Autophagy in Plant Cell Death

Subjects: Plant Sciences

Contributor: Karolina Wleklik, Sławomir Borek

Autophagy is considered as a two-faced process: it can ensure cell survival as well as promote cell death. Autophagic cell death (ACD) is the second form of animal PCD. It is associated with increased numbers of autophagosomes, autolysosomes, and small lytic vacuoles. Autophagic death is a controversial idea that has been discussed and debated many times. Although our knowledge of this subject in plants is limited, a few examples of cell death with autophagy have been described.

Keywords: Programmed Cell Death ; Vacuolar Processing Enzymes ; Atg genes ; Atg proteins

1. Autophagy - a housekeeping process in plant cell

Autophagy is the evolutionarily well-conserved process of cell self-eating occurring in yeasts, animals, and plants. Through the autophagy pathway, cellular components such as protein complexes and organelles are degraded. Moreover, bacteria and viruses can also be degraded in the infected cells through this process [1]. Autophagy takes place in all life stages of the plant, including development, senescence, and cell death [2][3]. Under normal development and growth conditions, the insensitivity of autophagy is relatively low—basal. Then, it works as a quality control mechanism to degrade and recycle unwanted or damaged cellular components [4]. However, it remarkably increases during biotic and abiotic stresses such as nutrient deficiency, drought, salinity, heat, oxidation, and pathogen attack [2]. In yeast, autophagy is regulated by over forty AuTophaGy-related (Atg) genes, which have also been found in animals and plants [5]. These genes encode Atg proteins, which play many roles during autophagy processes. For example, the Atg1/Atg13 kinase complex is essential for autophagy initiation by the target of rapamycin (TOR) signaling pathway [6]. TOR, a serine/threonine kinase, negatively regulates autophagy in response to many environmental stimuli [7], whereas the sucrose nonfermenting-1-related protein kinase 1 (SnRK1) is the central kinase complex, which positively regulates autophagy by activation of Atg1 kinase [8]. Autophagy occurs in both selective and non-selective ways. The selective form of autophagy takes place when only particular cell components are degraded, for example, mitochondria (mitophagy) or peroxisomes (pexophagy) [9][10][11]. Due to the differences in the delivery of the cargo intended for autophagic degradation, the following types of autophagy in plants are distinguished: macroautophagy, microautophagy, and mega-autophagy [12][13][14][15]. Macroautophagy (**Figure 1**a) is the best-known type of autophagy. It starts with the formation in the cytoplasm of a cup-shaped structure named a phagophore. The phagophore elongates until it is surrounded by cell components intended for degradation. A vesicle with a bilayer double-membrane, containing cargo intended for degradation, is called an autophagosome. These stages of macroautophagy are similar in yeasts, plants, and animals. The next stage, which is directing the autophagosome to the lytic cell compartments, is similar in yeasts and plants but distinguished from animals. Namely, in yeasts and plants, the autophagosome is directed to the vacuole, where it fuses with the tonoplast by its outer membrane. The unaffected internal membrane of the autophagosome with the cargo inside creates an autophagic body inside the vacuole. In animals, the autophagosome is directed to the lysosome, where they fuse, creating an autolysosome. Finally, in both the vacuole and the autolysosome, cargo is degraded by lytic enzymes [11][12][16]. Microautophagy (**Figure 1**b) is an autophagy pathway in which autophagosomes are not formed. Cell elements intended for degradation enter the lysosome or vacuole by membrane invagination or protrusion of these organelles [11][12]. Mega-autophagy (**Figure 1**c) has only been observed in plants, and it is perceived as massive cytoplasm destruction that occurs during dPCD and abiotic stress-induced ePCD. Nonetheless, none of the Atg genes are involved in mega-autophagy, and cellular components are not directed to the vacuole for degradation [17][18]

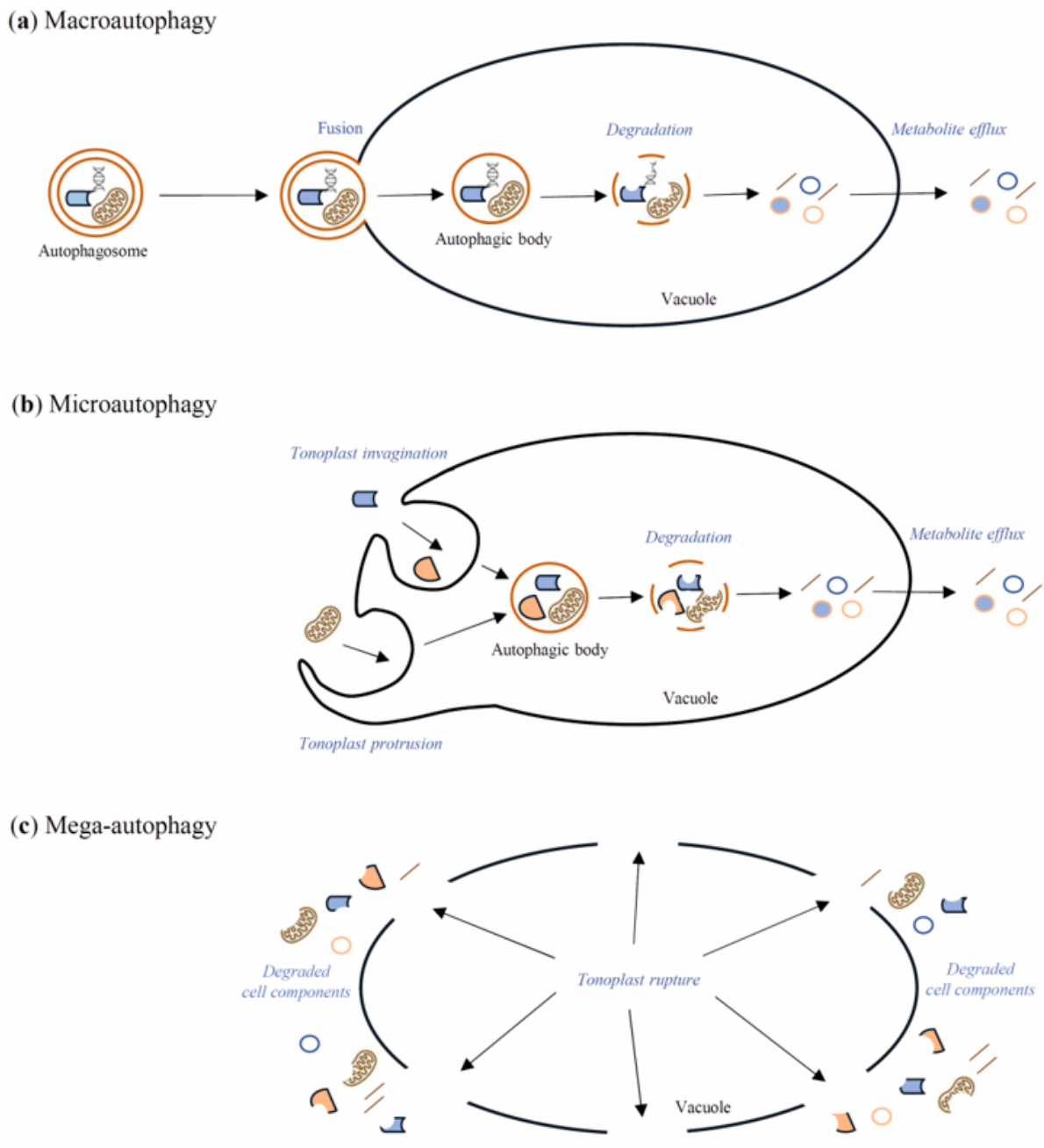

Figure 1. Schematic diagram of macroautophagy (**a**), microautophagy (**b**), and mega-autophagy (**c**) in plants. During macroautophagy, cargo intended for degradation is transported to the vacuole inside an autophagosome. The outer membrane of the autophagosome fuses with the tonoplast, while the internal autophagosome membrane and the cargo create an autophagic body inside the vacuole. The autophagic body is rapidly degraded by vacuolar hydrolases, which allow for the recycling of metabolites. During microautophagy, the autophagosome is not formed, but cell components intended for degradation enter the vacuole through the tonoplast invagination or tonoplast protrusion. Inside the vacuole, there arise the autophagic bodies, which, as in macroautophagy, are degraded by vacuolar hydrolases. Mega-autophagy differs significantly from macro- and microautophagy, as cell elements are not transported to the vacuole for degradation. Instead, the vacuole membrane is destroyed, and subsequently cell death occurs.

2. "Autophagic death" or "Death with autophagy"?

Autophagic cell death (ACD) is the second form of animal PCD. It is associated with increased numbers of autophagosomes, autolysosomes, and small lytic vacuoles [19]. Autophagic death is a controversial idea that has been discussed and debated many times. Autophagy is considered as a two-faced process: it can ensure cell survival as well as promote cell death [20]. However, it is difficult to distinguish when the occurrence of autophagic-related structures and recruitment of Atg genes function with the aim of cell survival and, conversely, when the aim is cell death. To solve this problem, it has been proposed to define “autophagic death” as when inhibition of autophagy contributes to long-term cell survival. In contrast, “cell death with autophagy” should be defined when inhibition of autophagy does not determine the subsequent death of the cell, but may change its morphology and delay the process (**Figure 2**) [21]. Therefore, crosstalk between these two processes remains important to study. Many genes are involved in both autophagy and cell death in

animal models [20]. Although our knowledge of this subject in plants is limited, a few examples of cell death with autophagy have been described, and VPEs, as proteases associated with cell death execution in plants, may be an important factor connected with the pro-death or pro-survival role of plant autophagy [22][23].

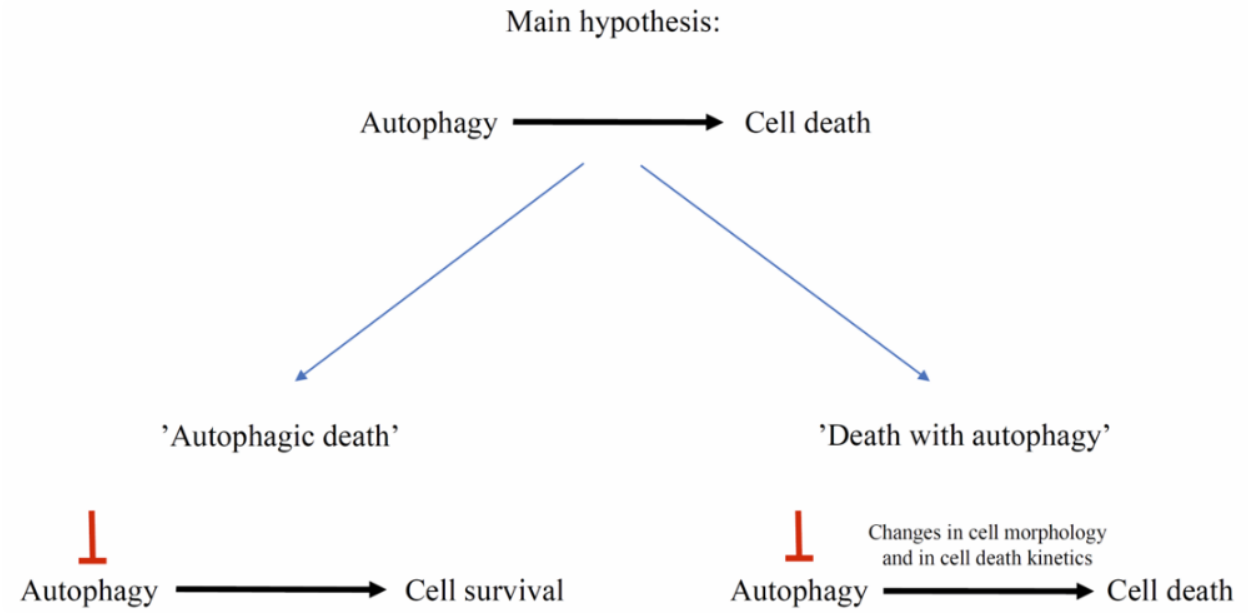

Figure 2. To determine whether autophagy acts in a cell pro-death or pro-survival manner, an experimental approach based on inhibition of autophagy is needed. "Autophagic death" occurs when inhibition of autophagy contributes to cell survival, whereas "death with autophagy" occurs when inhibition of autophagy, for example, delays cell death, but finally it will occur.

The initial degradation of cellular components by autophagy may be important for subsequent dPCD in plants. During PCD-dependent development of the root velamen radicum in the epiphytic orchid *Cymbidium tracyanum*, five genes of VPE, eight genes related to autophagy, and two genes of metacaspases were upregulated [22]. The differentiation of tracheary elements of the xylem is also a process in which dPCD and autophagy come together. It has been found that autophagy-related small GTP binding protein RabG3b and atg5 may be involved in xylem development of *Arabidopsis thaliana* [24]. The potential involvement of Atg genes in dPCD during xylogenesis has also been evaluated in the root of *Populus trichocarpa*. Increased expression levels of Atg8h, Atg11, and Atg18d genes were found in the isolated secondary xylem cells in comparison to the primary stem cells, implying that activation of these genes may be significant to dPCD [25]. Similarly, autophagy and PCD coexist in senescing barley (*Hordeum vulgare*) leaves. Among two VPEs (*αVPE* and *VPE2c*) and four Atg genes (*Atg4*, *Atg6*, *Atg8*, *Atg9*), the expression of αVPE and all Atg genes increased after ten days of senescence [26]. The involvement of autophagy in dPCD was also found in the root cap of *Arabidopsis thaliana*. Mutation of key autophagy genes Atg2, Atg5, and Atg7 contributed to the delay of dPCD and subsequent protoplast clearance in some cells of the root cap [27].

The potential involvement of autophagy in cell death is not only characteristic for dPCD. Atg6/BECLIN-like protein is required to limit HR to infected tissues in *Arabidopsis thaliana* attacked by *Pseudomonas syringae* pv. *tomato* (Pst). In yeast, Atg6/Vps30 is one of the key autophagy proteins, as it is involved in autophagosome formation [28]. On the other hand, *atg7-1* and *atg9-1* knockout mutants of *Arabidopsis thaliana* showed the pro-death function of autophagy during HR, as such manipulation contributed to cell death inhibition [29]. In addition, it has been shown that pathogen effectors, for example, HopF3, affect Atg proteins and through that action modulate autophagy to enhance virulence [30].

3. VPEs as a crosstalk point between autophagy and PCD

VPEs may be the point of crosstalk between autophagy and PCD. Simultaneous carbon starvation and treatment with the autophagy inhibitor concanamycin A of tobacco BY-2 cells expressing *StVPE1*-GFP resulted in accumulation in the vacuole of both autophagic bodies and labeled *StVPE1*. Moreover, colocalization of VPE and Atg8IL anchored in the outer membrane of autophagosome has been demonstrated. Silencing of Atg4, which is essential for Atg8 processing, contributed to decreased VPE activity and cell death rate. Taken together, the evidence implies that VPE translocates through the autophagy pathway to the vacuole, where it executes cell death (**Figure 3**) [23]. On the other hand, it has previously been shown that γVPE can be translocated through ER bodies (**Figure 3**) to the vacuole to promote stress-induced cell death in young seedlings of *Arabidopsis thaliana* [31][32]. Nevertheless, it has also been found that dPCD of pericarp cells in wheat (*Triticum*) grains coexists with the autophagy pathway, as silencing Atg8 inhibited dPCD and

caused the formation of small premature grains with a thick pericarp layer [33]. However, by the manipulation of autophagy with inhibitors and accelerants such as concanamycin A, wortmannin, and rapamycin, autophagy was found to promote cell survival rather than cell death in the lace plant (*Aponogeton madagascariensis*). Direct involvement of autophagy in dPCD has not been implicated, but on the other hand, the number of Atg8-positive points in the cells increased as cell death progressed [34]. In conclusion, it seems that ePCD and dPCD pathways may be strongly associated with autophagy processes, and VPE activity may be dependent on autophagy regulators such as Atg8. The results described here do not explain fully the dependencies between autophagy and cell death, but rather constitute an introduction to future research.

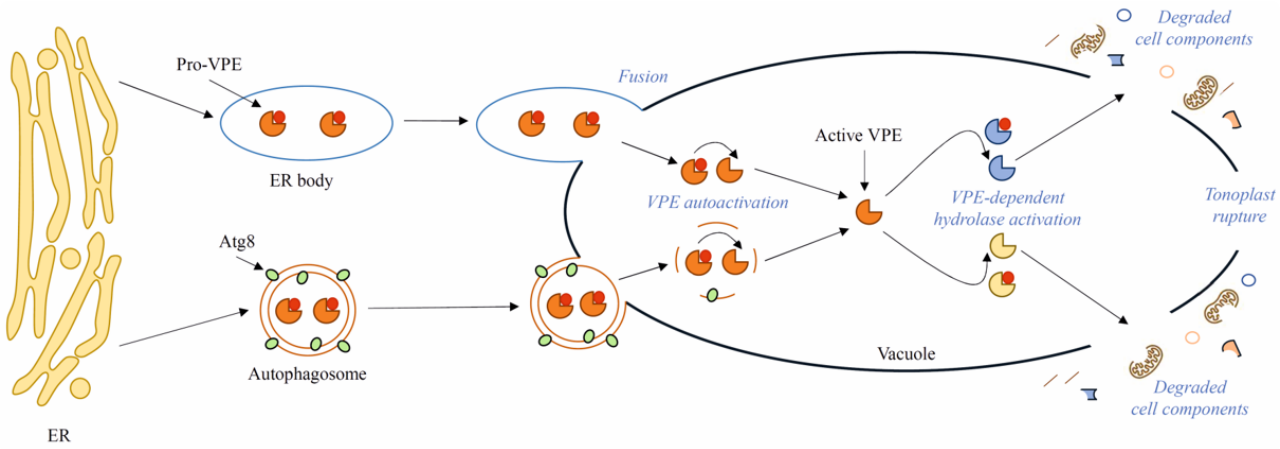

Figure 3. Possible ways of transport of VPEs in plant cells. Premature VPEs are synthesized in the endoplasmic reticulum (ER) and then are translocated to the vacuole through spindle-shaped ER bodies with single membranes or through autophagy inside autophagosomes tagged with Atg8. After fusion with the tonoplast, pro-VPEs are subjected to autoactivation in acidic pH inside the vacuole. It is presumed that mature VPEs process other vacuolar hydrolases, which contribute to tonoplast rupture and cell death.

4. Conclusions and Future Perspectives

Autophagy was first observed in the 1950s [35]. Decades of research have revealed the key importance of autophagy in plant development and responses to internal and external stimuli. Nonetheless, many aspects, for example, the late stages of autophagy, i.e., degradation of autophagic bodies and metabolite efflux from the vacuole to the cytoplasm, have been overlooked in the research, and the knowledge about these stages of autophagy in plants is vestigial. The process of degradation of autophagic bodies in yeast occurs with the participation of several known enzymes, whereas in plants, only VPEs are taken into consideration as potentially involved in this process. However, it is not clear how VPEs would distinguish their pro-death activity during PCD from pro-survival activity during autophagy and limit their role only to the initiation of autophagic body degradation. Moreover, the links between autophagy and PCD are still poorly understood. The evidence that VPEs may be delivered to the vacuole by the autophagy pathway seems to be a good reference point for future investigations. The Atg8 gene family, which encodes ubiquitin-like proteins required for autophagosome formation, should also be examined as a potential point of crosstalk between autophagy and cell death.

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