The programme of cell death in plants and animals – A comparison

K. V. Krishnamurthy[†,*], R. Krishnaraj[#], R. Chozhavendan[†] and F. Samuel Christopher[†]

[†]Department of Plant Sciences, Bharathidasan University, Thiruchirapalli 620 024, India
[#]Present address: Max Planck Institute for Infectious Biology, Berlin, Germany

A critical review of the phenomenon of programmed cell death (PCD) in plants has been attempted. PCD and its mechanism are fairly well known in animals than in plants. The available information indicates that out of eight categories of cells that undergo PCD, only six are known in plants. The mechanism of PCD in the different categories of plant cells appears to be different, at least in some important features, suggesting that more than one pathway of PCD is likely to operate in plants in comparison to animals, where there seems to be only one programme. The differentiation of tracheary elements, fibres, scelerids and cork cells appears to follow a very unique mechanism of PCD. Animal PCD involves a syndrome of processes involving effectors, adaptors, regulators, and signals as well as the activity of specific endonucleases, while available evidences in plant PCD do not indicate the involvement of regulators and adaptors; also the data on the effector proteinases and their substrates are not sufficient enough to unequivocally compare them with the animal caspases and their substrates. The involvement of the various signalling substances in plant PCD has not been proved beyond doubt, unlike in animals; neither the specific involvement of endonucleases in the fragmentation of DNA demonstrated. In view of the above, it is premature to assume that animal and plant PCDs are similar, and more work is needed to prove it otherwise.

THERE have been many scattered reports on the topic of cell death for more than a century. However, during the last five years more than 20,000 publications have appeared on this topic emphasizing the 'contemporary fascination' that the topic enjoys today[1,2]. Programmed cell death (PCD), a usage coined by Lockshin (Lockshin and Williams[3]), is a physiological process that leads to the selective elimination of unwanted cells in multicellular animals[4,5]. PCD is characterized by the invariable involvement of the dying cells' own machinery as an 'executioner', i.e. it is a case of cell suicide. It is reported to play a major role in controlling cell number and turnover especially in the skin, gut and reproductive tract, tissue homeostasis and specialization, tissue sculpting and pattern formation, oncogenesis and other disease incidents, defence reactions, and above all, in maintaining the overall integrity of the animal. PCD is distinct from necrosis. While the former is an energy-dependent physiological process that is genetically programmed with the involvement of regulatory genes, stimulatory events, signalling pathways and morphologically expressed distinctive features, the latter is a non-physiological process, dissociated from developmental and morphogenetic events and involving cell swelling, lysis and leakage of cell contents[6] with no obvious changes in DNA (i.e. no chromatin condensation and DNA fragmentation).

PCD has also been reported in plants[7], although plant PCD studies have been initiated with greater vigour only in very recent years. The limited details that we have on plant PCD are believed to be sufficient enough to conclude that PCD is as important in plants as in animals[8–10] and that it can occur on a local or systemic scale. The molecular mechanisms underlying PCD in the animal system are comparatively better known than in plants, although the relationships between the mechanisms of PCD in the two systems are far from clear. No plant system is yet described which shows all the features common to animal PCD. To date, no critical component of PCD execution has been identified in plants, as also the regulators of cell death[11]. Moreover, more than one distinct cell death pathways are likely to operate in plants, unlike in animals where there is more uniformity[12–14].

The present review will, therefore, address the following questions: Are PCDs, in animal and plant systems comparable? If comparable, how similar are the two? If not, in what respects are they different? Based on the analysis, the probable lines of future research on plant PCD to be undertaken would be indicated.

Categories of cells that undergo death

A number of categories of cells can be recognized within those that undergo death in multicellular animals[10,15]. The objective of this section of the review is to find out whether all these categories of cells also exist in plants.

*For correspondence. (e-mail: kvk@bdu.ernet.in)

Category (i): Cells that have served their functions

As examples of cells of animals that belong to this category, the tadpole tail cells and the cells lining the intestinal and reproductive tracts may be mentioned. A number of plant cell types belong to this category: root cap cells, cells undergoing senescence[16], cells involved in abscission of plant parts, vegetative cell of pollen, synergids, antipodals, anther tapetal and wall cells, transmitting cells of stigma, style and ovary wall, endosperm cells, including haustoria, aleurone cells of grass endosperm, suspensor cells of developing embryo, etc. The cells of this category, whether in animals or in plants, lose their earlier identity, morphology and functions and are completely eliminated in animals, while in plants their remnants or corpses may persist. Available evidence strongly indicates that these cells in plants or animals undergo a physiological PCD process. It may, however, be indicated that not all cells of a plant organ are about to abscise undergo PCD; cells at the line of abscission may undergo PCD while the other cells of the organ may die indirectly because of not receiving nutrients, water or growth regulators.

Category (ii): Cells that are unwanted from the beginning

The muellarian duct cells that are required in females but not in males may be cited as an example of this category in the animal system. The stamen and carpel primordial cells, respectively, in female and male flowers,[17] nonfunctional megaspores in monosporic and bisporic embryo sac categories[18], nonfunctional microspores in members of Cyperaceace and Epacridaceae,[19] nonfunctional cells formed during embryoid initiation in cultured cells[20,21], etc. are examples of plant cells belonging to this category.

Category (iii): Cells that die during redifferentiation into specialized cell types

Examples of cells of this category are rather rare in the animal system. The keratinocytes at the skin surface of the vertebrate animals are the typical examples from the animal system. These cells extrude their nuclei as part of their normal programme of redifferentiation. Xylem tracheary elements (TEs)[22–25], cells of certain trichomes, thorns and spines, scelerenchyma (sclereids and fibres), cork cells, etc. are some of the plant cells belonging to this category. The most important characteristic of these cells is that they all start functioning only after their death. The other characteristic feature of these cells is that significant changes (morphological and chemical) take place in their cell walls. In other words, cell death leads to terminal differentiation in this category of cells.

A sub-category under this includes those cells which undergo 'partial' (?) death during differentiation. The red blood corpuscles and the phloem sieve elements belong to this sub-category. (Nuclei persist in mature sieve elements, albeit with a lot of structural changes and loss of transcriptional activity in species of *Selaginella* and *Isoetes* and in some ferns[26].) Here the nucleus is lost, but not the cytoplasm and its integrity (?). The functional life span of the cell is, however, fixed; RBC functions for 120 days after it has lost its nucleus, while a sieve tube element functions from a few days to a few years depending upon the plant[26].

Category (iv): Cells that are subjected to hypersensitive response

Cells subjected to hypersensitive response (HR) due to pathogenesis and other environmental and abiotic stresses such as osmotic, oxidative (H_2O_2 and salicylic acid), temperature, salt, water, heavy metals, UV, nutrient deprivation, toxins, chemicals, etc. often undergo PCD[10,27]. This category of cells is commonly reported in animals as well as in plants. Here cell death is used as a defence against the stress or as a means of emanating signals for the other nearby cells to build up defence/immune reactions[28]. PCD is reported to be employed in these instances not only to kill the host cells (to result in HR lesions in plants but not in animals) to prevent further infection, but also the invading pathogens. In many instances, cells experiencing lethal stress doses often react by activating their PCD mechanisms to commit suicide before they are killed[5]. A critical survey of the available literature indicates that not all cells subjected to stress undergo PCD; there are cells which may undergo necrosis, especially if the dosage of stress is beyond a particular level.

Category (v): Cells that are damaged and unable to function further

Death of this category of cells removes the potentially harmful cells and prevents their multiplication and spread in the animals[15]. It should, however, be made clear that not all damaged cells undergo PCD in animals and they may recover after repair or the damaged cells may undergo necrosis[29]. In plants also, cell death due to injury and other reasons may occur, but it is not known whether such deaths are programmed. It is often difficult to distinguish this category of plant cells from the previous category.

Category (vi): Cells that are produced in excess

The vertebrate neurons can be cited as examples of this category. There is no instance in plants where cells are

produced in excess and are eliminated because of that reason.

Category (vii): Cells that are present in wrong places

The cells present between the developing digits in vertebrates may be mentioned as examples of this category. Cells that die to result in leaf lobe formation in lobed leaves and leaflets in compound leaves or to result in holes in the lamina of taxa like *Monstera*[7] and some Croton varieties are typical plant examples of this category.

Category (viii): Cells which through their death give rise to diseases

The death of helper T cells of the animal immune system results in AIDS; similarly death of selected brain neurons gives rise to Alzheimer's, Huttington's, Parkinson's and Lou Gehrig's diseases. There are no examples of plant cells for this category.

Processes associated with PCD in the animal system

PCD in the animal system is reported to result in the disassembly of cells involving the condensation, shrinkage and fragmentation of the cytoplasm and nuclei into several sealed packets (often called the apoptotic bodies), which are then phagocytosed by the neighbouring cells or the macrophages. Thus, there are no remnants of cell corpses left. Nuclear fragmentation is preceded by chromatin condensation and marginalization in the nucleus. Fragmentation of DNA at the nucleosome linker sites then takes place. When all these events are combined to result in a distinct morphological expression then the PCD is termed as apoptosis[6], a word coined by Kerr *et al.*[30] in the year 1972. In other words, apoptosis is a distinct form of PCD[31,32]. However, there are others who consider apoptosis and PCD as one and the same[33–36]. The apoptotic PCD involves a syndrome of mechanisms with effectors, adaptors, regulators and signals as well as the activity of endonucleases.

Effectors

The key effector molecules of animal PCD are the cysteine aspartate-specific proteases (caspases) and granzymes. The former are the conserved cysteine proteases, while the latter are serine proteases; both specifically cleave after the aspartate residues of many proteins. These proteins include nuclear lamins, Poly ADP Ribose Polymerase (PARP), Inhibitor of Caspase Activated DNase (ICAD), DNA-PK, SRE/BP, PAK2, 70 kDa components of U1sn-RNP, procaspases and so on[37]. Caspases act on many proteins such as the above and bring about cell condensation, shrinkage and disassembly into apoptotic bodies[38]. The most important protein substrate is CAD–ICAD complex which on cleavage releases Caspase Activated DNase (CAD); subsequently CAD activity results in the fragmentation of DNA. Similarly, there is a specific proteolytic cleavage of PARP to the signature of 89 kDa apoptotic fragments during cell death. The first known caspase is the worm caspase CED-3 and till date 14–15 different caspases that play a role in inflammation (group 1 caspases) and apoptosis (group 2 caspases) have been identified in animals[37]. All these are believed to share a high level of similarity in sequence structure and substrate specificity[37–39]. Initially all the caspases remain as inactive zymogens (proenzymes), but get activated during PCD when proteolytic processing at a few specific sites of these zymogens unleashes their latent enzymatic activity. Production and functioning of caspases during PCD depend on the sequential evocation and action of specific genes[4,40]. In animals such as *Caenorhabditis elegans*, specific genes such as *Ced-3*, *Ced-4* and *Ced-9* have been implicated in cell death[41]. All the three genes have their mammalian counterparts[33]. Among the animal cell types that undergo death, RBC and keratinocytes are described as caspase independent cell deaths[9], while in all PCDs the involvement of some caspase or the other has been implicated.

Adaptors

The activation of caspase precursors (zymogens) is achieved by adaptor proteins that bind to them via shared motifs. TNF receptor superfamily or the apoptogenic co-factors released by the mitochondria can be mentioned as examples of adaptors. Caspase 8 is activated when death effector domains (DEDs) in its prodomain bind to the C-terminal DED in the adaptor molecule Fas-associated Death Domain (FADD); similarly caspase 9 is activated after the association of the caspase recruitment domain (CARD) in its prodomain with the CARD in another co-factor protein, Apoptosis protease activating factor-1 (Apaf-1)[5,39]. In the worm *C. elegans*, CED-4 acts as the adaptor molecule.

Regulators

The ability of the adaptors to activate the effector caspases is regulated by other proteins that appear to directly interact with the adaptors/co-factors. For instance, the FLIP can inhibit caspase activation by binding to FADD in the vertebrates; similarly CED-9 can prevent caspase activation by binding to the adaptor CED-4 (ref. 5) in the worms. The Bcl-2 family of proteins include both the

proapoptotic (Bax, Bak, Bok, etc.) as well as the anti-apoptotic (Bcl-2, Bcl-x_L, Bcl-w, etc.) members which function primarily with the mitochondria in controlling cell death in vertebrates. It is now an established fact that mitochondria, which are called the 'power houses' of the cell, not only generate energy for cellular activities but also play an important role in cell death[42,43] in animals. They release several death-promoting factors such as cytochrome c (which contributes to caspase activation), Apaf-1, apoptosis-inducing factor (AIF), procaspase-3, Ca^{2+}, and reactive oxygen species (ROS) in response to various stimuli. In animal PCD, the outer mitochondrial membrane is physically disrupted either as a result of hyperpolarization of the inner membrane or by the collapse of the membrane potential (Ψ_m) by the opening up of the permeability transition pore (PTP). This pore is seen at the apposed regions where the voltage dependent anion channel (VDAC) or the mitochondrial porin in the outer mitochondrial membrane and the adenine nucleotide translocator (ANT) in the inner membrane come in contact. The pore is regulated by the Bcl-2 proteins and the intracellular ATP levels[42,44]. The Bcl-2 proteins are membrane spanning and have at least one of the four Bcl-2 homology (BH) domains. It is shown that the proapoptotic Bax interacts with the VDAC and ANT and brings about a conformational alteration to form a megachannel leading to the release of cytochrome c (ref. 45). It appears that Bcl-x_L suppresses PCD in many animal cells[4] by closing the PTP and sustaining the ADP/ATP exchange by an unknown mechanism.

Another well-studied apoptosis regulator is the 35 kDa baculovirus protein encoded by the *p35* gene from the *Autographa californica* nuclear polyhedrosis virus (AcNPV). *p35* has been shown to be a broad spectrum caspase inhibitor, including caspases 1–4 and CED-3 in various systems. It functionally complements Bcl-2 in every given system. It blocks apoptosis induced by potent apoptotic inducers such as stauroporine, actinomycin D, growth factor withdrawal, oxidative stress, etc. in various systems[46,47].

Signals

The whole process of animal PCD is exemplified by the involvement of a number of cell signalling (often characterized as 'unbalanced cell signalling') events which may impinge upon the cell death mechanism at any level[5]. The decision of a cell to initiate the act to kill itself is determined by the nature of the signals received from outside the cell, or by the generation of its own signal molecules or a combination of both. The following signalling events are reported to be involved in animal PCD: (a) an increase in free cytosolic calcium concentration; (b) an oxidative burst manifested as an increase in free radical activity; (c) an increase in reduced glutathione; (d) a collapse in the mitochondrial membrane potential, as already discussed; (e) an increased exposure of the acidic phospholipid phosphotidyl serine (PS) on the outer surface of the plasma membrane; binding of annexin V to PS has proved to be a reliable and useful indicator of apoptosis[48], and (f) an increase in the intracellular ceramide levels.

The requirement of ROS (such as O_2^-, H_2O_2 or HO) for signalling cell death in animals has been demonstrated[49], although PCD in animals can also take place in the absence of O_2 (ref. 50). ROS production is due to the mitochondrial action or due to plasma membrane NADPH oxidase, whose activity in turn depends on protein phosphorylation/dephosphorylation (see later). It is suggested that certain environmental/developmental signals initiate early signalling including protein phosphorylation, which then leads to the activation of NADPH oxidase. It has also been reported recently[47] that Ros besides being quenched by antioxidants can be quenched by *p35*, an apoptosis regulator mentioned earlier. The cytosolic calcium concentration increases in order to signal the onset of PCD in the animal system[51,52]. It is believed that one role of calcium is the activation of PCD endonucleases[53] leading to chromatin condensation and DNA fragmentation[54] and the other is to activate caspases, especially calpain.

There is not much information on the possible significance of the increase in reduced glutathione as a signalling event in animal PCD. An increased exposure of PS on the outer surface of the plasma membrane is one important signalling event in animal PCD. This is the result of plasma membrane blebbing. As already stated, PCD in the animal system requires dephosphorylation[52]. Growth factor deprivation (GFD) is shown to be a signalling factor in initiating cell death in the animal system[55,56]. This is substantiated by the fact that autocrine signals suppress PCD in animals[56]. Ceramide is a 'second messenger' implicated in apoptosis signalling[42]. It disrupts the electron transport system (drop in ATP production) leading to cell death and activates prICE.

Endonucleases and nuclear fragmentation

PCD in animals is often morphologically expressed through the condensation, shrinkage and fragmentation of the nucleus and the cytoplasm, resulting in the formation of several sealed packets called apoptotic bodies, each with some cytoplasm and nuclear material. Nuclear fragmentation is preceded by the condensation and marginalization of chromatin in the nucleus. Fragmentation of DNA takes place at the nucleosome-linker sites and the fragmented oligonucleosomal bits are reported to be of 180 base pairs[32]. Fragmentation is effected by endonucleases such as NUC18, DNaseI, and DNaseII[57,58], which are present in the nucleus and are activated by Ca^{2+} and

Mg^{2+} but inhibited by Zn^{2+}. Electrophoretic separation exhibits these DNA fragments as a 'ladder formation'; the rungs of the ladder are multiples of 180 bp. The DNA fragments can be cytochemically determined by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) of DNA 3′-OH groups[59].

Is DNA fragmentation a necessary prerequisite for cell death? In almost all apoptotic animal cell deaths it is a marker feature. Studies carried out on *C. elegans* lacking nuc-1, on enucleate cells such as RBCs and knockout mice unable to produce active CAD indicate that DNA fragmentation is not absolutely required for cell death[5].

Processes associated with PCD in plants

Cytoplasmic condensation, shrinkage and fragmentation

Condensation and shrinkage of the cytoplasm and its subsequent fragmentation to form apoptotic bodies are very important processes in animal PCD. These changes cause the shrinkage of the dying cell as well. Although several plant systems have been studied to understand the mechanism of PCD, very little attention has been paid to the details of cytoplasmic changes, if any, during cell death. Condensation and shrinkage of the cytoplasm were reported in dying aleurone cells[60–62], root cap cells[63], sister cell of the embryoid initial[10,64,65], and HR lesion cells of *Arabidopsis*[65]. In the embryoid sister cell, the breakdown products of the cytoplasm are reported to form several small membrane-sealed packets, but an electron micrograph of a dead cell reproduced as figure 2B in Pennell and Lamb[10] does not show breakage of the cytoplasm. The cytoplasm of differentiating TEs of intact plants is reported to become lobed, condensed, shrunken and finally gets broken into small packets[67–69]. Our own experience in TE differentiation (both from procambial and vascular cambial derivatives), as well as that of others (see Barnett[70]) indicates that what is reported above may be an artefact induced during specimen preparation. In fact, differentiating TEs often elongate and enlarge rather than shrinking[25]. What really happens in differentiating TEs is that the cytoplasm shrinks but does not break into bits and is abruptly lost due to vacuolar autophagy. A critical perusal of hundreds of published micrographs of dying plant cells belonging to megaspore dyads and tetrads, nucellus, endosperm, embryo suspensor, anther tapetum, root cap, etc. indicates the occurrence of cytoplasmic condensation and shrinkage but not its breakage into bits; there are evidences of vacuolar autophagy in most cases which accounts for the elimination of the cytoplasm.

A different mechanism of programmed autolysis was first suggested by Nagl[71,72] with reference to the death of embryo suspensor cells. The filiform leucoplasts of the suspensor cells are transformed into cytosome-like structures displaying high acid phosphatase activity. During transformation, polar swelling of the plastids takes place incorporating into it the invaginations of the degenerating cytoplasm to form spoon-like structures, which may later on become 'ball-like'. These structures were termed 'plastolysomes'. They form the characteristic elements of the lytic compartment. Plastolysomes include various organelles and cytoplasmic components, particularly ER, polysomes and lipid bodies. At a later stage, the stroma of these plastolysomes disappears completely and finally the bodies are transformed into autophagic vacuoles surrounded by a single membrane; all the included cytoplasmic components also degenerate. Whether it be by vacuolar autophagy or by plastolysomy, the plant cells that died are always evident as corpses and these are invariably represented by the remnants of the cell wall.

Effector caspases

It was mentioned earlier that animal PCD is due to a syndrome of well organized and executed mechanisms involving effectors, adaptors, regulators and signals. The chief effector molecules are the caspases. No caspase has yet been cloned from plants. But there is a growing information on the family of caspase-like proteinases and their involvement in plant PCD[73,74]. The expression of cysteine proteinases (CyPs) is reported during cell death in *Solanum melongena*[75]. Several types of cysteine proteinases such as actinidin, oryzain, barley aleurain, etc. are known from the plant system. Phytepsin D, a barley vacoular aspartate proteinase, is reported to be expressed very highly during autolysis and development of TEs and sieve elements[74]. It is, however, interesting to note that these proteinases are expressed not only during cell death but also during other cell events. There is still only a speculation that the proteinases reported in plant PCD are involved in a function analogous to that of the cysteine proteases of the interleukin 1β converting enzyme (ICE) family reported in the animal system[10].

The substrates for almost all these plant proteinases are yet to be identified. It is quite possible that the plant proteinases are involved in the clearance of cytoplasmic and nuclear proteins from dying/already dead cells such as TEs, i.e. they may merely be doing a scavenging job, thereby helping the recycling of nitrogenous compounds. Moreover, the results of Xu and Chye[75] in brinjal on the isolation and characterization of cDNA encoding CyPs and their expression indicate their coincidence with several rather than specific events in cell death in many organs/tissues such as ovules, xylem, placenta, receptacle, anther, epidermis, endothecium, nucellus, etc. Although CyPs of brinjal has 90–92% amino acid identity to the other CyPs from Solanaceae family (tobacco CyP 8 and tomato CyP 2), they show only 39% homo-

logy to cathepsin H, an animal protease. Caspase inhibitors, such as the choloromethylketone Ac-YVAD-CMK or aldehyde Ac-DEVD-CHO, are reported to completely abolish HR cell death in tobacco[76]. This is cited as an evidence for the presence of caspase-like plant proteases that participate in HR cell death. But it is not yet clear whether these inhibitors are targetting the proteases that are evolutionarily related to the metazoan caspases[5]. The results obtained by D'Silva *et al.*[31] indicate that HR in the cowpea–cowpea rust fungus system, involves the activation of cysteine proteases, some of which are similar to caspases in having specificity for Asp residues, while others do not have a defined specificity. They further argued that the proteolytic activity during PCD in plants and particularly during HR, need not necessarily be identical to that seen during animal apoptosis, given the fact that plants have evolved independently from animals. In the light of the discussion made above, it is premature to call the plant CyPs and related enzymes as equivalent to animal caspases. It is also premature to speak of their involvement in 'apoptosis-like' cell death of plants[76]. In other words, available evidence is not enough to conclude beyond doubt that plants and animals turn out to share the same cell death effector mechanism[5].

There are reports of a gene implicated in 'caspase' production and function in plants. *SAG12*, an *Arabidopsis* gene, is reported to encode a putative cysteine protease[77] involved in senescence. *HSR 203J*, a tobacco gene, is activated during HR[78] specifically, while *HIN1*[79], a tobacco gene is activated during HR as well as lately in senescence, but the last two genes are reported to be activated only after the switching of 'cysteine proteases'.

Attention was earlier drawn to the fact that the common substrates of caspases so well deciphered in the animal system are also not known in the plant system during cell death. For example, as already indicated, in the animal system the specific proteolytic cleavage of PARP to the signature of 89 kDa apoptotic fragment is a hallmark of PCD. PARP is a highly conserved nuclear enzymatic protein that is tightly bound to the chromatin or nuclear matrix. It is with three principal domains, a 46 kDa N-terminal DNA binding domain, a 22 kDa central automodification domain, and a 54 kDa C-terminal catalytic domain. There is a single type of PARP in animals while the available studies indicate that in plants two forms of PARP genes occur: one highly similar to the PARP gene of animals and recently discovered in *Zea mays* (EMBL accession number: AJ222589)[31] and the other a full length *Arabidopsis thaliana* cDNA encoding a short PARP homology (72–73 kDa) with 60% similarity to the catalytic domain of vertebrate PARP[80]. In plants, although PARP genes have been cloned from these two taxa and PARP activity has been identified in a few species[81], there have been no reports yet of the cleavage of PARP during cell death[31]. Abundance of PARP is also rather limited in plants[82]. Because of the fact that PARP is involved in DNA repair and replication also, a process contrary to cell death[82], its mere presence alone cannot be cited as indicative of cell death. Perhaps, it may be involved in DNA repair and maintaining genome integrity. Mild DNA damage caused by stress would be repaired, while higher doses of stress may promote PCD and in both PARP may be involved. It is also likely that PARP has a role in the activation of phenylalanine ammonia lyase (PAL) enzyme. There is also a suggestion that cleavage of PARP may not be an absolute requirement for cell death in HR response[31].

Adaptor molecules

Neither adaptor molecules nor the cascade of events leading to the activation of the CyPs which are believed to be the effector proteases of the plant PCD, have been reported so far in any plant cell undergoing PCD.

Regulator molecules

None of the regulator molecules so far reported in animal PCD is yet to be reported in plant PCD. In plants the extraneous supply of antiapoptotic protein Bcl-x_L does not suppress HR cell death[66], although it does so in animals[6].

Signal molecules

Oxidative stress, as a signal event, is shown to trigger cell death in plants; the involvement of O_2^- in plant HR responses[83] and of H_2O_2 in cell death mediated by pathogens[66] are reported. However, we do not have a clear idea about the exact role of these two ROS in cell death process in both animals and plants, although H_2O_2 is likely to induce DNA fragmentation and an influx of extracellular calcium. In plants, H_2O_2 has been reported not only in cells infected with pathogens and subjected to stress but is also regularly produced when there is an activity of peroxidase and polyaminic oxidase, lignification and cross-linking of extensins in the cell wall[84]. It is true that H_2O_2 is present not only in living cells but also in cells that undergo death due to wounding, pathogen entry and xylogenesis, but available evidence is not sufficient enough to unequivocally implicate ROS species in PCD of these cells; they are more likely to be involved in necrotic cell death which is found hand in hand with PCD in systems subjected to biotic and abiotic stresses, especially at a level more than the threshold.

Like in animal PCD, in plant cells undergoing death an increase in cytosolic calcium is reported to be an important signalling event. The probable role of calcium is the

activation of endonucleases in both PCDs. However, in plants a Zn^{2+}-dependent endonuclease is known in addition to the Ca^{2+}-dependent endonuclease. The former is known in cells of wheat leaf undergoing senescence, while the latter is known in differentiating TEs, aleurone cells and in HR lesion cells[60,66,85]. There is no categorical report of Ca^{2+} activated CyPs in plant PCD, in contrast to animals.

The three, probably interrelated, general mechanisms of mitochondrial control of animal PCD[42], viz. (i) Disruption of electron transport and ATP production, (ii) Release of caspase-activating proteins such as cytochrome c, and (iii) alteration of cellular redox potential through ROS are not yet fully worked out in plants. There are a few recent reports which mention about the role of cytochrome c released during PCD in plants[86-88]. The mitochondria in most plant cells undergoing death have been shown to be normal[70,89,90], but they are reported to be slightly inflated in dying suspensor cells[71,72], and with a slightly deformed membrane system in dying synergids[91,92].

Plant cells subjected to oxidative stress (i.e. treatment of H_2O_2) showed an increase in intracellular reduced glutathione[54,93]. Plasma membrane blebbing has not so far been reported in plant cells undergoing death, although PS exposure has been demonstrated[54] through Annexin V binding assay in the protoplasts of tobacco subjected to abiotic stresses. Whether this has any relation to the presence of the cell wall in the plant system is not clear.

PCD in plants also is reported to require phosphorylation/dephosphorylation changes in hypoxia[10], HR reactions and aleurone[61,62] cell death. For example, in aleurone cells the supply of okadaic acid, an inhibitor of protein phosphatase, inhibits cell death. In HR reaction also, plant cells are reported to exhibit protein phosphorylation/dephosphorylation changes in cell death[93]. Phosphorylation/dephosphorylation events are also important in the regulation of ROS production[93]. Whether such a change is really related to death is also debatable and more work should be directed towards the role of phosphorylation and dephosphorylation in PCD.

GFD is known to promote PCD in plants as well[94]. The autocrine signals believed to be involved in PCD include growth regulators like cytokinins. Cytokinins appear to be a common suppressor of certain kinds of HR and a well-known suppressor of senescence (see full literature in Pontier *et al.*[11]). However, the results should be viewed with caution because some of the allocrine signals in the form of gibberellins and ethylene are known to promote/stimulate cell death in cereal endosperm[95,96], in xylem element differentiation and heart wood formation and in senescence and abscission. Ethylene is believed to regulate nuclease activation and cell death[95,96]. ABA prevents PCD in anther wall cells[97] as well as in barley[62] and wheat[61] aleurone cells. In all likelihood GFD may be a secondary or even tertiary factor in cell death.

Chromatin condensation, endonucleases and DNA fragmentation

The DNA processing reported earlier for animal PCD is believed to exist in the dying cells of plants as well[62,63,98,99]. In aleurone cells of the grass species such as barley, in dying root cap cells and in tobacco cells subjected to HR, nuclear condensation and shrinkage as well as oligonucleosome sized DNA fragments have been recorded through the presence of 3′OH detected by TUNEL experiments[10,100]. The major problem relating to nuclear changes in plant PCD is that there is no consistency regarding the size of the DNA fragments formed during DNA fragmentation: fragments of more or less 50 kb (50,000 bp) in some cases[100] and as small as 0.14 kb (140 bp) in others[6,54]. It is believed that the activation of some endonucleases leads to 50 kb DNA fragments followed later by a different set of endonucleases causing the production of oligonucleosomal length of DNA fragments[101]. The first type of cleavage is believed to be the result of the release of the chromatin loops and is observed in almost all cases of apoptosis and the subsequent nucleosomal laddering occurs less often and is considered to be not essential for apoptosis[102]. In certain plant cells such as the TEs derived from procambium or vascular cambium and sclerenchyma fibres the nucleus undergoes a dramatic increase in nuclear volume and alterations in structure[69,103–105] and these changes are unlike in any animal cell undergoing PCD. Multinucleate condition as well as the occurrence of endopolyploidy (up to 64 C level of DNA in some cases) is common in the TEs and fibres. Endopolyploidy is also a very common feature in endosperm haustoria, tapetal cells, embryo suspensor and quite a number of other specialized plant cells before they undergo death. Thus, DNA fragmentation may be a marker feature in certain cell deaths of plants such as those cells that die after performing specific functions (for example, root cap cells, aleurone cells, etc.), but is not likely to be involved in the differentiation of TEs and fibres. In enucleated sieve elements also, nuclear fragmentation has not been shown in any of the ultrastructurally investigated species[90].

DNA fragmentation due to endonuclease activity is very categorically established in apoptotic animal cells. Almost all the reports of the presence of endonucleases in plant systems are either indirect or indefinite with reference to their role in fragmenting DNA. Two prominent and several minor nuclease activities were detected in dying pericarp and nucellar cells of maize[96]. Nuclease activity was also detected in dying endosperm cells. It remains to be determined whether one or more of the observed nuclease activities is responsible for the internucleosomal degradation of endosperm DNA[96]. There is no conclusive evidence that any of these nucleases are responsible for the internucleosomal fragmentation associated with PCD[96]. A single-stranded DNA nuclease is

induced in differentiating TEs of Zinnia[106]. This nuclease is a 43 kDa protein and has endonuclease activity on ds-DNA, ss-DNA and RNA either in the presence of Ca^{2+} or Zn^{2+}. This endonuclease, regarded as likely to be responsible for the observed DNA fragmentation and accumulation of 3′-OH groups[22,63], is predominantly active just prior to the onset of differentiation whereas during xylem differentiation, a lower-molecular-weight activity appeared and increased as the level of the former nuclease activity decreased[106]. Nucleases involved in TEs differentiation are secreted into the apoplast from the neighbouring cells[106] or have their genesis in the vacuole of the TEs. The endonuclease reported from barley aleurone cells is a 35 kDa protein, and has activity on ds-DNA, ss-DNA and RNA but only in the presence of Zn^{2+}; the nucleases from other plants require Ca^{2+} and yet others are activated by both[107]. In tobacco HR, at least three Ca^{2+}-induced endonucleases are known[100]. It is likely that the endonucleases reported in at least some cell types of plants such as TEs may alternately be functioning in the clearance of DNA from the cells which have entered into death, i.e. acting more like scavengers and helping in the recycling of DNA components. To discriminate between the two possibilities of involvement of the nucleases in PCD and clearance of DNA, it is necessary first to determine whether these nucleases mediate the processing of DNA into oligonucleosome-sized pieces. But such studies are yet to be carried out in plants.

Stress proteins

One very interesting feature about the death of some types of plant cells is the active production of special categories of structural proteins rich in hydroxyproline, proline, glycine, arabino galactan and occasionally threonine. These are actively synthesized in the endoplasmic reticulum, packed in the golgi and transported to the cell wall where they become integral components. In sclerenchyma cells and xylem elements such proteins have been repeatedly reported during the process of cell death (see ref. 84). The production of such proteins, to the best of our knowledge, is not encountered in any animal cell type undergoing death.

The role of cell wall

Cell walls are absent in animals and present in plants. Their role in, as well as changes in their morphology and chemistry during cell death must be critical, but yet a study of this aspect is totally neglected in plant cell death. Many plant cells, as already indicated, survive after death only as cell walls and perform specialized functions such as mechanical support and water transport only because of the possession of the cell wall. Secondary wall deposition often takes place, depending on the cell type, with specific types of chemicals such as lignin, suberin, cutin, etc. in addition to changes in cell wall carbohydrates and structural proteins, especially stress-related proteins.

PCD in different categories of plant cells

Of the eight categories of cells that undergo cell death, six categories are represented in plants. Out of these, cell death mechanism has been studied, at least to a limited extent, in four categories: cells that die after performing specific function(s), cells that are unwanted from the beginning, cells that die during terminal differentiation into specialized cell types, and cells that are subjected to abiotic and biotic stresses. A critical analysis of the results so far obtained pertaining to the processes associated with cell death in these four categories of cells reveals the following (Table 1):

(i) Cells that die after performing their functions undergo cell death whose mechanism is almost similar, if not totally, to the PCD events of animal cells. They are reported to share events such as cytoplasmic condensation and shrinkage, cell shrinkage, probable presence of effector proteases and signalling events such as ROS production, increase in cytosolic calcium, phosphorylation/dephosphorylation changes, chromatin condensation, probable presence and activation of endonucleases, and DNA fragmentation as revealed through electrophoretic ladder formation and TUNEL staining; the size of the DNA fragments obtained is also around 180 bp.
(ii) There is not much work on the cell death mechanism in the second category of cells and so definite conclusions cannot be made.
(iii) The third category of cells, which includes TEs, sclerenchyma fibres, sclerids and cork cells, undergo very marked changes during cell death and this can be considered as a unique type of PCD. These cells die to function and consequently the cytological events accompanying cell death in these cells are oriented towards making them persist and function till the end of the life of the plant. They do not show cell shrinkage common in all other cells undergoing PCD, but instead often enlarge and elongate during differentiation. The nuclei of the cells are not only metabolically very active but often undergo endoduplication and polytenic changes during the differentiation process. They are transcriptionally very active and are responsible for the production of several chemicals that form part of the persisting specialized secondary cell wall. The presence of some types of proteases in these cells is perhaps related to the cleaning up of the cytoplasmic proteins and to recycle them for use in organizing the specialized secondary wall. The same may be said of the endonucleases reported and the TUNEL positive reaction shown in the cells. Fukuda[25] considers

Table 1. PCD in different categories of plant cells

Events in cell death	Category 1	Category 2	Category 3	Category 4
Types of cells studied	Endosperm and aleurone cells, anther wall cells, including tapetum, senescing cells, root cap cells and embryo suspensor	Sister cell of embryoid initial	Tracheary elements	Cells subjected to various abiotic and biotic stresses (HR response), lesion mimics, cells subjected to hypoxia
Cytoplasmic condensation and shrinkage	+	+	+	+ in most cases
Cytoplasmic fragmentation	–	–	–	–
Cell shrinkage	+	+	–	+ in most cases
Cysteine proteinases (CyPs)	+ in some, NS in some; in the former, their involvement in PCD not proved beyond doubt	NS	A 30 kDa CyP, a 145 kDa serine protease and a 60 kDa protease are known. However, the substrates, cellular localization, mode of induction, etc. are not known	CyPs + in HR, some of which cleave after asp residues while others do not
PARP involvement	NS	NS	NS	+ in some but whether its role is to repair DNA, cause PCD or to activate PAL is not known
PARP cleavage	NS	NS	NS	–
Adaptor molecules	–	–	–	–
Regulator molecules	–	–	–	–
Signalling events:				
a) Oxidative burst and ROS production (O_2 and H_2O_2)	NS in some and + in others	NS	+ (?)	+ (?) in some, NS in others
b) Increase in cytosolic Ca^{2+}	+ in some and NS in some	NS	+	+ in some, but only proved through channel blockers; NS in others
c) Mitochondrial involvement and collapse of membrane potential	NS	NS	NS	NS
d) PS exposure on the plasma membrane	NS	NS	NS	+ in one system studied, NS in others
e) Phosphorylation/ dephosphorylation	+ in one system, NS in others	NS	NS	+ in some systems, NS in others
f) GFD	+ in senescent cells, NS in others	NS	NS	+ in HR, NS in others
g) Ethylene	+ in endosperm cells and senescent cells, NS in others	NS	+ (?)	+ in most cases
Chromatin condensation	+	NS	?	+ in some, – in some, NS in hypoxia
Endonucleases	NS in most, + (?) in senescing cells and endosperm cells. Zn^{2+} activated	NS	A 43 kDa enzyme is reported; Zn^{2+} activated enzyme; Involvement in PCD not established	A 36 kDa enzyme is known in tobacco HR cells activated by Ca^{2+}, but inhibited by Zn^{2+}
DNA fragmentation	+, ladder formation shown, TUNEL +	Ladder formation ?, TUNEL +	?, probably absent; TUNEL +, but Fukuda[24] considers this as due to the activity of a general nuclease rather than an apoptotic nuclease	NS in hypoxia, ladder in some but not all, TUNEL + in some and negative in others
Size of the DNA fragments	180 bp in endosperm cells. 200 bp in anther cells; NS in others	140 bp	–	180 bp in UV stressed cells, 50,000 bp in some HR cells

+, detected; NS, not studied; –, not detected; ?, doubtful.

these endonucleases as of general nuclease category and the TUNEL positive reaction observed as due to these general nucleases. It is significant to mention here that in many instances even fully mature and functioning TEs and fibres possess intact nuclei. In the light of the above, it may be emphasized that the third category cells undergo a unique type of PCD probably unknown in any other cell type.

(iv) The fourth category of cells includes those that are subjected to various abiotic and biotic stresses causing HR response. In fact, this category of plant cells is the most exhaustively studied one. A critical assessment of these studies reveals that among a population of cells subjected to any of these stresses, there may be cells that are undergoing PCD and others undergoing necrosis depending upon the intensity of the stress and the proximity of the cell to the stress agent. This is reflected in the extent of cytological variations shown by these cells following the application of the stress factor. Barring a few papers where the above distinctions have been taken care of during the presentation of results and discussion, in other stress-related PCD works the overall changes noticed have been reported, some of which, as said earlier, may be due to PCD, some due to necrosis and some due to a combination of both.

PCD in plants and animals – A comparison

Table 2 provides a detailed comparison of PCD in animals and plants. It is evident from this table that PCD does occur in plants as well, but a number of differences are noticed. Cytoplasmic condensation and shrinkage appear to be common between plants and animals irrespective of the type of cell undergoing PCD. The condensation of chromatin and nuclear fragmentation observed in animal PCD are also shared by some categories of plant cells undergoing PCD but not all. The characteristic apoptotic bodies formed in animal PCD are not noticed in any plant cell and this is likely to be due to the manner of disposal of dead cells; in animal apoptosis, the apoptotic bodies facilitate their engulfment by the neighbouring cells or macrophages. Since phagocytes are absent in plants, the degenerated cytoplasm and the nucleus have to be eliminated by a different process of vacuolar autophagy and plastolysomy. Future work should be undertaken to find out the probable role of plastids, mitochondria and other cytoplasmic organelles as well as the detailed mechanism of the vacuolar autophagy in eliminating the degenerated cytoplasm and nucleus in plant cells undergoing PCD. The lack of cell wall is the reason for the non-persistence of cell corpses in animals unlike in plant cells. In addition, in plants a number of cell types start functioning only after the cell death and the function is essentially facilitated by the persisting cell wall which may undergo additional morphological, structural, physical and chemical changes in consonance with their function. Consequently, these cells do not also shrink, and may even expand and elongate during cell death, a feature not noticed in animal PCD.

Animal PCD is invariably due to a syndrome of well organized and executed mechanisms involving effectors, adaptors, regulators and signals. Whether it be a worm, insect or a mammal, PCD appears to follow an almost fixed line of changes. In plants, the mechanism underlying PCD is still incompletely known and so far no adaptor or regulator molecule has been shown to be involved. Even with reference to the effector and signal molecules reported in plants, there are many problems which have to be sorted out before coming to a conclusion that they are similar or at least homologous to the effector and signal molecules of animal PCD. The expression of CyPs in some plant cells has not been proved to be exclusively operative during cell death. Neither have their substrates been identified beyond doubt. Their specificity is also open to question. Moreover, their homology to the animal caspases is also very low. One of the common substrates of animal caspases, the PARP, has not been shown to undergo cleavage in any plant PCD so far. Moreover, two types of PARPs are so far known in plants unlike in animals where there is only one type. These PARPs are not shown to be definitely involved in PCD; they are likely to be responsible for the DNA repair, and perhaps also in secondary metabolism, especially in the induction and activity of phenylalanine ammonia lyase (PAL) activity. PAL is a very vital enzyme involved in lignification, stress metabolism, necrosis and wound healing. The same may be said of the ROS like H_2O_2, which is a key substance during lignification, peroxidase and polyamine oxidase activation, wound healing and necrosis.

Increase in cytosolic calcium concentration is a feature commonly reported for both plant and animal PCD. It has been claimed that Ca^{2+} activates endonucleases and caspases in animals. While it is possible for the involvement of Ca^{2+} in the activation of endonucleases in plants, there is no report yet of Ca^{2+}-activated protease in plant PCD. The endonuclease that is involved in animal PCD is Ca^{2+} and/or Mg^{2+}-dependent only, but the endonucleases so far reported in plants are activated by Ca^{2+} or Zn^{2+} or by both.

Endonucleases of animal PCD act on DNA of dying cells and result in DNA fragmentation of more or less 180 bp units (oligonucleasomal fragments). In some categories of plant PCD, DNA ladder formation as well as TUNEL staining were reported, while in others they were not. There are also discrepancies in the size of the DNA fragments obtained. There are reports of 180 bp, 140 bp and 50,000 bp in different types of dying cells in plants. In TEs and fibres the nucleus instead of undergoing DNA fragmentation often breaks into two or more nuclei. All these indicate that there is a spectrum of variation in the

Table 2. Comparison of PCD in animals and plants

Animals	Plants
All the eight categories of cells that undergo cell death are known in animals	Of the eight categories recognized, only six are represented in plants. Cells that are produced in excess and cells which through their death give rise to diseases are absent in plants
Cytoplasmic condensation, shrinkage and fragmentation are always noticed	Condensation and shrinkage of cytoplasm noticed, but not fragmentation. Reports of fragmentation are either due to artefacts or uncritical observation
Cells shrink	Cells shrink in most plant cell categories but not in differentiating TEs, fibres and sclerids
Cell corpses are engulfed and eliminated through phagocytosis by neighbouring cells or macrophages	Cell corpses persist due to the persistence of cell wall; in TEs, fibres and sclerids cell corpses not only persist in a distinctive manner, but start functioning only then. Dead cytoplasm almost always eliminated by vacuolar autophagy; elimination by plastolysomes is also likely
PCD is due to a syndrome of well organized and executed mechanisms involving effectors, adaptors, regulators and signals	The mechanism underlying plant PCD is incompletely known; so far no adaptor or regulator molecule has been known; with regard to effector and signal molecules there are many problems
Effector caspases (cysteine proteases) and Granzymes are activated and expressed; they specifically cleave after the aspartate residues in proteins	Expression of cysteine proteinases is reported in some cases, but not exclusively during cell death; their role in scavenging the proteins of dead cytoplasm cannot be ruled out; substrates for some of the expressed cysteine proteinases are not yet definitely shown; their specificity is also a question; their homology to animal caspases is also very low
Cleavage of PARP to the signature of 89 kDa apoptotic fragment is a hallmark; there is only one type of PARP in animals	There have been no reports yet of the cleavage of PARP. Two types of PARPs are so far known respectively in *Zea mays* and *Arabidopsis thaliana*, although abundance of PARP is generally very limited in plants
The antiapoptotic protein BCl-x_L suppresses PCD at least in some cells	Bcl-x_L does not suppress PCD associated with HR
ROS like O_2 and H_2O_2 as signalling molecules are required to activate PCD	O_2 and H_2O_2 are implicated in cell death, especially in HR responses due to biotic and abiotic stresses; evidences, however, are not conclusive enough. The reported involvement of H_2O_2 in TE death and HR should be viewed with caution
Increase in cytosolic Ca^{2+} can activate PCD through the activation of endonucleases and caspases	Increase in cytosolic Ca^{2+} can activate PCD, probably through the activation of endonucleases; there is no report yet of Ca^{2+} activated proteinase in plant PCD
The role of mitochondria in executing PCD is well deciphered	The role, if any, of mitochondria in PCD is to be substantiated, although there are one or two reports implicating it
Plasma membrane blebbing is common	Plasma membrane blebbing has so far not been reported
There is an increased exposure of the acidic phospholipid phosphotidyl serine (PS) on the outer surface of the plasma membrane, which can be demonstrated by Annexin V binding	So far PS exposure has been shown through Annexin V binding, only in the protoplasts of tobacco subjected to abiotic stresses
Protein phosphorylation/dephosphorylation changes common	Protein phosphorylation/dephosphorylation changes reported only in cells subjected to hypoxia and HR, and in aleurone cells
Growth factor deprivation (GFD) promotes cell death	There are reports of GFD-promoting cell death in some categories of plant cells; contradictory results are obtained in some cells (where supply of growth regulators promotes cell death)
Chromatin condensation noticed	Chromatin condensation noticed in some categories of dying cells, but not in all
Systematic DNA cleavage and fragmentation demonstrated through electrophoretic ladder formation and through TUNEL staining; Fragmentation takes place at the nucleosome linker sites to result in oligonucleosomal fragments	DNA cleavage and fragmentation demonstrated through electrophoretic ladder formation and through TUNEL staining only in some categories of cells but not in all
DNA fragments of more or less 180 bp	DNA fragments of 50 kb (50,000 bp) in some cells and 0.14 kb (140 bp) in some cells are reported. DNA fragmentation is not likely to occur in differentiating TEs, fibres and sclerids
Ca^{2+}-dependent endonucleases are shown to be responsible for DNA processing and fragmentation; in almost all instances (excepting one case in *C. elegans*) the nuclease is the product of the dying cell itself	Nucleases reported in some dying plant cells; but there is yet no direct evidence of their involvement in PCD. Plant nucleases are either Ca^{2+} or Zn^{2+}-dependent. Some nucleases are activated by both Ca^{2+} and Zn^{2+}. Nucleases may be the product of the dying cell itself or may be transported from adjacent cells
Typical apoptotic bodies each consisting of some cytoplasm and an oligonucleosomal DNA bit are formed	There is no instance where typical apoptoic bodies are yet reported
Stress proteins are not synthesized during cell death	Stress proteins such as hydroxyproline, glycine, arabinogalactan, and theonine-rich proteins are often synthesized and become integral components of cell walls of some categories of cells undergoing death.

size of the fragmented DNA material in plants, although in animal PCD, a very great uniformity exists in the size of DNA fragments.

There are also problems relating to other aspects of PCD. The role of mitochondria in executing PCD in animal cells is not elucidated so far in plants. Plasma membrane blebbing common in animal PCD has not been shown in plants so far.

Thus a few conclusions can be made. There are likely to be inherent differences in the operational mechanism of PCD between plants and animals; there is also the possibility for the involvement of different operational mechanisms of PCD in different plant cell types, i.e. more than one pathway of PCD is likely to be operative in plants, while in animals there seems to be only one programme. No plant system is yet described which shows all features common to animal PCD. There are few similarities between the two, particularly the conservation of the apoptotic ATPases[108], indicating either the derivation of both PCD mechanisms from a common ancestor such as unicellular eukaryotes or even bacteria[108], or a parallel evolution of PCD mechanisms in the two groups. Plant PCD work is at an infant stage and much more has to be done before coming to any valid conclusion regarding the relationships between the plant and animal PCDs.

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