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# Functional diversity of Toll/interleukin-1 receptor domains in flowering plants and its translational potential

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**Abstract**

Across the Tree of Life, innate immunity and cell death mechanisms protect hosts from potential pathogens. In prokaryotes, animals, and flowering plants, these functions are often mediated by Toll/interleukin-1 receptor (TIR) domain proteins. Here, we discuss recent analyses of TIR biology in flowering plants, revealing (i) TIR functions beyond pathogen recognition, e.g. in the spatial control of immunity, and (ii) the existence of at least two pathways for TIR signaling in plants. Also, we discuss TIR-based strategies for crop improvement and argue for a need to better understand TIR functions outside of commonly studied dicot pathways for future translational work. Opinions of experts on emerging topics in basic and translational plant TIR research are presented in supplementary video interviews.

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## Flowering plants deploy Toll/interleukin-1 receptor (TIR) domains to detect and respond to pathogens

Plants use cell surface and intracellular immune receptors to detect microbes. The surface immune receptors are membrane-spanning modular proteins with extracellular sensor domains such as leucine-rich repeats (LRRs). These pattern recognition receptors (PRRs) recognize conserved pathogen-associated molecular patterns (PAMPs), for instance, fragments of bacterial flagella [1]. Extracellular perception of microbial signals by PRRs initiates a robust immune response that must be overcome by successful pathogens. To achieve this, pathogens often deliver virulence proteins, or effectors, into the host cytoplasm. Once inside the cytoplasm, pathogen effectors can suppress host immunity and rewire metabolism to benefit the pathogen [2,3]. In response to this challenge, plants have evolved intracellular receptors to directly or indirectly detect pathogen effectors [1].

Canonical intracellular immune receptors are named nucleotide-binding site and LRR domain receptors (NLRs) for their domain structure, which includes a central nucleotide-binding site domain (NBS) and a C-terminal LRR domain [4]. Non-canonical or "truncated" NLRs lacking one or more domains also exist as functional immune receptors [5,6]. NLR receptors are divided into two classes based on their N-terminal signaling domains: (1) a coiled-coil (CC) domain NLRs (CNLs) and (2) Toll/interleukin-1 receptor (TIR) domain NLRs, or TNLs [7]. Effector activation of CNLs results in the formation of a pentameric CNL "resistosome" that acts as a calcium channel to promote immunity [8,9]. Effector-activated TNLs oligomerize into a tetrameric resistosome, which allows them to function as nicotinamide adenine dinucleotide ($NAD^+$)-consuming enzymes [10–15].

A few "helper" NLRs function downstream of other immune receptors. This particular class of NLRs has a phylogenetically distinct N-terminal $CC_R$ domain initially described in the CC-only *Arabidopsis thaliana* protein Resistance to Powdery Mildew 8 (RPW8) [4,7,16]. Following the convention of CNL and TNL, these helper NLRs are referred to as RNLs based on the presence of the $CC_R$ domain. So far, RNLs are not implicated in direct effector recognition, but they are critical to converting TIR enzymatic activities into cell

death and resistance responses, likely via their CNL-like resistosome enabling $Ca^{2+}$ influx [10,16–20].

PRR plasma membrane receptors and NLR intracellular receptors are required for each other's efficient functioning during an immune response [21,22]. Research in *Arabidopsis* suggests that plant TIRs or TNLs function closely with plasma membrane receptors to transmit downstream signals or monitor their modification by effectors [23–25]. The function of some PRR receptors, such as receptor-like protein 23 (RLP23), depends on the Enhanced Disease Susceptibility 1 (EDS1) pathway, which is the downstream mediator of TIR/TNL signaling [23,24,26]. The converse is also true, as TNL pathways can depend on the PRR function for full output [21,22,27]. In short, evidence from *Arabidopsis* and a few other species shows that TIR-containing proteins help flowering plants detect and respond to pathogens.

## Plant TIRs exhibit intrinsic enzymatic activities

TIRs can function via at least three mechanisms. The first described mechanism was an inducible scaffold function in animal Toll-like receptors and their cytoplasmic TIR adapters [28]. Physical interactions between these TIRs can result in high-order structures that concentrate signaling proteins such as kinases in a cell [28–30]. This model might also apply to plants, as proximity labeling of a TNL N suggests physical interactions between its TIR domain and CNLs in *Nicotiana benthamiana*, which may contribute to the TNL-dependent resistance [31].

The second mechanism of TIR function is a recently discovered intrinsic TIR $NAD^+$-degrading activity (Figure 1) [32]. Such activity was initially found in the animal TIR protein Sterile Alpha and TIR motif containing 1 (SARM1), which induces neuronal cell death via $NAD^+$ depletion in response to injury [33]. Subsequently, TIR NADase activity was detected in plant and prokaryotic TIRs activating immunity [34–36]. In the case of prokaryotes, this TIR-produced signal can stimulate non-TIR NADases to substantially deplete $NAD^+$ [37]. By contrast, in plants, TIR $NAD^+$-derivatives activate EDS1-dependent immunity without a significant depletion of $NAD^+$ [35,36,38,39] (Figure 1a, red box).

In the third mechanism, plant TIRs function as dsDNA/RNA hydrolases to generate 2',3' cyclic adenosine/guanosine monophosphates (2',3'-cAMP/cGMP, Figure 1b, blue box). This mechanism is associated with oligomeric TIR filaments of indeterminant length rather than head-to-tail tetrameric complexes that hydrolyze $NAD^+$ [15,40]. This synthetase activity might not be directly relevant to TNL cell death activity [41]. Instead, 2',3'-cAMP/cGMP probably act as secondary messengers in plants to activate stress-related transcriptional reprogramming [42]. The role of these metabolites in immunity is supported by the observation that expression of *Arabidopsis* nudix hydroxylase homolog 7 (NUDT7) and other phosphodiesterases that degrade 2'3'-cAMP/cGMP suppresses TIR cell death in *N. benthamiana* [40]. Similarly, a *nudt7* loss-of-function *Arabidopsis* mutant displays autoimmunity [43], and at least one pathogen effector can degrade 2',3'-cAMP/cGMP [40]. Although the 2',3'-cAMP/cGMP synthetase function has been demonstrated for TIRs of flowering plants including monocots, NUDT7 is *Brassicaceae*-specific (phylogenes.org; PTHR13994). Thus, containment mechanisms of the TIR 2',3'-cAMP/cGMP synthetase activity likely vary across phylogeny.

## Plants use EDS1 family complexes to link TIR enzymatic activity with cell death and resistance

Signaling by TIRs in flowering plants depends on a small family of EDS1 and EDS1-related proteins. (Figure 1a). These proteins have an N-terminal lipase-like domain connected to a plant-specific C-terminal α-helical "EP" ('EDS1-PAD4') domain [26]. EDS1 forms exclusive heterodimers with one of its paralogs, phytoalexin-deficient 4 (PAD4) or senescence-associated gene 101 (SAG101) [44–46]. The heterodimerized EP domains create a binding site for a subset of TIR-produced $NAD^+$-derived small molecules (pRib-AMP, pRib-ADP, ADPr-ATP, and diADPR) (Figure 1) [38,39]. Once activated by pRib-AMP or pRib-ADP, the heterodimer EDS1-PAD4 interacts specifically with RNL activated disease resistance 1 (ADR1). Similarly, ADPr-ATP or diADPR interact with EDS1-SAG101 and facilitate their interaction with RNL N requirement gene 1 (NRG1) [18,38,39,47]. In addition to RNLs, EDS1 complexes may include proteins such as TIR-containing proteins or transcription factors [18,47].

## Why do plant TIRs and pathogen TIR effectors produce an overlapping spectrum of NADase products?

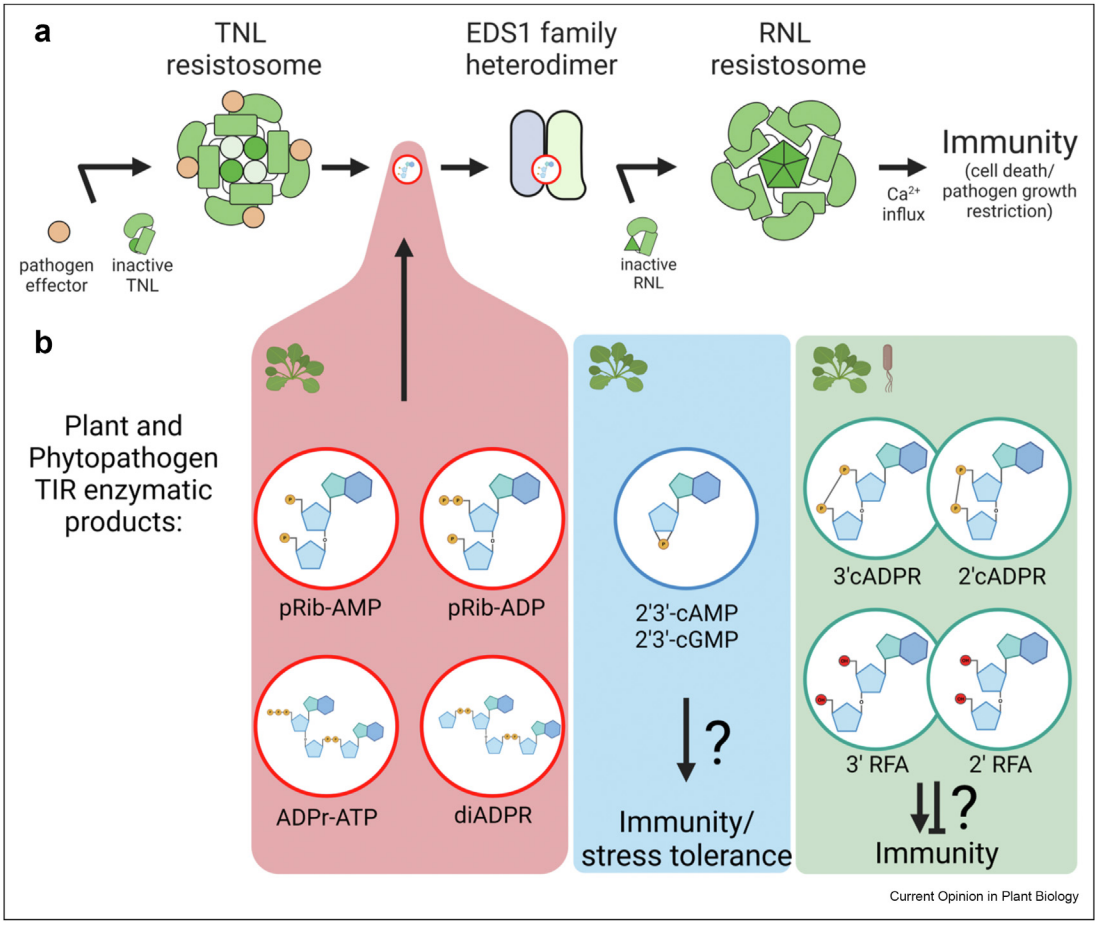
As seen above, TIRs of flowering plants generate a diverse set of enzymatic products. Four of them (pRib-AMP, pRib-ADP, ADPr-ATP, and diADPR) are immunogenic, bind EDS1 heterodimers both *in* vitro and *in planta*, and activate $Ca^{2+}$ influx via RNLs (Figure 1) [17,38,39]. Plant TIRs can also produce stable isomers of cyclic ADPR (2'-cADPR and 3'-cADPR) that are, surprisingly, identical to those produced by the bacterial TIR and TIR-like effectors HopAM1 and HopBY1 (Figure 1b, green box) [48–51]. Plant TIRs (and likely HopAM1) also produce 3'-O-β-D-ribofuranosyladenosine (RFA), a molecule equivalent to a dephosphorylated version of the EDS1-activating TIR product pRib-AMP [52,53]. Why would a plant produce the same TIR products as the pathogen? Assuming that

effector products are negative regulators of immunity, plant-produced cADPR isomers could be negative regulators of immunity [49–51,54]. Perhaps plant TIRs are making both positive regulators of defense (i.e. EDS1-activating pRib, et al.) and negative regulators (cADPR isomers) to limit inappropriate immune activation or the cost of defense. Alternatively, cADPR isomers and RFA produced by plant and pathogen TIRs may be enzymatic side-products or metabolites of EDS1 signals [48,52,53]. Finally, it seems possible that NADase effectors function by depleting $NAD^+$ to disrupt host metabolism rather than by generating signaling molecules [48,49]. Differentiating between these hypotheses will require new bioassays to deliver small molecules into the plant cell or new TIR mutants that decouple the production of the various TIR enzymatic products from each other.

## TIRs of flowering plants functioning as immunity boosters beyond effector recognition

Recent work using pharmacological inhibitors of TIR signaling in *Arabidopsis* has demonstrated that TIR enzymatic activity can be required for effector-triggered immunity even when CNLs initiate the effector-triggered immune responses (Figure 2) [53]. Many *TIR* loci are among the earliest transcriptionally activated defense genes in flowering plants [23,55–57]. Together, these data implicate TIRs in plant immunity beyond TIR or TNL-mediated effector recognition.

**Figure 1**




**Plant and plant-pathogen TIR proteins generate diverse NADase products to regulate immunity. (a)** Intracellular TNL (TIR-NLR) immune receptors oligomerize into tetrameric resistosomes in response to pathogen effectors. Oligomerization of the TIR domains (green circles) activates their enzymatic activity to produce small molecules (red circle). TIR products signal downstream by activating the EDS1 heterodimer, which then promotes the oligomerization of an RNL resistosome. RNL resistosomes function as calcium channels to activate immunity via an unknown mechanism. **(b)** Plant and plant pathogen TIRs produce a spectrum of nucleotide-related small molecules. Several TIR products (e.g. pRib-AMP, red) have been shown to function by activating the EDS1 complex. Plant TIRs can also degrade dsDNA/RNA to produce 2',3'-cAMP/cGMP previously linked to stress tolerance (blue). Plant and plant pathogen TIRs also produce isomers of cyclic ADPR and pRib-like RFA molecules (green). These molecules have been proposed to function as regulators of immunity, but any functions remain to be demonstrated. Small-molecule structures are graphically simplified to highlight their similarities and differences.

One hypothesis that may explain these results is that certain TIR proteins act as "immune boosters" to enhance plant immune responses at a transcriptional level (Figure 2, infected cell) [40]. This model would also explain the importance of EDS1 and PAD4, receptors of some TIR enzymatic products, for signaling by the defense hormone salicylic acid [58].

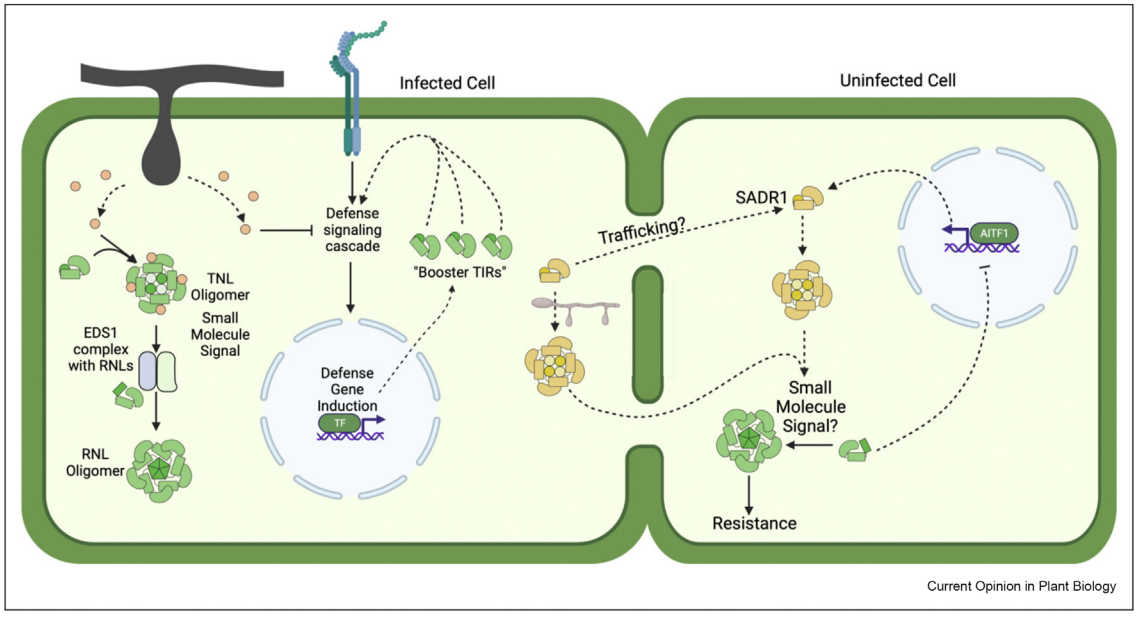
## Emerging roles of TIRs in local acquired immunity in flowering plants

TIRs/TNLs have primarily been studied as initiators of a cell-autonomous EDS1-dependent response. However, recent studies of the TNL suppressor of ADR1-L2 1, (SADR1) also named SAA1, suppressor of AITF1-induced autoimmunity 1, suggest that TIRs can have EDS1-independent functions essential for immune responses in areas next to the pathogen recognition site referred to as 'local acquired resistance' (LAR; [14,53,59]). SADR1 was identified as a suppressor of an autoactive D484V variant of the helper RNL ADR1-L2. Surprisingly, *sadr1*'s suppression of ADR1-L2$^{D484V}$ autoactivity was not fully phenocopied by an *eds1* mutant, indicating that at least some SADR1 function is EDS1-independent. Intriguingly, TIR functions supplied by two other autoactive TNLs were able to partially restore autoimmunity of the suppressed *ADR1-L2*$^{D484V}$ *sadr1* double mutant, indicating that other TNLs share this SADR1 function [53]. SADR1 was also required to express the defense marker gene *pathogenesis-related gene 1 (PR-1)* in a zone around the infected tissue. This non-cell autonomous expression was lost in an RNL polymutant that lacked *ADR1* and *NRG1* functions [53]. One could speculate that TIR-catalyzed signaling products or the downstream signals move from infected cells to induce immunity in surrounding uninfected cells. A biomarker for TIR signaling activity identified in this study, 2′/3′-O-β-D-ribofuranosyladenosine, could be a candidate for a mobile TIR-produced non-autonomous immune signal. These signals could plausibly travel intracellularly through plasmodesmata or function through an apoplastic route as phytocytokines perceived by PRRs (Figure 2).

## TIRs in flowering plants differ in distribution across species and signaling requirements

Plant TIR differentiation in function and signaling was also elaborated using phylogenomics methods. They revealed two primary plant TIR classes (Figure 3) [56,60,61]. The first class was introduced above as associated with NBS and LRR domains (Figure 3a). This class also includes "truncated" TIRs without LRR or NBS-LRR (i.e. TIR-NBS or TIR-only, respectively). We refer to this first class as "TNL" class TIRs [56,61]. This class is found in receptor TNLs and TIR-only proteins, putative booster TIR proteins, and TNL SADR1 acting in LAR (Figure 3a). The second class includes TIR proteins with an NBS domain and a C-terminal

**Figure 2**

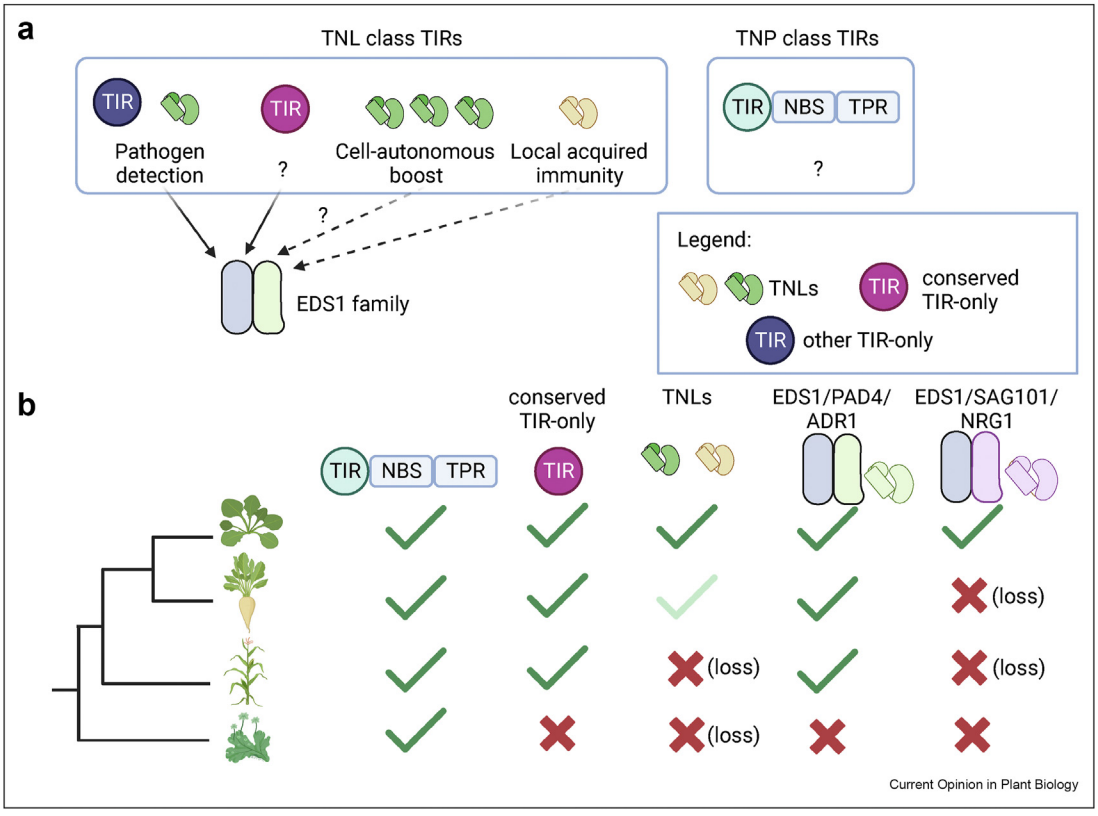



**TIR proteins function in immune responses beyond effector recognition.** Upon activation of pattern-triggered immunity, "Booster TIRs" may be transcriptionally activated to further potentiate early immune responses. SADR1 would be activated in response to these same signals to regulate immune responses in neighboring uninfected cells, enhancing RNL-mediated immune responses to prevent pathogen spread. SADR1 could also be trafficked directly from pathogen-infected cells to neighboring cells, or small-molecule signals produced by SADR1 could move between cells through the apoplast or plasmodesmata to propagate a non-autonomous immune response.

tetratricopeptide repeat (TPR) rather than an LRR (Figure 3a, right). This class has been named TIR-NBS-TPR (TNP) TIRs [55,56,62]. TNP-encoding genes are present in plant genomes in relatively low numbers, but they are the most widely distributed plant TIRs [56,62]. By contrast, the TNL-TIR class shows lineage-specific expansions and contractions. TNLs often have a C-terminal extension called C-JID (C-terminal jelly roll/immunoglobulin-like domain), which binds effectors via a variable loop surface [13,55,63]. These observations support the notion that many TNLs recognize variable effectors. However, predicting more specific functions (e.g. as booster TIRs or LARs) based on the sequence seems difficult. For instance, SADR1 also has a C-JID domain and thus may bind an unknown ligand in addition to, or as part of, its EDS1-independent and LAR functions [53].

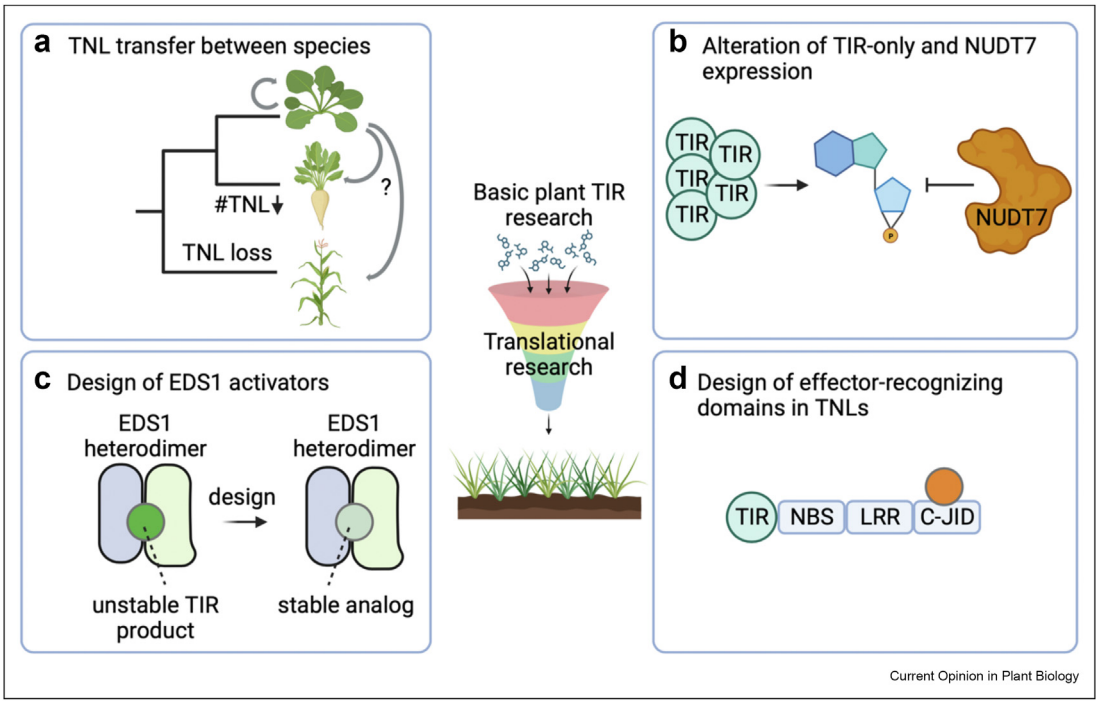
The TNL-TIR class shows clade-specific expansions and contractions. In contrast to dicots, monocots have lost nearly all TNL TIRs except for a low- or single-copy number TIR-only shared by most flowering plants (Figure 3b) [56]. The EDS1 paralog SAG101 and SAG101-interacting RNL helper NRG1 were also lost in these genomes, indicating coevolution between TNLs, SAG101, and the helper NRG1 (Figure 3b) [45,64]. Still, many known TIR signaling components are retained in monocot genomes, including EDS1, PAD4, and ADR1 (Figure 3b) [56], suggesting they function with the remaining TIR-only proteins. The physiological functions of this particular TIR-only group remain unknown, but it seems more parsimonious to hypothesize that they broadly regulate immunity rather than recognize a specific pathogen across such a wide phylogenetic distance.

**Figure 3**




**TIRs of flowering plants differ in conservation and function. (a)** Two major TIR classes are found in plants: TIR in full-length or truncated TNLs ('TNL class TIRs') and TNP TIRs. The first class is implicated in pathogen detection, cell-autonomous boosting of immunity, and local acquired immunity. Functions of the phylogenetically shared conserved TIR-only (purple circle; TNL class TIR) and TNPs are unknown. The EDS1 family is required for the activity of TIR-containing pathogen receptors and cell death by the conserved TIR-only proteins. The dependence of other groups of TIR proteins on EDS1 is either unknown (shown as '?', dashed line), limited (dashed line), or not detected (no arrow from TNP TIRs). **(b)** While TNPs are found across plant phylogeny including early land plants, conserved TIR-only are limited to flowering plants. Some lineages of flowering plants lost TNLs (monocots) or significantly reduced their numbers (*Caryophyllales*, *Lamiales*). The expansion of TNLs correlates with the presence of the EDS1/SAG101/NRG1 signaling node; however, the EDS1/PAD4/ADR1 node is still present in monocots, retaining conserved TIR-only proteins as the only members of TIRs from the TNL class. Since TNLs and EDS1/SAG101/NRG1 are present in Nymphaeales, a sister clade of dicots and monocots [55], monocots have likely lost SAG101 and NRG1.

**Figure 4**




**Possible strategies to improve crop resistance to pathogens and general stress resilience using leads from the fundamental plant TIR research. (a)** There are likely no limitations on the side of biology in the transfer of TNLs between species with large TNL repertoires (Brassicaceae, Solanaceae). However, their transfer to species with no or reduced TNL sets (monocots, *Caryophyllales*, and *Lamiales*) would require a better understanding of TIR signaling in these species. **(b)** 2',3'-cAMP/cGMP can activate defense and stress-related transcriptional reprogramming, and their levels could be controlled by changing the expression of genes encoding for TIR-only and NUDT7 that regulate the 2',3'-cAMP/cGMP production. **(c)** TIR products activating EDS1 complexes are not stable in plant cells. Searching for their more stable synthetic analogs can provide ways to boost immunity signaling. **(d)** The C-JID domain can function as a module recognizing effectors. Structural information enables the design of new C-JID's; however, finding an appropriate design might prove difficult.

In contrast to TNL TIRs, TNP TIRs are present in plants without the EDS1 family and RNLs (Figure 3b) suggesting TNP activities are independent of them. Indeed, cell death by maize (*Zea mays*) TNP in *Nicotiana tabacum* requiring the TIR catalytic glutamate was not compromised in the *eds1* silencing line. The *tnp* mutants of *N. benthamiana* behaved like wild-type plants at the level of growth, early PRR receptor-like kinase signaling, and TNL cell death and resistance [56], which leaves an open question about TNP physiological function(s).

## Implications of TIR basic research for crop improvement

In the final part of this opinion article, we display four strategies for how basic knowledge about plant TIRs could facilitate crop improvement (Figure 4). These strategies include (A) transferring TNLs between plant species, (B) engineering *TIR-only* and *NUDT7* expression to increase basal 2',3'-cAMP/cGMP levels and thereby prime crops for stress tolerance [40,42], (C) designing small molecules activating EDS1 complexes but more stable *in planta* than TIR enzymatic products [38,39], and (D) engineering the C-JID domain in TNLs to design receptors for specific effectors. Implementing these strategies would require additional fundamental and translational research. For instance, TNL transfer between plant species is likely possible between plants separated by large evolutionary distances [45,65–67], but limitations might occur when the recipient species such as monocots and some dicots, have eliminated or significantly reduced their TNL repertoires. Thus, the transfer of TNL receptors to these genomes may require engineering of compatible EDS1/RNL pathways [45]. Similarly, rational engineering of novel C-JID specificities will require advances in *in silico* prediction of effector binding or high-throughput functional assays. Additional discussion can be found in the interview with an invited expert (Supplementary Video 1).

Supplementary videos related to this article can be found at https://doi.org/10.1016/j.pbi.2023.102481

## Conclusion and perspectives

Research on plant TIRs has revealed diverse enzymatic activities and products. Some of these products have connected TIR proteins to the downstream EDS1/RNL pathway, while others remain more obscure. One of the burning questions is the identity of receptors and their functions for these plant and phytopathogen TIR products. Also, booster TIR/TNL and LAR open questions about the role of plant TIRs in spatially controlled tissue-level immune responses. Finally, the major current limitation is that most plant TIR research has been conducted on a handful of proteins, mostly in non-crops, but TIR signaling execution mechanisms differ even in dicot plants. Thus, more appreciation is needed for translational TIR research if we want current fundamental advances to have an impact on agriculture. For more opinions on the present issues in plant TIR biology, please refer to an interview with another invited expert (Supplementary Video 2).

Supplementary videos related to this article can be found at https://doi.org/10.1016/j.pbi.2023.102481

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dmitry Lapin reports a relationship with Max Planck Institute for Plant Breeding Research that includes: employment. DL was a member of the laboratory of Jane E. Parker whose interview is shown in Supplementary Video 2.

## Data availability

No original experimental data was used for the research described in the article.

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## References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Ngou BPM, Ding P, Jones JDG: **Thirty years of resistance: zig-zag through the plant immune system**. *Plant Cell* 2022, **34**: 1447–1478.

2. Toruño TY, Stergiopoulos I, Coaker G: **Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners**. *Annu Rev Phytopathol* 2016, **54**: 419–441.

3. Ceulemans E, Ibrahim HMM, De Coninck B, Goossens A: **Pathogen effectors: exploiting the promiscuity of plant signaling hubs**. *Trends Plant Sci* 2021, **26**:780–795.

4. Tamborski J, Krasileva KV: **Evolution of plant NLRs: from natural history to precise modifications**. *Annu Rev Plant Biol* 2020, **71**:355–378.

5. Santamaría ME, Martínez M, Arnaiz A, Rioja C, Burow M, Grbic V, Díaz I: **An Arabidopsis TIR-lectin two-domain protein confers defense properties against tetranychus urticae**. *Plant Physiol* 2019, **179**:1298–1314.

6. Nishimura MT, Anderson RG, Cherkis KA, Law TF, Liu QL, Machius M, Nimchuk ZL, Yang L, Chung E-H, El Kasmi F, *et al.*: **TIR-only protein RBA1 recognizes a pathogen effector to regulate cell death in Arabidopsis**. *Proc Natl Acad Sci USA* 2017, **114**:E2053–E2062.

7. Shao Z-Q, Xue J-Y, Wu P, Zhang Y-M, Wu Y, Hang Y-Y, Wang B, Chen J-Q: **Large-scale analyses of angiosperm nucleotide-binding site-leucine-rich repeat genes reveal three anciently diverged classes with distinct evolutionary patterns**. *Plant Physiol* 2016, **170**:2095–2109.

8. Bi G, Su M, Li N, Liang Y, Dang S, Xu J, Hu M, Wang J, Zou M, Deng Y, *et al.*: **The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling**. *Cell* 2021, **184**: 3528–3541.e3512.

9. Förderer A, Li E, Lawson AW, Deng Y-n, Sun Y, Logemann E, Zhang X, Wen J, Han Z, Chang J, *et al.*: **A wheat resistosome defines common principles of immune receptor channels**. *Nature* 2022, **610**:532–539.

10. Wang J, Song W, Chai J: **Structure, biochemical function, and signaling mechanism of plant NLRs**. *Mol Plant* 2023, **16**: 75–95.

11. Maruta N, Burdett H, Lim BYJ, Hu X, Desa S, Manik MK, Kobe B: **Structural basis of NLR activation and innate immune signalling in plants**. *Immunogenetics* 2022, **74**:5–26.

12. Martin R, Qi T, Zhang H, Liu F, King M, Toth C, Nogales E, Staskawicz BJ: **Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ**. *Science* 2020, **370**.

13. Ma S, Lapin D, Liu L, Sun Y, Song W, Zhang X, Logemann E, Yu D, Wang J, Jirschitzka J, *et al.*: **Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme**. *Science* 2020, **370**.

14. * Wang Y, Tang M, Zhang Y, Huang M, Wei L, Lin Y, Xie J, Cheng J, Fu Y, Jiang D, *et al.*: **Coordinated regulation of plant defense and autoimmunity by paired trihelix transcription factors ASR3/AITF1 in Arabidopsis**. *New Phytol* 2023, **237**: 914–929.
Identifies AITF1, a member of the trihelix transcription factors in Arabidopsis, as a regulator of the TNL SADR1/SAA1 expression.

15. Maruta N, Sorbello M, Lim BYJ, McGuinness HY, Shi Y, Ve T, Kobe B: **TIR domain-associated nucleotides with functions in plant immunity and beyond**. *Curr Opin Plant Biol* 2023, **73**: 102364.

16. Jubic LM, Saile S, Furzer OJ, El Kasmi F, Dangl JL: **Help wanted: helper NLRs and plant immune responses**. *Curr Opin Plant Biol* 2019, **50**:82–94.

17. Jacob P, Kim NH, Wu F, El-Kasmi F, Chi Y, Walton WG, Furzer OJ, Lietzan AD, Sunil S, Kempthorn K, *et al.*: **Plant "helper" immune receptors are Ca2+-permeable nonselective cation channels**. *Science* 2021, **373**:420–425.

18. Sun X, Lapin D, Feehan JM, Stolze SC, Kramer K, Dongus JA, Rzemieniewski J, Blanvillain-Baufumé S, Harzen A, Bautor J, *et al.*: **Pathogen effector recognition-dependent association of NRG1 with EDS1 and SAG101 in TNL receptor immunity**. *Nat Commun* 2021, **12**:3335.

19. Saile SC, Jacob P, Castel B, Jubic LM, Salas-Gonzáles I, Bäcker M, Jones JDG, Dangl JL, El Kasmi F: **Two unequally redundant "helper" immune receptor families mediate Arabidopsis thaliana intracellular "sensor" immune receptor functions**. *PLoS Biol* 2020, **18**, e3000783.

20. Wu Z, Tian L, Liu X, Zhang Y, Li X: **TIR signal promotes interactions between lipase-like proteins and ADR1-L1 receptor and ADR1-L1 oligomerization**. *Plant Physiol* 2021, **187**:681–686.

21. Ngou BPM, Ahn H-K, Ding P, Jones JDG: **Mutual potentiation of plant immunity by cell-surface and intracellular receptors**. *Nature* 2021, **592**:110–115.

22. Yuan M, Jiang Z, Bi G, Nomura K, Liu M, Wang Y, Cai B, Zhou J-M, He SY, Xin X-F: **Pattern-recognition receptors are required for NLR-mediated plant immunity**. *Nature* 2021, **592**:105–109.

23. Tian H, Wu Z, Chen S, Ao K, Huang W, Yaghmaiean H, Sun T, Xu F, Zhang Y, Wang S, *et al.*: **Activation of TIR signalling boosts pattern-triggered immunity**. *Nature* 2021, **598**: 500–503.

24. Pruitt RN, Locci F, Wanke F, Zhang L, Saile SC, Joe A, Karelina D, Hua C, Fröhlich K, Wan W-L, *et al.*: **The EDS1–PAD4–ADR1 node mediates Arabidopsis pattern-triggered immunity**. *Nature* 2021, **598**:495–499.

25. * Ming-Yu W, Jun-Bin C, Rui W, Hai-Long G, Yan C, Zhen-Ju L, Lu-Yang W, Chuang L, Sheng-Feng H, Mei-Da D, *et al.*: **The immune receptor SNC1 monitors helper NLRs targeted by a bacterial effector**. *bioRxiv* 2023. 2023.2003.2023.533910.
Identifies the long-sought function for the *Arabidopsis* TNL protein SNC1 as a sensor for PRR manipulation via effectors.

26. Lapin D, Bhandari DD, Parker JE: **Origins and immunity networking functions of EDS1 family proteins**. *Annu Rev Phytopathol* 2020, **58**:253–276.

27. ** Feehan JM, Wang J, Sun X, Choi J, Ahn H-K, Ngou BPM, Parker JE, Jones JDG: **Oligomerization of a plant helper NLR requires cell-surface and intracellular immune receptor activation**. *Proc Natl Acad Sci USA* 2023, **120**, e2210406120.
Shows that the formation of the stable EDS1-SAG101-NRG1 complex depends on both TIR enzymatic activity and the activation of PRRs. Since EDS1-SAG101-NRG1 can be reconstituted in vitro using purified TIRs and TNL (Jia et al., 2022 and Huang et al., 2022), the study by Feehan et al., 2023, supports the role of booster TIRs in reinforcing EDS1 signaling via PRRs.

28. Nanson JD, Kobe B, Ve T, Death TIR, RHIM: **Self-assembling domains involved in innate immunity and cell-death signaling**. *J Leukoc Biol* 2019, **105**:363–375.

29. Clabbers MTB, Holmes S, Muusse TW, Vajjhala PR, Thygesen SJ, Malde AK, Hunter DJB, Croll TI, Flueckiger L, Nanson JD, *et al.*: **MyD88 TIR domain higher-order assembly interactions revealed by microcrystal electron diffraction and serial femtosecond crystallography**. *Nat Commun* 2021, **12**: 2578.

30. Ve T, Vajjhala PR, Hedger A, Croll T, DiMaio F, Horsefield S, Yu X, Lavrencic P, Hassan Z, Morgan GP, *et al.*: **Structural basis of TIR-domain-assembly formation in MAL- and MyD88-dependent TLR4 signaling**. *Nat Struct Mol Biol* 2017, **24**: 743–751.

31. Zhang Y, Song G, Lal NK, Nagalakshmi U, Li Y, Zheng W, Huang P-j, Branon TC, Ting AY, Walley JW, *et al.*: **TurboID-based proximity labeling reveals that UBR7 is a regulator of N NLR immune receptor-mediated immunity**. *Nat Commun* 2019, **10**:3252.

32. Essuman K, Milbrandt J, Dangl JL, Nishimura MT: **Shared TIR enzymatic functions regulate cell death and immunity across the tree of life**. *Science* 2022, **377**.

33. Essuman K, Summers DW, Sasaki Y, Mao X, DiAntonio A, Milbrandt J: **The SARM1 toll/interleukin-1 receptor domain possesses intrinsic NAD+ cleavage activity that promotes pathological axonal degeneration**. *Neuron* 2017, **93**: 1334–1343.e1335.

34. Essuman K, Summers DW, Sasaki Y, Mao X, Yim AKY, DiAntonio A, Milbrandt J: **TIR domain proteins are an ancient family of NAD+-Consuming enzymes**. *Curr Biol* 2018, **28**: 421–430.e424.

35. Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, Chung EH, Nishimura EO, DiAntonio A, Milbrandt J, Dangl JL, *et al.*: **TIR domains of plant immune receptors are NAD+-cleaving enzymes that promote cell death**. *Science* 2019, **365**: 799–803.

36. Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, Chen J, Qi T, Gilley J, Lai JS, Rank MX, *et al.*: **NAD+ cleavage activity by animal and plant TIR domains in cell death pathways**. *Science* 2019, **365**:793–799.

37. Ofir G, Herbst E, Baroz M, Cohen D, Millman A, Doron S, Tal N, Malheiro DBA, Malitsky S, Amitai G, *et al.*: **Antiviral activity of bacterial TIR domains via immune signalling molecules**. *Nature* 2021, **600**:116–120.

38. ** Huang S, Jia A, Song W, Hessler G, Meng Y, Sun Y, Xu L, Laessle H, Jirschitzka J, Ma S, *et al.*: **Identification and receptor mechanism of TIR-catalyzed small molecules in plant immunity**. *Science* 2022, **377**.
Uses cryogenic electron microscopy (Cryo-EM) of active EDS1-PAD4 heterodimers to identify the structure of the TIR-produced signaling molecule required for its signaling, termed pRIB-AMP, which reconstitutes specific associations of EDS1 heterodimers with RNLs.

39. ** Jia A, Huang S, Song W, Wang J, Meng Y, Sun Y, Xu L, Laessle H, Jirschitzka J, Hou J, *et al.*: **TIR-catalyzed ADP-ribosylation reactions produce signaling molecules for plant immunity**. *Science* 2022, **377**.
Uses Cryo-EM of active EDS1-SAG101 heterodimers to identify the structure of the TIR-produced signaling molecules required for EDS1-SAG101 signaling, termed ADPR-ATP and di-ADPR. The study demonstrates that 2',3'-cNMPs are likely insufficient to activate the EDS1 heterodimers to interact with RNLs. The analysis also demonstrates that the conserved TIR-only *Bd*TIR promotes EDS1-SAG101 dimer interaction with NRG1.

40. ** Yu D, Song W, Tan EYJ, Liu L, Cao Y, Jirschitzka J, Li E, Logemann E, Xu C, Huang S, *et al.*: **TIR domains of plant immune receptors are 2′,3′-cAMP/cGMP synthetases mediating cell death**. *Cell* 2022, **185**:2370–2386.e2318.
Identifies the 2',3'-cAMP/cGMP-synthetase activity of TIRs dependent on their high-order complex formation. This study implies the amplification of immunity signaling by TIRs and the connection of defense to the general priming of plants for stress.

41. Tian L, Lu J, Li X: **Differential requirement of TIR enzymatic activities in TIR-type immune receptor SNC1-mediated immunity**. *Plant Physiol* 2022, **190**:2094–2098.

42. * Chodasiewicz M, Kerber O, Gorka M, Moreno JC, Maruri-Lopez I, Minen RI, Sampathkumar A, Nelson ADL, Skirycz A: **2′,3′-cAMP treatment mimics the stress molecular response in Arabidopsis thaliana**. *Plant Physiol* 2022, **188**:1966–1978.
shows that exposure of *Arabidopsis* to 2',3'-cAMP activates stress-related transcriptional reprogramming

43. Bartsch M, Gobbato E, Bednarek P, Debey S, Schultze JL, Bautor J, Parker JE: **Salicylic acid–independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in Arabidopsis immunity and cell death is regulated by the monooxygenase FMO1 and the nudix hydrolase NUDT7**. *Plant Cell* 2006, **18**: 1038–1051.

44. Wagner S, Stuttmann J, Rietz S, Guerois R, Brunstein E, Bautor J, Niefind K, Parker Jane E: **Structural basis for signaling by exclusive EDS1 heteromeric complexes with SAG101 or PAD4 in plant innate immunity**. *Cell Host Microbe* 2013, **14**:619–630.

45. Lapin D, Kovacova V, Sun X, Dongus JA, Bhandari D, von Born P, Bautor J, Guarneri N, Rzemieniewski J, Stuttmann J, *et al.*: **A coevolved EDS1-SAG101-NRG1 module mediates cell death signaling by TIR-domain immune receptors**. *Plant Cell* 2019, **31**:2430–2455.

46. Bhandari DD, Lapin D, Kracher B, von Born P, Bautor J, Niefind K, Parker JE: **An EDS1 heterodimer signalling surface enforces timely reprogramming of immunity genes in Arabidopsis**. *Nat Commun* 2019, **10**:772.

47. * Dongus JA, Bhandari DD, Penner E, Lapin D, Stolze SC, Harzen A, Patel M, Archer L, Dijkgraaf L, Shah J, *et al.*: **Cavity surface residues of PAD4 and SAG101 contribute to EDS1 dimer signaling specificity in plant immunity**. *Plant J* 2022, **110**:1415–1432.
Mutational analysis of *Arabidopsis* PAD4 and SAG101 identifies requirements on the EP domain side for EDS1 heterodimer association with RNLs from the ADR1 and NRG1 groups.

48. ** Bayless AM, Chen S, Ogden SC, Xu X, Sidda JD, Manik MK, Li S, Kobe B, Ve T, Song L, *et al.*: **Plant and prokaryotic TIR domains generate distinct cyclic ADPR NADase products**. *Sci Adv* 2023, **9**.
By analyzing cross-signaling by TIRs from bacteria and plants in innate immunity, the study shows that these TIRs have overlapping sets of products. Also, this work demonstrates that TNL Recognition of Peronospora parasitica 1 (RPP1) and the conserved plant TIR-only *Bd*TIR have quantitatively different patterns of enzymatic products.

49. ** Hulin MT, Hill L, Jones JDG, Ma W: **Pangenomic analysis reveals plant NAD+ manipulation as an important virulence activity of bacterial pathogen effectors**. *Proc Natl Acad Sci USA* 2023, **120**, e2217114120.
reveals that bacterial pathogens have a range of effectors to manipulate NAD+ and nucleotide metabolism in *Arabidopsis*

50. * Manik MK, Shi Y, Li S, Zaydman MA, Damaraju N, Eastman S, Smith TG, Gu W, Masic V, Mosaiab T, *et al.*: **Cyclic ADP ribose isomers: production, chemical structures, and immune signaling**. *Science* 2022, **377**.
Defines the structures of TIR-catalyzed cADPR products produced by TIRs and demonstrates functional differences in plant and bacterial cADPR isomers.

51. * Eastman S, Smith T, Zaydman MA, Kim P, Martinez S, Damaraju N, DiAntonio A, Milbrandt J, Clemente TE, Alfano JR, *et al.*: **A phytobacterial TIR domain effector manipulates NAD+ to promote virulence**. *New Phytol* 2022, **233**:890–904.
Demonstrates the enzymatic activity of a TIR from a plant pathogen and overlapping enzymatic products with plant TIR domains.

52. Drenichev MS, Bennett M, Novikov RA, Mansfield J, Smirnoff N, Grant M, Mikhailov SN: **A role for 3′-O-β-D-ribofuranosyladenosine in altering plant immunity**. *Phytochemistry* 2019, **157**:128–134.

53. ** Jacob P, Hige J, Song L, Bayless A, Russ D, Bonardi V, El Kasmi F, Wünsch L, Yang Y, Fitzpatrick Connor R, *et al.*: **Broader functions of TIR domains in Arabidopsis immunity**. *Proc Natl Acad Sci USA* 2023, **120**, e2220921120.
Uses pathogen growth assays and genetic dissection of TIR signaling pathways to establish a role for the TIR-containing SADR1 protein in limiting pathogen spread throughout plant tissues in an EDS1-independent manner. Also, this study defines 2′/3′-O-β-D-ribofuranosyladenosine as a biochemical marker of TIR activity.

54. Duxbury Z, Wang S, MacKenzie CI, Tenthorey JL, Zhang X, Huh SU, Hu L, Hill L, Ngou PM, Ding P, *et al.*: **Induced proximity of a TIR signaling domain on a plant-mammalian NLR chimera activates defense in plants**. *Proc Natl Acad Sci USA* 2020, **117**:18832–18839.

55. Lapin D, Johanndrees O, Wu Z, Li X, Parker JE: **Molecular innovations in plant TIR-based immunity signaling**. *Plant Cell* 2022, **34**:1479–1496.

56. * Johanndrees O, Baggs EL, Uhlmann C, Locci F, Läßle HL, Melkonian K, Käufer K, Dongus JA, Nakagami H, Krasileva KV, *et al.*: **Variation in plant Toll/Interleukin-1 receptor domain protein dependence on ENHANCED DISEASE SUSCEPTIBILITY 1**. *Plant Physiol* 2023, **191**:626–642.
Surveys the diversity of angiosperm TIR proteins and identifies novel EDS1-independent plant TIR signaling activity.

57. Bjornson M, Pimprikar P, Nürnberger T, Zipfel C: **The transcriptional landscape of Arabidopsis thaliana pattern-triggered immunity**. *Nat Plants* 2021, **7**:579–586.

58. Cui H, Gobbato E, Kracher B, Qiu J, Bautor J, Parker JE: **A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in Arabidopsis immunity**. *New Phytol* 2017, **213**:1802–1817.

59. ** Jacob P, Hige J, Dangl JL: **Is localized acquired resistance the mechanism for effector-triggered disease resistance in plants?** *Nat Plants* 2023, **9**:1184–1190.
Proposes a new model of how NLR-effector-triggered immunity arrests pathogen spread.

60. Toshchakov VY, Neuwald AF: **A survey of TIR domain sequence and structure divergence**. *Immunogenetics* 2020, **72**:181–203.

61. Meyers BC, Morgante M, Michelmore RW: **TIR-X and TIR-NBS proteins: two new families related to disease resistance TIR-NBS-LRR proteins encoded in Arabidopsis and other plant genomes**. *Plant J* 2002, **32**:77–92.

62. Zhang Y-M, Xue J-Y, Liu L-W, Sun X-Q, Zhou G-C, Chen M, Shao Z-Q, Hang Y-Y: **Divergence and conservative evolution of XTNX genes in land plants**. *Front Plant Sci* 2017, **8**.

63. Dodds PN, Lawrence GJ, Ellis JG: **Six amino acid changes confined to the leucine-rich repeat β-Strand/β-Turn motif determine the difference between the P and P2 rust resistance specificities in flax**. *Plant Cell* 2001, **13**:163–178.

64. Collier SM, Hamel L-P, Moffett P: **Cell death mediated by the N-terminal domains of a unique and highly conserved class of NB-LRR protein**. *MPMI (Mol Plant-Microbe Interact)* 2011, **24**:918–931.

65. Borhan MH, Holub EB, Kindrachuk C, Omidi M, Bozorgmanesh-Frad G, Rimmer SR: **WRR4, a broad-spectrum TIR-NB-LRR gene from Arabidopsis thaliana that confers white rust resistance in transgenic oilseed brassica crops**. *Mol Plant Pathol* 2010, **11**:283–291.

66. Gantner J, Ordon J, Kretschmer C, Guerois R, Stuttmann J: **An EDS1-SAG101 complex is essential for TNL-mediated immunity in Nicotiana benthamiana**. *Plant Cell* 2019, **31**:2456–2474.

67. Schultink A, Qi T, Lee A, Steinbrenner AD, Staskawicz B: **Roq1 mediates recognition of the Xanthomonas and Pseudomonas effector proteins XopQ and HopQ1**. *Plant J* 2017, **92**:787–795.