Trends in Plant Science

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PRIMER cells: immune hotspots in plants

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Advances in single-cell and spatial biology have transformed the study of plant immunity, revealing distinct immune cell states such as primary immune responder (PRIMER) cells and offering a deeper understanding of defense mechanisms. These insights offer opportunities for the development of advanced strategies for crop protection and disease resistance.

A cellular revolution in plant immunity studies: decoding transcriptome signatures in all versus PRIMER cells

Understanding plant pathogen evasion, defense signaling, and resistance strategies has been studied over four decades. A fundamental framework was established by Jones and Dangl's zigzag model of plant immunity, which described pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) [1]. However, much research has relied on biochemical assays and bulk-tissue transcriptomics, which inadequately captures cellular-level immune response diversity. Recent developments in single-cell and spatial biology have answered critical questions and offered new avenues for cell type-specific plant immunity research fields (Figure 1A) [2–4]. Nonetheless, how these advancements can reveal the signaling pathways and cell-to-cell communications in defense priming and what role spatial heterogeneity plays in pathogen resistance, remains to be answered. A recent study by Nobori *et al*. [5] addresses some of these questions elaborately. This led to the construction of a comprehensive, time-resolved, single-cell multi-omics atlas of immune responses in *Arabidopsis thaliana*, providing an exclusive insight into the cellular mechanisms under virulent *Pseudomonas syringae* pv. tomato DC3000 and avirulent, *avrRpt2* and *avrRpm1* bacterial infections. This study highlights the spatiotemporal dynamics of immune responses and unveiled a rare, specialized immune cell population, the PRIMER cells.

Dissecting immunity at the single-cell level: unraveling the secrets of plant defense

Bulk transcriptomics provides an informative averaged interpretation of gene expression, masking cell type-specific responses and cellular interactions during infection [3–5]. To address this, investigators applied both single-nucleus RNA sequencing (snRNA-seq) and single-nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq) to the same set of single nuclei from 15 samples. This approach generated a time-resolved single-nucleus multiomic atlas of 65 061 cells from *A. thaliana* leaves under various virulent and avirulent *P. syringae* strains infection at different time intervals. This extensive dataset identified the specific cell states with distinct roles in plant immunity. Notably, it uncovered major immune-active cell clusters in key cell types, such as the mesophyll and epidermis under ETI response through gene ontology (GO) enrichment analysis. Furthermore, these cell populations were identified as enriched with defense-related genes, emphasizing the immune response, including activation of the salicylic acid (SA) biosynthesis pathway. A significant finding was the identification of 429 transcriptionally diverse subclusters, a subset of which displayed immune-related signatures, highlighting the cellular complexity and heterogeneity of the plant immune response. The integration of chromatin accessibility data with gene expression profiles revealed several chromatin regions, including immune-related genes like *ICS1*, a key gene for pathogen-induced SA biosynthesis that becomes more accessible to transcription factors (TFs) upon pathogen infection. This epigenomic landscape facilitated the identification of WRKY46 and CAMTAs TFs, that play crucial roles in regulating immune responses. To further elucidate the spatial dynamics of immune responses, the study employed multiplexed error-robust fluorescence *in situ* hybridization (MERFISH), enabling high-resolution spatial mapping of gene expression within intact plant tissues. This approach deciphered the expansion of immune-active zones over time during infection, illustrating a spatial context to the temporal dynamics captured by single-cell analyses (Figure 1B).

Unveiling a specialized immune frontline: PRIMER cell

Among the novel breakthroughs was the identification of a rare cell population termed PRIMER cells. PRIMER cells are found at immune-active hotspots that exhibit unique traits. Their roles include activation of novel TFs such as *GT-3A*, crucial for plant immunity against bacterial infections (Figure 1C). The neighboring non-infected cells of PRIMER, known as bystander cells, were found to express the immune-associated genes *ALD1* and *FMO1*. While their expression suggests a potential role in long-distance signaling and systemic acquired resistance, direct evidence for their involvement in cell-to-cell communication remains to be established. This research establishes a new standard in plant spatial biology by combining multi-omics methods to provide a spatiotemporal framework for understanding plant immunity. Another remarkable characteristic of PRIMER cells is their distinct chromatin accessibility landscape, indicating they undergo rapid epigenetic reprogramming upon pathogen

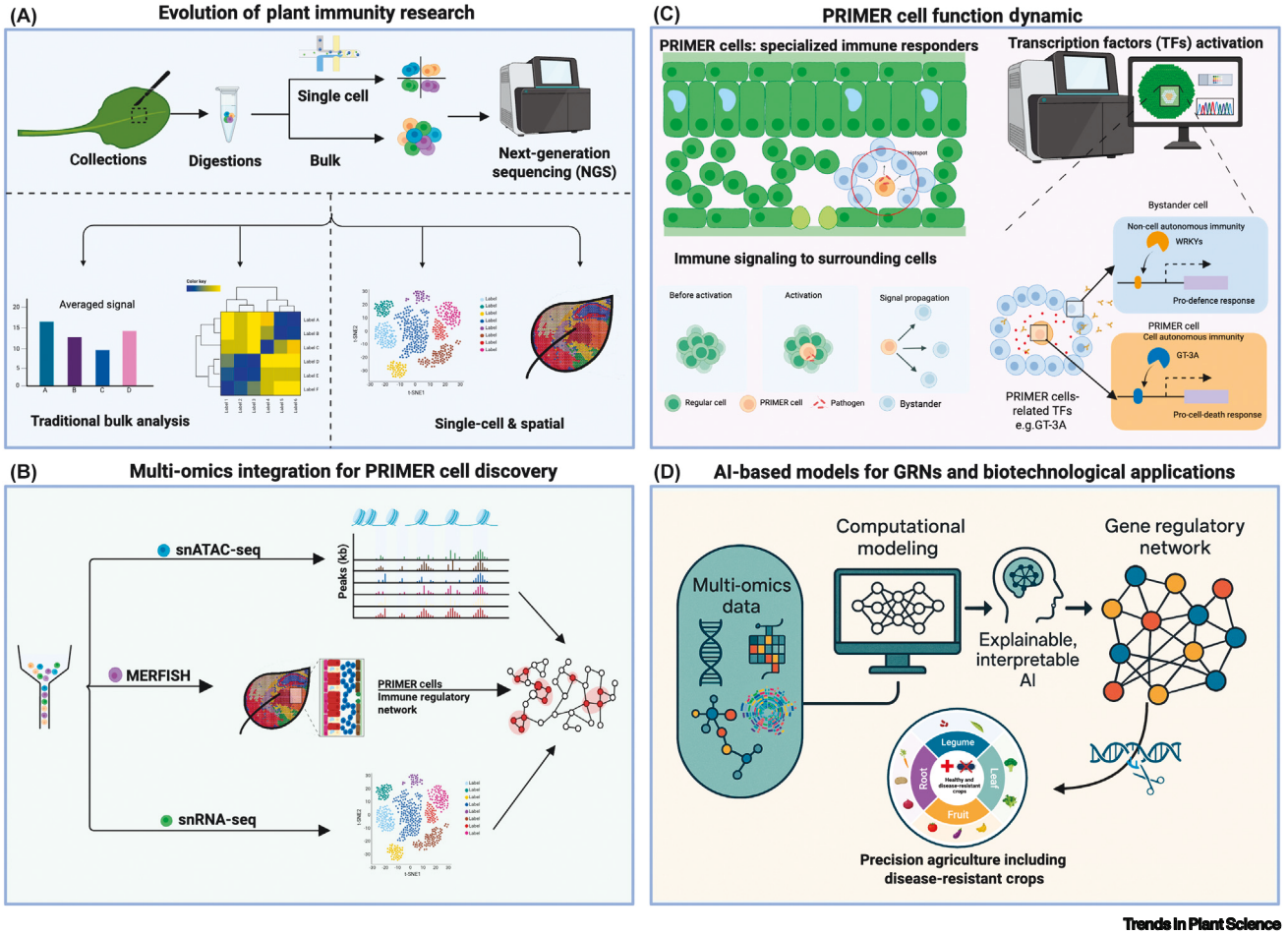

Figure 1. Advances in plant immunity research and the role of primary immune responder (PRIMER) cells. (A) The evolution of plant immunity research is illustrated, highlighting the transition from averaged signals via traditional bulk analysis to high-resolution single-cell and spatial analyses, enabling the dissection of cellular heterogeneity in plant immune responses. (B) An integrative multi-omics framework, incorporating methods to study chromatin accessibility (snATAC-seq), transcriptome profiling (snRNA-seq), and spatial gene expression mapping (MERFISH), facilitating the identification and characterization of PRIMER cells. (C) PRIMER cells function as distinct immune responders, detecting pathogens and activating transcription factors such as *GT-3A*, to trigger immune signaling cascades. These signals propagate to neighboring cells, including bystander cells, assembling a coordinated defense response. (D) Computational modeling of multi-omics data, associated with explainable and interpretable artificial intelligence (AI), allows the analysis of gene regulatory networks (GRNs) and the prediction of gene expression dynamics, offering novel insights into plant immunity. This integrative biotechnological and systems biology approach may hold promise for enhancing disease resistance and improving crop health, particularly in legumes and fruits. The figure was prepared using BioRender. Abbreviations: MERFISH, multiplexed error-robust fluorescence *in situ hybridization; snATAC-seq, single-nucleus assay for transposase-accessible chromatin sequencing; snRNA-seq, single-nucleus RNA sequencing.*

detection. This supports existing findings demonstrating that chromatin modifications contribute to immune memory and defense priming in plants [6]. The discovery of PRIMER cells reveals a transient, immune-activated state with distinct transcriptomic and epigenomic features, suggesting that plant immune responses involve dynamic cellular reprogramming. These findings indicate that different types of plant cells can achieve PRIMER-like states when responding to pathogen challenges rather than relying on a fixed population of specialized immune cells. The methodologies and understandings from these studies hold transformative potential for improving plant resilience and advancing sustainable agricultural practices. Future research could build on these findings by investigating how PRIMER cells function across different plants, unraveling additional regulatory networks and exploring their potential for engineering disease-resistant crops.

Bridging systems biology and agricultural innovation: future perspectives and opportunities

The identification of PRIMER cells represents a paradigm shift in plant immunity

research, enabling systems and network biology approaches to study plant defense mechanisms. This discovery redefines ETI beyond its traditional view as an amplified PTI response, highlighting the role of diverse immune clusters and PRIMER cells. Simultaneously, this study uncovers new research avenues, raising key questions: do the same TFs regulate PTI and ETI? How do pathogens evade these responses across cell types over time and establish effector-triggered susceptibility (ETS)? Additionally, understanding pathogen entry through stomata remains crucial, as a limitation of this study is the use of syringe infiltration, which circumvents guard cell defenses.

Recently applied in mammalian gene regulatory networks (GRNs), explainable and interpretable artificial intelligence (AI) can be used to integrate multiomics data to model PTI and ETI, offering significant potential for plant immunity research (Figure 1D). Probing deeper into these mechanisms raises numerous questions. How can high-plex spatial multiomics techniques, such as Spatial ATAC-seq, 10x Genomics Xenium, or Stereo-Seq, be optimized for plant tissues to simultaneously map chromatin accessibility, RNA, and protein *in situ*? What unique insights might this multidimensional approach reveal about the cellular state of PRIMER cells and their surrounding cells? ETS is another area that requires further investigation to mechanistically understand how diverse effectors (protein or RNA) manipulate PRIMER cells. CRISPR-based live-cell imaging techniques are bringing dynamic progresses into focus to track real-time gene expression and TF activity in PRIMER cells during pathogen infection [7]. How can we further optimize these tools to uncover the temporal dynamics of immune activation or disease progression? By integrating fluorescent reporters with CRISPR guide RNAs, we can visualize the activation and localization of these factors in real time within PRIMER cells, providing invaluable insights into the rapid immune response [8]. We can expect to observe the rapid activation of PRIMER cells within minutes of pathogen detection, followed by waves of signaling to surrounding bystander cells.

With deep learning algorithms, we can develop neural networks based on large datasets of plant tissue images and gene expression profiles to accurately predict cell–cell communication patterns or infer 3D tissue architecture from 2D MERFISH data [9–12]. With this network system, complex signaling networks may be revealed between PRIMER cells and bystander cells, indicating propagation of immune responses throughout tissues. This will help to identify key mediators of cell–cell communication and discover the influence of spatial arrangement of cells on spreading immunity. With the combination of developing plant-specific CRISPR libraries targeting potential immune regulators and single-cell multiomics, we may validate the function of regulators such as *GT-3A* by assessing the effects of gene knockouts on transcriptome and chromatin accessibility in PRIMER cells [8–10]. What improvements in spatial resolution and multi-omics integration are needed to capture the complexity of plant immune responses? Techniques like PHYTOMap might hold great promise for studying PRIMER cells in their native context [11]. As the technologies continue to evolve, we can adapt PHYTOMap by developing plant-specific probe sets targeting PRIMER cell markers and immune response genes. What discoveries about the role of PRIMER cells and other specialized immune components in plant defense mechanisms can we anticipate? How will this knowledge translate into the development of more resilient crops and innovative strategies for plant protection against pathogens? Exploring transfer learning – a machine learning technique where a model trained on one task is repurposed and fine-tuned for a new one – could be a promising approach for predicting immune signaling pathways in agronomically important crops. Moreover, implementing single-cell and spatial omics technologies could advance precision breeding and crop protection strategies. The identification of PRIMER cells as key immune regulators suggests that targeted genome editing of PRIMER-associated immune pathways could enhance disease resistance against bacterial and fungal pathogens. Furthermore, spatially resolved metagenomics could identify beneficial microbiomes that prime immune responses, guiding the development of microbial biofertilizers to strengthen plant resilience.

Additionally, integrating high-throughput, AI-assisted phenotyping with spatial multi-omics biomarkers would accelerate disease resistance screening in crops. Predicting early immune activation patterns through chromatin remodeling signatures might facilitate real-time pathogen detection in the field, optimize pesticide use, and reduce environmental impact. Multiomics datasets across plant species could also enable comparative analyses to determine the existence of PRIMER-like immune cells in staple crops such as rice, maize, and wheat. Expanding network modeling of immune signaling pathways across diverse species would facilitate dynamic immune engineering strategies specific to agricultural needs, ensuring climate-resilient, disease-resistant crops for global food security.

Conclusion

The recent advancements in single-cell and spatial biology techniques have revolutionized our understanding of plant immunity, addressing long-standing questions and opening new avenues for research. We will likely discover additional specialized immune cell states beyond PRIMER cells, gain a more comprehensive understanding of the molecular mechanisms driving rapid immune activation, and uncover insights into how plants coordinate systemic

immune responses across tissues. These have enabled in-depth exploration of cellular heterogeneity and spatiotemporal dynamics in plant–pathogen interactions, surpassing limitations of traditional bulk tissue analyses. The convergence of multi-omics, AI, and precision genome editing presents an unprecedented opportunity to transform plant immunity research and agricultural sustainability.

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Declaration of interests

The authors declare no competing interests.

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