REVIEW SUMMARY

INNATE IMMUNITY

Shared TIR enzymatic functions regulate cell death and immunity across the tree of life

Kow Essuman, Jeffrey Milbrandt, Jeffery L. Dangl, Marc T. Nishimura*

BACKGROUND: Diverse organisms, from archaea and bacteria to plants and humans, use receptor systems to recognize both pathogens and dangerous self-derived or environmentally derived stimuli. These intricate, well-coordinated immune systems, composed of innate and adaptive components, ensure host survival. In the late 20th century, researchers identified the Toll/interleukin-1/resistance gene (TIR) domain as an evolutionarily conserved component of animal and plant innate immune systems. Today, TIR-domain proteins are known to be broadly distributed across the tree of life. The TIR domain was first recognized as an adaptor for the assembly of macromolecular signaling complexes in mammalian innate immune pathways. Work on axon degeneration in animals—as well as on plant, archaeal, and bacterial immune systems—has uncovered additional enzymatic activities for TIR domains.

ADVANCES: Mammalian axons initiate a self-destruct program upon injury and during disease that is mediated by the sterile alpha and TIR motif containing 1 (SARM1) protein. The SARM1 TIR domain enzymatically consumes the essential metabolic cofactor nicotinamide adenine dinucleotide (NAD^+) to promote axonal death. Identification of the SARM1 NAD^+-consuming enzyme (NADase) revealed that TIR domains can function as enzymes. Given the evolutionary conservation of TIR domains, studies investigated whether the SARM1 TIR NADase was also conserved. Indeed, bacteria, archaea, and plant TIR domains possess NADase activity. In prokaryotes, TIR NADase activity is found in an ancient antiphage immune system. In plants, identification of TIR NADase activity and linkage of TIR enzymatic products to downstream signaling components addressed the question of how nucleotide-binding, leucine-rich repeat (NLR) receptors trigger hypersensitive cell death during an immune response. Studies in plants show that their TIR domains can cleave nucleic acids and possess 2′,3′ cyclic adenosine monophosphate (2′,3′-cAMP) and 2′,3′ cyclic guanosine monophosphate (2′,3′-cGMP) synthetase activity that aids cell death programs in plant innate immunity. Thus, TIR domains constitute an ancient family of enzymes that are activated in immune and cell death pathways.

OUTLOOK: The discovery of TIR-domain enzyme activities carries implications for innate immunity and neurodegeneration. The identification of the SARM1 NADase defined a drug target for a wide number of neurodegenerative diseases that is being exploited in both preclinical and clinical studies. Hyperactive mutations in the SARM1 NADase have been discovered in amyotrophic lateral sclerosis (ALS) patients. Future work will seek to clarify the contribution of the SARM1 axon degeneration pathway to ALS pathogenesis. NAD^+ biology influences cellular processes from metabolism to DNA repair to aging. How TIR enzymes influence the NAD^+ metabolome and its associated pathways in bacteria, archaea, plants, and animals will be an exciting area for upcoming investigation. The discovery of the diversity of TIR enzymatic products is revealing signaling pathways across kingdoms. Discovery of TIR enzymatic function in plants and animals may yet inspire studies of enzymatic functions for Toll-like receptors in animals. We anticipate that cross-kingdom studies of TIR-domain function will guide interventions that will span the tree of life, from treating human neurodegenerative disorders and bacterial infections to preventing plant diseases. ■

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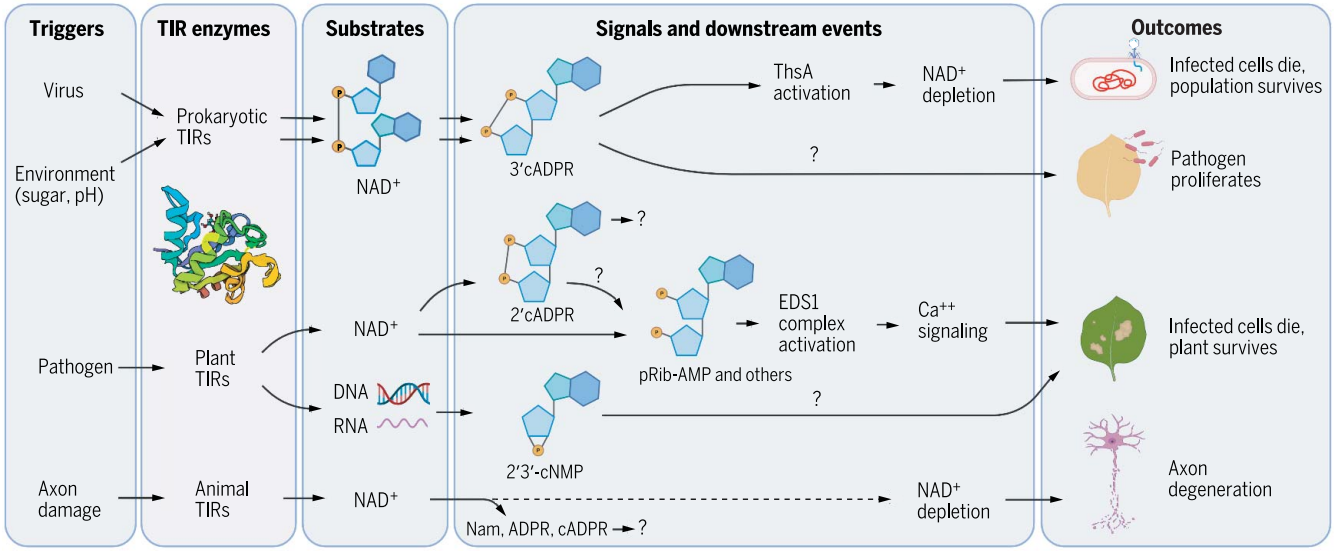

Conserved TIR-domain enzymatic activity. TIR-domain proteins from prokaryotes and eukaryotes cleave NAD^+ into nicotinamide (Nam), ADP-ribose (ADPR), cyclic ADP-ribose (cADPR), isomers of cyclic ADP-ribose (2′ or 3′cADPR), and related molecules [e.g., phosphoribosyl adenosine monophosphate (pRib-AMP)]. Plant TIR domains also possess a nuclease activity, can degrade DNA and RNA, and can function as a 2′,3′-cAMP or 2′,3′-cGMP synthetase. TIR enzymatic activity drives cell death and immune pathways across kingdoms. TIR activity can kill cells directly through NAD^+ depletion or indirectly using enzymatic products as signal molecules. The representative TIR domain structure shown here is Protein Data Bank ID 6O0Q. EDS1, enhanced disease susceptibility 1; ThsA, Thoeris A.

REVIEW

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Shared TIR enzymatic functions regulate cell death and immunity across the tree of life

Kow Essuman[1], Jeffrey Milbrandt[2,3,4], Jeffery L. Dangl[5,6], Marc T. Nishimura[7]*

In the 20th century, researchers studying animal and plant signaling pathways discovered a protein domain that is shared across diverse innate immune systems: the Toll/interleukin-1/resistance gene (TIR) domain. The TIR domain is found in several protein architectures and was defined as an adaptor that mediates protein-protein interactions in animal innate immunity and developmental signaling pathways. However, studies of nerve degeneration in animals—and subsequent breakthroughs in plant, bacterial, and archaeal systems—revealed that TIR domains possess enzymatic activities. We provide a synthesis of TIR functions and the role of various related TIR enzymatic products in evolutionarily diverse immune systems. These studies may ultimately guide interventions that would span the tree of life, from treating human neurodegenerative disorders and bacterial infections to preventing plant diseases.

Innate immune systems of living organisms comprise a set of molecular, cellular, and physiological responses that are rapidly mobilized in response to pathogens and harmful stimuli derived from damaged host cells (*1*, *2*). Studies of animal and plant innate immune responses identified the evolutionarily conserved Toll/interleukin-1/resistance gene (TIR) domain as a common element in proteins involved in innate immunity (Fig. 1) (*3*). In animal innate immune systems, TIR domains provide the signaling motif of Toll-like receptors (TLRs) and interleukin-1 receptors (IL-1Rs) (*4*). These TIR domains function as scaffolds to assemble signaling complexes and transduce defense responses that include activation of nuclear factor κB (NF-κB) signaling and interferon production (*4*). Activation of mammalian TIR protein complexes also stimulates aerobic glycolysis in functional immune cells (*5*, *6*). In plants, TIR domains are typically found at the amino terminus of nucleotide-binding leucine-rich repeat receptor (NLR) proteins (Fig. 1) (*7*, *8*). Canonical plant NLRs possess a nucleotide binding site (NBS), leucine-rich repeat (LRR) domain, and a TIR domain or coiled-coil (CC) domain at their N terminus, giving rise to TIR-NLRs (TNLs) and CC-NLRs (CNLs), respectively. NLRs are the key intracellular receptors in the plant innate immune system (*7*, *8*) and are structurally similar to animal NLR proteins that signal by oligomerizing into inflammasomes (*7*). In response to pathogenic and endogenous danger signals, plant NLRs trigger a rapid immune response that initiates a transcriptional reprogramming that halts pathogen growth and often culminates in localized cell death (*9*). TIR domains are also encoded (more rarely) in some fungal and protozoal genomes where their function remains to be elucidated (*10*, *11*). More recently, TIR domain proteins were discovered to be widely found in bacteria and archaea (Fig. 1) (*12*–*17*). Initial characterization of these bacterial proteins indicated that they function as virulence factors to interrupt TLR signaling in the mammalian host, but recent work also defines intracellular signaling roles in bacterial antiphage defense (*12*, *13*, *16*, *18*). Thus, TIR domain immune function is found across kingdoms in diverse systems.

The original molecular function of TIR domains as signaling scaffolds is well documented for innate immune responses in animals (*4*, *19*–*21*). Recent work, however, demonstrated that TIR proteins are also an ancient enzyme family across all domains of life (*15*, *18*, *22*–*26*). TIR domains can act as nicotinamide adenine dinucleotide (NAD^+) hydrolases (*15*, *22*–*26*). Additionally, at least some plant TIR domains are bifunctional enzymes that are also able to hydrolyze both NAD^+ and DNA and RNA, thus also acting as 2′,3′ cyclic adenosine monophosphate (2′,3′-cAMP) and 2′,3′ cyclic guanosine monophosphate (2′,3′-cGMP) synthetases (*23*, *24*, *27*). In this review, we discuss the biology of TIR domains with an emphasis on enzymatic functions in immune and cell death pathways (summarized in Fig. 1B).

Discovery of the TIR domain

The identification of the TIR domain began with the characterization of three key genes that encode the mammalian IL-1R, *Drosophila* Toll, and the tobacco N protein, which conditions resistance to tobacco mosaic virus (*19*). In the late 1980s, IL-1R was cloned (*28*). A few years later, its cytosolic domain was found to be homologous to the cytosolic domain of *Drosophila* Toll (*29*, *30*). Toll, at that time, was implicated in the dorsoventral patterning developmental process of *Drosophila* that involved signaling by the NF-κB factor Dorsal (*31*, *32*). IL-1 was also shown to activate NF-κB (*29*, *33*). In 1994, researchers studying the plant immune system discovered that the gene *N* encodes an amino-terminal domain with substantial homology to the carboxyl-terminal domain of Toll and IL-1R (*34*). Because of its N-terminal TIR domain, N is a TNL receptor. Its activation by recognition of a protein from the tobacco mosaic virus leads to hypersensitive programmed cell death and restriction of viral replication (*34*). Functions for Toll were later extended beyond development to immune activation during bacterial and fungal infections (*35*–*37*). These discoveries led to the identification of several mammalian TLR proteins harboring TIR domains and a large superfamily of intracellular plant TNL receptors that are widely distributed across dicotyledonous plant genomes (*4*, *7*). Single-domain plant TIR proteins and "TIR-X" proteins (where X is a domain of unknown function) are also common and functional (Fig. 1) (*38*–*40*).

TIR domains in animal immune signaling

The realization that TIR domains function as scaffolds emerged from extensive work in animal innate immune signaling (*19*). In animals, TIR domains are found in Toll and TLRs, their cytosolic adaptor proteins, and the IL-1R family (*4*, *19*) (Fig. 1). TLR receptors are pattern recognition receptors, which detect pathogen- or microbe-associated molecular patterns (PAMPs and MAMPs) and activate downstream signaling pathways (*4*). The detection of PAMPs or MAMPs occurs through the N-terminal domain of TLR dimers at the cell surface or within endosomal membranes (*4*). This triggers TLR C-terminal TIR domain dimerization, recruitment of cytosolic TIR adaptors (*4*, *21*, *41*), and TIR-mediated cooperative assembly of a supramolecular organizing complex (*19*–*21*, *42*, *43*). Complex formation leads to signaling through activation of downstream protein kinases and transcription factors, and then to host inflammatory

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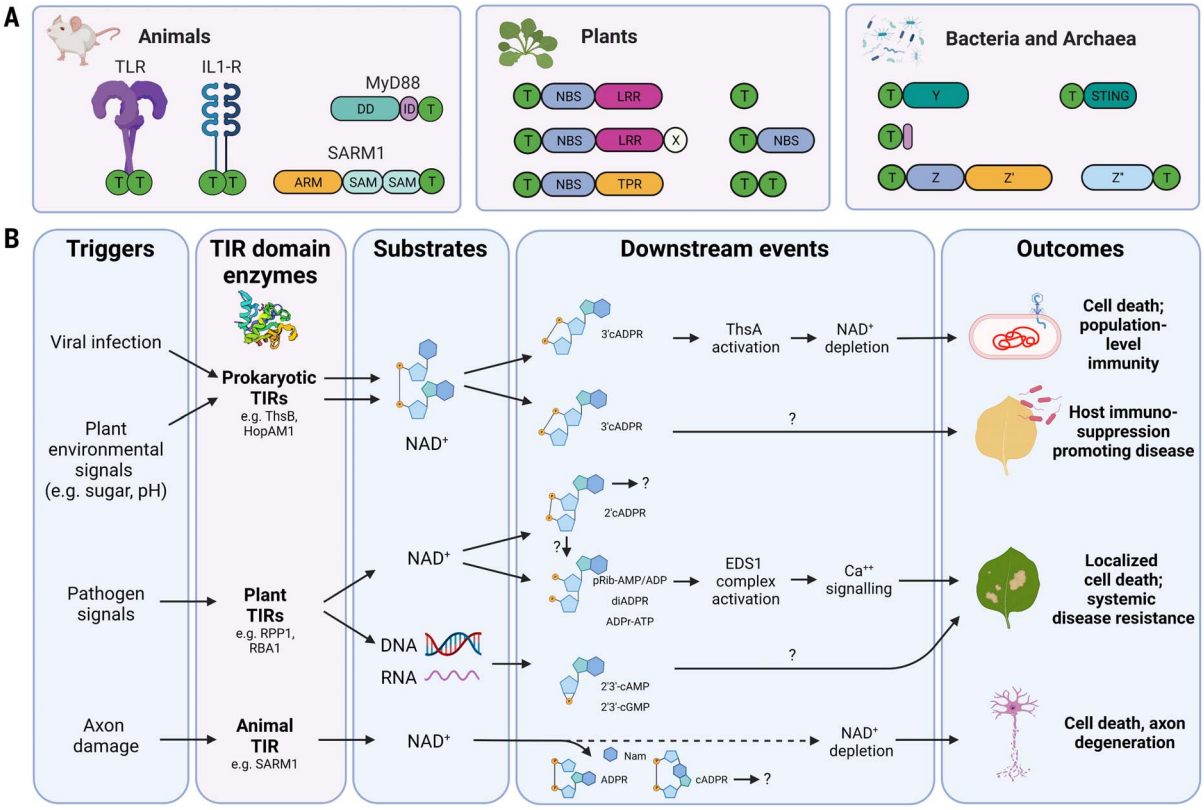

Fig. 1. Diverse TIR domain proteins across the tree of life share related enzymatic functions to regulate cell death and immunity. (**A**) TIR domains (labeled T, green circle) are present in diverse domain configurations from animal, plants, and prokaryotes. Many domain configurations are not shown. Plant genomes encode TIR domain proteins with diverse domain architectures. Bacteria and archaea also possess TIR domain proteins with diverse domain architectures, including TIR domains located at either the N or C termini. Other domains present in prokaryotic TIR proteins that are represented by Y-Z″ in the figure include CC domains, STING, WD 40 repeats, and the Mpr1p Pad1p N-terminal domain. DD, death domain; ID, intermediate domain; MyD88, myeloid differentiation primary response protein 88; TPR, tetratricopeptide repeat. (**B**) Across the tree of life, TIR domains function as enzymes to process nucleotide-containing molecules, either to generate signals or to deplete them from the cell. TIR products or NAD^+ depletion are often associated with beneficial cell death or immune activation.

and defense responses (*4*, *42*, *44*). TIR domains in the IL-1R family share similar intracellular signaling mechanisms as TLRs and influence myriad innate and adaptive immune responses (*4*, *45*–*47*).

Although animal TIR adaptor proteins generally promote TLR signaling, the discovery that the TIR "adaptor" protein, sterile alpha and TIR motif containing 1 (SARM1), inhibits TLR signaling indicated that it might have distinct functions (*48*). Indeed, the *Caenorhabditis elegans* SARM1 ortholog (called TIR-1) has essential roles in both immunity and development (*49*–*51*). TIR-1 is required for resistance to certain fungal and bacterial infections (*49*) and specification of asymmetric odorant receptor expression during neuronal development (*50*). Thus, *C. elegans* TIR-1 function is reminiscent of Toll function in dorsoventral patterning in development and innate immunity (*31*, *35*–*37*). Another distinguishing feature of SARM1 among TIR adaptors is its enzymatic role in driving axon degeneration (*22*, *52*, *53*) (Fig. 2), which laid the groundwork for the discovery of TIR domain enzymatic function across kingdoms.

TIR domains function in neuronal cell death

In the mid-19th century, neurophysiologist Augustus Waller described the degeneration of injured frog hypoglossal and glossopharyngeal nerves (*54*). Wallerian degeneration initially was thought to be a passive wasting of the distal segment of a damaged axon, but this view was challenged by the serendipitous discovery of a naturally occurring spontaneous mutant mouse, the *Wallerian degeneration slow* (*Wld*^{S}) mutant (*55*). In *Wld*^{S} mice, transected distal axons remain intact for several days after injury and continue to conduct action potentials and remain metabolically active (*55*, *56*). The axonal protection observed in the *Wld*^{S} mice is caused by a gain-of-function mutation that results in a fusion protein (Wld^S) composed of portions of the ubiquitination factor UBE4B and nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1) (*56*, *57*). The enzymatic activity of NMNAT1, which converts nicotinamide mononucleotide (NMN) to NAD^+ (Fig. 3), is required for Wld^S to protect axons (*58*). Unraveling the mechanism of this hypermorphic mutation revealed an active, genetically encoded death program in axons after injury (*53*, *59*–*63*).

Genetic screens in *Drosophila* and in mouse dorsal root ganglia neurons identified SARM1 as a required component of a conserved axon death program (*61*, *62*). Subsequent experiments using SARM1 knockout mice showed robust axonal protection comparable to that observed in *Wld*^{S} mice (*56*, *61*, *62*). Transected axons from *Drosophila* SARM1 mutants can remain intact for at least 6 weeks, a duration that approximates the life span of the fly (*61*). Loss of SARM1 delays axon degeneration and improves functional outcomes in several neurodegenerative disease mouse models, including chemotherapeutic and diabetic peripheral neuropathy, traumatic brain injury, glaucoma, retinopathy, and amyotrophic lateral sclerosis (ALS) (*64*–*70*). Loss of SARM1 also blocks axon degeneration in human induced pluripotent stem cell–derived sensory neurons (*71*). In the absence of injury, SARM1 knockout mice display no overt

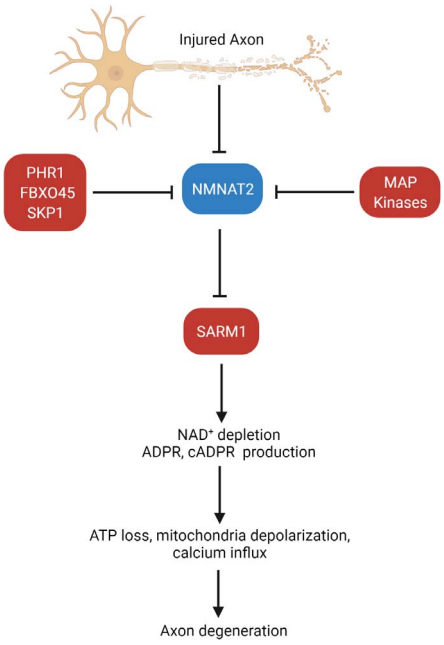

Fig. 2. Working model of the Wallerian axon degeneration pathway. Activation of the Wallerian axon degeneration pathway during injury involves the reduction of axon protective factors, primarily nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2). The level of NMNAT2 is regulated by multiple mitogen-activated protein (MAP) kinases, PAM-Highwire-Rpm-1 (PHR1), F Box protein 45 (FBXO45), and S phase kinase associated protein 1 (SKP1). Low levels of NMNAT2 lead to an increase in NMN and a decrease in NAD^+. Both NMN and NAD^+ bind to SARM1, where a high NMN:NAD^+ ratio is responsible for potent SARM1 NADase enzyme activation. Activated SARM1 NADase cleaves NAD^+ into Nam, ADPR, and cADPR. SARM1-mediated NAD^+ cleavage leads to energetic failure through axonal ATP loss and subsequent mitochondrial depolarization, calcium influx, and, ultimately, axon fragmentation.

phenotype, suggesting that SARM1 inhibition may be an attractive therapeutic strategy for neurodegenerative diseases (*72*–*74*).

SARM1 reveals an enzymatic TIR function

SARM1 is a multidomain protein with an N-terminal regulatory region that contains multiple Armadillo repeats, a tandem sterile alpha motif (SAM) domain that mediates SARM1 multimerization, and a C-terminal TIR domain (*52*, *62*) (Fig. 1). Forced dimerization of the SARM1 TIR domains leads to rapid NAD^+ depletion that is sufficient to trigger axon degeneration (*75*). Because TIR domains function as molecular scaffolds to promote the formation of signaling complexes, it was hypothesized that axon injury promoted dimerization of SARM1 TIR domains that resulted in the interaction and activation of an

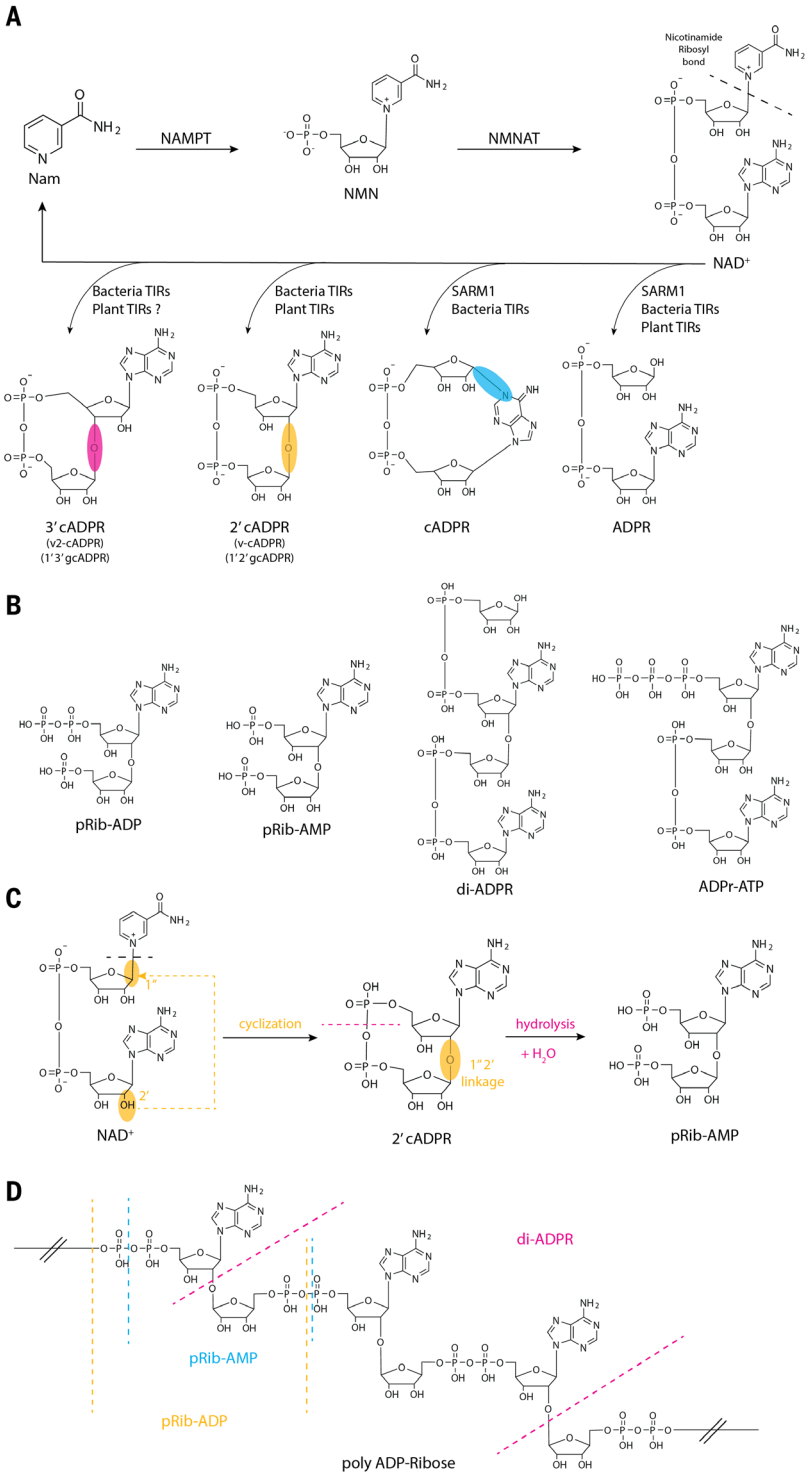

Fig. 3. Select pathways of NAD^+ synthesis and TIR-mediated NAD^+ degradation. (**A**) Nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in NAD^+ biosynthesis, produces NMN from Nam and 5-phosphoribosyl-1-pyrophosphate (PRPP) (not shown). NMNAT synthesizes NAD^+ from NMN and ATP. NAD^+ can be cleaved by prokaryotic and eukaryotic TIR domains. The SARM1 TIR domain cleaves NAD^+ into Nam, ADPR, and cADPR. Bacterial TIRs can cleave NAD^+ into Nam, ADPR, cADPR, and v-cADPRs. Plant TIRs can also cleave NAD^+ into Nam, ADPR, and v-cADPR. Two linkages of v-cADPR have been described: 2′cADPR and 3′cADPR. (**B**) Plant TIRs generate small-molecule signals that activate the EDS1 complex. pRib-AMP and pRib-ADP promote the EDS1-PAD 4 interaction with ADR1, whereas ADPr-ATP and di-ADPR promote EDS1/SAG101 interaction with NRG1. (**C**) 2′cADPR and TIR-derived EDS1 signals share a 1″-2′ ribose-ribose linkage. (**D**) Hypothetical cleavage sites in poly(ADP-ribose) that could produce plant-derived TIR molecules.

NAD^+-consuming enzyme in the axon (*22*, *75*). However, biochemical experiments using purified proteins established that the SARM1 TIR domain itself possesses NAD^+ hydrolase activity (*22*). SARM1 hydrolyzes NAD^+ through a reaction that requires a glutamate residue [Glu^{642} (E642) in human SARM1] in the TIR catalytic pocket (*22*, *24*). This NAD^+ hydrolase activity and conserved glutamate is present in SARM1 homologs from multiple species, including humans, mice, flies, and worms (*22*, *24*, *26*).

SARM1 TIR cleaves NAD^+ at the nicotinamide ribosyl bond to produce nicotinamide (Nam), adenosine diphosphate ribose (ADPR), and cyclic ADPR (cADPR) (*22*, *24*, *26*) (Fig. 3). SARM1 also possesses base-exchange activity that can generate nicotinic acid adenine dinucleotide phosphate (NAADP) (*26*, *76*) as well as modifications of other compounds (*77*). SARM1 activation in axons leads to depletion of NAD^+ and adenosine triphosphate (ATP), followed by calcium influx, mitochondrial depolarization, and axon degeneration (*60*, *75*, *78*, *79*) (Fig. 2). The discovery of the SARM1 NAD^+-consuming enzyme (NADase) reemphasized the importance of NAD^+ in axon degeneration. Later studies showed that the Wld^S functional moiety, NMNAT1, confers axonal protection by inhibiting SARM1-mediated NAD^+ depletion (*80*).

Shared TIR enzymatic function across the tree of life

The discovery that the SARM1 TIR domain is an NAD^+ hydrolase suggested that TIR domains in proteins that are present in different kingdoms might also possess enzymatic activity. The SARM1 TIR domain is phylogenetically and structurally more related to bacterial, archaeal, and plant TIR domains than to TIR domains implicated in animal innate immunity (*14*, *24*). Indeed, purified TIR domain proteins of human TLR4 and other animal TIR adaptors failed to cleave NAD^+ in vitro (*22*, *24*, *81*). This result reinforced phylogenetic analyses and suggested that SARM1 may have functions distinct from other mammalian TIR domains. Biochemical assays using bacterial, archaeal, and plant TIR domains demonstrated that many of them can function as NADases (*15*, *17*, *18*, *23*–*26*). A conserved glutamate is present in plant, bacterial, and archaeal TIR domains and is required for their NAD^+ hydrolase activity (*15*, *16*, *23*, *24*). Other closely related analogs of NAD^+, such as nicotinamide adenine dinucleotide phosphate ($NADP^+$), can be cleaved by SARM1, plant, and bacterial TIR proteins (*15*, *23*, *24*, *76*). TLRs and their adaptors have not been shown to possess TIR enzymatic function thus far, but their ability to form complexes with other enzymes suggests that TIR-associated enzymatic activation may be a conserved mechanism across kingdoms (*6*, *19*, *42*). Moreover, TLRs can activate aerobic glycolysis and metabolic reprogramming (*5*, *6*, *82*). This, coupled with NAD^+ being an indispensable and ubiquitous metabolic factor (*83*), implicates TIRs as indirect mediators of metabolism. Future studies should clarify if mammalian TIRs that lack NAD^+ cleavage activity might still bind NAD^+ and thus also influence signaling pathways in this manner.

Bacterial and archaeal TIR domains are enzymes

TIR domain NADases are present and active in diverse prokaryotes from a range of habitats (*15*–*18*, *25*, *84*). Prokaryotic TIRs also cleave NAD^+ at the nicotinamide ribosyl bond (*15*, *25*) (Fig. 3) but can create multiple types of products. For example, TIR proteins from *Staphylococcus aureus* (TirS) and uropathogenic *Escherichia coli* (TcpC) generate Nam and ADPR (*15*). By contrast, TIR proteins BtpA (from *Brucella*), AbTir (from *Acinetobacter baumannii*), TcpO (from the archaea *Methanobrevibacter olleyae*), and other prokaryotic TIR domains generate a variant of cADPR (v-cADPR) (*15*, *18*, *84*) (Fig. 3). v-cADPR was also detected in gnotobiotic mice colonized with human gut bacterial strains (*17*). Plant TIRs also produce v-cADPR in vitro and in vivo (*23*). Finally, yet another variant of cADPR (v2-cADPR) is produced by the bacterial TIR virulence factor HopAM1 and other bacterial TIR proteins (*17*, *84*, *85*). The cADPR isomers produced by plant TIRs and several bacterial TIRs have the same molecular weight as cADPR but are distinct, as shown by retention times in chromatography experiments (*15*, *18*, *23*, *85*). The first chemical structures of plant and prokaryotic v-cADPR and v2-cADPR molecules indicate that they are circularized by means of ribose-ribose linkages (*84*, *86*) (Fig. 3). v-cADPR is circularized by means of a 1″-2′ glycosidic linkage between riboses (hereafter, 2′cADPR), whereas v2-cADPR is circularized by means of a 1″-3′ glycosidic linkage (hereafter, 3′cADPR) (*84*, *86*). It remains to be seen whether TIRs are capable of other linkages or whether there are isomeric differences between cADPR molecules that share the same linkage.

Bacterial TIR domain proteins were first classified as virulence factors and thought to act by interacting with mammalian host TLRs to disrupt innate immune pathways (*12*, *13*, *87*–*89*). BtpA is a bacterial virulence effector protein from *Brucella abortus* that reduces total intracellular NAD^+, an activity that requires the catalytic glutamate (*89*). Similar strategies are used by plant pathogens. The plant pathogen *Pseudomonas syringae* pv. (pathovar) tomato strain DC3000 encodes a TIR domain–containing virulence protein called HopAM1. HopAM1 virulence phenotypes are dependent on its conserved catalytic glutamate (*84*, *85*). The mechanism by which HopAM1's enzymatic activity suppresses plant immunity remains unknown. One hypothesis is that the HopAM1 TIR competes for a plant TIR substrate that is required to promote immunity. An alternate hypothesis is that HopAM1-produced 3′cADPR, or related enzymatic products, interfere with the host's immune system.

Bacterial TIR enzymes in antiphage defense responses

Global surveys of bacterial genomes revealed that TIR domain–encoding genes are enriched in genomic regions that contain phage defense genes, suggesting that they may function in suppressing phage infections (*16*). One system, Thoeris, comprises an operon that is composed of ThsA and ThsB. ThsA usually encodes an NAD^+ binding and cleavage domain at its N terminus (*18*, *90*). ThsB encodes a TIR-domain protein (*16*). The Thoeris system is found in a wide number bacterial and archaeal genomes and restricts viral replication upon activation (*16*). Mutations of the conserved catalytic glutamate in the TIR domain of ThsB, or of residues that affect NAD^+ binding in ThsA, abolish phage protection (*16*, *18*).

Upon bacteriophage infection, ThsB TIR domains produce a cADPR isomer (presumably 3′cADPR) (*18*, *84*), which then acts as a signaling molecule to activate ThsA (*18*). ThsA consequently consumes bacterial NAD^+, promoting bacterial growth inhibition and/or cell death (Fig. 4). This process of metabolic arrest and cell death, termed abortive infection, is thought to curb the spread of the infecting phage at a population level (*91*, *92*). Abortive infection is reminiscent of the axon self-destruct mechanism that is mediated by SARM1 activation in damaged axons and the hypersensitive cell death response in plants after successful pathogen recognition (*7*, *52*). The discovery that bacterial TIR domain–containing proteins mediate antiphage defense systems is an impressive example of the continuity of TIR immune function, extending from phage defense in bacteria to pathogen responses in plants and animals. In addition to ThsB, other phage defense proteins are TIR-domain NAD^+ hydrolases regulated by cyclic nucleotides (Fig. 4) (*25*, *93*). Similarly, detection of foreign DNA by a prokaryotic Argonaute protein via guide RNAs activates TIR NADase activity in the SPARTA (short prokaryotic Argonaute and TIR-APAZ) immune system (*94*).

TIR domains as enzymes in plant immune receptor function

Plants deploy an intricate innate immune system that prevents disease (*7*, *9*). Initial hypotheses imagined that similar to animal TIR proteins, plant immune receptors containing TIR domains could function through

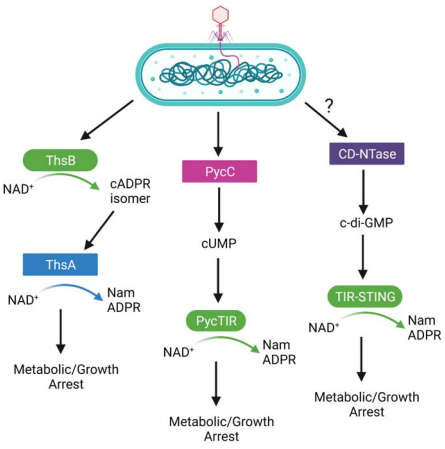

Fig. 4. Bacterial TIR NADases in antiphage, abortive infection defense programs. Shown are the Thoeris (left), Pycsar (middle), and CBASS (cyclic oligonucleotide–based antiphage signaling system) (right) defense systems. In the Thoeris system, bacteriophage infection activates the host ThsB TIR NADase to produce an isomer of cADPR, which then activates ThsA, leading to NAD^+ depletion and subsequent metabolic crisis and growth arrest. In the Pycsar system, phage infection activates the PycC uridylate cyclase to produce cyclic uridine monophosphate (cUMP). cUMP activates the PycTIR NADase effector to promote abortive infection through NAD^+ depletion. In CBASS, CD-NTase (cGAS/DncV-like nucleotidyltransferase) enzymes generate cyclic oligonucleotides. In the example presented here, c-di-GMP is generated by a member of the CD-NTase family. The ci-di-GMP activates the NADase activity of the TIR-STING protein to cause bacterial growth arrest. Although the genomic location of CD-NTase and TIR-STING within "defense islands" suggests a role in bacterial defense, the phages that activate the TIR-STING system remain unknown.

assembly of signaling complexes. The observation that the animal SARM1 TIR domain expressed an enzymatic function prompted the discovery of NAD^+ hydrolase activity in both plant TIR-only proteins and TNL proteins (*23*, *24*, *95*). Although both animal SARM1 and plant TIR domain proteins trigger cell death after activation, they achieve this through distinct mechanisms. SARM1 possesses high enzymatic activity and rapidly depletes the cell of NAD^+, resulting in energetic catastrophe and death (*22*, *23*, *75*). Plant TIRs are less-active enzymes in biochemical assays, and in vivo TIR activity does not result in severe NAD^+ depletion (*23*). This raises the question of how TIR activity results in cell death if it does not induce energetic catastrophe.

Enhanced disease susceptibility 1 (EDS1) is a conserved immune hub required for plant TIR-dependent cell death and disease resistance phenotypes across plant phylogeny (*96*). Downstream of EDS1 is a small set of ancient and conserved resistance to powdery mildew 8 (RPW8)–like NLRs, called RNL "helper NLRs" that are also required for TIR-mediated cell death and immunity (*97*). RNL helper NLRs transduce TIR-EDS1–dependent signals into direct calcium channel formation and subsequent signaling (*98*, *99*). If plant TIRs functioned through direct NAD^+ depletion, one might expect that they would not require downstream signaling components. Indeed, ectopic expression of SARM1 TIR triggers EDS1-independent cell death in plants (*23*). This is consistent with the hypothesis that strong NADases can kill cells through metabolic dysregulation. Most plant TIRs, by contrast, are EDS1 dependent, even though they are enzymatically active in *eds1* mutants, as measured by the production of v-cADPR (*23*). Thus, plant TIR NADase function is upstream of EDS1 (Fig. 5). A class of TIR proteins is encoded in some plant genomes that lack EDS1 (*100*). It is unclear how these EDS1-independent TIRs function or whether they are producing small-molecule signals.

How, then, does TIR enzymatic activity activate downstream signaling? A pair of studies demonstrated a mechanistic link between TIR enzymatic products and EDS1 complex function (*101*, *102*). Huang *et al.* (*101*) identified a small molecule after coexpressing an active TIR enzyme along with the downstream components EDS1 and PHYTOALEXIN DEFICIENT 4 (PAD4) in insect cells (*101*). EDS1 functions as a heterodimer with one of two structurally related proteins, either PAD4 or SENESCENCE-ASSOCIATED GENE 101 (SAG101). EDS1, PAD4, and SAG101 share a similar domain architecture, with an N-terminal lipase-like domain and a C-terminal domain called the "EP domain" for EDS1 and PAD4. SAG101 also contains an EP domain (Fig. 5). The EDS1-SAG101 crystal structure revealed a cavity formed in the heterodimer, and EP domain residues that face this cavity are required for EDS1 complex function (*103*, *104*). By purifying and crystalizing the TIR-activated EDS1-PAD4 complex, Huang *et al.* found electron density in the EP domain pocket consistent with an ADPR-related molecule that they named phosphoribosyl adenosine diphosphate (pRib-ADP) (*101*). TIR activity induces preferential EDS1-PAD4 heterodimer formation followed by recruitment and activation of downstream ACTIVATED DISEASE RESISTANCE (ADR) RNLs in vivo (*104*–*106*). Using an in vitro assay, Huang *et al.* found that synthetic pRib-ADP and a phosphoribosyl adenosine monophosphate (pRib-AMP) can replace TIR activity that drives the interaction of EDS1-PAD4 heterodimers with downstream ADR RNLs (*101*). The putative TIR catalytic glutamate was required both in vitro to drive EDS1 complex oligomerization and in vivo for the TIR-only protein RBA1 (response to the bacterial type III effector protein HopBA1) to promote pRib-AMP accumulation (*38*, *101*). Thus, this study links TIR enzymatic function mechanistically to EDS1 and downstream events, which likely induce RNLs to form active resistosome-like calcium channels (*98*, *99*) (Fig. 5). In an accompanying research article (*102*), plant TIRs were demonstrated to generate ADP-ribosylated ADPR (di-ADPR) and ADP-ribosylated ATP (ADPr-ATP) (Fig. 3B). These molecules are structurally related to pRib-AMP and pRib-ADP but preferentially activate the EDS1-SAG101 heterodimer via a binding site overlapping that of the pRib molecules (*102*). In vitro, the RPS4 TIR domain was capable of producing all four EDS1 complex signals from NAD^+ and ATP (*102*). These results provide a compelling model that explains how PAD4 or SAG101 form specific EDS1 heterodimeric complexes that selectively recruit and signal through the two subclasses of RNL helper NLRs, ADRs, or NRGs, respectively. This results in distinct outputs, one biased toward cell death and the other toward a transcriptional immune output by means of as-yet-unknown mechanisms (*107*).

The plant protein BdTIR activates ThsA in the Thoeris system (*18*). Thus, plant TIRs can produce a conserved signal (or signals) recognized by downstream signaling molecules across a considerable phylogenetic distance. Similar experiments were performed in the opposite direction by expressing bacterial TIRs in plants (*108*). The human inflammasome perceives intracellular pathogen signals and oligomerizes into a wheel-like disc (*109*, *110*). Using this chassis, Duxbury *et al.* generated a hybrid inflammasome that fused TIR domains to the oligomerization domains of an animal inflammasome (*108*). The hybrid receptor with a plant TIR domain activated cell death in planta in a ligand-specific manner. This death was EDS1 dependent, consistent with activation of the endogenous TIR cell death pathway. Similar fusions with bacterial AbTir, however, failed to trigger cell death but did produce measurable v-cADPR (2′cADPR) (*108*). Duxbury *et al.* concluded that TIR NADase activity and v-cADPR production appear necessary, but not sufficient, for plant cell death (but see section "Plant TIR domains also cleave DNA and RNA" for 2′,3′-cNMP synthetase activity).

One outstanding question is the relevance of plant TIR-produced 2′cADPR (Fig. 3). Is this molecule an intermediate in the production of the ligands that drive specific EDS1 complex formation, does it have distinct functions, or is it immunologically irrelevant enzymatic noise? Could it have a function related to the 3′cADPR produced in the plant cell by phytopathogens via HopAM1? The 2′cADPR molecule was originally described as a "biomarker" for TIR

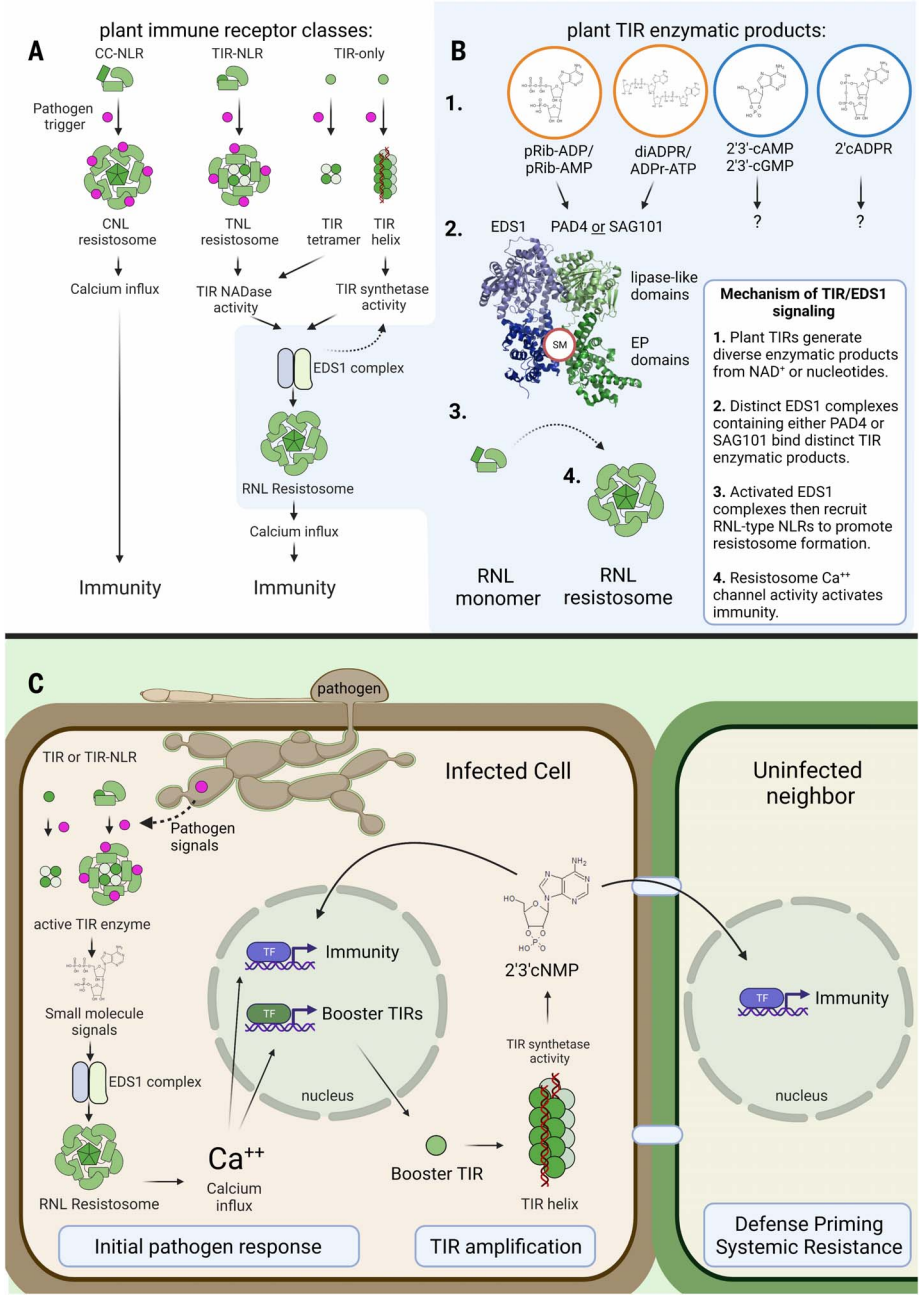

Fig. 5. Intracellular plant immune receptor pathways. (**A**) Plant immune receptors are shown in green. Both CC-NLRs and TIR-NLRs have been shown to bind pathogen triggers (pink circles) and oligomerize into wheel-like resistosomes. For CC-NLRs, the pentameric resistosome generates an ion channel that allows an influx of Ca^{++} and potentially other ions into the cytoplasm. TIR-NLRs form a tetrameric oligomer that activates the NADase activity of the TIR domain. TIR-only proteins can be activated by pathogen triggers to form a presumed NADase tetramer, which produces small-molecule signals (e.g., pRib-ADP) or a helical assembly that expresses both nuclease and 2′3′-cNMP synthetase activity. TNL and TIR-only pathways are dependent on the downstream EDS1 complex and CC_{RPW8}-NLRs (RNL "helper NLRs"). (**B**) The EDS1 complex binds TIR enzymatic products, and this activated complex interacts selectively with ADR or NRG helper RNLs to activate immunity (see inset). SM, small molecules. (**C**) A hypothetical model for a two-tiered TIR immune system. The initial TIR-NLR response is triggered when a TIR-NLR or TIR-only protein recognizes the presence of a pathogen-dependent trigger. This results in production of enzymatic products and activation of the EDS1 complex and RNLs, as shown in (A) and (B). TIR amplification occurs when the initial pathogen response results in the transcription of a large number of "booster TIRs." Booster TIRs amplify the initial response and result in the production of large amounts of 2′3′-cNMP, which then goes on to activate further immune response in both infected and neighboring uninfected cells. TF, transcription factor.

enzymatic function in planta because its presence was correlated with TIR immune function (*23*). It is notable that hydrolysis of the pyrophosphate linkage of 2′cADPR would yield pRib-AMP (Fig. 3C). pRib-ADP has an additional phosphate that is not present in 2′cADPR. Generation of pRib-ADP from 2′cADPR would require either phosphorolysis of the pyrophosphate bond in 2′cADPR (*111*) or phosphorylation of pRib-AMP. 2′cADPR's characteristic 1″-2′ ribose-ribose linkage is also present in (poly)ADP-ribose, a molecule that is induced during plant immune responses (*112*). Cleavage of linear (poly)ADP-ribose could directly produce three of the EDS1-complex signals: pRib-AMP, pRib-ADP, and ADP-ribosylated ADPR (Fig. 3D). It will be interesting to determine whether TIRs can also use polyADP-ribose as a substrate, potentially releasing diADPR through hydrolysis of the 1″-2′ linkage.

Structural basis of TIR-domain enzymatic function

The crystal structures of many animal, plant, and bacterial TIR domains have been determined (*43*, *113*–*117*). A conserved core fold is present in TIR domains that consists of a conserved central five-stranded β sheet (βA to βE), typically with five surrounding helical regions (αA to αE) separated by loops (*10*, *116*). These TIR-domain structures share similarities with the Rossman fold, an ancient motif with alternating β strands and α helices that can bind nucleotides such as NAD^+ (*90*, *118*, *119*). As noted above, mutation of the conserved glutamate residue in TIR domains abolishes enzymatic activity and function in animal, plant, and bacterial systems. Additionally, a conserved aromatic amino acid was identified that is required for generating a cyclized ADPR product (cADPR, 2′cADPR, or 3′cADPR) (*84*). Thus, a shared TIR catalytic mechanism has been conserved throughout evolution.

TIR activation is often driven by oligomerization. Two groups reported cryo–electron

microscopy (cryo-EM) structures for ligand-bound, activated plant TNL receptors (*120*, *121*). The oligomerization state of activated TNL immune receptors bound to their ligands is a tetramer (Fig. 6). The pathogen effector ligand binds primarily to the LRRs and a downstream C-terminal domain and causes the NBS-LRR domains to oligomerize into a ring-like structure. This structure brings four TIR domains into proximity and is reminiscent of the pentameric ZAR1 CNL resistosome (*122*) and several animal inflammasomes. The interactions between these TIR domains in their full-length protein context leverage interfaces predicted from truncated TIR-domain crystal structures to produce a tetramer composed of two pairs of TIRs (*120*, *121*). Within each TIR pair, one of the TIR domains undergoes a conformational change in which the "BB loop" is rearranged to expose the putative catalytic glutamate residue. This transformation upon tetramerization would allow cleavage of NAD^+ and promote production of TIR-derived signal molecules. Cryo-EM studies of SARM1 in its active conformation revealed similar findings, with two SARM1 TIR molecules forming a substrate binding site (*77*). The BB loop is present in this proposed active site for NAD^+ cleavage (Fig. 6), and mutations in the BB loop inhibit NADase activity and axon degeneration (*22*, *24*, *123*). Similar to SARM1, a substrate binding site in bacterial AbTir is formed by two TIR molecules. Mutations in the BB loop also impair enzymatic function (*77*, *84*).

The autoinhibitory regulation of plant NLRs and animal SARM1 share some common features. SARM1 exists as an octamer in a ring shape, with the tandem SAM domains forming the central core of the ring, the N-terminal armadillo repeat (ARM) domains wrapping around the ring, and the TIR domains lodged between the ARM domains on the outside of the ring (*77*, *114*, *115*, *124*–*127*). The N-terminal autoinhibitory ARM domain in SARM1 interacts with the TIR domain (*77*, *114*, *115*, *123*–*126*). This is analogous to the NBS and LRR domains in plant NLRs (*7*). After activation, the eight SARM1 TIR domains move above the plane of the ARM-SAM ring and reorganize in a two-by-four grid (*77*). The organization of the SARM1 TIRs is notably similar to that of the plant tetrameric TIR, using the same interfaces and overall geometry (*77*).

During activation of SARM1, both NAD^+ and NMN compete for an allosteric binding site within the autoinhibitory N-terminal domain of SARM1, with the ratio of bound NMN to NAD^+ dictating the activation state of the enzyme (*114*, *115*, *125*, *126*, *128*). Higher NMN-to-NAD^+ ratios result in an activated enzyme that consumes NAD^+ and promotes axon death, whereas low ratios keep the enzyme in the off state (*114*). Mutations in the NMN-NAD^+ binding pocket that disrupt NMN binding block both activation of NAD^+ hydrolase activity and injury-induced axon degeneration (*114*). Conversely, hypermorphic mutations in the NMN binding pocket produce an enhanced or constitutively active form of the enzyme (*114*). These structural studies of SARM1 clarify the activation mechanism of the TIR enzymatic activity and provide a blueprint for how other proteins with TIR-domain NAD^+ hydrolases may be regulated.

Although plant TNLs and animal SARM1 have convergent mechanisms for TIR activation, not all TIRs follow their example as closely. The bacterial TIR-STING (TIR–stimulator of interferon genes) sensor is present in both prokaryotes and basal eukaryotes (*25*). TIR domains of TIR-STING proteins use a proline-rich loop to mediate TIR-TIR interactions but rely on the same conserved catalytic glutamate for NADase activity (Fig. 6) (*25*). The binding of cyclic-di-guanine monophosphate (c-di-GMP) to the STING domain of TIR-STING proteins drives oligomerization into filamentous

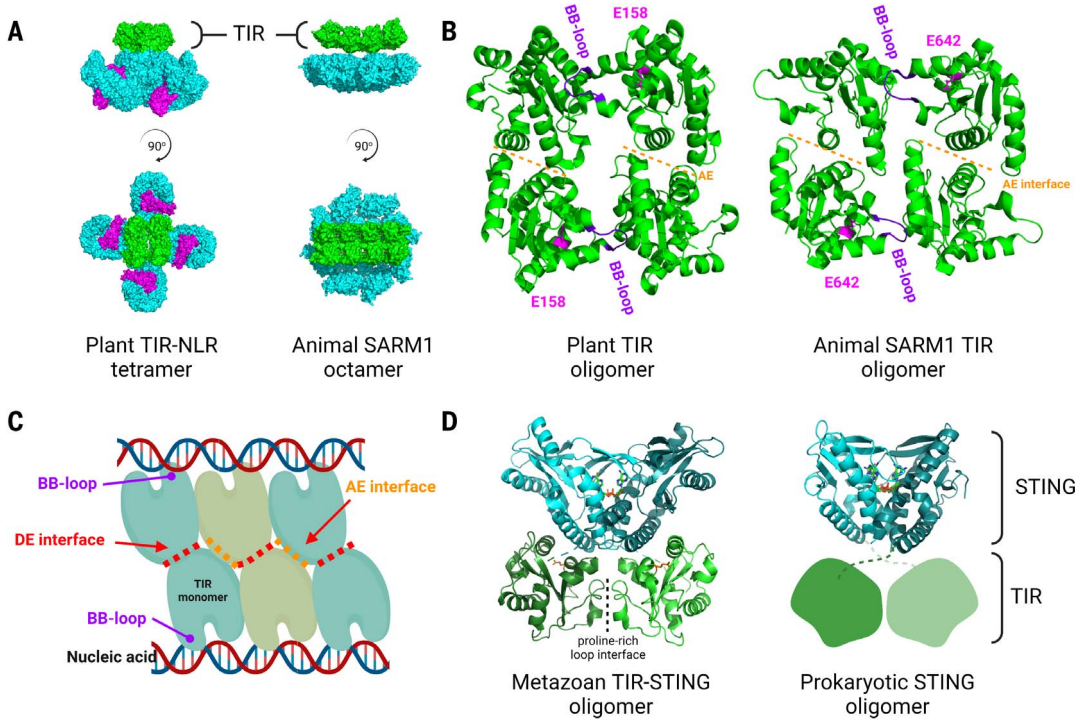

Fig. 6. TIR protein oligomerization strategies. (**A**) Activated plant TIR-NLR immune receptors and animal SARM1 function as oligomeric proteins. TIR domains are shown in green, the oligomeric chassis is in teal, and, for the plant complex, the bacterial ligand is in magenta. The oligomerized plant TIR complex is tetrameric [RPP1—Protein Data Bank (PDB) ID 7CRC—is shown; also see ROQ1 (PDB ID 7JLX)], and the animal SARM1 is octameric (PDB IDs 7NAK and 7NAL). (**B**) Convergent oligomerization strategies shown in (A) result in similar TIR interfaces for plant TIRs (PDB ID 7CRC is shown) and SARM1. Putative catalytic glutamates are shown in magenta. The BB loop is shown in purple, and the interaction interface mediated by the αA and αE helices (AE) is shown in orange. Graphics in (A) and (B) are adapted from Shi *et al*. (*77*). (**C**) Schematic cartoon of a plant TIR–nucleic acid helical oligomer. In contrast to the tetrameric TIR-NLR structure, helical TIRs are interconnected with repeating AE (orange) and DE (red) interfaces. The DE interaction interface is mediated by the αD and αE helices. The BB loop interacts with the minor groove of DNA. The cartoon is adapted from Yu *et al*. (*27*). (**D**) Both metazoans and prokaryotes have TIR NADases that are regulated by C-terminal STING domains. Oligomerization of TIR-STING proteins is controlled by cyclic-dinucleotide (sticks) binding to the STING domain (teal). The two monomers cross perpendicular to each other, indicated by dark and light shading. Green TIR domains associate through a distinctive proline-rich loop interaction surface (black dashed line). Putative catalytic glutamate is shown in orange. The prokaryotic TIR domain was not crystalized but is potentially similar to the metazoan structure. The metazoan TIR-STING structure is PDB ID 6WT7, and the prokaryotic STING structure is PDB ID 6WT4. The graphic in (D) is adapted from Morehouse *et al*. (*25*).

structures, and mutations that disrupt either c-di-GMP binding or oligomerization into filaments disrupt NAD^+ cleavage (*25*). The formation of filamentous structures was also observed upon binding of small molecules to additional bacterial TIR NADases (*84*, *129*). The function of these higher-order structures remains to be elucidated but is reminiscent of prion-like protein activation seen in other immune defense proteins (*130*, *131*). Thus, there are diverse, independently evolved strategies to oligomerize and/or activate TIR NADase activity.

Plant TIR domains also cleave DNA and RNA

Plant TIR enzymatic activity that produces small-molecule signals to activate either of the EDS1 RNL complexes is essential for function. A second enzymatic function was described for plant TIRs (*27*). Yu *et al.* found that both TIR-only proteins and TIR domains derived from TNL proteins could degrade nucleotides, DNA, and RNA in vitro. Plant TIR domains produce cyclic nucleotide monophosphates (cNMPs) such as 2′,3′-cAMP and 2′,3′-cGMP, molecules that are stress signals in plants and animals (*132*, *133*).

Whereas the first plant TNL cryo-EM structures revealed a tetrameric resistosome, new cryo-EM structures show that a plant TIR domain (lacking NBS-LRR domains) is capable of oligomerizing into helical structures (*27*). The purified L7 TIR domain is a C-terminal truncation that lacks the NBS-LRR domains that are found in the full-length L7 TNL protein. The L7 TIR domain structure revealed two protofilaments that twist into a superhelix that also appears to contain nucleic acid (Fig. 6). This structure supports the hypothesis that plant TIRs can also act as nucleases and defines basic arginine and lysine residues that likely contact the nucleic acid. Indeed, the mutation of these residues completely blocks 2′,3′-cNMP synthetase activity and TIR-triggered cell death but only partially impairs NADase function. Thus, NADase and synthetase activity are separable, and partial NADase activity alone is insufficient for immune function as assayed by cell death. Although the structural details of catalysis are unclear, TIR nuclease activity and 2′,3′ synthetase activity both share the requirement for the putative catalytic glutamic acid with NADase activity, indicating functional overlap.

In planta experiments that genetically reduce levels of 2′,3′-cNMP also blocked TIR cell death, consistent with an immune signaling function for these cyclic nucleotide products. Is 2′,3′-cNMP another signal that activates EDS1? Or does this product have other signaling functions? Accumulation of 2′,3′-cNMP in planta requires the EDS1 complex, which would place its generation downstream of EDS1, at odds with data showing that TIR enzymes act upstream of EDS1 (*27*). To explain this, Yu *et al.* suggest a feedback loop in which the putative 2′,3′-cNMP EDS1-activating signal must be amplified in an EDS1-dependent manner (Fig. 5) (*27*). Yu *et al.* also found that bacterial AbTir does not have 2′,3′-cNMP synthetase activity. The lack of AbTir synthetase activity could potentially explain why this 2′cADPR-producing TIR did not activate the EDS1-dependent plant cell death pathway as a chimeric fusion to the NLRC4 chassis, as described above (*27*, *108*). The authors conclude that plant TIRs can exist in two distinct oligomeric complexes: as a tetramer with NADase activity and as a helical synthetase. Many details of this dual-specificity enzymatic system remain to be clarified. Are full-length TIR-NBS-LRR proteins capable of forming the helical synthetase given potential steric issues? Do alternatively spliced TIRs related to the truncated TIR-only isoform generate 2′3′-cNMP? Is there selective catalysis to produce both the EDS1-complex signals and 2′,3′-cNMP products? And what are the relevant in vivo substrates? Immune activation leads to transcriptional activation of many TIR domain–encoding genes, and overexpression of these genes can cause cell death (*39*, *134*–*136*). Thus, one model is that a primary TNL or TIR-only receptor is activated by a pathogen-encoded ligand to promote oligomerization that favors NAD^+ hydrolase activity; the consequent EDS1 complex–dependent transcriptional output generally raises TIR domain levels through new transcription (Fig. 5C). These "booster TIRs," in turn, drive helical oligomerization that favors 2′3′-cNMP synthetase activity to stimulate defense responses, perhaps in different subcellular compartments and/or in cells directly neighboring the infection site (Fig. 5C).

Conclusion

The study of TIR domain proteins as an enzyme family has transformed our understanding of this evolutionarily ancient signaling domain. The discovery of the SARM1 NADase has directed therapeutic approaches in neurodegeneration (*137*–*140*). Constitutively hyperactive SARM1 NADase variants were found in ALS patients (*141*, *142*), suggesting that SARM1 alleles may contribute to disease risk in ALS. Hence, inhibiting SARM1 could alleviate certain neurodegenerative diseases. The role of SARM1 and NAD^+ metabolism during development and aging is also emerging (*143*–*147*). Future studies will help strengthen this link, with implications for neurodevelopmental genetic disorders.

Do TLRs and other animal TIR domains besides SARM1 truly lack enzymatic function? A few published reports suggest this (*22*, *24*, *81*). Yet some of these proteins possess the conserved glutamic acid residue necessary for NAD^+ cleavage in SARM1, plant TIRs, and bacterial TIRs. Structural studies indicate these glutamate residues are in suboptimal positions to perform the necessary catalytic functions (*24*, *81*). It is possible that mammalian TIR domains retain enzymatic activities but that their cognate substrates have not yet been identified. Prior biochemical experiments with purified proteins may have failed to recapitulate the true biologically active conformation of these mammalian proteins and hence missed their enzymatic activity.

The discovery of new cADPR isomers generated by plant and bacterial TIR proteins (Fig. 3) is another advance emerging from the work on TIR enzymatic functions. We now know that there are at least two distinct linkages: 2′cADPR and 3′cADPR. These small molecules can bind to and activate other effector enzymes in bacterial antiphage defense programs. Moreover, this ability to activate effector proteins to trigger bacterial suicide can be leveraged to develop a class of antibiotics that will target bacterial TIR proteins during infections. The structural similarities of the cADPR isomers also raise the possibility that these molecules, whether in their native biological context or whether repurposed as drugs, can function as competitive inhibitors of immune pathway proteins to subvert host immune responses. It also remains to be determined if these cADPR isomers possess calcium-mobilizing effects.

Understanding the role of TIR-derived small-molecule products in the plant immune system should clarify gaps in our understanding of plant immunity. Discovery of 2′-linked ribose-ribose small molecules as the connection between TIR enzymatic activity and EDS1 complex association with downstream RNLs is a landmark discovery and may provide new natural chemicals to control disease resistance. Yu *et al.* (*27*) propose that the protein structures produced by TIR-only and TNL proteins have distinct enzymatic activities. How and where these protein structures and activities are occurring in vivo remains an open question for which we have proposed a speculative model (Fig. 5C). Similarly, it is unknown whether full-length TNL proteins (and alternatively spliced products) form both the tetramer NADase and the helical 2′,3′-cNMP synthetase. Equally curious is the potential for separation of functions encoded by full-length TNLs and TIR domain–only proteins like RBA1.

The revelation that TIR proteins are performing similar functions in cell death and disease resistance across kingdoms has had an energizing effect across many fields. Discoveries in animals, plants, and prokaryotes are generating hypotheses and driving research and applications across kingdoms as we learn more about the common and distinctive features of members of this broad and ancient protein family.

REFERENCES AND NOTES

1. S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity. *Cell* **124**, 783–801 (2006). doi: 10.1016/j.cell.2006.02.015; pmid: 16497588
2. P. C. Ronald, B. Beutler, Plant and animal sensors of conserved microbial signatures. *Science* **330**, 1061–1064 (2010). doi: 10.1126/science.1189468; pmid: 21097929
3. R. Medzhitov, P. Preston-Hurlburt, C. A. Janeway Jr., A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **388**, 394–397 (1997). doi: 10.1038/41131; pmid: 9237759
4. K. A. Fitzgerald, J. C. Kagan, Toll-like receptors and the control of immunity. *Cell* **180**, 1044–1066 (2020). doi: 10.1016/j.cell.2020.02.041; pmid: 32164908
5. B. Everts *et al*., TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* **15**, 323–332 (2014). doi: 10.1038/ni.2833; pmid: 24562310
6. Y. Tan, J. C. Kagan, Innate immune signaling organelles display natural and programmable signaling flexibility. *Cell* **177**, 384–398.e11 (2019). doi: 10.1016/j.cell.2019.01.039; pmid: 30853218
7. J. D. G. Jones, R. E. Vance, J. L. Dangl, Intracellular innate immune surveillance devices in plants and animals. *Science* **354**, aaf6395 (2016). doi: 10.1126/science.aaf6395; pmid: 27934708
8. A.-L. Van de Weyer *et al*., A species-wide inventory of NLR genes and alleles in *Arabidopsis thaliana*. *Cell* **178**, 1260–1272.e14 (2019). doi: 10.1016/j.cell.2019.07.038; pmid: 31442410
9. J. D. G. Jones, J. L. Dangl, The plant immune system. *Nature* **444**, 323–329 (2006). doi: 10.1038/nature05286; pmid: 17108957
10. V. Y. Toshchakov, A. F. Neuwald, A survey of TIR domain sequence and structure divergence. *Immunogenetics* **72**, 181–203 (2020). doi: 10.1007/s00251-020-01157-7; pmid: 32002590
11. A. M. Spear, N. J. Loman, H. S. Atkins, M. J. Pallen, Microbial TIR domains: Not necessarily agents of subversion? *Trends Microbiol.* **17**, 393–398 (2009). doi: 10.1016/j.tim.2009.06.005; pmid: 19716705
12. R. M. Newman, P. Salunkhe, A. Godzik, J. C. Reed, Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. *Infect. Immun.* **74**, 594–601 (2006). doi: 10.1128/IAI.74.1.594-601.2006; pmid: 16369016
13. C. Cirl *et al*., Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. *Nat. Med.* **14**, 399–406 (2008). doi: 10.1038/nm1734; pmid: 18327267
14. Q. Zhang, C. M. Zmasek, X. Cai, A. Godzik, TIR domain-containing adaptor SARM is a late addition to the ongoing microbe-host dialog. *Dev. Comp. Immunol.* **35**, 461–468 (2011). doi: 10.1016/j.dci.2010.11.013; pmid: 21110998
15. K. Essuman *et al*., TIR domain proteins are an ancient family of NAD$^+$-consuming enzymes. *Curr. Biol.* **28**, 421–430.e4 (2018). doi: 10.1016/j.cub.2017.12.024; pmid: 29395922
16. S. Doron *et al*., Systematic discovery of antiphage defense systems in the microbial pangenome. *Science* **359**, eaar4120 (2018). doi: 10.1126/science.aar4120; pmid: 29371424
17. J. S. Weagley *et al*., Products of gut microbial Toll/interleukin-1 receptor domain NADase activities in gnotobiotic mice and Bangladeshi children with malnutrition. *Cell Rep.* **39**, 110738 (2022). doi: 10.1016/j.celrep.2022.110738; pmid: 35476981
18. G. Ofir *et al*., Antiviral activity of bacterial TIR domains via immune signalling molecules. *Nature* **600**, 116–120 (2021). doi: 10.1038/s41586-021-04098-7; pmid: 34853457
19. L. A. J. O'Neill, D. Golenbock, A. G. Bowie, The history of Toll-like receptors – redefining innate immunity. *Nat. Rev. Immunol.* **13**, 453–460 (2013). doi: 10.1038/nri3446; pmid: 23681101
20. J. D. Nanson, B. Kobe, T. Ve, Death, TIR, and RHIM: Self-assembling domains involved in innate immunity and cell-death signaling. *J. Leukoc. Biol.* **105**, 363–375 (2019). doi: 10.1002/JLB.MR0318-123R; pmid: 30517972
21. S. Nimma, T. Ve, S. J. Williams, B. Kobe, Towards the structure of the TIR-domain signalosome. *Curr. Opin. Struct. Biol.* **43**, 122–130 (2017). doi: 10.1016/j.sbi.2016.12.014; pmid: 28092811
22. K. Essuman *et al*., The SARM1 Toll/interleukin-1 receptor domain possesses intrinsic NAD$^+$ cleavage activity that promotes pathological axonal degeneration. *Neuron* **93**, 1334–1343.e5 (2017). doi: 10.1016/j.neuron.2017.02.022; pmid: 28334607
23. L. Wan *et al*., TIR domains of plant immune receptors are NAD$^+$-cleaving enzymes that promote cell death. *Science* **365**, 799–803 (2019). doi: 10.1126/science.aax1771; pmid: 31439793
24. S. Horsefield *et al*., NAD$^+$ cleavage activity by animal and plant TIR domains in cell death pathways. *Science* **365**, 793–799 (2019). doi: 10.1126/science.aax1911; pmid: 31439792
25. B. R. Morehouse *et al*., STING cyclic dinucleotide sensing originated in bacteria. *Nature* **586**, 429–433 (2020). doi: 10.1038/s41586-020-2719-5; pmid: 32877915
26. Z. Y. Zhao *et al*., A cell-permeant mimetic of NMN activates SARM1 to produce cyclic ADP-ribose and induce non-apoptotic cell death. *iScience* **15**, 452–466 (2019). doi: 10.1016/j.isci.2019.05.001; pmid: 31128467
27. D. Yu *et al*., TIR domains of plant immune receptors are 2′,3′-cAMP/cGMP synthetases mediating cell death. *Cell* **185**, 2370–2386.e18 (2022). doi: 10.1016/j.cell.2022.04.032; pmid: 35597242
28. J. E. Sims *et al*., cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science* **241**, 585–589 (1988). doi: 10.1126/science.2969618; pmid: 2969618
29. N. J. Gay, F. J. Keith, *Drosophila* Toll and IL-1 receptor. *Nature* **351**, 355–356 (1991). doi: 10.1038/351355b0; pmid: 1851964
30. D. S. Schneider, K. L. Hudson, T. Y. Lin, K. V. Anderson, Dominant and recessive mutations define functional domains of Toll, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo. *Genes Dev.* **5**, 797–807 (1991). doi: 10.1101/gad.5.5.797; pmid: 1827421
31. K. V. Anderson, G. Jürgens, C. Nüsslein-Volhard, Establishment of dorsal-ventral polarity in the Drosophila embryo: Genetic studies on the role of the *Toll* gene product. *Cell* **42**, 779–789 (1985). doi: 10.1016/0092-8674(85)90274-0; pmid: 3931918
32. R. Steward, *Dorsal*, an embryonic polarity gene in *Drosophila*, is homologous to the vertebrate proto-oncogene, c-*rel*. *Science* **238**, 692–694 (1987). doi: 10.1126/science.3118464; pmid: 3118464
33. E. Stylianou *et al*., Interleukin 1 induces NF-κB through its type I but not its type II receptor in lymphocytes. *J. Biol. Chem.* **267**, 15836–15841 (1992). doi: 10.1016/S0021-9258(19)49611-7; pmid: 1386364
34. S. Whitham *et al*., The product of the tobacco mosaic virus resistance gene *N*: Similarity to toll and the interleukin-1 receptor. *Cell* **78**, 1101–1115 (1994). doi: 10.1016/0092-8674(94)90283-6; pmid: 7923359
35. Y. T. Ip *et al*., *Dif*, a *dorsal*-related gene that mediates an immune response in *Drosophila*. *Cell* **75**, 753–763 (1993). doi: 10.1016/0092-8674(93)90495-C; pmid: 8242747
36. B. Lemaitre, E. Nicolas, L. Michaut, J. M. Reichhart, J. A. Hoffmann, The dorsoventral regulatory gene cassette *spätzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* **86**, 973–983 (1996). doi: 10.1016/S0092-8674(00)80172-5; pmid: 8808632
37. S. Rutschmann *et al*., The Rel protein DIF mediates the antifungal but not the antibacterial host defense in *Drosophila*. *Immunity* **12**, 569–580 (2000). doi: 10.1016/S1074-7613(00)80208-3; pmid: 10843389
38. M. T. Nishimura *et al*., TIR-only protein RBA1 recognizes a pathogen effector to regulate cell death in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E2053–E2062 (2017). doi: 10.1073/pnas.1620973114; pmid: 28137883
39. R. S. Nandety *et al*., The role of TIR-NBS and TIR-X proteins in plant basal defense responses. *Plant Physiol.* **162**, 1459–1472 (2013). doi: 10.1104/pp.113.219162; pmid: 23735504
40. M. E. Santamaría *et al*., An Arabidopsis TIR-lectin two-domain protein confers defense properties against *Tetranychus urticae*. *Plant Physiol.* **179**, 1298–1314 (2019). doi: 10.1104/pp.18.00951; pmid: 30765478
41. P. R. Vajjhala, T. Ve, A. Bentham, K. J. Stacey, B. Kobe, The molecular mechanisms of signaling by cooperative assembly formation in innate immunity pathways. *Mol. Immunol.* **86**, 23–37 (2017). doi: 10.1016/j.molimm.2017.02.012; pmid: 28249680
42. J. C. Kagan, V. G. Magupalli, H. Wu, SMOCs: Supramolecular organizing centres that control innate immunity. *Nat. Rev. Immunol.* **14**, 821–826 (2014). doi: 10.1038/nri3757; pmid: 25359439
43. T. Ve *et al*., Structural basis of TIR-domain-assembly formation in MAL- and MyD88-dependent TLR4 signaling. *Nat. Struct. Mol. Biol.* **24**, 743–751 (2017). doi: 10.1038/nsmb.3444; pmid: 28759049
44. S. Nimma *et al*., Structural evolution of TIR-domain signalosomes. *Front. Immunol.* **12**, 784484 (2021). doi: 10.3389/fimmu.2021.784484; pmid: 34868065
45. C. Garlanda, C. A. Dinarello, A. Mantovani, The interleukin-1 family: Back to the future. *Immunity* **39**, 1003–1018 (2013). doi: 10.1016/j.immuni.2013.11.010; pmid: 24332029
46. C. A. Dinarello, The IL-1 family of cytokines and receptors in rheumatic diseases. *Nat. Rev. Rheumatol.* **15**, 612–632 (2019). doi: 10.1038/s41584-019-0277-8; pmid: 31515542
47. J. E. Sims, D. E. Smith, The IL-1 family: Regulators of immunity. *Nat. Rev. Immunol.* **10**, 89–102 (2010). doi: 10.1038/nri2691; pmid: 20081871
48. M. Carty *et al*., The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nat. Immunol.* **7**, 1074–1081 (2006). doi: 10.1038/ni1382; pmid: 16964262
49. C. Couillault *et al*., TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat. Immunol.* **5**, 488–494 (2004). doi: 10.1038/ni1060; pmid: 15048112
50. C.-F. Chuang, C. I. Bargmann, A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. *Genes Dev.* **19**, 270–281 (2005). doi: 10.1101/gad.1276505; pmid: 15625192
51. J. E. Irazoqui, J. M. Urbach, F. M. Ausubel, Evolution of host innate defence: Insights from *Caenorhabditis elegans* and primitive invertebrates. *Nat. Rev. Immunol.* **10**, 47–58 (2010). doi: 10.1038/nri2689; pmid: 20029447
52. J. Gerdts, D. W. Summers, J. Milbrandt, A. DiAntonio, Axon self-destruction: New links among SARM1, MAPKs, and NAD$^+$ metabolism. *Neuron* **89**, 449–460 (2016). doi: 10.1016/j.neuron.2015.12.023; pmid: 26844829
53. M. D. Figley, A. DiAntonio, The SARM1 axon degeneration pathway: Control of the NAD$^+$ metabolome regulates axon survival in health and disease. *Curr. Opin. Neurobiol.* **63**, 59–66 (2020). doi: 10.1016/j.conb.2020.02.012; pmid: 32311648
54. A. Waller, Experiments on the section of the glosso-pharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Edinb. Med. Surg. J.* **76**, 369–376 (1851). pmid: 30332247
55. E. R. Lunn, V. H. Perry, M. C. Brown, H. Rosen, S. Gordon, Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. *Eur. J. Neurosci.* **1**, 27–33 (1989). doi: 10.1111/j.1460-9568.1989.tb00771.x; pmid: 12106171
56. T. G. A. Mack *et al*., Wallerian degeneration of injured axons and synapses is delayed by a *Ube4b/Nmnat* chimeric gene. *Nat. Neurosci.* **4**, 1199–1206 (2001). doi: 10.1038/nn770; pmid: 11770485
57. L. Conforti *et al*., A *Ufd2/D4Cole1e* chimeric protein and overexpression of *Rbp7* in the slow Wallerian degeneration (*Wld*^{S}) mouse. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11377–11382 (2000). doi: 10.1073/pnas.97.21.11377; pmid: 11027338
58. T. Araki, Y. Sasaki, J. Milbrandt, Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* **305**, 1010–1013 (2004). doi: 10.1126/science.1098014; pmid: 15310905
59. B. R. Miller *et al*., A dual leucine kinase-dependent axon self-destruction program promotes Wallerian degeneration. *Nat. Neurosci.* **12**, 387–389 (2009). doi: 10.1038/nn.2290; pmid: 19287387
60. J. Yang *et al*., Pathological axonal death through a MAPK cascade that triggers a local energy deficit. *Cell* **160**, 161–176 (2015). doi: 10.1016/j.cell.2014.11.053; pmid: 25594179
61. J. M. Osterloh *et al*., dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. *Science* **337**, 481–484 (2012). doi: 10.1126/science.1223899; pmid: 22678360
62. J. Gerdts, D. W. Summers, Y. Sasaki, A. DiAntonio, J. Milbrandt, Sarm1-mediated axon degeneration requires both SAM and TIR interactions. *J. Neurosci.* **33**, 13569–13580 (2013). doi: 10.1523/JNEUROSCI.1197-13.2013; pmid: 23946415
63. M. P. Coleman, A. Höke, Programmed axon degeneration: From mouse to mechanism to medicine. *Nat. Rev. Neurosci.*

21, 183–196 (2020). doi: 10.1038/s41583-020-0269-3; pmid: 32152523

64. N. Henninger *et al*., Attenuated traumatic axonal injury and improved functional outcome after traumatic brain injury in mice lacking *Sarm1*. *Brain* **139**, 1094–1105 (2016). doi: 10.1093/brain/aww001; pmid: 26912636
65. S. Geisler *et al*., Prevention of vincristine-induced peripheral neuropathy by genetic deletion of SARM1 in mice. *Brain* **139**, 3092–3108 (2016). doi: 10.1093/brain/aww251; pmid: 27797810
66. M. A. White *et al*., *Sarm1* deletion suppresses TDP-43-linked motor neuron degeneration and cortical spine loss. *Acta Neuropathol. Commun.* **7**, 166 (2019). doi: 10.1186/s40478-019-0800-9; pmid: 31661035
67. Y. Cheng *et al*., *Sarm1* gene deficiency attenuates diabetic peripheral neuropathy in mice. *Diabetes* **68**, 2120–2130 (2019). doi: 10.2337/db18-1233; pmid: 31439642
68. E. Turkiew, D. Falconer, N. Reed, A. Höke, Deletion of Sarm1 gene is neuroprotective in two models of peripheral neuropathy. *J. Peripher. Nerv. Syst.* **22**, 162–171 (2017). doi: 10.1111/jns.12219; pmid: 28485482
69. Y. Sasaki *et al*., SARM1 depletion rescues NMNAT1-dependent photoreceptor cell death and retinal degeneration. *eLife* **9**, e62027 (2020). doi: 10.7554/eLife.62027; pmid: 33107823
70. K. W. Ko, J. Milbrandt, A. DiAntonio, SARM1 acts downstream of neuroinflammatory and necroptotic signaling to induce axon degeneration. *J. Cell Biol.* **219**, e201912047 (2020). doi: 10.1083/jcb.201912047; pmid: 32609299
71. Y.-H. Chen, Y. Sasaki, A. DiAntonio, J. Milbrandt, SARM1 is required in human derived sensory neurons for injury-induced and neurotoxic axon degeneration. *Exp. Neurol.* **339**, 113636 (2021). doi: 10.1016/j.expneurol.2021.113636; pmid: 33548217
72. K. J. Szretter *et al*., The immune adaptor molecule SARM modulates tumor necrosis factor alpha production and microglia activation in the brainstem and restricts West Nile virus pathogenesis. *J. Virol.* **83**, 9329–9338 (2009). doi: 10.1128/JVI.00836-09; pmid: 19587044
73. C. G. Doran *et al*., CRISPR/Cas9-mediated SARM1 knockout and epitope-tagged mice reveal that SARM1 does not regulate nuclear transcription, but is expressed in macrophages. *J. Biol. Chem.* **297**, 101417 (2021). doi: 10.1016/j.jbc.2021.101417; pmid: 34793837
74. R. Krauss, T. Bosanac, R. Devraj, T. Engber, R. O. Hughes, Axons matter: The promise of treating neurodegenerative disorders by targeting SARM1-mediated axonal degeneration. *Trends Pharmacol. Sci.* **41**, 281–293 (2020). doi: 10.1016/j.tips.2020.01.006; pmid: 32107050
75. J. Gerdts, E. J. Brace, Y. Sasaki, A. DiAntonio, J. Milbrandt, SARM1 activation triggers axon degeneration locally via NAD^+ destruction. *Science* **348**, 453–457 (2015). doi: 10.1126/science.1258366; pmid: 25908823
76. C. Angeletti *et al*., SARM1 is a multi-functional NAD(P)ase with prominent base exchange activity, all regulated bymultiple physiologically relevant NAD metabolites. *iScience* **25**, 103812 (2022). doi: 10.1016/j.isci.2022.103812; pmid: 35198877
77. Y. Shi *et al*., Structural basis of SARM1 activation, substrate recognition, and inhibition by small molecules. *Mol. Cell* **82**, 1643–1659.e10 (2022). doi: 10.1016/j.molcel.2022.03.007; pmid: 35334231
78. K. W. Ko, L. Devault, Y. Sasaki, J. Milbrandt, A. DiAntonio, Live imaging reveals the cellular events downstream of SARM1 activation. *eLife* **10**, e71148 (2021). doi: 10.7554/eLife.71148; pmid: 34779400
79. Y. Li *et al*., Sarm1 activation produces cADPR to increase intra-axonal Ca^{++} and promote axon degeneration in PIPN. *J. Cell Biol.* **221**, e202106080 (2022). doi: 10.1083/jcb.202106080; pmid: 34935867
80. Y. Sasaki, T. Nakagawa, X. Mao, A. DiAntonio, J. Milbrandt, NMNAT1 inhibits axon degeneration via blockade of SARM1-mediated NAD^+ depletion. *eLife* **5**, e19749 (2016). doi: 10.7554/eLife.19749; pmid: 27735788
81. S. Nimma *et al*., Crystal structure of the Toll/interleukin-1 receptor (TIR) domain of IL-1R10 provides structural insights into TIR domain signalling. *FEBS Lett.* **596**, 886–897 (2022). doi: 10.1002/1873-3468.14288; pmid: 35038778
82. L. A. J. O'Neill, R. J. Kishton, J. Rathmell, A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* **16**, 553–565 (2016). doi: 10.1038/nri.2016.70; pmid: 27396447
83. C. Cantó, K. J. Menzies, J. Auwerx, NAD^+ metabolism and the control of energy homeostasis: A balancing act between mitochondria and the nucleus. *Cell Metab.* **22**, 31–53 (2015). doi: 10.1016/j.cmet.2015.05.023; pmid: 26118927
84. M. K. Manik *et al*., Chemical structures of cyclic ADP ribose (cADPR) isomers and the molecular basis of their production and signaling. bioRxiv 2022.05.07.491051 [Preprint] (2022); doi: 10.1101/2022.05.07.491051
85. S. Eastman *et al*., A phytobacterial TIR domain effector manipulates NAD^+ to promote virulence. *New Phytol.* **233**, 890–904 (2022). doi: 10.1111/nph.17805; pmid: 34657283
86. A. Leavitt *et al*., Viruses inhibit TIR gcADPR signaling to overcome bacterial defense. bioRxiv 2022.05.03.490397 [Preprint] (2022). doi: 10.1101/2022.05.03.490397
87. A. Waldhuber *et al*., A comparative analysis of the mechanism of Toll-like receptor-disruption by TIR-containing protein C from uropathogenic *Escherichia coli*. *Pathogens* **5**, 25 (2016). doi: 10.3390/pathogens5010025; pmid: 26938564
88. F. Askarian *et al*., A *Staphylococcus aureus* TIR domain protein virulence factor blocks TLR2-mediated NF-κB signaling. *J. Innate Immun.* **6**, 485–498 (2014). doi: 10.1159/000357618; pmid: 24481289
89. J. M. Coronas-Serna *et al*., The TIR-domain containing effectors BtpA and BtpB from *Brucella abortus* impact NAD metabolism. *PLOS Pathog.* **16**, e1007979 (2020). doi: 10.1371/journal.ppat.1007979; pmid: 32298382
90. D. Ka, H. Oh, E. Park, J.-H. Kim, E. Bae, Structural and functional evidence of bacterial antiphage protection by Thoeris defense system via NAD^+ degradation. *Nat. Commun.* **11**, 2816 (2020). doi: 10.1038/s41467-020-16703-w; pmid: 32499527
91. A. Lopatina, N. Tal, R. Sorek, Abortive infection: Bacterial suicide as an antiviral immune strategy. *Annu. Rev. Virol.* **7**, 371–384 (2020). doi: 10.1146/annurev-virology-011620-040628; pmid: 32559405
92. N. Tal, R. Sorek, SnapShot: Bacterial immunity. *Cell* **185**, 578–578.e1 (2022). doi: 10.1016/j.cell.2021.12.029; pmid: 35120666
93. N. Tal *et al*., Cyclic CMP and cyclic UMP mediate bacterial immunity against phages. *Cell* **184**, 5728–5739.e16 (2021). doi: 10.1016/j.cell.2021.09.031; pmid: 34644530
94. B. Koopal *et al*., Short prokaryotic Argonaute systems trigger cell death upon detection of invading DNA. *Cell* **185**, 1471–1486.e19 (2022). doi: 10.1016/j.cell.2022.03.012; pmid: 35381200
95. A. M. Bayless, M. T. Nishimura, Enzymatic functions for Toll/interleukin-1 receptor domain proteins in the plant immune system. *Front. Genet.* **11**, 539 (2020). doi: 10.3389/fgene.2020.00539; pmid: 32582284
96. D. Lapin, D. D. Bhandari, J. E. Parker, Origins and immunity networking functions of EDS1 family proteins. *Annu. Rev. Phytopathol.* **58**, 253–276 (2020). doi: 10.1146/annurev-phyto-010820-012840; pmid: 32396762
97. L. M. Jubic, S. Saile, O. J. Furzer, F. El Kasmi, J. L. Dangl, Help wanted: Helper NLRs and plant immune responses. *Curr. Opin. Plant Biol.* **50**, 82–94 (2019). doi: 10.1016/j.pbi.2019.03.013; pmid: 31063902
98. G. Bi *et al*., The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. *Cell* **184**, 3528–3541.e12 (2021). doi: 10.1016/j.cell.2021.05.003; pmid: 33984278
99. P. Jacob *et al*., Plant "helper" immune receptors are Ca^{2+}-permeable nonselective cation channels. *Science* **373**, 420–425 (2021). doi: 10.1126/science.abg7917; pmid: 34140391
100. O. Johanndrees *et al*., Differential EDS1 requirement for cell death activities of plant TIR-domain proteins. bioRxiv 2021.11.29.470438 [Preprint] (2021). doi: 10.1101/2021.11.29.470438
101. S. Huang *et al*., Identification and receptor mechanism of TIR-catalyzed small molecules in plant immunity. *Science* **377**, eabq3297 (2022). doi: 10.1126/science.abq3297
102. A. Jia *et al*., TIR-catalyzed ADP-ribosylation reactions produce signaling molecules for plant immunity. *Science* **377**, eabq8180 (2022). doi: 10.1126/science.abq8180
103. D. D. Bhandari *et al*., An EDS1 heterodimer signalling surface enforces timely reprogramming of immunity genes in Arabidopsis. *Nat. Commun.* **10**, 772 (2019). doi: 10.1038/s41467-019-08783-0; pmid: 30770836
104. J. A. Dongus *et al*., Cavity surface residues of PAD4 and SAG101 contribute to EDS1 dimer signaling specificity in plant immunity. *Plant J.* **110**, 1415–1432 (2022). doi: 10.1111/tpj.15747; pmid: 35324052
105. X. Sun *et al*., Pathogen effector recognition-dependent association of NRG1 with EDS1 and SAG101 in TNL receptor immunity. *Nat. Commun.* **12**, 3335 (2021). doi: 10.1038/s41467-021-23614-x; pmid: 34099661
106. Z. Wu, L. Tian, X. Liu, Y. Zhang, X. Li, TIR signal promotes interactions between lipase-like proteins and ADR1-L1 receptor and ADR1-L1 oligomerization. *Plant Physiol.* **187**, 681–686 (2021). doi: 10.1093/plphys/kiab305; pmid: 34608964
107. D. Lapin *et al*., A coevolved EDS1-SAG101-NRG1 module mediates cell death signaling by TIR-domain immune receptors. *Plant Cell* **31**, 2430–2455 (2019). doi: 10.1105/tpc.19.00118; pmid: 31311833
108. Z. Duxbury *et al*., Induced proximity of a TIR signaling domain on a plant-mammalian NLR chimera activates defense in plants. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 18832–18839 (2020). doi: 10.1073/pnas.2001185117; pmid: 32709746
109. Z. Hu *et al*., Structural and biochemical basis for induced self-propagation of NLRC4. *Science* **350**, 399–404 (2015). doi: 10.1126/science.aac5489; pmid: 26449475
110. L. Zhang *et al*., Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. *Science* **350**, 404–409 (2015). doi: 10.1126/science.aac5789; pmid: 26449474
111. H. Carminatti, E. Cabib, Phosphorolysis of the pyrophosphate bond of some nucleotides. *Biochim. Biophys. Acta* **53**, 417–419 (1961). doi: 10.1016/0006-3002(61)90458-9; pmid: 13876695
112. L. Adams-Phillips, A. G. Briggs, A. F. Bent, Disruption of poly (ADP-ribosyl)ation mechanisms alters responses of *Arabidopsis* to biotic stress. *Plant Physiol.* **152**, 267–280 (2010). doi: 10.1104/pp.109.148049; pmid: 19889874
113. N. Maruta *et al*., Structural basis of NLR activation and innate immune signalling in plants. *Immunogenetics* **74**, 5–26 (2022). doi: 10.1007/s00251-021-01242-5; pmid: 34981187
114. M. D. Figley *et al*., SARM1 is a metabolic sensor activated by an increased NMN/NAD^+ ratio to trigger axon degeneration. *Neuron* **109**, 1118–1136.e11 (2021). doi: 10.1016/j.neuron.2021.02.009; pmid: 33657413
115. M. Sporny *et al*., Structural basis for SARM1 inhibition and activation under energetic stress. *eLife* **9**, e62021 (2020). doi: 10.7554/eLife.62021; pmid: 33185189
116. Y. Xu *et al*., Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* **408**, 111–115 (2000). doi: 10.1038/35040600; pmid: 11081518
117. T. Ve, S. J. Williams, B. Kobe, Structure and function of Toll/interleukin-1 receptor/resistance protein (TIR) domains. *Apoptosis* **20**, 250–261 (2015). doi: 10.1007/s10495-014-1064-2; pmid: 25451009
118. A. M. Burroughs, D. Zhang, D. E. Schäffer, L. M. Iyer, L. Aravind, Comparative genomic analyses reveal a vast, novel network of nucleotide-centric systems in biological conflicts, immunity and signaling. *Nucleic Acids Res.* **43**, 10633–10654 (2015). doi: 10.1093/nar/gkv1267; pmid: 26590262
119. P. Laurino *et al*., An ancient fingerprint indicates the common ancestry of Rossmann-fold enzymes utilizing different ribose-based cofactors. *PLOS Biol.* **14**, e1002396 (2016). doi: 10.1371/journal.pbio.1002396; pmid: 26938925
120. R. Martin *et al*., Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. *Science* **370**, eabd9993 (2020). doi: 10.1126/science.abd9993; pmid: 33273074
121. S. Ma *et al*., Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. *Science* **370**, eabe3069 (2020). doi: 10.1126/science.abe3069; pmid: 33273071
122. J. Wang *et al*., Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science* **364**, eaav5870 (2019). doi: 10.1126/science.aav5870; pmid: 30948527
123. D. W. Summers, D. A. Gibson, A. DiAntonio, J. Milbrandt, SARM1-specific motifs in the TIR domain enable NAD^+ loss and regulate injury-induced SARM1 activation. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E6271–E6280 (2016). doi: 10.1073/pnas.1601506113; pmid: 27671644
124. C. Shen *et al*., Multiple domain interfaces mediate SARM1 autoinhibition. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2023151118 (2021). doi: 10.1073/pnas.2023151118; pmid: 33468661
125. M. Bratkowski *et al*., Structural and mechanistic regulation of the pro-degenerative NAD hydrolase SARM1. *Cell Rep.* **32**, 107999 (2020). doi: 10.1016/j.celrep.2020.107999; pmid: 32755591

126. Y. Jiang *et al*., The NAD^+-mediated self-inhibition mechanism of pro-neurodegenerative SARM1. *Nature* **588**, 658–663 (2020). doi: 10.1038/s41586-020-2862-z; pmid: 33053563
127. M. Sporny *et al*., Structural evidence for an octameric ring arrangement of SARM1. *J. Mol. Biol.* **431**, 3591–3605 (2019). doi: 10.1016/j.jmb.2019.06.030; pmid: 31278906
128. M. Di Stefano *et al*., A rise in NAD precursor nicotinamide mononucleotide (NMN) after injury promotes axon degeneration. *Cell Death Differ.* **22**, 731–742 (2015). doi: 10.1038/cdd.2014.164; pmid: 25323584
129. G. Hogrel *et al*., Cyclic nucleotide-induced superhelical structure activates a bacterial TIR immune effector. bioRxiv 2022.05.04.490601 [Preprint] (2022); doi: 10.1101/2022.05.04.490601
130. X. Cai, H. Xu, Z. J. Chen, Prion-like polymerization in immunity and inflammation. *Cold Spring Harb. Perspect. Biol.* **9**, a023580 (2017). doi: 10.1101/cshperspect.a023580; pmid: 27881448
131. H. Xu *et al*., Structural basis for the prion-like MAVS filaments in antiviral innate immunity. *eLife* **3**, e01489 (2014). doi: 10.7554/eLife.01489; pmid: 24569476
132. T. Van Damme *et al*., Wounding stress causes rapid increase in concentration of the naturally occurring 2′,3′-isomers of cyclic guanosine- and cyclic adenosine monophosphate (cGMP and cAMP) in plant tissues. *Phytochemistry* **103**, 59–66 (2014). doi: 10.1016/j.phytochem.2014.03.013; pmid: 24735826
133. E. K. Jackson, Discovery and roles of 2′,3′-cAMP in biological systems. *Handb. Exp. Pharmacol.* **238**, 229–252 (2017). doi: 10.1007/164_2015_40; pmid: 26721674
134. H. Tian *et al*., Activation of TIR signalling boosts pattern-triggered immunity. *Nature* **598**, 500–503 (2021). doi: 10.1038/s41586-021-03987-1; pmid: 34544113
135. M. Bjornson, P. Pimprikar, T. Nürnberger, C. Zipfel, The transcriptional landscape of *Arabidopsis thaliana* pattern-triggered immunity. *Nat. Plants* **7**, 579–586 (2021). doi: 10.1038/s41477-021-00874-5; pmid: 33723429
136. B. P. M. Ngou, P. Ding, J. D. G. Jones, Thirty years of resistance: Zig-zag through the plant immune system. *Plant Cell* **34**, 1447–1478 (2022). doi: 10.1093/plcell/koac041; pmid: 35167697
137. T. Bosanac *et al*., Pharmacological SARM1 inhibition protects axon structure and function in paclitaxel-induced peripheral neuropathy. *Brain* **144**, 3226–3238 (2021). doi: 10.1093/brain/awab184; pmid: 33964142
138. R. O. Hughes *et al*., Small molecule SARM1 inhibitors recapitulate the $SARM1^{-/-}$ phenotype and allow recovery of a metastable pool of axons fated to degenerate. *Cell Rep.* **34**, 108588 (2021). doi: 10.1016/j.celrep.2020.108588; pmid: 33406435
139. S. Geisler *et al*., Gene therapy targeting SARM1 blocks pathological axon degeneration in mice. *J. Exp. Med.* **216**, 294–303 (2019). doi: 10.1084/jem.20181040; pmid: 30642945
140. S. A. Gould *et al*., *Sarm1* haploinsufficiency or low expression levels after antisense oligonucleotides delay programmed axon degeneration. *Cell Rep.* **37**, 110108 (2021). doi: 10.1016/j.celrep.2021.110108; pmid: 34910914
141. J. Gilley *et al*., Enrichment of *SARM1* alleles encoding variants with constitutively hyperactive NADase in patients with ALS and other motor nerve disorders. *eLife* **10**, e70905 (2021). doi: 10.7554/eLife.70905; pmid: 34796871
142. A. J. Bloom *et al*., Constitutively active SARM1 variants that induce neuropathy are enriched in ALS patients. *Mol. Neurodegener.* **17**, 1 (2022). doi: 10.1186/s13024-021-00511-x; pmid: 34991663
143. E. J. Brace *et al*., Distinct developmental and degenerative functions of SARM1 require NAD^+ hydrolase activity. *PLOS Genet.* **18**, e1010246 (2022). doi: 10.1371/journal.pgen.1010246; pmid: 35737728
144. K. A. Herrmann *et al*., Divergent signaling requirements of dSARM in injury-induced degeneration and developmental glial phagocytosis. *PLOS Genet.* **18**, e1010257 (2022). doi: 10.1371/journal.pgen.1010257; pmid: 35737721
145. N. D. Peterson *et al*., Pathogen infection and cholesterol deficiency activate the *C. elegans* p38 immune pathway through a TIR-1/SARM1 phase transition. *eLife* **11**, e74206 (2022). doi: 10.7554/eLife.74206; pmid: 35098926
146. M. Lukacs *et al*., Severe biallelic loss-of-function mutations in nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) in two fetuses with fetal akinesia deformation sequence. *Exp. Neurol.* **320**, 112961 (2019). doi: 10.1016/j.expneurol.2019.112961; pmid: 31136762
147. P. Huppke *et al*., Homozygous *NMNAT2* mutation in sisters with polyneuropathy and erythromelalgia. *Exp. Neurol.* **320**, 112958 (2019). doi: 10.1016/j.expneurol.2019.112958; pmid: 31132363

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Shared TIR enzymatic functions regulate cell death and immunity across the tree of life

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A common core of immune responses

Dealing with pathogens is a constant struggle for many life-forms, and innate and adaptive immune systems are needed to support survival. Essuman *et al*. reviewed the latest insights into the Toll/interleukin-1 resistance/receptor (TIR) domain proteins, which support immune responses across the tree of life, from archaea to bacteria to plants to humans. TIR domains function in innate immune signaling pathways, as well as in axon degeneration in animals. Some, but not all, TIR domains have enzymatic activity. TIR nicotinamide adenine dinucleotide hydrolase activity starves phages as they infect prokaryotes and promotes hypersensitive cell death in a plant's response to pathogens. Plant TIR domains can also synthesize small, nucleotide-based second messengers that initiate an immune response. —PJH