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Spotlight

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TIRggering cell death via two enzymatic reactions

EFFECTOR-TRIGGERED IMMUNITY AND TIR DOMAINS

Plant pathogens rely on effectors that are delivered into host cells to cause diseases. To fight back, plants acquired nucleotide-binding site–leucine-rich repeat (NB-LRR or NLR) resistance proteins to recognize these pathogen effectors directly or to detect the activities of these effectors indirectly, triggering effector-triggered immunity (ETI) (Cui et al., 2015). These sensor NLRs also carry a variable toll/interleukin-1 receptor (TIR) or coiled-coil domain on their N termini.

DISTRIBUTIONS OF TIR DOMAINS

In addition to canonical TIR-NB-LRR (TNL) resistance proteins, plant genome encodes many proteins consisting of only a TIR domain (TIR only) or both TIR and NB (TIR-NB). TIR is short for toll/interleukin-1 receptor. Interleukin-1 (IL-1) is a cytokine that induces inflammation through cell surface type I IL-1 receptor (IL-1RI). Interestingly, the cytosolic region of IL-1RI shares similarity with the *Drosophila melanogaster* protein Toll. Therefore, this domain was termed Toll/IL-1R (TIR) (O'Neill, 2008). TIR is a protein–protein interaction domain that is widely distributed in animals, plants, and bacteria, but TIR has not been found in fungi, archaea, and viruses (Ve et al., 2015). The major function of TIR-domain-containing proteins in animals and plants is to induce immune responses. On the other hand, some bacterial TIR-domain-containing proteins have been shown to interfere with host-defense pathways or activate antiviral responses (Ve et al., 2015; Ofir et al., 2021).

SOS: LETS BREAK DOWN NAD^+

In plants, NLR resistance proteins play a key role in plant defense against pathogen infection. Since the first plant TNL resistance protein was reported in 1994 (Whitham et al., 1994), the exact biochemical functions of TIR domains remained obscure until 3 years ago. In 2019, two papers, which were published in *Science* back to back (Horsefield et al., 2019; Wan et al., 2019), revealed that the plant TIR domain functions as an NAD^+-cleaving enzyme to produce nicotinamide, ADP-ribose, and a special variant of cyclic ADP-ribose (v-cADPR) (Figure 1A). Facing elevated threats from pathogen effectors, plants have already made a tough choice by breaking down NAD^+, which is one of the most important coenzymes in cells. The NADase activity is required, but not sufficient, for plant TIR-domain-containing NLR proteins to induce cell death, suggesting the involvement of additional signaling molecules (Horsefield et al., 2019; Wan et al., 2019; Duxbury et al., 2020).

2^03^0-cAMP/cGMP SYNTHETASE: FIRING ANOTHER SHOT

When Yu et al. examined the NAD^+-cleaving activity of plant TIR-only protein RBA1, they discovered that RBA1 could hydrolyze ATP, GTP, DNA, and RNA molecules besides NAD^+ *in vitro* (Yu et al., 2022). Using liquid chromatography-mass spectrometry, the authors identified 2^03^0-cAMP and 2^03^0-cGMP as the enzymatic products of RNA or DNA by $L7^{TIR}$ and RBA1 (Figure 1B). Therefore, TIR domains display an additional 2^03^0-cAMP/cGMP synthetase activity using DNA or RNA molecules as substrates. Similar to TIR's NADase activity, 2^03^0-cAMP/cGMP synthetase activity is necessary for plant TIRs to confer ETI.

NON-CANONICAL PRODUCTS

Instead of 3^05^0-cAMP and 3^05^0-cGMP, which function as classical second messengers, TIR proteins produce 2^03^0-cAMP/cGMP through their 2^03^0-cAMP/cGMP synthetase activities (Yu et al., 2022). In contrast to animal TIR counterparts, which only produce ADP-ribose, plant TIR proteins produce an extra v-cADPR through their NADase functions (Figure 1A) (Wan et al., 2019). Hence, the plant TIR proteins can yield non-canonical products via two separate enzymatic activities.

DEGRADATION OF 2^03^0-cAMP/cGMP BY PHOSPHODIESTERASES

The classical second messengers 3^05^0-cAMP and 3^05^0-cGMP are produced from ATP and GTP by adenylate cyclase and guanylate cyclase, respectively, and are metabolized by phosphodiesterase.

EDS1 is required for TNLs to confer ETI (Cui et al., 2015). Previous studies found that the nudix hydrolase AtNUDT7 suppresses EDS1 signaling dependent on its catalytic site and that nudix hydrolase effectors XopQ from the plant bacterial pathogen *Xanthomonas euvesicatoria* and Avr3b from the oomycete pathogen *Phytophthora sojae* act as important virulence factors (Yu et al., 2022). Yu et al. showed that AtNUD7, XopQ, and Avr3b possess 2^03^0-cAMP/cGMP phosphodiesterase activities to degrade these cNMPs and suppress cell death induced by TIRs (Figures 1C and 1D), further supporting the crucial functions of cNMPs in ETI (Yu et al., 2022).

PERSPECTIVES

It was recently reported that TIR proteins are induced by pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) (Chang et al., 2022). It would be interesting to find out whether and how the NADase and 2^03^0-cAMP/cGMP synthetase activities of TIRs contribute to PTI.

New structural and biochemical data show that TIR domains can adopt different oligomers with mutually exclusive NADase and synthetase activity (Figures 1A and 1B). The TIR domain represents an unprecedented example of two unrelated

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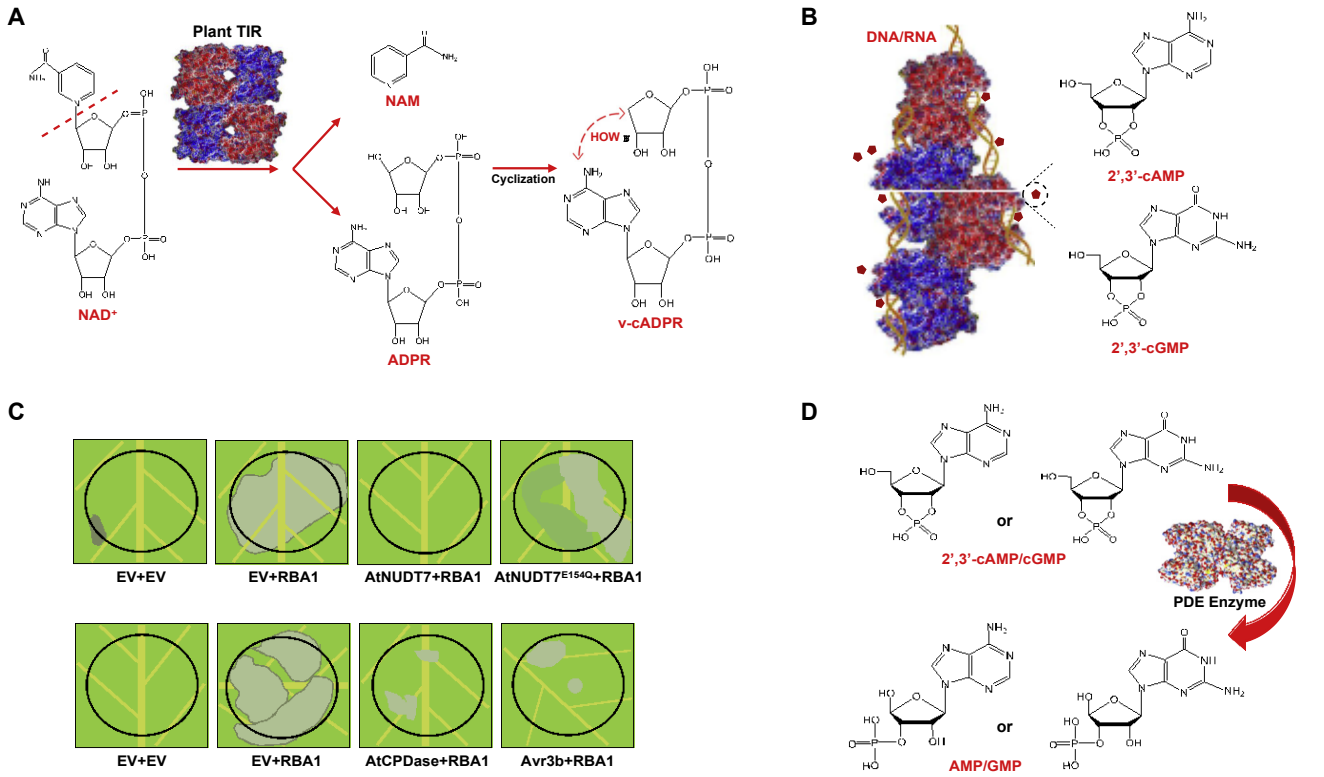

Figure 1. Plant TIR proteins display two enzymatic activities and the role of TIR's $2^0 3^0$-cAMP/cGMP synthetase activity in cell death.
(A) Plant TIR proteins function as NADase to degrade NAD^+ into nicotinamide, ADP-ribose, and a special variant of cyclic ADP-ribose.
(B) Plant TIRs exhibit $2^0 3^0$-cAMP/cGMP synthetase activity to generate $2^0 3^0$-cAMP/cGMP using DNA/RNA as substrates.
(C) The $2^0 3^0$-cAMP/cGMP synthetase activity is required for the TIR-only protein RBA1 to activate cell death in *Nicotiana benthamiana* plants. AtNudix7, AtCPDase, and the pathogen effector Avr3b from the oomycete pathogen *Phytophthora sojae* suppress RBA1-induced cell death because they function as phosphodiesterase to degrade $2^0 3^0$-cAMP and $2^0 3^0$-cGMP into AMP and GMP, respectively.
(D) Substitution of E154 with Q, which results in the loss of phosphodiesterase activity, compromises the ability of AtNUDT7 to suppress RBA-dependent cell death. The crystal structures of the TIR domain and phosphodiesterase were downloaded from Protein Data Bank (www.rcsb.org) and surfaced by Swiss-PdbViewer.

enzymatic activities within a single domain. The different oligomeric TIR structures, mediated through TIR–TIR interfaces, explain how a single domain can confer different enzymatic activities. It is not immediately clear why both enzymatic activities are needed and what would be the exact role of NADase and $2^0 3^0$-cAMP/cGMP synthetase in PTI, ETI, and abiotic stresses. The molecular and structural bases for the regulation and coordination of these two enzymatic activities will deepen our understanding of how plants cope with biotic and abiotic stresses. Whether and how the various enzymatic TIR nucleotide-based signaling molecules are generated *in planta* in a spatiotemporal sequence at attempted infection sites, e.g., in dying host cells and surrounding bystander cells, remain to be investigated.

TNLs require ADR1 and NRG1 families of helper NLRs to activate ETI. Both ADR1 and NRG1 helper NLRs function as calcium channels (Chen et al., 2021). Upon the activation of TIR-NLR (TNL), the EDS1-PAD4-ARD1 and EDS1-SAG101-NRG1 nodes will promote Ca^{2+} influx through Ca^{2+} channels formed by ADR1 and NRG1 families of helper NLRs (Sun et al., 2021). Both the enzymatic activities of TIRs and the channel activity of ADR1/NRG1 are required for TNL-mediated ETI. TIRs function upstream of ADR1/NRG1. Whether and how the NADase activity and/or $2^0 3^0$-cAMP/cGMP synthetase activity of TIRs contribute to the formation of Ca^{2+} channels remain to be investigated. $3^0 5^0$-cAMP and $3^0 5^0$-cGMP, especially $3^0 5^0$-cAMP, function as important second messengers, regulating many important biological processes in plants and mammals. Both $3^0 5^0$-cAMP and cADPR are known to cause calcium influx (Kurosaki and Nishi, 1993; Li et al., 2022).

Wounding, heat, and dark all induce the accumulation of $2^0 3^0$-cAMP/cGMP (Yu et al., 2022). Hence, it is likely that TIR proteins also mediate abiotic stresses in plants. This notion is also supported by the formation of stress granules induced by $2^0 3^0$-cAMP (Yu et al., 2022). Whether TIRs induce the formation of stress granules through $2^0 3^0$-cAMP/cGMP needs to be determined. The identification of the exact components in these putative ETI-induced stress granules will help us gain knowledge of plant immune responses.

Previous studies have already shown that both animal and plant TIRs exhibit NADase activities (Horsefield et al., 2019). Recently, a very exciting paper demonstrated that a bacterial TIR protein is involved in cell death and antiviral responses through its NADase

activity (Ofir et al., 2021). Thus, TIRs from animal, plants, and bacterial all can activate cell death dependent on their NADase activities. However, it has been shown both animal and bacterial TIRs cause NAD^+ depletion, while plant TIRs do not. Therefore, bacterial, animal, and plant TIR-induced responses share striking similarities and differences, suggesting that plant and animal TIRs are evolved from bacterial TIRs. More work is required to elucidate how bacteria recognize virus pathogens to activate TIR's NADase activity.

TIR domains are also widely distributed in animals and bacteria. Although Yu et al. have already shown that a bacterial TIR protein *Acinetobacter baumannii* TIR displayed no nuclease and 2^03^0-cAMP/cGMP synthetase activity (Yu et al., 2022), it remains to be determined whether animal and other bacterial TIR proteins possess 2^03^0-cAMP/cGMP synthetase activity.

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