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The role of vacuolar processing enzymes in plant immunity

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Proteases play important roles in plant innate immunity. In this mini-review, we describe the current view on the role of a plant protease, vacuolar processing enzyme (VPE), and the first identified plant caspase-1-like protein, in plant immunity. In the past several years, VPEs were determined to play important roles in various types of cell death in plants. Early studies demonstrated the identification of VPE as a vacuolar hydrolytic protein responsible for maturation of vacuolar proteins. Later, *Nicotiana benthamiana* VPE was reported to mediate virus-induced hypersensitive response by regulating membrane collapse. The ortholog of VPE in Arabidopsis is also suggested to be involved in both mycotoxin-induced cell death and developmental cell death. However, the role of VPE in elicitor-signaling is still unclear. Our recent studies demonstrated the involvement of VPE in elicitor signal transduction to induce stomatal closure and defense responses, including defense gene expression and hypersensitive cell death.

In the course of their development, plants have had to face a wide range of potential pathogens, including viral, bacterial, fungal and oomycete pathogens. Plants, unlike animals, which have specialized defender cells and an adaptive immune system, have an innate immunity of each cell and produce systemic signals emanating from the infection site. The plant innate immunity (PTI) is induced by pathogen-associated molecular patterns (PAMPs)[1] and elicitors.[2,3] However, some pathogens deliver virulence proteins that target host protein to overcome the plant immunity response. Most plants have evolved the corresponding resistance (R) protein to recognize effector activity, which will trigger plant resistance through effector-triggered immunity (ETI).[4] Natural selection drives evolution of new pathogen effector proteins and plant R proteins. This tug-of-war between plants and pathogens is represented as a zig-zag-zig model.[5-7] Both PTI and ETI cause stomatal closure and hypersensitive response (HR), a programmed host cell death (PCD) to limit pathogen development.[5,8] In plants, HR is caused by proteases with caspase activity. At least eight caspase activities have now been measured in plant extracts, which were found using caspase substrates, and various caspase inhibitors can block many forms of plant programmed cell death.[9]

In the past several years, vacuolar-processing enzyme (VPE) has been determined to play important roles in plant immunity responses. In this review paper, I describe the current view on the role of VPE in plant immunity, based on our own research and recent developments in this field.

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Role of VPE in PCD

Plant vacuoles play fundamental roles in controlling turgor pressure, molecular degradation and storage, as well as timely execution of PCD in, e.g., plant disease resistance, tissue senescence and seed development.[10,11] A paper in *Science* reported that VPE localizes in the vacuolar membrane and mediates virus-induced hypersensitive cell death by regulating the collapse of this membrane.[12] VPEs, including seed-type VPE and vegetative-type VPE, were originally discovered as cysteine proteinases responsible for the maturation of vacuolar proteins.[13,14] Elicitors can activate plant innate immunity. In addition, components identified in elicitor signaling can be applied in transgenic plants to activate defense responses to limit plant disease.[3]

We studied the elicitor signal-transduction pathway in plants using three elicitors (bacterial harpin, fungal Nep1 and oomycete boehmerin) in *Nicotiana benthamiana* because such a simple experimental system provides an excellent model based on virus-induced gene silencing.[15-17] These three elicitors induce a diverse array of defense responses, including elevation of cytosolic calcium, nitric oxide (NO) and hydrogen peroxide (H_2O_2) accumulation; an active oxygen oxidative burst; expression of a subset of defense-related genes; and finally hypersensitive cell death and stomatal closure.[15,16] A study demonstrated that elicitors induce YVADase activity in cell extracts of PCD-induced cells and that this activity is blocked by caspase inhibitors.[18] However, no caspase homolog has been found in plants.[9] Therefore, the molecular identification of protein kinases with caspase activity will help us elucidate elicitor signal transduction. As VPE was identified to have YVADase activity and to be involved in various cell death responses,[12,19] we postulated that elicitor signals are transduced via VPEs in plants.

Nicotiana benthamiana plants show typical cell death in elicitor-infected regions of leaves. *VPE1a* gene silencing completely suppresses cell death formation induced by harpin, but not by boehmerin or Nep1. *VPE1b* silencing does not affect elicitor-induced cell death. Furthermore, the phenotype was confirmed using trypan blue staining, which detects dead cells.[16] The results suggest that pathogen elicitor-triggered cell death can be dependent or independent of VPE. This is similar to a previous study reporting that animal PCD may be dependent or independent of caspase activity.[20]

VPE1a deficiency prevents the typical characteristics of harpin-induced cell death, whereas it does not interfere with H_2O_2 accumulation in response to boehmerin, harpin, or Nep1.[16] This means that independent processes regulate PCD and H_2O_2 accumulation triggered by harpin. Additionally, *VPE* silencing does not abolish H_2O_2 accumulation or cell death in response to boehmerin or Nep1, indicating that VPE is not involved in H_2O_2 production or cell death after boehmerin and Nep1 treatment, and that H_2O_2 plays different roles in cell death induced by various elicitors. Moreover, the expression levels of pathogenesis-related (*PR*) genes were reduced by *VPE* silencing in elicitor signaling.[16] These findings demonstrate that VPE exhibiting caspase-1-like activity is essential for bacterial elicitor-induced PCD.

VPE exhibits caspase-1 activity in TMV-infected tobacco leaves.[12] Both the caspase-1 inhibitor and VPE inhibitor abolish TMV-induced PCD. A combination of pull-down and immunoblot analysis showed that the VPE interacted with the caspase-1 substrate YVAD.[12] This suggests VPE has YVADase activity to mediate virus-induced PCD. From ultrastructural observations, vacuolar membrane collapse was detected before cell death occurred. An immunoblot result showed that the virus was produced more abundantly in *VPE* RNAi lines.[12] Together, these results indicate that VPE mediates the release of vacuolar hydrolytic enzymes for attacking the virus by regulating vacuolar membrane collapse in response to virus infection.

Some compatible pathogens secrete toxins to kill host cells and promote pathogen growth. A study showed that mycotoxin-induced cell death was accompanied by disintegration of the vacuolar membrane, followed by lesion formation, and that cell death was completely abolished in the Arabidopsis VPE-null mutant and the caspase-1 inhibitor.[21] These findings suggest that a susceptible response to toxin-induced cell death is caused by a VPE-mediated vacuolar mechanism similar to the resistance response of hypersensitive cell death induced by the virus and PAMP.

During the early stages of seed development, δVPE was expressed in the inner integuments of the seed coat; the thickness of the inner integuments was reduced in wild-type Arabidopsis plants. However, in a *δVPE*-deficient mutant, the inner integuments remained and cell death of these inner integuments of the seed coat was delayed.[22] This suggests that δVPE is a key player in the developmental PCD of limited cell layers during the formation of the seed coat.

Role of VPE in Stomatal Movement

Increasing numbers of plant studies have strongly confirmed the importance of the stoma in plant immunity. Stomata, specialized epidermal structures formed by two guard cells surrounding a pore, control photosynthesis and the water status of the plant.[23,24] Stomatal closure can be triggered by pathogens, PAMPs and elicitors.[15,16,25,26] Therefore, the stomatal closure observed in such situations is an output of PTI.

Because mature guard cells lack plasmodesmata, all solute uptake and efflux must occur via the plasma membrane and vacuole.[27] Determining the vacuole-localized VPE in controlling stomatal closure during PTI is of great interest. Our recent study addressed this question and found that VPE is essential for elicitor-induced stomatal closure in *N. benthamiana*:[16] elicitors induced stomatal closure in control plants. Unexpectedly, stomatal closure was significantly inhibited in the *VPE* RNAi lines after elicitor inoculation.[16] Additionally, *VPE* deficiency suppresses NO accumulation in guard cells triggered by elicitors.[16] Thus, the results indicate that VPE mediates elicitor-induced stomatal closure by regulating NO accumulation in guard cells. This finding suggests that *VPE* silencing may affect vacuolar membrane function and subsequently inhibit elicitor-induced stomatal closure.

Other studies have shown that membrane-associated proteins are involved in guard cell signal transduction. The NtrbohD protein,[28] G protein-coupled receptors (GPCRs),[29] fast vacuolar (FV) channels, K^+-selective vacuolar (VK) cation channels,[30] slow vacuolar (SV) channels, slow anion channel-associated 1 (SLAC1)[31,32] and H^+-ATPases (AHA1 and AHA2)[33] are localized to the membrane and function during abiotic or biotic responses in guard cells.[34] These findings also demonstrate that the guard cell signal transduction in response to biotic and abiotic signals shares some common steps, including NO accumulation in guard cells. Furthermore, Arabidopsis protein RIN4, a negative regulator of plant immunity, interacts with AHA1 and AHA2 to regulate stomatal aperture, inhibiting the entry of bacterial pathogens into the plant leaf during infection.[33] The *coronatine-insensitive* 1 mutation shows impaired methyl jasmonate-induced stomatal closing, but not ABA-induced stomatal closing.[35]

VPE-dependent stomatal closure triggered by elicitors may play important roles in plant PTI. The evidence suggests that pathogens secrete or deliver a group of effectors into the plant cell to suppress PTI, which then inhibits stomatal closure. Thus, VPE or its dependent pathway may be a target for pathogen virulence proteins. Recently, a study reported that the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 uses the virulence factor coronatine (COR) to actively open stomata.[25] However, how COR promotes stomatal opening remains to be elucidated. Pretreatment with extracellular high Ca^{2+} elicited S-type anion currents both in wild-type GCPs and in COI1 GCPs, suggesting that COI1 could function upstream of Ca^{2+} sensor priming and $[Ca^{2+}]_{cyt}$ elevation for S-type anion channel activation.[35] Another virulent factor secreted by the bacterial phytopathogen

Xanthomonas campestris pv. *campestris* (*Xcc*) can interfere with stomatal closure induced by bacteria.[36] In addition, the virulence protein can complement the infectivity of *Pseudomonas syringae* pv. *tomato* (*Pst*) mutants deficient in production of the COR toxin, and the signal pathway of suppression of stomatal response requires an intact rpf/diffusible signal factor system.[36] Moreover, the pathogenic fungi *Rhynchosporium secalis* and *Plasmopara viticola* induce stomatal closure; however, a virulence factor (oxalate) produced by many fungi can promote stomatal opening, which might be due to an increase in osmotically active solutes.[37]

Collectively, descriptions of the toxins coronatine, oxalate and the factor reported capable of overcoming stomatal defense suggest that mechanisms to disable stomatal innate defense might be a widespread strategy that might have evolved independently in different pathogen species. Identification of more pathogen virulence effectors involved in regulation of stomatal defense and the target of action inside guard cells might improve our understanding of bacterial pathogenesis and stomatal physiology.

Perspectives

VPEs have been suggested to play a central role in plant signal transduction. During recent years, the direct involvement of VPEs in defense responses has been evident as a result of elegant biochemical and genetic analyses, although the role of VPEs in plant defense signaling and systematic analysis of the genes involved in modules in the pathway are still unclear. Therefore, combining various genetic, reverse genetic and biochemical techniques is important to identify receptors, scaffold proteins, negative regulators and substrates of VPEs, and to target genes that will help us to fully understand these plant defense mechanisms.

Current results suggest that PCD and stomatal closure are integral parts of plant immunity. Pathogens can produce virulence protein to suppress PCD and stomatal closure, and plants in turn evolve disease resistance proteins to recognize some of these virulence effectors. In the future, elucidating how different pathogen virulence factors inhibit PCD and stomatal closure will be important. An in-depth investigation resulting from such studies will greatly facilitate our understanding of innate immune signaling, as well as helping in the discovery of methods for managing plant disease.

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References

1. Hématy K, Cherk C, Somerville S. Host-pathogen warfare at the plant cell wall. Curr Opin Plant Biol 2009; 12:406-13.
2. Zhao JT, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 2005; 23:283-333.
3. Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, David L, Benoit P, et al. Early signaling events induced by elicitors of plant defenses. Mol Plant Microbe Interact 2006; 19:711-24.
4. Chisholm ST, Coaker G, Day B, Staskawicz BJ. Host-microbe interactions: shaping the evolution of the plant immune response. Cell 2006; 124:803-14.
5. Jones JD, Dangl JL. The plant immune system. Nature 2006; 444:323-9.
6. Hok S, Attard A, Keller H. Getting the most from the host: How pathogens force plants to cooperate in disease. Mol Plant Microbe Interact 2010; 23:1253-9.
7. Nishimura MT, Dangl JL. Arabidopsis and the plant immune system. Plant J 2010; 61:1053-66.
8. Zeng W, Melotto M, He SY. Plant stomata: a checkpoint of host immunity and pathogen virulence. Curr Opin Biotech 2010; 21:1-5.
9. Bonneau L, Ge Y, Drury GE, Gallois P. What happened to plant caspases? J Exp Bot 2008; 59:491-9.
10. Marty F. Plant vacuoles. Plant Cell 1999; 11:587-600.
11. Reisen D, Marty F, Leborgne-Castel N. New insights into the tonoplast architecture of plant vacuoles and vacuolar dynamics during osmotic stress. BMC Plant Biol 2005; 5:13.
12. Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, Kondo M, et al. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. Science 2004; 305:855-8.
13. Hara-Nishimura I, Inoue K, Nishimura M. A unique vacuolar processing enzyme responsible for conversion of several proprotein precursors into the mature forms. FEBS Lett 1991; 294:89-93.
14. Kinoshita T, Nishimura M, Hara-Nishimura I. Homologues of a vacuolar processing enzyme that are expressed in different organs in *Arabidopsis thaliana*. Plant Mol Biol 1995; 29:81-9.
15. Zhang HJ, Fang Q, Zhang ZG, Wang YC, Zheng XB. The role of respiratory burst oxidase homologs in elicitor-induced stomatal closure and hypersensitive response in *Nicotiana benthamiana*. J Exp Bot 2009; 60:3109-22.
16. Zhang HJ, Dong SM, Wang MF, Wang W, Song WW, Dou XY, et al. The role of *vacuolar processing enzyme* (*VPE*) from *Nicotiana benthamiana* in elicitor-triggered hypersensitive response and stomatal closure. J Exp Bot 2010; 61:3799-812.
17. Goodin MM, Zaitlin D, Naidu RA, Lommel SA. *Nicotiana benthamiana*: its history and future as a model for plant-pathogen interactions. Mol Plant Microbe Interact 2008; 21:1015-26.
18. Elbaz M, Avni A, Weil M. Constitutive caspase-like machinery executes programmed cell death in plant cells. Cell Death Differ 2002; 9:726-33.
19. Hatsugai N, Kuroyanagi M, Nishimura M, Hara-Nishimura I. A cellular suicide strategy of plants: vacuole-mediated cell death. Apoptosis 2006; 11:905-11.
20. Lavrik IN, Golks A, Krammer PH. Caspases: pharmacological manipulation of cell death. J Clin Invest 2005; 115:2665-72.
21. Kuroyanagi M, Yamada Kenji, Hatsugai N, Kondo M, Nishimura M, Hara-Nishimura I. Vacuolar processing enzyme is essential for mycotoxin induced cell death in *Arabidopsis thaliana*. J Biol Chem 2005; 280:32914-20.
22. Nakaune S, Yamada K, Kondo M, Kato T, Tabata S, Nishimura M, et al. A vacuolar processing enzyme, δVPE, is involved in seed coat formation at the early stage of seed development. Plant Cell 2005; 17:876-87.
23. Fan LM, Zhao Z, Assmann SM. Guard cells: a dynamic signaling model. Curr Opin Plant Biol 2004; 7:537-46.
24. Nadeau JA. Stomatal development: new signals and fate determinants. Curr Opin Plant Biol 2008; 12:1-7.
25. Melotto M, Underwood W, Koczan J, Nomura K, He SY. Plant Stomata function in innate immunity against bacterial invasion. Cell 2006; 126:969-80.
26. Zeng W, He SY. A prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to *Pseudomonas syringae* pv. *tomato* DC3000 in Arabidopsis. Plant Physiol 2010; 153:1188-98.
27. Pandey S, Zhang W, Assmann SM. Roles of ion channels and transporters in guard cell signal transduction. FEBS Lett 2007; 581:2325-36.
28. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, et al. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J 2003; 22:2623-33.
29. Pandey S, Assmann SM. The Arabidopsis putative G protein-coupled receptor GCR1 interacts with the G protein a subunit GPA1 and regulates abscisic acid signaling. Plant Cell 2004; 16:1616-32.
30. Allen GJ, Sanders D. Calcineurin, a type 2B protein phosphatase, modulates the Ca^{2+}-permeable slow vacuolar ion channel of stomatal guard cells. Plant Cell 1995; 7:1473-83.
31. Negi J, Matsuda O, Nagasawa T, Oba Y, Takahashi H, Kawai-Yamada M, et al. CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature 2008; 452:483-6.
32. Vahisalu T, Kollist H, Wang Y, Nishimura N, Chan WYi, Valerio G, et al. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature 2008; 452:487-91.
33. Liu J, Elmore JM, Fuglsang AT, Palmgren MG, Staskawicz BJ, Coaker G. RIN4 functions with plasma membrane H^+-ATPases to regulate stomatal apertures during pathogen attack. PLoS Biol 2009; 7:1000139.
34. MacRobbie EAC. Control of volume and turgor in stomatal guard cells. J Membr Biol 2006; 210:131.
35. Munemasa S, Oda K, Watanabe-Sugimoto M, Nakamura Y, Shimoishi Y, Murata Y. The coronatine-insensitive 1 mutation reveals the hormonal signaling interaction between abscisic acid and methyl jasmonate in Arabidopsis guard cells. Specific impairment of ion channel activation and second messenger production. Plant Physiol 2007; 143:1398-407.
36. Gudesblat GE, Torres PS, Vojnov AA. *Xanthomonas campestris* overcomes Arabidopsis stomatal innate immunity through a DSF cell-to-cell signal-regulated virulence factor. Plant Physiol 2009; 149:1017-27.
37. Guimaraes RL, Stotz HU. Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. Plant Physiol 2004; 136:3703-11.