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NB-LRR proteins: pairs, pieces, perception, partners, and pathways

Timothy K Eitas[1] and Jeffery L Dangl[1,2,3,4]

In plants, many of the innate immune receptors or disease resistance (R) proteins contain a NB-LRR (Nucleotide-binding site, Leucine-rich repeat) structure. The recent findings regarding NB-LRR signaling are summarized in this article. An emerging theme is that two NB-LRRs can function together to mediate disease resistance against pathogen isolates. Also, recent results delineate the NB-LRR protein fragments that are sufficient to initiate defense signaling. Importantly, distinct fragments of different NB-LRRs are sufficient for function. Finally, we describe the new roles of accessory proteins and downstream host genes in NB-LRR signaling.

Addresses
[1] Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC 27599, USA
[2] Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA
[3] Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599, USA
[4] Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC 27599, USA

Corresponding authors: Dangl, Jeffery L (dangl@email.unc.edu)

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Introduction

Plants have evolved mechanisms to resist attack by pathogens. The first level of defense consists of Pattern Recognition Receptors (PRRs) that perceive Pathogen Associated Molecular Patterns (PAMPs) and initiate PAMP Triggered Immunity or PTI [1]. Pathogens have evolved the ability to suppress PTI, in many cases through the deployment of proteins generically termed effectors [1]. In response to effectors, plants have evolved NB-LRR (Nucleotide-binding site, Leucine-rich repeat) proteins that are the most common disease resistance (*R*) genes. NB-LRR proteins recognize effectors and initiate Effector Triggered Immunity or ETI [1]. The perception of effectors by NB-LRR proteins can occur directly or indirectly through an intermediate protein called a 'Guardee' [1]. Proteins analogous to NB-LRR proteins, called NLR proteins, initiate cell death, inflammation, and responses to pathogens in mammalian cells [2].

In plants, NB-LRR proteins are divided into two subclasses on the basis of the presence of an N-terminal Coiled-coil (CC) or Toll and human interleukin receptor (TIR) domain [3]. The presence of either a CC or TIR domain typically determines whether an NB-LRR-mediated resistance response requires either NDR1 (Non-race-specific Disease Resistance) or the EDS1 (Enhanced Disease Susceptibility 1)/PAD4 (Phytoalexin Deficient 4)/SAG101 (Senescence Associated Gene 101) complex, respectively [4,5]. A molecular linkage between NB-LRR protein signaling and NDR1 or the EDS1/PAD4/SAG101 complex remains elusive, although some progress has been made [5,6].

The precise mechanism of NB-LRR protein activation and the subsequent signaling in ETI remains largely an open question. NB-LRR proteins are a subgroup of the STAND (Signal Transduction ATPase with Numerous Domains) family of proteins [7•]. These proteins are regulated by nucleotide binding, nucleotide hydrolysis, and intramolecular domain interactions [7•]. Additionally, NB-LRR proteins can undergo homotypic interactions and associate with accessory proteins including chaperones and 'Guardees' [7•,8••,9,10]. These topics are extensively reviewed elsewhere [1,7•,10,11]. Also, some recent studies have linked NB-LRR function to nuclear protein accumulation. Although this topic will be briefly addressed in this article, readers are referred to [12] for a comprehensive review. Our focus in this article is on papers published in the past 18 months that describe NB-LRR signaling.

It takes two to tango: disease resistance mediated by NB-LRR pairs

Early research in plant pathology characterizing the interaction between the fungal pathogen flax rust (*Melampsora lini*) and flax (*Linum usitatissimum*) revealed the gene-for-gene relationship, in which the outcome of a pathogen–plant interaction is determined by whether a pathogen avirulence gene (*avr*) coincides with a corresponding plant resistance gene (*R*) [13]. However, an emerging theme from both model and agriculturally important plants is that disease resistance against a pathogen isolate, or response to a single *avr* gene product, can require pairs of *NB-LRR* genes. Interestingly, these *NB-LRR* pairs differ in their (1) encoded protein domain structures, (2) pathogen isolate, and (3) genomic location (Figure 1 and Table 1).

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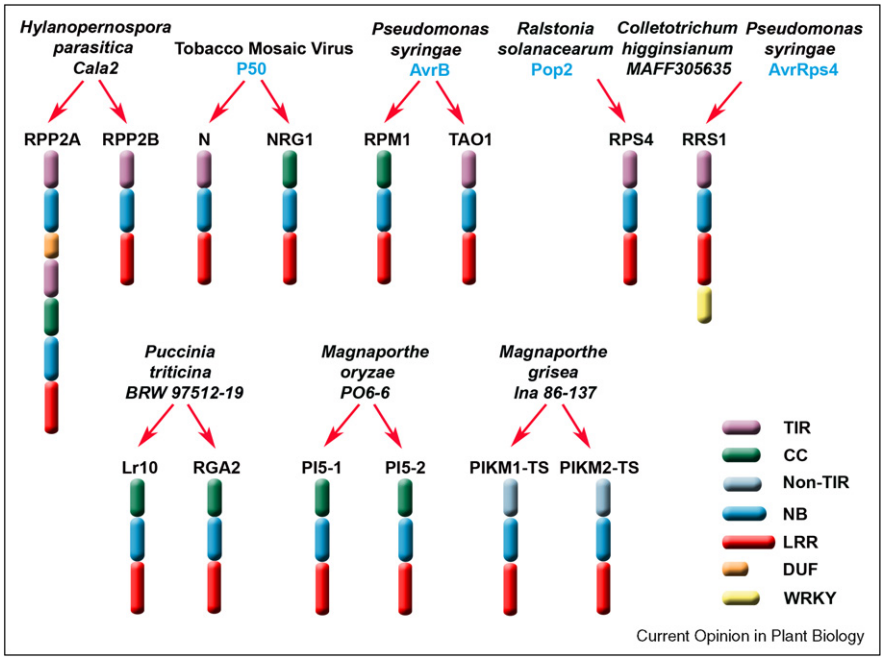
Figure 1



Domain structure and pathogen isolates of NB-LRR pairs. *Top row*: NB-LRR pairs in Arabidopsis and Tobacco. Black lettering represents pathogen isolate. Blue lettering represents *avr* gene product. *Bottom row*: NB-LRR pairs in Wheat and Rice. Black lettering represents pathogen isolate.

The first demonstration that a pair of *NB-LRR* genes function together in disease resistance against a pathogen isolate was the finding that both *RPP2A* and *RPP2B* are required for disease resistance to an oomycete pathogen isolate [14]. Since there was no evidence that RPP2A and RPP2B perceived the product of a single *avr* gene, RPP2A and RPP2B may become activated by multiple *avr* products. Characterization of *N-NRG1* and *RPM1-TAO1* revealed that disease resistance to viral and bacterial pathogens expressing a single *avr* product (p50-Tobacco Mosaic Virus, AvrB-*Pseudomonas syringae*, respectively) can be mediated by an *NB-LRR* pair encoding proteins of the TIR and CC subclasses [15,16]. Recent investigation of *RRS1* and *RPS4* demonstrated that this *TIR-NB-LRR* pair is required for disease resistance against multiple pathogen isolates [17–19]. Examples of CC-NB-LRR-encoding gene pairs mediating disease resistance to fungal pathogen isolates came from the identification of *Lr10–RGA2* and *Pi5-1–Pi5-2* [20,21]. Finally, characterization of *Pikm1-TS* and *Pikm2-TS* demonstrated that two *NB-LRR* genes encoding N-terminal non-TIR domains are required for disease resistance against a fungal pathogen isolate [22]. Similar to RPP2A–RPP2B, it is unclear whether the Lr10–RGA2, Pi5-1–Pi5-2, and Pikm-1-TS–Pikm2-TS pairs are activated by a single or multiple *avr* gene products.

Table 1

Characteristics of *NB-LRR* pairs

NB-LRR pair	Plant	Pathogen isolate	Linkage
RPP2A/RPP2B	Arabidopsis	Oomycete	Yes
TAO1/RPM1	Arabidopsis	Bacterial	No
N/NRG1	Tobacco	Viral	NA
RPS4/RRS1	Arabidopsis	Bacterial Fungal	Yes
Lr10/RGA2	Wheat	Fungal	Yes
Pi5-1/Pi5-2	Rice	Fungal	Yes
Pikm1-TS/Pikm2-TS	Rice	Fungal	Yes

Since *NB-LRR* gene families can exist in genomic clusters, a possibility is that a *NB-LRR* pair may reside within a single locus. In fact, many of the *NB-LRR* pairs are linked (*RPP2A–RPP2B*, *RRS1–RPS4*, *Lr10–RGA2*, *Pikm-1-TS–Pikm-2-TS*, *Pi5-1–Pi5-2*). For all of these linked *NB-LRR* pairs, both NB-LRR proteins are required for disease resistance [14,19–23]. However, overexpression of NRG1 or an RPS4 truncation can initiate ectopic cell death in the absence of N or RRS1 activation, respectively [15,24••]. These data demonstrate that NRG1 or RPS4 either signal downstream of their respective partner NB-LRR, or that overexpression of these NB-LRRs can overcome the requirement for the partner NB-LRR. Interestingly, the *TAO1–RPM1* pair is not linked and these NB-LRR proteins can independently produce defense responses following recognition of AvrB [16] (Figure 1). These results collectively indicate that the function of one NB-LRR does not always require the partner NB-LRR.

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Pieces: modularity in NB-LRR signaling

Given that NB-LRR proteins are modular [7•], two reasonable questions are which portion(s) of the protein mediates downstream signaling, and whether these requirements are generalizable across the NB-LRR superfamily. Swiderski *et al.* (2009) demonstrated that two N-terminal protein fragments of the TIR-NB-LRR protein RPS4, TIR+45 (AA1-205) and TIR+80 (AA1-240), were sufficient to induce cell death. The TIR+80-induced cell death required EDS1, SGT1, and HSP90, indicating that cell death mediated by this fragment had the same genetic requirements as cell death induced by the full-length protein [25,26]. Interestingly, cell death was also induced by a TIR+80 fragment of RPP1A but not RPP2A or RPP2B [24••]. Collectively, these data showed that the TIR+80 fragment was sufficient to initiate cell death induced by some but not all TIR-NB-LRR proteins.

Recent evidence suggests that full-length RPS4 requires nuclear accumulation for cell death [25]. Furthermore, it was shown that residues in the C-terminal extension domain of RPS4 are required for both nuclear accumulation and cell death [25]. Since the RPS4 TIR+80 fragment lacks this C-terminal extension domain, an important extension of Swiderski *et al.* (2009) would be to describe the localization pattern for the RPS4 TIR+80 fragment.

Characterization of the CC-NB-LRR protein Rx revealed that a fragment of the NB domain (AA139-293) was sufficient to produce cell death [27••]. Strikingly, the NB-mediated cell death occurred with a variant that contained multiple mutations in the highly conserved Walker A motif [27••]. Therefore, ectopic cell death activity of this fragment was probably independent of nucleotide binding. NB domain-induced cell death was dependent on SGT1, consistent with previous data for cell death induced by the full-length Rx protein [27••,28].

These studies demonstrated that overexpression of fragments from both TIR and CC-containing NB-LRR proteins can initiate cell death. Notably, cell death does not always correlate with disease resistance [29,30]. Therefore, it will be important to evaluate if expression of the TIR+80 fragments or the NB domain fragment of Rx is also sufficient for ectopic disease resistance. Signaling by these fragments (TIR+80 (RPS4, RPP1A), NB (Rx)) is probably independent of nucleotide binding. Nucleotide binding and hydrolysis regulate the on–off states and stabilization for some NB-LRR proteins [25,31]. Therefore, the recent results for the TIR+80 (RPS4, RPP1A) and NB (Rx) fragments may indicate that these fragments bypass regulation at the resting state, and thus represent the exposed signaling platform normally unleashed by activation. Notably, NB-containing protein fragments of RPS2 and RPS5 require the CC domain in order to initiate ectopic cell death [32,33]. The CC domain is also required for ectopic cell death and disease resistance mediated by the CC-NB-LRR protein NRG1 [15].

Collectively, these data demonstrate a lack of uniformity for NB-LRR fragment-mediated cell death. This suggests that the mechanism for unleashing NB-LRR activity, likely to require intramolecular conformational changes, might be particular to each NB-LRR protein. Such specificities could be driven evolutionarily by a general requirement for NB-LRRs to recognize variously shaped effector-dependent modified self molecules, or to directly interact with specifically shaped effector molecules. NB-LRR function thus needs to be buffered against strict structural constraints. It will be interesting in the future to re-interpret the data summarized above in light of the varying requirement for the HSP90/SGT1/RAR1 co-chaperone triumvirate in NB-LRR protein regulation.

Perception and partners: roles of accessory proteins in NB-LRR signaling

NRIP (N-receptor-interacting protein) was recently demonstrated to be required for disease resistance mediated by the TIR-NB-LRR protein N [34]. NRIP interacted with both N and the corresponding viral *avr* product, p50 [34]. Expression of p50 *in planta* caused NRIP to move from chloroplasts to the cytoplasm and nucleus, resulting in association with N [34]. The NRIP–N relationship is unique since the association of the full-length proteins occurred only in the presence of p50 [34]. Further studies may reveal whether NRIP induces N activation or if NRIP has a role in N signaling downstream of initial activation. It is also possible that NRIP is important for both aspects of N-mediated defense.

The accessory protein Pto and highly related Pto-like kinases regulate the function of the N-term-SD (Solanaceous Domain)-CC-NB-LRR protein Prf [8••]. In the absence of pathogen, Pto is required for Prf to self-associate into a signaling competent protein complex of ~600 kDA [8••]. The Pto–Prf complex is targeted by the bacterial effectors AvrPto and AvrPtoB, leading to Prf-mediated cell death and disease resistance [8••]. In the absence of AvrPto and AvrPtoB, co-expression of an N-terminal domain fragment (AA1-537) and a SD-CC-NB-LRR fragment (AA537-1824) of Prf caused weak cell death, suggesting that the split domains could interact but in a manner that did not fully recapitulate the 'off' state [8••]. Interestingly, this Prf-mediated cell death was dependent on Pto, revealing that Pto had a positive role in ectopic Prf signaling [8••]. Co-expression of AvrPtoB, Pto, the N-term domain Prf fragment, and the SD-CC-NB-LRR Prf fragment also caused cell death, indicating that elicitor-mediated Prf signaling was functional [8••]. Surprisingly, Prf could perceive AvrPto and/or AvrPtoB through association with the Pto-like kinases Fen, Pth2, and Pth3 in the absence of Pto [8••]. These data suggest an intriguing model where NB-LRR proteins can

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associate with closely related, effector-targeted accessory proteins. Different NB-LRR-accessory protein interactions could provide greater flexibility in both pathogen perception and subsequent signaling. This could be especially beneficial if the pathogen effectors evolve to lose association with the 'original' accessory protein. Duplication and divergence of a new, related accessory protein could allow the host to regain recognition of pathogen effectors. This model would be especially useful to plants in those cases where the effectors are perceived through their action on 'decoys' (defined as proteins that serve no other function except binding to pathogen effectors to trigger NB-LRR action). This has been suggested for Pto and Prf, where the virulence target for AvrPto and AvrPtoB is likely to be the BAK1 PRR co-receptor [11].

Pathways: downstream requirements for NB-LRR function

The Arabidopsis *snc1-1* (*suppressor of npr1-1*, *constitutive*) mutation lies in the linker region between the NB and LRR domains of a TIR-NB-LRR protein, resulting in constitutive defense activation [35]. A forward genetic screen for suppressors of *snc1-1* resulted in the isolation of *mos7-1* [36••]. *mos7-1* partially suppressed the *snc1-1*-conferred phenotypes of dwarf morphology, constitutive PR-1 expression, and enhanced basal resistance [36••]. In the absence of the *snc1-1* mutation, *mos7-1* plants were compromised for basal defense and SAR (Systemic Acquired Resistance) [36••]. The *mos7-1* phenotypes correlated with lower protein accumulation of the defense-associated proteins EDS1 and NPR1 (Nonexpressor of *PR* genes 1) [36••]. Interestingly, the *mos7-1* mutant was also partially compromised for disease resistance mediated by multiple TIR-NB-LRR and CC-NB-LRR proteins [36••].

MOS7 has homology to the Drosophila and human nucleoporin protein Nup88 and is localized to the nuclear envelope [36••]. Importantly, suppression of the *snc1-1* phenotypes by *mos7-1* correlated with a loss of SNC1 nuclear accumulation [36••]. These data led the authors to propose that MOS7-mediated nuclear export pathway has a crucial role in NB-LRR function [36••]. Additionally, these data could indicate that MOS7 acts a nuclear chaperone for NB-LRR proteins. Regardless of the biochemical activity of MOS7, characterization of *mos7-1* agrees with previous studies (reviewed in [12]) demonstrating that nuclear accumulation is important for signaling of some NB-LRR proteins.

An important genetic redundancy involving SA (Salicylic Acid) and EDS1 (Enhanced Disease Resistance) for NB-LRR function was recently uncovered [37••]. This study demonstrated that *sid2* (SA induction deficient) or *eds1* mutants did not alter NB-LRR (HRT, RPS2, or RPP8)-mediated disease resistance whereas disease resistance was compromised in the double *sid2 eds1* mutant [37••]. For the CC-NB-LRR protein RPS2, loss of disease resistance in the *sid2 eds1* line was not attributed to the loss of RPS2 protein accumulation [37••]. These data led to the conclusion that SA and EDS1 have redundant but crucial roles in NB-LRR signaling. Data showing a loss of NB-LRR-mediated cell death (RPM1, RPS2, RPS5) in the *mos7-1* or *eds1 sid2* double mutants would further indicate a direct involvement of MOS7, EDS1, and SID2 in NB-LRR signaling.

Parting shots: perspectives

A number of recent reports have demonstrated that pairs of *NB-LRR* genes are required for disease resistance to a pathogen isolate or a single *avr* product. These NB-LRR pairs function in disease resistance against multiple pathogens, include homotypic and heterotypic N-terminal domain pairs, and can be genetically linked or unlinked (Figure 1 and Table 1). When both *NB-LRR* genes of a pair are required for defense, a possible model is that these NB-LRR pairs form hetero-multimers that allow for pathogen detection. Heterotypic interactions of both Toll-like receptors (TLRs) and NLRs have been demonstrated in mammals [38,39,40•]. Downstream of *avr* product perception, activation of multiple NB-LRR proteins may lead to an increase or diversity of signal(s) that is required for an effective defense response.

The recent work characterizing NB-LRR signaling provokes some compelling questions. First, why is there a lack of uniformity for signaling among fragments of TIR-NB-LRR and CC-NB-LRR proteins? Second, how do these NB-LRR fragments biochemically initiate cell death? Third, are the same NB-LRR fragments required for both cell death and disease resistance? Fourth, does signaling leading to cell death and pathogen growth restriction occur in the same subcellular compartment? Fifth, how do accessory proteins influence effector-mediated NB-LRR signaling? Finally, what is the molecular mechanism underlying the loss of NB-LRR-mediated disease resistance in the *mos7-1* and *eds1 sid2* mutants? As is typically the case in science, these initial findings have provided fodder for further investigation.

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