Plant caspase, a missing link in plant apoptosis

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Programmed cell death (PCD), or apoptosis, is a fundamentally important process that maintains the integrity and homeostasis of organisms, regulates their growth, development and responses to pathogen attacks and abiotic stresses. Caspases (cysteinyl aspartate-specific proteinases) have been identified as the main "executioner elements" in cell-suicide machinery, and have been shown to play a critical role in mammalian PCD (Fig. 1). Caspases are responsible for the proteolysis of key proteins that are known to be selectively cleaved at the onset of apoptosis. Caspase-mediated protein fragmentation eventually leads to cell death and caspase knockouts or caspase-specific peptide inhibitors, based on sequences cleaved by caspases, counteract apoptosis in animals. In plants, several tissues or whole organs undergo PCD as part of normal development or in response to environmental stresses. Moreover, the hypersensitive response (HR) is a form of rapid, localised PCD that prevents the spread of pathogens in resistant (incompatible) interactions. Morphological features of apoptosis, such as membrane blebbing, chromatin condensation and DNA fragmentation can be observed in the HR. However, in spite of the striking similarities between PCD pathways in animals and plants, the case for any existence of caspases in plants has been controversial. Although some specific inhibitors of animal caspases have been shown to affect development of PCD in plants, no direct homologues of animal caspase genes have been identified in plants.

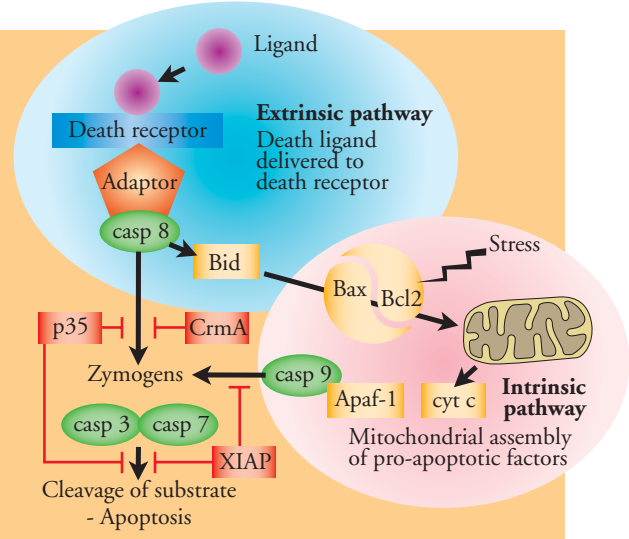

Figure 1 Biochemical events governing apoptotic cell suicide in mammalian PCD. Two initiation pathways (intrinsic and extrinsic), triggered by separate events, converge at a common point to execute apoptosis. Natural inhibitors (red boxes) affect different points on the pathways. The extrinsic pathway is triggered through extracellular ligation of death receptors and their ligands. The intrinsic pathway responds primarily to cellular stress with a mitochondrion acting as an important integrator. Pro- and anti-apoptotic members of Bcl2 family (Bid, Bax, Bcl2) regulate release of cytochrome c (cyt c) leading to activation of caspase 9. Both pathways activate the executioner proteases, caspases 3 and 7 (active forms of caspases shown in green).

Recently in collaboration with Moscow State University (Russia) and Beckman Research Institute (California, USA) we have found this "missing link" in the plant PCD pathway and identified a caspase-like protease, which is activated and counteracts PCD in tobacco plants during *N*-gene mediated HR triggered by *Tobacco mosaic virus* (TMV)[1]. In our work, the nuclear *Agrobacterium tumefaciens* VirD2 protein was used for detection, identification, and purification of a tobacco caspase, based on our prediction that this protein might represent a genuine caspase target. Indeed, we demonstrated that this protein could be specifically cleaved at two sites (TATD and GEQD) by human caspase-3. The VirD2 protein was fused with green fluorescent protein (GFP) and expressed from a TMV-based vector. In these experiments TMV also played the role of inducer of the HR. When the HR was induced, rapid re-localization of the target GFP-VirD2 derivatives from the nucleus to the cytoplasm occurred because the nuclear localisation signal (NLS) became detached from GFP (Fig. 2). Mutational analysis of potential cleavage sites and MALDI mass spectrometry of the cleavage products have identified two sites at which the GFP-VirD2 protein is cleaved by a plant enzyme activated during TMV-mediated HR *in vivo* (TATD and GEQD) that are identical to those identified for the caspase-3 in the experiments *in vitro*[1]. A proteolytic activity capable of specifically cleaving the model substrate at TATD was purified from these leaves. A tetrapeptide aldehyde designed and synthesized on the basis of the elucidated plant caspase cleavage site prevented fragmentation of the substrate protein by plant and

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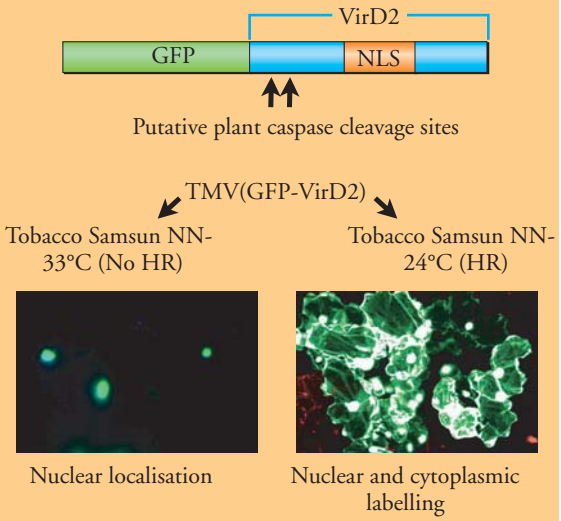

Figure 2 Re-localisation of the target GFP-VirD2 protein from the nucleus to the cytoplasm during the HR as a result of plant caspase activation.

human caspases *in vitro* and counteracted TMV-triggered HR *in vivo* (Fig. 3).

Conclusions and prospects Our data provide a first characterization of caspase-specific protein fragmentation in apoptotic plant cells, with implications for the importance of such an activity for implementation of plant PCD. The plant enzyme identified in our work

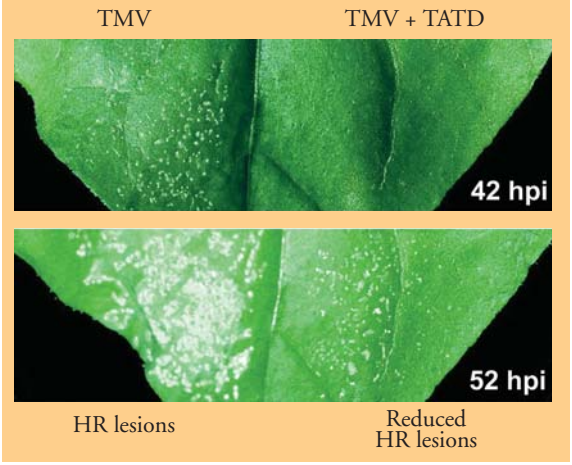

Figure 3 A tetrapeptide aldehyde, biotinyl-TATD-CHO partially inhibits formation of necrotic lesions in tobacco plants infected with TMV (hpi = hours post-inoculation).

represents a novel functional analogue of animal caspases that may contribute to plant resistance to pathogens and abiotic stresses. Future work will provide novel "plant caspase" genes that may be directly deployed to develop durable disease resistance in different crops including barley and potato.

Reference

[1] Chichkova , N.V., Kim, S.H., Titova, E.S., Kalkum, M., Morozov, V.S., Rubtsov, Yu.P., Kalinina, N.O., Taliansky, M.E. & Vartapetian, A.B. (2004) *Plant Cell* **16**, 157-171.