nature plants

Perspective

https://doi.org/10.1038/s41477-023-01466-1

Is localized acquired resistance the mechanism for effector-triggered disease resistance in plants?

Received: 30 January 2023

Accepted: 23 June 2023

Published online: 03 August 2023

Pierre Jacob [1,2], Junko Hige[1,2] & Jeffery L. Dangl [1,2] ✉

Plant nucleotide-binding leucine-rich repeat receptors (NLRs) are intracellular immune receptors that are activated by their direct or indirect interactions with virulence effectors. NLR activation triggers a strong immune response and consequent disease resistance. However, the NLR-driven immune response can be targeted by virulence effectors. It is thus unclear how immune activation can occur concomitantly with virulence effector suppression of immunity. Recent observations suggest that the activation of effector-triggered immunity does not sustain defence gene expression in tissues in contact with the hemi-biotrophic pathogen *Pseudomonas syringae* pv. *tomato*. Instead, strong defence was observed on the border of the infection area. This response is reminiscent of localized acquired resistance (LAR). LAR is a strong defence response occurring in a ~2 mm area around cells in contact with the pathogen and probably serves to prevent the spread of pathogens. Here we propose that effector-triggered immunity is essentially a quarantining mechanism to prevent systemic pathogen spread and disease, and that the induction of LAR is a key component of this mechanism.

Plants survey their microbial environment using plasma-membrane pattern-recognition receptors (PRRs). The perception of pathogen-associated molecular patterns leads to pattern-triggered immunity (PTI), which restricts microbial growth and prevents dysbiosis. To be pathogens, micro-organisms need to dampen or block the plant immune response. Pathogens deploy virulence effectors to host cells; these effectors modulate immunity and render the plants susceptible to disease in a process called effector-triggered susceptibility (ETS)[1,2]. Plants recognize effectors directly or indirectly via intracellular NLRs[2]. NLRs trigger strong defence responses that can be accompanied by hypersensitive cell death and can protect against disease in the field[1,3]. Although the exact nature of the disease resistance mechanisms deployed by NLRs that halt pathogen ingress is still unclear, NLR activation can determine disease resistance.

NLRs are divided into three main categories on the basis of their amino-terminal signalling domains: the CC-NLRs (CNLs), the Toll/Interleukin-like Resistance genes (TIR)-NLRs (TNLs) and the CC-RPW8 NLRs (RNLs)[4]. TNLs form enzymes that degrade NAD^+ to produce a mixture of signal molecules[5–9], whereas RNLs and some CNLs function as calcium influx channels[10–12]. Calcium influx is a major regulator of defence and is required and sufficient for immune activation[13]. TNLs possess at least two functions in *Arabidopsis* immunity. First, they activate RNL-driven calcium influx via two types of Enhanced Disease Susceptibility 1 heterodimer[5,9]. This signalling branch is essential for disease resistance and cell death during TNL-initiated effector-triggered immunity (ETI). Second, TNLs express an emerging broader function in potentiating or boosting the increase in cytoplasmic $[Ca^{2+}]$ following RNL and CNL activation[8,14]. TIR-domain-containing genes are transcriptionally upregulated early during the PTI phase of infection and participate in a positive feedback loop. Defence amplification by TNLs is important for PRR and NLR signalling[7,8,14,15].

[1]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [2]Howard Hughes Medical Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. ✉e-mail: dangl@email.unc.edu

https://doi.org/10.1038/s41477-023-01466-1

How does NLR signalling escape immune suppression by virulence effectors?

PRR signalling potentiates ETI, indicating that PRRs prime the cell for subsequent defence activation by NLRs[16,17]. In addition, ETI and PTI activate a globally similar transcriptional output; NLRs overall induce an enhanced PTI response[15,18,19]. Thus, the collective action of virulence effectors that suppress defence should, theoretically, affect both ETI and PTI[20–22]. This issue is further highlighted by the 'guard hypothesis'. Plants have a fixed number of NLR receptors and therefore limited recognition capacities[4]. To overcome these limitations, in a subtantial number of cases (particularly involving resistance to bacterial pathogens), NLRs do not recognize virulence effectors directly but rather survey the cellular environment to detect inhibition of the immune system by virulence effectors, which activates NLRs associated with the virulence target[1].

Therefore, at least for guard NLRs, attempted PTI inhibition should be a prerequisite for NLR activation. How NLRs restore an efficient defence response in the presence of virulence effectors is a major paradox of plant immunology. Previous studies speculated that ETI signalling would overcome ETS by inducing a stronger PTI response and renew PTI signalling components, making them available for inducing immunity[16]. Alternatively, in 1961, the hypersensitive response was linked to a specific, spatially organized defence response called LAR[23]. Although little is known about the spatial regulation of defence, it could explain how NLR signalling escapes from effector activity. Here we discuss how quantitative, temporal and spatial aspects of NLR signalling could help us understand how NLRs trigger immunity in the context of immune suppression. We propose that LAR is a major mechanism for ETI-driven disease resistance.

How does ETI overcome ETS?

Disease resistance is a quantitative phenotype. The zig-zag model suggests that disease susceptibility or resistance results from the balance between factors that inhibit defence (virulence effectors) and factors that activate or reactivate defence (immune receptors)[2]. This implies that ETI overactivates PTI, which in turn overcomes ETS and induces disease resistance.

In support of this quantitative view of defence, NLRs are often semi-dominant[24,25]. In addition, ETI triggered by different NLRs varies in its capability to limit pathogen growth, indicating that NLRs act quantitatively[3]. RNL Activated Disease Resistance 1 (ADR1s) and N requirement gene 1 (NRG1s) contribute quantitatively to TNL ETI[26]. Basal defence against the virulent pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) involves the activation of weak ETI and is dependent on the TNL signalling partner Enhanced Disease Susceptibility 1 (ref. 27). This further indicates that TNLs contribute quantitatively to the limitation of pathogen growth during basal defence. Inversely, virulence is typically not conferred by individual effectors but emerges from the collective action of the pathogen's effector repertoire[22].

In addition, NLRs signal through PTI signalling components[15–17], and single effectors can inhibit NLR-driven cell death when overexpressed in *Nicotiana benthamiana*[20,21]. The timely induction of defence should therefore be important for strong defence signalling, and this is consistent with the view that ETI overcomes ETS by triggering stronger defence earlier[28]. Indeed, the speed of local ETI induction seems to be correlated with the strength of the ETI response. This observation is particularly relevant for obligate biotrophic pathogens such as *Hyaloperonospora arabidopsidis*, for which slower ETI responses are associated with trailing necrosis and overall lower disease resistance[29]. However, in the case of obligate biotrophs, cell death of, for example, haustoriated cells is a major determinant of disease resistance, contrary to hemibiotrophs, which can feed from dead tissue. Overall, some evidence argues in favour of ETI triggering disease resistance by overcoming ETS through the quantitative and temporal control of immunity.

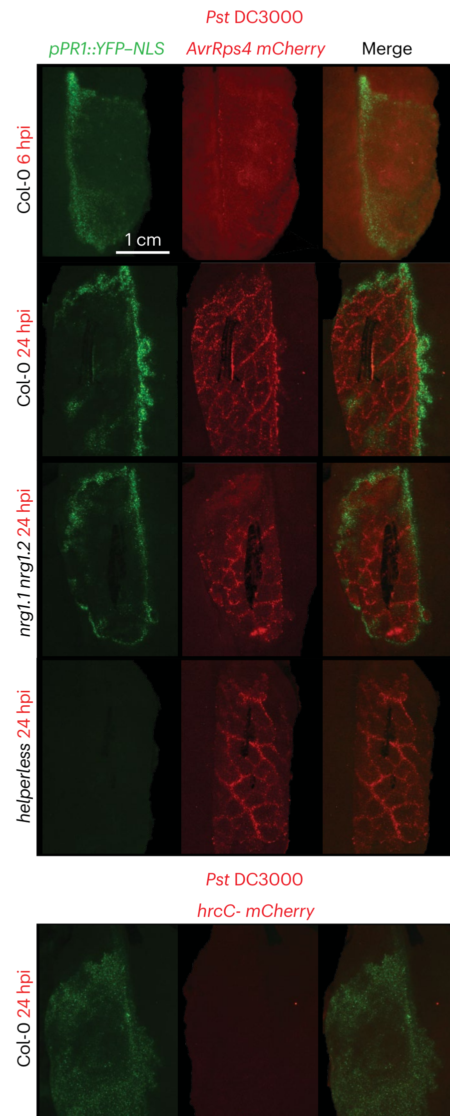

Fig. 1 | ETI does not restore defence gene expression that has been suppressed by virulence effectors. Close-up epifluorescence photographs of a reporter plant expressing *pPR1::YFP–NLS* in leaves inoculated with mCherry-tagged bacteria and observed at the indicated times. Approximately two-thirds of a half leaf was inoculated. The acquisition time was increased for pictures taken at six hours post infection (hpi). Notably, virulence effectors delivered by *Pst* DC3000 *AvrRps4* inhibit *PR1* expression (compare with *Pst* DC3000 *hrcC-*, which cannot deliver effectors), even though *AvrRps4* induces RPS4 ETI. Figure adapted with permission from ref. 14, National Academies.

However, recent observations suggest that spatial regulation of defence generated during ETI in cells surrounding the infection site is a critical component of pathogen restriction. We noted that ETI does not restore *Pathogenesis Related 1* (*PR1*) expression during infection with a virulent pathogen (Fig. 1). We used *PR1* reporter plants to assess the spatial regulation of defence during infection and observed that infection with *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 *AvrRps4* inhibits *PR1* expression locally in the context of ETI (Fig. 1). In other words, this observation suggests that ETI induced by the TNL Resistance to *Pseudomonas Syringae* 4 (RPS4) does not fully restore defence gene expression in cells that are in contact with effectors and subjected to ETS. Instead, we observed a pattern of *PR1* expression around the infection site. Notably, areas where *PR1* was expressed were different from areas with high mCherry signal from the fluorescent bacteria, suggesting that ETS prevented *PR1* expression in this area (Fig. 1).

https://doi.org/10.1038/s41477-023-01466-1

Consistent with this observation, plants inoculated with *Pst* DC3000 *hrcC-* bacteria, which cannot inject virulence effectors into the host cytoplasm, induced *PR1* expression throughout the inoculated area, and bacterial growth was effectively halted, as evidenced by the lack of strong mCherry signal (Fig. 1). This pattern of *PR1* expression did not result from tissue collapse or cell death induction by RPS4 as it was observed at six hours post infiltration, long before cell death, and was also observed in the cell-death-deficient *N requirement gene 1.1* and *1.2* (*nrg1.1 nrg1.2*) double mutant[30]. In addition, the ETI-deficient *helperless* mutant lacking all five active RNLs (ADR1, ADR1-L1, ADR1-L2, NRG1.1 and NRG1.2 (refs. 26,31,32)) did not exhibit a strong increase in bacterial growth compared with Col-0.

These results suggest that ETI may not restore defence signalling that has been inhibited by effectors, but rather bypasses the effects of ETS by inducing defence in cells that are not in contact with the pathogen. We tested this hypothesis by inoculating *Arabidopsis* Col-0 with a high-concentration inoculum of virulent or avirulent bacteria, to ensure that all cells in the infiltration area were in contact with the pathogen and thus subjected to ETS. We thereby aimed to reproduce at the macroscopic scale what should happen during infection at the microscopic scale. While the use of such a highly concentrated inoculum might not seem physiologically relevant, *Pst* DC3000 inoculated in low doses still grows to very high concentrations in apoplastic microcolonies during disease development[33]. Consistent with this, the inoculation of high- and low-dose inocula of avirulent bacteria trigger similar responses: defence-related transcript accumulation, reactive oxygen species (ROS) production and cell death induction[34]. In addition, *Pst* DC3000 is still able to grow substantially even if inoculated at a high concentration, as evidenced by the large increase in mCherry fluorescence (Fig. 1). A high-dose inoculum thus reflects physiologically relevant infection processes to a large extent.

We measured bacterial growth in the infiltration area and outside the infiltration area after three days (Fig. 2). We found that ETI triggered by RPS4 or Resistance to *Pseudomonas Syringae* pv. *maculicola* 1 (RPM1) had little to no effect on bacterial growth within the inoculated site. In the infection area, *Pst* DC3000 empty vector (EV), *AvrRps4* or *AvrRpm1* grew to overall similar levels in Col-0 (Fig. 2). These results are consistent with the observation that avirulent bacteria can grow and form microcolonies of clonal origin despite the activation of ETI[33]. Interestingly, we observed a weak increase in pathogen growth inside the inoculation site in *helperless* plants infected by either *Pst* DC3000 EV or *AvrRps4*, consistent with the fact that RNLs are required for PTI[15,18]. By contrast, RPS4 and RPM1 efficiently contained bacteria to the infiltration area. Bacterial titres outside the inoculation site were 100 to 1,000 times lower in plants infected with *Pst* DC3000 *AvrRps4* or *AvrRpm1* than in those infected with *Pst* DC3000 EV (Fig. 2). This containment mechanism required RNLs for activation of the TNL RPS4, as expected[26,31,32]. Thus, ETI does not overcome ETS, and (at least for this hemibiotrophic pathogen) it does not inhibit pathogen growth inside the initial infection site. However, obligate biotrophs that form haustoria, and viruses, can be directly affected by cell death, contrary to hemibiotrophs, which can feed from dead tissue.

Spatial partitioning of ETI signalling and effector activity provides a harmonious explanation of how ETI signalling escapes effector-driven suppression of defence and why ETI requires PTI components for signalling[15,17]. In this model, NLR activation in one cell would provide disease resistance by generating cell non-autonomous danger signals that induce defence around the infection site and outward several tens of cells, in cells that have not been corrupted by the action of virulence effectors. This would eventually block further invasion of new tissues by the pathogen and thus minimize the impact of the pathogen on plant fitness. In other words, it would stop disease. This would be particularly important for pathogens that are not obligate biotrophs and can feed from dead tissue.

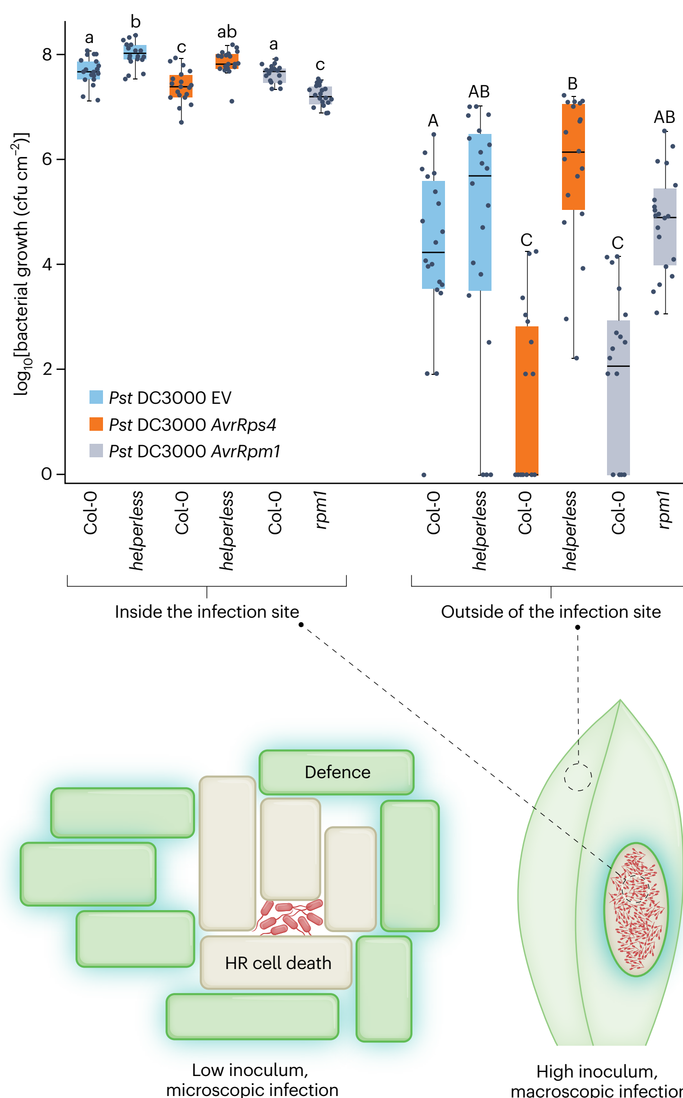

Fig. 2 | ETI does not inhibit pathogen growth locally but limits pathogen propagation. Plants were inoculated on approximately two-thirds of a half leaf with ETI-inducing bacteria (*Pst* DC3000 *AvrRps4* and *AvrRpm1*) or not (*Pst* DC3000 EV), using high inoculum (OD_{600} = 0.2) to mimic microscopic infection at the macroscopic scale. Bacterial growth was measured after three days on the infection site or outside the infection site on the same leaf. The letters indicate statistical significance (analysis of variance with post-hoc Tukey, $P < 0.05$, $n = 20$). Statistical analyses of the data from 'inside' and 'outside' were performed separately. The data are from five independent experiments. HR, hypersensitive response.

ETI regulates LAR to prevent systemic pathogen spread and disease

Defence gene expression is spatially regulated during ETI in a process similar or identical to LAR[23]. LAR is induced by NLR activity as it is associated with the hypersensitive response, and it consists of the induction of a very strong defence response in a 2 mm area surrounding cells in contact with the elicitor[23,35,36]. The expression of *PR1* is typical of LAR-induced defence, indicating that salicylic acid (SA) plays a major role in LAR[36]. This mechanism is distinct from systemic acquired resistance (SAR), which primes defence in distal tissues to prevent secondary infections[37]. Contrary to SAR, LAR is acute and short-lasting. LAR was

 https://doi.org/10.1038/s41477-023-01466-1

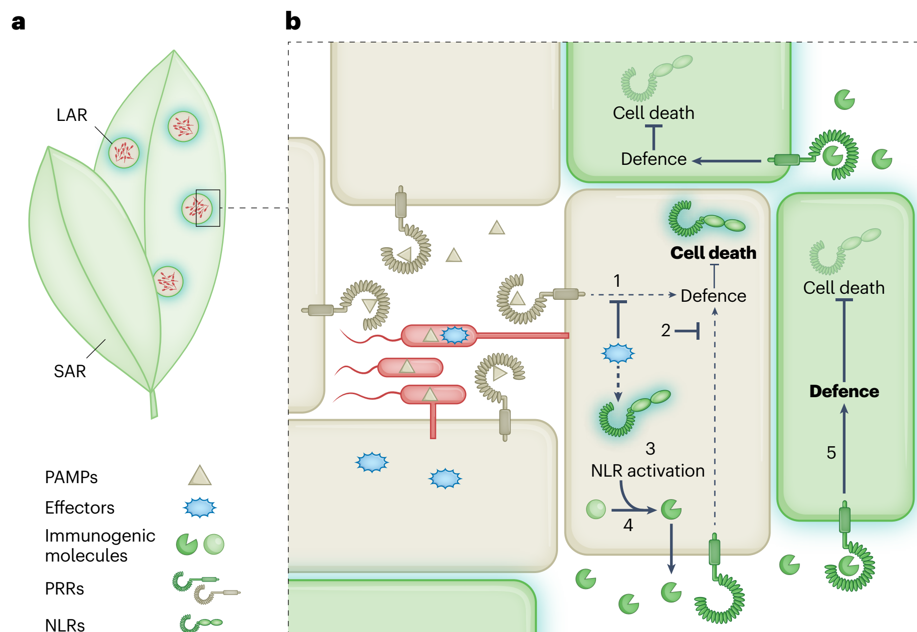

Fig. 3 | Does ETI induce LAR to prevent systemic pathogen spread?. a, Invasion of a plant host by an avirulent *Pst* DC3000 leads to spatially organized defence responses. LAR around the infection area stops the spread of the pathogen, and SAR prevents subsequent colonization events in distal tissues. **b**, Events occurring after the invasion of an avirulent *Pst* DC3000. Plasma membrane PRRs are activated by the perception of pathogen-associated molecular patterns (PAMPs) (1). Virulence effectors then inhibit defence (2). Virulence effectors trigger the activation of intracellular NLR receptors, ultimately leading to cell death in the absence of negative feedback from immune signalling (3). Immunogenic molecules or pro-immune-response factors are activated or produced and then released (4). Finally, immunogenic molecules activate defence in neighbouring cells, leading to pathogen containment (5).

first described in *Nicotiana tabacum* following infection with various viruses and was interpreted as a barrier to stop the progression of the viruses in the host[23]. Importantly, the hypersensitive response triggered by an elicitor (that is, NLR activity) was found to activate defence in the surrounding cells without them being in direct contact with the elicitor[36]. NLR activity in one cell thus triggers defence gene expression in the surrounding cells. Later, it was observed that following activation of the RPS2 CNL, cells expressing the defence gene *PR1* are distinct from the cells undergoing cell death as evidenced by chlorophyll degradation, suggesting that they are not in direct contact with the pathogen[38]. Cell non-autonomous ETI signalling has also been observed, unequivocally, at single-cell resolution in the context of *H. arabidopsidis* Emwa1 infection[39]. During an incompatible interaction with *H. arabidopsidis* Emwa1 (which triggers the TNL RPP4), cells that are not in contact with the pathogen trigger the highest *PR1* expression. Similar observations were made using the β-glucuronidase (GUS) reporter gene fused to the chitinase A (*CHIA1*) promoter. *CHIAp* was highly induced in a small area surrounding cell death lesions following infection with *Rhizoctonia solani* in *Arabidopsis* or with *Alternaria solani* and *Phytophthora infestans* in tomato[40]. This further indicates that ETI in one cell triggers immune signalling in the surrounding cells.

This cell non-autonomous signalling has been linked to NLR activity. In the *lesion simulating disease 1* (*lsd1*) mutant, defence signalling triggers cell death dependent on ADR1-L1 and ADR1-L2 NLRs[41,42]. Most importantly, in the *lsd1* mutant, cell death induction in one cell triggers cell death in neighbouring cells, in a superoxide-dependent process called runaway cell death[43]. NLR activity in one cell can thus evoke immune signalling in neighbouring cells, similar to damage-associated molecular pattern (DAMP) signalling[44].

Cell non-autonomous signalling is also visible at the transcriptomic level. It brings heterogeneity in the transcriptomic profiles of host cells during infection[45–47]. Transcriptomic analyses demonstrated the existence of bi-phasic or echoing defence response during ETI, reminiscent of a cell non-autonomous relay of defence gene expression[48]. Spatial mapping of the whole transcriptome during infection showed that 'older' immune active cells are surrounded by 'early' immune active cells, further suggesting that cells in contact with the pathogen activate immunity in bystander cells[49]. This signalling leads to the formation of a pattern of *PR1* expression around the infection area[14,38,39]. The presence of this pattern correlates with the ability of the virulent *Pst* DC3000 to spread and cause systemic disease symptoms in *Arabidopsis*, even though *Pst* DC3000 effectors can suppress immunity locally[14]. ETI was sufficient to stop the spread of pathogens in *Nicotiana benthamiana* and *Phaseolus vulgaris*[33,50]. The ultimate outcome of viral infections is dependent on LAR. Ectopic expression of the TNL N in *N. benthamiana* causes tobacco mosaic virus resistance by stopping viral spread[51]. However, the virus is not eliminated locally, at the infection site. SA signalling is not required for cell death induction but stops the spread of tobacco mosaic virus in *N. benthamiana*, turnip crinkle virus in *Arabidopsis* and potato virus X and Y in *Solanum tuberosum*[52–56]. Overall, NLR signalling can trigger defence around the infection area, and this mechanism is correlated with decreased spread of pathogens and disease resistance, suggesting that LAR is a major mechanism used by NLRs to prevent disease.

Many open questions remain, in particular regarding the identity of the immunogenic molecules triggering LAR. These molecules should be regulated directly or indirectly by NLRs. DAMPs are strong candidates, as NLR activation leads to apoplastic reactive oxygen bursts that could act directly on uninfected surrounding cells and calcium influx that could orchestrate the maturation and the release of DAMPs, as seen in the case of PEP1 (ref. 44). A gradient of SA was ruled out as a possible regulatory mechanism even though LAR triggers *PR1* expression, which is a hallmark of the SA response[35,36]. ROS are probably involved in LAR since they are NLR-regulated and are required for the spread of cell death in *lsd1*, indicating that they function in cell non-autonomous ways[57]. ROS may play multiple roles in LAR. ROS act as regulators of the host defence response and cell death but may also directly inhibit pathogen growth and act as a barrier limiting pathogen

spread[58,59]. Additionally, local sustained ROS production is sufficient to trigger long-distance defence signalling, indicating that ROS are major regulators of non-autonomous defence signalling[34,59,60].

Cell death and spatial regulation of defence

It is unclear to what extent cell death contributes to the regulation of LAR. We observed LAR during RPS4-driven ETI before the induction of cell death and in mutants that cannot trigger RPS4-driven cell death (Fig. 1). The NLR Rx also stops potato virus X without triggering cell death, although Rx can trigger cell death if overstimulated by high amounts of viral coat protein[61]. Cell death is also unnecessary for LAR in parsley cells challenged with *Phytophtora infestans*[62]. In contrast, cell death is required for LAR triggered by a fungal glycoprotein elicitor[35]. Cell death is thus associated with but not strictly required for LAR.

Increased or unrestricted NLR activity results in cell death, as seen in hybrid necrosis[63,64] or in mutants that overaccumulate NLRs[65]. The occurrence of NLR-driven cell death is associated with increased pathogen growth restriction[3]. It is important to note that cell-autonomous cell death is probably the major disease resistance mechanism against obligate biotrophs. Our model does not exclude the contribution of hypersensitive cell death to disease resistance but rather emphasizes the importance of non-autonomous signalling. Indeed, it is difficult to understand how dead cells, where high NLR activity occurred, can mount an effective immune response against hemibiotrophic pathogens, at least on their own. Higher disease resistance from cell-death-inducing ETI presupposes that bystander cells are affected by the strong ETI signalling in the dying cells. NLR-triggered cell death can be inhibited by prior induction of defence by SA[66], indicating that defence induction inhibits NLR signalling in a negative feedback loop. Indeed, SNIPER1 and 2, which regulate the proteasomal degradation of many NLRs, are upregulated during PTI and ETI (AtGeneExpress[63]). NLR-driven cell death could thus result from unrestricted NLR activation in cells directly in contact with the pathogen and subjected to the action of virulence effectors.

Conclusion

Plants continuously develop new organs. Localized infections are therefore not a major threat to plant health, as long as the pathogen cannot spread systemically. Here we propose that during infections by hemibiotrophic pathogens, ETI prevents disease by stopping the systemic spread of the pathogen through the mechanism of LAR. We further propose that plants use the spatial regulation of immune responses to counter the actions of pathogen effectors and the occurrence of ETS (Fig. 3). In this model, pathogens are first recognized at the level of the plasma membrane by PRRs, which trigger an initial activation of defence that is sufficient to limit the growth of most micro-organisms and enhances eventual ETI activation (1). It is worth recalling that the expression and delivery of virulence effectors are temporally delayed compared with the triggering of PRRs. Second, pathogens inhibit defence activation in infected cells via ETS, which enables initial growth and niche colonization (2). The action of effectors then triggers NLR activity in the immune-suppressed cells (3). NLR activity orchestrates enhanced activation and release of immunogenic molecules or pro-immune response signals such as reactive oxygen intermediates previously induced by PTI, eventually leading to cell-autonomous cell death in the absence of a negative feedback loop (4). Finally, LAR is triggered. PRRs in the neighbouring cells perceive the immunogenic signals from ETI-induced, ETS-influenced cells and activate strong immune responses (5). This induction effectively quarantines the infected area and stops further colonization of the plant host. Future studies should aim at confirming or disproving this model. In particular, detailed analyses of the spatial regulation of defence during ETI should be performed, using low-dose inoculum that more closely resembles plant–pathogen interaction in the field. In addition, the contributions of autonomous and non-autonomous signalling to disease resistance as well as the mechanisms underlying pathogen containment should be examined. Indeed, while the *pPR1*::*YFP–NLS* reporter allows us to visualize one defence response deployed by the plant, *PR1* expression does not represent the only possible mechanism for pathogen containment. ROS burst, cell wall modifications, and nutrient and secondary metabolite release, among other mechanisms, are likely to play major roles in pathogen containment.

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Acknowledgements

We thank T. Nobori, J. G. Ellis, G. L. Coaker and T. Nuernberger for constructive critical reading of the manuscript and members of the Dangl lab for useful discussions. This research was supported by the National Science Foundation (grant no. IOS-1758400 to J.L.D.) and the Howard Hughes Medical Institute (HHMI). J.L.D. is an HHMI Investigator. This article is subject to HHMI's Open Access to Publications policy. HHMI lab heads have previously granted a non-exclusive CC BY 4.0 licence to the public and a sublicensable licence to HHMI in their research articles. Pursuant to those licences, the author-accepted manuscript of this article can be made freely available under a CC BY4.0 licence immediately upon publication.

Author contributions

P.J. conceptualized the project, wrote the original draft, reviewed and edited the manuscript, and conducted the formal analysis. J.H. conducted the investigation. J.L.D. conceptualized the project, reviewed and edited the manuscript, acquired the funding and supervised the project.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to Jeffery L. Dangl.

Peer review information *Nature Plants* thanks Matthieu Joosten and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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