Developmental programmed cell death in plants

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Mechanisms of plant developmental programmed cell death (PCD) have been intensively studied in recent years. Most plant developmental PCD is triggered by plant hormones, and the 'death signal' may be transduced by hormonal signaling pathways. Although there are some fundamental differences in the regulation of developmental PCD in various eukaryotes of different kingdoms, hormonal control and death signal transduction via pleiotropic signaling pathways constitute a common framework. However, plants possess a unique process of PCD execution that depends on vacuolar lytic function. Comparisons of the developmental PCD mechanisms of plants and other organisms are providing important insights into the detailed characteristics of developmental PCD in plants.

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Abbreviations

ABA	abscisic acid
GA	gibberellic acid
JA	jasmonic acid
MAPK	mitogen-activated protein kinase
PCD	programmed cell death
ROS	reactive oxygen species
TE	tracheary element

Introduction

Programmed cell death (PCD) is indispensable for the development of most multicellular organisms [1]. Mechanisms for apoptosis occur extensively in metazoans and govern their developmental PCD [2]. The actions of most factors that regulate developmental PCD converge in the initiation of apoptosis in these organisms. Despite sustained efforts to understand this type of apoptotic machinery in plants, however, it has been difficult to determine whether it really exists. The simple application of analytical methods that are analogous to those used in studies of metazoan apoptosis have revealed little about PCD systems in plants.

On the other hand, recent studies of developmentally regulated PCD programs have revealed important insights into their regulatory mechanisms [3••–6••]. Here, we focus on the results of these studies, and those of several other well-studied plant, animal, and fungal systems, and discuss their similarities and differences to identify common mechanisms of developmental PCD.

Developmental PCD

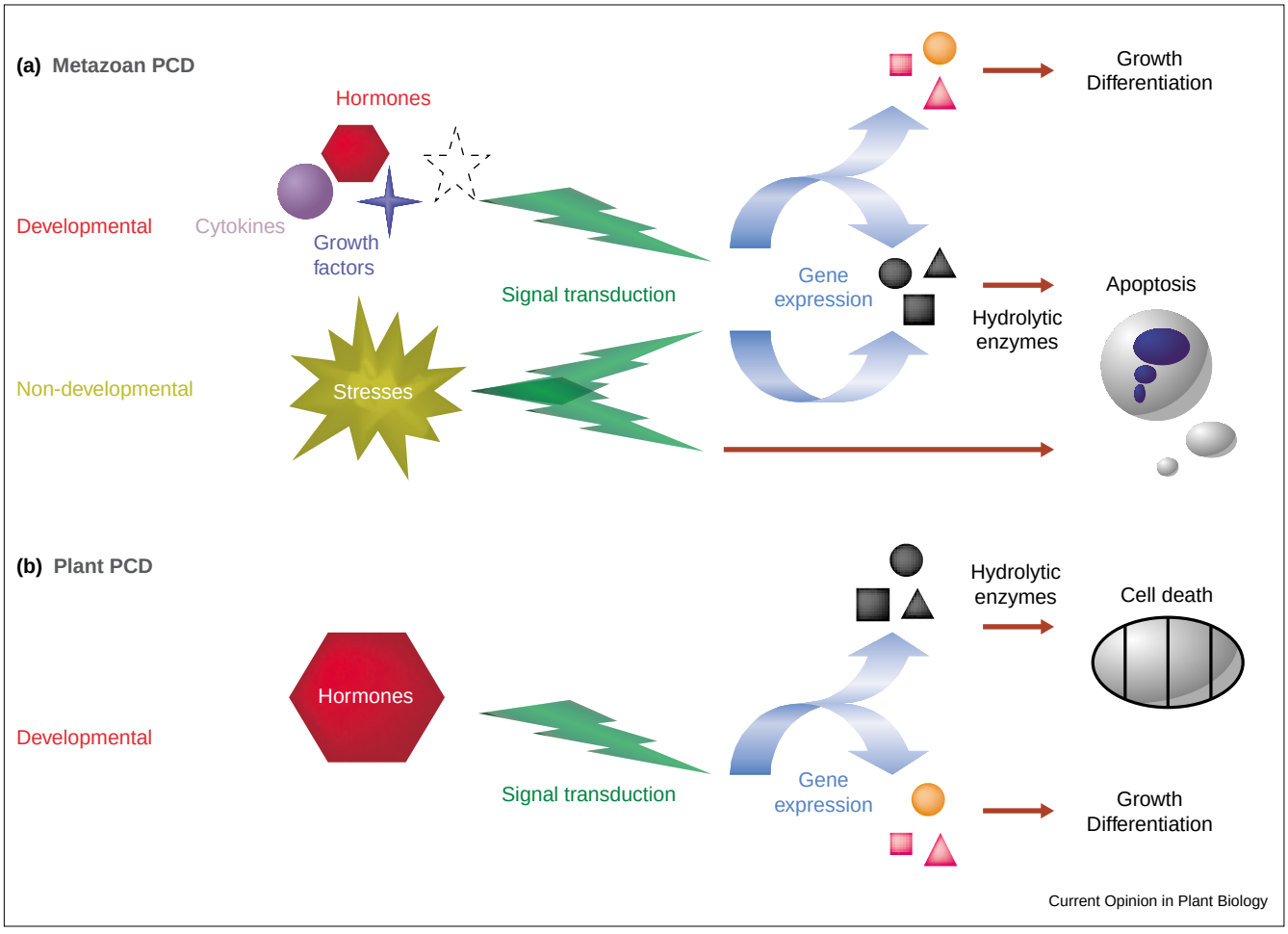
Plants employ PCD mechanisms for the expression of tissue and organ functions, and for efficient nutrition and reproduction [7]. Plant PCD occurs in senescent leaves and petals, in germinating seed tissues (such as aleurone layers and embryonic suspensors), in the xylem of vascular bundles, in the tissues of reproductive organs (such as stomium, tapeta, and ovaries), in the reproductive organ primordia of dioecious plants, in root caps, and in cortex that is forming aerenchyma [7]. The cells of these tissues or organs display characteristic features as their PCD progresses. The activation of cell-death-associated hydrolytic enzymes, protein degradation, and breakage of nuclear DNA strands are observed frequently [8,9••,10–12]. However, little was known about the regulatory mechanisms that control these events until recent progress was made in several areas.

Leaf senescence culminates in the death of component cells on a massive scale. Recent studies have identified several *Arabidopsis* mutants and transformants in which senescence processes are delayed [5••,13]. In particular, the *ore9* mutant has a marked delay in the progression of senescence, which results from defects in ethylene, abscisic acid (ABA), and methyl jasmonic acid (methyl JA) signaling. The gene *ORE9* encodes a protein that contains an F-box domain and leucine-rich repeats. The interaction of ORE9 with a Skp1, cullin and F-box (SCF)-complex protein is most likely involved in the transduction of a senescence signal via ubiquitin-mediated protein degradation [5••].

Following seed germination, cereal aleurone cells produce large amounts of α-amylases, which hydrolyze and mobilize starch to supply an energy source for embryos, and then undergo PCD. Using a model system of barley aleurone protoplasts, Jones and colleagues [8] revealed that this PCD occurs in a gibberellic acid (GA)-dependent manner. GA exerts its effect on the downregulation of expression levels and/or on the activities of enzymes that scavenge reactive oxygen species (ROS), thereby lowering the cellular capacity for metabolizing ROS [3••]. As a result, the protoplasts become unable to detoxify intrinsic ROS and die from ROS-induced injury to their structural components.

Vessels and tracheids, which are conductive tissues in vascular plants, are constructed from aligned dead tracheary elements (TEs). The PCD of TEs is tightly coupled with the formation of secondary walls. At present, no developmental factor is known that initiates only PCD. However, brassinosteroids are a factor in the initiation of both PCD and secondary-wall formation [9••,14,15]. It was found recently that the brassinosteroid biosynthetic pathway is activated before TE PCD, and that the synthesized brassinosteroids induce PCD and the formation of secondary walls [6••].

Figure 1



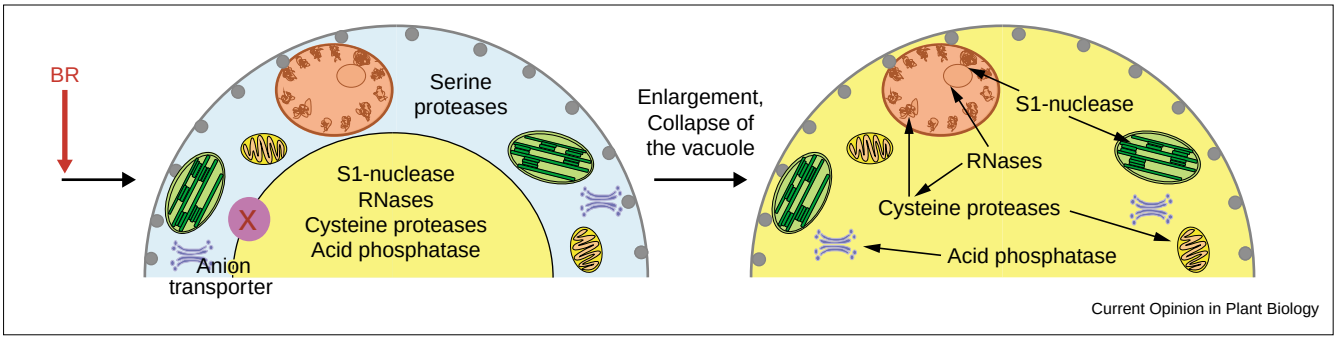
A schematic diagram illustrating metazoan and plant PCD mechanisms. (a) In metazoan developmental PCD, hormone, cytokine, and growth factor signals are transduced to invoke gene expression that results in the activation of apoptotic machinery. These signals can induce growth and differentiation under other conditions. By contrast, oxidative stresses or ionized radiation cause non-developmental apoptosis through either signal transduction and gene expression or signal transduction alone. (b) Plant cells undergo developmental PCD in response to hormone-regulated signaling and gene expression. Interestingly, these signals can also regulate growth and differentiation in certain specific cases.

Hormonal control of plant PCD is also evident during the ethylene-regulated formation of aerenchyma [16] and during JA-regulated stomium degeneration [17]. These examples imply that the transduction of PCD signals is mediated by hormone signaling pathways. Such pathways include changes in cellular Ca^{2+} concentrations and protein kinase activities [18], which are reported to function in several cases of developmental PCD [8,9••,16,19].

Apart from induction mechanisms, signaling in cell death that is related to the plant hypersensitive response (HR) involves the activation of calcium channel(s) and of receptor-like and mitogen-activated protein kinases (MAPKs), alteration of cellular ion content, and changes in the functions of small GTP-binding proteins [20,21••]. Although such evidence suggests that some homologous components work in signaling both developmental and pathogen-induced 'cell death', the available information is still fragmented, making it difficult to envision whole signaling mechanisms.

The developmental PCD of metazoan cells is based on apoptosis that is controlled by highly conserved cellular machinery [2]. Mammalian lymphocytes communicate with each other via apoptogenic receptor–ligand-binding reactions at the cell surface to eliminate selectively other lymphocytes that harbor autoimmune activity. Complex cytokine-mediated gene regulation underlies this cell–cell communication [22]. Neurons that have failed to form a proper nerve circuit cannot obtain sufficient nerve growth factors (NGFs) and die by apoptosis [23]. NGF deprivation causes the phosphorylation of c-Jun by c-Jun amino-terminal kinase (JNK; a MAPK), which leads to the expression of the pro-apoptotic Bid-like protein DP5/Hrk, which then initiates the action of the apoptotic machinery [23]. Transforming growth factor β (TGF-β) and its signaling

Figure 2



TE PCD-specific hydrolytic enzymes. Brassinosteroids (BRs) induce PCD, as well as the formation of secondary walls. PCD-specific hydrolytic enzymes, such as an S1-nuclease, RNases and cysteine proteases, are synthesized and accumulate in the vacuole. The transport of organic anions into the vacuole is inhibited, in association with the enlargement of the vacuole. The enlarged vacuole bursts, then shrinks and fragments. The collapse of the vacuole causes hydrolytic enzymes to invade the cytoplasm and to attack various organelles, resulting in the degradation of cell contents and part of the cell walls. Finally, perforation of the wall causes TEs to lose all of their cell contents and to form mature hollow tubes that are reinforced by secondary walls. It takes 20 minutes to lose nuclear DNA after the collapse of the vacuole. Note that hydrolytic enzymes that are located in the cytoplasm are also expressed in association with TE PCD.

mechanisms, as well as MAPK pathways, regulate PCD in the inter-digital region [24]. In *Drosophila melanogaster*, larval mid-gut and salivary gland cells undergo apoptosis upon metamorphosis. This PCD is regulated by an insect steroid hormone, 20-hydroxyecdysone, which invokes a cascade of transcription factor gene expression that leads to the *de novo* synthesis of a *Drosophila* caspase (i.e. Dronc), an Apaf-4-like adaptor (i.e. Dark), and inhibitor of apoptosis (IAP) antagonists (i.e. Hid and Reaper) [25]. In light of these results, it is clear that most cases of metazoan developmental PCD depend on the actions of cytokines, growth factors, or hormones.

Comparisons of developmental PCD in plants and other organisms

Because many fundamental biological mechanisms are well conserved among organisms of different kingdoms, those related to developmental PCD are also expected to share common cytological and molecular biological aspects in different organisms [20]. This seems true for at least three aspects (Figure 1). First, developmental PCD is regulated by hormones or intrinsic biologically active molecules. Developmental PCD must be coordinated with other physiological processes, such as growth and differentiation, occurring in the tissue, organ, or individual. This type of regulation may be helpful in orchestrating different physiological processes. Second, developmental 'death signals' are mediated by pleiotropic signal transduction machinery. These pathways also have roles in cell proliferation and specification, and to date, no pathways appear to transduce only developmental death signals. The details of such pleiotropic signal transduction pathways, which are ultimately reflected in different kinds of cellular responses, are largely unknown, but they may also have a role in the coordinated progression of cell proliferation, differentiation, and death during development. Third, PCD is accompanied by the activation of specific hydrolytic enzymes, the actions of which often result in apparently shared cytological phenotypes of dying cells. Some of these phenotypes are regarded as hallmarks of apoptosis [26].

In view of this, the following question may be posed: is the apoptotic machinery ubiquitous, and does it also regulate plant and fungal developmental PCD? Although various apoptogenic chemicals and stresses can kill plant and fungal cells [27], such evidence is insufficient to implicate these factors as the cause of apoptosis. Not all components of the metazoan apoptotic machinery have orthologs in organisms of other kingdoms. For example, highly homologous sequences of caspases and the Bcl-2 family proteins, both of which are 'core' members of metazoan apoptotic machinery, are missing from *Arabidopsis thaliana*, *Dictyostelium discoideum*, and *Saccharomyces cerevisiae* [28,29]. Of course, it is nonetheless possible that proteins that are structurally non-orthologous but functionally equivalent to those working in metazoan apoptosis are involved in plant and fungal PCD. For example, a metacaspase plays a role in H_2O_2-induced apoptosis in yeast [30•] and the *A. thaliana* genome contains more than 10 metacaspase homologs [28]. However, only partially homologous domain sequences of the protein CED-4/Apaf-1 are found in plants and fungi [20]. Usually, the absence of such component proteins or the significant alteration of their primary structures accompanies inevitable and drastic changes in the mode of operation of the molecular system. The analysis of *D. discoideum* PCD supported this concept [31•]. Indeed, even though release of cytochrome *c* from mitochondria precedes TE PCD in plants, this release does not induce PCD [32].

Lytic vacuole-initiating plant PCD

Instead, plants have a unique PCD process that includes the large lytic vacuole as a main player, as typically

observed in TE PCD [9••]. Figure 2 illustrates the process: the degradation of cell contents in TE PCD starts at vacuolar collapse, which causes the release of insulated hydrolytic enzymes and allows them to attack organelles. For example, degradation of nuclear and chloroplast DNA can be completed within 20 minutes of vacuole rupture, whereas chlorophyll is degraded much more slowly [4••]. Although Kuriyama [33] proposed that a change in the organic anion permeability of the tonoplast initiates vacuolar collapse in TEs *in vivo*, the true molecular mechanism for vacuolar collapse has not yet been confirmed.

The expression of PCD-specific hydrolytic enzymes, including nucleases and proteases, is associated with TE PCD. Many of these enzymes accumulate in the vacuole of differentiating TEs. ZEN1, an S1-type Zn^{2+}-dependent nuclease that plays a major role in nuclear DNA degradation, is first insulated in the vacuole and functions in hydrolyzing nuclear and organellar DNA after the collapse of the vacuole ([34]; J Ito, H Fukuda, unpublished data). Indeed, the acidic pH optimum of ZEN1 facilitates its activity under such conditions. Likewise, two similar TE PCD-specific papain-like cysteine proteases with acidic pH optima accumulate in the vacuole of differentiating TEs [11,35,36]. These results clearly indicate that the vacuole strengthens its function as a lytic organelle by accumulating and releasing a variety of PCD-specific hydrolases during TE PCD. On the other hand, the TE PCD-specific appearance of serine proteases with neutral pH optima [36,37] suggests that a partial autolytic pathway is involved in the cytoplasm before comprehensive autolysis is caused by the collapse of the lytic vacuole.

PCD should also be organized well in relation to neighboring cells. Cell perforation is restricted to the longitudinal ends of TEs [38]. The middle lamella between neighboring cells that are in contact, of which only one has differentiated, is resistant to autolysis [39]. By contrast, the middle lamella between two differentiated neighboring TEs is digested completely [39], implying that selected cell wall degradation is programmed in TE PCD. This event may result from the site-specific targeting of wall-degrading enzymes and/or the site-specific modification of cell walls, which probably starts before vacuolar collapse. In fact, TE-specific pectin-degrading activity appears on cell walls before vacuole rapture [40••]. At the final stage of TE maturation, digested cell contents that contain proteases and nucleases are released to the extracellular spaces, usually into a neighboring hollow TE. Endo *et al.* [41••] revealed that differentiating TEs and other xylem cells secrete a proteinaceous inhibitor that impairs the activity of a proteasome that is released from dying TEs. This suggests that a safety mechanism prevents the actions of harmful cell contents released from TEs that are dying as a result of PCD.

The lysosome of animal cells is the counterpart of the plant lytic vacuole. Recently, the lysosome was reported to participate in caspase-dependent apoptosis [42]. Oxidative stress [43] and the tumor suppressor protein p53 [44•] cause lysosomal breakage. Sphingosine, a well-known pro-apoptotic molecule, stimulates the activity of some lysosomal proteases, including cathepsin B and D [42,45], which in turn activate caspases by the cleavage of their pro-domains [42]. Surprisingly, such apoptotic lysosome disintegration precedes even the decrease in mitochondrial membrane potential that is one of the earliest events of apoptosis, thus suggesting that lysosomes have a regulatory role in starting the action of apoptotic machinery [44•].

Because the vacuole also disintegrates upon TE PCD, vacuolar collapse might trigger apoptosis-like signaling mechanisms. However, the central vacuole of plant cells is often large and occupies most of the cell volume. If such a large acidic compartment disintegrates, then the cytosol immediately becomes acidified and the cell dies within a few minutes, as shown in TE PCD in *Zinnia* cell culture [4••,14]. In such a situation, cytosolic hydrolases (e.g. proteases) that have neutral pH optima are unlikely to be fully activated. Therefore, even if proteolytic signals that are derived from the vacuole exist in dying TEs, they must be generated before the rupture of the large central vacuole.

Conclusions

Given these considerations, plants should develop PCD execution mechanisms that are at least partially distinct from those of other organisms. Efforts to analyze hormone-based regulatory mechanisms, especially pathways that transduce the death signal, and to examine the roles of plant-specific organelles are necessary to obtain a comprehensive understanding of plant developmental PCD. Current innovative technologies and expanding analytical resources will provide powerful tools with which to address these problems.

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