



Review Paper

Autophagy: Molecular Insight and Role in Plant Programmed Cell Death and Defense Mechanism.

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Abstract

Autophagy is an evolutionary conserved pathway of vacuolar degradation of cytoplasmic constituents. The characteristic feature of the pathway is double membrane bound autophagosome which transfer the damaged and excessive cell components to the lysosome or vacuoles for degradation and recycling. Autophagy allows the cell to survive under nutrient starvation and various biotic and abiotic stresses. The molecular mechanism of autophagy has been studied in yeast to mammals and also in plants. Many Experimental results suggested that the basic molecular mechanism and pathways are conserved in yeast, mammals and plants to a large extent. This review highlights basic molecular autophagy and its role in defense mechanism and programmed cell death in plants.

Keywords: Autophagosome, Beclin1, Rapamycin, Necrosis, Arabidopsis, Phagophore.

Introduction

Autophagy is a catabolic process during which cytoplasmic content undergoes the degradation either by lysosome (animal cells) or by vacuole (yeast and plants). It is a turn over process of organelles and macromolecules, allowing cells to survive during nutrient starvation. The main feature of autophagy is formation of autophagosome¹. Autophagosome is a double membranous vesicle, engulfs the cytoplasmic material and degrades it. After the degradation, new macromolecules are synthesized via the recycling of degraded products².

The autophagy is classified into macro, micro and chaperone dependent autophagy. In Microautophagy, cytoplasmic content are directly engulfed by lysosomal membrane by self invagination. While in macroautophagy a double membrane bound autophagosome is formed and fuses with lysosome/vacuole, dissolve the cell content and the products are recycled back into the cell cytoplasm for reuse. Whereas, in chaperon mediated autophagy heat shock proteins are involved in the translocation of misfolded and damaged proteins³.

Autophagy is induced under various abiotic and biotic stress conditions including heat, temperature, nutrient starvation, oxidative and ER induced stresses. Cell development and differentiation is highly dependent on autophagy⁴. Autophagy is essential in eliminating pathogens and for disease progression of non communicable diseases in animals. In budding yeast *Saccharomyces cerevisiae*, to date 34 Autophagy Related Genes (ATG) have been identified⁵, and in which at least 18 are involved in formation of autophagosome.

Most of the autophagy genes are discovered in yeast and homologs genes are identified in other eukaryotes suggesting that autophagy is an evolutionary conserved mechanism across various kingdom⁶. Autophagosome formation is common in all types of macroautophagy. In all autophagic pathways core autophagy machinery is involved and encoded by specific genes in each step of the autophagy⁷.

In this review, we discuss the mechanism of complex pathways of autophagy, role of autophagy in defense mechanism of plants and also in the programmed cell death.

Molecular Mechanism of Autophagy: Autophagy is a highly regulated process and their induction and regulation is extensively studied from lower to higher eukaryotes like yeast, drosophila, mammals and plants. The signaling pathways involved in autophagy induction are summarized below.

Induction and Regulation

Role of TOR in Yeast: Cells receive and transmit the stimulus by a complex system to control cellular growth. TOR is well studied in eukaryotes including yeast, mammals, fungi and worms that induce the cell signals during nutritional requirement of cell for the cell growth. Target of Rapamycin has also been reported in plants and algae. Target of Rapamycin is 270 kDa protein composed of two subunits including Target of Rapamycin C1 and Target of Rapamycin C2⁸.

TORC1 modulates temporal cell growth whereas TORC2 controls spatial cell growth. Anabolic processes like protein

synthesis, transcription and ribosome biogenesis and catabolic process such as TOR signaling pathways. TORC1 regulation is dependent on nutrient conditions; for example autophagy is stimulated by TORC1 under nutrient starvation.

It was studied at molecular level in the yeast on how the cells receive signals. In yeast it has been identified that TORC1 inhibited under nutrient starvation condition due to inactivation of TOR by rapamycin⁹. In yeast TORC1 regulate the ATG1- ATG13 and ATG 17, kinase complex. ATG1 is a kinase protein and plays an important role in controlling autophagy and Cytoplasm to Vacuole Transport pathway¹⁰ which is conserved among eukaryotes.

Role of TOR in Plants

TOR protein is conserved in plants, three putative ATG1 homologues and two putative ATG13 homologues have been identified in Arabidopsis but the role and interaction with TOR are still unknown. In Arabidopsis RAPTOR (Regulatory-Associated Protein of TOR) homologues have also been identified. This protein has been shown to mediate the activation of TOR kinase and recruited substrate for TOR¹¹. Recent studies suggested that TOR kinase activity inhibited due to dissociation of RAPTOR by rapamycin in mammals⁹.

In Arabidopsis, it has been analyzed that TOR interacts with RAPTOR, that regulates the Activity of S6 Kinase (a putative substrate of TOR) in response to Osmotic Stress Signals. Along with the plant, regulation of autophagy may also have been studied in photosynthetic algal species. A recent studies in *Chlamydomonas reinhardtii* showed that autophagy is induced by rapamycin treatment¹³.

Role of TOR in Mammals

The target of rapamycin (TOR) protein is highly conserved in mammals and regulates the autophagy by sensing the nutrient conditions. The main regulators of mTOR includes insulin receptor and 1 and 2 substrates, class-I PtdIns3K, 3-phosphoinositide-dependent protein kinase 1 (PDK1), and protein kinase B¹⁴. The heterodimer tuberous sclerosis 1 protein 1 and tuberous sclerosis 1 protein 2 regulate the mTOR activity as an activator of GTPase for the GTPase Rheb which controls mTOR signaling. The ribosomal subunit S6 (p70S6K) kinase located downstream of mTOR negatively regulates mTOR signaling by phosphorylating IRS1, which down regulate the insulin signaling, that leads to a decline in PtdIns 3, 4 and 5 and regulate autophagy even under nutrient rich conditions¹⁵.

ATG 9 Cycling System

The ATG9 is the only identified integral membrane protein

required for autophagosome formation, and it is thought to cycle between the membrane sources and the phagophore assembly site (PAS). Thus, ATG9 plays an important role as a membrane carrier.

In *S. cerevisiae*, ATG9 cycles between peripheral sites and the pre-autophagosomal structure/phagophore assembly site (PAS). A binding partner of ATG9 is ATG11 identified by the yeast two hybrid screen which suggests that ATG11 mediates the anterograde transport of ATG9 to the PAS along the actin cytoskeleton^{17,18}. Most of the ATG proteins, primarily display single punctate localization at the PAS while ATG9 localizes to multiple punctate structures, including the PAS^{16,17}. In the absence of ATG11, the transport of ATG9 to the PAS is blocked¹⁶. The transport of ATG9 to the PAS is regulated by ATG11 and ATG23 and ATG27.

Phosphatidylinositol 3 -Kinase Complex

The class III Phosphatidylinositol 3-kinase (PtdIns3K) also known as Vacuolar Protein Sorting 34 (VPS34) protein, first identified in yeast is responsible for the synthesis of phosphatidylinositol 3-phosphate. It constitutes the membrane-associated signal transduction complex.

It forms two complexes, complex I and complex II in yeast. Each complex consists of VPS34, VPS15 and VPS30/Atg6 proteins. VPS15 is essential for association with VPS 34. The ATG6 in PtdIns3K complex is reported as an important regulator of autophagy, vesicular trafficking, G protein signaling and nutrient sensing mechanism¹⁹.

Mammalian cells have two types of PtdIns3K and human VPS34 which are essential to generate PtdIns3 and to stimulate autophagy. In mammalian cells, class III PI3K/P150 is associated with the protein Beclin 1 and forms a functional cluster with VPS15¹⁹.

The protein PtdIns3k has important physiological roles in plants²⁰ and it is more homologous to yeast PtdIns3k (VPS34) when compared to mammalian protein P110. It has also been reported by genetic transmission analysis that At VPS34 is essential for pollen development and vacuole reorganization²⁰. It has been reported²¹ that plants deficient in VPS30/ATG6 has short roots, early leaf senescence, increased anthocyanin production, developed dwarfism, with fewer flowers and low fertility. Furthermore, antisense plants of AtATG6 are failed to limit the pathogen associated cell death response²².

Ubiquitin-like Protein Conjugation System

In eukaryotes, Ubiquitin is a small 8.5 kDa regulatory protein ubiquitous in all the tissues. These proteins used in post transcriptional modification and binds with the substrate protein and modify the proteins in three steps like activation, conjugation and ligation. Modification can be achieved either

by single ubiquitin or by chain of ubiquitin²³.

In selective and non selective autophagy two types of conjugation systems are involved including ubiquitin-like proteins ATG12 and ATG8²³. These are evolutionary conserved process from yeast to human. In autophagy ATG12 involves in covalent conjugation with ATG5 through an isopeptide bond. To initiate this conjugation additional proteins are required that are ATG 10, and ATG7, ATG7 act as E1 ubiquitin-activating enzyme and ATG10 functions as an E2 Ubiquitin-conjugating enzyme²⁴.

In ATG12-ATG5 conjugate, ATG5 non covalently binds with a coiled protein ATG16 and forms a trimeric complex which is important to induce autophagy²⁴. ATG8 binds to phosphatidyl ethanolamine (PE)²⁵ and functioned as an integral membrane protein. But this conjugation is just reversible to ATG12-ATG5 conjugate where ATG8 is released from lipid by ATG4 which in turn is utilized in CVT pathway²⁶. The ATG12 and ATG8 systems are conserved among the organisms. In mammals the yeast homologs ATG5 and ATG12 are present and performing similar function as in

yeast²⁶. In contrast to yeast, that has a single gene, Arabidopsis has gene families, for example AtATG8 has 9 gene families including AtATG8a to AtATG8i²⁷.

Role of Autophagy in Defense Mechanism and Programmed Cell Death: The autophagy can play dual roles of “Prosurvival” and “Prodeath” during pathogen infection in plants. The pathogens infecting plants can be categorized as biotrophic and necrotrophic pathogens. The necrotrophic pathogen kills the host cells while biotrophic rely on host for survival. It was reported²⁸ that necrotrophic fungal pathogen *Botrytis cinerea* infect the Arabidopsis and accelerates autophagy. In Arabidopsis infection with necrotrophic and biotrophic pathogen in autophagy mutant (ATG5, ATG10 and ATG18) showed higher rate of fungal proliferation compared to wild type plants and this suggests that autophagy genes are playing critical role in pathogenesis²⁸.

The plant immunity is also associated with Pathogen-associated molecular pattern molecules (PAMPs) recognized by pattern

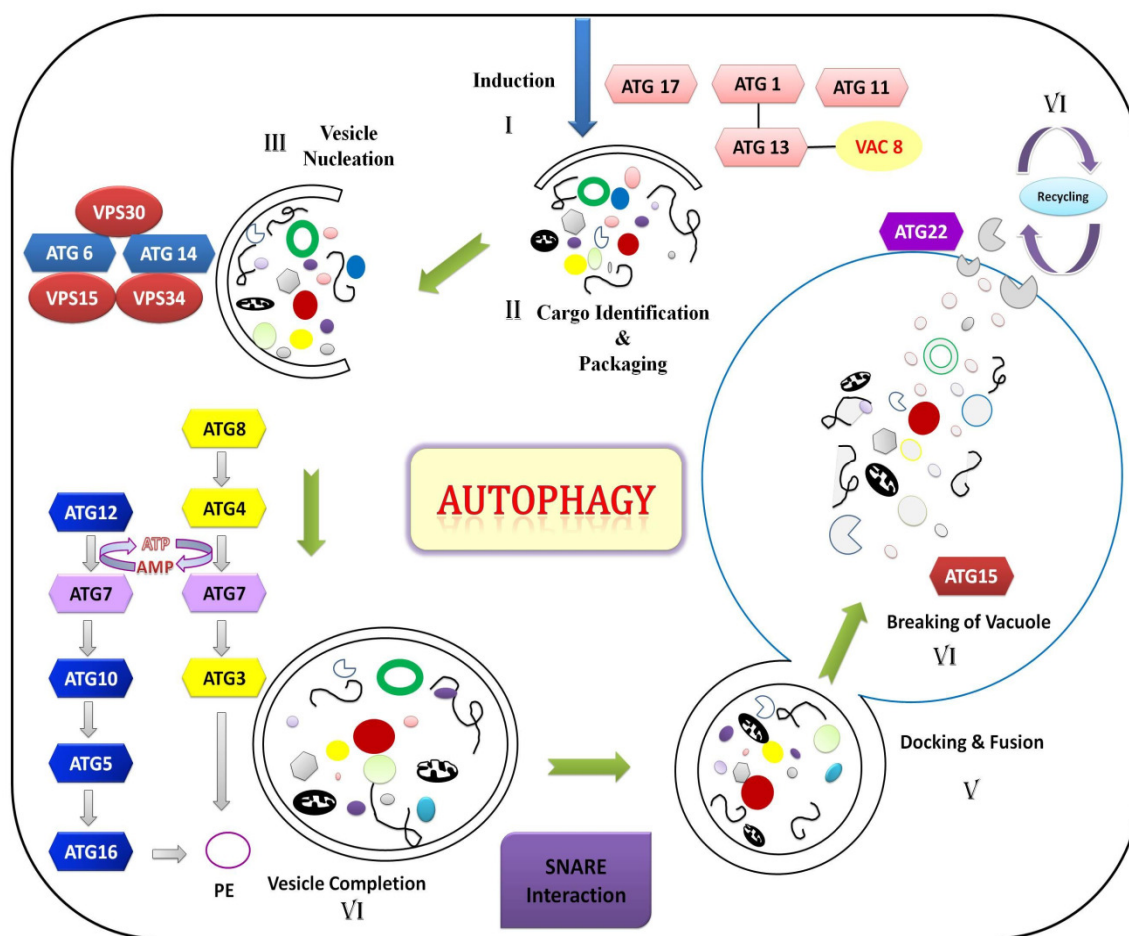


Figure-1

The Steps involved in autophagy pathway. It includes a series of steps like induction, cargo identification, packaging, vesicle nucleation, vesicle completion and vesicle fusion with vacuole/Lysosome. Different classes of ATG proteins are governing the functions at various steps in the autophagic pathway

recognition receptor (PRR) and resistance proteins that identifies the microbial infection or attack referred as Effector Mediated Immunity (ETI). At the site of microbial attack cells undergoes the death with ETI and known as hypersensitive response (HR). How the cells undergo in cell death with diverse form of immunity and their relationship is not well known in plants. Previous studies³⁰ have observed the upregulation of ATG genes in microbe-infected Arabidopsis plants and suggested that autophagy is important for Plant disease control and immunity.

In another study¹⁹ demonstrated that knockdown mutant Beclin1 or ATG6 of Arabidopsis infected with bacterial pathogen *Pseudomonas syringae* pv. Tomato (Pst) DC3000 (avrRPM1), showed the ETI-associated spreading of PCD. All of these data suggested autophagy is critical mechanism for survival to control the HR mediated PCD by reducing the death signals.

The mechanism of programmed death induced by autophagy in plants during stress and development is comparable to apoptosis in animals. Two types of cell death processes have been identified in plants including vacuolar cell death and necrosis. Vacuolar death occurs during differentiation and development of plant tissues and organs while necrosis is initiated as a result of abiotic stress, HR–Cell death and necrotrophic pathogen evoked cell death³⁰. Many plant hormones are also involved in autophagic processes and they show variable responses to necrotrophic and biotrophic pathogens.

It was reported that defense responses against the necrotrophic pathogens contingent upon the jasmonic acid (JA) and ethylene signals, whereas against biotrophic pathogens intricate on SA signaling²⁹. It was analyzed that autophagy mutant of Atg2 and Atg5 in Arabidopsis displayed early senescent phenotypes under nutrient rich conditions. The salicylic acid (SA) accumulated in early senescent ATG2 and ATG5 mutants. Blocking the SA signaling pathways by NahG, sid2 and npr1 in ATG 5 and ATG2 mutants reveal autophagy operates a negative feedback loop modulating SA signaling and that this negative feedback limits senescence and immunity-related PCD in plants³⁰.

Conclusion

Autophagy plays important role in defense to limit the pathogen spread and infection: The production of ROS is enhanced by autophagy to prevent death and to increase the survival. Autophagy is a conserved mechanism in yeast, plants, animals and humans. Further research is needed to identify the target of the pathogens and interaction of autophagy genes with the targets. By identifying the targets, inhibitors can be designed to increase immunity and disease resistance in plants. The critical roles played by the genes involved in autophagy can be identified by functional and comparative genomics and will shed light on molecular mechanisms. This can be utilized in

crop improvement, production and protection.

Abbreviations:

ATG: Autophagy related Gene
CMA: Chaperone Mediated Autophagy
ER: Endoplasmic Reticulum
TOR: Target of Rapamycin
mTOR: mammalian Target of Rapamycin
TORC1: Target of Rapamycin Complex 1
CVT: Cytoplasm to Vacuole Transport
RAPTOR: Regulatory Associated Protein of TOR
PtdIns3K: Phosphoinositide 3-kinase
PDK1: 3-Phosphoinositide-Dependent Protein Kinase 1
PKB: Protein Kinase B
PAS: Phagophore Assembly Site
VPS34: Vacuolar Protein Sorting 34
AtVPS34: Arabidopsis Vacuolar Protein Sorting 34
PAMPs: Pathogen Associated Molecular Pattern

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