phenotype and the unrestricted PCD during *Pst avrRpm1* infection were rescued (143). Crossing the *atg* mutants to JA-related mutants did not produce the same result, suggesting a JA-independent mechanism (143). Upon treatment with the SA agonist benzo-(1,2,3)-thiadiazole-7-carbothioic acid, autophagy was induced in WT plants but not in the *npr1* mutant (a downstream component of SA signaling), indicating that SA-induced autophagy is dependent on NPR1 (143). Collectively, these results demonstrate that the premature senescence and the infection-induced spreading of HR-PCD are the result of an increased basal SA level in the *atg* mutants, and that in WT plants autophagy functions to eliminate SA via a negative-feedback loop, which in turn suppresses the senescence and unrestricted PCD (141, 143).

The discovery of the involvement of SA signaling may help to explain three phenomena.

TOR (target of rapamycin): a PtdIns3K-related kinase that functions as a serine/threonine protein kinase

First, the increased resistance to biotrophic pathogen infection in *atg* mutants may result from the constitutively slightly enhanced SA level and therefore increased expression of SA-induced defense genes (67). Second, as mentioned above, the spreading of HR-PCD in *atg* mutants has been observed only in the older leaves of older plants, and the increased resistance has been observed in younger leaves and younger plants (42, 94, 143). It is possible that in the younger plants, the *atg* mutants have only a slightly increased SA level, which is responsible for the increased resistance during pathogen infection. However, the older *atg* mutants generate more SA, which leads to the phenotypes of early senescence and hypersusceptibility to infection (38, 144). Autophagy can serve both a prodeath and a prosurvival role during biotrophic pathogen infection, and this determination is age dependent. Third, the role of autophagy during starvation stress is well understood: It helps to recycle nutrients, leading to increased sensitivity of *atg* mutants to starvation stress. However, the role of autophagy in nutrient-rich conditions is still under investigation. It has long been known that *atg* mutants have an early-senescence phenotype, but the underlying mechanism is unclear. Now it has been shown that senescence is modulated by the SA level, and autophagy functions to eliminate excessive SA, thus preventing premature senescence (140, 141).

REGULATION OF AUTOPHAGY IN PLANTS

A milestone in our understanding of the regulation of autophagy was the identification of the TOR (target of rapamycin) kinase (13, 60). TOR is a PtdIns3K-related kinase that functions as a serine/threonine protein kinase and as a nutrient sensor that integrates multiple upstream signals (45). The antibiotic rapamycin inhibits TOR kinase activity by forming a ternary complex with the FRB (FKBP12 and rapamycin binding) domain of TOR and the FKBP12 protein (15). In yeast, two TOR genes have been identified; plants, mammals, and other eukaryotes have only one TOR gene (95). In both yeast and animals, TOR forms two different functional complexes, TORC1 and TORC2, each of which contains distinct TOR binding partners (75). TORC2 controls spatial cell growth by regulating polarization of the actin cytoskeleton in a rapamycin-insensitive manner, whereas TORC1 is sensitive to rapamycin and controls temporal cell growth by promoting translation, transcription, ribosome biogenesis, and nutrient transport and negatively regulating autophagy (25, 75, 135). In yeast, TORC1 regulates the Atg1-Atg13-Atg17 complex depending on nutritional status. Under normal conditions, TORC1 is activated and causes the phosphorylation of Atg13; this hyperphosphorylated form of Atg13 has a low affinity for Atg1 and Atg17. Upon starvation or rapamycin treatment, TORC1 is inactivated and causes the dephosphorylation of Atg13; this hypophosphorylated form of Atg13 has a higher affinity for Atg1 and Atg17. The Atg1 kinase activity is activated after its interaction with Atg13 and Atg17, leading to the induction of autophagy (54, 135). Surprisingly, even though the key components of this regulatory pathway are conserved, in mammals and *Drosophila* the regulation and relationships between TORC1, Atg1, and Atg13 are divergent (40). Intriguingly, despite TOR being a key regulator for nutrient stress–induced autophagy, studies in HeLa cells and primary fibroblasts suggest that autophagy induced upon pathogen infection may be TOR independent (126).

The TOR homolog in *Arabidopsis* has been identified (83), but the investigation of TOR function is impeded for two reasons: First, disruption of the *AtTOR* gene is embryonic lethal (83), making the study of its postembryonic function difficult; and second, the *Arabidopsis* FKBP12 protein does not bind to rapamycin, making it insensitive to rapamycin, which means that this inhibitor cannot be used to study the TOR pathway in this species (77). Several strategies have been developed to overcome these problems. Transgenic *Arabidopsis* plants expressing yeast ScFKBP12 showed rapamycin sensitivity and displayed

rapamycin-dependent arrest of root growth (110). *Arabidopsis* transfer-DNA insertion lines with increased *AtTOR* expression and RNAi lines with decreased *AtTOR* expression showed that root and shoot growth are correlated with TOR expression level, indicating a role in growth regulation (23).

Recently, a mutant screen for suppressors of the root-hair cell-wall-formation mutant *lrx1* led to identification of the *rol5* mutant (66). ROL5 is structurally and functionally similar to the yeast Ncs6 protein, which is a component of the yeast TOR pathway. The authors suggested that the suppression of *lrx1* by *rol5* is based on alteration of TOR signaling, because treating *lrx1* plants expressing yeast FKBP12 with rapamycin also relieves the *lrx1* phenotype. This finding reveals that the TOR pathway is involved in cell wall formation in *Arabidopsis* (66).

Two studies provided direct evidence that TOR is a regulator of autophagy in photosynthetic species (72, 96). The green alga *Chlamydomonas reinhardtii* is sensitive to rapamycin, and autophagy was induced upon rapamycin treatment (96). In *Arabidopsis*, RNAi-*AtTOR* plants showed constitutive activation of autophagy (72). These results indicate that TOR function is conserved and that this factor serves as a negative regulator of autophagy in plants (**Figure 5**).

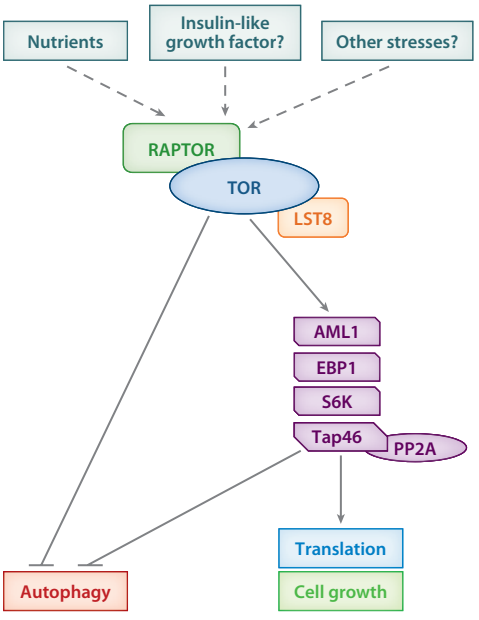

Figure 5

Potential TOR signaling pathways in plants. The TORC1 complex, including TOR, RAPTOR, and LST8, senses and integrates multiple upstream signals such as nutrient starvation, insulin-like growth factors, or other stresses (*dashed arrows*). TOR may serve as a negative regulator of autophagy. Some TORC1 substrates have been identified, including AML1, EBP1, Tap46 (a regulatory subunit of PP2A), and S6K. These substrates may function to control translation, cell growth, and possibly autophagy.

As mentioned above, TOR works in two different complexes. Although the TORC2 subunits remain to be identified in plants, some of the TORC1 binding partners have been identified (25). These include Raptor (35), which recruits substrates and presents them to TOR for phosphorylation, and LST8 (128), which stabilizes the TOR complex. In animal cells, rapamycin inhibits TOR activity partially by uncoupling the TOR/RAPTOR interaction (93). Two *RAPTOR* homologs have been identified in *Arabidopsis*: *AtRAPTOR1A* and *AtRAPTOR1B* (4, 22). Both of them are expressed in growing tissues throughout the plant, although *AtRAPTOR1B* is the most highly expressed isoform (4, 22). Disruption of *AtRAPTOR1A* has no obvious phenotype, whereas disruption of *AtRAPTOR1B* has been reported to have two contradictory phenotypes. One study showed that loss of AtRAPTOR1B causes seed abortion and a complete arrest of embryo development at a preglobular stage (22). Another showed that an *Atraptor1B* mutant is viable but has defects in both root and shoot growth, resulting in delayed development (4). An *Atraptor1A Atraptor1B* double mutant exhibited normal embryonic development but was unable to maintain postembryonic meristem-driven growth (4). This phenotype discrepancy can potentially be explained by a variation in growth conditions, because the addition of 1% sucrose to the growth medium partially rescued this phenotype (4). Another TOR binding partner,

LST8, has been identified in *Chlamydomonas reinhardtii* (24). CrTOR and CrLST8 exist in a large complex in which CrLST8 interacts with the CrTOR kinase domain; together, they colocalize to ER membranes. *CrLST8* is able to complement the yeast *lst8* mutant, implying a conserved function for this protein (24).

As a kinase, TOR signals through a combination of direct phosphorylation of downstream targets and repression of phosphatase activity (99). Recent research has shown that TOR controls embryogenesis and postembryonic development through its kinase domain in *Arabidopsis* (102). To date, several potential TOR substrates have been identified in plants. As mentioned above, the Atg1-Atg13-Atg17 complex is a TORC1 substrate, and in yeast and animals it is regulated by TORC1 depending on the nutritional status. In *Arabidopsis*, three putative ATG1 homologs and two putative ATG13 homologs have been identified (25); however, their functions and interactions have not been experimentally studied. In yeast, autophagy is regulated through PP2A (protein phosphatase type 2A), and a regulatory subunit of PP2A, Tap42/α4, has been shown to be a downstream substrate of the Tor protein. Tap42 is phosphorylated and tightly associates with PP2A under nutrient-rich conditions; upon starvation or rapamycin treatment, Tap42 is dephosphorylated and dissociates from PP2A (135). A plant homolog of Tap42/α4, Tap46, has been identified in both *Arabidopsis* and tobacco (*Nicotiana tabacum*) (2). Tap46 interacts with PP2A and is phosphorylated by TOR, suggesting that Tap46 is a direct substrate of TOR. Loss of Tap46 function results in growth arrest and activation of autophagy. These findings suggest that Tap46 functions as a component of the TOR signaling pathway (2).

Mei2 is a meiosis signaling molecule that has been suggested to be a potential TOR substrate in yeast (127). In *Arabidopsis*, the Mei2 homolog AML1 (*Arabidopsis* Mei2-like1) interacts with RAPTOR1B in a yeast two-hybrid assay, which implicates AML1 as a candidate substrate of TOR (3). EBP1 (ErbB-3 epidermal growth factor receptor binding protein) is a nucleolar and cytoplasmic regulator of ribosome assembly and translation; it has been shown to regulate cell growth and proliferation in plants (44). In *Arabidopsis*, the expression of the *EBP1* gene is correlated with TOR expression level, which indicates that EBP1 might be a possible substrate that functions downstream of TOR (23). S6K (ribosomal p70 S6 kinase) is a translational regulator that interacts with RAPTOR in *Arabidopsis*, which also makes it a potential TOR substrate (77). However, the relationship of these potential TOR substrates to autophagy induction has not been tested.

In animals, insulin is a key upstream regulator of the TOR pathway (7), and a maize insulin-related peptide, ZmIGF (*Zea mays* insulin-like growth factor), promotes cell growth via ZmTOR kinase activity (26). Additionally, maize has been shown to be responsive to rapamycin treatment (1) and the TOR-S6K pathway seems to be conserved (26), making maize a possibly superior model for studying the TOR-autophagy pathway in plants.

In yeast and animals, other signaling pathways that control autophagy have been identified. For example, the mammalian AMPK (AMP-activated protein kinase) and its yeast homolog SNF1 (sucrose nonfermenting 1) have been shown to regulate autophagy induction (135). In plants, SNF1/AMPK-related kinases (SNRKs) have been identified as SNF1/AMPK homologs. The *Arabidopsis* SNRK AKIN10 has been shown to induce several autophagy genes (8), suggesting a conserved role for AMPK in the regulation of autophagy in plants. However, further analysis is needed to confirm this interpretation.

EVIDENCE FOR SELECTIVE AUTOPHAGY

In general, autophagy is considered to be a nonselective degradation process. However, in yeast and mammals, several studies have shown that certain organelles or protein aggregates can be selectively targeted by autophagy (for a more comprehensive review on selective autophagy, see 51, 103, 135). In

yeast, the biosynthetic cytoplasm-to-vacuole targeting pathway is a unique type of selective autophagy. The precursors of the vacuolar hydrolases Ape1 (aminopeptidase 1) and Ams1 (alpha-mannosidase 1) are delivered into the vacuole via double-membrane vesicles. The cargo receptor Atg19 recognizes and interacts with both the cargo and Atg8, and together they are transported to the PAS (135). Atg19 recognizes Atg8 through the AIM (see above) (88); this process also requires Atg11 as an adaptor. Various organelle-specific autophagy types have also been described in yeast (103)—for example, the selective degradation of mitochondria (mitophagy), peroxisomes (pexophagy), and ribosomes (ribophagy). In animals, two autophagic adaptors, p62/SQSTM1 (sequestosome 1) and NBR1, have been studied extensively and seem to play an important role during selective autophagy (51). Both p62 and NBR1 harbor a ubiquitin-associated domain that can bind ubiquitinated proteins and an AIM that can interact with Atg8/LC3. It has been proposed that both p62 and NBR1 are selective autophagy substrates and work as cargo receptors: First, protein aggregates are ubiquitinated, and then p62 and NBR1 are recruited to the ubiquitinated substrates; this is followed by interaction with Atg8, leading to the formation of autophagosomes around the cargo (51).

Plant NBR1 homologs were recently identified. In *Arabidopsis*, a single *AtNBR1* (At4g24690) gene has been identified; it binds ubiquitinated proteins via a C-terminal ubiquitin-associated domain and interacts with AtATG8 through the AIM motif (116). In tobacco (*Nicotiana tabacum*), Joka2 has been identified as a structural and possibly a functional homolog of p62 and NBR1 proteins. Joka2 interacts with NtATG8f, and its expression level increases during nutrient deprivation conditions (146). These findings suggest that the selective autophagy machinery is conserved to some extent from yeast to animals and plants.

In plants, mitophagy and pexophagy have not been studied, although some research indicates that autophagy is capable of selectively removing certain proteins or structures. In BY-2 cells, a fusion protein between cytochrome b5 (Cyt b5) and red fluorescent protein (RFP) is transported to the vacuole for processing during nitrogen starvation (118). Interestingly, the vacuolar degradation rate of Cyt b5–RFP appears to be faster than that of other proteins, and the percentage of colocalization between Cyt b5–RFP and yellow fluorescent protein (YFP)–Atg8 is higher than with mitochondria, indicating that the Cyt b5–RFP proteins are engulfed by autophagosomes at a higher frequency. This study suggests that there is some autophagy selectivity toward the Cyt b5–RFP proteins (118).

In *Arabidopsis*, RNS2 is a conserved ribonuclease of the *RNase T2* gene family, and is essential for normal ribosomal RNA (rRNA) decay. *Arabidopsis* plants lacking RNS2 activity have longer-lived rRNA, accumulate RNA in the vacuole, and have constitutive autophagy (41). It has been proposed that RNS2 may participate in a possible "ribophagy"-like mechanism in plants, functioning in ribosome turnover under normal growth conditions. The absence of RNS2 disrupts cell homeostasis, resulting in constitutive autophagy to restore the housekeeping role of the ribophagy-like process (41, 76).

As mentioned above, plants recycle nutrients from senescing leaf chloroplasts to newly forming organs at least partially through the autophagy pathway. During leaf senescence, small vesicles containing only stromal components (RCBs) pinch off from the chloroplasts and are delivered to the vacuole. GFP-AtATG8 colocalizes with stroma-targeted DsRed in spherical bodies in the vacuole, and in an *atg5* mutant this vacuolar RCB accumulation is compromised. These data suggest that after the RCBs form, they are enclosed within autophagosomes and delivered to the vacuole for degradation (14, 49, 50, 124). The discovery of RCBs demonstrates that autophagy selectively degrades stromal fractions of chloroplasts during leaf senescence. This exciting finding indicates that autophagy can thereby degrade certain components of an organelle and not others.

Despite the emerging evidence for organelle-specific autophagy in plants, its underlying mechanism is still unclear. As mentioned above, cytoplasmic ubiquitinated proteins may be recognized by p62 and NBR1 and thus selectively incorporated into autophagosomes. However, how organelles or parts of organelles are recognized for degradation is unknown. In yeast, the mitophagy-specific protein Atg32 serves as a tag for mitochondrial degradation (56, 91). Atg32 localizes on the mitochondrial outer membrane, binds to the selective autophagy adaptor Atg11, and along with mitochondria is further recruited to the vacuole for degradation. However, Atg32 and another mitophagy-specific protein, Atg33 (55), do not seem to have corresponding plant homologs (103). This raises the question of whether plants have other types of organelle-specific autophagy, such as mitophagy or pexophagy. If they do, do plants use a different set of genes or mechanisms? Future studies are anticipated to address these questions.

SUMMARY POINTS

1. The autophagy core machinery is conserved from yeast to animals and plants, although some of the identified yeast autophagy (*ATG*) genes are present as gene families in plants. Several autophagy-defective plants with impaired *ATG* genes have been characterized; most of them are able to complete their life cycles but display early senescence and hypersensitivity to starvation conditions.
2. Plant autophagy functions during various abiotic stresses, including nitrogen and carbon starvation as well as oxidative, salt, and osmotic stresses.
3. Plants have a basal level of housekeeping autophagy even under favorable growth conditions, which may function during vacuole biogenesis, help in the elimination of damaged proteins and organelles, and remobilize nutrients during leaf senescence and seed germination.
4. Plant autophagy is involved in PCD during pathogen infection. However, its exact role is unclear. The SA signal may mediate this process.
5. Just as in yeast and animals, evidence suggests that the TOR kinase serves as a negative regulator of autophagy in plants. Some components of the TOR complexes and TOR substrates have also been identified in plants.

FUTURE ISSUES

1. Although many yeast *ATG* gene homologs have been identified in plants, a few genes are missing from plant genomes, and some genes have expanded to gene families. It will be interesting to determine whether novel autophagy genes exist in plants and whether the genes within families have different roles under different circumstances.
2. The role of autophagy upon pathogen infection is still unclear. Several studies show that autophagy can function in both a prosurvival and a prodeath role. It seems that both pathogen type and plant age can affect the plant response, and the hormone SA is implicated. The role of autophagy and the signals that determine autophagy function during the immune response still need to be resolved.

3. Although TOR probably serves as an autophagy regulator in plants, its upstream signals and downstream substrates that control the autophagy pathway still need to be investigated. Also, TOR-independent regulatory pathways are yet to be unveiled.
4. Do plants also have organelle-specific autophagy? If so, do they use the same mechanisms as in yeast?
5. Despite the important role of plant autophagy, autophagy-defective plants are able to complete their life cycles. Whether a compensatory pathway exists that ensures survival when the autophagy pathway is impaired remains an open question.

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