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PERSPECTIVES

Rice plants infected with the fungal pathogen *Magnaporthe oryzae* exhibit reduced grain yield. New results reveal an immune mechanism shared among all flowering plants.

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PLANT IMMUNITY

A common immune response node in diverse plants

Small molecules activate a defense mechanism shared by all flowering plants

By **Jeffery L. Dangl**[1,2] *and* **Jonathan D. G. Jones**[3]

Plant diseases cause losses of up to 30% of worldwide food production (*1*). Crop monocultures are particularly vulnerable—a pathogen strain that grows on one plant in a field can grow on them all. Methods to combat plant diseases include chemical intervention and bolstering the plant's immune system. The latter can require creating varieties with repertoires of innate immune receptor variants. These receptors recognize virulence proteins that invading bacteria or fungi deliver into the plant cell, where their recognition triggers an immune response. Despite remarkable progress in unraveling plant immunity during the past 50 years (*2*), durable crop disease resistance remains elusive. On pages 1413 and 1405 of this issue, Yu *et al.* (*3*) and Wu *et al.* (*4*), respectively, substantially advance our understanding of how disease resistance is activated in all flowering plants (angiosperms), the most diverse group of land plants.

Plant intracellular nucleotide-binding leucine-rich repeat (NLR) proteins with an N-terminal Toll/interleukin-1 receptor (TIR) domain are a class of innate immune receptor (TNLs) whose mechanisms are broadly understood (*2*). TNLs bind pathogen virulence effectors, oligomerize, and form TIR domain–containing nicotinamide adenine

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dinucleotide glycohydrolase (NADase) enzymes that produce several nucleotide-derived signaling molecules (*5*, *6*). These derivatives are further processed into short-lived products that act as selective ligands, depending on their structure, to drive a specific rearrangement on preformed protein heterodimers comprising the lipase-like protein EDS1 (enhanced disease susceptibility 1) and either SAG101 (senescence-associated gene 101) or PAD4 (phytoalexin-deficient 4) (*5*, *6*). The two types of EDS1 heterodimers then selectively recruit so-called "helper NLRs" called ADR1 (activated disease resistance 1), in the case of EDS1-PAD4, or NRG1 (N required gene 1) in the case of EDS1-SAG101. NRG1 and ADR1 then oligomerize and form membrane-localized calcium channels that activate downstream immune responses and, particularly for NRG1, death of the infected plant cell (*7*, *8*).

The mechanism by which TIR-dependent NADase products bind EDS1-SAG101 or EDS1-PAD4 and activate the helper NLR has not been clear until now. Ligand binding causes a rotation of a particular α helix located in the C terminus of either SAG101 or PAD4 (*9*). TIR-dependent small-molecule specificity thus determines which structural rearrangement takes place.

Yu *et al.* report the structure of the EDS1-PAD4-ADR1 heterotrimer from the flowering plant *Arabidopsis thaliana*. As anticipated, the authors observed that TIR-derived small-molecule binding drives rotation of the C-terminal PAD4 α helix in the preformed EDS1-PAD4 heterodimer to create a docking site for ADR1. The authors used mutagenesis and functional assays to verify the role of structurally inferred key amino acids in ADR1, PAD4, and EDS1.

What is the functional relationship between the immediate TIR-dependent enzymatic products [these are 2′-(5′-phosphoribosyl)-5′-adenosine monophosphate isomers called 2′cADPR and 3′cADPR] and their isomers (called pRib-AMP/ADP)? Yu *et al.* drove production of either 2′cADPR or 3′cADPR by transiently coexpressing bacterial TIR domain virulence factors that make only one or the other product in plant leaf tissue. Only those TIR domains that produced 2′cADPR drove formation of the EDS1-PAD4-ADR1 heterotrimer in vivo. The authors produced and purified 2′cADPR in vitro and found it sufficient to drive heterotrimer formation of purified EDS1, PAD4, and ADR1 proteins in vitro.

[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. [3]Sainsbury Lab, University of East Anglia, Colney Lane, Norwich, UK. Email: dangl@email.unc.edu; jonathan.jones@tsl.ac.uk

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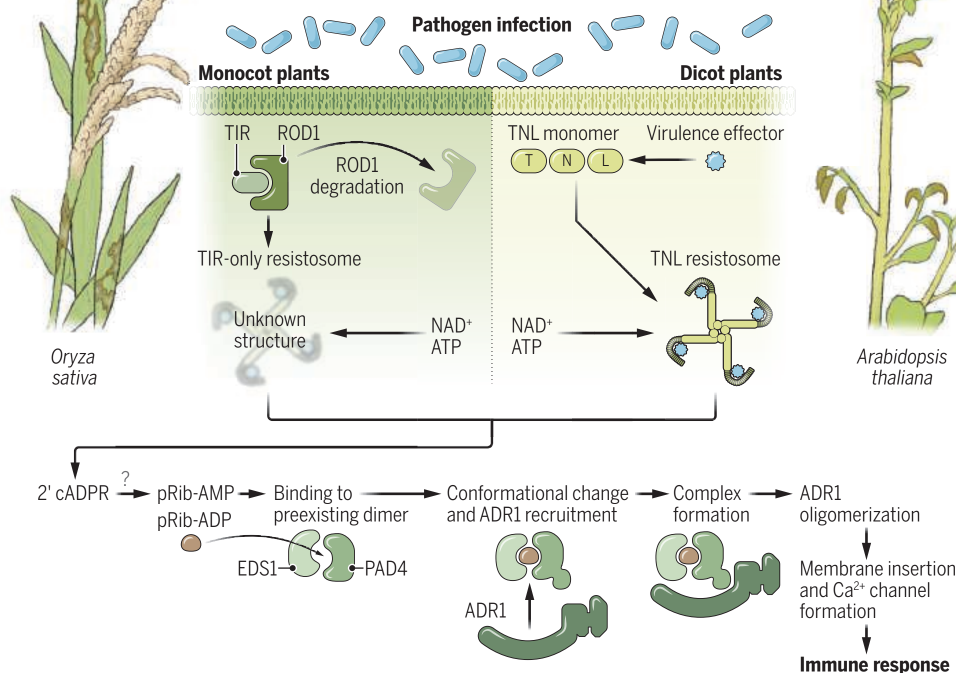
Plant defense across flowering plants

In monocots and dicots, small nucleotide derivatives (pRib-AMP and pRib-ADP) drive the formation of similar protein complexes that trigger immune responses to pathogen infection. The rice TIR-only resistosome functions like the TNL resistosome, indicating conservation of the mechanism across angiosperms.



2′cADPR, 2′-(5′′-phosphoribosyl)-5′-adenosine monophosphate; ATP, adenosine triphosphate; ADR1, activated disease resistance 1; EDS1, enhanced disease susceptibility 1; NAD^+, nicotinamide adenine dinucleotide; PAD4, phytoalexin-deficient 4; pRib-AMP, phosphoribosyl adenosine monophosphate; pRib-ADP, phosphoribosyl adenosine diphosphate; ROD1, resistance of rice to diseases 1; TIR, Toll/interleukin-1 receptor; TNL, TIR nucleotide-binding leucine-rich repeat receptor.

Yu *et al.* also showed that 2′cADPR is converted to pRib-AMP/ADP. When applied to plant leaves in high doses, 2′′cADPR activated the expression of immune response genes (though how such a charged molecule traverses membranes remains puzzling). Transcriptional immune response activation was genetically dependent on EDS1, PAD4, and the three-member ADR gene family. Yu *et al.* propose that 2′cADPR converts into the EDS1 ligand that drives heterotrimer formation with PAD4 and the ADR proteins. However, the notion of 2′cADPR as a positive regulator of EDS1-PAD4-ADR1 heterotrimer formation and consequent immune response gene activation is at odds with previous work showing that TIR domain bacterial virulence effectors that produce 2′cADPR can suppress immune responses (*10*, *11*) or at least do not activate cell death (*12*). Further work is required to resolve these differences.

Monocot plant (such as cereal) genomes lack TNL-encoding genes but encode several "TIR-only" proteins. They also encode EDS1, PAD4, and ADR1 proteins, whose contribution to innate immunity has been obscure. Wu *et al.* conducted a rice genetic analysis to address this question. Their study began, however, with an earlier report of an induced mutation in the rice *ROD1* (*resistance of rice to diseases1*) gene, which encodes a Ca^{2+}-binding C2 class protein (*13*). *ROD1* mutation results, through unknown mechanisms, in ectopic lesions and cell death, a sign of constitutive immune defense activation. To understand why, Wu *et al.* searched for mutations that abolished or attenuated the effect that results from ROD1 loss.

Notably, mutants that abrogated the phenotype of ROD1 mutants fell into four classes. Three involved mutations in genes encoding the rice homologs OsEDS1, OsPAD4, and OsADR1. These mutations also increased susceptibility to a rice blast fungus, and all three mutations abolished the enhanced disease resistance of *rod1* plants. The fourth class mapped to a gene that encodes a TIR domain-only protein, OsTIR, which reveals the likely source of the signaling molecules perceived by OsEDS1, OsPAD4, and OsADR1.

Upon pathogen infection, ROD1 is targeted by E3 ubiquitin ligases RIP1 and APIP6 for degradation, which relieves its brake on immunity. OsTIR catalyzes the production of pRib-AMP/ADP, which triggers formation of an OsEDS1-OsPAD4-OsADR1 immune complex. Wu *et al.* expressed OsEDS1, OsPAD4, and OsADR1 in

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insect cells, activated their oligomerization, and determined the structure of OsEDS1, OsPAD4, and OsADR1 bound to pRib-ADP by cryo–electron microscopy. The findings of Wu *et al.* confirm those of Yu *et al.*, that ROD1 interacts with OsTIR and inhibits its enzymatic activity. Mutation of ROD1 leads to constitutive activation of this complex.

How do OsEDS1, OsPAD4, and OsADR1 contribute to rice immunity? Mutations in these proteins result in compromised immune responses in rice, such as the production of reactive oxygen species in response to chitin, a component of fungal cell walls. Expression of the *OsTIR* gene was induced by pathogen infection, and rice plants with *OsTIR* mutations were also compromised in rice blast resistance. The approach of Wu *et al.* is a salutary reminder that forward genetic screens continue to be a wonderful source of new discoveries.

The studies of Yu *et al.* and Wu *et al.* unify an important component of plant immunity (see the figure). The EDS1, PAD4, and ADR1 components likely contribute to defense through TIR-dependent heterotrimer formation in nearly all angiosperms (although some aquatic plants with very few NLRs may lack them). However, this event is initiated by the relief of repression of TIR-only enzymatic activity in rice, in contrast to ligand-dependent activation of full-length TNL receptors in *Arabidopsis*. Thus, despite the absence of TNLs in monocots, the ultimate outcome of the TIR enzymatic function is maintained across ~200 million years of evolution. It remains to be seen how ADR1 activation leads to a transcriptional immune response. These studies are important confirmations of foundational concepts for all plant innate immunity because TIR domain signaling underpins innate immunity from bacteria to humans. ■

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This commentary is dedicated to the memory of Joanne Chory.

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IMMUNOLOGY

Platform influencers—host RNA control of antiviral immunity

Cellular RNAs directly regulate the activity of an antiviral immune signaling complex

By **Sonja M. Best** and **Adam Hage**

The ability of a cell to rapidly respond to viral infection by detecting invading nucleic acids and mounting an immune response is critical to host survival. In mammalian cells, type I interferon (IFN-I) directs the intrinsic antiviral response in tissues and orchestrates adaptive responses to promote virus clearance and establish immune memory (*1*). However, dysregulated expression of IFN-I can exacerbate inflammatory responses in the context of viral infection and otherwise promote autoimmunity (*2*). The central importance of these pathways to human health makes it imperative to fully define cellular signaling events that are required for innate antiviral and inflammatory responses. On page 1362 of this issue, Gokhale *et al.* (*3*) report that cellular mRNAs directly regulate protein complex assembly to enable transcriptional responses to immunostimulatory RNA.

"The finding... expands the known roles of RNAs as architects of higher-order protein scaffolds in innate immunity..."

Retinoic acid–inducible gene I (RIG-I)–like receptors (RLRs) are RNA helicases that initiate the central response to immunostimulatory RNA, such as viral RNA, in the cytoplasm (*2*). This protein family includes RIG-I and MDA5 (melanoma differentiation-associated protein 5), which are maintained in inactive conformations under normal conditions. Upon recognition and binding of viral RNA, however, RIG-I and MDA5 undergo conformational changes that expose their caspase activation and recruitment domains (CARDs). These CARDs interact with the CARD within mitochondrial antiviral signaling protein (MAVS), which is the sole adaptor protein responsible for coordinating RLR signal transduction. MAVS function is dependent on its anchoring to membranes of mitochondria or peroxisomes, where RLR CARD binding induces MAVS oligomerization and the recruitment of numerous signaling intermediates (*4*). Assembly of this higher-order signaling platform, called the MAVS signalosome, leads to activation of key transcription factors, including interferon regulatory factor 3 (IRF3) and nuclear factor κB (NF-κB), which drive IFN-I and cytokine expression (see the figure).

Previous studies have detailed the types and molecular properties of RNA molecules that activate RLRs. These triggers include single- and double-stranded viral RNAs as well as endogenous cellular RNAs that have been mislocalized or misprocessed (*5*–*8*). Gokhale *et al.* demonstrate that RLRs are not the only proteins to bind RNAs to activate the IFN-I response. Unexpectedly, their study shows that cellular mRNAs are coopted by MAVS independently of RLRs to facilitate higher-order assembly of the MAVS signalosome.

To elucidate mechanisms of MAVS signalosome assembly, Gokhale *et al.* stimulated MAVS oligomerization independently of viral RNAs by expressing dimer-inducible CARDs of RIG-I in human cells deficient in IRF3. The ability of the MAVS signalosome to activate transcription factors was then determined by mixing mitochondrial extracts from these cells with lysates from normal, unstimulated cells to provide a source of IRF3. Mitochondrial extracts were treated with enzymes to eliminate various forms of nucleic acids. IRF3 activation (phosphorylation) by the MAVS signalosome was prevented only when single-stranded RNA was destroyed, even in the absence of RLRs. Elimination of single-stranded RNA did not prevent MAVS oligomerization. Instead, it disrupted higher-order protein complexes containing tumor necrosis factor (TNF) receptor–associated factor (TRAF) proteins, which bind to MAVS oligomers and activate enzymes that phosphorylate IRF3. Further examination of the protein complex bound

Innate Immunity and Pathogenesis Section, Laboratory of Neurological Infections and Immunity, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA. Email: sbest@niaid.nih.gov

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