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TIR-domain enzymatic activities at the heart of plant immunity

Federica Locci[1], Junli Wang[1] and Jane E. Parker[1,2]

Abstract

Toll/interleukin-1/resistance (TIR) domain proteins contribute to innate immunity in all cellular kingdoms. TIR modules are activated by self-association and in plants, mammals and bacteria, some TIRs have enzymatic functions that are crucial for disease resistance and/or cell death. Many plant TIR-only proteins and pathogen effector-activated TIR-domain NLR receptors are NAD^+ hydrolysing enzymes. Biochemical, structural and functional studies established that for both plant TIR-protein types, and certain bacterial TIRs, NADase activity generates bioactive signalling intermediates which promote resistance. A set of plant TIR-catalysed nucleotide isomers was discovered which bind to and activate EDS1 complexes, promoting their interactions with co-functioning helper NLRs. Analysis of TIR enzymes across kingdoms fills an important gap in understanding how pathogen disturbance induces TIR-regulated immune responses.

Addresses

[1] Department of Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, Cologne, 50829, Germany

[2] Cologne-Düsseldorf Cluster of Excellence on Plant Sciences (CEPLAS), 40225, Düsseldorf, Germany

Corresponding author: Parker, Jane E. (parker@mpipz.mpg.de)

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Disease resistance, Cell death, TIR protein, TNL receptor, NADase, EDS1, Helper NLR, Host-pathogen interaction.

Introduction

Animals and plants are infected by a wide range of pathogens that have evolved virulence strategies to disable host defence pathways and divert metabolism in order to grow. To counter pathogen attack, hosts deploy innate immune receptors at the cell surface and inside cells which detect pathogen molecules or damage and trigger disease resistance mechanisms. Unlike mammals, plants rely entirely on their innate immune receptor repertoires to combat disease [1,2]. This is reflected in the numbers and diversity of plant genes encoding cell-surface pattern recognition receptors (receptor-like kinases; RLKs or receptor-like proteins; RLPs) conferring pattern-triggered immunity (PTI), and intracellular nucleotide-binding/leucine-rich repeat (NLR) receptors conferring pathogen effector-triggered immunity (ETI). Effectors delivered by pathogen strains and effector-sensing host NLRs can be highly variable, driven by host-pathogen co—evolutionary cycles of defence and counter-defence [3].

Studies in *Arabidopsis* show that defence signalling cascades triggered by PTI and ETI receptor systems cross-potentiate each other to mediate a full immune response and ETI-associated localized host cell death (the hypersensitive response) [4,5]. Consistent with this model, PTI and ETI employ a network of NLR proteins (called helper NLRs) for immunity signalling downstream of pathogen detection [3,6]. Also, PTI and ETI converge on the same machineries - such as NADPH oxidases producing reactive oxygen species (ROS), mitogen-activated protein (MAP) kinase cascades, Ca^2-permeable channels, and Ca^{2+}-decoding kinases and transcription factors, to mobilize defences [4,5,7—10].

Here we consider the emerging roles of Toll/Interleukin-1 Receptor (TIR)-domain protein enzymes as mediators of immunity signalling and as PTI-ETI 'connectors' in plants. TIR-domain NADase modules regulating cell death were first discovered and biochemically characterized in mammals and bacteria [11,12]. Studies in plants revealed that TIR-domain enzymatic modules also contribute to PTI and ETI [13—17]. We discuss newly defined biochemical principles and signalling functions of plant TIR-domain enzymes and some interesting mechanistic parallels, but also distinctions, with mammalian and bacterial immune systems.

Insights from a mammalian TIR NADase enzyme regulating cell death

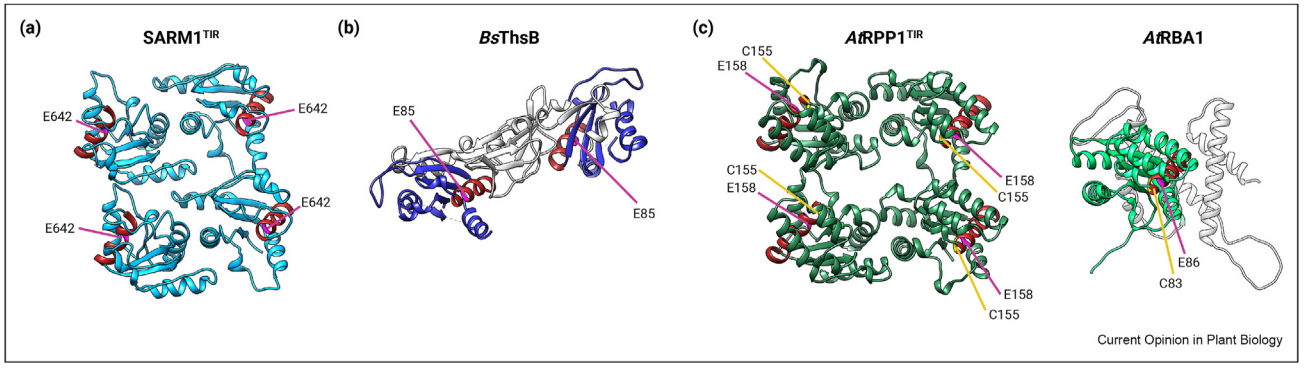
In animals, TIR domains are well known for their roles as non-enzymatic adaptors which, via TIR—TIR

Abbreviations

RLKs	receptor-like kinases
RLPs	receptor-like proteins
PTI	pattern-triggered immunity
NLRs	nucleotide oligomerization domain (NOD) leucine-reach repeats
ETI	effector-triggered immunity
HR	hypersensitive response
TIR	Toll/Interleukin-1 Receptor
SARM1	human protein sterile alpha and Toll/ interleukin-1 receptor motif-containing 1
$SARM1^{TIR}$	TIR domain of human SARM1
NMN	nicotinamide mononucleotide
NAD^+	Nicotinamide Adenine Dinucleotide +
cADPR	cyclic adenosine diphosphate-ribose
TNL	TIR - nuclear-binding site (NBS) - leucine-rich repeats (LRRs)
PAMPs	pathogen associated molecular patterns
CC/HeLo	coiled coil HET-S/LOP-B domain
EDS1	enhanced disease susceptibility 1
PAD4	phytoalexin deficient 4
SAG101	senescence associated gene 101
ADR1	activated disease resistance 1
NRG1	N requirement gene 1
RPP1	resistance to *Peronospora parasitica* 1
ATR1	*Arabidopsis thaliana* Recognized 1
di-ADPR	ADP-ribosylated ADPR
ADPr-ATP	ADP-ribosylated adenosine tri-phosphate
pRib-AMP/pRib-ADP	2'-(5''-phosphoribosyl)-5'-adenosine mono-/ di-phosphate

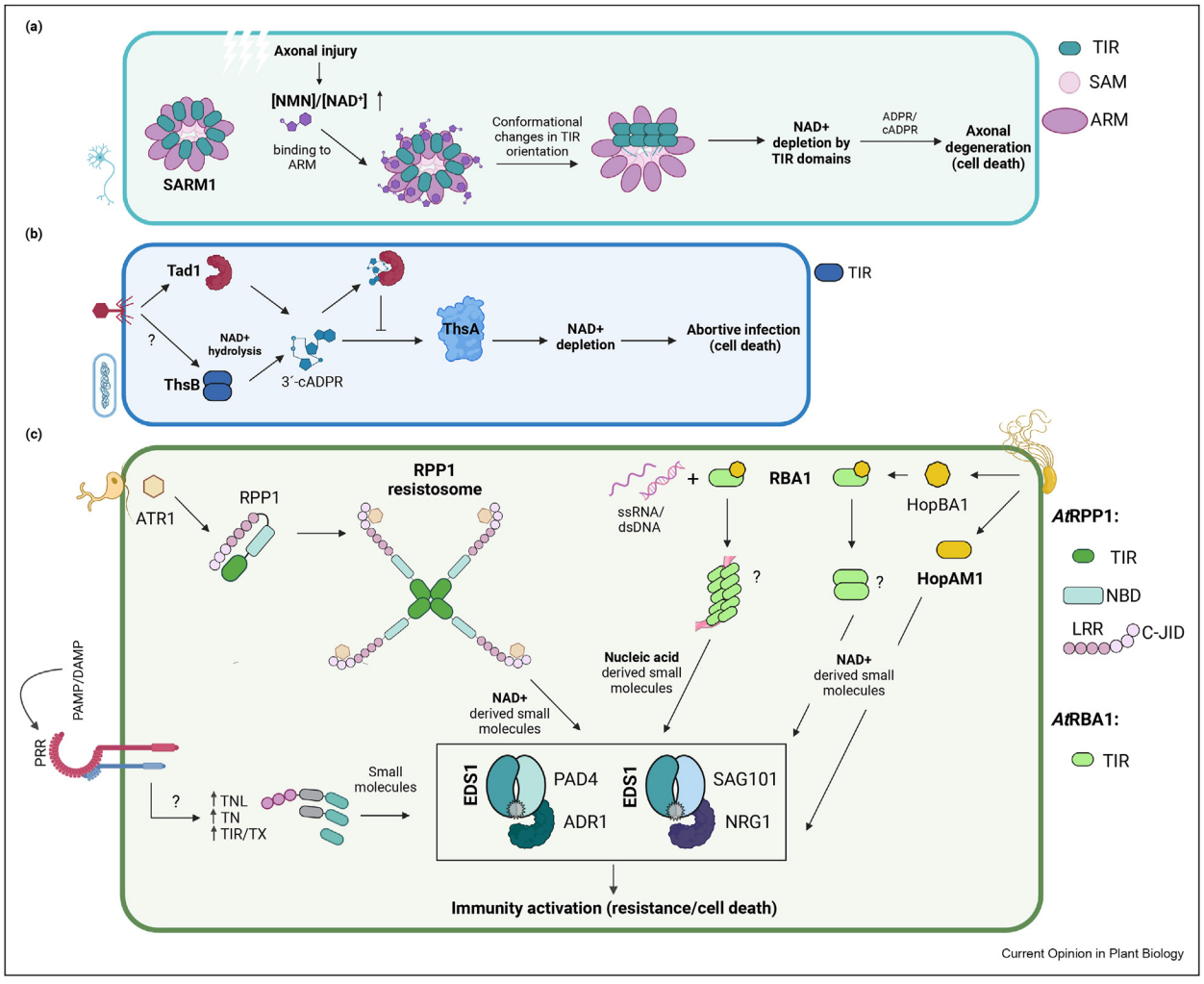
interactions, transduce cell-surface TIR-containing immune receptor activation to intracellular signalling complexes mediating inflammatory responses and/or cell death [18,19]. By contrast, the human SARM1 protein, which regulates neurodegeneration, functions as a nicotinamide adenine dinucleotide$^+$ (NAD^+) hydrolysing enzyme [11]. Self-association of the $SARM1^{TIR}$ domains initiates NAD^+ cleavage activity which promotes axonal cell death [11] (Figure 1a, Figure 2a). Under non-induced conditions, Armadillo (ARM) domains of SARM1 form an octamer configuration which prevents its eight TIR domains from interacting with each other [20]. A cellular metabolic change increases nicotinamide mononucleotide (NMN) levels which out-compete ARM-domain binding of NAD^+ [21,22] (Figure 2a). This causes an allosteric re-orientation of the $SARM1^{TIR}$ octamer, allowing TIR–TIR interactions in a two-stranded assembly with NAD^+ hydrolysing activity (Figure 2a). Current evidence shows that SARM1-induced neuronal cell death is caused by NAD^+ depletion in the cell [23], together with production of an NAD^+-derived product, cyclic adenosine diphosphate-

Figure 1



TIR-domain structures across kingdoms. Examples of Toll/Interleukin-1 (TIR) domain proteins and their structures. **(a)** Cryo-EM structure of a human $SARM1^{TIR}$ representative tetramer from the SARM1 octameric activated form (PDB: 7NAK). Self-association of the TIR-domains confers NAD^+ hydrolysing activity via the conserved catalytic glutamate (E642, magenta). **(b)** Crystal structure of two TIR-domains of anti-phage Thoeris component ThsB from *Bacillus subtilis* (*Bs*) (PDB: 6LHY). A putative active TIR *Bs*ThsB dimer hydrolyses NAD^+ (via conserved E85, magenta). An additional antiparallel β-sheet which is absent in the other TIR proteins is coloured light grey. **(c)** (Left) Cryo-EM structure of activated TIR domains in the *Arabidopsis thaliana* (*At*) RPP1 ($RPP1^{TIR}$) resistosome (PDB: 7CRC) and (right) an AlphaFold model of TIR-only protein *At*RBA1. Effector recognition by TNL receptor *At*RPP1 induces assembly of a tetramer with TIR-domain NADase activity. *At*RBA1 can be a cyclic nucleotide synthase in addition to an NADase. Conserved amino acids in the TIR domains essential for NADase (E158 and E86, magenta) or synthase (C155 and C83, yellow) activities are shown. *At*RBA1 is shown as a monomer because there is no structure of the activated form. The *At*RBA1 C-terminus is shown in dark grey due to low confidence modelling of this domain. In all structures, a central α-helix with conserved catalytic residues is coloured in red. UCSF Chimera software (https://www.rbvi.ucsf.edu/chimera) was used to align the different TIR-domains to the same orientation as $AtRPP1^{TIR}$ (Chain A, bottom right).

Figure 2



TIR-domain enzymatic modes of action in animals, bacteria and plants. Examples of Toll/Interleukin-1 (TIR) domain proteins in (a) animals, (b) bacteria and (c) plants and their roles in promoting defence and/or cell death via NAD^+ hydrolysis. **(a)** Upon axonal damage, an increase of cytosolic NMN with consequent NAD^+ depletion occurs through a mechanism which is still unclear. NMN functions as an allosteric activator of SARM1 by binding to the protein ARM domains. This leads to self-association of the TIR domains into a two-stranded (antiparallel) octameric assembly. The new SARM1 conformation creates TIR domain active sites for NAD^+ hydrolysis. NAD^+ depletion, accompanied by ADPR/cADPR production, leads to axonal cell death. **(b)** Anti-phage Thoeris system in *Bacillus subtilis* (*Bs*). A phage infection-activated *Bs*ThsB TIR-domain protein hydrolyses NAD^+ to produce a cADPR isomer (3′-cADPR) which is sensed by the cell death "executioner" NADase protein *Bs*ThsA, leading to abortive infection. Phages can deliver Tad1 which sequesters *Bs*ThsB-derived 3′-cADPR, inhibiting the activation of *Bs*ThsA. **(c)** In *Arabidopsis thaliana*, perception of the *Hpa* effector ATR1 by TNL receptor RPP1 induces assembly of a tetrameric resistosome with TIR-domain NADase activity. This generates NAD^+-derived small molecules which become bound by EDS1-PAD4 and EDS1-SAG101 dimers to promote their associations with co-functioning helper NLRs (ADR1 or NRG1). Some plant TIR-only proteins (e.g., RBA1 recognizing *P. syringae* (*Ps*) effector HopBA1) can also bind double-stranded RNA/DNA *in vitro* and act as a cyclic nucleotide synthetase producing 2′,3′-cNMPs which, probably indirectly, stimulate *EDS1*-dependent defence. Both TIR enzymatic activities are necessary for fully establishing host cell death. *Ps*TIR-domain effector protein HopAM1 hydrolyses NAD^+ but inhibits host immunity and cell death. Plant TIR-encoding genes are induced in PTI, potentially generating small molecules which signal via EDS1 dimers. Figure generated with Biorender. com.

ribose (cADPR) [24] (Figure 2a). SARM1-derived cADPR promotes intra-axonal Ca^{2+} fluxes from intracellular and extracellular calcium stores which contribute to axonal degeneration [24]. Elucidating the $SARM1^{TIR}$ mode of action prompted a redefinition of certain TIR-domain proteins as metabolic regulatory enzymes [12], and laid the path for examining TIR enzymatic activities in other organisms.

TIR NADases conferring immunity in bacteria

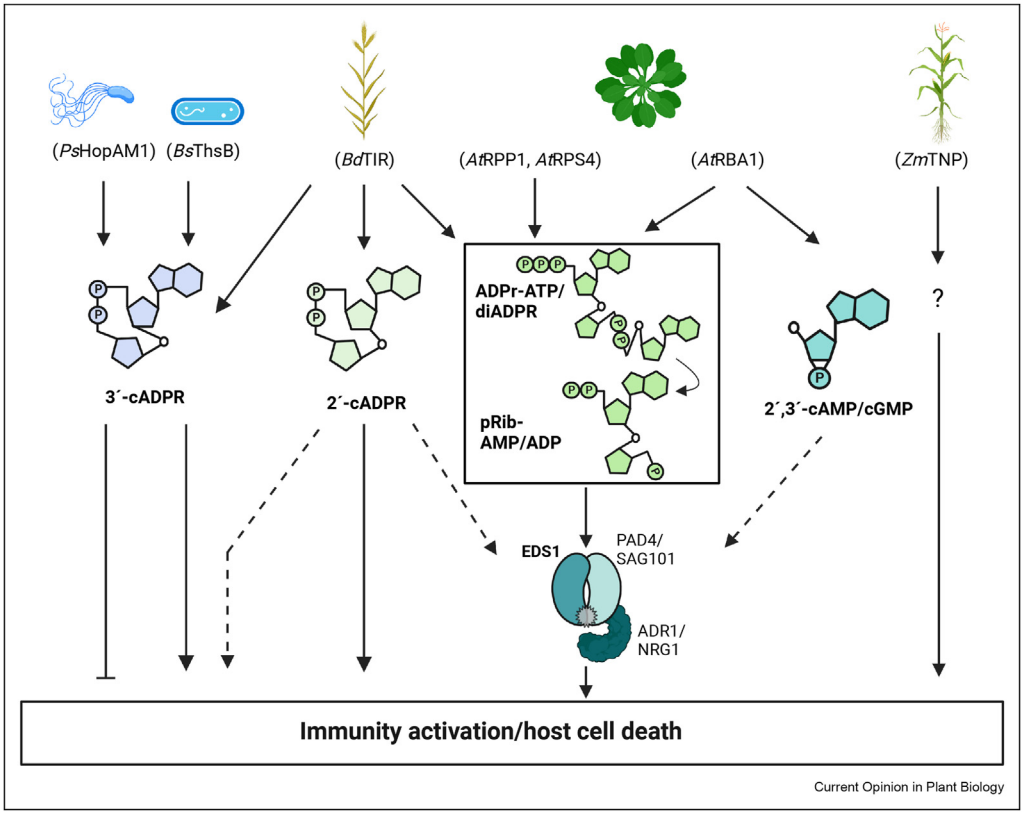
Further insights to the roles of TIR enzymatic modules were gained from studies of bacterial immune responses to bacteriophage infection [12,25]. The occurrence of bacterial cell death following phage infection is known as "abortive infection" which prevents the spread of viral progeny to other cells. Components of a newly

identified anti-phage resistance mechanism called 'Thoeris' are encoded in numerous microbial genomes [26,27]. Thoeris defence involves a sensor TIR-containing protein, ThsB, and a non-TIR sirtuin2-type (SIR2) NADase, ThsA, both with the capacity to hydrolase NAD^+ (Figure 1b, Figure 2b). Analysis of the *Bacillus subtilis Bs*ThsB crystal structure reveals its core TIR-domain and possible activation by dimerization, although the phage stimulating mechanism remains unclear (Figure 1b, Figure 2b). Once activated, *Bs*ThsB generates immune signals by NAD^+ hydrolysis requiring its TIR catalytic site glutamate residue (E45) (Figure 1b) [25]. The *Bs*ThsB TIR NADase enzyme produces an isomer of cADPR that was previously identified by mass spectrometry as a variant (v2)-cADPR [14,25,28]. Recent protein biochemical and structural studies revealed v2-cADPR to be 3′-cADPR (Figure 2b, Figure 3), which binds with high potency to a pocket in ThsA, thereby activating ThsA NADase activity and resulting in cell death [28–30]. Hence, *Bs*ThsB-catalysed NAD^+ derived 3′-cADPR is a bioactive small molecular intermediate and *Bs*ThsA its receptor for bacterial abortive cell death against phage infection (Figure 2b).

TIR enzymes at the host defence - pathogen infection interface

The bacterial Thoeris - phage pathosystem has signatures of host-pathogen coevolution [29]. A small phage protein, Tad1 with homologues in many phage genomes, inhibits *Bs*Thoeris anti-phage defence by binding ThsB-generated 3′-cADPR, thereby sequestering the small molecule away from ThsA to block abortive cell death [28,29] (Figure 2b). Tad1-mediated small molecule

Figure 3



TIR-produced cyclic and non-cyclic nucleotide-based immunity signals. Bacterial *Ps*HopAM1 and *Bs*ThsB TIR proteins catalyse production of 3′-cADPR. *Ps*HopAM1 generated 3′-cADPR inhibits immunity in plant hosts. Plant *Bd*TIR can hydrolyse NAD^+ to 2′-cADPR to induce cell death in plants and bacteria. *Bd*TIR triggers *EDS1*-dependent cell death in dicot plants, indicating that the 2′-cADPR isomer might generate nucleotide molecules that are sensed by EDS1 dimers (dashed arrow). Plant TIR (*At*RBA1, *Bd*TIR) and TNL (*At*RPP1, *At*RPS4) proteins generate ADPr-ATP/diADPR which can undergo hydrolysis to form pRib-AMP/ADP. The box surrounds two different ADP-ribosylated nucleotide forms so far identified to bind to EDS1 dimer C-terminal domain pockets: ADPr-ATP/diADPR are bound by EDS1-SAG101 dimers, and pRib-AMP/ADP by EDS1-PAD4, to conformationally activate helper NLR (respectively, NRG1 and ADR1) mediated host cell death and pathogen resistance. Since pRib-AMP/ADP likely derive from ADPr-ATP/diADPR, we cannot exclude the possibility that other related bioactive molecules are produced in plants which have not been identified due to their instability and/or limited detection power. *At*RBA1 TIR can also catalyse production of 2′,3′-cAMP/cGMP metabolites *in vitro* and *in vivo*. These cyclic nucleotide signals potentiate immune responses via EDS1, probably indirectly (dashed arrow). An NLR-like protein *Zm*TNP promotes *EDS1*-independent cell death via a catalytic site glutamate in a tobacco transient assay. It is unclear whether *Zm*TNP oligomerizes to synthesize the same or a different suite of nucleotide-based signalling molecules. Figure generated with Biorender. com.

sequestration likely represents an evolutionary step in evading bacterial resistance [27,29]. In this regard, it is striking that bacteria and archaea contain diverse families of NLR-like proteins, some of which bind phage proteins to activate resistance [31]. Bacterial NLR-like fusions with various domains, including TIRs, might have been the early building blocks for NLR immune receptors functioning in plants and mammals, although phylogenetic evidence suggests that plant and metazoan NLR immune receptor architectures arose independently [32,33].

A network of TIR NADases potentiates plant immune responses

In plants, TIR-domain proteins consisting of TIR-only, TIR-NB and TNL modules are important mediators of pathogen immunity [17,34,35]. The TNL receptors constitute a large sub-class of pathogen effector-sensing NLRs conferring ETI in eudicot plants [3,17]. Plant TIR-only forms (such as *Bd*TIR and *At*RBA1) or TIR-domains derived from TNLs (such as *At*RPP1, *At*RPS4, flax (*Linum usitatissimum*) *Lu*L7 and *Nb*Roq1) have been biochemically and structurally characterized [17,34,36,37]. Many of the analysed plant TIR-domains have NADase activity that is dependent on their conserved catalytic site glutamate and a TIR—TIR topology that can be almost perfectly superimposed on the active TIRs of $SARM1^{TIR}$ [13,17] (Figure 1). *In vitro* and *in vivo* assays of a number of plant TIRs showed that these NADases cleave NAD^+ or $NADP^+$ to produce ADPR, cADPR and v-cADPR [13,14]. The v2-cADPR was recently identified as a 3′-cADPR isomer also generated by bacterial TIRs for Thoeris defence, and v-cADPR likely corresponds to *Bd*TIR-generated 2′-cADPR [25,28,29,38].

The cryo-EM structures of pathogen effector-activated TNL receptors *At*RPP1 and *Nb*Roq1 reveal how effector binding to the LRR domains creates a potent NADase enzyme through receptor tetramerization [15,16]. In the induced TNL tetramers, two asymmetrically aligned TIR domain pairs create two NADase catalytic sites [15,36,39,40] (Figure 1c). Therefore, TNL receptors are pathogen-activated NADase enzymes (Figure 2c). Further *in vitro* biochemical analysis of the *At*RPP1 induced oligomer (called a resistosome) indicated that this tetrameric TIR-domain organization creates a combined NADase and ADP/ATP-ribosylation catalytic function generating nucleotide-based signalling intermediates for immunity [39,40] (Figure 2c; Figure 3).

By contrast, *in vitro* testing of the TIR domain from flax L7 TNL receptor or TIR-only protein *AtRBA1*, showed that besides being NADases, these TIRs can display a nuclease/cyclic nucleotide synthetase activity when provided with a nucleic acid substrate [41]. This TIR activity relies on different motifs and TIR—TIR interfaces, leading to a TIR filament-like assembly and generation of the small molecules 2′,3′-cAMP/cGMP (Figure 2c, Figure 3) [41]. Studies suggest that 2′, 3′-cAMP/cGMP are important signals which potentiate plant responses to biotic and abiotic stresses [42,43]. Given evidence that cell-surface receptor signalling in PTI transcriptionally up-regulates various TIRs, TIR-NBs and TNLs [8], it is plausible that a network of induced TIR and TNL enzymatic modules orchestrates PTI - ETI potentiation in *Arabidopsis* [8,9].

Plant EDS1 dimers are receptors for non-cyclic TIR NADase products

Heterodimers formed by the seed plant lipase-like proteins EDS1-SAG101 and EDS1-PAD4, function together with two conserved sub-groups of coiled-coil (CC) domain helper NLRs (respectively, NRG1s and ADR1s), as essential components of TNL and TIR mediated immunity [17,44,45]. Phylogenetic evidence suggests that quite conserved EDS1 and NRG1/ADR1 families arose early in seed plant evolution, post-dating the origins of TIRs and TNLs [35,44]. It was proposed that these new elements, consisting of a fusion between an N-terminal class-3 lipase domain and a unique α-helical bundle C-terminal signalling domain, persist in seed plants because they transduce TIR signals and thus increase functional connectivity between PTI and ETI receptor systems [46]. Whereas TNLs found in dicot species appear to have coevolved with SAG101 and NRG1s principally for ETI, PAD4 and ADR1s occur more widely across seed plants and have a broader role in promoting transcriptional defence downstream of TNL and TIR enzymes [8,9,44,46—49].

In TNL-activated leaves, EDS1-SAG101 dimers form induced complexes specifically with NRG1s, and EDS1-PAD4 dimers with ADR1s [50,51], consistent with EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 constituting distinct immune signalling branches [9,47,48,50]. Structure—function studies of the *Arabidopsis* EDS1 dimers identified similar but non-identical positively charged pocket surfaces formed by the partner C-terminal domains [44,49]. These domains were postulated to bind one or more of the identified TIR/TNL enzymatic products, thereby potentially stabilizing EDS1 dimer interactions with corresponding helper NLRs to execute immunity [45].

The co-expression of NRG1 or ADR1 together with an NADase-active TNL (RPP1) and EDS1-SAG101 or EDS1-PAD4 in insect cells demonstrated that the TNL-induced EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 specific interactions observed in plants could be reconstituted in a non-plant heterologous system [39,40]. Purification and structural analysis of polar small molecules bound by TNL-activated recombinant

EDS1-SAG101 or EDS1-PAD4 dimers led to the discovery of two sets of non-cyclic TIR NADase products [39,40]. It emerged that TIR-only or TNL proteins catalyse an ADP-ribose transfer reaction using NAD^+ or NAD^+ with ATP as substrates to form ADP-ribosylated ADPR (di-ADPR) and ADP-ribosylated ATP (ADPr-ATP), respectively [39]. ADPr-ATP or di-ADPR binding by EDS1-SAG101 dimers at the C-terminal pocket leads to a conformational change in SAG101 which promotes NRG1 association (Figure 3) [39]. Through a similar mode of action, binding of two less bulky TIR NADase products, pRib-AMP and pRib-ADP (2'-(5"-phosphoribosyl)-5′-adenosine mono-/di-phosphate) by the EDS1-PAD4 dimer pocket, induces a conformational change in PAD4 which promotes ADR1 association (Figure 3) [40]. Hence, plant EDS1 dimers are receptors for TIR-generated ribosylated nucleotide signals, thereby linking TNLs and TIR NADase enzymes to helper NLRs to execute immune responses [10].

The above findings raise questions of how TNL and TIR 'activated' EDS1 dimer — helper NLR complexes confer pathogen resistance and host cell death. An autoactive *Arabidopsis* NRG1 oligomer was reported to form a plasma membrane-localized Ca^{2+}-permeable pore or channel, potentially resembling a CNL pentameric resistosome [52]. Therefore, one likely immunity output of TIR/EDS1 dimer-activated helper NLRs is their promotion of Ca^{2+} influx into the cytoplasm [53,54]. A recent study of complexes formed between native expressed EDS1-SAG101 dimers and NRG1 detected oligomeric NRG1 bound to EDS1-SAG101 at the plasma membrane but also a clear nuclear EDS1-SAG101-NRG1 pool in ETI-activated cells [53]. It is possible that EDS1 — helper NLR nuclear complexes help to release Ca^{2+} directly into nuclei, perhaps by forming channels at the nuclear membrane or through a different nuclear action in transcriptional defence [53].

Are TIR-generated bioactive nucleotides shared between kingdoms?

We have described the capacities of plant and bacterial TIRs to produce bioactive cyclic or non-cyclic ADP-ribosylated nucleotide molecules. Do these compounds have dedicated roles that have evolved in particular organisms or is there cross-use of nucleotides between kingdoms? Cross-activation between cADPRs produced by different organisms appears to be partial (Figure 3). For example, a 2′-cADPR isomer generated by the monocot plant *Brachypodium distachyon* TIR-only protein *Bd*TIR *in vitro* can bind to phage Tad1 with lower affinity than 3′-cADPR, and weakly activate bacterial ThsA (Figure 3) [29]. However, *Bs*ThsB (producing 3′-cADPR) does not activate a plant *EDS1*-dependent immune response [29,30]. These findings suggest that the enzymatic products and signalling requirements for plant and bacterial Thoeris pathways are different (Figure 3). Supporting this notion, activated TNL receptor *At*RPP1 did not stimulate ThsA-mediated chlorosis or NAD^+-depletion in *Nicotiana benthamiana (Nb)* [30]. Also, the plant pathogen TIR NADase effector *Pseudomonas syringae* (*Ps*) HopAM1 which can synthesize 3′-cADPR did not activate EDS1 - RNL complexes *in vitro* [39,40]. Therefore, input signals for plant and prokaryotic TIR systems are likely not conserved (Figure 3).

The TIRs of SARM1 or bacterial HopAM1 induce *EDS1*-independent necrosis in *Nb* transient expression assays, and therefore the nucleotide intermediates they produce are unlikely to activate canonical plant immune responses. A conserved family of non-canonical TIR/nucleotide-binding/tetra-tricopeptide repeat proteins (called TNPs) are also present in plant lineages that do not contain *EDS1*-family genes [35]. Transient expression of a maize TNP member in *N. tabacuum* produced necrosis independently of *EDS1* but requiring its catalytic site glutamate and intact ATP-ADP binding (p-loop) motif for potential oligomerization [35] (Figure 3). Thus, TNPs might constitute a different NLR-like TIR-domain enzyme. Whether TNPs produce a suite of known or different TIR enzymatic products remains to be seen.

Outlook

Elucidating the possible synthetic routes and biochemical modes of action of TIR-catalysed molecules in mammals, bacteria and plants puts TIR enzymes centre-stage in immunity and cell death signalling across kingdoms. The versatility of TIR enzymatic modules is remarkable [17,30,35,36] and suggests that more nucleotide-based compounds working at the interface between hosts and pathogens will be found. With regard to the plant immunity mechanisms discussed here, a number of questions remain open. One is whether and how induced TNL and TIR-only enzymes might cooperate in defence signal relay, maybe as a defining intersection between PTI and ETI. Would this cooperation involve TIR NADase and cyclic—synthetase activities, given that TIR-only proteins have an additional capacity to process nucleic acid substrates [41]? The half-lives, dynamics and sub-cellular locations of TIR substrates and products, as well as their targets are not known, but will likely shape immunity strength and outputs. Related to this question, phosphorylation of EDS1, the scaffold protein for binding of TIR-generated nucleotide molecules [39,40], was found to impact EDS1 subcellular localisation and function in *Arabidopsis* [55]. How would this fit with potential actions of EDS1 dimer — helper NLR complexes in nuclei or at the plasma membrane? It will also be fascinating to learn how pathogen NADase effectors such as *Pst* HopAM1 disable plant immune responses, possibly by subverting host TIR catalytic defences. It seems that comparisons

between innate immunity systems in plants, bacteria and mammals bring unexpected rewards and added value for deciphering core immunity mechanisms and lineage-specific innovations.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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