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Commentary

New insights into cell death induced by long chain bases in *Arabidopsis*

Programmed cell death (PCD) is an essential process that multicellular organisms undertake to regulate growth and development and to respond to the environment. Many different factors are involved in coordinating PCD and over recent years it has been shown that sphingolipids can act as mediators. PCD has been well studied in animal systems and in yeast but in plants our understanding of the genetic and biochemical mechanisms which regulate and execute PCD is limited. A study by Saucedo-García *et al.* in this issue of *New Phytologist* (pp. 943–957) provides new insight into cell death transduction pathways in plants.

'Excitingly, MPK6 was shown to have a regulatory role in PCD induced by LCBs.'

Sphingolipids are ubiquitous membrane lipids that have been shown to be essential in many different eukaryotes (Dunn *et al.*, 2004). Until relatively recently plant sphingolipids were generally considered to be cellular components of limited importance; however, research over the last decade has shown that they undertake key roles in many aspects of plant biology, including reproduction, development and also biotic and abiotic stress, in addition to having an important role as structural components of membranes.

Sphingolipids have considerable natural diversity and can differ in the composition of their head groups, degree of hydroxylation and number and positions of double bonds. The functional significance of this immense structural heterogeneity of plant sphingolipids is largely unknown, but recent advances in analytical tools have started to bear fruit (Markham & Jaworski, 2007). For example tri-hydroxy long-chain base (LCB) synthesis is essential for maintaining

growth and mediating the total content and fatty acid composition of sphingolipids in *Arabidopsis* (Chen *et al.*, 2008), but the Δ^4 desaturation of the LCB has a more limited effect in *Arabidopsis* (Michaelson *et al.*, 2009).

Plant PCD serves a fundamental function during developmental processes, including embryo formation, floral organ abscission, pollen self-incompatibility, remodeling of some types of leaf shape and leaf senescence (Gadjev *et al.*, 2008). PCD is intimately involved with plant immunity to pathogens and is therefore an essential mechanism for plant defence; this is often referred to as the hypersensitive response (HR-PCD). Sphingolipid involvement in PCD has been known for some time, initially through the use of mycotoxins such as AAL (a dihydrosphingosine-analog mycotoxin from *Alternaria alternata* f. sp. *lycopersici*) and fumonisin B1 (FB1) as an experimental tool to disrupt sphingolipid metabolism (Wang *et al.*, 1996; Shi *et al.*, 2007).

Work on an *Arabidopsis* mutant disrupted in the LCB1 subunit of serine palmitoyltransferase (SPT) has shown that it fails to generate reactive oxygen species (ROS) and is incapable of initiating programmed cell death or apoptosis when the mutant is challenged by inhibitors of ceramide synthesis such as FB1 (Shi *et al.*, 2007). The free LCB bases dihydrosphingosine, phytosphingosine and sphingosine induce ROS generation followed by cell death. ROS generation and cell death induced by dihydrosphingosine were shown to be blocked by its phosphorylated form, dihydrosphingosine-1-phosphate, in a dose-dependent manner, suggesting that the maintenance of homeostasis between a free LCB and its phosphorylated derivative is critical to determining the cell fate. Further evidence for the importance of this relationship between free LCBs and its phosphorylated derivative has been shown by the targeting and stimulation of the *Arabidopsis* sphingosine kinases by phosphatidic acid (Guo *et al.*, 2011). AAL was shown to promote apoptosis during cell death in tomato. AAL toxin prevents ceramide synthesis and therefore complex sphingolipid biosynthesis, causing the accumulation of LCBs and leading to cell death (Wang *et al.*, 1996). If LCB synthesis via SPT is inhibited, some of these toxic effects are reduced (Spassieva *et al.*, 2002). The *Arabidopsis* SPT is a heteromeric enzyme comprised of two subunits similar to that found in yeast and mammals. *Arabidopsis* plants disrupted in the gene encoding the LCB1 subunit and LCB2 subunit caused the formation of abortive microspores and initiated apoptotic cell death in binucleated microspores (Dietrich *et al.*, 2008; Teng *et al.*, 2008). These data suggest that

SPT-modulated programmed cell death plays an important role in the regulation of male gametophyte development (Dietrich *et al.*, 2008; Teng *et al.*, 2008).

LCB metabolism has been shown to have a role in HR-PCD in *Arabidopsis* (Brodersen *et al.*, 2002). The accelerated-cell-death-11 *Arabidopsis* mutant (*acd11*) constitutively expresses defence-related genes that accompany the hypersensitive response normally triggered by pathogens. *ACD11* encodes a protein that may play a role in the transfer of LCBs, but not glycosphingolipids between membranes *in vitro*. The *Arabidopsis* IPC synthase, which converts ceramide to inositol phosphoceramide (IPC), has been identified and shown to be involved in enhancing the hypersensitive response triggered by the RPW8 gene (Wang *et al.*, 2008). RPW8 triggers the hypersensitive response to limit powdery mildew infection via the salicylic acid-dependent signalling pathway and mutation in the IPC synthase causes ceramide accumulation in plants expressing RPW8. An *Arabidopsis* ceramide kinase mutant (*acd5*; accelerated-cell-death-5) shows enhanced disease symptoms during pathogen attack and apoptotic-like cell death which is dependent on defence signalling late in development (Liang *et al.*, 2003). PCD was found to be induced by the over accumulation of ceramide, whereas its phosphorylated derivative partially blocked this response. This supports a role for ceramide phosphorylation in modulating cell death in plants. These results collectively suggest that sphingolipids and their metabolites play important roles in plant defence and cell death and that their tight control is essential.

These observations of sphingolipid involvement in PCD and HR-PCD have led researchers to search for mechanisms by which this process takes place. The article by Saucedo-García *et al.* answers some of the questions that have been generated by previous work on sphingolipid involvement in PCD. These authors tested the hypothesis that LCBs could induce mitogen activated protein kinase (MAPK) cascades leading to PCD induced by FB1. PCD conditions in *Arabidopsis* mutants disrupted in the LCB2 subunit of serine palmitoyltransferase, mitogen-activated protein kinase (*mpk*), mitogen-activated protein phosphatase (*mkip1*) and LCB-hydroxylase were induced using FB1.

The *lcb2a-1* mutant was unable to mount an effective PCD in response to the FB1 which revealed that the *LCB2a* gene is central to PCD. Detailed analysis of the free LCBs and LCB-Ps showed that dihydroxy-LCBs may be the primary mediators for LCB-induced PCD and that trihydroxy-LCBs are not essential triggers. Additional evidence for this was shown by some elegant experiments which showed that exogenous d18:0 (dihydrosphingosine) induced PCD in wild-type seedlings. This was not evident when t18:0 (phytosphingosine) or d18:0-P (dihydrosphingosine-1-phosphate) were used as supplements.

Excitingly, MPK6 was shown to have a regulatory role in PCD induced by LCBs. The increase of endogenous LCBs induced by FB1 did not produce severe changes in the *mpk6* seedling phenotype and the *mpk6* seedlings were also resistant to the exogenous d18:0. An MPK activity assay was performed and showed that wild-type seedlings exposed to exogenously added FB1, d18:0 or to t18:0 showed heavy myelin basic protein labelling corresponding only to MPK6 activity and not to MPK3. Direct evidence for involvement in the HR-PCD response was obtained when wild-type, *lcb2a-1* and *mpk6* seedlings were exposed to FB1 and then to *Pseudomonas syringae* pv. *Tomato avrRpm1*, a bacterial strain that is avirulent towards *Arabidopsis*. The FB1 treatment caused a decrease in bacterial proliferation in the wild-type and *lcb2a-1* seedlings and showed no such effect on the *mpk6* seedlings. The resistance of the *mpk6* seedlings and the altered response to the bacteria is very interesting as it indicates that MPK6 mediates PCD downstream of LCBs. MPK6 is the first kinase described as a transducer in LCB-mediated PCD in plants.

In addition to these observations the paper describes the novel features of the cells undergoing PCD as a consequence of LCB accumulation. The authors observed DNA fragmentation and the loss of integrity of organelle membranes. The increased levels of free –LCBs and a decrease in complex sphingolipids may be responsible for the membrane alterations but it could also be the generation of ROS as described by Shi *et al.* (2007).

Recent studies on *Arabidopsis* are beginning to decipher the connections between sphingolipid metabolism and PCD, though the understanding of sphingolipid signalling and its regulation in plants is still in its infancy. The growing collections of *Arabidopsis* lines with specific alterations in the LCB composition provide a 'toolkit' to elucidate the molecular and genetic features of this signalling pathway as shown by Saucedo-García *et al.* and will also shed light on the compartmentation of biosynthetic activities. It will be interesting to see if other plants show similar trends and responses as the sphingolipid profiles from different species can be very different and may have an effect on which sphingolipids are used in the pathways that mediate PCD. It is evident that sphingolipids play an important role, as bioactive molecules in development and metabolism, and it is expected that new functions and roles will become known to elucidate the mechanisms that are used for the transmission and integration of environmental and developmental cues.

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Key words: *Arabidopsis*, fumonisins B1 (FB1), long chain base (LCB), MPK6, programmed cell death (PCD), sphingolipids.

Gone with the wind: understanding evolutionary transitions between wind and animal pollination in the angiosperms

Flowering plants exhibit spectacular diversity in flower design and display, and much of the functional basis of this variation is associated with the evolution of pollination systems. Transitions between different pollination systems among closely related species can shed light on the key characters involved in facilitating and responding to the shift. In contrast to well-studied transitions such as bee to bird pollination, we are just beginning to understand the transition from insect to wind pollination (Friedman & Barrett, 2009). The evolution of wind pollination from animal pollinated ancestors in the angiosperms is fairly common, occurring at least 65 times (Linder, 1998), and is sometimes considered irreversible (Cox, 1991; Dodd *et al.*, 1999). Nonetheless, there are some examples of reversals from wind pollination to insect pollination in flowering plants. Although these are remarkably rare, they are important because they expose key characters that may have been involved in the early radiation of the flowering plants as they co-evolved with their animal pollinators. In one of the most convincing examinations of the evolution of animal pollination from wind pollination in the predominantly wind-pollinated sedges (Cyperaceae), Wragg & Johnson (pp. 1128–1140) in this issue of *New Phytologist*, describe differences in color and scent between closely related species that are, and are not, visited by insects.

‘... under the appropriate ecological conditions, small changes might be sufficient to achieve pollination transitions.’

In some wind-pollinated species, the presence of insects on inflorescences is simply opportunistic (e.g. the frequent observation of pollen collecting insects on *Plantago lanceolata* and many grasses), and it is unclear in these cases how much pollen is actually transported by insects between plants. Wragg & Johnson conducted detailed and thorough experiments on putatively insect-pollinated *Cyperus* species and their closest co-occurring wind-pollinated sister species.