Journal of Experimental Botany, Vol. 69, No. 6 pp. 1335–1353, 2018
doi:10.1093/jxb/ery069 Advance Access publication 21 February 2018

REVIEW PAPER

Autophagy-related approaches for improving nutrient use efficiency and crop yield protection

Tamar Avin-Wittenberg[1], Frantisek Baluška[2], Peter V. Bozhkov[3], Pernilla H. Elander[3], Alisdair R. Fernie[4], Gad Galili[5], Ammar Hassan[2], Daniel Hofius[6], Erika Isono[7], Romain Le Bars[8], Céline Masclaux-Daubresse[9], Elena A. Minina[3], Hadas Peled-Zehavi[5], Núria S. Coll[10], Luisa M. Sandalio[11], Béatrice Satiat-Jeunemaitre[8], Agnieszka Sirko[12], Pilar S. Testillano[13] and Henri Batoko[14,*]

[1] Department of Plant and Environmental Sciences, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Givat Ram, Jerusalem 9190401, Israel
[2] Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany
[3] Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, PO Box 7015, SE-75007 Uppsala, Sweden
[4] Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany
[5] Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot 76100 Israel
[6] Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center of Plant Biology, SE-75007 Uppsala, Sweden
[7] Department of Biology, University of Konstanz, Universitätsstrasse 10, D-78464 Konstanz, Germany
[8] Cell Biology Pôle Imagerie-Gif, Institute of Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, 91 198, Gif-sur-Yvette, France
[9] INRA-AgroParisTech, Institut Jean-Pierre Bourgin, UMR1318, ERL CNRS 3559, Saclay Plant Sciences, Versailles, France
[10] Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra-Cerdanyola del Valles 08193, Catalonia, Spain
[11] Departmento de Bioquímica, Biología Celular y Molecular de Plantas Experimental del Zaidín, CSIC, 18008 Granada, Spain
[12] Institute of Biochemistry and Biophysics Polish Academy of Sciences, ul. Pawinskiego 5A, 02-106 Warsaw, Poland
[13] Pollen Biotechnology of Crop Plants group, Centro de Investigaciones Biológicas, Biological Research Centre (CIB), CSIC, Ramiro de Maeztu, 9, 28040 Madrid, Spain
[14] Université Catholique de Louvain, Institute of Life Sciences, Croix du Sud 4, L7.07.14, 1348 Louvain-la-Neuve, Belgium

*Correspondence: henri.batoko@uclouvain.be

Received 20 November 2017; Editorial decision 16 February 2018; Accepted 16 February 2018

Editor: Chris Hawes, Oxford Brookes University, UK

Abstract

Autophagy is a eukaryotic catabolic pathway essential for growth and development. In plants, it is activated in response to environmental cues or developmental stimuli. However, in contrast to other eukaryotic systems, we know relatively little regarding the molecular players involved in autophagy and the regulation of this complex pathway. In the framework of the COST (European Cooperation in Science and Technology) action TRANSAUTOPHAGY (2016–2020), we decided to review our current knowledge of autophagy responses in higher plants, with emphasis on knowledge gaps. We also assess here the potential of translating the acquired knowledge to improve crop plant growth and development in a context of growing social and environmental challenges for agriculture in the near future.

Keywords: Abiotic stress, autophagy regulation, biotic stress, nutrient deficiency, phagophore initiation, plant autophagy

Introduction

During the lifespan of a eukaryotic cell, a catabolic pathway known as autophagy degrades dysfunctional or unnecessary cellular components as a way of recycling macromolecules and ensuring cellular homeostasis (Klionsky *et al.*, 2016). Essentially, autophagy consists of the translocation of cytoplasmic components (cargo) into the vacuole (yeast and plant) or the lysosome (animal), and their subsequent degradation (Li and Vierstra, 2012). In plants, autophagy functions in regulating fitness, longevity, and fecundity, underpinning plant tolerance to various biotic and abiotic stresses (Minina *et al.*, 2018). Plant cells decrease their dependency on external nutrient sources by recycling their contents via autophagy (Guiboileau *et al.*, 2013; Minina *et al.*, 2013*b*). Furthermore, autophagy increases cell viability under stress conditions by a quick removal of damaged macromolecules and organelles (Bassham *et al.*, 2006; Li and Vierstra, 2012; Michaeli *et al.*, 2016), modulation of immune response, and targeting of virulence factors or entire pathogens (Lenz *et al.*, 2011; Hafrén *et al.*, 2017; Haxim *et al.*, 2017). Thus, autophagy may have an effect on important agricultural traits, namely tolerance to macronutrient depletion, drought, heat, oxidative, and salt stress, as well as immune response to pathogen infection. Although most of the research has been performed in the model plant *Arabidopsis thaliana* thus far, the involvement of autophagy in a variety of agricultural traits generates interest in the development of tools for efficient modulation of autophagy in plants. Here we will review the current knowledge regarding autophagy in plants, its functional mechanisms, and physiological roles, and highlight possible uses for autophagy manipulation as potential enhancers of plant yield and stress tolerance.

Types of autophagy in plants

Autophagy can be broadly divided into microautophagy and macroautophagy (Galluzzi *et al.*, 2017). Other variants of autophagy such as chaperone-mediated autophagy (CMA) (Kaushik and Cuervo, 2012), secretory autophagy (Ponpuak *et al.*, 2015) in mammalian cells, and cytoplasm to vacuole transport (CVT) in yeast (Reggiori *et al.*, 2004) are cell type specific and have not yet been described in plants. Both microautophagy and macroautophagy can be selective or non-selective in plants. Microautophagy is characterized by a direct invagination of the tonoplast (vacuolar membrane) to take up the cellular components to be degraded. A well-described example in plants is the functional accumulation of anthocyanins through microautophagy-derived inclusion bodies in the plant vacuole (Chanoca *et al.*, 2015). Anthocyanins are a diverse family of flavonoid pigments synthesized in the cytoplasm, acting as antioxidants and involved in plant tissue responses to environmental cues. These pigments are stored in the vacuole as densely packed 3–10 µm vesicles generated through a microautophagy process (Chanoca *et al.*, 2015). The molecular mechanisms of membrane dynamics driving microautophagy are not well understood in plants, but seem not to require any of the gene products involved in macroautophagy. Macroautophagy (hereafter referred to as autophagy) is characterized by the *de novo* formation of a double-membrane organelle, the autophagosome, wrapping defined cytoplasmic components for degradation. A conserved set of proteins encoded by autophagy-related (*ATG*) genes facilitate the initiation, elongation, maturation, and fusion of the autophagosome with the vacuole (Tsukada and Ohsumi, 1993). Plant-specific autophagic pathways defined by their cargo type exist as well. A well-described example is chlorophagy or the autophagic degradation of whole chloroplasts (up to 5–10 µm in size, mean volume of 20 μm^3) damaged by UV light (Izumi *et al.*, 2017). The molecular mechanism and the complement of ATG proteins involved in the formation of these uncommonly large autophagosomes may be specific to the plant kingdom.

Core autophagy complexes and their regulation in plants

Autophagy is a tightly regulated cellular process, which can be activated rapidly and transiently in eukaryotic cells. The formation of the autophagosome is a complex, dynamic, and stepwise process resulting in the engulfment of cytoplasmic material and its translocation to the vacuole. The molecular machinery that executes and regulates autophagy was first characterized in yeast (Tsukada and Ohsumi, 1993). More than 36 *ATG* genes have been characterized to date, half of them encoding core autophagy proteins, and appear to be well conserved in most studied multicellular organisms, including plants (Klionsky *et al.*, 2016; Galluzzi *et al.*, 2017). Autophagy needs to be quickly fine-tuned to fit the temporary requirements of cells under variable conditions. In animal cells, autophagy seems to oscillate with surprisingly high frequency (Nazio *et al.*, 2016). Most of the ATG proteins are synthesized in an inactive form to prevent unwanted autophagic activity, and require activation by post-translational modification and recruitment into complexes (Ohsumi, 2014). Activation of autophagy is regulated by sensors of the cellular nutrient state (Liu and Bassham, 2010) and stress (Minina *et al.*, 2013*a*; Wang *et al.*, 2015*a*; Yang *et al.*, 2016). The pre-autophagosomal membrane or phagophore is initiated in response to an internal or external cellular stimulus, then elongates and enwraps the cytoplasmic cargo. The closed phagophore matures into an autophagosome and then fuses with the vacuole/lysosome (Enrique Gomez *et al.*, 2017). Each of the mentioned steps is under the control of specific autophagy complexes made of core autophagy proteins, whose assembly, subsequent subcellular localization, and activity are directly or indirectly regulated by stress signaling pathways (He and Klionsky, 2009). Four main complexes are known to be required for autophagosome initiation and formation, namely the ATG1 complex, the VPS34 complex, the ATG9 complex, and the ATG8 conjugation systems (Fig. 1).

The ATG1 complex is thought to function in transmitting stress signals to the site where the autophagosome will be formed, most probably at an organelle contact site involving

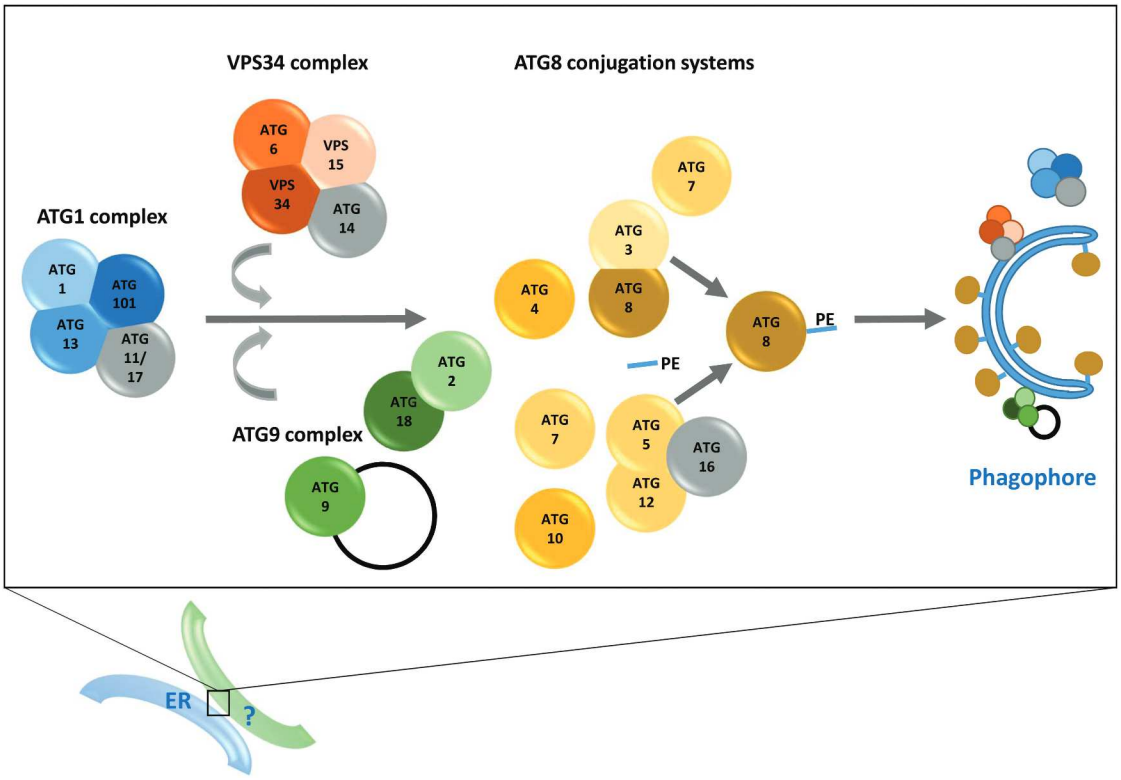

Fig. 1. Autophagic response initiation in plants: complexes in complexity. In response to a stimulus, the ATG1 complex is formed and targeted to an organelle contact site involving the endoplasmic reticulum (ER) and an unknown organelle (?). The ATG1 complex activates and recruits the VPS34 complex, resulting in local PI3P (phosphatidylinositol-3-phosphate) synthesis and enrichment within the organelle contact site. ATG9-containing vesicles (black circle) are docked to the contact site by ATG9 interaction with ATG2–ATG18 dimers, and site-localized through ATG18 binding to PI3P, the input of membrane lipids and defined proteins contributing in the formation of the phagophore. The phagophore membranes are decorated with enzymatically processed and lipidated (conjugation to PE, phosphatidylethanolamine) ATG8. This process is facilitated by components of the ATG8 conjugation systems. Putative subunits of the various complexes not yet characterized in plant are illustrated in gray.

the endoplasmic reticulum (ER) (He and Klionsky, 2009; Antonioli *et al.*, 2017; Nascimbeni *et al.*, 2017). In yeast and mammalian cells, the ATG1 complex is a trimeric heterocomplex made of a catalytic subunit (ATG1/ULK, a serine/threonine kinase), regulatory subunits (ATG13 and ATG101), and scaffold subunits (ATG11 or ATG17 in yeast, and FIP200/RB1CC1 RB1 inducible coiled-coil 1 in animals) (Galluzzi *et al.*, 2017). The structure, function, and regulation of the ATG1 complex in plants are not well understood. The Arabidopsis genome, for example, encodes three full-length ATG1 proteins (ATG1a, locus AT3G61960; ATG1b, locus AT3G53960; and ATG1c, locus AT2G37840), and a C-terminal truncated ATG1 variant called ATG1t (locus AT1G49180) (Suttangkakul *et al.*, 2011), whose function is not yet clear. The Arabidopsis genome also encodes two functional ATG13 homologs, ATG13a and ATG13b, and a single ATG101 (Suttangkakul *et al.*, 2011). Interestingly, no functional or structural homolog of ATG17/FIP200 has been found yet in deciphered plant genomes. A potentially bifunctional protein containing structural domains related to yeast ATG11 and ATG17 is present in plants. In Arabidopsis, this protein was named an ATG11 homolog since it is required for selective degradation of mitochondria via autophagy (Li *et al.*, 2014). However, whether the plant ATG11-related protein acts as a scaffold protein within a *bona fide* ATG1 complex remains to be clarified. If the plant ATG11-related protein functions only in selective autophagy, the plant scaffold protein required for non-selective autophagy is still to be identified.

The clustering and activation of the ATG1 complex at the phagophore initiate the recruitment of other autophagy complexes, in particular the class III VPS34 complex. The class III VPS34 complex involved in autophagy contains the catalytic subunit phosphoinositide 3-kinase (PI3K), the regulatory subunits ATG6/Beclin-1 and ATG14, and the scaffold subunit VPS15. As compared with other multicellular organisms, plants surprisingly express only one, essential PI3K of the class III type. The structure of the VPS34 complex involved in autophagy is not known in plants. Remarkably, ATG14 is absent in the plant lineage. Yeast ATG14 and its functional homologs in other eukaryotic systems only share resemblance at their N-terminal coil-coiled domain (~200 first amino acids), whereas the C-terminus of these proteins is highly divergent (Itakura *et al.*, 2008). ATG14 is known to determine the localization of the VPS34 complex, and to be required for both basal and induced autophagy in yeast and animals (Fan *et al.*, 2011; Diao *et al.*, 2015). Phosphorylation of ATG14 by the ATG1 kinase activates the catalytic activity of PI3K, which catalyzes the production of the membrane lipid PI3P (phosphatidylinositol-3-phosphate) essential for

phagophore initiation and expansion (Baskaran *et al.*, 2014). Whether a functional counterpart of ATG14 exists in plants awaits further research.

Phagophore initiation and expansion require the input of specific lipids and proteins. The membrane source of these materials is still under debate, but they are most probably channeled to the site of autophagosome formation through ATG9-containing vesicles (Abada and Elazar, 2014; Karanasios *et al.*, 2016). ATG9 is the only transmembrane protein among all known ATG proteins (Reggiori *et al.*, 2004). The heterodimer complex ATG2–ATG18 regulates ATG9 vesicle-mediated cycling and tethering to and from the growing phagophore. Although most plants seem to encode a single functional ATG9 and ATG2 homologs, a multigenic family encodes the PI3P-binding ATG18-related proteins (up to eight in Arabidopsis as compared with one in yeast and four in mammals). The resting cellular localization of ATG9 and the full complement of its interacting partners during autophagy-dependent membrane dynamics are not yet understood in plants.

Phagophore membrane expansion also requires the recruitment of lipidated ATG8-related protein. Soluble ubiquitin-like ATG8 becomes membrane anchored through conjugation to the membrane lipid PE (phosphatidylethanolamine) at its C-terminus. This modification occurs through a ubiquitylation-like cascade regulated by the protease ATG4, the E1 activating enzyme ATG7, the E2 conjugating enzyme ATG3, and the E3 ligase complex comprising ATG5/ATG12/ATG16 (Pengo *et al.*, 2017; Sánchez-Wandelmer *et al.*, 2017). Apart from *bona fide* ATG16, whose plant ortholog has not yet been characterized, all the other components of this cascade are expressed and active in plants. ATG8-related members are relatively more varied in plants, with some C-terminally truncated isoforms that are unique to plants (Bassham *et al.*, 2006; Li *et al.*, 2016).

Autophagy is regulated in various eukaryotic systems at many steps through post-translational modifications such as ubiquitylation, phosphorylation, acetylation, glycosylation, and lipidation of ATG proteins. ATG proteins can be modified differently at multiple sites, and one modification may be dependent on other modifications. An example is phosphorylation-dependent ubiquitylation that leads to degradation of target proteins (Lin *et al.*, 2002). By acting as a degradation signal, ubiquitylation not only marks the cargo of selective autophagy, but also regulates the autophagy machinery itself. The abundances of mammalian ATG1/ULK1, ATG6/VPS30/beclin-1 (BECN1), and ATG12, for example, were reported to be regulated by ubiquitylation. ULK1 controls the autophagic flux together with ATG13, and is ubiquitylated by the E3 ligase NEDD4L (Nazio *et al.*, 2016), making NEDD4L a negative regulator of autophagy. BECN1, a positive regulator of autophagy induction, is a target of multiple E3 ligases (Shi and Kehrl, 2010; Xia *et al.*, 2013; Xu *et al.*, 2014). By removing the ubiquitin chains, deubiquitylating enzymes such as USP10, USP13, USP14, and USP19 can counteract the E3 ligase activity and rescue BECN1 from degradation, and thus act as positive regulators of autophagy (Liu *et al.*, 2011; Jin *et al.*, 2016; Xu *et al.*, 2016). These recent studies reveal the molecular details of a tight regulation of autophagy through post-translational modification. Since not all of these regulators are conserved in plants, future research is needed to determine whether and how post-translational modification regulates plant ATG proteins.

Maturation and fusion of the autophagosome with the lytic compartment involve the movement of the matured autophagosome toward the vacuole in plants and specific tethering of the autophagosome to the tonoplast. The F-actin nucleating and branching ARP2/3 complex was shown in yeast to be associated with the autophagosome (Reggiori *et al.*, 2005). In mammalian cells, WASP homolog associated with actin, membranes, and microtubules (WHAMM), WAS protein family homolog (WASH), and junction-mediating and regulatory protein (JMY) were reported to regulate autophagy (King *et al.*, 2013; Xia *et al.*, 2013; Zavodszky *et al.*, 2014; Coutts and La Thangue, 2015; Kast *et al.*, 2015). In plants, only the WASP family verprolin (WAVE) homologous complex has been shown to be involved in autophagosome movement within the cytoplasm (Wang *et al.*, 2016*b*). One of the WAVE subunits, NAP1, changes its localization from the cytoplasm to ER membrane under mechanical stress (Wang *et al.*, 2016*a*). This localization change triggers the ARP2/3-dependent F-actin nucleation on the phagophore, facilitating phagophore expansion and autophagosome maturation (Wang et al., 2016*b*, 2017*a*). Loss-of-function *nap1* mutant Arabidopsis seedlings (lacking a functional WAVE complex) form fewer autophagosomes and are more sensitive to salt and nitrogen deficiency stresses (Wang *et al.*, 2016*b*, 2017*a*).

Selective autophagy in plants

Autophagy was initially considered as a bulk, non-selective process. It later became evident that autophagy selectively degrades cellular components under various conditions (Li and Vierstra, 2012; Veljanovski and Batoko, 2014; Yang and Bassham, 2015; Michaeli *et al.*, 2016; Anding and Baehrecke, 2017). Selective autophagy typically utilizes cargo receptors that directly or indirectly bind specific cargo destined for degradation, and tether it to the forming autophagosome via interaction with core autophagy proteins (mainly ATG8) (Farré and Subramani, 2016; Zaffagnini and Martens, 2016; Kellner *et al.*, 2017). In mammals, multiple cargo receptors were identified, including p62/SQSTM1 and NBR1 that were implicated in the selective autophagy of protein aggregates and organelles (Zaffagnini and Martens, 2016; Anding and Baehrecke, 2017). p62 and NBR1 bind both ubiquitin and the mammalian ATG8 homolog, LC3, docking ubiquitinated substrates to the autophagosome. Arabidopsis NBR1 and its tobacco homolog JOKA2 are functional hybrids of mammalian p62 and NBR1, capable of binding ATG8 and ubiquitin. Both were shown to play a role in nutrient deficiency and abiotic stress tolerance (Svenning *et al.*, 2011; Zientara-Rytter *et al.*, 2011; Zhou *et al.*, 2013, 2014*a*; Hafrén *et al.*, 2017).

A crosstalk between the major cellular degradation pathways, autophagy and the proteasome, was uncovered with the discovery of both non-selective (starvation- induced) and

selective autophagy-mediated degradation of the 26S proteasome in Arabidopsis (Marshall *et al.*, 2015). While the proteasome subunit RPN10 was previously shown to facilitate the recognition of ubiquitinated targets, Marshall *et al.* (2015) found that it can also bind ATG8. They further demonstrated that RPN10 is needed for inhibition-induced selective degradation of inactive 26S proteasome complexes (proteaphagy), suggesting a role for RPN10 as an autophagy cargo receptor. Intriguingly, mammalian p62 was shown to mediate selective starvation-induced autophagosomal uptake of proteasomes (Cohen-Kaplan *et al.*, 2016; Marshall *et al.*, 2016). Whether NBR1 or other plant cargo receptors have similar functions awaits further research.

Chloroplasts represent an interesting case study for selective autophagy in plants, as they have unique turnover demands due to the photosynthetic electron chain and its oxidative by-products. In addition, chloroplasts are the major nitrogen reservoir in mesophyll cells and thus important for nutrient recycling (Ishida *et al.*, 2014). Early studies suggested that the vacuole plays a role in chloroplast recycling (Wittenbach *et al.*, 1982; Minamikawa *et al.*, 2001), and autophagy-related and -unrelated pathways were later implicated in the degradation of chloroplast components (chlorophagy) (Martínez *et al.*, 2008; Wang *et al.*, 2013; Ishida *et al.*, 2014; Michaeli *et al.*, 2014; Wang and Blumwald, 2014; Xie *et al.*, 2016; Izumi *et al.*, 2017). Two types of vesicles, Rubisco-containing bodies (RCBs) and ATG8-interacting protein1 (ATI1) bodies, were shown to participate in chlorophagy and are induced during senescence and abiotic stresses (Chiba *et al.*, 2003; Ishida *et al.*, 2008; Wada *et al.*, 2009; Honig *et al.*, 2012; Yamane *et al.*, 2012; Dong and Chen, 2013; Michaeli *et al.*, 2014). Autophagy is also involved in the remobilization of transitory starch from chloroplasts to vacuoles via small starch granule-like structures (SSGLs) (Wang *et al.*, 2013). RCBs were characterized in Arabidopsis, tobacco, wheat, and rice (Chiba *et al.*, 2003; Ishida *et al.*, 2008; Prins *et al.*, 2008; Wada *et al.*, 2009; Ono *et al.*, 2013; Izumi *et al.*, 2015). They were shown to deliver Rubisco and other stromal proteins to the vacuole, though their mode of cargo recognition is not known (Chiba *et al.*, 2003). ATI1 is a plant-specific ATG8-binding protein localized to the ER and chloroplasts (Honig *et al.*, 2012; Michaeli *et al.*, 2014). In Arabidopsis, ATI1-labeled vesicles (ATI1-bodies) were shown to deliver plastid-targeted green fluorescent protein (GFP) to the vacuole. ATI1 can bind both stromal and membrane-bound chloroplast proteins, suggesting that the cargo of ATI1 bodies differ from that of RCBs (Michaeli *et al.*, 2014). Another difference is that RCBs are associated with chloroplast stromules, while ATI1 bodies initiate inside the chloroplast. Also, the release of RCBs from the chloroplast is dependent on the ATG machinery, while ATI1 bodies bud from it in an ATG-independent manner, though their delivery to the vacuole requires an active autophagy machinery (Ishida *et al.*, 2014; Michaeli *et al.*, 2014). Two ESCRT-III subunit paralogs were implicated in the delivery of RCBs to the vacuole, suggesting a crosstalk between chlorophagy and endomembrane trafficking events (Spitzer *et al.*, 2015; Kalinowska and Isono, 2018). Another chlorophagy pathway involves the vacuolar delivery of entire shrunken chloroplasts (Wada *et al.*, 2009). This pathway is induced upon UV-B or high light treatments (Izumi *et al.*, 2017). Information regarding selective autophagy of other types of plastids is still limited, but there is evidence for RCB-like and entire plastid autophagy in roots of Arabidopsis and rice (Nakayama *et al.*, 2012; Izumi *et al.*, 2015).

Selective autophagy of other organelles was demonstrated in plants, as well as yeast and mammalian systems. Mitophagy, the selective degradation of mitochondria by autophagy, has only recently been identified in plants with the characterization of an Arabidopsis ATG11-related protein. As in yeast, the Arabidopsis ATG11-related protein participates in the selective clearance of mitochondria. ATG11 mutant Arabidopsis plants display mitochondrial accumulation (Li *et al.*, 2014). However, a plant homolog of the yeast ATG32, which recruits ATG11 to damaged mitochondria, has not been identified (Li *et al.*, 2014; Anding and Baehrecke, 2017). Plants also lack homologs of animal mitophagy receptors such as the BCL2-interacting protein (BNIP) family members. ER-phagy (reticulophagy), the selective degradation of ER by autophagy, is induced by ER stress resulting from the accumulation of unfolded or misfolded proteins in the ER, similarly to yeast and mammals (Liu *et al.*, 2012; Yang *et al.*, 2016; Anding and Baehrecke, 2017; Dikic, 2017). This process requires the ER stress sensor IRE1b, but the downstream factors remain unknown (Liu *et al.*, 2012). In Arabidopsis, as in other organisms, ribophagy, the autophagic degradation of rRNA, requires the non-specific T2 endoribunuclease RNS2 (Hillwig *et al.*, 2011; Floyd *et al.*, 2015, 2017; Bassham and MacIntosh, 2017). A differential role was suggested for ATG5 and ATG9 in this process, but the exact mechanism of the selection of rRNA for degradation is still unknown (Floyd *et al.*, 2015).

Recent studies have demonstrated the selective degradation of peroxisomes by autophagy (pexophagy) in plants, a process previously characterized in yeast and mammalian systems (Cho *et al.*, 2018). Peroxisomes are highly dynamic organelles, housing oxidative metabolic pathways, such as photorespiration and fatty acid β-oxidation, produce reactive oxygen species, and contain antioxidative components (Sandalio and Romero-Puertas, 2015). During seedling establishment, in a light-dependent manner, there is a functional transition from glyoxysomes, peroxisomes present in seeds and containing the glyoxylate cycle and β-oxidation, to leaf-type peroxisomes, containing photorespiration enzymes. Recent evidence shows that pexophagy takes place during this metabolic remodeling combined with peroxisomal protease LON2 activity (Young and Bartel, 2016). Pexophagy also mediates the turnover of peroxisomes damaged by H_2O_2 accumulation in old tissues, under favorable and stress conditions, regulating the quality and number of peroxisomes (Shibata *et al.*, 2013, 2014). Pexophagy occurs at a higher rate in green tissues, and appears to be more substantial than other types of selective autophagy due to the highly oxidative peroxisomal metabolism (Yoshimoto *et al.*, 2014). Autophagy receptors facilitate pexophagy, although the plant autophagy receptor/adaptor protein linking ATG8 to damaged peroxisomes has not been identified. Some evidence, though controversial, suggests the

involvement of NBR1 (Zhou *et al.*, 2013). Recently, nine peroxines (PEXs; peroxisomal membrane proteins) have been identified as possible ATG8-binding proteins, two of which, AtPEX6 and AtPEX10, were shown to interact with ATG8 by bimolecular fluorescence complementation (BiFC) (Xie *et al.*, 2016). The signal involved in triggering pexophagy has not yet been identified, although oxidized catalase has been proposed as a possible candidate (Shibata *et al.*, 2013).

Methods of monitoring and manipulating autophagy in plants

Monitoring autophagy in various systems has been previously described (Klionsky *et al.*, 2016). However, plant systems pose unique challenges requiring specific modifications. Here we summarize some of the methods commonly used to assess and modulate autophagy in plants, adding to some other excellent reviews on the topic (Bassham, 2015).

Monitoring autophagy in plants by biochemical analysis

Assessing the formation and degradation of autophagosomes can be performed using western blot analysis. Two main approaches exist for this analysis: (i) ATG8 lipidation assay (autophagosome formation) and (ii) free GFP release assay from the expressed GFP–ATG8 chimera (autophagosome degradation in the vacuole).

ATG8 is incorporated into the growing phagophore membranes through a C-terminal post-translational modification (processing followed by lipidation). Assessing the rate of ATG8 lipidation can be used as a measure of autophagosome formation. The lipidated and non-lipidated forms of ATG8 can be separated by SDS–PAGE in the presence of 6 M urea followed by western blotting (Thompson *et al.*, 2005; Chung *et al.*, 2009). Expression of GFP–ATG8 can be used to visualize autophagosomes using confocal microscopy, as discussed later in this section. It is also possible to monitor the release of free GFP after proteolysis of GFP–ATG8 in the vacuole. The level of free GFP released from ATG8 indicates the relative rate of autophagosome degradation and can be used as a measure of autophagic flux (Slavikova *et al.*, 2008; Li *et al.*, 2015). Concanamycin A (ConcA) treatment drastically reduces GFP–ATG8 degradation in the vacuole. ConcA increases the pH of the vacuolar lumen by inhibiting the activity of the vacuolar H^+-ATPase. Therefore, ConcA treatment can result in autophagosomal bodies accumulating in the vacuole, hence reducing the proteolysis of expressed GFP–ATG8.

Imaging approaches to study plant autophagy

Live imaging of autophagosomes in plants requires both specific reporters and an adequate light microscope configuration. Multiple organic dyes, such as LysoTracker (Liu *et al.*, 2005) and monodansylcadaverin (MDC) (Contento *et al.*, 2005), have been used to label autophagosomes, based on the presumed acidity of the inner part of the autophagosome. However, their selectivity for autophagic compartments is still under debate (Mizushima, 2004; Klionsky *et al.*, 2016). Fluorescently tagged ATG proteins are more frequently used as autophagosome markers, allowing specific identification of autophagosomes at different stages of their maturation (Suttangkakul *et al.*, 2011; Le Bars *et al.*, 2014).

Tracking autophagosome formation and dynamics within plant cells may be complicated because (i) the lifetime of the process is very short; (ii) ATG proteins are only transiently associated with the autophagosomal membranes; and (iii) low expression levels of potential marker proteins and their high dynamics in some cell types. One possibility to circumvent these limitations is to use a light microscope equipped with highly sensitive detectors and image acquisition at a high frame rate. These conditions are met using confocal laser scanning microscopes with a resonant scanner, or a spinning disk microscope whose high-speed acquisition rate can also lower the phototoxic effect of the imaging process (Fig. 2a).

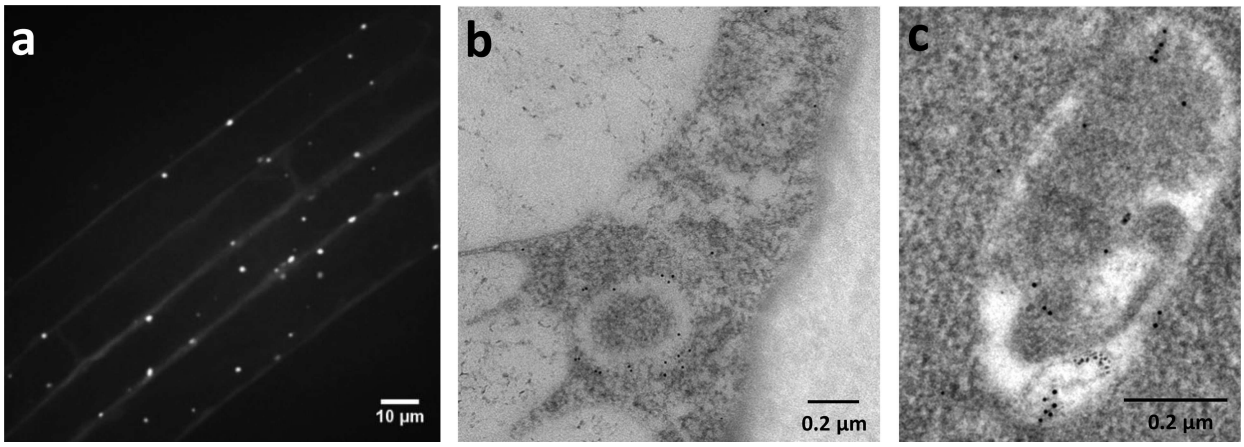

Fig. 2. Imaging of plant autophagic structures and subcellular localization of ATG8 by different microscopy approaches. (a) Live imaging of ATG8–GFP reporter proteins in Arabidopsis roots observed by spinning disk confocal microscopy. (b) TEM micrograph of the same plant tissues immunolabeled for ATG8–GFP detection using anti-GFP antibodies. Note the gold particles on the autophagosome membrane. (c) TEM immunogold labeling with anti-ATG8 antibodies of a tapetal cell of *Brassica napus*.

Higher resolution autophagic structures can be visualized with TEM (Fig. 2b, c). Correlative light and electron microscopy (CLEM) protocols, allowing light microscopy and TEM observations of the same sample can be used to combine the localization of ATG proteins with light microscopy and the identification of the labeled membranes with TEM (Marion *et al.*, 2017).

Indirect (using anti-GFP antibodies) (Fig. 2b) or direct (using specific antibodies against plant ATG8) (Chung *et al.*, 2010) TEM immunogold labeling of ATG8 can provide ultrastructural details of ATG8 membrane-bound structures including autophagosomes. A convenient and feasible processing method for ATG8 immunogold labeling is freeze-substitution (FS) followed by cryoembedding in acrylic resin (Fig. 2c). Several protocols of FS have been developed, providing good ultrastructure and high sensitivity immunogold labeling of various antigens, including membrane-bound molecules (Seguí-Simarro *et al.*, 2003, 2011; Andreu *et al.*, 2007; Bernal *et al.*, 2007; Derrien *et al.*, 2012). This strategy has revealed the localization of ATG8 in autophagosomes in various plant cells and tissues such as maize aleurone (Reyes *et al.*, 2011), Arabidopsis root (Zhuang *et al.*, 2013), or *Brassica napus* tapetum (Fig. 2c), a tissue with high autophagy activity during late pollen development (Hanamata *et al.*, 2014; Papini *et al.*, 2014). The development of antibodies against plant ATG proteins, with high specificity and sensitivity, will help to identify the components and ultrastructural organization of autophagic structures in various plant cells and systems.

Approaches for manipulating plant autophagy

Autophagy is a very dynamic process, and is therefore tightly regulated at multiple levels: transcriptional, post-transcriptional, translational, and post-translational (Kraft *et al.*, 2008; Feng *et al.*, 2015). This provides many opportunities for manipulating autophagy at various regulation levels.

Targeting transcriptional regulation of plant ATG *genes*

Some ATG proteins are either actively incorporated into autophagosomes or are engulfed together with the cargo destined for degradation (Nakatogawa *et al.*, 2012; Nakatogawa, 2013). Autophagy is constitutively active at the basal level in most types of plant cells, playing a role in cell maintenance. Hence, *ATG* genes are constitutively transcribed (Pu *et al.*, 2017). Interestingly, expression of multiple plant *ATG* genes increases under stress, (e.g. under starvation conditions), coinciding with up-regulation of autophagic activity (Rose *et al.*, 2006; Chung *et al.*, 2010; Minina *et al.*, 2013*b*). Thus, identification of master regulators influencing the expression of *ATG* genes is an essential step towards the development of autophagy-modulating tools. Multiple transcription factors regulating *ATG* gene expression in animal cells have already been identified (Feng *et al.*, 2015). Although it is expected that such transcription factors also exist in plants, information about them is still very scarce. For example, it has been demonstrated that induced expression of Arabidopsis *ATG* genes upon *Botrytis cinerea* infection is directly mediated by the transcriptional activator AtWRKY33 (Lai *et al.*, 2011). Also, the tomato transcription factor HsFA1a binds the promoters of *ATG10* and *ATG18f* to activate their transcription upon drought stress (Wang *et al.*, 2015*a*).

To date, phenotypic studies of the role of autophagy in plants have been based on comparing the performance of either *ATG*-knockout or knockdown lines with that of wild-type plants under various unfavorable conditions (Kim *et al.*, 2012). All these studies collectively indicate a potential benefit of up-regulated autophagy for stress tolerance and plant fitness. While ectopic overexpression of *ATG* genes in yeast did not seem to have an effect on autophagic activity (Ma *et al.*, 2007), a growing body of evidence indicates that overexpression of *ATG* genes successfully up-regulates autophagy in other model organisms, including plants (Scott *et al.*, 2007; Xia *et al.*, 2012; Pyo *et al.*, 2013; Wang *et al.*, 2016*a*, 2017*b*; Minina *et al.*, 2018; Sun *et al.*, 2018*a*, *b*). These results indicate that the level of the core ATG proteins is a limiting factor of autophagic activity in plant and animal cells, but not in yeast. A possible explanation of this phenomenon is the difference in the number of phagophore assembly sites (PASs), where the core ATG proteins are active. While yeast cells contain a single PAS, animal and plant cells do not seem to have a limit on the number of PASs. Thus, availability of a higher amount of the core ATG proteins might stimulate the formation of a higher number of PASs, leading to increased formation of autophagosomes (Minina *et al.*, 2018). The predicted benefit of enhanced autophagy for plant fitness, fecundity, biomass, and stress tolerance has been described in recent studies (Xia *et al.*, 2012; W. Li *et al.*, 2016; Wang *et al.*, 2016*c*; Minina *et al.*, 2018; Sun *et al.*, 2018*a*, *b*). Further development of this approach is required as there might be penalties for constitutive up-regulation of plant autophagy in most of the tissues as well as benefits of tissue-/organ-specific stimulation of autophagic activity.

Targeting post-transcriptional regulation of plant ATG *genes*

Although multiple examples of miRNA regulating autophagy are known for animal models (Feng *et al.*, 2015), almost nothing is known about the post-transcriptional regulation of autophagy in plants. Indirect evidence of possible regulation of autophagy by miRNA via the stress sensor SnRK1 was demonstrated in the study by Confraria *et al.* (2013). So far, post-transcriptional silencing of plant *ATG* genes has only been implemented by using artificial *ATG*-specific RNAi constructs (Kim *et al.*, 2012).

Targeting translational regulation of plant ATG *genes*

Under stress conditions, autophagy degrades cytoplasmic content together with ribosomes, thus down-regulating the translation of most mRNAs, including ATG mRNAs (Kraft *et al.*, 2008; Bassham and MacIntosh, 2017). Importantly, selective degradation of ribosomes, ribophagy, under non-stress conditions positively affects the efficacy of the translational machinery by controlling ribosome quality (Mathis *et al.*, 2017). Artificial modulation of autophagy at the translational level has not yet been attempted due to numerous challenges regarding the specificity of this approach.

Pharmacological modulation of plant autophagy

As compared with animals, pharmacological manipulation of autophagy in plants has not been comprehensively tested, in part due to the poor cellular accessibility of many of the described chemical modulators. Paradoxically, some of the natural chemicals tested for their modulation of animal cell autophagy are plant derived, and we know nothing about their potential effect on plant autophagy (Fleming *et al.*, 2011; Vakifahmetoglu-Norberg *et al.*, 2015; Wang *et al.*, 2017*c*). Several compounds have been demonstrated either to inhibit or to stimulate plant autophagy (Klionsky *et al.*, 2016; Table 1). Drug treatment can be a quick and a relatively easy method to modulate autophagy activity in plants. The disadvantages of pharmacological modulation of plant autophagy are potential off-target effects of the drugs currently available (Table 1), issues with drug stability, and tissue/cell permeability. Nevertheless, this approach has a practical benefit, as it may be applicable for agricultural purposes in the countries that do not allow cultivation of genetically modified organisms.

Autophagy responses to abiotic stress in plant

Plant stress has been defined by Lichtenthaler (1996) as 'any unfavorable condition or substrate that affects or blocks a plant metabolism, growth or development'. A common feature of abiotic stresses such as high salinity, drought, and osmotic stress is their ability to induce, at the cellular level, a transient or permanent physiological water deficit, conducive to energy limitation in plants. A low energy level in plant tissues is sensed by a subfamily of serine/threonine kinases known as SnRK1 (SNF1-related kinase), homologous to the yeast SNF1 (sucrose non-fermenting-1) and the animal AMPK (AMP-activated protein kinase). Plant SnRK1s act as metabolite sensors to adapt metabolism constantly to the energy supply demand, and are central integrators of a transcriptional network for stress and energy signaling (Jossier *et al.*, 2009; Emanuelle *et al.*, 2015; Nukarinen *et al.*, 2016; Bakshi *et al.*, 2017). SnRK1-dependent re-establishment of energy homeostasis and promotion of tolerance to adverse conditions is partly achieved through induction of catabolic processes and general repression of anabolism (Emanuelle *et al.*, 2015; Soto-Burgos and Bassham, 2017). Autophagy can be induced by a multitude of stimuli (Liu *et al.*, 2009; Avin-Wittenberg *et al.*, 2015; Li *et al.*, 2015; Yang *et al.*, 2016). Indeed, activated SnRK1 induces autophagy by inhibiting its negative regulator TOR (target of rapamycin) complex in plants (Chen *et al.*, 2017; Soto-Burgos and Bassham, 2017). A crucial feature of autophagy is that it is a highly regulated and dynamic process, able to respond quickly to cellular stress (Antonioli *et al.*, 2017). High salinity and osmotic stress induce autophagy in plant tissues within a couple of hours of incubation in stress induction medium (Liu *et al.*, 2009; Vanhee *et al.*, 2011*b*). Accordingly, many core *ATG* genes are transcriptionally up-regulated by various abiotic stresses (Bassham *et al.*, 2006; Zhou *et al.*, 2014*a*; Wang *et al.*, 2015*a*). Conversely, autophagy-deficient plants are more sensitive to abiotic stresses (Liu *et al.*, 2009). Recent evidence also suggests that ectopic overexpression of defined plant core *ATG* genes can confer tolerance to various types of stresses and improve growth performance under nutrient starvation conditions (Wang *et al.*, 2017*b*; Minina *et al.*, 2018)

The plant adaptation responses to abiotic stresses involve phytohormone-dependent signaling cascades, including that of the stress hormone abscisic acid (ABA; a growth inhibitor) and that of brassinosteroid (BR; a growth-promoting regulator) (Mair *et al.*, 2015). When subjected to abiotic stress, plants have to balance between maintaining growth and competitiveness on the one hand and ensuring survival on the other hand (Claeys and Inzé, 2013). This delicate and vital process involves hormone-regulated master regulators, some of which have been characterized recently.

ABI1 (ABA insensitive 1) and PP2CA (protein phosphatase 2C-A) are negative regulators of ABA-dependent signaling, and the two phosphatases were shown to dephosphorylate and inactivate SnRK1. This repressive action of the ABA signaling pathway is blocked by the ABA-dependent interaction of the phosphatases with ABA receptors (Emanuelle *et al.*, 2015). ABA-dependent signaling results in the expression of effector proteins regulating different aspects of plant physiology. The polytopic transmembrane protein TSPO is a multistress regulator, transiently induced by water-related stress and ABA treatment in plants (Guillaumot *et al.*, 2009). The expressed Arabidopsis TSPO protein is also rapidly (within 48 h) degraded, suggesting a time-limited role for it during stress. Plant TSPO may act as an autophagy cargo receptor for a diverse set of cargo such as cytoplasmic free porphyrins and defined water channels (Veljanovski and Batoko, 2014). TSPO interacts with a highly expressed plasma membrane water channel, aquaporin PIP2;7, during osmotic stress. The aquaporin–TSPO complex is targeted by autophagy for degradation in the vacuole, thus preventing PIP2;7 from reaching the plasma membrane and possibly protecting the cell from water loss (Hachez *et al.*, 2014). However, constitutive expression of TSPO can be detrimental to plant growth and development (Guillaumot *et al.*, 2009; Vanhee *et al.*, 2011*a*). This effect is probably due to its intrinsic free heme binding capacity, and the consequence of this cytoplasmic heme titration on reactive oxygen species (ROS) scavenger enzyme activity (Batoko *et al.*, 2015; Vanhee *et al.*, 2011*b*). Enhanced ROS accumulation could generate ER stress and chronic UPR (unfolded protein response) followed by cell death (Petrov *et al.*, 2015). The free heme/porphyrin detoxification function of TSPO may only be required transiently when the plant cell needs to manage stress-induced ROS, and probably ROS-dependent signaling events (Batoko *et al.*, 2015).

Plant TSPO is also up-regulated by the growth-promoting hormone BR (Nolan *et al.*, 2017). The BR plasma membrane receptor BRI1 (brassinosteroid insensitive 1) and the downstream signaling components regulate the activity of the transcription factor BES1 (BRI1-EMS suppressor 1) (Li and Nam, 2002). BR inhibits the activity of the kinase BIN2 that negatively regulates BES1 by phosphorylation. BES1 master transcriptional activity promotes plant growth, and

Table 1. *Tools for plant autophagy modulation*

			Confirmation of the expected effect on autophagy		
Type of regulation	**Effect on autophagy**	**Suggest mechanism of action/target**	**Algae**	**Mosses**	**Seed plants**
Genetic regulation					
Knockout of *ATG* gene(s)	Inhibition		Possibly (Zhang *et al.*, 2014b)	Yes (Mukae *et al.*, 2015; Sanchez-Vera *et al.*, 2017)	Yes (Kim *et al.*, 2012)
Knockdown of *ATG* gene(s)	Inhibition		–	–	Yes (Kim *et al.*, 2012)
Overexpression of *ATG* gene(s)	Stimulation		–	–	Possibly (Wang *et al.*, 2017*b*; Wang *et al.*, 2016*a*, *c*; Xia *et al.*, 2012)
Pharmacological regulation					
Rapamycin	Stimulation	An inhibitor of TOR kinase	Yes (Crespo *et al.*, 2005)	Reference to personal communication in Menand *et al.* (2002)	In Arabidopsis it requires the presence of FKBP12 (Ren *et al.*, 2011; Zhang *et al.*, 2014*a*)
AZD8055	Stimulation	TOR kinase active site inhibitor	Yes (Imamura *et al.*, 2016)	–	Yes (Dong *et al.*, 2015)
Torin1	Stimulation	TOR active site inhibitor	Yes (Imamura *et al.*, 2016)	–	Yes (Montané and Menand, 2013)
KU63794	Stimulation	TOR active site inhibitor	–	–	Yes, especially in combination with rapamycin (Deng *et al.*, 2017)
3-MA (3-methyladenine)	Inhibition, but also might lead to enhancement	A pan phosphatidylinosytol-3 kinase (PI3K) inhibitor. Can persistently inhibit class III PI3K and transiently inhibit class I PI3K.	Yes (Jiang *et al.*, 2012)	–	Yes (Takatsuka *et al.*, 2004; Wang *et al.*, 2013; Barany *et al.*, 2018)
Wortmannin	Inhibition	A pan phosphatidylinosytol-3 kinase (PI3K) inhibitor. Inhibits class I and III PI3K with the same efficacy.	–	–	Yes (Takatsuka *et al.*, 2004)
LY294002	Inhibition, but also might lead to enhancement	A pan phosphatidylinosytol-3 kinase (PI3K) inhibitor. Inhibits activity of class I and class III PI3Ks and additionally influences Ca^{2+} homeostasis.	–	–	Yes (Takatsuka *et al.*, 2004)
Bafilomycin A1	Inhibition	A specific inhibitor of vacuolar H^+-ATPase	–	–	Yes. In BY-2 it gives a weaker effect than concanamycin A (Matsuoka *et al.*, 1997)
Concanamycin A	Inhibition	A specific inhibitor of vacuolar H^+-ATPase	–	Yes (Mukae *et al.*, 2015)	Yes (Matsuoka *et al.*, 1997; Yano *et al.*, 2015; Barany *et al.*, 2018)
E-64c/d	Inhibition	A cysteine-protease inhibitor, blocks degradation of autophagic cargo and ATG4 activity	Possibly (Moriyasu, 1995)	Yes (Mukae *et al.*, 2015)	Yes (Oh-ye *et al.*, 2011; Yano *et al.*, 2015; Barany *et al.*, 2018)
BTH (*benzothi*adiazole)	Stimulation	Acts as an analog of salicylic acid	–	–	Yes (Yoshimoto *et al.*, 2009)
Fumonisin B1	Stimulation	An inhibitor of sphingosine *N*-acyltransferase	–	–	Possibly (Qin *et al.*, 2017)
Tunicamycin	Stimulation	Induces ER stress	Possibly (Díaz-Troya *et al.*, 2011)	-	Yes (Yang *et al.*, 2016)
Polyamines	Stimulation	Spermidine was suggested to influence expression of *ATG* genes by changing chromatin structure	–	–	Possibly (Sequera-Mutiozabal *et al.*, 2016)

–, no published data are yet available.

its deregulation was shown recently to enhance plant survival instead of growth during abiotic stress (Nolan *et al.*, 2017). During osmotic stress, for example, BES1 is ubiquitylated and interacts with the ubiquitin-binding receptor protein DSK2 (dominant suppressor of Kar2), a known autophagy cargo receptor in higher eukaryotes (Lee *et al.*, 2013). BES1 is therefore targeted for autophagy-mediated degradation as a response to abiotic stress. Phosphorylation regulates DSK2's autophagy receptor activity, the latter being catalyzed by the BIN2 kinase. Loss-of-function *dsk2* mutant plants accumulate BES1 proteins, and have altered global gene expression profiles and compromised survival during abiotic stresses (Nolan *et al.*, 2017). Consistently, constitutively active BR signaling mutant plants are more sensitive to abiotic stress, suggesting that reducing growth during abiotic stress is a vital mechanism for the plant's survival. Although BES1 abundance can be regulated by the ubiquitin–proteasome system (Lin *et al.*, 2011), autophagy appears to be an essential pathway in achieving this tricky physiological and metabolic balance between growth and survival.

The role of autophagy in plant–pathogen interactions

Autophagy plays a role in plant innate immunity. It can act either as a survival or cell death pathway in response to invading microbes with different pathogenic (i.e. biotrophic or necrotrophic) lifestyles (Üstün *et al.*, 2017). Because of the co-evolutionary battle with their hosts, several pathogens have also developed various counter-measures to suppress, evade, or subvert autophagy processes to the benefit of infection. In addition, some eukaryotic microbes require their own autophagy machinery for successful pathogenesis (Hofius *et al.*, 2017). Most studies demonstrating the role of autophagy in plant–microbe interactions have considered autophagy as a mostly unspecific ('bulk') process. However, recent reports indicate that plants can exploit selective autophagy to fend off microbial intruders efficiently, whereas some pathogens overcome plant immunity by hijacking autophagy pathways for selective removal of host components (Dagdas *et al.*, 2016; Clavel *et al.*, 2017; Hafrén *et al.*, 2017; Haxim *et al.*, 2017). In this section, we will briefly discuss the role of autophagy in different immunity- and disease-related contexts. More comprehensive reviews on the topic are available (Minina *et al.*, 2014; Zhou *et al.*, 2014*b*; Hofius *et al.*, 2017; Leary *et al.*, 2018).

Pathogen recognition by the plant immune system often results in hypersensitive response (HR), a localized form of programmed cell death (PCD) activated by intracellular immune receptors [known as resistance (*R*) genes] (Coll *et al.*, 2011). HR levels were reduced in autophagy-deficient mutants infected with avirulent bacteria and oomycetes, or enhanced in autophagy-stimulated transgenic plants upon virus challenge (Coll *et al.*, 2011; Hackenberg *et al.*, 2013; Munch *et al.*, 2014, 2015; Han *et al.*, 2015). Hence, autophagy acts locally as a positive regulator of HR. Autophagy mutants were also shown to display unrestricted cell death upon HR induction (Liu *et al.*, 2005; Yoshimoto *et al.*, 2009), suggesting that autophagy can contribute to the confinement of HR, thus minimizing damage to healthy, non-infected tissue (Hofius *et al.*, 2011). This pro-survival effect of autophagy might be linked to its homeostatic role in eliminating potentially toxic by-products of systemic responses triggered during infection (Yoshimoto *et al.*, 2009; Hofius *et al.*, 2011; Coll *et al.*, 2014; Munch *et al.*, 2014).

An additional pro-survival role of autophagy in immunity has been revealed in the context of plant defense against necrotrophs, which deliberately activate cell death to retrieve nutrients from the host. Autophagy-deficient mutants displayed enhanced disease-associated cell death and pathogen growth upon infection with different necrotrophic fungi (Lai *et al.*, 2011; Lenz *et al.*, 2011; Katsiarimpa *et al.*, 2013; Y. Li *et al.*, 2016), whereas plants with an elevated level of autophagy showed increased resistance (Minina *et al.*, 2018). Besides restricting disease-associated necrotic cell death, autophagy may also contribute to basal defense against necrotrophs by modulating hormone levels or eliminating toxic cellular constituents induced as part of the disease response (Lai *et al.*, 2011). Some necrotrophic fungi have therefore evolved mechanisms to overcome autophagy-mediated defenses in plants. For example, secretion of the phytotoxin oxalic acid by *Sclerotinia sclerotiorum* results in unrestricted host cell death via autophagy inhibition (Kabbage *et al.*, 2013).

Intracellular pathogens in animals are often subject to direct targeting and elimination by the autophagy machinery in a process referred to as xenophagy (Levine *et al.*, 2011; Mostowy, 2013; Paul and Münz, 2016). In plants, viruses are the only pathogens with intracellular replication, but the anti- and pro-viral functions of autophagy in host immunity and viral pathogenesis have only recently begun to emerge (Clavel *et al.*, 2017). Selective autophagy mechanisms were discovered as an integral part of the innate immune response against different DNA viruses. The cargo receptor NBR1 mediates autophagic degradation of non-assembled capsid proteins and viral particles of *Cauliflower mosaic virus* (CaMV), providing the first example of xenophagy in plants (Hafrén *et al.*, 2017). Likewise, the virulence factor βC1 of *Cotton leaf curl Multan virus* (CLCuMuV) is selectively targeted during infection (Haxim *et al.*, 2017). However, recruitment of this viral suppressor of RNA silencing (VSR) to autophagosomes seems to involve direct interaction with ATG8 rather than distinct cargo receptors. The potyviral HCpro and cucumoviral 2b proteins, representing VSRs of RNA viruses, were also shown to undergo autophagic clearance, but the link between their binding to the host protein rgsCaM and the autophagy machinery remained unclear (Nakahara *et al.*, 2012). A recent report demonstrated that NBR1 specifically targets the HCpro of *Turnip mosaic virus* (TuMV) to suppress viral accumulation, indicating the integration of selective autophagy also into immunity against RNA viruses (Hafrén *et al.*, 2018). However, TuMV has evolved viral protein functions to antagonize NBR1-dependent autophagy, thereby limiting its antiviral capacity efficiently. The mechanistic basis of how TuMV proteins block NBR1 flux and HCpro degradation remains to be further established (Hafrén *et al.*, 2018).

As part of their counter-defense, some viruses trigger the autophagic degradation of host antiviral RNA silencing pathway components (Derrien *et al.*, 2012; Cheng and Wang, 2017). Also, virus-induced activation of bulk autophagy seems to benefit virus survival and particle production via suppression of disease-associated cell death and promotion of plant fitness (Hafrén *et al.*, 2017, 2018). Hence, the steps taken by viruses to interfere with autophagic targeting may influence the pro-viral effects of bulk autophagy, implying a potential trade-off between suppression of antiviral autophagy and host survival.

The hemibiotrophic oomycete pathogen *Phytophthora infestans* was also shown to be targeted by NBR1-dependent autophagy processes as part of the host defense (Dagdas *et al.*, 2016). In turn, the *P. infestans* effector protein PexRD54 can outcompete the interaction of the NBR1 tobacco homolog Joka 2 with an ATG8 protein, which led to the speculation that *P. infestans* hijacks the autophagy pathway to remove defense components selectively or to recycle and deviate nutrients to the intracellular infection structures (Dagdas *et al.*, 2016). The role of autophagy during infection with the strictly biotrophic downy mildew oomycete and powdery mildew fungal species remains unclear, probably because of the use of different autophagy-deficient mutant backgrounds and pathogen species or plant age-dependent alterations in cellular homeostasis and hormone signaling (Hofius *et al.*, 2009; Lenz *et al.*, 2011).

Similarly, the functions of autophagy during virulent bacterial infection are not well understood. There is the prevailing view that autophagy promotes plant susceptibility to infection with *Pseudomonas syringae* (Lenz *et al.*, 2011; Kwon *et al.*, 2013; Hofius *et al.*, 2017). The recent identification of the *Ralstonia solanacearum* AWR5 effector, which inhibits the negative autophagy regulator TOR, further suggests that bacteria can exploit autophagy activation to enhance virulence (Popa *et al.*, 2016)

Autophagy as a facilitator of nutrient recycling and remobilization in plants

It is generally accepted that autophagy is involved in nutrient recycling and that it is induced under nutrient starvation. This role has been suspected since the early stages of autophagy research, when de Duve observed autophagosome structures in the livers of rats subjected to nutrient starvation (Deter *et al.*, 1967). Further, the possibility to induce autophagy for nutrient recycling in yeast using starvation was used by Oshumi and colleagues to set up a mutant screening strategy that permitted the discovery of the *ATG* genes (Takeshige *et al.*, 1992). In mice, the importance of autophagy in nutrient recycling was demonstrated by the substantial impact of autophagic activity on newborn survival (Kuma *et al.*, 2004). In plants, hypersensitivity to carbon and nitrogen starvation has been established as a primary phenotype of *atg* mutants, initially characterized in Arabidopsis (Doelling *et al.*, 2002; Ishizaki *et al.*, 2005; Thompson *et al.*, 2005; Phillips *et al.*, 2008), but also shown in maize (Li *et al.*, 2015). However, our knowledge of the underlying molecular details of such interplay is limited.

Both carbon and nitrogen starvation are known to induce autophagy (Rose *et al.*, 2006; Avila-Ospina *et al.*, 2016). Expression of *ATG* genes was shown to increase upon carbon and nitrogen starvation in many plant species, including Arabidopsis, maize, tobacco, wheat, and the model algae *Chlamydomonas reinhardtii*, as well as increased lipidation of ATG8 (Thompson *et al.*, 2005; Pérez-Pérez *et al.*, 2010; Caldana *et al.*, 2011; Zientara-Rytter *et al.*, 2011; Pei *et al.*, 2014; Li *et al.*, 2015). In addition, crossing *atg* mutants with starch-deficient mutants was shown to exacerbate their starvation phenotype, demonstrating the tight connection between autophagy and carbon supply under starvation (Izumi *et al.*, 2013). Links between autophagy and other nutrient deficiencies are less documented. Induction of some autophagy-related genes (*ATG8* and *Joka2*) in roots of tobacco plants grown in sulfur-deficient conditions (Zientara-Rytter *et al.*, 2011) suggested that sulfur starvation induces autophagy activity. Indeed, it was recently shown in Arabidopsis that limited sulfur supply decreases soluble sugars, down-regulates TOR activity, as demonstrated by down-regulation of its downstream target S6K, and increases the level of the lipidated ATG8a (Dong *et al.*, 2017). Induction of autophagy under phosphorus starvation has also been suggested in the model algae *C. reinhardtii* and the marine algae *Emiliania huxleyi* (Shemi *et al.*, 2016; Couso *et al.*, 2017). In Arabidopsis, it has been proposed that in the absence of phosphate, selective autophagy (with PUB9 as E3 ligase) is involved in degradation of an auxin accumulation repressor, leading to auxin accumulation and growth of lateral roots (Deb *et al.*, 2014).

We do not know what triggers the induction of autophagy-related genes during limitation of specific nutrients. However, the signal, at least for nitrogen, carbon, and sulfur starvation, is probably TOR dependent (Rexin *et al.*, 2015; Dong *et al.*, 2017; Pu *et al.*, 2017). Interestingly, the level of hydrogen sulfide, a recently identified negative regulator of autophagy, drops during sulfur limitation, and, at least, in such conditions might be one of the triggers (Gotor *et al.*, 2013; Laureano-Marín *et al.*, 2016).

It is considered that starvation induces bulk autophagy of cytoplasmic components for nutrient remobilization. In mammals, however, the selective degradation of lipid bodies under starvation was demonstrated in hepatocytes (Singh *et al.*, 2009). Also, selective degradation of ald6p, an acetaldehyde dehydrogenase which catalyzes the conversion of acetaldehyde to acetate in the cytosol, has been demonstrated in yeast under nitrogen starvation (Onodera and Ohsumi, 2004). The diversity of the cytoplasmic components destined for degradation by autophagy suggests that in addition to C and N molecules, many other mineral nutrients could be released from the process. These compounds can then be used for the cell's metabolism, to sustain respiration, for example (Barros *et al.*, 2017), or dedicated for the whole organism after remobilization. It is not yet documented whether phosphate, iron, zinc, sulfur, or potassium can be recycled through. In addition, it is unknown whether some selectivity exists in the cargos degraded by autophagy under starvation conditions.

It is likely, for example, that under dark conditions, chlorophagy and selective starch degradation will be activated (Wang and Liu, 2013; Wang *et al.*, 2015*b*). Under low nitrate availability, autophagy would mainly function in protein degradation but not starch degradation, as proteins accumulated in *atg* mutants while starch was depleted (Guiboileau *et al.*, 2013). Under carbon starvation, the situation is the opposite, with increased usage of free amino acids, presumably as an alternative carbon source for respiration (Avin-Wittenberg *et al.*, 2015).

At the whole-plant level, autophagy is an essential process for nitrogen remobilization from leaves to seeds, as shown by the ^{15}N pulse–chase experiments performed in Arabidopsis (Guiboileau *et al.*, 2012). Based on this Arabidopsis study, the pulse–chase labeling strategy was used on maize *atg12* mutants that showed lower N mobilization to the seeds (Li *et al.*, 2015) accordingly. Both studies thus confirmed that autophagy manages nutrient resources in source leaves and that its role for seed formation and seed filling is fundamental. In Arabidopsis, the composition of *atg* mutant seeds is strongly modified as their nitrogen content mainly relies on the post-anthesis nitrate uptake rather than N remobilization from leaves (Guiboileau *et al.*, 2012). Because of their reduced N remobilization capacity, *atg* mutants display lower yield and lower harvest index. On the other hand, overexpression of *ATG* genes in Arabidopsis increased seed yield and fatty acid content (Minina *et al.*, 2018). However, this overexpression was general and not only limited to source leaves.

While several studies have performed metabolic profiling of *atg* mutants (Masclaux-Daubresse *et al.*, 2014; Avin-Wittenberg *et al.*, 2015; Barros *et al.*, 2017), analysis of the changes in the metabolic fluxes are considerably less frequent. Metabolic flux analysis relies on determining the redistribution of label over time to estimate the atomic flux between pools of different metabolic species. Two approaches are commonly used: (i) radiolabeled isotopes, namely ^{14}C, ^{35}S, and ^{3}H; and (ii) stable isotopes, such as ^{13}C and ^{15}N (Batista Silva *et al.*, 2016). Few works investigated the effect of autophagy on primary metabolic fluxes in plants. In the first study, etiolated wild-type and *atg* mutant Arabidopsis seedlings were incubated in the presence of either uniformly labeled [^{14}C]glucose, positionally labeled [^{14}C]glucose, or [^{13}C]lysine to characterize the respiratory metabolism of these mutants (Avin-Wittenberg *et al.*, 2015). This revealed various effects, including lower protein synthesis and increased labeling in specific amino acids and tricarboxylic acid (TCA) cycle intermediates. As mentioned above, the change in amino acid levels was different from that reported during nitrogen deficiency (Masclaux-Daubresse *et al.*, 2014). It would therefore be interesting to examine the impact of autophagy deficiency on metabolic fluxes in a range of conditions/tissues other than the etiolated seedling. These examples highlight the power of incorporating flux analyses into studies on plant autophagy, suggesting that their greater adoption will yield further insights into molecular and energetic mechanisms regulating and being modulated by autophagy.

Targeting autophagy in plant oil production

Plant oils play a pivotal role in human nutrition, and the potential for plant oils to replace fossil oil in the chemical industry is also promising. In order to realize the full potential of using plant oils, it is crucial to optimize quantity and quality of the oil *in planta* using genetic and metabolic engineering (Tao, 2007). As in all eukaryotes, plants store their lipid reserves in specialized organelles, lipid droplets (LDs), which are abundant in seeds of oilseed crops (Elander *et al.*, 2017). Recent research using animal and yeast systems has established that autophagy has functions in both breakdown and biogenesis of LDs (Singh *et al.*, 2009; Zhang *et al.*, 2009) and that LDs in return can regulate autophagy (Shpilka *et al.*, 2015). The process of autophagic degradation of LDs in the lysosome or lytic vacuole has been named ‘lipophagy’ and shown to crosstalk in a number of ways with cytosolic lipolysis (Zechner *et al.*, 2017).

It has been shown that Arabidopsis mutants in β-oxidation of fatty acids have greatly reduced seed oil content, demonstrating that lipid turnover is an essential component for efficient seed oil accumulation (Lin *et al.*, 2004). Therefore, can manipulation of autophagy be used as a tool to improve oil crops? To date, the evidence for the role of autophagy in biogenesis or degradation of LDs in plants is somewhat scarce and fragmented, including only a few species. Accordingly, autophagy is required for the formation of LDs in tapetal cells and phospholipid editing in rice pollen (Kurusu *et al.*, 2014), as well as for the synthesis of triacylglycerols and formation of LDs in nitrogen- or phosphate-starved *Chlamydomonas* cells (Couso *et al.*, 2017. Two cytological studies using electron microscopy have revealed micro- and macroautophagy-mediated engulfment of LDs in the algae *Auxenochlorella protothecoides* (Zhao *et al.*, 2014) and *Micrasterias denticulata* (Schwarz *et al.*, 2017), respectively. Finally, although autophagy does not seem to be critically required for Arabidopsis seed development, efficient mobilization of lipids upon seed germination under carbon-deprived conditions is at least partly dependent on autophagy (Avin-Wittenberg *et al.*, 2015). More research is needed to establish a robust platform for biotechnological application of autophagy in regulating plant oil reserves (Elander *et al.*, 2017).

Future perspectives

The study of autophagy in plants has boomed in the last few years, and our understanding of the function and regulation of this complex mechanism is steadily expanding. However, much work is still needed to understand the many facets of autophagy and utilize it for agricultural use. For example, information on the role of selective autophagy in plant development is still scarce. It is also essential to continue deciphering the mechanisms regulating autophagy in plants, as these are still only partially understood. In the field of selective autophagy, cargo receptors or other specificity factors need to be further identified and characterized, and the role of ubiquitin tagging in organelle degradation elucidated. As a

complementary approach, understanding the functional differences between the different plant ATG8 isoforms would be very useful. A better understanding of the molecular mechanisms that pathogens use to exploit plant autophagy for their benefit, as well as the autophagic components and pathways contributing to plant innate immune responses, is also of paramount importance.

Manipulation of autophagic pathways is a promising strategy for the improvement of crop fitness under changing climate conditions, as it could result in plants with increased nutrient remobilization, able to be more resistant to nutrient starvation, as well as plants better able to cope with high pathogen pressure in the field. Advances in CRISP/Cas9-based genetic editing tools and high-throughput drug screens are likely to facilitate the manipulation of autophagy in crops and lessen the need for laborious and time-consuming breeding programs. However, most of our understanding of selective autophagy pathways is currently based on studies in model plants, mainly Arabidopsis. Hence, more emphasis should be given to expanding the research to crops, and to possible species-specific differences in autophagy pathways and responses.

Acknowledgements

The support of the COST action TRANSAUTOPHAGY (CA15138) is recognized. We would like to acknowledge Dr Liliana Avila-Ospina (IPS2, Orsay, France) for her assistance in the composition of the manuscript. Research on autophagy in the Bozhkov and Minina laboratory is supported by The Swedish Foundation for Strategic Research (SSF), The Swedish Research Council (VR), The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas), the Knut and Alice Wallenberg Foundation, and the Research Programme 'Trees and Crops for the Future'. Research in the Hofius laboratory is supported by the Swedish University of Agricultural Sciences (SLU), the Swedish Research Councils VR and FORMAS, the Knut and Alice Wallenberg foundation, and Carl-Tryggers Stiftelse (CTS). Research of the Avin-Wittenberg group is supported by the Israeli Science Foundation, grant number 1899/16. The Testillano group is supported by projects AGL2014-52028-R and AGL2017-82447-R funded by the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF/FEDER). Autophagy research at the Galili laboratory is supported by The Israel Science Foundation (grant 612/16). Research in the Batoko laboratory was funded by the Wallonia–Brussels Federation Joint Research Action (ARC grant #11/16-036), the Belgian Funds for Scientific Research (FRS-FNRS) (CDR grant #19516174, and an FRFC grant #6794930). Research in the Coll group is supported by grants 2016-78002-R (AGL) and RyC 2014-16158 from the Spanish Ministry of Economy and Competitiveness (MINECO).

References

Abada A, Elazar Z. 2014. Getting ready for building: signaling and autophagosome biogenesis. EMBO Reports **15,** 839–852.

Anding AL, Baehrecke EH. 2017. Cleaning house: selective autophagy of organelles. Developmental Cell **41,** 10–22.

Andreu V, Collados R, Testillano PS, Risueño Mdel C, Picorel R, Alfonso M. 2007. In situ molecular identification of the plastid omega3 fatty acid desaturase FAD7 from soybean: evidence of thylakoid membrane localization. Plant Physiology **145,** 1336–1344.

Antonioli M, Di Rienzo M, Piacentini M, Fimia GM. 2017. Emerging mechanisms in initiating and terminating autophagy. Trends in Biochemical Sciences **42,** 28–41.

Avila-Ospina L, Marmagne A, Soulay F, Masclaux-Daubresse C. 2016. Identification of barley (*Hordeum vulgare* L.) autophagy genes and their expression levels during leaf senescence, chronic nitrogen limitation and in response to dark exposure. Agronomy **6,** 15.

Avin-Wittenberg T, Bajdzienko K, Wittenberg G, Alseekh S, Tohge T, Bock R, Giavalisco P, Fernie AR. 2015. Global analysis of the role of autophagy in cellular metabolism and energy homeostasis in Arabidopsis seedlings under carbon starvation. The Plant Cell **27,** 306–322.

Bakshi A, Moin M, Kumar MU, Reddy AB, Ren M, Datla R, Siddiq EA, Kirti PB. 2017. Ectopic expression of Arabidopsis Target of Rapamycin (AtTOR) improves water-use efficiency and yield potential in rice. Scientific Reports **7,** 42835.

Barany I, Berenguer E, Solis MT, Perez-Perez Y, Santamaria ME, Crespo JL, Risueno MC, Diaz I, Testillano PS. 2018. Autophagy is activated and involved in cell death with participation of cathepsins during stress-induced microspore embryogenesis in barley. Journal of Experimental Botany **69,** 1387–1402.

Barros JAS, Cavalcanti JHF, Medeiros DB, Nunes-Nesi A, Avin-Wittenberg T, Fernie AR, Araújo WL. 2017. Autophagy deficiency compromises alternative pathways of respiration following energy deprivation in *Arabidopsis thaliana*. Plant Physiology **175,** 62–76.

Baskaran S, Carlson LA, Stjepanovic G, Young LN, Kim DJ, Grob P, Stanley RE, Nogales E, Hurley JH. 2014. Architecture and dynamics of the autophagic phosphatidylinositol 3-kinase complex. eLife **3,** e05115.

Bassham DC. 2015. Methods for analysis of autophagy in plants. Methods **75,** 181–188.

Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K. 2006. Autophagy in development and stress responses of plants. Autophagy **2,** 2–11.

Bassham DC, MacIntosh GC. 2017. Degradation of cytosolic ribosomes by autophagy-related pathways. Plant Science **262,** 169–174.

Batista Silva W, Daloso DM, Fernie AR, Nunes-Nesi A, Araujo WL. 2016. Can stable isotope mass spectrometry replace radiolabelled approaches in metabolic studies? Plant Science **249,** 59–69.

Batoko H, Veljanovski V, Jurkiewicz P. 2015. Enigmatic translocator protein (TSPO) and cellular stress regulation. Trends in Biochemical Sciences **40,** 497–503.

Bernal M, Testillano PS, Alfonso M, del Carmen Risueño M, Picorel R, Yruela I. 2007. Identification and subcellular localization of the soybean copper P1B-ATPase GmHMA8 transporter. Journal of Structural Biology **158,** 46–58.

Caldana C, Degenkolbe T, Cuadros-Inostroza A, et al. 2011. High-density kinetic analysis of the metabolomic and transcriptomic response of Arabidopsis to eight environmental conditions. The Plant Journal **67,** 869–884.

Chanoca A, Kovinich N, Burkel B, Stecha S, Bohorquez-Restrepo A, Ueda T, Eliceiri KW, Grotewold E, Otegui MS. 2015. Anthocyanin vacuolar inclusions form by a microautophagy mechanism. The Plant Cell **27,** 2545–2559.

Chen L, Su ZZ, Huang L, Xia FN, Qi H, Xie LJ, Xiao S, Chen QF. 2017. The AMP-activated protein kinase KIN10 is involved in the regulation of autophagy in Arabidopsis. Frontiers in Plant Science **8,** 1201.

Cheng X, Wang A. 2017. The potyvirus silencing suppressor protein VPg mediates degradation of SGS3 via ubiquitination and autophagy pathways. Journal of Virology **91,** e01478-16.

Chiba A, Ishida H, Nishizawa NK, Makino A, Mae T. 2003. Exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts by specific bodies in naturally senescing leaves of wheat. Plant and Cell Physiology **44,** 914–921.

Cho DH, Kim YS, Jo DS, Choe SK, Jo EK. 2018. Pexophagy: molecular mechanisms and implications for health and diseases. Molecules and Cells **41,** 55–64.

Chung T, Phillips AR, Vierstra RD. 2010. ATG8 lipidation and ATG8-mediated autophagy in Arabidopsis require ATG12 expressed from the differentially controlled ATG12A and ATG12B loci. The Plant Journal **62,** 483–493.

Chung T, Suttangkakul A, Vierstra RD. 2009. The ATG autophagic conjugation system in maize: ATG transcripts and abundance of the ATG8–lipid adduct are regulated by development and nutrient availability. Plant Physiology **149,** 220–234.

Claeys H, Inzé D. 2013. The agony of choice: how plants balance growth and survival under water-limiting conditions. Plant Physiology **162,** 1768–1779.

Clavel M, Michaeli S, Genschik P. 2017. Autophagy: a double-edged sword to fight plant viruses. Trends in Plant Science **22,** 646–648.

Cohen-Kaplan V, Livneh I, Avni N, Fabre B, Ziv T, Kwon YT, Ciechanover A. 2016. p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome. Proceedings of the National Academy of Sciences, USA **113,** E7490–E7499.

Coll NS, Epple P, Dangl JL. 2011. Programmed cell death in the plant immune system. Cell Death and Differentiation **18,** 1247–1256.

Coll NS, Smidler A, Puigvert M, Popa C, Valls M, Dangl JL. 2014. The plant metacaspase AtMC1 in pathogen-triggered programmed cell death and aging: functional linkage with autophagy. Cell Death and Differentiation **21,** 1399–1408.

Confraria A, Martinho C, Elias A, Rubio-Somoza I, Baena-González E. 2013. miRNAs mediate SnRK1-dependent energy signaling in Arabidopsis. Frontiers in Plant Science **4,** 197.

Contento AL, Xiong Y, Bassham DC. 2005. Visualization of autophagy in Arabidopsis using the fluorescent dye monodansylcadaverine and a GFP–AtATG8e fusion protein. The Plant Journal **42,** 598–608.

Couso I, Perez-Perez ME, Martinez-Force E, Kim HS, He Y, Umen JG, Crespo JL. 2017. Autophagic flux is required for the synthesis of triacylglycerols and ribosomal protein turnover in *Chlamydomonas*. Journal of Experimental Botany **69,** 1355–1367.

Coutts AS, La Thangue NB. 2015. Actin nucleation by WH2 domains at the autophagosome. Nature Communications **6,** 7888.

Crespo JL, Díaz-Troya S, Florencio FJ. 2005. Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga *Chlamydomonas reinhardtii*. Plant Physiology **139,** 1736–1749.

Dagdas YF, Belhaj K, Maqbool A, et al. 2016. An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. eLife **5,** e10856.

Deb S, Sankaranarayanan S, Wewala G, Widdup E, Samuel MA. 2014. The S-domain receptor kinase arabidopsis receptor kinase2 and the U box/armadillo repeat-containing E3 ubiquitin ligase9 module mediates lateral root development under phosphate starvation in Arabidopsis. Plant Physiology **165,** 1647–1656.

Deng K, Dong P, Wang W, et al. 2017. The TOR pathway is involved in adventitious root formation in Arabidopsis and potato. Frontiers in Plant Science **8,** 784.

Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P. 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. Proceedings of the National Academy of Sciences, USA **109,** 15942–15946.

Deter RL, Baudhuin P, De Duve C. 1967. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. Journal of Cell Biology **35,** C11–C16.

Diao J, Liu R, Rong Y, et al. 2015. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. Nature **520,** 563–566.

Díaz-Troya S, Pérez-Pérez ME, Pérez-Martín M, Moes S, Jeno P, Florencio FJ, Crespo JL. 2011. Inhibition of protein synthesis by TOR inactivation revealed a conserved regulatory mechanism of the BiP chaperone in *Chlamydomonas*. Plant Physiology **157,** 730–741.

Dikic I. 2017. Proteasomal and autophagic degradation systems. Annual Review of Biochemistry **86,** 193–224.

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*. Journal of Biological Chemistry **277,** 33105–33114.

Dong J, Chen W. 2013. The role of autophagy in chloroplast degradation and chlorophagy in immune defenses during Pst DC3000 (AvrRps4) infection. PLoS One **8,** e73091.

Dong P, Xiong F, Que Y, Wang K, Yu L, Li Z, Ren M. 2015. Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in Arabidopsis. Frontiers in Plant Science **6,** 677.

Dong Y, Silbermann M, Speiser A, et al. 2017. Sulfur availability regulates plant growth via glucose–TOR signaling. Nature Communications **8,** 1174.

Elander PH, Minina EA, Bozhkov PV. 2017. Autophagy in turnover of lipid stores: trans-kingdom comparison. Journal of Experimental Botany **69,** 1301–1311.

Emanuelle S, Hossain MI, Moller IE, et al. 2015. SnRK1 from *Arabidopsis thaliana* is an atypical AMPK. The Plant Journal **82,** 183–192.

Enrique Gomez R, Joubes J, Valentin N, Batoko H, Satiat-Jeunemaitre B, Bernard A. 2017. Lipids in membrane dynamics during autophagy in plants. Journal of Experimental Botany **69,** 1287–1299.

Fan W, Nassiri A, Zhong Q. 2011. Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). Proceedings of the National Academy of Sciences, USA **108,** 7769–7774.

Farré JC, Subramani S. 2016. Mechanistic insights into selective autophagy pathways: lessons from yeast. Nature Reviews. Molecular Cell Biology **17,** 537–552.

Feng Y, Yao Z, Klionsky DJ. 2015. How to control self-digestion: transcriptional, post-transcriptional, and post-translational regulation of autophagy. Trends in Cell Biology **25,** 354–363.

Fleming A, Noda T, Yoshimori T, Rubinsztein DC. 2011. Chemical modulators of autophagy as biological probes and potential therapeutics. Nature Chemical Biology **7,** 9–17.

Floyd BE, Morriss SC, MacIntosh GC, Bassham DC. 2015. Evidence for autophagy-dependent pathways of rRNA turnover in Arabidopsis. Autophagy **11,** 2199–2212.

Floyd BE, Mugume Y, Morriss SC, MacIntosh GC, Bassham DC. 2017. Localization of RNS2 ribonuclease to the vacuole is required for its role in cellular homeostasis. Planta **245,** 779–792.

Galluzzi L, Baehrecke EH, Ballabio A, et al. 2017. Molecular definitions of autophagy and related processes. EMBO Journal **36,** 1811–1836.

Gotor C, García I, Crespo JL, Romero LC. 2013. Sulfide as a signaling molecule in autophagy. Autophagy **9,** 609–611.

Guiboileau A, Avila-Ospina L, Yoshimoto K, Soulay F, Azzopardi M, Marmagne A, Lothier J, Masclaux-Daubresse C. 2013. Physiological and metabolic consequences of autophagy deficiency for the management of nitrogen and protein resources in Arabidopsis leaves depending on nitrate availability. New Phytologist **199,** 683–694.

Guiboileau A, Yoshimoto K, Soulay F, Bataillé MP, Avice JC, Masclaux-Daubresse C. 2012. Autophagy machinery controls nitrogen remobilization at the whole-plant level under both limiting and ample nitrate conditions in Arabidopsis. New Phytologist **194,** 732–740.

Guillaumot D, Guillon S, Déplanque T, Vanhee C, Gumy C, Masquelier D, Morsomme P, Batoko H. 2009. The Arabidopsis TSPO-related protein is a stress and abscisic acid-regulated, endoplasmic reticulum–Golgi-localized membrane protein. The Plant Journal **60,** 242–256.

Hachez C, Veljanovski V, Reinhardt H, Guillaumot D, Vanhee C, Chaumont F, Batoko H. 2014. The Arabidopsis abiotic stress-induced TSPO-related protein reduces cell-surface expression of the aquaporin PIP2;7 through protein–protein interactions and autophagic degradation. The Plant Cell **26,** 4974–4990.

Hackenberg T, Juul T, Auzina A, et al. 2013. Catalase and NO CATALASE ACTIVITY1 promote autophagy-dependent cell death in Arabidopsis. The Plant Cell **25,** 4616–4626.

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. Proceedings of the National Academy of Sciences, USA **114,** E2026–E2035.

Hafrén A, Üstün S, Hochmuth A, Svenning S, Johansen T, Hofius D. 2018. Turnip mosaic virus counteracts selective autophagy of the viral silencing suppressor HCpro. Plant Physiology **176,** 649–662.

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*. The Plant Cell **27,** 1316–1331.

Hanamata S, Kurusu T, Kuchitsu K. 2014. Roles of autophagy in male reproductive development in plants. Frontiers in Plant Science **5,** 457.

Haxim Y, Ismayil A, Jia Q, et al. 2017. Autophagy functions as an antiviral mechanism against geminiviruses in plants. eLife **6,** e23897.

He C, Klionsky DJ. 2009. Regulation mechanisms and signaling pathways of autophagy. Annual Review of Genetics **43,** 67–93.

Hillwig MS, Contento AL, Meyer A, Ebany D, Bassham DC, Macintosh GC. 2011. RNS2, a conserved member of the RNase T2

family, is necessary for ribosomal RNA decay in plants. Proceedings of the National Academy of Sciences, USA **108,** 1093–1098.

Hofius D, Li L, Hafrén A, Coll NS. 2017. Autophagy as an emerging arena for plant–pathogen interactions. Current Opinion in Plant Biology **38,** 117–123.

Hofius D, Munch D, Bressendorff S, Mundy J, Petersen M. 2011. Role of autophagy in disease resistance and hypersensitive response-associated cell death. Cell Death and Differentiation **18,** 1257–1262.

Hofius D, Schultz-Larsen T, Joensen J, et al. 2009. Autophagic components contribute to hypersensitive cell death in Arabidopsis. Cell **137,** 773–783.

Honig A, Avin-Wittenberg T, Ufaz S, Galili G. 2012. A new type of compartment, defined by plant-specific Atg8-interacting proteins, is induced upon exposure of Arabidopsis plants to carbon starvation. The Plant Cell **24,** 288–303.

Imamura S, Kawase Y, Kobayashi I, Shimojima M, Ohta H, Tanaka K. 2016. TOR (target of rapamycin) is a key regulator of triacylglycerol accumulation in microalgae. Plant Signaling and Behavior **11,** e1149285.

Ishida H, Izumi M, Wada S, Makino A. 2014. Roles of autophagy in chloroplast recycling. Biochimica et Biophysica Acta **1837,** 512–521.

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 Physiology **148,** 142–155.

Ishizaki K, Larson TR, Schauer N, Fernie AR, Graham IA, Leaver CJ. 2005. The critical role of Arabidopsis electron-transfer flavoprotein:ubiquinone oxidoreductase during dark-induced starvation. The Plant Cell **17,** 2587–2600.

Itakura E, Kishi C, Inoue K, Mizushima N. 2008. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. Molecular Biology of the Cell **19,** 5360–5372.

Izumi M, Hidema J, Makino A, Ishida H. 2013. Autophagy contributes to nighttime energy availability for growth in Arabidopsis. Plant Physiology **161,** 1682–1693.

Izumi M, Hidema J, Wada S, Kondo E, Kurusu T, Kuchitsu K, Makino A, Ishida H. 2015. Establishment of monitoring methods for autophagy in rice reveals autophagic recycling of chloroplasts and root plastids during energy limitation. Plant Physiology **167,** 1307–1320.

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

Jiang Q, Zhao L, Dai J, Wu Q. 2012. Analysis of autophagy genes in microalgae: *Chlorella* as a potential model to study mechanism of autophagy. PLoS One **7,** e41826.

Jin S, Tian S, Chen Y, Zhang C, Xie W, Xia X, Cui J, Wang RF. 2016. USP19 modulates autophagy and antiviral immune responses by deubiquitinating Beclin-1. EMBO Journal **35,** 866–880.

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*. The Plant Journal **59,** 316–328.

Kabbage M, Williams B, Dickman MB. 2013. Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of *Sclerotinia sclerotiorum*. PLoS Pathogens **9,** e1003287.

Kalinowska K, Isono E. 2018. All roads lead to the vacuole-autophagic transport as part of the endomembrane trafficking network in plants. Journal of Experimental Botany **69,** 1313–1324.

Karanasios E, Walker SA, Okkenhaug H, et al. 2016. Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. Nature Communications **7,** 12420.

Kast DJ, Zajac AL, Holzbaur EL, Ostap EM, Dominguez R. 2015. WHAMM directs the Arp2/3 complex to the ER for autophagosome biogenesis through an actin comet tail mechanism. Current Biology **25,** 1791–1797.

Katsiarimpa A, Kalinowska K, Anzenberger F, et al. 2013. The deubiquitinating enzyme AMSH1 and the ESCRT-III subunit VPS2.1 are required for autophagic degradation in Arabidopsis. The Plant Cell **25,** 2236–2252.

Kaushik S, Cuervo AM. 2012. Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends in Cell Biology **22,** 407–417.

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

Kim SH, Kwon C, Lee JH, Chung T. 2012. Genes for plant autophagy: functions and interactions. Molecules and Cells **34,** 413–423.

King JS, Gueho A, Hagedorn M, Gopaldass N, Leuba F, Soldati T, Insall RH. 2013. WASH is required for lysosomal recycling and efficient autophagic and phagocytic digestion. Molecular Biology of the Cell **24,** 2714–2726.

Klionsky DJ, Abdelmohsen K, Abe A, et al. 2016. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy **12,** 1–222.

Kraft C, Deplazes A, Sohrmann M, Peter M. 2008. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nature Cell Biology **10,** 602–610.

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N. 2004. The role of autophagy during the early neonatal starvation period. Nature **432,** 1032–1036.

Kurusu T, Koyano T, Hanamata S, et al. 2014. OsATG7 is required for autophagy-dependent lipid metabolism in rice postmeiotic anther development. Autophagy **10,** 878–888.

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 Physiology **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. The Plant Journal **66,** 953–968.

Laureano-Marín AM, Moreno I, Romero LC, Gotor C. 2016. Negative regulation of autophagy by sulfide is independent of reactive oxygen species. Plant Physiology **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. Nature Communications **5,** 4121.

Leary AY, Sanguankiattichai N, Duggan C, et al. 2018. Modulation of plant autophagy during pathogen attack. Journal of Experimental Botany **69,** 1325–1333.

Lee DY, Arnott D, Brown EJ. 2013. Ubiquilin4 is an adaptor protein that recruits Ubiquilin1 to the autophagy machinery. EMBO Reports **14,** 373–381.

Lenz HD, Haller E, Melzer E, et al. 2011. Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. The Plant Journal **66,** 818–830.

Levine B, Mizushima N, Virgin HW. 2011. Autophagy in immunity and inflammation. Nature **469,** 323–335.

Li F, Chung T, Pennington JG, Federico ML, Kaeppler HF, Kaeppler SM, Otegui MS, Vierstra RD. 2015. Autophagic recycling plays a central role in maize nitrogen remobilization. The Plant Cell **27,** 1389–1408.

Li F, Chung T, Vierstra RD. 2014. AUTOPHAGY-RELATED11 plays a critical role in general autophagy- and senescence-induced mitophagy in Arabidopsis. The Plant Cell **26,** 788–807.

Li F, Vierstra RD. 2012. Autophagy: a multifaceted intracellular system for bulk and selective recycling. Trends in Plant Science **17,** 526–537.

Li J, Nam KH. 2002. Regulation of brassinosteroid signaling by a GSK3/SHAGGY-like kinase. Science **295,** 1299–1301.

Li W, Chen M, Wang E, et al. 2016. Genome-wide analysis of autophagy-associated genes in foxtail millet (*Setaria italica* L.) and characterization of the function of SiATG8a in conferring tolerance to nitrogen starvation in rice. BMC Genomics **17,** 797.

Li Y, Kabbage M, Liu W, Dickman MB. 2016. Aspartyl protease-mediated cleavage of BAG6 is necessary for autophagy and fungal resistance in plants. The Plant Cell **28,** 233–247.

Lichtenthaler HK. 1996. Vegetation stress: an introduction to the stress concept in plants. Journal of Plant Physiology **148,** 4–14.

Lin HK, Wang L, Hu YC, Altuwaijri S, Chang C. 2002. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. EMBO Journal **21,** 4037–4048.

Lin Y, Cluette-Brown JE, Goodman HM. 2004. The peroxisome deficient Arabidopsis mutant sse1 exhibits impaired fatty acid synthesis. Plant Physiology **135,** 814–827.

Lin YL, Sung SC, Tsai HL, et al. 2011. The defective proteasome but not substrate recognition function is responsible for the null phenotypes of the Arabidopsis proteasome subunit RPN10. The Plant Cell **23,** 2754–2773.

Liu J, Xia H, Kim M, et al. 2011. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell **147,** 223–234.

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

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. The 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.

Ma J, Jin R, Dobry CJ, Lawson SK, Kumar A. 2007. Overexpression of autophagy-related genes inhibits yeast filamentous growth. Autophagy **3,** 604–609.

Mair A, Pedrotti L, Wurzinger B, et al. 2015. SnRK1-triggered switch of bZIP63 dimerization mediates the low-energy response in plants. eLife **4,** e05828.

Marion J, Le Bars R, Satiat-Jeunemaitre B, Boulogne C. 2017. Optimizing CLEM protocols for plants cells: GMA embedding and cryosections as alternatives for preservation of GFP fluorescence in Arabidopsis roots. Journal of Structural Biology **198,** 196–202.

Marshall RS, Li F, Gemperline DC, Book AJ, Vierstra RD. 2015. Autophagic degradation of the 26S proteasome is mediated by the dual ATG8/ubiquitin receptor RPN10 in Arabidopsis. Molecular Cell **58,** 1053–1066.

Marshall RS, McLoughlin F, Vierstra RD. 2016. Autophagic turnover of inactive 26S proteasomes in yeast is directed by the ubiquitin receptor Cue5 and the Hsp42 chaperone. Cell Reports **16,** 1717–1732.

Martínez DE, Costa ML, Gomez FM, Otegui MS, Guiamet JJ. 2008. 'Senescence-associated vacuoles' are involved in the degradation of chloroplast proteins in tobacco leaves. The Plant Journal **56,** 196–206.

Masclaux-Daubresse C, Clément G, Anne P, Routaboul JM, Guiboileau A, Soulay F, Shirasu K, Yoshimoto K. 2014. Stitching together the multiple dimensions of autophagy using metabolomics and transcriptomics reveals impacts on metabolism, development, and plant responses to the environment in Arabidopsis. The Plant Cell **26,** 1857–1877.

Mathis AD, Naylor BC, Carson RH, et al. 2017. Mechanisms of in vivo ribosome maintenance change in response to nutrient signals. Molecular and Cellular Proteomics **16,** 243–254.

Matsuoka K, Higuchi T, Maeshima M, Nakamura K. 1997. A vacuolar-type H^+-ATPase in a nonvacuolar organelle is required for the sorting of soluble vacuolar protein precursors in tobacco cells. The Plant Cell **9,** 533–546.

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. Proceedings of the National Academy of Sciences, USA **99,** 6422–6427.

Michaeli S, Galili G, Genschik P, Fernie AR, Avin-Wittenberg T. 2016. Autophagy in plants—what's new on the menu? Trends in Plant Science **21,** 134–144.

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. The Plant Cell **26,** 4084–4101.

Minamikawa T, Toyooka K, Okamoto T, Hara-Nishimura I, Nishimura M. 2001. Degradation of ribulose-bisphosphate carboxylase by vacuolar enzymes of senescing French bean leaves: immunocytochemical and ultrastructural observations. Protoplasma **218,** 144–153.

Minina EA, Bozhkov PV, Hofius D. 2014. Autophagy as initiator or executioner of cell death. Trends in Plant Science **19,** 692–697.

Minina EA, Filonova LH, Fukada K, et al. 2013*a*. Autophagy and metacaspase determine the mode of cell death in plants. Journal of Cell Biology **203,** 917–927.

Minina EA, Moschou PN, Vetukuri RR, et al. 2018. Transcriptional stimulation of rate-limiting components of the autophagic pathway improves plant fitness. Journal of Experimental Botany **69,** 1415–1432.

Minina EA, Sanchez-Vera V, Moschou PN, Suarez MF, Sundberg E, Weih M, Bozhkov PV. 2013*b*. Autophagy mediates caloric restriction-induced lifespan extension in Arabidopsis. Aging Cell **12,** 327–329.

Mizushima N. 2004. Methods for monitoring autophagy. International Journal of Biochemistry and Cell Biology **36,** 2491–2502.

Mostowy S. 2013. Autophagy and bacterial clearance: a not so clear picture. Cellular Microbiology **15,** 395–402.

Montané MH, Menand B. 2013. ATP-competitive mTOR kinase inhibitors delay plant growth by triggering early differentiation of meristematic cells but no developmental patterning change. Journal of Experimental Botany **64,** 4361–4374.

Moriyasu Y. 1995. Examination of the contribution of vacuolar proteases to intracellular protein degradation in *Chara corallina*. Plant Physiology **109,** 1309–1315.

Mukae K, Inoue Y, Moriyasu Y. 2015. ATG5-knockout mutants of *Physcomitrella* provide a platform for analyzing the involvement of autophagy in senescence processes in plant cells. Plant Signaling and Behavior **10,** e1086859.

Munch D, Rodriguez E, Bressendorff S, Park OK, Hofius D, Petersen M. 2014. Autophagy deficiency leads to accumulation of ubiquitinated proteins, ER stress, and cell death in Arabidopsis. Autophagy **10,** 1579–1587.

Munch D, Teh OK, Malinovsky FG, et al. 2015. Retromer contributes to immunity-associated cell death in Arabidopsis. The Plant Cell **27,** 463–479.

Nakahara KS, Masuta C, Yamada S, et al. 2012. Tobacco calmodulin-like protein provides secondary defense by binding to and directing degradation of virus RNA silencing suppressors. Proceedings of the National Academy of Sciences, USA **109,** 10113–10118.

Nakatogawa H. 2013. Two ubiquitin-like conjugation systems that mediate membrane formation during autophagy. Essays In Biochemistry **55,** 39–50.

Nakatogawa H, Ohbayashi S, Sakoh-Nakatogawa M, et al. 2012. The autophagy-related protein kinase Atg1 interacts with the ubiquitin-like protein Atg8 via the Atg8 family interacting motif to facilitate autophagosome formation. Journal of Biological Chemistry **287,** 28503–28507.

Nakayama M, Kaneko Y, Miyazawa Y, et al. 2012. A possible involvement of autophagy in amyloplast degradation in columella cells during hydrotropic response of Arabidopsis roots. Planta **236,** 999–1012.

Nascimbeni AC, Giordano F, Dupont N, Grasso D, Vaccaro MI, Codogno P, Morel E. 2017. ER–plasma membrane contact sites contribute to autophagosome biogenesis by regulation of local PI3P synthesis. EMBO Journal **36,** 2018–2033.

Nazio F, Carinci M, Valacca C, et al. 2016. Fine-tuning of ULK1 mRNA and protein levels is required for autophagy oscillation. Journal of Cell Biology **215,** 841–856.

Nolan TM, Brennan B, et al. 2017. Selective autophagy of BES1 mediated by DSK2 balances plant growth and survival. Developmental Cell **41,** 33–46.e7.

Nukarinen E, Nägele T, Pedrotti L, et al. 2016. Quantitative phosphoproteomics reveals the role of the AMPK plant ortholog SnRK1 as a metabolic master regulator under energy deprivation. Scientific Reports **6,** 31697.

Oh-ye Y, Inoue Y, Moriyasu Y. 2011. Detecting autophagy in Arabidopsis roots by membrane-permeable cysteine protease inhibitor E-64d and endocytosis tracer FM4-64. Plant Signaling and Behavior **6,** 1946–1949.

Ohsumi Y. 2014. Historical landmarks of autophagy research. Cell Research **24,** 9–23.

Ono Y, Wada S, Izumi M, Makino A, Ishida H. 2013. Evidence for contribution of autophagy to rubisco degradation during leaf senescence in *Arabidopsis thaliana*. Plant, Cell and Environment **36,** 1147–1159.

Onodera J, Ohsumi Y. 2004. Ald6p is a preferred target for autophagy in yeast, *Saccharomyces cerevisiae*. Journal of Biological Chemistry **279,** 16071–16076.

Papini A, Mosti S, van Doorn WG. 2014. Classical macroautophagy in *Lobivia rauschii* (Cactaceae) and possible plastidial autophagy in *Tillandsia albida* (Bromeliaceae) tapetum cells. Protoplasma **251,** 719–725.

Paul P, Münz C. 2016. Autophagy and mammalian viruses: roles in immune response, viral replication, and beyond. Advances in Virus Research **95,** 149–195.

Pei D, Zhang W, Sun H, Wei X, Yue J, Wang H. 2014. Identification of autophagy-related genes ATG4 and ATG8 from wheat (*Triticum aestivum* L.) and profiling of their expression patterns responding to biotic and abiotic stresses. Plant Cell Reports **33,** 1697–1710.

Pengo N, Agrotis A, Prak K, Jones J, Ketteler R. 2017. A reversible phospho-switch mediated by ULK1 regulates the activity of autophagy protease ATG4B. Nature Communications **8,** 294.

Pérez-Pérez ME, Florencio FJ, Crespo JL. 2010. Inhibition of target of rapamycin signaling and stress activate autophagy in *Chlamydomonas reinhardtii*. Plant Physiology **152,** 1874–1888.

Petrov V, Hille J, Mueller-Roeber B, Gechev TS. 2015. ROS-mediated abiotic stress-induced programmed cell death in plants. Frontiers in Plant Science **6,** 69.

Phillips AR, Suttangkakul A, Vierstra RD. 2008. The ATG12-conjugating enzyme ATG10 Is essential for autophagic vesicle formation in *Arabidopsis thaliana*. Genetics **178,** 1339–1353.

Ponpuak M, Mandell MA, Kimura T, Chauhan S, Cleyrat C, Deretic V. 2015. Secretory autophagy. Current Opinion in Cell Biology **35,** 106–116.

Popa C, Li L, Gil S, Tatjer L, Hashii K, Tabuchi M, Coll NS, Ariño J, Valls M. 2016. The effector AWR5 from the plant pathogen *Ralstonia solanacearum* is an inhibitor of the TOR signalling pathway. Scientific Reports **6,** 27058.

Prins A, van Heerden PD, Olmos E, Kunert KJ, Foyer CH. 2008. Cysteine proteinases regulate chloroplast protein content and composition in tobacco leaves: a model for dynamic interactions with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) vesicular bodies. Journal of Experimental Botany **59,** 1935–1950.

Pu YT, Luo XJ, Bassham DC. 2017. TOR-dependent and -independent pathways regulate autophagy in *Arabidopsis thaliana*. Frontiers in Plant Science **8,** 1204.

Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, Jung S, Jung YK. 2013. Overexpression of Atg5 in mice activates autophagy and extends lifespan. Nature Communications **4,** 2300.

Qin X, Zhang RX, Ge S, Zhou T, Liang YK. 2017. Sphingosine kinase AtSPHK1 functions in fumonisin B1-triggered cell death in Arabidopsis. Plant Physiology and Biochemistry **119,** 70–80.

Reggiori F, Monastyrska I, Shintani T, Klionsky DJ. 2005. The actin cytoskeleton is required for selective types of autophagy, but not nonspecific autophagy, in the yeast *Saccharomyces cerevisiae*. Molecular Biology of the Cell **16,** 5843–5856.

Reggiori F, Wang CW, Nair U, Shintani T, Abeliovich H, Klionsky DJ. 2004. Early stages of the secretory pathway, but not endosomes, are required for Cvt vesicle and autophagosome assembly in *Saccharomyces cerevisiae*. Molecular Biology of the Cell **15,** 2189–2204.

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 Physiology **155,** 1367–1382.

Rexin D, Meyer C, Robaglia C, Veit B. 2015. TOR signalling in plants. Biochemical Journal **470,** 1–14.

Reyes FC, Chung T, Holding D, Jung R, Vierstra R, Otegui MS. 2011. Delivery of prolamins to the protein storage vacuole in maize aleurone cells. The Plant Cell **23,** 769–784.

Rose TL, Bonneau L, Der C, Marty-Mazars D, Marty F. 2006. Starvation-induced expression of autophagy-related genes in Arabidopsis. Biology of the Cell **98,** 53–67.

Sanchez-Vera V, Kenchappa CS, Landberg K, et al. 2017. Autophagy is required for gamete differentiation in the moss *Physcomitrella patens*. Autophagy **13,** 1939–1951.

Sánchez-Wandelmer J, Kriegenburg F, Rohringer S, et al. 2017. Atg4 proteolytic activity can be inhibited by Atg1 phosphorylation. Nature Communications **8,** 295.

Sandalio LM, Romero-Puertas MC. 2015. Peroxisomes sense and respond to environmental cues by regulating ROS and RNS signalling networks. Annals of Botany **116,** 475–485.

Schwarz V, Andosch A, Geretschläger A, Affenzeller M, Lütz-Meindl U. 2017. Carbon starvation induces lipid degradation via autophagy in the model alga *Micrasterias*. Journal of Plant Physiology **208,** 115–127.

Scott RC, Juhász G, Neufeld TP. 2007. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Current Biology **17,** 1–11.

Seguí-Simarro JM, Corral-Martínez P, Corredor E, Raska I, Testillano PS, Risueño MC. 2011. A change of developmental program induces the remodeling of the interchromatin domain during microspore embryogenesis in *Brassica napus* L. Journal of Plant Physiology **168,** 746–757.

Seguí-Simarro JM, Testillano PS, Risueño MC. 2003. Hsp70 and Hsp90 change their expression and subcellular localization after microspore embryogenesis induction in *Brassica napus* L. Journal of Structural Biology **142,** 379–391.

Sequera-Mutiozabal MI, Erban A, Kopka J, Atanasov KE, Bastida J, Fotopoulos V, Alcázar R, Tiburcio AF. 2016. Global metabolic profiling of arabidopsis polyamine oxidase 4 (AtPAO4) loss-of-function mutants exhibiting delayed dark-induced senescence. Frontiers in Plant Science **7,** 173.

Shemi A, Schatz D, Fredricks HF, Van Mooy BA, Porat Z, Vardi A. 2016. Phosphorus starvation induces membrane remodeling and recycling in *Emiliania huxleyi*. New Phytologist **211,** 886–898.

Shi CS, Kehrl JH. 2010. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. Science Signaling **3,** ra42.

Shibata M, Oikawa K, Yoshimoto K, et al. 2013. Highly oxidized peroxisomes are selectively degraded via autophagy in Arabidopsis. The Plant Cell **25,** 4967–4983.

Shibata M, Oikawa K, Yoshimoto K, et al. 2014. Plant autophagy is responsible for peroxisomal transition and plays an important role in the maintenance of peroxisomal quality. Autophagy **10,** 936–937.

Shpilka T, Welter E, Borovsky N, Amar N, Mari M, Reggiori F, Elazar Z. 2015. Lipid droplets and their component triglycerides and steryl esters regulate autophagosome biogenesis. EMBO Journal **34,** 2117–2131.

Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. 2009. Autophagy regulates lipid metabolism. Nature **458,** 1131–1135.

Slavikova S, Ufaz S, Avin-Wittenberg T, Levanony H, Galili G. 2008. An autophagy-associated Atg8 protein is involved in the responses of Arabidopsis seedlings to hormonal controls and abiotic stresses. Journal of Experimental Botany **59,** 4029–4043.

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. The Plant Cell **27,** 391–402.

Sun X, Jia X, Huo L, Che R, Gong X, Wang P, Ma F. 2018*a*. MdATG18a overexpression improves tolerance to nitrogen deficiency and regulates anthocyanin accumulation through increased autophagy in transgenic apple. Plant, Cell & Environment **41,** 469–480.

Sun X, Wang P, Jia X, Huo L, Che R, Ma F. 2018*b*. Improvement of drought tolerance by overexpressing MdATG18a is mediated by modified antioxidant system and activated autophagy in transgenic apple. Plant Biotechnology Journal **16,** 545–557.

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. The Plant Cell **23,** 3761–3779.

Svenning S, Lamark T, Krause K, Johansen T. 2011. Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. Autophagy **7,** 993–1010.

Takatsuka C, Inoue Y, Matsuoka K, Moriyasu Y. 2004. 3-Methyladenine inhibits autophagy in tobacco culture cells under sucrose starvation conditions. Plant and Cell Physiology **45,** 265–274.

Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. 1992. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. Journal of Cell Biology **119,** 301–311.

Tao BY. 2007. Industrial applications for plant oils and lipids. In: **Yang S-T**, ed. Bioprocessing for value-added products from renewable resources. Amsterdam: Elsevier Science, 611–627.

Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD. 2005. Autophagic nutrient recycling in Arabidopsis directed by the ATG8 and ATG12 conjugation pathways. Plant Physiology **138,** 2097–2110.

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

Üstün S, Hafrén A, Hofius D. 2017. Autophagy as a mediator of life and death in plants. Current Opinion in Plant Biology **40,** 122–130.

Vakifahmetoglu-Norberg H, Xia HG, Yuan J. 2015. Pharmacologic agents targeting autophagy. Journal of Clinical Investigation **125,** 5–13.

Vanhee C, Guillon S, Masquelier D, Degand H, Deleu M, Morsomme P, Batoko H. 2011*a*. A TSPO-related protein localizes to the early secretory pathway in Arabidopsis, but is targeted to mitochondria when expressed in yeast. Journal of Experimental Botany **62,** 497–508.

Vanhee C, Zapotoczny G, Masquelier D, Ghislain M, Batoko H. 2011*b*. The Arabidopsis multistress regulator TSPO is a heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism. The Plant Cell **23,** 785–805.

Veljanovski V, Batoko H. 2014. Selective autophagy of non-ubiquitylated targets in plants: looking for cognate receptor/adaptor proteins. Frontiers in Plant Science **5,** 308.

Wada S, Ishida H, Izumi M, Yoshimoto K, Ohsumi Y, Mae T, Makino A. 2009. Autophagy plays a role in chloroplast degradation during senescence in individually darkened leaves. Plant Physiology **149,** 885–893.

Wang P, Hawkins TJ, Hussey PJ. 2017*a*. Connecting membranes to the actin cytoskeleton. Current Opinion in Plant Biology **40,** 71–76.

Wang P, Richardson C, Hawes C, Hussey PJ. 2016*b*. Arabidopsis NAP1 regulates the formation of autophagosomes. Current Biology **26,** 2060–2069.

Wang P, Sun X, Jia X, Ma F. 2017*b*. Apple autophagy-related protein MdATG3s afford tolerance to multiple abiotic stresses. Plant Science **256,** 53–64.

Wang P, Sun X, Jia X, Wang N, Gong X, Ma F. 2016*c*. Characterization of an autophagy-related gene MdATG8i from apple. Frontiers in Plant Science **7,** 720.

Wang P, Sun X, Wang N, Jia X, Ma F. 2016*a*. Ectopic expression of an autophagy-associated MdATG7b gene from apple alters growth and tolerance to nutrient stress in *Arabidopsis thaliana*. Plant Cell, Tissue and Organ Culture, 1–15.

Wang P, Zhu L, Sun D, Gan F, Gao S, Yin Y, Chen L. 2017*c*. Natural products as modulator of autophagy with potential clinical prospects. Apoptosis **22,** 325–356.

Wang S, Blumwald E. 2014. Stress-induced chloroplast degradation in Arabidopsis is regulated via a process independent of autophagy and senescence-associated vacuoles. The Plant Cell **26,** 4875–4888.

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

Wang Y, Liu Y. 2013. Autophagic degradation of leaf starch in plants. Autophagy **9,** 1247–1248.

Wang Y, Yu B, Zhao J, et al. 2013. Autophagy contributes to leaf starch degradation. The Plant Cell **25,** 1383–1399.

Wang Y, Zheng XY, Yu BJ, et al. 2015*b*. Disruption of microtubules in plants suppresses macroautophagy and triggers starch excess-associated chloroplast autophagy. Autophagy **11,** 2259–2274.

Wittenbach VA, Lin W, Hebert RR. 1982. Vacuolar localization of proteases and degradation of chloroplasts in mesophyll protoplasts from senescing primary wheat leaves. Plant Physiology **69,** 98–102.

Xia P, Wang S, Du Y, et al. 2013. WASH inhibits autophagy through suppression of Beclin 1 ubiquitination. EMBO Journal **32,** 2685–2696.

Xia T, Xiao D, Liu D, Chai W, Gong Q, Wang NN. 2012. Heterologous expression of ATG8c from soybean confers tolerance to nitrogen deficiency and increases yield in Arabidopsis. PLoS One **7,** e37217.

Xie Q, Tzfadia O, Levy M, Weithorn E, Peled-Zehavi H, Van Parys T, Van de Peer Y, Galili G. 2016. hfAIM: a reliable bioinformatics approach for in silico genome-wide identification of autophagy-associated Atg8-interacting motifs in various organisms. Autophagy **12,** 876–887.

Xu C, Feng K, Zhao X, et al. 2014. Regulation of autophagy by E3 ubiquitin ligase RNF216 through BECN1 ubiquitination. Autophagy **10,** 2239–2250.

Xu D, Shan B, Sun H, et al. 2016. USP14 regulates autophagy by suppressing K63 ubiquitination of Beclin 1. Genes and Development **30,** 1718–1730.

Yamane K, Mitsuya S, Taniguchi M, Miyake H. 2012. Salt-induced chloroplast protrusion is the process of exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts into cytoplasm in leaves of rice. Plant, Cell and Environment **35,** 1663–1671.

Yang X, Bassham DC. 2015. New insight into the mechanism and function of autophagy in plant cells. International Review of Cell and Molecular Biology **320,** 1–40.

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

Yano K, Yanagisawa T, Mukae K, Niwa Y, Inoue Y, Moriyasu Y. 2015. Dissection of autophagy in tobacco BY-2 cells under sucrose starvation conditions using the vacuolar H(+)-ATPase inhibitor concanamycin A and the autophagy-related protein Atg8. Plant Signaling and Behavior **10,** e1082699.

Yoshimoto K, Jikumaru Y, Kamiya Y, Kusano M, Consonni C, Panstruga R, Ohsumi Y, Shirasu K. 2009. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in Arabidopsis. The Plant Cell **21,** 2914–2927.

Yoshimoto K, Shibata M, Kondo M, Oikawa K, Sato M, Toyooka K, Shirasu K, Nishimura M, Ohsumi Y. 2014. Organ-specific quality control of plant peroxisomes is mediated by autophagy. Journal of Cell Science **127,** 1161–1168.

Young PG, Bartel B. 2016. Pexophagy and peroxisomal protein turnover in plants. Biochimica et Biophysica Acta **1863,** 999–1005.

Zaffagnini G, Martens S. 2016. Mechanisms of selective autophagy. Journal of Molecular Biology **428,** 1714–1724.

Zavodszky E, Seaman MN, Moreau K, Jimenez-Sanchez M, Breusegem SY, Harbour ME, Rubinsztein DC. 2014. Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. Nature Communications **5,** 3828.

Zechner R, Madeo F, Kratky D. 2017. Cytosolic lipolysis and lipophagy: two sides of the same coin. Nature Reviews. Molecular Cell Biology **18,** 671–684.

Zhang R, Meng Z, Zhou T, Deng Y, Feng L, Wang Y, Sun G, Guo S, Ren M. 2014*a*. ScFKBP12 bridges rapamycin and AtTOR in Arabidopsis. Plant Signaling and Behavior **8,** e26115.

Zhang R, Patena W, Armbruster U, Gang SS, Blum SR, Jonikas MC. 2014*b*. High-throughput genotyping of green algal mutants reveals random distribution of mutagenic insertion sites and endonucleolytic cleavage of transforming DNA. The Plant Cell **26,** 1398–1409.

Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, Jin S. 2009. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. Proceedings of the National Academy of Sciences, USA **106,** 19860–19865.

Zhao L, Dai J, Wu Q. 2014. Autophagy-like processes are involved in lipid droplet degradation in *Auxenochlorella protothecoides* during the heterotrophy–autotrophy transition. Frontiers in Plant Science **5,** 400.

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 Genetics **9,** e1003196.

Zhou J, Wang J, Yu JQ, Chen Z. 2014*a*. Role and regulation of autophagy in heat stress responses of tomato plants. Frontiers in Plant Science **5,** 174.

Zhou J, Yu JQ, Chen Z. 2014*b*. The perplexing role of autophagy in plant innate immune responses. Molecular Plant Pathology **15,** 637–645.

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. The Plant Cell **25,** 4596–4615.

Zientara-Rytter K, Lukomska J, Moniuszko G, Gwozdecki R, Surowiecki P, Lewandowska M, Liszewska F, Wawrzyńska A, Sirko A. 2011. Identification and functional analysis of Joka2, a tobacco member of the family of selective autophagy cargo receptors. Autophagy **7,** 1145–1158.