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Review

Tansley insight

A new biochemistry connecting pathogen detection to induced defense in plants

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Summary

Plant cell surface and intracellular immune receptors recognizing pathogen attack utilize the same defense machineries to mobilize resistance. New genetic, protein structural and biochemical information on receptor activation and signaling is transforming understanding of how their shared defense network operates. We discuss the biochemical activities of two classes of intracellular nucleotide-binding/leucine-rich repeat (NLR) receptor – one forming a Ca^{2+} channel, the other an NADase enzyme – which define engagement of enhanced disease susceptibility 1 (EDS1)-family heterodimers and cofunctioning helper NLRs (RNLs) to connect receptor systems and amplify defenses. Toll-interleukin-1 receptor (TIR) domain NLR receptors and TIR-domain proteins, with a capacity to produce NAD+-derived small molecules, require EDS1 dimers and RNLs for defense induction. The TIR-driven EDS1/RNL modules emerge as central elements in Ca^{2+}-based immunity signaling initiated by receptors outside and inside host cells.

I. Introduction

The plant immune system deploys two interconnected receptor layers which detect microbial molecules or host 'damage' signals (patterns) and trigger resistance to disease. Panels of plasma membrane (PM)-anchored pattern recognition receptors (PRRs), including receptor-like kinases (RLKs) and receptor-like proteins (RLPs), activate a basal resistance response called pattern-triggered immunity (PTI), which is often sufficient to prevent invasion by non- or poorly adapted microbes. Inside cells, nucleotide-binding/leucine-rich repeat (NLR) receptors sense activities of virulence factors (effectors) that are delivered into host cells by adapted pathogen strains, often to disable PTI. NLR-effector recognition leads to effector-triggered immunity (ETI), which frequently

culminates in host localized cell death (the hypersensitive response).

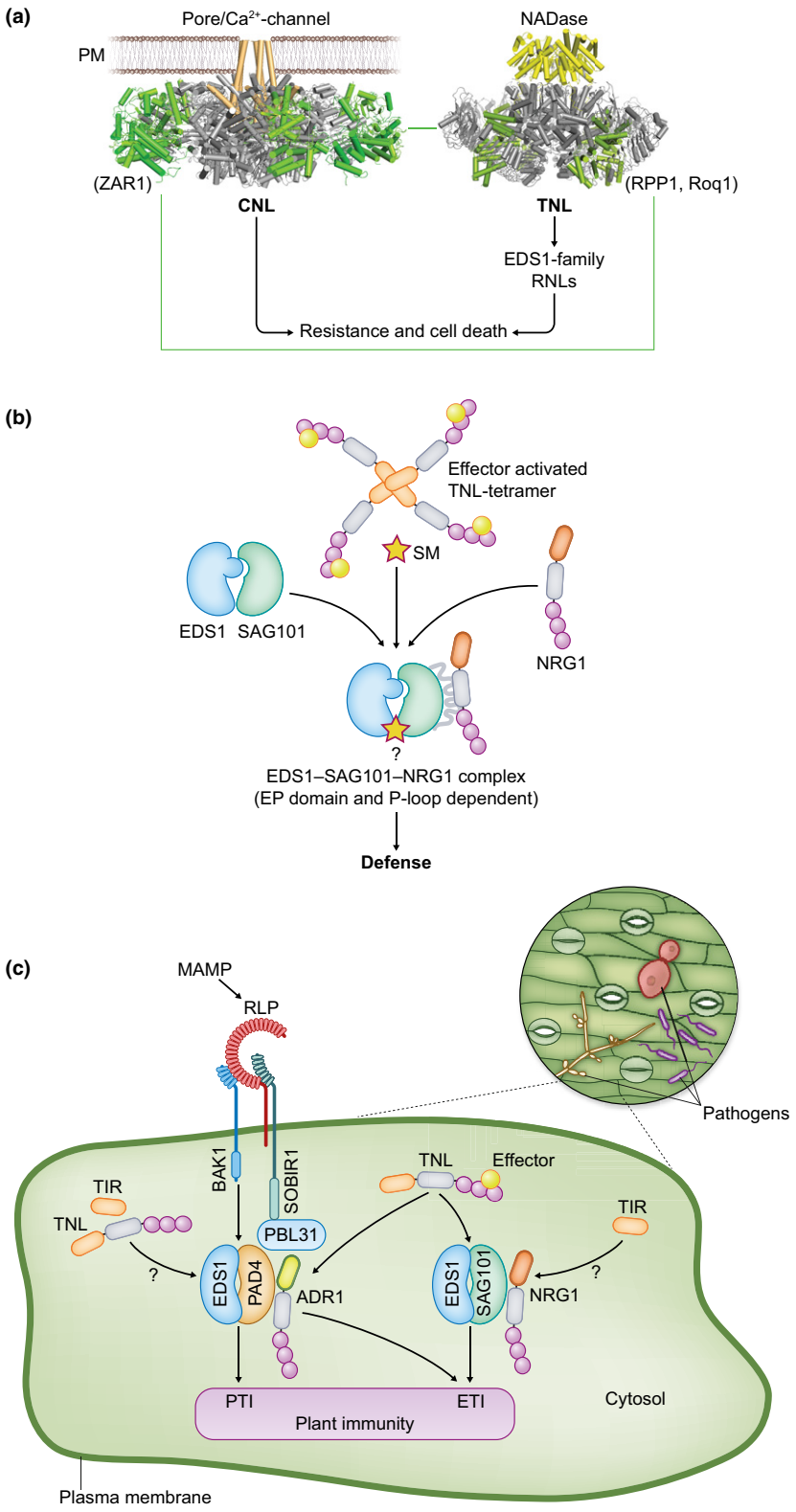
There has been a surge of new information over the last 3 yr on the biochemical processes underpinning PRR and NLR activation and downstream signaling. PRRs trigger early defenses to limit microbe colonization, and NLRs provide a mechanism to reinstate and transcriptionally amplify PTI-related defenses that are breached during infection. Hence, PRRs and NLRs converge on qualitatively similar transcriptional and metabolic outputs, such as NADPH-oxidase generated reactive oxygen species (ROS), Ca^{2+} ion fluxes, mitogen-activated protein kinase (MAPK) cascades and stress hormone network reprograming, the latter often leading to boosted salicylic acid (SA) defense (Yuan *et al.*, 2021b). Recent studies in *Arabidopsis* show that these two receptor layers potentiate each other to strengthen the immune response and therefore should be viewed more as interlinked barriers to disease than separate entities (Ngou *et al.*, 2021; Yuan *et al.*, 2021a). Here we consider some of the newest molecular and biochemical insights to signaling in ETI and its connectivity to PTI. A clearer picture emerges of intersecting defense pathways downstream of pathogen receptors activated in different cell compartments.

II. Functional significance of NLR oligomeric scaffolds

NLR receptor activation, via direct pathogen effector binding or indirect detection of effector-induced host modifications, defines current plant ETI models (Saur *et al.*, 2021). Fusion of a central nucleotide-binding (NB)/oligomerization domain with C-terminal LRRs (of varying lengths) and N-terminal coiled-coil (CC) or Toll-interleukin-1 receptor (TIR) domains creates an effective ADP/ATP-driven conformational switch. It is therefore perhaps not surprising that similar NLR architectures evolved in animals for innate immunity and cell death regulation (Xiong *et al.*, 2020; Saur *et al.*, 2021). In 2019, the cryoelectron (cryo-EM) microscopy structures of *Arabidopsis* ZAR1 (a CC-domain NLR, abbreviated to CNL) before and after indirect bacterial effector activation set the stage for understanding plant NLR modes of action (Wang *et al.*, 2019a,b). Activated ZAR1 assembles into a wheel-like pentamer in which the first α-helices of five CC-domains are exposed to form a funnel that contacts the PM (Fig. 1a). Single-molecule imaging and ion flux measurements of a preassembled ZAR1 oligomer suggest it forms an autonomous Ca^{2+}-permeable cation channel at the PM (Bi *et al.*, 2021). This has huge significance for the field because it implicates a direct CNL receptor mechanism for promoting Ca^{2+} influx into cells, thereby probably increasing Ca^{2+}-regulated kinase and transcription factor activities to mediate host defense and cell death (Seybold *et al.*, 2014; Yuan *et al.*, 2021b). In contrast to transient cytosolic Ca^{2+} elevation, which occurs within minutes of cell-surface PRR activation in PTI, CNL-triggered Ca^{2+} influx can last several hours (Grant *et al.*, 2000; Bi *et al.*, 2021), similar to sustained MAPK signaling which orchestrates ETI transcriptional defense (Tsuda *et al.*, 2013; Yuan *et al.*, 2021b). Hence, a major CNL sensor output is elevated Ca^{2+} inside host cells, which induces antipathogen defenses and, ultimately, cell death (see Box 1).

Recently, the cryo-EM structures of two pathogen-activated TIR-domain NLR receptors (called TNLs) – *Arabidopsis* RPP1 which directly binds an oomycete effector, and wild tobacco (*Nicotiana benthamiana*; *Nb*) Roq1 which binds a bacterial effector – were resolved and functionally characterized (Ma *et al.*, 2020; Martin *et al.*, 2020). The activated TNLs oligomerize into stable tetramers which, in contrast to CNL ZAR1, assemble two asymmetrically aligned N-terminal TIR-domain pairs. This creates an NAD+ hydrolyzing enzyme which initiates signaling (Ma *et al.*, 2020; Martin *et al.*, 2020) (Fig. 1a). The contrasting outputs of CNL and TNL activation – one an induced membrane pore with channel activity, the other a holoenzyme – help to explain why these two NLR classes have different signaling requirements in disease resistance. Genetic studies in *Arabidopsis* and *Nb* tobacco show that

Fig. 1 Modes of NLR and PRR activation and signaling. (a) Pathogen-activated coiled-coil (CC)-domain NLR (CNL) ZAR1 assembles into a pentamer with five exposed CC-domain α-helices forming a funnel (orange) that contacts the plasma membrane (PM) and creates a pore with Ca^{2+}-permeable inward cation channel activity (Wang *et al*., 2019a; Bi *et al*., 2021). Pathogen-activated toll-interleukin-1 receptor (TIR)-domain NLR (TNL) receptors RPP1 and Roq1 oligomerize into similar tetramers, in which two asymmetrically aligned TIR-domain pairs form an active NAD+ hydrolase (NADase) enzyme (yellow) (Ma *et al*., 2020; Martin *et al*., 2020). In both NLR oligomers, the central nucleotide-binding/oligomerization (NB)- and C-terminal leucine-rich repeat (LRR)-containing domains are shown in gray. ZAR1-activating host proteins $PBL2^{UMP}$ and RSK1 and RPP1-activating pathogen effector ATR1 are in green. The EDS1-family of lipase-like proteins and HeLo-domain helper NLRs (RNLs) are necessary for signaling downstream of TNL receptors to confer resistance and cell death in effector-triggered immunity (ETI). By contrast, the membrane-bound CNL ZAR1 oligomer might autonomously induce local resistance and cell death by promoting Ca^{2+}-dependent processes and transcriptional defense inside host cells. Protein structures were drawn from protein database PDBs 7CRC (RPP1: https://www.rcsb.org/3d-view/7CRC) and 6J5T (ZAR1: https://www.rcsb.org/structure/6J5T). (b) Direct pathogen effector binding leads to TNL (RPP1 and Roq1) receptor tetramerization, creating an active NADase enzyme which produces an NAD+ derived small molecule (SM, yellow and purple star) (Ma *et al*., 2020; Martin *et al*., 2020). In the speculative model shown, an SM binds to the EP-domain cavity surface of an EDS1-SAG101 dimer which promotes its association with oligomerization-competent NRG1-family RNLs. It is possible that an RNL oligomer associates with multiple EDS1-SAG101 dimers. Induced EDS1-RNL interaction is a necessary but perhaps transient step in TNL-mediated ETI signaling (Lapin *et al*., 2019; Sun *et al*., 2021; Wu *et al*., 2021). (c) A model depicting the EDS1-PAD4-ADR1 node in *Arabidopsis* operating at an intersection between ETI initiated by TNL receptors inside cells and pattern-triggered immunity (PTI) initiated by certain pattern recognition receptors (PRRs) at the cell surface. *Arabidopsis* PRR RLP23 forms a complex with RLKs SOBIR1 and BAK1 (Albert *et al*., 2015; W. L. Wan *et al*., 2019) and signals primarily via cytoplasmic receptor-like kinase PBL31 and the EDS1-PAD4-ADR1 node to promote defenses and pathogen immunity (Pruitt *et al*., 2021; Tian *et al*., 2021). Pools of EDS1-PAD4, ADR1 and PBL31 are found in close proximity to the plasma membrane and together with SOBIR, suggesting that EDS1-PAD4-ADR1-controlled basal immunity might be launched from PRR receptor sites at the plasma membrane (Pruitt *et al*., 2021). *Arabidopsis* TNL receptors utilize the EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 nodes to varying extents in ETI signaling. As indicated, it is likely that PRR-induced TNLs and/or truncated TIR-domain proteins contribute to defense amplification via EDS1-RNL nodes (Yuan *et al*., 2021b). MAMP, microbe-associated molecular pattern; RLP, receptor-like protein.



TNL, but not CNL sensor NLRs, require a small conserved family of HET-S/LOB-B (HeLo)-domain NLRs (referred to as helper NLRs, or RNLs) and the EDS1-family of three lipase-like proteins to confer local pathogen resistance and host cell death (Feehan *et al.*, 2020; Dongus & Parker, 2021) (Fig. 1a).

Oligomerization of CNL and TNL receptors into multimeric platforms broadly resembles mammalian NLR inflammasome assemblies which serve as scaffolds for concentrating signaling components, such as caspase enzymes, to initiate proinflammatory and cell death responses (Xiong *et al.*, 2020). Hence, a shared

Box 1 Calcium (Ca^{2+}) channels in immunity.

One of the earliest events in pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) is a rapid increase of cytosolic Ca^{2+} (Yuan *et al*., 2021b). Inside the cell, Ca^{2+} ions function as second messengers to transmit and amplify defense cascades mediated by Ca^{2+}-binding proteins and their downstream targets. Immune-related transport of Ca^{2+} into plant cells is controlled by several types of Ca^{2+}-permeable channel, including cyclic nucleotide-gated channels (CNGCs), OSCA1.3 and 1.7, and glutamate-like receptors (GLRs). *Arabidopsis* CNGC2 and CNGC4 mediate PAMP-induced increases in cytosolic Ca^{2+} in *Arabidopsis* leaves under conditions of high external [Ca^{2+}] (Tian *et al*., 2019). A misregulated *Arabidopsis* CNGC20 channel leads to increased Ca^{2+} inside cells and both PTI and ETI responses (Zhao *et al*., 2021). OSCA1.3 and 1.7 are essential mediators of PAMP-induced stomatal immunity (Thor *et al*., 2020). Members of a GLR2 clade (GLR2.7, 2.8 and 2.9) also contribute to PAMP-induced Ca^{2+} influx in *Arabidopsis* (Bjornson *et al*., 2021). In contrast to the transient rise in cytosolic Ca^{2+} induced by PAMPs, a sustained increase in cytosolic Ca^{2+} is observed in NLR-mediated ETI (Grant *et al*., 2000; Bi *et al*., 2021). These distinctive cytosolic Ca^{2+} signatures might be key factors determining ETI strength compared to PTI. They also suggest that different Ca^{2+} channel types contribute to NLR-mediated ETI. Indeed, cryo-EM structural studies and biochemical activity assays of the *Arabidopsis* activated CNL receptor ZAR1 suggest that it makes a pore at the plasma membrane (PM) with cation-selective Ca^{2+}-permeable inward ion channel activity (Bi *et al*., 2021). Protein structural and biochemical evidence also point to ADR1- and NRG1-type RNLs oligomerizing and forming pores associated with increased Ca^{2+}-permeable cation channel activity at the PM (Jacob *et al*., 2021). ZAR1 and NRG1 promote a sustained increase of cytosolic Ca^{2+}, fitting with Ca^{2+} signatures observed in ETI. Possibly, most CNLs and RNLs have the capacity to form Ca^{2+}-permeable channels to directly regulate cytosolic Ca^{2+} levels. Nevertheless, mutation of *CNGC2* or *CNGC4* genes in *Arabidopsis* impairs NLR-triggered HR cell death (Clough *et al*., 2000; Balague *et al*., 2003), indicating that various Ca^{2+} channels involved in PTI also contribute to Ca^{2+} influx in ETI. It will be important to determine how different ion channel mechanisms are regulated and coordinated for immune responses across host cells and tissues.

mechanistic principle for NLR oligomers across kingdoms appears to be the construction of a multimeric signaling scaffold. The same logic applies to the need for tight NLR constraint when not required, since their misactivation leads to autoimmunity with serious fitness consequences.

III. TNL and TIR-domain small molecule products

TIR domains have an ancient origin and self-association properties (Bayless & Nishimura, 2020). In animal immunity they are often employed as homotypic signaling adaptors which lack NADase activity. However, the mammalian TIR-domain-containing protein SARM1 has NADase activity which causes neuronal death by hydrolyzing and depleting cellular NAD+ (Essuman *et al*., 2017; Bayless & Nishimura, 2020). TIR domains of many plant TNL receptors and truncated TIR-domain proteins contain a conserved NADase catalytic glutamic acid (Glu) residue, and a number of these proteins possess Glu-dependent NADase activity after self-association (Horsefield *et al*., 2019; L. Wan *et al*., 2019). In contrast to SARM1, *in vitro* biochemical assays and *in vivo* cell death phenotyping suggest that plant TIR domains signal by generating one or more NAD+ hydrolysis products (Horsefield *et al*., 2019; L. Wan *et al*., 2019; Bayless & Nishimura, 2020). Several candidate TIR-domain NAD+-derived molecules, such as c-ADPR and a variant form v-cADPR, accumulate in plant tissues, but these are not sufficient to elicit cell death (Bayless & Nishimura, 2020; Duxbury *et al*., 2020). The fact that NADase-active TNLs and TIR-only proteins require *EDS1* to elicit plant cell death (L. Wan *et al*., 2019; Bayless & Nishimura, 2020; Wu *et al*., 2021) is in line with a TIR/NAD+ produced SM signaling via EDS1 to promote immunity. A new set of TIR-domain synthesized cyclic nucleotides (2′,3′-cAMP and 2,3-cGMP) were identified recently which lead to immune-related cell death (Yu *et al*., 2021), suggesting that multiple TIR-generated SMs might converge on EDS1. In various studies, EDS1 was found to associate with TNLs (Lapin *et al*., 2020). The interaction perhaps creates a concentrated microenvironment for EDS1 and/or other components to receive a TIR-generated SM and amplify the defense signal. In this context, it is interesting that TNL RPP1 NADase activity is itself stimulated by Ca^{2+} ions *in vitro* (Ma *et al*., 2020) and therefore TNL/SM generation could be further boosted by calcium influx to cells.

IV. EDS1-family dimers transduce NLR signals: connecting some dots

A plausible link between TNL and EDS1 via one or more SM intermediates is brought into sharp focus by recent structure–function insights to the EDS1-family of three proteins: EDS1, PAD4 and SAG101. *EDS1*-family genes are found in gymnosperms and angiosperms (seed plants) and therefore postdate the origins of NLRs, RNLs and core phytohormone pathways in plant evolution (Lapin *et al*., 2020) (see Fig. 2). The EDS1-family is characterized by fusion of an N-terminal α/β-hydrolase (class 3-lipase) domain with a unique C-terminal α-helical bundle 'EP' domain (Lapin *et al*., 2020). *Arabidopsis* EDS1 forms exclusive, stable heterodimers with SAG101 or PAD4 mediated by similar N-terminal domain interactions and independent of lipase catalytic residues (Wagner *et al*., 2013). The dimer N-terminal interfaces stabilize weaker associations between partner EP-domains to create an essential signaling surface around a cavity (Wagner *et al*., 2013; Bhandari *et al*., 2019; Dongus & Parker, 2021).

Although EDS1-SAG101 and EDS1-PAD4 dimers are similar, they do not have identical roles in the plant immune response. In *Arabidopsis* and *Nb* tobacco, EDS1-SAG101 signals preferentially in ETI conferred by TNL receptors where it promotes defense and host cell death (Castel *et al*., 2019; Lapin *et al*., 2019; Wu *et al*., 2019; Sun *et al*., 2021). By contrast, EDS1-PAD4 promotes a PTI-like basal immune response which contributes to varying extents to TNL and CNL ETI (Cui *et al*., 2017, 2018). *Arabidopsis* EDS1-PAD4-controlled basal immunity slows infection of virulent pathogens in the absence of ETI-related host cell death (Cui *et al*., 2017). Closer analysis of ETI conferred by an *Arabidopsis* TNL pair (RRS1–RPS4) to *Pseudomonas* bacteria revealed that EDS1-PAD4 (via its EP-domains) promotes rapid transcriptional upregulation

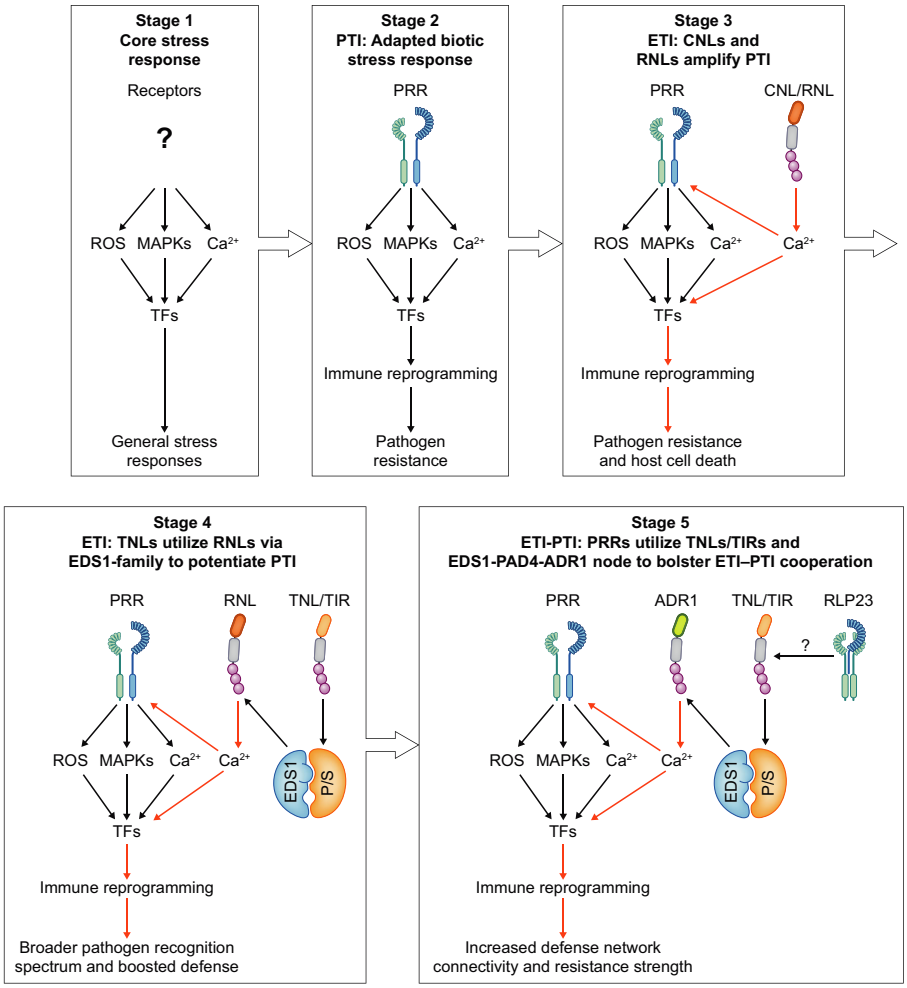


Fig. 2 Incremental stages in development of a plant immune receptor signaling network. We present here a five-stage process depicting the gradual elaboration of an immune receptor signaling network that now functions in seed plants. Stage 1: reactive oxygen species (ROS), mitogen-activated protein kinases (MAPKs), Ca^{2+} and transcription factors (TFs) are general stress components constituting a core system for responding to plant stress. Stage 2: pattern recognition receptors (PRRs) evolved in plants to activate existing ROS, MAPK and Ca^{2+} signaling and adapt or tune the core stress response machinery for antipathogen immunity. This feature is supported by PRR general stress-related transcriptomes (Jacob *et al*., 2018; Bjornson *et al*., 2021). Stage 3: in response to pathogen effector manipulation, plants evolved CC-domain NLRs (CNL) and HeLo-domain RNLs to form effector-triggered oligomeric pores with Ca^{2+}-permeable ion channel activity to boost Ca^{2+} cascades and amplify PRR immunity, resulting in a more robust response called effector-triggered immunity (ETI). Stage 4: in seed plants (e.g. *Arabidopsis*), RNLs became recruited by TNL receptors via EDS1-PAD4 or EDS1-SAG101 dimers to broaden the pathogen resistance spectrum and assist ETI-PTI cross-potentiation. Stage 5: in *Arabidopsis*, LRR-RP receptor RLP23 (potentially representing multiple PRRs) recruited the EDS1-PAD4-ADR1 module through unknown TIR/TNL components to extend the NLR–PRR defense network and strengthen resistance. P/S, PAD4 or SAG101. Black arrows indicate positive regulation and orange arrows indicate enhanced activation.

of defense genes (Bhandari *et al*., 2019). In addition to amplifying expression of numerous NLRs, RNLs and immunity components, *Arabidopsis* EDS1-PAD4 effectively prioritizes SA resistance over SA-antagonizing jasmonic acid (JA) pathways (Cui *et al*., 2018; Bhandari *et al*., 2019). In both EDS1 dimers, several positively charged EP-domain residues bordering the cavity are crucial for function (Wagner *et al*., 2013; Gantner *et al*., 2019; Lapin *et al*., 2019), consistent with the dimer EP-domains potentially accommodating a TIR-generated polar SM (Fig. 1b).

V. Rationalizing pathogen-induced EDS1 dimer–RNL associations

At this point it is useful to revisit the HeLo-domain-containing RNLs (helper NLRs) mentioned above, which in *Arabidopsis* and *Nb* tobacco are additional essential elements in TNL-mediated ETI (Qi *et al*., 2018; Feehan *et al*., 2020). RNLs divide into two small subfamilies, ADR1s and NRG1s. HeLo-domains are present in fungal cell death regulators and mammalian mixed lineage kinase-like (MLKL) immunity executors which can form pores at host membranes (Feehan *et al*., 2020; Mahdi *et al*., 2020). Moreover, HeLo-domains have a similar topology to the N-terminal coiled-coils of plant CNL receptors (Feehan *et al*., 2020; Jacob *et al*., 2021). In fact, an *Arabidopsis* NRG1 autoactive variant forms an oligomer which associates with cell membranes in a mode potentially similar to the activated CNL ZAR1 pore with Ca^{2+}-permeable ion channel activity (Fig. 1a) (Jacob *et al*., 2021). Does this mean that RNLs are functional equivalents of CNL oligomers for promoting Ca^{2+} influx into cells?

The answer might be more nuanced. Studies of *Arabidopsis ADR1*- and *NRG1*-family CRISPR mutants show that NRG1-type RNLs cofunction with EDS1-SAG101 in TNL ETI, and ADR1s with EDS1-PAD4 in TNL ETI and basal immunity, as two genetically separate branches or nodes (Lapin *et al.*, 2019; Feehan *et al.*, 2020; Saile *et al.*, 2020; Sun *et al.*, 2021). The co-occurrence of *NRG1s* with *SAG101*, and of *ADR1s* with *EDS1* and *PAD4* genes, in seed plant genomes lends further support to EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 cooperation as two nodes (Van Ghelder *et al.*, 2019; Lapin *et al.*, 2020). Indeed, TNL-triggered immunity and cell death could be reconstituted in an *Nb* tobacco CRISPR mutant lacking all *EDS1*-family members by transiently coexpressing an *Arabidopsis* EDS1-SAG101 dimer with *Arabidopsis* NRG1 (Lapin *et al.*, 2019). Since *Arabidopsis* NRG1 could not be substituted by tobacco or tomato (solanaceous) NRG1 or *Arabidopsis* ADR1 in these assays, it seems that within clade molecular cofunctions of particular RNL subtypes with EDS1 dimers underpin two genetically hard-wired resistance mechanisms. Importantly, EDS1 dimer – RNL cooperation depends on specific associations between node components, as borne out by immunoprecipitation studies in TNL-induced *Arabidopsis* and tobacco (*Nb*) leaf extracts (Sun *et al.*, 2021; Wu *et al.*, 2021). It is striking that induced interaction of EDS1-SAG101 with NRG1s and an immune response require TNL activation, a functional EDS1-SAG101 dimer EP-domain 'cavity' surface and an oligomerization-competent form of NRG1 (Sun *et al.*, 2021) (Fig. 1b). Hence, pathogen-activated TNL receptors mobilize ETI defense through induced EDS1 dimer–RNL complexes.

EDS1-family/RNL cooperation and association needs to be rationalized with proposed RNL activities as Ca^{2+}-permeable channels at host membranes (Jacob *et al.*, 2021). If the prime function of ADR1- and NRG1-family RNLs is as CNL receptor-like oligomers forming membrane pores for Ca^{2+} signal relay, we speculate that: an EDS1-family heterocomplex binds to and induces RNL oligomerization to enhance or alter its channel properties; or EDS1 dimer–RNL complex formation is a transient but essential step, after which a mature RNL oligomer is released to function at membranes. Both scenarios prompt the question of whether an EDS1-assisted RNL pore is equivalent to that made by CNL sensor NLRs or has different properties. Analysis of membrane pores in mammalian immune responses reveals a considerable range of sizes and signaling outcomes, as well as recruitment of additional components for membrane permeabilization (Ros *et al.*, 2017; Flores-Romero *et al.*, 2020; Kayagaki *et al.*, 2021). Another unknown is the extent to which RNL, and indeed CNL, ion channel activities relate to other Ca^{2+} channel families contributing to PTI and/or ETI responses (Box 1).

VI. Convergence of NLR and PRR signaling at the EDS1-PAD4-ADR1 node

Two recent studies of *Arabidopsis* cell-surface PRR (RLP23) basal immunity (PTI) reinforce the view that the EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 nodes make distinctive contributions to plant immunity (Pruitt *et al.*, 2021; Tian *et al.*, 2021). RLP23 is activated by an oomycete PAMP and signals via two membrane-bound RLKs, SOBIR1 and BAK1, and a small family of mobile receptor-like cytoplasmic kinase (RLCKs) (Albert *et al.*, 2015; W. L. Wan *et al.*, 2019), of which PBL31 is the most prominent (Pruitt *et al.*, 2021). RLP23-induced pathogen immunity has a strong genetic requirement for *EDS1-PAD4* (but much less for *EDS1-SAG101*), and uses the same dimer EP-domain cavity surface as engaged by NLRs in ETI (Pruitt *et al.*, 2021). These findings highlight a role of the EDS1-PAD4 dimer at an intersection between NLR and PRR signaling, perhaps to mediate ETI–PTI potentiation (Fig. 1c) (Dongus & Parker, 2021; Yuan *et al.*, 2021b).

Protein interaction assays suggest that the RLP23-SOBIR1 coreceptor associates constitutively with pools of EDS1-PAD4 and ADR1s at the inner side of the plasma membrane (Pruitt *et al.*, 2021), perhaps to launch signaling from the host cell surface. A related study implicates several transcriptionally induced TIR-domain and TNL genes as components of *EDS1-PAD4*-dependent PRR basal immunity, so TNLs and/or TIR-domain proteins are potential amplifiers of PRR-triggered defense (Tian *et al.*, 2021) (Fig. 1c). The close structural relatedness of the two EDS1 dimers, combined with evidence that TNL and/or TIR-protein-generated SMs drive EDS1 dimer–RNL associations, prompts the speculation that RNLs have become effective NLR immune receptors for TIR/SM-activated EDS1 dimers during seed plant evolution (Fig. 2).

VII. Conclusions

Advances made over the last 2–3 yr have transformed the picture of immune receptor functions and defense network evolution in land plants. We present our impression of this in five incremental stages from an early 'core' general stress response system, through PRRs that became recruited and tuned to biotic stress, to an increasingly elaborate defense network involving CNLs, TNLs and RNLs connected by EDS1-family proteins which potentiate ETI–PTI outputs (Fig. 2). This new information also brings a fresh set of challenges. Of these, the most important seems to be determining whether one or more SMs generated by TIR-domains of TNLs and TIR proteins define recruitment of the two EDS1 nodes in NLR- and PRR-triggered immunity. A recent preprint article identifies additional TIR-domain enzymatic activities and nucleotide products which cause immune-related cell death (Yu *et al.*, 2021). A further question is whether the role of EDS1 dimers is simply to activate RNLs, which then proceed alone or as a large multiprotein complex to signal as CNL-like membrane-associated ion channels. Alternatives we have considered are that the EDS1–RNL association changes the character of the RNL oligomer and/or that EDS1–RNL complexes have additional roles in the plant immune response. Although not covered in this perspective, we should not ignore that fact that in *Arabidopsis* a nuclear EDS1 pool confers NLR signaling (Lapin *et al.*, 2020). Is this resistance function tied to RNLs or do nuclear EDS1 dimers have a different activity? Certainly, *Arabidopsis* PAD4 can multitask in biotic stress signaling (Dongus *et al.*, 2020), suggesting these regulators have more than one mode of action, perhaps defined by their subcellular locations and partners. Finally, it is

tempting to speculate that TNLs and TIR-domain proteins all signal through EDS1-family members in ETI and PTI, but it remains to be established whether this is the case and how, molecularly and spatially, it would work.

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References

Albert I, Bohm H, Albert M, Feiler CE, Imkampe J, Wallmeroth N, Brancato C, Raaymakers TM, Oome S, Zhang HQ *et al.* 2015. An RLP23-SOBIR1-BAK1 complex mediates NLP-triggered immunity. *Nature Plants* **1**: 140.

Balague C, Lin BQ, Alcon C, Flottes G, Malmstrom S, Kohler C, Neuhaus G, Pelletier G, Gaymard F, Roby D. 2003. HLM1, an essential signaling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel ion channel family. *Plant Cell* **15**: 365–379.

Bayless AM, Nishimura MT. 2020. Enzymatic functions for Toll/Interleukin-1 receptor domain proteins in the plant immune system. *Frontiers in Genetics* **11**: 539.

Bhandari DD, Lapin D, Kracher B, von Born P, Bautor J, Niefind K, Parker JE. 2019. An EDS1 heterodimer signalling surface enforces timely reprogramming of immunity genes in Arabidopsis. *Nature Communications* **10**: 772.

Bi GZ, Su M, Li N, Liang Y, Dang S, Xu JC, Hu MJ, Wang JZ, Zou MX, Deng YA *et al.* 2021. The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. *Cell* **184**: 3528–3541.

Bjornson M, Pimprikar P, Nurnberger T, Zipfel C. 2021. The transcriptional landscape of *Arabidopsis thaliana* pattern-triggered immunity. *Nature Plants* **7**: 579–586.

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

Clough SJ, Fengler KA, Yu IC, Lippok B, Smith RK, Bent AF. 2000. The Arabidopsis dnd1 "defense, no death" gene encodes a mutated cyclic nucleotide-gated ion channel. *Proceedings of the National Academy of Sciences, USA* **97**: 9323–9328.

Cui H, Gobbato E, Kracher B, Qiu J, Bautor J, Parker JE. 2017. A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in Arabidopsis immunity. *New Phytologist* **213**: 1802–1817.

Cui H, Qiu J, Zhou Y, Bhandari DD, Zhao C, Bautor J, Parker JE. 2018. Antagonism of transcription factor MYC2 by EDS1/PAD4 complexes bolsters salicylic acid defense in Arabidopsis effector-triggered immunity. *Molecular Plant* **11**: 1053–1066.

Dongus JA, Bhandari DD, Patel M, Archer L, Dijkgraaf L, Deslandes L, Shah J, Parker JE. 2020. The Arabidopsis PAD4 lipase-like domain is sufficient for resistance to Green Peach aphid. *Molecular Plant–Microbe Interactions* **33**: 328–335.

Dongus JA, Parker JE. 2021. EDS1 signalling: at the nexus of intracellular and surface receptor immunity. *Current Opinion in Plant Biology* **62**: 102039.

Duxbury Z, Wang SS, MacKenzie CI, Tenthorey JL, Zhang XX, Huh SU, Hu LX, Hill L, Ngou PN, Ding PT *et al.* 2020. Induced proximity of a TIR signaling domain on a plant-mammalian NLR chimera activates defense in plants. *Proceedings of the National Academy of Sciences, USA* **117**: 18832–18839.

Essuman K, Summers DW, Sasaki Y, Mao XR, DiAntonio A, Milbrandt J. 2017. The SARM1 Toll/Interleukin-1 receptor domain possesses intrinsic NAD^+ cleavage activity that promotes pathological axonal degeneration. *Neuron* **93**: 1334–1343.

Feehan JM, Castel B, Bentham AR, Jones JDG. 2020. Plant NLRs get by with a little help from their friends. *Current Opinion in Plant Biology* **56**: 99–108.

Flores-Romero H, Ros U, Garcia-Saez AJ. 2020. Pore formation in regulated cell death. *EMBO Journal* **39**: e105753.

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**: 2456–2474.

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. *The Plant Journal* **23**: 441–450.

Horsefield S, Burdett H, Zhang XX, Manik MK, Shi Y, Chen J, Qi TC, Gilley J, Lai JS, Rank MX *et al.* 2019. NAD^+ cleavage activity by animal an plant TIR domains in cell death pathways. *Science* **365**: 793–799.

Jacob F, Kracher B, Mine A, Seyfferth C, Blanvillain-Baufume S, Parker JE, Tsuda K, Schulze-Lefert P, Maekawa T. 2018. A dominant-interfering *camta3* mutation compromises primary transcriptional outputs mediated by both cell surface and intracellular immune receptors in *Arabidopsis thaliana*. *New Phytologist* **217**: 1667–1680.

Jacob P, Kim NH, Wu FH, El Kasmr F, Chi Y, Walton WG, Furzer OJ, Lietzan AD, Sunil S, Kempthorn K *et al.* 2021. Plant "helper" immune receptors are Ca^{2+}-permeable nonselective cation channels. *Science* **373**: 420–425.

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

Lapin D, Bhandari DD, Parker JE. 2020. Origins and immunity networking functions of EDS1 family proteins. *Annual Review of Phytopathology* **58**: 253–276.

Lapin D, Kovacova V, Sun X, Dongus JA, Bhandari D, von Born P, Bautor J, Guarneri N, Rzemieniewski J, Stuttmann J *et al.* 2019. A coevolved EDS1-SAG101-NRG1 module mediates cell death signaling by TIR-domain immune receptors. *Plant Cell* **31**: 2430–2455.

Ma S, Lapin D, Liu L, Sun Y, Song W, Zhang X, Logemann E, Yu D, Wang J, Jirschitzka J *et al.* 2020. Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. *Science* **370**: eabe3069.

Mahdi LK, Huang M, Zhang X, Nakano RT, Kopp LB, Saur IML, Jacob F, Kovacova V, Lapin D, Parker JE *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**: 813–824.

Martin R, Qi T, Zhang H, Liu F, King M, Toth C, Nogales E, Staskawicz BJ. 2020. Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. *Science* **370**: eabd9993.

Ngou BPM, Ahn HK, Ding PT, Jones JDG. 2021. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature* **592**: 110–115.

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

Qi T, Seong K, Thomazella DPT, Kim JR, Pham J, Seo E, Cho MJ, Schultink A, Staskawicz BJ. 2018. NRG1 functions downstream of EDS1 to regulate TIR-NLR-mediated plant immunity in *Nicotiana benthamiana*. *Proceedings of the National Academy of Sciences, USA* **115**: E10979–E10987.

Ros U, Pena-Blanco A, Hanggi K, Kunzendorf U, Krautwald S, Wong WW-L, Garcia-Saez AJ. 2017. Necroptosis execution is mediated by plasma membrane nanopores independent of calcium. *Cell Reports* **19**: 175–187.

Saile SC, Jacob P, Castel B, Jubic LM, Salas-Gonzales I, Backer M, Jones JDG, Dangl JL, El Kasmi F. 2020. Two unequally redundant "helper" immune receptor families mediate *Arabidopsis thaliana* intracellular "sensor" immune receptor functions. *PLoS Biology* **18**: e3000783.

Saur IML, Panstruga R, Schulze-Lefert P. 2021. NOD-like receptor-mediated plant immunity: from structure to cell death. *Nature Reviews Immunology* **21**: 305–318.

Seybold H, Trempel F, Ranf S, Scheel D, Romeis T, Lee J. 2014. Ca^{2+} signalling in plant immune response: from pattern recognition receptors to Ca^{2+} decoding mechanisms. *New Phytologist* **204**: 782–790.

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

Thor K, Jiang SS, Michard E, George J, Scherzer S, Huang SG, Dindas J, Derbyshire P, Leitao N, DeFalco TA *et al.* 2020. The calcium-permeable channel OSCA1.3 regulates plant stomatal immunity. *Nature* **585**: 569–573.

Tian HN, Wu ZS, Chen SY, Ao KV, Huang WJ, Yaghmaiean H, Sun TJ, Xu F, Zhang YN, Wang SC *et al.* 2021. Activation of TIR signalling boosts pattern-triggered immunity. *Nature* **598**: 500–503.

Tian W, Hou CC, Ren ZJ, Wang C, Zhao FG, Dahlbeck D, Hu SP, Zhang LY, Niu Q, Li LG *et al.* 2019. A calmodulin-gated calcium channel links pathogen patterns to plant immunity. *Nature* **572**: 131–135.

Tsuda K, Mine A, Bethke G, Igarashi D, Botanga CJ, Tsuda Y, Glazebrook J, Sato M, Katagiri F. 2013. Dual regulation of gene expression mediated by extended MAPK activation and salicylic acid contributes to robust innate immunity in *Arabidopsis thaliana*. *PLoS Genetics* **9**: e1004015.

Van Ghelder C, Parent GJ, Rigault P, Prunier J, Giguere I, Caron S, Sena JS, Deslauriers A, Bousquet J, Esmenjaud D *et al.* 2019. The large repertoire of conifer NLR resistance genes includes drought responsive and highly diversified RNLs. *Scientific Reports* **9**: 11614.

Wagner S, Stuttmann J, Rietz S, Guerois R, Brunstein E, Bautor J, Niefind K, Parker JE. 2013. Structural basis for signaling by exclusive EDS1 heteromeric complexes with SAG101 or PAD4 in plant innate immunity. *Cell Host & Microbe* **14**: 619–630.

Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, Chung EH, Nishimura EO, DiAntonio A, Milbrandt J, Dangl JL *et al.* 2019. TIR domains of plant immune receptors are NAD^+-cleaving enzymes that promote cell death. *Science* **365**: 799–803.

Wan WL, Zhang LS, Pruitt R, Zaidem M, Brugman R, Ma XY, Krol E, Perraki A, Kilian J, Grossmann G *et al.* 2019. Comparing Arabidopsis receptor kinase and receptor protein-mediated immune signaling reveals BIK1-dependent differences. *New Phytologist* **221**: 2080–2095.

Wang JZ, Hu MJ, Wang J, Qi JF, Han ZF, Wang GX, Qi YJ, Wang HW, Zhou JM, Chai JJ. 2019a. Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science* **364**: eaav5870.

Wang JZ, Wang J, Hu MJ, Wu S, Qi JF, Wang GX, Han ZF, Qi YJ, Gao N, Wang HW *et al.* 2019b. Ligand-triggered allosteric ADP release primes a plant NLR complex. *Science* **364**: eaav5868.

Wu ZS, Li M, Dong OX, Xia ST, Liang WW, Bao YK, Wasteneys G, Li X. 2019. Differential regulation of TNL-mediated immune signaling by redundant helper CNLs. *New Phytologist* **222**: 938–953.

Wu ZS, Tian L, Liu XR, Zhang YL, Li X. 2021. TIR signal promotes interactions between lipase-like proteins and ADR1-L1 receptor and ADR1-L1 oligomerization. *Plant Physiology* **187**: 681–686.

Xiong YH, Han ZF, Chai JJ. 2020. Resistosome and inflammasome: platforms mediating innate immunity. *Current Opinion in Plant Biology* **56**: 47–55.

Yu D, Song W, Tan E, Liu L, Cao Y, Jirschitzka J, Li E, Logemann E, Xu C, Huang S *et al.* 2021. TIR domains of plant immune receptors are 2′,3′-cAMP/cGMP synthetases mediating cell death. *bioRxiv*. doi: 10.1101/2021.11.09.467869.

Yuan MH, Jiang ZY, Bi GZ, Nomura K, Liu MH, Wang YP, Cai BY, Zhou JM, He SY, Xin XF. 2021a. Pattern-recognition receptors are required for NLR-mediated plant immunity. *Nature* **592**: 105–109.

Yuan MH, Ngou BPM, Ding PT, Xiu-Fan X. 2021b. PTI-ETI crosstalk: an integrative view of plant immunity. *Current Opinion in Plant Biology* **62**: 102030.

Zhao C, Tang Y, Wang J, Zeng Y, Sun H, Zheng Z, Su R, Schneeberger K, Parker JE, Cui H. 2021. A mis-regulated cyclic nucleotide-gated channel mediates cytosolic calcium elevation and activates immunity in Arabidopsis. *New Phytologist* **230**: 1078–1094.

See also the Commentary on this article by Lee & Romeis, **234**: 769–772.