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Programmed cell death in plants: distinguishing between different modes

Theresa J. Reape, Elizabeth M. Molony and Paul F. McCabe*

School of Biology and Environmental Science, University College Dublin, Dublin 4, Republic of Ireland

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Abstract

Programmed cell death (PCD) in plants is a crucial component of development and defence mechanisms. In animals, different types of cell death (apoptosis, autophagy, and necrosis) have been distinguished morphologically and discussed in these morphological terms. PCD is largely used to describe the processes of apoptosis and autophagy (although some use PCD and apoptosis interchangeably) while necrosis is generally described as a chaotic and uncontrolled mode of death. In plants, the term PCD is widely used to describe most instances of death observed. At present, there is a vast array of plant cell culture models and developmental systems being studied by different research groups and it is clear from what is described in this mass of literature that, as with animals, there does not appear to be just one type of PCD in plants. It is fundamentally important to be able to distinguish between different types of cell death for several reasons. For example, it is clear that, in cell culture systems, the window of time in which ‘PCD’ is studied by different groups varies hugely and this can have profound effects on the interpretation of data and complicates attempts to compare different researcher’s data. In addition, different types of PCD will probably have different regulators and modes of death. For this reason, in plant cell cultures an apoptotic-like PCD (AL-PCD) has been identified that is fairly rapid and results in a distinct corpse morphology which is visible 4–6 h after release of cytochrome c and other apoptogenic proteins. This type of morphology, distinct from autophagy and from necrosis, has also been observed in examples of plant development. In this review, our model system and how it is used to distinguish specifically between AL-PCD and necrosis will be discussed. The different types of PCD observed in plants will also be discussed and the importance of distinguishing between different forms of cell death will be highlighted.

Key words: Apoptosis, apoptosis-like programmed cell death (AL-PCD), Arabidopsis, autophagy, mitochondria, necrosis, programmed cell death (PCD).

Introduction

Programmed cell death (PCD) has been defined as a sequence of (potentially interruptible) events that lead to the controlled and organized destruction of the cell (Lockshin and Zakeri, 2004). It is crucial for defence responses to restrict the spread of pathogens and for proper development of the multicellular body plan (Lam, 2004). Ultrastructural studies by Kerr and co-workers originally described two distinct forms of cell death (Kerr et al., 1972): apoptosis and necrosis. Apoptosis was initially described in very specific morphological terms (Kerr et al., 1972) and still is characterized by cell shrinkage, nuclear condensation and fragmentation, and eventually the breakup of the cell into ‘apoptotic bodies’ (Adrain and Martin, 2001). The term apoptosis was specifically used to distinguish between this form of controlled cell death and necrosis. A great deal is now known about the biochemical events that occur during apoptosis and it remains the best defined form of animal PCD. For example, apoptotic cellular destruction is driven by activation of a family of cysteine proteases known as caspases (Wolf and Green, 1999). Activation of these caspases can occur through extrinsic death receptors, or alternatively, intrinsic pathways, controlled in part by the mitochondria and the release of cytochrome c. This release of cytochrome c drives the assembly of the

* To whom correspondence should be addressed. E-mail: paul.mccabe@ucd.ie

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apoptosome, a caspase activating complex in the cytoplasm. Many key regulators of apoptosis have been identified and shown to promote or inhibit the loss of mitochondrial integrity (Green and Reed, 1998; Adrain and Martin, 2001). In contrast to apoptosis, necrosis has been described as an uncontrolled form of cell death which often follows overwhelming cellular stress where the cell is unable to activate its apoptotic pathways. During necrosis, swelling rather than shrinkage is the defining feature of the morphological change. This swelling is due to the cell losing the ability to osmoregulate, resulting in water and ions flooding into the cell (Lennon et al., 1991).

More recently, cell death in animal systems has been subdivided into three categories: apoptosis (Type I), autophagic cell death (Type II), and necrosis (Type III) (Lockshin and Zakeri, 2004; Bras et al., 2005). This system of classification provides an important framework for the study of PCD. Autophagy, or Type II cell death, appears distinct from apoptosis or necrosis (Schweichel and Merker, 1973). The term autophagy is derived from the Latin meaning ‘self-eating’ and describes the process whereby cytoplasmic materials are degraded through the lysosomal machinery within the cell. It is characterized mainly by the formation of autophagic vacuoles and also dilatation of the mitochondria and endoplasmic reticulum and slight enlargement of the Golgi (Bras et al., 2005). However, cells with enlarged vacuoles and undergoing autophagy do not necessarily die as the process is involved in the routine turnover of cell constituents. It is important, therefore, to use the term ‘autophagy/Type II cell death’ only when there is a fatal destruction of the cell.

In plants, PCD is a facet of a wide range of developmental programmes which vary from the beginning of the plant’s life cycle, through essential development (xylogenesis), right until the end of the plant life cycle (senescence) (Beers, 1997; Rogers, 2005). As with animals, it is involved in pathogen (hypersensitive response) and stress (e.g. aerenchyma formation) responses (Pennell and Lamb, 1997; van Doorn and Woltering, 2005). Indeed, as with animals it may be that cell death is as fundamentally important to plants as cell division. PCD is used to describe nearly all instances of cell death observed in plants, invariably leading to much confusion. Understanding research in the field of plant PCD would benefit from well-defined cell death classifications, while being aware that there is probably much overlap between the different forms of cell death. The importance of this overlap between the different cell death types and the decisions involved in determining the cell’s fate is being re-highlighted (Lockshin and Zakeri, 2004; Bras et al., 2005). This review will concentrate on comparing and contrasting the different forms of PCD that occur in plant cells.

Recognizing different modes of death in cell culture

The distinction between apoptosis and necrosis in cell culture is one of timing and severity of insult (Lockshin and Zakeri, 2004). Indeed, Lennon et al. (1991) showed in animal cells that either apoptosis or necrosis can be experimentally induced by varying the levels of stress used to induce death. They subjected tumour cells to ranges of noxious stimuli and were able to show that at a low level of stress, cells repair cell damage, while higher levels lead to organized controlled apoptosis (the plasma membrane retains its integrity and the cell shrinks) and higher levels again lead to uncontrolled chaotic necrosis (the plasma membrane loses its ability to osmoregulate, water enters the cell, and the cell bursts). Similarly, low doses of ultraviolet-B irradiation induce apoptosis in keratinocyte cells while higher doses cause necrosis (Mammone et al., 2000).

In plants, a similar approach was taken by McCabe et al. (1997) where carrot cells were subjected to various levels of heat stress. The authors noted that cell death at temperatures up to 55 °C resulted in a cell corpse with a very specific cellular morphology. The most obvious feature of this morphology consisted of a retraction of the protoplast away from the cell wall. If the findings of Lennon et al. (1991) applied to plant cells, then it followed that if this retraction was a hallmark of PCD, it should be absent at higher necrotic stress levels. This was indeed the case, and at temperatures above 55 °C the incidence of this morphology declined markedly and above 75 °C cells died without any cytoplasmic withdrawal. This demonstrated that the withdrawal of the cytoplasm was an active result of PCD rather than a mere collapse of the cell in on itself.

The strict definition for apoptosis states that the cell eventually breaks up into apoptotic bodies that can then be engulfed by phagocytes. While there are recorded instances of plant cells breaking up into apoptotic bodies it is not a common feature of plant cell death (McCabe and Pennell, 1996). However, other hallmarks of apoptosis are evident during some plant cell deaths. For example, while there is no evidence for classical caspases in the Arabidopsis genome, caspase-like molecules are being discovered, and caspase substrates are cleaved during plant PCD (reviewed by Woltering, 2004; Sanmartín et al., 2005). In addition, DNA is cleaved and several genes implicated in apoptosis seem to function in plant PCD (Danon et al., 2000). So, during some plant cell deaths there are striking similarities with apoptosis, but at the same time there are also differences. Therefore it has been suggested that this morphologically distinct type of PCD in plants should be termed apoptotic-like PCD (AL-PCD) (Danon et al., 2000). If this term is used it acknowledges that there are similarities (that may have
The importance of recognizing a morphologically defined PCD in plants

The primary importance of a morphologically defined PCD is that it provides researchers with a common reference point. Our studies suggest AL-PCD, while slower than animal apoptosis, is still a fairly rapid process, being initiated and finishing in as little as 6 h (McCabe et al., 1997; Balk et al., 2003; Burbridge et al., 2007). Research findings from different groups are much easier to compare if they are defined by this 6 h window of apoptotic-like morphological change. In addition, using the morphological change as a marker of PCD allows researchers to differentiate between apoptotic-like and necrotic or autophagic cell death. This is important as AL-PCD may respond to specific signalling molecules, gene regulation, caspase inhibitors, etc, while necrotic or autophagic cell death may not. If studies record death without differentiating between modes of cell death they may develop contradictory conclusions as to the role of certain molecules in the pathway of AL-PCD. A scan through recent literature describing agents/compounds, which have effects on plant ‘PCD’ reveals many researchers’ endpoint measure of ‘PCD’ to be merely a lack of cell viability. Viability stains will not differentiate between AL-PCD and necrosis. In addition, when PCD is being investigated in tissue culture systems, it is crucial to recognize the fact that the eventual fate of an apoptotic or autophagic cell will always be necrosis as it is not being consumed by phagocytes and so it will ultimately lyse (Lockshin and Zakeri, 2004). Therefore, as is the case of animal systems, the time point at which you examine the effect of your insult and the severity of that insult will govern what type of PCD is being investigated. On the other hand, it is argued by some that necrosis is not merely an uncontrolled chaotic event but a programmed one (Proskuryakov et al., 2003; Golstein and Kroemer, 2006). This concept requires further investigation in plant systems that can distinguish between different types of PCD. It is therefore essential that there are defined markers that can be used to demonstrate what type of PCD has been triggered in the cells under investigation.

Distinguishing AL-PCD and necrosis in Arabidopsis cells

Obviously, understanding the control mechanisms of PCD in plants has become the focus of intensive research. However, studying the mechanism of PCD in whole plants can be difficult as it often occurs in a small group of inaccessible cells buried in a bulk of surrounding healthy tissue, for example, tapetal cell death in anther tissues (Balk and Leaver, 2001). Instead of studying PCD in vivo Arabidopsis suspension cultures have been chosen as the cell model to study plant PCD, and some time has been spent characterizing this particular mode of PCD. This cell culture-induced PCD is often referred to as AL-PCD, as there is manifestation of some of the hallmarks of mammalian apoptosis, such as the release of cytochrome c, DNA degradation, and distinct morphology (Balk et al., 1999, 2003; McCabe and Leaver, 2000). There are a number of reasons that make cell cultures an important model system with which to study the mechanism of PCD, for example, (i) uniformity of cultures: which are rapidly dividing homogeneous collections of cells, (ii) accessibility of the cultures: as it is easy synchronously to activate cell death in the whole population, and then, using a microscope and markers of cell death, follow the progression of the cell death process in real time, and (iii) reduced complexity: the fact that they are undifferentiated and can be reduced to single cells or protoplasts with ease. The question as to whether PCD utilizes a ‘core’ mechanism whether activated in vivo or in vitro remains unanswered, however, as, with animal cells, it is most probable that PCD activated in a cell culture shares fundamental regulatory mechanisms with whole plant developmental or defence-related PCD.

As mentioned earlier, agents that cause apoptosis can also cause necrosis, and the distinction between the two forms of cell death in cultures depends on the severity of the insult (Lennon et al., 1991; McCabe et al., 1997; O’Brien et al., 1998; Mammone et al., 2000). It is therefore crucial to be able to distinguish between these two forms of cell death in a specified time frame if meaningful results are to be obtained. McCabe and co-workers have developed a heat-inducible cell death assay within which they can distinguish between living cells, cells undergoing AL-PCD, and those undergoing necrosis (McCabe et al., 1997; McCabe and Leaver, 2000; Burbridge et al., 2007). A moderate heat stress causes most of the cells to die via AL-PCD while higher temperatures cause cells to die via necrosis (Fig. 1A). The most obvious morphological change during AL-PCD is...
the condensation of the protoplast away from the cell wall. Fluorescein diacetate (FDA) fluoresces in living cells, and an absence of fluorescence indicates cell death. FDA can be used in conjunction with morphology scores to discern the mode of cell death. No fluorescence along with cytoplasmic condensation implies that the cell has undergone PCD. No fluorescence, and no cytoplasmic retraction indicates that the cell has died necrotically without...
activating PCD (Fig. 1B). DNA cleavage is also a marker of AL-PCD as PCD-activated nuclease cleaves DNA at linker sites between nucleosomes, resulting in DNA fragments that are multimers of approximately 180 bp and run as a ‘ladder’ pattern when separated by electrophoresis in agarose gels (Mittler and Lam, 1997; Danon and Gallois, 1998). This method can also be used to distinguish between AL-PCD and necrosis. Carrot cells subjected to heat shock at 55 °C do not display DNA fragmentation immediately following heat shock. DNA fragmentation can only be detected 3–5 h after heat treatment, presumably because nucleases have to be activated and take time to degrade DNA. However, in cells exposed to higher temperatures which cause necrosis, DNA is degraded immediately (due to temperature effects rather than nuclease activation) and runs as a smear on an agarose gel (McCabe et al., 1997). While DNA fragmentation provides a useful indicator of PCD, the morphology/FDA approach is much more rapid and convenient for scoring large numbers of samples and treatments. This method has been used successfully in carrot (McCabe et al., 1997), Arabidopsis (McCabe and Leaver, 2000), and tobacco cells (Burbridge et al., 2007).

Apoptotic-like PCD and necrotic cell death thresholds

As discussed earlier, the level of stress that a cell is exposed to can be a critical determinant on the ultimate fate of the cell. In a population of cells subjected to low level stresses the majority will survive, when subjected to moderate levels of stress the majority execute PCD, while high levels of stress induce widespread necrosis. Being able accurately to report the percentage of cells remaining alive or undergoing PCD or necrosis is essential in determining the effect various treatments have on stress or PCD responses. To illustrate this, three hypothetical experiments are now discussed (Fig. 2).

In scenario A (A1–wild-type cells, and A2–transgenic overexpression gene that confers stress tolerance) cells are exposed to moderate stress (e.g. 50 °C for 10 min). Most of the wild-type cells undergo PCD, however, a sub-population undergo necrosis, while a percentage survive the treatment (Fig. 2, A1). A2 is a transgenic cell line which is overexpressing a gene conferring stress tolerance on the entire population. When these cells are exposed to the same moderate stress (50 °C for 10 min) a greater number of cells survive compared with the wild-type, while necrotic levels fall. In this scenario PCD numbers stay unchanged (cells surviving rather than undergoing PCD equal the number of cells undergoing PCD instead of necrosis). In this experiment if you were to only assess the number of cells undergoing PCD (e.g. an assay for caspase substrate cleavage) there would be no difference between the A1 and A2 cell lines, suggesting that the gene has no effect on stress levels. However, counting all three (living/PCD/necrosis) states show there is a significant difference between the wild type and the transgenic line.

Scenario B (B1 and B2 cells) examines the same two cell lines (wild type and transgenic overexpressing gene that confers stress tolerance) having been subjected to a high stress level (e.g. 60 °C for 10 min). The stress treatment results in cells undergoing either PCD or necrosis only (no survivors), and adding a factor (e.g. transgenic gene product) that reduces the stress may simply shift cells from necrosis to PCD. In this scenario, if cell viability (e.g. FDA staining) is the only way in which cells are assessed there is no difference between absolute death levels in the cell lines (Fig. 2, B1 and B2). However, scoring necrosis and AL-PCD rates (e.g. FDA and morphology) would demonstrate that the transgenic line is responding significantly differently to the stress than the wild-type cell line.

An example of the above scenario was demonstrated in a study by Burbridge et al. (2007). They used both FDA and morphological changes associated with AL-PCD to monitor changes in transgenic cultures of tobacco with high levels of peroxidase activity. Peroxidases can regulate ROS within a cell and ROS has been implicated in both signalling and triggering of PCD (Burbridge et al., 2007). Using the heat shock assay, the authors showed that, compared with wild-type cells, transgenic cultures with elevated levels of peroxidase have higher PCD rates up to 55 °C, and lower PCD rates above 55 °C. The different threshold effects can explain this apparent contradiction. Presumably over-expression of a peroxidase gene has a significant effect on reactive oxygen species (ROS).
levels in the cell and altering ROS levels can cause alive/PCD/necrosis threshold changes as it alters cellular stress levels. If peroxidase overproducing cells have higher ROS levels than wild-type cells, they will be more sensitive than the wild-type cell to PCD-inducing stresses. At temperatures below 55 °C the effect of peroxidase overexpression will be that more transgenic cells die and so more cells undergo AL-PCD than in wild-type cultures. However, at temperatures above 55 °C, all cells will die either by necrosis or AL-PCD. Higher ROS levels will cause more of the transgenic population to undergo necrosis rather than AL-PCD so PCD rates are seen to fall compared with the wild type above 55 °C. This conclusion could not be arrived at if cell viability (FDA alone) had been the endpoint measure of PCD.

Being able to identify whether a cell has died via PCD or necrosis can also determine whether treatments change PCD rates by acting specifically on the PCD pathway. Altering the general stress levels will alter the rates of PCD/necrosis, however, if the treatment is more specific and interacts directly with the PCD pathway, then PCD numbers will change, but there should be no effect on necrosis levels. This is illustrated in scenario C (Fig. 2, C1 and C2), where the transgenic (Fig. 2, C2) is expressing a gene product that specifically interacts with the cell death programme rather than affecting general stress responses. In this case, scoring all three cell states shows that necrotic death levels are unaffected by expression of this gene whereas PCD levels fall significantly and living cells increase. Monitoring this uncoupling of PCD and necrosis can therefore be used as an indicator of whether a treatment interacts specifically with the cell death programme or has a more general effect on a cells stress status.

Countdown to death: the timing of apoptotic-like PCD-associated events following initiation of death

As all the components in the plant cell death process have not yet been identified it is difficult precisely to track the events that occur following the triggering of AL-PCD and subsequent degradation of the cell. However, there have been a few studies that have used biotic and abiotic stresses synchronously to initiate AL-PCD in plant cells and have monitored the timings of the key events that lead to cell death. Lanthanum chloride can block AL-PCD if added before the heat shock (McCabe et al., 1997), but recent results from our laboratory have shown that it has no effect if added after the heat stress, suggesting that a calcium influx is an early event (and possible trigger) in the cell death process (EM Molony, PF McCabe, unpublished results).

Release of apoptogenic proteins from the mitochondria is regarded as a hallmark feature of animal apoptosis and is also observed in AL-PCD. Cytochrome c is released from the mitochondria almost immediately following heat stress in cucumber cotyledons (Balk et al., 1999), with almost all the cytochrome c found in the cytoplasm after 1 h and no evidence of any cytochrome c remaining in the mitochondria 3 h after the heat stress. Similarly, cytochrome c relocation occurs almost immediately following self-incompatibility induction (to prevent self fertilization) in incompatible pollen tubes (Thomas and Franklin-Tong, 2004).

Caspase-like activity is also associated with AL-PCD and Danon et al. (2004) were able to show that a protease (DEVDase) was activated within 1 h of PCD induced by ultraviolet-C overexposure. Similarly, a caspase-like activity was detected within 1 h of induction of the hypersensitive response (a cellular response to avirulent pathogens that terminates in the rapid death of infected or challenged cells) in tobacco cells infected with tobacco mosaic virus (Chichkova et al., 2004).

In animal cells, loss of mitochondrial transmembrane potential is often proposed as the committal step during PCD (Curtis and Wolpert, 2004). Curtis and Wolpert (2004) determined the timing of cell death events following the treatment of oat seedlings with victorin, a host-selective toxin produced by Cochliobolus victoriae. They were able to show that a victorin-induced collapse in mitochondrial transmembrane potential (∆ψm), indicative of a mitochondrial permeability transition, occurred in a significant population of the cells’ mitochondria. This collapse in mitochondrial transmembrane potential occurred over 2–4 h following treatment of cells in the abaxial mesophyll layer. The authors speculated that the loss of ∆ψm in one subpopulation of mitochondria could activate PCD through the release of apoptogenic proteins, while a second subpopulation of mitochondria, which does not lose ∆ψm, may generate the energy necessary for PCD to proceed.

DNA degradation is also a feature of AL-PCD, and poly(ADP-ribose) polymerase (PARP), which is involved in DNA repair and is a classic substrate for caspase-3 activity, is cleaved within 2 h of self-incompatibility induction (Thomas and Franklin-Tong, 2004) while there is DNA fragmentation after 4 h (Jordan et al., 2000). Using a cell free system, Balk et al. (2003) demonstrated that a Mg2+-dependent nuclease activity, relocated from the mitochondrial intermembrane space, was responsible for DNA degradation. This degradation began within 3 h, with the generation of 30 kb fragments and subsequent chromatin condensation occurred within 6 h. In the cell free system, the appearance of internucleosomal fragments is a slower process, taking place after 12 h (Balk et al., 2003).

Morphologically, retraction of the protoplast from the cell wall in carrot begins within 3 h of heat stress and is essentially complete within 6 h (McCabe et al., 1997).
Similarly, cytoplasmic condensation is detected within 4 h of a heat stress in tobacco cells (Burbridge et al., 2007), and protoplast shrinkage of victorin-treated oat abaxial mesophyll cells occurs within 4 h of treatment (Curtis and Wolpert, 2004). In addition, the latter authors also showed that the loss of $\Delta\psi_m$ in the predominant population of mitochondria preceded shrinkage by 20–35 min.

**Morphologies associated with developmental cell death**

Although AL-PCD morphology is commonly described during the hypersensitive response in plants (Levine et al., 1996; Yano et al., 1998; Curtis and Wolpert, 2004), during normal plant growth and developmental PCD much of the morphology described in the literature conforms to the mammalian Type II/autophagic PCD (i.e. formation of large vacuole which ruptures to release hydrolytic enzymes that degrade cellular contents: see reviews by Beers, 1997; Rogers, 2005; van Doorn and Woltering, 2005). Some authors would go as far as to say that autophagic PCD is the only form of PCD in plants (Bozhkov and Jansson, 2007). However, in animals it is clear that the distinction between apoptosis and autophagic PCD may not be so straightforward. For example, during the PCD of the salivary gland in *Drosophila* the first 90% of cell collapse is autophagic, but once the cytoplasm has almost entirely been removed, the cell displays many apoptotic features including mitochondrial depolarization and caspase-like enzyme activation (Lockshin and Zakeri, 2004). This is also observed during PCD of metamorphosing labial glands in *Manducas sexta*. In addition, high doses of tamoxifen induces necrotic cell death in human mammary carcinoma cells while, at lower doses, an autophagic cell death (with vacuole formation) was found to precede apoptotic-like nuclear condensation (Bursch et al., 1996).

In several cases of developmental cell death in plants, morphological descriptions of dead or dying cells are not typical of autophagy but rather conform to AL-PCD. For example, tapetum development of angiosperms results in extremely late in the cell death process. Suspensor-like cells in embrogenic carrot cultures also display the condensed morphology associated with AL-PCD (Havel and Durzan, 1996; McCabe et al., 1997), and a recent study by Lombardi et al. (2007), indicates that degeneration of the finally differentiated *Phaseolus coccineus* suspensor involves cytochrome c release and activation of caspase-like proteases. Cytoplasmic retraction occurs in 14-d-old senescing *Arabidopsis* cell cultures (Swidinski et al., 2002) and it is also a feature of senescence cucumber cotyledon cells in whole plants (Delorme et al., 2000). Interestingly, no AL-PCD morphology or DNA laddering was found until 12 h before the death of the organ. When AL-PCD was activated it was under similar timelines to AL-PCD after heat stress suggesting that AL-PCD operates as either the final instalment of senescence, or is initiated as soon as senescence has finished (Delorme et al., 2000).

**Mitochondria: central regulators in all types of cell death?**

The heat shock graph (Fig. 1A) shows a very dramatic switch from live cells to PCD to necrosis. How can this be regulated in plant cells? One possible regulator of all three states is the mitochondrion and more specifically the permeability of the outer mitochondrial membrane. It is now well established that the mitochondria have a central regulatory role in integrating stress and/or PCD signals in both plant and animal PCD (see reviews by Green and Reed, 1998; Jones, 2000; Bras et al., 2005; Diamond and McCabe, 2007). The release of apoptogenic proteins from the mitochondrial inner membrane space (IMS) triggers apoptosis in mammalian cells (Wang, 2001; Adrain and Martin, 2001) and release of these apoptogenic proteins occurs via a Bax/Bcl-2 controlled pore (Shimizu et al., 1999) or the opening of the permeability transition (PT) pore (Green and Reed, 1998). In mammalian cells the Bax/Bcl-2 pore can selectively release IMS proteins that activate caspases. However, there is no evidence that the Bax/Bcl-2 family normally operates in plant cells although there is evidence for a mitochondrial release of PCD activating molecules following a moderate level 55 °C heat shock (Balk et al., 2003). The other way that mitochondria may release PCD activating molecules in animal cells is through the formation of the PT pore (Green and Reed, 1998). So, while it appears that plants lack the classical caspase family required for true apoptotic death, there is strong evidence that the PT pore operates in plant cells (Arpagaus et al., 2002; Maxwell et al., 2002; Yu et al., 2002), and three important components of the PT pore in animals (voltage-dependent anion channel, adenine nucleotide transporter, and cyclophilin D) are present in plants (Yao et al., 2004). It has been proposed that, in animal cells, a moderate loss of membrane potential would lead to a release of mitochondrial IMS proteins such as cytochrome c, leading to activation of caspase-like molecules and resulting in apoptosis. However, a more severe loss of membrane potential would lead to catastrophic damage to the cell.
and result in necrosis (Hirsch et al., 1997). The activation of the PT pore in plant cells would explain the abrupt switch from PCD to necrosis seen in the heat-shocked cells when the level of stress is increased, as the thresholds for living/PCD/necrotic states would be regulated by opening of a mitochondrial pore. Another hypothesis by Lemasters et al. (1998) suggests that cells respond to mitochondrial permeability transition (MPT) in a graded manner, with autophagy resulting when MPT occurs only in small number of cells leading to lysosomal degradation of the affected mitochondria and removal of the signals required for stimulation of autophagy. Apoptosis occurs when larger numbers of mitochondria are permeabilized, probably through increased release of apoptogenic factors such as cytochrome c and apoptosis-inducing factor (AIF). When virtually all mitochondria are permeabilized and ATP becomes depleted, necrotic death is the outcome. In plants, with apoptotic machinery not available, alternative modes of cell death are switched on. Permeabilization of mitochondria during a moderate stress in plant cells, resulting in the release of apoptogenic factors (but no canonical caspases), could explain why the release of cytochrome c has been widely observed during PCD in plant systems (Balk et al., 1999, 2003; Stein and Hansen, 1999; Sun et al., 1999; Balk and Leaver, 2001; Krause and Durner, 2004; Thomas and Franklin-Tong, 2004; Yao et al., 2004; Vacca et al., 2006), but there is little evidence supporting cytochrome c as a direct activator of plant PCD (Yu et al., 2002; Balk et al., 2003; Vacca et al., 2006). Interestingly, caspase-independent cell death (CICD) in mammalian systems resulting in alternative death morphotypes is the focus of much attention (Chipuk and Green, 2005; Kroemer and Martin, 2005) as it has become clear that pharmacological inhibitors of caspases do not always prevent cell death. In plants, unravelling the pathways of PCD may reveal an evolutionary conserved pathway in eukaryotic cells and aid in the understanding of CICD in animals.

Conclusions

There is no doubt as to the importance of PCD to plant growth and development and finding ways to manipulate plant PCD pathways may have agronomic benefits. Moving the field of plant PCD forward involves giving serious thought as to what form of cell death is actually being studied in a chosen system while remaining aware that these pathways may overlap. The elucidation of molecular pathways of caspase-dependent apoptotic cell death and the domination of this research in studying eukaryotic PCD has led to the term apoptosis being used interchangeably with PCD. However, in plants, where true apoptosis doesn’t appear to exist, this has led many researchers to use PCD as the ‘blanket’ term for different modes of cell death, even though it is clear from the literature that different morphologies exist. Having reviewed the field of plant ‘PCD’ it is apparent that at least three forms of cell death are described: autophagy, AL-PCD, and necrosis. The most common form of PCD induced in cell culture systems is AL-PCD, with necrosis occurring at higher levels of insult. Autophagy appears to be associated with the starvation response in cultured cells. While AL-PCD has been shown to occur in both developmental death and hypersensitive response-induced death, it is not known if it occurs during all developmental deaths. Is autophagy the more common form of death during development? This may make sense during pre-programmed events during the life cycle of the plant where there is time to recycle the cell’s nutrients before death. Or are autophagy and AL-PCD more closely related as seen during the PCD of the Drosophila salivary gland? Are all of these deaths regulated by manipulation of the PT pore in mitochondria? These questions remain to be answered but in order to be answered, we first need to recognize and confidently identify different modes of dying in plants.

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