Plant cell death: **Unmasking the gatekeepers**

Gary Loake

Cell death is an important aspect of plant resistance to pathogen infection. Recent results have shed new light on the mechanisms that control this cell death following attempted pathogen infection.

Address: Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh EH9 3JH, UK.
E-mail: gloake@ed.ac.uk

Current Biology 2001, 11:R1028–R1031

0960-9822/01/$ – see front matter

Since antiquity the human race has been acutely aware of the potential impact of plant diseases. Fortunately, however, disease is the exception, because plants possess a complex set of molecular defences which are sufficient to repel most potential microbial pathogens. Chief among these is an extensive repertoire of resistance (*R*) genes, the products of which effectively scan the cellular environment for the presence of pathogen-derived effectors, encoded by so-called avirulence (*avr*) genes. Interestingly, *R* gene products turn out to have domains similar to animal Toll-like receptors, which function in the innate immune system (reviewed in [1]). In the model plant *Arabidopsis thaliana*, R proteins are thought to signal via several distinct pathways, one of which is defined by the NDR1 protein, and a second of which is defined by the EDS1 and PAD4 proteins (Figure 1). The structure of the particular R protein activated by a pathogen is thought to determine which route is followed [2].

When a plant recognises attempted infection by a pathogen, a complex signalling network is engaged that results in deployment of a plethora of inducible defence responses (reviewed in [3]). One of the most prominent of these responses is the production of reactive oxygen intermediates, primarily superoxide (O_2^-) and hydrogen peroxide (H_2O_2) at the point of attempted pathogen invasion (reviewed in [4]) (Figure 1). Notably, nitric oxide (NO) also accumulates during the plant oxidative burst — another parallel between plant and animal pathogen responses, as NO is known to act as a signal molecule in the animal immune, nervous and vascular systems [5,6]. Importantly, the production of these reactive oxygen intermediates is thought to be necessary for the initiation of host cell death in the plant hypersensitive disease-resistance response [6].

A number of mutations have been identified in crop plants as well as *Arabidopsis* which lead to spontaneous hypersensitive response-like cell death in the absence of pathogen. The mutants have been christened 'paranoid plants' by the *cognoscenti* (reviewed in [7]). Interestingly, the sites of cell death in these mutants frequently bear features characteristic of programmed cell death in animals. The recessive *lsd1* mutant is the prototypic example of the cell-death propagation subclass of such mutants. Importantly, while *lsd1* plants initially produce a typical hypersensitive-response lesion in response to attempted pathogen invasion, runaway cell death is subsequently initiated from the borders of the developed hypersensitive response and rapidly spreads to consume the whole leaf. Interestingly, O_2^- was shown to cue the initiation of runaway cell death in *lsd1* plants, suggesting that LSD1 negatively regulates a cell death pathway engaged by O_2-dependent signals released from pathogen-challenged plant cells [8]. *LSD1* was found to encode a novel zinc finger protein, with homology to GATA-type transcription factors, which may act either to repress a prodeath pathway or activate an anti-death pathway [9]. The cellular component(s) that function in concert with LSD1 to effect runaway cell death have, however, remained enigmatic.

Enter the Dangl and Parker groups, who in two landmark papers [10,11] have unmasked the identity of key regulators of runaway cell death. They generated a panel of *Arabidopsis* double mutants defective in LSD1 function and also the activity of one of EDS1, PAD4 or NDR1, three known positive regulators of R signalling. The aim was thus to discover whether genes required for prototypic R signalling are also involved in runaway cell death in *lsd1* mutants. This analysis also exploited recombinant *Pseudomonas syringae* pv. *tomato* (*Pst*) strains that express either the *avrRps4* or *avrRpm1* avirulence genes, which are recognised by the R proteins RPS4 and RPM1, respectively (Figure 1). While RPS4 requires both EDS1 and PAD4 to establish resistance, RPM1 requires only NDR1 (Figure 1). This was important, because it facilitated an assessment of the impact of *eds1*, *pad4* and *ndr1* mutations on the *lsd1* phenotype in the context of both intact and defective R signalling.

As expected, runaway cell death was initiated in *lsd1* plants in response to *Pst* strains expressing either *avr* gene. Surprisingly, however, this phenomenon was completely abolished in both *eds1*, *lsd1* and *pad4*, *lsd1* double mutant plants, in response to either *Pst* strain, while it was partially suppressed in *ndr1*, *lsd1* double mutants. Crucially, the striking requirement for *EDS1* and *PAD4* in runaway cell death was therefore independent of their R signalling functions. For example, neither EDS1 nor PAD4 is required for RPM1 mediated-resistance, but both are required for runaway cell death initiated via RPM1. This

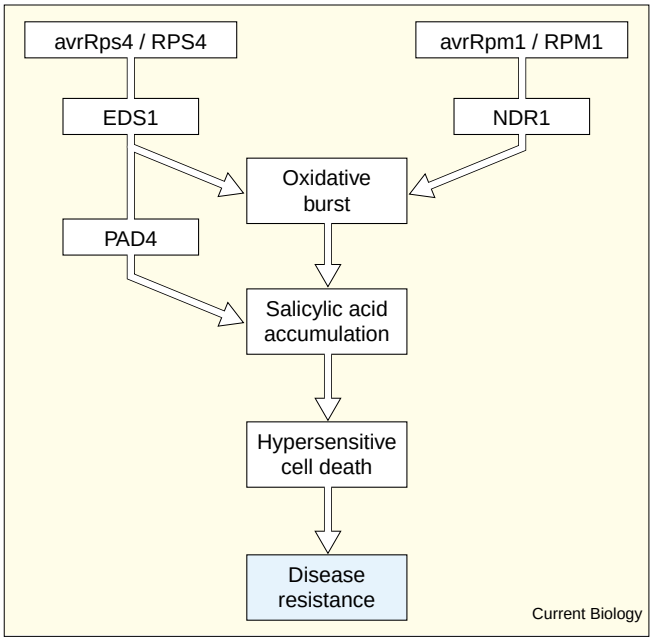
important conclusion was extended further by experiments employing distinct avirulent isolates of an oomycete pathogen, which has a completely different lifestyle to Pseudomonads, with similar results).

As reactive oxygen intermediates cue the initiation of runaway cell death in *lsd1* plants, the investigators next examined if the impact of *eds1*, *pad4* and *ndr1* on runaway cell death was a consequence of a compromised oxidative burst. While both EDS1 and PAD4 were required for runaway cell death, neither was found necessary for the production of reactive oxygen intermediates. In contrast, however, the oxidative burst was significantly misregulated in the absence of NDR1. Of particular significance was the discovery that H_2O_2 accumulation was absent at the boundaries of developing runaway cell death lesions. This was totally unexpected because O_2^- accumulation at runaway cell death boundaries has been well documented and this would have been expected to dismutate to H_2O_2. Conversely, O_2^- was not found to accumulate at any point during the development of hypersensitive response lesions. This again was surprising, because overwhelming evidence suggests that O_2^- production is the proximal event of the oxidative burst (reviewed in [4]).

The fate of O_2^- during the development of hypersensitive response lesions is therefore distinct from that during runaway cell death. How might this be explained? Rustérucci *et al.* [10] mention two possibilities. As O_2^- is very transient in nature, because of its inherent instability and rapid enzymatic conversion to H_2O_2, this observation may reflect a differential deployment of antioxidants during the hypersensitive response and runaway cell death. Alternatively, the O_2^- generated at the boundaries of runaway cell death lesions could be rapidly converted to something other than H_2O_2. In animal cells, for example, NO has been well documented to antagonise the action of O_2^-, by converting this reactive oxygen intermediate to peroxynitrite ($ONOO^-$). Indeed, recent data suggest that the hypersensitive response may be controlled by a balance between NO and H_2O_2, following its dismutation from O_2^- [12].

These results suggest that the cell-nonautonomous signalling by directly challenged plant cells may be distinct from that in cells bordering the site of attempted infection. How, then, might this signalling lead to runaway cell death in unchallenged neighbouring cells? Exogenous application of the plant metabolite salicylic acid, or one of its analogues, was known to cue runaway cell death in *lsd1* plants [13]. Furthermore, Aviv *et al.* [11] found this response was dependent on *EDS1* and *PAD4*, but not on *NDR1*. Numerous studies have suggested that salicylic acid functions at multiple nodes in the defence signalling network to potentiate a variety of plant defence responses, including

Figure 1



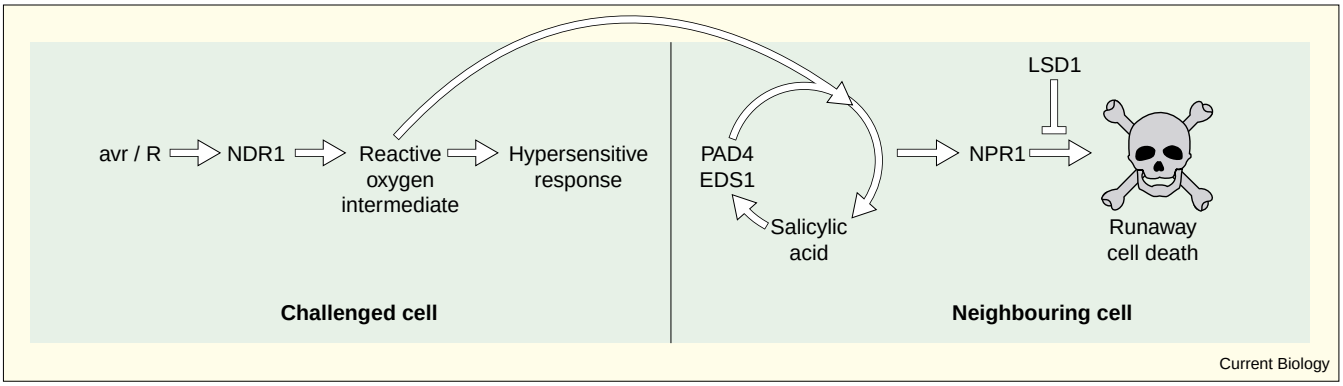
Recognition by *Arabidopsis* plants of *Pseudomonas syringae* pv. *tomato* (*Pst*) strains expressing either the *avrRps4* or *avrRpm1* avirulence genes. AvrRps4 is recognised specifically by the R protein RPS4. Subsequent RPS4 signalling is dependent on EDS1 and PAD4. In contrast, AvrRpm1 is recognised by RPM1, which requires NDR1 for the establishment of disease resistance. Recognition of either avirulence protein engages a rapid oxidative burst, salicylic acid accumulation and cell death which, in conjunction with other defence responses, establish disease resistance.

the oxidative burst [14]. In this context, *EDS1* and *PAD4* are both transcriptionally activated by salicylic acid [15,16].

To explore this idea further, Aviv *et al.* [11] generated plants combining the *lsd1* mutation with either a transgene causing depletion of salicylic acid or a second mutation causing a defect in NIM1/NPR1, a global salicylic acid response regulator [17,18]. Informatively, in the absence of salicylic acid accumulation or NIM1/NPR1 function, *lds1*-mediated runaway cell death was found to be significantly blunted in response to both exogenous salicylic acid and avirulent *Pst* strains. LSD1 thus both generates and responds to salicylic acid-mediated signals. These observations provide compelling evidence for the existence of a NPR1-dependent, salicylic acid-potentiation loop in the initiation of runaway cell death (Figure 2).

So what are the biochemical activities of these key signalling proteins, and how do they fit into this emerging conceptual framework? NDR1 is a small membrane-spanning protein of unknown function, which modulates production of reactive oxygen intermediates but is not required for salicylic acid-initiated runaway cell death [19]. Hence, NDR1 may

Figure 2



A model for the cell death pathway regulated by LSD1. Pathogen recognition in directly challenged cells activates the oxidative burst which is modulated by NDR1. Cell-nonautonomous reactive oxygen intermediates stimulate EDS1 and PAD4 activities in neighbouring unchallenged cells, which are amplified via a salicylic acid-potentiation loop. The resulting signals are interpreted by NPR1, which engages a prodeath pathway. LSD1 negatively regulates this pathway by attenuating NPR1 activity, raising the signal threshold for engagement of the cell death machinery.

function early in this pathway, possibly in the regulation or interpretation of signals in directly challenged cells. EDS1 and PAD4 both exhibit sequence similar to the catalytic domains of eukaryotic lipases [15,16], so their activities may be directed towards the generation of lipid signals, cued by cell-nonautonomous reactive oxygen intermediates. It is well documented that distinct lipid-based signals can function as powerful effectors of cell death in both plants and animals [20,21]. Moreover, a redox cue is known to directly regulate the production of animal prostaglandins, lipid-based stimuli produced during the inflammatory response [22].

The salicylic acid-dependent potentiation loop may function to amplify the production of these EDS1- and PAD4-dependent signals, within plant cells surrounding the immediate site of attempted infection. The concentration of these signals may ultimately exceed a threshold value required to engage cellular execution. In the absence of LSD1, this threshold may be significantly reduced, resulting in self-perpetuating ectopic cell death, as manifested in *lsd1* mutants. But how might these amplified signals engage the cell death machinery? Recent evidence has suggested that NIM1/NPR1 function may positively regulate a cell death pathway, at least in some contexts [23]. Moreover, this global salicylic acid-response regulator, which may function to modulate transcription, is clearly required for complete runaway cell death. So one fascinating possibility proposed by Aviv *et al.* [11] is that LSD1 may antagonise NPR1 function, possibly at the level of gene regulation, suppressing the engagement of a prodeath signalling pathway (Figure 2).

The discovery that EDS1 and PAD4 function to control cell death within a salicylic acid- and NPR1-dependent pathway raises new questions. What are the biochemical activities of EDS1 and PAD4? And how are these activities modulated by reactive oxygen intermediates- and salicylic acid-generated signals? Does LSD1 negatively regulate this pathway by attenuating transcription? How might NPR1 engage the cell death machinery? Like primed *lsd1* plants, the field has now been cued for a rapid burst of activity.

Acknowledgements
Research on plant disease resistance in the Loake lab is supported by the BBSRC.

References

1. Dangl JL, Jones JDG: Plant pathogens and integrated defence responses to infection. *Nature* 2001, 411:826-833.
2. Aarts N, Metz M, Holub E, Staskawicz BJ, Danels MJD, Parker JE: Different requirements for ***EDS1*** and ***NDR1*** by disease resistance genes define at least two ***R*** gene-mediated signalling pathways in ***Arabidopsis***. *Proc Natl Acad Sci USA* 1998, 95:10306-10311.
3. Glazebrook J: Genes controlling expression of defence responses in ***Arabidopsis*** - 2001 status. *Curr Opin Plant Biol* 4:301-308.
4. Grant JJ, Loake GJ: Role of reactive oxygen intermediates and cognate redox signalling in disease resistance. *Plant Physiol* 2000, 124:21-29.
5. Delladonne M, Xia YJ, Dixon RA, Lamb C: Nitric oxide functions as a signal in plant disease resistance. *Nature* 1998, 394:585-588.
6. Durner J, Wendehenne D, Klessig DF: Defence gene induction in tobacco by nitric oxide, cyclic GMP and cyclic ADP-ribose. *Proc Natl Acad Sci USA* 1998, 96:3292-3297.
7. Greenberg JT: Programmed cell death in plant:pathogen interactions. *Annu Rev Plant Physiol Plant Mol Biol* 1997, 48:525-545.
8. Jabs T, Dietrich RA, Dangl JL: Initiation of runaway cell death in an ***Arabidopsis*** mutant by extracellular superoxide. *Science* 1996, 273:1853-1856.
9. Dietrich RA, Richberg MH, Schimdt R, Dean C, Dangl JL: A novel zinc finger protein is encoded by the ***Arabidopsis LSD1*** gene and functions as a negative regulator of plant cell death. *Cell* 1997, 88:685-694.
10. Rustérucci C, Aviv DH, Holt BF, Dangl JL, Parker JE: The disease resistance signalling components EDS1 and PAD4 are essential regulators of the cell death pathway controlled by ***LSD***1 in ***Arabidopsis***. *Plant Cell* 2001, in press.

11. Aviv DH, Rustérucci C, Holt BF, Dietrich RA, Parker JE, Dangl JL: Runaway cell death, but not basal resistance, in ***lsd1*** is SA- and NIM1/NPR1-dependent. *Plant J* 2001, in press.
12. Delladonne M, Zeier J, Marocco A, Lamb C: Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proc Natl Acad Sci USA*, in press.
13. Dietrich RA, Delaney TP, Uknes SJ, Ward EJ, Ryals JA, Dangl JL ***Arabidopsis*** mutants simulating disease resistance response. *Cell* 77:565-578.
14. Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb CJ: Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defence mechanisms. *Plant Cell* 1997, 9:261-270.
15. Falk A, Feys B, Frost LN, Jones JDG, Danels MJD, Parker JE: ***EDS1***, an essential component of ***R*** gene-mediated disease resistance in ***Arabidopsis*** has homology to eukaryotic lipases. *Proc Natl Acad Sci USA* 1999, 96:3292-3297.
16. Jirage D, Tootle TL, Reuber TL, Frost LN, Feys BJ, Parker JE, Ausubel FM, Glazebrook J: ***Arabidopsis thaliana PAD4*** encodes a lipase-like gene that is important for salicylic acid signaling. *Proc Natl Acad Sci USA* 1999, 96:13583-13588.
17. Delaney TP, Friedrich L, Ryals JA: ***Arabidopsis*** signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc Natl Acad Sci USA* 1995, 92:6602-6606.
18. Cao H, Bowling SA, Gordon S, Dong X: Characterisation of an ***Arabidopsis*** mutant that is non-responsive to inducers of systemic acquired resistance. *Plant Cell* 1994, 6:1583-1592.
19. Century KS, Shapiro AD, Repetii PP, Dahlbeck D, Holub E, Staskawicz BJ: ***NDR1***, a pathogen-induced component required for ***Arabidopsis*** disease resistance. *Science* 1997, 278:1963-1965.
20. Wang H, Li J, Bostock RM, Gilchrist DG: Apoptosis: a functional paradigm for programmed cell death induced by a host-selective phytotoxin and invoked during development. *Plant Cell* 1996, 8:375-391.
21. Rustérucci C, Montillet J-L, Agnel J-P, Battesti C, Alonso B, Knoll A, Bessoule J-J, Etienne P, Suty L, Blein J-P, Triantaphylides C: Involvement of lipoxygenas-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptogein on tobacco leaves. *J Biol Chem* 1999, 274:36446-36455.
22. Salvemini D, Misko TP, Maserer JL, Seibert K, Currie MG, Needleman P: Nitric-oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci USA* 1993, 90:7240-7244.
23. Greenberg JT, Silverman FP, Liang H: Uncoupling salicylic acid-dependent cell death and defence-related responses from disease resistance in the ***Arabidopsis*** mutant ***acd5***. *Genetics* 2000, 156:341-350.