Protoplasma (2012) 249:285–299
DOI 10.1007/s00709-011-0296-z

REVIEW ARTICLE

Variations on a theme: plant autophagy in comparison to yeast and mammals

Tamar Avin-Wittenberg · Arik Honig · Gad Galili

Received: 20 May 2011 / Accepted: 22 May 2011 / Published online: 10 June 2011

Abstract Autophagy is an evolutionary conserved process of bulk degradation and nutrient sequestration that occurs in all eukaryotic cells. Yet, in recent years, autophagy has also been shown to play a role in the specific degradation of individual proteins or protein aggregates as well as of damaged organelles. The process was initially discovered in yeast and has also been very well studied in mammals and, to a lesser extent, in plants. In this review, we summarize what is known regarding the various functions of autophagy in plants but also attempt to address some specific issues concerning plant autophagy, such as the insufficient knowledge regarding autophagy in various plant species other than *Arabidopsis*, the fact that some genes belonging to the core autophagy machinery in various organisms are still missing in plants, the existence of autophagy multigene families in plants and the possible operation of selective autophagy in plants, a study that is still in its infancy. In addition, we point to plant-specific autophagy processes, such as the participation of autophagy during development and germination of the seed, a unique plant organ. Throughout this review, we demonstrate that the use of innovative bioinformatic resources, together with recent biological discoveries (such as the ATG8-interacting motif), should pave the way to a more comprehensive understanding of the multiple functions of plant autophagy.

Handling Editor: David Robinson

Avin-Wittenberg and Honig contributed equally to this review.

T. Avin-Wittenberg · A. Honig · G. Galili (✉)
Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel
e-mail: Gad.Galili@weizmann.ac.il

Keywords Autophagy · Plants · ATG8 · ATG18 · ATG8-interacting motif

Abbreviations

AIM	ATG8-interacting motif
ATG	Autophagy
BLAST	Basic local alignment search tool
ER	Endoplasmic reticulum
GABARAP	GABA receptor-associated protein
GATE-16	Golgi-associated ATPase enhancer of 16 kDa
GFP	Green fluorescent protein
LC3	Light chain 3 microtubule-associated protein
LIR	LC3-interacting region
NBR1	Neighbor of BRCA1
p62/SQSTM1	Sequestosome 1
PAS	Phagophore assembly site
PCD	Programmed cell death
PI3 kinase	Phosphoinositide 3 kinase
PI(3)P	Phosphatidylinositol 3 phosphate
PSV	Protein storage vacuole
RNAi	RNA interference
ROS	Reactive oxygen species
TOR	Target of rapamycin
RT-PCR	Real-time polymerase chain reaction
RUBISCO	Ribulose-1,5-bisphosphate carboxylase oxygenase
TSPO	Tryptophan-rich sensory protein
Ub	Ubiquitin
WIPI	WD repeat protein-interacting phosphoinositides

Introduction

Macroautophagy (hereafter referred to as "autophagy") is a conserved eukaryotic mechanism, which is classically defined as the degradation of cytoplasmic constituents in the lytic organelle (vacuoles in yeast and plants and lysosomes in mammals) (Xie and Klionsky 2007). The general targets of autophagy vary from long-lived proteins to protein complexes and even organelles (Reumann et al. 2010). Morphologically, autophagy begins in the formation of cup-shaped double membranes, which expand to form autophagosomes engulfing malfunctioning or unneeded macromolecules and organelles and transport them for degradation inside the vacuole. Upon arrival of the autophagosomes to the vacuoles, their outer membranes fuse with the tonoplast, creating single-membrane vesicles inside the vacuole, termed "autophagic bodies." The autophagic bodies and their contents are then degraded inside the vacuole, providing recycled materials to build new macromolecules (Bassham 2009). The genes participating in the autophagic process (termed *AuTophaGy*-related or ATG genes) were originally discovered in yeast (*Saccharomyces cerevisiae*), using autophagy-defective mutants whose cells show little or no accumulation of autophagic bodies during nutrient starvation (Xie and Klionsky 2007). Many of the ATG genes are conserved in evolution, and homologs to the yeast genes have been found in many organisms including mammals and plants (Reumann et al. 2010; Tanida 2011). The core autophagy machinery is divided into three groups: (i) the ATG9 cycling system including ATG1, ATG2, ATG9, ATG13, ATG18, and ATG27; (ii) the PI3 kinase complex comprised of ATG6/VPS30/BECLIN1, ATG14, VPS15, and VPS34; and (iii) the ubiquitin (Ub)-like protein system comprised of ATG3, ATG4, ATG5, ATG7, ATG8, ATG10, ATG12, and ATG16 (for examples, see the following reviews: Xie and Klionsky 2007; Farré et al. 2009; Suzuki and Ohsumi 2010; Tanida 2011). This review focuses on selected aspects of plant autophagy, which have not been extensively reviewed before. A more comprehensive view of the plant autophagy machinery and its multiple functions can be obtained in the following representative reviews as examples (Thompson and Vierstra 2005; Bassham et al. 2006; Bassham 2007, 2009; Kwon and Park 2008; Hayward and Dinesh-Kumar 2010; Reumann et al. 2010; Yoshimoto et al. 2010).

The impact of autophagy on plant growth and response to stress

Autophagy studies in plants initially focused on nutrient starvation and senescence. Homologs of many of the yeast ATG genes were found in plants and studies of T-DNA knockout mutants in these genes existing in the model plant *Arabidopsis thaliana* (such as *atg7*, *atg5*, *atg4a4b*, *atg9*, *atg10*, *atg12*) revealed increased sensitivity to carbon and nitrogen starvation (Doelling et al. 2002; Hanaoka et al. 2002; Yoshimoto et al. 2004; Thompson et al. 2005; Phillips et al. 2008; Chung et al. 2010). In addition, under short-day conditions, the autophagy knockout *Arabidopsis* mutants exhibited early senescence of rosette leaves and cotyledons and also had a lower seed set than wild-type plants. Under favorable (nonstress) growth conditions, most of the *Arabidopsis* ATG knockout lines examined so far develop relatively normally (Doelling et al. 2002; Hanaoka et al. 2002; Yoshimoto et al. 2004; Thompson et al. 2005; Phillips et al. 2008; Chung et al. 2010). The normal development of ATG knockout plants is surprising since, in mammals, knockout of ATG genes may lead to severe developmental phenotypes such as embryo lethality (Kuma et al. 2004; Komatsu et al. 2005). One exception to this rule is the ATG6 knockout plant which displays aberrant pollen germination, and therefore homozygous knockout plants were not achieved (Fujiki et al. 2007; Qin et al. 2007). It has been argued that this phenotype stems from the possible involvement of ATG6 in cellular processes and is different than the classical autophagy process of nutrient recycling (Fujiki et al. 2007).

In the past few years, our knowledge of the functions of autophagy in plants has been greatly expanded. Autophagy in plants has been shown to occur at basal levels under favorable (nonstress) growth conditions (Sláviková et al. 2005; Inoue et al. 2006) and was also shown to be involved in the response of plants to various abiotic and biotic stresses as well as in hormonal control (Xiong et al. 2007; Slavikova et al. 2008; Liu et al. 2009; Hayward and Dinesh-Kumar 2010; Vanhee et al. 2011) . It is also important to note that degradation of the major plastid protein ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO) as well as entire chloroplasts during senescence was shown to be performed by autophagy (Ishida et al. 2008; Wada et al. 2009).

Another fascinating aspect is the role of autophagy in plant–pathogen interactions (Cacas 2010; Hayward and Dinesh-Kumar 2010). Initially, autophagy was suggested to function as an "anti-death" mechanism, limiting the extent of programmed cell death (PCD) during the hypersensitive response (Liu et al. 2005). Later, however, autophagy was suggested to take part in an opposite "pro-death" activity, since ATG mutants seemed to exhibit lower levels of PCD in response to pathogen infection (Hofius et al. 2009). A recent study (Lenz et al. 2011) attempted to settle this contradiction by suggesting that autophagy is involved in limiting necrosis, a form of disease-associated cell death. In addition, autophagy has been shown to be involved in plant–pathogen resistance by fine-tuning the salicylic acid-

mediated immune response, although the specific functions of autophagy in these processes are still a matter of debate (Love et al. 2008; Cacas and Diamond 2009).

The conservation of the autophagy machinery within the plant kingdom: comparing *Arabidopsis*, *Chlamydomonas*, and rice

The proteins composing the core autophagy machinery in plants (particularly studied in the model plant *Arabidopsis*) have been extensively studied and reviewed (see, for example, Bassham 2007; Kwon and Park 2008). In contrast, studies on the evolution of the autophagy machinery among photosynthetic organisms, ranging from lower species, such as *Chlamydomonas reinhardtii*, to major crop plants, such as cereals, are still in their infancy and have not been extensively reviewed. The morphological conservation of starvation-induced autophagy structures and the published sequence conservation of ATG genes and proteins in fully sequenced genomes of various photosynthetic organisms allow us to predict: (i) which autophagy-associated mechanisms and core signal transduction cascades might have been conserved between ancient photosynthetic organisms and different species of higher plants; and (ii) which autophagy-associated biological processes evolved plant-specific components and strategies required for the adaptation of plant physiology to various ecological needs (Reumann et al. 2010). To address this issue, we discuss in the following the comparative evolution of autophagy in the photosynthetic alga *C. reinhardtii* and in the monocot cereal rice (*Oryza sativa*) in comparison to *Arabidopsis*. All of these organisms have fully sequenced genomes and are also amongst the few members of the plant kingdom that already have relatively detailed experimental evidence for the existence of ATG genes (Crespo et al. 2005; Su et al. 2006; Díaz-Troya et al. 2008a; Shin et al. 2009; Pérez-Pérez et al. 2010). To address this issue, we rely on published data (Bassham et al. 2006; Su et al. 2006; Meijer et al. 2007; Díaz-Troya et al 2008a; Shin et al. 2009) and on our own basic local alignment search tool (BLAST)-generated analyses of sequence homology comparisons of the core autophagy genes of *Chlamydomonas*, rice, *Arabidopsis*, and the yeast *Sacharomyces cerevisae*. A schematic representation summerizing the existence of core autophagy genes in these photosynthetic model organisms is provided in Fig. 1, while Table 1 provides a more detailed description of the ATG gene terminology in the chosen organisms compared to yeast.

The alga *C. reinhardtii* is an excellent example of a simple single-celled model of a lower photosynthetic

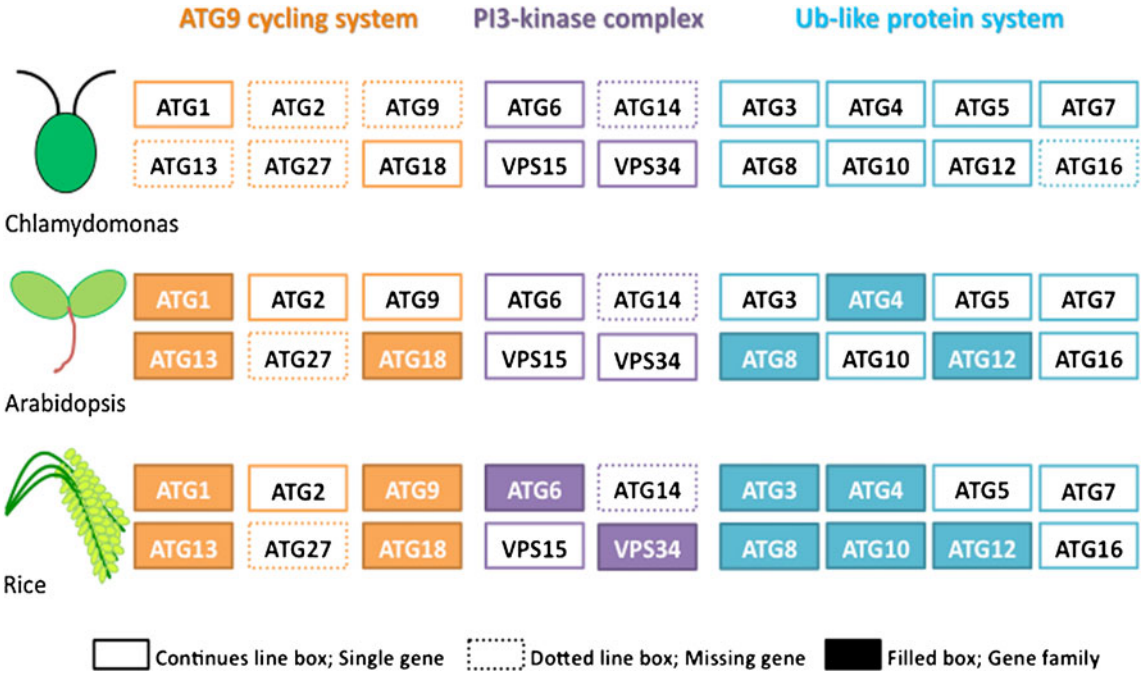

Fig. 1 The core autophagy machinery genes in three different photosynthetic model organisms: *Chlamydomonas*, *Arabidopsis*, and Rice, compared to yeast. The yeast 18 core autophagy genes were divided into three functional groups according to latest reviews (Xie and Klionsky 2007; Farré et al. 2009; Suzuki and Ohsumi 2010; Tanida 2011): Atg9 cycling system (*orange*), PI3 kinase complex (*purple*) and Ub-like protein system (*blue*). Continues line boxes stand for single genes, dotted line boxes stand for missing genes, and filled boxes stand for gene families. *Upper panel Chlamydomonas*, *middle panel Arabidopsis*, and *lower panel* rice (*Oryza sativa*). Full gene terminology is given in Table 1

Table 1 Core autophagy machinery orthologs in the model organisms: yeast, algae, and plants[a,b]

Saccharomyces cerevisiae	*Arabidopsis thaliana*	*Chlamydomonas reinhardtii*	*Oryza sativa*
*ATG*1	Small gene family: *At*2g37840, *At*3g53930, *At*3g61960	Single gene: CHLREDRAFT_195647*	Small gene family: *Os*01g60910, *Os*03g02980, *Os*03g16130, *Os*07g48100
*ATG*2	Single gene: *At*3g19190	No homolog	Single gene: *Os*06g15700
*ATG*3	Single gene: *At*5g61500	Single gene: CHLREDRAFT_186904*	Two genes: *Os*01g10290, *Os*10g41110
*ATG*4	Two genes: *At*2g44140, *At*3g59950	Single gene: CHLREDRAFT_169763*	Two genes: *Os*03g27350, *Os*04g58560 **
*ATG*5	Single gene: *At*5g17290	Single gene: CHLREDRAFT_196810*	Single gene: *Os*02g02570
*ATG*6	Single gene: *At*3g61710	Single gene: CHLREDRAFT_195652*	Small gene family: *Os*03g15290, *Os*03g44200, *Os*01g48920
*ATG*7	Single gene: *At*5g45900	Single gene: CHLREDRAFT_114160*	Single gene: *Os*01g42850
*ATG*8	Large gene family: *At*4g21980, *At*4g04620, *At*1g62040, *At*2g05630, *At*2g45170, *At*4g16520, *At*3g60640, *At*3g06420, *At*3g15580.	Single gene: CHLREDRAFT_59275*	Large gene family: *Os*07g32800**, *Os*04g53240, *Os*08g09240, *Os*02g32700, *Os*11g01010
*ATG*9	Single gene: *At*2g31260	No homolog	Two genes: *Os*03g14380, *Os*10g07994
*ATG*10	Single gene: *At*3g07525	Single gene: CHLREDRAFT_206226*	Two genes: *Os*04g41990***, *Os*12g32210***
*ATG*12	Two genes: *At*1g54210, *At*3g13970	Single gene: CHLREDRAFT_194981*	Small gene family: *Os*03g37140, *Os*06g10340, *Os*09g27230
*ATG*13	Two genes: *At*3g49590, *At*3g18770	No homolog	Two genes: *Os*02g43040, *Os*11g06320
*ATG*14	No homolog	No homolog	No homolog
*ATG*16	Single gene: *At*5g50230	No homolog	Single gene: *Os*03g53510
*ATG*18	Large gene family: *At*3g62770, *At*4g30510, *At*2g40810, *At*3g56440, *At*5g05150, *At*5g54730, *At*1g03380, *At*1g54710	Single gene: CHLREDRAFT_187711*	Large gene family: *Os*01g57720, *Os*01g70780, *Os*01g07400, *Os*05g07710, *Os*02g54910, *Os*05g33610
*ATG*27	No homolog	No homolog	No homolog
*VPS*15	Single gene: *At*4g29380	Single gene: CHLREDRAFT_144070	Single gene: *Os*02g55340
*VPS*34	Single gene: *At*1g60490	Single gene: CHLREDRAFT_196943	Two genes: *Os*05g08810, *Os*08g21590

[a] Gene terminology used: *Arabidopsis* AGI code, *Chlamydomonas* NCBI terminology, *rice* TIGR code. Yeast ATG homologs in *Arabidopsis* partition is based on Bassham et al. (2006) and Meijer et al. (2007)

[b] Asterisks mark previously identified homolog genes: *Díaz-Troya et al. 2008b, **Su et al. 2006, ***Shin et al. 2009

organism whose full genome sequence is available (Merchant et al. 2007). As can be seen in Table 1, the *Chlamydomonas* genome contains homologous genes for most of the core autophagy machinery genes existing both in yeast and *Arabidopsis*. Similarly to yeast, all predicted *Chlamydomonas* core ATG genes are single-copy genes, and this organism possesses no unique ATG genes that are missing in *Arabidopsis*. In contrast, some of the ATG genes present in *Arabidopsis* possess no homologs in *Chlamydomonas* (*ATG*2, *ATG*9, *ATG*13, *ATG*16), suggesting that either these ATG genes do not exist in *Chlamydomonas*, or that functional homologs of those genes exist in *Chlamydomonas* but possess sufficient diversification that render them unidentified in a regular BLAST search (Fig. 1). Moreover, higher plants evolved either small or large families of specific ATG genes to perform specialized functions that are apparently not present and not needed in ancient algae. Pioneering work identified and character-

ized the *Chlamydomonas* ortholog of target of rapamycin (TOR) kinase, *Cr*TOR (Crespo et al. 2005). In yeast and mammals, rapamycin treatment is used to induce autophagy via inhibition of the TOR kinase (Cebollero and Reggiori 2009; Jung et al. 2010). A significant advantage in studying autophagy regulation in *Chlamydomonas* is the fact that it is sensitive to rapamycin, in contrast to higher plants that are rapamycin-resistant (Menand et al. 2002; Díaz-Troya et al. 2008b). Working with *Chlamydomonas* may present the unique opportunity to induce autophagy in photosynthetic organisms using rapamycin. Moreover, a single *Chlamydomonas* ortholog to the ATG8 gene family of higher plants, *CrATG*8, has been discovered (Pérez-Pérez et al. 2010). Utilization of the *Cr*ATG8 protein as an in vivo marker for various autophagy-associated processes in this algal species looks to be a promising approach for future discoveries of autophagy-associated processes in *Chlamydomonas*, rendering this organism an emerging new model system for autophagy research.

Rice is a staple monocot crop plant, and the availability of its full-genome sequence renders it an additional important model plant for studying various biological processes including those associated with autophagy. Nevertheless, compared to the relatively significant identification and functional analysis of the *Arabidopsis* ATG orthologs, elucidation of the core autophagy genes and their functions in rice has, in general, not been extensively studied. As can be deduced from Table 1, based on DNA and protein sequence homology, rice possesses, as expected, all the genetic potential for fully active core autophagy machinery. In addition, expansion of the core ATG genes into gene families occured in rice quite extensively compared to *Arabidopsis* (Fig. 1). The operation of autophagy in rice suspension culture cells under sucrose starvation has been demonstrated some time ago (Chen et al. 1994). Yet, so far, only four ATG genes have been identified and characterized in rice. Su et al. (2006) had cloned two of the rice *ATG*4 and *ATG*8 orthologs based on sequence homology with *Arabidopsis ATG*4b and *ATG*8a genes. The *Os*ATG4 protease was also shown to interact with the *Os*ATG8 protein in a yeast-two-hybrid analysis, resulting in the cleavage of the C-terminal tail of *Os*ATG8, implying a functional conservation of the autophagy process in dicotyledonous and monocotyledous plant species (Su et al. 2006). Similarly, Shin and coworkers had cloned two rice orthologs for the *Arabidopsis ATG*10 genes based on their DNA sequence homology. The two rice ATG10 proteins are 75% identical to each other, but possess only 39–43% protein sequence homology to the single *Arabidopsis* ATG10 (Shin et al. 2009). Moreover, the C terminal domain of the rice ATG10, especially the cysteine residue that is necessary for binding to ATG12, is conserved in the *Arabidopsis* and rice homologs (Shintani et al. 1999; Shin et al. 2009).

A rice mutant lacking the ATG10b protein (the more abundantly expressed of the two rice ATG10 proteins) was more sensitive than the wild-type plant to salt and oxidative stresses, had decreased number of autophagosomes and showed accumulation of oxidized proteins (Shin et al. 2009). Similar phenotypes were also demonstrated in autophagy-defective *Arabidopsis* ATG18a RNA interface (RNAi) plants (Xiong et al. 2007). *Arabidopsis atg10* plants were additionally hypersensitive to nitrogen and carbon starvation, initiated senescence more quickly than the wild type and failed to accumulate autophagic bodies inside the vacuole (Phillips et al. 2008), all phenotypes that were not examined in the rice *atg10* mutant. Even though different ATG8 proteins were used in these experiments, the fact that both ATG10 and ATG18 are central to the operation of autophagy supports a functional conservation of autophagy in dicotyledonous and monocotyledonous plant species. Interestingly, a functional rice *ATG*10a homolog does not compensate for the phenotype of the absence of *ATG*10b, suggesting that these two genes are not functionally redundant in rice (Shin et al. 2009). It is important to note that the autophagy machinery has also been demonstrated in maize, another staple crop plant which was not discussed in this review (Chung et al. 2009).

Playing hide and seek — the missing links in the plant core autophgay machinery

In yeast, 18 out of 35 ATG genes comprise the core autophagic machinery (Meijer et al. 2007; Xie and Klionsky 2007). The *Arabidopsis* genome has homologs to almost all of the yeast ATG genes, some of which are present in more than one isoform, particularly *ATG*8 and *ATG*18 (see discussion below; Table 1 and Fig. 1). Seven of the yeast core ATG genes (*ATG*1, *ATG*2, *ATG*3, *ATG*13, *ATG*16, *VPS*15, *VPS*34) have only predicted *Arabidopsis* orthologs that were found on the basis of sequence similarity but were never functionally studied (Hanaoka et al. 2002; Meijer et al. 2007). Interestingly, two well studied and characterized yeast genes of the core autophagy machinery, namely, *ATG*14 and *ATG*27, have no known or predicted orthologs in *Arabidopsis* plants.

The yeast ATG14 is a hydrophilic protein with a coiled-coil motif in its N-terminus. It was shown to be essential for proper autophagy in *S. cerevisiae* and was also shown to function as a part of an autophagy-specific phosphoinositide 3 (PI3) kinase complex consisting of VPS34, VPS15, ATG6, and ATG14 (Kametaka et al. 1998; Kihara et al. 2001). Basic BLAST analysis could not clearly identify an *ATG*14 gene homolog in mammals and plants. Yet, a

functional mammalian ortholog for yeast ATG14 was independently identified in human and mouse by two specifically dedicated approaches. First, bioinformatic analysis comparing the yeast and *Candida glabrata* ATG14 protein sequence to the protein databases of mouse and human: the analysis revealed a weak homolog to yeast ATG14 (13% identity and 37% similarity; Itakura et al. 2008). Second, in vivo isolation of Beclin-1 complexes from various mouse tissues and identification of their components using mass spectrometry: this yielded in a mouse ATG14 homolog (Zhong et al. 2009). Furthermore, similarly to yeast, the ATG14-Beclin1-VPS15-VPS34 complex was identified in mammals as an autophagy-specific PI3 kinase complex (Itakura et al. 2008; Sun et al. 2008; Matsunaga et al. 2009, 2010; Zhong et al. 2009). In contrast to yeast and mammalians, no ATG14 homolog has so far been identified in plants. Yet, the important principal role of ATG14 in yeast and its homologs in mammalian cells, which is expected also to operate in plant cells, calls for additional efforts to search for a putative plant protein carrying a comparable function to ATG14 from yeast and mammalian cells.

ATG27 is a second integral membrane protein of the core autophagy machinery (the first is ATG9). It is also a PI3P-binding protein. Similarly to ATG9, the ATG27 protein cycles between two different membrane compartments in yeast cells, the mitochondria and phagophore assembly site (PAS), but its additional localization to the Golgi apparatus suggests the involvement of the Golgi complex itself in the autophagy pathway, perhaps by lipid delivery from the Golgi to the forming double-membrane autophagosome vesicles (Yen et al. 2007; Ohashi and Munro 2010). When ATG27 is depleted in yeast cells, autophagy still occurs, but at a substantially reduced level (Yen et al. 2007). To our knowledge, there is no mammalian or plant ortholog to ATG27. We propose that the absence of an ATG27 ortholog in plant and mammalian genomes makes biological sense as the yeast ATG27 protein is localized to the PAS, a compartment missing in mammalians and plants. In addition, alternative membrane sources other than the Golgi are used for autophagosome assembly in mammalian and plant cells such as the ER (Hayashi-Nishino et al. 2009; Ylä-Anttila et al. 2009). ATG27 may thus be a protein that was specifically evolved in yeast cells to fulfill yeast-specific functions, or, on the contrary, the ancestor ATG27 protein was lost during mammalian and plant evolution.

The multigene families of plant autophagy — *ATG*8 and *ATG*18

Two of the proteins belonging to the core autophagy machinery, namely, ATG8 and ATG18, are encoded in higher plants by multigene families. The model plant *Arabidopsis* possesses nine genes encoding ATG8 isoforms and eight genes encoding ATG18 isoforms (Doelling et al. 2002; Xiong et al. 2005). Mammalian cells possess eight *ATG*8 genes, but in sharp contrast to higher plants, they possess only two to four genes encoding ATG18 isoforms (Polson et al. 2010; Weidberg et al. 2010). These two gene families, *ATG*8 and *ATG*18, were suggested as candidates for functional specification of autophagy in plants (Hayward et al. 2009). The reasons for the existence of these two large gene families in plants is yet unknown, but are apparently related to the multiple functions of these proteins in plants as will be discussed later on during this review.

ATG8

ATG8 is one of two proteins containing a Ub-fold in the autophagy core machinery. It is synthesized as a pro-protein that is cleaved by ATG4 to expose a glycine at the C-terminus of the protein. In a Ub-like conjugation system, the processed ATG8 protein then binds through the exposed glycine to phosphatidylethanolamine (PE) molecules on mvembranes that are programmed to differentiate into autophagosomes (Tanida 2011). ATG8-PE located on the outer membrane of the autophagosome is cleaved off during autophagosome deposition by ATG4. ATG8-PE located on the inner membrane of the autophagosome enters the vacuole with the autophagic body and is degraded (Chung et al. 2010). Yeast possesses one *ATG*8 gene, that has been shown to be involved in autophagosome formation and membrane expansion, possibly by mediating membrane tethering and hemifusion (Nakatogawa et al. 2007).

In contrast to yeast, mammals possess eight ATG8 isoforms, while *Arabidopsis* plants possess nine ATG8 isoforms (Doelling et al. 2002; Yoshimoto et al. 2004). The mammalian ATG8 proteins are principally divided into two subfamilies according to protein sequence homology, namely, LC3 subfamily (four members) and GABARAP/GATE-16 subfamily (four members) (Weidberg et al. 2010). The *Arabidopsis* ATG8 homologs all display high sequence similarity to the yeast ATG8 (between 73% and 90%), and the processed proteins following the C-terminal cleavage by the plant ATG4 proteins contain a glycine residue at their C-terminus (Doelling et al. 2002; Hanaoka et al. 2002). They are divided into three subfamilies according to protein sequence similarity: *At*ATG8a, *At*ATG8c, *At*ATG8d, and *At*ATG8f (four members); *At*ATG8b, *At*ATG8e, and *At*ATG8g (three members); and *At*ATG8h and *At*ATG8i (two members; Doelling et al. 2002). *At*ATG8h and *At*ATG8i differ from the other *At*ATG8s since they lack extra residues at the C-terminus

following the glycine residue. The ATG8 Ub-like conjugation system, existing in yeast and mammals, also exists in plants and ATG8 cleavage by the ATG4 protease as well as its lipidation has been demonstrated in many independent studies (Doelling et al. 2002; Yoshimoto et al. 2004; Thompson et al. 2005; Fujioka et al. 2008; Chung et al. 2009, 2010).

A number of reports also implied multiple functions for ATG8 proteins in both mammalians and plants, which appear to be unrelated to the basic process of autophagosome formation and may be differentially executed by the different ATG8 isoforms. The mammalian and plant ATG8 homologs were shown to bind microtubules, implying a connection between autophagy and the cytoskeleton (Mann and Hammarback 1994; Wang et al. 1999; Ketelaar et al. 2004). In addition, the mammalian ATG8 homologs were shown to be connected to intracellular trafficking unrelated to autophagosomes (Wang et al. 1999; Sagiv et al. 2000; Kittler et al. 2001). Work previously published by our group has also demonstrated a connection between overexpression of an *Arabidopsis* green fluorescent protein (GFP)-ATG8f fusion protein and an altered response to plant hormones, suggesting a role for ATG8 or autophagy in regulation of hormonal signaling (Slavikova et al. 2008). Furthermore, ATG8 in yeast and mammals has been shown to mediate target recognition during selective autophagy by specific binding to protein targets (Noda et al. 2010). This target recognition is discussed further in a later section of this review.

The reason for the presence of such large ATG8 families in mammalians and plants is not entirely clear, but may imply that the different ATG8 isforms possess multiple nonparallel functions in the various biological processes associated with Autophagy. Interestingly, the different mammalian ATG8 subfamilies have been suggested to play nonparallel roles in the different stages of autophagosome formation, namely, the LC3 subfamily being involved in phagophore membrane elongation, while members of the GABARAP/GATE-16 subfamily take part in autophagosome maturation (Weidberg et al. 2010). In *Arabidopsis*, previous studies conducted by our group as well as by others have shown differential expression of diverse *At*ATG8 homologs in various plant organs and plant tissues (Yoshimoto et al. 2004; Sláviková et al. 2005; Thompson et al. 2005). This nonuniform expression pattern may suggest specific roles for each *At*ATG8 homolog. Interestingly, although expression of all the *Arabidopsis* ATG8 homologs is induced by nitrogen starvation, each homolog shows a different pattern of induction (Yoshimoto et al. 2004). This may suggest discrete roles in autophagosome formation for the various *At*ATG8 proteins, as previously seen for mammalian ATG8 homologs (Weidberg et al. 2010). To test the expression patterns of the different *Arabidopsis At*ATG8 isoforms in response to various stresses, hormonal and nutritional cues, we used the public microarray database located in the Nottingham *Arabidopsis* Stock Centre (http://affymetrix.arabidopsis.info/AffyWatch.html). The gene expression of the different *At*ATG8 isoforms is differentially induced in response to the various cues, implying the specific involvement of autophagy, or the different *At*ATG8 isoforms, in the various conditions. In addition, only four of the nine ATG8 homologs displayed a considerable change in expression over the cues examined (Fig. 2A). The lack of change in expression in five other ATG8 homologs can be explained either by a lack of response of these specific homologs to the treatments examined in the database or by the relatively limited expression of these homologs in specific tissues or developmental stages. It is important to note that the homologs that showed marked differences in expression belong to all three *At*ATG8 subfamilies, so the ability to respond to stresses at the level of gene expression is not limited to one subfamily. It can also be seen in Fig. 2 that the expression pattern varies between the homologs in both regulation type (induction or repression) and also in the actual level of induction of the genes. These results may imply differential functions of the various ATG8 proteins in plants, similarly to what has been observed in mammals.

ATG18

Similarly to ATG8, yeast possesses only one *ATG*18 encoding gene. The yeast ATG18 is a WD-40 repeat containing protein, which specifically recognizes PI3P (Tooze and Yoshimori 2010). Yeast contains two more WD repeat proteins, ATG21 and Ygr223c, that together, with ATG18, make up a family of three PI-binding proteins. WD repeat protein-interacting phosphoinositides 1 and 2 (WIPI1 and WIPI2, respectively) are both mammalian orthologs proteins of the yeast ATG18 (Polson et al. 2010). Both WIPI proteins were shown to be involved in the initiation of autophagy in mammalian cells (Burman and Ktistakis 2010; Polson et al. 2010).

Like ATG8, ATG18 has undergone diversification in plants. In *Arabidopsis*, there is an eight member family of *ATG*18 genes with multiple splice variants named ATG18 a-to-h (Xiong et al. 2005; Bassham et al. 2006). Those genes were divided to three major subgroups: *At*ATG18a, *At*ATG18c, *At*ATG18d, and *At*ATG18e are surprisingly more similar to the yeast Ygr223c gene; *At*ATG18b is the sole gene most similar to yeast *ATG*18 itself; and *At*ATG18f, *At*ATG18g, and *At*ATG18h are more divergent, forming a less conserved subgroup with longer peptide sequences (Xiong et al. 2005). Also, plant *ATG*18 genes are characterized by a great variety of expression patterns upon exposure to stress conditions (Hayward et al. 2009). Very

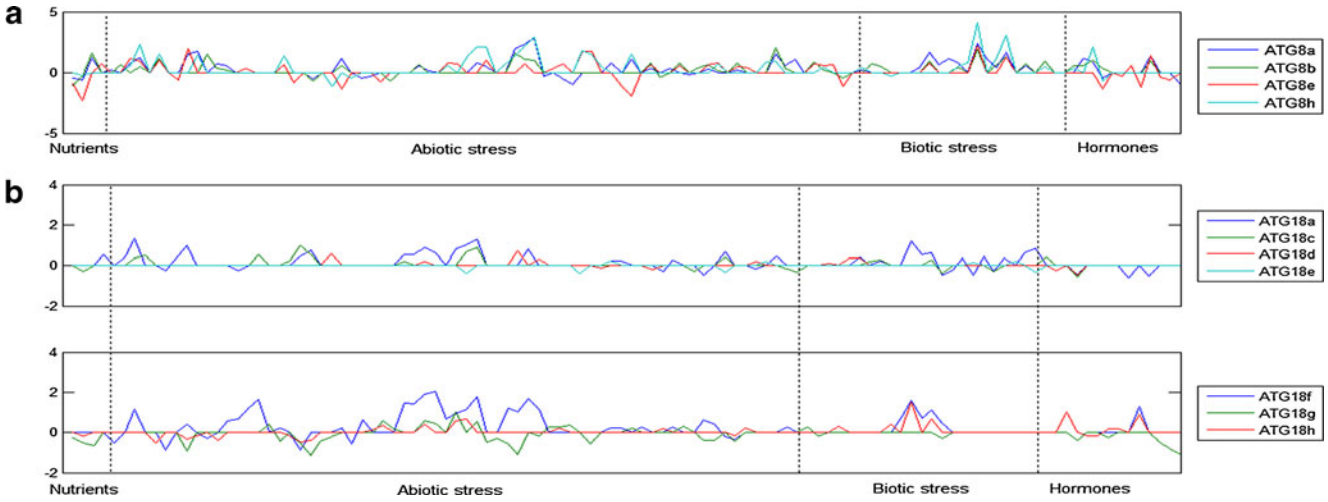

Fig. 2 Variable expression pattern of *At*ATG8 and *At*ATG18 family members under various treatments using a database of publically available microarray data (Nottingham *Arabidopsis* Stock Centre, http://affymetrix.arabidopsis.info/AffyWatch.html). We analyzed the expression levels of the *At*ATG8 (**A**) and *At*ATG18 (**B**) homologs. The treatments displayed are ones in which at least one of genes tested demonstrated a difference in expression. The *At*ATG8 homologs displayed are the ones who showed a massive change in expression. *At*ATG18b does not have a probset and therefore was not included in the analysis

few studies have tried to elucidate the functions of the different ATG18 isoforms in *Arabidopsis*. Xiong and coworkers (2005) showed significant differences in the expression of *AtATG*18 genes in response to various treatments and in different plant organs. Apparently, *AtATG*18a, which is closer in sequence homology to the yeast Ygr223c than to yeast ATG18, was the only isoform that showed increased transcription in response to both starvation and senescence and was also detected in all plant organs (Xiong et al. 2005). Later studies showed that *At*ATG18a is upregulated in response to oxidative, salt and drought stresses as well (Xiong et al. 2007; Liu et al. 2009). The rest of the *AtATG*18 genes were involved either in starvation or in senescence or were partially expressed in the different plant organs. The *At*ATG18e isoform was not detected in any of the plant organs or in the stress conditions tested. The changes in expression of the various *AtATG*18 genes in response to various treatments and in different plant organs did not follow the subgroup division made according to sequence homology, suggesting that in the *ATG*18 gene family, sequence similarity does not necessarily correspond to protein functions. The Bassham group generated unique *At*ATG18a RNAi plants with reduced expression levels of *AtATG*18a gene (Xiong et al. 2005). Surprisingly, in spite of the high sequence similarity with other members of the family, the reduction in *AtATG*18a transcript level was gene-specific and did not affect the transcript level of closer or farther homologs (Xiong et al. 2005). In addition, despite the extensive number of *At*ATG18 isoforms, *At*ATG18a RNAi plants displayed a similar phenotype to classical ATG knockout plants (Xiong et al. 2005), as well as higher sensitivity to oxidative stress conditions and higher production of oxidized proteins and reactive oxygen species (ROS) then wild-type plants (Xiong et al. 2007). *At*ATG18a RNAi plants exhibited also higher sensitivity to salt and osmotic/drought conditions than wild-type plants (Liu et al. 2009). At the cellular level, The *At*ATG18a RNAi plants had no detectable autophagosomes, during nitrogen and carbon starvation (Xiong et al. 2005) oxidative stress (Xiong et al. 2007) and salt and osmotic stresses (Liu et al. 2009). These results indicate that *At*ATG18a is not functionally redundant with other members of the ATG18 family and is critical for autophagosome formation under different starvation and stress conditions in *Arabidopsis*. It will be interesting to test whether other *At*ATG18 isoforms also possess isoform-specific functions. The *AtATG*18f, *AtATG*18g, and *AtATG*18h genes, belonging to a divergent subgroup in the ATG18 family, were all upregulated during nutrient starvation but had some differences in expression under various abiotic stresses (Rizhsky et al. 2004).

In order to get some clues concerning specialized functions of different ATG18 isoforms, we analyzed the gene expression of *ATG*18 genes from *Arabidopsis* under various treatments using data taken from Nottingham *Arabidopsis* Stock Center in a similar way to the analysis performed on the *ATG*8 gene family (see above). The genes were divided to three subgroups according to (Xiong et al. 2005), but due to a missing probeset of *AtATG*18b, only two subgroups of genes were analyzed (Fig. 2B). Our expression analysis together with previous works suggest that different *At*ATG18 isoforms have specialized in plants to participate in different processes that involve autophagy

activation, some of them probably plant-specific processes, which are triggered by different stimuli. Those processes can be different in time and space, because diverse *AtATG*18 gene isoforms were expressed differently in various plant tissues (Xiong et al. 2005). Interestingly, the *At*ATG18e isoform was not detected at all by real-time polymerase chain reaction (RT-PCR) in any of the organs/treatments checked (Xiong et al. 2005), but public microarray data showed very low but noticeable changes in the level of expression under certain conditions. *At*ATG18e is the closest homolog to *At*ATG18a, the most responsive *At*ATG18 homolog. It is therefore possible that *At*ATG18e either serves a very specific function or is expressed in discrete tissues or developmental stages. *At*ATG18a is the only well-studied isoform in plants whose absence shows clear phenotype and, as far as the experimental data shows, is the closest plant ortholog to the yeast ATG18 and the mammalian WIPI2 protein. Elucidating the physiological effects of the absence of the rest of the *AtATG*18 gene family on the plant phenotype is a challenging issue for future research.

In contrast to the case of *At*ATG8 proteins, so far, no GFP-tagged *At*ATG18 proteins have been employed to elucidate the intracellular localization of these proteins and to search for other plant proteins that might be colocalized with them. Such studies may aid in better understanding of the compound cellular and physiological roles of ATG18 in plants.

The regulatory role of ATG8 in the selective degradation of specific cellular components —ATG8-interacting motif (AIM)

The operation of ATG8 in the selective degradation of various protein substrates requires specific interaction of ATG8 with these proteins, and the way ATG8 executes this function has only recently been addressed. Noda et al. (2008) identified by structural analysis a common tetrapeptide motif "WXXL" (X is any amino acid) in two totally different proteins, the yeast ATG19 and the mammalian sequestosome 1 (p62/SQSTM1), previously shown to interact with ATG8 and LC3, respectively (Shintani et al. 2002; Pankiv et al. 2007;). This original ATG8-interacting motif (AIM) was located within the longer (22 amino acids) LC3-interacting region (LIR) identified in the p62/SQSTM1 protein (Pankiv 2007). Later, three additional proteins, namely, the yeast ATG32 and the mammalian neighbor of BRCA1 (NBR1) and Nix, were also identified as ATG8-binding proteins in different studies. Those proteins also contained a motif similar to AIM with some minor alterations (Kirkin et al. 2009a; Okamoto et al. 2009; Schwarten et al. 2009). This observation triggered a research to identify additional AIM-containing proteins (Behrends et al. 2010; Pankiv et al. 2010; Johansen and Lamark 2011). AIMs are evolutionary conserved among the large family of ATG8-binding proteins in various species (Noda et al. 2010). The initial longer definition of the AIM was $X_{-1}X_{-2}X_{-3}W_0X_1X_2LX_3$ (Noda et al. 2008). Later studies have shown relative "flexibility and demands" needed for functional AIMs (Noda et al. 2010; Johansen and Lamark 2011). In light of the fast progress in the field of AIM study, one can speculate that more flexibility in the AIM will be added in parallel to the discovery of new ATG8-binding proteins. This quite extensive flexibility of the AIM makes the use of this predictive approach quite challenging in the identification of novel ATG8-interacting proteins. Thus, in the discovery of novel ATG8-interacting proteins, biochemical or genetic methods generally precede the identification of an AIM in the target protein sequence (Behrends et al. 2010; Vanhee et al. 2011). The interaction between autophagic receptors and ATG8-family proteins through AIMs contributes, at least partially, to the selection of specific cargoes, possibly by linking the cargoes to autophagic membranes and/or to their forming machineries (Noda et al. 2010). In some cases, aggregation/polymerization is also required in addition to the AIM-dependent binding (Johansen and Lamark 2011). Functional AIMs have been found in various cellular proteins such as calreticulin and clathrin heavy chain (Mohrlüder et al. 2007a, b) as well as ATG8 modifying enzymes belonging to the core autophagy machinery (Yamada et al. 2007; Satoo et al. 2009; Yamaguchi et al. 2010), suggesting that ATG8 binding is involved in many biological processes.

Despite the extensive identification of AIM-containing ATG8-binding proteins in mammals and yeast, there are so far only two recent publications on AIM-containing ATG8-binding proteins in plants (Svenning et al. 2011; Vanhee et al. 2011). The first has identified an *Arabidopsis* AtNBR1 homolog as a selective autophagy substrate (Svenning et al. 2011). The AtNBR1 was also capable of binding Ub (see the section discussing autophagy and the Ub system). The second publication has shown that the *Arabidopsis* tryptophan-rich sensory protein (TSPO), a sensory protein localized to the cell plasma membrane, possesses an AIM and is degraded through selective autophagy and not through the proteasome pathway (Vanhee et al. 2011). Interestingly, TSPO binds heme, which is a biologically important metabolite, but also a potentially toxic compound that can generate ROS. Thus, under physiological conditions (such as abiotic stresses) in which heme may have toxic effects, TSPO binds to heme and targets it for selective degradation in the vacuoles by using the ATG8-mediated selective autophagy process (Hofmann 2011). Our laboratory (unpublished results) has recently identified a

number of other *Arabidopsis* plant-specific proteins that interact with ATG8 and possess AIMs, implying that plant ATG8 proteins specifically interact with multiple other plant proteins, containing AIMs, which apparently possess multiple biological functions, some of which appear to be plant-specific.

Is there a connection between autophagy and the Ub machinery in plants?

The Ub protein is a central part of the proteasomal degradation machinery. The covalent attachment of a Ub tag to a substrate protein generally leads to its degradation by the proteasome (Clague and Urbé 2010). Yet, Ub has also been shown to participate in various other cellular processes such as endocytosis, signal transduction, and DNA repair (Kirkin et al. 2009b). In the past few years, emerging evidence from mammalian cells has demonstrated an additional role for Ub in targeting proteins for degradation by selective autophagy (reviewed in Kirkin et al. 2009b; Clague and Urbé 2010; Lamark and Johansen 2010). The connection between Ub and autophagy is facilitated by so-called "adapter" proteins able to bind Ub but also capable of binding proteins in the autophagy machinery (Johansen and Lamark 2011). For example, the protein p62/SQSTM1 and its interaction partner NBR1 were shown to be involved in the degradation of protein aggregates by binding to both poly-Ub chains and LC3. This binding of LC3 occurs through an AIM located in these proteins (Bjørkøy et al. 2005; Pankiv et al. 2007; Kirkin et al. 2009a).

Even though a connection between the ubiqutination and the autophagy machineries has not yet been reported in plants, the conserved pattern of operation of both these systems in plants implies that an analogous connection between autophagy and Ub may also exist in plants. To get some clues about this possibility, we conducted a coexpression analysis of the *ATG*8 gene family and the *ATG*18 gene family of *Arabidopsis*, using the ATTED-II database (http://atted.jp). This analysis showed that genes of the plant autophagy machinery are coexpressed with genes belonging to the plant Ub conjugation system, mainly E3 genes (data not shown). This data may suggest the plant autophagy and ubiquitination machineries that interact with each other. It should indeed prove fascinating to elucidate the ubiquitinated targets of selective autophagy in plants and also the Ub-binding proteins that connect the targets to the autophagy machinery. While NBR1 homologs are found throughout the eukaryotic kingdom including plants (see above), p62/SQSTM1 homologs are confined to the metazoans (Svenning et al. 2011). Interestingly, the *Arabidopsis* AtNBR1 contains similar functional domains of the mammalian p62/SQSTM1 and hence, AtNBR1 was suggested to be a functional hybrid of the mammalian autophagic adapters p62/SQSTM1 and NBR1 (Svenning et al. 2011). Deciphering the interplay between Ub and autophagy in plants may shed light on the likely existing multiple protein targets of selective autophagy in plants.

Autophagy in seed development and germination

Although the autophagy machinery is relatively conserved between plants, yeast, and mammals, plants, being sessile organisms that are highly sensitive to changing environments, may be expected to possess many unique features that result in dedicated specializations of the autophagy machinery. For instance, to ensure survival, developing seeds of a many dicotyledonuous and monocotyledonous plant species efficiently synthesize massive amounts of storage proteins and deposit them as protein bodies inside protein storage vacuoles (PSVs), Then upon early germination, these storage proteins are degraded to enable the accumulation of sufficient energy and amino acids to synthesize new plant organs and to commence photosynthesis. During early stages of seed development, the seed storage proteins are transported to the PSVs via the Golgi (Robinson et al. 1997; Robinson and Hinz 1999; Hillmer et al. 2001; Winter and Stoger 2011). Yet, later, during seed development, when massive amounts of storage proteins are synthesized, they are transported directly from the ER to the PSVs by an intracellular process that resembles autophagy in its microscopic appearance (reviewed by Galili et al. 1993; Robinson et al. 1998; Herman and Larkins 1999; Chrispeels and Herman 2000; Bassham 2002). This process is also associated with enhanced expression of genes encoding ATG proteins of the core autophagy machinery. Our previous seed gene expression data (Angelovici et al. 2009) showed a coordinated upregulation of many of the core *Arabidopsis* ATG genes during seed maturation and desiccation (Fig. 3). The highest expression level is observed in dry seeds.

During early germination, plant seeds synthesize proteases and efficiently target them from the ER to the vacuole to mobilize the storage proteins that were accumulated during seed development (Toyooka et al. 2000). Interestingly, some of these vacuolar proteases that degrade storage proteins inside the vacuole posses a C-terminal K/HDEL signal sequence that generally functions in the retention of ER resident proteins within the ER. Moreover, removal of the K/HDEL signal causes the transport of these proteases to the plasma membrane (Toyooka et al. 2000, 2001), implying that this signal possesses a novel plant-specific regulatory role in the massive transport of the

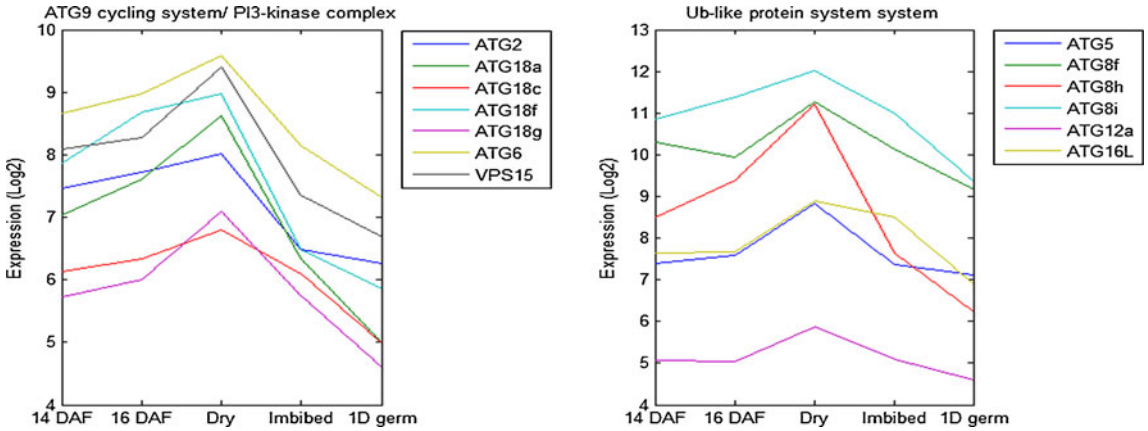

Fig. 3 Expression of depicted *Arabidopsis* ATG genes during seed development and early germination. The data was taken from microarray analysis performed by our group (Angelovici et al. 2009). *DAF* Days after flowering, *DRY* dry seeds, *imbibed* 3 days imbibition at 4°C in the dark, *1D germ* 1 day under long-day conditions after imbibition

proteases directly from the ER to the vacuole, bypassing the Golgi apparatus. In addition, upon the transport of these newly synthesized proteases to the vacuole, their C-terminus appears to be removed by a yet uncharacterized process (Okamoto et al. 2001). This unique mechanism of the transport of K/HDEL-containing proteins from the ER directly to the lytic vacuole has so far not been demonstrated in mammalian cells. The mechanism mediating this transport is still not clear. A simple likely explanation is that the PSVs develop directly out of the ER, although other routes cannot be rulled out. It will also be interesting to test whether the autophagy machinery is involved in this transport process.

Recently, the delivery of different prolamin storage proteins to the storage vacuole of aleurone cells in developing maize seeds was suggested to be associated with an atypical autophagic process, that delivers ER material directly to PSVs independently of ATG8 lipidation (Reyes et al. 2011). The authors identified autophagosome-like structures involved in transport to PSVs in maize aleurone cells. However, these structures were neither surrounded by a double-membrane nor decorated by ATG8, preventing their identification as classical autophagosomes. The authors suggested that these structures might be a specialized case of ATG8-independent selective autophagy used to deliver storage protein aggregates from ER to the storage vacuole, a hypothesis that is supported by the recent identification of an analogous ATG8-independent subcellular route in mammalian cells (Nishida et al. 2009). Additional research is needed to clearly link the above findings to the autophagy process and to elucidate whether it is a unique delivery mechanism of storage proteins in developing and germinating seeds of various plant species. The generation of *atg* mutants in cereal seeds in the future will be an important advance in solving these challenging issues (Chung et al. 2009).

Future prospects

In this review, we attempted to summarize the recent knowledge regarding plant autophagy while addressing issues that have not been extensively reviewed previously and that we believe will take center stage in plant autophagy research in the coming years. In order to promote the plant autophagy field, we believe that the following approaches should be taken. First, the marker collection available for cellular studies of plant autophagy should be widened. The current markers are solely based on different ATG8 isoforms tagged with GFP (Yoshimoto et al. 2004; Sláviková et al. 2005; Thompson et al. 2005; Xiong et al. 2007), while in yeast and mammalian cells, many more ATG markers are used (see, for example, Suzuki et al. 2001; Krick et al. 2008; Gao et al. 2010), enabling a better understanding of the cellular processes involving autophagy. Second, the pool of available autophagy mutants should be widened, preferably generating mutants involved in autophagy initiation, which, according to recent opinion (Hayward and Dinesh-Kumar 2010), are predicted to display a more severe developmental phenotype that the current *atg* mutants. Third, the autophagy machinery in species other than *Arabidopsis* should be investigated. Emerging data from the last years regarding *Chlamydomonas* autopgahy genes (Díaz-Troya et al. 2008b; Pérez-Pérez et al. 2010) render it a novel model organism for studying the cellular autophagy mechanism in photosynthetic organisms. In addition, studying autophagy in crop plants such as rice may pave the way for the utilization of autophagy for agricultural purposes. Fourth, the specific functions of the different family members in the multiple-member gene families (*ATG8*, *ATG18*) should be elucidated. This can be achieved by either testing for specific treatments or analyzing specific plant organs and tissues. Fifth, plant-specific autophagy-associated proteins using the core

autophagy proteins as baits should be searched. This may help elucidate plant-specific targets of selective autophagy. Sixth, autophagy in plant-specific organs and organelles must be investigated. Recent findings regarding sequestration of chloroplasts for degradation (Ishida et al. 2008; Wada et al. 2009) demonstrate an exciting plant-unique feature of autophagy. Finally, autophagy during seed development and germination still requires further studies. The great advances made in plant autophagy research in the last years could be utilized to better investigate these issues.

Acknowledgments We thank the J & R Center for Scientific Research at the Weizmann Institute of Science and the Israeli Ministry of Agriculture for supporting our research on autophagy. GG is the incumbent of the Bronfman Chair of Plant Sciences.

Conflicts of Interest None

References

Angelovici R, Fait A, Zhu X, Szymanski J, Feldmesser E, Fernie AR, Galili G (2009) Deciphering transcriptional and metabolic networks associated with lysine metabolism during *Arabidopsis* seed development. Plant Physiol 151(4):2058–2072

Bassham DC (2002) Golgi-independent trafficking of macromolecules to the plant vacuole. Adv Bot Res 38:65–91

Bassham DC (2007) Plant autophagy-more than a starvation response. Curr Opin Plant Biol 10(6):587–593

Bassham DC (2009) Function and regulation of macroautophagy in plants. Biochim Biophys Acta 1793(9):1397–1403

Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K (2006) Autophagy in development and stress responses of plants. Autophagy 2(1):2–11

Behrends C, Sowa ME, Gygi SP, Harper JW (2010) Network organization of the human autophagy system. Nature 466 (703):68–76

Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T (2005) p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 171(4):603–614

Burman C, Ktistakis NT (2010) Regulation of autophagy by phosphatidylinositol 3-phosphate. FEBS Lett 584(7):1302–1312

Cacas JL (2010) Devil inside: does plant programmed cell death involve the endomembrane system? Plant Cell Environ 33 (9):1453–1473

Cacas JL, Diamond M (2009) Is the autophagy machinery an executioner of programmed cell death in plants? Trends Plant Sci 14(6):299–300

Cebollero E, Reggiori F (2009) Regulation of autophagy in yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta 1793(9):1413–1421

Chen MH, Liu LF, Chen YR, Wu HK, Yu SM (1994) Expression of alpha-amylases, carbohydrate metabolism, and autophagy in cultured rice cells is coordinately regulated by sugar nutrient. Plant J 6(5):625–636

Chrispeels MJ, Herman EM (2000) Endoplasmic reticulum-derived compartments function in storage and as mediators of vacuolar remodeling via a new type of organelle, precursor protease vesicles. Plant Physiol 123(4):1227–1234

Chung T, Suttangkakul A, Vierstra RD (2009) The ATG autophagic conjugation system in maize: ATG transcripts and abundance of the ATG8-lipid adduct are regulated by development and nutrient availability. Plant Physiol 149(1):220–234

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(3):483–493

Clague MJ, Urbé S (2010) Ubiquitin: same molecule, different degradation pathways. Cell 143(5):682–685

Crespo JL, Díaz-Troya S, Florencio FJ (2005) Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga *Chlamydomonas reinhardtii*. Plant Physiol 139(4):1736–1749

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(36):33105–33114

Díaz-Troya S, Florencio FJ, Crespo JL (2008a) Target of rapamycin and LST8 proteins associate with membranes from the endoplasmic reticulum in the unicellular green alga *Chlamydomonas reinhardtii*. Eukaryot Cell 7(2):212–222

Díaz-Troya S, Pérez-Pérez ME, Florencio FJ, Crespo JL (2008b) The role of TOR in autophagy regulation from yeast to plants and mammals. Autophagy 4(7):851–865

Farré JC, Krick R, Subramani S, Thumm M (2009) Turnover of organelles by autophagy in yeast. Curr Opin Cell Biol 21(4):522–530

Fujiki Y, Yoshimoto K, Ohsumi Y (2007) An *Arabidopsis* homolog of yeast ATG6/VPS30 is essential for pollen germination. Plant Physiol 143(3):1132–1139

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(4):1921–1928

Galili G, Altschuler Y, Levanony H (1993) Assembly and transport of seed storage proteins. Trends Cell Biol 3(12):437–442

Gao W, Kang JH, Liao Y, Ding WX, Gambotto AA, Watkins SC, Liu YJ, Stolz DB, Yin XM (2010) Biochemical isolation and characterization of the tubulovesicular LC3-positive autophagosomal compartment. J Biol Chem 285(2):1371–1383

Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, Shibata D, Tabata S, Ohsumi Y (2002) Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an *Arabidopsis* autophagy gene. Plant Physiol 129(3):1181–1193

Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A (2009) A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat Cell Biol 11 (12):1433–1437

Hayward AP, Dinesh-Kumar SP (2010) What can plant autophagy do for an innate immune response? Annu Rev Phytopathol 49:4.1–4.20

Hayward AP, Tsao J, Dinesh-Kumar SP (2009) Autophagy and plant innate immunity: defense through degradation. Semin Cell Dev Biol 20(9):1041–1047

Herman EM, Larkins BA (1999) Protein storage bodies and vacuoles. Plant Cell 11(4):601–614

Hillmer S, Movafeghi A, Robinson DG, Hinz G (2001) Vacuolar storage proteins are sorted in the cis-cisternae of the pea cotyledon Golgi apparatus. J Cell Biol 152(1):41–50

Hofius D, Schultz-Larsen T, Joensen J, Tsitsigiannis DI, Petersen NH, Mattsson O, Jørgensen LB, Jones JD, Mundy J, Petersen M (2009) Autophagic components contribute to hypersensitive cell death in *Arabidopsis*. Cell 137(4):773–783

Hofmann NR (2011) How a transient response becomes transient: autophagy cleans up after abscisic acid. Plant Cell 23 (2):429

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(12):1641–1652

Ishida H, Yoshimoto K, Izumi M, Reisen D, Yano Y, Makino A, Ohsumi Y, Hanson MR, Mae T (2008) Mobilization of rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an ATG gene-dependent autophagic process. Plant Physiol 148(1):142–155

Itakura E, Kishi C, Inoue K, Mizushima N (2008) Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. Mol Biol Cell 19(12):5360–5372

Johansen T, Lamark T (2011) Selective autophagy mediated by autophagic adapter proteins. Autophagy 7(3):279–296

Jung CH, Ro SH, Cao J, Otto NM, Kim DH (2010) mTOR regulation of autophagy. FEBS Lett 584(7):1287–1295

Kametaka S, Okano T, Ohsumi M, Ohsumi Y (1998) Apg14p and Apg6/Vps30p form a protein complex essential for autophagy in the yeast, *Saccharomyces cerevisiae*. J Biol Chem 273 (35):22284–22291

Ketelaar T, Voss C, Dimmock SA, Thumm M, Hussey PJ (2004) *Arabidopsis* homologues of the autophagy protein Atg8 are a novel family of microtubule binding proteins. FEBS Lett 567 (2–3):302–306

Kihara A, Noda T, Ishihara N, Ohsumi Y (2001) Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*. J Cell Biol 152(3):519–530

Kirkin V, Lamark T, Sou YS, Bjørkøy G, Nunn JL, Bruun JA, Shvets E, McEwan DG, Clausen TH, Wild P, Bilusic I, Theurillat JP, Øvervatn A, Ishii T, Elazar Z, Komatsu M, Dikic I, Johansen T (2009a) A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell 33(4):505–516

Kirkin V, McEwan DG, Novak I, Dikic I (2009b) A role for ubiquitin in selective autophagy. Mol Cell 34(3):259–269

Kittler JT, Rostaing P, Schiavo G, Fritschy JM, Olsen R, Triller A, Moss SJ (2001) The subcellular distribution of GABARAP and its ability to interact with NSF suggest a role for this protein in the intracellular transport of GABA(A) receptors. Mol Cell Neurosci 18(1):13–25

Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, Chiba T (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol 169(3):425–434

Krick R, Henke S, Tolstrup J, Thumm M (2008) Dissecting the localization and function of Atg18, Atg21 and Ygr223c. Autophagy 4(7):896–910

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N (2004) The role of autophagy during the early neonatal starvation period. Nature 432(7020):1032–1036

Kwon SI, Park OK (2008) Autophagy in plants. J Plant Biol 51 (5):313–320

Lamark T, Johansen T (2010) Autophagy: links with the proteasome. Curr Opin Cell Biol 22(2):192–198

Lenz HD, Haller E, Melzer E, Kober K, Wurster K, Stahl M, Bassham DC, Vierstra RD, Parker JE, Bautor J, Molina A, Escudero V, Shindo T, van der Hoorn RA, Gust AA, Nürnberger T (2011) Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. Plant J 66(5):818–830

Liu Y, Schiff M, Czymmek K, Tallóczy Z, Levine B, Dinesh-Kumar SP (2005) Autophagy regulates programmed cell death during the plant innate immune response. Cell 121(4):567–577

Liu Y, Xiong Y, Bassham DC (2009) Autophagy is required for tolerance of drought and salt stress in plants. Autophagy 5 (7):954–963

Love AJ, Milner JJ, Sadanandom A (2008) Timing is everything: regulatory overlap in plant cell death. Trends Plant Sci 13 (11):589–595

Mann SS, Hammarback JA (1994) Molecular characterization of light chain 3. A microtubule binding subunit of MAP1A and MAP1B. J Biol Chem 269(15):11492–11497

Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, Maejima I, Shirahama-Noda K, Ichimura T, Isobe T, Akira S, Noda T, Yoshimori T (2009) Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol 11(4):385–396

Matsunaga K, Morita E, Saitoh T, Akira S, Ktistakis NT, Izumi T, Noda T, Yoshimori T (2010) Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. J Cell Biol 190(4):511–521

Meijer WH, van der Klei IJ, Veenhuis M, Kiel JA (2007) ATG genes involved in non-selective autophagy are conserved from yeast to man, but the selective Cvt and pexophagy pathways also require organism-specific genes. Autophagy 3(2):106–116

Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C (2002) Expression and disruption of the *Arabidopsis* TOR (target of rapamycin) gene. Proc Natl Acad Sci U S A 99 (9):6422–6427

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Maréchal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R, Dutcher S, Fernández E, Fukuzawa H, González-Ballester D, González-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riaño-Pachón DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, Dubchak I, Goodstein D, Hornick L, Huang YW, Jhaveri J, Luo Y, Martínez D, Ngau WC, Otillar B, Poliakov A, Porter A, Szajkowski L, Werner G, Zhou K, Grigoriev IV, Rokhsar DS, Grossman AR (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 318 (5848):245–250

Mohrlüder J, Hoffmann Y, Stangler T, Hänel K, Willbold D (2007a) Identification of clathrin heavy chain as a direct interaction partner for the gamma-aminobutyric acid type A receptor associated protein. Biochemistry 46(50):14537–14543

Mohrlüder J, Stangler T, Hoffmann Y, Wiesehan K, Mataruga A, Willbold D (2007b) Identification of calreticulin as a ligand of GABARAP by phage display screening of a peptide library. FEBS J 274(21):5543–5555

Nakatogawa H, Ichimura Y, Ohsumi Y (2007) Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell 130(1):165–178

Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, Komatsu M, Otsu K, Tsujimoto Y, Shimizu S (2009) Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature 461(7264):654–658

Noda NN, Kumeta H, Nakatogawa H, Satoo K, Adachi W, Ishii J, Fujioka Y, Ohsumi Y, Inagaki F (2008) Structural basis of target recognition by Atg8/LC3 during selective autophagy. Genes Cells 13(12):1211–1218

Noda NN, Ohsumi Y, Inagaki F (2010) Atg8-family interacting motif crucial for selective autophagy. FEBS Lett 584(7):1379–1385

Ohashi Y, Munro S (2010) Membrane delivery to the yeast autophagosome from the Golgi-endosomal system. Mol Biol Cell 21(22):3998–4008

Okamoto T, Toyooka K, Minamikawa T (2001) Identification of a membrane-associated cysteine protease with possible dual roles in the endoplasmic reticulum and protein storage vacuole. J Biol Chem 276(1):742–751

Okamoto K, Kondo-Okamoto N, Ohsumi Y (2009) Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev Cell 17(1):87–97

Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, Johansen T (2007) p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282(33):24131–24145

Pankiv S, Alemu EA, Brech A, Bruun JA, Lamark T, Overvatn A, Bjørkøy G, Johansen T (2010) FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. J Cell Biol 188(2):253–269

Pérez-Pérez ME, Florencio FJ, Crespo JL (2010) Inhibition of target of rapamycin signaling and stress activate autophagy in *Chlamydomonas reinhardtii*. Plant Physiol 152(4):1874–1888

Phillips AR, Suttangkakul A, Vierstra RD (2008) The ATG12-conjugating enzyme ATG10 Is essential for autophagic vesicle formation in *Arabidopsis thaliana*. Genetics 178(3):1339–1353

Polson HE, de Lartigue J, Rigden DJ, Reedijk M, Urbé S, Clague MJ, Tooze SA (2010) Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy 6(4):506–522

Qin G, Ma Z, Zhang L, Xing S, Hou X, Deng J, Liu J, Chen Z, Qu LJ, Gu H (2007) *Arabidopsis* AtBECLIN 1/AtAtg6/AtVps30 is essential for pollen germination and plant development. Cell Res 17(3):249–263

Reumann S, Voitsekhovskaja O, Lillo C (2010) From signal transduction to autophagy of plant cell organelles: lessons from yeast and mammals and plant-specific features. Protoplasma 247 (3–4):233–256

Reyes FC, Chung T, Holding D, Jung R, Vierstra R, Otegui MS (2011) Delivery of prolamins to the protein storage vacuole in maize aleurone cells. Plant Cell 23(2):769–784

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol 134(4):1683–1696

Robinson D, Hinz G (1999) Golgi-mediated transport of seed storage proteins. Seed Sci Res 9:267–283

Robinson DG, Bäumer M, Hinz G, Hohl I (1997) Ultrastructure of the pea cotyledon Golgi apparatus: origin of dense vesicles and the action of brefeldin A. Protolplasma 200:198–209

Robinson D, Galili G, Herman E, Hillmer S (1998) Topical aspects of vacuolar protein transport: autophagy and prevacuolar compartments. J Exp Bot 49(325):1263–1270

Sagiv Y, Legesse-Miller A, Porat A, Elazar Z (2000) GATE-16, a membrane transport modulator, interacts with NSF and the Golgi v-SNARE GOS-28. EMBO J 19(4):1494–1504

Satoo K, Noda NN, Kumeta H, Fujioka Y, Mizushima N, Ohsumi Y, Inagaki F (2009) The structure of Atg4B-LC3 complex reveals the mechanism of LC3 processing and delipidation during autophagy. EMBO J 28(9):1341–1350

Schwarten M, Mohrlüder J, Ma P, Stoldt M, Thielmann Y, Stangler T, Hersch N, Hoffmann B, Merkel R, Willbold D (2009) Nix directly binds to GABARAP: a possible crosstalk between apoptosis and autophagy. Autophagy 5(5):690–698

Shin JH, Yoshimoto K, Ohsumi Y, Jeon JS, An G (2009) OsATG10b, an autophagosome component, is needed for cell survival against oxidative stresses in rice. Mol Cells 27(1):67–74

Shintani T, Huang WP, Stromhaug PE, Klionsky DJ (2002) Mechanism of cargo selection in the cytoplasm to vacuole targeting pathway. Dev. Cell 3:825–837

Shintani T, Mizushima N, Ogawa Y, Matsuura A, Noda T, Ohsumi Y (1999) Apg10p, a novel protein-conjugating enzyme essential for autophagy in yeast. EMBO J 18(19):5234–5241

Sláviková S, Shy G, Yao Y, Glozman R, Levanony H, Pietrokovski S, Elazar Z, Galili G (2005) The autophagy-associated Atg8 gene family operates both under favourable growth conditions and under starvation stresses in *Arabidopsis* plants. J Exp Bot 56 (421):2839–2849

Sláviková S, Ufaz S, Avin-Wittenberg T, Levanony H, Galili G (2008) An autophagy-associated Atg8 protein is involved in the responses of *Arabidopsis* seedlings to hormonal controls and abiotic stresses. J Exp Bot 59(14):4029–4043

Su W, Ma H, Liu C, Wu J, Yang J (2006) Identification and characterization of two rice autophagy associated genes, *Os*Atg8 and *Os*Atg4. Mol Biol Rep 33(4):273–278

Sun Q, Fan W, Chen K, Ding X, Chen S, Zhong Q (2008) Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 105(49):19211–19216

Suzuki K, Ohsumi Y (2010) Current knowledge of the pre-autophagosomal structure (PAS). FEBS Lett 584(7):1280–1286

Suzuki K, Kirisako T, Kamada Y, Mizushima N, Noda T, Ohsumi Y (2001) The pre-autophagosomal structure organized by concerted functions of APG genes is essential for autophagosome formation. EMBO J 20(21):5971–5981

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(9):1–18.

Tanida I (2011) Autophagosome formation and molecular mechanism of autophagy. Antioxid Redox Signal 14(11):2201–2214

Thompson AR, Vierstra RD (2005) Autophagic recycling: lessons from yeast help define the process in plants. Curr Opin Plant Biol 8(2):165–173

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 (4):2097–2110

Tooze SA, Yoshimori T (2010) The origin of the autophagosomal membrane. Nat Cell Biol 12(9):831–835

Toyooka K, Okamoto T, Minamikawa T (2000) Mass transport of proform of a KDEL-tailed cysteine proteinase (SH-EP) to protein storage vacuoles by endoplasmic reticulum-derived vesicle is involved in protein mobilization in germinating seeds. J Cell Biol 148(3):453–464

Toyooka K, Okamoto T, Minamikawa T (2001) Cotyledon cells of Vigna mungo seedlings use at least two distinct autophagic machineries for degradation of starch granules and cellular components. J Cell Biol 154(5):973–982

Vanhee C, Zapotoczny G, Masquelier D, Ghislain M, Batoko H (2011) The *Arabidopsis* multistress regulator TSPO is a heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism. Plant Cell 23 (2):785–805

Wada S, Ishida H, Izumi M, Yoshimoto K, Ohsumi Y, Mae T, Makino A (2009) Autophagy plays a role in chloroplast degradation during senescence in individually darkened leaves. Plant Physiol 149(2):885–893

Wang H, Bedford FK, Brandon NJ, Moss SJ, Olsen RW (1999) GABA(A)-receptor-associated protein links GABA(A) receptors and the cytoskeleton. Nature 397(6714):69–72

Weidberg H, Shvets E, Shpilka T, Shimron F, Shinder V, Elazar Z (2010) LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. EMBO J 29(11):1792–1802

Winter V, Stoger E (2011) The formation, function and fate of protein storage compartments in seeds. Protoplasma (in press)

Xie Z, Klionsky DJ (2007) Autophagosome formation: core machinery and adaptations. Nat Cell Biol 9(10):1102–1109

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(4):535–546

Xiong Y, Contento AL, Nguyen PQ, Bassham DC (2007) Degradation of oxidized proteins by autophagy during oxidative stress in *Arabidopsis*. Plant Physiol 143(1):291–299

Yamada Y, Suzuki NN, Hanada T, Ichimura Y, Kumeta H, Fujioka Y, Ohsumi Y, Inagaki F (2007) The crystal structure of Atg3, an autophagy-related ubiquitin carrier protein (E2) enzyme that mediates Atg8 lipidation. J Biol Chem 282(11):8036–8043

Yamaguchi M, Noda NN, Nakatogawa H, Kumeta H, Ohsumi Y, Inagaki F (2010) Autophagy-related protein 8 (Atg8) family interacting motif in Atg3 mediates the Atg3–Atg8 interaction and is crucial for the cytoplasm-to-vacuole targeting pathway. J Biol Chem 285(38):29599–29607

Yen WL, Legakis JE, Nair U, Klionsky DJ (2007) Atg27 is required for autophagy-dependent cycling of Atg9. Mol Biol Cell 18 (2):581–593

Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL (2009) 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 5(8):1180–1185

Yoshimoto K, Hanaoka H, Sato S, Kato T, Tabata S, Noda T, Ohsumi Y (2004) Processing of ATG8s, ubiquitin-like proteins, and their deconjugation by ATG4s are essential for plant autophagy. Plant Cell 16(11):2967–2983

Yoshimoto K, Takano Y, Sakai Y (2010) Autophagy in plants and phytopathogens. FEBS Lett 584(7):1350–1358

Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, Heintz N, Yue Z (2009) Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1–phosphatidylinositol-3-kinase complex. Nat Cell Biol 11(4):468–476