Dynamics of Autophagosome Formation[1][OPEN]

Junmarie Soto-Burgos,[a,2] Xiaohong Zhuang,[b,2] Liwen Jiang,[b] and Diane C. Bassham[a,3]

[a]Department of Genetics, Development and Cell Biology, Iowa State University, Ames, Iowa 50011
[b]Centre for Cell and Developmental Biology, State Key Laboratory of Agrobiotechnology, School of Life Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

ORCID IDs: 0000-0002-2480-8210 (X.Z.); 0000-0001-7411-9360 (D.C.B.).

Autophagy, literally defined as "self-eating," functions as a degradation process by recycling cytoplasmic contents under stress conditions or during development. Upon activation of autophagy, a membrane structure known as a phagophore forms and expands, finally closing to form a double-membrane vesicle called an autophagosome (Fig. 1; Lamb et al., 2013; Yin et al., 2016). The completed autophagosome, which contains the autophagic cargo, is delivered to the vacuole (plants and yeast) or lysosome (animals). The outer membrane fuses with the vacuolar/lysosomal membrane, and the inner membrane and contents are released into the vacuole/lysosome as an autophagic body and are degraded by hydrolases. The breakdown products are transported back into the cytoplasm for reuse by the cell (Yang and Bassham, 2015).

The initial identification of many autophagy-related (*ATG*) genes in yeast (Tsukada and Ohsumi, 1993; Thumm et al., 1994; Harding et al., 1995) was key in beginning to understand the mechanism by which autophagy occurs. The core machinery for autophagosome formation includes ATG1, which forms a complex with ATG13 for the induction of autophagy (Kamada et al., 2000); two ubiquitin-like conjugates, ATG12-ATG5 and ATG8-PE, which are recruited to the phagophore assembly site and play an important role in autophagosome formation (Yin et al., 2016); and ATG9, which may function in the recruitment of other ATG components and membrane to the forming autophagosome (Reggiori et al., 2005). In plants, autophagy has been well studied as a response to stress conditions, including nutrient deficiency (Doelling et al., 2002; Hanaoka et al., 2002), salt and drought stress (Liu et al., 2009), heat stress (Zhou et al., 2013; Yang et al., 2016), oxidative stress (Xiong et al., 2007), hypoxia (Chen et al., 2015), pathogen attack (Liu et al., 2005; Lai et al., 2011), and endoplasmic reticulum (ER) stress (Liu et al., 2012; Fig. 2). In this review, we summarize recent advances in our understanding of the dynamics of plant autophagy, focusing on regulation of autophagy and mechanisms of autophagosome formation.

REGULATION OF AUTOPHAGY

The autophagy pathway is highly conserved among all eukaryotes. In plants, it is activated during development and in response to stress, and a basal level of autophagy is important for cellular homeostasis (Wang et al., 2017). Appropriate activation of autophagy is critical in balancing growth with stress tolerance, and better understanding and

ADVANCES

- Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isoforms and the sulfide molecule have been identified as negative regulators of autophagy.
- Autophagy can be regulated by TOR-dependent or -independent pathways, depending on the environmental conditions.
- The Snf1-related protein kinase 1 (SnRK1) activates autophagy by two pathways, direct phosphorylation of ATG1 or through TOR.
- ATG9, SH3P2, and NAP1 function in membrane dynamics during autophagosome formation.
- Plastids and plastid fragments can be degraded by multiple autophagy-related pathways.

[1] This work was financially supported by the National Institutes of Health (grant no. 1R01GM120316-01A1 to D.C.B.) and by the Iowa State University Walter E. and Helen Parke Loomis fund, and grants from the Research Grants Council (RGC) of Hong Kong (CUHK14130716, C4011-14R, C4012-16E, and AoE/M-05/12) to L.J.

[2] These authors contributed equally to this work.

[3] Address correspondence to bassham@iastate.edu.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Diane C. Bassham (bassham@iastate.edu).

L.J. and D.C.B. conceived and outlined the article; J.S.-B. and X.Z. drafted the article; L.J. and D.C.B. revised the article.

[OPEN] Articles can be viewed without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.17.01236

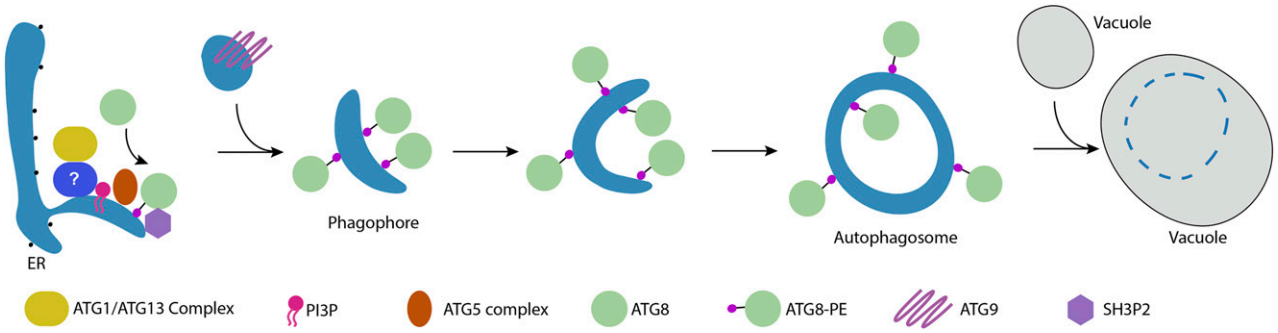

Figure 1. A proposed model for autophagosome formation in plant cells. Once autophagy is induced, a crescent-shaped isolation membrane named a phagophore is assembled on its membrane origin (e.g. ER). During this process, the ATG1 complex is activated, and downstream regulators (question mark) are recruited onto the initiation site. PI3P is generated on the phagophore assembly site, and ATG8 is conjugated onto the membrane to become the ATG8-PE form. Also, ATG5 and SH3P2 have been shown to localize on the phagophore structures. In addition, ATG9 vesicles are required for the efficient budding of the phagophore from the ER platform. In the subsequent steps, more ATG8 lipidation occurs, and the isolation membrane will elongate and close to form the completed double-membrane autophagosome. Finally, the autophagosome will deliver the cargos into the vacuole by fusion with the vacuole. With the help of the acidic environment and hydrolysis enzymes within the vacuole, the cargos will be degraded. Other regulators involved in the later steps after phagophore initiation are not listed.

subsequent modification of the pathway could lead to improvements in crop growth and yield. In this section, we will discuss known regulators of the autophagy pathway in plants.

SnRK1 Activates Autophagy in Response to Abiotic Stress

Autophagy is induced by nutrient depletion, most likely as a mechanism for nutrient recycling and generation of substrates for energy metabolism (Doelling et al., 2002; Hanaoka et al., 2002). The energy sensor Snf1-related protein kinase 1 (SnRK1) is a heterotrimeric complex that has been suggested to be a master regulator of metabolism in plants in response to nutrient and energy deficiency (Sugden et al., 1999; Baena-González et al., 2007; Crozet et al., 2014). The animal and yeast orthologs of SnRK1, AMP-activated kinase (AMPK) and Suc nonfermenting 1 (Snf1), respectively, are energy and metabolic sensors that maintain cellular homeostasis and activate autophagy under low-energy conditions (Hardie, 2011; Carroll and Dunlop, 2017). AMPK/Snf1 can regulate autophagy by inhibiting the target of rapamycin (TOR) complex (Lee et al., 2010), a negative regulator of autophagy, therefore allowing autophagy to become active, or by direct phosphorylation of ATG1, which also leads to the activation of autophagy (Wang et al., 2001; Egan et al., 2011; Kim et al., 2011). In Arabidopsis (*Arabidopsis thaliana*), there are two isoforms of the SnRK1 complex catalytic subunit, KIN10 and KIN11, with KIN10 being responsible for most of the SnRK1 activity (Baena-González et al., 2007; Jossier et al., 2009; Crozet et al., 2014). A *kin10 kin11* double mutant is lethal, and reduced expression via virus-induced gene silencing leads to decreased activation of stress and starvation genes and to deformed leaves, flowers, and inflorescence (Baena-González et al., 2007), indicating that SnRK1 functions in development and stress responses.

Overexpression of the *KIN10* gene in Arabidopsis leads to constitutive activation of autophagy (Chen et al., 2017; Soto-Burgos and Bassham, 2017), suggesting a positive role in the regulation of autophagy. KIN10 overexpression led to increased phosphorylation of ATG1 during Suc starvation, suggesting that KIN10 regulates autophagy by affecting the phosphorylation of ATG1 (Chen et al., 2017), as in mammals and yeast. Autophagy is activated during a wide range of abiotic stresses, and a *kin10* knockout mutant failed to activate autophagy during most of these stresses (Soto-Burgos and Bassham, 2017). This indicates that KIN10 activates autophagy not just in response to nutrient deficiency or energy depletion as predicted, but also during other abiotic stresses, indicating a wider role for SnRK1 in regulation of autophagy than previously expected.

TOR Is a Negative Regulator of Autophagy

The TOR complex is a key regulator of the balance between growth and autophagy in all eukaryotes tested (Noda and Ohsumi, 1998; Pattingre et al., 2008; Liu and Bassham, 2010). In plants, TOR is activated when nutrients are abundant, in turn enhancing mRNA translation initiation, ribosome biogenesis, cell wall synthesis and growth and inhibiting autophagy (Deprost et al., 2007; Ren et al., 2011; Xiong and Sheen, 2015). Upon nutrient deficiency, TOR is inactivated, reducing growth and allowing the activation of autophagy (Pattingre et al., 2008).

The Arabidopsis TOR complex consists of TOR itself, a Ser/Thr kinase (Menand et al., 2002), the Regulatory Associated Protein of TOR, RAPTOR (Anderson et al., 2005; Deprost et al., 2005), which presents substrates to TOR for phosphorylation (Hara et al., 2002), and Lethal with Sec Thirteen 8, which stabilizes the complex (Moreau et al., 2012). A complete knockout of *TOR* is embryo-lethal (Menand et al., 2002), while knockdown by RNA interference

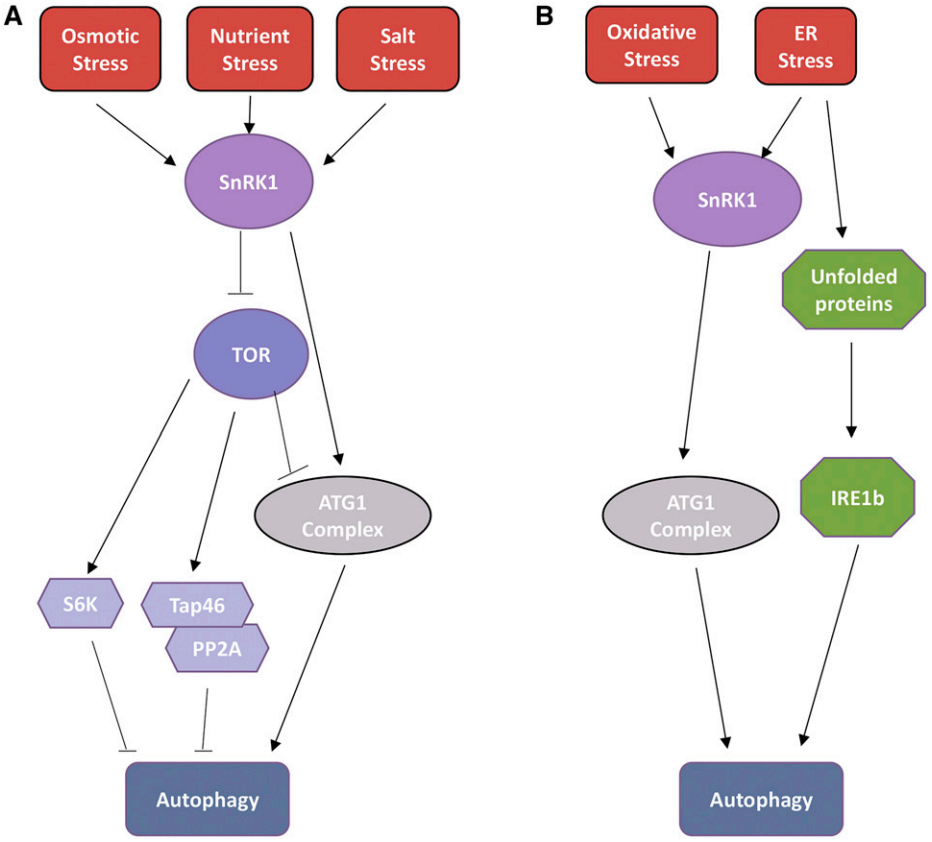

Figure 2. TOR-dependent and -independent regulatory pathways for autophagy in plants. Autophagy can be activated by abiotic stress, including osmotic, nutrient, salt, oxidative, and ER stress. This activation can be regulated in a TOR-dependent or -independent manner. A, Upon osmotic, nutrient, or salt stress, the SnRK1 complex can inhibit TOR, leading to activation of the ATG1 complex or deactivation of S6K and Tap46, in turn activating autophagy. SnRK1 can also activate the ATG1 complex by phosphorylation of ATG1, leading to activation of autophagy. B, Upon oxidative or ER stress, SnRK1 activates the ATG1 complex, leading to the activation of autophagy. Upon ER stress, unfolded proteins accumulate and activate IRE1b, leading to autophagy. Ovals represent kinase complexes, hexagons represent TOR targets, and octagons represent components of the ER stress response pathway.

leads to arrest of plant growth and development and constitutive autophagy (Deprost et al., 2007; Liu and Bassham, 2010). Disruption of *RAPTOR* in Arabidopsis similarly leads to defects in plant growth and development, although less severe than those of a TOR knockout, as well as constitutive autophagy (Anderson et al., 2005; Deprost et al., 2005; Pu et al., 2017). Overexpression of TOR blocks autophagy induced by nutrient starvation, salt, and osmotic stresses, while autophagy induced by oxidative and ER stress is not affected (Pu et al., 2017). Regulation of autophagy can therefore be TOR dependent or independent (Fig. 2) depending on the environmental stress to which the plant is subjected.

RAPTOR interacts with ribosomal p70 S6 kinase (S6K) in response to osmotic stress signals, suggesting a role for S6K in the TOR signaling pathway and plant stress responses (Mahfouz et al., 2006). Several other proteins have also been shown to interact with RAPTOR or TOR in vitro, including Arabidopsis Mei2-like1 (Anderson et al., 2005) and transcription factor E2Fa. Tap42/α4 is an effector of TOR in yeast and mammals, and its Arabidopsis homolog, Tap46, has been identified as a downstream target of TOR. Tap46 is phosphorylated by TOR and interacts with protein phosphatase type 2A, a regulator of autophagy in yeast (Ahn et al., 2011). Overexpression or reduction of expression of Tap46 correlates with TOR activity (Ahn et al., 2015), suggesting that Tap46 is a positive regulator of the TOR pathway. Silencing of the *TAP46* gene using virus-induced gene silencing in tobacco (*Nicotiana tabacum*) led to induction of autophagy, as in the *TOR* RNAi plants (Ahn et al., 2011), indicating that it can negatively regulate autophagy. This suggests that Tap46 acts as a positive effector in the TOR signaling pathway, leading to the regulation of autophagy.

Recently, a connection between the TOR signaling pathway and SnRK1 complex has been demonstrated. KIN10 interacts with RAPTOR in vivo and can phosphorylate RAPTOR in vitro, like its mammalian orthologs (Nukarinen et al., 2016). Blocking TOR activity in a *kin10* mutant led to activation of autophagy, while inhibition of SnRK1 activity in a *raptor1b* mutant failed to block the constitutive autophagy observed in this mutant (Soto-Burgos and Bassham, 2017). SnRK1 therefore acts upstream of the TOR signaling pathway in the regulation of autophagy (Soto-Burgos and Bassham, 2017), at least under conditions in which activation of autophagy is TOR dependent.

The ATG1 Kinase Complex

A major regulator of autophagy and a downstream substrate of TOR in yeast and animals is the ATG1/ATG13 kinase complex. ATG1 is the catalytic subunit of the

complex and activates autophagy in response to nutrient depletion (Diaz-Troya et al., 2008; Mizushima 2010). In mammals, AMPK promotes autophagy by phosphorylating Ulk1 (ATG1 homolog) upon Glc starvation (Kim et al., 2011). In Arabidopsis, KIN10 overexpression results in an increase in ATG1 phosphorylation, suggesting that this mode of regulation is conserved (Chen et al., 2017). In yeast, TOR phosphorylates ATG13 in nutrient-rich conditions, causing a decrease in its affinity for ATG1, preventing their association and therefore repressing autophagy. Under starvation conditions, TOR becomes inactive, leading to the dephosphorylation of ATG13, allowing ATG1 to associate with ATG13 and activate autophagy (Nakatogawa et al., 2009; Kamada et al., 2010). In mammals, unlike in yeast, ATG1 associates with ATG13 under all conditions, indicating that the regulatory mechanism of ATG1/ATG13 differs between mammals and yeast (Lee et al., 2007).

In Arabidopsis, ATG1 and ATG13 are present in multiple copies, which are most likely functionally redundant. During nutrient starvation, ATG1a is hyperphosphorylated, potentially by SnRK1 (Chen et al., 2017), while ATG13a is hypo-phosphorylated (Suttangkakul et al., 2011), suggesting that the ATG1 complex functions in a manner similar to yeast in terms of autophagy regulation. Disruption of ATG13 leads to phenotypes similar to those of mutants in other core autophagy genes, with hypersensitivity to nutrient starvation and accelerated senescence. *atg13* mutants have defects in the formation of autophagic bodies, suggesting that the complex acts upstream of autophagosome formation (Suttangkakul et al., 2011). ATG1a associates with autophagic bodies and is delivered to the vacuole for degradation, indicating that the ATG1 complex is a substrate of autophagy. A negative feedback mechanism is therefore proposed to exist to reduce activated ATG1 complex levels after induction of autophagy by nutrient deficiency (Suttangkakul et al., 2011). This turnover might be an attempt to reset autophagic induction by requiring the incorporation of freshly activated ATG1 kinase during each round of phagophore assembly (Suttangkakul et al., 2011). Based on this evidence, and by comparison with ATG1 complex functions in animals and yeast, we hypothesize that the ATG1 complex may regulate autophagy in Arabidopsis via its phosphorylation by TOR and/or SnRK1 (Fig. 2).

Regulation of Autophagy by IRE1 during ER Stress

Autophagy is induced by ER stress, in which accumulation of unfolded and misfolded proteins within the ER activates the unfolded protein response (UPR; Liu et al., 2012; Liu and Howell, 2016). Although repression of TOR activity leads to activation of autophagy during some abiotic stresses, autophagy induced by ER stress seems to be independent of TOR (Pu et al., 2017), as autophagosomes are formed normally during ER stress in *TOR* overexpression lines. Instead, ER stress-induced autophagy depends on inositol-requiring enzyme-1 (IRE1), an ER stress sensor that activates the UPR (Cox and Walter, 1996; Mori et al., 1996; Chen and Brandizzi, 2013). During ER stress, IRE1 is activated by oligomerization and autophosphorylation (Korennykh et al., 2009). After activation, IRE1 splices an mRNA encoding a membrane-associated basic Leu zipper transcription factor (bZIP60; Nagashima et al., 2011). The spliced *bZIP60* mRNA is translated, producing an active protein that is translocated into the nucleus and upregulates UPR genes such as *BIP* (Iwata and Koizumi, 2005; Deng et al., 2011).

Two *IRE1* genes have been identified in Arabidopsis, *IRE1a* and *IRE1b* (Koizumi et al., 2001; Deng et al., 2011; Moreno et al., 2012), and a mutant defective in *IRE1b* is unable to form autophagosomes after inducing ER stress using dithiothreitol (DTT) or tunicamycin, indicating that *IRE1b* is required for the induction of autophagy by ER stress (Liu et al., 2012). Mutations in either *IRE1a* or *bZIP60* have no effect on autophagy during ER stress, suggesting that only IRE1b is involved in the regulation of autophagy and that its *bZIP60* splicing activity is not required (Liu et al., 2012). The addition of chemical chaperones or overexpression of molecular chaperones inhibited activation of autophagy by DTT or tunicamycin, and expression of a misfolded protein mimic in the ER was sufficient to induce autophagy via IRE1b activity. The accumulation of unfolded proteins in the ER, presumably recognized by IRE1b, is therefore a key event in activating autophagy during ER stress (Yang et al., 2016).

Excessive heat is a major factor that causes ER stress, as indicated by the splicing of *bZIP60* mRNA by IRE1b (Deng et al., 2011) and by up-regulation of BiP (Leborgne-Castel et al., 1999). The induction of autophagy by heat stress is also mainly due to the accumulation of misfolded proteins (Yang et al., 2016). Autophagy activation is significantly reduced in an *ire1b* mutant during heat stress, compared to the wild type, indicating that the autophagy response during heat stress is dependent on IRE1b (Yang et al., 2016) and is most likely primarily acting as an ER stress response.

Other Possible Regulators

Most of the identified regulators of plant autophagy act posttranslationally, and relatively little is known about transcriptional regulation of autophagy-related genes. In tomato (*Solanum lycopersicum*), the transcription factor HsfA1a has been shown to induce drought tolerance by the activation of *ATG* genes and the induction of autophagy (Wang et al., 2015a). The Arabidopsis WRKY33 transcription factor has also been suggested to regulate autophagy. WRKY33 is important for plant resistance to necrotrophic pathogens (Zheng et al., 2006), and a yeast two-hybrid screen showed that WRKY33 interacts with ATG18a, a core autophagy component (Lai et al., 2011). Furthermore, a *wrky33* mutant was defective in up-regulation of *ATG18a* and induction of autophagy upon infection with *Botrytis cinerea* (Lai et al., 2011). Silencing of *WRKY33* in tomato led to reduced *ATG* gene expression and autophagosome accumulation during heat stress (Zhou et al., 2014), suggesting that it also functions in abiotic stress responses.

Several new pathways for regulation of autophagy in plants have been identified recently. First, the glyceraldehyde-3-phosphate dehydrogenases (GAPDH) have been shown to negatively regulate autophagy (Han et al., 2015; Henry et al., 2015). In Arabidopsis, there are multiple isoforms of GADPH, including chloroplastic photosynthetic (*GAPA1*, *GAPA2*, and *GAPB*) and cytosolic glycolytic (*GAPC1* and *GAPC2*) enzymes (Zaffagnini et al., 2013). Mutants in the GADPH isoforms *GAPA1* and *GAPC1* have constitutive autophagy, suggesting that GAPDH can negatively regulate autophagy (Henry et al., 2015). In tobacco, silencing of GAPCs activated autophagy, whereas overexpression of GAPCs inhibited oxidative stress-induced autophagy (Han et al., 2015). Furthermore, GAPCs interact with ATG3 in vivo and in vitro, but upon oxidative stress this interaction weakens (Han et al., 2015). Disruption of GAPDHs led to enhanced disease resistance (Han et al., 2015; Henry et al., 2015), although whether this is related to autophagy is unclear. Together, these data suggest that GADPH negatively regulates autophagy through interaction with ATG3.

Second, hydrogen sulfide has been linked to the regulation of autophagy. Hydrogen sulfide is an important signaling molecule in mammalian systems, and emerging data suggest that this is also true in plants. It has been identified as a component of the ABA signaling pathway (García-Mata and Lamattina, 2010) and has roles in regulation of photosynthesis (Chen et al., 2011) and tolerance to copper (Zhang et al., 2008), aluminum (Zhang et al., 2010), and boron (Wang et al., 2010) stress. DES1 is an L-Cys desulfhydrase that is involved in the production of hydrogen sulfide and the degradation of Cys (Alvarez et al., 2010). A mutation in the *DES1* gene impedes sulfide generation in the cytosol and promotes the accumulation of ATG8 and ATG8-PE, indicating activation of autophagy. Furthermore, addition of exogenous sulfide to a *des1* mutant or genetic complementation of *DES1* gene prevented the accumulation and lipidation of ATG8 proteins (Álvarez et al., 2012). Recently, it was demonstrated that the negative regulation of autophagy by sulfide is independent of reactive oxygen species, and sulfide therefore probably regulates autophagy by an alternative pathway (Laureano-Marín et al., 2016).

Third, the plant Bax inhibitor-1 (BI-1) has recently been shown to interact with ATG6 in vivo and in vitro and to positively regulate autophagy (Xu et al., 2017). Silencing of tobacco BI-1 reduced the autophagy activity induced by virus infection or oxidative stress, while overexpression of BI-1 increased autophagy activity and caused autophagy-dependent cell death (Xu et al., 2017). BI-1 therefore has both autophagy-dependent prosurvival and prodeath effects, depending on the conditions.

As research progresses, more information becomes available about how autophagy is regulated in plants. Although new discoveries have been made, further research is needed to fully understand how the autophagy pathway is controlled under different conditions and how the regulatory components are coordinated to determine the degree of autophagy activation (see Outstanding Questions).

AUTOPHAGOSOME MEMBRANE DYNAMICS

After autophagy is activated, a conserved autophagosome formation process has been observed in plant cells, which involves several steps: initiation, expansion, maturation, and degradation (Fig. 1; Liu and Bassham, 2012). Autophagosomes may fuse with endosomes for further maturation prior to reaching the final destination, the vacuole, to acquire degradative enzymes, including proteases and lipases, for cargo degradation (Cui et al., 2016). Each step requires a dynamic membrane deformation process to give rise to the newly formed membrane, elongate the membrane for cargo sequestration, and fuse with other endomembrane compartments like endosomes and finally the vacuole/lysosome. In the following, we will provide an update on membrane dynamics during autophagosome formation, and we apologize to authors whose primary work cannot be cited here due to the space limitation.

Phagophore Initiation

Recent studies on yeast and mammalian cells have greatly advanced our understanding of the underlying mechanisms of autophagosome formation, and multiple membrane sources have been reported (Lamb et al., 2013). A typical preautophagosome structure, which is characterized by an open cup-like double membrane with highly curved edges, is called a phagophore or isolation membrane. It has been shown that the phagophore arises from an omega-shaped structure on an ER subdomain, called the omegasome (Axe et al., 2008). The highly curved shape of the phagophore membrane can be achieved by lipid composition and/or asymmetric distribution or by scaffolding through membrane curvature sensing proteins. So far, a general model for phagophore initiation can be described by the following sequence of events: ATG1 and PI3K complexes are initially recruited to the omegasome, leading to phosphatidylinositol 3-phosphate (PI3P) production, as well as recruitment of the ATG12-ATG5-ATG16 complex and other downstream regulators, which further facilitates the conjugation of ATG8 to phosphatidylethanolamine on the nascent phagophore membrane and its detachment from the ER platform (Lamb et al., 2013).

Although a number of images collected by electron microscopy provide a detailed morphological description of autophagosomal structures in plant cells, studies focusing on the mechanism of phagophore initiation are relatively rare (van Doorn and Papini, 2013). Similar to the complex in yeast and animal cells, the ATG1 complex functions at an early step for phagophore formation in plants, as an *atg13* mutant fails to form autophagosomal structures (Suttangkakul et al., 2011). PI3P is also crucial for autophagosome initiation, as autophagosome formation is completely blocked after PI3K inhibitor treatment (Zhuang et al., 2013; Le Bars et al., 2014), while the autophagic defect in a yeast mutant in the PI3K component *atg6/vps30* can be restored by expressing its Arabidopsis homolog ATG6 (Fujiki et al., 2007). However, the connection between the ATG1 complex and the PI3K complex as well as other

downstream regulators remains unknown, hindering our further understanding of the current model for phagophore initiation in plants.

A recent study focusing on ATG5 dynamics provides a new model for phagophore formation in Arabidopsis (Le Bars et al., 2014). In this paper, early autophagosomal structures were labeled by ATG5-GFP fluorescent proteins and exhibit a tight connection with the ER network. Interestingly, ATG5 fusion proteins decorate the high curvature domain of the phagophore at all stages of its differentiation and finally detach from the phagophore once it is sealed. By detailed real-time and 3D imaging analysis of the growing phagophore, it was shown that ATG5-GFP is located on the growing phagophore with a toroidal disposition, raising the possibility that ATG5 and/or its related complex (ATG12-ATG16) may sense or promote the membrane deformation. This observation is consistent with that in animal cells, in which when ATG16 is artificially targeted to the plasma membrane, LC3 (animal ATG8 homolog) lipidation also occurs on the plasma membrane (Fujita et al., 2008). However, a counterpart for ATG16 in Arabidopsis has not been identified.

In another study, by tracking SH3P2, it was also shown that omegasome-like structures may be employed for phagophore formation (Zhuang et al., 2013). It was clearly observed that SH3P2 was predominantly distributed on a highly curved domain in omegasome-like structures as well as on the nascent phagophore, which is closely associated with the ER membranes (Zhuang et al., 2013). In addition, the fusion process has also been visualized through labeling with SH3P2, while SH3P2 still displays an asymmetrical distribution on the membrane. It is noteworthy that SH3P2 contains a BAR domain, a well-established membrane curvature sensor, and directly interacts with ATG8; thereby, it is also speculated that SH3P2 may function as a membrane sensor during the membrane remodeling process. It will be interesting to investigate how the interaction between ATG8 and SH3P2 regulates autophagosome membrane remodeling.

ATG9 Vesicles

In contrast to most other ATG proteins, ATG9 is the only transmembrane protein and moves rapidly as numerous distinct compartments throughout the cytoplasm (Yamamoto et al., 2012; Karanasios et al., 2016; Rao et al., 2016). Therefore, ATG9 vesicles have been postulated as a key contributor to deliver the membrane source or other regulators to the phagophore membrane. In yeast, autophagy is completely blocked in the *atg9* mutant (Yamamoto et al., 2012). In mice, deficiency in ATG9 only leads to fewer autophagosome structures upon induction and decreased autophagic activity, suggesting that mammalian ATG9 is not crucial in basal conditions (Orsi et al., 2012). Furthermore, interaction data also show that ATG9 is associated with ATG and non-ATG regulators for phagophore initiation (Karanasios et al., 2016; Rao et al., 2016).

In plants, analysis of Arabidopsis *atg9* mutants shows that autophagy is less severely blocked when compared with other *atg* mutants like *atg5* and *atg7* (Hanaoka et al., 2002). Recently, advanced-imaging analysis has provided novel insights into the roles of ATG9 in the formation of early autophagosomal structures (Zhuang et al., 2017). In Arabidopsis, ATG9 vesicles are observed as distinct mobile compartments and show transient interactions with the autophagosomal membrane after autophagic induction. Interestingly, upon benzothiadiazole and DTT treatments, highly dynamic extending tubules labeled by YFP-ATG8e are captured in the *atg9* mutants. Moreover, 3D electronic tomography as well as dynamic confocal microscopy analysis demonstrated a direct contact between these abnormal autophagosomal structures and the ER membrane, providing clear evidence for the ER origin of autophagosomes in Arabidopsis. In addition, the PI3P effector ATG18, an ATG9-interacting protein, is also trapped on extending tubules in *atg9* mutants, suggesting that in plants, ATG9 acts as a carrier to recycle regulators from the newly formed phagophore to control the elongation of autophagosomal membrane. ATG9 may also interact with other membrane remodeling proteins for the fission of the phagophore from the membrane of origin, which has also been indicated in other eukaryotic cells, including Trs85, a specific subunit of the transport protein particle III complex (Kakuta et al., 2012). It should be pointed out that such a defect has not been observed in other model organisms or other Arabidopsis *atg* mutants like *atg5* and *atg7* under the same conditions, thus revealing a unique role of ATG9 for autophagosome development on the ER membrane. However, it remains to be seen whether this is true in all types of ER-dependent autophagy or whether it is specific to benzothiadiazole- and DTT-induced autophagy, both drugs being able to induce ER stress. More importantly, future efforts in identifying and characterizing ATG9-interacting proteins would certainty facilitate our understanding of how ATG9 is involved in this process.

Autophagosome Expansion, Maturation, and Degradation

Once the phagophore is formed, it will undergo a series of steps for expansion, maturation, and finally degradation in the vacuole by fusion with endosomes and vacuole, which requires additional driving forces for membrane deformation and fusion. Recent exciting studies have uncovered several non-ATG regulators in this process, particularly the endocytic components that function in endomembrane trafficking (Zhuang et al., 2015).

The cytoskeleton may drive the membrane shaping during autophagosome formation in both yeast and mammalian cells (Kast and Dominguez, 2017). Studies demonstrating the colocalization of the autophagy markers ATG8 and JOKA2 with cytoskeletal components (Ketelaar et al., 2004; Zientara-Rytter and Sirko, 2014) have provided evidence of links between the cytoskeleton and plant autophagy. Another study also reported a role for a

subunit of the exocyst complex in autophagic membrane transport to the vacuole, as an *exo70B1* mutant showed decreased amounts of intravacuolar autophagic bodies (Kulich et al., 2013), while the exocyst complex has been implicated to function in coordination of vesicle trafficking with the cytoskeleton (Synek, et al., 2014). In addition, disruption of the microtubule cytoskeleton compromised autophagosome formation upon autophagic induction (Wang et al., 2015b). What is more, when a component of the SCAR/WAVE complex named NAP1 is defective, autophagosome formation is reduced (Wang et al., 2016). NAP1 is initially ER associated and coaligns with the cytoskeleton, but when treated with constant pressure, NAP1-labeled punctae are induced and colocalize with an autophagosome marker. It is proposed that ER-associated NAP1 may activate actin polymerization to promote membrane deformation for phagophore formation and expansion. However, future work should clarify how cytoskeleton activities are coordinated for autophagosome formation.

In contrast, abnormal autophagosomal structures have been shown to accumulate in several mutants defective in endosome or vacuole trafficking, particularly the ESCRT complex, which is essential for MVB and vacuole biogenesis (Surpin et al., 2003; Katsiarimpa et al., 2013; Kwon et al., 2013; Gao et al., 2015; Zhuang et al., 2015). It is possible that a failure in fusion with endosomes or the vacuole leads to these defects. The importance of autophagosomal fusion with endosomes was supported by other studies. A plant-specific ESCRT component, FYVE domain protein required for endosomal sorting 1 (FREE1), has been reported to associate with the ESCRT components and participate in regulating vacuolar protein transport (Gao et al., 2014; Zhao et al., 2015; Belda-Palazon et al., 2016). On the other hand, FREE1 interacts with SH3P2, and an Arabidopsis *free1* mutant accumulates abnormal autophagosome-like structures, which display a higher association with the late endosome and failure in delivery of autophagosomes to the vacuole (Gao et al., 2015). Since SH3P2 binds to the autophagosome membrane and ATG8 (Zhuang et al., 2013), it is hypothesized that FREE1-SH3P2 serves as a bridge for autophagosome fusion with the endosome/vacuole. Several studies have shown that autophagosomes share the membrane tethering machineries with the endomembrane system in yeast and animal cells (Tooze et al., 2014). Therefore, identification of the components in the fusion process will be an important first step to fully understand the membrane fusion mechanism.

Membrane Dynamics in Selective Autophagy

Selective autophagy involves the engulfment of specific proteins or organelles into autophagosomes, which requires receptors or adaptor proteins that bind the cargo and also interact with the ATG component(s) for the recruitment of the cargo into autophagosomes (Floyd et al., 2012; Zhou et al., 2013; Hafren et al., 2017). In this process, ATG8 is a central player for selective autophagy that decorates autophagosomes and binds to various cargo receptors (Kellner et al., 2017). Autophagosomes may develop into multiple sizes to sequester different cargos, including protein aggregates and organelles, to avoid excess damage to the cell. As such, autophagosomes will undergo drastic membrane expansion to engulf specific cargos efficiently and selectively. Here we

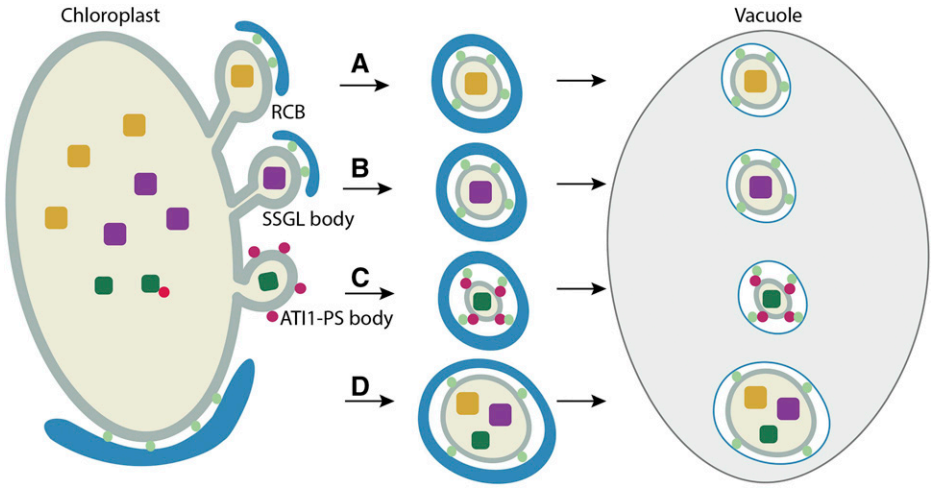

Figure 3. Summary of the characterized autophagy-mediated pathways for chloroplast material degradation. Three types of structures, RCB (A), SSGL body (B), and ATI1-PS body (C), bud off from chloroplasts with different cargos and are sequestered into ATG8-coated membranes (blue color). The receptors for engulfment of RCB and SSGL into autophagosomal membranes are presently unidentified. In addition, when cells are exposed to light-induced damage, whole damaged, dysfunctional chloroplasts can be targeted by ATG8-decorated autophagosome structures (D) to be delivered into the vacuole, but the underlying mechanism remains unknown. ATG8 and ATI1 are labeled with green and red dots, respectively.

OUTSTANDING QUESTIONS

- How is transcription of autophagy-related genes regulated?
- What upstream signals affect SnRK1 and TOR activity to regulate autophagy in response to stress?
- What is the molecular mechanism of membrane remodeling of the phagophore from its membrane origin in plant cells?
- Does ATG9 play a specific role in ER stress-induced autophagy or ER-derived autophagosome biogenesis? Are there any other membrane sources for plant autophagosomes?
- How does autophagy recognize its cargos in the plant cell and how is the ATG machinery coordinated?

will focus on one plant-specific type of selective autophagy, chlorophagy (Fig. 3). New data show that autophagy is involved in chloroplast degradation in different manners for specific chloroplast contents or the entire chloroplast, including: Rubisco-containing bodies (RCBs), small starch granule-like structure (SSGL) bodies, and ATG8-interacting Protein 1 (ATI1-PS) bodies (Michaeli and Galili, 2014).

Stromal proteins are imported into the small double membrane structures termed RCBs and eventually transported to the vacuole for degradation (Ishida et al., 2008). It has been observed that RCBs labeled by a chloroplast-targeted DsRed fluorescent protein colocalized with the GFP-ATG8 autophagosome marker. A recent study demonstrates that the ESCRT components CHMP1A and B play a direct role in the delivery of RCB cargos into the vacuole, as the *chmp1* mutant accumulates plastid clusters with plastid proteins (Spitzer et al., 2015). Notably, a defect in plastid morphology is also observed in *atg5* and *atg7* mutants, with the accumulation of long plastid bridges and extensions. In this study, vacuolar turnover of free GFP cleaved from GFP-ATG8 is also increased in the *chmp1* mutant, implying a possible role of CHMP1 in promoting the efficient sequestration of cargo from plastids into autophagosomes. Similar to RCB bodies, SSGL bodies are another type of plastid-derived small spherical structures, which are responsible for the delivery of small starch granules from chloroplasts to vacuoles in an autophagy-dependent mechanism (Wang et al., 2013). ATI1-PS bodies require a membrane-spanning protein, ATI1, which can interact with chloroplast proteins and ATG8, for the targeting of plastid proteins into the vacuole (Michaeli et al., 2014). The ATI1-PS bodies are detected in the periphery and inside of plastids, which will finally bud off from plastids into the cytoplasm independent of the core ATG machinery such as ATG5. Apart from these different pathways for chloroplast degradation, it is also of note that entire chloroplasts can be engulfed by autophagosomal structures when cells are exposed to UV light-induced damage (Izumi et al., 2017). However, how the ATG proteins sense the targeted chloroplast cargos to initiate the formation of various types of structures remains unclear.

CONCLUSIONS

Accumulating studies have begun to address the essential roles of autophagy in plant development and growth. It is apparent that plants may exhibit specific types of autophagy and autophagosomal structures. Exciting findings such as the identification of novel regulators to sense and shape the unique double membrane structures in yeast and animal cells have provided great advances in our understanding of autophagy regulation and autophagosome formation. It is very likely that unique mechanisms for autophagy regulation will be uncovered in the near future and more plant-specific cellular functions will be unraveled (see Outstanding Questions).

Received August 31, 2017; accepted October 18, 2017; published October 23, 2017.

LITERATURE CITED

Ahn CS, Ahn HK, Pai HS (2015) Overexpression of the PP2A regulatory subunit Tap46 leads to enhanced plant growth through stimulation of the TOR signalling pathway. J Exp Bot **66:** 827–840

Ahn CS, Han JA, Lee HS, Lee S, Pai HS (2011) The PP2A regulatory subunit Tap46, a component of the TOR signaling pathway, modulates growth and metabolism in plants. Plant Cell **23:** 185–209

Alvarez C, Calo L, Romero LC, García I, Gotor C (2010) An O-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis. Plant Physiol **152:** 656–669

Álvarez C, García I, Moreno I, Pérez-Pérez ME, Crespo JL, Romero LC, Gotor C (2012) Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in Arabidopsis. Plant Cell **24:** 4621–4634

Anderson GH, Veit B, Hanson MR (2005) The Arabidopsis AtRaptor genes are essential for post-embryonic plant growth. BMC Biol **3:** 12

Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol **182:** 685–701

Baena-González E, Rolland F, Thevelein JM, Sheen J (2007) A central integrator of transcription networks in plant stress and energy signalling. Nature **448:** 938–942

Belda-Palazon B, Rodriguez L, Fernandez MA, Castillo MC, Anderson EA, Gao C, González-Guzmán M, Peirats-Llobet M, Zhao Q, De Winne N, et al (2016) FYVE1/FREE1 interacts with the PYL4 ABA receptor and mediates its delivery to the vacuolar degradation pathway. Plant Cell **28:** 2291–2311

Carroll B, Dunlop EA (2017) The lysosome: a crucial hub for AMPK and mTORC1 signalling. Biochem J **474:** 1453–1466

Chen J, Wu FH, Wang WH, Zheng CJ, Lin GH, Dong XJ, He JX, Pei ZM, Zheng HL (2011) Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. J Exp Bot **62:** 4481–4493

Chen L, Liao B, Qi H, Xie LJ, Huang L, Tan WJ, Zhai N, Yuan LB, Zhou Y, Yu LJ, et al (2015) Autophagy contributes to regulation of the hypoxia response during submergence in Arabidopsis thaliana. Autophagy **11:** 2233–2246

Chen L, Su Z-Z, Huang L, Xia F-N, Qi H, Xie L-J, Xiao S, Chen Q-F (2017) The AMP-activated protein kinase KIN10 is involved in the regulation of autophagy in Arabidopsis. Front Plant Sci **8:** 1201

Chen Y, Brandizzi F (2013) IRE1: ER stress sensor and cell fate executor. Trends Cell Biol **23:** 547–555

Cox JS, Walter P (1996) A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. Cell **87:** 391–404

Crozet P, Margalha L, Confraria A, Rodrigues A, Martinho C, Adamo M, Elias CA, Baena-González E (2014) Mechanisms of regulation of SNF1/AMPK/SnRK1 protein kinases. Front Plant Sci **5:** 190

Cui Y, Shen J, Gao C, Zhuang X, Wang J, Jiang L (2016) Biogenesis of plant prevacuolar multivesicular bodies. Mol Plant **9:** 774–786

Deng Y, Humbert S, Liu JX, Srivastava R, Rothstein SJ, Howell SH (2011) Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in Arabidopsis. Proc Natl Acad Sci USA **108:** 7247–7252

Deprost D, Truong HN, Robaglia C, Meyer C (2005) An Arabidopsis homolog of RAPTOR/KOG1 is essential for early embryo development. Biochem Biophys Res Commun **326:** 844–850

Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, Nicolaï M, Bedu M, Robaglia C, Meyer C (2007) The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. EMBO Rep **8:** 864–870

Díaz-Troya S, Pérez-Pérez ME, Florencio FJ, Crespo JL (2008) The role of TOR in autophagy regulation from yeast to plants and mammals. Autophagy **4:** 851–865

Doelling JH, Walker JM, Friedman EM, Thompson AR, Vierstra RD (2002) The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in Arabidopsis thaliana. J Biol Chem **277:** 33105–33114

Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, et al (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science **331:** 456–461

Floyd BE, Morriss SC, Macintosh GC, Bassham DC (2012) What to eat: evidence for selective autophagy in plants. J Integr Plant Biol **54:** 907–920

Fujiki Y, Yoshimoto K, Ohsumi Y (2007) An Arabidopsis homolog of yeast ATG6/VPS30 is essential for pollen germination. Plant Physiol **143:** 1132–1139

Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T (2008) The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol Biol Cell **19:** 2092–2100

Gao C, Luo M, Zhao Q, Yang R, Cui Y, Zeng Y, Xia J, Jiang L (2014) A unique plant ESCRT component, FREE1, regulates multivesicular body protein sorting and plant growth. Curr Biol **24:** 2556–2563

Gao C, Zhuang X, Cui Y, Fu X, He Y, Zhao Q, Zeng Y, Shen J, Luo M, Jiang L (2015) Dual roles of an Arabidopsis ESCRT component FREE1 in regulating vacuolar protein transport and autophagic degradation. Proc Natl Acad Sci USA **112:** 1886–1891

García-Mata C, Lamattina L (2010) Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. New Phytol **188:** 977–984

Hafrén A, Macia JL, Love AJ, Milner JJ, Drucker M, Hofius D (2017) Selective autophagy limits cauliflower mosaic virus infection by NBR1-mediated targeting of viral capsid protein and particles. Proc Natl Acad Sci USA **114:** E2026–E2035

Han S, Wang Y, Zheng X, Jia Q, Zhao J, Bai F, Hong Y, Liu Y (2015) Cytoplastic glyceraldehyde-3-phosphate dehydrogenases interact with ATG3 to negatively regulate autophagy and immunity in Nicotiana benthamiana. Plant Cell **27:** 1316–1331

Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, Shibata D, Tabata S, Ohsumi Y (2002) Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an Arabidopsis autophagy gene. Plant Physiol **129:** 1181–1193

Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. Cell **110:** 177–189

Hardie DG (2011) AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev **25:** 1895–1908

Harding TM, Morano KA, Scott SV, Klionsky DJ (1995) Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. J Cell Biol **131:** 591–602

Henry E, Fung N, Liu J, Drakakaki G, Coaker G (2015) Beyond glycolysis: GAPDHs are multi-functional enzymes involved in regulation of ROS, autophagy, and plant immune responses. PLoS Genet **11:** e1005199

Ishida H, Yoshimoto K, Izumi M, Reisen D, Yano Y, Makino A, Ohsumi Y, Hanson MR, Mae T (2008) Mobilization of rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an ATG gene-dependent autophagic process. Plant Physiol **148:** 142–155

Iwata Y, Koizumi N (2005) An Arabidopsis transcription factor, AtbZIP60, regulates the endoplasmic reticulum stress response in a manner unique to plants. Proc Natl Acad Sci USA **102:** 5280–5285

Izumi M, Ishida H, Nakamura S, Hidema J (2017) Entire photodamaged chloroplasts are transported to the central vacuole by autophagy. Plant Cell **29:** 377–394

Jossier M, Bouly JP, Meimoun P, Arjmand A, Lessard P, Hawley S, Grahame Hardie D, Thomas M (2009) SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. Plant J **59:** 316–328

Kakuta S, Yamamoto H, Negishi L, Kondo-Kakuta C, Hayashi N, Ohsumi Y (2012) Atg9 vesicles recruit vesicle-tethering proteins Trs85 and Ypt1 to the autophagosome formation site. J Biol Chem **287:** 44261–44269

Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, Ohsumi Y (2000) Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J Cell Biol **150:** 1507–1513

Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, Yonezawa K, Ohsumi Y (2010) TOR directly controls the Atg1 kinase complex to regulate autophagy. Mol Cell Biol **30:** 1049–1058

Karanasios E, Walker SA, Okkenhaug H, Manifava M, Hummel E, Zimmermann H, Ahmed Q, Domart MC, Collinson L, Ktistakis NT (2016) Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. Nat Commun **7:** 12420

Kast DJ, Dominguez R (2017) The cytoskeleton-autophagy connection. Curr Biol **27:** R318–R326

Katsiarimpa A, Kalinowska K, Anzenberger F, Weis C, Ostertag M, Tsutsumi C, Schwechheimer C, Brunner F, Hückelhoven R, Isono E (2013) The deubiquitinating enzyme AMSH1 and the ESCRT-III subunit VPS2.1 are required for autophagic degradation in Arabidopsis. Plant Cell **25:** 2236–2252

Kellner R, De la Concepcion JC, Maqbool A, Kamoun S, Dagdas YF (2017) ATG8 expansion: a driver of selective autophagy diversification? Trends Plant Sci **22:** 204–214

Ketelaar T, Voss C, Dimmock SA, Thumm M, Hussey PJ (2004) Arabidopsis homologues of the autophagy protein Atg8 are a novel family of microtubule binding proteins. FEBS Lett **567:** 302–306

Kim J, Kundu M, Viollet B, Guan KL (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol **13:** 132–141

Koizumi N, Martinez IM, Kimata Y, Kohno K, Sano H, Chrispeels MJ (2001) Molecular characterization of two Arabidopsis Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases. Plant Physiol **127:** 949–962

Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, Stroud RM, Walter P (2009) The unfolded protein response signals through high-order assembly of Ire1. Nature **457:** 687–693

Kulich I, Pečenková T, Sekereš J, Smetana O, Fendrych M, Foissner I, Höftberger M, Zárský V (2013) Arabidopsis exocyst subcomplex containing subunit EXO70B1 is involved in autophagy-related transport to the vacuole. Traffic **14:** 1155–1165

Kwon SI, Cho HJ, Kim SR, Park OK (2013) The Rab GTPase RabG3b positively regulates autophagy and immunity-associated hypersensitive cell death in Arabidopsis. Plant Physiol **161:** 1722–1736

Lai Z, Wang F, Zheng Z, Fan B, Chen Z (2011) A critical role of autophagy in plant resistance to necrotrophic fungal pathogens. Plant J **66:** 953–968

Lamb CA, Yoshimori T, Tooze SA (2013) The autophagosome: origins unknown, biogenesis complex. Nat Rev Mol Cell Biol **14:** 759–774

Laureano-Marín AM, Moreno I, Romero LC, Gotor C (2016) Negative regulation of autophagy by sulfide is independent of reactive oxygen species. Plant Physiol **171:** 1378–1391

Le Bars R, Marion J, Le Borgne R, Satiat-Jeunemaitre B, Bianchi MW (2014) ATG5 defines a phagophore domain connected to the endoplasmic reticulum during autophagosome formation in plants. Nat Commun **5:** 4121

Leborgne-Castel N, Jelitto-Van Dooren EP, Crofts AJ, Denecke J (1999) Overexpression of BiP in tobacco alleviates endoplasmic reticulum stress. Plant Cell **11:** 459–470

Lee SB, Kim S, Lee J, Park J, Lee G, Kim Y, Kim JM, Chung J (2007) ATG1, an autophagy regulator, inhibits cell growth by negatively regulating S6 kinase. EMBO Rep **8:** 360–365

Lee JW, Park S, Takahashi Y, Wang HG (2010) The association of AMPK with ULK1 regulates autophagy. PLoS One **5:** e15394

Liu JX, Howell SH (2016) Managing the protein folding demands in the endoplasmic reticulum of plants. New Phytol **211:** 418–428

Liu Y, Bassham DC (2010) TOR is a negative regulator of autophagy in Arabidopsis thaliana. PLoS One **5:** e11883

Liu Y, Bassham DC (2012) Autophagy: pathways for self-eating in plant cells. Annu Rev Plant Biol **63:** 215–237

Liu Y, Burgos JS, Deng Y, Srivastava R, Howell SH, Bassham DC (2012) Degradation of the endoplasmic reticulum by autophagy during endoplasmic reticulum stress in Arabidopsis. Plant Cell **24:** 4635–4651

Liu Y, Schiff M, Czymmek K, Tallóczy Z, Levine B, Dinesh-Kumar SP (2005) Autophagy regulates programmed cell death during the plant innate immune response. Cell **121:** 567–577

Liu Y, Xiong Y, Bassham DC (2009) Autophagy is required for tolerance of drought and salt stress in plants. Autophagy **5:** 954–963

Mahfouz MM, Kim S, Delauney AJ, Verma DPS (2006) Arabidopsis TARGET OF RAPAMYCIN interacts with RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals. Plant Cell **18:** 477–490

Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C (2002) Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proc Natl Acad Sci USA **99:** 6422–6427

Michaeli S, Galili G (2014) Degradation of organelles or specific organelle components via selective autophagy in plant cells. Int J Mol Sci **15:** 7624–7638

Michaeli S, Honig A, Levanony H, Peled-Zehavi H, Galili G (2014) Arabidopsis ATG8-INTERACTING PROTEIN1 is involved in autophagy-dependent vesicular trafficking of plastid proteins to the vacuole. Plant Cell **26:** 4084–4101

Mizushima N (2010) The role of the Atg1/ULK1 complex in autophagy regulation. Curr Opin Cell Biol **22:** 132–139

Moreau M, Azzopardi M, Clément G, Dobrenel T, Marchive C, Renne C, Martin-Magniette ML, Taconnat L, Renou JP, Robaglia C, et al (2012) Mutations in the Arabidopsis homolog of LST8/GβL, a partner of the target of Rapamycin kinase, impair plant growth, flowering, and metabolic adaptation to long days. Plant Cell **24:** 463–481

Moreno AA, Mukhtar MS, Blanco F, Boatwright JL, Moreno I, Jordan MR, Chen Y, Brandizzi F, Dong X, Orellana A, et al (2012) IRE1/bZIP60-mediated unfolded protein response plays distinct roles in plant immunity and abiotic stress responses. PLoS One **7:** e31944

Mori K, Kawahara T, Yoshida H, Yanagi H, Yura T (1996) Signalling from endoplasmic reticulum to nucleus: transcription factor with a basic-leucine zipper motif is required for the unfolded protein-response pathway. Genes Cells **1:** 803–817

Nagashima Y, Mishiba K, Suzuki E, Shimada Y, Iwata Y, Koizumi N (2011) Arabidopsis IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor. Sci Rep **1:** 29

Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol **10:** 459–467

Noda T, Ohsumi Y (1998) Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J Biol Chem **273:** 3963–3966

Nukarinen E, Nägele T, Pedrotti L, Wurzinger B, Mair A, Landgraf R, Börnke F, Hanson J, Teige M, Baena-Gonzalez E, et al (2016) Quantitative phosphoproteomics reveals the role of the AMPK plant ortholog SnRK1 as a metabolic master regulator under energy deprivation. Sci Rep **6:** 31697

Orsi A, Razi M, Dooley HC, Robinson D, Weston AE, Collinson LM, Tooze SA (2012) Dynamic and transient interactions of Atg9 with autophagosomes, but not membrane integration, are required for autophagy. Mol Biol Cell **23:** 1860–1873

Pattingre S, Espert L, Biard-Piechaczyk M, Codogno P (2008) Regulation of macroautophagy by mTOR and Beclin 1 complexes. Biochimie **90:** 313–323

Pu Y, Luo X, Bassham DC (2017) TOR-dependent and -independent pathways regulate autophagy in Arabidopsis thaliana. Front Plant Sci **8:** 1204

Rao Y, Perna MG, Hofmann B, Beier V, Wollert T (2016) The Atg1-kinase complex tethers Atg9-vesicles to initiate autophagy. Nat Commun **7:** 10338

Reggiori F, Shintani T, Nair U, Klionsky DJ (2005) Atg9 cycles between mitochondria and the pre-autophagosomal structure in yeasts. Autophagy **1:** 101–109

Ren M, Qiu S, Venglat P, Xiang D, Feng L, Selvaraj G, Datla R (2011) Target of rapamycin regulates development and ribosomal RNA expression through kinase domain in Arabidopsis. Plant Physiol **155:** 1367–1382

Soto-Burgos J, Bassham DC (2017) SnRK1 activates autophagy via the TOR signaling pathway in Arabidopsis thaliana. PLoS One **12:** e0182591

Spitzer C, Li F, Buono R, Roschzttardtz H, Chung T, Zhang M, Osteryoung KW, Vierstra RD, Otegui MS (2015) The endosomal protein CHARGED MULTIVESICULAR BODY PROTEIN1 regulates the autophagic turnover of plastids in Arabidopsis. Plant Cell **27:** 391–402

Sugden C, Crawford RM, Halford NG, Hardie DG (1999) Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 5′-AMP. Plant J **19:** 433–439

Surpin M, Zheng H, Morita MT, Saito C, Avila E, Blakeslee JJ, Bandyopadhyay A, Kovaleva V, Carter D, Murphy A, et al (2003) The VTI family of SNARE proteins is necessary for plant viability and mediates different protein transport pathways. Plant Cell **15:** 2885–2899

Suttangkakul A, Li F, Chung T, Vierstra RD (2011) The ATG1/ATG13 protein kinase complex is both a regulator and a target of autophagic recycling in Arabidopsis. Plant Cell **23:** 3761–3779

Synek L, Sekereš J, Zárský V (2014) The exocyst at the interface between cytoskeleton and membranes in eukaryotic cells. Front Plant Sci **4:** 543

Thumm M, Egner R, Koch B, Schlumpberger M, Straub M, Veenhuis M, Wolf DH (1994) Isolation of autophagocytosis mutants of Saccharomyces cerevisiae. FEBS Lett **349:** 275–280

Tooze SA, Abada A, Elazar Z (2014) Endocytosis and autophagy: exploitation or cooperation? Cold Spring Harb Perspect Biol **6:** a018358

Tsukada M, Ohsumi Y (1993) Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett **333:** 169–174

van Doorn WG, Papini A (2013) Ultrastructure of autophagy in plant cells: a review. Autophagy **9:** 1922–1936

Wang BL, Shi L, Li YX, Zhang WH (2010) Boron toxicity is alleviated by hydrogen sulfide in cucumber (Cucumis sativus L.) seedlings. Planta **231:** 1301–1309

Wang P, Mugume Y, Bassham DC (2017) New advances in autophagy in plants: regulation, selectivity and function. Semin Cell Dev Biol S1084-9521(17)30129-5 (in press) http://doi.org/10.1016/j.semcdb.2017.07.018

Wang P, Richardson C, Hawes C, Hussey PJ (2016) Arabidopsis NAP1 regulates the formation of autophagosomes. Curr Biol **26:** 2060–2069

Wang Y, Cai S, Yin L, Shi K, Xia X, Zhou Y, Yu J, Zhou J (2015a) Tomato HsfA1a plays a critical role in plant drought tolerance by activating ATG genes and inducing autophagy. Autophagy **11:** 2033–2047

Wang Y, Yu B, Zhao J, Guo J, Li Y, Han S, Huang L, Du Y, Hong Y, Tang D, et al (2013) Autophagy contributes to leaf starch degradation. Plant Cell **25:** 1383–1399

Wang Y, Zheng X, Yu B, Han S, Guo J, Tang H, Yu AY, Deng H, Hong Y, Liu Y (2015b) Disruption of microtubules in plants suppresses macroautophagy and triggers starch excess-associated chloroplast autophagy. Autophagy **11:** 2259–2274

Wang Z, Wilson WA, Fujino MA, Roach PJ (2001) Antagonistic controls of autophagy and glycogen accumulation by Snf1p, the yeast homolog of

AMP-activated protein kinase, and the cyclin-dependent kinase Pho85p. Mol Cell Biol **21:** 5742–5752

Xiong Y, Contento AL, Nguyen PQ, Bassham DC (2007) Degradation of oxidized proteins by autophagy during oxidative stress in Arabidopsis. Plant Physiol **143:** 291–299

Xiong Y, Sheen J (2015) Novel links in the plant TOR kinase signaling network. Curr Opin Plant Biol **28:** 83–91

Xu G, Wang S, Han S, Xie K, Wang Y, Li J, Liu Y (2017) Plant Bax Inhibitor-1 interacts with ATG6 to regulate autophagy and programmed cell death. Autophagy **13:** 1161–1175

Yamamoto H, Kakuta S, Watanabe TM, Kitamura A, Sekito T, Kondo-Kakuta C, Ichikawa R, Kinjo M, Ohsumi Y (2012) Atg9 vesicles are an important membrane source during early steps of autophagosome formation. J Cell Biol **198:** 219–233

Yang X, Bassham DC (2015) New insight into the mechanism and function of autophagy in plant cells. Int Rev Cell Mol Biol **320:** 1–40

Yang X, Srivastava R, Howell SH, Bassham DC (2016) Activation of autophagy by unfolded proteins during endoplasmic reticulum stress. Plant J **85:** 83–95

Yin Z, Pascual C, Klionsky DJ (2016) Autophagy: machinery and regulation. Microb Cell **3:** 588–596

Zaffagnini M, Fermani S, Costa A, Lemaire SD, Trost P (2013) Plant cytoplasmic GAPDH: redox post-translational modifications and moonlighting properties. Front Plant Sci **4:** 450

Zhang H, Hu LY, Hu KD, He YD, Wang SH, Luo JP (2008) Hydrogen sulfide promotes wheat seed germination and alleviates oxidative damage against copper stress. J Integr Plant Biol **50:** 1518–1529

Zhang H, Tan ZQ, Hu LY, Wang SH, Luo JP, Jones RL (2010) Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. J Integr Plant Biol **52:** 556–567

Zhao Q, Gao C, Lee P, Liu L, Li S, Hu T, Shen J, Pan S, Ye H, Chen Y, et al (2015) Fast-suppressor screening for new components in protein trafficking, organelle biogenesis and silencing pathway in Arabidopsis thaliana using DEX-inducible FREE1-RNAi plants. J Genet Genomics **42:** 319–330

Zheng Z, Qamar SA, Chen Z, Mengiste T (2006) Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J **48:** 592–605

Zhou J, Wang J, Cheng Y, Chi YJ, Fan B, Yu JQ, Chen Z (2013) NBR1-mediated selective autophagy targets insoluble ubiquitinated protein aggregates in plant stress responses. PLoS Genet **9:** e1003196

Zhou J, Wang J, Yu JQ, Chen Z (2014) Role and regulation of autophagy in heat stress responses of tomato plants. Front Plant Sci **5:** 174

Zhuang X, Chung KP, Cui Y, Lin W, Gao C, Kang BH, Jiang L (2017) ATG9 regulates autophagosome progression from the endoplasmic reticulum in Arabidopsis. Proc Natl Acad Sci USA **114:** E426–E435

Zhuang X, Cui Y, Gao C, Jiang L (2015) Endocytic and autophagic pathways crosstalk in plants. Curr Opin Plant Biol **28:** 39–47

Zhuang X, Wang H, Lam SK, Gao C, Wang X, Cai Y, Jiang L (2013) A BAR-domain protein SH3P2, which binds to phosphatidylinositol 3-phosphate and ATG8, regulates autophagosome formation in Arabidopsis. Plant Cell **25:** 4596–4615

Zientara-Rytter K, Sirko A (2014) Selective autophagy receptor Joka2 co-localizes with cytoskeleton in plant cells. Plant Signal Behav **9:** e28523