abdominal tip, through which they discharge chemicals. The researchers first showed that survival after exposure to fire ants dramatically decreased in tawny crazy ants that had their acidopore experimentally sealed. Sealing the acidopore in itself, on the other hand, did not adversely affect the ants. This suggested that tawny crazy ants indeed apply some glandular product from the acidopore to their mandibles before spreading it to their entire body surface.

Two exocrine glands open into the crazy ant acidopore, the Dufour's gland, which is the source of alarm pheromone, and the venom gland, which produces venom that primarily consists of the ant classic formic acid. In a second experiment, the researchers showed that only the content of the crazy ant venom gland, but not of the Dufour's gland, detoxifies fire ant venom. They then demonstrated that the same detoxifying effect can also be observed when applying formic acid directly, strongly suggesting that formic acid itself serves as the detoxifying agent. Although the precise action of formic acid in this context is unknown, LeBrun and colleagues [7] surmise that formic acid might detoxify fire ant venom as a topical insecticide by denaturing venom enzymes that are required to disrupt cell membranes and allow the alkaloid fraction of the venom to penetrate.

In a final experiment, the researchers showed that the behavioral response displayed by crazy ants is specific to interactions with *S. invicta*. While crazy ants showed some level of detoxification behavior in response to confrontations with other ants that employ defensive compounds, including several close relatives of *S. invicta*, their detoxification response increased markedly when exposed to *S. invicta*.

The triumphal procession of crazy ants into the U.S. began in the early 2000s, nearly a century after the introduction of fire ants. Despite the fact that the crazy ant infestation went anything but unnoticed, the crazy ants' taxonomic identity, and therefore their native range, remained controversial. This controversy was resolved recently in an effort by Dietrich Gotzek and colleagues that employed a combination of morphometrics and molecular data [15]. Once the species had been unequivocally identified as *Nylanderia fulva*, it became clear that the likely source of the crazy ant invasion was the watershed area of the Paraná River in northern Argentina, Paraguay, and southern Brazil. This region is infamous as a prolific cradle of invasive ants, including other major pests like the Argentine ant and, who would have guessed, the red imported fire ant. And here lies the crux of the story: crazy ants and fire ants are old acquaintances. In other words, crazy ants and fire ants probably share millions of years of evolutionary history, sufficient time for crazy ants to evolve specific behavioral responses that allow them to co-exist and compete with fire ants in their shared native range. In fact, the competition with crazy ants might be an important factor in restricting fire ants in their native range. In that sense, the study by LeBrun and colleagues [7] makes a strong case for the enemy release hypothesis, and provides a fascinating example of what happens when the evolutionary past of invasive species finally catches up with them.

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Programmed Cell Death: New Role in Trimming the Root Tips

How is a rapid cellular turnover of the lateral root cap achieved in plants to control cap size in the growing root tips? Downstream of *ANAC033/SOMBRERO*, a highly organized and temporally coordinated cell death program involving BFN1 nuclease-mediated rapid corpse clearance eliminates these cells.

Shri Ram Yadav and Ykä Helariutta*

Programmed cell death (PCD), an intracellular program for death, plays a fundamental role in various biological processes, including growth and development, in almost all eukaryotes. In plants, PCD can either be developmentally regulated or induced by abiotic and biotic factors [1–3]. During development, PCD is known to control some specialized differentiation programs of certain plant tissues, such as the pollen

tapetum and xylem tracheary elements, and to be required for their function [3–5]. Despite being fundamentally conserved in eukaryotes, PCD processes show only limited similarities at the molecular level between animals and plants [3]. Unlike animals, plants have a rigid cell wall and lack phagocytic processes; they therefore require a specific cell-autonomous process to remove the cell corpse after autolytic PCD during development.

Plant root tips contain a stem cell pool which is responsible for indeterminate root growth. Growing root tips are protected from damage during soil penetration by a specialized tissue called the root cap. The root tip is also a site of interactions with the rhizosphere, as well as for sensing signals for gravity, pressure, and moisture [6]. The root cap consists of two parts, the central columella and the lateral root cap (LRC). Both of these tissues are formed by synchronous periclinal cell divisions, though they develop from entirely different groups of initial cells [6,7]. The columella arises from the 'columella initials' located at the base of columella, whereas the LRC is derived from epidermal/LRC stem cells [7]. LRC daughter cells undergo several rounds of anticlinal cell divisions before they enter into the differentiation program. Unlike other organs, mature LRC cells are sloughed off of the root after terminal differentiation. Although this cellular turnover contributes to maintaining the root cap at a constant size, the cellular mechanism executing cell elimination is not entirely clear. In this issue of *Current Biology*, Fendrych *et al.* [8] report the discovery of a highly organized and temporally coordinated cell death program that removes the LRC cells before they enter the root elongation zone. They demonstrate that the *Arabidopsis* transcription factor *ANAC033/SOMBRERO* initiates a temporally regulated PCD program in the LRC, which is then followed by rapid clearance of the cell corpse mediated by the S1-P1 type nuclease BFN1 [8].

In order to demonstrate that cell death is involved in LRC differentiation, Fendrych *et al.* [8] performed a meta-analysis to compare the transcript profile of the LRC with maturing xylem, which is also known to undergo PCD, and identified two highly co-regulated genes, the S1-P1 nuclease *BFN1* [9,10] and the aspartate protease *PASPA3* [11]. Both of these genes are expressed in a specific zone of the LRC named the PCD buildup zone (Figure 1A) where cells prepare themselves for subsequent PCD. By analyzing a tonoplast integrity marker in the *PASPA3* expression domain, they showed that PCD is established in the most distal LRC cells of this domain, located at the transition zone of the root meristem (called PCD site I), and involves tonoplast rupture and abrupt vacuole collapse during LRC elimination. Data from live-cell imaging were used to show that cells increase *PASPA3* expression as they approach the end of the transition zone and then die at PCD site I (Figure 1B). The authors also suggested that once the PCD is established, cell death progresses towards the root tip, forming PCD site II; finally, the cells lose their contact with the root at the proximal end of the columella. This entire process is re-capitulated in the next-younger LRC cell layer once it reaches the transition zone (Figure 1B).

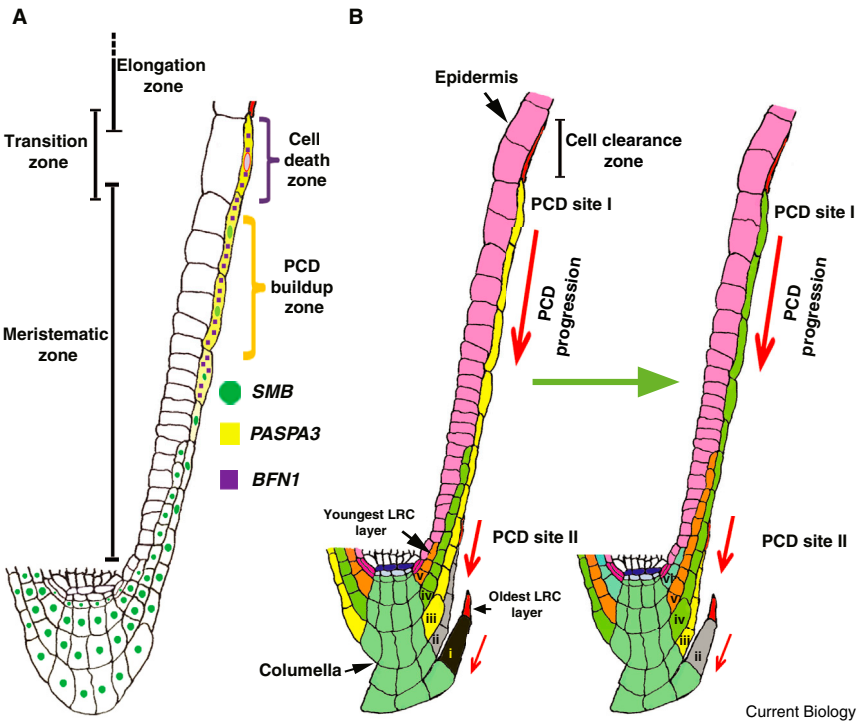

Figure 1. *SMB*-regulated PCD controls *Arabidopsis* root cap development.
(A) A schematic diagram showing the expression pattern/protein distribution of *SMB* (green), *PASPA3* (yellow) and *BFN1* (purple) in developing LRC cells. The cells prepare themselves for death in the 'PCD buildup zone' and finally die in the 'cell death zone'. *PASPA3* expression increases in elongating cells which subsequently die. The BFN1 protein is localized to the ER prior to cell death (purple boxes), but upon cell death the protein is released into the cell, including the nucleus (green/purple merged color in nucleus). (B) PCD progression across the LRC cell layers of the root tip. Various layers of the LRC are highlighted with different colors. In the left panel, five LRC layers (marked as i–v) are shown. In the right panel, the oldest layer (i) has been detached from the root cap and the youngest layer (vi) is formed. PCD is established at PCD site I of layer (iii) of the left picture, progresses cell-by-cell via PCD site II towards the root tip. Meanwhile, the underlying cell layer (layer iv) reaches the transition zone in the right picture and re-initiates a new round of PCD in the cell death zone. Cell clearance follows the progressing PCD front. The schematic diagram was generated using Figure 1E as a template from an article by Wenzel and Rost [20].

Next, Fendrych *et al.* [8] studied subcellular PCD hallmarks at a high temporal resolution during LRC terminal differentiation. Cytoplasmic acidification and release of hydrolases have been previously observed in several cases of developmental PCD, but are generally believed to be a consequence of vacuolar collapse during cell death [12,13]. Using a pH-sensitive green fluorescent protein (GFP) variant, the authors demonstrated that a sharp decrease in cytoplasmic pH occurs well before propidium iodide (PI) enters the cell and that the tonoplast ruptures

subsequently. These observations allowed them to define a new sequence of events during PCD in LRC cells; an initial cytoplasmic pH drop is followed by plasma membrane permeabilization and subsequent tonoplast rupture. They further confirmed the significance of the pH drop in cell death progression by studying the effects of extra- and intra-cellular pH manipulations on the frequency of LRC deaths. Together, these data show that a pH-activated cell death program is necessary and sufficient to trigger PCD in LRC cells.

The next hallmark of PCD is clearance of the cell corpse; in animals, this is largely achieved by phagocytosis [14], but in plants it is accomplished by the activity of hydrolytic enzymes in a cell-autonomous fashion [15]. In order to address this in LRC cells, Fendrych *et al.* [8] analyzed whether BFN1 and PASPA3 hydrolases are involved in LRC cell clearance. Time-lapse and serial block-face scanning electron microscopy imaging showed that while the *paspa3* loss-of-function mutant did not have any phenotype, nucleus degradation was significantly delayed in the *bfn1* null mutant. Furthermore, they show that BFN1 is compartmentalized to the endoplasmic reticulum of LRC cells before cell death, as reported earlier in other tissues [16], and is released only at the time of cell death to bring about a rapid and irreversible degradation of nucleic acids. Interestingly, despite broad expression of *BFN1* in other tissues, the nuclear degradation phenotype was only seen in the LRC, suggesting that it is a key enzyme for cell clearance in the LRC cells.

To further investigate whether cell death is the terminal differentiation step and an inherent part of LRC development, Fendrych *et al.* [8] analyzed cell death in the *tornado2* (*trn2*) and *sombrero* (*smb*) mutants. *TRN2* encodes a tetraspanin-type protein, and *trn2* mutants develop ectopic LRC cells in the epidermal layer which eventually die in the elongation zone [17]. Conversely, *SMB* is a root cap-specific *NAC* domain transcription factor, and *smb* mutants display delayed LRC differentiation [18]. The authors showed that the ectopic LRC cells of *trn2* express *PASPA3* in the elongation zone and also follow a PCD pattern identical to LRC cells, confirming that cell death is a context-independent feature inherent to LRC cells. However, their complementary analysis of the *smb* mutant demonstrated that expression of *PASPA3* and *BFN1* is completely absent from the distal cell zone and overall cell death is delayed in *smb* mutants. This indicates that *SMB* transcriptionally regulates the preparation of cell death during the final stages of LRC differentiation. Interestingly, the delayed cell death in *smb* mutants does not follow the normal pattern of LCR PCD. It occurs without preceding *PASPA3* expression and cell death does not include subsequent cell clearance. In order to understand how these *smb* LRC cells eventually die in the elongation zone, Fendrych *et al.* [8] tested the hypothesis that the massive physical strain generated from the direct connection with an elongating epidermal cell could be the cause of LRC cell death in this mutant. They showed that inhibition of cell expansion in the root elongation zone by brassinazole (Brz) treatment strongly reduced the aberrant cell death in the *smb* mutant, suggesting that cell death in the *smb* mutant may be passive, in contrast to a highly organized death program in wild-type roots.

To summarize, the current study by Fendrych *et al.* [8] provides interesting insights into the role of PCD in controlling root cap size. The role of PCD in controlling organ size has been well documented in animals [19]. A temporally coordinated PCD at the distal part of the root cap together with shedding of cells at the very tip of the root [18] ensures the maintenance of the root cap size in *Arabidopsis*. The role of *PASPA3* in this process suggests that it may serve as a marker for cell death in future studies. Finally, given the demonstration that a sharp pH drop can trigger PCD in LRC cells, it will be interesting to explore the mechanistic basis for the pH-activated cell death program in future research.

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