BOTANICAL BRIEFING

Programmed Cell Death in Floral Organs: How and Why do Flowers Die?

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INTRODUCTION: WHAT DO WE MEAN BY PROGRAMMED CELL DEATH IN FLORAL ORGANS?

Recently there has been much controversy over the use of the terms ‘senescence’ and ‘programmed cell death’ (PCD), especially with regard to leaves (Thomas et al., 2003; van Doorn and Woltering, 2004). In flowers it seems to me that the distinction is largely unnecessary. The deterioration of a flower is certainly programmed, is not a reversible process and inevitably leads to cell death. Thus, I have used the terms essentially interchangeably, using PCD more often when discussing the death of individual cell types, and senescence for whole organs.

WHY DO FLOWERS DIE, AND WHY DO THEY LAST LONGER IN SOME SPECIES THAN OTHERS?

Selective removal of reproductive structures is not unique to plants. Although sperm cells continue to be produced throughout male adult life, 99-9% of human oocytes are removed by PCD (Tilly, 2001), perhaps ensuring that the costs of female reproduction are tightly regulated to benefit the survival of progeny to adulthood. However, unlike in mammals, both male and female reproductive structures in plants are only retained while they are needed, and are developed de novo, in perennial species, the following season. The duration of the flower is species-specific and carefully tailored to its ecological requirements. This is important because firstly the flower can be a substantial sink on the plant’s resources, and as such is energetically expensive to maintain beyond its useful life (Ashman and Schoen, 1994). In addition, its architecture has been exploited by pathogens that use the stigma as a point of entry, and thus the flower poses an added risk of pathogen attack (Shykoff et al., 1996). Another important reason for floral death after pollination is to remove it from the population so that it does not compete for pollinators with the remaining blooms. One of the key triggers for petal death is pollination, which initiates a series of physiological events, orchestrated by plant growth regulators (PGRs). Ethylene is a clear regulator of petal senescence in some species (Stead and van Doorn, 1994); however, in other species including lilies such as Hemerocallis (daylilies) and Alstroemeria it appears to play little or no part (Woltering and van Doorn, 1988; Wagstaff et al., 2005).

How petal senescence in these species is triggered and orchestrated remains unknown. Given the failure to find a common regulator for these species, and their taxonomic diversity, it seems likely that several inter-related mechanisms may be at play. Resource allocation has been one trigger proposed, and indeed removal of lower flowers in a cyme can lead to increased longevity of the first flower (Chanasut et al., 2003). However, this is clearly not a full explanation for all ethylene-insensitive species.

Key words: Programmed cell death, flowers, petal, tapetum, pollen-tubes, senescence, ethylene, apoptosis, autophagy, ricinosomes, metacaspases, chromoplasts.

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An important feature of floral death is that the different floral organs play very different roles. Hence, their life span needs to be appropriately co-ordinated. Likewise, the purpose and fate of the dying cells depends on the organ and tissues involved. At a whole organ level, petals, anthers and stigma are no longer required following pollination, whereas the ovary will mature to contain the developing seeds. In many species there is also a mechanism for rescuing resources from the degenerating organs such as petals, and diverting them to other parts of the plant such as the developing ovary (Stead and van Doorn, 1994). At a tissue and cell level, the situation is even more complex as there is a requirement for some reproductive tissues and cells to die to ensure correct development. For example, the tapetum must degenerate for pollen to develop properly, and synergid cells must die to allow fertilization. However, the fate of the dead cells is very different. In the case of the tapetum, cell contents are used to form the coat of the pollen grains, whereas removal of synergid cells is required for fertilization to occur (Christensen et al., 2002). Some types of cell death in floral organs also depend on specific genetic interactions. PCD occurs as a result of incompatible pollination events (Thomas and Franklin-Tong, 2004), and also as a result of defects in pollen development displayed in cytoplasmic male sterile lines (Balk and Leaver, 2001). Thus, important questions with regard to cell death in reproductive organs are: How do the cells perceive and respond to death signals, or, put more teleologically, how do they know when to die? Are the primary signals processed in the same way by the different organs and cells? Is the type of PCD in floral organs also found in other plant tissues and organs? I restrict myself here to considering the organs that make up the mature flower (Fig. 1). Although fruit ripening and seed maturation include further examples of PCD, these will not be considered here.

**HOW DO THE CELLS KNOW WHEN TO DIE?**

In some species pollination dramatically shortens floral life span. For example, orchid flowers will last several months but senesce rapidly once pollinated. In several species, including *Petunia*, tobacco, carnation and orchids, senescence is mediated by the evolution of ethylene following contact between pollen and the stigmatal surface, which precedes fertilization (O’Neill, 1997). However, the exact nature of the primary signal resulting in ethylene evolution has not been established, although other PGRs and low-molecular-weight compounds have been implicated (O’Neill, 1997). In carnations, ethylene produced from the pollinated stigma is translocated, via the style and ovary, to the petals. Here it up-regulates ethylene biosynthetic genes and induces the production of ethylene in

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**Fig. 1.** Sites of programmed cell death in floral organs. SI, self-incompatibility; CMS, cytoplasmic male sterility; AU, autophagous-like mechanism; AP, apoptotic-like mechanism; square indicates strong evidence, circle indicates weaker evidence. Bold text indicates cells and tissues discussed in the text.
the petals (ten Have and Woltering, 1997). Once initiated, the evolution of ethylene becomes autocatalytic (Woodson and Lawton, 1988). This strongly suggests that promoters of the ethylene biosynthetic genes respond to ethylene and contain ethylene-responsive elements (EREs). To date, this has not been verified although an ERE from a senescence- and ethylene-regulated gene in carnation bears similarities to the ERE from an ethylene-responsive fruit-ripening gene. E4, suggesting commonality of transcription factors in these two processes (Deikman, 1997).

The response to ethylene is regulated by the production of ethylene receptors but how this regulation is achieved is not clear. In tomato an ETR1 (‘ethylene-resistant’) -type ethylene receptor was not transcribed in young flowers or senescent flowers, but only in mature flowers (Payton et al., 1996). Furthermore, ethylene receptor expression may itself be regulated by ethylene production. In pea, transcripts of an ERS (‘ethylene response sensor’)-type ethylene receptor were reduced when un-pollinated flowers were treated with an inhibitor of ethylene biosynthesis (Orzaez et al., 1999). So the balance between receptor production and ethylene sensitivity is clearly regulated at several levels.

Notably, in species in which ethylene is a major regulator, ethylene-independent signals are also present. Disruption of ethylene signalling or biosynthesis in carnation and petunia results in delayed floral death, but the flowers do eventually die (Michael et al., 1993). Perhaps it is these endogenous signals that are active in species where ethylene is not a major regulator. Several global transcriptomic studies (e.g. Alstroemeria: Breeze et al., 2004; Iris: van Doorn et al., 2003) have attempted to reveal the genes or pathways regulating floral degeneration in these species; however, no clear patterns have yet emerged. Verifying the role of genes in these species is hampered by the lack of genome sequences and, often, lack of efficient transformation systems. Another possibility is that senescence and PCD are regulated post transcriptionally, as argued by Thomas et al. (2003). Perhaps a complex network of both transcriptional and post-transcriptional control is involved, as is found in other fundamental cellular processes such as the cell cycle. If the underlying ethylene-independent life span control in ethylene-sensitive species is common to ethylene-insensitive species, then models such as Arabidopsis and Brassica or tomato and petunia may offer better species in which to investigate these control networks. This would be a neat solution to a difficult problem. Langston et al. (2005) used this approach to study DNA fragmentation in petunia, showing that the ethylene induction of a 43-kDa nuclease (PhNUC1) was delayed in 35S:etr1-1 plants but not eliminated. Nine thousand expressed sequence tags (ESTs) have been recently generated from a global transcriptomic analysis of petunia floral senescence (D. Clarke, University of Florida, pers. comm.) and it will be interesting to see what proportion of these genes are up-regulated in 35S:etr1-1 lines. Likewise, in Arabidopsis, transcriptomic and perhaps proteomic analysis of petal senescence in etr1-1 lines may be a fruitful line of enquiry. However, if ethylene-independent regulation turns out to be species-specific then it is important to continue work with the diverse species currently being studied to appreciate the range of networks employed.

Another important question is whether the same signal differentially regulates PCD in floral organs. In some cases the answer is yes: for example in tobacco, ethylene regulates petal senescence (Rieu et al., 2003); however, at the same time ovary tissues continue to develop. So how is a primary signal such as ethylene transduced to ensure the co-ordinated life and death of different floral organs? Presumably this is through differential signal translocation or differential signal perception. Petal margins often degenerate before the centre and cross-sections of developing petals reveal that while the epidermal cells are still functional, mesophyll cells have largely degenerated even before the flower is fully open (Wagstaff et al., 2003). So is there a gradient here of a diffusible signal, or of receptors or other intracellular mediators of the cell-death signal? In some cases this signalling differential is very distinct: in the Arabidopsis gla2 mutant, synergid fail to undergo PCD, but antipodal PCD is not affected (Christensen et al., 2002).

Ethylene is not the only PGR stimulating PCD in floral organs: some links to other PGRs are reviewed in Wu and Cheung (2000). Mutation of gibberellic acid biosynthetic genes anther ear 1 and dwarf results in failure to abort stamens on maize female flowers. Mutation of a gene associated with brassinosteroids (TS) (‘tasselseed’) results in feminization of male flowers, and application of jasmonate (JA) enhances petal senescence in some species (Porat and Haley, 1993), although this effect may be indirect, through ethylene signalling (Stead et al., in press). Elevating cytokinin levels in petunia delayed flower senescence; however, this may also be indirect through changes in sugar transport (Lara et al., 2004). So are all these PGRs involved in floral PCD in all species? Or are there important quantitative or even qualitative species-specific differences in their effects? Perhaps metabolomic approaches to measure endogenous levels of PGRs, coupled with a more extensive use of mutants, may begin to address these questions.

IS THERE JUST ONE PCD MECHANISM OPERATING IN FLORAL ORGANS?

van Doorn and Woltering (2005) have recently categorized plant PCD into three types: apoptotic, autophagic and neither apoptotic nor autophagic. In animal cells four types of apoptosis have been described (Orrenius et al., 2003), three of which involve cytochrome c release from the mitochondrion controlled by a family of proteins (Bcl-2) that interact with the mitochondrial membrane to facilitate or inhibit this process. Cytochrome c then activates a family of cysteine aspartate-specific proteases (caspases), which both regulate and effect PCD. Apoptotic PCD in animals is characterized by cytological features, including chromatin and nuclear condensation and marginalization followed by DNA fragmentation into nucleosomal units known as DNA laddering, nuclear blebbing and formation of membrane inclusions known as apoptotic
bodies (Cohen, 1993). The apoptotic bodies are then engulfed by neighbouring living cells.

In the tapetum and pollen-tubes, there is compelling evidence to support an important role for the mitochondrion and involvement of caspases. This suggests a mechanism similar to animal apoptosis, although caution must be exercised in drawing too close a parallel, as engulfment of cellular remains by other cells does not occur in plants (van Doorn and Woltering, 2005). Following its nutritive role during pollen development, the tapetum degenerates. This is characterized by chromatin condensation in *Lobivia rauschii* and *Tillandsia albida* (Papini et al., 1999), and by DNA fragmentation in barley anthers (Wang et al., 1999). In *Brassica oleracea*, *Brassica napus*, *Digitalis purpurea* and a cultivated form of *Fuchsia* there is also nuclear blebbing (A. D. Stead, Royal Holloway, University of London, unpubl. data). Furthermore, in *PETI* cytoplasmic male sterility (CMS) of sunflower, there is cytochrome c release into the cytosol followed by changes in cell morphology, loss of outer mitochondrial membrane integrity and a fall in the respiratory control ratio (Balk and Leaver, 2001). Assuming that CMS is just anticipating a normal event (quite a major assumption), it might be concluded that PCD in the tapetum is apoptotic; however, we still do not know how it is triggered. Studies of nuclear mutations resulting in tepal degeneration may be helpful: morphological changes were charted in a rice male sterile mutant (Ku et al., 2003), including cytoplasmic shrinkage, membrane blebbing, vacuolation, changes in mitochondrial morphology and early DNA fragmentation. However, cytochrome c leakage and respiratory control ratio were not measured in this system. Further studies on PCD in non-CMS tepal cells would be helpful in this context.

Another example of apoptotic-like PCD in floral organs is in the death of the pollen-tube during self-incompatible tapetal cells would be helpful in this context. Further studies on PCD in non-CMS tissues. Brining together the different strands of evidence, there seems to me to be a good argument for VPEs performing a regulatory role in many if not all the forms of PCD seen in floral organs.

Similar organelles to PPVs have been isolated from *Ricinus communis* endosperm known as ricinosomes. These organelles develop as the cells undergo PCD, and rupture releasing their cargo of proteases directly into the cytosol. Acidification triggers ricinsonome rupture; thus, release of proteases from ricinosomes may be activated after acidification of the cytosol following vacuolar leakage (Schmid et al., 1999). Ricinosomes contain CysEP, a type of cysteine endoprotease with a C-terminal KDEL motif (Gietl et al., 1997), which directs proteins to the ER lumen. KDEL-containing cysteine proteases have been identified in a number of senescent floral organs including *Hemerocallis* (daylily) petals (Valpuesta et al., 1995) and in *Pisum sativum* senescent ovaries (Cercos et al., 1999). In senescent daylily petals the KDEL-containing protease is within vesicles similar to ricinosomes (Schmid et al., 1999) so ricinosome-mediated PCD may be a feature of petal PCD. However, ricinosome-like vesicles are clearly distinct from the PPVs in that they are presumed to act downstream of the vacuolar leakage, and to deliver their cargo into the cytosol rather than the vacuole (Fig. 2). At least two metacaspases (*AtMCP2f* and *AtMCP1a*) are also thought, based on array data, to be induced in senescing flowers (Sanmartin et al., 2005), but their location is unknown. Confirmation of their role in planta will be important.

**DO FLORAL ORGANS AND OTHER PLANT TISSUES DIE IN THE SAME WAY?**

A functional categorization of PCD can be made on the basis of the fate of the cell contents. Remobilization is central to leaf, sepal and petal senescence (Thomas et al., 2003) and in a different way also to tepetal PCD. But
endothecium, synergid, antipodal cell or pollen-tube PCD is the selective death of unwanted cells.

In green tissues, the chloroplast is seen by some (Thomas et al., 2003) as the key participant in the senescence process, and an early sign of senescence in green tissues is conversion of chloroplasts to gerontoplasts. Sepals, the floral organ that most closely resembles leaves, senesce in a similar way: in broccoli, sepal chlorophyll degradation is the first visual sign of senescence (Page et al., 2001). Petals are, however, not usually green, and an early step in their development is a conversion of chloroplasts to chromoplasts. This conversion has been compared with the chromoplast/gerontoplast transition (Thomas et al., 2003) with the inference that petals are most similar to senescent leaves. This agrees with the very early cell death seen in flowers (Wagstaff et al., 2003) presumably associated with nutrient remobilization. However, in-silico comparison of transcriptome changes in senescent Arabidopsis leaves and petals indicates that 25–30% of genes share similar patterns of expression (Stead et al., in press). A comparison of transcriptome changes during petal senescence and fruit ripening would be interesting. Complex networks of ethylene, JA and salicylic acid operate in leaves (He et al., 2001), which may differ from those in petals, as ethylene does not have the same dramatic effects on leaves seen in petals (Grbić and Bleeker, 1995). At a subcellular level morphological changes to subcellular compartments during PCD are shared by many different cell types and tissues (Rogers, 2005). VPEs are found in leaves, roots and flowers; ricinosomes are seen in seed and petal tissues; and caspase activity is detected in pollen-tubes undergoing SI, and in many non-floral tissues during natural senescence and also during pathogen responses (Sanmartín et al., 2005).

CONCLUSIONS: WHAT DO WE REALLY WANT TO KNOW?

Progress in our understanding of PCD in plants has been rapid in the last 10 years, but the key regulators of some types of floral organ senescence, such as petal senescence in ethylene-insensitive species, remain obscure. It is also unclear whether regulation of petal senescence and PCD in these species is similar or divergent. The latter is an important question to resolve before a good model for these species can be developed. Even in ethylene-regulated petal senescence, the primary signal initiating the ethylene cascade remains elusive and needs pinning down, and again it is not at all certain that it will prove to be common to all species. Work on leaf senescence is suggesting that the search for a ‘master-switch’ gene or genes of plant senescence may be futile, and that a complex network of endogenous and exogenous signals tips the balance towards death. Floral senescence appears much more tightly regulated developmentally, so should we be looking for a master regulator here? Transcriptome analyses have not revealed any obvious candidates, but perhaps we should not stop looking yet. Another area that seems under-researched is our understanding of the promoters of floral-senescence genes. Genomic approaches and new developments in bio-informatics are powerful tools for developing this area and exploring how far transcription factors are shared between leaf senescence, floral senescence, fruit ripening and localized PCD. This will help to reveal the upstream regulatory networks, even if a master-switch is not the answer. Proteomics and metabolomics may also help to understand post-transcriptional regulatory networks, and define better the role of PGRs. Transgenics and mutants
Table 1. Comparison of signalling and possible mechanisms of programmed cell death (PCD) in floral organs

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<td>Tapetum in cytoplasmic male sterility lines</td>
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<td>Pollen-tube</td>
<td>During self-incompatibility interactions</td>
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are powerful tools, and perturbation of specific pathways such as manipulation of PGR levels using inducible promoters will also contribute to our understanding of the roles of PGRs in floral senescence and PCD. At a cellular level plant PCD seems poised on the edge of major advances in unravelling protease cascades and intracellular signalling events. We can start to build a picture of the types of mechanisms operating in different cell types (Fig. 1, Table 1); however, it is far from complete. So we are still some way from a systems biology approach that might describe the whole process.

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LITERATURE CITED


