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Regulation of Cell Death and Signaling by Pore-Forming Resistosomes

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Abstract

Nucleotide-binding leucine-rich repeat receptors (NLRs) are the largest class of immune receptors in plants. They play a key role in the plant surveillance system by monitoring pathogen effectors that are delivered into the plant cell. Recent structural biology and biochemical analyses have uncovered how NLRs are activated to form oligomeric resistosomes upon the recognition of pathogen effectors. In the resistosome, the signaling domain of the NLR is brought to the center of a ringed structure to initiate immune signaling and regulated cell death (RCD). The N terminus of the coiled-coil (CC) domain of the NLR protein HOPZ-ACTIVATED RESISTANCE 1 likely forms a pore in the plasma membrane to trigger RCD in a way analogous to animal pore-forming proteins that trigger necroptosis or pyroptosis. NLRs that carry TOLL-INTERLEUKIN1-RECEPTOR as a signaling domain may also employ pore-forming resistosomes for RCD execution. In addition, increasing evidence supports intimate connections between NLRs and surface receptors in immune signaling. These new findings are rapidly advancing our understanding of the plant immune system.

INTRODUCTION

Disease resistance genes protect plants from assault by a variety of pathogenic microbes and are of great value in the breeding of crop plants. The isolation and characterization of various resistance genes in the 1990s led to the realization that plants possess an innate immune system similar to that of animals (4). The plant immunity field was further advanced by studies on elicitors, also referred to as pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) (10), cell surface immune receptors, and pathogen effector proteins (36, 98). These advances establish a framework of a two-tiered plant immune system that monitors threats from pathogens and pests (35, 70). Thus, cell surface immune receptors, consisting of transmembrane receptor kinases (RKs) or receptor-like proteins (RLPs), form the first tier by detecting PAMPs or host-derived damage-associated patterns to activate defenses, and this is called pattern-triggered immunity (PTI). Adapted pathogens have evolved various effector proteins that are targeted to the host cell to promote pathogenesis. Plants have evolved intracellular immune receptors, nucleotide-binding (NB) leucine-rich repeat (LRR) receptors (NLRs), to intercept the activities of effector proteins that are delivered inside the host cell and activate defenses, forming the second-tier immunity referred to as effector-triggered immunity (ETI). ETI is often associated with hypersensitive response (HR), a form of regulated cell death (RCD) at the site of infection (**Figure 1**). Increasing evidence supports the idea that ETI is an important part of the animal innate immune

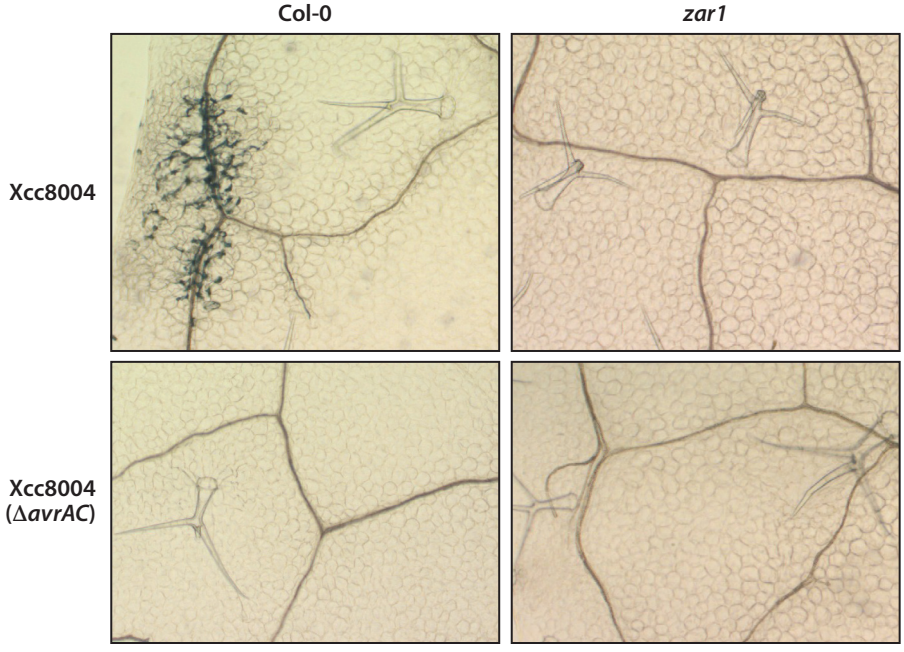

Figure 1

Microscopic cell death triggered by the activation of the NLR protein ZAR1. The main leaf veins of *Arabidopsis* were inoculated by piercing with suspensions of *Xanthomonas campestris* pv. *campestris* strains Xcc8004 and Xcc8004 (Δ*avrAC*). Two days later, the leaves were stained with trypan blue and photographed under a microscope. Positive trypan blue staining indicative of cell death was observed in Col-0, which carries ZAR1 that recognizes the effector protein AvrAC delivered by Xcc8004 bacteria. Deleting *avrAC* (Δ*avrAC*) from the bacterium or knocking out *ZAR1* from plants (*zar1*) abolished the cell death induction.

system (43) as well, indicating a similar logic in the evolution of surveillance systems in plants and animals.

The mechanism by which surface receptors activate plant immune responses is relatively well understood and mostly involves activation of receptor-associated kinases, which further activate downstream signaling components, including calcium channels, NADPH oxidases, mitogen-activated protein kinases, heterotrimeric G proteins, and transcription factors (90, 95, 150, 187). Our understanding of ETI in plants has until recently been largely limited to how NLRs perceive pathogen effector proteins (26, 71).

In the past two years, the field of plant NLR studies has advanced tremendously, spearheaded by structural biology. The structures of three full-length NLRs in their active forms have been solved (102, 110, 158, 159). Among these, studies on the NLR protein ZAR1 provide a unique paradigm for our understanding of the plant immune system. We now know that ligand-triggered formation of NLR resistosomes, in a way similar to inflammasome formation in animals, is critical for the initiation of plant immunity. Furthermore, recent molecular studies indicated that the link between PTI and ETI is much more intimate than previously known (115, 123, 176). This review discusses new insights brought about by relevant studies and future challenges on effector recognition, regulation of NLR activation, cell death control, and immune signaling.

REPRESENTATIVE EXAMPLES OF PLANT NLRs

NLR-coding genes first appeared in charophytes and exist in all land plants, with each species possessing dozens, hundreds, or even more than 1,000 genes in the pangenome (49, 71, 143, 153). In the past two decades, many plant NLR genes and their cognate effectors from diverse pathogens have been characterized (79). Although they evolved independently, plant and animal NLRs share a tripartite domain structure, including an N-terminal domain, a central nucleotide-binding and oligomerization domain (NOD), and a C-terminal LRR domain. The NOD is believed to function as a molecular switch, with an adenosine 5′-diphosphate (ADP)-bound inactive state and an adenosine 5′-triphosphate (ATP)-bound active state. Based on their N-terminal domains, Toll/interleukin-1 receptor (TIR), CC, and RPW8-like CC (CC_R), NLRs can be divided into three subclasses: TNLs (TIR-NB-LRRs), CNLs (CC-NB-LRRs), and RNLs (RPW8-NB-LRRs) (71). The CC, CC_R, and TIR domains are predicted to initiate downstream signaling, as expression of these domains alone often triggers immune responses and HR (18, 24, 38, 45, 106).

Plant NLRs can be classified based on their roles in sensing effectors or the execution of immune signaling. NLRs that play a dual role in effector sensing and immune signaling are called singleton NLRs. Those specialized in effector sensing are referred to as sensor NLRs. Some NLRs that execute immune signaling are genetically linked to specific sensor NLRs and are referred to as executor NLRs, whereas those unlinked to sensor NLRs are referred to as helper NLRs. Sensor NLRs and singleton NLRs recognize pathogen effectors either directly through a physical interaction with cognate effectors or indirectly through additional host proteins that are associated with NLRs. Readers are referred to several excellent reviews for relevant concepts (26, 72, 79). Representative NLRs are discussed in the following sections.

NLRs That Directly Recognize Effectors

PERONOSPORA PARASITICA 1 (RPP1) is a TNL that specifically recognizes the *Hyaloperonospora parasitica* effector ATR1 (12, 142). The RPP1 C-terminal LRR domain directly binds ATR1 to trigger immunity (52, 142). Similarly, the TNL protein RECOGNITION OF XOPQ

1 (Roq1) directly associates with *Xanthomonas* XopQ and *Pseudomonas* HopQ1, which are homologous effector proteins (134, 135, 160).

NLRs That Indirectly Recognize Effectors

Most of the reported NLRs that indirectly recognize effectors interact with a single sensor protein to perceive biochemical activities of pathogen effectors. For instance, the *Arabidopsis* CNL proteins RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1 (RPM1) and RESISTANCE TO PSEUDOMONAS SYRINGAE 2 (RPS2) interact with the sensor protein RPM1-INTERACTING PROTEIN 4 (RIN4) (3, 104, 105). The RPS2-RIN4 complex recognizes the *P. syringae* effector AvrRpt2, whereas the RPM1-RIN4 complex recognizes the *P. syringae* effectors AvrRpm1 and AvrB.

In contrast, the *Arabidopsis* ZAR1 protein, first identified as a CNL conferring resistance to *P. syringae* carrying the effector protein HopZ1a (88), recognizes the *Xanthomonas campestris* pv. *campestris* effector protein AvrAC through an adaptor protein RESISTANCE-RELATED KINASE 1/ZED1-RELATED KINASE 1 (RKS1/ZRK1) and a sensory protein PBS1-LIKE 2 (PBL2) (156). RKS1 is a pseudokinase belonging to the RLCK XII clade, whereas PBL2 belongs to the RLCK VII clade. The *Arabidopsis* ZAR1 additionally confers resistance to *P. syringae* carrying effector proteins HopF1, HopX1, HopO1, and HopBA1 (83, 136). The *Nicotiana benthamiana* ZAR1 confers resistance to *Xanthomonas perforans* carrying the effector protein XopJ4 (133). A pangenome analysis showed that ETI-eliciting alleles of *P. syringae* effector are collectively distributed in numerous *P. syringae* strains, and the *Arabidopsis* ZAR1 in a single ecotype Col-0 is predicted to recognize 40% of the 494 tested *P. syringae* strains (83). ZAR1 is conserved among 1,135 sequenced *Arabidopsis* ecotypes, indicating that *ZAR1* is a broad-spectrum, durable disease resistance gene.

Paired NLRs

In several cases, genetically linked sensor and executor NLRs have been shown to directly interact for pathogen perception and disease resistance (94). Some of these sensor NLRs carry an integrated domain (ID) for effector recognition. The TNL RESISTANCE TO RALSTONIA SOLANACEARUM 1 (RRS1), which carries a DNA-binding domain of WRKY transcription factors as an ID, is a sensor NLR that recognizes two unrelated effector proteins, *P. syringae* AvrRps4 and *R. solanacearum* PopP2. RRS1 constitutively interacts with the executor TNL RESISTANCE TO PSEUDOMONAS SYRINGAE 4 (RPS4) to activate disease resistance upon effector recognition (30, 51). Both AvrRps4 and PopP2 target the WRKY domain of RRS1, leading to RPS4-dependent immune responses (86, 132). Likewise, the rice sensor CNL RGA5, which carries a heavy-metal associated domain (HMA, also called RATX1), recognizes two unrelated effectors, AVR-Pia and AVR1-CO39, from *Magnaporthe oryzae* and heterodimerizes with executor CNL RGA4 to confer disease resistance (19, 116). Another rice sensor, CNL Pik-1, also contains an integrated HMA to recognize the effector AVR-Pik from *M. oryzae* and triggers immunity through the executor CNL Pik-2 (2, 175).

Helper NLRs

The SOLANACEAE-SPECIFIC NLR REQUIRED FOR CELL DEATH (NRC) is a conserved clade of CNLs required for disease resistance and HR mediated by some sensor CNLs (163). As such, NRC4 is required for the function of sensor CNLs Rbi-blb2, Mi-1.2, R1, and CNL-11990, which recognize effectors from oomycetes and nematodes, whereas NRC2 and NRC3 are

required for the function of sensor CNL Prf. In addition, NRC2, NRC3, and NRC4 are redundantly required for the function of sensor CNLs Sw5b, R8, Rx, and Bs2, which recognize effectors from viruses, oomycetes, and bacteria. Interestingly, the sensor CNLs requiring NRCs to function belong to sister clades of the NRC clade, and several tested CNLs that do not belong to sister clades do not appear to require NRCs for function. The NRC clade and sister clades emerged ~100 million years ago and exist in caryophyllales and asterids but not rosids. It is proposed that NRCs and their sister CNL clades evolved from an ancestor singleton CNL after gene duplication and subsequent specialization (165).

RNLs form an evolutionarily conserved clade and include members of the ACTIVATED DISEASE RESISTANCE 1 (ADR1) subclade and the N REQUIRED GENE 1 (NRG1) subclade (24, 53, 119). These RNLs are differentially required for HR cell death and immune signaling by acting downstream of all tested TNLs and some CNLs (11, 24, 47, 85, 126, 131).

MECHANISMS OF EFFECTOR RECOGNITION

Knowledge of how NLRs sense pathogen effectors is important not only for our understanding of host–pathogen coevolution but also for the efficacy of our effort to identify or engineer new resistance genes. Both RPP1 in *Arabidopsis* natural population and ATR1 in *H. parasitica* populations are highly polymorphic, and different RPP1 alleles confer resistance to *H. parasitica* strains carrying different ATR1 variants, indicating a host–pathogen arms race (12, 80, 128, 142). Similar observations have been made in other NLRs that directly recognize effectors (16, 34, 101). The structures revealed by cryo-EM (electron microscopy) of the RPP1-ATR1 and Roq1-XopQ complexes show precisely how these NLRs interact with effectors (102, 110). A distinct domain, called the C-terminal jelly roll and Ig-like domain (C-JID) or Post-LRR (PL) domain, at the C termini of both RPP1 and Roq1 directly interacts with their cognate effectors. In addition to C-JID/PL, the inner surface of LRR also contributes to the interaction with the two effectors. The C-JID/PL is not predicted by the primary sequence but can be predicted by a hidden Markov model. Importantly, structure-guided mutagenesis experiments indicate that residues determining the recognition specificity among different RPP1 alleles are located in LRR and C-JID/PL (102). Interestingly, C-JID/PL exists in a large number of TNLs, including RPS4, suggesting that the variable C-JID/PL domain sequence is uniquely important for TNLs.

IDs in NLRs are important determinants of recognition specificity. The RRS1-R allele from certain *Arabidopsis* accessions recognizes two bacterial effectors, PopP2 and AvrRps4, whereas the RRS1-S allele recognizes AvrRps4 only (30). Although both RRS1-S and RRS1-R contain a WRKY domain essential for effector recognition, RRS1-R additionally contains a C-terminal extension required for the recognition of PopP2, although the precise mechanism remains unknown (103, 141). The recognition of Avr-Pik alleles in the blast fungus by Pik alleles in rice provides another model for the understanding of specificity determinants (25, 75, 175). Crystal structure reveals that Pikp-HMA and Avr-PikD form an intimate interface. Interestingly, all the polymorphic residues in Avr-Pik alleles are localized in the binding interface, indicating that *M. oryzae* has evolved to evade recognition (108). Conversely, Pikp-HMA residues that may mediate recognition are also variable among different alleles, suggesting coevolution between *M. oryzae* and rice.

Diverse recognition specificity of ZAR1 is determined by different adaptor proteins of the ZRK clade and sensor proteins of the RLCK VII clade (89, 109, 133, 156). HOPZ-ETI-DEFICIENT 1 (ZED1), the founding member of ZRKs, constitutively interacts with ZAR1 and is indispensable for HopZ1a recognition (89). Subsequent work demonstrated that multiple ZRKs interact with ZAR1 to monitor different effectors (76, 109, 133, 156). Thus, RKS1, ZRK2, and ZRK3 associate with the *Arabidopsis* ZAR1 to sense AvrAC, HopBA1, and HopF1, respectively (109, 136, 156). In

addition to HopZ1a, ZED1 determines recognition of HopX1, whereas ZRK3 confers recognition of both HopF1 and HopO1 (109). The *N. benthamiana* ZRK protein XOPJ4 IMMUNITY 2 (JIM2) associates with *Nb*ZAR1 to recognize XopJ4 (133). Thus, the association with different ZRKs greatly expands recognition specificity of a single NLR protein ZAR1, which is of interest to engineering broad-spectrum and durable disease resistance in crop plants.

Recognition of different effectors by ZAR1 additionally requires sensor proteins of the RLCK VII clade. The sensor protein detects the enzymatic activity of AvrAC to trigger ZAR1 resistance (156). AvrAC is an uridylylate transferase that modifies several RLCK VII members including BOTRYTIS-INDUCED KINASE 1 (BIK1) and PBL2 (42, 156). $PBL2^{UMP}$ acts as a ligand to interact with the ZAR1-RKS1 complex, leading to immune activation (156). PBL2 apparently acts as a decoy, because the virulence function of AvrAC depends on uridylylation of BIK1 but not PBL2. BIK1 plays a central role in cell surface immune receptor–mediated signaling (95, 100, 179), and the uridylylation by AvrAC inhibits BIK1 kinase activity and blocks cell surface receptor-mediated immune signaling (42). Similar to the PBL2-dependent recognition of AvrAC, the recognition of HopX1 by ZAR1 also requires an RLCK VII member, SUPPRESSOR OF ZED1-D1 (SZE1), in addition to the adaptor protein ZED1 (96, 109). In yeast, the presence of HopX1 induces the interaction between ZED1 and SZE1, although the precise post-translational modification that triggers the ZED1-SZE1 interaction is not known (109). Likewise, HopZ1a has been shown to acetylate multiple RLCK VII members and promote the interaction with ZED1 to trigger immune responses (5, 109). Loss of closely related SZE1 and SZE2 impairs HopZ1a-induced disease resistance (5, 96). Thus, the combination of different ZRK adaptors with RLCK VII sensors further expands recognition capacity of ZAR1.

The recent cryo-EM derived structure of ZAR1-RKS1-$PBL2^{UMP}$ tertiary complex uncovered mechanisms underlying the ZRK-mediated recognition of diverse effectors (159). RKS1 interacts with ZAR1 and $PBL2^{UMP}$ through different surfaces. Remarkably, RKS1 residues that interact with $ZAR1^{LRR}$ are highly conserved among ZRKs, whereas RKS1 residues that interact with the $PBL2^{UMP}$ ligand are diverged among ZRKs. These findings illustrate how ZRKs act as adaptor proteins to recognize distinct effector targets (PBLs) while at the same time interacting with the same NLR protein, ZAR1. The detailed structural understanding of ZRK- and PBL-mediated pathogen recognition thus informs future genetic engineering of disease resistance in crop plants.

AUTOINHIBITION AND ACTIVATION OF AN NLR

In animals, activated NOD-containing proteins often form oligomeric complexes, such as inflammasomes composed of CARD DOMAIN–CONTAINING PROTEIN 4 (NLRC4) and NLR FAMILY APOPTOSIS INHIBITORY PROTEINs (NAIPs), to initiate downstream signaling (67, 180). Plant NLRs also form oligomeric complexes upon recognition of effectors. The active ZAR1 complex reconstituted in vitro is a pentamer in which ZAR1, RKS1, and $PBL2^{UMP}$ exist in a 1:1:1 ratio (158). The RPP1-ATR1 complex expressed in insect cells and the Roq1-XopQ complex purified from *N. benthamiana* plants are tetramers with RPP1 and Roq1 bound to their cognate effector proteins in a 1:1 ratio (102, 110). These remarkable studies show that both CNLs and TNLs form oligomeric ringed complexes called resistosomes in a way similar to animal NLR inflammasomes (**Figure 2**). Both AvrAC and HopZ1a induce ZAR1 oligomerization in *Arabidopsis* protoplasts, indicating that the ZAR1 resistosome indeed forms in the plant cell upon effector recognition (65). Structure-guided mutagenesis demonstrated that the formation of resistosomes is essential for immune activation by these NLRs (102, 110, 158). In addition, blue native gel assays showed that the *Arabidopsis* CNL RPP7 interacts with an immune-activating allele of RPW8 to form an oligomeric complex, which is responsible for autoimmune phenotypes in hybrid plants

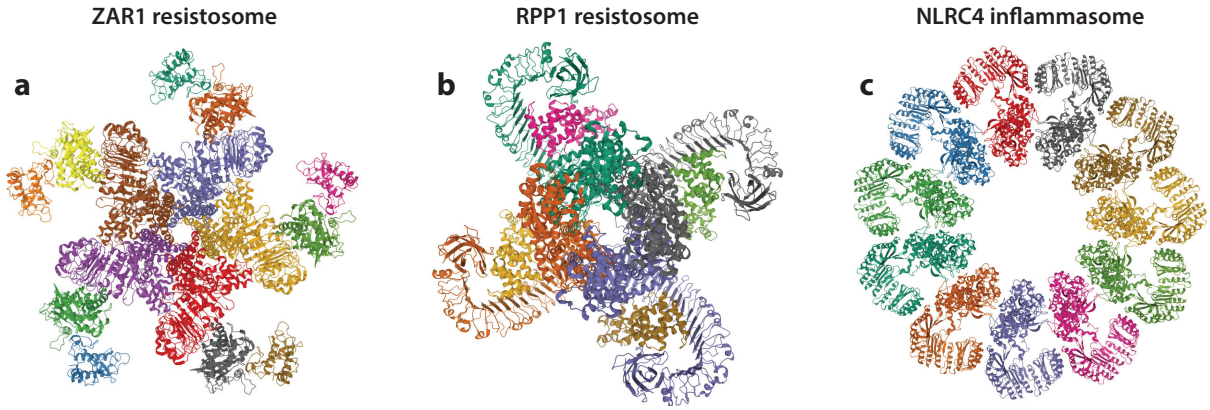

Figure 2

Structures of ZAR1 and RPP1 resistosomes and NLRC4 inflammasome. Shown are top views of the structures of the (*a*) ZAR1 resistosome (PDB ID: 6J5T), (*b*) RPP1 resistosome (PDB ID: 7CRC), and (*c*) NLRC4 inflammasome (PDB ID: 3JBL).

carrying incompatible RPP7 and RPW8 alleles (91). Furthermore, cognate effectors can induce self-association of several NLRs, including N and Tm-2^2, although it is unknown whether these reflect oligomerization of NLRs (111, 157). In a recent study, coexpression of the TIR^{RPS4}-NAIP5 chimeric protein and NLRC4, which enables flagellin-triggered oligomerization, results in TIR^{RPS4}-dependent cell death in plants, suggesting that oligomerization is involved in RPS4 activation (39). Thus, increasing evidence indicates that effector-triggered oligomerization of plant NLRs is critical for immune activation. However, NLRs must be tightly regulated to avoid autoimmunity in the absence of cognate pathogen effectors as inappropriate activation results in plant growth defects and even lethality (154). A detailed understanding of the control of NLR autoinhibition as well as effector-triggered activation is important for future engineering of new NLRs.

Autoinhibition of NLRs in a Resting State

Autoinhibition is primarily mediated by intramolecular interactions between various domains of NLR proteins. However, as many NLRs are organized in protein complexes with sensor proteins or paired NLRs, intermolecular interactions can also contribute to autoinhibition.

Conformation of NOD of plant NLRs is crucial for autoinhibition in the resting state and activation upon effector recognition. Early studies showed that mutations in specific residues in NOD can lead to constitutive activation of NLRs (93, 140). The canonical NOD module of plant NLRs belongs to the AAA+ superfamily and can be subdivided into an NB domain (NBD), a helical domain (HD1), and a winged-helix domain (WHD). The structure of an inactive ZAR1-RKS1 complex showed that intramolecular interactions among various domains within ZAR1 play a key role in maintaining ZAR1 in an inactive state (159). Among these, the extensive interaction between LRR and WHD is particularly important, which explains autoimmunity conferred by mutations in these domains and autoactivation of chimeric NLRs that carry an inappropriate combination of WHD and LRR domains (7, 63, 68, 124, 127, 146, 183). The inhibitory role of LRR is similar to that of NLRC4 (66, 78). In addition to the LRR-WHD interaction, the ZAR1 CC domain forms a four-helix bundle and interacts with HD1 and WHD, which is consistent with a previous observation that RPM1 CC interacts with multiple domains of RPM1 (40, 159). The interaction may be needed to keep the CC domain in an inactive state.

The intramolecular interactions that autoinhibit ZAR1 are further stabilized by ADP binding, which is consistent with previous observations that full-length plant NLR proteins, such as M, L6, L7, and MLA27, are preferentially bound with ADP in vitro (106, 162). ADP interacts with NBD, HD1, and WHD to stabilize ZAR1 in the inactive state, and some of the previously observed mutations that autoactivate NLRs are expected to perturb the ADP-WHD interaction (7). In the inactive state, the animal NLRs NLRC4, NLR FAMILY PYRIN DOMAIN CONTAINING 3 (NLRP3), and NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN CONTAINING PROTEIN 2 (NOD2) also bind ADP in a similar manner, indicating a conserved mechanism for NLR autoinhibition in plants and animals (171).

For paired NLRs, the interaction between sensor NLR and executor NLR is important for autoinhibition. For immunity governed by the RRS1-RPS4 complex, the TIR domain of RPS4 possesses signaling activity, whereas that of RRS1 does not (161). Intermolecular interaction between TIR^{RRS1} and TIR^{RPS4} inhibits RPS4 activation. A recent study shows that the phosphorylation of a specific site in the C-terminal extension of RRS1 is required for autoinhibition, presumably by promoting inhibitory interactions (55). Because RPS4 is also expected to form an oligomeric resistosome after activation, additional intramolecular interactions within RRS1 and/or RPS4 are likely needed to prevent oligomerization.

Similar to the RRS1-RPS4 model, the sensor CNL RGA5 inhibits cell death triggered by executor CNL RGA4, although the mechanism remains unclear (17). The rice CNL PigmR confers broad-spectrum resistance to *M. oryzae* and triggers cell death when expressed in *N. benthamiana* leaves (29). A genetically linked CNL PigmS interacts with PigmR and inhibits PigmR-mediated resistance and cell death. Interestingly, expression of PigmS counteracts PigmR-induced yield cost, providing a mechanism for balancing resistance with yield.

NLRs that indirectly recognize effectors recruit additional host proteins into a preformed complex in the resting state (26). For instance, constitutive interactions of RIN4 with RPM1 and RPS2 are required for the autoinhibition of the two CNLs (3, 77, 104). Transient expression of RPS2 in *N. benthamiana* leaves triggers cell death that is blocked when coexpressed with RIN4 (28). RIN4 also inhibits cell death triggered by an autoactive allele of RPM1 in *N. benthamiana* leaves (50). The AvrB-triggered RPM1 activation is mediated by induced phosphorylation on RIN4 Thr166 (22, 97). The *Arabidopsis* rotamase CYP1 (ROC1) protein, a prolyl-peptidyl isomerase, regulates the configuration of RIN4 at Pro149, and this inhibits the RIN4 Thr166 phosphorylation and contributes to autoinhibition of RPM1 and RPS2 (92).

Nucleotide Exchange in NLR Activation

NLRs bind ADP or ATP through the P-loop, a highly conserved motif in the NBD. Mutations of the P-loop frequently abolish NLR function (99, 148). As discussed earlier, exchange of ADP with ATP is thought to switch the NOD-containing proteins to an active state. The mechanism of this transition is best illustrated by structural comparisons of inactive, intermediate, and active ZAR1 complexes (158, 159). In the ZAR1-RKS1-$PBL2^{UMP}$ tertiary complex, called an intermediate complex, $PBL2^{UMP}$ stabilizes an activation segment of RKS1 to promote the release of ADP from NBD, indicating a role of effector-triggered nucleotide exchange for NLR activation (159). This observation also supports the equilibrium model in which effectors shift the balance toward an ATP-bound state (9, 181).

The structure of ZAR1 resistosome, which was reconstituted in vitro in the presence of dATP, shows that dATP directly binds to the interface of NBD and HD1, and this triggers major conformational changes in multiple ZAR1 domains and exposes surfaces required for ZAR1 oligomerization (158). These likely reflect ZAR1 activation upon ATP binding in the plant cell, as a double mutation of dATP binding sites abolishes AvrAC-induced ZAR1 oligomerization in

vitro, cell death in protoplasts, and disease resistance to *X. campestris* carrying AvrAC. In addition, these two ZAR1 residues are also essential for HopZ1a-induced cell death and disease resistance (65). The Roq1 resistosome, which was isolated from *N. benthamiana* plants, contains an ATP molecule that is bound to the NBD and HD1 interface in a similar fashion, suggesting a similar mechanism in activation (110).

ATP-Independent Activation of NLRs

Although the ADP-ATP exchange appears to be a common mechanism for NLR oligomerization, the RPP1-ATR1 resistosome contains an ADP molecule instead of ATP (102). The conformational switch that leads to RPP1 oligomerization is probably caused by an interaction between RPP1 and ATR1 instead of ATP binding. This possibility is consistent with the observation that the P-loop of NAIP5 is dispensable for the flagellin-induced formation of the NAIP5-NLRC4 inflammasome and caspase-dependent cell death (56). Flagellin binds multiple NAIP5 domains, which likely induces an active NAIP5 conformation (151, 174). ATP-independent activation is also known in several other plant NLRs. P-loop mutants of the sensor NLRs RRS1 and RGA5 are fully functional, although P-loop mutations abolish the function of their executor NLRs, RPS4 and RGA4 (17, 161). A recent study showed that the PopP2-triggered immune activation is mediated by enhanced proximity between the TIR and C-terminal domains of RRS1-R, which relieves the inhibition of TIR^{RPS4} by TIR^{RRS1} (55). Furthermore, the P-loop is absent in rice CNL PANICLE BLAST 1 (Pb1), which confers broad-spectrum resistance to the blast fungus (59).

Role of Oligomerization

The three reported plant resistosomes are similar to the animal NLRC4 inflammasome in that they all adopt a ringed structure with the N-terminal signaling domain positioned in the center, indicating a shared logic for the triggering of RCD and signaling (**Figure 2**). NLRC4 contains a caspase recruitment domain (CARD) at the N terminus required for RCD and downstream signaling. In the NLRC4 inflammasome, the CARD domain is positioned in the center to recruit caspase-1 directly or indirectly by an apoptosis-associated speck-like protein, ASC (44, 67, 180).

In the ZAR1 resistosome, the N-terminal α1 helices of five ZAR1 protomers form a funnel-shaped structure required for immune signaling (158). This mechanism appears to be shared by many, but not all, CNLs. A recent study uncovered an N-terminal segment termed the MADA motif that is conserved in 20% of CNLs with canonical CC domains, including NRC4 and ZAR1 (1). This motif is required for cell death induction and functionally interchangeable among various CNLs carrying this motif. Interestingly, sensor CNLs that require NRC for function lack the MADA motif, indicating a functional differentiation of these sensor NLRs and NRCs. The function of the funnel-shaped structure is discussed in detail in the next section.

In the RPP1 and Roq1 resistosomes, the TIR domain is clustered in the center ring as a tetramer (102, 110). Recent studies show that the TIR domain of several plant TNLs is structurally and functionally similar to the animal TIR domain of the STERILE ALPHA AND TIR MOTIF CONTAINING 1 (SARM1) protein, which functions as an NAD^+-cleaving enzyme (NADase) (41, 61, 155). Dimerization of TIRs and a catalytic residue are required for the enzymatic activity and immune responses conferred by these TNLs. Importantly, the RPP1-ATR1 complex possesses strong NADase activity, whereas the RPP1 protein alone does not, indicating that oligomerization of RPP1 is required to transform the TIR domain into an active enzyme (102). The TIR domain in the RPP1 and Roq1 resistosomes adopts a conformation that opens the NADase active site, explaining the indispensable role of oligomerization in NADase activity (102, 110). This is in agreement with the observation that an NLRC4-mediated oligomerization

of chimeric RPS4^{TIR} protein can trigger cell death in plants (39). Thus, the oligomerization of these TNLs assembles TIR into an active enzyme for immune signaling. Readers are referred to two excellent reviews for explanation of the signaling mechanisms downstream of TIR NADase activity (6, 84).

PORE FORMING AND PROGRAMMED CELL DEATH

HR cell death is thought to limit the growth of biotrophic and semibiotrophic pathogens (82). In addition to local resistance, HR also confers systemic acquired resistance, which activates a stronger immune response to secondary infection (37). However, RCD in plants is ill-defined, and it is not clear how the cell death is executed.

RCD is much better defined in animals. Besides apoptosis, which was first discovered nearly four decades ago, RCD also includes necroptosis, pyroptosis, ferroptosis, and autophagy-dependent cell death, to name a few (149). Apoptosis, necroptosis, pyroptosis, and autophagy-dependent cell death are potentially linked to HR in plants, as discussed by Dickman & Fluhr (31). The pore-forming activity of the ZAR1 resistosome suggests a resemblance of plant HR cell death to necroptosis or pyroptosis, both of which involve pore-forming proteins and are intimately linked to immune responses (**Figure 3**). It is thus useful to discuss and compare HR with necroptosis and pyroptosis.

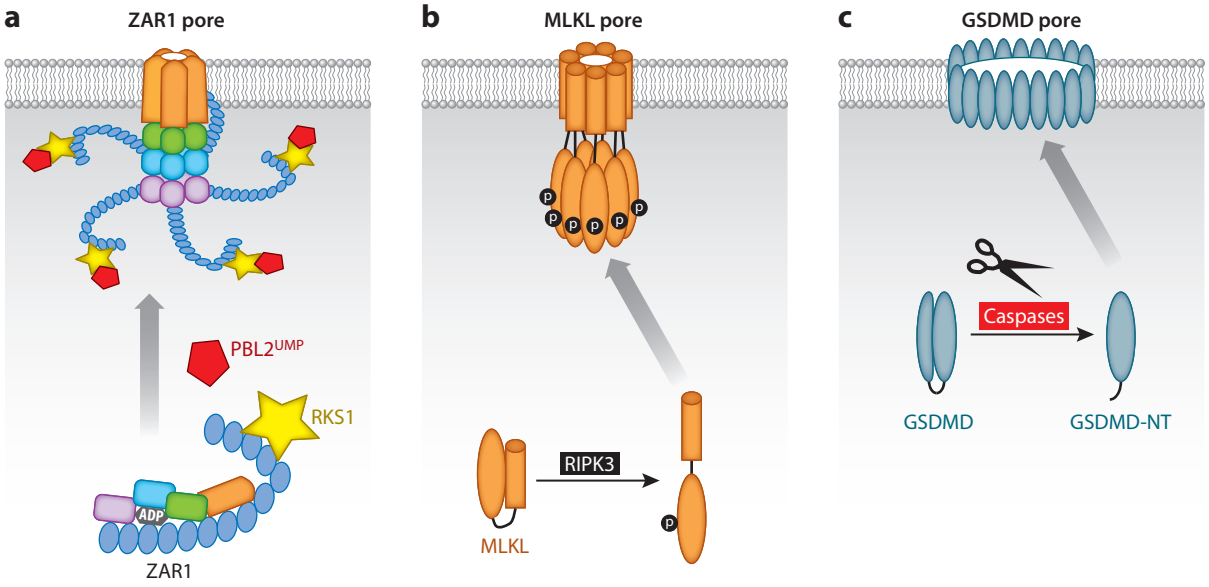

Figure 3

Pore forming by ZAR1 resistosome, MIXED LINEAGE KINASE LIKE (MLKL), and gasdermin D (GSDMD). (*a*) RKS1 directly binds to a leucine-rich repeat domain of ZAR1 in the resting state. Uridylylated PBL2 (PBL2^{UMP}) is recruited specifically to the ZAR1-RKS1 complex to induce oligomerization of ZAR1 and expose an N-terminal α1 helix for plasma membrane (PM) association. (*b*) In the resting state, the MLKL protein exists as a monomer in animal cells. Upon activation by upstream stimuli, RECEPTOR-INTERACTING SERINE/THREONINE KINASE 3 (RIPK3) phosphorylates the pseudokinase domain of MLKL, allowing MLKL to oligomerize through the four-helix bundle and form pores in the PM. (*c*) In the resting state, the C terminus of GSDMD binds with the N terminus (GSDMD-NT) to inhibit the pore-forming activity. Caspases activated by inflammasomes cleave the linker between two domains and release GSDMD-NT, which then oligomerizes in the PM to form pores. Abbreviation: p, phosphorylation.

MLKL and Necroptosis

In necroptosis and pyroptosis, the dying cell loses membrane integrity, lyses, and releases cellular contents and cytokines. This is in contrast to apoptosis, during which the dying cell forms apoptotic bodies enclosed with intact membranes that are then engulfed by phagocytic processes (149).

Necroptosis can be triggered by multiple stimuli, including those that activate transmembrane and intracellular immune receptors (149). Necroptosis is regulated by RECEPTOR-INTERACTING SERINE/THREONINE KINASE 3 (RIPK3) and various upstream proteins, depending on specific stimuli, and is executed by the MIXED LINEAGE KINASE LIKE (MLKL) protein. For example, stimulation of TUMOR NECROSIS FACTOR RECEPTOR activates RIPK1, which then interacts with RIPK3 to form a complex called a necrosome. RIPK3 then phosphorylates the MLKL protein to stimulate the oligomerization of the latter protein (60, 178). The oligomerized MLKL inserts into the plasma membrane (PM) to form a pore and trigger necroptosis (**Figure 4**). RIPK3-dependent necroptosis can also be activated by Toll-like receptors through TIR-DOMAIN-CONTAINING ADAPTER-INDUCING INTERFERON-β.

The mammalian MLKL protein contains an executor domain at the N terminus, a brace region containing two brace helices, and a C-terminal pseudokinase domain. The executioner domain forms a four-helix bundle as observed in the mouse MLKL structure, whereas an additional helix at the top of the four-helix bundle exists in human MLKL (114, 145). Although

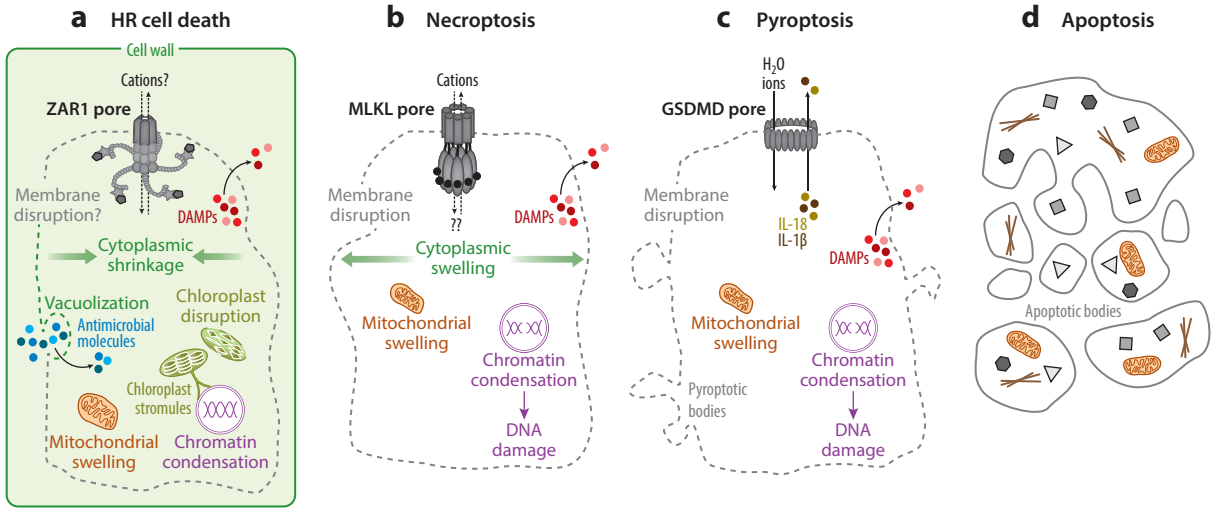

Figure 4

Comparison of hypersensitive response (HR) cell death with necroptosis, pyroptosis, and apoptosis. (*a*) The resistosome pore in the plasma membrane (PM) (or other cellular membrane) may act as an ion channel to regulate the execution of regulated cell death. Nucleotide-binding leucine-rich repeat receptor (NLR)-mediated HR cell death is associated with morphological changes, including cytoplasmic shrinkage, mitochondrial swelling, chromatin condensation, the release of damage-associated molecular patterns (DAMPs), perturbed membrane integrity, and the development of chloroplastic stromules. (*b*) In necroptosis, the MIXED LINEAGE KINASE LIKE (MLKL) pore formed in the PM may act as an ion channel to regulate unknown components that execute cell killing. The dying cell swells gradually and eventually explodes, which likely involves an alteration of turgor pressure. The dead cell displays membrane disruption, mitochondrial swelling, chromatin condensation, DNA damage, and the release of DAMPs. (*c*) The formation of a gasdermin D (GSDMD) pore initiates pyroptosis. The dying cell forms pyroptotic bodies and releases small proteins such as IL-18 and IL-1β before cell lysis. In addition, the cell shows mitochondrial swelling, chromatin condensation, DNA damage, and the release of DAMPs. (*d*) In apoptosis, the cell forms membrane bubbles called apoptotic bodies, which are enclosed by intact cell membrane. Intracellular contents are not released during apoptosis.

the oligomer of the four-helix bundle is thought to form a pore in the PM, biochemical studies have suggested that the MLKL pore is composed of a trimer, tetramer, hexamer, octamer, or higher-order polymer of the MLKL protein (121, 122, 170). Structure studies are needed to advance our understanding of the MLKL-dependent pore-forming mechanism.

In MLKL-mediated necroptosis, the cell swells gradually and consequently explodes like an overinflated balloon (20, 182) (**Figure 4**). Several competing models have been proposed for cell death triggered by the MLKL pore (184). The MLKL pore may directly cause PM lysis. Alternatively, MLKL may function as an ion channel or activate downstream ion channels to alter osmotic pressure, leading to PM rupture. It was reported that MLKL assembled in vitro possesses cation channel activity, which is preferentially permeable to Mg^{2+} (168). However, it is unknown whether the Mg^{2+} channel activity is required for necroptosis and how Mg^{2+} influx may trigger necroptosis.

A secondary structure–based search identified three *Arabidopsis* proteins with domains similar to the MLKL pore-forming domain (107). These are subdivided into subfamily I (*At*MLKL1 and *At*MLKL2) and subfamily II (*At*MLKL3). The structure of *At*MLKL3 showed that it indeed adopts a similar fold as the mouse and human counterparts. Both *At*MLKL2 and *At*MLKL3 form tetramers in vitro in which pore-forming domains are completely buried, suggesting an inactive oligomer. The *Atmlkl* triple mutant is impaired in disease resistance mediated by the TNLs RPP4 and RRS1-RPS4 but shows normal disease resistance mediated by CNLs RPS2 and RPM1. The RPS4- and RPM1-triggered HR, however, remains normal in triple mutants. Nonetheless, overexpression of the *At*MLKL pore-forming domain in protoplasts can induce cell death. It remains unknown whether any of the *At*MLKLs function as an executor in HR during ETI.

Gasdermins and Pyroptosis

Pyroptosis is triggered by inflammasomes and executed by a family of pore-forming proteins called gasdermins (GSDMs; **Figure 3**), which exist in vertebrates only (13). Among these, GSDMD plays a central role in RCD, which is mediated by canonical and noncanonical inflammasomes. *gsdmd* mutants are completely blocked in LPS-triggered pyroptosis in HeLa and iBMDM cells (139).

All GSDM members except DFNB59 contain a highly conserved pyroptosis-triggering domain at the N terminus (GSDM-NT), a repressor domain at the C terminus (GSDM-CT), and a variable linker connecting the two domains. The activation of the NLRP3 inflammasome by the bacterial toxin nigericin and of the NLRC4 inflammasome by bacterial flagellin recruit caspase 1, which cleaves GSDMD to release GSDMD-NT. The latter localizes to the PM by anchoring to phosphatidylinositol 4-biphosphate (PI4P) and phosphatidylinositol 4,5-biphosphate (PI4,5P) on the inner leaflet of the cell membrane to form a pore (32, 139) (**Figure 3**).

The high-resolution structure of the GSDMA3-NT membrane pore provides the pore-forming mechanism of GSDMs (130). The GSDMA3-NT pore contains 26–28 protomers with two rings, of which the top ring inserts into the membrane and the bottom ring is soluble. The size of the inner diameter is approximately 180 Å, which may permit the exchange of contents between the cytoplasm and extracellular spaces. The proinflammatory cytokine IL-1β with a molecular diameter of ~5 nm is secreted into the extracellular space in a GSDMD-dependent manner before membrane rupture (74, 169). Furthermore, expression of GSDMD-NT rapidly induces an influx of Ca^{2+} and Na^{+} and an efflux of K^{+} (20). Nonselective ion flux is not expected to increase osmotic pressure inside the cell, and ion flux mediated by GSDMD-NT does not appear to directly kill the cell. It should be noted, however, that efflux of K^{+} is known to secondarily activate the NLRP3 inflammasome that may contribute to cell death (112), and this is also relevant to ion flux triggered by the MLKL pore discussed above.

Compared to necroptosis, GSDMD-mediated pyroptosis displays modest cell swelling and forms membrane bubbles called pyroptotic bodies before cell lysis (20, 31, 182) (**Figure 4**). How GSDMs trigger cell rupture requires future investigation.

The ZAR1 Resistosome Pore and Hypersensitive Response

The funnel-shaped structure formed by the α1 helix of ZAR1 is amphipathic in nature, which is suggestive of membrane association (158). Protein fractionation experiments indeed supported the idea that the funnel structure is associated with the PM. Mutations that abolish ZAR1 oligomerization or alter the hydrophobic exterior of the α1 helix can all disrupt PM localization, cell death triggering, and immune function of ZAR1, supporting the idea that the ZAR1 resistosome forms a pore in the PM to trigger RCD and signaling (**Figure 3**). Although the ZAR1 protein fractionation experiments support a PM association, further experiments are needed to determine the dynamic of resistosome assembly in the plant PM and test whether an interaction with specific phospholipids in the PM is involved.

The ZAR1 resistosome pore predicted by the structure has an inner diameter of 10 Å, and the interior contains carboxylate rings formed by glutamate residues at positions 11 and 18. Simultaneous mutation of the two residues compromises ZAR1-triggered cell death and disease resistance, which indicates that the interior space of the pore plays a key role in ZAR1-triggered immunity (65, 158). Two additional glutamate residues at the base of the pore (positions 130 and 140) also appear to form carboxylate rings (14), but their functional importance is unknown. The negatively charged residues in the ZAR1 pore are positioned in a way similar to that of the mitochondrial calcium uniporter, the calcium release-activated calcium channel ORAI, and the calcium channel ryanodine receptor RyR2, as noted by Kobe and colleagues (14, 62, 117, 120). Although these findings point to an ion channel function of the ZAR1 resistosome pore, electrophysiological experiments are urgently needed to establish such a role.

Modeling of secondary structures showed that the MLKL four-helix bundle is similar to the pore-forming domain of the fungal HET-S protein, which triggers RCD that prevents fusion of mycelia from incompatible strains (27). Similar modeling suggested that the CC and CC_R domains of several plant NLRs, including ZAR1, ADR1s, and NRG1s, also adopt similar folds as found in the MLKL and HET-S pore-forming domains (8, 72). These observations suggest a common mechanism of cell death control across different kingdoms and imply that the mechanism underlying ZAR1 HR also applies to RNLs (6, 72, 84), which mediate TNL HR and signaling. This raises a question as to whether RNLs oligomerize in HR execution. Consistent with this possibility, mutations that perturb the ADP-mediated stabilization of inactive conformation in *Arabidopsis* ADR1-L2 and NRG1 result in autoactivation and trigger cell death (119, 129). This autoactivation requires the P-loop, which is consistent with the role of ATP-induced oligomerization. Furthermore, the *N. benthamiana* NRG1 requires the P-loop for the helper function downstream of Roq1 (126). NRG1s are reported to localize to the endoplasmic reticulum (ER) (167), whereas ADR1s have been implicated to associate with PM-localized surface receptors (123). An outstanding question is whether RNLs form pores in the PM or ER for HR execution. The similarity of the ZAR1 CC and RNL CC_R domains to the pore-forming domains of HET-S and MLKL proteins implies a cell death mechanism similar to necroptosis.

HR is associated with morphological changes such as cytoplasmic shrinkage, chromatin condensation, organelle dynamics, and perturbed membrane integrity (113) (**Figure 4**). The final stage of HR involves cell membrane rupture, as indicated by electrolyte leakage associated with ETI and staining of dead cells by cell membrane–impermeable dyes. As with necroptosis and pyroptosis, the resistosome pore may or may not directly cause membrane lysis. Morphological

features of the dying cell at early phases of HR need to be documented in real-time. It remains to be determined whether RCD during HR is also preceded by cell swelling in a way similar to necroptosis and pyroptosis.

The possibility that the ZAR1 resistosome pore acts as an ion channel is particularly relevant to the onset of HR (**Figure 4**), as Ca^{2+} serves as a secondary messenger and plays a central role in plant NLR-triggered immune responses. An early study showed that the recognition of AvrRpm1 and AvrB triggers a prolonged Ca^{2+} influx mediated by RPM1 (54). Furthermore, $LaCl_3$, a channel blocker, is sufficient to prevent cell death triggered by RPM1 and ZAR1 (40, 54, 158). The increase of cytosolic Ca^{2+} has been shown to regulate several components in ETI. Loss of two Ca^{2+}-dependent protein kinases, CPK1 and CPK2, compromised RPM1- and RPS2-triggered cell death (48). Reactive oxygen species (ROS) produced by two NADPH oxidases, RbohD and RbohF, are a major source of pathogen-triggered apoplastic ROS, which contribute to NLR-mediated cell death (152). A prolonged elevation of cytosolic Ca^{2+} concentration can potentially activate ROS production, which in turn enhances cell death (125). It remains unknown whether ROS act as a signal or executor of RCD during HR.

It should be noted that NLR-triggered cell death is unlikely to be limited to activity in the PM, as different subcellular localizations have been reported for various NLR proteins (167). It has been reported that viral and bacterial effectors can trigger the fusion of the central vacuole membrane with the PM in the early phases of HR (58). Pharmacological experiments suggested that the vacuole–PM fusion is required for HR. It is interesting to note that vacuolar proteins are detectable in the extracellular spaces after pathogen inoculation, suggesting a possible role of membrane fusion in the release of vacuolar contents to extracellular spaces. After effector recognition, multiple NLRs trigger a prolonged activation of MPK3/6, which inhibits photosynthetic activity and promotes HR (144). In addition to photosynthetic inhibition, chloroplastic stromules are induced at the beginning of HR, which is triggered by viral and bacterial effectors (15, 33). These stromules connect chloroplasts to the nucleus, which may transfer proteins and H_2O_2 into the nucleus (113). Whether and how a resistosome activates the aforementioned processes await future investigation.

SIGNALING BY RESISTOSOME

Although the pore-forming activity of the ZAR1 resistosome provides a mechanism for RCD, whether and how this triggers a complex array of downstream responses remain unknown. In this section, we discuss insights from the most recent studies and issues that require attention.

Signals Generated by the Resistosome Pore

The ZAR1 resistosome may trigger a calcium flux by itself or through other calcium channels to regulate downstream signaling. The transcription factor calmodulin binding protein 60-like g (CBP60g) binds with calmodulins to promote the expression of salicylic acid biosynthesis pathway genes, which constitutes a link between Ca^{2+} and disease resistance (147, 185). *Arabidopsis* metacaspases contain nine members, which are further divided into types I (AtMC1–AtMC3) and II (AtMC4–AtMC9). MC1 and MC2 antagonistically regulate RPM1-triggered cell death (23). Type II MCs promote the maturation of plant elicitor peptides (Peps) by cleaving precursors of Peps (PROPEPs) in a Ca^{2+}-dependent manner during cell damage or upon treatment of the bacterial flagellin epitope flg22, although the role of type II MCs in NLR-mediated signaling remains unknown (57, 138).

After cell membrane rupture, animal cells release DAMPs such as calreticulin, high-mobility group box 1 (HMGB1), ATP, heat-shock proteins, peptides, and DNA, which trigger inflammatory

responses (81). During pathogen infection, plants also release DAMPs, including HMGB3, ATP, NAD(P)H, DNA, sucrose, peptides, and oligogalacturonides, some of which are recognized by cell surface receptors to enhance immune responses (21). For instance, genetic depletion of BRASSINOSTEROID INSENSITIVE 1 (BRI1)-ASSOCIATED RECEPTOR KINASE 1 (BAK1) and its closest paralog BKK1, which are coreceptors of surface immune receptors, leads to autoimmunity in a manner dependent on ADR1s (166), suggesting that these coreceptors are monitored by unknown sensor NLRs. Interestingly, the autoimmune phenotype of the *bak1 bkk1* mutant requires perception of Peps by cell surface receptors PEPR1/2 (173). Whether Peps serve as DAMPs in bona fide ETI signaling remains unknown.

Transcriptional Regulation by NLRs

During ETI, some NLRs localize or relocalize into the nucleus to activate transcriptional reprogramming. For example, the rice CNL PigmR interacts with transcription factor PigmR-INTERACTING and BLAST RESISTANCE PROTEIN 1 (PIBP1) in the nucleus to trigger defense-related gene expression (177). In addition, CNLs, such as Pb1, BPH14, MLA10, Pi9, Piz-t, PID3 and Rx, and TNLs, such as RPS4, SNC1, and N, have been reported to localize in the nucleus and/or associate with transcription factors (64, 69, 118, 137, 172, 186). It remains to be determined whether resistosome formation is required for the nuclear action of these NLRs. A recent study showed a key role of RNLs in transcriptional regulation downstream of sensor NLRs, including both CNLs and TNLs (131). How RNLs regulate defense gene transcription remains to be elucidated. It is not clear whether some of the RNL proteins are localized in the nucleus after activation by upstream sensor NLRs.

Cross Talk with Cell Surface Receptors

ETI and PTI are known to share similar immune responses, although time and intensity differ. Several key immune components display a similar phosphorylation change during PTI and ETI, although the underlying mechanism remains unclear (73). Recently, two independent studies show that cell-surface receptors synergistically function with NLRs to fully activate disease resistance and immune responses, including ROS accumulation, callose deposition, MAPK activation, defense gene induction, and HR (115, 176). The mechanism underlying this synergistic action is not clear but likely involves NLR-dependent transcriptional activation of PTI pathway genes and BIK1-mediated phosphorylation of RBOHD.

Increasing evidence indicates that helper NLRs are involved in not only ETI but also PTI. Among these, NRC2 and NRC3 from *N. benthamiana* are required for immune signaling of the cell surface immune receptors Cf-4 and *Le*EIX2, which recognize the apoplastic effector Avr4 of *Cladosporium fulvum* and ethylene-inducing xylanase (EIX) from multiple fungi, respectively (46, 164). *Sl*NRC4a can associate with *Le*EIX2, suggesting a potential link between NLRs and cell surface receptors (87). Both Cf-4 and *Le*EIX2 are RLPs known to trigger HR upon activation. Their link to NRCs provides an explanation for HR activation, likely through pore-forming activity of the MADA motif. A more recent study showed that *Arabidopsis* ADR1s and several RLCK VII members are required for immune responses mediated by RLPs, such as RLP23, RLP32, and RLP42, which recognize ethylene-inducing peptide 1–like proteins, proteobacterial translation initiation factor 1 (IF1), and fungal polygalacturonases, respectively (123). Similar to the interaction of *Sl*NRC4a and *Le*EIX2, ADR1s associate with multiple RLP complexes. However, ADR1s are dispensable for immune signaling of the RKs FLAGELLIN SENSING 2 (FLS2) and ELONGATION FACTOR RECEPTOR (EFR), suggesting a specific link between RNLs and RLPs (123). These exciting findings point to a central role of helper NLRs in the integration

of PTI and ETI. Future studies are needed to elucidate the interplay between helper NLRs and canonical PTI components.

SUMMARY POINTS

1. Ligand-induced oligomerization is a common mechanism for the activation of plant NLR proteins.
2. Structures of the ZAR1, RPP1, and Roq1 resistosomes provide a multitude of insights into plant NLR activation and clues to downstream signaling.
3. Clustering of plant NLR signaling domains, including CC and TIR, in the center of the ringed structure of resistosomes initiates immune signaling.
4. The N terminus of ZAR1, and likely many other CNLs, forms a funnel structure that creates pores in cellular membranes.
5. The ZAR1 resistosome pore triggers cell death in a way analogous to animal pore-forming proteins that trigger necroptosis and pyroptosis.
6. NLRs and surface receptors are intimately linked in the activation of immune signaling.
7. Helper NLRs function in both RLP-mediated PTI and sensor NLR-mediated ETI.

FUTURE ISSUES

1. The association of the ZAR1 resistosome with the plasma membrane needs to be fully established.
2. Electrophysiology experiments are needed to test whether the ZAR1 resistosome indeed possesses ion channel activity.
3. Pore forming does not appear to disrupt the membrane directly, and the mechanism underlying RCD requires further investigation.
4. How pore-forming resistosomes activate immune signaling, particularly transcriptome programming, remains to be elucidated.
5. It is urgently needed to identify proteins that interact with the activated resistosome and elucidate their role in RCD and immune signaling.
6. Mechanistic details are needed to fully understand the PTI-ETI cross talk.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Adachi H, Contreras MP, Harant A, Wu CH, Derevnina L, et al. 2019. An N-terminal motif in NLR immune receptors is functionally conserved across distantly related plant species. *eLife* 8:e49956
2. Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, et al. 2008. Two adjacent nucleotide-binding site–leucine-rich repeat class genes are required to confer Pikm-specific rice blast resistance. *Genetics* 180:2267–76
3. Axtell MJ, Staskawicz BJ. 2003. Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. *Cell* 112:369–77
4. Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar S. 1997. Signaling in plant-microbe interactions. *Science* 276:726–33
5. Bastedo DP, Khan M, Martel A, Seto D, Kireeva I, et al. 2019. Perturbations of the ZED1 pseudokinase activate plant immunity. *PLOS Pathog.* 15:e1007900
6. Bayless AM, Nishimura MT. 2020. Enzymatic functions for Toll/interleukin-1 receptor domain proteins in the plant immune system. *Front. Genet.* 11:539
7. Bendahmane A, Farnham G, Moffett P, Baulcombe DC. 2002. Constitutive gain-of-function mutants in a nucleotide binding site–leucine rich repeat protein encoded at the Rx locus of potato. *Plant J.* 32:195–204
8. Bentham AR, Zdrzalek R, De la Concepcion JC, Banfield MJ. 2018. Uncoiling CNLs: structure/function approaches to understanding CC domain function in plant NLRs. *Plant Cell Physiol.* 59:2398–408
9. Bernoux M, Burdett H, Williams SJ, Zhang X, Chen C, et al. 2016. Comparative analysis of the flax immune receptors L6 and L7 suggests an equilibrium-based switch activation model. *Plant Cell* 28:146–59
10. Boller T, Felix G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60:379–406
11. Bonardi V, Tang S, Stallmann A, Roberts M, Cherkis K, Dangl JL. 2011. Expanded functions for a family of plant intracellular immune receptors beyond specific recognition of pathogen effectors. *PNAS* 108:16463–68
12. Botella MA, Parker JE, Frost LN, Bittner-Eddy PD, Beynon JL, et al. 1998. Three genes of the *Arabidopsis* RPP1 complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. *Plant Cell* 10:1847–60
13. Broz P, Pelegrin P, Shao F. 2020. The gasdermins, a protein family executing cell death and inflammation. *Nat. Rev. Immunol.* 20:143–57
14. Burdett H, Bentham AR, Williams SJ, Dodds PN, Anderson PA, et al. 2019. The plant "resistosome": structural insights into immune signaling. *Cell Host Microbe* 26:193–201
15. Caplan JL, Kumar AS, Park E, Padmanabhan MS, Hoban K, et al. 2015. Chloroplast stromules function during innate immunity. *Dev. Cell* 34:45–57
16. Catanzariti A-M, Dodds PN, Ve T, Kobe B, Ellis JG, Staskawicz BJ. 2010. The AvrM effector from flax rust has a structured C-terminal domain and interacts directly with the M resistance protein. *Mol. Plant-Microbe Interact.* 23:49–57
17. Cesari S, Kanzaki H, Fujiwara T, Bernoux M, Chalvon V, et al. 2014. The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. *EMBO J.* 33:1941–59
18. Cesari S, Moore J, Chen C, Webb D, Periyannan S, et al. 2016. Cytosolic activation of cell death and stem rust resistance by cereal MLA-family CC-NLR proteins. *PNAS* 113:10204–9
19. Cesari S, Thilliez G, Ribot C, Chalvon V, Michel C, et al. 2013. The rice resistance protein pair RGA4/RGA5 recognizes the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by direct binding. *Plant Cell* 25:1463–81
20. Chen X, He W-T, Hu L, Li J, Fang Y, et al. 2016. Pyroptosis is driven by non-selective gasdermin-D pore and its morphology is different from MLKL channel-mediated necroptosis. *Cell Res.* 26:1007–20
21. Choi HW, Klessig DF. 2016. DAMPs, MAMPs, and NAMPs in plant innate immunity. *BMC Plant Biol.* 16:232

22. Chung E-H, da Cunha L, Wu A-J, Gao Z, Cherkis K, et al. 2011. Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. *Cell Host Microbe* 9:125–36
23. Coll NS, Epple P, Dangl JL. 2011. Programmed cell death in the plant immune system. *Cell Death Differ*. 18:1247–56
24. Collier SM, Hamel L-P, Moffett P. 2011. Cell death mediated by the N-terminal domains of a unique and highly conserved class of NB-LRR protein. *Mol. Plant-Microbe Interact.* 24:918–31
25. Costanzo S, Jia Y. 2010. Sequence variation at the rice blast resistance gene *Pi-km* locus: implications for the development of allele specific markers. *Plant Sci*. 178:523–30
26. Cui H, Tsuda K, Parker JE. 2015. Effector-triggered immunity: from pathogen perception to robust defense. *Annu. Rev. Plant Biol.* 66:487–511
27. Daskalov A, Habenstein B, Sabaté R, Berbon M, Martinez D, et al. 2016. Identification of a novel cell death-inducing domain reveals that fungal amyloid-controlled programmed cell death is related to necroptosis. *PNAS* 113:2720–25
28. Day B, Dahlbeck D, Huang J, Chisholm ST, Li D, Staskawicz BJ. 2005. Molecular basis for the RIN4 negative regulation of RPS2 disease resistance. *Plant Cell* 17:1292–305
29. Deng Y, Zhai K, Xie Z, Yang D, Zhu X, et al. 2017. Epigenetic regulation of antagonistic receptors confers rice blast resistance with yield balance. *Science* 355:962–65
30. Deslandes L, Olivier J, Peeters N, Feng DX, Khounlotham M, et al. 2003. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *PNAS* 100:8024–29
31. Dickman MB, Fluhr R. 2013. Centrality of host cell death in plant-microbe interactions. *Annu. Rev. Phytopathol.* 51:543–70
32. Ding J, Wang K, Liu W, She Y, Sun Q, et al. 2016. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535:111–16
33. Ding X, Jimenez-Gongora T, Krenz B, Lozano-Duran R. 2019. Chloroplast clustering around the nucleus is a general response to pathogen perception in *Nicotiana benthamiana*. *Mol. Plant Pathol.* 20:1298–306
34. Dodds PN, Lawrence GJ, Catanzariti A-M, Teh T, Wang C-I, et al. 2006. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *PNAS* 103:8888–93
35. Dodds PN, Rathjen JP. 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat. Rev. Genet.* 11:539–48
36. Dou D, Zhou J-M. 2012. Phytopathogen effectors subverting host immunity: different foes, similar battleground. *Cell Host Microbe* 12:484–95
37. Durrant WE, Dong X. 2004. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 42:185–209
38. Duxbury Z, Ma Y, Furzer OJ, Huh SU, Cevik V, et al. 2016. Pathogen perception by NLRs in plants and animals: parallel worlds. *BioEssays* 38:769–81
39. Duxbury Z, Wang S, MacKenzie CI, Tenthorey JL, Zhang X, et al. 2020. Induced proximity of a TIR signaling domain on a plant-mammalian NLR chimera activates defense in plants. *PNAS* 117:18832–39
40. El Kasmi F, Chung E-H, Anderson RG, Li J, Wan L, et al. 2017. Signaling from the plasma-membrane localized plant immune receptor RPM1 requires self-association of the full-length protein. *PNAS* 114:E7385–94
41. Essuman K, Summers DW, Sasaki Y, Mao X, DiAntonio A, Milbrandt J. 2017. The SARM1 Toll/interleukin-1 receptor domain possesses intrinsic NAD^+ cleavage activity that promotes pathological axonal degeneration. *Neuron* 93:1334–43.e5
42. Feng F, Yang F, Rong W, Wu X, Zhang J, et al. 2012. A *Xanthomonas* uridine 5′-monophosphate transferase inhibits plant immune kinases. *Nature* 485:114–18
43. Fischer NL, Naseer N, Shin S, Brodsky IE. 2020. Effector-triggered immunity and pathogen sensing in metazoans. *Nat. Microbiol.* 5:14–26
44. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. 2009. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat. Immunol.* 10:241–47

45. Frost D, Way H, Howles P, Luck J, Manners J, et al. 2004. Tobacco transgenic for the flax rust resistance gene *L* expresses allele-specific activation of defense responses. *Mol. Plant-Microbe Interact.* 17:224–32
46. Gabriels SH, Vossen JH, Ekengren SK, van Ooijen G, Abd-El-Haliem AM, et al. 2007. An NB-LRR protein required for HR signalling mediated by both extra- and intracellular resistance proteins. *Plant J.* 50:14–28
47. Gantner J, Ordon J, Kretschmer C, Guerois R, Stuttmann J. 2019. An EDS1-SAG101 complex is essential for TNL-mediated immunity in *Nicotiana benthamiana*. *Plant Cell* 31:2456–74
48. Gao X, Chen X, Lin W, Chen S, Lu D, et al. 2013. Bifurcation of *Arabidopsis* NLR immune signaling via Ca^{2+}-dependent protein kinases. *PLOS Pathog.* 9:e1003127
49. Gao Y, Wang W, Zhang T, Gong Z, Zhao H, Han G-Z. 2018. Out of water: the origin and early diversification of plant R-genes. *Plant Physiol.* 177:82–89
50. Gao Z, Chung E-H, Eitas TK, Dangl JL. 2011. Plant intracellular innate immune receptor Resistance to *Pseudomonas syringae* pv. *maculicola* 1 (RPM1) is activated at, and functions on, the plasma membrane. *PNAS* 108:7619–24
51. Gassmann W, Hinsch ME, Staskawicz BJ. 1999. The *Arabidopsis* RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. *Plant J.* 20:265–77
52. Goritschnig S, Steinbrenner AD, Grunwald DJ, Staskawicz BJ. 2016. Structurally distinct *Arabidopsis thaliana* NLR immune receptors recognize tandem WY domains of an oomycete effector. *New Phytol.* 210:984–96
53. Grant JJ, Chini A, Basu D, Loake GJ. 2003. Targeted activation tagging of the *Arabidopsis* NBS-LRR gene, *ADR1*, conveys resistance to virulent pathogens. *Mol. Plant-Microbe Interact.* 16:669–80
54. Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J. 2000. The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. *Plant J.* 23:441–50
55. Guo H, Ahn HK, Sklenar J, Huang J, Ma Y, et al. 2020. Phosphorylation-regulated activation of the *Arabidopsis* RRS1-R/RPS4 immune receptor complex reveals two distinct effector recognition mechanisms. *Cell Host Microbe* 27:769–81.e6
56. Halff EF, Diebolder CA, Versteeg M, Schouten A, Brondijk TH, Huizinga EG. 2012. Formation and structure of a NAIP5-NLRC4 inflammasome induced by direct interactions with conserved N- and C-terminal regions of flagellin. *J. Appl. Biol. Chem.* 287:38460–72
57. Hander T, Fernandez-Fernandez AD, Kumpf RP, Willems P, Schatowitz H, et al. 2019. Damage on plants activates Ca^{2+}-dependent metacaspases for release of immunomodulatory peptides. *Science* 363(6433):eaar7486
58. Hatsugai N, Iwasaki S, Tamura K, Kondo M, Fuji K, et al. 2009. A novel membrane fusion-mediated plant immunity against bacterial pathogens. *Genes Dev.* 23:2496–506
59. Hayashi N, Inoue H, Kato T, Funao T, Shirota M, et al. 2010. Durable panicle blast-resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J.* 64:498–510
60. He S, Wang L, Miao L, Wang T, Du F, et al. 2009. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-α. *Cell* 137:1100–11
61. Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, et al. 2019. NAD^+ cleavage activity by animal and plant TIR domains in cell death pathways. *Science* 365:793–99
62. Hou X, Pedi L, Diver MM, Long SB. 2012. Crystal structure of the calcium release–activated calcium channel Orai. *Science* 338:1308–13
63. Howles P, Lawrence G, Finnegan J, McFadden H, Ayliffe M, et al. 2005. Autoactive alleles of the flax *L6* rust resistance gene induce non-race-specific rust resistance associated with the hypersensitive response. *Mol. Plant-Microbe Interact.* 18:570–82
64. Hu L, Wu Y, Wu D, Rao W, Guo J, et al. 2017. The coiled-coil and nucleotide binding domains of BROWN PLANTHOPPER RESISTANCE14 function in signaling and resistance against planthopper in rice. *Plant Cell* 29:3157–85
65. Hu M, Qi J, Bi G, Zhou JM. 2020. Bacterial effectors induce oligomerization of immune receptor ZAR1 in vivo. *Mol. Plant* 13:793–801

66. Hu Z, Yan C, Liu P, Huang Z, Ma R, et al. 2013. Crystal structure of NLRC4 reveals its autoinhibition mechanism. *Science* 341:172–75
67. Hu Z, Zhou Q, Zhang C, Fan S, Cheng W, et al. 2015. Structural and biochemical basis for induced self-propagation of NLRC4. *Science* 350:399–404
68. Hwang C-F, Bhakta AV, Truesdell GM, Pudlo WM, Williamson VM. 2000. Evidence for a role of the N terminus and leucine-rich repeat region of the *Mi* gene product in regulation of localized cell death. *Plant Cell* 12:1319–29
69. Inoue H, Hayashi N, Matsushita A, Xinqiong L, Nakayama A, et al. 2013. Blast resistance of CC-NB-LRR protein Pb1 is mediated by WRKY45 through protein-protein interaction. *PNAS* 110:9577–82
70. Jones JD, Dangl JL. 2006. The plant immune system. *Nature* 444:323–29
71. Jones JD, Vance RE, Dangl JL. 2016. Intracellular innate immune surveillance devices in plants and animals. *Science* 354(6316):aaf6395
72. Jubic LM, Saile S, Furzer OJ, El Kasmi F, Dangl JL. 2019. Help wanted: helper NLRs and plant immune responses. *Curr. Opin. Plant Biol.* 50:82–94
73. Kadota Y, Liebrand TWH, Goto Y, Sklenar J, Derbyshire P, et al. 2019. Quantitative phosphoproteomic analysis reveals common regulatory mechanisms between effector- and PAMP-triggered immunity in plants. *New Phytol.* 221:2160–75
74. Kanneganti A, Malireddi R, Saavedra PH, Vande Walle L, Van Gorp H, et al. 2018. GSDMD is critical for autoinflammatory pathology in a mouse model of familial Mediterranean fever. *J. Exp. Med.* 215:1519–29
75. Kanzaki H, Yoshida K, Saitoh H, Fujisaki K, Hirabuchi A, et al. 2012. Arms race co-evolution of *Magnaporthe oryzae AVR-Pik* and rice *Pik* genes driven by their physical interactions. *Plant J.* 72:894–907
76. Khan M, Subramaniam R, Desveaux D. 2016. Of guards, decoys, baits and traps: pathogen perception in plants by type III effector sensors. *Curr. Opin. Microbiol.* 29:49–55
77. Kim MG, da Cunha L, McFall AJ, Belkhadir Y, DebRoy S, et al. 2005. Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. *Cell* 121:749–59
78. Kofoed EM, Vance RE. 2011. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature* 477:592–95
79. Kourelis J, van der Hoorn RAL. 2018. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell* 30:285–99
80. Krasileva KV, Dahlbeck D, Staskawicz BJ. 2010. Activation of an *Arabidopsis* resistance protein is specified by the in planta association of its leucine-rich repeat domain with the cognate oomycete effector. *Plant Cell* 22:2444–58
81. Krysko O, Love Aaes T, Bachert C, Vandenabeele P, Krysko DV. 2013. Many faces of DAMPs in cancer therapy. *Cell Death Dis.* 4:e631
82. Künstler A, Bacsó R, Gullner G, Hafez YM, Király L. 2016. Staying alive: Is cell death dispensable for plant disease resistance during the hypersensitive response? *Physiol. Mol. Plant Pathol.* 93:75–84
83. Laflamme B, Dillon MM, Martel A, Almeida RN, Desveaux D, Guttman DS. 2020. The pan-genome effector-triggered immunity landscape of a host-pathogen interaction. *Science* 367:763–68
84. Lapin D, Bhandari DD, Parker JE. 2020. Origins and immunity networking functions of EDS1 family proteins. *Annu. Rev. Phytopathol.* 58:253–76
85. Lapin D, Kovacova V, Sun X, Dongus JA, Bhandari D, et al. 2019. A coevolved EDS1-SAG101-NRG1 module mediates cell death signaling by TIR-domain immune receptors. *Plant Cell* 31:2430–55
86. Le Roux C, Huet G, Jauneau A, Camborde L, Tremousaygue D, et al. 2015. A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. *Cell* 161:1074–88
87. Leibman-Markus M, Pizarro L, Schuster S, Lin ZJD, Gershony O, et al. 2018. The intracellular nucleotide-binding leucine-rich repeat receptor (SlNRC4a) enhances immune signalling elicited by extracellular perception. *Plant Cell Environ.* 41:2313–27
88. Lewis JD, Abada W, Ma W, Guttman DS, Desveaux D. 2008. The HopZ family of *Pseudomonas syringae* type III effectors require myristoylation for virulence and avirulence functions in *Arabidopsis thaliana*. *J. Bacteriol. Res.* 190:2880–91

89. Lewis JD, Lee AH-Y, Hassan JA, Wan J, Hurley B, et al. 2013. The *Arabidopsis* ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a. *PNAS* 110:18722–27
90. Li B, Meng X, Shan L, He P. 2016. Transcriptional regulation of pattern-triggered immunity in plants. *Cell Host Microbe* 19:641–50
91. Li L, Habring A, Wang K, Weigel D. 2020. Atypical resistance protein RPW8/HR triggers oligomerization of the NLR immune receptor RPP7 and autoimmunity. *Cell Host Microbe* 27:405–17.e6
92. Li M, Ma X, Chiang Y-H, Yadeta KA, Ding P, et al. 2014. Proline isomerization of the immune receptor-interacting protein RIN4 by a cyclophilin inhibits effector-triggered immunity in *Arabidopsis*. *Cell Host Microbe* 16:473–83
93. Li X, Clarke JD, Zhang Y, Dong X. 2001. Activation of an EDS1-mediated *R*-gene pathway in the *snc1* mutant leads to constitutive, NPR1-independent pathogen resistance. *Mol. Plant-Microbe Interact.* 14:1131–39
94. Li X, Kapos P, Zhang Y. 2015. NLRs in plants. *Curr. Opin. Immunol.* 32:114–21
95. Liang X, Zhou J-M. 2018. Receptor-like cytoplasmic kinases: central players in plant receptor kinase–mediated signaling. *Annu. Rev. Plant Biol.* 69:267–99
96. Liu C, Cui D, Zhao J, Liu N, Wang B, et al. 2019. Two *Arabidopsis* receptor-like cytoplasmic kinases SZE1 and SZE2 associate with the ZAR1–ZED1 complex and are required for effector-triggered immunity. *Mol. Plant* 12:967–83
97. Liu J, Elmore JM, Lin Z-JD, Coaker G. 2011. A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. *Cell Host Microbe* 9:137–46
98. Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, et al. 2015. Fungal effectors and plant susceptibility. *Annu. Rev. Plant Biol.* 66:513–45
99. Lolle S, Greeff C, Petersen K, Roux M, Jensen MK, et al. 2017. Matching NLR immune receptors to autoimmunity in *camta3* mutants using antimorphic NLR alleles. *Cell Host Microbe* 21:518–29.e4
100. Lu D, Wu S, Gao X, Zhang Y, Shan L, He P. 2010. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *PNAS* 107:496–501
101. Lu X, Kracher B, Saur IM, Bauer S, Ellwood SR, et al. 2016. Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen. *PNAS* 113:E6486–95
102. Ma S, Lapin D, Liu L, Sun Y, Song W, et al. 2020. Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. *Science* 370(6521):eabe3069
103. Ma Y, Guo H, Hu L, Martinez PP, Moschou PN, et al. 2018. Distinct modes of derepression of an *Arabidopsis* immune receptor complex by two different bacterial effectors. *PNAS* 115:10218–27
104. Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL. 2003. *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell* 112:379–89
105. Mackey D, Holt BF III, Wiig A, Dangl JL. 2002. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell* 108:743–54
106. Maekawa T, Cheng W, Spiridon LN, Toller A, Lukasik E, et al. 2011. Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. *Cell Host Microbe* 9:187–99
107. Mahdi LK, Huang M, Zhang X, Nakano RT, Kopp LB, et al. 2020. Discovery of a family of mixed lineage kinase domain-like proteins in plants and their role in innate immune signaling. *Cell Host Microbe* 28(6):813–24.e6
108. Maqbool A, Saitoh H, Franceschetti M, Stevenson C, Uemura A, et al. 2015. Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. *eLife* 4:e08709
109. Martel A, Laflamme B, Seto D, Bastedo DP, Dillon MM, et al. 2020. Immunodiversity of the *Arabidopsis* ZAR1 NLR is conveyed by receptor-like cytoplasmic kinase sensors. *Front. Plant Sci.* 11:1290
110. Martin R, Qi T, Zhang H, Liu F, King M, et al. 2020. Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. *Science* 370(6521):eabd9993
111. Mestre P, Baulcombe DC. 2006. Elicitor-mediated oligomerization of the tobacco N disease resistance protein. *Plant Cell* 18:491–501

112. Muñoz-Planillo R, Kuffa P, Martínez-Colón G, Smith BL, Rajendiran TM, Núñez G. 2013. K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* 38:1142–53
113. Mur LA, Kenton P, Lloyd AJ, Ougham H, Prats E. 2008. The hypersensitive response; the centenary is upon us but how much do we know? *J. Exp. Bot.* 59:501–20
114. Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, et al. 2013. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* 39:443–53
115. Ngou BPM, Ahn H-K, Ding P, Jones JDG. 2020. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. bioRxiv 034173. **https://doi.org/10.1101/2020.04.10.034173**
116. Okuyama Y, Kanzaki H, Abe A, Yoshida K, Tamiru M, et al. 2011. A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J.* 66:467–79
117. Oxenoid K, Dong Y, Cao C, Cui T, Sancak Y, et al. 2016. Architecture of the mitochondrial calcium uniporter. *Nature* 533:269–73
118. Padmanabhan MS, Ma S, Burch-Smith TM, Czymmek K, Huijser P, Dinesh-Kumar SP. 2013. Novel positive regulatory role for the SPL6 transcription factor in the N TIR-NB-LRR receptor-mediated plant innate immunity. *PLOS Pathog.* 9:e1003235
119. Peart JR, Mestre P, Lu R, Malcuit I, Baulcombe DC. 2005. NRG1, a CC-NB-LRR protein, together with N, a TIR-NB-LRR protein, mediates resistance against tobacco mosaic virus. *Curr. Biol.* 15:968–73
120. Peng W, Shen H, Wu J, Guo W, Pan X, et al. 2016. Structural basis for the gating mechanism of the type 2 ryanodine receptor RyR2. *Science* 354(6310):aah5324
121. Petrie EJ, Czabotar PE, Murphy JM. 2019. The structural basis of necroptotic cell death signaling. *Trends Biochem. Sci.* 44:53–63
122. Petrie EJ, Sandow JJ, Jacobsen AV, Smith BJ, Griffin MDW, et al. 2018. Conformational switching of the pseudokinase domain promotes human MLKL tetramerization and cell death by necroptosis. *Nat. Commun.* 9:2422
123. Pruitt RN, Zhang L, Saile SC, Karelina D, Fröhlich K, et al. 2020. *Arabidopsis* cell surface LRR immune receptor signaling through the EDS1-PAD4-ADR1 node. bioRxiv 391516. **https://doi.org/10.1101/2020.11.23.391516**
124. Qi D, DeYoung BJ, Innes RW. 2012. Structure-function analysis of the coiled-coil and leucine-rich repeat domains of the RPS5 disease resistance protein. *Plant Physiol.* 158:1819–32
125. Qi J, Wang J, Gong Z, Zhou J-M. 2017. Apoplastic ROS signaling in plant immunity. *Curr. Opin. Plant Biol.* 38:92–100
126. Qi T, Seong K, Thomazella DPT, Kim JR, Pham J, et al. 2018. NRG1 functions downstream of EDS1 to regulate TIR-NLR-mediated plant immunity in *Nicotiana benthamiana*. *PNAS* 115:E10979–87
127. Rairdan GJ, Moffett P. 2006. Distinct domains in the ARC region of the potato resistance protein Rx mediate LRR binding and inhibition of activation. *Plant Cell* 18:2082–93
128. Rehmany AP, Gordon A, Rose LE, Allen RL, Armstrong MR, et al. 2005. Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two *Arabidopsis* lines. *Plant Cell* 17:1839–50
129. Roberts M, Tang S, Stallmann A, Dangl JL, Bonardi V. 2013. Genetic requirements for signaling from an autoactive plant NB-LRR intracellular innate immune receptor. *PLOS Genet.* 9:e1003465
130. Ruan J, Xia S, Liu X, Lieberman J, Wu H. 2018. Cryo-EM structure of the gasdermin A3 membrane pore. *Nature* 557:62–67
131. Saile SC, Jacob P, Castel B, Jubic LM, Salas-Gonzales I, et al. 2020. Two unequally redundant "helper" immune receptor families mediate *Arabidopsis thaliana* intracellular "sensor" immune receptor functions. *PLOS Biol.* 18:e3000783
132. Sarris PF, Duxbury Z, Huh SU, Ma Y, Segonzac C, et al. 2015. A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell* 161:1089–100
133. Schultink A, Qi T, Bally J, Staskawicz B. 2019. Using forward genetics in *Nicotiana benthamiana* to uncover the immune signaling pathway mediating recognition of the *Xanthomonas perforans* effector XopJ4. *New Phytol.* 221:1001–9

134. Schultink A, Qi T, Lee A, Steinbrenner AD, Staskawicz B. 2017. Roq1 mediates recognition of the *Xanthomonas* and *Pseudomonas* effector proteins XopQ and HopQ1. *Plant J.* 92:787–95
135. Schwartz AR, Potnis N, Timilsina S, Wilson M, Patané J, et al. 2015. Phylogenomics of *Xanthomonas* field strains infecting pepper and tomato reveals diversity in effector repertoires and identifies determinants of host specificity. *Front. Microbiol.* 6:535
136. Seto D, Koulena N, Lo T, Menna A, Guttman DS, Desveaux D. 2017. Expanded type III effector recognition by the ZAR1 NLR protein using ZED1-related kinases. *Nat. Plants* 3:17027
137. Shen Q-H, Saijo Y, Mauch S, Biskup C, Bieri S, et al. 2007. Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* 315:1098–103
138. Shen W, Liu J, Li JF. 2019. Type-II metacaspases mediate the processing of plant elicitor peptides in *Arabidopsis*. *Mol. Plant* 12:1524–33
139. Shi J, Zhao Y, Wang K, Shi X, Wang Y, et al. 2015. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526:660–65
140. Shirano Y, Kachroo P, Shah J, Klessig DF. 2002. A gain-of-function mutation in an *Arabidopsis* Toll interleukin1 receptor-nucleotide binding site-leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. *Plant Cell* 14:3149–62
141. Sohn KH, Segonzac C, Rallapalli G, Sarris PF, Woo JY, et al. 2014. The nuclear immune receptor RPS4 is required for RRS1 SLH1-dependent constitutive defense activation in *Arabidopsis thaliana*. *PLOS Genet.* 10:e1004655
142. Steinbrenner AD, Goritschnig S, Staskawicz BJ. 2015. Recognition and activation domains contribute to allele-specific responses of an *Arabidopsis* NLR receptor to an oomycete effector protein. *PLOS Pathog.* 11:e1004665
143. Steuernagel B, Witek K, Krattinger S, Ramirez-Gonzalez R, Schoonbeek H, et al. 2018. Physical and transcriptional organisation of the bread wheat intracellular immune receptor repertoire. bioRxiv 339424. **https://doi.org/10.1101/339424**
144. Su J, Yang L, Zhu Q, Wu H, He Y, et al. 2018. Active photosynthetic inhibition mediated by MPK3/MPK6 is critical to effector-triggered immunity. *PLOS Biol.* 16:e2004122
145. Su L, Quade B, Wang H, Sun L, Wang X, Rizo J. 2014. A plug release mechanism for membrane permeation by MLKL. *Structure* 22:1489–500
146. Sun Q, Collins NC, Ayliffe M, Smith SM, Drake J, et al. 2001. Recombination between paralogues at the Rp1 rust resistance locus in maize. *Genetics* 158:423–38
147. Sun T, Zhang Y, Li Y, Zhang Q, Ding Y, Zhang Y. 2015. ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity. *Nat. Commun.* 6:10159
148. Tameling WI, Elzinga SD, Darmin PS, Vossen JH, Takken FL, et al. 2002. The tomato R gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. *Plant Cell* 14:2929–39
149. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. 2019. The molecular machinery of regulated cell death. *Cell Res.* 29:347–64
150. Tang D, Wang G, Zhou J-M. 2017. Receptor kinases in plant-pathogen interactions: more than pattern recognition. *Plant Cell* 29:618–37
151. Tenthorey JL, Haloupek N, López-Blanco JR, Grob P, Adamson E, et al. 2017. The structural basis of flagellin detection by NAIP5: a strategy to limit pathogen immune evasion. *Science* 358:888–93
152. Torres MA, Dangl JL, Jones JD. 2002. *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *PNAS* 99:517–22
153. Van de Weyer A-L, Monteiro F, Furzer OJ, Nishimura MT, Cevik V, et al. 2019. A species-wide inventory of NLR genes and alleles in *Arabidopsis thaliana*. *Cell* 178:1260–72.e14
154. van Wersch R, Li X, Zhang Y. 2016. Mighty dwarfs: *Arabidopsis* autoimmune mutants and their usages in genetic dissection of plant immunity. *Front. Plant Sci.* 7:1717
155. Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, et al. 2019. TIR domains of plant immune receptors are NAD^+-cleaving enzymes that promote cell death. *Science* 365:799–803
156. Wang G, Roux B, Feng F, Guy E, Li L, et al. 2015. The decoy substrate of a pathogen effector and a pseudokinase specify pathogen-induced modified-self recognition and immunity in plants. *Cell Host Microbe* 18:285–95

157. Wang J, Chen T, Han M, Qian L, Li J, et al. 2020. Plant NLR immune receptor Tm-22 activation requires NB-ARC domain-mediated self-association of CC domain. *PLOS Pathog*. 16:e1008475
158. Wang J, Hu M, Wang J, Qi J, Han Z, et al. 2019. Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science* 364(6435):eaav5870
159. Wang J, Wang J, Hu M, Wu S, Qi J, et al. 2019. Ligand-triggered allosteric ADP release primes a plant NLR complex. *Science* 364(6435):eaav5868
160. Wei CF, Kvitko BH, Shimizu R, Crabill E, Alfano JR, et al. 2007. A *Pseudomonas syringae* pv. *tomato* DC3000 mutant lacking the type III effector HopQ1-1 is able to cause disease in the model plant *Nicotiana benthamiana*. *Plant J*. 51:32–46
161. Williams SJ, Sohn KH, Wan L, Bernoux M, Sarris PF, et al. 2014. Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science* 344:299–303
162. Williams SJ, Sornaraj P, deCourcy-Ireland E, Menz RI, Kobe B, et al. 2011. An autoactive mutant of the M flax rust resistance protein has a preference for binding ATP, whereas wild-type M protein binds ADP. *Mol. Plant-Microbe Interact.* 24:897–906
163. Wu C-H, Abd-El-Haliem A, Bozkurt TO, Belhaj K, Terauchi R, et al. 2017. NLR network mediates immunity to diverse plant pathogens. *PNAS* 114:8113–18
164. Wu C-H, Belhaj K, Bozkurt TO, Birk MS, Kamoun S. 2016. Helper NLR proteins NRC 2a/b and NRC 3 but not NRC 1 are required for Pto-mediated cell death and resistance in *Nicotiana benthamiana*. *New Phytol.* 209:1344–52
165. Wu C-H, Derevnina L, Kamoun S. 2018. Receptor networks underpin plant immunity. *Science* 360:1300–1
166. Wu Y, Gao Y, Zhan Y, Kui H, Liu H, et al. 2020. Loss of the common immune coreceptor BAK1 leads to NLR-dependent cell death. *PNAS* 117:27044–53
167. Wu Z, Li M, Dong OX, Xia S, Liang W, et al. 2019. Differential regulation of TNL-mediated immune signaling by redundant helper CNLs. *New Phytol*. 222:938–53
168. Xia B, Fang S, Chen X, Hu H, Chen P, et al. 2016. MLKL forms cation channels. *Cell Res*. 26:517–28
169. Xiao J, Wang C, Yao J-C, Alippe Y, Xu C, et al. 2018. Gasdermin D mediates the pathogenesis of neonatal-onset multisystem inflammatory disease in mice. *PLOS Biol*. 16:e3000047
170. Xie T, Peng W, Yan C, Wu J, Gong X, Shi Y. 2013. Structural insights into RIP3-mediated necroptotic signaling. *Cell Rep*. 5:70–78
171. Xiong Y, Han Z, Chai J. 2020. Resistosome and inflammasome: platforms mediating innate immunity. *Curr. Opin. Plant Biol.* 56:47–55
172. Xu F, Kapos P, Cheng YT, Li M, Zhang Y, Li X. 2014. NLR-associating transcription factor bHLH84 and its paralogs function redundantly in plant immunity. *PLOS Pathog*. 10:e1004312
173. Yamada K, Yamashita-Yamada M, Hirase T, Fujiwara T, Tsuda K, et al. 2016. Danger peptide receptor signaling in plants ensures basal immunity upon pathogen-induced depletion of BAK 1. *EMBO J*. 35:46–61
174. Yang X, Yang F, Wang W, Lin G, Hu Z, et al. 2018. Structural basis for specific flagellin recognition by the NLR protein NAIP5. *Cell Res*. 28:35–47
175. Yoshida K, Saitoh H, Fujisawa S, Kanzaki H, Matsumura H, et al. 2009. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *Plant Cell* 21:1573–91
176. Yuan M, Jiang Z, Bi G, Nomura K, Liu M, et al. 2020. Pattern-recognition receptors are required for NLR-mediated plant immunity. bioRxiv 031294. **https://doi.org/10.1101/2020.04.10.031294**
177. Zhai K, Deng Y, Liang D, Tang J, Liu J, et al. 2019. RRM transcription factors interact with NLRs and regulate broad-spectrum blast resistance in rice. *Mol. Cell* 74:996–1009.e7
178. Zhang D-W, Shao J, Lin J, Zhang N, Lu B-J, et al. 2009. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* 325:332–36
179. Zhang J, Li W, Xiang T, Liu Z, Laluk K, et al. 2010. Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a *Pseudomonas syringae* effector. *Cell Host Microbe* 7:290–301
180. Zhang L, Chen S, Ruan J, Wu J, Tong AB, et al. 2015. Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. *Science* 350:404–9

181. Zhang X, Dodds PN, Bernoux M. 2017. What do we know about NOD-like receptors in plant immunity? *Annu. Rev. Phytopathol.* 55:205–29
182. Zhang Y, Chen X, Gueydan C, Han J. 2018. Plasma membrane changes during programmed cell deaths. *Cell Res.* 28:9–21
183. Zhang Y, Goritschnig S, Dong X, Li X. 2003. A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npr1–1, constitutive 1. *Plant Cell* 15:2636–46
184. Zhang Y, Han J. 2016. Electrophysiologist shows a cation channel function of MLKL. *Cell Res.* 26:643–44
185. Zhang Y, Xu S, Ding P, Wang D, Cheng YT, et al. 2010. Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *PNAS* 107:18220–25
186. Zhu Z, Xu F, Zhang Y, Cheng YT, Wiermer M, et al. 2010. *Arabidopsis* resistance protein SNC1 activates immune responses through association with a transcriptional corepressor. *PNAS* 107:13960–65
187. Zipfel C, Oldroyd GE. 2017. Plant signalling in symbiosis and immunity. *Nature* 543:328–36