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FOCUSED REVIEW

Plant NLRs are getting into higher-order architectures

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SUMMARY

Nucleotide-binding leucine-rich repeat (NLR) proteins are central components of the plant immune system that detect pathogen-derived effectors or modifications of host proteins within the cell to activate immune responses. NLRs have co-evolved with pathogens in an ongoing evolutionary arms race, leading to diversification of NLR structures and the establishment of highly interconnected immune networks. Recent studies have shown that NLRs dynamically assemble into higher-order structures, including multimeric complexes, filaments, and biomolecular condensates. These higher-order architectures are essential for NLR activation, intracellular signaling, and interactions with host proteins. Structural and biochemical analyses have revealed both conserved and divergent mechanisms underlying these assemblies. In this review, we summarize recent advances in understanding the formation and functional significance of higher-order NLR assemblies.

Keywords: effector-triggered immunity, nucleotide-binding leucine-rich repeat, high-order structures, resistosome, filament, biomolecular condensates.

INTRODUCTION

Plants are immobile organisms that are constantly exposed to diverse pathogens in their environment. Lacking an acquired immune system, plants rely solely on innate immunity to defend against pathogenic invasion (Dangl & Jones, 2001; Han, 2019). Plant innate immunity is broadly categorized into pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) based on the mode of pathogen recognition (Chisholm et al., 2006; Jones et al., 2024; Jones & Dangl, 2006; Ngou et al., 2022). PTI is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) through extracellular domains of membrane-localized receptor proteins, whereas ETI is triggered by the pathogen-secreted effectors that are recognized by intracellular resistance (R) proteins, most of which are nucleotide-binding leucine-rich repeat (NLR) proteins. NLRs have undergone extensive diversification during evolution as an adaptation to the wide variety of pathogens encountered by plants (Baggs et al., 2017; Barragan & Weigel, 2021) (Figure 1). NLRs are composed of three modules: an N-terminal domain, a nucleotide-binding adaptor shared by Apaf-1, R-protein, and CED-4 (NB-ARC), and a C-terminal leucine-rich repeat (LRR) domain (Dangl & Jones, 2001; Duxbury et al., 2021). These domains mediate signal transduction, oligomerization, and effector recognition, respectively (Förderer, Yu, et al., 2022).

NLRs are commonly classified based on their N-terminal domains into coiled-coil (CC)-NLRs (CNLs) and Toll/interleukin-1 receptor (TIR)-NLRs (TNLs) (Meyers et al., 2003). CNLs are phylogenetically divergent and subdivided into several clades, including singleton MADA-type CNLs, G10-type CC (CC_{G10})-NLRs, resistance to powdery mildew 8 (RPW8)-like CNLs (RNLs), and NLRs required for cell death (NRCs) (Contreras, Lüdke, Pai, et al., 2023; Guo et al., 2025; Lee et al., 2021; Shao et al., 2016). Upon activation, diverse CC domains of CNLs function as calcium-permeable ion channels, representing a conserved feature across divergent clades. This feature has been demonstrated in multiple CNL subclasses, including singleton MADA-CNLs (e.g., HOPZ-activated resistance 1, ZAR1;

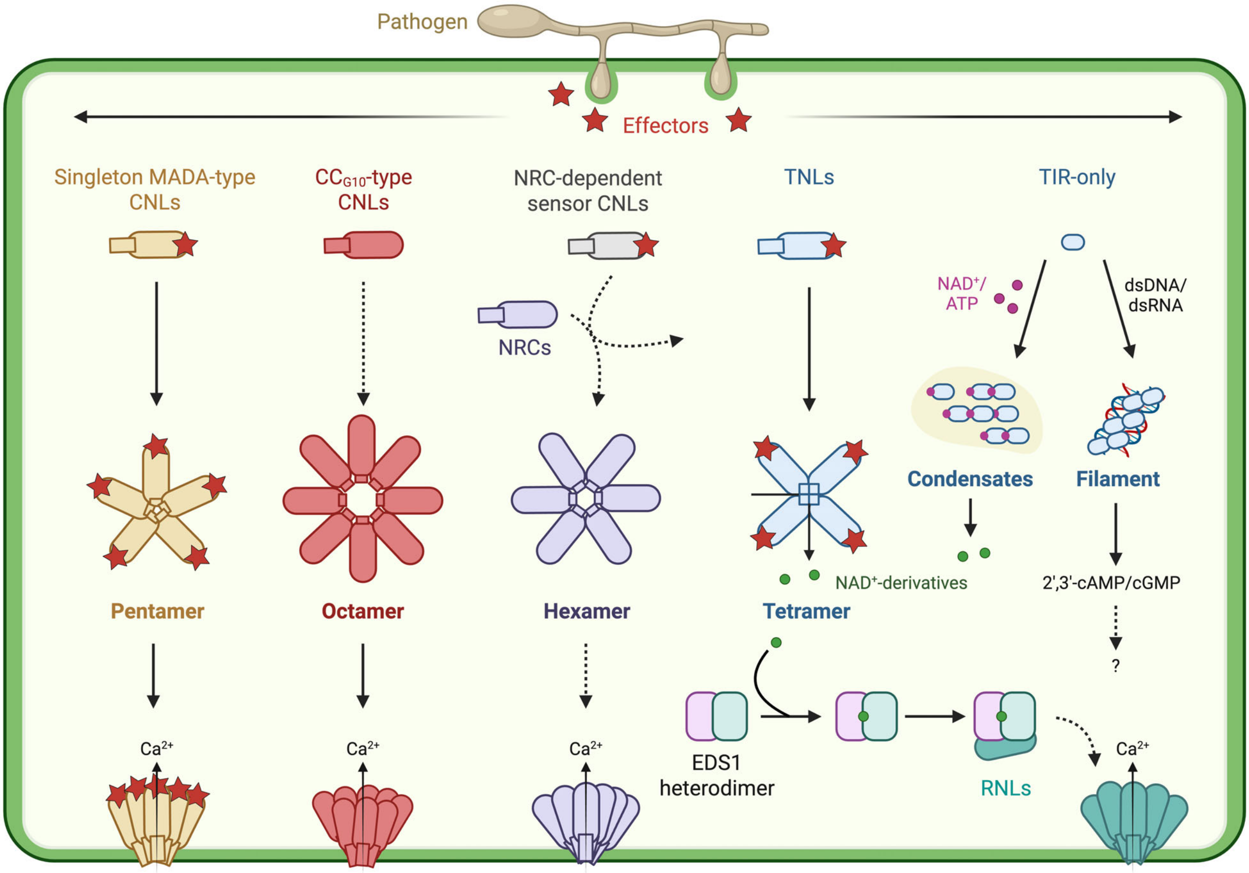

Figure 1. Overview of plant NLR-mediated immune signaling.
Plant NLRs assemble into diverse resistosomes to initiate effector-triggered immunity (ETI). Upon activation, singleton MADA-type CNLs assemble into pentameric resistosomes, whereas $\mathrm{CC}_{\mathrm{G10}}$-type CNLs form octameric resistosomes. In the NRC network, NRC-dependent sensor CNLs trigger the oligomerization of NRCs into hexameric resistosomes. These CNL assemblies function as Ca^{2+}-permeable channels to mediate calcium influx. Activated TNLs form tetrameric resistosomes that function as NADase holoenzymes. TIR-only proteins form condensates in the presence of NAD^{+}/ATP, enabling NADase activity. The NAD^{+}-derived small molecules produced by TNLs or TIR-only proteins bind to EDS1 heterodimers, which subsequently recruit helper RNLs to form Ca^{2+} channels. TIR-only proteins can also assemble into filaments upon binding dsDNA or dsRNA, functioning as 2′,3′-cAMP/cGMP synthetases. Dash lines indicate steps that remain to be elucidated. EDS1, enhanced disease susceptibility 1; NLR, nucleotide-binding leucine-rich repeat; NRC, NLRs required for cell death; TIR, toll/interleukin-1 receptor.

stem rust resistance 35, Sr35), $\mathrm{CC}_{\mathrm{G10}}$-NLRs (e.g., Wheat autoimmunity 3, WAI3; resistance to *Pseudomonas syringae* 2, RPS2), RNLs (e.g., activated disease resistance 1, ADR1; N-requirement gene 1.1, NRG1.1), and NRCs (e.g., NRC3, NRC4) (Bi et al., 2021; Förderer, Li, et al., 2022; Guo et al., 2026; Jacob et al., 2021; Liu et al., 2024). By contrast, the TIR domain of TNLs acts as an enzyme to produce signaling molecules (Horsefield et al., 2019; Wan et al., 2019). Structural studies have defined that the NB-ARC domain consists of three sub-domains: the nucleotide-binding domain (NBD), helical domain 1 (HD1), and winged-helix domain (WHD) (Albrecht & Takken, 2006; Wang, Hu, et al., 2019; Wang, Wang, et al., 2019). The NB-ARC domain binds to a nucleotide and functions as a molecular switch, mediating overall conformational changes that drive NLR activation (Tameling et al., 2006; Wang, Wang, et al., 2019). The LRR domain adopts a horseshoe-shaped structure that mediates direct or indirect effector recognition (Ade et al., 2007; Chen et al., 2022; Jia et al., 2000; Kobe & Deisenhofer, 1993). In some TNLs, an additional subdomain, such as a C-terminal jelly-roll/Ig-like domain (C-JID), is present following the LRR domain, which is involved in effector recognition (Ma et al., 2020; Martin et al., 2020; Saucet et al., 2021).

Functionally, NLRs can also be classified into sensor and helper NLRs (Bonardi et al., 2011). The sensor NLRs directly or indirectly recognize effectors, while the helper NLRs receive signals generated from the sensor NLRs, eventually triggering cell death (Contreras, Pai, Tumtas, et al., 2023). Sensor NLRs require helper NLRs to fully signal and execute cell death and mount disease resistance, while some CNLs function as standalone NLRs (singleton CNL) both sensing and leading to cell death.

Recent structural studies have revealed that multimerization plays a critical role in NLR-mediated immune signaling (Förderer & Kourelis, 2023; Wang, Chen, et al., 2023). In this review, we focus on recent structural studies of plant NLRs over the past decade, highlighting how NLRs adopt distinct inactive and active conformations (Figure 2) and assemble into diverse higher-order architectures.

DIVERSE ASSEMBLY OF SENSOR NLRS FOR IMMUNE ONSET

Upon immune response, NLRs undergo conformational changes and form into higher-order oligomeric complexes called 'resistosome' (Wang, Hu, et al., 2019). This precise structural rearrangement of NLRs is required for the N-terminal domain to become functional ion channels or active enzymes by bringing key residues into close spatial proximity (Bi et al., 2021; Ma et al., 2020). Recent advances in cryo-electron microscopy (cryo-EM) resolved the structures of diverse oligomeric complexes in high resolution and visualized their dynamic conformational transitions, revealing how distinct NLR subtypes initiate immune responses by forming higher-order structures (Table 1).

ZAR1 was the first plant CNL resistosome whose structure was elucidated (Wang, Hu, et al., 2019). The structural analysis of ZAR1 revealed well-defined conformational changes from the resting to the activated states. In the resting state, ZAR1 posits an auto-inhibited conformation by associating with members of the ZED1-related

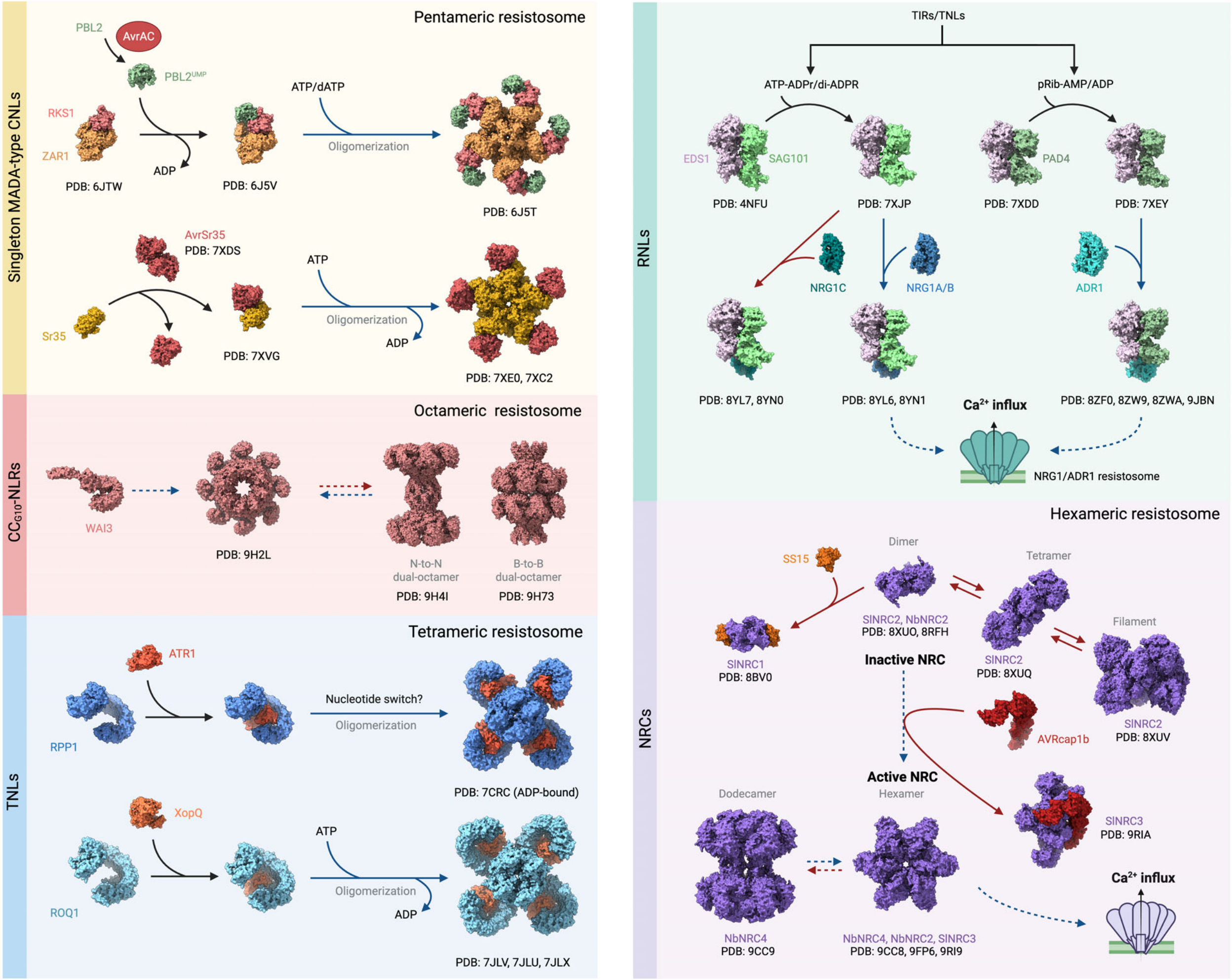

Figure 2. Structural diversity and assembly of plant NLR resistosomes.
The figure outlines activation mechanisms of major structurally characterized plant NLR classes. Activation induces NLR oligomerization, leading to the formation of diverse resistosomes. Blue and red arrows indicate activation and inactivation processes, respectively. PDB accession codes for the depicted structures are provided. Dashed lines indicate steps that remain to be elucidated.

Table 1 Structurally characterized plant NLRs

Functional types	Subtypes	Protein	Organisms	Oligomeric state	PDB ID	Components	References
Sensor NLRs	Singleton MADA-type CNLs	ZAR1	*Arabidopsis thaliana*	Heterodimer	6J5W	ZAR1-RKS1	Wang, Wang, et al. (2019)
		ZAR1	*Arabidopsis thaliana*	Heterotrimer	6J5V	ZAR1-RKS1-PBL2^{UMP}	Wang, Wang, et al. (2019)
		ZAR1	*Arabidopsis thaliana*	Pentamer	6J5T	ZAR1-RKS1-PBL2^{UMP}	Wang, Hu, et al. (2019)
		Sr35	*Triticum monococcum*	Heterodimer	7XVG	Sr35-AvrSr35	Zhao et al. (2022)
		Sr35	*Triticum monococcum*	Pentamer	7XE0, 7XC2	Sr35-AvrSr35	Zhao et al. (2022), Förderer and Kourelis (2023)
		MLA13	*Hordeum vulgare*	Heterodimer	9YFC	MLA13-AVRA13	Lawson et al. (2025)
	CC_{G10}-NLRs	WAI3	*Triticum aestivum*	Octamer	9H2L		Guo et al. (2026)
		WAI3	*Triticum aestivum*	Hexadecamer (N-to-N)	9H4I		Guo et al. (2026)
		WAI3	*Triticum aestivum*	Hexadecamer (B-to-B)	9H73		Guo et al. (2026)
	TNLs	RPP1	*Arabidopsis thaliana*	Tetramer	7CRC	RPP1-ATR1	Ma et al. (2020)
		ROQ1	*Nicotiana benthamiana*	Tetramer	7JLV, 7JLU, 7JLX	ROQ1-XopQ	Martin et al. (2020)
Helper NLRs	RNLs	NRG1C	*Arabidopsis thaliana*	Heterotrimer	8YL7, 8YN0	EDS1-SAG101-$\text{NRG1C}^{\text{WHD-LRR}}$	Xiao et al. (2025), Huang et al. (2025)
		NRG1A	*Arabidopsis thaliana*	Heterotrimer	8YL6, 8YN1	EDS1-SAG101-$\text{NRG1A}^{\text{WHD-LRR}}$	Xiao et al. (2025), Huang et al. (2025)
		ADR1	*Oryza sativa*	Heterotrimer	8ZF0	EDS1-PAD4-$\text{ADR1}^{\text{WHD-LRR}}$	Wu et al. (2024)
		ADR1	*Arabidopsis thaliana*	Heterotrimer	8ZW9, 8ZWA	EDS1-PAD4-$\text{ADR1}^{\text{WHD-LRR}}$	Yu et al. (2024)
		ADR1-L2	*Arabidopsis thaliana*	Heterotrimer	9JBN	EDS1-PAD4-$\text{ADR1-L2}^{\text{WHD-LRR}}$	Wang, Tan, et al. (2024)
	NRCs	SlNRC2	*Solanum lycopersicum*	Dimer	8XUO		Ma et al. (2024)
		SlNRC2	*Solanum lycopersicum*	Tetramer	8XUQ		Ma et al. (2024)
		SlNRC2	*Solanum lycopersicum*	Filament	8XUV		Ma et al. (2024)
		SlNRC2	*Solanum lycopersicum*	Heterodimer	8BV0	$\text{SlNRC2}^{\text{NB-ARC}}$-SS15	Contreras, Pai, Selvaraj, et al. (2023)
		NbNRC2	*Nicotiana benthamiana*	Dimer	8RFH		Selvaraj et al. (2024)
		NbNRC2	*Nicotiana benthamiana*	Hexamer	9FP6		Madhuprakash et al. (2024)
		SlNRC3	*Solanum lycopersicum*	Heterotetramer	9RIA	SlNRC3-AVRcap1b	Seager et al. (2025)
		SlNRC3	*Solanum lycopersicum*	Hexamer	9RI9		Seager et al. (2025)
		NbNRC4	*Nicotiana benthamiana*	Hexamer	9CC8		Liu et al. (2024)
		NbNRC4	*Nicotiana benthamiana*	Dodecamer	9CC9		Liu et al. (2024)

kinase (ZRK) family, such as resistance-related kinase 1 (RKS1) (Baudin et al., 2020). These associations are shown to be in a dynamic equilibrium state *in planta*. The heterodimeric structure of ZAR1 bound to RKS1 (PDB: 6J5W) revealed that the first α-helix of the CC domain is buried between the ADP-bound NBD and the LRR, making ZAR1 inactive (Wang, Wang, et al., 2019). Upon infection by *Xanthomonas campestris*, ZAR1 becomes fully activated by sensing the presence of the effector AvrAC (Wang et al., 2015). AvrAC uridylates a host protein, PBL1-like kinase 2 (PBL2), which is a member of the receptor-like cytoplasmic kinases (RLCK) family. The uridylated PBL2 (PBL2$^{\mathrm{UMP}}$) is then recognized by RKS1 within the ZAR1-RKS1 complex (Wang, Wang, et al., 2019). The binding of the uridylated PBL2 stabilizes an activation segment loop of RKS1, leading to a steric clash with the NBD. This clash induces an outward rotation of the NBD, facilitating the exchange of ADP with ATP (PDB ID: 6J5V). In the presence of ATP/dATP, extensive conformational changes occur in the ZAR1-RKS1-PBL2$^{\mathrm{UMP}}$ complex to form a wheel-shaped pentameric resistosome (PDB: 6J5T) (Wang, Hu, et al., 2019). In the activated state of ZAR1, a pentameric resistosome, the first α-helix of the CC domain, which was buried in the resting state, becomes exposed and forms a channel with a highly acidic inner surface (Bi et al., 2021). This acidic channel is inserted into the plasma membrane and functions as a calcium-permeable cation channel.

Another example of CNL forming into a pentameric resistosome is a wheat immune receptor Sr35, which also functions as a calcium-permeable cation channel (PDB: 7XE0, 7XC2) (Förderer, Li, et al., 2022; Zhao et al., 2022). While ZAR1 indirectly recognizes an effector via RKS1, Sr35 directly recognizes an AvrSr35 effector from *Puccinia graminis* f. sp. *tritici* (Bolus et al., 2020). AvrSr35 forms a homodimer (PBD: 7XDS), but notably, the structure of Sr35-AvrSr35 showed that Sr35 interacts with the dimerization interface of AvrSr35 (PDB: 7XVG), disrupting the dimerization of AvrSr35 (Zhao et al., 2022). The binding of AvrSr35 to the LRR domain of Sr35 leads to a steric hinderance with the NBD domain, allosterically activating the NBD domain to release ADP and bind to ATP. Through this process, Sr35-AvrSr35 forms a pentameric resistosome functioning as a channel, triggering immune activation (Förderer, Li, et al., 2022). This pentameric resistosome is self-sufficient to induce cell death, as expressing Sr35 in insect cells results in cell death.

ZAR1 and Sr35 harbor a conserved EDVID motif in the CC domain (Förderer, Li, et al., 2022). In the resistosome structures, the EDVID-mediated CC–LRR interaction contributes to positioning the LRR domain between the CC domain and the NB-ARC domain, resulting in an h-shaped configuration (Förderer, Li, et al., 2022; Guo et al., 2026; Wang, Hu, et al., 2019; Zhao et al., 2022).

The CC$_{\mathrm{G10}}$-NLRs, a phylogenetically distinct CNL clade, assemble into an octameric resistosome, as revealed by EM analysis of WAI3 (PDB: 9H2L) and RPS2 (Guo et al., 2026). Interestingly, CC$_{\mathrm{G10}}$-NLRs lack the EDVID motif (Guo et al., 2026; Seo et al., 2016). The structure of WAI3 resistosome revealed that the absence of the EDVID-mediated CC–LRR interaction allows the NBD to rotate substantially around a hinge connecting the CC and NBD domains, adopting an extended, j-shaped configuration (Guo et al., 2026). The active octameric WAI3 resistosome bound with ATP exhibits calcium channel activity, triggering cell death. WAI3 was also observed forming double-octamer assemblies. These double-octamer assemblies adopt two distinct arrangements: an N-to-N configuration (PDB: 9H4I) formed through the N-terminal CC domains of two octamers and a back-to-back (B-to-B) configuration (PDB:9H73) formed through the C-terminal LRR domains. Similar double-ring assemblies have been observed in other plant NLRs, including Sr35, NbNRC2, NbNRC4 (PDB: 9CC9), and RPS2 (Guo et al., 2026; Liu et al., 2024; Madhuprakash et al., 2024; Zhao et al., 2022). In other kingdoms, the *Drosophila* Apaf-1-related killer (Dark) apoptosome and the mammalian inflammasome formed by the NLR family apoptosis inhibitory protein (NAIP) together with the NLR family caspase recruitment domain-containing protein 4 (NLRC4) also exhibit double-ring assemblies, which are mediated by caspase recruitment domain (CARD)–CARD interactions (Halff et al., 2012; Matico et al., 2024; Yu et al., 2006). These double-ring assemblies are typically observed within the pool of activated resistosome, particularly at high protein concentrations (Guo et al., 2026; Halff et al., 2012; Liu et al., 2024; Yuan et al., 2011). The physiological role of these double-ring assemblies remains unclear. The N-to-N assemblies may represent a self-suppressive mechanism, thereby preventing excessive resistosome activation, as the N-terminal signaling domains are occluded in this configuration. However, it is also possible that these assemblies are artifacts due to high protein concentration, as they have not been observed in a native cellular environment.

Whereas CNL resistosomes promote ion flux through channel activity, in TNL resistosome, the enzymatic function residing in the TIR domains is activated upon oligomerization mediated by the effector recognition (Ma et al., 2020; Martin et al., 2020). Recent studies showed that TNLs, such as recognition of *Peronospora parasitica* 1 (RPP1) and recognition of XopQ 1 (ROQ1), recognize effectors via their LRR and C-JID domains and undergo conformational change, leading to the tetramerization of NB-ARC domains. The NB-ARC tetramerization re-positions the TIR domain in close proximity. Interestingly, the NB-ARC tetramer has a four-fold rotational symmetry, while the TIR domains connected with NB-ARC via a flexible linker forms an asymmetric tetramer with a two-fold rotational symmetry where the TIR domain assemble into a dimer of a dimer configuration, creating catalytic sites in the two of four dimeric interfaces (a.k.a. BE interface) in the TIR tetramer. The tetramer TNL resistosomes show full NADase activity residing in the TIR domains, hydrolyzing NAD^{+} to generate diverse NAD^{+}-derivatives as second messengers (Bayless et al., 2023, 2025; Huang et al., 2022; Jia et al., 2022; Wan et al., 2019; Yu et al., 2024).

ROQ1 possesses a conserved TTR motif in its NBD domain, in which the arginine residue (R329) strongly interacts with γ-phosphate of ATP, stabilizing the active state (Martin et al., 2020). While ROQ1 binds ATP/dATP upon activation, RPP1 is found to bind with ADP in its active state (Ma et al., 2020; Martin et al., 2020). RPP1 contains a negatively-charged glutamate (E400) instead of arginine, unable to hold the γ-phosphate (Ma et al., 2020). Instead, RPP1 possesses a long β2-α2 loop within the NBD which interacts with the groove between the NBD and WHD of an adjacent protomer, stabilizing the resistosome assembly. The presence of ADP in the RPP1 resistosome could be due to rapid hydrolysis of ATP by its high intrinsic ATPase activity. Alternatively, the canonical nucleotide switch from ADP to ATP does not occur during RPP1 activation. However, further investigation is required to determine the mechanism of nucleotide exchange and hydrolysis in RPP1.

While singleton NLRs such as ZAR1 and Sr35 are capable of both sensing effectors and triggering cell death on their own, numerous sensor NLRs require additional NLRs, broadly categorized under CNLs, as helper NLRs (Bonardi et al., 2011; Contreras, Lüdke, Pai, et al., 2023).

DIVERSE ASSEMBLY OF HELPER NLRS TO DRIVE IMMUNE ACTIVATION

Sensor-helper NLRs form a network with a many-to-one and one-to-many organization (Contreras, Lüdke, Pai, et al., 2023; Wu et al., 2017, 2018). In this network, sensor and helper NLRs are functionally specialized for effector recognition and immune signaling execution, respectively. In the many-to-one mechanism, plants use diverse sensor NLRs that exhibit evolvability to cope with a wide range of pathogens and can integrate signals from several sensor NLRs via a small set of core helper NLRs (Adachi et al., 2019; Wu et al., 2017). However, this mechanism has a potential vulnerability in that the immune signals from multiple upstream sensor NLR pathways can be disrupted by pathogen targeting core helper NLRs (Contreras, Pai, Selvaraj, et al., 2023; Derevnina et al., 2021; Seager et al., 2025). In one-to-many organization, plants convey signals from one sensor NLR to multiple helper NLRs, providing immune robustness (Wang, Liu, et al., 2023; Wu et al., 2017). Sensor-helper NLRs represent one of the evolutionary strategies adopted in plant immunity, alongside singleton NLRs.

The major helper NLR families include N-requirement gene 1 (NRG1) and activated disease resistance 1 (ADR1), which are involved in signaling downstream of TIR-assemblies (Bonardi et al., 2011; Peart et al., 2005). NRG1 and ADR1 contain the RPW8-like CC domain at the N-terminus and are classified as RNLs (Collier et al., 2011). Those RNLs work together with the lipase-like protein enhanced disease susceptibility 1 (EDS1), which forms exclusive complexes with phytoalexin deficient 4 (PAD4) or senescence-associated gene 101 (SAG101) to mediate immune signaling (Sun et al., 2021). NAD$^+$-derivatives generated by TIR-assemblies bind to EDS1 complexes (Huang et al., 2022; Jia et al., 2022). The EDS1-SAG101 heterodimeric complex recognizes NAD$^+$ derivatives such as ADPr-ATP and di-ADPR, and undergoes a conformational change that enables the EDS1 complex to associate with NRG1 (Huang et al., 2025; Jia et al., 2022; Xiao et al., 2025). Structural studies on the NRG1 WHD-LRR domain show that it utilizes LRR domain to interact with the C-terminal α-helical domain of the EDS1-SAG101 complex, known as the EP domain. This interaction is crucial for NRG1 oligomerization, which triggers cell death (Huang et al., 2025; Xiao et al., 2025). The EDS1-SAG101 complex binds and promote the oligomerization of NRG1. However, the full conformational trajectory of NRG1 activation remains unknown, as such the mechanism by which NBDs become exposed to drive oligomerization of NRG1 and the final stoichiometry of the finally activated resistosome. It is currently unclear whether the EDS1-SAG101 complex is part of the final NRG1 resistosome. Some studies have observed its association with NRG1 oligomers at early stages of assembly or under conditions where ETI and PTI are co-activated (Feehan et al., 2023; Huang et al., 2025), whereas other studies could not detect the EDS1-SAG101 complex in the final NRG1 oligomers (Wang, Song, & Chai, 2023; Xiao et al., 2025). This difference may be attributed to variations in the time points at which the process was captured and in the experimental conditions (Huang et al., 2025; Xiao et al., 2025). Further investigation is required to clarify the composition and dynamics of the NRG1 resistosome. Time-resolved analysis coupled with cross-linking or stabilizing mutations would be helpful to trace the sequential compositional changes of the NRG1 complex, and structural analysis using plasma membrane fractions may allow for the characterization of the final, functional oligomeric complex.

Another EDS1-PAD4 heterodimeric complex binds to pRib-AMP/ADP and induces the assembly of another helper NLR, ADR1, in a similar manner to NRG1 (Huang et al., 2022; Wang, Tan, et al., 2024; Wu et al., 2024; Yu et al., 2024). Oligomerized RNLs via EDS1 complexes localize to the plasma membrane and function as calcium-permeable cation channels (Jacob et al., 2021; Wang, Song, & Chai, 2023). Intriguingly, the P-loop motif of ADR1-L2, which is typically essential for ATP binding and oligomerization of NLRs, is dispensable for its function in RPS2-mediated resistance (Bonardi et al., 2011). This P-loop independent activity suggests that ADR1 may also acts as a signaling scaffold to associate with EDS1-PAD4 for transcriptional reprogramming via a non-resistosome mechanism, similar to the barley NLR mildew A (MLA) which interacts with transcription factors via its CC domain (Bonardi et al., 2011; Chang et al., 2013; Wang, Song, et al., 2024).

There is another type of CNL working as a helper NLR, called 'NLRs required for cell death (NRC)', which is found in *Solanaceae* plants and operates independently of the EDS1 complex (Wu et al., 2017). Unlike typical singleton CNLs, which form pentameric resistosomes, structural studies on several NRCs including NRC0, NbNRC2, SlNRC3, and NbNRC4 revealed that these NRCs form hexameric resistosomes (PDB: 9CC8, 9FP6, 9RI9) (Liu et al., 2024; Madhuprakash et al., 2024; Seager et al., 2025). These hexameric resistosomes are in their active states and drive calcium influx to activate immunity (Liu et al., 2024). The activated NRC4 forms punctate structures at the plant cell plasma membrane, suggesting that these resistosomes insert into the plasma membrane to trigger programmed cell death (Duggan et al., 2021). However, the NRC mutants (NRC2, NRC3, NRC4) mimicking the active state failed to induce calcium influx or cell death in animal cells (Liu et al., 2024), while other NLRs such as ZAR1, Sr35, and NRG1A can function autonomously in these heterologous systems (Bi et al., 2021; Förderer, Li, et al., 2022; Jacob et al., 2021). This observation suggests that other plant components may be required for the full activation of NRCs (Liu et al., 2024).

As the activation of NRC causes cell death, cells would need to devise multiple ways to regulate the NRC function to prevent unwanted immune responses. Structures of NRC in diverse multimeric forms were reported. These multimeric forms seem to be inactive states, as the disruption the multimeric interface leads to hyper-activation of NRC (Ma et al., 2024; Selvaraj et al., 2024). In case of SlNRC2, it forms a homodimer stabilized by electrostatic interactions between the LRR–LRR and LRR–NBD interfaces, forming a head-to-head arrangement (PDB: 8XUO) (Ma et al., 2024). In this dimer structure, the N-terminal coiled-coil domains were not visible in the cryo-EM structure. In the same study, a tetrameric SlNRC2 structure was reported, showing that SlNRC2 dimers associate in a back-to-back configuration (PDB: 8XUQ). In this tetrameric SlNRC2, the CC domains interact with the LRR domains from the adjacent dimer. Furthermore, higher-order filamentous form of SlNRC2 was also observed as an inactive form (to be further discussed in later section). Such higher-order assemblies may serve as structural safeguards that stabilize the inactive state. Compared with the fully-active hexameric NbNRC2 structure (PDB: 9FP6), the interfaces observed in the inactive dimer of NbNRC2 (PDB: 8RFH) are substantially different to the interfaces in hexameric resistosome (Madhuprakash et al., 2024; Selvaraj et al., 2024). This observation suggests that cells prevent NRC from forming an active hexamer by sequestering NRC into distinct oligomer structures. In addition, NbNRC4 were also reported to exist as inactive homodimers (Liu et al., 2024). The formation of an auto-inhibited structure through LRR–LRR interactions in the resting state is also observed in the animal NLR, NLR family pyrin domain-containing 3 (NLRP3) (Andreeva et al., 2021; Ma et al., 2024). In its inactive structure, NLRP3 assembles into a cage structure through face-to-face interactions, in which the concave inner surfaces of the LRR domains interlock like hooks and back-to-back interactions between their convex outer surfaces (Andreeva et al., 2021; Hochheiser et al., 2022). This arrangement traps the N-terminal pyrin domain (PYD), which serves as the signaling domain, within the inner cavity of the cage. This is similar to the inhibitory strategy of SlNRC2, in which LRR–LRR interactions drive inhibitory oligomerization that sequesters the signaling CC domain via LRR-CC contacts (Ma et al., 2024).

The NRCs receive activation signals from several different sensor NLRs including Resistance to Potato Virus X (Rx), Bacterial Spot resistance gene 2 (Bs2), Tomato Spotted Wilt Virus resistance gene 5b (Sw5b), *Meloidogyne incognita* resistance gene 1.2 (Mi-1.2), and Pseudomonas resistance and fenthion sensitivity (Prf) (Contreras, Lüdke, Pai, et al., 2023; Wu et al., 2015, 2017). Rx and Bs2 are CNLs lacking the MADA motif, and Sw5b, Mi-1.2, and Prf are CNLs harboring an N-terminal solanaceous domain (SD). NRCs appear to undergo oligomerization through transient interactions with sensor NLRs, as sensor NLRs are not detected in the NRC resistosomes (Ahn et al., 2023; Huang et al., 2023; Liu et al., 2024; Madhuprakash et al., 2024; Seager et al., 2025). The finding that the NBD of the sensor NLR Rx is sufficient to induce NRC2 oligomerization, while this activity is abolished by P-loop mutation in the NBD, suggests that sensor NLRs undergo a conformational change to expose a primed NBD surface in a nucleotide-dependent manner to activate NRCs (Contreras et al., 2024). This observation further suggests that a primed NBD surface of the sensor NLR may act as the activating signal, promoting the helper NRC oligomerization through the interaction between NBDs of the sensor NLR and NRC.

However, since a stable complex is not observed between sensor NLRs and NRCs, the molecular mechanism by which sensor NLRs transmit signals to NRCs remains unknown. To investigate the sensor NLR–NRC interaction, various approaches need to be attempted to capture transient protein–protein interactions, among which chemical cross-linking or mutants that enhance the sensor NLR–NRC interaction can be considered. In addition, it is worthwhile to apply time-resolved cryo-EM to capture dynamic transitions.

Oligomerization of helper NLRs is crucial for regulating their activation state, and pathogens target this process to suppress immune responses (Derevnina et al., 2021). SPRYSEC15 (SS15), an effector of *Globodera rostochiensis*, binds to the HD1 of NRCs and prevents the oligomerization of NRCs, keeping them in an inactive state (PDB: 8BV0) (Contreras, Pai, Selvaraj, et al., 2023). Another effector protein, AvrCap1b from *Phytophthora infestans*, binds to the intermediate oligomeric state of NRCs and prevents it from further oligomerization to become the active NRC resistosome (Seager et al., 2025). The recent cryo-EM structure of AVRcap1b-SlNRC3 complex (PDB: 9RIA) shows that AVRcap1b adopts an L-shaped structure and interacts with the trimeric form of SlNRC3. AVRcap1b masks the oligomerization interface of the SlNRC3, therefore preventing further oligomerization. Furthermore, AVRcap1b also forms a complex with the epsin N-terminal homology (ENTH) domain of NbTOL9a (PDB: 9RDC), a host protein involved in the endosomal sorting complexes required for transport (ESCRT) pathway (Madhuprakash et al., 2025). The interface between AVRcap1b and NbTOL9a is distinct from the one observed in the AVRcap1b-NbNRC2 complex, suggesting the potential formation of a ternary complex consisting of the effector, NLR, and host protein. These observations imply the possibility that NRC intermediate could be hijacked by the host cell machinery and targeted for degradation, which could suppress NRC-mediated cell death. Notably, depletion of NbTOL9a results in an enhanced cell-death phenotype mediated by auto-active NRC2/NRC3 in *Nicotiana benthamiana* leaves (Derevnina et al., 2021).

Helper NLR function (NRG1A/NRG1B) can also be suppressed through the host-intrinsic negative regulator, NRG1C (Huang et al., 2025; Wu et al., 2022; Xiao et al., 2025). NRG1C is a helper NLR lacking the N-terminal region, which is critical for helper NLR function. NRG1C has a higher binding affinity than NRG1A and NRG1B toward the EDS1-SAG101 complex, leading to formation of inactive NRG1C-EDS1-SAG101 complex (Huang et al., 2025; Xiao et al., 2025). The expression of NRG1C is highly induced in response to ETI signaling triggered in autoimmune mutants or by pathogen infection, which is thought to represent a host self-protective mechanism that prevents excessive immune activation (Wu et al., 2022). These observations suggest that pathogens may take advantage of the host-intrinsic immune regulatory mechanisms.

In addition to helper NLR-mediated networks, plants have evolved another regulatory strategy to control NLR activation in the form of paired NLR systems (Adachi et al., 2019; Cesari et al., 2014). In these systems, two genetically linked NLRs are functionally specialized into a sensor and an executor that act together to regulate immune responses (Ashikawa et al., 2008; Narusaka et al., 2009; Okuyama et al., 2011; Xu et al., 2015). The sensor regulates the executor's activity in negative or cooperative manners (Césari et al., 2014; Williams et al., 2014; Yang et al., 2022; Zdrzałek et al., 2020). For example, in the cases of resistance gene analog 5/resistance gene analog 4 (RGA5/RGA4), resistance to *Ralstonia solanacearum* 1/resistance to *Pseudomonas syringae* 4 (RRS1/RPS4), and chilling sensitive 3/constitutive shade-avoidance 1 (CHS3/CSA1) (specifically clade 1 CHS3 variants) pairs, the sensor NLR functions as a negative regulator, suppressing the auto-activity of the executor within the pre-activation heterocomplex (Césari et al., 2014; Williams et al., 2014; Yang et al., 2022). By contrast, the Pik-1/Pik-2 pair functions cooperatively, as the Pik-2 executor is not auto-active and requires the Pik-1 sensor to trigger immune responses (Maqbool et al., 2015; Zdrzałek et al., 2020). These paired NLRs are likely to exist as pre-assembled complexes in the resting state, which may undergo conformational changes upon activation to become fully active (Bentham et al., 2023; De la Concepcion et al., 2021; Zdrzałek et al., 2020). However, the structure of paired NLR heterocomplexes remains poorly characterized. Therefore, future structural studies determining the structures of paired NLR heterocomplexes in both their inactive and active states will be essential for elucidating the molecular mechanisms underlying heteromeric NLR signaling.

Collectively, NLRs regulate immune responses through diverse oligomeric states. A wide range of NLRs exist as pre-assembled heterocomplexes with host proteins (e.g., ZAR1-RKS1) or partner NLRs (paired NLRs), in their resting state (Bentham et al., 2023; Tran et al., 2017; Wang, Wang, et al., 2019; Williams et al., 2014), whereas others, like NRC2, form homo-complexes (Ma et al., 2024; Selvaraj et al., 2024). These complexes remain inactive until specific immune cues are recognized, such as direct effector binding or the perception of modified host proteins. These mechanisms ensure that NLR-mediated immune responses are triggered only when sufficient signals are present. The differences in oligomeric states are closely linked to the functional requirements of each NLR. TNLs typically form a tetramer to cluster at least two TIR domains for efficient NAD^+ hydrolysis, whereas CNLs assemble into oligomers ranging from pentamers to octamers to form an ion channel. These diverse oligomeric states are determined by the intrinsic structural features of NLRs. For instance, the flexibility of the linker between the N-terminal and NB-ARC domains and the angle between the HD1 and WHD domains appear to be major determinants of the number of subunits in the resistosomes (Guo et al., 2026; Martin et al., 2020). This structural diversity represents an evolutionarily optimized adaptation that allows NLRs to perform their immune functions efficiently and accurately.

FILAMENTOUS ASSEMBLIES OF IMMUNE PROTEINS TO REGULATE IMMUNE RESPONSE

Certain plant NLR can assemble into filamentous structures. SlNRC2 forms into filaments (PDB: 8XUV) under high concentration and a filament is composed of three protofilaments twisting helically around each other (Ma et al., 2024). Each protofilament elongates through the sequential addition of dimeric units of SlNRC2. In these protofilaments, the inactive form of SlNRC2 in dimers and tetramers is observed, suggesting that the filamentous SlNRC2 is also in an inactive form. This filamentous form of NRC2 was also observed in *N. benthamiana* cells through live-cell imaging (Duggan et al., 2021). These observations raise the possibility that filament formation may serve as a regulatory strategy in certain members of the NRC family.

Some plant TIR domains are capable of assembling into filamentous structures (Yu et al., 2022). The TIR domain of flax L7 (L7^{TIR}) forms into filaments in the presence of double strand DNA (dsDNA) or RNA (dsRNA) under *in-vitro* conditions. A filament is formed with two L7^{TIR} protofilaments with the dsDNA/RNA mediating two protofilaments. In the absence of the nucleic acids, L7^{TIR} forms a low-molecular-weight oligomeric form. The L7^{TIR} exhibited dual enzymatic activities *in-vitro*: NADase and 2′,3′-cAMP/cGMP synthetase. These enzymatic activities seem to be modulated by its assembly modes. While the low-molecular-weight form of L7^{TIR} exhibits a NADase activity, presumably via an asymmetric TIR arrangement, L7^{TIR} domains in the filamentous form are arranged symmetrically and lack NADase activity. Instead, the filamentous form shows a 2′,3′-cAMP/cGMP synthetase using nucleotides hydrolyzed from the L7^{TIR} bound dsDNA/RNA. When the interfaces required for L7^{TIR} filament formation are mutated without altering the catalytic residues, L7^{TIR} retains NADase activity but loses 2′,3′-cAMP/cGMP synthetase activity, suggesting these two enzymatic activities are separable and the oligomerization mode switches these two activities. However, the catalytic mechanism by which L7^{TIR} hydrolyzes nucleotides and cyclizes them into cyclic nucleotide is not well defined. While the physiological role of the 2′,3′-cAMP/cGMP synthetase activity is not clear, it has been shown that 2′,3′-cAMP/cGMP is essential for TIR-mediated cell death. The filament formation is also observed in other plant TIR domains such as L6^{TIR}, recognition of HopBA1 (RBA1), and TX0, suggesting that filament formation may represent a common feature of plant TIR proteins, although its functional relevance remains to be elucidated.

Filament formation seems to be one of general strategies to regulate immune response in different kingdoms (Kobe et al., 2025; Park & Horton, 2019). The N-terminal PYD and CARD of animal NLRs self-oligomerize into filaments (Li et al., 2018; Lu et al., 2014). PYD filaments function as nucleation sites for inflammasome assembly, and CARD filaments act as platforms to recruit and activate host proteins such as caspase-1. Interestingly, other TIR domain-containing immune proteins, such as CRISPR-associated TIR protein 1 (Cat1), TIR-SMODS-associated with variable effector domains (SAVED), TIR-stimulator of interferon genes (STING), TIR-containing protein from *Acinetobacter baumannii* (AbTIR), and the animal sterile alpha and TIR motif-containing 1 (SARM1), exhibit NADase activity when assembled into filaments (Baca et al., 2025; Hogrel et al., 2022; Manik et al., 2022; Morehouse et al., 2022; Zhang et al., 2026). Unlike plant L7^{TIR} filament, which forms symmetric assemblies on nucleic acid scaffolds to function as a synthetase (Yu et al., 2022), these TIR filaments exhibiting NADase activity adopt an asymmetric head-to-tail arrangement (Baca et al., 2025; Hogrel et al., 2022; Horsefield et al., 2019; Manik et al., 2022; Morehouse et al., 2022). Mechanistically, this asymmetric arrange is critical for forming a composite active site essential for NADase activity by enabling the BB-loop to interact with the adjacent TIR domain. Taken together, these observations suggest that TIR domains possess an intrinsic structural potential to adopt either symmetric or asymmetric assemblies, and their enzymatic function appears to depend on the scaffold that enforces a particular mode of arrangement. Plant TIR domains may utilize negatively-charged nucleic acids as assembly scaffolds using a conserved positively charged surface on the αD helix (Yu et al., 2022), whereas TIRs in a different arrangement rely on adjacent oligomerization domains (Hogrel et al., 2022; Manik et al., 2022; Morehouse et al., 2022).

Filamentation confines the distribution of proteins to localize their activities and promotes the formation of a stable complex with repeating interaction sites, functioning as a scaffold. In addition, it allows proteins to interact in a specific arrangement to gain catalytic activity, which a monomer does not have. In particular, plant TIR-only proteins may more readily form higher-order assemblies than full-length NLRs having large structural domains. Thus, the TIR-only proteins could adopt filamentous structures to enhance or modulate their enzymatic activity. Moreover, filament formation can be triggered by physiological conditions, such as the presence of specific metabolites, and can also serve to sequester proteins in an inactive state. Altogether, filamentation emerges as a widespread strategy to fine-tune protein localization and functional regulation.

BIOMOLECULAR CONDENSATES OF IMMUNE PROTEINS FOR EFFICIENT IMMUNE RESPONSE

Biomolecular condensates are membraneless organelles, ranging from nanometer to micrometer scales, that enable efficient and selective regulation of biochemical reactions through internal compartmentalization (Banani et al., 2017). Condensates typically form through liquid–liquid phase separation (LLPS), maintained by multivalent interactions such as electrostatic and hydrophobic interactions, which often occur through intrinsically disordered regions (IDRs) of proteins. The characteristics of these weak multivalent interactions allow dynamic molecular exchange (Das et al., 2020; Li et al., 2012). Biomolecular condensates can exist not only in liquid-like states, but also as gel-like and solid-like states (Murakami et al., 2015; Shen et al., 2023). A liquid-like state can transition to a gel-like state through a process called maturation, in which transient interactions gradually strengthen over time or under specific cellular conditions, leading to a more rigid and gel-like network (Lin et al., 2015; Monahan et al., 2017; Ranganathan et al., 2022). This network reduces molecular mobility but still retains some flexibility. However, molecular interactions of condensates become highly ordered to form a solid-like state, often resulting in irreversible structures. Biomolecular condensates are found in various subcellular locations, including the nucleus, cytoplasm, and plasma membrane (Banani et al., 2017). These condensates display distinct chemical properties such as charge densities, creating a unique microenvironment that can modulate biochemical reactions (Posey et al., 2024). Recent studies have shown that plant NLR-related proteins are finely regulated through the formation of biomolecular condensates (Figure 3) (Song et al., 2024; Wan et al., 2025; Wang, Tan, et al., 2024).

The TIR domains from RPP1 (RPP1^{TIR}) and the TIR-only proteins RBA1 and TX14 form phase-separated condensate *in vitro* in the presence of substrates such as NAD$^+$ and ATP (Song et al., 2024). Fluorescence recovery after photobleaching (FRAP) experiments showed that RPP1^{TIR} displays characteristics of LLPS, whereas RBA1 and TX14 form gel-like condensates with relatively lower dynamics than RPP1 condensate. These condensate formations likely provide a physical environment that favors the enzymatic activities by clouding the TIR domains. The physiological role of the biomolecular condensate has been shown in a TIR-only immune protein, RBA1. Introducing the effector HopBA1 in *Arabidopsis* induces RBA1 condensation, demonstrating that TIR condensate is involved in immune responses. In addition, TIR proteins such as RPP1^{TIR} and TX14 overexpressed in *N. benthamiana* leaf cells form nuclear and peri-nuclear puncta and nuclear-cytoplasmic puncta, respectively. Another TIR-only protein, *N. benthamiana* stomatal TIR 1 (NbSTIR1), has also been reported to form condensates *in vitro* in the presence of NAD$^+$ (Wang, Tan, et al., 2024). *In planta*, NbSTIR1 forms an inactive complex with *N. benthamiana* inhibitor of stomatal immunity C2-domain protein 1 (NbISIC1), and the cytosolic calcium influx releases the inhibitory effect of NbISIC1, allowing NbSTIR1 to form condensates, which

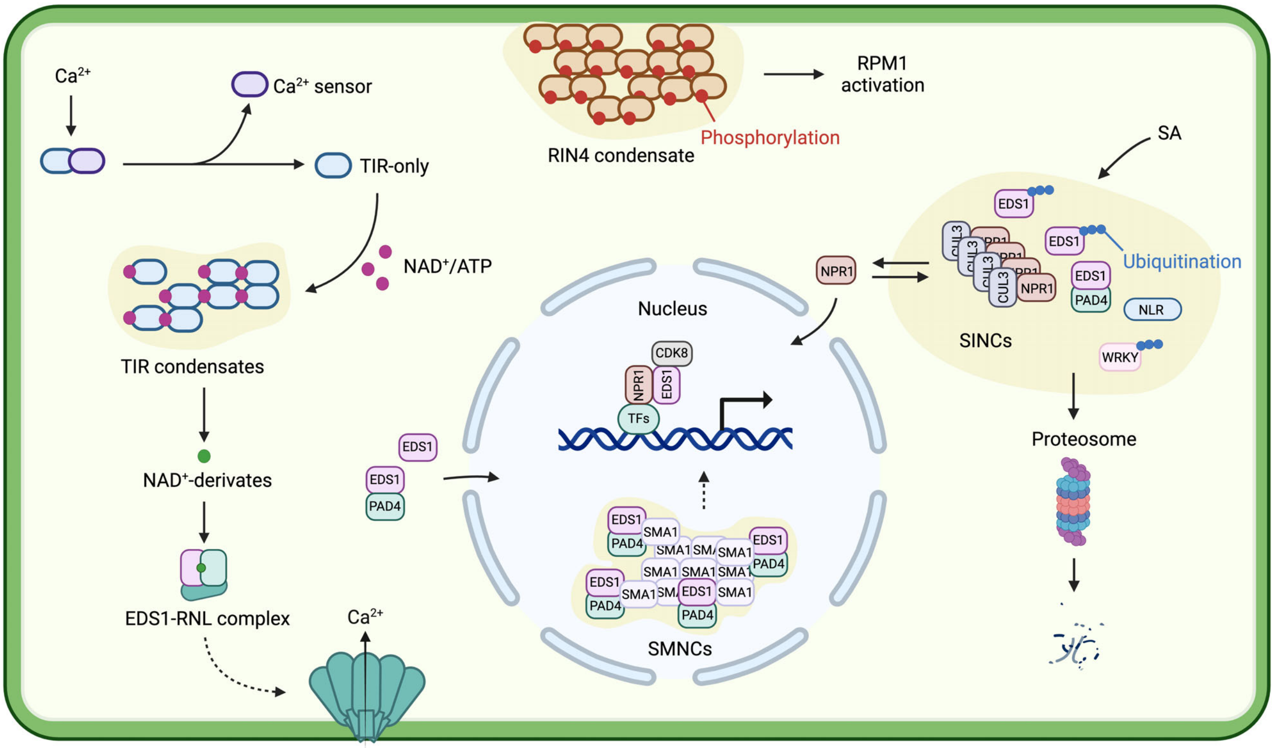

Figure 3. Biomolecular condensates mediate plant immune signaling.

Certain TIR-only proteins exist in an inhibited state bound to Ca^{2+} sensors but are dissociated and activated upon pathogen-triggered Ca^{2+} influx. These activated TIR-only proteins undergo phase separation in the presence of NAD$^+$ and ATP, forming biomolecular condensates with NADase activity. RIN4 forms condensates that facilitate its phosphorylation, thereby activating RPM1. Other immune proteins, such as NPR1 and SMA1, co-condense with EDS1 to fine-tune immune activity and homeostasis. In the nucleus, EDS1 functions as a transcriptional coactivator that cooperates with NPR1 and cyclin-dependent kinase 8 (CDK8) to promote the transcription of defense genes (Chen et al., 2021). EDS1, enhanced disease susceptibility 1; TIR, toll/interleukin-1 receptor.

are essential for its NADase activity and triggering cell death. This calcium-dependent inhibitory-release mechanism appears conserved across plant species. For instance, the *Oryza sativa* TIR-only protein (OsTIR) is inhibited by the calcium sensor resistance of rice to diseases 1 (ROD1), but this inhibition is relieved upon calcium influx, enabling OsTIR to activate (Wu et al., 2024). In addition to TIR-only proteins, a recent study has shown that the full-length TNL DM2h/RPP1 forms condensates in the nucleus in the presence of its immune-triggered partner, DM3$^{\text{Hh-0}}$ (Wan et al., 2025). This finding suggests that not only TIR domains but also full-length TNLs may utilize phase separation as a mechanism for activation.

Interestingly, the EDS1/PAD4 complex, the central hub of TIR/TNL signaling, forms biomolecular condensates with host regulators, including non-expressor of pathogenesis-related PR 1 (NPR1) and the DEAD-box protein SMALL 1 (SMA1) upon salicylic acid (SA) induction (Li et al., 2025; Zavaliev et al., 2020). These condensates are termed SA-induced NPR1 condensates (SINCs) and SMA1-mediated nuclear condensates (SMNCs), respectively. These biomolecular condensates play critical roles in regulating the TIR/TNL signaling. SINCs are formed in the cytoplasm and contain several NLRs, such as RPP1 and RPP5, as well as the E3 ubiquitin ligase Cullin 3 (CUL3), which ubiquitylate EDS1 leading to its degradation (Zavaliev et al., 2020). It would be interesting to investigate whether the NLRs within SINCs are also targeted for degradation by CUL3. While SINC functions in the cytoplasm, SMNC recruits EDS1 to the nucleus, leading to the expression of defense genes (Li et al., 2025).

RPM1-interacting protein 4 (RIN4), which interacts with NLRs such as RPM1, also forms nanoscale condensates at the plasma membrane, facilitating the phosphorylation of RIN4 by RPM1-interacting protein kinase (RIPK) (Zhu et al., 2025). This RIN4 condensate acts as a hub for immune response activation and is targeted by a pathogen to compromise immune response. XopR effector infiltrates and disrupts the RIN4 condensates, compromising RPM1-mediated immune response. This observation suggests that phase separation provides a favorable environment for the function of immune proteins and that a pathogen has co-evolved to target this biomolecular condensate.

Biomolecular condensates and filaments have distinct properties regarding their organization, but recent studies suggest that they are closely interconnected. For example, the bacterial polar organizing protein Z (PopZ), which regulates asymmetric cell division, forms condensates via LLPS that contain filamentous structures within (Scholl et al., 2026). This filament structure increases the viscosity and surface tension of the condensates, enhancing their structural stability. Another example is vimentin, which forms liquid-like condensates suggested to serve as precursors for filament formation (Basu et al., 2025; Prahlad et al., 1998). Vimentin forms punctate structures having liquid-like properties and these condensates eventually develop into filamentous assemblies (Basu et al., 2025). Unlike the biomolecular condensates, the filamentous form remains stable even under conditions that dissolve the condensate. Crosstalk between biomolecular condensates and filaments suggests that these assemblies may be interconnected processes, in which increased local concentration of subunits within condensates facilitates their transition into another oligomeric form: filament formation.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Over the past decade, structural elucidation of plant NLRs has provided crucial insights into their modes of action (Box 1). However, there are several questions that remain to be elucidated: how sensor NLRs induce helper NLRs oligomerization, what plant factors mediate the insertion of the cell-death executing resistosome into the plasma membrane, and how RNLs oligomerize to form the final resistosomes (Ahn et al., 2023; Liu et al., 2024; Wang, Song, & Chai, 2023). In addition, it is not clear how an inactive NLR undergoes a conformational change to become activated as there is little structure available in an inactive state. Therefore, structural analysis on the inactive form of NLR will be essential to understand the fine-tuning of either membrane function or TIR-enzymatic function.

Despite the presence of various NLRs, the structural information is limited to a subset of NLRs, largely due to their intrinsic conformational dynamic property. The absence of endogenous factors, which may stabilize NLRs, and the heterologous expression system, which does not reflect the *in-planta* environment, make it further difficult

Box 1. Summary

- Plant NLRs dynamically assemble into higher-order structures, including multimeric complexes, filaments, and biomolecular condensates.
- Structurally characterized plant NLRs exhibit distinct oligomeric stoichiometries depending on their type: TNLs assemble as tetramers, while CNLs form pentamers, hexamers, or octamers.
- Pathogen effectors have evolved to target and inhibit the NLR oligomeric assembly to suppress immunity.
- Immune proteins can form filamentous structures to regulate enzymatic activity or mediate autoinhibition.
- Biomolecular condensates are formed to concentrate immune-related components, providing a favorable physical environment for their function.

to generate samples suitable for structural investigation. Therefore, to overcome these caveats, recent structural studies utilized the proteins expressed *in planta* (Lawson et al., 2026; Martin et al., 2022).

Some NLRs contain atypical N-terminal domains, such as alpha-beta hydrolase domain or protein kinase domains (Chia & Carella, 2023). However, the function and role of these atypical NLRs in plant immune response are completely unknown. So far, all the structures of NLRs available are homo-oligomeric NLRs. Interestingly, however, many other NLRs also function as hetero-pairs or act together with non-NLR proteins to trigger immune response (Cesari et al., 2014; Li et al., 2020; Tran et al., 2017; Wan et al., 2025; Yang et al., 2024; Zdrzałek et al., 2020). Further biochemical and structural studies on diverse NLRs may provide hints on their functions. Recent studies on animal NLRP3-activated ASC (apoptosis-associated speck-like protein containing a CARD) complexes using cryo-electron tomography (cryo-ET) show the organization of ASC puncta and their interactions with surrounding organelles (Liu et al., 2023), highlighting the contribution of other subcellular compartments to immunity. *In situ* structural analysis on plant NLRs using cryo-ET will likely provide comprehensive understanding on the structure and organization of NLRs.

Understanding the structure of NLR proteins provides not only valuable insights into plant immune mechanisms but also enables us to engineer NLRs to develop crops with enhanced disease resistance (Liu et al., 2021; Marchal et al., 2022). For instance, structural insights into NLR (in)compatibility in higher-oligomeric assemblies would allow for the fine-tuning the activation thresholds. This would certainly enable genome prediction platforms not only to prevent hybrid necrosis but also to guide the targeted substitutions of incompatible NLR residues to overcome the combining ability barriers in breeding (Bentham et al., 2023). Furthermore, structurally characterizing the effector-binding surfaces of NLRs facilitates engineering to expand recognition ranges against evolving pathogens, or to design NLRs that evade pathogen-mediated suppression (Contreras, Pai, Selvaraj, et al., 2023; De la Concepcion et al., 2019; Maidment et al., 2023). Once our knowledge accumulates which NLR residues are sensitized to environmental conditions toward activation, deployment of germplasm into different environmental conditions based on the NLRome will be made possible. These structure-guided strategies provide a framework for developing crops with tightly controlled immune activation and enhanced disease resistance.

The presence of diverse NLRs and mode of action suggests that NLR-mediated immune activation in plants is more complex than what is currently known. Future research using structural and biochemical approaches is expected to shed light on the remaining questions in plant immunity (Box 2).

Box 2. Open questions

- What is the molecular mechanism by which sensor NLRs induce helper NLRs oligomerization, and how is immune signaling specificity achieved in this process?
- What plant factors mediate the insertion of the cell-death executing resistosome into the plasma membrane?
- What are the molecular mechanisms and functional roles of NLRs with atypical N-terminal domains?
- What are the roles and physiological states of NLR hetero-pairs and NLR-non-NLR protein complexes in plant immunity?
- How are NLRs subcellularly localized, and how do they interact with organelles *in planta*?

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AUTHOR CONTRIBUTIONS

All authors contributed to writing and editing the manuscript.

CONFLICT OF INTEREST

J-JS is a co-founder and CTO of Epinogen.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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