Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Life-or-death decisions in plant immunity

Xing Zhang[1,2] and Xinnian Dong[1,2]

Upon pathogen challenge, plant cells can mount defense not only by triggering programmed cell death (PCD) to limit pathogen growth, but also by secreting immune signals to activate subsequent organism-scale defense responses. Recent advances in the study of plant immune mechanisms have found that pathogen-induced oligomerization of immune receptors is a common 'on' switch for the normally self-inhibitory proteins. The resulting 'resistosome' triggers PCD through the formation of a calcium channel or a NADase. Synergy between different receptor-mediated signaling pathways appears to be required for sustained immune induction to trigger PCD of infected cells. In the neighboring cells, PCD is inhibited through the production of immune signal salicylic acid (SA) which mediates degradation of PCD-inducing immune components in biomolecular condensates. Future work is required to connect the resistosome-mediated channel formation and the NADase activity to the downstream regulation of immune execution.

Addresses
[1] Howard Hughes Medical Institute, Duke University, Durham, NC 27708, USA
[2] Department of Biology, Duke University, Durham, NC 27708, USA

Corresponding author: Dong, Xinnian (xdong@duke.edu)

Current Opinion in Immunology 2022, **75**:xx–yy

This review comes from a themed issue on **Innate immunity**

Edited by **Dusan Bogunovic** and **Zhijian James Chen**

https://doi.org/10.1016/j.coi.2022.102169

The advent of molecular study of innate immune mechanisms in both plants and animals was marked by the cloning of innate immune receptors in 1990s. The surprising conservation of specific domains found in these receptors from both kingdoms, such as the Toll/interleukin-1 receptor (TIR) domain and the leucine-rich repeat (LRR) domain, has inspired scientists to search for potential commonalities in pathogen sensing, signal transduction and immune execution in organisms across kingdoms. However, unlike the animal immune systems, plants do not have specialized immune cells, a plant immune response has to be balanced with normal physiological activities. There are also distinctions in the downstream signaling transduction. Moreover, direct homologs of caspases critical in the execution of immunogenic PCD (e.g. pyroptosis) in animals are missing in the plant lineage, begging for the question how PCD of the infected plant cell is mediated by the intracellular nucleotide-binding domain (NBD) and LRR receptors (NLRs). Here we highlight some of the recent breakthroughs in the study of plant immune mechanisms which provide us a much clearer view on the basic elements in mounting a full immune response.

Oligomerization of immune receptors as a common activation mechanism

The plant immune receptors can be roughly distinguished by the signals that they recognize, with the membrane-localized pattern-recognition receptors (PRRs) recognizing functionally conserved microbe-associated molecular patterns (MAMPs) leading to pattern-triggered immunity (PTI), whereas the intracellular NLRs detecting either the pathogen-specific effectors delivered through various secretory systems or the perturbations of host targets caused by the effectors resulting in effector-triggered immunity (ETI). This is different from the animal immune receptors. With the Toll-like receptors (TLRs) recognizing MAMPs on the membrane, the cytosolic NLRs have members to perceive MAMPs as PRRs and also members to detect the invasion of pathogen effectors [1,2].

Regardless of the signals that they recognize, the structural similarities in NLRs from both lineages are intriguing and suggestive. Besides the C-terminal LRR domain that provides the ligand specificity, and the middle NBD, later named as nucleotide-binding oligomerization domain (NOD), the plant NLRs also have a variable N-terminus, based on which they are classified into three groups: coiled-coil domain (CC)-containing NLR (CNL), TIR domain-containing NLR (TNL) and resistance to powdery mildew 8 (RPW8)-like CC domain (CC-R)-containing NLR (RNL) [3].

Studies of animal receptors discovered a tendency of interaction between the same type of domains leading to the assembly of novel functional modules or proximity of working domains such as caspase recruitment domain (CARD) to enhance sensitivity for a limited level of pathogen stimuli [4]. For example, the mammalian intracellular NLRs can form inflammasomes through the NODs to allow N-terminal recruitment of pro-caspase-1 via a direct interaction between CARD domains in the case of the CARD-containing NLRs (i.e. NLRC4) or with the help of the apoptosis-associated speck-like protein containing a CARD (ASC) for other NLRs [1,5].

Recent breakthroughs in cryo-EM structural studies of plant NLRs discovered that they switch from the auto-inhibitory monomer to the active oligomer following effector-mediated activation of the LRR domains [6–8,9••,10••,11••,12••]. The first structure reported by Wang *et al.* [9••] showed that the *Arabidopsis* PBL1-like protein 2 (PBL2) uridylated by the *Xanthomonas* effector AvrAC becomes associated with resistance-related kinase 1 (RKS1) to activate the CNL receptor ZAR1 (HOPZ-activated resistance 1) by forming the ZAR1-RKS1-PBL2^{UMP} pentameric 'ZAR1 resistosome' (Figure 1). This is accomplished by repositioning of the NOD in ZAR1 to allow oligomerization, followed by the CC domain self-association with a dramatic allosteric change at the very N-terminal helix to form a funnel-shaped structure that penetrates the plasma membrane. Based on the chemical nature of residues involved and the diameter of this pentameric funnel (10−30 Å), the authors proposed that it may serve as a cation channel [9••,10••,13••].

If the funnel structure formed by the CC domain of ZAR1 is functionally important, the logical question to ask is how does the TNL class of NLRs initiate immune signaling in the absence of the CC domain? The back-to-back publications by Ma *et al.* [11••] and Martin *et al.* [12••] showed that two TNL receptors, the *Arabidopsis* RPP1 (recognition of *Peronospora parasitica* 1) against the *Hyaloperonospora* effector ATR1 and the *Nicotiana benthamiana* ROQ1 (Recognition of Xopq 1) against the *Xanthomonas* effector XopQ, are activated through direct binding of the effectors to the C-terminal LRR domain and the polymorphic jelly roll/Ig-like domain (C-JID). The result is the formation of the tetrameric 'RPP1 resistosome' and 'ROQ1 resistosome', respectively. The conformational change that occurs within the NOD brings the functional end of the NLRs (i.e. the TIR domain) together to form an active NADase enzymatic pocket (Figure 1) [11••,12••]. This provided the structural basis for the detection of NADase activity from the TIR domain-containing proteins such as the mammalian SARM1 protein and the plant immune receptors RBA1 (Response to the bacterial type III effector protein HopBA 1), RPS4 (Resistant to *Pseudomonas syringae* 4) and RPP1, and the requirement of the enzymatic activity for triggering PCD by these proteins [14••,15••].

Though not all TIR domains have the NADase enzymatic activity, they all have the ability to oligomerize. For example, the TIR domain of TLRs in the mammalian innate immunity dimerizes upon MAMP recognition and recruits more TIR domain-containing adaptors to oligomerize for the assembly of the downstream functional modules such as the death domain (DD) or kinase domain (K) to activate the kinase cascade and subsequent transcription factors (TFs) for the production of proinflammatory cytokines (Figure 1) [16–18]. Instead of forming higher order oligomer, plant PRRs are activated by forming heterodimer with the co-receptors to initiate the kinase cascade and transduce the signal [19].

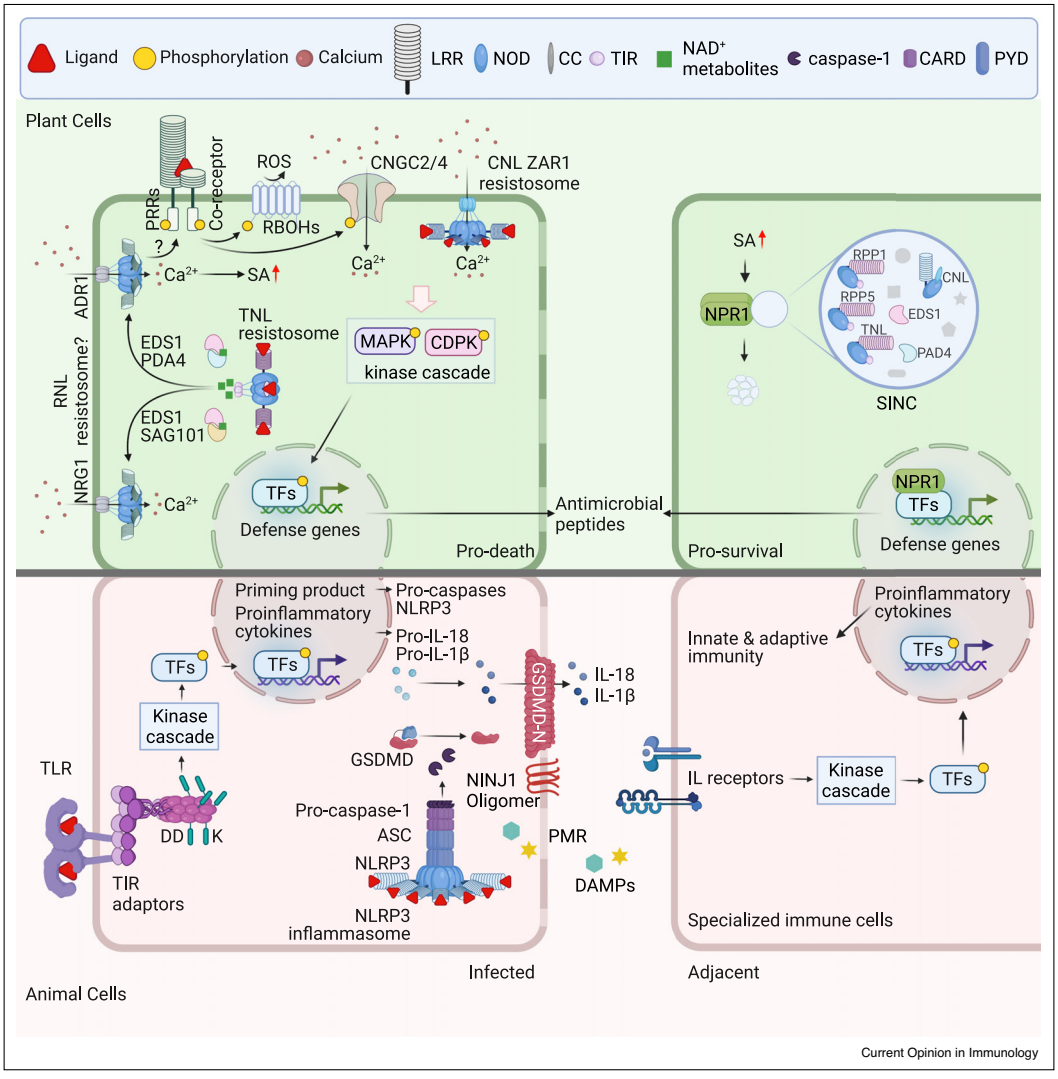
The significance of using oligomerization as a common activation mechanism for innate immune induction is the enhanced sensitivity to pathogen signals which are likely to be present in a meager amount. The simultaneous involvement of multiple components may also ensure that the response, which often leads to cell death, is tightly controlled and robust.

Pore formation as the trigger, instead of the executor, of immune-associated PCD in plants

In both plant and animal innate immune responses, PCD of the infected cell is a common strategy used to restrict pathogen growth as well as to release immune signals for the subsequent defense responses in the organisms. However, NLR-triggered PCD in plants occurs without direct homologs of caspases as in mammalian cells where inflammatory caspases, such as caspase-1 activated by the inflammasome, cleave gasdermin D (GSDMD) to release its N-terminal fragment (GSDMD-N) to form membrane pores, leading to pyroptosis [20]. The plasma membrane anchoring of the plant ZAR1 resistosome through its N-terminal funnel suggests that in plants, NLR-induced PCD also involves the formation of membrane pores. However, the size of this pore is much smaller than that formed by GSDMD-N (~215 Å) [21] and there is limited cell swelling, suggesting that the pore formed by the ZAR1 resistosome is unlikely the final executor of cell death, but rather a cation-permeable channel that triggers cell death-inducing signaling (Figure 1). To follow-up on the structural breakthrough, Bi *et al.* showed that activation of ZAR1 indeed leads to a calcium influx that is dependent on the key channel residue Glu11 and that calcium serves as an essential messenger for the subsequent reactive oxygen species (ROS) burst, organelle disturbance and the final plasma membrane rupture (PMR) [13••]. The massive transcriptional reprogramming due to an increase in the nuclear pore permeability has been proposed to be involved in PCD execution [22]. Intriguingly, recent identification of Ninjurin 1 (NINJ1) in macrophage PMR, whose mutant exhibited a loss of cell viability but persistent ballooned morphology, genetically separated GSDMD pore-triggered pyroptotic cell death from the ultimate cell lysis accompanied by the massive release of danger-associated molecular patterns (DAMPs) [23]. Whether a NINJ1-like protein is required for PMR in plants needs future investigation.

Although the TNL resistosome does not lead to pore formation directly, the NAD^+ metabolic products generated by their NADase activity, such as variant of cyclic adenosine diphosphate ribose (v-cADPR), may serve as signals to activate RNLs to form membrane pores. This

Figure 1



An overview of plant and animal immune mechanisms.
In plant, a potential pathogen is first recognized by the cell surface-localized PRR/co-receptor complex to induce PTI. An adapted pathogen can also deliver virulence-promoting effectors into the host cells, which can be detected by the intracellular NLRs to induce ETI. These immune responses share some cellular events with distinct temporal patterns and intensities which include the PTI-mediated calcium influx channeled by CNGC2/4 upon PRR-induced phosphorylation and the ETI-mediated calcium increase due to the oligomerization of CNLs (e.g. ZAR1 resistosome) triggered directly by the effector or RNLs (NRG1 and ADR1) activated indirectly via TNL resistosome-generated NAD^+ metabolites and EDS1-SAG101 or EDS1-PAD4 through an unknown mechanism. Elevated calcium level in the cytoplasm activates the MAPK and CDPK cascades leading to the phosphorylation of RBOHs (NADPH oxidase/respiratory burst oxidase homologs) and TFs to induce ROS production and defense gene expression, respectively. EDS1-PAD4, together with the RNL ADR1, have been found to be required for the production of the immune signal SA and defense gene expression to confer resistance. This complex is also reported to be associated with specific PRR complexes to boost PTI through SA synthesis and induction of TNL genes. Prolonged cytosolic calcium increase and ROS production are required for PCD of the infected cell during ETI. Beyond the infection site, cell death is inhibited through the SA-responsive NPR1 protein which promotes cell survival through induction of antimicrobial genes in the nucleus and the formation of SA-induced NPR1 condensates (SINCs) in the cytoplasm to sequester and degrade multiple signaling components involved in cell death to switch on the pro-survival immune strategy.
In animals, cell surface-localized TLRs detect extracellular MAMPs and trigger transcriptional changes through sequential and co-operative assembly of protein domains such as TIR and DD to bring the kinase domain (K) into proximity to initiate the kinase cascade that ultimately leads to the activation of TFs. The increases in pro-caspases and NLRP3 expression prime the cell for agonist-triggered formation of inflammasomes, such as by the intracellular NLRP3, the CARD-containing and pyrin domain (PYD)-containing adaptor ASC, and pro-caspase-1. The matured caspase-1 not only processes the proinflammatory cytokines but also cleaves GSDMD to release GSDMD-N to form membrane pores causing pyroptotic cell death. The secreted proinflammatory cytokines, such as IL-18 and IL-1β, bind to their receptors on the surface of adjacent specialized immune cells to induce further innate or adaptive immune responses. NINJ1-mediated plasma membrane rupture (PMR) is the last step of pyroptotic cell lysis, through which DAMPs are massively released.
Common features are as noted at the top of the graph.

hypothesis is based on the observation that CC-R domain-containing RNLs are essential for TNL-mediated cell death [24,25]. In the recent work by Jacob *et al.* [26••], the authors performed structural modeling of the *Arabidopsis* RNL, N requirement gene 1.1 (NRG1.1), and found that this 'helper NLR' shares a structural similarity with the N-terminal 4-helix bundle of the mixed lineage kinase domain-like (MLKL) protein which has been shown to oligomerize and serve as a cation permeable channel in mammalian cell necroptosis [27,28]. Based on the structural model, genetic and functional analyses of key residues in NRG1.1 and another RNL, activated disease resistance 1 (ADR1), supported the prediction that oligomerization and channel function of these helper NLRs are critical for direct regulation of cytoplasmic Ca^{2+} levels and subsequent cell death (Figure 1). In addition to their essential roles for TNL resistance, helper NLRs also facilitate resistance mediated by several CNLs. The level of the immune response is modulated between the 'sensor NLRs' and the helper NLRs. Whereas the helper NLR mutant *nrg1* completely abolishes cell death induced by the sensor TNLs, it only attenuates cell death triggered by the sensor CNL RPS2 and has no effect on the CNL RPS5-mediated or MLA7-mediated PCD [24], indicating an interplay between the NLRs in reaching the signaling threshold and that NLR signaling pathways converge on the formation of pores as well as the subsequent calcium flux for cell death commitment.

What the activation signal is for the helper NLRs remains to be investigated. The secondary position of these NLRs in the signaling cascade is reminiscent of that for the animal NLRP3 inflammasome which is activated by a loss of host cell homeostasis such as K^+ efflux, lysosomal rupture, calcium mobilization and production of ROS [29]. NAD^+ metabolites, such as v-cADPR, produced by TNL resistosome may be good candidates for signals that activate helper NLRs. Moreover, the signaling protein enhanced disease susceptibility 1 (EDS1) has long been known to be required for TNL-mediated immune response. Its complexes with homologs senescence associated gene 101 (SAG101) and phytoalexin deficient 4 (PAD4) contribute to ETI-cell death and SA-mediated inhibition of pathogen growth by preferential interaction with the two helper NLRs, NRG1 and ADR1, respectively (Figure 1) [30,31]. The discoveries of AvrRPS4 effector-dependent association between EDS1-SAG101 and NRG1 and the stimulation of the association between EDS1-PAD4 with ADR1-L1 by the overexpression of the TIR-containing receptor RBA1 immediately bring on the following questions: (1) What other sensor NLRs can trigger EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 assembly? (2) What is the structural basis for triggering the complexes assembly and the RNL resistosome formation? (3) How does the interplay between the activated sensor CNLs and the helper NLRs happen mechanistically? and (4) Whether NAD^+ metabolites generated by TNL resistosome are directly involved in the complex assembly or indirectly through cellular changes such as calcium flux [32,33]?

Crosstalk between different receptor-mediated pathways in mounting a full immune response

A major question in the innate immune study is how the system distinguishes pathogens from commensal and beneficial microbes through recognizing MAMPs which have conserved functions. One possible way to prevent false alarms is through the crosstalk between PTI mediated by the cell surface PRRs and ETI by the intracellular NLRs. Overlapping cellular events between PTI and ETI have been found in calcium mobilization, the involvement of kinase cascade including both mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK), ROS production, as well as defense gene induction (Figure 1), although with distinct temporal patterns and intensities [34]. Calcium influx is considered the initial event which is channeled by cyclic nucleotide-gated channel 2/4 (CNGC2/4) in PTI and activated by a CC-containing NLR resistosome in ETI [13••,26••,35]. More calcium-permeable channels have been reported in establishing the PTI-calcium signature, such as the hyperosmolality-induced calcium increase 1.3/1.7 (OSCA1.3/1.7) in guard-cells to trigger their closure to block pathogen entry [36], as well as the glutamate receptors (GLRs), inhibition of which with antagonists compromised MAMP-induced calcium influx [37]. Whether additional channels participate in the ETI-calcium signature needs further exploration. Once PTI is triggered by MAMPs, ETI is enhanced as well, and *vice versa*. Recent studies by Ngou *et al.* and Yuan *et al.* more firmly established the reliance of full ETI on the early signaling components of PTI and the feedback boost of PTI by ETI [38••,39••]. Studies from Pruitt *et al.*, and Tian *et al.* further linked the TNL signaling components, EDS1, PAD4 and ADRs, to PTI [40,41]. These findings indicate that only pathogens carrying the MAMP signals and having the ability to deliver effectors into the plant cell can trigger a full immune response. In animals, extracellular lipopolysaccharide (LPS) detection by TLR4 on the cell surface only serves the priming function through induced gene expression of proinflammatory cytokines, pro-caspases, and also NLRP3, whereas detection of cytosolic LPS, as a result of bacterial invasion, leads to the inflammasome activation, assembly of GSDMD-N membrane pores, execution of pyroptotic cell death, as well as maturation and secretion of active cytokines (Figure 1) [42–45]. With development of single-cell level analyses and application of transient gene function perturbation in plants, more spatial and temporal information will be provided to explain whether the crosstalks observed in the genetic experiments occur within the same cells or between cells.

Biomolecular condensates in promoting cell survival

Oligomerization of immune receptors is the trigger for immune-associated PCD. However, PCD of infected cells is not the final 'goal', but rather a commonly used sacrifice to promote survival of the organism through induction of other immune responses. In animals, GSDMD-formed pore in pyroptosis is required for the release of proinflammatory cytokines such as interleukin (IL)-1β and IL-18 [20] which bind to their receptors and transduce the signal through a kinase cascade to systemically activate and regulate specialized immune cells including T cells and B cells involved in adaptive immunity (Figure 1) [46], while the final cell lysis leads the massive release of DAMPs to alert the bystander cells [23]. However, even activation of GSDMD is not an irreversible path to cell death because the calcium influx through the pore can also activate the ESCRT (endosomal sorting complexes required for transport)-mediated membrane repairment of the infected cells [20], indicating that even for specialized immune cells, death is tightly controlled.

In the absence of professional immune cells, plants synthesize and release immune signals such as SA during the local PTI and ETI responses to promote survival of neighboring cells and activate broad-spectrum systemic acquired resistance (SAR) through secreting an array of antimicrobial peptides [47]. A recent study by Zavaliev *et al.*, found that the master immune regulator, nonexpressor of pathogenesis-related gene 1 (NPR1) is not only required in the nucleus for expressing genes in defense peptide production and secretion, but also in the cytoplasm for the formation of SA-induced NPR1 condensates (SINCs) enriched with many NLRs such as RPP1 and RPP5, signaling components such as EDS1 and PAD4, together with protein degradation machineries in cells surrounding the PCD site [48••]. Therefore, in addition to the mechanisms in controlling NLR homeostasis that have been reported previously, from chromatin structure of the NLR gene loci [49,50], the nucleocytoplasmic distributions of NLR proteins [51] to ubiquitin/proteasome-mediated degradation [52,53], sequestration and degradation of the entire signaling pathway components through the formation of SINCs may be a more effective mechanism to shut down PCD and switch on the survival-promoting immune strategy, SAR (Figure 1). A similar live-or-die checkpoint has been reported by Samir *et al.* in the animal system where competition for the ATP-dependent RNA helicase DDX3X (DEAD-box helicase 3X-linked) required for the formation of the cytosolic stress granules and the NLRP3 inflammasome-associated ASC specks determines whether the stressed cell survives or undergoes pyroptotic cell death [54]. Prior induction of stress granule formation leads to a decrease in NLRP3 inflammasome assembly and a reduction in active caspase-1 and secreted cytokine levels. Similarly, pre-treating plants with SA blocks NLR-triggered PCD [48••].

In the past few years, several major missing pieces have been found in our understanding of plant immune mechanisms (Figure 1). With the big picture coming into focus, some of the immanent questions to address are: (1) How do TNL resistosomes activate helper NLRs? What is the molecular activity of EDS1/SAG101 and EDS1/PAD4 in this signal relay? (2) What are the signaling events downstream of resistosome-induced calcium influx? (3) How is PCD executed during ETI? (4) What are the biogenesis mechanism and the structure of SINC in promoting cell survival? More than ever, we are closer to understanding how plants thrive under constant pathogen challenges.

Funding

The work in XD's lab is supported by grants from National Institutes of Health5R35-GM118036-06, National Science FoundationIOS-2041378, and Howard Hughes Medical Institute to XD.

Conflict of interest statement

X.D. is a cofounder of Upstream Biotechnology, Inc., and a Scientific Advisory Board member of Inari Agriculture.

Acknowledgement

We thank Dr Yuan Zhuang and all the Dong lab members for their critical reading of the manuscript. The figure is created using BioRender.com.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- •• of outstanding interest

1. Platnich JM, Muruve DA: **NOD-like receptors and inflammasomes: a review of their canonical and non-canonical signaling pathways**. *Arch Biochem Biophys* 2019, **670**:4-14.
2. Fischer NL, Naseer N, Shin S, Brodsky IE: **Effector-triggered immunity and pathogen sensing in metazoans**. *Nat Microbiol* 2020, **5**:14-26.
3. Shao ZQ, Xue JY, Wu P, Zhang YM, Wu Y, Hang YY, Wang B, Chen JQ: **Large-scale analyses of angiosperm nucleotide-binding site-leucine-rich repeat genes reveal three anciently diverged classes with distinct evolutionary patterns**. *Plant Physiol* 2016, **170**:2095-2109.
4. Vajjhala PR, Ve T, Bentham A, Stacey KJ, Kobe B: **The molecular mechanisms of signaling by cooperative assembly formation in innate immunity pathways**. *Mol Immunol* 2017, **86**:23-37.
5. Mariathasan S, Newton K, Monack DM, Vucic D, French DM, Lee WP, Roose-Girma M, Erickson S, Dixit VM: **Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf**. *Nature* 2004, **430**:213-218.
6. Hu Z, Yan C, Liu P, Huang Z, Ma R, Zhang C, Wang R, Zhang Y, Martinon F, Miao D *et al.*: **Crystal structure of NLRC4 reveals its autoinhibition mechanism**. *Science* 2013, **341**:172-175.
7. Hu Z, Zhou Q, Zhang C, Fan S, Cheng W, Zhao Y, Shao F, Wang HW, Sui SF, Chai J: **Structural and biochemical basis for induced self-propagation of NLRC4**. *Science* 2015, **350**:399-404.

8. Zhang L, Chen S, Ruan J, Wu J, Tong AB, Yin Q, Li Y, David L, Lu A, Wang WL *et al.*: **Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization**. *Science* 2015, **350**:404-409.

9. •• Wang J, Hu M, Wang J, Qi J, Han Z, Wang G, Qi Y, Wang HW, Zhou JM, Chai J: **Reconstitution and structure of a plant NLR resistosome conferring immunity**. *Science* 2019, **364**:aav5870
The authors reconstituted the structure of a wheel-like pentameric 'ZAR1 resistosome' from the pre-existing ZAR1-RKS1 complex, the effector AvrAC-uridylated PBL2 (PBL2^{UMP}) and dATP, in which the very N-terminal helix of CNL ZAR1 form a funnel-shaped structure that is required for cell death in effector-triggered immunity.

10. •• Wang J, Wang J, Hu M, Wu S, Qi J, Wang G, Han Z, Qi Y, Gao N, Wang HW *et al.*: **Ligand-triggered allosteric ADP release primes a plant NLR complex**. *Science* 2019, **364**:aav5868
The authors reported the cryo-EM structures of the inactive ZAR1-RKS1 and the intermediate ZAR1-RKS1-PBL2^{UMP} complexes which revealed extensive intramolecular interactions within ZAR1 in maintaining the inactive state and PBL2^{UMP} binding in inducing ZAR1 conformational change as well as ADP release in the intermediate state.

11. •• Ma S, Lapin D, Liu L, Sun Y, Song W, Zhang X, Logemann E, Yu D, Wang J, Jirschitzka J *et al.*: **Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme**. *Science* 2020, **370**:abe3069
The authors showed the structural detail of the *Arabidopsis* TNL RPP1 resistosome assembly upon recognition of the pathogen effector ATR1 directly through LRR and C-JID, which releases the intramolecular auto-inhibitory contacts and allows NOD to oligomerize. The resulting tetramer facilitates the N-terminal TIR domain self-association and the formation of the NADase active pocket.

12. •• Martin R, Qi T, Zhang H, Liu F, King M, Toth C, Nogales E, Staskawicz BJ: **Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ**. *Science* 2020, **370**:abd9993
The authors provided the structural detail for the TNL ROQ1 resistosome upon direct recognition of pathogen effector XopQ where the C-terminal LRR and C-JID confer ligand-binding specificity. Switching from auto-inhibitory monomer into the active tetramer within NOD brings the N-terminal TIR domain in close contact and leads to the formation of the NADase active site.

13. •• Bi G, Su M, Li N, Liang Y, Dang S, Xu J, Hu M, Wang J, Zou M, Deng Y *et al.*: **The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling**. *Cell* 2021, **184**:3528-3541
The authors showed that upon induction, the pentameric ZAR1 resistosome localizes to the plasma membrane and forms a calcium-permeable channel leading to the ROS burst, organelle disturbance and plasma membrane rupture.

14. •• Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, Chen J, Qi T, Gilley J, Lai JS, Rank MX *et al.*: **NAD$^+$ cleavage activity by animal and plant TIR domains in cell death pathways**. *Science* 2019, **365**:793-799
The authors discovered that cleavage of NAD$^+$ is a conserved activity for TIR domain-containing proteins in triggering cell death in animals and in plants. The NADase activity is dependent on self-association of TIR domains.

15. •• Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, Chung EH, Osborne Nishimura E, DiAntonio A, Milbrandt J, Dangl JL *et al.*: **TIR domains of plant immune receptors are NAD$^+$-cleaving enzymes that promote cell death**. *Science* 2019, **365**:799-803
The authors showed that the NADase activity is essential for TNL-mediated cell death in plant immunity and the product, v-cADPR, is an *in planta* biomarker of the plant TIR-domain enzymatic function.

16. Takeda K, Akira S: **Toll-like receptors in innate immunity**. *Int Immunol* 2005, **17**:1-14.

17. Lin SC, Lo YC, Wu H: **Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling**. *Nature* 2010, **465** 885-U882.

18. Clabbers MTB, Holmes S, Muusse TW, Vajjhala PR, Thygesen SJ, Malde AK, Hunter DJB, Croll TI, Flueckiger L, Nanson JD *et al.*: **MyD88 TIR domain higher-order assembly interactions revealed by microcrystal electron diffraction and serial femtosecond crystallography**. *Nat Commun* 2021, **12**:2578.

19. Tang D, Wang G, Zhou JM: **Receptor kinases in plant-pathogen interactions: more than pattern recognition**. *Plant Cell* 2017, **29**:618-637.

20. Xia S, Hollingsworth LR, Wu H: **Mechanism and regulation of gasdermin-mediated cell death**. *Cold Spring Harb Perspect Biol* 2020, **12**.

21. Xia S, Zhang Z, Magupalli VG, Pablo JL, Dong Y, Vora SM, Wang L, Fu TM, Jacobson MP, Greka A *et al.*: **Gasdermin D pore structure reveals preferential release of mature interleukin-1**. *Nature* 2021, **593**:607-611.

22. Gu Y, Zebell SG, Liang Z, Wang S, Kang BH, Dong X: **Nuclear pore permeabilization is a convergent signaling event in effector-triggered immunity**. *Cell* 2016, **166**:1526-1538 e1511.

23. Kayagaki N, Kornfeld OS, Lee BL, Stowe IB, O'Rourke K, Li Q, Sandoval W, Yan D, Kang J, Xu M *et al.*: **NINJ1 mediates plasma membrane rupture during lytic cell death**. *Nature* 2021, **591**:131-136.

24. Castel B, Ngou PM, Cevik V, Redkar A, Kim DS, Yang Y, Ding PT, Jones JDG: **Diverse NLR immune receptors activate defence via the RPW8-NLR NRG1**. *New Phytol* 2019, **222**:966-980.

25. Wu Z, Li M, Dong OX, Xia S, Liang W, Bao Y, Wasteneys G, Li X: **Differential regulation of TNL-mediated immune signaling by redundant helper CNLs**. *New Phytol* 2019, **222**:938-953.

26. •• Jacob P, Kim NH, Wu F, El-Kasmi F, Chi Y, Walton WG, Furzer OJ, Lietzan AD, Sunil S, Kempthorn K *et al.*: **Plant "helper" immune receptors are Ca^{2+}-permeable nonselective cation channels**. *Science* 2021, **373**:420-425
The authors discovered the structural similarity between the CC-R domains of RNL NRG1.1 and that of the ZAR1 complex in the resting state as well as the cation channel forming protein MLKL in mammalian cells. They showed that ectopic expression of the RNL in human HeLa cells leads to pore formation and calcium influx. The conserved negatively charged residues in the N-terminus are required for RNL-mediated calcium influx and the subsequent cell death.

27. Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, Lewis R, Lalaoui N, Metcalf D, Webb AI *et al.*: **The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism**. *Immunity* 2013, **39**:443-453.

28. Xia B, Fang S, Chen X, Hu H, Chen P, Wang H, Gao Z: **MLKL forms cation channels**. *Cell Res* 2016, **26**:517-528.

29. Munoz-Planillo R, Kuffa P, Martinez-Colon G, Smith BL, Rajendiran TM, Nunez G: **K$^+$ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter**. *Immunity* 2013, **38**:1142-1153.

30. Sun XH, Lapin D, Feehan JM, Stolze SC, Kramer K, Dongus JA, Rzemieniewski J, Blanvillain-Baufume S, Harzen A, Bautor J *et al.*: **Pathogen effector recognition-dependent association of NRG1 with EDS1 and SAG101 in TNL receptor immunity**. *Nat Commun* 2021, **12**:3335.

31. Wu Z, Tian L, Liu X, Zhang Y, Li X: **TIR signal promotes interactions between lipase-like proteins and ADR1-L1 receptor and ADR1-L1 oligomerization**. *Plant Physiol* 2021, **187**:681-686.

32. Wu Y, Kuzma J, Marechal E, Graeff R, Lee HC, Foster R, Chua NH: **Abscisic acid signaling through cyclic ADP-Ribose in plants**. *Science* 1997, **278**:2126-2130.

33. Fliegert R, Gasser A, Guse AH: **Regulation of calcium signalling by adenine-based second messengers**. *Biochem Soc Trans* 2007, **35**:109-114.

34. Yuan M, Ngou BPM, Ding P, Xin XF: **PTI-ETI crosstalk: an integrative view of plant immunity**. *Curr Opin Plant Biol* 2021, **62**:102030.

35. Tian W, Hou C, Ren Z, Wang C, Zhao F, Dahlbeck D, Hu S, Zhang L, Niu Q, Li L *et al.*: **A calmodulin-gated calcium channel links pathogen patterns to plant immunity**. *Nature* 2019, **572**:131-135.

36. Thor K, Jiang S, Michard E, George J, Scherzer S, Huang S, Dindas J, Derbyshire P, Leitao N, DeFalco TA *et al.*: **The calcium-**

permeable channel OSCA1.3 regulates plant stomatal immunity. *Nature* 2020, **585**:569-573.

37. Kwaaitaal M, Huisman R, Maintz J, Reinstadler A, Panstruga R: **Ionotropic glutamate receptor (iGluR)-like channels mediate MAMP-induced calcium influx in *Arabidopsis thaliana***. *Biochem J* 2011, **440**:355-365.

38. •• Ngou BPM, Ahn HK, Ding P, Jones JDG: **Mutual potentiation of plant immunity by cell-surface and intracellular receptors**. *Nature* 2021, **592**:110-115
The authors demonstrated the reliance of full ETI response on the early signaling components of PTI and the feedback boost of PTI by ETI. They found that NLR signaling rapidly augments the transcript and/or protein levels of key PTI components. Activation of either immune system alone is insufficient to provide effective resistance.

39. •• Yuan M, Jiang Z, Bi G, Nomura K, Liu M, Wang Y, Cai B, Zhou JM, He SY, Xin XF: **Pattern-recognition receptors are required for NLR-mediated plant immunity**. *Nature* 2021, **592**:105-109
The authors demonstrated the reliance of full ETI response on the early signaling components of PTI and the feedback boost of PTI by ETI. They found that PRR and PRR co-receptor mutants are markedly impaired in the ETI response, whereas NLR signaling rapidly augments the transcript and/or protein levels of key PTI components. Activation of either immune system alone is insufficient to provide effective resistance.

40. Pruitt RN, Locci F, Wanke F, Zhang L, Saile SC, Joe A, Karelina D, Hua C, Frohlich K, Wan WL *et al.*: **The EDS1-PAD4-ADR1 node mediates *Arabidopsis* pattern-triggered immunity**. *Nature* 2021, **598**:495-499.

41. Tian H, Wu Z, Chen S, Ao K, Huang W, Yaghmaiean H, Sun T, Xu F, Zhang Y, Wang S *et al.*: **Activation of TIR signalling boosts pattern-triggered immunity**. *Nature* 2021, **598**:500-503.

42. Hagar JA, Powell DA, Aachoui Y, Ernst RK, Miao EA: **Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock**. *Science* 2013, **341**:1250-1253.

43. Kayagaki N, Wong MT, Stowe IB, Ramani SR, Gonzalez LC, Akashi-Takamura S, Miyake K, Zhang J, Lee WP, Muszynski A *et al.*: **Noncanonical inflammasome activation by intracellular LPS independent of TLR4**. *Science* 2013, **341**:1246-1249.

44. Gurung P, Malireddi RK, Anand PK, Demon D, Vande Walle L, Liu Z, Vogel P, Lamkanfi M, Kanneganti TD: **Toll or interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-beta (TRIF)-mediated caspase-11 protease production integrates Toll-like receptor 4 (TLR4) protein- and Nlrp3 inflammasome-mediated host defense against enteropathogens**. *J Biol Chem* 2012, **287**:34474-34483.

45. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L, Shao F: **Inflammatory caspases are innate immune receptors for intracellular LPS**. *Nature* 2014, **514**:187-192.

46. Arango Duque G, Descoteaux A: **Macrophage cytokines: involvement in immunity and infectious diseases**. *Front Immunol* 2014, **5**:491.

47. Fu ZQ, Dong X: **Systemic acquired resistance: turning local infection into global defense**. *Annu Rev Plant Biol* 2013, **64**:839-863.

48. •• Zavaliev R, Mohan R, Chen T, Dong X: **Formation of NPR1 condensates promotes cell survival during the plant immune response**. *Cell* 2020, **182**:1093-1108
The authors discovered that the master immune regulator NPR1 promotes plant cell survival in response to a broad-spectrum of pathogens and abiotic stresses through its transcriptional function in the nucleus in inducing defense gene expression as well as in the cytoplasm through the formation of condensates with stress proteins to control their homeostasis.

49. Hu B, Wang N, Bi X, Karaaslan ES, Weber AL, Zhu W, Berendzen KW, Liu C: **Plant lamin-like proteins mediate chromatin tethering at the nuclear periphery**. *Genome Biol* 2019, **20**:87.

50. Huang CY, Rangel DS, Qin X, Bui C, Li R, Jia Z, Cui X, Jin H: **The chromatin-remodeling protein BAF60/SWP73A regulates the plant immune receptor NLRs**. *Cell Host Microbe* 2021, **29**:425-434.

51. Jia M, Shen X, Tang Y, Shi X, Gu Y: **A karyopherin constrains nuclear activity of the NLR protein SNC1 and is essential to prevent autoimmunity in *Arabidopsis***. *Mol Plant* 2021, **14**:1733-1744.

52. van Wersch S, Tian L, Hoy R, Li X: **Plant NLRs: the whistleblowers of plant immunity**. *Plant Commun* 2020, **1**:100016.

53. Wu Z, Tong M, Tian L, Zhu C, Liu X, Zhang Y, Li X: **Plant E3 ligases SNIPER1 and SNIPER2 broadly regulate the homeostasis of sensor NLR immune receptors**. *EMBO J* 2020, **39**:e104915.

54. Samir P, Kesavardhana S, Patmore DM, Gingras S, Malireddi RKS, Karki R, Guy CS, Briard B, Place DE, Bhattacharya A *et al.*: **DDX3X acts as a live-or-die checkpoint in stressed cells by regulating NLRP3 inflammasome**. *Nature* 2019, **573**:590-594.