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Autophagy as initiator or executioner of cell death

Elena A. Minina, Peter V. Bozhkov, and Daniel Hofius

Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Box 7080, 75007 Uppsala, Sweden

Autophagy plays multiple, often antagonistic roles in plants. In particular, cytoprotective functions of autophagy are well balanced by cell death functions to compensate for the absence of apoptosis culminating in phagocytic clearance of dead cells. If autophagy is indeed required for plant programmed cell death (PCD), then what place does it occupy in the PCD pathways? Recent studies have examined the effects of impaired autophagy on pathogen-induced hypersensitive response (HR) and developmental PCD. While HR death was efficiently suppressed, inhibition of autophagy induced a switch from vacuolar PCD essential for development to necrosis. We therefore propose a dual role for autophagy in plant PCD: as an effector of HR PCD lying upstream of the 'point-of-no-return', and also as a downstream mechanism for clearance of terminally differentiated cells during developmental PCD.

Lessons from animal research

In animals, autophagy (see Glossary) can inhibit, promote, alter, or have no effect on cell death, the actual outcome depending on several factors such as cell type, cell death stimulus, stress intensity, and the basal level of autophagy [1]. This ambiguity in autophagy–cell death relationships is brought about by a plethora of mechanistic intersections between the two processes. The best-understood of these are the activation of both autophagy and cell death by the same signal transduction pathways [2–4], selective autophagic degradation of pro- or anti-death components [4,5], conversion of AuTophaGy-related (ATG) proteins into cytotoxic molecules by, for example, proteolytic cleavage [6,7], and the requirement for autophagy in heterophagic clearance of dead cells [8]. Furthermore, autophagy *per se* can sometimes (almost exclusively during nematode and insect development) operate as a cell death effector mechanism, a phenomenon termed 'autophagic cell death' [9,10]. In this case, suppression of autophagy leads to complete or substantial inhibition of cell death, demonstrating that cells die by (rather than with) autophagy.

Corresponding authors: Bozhkov, P.V. (peter.bozhkov@slu.se); Hofius, D. (daniel.hofius@slu.se).

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Autophagy is involved in plant cell death

Similarly to the situation in animals, both autophagy and cell death play central roles in plant disease and development [11–13], although they have for a long time been studied as separate processes. However, morpho-physiological evidence for involvement of autophagy in plant cell death was available well before the first characterization of autophagy-deficient mutants in *Arabidopsis thaliana*

Glossary

Autophagic flux: the dynamic process of autophagosome formation, delivery of autophagic substrates to the lysosome (or vacuole), and degradation of autophagic substrates inside the lysosome (or vacuole).
Autophagosome: a cytosolic membrane-bound compartment denoted by a limiting double membrane.
Autophagy: from Greek, 'eat oneself'; a major catabolic process in eukaryotic cells wherein a portion of the cytoplasm is engulfed by a specific membrane, delivered to lysosome (in animals) or vacuole (in fungi or plants), and is finally digested by hydrolytic enzymes.
AuTophaGy-related (ATG) proteins: a group of proteins from different families, required for progression through specific stages of the autophagy pathway.
Effector-triggered immunity (ETI): a complex, high-amplitude resistance response induced by intracellular immune receptors of the nucleotide-binding leucine-rich (NB-LRR) protein family upon recognition of pathogen-secreted effector proteins or their activities on host cellular targets. ETI is typically associated with the development of HR and SAR.
Heterophagy: the digestion within a cell of a material taken in by phagocytosis from the cellular environment. At the final stages of apoptotic cell death, apoptotic bodies are removed by heterophagy.
Hypersensitive response (HR): a rapid and locally restricted PCD reaction as a result of ETI. HR cell death is often but not always dispensable for ETI-mediated limitation of pathogen proliferation and spread.
Metacaspases: a group of cysteine-dependent proteases belonging to the C14 family that are found in protozoa, fungi, and plants. Two types of metacaspases are distinguished: (i) type I metacaspases have a N-terminal prodomain containing a proline-rich repeat motif and, in plant members, also a zinc-finger motif, (ii) type II metacaspases lack such a prodomain but harbor a linker region between the putative large (p20) and small (p10) caspase-like subunits. Unlike aspartate-specific caspases, metacaspases possess arginine/lysine substrate cleavage specificity.
Necrosis: one of the two major types of cell death in plants, characterized by mitochondrial dysfunction, energetic catastrophe, and early rupture of plasma membrane. Necrosis is typically found under abiotic stress, whereas pathogen-induced HR cell death displays features of both necrosis and vacuolar cell death. Most morphological and biochemical characteristics of necrosis are conserved between animals and plants.
Suspensor: a part of the plant embryo that functions as a conduit of nutrients and hormones to the embryo proper. The suspensor becomes obsolete at late stages of embryogenesis and undergoes slow degradation by vacuolar cell death.
Systemic acquired resistance (SAR): a broad-spectrum resistance response in uninfected tissue following local activation of ETI. SAR is usually characterized by salicylic acid (SA)- and NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent induction of pathogenesis-related (PR) proteins.
Vacuolar cell death: one of the two major types of cell death in plants, wherein the content of dying cell is gradually engulfed by growing lytic vacuoles without loss of protoplast turgor, and culminating in vacuolar collapse. Vacuolar cell death is commonly observed during plant development, for example in the embryo-suspensor and xylem elements.

(referred to here as *Arabidopsis*). First, massive formation of autophagosomes, and delivery of the cytoplasm and organelles to the lytic vacuoles in the terminally differentiated plant cells, were shown to underlie vacuole expansion and complete protoplast clearance following tonoplast rupture [14–18]. Second, it has been suggested [19] that autophagic degradation of the chloroplasts may take place during natural leaf senescence (a paradigm of developmental PCD), which has more recently been confirmed using reverse genetics [20,21]. Finally, Wilson [22] put forward the pioneering hypothesis that autolytic machinery should play a central role in HR cell death under pathogen attack.

Recent progress in studying the genetic and biochemical regulation of plant autophagy and PCD, and advances in the development of methods for their detection and measurement, have instigated significant interest in bridging both processes and, particularly, in unraveling the mechanistic role of autophagy in PCD. In this Opinion article we use knowledge from recent studies to propose coexistence of two fundamentally distinct roles of autophagy in plant PCD, in which autophagy acts either as an initiator or executioner of cell demise.

Autophagy as an initiator of cell death

Autophagy has emerged as a central process in pathogen-triggered plant disease and immunity. However, the exact roles and mechanisms of autophagy, particularly during immunity-associated HR, are a matter of ongoing debate [23,24]. Initially, autophagy was considered to function as a cytoprotective mechanism during pathogen invasion (Table 1). Leaves of *Nicotiana benthamiana* and *Arabidopsis* plants with silenced or knocked-out *ATG* genes displayed spreading of cell death symptoms far beyond HR lesions following challenge with avirulent tobacco mosaic virus or with the bacterium *Pseudomonas syringae* pathovar (pv) tomato (*Pst* DC3000) harboring the effector protein AvrRpm1 [avirulence (Avr) gene product recognized by *Arabidopsis* RPM1] [25–27], respectively. Similarly, *Arabidopsis* loss-of-function *atg* mutants failed to contain disease-associated cell death following infection with the necrotrophic fungi *Alternaria brassicicola* or *Botrytis cinerea* [28,29].

Autophagy was also shown to play an opposite, pro-death role in primary infection sites during the onset of HR [30] (Table 1). Several *atg* mutants (e.g., *atg7, atg9*) displayed considerable suppression of HR-associated cell death triggered by AvrRpm1- and AvrRps4-containing *Pst* DC3000, and by an avirulent isolate of the oomycete *Hyaloperonospora arabidopsidis*. Notably, the suppressive effect of autophagy deficiency was most pronounced when HR was conditioned by activated Toll-interleukin 1 receptor (TIR)-type nucleotide-binding leucine-rich repeat (NB-LRR) proteins (i.e., RPS4 and RPP1) via the defense regulator ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) [30]. Consistent with this finding, *eds1* and *atg7* mutants showed comparable inhibition of both autophagy and cell

Table 1. Examples of autophagy gene-regulated cell death in plants

Species	Reverse genetics	Setting	Tissue/organ/cell type	Result of suppressed autophagy	Refs
Nicotiana benthamiana	VIGS[a] of *ATG3, ATG6, ATG7, VPS34*	Pathogen infection (TMV[b])	Leaves	Unrestricted cell death into non-infected tissue several days after infection	[25]
Arabidopsis thaliana	KO[c] of *ATG5*	Pathogen infection (avirulent strain of *Pst*[d])	Leaves of older plants	Unrestricted cell death into non-infected tissue several days after infection	[27]
Arabidopsis thaliana	KO of *ATG5, ATG10, ATG18a*	Pathogen infection (necrotrophic *Alternaria brassicicola*)	Leaves	Spreading of cell death, enhanced disease susceptibility	[28]
Arabidopsis thaliana	KO of *ATG5, ATG7, ATG18a*; RNAi of *ATG18a*	Pathogen infection (necrotrophic *Botrytis cinerea*)	Leaves	Spreading of cell death, enhanced disease susceptibility	[29]
Arabidopsis thaliana	KO of *ATG7, ATG9*	Pathogen infection (avirulent strains of *Pst* and *Hpa*[e])	Leaves of younger plants (*Pst*), and cotyledons (*Hpa*)	Suppressed HR cell death in infected tissue	[30]
Arabidopsis thaliana	KO of *ATG2*	Pathogen infection (avirulent strain of *Pst*)	Leaves of younger plants	Suppressed HR cell death in infected tissue	[38]
Arabidopsis thaliana	KO of *ATG18a*	Pathogen infection (avirulent strain of *Pst*)	Leaves of younger plants	Suppressed HR cell death in infected tissue	[61]
Arabidopsis thaliana	KO of *ATG2, ATG4a/4b, ATG5, ATG7*	Drug treatment (hydroxyurea)	Seedlings	Suppressed cell death, seedling survival	[38]
Arabidopsis thaliana	KO of *ATG5*	Formation of tracheary elements	Xylogenic cell culture	Suppressed vacuolization, cell clearance, and formation of tracheary elements	[42]
Picea abies	RNAi[f] of *ATG5, ATG6*	Embryogenesis	Suspensor	Switch from vacuolar to necrotic death, developmental arrest	[49]

[a]VIGS, virus-induced gene silencing
[b]TMV, tobacco mosaic virus
[c]KO, knockout
[d]*Pst, Pseudomonas syringae* pv. *tomato*
[e]*Hpa, Hyaloperonospora arabidopsidis*
[f]RNAi, RNA interference