Plant Immune Networks

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Abstract

Plants have both cell-surface and intracellular receptors to recognize diverse self- and non-self-molecules. Cell-surface pattern recognition receptors (PRRs) recognize extracellular pathogen-/damage-derived molecules or apoplastic pathogen-derived effectors. Intracellular nucleotide-binding leucine-rich repeat proteins (NLRs) recognize pathogen effectors. Activation of both PRRs and NLRs elevates defense gene expression and accumulation of the phytohormone salicylic acid (SA), which results in SA-dependent transcriptional reprogramming. These receptors, together with their co-receptors, form networks to mediate downstream immune responses. In addition, cell-surface and intracellular immune systems are interdependent and function synergistically to provide robust resistance against pathogens. Here, we summarize the interactions between these immune systems and attempt to provide a holistic picture of plant immune networks. We highlight current challenges and discuss potential new research directions.

Plant Immunity

To confer full protection to pathogen attack, plant immunity requires the functions of multiple classes of receptors and ligands. Cell-surface pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). This leads to PRR-mediated immunity, commonly known as pattern-triggered immunity (PTI). Pathogens secrete virulence molecules termed effectors to inhibit PTI or

interfere with plant physiological responses. Some effectors are recognized by intracellular nucleotide-binding domain, leucine-rich-repeat containing receptors (NLRs). This results in NLR-mediated immunity, commonly known as effector-triggered immunity (ETI). Both PTI and ETI can elevate the biosynthesis of salicylic acid (SA) and N-hydroxyl-pipecolic acid (NHP), defense phytohormones which mediate systemic acquired resistance (SAR) [1–5]. PRR-, NLR- and SA-mediated immunity have been extensively studied for the past 30 years. Here, we highlight some major discoveries and current challenges in these three areas in plant immunity (Box 1).

Overviews of PRR-, NLR- and SA-mediated Immunity

PRR-mediated Immunity

PRRs comprise both receptor kinase (RLKs) and receptor-like proteins (RLPs) [6]. In 1994, researchers identified the first PRR-encoding gene in tomato, *Cf-9* (an RLP), which recognizes an apoplastic effector, Avr9, from the fungal pathogen *Cladosporium fulvum* [7]. Multiple RLPs that recognize apoplastic effectors, such as *Cf-4* and *Cf-2*, were identified afterwards [8,9]. The RLK FLAGELLIN SENSING 2 (FLS2) is the first PRR identified in *Arabidopsis thaliana* (arabidopsis thereafter), which recognizes the bacterial flagellin and its conserved 22-amino-acid peptide, flg22 [10,11]. Following the identification of PRRs, the downstream responses triggered by PRRs and the signaling components that activate them were explored. In 2002, the arabidopsis mitogen-activated protein kinase (MAPK) signaling cascade triggered by PAMPs was identified [12]. The arabidopsis MAPKs, MPK3 and MPK6, are orthologs of the tobacco WOUNDING-INDUCED PROTEIN KINASE (WIPK) and SALICYLIC ACID-INDUCED PROTEIN KINASE (SIPK), respectively [13,14]. In the same year, the arabidopsis NADPH oxidases RESPIRATORY BURST NADPH OXIDASE HOMOLOG D (RbohD) and RbohF are shown to be required for reactive oxygen species (ROS) production during immunity [15]. In 2005, tomato ACIK1 was identified as an essential signaling component required for Cf-9-mediated resistance, which was the first RECEPTOR-LIKE CYTOPLASMIC KINASES (RLCKs) reported to contribute to cell-surface receptor initiated immunity [16]. The arabidopsis RLCK, BIK1, was later identified as a central signaling component in PTI signaling [17,18]. BIK1 phosphorylates and activates downstream signaling components, such as RbohD [19,20]. Multiple calcium channels, such as CNGC2, CNGC4 and OSCA1.3, are also phosphorylated by BIK1 to induce calcium influxes during PTI [21,22]. Many PRRs require co-receptors to mediate downstream responses. In 2007, the arabidopsis RLK BAK1 was identified as a co-receptor essential for FLS2-mediated resistance [23] and the structure of the FLS2/BAK1 receptor complex with flg22 has been defined [24]. The RLK SUPPRESSOR OF BIR1-1 (SOBIR1) was found to be a co-receptor of RLPs, such as Cf-4, RLP23 and RLP30 [25]. It was then proposed that PRRs form networks to modulate signaling in response to

different extracellular ligands. In 2018, an analysis of interactions between arabidopsis leucine-rich repeat receptor-like kinases (LRR-RLKs) was reported, suggesting that PRRs interact with each other and form receptor networks [26] (Figure 1).

NLR-mediated Immunity

NLR-mediated immunity is triggered by intracellular nucleotide-binding, leucine-rich repeat (NB-LRR) receptor (NLR) proteins. The major three classes of NLRs are: the helical coiled-coil (CC) NLRs (CNLs), Toll/Interleukin-1 receptor/Resistance protein (TIR) NLRs (TNLs) and RPW8-like coiled-coil domain (RPW8) NLRs (RNLs) [27]. In 1994, the arabidopsis *RESISTANCE TO PSEUDOMONAS SYRINGAE PROTEIN 2* (*RPS2*, CNL) and the tobacco *N* gene (TNL) were reported [28–30]. Many other NLRs that recognize intracellular effectors have now been identified [31,32]. Following the cloning of multiple NLRs, attention turned to investigating NLR-mediated responses and the identification of signaling components that activate these responses. ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), a lipase-like (EP) protein required for TIR-NLR-mediated resistance plays a crucial role [33,34], and co-functions with another EP protein PHYTOALEXIN DEFICIENT 4 (PAD4) [35,36]. In 2005, SENESCENCE-ASSOCIATED GENE101 (SAG101) was found to interact with both EDS1 and PAD4 to mediate resistance and hypersensitive cell death responses (HR) mediated by TNLs [37–39]. The RNL N REQUIREMENT GENE 1 (NRG1) is required for resistance against tobacco mosaic virus (TMV) mediated by the *N* gene [40]. A distinct class of RNLs, from the ACTIVATED DISEASE RESISTANCE 1 class (collectively known as ADR1s, which includes ADR1, ADR1-L1 and ADR1-L2) also contribute to sensor NLR (RPS2 and RPP4)-dependent resistance [41]. In 2017, an additional class of helper NLRs, the NRCs, was discovered in the Solanaceae where they support the function of many sensor NLRs [42]. In arabidopsis, the NRG1 and ADR1 RNLs function downstream of multiple sensor NLRs to mediate HR and resistance [43–45]. In 2019, a new insight into the function of TIR-NLRs was provided by the discovery that the TIR domains in TNLs exhibit NADase activity which leads to the production of variant-cyclic-ADP-ribose (v-cADPR) [46,47]. V-cADPR was proposed to activate downstream signaling components such as the EP proteins. Within the same year, the full-length structure of the CNL HOPZ-ACTIVATED RESISTANCE 1 (ZAR1)-mediated recognition complex was solved [48]. In 2020, the structures of the TNL RESISTANCE TO PERONOSPORA PARASITICA 1 (RPP1) and RECOGNITION OF XOPQ 1 (ROQ1) recognition complexes were also solved [49,50]. An important insight into processes activated by ETI was recently reported; a key output from NLR activation is the replenishment and potentiation of PRR signaling components, restoring PTI after its attenuation by pathogen effectors [51,52]. Recently, the CNL ZAR1 and helper NLRs have been proposed to function as cation channels to induce cell death [53,54] (Figure 1).

SA-mediated Immunity

SA is a beta-hydroxy phenolic acid that has long been known to be a defense-related phytohormone [2,3]. Following the discovery of the roles of SA in SAR, researchers focused on characterizing SA biosynthesis and identifying the enzymes that are required for SA accumulation. *ISOCHORISMATE SYNTHASE 1* (*ICS1*, also known as *SID2* or *EDS16*) was identified from two independent genetic screens [55–57]. ICS1 converts chorismate into isochorismate [58]. The same genetics screens revealed ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) [59]. EDS5 was characterized as a MULTIDRUG AND TOXIN EXTRUSION (MATE) transporter family protein which likely transports isochorismate from the plastids to the cytosol [60]. Two other genes, *AVRPPHB SUSCEPTIBLE 3* (*PBS3*) and *ENHANCED PSEUDOMONAS SUSCEPTIBILTY 1* (*EPS1*), encode enzymes involved in SA biosynthesis [61–63]. Recently, it was found that isochorismate is adenylated and then conjugated with glutamate by PBS3, which produces isochorismoyl-9-glutamate (IC-9-Glu) [64,65]. IC-9-Glu then spontaneously break down into SA, or be converted into SA by EPS1 [64,65]. Other than the isochorismate pathway, SA can also be synthesized from phenylalanine by PHE AMMONIA-LYASES (PALs) [4].

Following pathogen recognition, the transcription factors SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1) and CALMODULIN-BINDING PROTEIN 60G (CBP60g) positively regulate SA biosynthesis by activating the expression of *ICS1*, *EDS5* and *PBS3* [66,67]. The increased concentration of cytosolic SA is then perceived by SA receptors in plants. In 1994, the first SA receptor encoding gene, *NONEXPRESSER OF PR GENE 1* (*NPR1*), was identified from a SA-insensitive mutant screening, though the SA-binding activity of NPR1 was not known [68–70]. In 2012, another two SA receptors NPR3 and NPR4, were reported to act as negative regulators in SA signaling via degradation of NPR1 upon their binding to SA [71]. In 2018, it was further shown that both positive immune regulator NPR1 and negative immune regulators NPR3/4 can bind to SA and function in parallel to regulate SA-dependent immunity [72]. This is further supported by the recently resolved structure of NRP4 C-terminus [73]. NPR1, NPR3 and NPR4 regulate SA-induced gene expression via their direct interactions with the TGACG-binding transcription factors TGA2, TGA5 and TGA6 [74,75]. The perception of SA also induces the biosynthesis of N-hydroxy-pipecolic acid (NHP), a putative mobile signal molecule that is involved in SAR establishment [76–78] (Figure 1). It was noted that NHP biosynthesis genes are highly induced upon ETI activation in the absence of cell-surface-receptor-initiated immunity and prior to the ETI-induced SA accumulation [51,79], indicating ETI activates NHP biosynthesis without SA.

The Plant Immune Receptor Network

PRRs and NLR immune receptor genes were first isolated in 1994 [7,28–30]. Subsequently, it was found that both NLRs and PRRs require other functionally linked NLRs and PRRs as helpers/co-receptors, respectively, to initiate immune responses [23,25,40,41]. Recently, the concept of 'receptor network' was proposed and is becoming gradually accepted. The first NLR network was proposed in 2017, shortly followed by the PRR network proposed in 2018 [26,42]. In addition, the phytohormone signaling pathways are also highly interconnected [80]. Here, we summarize the features of molecular pattern, effector and SA perception in plants, and then compare the PRR, NLR and SA receptor networks.

Pattern Recognition: Mostly One-to-one

Most characterized PRRs have been shown to bind to one specific ligand, which leads to the activation PTI. Examples include the binding of flg22 to FLS2; epitope of the bacterial elongation factor Tu (elf18) to ELONGATION FACTOR-THERMO UNSTABLE RECEPTOR (EFR); proteinaceous plant elicitor peptide 1 (AtPep1) to PEP1 RECEPTOR 1 (PEPR1) and PEPR2; SERINE RICH ENDOGENOUS PEPTIDE (SCOOP) phytocytokines and Fusarium-derived SCOOP-like peptides to MALE DISCOVERER 1-INTERACTING RECEPTOR LIKE KINASE 2 (MIK2); fragments of the N-acetylglucosamine-containing glycan chitin to LYSIN MOTIF RECEPTOR KINASES (LYKs); bacterial peptidoglycan (PGN) to LysM DOMAIN-CONTAINING GPI-ANCHORED PROTEINS (LYMs); NECROSIS AND ETHYLENE-INDUCING PEPTIDE1-LIKE PROTEIN 20 (NLP20) to arabidopsis RECEPTOR-LIKE PROTEIN 23 (RLP23), bacterial medium-chain 3-hydroxy fatty acid (mc-3-OH-FA) to the G-type lectin RLK LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) and sulfated peptide REQUIRED FOR ACTIVATION OF XA21-MEDIATED IMMUNITY X (RaxX) to rice immune receptor XA21 [11,81–91]. Since the majority of PRRs perceive PAMPs/DAMPs through direct binding, it is likely that most PRRs confers recognition to one distinct and relatively conserved ligand (Figure 2). However, two recent publications suggested that the arabidopsis RLK HPCA1/CARD1 (HYDROGEN-PEROXIDE-INDUCED CA^{2+} INCREASES 1/ CANNOT RESPOND TO DMBQ 1) is required for the perception of both hydrogen peroxide and 2,6-dimethoxy-1,4-benzoquinone (DMBQ) [92,93]. Similarly, the *Nicotiana benthamiana* RLP NbCSPR was reported to perceive the bacterial cold shock protein peptide csp22 and a small cysteine-rich protein VmE02 from both fungi and oomycetes [94,95]. In addition, the tomato RLP Cf-2 recognizes apoplastic effectors that targets the cysteine protease Rcr3 [9,96]. Thus, some PRRs might be able perceive multiple elicitors through distinctive mechanisms.

The PRR Network

Many PRRs function with co-receptors to transduce downstream signals. In arabidopsis, FLS2, EFR and PEPRs require the co-receptors BAK1 and BKK1; LYKs and LYMs require the co-receptor CERK1 and RLP23 requires BAK1 and SOBIR1 [23,25,85,86,97]. The binding of ligands to the LRR domains leads to heteromeric receptor complex formation between these PRRs and their co-receptors. This induces the proximity of the cytoplasmic domains between these PRRs, which leads to the phosphorylation of the kinase domains and subsequent activation of RLCKs [98]. Some PRRs, such as LORE, might not require co-receptors to downstream responses. In addition, it has been suggested that some PRRs, such as RLP23, might require ADR1s, PAD4 and EDS1 to activate some downstream immune responses [99,100]. Whether helper NLRs and EP proteins function as a complex with PRR co-receptors remains to be determined.

Some RLKs also negatively regulate PRR-signaling. BAK1-INTERACTING RECEPTOR-LIKE KINASE (BIR) family proteins associate with and sequester SOBIR1 and BAK1 to prevent auto-activation [101,102]. Other RLKs, such as FERONIA (FER), APEX and the NUCLEAR SHUTTLE PROTEIN (NSP)-INTERACTING KINASE 1 (NIK1), have also been reported to negatively regulate the association between FLS2 and BAK1 [26,103]. Thus, association of PRRs can lead to both activation and inhibition of downstream immune responses. Furthermore, the arabidopsis LRR-RLK interactome data suggest that small LRR-RLKs, such as BAK1 and APEX, might act as scaffolds to organize the PRR signaling network [26]. The relationship and regulatory interactions between different PRRs and co-receptors within this receptor network remain a topic of active investigation.

Effector Recognition: One-to-one, Many-to-one and One-to-many

Intracellular NLRs detect pathogen-secreted effectors either through i) direct binding to the effectors, ii) guarding host proteins targeted by effectors or iii) guarding decoys targeted by effectors [104]. As a result, some NLRs can perceive a specific effector, while other NLRs can detect multiple effectors and some effectors can be detected by multiple NLRs. In arabidopsis, the TNL RESISTANCE TO PERONOSPORA PARASITICA 1 (RPP1) recognizes the *Hyaloperonospora arabidopsidis* (*Hpa*) effector ATR1 through direct binding (one receptor to one ligand) [105,106]. The CNL ZAR1 guards the RLCK-mimicking pseudokinases such as ZED1 and RKS1. ZAR1 recognizes multiple effectors, including AvrAC from *Xanthomonas campestris* and HopZ1a from *Pseudomonas syringae* [107,108]. A remarkable feature of ZAR1 is that is one of very few sensor NLRs for which orthologs can be identified between arabidopsis and the Solanaceae [109]. The NLR paralogs WRR4A and WRR4B can each recognize multiple and different *Albugo candida* CX_2CX_5G (CCG) effectors (one receptor to many ligands) [110]. The arabidopsis TNL pair RRS1/RPS4 can recognize AvrRps4 from

Pseudomonas syringae, PopP2 from *Ralstonia solanacearum* and an unknown effector from *Colletotrichum higginsianum* [111,112]. AvrRps4 is also recognized by two functionally-independent arabidopsis TNL pairs, RRS1/RPS4 and RRS1B/RPS4B (many receptors to one ligand) [113]. In addition, AvrRpm1 from *Pseudomonas syringae* is recognized by two arabidopsis CNLs, RPM1 and RPS2 [114,115] (Figure 2).

The NLR Network

The NB-LRR REQUIRED FOR HR-ASSOCIATED CELL DEATH-2 (NRC2), NRC3 and NRC4 proteins function as helper NLRs for multiple sensor NLRs in solanaceous and likely in other asterid, but not rosid plants [42]. Helper NLRs were proposed to interact with sensor NLRs to mediate downstream immune responses [42,116]. In arabidopsis, multiple sensor NLRs also require helper NLRs (RNLs) to mediate downstream signaling. RRS1/RPS4-, RPS2- and RPS5-mediated bacterial resistance is dependent on the RNLs ADR1s, NRG1A and NRG1B (collectively known as NRG1s) [41,43,44,117]. On the other hand, RRS1/RPS4-, but not RPS2- or RPS5-, mediated HR is dependent on NRG1s but not ADR1s [43,44,117]. Thus, there is unequal redundancy between the NRG1s and ADR1s when mediating immune responses from different sensor NLRs. It is unclear how sensor NLRs activate RNLs. Conceivably, sensor NLRs directly associate with helper NLRs to mediate downstream responses, while others can signal via indirect actions on RNLs. For example the vc-ADPRs produced by the NADase activity of most TNLs could trigger the activation of downstream RNLs [46,47]. Interestingly, neither bacterial resistance nor HR mediated by RPM1 and ZAR1 are dependent on RNLs [43,44,117]. These NLRs are classified as singletons and function through their N-terminal domain containing the conserved MADA motif to induce HR [118,119].

The RPW8-like domain in RNLs is highly similar to the HeLo domain in the human mixed-lineage kinases (MLKLs) and the fungal HeLo/HeLo-Like (HELL) domain [120]. It has therefore been proposed that RPW8-like domains might function similarly to the HeLo domains of MLKLs, which trigger cell death by forming pores in the membrane [121–123]. Recently, it has been reported that the arabidopsis MLKLs (AtMLKLs) are required for full TNL-mediated resistance [120]. In addition, NRG1 and ADR1 were proposed to function as calcium channels to activate HR [124]. The mechanism by which RNLs oligomerize to form ion channels remains to be tested. In addition to helper NLRs, EP proteins are also required for sensor NLR-mediated responses. In arabidopsis, SAG101 is required for TNL-mediated HR but not bacterial resistance, while EDS1 and PAD4 are required for TNL-induced SA biosynthesis and resistance, but not HR [125,126]. The '***helperless'*** mutant that lacks both ADR1s and NRG1s phenocopies *eds1* and *pad4/sag101* [43,117]. Emerging data suggests that NRG1s function in association with the EP proteins SAG101 and EDS1 to mediate HR, while ADR1s might

associate with PAD4 and EDS1 to mediate resistance [125,127–129]. Furthermore, recent data suggest effector recognition-dependent association of helper NLRs with EP proteins [128,130]. The mechanisms by which helper NLRs modulate downstream immune responses remain to be investigated.

SA Perception: A Single Type of Receptors with Different Actions

SA is perceived by multiple receptors in plants. There are five NPR1 paralogs in arabidopsis (NPR2/3/4/5/6). NPR1 and NPR2 are positive regulators in SA signaling, while NPR3 and NPR4 act as negative regulators [68,131]. NPR5 and NPR6 are also known as BLADE ON PETIOLE 1 (BOP1) and BOP2. Arabidopsis NPR proteins contain BROAD-COMPLEX, TRAMTRACK, AND BRIC-À-BRAC (BTB) domain and a ANKYRIN repeats (ANKs) region [4]. SA can bind to all the six NPR paralogs in arabidopsis, with relatively stronger affinity towards NPR1/2/3/4 compared to BOP1 and BOP2, possibly due the lack of C-terminal SA-binding domain present in NPR1/3/4 [132]. With low SA concentration, NPR1 exists mostly as oligomers outside the nucleus [133]. At high SA concentration, NPR1 oligomers are reduced to monomers which then accumulate in the nucleus [133]. The ANKs region of NPR1 interacts with transcription factors TGA2, TGA5 and TGA6 to upregulate SA-responsive genes [75,134]. SA also binds to NPR3/4 to derepress SA-responsive genes [71,72]. While *bop1 bop2* has no defects in SA perception compared to WT, *npr3 npr4 bop1 bop2* exhibits stronger response to SA compared to the double mutants *npr3 npr4* and *bop1 bop2* [135]. Thus, BOP1 AND BOP2 might function redundantly with NPR3/4 as negative regulators in SA signaling (Figure 2). In addition to the NPR proteins, there are multiple SA-binding proteins (SABPs), such as catalase and glutathione peroxidase [136]. These indicate that SA is perceived by multiple receptors to regulate diverse biological processes, including defense and cellular redox regulation. Recently it has been reported that both NPR1 and NPR4 (redundant with NPR3) are required for SAR and transcriptional reprogramming induced by NHP [78,137]. Thus, NPR proteins might be involved in the perception of other defense-related phytohormones to induce immunity.

The SA-receptor Network

While SA has been reported to be perceived by multiple NPR proteins, the function and relationship between these receptors are rather complex. Currently there are two models of how NPR1 and NPR3/4 perceive SA and regulate SA-induced transcriptional reprogramming: Model-1) NPR1 and NPR3/4 function independently to activate and derepress SA-induced gene expression [72]. During infection, SA binds to and activates NPR1 to induce transcriptional reprogramming. In contrast, binding of SA inhibits the transcriptional repression activities of NPR3/4 [72]. This is further supported by the fact that *npr1-1* and gain-of-function

npr4-4D mutants have additive effects on the suppression of SA responses [72]. Model-2) At low SA concentration, NPR3/4 interacts with the Cullin-RING ubiquitin E3 ligase CUL3 to degrade NPR1. At high SA concentration, NPR3/4 is inhibited by SA which leads to NPR1 accumulation [71]. The physical interactions between NPR1 and NPR3/4 are inconsistent between different reports [71–73,132]. However, it is important to note that these are not mutually exclusive models and both mechanisms might contribute to SA-mediated responses. As mentioned, BOP1 and BOP2 might also function as negative regulators in SA-signaling [135]. Whether BOP1 and BOP2 interact with NPR3/4 is unclear. In addition, over-expression of NPR2 can complement the SA-insensitivity in an *npr1* mutant, indicating that NPR2 might also function as a positive regulator in SA-signaling [132]. The interaction between different NPR proteins in the absence and presence of SA remains to be fully defined.

The reciprocal antagonism between SA and JA pathways has been well characterized across several plant species [138]. In arabidopsis, exogenous application of SA leads to NPR1-dependent downregulation of JA-mediated gene expression [139]. On the other hand, the JA analogue coronatine produced by *Pseudomonas syringae* suppresses SA-signaling pathway [140,141]. Despite much evidence showing the antagonism between SA and JA, SA perception by NPR3/4 may lead to the degradation of JAZ, which derepresses the JA pathway to trigger HR and resistance against *Pseudomonas syringae* [142]. Thus, the interaction between JA and SA signaling might orchestrate immunity against both biotrophic and necrotrophic pathogens simultaneously [143]. Indole acetic acid (IAA or auxin) and gibberellic acid (GA) are phytohormones that regulate growth and development [144,145]. Exogenous application of SA suppresses the expression of auxin-related genes, while exogenous application GAs can lead to upregulation of *ICS1* and SA accumulation [146,147]. Thus, there is extensive crosstalk between SA and other phytohormone signaling pathways, which was further validated by the recently published phytohormone signaling network [80]. The intricate relationship between different phytohormone pathways remains to be investigated. In particular, the interactions and mutual potentiation of SA and NHP responses remains to be fully defined.

The Crosstalk between PRR-, NLR- and SA-mediated Immunity

The interaction between PRR-, NLR- and SA-mediated immunity has recently received more attention. PRR- and SA-mediated immunity have been usually investigated on their own. NLR-mediated immunity is usually investigated in the presence of PAMPs or microbes, which introduces interference from PRR-mediated immunity. Here, we summarize reports on the crosstalk between immune systems in plants and dissect those interactions at both local and systemic levels.

The Crosstalk between Immunity Mediated by Different PRRs

The crosstalk between PRRs can lead to enhanced activation of immune responses. Perception of flg22, elf18 and Atpep1 lead to the juxta-membrane (JM) phosphorylation of CERK1, which primes CERK1 and results in enhanced resistance against fungal pathogens [148]. JM phosphorylation of CERK1 is directly mediated by BAK1, indicating that the activation of multiple RLKs might also prime CERK1 [148]. Interestingly, CERK1 activation induced by chitin does not lead to phosphorylation of BAK1, indicating that CERK1 might not be able to prime BAK1 [148]. In addition, an *fls2* mutant exhibits reduced pep3-induced responses and a *pepr1/2* mutant shows reduced flg22-induced responses [149]. This indicates inter-dependency and potential crosstalk between these RLKs. Multiple PRRs are activated during natural infection. The crosstalk and simultaneous activation of multiple PRRs provide robust defence response against diverse pathogens.

BIR proteins and FER can negatively regulate PRR-signaling. The BIR family contains four RLKs: BIR1, BIR2, BIR3 and BIR4 [102]. These RLKs associate with and sequester BAK1 from FLS2 [101,102,150–152]. Ligand-bound PRRs (such as flg22-bound FLS2) can displace BIRs from BAK1 to form a receptor complex [101]. Following PAMP perception, SUBTILISIN-LIKE PROTEASE SBT6.1 cleaves the endogenous PRO-RAPID ALKALINIZATION FACTOR 23 (PRO-RALF23) into RALF23 [153]. RALF23 is perceived by the FER and the LORELEI-LIKE-GPI ANCHORED PROTEIN 1 (LLG1). The perception of RALF23 by FER negatively regulates the formation of the FLS2-BAK1 complex [153,154]. To summarize, activation of some RLKs can prime other PRRs to restrict further infections, while some RLKs modulate other PRRs to prevent prolonged immune responses (Figure 3a-b).

The Crosstalk between PRR- and NLR-mediated Immunity

NLR-mediated immunity was rarely investigated in the absence of PRR-mediated immunity. It was assumed that PRR-and NLR-mediated immunity are independent and do not affect each other. Two recent publications showed that these two systems mutually potentiate each other [51,52]. Activation of NLRs leads to transcript and protein accumulation for multiple PRR-signaling components, which in turn enhance and prolong the activation of PRR-mediated immune responses [51,52]. This is further supported by the fact that NLR-mediated resistance against *Pseudomonas syringae* is ineffective in PRR and PRR co-receptor mutants [51,52]. Thus, activation of NLRs potentiates PRR-mediated immunity.

Reciprocally, activation of PRRs enhances NLR-mediated HR [51]. HR triggered by *Pseudomonas syringae* delivering AvrRpt2 (activates RPS2) is compromised in *fls2*, *pepr3*,

fls efr cerk1 and *bak1-5 bkk1 cerk1* mutants [52,149]. MAPKs and NADPH oxidases mutants also exhibit compromised NLR-mediated resistance and HR compared to Col-0 [15,51,52,155,156]. These data imply that enhanced activation of PRR-signaling components following NLR activation contributes to both HR and resistance against pathogens. Furthermore, activation of PRRs leads to transcript accumulation of multiple NLRs and EP proteins [99,157,158]. PRR-mediated immunity is also partially dependent on EP-proteins and helper NLRs [99,100]. Thus, activation of PRRs might also prime NLR-mediated immunity through upregulation of NLR-signaling components. The crosstalk between PRRs and NLRs is essential to confer effective disease resistance and the mechanisms by which they cooperate with one another remain to be investigated (Figure 3c-d).

The crosstalk between Immunity Mediated by Different NLRs

While mechanisms of individual NLR activation have been extensively studied, it is unclear whether the activation of an NLR can influence other NLRs. Recently published pan-genome analysis on NLR-mediated immunity reveals that 70% *Pseudomonas syringae* strains carry more than one effector that can be recognized by NLRs in arabidopsis accession Col-0 [108]. This indicates that during natural infection, multiple NLRs are likely to be activated simultaneously. Furthermore, the fact that many *NLR* genes are semi-dominant suggests that coactivation of multiple NLRs can result in more robust resistance against pathogens [159]. Indeed, 'stacks' of NLRs provide stronger and more durable resistance against pathogens in the field [160–162]. Since activation of NLRs leads to transcriptional upregulation of NLRs and EP proteins, we can expect that NLR activation can potentiate subsequential activation of other NLRs [51]. Whether coactivation of NLRs has additive or synergistic effects on resistance against pathogens remains to be determined (Figure 3e).

While most helper NLRs have been reported to function as positive regulators, some helper NLR homologs might act as negative regulators to modulate NLR-mediated immunity. The overexpression of NRG1C leads to compromised HR and resistance triggered by multiple TNLs [163]. All three orthologs of arabidopsis NRG1 can also associate with EDS1 and SAG101 [128,163]. Thus, NRG1C might associate with and disrupt the interaction of EDS1 and SAG101 with NRG1A/B (Figure 3f).

The Crosstalk between PRR- and SA-mediated Immunity

PRR activation leads to SARD1/CBP60G-dependent upregulation of SA-biosynthesis genes [63,67]. Exogenous application of SA leads to accumulation of PRR-signaling components, such as FLS2, BAK1, MPK3 and RbohD, which results in enhanced physiological responses triggered by PAMPs [164–169]. Resistance against *Pseudomonas* DC3000 *hrcC*$^{-}$ and flg22-

induced immunity is compromised in the *npr1-1 npr4-4D* mutant, indicating that SA perception is required for PRR-mediated immunity [78]. Thus, SA biosynthesis upon PAMP recognition leads to NPR1/3/4-depedent upregulation of PRR-signaling components, which results in a positive feedback to amplify PRR-mediated immunity (Figure 3g).

While NLR activation also leads to robust accumulation of these PRR-signaling components, transcriptional upregulation of these genes during NLR activation is unaffected in the *ics1*/*sid2* mutant [51,52]. This indicates that both SA-dependent and SA-independent pathways can contribute to the accumulation of PRR-signaling components. In addition, HR triggered by *Pseudomonas* DC3000 delivering AvrRpt2 (coactivation of PRRs and NLR), but not by inducible expression of AvrRpt2 (activation of NLR only), is compromised in the arabidopsis quadruple mutant *pad4 dde2 ein2 sid2* (*peds*) [170]. Notably, upregulation of PRR-signaling components, such as MKK4, is compromised in *peds* following PAMP recognition [170]. This indicates that the PRR-mediated positive feedback is compromised in the *peds* mutant and thus is unable to potentiate HR mediated by NLRs.

The Crosstalk between NLR- and SA-mediated Immunity

Similar to PRRs, activation of NLRs also leads to SARD1/CBP60G-dependent upregulation of SA-biosynthesis genes [63,67,171,172]. The upregulation of these genes is also dependent on EDS1 and PAD4 during TNL activation [128,173]. Exogenous application of SA also leads to upregulation of both NLRs and EP proteins [36,72,174]. In addition, resistance against *Pseudomonas syringae* DC3000 delivering AvrRpt2 and AvrRps4 (which activates RPS2 and RRS1/RPS4) is largely compromised in both *sid2* and *npr1-1 npr4-4D* mutants, indicating that SA biosynthesis and perception are both required for NLR-mediated immunity [78]. Thus, NLRs and SA also form a positive feedback loop to amplify each other's immune responses.

While NLR-mediated immunity requires SA, NLR-induced HR can also be negatively regulated by SA [78,175]. *P. syringae* DC3000 delivering AvrRpt2 induces stronger HR in *eds5-3* and *npr1-1 npr4-4D* mutants compared to WT [175]. Furthermore, exogenous application of SA also suppresses HR induced by *P. syringae* DC3000 delivering AvrRpt2 [176]. A recent report suggested that high SA concentration in cells adjacent to infected tissues facilitates the formation of cytosolic NPR1 condensates, which sequester and degrade NLRs, EP proteins and WRKY transcription factors to promote cell survival [176]. Thus, different SA concentrations might lead to positive or negative regulation in NLR-mediated immunity (Figure 3h-i). The mechanism by which SA concentration is maintained in different tissues remains to be determined.

Local and Systemic Interactions between Different Immune Systems

Since PRRs physically associate to enhance or inhibit each other, the crosstalk between PRRs is most likely to be local or cell autonomous. Similarly, the crosstalk between NLRs is likely to be cell autonomous (Figure 4a). Potentiation of RbohD activation by PRR and NLR occurs in both leaf tissues and protoplast [51,52]. Thus, the mutual potentiation of PRR and NLR is cell autonomous and potentially also occurs systemically. Furthermore, mRNA of *FLS2*, *PEPR1*, *RbohD*, *MKK4* and *MPK3* can move cell-to-cell [177]. Thus, PRR-signaling component transcripts induced by NLR activation might move to neighboring tissues to prime PRR-mediated immunity. Similarly, mRNA of *PAD4* and multiple TNLs, such as *WRR4* and *RPS6*, are also cell-to-cell mobile [177]. Thus, NLR transcripts induced by PRR activation might move to adjacent cells to prime NLR-mediated immunity (Figure 4b). Perception of SA via NPR1 and NPR3/4 leads to upregulation of *FLAVIN-DEPENDENT MONOOXYGENASE 1 (FMO1)*, *AGD2-LIKE DEFENSE RESPONSE PROTEIN 1 (ALD1)* and *SARD4*, which leads to biosynthesis and accumulation of the putative SAR mobile signal molecule NHP [77,78,178,179]. NHP induces the biosynthesis and accumulation of SA in distal tissue via upregulation of SARD1 and CBP60g [76,77]. Thus, SA can potentiate or regulate both PRR-/NLR-mediated immunity in distal tissues (Figure 4c). In addition, perception of ligands by different receptors can vary in different tissues and cell types, because these receptors have different expression patterns under stress conditions [180].

Conclusion Remarks and Future Perspective

Plants perceive a range of self- and non-self-molecules as triggers to activate resistance against pathogens. Signaling initiated by any of these receptor classes, such as PRRs, NLRs and the hormone receptor NPRs, can influence the signaling initiated by other receptor classes. Although some receptors, like LORE, RPM1 and ZAR1, may act without helper signaling proteins, the majority of sensor PRRs and NLRs function through interacting with other co-receptors and form receptor networks. These interactions between receptor signaling components perhaps provide plants a better capacity, flexibility and adaptation for recognition of fast-evolving pathogens, and for creating appropriate responses to the combinations of biotic challenges that arise in nature [116]. In addition, receptor networks are less vulnerable to pathogens' manipulation due to genetic redundancy of co-receptors [116]. On the other hand, it is perhaps more efficient for the pathogens to directly target the 'hub'-like co-receptors than individual sensor receptors during invasion. For example, multiple pathogen effectors target the central nodes of plant receptor networks, such as BAK1 and NRCs [181,182].

Other than receptor networks, immune systems also interact with each other to potentiate or modulate downstream responses. Emerging evidence suggests that plant immune systems

are dependent on each other. For example, NLR-mediated immunity is dependent on PRRs, some PRR-mediated signaling requires NLR-signaling components, and the perception of SA is required for both PRR- and NLR-mediated immunity [51,52,78,99,100]. The plant immune system should be considered as an integrated network instead of individual "stand-alone" pathways. These networks integrate information from sensor receptors and fine-tune appropriate immune responses to maximize fitness. The interdependency between immune systems implies that pathogens might target hubs in these networks. Whether pathogens suppress the crosstalk between PRRs, NLRs and SA remains to be determined. Future research should address this crosstalk in other plants species during diverse plant-biotic interactions. In the future, we might be able to edit or engineer not just immune receptor repertoires, but also plant immune networks in crops to provide robust and durable protection diverse pathogens (see Outstanding Questions).

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Box1. Current Challenges of Research in PRR-, NLR- and SA-mediated Immunity.

Cytosolic calcium influx is one of the first physiological responses triggered by PRRs and contributes to multiple downstream responses [6]. CNGC, OSCA and GLUTAMATE RECEPTOR-LIKE (GLR) family members have been shown to induce calcium influxes following PAMP recognition [21,22,157,183,184]. Whether other calcium channels are involved in PRR-induced calcium influxes remains to be determined. Other than calcium influxes, PRR activation also induces MAPK activation, ROS production, callose deposition, sugar efflux and production of antimicrobial compounds [185]. The mechanisms by which PRR-induced physiological responses halt pathogens remain to be determined. Recent evidence suggests that some PRRs might require helper NLRs and lipase-like proteins (EP proteins) to induce downstream responses, the mechanism by which PRRs connect to these proteins remains to be determined [99,100].

Although the NLR signaling pathway has been extensively studied over the last 25 years, it remains unclear how NLR induces downstream responses, such as transcriptional reprogramming and the activation of HR. It is also not clear how the EP proteins and helper NLRs function together to mediate these downstream responses [117,130,186]. Moreover, how v-cADPR leads to activation of EP proteins and helper NLRs upon activation of TNLs is unknown. It has been recently proposed that ZAR1 and some helper NLRs function as calcium

channels [53,54,187]. However, the mechanism by which plant cells distinguish different types of calcium influxes and mediate HR and gene expression remains to be determined [187]. It was shown recently that NLR-mediated HR and bacterial resistance is dependent on functional PRRs [51,52,188–190], which added up more complexity to the understanding of NLR signaling.

SARD1 and CBP60g are required for the upregulation of ICS1, EDS5 and PBS3 during both PTI and ETI [63,67]. How PRRs and NLRs activate these transcription factors is unclear. In addition to the induction of SAR, SA also contributes to HR. Exogenous application of SA can suppress HR triggered by NLRs [176,191]. Furthermore, HR induced by NLRs is also enhanced in SA-deficient mutants [175]. The role of SA in regulating HR locally and systemically remains to be determined. In addition, SA-mediated responses interact with other phytohormone-mediated pathways, such as those mediated by jasmonic acid (JA) and ethylene (ET), to regulate the defence against herbivores and necrotrophic pathogens [143]. Recent data suggested that the arabidopsis phytohormone signaling network is highly interconnected. The crosstalk mechanisms between SA- and other phytohormone-signaling pathways remain to be investigated [80].

Figure 1. Historical timeline of discoveries in PRR-, NLR- and SA-mediated immunity. (Red timeline; top) In 1994, the first plant PRR-encoding gene, *Cf-9*, was identified in tomato. The first PRR from *Arabidopsis thaliana* (thereafter arabidopsis), FLS2, was identified in 2000. In 2002, the arabidopsis MAPK signaling cascade triggered by PTI was identified. The NADPH oxidases required for ROS production during PTI, RbohD and RbohF, were also identified in the same year. In 2005, the RLCK ACIK1 was identified as an essential signaling component required for Cf-9-mediated resistance. In 2010, the arabidopsis RLCK, BIK1, was also identified as a central signaling component for PTI. In 2007, the arabidopsis LRR-RLK BAK1 was identified as a co-receptor essential for FLS2-mediated immunity. Later in 2013, the structure of the FLS2/BAK1 receptor complex was solved. In 2018, the arabidopsis LRR-RLKs network was reported. Recently, multiple calcium channels have been shown to be involved in PAMP-triggered calcium influx. (Blue timeline; middle) In 1994, researchers identified the first two NLR-encoding genes, the arabidopsis *RPS2* and the tobacco *N* gene. In 1996, EDS1, an EP protein required for NLR-mediated resistance, was identified. In 1998, another EP protein PAD4 was identified. In 2005, SAG101 was found to interact with both EDS1 and PAD4 to mediate resistance and HR mediated by NLRs. Within the same year, the RNL NRG1 was reported to be required for resistance mediated by the *N* gene. In 2011, the RNLs ADR1, ADR1-L1 and ADR1-L2 were shown to be required for resistance mediated by RPS2. In 2017, the NRCs in the Solanaceae were reported to support the function of multiple sensor NLRs. In 2019, TIR domains in TNLs were shown to exhibit NADase activity which leads to the

production of v-cADPR. Within the same year, the structure of ZAR1 resistosome was solved. In 2020, the structures of the TNLs RPP1 and ROQ1 were also solved. Recently, it was shown that PTI and ETI mutually potentiate each other to mediate robust resistance. (Yellow timeline; bottom) SA is a defense-related phytohormone that was shown to induce SAR in 1990. In 1994, the first SA receptor encoding gene, *NPR1*, was identified. Multiple enzyme-encoding genes involved in SA biosynthesis were identified afterwards. In 1997, EDS5 was isolated. ICS1 was identified from two independent genetic screenings in 1998 and 1999. PBS3 and EPS1 were isolated in 1999 and 2009, respectively. In 2009 and 2010, the transcription factors SARD1 and CBP60g were reported to regulate SA biosynthesis by activating the expression of ICS1, EDS5 and PBS3. In 2012, another two SA receptors NPR3 and NPR4, were reported to act as negative regulators in SA-signaling. In 2018, it was shown that both NPR1 and NPR3/4 can bind to SA and function in parallel to regulated SA-mediated immunity. This is further supported by the recently resolved NPR4 structure.

Figure 2. PRR-, NLR- and SA-perception network. (Red shade; left) PRR network. LORE perceives the bacterial medium-chain 3-hydroxy fatty acid (C10:0). LYKs (LYK2/4/5) perceives the N-acetylglucosamine-containing glycan chitin. LYMs (LYM1/3) perceives bacterial peptidoglycan. Both LYKs and LYMs signal through the co-receptor CERK1. FLS2 perceives recognizes the 22-amino-acid peptide, flg22 from bacterial flagellin. EFR perceives the bacterial elongation factor Tu (elf18) and PEPR1 perceives the proteinaceous plant elicitor peptides (AtPep). FLS2, EFR and PEPR function with the co-receptor BAK1 to mediate downstream immune responses. RLP30 perceives the proteinaceous elicitor SCLEROTINIA CULTURE FILTRATE ELICITOR1 (SCFE1) from the necrotrophic fungal pathogen *Sclerotinia sclerotiorum* [192]. RLP23 perceives the NECROSIS AND ETHYLENE-INDUCING PEPTIDE1-LIKE PROTEIN 20 (NLP20). RLP30 and RLP23 function through BAK1 and SOBIR1 to mediate immunity. Recently, it has been suggested that ADR1, EDS1 and PAD4 might also be required for RLP-mediated immunity. (Blue shade; middle) NLR network. The TNL pairs, RRS1/RPS4 and RRS1B/RPS4B recognize AvrRps4 from *Pseudomonas syringae*, PopP2 from *Ralstonia solanacearum* and an unknown effector from *Colletotrichum higginsianum*. the TNL RPP1 recognizes the *Hyaloperonospora arabidopsidis* effector ATR1. The NLR paralogs WRR4A and WRR4B (TNLs) can recognize multiple *Albugo candida* CX2CX5G (CCG) effectors. TNLs signal through ADR1 (ADR1, ADR1-L1 and ADR1-L2), NRG1A/B, EDS1, PAD4 and SAG101 to mediate HR and resistance. The CNL RPS5 recognizes AvrPphB from *Pseudomonas syringae* and RPS2 recognizes both AvrRpt2 and AvrRpm1 from *Pseudomonas syringae*. RPS2 and RPS5 require ADR1 and NRG1A/B to mediate full resistance. The CNL RPM1 recognizes AvrRpm1 from *Pseudomonas syringae*. The CNL ZAR1 recognizes multiple effectors, including AvrAC from the *Xanthomonas*

campestris and HopZ1a from *Pseudomonas syringae*. RPM1 and ZAR1 does not require helper NLRs or EP proteins to mediate immunity. (Yellow shade; right) SA perception network. SA is perceived by NPR1/2/3/4 and BOP1/2 (NPR5/6). Perception of SA by NPR1 leads to SA-induced transcriptional reprogramming. NPR2 also positively regulates SA-mediated immunity. Binding of SA inhibits the transcriptional repression activities of NPR3/4. In addition, degradation of NPR1 by NPR3/4 and CUL3 is inhibited by high SA concentration. BOP1/BOP2 might function together with NPR3/4 as negative regulators in SA-signaling. It is unclear whether other NPRs interact with each other to modulate SA-mediated immunity.

Figure 3. Crosstalk between PRRs, NLRs and SA. (a) Potentiation of PRRs by PRRs. Activation of BAK1 by different PAMPs leads to juxta-membrane (JM) phosphorylation of CERK1. Priming of CERK1 enhances resistance against fungal pathogens. (b) Inhibition of PRRs by other PRRs. BIR proteins sequester BAK1 from FLS2 and inhibits flg22-induced immunity. Perception of the endogenous peptide RALF23 by FER negatively regulates the formation of the FLS2-BAK1 complex. (c) Potentiation of PRRs by NLRs. Activation of NLRs leads to upregulation of PRR-signaling components, which primes PRR-mediated immunity. (d) Potentiation of NLRs by PRRs. Activation of PRRs potentiate NLR-induced HR through an unknow mechanism. (e) Coactivation of multiple NLRs might have synergistic effect on resistance against pathogens. (f) Inhibition of NLRs by other NLRs. Negative regulation of NRG1A/B-induced HR by NRG1C. (g) Priming of PRRs by SA. Perception of SA by NPR proteins (NPR1/3/4) leads to upregulation of PRR-signaling components, which primes PRR-mediated immune responses. (h) Priming of NLRs by SA. Perception of SA also induces leads to upregulation of NLR-signaling components, which primes NLR-mediated immunity. (i) Inhibition of NLRs by SA. High SA concentration facilitates the formation of cytosolic NPR1 condensates, which leads to sequestering and degradation of NLRs, EP proteins and WRKY transcription factors to promote cell survival.

Figure 4. Local and systemic interactions between PRRs, NLRs and SA. (a) Cell-autonomous interactions between PRRs and NLRs. Physical interactions between PRRs and NLRs are likely to occur within the same cell. (b) Activation of PRRs and NLRs leads to upregulation of defense-related transcripts. Some of these transcripts, such as *FLS2*, *RbohD*, *MPK3*, *PAD4* and *WRR4A*, are cell-to-cell mobile. Thus, activation of PRR or NLR might prime immune responses in adjacent cells. (c) Activation of PRRs and NLRs leads to SARD1/CBP60g-dependent upregulation of ICS1 and EDS5, which leads to the biosynthesis of SA. Perception of SA by NPR1 and NPR3/4 leads to biosynthesis of NHP, a mobile signal which induces SAR and primes PRR-/NLR-immunity in distal tissues.

Graphical summary of the review. Each panel represents a section (or figure) of the review and is linked to the previous one.

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