Review

CellPress

NOD-like receptor cooperativity in effector-triggered immunity

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Intracellular nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are basic elements of innate immunity in plants and animals. Whereas animal NLRs react to conserved microbe- or damage-associated molecular patterns, plant NLRs intercept the actions of diverse pathogen virulence factors (effectors). In this review, we discuss recent genetic and molecular evidence for functional NLR pairs, and discuss the significance of NLR self-association and heteromeric NLR assemblies in the triggering of downstream signaling pathways. We highlight the versatility and impact of cooperating NLR pairs that combine pathogen sensing with the initiation of defense signaling in both plant and animal immunity. We propose that different NLR receptor molecular configurations provide opportunities for fine-tuning resistance pathways and enhancing the host's pathogen recognition spectrum to keep pace with rapidly evolving microbial populations.

Role of NLRs in innate immunity

The capacity of individual cells to sense pathogen interference and trigger defense responses – referred to as innate immunity – is fundamental for the survival of plants and animals. In mammals, innate immunity provides an initial and major barrier against microbial infection, being generally followed by the triggering of the adaptive immune response [1]. By contrast, plant recognition of diverse pathogen virulence factors (effectors) is accomplished entirely by a panel of germ line-encoded receptors [2]. Conserved microbe-associated molecular patterns (MAMPs) or host-derived molecules that are modified during pathogen infection (damage-associated molecular patterns, DAMPs) are detected by pattern-recognition receptors (PRRs) in a process called pattern-triggered immunity (PTI) [3]. In plants, PRRs are usually membrane-resident receptor kinases or receptor-like proteins that become activated by extracellular MAMPs [3]. In mammals, both cell surface and cytoplasmic Toll-like receptors (TLRs), and intracellular NOD-like (nucleotide-binding oligomerization domain leucine rich repeat) receptors (NLRs) intercept MAMP signals [4].

An emerging feature of plant and animal NLRs is the capacity for receptor self-association as well as the formation of NLR heteromeric complexes. Whereas only a few functional NLR heterocomplexes have been reported to date, these findings suggest a common mechanistic framework in which co-acting NLR heteromeric molecules combine the attributes of specific pathogen recognition with activation of downstream defense signaling. In this review, we discuss recent findings and, in the context of the broader understanding of NLR structure and function, discuss the significance of receptor interactions in innate immunity. The expansion and diversification of *NLR* genes (Box 1), together with evidence of further co-acting gene pairs in plant genomes, lead us to propose that different NLR pairings might further increase the NLR repertoire for combating disease.

Plant NLRs in effector-triggered immunity

Infectious pathogens use secreted effectors to suppress host defenses activated by PTI, and this leads to effector-triggered susceptibility. The ability of hosts, in turn, to sense pathogen effectors and induce a robust cellular immune response is referred to as effector-triggered immunity (ETI) [1,5]. In plants, ETI involves a 'rebooting' and amplification of the PTI defense pathways [6,7]. A genetic framework for ETI in plants was first formulated in the 1940s as the 'gene-for-gene' resistance model, based on inheritance of complementary gene pairs in the host plant (*Resistance*, *R* genes) and pathogen (*Avirulence*, *Avr* genes) determining disease resistance [8]. It was not until pathogen *Avr* and plant *R* genes were cloned and protein interactions characterized that a molecular underpinning for plant disease resistance specificity in ETI began to take shape. *Avr* genes encode disease-promoting effectors and many *R* gene products are, in fact, intracellular NLRs recognizing particular effectors [5]. Recent developments in mammalian immunity reveal that ETI is not a protection strategy peculiar to plants but is also employed by animal cells [1].

Plant NLRs can recognize intracellular effector proteins directly, as a receptor-ligand couple [9,10], or indirectly through modifications of key host proteins that are constitutively part of an NLR receptor complex [11–14], or become available for NLR association upon pathogen interference [15]. Some host NLR cofactors are components of PRR-mediated immune signaling and as such are attractive

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Keywords: plant defense; innate immunity; NLR pairs; transcriptional reprogramming.
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1471-4906/
 http://dx.doi.org/10.1016/j.it.2014.09.005

Box 1. Plant NLR diversification

A distinctive characteristic of the plant NLR family is their expansion and diversification in different lineages, probably driven by pathogen infection pressure, so that a single plant germ line can contain a few hundred *NLR* genes [77,93]. Within a natural plant population, the *NLR* complement is likely to be even greater [94]. This contrasts with vertebrate NLR repertoires which typically comprise ~ 20 members [95]. However, early metazoans and some fish species have larger *NLR* panels, as in the sea urchin genome with over 200 *NLR* genes [96]. Differences in *NLR* numbers between animal lineages might in part be determined by the efficiency with which functional adaptive immune systems become mobilized. Plant host–pathogen coevolution is driven by reiterating cycles of host defense and pathogen counter-defense, and this is viewed as a major force promoting variation in NLR recognition and resistance functions [5,94]. NLR monitoring of effector interference with certain protein 'hubs' that are highly connected within the cellular defense network might help to further increase the plant NLR recognition spectrum [97], since NLR activation often appears to rely on sensing effector interference with host processes rather than direct recognition of the effector molecule [16].

Box 2. N-terminal structures of plant NLRs

TOLL/interleukin 1 receptor (TIR) or coiled-coil (CC) domains define the two major N-terminal modules in plant NLRs. Strikingly, TNLs have been lost from monocot lineages (e.g., wheat, rice) and reduced in *Magnoliids* (e.g., Avocado) during evolution [77]. The ADR1 NLR family, containing a CC_R-domain resembling the RPW8 protein, is exceptionally conserved across monocot and dicot plant species [98]. In several cases, a penta-amino acid motif EDVID, involved in intra-molecular interactions, is found in CNLs [59], but not in ADR1 family proteins [98]. In addition to the TIR-, CC-, CC_{EDVID}-, and CC_R-domains, other N-terminal domains have been discovered in various plant genomes including mosses and trees. For example, protein kinase (PK), an α/β-hydrolase, a DNA-binding zinc-finger, and WRKY domains occur, although their functional significance has not been established in many cases [99–105]. The structural diversity and/or ambiguity of N-terminal domains for a significant proportion of plant NLRs have led to the designation of NLRs which do not contain an N-terminal TIR domain as non-TIR-type NLRs. Four TIR crystal structures are now available. The general fold topologies are similar between RPS4, AtTIR, L6, and RRS1 [54,66,106]. However, a slight variation in the RRS1 crystal was observed, consistent with a 22-amino acid deletion in RRS1 [66]. So far, two contrasting crystal structures of CC_{EDVID}-domains have been reported [53,107]. The barley MLA10 CC forms an anti-parallel homodimer, resembling two springs slammed against each other, whereas the Rx CC in co-crystal with a part of its cofactor, RanGAP2 (Ran GTPase-activating protein 2), is a four-helix bundle fold monomer [53,107]. It is unclear whether the resolved Rx structure represents a state prior to transition to a homo-dimer, as in MLA10 CC.

effector operational targets [2]. Others lack obvious resistance signaling functions, but serve as molecular traps (decoys) for effectors [14,16]. As an amplified resistance response, ETI is associated with the generation of reactive oxygen species, a sustained increase in cytosolic Ca^{2+}, activation of calcium-dependent protein kinases (CPKs) and mitogen-activated protein kinase (MAPK) cascades, as well as transcriptional reprogramming, and in many instances, localized host cell death at sites of pathogen challenge [4,16]. The relative strength and robustness of ETI compared to PTI appear to be accomplished by the host employing compensatory pathways and amplification systems within a defense signaling network [17].

Building blocks and molecular architecture of plant NLRs

Plant and animal NLRs belong to a subclass of the STAND (signal transduction ATPases with numerous domains) superfamily of proteins [18] which possess variable N-terminal domains, a central conserved NB-ARC [Nucleotide-Binding, shared by Apoptotic protease activating factor 1 (Apaf-1), certain R-proteins, and Cell death protein 4 (CED-4)] domain, and a C-terminal leucine-rich repeat (LRR) domain of varying repeat number [18,19]. Recruitment of either a TOLL/interleukin 1 receptor (TIR)-like domain or a coiled-coil (CC) domain at the N terminus is specific to plants and defines two major classes of NLRs, TNLs, and CNLs, respectively [4] (Box 2).

Current evidence suggests that NLR N-terminal portions are important for initiating downstream signaling (discussed further below). The central domains of NLRs from both phyla are subdivided into a nucleotide-binding (p-loop), a four-helix bundle (ARC1), a winged-helix fold (ARC2), and an animal-specific helical bundle domain (ARC3, also known as helical domain 2) [20–22]. The C-terminal LRR domain serves a negative regulatory role in NLR action though intramolecular interactions with the other NLR domains [23,24]. A general scheme for specific NLR activation was recently described, in which the receptor is held in an autoinhibited state via inter-domain interactions and, often, associations with host proteins [4,19]. The recognized pathogen effector induces a series of NLR conformational changes that expose the NB-ARC domain, allowing replacement of ADP by ATP and transition to an activated receptor form [19].

The ARC3 segment has been shown to mediate homo-oligomerization of most animal NLRs [18,21,25]. Plant NLRs, however, lack an ARC3 portion [22], and thus, the relevance of this domain for plant NLR oligomerization has not been established. Higher order macromolecular NLR assemblies, such as the inflammasome, have been reported in mammals (reviewed in [25]), but not yet in plants. Therefore, we begin by discussing the mechanisms of NLR assembly and their impact in the context of mammalian innate immunity.

NLR cooperativity in effector recognition and resistance signaling

The NLRC4–NAIP inflammasome: an NLR signaling platform in mammals

Mechanisms underlying functional cooperativity between NLRs have been uncovered in mammalian innate immunity. In mouse, NLRC4 (NLR family, CARD domain containing 4) confers immunity to two different bacterial proteins [26,27]. For this, NLRC4 requires partner NLRs, designated as NAIPs (NLR family, apoptosis inhibitory protein), which provide recognition specificity for a distinct pathogen-derived ligand [26]. NLRC4 activation in response to infection by *Legionella pneumophila* or treatment with the C-terminal portion of the MAMP flagellin is dependent on NAIP5 [28,29]. NAIP5 is not required for NLRC4 activation upon infection by a different bacterial pathogen, *Salmonella typhimurium*, or in response to