GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: Global Institute for Research & Education)
www.gifre.org

AUTOPHAGY IS THE EMERGING ROLE IN PLANT DEFENSE AGAINST PATHOGEN ATTACK

Chetan, S. Janawad, Jyothi, G[2]., Manu, T. G[3]. and Murali, R[4].
[1-4]Department of Plant Pathology, UAS, GKVK, Bangalore, Karnataka, India-560065

Abstract

Plants and plant-associated microorganisms including phytopathogens have to adapt to drastic changes in environmental conditions. Because of their immobility, plants must cope with various types of environmental stresses such as starvation, oxidative stress, drought stress and invasion by phytopathogens during their differentiation, development, and aging processes. Autophagy regulates senescence and pathogen-induced cell death in plants and autophagy and pexophagy play critical roles in differentiation and the invasion of host cells by phytopathogenic fungi. Ancient autophagy pathways are emerging as key defense modules in host eukaryotic cells against microbial pathogens.

Key Words: Autophagy, hypersensitive reaction, phytopathogens, ATG gene

Introduction

Unlike animals, plants are sessile organisms that must endure or overcome variable and severe environmental conditions. For example, the growth of seedlings in nitrogen-poor soils or in the shade indicates that plants have mechanisms for coping with nitrogen and carbon starvation. Under nutrient poor conditions the bulk degradation and recycling of macromolecules is integral to the plant's ability to adapt to its environment. Autophagy is the major system responsible for the degradation of organelles and recycling of proteins, organelles, cytoplasm, cytosolic macromolecules in the vacuole and is therefore assumed to be an extremely important function in plants. Conversely, autophagic processes are also important in the defense responses of plants that are able to perceive and react to invading pathogens.

Autophagy is emerging as an important process in plant infection by pathogenic fungi, which develop differentiated infection cells to breach the plant cuticle. The pivotal role of autophagy in both fungal pathogenesis and disease resistance is linked to its function in the regulation of programmed cell death which is a key component of plant immunity responses and fungal infection related development. Autophagy can be broadly divided into two processes, which share some common components, but have quite distinct cellular functions.

The importance of autophagy in innate immunity in mammals is well documented but how autophagy contributes to plant innate immunity and cell death is not that clear. Here we briefly describe the early studies of plant autophagy, summarize recent studies on the molecular functions of ATG genes, and speculate on the role of autophagy in plants and phytopathogens.

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 and Nakatogawa *et al*., 2009). 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 and Bassham, 2007). Several laboratories originally used their own nomenclature to describe autophagy and autophagy related pathways, including APG for autophagy (Tsukada and Ohsumi, 1993), AUT for autophagy (Thumm *et al*., 1994), CVT for cytoplasm to vacuole targeting (Harding *et al*., 1995, 1996), GSA for glucose-induced selective autophagy (Yuan *et al*., 1997), PAG for peroxisome degradation via autophagy (Sakai *et al*., 1998), PAZ for pexophagy zeocin-resistant (Mukaiyama *et al*., 2002), and PDD for peroxisome degradation deficient (Titorenko *et al*., 1995).

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.

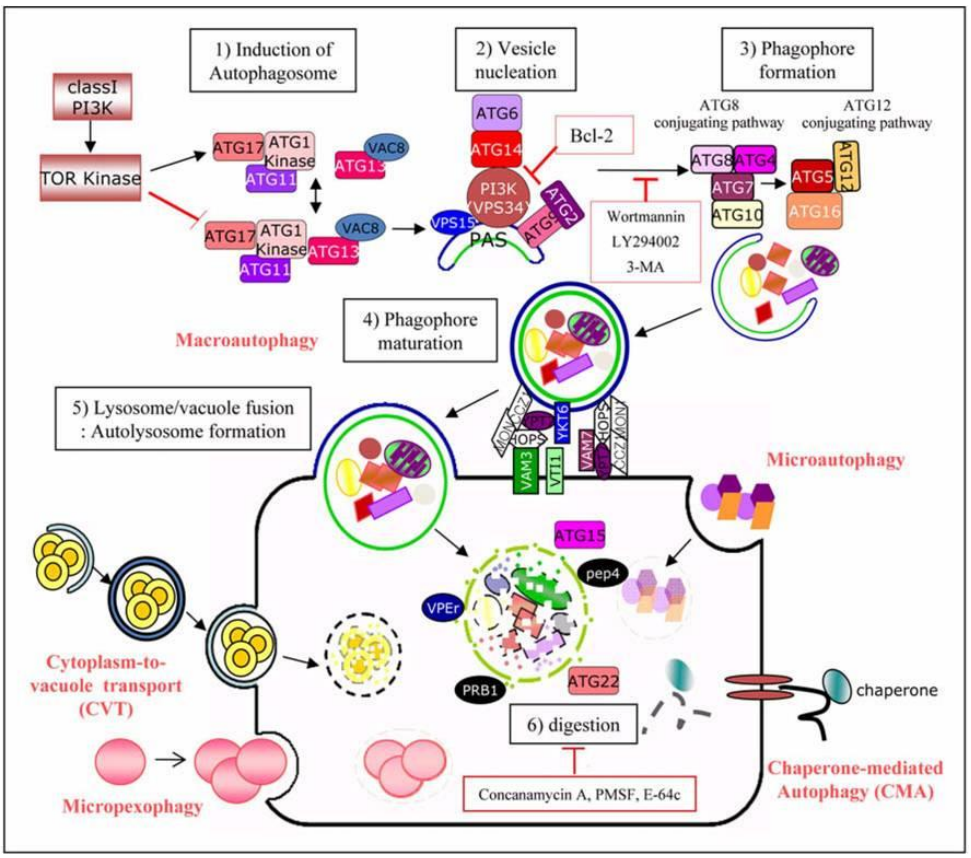

Figure 1: Schematic overview of ATG and ATG-associated components in autophagy and autophagy-related pathways. Micropexophagy and microautophagy include process of tonoplast invagination to engulf cytosolic materials and peroxisomes, making autophagic bodies within the lysosome/vacuole. Macroautophagy 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.

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 Plants

Much has been learned about the requirement for specific ATG genes in the model plant Arabidopsis. Loss of function mutations in ATG genes such as ATG7 and ATG5 implicate autophagy as a central player in cellular homeostasis. Processing and delivery of ATG8 to the vacuole under nitrogen-starved condition requires the cysteine protease ATG4 and the ATG12-ATG5 conjugate and atg5, atg7, atg10, as well as atg12a/b double mutants are hypersensitive to both nitrogen and carbon starvation. Thus, both autophagic related conjugation pathways seem to be required for autophagy in plants and as in yeast and other models, the process is required to recycle nutrients during starvation. Several reports have documented the roles of autophagy in plant development and under stress conditions. During senescence of Arabidopsis leaves kept in darkness (a form of carbon starvation for photosynthetic autotrophs), autophagy seems to be responsible for degradation of the chloroplasts and root development also becomes impaired in different atg mutants during nitrogen starvation.Perhaps not surprisingly, autophagy functions in the removal of oxidized proteins during oxidative stress in Arabidopsis, and downregulation of ATG18a using interference RNA (RNAi) renders plants more sensitive to salt and drought stress.26 Collectively, these reports demonstrate that autophagy affects plants in many aspects of their life cycle. In contrast to autophagy mechanisms in yeast and mammals, information about the signaling pathways triggering the induction of plant autophagy in response to developmental, nutritional and environmental cues is largely lacking. Only recently, direct genetic evidence has been provided that the TOR kinase is a negative regulator of autophagy in higher plants.27 Although knockout of the single TOR gene in Arabidopsis proved to be embryo-lethal,28,29 knockdown by RNAi resulted in constitutive autophagy under non-stressed conditions in an ATG18-dependent fashion.27 In addition, Tap46, the regulatory subunit of protein phosphatase 2A, was recently identified as a downstream effector of the TOR signaling pathway. Depletion of Tap46 reproduced the signature phenotypes of TOR inactivation, including autophagy induction.

1. 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 and 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.

2. 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).

3. 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).

4. 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 and 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 and 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).

The Role of Autophagy in Plant Defense

Autophagy has recently been identified as a significant component of the plant defense mechanism deployed by plants. The majority of plant autophagy associated ATG genes are, for instance, expressed preferentially upon challenge with the aphid *Myzus persicae* or the bacterium *Pseudomonas syringae*. Plant defense against potential pathogens involves the recognition of microbial proteins at the cell surface often termed pathogen associated molecular patterns (PAMPs) that trigger defense signaling and responses such as secondary cell wall thickening and production of anti-microbial compounds. Pathogenic micro-organisms, however, target plant defense signaling directly using secreted effector proteins and thereby suppress plant defense, facilitating their entry and colonization of plant tissue. As a response to the action of microbial effectors, plants have developed immunity from disease by the recognition of specific pathogen effectors. This triggers a hypersensitive response (HR) in which plant cells at the site of infection are killed by a form of programmed cell death, thereby preventing the invading pathogen from further spread. How plants are able to restrict the HR to cells in the immediate area surrounding an infection has, until recently, been unclear but autophagy appears to be necessary for the spatial restriction of programmed cell death. An orthologue of the ATG6 (Beclin1) gene was studied in *Nicotiana benthamiana*. Silencing of the NbBECLIN1 gene by RNA interference in plants that contained the N resistance gene resulted in reduced autophagy and uncontrolled HR upon infection with tobacco mosaic virus. N belongs to the family of TIR–NBS–LRR immune receptor proteins (Toll/interleukin-1 receptor/nucleotide binding site/leucine-rich repeat protein) and recognizes the helicase domain of the TMV replicase protein, which triggers HR and disease resistance. The uncontrolled HR observed in NbBECLIN1 silenced plants was completely dependent on the action of N-mediated immunity, because no cell death was observed when the silenced plants were challenged with TMV in the absence in lines not containing the N gene. The role of ATG6/Beclin1 in HR regulation has also been confirmed in *Arabidopsis thaliana* where AtATG6 antisense plants were found to show a spreading HR phenotype when infected with an incompatible *P. syringae* DC3000 bacterial pathogen expressing the AvrRpm1 effector protein in plants carrying the corresponding RPM1 resistance gene. AtATG6 furthermore showed enhanced expression upon infection with either virulent, or avirulent *P. syringae* bacteria and plant cell death caused by virulent bacteria was also affected by the reduced expression of AtATG6 in antisense lines. These results indicate that autophagy is vital to.

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).

Conclusion

Recent studies have shown that autophagy is likely to be pivotal both to plant infection by pathogenic fungi and also to host defense responses. This highlights the importance of autophagic processes to developmental biology and extracellular responses in multi-cellular eukaryotes as a means of rapidly redeploying cellular resources under times of

starvation stress or acute developmental need. Autophagy monitoring systems allow us to know when and where autophagy occurs and under which conditions autophagy is induced. These systems will contribute to a further understanding of the physiological roles of autophagy in these organisms.

References

Aubert S, Gout E, Bligny R, Marty Mazars D, Barrieu F, Alabouvette J, Marty F, Douce R. (1996). Ultrastructural and biochemical characterization of autophagy in higher plant cells subjected to carbon deprivation: control by the supply of mitochondria with respiratory substrates. J. Cell Biol., 133:1251-1263.

Baehrecke E.H. (2005). Autophagy: dual roles in life and death? Nat. Rev. Mol. Cell, 6:505-510

Bassham D.C., Laporte M., Marty F., Moriyasu Y., Ohsumi Y., Olsen L.J., Yoshimoto K. (2006). Autophagy in development and stress responses of plants. Autophagy, 2:2-11.

Bassham D.C. (2007). Plant autophagy more than a starvation response. Curr. Opin. Plant Biol., 10:586-593.

Bursch W. (2001). The autophagosomal-lysosomal compartment in programmed cell death. Cell Death Differ, 8:569-581.

Chen M.H., Liu L.F., Chen Y.R., Wu H.K., Yu S.M. (1994). Expression of áamylases, carbohydrate metabolism, and autophagy in cultured rice cells is coordinately regulated by sugar nutrient. Plant J., 6:625-636.

Contento A.L., Kim S.J., Bassham D.C. (2004). Transcription profiling of the response of Arabidopsis suspension culture cells to Suc. starvation. Plant Physiol.,135:2330-2347.

Dangl J.L. and Jones J.D.G. (2001). Plant pathogens and integrated defense responses to infection. Nature, 411:826-833.

Doelling J.H., Walker J.M., Friedman E.M., Thompson A.R., Vierstra R.D. (2002). The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in *Arabidopsis thaliana*. J. Biol. Chem., 277:33105-33114.

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

Fukuda H. (2004). Signals that control plant vascular cell differentiation. Nat. Rev. Mol. Cell Biol., 5:379-391.

Gozuacik D. and Kimchi A. (2007). Autophagy and cell death. Curr. Top. Dev. Biol., 78:217-245.

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

Harding T.M., Hefner-Gravink A., Thumm M. and Klionsky D.J. (1996). Genetic and phenotypic overlap between autophagy and the cytoplasm to vacuole protein targeting pathway. J. Biol. Chem., 271:17621-17624.

Harding T.M., Morano K.A., Scott S.V. and Klionsky D.J. (1995). Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. J. Cell Biol., 131:591-602.

Harrison-Lowe N.J. and Olsen L.J. (2008). Autophagy protein 6 (ATG6) is required for pollen germination in *Arabidopsis thaliana*. Autophagy, 4:339-348.

Inoue Y., Suzuki T., Hattori M., Yoshimoto K., Ohsumi Y. and Moriyasu Y. (2006). AtATG genes, homologs of yeast autophagy genes, are involved in constitutive autophagy in Arabidopsis root tip cells. Plant Cell Physiol., 47:1641-1652.

Jones J.D.G. and Dangl J.L. (2006). The plant immune system. Nature, 444:323-329.

Kang C., You Y. and Avery L. (2007). Dual roles of autophagy in the survival of Caenorhabditis elegans during starvation. Genes Dev., 21:2161-2171.

Klionsky D.J. (2007). Autophagy from phenomenology to molecular understanding in less than a decade. Nat. Rev. Mol. Cell Biol., 8:931-937.

Lamb E., Kato N. and Lawton M. (2001). Programmed cell death, mitochondria and the plant hypersensitive response. Nature, 411: 848-853.

Levine B. and Kroemer G. (2008). Autophagy in the pathogenesis of disease. Cell, 132:27-42.

Levine B. and Yuan J. (2005). Autophagy in cell death: An innocent convict? J. Clin. Invest., 115:2679-2688.

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

Maiuri M.C., Zalckvar E., Kimchi A. and Kroemer G. (2007). Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat. Rev. Mol. Cell. Biol., 8:741-752.

Maiuri M.C., Zalckvar E., Kimchi A. and Kroemer G. (2007). Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat. Rev. Mol. Cell Biol., 8:741-752.

Massey A.C., Zhang C. and Cuervo A.M. (2006). Chaperone-mediated autophagy in aging and disease. Curr. Top Dev. Biol., 73:205-235.

Mittler R., Vanderauwera S., Gollery M. and Van Breusegem F. (2004). Reactive oxygen gene network of plants. Trends Plant Sci., 9:490-498.

Mizushima N., Levine B., Cuervo A.M. and Klionsky D.J. (2008). Autophagy fights disease through cellular self-digestion. Nature, 451:1069-1075.

Mizushima N., Levine B., Cuervo A.M. and Klionsky D.J. (2008). Autophagy fights disease through cellular self-digestion. Nature, 451:1069-1075.

Mizushima N., Levine B., Cuervo A.M. and Klionsky D.J. (2008). Autophagy fights disease through cellular self-digestion. Nature, 451:1069-1075.

Mizushima N., Levine B., Cuervo A.M. and Klionsky D.J. (2008). Autophagyfights disease through cellular self-digestion. Nature, 451:1069-1075.

Mizushima N., Noda T., Yoshimori T., Tanaka Y., Ishii T., George M.D., Klionsky D.J., Ohsumi M. and Ohsumi Y. (1998). A protein conjugation system essential for autophagy. Nature, 395:395–398.

Moriyasu Y. and Ohsumi Y. (1996). Autophagy in tobacco suspension cultured cells in response to sucrose starvation. Plant Physiol., 111:1233-1241.

Moriyasu Y. and Ohsumi Y. (1996). Autophagy in tobacco suspension-cultured cells in response to sucrose starvation. Plant Physiol., 111:1233–1241.

Mukaiyama H., Oku M., Baba M., Samizo T., Hammond A.T., Glick B.S., Kato N. and Sakai Y. (2002). Paz2 and 13 other PAZ gene products regulate vacuolar engulfment of peroxisomes during micropexophagy. Genes Cells, 7:75-90.

Nakatogawa H., Suzuki K., Kamada Y. and Ohsumi Y. (2009). Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat. Rev. Mol. Cell Biol., 10:458–467.

Ohsumi Y. (2001). Molecular dissection of autophagy: two ubiquitin-like systems. Nat. Rev. Mol. Cell Biol., 2:211–6.

Patel S., Caplan J. and Dinesh-Kumar S.P. (2006). Autophagy in the control of programmed cell death. Curr. Opin. Plant Biol., 9:391- 396.

Patel S. and Dinesh-Kumar S.P. (2008). Arabidopsis ATG6 is required to limit the pathogen-associated cell death response. Autophagy, 4:20-27.

Pattingre S., Tassa A., Qu X., Garuti R., Liang X.H., Mizushima N., Packer M., Schneider M.D. and Levine B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell, 122:927-939.

Pattingre S., Tassa A., Qu X., Garuti R., Liang X.H., Mizushima N., Packer M., Schneider M.D. and Levine B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell, 122:927- 939.

Pennell R.I. and Lamb C. (1997). Programmed cell death. Plant Cell, 9:1157-1168.

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

Rose T.L., Bonneau L., Der C., Marty-Mazars D. and Marty F. (2006). Starvation- induced expression of autophagy-related genes in Arabidopsis. Biol. Cell, 98:53-67.

Sakai Y., Koller A., Rangell L.K., Keller G.A. and Subramani S. (1998). Peroxisome degradation by microautophagy in Pichia pastoris: identification of specific steps and morphological intermediates. J. Cell Biol., 141:625-636.

Scherz-Shouval R. and Elazar Z. (2007). ROS, mitochondria and the regulation of autophagy. Trends Cell Biol., 17:422-427.

Scherz-Shouval R., Shvets E., Fass E., Shorer H., Gil L. and Elazar Z. (2007). Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO. J., 26:1749-1760.

Scott R.C., Juhasz G. and Neufeld T.P. (2007). Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr. Biol., 17:1-11.

Seay M.D. and Dinesh-Kumar S.P. (2005). Life after death: are autophagy genes involved in cell death and survival during plant innate immune responses? Autophagy, 1:185-186.

Seay M.D., Patel S. and Dinesh-Kumar S.P. (2006). Autophagy and plant innate immunity. Cellular Microbiology, 8:899-906.

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

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

Thompson A.R. and Vierstra R.D. (2005). Autophagic recycling: lessons from yeast help define the process in plants. Curr. Opin. Plant Biol., 8:165-173.

Thumm M., Egner R., Koch M., Schlumpberger M., Straub M., Veenhuis M. and Wolf D.H. (1994). Isolation of autophagocytosis mutants. Nat. Rev. Mol. Cell Biol., 8:741-752.

Titorenko V.I., Keizer I., Harder W. and Veenhuis M. (1995). Isolation and characterization of mutants impaired in the selective degradation of peroxisomes in the yeast *Hansenula polymorpha*. J. Bacteriol., 177:357-363.

Toyooka K., Moriyasu Y., Goto Y., Takeuchi M., Fukuda H. and Matsuoka K. (2006). Protein aggregates are transported to vacuoles by a macroautophagic mechanism in nutrient-starved plant cells. Autophagy, 2:96-106

Toyooka K., Okamoto T. and 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., 154:973-982.

Tsukada M. and Ohsumi Y. (1993). Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett., 333:169–74.

Van der Wilden W., Herman E.M. and Chrispeels M.J. (1980). Protein bodies of mung bean cotyledons as autophagic organelles. Proc. Natl. Acad. Sci. USA, 77:428-432.

Van Doorn W.G. and Woltering E.J. (2005). Many ways to exit? Cell death categories in plants. Trends Plant Sci., 10:117-122.

Xiong Y., Contento A.L. and Bassham D.C. (2005). AtATG18a is required for the formation of autophagosomes during nutrient stress and senescence in *Arabidopsis thaliana*. Plant J., 42:535-546.

Xiong Y., Contento A.L. and Bassham D.C. (2007a). Disruption of autophagy results in constitutive oxidative stress in Arabidopsis. Autophagy, 3:257-258.

Xiong Y., Contento A.L., Nguyen P.Q. and Bassham D.C. (2007b). Degradation of oxidized proteins by autophagy during oxidative stress in Arabidopsis. Plant Physiol., 143:291-299.

Yano K., Suzuki T. and Moriyasu Y. (2007). Constitutive autophagy in plant root cells. Autophagy, 3:360-362.

Yoshimori T. (2007). Autophagy: Paying Charon's toll. Cell, 128:833-836.

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

Yuan W., Tuttle D.L., Shi Y.J., Ralph G.S. and Dunn Jr. W.A. (1997). Glucoseinduced microautophagy in Pichia pastoris requires the α-subunit of phosphofructokinase. J. Cell Sci., 110:1935-1945.