

# Caspases. Regulating Death Since the Origin of Life<sup>1</sup>

Maite Sanmartín, Lukasz Jaroszewski, Natasha V. Raikhel, and Enrique Rojo\*

Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, CSIC, E-28049 Madrid, Spain (M.S., E.R.); Joint Center for Structural Genomics, Scripps Research Institute, La Jolla, California 92037 (L.J.); and Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, California 92521 (N.V.R.)

## PROGRAMMED CELL DEATH IN EUKARYOTES

Programmed cell death (PCD) is the genetically controlled suicide of cells. The tight regulation of this program is essential to ensure that it is only activated in the required cells at the proper moment. Deregulation of apoptosis, the main form of PCD in animals, is associated with diseases such as cancer, autoimmune diseases, and neurodegenerative disorders (Shi, 2002). In plants, deregulation of PCD is often associated with pleiotropic developmental defects and lethality (Lorrain et al., 2003). PCD occurs in all eukaryotic kingdoms, both in unicellular and multicellular organisms, and is involved in many aspects of development as well as in responses to external stimuli. PCD has morphological similarities in animals and plants, and it regulates common processes such as morphogenesis of the embryo, organ abscission, formation of hollow tubular structures (vessels), and defense against pathogens (Woltering et al., 2002). In view of the ubiquitous occurrence of PCD throughout nature and the coincident morphological and functional features in eukaryotes, a question arises: Has PCD developed independently in the different kingdoms or has it evolved from a common ancestral cell death process and thus plants, fungi, and animals may share common regulatory mechanisms?

The most convincing evidence for a common origin of PCD is that some of the molecular components of the program are conserved in the different kingdoms. Proteins that activate or inhibit PCD in animals, such as the Bcl2 family of pro- and antiapoptotic proteins, or the baculovirus antiapoptotic proteins p35 and IAP, have a similar effect on PCD when expressed in plants or yeast (*Saccharomyces cerevisiae*; Lacomme and Santa Cruz, 1999; Mitsuhara et al., 1999; Sanchez et al., 2000; Lincoln et al., 2002; Danon et al., 2004). Moreover, homologs of genes that control apoptosis in animals, such as *Defender against Apoptotic Death-1* and *Bax-inhibitor-1*, are encoded in plant genomes and serve the same function in PCD control (Matsumura et al., 2003; Danon et al., 2004). In contrast, there are no evident

homologs in plant or fungal genomes of caspases, arguably the key regulators of PCD in animals. Caspases are a family of Cys proteases with specificity for Asp (hence the name) that function as molecular switches to activate the cell death program in metazoans as diverse as mammals, nematodes, or insects (Shi, 2002). PCD promoting signals induce the self-processing of inactive caspase zymogens. This irreversible activation triggers a proteolytic cascade that turns on enzymes involved in killing the cell. Paradoxically, although genome mining has not yielded candidate caspases, caspase inhibitors can block PCD in plants, and most instances of plant PCD are associated with the induction of caspase-like activities (del Pozo and Lam, 1998; Watanabe and Lam, 2004). Are these activities encoded by enzymes phylogenetically unrelated to caspases? Do they regulate PCD in a similar manner to animal caspases? We will address these questions in light of the recent identification of plant enzymes displaying caspase activity and regulating PCD.

## PLANT CASPASES

Two groups of proteases have been proposed as candidates to encode caspase-like activities in plants: the vacuolar processing enzymes (VPEs) and the metacaspases (Woltering et al., 2002). They are related in sequence and in tertiary structure to animal caspases (Aravind and Koonin, 2002) and have been associated with PCD processes in plants (Hoerberichts et al., 2003; Rojo et al., 2003). Recent work has provided evidence that VPEs and metacaspases have caspase activity and function in regulating PCD in plants, suggesting that they are true caspase orthologs (Hatsugai et al., 2004; Rojo et al., 2004; Suarez et al., 2004). In addition, a Ser protease displaying caspase-like activity and possibly controlling PCD has been identified in oat (*Avena sativa*; Coffeen and Wolpert, 2004).

## VACUOLAR PROCESSING ENZYMES

VPEs have been extensively studied for their role in the maturation of proteins in seed storage vacuoles, where they account for the bulk of processing activity

<sup>1</sup> This work was supported in part by the Spanish Ministerio de Educación y Ciencia (grant no. MBC2003-08039 to E.R.).

\* Corresponding author; e-mail erojo@cnb.uam.es; fax 34-91-5854674.

[www.plantphysiol.org/cgi/doi/10.1104/pp.104.058552](http://www.plantphysiol.org/cgi/doi/10.1104/pp.104.058552).

(Shimada et al., 2003; Gruis et al., 2004). Recently, it was reported that members of this family are expressed in vegetative tissues where they are localized in protease precursor vesicles (PPVs) and in vacuoles (Kinoshita et al., 1999; Hayashi et al., 2001), organelles intimately associated with PCD in plants. In addition, the solved structures that are most similar to the folding predicted for VPEs are those of caspases, and modeling of the three-dimensional structure of *Arabidopsis thaliana* VPE $\gamma$  predicts a close alignment of its catalytic residues with those of caspase-8 (Fig. 1). Furthermore, VPE $\gamma$  has been shown to regulate protein degradation during senescence, a type of PCD (Rojo et al., 2003). Taken together, these data suggest that VPEs may encode caspase activities associated with PCD progression in plants.

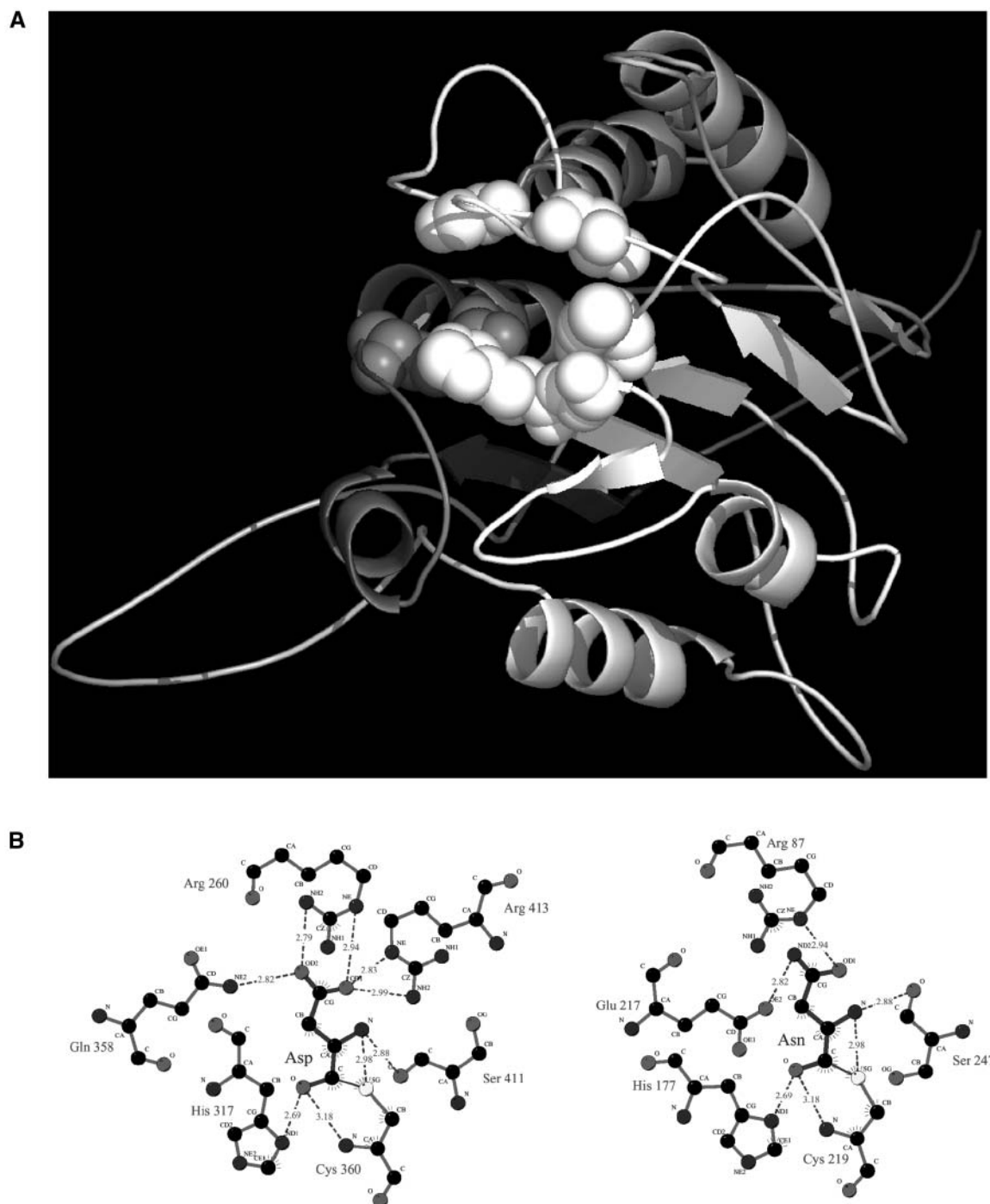
Two recent reports have provided direct evidence that VPEs have caspase-like activity and regulate cell death in *Nicotiana* and *Arabidopsis* plants (Hatsugai et al., 2004; Rojo et al., 2004). Tobacco (*Nicotiana tabacum*) plants carrying the N resistance gene activate an acute PCD response when infected by tobacco mosaic virus (TMV), which is blocked by treatment with VPE-inhibitors or caspase1-inhibitors. A caspase activity induced during TMV infection is sensitive to VPE inhibitors, and, conversely, VPE activity in infected plants is inhibited by caspase1-inhibitors. In VPE-silenced *Nicotiana benthamiana* plants, the induction of caspase activity in response to TMV is suppressed, vacuolar collapse and PCD are blocked, and TMV proliferation increases (Hatsugai et al., 2004). These results suggest that the VPEs from *Nicotiana* may display caspase activity and initiate PCD during TMV infection by promoting vacuolar rupture. However, since many VPEs were silenced simultaneously, the activity and function of the individual *N. benthamiana* VPEs remains unknown. A study of the *Arabidopsis* VPE $\gamma$  gene has provided direct in vivo evidence of its caspase-like activity and genetic evidence for the involvement of VPE $\gamma$  in disease resistance and cell death progression (Rojo et al., 2004). VPE $\gamma$  binds to caspase inhibitors that block self-maturation of this enzyme and all its downstream in vivo activities. An increase in caspase activity, concurrent with the initiation of a rapid PCD response, is observed in *Arabidopsis* plants infected with an incompatible strain of *Pseudomonas syringae* pv tomato DC3000 (*Pst*). The early induction of this caspase activity is compromised in *vpe $\gamma$*  mutants, and *vpe $\gamma$*  mutants are more susceptible to infection with the incompatible strain of *Pst*. The *vpe $\gamma$*  mutants are also more susceptible to infection with turnip mosaic virus (TuMV), a pathogen that does not induce a classical PCD but rather affects the viability of infected cells. Thus, VPEs negatively regulate the growth of several biotrophic pathogens (TMV, *Pst*, TuMV), most likely by promoting cell death progression. What is their effect on the growth of necrotrophic pathogens that thrive on dead tissues? Interestingly, *vpe $\gamma$*  plants are more susceptible to *Botrytis cinerea*, although this

pathogen benefits from PCD in other conditions (Govrin and Levine, 2000; Dickman et al., 2001).

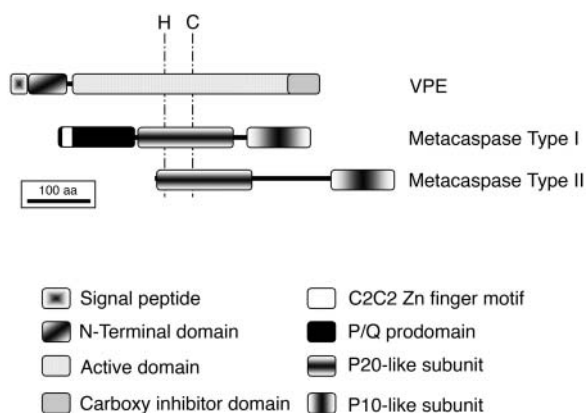
The picture that emerges from these studies is that VPEs are caspase orthologs that activate PCD in plants and influence the outcome of a wide range of interactions with pathogens.

## METACASPASES

Iterative PSI-BLAST searches revealed the presence of a caspase-related family of proteases termed metacaspases in plant and fungal genomes (Uren et al., 2000). The yeast metacaspase YCA1 is required for the increased caspase-like activity and the subsequent PCD observed in H<sub>2</sub>O<sub>2</sub>-treated or in aging yeast cells. Moreover, YCA1 overexpression results in higher caspase activity and PCD in those conditions, suggesting that YCA1 encodes a caspase that activates PCD in yeast (Madeo et al., 2002). Plant genomes contain an extended complement of metacaspases, which have been classified as type I and type II based on their sequence and structural features (Fig. 2). *Arabidopsis* contains 3 type I (*AtMCP1a–1c*) and 6 type II (*AtMCP2a–2f*) metacaspases. *AtMCP2d* and *AtMCP2f* are Cys proteases that in vitro do not have caspase activity, instead cleaving substrates after P1 Arg/Lys residues (Vercammen et al., 2004). The in vitro or in vivo activity of the other metacaspases has not been tested. A type II metacaspase, *mcII-Pa* from *Picea abies*, is required for a burst of caspase activity that occurs during somatic embryogenesis in cells undergoing PCD (Suarez et al., 2004). The *mcII-Pa* transcript is expressed in embryonic tissues that are committed to death (susensor and procambial strands), and its silencing by RNAi blocks the induction of caspase-like activity, inhibiting PCD and somatic embryogenesis (Suarez et al., 2004). These results suggest that *mcII-Pa* displays caspase-like activity and activates PCD, which is essential for embryo development (Bozhkov et al., 2004). A caveat of these studies in *Picea* is that the specificity of the RNAi for *mcII-Pa* was not analyzed, and the effects observed may be due to the silencing of related genes. Genetic analysis of an orthologous *Arabidopsis* gene could give further insights on the function of this gene. Unfortunately, *Arabidopsis* contains 4 genes highly similar to *mcII-Pa*, which may fulfill redundant functions and are located contiguously in chromosome 1 (*AtMCP2a–2d*), complicating the genetic analysis of their function. However, since all metacaspases have been identified in *Arabidopsis*, gene-specific silencing may be attempted and the specificity determined. The genetic analysis of the five remaining metacaspases in *Arabidopsis* should be more straightforward. Analysis of their expression may provide hints on their function (Table I). A tomato type II metacaspase (*LeMCA1*), which is most similar to the *AtMCP2a* to *2d* gene cluster, is induced during infection with *B. cinerea* (Hoerberichts et al., 2003), suggesting that these genes may play a role in the PCD induced by this pathogen. We have performed in silico northern of the



**Figure 1.** The model VPE $\gamma$  based on human caspase-8. A, The sequence of VPE $\gamma$  was submitted to FFAS03 (Jaroszewski et al., 2000) and SUPERFAMILY (Gough et al., 2001) fold prediction servers. Both FFAS03 and SUPERFAMILY listed caspase fold as the most probable fold for the central region of VPE $\gamma$  sequence (residues 50–330), and selected caspase-8 as the structure most similar to VPE $\gamma$ . The alignment from FFAS03 was used for modeling using caspase-8 as a structural template. The model of the VPE $\gamma$  molecule was built with the MODELER program. Residues aligned with binding site residues of caspase-8 are shown as spheres. B, The active site interactions in human caspase-8 (left) and predicted active site interactions in VPE $\gamma$  (right). Replacement of Gln residue with Glu residue is connected with difference in specificity between caspases and VPEs (Asp and Asn residues in P1 position of the substrate, respectively).



**Figure 2.** Domain structure of plant VPEs and metacaspases. The position of the His-Cys catalytic dyad of these enzymes is shown. VPEs are synthesized as inactive zymogens with a signal peptide for insertion into the endomembrane system. The inhibitory C-terminal propeptide and the N-terminal propeptide of VPEs are sequentially removed, most likely in the acidic environment of the vacuole. Metacaspases are probably cytosolic enzymes that may be autoprocessed into 2 subunits, p20 and p10. In addition, type I metacaspases contain a prodomain rich in Pro and Gln residues, which contain a zinc finger motif similar to the ones present in *LSD1*, a negative regulator of PCD in Arabidopsis.

Arabidopsis metacaspases in the GENEVESTIGATOR database (Zimmermann et al., 2004). The data retrieved shows that *AtMCP2d* is the most abundantly expressed metacaspase, and *AtMCP1b* expression is induced during compatible and incompatible interactions with *Pst*. These results corroborate previously reported data (Watanabe and Lam, 2004), indicating that the information obtained in the database for these probes is reliable. Other interesting in silico results have not been previously published (Table I). *AtMCP1c* is also induced in response to infection with compatible and incompatible strains of *Pst* at a higher-fold level than *AtMCP1b*. Interestingly, both *AtMCP1b* and *AtMCP1c* are induced when infected by a type III-secretion HrcC mutant of *Pst* and during a nonhost interaction with *P. syringae* pv phaseolicola, indicating that their expression is responsive to pathogen-associated molecular patterns (PAMPs) present in the bacterial surface rather than to virulent factors injected into the plant cell. Consistent with this, treatment with the flagellin peptide Flg22, a well-characterized bacterial PAMP (Zipfel et al., 2004), induces the expression of *AtMCP1b* and *AtMCP1c*. *AtMCP2e*, for which there was no previous evidence of expression (Vercammen et al., 2004; Watanabe and Lam, 2004), is also induced by *Pseudomonas* infection or by treatment with Flg22 and HrpZ. Moreover, infection with *Phytophthora infestans* or treatment with NPP1, a peptide PAMP from *Phytophthora* (Felbrich et al., 2002), induces the expression of *AtMCP1b*, *AtMCP1c*, and *AtMCP2e*. Thus, these three genes are coordinately induced by PAMPs from different pathogens and may be part of the innate immune response of plants, which in some cases includes the activation of cell death processes. *AtMCP2f* is induced in senescing

organs of the flower and in senescing cell cultures, indicating that it may play a role in this type of PCD. Interestingly, 3 genes from the chromosome 1 cluster, *AtMCP2a*, *AtMCP2c*, and *AtMCP2d*, are expressed at higher levels in roots than in aerial organs, suggesting they may play a specialized role in these organs. Moreover, *AtMCP2a* is induced in roots under conditions (salt, drought, and genotoxic stress) that activate cell death. In summary, the expression data is consistent with a role of metacaspases in developmental or stress-induced PCD.

## SER PROTEASE WITH SPECIFICITY TOWARD ASPARTATE

Isolates of the necrotrophic fungus *Cochliobolus victoriae* that produce the toxin victorin activate a PCD response in susceptible oat cultivars, leading to disease progression (Navarre and Wolpert, 1999). Victorin-induced PCD is associated with two caspase-like activities that can be differentiated by their sensitivity to caspase inhibitors (Coffeen and Wolpert, 2004). One of these caspase-like enzymes is translocated to the apoplast in response to victorin treatment or heat shock, correlating with the induction of PCD by these stimuli. This caspase activity was purified and partial sequence of the coeluting protein showed that it was homologous to subtilisin-like Ser proteases, thus its name saspase. Extensive biochemical characterization of the purified saspase showed that its substrate specificity is strict for Asp at the P1 position and thus it is distinct from all other known Ser proteases. Once the gene encoding the saspase is cloned, it will be possible to test its role in PCD activation.

## PLANT AND ANIMAL CASPASES REGULATE PCD FROM DIFFERENT SUBCELLULAR COMPARTMENTS

Most animal caspases are soluble cytoplasmic proteins. Two exceptions are caspase-12, which is associated with the endoplasmic reticulum (ER) membrane from the cytosolic side and mediates ER-stress induced PCD (Nakagawa et al., 2000), and caspase-2, which is partially localized in the nucleus and mediates PCD triggered by DNA damage (Lassus et al., 2002). However, once activated, all animal caspases initiate a proteolytic cascade in the cytosol that results in the activation of numerous enzymes that degrade cellular components and kill the cell.

In plants, the enzymes displaying caspase activity may regulate PCD from several compartments, including the cytosol (Table I). Type II metacaspases do not contain signal peptides or transmembrane domains (Fig. 2), so they are predicted to be cytosolic and thus would function in the same location as animal caspases. Type I metacaspases are predicted to be localized in mitochondria and chloroplasts

**Table 1.** Summary of *Arabidopsis* VPEs and metacaspases

The expression data from VPEs is from published works, whereas the data on metacaspases was obtained from the GENEVESTIGATOR database (Zimmermann et al., 2004) on December 5, 2004. Psp, *P. syringae* pv phaseolicola; Pi, *P. infestans*; HrpZ, harpin from *P. syringae* pv tomato DC3000; NPP1, cell wall elicitor protein from *P. infestans*.

Gene	AGI-TIGR Locus	Signal Peptide	EST + cDNAs	T-DNA Mutants	Subcellular Location	Expression
<b>VPEs</b>						
VPE $\alpha$	At2g25940	Amino acids 1–20	13 + 2	Available	Secretory pathway <sup>a</sup> , plasma membrane <sup>a</sup>	Vegetative tissues. Induced in leaves by ethylene, salicylic acid (SA), wounding, and senescence.
VPE $\beta$	At1g62710	Amino acids 1–21	33 + 2	Available	Secretory pathway <sup>a</sup> , vacuole <sup>a</sup>	Seeds, flowers, root tips.
VPE $\gamma$	At4g32940	Amino acids 1–25	129 + 5	Available	PPV, vacuole	Vegetative tissues, induced by ethylene, SA, wounding, and senescence. Induced during infection with Pst avrRpm1, TuMV, <i>B. cinerea</i> .
VPE $\delta$	At3g20210	Amino acids 1–24	62 + 2	Available	Secretory pathway <sup>a</sup> , apoplasm <sup>a</sup>	All stages in developing seeds, most abundant in cell division stage and germinating seeds.
<b>Metacaspases</b>						
AtMCP1a	At5g64240		25 + 1	Available	Mitochondrion <sup>a</sup>	Ubiquitously expressed. Induced in senescing flowers.
AtMCP1b	At1g02170		3	Available	Chloroplast <sup>a</sup>	Induced in leaves by Pst, Pst avrRpm1, Psp, Pi, Flg22, HrpZ, and NPP1.
AtMCP1c	At4g25110		4	Available	Mitochondrion <sup>a</sup>	Induced in leaves by Pst, Pst avrRpm1, Psp, Pi, Flg22, HrpZ, NPP1, SA, and AgNO <sub>3</sub> .
AtMCP2a	At1g79310		1	Available	Cytosol <sup>a</sup>	Higher expression in roots than aerial organs. Induced in wounded roots or in roots under salt, osmotic, or genotoxic stress.
AtMCP2b	At1g79330		2	Available	Cytosol <sup>a</sup>	Seed specific expression.
AtMCP2c	At1g79320		None	Available	Cytosol <sup>a</sup>	Very low expression. Higher in roots than aerial organs
AtMCP2d	At1g79340		49 + 2	Available	Cytosol <sup>a</sup>	Ubiquitously expressed. Higher expression in roots than aerial organs.
AtMCP2e	At1g16420		None	None	Cytosol <sup>a</sup>	Induced in leaves by Pst, Pst avrRpm1, Psp, Pi, Flg22, HrpZ, and NPP1. Higher expression in roots than in aerial organs.
AtMCP2f	At5g04200		9 + 2	Available	Cytosol <sup>a</sup>	Induced in senescing cell cultures and flowers. Induced in roots by osmotic stress.

<sup>a</sup>Localization predicted by TargetP.

based on the presence of putative transit peptides at their N terminus of the proteins. However, the confidence level of these predictions is low and thus they may also be cytosolic enzymes. In contrast, VPEs and saspases probably function in other subcellular compartments. VPEs contain signal peptides for insertion into the endomembrane system (Fig. 2), and they have been localized to PPVs or vacuoles (Rojo et al., 2003). VPEs in *N. benthamiana* are required for the disruption of the tonoplast, a process that is common to most cases of PCD in plants and possibly constitutes an irreversible step in cell death commitment. Although the localization of *N. benthamiana* VPEs has not been reported, they are likely localized in vacuoles, and would activate PCD from the vacuolar lumen. In addition, VPEs may act after disruption of the tonoplast by processing cytosolic enzymes involved in PCD execution. This function would be similar to the role of animal cathepsins, which are lysosomal proteases that when released from damaged lysosomes activate caspase cascades in the cytosol, triggering apoptosis (Ferri and Kroemer, 2001). Subtilisin Ser proteases homologous to the saspase identified by Coffeen and Wolpert (2004) contain signal peptides and have been detected in vacuoles or in the extracellular space. The oat saspase is localized intracellularly in the absence of PCD-promoting stimuli, possibly retained in the ER or the Golgi apparatus. Secretion of the saspase to the apoplast correlates with the initiation of PCD, suggesting that it activates cell death from the apoplast.

## REGULATION OF CASPASE ACTIVITY IN PLANTS

Animal caspases are expressed constitutively, but in a latent precursor form. The zymogens are processed and PCD is irreversibly initiated only in response to cell death promoting signals. Thus, in animals, the regulation of caspase activity is mainly posttranslational via proteolytic digestion of the zymogens.

This situation may be different in plants. For instance, the expression of VPEs is induced in conditions that activate PCD, such as TMV infection in tobacco (Hatsugai et al., 2004) or senescence and pathogen attack in Arabidopsis (Rojo et al., 2004), indicating that the regulation of their activity may be partly at the level of transcription. Similarly, metacaspases are induced in conditions that activate PCD (Table I). In addition, posttranslational regulation of VPEs by maturation of the zymogens may also occur. It has been postulated that VPEs are self-processed in the acidic environment of the vacuole. Therefore, VPEs may be maintained in a latent unprocessed form by keeping them in intermediate nonacidic compartments of the secretory pathway, such as PPVs (Rojo et al., 2003). In conditions inducing PCD, VPEs may be transferred to the vacuole where they are activated and promote vacuolar collapse. Whether this is

achieved through a vacuolar proteolytic cascade similar to the cytosolic cascade activated by caspases of metazoans is not yet known. VPE $\gamma$  has been shown to process a vacuolar protease and to regulate the degradation of vacuolar proteins, including a cystatin that may inhibit PCD progression (Rojo et al., 2004), suggesting that proteolytic events downstream of VPE activation may be important for PCD initiation. In contrast to VPEs and metacaspases, the oat saspase is expressed constitutively, most likely as an active enzyme (Coffeen and Wolpert, 2004). It has been proposed that this saspase is maintained inactive by its retention in the secretory pathway and is activated by secretion to the apoplast where it may gain access to effector substrates that activate PCD. What are these effector proteins? P69, a pathogen-induced Ser protease from tomato, has been shown to cleave an extracellular Leu-rich repeat containing protein (LRP) during PCD induced by bacterial infection (Tornero et al., 1996). LRPs are in many cases signaling molecules, and some like the tomato Cf-2 are involved in disease resistance through the regulation of PCD. It is tempting to speculate that the oat saspase may cleave an LRP and activate a signaling cascade inducing PCD. In summary, several levels of regulation of caspase activity are functional in plants. The expression of caspase-like enzymes may be induced prior to PCD and their activity may be regulated by maturation of inactive zymogens or by subcellular relocalization.

A challenge for the future is to understand how developmental cues or external stimuli feed into these regulatory systems to activate plant caspases, and how they in turn promote cell death execution. As we have discussed, some of the plant caspases are functionally and evolutionarily related to animal caspases, suggesting that part of the information obtained from animal systems may be useful to further characterize PCD and caspase cascades in plants. However, fundamental differences in regulation and subcellular compartmentalization of plant and animal caspases have also been reported, indicating that many features of PCD will be plant specific.

## ACKNOWLEDGMENT

We thank Dr. José J. Sánchez-Serrano and Dr. Raquel Martín for critically reading the manuscript.

Received December 17, 2004; returned for revision December 23, 2004; accepted December 23, 2004.

## LITERATURE CITED

- Aravind L, Koonin E (2002) Classification of the caspase-hemoglobinase fold: detection of new families and implications for the origin of the eukaryotic separins. *Proteins* **46**: 355–367
- Bozhkov PV, Filonova LH, Suarez MF, Helmersson A, Smertenko AP, Zhivotovsky B, von Arnold S (2004) VEIDase is a principal caspase-like activity involved in plant programmed cell death and essential for embryonic pattern formation. *Cell Death Differ* **11**: 175–182

- Coffeen WC, Wolpert TJ** (2004) Purification and characterization of serine proteases that exhibit caspase-like activity and are associated with programmed cell death in *Avena sativa*. *Plant Cell* **16**: 857–873
- Danon A, Rotari VI, Gordon A, Mailhac N, Gallois P** (2004) Ultraviolet-C overexposure induces programmed cell death in *Arabidopsis*, which is mediated by caspase-like activities and which can be suppressed by caspase inhibitors, p35 and Defender against Apoptotic Death. *J Biol Chem* **279**: 779–787
- Dickman MB, Park YK, Oltersdorf T, Li W, Clemente T, French R** (2001) Abrogation of disease development in plants expressing animal anti-apoptotic genes. *Proc Natl Acad Sci USA* **98**: 6957–6962
- Felbrich G, Romanski A, Varet A, Blume B, Brunner F, Engelhardt S, Felix G, Kemmerling B, Krzymowska M, Nürnberger T** (2002) NPP1, a Phytophthora-associated trigger of plant defense in parsley and *Arabidopsis*. *Plant J* **32**: 375–390
- Ferri KF, Kroemer G** (2001) Organelle-specific initiation of cell death pathways. *Nat Cell Biol* **3**: 255–263
- Gough J, Karplus K, Hughey R, Chothia C** (2001) Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure. *J Mol Biol* **313**: 903–919
- Govrin EM, Levine A** (2000) The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr Biol* **10**: 751–757
- Gruis D, Schulze J, Jung R** (2004) Storage protein accumulation in the absence of the vacuolar processing enzyme family of cysteine proteases. *Plant Cell* **16**: 270–290
- Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, Kondo M, Nishimura M, Hara-Nishimura I** (2004) A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science* **305**: 855–858
- Hayashi Y, Yamada K, Shimada T, Matsushima R, Nishizawa NK, Nishimura M, Hara-Nishimura I** (2001) A proteinase-storing body that prepares for cell death or stresses in the epidermal cells of *Arabidopsis*. *Plant Cell Physiol* **42**: 894–899
- Hoebrechts FA, ten Have A, Woltering EJ** (2003) A tomato metacaspase gene is upregulated during programmed cell death in *Botrytis cinerea*-infected leaves. *Planta* **217**: 517–522
- Jaroszewski L, Rychlewski L, Godzik A** (2000) Improving the quality of twilight-zone alignments. *Protein Sci* **9**: 1487–1496
- Kinoshita T, Yamada K, Hiraiwa N, Kondo M, Nishimura M, Hara-Nishimura I** (1999) Vacuolar processing enzyme is up-regulated in the lytic vacuoles of vegetative tissues during senescence and under various stressed conditions. *Plant J* **19**: 43–53
- Lacomme C, Santa Cruz S** (1999) Bax-induced cell death in tobacco is similar to the hypersensitive response. *Proc Natl Acad Sci USA* **96**: 7956–7961
- Lassus P, Opatz-Araya X, Lazebnik Y** (2002) Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. *Science* **297**: 1352–1354
- Lincoln JE, Richael C, Overduin B, Smith K, Bostock R, Gilchrist DG** (2002) Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. *Proc Natl Acad Sci USA* **99**: 15217–15221
- Lorrain S, Vaillau F, Balagué C, Roby D** (2003) Lesion mimic mutants: keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci* **8**: 263–271
- Madeo F, Herker E, Maldener C, Wissing S, Lachelt S, Herlan M, Fehr M, Lauber K, Sigrist SJ, Wesselborg S, et al** (2002) A caspase-related protease regulates apoptosis in yeast. *Mol Cell* **9**: 911–917
- Matsumura H, Nirasawa S, Kiba A, Urasaki N, Saitoh H, Ito M, Kawai Yamada M, Uchimiya H, Terauchi R** (2003) Overexpression of Bax inhibitor suppresses the fungal elicitor-induced cell death in rice (*Oryza sativa* L) cells. *Plant J* **33**: 425–434
- Mitsuhara I, Malik KA, Miura M, Ohashi Y** (1999) Animal cell-death suppressors Bcl-x(L) and Ced-9 inhibit cell death in tobacco plants. *Curr Biol* **9**: 775–778
- Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J** (2000) Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. *Nature* **403**: 98–103
- Navarre DA, Wolpert TJ** (1999) Victorin induction of an apoptotic/senescence-like response in oats. *Plant Cell* **11**: 237–249
- del Pozo O, Lam E** (1998) Caspases and programmed cell death in the hypersensitive response of plants to pathogens. *Curr Biol* **8**: 1129–1132
- Rojo E, Martin R, Carter C, Zouhar J, Pan S, Plotnikova J, Jin H, Paneque M, Sanchez-Serrano JJ, Baker B, et al** (2004) VPEgamma exhibits a caspase-like activity that contributes to defense against pathogens. *Curr Biol* **14**: 1897–1906
- Rojo E, Zouhar J, Carter C, Kovaleva V, Raikhel NV** (2003) A unique mechanism for protein processing and degradation in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* **100**: 7389–7394
- Sanchez P, de Torres Zabala M, Grant M** (2000) AtBI-1, a plant homologue of Bax inhibitor-1, suppresses Bax-induced cell death in yeast and is rapidly upregulated during wounding and pathogen challenge. *Plant J* **21**: 393–399
- Shi Y** (2002) Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell* **9**: 459–470
- Shimada T, Yamada K, Kataoka M, Nakaune S, Koumoto Y, Kuroyanagi M, Tabata S, Kato T, Shinozaki K, Seki M, et al** (2003) Vacuolar processing enzymes are essential for proper processing of seed storage proteins in *Arabidopsis thaliana*. *J Biol Chem* **278**: 32292–32299
- Suarez ME, Filonova LH, Smertenko A, Savenkov EI, Clapham DH, von Arnold S, Zhivotovsky B, Bozhkov PV** (2004) Metacaspase-dependent programmed cell death is essential for plant embryogenesis. *Curr Biol* **14**: 339–340
- Tornero P, Conejero V, Vera P** (1996) Primary structure and expression of a pathogen-induced protease (PR-P69) in tomato plants: similarity of functional domains to subtilisin-like endoproteases. *Proc Natl Acad Sci USA* **93**: 6332–6337
- Uren AG, O'Rourke K, Aravind L, Pisabarro MT, Seshagiri S, Koonin EV, Dixit VM** (2000) Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol Cell* **6**: 961–967
- 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** (2004) Type II metacaspases Atmc4 and Atmc9 of *Arabidopsis thaliana* cleave substrates after arginine and lysine. *J Biol Chem* **279**: 45329–45336
- Watanabe N, Lam E** (2004) Recent advance in the study of caspase-like proteases and Bax inhibitor-1 in plants: their possible roles as regulator of programmed cell death. *Mol Plant Pathol* **5**: 65–70
- Woltering EJ, van der Bent A, Hoebrechts FA** (2002) Do plant caspases exist? *Plant Physiol* **130**: 1764–1769
- Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W** (2004) GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol* **136**: 2621–2632
- Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T** (2004) Bacterial resistance in *Arabidopsis* through flagellin perception. *Nature* **431**: 764–767