Both animal and plant cells rely on innate immune systems to recognize and appropriately respond to pathogens. These systems have many similar themes and surprising commonalities, both in the protein domains used and in the mechanisms of larger immune complexes (1). In PNAS, Duxbury et al. (2) explore these commonalities by generating functional plant–animal hybrid immune receptors to ask questions about how plant immune receptors function.

In animals, the innate immune system is supported by adaptive immunity. Recombination of animal adaptive immune receptors greatly expands the ability of animals to recognize and respond to any pathogen (3). In contrast, plants lack adaptive immune cells; the innate immune system is on its own (1). The plant immune system must somehow be able to respond to all pathogens with an elaborated, but limited, set of genomically encoded receptors. So how do plants do it? Many details are still vague, but downstream of pathogen recognition, plant immune receptors activate both cell death and transcriptional outputs to promote defense. Despite their agronomic significance, and over 25 y of study, how plant immune receptors are able to activate defense responses remains a mystery (4, 5).

**Discovery of NLRs in Plants and Animals**

Propelled by the introduction of plant molecular biology in the 1980–90s and the sequencing of the first plant genome in 2001, researchers realized that plants share just a few common types of immune receptors to monitor the outside and inside of cells (6). One major class, the nucleotide-binding site, leucine-rich repeat (“NBS-LRR,” or simply “NLR”) proteins, detects intracellular signals of pathogens. This class of receptors was named for its stereotypical domains: a N-terminal coiled-coil (CC/CCp) or Toll–interleukin-1 receptor (TIR) domain, a central nucleotide binding site domain (NBS), and a C-terminal leucine-rich repeat (LRR) (6). Plant genomes typically have hundreds of these proteins, and they can detect pathogens from all kingdoms of life. Animal nucleotide-binding oligomerization domain-like receptors, or NOD-like receptors (also “NLRs”), like plant NLRs, recognize intracellular signals of pathogenesis and activate defense. Animal NLRs have an N-terminal domain, a central NBS-like NACHT domain, and a C-terminal LRR. Despite such architectural and functional similarities, plant and animal NLRs are products of convergent evolution (7). Across kingdoms, evolution has converged on this solution, using oligomerization as a driver of “induced proximity” of diverse N-terminal signaling domains.

**Animal and Plant NLRs Oligomerize into Wheels**

The functional similarities between plant and animal NLRs are striking. The animal inflammasome is a complex required to activate caspase-dependent inflammation, often in response to pathogens. In 2015, the inflammasome’s primary mechanism was revealed to be NLR oligomerization into a wheel complex similar to the apoptosome (8, 9). While the apoptosome is made of identical subunits, the inflammasome uses two distinct subunits to build its wheel (Fig. 1). Single monomers of an adaptor NLR (called NAIPs) are activated by bacterial ligands. Activated NAIP nucleates binding of a second NLR, in this case NLRC4. A wheel of 11 spokes forms: the initiating NAIP and 10 nucleated NLRC4 molecules. Different NAIPs bind specific ligands, and all can initiate inflammasomes. This NAIP/NLRC4 system is an elegant strategy to expand the repertoire of a limited innate immune system.

In 2019, the first plant “resistosome” structure was generated by cryogenic electron microscopy of the NLR ZAR1, and the picture became more clear: in both animals and plants, NBS and NLR-based proteins were oligomerizing into wheels (10, 11). In both plants and animals, NLR oligomerization results in concentrating their N-terminal domains into a central hub. In the case of the inflammasome, induced proximity of the N-terminal caspase activation and recruitment domain (CARD) activates Caspase-1 which then cleaves...
downstream targets. One of these cleaved targets, Gasdermin D, then forms pores in the plasma membrane to trigger pyroptotic cell death and release of inflammatory cytokines (12). In the plant resistosome structure, induced proximity of the ZAR1 N-terminal CC domain results in formation of a putative pore-forming structure similar to bacterial toxins (11). Membrane disruption would be a plausible mechanism for hypersensitive cell death, a hallmark of plant disease resistance (13).

What Are TIR Domains Doing in Plants?

What about the other class of plant NLRs, the TIR-NLRs? There is not yet a TIR-NLR resistosome structure, but TIR domain structures are quite distinct from CC structures. Unlike the putative pore formation of the ZAR1 N-terminal CC, plant TIRs have recently been shown to be enzymes that cleave NAD$^+$ and NADP$^+$ (14, 15). TIR NADase function seems widely conserved across kingdoms and is found in a variety of proteins with cell death and immune functions (16). How this putative enzymatic NADase function activates downstream events is a major unanswered question for the plant immune system.

Reinventing the Wheel

Structural and genetic analyses indicate that plant TIR domain function requires at least two oligomerization interfaces, but how they are organized is unknown (17, 18). To learn more about the structural requirements for TIR signaling, Duxbury et al. generate a remarkable hybrid immune receptor. They fuse the TIR domain of the plant immune receptor RPS4 onto the N terminus of mammalian NLRC4, generating a “plant inflammasome.” They demonstrate that the TIR$^{\text{RPS4}}$-NLRC4 hybrid oligomerizes in planta in response to NAIPs and cognate ligands, like bacterial flagellin. Remarkably, the ligand/receptor logic of NAIP/NLRC4 is retained, consistent with the hybrid receptor forming a proper inflammasome. Next, they ask whether TIR$^{\text{RPS4}}$-NLRC4 can activate TIR-mediated hypersensitive-like cell death when transiently expressed in tobacco. They find induced proximity of TIR domains driven by NLRC4 oligomerization is sufficient to cause cell death. This TIR$^{\text{RPS4}}$-NLRC4 death is dependent on residues that are known to block inflammasome function, and on residues that are required for TIR oligomerization and enzymatic function. The downstream requirements are also conserved, so the TIR domain signals through the known plant pathways. These findings indicate that the positional and stoichiometric constraints of the animal inflammasome are sufficient to promote the activities of a plant immune domain. Thus, plant TIRs have conserved self-association surfaces that require induced proximity, but they seem to have substantial flexibility in how that proximity is promoted.

Implications of NADase Activity from Non-Plant TIRs

TIR domains appear to have conserved enzymatic functions in plants, animals, and prokaryotes (14, 15, 19, 20). Recently, plant TIRs have been shown to cleave NAD$^*$, releasing Nam and ADPR. TIRs from different kingdoms also release cyclized versions of ADPR, of the same mass as cADPR, but likely with differences in cyclization (15, 20). The exact structure(s) of these molecules remain mysterious. Wan et al. (15) found that so-called “variant cADPR” (v-cADPR), was produced both in vitro and in vivo by plant TIR proteins and proposed it as a “biomarker” for plant TIR

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Fig. 1. Animal and plant NLRs form oligomeric wheels. (Left) The animal inflammasome is initiated when a single NAIP molecule (tan) is activated by a pathogen ligand (orange). Activated NAIP nucleates the formation of a wheel from NLRC monomers. Induced proximity of NLRC N termini activates caspase-1 (not shown) to initiate pyroptosis. (Middle) Plant CC-NLRs (e.g., ZAR1, green) can be activated by pathogen modification of host proteins (orange). Conformational change of ZAR1 leads to the formation of a pentameric wheel with a pyramidal N terminus proposed to be a pore-forming needle. A structure for a TIR-NLR oligomeric wheel has not been determined, but may be similar to ZAR1. The TIR domain (green circle) oligomerization is required for NADase function and cell death/immunity. (Right) A hybrid TIR-NLRC4 receptor can respond appropriately to NAIPS activated by pathogen ligands to activate TIR enzymatic function and cell death.
activity and a potential signaling molecule. Plant TIR-produced v-cADPR appears identical to a product produced by a bacterial AbTIR protein (15, 20). Would production of v-cADPR by AbTIR result in cell death in plants? Using their NLRC4 chassis as a system to induce AbTIR oligomerization, the authors could answer this important question. What they found is that in contrast to plant TIR-NLRC4 hybrids, AbTIR-NLRC4 proteins did not activate cell death. However, they did produce detectable amounts of v-cADPR measured by mass spectroscopy. The authors concluded that v-cADPR may be necessary, but not sufficient to activate cell death. They hypothesize that plant-specific coordination between TIR enzymatic function and essential downstream components (such as EDS1) could be required. Could plant TIRs have other substrates and products? This seems plausible, as plant TIRs are also reported to cleave NADP+ (15). It is also possible that the plant and prokaryotic TIRs produce subtly different products that will only be clarified by the determination of their structures.

**Prospects for Engineered Disease Resistance?**

The authors also explore whether TIR<sup>RPS4</sup>-NLRC4 can recognize ligands from plant pathogens and potentially act as artificial immune receptors. Many plant bacterial pathogens also use flagella while infecting plants, so the authors tested whether plant pathogen flagellin would be able to activate a TIR<sup>RPS4</sup>-NLRC4/NAIP5 pair that recognizes flagellin from animal pathogens. Despite divergent sequences, amazingly, coexpressed flagellin from plant pathogenic bacteria was able to trigger both cell death and oligomerization of TIR<sup>RPS4</sup>-NLRC4 in a proof-of-principle transient expression assay. Tests of real disease resistance in transgenic Arabidopsis were less successful, but optimizing the expression of the system, or tuning NAIPs for plant pathogen ligands, could lead to more functional artificial receptors.

Engineering the plant immune system will continue to be an important goal to improve crop yields in the face of increasing need and climate change. By understanding how immune receptors function at a mechanistic level, we will be able to better deploy existing receptors, tune the balance of cell death and disease resistance, or even generate new specificities. There are likely many roads to these destinations; here in PNAS, Duxbury et al. show that reinventing the wheel may help get us there.

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