Plant Pathol. J. 21(2) : 99-101 (2005)

The Plant Pathology Journal

Mini-Review

The Hypersensitive Response. A Cell Death during Disease Resistance

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(Received on April 11, 2005; Accepted on April 29, 2005)

Host cell death occurs during many, but not all, interactions between plants and the pathogens that infect them. This cell death can be associated with disease resistance or susceptibility, depending on the nature of the pathogen. The most well-known cell death response in plants is the hypersensitive response (HR) associated with a resistance response. HR is commonly regulated by direct or indirect interactions between avirulence proteins from pathogen and resistance proteins from plant and it can be the result of multiple signaling pathways. Ion fluxes and the generation of reactive oxygen species commonly precede cell death, but a direct involvement of the latter seems to vary with the plant-pathogen combination. Exciting advances have been made in the identification of cellular protective components and cell death suppressors that might operate in HR. In this review, recent progress in the mechanisms by which plant programmed cell death (PCD) occurs during disease resistance will be discussed.

Keywords **:** hypersensitive response, plant-pathogen interactions, programmed cell death, resistance

Recognition of a diverse range of pathogens, followed by an appropriate defense response, is crucial for the survival of plants. It is conditioned by initial recognition events between plant host and pathogen, which are often mediated by plant resistance (R) gene and a corresponding microbial avirulence (Avr) gene (Flor, 1971). Avr-R interactions lead to activation of various host defense responses, including a specialized type of programmed cell death known as a hypersensitive response. Accumulating evidence indicates that *R* gene products function either directly as the receptor for the products of *avr* genes (Bent, 1996; Ellingboe, 1980; Yang et al., 1997) or recognizes the Avr factor indirectly through a coreceptor (Dixon et al., 1998). To date, direct interaction between an R protein and an Avr factor has been demonstrated only for the tomato Pto and the *Pseudomonas syringae* AvrPto proteins (Scofield et al., 1996; Tang et al., 1996) and between the rice Pi-ta and the *Magnaporthe grisea* Avr-Pita proteins (Jia et al., 1999). Additionally, one interaction was shown to occur via formation of a complex probably involving an R protein, an Avr protein and an additional host protein(s) (Leister and Katagiri, 2000). Finally, an R-Avr interaction was recently found to result from the enzymatic activity of the Avr protein (Shao et al., 2003). Although resistance responses are often associated with the HR, in some cases, they can occur without or with very little cell death (for example, see Bendahmane et al., 1999).

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Cell death mechanisms during the HR. Cells killed via the HR usually produce autofluorescence under UV and then dark brown, attributable to the accumulation and oxidation of phenolic compounds (Nicholson and Hammerschmidt, 1992) and it is these features that are commonly used to identify the HR. However, autofluorescence and browning are the final steps in the death process, and they may occur hours after the death has become irreversible. Although apoptosis was first recognized in mammalian cells by its strikingly distinctive morphology, particularly as seen by electron microscopy (Heath, 1998), no equivalently consistent morphology has been recognized for the HR (Bestwick et al., 1995). In particular, apoptotic-like bodies with avirulent *Pseudomonas syringae* infections were observed (Levine et al., 1996). Bestwick et al. (1995) found early changes in mitochondrial morphology (swelling and cristae disorganization) in avirulent *P. syringae*-infected lettuce, similar to what occurs in animal cells undergoing apoptosis (Wakabayashi and Karbowski, 2001). Later stages of the infection were accompanied by membrane dysfunction (loss of ability to be plasmolysed) and progressive vacuolization of the cytoplasm. Membrane disruption was proposed to be the critical event for cell death. Another hallmark of apoptosis is the cleavage of nuclear DNA into oligonucleosomal fragments, producing a 'ladder' of DNA when run on agarose gels. Plant DNA cleavage has been reported for fungus-, bacteria- and virus-induced HR (Levine et al., 1996; Mittler et al., 1997; Ryerson and Heath, 1996) but its timing during the death process varies and it results in a DNA ladder only during the fungus-induced response (Ryerson and Heath, 1996). In animals, apoptosis often

involves proteases called caspases (Green and Reed, 1998). Although clear homologues of caspases have not been found in the complete genome of the model plant *Arabidopsis*, caspase-like activities in plants have been documented biochemically or inferred from inhibitor studies (del Pozo and Lam, 2003; Elbaz et al., 2002; Lincoln et al., 2002). Lam et al. (2001) have suggested that, as in animals, mitochondria could have a role in controlling the HR. Evidence for a role of plastids in virus-induced HR is indicated by the change in the amount of cell death during infection in plants when levels of the plastid protease FtsH are increased or decreased (Seo et al., 2000).

Signaling during the HR. In particular, pathogen-derived molecules, called elicitors, that induce HR-like cell death reactions have been used either by applying them directly to plant cells or by expressing the genes for these elicitors directly in plant cells. Some researchers have also used pathogen infections of plant cell cultures. Using such approaches, an oxidative burst (Heath, 2000), ion channels (Atkinson et al., 1996; Wendehenne et al., 2002), nitric oxide (Delledonne et al., 2001) and the interaction between some of these different signals (Delledonne et al., 2001) were implicated in HR. The exact role of these molecules is still the subject of intense investigations by many researchers. It is possible that cells in an infection zone undergoing resistance responses may not all die from the same signals. In particular, the HR has been suggested to require the correct relative levels of both NO and H_2O_2 to be induced in the host (Delledonne et al., 2001). However, NO was found to be generated first at cell surfaces in avirulent *P. syringae*-infected *Arabidopsis* at a time too late to be causal to the first cell death events during the HR. The pattern of NO generation suggests that it has a role in cell-cell signaling and the spreading of cell death as an infection proceeds (Zhang et al., 2003). Furthermore, inhibition of NO synthesis or action only attenuates the HR, in support of this role. Interestingly, an *Arabidopsis AtbohF/AtbohD* double mutant in NADPH oxidase complex components, thought to generate an oxidative burst during the HR, lacks detectable H_2O_2 accumulation during a resistance (Torres et al., 2002). This double mutant still activated some early cell death. Torres et al. (2002) suggested that the first cell deaths occur independently of H_2O_2 and subsequent death requires H_2O_2 generation. Thus, initial cell deaths during the HR may be both NO and H_2O_2 independent.

Functions of the HR. A number of attempts have been made to address the role of cell death in disease resistance. Many experiments have involved using pharmacology and genetics combined with an analysis of the timing of pathogen arrest relative to cell death. Interpretations of these experiments can be difficult as a number of genes and signal molecules affect more than just cell death. For pathogens that require living host cells for their survival, it is possible that cell death alone is a defense mechanism, but even this assumption is not always supported by experimental data (Richael and Gilchrist, 1999).

To evaluate rigorously the contribution of the HR to the resistance response, the ideal experiment would involve the selective inhibition of the HR. To do this, one needs to know the components of the cell death machinery and the selectivity of the reagent used to inhibit the machinery. There is some evidence that the HR involves the activation of caspase-like activities in plants. If in plants these caspase-like activities are truly specifically involved in activating PCD, then they provide an ideal target to disrupt in order to test the involvement of the HR in resistance. The identification of the basal cell death machinery in plants will hopefully lead to additional rigorous experiments to test the role of the HR in resistance.

Hypersensitive cell death also has been suggested to release signals that condition adjacent cells to become responsive to pathogen elicitors (Graham and Graham, 1999) and that activate systemic resistance throughout the plant. Alvarez et al. (1998) have suggested that this systemic acquired resistance (SAR) in *Arabidopsis* depends on secondary oxidative bursts in distant tissues and the formation of 'micro-HRs'. Such data suggest that the cell death component of the HR may function more as a signaling system than as a direct defense mechanism.

Conclusion

Many questions about the role, regulation and mechanism of PCD during host-plant interactions remain unanswered. Some defense genes may be activated during leaf senescence (Buchanan-Wollaston, 1997) and the *SAG12* marker gene for senescence in *Arabidopsis* is expressed in low levels in cells surrounding TMV- and bacteria induced HR lesions in transgenic tobacco (Pontier et al., 1999). Similarly, the putative HR marker gene, *HIN1*, is expressed in a late stage of leaf senescence (Pontier et al., 1999). These raise the possibility that there is basal cell death machinery engaged during the different responses.

Further analysis of PCD mechanisms in different conditions will be necessary to resolve this question. For future evaluation of the role of cell death in plant disease resistance, it will be important to try to inhibit the cell death machinery selectively and simultaneously to monitor other defense- and pathogenesis-related events. Using this approach, it should be possible to determine whether cell death can be uncoupled from other responses and, if so, what its contribution to resistance is.

Acknowledgement

This research was supported by grants from Plant Diversity Research Center (21st Century Frontier Research Program funded of MOST project No. PF0330509-00) to JMP.

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