Programmed Cell Death in Development and Defense

Alan M. Jones*

Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599–3280

THE CONCEPT OF PROGRAMMED CELL DEATH (PCD) CAME FROM PLANTS

Around the time of this journal's first volume, the concept of PCD, i.e. the cell's active participation in its own demise, was introduced using the example of a plant cell infected by a fungus (1). This was 7 decades before the flurry of apoptosis research in animals. Death during an incompatible interaction between a plant and a pathogen was proposed to function as a physical block to further pathogen ingress. This "program" concept profoundly influenced the mindset of a large number of physiologists studying cell death in various contexts for the rest of the century. Plant physiologists knew that cell death is essential for normal development. Carl Leopold made this point to the general scientific audience in his influential 1961 paper (16) by enumerating the evidence for the selective ecological and evolutionary fitness conferred by cell death in plants, its importance for normal plant physiology, and its control by the balance between both survival and death signals. His publication marked the revival of interest in PCD in the modern era, a decade before Kerr et al. (13) coined the term "apoptosis" to describe the first cell death morphotype in animal cells.

Three strands of research came together to shape today's research agenda about plant PCD: terminal differentiation, senescence, and disease resistance. Cell death fulfills several essential functions in plant development: (a) Senescence removes cells by recycling much of its carbon, nitrogen, and phosphorous; (b) cell death is important in sculpting tissues such as the formation of lysigenous aerenchyma, flower primordia during floral abortion, and aleurone layers during germination; (c) cells invaded by pathogens may be self eliminated as part of a hypersensitive response against the pathogen; (d) cell death also occurs during terminal differentiation and the classic example is the formation of vessel members and tracheids, collectively termed tracheary elements (TE); and (e) cell death is programmed when the metabolism of cells is perturbed either by coping with abiotic stresses imposed upon it or by bioengineering. Research up to the 1970s focused primarily on the cytology of death, biochemical characterization of dying cells, and the discovery of survival- and death-inducing signals. The 1980s saw the introduc-tion of genetics to study cell death and the last decade focused primarily on identifying the signal intermediates in this pathway. I intend to take a broad view of plant cell death, and from it, identify features of cell death that are shared among all PCDs. The conclusion will be a testable model on how death is triggered and the corpse managed.

CELL DEATH IS AN ACTIVE PROGRAM INDUCED BY SIGNALS

The evidence that death is an active program came first and primarily from work on leaf senescence. Yoshio Yoshida elegantly showed that the nucleus is required for cellular disassembly (21) and so it is not surprising that many labs independently showed that inhibitors of protein translation block leaf senescence. Non-senescencing (stay-green) mutants have been isolated indicating that components of plant PCD are genetically programmed and senescence has been shown to be reversible and regulated by signals such as hormones and light (18). For example, it is long known that cytokinin blocks senescence. This was shown more recently by a clever strategy from Amasino's group at the University of Wisconsin (Madison). They generated plants that do not senesce by simply engineering a cytokinin synthesis gene driven by a senescence-inducible promoter (7). In contrast to cytokinin, ethylene accelerates senescence consistent with the observed delay in senescence by ethylene-insensitive plants (8). In lesion formation of the hypersensitive response, evidence suggests that salicylic acid plays an early and a later potentiating role (19). These results taken together indicate unequivocally that plant PCD defines an active process of death, genetically dissectable and cytoplasmically driven. Moreover, it is now clear that plant cells integrate death and survival signals to make decisions when to die. As will be discussed further below, we now know that these signals also instruct cells how to process their own corpse.

An active program of death has also been shown for terminal differentiation, lysigenous aerenchyma formation, and aleurone degradation, although in all cases with much less rigor than by researchers working on senescence. Most of the evidence was published in the 1990s in the form of pharmacological studies showing that death can be inhibited, thus eliminating the possibility that death is a consequence of metabolic run-down. Moreover, these

---

* E-mail alan_jones@unc.edu; fax 919–962–1625.

studies have all pointed to a common role for calcium in every death pathway.

An in vitro culture of synchronously differentiating (and dying) cells developed by Hiroo Fukuda has had a major impact in our understanding of PCD during tracheary element differentiation (6). Andrew Groover found that death occurring in these cells is initiated by auxin and cytokinin and utilizes a defined signal transduction pathway that includes a calcium flux shown to be necessary and sufficient to initiate death (9). An extracellular signal triggers this calcium influx which leads toward the disruption of the vacuole as described further below. Malcolm Drew's group at Texas A&M University (College Station) has shown that ethylene is important for inducing cortical root cells to die and autolyze during lysigenous aerenchyma formation (11). Ethylene also evokes a signaling pathway that involves a calcium influx during aerenchyma formation that is necessary for this type of death. Doug Bush's lab at Rutgers University (Newark, NJ) has shown calcium increases prior to gibberellin-induced death of aleurone cells (15). Michele Heath of the University of Toronto has shown that calcium is also necessary for cell death induced by a fungus (20). It is clear that a major contribution from numerous labs during the 1990s is the preliminary characterization of signal elements within different plant PCD pathways and the consensus that calcium plays a common central role in death execution.

CELL DEATH, CORPSE MANAGEMENT, AND THE ROLE OF THE VACUOLE

Today, the concepts of cell death and cell corpse processing in animals are confused because the morphology of the dead (dying) cell is often used to describe the "type of cell death." For example, the presence of apoptotic bodies and DNA laddering are two hallmarks of apoptotic death, but they represent time points well beyond the "point of no return" and the moment of death. These features only define corpse processing and do not say anything about the execution of death. Decisions about corpse management based on the integration of various signals such as auxin, cytokinins, ethylene, and elicitors are probably made by the living cell long before the moment of death and probably even well before the point of no return. The ability to make these decisions is perhaps especially relevant to plant cells because corpse processing in plants is autolytic: The plant does not have the various macrophages and neutrophil cells to make these decisions for them. The last 75 years have yielded insight into the moment of death and how death is triggered in the various plant PCDs. From a compilation of cytological characterization of cell death in the hypersensitive response, terminal differentiation, and senescence, it becomes clear that a singular event is shared by all: the action of the vacuole.

The vacuole is a remarkably versatile organelle. By 1979, Boller and Kende confirmed Philippe Matile's hypothesis that the plant cell vacuole can transform into a large hydrolytic compartment (2). In the prior 2 decades, dramatic changes in the vacuole had been observed during senescence, the hypersensitive response, and terminal differentiation. To my knowledge, Cronshaw and Bouck (5) were the first to propose that the vacuole played a primary role in PCD of the developing tracheary element, but it was Andrew Groover (10), through his videomicroscopy, who clearly showed that collapse of the vacuole coincides with cessation of cytoplasmic streaming (moment of death?) and that this collapse marks the onset of autolysis during TE differentiation (see http://www.unc.edu/depts/joneslhp/pcd/). Vacuole collapse is regulated by the cell and not a result of metabolic rundown. For example, it does not occur during necrotic death. It is as if the cell has some molecular pin that "pops" the vacuole to trigger the release of sequestered hydrolases, effectively acting as a large suicide bomb detonated by calcium flux. Vacuole collapse and chromatin degradation revealed by TUNEL (TdT-mediated dUTP nick-and labeling) analysis turned out to be such robust markers of PCD in these cells (i.e. these markers confidently distinguish necrosis from PCD) that elements in the signal transduction of PCD were worked out. The vacuole collapse requires a calcium flux and artificial calcium influx prematurely triggered collapse followed by chromatin degradation in cells competent to undergo PCD. Malcolm Drew concluded similarly that loss of tonoplast integrity and calcium flux are two early events in lysigenous aerenchyma formation, which can also be viewed as a type of terminal differentiation even though the process leaves behind no corpse. Butler and Simon (3) compiled all ultrastructural information concerning dying cells that was published prior to 1971, essentially the bulk of what is known today about cytoplasmic changes during different PCDs. They noted that in all types of death, including senescence, the breakdown of the tonoplast is an early event. Peter Hepler and coworkers recently have documented the calcium flux in senescing cells (12). Tomiyama's lab, at Hokkaido National Agriculture Station (Japan), contributed tremendously to our understanding of the cytoplasmic events that occur leading up to death in the hypersensitive response (14). Michelle Heath, Elmon Schmelzer (Max-Planck Institute, Köln, Germany), and others have built on Tomiyama's original model by resolving in greater detail the spatial and temporal changes in the pathogen-challenged cytoplasm. Cells infected with fungal hyphae undergo cytoplasmic changes of which some are similar to changes occurring in senescing leaves. Cytoplasmic streaming slows during infection and eventually stops prior to protoplast collapse, which is interpreted by me to be the moment tonoplast integrity is breached. As al-
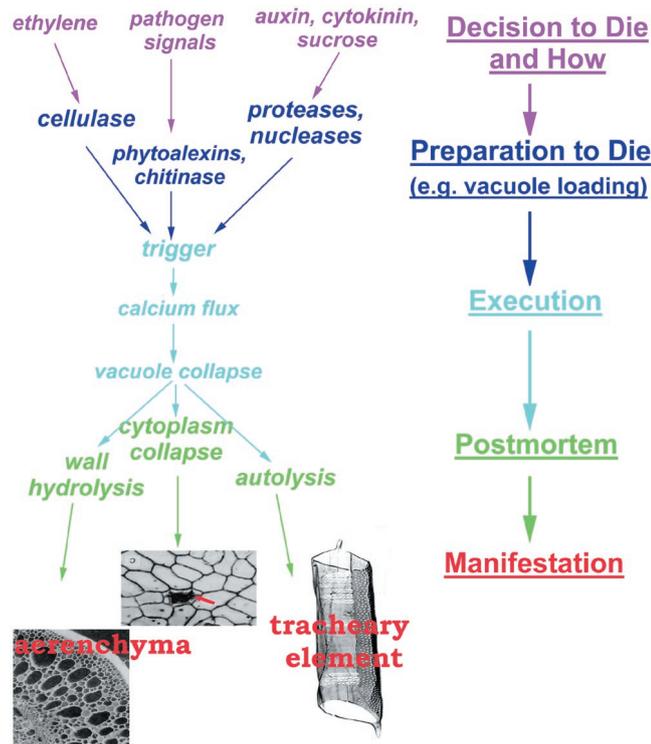
Figure 1. A model of the general mechanism of three PCDs in plants. Cells integrate various combinations of survival and death signals to decide whether to die and subsequently how the corpse will be managed. This acquired program of death (and corpse management) begins well before cells die. How the cell corpse is managed is a function of the profile of vacuole hydrolases (and toxins) that are loaded into the vacuole and these profiles are established by the original set of signals. Death is triggered and two events are shared among most plant cell deaths: calcium flux and vacuole collapse. Collapse of the vacuole marks the beginning of corpse management. The different profiles of hydrolases loaded into the vacuole determine the manifestation of death. For tracheary elements, the protoplasm but not secondary cell walls are autolyzed. During the formation of lysigenous aerenchyma, the entire corpse is removed, whereas the corpse from the hypersensitive response is left to be crushed by expanding tissues. Not shown is death and corpse management for senescing cells which shares all these features. However, many obvious signs of cell disassembly occur before vacuole disruption in senescent cells. This skeletal model is intended to serve as a unifying theory of many, but not all, PCDs in plants and represents those features of PCD that are in common. It acknowledges the fact that many of the cytoplasmic changes that are occurring during the "preparation to die" stage differ between programs and that these changes are an integral part of the manifestation of death (corpse management). It is expected that more complex models that include the multiple signal pathways and feedback regulation will be overlaid on the model shown. With this in mind, senescence, for which much more detail of its signal transduction and cytoplasmic disassembly is known, could be integrated into the three PCDs shown above as well.

ready mentioned, calcium influx is also required for this event.

Butler and Simon concluded that ". . . death of the cell, whatever the cause, follows a characteristic and repeatable pattern." Part of these patterns is shared among all cell deaths, namely vacuole collapse and calcium flux. It is interesting that Tom Wolpert of Oregon State University (Corvallis) concluded that death induced by a fungal toxin recapitulates some of the observed molecular changes during death by senescence (17). In addition, the expression of a number of genes is shared by these types of PCDs in plants, suggesting that some of the underlying molecular mechanisms are shared (4). But there are important differences in ways that the cell manages its corpse that must not be glossed over. During terminal differentiation, there is little structural evidence of cytoplasmic degeneration prior to vacuole collapse. However, in senescing cells, chloroplast degradation occurs well prior to vacuole collapse and during the hypersensitive response, cytoplasmic streaming declines exponentially up to the point of collapse. Even within a specific type of cell death, it has been proposed that multiple pathways are present.

A UNIVERSAL DEATH INDUCTION AND A CUSTOMIZED CORPSE PROCESSING

Three-quarters of a century of research on plant cell death have brought us an understanding of the general mechanism of death and corpse management. It appears that collapse of the vacuole may be the universal trigger of plant cell death; however, the differences in the way death is manifested results from different mechanisms for processing the cell corpse. Figure 1 illustrates a model for death and corpse processing that incorporates the similarities and differences between three plant PCDs. In this model, information from initiating signals sets the outcome but all cells progress through a single mechanism to trigger death but then diverge again depending on the predetermined mode of corpse processing.

The vacuole solves a dilemma for the cell that must actively process its own corpse. The cell must be metabolically active to synthesize the destructive hydrolases it needs to process its corpse; therefore, it sequesters these hydrolases and toxins into the vacuole and releases them when the vacuole collapses. Based on the integration of signals, the cell creates a profile of hydrolases in the vacuole that establishes the way the corpse is processed. Thus, loading of the vacuole at a time well before death determines how the corpse will be processed. For example, auxin and cytokinin induce the de novo synthesis of vacuole-sequestered nucleases and proteases but obviously not the hydrolytic activity that would remove the secondary wall that a tracheary element builds prior to its death. In contrast, during lysigenous aerenchyma formation induced by ethylene, cell wall hydrolases such as cellulase are included to fulfill theneed to remove not only the protoplasm but the extracellular matrix as well. In the hypersensitive response, signals from pathogens in most cases in-

duce the production of toxic phytoalexins, polyphenols, and chitinases, and these are released when the vacuole collapses, but otherwise the corpse is not significantly autolyzed (minimal corpse processing).

Cell death is triggered after the cell has prepared to carry out the postmortem events. This component of plant PCD may be shared by all or most forms to date, namely vacuole collapse mediated by a calcium flux. Release of the vacuole contents marks the beginning of the postmortem events. For aerenchyma formation, complete hydrolysis of the cell results in gas spaces, whereas tracheary elements remove only the protoplasm. Death in the hypersensitive response does not remove the corpse but does release toxins directed against the pathogen. The corpse subsequently is crushed by expanding tissues. Such a "messy" death might be part of the cell's strategy for defense.

FUTURE

Although the working concepts of PCD originated with plants, it is unfortunate that today's plant researchers too often try to force animal paradigms onto plant PCD and are not focused enough on understanding plant PCD within a biological context. Thus, it might appear hopeless that plant PCD research can ever retake the lead that it established 75 years ago. How can research in plants contribute to our understanding of PCD in eukaryotic cells? It is clear that we will understand the evolution of PCD by determining the mechanism in plants. If aspects of PCD are found to be shared between plant and animal cells, insight into the primordial pathway may be revealed. But the major contributions will come from research focused specifically on how cell death occurs within unique biological contexts (hypersensitive response, tracheary element differentiation, etc.) that are of interest to plant biologists and important for improving agriculture.

LITERATURE CITED

1. **Allen PJ** (1923) J Agric Res **23:** 131–152
2. **Boller T, Kende H** (1979) Plant Physiol **63:** 1123–1132
3. **Butler RD, Simon EW** (1971) Adv Gerontol Res **3:** 73–129
4. **Butt A, Mousley C, Morris K, Beynon J, Can C, Holub E, Greenberg JT, Buchanan-Wollaston V** (1998) Plant J **16:** 209–221
5. **Cronshaw J, Bouck B** (1965) J Cell Biol **24:** 414–431
6. **Fukuda H, Komamine A** (1980) Plant Physiol **52:** 57–60
7. **Gan S, Amasino RM** (1995) Science **270:** 1986–1988
8. **Grbic V, Bleeker AB** (1995) Plant J **8:** 595–602
9. **Groover A, Jones AM** (1999) Plant Physiol **119:** 375–384
10. **Groover AT, DeWitt NG, Heidel AJ, Jones AM** (1997) Protoplasma **196:** 197–211
11. **He C-J, Morgan PW, Drew MC** (1996) Plant Physiol **112:** 463–472
12. **Huang F-Y, Philosoph-Hadas S, Meir S, Callaham DA, Sabato R, Zelcer A, Hepler PK** (1997) Plant Physiol **115:** 51–60
13. **Kerr JFR, Wyllie AH, Currie AR** (1972) Br J Cancer **26:** 239–257
14. **Kitazawa K, Inagaki H, Tomiyama K** (1973) Phytopathol Z **76:** 80–86
15. **Kuo A, Cappelluti S, Cervantes-Cervantes M, Rodriguez M, Bush DS** (1996) Plant Cell **8:** 259–269
16. **Leopold AC** (1961) Science **134:** 1727–1732
17. **Navarre DA, Wolpert TJ** (1999) Plant Cell **11:** 237–249
18. **Thomas H, Stoddart JL** (1980) Annu Rev Plant Physiol **31:** 83–111
19. **Weymann K, Hunt M, Uknes S, Neuenschwander U, Lawton K, Steiner H-Y, Ryals J** (1995) Plant Cell **7:** 2013–2022
20. **Xu H, Heath MC** (1998) Plant Cell **10:** 585–597
21. **Yoshida Y** (1962) Protoplasma **54:** 476–492