Review

New Phytologist

Tansley insight

The power of patterns: new insights into pattern-triggered immunity

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Received: *22 April 2023*
Accepted: *16 June 2023*

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New Phytologist (2023) **240:** 960–967
doi: 10.1111/nph.19148

Key words: immune receptor, molecular pattern, pattern recognition receptor, pattern-triggered immunity, phytocytokine, plant innate immunity, plant–microbe interactions.

Summary

The plant immune system features numerous immune receptors localized on the cell surface to monitor the apoplastic space for danger signals from a broad range of plant colonizers. Recent discoveries shed light on the enormous complexity of molecular signals sensed by these receptors, how they are generated and removed to maintain cellular homeostasis and immunocompetence, and how they are shaped by host-imposed evolutionary constraints. Fine-tuning receptor sensing mechanisms at the molecular, cellular and physiological level is critical for maintaining a robust but adaptive host barrier to commensal, pathogenic, and symbiotic colonizers alike. These receptors are at the core of any plant-colonizer interaction and hold great potential for engineering disease resistance and harnessing beneficial microbiota to keep crops healthy.

I. Introduction

Plants possess various extracellular and intracellular immune receptors to detect molecular cues indicating colonization by other organisms ('non-self') or pathologic cellular alterations ('modified-self') as danger signals. Cell-surface pattern recognition receptors (PRRs) sense conserved epitopes, called 'molecular patterns' (MPs), released into the apoplast during plant-colonizer interactions or mechanical damage and activate pattern-triggered immunity (PTI). Colonizer-derived MPs are commonly subdivided by origin, for example as microbe- or herbivore-associated (Box 1; Ngou *et al.*, 2022). Host-derived MPs include damage-associated MPs (DAMPs) and immunomodulatory phytocytokines (Gust *et al.*, 2017). Plants possess numerous PRRs with distinct ectodomains to sense chemically diverse MPs (Fig. 1; Ngou *et al.*, 2022). Early PTI responses are remarkably congruent and include a 'general stress response' module (Bjornson *et al.*, 2021). PTI confers

Box 1 Some MPs do not fit our current narrow terminology

The term pathogen-associated MP was adopted from the field of animal immunity for plant general elicitors to underline conceptual commonalities of animal and plant innate immunity. While this spurred interest in this branch of plant immunity, researchers are struggling to find appropriate term(s) for all elicitor types discovered since. Microbe-, nematode-, herbivore-, egg- and parasitic plant-associated MPs subcategorize non-self-MPs by origin (Ngou *et al*., 2022). Plant endogenous general elicitors include genuine (primary/constitutive) DAMPs, passively released plant breakdown products, and actively secreted immunomodulatory (secondary/inducible) DAMPs, renamed 'phytocytokines' as they resemble metazoan cytokines (Gust *et al*., 2017). Yet many MPs do not fit these classifications, for example: (1) MPs present in hosts and colonizers, for example SCOOP phytocytokines and fungal/bacterial SCOOP-like MAMPs (Hou *et al*., 2021; Rhodes *et al*., 2021); (2) MPs perceived by hosts and colonizers but exerting different functions, for example quinone-induced immune activation in nonparasitic vs haustorium formation in parasitic plants (Laohavisit *et al*., 2020); (3) DAMPs that are also generated during plant growth and development, for example, OGs (Pontiggia *et al*., 2020); (4) general metabolites present in all organisms combining features (1–3), for example, ATP, NAD(P)$^+$, and quinones; and (5) MPs that are apoplastic effectors or part thereof, for example xyloglucanase XEG1 (Wang *et al*., 2018).

Subcategorization by the site of perception, rather than ambiguous origin, as extracellular or intracellular MPs allows researchers to specifically describe the different receptors and branches of immunity activated (van der Burgh & Joosten, 2019). The 'danger model' provides an even more holistic classification encompassing the full range of different plant-colonizer interactions and reflecting the multifaceted nature of plant immune sensing (Gust *et al*., 2017), including emerging 'noncanonical' danger sensing systems, for example mechano-sensing at the plasma membrane or by trichomes (Schellenberger *et al*., 2021; Matsumura *et al*., 2022).

broad-spectrum immunity locally and systemically (Ngou *et al*., 2022).

Successful colonizers must overcome PTI, for example, by manipulating the plant through apoplastic or intracellular effectors, resulting in effector-triggered susceptibility. Cell-surface-localized and intracellular plant receptors sense effectors or their manipulation and activate effector-triggered immunity (ETI; Ngou *et al*., 2022; Wang *et al*., 2022). Despite activating similar responses, PTI and ETI were long considered separate branches of immunity. In fact, they are intertwined, and ETI largely acts by potentiating PTI (Ngou *et al*., 2021; Pruitt *et al*., 2021; Tian *et al*., 2021; Yuan *et al*., 2021). This highlights PTI's pivotal role in plant immunity and raises the attention of researchers and breeders, as PTI has the potential to confer durable disease resistance in crops.

Molecular patterns and PRRs form the fundamental module underlying PTI. Here, we selected examples from recent literature to encapsulate new insights into PTI.

II. The complexity of molecular patterns: from macromolecules to small metabolites

Pattern recognition receptors sense diverse ligands varying remarkably in complexity and size (Fig. 1). Sensing of proteobacterial protein translation-initiation factor 1 (IF1) by RLP32, for example, requires the intact protein, suggesting its 3D-structure determines the elicitor activity (Fan *et al*., 2022). Similarly, sensing of xyloglucanase XEG1 from oomycete and fungal pathogens by the PRR RXEG1 in soybean and Solanaceae, requires the folded protein but not its enzymatic activity. Binding of XEG1 to RXEG1 not only activates PTI but also inhibits its glucanase activity (Wang *et al*., 2018; Sun *et al*., 2022).

For most non-self-proteins, however, short conserved peptides (*c*. 10–30 residues) are sufficient for PRR activation (Fig. 1). Since first demonstrated for sensing of bacterial flagellin epitope flg22 and elongation factor thermo unstable epitope elf18 by Leucine-rich-repeat (LRR)-type PRRs FLS2 and EFR, respectively, numerous peptides, mostly sensed by LRR-PRRs, from diverse colonizers were identified (Ngou *et al*., 2022). Similarly, phytocytokines are often processed from larger precursors. Their size varies from over 50, for example RALF23, to only a few residues, for example pentapeptide PSK (Fig. 1). Many phytocytokines require post-translational modifications, like proline hydroxylation and arabinosylation, tyrosine sulfation, or disulphide bridges, for activity (Olsson *et al*., 2019; Guillou *et al*., 2022).

Glycans are structural cell wall components in bacteria, fungi, oomycetes, and plants. N-acetylglucosamine-containing glycans like fungal chitin and bacterial peptidoglycan are sensed by different lysin-motif (LysM)-type PRRs (Fig. 1; Ngou *et al*., 2022). LysM-PRRs also sense other glycans, for example rice CERK1 binds β-1,3-1,4-glucan-tri-/tetramers released from Poaceae cell walls by a fungal pathogen (Yang *et al*., 2021), while Arabidopsis CERK1 senses nonbranched β-1,3-glucan-oligosaccharides from fungal and oomycete cell walls and possibly plant callose (Melida *et al*., 2018). Arabidopsis senses pectin-polymers and pectin-derived oligogalacturonides (OGs) from its cell wall through epidermal-growth-factor-like (EGF)-type PRRs WAK1/2 (Kohorn & Kohorn, 2012), while FER binds demethylesterified pectin (Lin *et al*., 2022). Notably, FER also binds peptides (Box 2).

Lipids are major membrane constituents, with compositions varying among organisms. Arabidopsis S-domain (SD)-type PRR RDA2 senses sphingoid bases, particularly 9-methyl-branched ones specific to fungal and oomycete ceramides, including ceramide-D of the pathogen *Phytophthora infestans*, but not plant ceramides (Kato *et al*., 2022). SD-PRR LORE senses (bacterial) medium-chain 3-hydroxy-fatty-acid (3-OH-FA)-metabolites, including 3-hydroxydecanoate and 3-hydroxyalkanoates (Fig. 1; Kutschera *et al*., 2019; Schellenberger *et al*., 2021).

Adenosine triphosphate (ATP), nicotinamide adenine dinucleotides NAD$^+$/NADP$^+$, and quinones are small key metabolic components in all organisms. ATP and NAD$^+$/NADP$^+$, present at

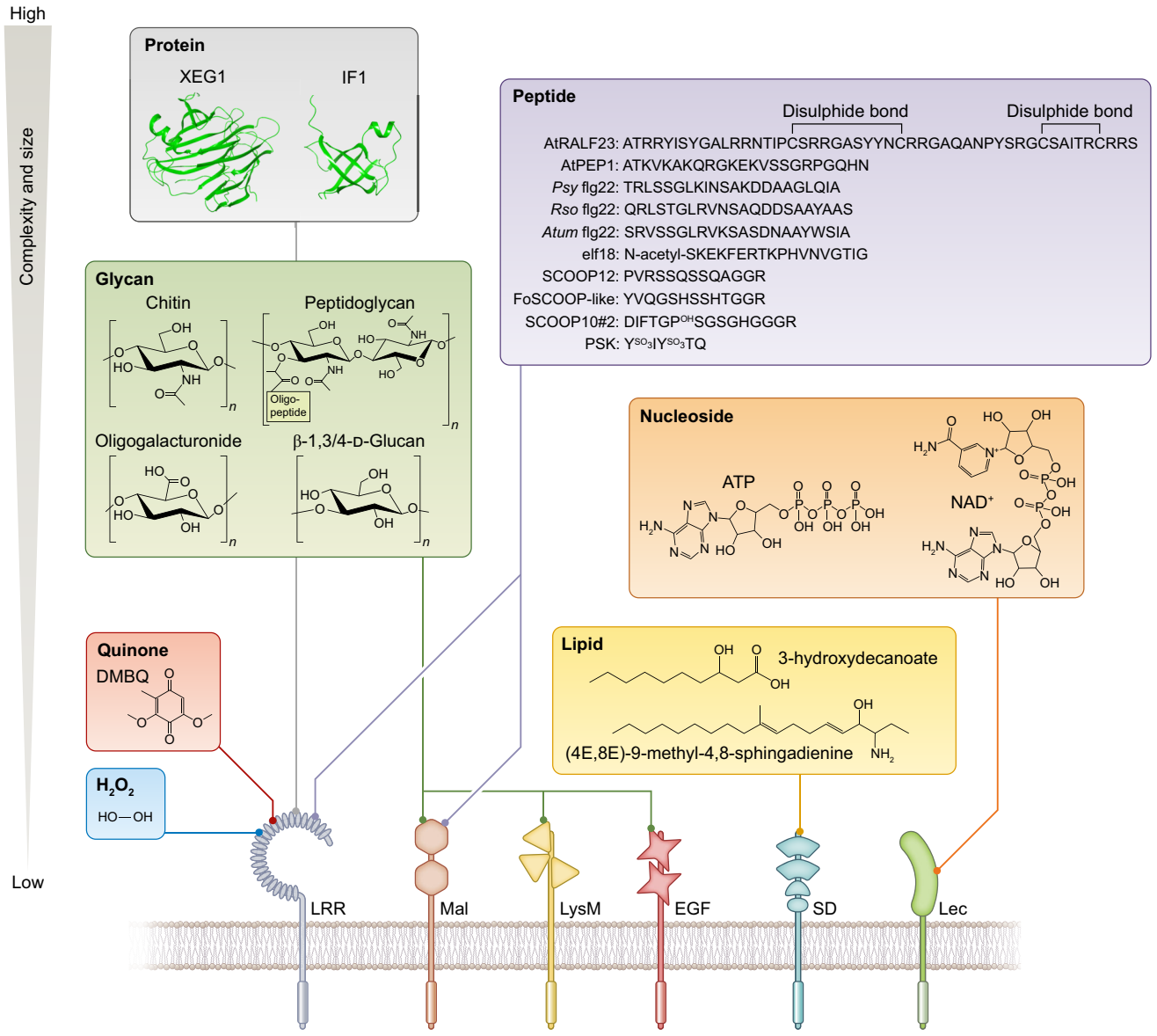

Fig. 1 Structural diversity and different complexity of molecular patterns. Molecular patterns (MPs) with known pattern recognition receptors (PRRs) are depicted with decreasing levels of complexity and size (top to bottom). Exemplary PRR families with different ectodomains, comprising Leucine-rich repeat (LRR), L-type lectin (Lec), S-domain (SD), tandem malectin (Mal), lysin-motif (LysM), and epidermal growth factor-like (EGF) domains, are shown on the plasma membrane. Protein: structurally intact and folded proteinaceous MPs show the highest level of structural complexity and size. Xyloglucan-specific endo-beta-1,4-glucanase XEG1 from *Phytophthora sojae*, for example, is sensed by LRR-PRR RXEG1. Tertiary-structure features also determine the eliciting activity of translation-initiation factor 1 (IF1) from proteobacteria sensed by LRR-PRR RLP32. Peptide: peptides strongly vary in structural complexity and size. RALF23 is a long peptide MP containing two pairs of disulphide bonds. The Mal-type receptor FER and glycosylphosphatidylinositol-anchored LLG1 form a binding complex for the recognition of RALF23. flg22 peptides released from bacterial flagellin bind to LRR-PRR FLS2, for example *Pseudomonas syringae Psy*flg22. Some deviant flg22 peptides are not sensed by most plant species (e.g. *Ralstonia solanacearum Rso*flg22, *Agrobacterium tumefaciens Atum*flg22). The N-acetylated elf18 peptide resides in the N-terminus of bacterial elongation factor thermo unstable and is perceived by LRR-PRR EFR. Phytocytokine PEP1 is sensed by LRR-PRR PEPR1/2, and SCOOP peptides from plants and SCOOP-like peptides from pathogens like *Fusarium oxysporum* f. sp. *lycopersici* (*Fo*) are perceived by LRR-PRR MIK2. The proline residue in SCOOP10#2 is often hydroxylated in Arabidopsis. The smallest member of the peptide MP class is the tyrosine-sulphated pentapeptide PSK sensed by the LRR-PRR PSKR. Glycan: chitin-oligomers released from fungi are perceived by LysM-PRRs LYK5/CERK1 and LYM2 in Arabidopsis, and CEBiP and LYP4/6 in rice. Bacterial peptidoglycan is sensed by LYM1/LYM3 in Arabidopsis. EGF-PRRs WAK1/2 sense pectin from the plant cell wall and oligogalacturonides released from pectin, while FER binds demethylesterified pectin. Rice CERK1 binds β-1,3-1,4-glucan-tri-/tetramers released from Poaceae cell walls, and Arabidopsis CERK1 senses nonbranched β-1,3-glucan-oligosaccharides derived from fungal cell walls and possibly plant callose. Nucleoside: extracellular adenosine triphosphate (ATP) is perceived by Lec-PRRs LecRK-I.9/DORN1/P2K1 and P2K2. Lec-PRRs LecRK-I.8 and LecRK-VI.2 detect extracellular nicotinamide adenine dinucleotide (NAD^+). Lipid: SD-PRR LORE senses bacterial medium-chain 3-hydroxy-fatty acid metabolites, such as 3-hydroxydecanoate. Sphingoid bases, including (4E,8E)-9-methyl-4,8-sphingadienine, are sensed by the SD-PRR RDA2. Quinone and H_2O_2: they are, so far, the smallest molecules known as MPs. Plant quinone 2,6-dimethoxy-1,4-benzoquinone (DMBQ) and extracellular H_2O_2 are sensed by the same LRR-PRR CARD1/HPCA1. Source of protein models: XEG1: 7DRB; IF1: AF-A0A093TP15-F1.

Box 2 Multifunctional receptors – Swiss army knives of plants?

Many receptors participate in different signalling pathways, often with very different outputs, raising the question of whether such receptors sense different ligands to activate different signalling pathways. The receptor kinase FERONIA (FER) is a prominent example of such a multifunctional receptor. FER is involved in a plethora of cellular processes, including fertility, root development, cell wall integrity sensing, and immunity (Zhu *et al*., 2021), and binds different ligands through its extracellular tandem malectin-like domains. For instance, peptides RALF33 and PCP-Bγ competitively bind to the malectin-B domain, while demethylesterified pectin binds to the malectin-A domain (Xiao *et al*., 2019; Liu *et al*., 2021; Lin *et al*., 2022).

The possibility that receptors sense multiple ligands, through the same and/or different binding sites, opens interesting perspectives on receptor specificity, regulation and crosstalk. Different ligands may guide the formation of different heterocomplexes or, vice versa, different heterocomplexes may create binding sites for different ligands. Alternatively, multiple ligands may compete for the same binding site, or allosteric crosstalk between multiple binding sites may influence ligand affinities, receptor activity and downstream outputs. Identification of the ligand binding sites at the molecular level will be required to functionally dissect the role of individual domains/sites in shaping the final response output. One may speculate that direct signal integration at the receptor input level provides evolutionary benefits for plant fitness, for example by generating fast and robust plant responses to multiple stresses or fine-tuning responses to different external and internal cues for optimal resource allocation.

high levels intracellularly, but usually not extracellularly, are perceived as DAMPs upon apoplastic release through L-lectin (Lec)-type PRRs (Fig. 1; Choi *et al*., 2014; Wang *et al*., 2017, 2019; Pham *et al*., 2020). Plant-derived and microbial quinones are sensed by LRR-PRR CARD1/HPCA1 through a conserved cysteine pair (Laohavisit *et al*., 2020). CARD1/HPCA1 also senses extracellular hydrogen peroxide (eH_2O_2) via different cysteine pairs. Extracellular hydrogen peroxide is considered a second messenger in numerous signalling pathways, but strictly speaking, second messengers are intracellular signalling molecules. Despite partial cytosol entry via aquaporins, eH_2O_2 mainly amplifies and systemically propagates apoplastic (immune) signalling like phytocytokines (Mittler *et al*., 2022). Many colonizers also produce eH_2O_2 during plant interactions (Segal & Wilson, 2018), likely activating CARD1/HPCA1. Indeed, eH_2O_2 may classify as the smallest MP (Fig. 1).

III. Release, recognize, remove and repeat

Release

Molecular patterns within macromolecules or insoluble polymers typically require release as smaller, soluble units to bind PRRs (Fig. 2a). Release may occur inadvertently during colonization or through processing by host lytic enzymes, many known as pathogenesis-related proteins. They often serve dual functions as antimicrobial agents and MP producers, for example chitinases that attack fungal hyphae to stop their growth and release elicitor-active chitooligomers (Wang *et al*., 2022). The MP flg22 is hidden inside the flagellin filament, which is protected from proteolytic degradation by *O*-glycosylation (Buscaill *et al*., 2019). Plant-secreted glycosidases remove the glycan shield, facilitating proteolytic flagellin degradation and the release of flg22-containing fragments. Different plant glycosidases hydrolyse specific *O*-glycans, and glycan variations facilitate host immune evasion (Fig. 2b; Buscaill *et al*., 2019). A specific plant ceramidase releases the 9-methyl-sphingoid base from ceramide-D for RDA2 sensing (Kato *et al*., 2022). Conversely, bacteria may inadvertently release 3-OH-FAs, for example during lipopolysaccharide biosynthesis, in their lipid secretome (Kutschera *et al*., 2019; Schellenberger *et al*., 2021). Bacterial surfactants like rhamnolipids may aid in releasing MPs, for example by shedding flagellin or lipopolysaccharide-derived lipids from bacteria (Al-Tahhan *et al*., 2000; Gerstel *et al*., 2009). Colonizers evolved various strategies to prevent MP release (Fig. 2b; Wang *et al*., 2022), emphasizing the importance of plant-governed MP processing in monitoring the apoplast.

Plant tissue-derived DAMPs are typically generated by colonizer-secreted lytic enzymes or mechanical damage. Plants have strategies to inhibit such enzymes, both to protect their cellular integrity and generate breakdown products of suitable size and structure for PRR binding (Pontiggia *et al*., 2020). Microbial polygalacturonases degrade plant pectin and are suppressed by plant polygalacturonase-inhibiting proteins to generate OGs comprising 10–15 units, which strongly activate immunity (De Lorenzo *et al*., 2018). *Ralstonia solanacearum* uses specific polygalacturonases to further degrade OGs to elicitor-inactive galacturonic acid-di-/monomers, which dampens immune activation and provides a carbon source for bacterial growth (Ke *et al*., 2023).

Most phytocytokines are proteolytically processed from precursors during or upon apoplastic secretion to release mature bioactive peptides (Gust *et al*., 2017). Many PRORALFs harbour a conserved motif for S1P-mediated cleavage to release bioactive RALFs, while others may not require processing (Stegmann *et al*., 2017; Abarca *et al*., 2021). Upon cleavage of PROSCOOPs by an unknown apoplastic protease, mature SCOOP peptides are sensed by the LRR-PRR MIK2 (Hou *et al*., 2021; Rhodes *et al*., 2021). PROPEPs, lacking N-terminal secretion signals, are cleaved upon cell damage by intracellular Ca^{2+}-dependent type-II-metacaspases, and PEPs leak into the apoplast to activate LRR-PRRs PEPR1/2 (Hander *et al*., 2019; Shen *et al*., 2019).

Recognition

Typically, binding of MPs by PRRs is mediated by intermolecular forces (Wang & Chai, 2020). In contrast to this 'canonical' receptor-ligand interaction, sensing of eH_2O_2 involves covalent cysteine modifications of the LRR-PRR CARD1/HPCA1. As redox carriers, quinones likely activate CARD1/HPCA1 through a similar mechanism (Laohavisit *et al*., 2020; Wu *et al*., 2020).

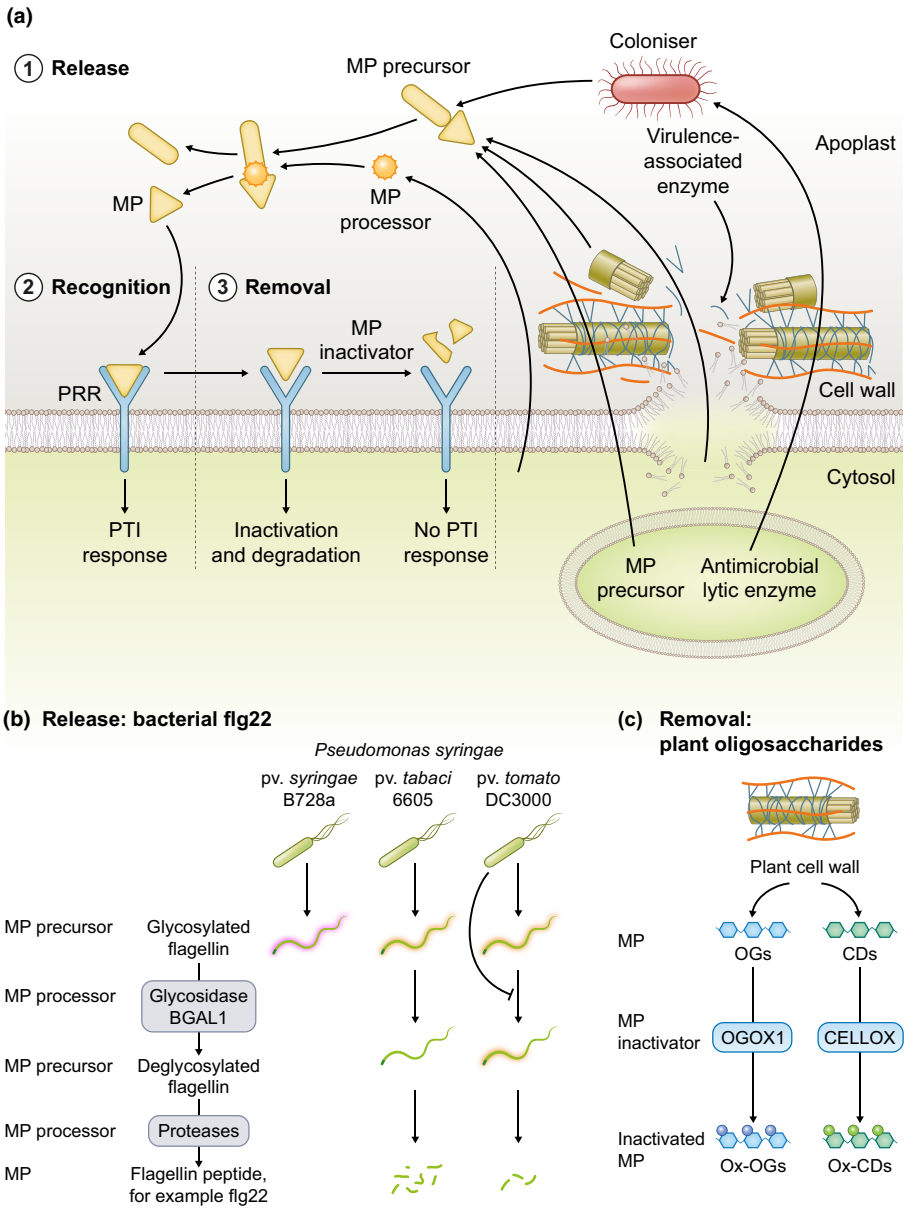

Fig. 2 '3R' process: release, recognition, and removal of molecular patterns in the apoplast. (a) Release: molecular patterns (MPs) are derived from colonizers and plants. They are actively secreted or passively released through the action of defence- and virulence-associated agents. Inactive precursors are processed to expose their eliciting MP structures. Recognize: MPs are recognized by their corresponding PRRs and activate PTI. Remove: MPs are removed from the apoplast to prevent continuous immune responses in the absence of danger. Plants employ various mechanisms to inactivate MPs, including modification or degradation of MPs by apoplastic enzymes, as well as endocytosis and subsequent degradation of MP-PRR complexes. Finally, to restore sensitivity to stimuli, PRRs are re-cycled or newly expressed (repeat). (b) The MP flg22 is hidden inside the flagellin polymer and shielded by flagellin *O*-glycosylation. The plant-secreted glycosidase β-galactosidase 1 (BGAL1) deglycosylates the terminal-modified viosamine glycan shield (orange) from flagellin of *Pseudomonas syringae* pv. *tabaci* 6605 and *P. syringae* pv. *tomato* DC3000, but not the putative 1,2-linked terminal N-acetylglucosamine shield (purple) from *P. syringae* pv. *syringae* B728a. DC3000 partially suppresses the activity of BGAL1 by secreting a β-galactosidase inhibitor. Deglycosylated flagellin is highly digested by plant apoplastic proteases (modified from Buscaill *et al.*, 2019). (c) Oligogalacturonides (OGs) and cellulose-oligosaccharides (CDs) are both sensed as DAMPs in plants. Oxidation of OGs (OX-OGs) and CDs (OX-CDs) by plant-secreted OG-oxidase 1 (OGOX1) and cellodextrin oxidase CELLOX, respectively, inactivates these DAMPs (Benedetti *et al.*, 2018; Locci *et al.*, 2019).

Removal

Immune signalling regulation is crucial to avoid detrimental growth-defence trade-offs. Negative regulation of PRRs includes interaction with inhibitory proteins, deactivation by protein phosphatases, and receptor degradation (Ngou *et al.*, 2022). Additionally, MPs must be cleared from the apoplast to prevent continuous PRR activation in the absence of danger (Fig. 2a). MP inactivation or degradation likely involves plant apoplastic enzymes. Oxidation of OG and cellulose-oligosaccharide DAMPs by plant oxidases OGOX1 and CELLOX, respectively, renders them elicitor-inactive and unsuitable as a carbon source for microbes (Fig. 2c; Benedetti *et al.*, 2018; Locci *et al.*, 2019). Interestingly, OGOX1-mediated oxidation produces eH_2O_2, which may activate CARD1/HPCA1. DAMPs also play roles in normal plant growth and development (Tanaka *et al.*, 2010; Pontiggia *et al.*, 2020). Controlling DAMP activity is, therefore, crucial for maintaining plant cellular and physiological homeostasis beyond immunity.

Eventually, clearing of MPs and replenishing of PRRs are required to restore sensitivity of plants to continuous or new stimuli ('repeat').

IV. Molecular patterns and receptors are shaped by plant-colonizer coevolution

Diversifying MPs is a common immune evasion strategy of colonizers, which is constrained by potential fitness costs (McCann *et al.*, 2012). This is intensely studied for flagellin sensing. Flagellin is required for bacterial motility, an important virulence factor of

pathogens. In Arabidopsis, flg22-responses vary among proteobacterial classes: flg22 epitopes of γ-/β-Proteobacteria, which include many phytopathogens, are overall more immunogenic than those of α-/δ-/ε-Proteobacteria and may have shaped FLS2 sensing specificities (Cheng *et al.*, 2021). Many prevalent Arabidopsis microbiota members have flg22 epitopes that are inactive, antagonistic or modulate FLS2 immune outputs (Colaianni *et al.*, 2021), reflecting the adaptation of these commensals to host PTI and the impact of PTI on microbiota composition. Most mutations of flg22 motifs impair bacterial motility but not the FLS2 interaction (Parys *et al.*, 2021), making it difficult for bacteria to evade FLS2 sensing without sacrificing motility. PRRs apparently evolve to target evolutionary constrained MPs of pathogens over adaptable MPs of beneficial commensals. This may explain why loss or gain of individual PRRs usually affects colonization with adapted phytopathogens but has no major impact on commensal community composition (Pfeilmeier *et al.*, 2021). Despite motility constraints, some phytopathogens evolved flg22 epitopes that are nonimmunogenic in most plants, for example α-Proteobacterium *Agrobacterium tumefaciens* $flg22^{Atum}$ or β-Proteobacterium *R. solanacearum* $flg22^{Rso}$ (Fig. 1; Fürst *et al.*, 2020; Wei *et al.*, 2020). Some plant species evolved FLS2 variants detecting these polymorphic flg22 sequences: $FLS2^{XL}$ from *Vitis riparia* binds flg22 and $flg22^{Atum}$ (Fürst *et al.*, 2020), while soybean FLS2 senses flg22 and $flg22^{Rso}$ (Wei *et al.*, 2020). Alternatively, plants acquire additional PRRs sensing distinct epitopes, for example flgII-28 by tomato FLS3 (Ngou *et al.*, 2022). Besides sequence polymorphisms, variations of O-glycan structures of flagellin prevent the release of flg22 and, presumably, flgII-28 epitopes. Different glycosidase specificities indicate host coevolution (Fig. 2b; Buscaill *et al.*, 2019). Within a wild tomato species, β-1,3-glucan responses vary quantitatively between populations, depending on geographical location, but also within populations, likely reflecting adaptations to local biotic and abiotic environments (Kahlon *et al.*, 2023). Symbiotic arbuscular mycorrhizal fungi (AMF) and their hosts employ other strategies to overcome PTI: additional PRR-like host receptors sense symbiont-specific signals to promote symbiosis. In *Medicago truncatula*, AMF-derived chitooligomer MPs induce both immunity and symbiosis signalling, while symbiont-specific lipochitooligosaccharides act synergistically with these MPs to amplify symbiosis over immune signalling and establish symbiosis (Feng *et al.*, 2019). In rice, activating symbiosis signalling by AMF-derived chitotetramers simultaneously inhibits AMF-derived chitooctamer-triggered immune signalling through out-competing PRR-coreceptor interaction (Zhang *et al.*, 2021). Overall, PTI exerts evolutionary pressure on pathogens, commensals, and symbionts, with the coevolution of MP sensing mechanisms depending on the type of host-colonizer interactions, their geographical distribution and environmental fine-tuning.

V. The spatiotemporal dimension of PTI

How do plants sense pathogen infection while being colonized by microbiota? One important factor is the adaptability and resilience of commensal communities. Commensals with nonimmunogenic flg22 epitopes are prevalent colonizers of Arabidopsis. Notably, many of these evasive flg22 epitopes are antagonistic. At community level, a balance between agonistic, inactive and antagonistic flg22 epitopes may prevent FLS2 activation, while an increase of agonistic and/or decrease of antagonistic epitopes, for example during pathogen infection, activates PTI, suggesting that plants monitor the relative, rather than the absolute abundance of immunogenic and nonimmunogenic variants (Colaianni *et al.*, 2021). Besides, commensals manipulate flg22 sensing by modulating FLS2-flg22 binding affinities through apoplast acidification (Yu *et al.*, 2019). Intriguingly, different commensal flg22 variants induce distinct immune outputs, suggesting that commensals can fine-tune PRR activation and signalling specificity (Colaianni *et al.*, 2021). Compromising PTI results in microbial dysbiosis and detrimental microbial proliferation, demonstrating that PTI controls the microbial load (Chen *et al.*, 2020; Pfeilmeier *et al.*, 2021).

Another important factor is the heterogeneity of plant tissues. Commensals do not colonize uniformly but persist in microhabitats. Adjustable spatiotemporal PRR expression patterns define local sensing thresholds to fine-tune immune surveillance sensitivity to locally control bacterial community composition and abundance. Whereas bacterial entry sites and prominent colonization niches, for example hydathodes, substomatal cavities, or vasculature, show constitutively high FLS2 expression, levels in other tissues are dynamically regulated to adjust sensitivity on demand, for example upon wounding (Beck *et al.*, 2014). Mechanisms to prevent unintended and/or constitutive immune activation are evident in roots, which are constantly exposed to MPs in microbe-rich soil. While vulnerable regions of roots are protected by constitutive PRR expression, immune activation in the differentiation zone is 'damage-gated', that is it requires coincident MAMP and DAMP sensing (Zhou *et al.*, 2020). Additionally, downstream immune outputs differ among tissues (Emonet *et al.*, 2021; Okada *et al.*, 2021), suggesting that tissue identity determines response competence. Tissue-specific FLS2 expression levels in roots are important to avoid over-activation of immunity by commensal bacteria, which interferes with developmental programmes and commensal colonization (Emonet *et al.*, 2021). A molecular 'growth-immunity' switch operates through apoplastic pH changes in the root meristem, where apoplast alkalinization during PTI differently affects peptide-receptor interactions, that is it disrupts RGF1/RGFR interaction to halt root growth, while it favours Pep1/PEPR interaction to enhance immune signalling (Liu *et al.*, 2022). Overall, PTI is less stereotypic than previously thought and embedded in an intricate network of different signalling pathways to integrate biotic, abiotic, and developmental signals for tissue-dependent adequate immune output.

Release of MPs varies depending on the kind of colonizer, infection strategy and colonization stage. Conceivably, expression patterns of different PRRs reflect sites/tissues where the cognate MPs are present/released during plant colonization. Future research on expression patterns of different PRRs will allow to better understand the important contribution of this spatiotemporal dimension to forming a robust, adaptive host immune barrier.

VI. Prospects

Evolution has created enormous genetic variations of MPs and PRRs, made accessible beyond model species by new sequencing technologies. This diversity provides an invaluable genetic toolbox of PRR alleles for plant disease control and adapted MP variants of prevalent commensal colonizers. A major bottleneck in PTI research and application is the molecular identification of matching MP-PRR pairs, which usually requires laborious biochemical and genetic screenings. Computational genome analysis and molecular modelling approaches are emerging as powerful tools to unlock this natural potential (McCann *et al.*, 2012; Del Hierro *et al.*, 2021; Lundstrom *et al.*, 2022; Snoeck *et al.*, 2022). The rapid advancement of these technologies will drive discoveries at unprecedented rates, with the prospect of successful deployment of PRR engineering in genetic crop protection, both in combating major pathogens and designing PRR-adapted commensals and symbionts conferring beneficial traits.

Acknowledgements

Work in the Ranf laboratory is funded by the German Research Foundation (grants SFB924/TP-B10, Priority Program 2125 'DECRyPT' RA2541/2 and Emmy Noether programme RA2541/1 to SR) and the Swiss National Science Foundation (grant 310030_208139 to SR). Schemes were created with BioRender.com. Protein models were visualized with Maestro, and chemical structures with ChemDraw 19.1. Open Access funding enabled and organized by Projekt DEAL.

Competing interests

None declared.

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