Trends in Plant Science

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Opinion

ATG8 Expansion: A Driver of Selective Autophagy Diversification?

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Selective autophagy is a conserved homeostatic pathway that involves engulfment of specific cargo molecules into specialized organelles called autophagosomes. The ubiquitin-like protein ATG8 is a central player of the autophagy network that decorates autophagosomes and binds to numerous cargo receptors. Although highly conserved across eukaryotes, ATG8 diversified from a single protein in algae to multiple isoforms in higher plants. We present a phylogenetic overview of 376 ATG8 proteins across the green plant lineage that revealed family-specific ATG8 clades. Because these clades differ in fixed amino acid polymorphisms, they provide a mechanistic framework to test whether distinct ATG8 clades are functionally specialized. We propose that ATG8 expansion may have contributed to the diversification of selective autophagy pathways in plants.

Trends

Selective autophagy is an ancient membrane-trafficking pathway that is essential for cellular homeostasis.

Selective autophagy involves engulfment of autophagic cargo within double-membrane vesicles called autophagosomes.

Autophagosomes are decorated by ATG8, a ubiquitin-like protein conserved across eukaryotes that is expanded in higher plants.

Selective cargo recruitment is mediated by autophagy receptors that interact with ATG8 via an ATG8 interaction motif (AIM). Specialization of autophagy receptors toward ATG8 variants contributes to selective autophagy.

Although selective autophagy plays important roles in development and stress tolerance, the molecular mechanisms underlying selectivity are currently elusive in plants.

Autophagy

Autophagy is an ancient membrane-trafficking pathway that mediates cellular recycling during stress and cellular differentiation. This conserved trafficking pathway carries cytoplasmic material to the vacuole for recycling or to the plasma membrane for secretion [1,2]. In contrast to the relatively immobile 26S proteasome system, autophagy is capable of long-distance transport of molecules, protein complexes, or even organelles. Initially, autophagy was discovered as a non-selective bulk degradation mechanism that provides energy and building blocks for survival during starvation stress. However, it is now well established that autophagy is highly selective and tightly regulated [2–6]. Receptors and scaffold proteins mediate the recruitment of specific cargo into double-membrane vesicles, the so-called **autophagosomes** (see Glossary), that function as autophagy organelles [7–10]. Autophagosomes and their cargos are then carried either to lytic compartments of the cell for recycling or to the plasma membrane for secretion [11–14]. The ever-growing inventory of **selective autophagy** cargo comprises a huge variety of cellular components, including damaged organelles, protein aggregates, and invading microbes [4].

Autophagy requires the concerted action of ~40 conserved proteins that are grouped into the autophagy-related (ATG) protein family [15,16]. They collectively integrate various autophagy-inducing signals and mediate the formation of autophagosomes. Autophagosome formation proceeds in three major steps: initiation, expansion, and maturation. Inducing signals such as starvation or infection converge on ATG1, a serine/threonine kinase. ATG1 in complex with ATG13 initiates the formation of a membranous structure named the phagophore [17–19]. The source of the phagophore is a matter of debate. Potential sources include the endoplasmic

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reticulum (ER) as well as ER–Golgi intermediate and ER–mitochondria contact sites [20–24]. ATG1 complex activates the phosphatidylinositol-3-kinase (PI3K) complex which is required for expansion of the phagophore. The PI3K complex localizes at the phagophore assembly site (PAS) [20,25,26]. The E1 ligase-like ATG7, E2 ligase-like ATG3, and E3 ligase-like ATG12–ATG5–ATG16 complex mediates insertion of the ubiquitin-like protein **ATG8** into the growing phagophore membrane [27,28]. The phagophore membrane grows by fusion of endosomes [29,30]. A cysteine protease, ATG4, cleaves the C-terminal part of ATG8, which exposes a terminal glycine residue. This glycine is then modified by phosphatidylethanolamine (PE) for membrane insertion [31]. Finally, cargo recruitment and membrane expansion lead to the formation of a mature autophagosome, a double-membrane vesicle which is decorated with ATG8 on both surfaces [3,32]. Mature autophagosomes are carried on cytoskeletal tracks to the tonoplast with which they fuse to deliver autophagic bodies for enzymatic recycling, or to the plasma membrane for secretion [15,28,33–36].

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The Crucial Role of ATG8

ATG8 is essential for all stages of autophagosome formation and selective cargo recruitment. ATG8 is a ubiquitin-like protein with a unique N-terminal extension [28] (Box 1). Accumulating evidence places ATG8 at the center of the autophagy process. ATG8 interacts with several proteins that are essential for autophagy signaling and cargo recruitment such as ATG1 [37,38], ATG6, and ATG7 [23,31,39]. ATG8 is also involved in trafficking and vacuolar fusion of autophagosomes. Autophagy adaptors FYCO1 and PLEKHM1 bind to ATG8 and mediate the trafficking and vacuolar fusion of autophagosomes, respectively [40,41]. Another group of ATG8-interacting proteins mediate selective cargo recruitment. These cargo receptors recognize autophagic labels, such as polyubiquitin chains or cytosolic lectins, and recruit tagged components into autophagosomes [42,43]. Interaction of **autophagy receptors** with ATG8 leads to the formation of supramolecular structures, and these facilitate the engulfment of high molecular weight cargo into autophagosomes [32,44].

Interaction of ATG8 with the core autophagy machinery, autophagy adaptors and receptors is mediated by a conserved motif called the **ATG8 interaction motif (AIM)** (or LIR–LC3II interacting region in mammals) [45]. The core AIM sequence is composed of an aromatic amino acid followed by two amino acids and a branched-chain amino acid, W/F/Y-XX-L/I/V, that is generally surrounded by negatively charged residues [45]. The first and last hydrophobic residues of AIM bind to the W and L pockets of ATG8, respectively, and the nearby acidic residues strengthen the interaction by forming non-covalent bonds with the residues surrounding the conserved W and L pockets [28] (Box 1). The AIM sequence or the neighboring residues can also be phosphorylated, enhancing the interaction with ATG8 [2,46]. Recently, two online tools were released for computational prediction of AIM in various organisms including *Arabidopsis thaliana* [47–49]. Even though the predicted AIM sequences need to be validated, these tools will facilitate the discovery of candidate AIM proteins. Nevertheless, spatiotemporal coordination of AIM-containing proteins with ATG8 is pivotal for all steps of autophagy, and is one of the key outstanding questions in the autophagy field.

The degree to which ATG8 variants have different functions is unclear. Although yeast and other fungi have a single ATG8, higher eukaryotes have several ATG8 isoforms [50,51]. Genetic characterization of ATG8 genes suggested that they are functionally redundant [52]. However, recent studies revealed functional specialization of ATG8 variants. For example, in humans, swapping three polymorphic residues between the two ATG8 homologs GABARAP and LC3B was sufficient to confer differential binding toward an autophagy-linked FYVE protein [53]. Similarly, four unique amino acids in the human ATG8 homolog LC3C govern specific binding to the antibacterial autophagy receptor NDP52 [54]. These examples highlight that subtle differences in ATG8 isoforms can provide different functional properties. However, studies

Box 1. Key Domains and Structure of Plant ATG8s

Recently Maqbool *et al.* solved the first crystal structure of a plant ATG8 protein, potato ATG8CL, in complex with a peptide containing AIM [64]. The structure revealed a similar domain organization to that seen in ATG8 proteins from mammals and yeast (reviewed in [67]). The core of the structure comprises four β-strands (β1–β4) and two α-helices (α3 and α4) adopting a β-grasp fold, which is similar to that found in ubiquitin. In addition, the structure has two α-helices (α1 and α2) that form an N-terminal α-helical domain, which is unique to ATG8 family (Figure IA). The AIM peptide sits on the surface of ATG8CL such that its hydrophobic residues are buried in two distinct pockets (W and L) of ATG8CL. The 'W' pocket is found in the region between the N-terminal α-helices and the β-grasp, and embraces the aromatic residue of AIM. The 'L' pocket is located within the ubiquitin-like fold and embraces the branched-chain amino acid in the core AIM (Figure IA).

Mapping consensus alignment sequences on the ATG8CL structure showed that residues forming the W and L pockets and the C-terminal glycine are highly conserved. Strikingly, N-terminal helices, unique to ATG8 and lacking in other ubiquitin-like proteins, are highly variable (Figure IB). These helices mediate the hemifusion of ATG8-containing vesicles with each other for expansion of autophagosomes. The variation at the N-terminus may contribute to the specialization of autophagosomes by preventing fusion of membranes containing different ATG8s.

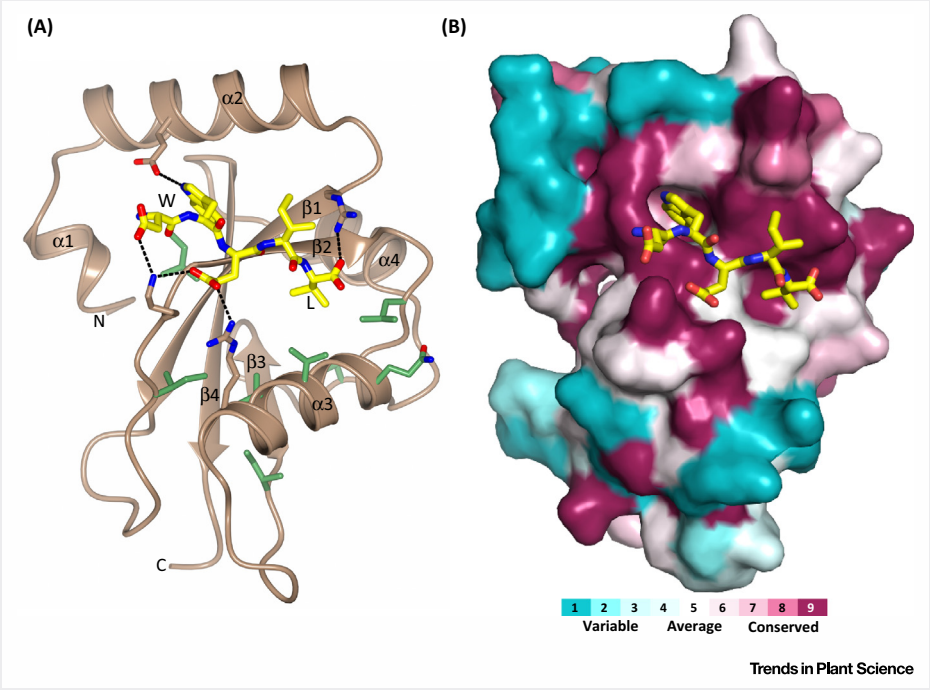

Figure I. N-Terminal Regions of Plant ATG8s Are Highly Variable. (A) ATG8CL in complex with the AIM peptide (DWEIV) derived from *Phytophthora infestans* PexRD54 virulence effector. α-Helices, β-strands, N-terminal region, and the C-terminal glycine residue (C) are shown. W and L pockets are marked by W and L, respectively. AIM peptide is shown in yellow. Eight residues that differentiate family-specific ATG8 clades are shown as green. (B) Structural modeling of plant ATG8 consensus alignment on potato ATG8CL. Conservation of each residue is mapped onto the ATG8CL structure with highly conserved amino acids being shown in magenta and the variable region depicted in light blue. The AIM-containing peptide is displayed as sticks with yellow carbon atoms.

Glossary

ATG8: a ubiquitin-like protein that plays a central role in autophagosome biogenesis and selective cargo recruitment.
ATG8 interaction motif (AIM): a conserved motif that facilitates the interaction between cargo receptors and other ATG8-interacting proteins with ATG8.
Autophagosomes: double-membrane vesicles that function as autophagy organelles.
Autophagy receptors: modular proteins that carry an AIM and recruit autophagic cargo to autophagosomes.
Monophyletic clade: a taxonomic group that includes an ancestral taxon and all of its descendants. Members of monophyletic clade share derived characteristics, so-called synapomorphies.
Neighbor-net network: a distance-based method for constructing phylogenetic networks that allow visualization of non-tree-like evolutionary history of genes resulting from recombination, hybridization, gene conversion, and gene transfer.
PexRD54: a secreted pathogen effector protein that binds to potato ATG8 proteins via an AIM and stimulates autophagosome formation.
Selective autophagy: a conserved endomembrane transport pathway that is crucial for maintaining cellular homeostasis.

on ATG8 specialization in plants are limited. **PexRD54**, a virulence effector protein of the Irish famine pathogen *Phytophthora infestans*, has ~10-fold higher binding affinity for potato ATG8C-like protein (ATG8CL) compared to ATG8I-like protein (ATG8IL) [55]. Concordantly, PexRD54 specifically stimulates the formation of ATG8CL-labeled autophagosomes but not of ATG8IL-labeled autophagosomes [55].

Higher plants have up to 22 different ATG8 isoforms (Table 1). We have investigated ATG8 diversity across green plants. Our phylogenetic analyses revealed distinct sets of ATG8 variants for single plant species and **monophyletic clades** of higher taxonomic order. Comparative

Table 1. Numbers of ATG8 Isoforms across the Green Plant Lineage

Species	Family	Number of sequences	Higher taxonomic group
Chlamydomonas reinhardtii	Chlamydomonadaceae	1	Green algae
Chlorella variabilis	Trebouxiophyceae	1	Green algae
Coccomyxa subellipsoidea	Coccomyxaceae	1	Green algae
Micromonas pusilla	Mamiellaceae	1	Green algae
Micromonas sp. *RCC299*	Mamiellaceae	1	Green algae
Ostreococcus lucimarinus	Bathycoccaceae	1	Green algae
Ostreococcus tauri	Bathycoccaceae	1	Green algae
Volvox carteri	Volvocaceae	1	Green algae
Physcomitrella patens	Funariaceae	6	Moss
Selaginella moellendorfii	Selaginellaceae	3	Lycophyte
Picea sitchensis	Pinaceae	3	Gymnosperm
Amborella trichopoda	Amborellaceae	2	Angiosperm
Arabidopsis lyrata	Brassicaceae	9	Angiosperm
Arabidopsis thaliana	Brassicaceae	9	Angiosperm
Arabis alpina	Brassicaceae	3	Angiosperm
Brassica napus	Brassicaceae	22	Angiosperm
Brassica rapa	Brassicaceae	13	Angiosperm
Capsella rubella	Brassicaceae	6	Angiosperm
Capsella sativa	Brassicaceae	22	Angiosperm
Eutrema salsugineum	Brassicaceae	10	Angiosperm
Tarenaya hassleriana	Brassicaceae	10	Angiosperm
Carica papaya	Caricaceae	4	Angiosperm
Manihot esculenta	Euphorbiaceae	7	Angiosperm
Ricinus communis	Euphorbiaceae	7	Angiosperm
Glycine max	Fabaceae	12	Angiosperm
Medicago truncatula	Fabaceae	4	Angiosperm
Phaseolus vulgaris	Fabaceae	6	Angiosperm
Linum usitatissimum	Linaceae	7	Angiosperm
Gossypium raimondii	Malvaceae	16	Angiosperm
Theobroma cacao	Malvaceae	5	Angiosperm
Eucalyptus grandis	Myrthaceae	10	Angiosperm
Mirabilis jalapa	Nyctaginaceae	5	Angiosperm
Mimulus guttatus	Phrymaceae	5	Angiosperm
Aegilops tauschii	Poaceae	2	Angiosperm
Brachypodium distachyon	Poaceae	4	Angiosperm
Hordeum vulgare ssp. *vulgare*	Poaceae	3	Angiosperm
Oryza sativa	Poaceae	4	Angiosperm
Panicum hallii	Poaceae	4	Angiosperm
Panicum virgatum	Poaceae	6	Angiosperm
Setaria italica	Poaceae	9	Angiosperm
Sorghum bicolor	Poaceae	3	Angiosperm
Triticum aestivum	Poaceae	14	Angiosperm

Table 1. (continued)

Species	Family	Number of sequences	Higher taxonomic group
Triticum urartu	Poaceae	2	Angiosperm
Zea mays	Poaceae	5	Angiosperm
Aquilegia coerulea	Ranunculaceae	4	Angiosperm
Coffea canephora	Rubiaceae	4	Angiosperm
Citrus clementina	Rutaceae	10	Angiosperm
Citrus sinensis	Rutaceae	8	Angiosperm
Populus trichocarpa	Salicaceae	14	Angiosperm
Capsicum annuum	Solanaceae	5	Angiosperm
Nicotiana bethamiana	Solanaceae	8	Angiosperm
Nicotiana sylvestris	Solanaceae	6	Angiosperm
Nicotiana tabacum	Solanaceae	12	Angiosperm
Nicotiana tomentosiformis	Solanaceae	6	Angiosperm
Solanum lycopersicum	Solanaceae	7	Angiosperm
Solanum pennelli	Solanaceae	7	Angiosperm
Solanum tuberosum	Solanaceae	9	Angiosperm
Vitis vinifera	Vitaceae	6	Angiosperm
Total = 58	Total = 18	Total = 386	

analysis revealed distinct ATG8 clades that are unique to particular plant families. Understanding the evolution of ATG8 proteins and the selective regimes that drove their diversification provides a framework for functional studies on selective autophagy in plants.

The *ATG8* Gene Family Is Expanded in Higher Plants

Flowering plants evolved multiple ATG8 isoforms, unlike early-diverged plant lineages and various other eukaryotes, such as fungi and oomycetes, which possess only a single ATG8 (Table 1). The model plant *A. thaliana* encodes nine isoforms (AtATG8a–i) [56–58]. To examine plant ATG8 diversity in the evolutionary context of eukaryotes we calculated a **neighbor-net network** of ATG8 homologs from *A. thaliana* and *Brachypodium distachyon* together with homologs from representative species from fungi (five), oomycetes (five), nematodes (one), insects (one) and mammals (one) (Figure 1A). The network supports two major clades (I and II) of plant ATG8s with differing similarities to other eukaryotes. Clade I groups together with ATG8 homologs from fungi and oomycetes whereas clade II is more similar to the ATG8 homologs of animals.

To further estimate ATG8 diversity across green plants we screened for ATG8 homologs by similarity searches for the nine *A. thaliana* isoforms in genome sequences of 58 plant species belonging to 18 different plant families. We included key representatives from the plant evolutionary tree, such as green algae (eight species), the moss *Physcomitrella patens*, the lycophyte *Selaginella moellendorfii*, the gymnosperm *Picea sitchensis*, and 51 angiosperm species including *Amborella trichopoda* (Table 1). For further analyses we only selected sequences that contain the C-terminal glycine and cover all ATG8-specific α-helices and β-sheets. Our screen identified 376 unique ATG8 sequences, with *Brassica napus* and *Capsella sativa* carrying the largest number (22 sequences; Table S1 in the supplemental information online). Analysis of ratios of nonsynonymous and synonymous substitutions revealed overall strong purifying selection of plant ATG8 homologs and no apparent evidence for positive selection acting on particular residues (Table S2 in the supplemental information online). Interestingly, some plant species lack

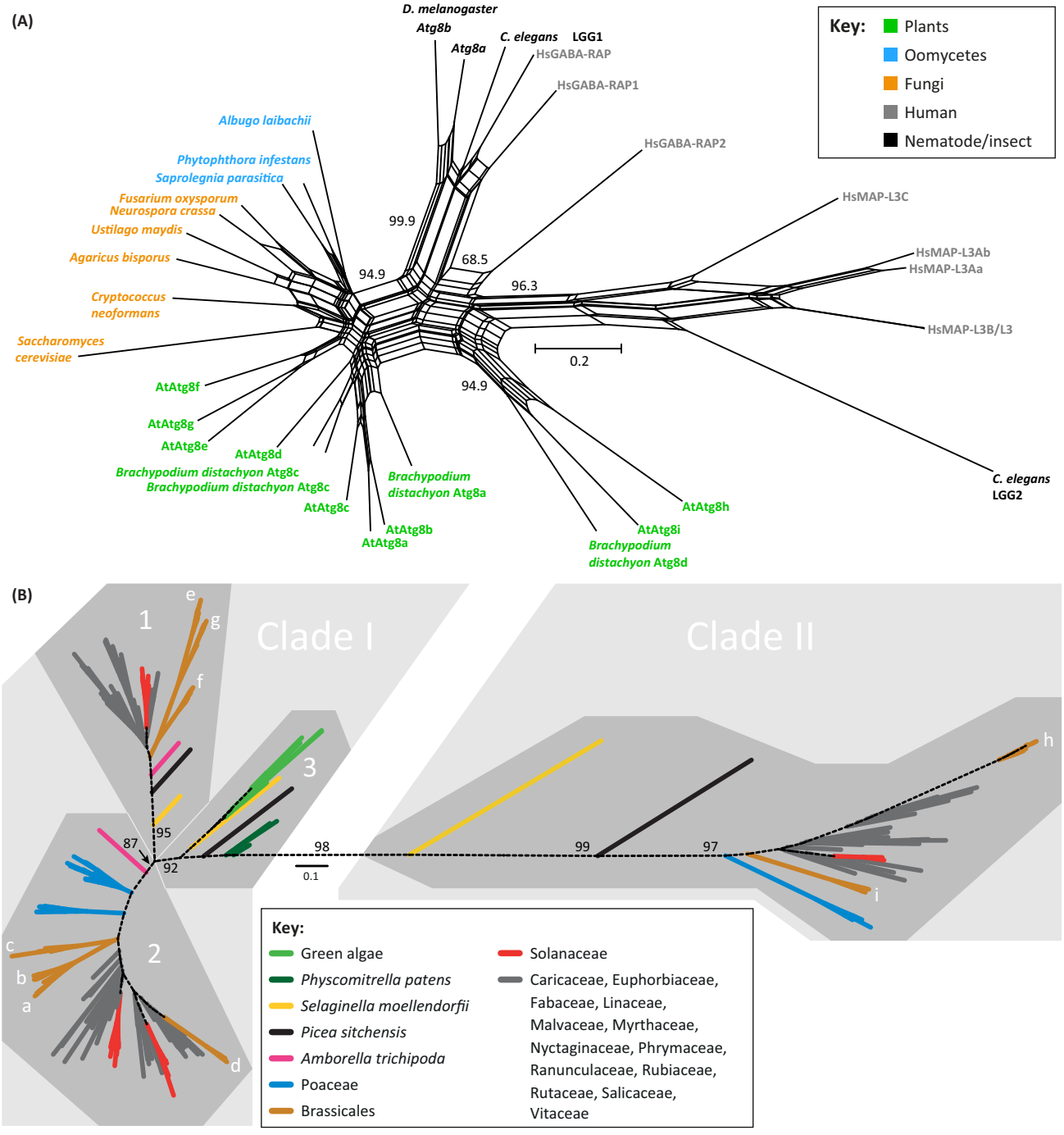

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Figure 1. The *ATG8* Gene Family Is Expanded in Higher Plants. (A) Neighbor-net analysis [68] of 34 *ATG8* nucleotide sequences from 14 eukaryotic species. The network was calculated with Splitstree4 from a 354 nt alignment (MUSCLE) applying the general time reversible (GTR) distance matrix. Bootstrap support values of 1000 replicates are given next to edges between major clades. (B) Unrooted maximum likelihood phylogram of 376 plant ATG8 homologs with gray boxes highlighting clades I and II, and colors indicating major plant lineages. The tree was calculated in PhyML from a 351 nt alignment (MUSCLE [69]). Branch lengths correspond to genetic distances as inferred from the GTR distance matrix. Approximate likelihood ratio test values of central branches are given in %. (a–i) Clades that contain the corresponding *A. thaliana* ATG8 variants AtATG8a–AtATG8i. (1–3) Three subclades of clade I.

clade II ATG8 sequences, for example *Capsicum annuum* and *Zea mays*, but we cannot rule out that this might be due to genome assembly issues [51] (Figures S1,S2 in the supplemental information online). For plant species that underwent multiple whole-genome duplications, we observed a general trend toward increased copy-numbers of *ATG8* genes per species, indicating the contribution of large-scale genome duplications to recent *ATG8* gene family expansions in plants (Table 1) [59–61].

To assess the evolutionary history of *ATG8* gene radiations in plants, we performed maximum likelihood analyses using an alignment of all 376 identified ATG8 sequences (Figure 1B). The phylogeny confirmed the previously described clades I and II. In addition, clade I divided into three subclades (1–3 in Figure 1B). Subclade I-1 contains sequences from flowering and non-flowering plants but lacks homologs from green algae and mosses. Subclade I-2 exclusively contains homologs from flowering plants and represents an angiosperm-specific ATG8 group. Homologs from green algae and mosses, together with one homolog of each from *S. moellendorfii* and *P. sitchensis*, form a polyphyletic sister clade to subclades I-1 and I-2. Because the topology of the major monophyletic clades of ATG8s reflects the chronology of plant evolution [62], we conclude that the presence of multiple distinct ATG8 isoforms is an ancient trait in plants. Hence, evolution of the plant *ATG8* gene family involved both the expansion and extinction of particular ATG8 lineages. However, ATG8 expansion dominates in higher plants,

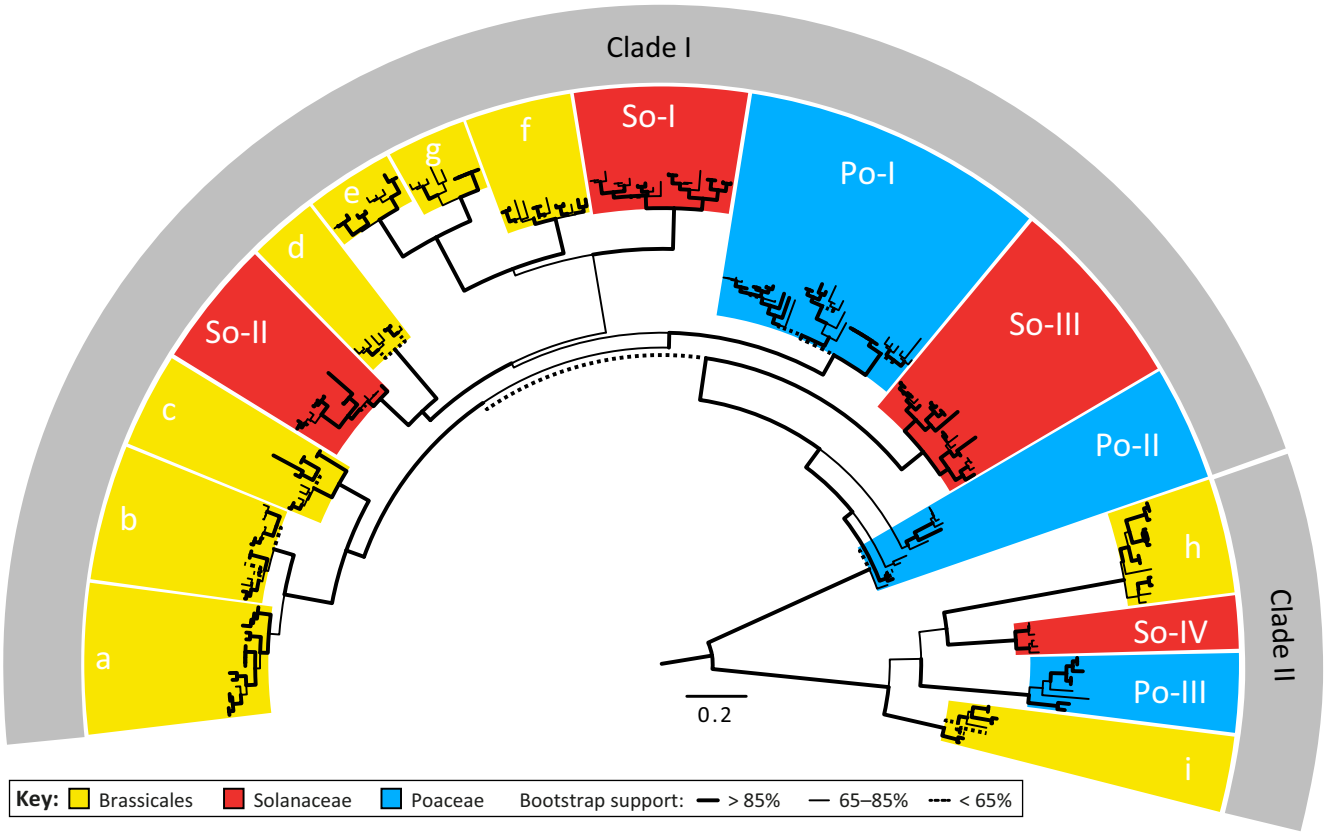

Figure 2. Plants Have Family-Specific ATG8 Clades. Neighbor-joining analysis of ATG8 sequences from three plant lineages (Brassicales, a–i; Solanaceae, So-I/II/III/IV; Poacea, PoI/II/III) showing monophyletic clades of higher taxonomic order. The tree was calculated in MEGA v6 from a 354 nt alignment (MUSCLE, codon-based) of 220 sequences applying the Kimura-2 distance matrix using transitions and transversions, gamma distributed rates among sites, and pairwise deletion [70]. Bootstrap support values of 1000 replicates are highlighted on branches.

possibly driven by different types of duplication events and at least three ancient radiation events (subclades I1–3) [51]. Interestingly, subclades I-1 and I-2 correlate with the emergence of vascular plants and angiosperms, respectively. Surprisingly, some ATG8 isoforms in clade II lack amino acid residues after the terminal glycine. Future experiments will determine whether these 'pre-activated' ATG8 proteins could be incorporated into autophagosome membranes without ATG4 processing [51].

Plants Have Family-Specific ATG8 Clades

We noticed that plant ATG8 isoforms formed well-supported monophyletic clades of higher taxonomic order (Figure 1B). A separate phylogenetic analysis of three plant lineages confirmed the presence of plant family-specific sets of ATG8 isoforms (Figure 2 and Figures S1-S3 in the supplemental information online). This includes nine clades in Brassicales (a–i), four clades in Solanaceae (So-I to IV), and at least two clades in Poaceae (Po-I and Po-III). Po-II is polyphyletic and intermediate to the major plant ATG8 clades I and II (Figure 2). The nine *A. thaliana* ATG8 variants, including closely related isoforms, have distinct spatial and temporal expression patterns consistent with functional specialization [63]. In summary, in contrast to previous studies [51], our extensive phylogenetic analyses revealed that each plant family has its own set of ATG8 isoforms that have been maintained over millions of years of evolution. Whether these family-specific ATG8 clades are functionally specialized is an exciting hypothesis that deserves to be tested in future.

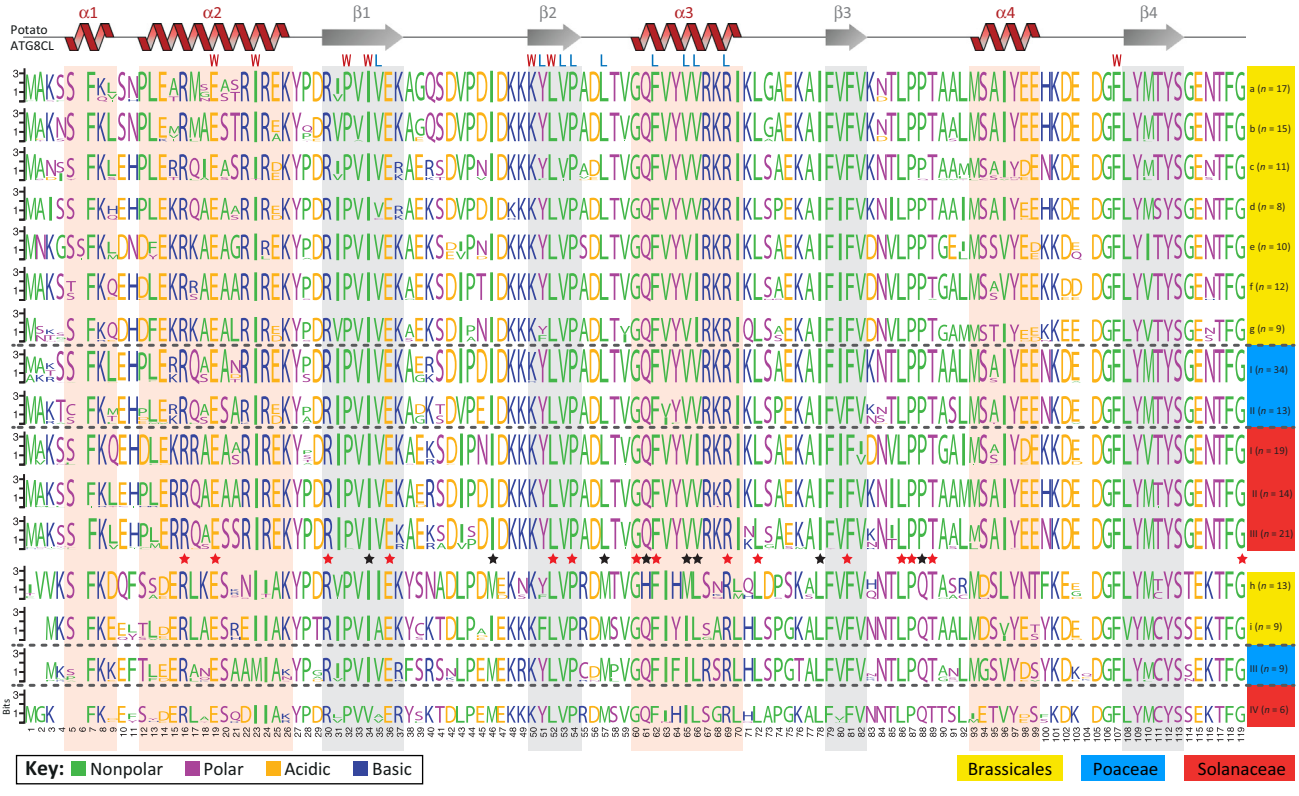

Figure 3. Distinct Amino Acid Polymorphisms Define Atg8 Subfamilies in Plants. MEME (multiple EM for motif elicitation [71]) sequence logos showing consensus amino acid sequences of plant family-specific clades of ATG8 isoforms from Brassicales (A–I), Solanaceae (So-I/II/III/IV), and Poaceae (Po-I/II/III). Color-coded sequences are aligned in accordance with the alignment of the phylogenetic analysis in Figure 2. Red asterisks depict residues that are conserved in all clades, and black asterisks highlight amino acid residues with fixed differences between clades. The protein model above is adopted from the potato homolog ATG8CL and corresponds to the structure shown in Box 1. W and L indicate key residues for the two hydrophobic pockets involved in AIM binding.

Distinct Amino Acid Polymorphisms Define ATG8 Subfamilies in Plants

To identify which polymorphisms define distinct subfamilies of ATG8, we generated a consensus sequence for each ATG8 clade and searched for unique conserved amino acid residues. This revealed eight polymorphic amino acid residues that are conserved in all members of a clade but differ between family-specific ATG8 clades (black asterisks in Figure 3). Taking advantage of the availability of the first crystal structure of a plant ATG8 protein [64], we mapped polymorphic residues on the structure and discovered that six of the eight family-specific ATG8 polymorphisms are in close proximity to the W and L pockets that bind to the AIM peptide (Box 1).

In addition, three of the four ATG8 residues that form electrostatic interactions with the AIM peptide, Glu19, Arg30, and Arg69, are invariant whereas residue Lys48 occurs as Lys or Arg (Figure 3 and Box 1). Also, the residues forming W and L pockets tend to be conserved, with 10 of the 15 residues being invariant across all clades (red asterisks in Figure 3). This suggests that residues neighboring the core pockets may determine ATG8 binding specificity [64]. Subtle differences between human ATG8 isoforms confer distinct binding affinities toward interacting proteins, indicating that polymorphisms in plant family-specific ATG8 clades could define selective autophagy pathways [53,54].

Concluding Remarks

Autophagy signaling is intricately linked with many processes, such as vesicle trafficking, secretion, organelle dynamics, cellular energy metabolism, protein synthesis, and the cell cycle [3,16,65,66]. This creates a complex evolutionary landscape, where the small ATG8 protein (~120 amino acids) connects with different pathways while retaining its primary function. We show that ATG8 has dramatically expanded and diversified in plants. We have defined multiple well-delimited ATG8 clades that tend to be specific to particular plant families. ATG8 expansion may have facilitated the connectivity of autophagy pathways and provided robustness to the autophagy network. Functional specialization of homeostatic processes such as autophagy may have provided selective advantages for the sessile lifestyle of plants.

Given that different plant families have maintained distinct sets of ATG8 isoforms, comparative studies should yield valuable insights into adaptive evolution of selective autophagy. ATG8 expansion may have been driven by functional diversification, for example interaction with different sets of AIM-containing proteins. ATG8 may also have coevolved with pathogen effectors. For additional issues that remain to be addressed see Outstanding Questions. Decoding the mechanistic underpinnings of ATG8 specialization will help to define the evolution and diversification of selective autophagy in plants.

Outstanding Questions

Are distinct clades of ATG8s functionally specialized? Do they bind to different sets of AIM-containing proteins?

What is the degree of functional redundancy between different ATG8 isoforms? Do ATG8s have family-specific functions?

What drove the diversification of ATG8s? Was it driven by an increase in developmental complexity or environmental adaptations?

How do the binding interfaces between ATG8 and AIM-containing proteins vary? Which residues define the binding specificity?

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Supplemental Information

Supplemental information associated with this article can be found online at doi:10.1016/j.tplants.2016.11.015.

References

1. He, C. and Klionsky, D.J. (2009) Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* 43, 67–93
2. Farré, J.C. and Subramani, S. (2016) Mechanistic insights into selective autophagy pathways: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* 17, 537–552
3. Zaffagnini, G. and Martens, S. (2016) Mechanisms of selective autophagy. *J. Mol. Biol.* 428, 1714–1724
4. Stolz, A. *et al.* (2014) Cargo recognition and trafficking in selective autophagy. *Nat. Cell Biol.* 16, 495–501
5. Green, D.R. and Levine, B. (2014) To be or not to be? How selective autophagy and cell death govern cell fate. *Cell* 157, 65–75
6. Jin, M. *et al.* (2013) SnapShot: selective autophagy. *Cell* 152, 368
7. Johansen, T. and Lamark, T. (2014) Selective autophagy goes exclusive. *Nat. Cell Biol.* 16, 395–397
8. Svenning, S. and Johansen, T. (2013) Selective autophagy. *Essays Biochem.* 55, 79–92

9. Kraft, C. *et al.* (2010) Selective autophagy: ubiquitin-mediated recognition and beyond. *Nat. Cell Biol.* 12, 836–841
10. Kadandale, P. and Kiger, A.A. (2010) Role of selective autophagy in cellular remodeling: 'self-eating' into shape. *Autophagy* 6, 1194–1195
11. Rubinsztein, D.C. *et al.* (2012) Mechanisms of autophagosome biogenesis. *Curr. Biol.* 22, R29–R34
12. Moreau, K. *et al.* (2011) Autophagosome precursor maturation requires homotypic fusion. *Cell* 146, 303–317
13. Jahreiss, L. *et al.* (2008) The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. *Traffic* 9, 574–587
14. Nakatogawa, H. *et al.* (2007) Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 130, 165–178
15. Wen, X. and Klionsky, D.J. (2016) An overview of macroautophagy in yeast. *J. Mol. Biol.* 428, 1681–1699
16. Kraft, C. and Martens, S. (2012) Mechanisms and regulation of autophagosome formation. *Curr. Opin. Cell Biol.* 24, 496–501
17. Yamamoto, H. *et al.* (2016) The intrinsically disordered protein Atg13 mediates supramolecular assembly of autophagy initiation complexes. *Dev. Cell.* 38, 86–99
18. Suzuki, S.W. *et al.* (2015) Atg13 HORMA domain recruits Atg9 vesicles during autophagosome formation. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3350–3355
19. Popelka, H. and Klionsky, D.J. (2015) One step closer to understanding mammalian macroautophagy initiation: interplay of 2 HORMA architectures in the ULK1 complex. *Autophagy* 11, 1953–1955
20. Vicinanza, M. *et al.* (2015) PI_5P regulates autophagosome biogenesis. *Mol. Cell* 57, 219–234
21. Sanchez-Wandelmer, J. *et al.* (2015) ERES: sites for autophagosome biogenesis and maturation? *J. Cell Sci.* 128, 185–192
22. Ge, L. *et al.* (2015) Biogenesis of autophagosomal precursors for LC3 lipidation from the ER–Golgi intermediate compartment. *Autophagy* 11, 2372–2374
23. Stanley, R.E. *et al.* (2014) The beginning of the end: how scaffolds nucleate autophagosome biogenesis. *Trends Cell Biol.* 24, 73–81
24. Ge, L. *et al.* (2013) The ER–Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. *Elife* 2, e00947
25. Ge, L. *et al.* (2014) Phosphatidylinositol 3-kinase and COPII generate LC3 lipidation vesicles from the ER–Golgi intermediate compartment. *Elife* 3, e04135
26. Sun, Q. *et al.* (2009) Regulation of Beclin 1 in autophagy. *Autophagy* 5, 713–716
27. Fujioka, Y. *et al.* (2008) In vitro reconstitution of plant Atg8 and Atg12 conjugation systems essential for autophagy. *J. Biol. Chem.* 283, 1921–1928
28. Klionsky, D.J. and Schulman, B.A. (2014) Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. *Nat. Struct. Mol. Biol.* 21, 336–345
29. Lamb, C.A. *et al.* (2013) The autophagosome: origins unknown, biogenesis complex. *Nat. Rev. Mol. Cell Biol.* 14, 759–774
30. Lamb, C.A. *et al.* (2013) Endocytosis and autophagy: shared machinery for degradation. *Bioessays* 35, 34–45
31. Kaufmann, A. *et al.* (2014) Molecular mechanism of autophagic membrane-scaffold assembly and disassembly. *Cell* 156, 469–481
32. Wurzer, B. *et al.* (2015) Oligomerization of p62 allows for selection of ubiquitinated cargo and isolation membrane during selective autophagy. *Elife* 4, e08941
33. Kruppa, A.J. *et al.* (2016) Myosins, actin and autophagy. *Traffic* 17, 878–890
34. Wang, Y. *et al.* (2015) Disruption of microtubules in plants suppresses macroautophagy and triggers starch excess-associated chloroplast autophagy. *Autophagy* 11, 2259–2274
35. Bernard, A. and Klionsky, D.J. (2015) Toward an understanding of autophagosome–lysosome fusion: the unsuspected role of ATG14. *Autophagy* 11, 583–584
36. Li, F. and Vierstra, R.D. (2012) Autophagy: a multifaceted intracellular system for bulk and selective recycling. *Trends Plant Sci.* 17, 526–537
37. Nakatogawa, H. *et al.* (2012) The autophagy-related protein kinase Atg1 interacts with the ubiquitin-like protein Atg8 via the Atg8 family interacting motif to facilitate autophagosome formation. *J. Biol. Chem.* 287, 28503–28507
38. Noda, N.N. *et al.* (2010) Atg8-family interacting motif crucial for selective autophagy. *FEBS Lett.* 584, 1379–1385
39. Kaufmann, A. and Wollert, T. (2014) Scaffolding the expansion of autophagosomes. *Autophagy* 10, 1343–1345
40. Pankiv, S. *et al.* (2010) FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. *J. Cell Biol.* 188, 253–269
41. McEwan, D.G. *et al.* (2015) PLEKHM1 regulates autophagosome–lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol. Cell* 57, 39–54
42. Randow, F. and Youle, R.J. (2014) Self and nonself: how autophagy targets mitochondria and bacteria. *Cell Host Microbe* 15, 403–411
43. Boyle, K.B. and Randow, F. (2013) The role of 'eat-me' signals and autophagy cargo receptors in innate immunity. *Curr. Opin. Microbiol.* 16, 339–348
44. Bertipaglia, C. *et al.* (2016) Higher-order assemblies of oligomeric cargo receptor complexes form the membrane scaffold of the Cvt vesicle. *EMBO Rep.* 17, 1044–1060
45. Birgisdottir, A.B. *et al.* (2013) The LIR motif – crucial for selective autophagy. *J. Cell Sci.* 126, 3237–3247
46. Matsumoto, G. *et al.* (2011) Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol. Cell* 44, 279–289
47. Kalvari, I. *et al.* (2014) iLIR: a web resource for prediction of Atg8-family interacting proteins. *Autophagy* 10, 913–925
48. Jacomin, A.C. *et al.* (2016) iLIR database: a web resource for LIR motif-containing proteins in eukaryotes. *Autophagy* 12, 1945–1953
49. Xie, Q. *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, 876–887
50. Popelka, H. and Klionsky, D.J. (2015) Post-translationally-modified structures in the autophagy machinery: an integrative perspective. *FEBS J.* 282, 3474–3488
51. Seo, E. *et al.* (2016) Comparative analyses of ubiquitin-like ATG8 and cysteine protease ATG4 autophagy genes in the plant lineage and cross-kingdom processing of ATG8 by ATG4. *Autophagy* 12, 2054–2068
52. Shpilka, T. *et al.* (2011) Atg8: an autophagy-related ubiquitin-like protein family. *Genome Biol.* 12, 226
53. Lystad, A.H. *et al.* (2014) Structural determinants in GABARAP required for the selective binding and recruitment of ALFY to LC3B-positive structures. *EMBO Rep.* 15, 557–565
54. von Muhlinen, N. *et al.* (2012) LC3C, bound selectively by a noncanonical LIR motif in NDP52, is required for antibacterial autophagy. *Mol. Cell* 48, 329–342
55. Dagdas, Y.F. *et al.* (2016) An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. *Elife* 5, e10856
56. Hanaoka, H. *et al.* (2002) Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an *Arabidopsis* autophagy gene. *Plant Physiol.* 129, 1181–1193
57. Doelling, J.H. *et al.* (2002) The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in *Arabidopsis thaliana*. *J. Biol. Chem.* 277, 33105–33114
58. Kellner, R. (2016) History of *A. thaliana* Atg8 isoform identifiers. *FigShare* Published online June 30, 2016. http://dx.doi.org/10.6084/m9.figshare.3468506.v1
59. Renny-Byfield, S. and Wendel, J.F. (2014) Doubling down on genomes: polyploidy and crop plants. *Am. J. Bot.* 101, 1711–1725
60. Lee, T.H. *et al.* (2013) PGDD: a database of gene and genome duplication in plants. *Nucleic Acids Res.* 41, D1152–D1158

61. Lysak, M.A. *et al.* (2005) Chromosome triplication found across the tribe Brassiceae. *Genome Res.* 15, 516–525
62. Magallon, S. *et al.* (2015) A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytol.* 207, 437–453
63. Winter, D. *et al.* (2007) An 'electronic fluorescent pictograph' browser for exploring and analyzing large-scale biological data sets. *PLoS One* 2, e718
64. Maqbool, A. *et al.* (2016) Structural basis of host autophagy-related protein 8 (ATG8) binding by the Irish potato famine pathogen effector protein PexRD54. *J. Biol. Chem.* 291, 20270–20282
65. Liu, J. and Debnath, J. (2016) The evolving, multifaceted roles of autophagy in cancer. *Adv. Cancer Res.* 130, 1–53
66. Martens, S. and Bachmair, A. (2015) How cells coordinate waste removal through their major proteolytic pathways. *Nat. Cell Biol.* 17, 841–842
67. Hurley, J.H. and Schulman, B.A. (2014) Atomistic autophagy: the structures of cellular self-digestion. *Cell* 157, 300–311
68. Huson, D.H. and Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* 23, 254–267
69. Dereeper, A. *et al.* (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 36, W465–W469
70. Tamura, K. *et al.* (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729
71. Bailey, T.L. *et al.* (2009) MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 37, W202–W208