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Plant NLR triggered immunity: From receptor activation to downstream signaling

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Abstract

Innate immune perception is the first line of inducible defense against invading pathogens. Plants lack specialized circulating immune cells. Therefore, diverse cell types are able to recognize and respond to pathogens. Surface localized and intracellular plant innate immune receptors are capable of recognizing diverse pathogen components. Intracellular nucleotide-binding leucine-rich repeat (NLR) receptors recognize pathogen effectors delivered inside host cells. Recent advances shed light onto NLR activation, phosphorylation of defense signaling nodes and overlap in transcriptional responses between pathogen perception and abiotic stress.

Keywords

plant innate immunity; NLR; pathogen effector; resistosome

Introduction

With the exception of viruses and specialized insect-vectored bacteria, most pathogens do not replicate inside plant cells. To cause disease and modify their hosts, pathogens secrete proteins, called effectors, into the extracellular space or directly into host cells [1]. Plant innate immune receptors include surface localized pattern recognition receptors (PRRs) as well as intracellular NLR receptors [2,3]. PRRs can recognize conserved microbe- or pathogen-associated molecular patterns (M/PAMPs), damage-associated molecular patterns (DAMPs) and extracellular effector proteins [1,3]. NLRs detect the presence or activity of effectors delivered into host cells during infection [2]. Both PRR- and NLR-triggered immunity (PTI and NTI) lead to a suite of downstream defense responses including the generation of reactive oxygen species (ROS), an influx of extracellular calcium, kinase

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Declaration of interests

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activation and global transcriptional reprogramming for defense [2,3] (Fig 1). After immune recognition, signals of pathogen perception are propagated from the initial infection site to distal tissues [4]. This systemic immune signaling primes naïve tissue against subsequent attack. Despite similarities, the timing, intensity and duration of defense can differ between PTI and NTI (Fig 1) [5]. NLR activation induces a quantitatively stronger, prolonged and robust response, frequently culminating in programmed cell death at the site of infection [2]. Here we will focus on recent advancements in NLR biology from receptor activation to downstream signaling.

NLR architecture and diverse modes of effector recognition

Plant genomes possess diverse NLR repertoires, with many species possessing hundreds of distinct NLRs that can be used to control pathogen infection in crops [6]. NLRs are composed of a central nucleotide-binding site (NBS) and C-terminal leucine-rich repeats (LRRs). They can be divided into two broad classes based on their N-termini, with CNLs carrying a coiled-coil (CC) domain and TNLs carrying a Toll-interleukin 1 receptor (TIR) domain (Fig 2) [2]. Both pathogen effectors and NLRs can localize to diverse subcellular locations including the cytoplasm, nucleus, plasma membrane, tonoplast and endoplasmic reticulum [2]. The barley CNL MLA10 and the *Arabidopsis* TNL RPS4 reside in the nucleus and cytoplasm, with both locations required for full resistance [7,8]. The *Arabidopsis* CNL RPM1 constitutively associates with the plasma membrane and recognizes a membrane-targeted *Pseudomonas* effector protein [9,10]. How NLRs with diverse subcellular localizations are able to trigger similar defense responses remains unknown.

Not only do NLRs localize to distinct cellular compartments, they also exhibit diversity in mechanisms of pathogen effector recognition. Some receptors can directly bind and recognize cognate pathogen effectors, while others monitor for effector-mediated perturbations of host targets [2]. For example, the *Nicotiana* TNL Roq1 confers resistance against *Xanthomonas* and is able to physically associate with the recognized effector XopQ [11]. In contrast, RPM1 recognizes effector-induced phosphorylation of the host protein RIN4 [12,13]. Animal NLRs recognize PAMPs as well as monitor for pathogen-mediated perturbations, such as the mouse NLR NOD1 that is activated by *Salmonella* SopE effector activity [14]. Plant NLRs can also act as pairs and can exhibit head-to-head chromosomal orientation to facilitate co-expression (Fig 2) [15,16]. These paired NLRs have been characterized from both monocots and dicots [17,18••]. Most NLR pairs consist of a canonical signaling NLR, such as RPS4, and a sensor NLR carrying an integrated domain that interacts with an effector target, such as RRS1-R with a WRKY domain (Fig 2) [15,16]. Finally, some receptors require downstream helper NLRs to form a functional unit for disease resistance [19••].

NLR activation and resistosome formation

NLR activity undergoes multilayered regulation, including self-inhibition, dimerization or oligomerization, epigenetic and transcriptional regulation, alternative splicing and proteasome- mediated degradation [20]. Their similar structure across diverse organisms, especially the presence of the NBS, indicates that nucleotide binding acts as a switch for

receptor activity. Early work in plants identified the first NLRs, demonstrated conservation of NBS domains and determined that the NBS is essential to their functionality [21–23]. If any of the multiple ATP-binding motifs within the NBS are mutated, this renders the NLR either locked in an activated state (ATP bound) or nonfunctional (ADP bound or unbound) in terms of its ability to elicit a defense response [22,24]. A longstanding model within the field of plant immunology posited NLRs are tightly folded and bound to ADP in an inactive state and upon effector perception exhibit conformational changes enabling ATP binding and higher order complex formation (Fig 2) [25,26].

The first structure of a plant NLR complex in inactive, intermediate and activated state was recently elucidated using cryo–electron microscopy [27••,28••] (Fig 2). This was accomplished with the CNL ZAR1, which recognizes the *Xanthomonas* effector AvrAC indirectly, through effector-mediated uridylation of the host kinase PBL2 [29]. When inactive, ZAR1 self-associates through inter-domain interactions and interacts with the pseudokinase RKS1 through its LRR domain [28••]. Upon uridylation, PBL2 recruits and binds to RKS1 [29]. The allosteric binding of PLB2 to RSK1 induces conformational changes in ZAR1's NBS domain, causing release of ADP and formation of a ZAR1-RSK1-PBL2 trimeric complex, likely representing a primed intermediate state [28••]. ZAR1 dATP or ATP binding induces conformational changes within the NBS domain, which in turn mediates oligomerization of the complex into a higher order wheel-like pentamer, called the resistosome [27••]. This multistep activation of ZAR1 may function to ensure appropriate activation of defense. When oligomerized, the N-terminal α-helices of the ZAR1 CC domains form a protruding funnel-like structure with similarity to pore-forming toxins [27••,30] (Fig 3). The N-terminal α-helix is essential for enhanced membrane association and signaling upon ZAR1 activation [27••]. Animal NLRs undergo higher order complex formation upon pathogen perception, forming inflammasomes and apoptosomes that trigger cell death [31]. Thus, ATP binding, oligomerization and cell death induction appears to be a common feature of NLR activation.

Resolving the resistosome structure is an important step in understanding mechanisms of NLR activation and opens new avenues for investigating downstream signaling. If activated CNLs are able to form pores in membranes, cell death may occur through disrupting selective membrane permeability in a similar manner to pore forming toxins. Membrane disruption could also induce DAMP signaling and be perceived by PRRs to amplify immune responses [3]. Alternatively, CNL resistosomes could form selective ion channels and transport signaling ions, such as Ca^{2+}, but this would need additional layers of regulation to control ion selectivity (Fig 2). Given the diverse and dynamic NLR sub-cellular localizations, it will be interesting to determine if other NLRs can form similar structures targeted to various membranes. CNL signaling may be two-fold, with pore/ion channel formation coupled to signaling initiated intracellularly through the resistosome complex.

Regulation of downstream signaling

Responses downstream of CNL receptors frequently require the NDR1 locus, while TNL receptors require a set of lipase-like proteins including EDS1 and SAG101 [2,32,33]. NDR1 is anchored to the plasma membrane, mediates plasma-membrane cell wall adhesions and

possess similarity to plant proteins involved in abiotic stress responses and mammalian integrins [34]. With the recent discovery that CNLs may form pore-like structures, it will be important to address the role of NDR1 and other immune signaling nodes for effects in plasma membrane integrity. While TNLs lack CC domains, they frequently require downstream helper NLRs of the CNL class, including ADR1, NRG1 and NRCs [19••,35,36]. For example, the TNLs Roq1 and RPP1 require the helper NLR NRG1 [37]. Furthermore, multiple CNLs in *Solanaceous* plants that require NRC helpers possess extended N-terminal regions before their CC domain, including the tomato CNLs Prf and Mi [19••,38]. Deletion of the N-terminal 13aa from the CC-domain of the NRG1 helper blocks its cell death inducing activity [27••]. It is possible that primary NLRs with diverse subcellular localizations or N-terminal domains unable to form pore-like structures partner with helper NLRs to achieve a robust NTI response.

Accumulation of extracellular ROS by NAPDH oxidases is a hallmark of both PTI and NTI responses [2,3]. Extracellular ROS also physically strengthens the plant cell wall, induces cell wall depositions and functions as a secondary signal required for both local and systemic innate immune responses [39]. In *Arabidopsis*, the primary NADPH oxidase required for ROS production, RBOHD, is activated by conformational changes induced by Ca^{2+} binding and N-terminal phosphorylation of conserved residues [40–43]. Phosphorylation of RBOHD S343 and S347 occurs during both PTI and NTI, but through distinct kinases [44••]. Downstream MAPK cascades are also similarly induced, but it is unknown if the upstream activating kinases are similar for both receptor types [5]. Thus, distinct kinases may converge upon critical signaling nodes with varying intensity to regulate the duration and magnitude of responses during PTI and NTI (Fig 1).

Transcriptional regulation of immunity: Overlap with general stress response

NLR recognition of pathogen effectors induces massive transcriptional reprogramming towards defense. Genetic studies have demonstrated that several transcription factor families play critical roles in innate immune and abiotic stress responses, including those in the AP2/ERF, bHLH, MYB, NAC, WRKY, bZIP and CAMTA families [45,46••]. Transcriptional profiling after activation of the barley CNL MLA1, the *Arabidopsis* TNL RPS4 and various PRRs recognizing bacterial and fungal PAMPs revealed significant overlap in early response genes [46••]. Early response genes are enriched in loci encoding signaling components, such as transcription factors, with CAMTA binding motifs enriched in their promoters [46••]. CAMTAs are a group of calmodulin binding transcription factors involved in both positive and negative regulation of various Ca^{2+} dependent stress responses [47]. Upon abiotic and biotic stress, CAMTAs rapidly and transiently induce gene expression by binding the Rapid Stress Response Element (RSRE). RSREs are overrepresented in the promoters of general stress response-associated genes [48]. The enrichment of CAMTA binding motifs in early response genes support the notion that both innate immune activation and abiotic stress induce a similar and rapid general stress response, with differential transcriptional outputs at later time points depending on pathogen or abiotic stimulus.

Systemic immunity and transgenerational resistance

After pathogen perception, immune signals are subsequently propagated within a tissue, systemically move to distal tissues, and prime the plant for heightened resistance against subsequent attack. Local immune priming can be established by NLR activation as well as crosstalk between PRRs and their co-receptors after MAMP perception [49]. After bacterial challenge, the flagellin co-receptor, BAK1, phosphorylates the receptor-like kinase for chitin perception, CERK1, which primes the plant and enhances defense activation upon subsequent fungal attack [49]. The plant hormone salicylic acid (SA) is required for defense in local and distal tissues. Systemic immunity in distal tissues induces transcriptional and metabolic reprogramming leading to heightened resistance against biotrophic pathogens that typically lasts for several weeks [50,51]. SA dependent immune priming can be propagated between individual *Arabidopsis* plants through monoterpene emissions [52,53]. This monoterpene-associated response depends on signals associated with systemic resistance, potentially mediating propagation of resistance at a population level [53].

SA application induces chromatin modifications, including acetylation and methylation, on the promoters of defense genes, which correlate with stronger and more robust expression upon pathogen challenge [54–56]. Progeny of *Arabidopsis* infected with *P. syringae* or treated with an SA analog displayed stronger induction of SA defense genes and enhanced resistance to *P. syringae* and the oomycete pathogen *Hyaloperonospora arabidopsidis* [54,55]. Furthermore, repeated pathogen challenge within a single generation increased the longevity of transgenerational resistance [54]. A greater mechanistic understanding of the interplay between defense priming, post-translational modifications, epigenetic changes and plant growth can be used to enhance disease resistance and minimize the growth penalty.

Conclusions

Plants represent excellent model systems to study NLR innate immune receptors. Recent evidence has revealed the structure of an NLR complex in various states of activation, demonstrating the formation of the first plant resistosome. Despite differences in defense timing and amplitude between innate immune receptor types, there is overlap in protein phosphorylation and early transcriptional responses. Future research focusing on how diverse NLR receptors induce cell death and resistance upon activation will significantly advance our understanding of this common protein family. Furthermore, given the impact of disease for agricultural production, a comprehensive understanding of NLR biology has significant translational applications for crop improvement.

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Highlights:

- Plant NLRs recognize intracellular effectors, inducing cell death and resistance
- NLRs exhibit diverse localization and modes of effector recognition
- The Arabidopsis ZAR1 NLR forms a pentameric resistosome complex upon activation
- Abiotic stress and innate immunity induce overlapping transcriptional reprogramming

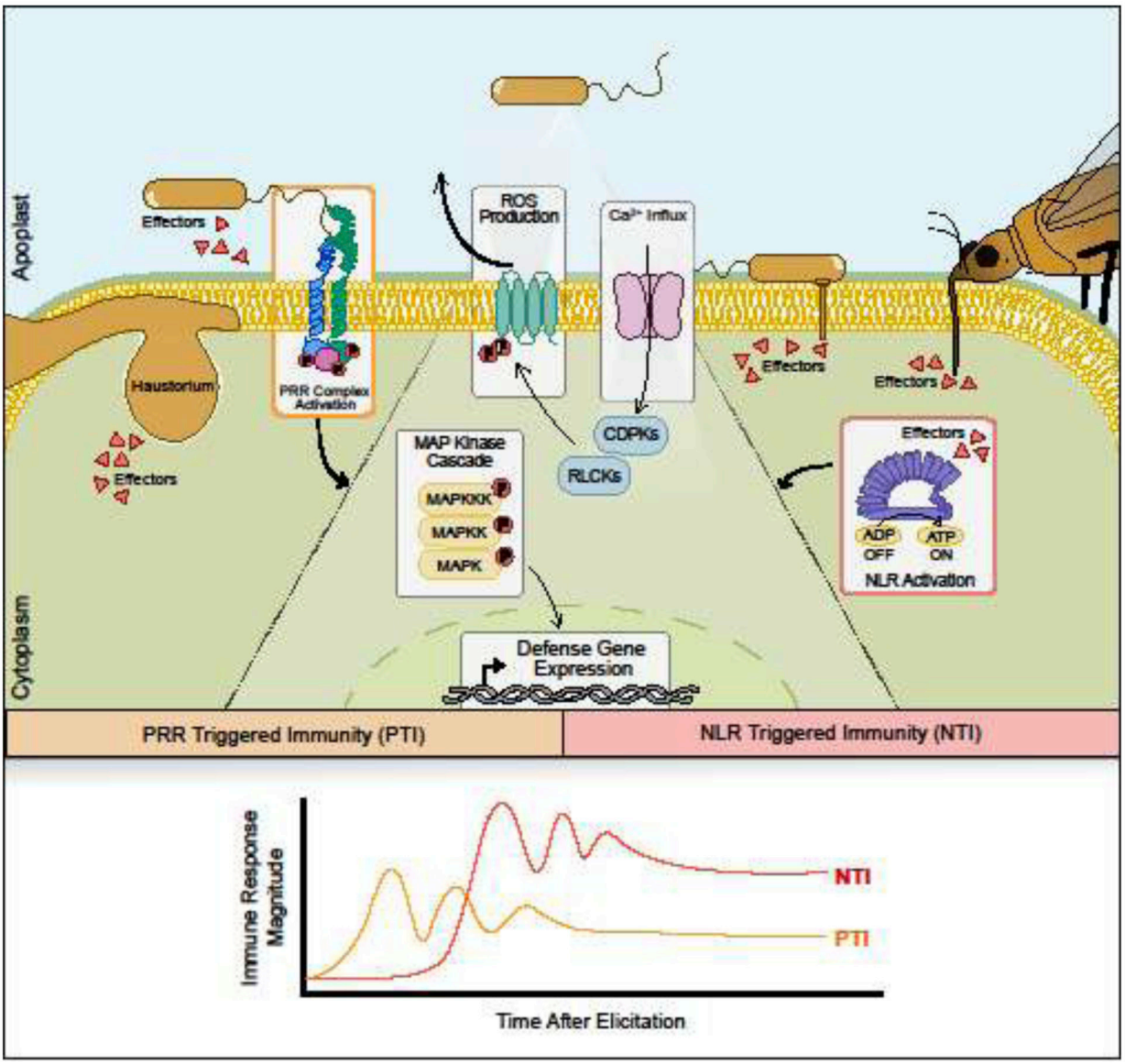

Figure 1: Surface localized and intracellular plant innate immune receptors recognize diverse pathogens.

Plant immune receptors include surface localized pattern recognition receptors (PRRs) and intracellular nucleotide binding leucine rich repeat (NLR) receptors. PRRs can recognize microbial features, damage associated molecular patterns, and extracellular receptors from insects, bacteria and filamentous pathogens. NLRs perceive pathogen effectors directly or through effector-mediated perturbations. Both PTI and NTI induce downstream defense responses including an influx of extracellular calcium, reactive oxygen species (ROS) production, kinase activation and transcriptional reprogramming for defense. While downstream defense responses are similar between PTI and NTI, the timing, amplitude and duration of responses differ.

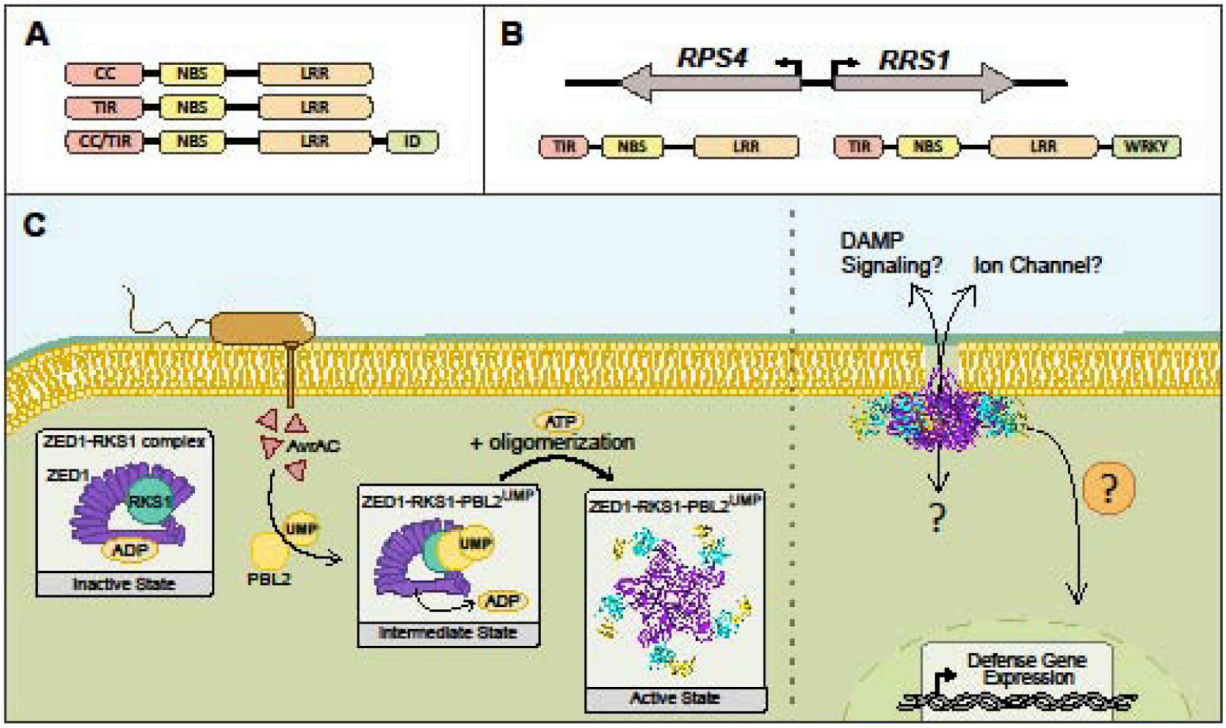

Figure 2: NLRs act as molecular switches to provide robust resistance against pathogens.

A. Plant NLR domain architecture includes an N-terminal coiled-coil (CC) or Toll/ interleukin-1 receptor (TIR) domain, a central nucleotide binding site (NBS) and C-terminal leucine-rich repeats (LRRs). Some NLRs carry an integrated domain (ID) that can directly sense pathogen effectors. **B**. NLRs can act in pairs, such as Arabidopsis *RPS4* and *RRS1.* Top: head-to-head genomic orientation, bottom: domain architecture from N- to C-termini. RRS1 is a sensor NLR with a WRKY ID. **C**. The *Arabidopsis* NLR ZAR1, pseudokinase RSK1, and kinase PBL2 can form a pentameric complex, or resistosome. Upon uridylation by the *Xanthomonas* effector AvrAC, PBL2 is recruited to the ZAR1-RKS1 complex (intermediate state). The active ZAR1 complex exhibits enhanced membrane affinity and the CC-domains of ZAR1 resemble a pore-like structure. Many questions remain regarding the activation of innate immune responses upon resistosome formation.