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Spotlight

Propagation of plant immunity via interactions between PRIMER and bystander cells

Diverse immune cells in the human body, such as neutrophils, macrophages, dendritic cells, T cells, and B cells, can move from one organ to another and respond to injuries, diseases, and other threats (Sender et al., 2023). However, plants do not have mobile, specialized immune cells like ours. Plant cells are immobile, and therefore, in addition to sustaining normal growth and development, they must also effectively manage their immune responses. Then how do plants enable each of their cells to recognize threats, communicate these threats, and respond appropriately without sacrificing other responsibilities? This has remained an enigma for a long time.

When plants, as multicellular organisms, face pathogen attacks, various cell types exhibit unique responses. For example, when *Arabidopsis* plants are infected by the fungal pathogen *Colletotrichum higginsianum*, a substantial number of intracellular Toll/interleukin-1 receptor nucleotide-binding domain leucine-rich repeat genes are primarily active in the vascular system. At the same time, genes related to abscisic acid signaling are specifically activated in guard cells responsible for closing stomata (Tang et al., 2023). Additionally, two studies have revealed both cell-autonomous and non-cell-autonomous responses to pathogen infection (Delannoy et al., 2023; Liu et al., 2024). However, because infected plants are complicated organisms that are challenging to study due to numerous variables, including timing, location, and reaction status, our comprehension of plant immune responses at the cellular level remains quite limited.

A recent study has addressed these issues using time-resolved single-nucleus multiomics (snMultiome) and spatial transcriptomics (Figure 1A) (Nobori et al., 2025). The authors introduced bacterial pathogens into the leaves of *Arabidopsis* plants and analyzed the plant's response by combining single-nucleus RNA sequencing, a single-nucleus assay for transposase-accessible chromatin followed by sequencing, and multiplexed error robust fluorescence *in situ* hybridization (MERFISH) (Figure 1B) (Chen et al., 2015; Marand and Schmitz, 2022). The pathogens used in this study include *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000), *P. syringae* pv. tomato DC3000 carrying the avirulent effector gene *avrRpt2*, and *P. syringae* pv. tomato DC3000 carrying the avirulent effector gene *avrRpm1* (Figure 1A). *P. syringae* pv. tomato DC3000 is a virulent pathogen that suppresses plant immunity and causes bacterial speck disease on *Arabidopsis* and tomato plants (Chen et al., 2022), while the avirulent effectors AvrRpt2 and AvrRpm1 are recognized by plant Toll/interleukin-1 receptor nucleotide-binding domain leucine-rich repeat receptors to activate effector-triggered immunity (Wu et al., 2014). A single-nucleus assay for transposase-accessible chromatin followed by sequencing is commonly employed to pinpoint potential *cis*-regulatory elements in specific cells and conditions. However, its application in researching plant immune responses remains unexplored (Marand and Schmitz, 2022). The MERFISH imagining technique allows for the visualization of thousands of RNA types within single cells. It combines FISH with sophisticated encoding strategies that detect and correct potential errors, resulting in highly accurate multiplexed RNA imaging within individual cells (Chen et al., 2015). These approaches have attained a groundbreaking level of spatiotemporal clarity in documenting a plant's immune reactions across various cells, uncovering unique immune responses specific to different cell types during pathogen invasion.

Nobori et al. (2025) created a detailed time-course single-cell atlas of *Arabidopsis thaliana* leaves infected with *P. syringae* pv. tomato DC3000 as well as DC3000 strains carrying *avrRpt2* or *avrRpm1*. They devised a method to quickly isolate nuclei, reducing alterations in the transcriptome and epigenome during sample processing (Figure 1A). Single-nucleus RNA sequencing data were used for dimensionality reduction and clustering. Gene ontology enrichment analysis of marker genes for each cluster identified mesophyll and epidermis cell populations enriched with defense-related genes. A closer examination showed that key genes responsible for systemic acquired resistance (SAR), such as *ALD1* and *FMO1*, which play a role in producing the SAR mobile signal N-hydroxy-pipecolic acid (Huang et al., 2020), were uniquely active in a sub-population of phloem companion cells. Intriguingly, Nobori et al. found that genes participating in various stages or pathways of synthesizing or secreting the tryptophan-derived defense metabolites camalexin and indole glucosinolate were distinctly active in immune-responsive mesophyll subpopulations, showcasing the diversity of immune cell states.

By combining snMultiome and MERFISH to systematically identify genes with significantly altered expression over pseudotime in the MERFISH data, Nobori et al. (2025) discovered two unique immune cell states through cell-type-specific analysis of differential expression. Pseudotime is a computational term that illustrates the progression of cells through a biological process. Among the *BON3*, *ALD1*, and *FMO1* genes, which exhibit increased expression near the core of the immune-active zone, *BON3*, which is a negative regulator of plant immunity, was identified as being significantly expressed in a confined region, a pattern that contrasts with *ALD1* and *FMO1*. *BON3* expression was most prominent in cells with the highest pseudotime scores, suggesting that these cells are initial responders to pathogen infection. Hence, Nobori et al. named these cells primary immune responder (PRIMER) cells. On the other hand, the cells surrounding PRIMER cells are termed bystander cells (Figure 1C). In

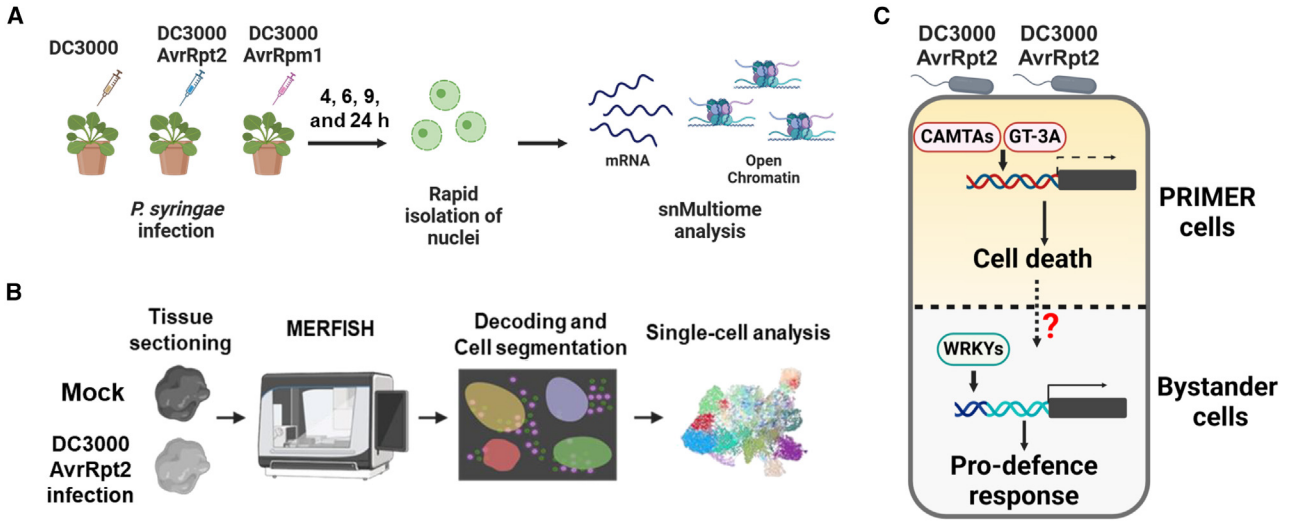

Figure 1. Identification of PRIMER and bystander cells by combining time-sensitive single-cell transcriptomics, epigenomics, and spatial transcriptomics analyses.

(A) Time-course snMultiome analysis of *A. thaliana* leaves infected by the virulent bacterial pathogen *P. syringae* pv. tomato DC3000 or the avirulent strains *P. synringae* pv. tomato DC3000 carrying either *avrRpt2* or *avrRpm1*.

(B) Schematic of the MERFISH procedures conducted in this study.

(C) A proposed model illustrating the potential roles of PRIMER and bystander cells in plant immunity upon infection by an avirulent bacterial pathogen. The figure was created with the software BioRender (BioRender.com).

contrast to *BON3*, the SAR genes *ALD1* and *FMO1*, which can help prevent cell death (Zavaliev et al., 2020), were expressed in bystander cells. These data suggest that PRIMER cells undergo cell death and subsequently send signals to adjacent cells to trigger plant immune responses (Figure 1C).

PRIMER and bystander cells exhibit distinct transcriptional and epigenetic features (Figure 1C) (Nobori et al., 2025). The study found that PRIMER cells are enriched for calmodulin-binding transcription activator (CAMTA) motifs and GT-3A motifs, while bystander cells are enriched for WRKY motifs. Compared with PRIMER cells, genes shown previously to be inhibited by CAMTA3 are significantly overexpressed in bystander cells, indicating that pathogen infection induces different cell states. The role of GT-3A, a trihelix DNA-binding transcription factor, in leaf immunity remains unexplored. The creation of transgenic overexpression lines showed that two independent GT-3A overexpression lines were more vulnerable to DC3000 infection, while *GT-3A* knockout mutant plants were more susceptible to DC3000 carrying *avrRpt2* infection compared to the wild type. These findings indicate that the precise expression of *GT-3A* in PRIMER cells is crucial for plant immunity. Earlier studies identified GT-3A as a suppressor of plant defenses against the root-knot nematode *Meloidogyne incognita* (Zhao et al., 2024). In alignment with these findings, the current research by Nobori et al. (2025) demonstrates that GT-3A overexpression plants show increased vulnerability to the fungal pathogen *C. higginsianum*. Taken together, these results indicate that GT-3A plays a role in controlling plant defense mechanisms against fungal, bacterial, and nematode pathogens.

Previously, most published studies in molecular plant–pathogen interactions concentrated on the tissue level rather than the cellular level. Only recently have several papers begun to explore the particular roles of different cell types in plant immune responses (Tang et al., 2023; Zhu et al., 2023). Nobori et al. (2025) discovered novel immune cell states, identifying specific populations of cells with distinct immune responses. In contrast, other single-cell studies have not achieved this level of precision in pinpointing cell states. The identification of PRIMER and bystander cells by Nobori et al. marks a pivotal moment in our comprehension of the multicellular network and communication during plant immune reactions. In addition, the detailed single-cell spatiotemporal atlas developed by Nobori et al. (2025) offers valuable functional and regulatory perspectives on the states of immune cells in plants. Furthermore, Nobori et al. (2025) have constructed a comprehensive database filled with valuable information. This resource can significantly enhance our comprehension of the processes and pathways that lead to cell death in PRIMER cells as well as the defense mechanisms activated in neighboring cells. Analyzing these data can also shed light on how signals are conveyed from PRIMER cells to bystander cells.

Since Nobori et al. (2025) utilized a leaf infiltration inoculation technique that circumvents guard cell closure of stomata to prevent DC3000 from entering the apoplast, future research employing spray or dip inoculation methods could uncover critical cellular reactions vital to stomatal plant immunity. The model illustrated in Figure 1G centers on the effector-triggered immunity response, leaving our comprehension of the PTI response in this context still wanting. Further studies will be required to pinpoint exact cell types that participate in the production of pathogenesis-related proteins, phytoalexins, the plant defense hormone salicylic acid, and mobile signals that play a role in SAR at various stages of plant–pathogen interactions. Which kinds of cells are responsible for the heritable immune memory or priming that can persist across several

generations in plants after encountering pathogens? What specific cell types are engaged in defending against necrotrophic pathogens and responding to soil-borne pathogens in roots? The published data probably just revealed the tip of the iceberg. We are at the dawn of an exciting era in single-cell plant pathology.

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