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Autophagy: The Master of Bulk and Selective Recycling

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Abstract

Plants have evolved sophisticated mechanisms to recycle intracellular constituents, which are essential for developmental and metabolic transitions; for efficient nutrient reuse; and for the proper disposal of proteins, protein complexes, and even entire organelles that become obsolete or dysfunctional. One major route is autophagy, which employs specialized vesicles to encapsulate and deliver cytoplasmic material to the vacuole for breakdown. In the past decade, the mechanics of autophagy and the scores of components involved in autophagic vesicle assembly have been documented. Now emerging is the importance of dedicated receptors that help recruit appropriate cargo, which in many cases exploit ubiquitylation as a signal. Although operating at a low constitutive level in all plant cells, autophagy is upregulated during senescence and various environmental challenges and is essential for proper nutrient allocation. Its importance to plant metabolism and energy balance in particular places autophagy at the nexus of robust crop performance, especially under suboptimal conditions.

Contents

INTRODUCTION

Ubiquitin (Ub): a 76-amino-acid protein that becomes attached to other proteins and facilitates their degradation via the proteasome or autophagy

Ubiquitin-proteasome system: a pathway for degrading proteins via the proteasome that first involves the selective attachment of one or more poly-ubiquitin chains to individual substrates

Proteasome: a 2.5-MDa, multicatalytic proteolytic complex that degrades proteins modified with poly-ubiquitin chains

Intracellular recycling is essential for the proper control of most, if not all, cellular events. In addition to modulating the levels of key regulators, it is the main housekeeper that removes cellular debris and replenishes essential nutrients needed for new growth (27, 119). In fact, cellular homeostasis and development are often based on recycled components. Arguably the most pervasive recycling system in eukaryotes is the ubiquitin (Ub)-proteasome system, but it is limited to catabolizing proteins individually (172). Ub-proteasome system substrates are first decorated with a poly-Ub chain that enables their recognition and turnover by the proteasome, a self-compartmentalized proteolytic machine that degrades the substrate but releases the Ub moieties intact for reuse (6, 17). As a testament to its complexity, as much as 6% of the *Arabidopsis* proteome is dedicated to the Ub-proteasome system, with much of that proportion devoted to the enzymes that direct the specificity of ubiquitylation (65).

As a complement, plants and other eukaryotes also employ autophagy (meaning self-eating), which is designed to handle cytoplasmic material in bulk, including large protein complexes, protein and nucleic acid aggregates, lipid bodies, and even entire organelles that become damaged or superfluous (107, 119, 176). Here, cytoplasmic constituents and cellular debris become sequestered into specialized autophagic vesicles that are delivered to the vacuole for breakdown. Although originally thought to be an unrestricted degradation system, autophagy may rival the selectivity of the Ub-proteasome system by engaging a wide array of receptors that recognize appropriate cargo and tether it to the enveloping autophagic membrane (27, 33, 107). In this way, specific proteins, macromolecular complexes (ribosomes and proteasomes), protein aggregates, fragments of endoplasmic reticulum (ER) or nuclei, whole organelles (mitochondria, peroxisomes, and chloroplasts), and possibly even invading pathogens can be selectively eliminated. In this review, we summarize our current understanding of the autophagic machinery in plants; describe recent advances in

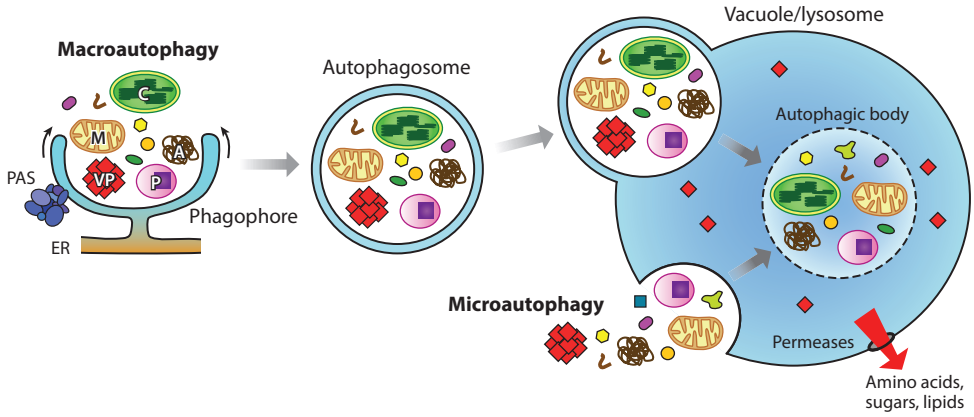


Figure 1

Morphological steps of microautophagy and macroautophagy in plants. Microautophagy proceeds by invagination of the tonoplast to engulf portions of the cytosol directly into autophagic bodies within the vacuole. Conversely, macroautophagy and related processes begin by assembling an engulfing membrane termed the phagophore, which likely arises from the endoplasmic reticulum (ER). The phagophore sequesters cytosolic components and then seals to form a double membrane–bound autophagosome. Phagophore initiation and expansion are directed by the pre-autophagosomal structure (PAS), which is generated by a hierarchical assembly of autophagy-related proteins. Autophagosomes fuse with the tonoplast to release the internal vesicle as an autophagic body into the vacuolar lumen, where its cargo is degraded by resident hydrolases. These digestion products are either stored in the vacuole or transported back to the cytosol for re-use. A possible cytoplasm-to-vacuole targeting pathway may transport functional cargo (*red diamonds*) to the vacuole. Abbreviations: A, protein aggregate; C, chloroplast; M, mitochondrion; P, peroxisome; VP, vacuole protein.

defining the receptors that determine its selectivity; and discuss the importance of autophagy to plant metabolism and growth, especially with regard to agronomic performance.

THE CYTOLOGICAL FEATURES OF AUTOPHAGY

Thus far, three distinct types of autophagy—microautophagy, macroautophagy, and mega-autophagy—have been described in plants (170). During microautophagy, cytoplasmic material congregates at the vacuole surface and becomes trapped by invagination of the tonoplast. The tonoplast then undergoes scission to release autophagic bodies, which are intravacuolar vesicles containing cytoplasmic material (**Figure 1**). By contrast, in macroautophagy, cargo is trapped in cytoplasmic vesicles formed de novo; such vesicles arise by expansion of a cup-shaped phagophore (or isolation membrane) that encircles cytoplasm and ultimately seals to generate the double membrane–bound autophagosome (**Figure 1**). The origin of the phagophore is unclear; in some cases it arises from the ER, whereas in others the membrane appears to emerge from a cage-like, tubular network that coalesces (99, 198). The outer membrane of the autophagosome then fuses with the tonoplast to release the internal vesicle as an autophagic body. For both micro- and macroautophagy, breakdown of the autophagic body membrane exposes the luminal contents to vacuolar hydrolases, which disassemble the cargo into its constituent parts for export back to the cytoplasm. Additionally, yeast (and possibly plants) use smaller autophagic vesicles to deliver functional cargo to the vacuole via a cytoplasm-to-vacuole targeting pathway (40) and use piece-meal autophagy that employs teardrop-like blebs to export lumenal material from the nucleus for breakdown in vacuoles (150).

Vacuole: a large, membrane-bound organelle found in plant and fungal cells; it houses hydrolytic enzymes that degrade autophagic bodies

Microautophagy: a degradative pathway mediated by direct vacuolar engulfment of cytoplasmic components by invagination and subsequent scission of the tonoplast

Macroautophagy: degradation of cytoplasmic components following engulfment by a phagophore and delivery of the resulting autophagosome to the vacuole

The most extreme form of autophagy is mega-autophagy. Here, the tonoplast permeabilizes or ruptures to release vacuolar hydrolases directly into the cytoplasm, where they degrade cytoplasmic material in situ (55, 170). Mega-autophagy often represents the final stage of programmed cell death (PCD) that occurs developmentally (e.g., during xylogenesis) or in response to pathogen invasion [e.g., the hypersensitive response (HR)]. Animals also use a chaperone-mediated autophagic route that is independent of vesicles: It employs dedicated transporters to import substrates directly into vacuoles (40). There is no evidence yet that such a route exists in plants, nor do plants appear to encode counterparts to the chaperone-assisted transporters found in animals (e.g., LAMP-2A).

Mega-autophagy: mass degradation of cytoplasmic components following permeabilization or rupture of the tonoplast and release of vacuolar hydrolases

Tonoplast: the membrane that surrounds the large central vacuole within plant cells

Phagophore: the cup-shaped, double membrane structure that expands, encloses, and isolates cytoplasmic components during macroautophagy

Autophagosome: the double membrane–bound vesicle formed during autophagy that engulfs cytoplasmic content and delivers it to the vacuole for degradation

Autophagic body: a single membrane–bound vesicle released into the vacuole during microautophagy or upon fusion of an autophagosome with the tonoplast during macroautophagy

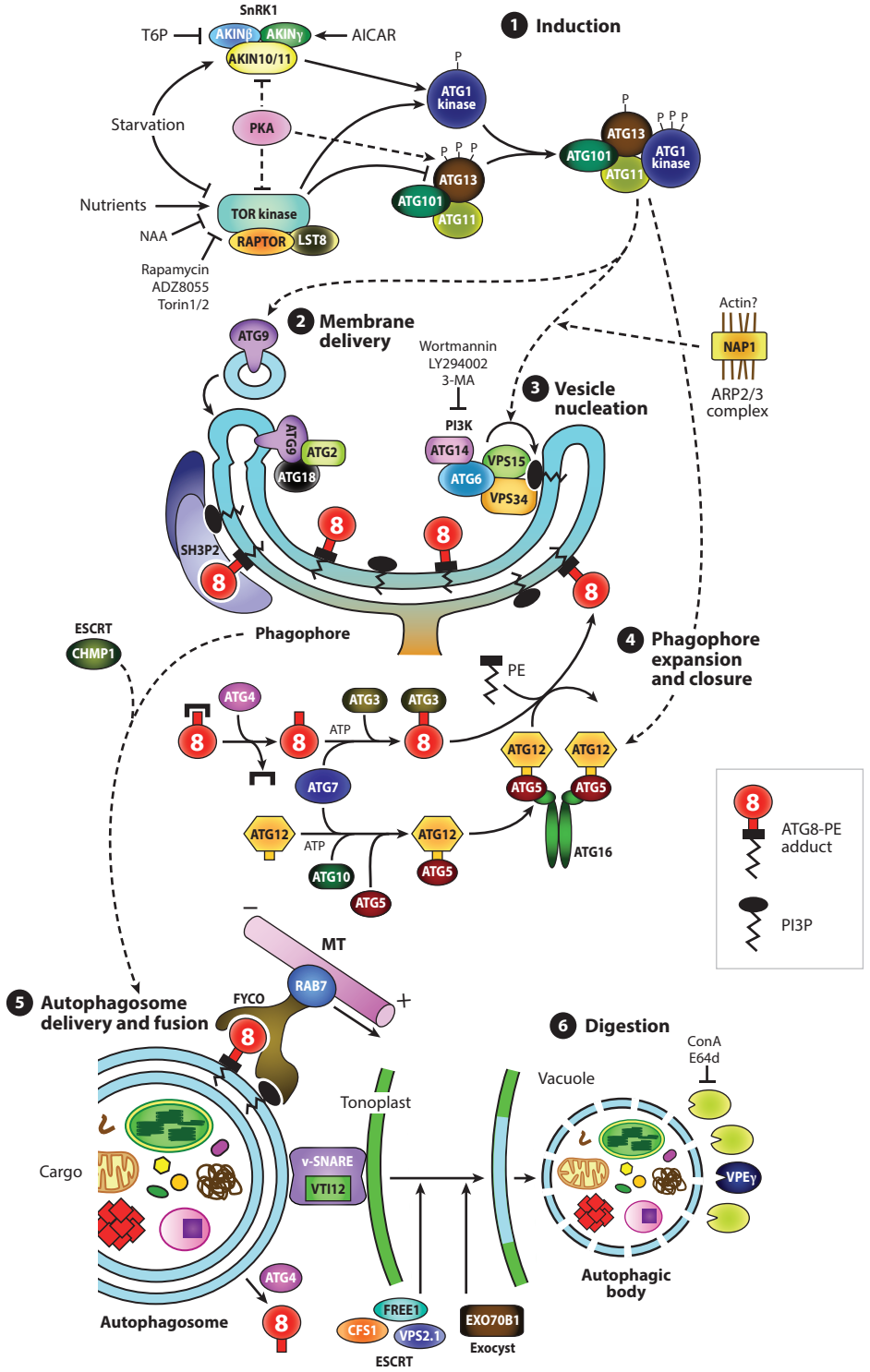
Pre-autophagosomal structure: a punctate structure localized in the vicinity of the vacuole that initiates phagophore formation and expansion

THE AUTOPHAGIC MACHINERY

Through studies on a variety of organisms over the past two decades, the underpinning autophagic machinery has emerged. Particularly illuminating were genetic screens developed by Yoshinori Ohsumi (awarded the Nobel Prize in Physiology or Medicine in 2016) and others to identify yeast *AUTOPHAGY-RELATED* (*ATG*) loci required for macro- and microautophagy, peroxisome turnover, and the cytoplasm-to-vacuole targeting pathway (137). Analysis of more than 40 Atg proteins revealed a canonical route for macroautophagy, with many components also shared with microautophagy. A number of these factors assemble into complexes that hierarchically promote autophagy induction based on the developmental and nutritional status of the cell, membrane delivery, vesicle nucleation, phagophore expansion and closure, delivery of the autophagosome to the vacuole, and finally breakdown of the autophagic membrane and digestion of its contents (**Figure 2**). Many macroautophagy factors congregate into a dynamic pre-autophagosomal structure that associates with the phagophore.

Seminal to our understanding of plant autophagy is that most yeast Atg proteins have obvious orthologs in species such as *Arabidopsis thaliana*, rice (*Oryza sativa*), maize (*Zea mays*), tobacco (*Nicotiana tabacum*), foxtail millet (*Setaria italica*), and the alga *Chlamydomonas reinhardtii*, which has enabled rapid understanding of the plant system based on the yeast paradigm (14, 84, 105, 108, 143, 167, 181, 197). Notably, a number of the plant proteins are encoded by small gene families, implying mechanistic expansion of the plant system and/or sub-functionalization of individual components (14, 29, 84, 105, 162, 184).

As shown in **Figure 2**, macroautophagy (henceforth termed autophagy) begins when both developmental and nutritional signals converge to promote assembly of the ATG1 kinase complex. Especially important is the negative regulator TARGET OF RAPAMYCIN (TOR). This essential serine/threonine kinase, together with its effectors RAPTOR and LETHAL WITH SEC THIRTEEN PROTEIN 8 (LST8), is active under nutrient-replete conditions—during which it upregulates cell growth and protein translation and suppresses autophagy—but is inactive upon nutrient deficiency (28). Active TOR blocks autophagy by phosphorylating the ATG1-activator ATG13, thus preventing its association with ATG1, whereas rapid dephosphorylation of ATG13 upon TOR inactivation permits ATG1 binding (162). Accordingly, overexpression of TOR in plants suppresses autophagy (147), whereas TOR inactivation via RNAi-mediated downregulation, chemical inhibitors such as rapamycin and AZD8055, or genetic elimination of RAPTOR or LST8 enhances autophagy (111, 147). PROTEIN KINASE A (PKA) and SUCROSE NONFERMENTING 1–RELATED KINASE 1 (SnRK1, known as AMP-ACTIVATED PROTEIN KINASE in animals) provide additional layers regulating autophagy induction upstream of TOR (40). For example, overexpression and RNAi-mediated downregulation of the AKIN10 catalytic subunit of *Arabidopsis* SnRK1 accelerate and repress autophagy induction, respectively, upon nutrient starvation (12, 158). Through additional hypo- and hyperphosphorylation events, an activated serine/threonine ATG1 kinase emerges that includes two additional subunits, ATG11 and



(Caption appears on following page)

Figure 2 (*Figure appears on preceding page*)

Schematic representation of known or proposed steps in macroautophagy. (❶) Induction of macroautophagy is regulated by the nutritional status of the cell. Under nutrient-rich conditions, TOR and other kinases induce hyperphosphorylation of the ATG13 subunit and hypophosphorylation of the ATG1 subunit within the ATG1 kinase complex, promoting its dissociation. Under nutrient-poor conditions, these steps are reversed, allowing ATG1, ATG13, and the accessory subunits ATG11 and ATG101 to assemble into an active complex. (❷) The activated ATG1 kinase promotes ATG9-mediated delivery of lipids to the developing phagophore, which promotes its (❸) nucleation and (❹) expansion via a process involving the VPS34 lipid kinase, which generates PI3P. PI3P decoration is accompanied by conjugation of ATG8 to PE, which is driven by the hexameric ATG5/ATG12/ATG16 E3 ligase complex. (❺) Sealed, ATG8- and PI3P-decorated autophagosomes are transported to the vacuole with the help of FYVE and coiled-coil domain–containing (FYCO) proteins that tether the autophagosome to the microtubule transport machinery. SNARE-mediated fusion of the autophagosomes with the tonoplast, with the help of ARP2/3 (NAP1), ESCRT (CFS1, CHMP1, FREE1, and VPS2.1), and exocyst (EXO70B1) components, then releases autophagic bodies into the vacuole. (❻) These vesicles are subsequently degraded by vacuolar hydrolases. Useful inhibitors are indicated next to the targeted reactions, while phenotypes and assays associated with several autophagy components in plants are shown in **Figure 4**. Almost all named components (except PKA and ATG16) have been formally investigated in plants. Key abbreviations: 3-MA, 3-methyladenine; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside monophosphate; ATG, autophagy-related; ConA, concanamycin A; ESCRT, endosomal sorting complexes required for transport; MT, microtubule; NAA, 1-naphthaleneacetic acid; PE, phosphatidylethanolamine; PI3K, phosphatidylinositol-3-kinase; PI3P, phosphatidylinositol-3-phosphate; PKA, protein kinase A; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; SnRK1, sucrose non-fermenting 1–related kinase 1; T6P, trehalose-6-phosphate; TOR, TARGET OF RAPAMYCIN.

TARGET OF RAPAMYCIN (TOR): a serine/threonine protein kinase that integrates multiple metabolic cues to negatively regulate the initiation of autophagy

Phosphatidylinositol-3-phosphate (PI3P): the phospholipid generated by the PI3K complex that recruits several binding partners such as SH3P2 and FREE1/FYVE1 during macroautophagy

Phosphatidylinositol-3-kinase (PI3K): an autophagy-related kinase complex consisting of ATG6/VPS30/Beclin1, ATG14 or VPS38, VPS15, and VPS34; is responsible for phosphorylating the lipid phosphatidylinositol to produce PI3P

ATG101, along with ATG13 (106, 162), which together stimulate several downstream autophagy steps, presumably via further phosphorylation events.

One such step involves delivery of lipids to the expanding phagophore, which is driven by the transmembrane protein ATG9 along with its cycling factors, ATG2 and ATG18 (184, 198) (**Figure 2**). Another step involves decoration of the phagophore with phosphatidylinositol-3-phosphate (PI3P) by a complex containing the class III phosphatidylinositol-3-kinase (PI3K) VACUOLAR PROTEIN SORTING 34 (VPS34), along with ATG/VPS30/Beclin1, VPS38, or ATG14, and VPS15 (**Figure 2**). *Arabidopsis* mutants lacking VPS34, ATG6/VPS30/Beclin1, and VPS15 are inviable (37, 102, 186), whereas those lacking the fourth subunit, either VPS38 or ATG14, display autophagy-related phenotypes and are compromised in autophagic body accumulation, albeit to a lesser extent than mutants impacting core components such as ATG7 (100; F. Liu, F. Li & R.D. Vierstra, unpublished manuscript). Other phenotypes are also evident, indicating that the class III PI3K has additional roles in plant development and that its product PI3P is required for processes other than autophagy. Posttranslational regulation of the PI3K complex appears to be important in modulating autophagic flux (148).

The next step centers on a signature conjugation pathway analogous to ubiquitylation that decorates the phagophore with the Ub-fold protein ATG8 [known as MICROTUBULE-ASSOCIATED PROTEIN 1 LIGHT CHAIN 3 (MAP1LC3) or GAMMA-AMINOBUTYRIC ACID RECEPTOR–ASSOCIATED PROTEIN (GABARAP) in animals] (137) (**Figure 3*a***). ATG8 is first processed from an inactive precursor by the ATG4 protease to expose a C-terminal glycine conserved among all family members (190). Mature ATG8 is then activated at this glycine by the ATP-dependent activating enzyme ATG7; is transferred to the conjugating enzyme ATG3; and is finally connected via an ether linkage to the lipid phosphatidylethanolamine (PE) by a hexameric ligase complex containing ATG5, ATG16, and a second Ub-fold protein, ATG12 (**Figure 2**). Assembly of this ligase uniquely requires conjugation of ATG12 to ATG5, using ATG7 and the ATG10 conjugating enzyme to isopeptide link the C-terminal glycine of ATG12 to a conserved lysine within ATG5. This ATG12-ATG5 conjugate then binds the dimeric scaffold protein ATG16,

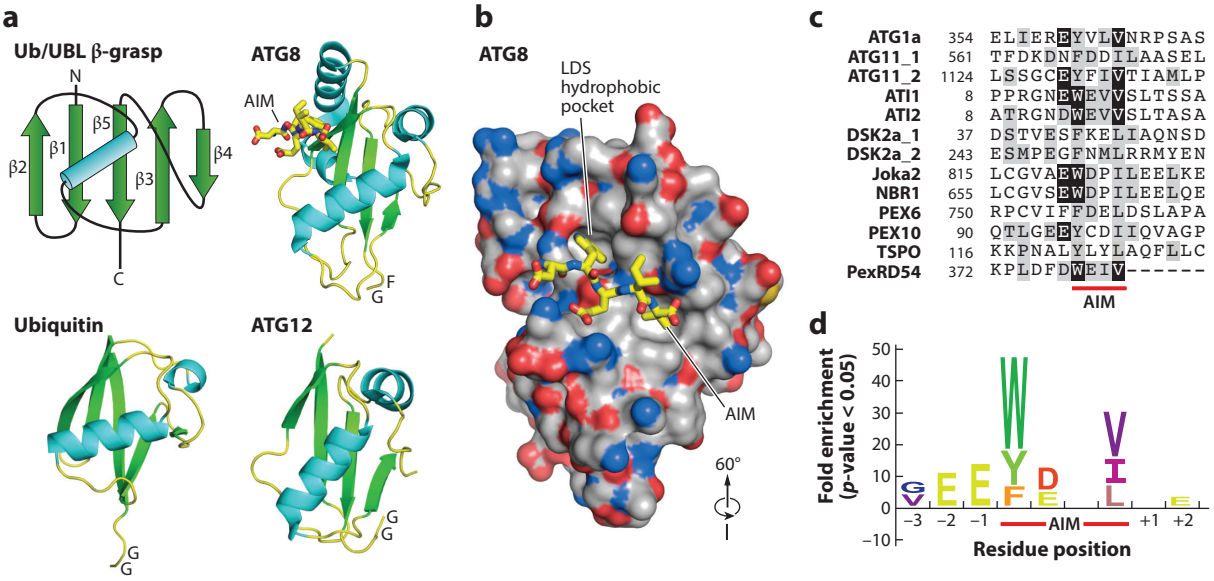


Figure 3

Structures and binding motifs of the autophagy-related ubiquitin (Ub)-fold proteins. (*a*) Ribbon diagrams of the β-grasp folds from Ub, ATG8, and ATG12. The processed forms are shown, and the exposed C-terminal amino acids are indicated. The schematic of the β-grasp fold at the top left shows the arrangement of the α-helix and β-strand secondary structures. The position of the ATG8-interacting motif (AIM) (residues 377–381) from *Phytophthora infestans* PexRD54 docked onto ATG8 is indicated by the yellow stick diagram (115). The Protein Data Bank accession numbers for the structures are 5L83 (*Solanum tuberosum* ATG8CL), 1WZ3 (*Arabidopsis thaliana* ATG12), and 1UBQ (*Avena sativa* Ub). Abbreviations: C, cysteine; F, phenylalanine; G, glycine. (*b*) Surface view of the three-dimensional structure of potato ATG8CL in complex with an AIM-containing peptide from PexRD54 shown in yellow stick form. Blue shading and red shading identify nitrogen and oxygen atoms, respectively. The position of the hydrophobic LIR/AIM docking site (LDS) is indicated. (*c*) Sequence alignment of known plant or plant pathogen AIMs generated with Clustal Omega and displayed using the BoxShade server. Residue numbers are shown on the left, whereas the position of the core AIM is indicated by a red line. Identical and similar amino acids are in black and gray boxes, respectively. The dashes denote gaps. (*d*) The consensus plant AIM sequence identified by iceLogo (16), using the 13 currently known AIMs shown in panel *c*.

which tethers the conjugate to the phagophore to promote ATG8 lipidation (13, 29, 39, 144, 166) (**Figure 2**).

The ATG8-PE adduct coats the expanding phagophore and helps seal the vesicle by recruiting BIN/AMPHIPHYSIN/RYS (BAR) domain– and SRC HOMOLOGY-3 (SH3) domain–containing proteins such as SH3P2 (199). Dimers of SH3P2 work cooperatively to stimulate phagophore curvature by using the SH3 domain to bind PI3P and the arched BAR domain to distort the membrane (199). Eventually, the ATG8-PE adducts lining the outer membrane are delipidated by ATG4 and released for reuse (190), whereas the ATG8-PE adducts trapped on the inner membrane are consumed in the vacuole. Autophagosome biogenesis also involves the ACTIN-RELATED PROTEIN 2/3 (ARP2/3) actin nucleation complex, with a key early activity provided by NCK-ASSOCIATED PROTEIN 1 (NAP1) (177).

Once formed, ATG8- and PI3P-coated autophagosomes are transported to the vacuole, possibly using the microtubule network controlled by the ENDOSOMAL SORTING COMPLEXES REQUIRED FOR TRANSPORT (ESCRT) machinery. Autophagosomes then fuse with the tonoplast by using the ESCRT-based VESICULAR SOLUBLE *N*-ETHYLMALEIMIDE-SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR (v-SNARE) factors (160),

AUTOPHAGY-RELATED 8 (ATG8): the ubiquitin-fold protein that becomes attached to phosphatidylethanolamine and acts as a docking platform for autophagic receptors and adaptors

thus releasing the autophagic body (**Figure 2**). The plant exocyst protein EXO70B1 and the ESCRT components CELL DEATH-RELATED ENDOSOMAL FYVE/SYLF PROTEIN 1 (CFS1), FYVE-DOMAIN PROTEIN REQUIRED FOR ENDOSOMAL SORTING 1 (FREE1), and VACUOLAR PROTEIN SORTING 2.1 (VPS2.1) have also been implicated in this transport and fusion (41, 79, 82, 95, 142, 161). Once autophagic bodies are deposited, their limiting membrane and contents are degraded by a host of vacuolar hydrolases active against lipids, proteins, nucleic acids, and carbohydrates (**Figure 2**). One prominent protease is the caspase-like VACUOLAR PROCESSING ENZYME γ (VPE-γ), which activates a number of vacuolar zymogens (152); it is upregulated during senescence and in cells undergoing mega-autophagy (18, 55).

Phosphatidylethanolamine (PE): the lipid to which ATG8 becomes attached via a ubiquitylation-like conjugation cascade during micro- and macroautophagy

METHODS FOR MONITORING AUTOPHAGY

Crucial to the appreciation of plant autophagy has been the development of assays to monitor the various steps and to screen for potential substrates. For example, both the conjugation of ATG12 to ATG5 and the lipidation of ATG8 cause changes in electrophoretic mobility that can be easily detected by immunoblot assays following SDS-PAGE (13, 14, 166, 190) (**Figure 4*a*,*b***). Detection of ATG8-PE adducts is often complicated by heterogeneity within the ATG8 family, by co-migration of the conjugates with unmodified ATG8, and by their low abundance. These problems can be solved by enriching for the membrane fraction by centrifugation, solubilizing the membranes with detergent, and then subjecting the samples to SDS-PAGE in the presence of 6 M urea, upon which lipidated ATG8 is seen as a faster-migrating species sensitive to phospholipase D treatment (13) (**Figure 4*b***). The ATG8-PE adduct can also be generated in vitro by using recombinant plant components (38). Interestingly, although the ATG8-PE adduct level typically varies to reflect autophagic flux, most ATG5 is constitutively modified with ATG12 regardless of the plant growth conditions, implying that this step is not regulatory (13, 125, 166).

An expanding list of chemical inhibitors and activators affecting various stages of autophagy has also provided invaluable tools (**Table 1** and **Figure 2**). One prominent inhibitor is concanamycin A (ConA), which blocks the v-ATPases on the tonoplast membrane responsible for vacuolar acidification (166, 190). As most vacuolar enzymes have low pH optima, the subsequent rise in pH after ConA treatment suppresses autophagic body breakdown, which both enhances microscopic detection of autophagic bodies and stabilizes their contents (**Figure 4*c***). Protease inhibitors such as E64d are also sometimes effective in stabilizing autophagic bodies (67, 128, 129). Other useful compounds include the TOR inhibitors rapamycin, Torin1/2, and AZD8055; the TOR activator 1-naphthaleneacetic acid; the SnRK1 inhibitor trehalose-6-phosphate; the SnRK1 activator AICAR; and the PI3K inhibitors wortmannin, LY294002, and 3-methyladenine (127, 147, 158, 165, 185, 194). Although controversy at first existed as to whether plant TOR was sensitive to rapamycin, more recent studies confirmed its efficacy (185).

By far the most helpful tools have been a suite of fluorescent reporters that enable both visual detection of autophagic structures, and quantitative measures of autophagic flux. For example, GFP-ATG8 fusions permit detection of autophagosomes within the cytoplasm and autophagic bodies within the vacuole by confocal fluorescence microscopy (19, 71, 105, 166, 190). Autophagic bodies in particular are easily observed as 1–2-μm vacuolar puncta stabilized by ConA or E64d (**Figure 4*c*,*d*,*e*,*g***). In addition, it is now appreciated that GFP and its derivatives CFP and YFP are uniquely stable fluorophores once inside the vacuole; whereas the fused protein is often rapidly released proteolytically and degraded, the freed fluorescent protein is substantially more stable and accumulates (13, 105, 106, 116, 159). Thus, the appearance of free GFP/YFP/CFP as detected by immunoblotting after SDS-PAGE, and their absence in autophagy-defective mutants, easily confirms that a potential autophagic substrate is degraded by autophagy, whereas changes in

the ratio of fused to free fluorescent protein provide a quantitative measure of autophagic flux (**Figure 4*f*,*h***).

Bimolecular fluorescence complementation, yeast two-hybrid screening, and co-immunoprecipitation have also been helpful in confirming both the assembly of various higher-order complexes and the binding of ATG8 to autophagic cargo receptors (e.g., 23, 106, 116). In addition, electron microscopy and three-dimensional tomographic imaging now offer unequivocal support for both micro- and macroautophagy, and have confirmed the autophagic transport of various organelles and ribosomes on the basis of their distinguishing morphological features (72, 149, 170). Interestingly, large cytoplasmic aggregates are often seen in plant

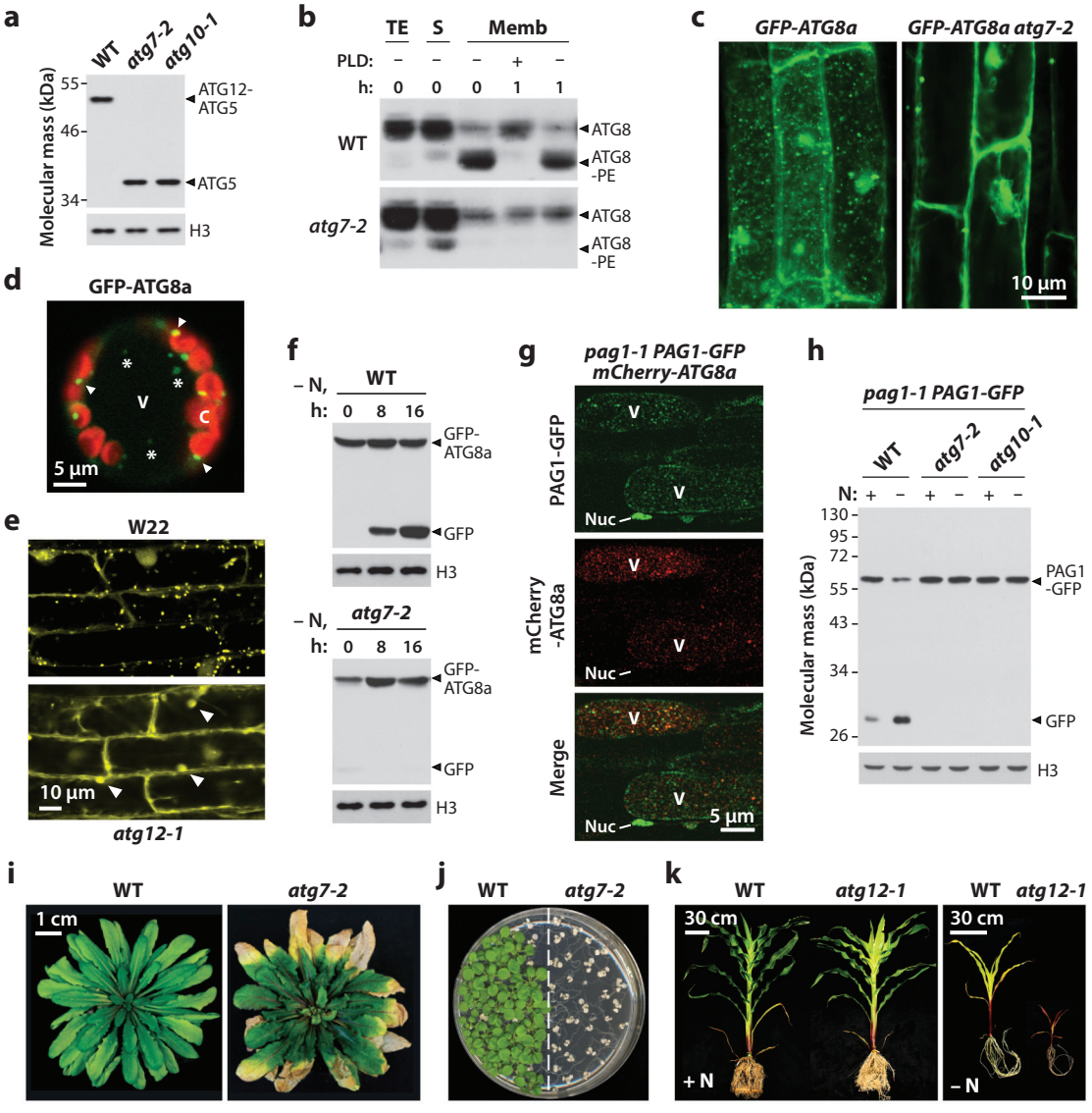


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Figure 4 (*Figure appears on preceding page*)

Common assays employed for monitoring autophagy in plants. (*a*) Immunoblot detection of the ATG12-ATG5 conjugate in crude extracts from 7-day-old wild-type (WT), *atg7-2*, and *atg10-1 Arabidopsis* seedlings. Arrowheads mark the ATG12-ATG5 conjugate and free ATG5. (*b*) Immunoblot detection of the ATG8-PE adduct. Total cell extracts (TE) from WT or *atg7-2* seedlings were centrifuged at 100,000 × *g* to separate the soluble (S) and membrane (Memb) fractions. The membranes were solubilized and incubated with or without phospholipase D (PLD). Samples were subjected to SDS-PAGE in the presence of 6 M urea and were immunoblotted with anti-ATG8a antibodies. Arrowheads indicate the ATG8-PE adduct and unmodified ATG8. (*c*) *Arabidopsis* autophagy-deficient mutants fail to accumulate autophagic bodies. The image shows confocal fluorescence microscopic analysis of root cells from 7-day-old WT or *atg7-2* seedlings stably expressing GFP-ATG8a. The seedlings were exposed to nitrogen (N)-deficient medium and 1 μM concanamycin A (ConA) for 16 h prior to imaging. (*d*) Transient expression of GFP-ATG8a in *Arabidopsis* protoplasts imaged 16 h after transfection. Arrowheads and asterisks locate possible autophagosomes in the cytoplasm (C) and autophagic bodies inside the vacuole (V), respectively. (*e*) Maize autophagy-deficient mutants also fail to accumulate autophagic bodies. The image shows confocal fluorescence microscopy of root cells from 10-day-old WT or *atg12-1* seedlings stably expressing YFP-ATG8a exposed to N-deficient medium for 36 h and then treated with 1 μM ConA for 16 h. Arrowheads indicate large cytoplasmic aggregates that accumulate in *atg12-1* plants. (*f*) Immunoblot detection of free GFP accumulation from GFP-ATG8a in crude extracts from 7-day-old WT, but not *atg7-2*, seedlings subjected to N starvation for the indicated periods of time. (*g*) Co-localization of a GFP-tagged autophagy substrate (in this case the proteasome subunit PAG1) in autophagic bodies with ATG8. PAG1 fused to GFP was imaged along with mCherry-ATG8a by confocal fluorescence microscopy of hypocotyl cells from 7-day-old seedlings exposed to N-deficient medium and 1 μM ConA for 16 h prior to imaging. Abbreviation: Nuc, nucleus. (*h*) Immunoblot detection of free GFP released from PAG1-GFP in 7-day-old WT, but not *atg7-2* or *atg10-1*, seedlings subjected to N starvation for 16 h. (*i*) Accelerated senescence of autophagy-deficient *Arabidopsis* plants. WT or *atg7-2* plants were imaged following growth for 56 days under a short-day photoperiod. (*j*) Enhanced sensitivity of autophagy-deficient *Arabidopsis* plants to fixed-carbon starvation. WT or *atg7-2* seedlings were grown in the absence of sucrose under a long-day (LD) photoperiod for 14 days, were placed in darkness for 10 days, and were returned to LD growth conditions for a further 14 days. (*k*) Enhanced sensitivity of autophagy-deficient maize plants to N starvation. WT or *atg12-1* plants were grown continuously for 49 days on high (15 mM) or low (0.1 mM) N-fertilized soil. Detection of histone H3 in panels *a*, *f*, and *h* was used to confirm near-equal protein loading. Panels *b*, *e*, and *k* were adapted with permission from Reference 105. Panels *c* and *j* were adapted with permission from Reference 13. Panel *d* was adapted with permission from Reference 162. Panels *g* and *h* were adapted with permission from Reference 116.

autophagy-defective mutants (105), which likely reflects a failure to clear this unwanted material (**Figure 4*e***). A number of studies have relied on the use of acidotropic dyes such as LysoTracker Red and monodansylcadaverine to identify autophagic vesicles (19); however, we caution against their use, as these dyes will stain any acidic compartment (123).

ROUTES FOR SELECTIVE AUTOPHAGY

Selective autophagy: an autophagic process that facilitates the degradation of specific cytoplasmic components such as protein complexes, aggregates, organelles, and pathogens

ATG8-interacting motif (AIM): a W/F/Y-X-X-V/I/L consensus amino acid sequence that permits binding of cargo receptors or other autophagy adaptors to ATG8

Autophagy appears to be active at a basal level in all plant cells at all developmental stages, during which it presumably removes cellular debris (67, 156). Transcriptional, cell biological, and proteomic data also show that this bulk turnover is strongly upregulated upon nutrient deprivation, likely via SnRK1, TOR, and ATG1 signaling (18, 111, 147, 158, 162). For the most part, this accelerated breakdown likely affects the cytoplasm in bulk as cells attempt to replenish needed pools of amino acids, carbohydrates, lipids, and nucleic acids.

Additionally, it has become increasingly clear that various routes for selective autophagy exist beyond those that solve metabolic emergencies. This selectivity works through lipidated ATG8, which drives specificity by associating with a collection of autophagic receptors with affinity for specific cargo (27, 33, 107). The primary interaction is through the ATG8-interacting motif (AIM) in plants and yeast [also known as the LC3-interacting region (LIR) in animals]. AIMs consist of a consensus core sequence, W/F/Y-X-X-V/I/L, which is often bracketed by acidic residues (33, 134) (**Figure 3*c*,*d***). As shown by a recent crystallographic structure of the potato (*Solanum tuberosum*) ATG8CL isoform together with the AIM sequence from the *Phytophthora infestans* effector PexRD54 (115), the AIM nestles within a hydrophobic patch on ATG8 known as the LIR/AIM docking site (LDS), with a prominent feature being two hydrophobic pockets that

Table 1 Common inhibitors and activators used in the study of plant autophagy

Inhibitor name	Mechanism of action	Effective concentrations[a]	Comments	References[b]
1-Naphthaleneacetic acid	TOR kinase activator	20 nM (*A. thaliana* seedlings)	May have pleiotropic effects related to nonautophagic roles of auxin/TOR signaling	147, 154, 158
3-Methyladenine	Phosphatidylinositol-3-kinase inhibitor	5 mM (*Nicotiana tabacum* suspension culture cells); 10 mM (*Arabidopsis thaliana* seedlings)	May have pleiotropic effects related to nonautophagic roles of the phosphatidylinositol-3-kinase complex	67, 165
5-Aminoimidazole-4-carboxamide ribonucleoside monophosphate (AICAR)	SnRK1 activator	10 mM (*A. thaliana* seedlings and leaves; *Spinacia oleracea* and *Pisum sativum* leaves)	Off-target effects include activation of glutamine synthase, NADH-nitrate reductase, and sucrose-phosphate synthase	66, 141, 158
AZD8055	TOR kinase inhibitor	1–5 μM (*A. thaliana* seedlings); 10 μM (*Lotus japonicas*, *Nicotiana benthamiana*, *Oryza sativa*, and *Panicum miliaceum* seedlings)	Has over 1,000-fold specificity for TOR compared to other kinases	30, 127, 147
Concanamycin A	Vacuolar H^+-ATPase inhibitor	100 nM (*N. tabacum* suspension culture cells); 1 μM (*A. thaliana* seedlings; *O. sativa* leaves; *Zea mays* roots)	Stabilizes autophagic bodies in the vacuole, aiding their detection; bafilomycin A can also be used	71, 105, 122, 166, 190
E64c/E64d	Cysteine protease inhibitors	10 μM (*N. tabacum* suspension culture cells); 100 μM (*A. thaliana* seedlings; *Hordeum vulgare* roots)	Stabilizes autophagic bodies in the vacuole, aiding their detection	67, 128, 129
LY294002	Phosphatidylinositol-3-kinase inhibitor	30 μM (*A. thaliana* seedlings; 100 μM (*N. tabacum* suspension culture cells; *Vicia faba* leaves)	Considered a more specific inhibitor than wortmannin; may have pleiotropic effects related to nonautophagic roles of the phosphatidylinositol-3-kinase complex	77, 101, 164, 165
Rapamycin	TOR kinase inhibitor	500 nM (*Chlamydomonas reinhardtii*); 10 μM (*Arabidopsis* seedlings)	Initially thought to be inactive in plants, but recent data suggest this is not the case	21, 185
Trehalose-6-phosphate	SnRK1 inhibitor	100 μM (*A. thaliana* seedlings)	May have pleiotropic effects related to nonautophagic roles of SnRK1	158, 194
Torin1/Torin2	TOR kinase inhibitors	250–1,000 nM (*A. thaliana* seedlings); 10 μM (*L. japonicas*, *N. benthamiana*, *O. sativa*, and *P. miliaceum* seedlings)	Considered less specific inhibitors than AZD8055 but still have over 300-fold specificity for TOR compared to other kinases	30, 127, 154

(*Continued*)

Table 1 (*Continued*)

Inhibitor name	Mechanism of action	Effective concentrations[a]	Comments	References[b]
Wortmannin	Phosphatidylinositol-3-kinase inhibitor	1–5 μM (*A. thaliana* seedlings; *O. sativa* leaves); 10 μM (*N. tabacum* suspension culture cells)	Can also inhibit phosphatidylinositol-4-kinases at higher concentrations; may have pleiotropic effects related to nonautophagic roles of the phosphatidylinositol-3-kinase complex	71, 94, 121, 165

[a]Concentrations typically used for a particular species and/or cell type are indicated, but use may vary depending on experimental requirements. Species examined in early studies are typically indicated; use in other species may also have been reported.
[b]Original citations describing the first use of an inhibitor are included where known, but in all cases numerous other examples can be found throughout the literature.

accommodate the bulky W/F/Y and V/I/L residues (**Figure 3*b***). In silico tools are now available to predict AIMs (80, 183), but these should be experimentally validated, given the degenerate nature of this short sequence.

From interaction screens with ATG8, an expanding catalog of AIM-containing ATG8-binding proteins, many of which are likely cargo receptors, is now available (**Table 2**). Notably, some of these receptors also have affinity for Ub, which strongly intertwines ubiquitylation with autophagy (27, 116, 135, 163). As described below, these autophagic receptors provide mechanisms (often labeled with the "-phagy" suffix) to eliminate specific proteins and protein complexes, remove

Table 2 Known ATG8-interacting proteins in plants

Locus[a]	Species[b]	Protein name	Method of detection[c]	Potential cargo[d]	Function of interaction	Reference(s)
Solyc01g090240	*Solanum lycopersicum*	ADI3	Y2H	Unknown	Regulation of programmed cell death?	25
At3g61960	*Arabidopsis thaliana*	ATG1a	Y2H, BiFC	Unknown; rest of ATG1 complex or PAS?	Initiation/regulation of autophagy?	106, 162
At5g61500	*A. thaliana*	ATG3	IVB	NA	ATG8 conjugation cascade	187
At3g59950	*A. thaliana*	ATG4	Y2H	NA	ATG8 processing and deconjugation	85
At5g45900	*A. thaliana*	ATG7	Y2H, IVB	NA	ATG8 conjugation cascade	14, 187
At4g30790	*A. thaliana*	ATG11	BiFC	Unknown; rest of ATG1 complex or PAS?	Initiation/regulation of autophagy?	106
At2g45980	*A. thaliana*	ATI1	Y2H, BiFC	Plastid-localized membrane proteins, including APE1, ATS2, BAS1, FNR1, LHCA4, NPQ1, and PMP22	Degradation of plastid components in response to salt stress or fixed-carbon starvation?	62, 124

(*Continued*)

Table 2 (*Continued*)

Locus[a]	Species[b]	Protein name	Method of detection[c]	Potential cargo[d]	Function of interaction	Reference(s)
At4g00355	*A. thaliana*	ATI2	Y2H, BiFC	Unknown; likely similar to ATI1?	Unknown	62
At5g65480	*A. thaliana*	CCI1	Y2H	Unknown	Unknown	8
At2g03670	*A. thaliana*	CDC48d	Y2H	Unknown	Unknown	90
At1g01340	*A. thaliana*	CNGC10	SUS	Unknown	Unknown	11
At1g13030	*A. thaliana*	COILIN	Y2H	Unknown	Unknown	8
At5g24930	*A. thaliana*	COL4	Y2H	Unknown	Unknown	8
At2g17190	*A. thaliana*	DSK2a	Y2H, BiFC, IVB	BES1	Degradation of brassinosteroid-responsive transcription factor BES1	135
At5g06780	*A. thaliana*	EML2	Y2H	Unknown	Unknown	8
At1g29800	*A. thaliana*	FYVE2	Y2H	Unknown	Links autophagosomes to microtubules?	8
At3g43230	*A. thaliana*	FYVE3	Y2H, BiFC	Unknown	Links autophagosomes to microtubules?	F. Li & R.D. Vierstra, unpublished manuscript
At2g20570	*A. thaliana*	GLK1	Y2H	Unknown	Unknown	8
At5g16150	*A. thaliana*	GLT1	SUS	Unknown	Unknown	11
NM_001325148	*Nicotiana tabacum*	Joka2	Y2H, IVB	Unknown; likely similar to AtNBR1?	Unknown	200
At4g39050	*A. thaliana*	KIN7.4	Y2H	Unknown	Unknown	8
At4g24690	*A. thaliana*	NBR1	Y2H, BiFC, IVB	Ubiquitylated protein aggregates; cauliflower mosaic virus P4 protein	Degradation of heat-induced protein aggregates and virus particles	51, 163, 195
At2g02040	*A. thaliana*	NTR1/ PTR2	SUS	Unknown	Unknown	8, 11
At1g03000	*A. thaliana*	PEX6	BiFC	Unknown; likely peroxisomes?	Degradation of peroxisomes?	183
At2g23650	*A. thaliana*	PEX10	BiFC	Unknown; likely peroxisomes?	Degradation of peroxisomes?	183
PITG_09316 and PGSC0003DMP400038670	*Phytophthora infestans* and *Solanum tuberosum*	PexRD54	GF, ITC, SPR, Co-IP	Unknown, likely none; interferes with host autophagy	Outcompetes Joka2; stimulates degradation of defense-related compound(s) or nutrient remobilization?	23, 115
At4g38630	*A. thaliana*	RPN10	Y2H, BiFC, IVB	26S proteasomes	Degradation of inactive 26S proteasomes	116

(*Continued*)

Table 2 (*Continued*)

Locus[a]	Species[b]	Protein name	Method of detection[c]	Potential cargo[d]	Function of interaction	Reference(s)
At4g34660	*A. thaliana*	SH3P2	Y2H, Co-IP	Unknown	Regulates autophagosome formation	199
At2g02860	*A. thaliana*	SUC3	SUS	Unknown	Unknown	11
At2g47770	*A. thaliana*	TSPO	Co-IP	PIP2;7 and other aquaporins; porphyrins	Degradation of aquaporins upon osmotic stress?	50, 171
At1g19450	*A. thaliana*	At1g19450	SUS	Unknown	Unknown	11
At2g35900	*A. thaliana*	At2g35900	Y2H	Unknown	Unknown	8
At2g46550	*A. thaliana*	At2g46550	Y2H	Unknown	Unknown	8
At4g15930	*A. thaliana*	At4g15930	Y2H	Unknown	Unknown	8
At4g23030	*A. thaliana*	At4g23030	SUS	Unknown	Unknown	11
At5g07730	*A. thaliana*	At5g07730	Y2H	Unknown	Unknown	8
At5g40690	*A. thaliana*	At5g40690	Y2H	Unknown	Unknown	8

[a]Locus identifiers are from the *Arabidopsis* Information Resource (**http://www.arabidopsis.org**), Phytozome (**http://www.phytozome.net**), or the Sol Genomics Network (**http://www.solgenomics.net**).
[b]Only species in which an interaction was first described are listed. Subsequent descriptions of the same interaction in additional species are not included.
[c]Abbreviations: BiFC, bimolecular fluorescence complementation; Co-IP, co-immunoprecipitation; GF, gel filtration; ITC, isothermal titration calorimetry; IVB, in vitro binding; PAS, pre-autophagosomal structure; SPR, surface plasmon resonance; SUS, split ubiquitin system; Y2H, yeast two-hybrid.
[d]NA, not applicable (proteins interact with ATG8 as part of its conjugation cascade; interactions of ATG8 with ATG5, ATG12, ATG13, and ATG16 that occur in *Saccharomyces cerevisiae* have not yet been reported in plants).

protein aggregates, degrade organelles, and possibly even clear invading pathogens (**Figure 5**). As some components of the pre-autophagosomal structure contain AIMs (ATG1, ATG3, and ATG11), the resulting interactions with ATG8 also help downregulate autophagy through self-digestion (106, 162).

Aggrephagy

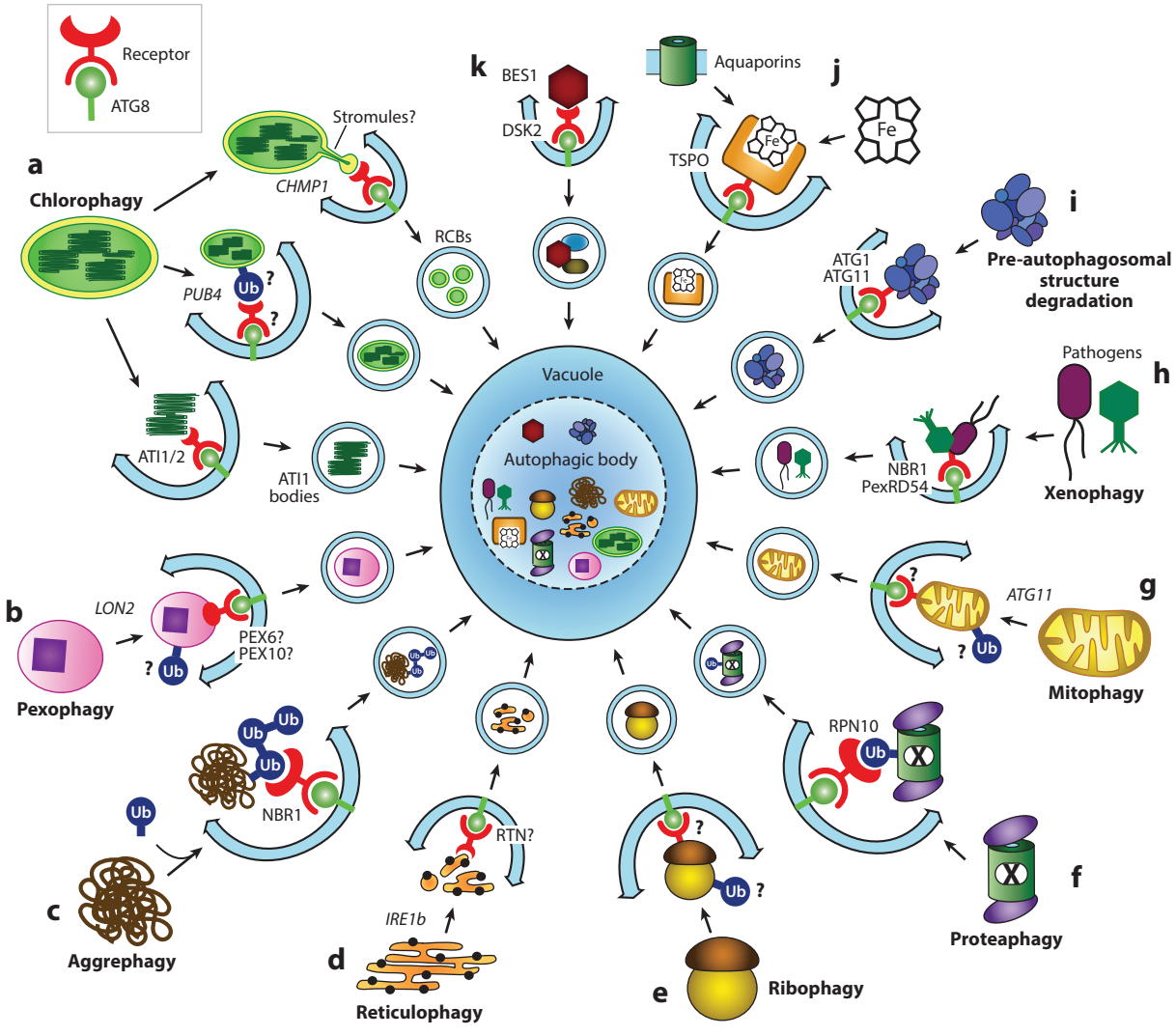
An essential aspect of protein homeostasis is the sequestration of nonfunctional and potentially cytotoxic proteins away from the cellular milieu, which is often achieved by their accumulation in distinct, spatially organized aggregates (157). These aggregates are sufficiently large to prevent their clearance by proteasomes and are instead degraded by a specialized autophagic route termed aggrephagy, which often exploits Ub as the signal. In fact, the first examples of autophagy receptors were mammalian p62/SEQUESTOSOME 1 (SQSTM1) and NEIGHBOR OF BRCA 1 (NBR1), which are responsible for the bulk of aggrephagy (89, 140). Subsequent studies identified yeast Cue5 as their functional equivalent (114). All of these receptors simultaneously bind ATG8 via an AIM and poly-Ub chains via an assortment of domains with strong affinity for Ub.

Similar forms of aggrephagy exist in plant cells, and accordingly ablation of autophagy and/or the corresponding aggrephagy receptors allow for the accumulation of large cytoplasmic aggregates presumably awaiting clearance (195). Sequence homologs of NBR1 and possibly Cue5 are evident in plants (163, 200; R.S. Marshall & R.D. Vierstra, unpublished manuscript), but counterparts for p62/SQSTM1 have not been found. However, like mammalian p62/SQSTM1, plant NBR1 proteins contain an N-terminal PB1 domain to promote receptor oligomerization,

possibly as a way to enhance aggregate binding (163, 180). Both *Arabidopsis* and tomato (*Solanum lycopersicum*) mutants lacking NBR1 have increased sensitivity to a spectrum of abiotic challenges, including heat and oxidative stress, which would be expected to accelerate protein misfolding, and they hyperaccumulate ubiquitylated protein aggregates in response to these treatments (195, 196). The observations that NBR1 protein levels increase substantially in *Arabidopsis* autophagy-defective mutants and that NBR1 enters vacuolar puncta along with ATG8 indicate that this receptor travels along with its aggregated cargo into the vacuole and is concomitantly consumed (163).

Chlorophagy

As the definitive photoautotrophic organelle, chloroplasts harbor the photosynthetic machinery and perform other essential metabolic functions (75). Chloroplast turnover through chlorophagy



(Caption appears on following page)

Figure 5 (*Figure appears on preceding page*)

Schematic representation of various selective autophagy pathways known in plants. The autophagic degradation routes for organelles, protein complexes, protein aggregates, and pathogens are shown (counterclockwise from top left), and unique features of each are highlighted. (*a*) Chlorophagy. Degradation of chloroplasts occurs through piecemeal-type degradation of stromal fragments in Rubisco-containing bodies (RCBs) during senescence or nutrient starvation, the formation of which may be mediated by ESCRT components such as CHMP1; through engulfment of whole chloroplasts in response to oxidative damage, possibly mediated by PUB4-dependent ubiquitylation; or through formation of ATI1/2 bodies containing thylakoid proteins. (*b*) Pexophagy. Degradation of peroxisomes occurs in young seedlings upon the onset of photomorphogenesis and in response to oxidative damage. No pexophagy receptors have yet been described in plants, although the LON2 chaperone likely plays a role in peroxisome stress sensing, whereas PEX6 and PEX10 interact with ATG8. (*c*) Aggrephagy. Degradation of intracellular protein aggregates that arise spontaneously or following abiotic perturbations that impair protein folding. Aggrephagy is triggered by aggregate ubiquitylation followed by binding of autophagy receptors such as NBR1 or Joka2. (*d*) Reticulophagy. Degradation of the endoplasmic reticulum occurs in response to the accumulation of unfolded proteins upon ER stress and depends on the IRE1b stress sensor. Reticulophagy receptors from the reticulon homology domain (RTN)–containing family have been described in mammals and yeast, but not yet in plants. (*e*) Ribophagy. Autophagic degradation of ribosomes has been implied by increased ribosomal protein levels in autophagy mutants and by the accumulation of ribosomal RNA (rRNA) in the vacuoles of an *rns2-2* mutant. RNS2 is a vacuolar RNase T2 enzyme required for the decay and recycling of rRNA in plants. (*f*) Proteaphagy. Autophagic degradation of proteasomes occurs in response to nitrogen starvation or proteasome inactivation. Inhibited proteasomes become ubiquitylated and are delivered to the expanding phagophore by the autophagic receptor RPN10. (*g*) Mitophagy. Degradation of mitochondria is induced during senescence. Although no proteins targeting plant mitochondria for autophagic clearance have been identified, ATG11 has been implicated. (*h*) Xenophagy. Numerous examples of intracellular pathogens being degraded by autophagy, such as NBR1-mediated elimination of the cauliflower mosaic virus P4 protein, in plants have been reported. Additionally, pathogens themselves can secrete effectors that interfere with the host autophagy machinery (e.g., PexRD54 from *Phytophthora infestans*). (*i*) Pre-autophagosomal structure degradation. Several pre-autophagosomal structure proteins, including the ATG1 and ATG11 subunits of the ATG1 kinase complex, contain ATG8-interacting motifs (AIMs). Degradation of the pre-autophagosomal structure provides a feedback mechanism to negatively regulate autophagic induction. (*j*, *k*) Selective autophagy of individual proteins. (*j*) Degradation of TRYPTOPHAN-RICH SENSORY PROTEIN (TSPO) by selective autophagy has been proposed as a mechanism to eliminate highly reactive porphyrin molecules and to control water transport by the selective removal of tonoplast and plasma membrane aquaporins in response to osmotic stress. (*k*) Additionally, the brassinosteroid-responsive transcription factor BES1 is degraded by autophagy upon ubiquitylation and delivery to the phagophore by DSK2, with binding of DSK2 to ATG8 regulated by BIN2-mediated phosphorylation around its AIMs. Adapted with permission from Reference 119. Other abbreviation: Ub, ubiquitin.

not only provides a quality control mechanism to eliminate nonfunctional chloroplasts, but also provides a major reservoir of nitrogen and fixed carbon that can be remobilized during senescence and starvation (57). Several types of chlorophagy operate, depending on the state of the chloroplast or nutritional needs (**Figure 5**). The most dramatic example is the encapsulation of entire chloroplasts into ATG8-decorated autophagic vesicles and their subsequent delivery to the vacuole via a route dependent on ATG8 lipidation (72). This process can be stimulated by strong photo-oxidative damage elicited by either UVB or high-intensity visible light, with the engulfed chloroplasts displaying a disorganized thylakoid structure and collapsed outer envelopes that are indicative of distress (72).

A link between Ub and this form of chlorophagy is possible on the basis of recent findings that mutants which accumulate the chlorophyll precursor protoporphyrinogen IX rapidly turn over chloroplasts in the light (179). A subsequent suppressor screen identified a key role for the cytosolic Ub ligase PLANT U-BOX PROTEIN 4 (PUB4), which ubiquitylates the chloroplast surface. No direct targets of PUB4 have been identified, but other studies have confirmed the ubiquitylation of chloroplast outer envelope proteins (109). A role for autophagy has not been demonstrated, but accumulation of protoporphyrinogen IX may stimulate excess reactive oxygen species (ROS) accumulation and chloroplast photodamage similar to those occurring under UVB or intense white light, which may then trigger chlorophagy (72).

Given that as much as 70–80% of leaf protein resides in chloroplasts, their breakdown during senescence offers a rich source of remobilized nitrogen to developing vegetative tissues and seeds

(57). The highly abundant Rubisco, in particular, is degraded rapidly via Rubisco-containing bodies. These small spherical structures become visible in the cytoplasm and vacuole during the early stages of senescence and contain stromal proteins such as Rubisco and glutamine synthetase, but not chloroplast envelope or thylakoid proteins (68). Upon treatment of *Arabidopsis* leaves with ConA, Rubisco-containing bodies decorated with GFP-ATG8 became abundant in vacuoles in a manner dependent on the autophagy machinery, thus confirming their identity as autophagic bodies (68, 73). Not surprisingly, Rubisco-containing body appearance is tightly linked to leaf carbon status. Their formation is suppressed by exogenous metabolic sugars (glucose, fructose, and sucrose), but not by inorganic nitrogen. They are largely absent in *Arabidopsis* mutants producing excess starch, but accumulate in increased numbers in several starchless mutants (73).

Rubisco-containing body: a small vesicle containing only stromal components; it pinches off from the chloroplast and enters the autophagy pathway during senescence

How Rubisco-containing bodies form is not yet clear. One possibility is that they arise by scission of stroma-filled tubules termed stromules that frequently protrude from plastids, using an ESCRT-type trafficking route. In particular, mutants affecting the ESCRT component CHARGED MULTI-VESICULAR BODY PROTEIN 1 (CHMP1) display aberrant stromule morphology; accumulate Rubisco-containing bodies; and degrade chloroplast proteins more slowly, including a synthetic chloroplast protein subject to autophagy (159). Small cup-shaped vesicles decorated with GFP-ATG8 and surrounding the stromule tips can be seen, which may predate the appearance of Rubisco-containing bodies.

A third route for chlorophagy involves two plant-specific proteins, termed ATG8-INTERACTING PROTEIN 1 (ATI1) and ATI2, which localize on the basis of GFP fusions to plastids and the ER, and to plastid-associated bodies that bud off into the cytoplasm during carbon starvation or salt stress (62, 124). Like Rubisco-containing bodies, delivery of these ATI1-decorated plastid bodies to the vacuole requires a functional autophagy system, but the cargo appears distinct. Whereas Rubisco-containing bodies exclusively contain stromal proteins, the ATI1-decorated bodies house plastid membrane proteins from either the outer envelope or thylakoids. Given that ATI1 also binds ATG8 through an AIM, it may act as a specialized chlorophagy cargo receptor (124).

Mitophagy

Mitochondria are the primary organelles responsible for energy generation in eukaryotes via the tricarboxylic acid cycle and oxidative phosphorylation. In addition, mitochondria play crucial roles in intracellular signaling, stress responses, and regulation of cell death (74). As such, maintenance of a healthy mitochondrial population is imperative, especially as mitochondria are a major source of ROS that can lead to oxidative damage if unsupervised.

The autophagic turnover of mitochondria (mitophagy) is best described in mammals, where ubiquitylation plays a prominent role via the Ub ligase PARKIN and its regulatory kinase PTEN-INDUCED PUTATIVE KINASE 1 (PINK1) (188). When mitochondria are damaged, PINK1 becomes stabilized on the mitochondrial surface, where it recruits and activates PARKIN to then rapidly ubiquitylate a myriad of mitochondrial outer membrane proteins. These ubiquitylated species recruit a collection of AIM-containing autophagy receptors such as NDP52, OPTN, and TAX1BP1, which tether the ubiquitylated mitochondria to the expanding phagophore (188). Several AIM-containing intrinsic mitochondrial proteins, including yeast Atg32, have also been connected to mitophagy (81, 138).

By contrast, little is known mechanistically about plant mitophagy, and almost all the major players crucial to animal and yeast mitophagy, including PARKIN, PINK1, Atg32, and the collection of mitophagy receptors, have no clear orthologs in plants. However, conditions that accelerate autophagy (fixed-carbon starvation and senescence) lead to a drop in mitochondria numbers in

Arabidopsis (83), and the degradation of mitochondrial proteins such as COXII and VDAC is blocked in autophagy-defective mutants (106). Mitophagy can also be seen cytologically; the fluorescent mitochondrial reporters CIB22-GFP and Mito-YFP track into vacuolar puncta decorated with mCherry-ATG8 (106). Prior studies with yeast implicated the ATG1 kinase subunit ATG11 in mitophagy (81); in concordance, Li et al. (106) showed that *Arabidopsis atg11* mutants have dampened mitophagy and weak autophagy-related phenotypes.

Pexophagy

Plant peroxisomes house various metabolic reactions, including the glyoxylate cycle, several steps in photorespiration, and the β-oxidation of fatty acids and hormone precursors (192). The oxidative nature of their metabolism predisposes peroxisomes to self-inflicted ROS damage, necessitating mechanisms for peroxisome disposal if sufficiently compromised. Additionally, the metabolic requirements of plant peroxisomes change throughout development, thus requiring the removal of obsolete peroxisome components (192). For example, germinating seeds, which depend on fatty acid β-oxidation and the glyoxylate cycle to metabolize stored lipids, develop glyoxysomes; they are specialized peroxisomes that are enriched in glyoxylate cycle enzymes such as isocitrate lyase and malate synthase. As seedlings age, peroxisomes eliminate these enzymes in favor of those required for photorespiration (110). Although part of this remodeling is driven by peroxisome matrix proteases such as LON2, genetic suppressor screens of an *Arabidopsis lon2-2* mutant also identified components of the autophagic machinery (ATG2, ATG3, and ATG7) as being important (31, 44).

In addition to developmental remodeling, pexophagy is also important for peroxisome quality control. Under nonstressed conditions, pexophagy likely limits plant peroxisome abundance, based on the observation that *Arabidopsis* autophagy mutants have increased peroxisome numbers relative to wild type (88, 155). Additionally, both carbon-starved and rapidly dividing tobacco cells hyperaccumulate peroxisomes when treated with the autophagy inhibitor 3-methyladenine (173). The co-localization of a peroxisome reporter (CFP tagged at its C terminus with the three-amino-acid peroxisome targeting motif, Ser-Lys-Leu) with GFP-ATG8 in vacuolar puncta in wild-type but not autophagy-defective *Arabidopsis* seedlings confirmed a role for autophagy (88). Although the abundance of these peroxisome-containing autophagic bodies is elevated during fixed-carbon starvation, they are reasonably abundant in nonstressed cells, implying that damaged peroxisomes continually appear and need to be recycled (88, 155, 173).

How peroxisomes are marked for turnover is not yet clear in plants. The chaperone function of LON2 can suppress pexophagy, suggesting that misfolded or aggregated matrix proteins may be signals (44). Such a signal would presumably traverse the peroxisome membrane and be recognized by a cytosolic pexophagy receptor. In yeast, two AIM-containing pexophagy receptors (Atg30 and Atg36) become attached to peroxisome surface proteins such as Pex3, Pex5, or Pex14 (33). Atg30 and Atg36 then recruit the autophagic machinery by interacting with Atg8 and Atg11 (32). Mammals do not have Atg30 or Atg36 but instead use p62/SQSTM1 or NBR1 as pexophagy adaptors that bind ubiquitylated forms of PEX5 or PMP70 (153, 193). Although plant peroxisome proteins are targets of ubiquitylation, plants do not have obvious orthologs of either Atg30 or Atg36, and there is no evidence that plant NBR1 is involved. However, the peroxisome proteins PEX6 and PEX10 were recently shown to interact with ATG8 via an AIM, suggesting that they could drive pexophagy (183).

Reticulophagy

The ER is the most abundant membranous structure within eukaryotic cells and is the entry point for the secretory system, where it directs the biosynthesis, folding, and delivery of most

secreted and membrane proteins. In adverse environments, the protein folding capacity of the ER can be overwhelmed, resulting in ER stress (63). As a first line of defense, cells induce (*a*) the unfolded protein response (UPR) to increase protein folding capacity within the ER and (*b*) the ER-associated protein degradation (ERAD) pathway to translocate misfolded luminal proteins back to the cytoplasm for clearance by the Ub-proteasome system. Under more extreme situations, compromised ER is degraded by a selective autophagic process termed reticulophagy (113, 189). ER stress–triggered reticulophagy in *Arabidopsis* is induced by hyperaccumulation of unfolded proteins and subsequent activation of the UPR factor INOSITOL-REQUIRING ENZYME 1b (IRE1b), a bifunctional kinase/ribonuclease (112). However, reticulophagy is independent of the alternative UPR factor bZIP28 and the downstream IRE1b splicing target bZIP60, implying that the reticulophagy and UPR signaling pathways diverge after IRE1b (63, 113).

It is currently unknown whether the kinase and/or ribonuclease activities of IRE1b are required for reticulophagy induction, nor is it clear whether plants have dedicated autophagy receptors for recruiting ER. Yeast employs Atg39 and Atg40 to mediate degradation of perinuclear ER and cortical ER, respectively (126), whereas in mammals, RETICULON 3 (RTN3) acts upon ER tubules and an Atg40 homolog, FAM134B, participates in the degradation of ER sheets (47, 86). Atg40, FAM134B, and RTN3 are members of the reticulon homology domain (RHD)-containing family, of which there are 21 possible relatives in *Arabidopsis* (136). Overexpression of *Arabidopsis* RTN isoforms disrupts ER morphology, as occurs during mammalian reticulophagy (47, 168), whereas maize mutants missing the RTN1 and RTN2 isoforms display reticulophagy defects (X. Zhang, X. Ding, R.S. Marshall, R.D. Vierstra & M.S. Otegui, unpublished manuscript). Consequently, further study of the RHD protein family might shed light on ER turnover in plants.

Ribophagy

The peptidyl transferase reaction of protein synthesis is catalyzed by the 80S ribosome, which is composed of the 40S small and 60S large subunits that collectively contain four ribosomal RNAs (rRNAs) and at least 80 proteins (9). Given that the biogenesis of ribosomes and subsequent protein translation are among the most energy consuming cellular processes, it is not surprising that eukaryotes employ a form of autophagy termed ribophagy to dampen translation and restore amino acid pools when limiting (1, 91). In fact, yeast ribosomes are degraded faster than other cytosolic proteins upon nitrogen starvation, implying a degree of specificity. Surprisingly, the 40S and 60S ribosomal subunits are separately targeted to the vacuole by distinct mechanisms, with the deubiquitylating enzyme Ubp3 and its cofactor Bre5 being essential for degradation of the 60S, but not the 40S, particles (91). Two Ub ligases have also been implicated, with Rsp5 being a positive regulator and Ltn1 being a negative regulator and antagonistic to Ubp3 (93, 139). Interestingly, Ubp3 is also important for the yeast mitophagy and proteaphagy pathways (130, 175, 202) and hence may participate in several selective autophagic routes regulated by Ub.

To date, the study of ribophagy in plants has focused principally on rRNA turnover. Autophagic degradation of *Arabidopsis* rRNA requires the T2 ribonuclease RNS2, with the *rns2-2* mutant accumulating rRNA inside the vacuole (34, 35, 59). The *rns2-2* seedlings also exhibit constitutive autophagy, leading to the hypothesis that RNS2 participates in a ribophagy-like route that degrades ribosomes as a housekeeping function (4). Surprisingly, rRNA turnover in *Arabidopsis* depends on ATG5 but not on ATG9 (34), suggesting that a unique form of autophagy is engaged, possibly either not involving phagophores (such as microautophagy) or restricted to unwanted rRNA (4). Whether ribosomal proteins are also subject to autophagic clearance, and whether Ub participates, remain to be demonstrated. Notably, *Arabidopsis* encodes counterparts of Ubp3, Rsp5, and Ltn1 that may be involved (R.S. Marshall & R.D. Vierstra, unpublished manuscript).

Proteaphagy

Through its ability to degrade ubiquitylated proteins via the Ub-proteasome system, the proteasome controls much of eukaryotic cell physiology, and consequently its activity and abundance are regulated at multiple levels, including transcriptionally (43), during assembly (64; R.S. Marshall, D.C. Gemperline & R.D. Vierstra, unpublished manuscript), and by various posttranslational modifications (6, 17). Recent studies showed that proteasome levels are also controlled by autophagy. This proteaphagy was first demonstrated in *Arabidopsis*, in which two routes were identified: one induced by nitrogen starvation and regulated by the ATG1 kinase, which may reflect a bulk starvation route, and another sensitive to proteasome inactivation (either by mutation or with proteasome inhibitors such as MG132) but independent of ATG1 (116, 118). Proteasome inhibition is accompanied by extensive ubiquitylation of the complex (6, 87, 116), which was speculated to permit recognition by an autophagy receptor with affinity for Ub. Surprisingly, the receptor was not NBR1 but was instead shown to be the Ub-binding proteasome subunit RPN10, which had previously been known to exist in a free form as well as part of the proteasome. In its proteaphagic role, RPN10 simultaneously binds (*a*) ubiquitylated proteasomes through a Ub-interacting motif (UIM) and (*b*) ATG8 via a second UIM-related sequence that is distinct from the canonical AIM (116) (**Figure 5**). Accordingly, inhibitor-induced proteaphagy was abolished in an *Arabidopsis* mutant expressing an RPN10 truncation missing the C-terminal region containing these UIMs (116).

Proteaphagy was subsequently described in both yeast (117, 132, 175) and mammals (15). As in *Arabidopsis*, yeast employs both starvation- and inhibitor-induced routes, with the inhibitor-induced route similarly accompanied by extensive proteasome ubiquitylation. However, yeast and human orthologs of RPN10 lack the UIM-related Atg8-binding motif and cannot bind Atg8, leading to the conclusion that an alternative proteaphagy receptor participates. Genetic analysis of the other known autophagy receptors in yeast, including ones that might bind Ub as well as Atg8, led to the discovery that Cue5 is employed instead (117). Unlike *Arabidopsis* RPN10, Cue5 and its functional human ortholog Tollip house both a canonical AIM that binds Atg8 and a CUE domain that recognizes Ub (114, 117). Proteaphagy thus represents an excellent example of convergent evolution in which different motifs (UIM-related versus AIM for ATG8 binding and UIM versus CUE for Ub binding) are co-opted for the same cellular outcome, in this case degradation of inactive proteasomes.

Because most proteasomes are localized in the nucleus, whereas autophagic engulfment likely occurs in the cytoplasm, mechanisms must exist to recognize inactive proteasomes, to promote their nuclear export, and finally to drive their ubiquitylation for subsequent autophagic recognition. As yeast proteasomes rapidly coalesce upon inactivation into puncta that resemble those containing cytoplasmic aggregates awaiting aggrephagy (117), an intriguing hypothesis is that this proteaphagy uses the same machinery. In support, the Hsp42 chaperone that sequesters misfolded proteins during aggrephagy (46, 157) is also required for the aggregation of inactivated proteasomes prior to autophagy (117). Clearly, the modification of proteasomes with Ub is a key step in inhibitor-induced proteaphagy; thus, finding the E3 Ub ligases that drive this ubiquitylation, knowing when and where during proteaphagy these ligases are active, and identifying the proteasome subunits that they modify are needed to fully appreciate the process. An intriguing side reaction is the aggregation of yeast and *Arabidopsis* proteasomes into cytoplasmic storage granules that protect these proteasomes from proteaphagy during fixed-carbon starvation (202). These granules likely arise via phase separation (36), and their formation acts to promote cell fitness by providing a cache of proteasomes that can be rapidly remobilized when carbon availability improves. Other protein complexes and/or organelles may have similar rescue strategies to delay elimination.

Xenophagy: Roles for Autophagy in Pathogen Defense

Plants are constantly exposed to a variety of bacterial, fungal, and viral pathogens, with often deadly consequences, and as a result have evolved sophisticated innate immune responses to protect themselves from attack (7, 22). For biotrophic pathogens that parasitize living tissue, perception of conserved pathogen-associated molecular patterns (PAMPs) by surface receptors activates basal defenses known as PAMP-triggered immunity (PTI). Adapted pathogens interfere with PTI by secreting effectors that block this process; these effectors are then recognized by a collection of host resistance proteins that initiate a second layer of defense termed effector-triggered immunity (ETI). ETI often culminates in a HR, seen as localized PCD at the infection site (7, 22). Less is known about plant immune responses against necrotrophic pathogens, which actively kill host cells before colonizing them.

In recent years it has become increasingly evident that selective autophagic routes, which are collectively termed xenophagy, aid in pathogen defense using either pro-death or pro-survival strategies (60). One of the first studies (112) showed that infection of *Nicotiana benthamiana* leaves with either *P. infestans* or *Pseudomonas syringae* pv. *tomato* DC3000 normally initiates a HR that is tightly restricted to the site of infection, but that this HR uncontrollably spreads to uninfected cells when core autophagy components (ATG3, ATG6, ATG7, and VPS34) are silenced, implying that autophagy is pro-survival (112). In contrast, *Arabidopsis* mutants deficient in autophagy were subsequently shown to have an attenuated PCD response upon infection with *P. syringae* harboring the avirulence gene *AvrRPS4*, suggesting that autophagy is pro-death in this situation (61). The relative contribution of autophagy to PCD appeared to depend on the type of immune receptor involved in effector recognition; PCD triggered by some receptors was independent of autophagy, whereas PCD triggered by other receptors was strictly dependent on autophagy (61). Autophagy also negatively regulates *Arabidopsis* immunity against powdery mildew fungal species, with autophagy-defective mutants showing enhanced resistance to *Golovinomyces cichoracearum* (178). Furthermore, *Arabidopsis* plants with a mutation in ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM 1 (AMSH1), which encodes a deubiquitylating enzyme required for autophagic degradation, were more resistant to the powdery mildew pathogen *Erysiphe cruciferarum* (82). Thus, autophagy plays both a positive and a negative role in basal immunity against bacterial, fungal, and oomycete species.

Unlike the variable results with biotrophic pathogens, several studies provided compelling evidence that autophagy plays a positive role in plant resistance to necrotrophic pathogens. Expression of many autophagy-related genes and accumulation of autophagosomes in *Arabidopsis* can be induced by infection with the fungal pathogen *Botrytis cinerea* (98). Furthermore, when autophagy-defective plants were inoculated with *B. cinerea* and other necrotrophic pathogens, they developed more extensive symptoms and supported more pathogen growth than did wild-type plants (82, 98, 103).

Given the perpetual arms race between plants and their pathogens, it is perhaps not surprising that successful microbes have also evolved sophisticated strategies to modulate autophagy for their benefit. For example, the necrotrophic pathogen *Sclerotinia sclerotiorum* enhances infection by secreting the phytotoxin oxalic acid to suppress autophagy (78). By contrast, other pathogens actually induce autophagy as part of their infection strategy. The secreted effector AWR5 from the bacterium *Ralstonia solanacearum* inhibits TOR, possibly as a mechanism to help feed the pathogen by upregulating autophagy (145). *P. syringae* also induces autophagy by NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent activation of salicylic acid signaling (191), and it can specifically stimulate the proteaphagy pathway by secreting the HopM1 effector that binds proteasomes (169). Another example comes from the *P. infestans* effector protein

PexRD54, which recognizes potato ATG8 through an AIM (115) (**Figure 3*b***). PexRD54 appears to enhance autophagosome assembly, possibly as a method to redistribute nutrients in favor of the pathogen (23). It also outcompetes binding of ATG8 with the tobacco NBR1-type autophagic cargo receptor Joka2, thus potentially blocking autophagic clearance of plant or pathogen proteins that negatively impact immunity (23).

As in animals, autophagy is also emerging as a central mechanism to suppress viral infection. In an early example, *N. benthamiana* plants were found to be more susceptible to tobacco mosaic virus infection when components of the autophagy machinery were silenced (112). Subsequently, it was shown that the tobacco calmodulin-like protein rgsCaM counteracts infection from viruses by inhibiting viral suppression of RNA silencing through autophagic breakdown of viral proteins such as cucumber mosaic virus 2b protein or turnip mosaic virus HCpro (52, 131). Autophagy in *N. benthamiana* also directs clearance of the virulence protein βC1 from cotton leaf curl Multan virus through its interaction with ATG8; ablation of this interaction prevents βC1 degradation and enhances disease symptoms (58). *N. benthamiana* silenced for ATG5 and ATG7 displayed accentuated leaf curling and increased viral DNA levels when infected by two other members of the Geminiviridae family (58), whereas the converse was observed when autophagy was activated through downregulation of cytosolic glyceraldehyde-3-phosphate dehydrogenase (53). Similar findings were reported for *Arabidopsis* infected with cauliflower, turnip or watermelon mosaic viruses; following infection, stronger disease symptoms were more evident in autophagy-deficient mutants (including *nbr1-2*) than in wild type (51, 52). The mutant plants also accumulated higher levels of cauliflower mosaic virus P4 protein, the major structural protein from this virus, which interacts directly with NBR1 without ubiquitylation, suggesting that NBR1-dependent clearance of P4 protects *Arabidopsis* during the early stages of viral infection (51).

Not surprisingly, viruses have also evolved mechanisms to counteract the host autophagy machinery (201) or to co-opt it by neutralizing frontline antiviral defenses such as RNAi-mediated gene silencing. The best example of the latter is the P0 protein from poleroviruses, which functions as a substrate-recognition F-box subunit of an SKP1-cullin-F-box (SCF) Ub ligase (5). This ligase ubiquitylates the core ARGONAUTE 1 (AGO1) subunit of the RNA-induced silencing complex and triggers its autophagic degradation, likely via a Ub-binding autophagy receptor (24).

One notable difference between plant and animal xenophagy is that some animal pathogens directly invade the cytoplasm, whereas plant counterparts are typically surrounded by a lipid barrier derived from the plasma membrane and are thus not directly accessible to autophagy. This situation in animals has permitted the evolution of AIM-containing autophagic receptors such as p62/SQSTM1, OPTN, NDP52, and TAX1BP1 that directly bind to the surface of the bacterial or fungal pathogen to facilitate their encapsulation (76). Although there is no evidence yet for similar xenophagy receptors in plants, such receptors may provide paradigms for engineering autophagic defenses against plant viruses as they enter the cytoplasm.

Additional Selective Autophagy Routes in Plants

Although our current appreciation of selective autophagy is centered on macromolecular complexes, organelles, and pathogens, emerging evidence indicates that autophagy can also eliminate individual proteins. Two excellent examples are the AIM-containing autophagy receptors TRYPTOPHAN-RICH SENSORY PROTEIN (TSPO) and DOMINANT SUPPRESSOR OF KAR2 (DSK2) (**Figure 5**). TSPO is a porphyrin-binding membrane protein that is expressed in response to abiotic stresses and resides within the Golgi apparatus. The accumulation of TSPO is strictly regulated, with its degradation driven by autophagy using a functional AIM (171). Degradation of TSPO was initially speculated to eliminate excess porphyrins; however, a more recent

study showed that TSPO also interacts with and facilitates the autophagic degradation of a variety of tonoplast and plasma membrane aquaporins, including PIP2;7 (50).

DSK2, in contrast, plays a role in brassinosteroid hormone signaling by targeting for degradation the transcription factor BRI1-EMS SUPPRESSOR 1 (BES1). This factor is downstream of the plasma membrane receptor kinase BRASSINOSTERIOID INSENSITIVE 1 (BRI1) that initiates brassinosteroid signaling. Both BES1 and another brassinosteroid-activated transcription factor, BRASSINAZOLE-RESISTANT 1 (BZR1), are degraded by the Ub-proteasome system upon BRI1 activation, but BES1 is also degraded by autophagy, as evidenced by the hyperaccumulation of the BES1 protein in autophagy-deficient backgrounds or upon exposure of wild-type plants to autophagy inhibitors such as ConA and E64d (135). A search for BES1 interactors discovered DSK2, an autophagy receptor containing two AIMs, a Ub-like (UBL) domain, and a Ub-associated (UBA) domain. Binding of DSK2 to ATG8 is regulated by the BRASSINOSTEROID INSENSITIVE 2 (BIN2) kinase; BIN2 transfers phosphate to residues adjacent to the AIMs, which enhances ATG8 binding (135). Ubiquitylation of BES1 by the Ub ligase SEVEN-IN-ABSENTIA 2 (SINAT2) in turn promotes binding of BES1 to DSK2, thus providing two independent regulatory loops to eliminate BES1 by autophagy (135). We expect that additional examples of individual autophagy targets will soon appear as the functions of more autophagic receptors become understood.

Selective Autophagy Helps Regulate Autophagy

As expected, a variety of starvation and stress conditions transcriptionally upregulate autophagy and, in some cases, autophagy components display co-regulation with the proteasome and other elements of the Ub-proteasome system (14, 18, 43, 56, 156, 181). One surprising feature of autophagy is its ability to auto-regulate through self-eating of key components. This step was first identified by studying the dynamics of the ATG1 kinase complex during both nitrogen and fixed-carbon starvation. Instead of seeing a rise in the levels of this integrator as autophagic demand increased, Suttangkakul et al. (162) observed the exact opposite: ATG1 and ATG13 protein levels dropped dramatically, and their levels were restored only when the plants were refed nitrogen or returned back to the light. Studies with several mutants revealed that autophagy was responsible and that inactivation of TOR might direct the process, as this degradation was accompanied by phosphorylation of ATG1 and dephosphorylation of ATG13 (162). ATG11 also participates by encouraging ATG1 phosphorylation (106). As both ATG1 and ATG11 (and their yeast and animal orthologs) contain an AIM, these ATG8-binding sequences likely connect the ATG1 complex to the phagophore, possibly along with other components of the pre-autophagosomal structure (92, 106, 162). These interactions may not only help direct pre-autophagosomal structure assembly but also promote its autophagic turnover. In agreement, GFP-labeled ATG1 complexes can be seen in ATG8-decorated autophagic bodies within vacuoles; this presence, at least for ATG11, depends on an intact AIM (106, 162). Presumably, ATG1 complex breakdown leads to suppression of starvation-induced autophagy by removing this central regulator.

PHYSIOLOGICAL AND DEVELOPMENTAL ROLES OF AUTOPHAGY

Numerous studies on a variety of autophagy-defective mutants provide a consistent picture of the roles of autophagy in plant growth and development. The most surprising is that ATG8-mediated autophagy is often not essential; although *Arabidopsis* and maize mutants null for core components display accelerated leaf senescence and reduced fecundity, they easily complete their life cycle and produce viable seeds under well-fed growth conditions. In fact, several developmental transitions for which mega-autophagy might be required proceed normally in planta, including xylem

biogenesis and other PCD events such as stomium and tapetum degeneration. In fact, only slight defects in xylem tracheary element differentiation were seen for *Arabidopsis* cell cultures missing ATG5 (97). This lack of effect is surprising given that poplar (*Populus trichocarpa*) upregulates a battery of autophagy-related genes during xylogenesis (20). Only in rice is an autophagy defect lethal, but this lethality is caused by a flaw in pollen development, and not by physiological challenges; in this context, an *atg*7 null mutant generates male sterility by compromising tapetum development (96). Reduced fecundity has also been observed in wheat (*Triticum aestivum*), but in this case accelerated growth caused by long-day photoperiods generated sugar starvation, which in turn was proposed to induce the premature autophagic cell death of florets (42).

Phenotypes typically emerge when autophagy-defective plants are grown under nutrient-deficient conditions such as inadequate supplies of nitrogen or fixed carbon; in such conditions, slow growth, enhanced senescence, lower fecundity, and reduced survival become evident (29, 54, 105, 166, 184) (**Figure 4*i,j,k***). Such assays make clear that mutants missing core components of the autophagy machinery (e.g., ATG3, ATG5, ATG7, and ATG10) are most compromised, whereas those affecting more peripheral components (e.g., the ATG1 and PI3K kinases and NBR1) are less impacted by these stresses (106, 162, 195). During leaf senescence, autophagy has opposing roles; one is to maintain longevity through nutrient recycling, while the other is to participate in the systematic degradation of tissue as nutrients are exported to areas of growth and storage (119). The latter role can be detected by ^{15}N tracing studies, which showed that mobilization of nitrogen from leaves to seeds was sharply decreased in autophagy-defective mutants of both *Arabidopsis* and maize (49, 105). A similar suppression of remobilization, in this case export of ^{15}N from senescing leaves, was seen in a rice *atg*7 mutant (174).

Reduced fecundity in autophagy-deficient plants may have two possible origins. One is the reduced remobilization of nitrogen to the filling grain (49, 105), whereas the other may reflect a lack of autophagic routes needed to deposit proteins into seed protein storage vacuoles. Initial studies in wheat—complemented by later studies in mung bean (*Vigna radiata*), pea (*Pisum sativum*), and pumpkin (*Cucurbita pepo*) seeds—revealed that direct transport of storage proteins from the ER into protein storage vacuoles involved a pathway that morphologically resembles autophagy (104, 151, 170). Electron tomography of maize aleurone cells provided evidence for an ATG8-independent autophagic route that delivers storage proteins into novel vacuoles (149), and a more recent study of *Arabidopsis* seeds found that *atg5* mutants had impaired processing of 12S globulin precursors, again implicating autophagy in the vacuolar delivery of storage proteins (26). Intriguingly, overexpression of *ATG5* or *ATG7* in *Arabidopsis*, or of different *ATG8* genes in both *Arabidopsis* and rice, increased seed yields; enhanced tolerance to drought, nitrogen starvation, and oxidative stress; and promoted resistance to necrotrophic pathogens, suggesting that boosting nutrient mobilization via autophagy could have agronomic benefits (108, 125, 182).

METABOLIC IMPACTS OF AUTOPHAGY

Although the phenotypic functions of autophagy mutants appear restricted, several metabolomics studies reveal a much more pervasive impact on plant metabolism, even under nutrient-rich growth conditions. The first such analysis compared rosette leaves from wild-type and autophagy-defective *Arabidopsis* grown under low- or high-nitrate conditions (120). Notably, the autophagy mutants accumulated fewer hexoses (glucose, fructose, mannose, and galactose) but more of their corresponding sugar alcohols (mannitol and sorbitol) and acids (gluconate, galacturonate, ribonate, and threonate), suggesting a defect in sugar redox management (48, 120). Numerous amino acids also hyperaccumulated in the autophagy-deficient mutants, including glutamate; aspartate; methionine; phenylalanine; branched-chain amino acids such as valine, leucine, and isoleucine; and

shikimate, which is the precursor of aromatic amino acids and a starting point for salicylic acid biosynthesis. Such accumulation implies that amino acid catabolism is modified, which may explain why autophagy mutants hyperaccumulate nitrogen-containing compounds such as ammonium and amino acids in their rosette leaves (48, 120). Indeed, shikimate accumulation was the most robust indicator of autophagy deficiency and was observed regardless of genetic background and nitrogen availability. Additionally, transcriptomic data showed that the pathways for glutathione, methionine, raffinose, and galacturonate production were perturbed (120). Unexpectedly, anthocyanin accumulation was also depressed in the *Arabidopsis* autophagy mutants; although such accumulation was first considered to reflect defects in the autophagic import of these pigments into vacuoles (10, 146), its root cause now appears related to an enigmatic connection between autophagy and the transcription of genes required for anthocyanin biosynthesis (120).

A second major study investigated etiolated *Arabidopsis* seedlings germinated without sucrose as a model for fixed-carbon starvation during seedling establishment. In this case, metabolic profiling in autophagy-deficient mutants uncovered reduced levels of free amino acids, in particular isoleucine, lysine, and valine, along with reduced steady-state levels of the organic acids malate, fumarate, and dehydroascorbate (2). Metabolic flux analysis suggested increased respiration and decreased net protein biosynthesis in the autophagy-deficient mutants relative to wild type. The lipid composition of the mutants also changed, with increases in triacylglycerols and free fatty acids being particularly evident in *atg5* mutants. Increased phosphatidylinositol levels were also seen in all autophagy mutants, possibly indicating an attempt to compensate for the lack of autophagy by providing more substrate for the VPS34 PI3K complex that promotes phagophore assembly (2).

A more recent metabolomics study comparing a maize *atg12* mutant with wild type grown under both nitrogen-rich and nitrogen-deficient conditions found dramatic increases in free fatty acids (e.g., palmitate and linoleate) and oxylipins (e.g., 9- and 13-hydroxyoctadecadienoic acid and 13-hydroxyoctadecatrienoic acid), implying strong perturbations in lipid metabolism in the absence of autophagy (F. McLoughlin, R.C. Augustine, R.S. Marshall, F. Li & R.D. Vierstra, unpublished manuscript). Such changes may reflect the enhanced turnover of lipids to supply the acetyl-CoA needed for β-oxidation and subsequent respiration and/or a heretofore undocumented role of autophagy in plant membrane turnover. The maize study also examined the proteome profile of *atg12* plants under normal and nitrogen-starved conditions. Increased levels of ribosomal, proteasomal, mitochondrial, and peroxisomal proteins were evident in the *atg12* plants relative to wild type, especially under nitrogen deficiency, thus linking starvation-induced autophagy with the bulk elimination of specific complexes and/or organelles (F. McLoughlin, R.C. Augustine, R.S. Marshall, F. Li & R.D. Vierstra, unpublished manuscript). Although these three omics analyses are a good start, more comprehensive and integrated analyses are needed to fully uncover the effects of nutrient starvation and autophagy deficiency on whole plant physiology.

Energy availability is a key determinant of plant growth and, accordingly, plants have evolved sophisticated mechanisms to maintain the pools of organic substrates needed for mitochondrial respiration. One involves modulating the levels of accumulated starch over day-night cycles to provide consistent diurnal supplies of hexoses (45). Evidence is emerging that autophagy contributes to this process when starch and hexose levels become insufficient by upregulating the degradation of proteins and lipids. The resulting breakdown products are then consumed in the mitochondrial tricarboxylic acid cycle or in an alternative respiratory pathway generated by the ELECTRON-TRANSFER FLAVOPROTEIN:UBIQUINONE OXIDOREDUCTASE (ETFQO) complex that consumes branched-chain amino acids and lysine as energy sources to reduce mitochondrial ubiquinone (69, 70). Notably, starchless *Arabidopsis* mutants have significantly increased levels of free amino acid pools, potentially due to autophagy, with these pools lessened when combined with autophagy-deficient backgrounds (70). Extended darkness, the absence of starch or sucrose, and

defects in autophagy also upregulate transcription of the *Arabidopsis* EFTQO pathway (3, 70), thus providing a strong feedback loop linking autophagy with this alternative respiratory route. The links between ETFQO and autophagy clearly require a closer look with regard to maintenance of a plant's energy balance.

CONCLUSIONS

The past decade has seen tremendous growth in our understanding of autophagy generally and of autophagy in plants specifically. Especially insightful has been the placement of ATG8 at a focal point, where it provides binding sites for AIM-containing proteins that either help assemble autophagic vesicles and fuse them with the vacuole or deliver specific cargo to the enveloping phagophore. The rapidly expanding collection of cargo receptors makes it evident that autophagy is not just a bulk recycling system but can target specific organelles, protein complexes, protein aggregates, and invading pathogens for breakdown. Both diversity within the plant ATG8 family (84) and recent observations that additional methods for binding ATG8 exist [e.g., *Arabidopsis* RPN10 (116)] raise the possibility that the catalog and influence of autophagy receptors could grow substantially. A number of these selective routes require prior ubiquitylation, thus uncovering a dynamic interplay between autophagy and the Ub-proteasome system (27). Whole proteome–based mass spectrometric approaches coupled with an increasing collection of plant autophagy mutants now offer shotgun approaches to assess this selective autophagy in depth for a number of plant species. The first glimpses into the metabolic consequences of autophagy defects also indicate that much of the plant metabolome is impacted by this recycling (2, 120; F. McLoughlin, R.C. Augustine, R.S. Marshall, F. Li & R.D. Vierstra, unpublished manuscript), implying that its redesign should offer opportunities for agronomic improvement. Furthermore, as pervasive as the ATG8-mediated system appears to be, it may not be the only autophagic route, based on emerging data that imply the existence of ATG8-independent autophagy (1, 133, 149).

SUMMARY POINTS

1. The core autophagy machinery is conserved among animals, yeast, and plants; however, many of the plant components are encoded by gene families, suggesting sub- or neo-functionalization.
2. Viable autophagy mutants are now available for several plant species; these mutants permit in-depth studies on the role of autophagy in plant growth, development, and survival under abiotic and biotic stresses.
3. Plant autophagy occurs at a basal level even under favorable conditions but is upregulated in response to nitrogen and fixed-carbon starvation and oxidative, osmotic, and proteotoxic stresses.
4. TOR kinase is a key negative regulator of starvation-induced autophagy in plants, whereas the AMPK/SnRK1 kinase appears to act as a positive regulator.
5. ATG8 is at the center of autophagy; it binds to proteins involved in autophagosome formation and provides a docking site for AIM-containing autophagic receptors that selectively recruit cargo.

6. Several pathways for selective autophagy have been described in plants, including pathways responsible for degradation of individual proteins, insoluble protein aggregates, large protein complexes, whole organelles, and invading pathogens.
7. Autophagy participates in defense against plant bacterial, fungal, oomycete, and viral pathogens, often by restricting pathogen growth via the HR, and is sometimes circumvented by a pathogen to enhance infection or block innate immunity.
8. Autophagy profoundly influences plant metabolism and is required for efficient remobilization of nutrients from leaves to developing seeds, an agronomically vital process in crop plants.

FUTURE ISSUES

1. Are the multiple ATG8 isoforms found in most plant species functionally redundant, or do they impart new functions to autophagy?
2. What are the phosphorylation targets of ATG1 in plants, and how do these modifications help initiate pre-autophagosomal structure assembly and activate autophagy?
3. Do selective autophagy receptors exist for plant chlorophagy, mitophagy, pexophagy, reticulophagy, and ribophagy, and if so, how are they activated, and what proteins do they recognize?
4. How is ubiquitylation integrated with the various types of selective autophagy? Which proteins are modified, and what are the Ub ligases responsible?
5. Are specific Ub attachment sites or poly-Ub chain topologies required to separately target cargo to either the Ub-proteasome system or autophagy?
6. How extensive is the suite of autophagy receptors, and do additional mechanisms exist beyond the AIM/LDS for the interaction of such receptors with ATG8?
7. Do ROS act as a common trigger for selective autophagy of plant organelles (e.g., chloroplasts, mitochondria, and peroxisomes), and if so, what are the mechanisms for sensing ROS production and activating autophagy?
8. Can autophagy be exploited to boost agronomic productivity in crop species by manipulating their metabolome, by altering the degradation of specific constituents, and/or by providing a new form of innate immunity?

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LITERATURE CITED

1. An H, Haper JW. 2018. Systematic analysis of ribophagy in human cells reveals bystander flux during selective autophagy. *Nat. Cell Biol.* 20:135–43
2. Avin-Wittenberg T, Bajdzienko K, Wittenberg G, Alseekh S, Tohge T, et al. 2015. Global analysis of the role of autophagy in cellular metabolism and energy homeostasis in *Arabidopsis* seedlings under carbon starvation. *Plant Cell* 27:306–22
3. Barros JA, Cavalcanti JHF, Medeiros DB, Nunes-Nesi A, Avin-Wittenberg T, et al. 2017. Autophagy deficiency compromises alternative pathways of respiration following energy deprivation. *Plant Physiol.* 175:62–76
4. Bassham DC, MacIntosh GC. 2017. Degradation of cytosolic ribosomes by autophagy-related pathways. *Plant Sci.* 262:169–74
5. Baumberger N, Tsai CH, Lie M, Havecker E, Baulcombe DC. 2007. The Polerovirus silencing suppressor P0 targets ARGONAUTE proteins for degradation. *Curr. Biol.* 17:1609–14
6. Book AJ, Gladman NP, Lee SS, Scalf M, Smith LM, Vierstra RD. 2010. Affinity purification of the *Arabidopsis* 26S proteasome reveals a diverse array of plant proteolytic complexes. *J. Biol. Chem.* 285:25554–69
7. Boutrot F, Zipfel C. 2017. Function, discovery and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. *Annu. Rev. Phytopathol.* 55:257–86
8. Braun P, Carvunis AR, Charloteaux B, Dreze M, Ecker JR, et al. 2011. Evidence for network evolution in an *Arabidopsis* interactome map. *Science* 333:601–7
9. Carroll AJ. 2013. The *Arabidopsis* cytosolic ribosomal proteome: from form to function. *Front. Plant Sci.* 4:32
10. Chanoca A, Kovinich N, Burkel B, Stecha S, Bohorquez-Restrepo A, et al. 2015. Anthocyanin vacuolar inclusions form by a microautophagy mechanism. *Plant Cell* 27:2545–59
11. Chen J, Lalonde S, Obrdlik P, Noorani-Vatani A, Parsa SA, et al. 2012. Uncovering the *Arabidopsis* membrane protein interactome enriched in transporters using mating-based split-ubiquitin assays and classification models. *Front. Plant Sci.* 3:124
12. Chen L, Su ZZ, Huang L, Xia FN, Qi H, et al. 2017. The AMP-activated protein kinase KIN10 is involved in the regulation of autophagy in *Arabidopsis*. *Front. Plant Sci.* 8:1201
13. 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. *Plant J.* 62:483–93
14. Chung T, Suttangkakul A, Vierstra RD. 2009. The ATG autophagic conjugation system in maize: ATG transcripts and abundance of the ATG8-PE adduct are regulated by development and nutrient availability. *Plant Physiol.* 149:220–34
15. Cohen-Kaplan V, Livneh I, Avni N, Fabre B, Ziv T, et al. 2016. p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome. *PNAS* 113:7490–99
16. Colaert N, Helsens K, Martens L, Vandekerckhove J, Gevaert K. 2009. Improved visualization of protein consensus sequences by iceLogo. *Nat. Methods* 6:786–87
17. Collins GA, Goldberg AL. 2017. The logic of the 26S proteasome. *Cell* 169:792–806
18. Contento AL, Kim SJ, Bassham DC. 2004. Transcriptome profiling of the response of *Arabidopsis* suspension culture cells to sucrose starvation. *Plant Physiol.* 135:2330–47
19. Contento AL, Xiong Y, Bassham DC. 2005. Visualization of autophagy in *Arabidopsis* using the fluorescent dye monodansylcadaverine and a GFP-AtATG8e fusion protein. *Plant J.* 42:598–608
20. Courtois-Moreau CL, Pesquet E, Sjödin A, Muñiz L, Bollhöner B, et al. 2009. A unique program for cell death in xylem fibers of *Populus* stem. *Plant J.* 58:260–74
21. Crespo JL, Diaz-Troya S, Florencio FJ. 2005. Inhibition of target of rapamycin signaling in the unicellular green alga *Chlamydomonas reinhardtii*. *Plant Physiol.* 139:1736–49

22. Cui H, Tsuda K, Parker JE. 2015. Effector-triggered immunity: from pathogen perception to robust defense. *Annu. Rev. Plant Biol.* 66:487–511
23. Dagdas YF, Belhaj K, Maqbool A, Chaparro-Garcia A, Pandey P, et al. 2016. An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. *eLife* 5:e10856
24. Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, et al. 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. *PNAS* 109:15942–46
25. Devarenne TP. 2011. The plant cell death suppressor Adi3 interacts with the autophagic protein Atg8h. *Biochem. Biophys. Res. Commun.* 412:699–703
26. Di Berardino J, Marmagne A, Berger A, Yoshimoto K, Cueff G, et al. 2018. Autophagy controls resource allocations and protein storage accumulation in *Arabidopsis* seeds. *J. Exp. Bot.* 69:1403–14
27. Dikic I. 2017. Proteasomal and autophagic degradation systems. *Annu. Rev. Biochem.* 86:193–224
28. Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincent M, et al. 2016. TOR signaling and nutrient sensing. *Annu. Rev. Plant Biol.* 67:261–85
29. Doelling JH, Walker JM, Friedman EM, Thompson AR, Vierstra RD. 2002. The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in *Arabidopsis thaliana*. *J. Biol. Chem.* 277:33105–14
30. Dong P, Xiong F, Que Y, Wang K, Yu L, et al. 2015. Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in *Arabidopsis*. *Front. Plant Sci.* 6:667
31. Farmer LM, Rinaldi MA, Young PG, Danan CH, Burkhart SE, Bartel B. 2013. Disrupting autophagy restores peroxisome function to an *Arabidopsis lon2* mutant and reveals a role for the LON2 protease in peroxisomal matrix protein degradation. *Plant Cell* 25:4085–100
32. Farré JC, Burkenroad A, Burnett SF, Subramani S. 2013. Phosphorylation of mitophagy and pexophagy receptors co-ordinates their interaction with Atg8 and Atg11. *EMBO Rep.* 14:441–49
33. Farré JC, Subramani S. 2016. Mechanistic insights into selective autophagy pathways: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* 17:537–52
34. Floyd BE, Morriss SC, MacIntosh GC, Bassham DC. 2015. Evidence for autophagy-dependent pathways of rRNA turnover in *Arabidopsis*. *Autophagy* 11:2199–212
35. 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* 245:779–92
36. Franzmann TM, Jahnel M, Pozniakovsky A, Mahamid J, Holehouse AS, et al. 2018. Phase separation of a yeast prion protein promotes cellular fitness. *Science* 359:5654
37. Fujiki Y, Yoshimoto K, Ohsumi Y. 2007. An *Arabidopsis* homolog of yeast *ATG6* is essential for pollen germination. *Plant Physiol.* 143:1132–39
38. Fujioka Y, Noda NN, Fujii K, Yoshimoto K, Ohsumi Y, Inagaki F. 2008. In vitro reconstitution of plant ATG8 and ATG12 conjugation systems essential for autophagy. *J. Biol. Chem.* 283:1921–28
39. Fujioka Y, Noda NN, Nakatogawa H, Ohsumi Y, Inagaki F. 2010. Dimeric coiled-coil structure of *Saccharomyces cerevisiae* Atg16 and its functional significance in autophagy. *J. Biol. Chem.* 285:1508–15
40. Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, et al. 2017. Molecular definitions of autophagy and related processes. *EMBO J.* 36:1811–36
41. Gao C, Zhuang X, Cui Y, Fu X, He Y, et al. 2015. Dual roles of an *Arabidopsis* ESCRT component FREE1 in regulating vacuolar protein transport and autophagic degradation. *PNAS* 112:1886–91
42. Ghiglione HO, Gonzalez FG, Serrago R, Maldonado SB, Chilcott C, et al. 2008. Autophagy regulated by day length determines the number of fertile florets in wheat. *Plant J.* 55:1010–24
43. Gladman NP, Marshall RS, Lee KH, Vierstra RD. 2016. The proteasome stress regulon is controlled by a pair of NAC transcription factors in *Arabidopsis*. *Plant Cell* 28:1279–96
44. Goto-Yamada S, Mano S, Nakamori C, Kondo M, Yamawaki R, et al. 2014. Chaperone and protease functions of LON2 modulate the peroxisomal transition and degradation via autophagy. *Plant Cell Physiol.* 55:482–96
45. Graf A, Schlereth A, Stitt M, Smith AM. 2010. Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. *PNAS* 107:9458–63

23. Describes the first example of a plant pathogen effector interfering with a host's autophagy machinery.

46. Grousl T, Ungelenk S, Miller S, Ho CT, Khokhrina M, et al. 2018. A prion-like domain in Hsp42 drives chaperone-facilitated aggregation of mis-folded proteins. *J. Cell Biol.* In press. **https://doi.org/10.1083/jcb.201708116**
47. Grumati P, Morozzi G, Hölper S, Mari M, Harwardt MI, et al. 2017. Full length RTN3 regulates turnover of tubular endoplasmic reticulum via selective autophagy. *eLife* 6:e25555
48. Guiboileau A, Avila-Ospina L, Yoshimoto K, Soulay F, Azzopardi M, et al. 2013. Physiological and metabolic consequences of autophagy deficiency for the management of nitrogen and protein resources in *Arabidopsis* leaves depending on nitrate availability. *New Phytol.* 199:683–94
49. Guiboileau A, Yoshimoto K, Soulay F, Bataillé MP, Avice JC, Masclaux-Daubresse C. 2012. The autophagy machinery controls nitrogen re-mobilization at the whole plant level under both limiting and ample nitrate conditions in *Arabidopsis*. *New Phytol.* 194:732–40
50. Hachez C, Veljanovski V, Reinhardt H, Guillaumot D, Vanhee C, et al. 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. *Plant Cell* 26:4974–90
51. 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. *PNAS* 114:2026–35
52. 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 Physiol.* 176:649–62
53. Han S, Wang Y, Zheng X, Jia Q, Zhao J, et al. 2015. Cytoplastic glyceraldehyde-3-phosphate dehydrogenases interact with ATG3 to negatively regulate autophagy and immunity in *Nicotiana benthamiana*. *Plant Cell* 27:1316–31
54. Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, et al. 2002. Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an *Arabidopsis* autophagy gene. *Plant Physiol.* 129:1181–93
55. Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, et al. 2004. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science* 305:855–58
56. Havé M, Balliau T, Cottyn-Boitte B, Dérond E, Cueff G, et al. 2018. Increase of proteasome and papain-like cysteine protease activities in autophagy mutants: back-up compensatory effect or pro-cell death effect. *J. Exp. Bot.* 69:1369–85
57. Havé M, Marmagne A, Chardon F, Masclaux-Daubresse C. 2017. Nitrogen re-mobilization during leaf senescence: lessons from *Arabidopsis* to crops. *J. Exp. Bot.* 68:2513–29
58. Haxim Y, Ismayil A, Jia Q, Wang Y, Zheng X, et al. 2017. Autophagy functions as an anti-viral mechanism against Geminiviruses in plants. *eLife* 6:e23897
59. 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. *PNAS* 108:1093–98
60. Hofius D, Li L, Hafrén A, Coll NS. 2017. Autophagy as an emerging arena for plant-pathogen interactions. *Curr. Opin. Plant Biol.* 38:117–23
61. Hofius D, Schultz-Larsen T, Jönsen J, Tsitsigiannis DI, Petersen NH, et al. 2009. Autophagic components contribute to hypersensitive cell death in *Arabidopsis*. *Cell* 137:773–83
62. 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. *Plant Cell* 24:288–303
63. Howell SH. 2013. Endoplasmic reticulum stress responses in plants. *Annu. Rev. Plant Biol.* 64:477–99
64. Howell LA, Tomko RJ, Kusmierczyk AR. 2017. Putting it all together: intrinsic and extrinsic mechanisms governing proteasome biogenesis. *Front. Biol.* 12:19–48
65. Hua Z, Vierstra RD. 2011. The cullin-RING ubiquitin-protein ligases. *Annu. Rev. Plant Biol.* 62:299–334
66. Huber SC, Kaiser WM. 1996. 5-Aminoimidazole-4-carboxamide riboside activates nitrate reductase in darkened spinach and pea leaves. *Physiol. Plant.* 98:833–37
67. Inoue Y, Suzuki T, Hattori M, Yoshimoto K, Ohsumi Y, Moriyasu Y. 2006. *AtATG* genes, homologs of yeast autophagy genes, are involved in constitutive autophagy in *Arabidopsis* root tip cells. *Plant Cell Physiol.* 47:1641–52

51. Together with Reference 58, provides the first evidence for autophagic degradation of specific viral proteins to limit infection.

68. Ishida H, Yoshimoto K, Izumi M, Reisen D, Yano Y, et al. 2008. Mobilization of Rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an *ATG* gene–dependent autophagic process. *Plant Physiol*. 148:142–55
69. 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. *Plant Cell* 17:2587–600
70. Izumi M, Hidema J, Makino A, Ishida H. 2013. Autophagy contributes to night-time energy availability for growth in *Arabidopsis. Plant Physiol*. 161:1682–93
71. Izumi M, Hidema J, Wada S, Kondo E, Kurusu T, et al. 2015. Establishment of monitoring methods for autophagy in rice reveals autophagic recycling of chloroplasts and root plastids during energy limitation. *Plant Physiol*. 167:1307–20
72. Izumi M, Ishida H, Nakamura S, Hidema J. 2017. Entire photo-damaged chloroplasts are transported to the central vacuole by autophagy. *Plant Cell* 29:377–94
73. Izumi M, Wada S, Makino A, Ishida H. 2010. The autophagic degradation of chloroplasts via Rubisco-containing bodies is specifically linked to leaf carbon status but not nitrogen status in *Arabidopsis. Plant Physiol*. 154:1196–209
74. Jacoby RP, Li L, Huang S, Lee CP, Millar AH, Taylor NL. 2012. Mitochondrial composition, function and stress response in plants. *J. Integr. Plant Biol.* 54:887–906
75. Jarvis P, López-Juez E. 2013. Biogenesis and homeostasis of chloroplasts and other plastids. *Nat. Rev. Mol. Cell Biol.* 14:787–802
76. Jo EK, Yuk JM, Shin DM, Sasakawa C. 2013. Roles of autophagy in elimination of intracellular bacterial pathogens. *Front. Immunol.* 4:97
77. Jung JY, Kim YW, Kwak JM, Hwang JU, Young J, et al. 2002. Phosphatidylinositol-3- and 4-phosphate are required for normal stomatal movements. *Plant Cell* 14:2399–412
78. Kabbage M, Williams B, Dickman MB. 2013. Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of *Sclerotinia sclerotiorum. PLOS Pathog*. 9:e1003287
79. Kalinowska K, Isono E. 2018. All roads lead to the vacuole: autophagic transport as part of the endomembrane trafficking network. *J. Exp. Bot.* 69:1313–24
80. Kalvari I, Tsompanis S, Mulakkal NC, Osgood R, Johansen T, et al. 2014. iLIR: a web resource for prediction of Atg8-family interacting proteins. *Autophagy* 10:913–25
81. Kanki T, Wang K, Cao Y, Baba M, Klionsky DJ. 2009. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev. Cell* 17:98–109
82. Katsiarimpa A, Kalinowska K, Anzenberger F, Weis C, Ostertag M, et al. 2013. The deubiquitinating enzyme AMSH1 and the ESCRT-III subunit VPS2.1 are required for autophagic degradation in *Arabidopsis. Plant Cell* 25:2236–52
83. Keech O, Pesquet E, Ahad A, Askne A, Nordvall D, et al. 2007. The different fates of mitochondria and chloroplasts during dark-induced senescence in *Arabidopsis* leaves. *Plant Cell Environ*. 30:1523–34
84. Kellner R, de la Concepcion JC, Maqbool A, Kamoun S, Dagdas YF. 2017. ATG8 expansion: a driver of selective autophagy diversification. *Trends Plant Sci*. 22:204–14
85. Ketelaar T, Voss C, Dimmock SA, Thumm M, Hussey PJ. 2004. *Arabidopsis* homologs of the autophagy protein Atg8 are a novel family of microtubule binding proteins. *FEBS Lett*. 567:302–6
86. Khaminets A, Heinrich T, Mari M, Grumati P, Hübner AK, et al. 2015. Regulation of endoplasmic reticulum turnover by selective autophagy. *Nature* 522:354–58
87. Kim DY, Scalf M, Smith LM, Vierstra RD. 2013. Advanced proteomic analyses yield a deep catalog of ubiquitylation targets in *Arabidopsis. Plant Cell* 25:1523–40
88. Kim J, Lee H, Lee HN, Kim SH, Shin KD, Chung T. 2013. Autophagy-related proteins are required for degradation of peroxisomes in *Arabidopsis* hypocotyls during seedling growth. *Plant Cell* 25:4956–66
89. Kirkin V, Lamark T, Sou YS, Bjørkøy G, Nunn JL, et al. 2009. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol. Cell* 33:505–16
90. Klopffleisch K, Phan N, Augustin K, Bayne RS, Booker KS, et al. 2011. The *Arabidopsis* G-protein interactome reveals connections to cell wall carbohydrates and morphogenesis. *Mol. Syst. Biol.* 7:532

91. 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. *Nat. Cell Biol.* 10:602–10
92. Kraft C, Kijanska M, Kalie E, Siergiejuk E, Lee SS, et al. 2012. Binding of the Atg1/ULK1 kinase to the ubiquitin-like protein Atg8 regulates autophagy. *EMBO J.* 31:3691–703
93. Kraft C, Peter M. 2008. Is the Rsp5 ubiquitin ligase involved in the regulation of ribophagy? *Autophagy* 4:838–40
94. Krinke O, Rülland E, Valentova O, Vergnolle C. Renou JP, et al. 2007. Phosphatidylinositol-4-kinase activation is an early response to salicylic acid in *Arabidopsis* suspension cells. *Plant Physiol.* 144:1347–59
95. Kulich I, Pečenková T, Sekereš J, Smetana O, Fendrych M, et al. 2013. An *Arabidopsis* exocyst subcomplex containing subunit EXO70B1 is involved in autophagy-related transport to the vacuole. *Traffic* 14:1155–65
96. Kurusu T, Koyano T, Hanamata S, Kubo T, Noguchi Y, et al. 2014. OsATG7 is required for autophagy-dependent lipid metabolism in rice post-meiotic anther development. *Autophagy* 10:878–88
97. Kwon SI, Cho HJ, Jung JH, Yoshimoto K, Shirasu K, Park OK. 2010. The Rab GTPase RabG3b functions in autophagy and contributes to tracheary element differentiation in *Arabidopsis*. *Plant J.* 64:151–64
98. Lai Z, Wang F, Zheng Z, Fan B, Chen Z. 2011. A critical role of autophagy in plant resistance to necrotrophic fungal pathogens. *Plant J.* 66:953–68

99. Reports that ATG5 defines a phagophore subdomain that is closely connected to the cortical ER.

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

100. Lee HN, Zarza X, Kim JH, Yoon MJ, Kim SH, et al. 2018. *Arabidopsis* VPS38 is required for vacuolar trafficking but is dispensable for autophagy. *Plant Physiol.* 176:1559–72
101. Lee Y, Bak G, Choi Y, Chuang WI, Cho HT, Lee Y. 2008. Roles of phosphatidylinositol-3-kinase in root hair growth. *Plant Physiol.* 147:624–35
102. Lee Y, Kim ES, Choi Y, Hwang I, Staiger CJ, et al. 2008. The *Arabidopsis* phosphatidylinositol-3-kinase is important for pollen development. *Plant Physiol.* 147:1886–97
103. Lenz HD, Haller E, Melzer E, Kober K, Wurster K, et al. 2011. Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. *Plant J.* 66:818–30
104. Levanony H, Rubin R, Altschuler Y, Galili G. 1992. Evidence for a novel route of wheat storage proteins to vacuoles. *J. Cell Biol.* 119:1117–28

105. Reports the first example of autophagy deficiency in maize and its role in nutrient remobilization to developing seeds.

105. Li F, Chung T, Pennington JG, Federico ML, Kaeppler HF, et al. 2015. Autophagic recycling plays a central role in maize nitrogen re-mobilization. *Plant Cell* 27:1389–408

106. Li F, Chung T, Vierstra RD. 2014. AUTOPHAGY-RELATED11 plays a critical role in general autophagy and senescence-induced mitophagy in *Arabidopsis*. *Plant Cell* 26:788–807
107. Li F, Vierstra RD. 2012. Autophagy: a multifaceted intracellular system for bulk and selective recycling. *Trends Plant Sci.* 17:526–37
108. Li W, Chen M, Wang E, Hu L, Hawkesford MJ, 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 Genom.* 17:797
109. Ling Q, Huang W, Baldwin A, Jarvis P. 2012. Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. *Science* 338:655–59
110. Lingard MJ, Monroe-Augustus M, Bartel B. 2009. Peroxisome-associated matrix protein degradation in *Arabidopsis*. *PNAS* 106:4561–66
111. Liu Y, Bassham DC. 2010. TOR is a negative regulator of autophagy in *Arabidopsis thaliana*. *PLOS ONE* 5:e11883
112. Liu Y, Schiff M, Czymmek K, Talloczy Z, Levine B, Dinesh-Kumar SP. 2005. Autophagy regulates programmed cell death during the plant innate immune response. *Cell* 121:567–77
113. Liu Y, Soto-Burgos J, Deng Y, Srivastava R, Howell SH, Bassham DC. 2012. Degradation of the endoplasmic reticulum by autophagy during endoplasmic reticulum stress in *Arabidopsis*. *Plant Cell* 24:4635–51
114. Lu K, Psakhye I, Jentsch S. 2014. Autophagic clearance of polyQ proteins mediated by ubiquitin-Atg8 adaptors of the conserved CUET protein family. *Cell* 158:549–63

115. Maqbool A, Hughes RK, Dagdas YF, Tregidgo N, Zess E, et al. 2016. Structural basis of host AUTOPHAGY-RELATED8 (ATG8) binding by the Irish potato famine pathogen effector protein PexRD54. *J. Biol. Chem.* 291:20270–82

116. 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*. *Mol. Cell* 58:1053–66

117. 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 Rep.* 16:1717–32

118. Marshall RS, Vierstra RD. 2015. Eat or be eaten: the autophagic plight of inactive 26S proteasomes. *Autophagy* 11:1927–28

119. Masclaux-Daubresse C, Chen Q, Havé M. 2017. Regulation of nutrient recycling via autophagy. *Curr. Opin. Plant Biol.* 39:8–17

120. Masclaux-Daubresse C, Clément G, Anne P, Routaboul JM, Guiboileau A, et al. 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*. *Plant Cell* 26:1857–77

121. Matsuoka K, Bassham DC, Raikhel NV, Nakamura K. 1995. Different sensitivity to wortmannin of two vacuolar sorting signals indicates the presence of distinct sorting machineries in tobacco cells. *J. Cell Biol.* 130:1307–18

122. Matsuoka K, Higuchi T, Maeshima M, Nakamura K. 1997. A vacuolar-type H^+-ATPase in a non-vacuolar organelle is required for the sorting of soluble vacuolar protein precursors in tobacco cells. *Plant Cell* 9:533–46

123. Merkolova EA, Guiboileau A, Naya L, Masclaux-Daubresse C, Yoshimoto K. 2014. Assessment and optimization of autophagy monitoring methods in *Arabidopsis* roots indicate direct fusion of autophagosomes with vacuoles. *Plant Cell Physiol.* 55:715–26

124. Michaeli S, Honig A, Levanony H, Peled-Zehavi H, Galili G. 2014. *Arabidopsis* ATG8-INTERACTING PROTEIN1 is involved in autophagy-dependent vesicular trafficking of plastid proteins to the vacuole. *Plant Cell* 26:4084–101

125. Minina EA, Moschou PN, Vetukuri RR, Sanches-Vera V, Cardoso C, et al. 2018. Transcriptional stimulation of rate-limiting components of the autophagic pathway improves plant fitness. *J. Exp. Bot.* 69:1415–32

126. Mochida K, Oikawa Y, Kimura Y, Kirisako H, Hirano H, et al. 2015. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature* 522:359–62

127. Montané MH, Menand B. 2013. ATP-competitive mTOR kinase inhibitors delay plant growth by triggering early differentiation of meristematic cells without changing developmental patterning. *J. Exp. Bot.* 64:4361–74

128. Moriyasu Y, Inoue Y. 2008. Use of protease inhibitors for detecting autophagy in plants. *Methods Enzymol.* 451:557–80

129. Moriyasu Y, Ohsumi Y. 1996. Autophagy in tobacco suspension-cultured cells in response to sucrose starvation. *Plant. Physiol.* 111:1233–41

130. Müller M, Kötter P, Behrendt C, Walter E, Scheckhuber CQ, et al. 2015. Synthetic quantitative array technology identifies the Ubp3-Bre5 deubiquitinase complex as a negative regulator of mitophagy. *Cell Rep.* 10:1215–25

131. Nakahara KS, Masuta C, Yamada S, Shimura H, Kashihara Y, et al. 2012. A tobacco calmodulin-like protein provides secondary defence by binding to and directing degradation of virus RNA silencing suppressors. *PNAS* 109:10113–18

132. Nemec AA, Howell LA, Peterson AK, Murray MA, Tomko RJ. 2017. Autophagic clearance of proteasomes in yeast requires the conserved sorting nexin Snx4. *J. Biol. Chem.* 292:21466–80

133. Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, et al. 2009. Discovery of Atg5/Atg7-independent alternative macroautophagy. *Nature* 461:654–58

134. Noda NN, Ohsumi Y, Inagaki F. 2010. An Atg8-interacting motif crucial for selective autophagy. *FEBS Lett.* 584:1379–85

116. Provides the first report of proteasomes being degraded by autophagy, via the Ub-binding RPN10 receptor.

120. Provides the first comprehensive view on the importance of autophagy to plant metabolism.

135. Demonstrates a role for receptor phosphorylation in regulating selective autophagic degradation of BES1 by DSK2.

135. Nolan TM, Brennan B, Yang M, Chen J, Zhang M, et al. 2017. Selective autophagy of BES1 mediated by DSK2 balances plant growth and survival. *Dev. Cell* 41:33–46

136. Nziengui H, Bouhidel K, Pillon D, Der C, Marty F, Schöefs B. 2007. Reticulon-like proteins in *Arabidopsis thaliana*: structural organization and ER localization. *FEBS Lett*. 581:3356–62
137. Ohsumi Y. 2001. Molecular dissection of autophagy: two ubiquitin-like systems. *Nat. Rev. Mol. Cell Biol.* 2:211–16
138. Okamoto K, Kondo-Okamoto N, Ohsumi Y. 2009. The mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev. Cell* 17:87–97
139. Ossareh-Nazari B, Niño CA, Bengtson MH, Lee JW, Joazeiro CA, Dargemont C. 2014. Ubiquitylation by the Ltn1 E3 ligase protects 60S ribosomes from starvation-induced selective autophagy. *J. Cell Biol.* 204:909–17
140. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, et al. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J. Biol. Chem.* 282:24131–45
141. Paul AL, Sehnke PC, Ferl RJ. 2005. Isoform-specific subcellular localization among 14-3-3 proteins in *Arabidopsis* seems to be driven by client interactions. *Mol. Biol. Cell* 16:1735–43
142. Pecenková T, Markovic V, Sabol P, Kulich I, Žárský V. 2017. Exocyst- and autophagy-related membrane trafficking in plants. *J. Exp. Bot.* 69:47–57
143. Pérez-Pérez ME, Florencio FJ, Crespo JL. 2010. Inhibition of target of rapamycin signaling and stress activate autophagy in *Chlamydomonas reinhardtii*. *Plant Physiol*. 152:1874–88
144. Phillips AR, Suttangkakul A, Vierstra RD. 2008. The ATG12-conjugating enzyme ATG10 is essential for autophagic vesicle formation in *Arabidopsis thaliana*. *Genetics* 178:1339–53
145. Popa C, Li L, Gil S, Tatjer L, Hashii K, et al. 2016. The effector AWR5 from the plant pathogen *Ralstonia solanacearum* is an inhibitor of the TOR signalling pathway. *Sci. Rep.* 6:27058
146. Pourcel L, Irani NG, Lu Y, Riedl K, Schwartz S, Grotewold E. 2010. The formation of anthocyanic vacuolar inclusions in *Arabidopsis thaliana* and implications for the sequestration of anthocyanin pigments. *Mol. Plant* 3:78–90
147. Pu Y, Luo X, Bassham DC. 2017. TOR-dependent and -independent pathways regulate autophagy in *Arabidopsis thaliana*. *Front. Plant Sci*. 8:1204
148. Qi H, Xia FN, Xie LJ, Yu LJ, Chen QF, et al. 2017. TRAF family proteins regulate autophagy dynamics by modulating AUTOPHAGY-RELATED6 stability in *Arabidopsis*. *Plant Cell* 29:890–911
149. Reyes FC, Chung T, Holding D, Jung R, Vierstra RD, Otegui MS. 2011. Delivery of prolamins to the protein storage vacuole in maize aleurone cells. *Plant Cell* 23:769–84
150. Roberts P, Moshitch-Moshkovitz S, Kvam E, O'Toole E, Winey M, Goldfarb DS. 2003. Piecemeal microautophagy of the nucleus in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 14:129–41
151. Robinson DG, Oliviusson P, Hinz G. 2005. Protein sorting to the storage vacuoles of plants: a critical appraisal. *Traffic* 6:615–25
152. Rojo E, Zouhar J, Carter C, Kovaleva V, Raikhel NV. 2003. A unique mechanism for protein processing and degradation in *Arabidopsis thaliana*. *PNAS* 100:7389–94
153. Sargent G, van Zutphen T, Shatseva T, Zhang L, Di Giovanni V, et al. 2016. PEX2 is the E3 ubiquitin ligase required for pexophagy during starvation. *J. Cell Biol.* 214:677–90
154. Schepetilnikov M, Dimitrova M, Mancera-Martinez E, Geldreich A, Keller M, Ryabova LA. 2013. TOR and S6K1 promote translation re-initiation of uORF-containing mRNAs via phosphorylation of eIF3h. *EMBO J.* 32:1087–102
155. Shibata M, Oikawa K, Yoshimoto K, Kondo M, Mano S, et al. 2013. Highly oxidized peroxisomes are selectively degraded via autophagy in *Arabidopsis*. *Plant Cell* 25:4967–83
156. Sláviková S, Shy G, Yao Y, Glozman R, Levanony H, et al. 2005. The autophagy-associated Atg8 gene family operates during both favourable growth conditions and starvation stress in *Arabidopsis*. *J. Exp. Bot.* 56:2839–49
157. Sontag EM, Samant RS, Frydman J. 2017. Mechanisms and functions of spatial protein quality control. *Annu. Rev. Biochem.* 86:97–122
158. Soto-Burgos J, Bassham DC. 2017. SnRK1 activates autophagy via the TOR signaling pathway in *Arabidopsis thaliana*. *PLOS ONE* 12:e0182591

159. Spitzer C, Li F, Buono R, Roschzttardtz H, Chung T, et al. 2015. The endosomal protein CHARGED MULTI-VESICULAR BODY PROTEIN1 regulates the autophagic turnover of plastids in *Arabidopsis*. *Plant Cell* 27:391–402
160. Surpin M, Zheng H, Morita MT, Saito C, Avila E, et al. 2003. The VTI family of SNARE proteins is necessary for plant viability and mediates different protein transport pathways. *Plant Cell* 15:2885–99
161. Sutipatanasomboon A, Herberth S, Alwood EG, Häweker H, Müller B, et al. 2017. Disruption of the plant-specific *CFS1* gene impairs autophagosome turnover and triggers EDS1-dependent cell death. *Sci. Rep.* 7:8677
162. Suttangkakul A, Li F, Chung T, Vierstra RD. 2011. The ATG1/ATG13 protein kinase complex is both a regulator and a target of autophagic recycling in *Arabidopsis*. *Plant Cell* 23:3761–79
163. 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
164. Takáč T, Pechan T, Šamajová O, Šamaj J. 2013. Vesicular trafficking and stress response coupled to PI3K inhibition by LY294002 as revealed by proteomic and cell biological analysis. *J. Proteome Res.* 12:4435–48
165. Takatsuka C, Inoue Y, Matsuoka K, Moriyasu Y. 2004. 3-Methyladenine inhibits autophagy in tobacco culture cells under sucrose starvation conditions. *Plant Cell Physiol.* 45:265–74
166. Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD. 2005. Autophagic nutrient recycling in *Arabidopsis* directed by the ATG8 and ATG12 conjugation pathways. *Plant Physiol.* 138:2097–110
167. Thompson AR, Vierstra RD. 2005. Autophagic recycling: Lessons from yeast help define the process in plants. *Curr. Opin. Plant Biol.* 8:165–73
168. Tolley N, Sparkes IA, Hunter PR, Craddock CP, Nuttall J, et al. 2008. Over-expression of a plant reticulon re-models the lumen of the cortical endoplasmic reticulum but does not perturb protein transport. *Traffic* 9:94–102
169. Üstün S, Hafrén A, Liu Q, Marshall RS, Minina EA, et al. 2018. Bacteria exploit autophagy for proteasome degradation and enhanced virulence in plants. *Plant Cell*. In press. **https://doi.org/10.1105/tpc.17.00815**
170. van Doorn WG, Papini A. 2013. The ultra-structure of autophagy in plant cells: a review. *Autophagy* 9:1922–36
171. Vanhee C, Zapotoczny G, Masquelier D, Ghislain M, Batoko H. 2011. The *Arabidopsis* multi-stress regulator TSPO is a heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism. *Plant Cell* 23:785–805
172. Vierstra RD. 2009. The ubiquitin-26S proteasome system at the nexus of plant biology. *Nat. Rev. Mol. Cell Biol.* 10:385–97
173. Voitsekhovskaja OV, Schiermeyer A, Reumann S. 2014. Plant peroxisomes are degraded by starvation-induced and constitutive autophagy in tobacco BY-2 suspension–cultured cells. *Front. Plant Sci.* 5:629
174. Wada S, Hayashida Y, Izumi M, Kurusu T, Hanamata S, et al. 2015. Autophagy supports biomass production and nitrogen use efficiency at the vegetative stage in rice. *Plant Physiol.* 168:60–73
175. Waite KA, De La Mota-Peynado A, Vontz G, Roelofs J. 2016. Starvation induces proteasome autophagy with different pathways for core and regulatory particles. *J. Biol. Chem.* 291:3239–53
176. Wang P, Mugume Y, Bassham DC. 2018. New advances in autophagy in plants: regulation, selectivity and function. *Semin. Cell Dev. Biol.* In press. **https://doi.org/10.1016/j.semcdb.2017.07.018**
177. Wang P, Richardson C, Hawes C, Hussey PJ. 2016. *Arabidopsis* NAP1 regulates the formation of autophagosomes. *Curr. Biol.* 26:2060–69
178. Wang Y, Nishimura MT, Zhao T, Tang D. 2011. ATG2, an autophagy-related protein, negatively affects powdery mildew resistance and mildew-induced cell death in *Arabidopsis*. *Plant J.* 68:74–87
179. Woodson JD, Joens MS, Sinson AB, Gilkerson J, Salomé PA, et al. 2015. Ubiquitin facilitates a quality-control pathway that removes damaged chloroplasts. *Science* 350:450–54
180. Wurzer B, Zaffagnini G, Fracchiolla D, Turco E, Abert C, et al. 2015. Oligomerization of p62 allows for selection of ubiquitylated cargo and isolation membrane during selective autophagy. *eLife* 4:e08941
181. Xia K, Liu T, Ouyang J, Wang R, Fan T, Zhang M. 2011. Genome-wide identification, classification and expression analysis of autophagy-associated gene homologs in rice (*Oryza sativa* L.). *DNA Res.* 18:363–77

163. Together with Reference 200, this paper describes the identification of NBR1/Joka2, the first selective autophagy receptor reported in plants.

182. 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
183. Xie Q, Tzfadia O, Levy M, Weithorn E, Peled-Zehavi H, et al. 2016. hfAIM: a reliable bioinformatics approach for in silico genome-wide identification of autophagy-associated Atg8-interacting motifs in various organisms. *Autophagy* 12:876–87
184. Xiong Y, Contento AL, Bassham DC. 2005. AtATG18a is required for the formation of autophagosomes during nutrient stress and senescence in *Arabidopsis thaliana*. *Plant J.* 42:535–46
185. Xiong Y, Sheen J. 2012. Rapamycin and glucose-target of rapamycin (TOR) protein signaling in plants. *J. Biol. Chem.* 287:2836–42
186. Xu N, Gao XQ, Zhao XY, Zhu DZ, Zhou LZ, Zhang XS. 2011. *Arabidopsis* VPS15 is essential for pollen development and germination through modulating phosphatidylinositol-3-phosphate formation. *Plant Mol. Biol.* 77:251–60
187. Yamaguchi M, Matoba K, Sawada R, Fujioka Y, Nakatogawa H, et al. 2012. Non-canonical recognition and UBL loading of distinct E2s by autophagy-essential Atg7. *Nat. Struct. Mol. Biol.* 19:1250–56
188. Yamano K, Matsuda N, Tanaka K. 2016. The ubiquitin signal and autophagy: an orchestrated dance leading to mitochondrial degradation. *EMBO Rep.* 17:300–16
189. Yang X, Srivastava R, Howell SH, Bassham DC. 2016. Activation of autophagy by unfolded proteins during endoplasmic reticulum stress. *Plant J.* 85:83–95
190. Yoshimoto K, Hanaoka H, Sato S, Kato T, Tabata S, et al. 2004. Processing of the ubiquitin-like ATG8 proteins by ATG4 is essential for plant autophagy. *Plant Cell* 16:2967–83
191. Yoshimoto K, Jikumaru Y, Kamiya Y, Kusano M, Consonni C, et al. 2009. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in *Arabidopsis*. *Plant Cell* 21:2914–27
192. Young PG, Bartel B. 2016. Pexophagy and peroxisomal protein turnover in plants. *Biochim. Biophys. Acta* 1863:999–1005
193. Zhang J, Tripathi DN, Jing J, Alexander A, Kim J, et al. 2015. ATM functions at the peroxisome to induce pexophagy in response to ROS. *Nat. Cell Biol.* 17:1259–69
194. Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RA, et al. 2009. Inhibition of SNF1-related protein kinase 1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiol.* 149:1860–71
195. Zhou J, Wang J, Cheng Y, Chi YJ, Fan B, et al. 2013. NBR1-mediated selective autophagy targets insoluble ubiquitinated protein aggregates in plant stress responses. *PLOS Genet.* 9:e1003196
196. Zhou J, Wang J, Yu JQ, Chen Z. 2014. Role and regulation of autophagy in heat stress responses of tomato plants. *Front. Plant Sci.* 5:174
197. Zhou XM, Zhao P, Wang W, Zou J, Cheng TH, et al. 2015. A comprehensive, genome-wide analysis of autophagy-related genes identified in tobacco suggests a central role of autophagy in plant response to various environmental cues. *DNA Res.* 22:245–57
198. Zhuang X, Chung KP, Cui Y, Lin W, Gao C, et al. 2017. ATG9 regulates autophagosome progression from the endoplasmic reticulum in *Arabidopsis*. *PNAS* 114:426–35
199. Zhuang X, Wang H, Lam SK, Gao C, Wang X, et al. 2013. A BAR-domain protein SH3P2, which binds to phosphatidylinositol-3-phosphate and ATG8, regulates autophagosome formation in *Arabidopsis*. *Plant Cell* 25:4596–615
200. Zientara-Rytter K, Lukomska J, Moniuszko G, Gwozdecki R, Surowiecki P, et al. 2011. Identification and functional analysis of Joka2, a tobacco member of the family of selective autophagy cargo receptors. *Autophagy* 7:1145–58
201. Zvereva AS, Golyaev V, Turco S, Gubaeva EG, Rajeswaran R, et al. 2016. A viral protein suppresses oxidative burst– and salicylic acid–dependent autophagy and facilitates bacterial growth on virus-infected plants. *New Phytol.* 211:1020–34
202. Marshall RS, Vierstra RD. 2018. Proteasome storage granules protect proteasomes from autophagic degradation upon carbon starvation. *eLife*. In press

198. Reports that the phagophore emerges from the endoplasmic reticulum and that this process requires ATG9.

199. Provides an early example of an ATG8-interacting protein regulating autophagosome formation rather than cargo recruitment.

202. Describes a novel route to protect substrates from autophagy by aggregating them into a membrane-less cytoplasmic compartment.