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REVIEW ARTICLE

Autophagy in Plants

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Autophagy is a highly conserved processing mechanism in eukaryotes whereby cytoplasmic components are engulfed in double-membrane vesicles called autophagosomes and are delivered into organelles such as lysosomes (mammal) or vacuoles (yeast/plant) for degradation and recycling of the resulting molecules. Isolation of yeast *AUTOPHAGY* (*ATG*) genes has facilitated the identification of corresponding *Arabidopsis ATG* genes based on sequence similarity. Genetic and molecular analyses using knockout and/or knockdown mutants of those genes have unraveled the biological functions of autophagy during plant development, nutrient recycling, and environmental stress responses. Additional roles for autophagy have been suggested in the degradation of oxidized proteins during oxidative stress and the regulation of hypersensitive response (HR)-programmed cell death (PCD) during innate immunity. Our review summarizes knowledge about the structure and function of autophagic pathways and *ATG* components, and the biological roles of autophagy in plants.

Keywords: autophagy, autophagosome, lysosome, vacuole, *Arabidopsis*

Autophagy is an important process for the turnover of damaged proteins and organelles, and is an adaptation response to environmental changes such as nutrient starvation. Autophagy is a universal pathway among eukaryotes, including yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, humans, and plants (Klionsky, 2007). During autophagic events, the damaged, aberrant, and/or toxic materials are wrapped into double-membrane vesicles and delivered into the lysosome and vacuole for breakdown and eventual recycling of the byproducts (Mizushima, 2007). Autophagy is induced by extracellular or intracellular factors and carries out pivotal roles in various biological processes such as development and environmental stress responses (Levine and Klionsky, 2004). Isolation of *ATG*-related mutants, in combination with the development of markers for detecting autophagy such as the acidotrophic fluorescent dyes lysoTracker Green/Red (Moriyasu et al., 2003; Liu et al., 2005) and monodansylcadaverine (Contento et al., 2005; Patel and Dinesh-Kumar, 2008), and GFP/RFP-ATG8 fusion proteins (Contento et al., 2005) has enhanced our understanding of autophagic pathways. Moreover, the use of inhibitors such as concanamycin A (V-ATPase inhibitor) and E-64d (cysteine protease inhibitor) has provided further insight into the mechanisms of autophagy (Yoshimoto et al., 2004; Bassham et al., 2006; Inoue et al., 2006; Bassham, 2007).

We strongly recommend that readers refer to previous review papers for detailed information about genetic, molecular, and cellular autophagy studies in eukaryotes (Ohsumi, 2001; Thompson and Vierstra, 2005; Bassham et al., 2006; Bassham, 2007; Mizushima, 2007; Klionsky et al., 2008; Levine and Kroemer, 2008; Mizushima et al., 2008). Here, we briefly summarize 1) the genetic and molecular isolation and characterization of ATG proteins, 2) the mechanistic regulation of autophagy and its associated components, and 3) the biological implications of autophagy in plants.

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THE HISTORY OF AUTOPHAGY

The term 'autophagy' was initially used to describe double membrane vesicles containing cytoplasmic material as observed under an electron microscope. Dr. Christian de Duve received a Novel Prize in 1974 for his pioneering achievements in the autophagy field that later led to the discovery of the lysosome. In the early 1990s, the laboratory of Dr. Yoshinori Ohsumi reported the first *ATG* mutants in yeast; this study was followed by the identification of about 30 *ATG* genes, some of which have now been characterized (Ohsumi, 2001; Nakatogawa et al., 2007). His laboratory also used a suspension of tobacco cells to show that autophagy is involved in the plant response to sucrose starvation (Moriyasu and Ohsumi, 1996). Since the early 2000s, comprehensive genome sequencing has allowed for the identification of *ATG* genes in yeast, mammals, and plants, which shows that autophagy is highly conserved among eukaryotes (Mizushima et al., 1998; Ohsumi, 2001; Doelling et al., 2002; Hanaoka et al., 2002; Bassham, 2007).

Several laboratories originally used their own nomenclature to describe autophagy and autophagy-related pathways, including *APG* for *aut*ophagy (Tsukada and Ohsumi, 1993), *AUT* for *aut*ophagy (Thumm et al., 1994), *CVT* for *c*ytoplasm-to-vacuole *t*argeting (Harding et al. 1995, 1996), *GSA* for *g*lucose-induced *s*elective *a*utophagy (Yuan et al., 1997), *PAG* for *p*eroxisome degradation via *a*utophagy (Sakai et al., 1998), *PAZ* for *p*exophagy zeocin-resistant (Mukaiyama et al., 2002), and *PDD* for *p*eroxisome *d*egradation-*d*eficient (Titorenko et al., 1995). This diversity in nomenclature confused people and thus, following discussions at the first Gordon Research Conference on "Autophagy in Stress, Development, and Disease", all terms were unified into one, *ATG*, to describe "*aut*ophagy-related".

ABNORMAL PROTEIN/CYTOSOLIC MATERIAL DEGRADATION PATHWAYS

Protein degradation is an important process for cellular

homeostasis and includes the removal of damaged proteins and recycling of amino acids. Another key role is the control of protein activity by regulating the formation of functional protein complexes and maintaining the correct distribution of enzymes and their cofactors. Thus, timely protein degradation is critical for modulating numerous biological processes such as development, differentiation, and stress responses.

Ubiquitin/Proteasome Pathways

A common protein degradation process, the ubiquitin/proteasome pathway, has been implicated in the signaling involved in photomorphogenesis and responses to phytohormones and pathogens (Ellis et al., 2002). In general, ubiquitination-mediated proteolysis can be divided into four steps: 1) activation of ubiquitin via the formation of a thioester bond between the target protein and a cysteine at the active site of a ubiquitin-activating enzyme (E1); 2) transfer of the activated ubiquitin to a ubiquitin-conjugated enzyme (E2); 3) recognition of the target protein as a substrate by a ubiquitin-protein ligase (E3); and 4) degradation of the ubiquitinated protein by the 26S proteasome (Smalle and Vierstra, 2004). E3 plays a key role in the ubiquitin/pro-

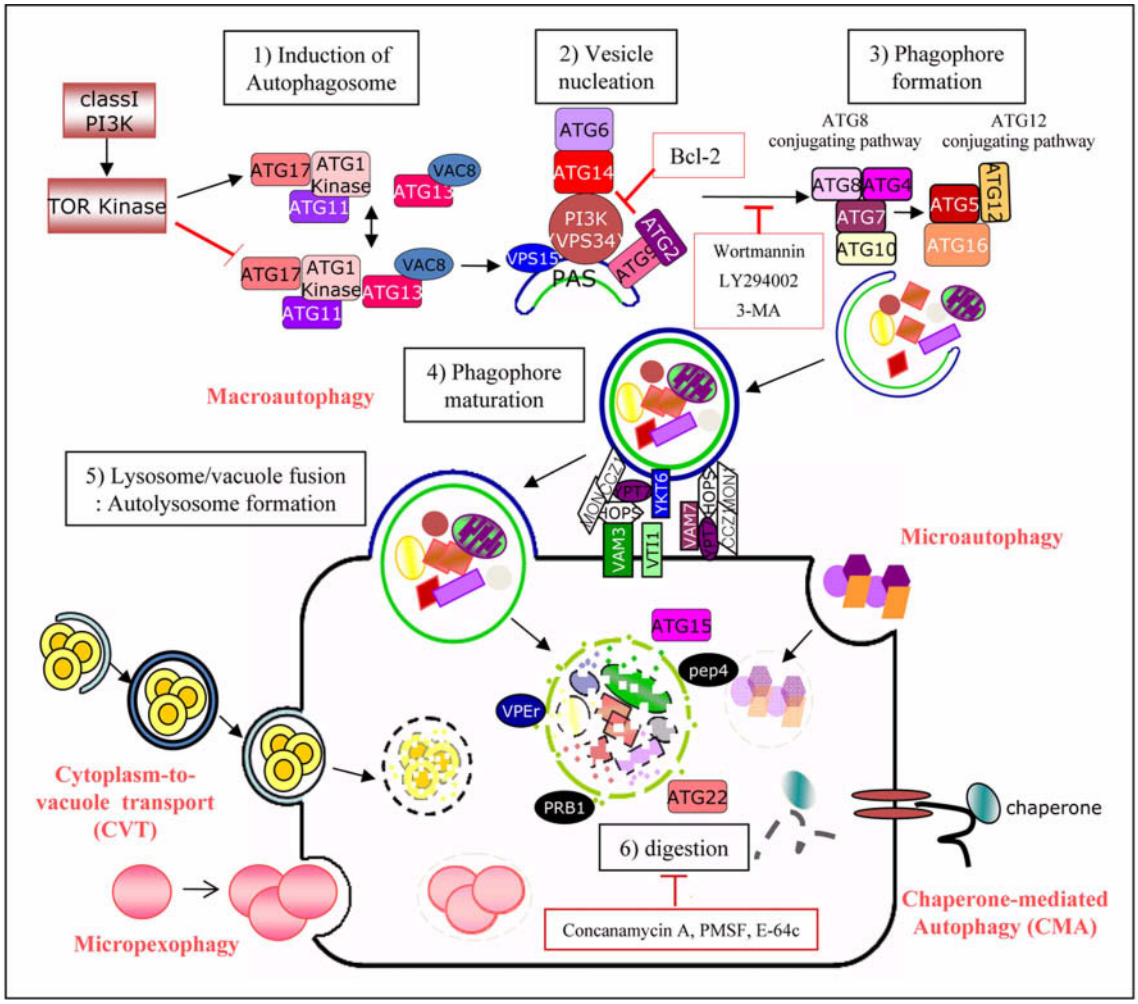

Figure 1. Schematic overview of ATG and ATG-associated components in autophagy and autophagy-related pathways. Micropexophagy and microautophagy include process of tonoplast invagination to engulf cytosolic materials and peroxisomes, making autophagic bodies within the lysosome/vacuole. Macroautophagy sequesters cytosolic constituents into vesicles with double membranes called autophagosomes which then fuse with tonoplast to degrade its contents in vacuolar lumen. CVT pathway also sequesters cytosolic components in double membrane vesicle that moves into lysosome/vacuole to degrade wrapped materials. CMA pathway directs translocation of unfolded proteins across lysosomal membrane via chaperone protein and membrane receptor. Autophagy signaling mechanism is regulated by TOR kinase and leads to the following: 1) under normal conditions, TOR kinase hyperphosphorylates ATG1 and ATG13, maintaining their dissociation from a complex that contains accessory components such as ATG11, ATG17, and VAC8. Under conditions of nutrient starvation, ATG1 and ATG13 are dephosphorylated, inducing re-association and activation of the complex; 2) for vesicle nucleation, ATG1-ATG13 kinase complex induces vesicle nucleation of phagophore assembly site (PAS) to form autophagosome by undergoing processing with PI3K (VPS34), ATG9, ATG6, and other ATG factors. This step is inhibited by such PI3K inhibitors as wortmannin; 3) and 4) phagophore formation on PAS is accomplished via combination of two ubiquitin-like conjugation systems that are composed of ATG8 and ATG12 as tags, E1 enzyme ATG7, and E2 enzymes ATG3 and ATG10. Both ATG8 and ATG12 conjugates associate with autophagosome formation; 5) maturation and fusion of autophagosome to lysosome/vacuole are achieved by vesicle trafficking system using v-SNARE complex, which includes v-SNARE VTI1, Rab-like GTP-binding protein (YKT6), and syntaxin (VAM3); and 6) breakdown of cytosolic materials within vacuole is completed by hydrolases, including lipase ATG15 and proteinases A (PEP4) and B (PRB1). This allows recycling of degraded components such as amino acids, fatty acids, sugars, and nucleotides. Degradation is affected by concanamycin A or protease inhibitors PMSF and E-64c.

teasome pathway, determining the target proteins for ubiquitination and ultimate degradation (Vierstra, 2003).

Autophagy and Autophagy-Related Pathways

Autophagy is represented by two autophagic pathways. Macroautophagy is the predominant pathway for degrading nonfunctional proteins and cellular organelles. It begins with the formation of an isolation membrane (vesicle nucleation) that then expands (vesicle elongation) into what is known as a phagophore. The ends of the phagophore combine to form an autophagosome, a double-membrane vesicle that includes degradative materials (vesicle completion). The autophagosome fuses with the lysosome/vacuole to produce an autolysosome, where the captured materials are degraded (Levine and Kroemer, 2008). Various functions of macroautophagy in several plant species have been reported in response to nutrient starvation, environmental stress, and senescence (Aubert et al., 1996; Moriyasu and Ohsumi, 1996; Doelling et al., 2002; Hanaoka et al., 2002; Bassham, 2007). The second autophagic pathway involves microautophagy, whereby cytoplasmic materials are directly engulfed by invagination of the tonoplast membrane, generating autophagic bodies in the vacuole lumen. Microautophagy is often used for the degradation of storage proteins during seed germination and developmental senescence (Van der Wilden et al., 1980; Toyooka et al., 2001, 2006).

A macroautophagy-derivative pathway, called the cytoplasm-to-vacuole targeting (CVT) pathway, also delivers functional proteins to the vacuole (Klionsky, 2007). Although this pathway has only been reported in yeast to date, a similar vacuolar transport pathway is likely to exist in plants (Seay et al., 2006). Furthermore, chaperone-mediated autophagy (CMA) directly transfers some unfolded substrate proteins into the lysosome, a process that is mediated by an interaction between the cytosolic and lysosomal chaperone hsc70 and the integral membrane receptor LAMP-2A protein (lysosome-associated membrane protein type 2A) in mammals (Massey et al., 2006; Mizushima et al., 2008). Apart from these pathways, several others that are morphologically similar have been identified (Thompson and Vierstra, 2005; Seay et al., 2006; Mizushima et al., 2008). An overall schematic diagram of autophagy-related pathways is shown in Figure 1.

IDENTIFICATION OF AUTOPHAGIC PATHWAYS USING A GENETIC APPROACH

High-throughput genetic approaches have led to the isolation of *ATG*-related mutants in yeast (Ohsumi, 2001). Analysis of these mutants has uncovered corresponding *ATG* genes that function in the essential steps of autophagy, e.g., cargo delivery to the vacuole. Following their identification in yeast, *ATG* orthologs have been found in various eukaryotic genomes, suggesting that autophagic pathways are highly conserved in eukaryotes (Thompson and Vierstra, 2005; Bassham et al., 2006; Bassham, 2007).

Yeast and *Arabidopsis*

ATG genes have been identified in *Arabidopsis*, based on their sequence similarity to yeast *ATG* genes (Bassham, 2007). ATG proteins are involved in a series of autophagy-related events and often function as complexes. For example, the phosphatidylinositol 3-kinase (PI3K) complex is active in autophagosome induction, the ubiquitin-like conjugation systems ATG8 and ATG12 are involved in the elongation of autophagic vesicles, and the ATG9 complex regulates membrane recruitment to the autophagosome. An overview of the putative *ATG* genes in *Arabidopsis* is shown in Table 1 (Bassham et al., 2006; Bassham, 2007).

Autophagy induction

Autophagy is induced by Target of Rampamycin (TOR) kinase signaling (Schmelzle and Hall, 2000). TOR-mediated phosphorylation of ATG13 prevents the association of ATG13 with ATG1 kinase. When TOR is inhibited, ATG13 associates with ATG1, which then undergoes phosphorylation and induces autophagy. The interaction of ATG13 with ATG1 is a pivotal step in the initiation of autophagy (Kamada et al., 2000; Klionsky, 2005). Only one *TOR* and two *ATG13* genes have been identified in *Arabidopsis* (Hanaoka et al., 2002; Menand et al., 2002); however, the *ATG1* genes (*AtATG1a* – *AtATG1d*) constitute a small gene family (Bassham et al., 2006) whose autophagy-related functions remain to be determined.

Vesicle nucleation

Vesicle nucleation is initiated by activation of the vacuolar-protein-sorting (VPS) 34 PI3K complex that includes the components ATG6 and VPS15 (Thompson and Vierstra, 2005). *ATG6* knockout or antisense mutant *Arabidopsis* plants display the following defective phenotypes, suggesting that ATG6/VPS30 is a multi-functional protein: 1) male sterility due to defect in pollen tube germination (Fujiki et al., 2007; Qin et al., 2007; Harrison-Lowe and Olsen, 2008); 2) early leaf senescence and a more sensitive response to nutrient starvation; 3) developmental abnormalities; and 4) uncontrolled hypersensitive response (HR)-programmed cell death (PCD) upon infection with avirulent pathogens (Patel and Dinesh-Kumar, 2008).

Autophagosome formation

Two ubiquitin-like conjugation pathways are involved in the elongation of autophagic vesicles (Ohsumi, 2001; Klionsky, 2005; Thompson and Vierstra, 2005). In these pathways, ATG8 and ATG12 function as ubiquitin-like tags and are conjugated to their targets, the lipid phosphatidylethanolamine (PE) and the ATG5 protein, respectively. The E1-like enzyme ATG7 activates both ATG8 and ATG12, and forms the thioester intermediates ATG8-ATG7 and ATG12-ATG7, which are transferred to the E2-like enzymes ATG3 and ATG10, and finally to PE and ATG5, respectively (Mizushima et al., 1998; Ichimura et al., 2000; Thompson and Vierstra, 2005; Phillips et al., 2008). The ATG8-PE conjugate requires the protease ATG4, whereas the ATG12-ATG5 conjugate forms a multimeric complex with ATG16 (Ohsumi, 2001; Kuma et al., 2002; Hanada et al., 2007). Fujioka et al. (2008) have successfully reconstituted the *Arabidopsis* ATG8 and ATG12 conjugation systems using recombinant proteins *in vitro*.

Table 1. *Arabidopsis* proteins potentially involved in autophagy.[a]

Protein complexes	Proteins	Gene accession numbers	Functions and characteristics in yeast
PI3K complex			**Autophagosome formation**
	ATG6	At3g61710	VPS30
	VPS15	At4g29380	Protein kinase
	VPS34	At1g60490	PI3K
Ubiquitin-like conjugation (ATG12)			**Conjugation of ATG12 and ATG5**
	ATG5	At5g17290	Target for ATG12 conjugation
	ATG7	At5g45900	E1 conjugating enzyme for ATG12 conjugation
	ATG10	At3g07525	E2 conjugating enzyme for ATG12 conjugation
	ATG12	At1g54210 (12a), At3g13970 (12b)	Ubiquitin-like modifier, ATG5 and ATG10 interaction
Ubiquitin-like conjugation (ATG8)			**Conjugation of ATG8 to phosphatidylethanolamine**
	ATG3	At5g61500	E2 conjugating enzyme for ATG8 conjugation
	ATG4	At2g44140 (4a), At3g59950 (4b)	Cysteine protease
	ATG7	At5g45900	E1 conjugating enzyme for ATG8 conjugation
	ATG8	At4g21980 (8a), At4g04620 (8b)	Ubiquitin-like modifier, ATG3 interaction
		At1g62040 (8c), At2g05630 (8d)	
		At5g05150 (8e), At4g16520 (8f)	
		At3g60640 (8g), At3g06420 (8h)	
		At3g15580 (8i)	
ATG9 complex			**Membrane recruitment to autophagosome**
	ATG9	At2g31260	Integral membrane protein
	ATG2	At3g19190	
	ATG18	At3g62770 (18a), At4g30510 (18b)	Function for ATG2 localization
		At2g40810 (18c), At3g56440 (18d)	ATG9 recycling from PAS
		At5g05150 (18e), At5g54730 (18f)	
		At1g03380 (18g), At1g54710 (18h)	
ATG1 complex			**Initiation of autophagy**
	TOR	At1g50030	Protein kinase, negative regulator of autophagy
	ATG1	At1g49180 (1a), At2g37840 (1b)	Serine/threonine kinase
		At3g53930 (1c), At3g61960 (1d)	
	ATG13	At3g18770 (13a), At3g49590 (13b)	Phosphoprotein
	ATG20	At5g06140	
SNARE			**Fusion of autophagososme with the vacuole**
	VTI12	At1g26670	

[a]See references (Thompson and Vierstra, 2005; Bassham et al., 2006; Seay et al., 2006; Bassham, 2007) for a more detailed description.

ATG7 is present as a single gene in *Arabidopsis*. T-DNA insertion *ATG7* mutant plants grow like the wild type under normal conditions, but show accelerated senescence and a highly sensitive response to nutrient limiting growth conditions (Doelling et al., 2002; Hanaoka et al., 2002). Two *ATG4* genes with different splice variants are present in the *Arabidopsis* genome. ATG4 exhibits cysteine protease activity that removes residues from ATG8 (Suzuki et al., 2005; Thompson et al., 2005; Bassham et al., 2006). Plants defective in ATG8/ATG12 conjugation systems display accelerated senescence and a hypersensitive response to nitrogen starvation (Doelling et al., 2002; Yoshimoto et al., 2004; Suzuki et al., 2005; Thompson et al., 2005). Additionally, the expression of *ATG3*, *ATG4a-ATG4b*, *ATG7*, and *ATG8a-ATG8i* is transiently up-regulated during nutrient starvation in *Arabidopsis* (Yoshimoto et al., 2004; Contento et al., 2005; Rose et al., 2006).

ATG8 and its orthologs are specifically localized to autophagosomes (Kirisako et al., 1999; Kabeya et al., 2000), thus making them excellent markers for autolysosomes/autophagosomes when expressed as GFP-ATG8 fusion proteins in cells (Contento et al., 2005; Xiong et al., 2005). The *Arabidopsis* genome contains 9 putative *ATG8* orthologs (Doelling et al., 2002; Hanaoka et al., 2002). One of these, *AtATG8e* (At2g45170), exhibits increased expression during sucrose starvation (Contento et al., 2004) and has been used as an autophagosomal marker in *Arabidopsis* (Yoshimoto et al., 2004).

ATG9 is an integral membrane protein that is required for the delivery of membranes for autophagosome formation (Nair and Klionsky, 2005). In yeast, the association of ATG9 with ATG2 and ATG18 is needed for the correct localization of the ATG9 complex (Wang et al., 2001). In *Arabidopsis*, components that comprise the ATG9 complex have been identified through genetic approaches. *Arabidopsis* T-DNA insertion *ATG9* mutant plants (*atatg9-1*) show phenotypic defects in autophagy-related processes (Hanaoka et al.,

2002), and *ATG2* T-DNA insertion mutant (*atatg2-1*) and *ATG18* RNAi transgenic (RNAi-*AtATG18a*) plants have difficulties in autophagosome formation (Xiong et al., 2005; Inoue et al., 2006).

VTI11 and *VTI12* belong to the *VTI* family of v-SNAREs in *Arabidopsis*. The VTI11 protein forms a SNARE complex at the prevacuolar compartment (PVC) with t-SNARE family members *SYP2* and *SYP5*, whereas the VTI12 protein forms a complex on the *trans*-Golgi network with t-SNARE family members *SYP4* and *SYP6* (Sanmartin et al., 2007). VTI11 plays a role in gravitropism and contributes to tissue identity. VTI12 regulates autophagosome formation and the docking and fusion of autophagosomes with vacuoles. VTI11 and VTI12 can replace and function for the other in SNARE complexes (Surpin et al., 2003; Sanmartin et al., 2007).

Rice

Genome analysis has demonstrated significant conservation among *ATG* genes in yeast and *Arabidopsis*, meaning that the molecular basis of autophagy is well conserved in eukaryotic systems. Rice autophagy-associated genes also appear to function in autophagy and in particular, *OsATG8* has been shown to be essential for autophagy as determined by mutational analysis (Su et al., 2006). Work in the laboratory of Dr. An (POSTECH, Korea) has identified the rice *ATG* gene *OsATG10b* as a component of autophagy because its mutation alters autophagosome formation and stress responses in rice (unpublished data and personal communication). Furthermore, the rice genome contains *ATG* orthologs with sequence similarities to yeast and *Arabidopsis ATG* genes.

BIOLOGICAL ROLES OF AUTOPHAGY IN PLANTS

Phenotypic analysis of *ATG* mutant plants indicates that autophagy plays a role in growth, development, and stress responses, as revealed by various phenotypic defects.

Autophagy in Nutrient Starvation

Because nutrient starvation (usually of sucrose, carbon, and nitrogen) can activate autophagy, this process has been used as a protocol condition for studying autophagy in plants (Chen et al., 1994; Doelling et al., 2002; Hanaoka et al., 2002; Contento et al., 2004; Xiong et al., 2005). During such starvation, autophagosome formation and degradation of cytoplasmic materials in lytic compartments occurs in cultured plant cells (Aubert et al., 1996; Moriyasu and Ohsumi, 1996; Takatsuka et al., 2004; Rose et al., 2006). Mutations in the *AtATG7*, *AtATG8*, and *AtATG9* induce the yellowing of leaves and the expression of *AtSEN1*, a senescence marker gene, indicating that nutrient-limiting conditions accelerate senescence (Doelling et al., 2002; Hanoaka et al., 2002; Slavikova et al., 2005; Xiong et al., 2005). These results suggest that autophagy serves as a core regulatory system to facilitate nutrient supply under conditions of starvation.

Autophagy in Development

A number of *Arabidopsis ATG* mutants have been identified and used for phenotypic analysis (Thompson and Vierstra, 2005; Bassham et al., 2006; Seay et al., 2006; Bassham, 2007). The *ATG4* mutant has shorter primary roots under nitrogen-depleted conditions (Yoshimoto et al., 2004) and autophagy induction is not observed in the root tips of *ATG2* and *ATG5* mutants in response to sucrose deprivation (Inoue et al., 2006). In general, autophagy appears to be involved in cell growth and differentiation such as root hair formation and root elongation under nutrient starvation conditions (Inoue et al., 2006; Yano et al., 2007). Three research groups also have recently reported that *Arabidopsis ATG6/VPS30* plays an important role in pollen germination (Fujiki et al., 2007; Qin et al., 2007; Harrison-Lowe and Olsen, 2008).

Autophagy in the Oxidative Stress Response

Reactive oxidative species (ROS) are highly toxic materials that accumulate in large amounts under various environmental stress conditions and/or during developmental stages. ROS can lead to cell death by causing damage to carbohydrates, DNA, lipids, and proteins (Mittler et al., 2004). Organisms have developed strategies, such as proteasome-dependent proteolysis and autophagy, to scavenge these damaged and oxidized proteins and cellular materials. *AtATG18a* knockdown transgenic plants (RNAi-*AtATG18a*) are defective in their induction and formation of autophagosomes and exhibit hypersensitivity to oxidative stress, suggesting a physiological function for autophagy in response to oxidative stress (Xiong et al., 2007b). Oxidized proteins also accumulate in those plants due to a reduction in degradation efficiency (Xiong et al., 2007a). These results demonstrate that oxidized and damaged cellular components produced during oxidative stress are transferred to the vacuole for autophagic degradation (Xiong et al., 2007b). Nutrient starvation also stimulates ROS formation and, thus, the oxidative stress conditions that are essential for inducing autophagy (Scherz-Shouval et al., 2007; Scherz-Shouval and Elazar, 2007).

Autophagy in Programmed Cell Death

PCD is an important mechanism that controls growth and development. It also acts as a defense response to various environmental stresses in eukaryotic organisms. In plants, PCD is essential for many developmental processes, including the specification of unisexual floral organs, the formation of tracheary elements in xylem differentiation, and leaf senescence (Pennell and Lamb, 1997; Lamb et al., 2001; Fukuda, 2004). A number of studies on plant-microbe interactions have demonstrated that the HR triggered by pathogen infections is also a form of PCD (Dangl and Jones, 2001; Jones and Dangl, 2006).

Autophagy is a mechanism that facilitates cell survival in response to abiotic and biotic stresses and controls PCD by degrading toxic cellular components (Van Doorn and Woltering, 2005). A pivotal role for autophagy in the response to pathogen infections has been defined in mammalian systems (Ogawa and Sasakawa, 2006). It also controls pathogen-induced HR-PCD in plants (Liu et al., 2005). Tobacco *ATG6/BECLIN1*, which was identified in a high-throughput

virus-induced gene silencing (VIGS) screen, is required for the restriction of PCD to TMV-infected sites (Seay and Dinesh-Kumar, 2005; Patel et al., 2006). In addition, *Arabidopsis ATG6* knockdown plants (*AtATG6-AS*) display impaired autophagy activity and accelerated leaf senescence, phenotypes that are similar to those of other *ATG* knockout mutants (Xiong et al., 2005; Patel and Dinesh-Kumar, 2008). Those knockdown plants also fail to regulate the HR-PCD that is initiated by infection with the avirulent bacteria *Pseudomonas syringae pv. tomato* DC3000 (*avrRpt2*).

DUAL ROLES OF AUTOPHAGY IN CELL DEATH AND CELL SURVIVAL

Autophagy is a survival mechanism that protects cells against unfavorable environmental conditions such as nutrient starvation, microbial pathogen infection, oxidative stress, and the aggregation of damaged proteins (Maiuri et al., 2007; Mizushima et al., 2008). However, autophagic structures are often observed within the dying cells in some eukaryotes (Bursch, 2001) and, when hyperactivated by the overexpression of ATG proteins, autophagy leads to cell death (Pattingre et al., 2005; Scott et al., 2007; Yoshimori, 2007). Although physiological levels of autophagy suppress PCD or apoptosis and thus promote cell survival and stress adaptation, excessive levels cause cell death termed autophagic cell death or Type II cell death (Kang et al., 2007). Thus, autophagy provides two opposing mechanisms for cell survival and cell death, both of which are a function of the degree of autophagy activation. Several pieces of evidence demonstrate that autophagy-dependent cell survival or cell death is subjected to tight regulation and depends on environmental conditions such as exposed stimuli, developmental stage, and cell type (Gozuacik and Kimchi, 2007; Maiuri et al., 2007; Mizushima et al., 2008). The signaling pathways of these two opposing responses and their crosstalk have been studied, revealing many of the constituent components shared. Autophagy is the core process that controls the cellular decision of survival and death, and is tightly regulated by complicated molecular mechanisms (Baehrecke, 2005; Levine and Yuan, 2005; Pattingre et al., 2005; Kang et al., 2007).

Concluding remarks

Autophagy has long been considered as simply a scavenging machinery in cells. However, accumulating data suggest that the autophagic pathway is much more complicated, regulating a variety of biological responses. The genetic identification of *ATG* genes in yeast and plants, along with the development of autophagy monitoring systems, has expedited the temporal and spatial analysis of autophagic processes and has greatly contributed to the dissection of the autophagy molecular mechanism. These studies have revealed the biological roles of autophagy in nutrient recycling, PCD response, adaptation to environmental stresses, and vacuole biogenesis. Although progress has been made in our understanding of autophagy, many questions must still be addressed. We need future studies on the structure and function of ATG proteins, the control of autophagy signaling, and the mechanistic basis of physiological defects associated with autophagy activation.

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