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News and Commentary

From the nematode and mammals back to the pine tree: on the diversity and evolution of programmed cell death

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Compared to animals, very little is known about the mechanisms of programmed cell death (PCD) in plants. This in large part is because until recently this area of cell biology was far removed from the focus of cell death researchers. However, it seems likely that PCD may play an even greater role during plant development than it does in animals, since plant cell corpses and their cytoplasmic components frequently serve very important functions.[1,2]

The literal meaning of the Greek word 'Apoptosis' is falling of the petals from a flower or leaves from a tree, signifying the naturally occurring abscission and death of plant organs.[3] Leaf senescence along with a host of other developmentally regulated and pathogen-induced plant cell deaths have received considerable attention during the last decade as researchers aim to understand any similarities between plant PCD regulatory mechanisms and animal apoptosis. In order to succeed in this endeavor, one apparently needs an adequate plant model system including a well-characterized sequence of developmental stages, either leading to terminal differentiation or involving PCD at one of the intermediate stages (akin to animal metamorphosis). Formation of tracheary elements and somatic embryogenesis in *Zinnia*[4] and Norway spruce[5] cell cultures, respectively, have already been shown to partly meet this criterion. Without diminishing the significance of these systems for investigation of plant PCD, their serious limitation is that in both situations the PCD pathways are regulated *in vitro*, i.e., under very artificial conditions, which are unlikely to reproduce the whole spectrum of factors regulating the natural counterparts of xylo- and embryogenesis.

Natural cell death, which is indispensable for embryogenesis and seed development in nonflowering (gymnosperm) plants, was described by a group from The Swedish University of Agricultural Sciences (see article in this issue[6]). Flowering plants usually initiate only one embryo per seed. In contrast, monozygotic polyembryony is featured by at least 20 genera of gymnosperms, and another type of polyembryony (when two or more egg cells are fertilised within the same ovule) is shared by almost all nonflowering plants.[7] In both types of polyembryony, only one embryo develops to maturity, giving rise to a plant; supernumerary embryos are aborted. This reproductive behaviour of gymnosperms has possibly evolved to survive the largest extinction event in the history of life, involving global climate fluctuations during the Permian period.

A study by Filonova *et al*.[6] revealed that the multiple subordinate embryos differentiated from a single zygote in Scots pine (*Pinus sylvestris*) undergo highly ordered self-destruction, while a single dominant embryo survives and develops to maturity. The morphotype of this form of PCD does not adhere to most of the apoptosis-like hallmarks (except for DNA fragmentation), but rather features classic signs of autophagic cell death. Although the primary signal triggering this PCD is as yet unknown, the authors provide indirect evidence for the maternal control of the demice of subordinate embryos in a pine seed, implicating a PCD-triggering role for the female gametophyte.[6] The latter is a haploid embryo-nourishing tissue being substituted for triploid endosperm in flowering plants during evolution.[8]

Plants represent one of the oldest phyla of eukaryotes. Molecular phylogenetic analyses place a rough approximations at best the common ancestor for plants and animals to about a billion years ago; this is about 45 million years earlier than divergence to slime molds, fungi and animals.[9] Non-flowering plants are often referrred to as living fossils; they have much older fossil records (approximately 320 million years ago)[10] than angiosperms (at almost 130 million years ago).[11] The history of the major discoveries in the field of mammalian PCD began with the use of the versatile *C. elegans* model system for genetic studies of the designated cell-suicide programme.[12] PCD in *C. elegans* is responsible for tissue and organ reconstruction at early stages of development.[13] *C. elegans* origin dates back about 760 million years ago. This is a hundred million years earlier than *Drosophila*, and the mammalian fossils do not exceed 170 million years in age.[14] However, molecular phylogenetic analyses places mammals as even more recently evolved grouping.[9] Thus, characterizing the signaling mechanisms regulating embryonic PCD in pine seed would be of considerable value to a better understanding of the origin and evolution of eukaryotic PCD machinery.[6]

The main examples of developmental PCD arranged in descending order of the organism's divergence time are summarised in Table 1. Autophagic PCD apparently evolved before apoptosis. Plants, slime molds and fungi do not show apoptosis-like cell dismantling. Rather all display autophagy-related processing of dying cells. It is plausible that autophagic PCD has been partially preserved during evolution by more advanced organisms as indicated by the occurrence of autophagy during metamorphosis in *Drosophila*.[15]

What is the signal required to induce autophagic PCD? In fact, in the last issue of The Journal of Cell Biology a group tried to address this question.[16] They found that

Table 1 Comparison of developmental PCD during embryogenesis in plants (*Pinus*) and animals (*C. elegans*, mouse and man), stalk formation in the slime mold (*Dictyostelium*), adjustment of population size in starving yeast (*Saccharomyces*) and during metamorphosis in *Drosophila*

Phyla	Model organisms	Developmental role of PCD	Trigger	Type of PCD	Mitochondrial involvement	Implication of caspases
Plants	*Pinus*	Embryogenesis	Uncharacterized signal yielded by female gametophyte	Autophagy	?	? (ancestral caspases)
Slime molds	*Dictyostelium*	Stalk formation	Starvation	Autophagy	yes	? (ancestral caspases)
Fungi	*Saccharomyces*	Control of population size	Starvation	Autophagy	yes	? (ancestral caspases)
Animals	*C. elegans*	Embryogenesis	Caspase effectors	Apoptosis	yes	yes
	Drosophila	Metamorphosis	Ecdysone	Autophagy	yes	yes
	Mouse	Embryogenesis	Bone morphogenetic protein signaling	Apoptosis	yes	yes
	Man	Embryogenesis	Various stimuli	Apoptosis	yes	yes
			DAPk family proteins	Autophagy	no	no

death-associated protein kinase (DAPk) and DAPk-related protein kinase (DRP)-1 proteins, which belong to the family of Ca^{2+}/calmodulin-regulated Ser/Thr death kinases, mediate the formation of autophagic vesicles as well as membrane blebbing during PCD. DRP-1 was localized to the lumen of autophagosomes as measured by immunogold staining, suggesting a direct involvement of this kinase in the process of autophagy. Interestingly, both events, membrane blebbing and extensive autophagy, were totally independent of caspase activity. Although both wild-type kinases can phosphorylate myosin light chain, which may induce cortical contractions leading to blebbing, the connection between this possible mechanism and autophagy remains unclear. It may be that evolution has retained the similar molecules for autophagy and PCD but used them in two completely different ways. It is interesting to note that a common feature for both autophagy and apoptosis is an active role for the mitochondrion.[17] Mitochondria permeabilization and disruption of mitochondrial transmembrane potential have been shown to occur during developmental autophagy-related PCD of tracheary elements in plants[18] as well as during the same type of PCD in the slime mold *Dictyostelium discoideum*.[19] In the absence of growth factors, superior ganglia neurons also undergo cell death. However, after inhibition of caspase activity their mitochondria are selectively eliminated and cells die by autophagy.[20] It has been suggested that autophagic death is a relatively slow process, in which the cells eventually die by the loss of essential organelles, such as mitochondria.[16] It is also possible that in higher organisms autophagy occurs in response to minimal damage and the cells require time to decide whether to repair this damage or to die. If the latter decision is made, cell metabolism effectively slows to enable intracellular double membrane structures to engulf large parts of cytoplasm, enclosing proteins and organelles for degradation. Thus, it seems that autophagic cells destroy their own components, whereas apoptotic cells depend on phagocytes to accomplish terminal degradation.

It is unknown which genes are involved in autophagy in plants, but many of the autophagy-associated genes are evolutionarily conserved among yeast and mammalian organisms and might represent the evolution link between autophagy and PCD.[21,22] In addition, some other proteins important for perpetuation of cell death process, e.g., apoptosis inducing factor (AIF),[23] appears to be a transitory from autophagy to caspase-dependent cell death. In plants, neither Bcl-2 family proteins nor AIF homologs have been identified.[24] Likewise, canonical caspases are not present in plants, although genes encoding proteins belonging to ancestral caspase/paracaspase/metacaspase superfamily have been identified in both flowering[25] and non-flowering (MF Suarez and PV Bozhkov, personal communication) plants. Thus, although autophagic death appears to be a phylogenetically old phenomenon, autophagic and apoptotic PCD should not be considered as mutually exclusive phenomena.

In conclusion, the pine seed model system[6] may provide answers to many intriguing questions, such as: How does deregulation of PCD affect embryonic pattern formation and seed development? Is mitochondrial permeability transition required for autophagy? What is the role for metacaspases in embryogenesis and associated PCD? With the progress that has been made in the last few years, it is likely that these and other important problems in the field of plant PCD will be solved in the near future.

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