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Review

Programmed cell death: A way of life for plants

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ABSTRACT **Cell death in higher plants has been widely observed in predictable patterns throughout development and in response to pathogenic infection. Genetic, biochemical, and morphological evidence suggests that these cell deaths occur as active processes and can be defined formally as examples of programmed cell death (PCD). Intriguingly, plants have at least two types of PCD, an observation that is also true of PCD in animals [Schwartz, L. M., Smith, W. W., Jones, M. E. E. & Osborne, B. A. (1993) *Proc. Natl. Acad. Sci. USA* 90, 980–984]. Thus, in plants, PCD resembles either a common form of PCD seen in animals called apoptosis or it resembles a morphologically distinct form of cell death. The ubiquitous occurrence and necessity of PCD for plant development and defense suggest that the underlying mechanisms of regulation and execution of these processes merit further examination.**

Programmed cell death (PCD) is the active process of cell death which occurs during development and in response to environmental cues. In plants, PCD is essential for development and survival—for example, xylem vessels are dead at maturity, the cells that are elements of the water, and nutrient conducting system live to make strong walls. In fact, 330 years ago the first cells ever observed microscopically were cork cells which had undergone PCD. All that remained of these differentiated plant cells was the surrounding cell wall (1). Plants also employ PCD in response to pathogens.

In animals, PCD is a way to rid the organism of unwanted cells (2). When dying animal cells exhibit certain morphological characteristics such as DNA strand breaks with 3′OH ends, condensation and fragmentation of the nucleus, membrane blebbing, and cytoplasmic condensation, the PCD is referred to as apoptosis (3, 4). PCD can serve as a mechanism to remove cells that have been damaged and it may be important for protection against pathogens [reviewed by Williams (5)]. Some pathogens have evolved to take advantage of PCD and can effectively trick the host into this process and thereby avoid the activation of host defenses (6). Thus, PCD in animals is important for normal development as well as in the manifestation of some diseases.

Despite the early recognition that PCD is required for growth of vascular plants, its study has been mostly neglected over the intervening years. Recent evidence suggests that plant cell death, in some cases at least, might be mechanistically similar to apoptosis in animal cells, since the dying plant cells appear morphologically similar to apoptotic cells in that they form apoptotic bodies (7, 8). In addition, some types of plant cell death are accompanied by DNA cleavage often with the characteristics of endonucleolytically processed DNA, one hallmark of apoptosis (7–10). Finally, a homologue of one gene, *dad1*, that is known to be involved in repressing PCD in animals (11, 12) has been found in plants (12, 13), but its function in plants remains to be determined. Despite these similarities between PCD in plants and animals, it is likely that some aspects of the function and mechanism of PCD in plants will differ from what is seen in animals. For example, plants cells do not engulf their dead neighbors. In some cases the dead plant cells become part of the very architecture of the plant performing crucial functions (see below). The purpose of this paper is to highlight a few examples of PCD in plants and to give a progress report on what is known about the mechanism and function of this process in plants.

Xylogenesis

Perhaps the most dramatic example of PCD in plants is that which occurs to form the water and nutrient conducting tubes that form the vascular system. Both xylem (the water-conducting cells) and phloem (the nutrient-conducting cells) undergo autolysis as they differentiate and mature. The study of xylem differentiation has been facilitated by the finding that mechanically isolated parenchyma cells can be induced to differentiate in culture [reviewed by Fukuda (14)]. In addition, it has long been known that wounding can also induce parenchyma cells near the wound site to redifferentiate into xylem if the vascular bundles are severed. In this case the function of PCD is obvious, since the resulting tracheary xylem elements function as long conducting tubes to bring water from the roots to the rest of the plants and they give the plant mechanical support. But how does the differentiation and cell death occur? At least in the culture system, xylogenesis requires RNA and protein synthesis and thus satisfies the criterion of being an active process (15). It is unlikely that the cell death is entirely cell autonomous since the dying cells can influence their neighbors to differentiate. Over the last several years, attempts to find genes involved in the differentiation process (including the cell death) have led to identification of a number of cDNAs which are preferentially expressed in the developing vasculature. One promising candidate for involvement in autolysis is a single-stranded nuclease (16). In animals PCD is associated with DNA degradation, thus the recent finding that developing xylem cells show evidence of DNA breaks with 3′OH ends (presumed to be the product of endonucleolytic cleavage) (9) suggests this may be an important aspect of the cell death process in plants. Another intriguing gene expressed in the developing vasculature is *TED2* (17) This gene has homology to crystallin, a quinone oxido-reductase (18). If oxidative signals trigger cell death in plants as they may in animals (19), this gene product could be the source of such signals.

It has also been suggested that arabinogalactan proteins play a role in triggering cell death in the developing vascular cells of corn coleoptiles (20). If the arabinogalactan proteins function to loosen the cell walls, this might in turn disrupt cell-matrix interactions. Since such a disruption causes PCD in animal cells (21), it will be interesting to determine if a similar disruption causes the same effect in plants.

Reproduction

Cell death has been observed to occur during many stages of plant reproduction. During somatic embryogenesis of Norway spruce, Havel and Durzan (22) have observed that nuclei fated to die display DNA strand brakes with 3′OH ends and

Abbreviations: PCD, programmed cell death; HR, hypersensitive response.

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apoptotic bodies. In *maize*, sex determination involves the selective killing of the female reproductive primordia in order that the male floral structures (the stamens) can develop in the tassel. DeLong *et al.* (23) recently described the cloning of the *Tasselseed2* gene which is required for cell death during sex determination. The Ts2 protein has sequence similarity with a hydroxy steroid reductase, although the *in vivo* substrate for the Ts2 protein is not yet known. It is likely that the *TS2* product generates a steroid-like molecule which might function as a signal to provoke a cell suicide program. Whether this signal is used as a global PCD trigger during development or pathogenesis remains to be determined.

Recent work by Cheung and colleagues (24) suggests that PCD also occurs in the transmitting tissue through which pollen tubes grow. This process is selective, since the tissues that surround the transmitting tissue in the pistil remain intact. The cell death appears to be correlated with pollen tube growth, since incompatible pollen (which may start to germinate but whose pollen tubes cannot elongate) does not elicit cell death. Interestingly, at least part of the cell death process requires the action of the hormone ethylene, since blocking the ethylene receptors causes the transmitting tissue to go only part way through the cell death process. Ethylene is also associated with other processes involving cell death such as ripening and leaf senescence (see below). The transmitting tissue that dies also shows a dramatic change in the size and stability of cellular RNAs. Many RNAs in the transmitting tissue are shortened and have a higher turnover rate. However, at least one RNA that is shortened persists in the cell, indicating that the there is a cellular mechanism for discriminating among RNAs in the tissue that is dying. The function of cell death during pollen tube elongation has not been established. One possibility is that the dying tissue provides nutrients to the growing tubes. Cell death may also be necessary to physically accommodate the growing pollen tubes. Herrero (25) has also suggested that the cell death might be a way to protect the tissue from pathogen invasion. This is likely be true only if the cell death is rapidly followed by tissue dehydration.

Senescence

Senescence in plants can refer to at least two distinct processes: the aging of various tissues and organs as the whole plant matures (best studied in leaves, petals and fruit) and the process of whole plant death that sometimes occurs after fertilization (called monocarpic senescence). The process of fruit aging or ripening has been extensively studied and has recently been reviewed (26–28). Senescence requires nuclear functions, suggesting that it is an active process (29–31). This makes sense for vegetative tissue, since plants must redistribute their nutrients as they develop, and an orderly turnover of macromolecules would facilitate an efficient use of resources. Since the elucidation of the genetic control of senescence in leaves has proved difficult (R. Amasino, personal communication), several groups have resorted to molecular approaches to identifying genes likely to be involved in senescence control. Several genes (termed *SAG* for senescence-associated genes) which show sequence similarity to cysteine proteases are induced early during senescence (32, 33). Because PCD during *Caenorhabditis elegans* development requires a cysteine protease [the product of the *ced-3* gene (34)], these plant proteases are good candidates for cell death initiation genes. It is not yet known whether the *SAG* genes are causally linked to senescence initiation and/or to macromolecular turnover. It has also been suggested that RNase (35) and lipoxygenase (36) activities might be involved in senescence control, since the activity of these enzymes increases during senescence, but again, no causal link between these activities and senescence has yet been established.

While it has been difficult to determine what molecular events are necessary for the control of senescence in leaves, Grbić and Bleecker (37) have recently shown that the hormone ethylene is a modulator of the senescence syndrome. Thus, mutants of *Arabidopsis* that are blocked in the perception of ethylene have greater leaf longevity probably because there is a delay in the onset of induction of the expression of *SAG* genes. A role for ethylene in modulating the rate of fruit ripening in tomato has also been suggested (38, 39).

Pathogenesis

Plants can recognize certain pathogens and activate defenses (called the resistance response) that result in the limitation of pathogen growth at the site of infection. One dramatic hallmark of the resistance response is the induction of a localized cell death response (the hypersensitive response or HR) at the site of the infection. The HR is likely to be important for limiting a pathogen's nutrient supply, since the dying tissue rapidly becomes dehydrated. The HR appears to be a form of PCD in plants. Firstly, the appearance of the HR is genetically controlled (see below) and second, purified HR-inducing factors from bacteria called harpins will not induce the HR unless the plant tissue is transcriptionally active (40). In addition, HR-inducing bacteria will not cause the HR if protein synthesis is blocked in the plant (41). In the search for a signal for HR induction, several groups have determined that H_2O_2 is rapidly produced by plant cells in culture during the HR in a phenomenon termed the oxidative burst [reviewed by Medhy (42)]. Levine *et al.* (43) showed that enhancing H_2O_2 production during the HR led to dramatic increases in the amount of cell death observed in a soybean cell culture system. The effects on cell death after trying to block H_2O_2 production were more modest. This observation in combination with the fact that bacterial mutants that fail to induce cell death in tobacco suspension cells yet still induce the oxidative burst has led to the suggestion that H_2O_2 is not sufficient to trigger the HR but may act in conjuction with other factors to activate cell death (44).

The induction of the HR by some pathogens and elicitors (molecules secreted by pathogens) may be mechanistically similar to apoptosis in animals, since apoptotic features such as DNA breaks with 3'OH ends, blebbing of the plasma membrane as well as nuclear and cytoplasmic condensation are present in some cells undergoing the HR (7–10). In some cases the HR is also accompanied by internucleosomic DNA cleavage, another apoptosis-associated event (8, 10). The HR is also correlated with the activation of K^+/H^+ exchange across the plasma membrane of plant cells in culture, an event which might lead to cell death and/or defense signaling (see, for example, refs. 45–47). Introduction of a gene which encodes a bacterial proton pump into tobacco plants causes the plants to undergo an apparent HR (9). If the bacterial protein really is functioning to translocate protons across the plasma membrane of plants, this suggests that the protein causes the HR by mimicking the K^+/H^+ exchange that occurs during the HR. This would provide the first compelling evidence that the exchange of ions is causally related to HR control.

The genetic control of the HR is beginning to be elucidated. Mutants of *Arabidopsis* called *acd2* (accelerated cell death 2) and *lsd* (lesions simulating disease) activate the HR and multiple defenses in the absence of any pathogen (48, 49). Since some of these mutations are recessive, one model is that the *ACD2* and some *LSD* genes negatively regulate the HR and multiple defense functions. The isolation and characterization of the *ACD2* and *LSD* genes are likely to lead to an understanding of how the HR is regulated. The existence of multiple genes that influence HR regulation brings up the question of whether the HR is controlled in one pathway or several pathways in response to different pathogens. It seems likely

that the mechanism of HR cell death can vary depending on the host and pathogen combination since some types of plant cells undergoing an HR do not show morphological features characteristic of apoptosis (50).

Many plant–pathogen interactions can lead to plant cell death that appears to be distinct from the HR either because it is not associated with resistance or it occurs late after infection and is not accompanied by tissue dehydration. It is not known if cell death that occurs in such susceptible interactions generally occurs by a programmed process. However, the existence of *maize* mutants (called Les) that resemble different diseases (51–53) and the recent discovery of *Arabidopsis* mutants that mimic specific diseases such as bacterial leaf spot and soft rot (unpublished observations) suggests that cell death associated with various diseases might be genetically programmed processes. It is not known whether any of the genes identified in *Arabidopsis* or *maize* act in the same pathway to control cell death. As these *Arabidopsis* and *maize* mutants become better characterized and some of the genes are cloned, it should become clearer how many pathogen-triggered cell death pathways there are. Interestingly, it has also been shown that certain fungal toxins can induce apoptosis in both animal and plant cells, although the gene products that control the cell death process in toxin-susceptible plants have not been identified (8, 54).

PCD: A Ubiquitous Process in Plants?

In addition to the examples given above, cell death also occurs predictably at specific sites and times throughout the life history of flowering plants. Even in the embryo, cells of the suspensor, the embryonic organ that attaches the embryo proper to the maternal tissues and supplies it with nutrients, die before embryo maturity (55). The surfaces of many plants are covered with a thick layer of dead unicellular hairs. These act to shield the photosynthetic apparatus from the damaging effects of high irradiance in certain environments, and provide a humidity trapping zone to reduce water loss in other cases. Root cap cells show evidence of endonuclease-generated DNA strand breaks, suggesting they die in a programmed process (8). Although morphogenesis in plants typically occurs exclusively by differential cell and tissue growth, there are a few plants in which cell death plays a role in the generation of leaf shape. In the genus *Monstera* patches of cells die at early stages of development of each leaf blade, generating holes or slits and resulting in leaves which at maturity contain a series of perforations or marginal lobes (56). The lobed leaves of *Monstera* contrast with the usual way in which lobed or compound leaves develop by differential growth at the leaf margins, and with the pseudocompound leaves of palms in which regular lines of cell separation result in the fragmentation of the previously entire leaf blade (56).

Cell death also occurs predictably during reproductive development in plants. In the tapetum, the cell layer that surrounds the developing pollen grains in the anther, cells undergo a precise program of breakdown that results in release of their contents which serve as nutrients for the pollen. Anther dehiscence which releases the mature pollen to the environment also results from the death of cells that occupy specific sites in the anther wall (57). In each ovule a single meiotic event results in four haploid cells, three of which degenerate, leaving the remaining one to produce the egg and associated cells of the embryo sac. Bell (58) has argued compellingly that this degeneration of megaspores occurs by apoptosis in seed plants. Cell death in reproductive structures may encompass whole organs, not just specific cell types (see above). In many unisexual flowers, both male and female organs are initiated and the inappropriate organs abort during flower development.

Following reproduction some plants undergo organismal senescence and death, but death of individual organs is a much more common phenomenon, the most obvious being the senescence and death of all mature leaves in deciduous perennial plants that are eliminated by formation of a zone of separation near the leaf base at which the abscission occurs (59).

In each of these examples, tissues or organs die at predictable times and places during development. However, whether these examples conform to the definition of PCD is not known for most of them. Indeed, while in many cases cytological details may be scanty or unknown and molecular information is absent, they are mentioned here to stimulate future inquiry into the mechanism and function of the observed cell death.

Concluding Remarks

The process of PCD is essential for ensuring the proper development of plants as well as ensuring a robust defense response against invading pathogens. Whether there are global mechanisms of PCD control and execution that are used by plants in all of the examples illustrated here remains an open question. It is intriguing that toxin-induced cell death, xylogenesis and some examples of the HR might occur by a mechanism that shares some common features with apoptosis of animal cells (7–10). If the execution of the HR uses many steps in common with apoptosis, it will be interesting to determine whether PCD arose independently for the plant and animal kingdoms, or if there is a common ancestor from which the process of PCD derived.

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