Review

New Phytologist

Tansley insight

Interplay between cell-surface receptor and intracellular NLR-mediated immune responses

Author for correspondence:
Jiorgos Kourelis
Email: jiorgos.kourelis@tsl.ac.uk

Jiorgos Kourelis

The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, NR4 7UH, Norwich, UK

Received: *7 April 2023*
Accepted: *28 June 2023*

Contents

New Phytologist (2023) **240:** 2218–2226
doi: 10.1111/nph.19212

Key words: cell-surface immunity, cellular signalling, intracellular NLR-mediated immunity, phosphorylation cascade, plant immunity.

Summary

The functional link between cell-surface receptors and intracellular NLR immune receptors is a critical aspect of plant immunity. To establish disease, successful pathogens have evolved mechanisms to suppress cell-surface immune signalling. In response, plants have adapted by evolving NLRs that recognize pathogen effectors involved in this suppression, thereby counteracting their immune-suppressing function. This ongoing co-evolutionary struggle has seemingly resulted in intertwined signalling pathways in some plant species, where NLRs form a separate signalling branch downstream of activated cell-surface receptor complexes essential for full immunity. Understanding these interconnected receptor networks could lead to novel strategies for developing durable disease resistance.

I. Introduction

Plants have an effective innate immune system which relies on the activation of cell-surface and intracellular immune receptors to recognize pathogen molecules to defend against a wide range of pathogens. Cell-surface immune receptors, including receptor-like kinases (RKs or RLKs) and receptor-like proteins (RPs or RLPs), detect conserved pathogen-derived or induced molecular signatures, often referred to as either pathogen-, microbe-, or damage-associated molecular patterns (PAMPs, MAMPs, or DAMPs, respectively), as well as specialized pathogen-secreted effectors (Albert *et al.*, 2020). These effectors manipulate the host environment to benefit the pathogen by suppressing immune responses or promoting nutrient production. Recognition is mediated by various extracellular ligand-binding domains, such as lectin domains, LysM domains, malectin-like domains, and leucine-rich repeat (LRR) domains (Dievart *et al.*, 2020). RKs and RPs contain a single helical transmembrane domain, with RKs having an intracellular protein kinase domain for signalling, while RPs have a small cytoplasmic tail (Dievart *et al.*, 2020). Both RKs and RPs play crucial roles in plant growth, development, abiotic stress responses, and symbiotic interactions, in addition to their role in biotic stress responses (Dievart *et al.*, 2020).

Jiorgos Kourelis is a finalist of the 2023 *New Phytologist* Tansley Medal competition for excellence in plant science. See Slater & Dolan (2023, 240: 2171–2172) for more details.

Pathogen-secreted effectors can also act within the host nucleocytoplasm. In response, plants have evolved a diverse repertoire of intracellular immune receptors, predominantly belonging to the nucleotide-binding domain and leucine-rich repeat-containing protein (NLR) family. Flowering plant NLRs share a multidomain architecture, characterized by a central NB-ARC (for nucleotide-binding adaptor shared by APAF-1, certain *R* gene products, and CED-4) domain, a C-terminal LRR domain, and an N-terminal domain involved in initiating downstream signalling (Kourelis *et al.*, 2021). Upon activation, various types of NLRs oligomerize into 'resistosome' structures, serving dual roles in pathogen recognition and signal transduction (Förderer *et al.*, 2022). Some NLRs have specialized functions in pathogen recognition or signal transduction as either 'sensor NLRs' or 'helper NLRs', which can operate in pairs or within more complex networks (Wu *et al.*, 2017).

Given the importance of cell-surface signalling for pathogen recognition and immunity, pathogens developed strategies to disrupt this signalling process at virtually all stages of the cascade. In response, intracellular NLR immune receptors evolved to specifically recognize this manipulation. A picture is now emerging of how this continuous co-evolutionary battle gave rise to intertwined cell-surface and NLR signalling pathways in plants (Table 1).

II. Convergence and diversification of intracellular NLR immune receptors in 'guarding' cell-surface receptor signalling in plants

Cell-surface receptors play a pivotal role in plant immunity against pathogens and pests by initiating a conserved signalling cascade upon ligand perception. Ligand recognition by receptor kinases (RKs) and receptor proteins (RPs) results in hetero-oligomerization with co-receptor RKs: LRR-containing RKs interact with SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 3 (SERK3 or BAK1), while LRR-containing RPs constitutively associate with SUPPRESSOR OF BAK1-INTERACTING RECEPTOR-LIKE KINASE 1 (BIR1; SOBIR1) and engage SERK3 upon activation. These receptor/co-receptor complexes then activate a conserved phosphorylation cascade which includes direct and indirect activation of PBS1-LIKE (PBL) receptor-like cytoplasmic kinases (RLCKs), which lack extracellular ligand-binding domains and are also known as RLCK subfamily VII, mitogen-activated protein kinase (MAPK) phosphorylation cascades, leading to a series of events including Ca^{2+} influx, activation of Ca^{2+}-dependent protein kinases, callose deposition, reactive oxygen species formation, and transcriptional reprogramming (DeFalco & Zipfel, 2021).

Due to the importance of this immune signalling, pathogens have evolved various mechanisms to disrupt this signalling, both extracellularly (Buscaill & van der Hoorn, 2021), and at virtually every intracellular signalling stage (Toruño *et al.*, 2016). In response to the manipulation of intracellular targets by pathogen effectors, plants have evolved intracellular NLR immune receptors capable of both direct and indirect effector recognition. Indirect recognition is described by the guard and decoy models, in which an intracellular effector target is either 'guarded' by an NLR as a 'guardee', or acts as a 'decoy' with the sole purpose of indirect effector recognition (van der Hoorn & Kamoun, 2008). There are many known examples of NLRs guarding signalling downstream of plant cell-surface receptor-mediated immunity (Table 1; Fig. 1).

NLRs, among the most rapidly diversifying genes in plant genomes, exhibit low conservation even between closely related species. However, some highly conserved NLRs are involved in recognizing the manipulation of signalling downstream of cell-surface receptors. For example, the Arabidopsis coiled-coil (CC)-type NLR ZAR1 indirectly recognizes multiple bacterial effectors through ZED1-related kinases (ZRKs, also known as RLCK subfamily XII-2) and their effector-induced interactions with various PBLs (Laflamme *et al.*, 2020; Martel *et al.*, 2020). ZAR1 is one of the most conserved NLRs found in flowering plants, present across eudicots, some lineages of basal flowering plants, and certain monocot species (Adachi *et al.*, 2022; Gong *et al.*, 2022). The requirement of ZRKs for ZAR1 function is also conserved across these species (Schultink *et al.*, 2019; Adachi *et al.*, 2022; Gong *et al.*, 2022). The ancient origin of ZAR1 suggests early convergence of intracellular NLRs on guarding components downstream of cell-surface receptors in flowering plant evolution. ZRKs appear to have originated from a wall-associated kinase (WAK)-RK, through the loss of extracellular ligand-binding domains and several key features required for kinase activity (Gong *et al.*, 2022). Effector-induced interaction of ZRKs with PBLs leads to the oligomerization of ZAR1 into a pentameric resistosome structure characteristic of activated plant CC-type NLRs, which acts as a Ca^{2+}-permeable pore on the plasma membrane (Wang *et al.*, 2019; Hu *et al.*, 2020; Bi *et al.*, 2021).

Similarly, the tomato NLR Prf, which has an ancient origin and is functionally conserved across the Solanaceae family, interacts with RLCKs encoded by genes in genomically co-occurring clusters (Salmeron *et al.*, 1996). For instance, the RLCK Pto is required for Prf-dependent recognition of *Pseudomonas* kinase-inhibiting effector AvrPto and E3 ubiquitin ligase effector AvrPtoB (Ntoukakis *et al.*, 2014). More recently evolved NLRs have also converged on guarding similar targets downstream of activated cell-surface receptors (Table 1). Both the unrelated Arabidopsis NLR RPS5 and the barley NLR Pbr1 interact with PBLs for the recognition of the *Pseudomonas* protease effector AvrPphB (Carter *et al.*, 2019). Meanwhile, the Arabidopsis NLR SUMM2 can perceive the perturbation of a MAPK signalling cascade (Zhang *et al.*, 2012).

Although NLRs often guard evolutionarily conserved components downstream of cell-surface receptors, it seems that in some instances, these guarding NLRs have evolved into a secondary signalling pathway and are now necessary for the full immune function of cell-surface receptors.

III. The EDS1–PAD4–ADR1 module enhances cell-surface immunity in Arabidopsis

In Arabidopsis, some NLRs have not only converged on guarding the signalling downstream of activated cell-surface receptors, but have also evolved to enhance the immunity mediated by these receptors (Fig. 2).

ADR1 and NRG1 type helper NLRs form networks that are required for immunity upon activation of Toll/Interleukin-1

Table 1 Summary of characterized NLRs guarding and/or enhancing immunity downstream of activated cell-surface receptors.

Type	Species	NLR	Receptor protein/ Guardee/Decoy	Guardee/Decoy type	Recognition of (biochemical function)
Guarding	*Arabidopsis thaliana*	CSA1 (CONSTITUTIVE SHADE AVOIDANCE 1) with CHS3 (CHILLING SENSITIVE 3)	BAK1 (BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1)	SERK3	HopB1 (Serine protease)[1–3]
			BAK1 and BIR3 (BAK1-INTERACTING RECEPTOR-LIKE KINASE 3)	SERK3 and LRR-X RK subfamily	Loss-of-function autoimmunity[3]
		RPS5 (RESISTANCE TO *PSEUDOMONAS SYRINGAE* 5)	PBS1 (AVRPPHB SUSCEPTIBLE 1)	PBL	AvrPphB (Cysteine protease)[4]
		SUMM2 (SUPPRESSOR OF MKK1 MKK2 2)	MEKK1 (MAPK KINASE KINASE 1)	MAPKKK	Loss-of-function autoimmunity[5]
			MKK1 and MKK2 (MAPK KINASE 1 and 2)	MAPKK	Loss-of-function autoimmunity[5]
			MPK4	MAPK	Loss-of-function autoimmunity[5] HopAI1 (Phosphothreonine lyase)[5]
			MEKK2 (MAPK KINASE KINASE 2)	MAPKKK	Required for *mekk1*, *mkk1/2*, *mpk4* autoimmunity[6]
			LLG1 (LORELEI-LIKE 1)	GPI-anchored protein	Required for *mekk1*, *mkk1/2*, *mpk4*, autoimmunity[7]
			LET1 and LET2 (LETHALITY SUPPRESSOR OF MEKK1 1 and 2)	CrRLK1L-RK	Required for *mekk1*, *mkk1/2*, *mpk4*, autoimmunity[7,8]
			CRCK3 (CALMODULIN-BINDING RLCK 3)	RLCK	Required for *mekk1*, *mkk1/2*, *mpk4*, and *MEKK2* overexpression autoimmunity[9]
		ZAR1 (HOPZ-ACTIVATED RESISTANCE 1)	RKS1 (RESISTANCE RELATED KINASE 1) and PBL2	ZRK-type RLCK and PBL	AvrAc (Uridine 5′-monophosphate transferase)[10]
			ZED1 (HOPZ-ETI-DEFICIENT 1) and SZE1 (SUPPRESSOR OF ZED1-D1)	ZRK-type RLCK and PBL	HopX1i and HopX1d (Unknown)[11,12]
			ZED1 and SZE1, SZE2, PBL4, PBL5, PBL15, PBL17 or PBL18	ZRK-type RLCK and PBLs	HopZ1a (Acetyltransferase)[13–15]
			Zed1-D and SZE1 or SZE2	ZRK-type RLCK and PBLs	Temperature-sensitive autoimmunity[15,16]
			ZRK2	ZRK-type RLCK	HopBA1a (Unknown)[11,12]
			ZRK3	ZRK-type RLCK	HopO1c (ADP-ribosyltransferase)[11,12]
			ZRK3 and PBL27	ZRK-type RLCK and PBL	HopF1r (ADP-ribosyltransferase)[17,18] Zaractin (Small molecule)[18]
	Hordeum vulgare subsp. *vulgare*	Pbr1 (AvrPphB Response 1)	HvPbs1-1 or HvPbs1-2	PBL	AvrPphB (Cysteine protease)[19]
	Nicotiana benthamiana	NbZAR1 (*Nicotiana benthamiana* ZAR1)	JIM2 (XOPJ4 IMMUNITY 2)	ZRK-type RLCK	XopJ4 (Acetyltransferase)[20] HopZ5 (Acetyltransferase)[21] AvrBsT (Acetyltransferase)[21]
	Solanum lycopersicum	Prf (*Pseudomonas* resistance and fenthion sensitivity)	Pto	RLCK	AvrPto (Kinase inhibitor)[22] AvrPtoB (E3 ubiquitin ligase/ kinase inhibitor)[22]
			Fen	RLCK	Fenthion (Insecticide)[23]
			LescPth5	RLCK	AvrPto (Kinase inhibitor)[24]

Table 1 (Continued)

Type	Species	NLR	Receptor protein/ Guardee/Decoy	Guardee/Decoy type	Recognition of (biochemical function)
Immunity-enhancing	*Arabidopsis thaliana*	EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1)–PAD4 (PHYTOALEXIN-DEFICIENT 4)–ADR1 (ACTIVATED DISEASE RESISTANCE 1) module and to a lesser extent EDS1–SAG101 (SENESCENCE-ASSOCIATED GENE 101)–NRG1 (N REQUIREMENT GENE 1) module	RLP23	LRR-RP	Microbial nlp20[25,26]
			RLP32	LRR-RP	Proteobacterial IF1[25]
			RLP1	LRR-RP	Bacterial eMax[25]
			FLS2 (FLAGELLIN-SENSITIVE 2)	LRR-RK	Bacterial flg22[26]
		CSA1 with CHS3. EDS1–PAD4–ADR1 module and to a lesser extent EDS1–SAG101–NRG1 module	RLP42	LRR-RP	Fungal pg13 and pg23[25,27]
	Solanaceae	NRC (NLR-REQUIRED FOR CELL DEATH)–dependent	REL (RESPONSIVE TO ELICITINS)	LRR-RP	Oomycete elicitins[28,29]
			Cf-2	LRR-RP	*Cladosporium fulvum* AVR2[30]
			Cf-5	LRR-RP	*Cladosporium fulvum* AVR5[30]
			Cf-9	LRR-RP	*Cladosporium fulvum* AVR9[30]
			LeEIX2	LRR-RP	Fungal ethylene-inducing xylanase (EIX)[31]
		NRC-dependent and EDS1-dependent*	Cf-4	LRR-RP	*Cladosporium fulvum* AVR4[30,32]
			Ve1	LRR-RP	Fungal Ave1 homologues[32,33]
		EDS1-dependent*	I	LRR-RP	*Fusarium oxysporum* AVR1[33,34]
			I-7	LRR-RP	Unknown *Fusarium oxysporum* effector[35]

[1]Li *et al*. (2016), [2]Wu *et al*. (2020), [3]Schulze *et al*. (2022), [4]Shao *et al*. (2003), [5]Zhang *et al*. (2012), [6]Kong *et al*. (2012), [7]Huang *et al*. (2020), [8]Liu *et al*. (2020), [9]Zhang *et al*. (2017), [10]Wang *et al*. (2015), [11]Laflamme *et al*. (2020), [12]Martel *et al*. (2020), [13]Lewis *et al*. (2013), [14]Bastedo *et al*. (2019), [15]Liu *et al*. (2019), [16]Wang *et al*. (2017), [17]Seto *et al*. (2017), [18]Seto *et al*. (2021), [19]Carter *et al*. (2019), [20]Schultink *et al*. (2019), [21]Ahn *et al*. (2022), [22]Kim *et al*. (2002), [23]Salmeron *et al*. (1996), [24]Chang *et al*. (2002), [25]Pruitt *et al*. (2021), [26]Tian *et al*. (2021), [27]Schulze *et al*. (2022), [28]Gabriëls *et al*. (2006), [29]Chen *et al*. (2023), [30]Kourelis *et al*. (2022), [31]Gabriëls *et al*. (2007), [32]Fradin *et al*. (2009), [33]Hu *et al*. (2005), [34]Catanzariti *et al*. (2017), [35]Gonzalez-Cendales *et al*. (2016).
*Not shown to require ADR1 or NRG1.

Receptor (TIR)-NLRs. This activation results in production of TIR-catalysed nucleotides which induce heterodimerization of the lipase-like proteins EDS1 with either PAD4 or SAG101 (Huang *et al*., 2022; Jia *et al*., 2022). This, in turn, induces formation of ADR1 and NRG1 oligomers which act as Ca^{2+} permeable pores at the plasma membrane (Jacob *et al*., 2021). In Arabidopsis, the EDS1–PAD4–ADR1 and, to a lesser degree, EDS1–SAG101–NRG1 modules are also genetically required for triggering a subset of immune responses by LRR-RPs and, to a lesser extent, LRR-RKs (Pruitt *et al*., 2021; Tian *et al*., 2021; Table 1). This might be mediated through activation of the TIR-NLR CSA1, as this TIR-NLR is required for cell-death induction upon ligand recognition by the LRR-RP RLP42, and the loss of *CSA1* or the *EDS1–PAD4–ADR1* module results in enhanced susceptibility to virulent *Pseudomonas syringae* (Pruitt *et al*., 2021; Tian *et al*., 2021; Schulze *et al*., 2022).

Additionally, partial loss of *BAK1* (*SERK3*) and *BAK1-LIKE 1* (*BKK1*) results in autoimmunity which can be alleviated by mutations in the *EDS1–PAD4–ADR1* module or *CSA1*, but complete loss of *BAK1*/*BKK1* is not suppressed by loss of *CSA1* (Wu *et al*., 2020; Schulze *et al*., 2022; Yang *et al*., 2022). Likewise, autoimmunity as the result of a combined loss of *BAK1* and the interacting RK *BIR3* leads to autoimmunity, which is alleviated in *CSA1* loss-of-function lines, and CSA1 directly interacts with BIR3 (Schulze *et al*., 2022; Yang *et al*., 2022). This suggests that cell-surface immunity might be enhanced by CSA1 through EDS1–PAD4–ADR1 and a mechanism that involves loss of BAK1. CSA1 and the genetically linked TIR–NLR CHS3 can also detect the activity of the *Pseudomonas* effector HopB1, an unconventional serine protease that cleaves BAK1 (Li *et al*., 2016; Schulze *et al*., 2022; Yang *et al*., 2022). CSA1 might have evolved to guard against BAK1 perturbation by HopB1 and later became crucial for full immunity triggered by cell-surface receptors (Fig. 2).

Finally, the partial suppression of the *BAK1*/*BKK1* phenotype by *CSA1* or *EDS1–PAD4–ADR1* loss suggests additional NLRs may have converged on this hub. Indeed, autoimmunity due to loss of another RK, *BIR1*, is partly suppressed by loss of *SOBIR1* or *PAD4*, but fully suppressed when both are absent (Gao *et al*., 2009). This indicates that multiple intracellular NLRs may converge on these hubs in Arabidopsis, contributing to both effector recognition and enhanced cell-surface receptor-mediated immunity.

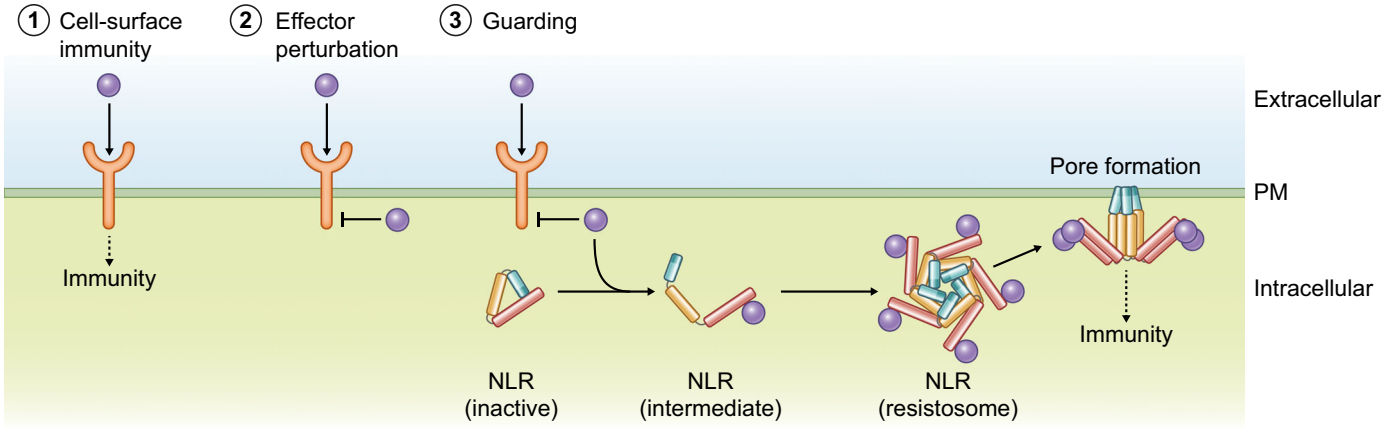

Fig. 1 Intracellular NLR immune receptors have converged on 'guarding' conserved components of the signalling cascade downstream of cell-surface receptors. (1) Ligand recognition by cell-surface receptors initiates a signalling cascade which ultimately results in immunity. (2) In response, pathogens have evolved effectors that act intracellularly and which interfere with the signalling downstream of these activated cell-surface receptors, aiming to suppress the immune response. (3) Countering this, plants have evolved intracellular NLR immune receptors capable of recognizing and responding to these pathogenic perturbations. Once activated, these NLRs directly or indirectly result in the formation of NLR resistosomes at the plasma membrane, a key step in triggering subsequent immune responses.

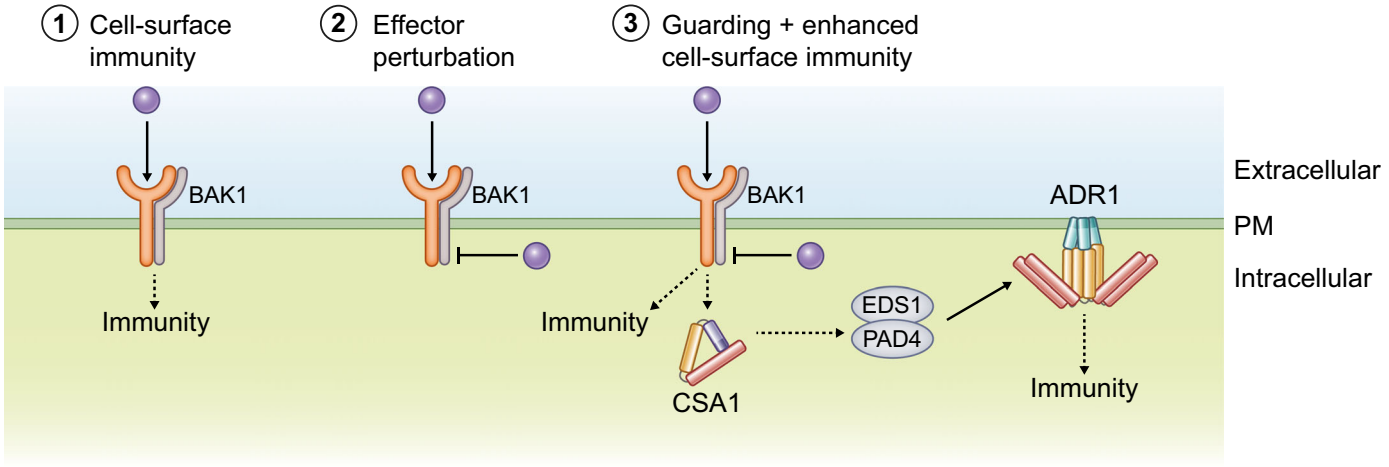

Fig. 2 BAK1 integrity is monitored through an EDS1–PAD4–ADR1 module to enhance cell-surface immunity and recognize pathogen effectors. (1) Ligand recognition by cell-surface receptors initiates a signalling cascade dependent on the co-receptor BAK1 (also known as SERK3) which ultimately results in immunity. (2) In response, pathogens have evolved intracellular effectors, such as the *Pseudomonas* effector HopB1. These effectors interfere with BAK1, aiming to suppress the immune response. (3) The TIR-NLR CSA1 may monitor the integrity of BAK1 through a ternary complex, involving BAK1 and other co-receptors. The loss of BAK1 – either due to cleavage by HopB1, or upon cell-surface receptor activation – likely results in CSA1 activation. The activation of CSA1 leads to the production of TIR-derived nucleotides. These induce dimerization of EDS1 with PAD4 and this heterocomplex in turn activates ADR1, resulting in the formation of ADR1 oligomers which act as Ca^{2+}-permeable pores at the plasma membrane.

IV. Multiple NLR networks connect to cell-surface receptors to mediate immune signalling in Solanaceous plants

Finally, in Solanaceous plants, a network of CC-type NLRs, known as the NRC network, is essential for the hypersensitive cell-death response triggered by numerous LRR-RPs (Table 1).

Within this network, NRCs function as helper NLRs for multiple sensor NLRs to mediate immune responses (Wu *et al.*, 2017). NRCs are also required for the hypersensitive cell-death response triggered by LRR-RP-encoding resistance genes recognizing fungal and oomycete pathogen effectors. This includes Cf-2, Cf-4, Cf-5, and Cf-9 (Gabriëls *et al.*, 2006, 2007; Kourelis *et al.*, 2022), which confer immunity to specific *Cladosporium fulvum* (syn. *Passalora fulva*) isolates, as well as the *Verticillium* resistance gene Ve1 (Fradin *et al.*, 2009), the fungal xylanase EIX responsive receptor LeEIX2 (Gabriëls *et al.*, 2007), and the oomycete elicitin-recognizing REL receptor (Gabriëls *et al.*, 2006; Chen *et al.*, 2023). While this NRC-dependency was initially identified through a virus-induced gene silencing strategy targeting *NRC1*, subsequent research has revealed that NRC3 is actually responsible for Cf-2, Cf-4, Cf-5, and Cf-9 mediated signalling (Kourelis *et al.*, 2022). It is likely that additional NRC-dependent LRR-RPs also use NRC3 for cell-death signalling. This NRC-dependent cell-death response likely requires NRC resistosome signalling and is required for full immunity by at least Cf-4 and Ve1

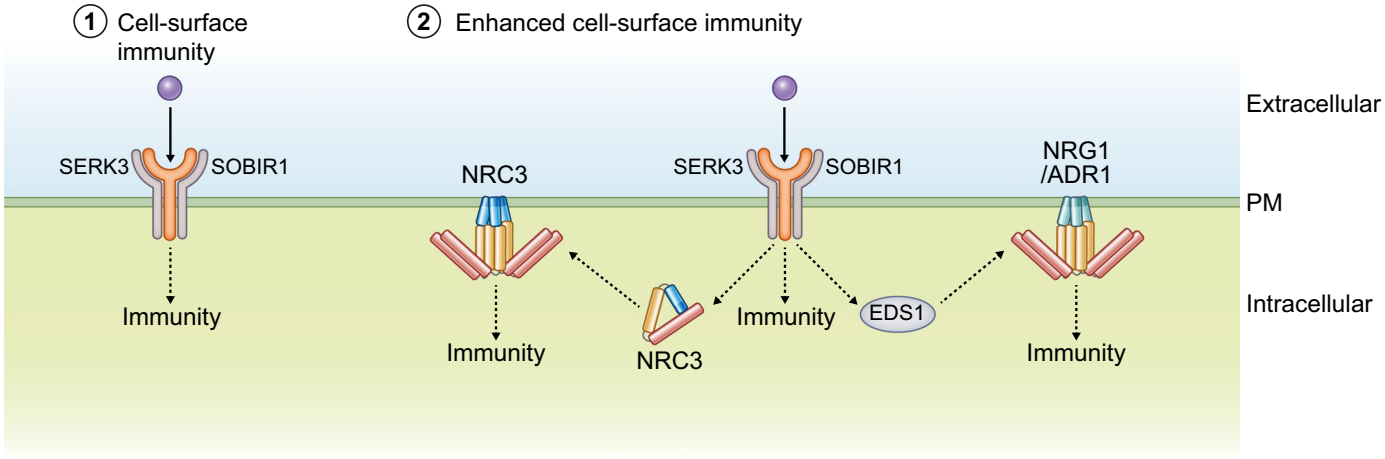

Fig. 3 Diverse NLR networks interact with cell-surface receptors to enhance immune signalling in Solanaceous plants. (1) Ligand recognition by cell-surface receptors triggers a signalling cascade, dependent on SOBIR1 and SERK3 co-receptors, leading to immunity. (2) Intracellular NLR immune receptors, such as CC-NLR NRC3, enhance this immunity. Upon activation by cell-surface receptors, NRC3 likely forms a resistosome at the plasma membrane, boosting immunity. EDS1 likely perceives TIR-derived nucleotides upon activation of cell-surface receptors, presumably activating NRG1 and/or ADR1 helper NLRs. Once activated, these NLRs function as Ca^{2+}-permeable resistosome pores at the plasma membrane, thereby enhancing cell-surface immunity.

(Gabriëls *et al.*, 2007; Fradin *et al.*, 2009; Kourelis *et al.*, 2022). Moreover, cell death triggered by these NRC-dependent LRR-RPs requires the LRR-RK co-receptors *SOBIR1* and *SERK3* (Fradin *et al.*, 2009; Liebrand *et al.*, 2013; Yin *et al.*, 2021). This suggests that in Solanaceae, an NRC-dependent sensor may be guarding a common component downstream of LRR-RP–SOBIR1–SERK3 complexes, resulting in NRC3 activation (Fig. 3). Other kinases, such as PBLs and MAPKs, have been identified as components in Cf and/or Ve1-mediated signalling and immunity (Gabriëls *et al.*, 2007; Fradin *et al.*, 2009). However, it is not known whether these kinases are necessary for NRC-dependent recognition, or downstream signalling following recognition.

Although *EDS1* is not required for cell-death signalling downstream of Cf-4 (Kourelis *et al.*, 2022; Zönnchen *et al.*, 2022), it is necessary for full immunity provided by Ve1 and Cf-4, as well as the LRR-RP encoding resistance genes I and I-7, which confer immunity towards specific *Fusarium oxysporum* isolates (Hu *et al.*, 2005; Fradin *et al.*, 2009; Gonzalez-Cendales *et al.*, 2016; Catanzariti *et al.*, 2017). Whether this process requires TIR-mediated signalling and the EDS1–PAD4–ADR1 module, like in Arabidopsis, remains unknown. The lack of conservation of TIR-NLRs between Arabidopsis and Solanaceous plants suggests that this signalling may have evolved independently in these species. Although Cf-gene orthologs appear to be exclusive to Solanaceae, the recognition of Ave1 by Ve1 homologues is conserved beyond this family (Song *et al.*, 2017; Kourelis *et al.*, 2020). This suggests that the NRC network may have evolved to enhance Ve1-mediated immunity in Solanaceae. In Arabidopsis, which lacks NRCs, transferring Ve1 results in EDS1-dependent immunity without cell death (Fradin *et al.*, 2011; Zhang *et al.*, 2013). This shows that in Solanaceous plants, at least two NLR networks contribute to enhancing immune signalling by cell-surface receptors, alongside NLR-independent signalling pathways (Fig. 3). Finally, the cell-death response induced by the recognition of *Verticillium* effector *Vd*EIX3 by the *N. benthamiana* receptor *Nb*EIX2 is *SOBIR1*- and *SERK3*-independent, although cell-death independent responses may still require these co-receptors (Yin *et al.*, 2021). This suggests that the pathway responsible for cell-death signalling downstream of this receptor differs from that employed by the Cf, Ve1, and REL receptors.

V. Conclusion

In summary, pathogens have converged on targeting intracellular components downstream of activated cell-surface receptors to establish infection. As a countermeasure, plants have evolved mechanisms to detect such perturbations using intracellular NLR immune receptors. In some instances, NLR-mediated signalling has become an integral part of the cell-surface-mediated immune response, contributing to overall immunity. Two potential evolutionary scenarios could explain this: either the integration evolved directly to enhance cell-surface-mediated immunity, or NLRs initially guarded common components downstream of these receptors and consequently enhanced immune signalling. In Arabidopsis, disruption of signalling components downstream of cell-surface receptor activation can trigger both effector recognition and an enhancement of surface immunity. In Solanaceous plants, however, cell-surface receptor-induced NRC-dependent cell death seems to solely augment cell-surface immunity. To what extent and to which pathogens these pathways contribute to disease resistance remains to be determined.

Because both the intracellular NLR immune repertoires and the cell-surface immune repertoires of plants exhibit considerable variability between species (Kourelis *et al.*, 2021; Ngou *et al.*, 2022), the interactions between cell-surface receptors and intracellular NLR immune receptors likely continuously arise (Ngou *et al.*, 2022). The distinct molecular mechanisms underpinning immune responses triggered by either cell-surface receptors or intracellular NLRs appear to be highly conserved among land plants (Chia *et al.*, 2022; Chu *et al.*, 2023). Meanwhile, the

re-emerging interactions between NLRs and various stages of the signalling cascade downstream of activated cell-surface receptors may offer a flexible enhancement to plant immunity. Although the molecular identities of the involved receptors may differ between species, the concept of leveraging NLR-mediated signalling to enhance cell-surface immunity remains constant. Several pathogens have evolved strategies to suppress components of NLR-mediated immunity that are utilized by cell-surface receptors (Wu & Derevnina, 2023), raising the possibility that this suppression is, at least partially, also necessary for inhibiting cell-surface-mediated immune responses.

Finally, in Arabidopsis, cell-surface immunity results in transcriptional regulation of components that are critical for the activation of some intracellular NLR immune responses, and vice-versa (Ngou *et al.*, 2021; Yuan *et al.*, 2021). This suggests a sophisticated level of interplay between these two mechanisms, adding an extra dimension to the intricacy of these interconnected immune systems. Uncovering the mechanisms by which intracellular NLR immune receptors reinforce signalling downstream of cell-surface receptors could pave the way for developing innovative strategies to enhance disease resistance.

Acknowledgements

I would like to acknowledge OpenAI's GPT-4, as implemented in ChatGPT, for its assistance in the editing process. I would like to thank members of the Kamoun group for the discussions which shaped this review. In particular, I would like to thank Andres Posbeyikian, Daniel Lüdke, Lida Derevnina, and Hiroaki Adachi for critically reading the manuscript and providing comments. I would also like to thank Sophien Kamoun for encouragement in writing this review. JK is funded by BASF Plant Science. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

JK receives funding from the industry on NLR biology and has filed patents on NLR biology.

Author contributions

JK contributed to the conceptualization, writing – original draft, writing – review and editing, visualization, and project administration.

ORCID

Jiorgos Kourelis https://orcid.org/0000-0002-9007-1333

References

Adachi H, Sakai T, Kourelis J, Pai H, Hernandez JLG, Maqbool A, Kamoun S. 2022. Jurassic NLR: conserved and dynamic evolutionary features of the atypically ancient immune receptor ZAR1. *bioRxiv*. doi: 10.1101/2020.10.12.333484.

Ahn YJ, Kim H, Choi S, Mazo-Molina C, Prokchorchik M, Zhang N, Kim B, Mang H, Yoon H, Segonzac C *et al.* 2022. Ptr1 and ZAR1 immune receptors confer overlapping and distinct bacterial pathogen effector specificities. *bioRxiv*. doi: 10.1101/2022.05.16.492216.

Albert I, Hua C, Nürnberger T, Pruitt RN, Zhang L. 2020. Surface sensor systems in plant immunity. *Plant Physiology* **182**: 1582–1596.

Bastedo DP, Khan M, Martel A, Seto D, Kireeva I, Zhang J, Masud W, Millar D, Lee JY, Lee AH-Y *et al.* 2019. Perturbations of the ZED1 pseudokinase activate plant immunity. *PLoS Pathogens* **15**: e1007900.

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

Buscaill P, van der Hoorn RAL. 2021. Defeated by the nines: nine extracellular strategies to avoid microbe-associated molecular patterns recognition in plants. *Plant Cell* **33**: 2116–2130.

Carter ME, Helm M, Chapman AVE, Wan E, Restrepo Sierra AM, Innes RW, Bogdanove AJ, Wise RP. 2019. Convergent evolution of effector protease recognition by *Arabidopsis* and barley. *Molecular Plant–Microbe Interactions* **32**: 550–565.

Catanzariti A-M, Do HTT, Bru P, de Sain M, Thatcher LF, Rep M, Jones DA. 2017. The tomato *I* gene for Fusarium wilt resistance encodes an atypical leucine-rich repeat receptor-like protein whose function is nevertheless dependent on SOBIR1 and SERK3/BAK1. *The Plant Journal* **89**: 1195–1209.

Chang JH, Tai Y-S, Bernal AJ, Lavelle DT, Staskawicz BJ, Michelmore RW. 2002. Functional analyses of the *Pto* resistance gene family in tomato and the identification of a minor resistance determinant in a susceptible haplotype. *Molecular Plant–Microbe Interactions* **15**: 281–291.

Chen Z, Liu F, Zeng M, Wang L, Liu H, Sun Y, Wang L, Zhang Z, Chen Z, Xu Y *et al.* 2023. Convergent evolution of immune receptors underpins distinct elicitin recognition in closely related Solanaceous plants. *Plant Cell* **35**: 1186–1201.

Chia K-S, Kourelis J, Vickers M, Sakai T, Kamoun S, Carella P. 2022. The N-terminal executioner domains of NLR immune receptors are functionally conserved across major plant lineages. *bioRxiv*. doi: 10.1101/2022.10.19.512840.

Chu J, Monte I, DeFalco TA, Köster P, Derbyshire P, Menke FLH, Zipfel C. 2023. Conservation of the PBL-RBOH immune module in land plants. *Current Biology* **33**: 1130–1137.

DeFalco TA, Zipfel C. 2021. Molecular mechanisms of early plant pattern-triggered immune signaling. *Molecular Cell* **81**: 3449–3467.

Dievart A, Gottin C, Périn C, Ranwez V, Chantret N. 2020. Origin and diversity of plant receptor-like kinases. *Annual Review of Plant Biology* **71**: 131–156.

Förderer A, Yu D, Li E, Chai J. 2022. Resistosomes at the interface of pathogens and plants. *Current Opinion in Plant Biology* **67**: 102212.

Fradin EF, Abd-El-Haliem A, Masini L, van den Berg GCM, Joosten MHAJ, Thomma BPHJ. 2011. Interfamily transfer of tomato *Ve1* mediates *Verticillium* resistance in Arabidopsis. *Plant Physiology* **156**: 2255–2265.

Fradin EF, Zhang Z, Ayala JCJ, Castroverde CDM, Nazar RN, Robb J, Liu C-M, Thomma BPHJ. 2009. Genetic dissection of *Verticillium* wilt resistance mediated by tomato *Ve1*. *Plant Physiology* **150**: 320–332.

Gabriëls SHEJ, Takken FLW, Vossen JH, de Jong CF, Liu Q, Turk SCHJ, Wachowski LK, Peters J, Witsenboer HMA, de Wit PJGM *et al.* 2006. cDNA-AFLP combined with functional analysis reveals novel genes involved in the hypersensitive response. *Molecular Plant–Microbe Interactions* **19**: 567–576.

Gabriëls SHEJ, Vossen JH, Ekengren SK, van Ooijen G, Abd-El-Haliem AM, van den Berg GCM, Rainey DY, Martin GB, Takken FLW, de Wit PJGM *et al.* 2007. An NB-LRR protein required for HR signalling mediated by both extra- and intracellular resistance proteins. *The Plant Journal* **50**: 14–28.

Gao M, Wang X, Wang D, Xu F, Ding X, Zhang Z, Bi D, Cheng YT, Chen S, Li X *et al.* 2009. Regulation of cell death and innate immunity by two receptor-like kinases in *Arabidopsis*. *Cell Host & Microbe* **6**: 34–44.

Gong Z, Qi J, Hu M, Bi G, Zhou J-M, Han G-Z. 2022. The origin and evolution of a plant resistosome. *Plant Cell* **34**: 1600–1620.

Gonzalez-Cendales Y, Catanzariti A-M, Baker B, Mcgrath DJ, Jones DA. 2016. Identification of *I-7* expands the repertoire of genes for resistance to Fusarium wilt in tomato to three resistance gene classes. *Molecular Plant Pathology* **17**: 448–463.

van der Hoorn RAL, Kamoun S. 2008. From guard to decoy: a new model for perception of plant pathogen effectors. *Plant Cell* **20**: 2009–2017.

Hu G, DeHart AKA, Li Y, Ustach C, Handley V, Navarre R, Hwang C-F, Aegerter BJ, Williamson VM, Baker B. 2005. *EDS1* in tomato is required for resistance mediated by TIR-class *R* genes and the receptor-like *R* gene *Ve*. *The Plant Journal* **42**: 376–391.

Hu M, Qi J, Bi G, Zhou J-M. 2020. Bacterial effectors induce oligomerization of immune receptor ZAR1 *in vivo*. *Molecular Plant* **13**: 793–801.

Huang S, Jia A, Song W, Hessler G, Meng Y, Sun Y, Xu L, Laessle H, Jirschitzka J, Ma S *et al.* 2022. Identification and receptor mechanism of TIR-catalyzed small molecules in plant immunity. *Science* **377**: eabq3297.

Huang Y, Yin C, Liu J, Feng B, Ge D, Kong L, Ortiz-Morea FA, Richter J, Hauser M-T, Wang W-M *et al.* 2020. A trimeric *Cr*RLK1L-LLG1 complex genetically modulates SUMM2-mediated autoimmunity. *Nature Communications* **11**: 4859.

Jacob P, Kim NH, Wu F, El-Kasmi 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.

Jia A, Huang S, Song W, Wang J, Meng Y, Sun Y, Xu L, Laessle H, Jirschitzka J, Hou J *et al.* 2022. TIR-catalyzed ADP-ribosylation reactions produce signaling molecules for plant immunity. *Science* **377**: eabq8180.

Kim YJ, Lin N-C, Martin GB. 2002. Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity. *Cell* **109**: 589–598.

Kong Q, Qu N, Gao M, Zhang Z, Ding X, Yang F, Li Y, Dong OX, Chen S, Li X *et al.* 2012. The MEKK1-MKK1/MKK2-MPK4 kinase cascade negatively regulates immunity mediated by a mitogen-activated protein kinase kinase kinase in Arabidopsis. *Plant Cell* **24**: 2225–2236.

Kourelis J, Contreras MP, Harant A, Pai H, Lüdke D, Adachi H, Derevnina L, Wu C-H, Kamoun S. 2022. The helper NLR immune protein NRC3 mediates the hypersensitive cell death caused by the cell-surface receptor Cf-4. *PLoS Genetics* **18**: e1010414.

Kourelis J, Malik S, Mattinson O, Krauter S, Kahlon PS, Paulus JK, van der Hoorn RAL. 2020. Evolution of a guarded decoy protease and its receptor in solanaceous plants. *Nature Communications* **11**: 4393.

Kourelis J, Sakai T, Adachi H, Kamoun S. 2021. RefPlantNLR is a comprehensive collection of experimentally validated plant disease resistance proteins from the NLR family. *PLoS Biology* **19**: e3001124.

Laflamme B, Dillon MM, Martel A, Almeida RND, Desveaux D, Guttman DS. 2020. The pan-genome effector-triggered immunity landscape of a host-pathogen interaction. *Science* **367**: 763–768.

Lewis JD, Lee AH-Y, Hassan JA, Wan J, Hurley B, Jhingree JR, Wang PW, Lo T, Youn J-Y, Guttman DS *et al.* 2013. The *Arabidopsis* ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a. *Proceedings of the National Academy of Sciences, USA* **110**: 18722–18727.

Li L, Kim P, Yu L, Cai G, Chen S, Alfano JR, Zhou J-M. 2016. Activation-dependent destruction of a co-receptor by a *Pseudomonas syringae* effector dampens plant immunity. *Cell Host & Microbe* **20**: 504–514.

Liebrand TWH, van den Berg GCM, Zhang Z, Smit P, Cordewener JHG, America AHP, Sklenar J, Jones AME, Tameling WIL, Robatzek S *et al.* 2013. Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection. *Proceedings of the National Academy of Sciences, USA* **110**: 10010–10015.

Liu C, Cui D, Zhao J, Liu N, Wang B, Liu J, Xu E, Hu Z, Ren D, Tang D *et al.* 2019. Two *Arabidopsis* receptor-like cytoplasmic kinases SZE1 and SZE2 associate with the ZAR1–ZED1 complex and are required for effector-triggered immunity. *Molecular Plant* **12**: 967–983.

Liu J, Huang Y, Kong L, Yu X, Feng B, Liu D, Zhao B, Mendes GC, Yuan P, Ge D *et al.* 2020. The malectin-like receptor-like kinase LETUM1 modulates NLR protein SUMM2 activation via MEKK2 scaffolding. *Nature Plants* **6**: 1106–1115.

Martel A, Laflamme B, Seto D, Bastedo DP, Dillon MM, Almeida RND, Guttman DS, Desveaux D. 2020. Immunodiversity of the *Arabidopsis* ZAR1 NLR Is conveyed by receptor-like cytoplasmic kinase sensors. *Frontiers in Plant Science* **11**: 1290.

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

Ngou BPM, Heal R, Wyler M, Schmid MW, Jones JDG. 2022. Concerted expansion and contraction of immune receptor gene repertoires in plant genomes. *Nature Plants* **8**: 1146–1152.

Ntoukakis V, Saur IM, Conlan B, Rathjen JP. 2014. The changing of the guard: the Pto/Prf receptor complex of tomato and pathogen recognition. *Current Opinion in Plant Biology* **20**: 69–74.

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.

Salmeron JM, Oldroyd GED, Rommens CMT, Scofield SR, Kim H-S, Lavelle DT, Dahlbeck D, Staskawicz BJ. 1996. Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. *Cell* **86**: 123–133.

Schultink A, Qi T, Bally J, Staskawicz B. 2019. Using forward genetics in *Nicotiana benthamiana* to uncover the immune signaling pathway mediating recognition of the *Xanthomonas perforans* effector XopJ4. *New Phytologist* **221**: 1001–1009.

Schulze S, Yu L, Hua C, Zhang L, Kolb D, Weber H, Ehinger A, Saile SC, Stahl M, Franz-Wachtel M *et al.* 2022. The *Arabidopsis* TIR-NBS-LRR protein CSA1 guards BAK1-BIR3 homeostasis and mediates convergence of pattern- and effector-induced immune responses. *Cell Host & Microbe* **30**: 1717–1731.

Seto D, Khan M, Bastedo DP, Martel A, Vo T, Guttman D, Subramaniam R, Desveaux D. 2021. The small molecule Zaractin activates ZAR1-mediated immunity in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **118**: e2116570118.

Seto D, Koulena N, Lo T, Menna A, Guttman DS, Desveaux D. 2017. Expanded type III effector recognition by the ZAR1 NLR protein using ZED1-related kinases. *Nature Plants* **3**: 17027.

Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW. 2003. Cleavage of *Arabidopsis* PBS1 by a bacterial type III effector. *Science* **301**: 1230–1233.

Song Y, Zhang Z, Seidl MF, Majer A, Jakse J, Javornik B, Thomma BPHJ. 2017. Broad taxonomic characterization of *Verticillium* wilt resistance genes reveals an ancient origin of the tomato Ve1 immune receptor. *Molecular Plant Pathology* **18**: 195–209.

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

Toruño TY, Stergiopoulos I, Coaker G. 2016. Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. *Annual Review of Phytopathology* **54**: 419–441.

Wang G, Roux B, Feng F, Guy E, Li L, Li N, Zhang X, Lautier M, Jardinaud M-F, Chabannes M *et al.* 2015. The decoy substrate of a pathogen effector and a pseudokinase specify pathogen-induced modified-self recognition and immunity in plants. *Cell Host & Microbe* **18**: 285–295.

Wang J, Hu M, Wang J, Qi J, Han Z, Wang G, Qi Y, Wang H-W, Zhou J-M, Chai J. 2019. Reconstitution and structure of a plant NLR resistosome conferring immunity. *Science* **364**: eaav5870.

Wang Z, Cui D, Liu J, Zhao J, Liu C, Xin W, Li Y, Liu N, Ren D, Tang D *et al.* 2017. Arabidopsis ZED1-related kinases mediate the temperature-sensitive intersection of immune response and growth homeostasis. *New Phytologist* **215**: 711–724.

Wu C-H, Abd-El-Haliem A, Bozkurt TO, Belhaj K, Terauchi R, Vossen JH, Kamoun S. 2017. NLR network mediates immunity to diverse plant pathogens. *Proceedings of the National Academy of Sciences, USA* **114**: 8113–8118.

Wu C-H, Derevnina L. 2023. The battle within: how pathogen effectors suppress NLR-mediated immunity. *Current Opinion in Plant Biology* **74**: 102396.

Wu Y, Gao Y, Zhan Y, Kui H, Liu H, Yan L, Kemmerling B, Zhou J-M, He K, Li J. 2020. Loss of the common immune coreceptor BAK1 leads to NLR-dependent cell death. *Proceedings of the National Academy of Sciences, USA* **117**: 27044–27053.

Yang Y, Kim NH, Cevik V, Jacob P, Wan L, Furzer OJ, Dangl JL. 2022. Allelic variation in the *Arabidopsis* TNL CHS3/CSA1 immune receptor pair reveals two functional cell-death regulatory modes. *Cell Host & Microbe* **30**: 1701–1716.

Yin Z, Wang N, Pi L, Li L, Duan W, Wang X, Dou D. 2021. *Nicotiana benthamiana* LRR-RLP NbEIX2 mediates the perception of an EIX-like protein from *Verticillium dahliae*. *Journal of Integrative Plant Biology* **63**: 949–960.

Yuan M, Jiang Z, Bi G, Nomura K, Liu M, Wang Y, Cai B, Zhou J-M, He SY, Xin X-F. 2021. Pattern-recognition receptors are required for NLR-mediated plant immunity. *Nature* **592**: 105–109.

Zhang Z, Liu Y, Huang H, Gao M, Wu D, Kong Q, Zhang Y. 2017. The NLR protein SUMM2 senses the disruption of an immune signaling MAP kinase cascade via CRCK3. *EMBO Reports* **18**: 292–302.

Zhang Z, van Esse HP, van Damme M, Fradin EF, Liu C-M, Thomma BPHJ. 2013. *Ve1*-mediated resistance against *Verticillium* does not involve a hypersensitive response in Arabidopsis. *Molecular Plant Pathology* **14**: 719–727.

Zhang Z, Wu Y, Gao M, Zhang J, Kong Q, Liu Y, Ba H, Zhou J, Zhang Y. 2012. Disruption of PAMP-induced MAP kinase cascade by a *Pseudomonas syringae* effector activates plant immunity mediated by the NB-LRR protein SUMM2. *Cell Host & Microbe* **11**: 253–263.

Zönnchen J, Gantner J, Lapin D, Barthel K, Eschen-Lippold L, Erickson JL, Villanueva SL, Zantop S, Kretschmer C, Joosten MHAJ *et al.* 2022. EDS1 complexes are not required for PRR responses and execute TNL-ETI from the nucleus in *Nicotiana benthamiana*. *New Phytologist* **236**: 2249–2264.