COMMENTARY

Two-faced TIRs trip the immune switch

Jonathan D. G. Jones[a,1] and Mark J. Banfield[b,1]

Both Plant and Animal Immune Receptors Can Carry a TIR Domain

Mammals, in addition to their adaptive immune system based on somatic evolution of antibodies, carry an innate immune system based on both cell surface and intracellular immune receptors (1). In animals ranging from insects to mammals, Toll-like receptors (TLRs), with extracellular leucine-rich repeats (LRRs) and an intracellular Toll/interleukin-1 receptor/resistance protein (TIR) domain, recognize extracellular ligands such as bacterial lipopolysaccharide or flagellin, and then activate immunity (2). Ligand perception triggers receptor oligomerization, leading to engagement of TIR domain-containing adapter proteins, activation of protein kinases, and stimulation of activity of NF-κB or related transcription factors that induce defense-related genes (2).

Plants also rely on innate immunity, and both plants and animals carry intracellular receptors of the nucleotide-binding, leucine-rich repeat (NLR) class (3, 4). In many plants, such NLRs can carry a TIR domain at their N termini (TNL proteins). Plant and animal TIR domains share a common structure comprising a flavodoxin-like fold with a central parallel β-sheet surrounded by α-helices, although plant TIRs have a longer αD helical region (5).

For plant and animal TIR domains, interaction with other TIR domain molecules is key to activation, and thus the interfaces between these domains are in principle key to understanding mechanism (5). However, in the last few years, analysis of plant TIR domain structure and function, has revealed an apparent inconsistency. The flax L6 gene encodes a TNL that confers resistance to flax rust. The structure of the L6 TIR domain revealed an interface involved in TIR–TIR self-association that involves the αD and αE helices (6). In a separate study, the TIR domains of the NLR gene pair RPS4 and RRS1, both of which are TNLs, interact via a different interface that involves helices αA and αE (7). Does this mean different plant TNL TIR domains function in different ways? In two papers published in PNAS (8, 9), signaling mediated via several different TIR domains is shown to require the function of both αD/αE and αA/αE interfaces.

Two-Faced Plant TIRs

In one of these papers, Zhang et al. (8) report that, for TIR domains from *Arabidopsis* TNLs SNC1 and RPP1, and for the RPS4 and L6 TIR domains, both αD/αE and αA/αE interfaces contribute to TIR–TIR interactions and are required for function. To show this, the authors first solved a crystal structure of TIR^{SNC1}, which revealed three molecules of the protein bound through both αD/αE and αA/αE interfaces. This demonstrated the capacity for the same TIR protein to oligomerize via both of these two interfaces. To explore their biological relevance, they exploited the fact that transient expression of TIR domains in *Nicotiana benthamiana* or *Nicotiana tabacum* leaves often activates a strong necrotic phenotype that may mimic the hypersensitive cell death response (HR) typically involved in NLR-mediated immune signaling. Mutations that perturb either the αD/αE or αA/αE interface of the $SNC1^{TIR}$, $L6^{TIR}$, or $RPS4^{TIR}$ abrogate this HR, and also appear to attenuate TIR–TIR interactions in solution (although these interactions are weak and transient in the wild-type proteins). A crystal structure of the TIR domain of the *Arabidopsis* RPP1 protein, which confers race-specific downy mildew resistance, also revealed a trimer involving both interfaces, although the interfaces are subtly different. Again, mutations in either of these interfaces abrogate the HR triggered upon transient expression of the RPP1-TIR in *N. benthamiana*. Interestingly, it appears that, in certain TIR–TIR interactions, the αA/αE surface forms the primary interface for interaction. Both αD/αE and αA/αE interfaces are also required for TIR domain function in the context of the full-length L6 protein that recognizes the flax rust effector AvrL567. A mutation in either interface abrogates AvrL567-dependent HR. The same is true for mutations in the αD/αE and αA/αE interfaces of $RPS4^{TIR}$, both for RRS1-dependent effector recognition and for transducing the activity of RRS1 autoactive alleles.

In a parallel study, Nishimura et al. (9) report a similar conclusion for a different and remarkable TIR domain protein from *Arabidopsis*, RBA1. They surveyed *Arabidopsis* diversity for recognition of an extensive set of

[a]The Sainsbury Laboratory, Norwich NR4 7UH, United Kingdom; and [b]Department of Biological Chemistry, John Innes Centre, Norwich NR4 7UH, United Kingdom

Author contributions: J.D.G.J. and M.J.B. wrote the paper.

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[1]To whom correspondence may be addressed. Email: jonathan.jones@tsl.ac.uk or mark.banfield@jic.ac.uk.

Pseudomonas syringae effectors and found that accession Ag-0, unlike the "lab rat" Col-0, activates cell death in response to transient delivery of effector HopBA1 from wheat and sugarbeet-infecting *P. syringae* strains. The predisposition to cause HR mapped to a highly unusual TIR-encoding gene that lacks the classical NB or LRR domains. They went on to define the structure of the recognized effector HopBA1, and its similarity to other effectors found in both plant and animal pathogens. Although HopBA1 coimmunoprecipitates with RBA1, it does not interact in yeast two-hybrid, suggesting any interaction between RBA1 and HopBA1 may be indirect. Provision of HopBA1 leads to posttranslational modification (likely, phosphorylation) of RBA1, but how this triggers ligand-dependent HR is unclear. One puzzle of this work is that, although RBA1 Ag-0 triggers HopBA1-dependent HR, it does not appear to condition enhanced effector-dependent disease resistance. Similar to the other TIRs above, overexpression of RBA1 in *N. benthamiana* or *N. tabacum* (and indeed *Arabidopsis*) results in HR. Although a crystal structure for RBA1 was unobtainable, its TIR domain was modeled based on the structure of $RPS4^{TIR}$, with which it shares 41% sequence identity. The authors then proceeded to predict putative αD/αE and αA/αE-like interfaces for RBA1 by docking their homology model on the $L6^{TIR}$ or $RPS4^{TIR}$ crystal structures, respectively. Consistent with Zhang et al.'s findings, mutations in both the putative αD/αE and αA/αE interfaces compromise the capacity of RBA1 to induce HR, and lost self-association as measured by in planta coimmunoprecipitation. Intriguingly, Nishimura et al. noted a correlation in localization of functional and nonfunctional versions of a YFP-RBA1 fusion.

Both papers conclude by discussing whether oligomerization of TIR domains via both the αD/αE and αA/αE domains may be key to the signaling function of both full-length plant TNLs, and plant TIR-only proteins—potentially via superhelical multi-TIR oligomeric structures (10). Such complexes have been proposed to be important for downstream signaling for certain TIR proteins in animals (11). However, although this is an intriguing hypothesis for how proximity-induced assembly may promote signaling in plant TIR-containing proteins, experimental data to support such a model are currently lacking. Looking to the future, it will be exciting to see how the TIR interfaces identified here may potentiate immunity-related signaling in response to pathogen elicitors at the structural level, and whether there is potential to engineer these interfaces to generate improved immune receptors.

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