ANNUAL REVIEWS

Annual Review of Plant Biology

A Comparative Overview of the Intracellular Guardians of Plants and Animals: NLRs in Innate Immunity and Beyond

Zane Duxbury,[1] Chih-hang Wu,[2] and Pingtao Ding[3,4]

[1]Jealott's Hill International Research Centre, Syngenta, Bracknell RG42 6EY, United Kingdom; email: zane.duxbury@syngenta.com

[2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan; email: wuchh@gate.sinica.edu.tw

[3]The Sainsbury Laboratory, University of East Anglia, Norwich NR4 7UH, United Kingdom

[4]Current affiliation: Institute of Biology Leiden, Leiden University, Leiden 2333 BE, The Netherlands; email: p.ding@biology.leidenuniv.nl

Annu. Rev. Plant Biol. 2021. 72:155–84

First published as a Review in Advance on March 9, 2021

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-080620-104948

Keywords

NLRs, plant immunity, resistosome, NLR networks, inflammasome

Abstract

Nucleotide-binding domain leucine-rich repeat receptors (NLRs) play important roles in the innate immune systems of both plants and animals. Recent breakthroughs in NLR biochemistry and biophysics have revolutionized our understanding of how NLR proteins function in plant immunity. In this review, we summarize the latest findings in plant NLR biology and draw direct comparisons to NLRs of animals. We discuss different mechanisms by which NLRs recognize their ligands in plants and animals. The discovery of plant NLR resistosomes that assemble in a comparable way to animal inflammasomes reinforces the striking similarities between the formation of plant and animal NLR complexes. Furthermore, we discuss the mechanisms by which plant NLRs mediate immune responses and draw comparisons to similar mechanisms identified in animals. Finally, we summarize the current knowledge of the complex genetic architecture formed by NLRs in plants and animals and the roles of NLRs beyond pathogen detection.

Contents

1. INTRODUCTION

LRR: leucine-rich repeat

NLR: nucleotide-binding domain and leucine-rich repeat receptor

HR: hypersensitive cell death response

The sophisticated innate immune system of plants prevents infection from diverse pathogens. Cell surface immune receptors, often termed pattern recognition receptors (PRRs), perceive conserved microbe-associated molecular patterns (MAMPs), pathogen-associated molecular patterns (PAMPs), or host-derived damage-associated molecular patterns (DAMPs). Upon ligand perception, PRRs activate immune responses that suppress the proliferation of nonadapted pathogens. These immune responses include the rapid production of reactive oxygen species (ROS), creating an oxidative burst, and the initiation of mitogen-activated protein kinase (MAPK) cascades (42, 89, 177). Pathogens use several mechanisms to facilitate infection, the foremost of these being the secretion of effector proteins that perturb the function of host PRR proteins and other immunity-related processes. To counteract the perturbations caused by effectors and stop disease progression, plants have evolved intracellular nucleotide-binding domain (NBD) leucine-rich repeat (LRR) receptors (NLRs) to perceive pathogen effectors and subsequently initiate robust immune responses (52, 89). NLR-mediated immune responses show molecular signatures that are both distinct from and similar to PRR-mediated responses, and they are often accompanied by a cell death phenomenon termed the hypersensitive response (HR) (134, 206). These molecular

surveillance systems confer resistance to plant diseases, including those caused by many devastating pathogens on major crops.

The innate immune system of animals similarly utilizes NLRs as intracellular immune receptors (57, 89). Different from plant NLRs, animal NLRs can detect both PAMPs and pathogen-secreted effector proteins (123). Several animal NLRs have been biochemically and biophysically characterized, some of which form multimeric complexes, named inflammasomes, once their cognate ligands are perceived. In this review, we summarize the latest insights into plant NLR biology and draw comparisons to animal NLR biology. In particular, we discuss the recent discovery of inflammasome-like complexes in plants and the striking similarity between the mechanisms of plant and animal NLR function. Furthermore, we summarize the current knowledge of the complex genetic architecture formed by NLRs in plants and animals. New horizons are appearing for NLR research, and we highlight these by discussing the role of NLRs beyond pathogen perception and drawing out enduring unanswered questions.

2. WHAT CHARACTERISTICS DO NLRS HAVE?

2.1. NLRs Are Modular Proteins with a Conserved Tripartite Architecture

The architecture of plant and animal NLRs is remarkably similar, with three core domains that have seemingly conserved functions: an N-terminal domain, a central NBD, and an LRR domain (**Figure 1**). The NBD of NLRs is in the signal transduction ATPases with numerous domains (STAND) AAA+ ATPase superfamily. Plant NLRs have an NB-ARC nucleotide-binding adaptor shared by apoptotic protease activating factor-1 (Apaf-1), resistance (R)-protein, and cell death abnormal (CED) family NBDs (109). Plant NB-ARC domains can be further divided into three conserved subdomains: the NB, ARC1, and ARC2 subdomains (164, 176) (**Figure 1*a***). The NB subdomain contains the conserved P-loop (or Walker A) motif required for nucleotide binding, and the Walker B motif required for Mg^{2+} coordination and adenosine triphosphate (ATP) hydrolysis (81, 144, 181). The ARC2 subdomain contains the methionine-histidine-aspartate (MHD) motif, which also binds nucleotides. The NBD in animal NLRs was found conserved in neuronal apoptosis inhibitory protein (NAIP), class II major histocompatibility complex (MHC) transactivator (CIITA), heterokaryon incompatibility loci E (HET-E), and telomerase protein component 1 (TP1), and is thus called the NACHT domain. The NACHT domain can similarly be divided into an NB domain, helical domain 1 (HD1, equivalent to ARC1), winged helical domain (WHD, equivalent to ARC2), and helical domain 2 (HD2 or ARC3) (107) (**Figure 1*b***). Although the NBDs in plant and animal NLRs display striking similarity, plant NB-ARCs lack an ARC3 and instead have a short linker connecting them to the LRR (**Figure 1*b***).

The LRR domain often functions as an autoinhibitory unit that regulates the activity of both plant and animal NLRs. For example, the plant NLRs Resistance to *Pseudomonas syringae* 5 (RPS5) and Recognition of *Peronospora parasitica* 1A (RPP1A) both become constitutively active when their LRR domains are deleted (137, 189). Similarly, in animals, the LRR of NLRC4 blocks its oligomerization interface when inactive, and truncated Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) mutants that lack their LRR are autoactive (81, 168). Furthermore, the LRR domain can also participate in direct binding of ligands during pathogen perception; for example, the LRR of RPP1 is necessary and sufficient to immunoprecipitate its cognate effector ATR1 (98). Two recent cryogenic electron microscopy (cryo-EM) studies showed that the LRR of Recognition of XopQ 1 (Roq1), a TIR-domain-containing NLR protein (TNL) identified in *Nicotiana benthamiana*, and the LRR of RPP1 from *Arabidopsis thaliana* directly interact with the corresponding effector proteins XopQ and ATR1, respectively (119, 128). These

Inflammasome: cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses, widely observed in vertebrates

NB-ARC: nucleotide-binding domains that are shared by apoptotic protease activating factor-1 (Apaf-1), resistance (R)-protein, and cell death abnormal (CED) family proteins

NAIP: neuronal apoptosis inhibitory protein

CIITA: class II major histocompatibility complex (MHC) transactivator

NACHT: nucleotide-binding domains conserved in neuronal apoptosis inhibitory protein (NAIP), class II major histocompatibility complex transactivator (CIITA), heterokaryon incompatibility loci E (HET-E), and telomerase protein component 1 (TP1)

NLRC4: NLR family CARD domain-containing protein 4

TNL: Toll/interleukin-1 receptor/resistance protein (TIR)-domain-containing NLR protein

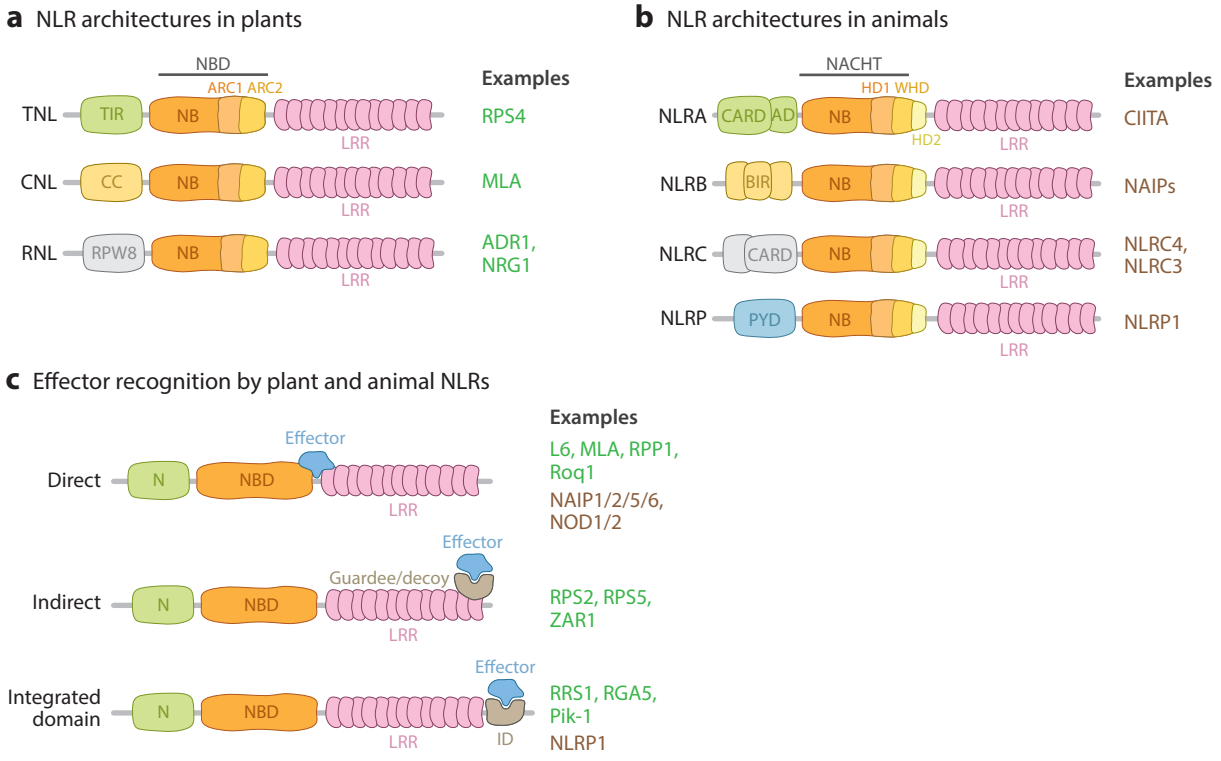


Figure 1

Domain architectures and models of effector recognition of NLRs in plants and animals. (*a*) Domain architectures of plant NLRs, classified into three major groups containing a TIR, CC, or RPW8-like CC domain fused to the NBD and LRR domains. The NBD can be further divided into NB, ARC1, and ARC2 subdomains. (*b*) Domain architectures of animal NLRs, classified into four major groups: NLRA contains a CARD followed by an AD, NLRB contains three N-terminal tandem repeats of BIR domains, NLRC contains a CARD, and NLRP contains a PYD at the N terminus. The NACHT (NBD) domain can further be divided into NB, HD1/ARC1, WHD/ARC2, and HD2/ARC3 subdomains. (*c*) Different models of effector recognition by plant and animal NLRs. Some plant and animal NLRs directly bind to the corresponding effector proteins or indirectly detect the pathogen effector through the guardee or decoy proteins. Some plant and animal NLRs have a noncanonical ID to mediate effector recognition. Plant examples are marked in green, whereas animal examples are marked in brown. Abbreviations: AD, acidic transactivation domain; BIR, baculovirus inhibitors of apoptosis repeat; CARD, caspase activation and recruitment domain; CC, coiled-coil; CIITA, class II major histocompatibility complex (MHC) transactivator; HD, helical domain; ID, integrated domain; LRR, leucine-rich repeat; NAIP, neuronal apoptosis inhibitory protein; NBD, nucleotide-binding domain; NLR, nucleotide-binding domain leucine-rich repeat receptor; PYD, pyrin domain; RPW8, resistance to powdery mildew 8; TIR, Toll/interleukin-1 receptor/R protein; WHD, winged helical domain.

observations suggest that the LRR may play multiple roles in ligand perception and activity regulation.

TIR: Toll/interleukin-1 receptor/resistance protein

CC: coiled-coil

2.2. NLR Classes Are Defined by Their N-Terminal Domains

NLRs can be classified into different groups based on their N-terminal domains. Plant NLRs are often grouped into three classes containing N-terminal Toll/interleukin-1 receptor/R protein (TIR), coiled-coil (CC), and resistance to powdery mildew 8 (RPW8)-like domains (**Figure 1*a***). In many cases, expressing truncated NLRs that contain only the N-terminal domain gives constitutive immune activation, such as HR cell death (41, 64, 98, 122, 163, 189). These truncated

domains often homo- and heterooligomerize, and mutations that disrupt oligomerization can suppress HR. For example, activation of the CC-NLR (CNL) MLA10 requires homodimerization via homophilic CC interactions (9). These observations indicate that downstream signaling is dependent on correct oligomerization and that other domains have a role in suppressing autoactivation in the full-length protein.

Similarly, TIR domains are found in the mammalian Toll-like receptors (TLRs), interleukin-1 receptors, and their downstream components such as MyD88 (47, 198). TLRs interact with MyD88 through homotypic TIR interactions, suggesting a mechanism by which plant NLRs may also signal. Further insights into plant NLR function were provided by the discovery that mammalian TIR-containing protein SARM1 has intrinsic nicotinamide adenine dinucleotide nucleosidase (NADase) activity (see Section 5.3).

The CC domain of plant NLRs can be further divided into two clades: Clades I and II are defined by the absence or presence of a conserved EDVID amino acid sequence, respectively. RPW8-like N-terminal domains are present in helper NLRs Activated disease resistance 1 (ADR1) and N requirement gene 1 (NRG1) (see Section 6.3) (16). Some other plant NLRs contain N-terminal domains that are unrelated to the above classes (97). For example, the Boundary element-associated factor (BEAF) and DNA replication-related element (DRE)-binding factor (DREF)-NLRs (BED-NLRs) were found to confer resistance to different pathogens, including *Xanthomonas oryzae* pv. *oryzae* and yellow rust, in poplar and several monocots (6, 44, 68, 73, 126, 204, 212). Another example is NLR Tan spot necrosis 1 (Tsn1) found in wheat, which contains an N-terminal serine/threonine protein kinase (S/TPK) domain and confers susceptibility to several fungal wheat pathogens, including *Stagonospora nodorum* and *Pyrenophora tritici-repentis* (60). However, it is not clear how BED or S/TPK domains signal downstream.

Animal NLRs can be classified into four groups: NLRA, containing a caspase activation and recruitment domain (CARD) and an acidic transactivation domain (ATD) or CIITA (94); NLRB, containing three N-terminal tandem baculovirus inhibitors of apoptosis repeat (BIR) domains; NLRC, containing an N-terminal CARD; and NLRP, containing a pyrin domain (PYD) (**Figure 1*b***). The best understood of these domains are the CARD and PYD, both of which establish multimeric interactions to recruit downstream molecules.

RPW8: resistance to powdery mildew 8

CNL: coiled-coil-domain-containing NLR

Nicotinamide adenine dinucleotide nucleosidase (NADase): participates in nicotinate and nicotinamide metabolism and the calcium signaling pathway; also called NAD^+ glycohydrolase

CARD: caspase activation and recruitment domain

2.3. Divergence and Convergence of NLRs Across the Plant and Animal Kingdoms

In the plant kingdom, *NLR* genes can be found in green algae, suggesting that plants employed *NLR* genes in their innate immune system prior to land colonization (67). In the animal kingdom, *NLR* genes are found in basal metazoans, such as in Cnidaria and Porifera, indicating that animal *NLRs* may originate from a common ancestor of metazoans (102, 175). Although *NLR* genes may have been lost in some metazoan lineages, such as in *Drosophila* or *Caenorhabditis*, the number of *NLR* genes has massively expanded in other lineages, such as in the sponge (*Amphimedon queenslandica*) and zebrafish (*Danio rerio*) (74, 77, 209). Unfortunately, functional studies of *NLRs* in basal metazoans and teleost fish are still missing, and hence the contribution of *NLRs* in the innate immune systems of these organisms is yet to be determined. Similar to what has been observed in the plant kingdom, the *NLR* gene family in animals displays striking lineage-specific expansion patterns, reflecting that the constant but diverse selection pressures on the immune system have driven the expansion and diversification of the *NLR* gene family across eukaryotic kingdoms (154, 175, 209).

Researchers have speculated that plant and animal *NLR* genes originated from the same common ancestor (85, 171). The NBD is highly conserved across taxa and likely has prokaryotic

origins. Interestingly, instead of a fusion of an LRR (e.g., NLRs), the C termini of NBDs in proteins in prokaryotes, fungi, and many examined nonmetazoan and nonplant eukaryotes are more often associated with tryptophan-aspartic-acid 40 (WD40, or β-transducin) repeats, tetratricopeptide repeats (TPR), or ankyrin (ANK) repeats (175). Furthermore, phylogenetic analysis of the NBD suggests that plant and metazoan NLR NBDs are not monophyletic, indicating that the architecture of NBD fusion to LRRs was acquired independently in the evolution of metazoans and plants (175). It is yet to be determined how both lineages converge in proteins with the same domain architecture as important players in innate immunity.

3. HOW DO NLRS RECOGNIZE EFFECTORS?

3.1. Direct Recognition

The simplest and most intuitive mechanism by which NLRs perceive effectors is through direct interaction (**Figure 1*c***). An emblematic example of direct recognition is the perception of variants of the flax rust fungus (*Melampsora lini*) effector AvrL567 by the flax (*Linum usitatissimum*) *L* locus-encoded TNLs L5, L6, and L7 (51). Recognition through direct interaction perhaps drove the diversifying selection on the *AvrL567* locus that has resulted in 12 variants, 5 of which have amino acid polymorphisms that evade binding and thwart recognition (51). This coevolutionary arms race also drove the diversification of the flax *L* locus, which contains 13 alleles of the same gene, including 3 NLRs that recognize variants of a single effector. Individual L TNLs can confer perception to multiple AvrL567 alleles. Specificity is probably provided by the LRR because substitution of 11 amino acids in the L6 LRR reduces the recognition spectrum from multiple effectors to one (51, 140). By better understanding how specificity is conferred in NLR allele series, new NLRs with novel specificities may be engineered. The allelic NLR series Pik in rice confers perception to multiple AVR-Pik effectors in *Magnaporthe oryzae* through direct binding. Pikm can recognize three AVR-Pik effectors, while Pikp can recognize only one. By studying the interactions between Pik and AVR-Pik proteins, researchers were able to use structure-guided engineering to create a novel Pik allele that bound a previously unrecognized AVR-Pik (46). Intriguingly, the Mildew-resistance locus A (Mla) CNLs of barley have evolved allelic variants that directly recognize sequence-unrelated effectors of powdery mildew fungi (148). Additional examples of plant NLRs detecting the presence of their cognate effectors through direct interaction include *Arabidopsis* TNL RPP1 binding to the effector ATR1 from *Hyaloperonospora arabidopsidis* and the rice CNL Pi-ta and its cognate fungal effector AVR-Pita from rice blast (87, 98). Two more sophisticated observations of direct recognition were reported in the recent cryo-EM structure of Roq1-XopQ and RPP1-ATR1 complexes, in which several side chains exposed on the surface of the LRR and an elongated linker between two LRRs bind effectors directly (119, 128).

Several mammalian NLRs also detect bacterial PAMPs through direct binding (**Figure 1*c***). NOD1/NLRC1 and NOD2/NLRC2 are mammalian NLRs in the NLRC class that confer recognition to bacterial cell wall components that are shed by bacteria during infection. NOD1 binds γ-D-glutamyl-*meso*-diaminopimelic acid, which is present in the cell walls of gram-negative bacteria, while NOD2 confers broad bacterial recognition through the recognition of muramyl dipeptide, which is a component of most bacterial cell walls (70, 104, 131). Ligand specificity is conferred by the LRR of both NOD1 and NOD2, although other domains could have a role in binding, for example, the NACHT domain of NOD2 (70, 104, 105, 131).

In mice, NAIP paralogs confer specificity to PAMP recognition by the NLRC4/NAIP inflammasome directly binding to PAMPs: NAIP1 and NAIP2 bind needle and inner rod components, respectively, and NAIP5 and NAIP6 bind flagellin, the protein subunit of the flagellum (72, 95,

200, 201, 214). Remarkably, the ligand specificity of NAIP2 and NAIP5/6 can be switched by substituting their NACHT domains in chimeras (170). The ability of the closely related NAIP paralogs to recognize different ligands is in part due to the structural similarity between the C-terminal portion of flagellin, rod, and needle proteins (213). Cryo-EM structural studies have revealed that flagellin forms intimate associations with the HD1 and HD2 of the NAIP5 NACHT, with other domains supporting these interactions, including the LRR and BIR1 (169, 203). Structure-guided mutagenesis and truncation confirmed the importance of the NAIP5 LRR for flagellin binding, but found that the NAIP2 LRR was dispensable for inner rod binding (169, 200).

Unlike mice, which have multiple *NAIP* genes that confer specificity to bacterial ligands, humans have a single *NAIP* gene. Initial studies reported that human NAIP confers recognition to type three secretion system (T3SS) needle proteins, but subsequent research uncovered that human NAIP is also a receptor for flagellin and T3SS rod proteins (96, 141, 143, 201, 214). Although mice have different NAIPs to confer recognition to different bacterial PAMPs, a synthetic NAIP2/5 chimera is able to confer dual recognition of T3SS rod protein and flagellin (170). Further investigations of the recognition specificity of different mammalian NAIPs may reveal how human and mice NAIPs adopted different strategies to perceive multiple PAMPs.

ZAR1: HopZ-activated resistance 1

Resistosome: a wheel-like pentamer formed by the active intermediate state of ZAR1

3.2. Indirect Recognition as a Guard or a Decoy

Many plant NLRs do not directly interact with the effectors to which they confer recognition (**Figure 1*c***). The guard hypothesis describes a model of effector recognition via indirect ligand-receptor interactions (43). Guard NLRs monitor the integrity of the molecular targets of effectors, or guardees, and activate an immune response upon the perception of a perturbation to the function of guardees. In *Arabidopsis*, RPM1-interacting protein 4 (RIN4) is a guardee that directly interacts with the guard NLRs Resistance to *P. syringae* 2 (RPS2) and Resistance to *P. syringae* pv. *maculicola* 1 (RPM1). Multiple effectors target RIN4, including the secreted protease AvrRpt2 from *P. syringae* via its T3SS, which cleaves RIN4, triggering RPS2-dependent immunity (8, 121). In addition, the *P. syringae* effector AvrRpm1 induces RIN4 adenosine diphosphate (ADP)-ribosylation, which in turn leads to RIN4 phosphorylation by host kinases, triggering immunity mediated by RPM1 (40, 115, 142). Another well-studied example of a guard NLR is RPS5, which monitors the protein kinase PBS1. The cleavage of PBS1 by the effector protease AvrPphB activates RPS5-mediated immunity (5, 153).

The decoy hypothesis, a derivation of the guard hypothesis, describes the scenario when guardee-like proteins are targeted by effectors and guarded by NLRs but have no discernible physiological role in immunity apart from being a decoy in effector recognition (178). A classical decoy is the pseudokinase HopZ1 ETI deficient 1 (ZED1), which is required for the activation of the CNL HopZ-activated resistance 1 (ZAR1). ZAR1 confers indirect recognition to multiple effectors, including *Xanthomonas campestris* pv. *campestris* effector AvrAC and *P. syringae* effector HopZ1a (101, 111, 150, 151, 184). AvrAC uridylylates and HopZ1a acetylates target receptor-like cytoplasmic kinases (RLCK) to confer virulence (62, 111). AvrAC uridylylates the RLCK PBL2 (resulting in PBL2^{UMP}), which subsequently interacts with the RLCK RKS1, which is in a preformed complex with ZAR1 (184). The ZAR1-RKS1-PBL2^{UMP} heterotrimer then assembles into a pentameric wheel-like structure called a resistosome, composed of five ZAR1-RKS1-PBL2^{UMP} protomers (186, 187). HopZ1a acetylates the pseudokinase ZED1, which is in a preformed complex with ZAR1, to trigger the assembly of a higher-order complex in planta presumed to be equivalent to a ZAR1-RKS1-PBL2^{UMP} resistosome (79, 111). Other RLCKs are targeted by HopZ1a and recognized by the ZAR1-ZED1 complex, where ZED1 performs an adaptor function reminiscent of RKS1 (14). Uridylation of PBL2 does not enhance AvrAC-mediated virulence,

and ZED1 has no kinase activity; hence, these host proteins are considered decoys, not guardees (111, 184).

ID: integrated domain

The guard hypothesis has been invoked in animals when researchers describe the non-NLR pyrin inflammasome that recruits the same downstream signaling partners as NLR inflammasomes (199). Pyrin confers recognition to effectors that perturb the function of Rho GTPases, yet it does not directly interact with Rho GTPases or these effectors (197). The Rho GTPase RhoA promotes the activity of the protein kinases PKN1 and PKN2. PKN1 and PKN2 phosphorylate Pyrin, which promotes the recruitment of 14-3-3 proteins that inhibit inflammasome formation (136). Upon abrogation of the function of RhoA by an effector, PKN1/2 activity decreases and nonphosphorylated pyrin dissociates from the inhibitory 14-3-3 proteins (66), forming an inflammasome and activating immunity. In this way, pyrin inflammasome assembly occurs when effectors target RhoA, providing an indirect mechanism by which pyrin guards RhoA.

3.3. Recognition with Integrated Domains in NLRs

Some NLRs contain noncanonical domains, called integrated domains (IDs), which provide recognition to corresponding pathogenic effectors (**Figure 1*c***). To confer effector perception, some IDs directly interact with their cognate effectors and are even enzymatically modified by the effectors. In these cases, the relationship between an ID and an NLR is reminiscent of a guardee being guarded by a corresponding NLR. Indeed, the genetic linkage resulting from the fusion of an ID to an NLR confers benefits; i.e., the shuffling of alleles that would result in a loss of compatibility between a guard and guardee is much less likely to happen in an integrated NLR-ID. The fusion of NLRs and IDs may have occurred via retrotransposition or ectopic recombination (10). The fusion of different NLRs to diverse noncanonical domains has occurred many times in divergent clades (10, 165).

The common mechanism by which several NLR-IDs recognize their cognate effectors has been revealed. One of the best-studied NLR-ID examples is Resistance to *Ralstonia solanacearum* 1 (RRS1), which contains a C-terminal WRKY as the ID (106, 147). WRKY domains bind DNA and many WRKY-containing transcription factors were found to regulate immune responses (135). The effector PopP2 from *R. solanacearum* is an acetyltransferase that binds and acetylates WRKY transcription factors, disrupting DNA binding and promoting virulence (106, 147). However, during the infection of plants expressing RRS1, the WRKY domain in RRS1 is acetylated by PopP2, and for resistance alleles of RRS1, WRKY acetylation is sufficient to activate an immune response in cooperation with its paired NLR Resistance to *P. syringae* 4 (RPS4) (106, 147). RRS1 confers recognition to other effectors, including the *P. syringae* effector AvrRps4, which also binds the RRS1 WRKY domain (147). Other well-studied plant NLR-IDs include the rice NLRs RGA5 and Pik-1, which contain integrated heavy metal–associated (HMA) domains (31).

The animal NLR NLRP1b appears to employ a similar integrated decoy strategy to confer pathogen perception. It has an atypical NLR architecture, with a CARD at the C terminus, connected to the LRR via a function-to-find domain (FIIND) (100, 196). The FIIND is constitutively autoproteolytic and cleaves itself to generate two NLRP1b peptides that remain noncovalently associated. NLRP1b confers recognition of *Bacillus anthracis*, the bacterium that causes anthrax. The infection strategy of *B. anthracis* includes the secretion of anthrax Lethal Toxin (LT) into cells, a multiprotein toxin that includes the endoprotease Lethal Factor (LF). LF cleaves the N terminus of host mitogen-activated protein kinase kinases (MAPKKs), inhibiting them and abrogating immunity (38, 55). LF also cleaves the N terminus of NLRP1b (34, 35), triggering the proteasomal degradation of the N-terminal fragment of NLRP1b and releasing the C-terminal fragment. The liberated fragment contains the CARD, which homooligomerizes and recruits downstream signaling molecules, including caspases, to form an inflammasome and activate immunity (39, 146).

NLRP1b shares the RRS1 trait of recognizing multiple effectors. In addition to LF, NLRP1b confers recognition to the *Shigella flexneri* effector IpaH7.8, an E3 ubiquitin ligase. IpaH7.8 ubiquitinates the N-terminal fragment of NLRP1b, designating it for proteasomal degradation and triggering inflammasome formation (146). Clearly, the diversity of effectors and their modes of action have given rise to diverse modes of effector perception, but the conservation of the underlying principles of direct and indirect recognition reveals their success.

4. HOW DO NLRS GET ACTIVATED?

4.1. ADP/ATP Exchange

The NBDs of plant and animal NLRs bind ADP and ATP, and the exchange of these nucleotides is associated with a switch on and off from the activation state of the NLRs (**Figure 2*a***). Many studies support the hypothesis that ADP-bound NLRs are in an inactive state, and ATP-bound NLRs

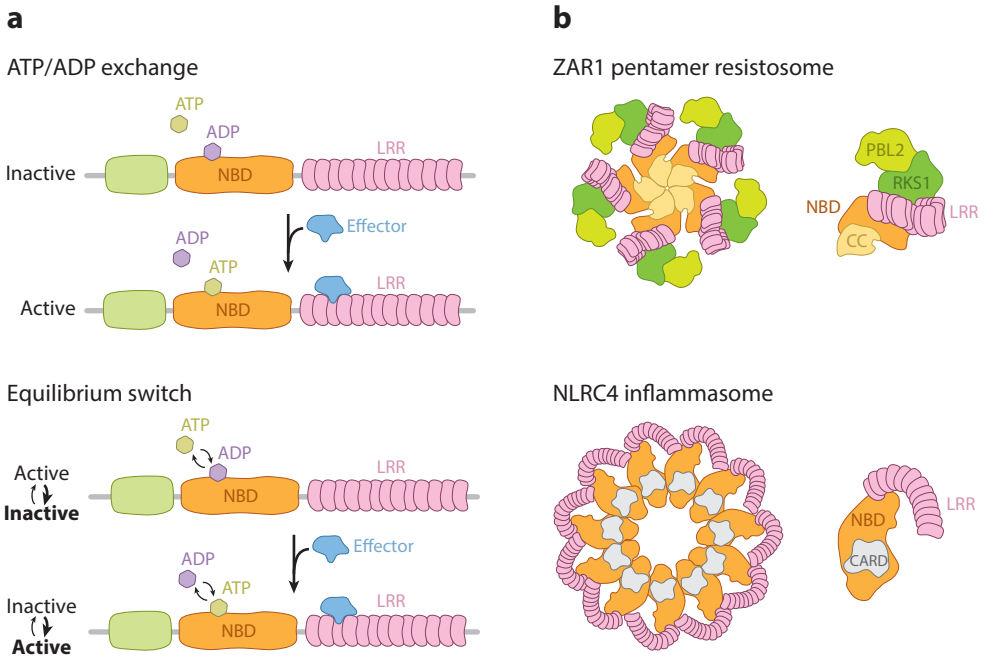


Figure 2

Plant and animal NLRs bind ATP and oligomerize upon activation. (*a*) Two different models of ATP/ADP binding of NLRs. In the ATP/ADP exchange model, NLRs in the resting state are ADP bound. Upon pathogen detection, the ADP is released and ATP enters the binding pocket to induce and stabilize conformational changes. In the equilibrium switch model, NLRs are cycling between an ADP-bound inactive state and an ATP-bound active state. Effector perception pushes the equilibrium toward the ATP-bound state, leading to the activation of NLR complexes. (*b*) Both plant and animal NLRs form high-order complexes upon activation. Plant NLR ZAR1 forms a pentamer resistosome complex consisting of ZAR1, RKS1, and PBL2. Animal NLR NLRC4 forms an inflammasome complex consisting of 9–11 NLRC4 and one NAIP upon pathogen detection. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; CARD, caspase activation and recruitment domain; CC, coiled-coil; LRR, leucine-rich repeat; NAIP, neuronal apoptosis inhibitory protein; NBD, nucleotide-binding domain; NLR, nucleotide-binding domain leucine-rich repeat receptor; PBL2, PBS1-like 2; PBS1, AvrPphB susceptible 1; RKS1, Resistance-related kinase 1; ZAR1, HopZ-activated resistance 1. Panel *b* adapted from References 186 and 210 with permission; copyright 2019 and 2015 American Association for the Advancement of Science.

are in an active state (11, 93, 122, 127, 138, 166, 167, 191). Indeed, mutations in the MHD motif in plant NLRs, or in the equivalent histidine (H) residue in NLRC4 that interacts with ADP, result in constitutively active NLRs bound to ATP (15, 78, 82, 159, 179, 185, 191, 202, 210). Although many NLRs require intact P-loops to bind nucleotides and transduce immune responses, some helper NLRs, which are required for downstream signaling of sensor NLRs (see Section 6.3), do not require functional P-loops to execute their signaling roles. For example, mutations in the P-loops of *Arabidopsis* helper NLRs AtADR1-L2, AtNRG1.1, and AtNRG1.2 do not abrogate their competence in immune signal transduction (90). In NLR pairs, sensor NLRs and their executor partner NLRs (see Section 6.2) may have distinct requirements for their P-loops. The *Arabidopsis* sensor NLR RRS1 and the rice sensor NLR RGA5 have dispensable P-loops, but their corresponding executors, RPS4 and RGA4, require P-loops to mediate immunity (31, 120). NLRP1b P-loop mutants are autoactive (114), but a P-loop mutation impairs downstream signaling of NLRP3, NOD1, NOD2, and NLRP1 in humans (56, 61, 133, 168). Although an NLRC4 P-loop mutant still induces normal immune signaling, P-loop mutation greatly reduces signaling activity induced by an LRR-truncated variant of NLRC4 that is constitutively active with no inflammasome formation (95). However, controversy has been reported, and P-loop mutation may affect the protein stability of NLRC4 (117). Thus, the contribution of the P-loop in NLRC4 function is still yet to be determined.

The recent elucidation of the cryo-EM structure of inactive ZAR1 reveals that ADP is buried in a binding pocket in the ZAR1-RKS1 complex and stabilizes ZAR1 in an inactive state. Recognition of uridylated PBL2 leads to a conformational change in the ZAR1-RKS1 complex that releases ADP and promotes the binding of ATP. ATP enters the binding pocket, further inducing and stabilizing conformational changes to an active state (187). Similarly, inactive NLRC4 is bound to ADP (81). However, ATP does not seem to be essential for NLRC4 inflammasome assembly (72), but, instead, flagellin can stabilize the NLRC4/NAIP5 inflammasome (169, 200). In general, ADP is associated with inactive NLRs and ATP with active NLRs, but the full requirement for ADP/ATP in each plant NLR remains to be elucidated.

The NBD can hydrolyze ATP to ADP (61, 81, 166, 174, 216), and this has been hypothesized as a mechanism of deactivating an ATP-bound NLR engaged in immune signaling (17, 22, 118, 215). Indeed, some Walker B mutations, which can impair ATP hydrolysis, impair NOD2 function, while others lead to autoactivation (216). ATP hydrolysis is required for NLRP1 and NOD1 function (56, 61, 216), whereas Walker B mutations with impaired ATP hydrolysis activity in tomato I-2 and *Arabidopsis* RPS5 confer autoactivity (5, 167). However, the requirement for ATP hydrolysis has not been clearly defined in a unifying model. It is possible that the status of direct binding to either ATP or ADP is more critical than the ATP hydrolysis activity. An equilibrium switch model, derived from observations of the flax NLRs L6 and L7, hypothesizes that NLRs exist in an equilibrium between an ADP-bound inactive state and an ATP-bound active state (17) (**Figure 2*a***). In the absence of pathogens, the equilibrium favors the inactive state. Effector perception pushes the equilibrium toward the active state, perhaps through stabilization of the ATP-bound protein. This is supported by observations that autoactive NLRs can trigger stronger immune responses in the presence of effectors.

4.2. Oligomerization

Several immune receptors form higher-order complexes during activation after the perception of pathogen-derived molecules. An iconic example is the inflammasome, a signaling platform assembled from NLR subunits (**Figure 2*b***). The structure of the NLRC4-NAIP inflammasomes has been resolved using cryo-EM. NAIP2 and NAIP5 undergo conformational changes upon binding of the inner rod protein from a bacterial T3SS or flagellin, respectively (48, 72, 82, 169, 203,

210). These changes release the autoinhibition of the NACHT domain by intramolecular interactions and reveal the donor surface of NAIPs, an oligomerization interface that recruits NLRC4. This induces conformational changes in NLRC4 that reveal a donor surface that recruits another NLRC4, which, in turn, recruits subsequent NLRC4 proteins perhaps in a stepwise manner, resulting in a wheel-like complex containing a ligand-bound NAIP and 9 to 11 NLRC4 subunits (82, 210). In this inflammasome, the N-terminal CARDs are at the center and the C-terminal LRRs are on the outside (82, 210). The CARDs form a high local density that induces the recruitment of downstream signaling proteins. Other animal NLRs form inflammasomes, including NLRP1 and NLRP3, but the platform they create and the downstream signaling adaptors differ from the NLRC4/NAIP inflammasome (88, 107, 155) (**Figure 2*b***). NOD1 and NOD2 both homooligomerize upon ligand perception via their NACHT and CARD domains and recruit downstream signaling proteins via CARD interactions (27, 132, 216), but the structure of these NLR complexes has not been resolved. The formation of these higher-order complexes enables NLRs to form signaling platforms to recruit downstream signaling proteins.

Until recently, the structure of activated plant NLRs has been mostly inferred from studies on structurally similar animal NLR-like proteins. The cryo-EM structure of the ZAR1-RKS1-PBL2^{UMP} resistosome complex has provided insights into the assembly of higher-order complexes during plant NLR immune signaling (186) (**Figure 2*b***). Upon pathogen perception, the TNL Roq1 oligomerizes into a tetrameric resistosome complex with a twofold symmetric dimer of dimers, providing an interesting contrast to the ZAR1 pentameric resistosome complex (**Figures 2*b*** and **3*d***) (128). Another TNL RPP1 assembles a very similar tetrameric resistosome upon effector perception. In addition to the cryo-EM structure of the active form of RPP1-ATR1, the authors were able to define a C-terminal jelly roll/Ig-like domain (C-JID) that follows the canonical LRR domain in the protein organization, and the C-JID is specific for effector recognition (**Figure 3*d***) (119). Though the primary sequences of C-JID from different TNLs are not shown in any consensus, structural prediction based on a hidden Markov model indicates that the sequence-diversified C-JIDs are perhaps shared by many TNLs in dicotyledonous plant species (119). Other plant NLRs also require oligomerization for immune signaling; for example, MLA, SNC1, and L6 homooligomerize, and RRS1 and RPS4, as well as RGA4 and RGA5, heterooligomerize (see Section 6.2) (18, 28, 31, 84, 122, 190, 211). Some plant NLRs assemble higher-order structures during activation. For example, the TNL protein N forms a ligand-dependent oligomer (130). The NLRs Dangerous mix 1 (DM1) and DM2d heterooligomerize to form complexes of approximately 500 kDa, suggestive of a high-order complex similar to the NLR resistosome in innate immunity (173). The paired NLRs RPS4 and RRS1 form a protein complex via direct interactions between RPS4 and RRS1, and a similar mechanism has also been reported with RGA4 and RGA5; however, it remains unclear whether they can form higher-order complexes with the corresponding pathogenic ligands (31, 83). Conformational changes that release autoinhibitory binding, allowing the N-terminal signaling domains of the NLRs in the complex to come into close proximity, are one of the possible mechanisms for why these paired NLRs do not rely on inducible complex formation for signaling activation. Further studies to resolve the structures of homo- and heterocomplexes of paired NLRs are required to understand the mechanism of activation after effector perception.

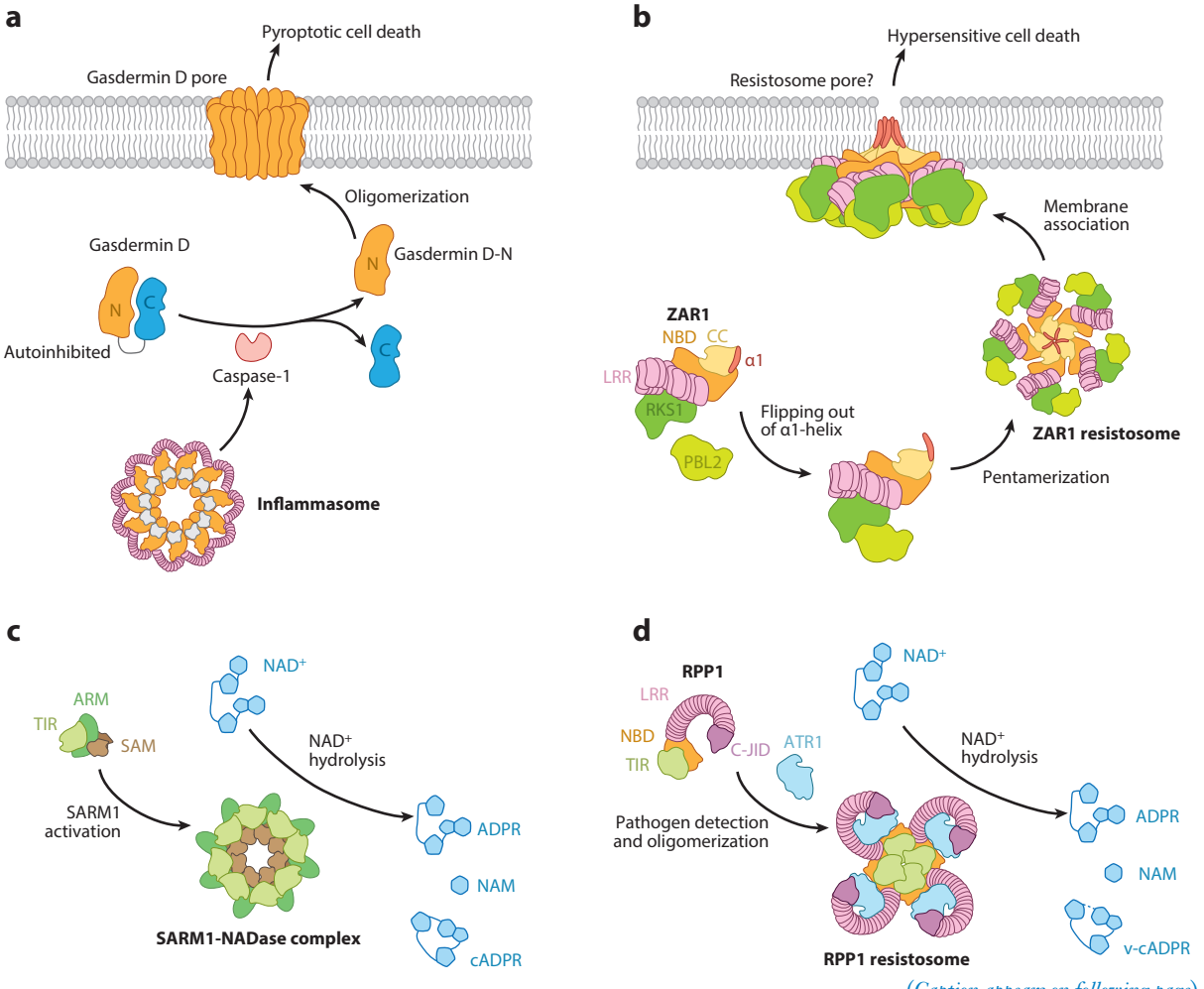
5. WHAT HAPPENS TO NLRS AFTER ACTIVATION?

5.1. Induced Proximity of NLRs

The assembly of large NLR complexes following pathogen perception facilitates downstream signaling. In the case of inflammasomes, the N-terminal domains (i.e., the CARD or PYD) are

brought together into the center of the wheel-like structure in a high local density (**Figure 2*b***). This induced proximity of signaling domains is sufficient to recruit downstream signaling proteins. NLRP-type NLRs that lack CARDs interact via homotypic PYD association with an adaptor protein called ASC, which contains a PYD and CARD. ASC subsequently recruits caspase-1 via homotypic CARD interactions. NLRs NLRC4 and NLRP1b, which contain CARDs, can directly interact with the protease caspase-1 without an adaptor. Caspase-1 is activated by autoproteolysis upon being brought into induced proximity when recruited to the inflammasome (36).

There is strong evidence supporting the induced proximity model of signaling activation in plant NLRs. Inflammasomes can be reconstituted in plants by transiently coexpressing NLRC4 with NAIPs and their cognate ligands in *N. benthamiana* (58). NLRC4 with the RPS4 TIR domain fused to its N terminus triggers cell death when induced to form an inflammasome in planta, indicating that induced proximity of TIR domains is sufficient to activate immunity and that TNLs employ induced proximity to mediate downstream immune signaling. Indeed, assembly of the ZAR1-RKS1-PBL2^{UMP} resistosome resembles inflammasome formation, and this may be because



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Comparison of mechanisms after NLR activation in plants and animals. (*a*) Activation of the inflammasome leads to pore formation by polymerized GSDMD on the plasma membrane. The CARDs in the inflammasome recruit and activate caspase-1, which then cleaves full-length GSDMD. After cleavage, the N terminus of GSDMD polymerizes and forms pores on the plasma membrane to release IL-1b and IL-18, driving pyroptosis. Panel *a* adapted with permission from Reference 210; copyright 2015 American Association for the Advancement of Science. (*b*) The ZAR1 resistosome may perturb the integrity of membranes. The α1-helix in the CC domain of ZAR1 flips out upon resistosome assembly, forming a wheel-like structure with the α1-helix sticking out. The complexes associate with the membrane and might function as a channel similar to GSDMD to trigger immune signaling. Panel *b* adapted with permission from Reference 186; copyright 2019 American Association for the Advancement of Science. (*c*) TIR domain–containing protein SARM1 has oligomerization-dependent NADase enzymatic activity. The SARM1 TIR domain cleaves NAD^+ to ADPR, cADPR, and NAM in in vitro biochemical assays. Panel *c* adapted with permission from Reference 24; copyright 2020 by Elsevier. (*d*) The RPP1 resistosome hydrolyzes NAD^+ to activate downstream immune responses. Upon activation, the TNL RPP1 forms a tetrameric resistosome complex with the exposed NADase active site. This allows the cleavage of NAD^+ to occur, hydrolyzing NAD^+ to ADPR, v-cADPR, and NAM, similar to the biochemical activities observed with the TIR domain of other plant TNLs. Panel *d* adapted with permission from Reference 119; copyright 2020 American Association for the Advancement of Science. Abbreviations: ADP, adenosine diphosphate; ADPR, ADP ribose; ATR1, *Arabidopsis thaliana* recognized 1; cADPR, cyclic ADP ribose; CC, coiled-coil; C-JID, C-terminal jelly roll/Ig-like domain; GSDMD, gasdermin D; LRR, leucine-rich repeat; NAD, nicotinamide adenine dinucleotide; NADase, nicotinamide adenine dinucleotide nucleosidase; NADP, nicotinamide adenine dinucleotide phosphate; NAM, nicotinamide; NBD, nucleotide-binding domain; RPP1, Recognition of *Peronospora parasitica* 1; SAM, sterile alpha motif; SARM1, sterile alpha and TIR motif-containing 1; TIR, Toll/interleukin-1 receptor/R protein; v-cADPR, variants of cyclic ADP ribose; ZAR1, HopZ-activated resistance 1.

induced proximity of ZAR1 N-terminal CC domains is required for downstream signaling (186). The fungal NLR-like protein NACHT and WD repeat domain-containing 2 (NWD2) also employs induced proximity of N-terminal signaling proteins to mediate downstream signaling. NWD2 contains an N-terminal heterokaryon incompatibility protein s (Het-s)-like prion-forming domain (PFD), a NACHT domain, and a C-terminal WD40 repeat domain and signals via the oligomerization of PFDs with HET-S protein (26). An NLRP3 chimera containing the NWD2 HET-S-like domain instead of a PYD maintained inflammasome signaling via a chimeric ASC that similarly had a Het-s prion-forming domain instead of a PYD (26). This observation demonstrated that, similar to the induced proximity of NLRP3 PYD during inflammasome formation, NWD2 employs induced proximity to initiate downstream signaling (26).

5.2. Membrane Perturbation

The signaling mechanism leading to cell death after induced proximity of NLRs has been a major research topic in NLR biology. One of the most promising mechanisms is via perturbing cell membranes, which leads to the formation of pores, allowing signaling molecules to move across the lipid bilayer. In the case of animal inflammasomes, mature caspase-1 cleaves the proinflammatory cytokines prointerleukin (pro-IL)-1b and pro-IL-18 and pore-forming gasdermin D (GSDMD) (91, 124, 156). The mature N-terminal fragment of GSDMD oligomerizes, disrupts the plasma membrane, and releases IL-1b and IL-18 to drive pyroptosis (116, 149, 156) (**Figure 3*a***).

The wheel-like ZAR1-RKS1-PBL2^{UMP} resistosome assembles into a funnel-shaped structure through its center formed from the CC domains and NBD. Because the α1-helix of the CC domain flips out during ZAR1 resistosome assembly, forming a protrusion that could perturb the integrity of a plasma membrane, researchers have hypothesized that it acts as a channel to activate immunity, perhaps by allowing the influx of calcium into the cell (25, 186) (**Figure 3*b***). In fact, the CNL RPM1 triggers Ca^{2+} influx when activated (69), lending some preliminary support to this hypothesis. In addition to the comparison with pore-forming GSDMD, parallels can be made with other systems: NWD2 induces HET-S to form a pore in the plasma membrane that causes cell death (152). Identifying the mechanism by which the resistosome mediates immunity is a leading priority in plant NLR research.

5.3. NADase Activity

As most reported TNLs in plants localize or function in the nucleus, it is unlikely that TIR domains adopt the same mechanism as CC domains to induce cell death. Studies of animal sterile alpha and TIR motif-containing 1 (SARM1), a TIR-containing protein that plays a role in axonal degeneration after the injury of the neuron, led to a breakthrough in the understanding of plant TNL function. Investigations to understand the mechanism by which SARM1 drives degradation discovered that the TIR domain in SARM1 has oligomerization-dependent NADase enzymatic activity, and purified SARM1 TIR domain ($SARM1^{TIR}$) in in vitro biochemical assays cleaves NAD^+ to ADP ribose (ADPR), cyclic ADPR (cADPR), and nicotinamide (**Figure 3*c***) (59).

The crystal structure of $SARM1^{TIR}$ resembles plant TIRs more than other animal TIRs, and plant TIRs also contain this conserved glutamate residue, leading to the landmark discoveries that several plant TNLs exhibit NADase activity, albeit with lower activity than SARM1 exhibits (**Figure 3*d***) (76, 182). Substitution of the conserved glutamate in the TIR domain from plant NLRs abolished NADase activity, as well as TIR-activated cell death, indicating that either NAD^+ depletion or the products of NADase activity are important for downstream immunity (76, 182). The fusion of $SARM1^{TIR}$ with sterile alpha motif (SAM) domains (the oligomerization domains of SARM1) ($SARM1^{TIR}$-$SARM1^{SAM}$) also causes cell death in plants. However, this cell death does not require the essential plant TNL signaling protein EDS1, suggesting that SARM1-induced plant cell death is distinct from plant TNL-mediated immunity (76, 182). Unlike $SARM1^{TIR}$-$SARM1^{SAM}$, the $SARM1^{TIR}$ fused with the NLRC4 inflammasome ($SARM1^{TIR}$-NLRC4) does not trigger cell death in plants (58). NAD^+ degradation and NADase products were detected in $SARM1^{TIR}$-NLRC4 upon ligand binding and in an inflammasome formation in planta. Therefore, NAD^+ degradation by NADase is not sufficient to induce cell death in plants, suggesting that cell death activated by TIRs in plants requires more than the products of NAD^+ cleavage (58).

Although the direct evidence is still missing, the products of NADase activity could play universal roles as direct links between plant TNLs and their downstream signaling components. Indeed, in the resistosome complexes of Roq1 and RPP1, the TIR domains were brought into proximity and the NADase active site was exposed, potentially allowing the cleavage of NAD^+ to take place (119, 128).

6. NLRS FUNCTION AS SINGLETONS, PAIRS, OR NETWORKS

Our conceptual understanding of plant disease resistance (*R*) gene function began with the gene-for-gene model proposed by Harold Henry Flor (63, 92). Studies over the last few decades revealed that most *R* genes in plants encode NLR proteins. Indeed, many NLRs function as single genetic determinants governing disease resistance to their corresponding pathogens. With increasing knowledge and depth of investigation in plant genetics and functional genomics, different levels of NLR networks, as well as PRR networks, have been recently revealed in different plant species (157, 194). Recent studies showed that some NLRs have evolved to function together, forming various degrees of genetic or biochemical complexes, leading to new understanding that NLRs can work as functional pairs and networks in addition to as a singleton. Here, we discuss how these concepts apply to NLRs in both plants and animals.

6.1. NLRs as Functional Singletons

Functional singleton NLRs can detect pathogen effectors directly or indirectly and initiate downstream responses without relying on an additional NLR (**Figure 4**). One of their key features is that they can induce immune responses when heterologously expressed together with

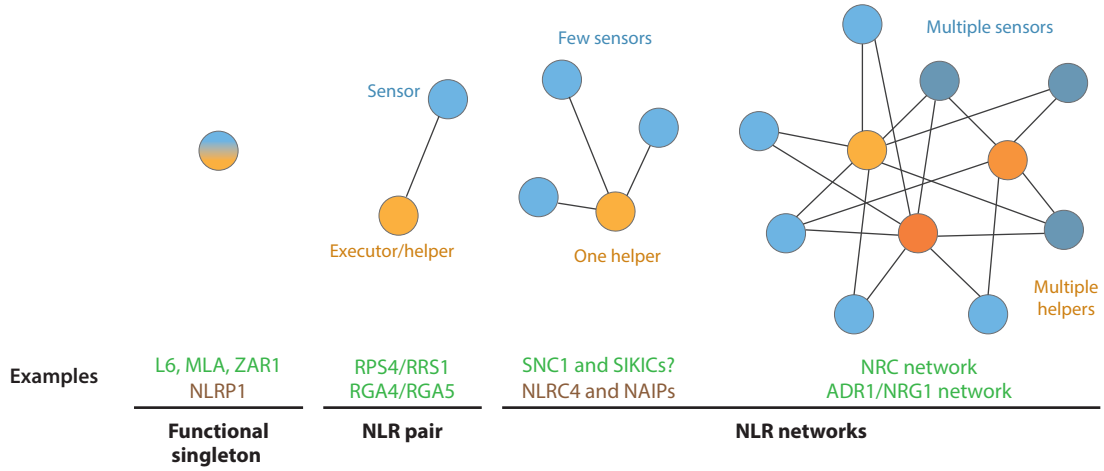


Figure 4

The complexity of NLR networks in both plants and animals. Plant and animal NLRs can work as functional singletons, pairs, or networks. Functional singleton NLRs detect pathogen effectors directly or indirectly and initiate immediate downstream responses without relying on an additional NLR. NLR pairs include both a sensor and an executor/helper, and they often function exclusively with each other. NLR networks link multiple sensor and helper NLRs in the same pathway. Depending on the complexity of the networks, some NLR networks involve multiple sensors and one helper whereas some other more complicated networks have multiple sensors and multiple helpers. Plant examples are marked in green, whereas animal examples are marked in brown. Abbreviations: ADR1, activated disease resistance 1; MLA, mildew resistance locus a; NAIP, neuronal apoptosis inhibitory protein; NLR, nucleotide-binding domain leucine-rich repeat receptor; NRC, NLR-required for cell death; NRG1, N requirement gene 1; RGA, R-gene analog; RPS4, Resistance to *Pseudomonas syringae* 4; RRS1, Resistance to *Ralstonia solanacearum* 1; SIKIC, sidekick SNC1; SNC1, Suppressor of *npr1-1*, constitutive 1; ZAR1, HopZ-activated resistance 1. Figure adapted with permission from Reference 4; copyright 2019 Elsevier.

the corresponding effectors in a distantly related species (4). The best-understood example of a plant singleton NLR is ZAR1, which detects effectors such as AvrAC through its RLCK partners RKS1 and PBL2 (184). As previously discussed, ZAR1 forms a pentamer resistosome complex, and the N-terminal CC domain likely involves membrane perturbation to trigger cell death responses (186). Roq1 and RPP1 resistosomes also function as singletons that directly detect the effector XopQ1 and ATR1, respectively, and form a tetrameric resistosome complex (119, 128). Many other plant NLRs, such as MLA, Sr50, RPP13, RPS5, and L6, were shown to induce cell death when expressed heterologously in *Nicotiana* spp., suggesting that these NLR proteins are likely to function as singletons (5, 17, 37, 110, 137, 140, 148). Human NLRP1 and its mouse ortholog NLRP1b also behave as functional singletons.

6.2. NLRs Function in Pairs

Many NLR proteins are known to function in pairs. These NLR pairs are distinguished by their head-to-head arrangement on chromosomes, which suggests that the two *NLR* genes are coregulated to optimize their functions in conferring disease resistance. Based on their activities, the two NLRs in an NLR pair are classified into sensor and executor/helper (4) (**Figure 4**). The sensor NLRs in NLR pairs often contain an ID that is believed to have originated from the targets of effectors (see Section 3.3) (30, 71). Two of the most-studied examples of NLR pairs are RPS4/RRS1 of *Arabidopsis* and RGA4/RGA5 (also known as Pia-1 and Pia-2) of rice. In the case of RPS4/RRS1, overexpressed RPS4 is constitutively autoactive and can be suppressed by coexpression with RRS1. The perception of effectors AvrRps4 and PopP2 by the WRKY domain of RRS1

initiates an immune response that requires direct interaction between RRS1 and RPS4 (106, 120, 147). Hence, RRS1 functions as a sensor NLR that suppresses the autoactivity of the executor RPS4 in the absence of effectors and activates RPS4 in the presence of effector proteins. The RGA4 and RGA5 NLR pair also detects two sequence-unrelated effector proteins, AVR-Pia and AVR1-CO39 from the rice blast fungus. Both effectors bind to the HMA domain integrated into RGA5 and then activate immune responses through RGA4 (31, 32). Similar domain integration was found in another rice NLR pair, Pik-1/Pik-2, in which the HMA domain integrated into Pik-1 and was able to bind effector protein AVR-Pik. Detection of AVR-Pik alleles by Pik-1 activates immune responses through the paired executor Pik-2 (7, 125). Interestingly, a recent study revealed that 20% of the NLRs in the Tetep rice genome are paired (188), suggesting that the paired NLR system may have a significant contribution to the disease resistance in some rice cultivars.

RNL: RPW8-type CC-domain-containing NLR

6.3. NLRs Function in Networks

Some NLRs in plants and animals have evolved more complex connections, which link multiple sensor NLRs in the same immune-signaling network (**Figure 4**). In this review, we define this scenario as NLR networks by which more than two NLRs are connected genetically, or, in some cases, biochemically, in an immunity pathway. The complexity of the NLR networks varies, depending on each case and hypothesis that have been tested thus far. Here, we summarize the major examples of NLR networks in both plants and animals.

6.3.1. The NRC network. The NLR-required for cell death (NRC) proteins are CNL proteins with typical domain architectures identified in solanaceous plants. They function as helper NLRs that mediate the immune responses of diverse sensor NLRs, which detect various pathogen effectors. In *N. benthamiana*, three NRCs (NRC2, NRC3, and NRC4) have been functionally characterized (192). These paralogous NRCs show degrees of genetic redundancy that increase the complexity and robustness of this NLR network. Interestingly, all reported NRC-dependent sensor NLRs fall into a phylogenetic superclade with the NRCs, indicating that the NRCs and their matched sensor NLRs are evolutionarily related. The NRC network perhaps originated from a gene cluster that predates the divergence of asterids and caryophyllenes. This ancestral NRC helper-sensor cluster greatly expanded and rearranged in the genome of asterid plants, constituting up to half of the NLRs in some species (192). It is not clear whether NRCs form heterocomplexes with the sensor NLRs that are similar to the NLRC4/NAIP inflammasome or form homocomplexes that are similar to the ZAR1 resistosome. It is also not clear whether NRCs function genetically downstream of other sensor NLRs, similar to the roles of RPW8-type CC-domain-containing NLRs (RNLs), such as ADR1 and NRG1. Interestingly, a recent report showed that the N terminus of NRC4 contains a MADA-motif that is conserved among NRCs, ZAR1, and several other singleton NLRs. This motif has degenerated in the NRC-dependent sensor NLRs, which may represent an important step during the evolution and diversification of the NRC-helper/sensor NLRs from their singleton ancestor. These results also suggest that NRC, ZAR1, and other singleton NLRs may share a conserved mechanism in inducing cell death (3). Some studies suggested that, in addition to participating in the NLR network described above, NRCs may, at least partially, contribute to immune responses mediated by PRRs, such as Cf-4 and LeEIX (65, 108, 193). Further studies may reveal how cell surface immune receptors mount responses to intracellular helper NLRs to achieve robust immunity.

6.3.2. SNC1 and SIKICs. The TNL protein SNC1 was identified in a suppressor screen of *npr1-1*, in which the identified allele *snc1* displayed a curly-leaved, dwarf autoimmune phenotype

(113). In a recent reverse genetic screen, three additional TNLs, named SIKICs, were identified as components involved in SNC1-mediated immune responses (53). The three SIKICs are genetically redundant in the growth defects caused by *snc1*, suggesting that SNC1 and SIKICs together form an NLR network composed of TNLs. Interestingly, SIKICs are in a gene cluster together with SNC1 and have high similarity (65%–72%) to SNC1, suggesting that this TNL network originated from a cluster that has been duplicated recently. The autoimmunity induced by *snc1* requires at least one SIKIC (53). However, whether immune responses induced by either one of the SIKICs require SNC1 is yet to be tested.

6.3.3. Network mediated by ADR1 and NRG1. Both ADR1 and NRG1 belong to the RNL clade, which is a class of ancient CNL with an N terminus similar to RPW8. Compared to the CNL and TNL clades that are extensively expanded in different plant lineages, the ADR1 and NRG1 families are relatively small (154). ADR1 family members are functionally redundant for some tested CNLs and TNLs, including the NLR pair RPS4/RRS1, whereas NRG1 family members act downstream of EDS1 to regulate the TNL signaling pathway (23, 29, 139, 195). Interestingly, the NRG1 family was lost in monocots and several dicot lineages. This phenomenon coincides with the loss of TNL gene families, reflecting the functional connection between TNLs and the NRG1 family (41, 154). Two recent studies using the helperless mutant (sextuple *adr1 nrg1* mutant) validated that ADR1 and NRG1 subfamilies contribute to plant basal defense and TNL-triggered immunity in a fully or unequally redundant manner; however, ADR1 and NRG1 subfamilies also have independent and nonredundant functions in immunity triggered by some NLRs, depending on the TNLs or CNLs tested (145, 195). Thus far, there is no evidence that ADR1 or NRG1 paralogs physically associate with sensor NLRs, and it is generally accepted that the ADR1 and NRG1 families are genetically downstream. The mechanisms by which an immune signal transduces from the sensor NLRs to ADR1/NRG1 and how they synergistically contribute to immunity are not clear.

6.3.4. NLRC4 and NAIPs. The only NLR pair/network example that has been clearly characterized in mammals thus far is the NAIP/NLRC4 inflammasome. Different NAIP sensor NLRs recognize bacterial protein ligands through direct binding and then recruit NLRC4 to form inflammasome complexes (180) (Section 3.1). NLRC4 does not bind to the ligand but functions as the helper/executor NLR that activates caspase-1 to launch inflammasome signaling. Whether or not plants have a resistosome heterocomplex similar to the inflammasomes that contain both a sensor and multiple executor/helper NLRs is yet to be determined.

7. NLRS BEYOND PATHOGEN DETECTION

Some studies suggest that NLRs and NLR-like proteins (NBD fusing to other domains instead of the LRR domain) participate in biological processes beyond pathogen detection. Here, we summarize related discoveries from different organisms.

7.1. NLRs and NLR-Like Proteins Involved in Surveillance of Cell Integrity

Apoptosis is controlled by the perception of cytochrome c, released from the mitochondria, by the NLR-like protein Apaf-1. In addition to an N-terminal CARD and a central NB-ARC, Apaf-1 contains two C-terminal WD40 domains. Upon binding cytochrome c, Apaf-1 assembles into a heptameric apoptosome that induces the proximity of its CARDs, which in turn recruits caspase-9 via homotypic interactions (54, 207). Before the structure of inflammasomes was resolved, the

wheel-like structure of the Apaf-1/cytochrome c apoptosome had been well-studied and guided hypotheses of inflammasome structure and function (2, 205, 208). For example, apoptosome formation requires ATP or deoxyadenosine triphosphate (dATP) binding but was also possible with nonhydrolyzable ATP, indicating that hydrolysis was not required for assembly, the WD40 sterically blocks oligomerization, and Apaf-1 with deleted WD40 domains is constitutively active (80, 144, 158).

The mammalian NLR NLRP3 is involved in an enormous range of surveillance activities. Activation of NLRP3 usually requires two steps: priming, which is mediated by a variety of convergent signaling pathways often mediated by PAMP perception by TLRs or NOD2, and activation, which can be in response to a staggering list of PAMPs and DAMPs (161, 162). It has been hypothesized that NLRP3 is a universal sensor of cellular stress, but it remains to be validated if there is a universal ligand downstream of each stress elicitor sensed by NLRP3.

7.2. NLRs in Mammalian Reproduction

Although NLRs are well-known in their roles in innate immunity, studies have suggested that several phylogenetically related mammalian NLRPs are involved in reproduction-related processes. NLRP5, also known as MATER, is an oocyte-specific gene that is essential for embryonic development beyond the two-cell stage (172). NLRP2 functions as a critical regulator of oocyte quality and perhaps contributes to age-dependent fertility loss in humans (99). NLRP7, an ortholog of NLRP2, is often found to be mutated in human patients with abnormal pregnancies (129). NLRP14 is also involved in egg development, as knocking down *NLRP14* arrests the embryo between the one-cell and eight-cell developmental stages (1). Orthologs of the *NLRP4* and *NLRP9* genes were also found to be specifically expressed in oocytes or testes, yet the functions of these two NLRPs are not clear (170a). Although NLRs have not been extensively studied outside of plants and mammals, these findings reveal that NLRs may have evolved to participate in different biological processes other than innate immunity.

7.3. NLRs Involved in Plant Hybrid Necrosis

Plant hybrid necrosis occurs when two incompatible parental plants are crossed, leading to inappropriate immune activation that causes deleterious effects in the progeny. Studies with *Arabidopsis* have found that many of these scenarios are caused by the activation of NLR genes (20, 33). For example, as mentioned previously, an NLR from the *DM1* locus interacts with NLRs from *DM2*, and, in progeny of an incompatible cross, this results in the formation of a resistosome-like complex and constitutive autoimmunity (33, 173). Furthermore, the oligomerization of the CNL RPP7 is induced when an incompatible allele of RPW8/HR is introduced into the same genetic background (13, 112). The TNL gene *DM10*, which perhaps has recently relocated from a larger NLR gene cluster, induces hybrid necrosis in combination with *DM11*, causing massive transcriptional changes overrepresented in immune signaling (12). The occurrence of hybrid incompatibility caused by NLR genes can be viewed as a by-product of the adaptive evolution of plant immunity, which then limits the combinations of NLRs that can be found in the same individual plant. Since hybrid incompatibility occurs in the progeny of some crosses, it may also indirectly affect the gene flow among plant populations, leading to the establishment or maintenance of species barriers. Thus, although the major function of plant NLRs lies in immunity, the incompatibility among NLRs or between NLRs and other genes in the genome may affect how a plant species evolves over time (19, 21, 183).

7.4. NLR-Like Proteins Involved in Nonself Recognition

NLR-like proteins in fungi can determine allorecognition outcomes by mediating cell death during a process called heterokaryon incompatibility. A recent example illustrates this with an NLR-like protein that employs many of the previously discussed mechanisms of plant and animal NLR function. Patatin-like phospholipase-1 (PLP-1) has a tripartite architecture similar to NLRs, including an N-terminal PLP domain, a central NB-ARC, and a C-terminal tetratricopeptide repeat domain (75). PLP-1 interacts with and guards the SNARE protein SEC-9. The *plp-1* and *sec-9* genes are highly polymorphic and are under balancing selection. Heterokaryon incompatibility is triggered in an incompatible cross between the distantly related fungi *Neurospora crassa* and *Podospora anserine* by the detection of SEC-9 polymorphisms by PLP-1 (75). NWD2, described in Section 5, responds to an as-yet-unknown ligand to mediate downstream signaling via amyloid templating of HET-S (45). These diverse signaling approaches by related NLR-like proteins demonstrate the utility of NLR-like proteins in fungi.

8. CONCLUSIONS AND PERSPECTIVE

In this review, we discussed major advances and landmark discoveries related to NLR-mediated plant immunity, including comparisons with NLRs or NLR-like proteins from other species to provide insights to better understand the characteristics of plant NLR proteins. However, due to space limitations, we are not able to cover many other interesting aspects of NLRs, for instance, transcriptional and translational machinery that regulate NLR homeostasis (160), as well as the degree to which the downstream signaling components, such as EDS1 family proteins and others, are conserved for various NLRs across different species (103).

The recent discoveries of the plant NLR ZAR1, Roq1, and RPP1 resistosomes and the helper NLRs NRG1/ADR1 as calcium channels have inspired many new questions. For instance, do other CNLs or TNLs form similar structures to those of the ZAR1, Roq1, or RPP1 resistosomes? Can paired NLRs (e.g., RRS1/RPS4) or NLRs forming networks (e.g., sensor NLRs with NRCs) also form structures like resistosomes or inflammasomes for activation? Can other helper NLRs (e.g., NRCs) form ADR1/NRG1-like calcium channels to mediate cell death and other downstream signaling? Or perhaps there are some other overlooked mechanisms involved. It appears that NADase activity requires TNL or TIR oligomerization, but whether TNL oligomerization also requires NADase activity is not known. How does TNL NADase activity link to downstream signaling, such as activation of transcription or activation of helper NLRs (e.g., ADR1/NRG1)? More broadly, is the complex from activated TNL or CNL homogeneous, or do they form complexes with different partner proteins even when only one specific pathogen-derived ligand has been recognized?

Activation of TNLs and CNLs can lead to similar transcriptional reprogramming (49, 86, 145), but the molecular pathways that converge to give similar transcriptional programs are not clear. Recently, a TNL-mediated gene regulatory network has been proposed (50), and whether this gene network is shared by all NLRs or the regulatory patterns are conserved across plant species remains to be discovered.

Another conceptual breakthrough was reported recently: NLR-mediated disease resistance is dependent on PRRs, and in turn, NLR activation potentiates and enhances PRR-mediated immune responses (134, 206). Future research will elucidate the general mechanism of immune responses mediated between cell surface and intracellular immune receptors. All of these recent discoveries provide important novel knowledge and insights on NLR-mediated immunity in plants, which will improve the breeding and engineering of novel and robust resistance of crops against diseases.

SUMMARY POINTS

1. Plant and animal nucleotide-binding domain leucine-rich repeat receptors (NLRs) share similar domain architectures and modular organizations, in which the nucleotide-binding domains may share the same ancestral origin.
2. Plant and animal NLRs have independently evolved mechanisms to detect pathogens directly or indirectly.
3. Both plant and animal NLRs bind adenosine diphosphate (ADP)/adenosine triphosphate (ATP) and oligomerize upon activation.
4. Plant ZAR1 resistosomes may perturb membrane integrity, assembling a similar mechanism that is mediated by gasdermin D upon inflammasome activation in animals.
5. Roq1 and RPP1 resistosomes induce close proximity of the TIR domain and activate the NADase activity of the TIR domain.
6. Some TIR domains from plant TNLs upon oligomerization hydrolyze NAD^+, similar to the NADase activity identified in the TIR domain of SARM1.
7. With increasing knowledge of the functional genomics of NLRs, more and more plant and animal NLRs are found to function in pairs and networks, which perhaps provides robustness of innate immunity against rapidly evolving pathogens.
8. NLR and NLR-like proteins also have functions beyond pathogen detection and innate immunity.

FUTURE ISSUES

1. What is the mechanism of cell death activated by plant NLRs? Do the ZAR1, RPP1, and Roq1 resistosome models apply to other plant NLRs?
2. How does a plant NLR activate downstream defense gene expression?
3. Is there a general mechanism by which NLRs and PPRs mutually potentiate each other to function?
4. How did NLR networks evolve and specialize in different plant lineages to confer resistance to different pathogens?
5. How does NLR-mediated immunity contribute to plant resistance to different types of pathogens?
6. Do different types of NLRs share similar downstream signaling?

NOTE ADDED IN PROOF

NADase activities of TIR domains are conserved across different kingdoms. Interestingly, in the Thoeris (Ths) bacterial antiphage immune system, TIR domains from ThsB are enzymatic, and they catalyze the production of a cADPR isomer or variant (v-cADPR) signaling molecule akin to the plant TIR domain (134a). In bacteria, this signal activates a downstream NADase, ThsA, that leads to NAD depletion and abortive infection, while in plants the exact role of the TIR enzymatic activity is not yet fully understood.

NLRs may function as ion channels upon activation. A recent study indicates that the N-terminal signaling domains of ADR1/NRG1 resemble the cation channel structures similar to those of ZAR1 and the animal Mixed lineage kinase domain-like (MLKL) protein (86a), and the autoactive alleles of ADR1/NRG1 can mediate Ca^{2+} influx and cell death in both plants and human HeLa cells (86a). However, it is still not clear whether this mechanism applies to the native conditions when the full-length ADR1/NRG1 are activated by the sensor NLRs upon recognition of effectors.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We sincerely apologize to colleagues whose work we were unable to cover in this review due to the word and page limits. We thank Dr. Sophien Kamoun (The Sainsbury Laboratory, Norwich) and Dr. Feng Shao (National Institute of Biological Sciences, Beijing) for feedback on an earlier draft of this article. P.D. acknowledges support from a Future Leader Fellowship funded by the Biotechnology and Biological Sciences Research Council (BB/R012172/1).

LITERATURE CITED

1. Abe T, Lee A, Sitharam R, Kesner J, Rabadan R, Shapira SD. 2017. Germ-cell-specific inflammasome component NLRP14 negatively regulates cytosolic nucleic acid sensing to promote fertilization. *Immunity* 46(4):621–34
2. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. 2002. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol. Cell* 9(2):423–32
3. Adachi H, Contreras MP, Harant A, Wu C-H, Derevnina L, et al. 2019. An N-terminal motif in NLR immune receptors is functionally conserved across distantly related plant species. *eLife* 8:e49956
4. Adachi H, Derevnina L, Kamoun S. 2019. NLR singletons, pairs, and networks: evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants. *Curr. Opin. Plant Biol.* 50:121–31
5. Ade J, DeYoung BJ, Golstein C, Innes RW. 2007. Indirect activation of a plant nucleotide binding site–leucine-rich repeat protein by a bacterial protease. *PNAS* 104(7):2531–36
6. Aravind L. 2000. The BED finger, a novel DNA-binding domain in chromatin-boundary-element-binding proteins and transposases. *Trends Biochem. Sci.* 25(9):421–23
7. Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, et al. 2008. Two adjacent nucleotide-binding site–leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics* 180(4):2267–76
8. Axtell MJ, Staskawicz BJ. 2003. Initiation of *RPS2*-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. *Cell* 112(3):369–77
9. Bai S, Liu J, Chang C, Zhang L, Maekawa T, et al. 2012. Structure-function analysis of barley NLR immune receptor MLA10 reveals its cell compartment specific activity in cell death and disease resistance. *PLOS Pathog.* 8(6):e1002752
10. Bailey PC, Schudoma C, Jackson W, Baggs E, Dagdas G, et al. 2018. Dominant integration locus drives continuous diversification of plant immune receptors with exogenous domain fusions. *Genome Biol.* 19(1):23
11. Bao Q, Riedl SJ, Shi Y. 2005. Structure of Apaf-1 in the auto-inhibited form: a critical role for ADP. *Cell Cycle* 4(8):1001–3
12. Barragan AC, Collenberg M, Wang J, Lee RRQ, Cher WY, et al. 2020. A truncated singleton NLR causes hybrid necrosis in *Arabidopsis thaliana*. *Mol. Biol. Evol.* 38(2):557–74

13. Barragan CA, Wu R, Kim S-T, Xi W, Habring A, et al. 2019. RPW8/HR repeats control NLR activation in *Arabidopsis thaliana*. *PLOS Genet*. 15(7):e1008313
14. Bastedo DP, Khan M, Martel A, Seto D, Kireeva I, et al. 2019. Perturbations of the ZED1 pseudokinase activate plant immunity. *PLOS Pathog*. 15(7):e1007900
15. Bendahmane A, Farnham G, Moffett P, Baulcombe DC. 2002. Constitutive gain-of-function mutants in a nucleotide binding site–leucine rich repeat protein encoded at the *Rx* locus of potato. *Plant J*. 32(2):195–204
16. Bentham AR, Zdrzalek R, De la Concepcion JC, Banfield MJ. 2018. Uncoiling CNLs: structure/function approaches to understanding CC domain function in plant NLRs. *Plant Cell Physiol*. 59(12):2398–408
17. Bernoux M, Burdett H, Williams SJ, Zhang X, Chen C, et al. 2016. Comparative analysis of the flax immune receptors L6 and L7 suggests an equilibrium-based switch activation model. *Plant Cell* 28(1):146–59
18. Bernoux M, Ve T, Williams S, Warren C, Hatters D, et al. 2011. Structural and functional analysis of a plant resistance protein TIR domain reveals interfaces for self-association, signaling, and autoregulation. *Cell Host Microbe* 9(3):200–11
19. Bomblies K. 2009. Too much of a good thing? Hybrid necrosis as a by-product of plant immune system diversification. *Botany* 87(11):1013–22
20. Bomblies K, Lempe J, Epple P, Warthmann N, Lanz C, et al. 2007. Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *PLOS Biol*. 5(9):e236
21. Bomblies K, Weigel D. 2007. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nat. Rev. Genet*. 8(5):382–93
22. Bonardi V, Cherkis K, Nishimura MT, Dangl JL. 2012. A new eye on NLR proteins: focused on clarity or diffused by complexity? *Curr. Opin. Immunol*. 24(1):41–50
23. Bonardi V, Tang S, Stallmann A, Roberts M, Cherkis K, Dangl JL. 2011. Expanded functions for a family of plant intracellular immune receptors beyond specific recognition of pathogen effectors. *PNAS* 108(39):16463–68
24. Bratkowsi M, Xie T, Thayer DA, Lad S, Mathur P, et al. 2020. Structural and mechanistic regulation of the pro-degenerative NAD hydrolase SARM1. *Cell Rep*. 32:107999
25. Burdett H, Bentham AR, Williams SJ, Dodds PN, Anderson PA, et al. 2019. The plant "resistosome": structural insights into immune signaling. *Cell Host Microbe* 26(2):193–201
26. Cai X, Chen J, Xu H, Liu S, Jiang Q-X, et al. 2014. Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. *Cell* 156(6):1207–22
27. Caruso R, Warner N, Inohara N, Núñez G. 2014. NOD1 and NOD2: signaling, host defense, and inflammatory disease. *Immunity* 41(6):898–908
28. Casey LW, Lavrencic P, Bentham AR, Cesari S, Ericsson DJ, et al. 2016. The CC domain structure from the wheat stem rust resistance protein Sr33 challenges paradigms for dimerization in plant NLR proteins. *PNAS* 113(45):12856–61
29. Castel B, Ngou P-M, Cevik V, Redkar A, Kim D-S, et al. 2019. Diverse NLR immune receptors activate defence via the RPW8-NLR NRG1. *New Phytol*. 222(2):966–80
30. Césari S, Bernoux M, Moncuquet P, Kroj T, Dodds PN. 2014. A novel conserved mechanism for plant NLR protein pairs: the "integrated decoy" hypothesis. *Front. Plant Sci*. 5:606
31. Césari S, Kanzaki H, Fujiwara T, Bernoux M, Chalvon V, et al. 2014. The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. *EMBO J*. 33(17):1941–59
32. Césari S, Thilliez G, Ribot C, Chalvon V, Michel C, et al. 2013. The rice resistance protein pair RGA4/RGA5 recognizes the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by direct binding. *Plant Cell* 25(4):1463–81
33. Chae E, Bomblies K, Kim S-T, Karelina D, Zaidem M, et al. 2014. Species-wide genetic incompatibility analysis identifies immune genes as hot spots of deleterious epistasis. *Cell* 159(6):1341–51
34. Chavarría-Smith J, Mitchell PS, Ho AM, Daugherty MD, Vance RE. 2016. Functional and evolutionary analyses identify proteolysis as a general mechanism for NLRP1 inflammasome activation. *PLOS Pathog*. 12(12):e1006052
35. Chavarría-Smith J, Vance RE. 2013. Direct proteolytic cleavage of NLRP1B is necessary and sufficient for inflammasome activation by anthrax lethal factor. *PLOS Pathog*. 9(6):e1003452

36. Chavarría-Smith J, Vance RE. 2015. The NLRP1 inflammasomes. *Immunol. Rev.* 265(1):22–34
37. Chen J, Upadhyaya NM, Ortiz D, Sperschneider J, Li F, et al. 2017. Loss of *AvrSr50* by somatic exchange in stem rust leads to virulence for *Sr50* resistance in wheat. *Science* 358(6370):1607–10
38. Chopra AP, Boone SA, Liang X, Duesbery NS. 2003. Anthrax lethal factor proteolysis and inactivation of MAPK kinase. *J. Biol. Chem.* 278(11):9402–6
39. Chui AJ, Okondo MC, Rao SD, Gai K, Griswold AR, et al. 2019. N-terminal degradation activates the NLRP1B inflammasome. *Science* 364(6435):82–85
40. Chung E-H, da Cunha L, Wu A-J, Gao Z, Cherkis K, et al. 2011. Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. *Cell Host Microbe* 9(2):125–36
41. Collier SM, Hamel L-P, Moffett P. 2011. Cell death mediated by the N-terminal domains of a unique and highly conserved class of NB-LRR protein. *Mol. Plant Microbe Interact.* 24(8):918–31
42. Couto D, Zipfel C. 2016. Regulation of pattern recognition receptor signalling in plants. *Nat. Rev. Immunol.* 16(9):537–52
43. Dangl JL, Jones JDG. 2001. Plant pathogens and integrated defence responses to infection. *Nature* 411(6839):826–33
44. Das B, Sengupta S, Prasad M, Ghose TK. 2014. Genetic diversity of the conserved motifs of six bacterial leaf blight resistance genes in a set of rice landraces. *BMC Genet*. 15:82
45. Daskalov A, Habenstein B, Martinez D, Debets AJM, Sabaté R, et al. 2015. Signal transduction by a fungal NOD-like receptor based on propagation of a prion amyloid fold. *PLOS Biol*. 13(2):e1002059
46. De la Concepcion JC, Franceschetti M, MacLean D, Terauchi R, Kamoun S, Banfield MJ. 2019. Protein engineering expands the effector recognition profile of a rice NLR immune receptor. *eLife* 8:e47713
47. Deguine J, Barton GM. 2014. MyD88: a central player in innate immune signaling. *F1000Prime Rep*. 6:97
48. Diebolder CA, Halff EF, Koster AJ, Huizinga EG, Koning RI. 2015. Cryoelectron tomography of the NAIP5/NLRC4 inflammasome: implications for NLR activation. *Structure* 23(12):2349–57
49. Ding P, Ngou BPM, Furzer OJ, Sakai T, Shrestha RK, et al. 2020. High-resolution expression profiling of selected gene sets during plant immune activation. *Plant Biotechnol. J.* 18:1610–19
50. Ding P, Sakai T, Shrestha RK, Perez NM, Guo W, et al. 2020. Chromatin accessibility landscapes activated by cell surface and intracellular immune receptors. bioRxiv 2020.06.17.157040. **https://doi.org/10.1101/2020.06.17.157040**
51. Dodds PN, Lawrence GJ, Catanzariti A-M, Teh T, Wang C-IA, et al. 2006. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *PNAS* 103(23):8888–93
52. Dodds PN, Rathjen JP. 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* 11(8):539–48
53. Dong OX, Ao K, Xu F, Johnson KCM, Wu Y, et al. 2018. Individual components of paired typical NLR immune receptors are regulated by distinct E3 ligases. *Nat. Plants* 4(9):699–710
54. Dorstyn L, Akey CW, Kumar S. 2018. New insights into apoptosome structure and function. *Cell Death Differ*. 25(7):1194–208
55. Duesbery NS, Webb CP, Leppla SH, Gordon VM, Klimpel KR, et al. 1998. Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science* 280(5364):734–37
56. Duncan JA, Bergstralht DT, Wang Y, Willingham SB, Ye Z, et al. 2007. Cryopyrin/NALP3 binds ATP/dATP, is an ATPase, and requires ATP binding to mediate inflammatory signaling. *PNAS* 104(19):8041–46
57. Duxbury Z, Ma Y, Furzer OJ, Huh SU, Cevik V, et al. 2016. Pathogen perception by NLRs in plants and animals: parallel worlds. *Bioessays* 38(8):769–81
58. Duxbury Z, Wang S, MacKenzie CI, Tenthorey JL, Zhang X, et al. 2020. Induced proximity of a TIR signaling domain on a plant-mammalian NLR chimera activates defense in plants. *PNAS* 117(31):18832–39
59. Essuman K, Summers DW, Sasaki Y, Mao X, DiAntonio A, Milbrandt J. 2017. The SARM1 Toll/interleukin-1 receptor domain possesses intrinsic NAD^+ cleavage activity that promotes pathological axonal degeneration. *Neuron* 93(6):1334–43.E5

58. Demonstrates that the NLRC4/NAIP inflammasome can assemble when heterologously expressed in plants; the activation of TIR domains was shown to be by induced proximity.

60. Faris JD, Zhang Z, Lu H, Lu S, Reddy L, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *PNAS* 107(30):13544–49
61. Faustin B, Lartigue L, Bruey J-M, Luciano F, Sergienko E, et al. 2007. Reconstituted NALP1 inflammasome reveals two-step mechanism of caspase-1 activation. *Mol. Cell* 25(5):713–24
62. Feng F, Yang F, Rong W, Wu X, Zhang J, et al. 2012. A *Xanthomonas* uridine 5′-monophosphate transferase inhibits plant immune kinases. *Nature* 485(7396):114–18
63. Flor HH. 1956. The complementary genic systems in flax and flax rust. *Adv. Genet.* 8:29–54
64. Frost D, Way H, Howles P, Luck J, Manners J, et al. 2004. Tobacco transgenic for the flax rust resistance gene *L* expresses allele-specific activation of defense responses. *Mol. Plant Microbe Interact.* 17(2):224–32
65. Gabriëls SHEJ, Vossen JH, Ekengren SK, van Ooijen G, Abd-El-Haliem AM, et al. 2007. An NB-LRR protein required for HR signalling mediated by both extra- and intracellular resistance proteins. *Plant J.* 50(1):14–28
66. Gao W, Yang J, Liu W, Wang Y, Shao F. 2016. Site-specific phosphorylation and microtubule dynamics control pyrin inflammasome activation. *PNAS* 113(33):E4857–66
67. Gao Y, Wang W, Zhang T, Gong Z, Zhao H, Han G-Z. 2018. Out of water: the origin and early diversification of plant *R*-genes. *Plant Physiol.* 177(1):82–89
68. Germain H, Séguin A. 2011. Innate immunity: Has poplar made its BED? *New Phytol.* 189(3):678–87
69. Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J. 2000. The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. *Plant J.* 23(4):441–50
70. Grimes CL, Ariyananda LDZ, Melnyk JE, O'Shea EK. 2012. The innate immune protein Nod2 binds directly to MDP, a bacterial cell wall fragment. *J. Am. Chem. Soc.* 134(33):13535–37
71. Grund E, Tremousaygue D, Deslandes L. 2019. Plant NLRs with integrated domains: Unity makes strength. *Plant Physiol.* 179(4):1227–35
72. Halff EF, Diebolder CA, Versteeg M, Schouten A, Brondijk THC, Huizinga EG. 2012. Formation and structure of a NAIP5-NLRC4 inflammasome induced by direct interactions with conserved N- and C-terminal regions of flagellin. *J. Biol. Chem.* 287(46):38460–72
73. Hart CM, Zhao K, Laemmli UK. 1997. The scs′ boundary element: characterization of boundary element-associated factors. *Mol. Cell. Biol.* 17(2):999–1009
74. Harton JA, Linhoff MW, Zhang J, Ting JP-Y. 2002. Cutting edge: CATERPILLER: a large family of mammalian genes containing CARD, pyrin, nucleotide-binding, and leucine-rich repeat domains. *J. Immunol.* 169(8):4088–93
75. Heller J, Clavé C, Gladieux P, Saupe SJ, Glass NL. 2018. NLR surveillance of essential SEC-9 SNARE proteins induces programmed cell death upon allorecognition in filamentous fungi. *PNAS* 115(10):E2292–301

76. Shows that NAD$^+$ cleavage by TIR domains is a conserved feature of animal and plant cell death signaling pathways (see also 182).

76. Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, et al. 2019. NAD$^+$ cleavage activity by animal and plant TIR domains in cell death pathways. *Science* 365(6455):793–99

77. Howe K, Schiffer PH, Zielinski J, Wiehe T, Laird GK, et al. 2016. Structure and evolutionary history of a large family of NLR proteins in the zebrafish. *Open Biol.* 6(4):160009
78. Howles P, Lawrence G, Finnegan J, McFadden H, Ayliffe M, et al. 2005. Autoactive alleles of the flax *L6* rust resistance gene induce non-race-specific rust resistance associated with the hypersensitive response. *Mol. Plant Microbe Interact.* 18(6):570–82
79. Hu M, Qi J, Bi G, Zhou J-M. 2020. Bacterial effectors induce oligomerization of immune receptor ZAR1 *in vivo*. *Mol. Plant* 13(5):793–801
80. Hu Y, Ding L, Spencer DM, Núñez G. 1998. WD-40 repeat region regulates Apaf-1 self-association and procaspase-9 activation. *J. Biol. Chem.* 273(50):33489–94
81. Hu Z, Yan C, Liu P, Huang Z, Ma R, et al. 2013. Crystal structure of NLRC4 reveals its autoinhibition mechanism. *Science* 341(6142):172–75
82. Hu Z, Zhou Q, Zhang C, Fan S, Cheng W, et al. 2015. Structural and biochemical basis for induced self-propagation of NLRC4. *Science* 350(6259):399–404
83. Huh SU, Cevik V, Ding P, Duxbury Z, Ma Y, et al. 2017. Protein-protein interactions in the RPS4/RRS1 immune receptor complex. *PLOS Pathog.* 13(5):e1006376

84. Hyun K-G, Lee Y, Yoon J, Yi H, Song J-J. 2016. Crystal structure of *Arabidopsis thaliana* SNC1 TIR domain. *Biochem. Biophys. Res. Commun.* 481(1–2):146–52
85. Inohara N, Nuñez G. 2001. The NOD: a signaling module that regulates apoptosis and host defense against pathogens. *Oncogene* 20(44):6473–81
86. Jacob F, Kracher B, Mine A, Seyfferth C, Blanvillain-Baufumé S, et al. 2018. A dominant-interfering *camta3* mutation compromises primary transcriptional outputs mediated by both cell surface and intracellular immune receptors in *Arabidopsis thaliana*. *New Phytol.* 217(4):1667–80
86a. Jacob PM, Kim NH, Wu F, El-Kasmi F, Walton WG, et al. 2021. The plant immune receptors NRG1.1 and ADR1 are calcium influx channels. bioRxiv 2021.02.25.431980. **https://doi.org/10.1101/2021.02.25.431980**
87. Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B. 2000. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J.* 19(15):4004–14
88. Jin T, Curry J, Smith P, Jiang J, Xiao TS. 2013. Structure of the NLRP1 caspase recruitment domain suggests potential mechanisms for its association with procaspase-1. *Proteins* 81(7):1266–70
89. Jones JDG, Vance RE, Dangl JL. 2016. Intracellular innate immune surveillance devices in plants and animals. *Science* 354(6316):aaf6395
90. Jubic LM, Saile S, Furzer OJ, El Kasmi F, Dangl JL. 2019. Help wanted: helper NLRs and plant immune responses. *Curr. Opin. Plant Biol.* 50:82–94
91. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, et al. 2015. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526(7575):666–71
92. Keen NT. 1990. Gene-for-gene complementarity in plant-pathogen interactions. *Annu. Rev. Genet.* 24:447–63
93. Kim H-E, Du F, Fang M, Wang X. 2005. Formation of apoptosome is initiated by cytochrome *c*-induced dATP hydrolysis and subsequent nucleotide exchange on Apaf-1. *PNAS* 102(49):17545–50
94. Kim YK, Shin JS, Nahm MH. 2016. NOD-like receptors in infection, immunity, and diseases. *Yonsei Med. J.* 57(1):5–14
95. Kofoed EM, Vance RE. 2011. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature* 477(7366):592–95
96. Kortmann J, Brubaker SW, Monack DM. 2015. Cutting edge: Inflammasome activation in primary human macrophages is dependent on flagellin. *J. Immunol.* 195(3):815–19
97. Kourelis J, Kamoun S. 2020. RefPlantNLR: a comprehensive collection of experimentally validated plant NLRs. bioRxiv 2020.07.08.193961. **https://doi.org/10.1101/2020.07.08.193961**
98. Krasileva KV, Dahlbeck D, Staskawicz BJ. 2010. Activation of an *Arabidopsis* resistance protein is specified by the in planta association of its leucine-rich repeat domain with the cognate oomycete effector. *Plant Cell* 22(7):2444–58
99. Kuchmiy AA, D'Hont J, Hochepied T, Lamkanfi M. 2016. NLRP2 controls age-associated maternal fertility. *J. Exp. Med.* 213(13):2851–60
100. Lacey CA, Miao EA. 2019. NLRP1–one NLR to guard them all. *EMBO J.* 38(13):e102494
101. Laflamme B, Dillon MM, Martel A, Almeida RND, Desveaux D, Guttman DS. 2020. The pan-genome effector-triggered immunity landscape of a host-pathogen interaction. *Science* 367(6479):763–68
102. Lange C, Hemmrich G, Klostermeier UC, López-Quintero JA, Miller DJ, et al. 2011. Defining the origins of the NOD-like receptor system at the base of animal evolution. *Mol. Biol. Evol.* 28(5):1687–702
103. Lapin D, Kovacova V, Sun X, Dongus JA, Bhandari D, et al. 2019. A coevolved EDS1-SAG101-NRG1 module mediates cell death signaling by TIR-domain immune receptors. *Plant Cell* 31(10):2430–55
104. Laroui H, Yan Y, Narui Y, Ingersoll SA, Ayyadurai S, et al. 2011. L-Ala-γ-D-Glu-*meso*-diaminopimelic acid (DAP) interacts directly with leucine-rich region domain of nucleotide-binding oligomerization domain 1, increasing phosphorylation activity of receptor-interacting serine/threonine-protein kinase 2 and its interaction with nucleotide-binding oligomerization domain 1. *J. Biol. Chem.* 286(35):31003–13
105. Lauro ML, D'Ambrosio EA, Bahnson BJ, Grimes CL. 2017. Molecular recognition of muramyl dipeptide occurs in the leucine-rich repeat domain of Nod2. *ACS Infect. Dis.* 3(4):264–70
106. Le Roux C, Huet G, Jauneau A, Camborde L, Trémousaygue D, et al. 2015. A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. *Cell* 161(5):1074–88

107. Lechtenberg BC, Mace PD, Riedl SJ. 2014. Structural mechanisms in NLR inflammasome signaling. *Curr. Opin. Struct. Biol.* 29:17–25
108. Leibman-Markus M, Pizarro L, Schuster S, Lin ZJD, Gershony O, et al. 2018. The intracellular nucleotide-binding leucine-rich repeat receptor (SlNRC4a) enhances immune signalling elicited by extracellular perception. *Plant Cell Environ*. 41(10):2313–27
109. Leipe DD, Koonin EV, Aravind L. 2004. STAND, a class of P-loop NTPases including animal and plant regulators of programmed cell death: multiple, complex domain architectures, unusual phyletic patterns, and evolution by horizontal gene transfer. *J. Mol. Biol.* 343(1):1–28
110. Leonelli L, Pelton J, Schoeffler A, Dahlbeck D, Berger J, et al. 2011. Structural elucidation and functional characterization of the *Hyaloperonospora arabidopsidis* effector protein ATR13. *PLOS Pathog*. 7(12):e1002428
111. Lewis JD, Lee AH-Y, Hassan JA, Wan J, Hurley B, et al. 2013. The *Arabidopsis* ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a. *PNAS* 110(46):18722–27
112. Li L, Habring A, Wang K, Weigel D. 2020. Atypical resistance protein RPW8/HR triggers oligomerization of the NLR immune receptor RPP7 and autoimmunity. *Cell Host Microbe* 27(3):405–17.e6
113. Li X, Zhang Y, Clarke JD, Li Y, Dong X. 1999. Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of *npr1-1*. *Cell* 98(3):329–39
114. Liao K-C, Mogridge J. 2013. Activation of the Nlrp1b inflammasome by reduction of cytosolic ATP. *Infect. Immun.* 81(2):570–79
115. Liu J, Elmore JM, Lin Z-JD, Coaker G. 2011. A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. *Cell Host Microbe* 9(2):137–46
116. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, et al. 2016. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 535(7610):153–58
117. Lu C, Wang A, Wang L, Dorsch M, Ocain TD, Xu Y. 2005. Nucleotide binding to CARD12 and its role in CARD12-mediated caspase-1 activation. *Biochem. Biophys. Res. Commun.* 331(4):1114–19
118. Lukasik E, Takken FLW. 2009. STANDing strong, resistance proteins instigators of plant defence. *Curr. Opin. Plant Biol.* 12(4):427–36
119. Ma S, Lapin D, Liu L, Sun Y, Song W, et al. 2020. Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. *Science* 370(6521):eabe3069
120. Ma Y, Guo H, Hu L, Martinez PP, Moschou PN, et al. 2018. Distinct modes of derepression of an *Arabidopsis* immune receptor complex by two different bacterial effectors. *PNAS* 115(41):10218–27
121. Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL. 2003. *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* 112(3):379–89
122. Maekawa T, Cheng W, Spiridon LN, Töller A, Lukasik E, et al. 2011. Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. *Cell Host Microbe* 9(3):187–99
123. Maekawa T, Kufer TA, Schulze-Lefert P. 2011. NLR functions in plant and animal immune systems: so far and yet so close. *Nat. Immunol.* 12(9):817–26
124. Man SM, Kanneganti T-D. 2016. Converging roles of caspases in inflammasome activation, cell death and innate immunity. *Nat. Rev. Immunol.* 16(1):7–21
125. Maqbool A, Saitoh H, Franceschetti M, Stevenson CEM, Uemura A, et al. 2015. Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. *eLife* 4:e08709
126. Marchal C, Zhang J, Zhang P, Fenwick P, Steuernagel B, et al. 2018. BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust. *Nat. Plants* 4(9):662–68
127. Marquenet E, Richet E. 2007. How integration of positive and negative regulatory signals by a STAND signaling protein depends on ATP hydrolysis. *Mol. Cell* 28(2):187–99
128. Martin R, Qi T, Zhang H, Liu F, King M, et al. 2020. Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. *Science* 370(6521):eabd9993
129. Messaed C, Chebaro W, Di Roberto RB, Rittore C, Cheung A, et al. 2011. *NLRP7* in the spectrum of reproductive wastage: Rare non-synonymous variants confer genetic susceptibility to recurrent reproductive wastage. *J. Med. Genet.* 48(8):540–48

119. Along with 128, provides the first structural insight into the plant TNL resistosome with direct recognition of the effector through the LRR domain.

130. Mestre P, Baulcombe DC. 2006. Elicitor-mediated oligomerization of the tobacco N disease resistance protein. *Plant Cell* 18(2):491–501
131. Mo J, Boyle JP, Howard CB, Monie TP, Davis BK, Duncan JA. 2012. Pathogen sensing by nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is mediated by direct binding to muramyl dipeptide and ATP. *J. Biol. Chem.* 287(27):23057–67
132. Moreira LO, Zamboni DS. 2012. NOD1 and NOD2 signaling in infection and inflammation. *Front. Immunol.* 3:328
133. Neiman-Zenevich J, Liao K-C, Mogridge J. 2014. Distinct regions of NLRP1B are required to respond to anthrax lethal toxin and metabolic inhibition. *Infect. Immun.* 82(9):3697–703
134. Ngou BPM, Ahn H-K, Ding P, Jones JDG. 2020. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. bioRxiv 2020.04.10.034173. **https://doi.org/10.1101/2020.04.10.034173**
134a. Ofir G, Herbst E, Baroz M, Cohen D, Millman A, et al. 2021. Antiviral activity of bacterial TIR domains via signaling molecules that trigger cell death. bioRxiv 2021.01.06.425286. **https://doi.org/10.1101/2021.01.06.425286**
135. Pandey SP, Somssich IE. 2009. The role of WRKY transcription factors in plant immunity. *Plant Physiol.* 150(4):1648–55
136. Park YH, Wood G, Kastner DL, Chae JJ. 2016. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. *Nat. Immunol.* 17(8):914–21
137. Qi D, DeYoung BJ, Innes RW. 2012. Structure-function analysis of the coiled-coil and leucine-rich repeat domains of the RPS5 disease resistance protein. *Plant Physiol.* 158(4):1819–32
138. Qi S, Pang Y, Hu Q, Liu Q, Li H, et al. 2010. Crystal structure of the *Caenorhabditis elegans* apoptosome reveals an octameric assembly of CED-4. *Cell* 141(3):446–57
139. Qi T, Seong K, Thomazella DPT, Kim JR, Pham J, et al. 2018. NRG1 functions downstream of EDS1 to regulate TIR-NLR-mediated plant immunity in *Nicotiana benthamiana*. *PNAS* 115(46):E10979–87
140. Ravensdale M, Bernoux M, Ve T, Kobe B, Thrall PH, et al. 2012. Intramolecular interaction influences binding of the flax L5 and L6 resistance proteins to their AvrL567 ligands. *PLOS Pathog.* 8(11):e1003004
141. Rayamajhi M, Zak DE, Chavarria-Smith J, Vance RE, Miao EA. 2013. Cutting edge: Mouse NAIP1 detects the type III secretion system needle protein. *J. Immunol.* 191(8):3986–89
142. Redditt TJ, Chung E-H, Karimi HZ, Rodibaugh N, Zhang Y, et al. 2019. AvrRpm1 functions as an ADP-ribosyl transferase to modify NOI domain-containing proteins, including Arabidopsis and soybean RPM1-interacting protein4. *Plant Cell* 31(11):2664–81
143. Reyes Ruiz VM, Ramirez J, Naseer N, Palacio NM, Siddarthan IJ, et al. 2017. Broad detection of bacterial type III secretion system and flagellin proteins by the human NAIP/NLRC4 inflammasome. *PNAS* 114(50):13242–47
144. Riedl SJ, Li W, Chao Y, Schwarzenbacher R, Shi Y. 2005. Structure of the apoptotic protease-activating factor 1 bound to ADP. *Nature* 434(7035):926–33
145. Saile SC, Jacob P, Castel B, Jubic LM, Salas-Gonzalez I, et al. 2020. Two unequally redundant "helper" immune receptor families mediate *Arabidopsis thaliana* intracellular "sensor" immune receptor functions. *PLOS Biol.* 18(9):e3000783
146. Sandstrom A, Mitchell PS, Goers L, Mu EW, Lesser CF, Vance RE. 2019. Functional degradation: a mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. *Science* 364(6435):eaau1330
147. Sarris PF, Duxbury Z, Huh SU, Ma Y, Segonzac C, et al. 2015. A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell* 161(5):1089–100
148. Saur IM, Bauer S, Kracher B, Lu X, Franzeskakis L, et al. 2019. Multiple pairs of allelic MLA immune receptor-powdery mildew AVR_A effectors argue for a direct recognition mechanism. *eLife* 8:e44471
149. Sborgi L, Rühl S, Mulvihill E, Pipercevic J, Heilig R, et al. 2016. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J.* 35(16):1766–78
150. Schultink A, Qi T, Bally J, Staskawicz B. 2019. Using forward genetics in *Nicotiana benthamiana* to uncover the immune signaling pathway mediating recognition of the *Xanthomonas perforans* effector XopJ4. *New Phytol.* 221(2):1001–9
151. Seto D, Koulena N, Lo T, Menna A, Guttman DS, Desveaux D. 2017. Expanded type III effector recognition by the ZAR1 NLR protein using ZED1-related kinases. *Nat. Plants* 3:17027

152. Seuring C, Greenwald J, Wasmer C, Wepf R, Saupe SJ, et al. 2012. The mechanism of toxicity in HET-S/HET-s prion incompatibility. *PLOS Biol.* 10(12):e1001451
153. Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW. 2003. Cleavage of *Arabidopsis* PBS1 by a bacterial type III effector. *Science* 301(5637):1230–33
154. Shao Z-Q, Xue J-Y, Wu P, Zhang Y-M, Wu Y, et al. 2016. Large-scale analyses of angiosperm nucleotide-binding site-leucine-rich repeat genes reveal three anciently diverged classes with distinct evolutionary patterns. *Plant Physiol.* 170(4):2095–109
155. Sharif H, Wang L, Wang WL, Magupalli VG, Andreeva L, et al. 2019. Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome. *Nature* 570(7761):338–43
156. Shi J, Zhao Y, Wang K, Shi X, Wang Y, et al. 2015. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526(7575):660–65
157. Smakowska-Luzan E, Mott GA, Parys K, Stegmann M, Howton TC, et al. 2018. An extracellular network of *Arabidopsis* leucine-rich repeat receptor kinases. *Nature* 553(7688):342–46
158. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. 1998. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. *Mol. Cell* 1(7):949–57
159. Stirnweis D, Milani SD, Jordan T, Keller B, Brunner S. 2014. Substitutions of two amino acids in the nucleotide-binding site domain of a resistance protein enhance the hypersensitive response and enlarge the PM3F resistance spectrum in wheat. *Mol. Plant Microbe Interact.* 27(3):265–76
160. Sun Y, Zhu Y-X, Balint-Kurti PJ, Wang G-F. 2020. Fine-tuning immunity: players and regulators for plant NLRs. *Trends Plant Sci.* 25(7):695–713
161. Sutterwala FS, Haasken S, Cassel SL. 2014. Mechanism of NLRP3 inflammasome activation. *Ann. N. Y. Acad. Sci.* 1319:82–95
162. Swanson KV, Deng M, Ting JP-Y. 2019. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* 19(8):477–89
163. Swiderski MR, Birker D, Jones JDG. 2009. The TIR domain of TIR-NB-LRR resistance proteins is a signaling domain involved in cell death induction. *Mol. Plant Microbe Interact.* 22(2):157–65
164. Takken FL, Albrecht M, Tameling WI. 2006. Resistance proteins: molecular switches of plant defence. *Curr. Opin. Plant Biol.* 9(4):383–90
165. Tamborski J, Krasileva KV. 2020. Evolution of plant NLRs: from natural history to precise modifications. *Annu. Rev. Plant Biol.* 71:355–78
166. Tameling WIL, Elzinga SDJ, Darmin PS, Vossen JH, Takken FLW, et al. 2002. The tomato *R* gene products I-2 and Mi-1 are functional ATP binding proteins with ATPase activity. *Plant Cell* 14(11):2929–39
167. Tameling WIL, Vossen JH, Albrecht M, Lengauer T, Berden JA, et al. 2006. Mutations in the NB-ARC domain of I-2 that impair ATP hydrolysis cause autoactivation. *Plant Physiol.* 140(4):1233–45
168. Tanabe T, Chamaillard M, Ogura Y, Zhu L, Qiu S, et al. 2004. Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J.* 23(7):1587–97
169. Tenthorey JL, Haloupek N, López-Blanco JR, Grob P, Adamson E, et al. 2017. The structural basis of flagellin detection by NAIP5: a strategy to limit pathogen immune evasion. *Science* 358(6365):888–93
170. Tenthorey JL, Kofoed EM, Daugherty MD, Malik HS, Vance RE. 2014. Molecular basis for specific recognition of bacterial ligands by NAIP/NLRC4 inflammasomes. *Mol. Cell* 54(1):17–29
170a. Tian X, Pascal G, Monget P. 2009. Evolution and functional divergence of NLRP genes in mammalian reproductive systems. *BMC Evol. Biol.* 9:202
171. Ting JP-Y, Davis BK. 2005. CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu. Rev. Immunol.* 23:387–414
172. Tong ZB, Gold L, Pfeifer KE, Dorward H, Lee E, et al. 2000. *Mater*, a maternal effect gene required for early embryonic development in mice. *Nat. Genet.* 26(3):267–68
173. Tran DTN, Chung E-H, Habring-Müller A, Demar M, Schwab R, et al. 2017. Activation of a plant NLR complex through heteromeric association with an autoimmune risk variant of another NLR. *Curr. Biol.* 27(8):1148–60

154. Systemic phylogenomics analysis of NLRs in angiosperms revealed the three major NLR groups and their evolutionary history.

169. Provides structural insights into ligand-induced NLR activation and inflammasome formation.

174. Ueda H, Yamaguchi Y, Sano H. 2006. Direct interaction between the tobacco mosaic virus helicase domain and the ATP-bound resistance protein, N factor during the hypersensitive response in tobacco plants. *Plant Mol. Biol.* 61(1–2):31–45

175. Urbach JM, Ausubel FM. 2017. The NBS-LRR architectures of plant R-proteins and metazoan NLRs evolved in independent events. *PNAS* 114(5):1063–68

176. Van Der Biezen EA, Jones JDG. 1998. Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* 23(12):454–56

177. van der Burgh AM, Joosten MHAJ. 2019. Plant immunity: thinking outside and inside the box. *Trends Plant Sci.* 24(7):587–601

178. van der Hoorn RAL, Kamoun S. 2008. From guard to decoy: a new model for perception of plant pathogen effectors. *Plant Cell* 20(8):2009–17

179. van Ooijen G, Mayr G, Albrecht M, Cornelissen BJC, Takken FLW. 2008. Transcomplementation, but not physical association of the CC-NB-ARC and LRR domains of tomato R protein Mi-1.2 is altered by mutations in the ARC2 subdomain. *Mol. Plant* 1(3):401–10

180. Vance RE. 2015. The NAIP/NLRC4 inflammasomes. *Curr. Opin. Immunol.* 32:84–89

181. Walker JE, Saraste M, Runswick MJ, Gay NJ. 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* 1(8):945–51

182. Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, et al. 2019. TIR domains of plant immune receptors are NAD$^+$-cleaving enzymes that promote cell death. *Science* 365(6455):799–803

183. Wan W-L, Kim S-T, Castel B, Charoennit N, Chae E. 2020. Genetics of autoimmunity in plants: an evolutionary genetics perspective. *New Phytol.* 229(3):1215–33

184. Wang G, Roux B, Feng F, Guy E, Li L, et al. 2015. The decoy substrate of a pathogen effector and a pseudokinase specify pathogen-induced modified-self recognition and immunity in plants. *Cell Host Microbe* 18(3):285–95

185. Wang G-F, Ji J, El-Kasmi F, Dangl JL, Johal G, Balint-Kurti PJ. 2015. Molecular and functional analyses of a maize autoactive NB-LRR protein identify precise structural requirements for activity. *PLOS Pathog.* 11(2):e1004674

186. Wang J, Hu M, Wang J, Qi J, Han Z, et al. 2019. Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science* 364(6435):eaav5870

187. Wang J, Wang J, Hu M, Wu S, Qi J, et al. 2019. Ligand-triggered allosteric ADP release primes a plant NLR complex. *Science* 364(6435):eaav5868

188. Wang L, Zhao L, Zhang X, Zhang Q, Jia Y, et al. 2019. Large-scale identification and functional analysis of *NLR* genes in blast resistance in the Tetep rice genome sequence. *PNAS* 116(37):18479–87

189. Weaver L, Swiderski MR, Li Y, Jones JDG. 2006. The *Arabidopsis thaliana* TIR-NB-LRR R-protein, RPP1A; protein localization and constitutive activation of defence by truncated alleles in tobacco and Arabidopsis. *Plant J.* 47(6):829–40

190. Williams SJ, Sohn KH, Wan L, Bernoux M, Sarris PF, et al. 2014. Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science* 344(6181):299–303

191. Williams SJ, Sornaraj P, deCourcy-Ireland E, Menz RI, Kobe B, et al. 2011. An autoactive mutant of the M flax rust resistance protein has a preference for binding ATP, whereas wild-type M protein binds ADP. *Mol. Plant Microbe Interact.* 24(8):897–906

192. Wu C-H, Abd-El-Haliem A, Bozkurt TO, Belhaj K, Terauchi R, et al. 2017. NLR network mediates immunity to diverse plant pathogens. *PNAS* 114(30):8113–18

193. Wu C-H, Belhaj K, Bozkurt TO, Birk MS, Kamoun S. 2016. Helper NLR proteins NRC2a/b and NRC3 but not NRC1 are required for Pto-mediated cell death and resistance in *Nicotiana benthamiana*. *New Phytol.* 209(4):1344–52

194. Wu C-H, Derevnina L, Kamoun S. 2018. Receptor networks underpin plant immunity. *Science* 360(6395):1300–1

182. Shows that plant TNLs require NADase function to transduce pathogen recognition into immune signaling leading to cell death responses (see also 76).

186. Provides the first structural insight into plant NLR oligomerization, resistosome formation, and potential mechanisms leading to cell death.

187. Provides structural and biochemical insights into plant NLR activation through an indirect recognition mechanism

192. Identifies a network composed of NRCs and matching sensor NLRs that confers robust immunity to diverse pathogens of solanaceous plants.

195. Wu Z, Li M, Dong OX, Xia S, Liang W, et al. 2019. Differential regulation of TNL-mediated immune signaling by redundant helper CNLs. *New Phytol.* 222(2):938–53

195. Demonstrates that TNLs use ADR1 and NRG1 to transduce differential downstream signals, forming a genetic network composed of TNL and RNL.

196. Xu H, Shi J, Gao H, Liu Y, Yang Z, et al. 2019. The N-end rule ubiquitin ligase UBR2 mediates NLRP1B inflammasome activation by anthrax lethal toxin. *EMBO J.* 38(13):e101996
197. Xu H, Yang J, Gao W, Li L, Li P, et al. 2014. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature* 513(7517):237–41
198. Xu Y, Tao X, Shen B, Horng T, Medzhitov R, et al. 2000. Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* 408(6808):111–15
199. Xue Y, Enosi Tuipulotu D, Tan WH, Kay C, Man SM. 2019. Emerging activators and regulators of inflammasomes and pyroptosis. *Trends Immunol.* 40(11):1035–52
200. Yang J, Zhao Y, Li P, Yang Y, Zhang E, et al. 2018. Sequence determinants of specific pattern-recognition of bacterial ligands by the NAIP-NLRC4 inflammasome. *Cell Discov.* 4:22
201. Yang J, Zhao Y, Shi J, Shao F. 2013. Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. *PNAS* 110(35):14408–13
202. Yang X, Lin G, Han Z, Chai J. 2019. Structural biology of NOD-like receptors. *Adv. Exp. Med. Biol.* 1172:119–41
203. Yang X, Yang F, Wang W, Lin G, Hu Z, et al. 2018. Structural basis for specific flagellin recognition by the NLR protein NAIP5. *Cell Res.* 28(1):35–47
204. Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, et al. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *PNAS* 95(4):1663–68
205. Yu X, Acehan D, Ménétret J-F, Booth CR, Ludtke SJ, et al. 2005. A structure of the human apoptosome at 12.8 Å resolution provides insights into this cell death platform. *Structure* 13(11):1725–35
206. Yuan M, Jiang Z, Bi G, Nomura K, Liu M, et al. 2020. Pattern-recognition receptors are required for NLR-mediated plant immunity. bioRxiv 2020.04.10.031294. **https://doi.org/10.1101/2020.04.10.031294**
207. Yuan S, Akey CW. 2013. Apoptosome structure, assembly, and procaspase activation. *Structure* 21(4):501–15
208. Yuan S, Yu X, Topf M, Ludtke SJ, Wang X, Akey CW. 2010. Structure of an apoptosome-procaspase-9 CARD complex. *Structure* 18(5):571–83
209. Yuen B, Bayes JM, Degnan SM. 2014. The characterization of sponge NLRs provides insight into the origin and evolution of this innate immune gene family in animals. *Mol. Biol. Evol.* 31(1):106–20
210. Zhang L, Chen S, Ruan J, Wu J, Tong AB, et al. 2015. Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. *Science* 350(6259):404–9
211. Zhang X, Bernoux M, Bentham AR, Newman TE, Ve T, et al. 2017. Multiple functional self-association interfaces in plant TIR domains. *PNAS* 114(10):E2046–52
212. Zhao K, Hart CM, Laemmli UK. 1995. Visualization of chromosomal domains with boundary element-associated factor BEAF-32. *Cell* 81(6):879–89
213. Zhao Y, Shao F. 2015. The NAIP-NLRC4 inflammasome in innate immune detection of bacterial flagellin and type III secretion apparatus. *Immunol. Rev.* 265(1):85–102
214. Zhao Y, Yang J, Shi J, Gong Y-N, Lu Q, et al. 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* 477(7366):596–600
215. Zurek B, Bielig H, Kufer TA. 2011. Cell-based reporter assay to analyze activation of Nod1 and Nod2. *Methods Mol. Biol.* 748:107–19
216. Zurek B, Proell M, Wagner RN, Schwarzenbacher R, Kufer TA. 2012. Mutational analysis of human NOD1 and NOD2 NACHT domains reveals different modes of activation. *Innate Immun.* 18(1):100–11