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Connecting the dots between cell surface- and intracellular-triggered immune pathways in plants

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Highlights

- NLRs oligomerize upon activation to function as ion channels or enzymes
- PRRs and NLRs converge on common immune responses, including direct Ca²⁺ influx
- PRR and NLR immunity share genetic requirements and potentiate each other
- Immune receptor networks and connections evolve differently in plant species

Abstract

Plants can detect microbial molecules *via* surface-localized pattern-recognition receptors (PRRs) and intracellular immune receptors from the nucleotide-binding, leucine-rich repeat receptor (NLR) family. The corresponding pattern-triggered (PTI) and effector-triggered (ETI) immunity were long considered as separate pathways, although they converge on largely similar cellular responses, such as calcium influx and overlapping gene reprogramming. A number of studies recently uncovered genetic and molecular interconnections between PTI and ETI, highlighting the complexity of the plant immune network. Notably, PRR- and NLR-mediated immune responses require and potentiate each other to reach an optimal immune output. How PTI and ETI connect to confer robust immunity in different plant species, including crops will be an exciting future research area.

Introduction

Plant pathogens and plant disease threaten global food supplies [1]. Genetic host disease resistance, as an economically efficient and ecologically sustainable approach to crop protection, is seen as a key strategy to reduce the use of pesticides while avoiding infection and ensuring high yields. At the molecular level, genetic resistance is mediated by the combined action of cell surface-resident and intracellular immune receptors, which sense the presence of microbe-derived or self-derived molecules to control invasion [2].

The plasma membrane-anchored surface receptors are either receptor-like kinases or receptor-like proteins (RLKs/RLPs), and are collectively termed pattern recognition receptors (PRRs). Many characterized PRRs recognize so called microbe/pathogen-associated molecular patterns (M/PAMPs), which are found in widely conserved molecules characteristic for a class of organisms, such as fragments of flagellin (flg22) or Elongation factor EF-Tu (elf18) from bacteria or fungal chitin. PRR-initiated resistance responses were therefore termed M/PAMP-triggered immunity, and are increasingly referred to as pattern-triggered immunity (PTI) [3]. Intracellular receptors from the nucleotide-binding, leucine-rich repeat receptor (NLR) family monitor the intracellular space for presence and activities of microbial virulence factors (effectors). NLR-induced immune responses were therefore termed effector-triggered immunity (ETI). However, this ligand-centered differentiation can be unclear [4]. Hence, PTI and ETI immune sectors are defined by underlying receptor types: PRRs (RLK/RLPs) and NLRs, respectively. In addition, immune responses induced by both types of receptors overlap, which received limited attention in the literature.

How PRRs and NLRs function and signal were covered in depth in several recent reviews [e.g., 5,6,7]. Here, we provide a broad overview of innate immune responses initiated at the cell surface or from within the cell, and focus on recent findings on the connections and mutual potentiation between these two pathways. We discuss how the mutual potentiation of PTI and ETI responses can be rationalized, and how PTI-ETI interconnectivity might be taken into account in future crop improvement.

Known knows: Activation modes of cell surface and intracellular immune receptors, and early signaling outputs

Immune signaling initiated at the cell surface: Activation of RLKs and RLPs

PRRs recognize MAMPs, self-derived danger-associated molecular patterns (DAMPs) or extracellular effectors to induce PTI. An overarching concept in PRR signaling is the formation of receptor complexes (Figure 1): Upon ligand recognition, PRRs associate with co-receptors of the SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family, most importantly SERK3/BAK1 [6]. For RLKs, receptor/co-receptor complexes create close-proximity between the cytoplasmic kinase domains, which engage into auto- and/or transphosphorylation. RLPs form bipartite receptor complexes with the adaptor RLK SOBIR1, which translate the signal by recruiting SERK-family co-receptors upon ligand binding [6].

PRR complexes then signal through receptor-like cytoplasmic kinases (RLCKs; Figure 1), which lack an ectodomain [8]. In Arabidopsis, there are close to 400 RLCKs. Especially subfamily VII members participate in immune signaling [8,9]. RLCKs phosphorylate and regulate a large panel of substrate proteins, such as the reactive oxygen species (ROS)-producing NADPH oxidase RbohD and cyclic nucleotide-gated calcium channels, leading to ROS production and Ca²⁺ influx [8,10,11].

RLCKs likely represent the link between activated PRR complexes and all downstream signaling responses. Besides ROS production and Ca²⁺ influx, early responses induced by PRR activation include production of the plant hormone ethylene, activation/regulation of MAP kinase cascades, calcium-dependent protein kinases (CDPKs), and ultimately transcriptional reprogramming through activation/phosphorylation of multiple transcription factors [12-14] (Figure 1).

Immune signaling initiated inside the cell: Activation of NLRs

NLR family members are multi-domain proteins found in both plant and animal immune receptors. They contain a C-terminal leucine-rich repeat domain, a central nucleotide binding/ oligomerization

domain and a variable N-terminal signaling domain [15]. Plant NLRs are subdivided in three major classes based on their N-terminal domains: TNLs display a Toll-interleukin 1 receptor-like (TIR) domain, CNLs a helical coiled-coil (CC) domain, and RNLs a CC_R domain, a subtype of the CC domain also found in the non-NLR immune regulator RPW8.

Most CNLs and TNLs act as sensor NLRs (sNLRs), i.e. they can sense, directly or indirectly, the presence of microbial effectors. RNLs are required as helper NLRs (hNLRs) in TNL downstream signaling [16,17]. In a resting state, NLRs are thought to stand in an equilibrium between inactive and active conformations. To prevent costly activation of unnecessary immune responses, the inactive state is predominant in absence of pathogen. Ligand recognition leads to a shift of the equilibrium towards the active state to rapidly activate immunity upon infection [18,19], which is often accompanied by the induction of cell death at infection sites, the hypersensitive response (HR) [2].

In the last three years, major breakthroughs and new concepts further defined how NLRs are activated and trigger intracellular immunity.

The Arabidopsis CNL ZAR1 was shown to assemble into pentameric resistosome complexes upon activation [20]. The resistosome complexes function as Ca²⁺-permeable channels at the plasma membrane *via* their N-terminal CC domains [21] (Figure 1). Structures of the TNLs Roq1 from *N. benthamiana* and RPP1 from Arabidopsis revealed that TNLs also oligomerize; the TIR domains assemble into holoenzymes with NADase activity within tetrameric TNL complexes [22,23].

In addition to NADase activity producing nicotinamide, ADP ribose and variants thereof, TIR domains also have 2',3'-cAMP/cGMP synthetase activity [24].

Whether *in planta* products of TIR-enzymatic activities (represented by a star in Figure 1) solely activate TNL-mediated immunity and what are the molecular mechanisms involved remain to be determined (see also "note added in proof").

All TNL-mediated immune responses converge on a small family of lipase-like proteins, called EP proteins or EDS1 (Enhanced Disease Susceptibility 1) family proteins [25]. Two lineages of RNLs, the NRG1 (N-required gene 1) and the ADR1 (Activated Disease Resistance 1) proteins, function downstream of the EP proteins (Figure 1) [17,26].

Convergence of PRRs and NLRs towards cation/Ca²⁺ influx and a common immune response

Both PRR- and NLR-mediated early immune responses include Ca²⁺ influx, ROS production, and MAP kinase cascade activation very briefly upon ligand recognition [27]. However, avirulent pathogen strains (recognized by NLRs) induce a quantitatively different response including a second and stronger wave of ROS [28], longer lasting MAPK activation [29,30] and sustained Ca²⁺ influx [31].

In cell surface-triggered immunity, Ca²⁺ influx is, at least in part, mediated by direct phosphorylation and activation of Ca²⁺-channels by RLCKs [6,11]. Recent analyses substantiated that ZAR1 resistosome complexes can function as ion channels and mediate Ca²⁺ influx into plant cells [21]. Similarly, cation channel activity was reported for Arabidopsis NRG1 and ADR1 hNLR proteins, functioning downstream of TNLs and EP proteins [32]. Hence, Ca²⁺ influx likely represents a common early output in PRR-, CNL- and TNL-initiated immunity (in the following, PTI, ETI^{CNL} and ETI^{TNL}), although distinct contributions of Ca²⁺ channels and resistosomes remain to be determined [33].

Similarly, transcriptional changes induced by pattern recognition (PTI only) or infection with virulent and avirulent pathogenic strains largely overlap, although with faster kinetics when ETI is activated by avirulent pathogens [34-37]. To summarize, PTI, ETI^{CNL} and ETI^{TNL} share Ca²⁺-influx as a common early output and converge on a common immune response (Figure 1).

Known unknowns: NLR signaling components EDS1 and NDR1

Consistent with their distinct molecular assemblies and early signaling modes, such as channel formation vs. enzymatic activity, two proteins, NDR1 (Non-race specific disease resistance 1) and EDS1, were recognized early on as differentially required for ETI^{CNL} and ETI^{TNL}, respectively [38]. The original observation of strict NDR1-CNL vs. EDS1-TNL bifurcation was partially breached, as numerous CNLs including ZAR1 do not require NDR1 [39], and also ETI^{CNL} may be modulated by EDS1 in some scenarios [40,41]. However, it is remarkable that the molecular functions of these broadly pathway-specific immune regulators remain obscure.

NDR1 belongs to a large family [45 members in Arabidopsis; 42] of NDR1/HIN1-like (*NHL*) genes. Some *NHL* genes are upregulated during leaf senescence and contribute to hormone responses. For example, *NHL29* is identical to HAB2 (Hypersensitive to ABA2), a regulator of the ABA response [43]. Loss or overexpression of *NHL* genes is frequently associated with altered pathogen resistance, but functional understanding is limited to the suggestion that NDR1 may contribute to the adhesion of the plasma membrane to the cell wall [44].

EDS1 forms mutually exclusive heterodimers with either of the further EP proteins, PAD4 (Phytoalexin-Deficient 4) or SAG101 (Senescence Associated Gene 101) [25]. All three proteins have homology to eukaryotic lipases (α/β -hydrolases) in their N-termini, and a lipase-like catalytic triad is conserved in EDS1 and PAD4. The discovery of TIR domain catalytic activities renewed the tempting hypothesis that EDS1 complexes could function as enzymes or small molecule receptors [45]. However, there is little support for a direct implication of the (conserved) catalytic triad regions in immune signaling [25,46]. Rather, the all-helical C-terminal domains of EP proteins appear to have critical functions in ETI^{TNL} and could represent a candidate surface for small molecule binding [46,47].

No TIR enzymatic products identified so far have been shown to be sufficient to activate immune signaling, suggesting that other or additional components may be involved [48]. Further understanding the function of TIR domains, including the identification of *in planta* immunity-promoting TIR domain enzymatic products, will provide new opportunities to address how EDS1 complexes are activated, notably by using biochemical rather than genetic approaches (see also “note added in proof”).

It is interesting to note that, while SAG101 is limited to genomes with expanded TIR repertoires, EDS1 and PAD4 are also present in many genomes lacking TNL genes [49]. Whether EP proteins execute immune functions in these species remains yet to be thoroughly addressed.

New knowns and familiar unknowns: Intertwined PTI and ETI responses mutually potentiate each other

Despite inducing a similar core immune transcriptome, PTI and ETI were long-considered as separate pathways. Indeed, PRRs and NLRs activate immunity from different cellular compartments, and early signaling following receptor activation relies on distinct signaling components (Figure 1). So, how do immune pathways initiated by different families of receptors converge on largely identical responses?

A large body of work recently untwisted and dissected PRR- and NLR-activated pathways and revealed that they mutually require and potentiate each other to achieve an optimal and robust immune response [50-53] (Figure 2).

Both PRR- and NLR-induced immunity genetically require canonical components of ETI and PTI. In Arabidopsis, PRR-induced immunity depends on appropriate NLR receptor homeostasis and NLR-associated signaling components [51,53,54]. Reducing NLR protein accumulation lowers PTI-related defense gene expression and bacterial resistance [51]. This observation originated from analyses of transgenic Arabidopsis lines overexpressing SNIPER1 (an E3 ubiquitin ligase), in which accumulation of sNLRs, but not hNLRs, is globally reduced [51,55].

In addition, NLR-associated signaling components such as RNL-type helper NLRs, EP proteins and, to a lesser extent, NDR1, were recently shown to be required for PRR signaling in Arabidopsis [51,53]. RLP- but not RLK-mediated immune responses and -induced resistance to virulent bacteria are compromised in mutants deficient for EP proteins EDS1-PAD4 and ADR1 hNLRs, demonstrating that this protein module plays an important role in RLP-mediated immune responses [51,53]. Similarly, defense marker gene induction (*PR1*) and ROS production upon PRR activation were reduced in *ndr1* mutant plants [51,54]. Together, these findings suggest that an adequate level of NLRs and intact NLR signaling components are required for an optimal PRR-mediated immune response.

Reciprocally, immunity induced by several NLRs, such as CNL RPS2 and TNL pair RRS1/RPS4, is impaired in Arabidopsis mutants deficient for PRRs/co-receptors and canonical components of PRR signaling, such as MAPKs, BIK1 and RbohD [29,50,52]. In these examples, the NLR-inducing effector was delivered via a microbial vector also expressing recognized patterns. Hence, it is difficult to determine whether the observed deleterious effects on immune responses were due to compromised NLR immunity or indirectly via weakened PRR-triggered immunity. One critical lever to activate NLR-induced immunity independently of PTI was the development of transgenic Arabidopsis lines expressing NLR-recognized effectors under the control of inducible promoters. Such experimental approach revealed that the activation of ETI-only (CNL or TNL) is sufficient to boost expression and protein accumulation of a number of PTI-related components (PRRs, co-receptors, BIK1, RBOHD, MPK3). This response is dependent on intact PRR signaling, and co-activation of PTI bolsters it, suggesting that ETI can compensate the attenuation of PTI response by effectors (effector-triggered susceptibility), in order to reinforce the immune system [50,52].

Strikingly, PTI-only also activates the expression of genes coding NLRs and TIR signaling components, and increases NLR protein accumulation [12,51,54]. Checks and balances are crucial for the regulation of immune receptors [reviewed in 56], and PTI appears to reshape this balance by reinforcing NLR signaling and boosting the immune system upon infection.

Co-activation of NLRs and PRRs is required to provide robust and sustained core immune responses, such as enhanced PRR-triggered cellular responses as well as NLR-mediated HR and disease resistance [50,52]. Inducible expression of the AvrRPS4 effector in transgenic Arabidopsis fails to trigger a visible HR in the presence of the matching NLR pair RRS1/RPS4. Co-activation of PRR signaling enhances effector-triggered HR mediated by RRS1/RPS4- and CNL RPS2 and boosts disease resistance to avirulent bacterial strains [50,52].

PRR-induced cellular responses such as callose deposition, ROS production, MAP kinase activation and RbohD phosphorylation are sustained or enhanced when PRRs and NLRs are co-activated [50,52,57]. Sustained production of ROS and MAP kinase activation are compromised in lines with impaired PTI or NLR signaling. Interestingly, while most tested defense marker genes are down regulated in *eds1*, *pad4*, *adr1*, and *ndr1* mutant backgrounds, early transient MAPK activation is intact [51,54]. This supports that both PRR- and NLR-signaling sectors contribute to the core immune response potentiation, but that the first wave of PRR-mediated immune response precedes this potentiation (Figure 2).

It is peculiar to note that the familiar unknowns, EDS1 and NDR1, may thus contribute to a large spectrum of immune responses, despite their initial identification as specific components of TNL and CNL-triggered immune pathways. Whether EDS1-PAD4-ADR1, and to lesser extent also NDR1, can have direct functions in PRR signaling, or whether TNLs and CNLs bolster PRR signaling (via EDS1,

NDR1) in an indirect manner, remains to be clarified [51,53,54]. Notably, several early PRR outputs, such as transient MAPK activation, are unaffected in lines with impaired ETI.

Multiple strategies to reinforce or protect plant immunity

The molecular mechanisms connecting immune pathways initiated at the cell surface or inside the cell remain unclear. We highlight several mechanisms/models that may explain how PRR- and NLR-mediated immune response impact and potentiate each other to confer robust immune responses (Figure 2).

A first explanation is that each pathway boosts the primary immune responses via a transcriptional feed forward loop [50-52,54]: A set of core immune genes including PTI and ETI receptors and signaling components, is induced during surface and intracellular immunity (Figure 2, box #1). An intriguing question is whether the upregulation of such genes is sufficient to boost immune responses. Interestingly, studies by Tian et al. and Lang et al. home in on a few NLR-coding genes (mainly *TIR/TNL* genes) strongly upregulated during PTI, in an EDS1- and NDR1-dependent manner [51,54]. Overexpression of some of these genes can trigger immune responses autonomously, which supports a direct enhancement of defences *via* the upregulation of these “signaling” NLR genes [51,54]. It will be interesting to test whether corresponding *nlr*-poly-mutants are impaired in PTI responses, which may help to solve upstream-downstream ambiguities.

Overexpression of well characterized sNLRs, autoimmune variants, or their isolated signaling domains, is sufficient to induce immune responses independently of PRR activation [58-61]. This response may mimic the activation of above-mentioned “signaling” NLRs. Alternatively, potentiation of NLR signaling by PTI may directly emerge from priming of sensor NLRs by early PRR-induced cellular responses (Figure 2; box #2). Given that Ca^{2+} influx occurs within minutes after PRR activation, Ca^{2+} could be involved in sensitizing NLRs (prior to effector recognition), possibly by shifting the receptor ON/OFF equilibrium towards the active state [18], and consequently increasing their sensitivity upon effector recognition.

Another way to reinforce the plant immune system is to bridge PRR- and NLR-induced responses via physical interactions between proteins involved in both pathways (Figure 2; box #3). Several studies have reported direct or indirect interaction of NLRs with PRRs or PTI signaling components. Further, microbial effectors often target hubs of the plant immune system to suppress defense responses and promote disease. Known effector targets include PTI-associated immune components, such as the PRR co-receptor BAK1, members of the RLCK family, MAP kinases [8,62], and also members of the NLR signaling pathways, such as helper NLRs [63]. To counteract hub manipulation, effector virulence targets or decoys are guarded by NLRs (Figure 2; box #3). A most perspicuous example is given by ZAR1-like NLRs: In different species, ZAR1 guards RLCKs and/or derived pathogen decoys, and can detect multiple bacterial effector proteins [64-66]. In several cases, NLR-dependent autoimmune phenotypes are induced by misregulation or modification of PTI components [67-69]. It is possible that some of these NLRs guard core immune components, but considering the intertwined PTI and ETI responses, PRRs may also control the activation of some NLRs. For example, CNL SUMM2 autoimmunity is induced by a disrupted MAP kinase cascade [67]. Recent studies suggest that RLKs are involved in the regulation of SUMM2 activation [70-72], which may support the hypothesis that early PRR-induced signaling can sensitize NLR activation (Figure 2, box #2).

Similarly, the EDS1-PAD4-ADR1 module bridges PRR and TNL sectors, as it is required for RLP- and, to minor extent, RLK-mediated responses [Figure 2; box #4; 51,53]. This may be mediated by physical connection of pathways through interaction of EDS1-PAD4-ADR1 with RLP complexes (box #4, blue arrows), or upregulation of TIR signaling upon PRR activation, which in turn requires EDS1-PAD4-EDS1 to translate into bolstered defense responses (box #4, green arrows) [51,53].

To summarize, PRR-mediated signaling is necessary for optimal NLR-mediated immune responses. Reciprocally, NLR-mediated signaling seems to both reinforce and/or protect PRR-mediated immunity that may be compromised by effector virulence functions (Figure 2).

Evolution of PTI-ETI immune sectors and connections

In Arabidopsis, PTI and ETI mutually potentiate each other, and the EDS1-PAD4-ADR1 module operates in ETI^{TNL} and supports PTI. Are such PTI-ETI connections a general feature of the plant innate immune system, are they conserved in other species? Experimental data to answer these questions are limited to some observations in the *Solanaceae* model species *N. benthamiana*, but evolutionary trajectories of immune sectors may provide additional perspectives.

Arabidopsis EDS1-PAD4-ADR1 have extensive “basal resistance” functions (weak ETI^{TNL}, bolstering of PTI or yet further distinct function). By contrast, in *N. benthamiana*, immune functions of EDS1-PAD4 were not detected [47], and ADR1 appears to have a minor role [26,73]. Rather, EDS1-SAG101-NRG1 execute immune functions limited to ETI^{TNL} [47]. At the same time, not the TNL sector, but CNL-type hNLRs of the NRC (NLR required for cell death) family appear to support PRR-mediated responses in *N. benthamiana* [46,74].

Predominance and connectivity of immune sectors go along with different evolutionary trajectories in these two species. Genomes of Arabidopsis accessions generally encode more TNLs than CNLs [75]. *Solanaceae* have reduced TNL repertoires, but evolved an extensive NRC sensor-helper receptor network that most likely encompasses half the NLRome in *N. benthamiana* [76,77]. Hence, it is tempting to speculate that expansion of distinct immune sectors results in different modes of connecting ETI and PTI.

CNL genes were the most represented NLRs in 268/305 angiosperm genomes recently surveyed [49]. The *Brassicaceae*, including Arabidopsis, make up half of the remaining <40 genomes encoding predominantly TNLs. Also, TNLs and downstream signaling components were repeatedly lost, and are absent in monocotyledonous plants [49,78]. Hence, reliance of PTI on TNLs and/or downstream signaling components EDS1-PAD4-ADR1 may not be widespread among angiosperms. The NRC network has evolved in asterids and expanded in *Solanaceae* [76,77], suggesting that also NRC-PTI connectivity is limited to this family. It is conceivable that immune sector connectivity may follow expansion and prioritization also in species in which immune receptor networks remain yet to be characterized in detail.

Future challenges: Immune receptor networks in resistance breeding

Global population, climate change, erosion of arable land and sustainable development goals place high demands on crop improvement. In respect to pathogen resistance, current breeding methods based on recombination still achieve steady improvements [79]. However, *Resistance (R)* genes are prone to boom-and-bust-cycles (adaptation of pathogens if cultivars carrying the same *R* gene(s) are widely used). This forces breeders to continuously seek new *R* gene donor lines. As in the long term, “new” genetic resistance donors might become limiting factors, strategies are required to avoid *R* gene erosion [80]. This can be achieved if breeders will be able to transition towards rational design of new plant varieties based on gene function knowledge, and their creation and stacking by precision breeding methods, in a fourth phase of plant breeding [81]. To efficiently implement such approaches into resistance breeding, it will be crucial to decipher the complexity of plant immune receptor networks and to identify co-evolving immunity modules. To this end, two Arabidopsis NLRs including ZAR1 were predicted to activate ETI upon infection with >90 % of *P. syringae* strains [64], suggesting these *NLRs* may confer broad-spectrum disease resistance. However, ZAR1 may be

activated in inter-specific pairings with independently evolved RLCKs (guardees) [65]. PTI-ETI connections bolstering immune reactions add yet another level of complexity; further analysis of network architecture will be required to put these to advantage.

Note added in proof

During reviewing of this manuscript, several Preprint articles describing NAD-derived molecules produced by TIR domain-containing proteins and/or binding to EDS1 complexes were released [82-85]. Analyzing a phage-encoded protein functioning as a sponge to suppress bacterial Thoiser defence, Leavitt et al. identified 1'–2' glycosyl cyclic adenosine diphosphate ribose as an immune-activating molecule produced by TIR domain-containing bacterial ThsB protein and a TIR-only protein from *Brachypodium distachyon* (*Bd*TIR). Manik et al. identified cADPR isomers termed 2'cADPR and 3'cADPR produced by bacterial TIR domains from *Acinetobacter baumannii* and *Aquimarina amphilecti*, respectively. 3'cADPR was also detected upon expression of the *Pseudomonas syringae* effector HopAM1 in *N. benthamiana*. Last, 2'-(5''-phosphoribosyl)-5'-adenosine mono-/di-phosphate and ADP-ribosylated ATP and di-ADPR were identified bound to isolated EDS1-PAD4 and EDS1-SAG101 complexes, respectively. Binding of respective small molecules promotes formation of EDS1-PAD4-ADR1 or EDS1-SAG101-NRG1 complexes [84-87]. These reports suggest that TIR domain proteins can produce a number of cADPR isomers functioning as second messengers in immune systems in different kingdoms. It is foreseeable that further analyses will soon promote EDS1 complexes to the “known knowns” category, thus resolving a key mechanism underpinning different branches of plant immunity.

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Figure Legends

Figure 1 : Core signaling modules in PRR- and NLR-initiated immune responses.

Left panel: PRR/co-receptor complexes (dark blue) detect microbe-derived patterns on the outside of the cell. Ligand binding induces auto- and transphosphorylation events, and phosphorylation of receptor-like cytoplasmic kinases (RLCKs). The NADPH oxidase RbohD (light blue) is activated by direct phosphorylation by RLCKs, which induces ROS production. PRR activation also induces transient Ca²⁺ influx through Ca²⁺ channels. This may be, in part, regulated by RLCK-mediated phosphorylation of CNGC2/4 (dashed arrow), but other Ca²⁺-channels also contribute; details remain to be elucidated (Kim et al., 2022). RLCKs and Ca²⁺ influx further activate or regulate MAP kinase cascades and calcium-dependent protein kinases (CDPKs), which in turn activate diverse transcription factors to regulate transcriptional reprogramming of core immunity genes.

Right panel: CNL- or TNL-type sensor NLRs (sNLRs) can detect microbial effectors secreted inside the host cell (blue circle). In the case of CNLs, this can induce the formation of pentameric resistosome complexes that function as Ca^{2+} -permeable channels, which may contribute to sustained Ca^{2+} influx. TNLs form tetrameric holoenzymes upon activation. A product of the TIR-mediated enzymatic activity (yellow star) may activate EDS1 complexes and induce interaction with RNL-type helper NLRs. Similar to CNLs, hNLRs may form resistosomes and mediate (sustained) Ca^{2+} influx. NLR-induced signaling leads to transcriptional reprogramming of core immunity genes, which broadly overlap with PRR-induced transcriptional changes, by yet unclear mechanisms (dashed arrow).

Figure 2: Conceptual representation of the connections and potentiation between PRR- and NLR-induced immune responses.

Left panel: PRR- and NLR-induced immune signaling have many connections (blue arrows and grey boxes) and potentiate each other (orange arrows) to induce sustained cellular responses, transcriptional reprogramming of core immunity genes and, consequently, a strong and robust immune response. A PRR-mediated transient early response is independent of the NLR immune sector, while a sustained response requires both PRR- and NLR-mediated signaling, including NLR signaling components NDR1, EDS1 protein family, and helper NLRs (RNLs). Both PRR and NLR activation converge on rapid Ca^{2+} influx (orange dots). How the Ca^{2+} signal is decoded (signal amplification, sNLR priming, others) remains to be elucidated.

Right panels: Graphical representation of the connections between PRR- and NLR-induced immunity as depicted in grey boxes in left panel. Box #1- PRR and NLR-mediated signaling amplify each other via a transcriptional feed forward amplification loop. Box #2- PRR-induced responses may prime sensor NLRs for effector recognition and/or activate signaling. Box #3- NLRs protect and reinforce early immune responses by monitoring immunity hubs, such as PRR complexes or PRR-induced signaling components (green circle) targeted by microbial effectors (blue circle). Box #4- PTI and ETI connect via TIR signaling. This connection can involve physical interaction between PRR complexes and NLR signaling components such as the EDS1-PAD4-ADR1 module (blue shaded). Also, PRR activation induces the expression of TIR proteins, which require the EDS1-PAD4-ADR1 module for signaling (green shaded).

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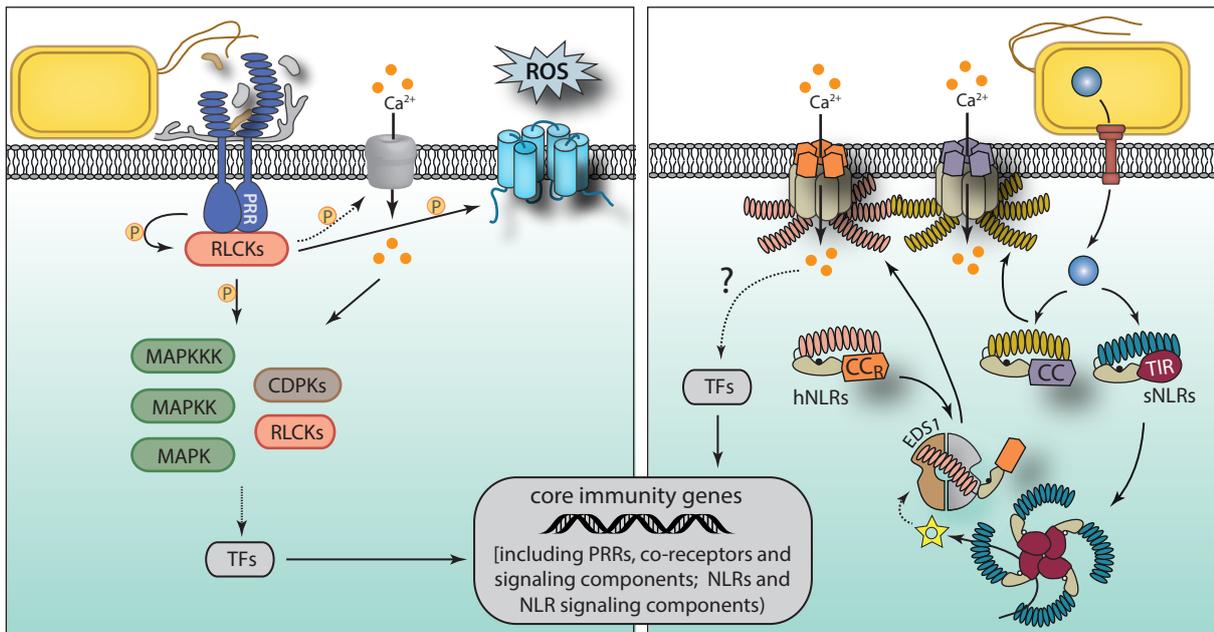


Figure 1 : Core signaling modules in PRR- and NLR-initiated immune responses.

Left panel: PRR/co-receptor complexes (dark blue) detect microbe-derived patterns on the outside of the cell. Ligand binding induces auto- and transphosphorylation events, and phosphorylation of receptor-like cytoplasmic kinases (RLCKs). The NADPH oxidase RbohD (light blue) is activated by direct phosphorylation by RLCKs, which induces ROS production. PRR activation also induces transient Ca^{2+} influx through Ca^{2+} channels. This may be, in part, regulated by RLCK-mediated phosphorylation of CNGC2/4 (dashed arrow), but other Ca^{2+} -channels also contribute; details remain to be elucidated (Kim et al., 2022). RLCKs and Ca^{2+} influx further activate or regulate MAP kinase cascades and calcium-dependent protein kinases (CDPKs), which in turn activate diverse transcription factors to regulate transcriptional reprogramming of core immunity genes.

Right panel: CNL- or TNL-type sensor NLRs (sNLRs) can detect microbial effectors secreted inside the host cell (blue circle). In the case of CNLs, this can induce the formation of pentameric resistosome complexes that function as Ca^{2+} -permeable channels, which may contribute to sustained Ca^{2+} influx. TNLs form tetrameric holoenzymes upon activation. A product of the TIR-mediated enzymatic activity (yellow star) may activate EDS1 complexes and induce interaction with RNL-type helper NLRs. Similar to CNLs, hNLRs may form resistosomes and mediate (sustained) Ca^{2+} influx. NLR-induced signaling leads to transcriptional reprogramming of core immunity genes, which broadly overlap with PRR-induced transcriptional changes, by yet unclear mechanisms (dashed arrow).

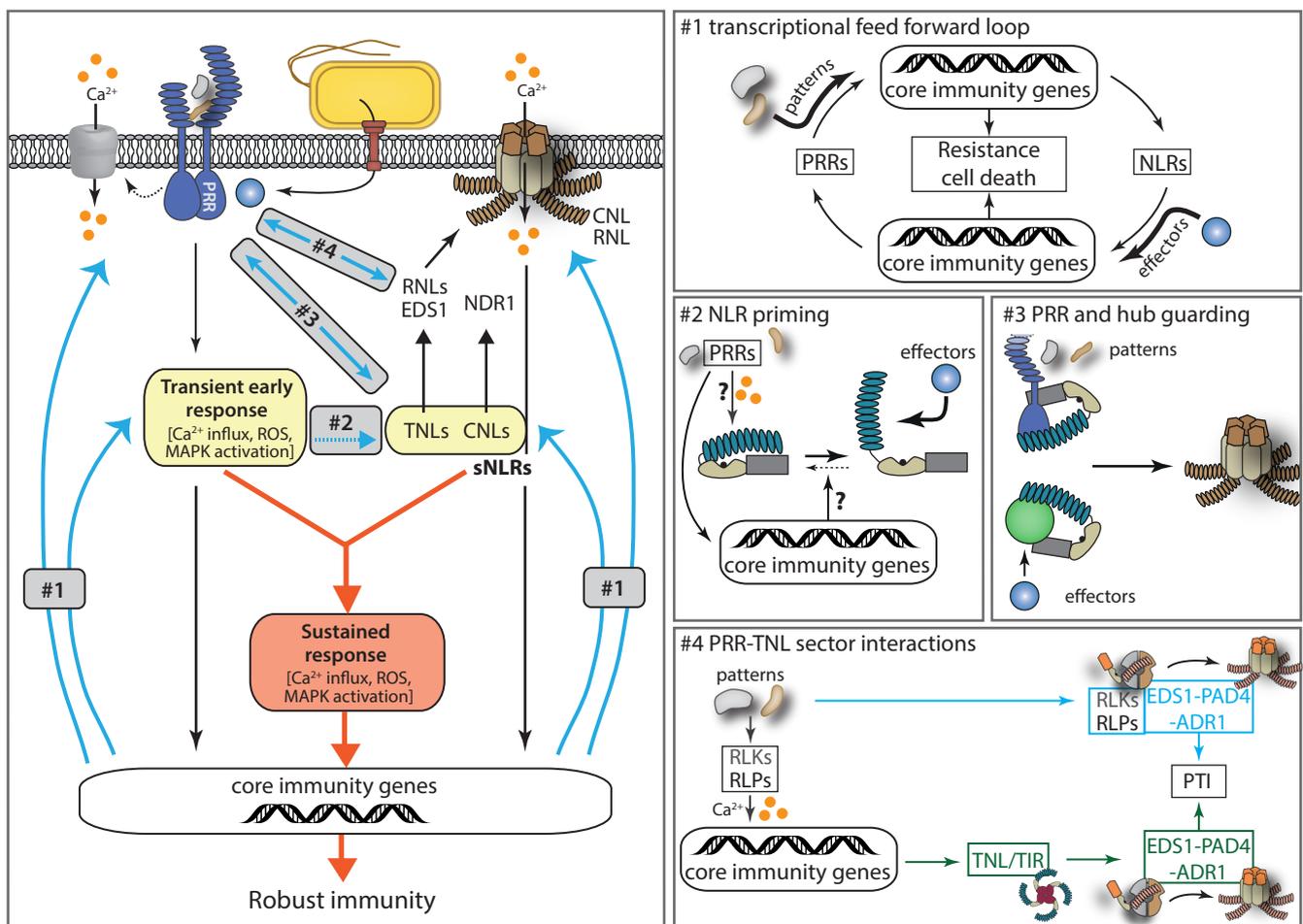


Figure 2: Conceptual representation of the connections and potentiation between PRR- and NLR-induced immune responses.

Left panel: PRR- and NLR-induced immune signaling have many connections (blue arrows and grey boxes) and potentiate each other (orange arrows) to induce sustained cellular responses, transcriptional reprogramming of core immunity genes and, consequently, a strong and robust immune response. A PRR-mediated transient early response is independent of the NLR immune sector, while a sustained response requires both PRR- and NLR-mediated signaling, including NLR signaling components NDR1, EDS1 protein family, and helper NLRs (RNLs). Both PRR and NLR activation converge on rapid Ca²⁺ influx (orange dots). How the Ca²⁺ signal is decoded (signal amplification, sNLR priming, others) remains to be elucidated. Right panels: Graphical representation of the connections between PRR- and NLR-induced immunity as depicted in grey boxes in left panel. Box #1- PRR and NLR-mediated signaling amplify each other via a transcriptional feed forward amplification loop. Box #2- PRR-induced responses may prime sensor NLRs for effector recognition and/or activate signaling. Box #3- NLRs protect and reinforce early immune responses by monitoring immunity hubs, such as PRR complexes or PRR-induced signaling components (green circle) targeted by microbial effectors (blue circle). Box #4- PTI and ETI connect via TIR signaling. This connection can involve physical interaction between PRR complexes and NLR signaling components such as the EDS1-PAD4-ADR1 module (blue shaded). Also, PRR activation induces the expression of TIR proteins, which require the EDS1-PAD4-ADR1 module for signaling (green shaded).