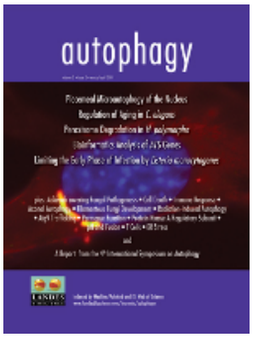

Autophagy

ISSN: 1554-8627 (Print) 1554-8635 (Online) Journal homepage: https://www.tandfonline.com/loi/kaup20

Autophagy and Cell-Death Proteases in Plants: Two Wheels of a Funeral Cart

Peter Bozhkov & Christer Jansson

To cite this article: Peter Bozhkov & Christer Jansson (2007) Autophagy and Cell-Death Proteases in Plants: Two Wheels of a Funeral Cart, Autophagy, 3:2, 136-138, DOI: 10.4161/auto.3600

To link to this article: https://doi.org/10.4161/auto.3600

Published online: 11 Dec 2006.

Submit your article to this journal

Article views: 250

View related articles

Citing articles: 2 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=kaup20

[Autophagy 3:2, 136-138; April/May/June 2007]; ©2007 Landes Bioscience

Addendum

Autophagy and Cell-Death Proteases in Plants

Two Wheels of a Funeral Cart

Peter Bozhkov*

Christer Jansson

Department of Plant Biology and Forest Genetics; Swedish University of Agricultural Sciences; Uppsala, Sweden

*Correspondence to: Peter Bozhkov; Department of Plant Biology and Forest Genetics; Swedish University of Agricultural Sciences; Box 7080; Uppsala SE-75007 Sweden; Tel.: 46.18.673228; Fax: 46.18.673389; Email: peter.bozhkov@vbsg.slu.se

Original manuscript submitted: 11/09/06
Manuscript accepted: 11/13/06

Previously published online as an *Autophagy* E-Publication:
http://www.landesbioscience.com/journals/autophagy/abstract.php?id=3600

KEY WORDS

autophagic programmed cell death, vacuolar processing enzyme, metacaspases, VEIDase, autophagosomes

ACKNOWLEDGEMENTS

We thank Dr. Budhi Sagar Tiwari for his dependable and stimulating exchange of ideas on the subject and his helpful comments. Cell death work in our labs is supported by the Carl Tryggers Foundation, the Swedish Foundation for International Cooperation in Research and Higher Education (STINT), the Wenner-Gren Foundations, the Royal Swedish Academy of Agriculture and Forestry (KSLA), the Swedish Research Council (VR) for Environment, Agricultural Sciences and Spatial Planning (Formas), the Nordic Joint Committee for Agricultural Research (NKJ), the Swedish Farmers' Foundation for Agricultural Research (SLF) and AgriFunGen Program at the Swedish University of Agricultural Sciences.

Addendum to:

Developmental Regulation of a VEIDase Caspase-Like Proteolytic Activity in Barley Caryopsis

M. Borén, A.S. Höglund, P. Bozhkov and C. Jansson

J Exp Bot 2006; 57:3747-3753.

ABSTRACT

Apoptosis is an evolutionarily young cell-death strategy evolved to disassemble animal cells through the action of the caspase family of proteases and phagocytic clearance. This strategy does not work in plants, which instead feature a phylogenetically older autophagic programmed cell death (PCD), as a bona fide type of cellular suicide. Recent work has begun to address the mechanistic roles for autophagic and proteolytic components, as well as their possible cooperation in plant PCD. A recent study has shown autophagosomal localization of a key cell-death proteolytic activity at the early stage of plant PCD. Here we focus on the relationship between autophagic and proteoloytic components in plant PCD at the cellular and organismal levels.

©2006 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

Programmed cell death (PCD) has evolved as an evolutionarily conserved strategy for controlling cell number and tissue differentiation, eliminating temporary organs and structures and guiding the development of many diseases and the immune response. There are many routes for committing suicide in eukaryotic cells. In animals, the best understood type of PCD is apoptosis, where the cells are taken apart into apoptotic bodies, which subsequently are engulfed and digested by the macrophages. Apoptosis does not operate in plants owing to the presence of rigid cell walls and a lack of macrophages. Instead, dying plant cells degrade themselves from inside by lytic vacuoles.[1-4] The latter are formed de novo via the fusion and growth of autophagosomes derived from Golgi,[5] proplastids[5,6] or endoplasmic reticulum[7-9] (Fig. 1A) providing an elegant paradigm for autophagic PCD.

Which molecules control autophagic PCD and degrade cellular content in plants devoid of apoptotic cell-death machinery still remains an open question. A lack of close homologues to caspase family proteases in plant genomes is particularly intriguing, as caspases and their numerous substrates constitute the major part of the animal PCD degradome.[10,11] The degradome of plant PCD remained elusive till a few years ago, when a trio of proteases, each being indispensable for the execution of cell death and exhibiting distinct substrate specificity and subcellular localization were identified (Fig. 1A). These proteases are vacuolar processing enzymes (VPEs), type II metacaspases and VEIDase (a protease(s) that cleaves the substrate Val-Glu-Ile-Asp).

VPEs are cysteine-dependent asparagine/aspartate-specific proteases from the legumain family (C13), with a high preference for the caspase-1 substrate YVAD (Tyr-Val-Ala-Asp).[12] Active VPEs reside in the lytic vacuoles and could mediate activation of other vacuolar enzymes and/or disruption of the tonoplast in the culminating phase of developmental and pathogen-induced PCD.[13-15] Metacaspases form a separate family of cysteine-dependent proteases found in protozoa, plants and fungi and are suggested to be ancestors of animal caspases.[16] Despite the conservation of caspase-specific catalytic residues in the metacaspases, they do not cleave peptides after aspartate, but display a strict preference for arginine or lysine residues at the P1 position in the substrate.[17-19] Type-II metacaspase, mcII-Pa from Norway spruce is activated in the terminally-differentiated cells, where it translocates from the cytoplasm to nuclei inducing nuclear envelope disassembly and DNA fragmentation,[18] whereas in tomato, expression of type-II metacaspase is up-regulated during fungal attack.[20] In contrast to molecularly characterized VPEs and metacaspases, a protease(s) causing VEIDase activity remains elusive. The VEIDase activity resembles the activity of mammalian caspase-6, and has been found as a principal caspase-like activity during PCD in varied eukaryotic systems ranging from yeast cells to Norway spruce embryos.[21-23] Originally described in Norway spruce embryos, this activity was shown to be localized in the cytoplasm of the terminally-differentiated suspensor cells.[22] Although a VEIDase

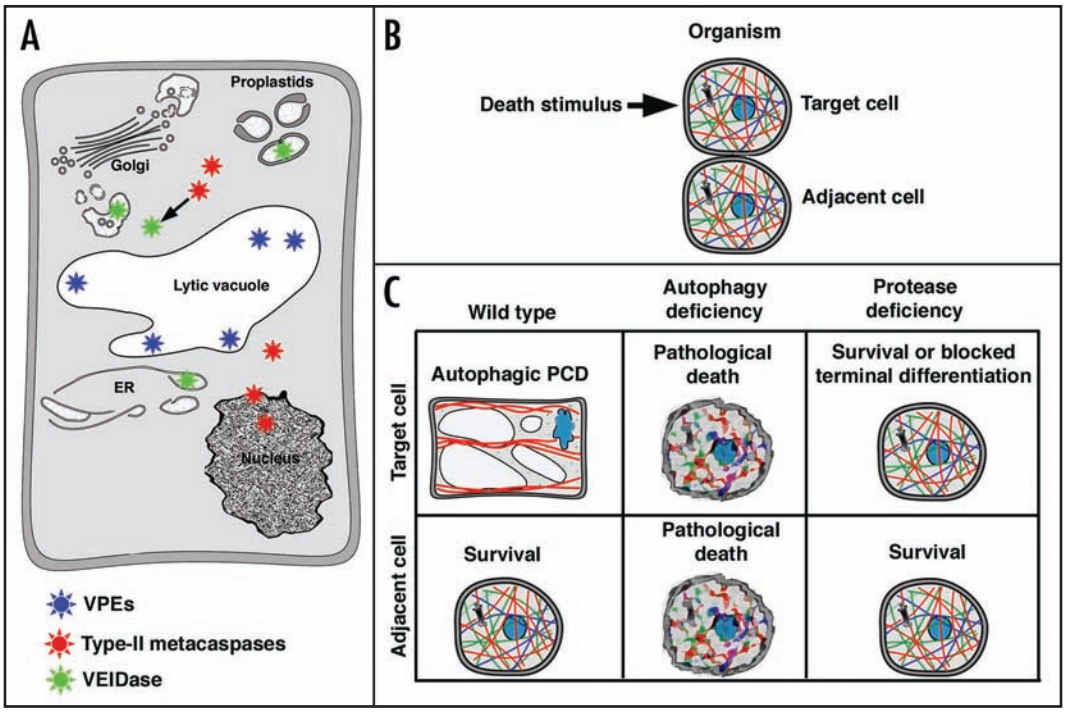

Figure 1. Relationship between autophagic and proteoloytic components in plant PCD at the cellular and organismal levels. (A) Model depicting subcellular distribution of a trio of key cell-death proteases (active forms). Note autophagosomal/cytosolic localization of the VEIDase,[22,24] which is activated by type-II metacaspases (arrow). Autophagosomes are derived from the Golgi, proplastids or endoplasmic reticulum (ER) and are the hallmark of the commitment phase of PCD.[3] VPE-dependent growth of lytic vacuoles[12-15] and metacaspase-mediated nuclear disassembly[18] are characteristic for the execution phase. (B) Multicellular development and viability are reliant on keeping a balance between PCD and cell survival; only a certain subset of cells (target cells) should commit suicide in response to a death stimulus. (C) This balance does not occur in plants deficient for autophagy or lacking one of the key proteolytic activities. Autophagy deficiency leads to unrestricted pathological cell death in response to pathogen,[27] whereas proteolytic dysfunction abrogates HR PCD during pathogen attack[13] and terminal differentiation-associated PCD during embryo patterning.[22,30]

enzyme per se has so far eluded all attempts at identification, we have found using biotinylated inhibitor VEID-acyloximethylketone that VEIDase is bound to metacaspase mcII-Pa in the cell lysates from Norway spruce embryos (Smertenko A, Bozhkov P, unpublished) providing strong mechanistic support for metacaspase-dependent activation of downstream protease(s) with VEIDase activity.[18,19,21] The establishment of two sets of proteolytic enzymes showing contrasting subcellular localization, with one set comprised by VPEs and another encompassing nucleo-cytoplasmic metacaspases and VEIDase, suggests a cooperation of two cell degradation pathways during execution of autophagic PCD in plants (Fig. 1A). However, these data do not link activation of proteases and autophagocytosis at the earliest stages of plant PCD when autophagosomes are just formed.

Our labs have recently demonstrated that the peak of the VEIDase activity occurred approximately one week ahead of nuclear DNA fragmentation and loss of plasma membrane integrity during PCD in barley endosperm.[24] Intriguingly, this proteolytic activity was strictly colocalized with monodansylcadaverine/LysoTracker Red-positive autophagosomes. In the well-defined sequence of cellular events underlying autophagic PCD of Norway spruce embryo-suspensor (a temporary structure that functions as a conduit of nutrients and growth factors at the early developmental stage), the formation of autophagosomes characterizes the commitment phase of PCD.[3,5,25] Therefore accumulation of active VEIDase in autophagosomes[24] might represent a convergence point for the autophagic and proteolytic pathways that is pivotal for the onset of the execution phase of plant PCD.

Degradation of cellular content during autophagic PCD is just one manifestation of the multifunctional process of autophagy recruited at diverse developmental and pathological settings to maintain homeostasis.[26] Accordingly, formation of autophagosomes per se is not sufficient to conclude that the cell will ultimately die. Hence, autophagy appears to be an obligatory but not sufficient component of plant PCD. Recent work on the genetic and pharmacological manipulations of the developmental and hypersensitive response-associated PCD (HR PCD) in plants gives an insight into the fate of the cells destined to die, and the reaction of the whole organism in response to specific inhibition of the autophagic or proteolytic pathway.

Under normal physiological conditions, only a group of cells or a certain tissue are targeted to destruction in response to the death stimulus, while adjacent cells survive (Fig. 1B and C, wild type). For example, elimination of the embryo-suspensor and survival of the embryonal mass is fundamental for plant embryogenesis, whereas localized cell death at the site of pathogen attack is an integral part of plant resistance. The contribution of autophagic machinery in virus-induced HR PCD has been elegantly shown using ATG6/BECLIN 1-silenced tobacco plants.[27] In nonsilenced plants, HR was associated with increased autophagic activity and upregulation of ATG6/BECLIN 1 both in infected target cells and in uninfected adjacent cells, resulting in localized PCD and plant survival (Fig. 1C, wild type). Silencing of ATG6/BECLIN 1 suppressed autophagosome formation in response to pathogen attack and led to unrestricted development of pathological cell death over the entire leaf tissue (Fig. 1C, autophagy deficiency) thus

compromising plant survival.[27] Interestingly, we had similar observations of unrestricted pathological death in Norway spruce embryos developed in the presence of the general autophagy inhibitor 3-methyladenine; the embryos did not differentiate suspensor cells (i.e., suppressed autophagic PCD) and possessed an abnormally high rate of embryo abortion (i.e., increased pathological death) (Filonova L, Bozhkov P, unpublished). Additional work is required to investigate how cells actually died in the both cases of autophagy deficiency.[3,28]

It is logical to conclude that autophagy has a dual role in plant ontogenesis, acting as a bona fide instrument in ordered cell dismantling during PCD (in contrast to animals, plants cannot choose between apoptotic and autophagic types of PCD[29]) and at the same time fulfilling its evolutionarily conserved pro-survival role for the whole organism. In this context the central task for future work is to identify the molecular switch, which guides the cellular choice between "metabolic or selective autophagy" in living cells, and "bulk autophagy" degrading the entire cellular content during PCD.

The proteolytic pathway, like autophagy, is essential for the execution of plant PCD. Tobacco plants lacking all four VPEs showed suppressed HR PCD in response to viral infection, with no signs of pathological unrestricted death or developmental aberrations[13] (Fig. 1C, protease deficiency). Likewise, silencing of mcII-Pa metacaspase or pharmacological inhibition of the VEIDase activity in Norway spruce embryos had a strong anti-cell death effect, but in contrast to VPE knockout, led to developmental arrest early during embryogenesis owing to a lack of terminally-differentiated suspensors (Fig. 1C, protease deficiency).[22,30] Although autophagy and protease deficiencies exert opposing effects on the fate of the cells targeted to PCD (Fig. 1C), both situations may negatively affect plant growth.

It becomes increasingly evident that the concerted action of autophagic and proteolytic machineries controls execution of PCD in plants. In order to understand how autophagic PCD operates in plants, we need to address the roles of distinct ATG proteins, and determine the molecular identity of the autophagosomal VEIDase, and all other missing components of the plant PCD degradome including a panel of as yet unknown natural targets for cell-death proteases. This is not a short-term goal, but when achieved, it will not only improve our understanding of plant biology but also the evolution of eukaryotic cell death - the way from the ancient and phylogenetically conserved autophagic PCD to the relatively young animal-specific apoptosis.

References

1. Beers EP, McDowell JM. Regulation and execution of programmed cell death in response to pathogens, stress and developmental cues. Curr Opin Plant Biol 2001; 4:561-7.
2. Zhivotovsky B. From the nematode and mammals back to the pine tree: On the diversity and evolution of programmed cell death. Cell Death Differ 2002; 9:867-9.
3. Bozhkov PV, Filonova LH, Suarez MF. Programmed cell death in plant embryogenesis. Curr Top Dev Biol 2005; 67:135-79.
4. Van Doorn WG, Woltering EJ. Many ways to exit? Cell death categories in plants. Trends Plant Sci 2005; 10:117-22.
5. Filonova LH, Bozhkov PV, Brukhin VB, Daniel G, Zhivotovsky B, von Arnold S. Two waves of programmed cell death occur during formation and development of somatic embryos in the gymnosperm, Norway spruce. J Cell Sci 2000; 113:4399-411.
6. Nagl W. 'Plastolysomes' - Plastids involved in the autolysis of the embryo-suspensor in *Phaseolus*. Z Pflanzenphysiol 1977; 85:45-51.
7. Gärtner PJ, Nagl W. Acid phosphatase activity in plastids (plastolysomes) of senescing embryo-suspensor cells. Planta 1980; 149:341-9.
8. Gietl C, Schmid M. Ricinosomes: An oranelle for developmentally regulated programmed cell death in senescing plant tissues. Naturwisssenschaften 2001; 88:49-58.
9. Wredle U. Autophagic programmed cell death in the suspensor and endosperm of *Vicia faba*: An ultrastructural study. PhD Thesis. Stockholm University (ISBN), 2004; 91:7265-893-2.
10. Lopez-Otin S, Overall CM. Protease degradomics: A new challenge for proteomics. Nature Rev Mol Cell Biol 2002; 3:509-19.
11. Fischer U, Jänicke RU, Schulze-Osthoff K. Many cuts to ruin: A comprehensive update of caspase substrates. Cell Death Differ 2003; 10:76-100.
12. Hara-Nishimura I, Hatsugai N, Nakaune S, Kuroyanagi M, Nishimura M. Vacuolar processing enzyme: An executor of plant cell death. Curr Opin Plant Biol 2005; 8:404-8.
13. Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, Kondo M, Nishimura M, Hara-Nishimura I. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. Science 2004; 305:855-8.
14. Rojo E, Martin R, Karter C, Zouhar J, Pan S, Plotnikova J, Jin H, Paneque M, Sanchez-Serrano JJ, Baker B, Ausubel FM, Raikhel NV. VPEγ exhibits a caspase-like activity that contributes to defense against pathogens. Curr Biol 2004; 21:1897-906.
15. Nakaune S, Yamada K, Kondo M, Kato T, Tabata S, Nishimura M, Hara-Nishimura I. A novel-type VPE, δVPE, is involved in seed coat formation at the early stage of seed development. Plant Cell 2005; 17:876-87.
16. Uren AG, O'Rourke K, Aravind L, Pisabarro TM, Seshagiri S, Koonin EV, Dixit VM. Identification of paracaspases and metacaspases: Two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell 2000; 6:961-7.
17. Vercammen D, van de Cotte B, de Jaeger G, Eeckhout D, Casteels P, Vandepoele K, Vandenberghe I, van Beeumen J, Inze D, van Breusegem F. Type II metacaspases Atmc4 and Atmc9 of *Arabidopsis thaliana* cleave substrates after arginine and lysine. J Biol Chem 2004; 279:45329-36.
18. Bozhkov PV, Suarez MF, Filonova LH, Daniel G, Zamyatnin Jr AA, Rodriguez-Nieto S, Zhivotovsky B, Smertenko A. Cysteine protease mcII-Pa executes programmed cell death during plant embryogenesis. Proc Natl Acad Sci USA 2005; 102:14463-8.
19. Watanabe N, Lam E. Two *Arabidopsis* metacaspases AtMCP1b and AtMCP2b are arginine/lysine-specific cysteine proteases and activate apoptosis-like cell death in yeast. J Biol Chem 2005; 280:14691-9.
20. Hoeberichts FA, ten Have A, Woltering EJ. A tomato metacaspase gene is upregulated during programmed cell death in *Botrytis cinerea*-infected leaves. Planta 2003; 217:517-22.
21. Madeo F, Herker E, Maldener C, Wissing S, Lächelt S, Herlan M, Fehr M, Lauber K, Sigrist SJ, Wesselborg S, Fröhlich KU. A caspase-related protease regulates apoptosis in yeast. Mol Cell 2002; 9:911-7.
22. Bozhkov PV, Filonova LH, Suarez MF, Helmersson A, Smertenko AP, Zhivotovsky B, von Arnold S. VEIDase is a principal caspase-like activity involved in plant programmed cell death and essential for embryonic pattern formation. Cell Death Differ 2004; 11:175-82.
23. Rotari VI, He R, Gallois P. Death by proteases in plants: Whodunit. Physiol Plant 2005; 123:376-85.
24. Borén M, Höglund AS, Bozhkov P, Jansson C. Developmental regulation of a VEIDase caspase-like proteolytic activity in barley caryopsis. J Exp Bot 2006; 57:3747-53.
25. Smertenko AP, Bozhkov PV, Filonova LH, von Arnold S, Hussey PJ. Reorganization of the cytoskeleton during developmental programmed cell death in *Picea abies* embryos. Plant J 2003; 33:813-24.
26. In: Klionsky D, ed. Autophagy. Georgetown, Austin: Eurekah.com, Landes Bioscience, 2004.
27. Liu Y, Schiff M, Czymmek K, Talloczy Z, Levine B, Dinesh-Kumar SP. Autophagy regulates programmed cell death during the plant innate immune response. Cell 2005; 121:567-77.
28. Greenberg TJ. Degrade or die: A dual function for autophagy in the plant immune response. Dev Cell 2005; 8:799-801.
29. Debnath J, Baehrecke EH, Kroemer G. Does autophagy contribute to cell death? Autophagy 2005; 1:66-74.
30. Suarez MF, Filonova LH, Smertenko A, Savenkov EI, Clapham DH, von Arnold S, Zhivotovsky B, Bozhkov PV. Metacaspase-dependent programmed cell death is essential for plant embryogenesis. Curr Biol 2004; 14:R339-R340.