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EDS1 signalling: At the nexus of intracellular and surface receptor immunity

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Abstract

The conserved lipase-like protein EDS1 transduces signals from pathogen-activated intracellular nucleotide-binding leucine-rich repeat (NLR) receptors to transcriptional defences and host cell death. In this pivotal NLR signalling role, EDS1 works as a heterodimer with each of its partners, SAG101 and PAD4. Different properties of EDS1-SAG101 and EDS1-PAD4 complexes and functional relationships to sensor and helper NLRs have emerged. EDS1-SAG101 dimers confer effector-triggered immunity mediated by intracellular TNL receptors. In contrast, EDS1-PAD4 dimers have a broader role promoting basal immune responses that can be initiated inside cells by TNL- or CNL-type NLRs, and at the cell surface by LRR-receptor proteins. Characterizing the essential elements of these two EDS1 modules will help to connect intracellular and surface receptor signalling networks in the plant immune system.

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Keywords

PAD4, SAG101, Basal immunity, ETI, CC_{HeLo}-NLR, Cell death, LRR-RP.

Abbreviations

ETI, effector-triggered immunity; PTI, pattern-triggered immunity; PRR, pattern recognition receptor; NLR, nucleotide-binding leucine-rich repeat; LRR, leucine-rich repeat; LRR-RK, leucine-rich repeat domain receptor-like kinase; LRR-RP, leucine-rich repeat domain receptor-like protein; TIR, Toll/Interleukin-1 Receptor/Resistance; CC, coiled coil; CC_{HeLo}, coiled coil HET-S/LOP-B domain; EDS1, enhanced disease susceptibility 1; PAD4, phytoalexin deficient 4; SAG101, senescence associated gene 101; LLD, lipase-like domain; EP-domain, EDS1 PAD4 domain.

Introduction

Microbes and pests encounter two plant innate immunity receptor barriers against infection. Plasma membrane-anchored pattern-recognition receptors (PRRs) detect microbial and modified host molecules outside cells to activate pattern triggered immunity (PTI) [1,2*]. Within cells, nucleotide-binding leucine-rich repeat (NLR) receptors intercept activities of virulence factors (effectors) delivered by pathogenic strains, often to disable or modulate PTI [3,4]. NLR-effector recognition amplifies generally weaker PTI defences in a process called effector-trigger immunity (ETI), which culminates in localized host cell death and pathogen resistance. Besides the clearly defined PTI and ETI immune pathways, a host response called basal immunity slows infection of virulent (host-adapted) pathogens. Basal immunity is thought to be the combined outcome of residual PTI (after effector interference) and weak ETI [5–7].

The EDS1 family of nucleocytoplasmic lipase-like proteins EDS1, PAD4 and SAG101 are well-known controllers of ETI and basal immune responses, in which they transcriptionally mobilize defence pathways and, in the case of ETI, promote host cell death [6*,8]. Here we examine information gained in recent years on the EDS1 immunity signalling node and distinct contributions of EDS1 family members to NLR and PRR networks. What emerges is a clearer view of cooperating surface and intracellular receptor signalling systems, and some important questions to answer as the field moves forward.

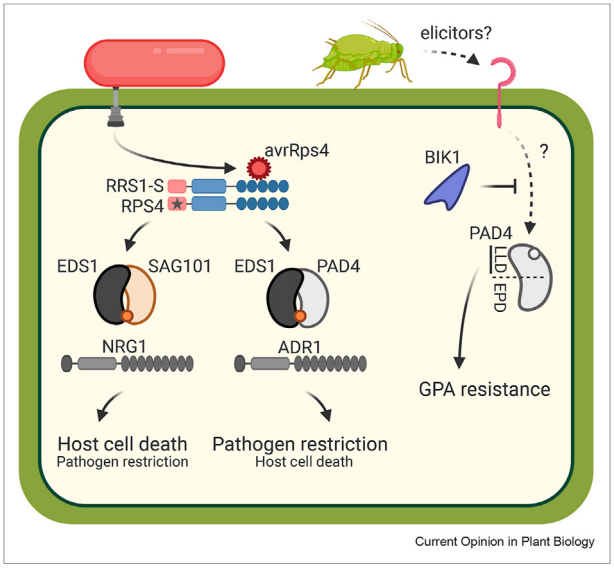
Surface and intracellular immune receptor networks

In seed plants, many immune-related PRRs possess leucine-rich repeat (LRR) ectodomains for ligand recognition. These PRR families are classified into LRR receptor-like kinases (LRR-RKs) and LRR receptor-like proteins (LRR-RPs) based on whether they possess or lack an intracellular kinase signalling domain [1,2*]. Upon ligand activation, PRR co-receptor complexes at the plasma membrane initiate phosphorylation cascades leading to apoplastic and transcriptional defences [1,2*]. The intracellular NLR receptors fall into two major sub-groups categorized by their N-terminal signalling domains: a Toll/Interleukin-1 Receptor/

Resistance (TIR) domain in TIR-type NLRs (often referred to as TNLs) and a coiled-coil (CC) domain in CC-type NLRs (CNLs) [4]. Increasing evidence shows that pathogen-sensing NLR receptors engage a network of related and unrelated NLR proteins (called helper NLRs) to promote immunity [9–13**].

Traditionally, surface and intracellular receptor systems have been regarded as being operationally independent, although both layers recognize a spectrum of conserved and polymorphic microbial and host molecules [14,15]. In addition, their signalling cascades converge on qualitatively similar and readily tuneable transcriptional outputs [3,15,16]. Further studies have reported evidence of cross-talk and synergy between NLR and PRR machineries [6*,17–19*]. Indeed in *Arabidopsis*, a fully effective NLR-triggered ETI response requires PTI receptor activation of key downstream mediators, such as cell-surface NADPH oxidases and MAP kinase cascades orchestrating apoplastic and transcriptional defences [20*,21*]. Reciprocally, certain surface LRR-RP receptors that can recognize general or strain-specific pathogen molecules, utilize the ETI signalling components *EDS1* and *PAD4* for resistance [22*,23*]. Hence, there is no longer a strict dichotomy between PTI initiated outside cells and intracellular ETI [24].

Figure 1



Recruitment of EDS1 family proteins in *Arabidopsis* RRS1-S/RPS4 immunity and resistance to green peach aphid. EDS1 forms exclusive, stable heterodimers with PAD4 or SAG101, which have different roles in pathogen immunity. In *Arabidopsis*, PAD4 contributes to GPA resistance without EDS1 or SAG101. On the left side, the image shows a well-studied TNL receptor pair (RRS1-S/RPS4) in *Arabidopsis* accession Columbia, which recognizes a bacterial effector avrRps4 after its delivery into host cells. Effector-activated RPS4 TIR domains have NAD^+ hydrolysis activity (indicated by black star) that is necessary for TNL signalling via the *EDS1* node. The RRS1-S/RPS4 ETI response is driven by two *EDS1* modules, *EDS1-SAG101* and *EDS1-PAD4*, functioning with different CC_{HeLo}-domain helper NLRs (RNLs), respectively, *NRG1* and *ADR1*. The two *EDS1* modules contribute to different extents to host cell death and pathogen resistance. Signalling by both heterodimers requires similar but non-identical surfaces in the C-terminal EP-domains (marked with an orange circle). On the right side, the image shows *Arabidopsis* PAD4 deploying its N-terminal LLD with an open α/β-hydrolase pocket (grey circle) to confer GPA resistance, possibly triggered by an aphid-derived elicitor or effector recognized at the host cell surface. A requirement for catalytic triad residues $PAD4^{S118/D178}$ but not $PAD4^{H229}$ in aphid resistance suggests binding of a ligand rather than enzymatic activity is important for PAD4 function. In the response to aphids the PAD4 EP-domain (EPD) is dispensable, revealing a degree of PAD4 multitasking in biotic stress signalling. Original images created with BioRender.com.

Diagnostic features of the EDS1 family

EDS1, PAD4 and SAG101 make up the EDS1 family that arose early in seed plant evolution on top of pre-existing sensor and helper NLR and core phytohormone systems [6*,25–27*]. The EDS1, PAD4 and SAG101 proteins are characterized by an N-terminal lipase-like domain (LLD) fused to a unique C-terminal α-helical bundle arrangement referred to as the EP-domain (PFAM database: PF18117) [6*,28]. Although EDS1 family LLDs have an α/β hydrolase architecture resembling eukaryotic class-3 lipase enzymes [29], genetic and structure–function analyses suggest that EDS1 family proteins behave as pseudo-enzymes [28,30–33]. Although the conserved *Arabidopsis* EDS1 and PAD4 catalytic Ser-Asp-His (S-D-H) triad residues are fully dispensable for pathogen basal immunity and ETI [28], *Arabidopsis* PAD4 employs its α/β hydrolase catalytic pocket to limit green peach aphid (GPA) proliferation [30,33]. Notably, the PAD4 LLD is able to confer GPA resistance without its EP-domain or *EDS1* and *SAG101* (Figure 1) [33], highlighting a distinctive *Arabidopsis* PAD4 LLD activity in biotic stress signalling. Phytohormone receptors such as *Arabidopsis* KAI2 and *Arabidopsis* and rice GID1 use modified α/β hydrolase pockets for hormone binding, which initiates a conformational change necessary for signalling [34]. The question remains whether PAD4 and/or other EDS1 family members bind specific ligands at their LLDs during signal relay.

Arabidopsis EDS1 forms mutually exclusive heterodimers with PAD4 and SAG101, which are essential for basal immunity and ETI against pathogens (Figure 1) [26**,28,32,35,36*]. Interrogation of *Arabidopsis* EDS1-SAG101 protein crystal and solution structures, and a derived EDS1-PAD4 model, shows how the juxtaposed partner LLDs confer dimerization through an EDS1 hydrophobic helix (αH) fitting into similar hydrophobic grooves of PAD4 or SAG101 [28,32]. This N-terminal interface stabilizes a weaker but crucial interaction between partner EP-domain α-helices, creating surfaces around a cavity that are essential for pathogen immunity [26**,28,35,37*]. EDS1 dimerization with PAD4 or SAG101 is probably a general feature of this immunity node since it was observed in orthologues from unrelated species [6*,26**,31,35].

EDS1 family coevolved functions with helper NLRs

Two conserved families of helper NLRs, ADR1s and NRG1s, mediate NLR receptor signalling and are especially important in TNL ETI and basal immunity [11*,12*,38–41*] (Figure 1). ADR1 and NRG1 proteins share a 4-helix bundle HET-S/LOP-B (HeLo) domain with fungal and mammalian cell death executors and an *Arabidopsis* immunity component, RPW8, and are therefore called RNLs (or CC_{HeLo}-NLRs) [11*,12*,42,43]. A further interesting family of HeLo-domain containing proteins was discovered in *Arabidopsis* that is structurally and functionally related to the mammalian inflammatory (necroptotic) cell death executor MLKL [44,45*] and promotes TNL ETI and basal immunity [46**]. Hence, the CC_{HeLo} domain appears to have been recruited in animals and plants for immunity and/or cell death-related functions.

Phylogenomic studies have shown that EDS1 and PAD4 orthologues are present across seed plant (Angiosperm and Gymnosperm) lineages, overlapping with the distribution of CNLs and ADR1 family RNL genes in seed plants. In contrast, SAG101 orthologues and NRG1-family RNLs are absent from Gymnosperms, monocots and some eudicot clades and thus show a similar occurrence pattern as TNLs, although TNL genes are found in conifer genomes [6*,26**,27*,47]. These occurrence patterns suggest cooperation between SAG101 and NRG1 proteins in TNL ETI, and a functional alliance between PAD4 and ADR1s that goes beyond TNL receptor signalling. Disease resistance phenotypes of *Arabidopsis* and *Nicotiana benthamiana* immunity pathway mutants indeed support a central role of NRG1 family RNLs in EDS1-SAG101 controlled TNL ETI responses [26**,35,38*–41*]. Strikingly, only within-clade combinations of *Arabidopsis* NRG1, EDS1 and SAG101 could reconstitute TNL immunity and host cell death in *N. benthamiana* transient assays [26**]. It therefore appears that coevolved proteins when present together form an effective EDS1-SAG101-NRG1 signalling module in TNL ETI [41*].

Linking TNL receptor activation to EDS1 signalling

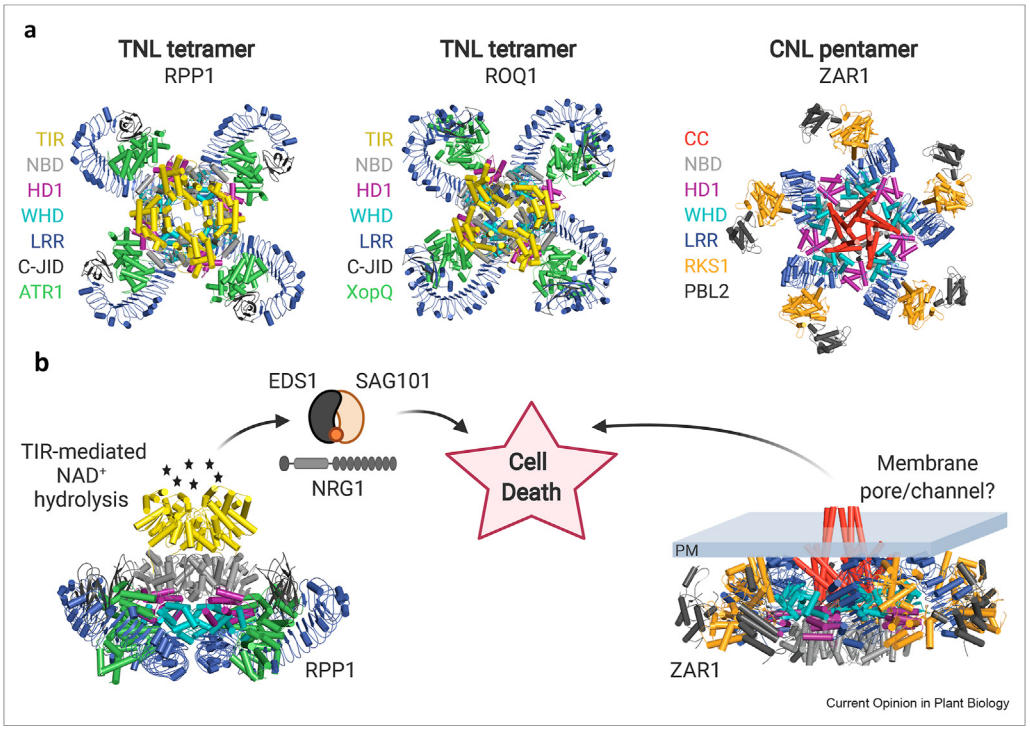
The fact that all studied pathogen-activated and autoactive TNLs converge on *EDS1* for immunity and cell death suggests that EDS1 bridges TNL receptor activation to downstream pathways [3,8]. Reinforcing earlier evidence of EDS1 association with several nucleocytoplasmic TNL proteins [6*], the tobacco TNL receptor N quantitatively enriched EDS1 by TurboID proximity labelling *in vivo* [13**]. It will be important to establish the TNL–EDS1 interaction dynamics and how they relate functionally to TNL post-activation events. A breakthrough in understanding the connectivity between TNLs and EDS1 was the demonstration that TIR self-association leads to hydrolysis of NAD^+, and in the case of plant TIR-domain proteins, *EDS1*-dependent immunity and cell death [48–52*]. The cryo-EM structures of two pathogen effector-activated TNL receptor complexes, *Arabidopsis* RPP1 and tobacco ROQ1, provided another major advance [53**,54**] (Figure 2a). Both TNLs form a stable tetramer in which the four TIR domains are orientated as two asymmetric pairs, creating a TIR NADase holoenzyme. The enzymatically active TNL RPP1 oligomer assembles *in vitro* without participation of EDS1 family proteins [53**]. It is therefore possible that one or more TIR-generated *in vivo* NAD^+ hydrolysis products enables EDS1-SAG101 downstream signalling, perhaps by binding to the essential heterodimer EP-domain cavity surface (Figure 2b) [26**,35,37*].

The cryo-EM structure of an activated CNL receptor, *Arabidopsis* ZAR1 [55**], is worth considering here because it reveals a different initial signalling mechanism compared to TNL receptors. In the induced ZAR1 pentamer, N-terminal α1 helices become exposed and assemble to form a potential membrane-associated pore or ion channel (Figure 2a,b) [55**,56]. Some sensor and helper CNLs have ZAR1-like α1 helix motifs [41*,57,60]. The sensor CNL *Arabidopsis* RPM1, ADR1-family RNLs and an autoactive form of NRG1.1, were found to interact functionally with the plasma membrane in a manner similar to CC_{HeLo}-containing MLKLs, where they might cause membrane permeabilization leading to Ca^{2+} influx [39*,46**,58–60]. Putting these data together suggests that CNL and TNL receptor-initiated signalling converges, in different ways, onto host membrane compartments as part of the ETI response (Figure 2b). A signature of mammalian pyroptotic and necroptotic immune responses is release of pro-inflammatory molecules through structured cell membrane pores, which potentiates resistance in surrounding cells and tissues [44,45*]. It will be important in the future to establish how plant NLR-mediated membrane disturbance or pore/ion channel formation relates to orchestrated nuclear transcriptional changes and to resistance signal propagation in surrounding cells and tissues.

EDS1-SAG101-NRG1 and EDS1-PAD4-ADR1 modules operate differently in immunity

In *Arabidopsis*, two genetically non-interchangeable *EDS1* modules signal in ETI. The first involves EDS1-SAG101 dimers with NRG1 family RNLs and is engaged by TNL receptors to mediate cell death and pathogen resistance (Figure 1) [26**,39*–41*]. In *Arabidopsis* and *N. benthamiana*, TNL activation-dependent association was detected between the EDS1-SAG101 dimer and NRG1 family proteins, underpinning their cooperation in ETI [41*]. Formation of

Figure 2



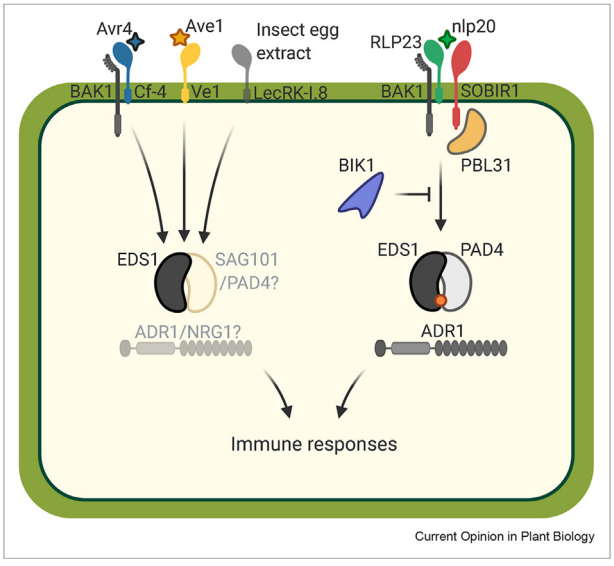
Pathogen-activated TNL and CNL receptor oligomers initiate signalling differently in ETI. **(a)** Left: Top view of *Arabidopsis* TNL RPP1 tetramer after activation by oomycete effector ATR1 direct binding to the LRR and C-JID domains (PDB: 7CRC). Middle: Top view of tobacco TNL ROQ1 tetramer after activation by *Xanthomonas* effector XOPQ direct binding to the LRR and C-terminal (C-JID) domains (Assembly of PDB: 7JLX, 7JLU and 7JLV). Right: Top view of *Arabidopsis* CNL ZAR1 pentamer with bound proteins RKS1 and PBL2 (PDB: 6J5T), activated by bacterial effector modification of PBL2. Different domains in the TNL RPP1, ROQ1 and CNL ZAR1 oligomers are colour-coded. In RPP1, ROQ1 and ZAR1, effector-induced conformational changes of the LRR, NBD, HD1 and WHD domains lead to a reorientation of respective N-terminal TIR and CC domains to initiate ETI signalling. **(b)** Left: Side view of the RPP1 tetramer. Effector-induced assembly orientates four TIR domains (yellow) as two asymmetric pairs, creating two holoenzyme active sites for hydrolysing NAD^+. In a model, the EDS1-SAG101 heterodimer in a TNL-induced complex with NRG1-family RNLs integrates predicted NAD^+-derived *in vivo* signal(s) (black stars) leading to host cell death and immunity. EDS1-NRG1.1 association depends on an intact NRG1.1 P-loop and the EDS1 EP domain cavity (orange circle in EDS1-SAG101 heterodimer). Right: Side view of the pathogen-activated ZAR1 pentamer. In contrast to RPP1 and ROQ1, ZAR1 induced cell death does not depend on EDS1 family proteins or RNLs. In a current model, the ZAR1 oligomer associates with the plasma membrane (PM) where exposed N-terminal α1 helices assemble into a membrane pore or channel, potentially releasing host defence-potentiating molecules and/or promoting inward Ca^{2+} ion fluxes to drive intracellular signalling cascades and transcription. Original images created with BioRender.com.

the EDS1-SAG101-NRG1 complex required both a functional EDS1 EP-domain and an intact NRG1 ADP/ATP-binding site (called the P-loop), but not essential N-terminal NRG1 residues modelled onto pore-forming α1-helices of the activated ZAR1 oligomer [41*,55**]. These data suggest that TNL-triggered EDS1-SAG101-NRG1 association is an important step in ETI signalling [41*]. In the second module, EDS1-PAD4 dimers with ADR1 family RNLs promote a basal immunity response that is not specific to TNL-initiated ETI [26**,37*,41*,61,62]. Although ADR1 RNLs were enriched with PAD4 in immune-activated *Arabidopsis* leaf extracts [41*], the interaction dynamics and co-functions of these components remain poorly understood. A hallmark of the *Arabidopsis* EDS1-PAD4-ADR1 node is rapid transcriptional mobilization of salicylic acid (SA)-dependent local and systemic resistance pathways [63], and dampening of SA-antagonizing jasmonic acid (JA) pathways [37*,40*,64–66*]. Genetic dissection of the *Arabidopsis* basal immunity network showed that *EDS1*, *PAD4* and *ADR1* family RNLs work in parallel with SA, enabling mutual reinforcement and protection against pathogen interference [41*,62,65,67]. Notably, in a rice basal immune response to virulent *Xanthomonas* bacteria, *EDS1* steered the phytohormone network towards JA signalling in resistance [31]. It will be interesting to dissect the roles of EDS1, PAD4 and ADR1 in monocot plant species, such as rice, which lack TNL receptor genes [25] and might use EDS1 and PAD4 to wire SA-JA phytohormone crosstalk and other antimicrobial pathways differently.

In *Arabidopsis*, the two EDS1 family - RNL modules contribute unequally to transcriptional reprogramming,

pathogen resistance and host cell death in ETI triggered by different TNLs [26**,39*,41*,66*] (Figure 1). For most *Arabidopsis* TNLs, signalling via *PAD4* and a family of three functionally redundant *ADR1* RNLs is sufficient to limit pathogen growth. In other cases, TNL immunity relies more on *SAG101* and two redundant *NRG1* RNLs [39*–41*]. It is revealing that a specific *Arabidopsis* TNL autoimmune variant, *chs3-2D*, signals via one of two *EDS1* paralogs (in accession Columbia) and *SAG101*, but not *PAD4* [68]. In *N. benthamiana*, various TNLs did not employ *PAD4* or *ADR1 RNLs* for ETI [26**,35], consistent with a curious absence of measurable basal immunity in this solanaceous species to tested virulent bacteria [38*]. Although the molecular basis for TNL preference for one or other EDS1 branch is not clear, we have proposed that strength of the initial TNL-effector signal is an important factor, with an EDS1-SAG101-NRG1 module dominating at high TNL amplitude in directly responding cells and EDS1-PAD4-ADR1s being engaged at lower amplitude, as observed around ETI foci [6*]. Molecular evidence also suggests that different surface properties of the EDS1-SAG101 and EDS1-PAD4 dimers, potentially binding distinct molecules, contribute to the decision-making for their recruitment in TNL ETI or basal immunity [26**,36*,37*].

Figure 3



Contributions of the EDS1 and PAD4 to cell-surface PRR signalling. In tomato, cell-surface LRR-RP Cf-4 together with LRR-RK co-receptor BAK1, and LRR-RP Ve1 recognize, respectively, fungal apoplastic effectors Avr4 and Ave1 and require *EDS1* for immunity signalling. In *Arabidopsis*, recognition of an insect egg extract requires the LRR-RK LecRKI-1.8, leading to *EDS1*-dependent immunity. It remains unclear whether these PRR systems in tomato and *Arabidopsis* signal via the EDS1-SAG101 or EDS1-PAD4 modules, potentially working with NRG1 or ADR1 family RNLs. In *Arabidopsis*, RLP23 specifically recognizes a pathogen elicitor peptide, nlp20. RLP23-nlp20 triggered defence signalling promoting pathogen resistance relies on RLK co-receptors SOBIR1 and BAK1, and a mobile receptor-like cytoplasmic kinase (RLCK), PBL31. Another RLCK, BIK1, antagonizes the RLP23-nlp20 response. In RLP23-triggered immunity, signals initiated at the plasma membrane converge mainly on the EDS1-PAD4 heterodimer EP-domain and ADR1s. Hence, the EDS1-PAD4-ADR1 module is a likely point of signal convergence between intracellular NLRs and extracellular PRRs. Actions of EDS1-PAD4 heterodimers with ADR1s in PRR-mediated defence potentiation might provide a mechanistic basis for host basal immunity which operates against virulent pathogens and reinforces NLR ETI foci. Original image created with BioRender.com.

EDS1-PAD4 as integrators of cell surface and intracellular receptor signalling

Given the evidence for cooperating NLR and PRR signalling systems in the potentiation of plant immune responses [14,20*,21*,69], it is worth examining more closely *EDS1*, *PAD4* and *ADR1 RNL* contributions to PRR responses elicited outside cells [2*,15]. A number of plasma membrane-anchored LRR-RPs signal via *EDS1* (Figure 3). For example, insect (*Pieris brassicae*) egg elicitor-induced cell death and defence gene expression in *Arabidopsis* required an LRR-RP *LecRK-I.8* and *EDS1* [70,71]. Tomato LRR-RPs Ve1 and Cf4 mediating ETI-like specific recognition of fungal apoplastic effectors, were found to signal via RLK co-receptor *BAK1* and *EDS1* to induce immune responses [6*].

Comparative studies of *Arabidopsis* LRR-RK and LRR-RP signalling via the mobile receptor-like cytoplasmic kinase (RLCK) BIK1 [72] and related RLCKs, established a positive BIK1 role in responses mediated by the LRR-RK PRRs, EFR and FLS2, but BIK1 antagonism of signalling by an LRR-RP, RLP23 [73*]. RLP23 is activated by a peptide ligand (nlp20) derived from a widespread family of variable pathogen elicitors [74,75]. RLP23-nlp20 induced transcriptional defences, which include a prominent ethylene response, required a different RLCK, *PBS31*, as well as *EDS1*, *PAD4* and *ADR1*, but not *SAG101* or *NRG1* [22*]. Another *Arabidopsis* study reports involvement of PRR-activated TIR-domain and TNL genes, as well as *PAD4* and *ADR1s*, in conferring PTI [23*]. Further dissection of RLP23 signalling shows that it utilizes the same EDS1-PAD4 EP-domain cavity surface that mediates the basal immunity branch of TNL ETI [22*,37*]. Interestingly, *Arabidopsis BIK1* also antagonizes the expression of *PAD4* and *PAD4*-dependent resistance to GPA infestation [76], suggesting that the host response to aphid feeding, which needs a PAD4 LLD function without EDS1 (Figure 1) [33], is part of a common regulatory network for cell surface-triggered anti-microbial and pest defences. Collectively, these findings make a strong case for recruitment of the EDS1-PAD4-ADR1 basal immunity node by both NLRs and PRRs in anti-pathogen defence. Hence, EDS1-PAD4 with ADR1 RNLs potentially represent a point of signal integration and

synergy between intracellular and surface receptor systems in plant innate immunity.

Conclusions

We present here recent advances in understanding how the EDS1 node works in plant immunity. There are many pressing questions. For example, it is not known if EDS1-SAG101 or EDS1-PAD4 complexes interact directly with their respective cooperating RNLs, NRG1 and ADR1. Establishing whether this is the case, where functional complexes locate in the cell, and what they do biochemically would be an important step forward. It will also be useful to determine the mechanisms by which cell surface and intracellular receptor systems converge on the EDS1-PAD4-ADR1 node and whether outputs in both cases define what we observe as basal immunity, potentially also recruiting TIR-domain proteins. Establishing how cross-talk and synergy between intracellular and extracellular receptor systems work will be a further goal. Moreover, while data suggest different spatial characteristics for the two EDS1 modules in cells and tissues, little is known about the dynamics of these response systems and other components or molecules directly involved. Another mechanistic question is whether authentically activated RNLs function at specific host membranes, and if so, do they form a structured pore or ion channel, which might permit ion influxes to orchestrate transcription. Related to this, it will be fascinating to establish the contribution of plant host cell death in ETI and whether formation of structured membrane pores or channels enables release of defence signals to surrounding cells as seen in mammalian pro-inflammatory death.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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