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Programmed Cell Death in Plants

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INTRODUCTION

Programmed cell death (PCD) is a physiological cell death process involved in the selective elimination of unwanted cells (Ellis et al., 1991). In animals, these unwanted cells include those that have served temporary functions, such as tadpole tail cells at metamorphosis; cells that are overproduced, such as vertebrate neurons; cells that are unwanted or present in inappropriate positions, such as cells between the developing digits and Müllerian duct cells required in females but not males; and cells that die during the process of cell specialization, such as keratinocytes at the surface of the skin (Jacobsen et al., 1997). PCD in specific cell types can also give rise to disease. These cell types include helper T cells, which undergo PCD in AIDS, and selected brain neurons, which die during Alzheimer's disease, Huntington's disease, Parkinson's disease, and Lou Gehrig's disease (Duke et al., 1996). Thus, PCD plays an important role in cell and tissue homeostasis and specialization, tissue sculpting, and disease.

PCD in *Caenorhabditis elegans* and other animals depends on the induction and action of specific genes that bring about the controlled disassembly of a cell (Wadewitz and Lockshin, 1988; Ellis et al., 1991). This disassembly involves the condensation, shrinkage, and fragmentation of the cytoplasm and nucleus and the fragmentation of the nuclear DNA into ~50-kb and, in some cases, ~0.14-kb pieces that represent oligonucleosomes (Cohen, 1993; Eastman et al., 1994). These DNA fragments can be detected by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) of DNA 3′-OH groups in sections of cells (Gavrieli et al., 1992). When PCD features marginalization of the chromatin in the nucleus, breakage of the cytoplasm and nucleus into small, sealed packets, and processing and fragmentation of the DNA at nucleosome linker sites, the process is termed apoptosis (Cohen, 1993). However, not all PCD involves all of these changes (Schwartz et al., 1993).

Many of the proteins responsible for PCD in animal cells have now been identified. Proteases of the interleukin-1β–converting enzyme (ICE) family bring about cell condensation and shrinkage (Martin and Green, 1995), and endonucleases cause the fragmentation of nuclear DNA (Eastman et al., 1994). The basic leucine zipper transcription factor *ces-2* (for cell death specification-2) is necessary for PCD in the sister cells of serotoninergic neurosecretory neurons in *C. elegans* (Metzstein et al., 1996), and oncoproteins in the Bcl-2 family regulate the onset of PCD in many animal cells (Farrow and Brown, 1996). Growth factor deprivation (Duke and Cohen, 1986; Raff, 1992) and reactive oxygen species (ROS) including O_2^- and H_2O_2 (Pierce et al., 1991; Jacobsen, 1996) can trigger signal pathways leading to PCD. However, PCD triggered by growth factor withdrawal can occur in near-anaerobic conditions, and the cell death program itself can operate in the absence of O_2 (Shimizu et al., 1995). Ca^{2+} and changes in protein phosphorylation also participate in PCD signaling pathways (Stewart, 1994; McConkey and Orrenius, 1995). One role of Ca^{2+} is in the activation of PCD endonucleases (Eastman et al., 1994).

Cells that are damaged and unable to function correctly can also undergo PCD. This removes potentially harmful cells and prevents them from multiplying and spreading (Jacobsen et al., 1997). However, not all damaged cells undergo PCD; cells that are damaged by stresses at overwhelming levels undergo necrosis instead (Lennon et al., 1991). Necrosis is a nonphysiological process involving cell swelling, lysis, and the inflammatory leakage of cell contents (Cohen, 1993). Moreover, whereas PCD occurs during development and is regulated by a complex mechanism, necrosis is not normally associated with development, does not require the activity of proteases and nucleases dedicated to controlled cell disassembly, does not operate through gene-dependent signal transduction pathways, and does not require Ca^{2+} or changes in protein phosphorylation.

In plants, selective cell death is necessary for growth and survival and can occur on a local or large scale (Barlow, 1982). As shown in Figure 1, cell death causes the deletion of the suspensor and aleurone cells, which serve temporary functions during development (Yeung and Meinke, 1993), and can also eliminate stamen primordia cells in female flowers of unisexual species (Dellaporta and Calderon-Urrea, 1994). Cell death may also cause the formation of certain leaf lobes and perforations (Greenberg, 1996), and cell death in xylem tracheary elements (TEs) and in some kinds of trichomes is essential for the specialization of these cells (Figure 1; Mittler and Lam, 1995a; Greenberg, 1996; see also Fukuda, 1997, in this issue). Senescence involves cell death on a large scale

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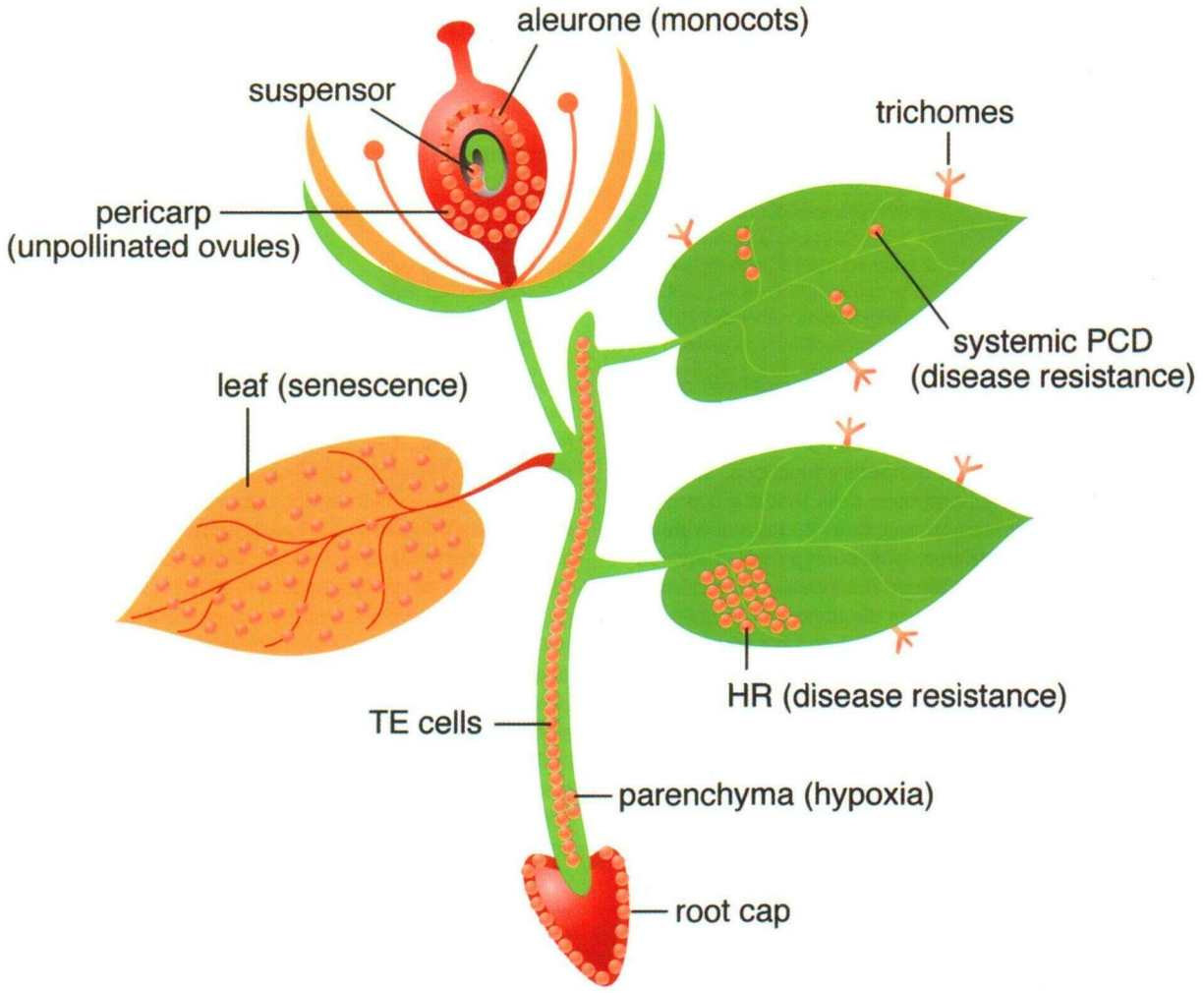

Figure 1. Sites of PCD in a Vascular Plant.

The orange spheres represent internal dead cells, and the branched structures on the leaves represent trichomes.

(Figure 1; Noodén, 1988; see also Bleecker and Patterson, 1997, in this issue). During interactions with the environment, cell death occurs in the so-called hypersensitive response (HR) to pathogen attack (Figure 1; Lamb and Dixon, 1997).

There is clear evidence that cell death during plant development and interactions with the environment involves PCD. Table 1 shows some of this evidence, whereas Table 2 shows the cells and tissues in which PCD in plants is thought to occur. In this review, we discuss the evidence for PCD during plant vegetative development. PCD is also likely to occur during the reproductive phase of plant development, for example, in the deletion of nonfunctional megaspores and other cells in the germ cell lines (Bell, 1996a). Although the relationship between the mechanisms underlying PCD in animals and plants is not known, we also consider the possible evolutionary origins of PCD in plants.

DEVELOPMENT

Aleurone Cells

In seeds of monocots, aleurone cells form a secretory tissue that releases hydrolases to digest the endosperm and nourish the embryo. Aleurone cells are unnecessary for postembryonic development and die as soon as germination is complete (Kuo et al., 1996). Several lines of physiological evidence

suggest that their death involves PCD. For example, in the aleurone, secretory processes and cell death are stimulated by gibberellin (GA), whereas abscisic acid (ABA) blocks the effects of GA and retards seed germination and cell death (Jones and Jacobsen, 1991). Moreover, an elevation in cytosolic Ca^{2+} occurs in aleurone cells treated with GA (Bush et al., 1989; Gilroy and Jones, 1992), suggesting that a signal transduction pathway controls secretion and cell death. This contention is supported by the finding that the protein phosphatase inhibitor okadaic acid also inhibits secretion and cell death in the aleurone (Kuo et al., 1996; M. Wang et al., 1996).

Morphological observations of dead and dying aleurone cells show that the cytoplasm and nuclei condense and shrink (Bush et al., 1989; Kuo et al., 1996; M. Wang et al., 1996), and electrophoresis of DNA from the dying aleurone of barley reveals oligonucleosome-sized DNA fragments, which are characteristic of apoptotic cell death (M. Wang et al., 1996). These data are consistent with TUNEL experiments showing that DNA 3′-OH groups accumulate in the nuclei of dying aleurone cells (M. Wang et al., 1996).

The apparent involvement of a specific signal pathway, the ensuing cell shrinkage, and the DNA processing and appearance of oligonucleosome-sized DNA fragments show that PCD occurs in aleurone tissues that are no longer necessary for embryo nourishment. The aleurone is eventually compressed between other ovule tissues, and the cell collapse that occurs during PCD may facilitate this compression.

Table 1. Comparison of PCD in Plants and Animals

Animals	Plants
Similarities	
Program can require O_2 (Shimizu et al., 1995)	HR requires O_2 (Mittler et al., 1996)
H_2O_2 can activate (Pierce et al., 1991)	H_2O_2 can activate (Levine et al., 1996)
Growth factor deprivation activates; autocrine signals suppress (Raff, 1992)	Growth factor deprivation activates (Kao and Michayluk, 1975); autocrine signals suppress (Jones et al., 1960; Stuart and Street, 1971; Schröder and Knoop, 1995)
Ca^{2+} can activate (McConkey and Orrenius, 1995)	Ca^{2+} can activate (Levine et al., 1996)
Can require transcription (Stewart, 1994)	Can require transcription (Thomas et al., 1992)
Can require dephosphorylation (Stewart, 1994)	Can require dephosphorylation (Kuo et al., 1996; M. Wang et al., 1996)
Cysteine proteases activated (Martin and Green, 1995)	Cysteine proteases expressed (Hensel et al., 1993; Minami and Fukuda, 1995)
DNA is processed (Eastman et al., 1994)	DNA is processed (H. Wang et al., 1996; M. Wang et al., 1996; McCabe et al., 1997; Orzáez and Granell, 1997)
Cells condense and shrink (Cohen, 1993)	Cells condense and shrink (Lai and Srivastava, 1976; Levine et al., 1996; McCabe and Pennell, 1996; H. Wang et al., 1996; McCabe et al., 1997)
Differences	
Bcl-xL suppresses in some cells (Ellis et al., 1991)	Bcl-xL does not suppress HR (Mittler et al., 1996)
Cell corpses engulfed (Cohen, 1993)	Cell corpses not engulfed (Levine et al., 1996)

Root Cap Cells

A cap of cells protects the root apical meristem during seed germination and seedling growth. Root cap cells are formed by initial cells in the meristem and are continually displaced to the root periphery by new cells (see Laux and Jürgens, 1997; Schiefelbein et al., 1997, in this issue). After several days, the peripheral cells die (Harkes, 1973). Cell death occurs in root caps when roots are grown in water, showing that cell death is a normal part of development and not a consequence of abrasion during soil penetration. Dying root cap cells shrink and adopt irregular profiles, and DNA staining shows that the nuclei in dying onion root cap cells become condensed (H. Wang et al., 1996). Moreover, because TUNEL experiments reveal the accumulation of 3′-OH groups in the DNA (H. Wang et al., 1996), these observations suggest that root cap cells die by PCD. Because root cap cells are exposed at the root surface, it would be relatively easy to apply inhibitors of PCD signal intermediates to developing root caps. Although changes in cell morphology suggest that PCD is an integral part of root cap development, it is important to confirm this biochemically.

TE Cells

Vascular plants transport water in columns of specialized dead cells termed TEs. Differentiation of TEs involves cell elongation, the deposition of cell wall components, including lignin, and autolysis (see Fukuda, 1997, in this issue). Autolysis begins as the cytoplasm and nuclei become lobed, condensed, and shrunken and ends as the cytoplasm breaks into small packets (Wodzicki and Humphreys, 1973, 1974; Lai and Srivastava, 1976). *Zinnia elegans* mesophyll cells can be cultured and induced to redifferentiate into TEs so that the cell death process can be studied biochemically as well as morphologically (Fukuda, 1994, 1997).

Treatment of differentiating *Zinnia* cells with actinomycin D or cycloheximide blocks cell death (Fukuda and Komamine, 1983). This suggests that cell death in TEs requires protein synthesis, and it is possible that among the proteins synthesized are the effector proteases and nucleases necessary for

Table 2. Evidence for PCD in Plant Cells and Tissues

Evidence	Cell Death	References
Requires gene expression	TE maturation; organ senescence	Fukuda and Komamine (1983); Thomas et al. (1992)
Suppressed by genetic interactions	Lesion mimic mutants	Walbot et al. (1983); Dangl et al. (1996)
Suppressed by autocrine signals	After cell dilution	Jones et al. (1960); Stuart and Street (1971); Schröder and Knoop (1995)
Requires Ca^{2+}	Aleurone deletion; HR	Bush et al. (1989); Levine et al. (1996)
Requires dephosphorylation	Aleurone deletion; hypoxia	He et al. (1996); Kuo et al. (1996); M. Wang et al. (1996)

cell disassembly. Indeed, one of the induced proteins is a cysteine protease (Demura and Fukuda, 1994; Minami and Fukuda, 1995) that could perform a function analagous to that of one of the ICE family proteases. However, it is also possible that the *Zinnia* cysteine protease is involved in the clearance of cytoplasm from TE cells that are already dead. A single-stranded DNA nuclease is also induced during xylogenesis in *Zinnia* (Thelen and Northcote, 1989). This nuclease is activated by Ca^{2+} (Thelen and Northcote, 1989) and is likely to be responsible for the observed fragmentation of DNA and accumulation of DNA 3′-OH groups (Mittler and Lam, 1995a; H. Wang et al., 1996), functions that have been ascribed to PCD-associated nucleases in animal cells (Gaido and Cidlowski, 1991). Alternatively, the *Zinnia* nuclease could function in the clearance of DNA from TEs that are already dead. To discriminate between these possibilities, it is necessary first to determine whether nuclease-mediated processing of DNA into oligonucleosome-sized pieces occurs during TE differentiation and then to link the single-stranded *Zinnia* nuclease to any such processing.

Somatic Embryogenesis

Cultured cells of some plant species can be induced to develop into somatic embryos. In embryogenic suspension cultures, totipotent cells divide asymmetrically into cell pairs, one member of which stops synthesizing DNA and dies, whereas the other member goes on to establish an embryo (Nomura and Komamine, 1985, 1986). Figure 2 shows electron microscope images of cells from an embryogenic suspension culture of carrot. Relative to the cytoplasm of living cells (Figure 2A), the cytoplasm in the dead cells is condensed and shrunken (Figure 2B). In some dead cells, the cytoplasm is broken into small, membrane-sealed packets, which suggests that the cells have undergone a form of PCD similar to apoptosis (Havel and Durzan, 1996; McCabe and Pennell, 1996). This contention is supported by the accumulation of 3′-OH groups in the DNA of these cells (McCabe et al., 1997).

The walls of dead and dying cells in carrot embryogenic suspension cultures can be labeled with monoclonal antibodies (Pennell et al., 1992; P.F. McCabe and R.I. Pennell, unpublished observations), and one such antibody has been used to separate the dead and dying cells from the rest of the cells in the culture (McCabe and Pennell, 1996; McCabe et al., 1997). DNA extracted from these cells contains fragments of ~0.14 kb. These fragments, which are not present in the DNA from living cells, may be the end products of DNA processing (McCabe et al., 1997). The condensation and shrinkage of the cytoplasm, the TUNEL reactions, and the small DNA fragments imply that these cells in embryogenic suspension cultures die by PCD rather than by necrosis. This conclusion has been reinforced by similar analyses of carrot cells that have been killed outright by incubation at very high temperatures (McCabe et al., 1997). Death of these cells involves swelling, lysis, and rapid DNA breakdown but without the formation of discrete-sized fragments (McCabe et al., 1997).

It is possible that many cells in suspension cultures undergo PCD because they have no function. Some of the cells that die may be the equivalent of the basal cells that develop into suspensors during zygotic embryogenesis, and they may die because they have no function in suspension cultures. However, cell death is a regular feature of the tissues

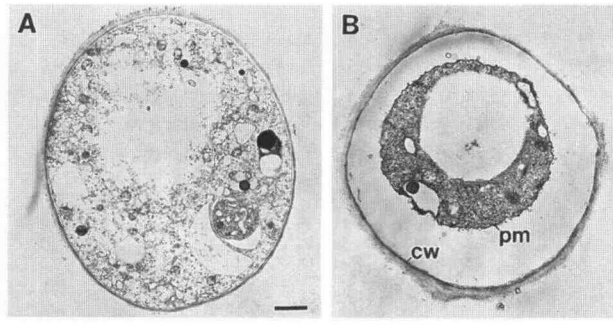

Figure 2. PCD in an Embryogenic Suspension Culture of Carrot.

(A) Electron micrograph of a living cell.

(B) Electron micrograph of a dead cell. In the dead cell, the plasma membrane (pm) is separated from the cell wall (cw), and the protoplast is shrunken and condensed.

Bar = 2.5 μm.

from which apomictic embryos originate (Haberlandt, 1922); therefore, it is also possible that PCD plays an active role in plant embryogenesis. For example, dying cells may somehow signal other cells to develop into embryos (Bell, 1996b).

Senescence

Senescence is the final phase of plant vegetative and reproductive development, preceding the widespread death of cells and organs. Senescence involves the active turnover and recapture of cellular material for use in other organs (Noodén, 1988; see also Bleecker and Patterson, 1997, in this issue). Membrane integrity and cellular compartmentalization are maintained until late into the senescence process, suggesting that there is little or no leakage of cellular contents (Noodén, 1988).

Senescence, which can be induced by ethylene (Grbić and Bleeker, 1995), requires nuclear functions (Thomas et al., 1992) and involves an increase in the generation of O_2^- and H_2O_2 (Pastori and del Rio, 1997). Also, natural senescence can be blocked by mutations in the ethylene-responsive (*ETR*) gene, by ethylene anatgonists, and by cytokinins (Bleecker et al., 1988). These observations suggest that senescence and cell death during senescence are under the control of a coordinated signaling pathway, consistent with the view that senescence involves PCD (Gan and Amasino, 1997).

In Arabidopsis, ethylene stimulates the expression of a battery of senescence-associated genes (*SAG*s; Thomas et al., 1992; Lohman et al., 1994), and nucleotide sequence comparisons suggest that two of them, *SAG2* and *SAG12*, encode cysteine proteases (Hensel et al., 1993; Lohman et al., 1994). Similarly, nucleotide sequence comparisons suggest that the tomato senescence upregulated genes *SENU2* and *SENU3* encode cysteine proteases (Drake et al., 1996). One possibility is that these plant proteases could function in PCD, somewhat like ICE family proteases, although it is also possible that they could be involved with the mobilization of cellular material. Nucleases also accumulate during senescence, and TUNEL experiments have shown that DNA in the pericarp of senescing rye seeds becomes fragmented (Cheah and Osborne, 1978). Electrophoresis of DNA from senescing pea carpels has also shown that seed DNA is processed into the oligonucleosome-sized pieces that are characteristic of PCD (Orzáez and Granell, 1997), which provides clear evidence that senescence involves PCD. It will be interesting to determine whether there is cell condensation and collapse during senescence in seeds and whether there is evidence from TUNEL, electrophoresis of DNA, and cell morphology to show that cell death that occurs on a very large scale in senescing leaves is PCD.

By confining the contents of dying and dead cells, PCD may help to prevent the infection of a senescing organ. Thus, PCD during senescence may help to prevent the spread of disease to living parts of the plant. Such control would not be possible during necrotic cell death, which involves the leakage of cell contents.

INTERACTIONS WITH THE ENVIRONMENT

Hypoxia

Cell death can occur in the cortex of the root and stem base in response to waterlogging and hypoxia. The aerated tissue so formed is termed aerenchyma, and the internal air spaces generated by cell death facilitate more efficient transfer of O_2 from aerial organs to waterlogged stem bases and roots (Armstrong, 1979).

Hypoxic conditions in maize trigger cell death and aerenchyma formation. Ethylene, which is generated in response to O_2 deprivation, is the trigger for a signal transduction pathway that leads to aerenchyma formation (Drew et al., 1981; Jackson et al., 1985). That this pathway involves Ca^{2+} and protein phosphorylation is suggested by data showing that the Ca^{2+} channel blocker thapsicargin and the protein phosphatase inhibitor okadaic acid promote cell death in maize roots at normal O_2 levels, whereas the Ca^{2+} chelator EGTA and the protein kinase inhibitor K252a prevent cell death in hypoxic roots (He et al., 1996). The existence of a signal transduction pathway suggests that cell death during hypoxia is PCD. It will be interesting to see whether aerenchyma formation also involves the cell condensation and shrinkage and DNA fragmentation that are characteristic of PCD.

Plant–Pathogen Interactions

Hypersensitive cell death occurs at the site of attempted attack by an avirulent pathogen, and the HR leads to the formation of a dry lesion that is clearly delimited from surrounding healthy tissue (Dangl et al., 1996). Attacks by virulent pathogens, which do not trigger the HR, lead instead to disease. Staining for dead cells shows that many of the cells that die during the HR lie close to the veins (Hammond-Kosack et al., 1996; M.E. Alvarez, R.I. Pennell, P.-J. Meijer, R.A. Dixon, and C. Lamb, unpublished observations). These observations suggest that the bundle sheath cells surrounding veins may be more susceptible to death-inducing signals than are mesophyll cells (see below). The localized deaths of bundle sheath cells may prevent pathogens from gaining entry to the vascular system and spreading systemically (Carrington et al., 1996).

HR cell death is an active process in which the accumulation of O_2^- and H_2O_2 leads to an elevation in cytosolic Ca^{2+} and triggers a protein kinase–mediated cell death process that is similar physiologically to PCD (Mehdy, 1994; Levine et al., 1996). Certain mutations in maize and Arabidopsis cause the spontaneous appearance of lesions resembling HR lesions (Walbot et al., 1983; Lamb and Dixon, 1997),

suggesting that the HR cell death itself is under genetic control. Some of these lesion mimic mutants may be compromised in essential cell functions that, when defective, trip the PCD program in specific cells. Further study of these lesion mimic mutants may therefore provide insight into the general mechanism of PCD in plants. By contrast, the *Rpm1* and *mlo* mutations of maize and barley, respectively, occur in disease resistance genes. Thus, further study of *Rpm1* and *mlo* may provide insight into the PCD signal pathway that is triggered specifically in the HR (Lamb and Dixon, 1997).

As shown in Figure 3, cells that die during the HR in Arabidopsis condense and shrink and ultimately look much like apoptotic cell corpses (Levine et al., 1996). A tobacco nuclease, NUC3, which is activated during the HR and is likely to participate in the fragmentation of DNA, has been isolated and characterized (Mittler and Lam, 1995b). Although NUC3 does not generate DNA fragments of discrete size (Mittler and Lam, 1995b), possibly related nucleases generate the ~50-kb and oligonucleosome-sized pieces of DNA that can appear during the HR in soybean and cowpea (Levine et al., 1996; Ryerson and Heath, 1996; H. Wang et al., 1996) as well as in tomato plants inoculated with host-selective *Alternaria alternata* phytotoxins (H. Wang et al., 1996).

Localized inoculation of Arabidopsis with an avirulent strain of *Pseudomonas syringae* leads to an HR and also triggers low-frequency cell death in uninoculated leaves (M.E. Alvarez, R.I. Pennell, P.-J. Meijer, and C. Lamb, unpublished observations). This cell death in uninoculated leaves begins at approximately the same time as the cell death in the HR and also occurs close to the veins. Cell morphology studies and TUNEL experiments suggest that the cell death in uninoculated leaves is also PCD. Similarly, low-frequency excision of a transposable element from the *Cf-9* disease resistance gene in tomato plants that are heterozygous for *Cf-9* and also express the corresponding *Cladosporium fulvum* avirulence gene *Avr9* results in low-frequency cell death that is associated with the appearance of "genetically acquired" resistance (Jones et al., 1994; Dangl et al., 1996). Thus, genetically acquired resistance may mimic the PCD that occurs in uninoculated leaves as an HR develops. The possibility that PCD occurs in uninfected leaves in many plants to contribute to systemic disease resistance would represent a novel biological function for PCD.

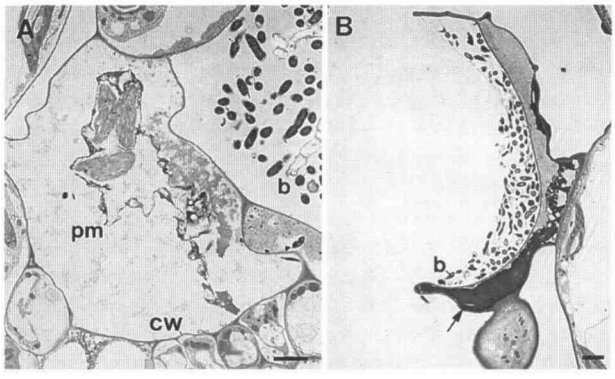

Figure 3. PCD during an HR.

Electron micrographs of Arabidopsis leaves inoculated with an avirulent strain of *Pseudomonas syringae*.

(A) Dead cell 60 hr after inoculation. The plasma membrane (pm) is separated from the cell wall (cw) and the protoplast is shrunken and condensed, but the organelles in the cytoplasm are intact.

(B) Dead cell 72 hr after inoculation. The arrow in **(B)** points to a small, highly condensed cell corpse. b, bacterium.

Bars in **(A)** and **(B)** = 2.5 μm.

CONTROL OF PCD IN PLANTS

Although there is clear evidence that PCD occurs during plant development and environmental responses, the signals that trigger PCD in plants are unknown. However, H_2O_2 accumulates in lettuce leaf TEs (Bestwick et al., 1997), and O_2^- and H_2O_2 accumulate in senescing pea leaves (Pastori and del Rio, 1997). Thus, it is possible that ROS are a general trigger for PCD in plants. Among the genes whose expression is upregulated when mesophyll cells of *Zinnia* are induced to differentiate into TEs is *TED2*, which encodes an NADPH oxidoreductase (Demura and Fukuda, 1994). Expression of a single gene is thought to be sufficient to trigger PCD in the megaspores of the fern *Marsilea* (Bell, 1996a), and it is possible that the product of such a gene could generate enough H_2O_2 to trigger PCD in several different kinds of plant cells (Demura and Fukuda, 1994). During the HR, a rapid oxidative burst also leads to the accumulation of O_2^- and H_2O_2 (Mehdy, 1994; Lamb and Dixon, 1997), and near-anaerobic conditions inhibit hypersensitive cell death in tobacco (Mittler et al., 1996). An NADPH oxidoreductase is also thought to generate O_2^- and H_2O_2 sufficient for PCD in the HR. This oxidoreductase seems to be activated after translocation of the putative regulatory subunits to the plasma membrane (Xing et al., 1997).

Antisense inactivation of 1-amino-cyclopropane-1-carboxylic acid oxidase, an ethylene biosynthetic enzyme, delays leaf senescence and cell death in tomato (Picton et al., 1993; John et al., 1995), and treatment of carpels with the ethylene antagonists silver thiosulfate or 2,5-norbornadiene also delays senescence and PCD in pea (Orzáez and Granell, 1997). Ethylene stimulates Ca^{2+} influx and alters protein phosphorylation patterns (Raz and Fluhr, 1992, 1993). Thus, ethylene-induced Ca^{2+} redistribution may be part of a general mechanism for the activation of PCD in plants. However, ethylene overproduction does not cause premature senescence (Guzmán and Ecker, 1990), and ethylene-insensitive Arabidopsis mutants and tobacco plants treated with

2,5-norbornadiene are able to condition a full defense response involving PCD (Bent et al., 1992; Silverman et al., 1993). Ethylene must therefore act in concert with other signal molecules to control PCD. Because GA can also stimulate PCD (Bush et al., 1989) and because many of the effects of ethylene and GA can be effectively countered by treatment with cytokinin or ABA (Gan and Amasino, 1996; Kuo et al., 1996; Orzáez and Granell, 1997), it is likely that other hormones are coregulators of PCD in plants.

It is not known how certain cells in plants die, whereas others, often very close to the dying cells, continue to live. One possibility is that the generation of O_2^-, H_2O_2, or other molecules that trigger PCD may be controlled at a supracellular level and accumulate only in cells that are fated to die. Another possibility is that the expression patterns of catalase and peroxidase genes, which can also be regulated at a supracellular level (Willekens et al., 1994; Cook et al., 1995; Peng et al., 1996), are controlled to degrade H_2O_2 and prevent PCD in cells that live. Indeed, human cells diluted into serum-free medium secrete catalases to degrade H_2O_2 and thereby prevent apoptosis (Sandstrom and Buttke, 1993).

A further possibility is that some plant cells are more prone to undergo PCD than others and that competence systems that predispose specific cells to undergo PCD operate in plants. For example, the constitutive expression of the avirulence gene *Avr9* in tomato plants carrying the corresponding *Cf-9* resistance gene does not cause widespread cell death until 13 days after germination (Hammond-Kosack et al., 1994). If *Cf-9* is in fact expressed during early seedling development, these results imply that cells in the *Avr9*-expressing seedlings do not become competent to undergo PCD for some time. Similarly, treatment of kangaroo berry cotyledons with 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine can cause cells around veins to differentiate into TEs and die several days before cells farther from veins do so (Gahan et al., 1994). This suggests that cotyledon periveinal cells, like bundle sheath cells in mature leaves during an HR (see above), are predisposed to undergo PCD. However, it is not known how such a predisposition to PCD may arise.

The survival of many animal cells depends on a supply of cell death–suppressing signals from other cells (Raff, 1992). Similarly, plant cells cultured below a critical cell density of $\sim 10^4$ cells mL^{-1} die (Kao and Michayluk, 1975; Street, 1977), and the cytoplasm and nuclei of such cells reveal many features characteristic of PCD (McCabe et al., 1997). Cell death in low-cell-density cultures of plant cells can be suppressed by nurse or feeder cells or by a cell-free, cell-conditioned growth medium (Muir et al., 1954; Jones et al., 1960; Stuart and Street, 1971; McCabe et al., 1997). These data suggest that plant cells deprived of death-suppressing signal molecules from other cells also undergo PCD.

Some progress toward the identification of these molecules has been made. For example, at concentrations of 10^{-8} to 10^{-9} M, a branched, ~1-kD oligosaccharide containing a backbone of three galacturonic acid residues and side chains of single glucose and fucose residues allows carrot and tobacco cells to survive at otherwise lethal dilutions (Schröder and Knoop, 1995). It is possible that the same or related oligosaccharides suppress PCD in whole plants. Competition for death-suppressing signal molecules may promote competition between cells and adjust cell numbers in a mechanism that is distinct from cell division. Operation of such a regulatory system could explain why some cells in apparently homogeneous cell populations suddenly die. Competition for death-suppressing signals could also lead to the selection of cells that are best able to compete for these factors (Raff, 1992). Because plant germ lines are determined from somatic cells late in development, genetic and epigenetic changes generated and selected for in the soma can be transmitted to the progeny (Walbot, 1985, 1996). Thus, the selection of cells in the soma could have heritable consequences for plant development.

Signals that activate cell death also occur in animals. For example, nerve growth factor triggers apoptosis in chick retina cells (Frade et al., 1996), and galectin-1, which is a β-galactoside binding protein, triggers apoptosis in activated T cells in humans (Perillo et al., 1995). Arabinogalactan proteins at the plasma membranes of plant cells also have lectin-like properties, and at least one such glycoprotein occurs specifically on dead and dying TEs in maize (Schindler et al., 1995). Because plant cells are thought to differentiate largely according to their positions (Irish, 1991; see also Clark, 1997; Kerstetter and Hake, 1997; Laux and Jürgens, 1997; Schiefelbein et al., 1997, in this issue), the induction of PCD by an endogenous lectin would represent a potential mechanism for activating PCD in a cell on the basis of its position.

EVOLUTION OF PCD

Processes that are similar to PCD have been described in prokaryotes and lower eukaryotes. For example, some strains of *Escherichia coli* harbor plasmid-encoded genes that trigger proteolysis and cell death after infection by bacteriophage (Yarmolinsky, 1995; Ameisen, 1996), and activation of the AMP pathway in nondifferentiating cells of *Dictyostelium discoides* induces a cell death process closely resembling PCD (Cornillon et al., 1994). Culture of several kinds of unicells at low cell density also induces a process resembling PCD (Welburn et al., 1996). *Dictyostelium*, plants, and animals are thought to have descended from a common unicellular eukaryote (Loomis and Smith, 1995), and because PCD is also thought to occur in the green alga *Volvox* and the fern *Marsilea* (Bell, 1996a), it is possible that the mechanisms underlying PCD are conserved throughout eukaryotes (Ameisen, 1996).

Molecular evidence for this has been presented in the form of plant and animal homologs of the yeast *Ost2* (for oligosaccharyltransferase-2) gene. A *C. elegans* homolog of

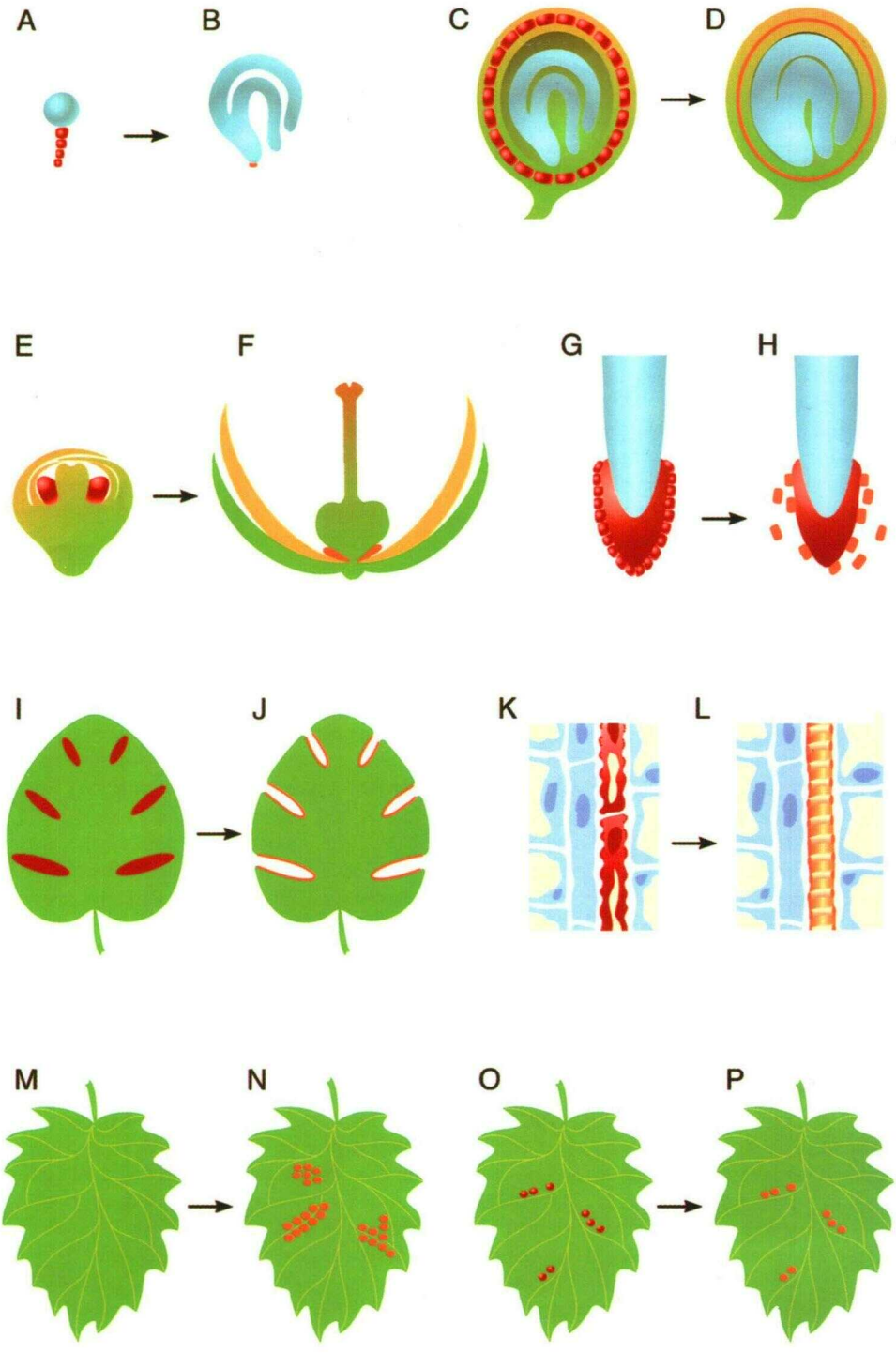

Figure 4. Functions of PCD in Plants.

(A) to **(D)** Deletion of cells with temporary functions. These include deletion of suspensor cells in embryos (**[A]** and **[B]**) and of aleurone cells in seeds (**[C]** and **[D]**).
(E) to **(H)** Deletion of unwanted cells. These include stamen primordia cells in unisexual flowers (**[E]** and **[F]**) and root cap cells (**[G]** and **[H]**).
(I) and **(J)** Deletion of cells during sculpting of the plant body **(I)** and deletion of leaf cells during leaf lobing **(J)**.
(K) and **(L)** Deletion of cells during cell specialization in TEs.
(M) to **(P)** Deletion of cells during plant interactions with pathogens. These include cells in an HR (**[M]** and **[N]**) and cells in uninfected leaves in response to HR-derived signals (**[O]** and **[P]**).
The red regions represent cells that have been targeted for PCD, and the orange regions represent cells that have died by PCD.

Ost2 can suppress PCD in a hamster cell line, and homologs in Arabidopsis and rice may also function as PCD suppressors (Sugimoto et al., 1995). However, because it is now known that the oligosaccharyltransferase encoded by *Ost2* is involved with the transfer of high-mannose oligosaccharides to endoplasmic reticulum proteins (Silberstein et al., 1995; Kelleher and Gilmore, 1997), it is unlikely that homologs of *Ost2* operate in a conserved PCD signal pathway.

Searches of expressed sequence tag databases and screening of cDNA and genomic libraries have not revealed evidence for any other plant homologs of genes known to participate in PCD. However, this could be because any homologous transcripts are low abundance or because they are not sufficiently conserved to be detected by sequence similarity. A further possibility is that the mechanisms of PCD are not conserved at the molecular level. In support of this contention, Bcl-xL blocks apoptosis in animals (Farrow and Brown, 1996), yet Mittler et al. (1996) have reported that human Bcl-xL does not block hypersensitive cell death in tobacco. However, Bcl-2 family proteins function as upstream regulators of PCD in animal cells and so are less likely to be conserved in plants. By contrast, ICE family proteases are an integral part of the cell death program itself, and in searching for details of the molecular mechanisms of PCD in plants, it may be more informative to study plants transgenic for a gene encoding an ICE family protease or to search for functionally equivalent plant proteases.

CONCLUSIONS

PCD is a cell suicide process involving cell condensation and shrinkage and ordered cell disassembly. Figure 4 summarizes what is known about the occurrence of PCD during plant development and interactions with the environment. PCD deletes cells that serve a temporary function, are unnecessary or unwanted, or give rise to specialized tissues. These include aleurone cells (Figures 4C and 4D), root cap cells (Figures 4G and 4H), and TEs (Figures 4K and 4L). During interactions with the environment, PCD deletes cells during hypoxia and after challenge with avirulent pathogens, both locally and systemically (Figures 4M to 4P). ROS such as H_2O_2 and phytohormones such as GA and ethylene can induce PCD in plants, whereas other phytohormones, including cytokinins and ABA, and signals from other cells can suppress it.

Some features of PCD in plants, including the changes in cell morphology and the processing of DNA into oligonucleosome-sized pieces, are similar to features characteristic of apoptosis in animals. That plant cells appear to default to PCD in the absence of PCD-suppressing signal molecules also suggests that the mechanisms controlling PCD in plants and animals may be similar. However, there is no molecular evidence for this, and oligosaccharides rather than proteins may be the PCD-suppressing signals in plants. Thus, it remains to be demonstrated conclusively that the mechanisms underlying PCD in plants and animals are conserved. If PCD did arise independently in plants, it would be necessary to identify, characterize, and manipulate the plant genes that control this process so that the role of PCD in plant development can be fully understood.

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