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Plant NBS-LRR proteins in pathogen sensing and host defense

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Abstract

Plant proteins belonging to the nucleotide-binding site–leucine-rich repeat (NBS-LRR) family are used for pathogen detection. Like the mammalian Nod-LRR protein 'sensors' that detect intracellular conserved pathogen-associated molecular patterns, plant NBS-LRR proteins detect pathogen-associated proteins, most often the effector molecules of pathogens responsible for virulence. Many virulence proteins are detected indirectly by plant NBS-LRR proteins from modifications the virulence proteins inflict on host target proteins. However, some NBS-LRR proteins directly bind pathogen proteins. Association with either a modified host protein or a pathogen protein leads to conformational changes in the amino-terminal and LRR domains of plant NBS-LRR proteins. Such conformational alterations are thought to promote the exchange of ADP for ATP by the NBS domain, which activates 'downstream' signaling, by an unknown mechanism, leading to pathogen resistance.

Plants lack the adaptive immunity that vertebrates rely on to respond to pathogens. To successfully detect and ward off pathogens, plants must rely solely on genes stably encoded in the genome. Although the exact mechanisms of pathogen detection differ, plants, like animals, use two distinct defense 'systems' to recognize and respond to pathogen challenge[1]. Pathogen-associated molecular patterns (PAMPs), such as bacterial flagellin, lipopolysaccharides and fungal-oomycete cellulose-binding elicitor proteins, are recognized by plant transmembrane receptors that activate basal defense, a first line of defense against pathogens that is reminiscent of innate immunity in vertebrates[2,3]. In both plants and animals, it is hypothesized that a biological 'arms race' is occurring, in which pathogens have acquired mechanisms to evade PAMP-triggered immunity by evolving effector molecules that modify the state of the host cell, thereby bypassing or disrupting the first line of defense. Plant evolution has countered with proteins that detect specific effector molecules, a mechanism called 'effector-triggered immunity'1 that amounts to a second line of defense. Plant effector-triggered immunity is more akin to mammalian adaptive immunity in that pathogen effectors, rather than conserved elements such as PAMPs, are specifically recognized. However, unlike the situation in mammalian adaptive immunity, the plant host specificity determinants of effector-triggered immunity are encoded in every cell of an organism.

The genes encoding the specificity determinants of effector-triggered immunity are known as resistance (*R*) genes. Most *R* genes encode proteins that contain a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs). NBS-LRR proteins are involved in the recognition of specialized pathogen effectors (also called avirulence (Avr) proteins) that are thought to provide virulence function in the absence of the cognate *R* gene[1]. NBS-LRR proteins are also important in animal innate immune systems; however, in animals they seem to be involved in PAMP recognition rather than recognition of pathogen effectors[3].

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Plant NBS-LRR proteins (also called NB-LRR or NB-ARC-LRR proteins) can be categorized into TIR and non-TIR classes based on the identity of the sequences that precede the NBS domain. The TIR class of plant NBS-LRR proteins contains an amino-terminal domain with homology to the Toll and interleukin 1 receptors. The non-TIR class is less well defined, but most NBS-LRR proteins of this class contain α-helical coiled-coil–like sequences in their amino-terminal domain[4]. Studies have begun to unravel the mechanisms that underlie plant NBS-LRR function and specificity; this review discusses those findings and their implications in the greater picture of disease resistance and NBS-LRR function in both plants and animals.

Distinct mechanisms of pathogen detection

The most straightforward explanation for the specificity of plant NBS-LRR proteins is that pathogens are detected through direct interaction of plant NBS-LRR proteins and pathogen-derived molecules. However, for direct detection of pathogens to remain a viable resistance mechanism over time, plants must balance the rapid evolution of microbial pathogens with equally rapid diversification of genes encoding NBS-LRR proteins. Although there is evidence that some plant NBS-LRR proteins have been under diversifying selection, the direct detection hypothesis for pathogen recognition fails to explain how a relatively limited number of plant resistance proteins can specifically recognize the vast array and diversity of potential pathogens and their effectors. That apparent disparity led to the ‘guard model’ of pathogen detection, which states that pathogens are detected indirectly through the action of their effectors[5]. Such an indirect detection mechanism allows the plant to monitor a limited number of key targets of pathogenesis and respond when those targets are perturbed, limiting the number of resistance proteins necessary for adequate resistance. Although only a few plant resistance proteins have been well characterized, there is mounting evidence that plants use both direct and indirect mechanisms of pathogen detection.

Evidence for direct detection

The first evidence for direct interactions between NBS-LRR proteins and pathogen effectors came from studies of *Pi-ta*, an *R* gene from rice that specifies resistance to strains of the rice blast fungus *Magnaporthe grisea*, which expresses the effector AVR-Pita. Yeast two-hybrid experiments detected interaction of the putative functional portion of AVR-Pita with the LRR-like domain of Pi-ta[6]. Although Pi-ta lacks the strict consensus of an LRR domain, it contains a leucine-rich domain that is similar in sequence and size to the LRR domains of other plant NBS-LRR proteins[7]. That observation marked the first example of the long-sought AVR–resistance protein interaction. The direct detection model was further supported by the observation that the *Arabidopsis thaliana* RRS1 protein interacts with the bacterial wilt pathogen protein PopP2 in a ‘split-ubiquitin’ yeast two-hybrid experiment[8]. RRS1 is an atypical member of the TIR-NBS-LRR class of resistance proteins because it contains a carboxy-terminal WRKY domain[9]. Notably, the inactive form of RRS1, RRS1-S, can also bind to PopP2 in that assay, suggesting that either the interaction in yeast does not recapitulate the interaction in plants or that steps in addition to ligand binding are necessary for the activation of signaling.

Finally, study of the *L* resistance locus of flax has shown that L protein interacts directly with the corresponding AvrL variants from the flax rust fungus *Melampsora lini*. Yeast two-hybrid experiments have indicated that the resistance proteins L5, L6 and L7 bind to specific variants of the flax rust AvrL567 effector. Those interactions recapitulate the *in vivo* specificity of the L isoforms, supporting the hypothesis that this interaction drives pathogen detection in plants[10].

Evidence for indirect detection

Support for an indirect detection mechanism can be found in many genetic studies and has been corroborated by experiments involving several well defined resistance pathways in plants. The effector proteins AvrRpm1 and AvrB, from the bacterial pathogen *Pseudomonas syringae*, are both detected by the *A. thaliana* NBS-LRR protein RPM1, whereas AvrRpt2, another *P. syringae* effector, is detected by the NBS-LRR protein RPS2 (refs. 11–14). Despite efforts to identify direct interaction between those proteins, binding of AvrRpm1 and AvrB to RPM1 or of AvrRpt2 to RPS2 has not been detected. However, AvrRpm1, AvrB and AvrRpt2 all physically associate with another plant protein, RIN4 (refs. 15–17). In addition, both RPM1 and RPS2 bind to RIN4, providing a means for indirect detection of AvrRpm1, AvrB and AvrRpt2 by surveillance of RIN4. Indeed, AvrRpm1, AvrB and AvrRpt2 not only bind to RIN4 but also function to modulate the state of the RIN4 protein. The presence of either AvrRpm1 or AvrB leads to phosphorylation of RIN4, whereas AvrRpt2 proteolytically cleaves RIN4 (refs. 15,17–19). RIN4 seems to be involved in negatively regulating basal defense in arabidopsis, but it is not yet apparent whether RIN4 is a chief target of those effectors, as AvrRpm1 and AvrRpt2 both enhance the virulence on arabidopsis in the absence of RIN4 (refs. 20,21). Regardless of which is true, virulence activity of those effectors has subsequently been converted into an avirulence activity by the evolution of the ‘guard function’ of both RPM1 and RPS2.

Another example of an indirect recognition mechanism is that of the arabidopsis proteins RPS5 and PBS1 in the detection of the *P. syringae* effector AvrPphB. RPS5 is a plant NBS-LRR, whereas PBS1 is a protein kinase with unknown substrates[22–24]. Both proteins are required for the recognition of *P. syringae* strains expressing AvrPphB. Direct interaction between RPS5 and AvrPphB has not been detected; however, PBS1 interacts with both AvrPphB and RPS5, forming a ternary complex (J. Ade and R.W.I., unpublished data). AvrPphB is a cysteine protease and functions in the plant cell to cleave PBS1 at a specific site[25,26]. Therefore, it seems that RPS5 functions to detect pathogen effectors such as AvrPphB by monitoring the status of PBS1.

The tomato protein Prf is yet another candidate NBS-LRR protein involved in the indirect detection of pathogen effectors, specifically AvrPto and AvrPtoB found in a subset of *P. syringae* pathovar tomato strains[27–29]. Genetic evidence indicates that Prf is required for the activation of defense in response to AvrPto and AvrPtoB, although direct interaction between Prf and AvrPto or AvrPtoB has not been detected. However, AvrPto and AvrPtoB bind to the tomato protein Pto, a serine-threonine protein kinase[29–32]. Furthermore, Pto directly interacts with Prf, suggesting that Prf indirectly detects the presence of AvrPto and AvrPtoB, probably as a result of their interaction with Pto[33].

The LRR as an effector-binding domain

LRR domains are located at the carboxy termini of plant NBS-LRR proteins and are composed of tandem LRRs. Several LRR domains from non-plant proteins have been crystallized; they form barrel-like structures with a parallel β-sheet lining the inner concave surface and α-helical structures comprising much of the rest of the domain[34]. The LRR domain of plant NBS-LRR proteins has long been hypothesized to be involved in specific recognition of pathogen effector molecules, and several lines of evidence support that hypothesis. LRRs are thought to be involved in mediating protein-protein interactions in animal systems[34]. In addition, the β-sheet portion of the LRR domain, which is proposed to be the ligand-binding interface, seems to be under diversifying selection in many plant NBS-LRR proteins[35]. Despite those points, there is only sparse experimental evidence supporting involvement of the LRR domain in binding to pathogen effector molecules.

The most compelling evidence for pathogen effector–LRR interactions comes from work on the flax *L* locus mentioned above, although that work used only full-length L clones rather than isolated LRR domains[10]. Many of the structural and sequence differences among the *L* alleles are confined to the LRR coding region, indicating involvement of the LRR domain in ligand specificity[36]. Additionally, substitution of the L2 LRR domain for the LRR domain of the L6 or L10 isoform results in L2 pathogen specificity[36]. However, some alleles with different pathogen specificity contain identical LRR domains, suggesting that sequences outside the LRR domain influence ligand specificity. Likewise, some chimeric L molecules have demonstrated previously unknown specificities, also suggesting that multiple domains contribute to pathogen-recognition specificity[36,37].

As mentioned above, direct interaction of effectors with the NBS-LRR proteins Pi-ta and RRS1-R has been detected in yeast two-hybrid experiments. The LRR-like domain of Pi-ta by itself is able to interact with the corresponding effector Avr-Pita, providing support for the hypothesis that the LRR domain is involved in effector binding. In addition, susceptible cultivars of rice contain an A918S substitution in Pi-ta, which is at the end of the LRR-like domain[6,7]. However, interaction of full-length Pi-ta and Avr-Pita could not be detected in the yeast two-hybrid assay, suggesting that this interaction may be more complex than simple binding of LRR to effector[6]. Binding of Avr-Pita to full-length Pi-ta has been detected in a protein-protein immunoblot analysis[6], but the specificity for avirulent Avr-Pita is lost in this assay. Conversely, RRS1-R interaction with PopP2 occurs only with the full-length RRS1-R construct and not with the LRR domain alone, suggesting that multiple domains may be necessary for effector-NBS-LRR interaction or simply that the interaction may require a specific NBS-LRR conformation not present in the domain constructs used in this experiment[8].

The LRR as a regulatory domain

Several lines of evidence support the idea of a regulatory function for the LRR domains of both plant and animal NBS-LRR proteins in signal transduction. For RPS2, RPS5 and RPP1A, the LRR domain seems to negatively regulate signaling, as deletion of the LRR domain causes constitutive activation of defense responses38,[39] (B.J.D. and R.W.I., unpublished data). Likewise, truncation of the LRR domain of the potato *R* gene *Rx* results in an increase in the hypersensitive response, a form of programmed cell death[3], although that is only seen when *Rx* is overexpressed[40]. Similar experiments with the vertebrate Nod2 receptor protein have shown that truncation of even part of the LRR domain results in a large increase in the basal activation of transcription factor NF-κB[41]. Likewise, overexpression of Nod1 results in enhanced signaling[42]. Furthermore, experiments using Rx-GPA2 chimeric proteins suggest that at least two regions in the LRR domain of Rx function to inhibit signaling by specifically interacting with a region of the Rx NBS domain[43].

Paradoxically, the LRR domain also seems to have a positive function in signaling, depending on the NBS-LRR protein assayed and the specific assay used. Substitution of the Mi-1.2 LRR domain or both the NBS and LRR domains for the corresponding domain(s) of the parologous Mi-1.1 protein results in a chimeric protein with apparent constitutive activity[44]. That constitutive activity can be eliminated by the introduction of substitutions in the LRR domain, suggesting that the activity is due to positive action of the LRR domain and not loss of negative regulation45. The LRR domain of Rx seems to be necessary for the function of several autoactivation mutants46. In addition, the ability of Rx to respond to the potato virus X coat protein is dependent on the carboxy-terminal portion of the LRR domain, further indicating a positive signaling function for this domain43. Finally, the

rps5-2 mutant contains a proline-to-serine substitution in the LRR domain and results in loss of RPS5 function rather than constitutive activity[23].

In contrast to RPS5 and Rx, truncation of the LRR domain of the tomato NBS-LRR protein I-2 does not seem to result in constitutive activity[47]. In addition, many splice variants and loci encoding LRR truncations in the genome do not demonstrate constitutive activity[47–52]. Furthermore, some full-length plant NBS-LRR proteins, such as Rx and RPS2, are constitutively active when overexpressed in plant cells, suggesting that the LRR domain is not sufficient to inhibit autoactivation in those conditions[14,38,40]. Therefore, it seems that the signaling status of the NBS-LRR proteins may be regulated by complex interactions among NBS and LRR domains and that overexpression can trigger signaling not detected at physiological concentrations.

The function of nucleotide binding

The NBS domain (also called the NB, NB-ARC, Nod or NACHT domain) contains blocks of sequence that are conserved in both plant and animal proteins[53,54]. Those include the canonical nucleotide-binding kinase 1a or P-loop and kinase 2 motifs (also called Walker's A and B boxes) and the kinase 3a motif, as well as several blocks of conserved motifs of unknown function (RNBS-A, RNBS-C, GLPL, RNBS-D and MHD)[53,55,56]. Although the biochemical function of those motifs is not well defined, they seem to be important as substitutions in these domains affect NBS-LRR function[10,40,47,52,57–60] (Table 1).

Publication of the crystal structures of the NBS domains of mammalian apoptotic protease-activating factor 1 (Apaf-1) and the *Caenorhabditis elegans* cell death protein CED-4 has enabled mapping of known plant NBS-LRR substitutions onto these structures[54,61,62], which is providing new insights into how NBS-LRR signaling is regulated. The NBS domain of Apaf-1 is composed of four subdomains: a three-layered α-β domain (which contains the P-loop), followed by a helical domain, an extended winged-helix domain and a second helical domain (also called ARC1, ARC2 and ARC3; Fig. 1). The structure of CED-4 is similar to that of Apaf-1, but it lacks ARC3. Sequence analysis suggests that plant NBS-LRR proteins have a domain structure similar to that of Apaf-1 and CED-4, but like CED-4 they lack ARC3 (ref.63).

The crystal structures of both CED-4 and Apaf-1 indicate that an ATP or ADP is bound in a pocket that is mostly buried in the NBS domain[61,62]. For Apaf-1, ADP seems to be 'preferentially' bound in the inactive form of the protein[61]. In that form, ADP coordinates many intramolecular interactions among the NBS subdomains, stabilizing this structure. In addition to the conserved P-loop residues involved in nucleotide binding, the histidine residue of the MHD motif is involved in coordinating the β-phosphate of bound ADP, indicating involvement of this motif in NBS function[61]. The MHD motif is highly conserved among plant NBS-LRR proteins but is less prevalent in animal NBS-LRR proteins[60]. Activation of caspase-9 and formation of the 'apoptosome' seem to require binding of ATP to Apaf-1. However, the function of ATP hydrolysis in activation is not clear, as the results from experiments testing ATPase activity have been interpreted in different ways[61,64,65]. Conversely, ATP is 'preferentially' bound to the CED-4 Nod domain in both active and inactive conformations, and ATPase activity does not seem to be necessary for CED-4 function[62].

In plants, both I-2 and Mi-1 are able to bind to and hydrolyze ATP[66]. Not unexpectedly, substitutions of conserved residues of the P-loop result in inactive proteins (Table 1 and Fig. 1). However, substitutions in I-2 that reduce ATP hydrolysis but not ATP binding result in constitutively active proteins, suggesting that ATP binding, not hydrolysis, is necessary for signaling in plant NBS-LRR proteins[47]. Thus, it may be that binding of ATP initiates a

conformational change in plant NBS-LRR proteins, resulting in its activation. That is similar to the mode of activation of small G proteins[67]. Notably, a variety of autoactivating substitutions in other plant NBS-LRR proteins also 'map' to the ADP binding pocket (Table 1). Although those residues are distributed throughout the NBS domain (notably, in many conserved NBS motifs), they are physically clustered around the terminal phosphates of ADP and may be involved in regulating or responding to nucleotide binding status (Fig. 1). Substitutions in human Nod-LRR proteins that lead to autoinflammatory disorders also 'map' to this region, suggesting that domain interactions mediated by nucleotide binding are crucial in the regulation of Nod-LRR activity[68].

Functions of the amino-terminal domain

In animal systems, the sequences located amino-terminal to the Nod domain are involved in binding to and activating 'downstream' signaling molecules. These so-called 'effector-binding domains' (referring here to downstream signaling molecules rather than pathogen effectors) typically contain protein-protein interaction motifs involved in homotypic interactions such as caspase-recruitment domains or pyrin domains, among others[3]. Thus, for animal Nod-LRR proteins, downstream activation is specified by the amino-terminal domains. In plants, the TIR domain of TIR-NBS-LRR proteins has been linked to downstream specificity based on the observation that TIR-class NBS-LRR proteins have a shared requirement for downstream genes that is different from those required for coiled-coil–class NBS-LRR protein signaling69. The TIR domain of vertebrate TLR proteins mediates homotypic protein-protein interactions with downstream signaling molecules also having a TIR domain70›71. In plants, there is no experimental evidence to support the idea of a similar function for the TIR domain, although homotypic TIR interactions have been identified in NBS-LRR oligomerization, as discussed in more detail below[72].

Many lines of evidence suggest alternative functions for the amino-terminal domains of plant NBS-LRR proteins. For example, domain-swapping experiments have shown that swapping portions of the TIR domain of the flax L protein can change the specificity of some *L* alleles, and that change is due to the difference of only a few amino acids[37]. In addition, a subdomain of the L protein TIR domain seems to have undergone diversifying selection, indicating that this domain may be involved in pathogen recognition[37].

The amino-terminal domain also seems to mediate the physical association between resistance proteins and pathogen effector targets, at least for those resistance proteins that use an indirect recognition mechanism. The first evidence of that came from yeast two-hybrid analyses of PM1-RIN4 interactions16. Subsequently, the amino-terminal portion of Prf was shown to be both necessary and sufficient for binding to Pto[33]. In addition, the coiled-coil domain of RPS5 is both necessary and sufficient for binding to PBS1 (J. Ade and R.W.I., unpublished data). However, the constitutively active truncated form of RPS5 lacking the LRR domain requires the coiled-coil domain for activity. These results collectively suggest that the amino-terminal domain of plant NBS-LRR proteins may be involved in both detection of the pathogen signal and activation of the downstream response.

Intramolecular interactions

Although much progress has been made in identifying components of the various pathogen-detection pathways, relatively little is known about the mechanisms that control NOD-LRR protein signaling. So far, none of the plant NBS-LRR proteins have been crystallized, so structural analyses have depended mainly on domain-swapping experiments as well as mutant analysis. In sum, it seems that plant NBS-LRR proteins have complex interactions among their domains; however, the function of those interactions in pathogen detection and

activation of signal transductions seems to vary to some degree among plant NBS-LRR proteins.

The first evidence for intramolecular interaction of plant NBS-LRR protein domains came from work on Rx and Bs2, an R protein from pepper (refs. 46,73). The coiled-coil domains of both Rx and Bs2 can complement the respective NBS-LRR domains when expressed *in trans*. Likewise, the LRR domain can complement the coiled-coil NBS domains when expressed *in trans*. Those complementation results have been further supported in Rx by coimmunoprecipitation experiments demonstrating physical interactions between the coiled-coil and NBS-LRR domains and between the coiled-coil–NBS and LRR domains. It is notable that interaction of Rx domains with domains from the closely related NBS-LRR proteins Bs2 and HRT (an arabidopsis R protein that confers resistance to turnip crinkle virus) has also been detected, suggesting some degree of 'promiscuity'; however, such physical interactions are not sufficient to reconstitute functional signaling molecules[43]. Furthermore, analysis of the signaling capacity of chimeric Rx-GPA2 proteins suggests that at least two regions in the Rx LRR interact with the winged-helix (ARC2) portion of the NBS domain.

Additional experiments have shown that a substitution that inactivates the P-loop of Rx disrupts the interaction of the coiled coil with the NBS-LRR domains but does not affect the interaction of the LRR domain with the coiled-coil–NBS domain[46]. That suggests that P-loop activity and presumably nucleotide-binding status affects the interaction of the coiled coil with the NBS domain. That conclusion is supported by the crystal structures of Apaf-1 and CED-4, which indicate that the bound nucleotide coordinates many intramolecular hydrogen bonds and van der Waals interactions, forming a molecular 'glue' that supports the structure of the Nod domain[61,62]. In both cases, the amino-terminal domain also packs tightly with the Nod domain, suggesting that perturbations in the Nod domain could affect that interaction.

The intramolecular interactions of RPS5 are similar but not identical to those reported for Rx and Bs2. Coimmunoprecipitation experiments have shown that the coiled-coil and LRR domains individually interact with the NBS domain, but not with each other (B.J.O. and R.W.I., unpublished data). That supports a model in which the NBS domain is central to intramolecular folding[46]. However, that model differs from what is suggested for the Mi protein from tomato, which confers resistance to both aphids and nematodes. It seems that the amino-terminal domain may function to negatively regulate LRR domain activity[44,45]. The Mi amino-terminal domain is much larger than those of RPS5 and Rx and seems to have additional domains involved in the regulation of Mi function.

The interaction of Rx domains is disrupted by viral coat protein, suggesting that the protein exists in a folded inactive state and that effector detection leads to conformational change, resulting in altered domain interactions[46]. In contrast to the interactions of Rx, the presence of the cognate bacterial effector does not disrupt Bs2 intramolecular interactions but seems to stabilize them[74]. In addition, AvrPto fails to disrupt Pto-Prf interactions[33]. Further investigation is needed to clarify the function of pathogen effectors and their targets in plant NBS-LRR activation.

NBS-LRR formation of oligomers

Many animal Nod-containing proteins seem to form oligomers, including Apaf-1, which forms a heptameric structure. That is not unexpected, given that portions of the Nod domain are related to the oligomerization domain of the 'AAA+' ATPases, which typically have sixfold or sevenfold symmetry[3]. There is evidence supporting the idea that several plant NBS-LRR proteins form oligomers; however, the function of the NBS domain in that

oligomerization remains unresolved. For example, several domains of Rx are able to interact when expressed *in trans*, as are domains of Bs2 (discussed above), although it seems that some of those interactions are 'preferentially' intramolecular[46,73]. Oligomer formation of full-length Rx in the absence of pathogen effector has not ben detected and direct interaction of NBS domains has not yet been tested for these proteins46.

The N protein of tobacco forms oligomers in the presence of the pathogen effector, but the TIR domain is the only domain directly associated with that oligomerization[72]. Notably, the amino-terminal coiled-coil domain of RPS5 also forms oligomers, suggesting that for plant NBS-LRR proteins, the amino-terminal domain be involved in the formation of oligomers, which differs from Apaf-1 and CED-4 (B.J.D. and R.W.I., unpublished data). An intact P-loop seems to be necessary for the formation of N oligomers, suggesting that the NBS domain is important for this function; however, difficulties with NBS domain expression have precluded direct testing of NBS oligomerization of N protein[72]. Additionally, substitutions in the kinase 1a domain of N protein result in loss of oligomer formation, further indicating involvement of the NBS domain in plant NBS-LRR oligomerization72.

Finally, work on tobacco N protein has shown that the formation of oligomers is necessary but not sufficient for disease resistance. In addition, downstream signal-transduction mutants do not affect the formation of oligomers, suggesting that the formation of oligomers is an early event in pathogen detection[72]. Notably, experimental evidence suggests that RPS5 may form oligomers before recognition of pathogen effectors. However, effector presence is necessary for activation of signal transduction (B.J.D. and R.W.I., unpublished data). That is not unlike the situation with CED-4, which is present as a dimer in an inactive complex with CED-9 but forms a tetramer when released from CED-9 (ref. 62).

Models for NBS-LRR function

The work discussed above suggests a general model for NBS-LRR function in the context of indirect pathogen recognition (Fig. 2). Proteins targeted by pathogen effectors exist in a complex with at least one plant NBS-LRR protein before modification by the effector. That pathogen target–NBS-LRR interaction is mediated by the amino-terminal domain of the NBS-LRR protein. However, the amino-terminal domain also seems to interact with the NBS domain, as does the LRR domain, giving rise to a tightly folded complex consisting of the effector target, the amino-terminal domain and the NBS and LRR domains. NBS domain conformation is regulated by the presence and identity of the adenine nucleotide that is bound. Those interactions are hypothesized to maintain the NBS-LRR protein in an inactive state. In particular, the interaction of the LRR domain with the NBS domain seems to have an important negative regulatory function.

Effector-induced change in host proteins induces conformational change in the associated NBS-LRR proteins, enabling the exchange of ADP for ATP. That change in bound nucleotide probably alters the structure of the NBS domain, which in turn can further alter the structural arrangement of the NBS-LRR domains (Fig. 2). Thus, NBS-LRR protein activation is a consequence of both structural changes as well as nucleotide-binding status. Those changes probably result in creation of new binding sites for downstream signaling molecules, resulting in the activation of signaling pathways that have not yet been well characterized. By analogy to Apaf-1 and CED-4, those conformational changes may initiate the formation of oligomers, bringing bound downstream signaling molecules near to each other. That could result in the activation of signaling through an 'induced proximity–type model'.

In the direct detection model, pathogen effector molecules are detected through direct binding to the NBS-LRR protein. It seems that the LRR domain may be involved in effector

binding; however, other domains such as the TIR domain of TIR-NBS-LRR proteins may also be important. It is likely that the three-dimensional structure of the NBS-LRR protein is crucial for specific interaction with the effector. After effector binding, conformational changes probably activate signal transduction in a way similar to that of the indirect detection model (Fig. 2). However, the structural changes that follow ligand binding have not been investigated for NBS-LRR proteins known to directly bind pathogen effectors.

Concluding remarks

It seems apparent that complex intra- and intermolecular interactions are necessary for the modulation of NBS-LRR signaling, but structural data describing those interactions are mostly absent. Determining the crystal structures of NBS-LRR proteins in the presence or absence of signaling partners will greatly facilitate understanding of the mechanisms of NBS-LRR function. Such crystal structures will also help to elucidate the function of nucleotide binding and hydrolysis in NBS-LRR activation. Work with Apaf-1 and CED-4 suggests that this may differ among plant NBS-LRR proteins, so it will be important to characterize many plant NBS-LRR proteins.

Additionally, direct downstream interacting partners of plant NBS-LRR proteins have not been identified, possibly because NBS-LRR proteins must adapt a specific activated conformation before engaging such partners. Using the present knowledge of NBS-LRR activation, yeast two-hybrid experiments or biochemical purification schemes may be designed to attempt to identify such interacting partners. Those approaches and others, such as *in vivo* localization of NBS-LRR complexes, should provide important research material for the foreseeable future, which will generate a greater understanding of NBS-LRR function in disease resistance for both plant and animal systems.

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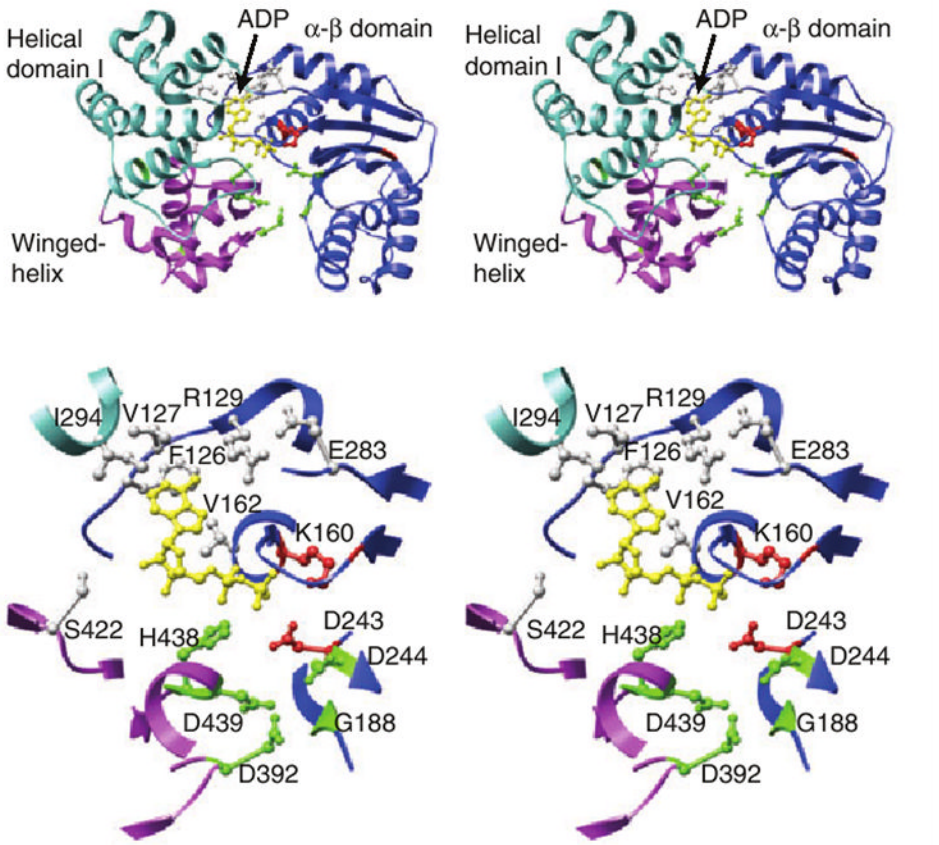

Figure 1.

NBS domain structure and location of informative substitutions in plant NBS-LRR proteins. Top, a 'stereo' view of the mammalian Apaf-1 Nod domain crystal structure bound to ADP (Molecular Modeling Database accession number, 33022)[61]. Helical domain II is not included because of the lack of complementarily to plant NBS domains. Bottom, a 'stereo' view of the ADP-binding pocket of mammalian Apaf-1, including side chains involved in coordinating ADP binding. The ADP-binding pocket is defined by helical domain I (cyan), the α-β domain (blue) and the winged helix domain (magenta). Amino acid substitutions of plant NBS domain mutants are mapped onto the Apaf-1 structure; autoactivating substitutions are green and loss-of-function substitutions are red. The side chains for the corresponding residues are in those colors as well, except where those residues differ among plant and Apaf-1 sequences. Images produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by National Institutes of Health P41 RR-01081)76.

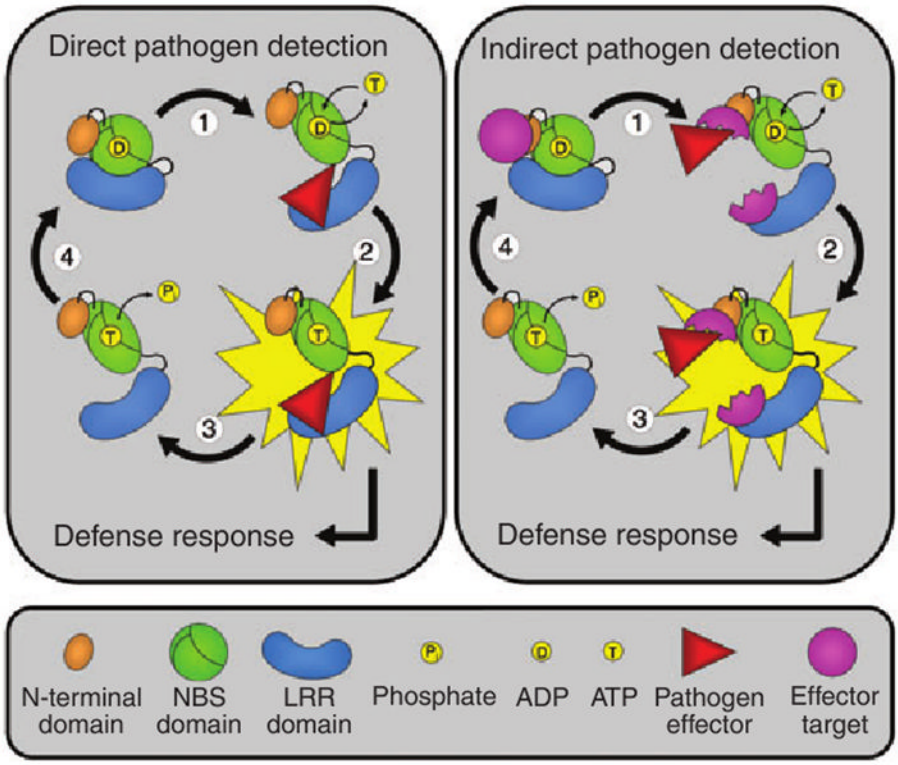

Figure 2.

Model for plant NBS-LRR activation. Signaling is activated in a similar way for both direct (left) and indirect (right) modes of pathogen detection. Presence of the pathogen effector(1) alters the structure of the NBS-LRR protein through direct binding (left) or modification of additional plant proteins (right), allowing exchange of ADP for ATP. Binding of ATP to the NBS domain (2) results in activation of signal transduction through the creation of binding sites for downstream signaling molecules and/or the formation of NBS-LRR protein multimers. Dissociation of the pathogen effector and modified effector targets (if present; 3) along with hydrolysis of ATP (4) returns the NBS-LRR protein to its inactive state.

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Table 1

NBS domain substitutions and related phenotypes

Substitution	Apaf-1[a]	Domain	Protein	Phenotype	Refs.
G200E	G154	P-loop	RPM1[b]	Loss of function	59
G216A, G216E, G216V, G216R	G154	P-loop	N	Loss of function	52
K222E/N	K160	P-loop	N	Loss of function	52
K207	K160	P-loop	I-2	Loss of function	66
K271M	K160	P-loop	L6	Loss of function	10,60
K188L	K160	P-loop	RPS2	Loss of function	38
S233F	G188	RNBS-A	I-2	Autoactiavation	47
D301H/N/Y	D243	Kinase 2	N	Loss of function	52
D283E	D244	Kinase 2	I-2	Autoactivation	47
R325Y/G	Q259	RNBS-B	N	Loss of function	52
G422R	S371	Between LPLss and RNBS-D	SSI4	Autoactivation	57
F393I	L386	RNBS-D	Rx	Autoactivation	40
D399V	D392	Near RNBS-D	Rx	Autoactivation	40
E400K	V393	Near RNBS-D	Rx	Autoactivation	40
H540A	H438	MHD	L6	Mild autoactivation	60
D460V	D439	MHD	Rx	Autoactivation	40
D541N/R/S	D439	MHD	L6	Mild autoactivation	60
D541V	D439	MHD	L6	Autoactiavation, no loss of ligand binding	10,60
D495V	D439	MHD	I-2	Autoactivation	58
E552K	NA	Between NBS and LRR	SNC1	Autoactivation	75

SSI4, suppressor of salicylic acid insensitivity of npr1-5; SNC1, suppressor of npr1-1, constitutive 1; NA, not applicable.

[a]Corresponding residue in Apaf-1.

[b]Reference includes a complete list of *RPM1* alleles and phenotype.