Paired Plant Immune Receptors

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series are correlated with values at another time) and were substantially different from predictions derived from a neutral theory of biodiversity that attributes trends in community dynamics to stochastic demographic processes.

The relationship between α diversity and β diversity needs further exploration, especially in the context of the formation and management of novel communities (see the figure) (*4*). As the species composition of an ecosystem changes in response to loss of species, it is important to understand not only what was lost, but also how that loss affects the emerging novel community. For example, reduction of fast-growing branching corals in the Caribbean (*5*) causes substantial loss in a major source of carbonate production and might lead to the inability of these reefs to continue accreting, even as sea level continues to rise. Species invasion, such as the introduction of avian malaria into Hawaii or zebra mussels to North American streams and lakes, can similarly impart a legacy of substantial community transformation.

Comprehensive as Dornelas *et al*.'s study is, ecosystem coverage is still patchy, with few data sets from the tropics. Those that do exist from the tropics are focused on terrestrial plants, reef fish, and birds. Inclusion of data sets for communities with large mammals and amphibians could lead to much worse α diversity trends for the tropics (*6*, *7*). The importance of long-term investment in monitoring in the tropics (such as the Smithsonian's Center for Tropical Forest Science) cannot be overemphasized.

In the new age of human impacts that is increasingly referred to as the Anthropocene (*8*), how can we reconcile warnings of the next mass extinction (*3*) with the observation that α diversity largely remains constant? Elucidation of the implications of the mass extinction caused by human impacts will have to be revised to consider the way in which whole communities will respond. Extinction is just one component of the way in which ecological communities will be transformed in the future. Managing the species loss involves building an understanding of species turnover in local communities.

The rapid rate of species turnover in ecological communities that Dornelas *et al*. document means that we can expect widespread emergence of novel communities. Identifying the causes of the biodiversity changes is challenging, but there is some evidence that large-scale drivers may influence regions differentially. For example, the composition of temperate communities can be strongly influenced by climate change (*9*, *10*), whereas tropical communities suffer disproportionately from other human activities (*11*). These changes in community composition may affect their resilience or ecosystem function. Ecosystem management approaches must anticipate the widespread emergence of novel ecological communities (*12*) and their consequences for dependent biota, including humans.

References

1. M. Dornelas *et al*., *Science* **344**, 296 (2014).
2. E. S. Poloczanska *et al*., *Nat. Clim. Change* 10.1038/nclimate1958 (2013).
3. A. D. Barnosky *et al*., *Nature* **471**, 51 (2011).
4. R. J. Hobbs, E. Higgs, J. A. Harris, *Trends Ecol. Evol.* **24**, 599 (2009).
5. D. R. Bellwood, T. P. Hughes, C. Folke, M. Nyström, *Nature* **429**, 827 (2004).
6. M. Hoffmann *et al*., *Philos. Trans. R. Soc. London Ser. B* **366**, 2598 (2011).
7. S. N. Stuart *et al*., *Science* **306**, 1783 (2004).
8. W. Steffen, J. Grinevald, P. Crutzen, J. McNeill, *Philos. Trans. R. Soc. London Ser. A* **369**, 842 (2011).
9. B. Sommer, P. L. Harrison, M. Beger, J. M. Pandolfi, *Ecology* 10.1890/13-1445.1 (2014).
10. K. C. Cavanaugh *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **111**, 723 (2014).
11. E. C. Ellis *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **110**, 7978 (2013).
12. N. A. Graham, J. E. Cinner, A. V. Norström, M. Nyström, *Curr. Opin. Environ. Sustain.* **7**, 9 (2014).

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PLANT SCIENCE

Paired Plant Immune Receptors

The ability of plant cell immune sensors to combine in different pairs could expand the host's defense against pathogens.

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Plants are constantly interpreting microbial signals from potential pathogens and potential commensals or mutualists. Because plants have no circulating cells dedicated to this task, every plant cell must, in principle, recognize any microbe as friend, foe, or irrelevant bystander. That tall order is mediated by an array of innate immune system receptors: pattern-recognition receptors outside the plant cell and nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs) inside the cell. Despite their importance for plant health, how NLRs function mechanistically has remained obscure. On page 299 of this issue, Williams *et al*. (*1*) reveal a role for heterodimerization between NLRs and show how the rather limited NLR repertoire of any plant genome might be enhanced by combinatorial diversity.

When first isolated 20 years ago, it was surprising to find that structurally similar NLRs could individually confer resistance to strains of plant pathogens from all kingdoms—insects, fungi, oomycetes, bacteria, and viruses (*2*, *3*). Plant NLRs are deployed to various intracellular addresses to monitor for pathogen virulence proteins ("effectors") that target host defense pathways. NLRs in plants and animals function as molecular switches, cycling between a closed "off" conformation bound to adenosine diphosphate (ADP) and an open "active" conformation bound to adenosine triphosphate (ATP). This switch is thought to be controlled by interactions between a C-terminal leucine-rich repeat (LRR) domain folded back across a central nucleotide-binding (NB) domain and an N-terminal dimerization output domain, which in plants is typically either a coiled-coil (CC) motif or a domain with homology to the Toll–interleukin-1 receptor (TIR) cytoplasmic domains (*4*). How the conformation of NLRs changes after effector recognition and during the activation cycle, and the consequences of these changes, are major unanswered questions in plant pathology. One hypothesis posits that the N-terminal TIR or CC domains can dimerize upon effector-mediated activation to transduce signals to the nucleus and reprogram the cell for disease resistance responses.

Williams *et al*. demonstrate that in addition to forming a signaling-competent homodimer, one TIR domain can heterodimerize with another (in the resting state) to suppress host defense signaling. The authors studied the TIR domains of an NLR pair (RPS4 and RRS1) that are encoded by genes linked head-to-head in the plant *Arabidopsis*. Both proteins are required for disease resistance to multiple pathogens (*5*, *6*). By

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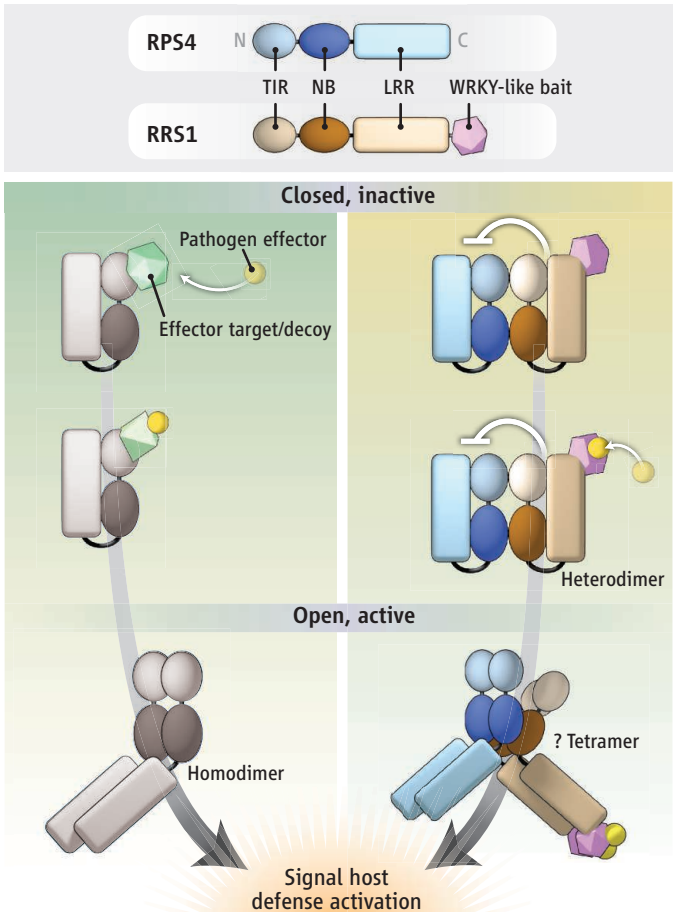
solving the crystal structure of the RPS4 dimer, the RRS1 dimer, and the RPS4-RRS1 heterodimer, Williams *et al*. identified amino acids required for both homodimer and heterodimer formation. Mutational analyses showed that this shared surface is required for RPS4 TIR homodimer signaling and also for RRS1 to suppress RPS4. Thus, RRS1 negatively regulates RPS4 through a shared TIR dimerization contact surface. Effector recognition likely drives a rearrangement of the resting heterodimer such that an RPS4 TIR homodimer is formed (see the figure).

NLR heterodimerization, if common, might enlarge the overall NLR receptor repertoire beyond the ~200 genes and fragments present in the reference *Arabidopsis* genome. Heterodimerization may also provide "protein complementation functions" for the many partial NLRs (those lacking either LRR or NB and LRR domains) that are beginning to be functionally appreciated (*7*, *8*).

Some plant NLRs bind directly to the pathogen effector that triggers their action; others, however, monitor the integrity of host proteins with which they associate. These NLRs "guard" host proteins that are targets of effectors, or act as decoys of those targets. Thus, a single NLR can efficiently detect multiple pathogen effectors, simplifying the problem of a limited receptor repertoire (*2*, *3*). One interpretation of the RPS4-RRS1 heterodimer is that RPS4 is guarding RRS1. Indeed, Williams *et al*. observed that two effectors can associate with the functional RPS4-RRS1 complex, as well as with RRS1 alone, to activate RPS4. Interaction of these two effectors with RPS4 cannot be excluded, but is thus far elusive. Also, RPS4 and RRS1 differ in their requirements for canonical NLR activation—that is, the ability of the NBS domain to bind and hydrolyze ATP via a phosphate binding (P-loop) motif. The RPS4 P-loop is required for disease resistance but the RRS1 P-loop is dispensable, which suggests that RRS1 has functionally diverged and is now a platform for binding effectors that activates disease resistance through RPS4. The most striking difference between RRS1 and RPS4 is the presence of a transcription factor DNA binding domain (WRKY) that is fused to the RRS1 C terminus. This domain can bind DNA; a mutation in RRS1 that abolishes DNA binding is constitutively active (*9*).



Partners in defense. In *Arabidopsis*, NLR sensors are activated by pathogen effectors. When some NLRs detect modification of a host cell protein and an effector, they form homodimers and activate defense responses **(left)**. RPS4 and RRS1 are "paired" TIR-NLR sensors **(right)**. RPS4 activity is normally blocked when it forms a heterodimer with RRS1. When an effector binds to RRS1, this suppression is released and RPS4 is activated.

There are three WRKY-NLR fusions encoded in the reference *Arabidopsis* genome. Additional plant NLRs contain unusual domains and/or combinations of TIR, NB, and LRR domains. WRKY transcription factors are a large family that are important for disease resistance and stress responses (*10*). It is thus reasonable for them to be targets of a variety of plant pathogen effectors. Could the RRS1 WRKY domain be an "effector bait" that mimics one or more effector virulence targets? One of the effectors that activates RRS1 is an acetyltransferase whose activity is required for RRS1 activation (*11*). This effector might acetylate RRS1 and remove it from DNA to drive RPS4 activation.

NLRs are the most rapidly evolving protein-encoding gene family across plant genomes, and new NLR proteins that have incorporated unusual domains are present in all examined plant genomes. Another functional NLR pair (the CC-NLRs RGA4 and RGA5) was described in rice (*12*). RGA5 has an unusual C-terminal effector-targeted domain with structural similarity to a ferredoxin fold found in the plant protein family related to ATX1 (RATX1), a yeast copper-binding chaperone (*12*). As with RPS4 and RRS1, one can predict that the RATX1-like domain in RGA5 is bait for effectors that target RATX1 proteins to promote pathogenicity. Exploring these oddities will help define the spectrum of important host machinery targeted by pathogens.

A major challenge for understanding NLR function is a structural description of the events before, during, and after NLR activation. How do two different effectors lead to an event recognized by RPS4? The events immediately downstream of RPS4 dimerization that activate defense responses are also not clear. Rational design of immune receptors is ultimately the goal of studying NLR function. Perhaps protein pairs like RPS4 and RRS1 may be useful starting substrates; in a modular guardee like RRS1, the WRKY domain could be replaced with a different effector-binding decoy that would result in RPS4 activation. The vistas opened by structural biology in a field rich with genetics and natural variation in both hosts and pathogens provide an exciting new view of plant immunology, 20 years after the first pathogen sensors were discovered.

References

1. S. J. Williams *et al*., *Science* **344**, 299 (2014).
2. J. D. Jones, J. L. Dangl, *Nature* **444**, 323 (2006).
3. P. N. Dodds, J. P. Rathjen, *Nat. Rev. Genet.* **11**, 539 (2010).
4. F. L. Takken, A. Goverse, *Curr. Opin. Plant Biol.* **15**, 375 (2012).
5. D. Birker *et al*., *Plant J.* **60**, 602 (2009).
6. M. Narusaka *et al*., *Plant J.* **60**, 218 (2009).
7. Y. Wang, Y. Zhang, Z. Wang, X. Zhang, S. Yang, *Plant J.* **75**, 553 (2013).
8. A. M. Zbierzak *et al*., *Plant J.* **75**, 539 (2013).
9. Y. Noutoshi *et al*., *Plant J.* **43**, 873 (2005).
10. P. J. Rushton, I. E. Somssich, P. Ringler, Q. J. Shen, *Trends Plant Sci.* **15**, 247 (2010).
11. C. Tasset *et al*., *PLOS Pathog.* **6**, e1001202 (2010).
12. S. Cesari *et al*., *Plant Cell* **25**, 1463 (2013).

10.1126/science.1253849