

NADases as weapons for both plant pathogens and their hosts

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In the coevolutionary arms race between pathogens and their hosts, an array of weapons has been assembled by both combatants. Plants have evolved a diverse set of innate immune receptors capable of recognizing and defeating microbial pathogens (1, 2). In response, plant pathogens deliver virulence proteins directly into the host cytoplasm to disarm the host immune system, rewire host metabolism, and promote virulence (3, 4). Both organisms evolve new weapons in response to current defenses over evolutionary time (5). In PNAS, Hulin et al. (6) survey pathogen diversity with pangenomics to discover pathogen virulence proteins that share a conserved enzymatic function with their host's immune receptors.

Host Cytoplasm as a Battleground for Plant–Pathogen Interactions

In the last 25 y, major advances have improved our mechanistic understanding of plant disease resistance in both model and agricultural systems (7). One common class of disease resistance genes in plants encode innate immune receptors known as nucleotide-binding, leucine-rich repeat receptors (NLRs) (1). These receptors reside in the plant cytoplasm where they detect pathogen virulence proteins. While pathogen virulence proteins attempt to disarm and reprogram the host, NLRs detect their presence or activity and reactivate immunity. Virulence protein–NLR interactions are the basis of many important disease resistance traits in the field (7).

Toll-interleukin1 receptor (TIR) NADases Regulate Cell Death and Immunity across the Tree of Life

Many plant NLR immune receptors contain a Toll-interleukin1 receptor (TIR) domain. Plant TIRs are signaling domains that activate downstream immune responses via a conserved NADase activity (8). Plant TIR proteins can cleave NAD⁺ and generate small molecules as products (9, 10). TIR domain enzymatic activity was first described in the context of neurodegeneration in animals, and TIR proteins are now known to function in prokaryotic antiphage defense systems (11, 12). In animals, the TIR protein SARM1 (sterile alpha and TIR motif containing 1) degrades NAD⁺ in damaged neurons, promoting cell death via NAD⁺ depletion and metabolic collapse. In plants, TIR immune receptors appear less active as NADases, but produce potent small molecule signals that can be recognized by the enhanced disease resistance 1 (EDS1) complex (13, 14). By binding these small molecule signals, EDS1 can activate downstream effectors of cell death and disease resistance to stop pathogen proliferation. Similarly, in the prokaryotic *Thoeris* system, TIR NADase-produced signals can activate strong

non-TIR NADases to drive NAD⁺ depletion and population-level viral resistance (12).

Plant TIR-Produced Small Molecule Signals Are Diverse

TIRs generate a variety of small molecules. These include v-cADPR (“variant cADPR”), a novel form of cyclic ADPR, first identified as a biomarker for TIR enzymatic function in plants and prokaryotes (10, 15). Subsequently, v-cADPR was structurally characterized and recognized to have at least two forms: 2’cADPR and 3’cADPR, differentiated by the position of the cyclic linkage at the ribose ring (16, 17). However, these molecules do not activate plant immunity via EDS1. The TIR-produced EDS1 signals (pRib-AMP, etc.) are structurally very similar to v-cADPR, sharing a common linkage at the 2’ position on the ribose, but are instead linear molecules (14, 16). 3’cADPR is a TIR-produced immune signal in the prokaryotic *Thoeris* antiviral immune system (16–18). Plant TIRs can also hydrolyze nucleic acids to produce cyclic nucleotides (e.g., 2’,3’ cyclic adenosine monophosphate) as potential immune signals, so the enzymology and array of potential signals produced by TIRs appear complex (19).

TIR NADases Are also Used by Plant Pathogens

However, how does this all relate to the story at hand? Surprisingly, plant pathogens also employ TIR proteins as weapons. Hulin et al. performed a pangenomic analysis to find bacterial virulence proteins that are potential NADases. This was prompted by the finding that the *Pseudomonas syringae* virulence protein HopAM1 is an enzymatically active TIR protein that is injected into the host cytoplasm as one of many Type III effector virulence proteins (20). After screening 35,000 orthogroups, Hulin et al. found 13 putative Type III effector families that had NADase activity. Most of the *Pseudomonas* genomes had at least one putative NADase Type III effector, and many had several. Hulin et al. focused on novel virulence proteins and described HopBY1 as a TIR-like protein family (distinct from HopAM1), that depended on NADase activity to

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promote virulence. So, it seems that manipulation of NAD^+ is a common strategy for *P. syringae* to subvert the plant immune system. Based on our current understanding of animal, prokaryote, and plant systems, TIRs can both deplete NAD^+ and generate small molecule signals. Hulin et al. investigated both signaling and NAD^+ depletion hypotheses to better understand the role of the TIR virulence protein HopBY1 (Fig. 1).

TIR-Produced Small-Molecule Immune Regulators?

The first plant pathogen TIR virulence protein described was HopAM1 (20). HopAM1 has a TIR-like fold and a conserved

putative catalytic glutamate that is required to suppress plant defense. HopAM1 was found to produce 3' cADPR, which has been proposed to be a plant immune suppressor (16). As characterized plant TIRs primarily produce 2' cADPR, it seems plausible that 2' cADPR and 3' cADPR could have opposing functions in plant immunity: Plant 2' cADPR would promote defense and prokaryotic 3' cADPR would inhibit defense. However, Hulin et al. find that HopBY1 actually produces the same 2' cADPR that is associated with plant immune receptors. This complicates correlative models where 2' cADPR was "the plant v-cADPR" and 3' cADPR was "the prokaryotic v-cADPR". While 3' cADPR functions as a TIR-produced signal in the prokaryotic *Thoeris* system, its function in plant cells has not been directly tested. Thus, HopBY1 challenges our

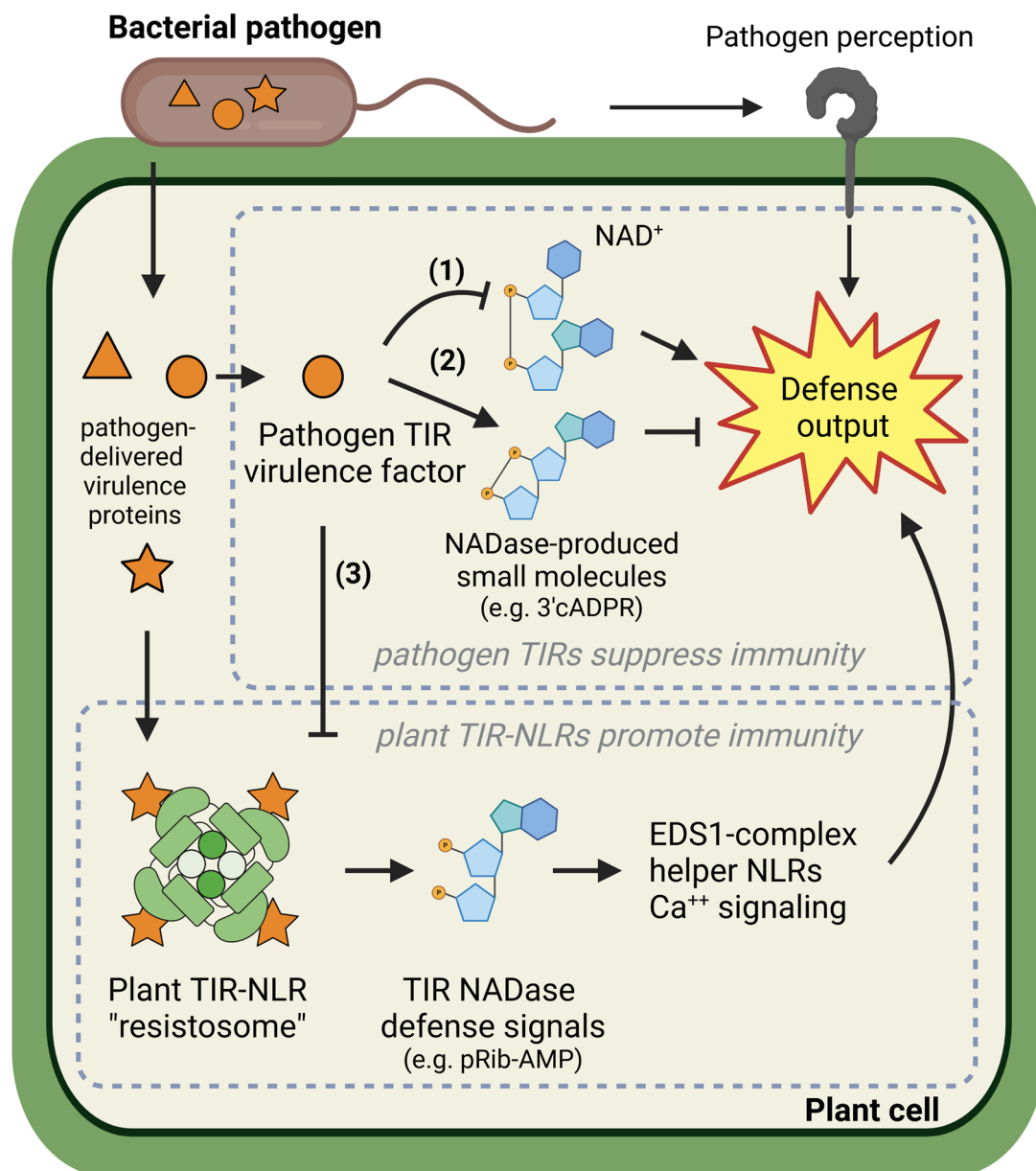


Fig. 1. Plant pathogen NADase virulence proteins suppress host immunity. Extracellular microbes are detected by plasma membrane receptors and result in the activation of diverse defense outputs that collectively generate disease resistance. Adapted pathogens, e.g., *P. syringae*, are able to suppress defenses, typically by the delivery of cytoplasmic virulence proteins (orange shapes). Some virulence proteins (orange star) can be recognized by plant NLR immune receptors and result in immune activation (*Bottom Inset*). TIR-NLR immune receptors oligomerize into a tetrameric "resistosome" and their TIR domains (green circles) produce small molecule signals to signal downstream. Hulin et al. have discovered that some pathogen virulence proteins (orange circle) are NAD^+ -manipulating enzymes that are structurally similar to the TIR NADase domains of plant TIR-NLRs (*Top Inset*). The mechanisms of NADase virulence proteins are unknown, but possible hypotheses include (1) NAD^+ depletion, (2) the NADase-dependent production of small molecule defense inhibitors, or (3) inhibition of TIR-NLR activity or signaling.

hypotheses for v-cADPR function and reinforces the importance of examining a diversity of TIR proteins and developing assays that directly test TIR-produced small molecules in vivo.

NAD⁺ Depletion as a Virulence Mechanism?

Metabolic collapse from NAD⁺ depletion is proposed to be the cell death mechanism in both animal SARM1 neurodegeneration and prokaryotic Thoeris antiviral TIR pathways. Hulin et al. found that HopBY1 also lowers the level of NAD⁺ in plant cells. In contrast, HopAM1 did not appreciably deplete NAD⁺ in planta. NAD⁺ depletion was previously observed in planta after ectopic overexpression of prokaryotic TIRs (18). Hulin et al. find that HopBY can deplete NAD⁺ even when delivered by *Pseudomonas* at biologically relevant levels, so this is not an overexpression artifact. Thus, pathogen-induced NAD⁺ depletion by HopBY1 is at least a plausible virulence mechanism, although the immune consequences of NAD⁺ depletion in plants are not understood. NAD⁺ depletion could increase virulence via a general dysregulation of metabolism or perhaps via downregulating a specific NAD⁺-dependent immune function. Hulin et al. hypothesize that pathogen TIR enzymatic activity could compete with plant TIRs by degrading shared substrates, potentially NAD⁺. Alternatively, they propose that pathogen TIRs could cleave plant TIR enzymatic products, thus blocking activation of immunity.

demonstrated, so perhaps HopBY1 was recruited as a *P. syringae* Type III effector for a similar function (21). The human pathogen *Brucella abortus* uses TIR proteins to disrupt host NAD⁺ homeostasis as it infects human cells, raising the possibility of shared mechanisms across diverse hosts (22).

NAD⁺ as a Common Target for Diverse Virulence Strategies

Researchers have characterized virulence proteins with NAD⁺-related mechanisms that appear structurally and functionally distinct from TIRs. AvrRxo1 from the plant pathogen *Xanthomonas oryzae* is reported to phosphorylate NAD⁺ on the 3' position of the ribose to generate 3'-NADP (Nicotinamide adenine dinucleotide phosphate) (23). Intriguingly, this is the same position as the cyclic linkage in 3'cADPR produced by HopAM1. Canonical NADP⁺ is phosphorylated on the 2' position of the ribose. 3'-NADP⁺ was previously only reported as a synthetic inhibitor of NAD⁺-dependent enzymes (23). A NUDIX hydrolase virulence protein called AvrM14 from a fungal plant pathogen can interfere with host NAD⁺-capping of mRNA, potentially to posttranscriptionally regulate gene expression (24). The other NADase families discovered by Hulin et al. may reveal additional novel virulence strategies.

Hulin et al.'s research focuses our attention on NAD⁺ as a molecule of interest in the battle between plants and pathogens. While plant TIR immune receptors promote disease resistance, pathogens have recruited TIR proteins to promote virulence via an unknown mechanism. Beyond plant-pathogen interactions, we see hosts and pathogens across the tree of life manipulating host NAD⁺ to gain an edge over their opposition. By understanding the molecular mechanisms of the weapons that pathogens and their hosts use in nature, scientists hope to discover new ways to improve agriculture and human health.

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Hulin et al. found that HopBY1 is similar to bacterial proteins predicted to be delivered by the Type VI secretion system. Prokaryotic Type VI systems inject toxins directly into competing microbes and sometimes into eukaryotic hosts. NAD⁺ depletion as a competitive Type VI strategy has been

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