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From Chaos to Harmony: Responses and Signaling upon Microbial Pattern Recognition

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Abstract

Pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) are detected as nonself by host pattern recognition receptors (PRRs) and activate pattern-triggered immunity (PTI). Microbial invasions often trigger the production of host-derived endogenous signals referred to as danger-or damage-associated molecular patterns (DAMPs), which are also perceived by PRRs to modulate PTI responses. Collectively, PTI contributes to host defense against infections by a broad range of pathogens. Remarkable progress has been made toward demonstrating the cellular and physiological responses upon pattern recognition, elucidating the molecular, biochemical, and genetic mechanisms of PRR activation, and dissecting the complex signaling networks that orchestrate PTI responses. In this review, we present an update on the current understanding of how plants recognize and respond to nonself patterns, a process from which the seemingly chaotic responses form into a harmonic defense.

Keywords

microbial elicitors; pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs); damage-associated molecular patterns (DAMPs); pattern recognition receptors (PRRs); pattern-triggered immunity (PTI); plant defense

INTRODUCTION

> "Knowing your enemy and yourself, you can fight a hundred battles without disaster."
>
> -*The Art of War*, Sun Tzu

The ability to discriminate self from nonself is the key for hosts to launch defense responses and win a war against microbes, which echoes the above saying from Sun Tzu. Compared to

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humans, sessile plants lack specialized immune cells and adaptive immunity but have developed a series of preformed defense barriers and the innate immune system to counteract nonself invasions (67, 145). The first line of plant innate immunity is triggered upon detection of microbial signatures, which were initially termed general elicitors and are now called pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) (6, 7, 14, 29, 116). Patterns originally referred to the structurally conserved and functionally essential characteristics commonly found in infectious agents but absent or disguised from the hosts in the studies of mammalian immunity (7, 116). Host-derived endogenous molecules released upon wounding or pathogen infections, named danger- or damage-associated molecular patterns (DAMPs), also trigger immune responses. These patterns are recognized by plasma membrane (PM)-resident pattern recognition receptors (PRRs) and activate immune responses against a broad spectrum of pathogens, collectively termed pattern-triggered immunity (PTI) (14, 29, 67). PTI contributes to host defense against nonadapted pathogens. Host-adapted pathogens secrete a myriad of effectors into plant cells, some of which are sensed by intracellular resistance (R) proteins, leading to effector-triggered immunity (ETI) in a pathogen race-specific manner (9, 67). Recent advances in the identification of plant PRRs and downstream signaling events suggest the blurred boundary between PTI and ETI. In addition, local induction of ETI and PTI could trigger plant resistance systemically against subsequent infections in distal tissues, a phenomenon called systemic acquired resistance (145). Recognition of nonself appears to trigger seemingly chaotic responses, yet hosts launch harmonic signaling events to warrant defense against invading pathogens. In this review, we aim to provide a current view of plant PTI, with a focus on the identification and characterization of microbial patterns and PRRs, physiological and cellular responses upon pattern recognition, and signaling events leading to PTI.

IDENTIFICATION AND CHARACTERIZATION OF PATTERNS

It has long been observed that microbial-derived compounds, including general and specific elicitors, could trigger defense responses in plants. As the genetic, molecular, and structural bases of these elicitors and the plant perception systems are being revealed, general elicitors perceived by cell-surface receptors were termed PAMPs or MAMPs, whereas pathogen-specific elicitors detected by intracellular immune sensors were often referred to as effectors (9). MAMPs from bacteria, fungi, and oomycetes, as well as plant-derived DAMPs, are diverse classes of biomolecules known to include peptides, lipophilic fatty acids, and oligosaccharides (Table 1). In this section, we review the origins and characteristics of MAMPs and DAMPs.

Bacterial Microbe-Associated Molecular Patterns

The flagellin monomer, the major component of flagella required for bacterial mobility, is a well-understood MAMP in plants and animals. The 22-amino-acid (aa) synthetic peptide flg22 corresponds to a conserved domain of the amino (N) terminus of flagellin from *Pseudomonas* species and acts as a potent elicitor in *Arabidopsis thaliana* and other plants (40). Interestingly, flgII-28, a synthetic peptide corresponding to a motif of flagellin distinct from flg22, elicits defense responses in certain *Solanaceae* species but not in *Arabidopsis*

(19). Polymorphisms in flagellin among different bacterial species appear to affect the inducibility of plant defense. A single amino acid variation in flg22 from *Xanthomonas campestris* pv. *campestris* (*Xcc*) leads to compromised defense responses in *Arabidopsis* (148). Flagellin monomers are known to be cleaved by the alkaline protease AprA secreted by *Pseudomonas*, which likely dampens PTI responses (120).

Cell walls of both Gram-positive and Gram-negative bacteria contain unique peptidoglycan (PGN) networks consisting of alternating sugar components N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid cross-linked by oligopeptides (52). PGN fragments are released by plant-derived lysozymes and act as MAMPs to elicit plant immunity (96). Treatment with PGN but not the breakdown molecules from *Staphylococcus aureus* activates defense responses in *Arabidopsis*(52). In addition, purified PGN and muropeptides from *Agrobacterium tumefaciens* and *Xcc* elicit defense responses in *Arabidopsis* (37). Lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, is a MAMP commonly used in triggering potent immune responses in mammals and also possesses elicitor activity in plants. LPS consists of lipid A, a core oligosaccharide, and an O-polysaccharide (167). Lipid A is relatively conserved and sufficient to trigger immune responses, and the core oligosaccharide is able to enhance defense in *Arabidopsis* (128).

The abundant bacterial protein elongation factor Tu (EF-Tu) triggers immune responses in various plant species (76). The conserved N-terminal 18-aa peptide (elf18) is sufficient to elicit responses in *Brassicaceae* species. In contrast, EFa50, a synthetic 50-aa peptide corresponding to the middle region of EF-Tu, functions as a MAMP in rice, suggesting that different EF-Tu recognition systems exist in different plant families (48). The N-terminal 22-aa peptide (cps22) of bacterial cold-shock proteins from *S. aureus* acts as a MAMP in tobacco (39). The serine protease PrpL secreted by *Pseudomonas aeruginosa* elicits defense responses in *Arabidopsis* (22). Notably, the catalytic triad required for its protease activity is essential for the elicitor activity. Whether PrpL itself or its product is perceived as a MAMP requires further investigation. An enigmatic proteinaceous eMax from *Xanthomonas axonopodis* pv. *citri* (*Xac*) triggers responses in several species of *Brassicaceae* (65). The tyrosine-sulfated protein RaxX secreted through the type I secretion system of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) acts as a MAMP in rice (124).

Fungal Microbe-Associated Molecular Patterns

Chitin, a homopolymer of β−1,4-linked GlcNAc, is a major component of fungal cell walls. Chitin fragments, N-acetylchitooligosaccharides consisting of more than five monomers and chitosan, a deacetylated chitin derivative, have been demonstrated as MAMPs in both dicot and monocot plants (141). β-glucans are the most abundant fungal cell wall polysaccharides. The β-glucans from *Magnaporthe oryzae* trigger production of phytoalexins, which are immune-related antimicrobial compounds, in rice (165). Chitin and glucans could be released by plant apoplastic chitinases and glucanases that decompose fungal cell walls. Ergosterol, the most abundant sterol in fungal cell membranes, is also recognized by plants and triggers immune responses (72).

Ethylene-inducing xylanase (EIX) from *Trichoderma viride* induces defense responses in tomato and potato independently of its enzymatic activity. The elicitation epitope was mapped to a pentapeptide on the exposed β-strand of EIX (131). Endopolygalacturonases (PGs), a class of fungal pectinases hydrolyzing plant pectin polysaccharides, trigger defense responses in grapes independently of their enzymatic activity, suggesting that these proteins themselves are perceived and activate defense responses (122). Furthermore, in tomato, tobacco, and *Arabidopsis*, the ceratoplatanin family protein *Bc*Spl1 secreted by *Botrytis cinerea* triggers the immunity-related cell death response called the hypersensitive response (HR) (47). In addition, the proteinaceous elicitor SCFE1 from *Sclerotinia sclerotiorum* culture filtrate induces typical PTI in *Arabidopsis* (170). The molecular identity of SCFE1 is still elusive.

Oomycete Microbe-Associated Molecular Patterns

Branched β−1,3- or β−1,6-glucans, the most abundant components of *Phytophthora* cell walls, trigger defense responses in soybean (44). The active fragment was identified as hepta-β-glucoside, a molecule that bears high binding affinity to the cell membranes of certain legumes. The essential fatty acids in oomycete species, such as eicosapentaenoic acid and arachidonic acid, are also able to trigger immune responses in plants (129).

Cellulose-binding elicitor lectin (CBEL) from *Phytophthora* cell walls is able to bind to crystalline cellulose and isolated plant cell walls. Infiltration of purified CBEL triggers necrosis and immune responses in tobacco and *Arabidopsis* (71). Cryptogein, a 10-kDa protein secreted by *Phytophthora cryptogea*, elicits HR and other defense responses in tobacco (11). Elicitins are structurally conserved lipid-binding proteins secreted by almost all pathogenic oomycetes that trigger defense responses in some species of *Solanaceae* and *Brassicaceae* (158). Pep-13, a conserved epitope derived from the calcium-dependent transglutaminase involved in cross-linking oomycete cell walls, activates defense responses in parsley and potato (17). Necrosis- and ethylene-inducing peptide 1–like proteins (NLPs), which are produced by a wide range of oomycetes, bacteria, and fungi, trigger necrosis and defense responses in dicot plants. Noncytotoxic NLPs discovered from oomycete and fungal species also confer immune activation ability (3). This elicitor activity was further pinpointed to a conserved 20-aa region (nlp20) (13). XEG1, a glycoside hydrolase 12 protein from *Phytophthora sojae*, is an important virulence factor and also acts as a MAMP in soybean (106).

Plant-Derived Damage-Associated Molecular Patterns

Molecules derived from the plant itself upon damage or pathogen infections are able to activate and/or amplify defense responses. Plant DAMPs include small peptides, nucleotides, and cell wall–derived oligosaccharides. Plant elicitor peptides (*At*PEPs) are 23-aa peptides derived from the precursor PROPEPs in *Arabidopsis*. Among six family members, *At*PEP1 is the first shown to be induced by wounding, jasmonic acid (JA), and ethylene (ET), and it activates defense responses(63). On the basis of transcriptional upregulation upon PTI elicitation, a group of small secreted peptides, designated PIPs, with 11 members in *Arabidopsis*, were proposed to function as DAMPs(62). Recently, the high mobility group box 3 (HMGB3) protein was shown to trigger PTI responses and mediate

resistance against *B. cinerea* in *Arabidopsis* (25). The intracellular HMGB3 is released to the extracellular matrix when cell membranes are damaged (25).

Extracellular adenosine-5′-triphosphate (eATP) is involved in various cellular processes and triggers immune responses in plants and animals (150). The extracellular pyridine nucleotides NAD or NADP induce defense responses and resistance against bacterial pathogens in *Arabidopsis* (172). Oligogalacturonides (OGs), oligomers of α−1,4-linked galacturonosyl residues derived from plant cell walls, activate plant defense responses and induce resistance against *B. cinerea* in *Arabidopsis* (43). OGs could be released by the hydrolytic activity of PGs from *B. cinerea*, which degrade polysaccharide homogalacturonan, a major component of pectins in plant cell walls (169). Interestingly, a small O-glycosylated peptide from a group of parasitic plants belonging to *Cuscuta* spp. elicits typical immune responses in tomato, suggesting that plants could sense danger not only from microbes but also from invading plants (58).

CELLULAR AND PHYSIOLOGICAL RESPONSES TRIGGERED BY PATTERNS

A series of coordinated cellular and physiological responses occur in plants upon pattern recognition (Figure 1). Distinct molecular and biochemical modules mediate differential cellular responses and integrate positive or negative feedback regulations to culminate in plant PTI, which collectively contributes to plant resistance against a broad spectrum of pathogens. These responses, dictated by spatial and temporal dynamics, occur instantaneously as early as seconds to minutes or as late as hours to days (Figure 2). Despite differing in thresholds, amplitudes, and timing, shared common responses upon pattern recognition represent hallmarks of PTI elicitation (Figures 1 and 2).

Increase of Cytosolic Ca^{2+} Concentration ($[Ca^{2+}]_{cyt}$)

The rapid and drastic increase of $[Ca^{2+}]_{cyt}$ is among the earliest cellular responses upon different MAMP or DAMP treatments. At the resting state, $[Ca^{2+}]_{cyt}$ is maintained at ~100–200 nanomoles, 10^4 times fewer than that in the apoplast or cellular organelles. Perception of MAMPs typically triggers the increase of $[Ca^{2+}]_{cyt}$ with a delay of ~30–40 sec and a peak at ~2–6 min, which then lasts for at least 30 min before declining to the resting state (127). The spatial and temporal changes in $[Ca^{2+}]_{cyt}$ vary in terms of the peak time, amplitude, and duration in response to different types and dosages of MAMPs (138). Cryptogein, flg22, and β-glucan trigger a biphasic $[Ca^{2+}]_{cyt}$ increase, whereas a chitin-induced $[Ca^{2+}]_{cyt}$ burst exhibits only a single peak. PGN triggers a relatively weaker $[Ca^{2+}]_{cyt}$ increase than flg22 (52). DAMPs, including OGs, eATP, and *At*PEPs, also induce typical $[Ca^{2+}]_{cyt}$ increase (78, 151).

$[Ca^{2+}]_{cyt}$ elevation can be due to an uptake of Ca^{2+} from the extracellular space and/or Ca^{2+} mobilization from organelles. Lines of evidence support that MAMP- triggered $[Ca^{2+}]_{cyt}$ increase comes from a sustained extracellular Ca^{2+} influx from the apoplast (138). Chelation of extracellular Ca^{2+} or pretreatment with Ca^{2+} surrogates abolishes $[Ca^{2+}]_{cyt}$ bursts, especially the first peak(78). However, the role of internal Ca^{2+} stores in MAMP-triggered

$[Ca^{2+}]_{cyt}$ increase remains ambiguous. Inhibition of Ca^{2+} release from internal stores by neomycin or U-73122 affects the second peak of a $[Ca^{2+}]_{cyt}$ burst triggered by β-glucans, OGs, or eATP (151). External Ca^{2+} influx may predominantly contribute to the first peak, whereas the release of Ca^{2+} from internal stores is required for the second peak of MAMP-induced $[Ca^{2+}]_{cyt}$ elevation.

$[Ca^{2+}]_{cyt}$ is regulated by the coordination of passive fluxes (Ca^{2+} channels) and active transporters (Ca^{2+}-ATPases and Ca^{2+}-antiporters) across the PM and endomembranes. However, the identity of Ca^{2+} channels mediating MAMP-induced $[Ca^{2+}]_{cyt}$ influx remains elusive. Mutation in *Arabidopsis* cyclic nucleotide-gated cation channel 2 (CNGC2) abolishes *At*PEP3- and LPS- but not flg22-induced $[Ca^{2+}]_{cyt}$ bursts (104). In addition, inhibiting the activity of ionotropic gluta-mate receptor type Ca^{2+} channels (iGluRs) blocks cryptogein- and flg22-induced $[Ca^{2+}]_{cyt}$ bursts in tobacco and *Arabidopsis* (138). Moreover, rice two-pore channel 1 (*Os*TPC1) is involved in xylanase-induced $[Ca^{2+}]_{cyt}$ bursts (56). Conversely, Ca^{2+}-ATPases, mediating Ca^{2+} efflux to the extracellular matrix and endomembrane compartments, also play certain roles in MAMP-induced $[Ca^{2+}]_{cyt}$ increase. Silencing of the endoplasmic reticulum (ER)-localized type 2B Ca^{2+}-ATPase (*Nb*CA1) in *Nicotiana benthamiana* increases cryptogein-triggered $[Ca^{2+}]_{cyt}$ bursts in both amplitude and duration (176). Furthermore, PM-resident autoinhibited Ca^{2+}-ATPases isoform 8 (ACA8) and ACA10 are essential for flg22-triggered $[Ca^{2+}]_{cyt}$ influx in *Arabidopsis* (46).

Plasma Membrane Depolarization and Extracellular Alkalinization

MAMP perception induces rapid Cl^-, NO_3^-, and K^+ effluxes, as well as H^+ influx across the PM, which often leads to membrane depolarization and extracellular alkalinization (66). Cytoplasmic acidification and PM depolarization are detected within 1 min in cryptogein-treated tobacco cells (125). Application of crude cell wall extracts from *P. sojae* to parsley cells also causes rapid alkalinization of the culture medium (64). Strong membrane potential depolarization and extracellular alkalinization in *Arabidopsis* mesophyll cells were recorded within 1 min after flg22 or elf18 treatment (66). Recovery of flg22-induced membrane potential to the resting state takes more than 1 h, a long-lasting process compared with that triggered by abiotic stresses (66). PGN induces a slower but more persistent increase in extracellular alkalinization than flg22 (52).

PM-resident H^+-ATPases (AHAs) are considered the primary pumps to transfer protons from cytosol to the extracellular matrix to establish the PM potential (36). Upon PTI elicitation, AHA activity is likely downregulated through the sequential phosphorylation of specific residues and dynamic localization to different PM microdomains (36). Upon MAMP perception, H^+ influx is accompanied by effluxes of Cl^- and K^+. Interestingly, flg22 treatment triggers a considerably higher peak value of Cl^- efflux than of H^+ influx, suggesting the involvement of anion channels in establishing PM depolarization (66). Integrin-linked kinase 1 (ILK1), which interacts with the high-affinity K^+ transporter HAK5, positively regulates flg22-induced PM depolarization, implicating the involvement of K^+ efflux in PM depolarization (16). Ca^{2+} signaling likely functions upstream of PM depolarization and extracellular alkalinization, as lanthanum, a PM Ca^{2+} channel blocker,

inhibits flg22-induced PM depolarization (66). The role of other ion channels in PTI signaling remains to be determined.

Apoplastic Reactive Oxygen Species Bursts

Reactive oxygen species (ROS) include partially reduced forms of oxygen such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$). Transient and rapid generation of apoplastic ROS, referred to as a ROS burst, is a hallmark of the early response to MAMP treatment (153). Typically, a ROS burst is initiated within ~4–6 min, reaches its peak ~10–15 min, then gradually declines to the resting state ~30 min after MAMP treatments in various plant species (64). ROS can act as a toxin barrier against subsequent pathogen infections. ROS are also involved in strengthening plant cell walls by oxidative cross-linking of polymers. In addition, ROS are versatile signaling molecules, mediating multiple responses (135, 153).

Both PM-localized NADPH oxidases and cell wall–associated peroxidases are involved in a MAMP-induced ROS burst. NADPH oxidases transfer electrons from cytosolic NADPH or NADH to apoplastic oxygen, leading to the production of O_2^-, which is then converted to H_2O_2 by superoxide dismutase (153). Among 10 respiratory burst oxidase homolog (*RBOH*) genes encoding NADPH oxidases in *Arabidopsis*, RBOHD is likely the major enzyme in MAMP-induced ROS production, as a MAMP-induced ROS burst is markedly compromised in the *rbohD* mutant (115). In addition, the class III apoplastic peroxidases that catalyze oxidoreduction between H_2O_2 and various reductants contribute to a MAMP-induced ROS burst. Mutations in *Arabidopsis* peroxidase 33 (*PRX33*) and *PRX34* diminish a MAMP-induced ROS burst (31). The relationship between peroxidase- and RBOH-mediated ROS production remains unknown.

As signaling molecules, ROS interact with other early responses triggered by MAMPs (Figure 1). It appears that $[Ca^{2+}]_{cyt}$ increase is a prerequisite for ROS production, as lanthanum abrogates flg22- and elf18-induced ROS bursts in *Arabidopsis* (127). A ROS burst is also likely downstream of ion fluxes, as inhibitors of ion channels block a ROS burst (64). In addition, the *rbohD* mutant does not affect flg22-induced PM depolarization (66). However, a feedback regulation of $[Ca^{2+}]_{cyt}$ by ROS likely exists because $[Ca^{2+}]_{cyt}$ increases in response to H_2O_2 treatment. MAMP-induced ROS burst is important for inducing the second peak or prolonged plateau of $[Ca^{2+}]_{cyt}$ (127). The causative relationship among these events is rather complex, and their genetic and biochemical bases await further elucidation.

Nitride Oxide Production

A rapid burst of nitric oxide (NO), a free radical gas that has an extremely short half-life and is a hallmark of animal innate immune responses, has also been suggested to act as an important secondary messenger and antimicrobial agent in plant defense (135). Through in vivo imaging coupled with fluorophore staining, an NO burst is detected within a few minutes upon treatment with cryptogein, xylanase, flg22, PGN, and LPS in various plant species (135). DAMPs, including eATP and *At*PEPs, have also been shown to induce an NO burst (45, 105).

In animals, NO is synthesized by NO synthases (NOS) through converting L-arginine to NO and L-citrulline. However, plants possess several distinct and unique pathways for NO production in different cellular compartments with interconnected activation and feedback regulations (135). The exact sources and enzymes mediating MAMP-induced NO biosynthesis remain elusive in plants. The NOS inhibitors L-NNA or L-NAME largely block MAMP- or DAMP-induced NO bursts in tobacco and *Arabidopsis*, suggesting the involvement of NOS-like enzymes (4). In *Arabidopsis*, mutation of *NOS1*, a plant homolog of snail *NOS*, significantly reduces LPS-induced NO production and gene expression, suggesting a role of NOS1 in PTI (167).

MAMP-induced NO production seems to be tightly connected with Ca^{2+} signaling. Ca^{2+} channel blockers inhibit MAMP-induced NO production (102). Mutation in *CNGC2*, which encodes a nonselective cation channel, inhibits LPS-induced NO production in *Arabidopsis* (4). Calmodulin-like protein 24 (CML24) is also required for LPS-induced NO production (102). During MAMP-triggered stomatal closure, NO acts downstream of the ROS burst but possesses a negative feedback regulation of NADPH oxidases through S-nitrosylation (Figure 1) (5).

Phosphatidic Acid Production

Phosphatidic acid (PA), an important intermediate in lipid biosynthesis, is also considered to be a key signaling molecule regulating various cellular activities and environmental responses. In plant immunity, PA is potentially involved in the regulation of ROS production, MAP kinase (MAPK) activation, defense gene induction, and actin remodeling (152). The levels of PA and its phosphor-ylated derivative diacylglycerol pyrophosphate (DGPP) are elevated in tomato suspension cells after treatments with xylanase, chitin, and flg22. The accumulation of PA occurs in 2 min and reaches its peak approximately 8 min after treatment (157). A rapid and transient PA induction was also detected in chitosan-treated rice suspension cells (164).

Two PA biosynthetic pathways exist in plants: the direct hydrolysis of phospholipids by phospholipase D (PLD) and the combined actions of phospholipase C (PLC) and diacylglycerol kinase (DGK) to phosphorylate diacylglycerol (DAG) to PA. The flg22- and chitosan-induced PA production is predominantly from the PLC/DGK pathway in tomato (157). Consistently, application of PLC or DGK inhibitors reduces xylanase-induced PA levels (77). In addition, xylanase also slightly activates the PLD pathway in both studies. Both PLC and PLD activities are elevated in rice cells upon chitosan treatment (164). It is possible that different MAMPs induce PA production via distinct pathways yet the genetic evidence for the involvement of PA in PTI signaling remains elusive. A mutation in PLD isoform PLDδ displays delayed defense gene induction upon chitin treatment and compromised resistance against barley powdery mildew (121). In addition, PA exhibits extensive cross-talk with other signaling molecules (Figure 1). An NO burst is required for PA production, whereas inhibition of either PLC or DGK activity decreases ROS production in xylanase-treated tobacco cells (77). Consistently, PA or DAG treatment directly induces ROS production in rice cells (164). PA stimulates RBOHD activity to promote ROS

production in plant hormone abscisic acid (ABA)-mediated stomatal closure (174). It remains unknown whether this is the case in plant PTI signaling.

Stomatal Closure

Stomata are the openings formed by two guard cells on the leaf surface for gas exchange and water transpiration. As the major routes for pathogen entry, stomatal opening and closure are regulated during pathogen infections. MAMP or DAMP treatment induces stomatal closure within 1 h, which serves as an important mechanism to limit pathogen entry (110). MAMP-induced stomatal closure is regulated by early signaling molecules, such as NO and ROS, as well as the plant hormone ET and several oxylipin molecules (5).

ABA is a key regulator of stomatal closure in abiotic stresses. It appears that flg22-induced stomatal closure shares common regulators with ABA-mediated stomatal closure (53). For example, the open stomata 1 (OST1) kinase regulates both ABA- and flg22-induced stomatal closure (110). ABA-activated OST1 subsequently phosphorylates S-type anion channel SLAC1, leading to SLAC1 activation and anion efflux, such as the efflux of Cl^-, a major contributor of stomatal closure. SLAC1 and its homolog 3 (SLAH3) are also required for flg22-induced stomatal closure. Stomata of the *slac1slah3* mutant are completely insensitive to flg22 stimulation (53). Flg22 inhibits inward K^+ channel and H^+-ATPase activity that regulates stomatal reopening (171). It remains unknown whether and how the OST1 kinase and anion channels are stimulated upon MAMP perception in guard cells.

Actin Filament Remodeling

The actin cytoskeleton, which supports cell rigidity and cytoplasmic streaming among different compartments, plays a central role in the activation of host defenses in animals. In plants, a growing body of evidence indicates that MAMP perception induces rapid and transient changes in actin organization, including an increase of both actin filament abundance and filament bundling (32). The increase in the actin filament abundance occurs as early as 15 min, whereas the enhanced filament bundling is detectable at 18 h after *Pseudomonas syringae* inoculation. Treatment of flg22 or chitin only triggers a rapid increase in actin filament abundance and does not enhance the extent of filament bundling (60). Detailed analyses of single-filament dynamics suggest that MAMP treatment increases the maximal length and lifetime of the growing actin filaments; meanwhile, it slows down the filament destruction process by inhibiting the severing activity, likely via actin depolymerization factor 4 (ADF4). It appears that the actin filament elongation rate and the regrowth frequency are not altered upon MAMP treatments (84).

Consistent with the above observations, inhibitors blocking actin polymerization promote *Arabidopsis* susceptibility to *P. syringae*, implicating an important role of actin cytoskeleton remodeling in plant immunity (60). Moreover, MAMP-triggered callose deposition is impaired when either the actin cytoskeleton or actin dynamics is perturbed (59). Interestingly, ADF4 is required for elf26 (a longer version of elf18) but not for chitin-induced actin filament abundance, suggesting that distinct signaling pathways contribute to different MAMP-induced actin dynamics (59). Capping proteins (CPs), key regulators in controlling the number of filament ends and the amount of filament-filament annealing, are

also involved in MAMP-induced actin cytoskeleton remodeling and callose deposition (84). Notably, PA binds and inhibits CP activity in vitro, and exogenous PA treatment increases the actin filament abundance. It is postulated that MAMP-induced actin remodeling is due to the negative regulation of CP by PA signaling (84). The detailed regulation between PA and CP in MAMP-induced actin dynamics still awaits elucidation.

Production of Antimicrobial Compounds and Phytohormones

Production of plant antimicrobial compounds referred to as phytoalexins has long been observed during plant-pathogen or -insect interactions. Camalexin, a characteristic indolic phytoalexin derived from tryptophan in *Arabidopsis* and related *Brassicaceae* species, is strongly induced by various pathogens, including bacteria, fungi, viruses, and oomycetes (1). However, MAMP-induced camalexin production is often not as pronounced as that induced by pathogen infections. It was reported that camalexin can be induced upon flg22 treatment in *Arabidopsis* roots (112). Indoleglucosinolates, another group of tryptophan-derived secondary metabolites, also participate in *Arabidopsis* defense against bacteria and fungi and are required for flg22-induced callose deposition (27). The tryptophan-derived cyanogenic compound, 4-hydroxyindole-3-carbonyl nitrile, is induced by flg22 treatment and contributes to defense responses in *Arabidopsis* (126). In rice, chitin elicits accumulation of terpenoids, the major phytoalexins in monocots (136). Phenolic compounds, such as phenylamides, are another type of phytoalexins that accumulate in response to MAMP treatment and mainly participate in cell wall reinforcement upon pathogen attacks in rice(24). With the advancement of mass spectrometry–based metabolomics and analytical chemistry, involvement of various types of phytoalexins in PTI will likely be elucidated.

Plant defense hormones, including salicylic acid (SA), ET, and JA, have been implicated in PTI responses (50). Consistently, the biosynthesis of these hormones is induced upon MAMP treatments. ET production has been established as a sensitive bioassay to monitor PTI responses triggered by MAMPs, including flg22, elf18, EIX, and nlp20 (3, 8, 40, 76) (Figure 2). Flg22 induces ET production at 1 h and peaks at 4 h in *Arabidopsis* seedlings (97). A moderate accumulation of SA was detected upon flg22 or LPS treatment in *Arabidopsis* (154). In addition, JA production, which is usually induced by necrotrophic pathogen infections, is elevated upon oomycete-derived Pep-13 treatment in potato (54). By analyzing the *Arabidopsis* mutants deficient in these three hormone pathways, it was shown that SA, JA, and ET act positively in flg22- and elf18-mediated PTI and mainly contribute to some of the late PTI responses (155). Additionally, other plant hormones, including brassinosteroids, auxins, ABA, cytokinins, and gibberellins, are also implicated in plant immunity (29).

Callose Deposition

Callose is a high molecular weight β−1,3 glucan polymer that strengthens weak or compromised sections of plant cell walls (101). Callose deposits in a timely manner at the site of infections, forming a prominent physical barrier for pathogen attacks and often leading to the formation of papillae. Various MAMPs or DAMPs, including flg22, elf18, chitin, PGN, and OG, induce callose deposits in plant roots, cotyledons, and leaves (101). Powdery mildew resistant 4 (PMR4), a glucan synthase-like protein, is involved in callose

synthesis and plays an important role in plant resistance against fungal infections. However, flg22- or chitosan-induced callose deposition is not completely blocked in the *pmr4* mutant, suggesting the involvement of other callose synthases in response to MAMPs (27, 101).

Callose deposition is regulated at multiple levels. Loss of RBOHD, a key enzyme in generating apoplastic ROS, results in compromised flg22- and OG-induced callose deposits (49). In addition, MAMP-induced callose deposition is almost completely abolished in the *prx33prx34* mutants(31), suggesting both NADPH oxidase– and peroxidase–produced ROS are required for MAMP-induced callose biosynthesis. Mutations in ethylene response 1 (ETR1) or ethylene insensitive 2 (EIN2), which block ET perception or signaling, respectively, largely diminish MAMP-induced callose deposition, pointing to a critical role of ET in MAMP-induced callose deposition (27).

Plasmodesmata Closure

Plasmodesmata (PD) are cytoplasmic channels that connect adjacent cells across the cell walls and mediate molecular exchanges among different cells in response to developmental, environmental, and pathogen signals (79). Regulation of PD permeability plays a significant role in plant-pathogen interactions (80). Chitin and flg22 induce PD closure by stimulating PD callose deposition in *Arabidopsis* (38). PD-located protein 5 (PDLP5), residing in the central region of PD channels, inhibits PD trafficking in *Arabidopsis*. Interestingly, *PDLP5* is induced upon bacterial infections and plays a positive role in plant defense against *P. syringae* infections, indicating the importance of PD closure in plant disease resistance (80). It is also plausible that signaling molecules and/or defense-related metabolites are transmitted via PD from infected cells. Recently, it has been shown that callose synthase 1 (CALS1) and CALS8 contribute to SA-mediated and ROS-dependent PD callose accumulations, respectively, both of which require PDLP5 (30). Apparently, plants employ specialized components and pathways to regulate PD dynamics in response to different signaling molecules.

PATTERN RECOGNITION RECEPTOR COMPLEXES PERCEIVING PATTERNS

Plants perceive patterns by cell surface–resident PRRs, which are mainly transmembrane receptor-like kinases (RLKs) or receptor-like proteins (RLPs). PRRs often dynamically complex with coreceptors and other regulatory proteins to ensure prompt signaling activation and attenuation (Figure 3). Both RLKs and RLPs comprise an extracellular domain and a transmembrane domain, but RLPs lack an intracellular kinase domain. The various extracellular domains, including leucine-rich repeats (LRRs), lysine motifs (LysMs), lectin motifs, and epidermal growth factor (EGF)-like domains, mainly determine the specificity of ligand binding (12, 29).

Leucine-Rich Repeat Domain–Containing Pattern Recognition Receptors

LRR domain–containing PRRs include both LRR-RLKs and LRR-RLPs, which primarily function in peptide ligand perception and signaling. LRR-RLKs constitute the largest group of RLKs, with more than 200 members in *Arabidopsis*, and include some well-studied

PRRs, such as flagellin-sensing 2 (FLS2) recognizing flg22, EF-Tu receptor (EFR) recognizing elf18, and PEP1 receptor 1 (PEPR1)/PEPR2 recognizing *At*PEPs (51, 75, 166, 177). Crystal structure analyses indicate that both N- and C-terminal segments of flg22 bind to the inner surface of the FLS2 LRR solenoid (149). Tomato LRR-RLK *Sl*FLS3 perceives flgII-28, a flagellin fragment distinct from flg22 (61), indicating that different regions of flagellin are recognized by different LRR-RLK receptors, likely to ensure the specificity of recognition. *Arabidopsis* RLK7 recognizes DAMPs PIP1 and PIP2 (62). Rice XA21 is the first cloned RLK that confers resistance against different X_{00} strains (144). The tyrosine-sulfated protein RaxX from X_{00} is a potential ligand of XA21 (124).

LRR-RLPs function in PTI signaling either as receptors or as signaling components. RLP23 is required for nlp20-triggered responses in *Arabidopsis* and specifically binds to nlp20 in vitro. Ec-topic expression of RLP23 causes potato plant sensitivity to nlp20 and enhances resistance against oomycete and fungal pathogens (3). Thus, RLP23 is a bona fide receptor of nlp20. Tobacco LRRRLP CSP receptor (*Nb*CSPR) associates with csp22 and is required for csp22-triggered defense responses (134). Recognition of elicitin INF1 from *Phytophthora infestans* is mediated by the potato LRR-RLP elicitin response (*St*ELR) (34). Tomato LRR-RLPs *Sl*EIX1 and *Sl*EIX2 associate with EIX and heterodimerize in a ligand-dependent manner. *Sl*EIX2 activates defense responses that are suppressed by *Sl*EIX1 (130). In addition, *Arabidopsis* RLP30, RLP1, and RLP42 and tomato LRR-RLP cuscuta receptor 1 (CuRe1) are genetically required for the defense responses triggered by SCFE1, eMAX, and PG and the parasitic plant *Cuscuta* peptide ligand, respectively (58, 65, 169, 170). Tomato LRR-RLPs, Cfs and Ve1, confer resistance against fungal pathogens *Cladosporium fulvum* and *Verticillium dahliae* carrying the cognate avirulence proteins, respectively (33, 133). This resistance occurs in a race-specific manner; thus, Cfs and Ve1 are considered as R proteins.

LysM Domain–Containing Pattern Recognition Receptors

Plant LysM domain–containing PRRs, including LysM-RLKs and LysM-RLPs, function in GlcNAc-containing microbial pattern perception and signaling (Figure 3b). Rice chitin elicitor-binding protein (*Os*CEBiP), a LysM-RLP, homodimerizes upon chitin binding and forms a sandwich-type receptor complex with two *Os*CEBiP molecules simultaneously binding to one chitin oligosaccharide (57). The second LysM domain (LysM2) of *Os*CEBiP directly binds to a chitin oligomer (94). Rice LysM-RLK chitin elicitor receptor kinase 1 (*Os*CERK1), which lacks detectable chitin binding ability, complexes with *Os*CEBiP and is involved in signaling activation (140). The *Arabidopsis* genome encodes 5 LysM-RLKs and 3 LysM-RLPs. In contrast, *Arabidopsis* CERK1 directly binds to chitin and homodimerizes for chitin-induced signaling activation (95). Interestingly, *Arabidopsis* LysM-RLK LYK5 without a detectable kinase activity possesses a significantly higher chitin-binding affinity than CERK1. Additionally, it heterodimerizes with CERK1 and is required for chitin-induced CERK1 phosphorylation, suggesting that LYK5 is a major chitin receptor in *Arabidopsis* (Figure 3b) (20). LysM-RLPs appear to not be involved in chitin-induced signaling in *Arabidopsis*, implicating that distinct chitin perception and signaling systems exist in different plant species.

Although lacking an apparent role in chitin-induced signaling, *Arabidopsis* LysM-RLPs LYM1 and LYM3 directly bind to PGNs and are required for PGN-mediated responses (160). Similarly, the rice LysM-RLPs *Os*LYP4 and *Os*LYP6 physically bind to PGN and chitin and mediate PGN-and chitin-triggered responses (92). Interestingly, CERK1 is also required in PGN-triggered signaling in *Arabidopsis* and rice, yet direct binding is not evident (92, 160). It is plausible that CERK1 complexes with LYM1 and LYM3 to mediate PGN-induced signaling. Apparently, CERK1 plays dual roles in chitin- and PGN-induced signaling in both monocots and dicots.

Lectin Domain–Containing and Epidermal Growth Factor Domain–Containing Receptor-Like Kinases

The *Arabidopsis* bulb-type lectin S-domain RLK lipooligosaccharide-specific reduced elicitation (LORE) mediates the sensitivity to the lipid A moiety of LPS from *Pseudomonas* and *Xanthomonas* (128). LPS-LORE recognition seems to be restricted to *Brassicaceae*. Transient expression of LORE in tobacco gains sensitivity to LPS, supporting the idea that LORE is the LPS receptor. The *Arabidopsis* legume-type lectin domain-containing RLK does not respond to nucleotides 1 (DORN1), binds to ATP and possibly other purine nucleotides, and is a receptor of eATP (26). In addition, *Arabidopsis* EGF motif-containing wall-associated kinase 1 (WAK1) was suggested to function as the receptor of DAMP OG (18). It is likely that other types of RLKs or RLPs with distinct ectodomains function as receptors of other MAMPs and DAMPs.

Pattern Recognition Receptor Complex Formation and Regulations

Ligand-induced dimerization of PRRs and their cognate coreceptors is a common and immediate event in initiating intracellular signaling (Figure 2). BRI1-associated receptor kinase 1 (BAK1) and other family members of somatic embryogenesis receptor-like kinases (SERKs) function as coreceptors of multiple LRR-RLKs involved in plant immunity and development (88, 103). BAK1 interacts with and acts as a coreceptor of FLS2, EFR, and PEPR1 (Figure 3a). The LRR-containing ectodomain of BAK1 directly contacts the C-terminus of FLS2-bound flg22, and flg22 appears to stabilize the FLS2-BAK1 complex as a molecular glue (149). Tomato FLS3 also complexes with BAK1 upon flgII-28 perception (61). In addition, upon the cognate ligand perception, BAK1 is recruited into multiple LRR-RLP complexes, including tomato Cfs, *Arabidopsis* RLP23, and tobacco *Nb*CSPR (3, 123, 134). However, BAK1 does not seem to be involved in the signaling pathway mediated by LysM- or lectin-domain containing RLKs, including LYK5, LYM1/LYM3, and LORE, which perceive chitin, PGN, and LPS, respectively (128). Suppressor of BIR1 (SOBIR1), another LRR-RLK with a short LRR ectodomain, constitutively complexes with various LRR-RLPs, including tomato Ve1 and Cfs and *Arabidopsis* RLP23 but not LRRRLKs, in a ligand-independent manner (3, 87). The aforementioned CERK1 heterodimerizes with LYK5 or *Os*CEBiP in chitin-triggered signaling (Figure 3b) and possibly with LYM1 and LYM3 in PGN-triggered signaling (20, 140, 160). It is possible that CERK1 functions as a shared coreceptor or signaling partner for LysM-domain containing PRRs.

PRR complexes associate with different regulatory proteins that orchestrate the timing, duration, and intensity of defense responses (Figure 3a). The pseudokinase BAK1-

interacting receptor-like kinase 2 (BIR2) associates with BAK1 and likely prevents the FLS2-BAK1 interaction in the resting state (55). Perception of flg22 by FLS2 leads to the release of BIR2 from BAK1, enabling the formation of FLS2-BAK1 complex (55). Specific subunits of protein serine/threonine phosphatase type 2A (PP2A) associate with BAK1 and negatively regulate the BAK1 basal phosphorylation (137). The phosphatase activity of PP2A is attenuated upon elf18 perception, thereby resulting in enhanced BAK1 kinase activity. Rice protein phosphatase 2C (PP2C) XB15 negatively regulates the XA21-mediated immune responses by dephosphorylating XA21 (118). However, rice ATPase XB24 promotes XA21 autophosphorylation via its ATPase activity but inhibits XA21-mediated resistance (21). The *Arabidopsis* E3 ubiquitin ligases PUB12 and PUB13 are recruited to FLS2 in a BAK1-dependent manner and directly ubiquitinate FLS2 for FLS2 degradation, leading to the attenuation of FLS2 signaling (99, 175). Ubiquitination of membrane receptors also leads to receptor endocytosis and lysosome/vacuole targeting. The ligand-activated PRRs, such as FLS2 and PEPR1, undergo clathrin-dependent internalization and enter an endocytic trafficking route from PM to vacuoles, which is likely required for the full activation of defense responses (109, 117). It remains an open question whether any E3 ligase–mediated ubiquitination is involved in PRR endocytosis.

EARLY PATTERN RECOGNITION RECEPTOR SIGNALING EVENTS

In contrast to the high recognition specificity of PRRs to the cognate MAMPs or DAMPs, the downstream signaling events triggered by PRRs converge at multiple modules, including the activation of receptor-like cytoplasmic kinases (RLCKs), G proteins, MAPK cascades, and calcium-dependent protein kinases (CDPKs/CPKs) (Figures 1 and 3). Apparently, the transcriptional reprogramming triggered by different MAMPs or DAMPs also largely overlaps (82).

Receptor-Like Cytoplasmic Kinases Complex with Pattern Recognition Receptors to Relay Pattern-Triggered Immunity Signaling

RLCKs belong to the same superfamily as RLKs but lack extracellular and transmembrane domains (143). Tomato *ACIK1*, which is induced upon Cf9 activation, is among the first RLCKs to be shown to have a role in disease resistance (133). Recent evidence indicates that RLCKs associate with PRR complexes to relay diverse intracellular signaling (Figure 3). The *Arabidopsis* RLCK, *Botrytis*-induced kinase 1 (BIK1), together with its close family member PBL1, interacts with multiple PRRs, including FLS2, EFR, PEPR1, and coreceptor BAK1 (98, 100, 168). BAK1 directly phosphorylates BIK1 at multiple serine, threonine, and tyrosine residues (89). Another RLCK, BR-signaling kinase 1 (BSK1), also associates with FLS2 and positively regulates certain PTI responses (139). In addition, PTI-compromised RLCK 1 (PCRK1) and PCRK2 associate with FLS2, and possibly other PRRs, and positively regulate multiple PTI responses, disease resistance, and SA biosynthesis (74, 146). Rice *Os*RLCK176 and *Os*RLCK185 interact with *Os*CERK1 and regulate both chitin- and PGN-mediated MAPK activation and defense gene induction (163). PBL27, the *Arabidopsis* ortholog of *Os*RLCK185, is required for chitin-triggered responses via direct interaction with and phosphorylation by CERK1 in *Arabidopsis* (Figure 3b) (142). Interestingly, CERK1 preferentially phosphorylates PBL27, whereas BAK1 preferentially

phosphorylates BIK1. It is likely that different RLCKs transduce signaling from specific PRRs via distinct phosphorylation by different coreceptors or receptors.

RLCK-mediated phosphorylation of different substrates appears to further relay PTI signaling to trigger different downstream events. BIK1 directly interacts with and phosphorylates RBOHD to positively regulate flg22- or elf18-triggered ROS bursts (68, 85). Interestingly, another BIK1 family member PBL13 also interacts with RBOHD but negatively regulates PTI responses (90). It is possible that distinct phosphorylation by BIK1 and PBL13 results in opposing activities of RBOHD or that the different interacting partners of BIK1 and PBL13 contribute to the differential regulations of RBOHD. Although loss-of-function of BIK1 and PBL1 does not significantly compromise flg22- or elf18-triggered MAPK activation, CERK1-activated PBL27 directly phosphorylates and activates a MAPK cascade consisting of MAPKKK5-MKK4/5-MPK3/6 in chitin-triggered signaling in *Arabidopsis* (162). Additional RLCK targets are likely involved in transducing signals into diverse intracellular outputs. RLCKs are also subjected to layered regulations. A phosphatase PP2C38 negatively regulates PTI responses by modulating BIK1 phosphorylation and activity toward RBOHD (28). BIK1 is phosphorylated by CPK28, which likely leads to 26S proteasome-dependent BIK1 turnover at the resting state (114). In addition, a heterotrimeric G-protein complex regulates BIK1 protein stability using an elusive mechanism (86). The trigger and mechanisms for RLCKs turnover require further investigations.

G Proteins Relay Signaling from Pattern Recognition Receptor Complexes

Despite the lack of G protein–coupled receptors (GPCRs) found in animals, plants do possess both small G proteins and heterotrimeric G-protein complexes that play critical roles in PTI signaling via direct association with PRR complexes and downstream components. Chitin treatment activates rice small GTPase *Os*Rac1, which interacts with the guanine nucleotide exchange factor *Os*RacGEF1 (2). Chitin-activated *Os*CERK1 directly phosphorylates and activates *Os*RacGEF1. Both *Os*Rac1 and *Os*RacGEF1 positively regulate chitin-triggered defense responses and resistance against the rice blast fungus *M. oryzae*. In addition, *Os*Rac1 interacts with the N-terminus of NADPH oxidase to regulate ROS bursts (Figure 3b) (161). Notably, *Os*RacGEF1 also interacts with *Os*FLS2, suggesting its broad involvement in different PRR complexes.

The canonical heterotrimeric G-protein complex includes one Gα (GPA1), one Gβ (AGB1), and three Gγ (AGG1, AGG2, and AGG3) in *Arabidopsis*. AGB1 connects a MAPK cascade activated by the serine protease PrpL secreted from *P. aeruginosa* or ArgC from *X. campestris* via the scaffold protein RACK1 (22). Although RACK1 and Gα do not appear to be involved in flg22-or elf18-triggered signaling, Gβ and Gγ are required for flg22- or elf18-induced ROS bursts and defense gene expression but not for MAPK activation (93). The *Arabidopsis* genome also contains three Gα-like extra-large G proteins (XLG1, XLG2, and XLG3), of which XLG2 is important in pathogen resistance and flg22- or elf18-triggered ROS bursts (108). Apparently, XLG2-Gβ-Gγ forms a noncanonical heterotrimeric G-protein complex to transduce flg22- or elf18-triggered signaling independent of MAPK activation. In addition, XLG2 interacts with FLS2 and BIK1 and attenuates BIK1

degradation for potentiating immune activation. Flg22 perception leads to BIK1-mediated phosphorylation of XLG2, which is subsequently released from Gβ to activate downstream proteins, such as RBOHD (86). Furthermore, BAK1 directly phosphorylates regulator of G-protein signaling 1 (RGS1), which keeps the G-protein complex at the resting state (156). Therefore, heterotrimeric G-protein complex emerges as an additional component downstream of PRR complexes to transduce PTI signaling.

Mitogen-Activated Protein Kinases and Calcium-Dependent Protein Kinases Are Convergent Components Downstream of Pattern Recognition Receptor Complexes

Rapid and transient activation of MAPKs is a hallmark of PTI responses (Figure 2) (6, 111). A MAPK cascade typically contains three sequentially activated kinases of a MAPK kinase kinase (MAPKKK or MEKK), a MAPK kinase (MAPKK or MKK), and a MAPK (MPK). At least two canonical MAPK cascades consisting of MEKK1/MEKK-MKK4/MKK5-MPK3/MPK6 and MEKK1-MKK1/MKK2-MPK4 in *Arabidopsis* have been indicated to exert opposing roles in plant defense, with the MPK3/MPK6 cascade having a positive role and the MPK4 cascade having a negative role (6, 73, 111). Chitin-activated CERK1-PBL27 phosphorylation relay activates another MAPK cascade consisting of MAPKKK5-MKK4/MKK5-MPK3/MPK6 (162). Interestingly, in contrast to the compromised MPK3/MPK6 activation triggered by chitin, the *mapkkk5* mutant shows elevated MPK3/MPK6 activation stimulated by flg22, suggesting that MAPKKK5 inversely regulates chitin- and flg22-triggered signaling (162). In contrast, the MAPKKK MKKK7, associated with FLS2 and phosphorylated upon flg22 treatment, negatively regulates flg22-triggered MPK6 activation and the ROS burst through unknown mechanisms (113).

In parallel, activation of CDPKs, which are unique Ca^{2+} sensor protein kinases, has been observed upon MAMP perception (15). *Arabidopsis* CPK4, CPK5, CPK6, and CPK11 are transiently activated in response to flg22 treatment and redundantly regulate expression of a subset of MAMP-responsive genes distinct from or overlapping with those controlled by MAPKs (15). In addition, CPK5 positively regulates flg22-induced ROS bursts via direct phosphorylation of RBOHD at specific residues different from those phosphorylated by BIK1 (35, 68). In contrast, CPK28 negatively regulates PTI signaling by modulating the BIK1 protein level through phosphorylation (114). The mechanism by which CDPKs regulate MAMP-responsive gene expression remains poorly understood.

Dynamic phosphorylation of different substrates by MAPKs and CDPKs further bifurcates PTI signaling. For instance, MPK3 and MPK6 phosphorylate and activate 1-aminocyclopropane-1-carboxylic acid synthase 2 (ACS2) and ACS6, the rate-limiting enzymes in ET biosynthesis, to regulate flg22-triggered ET production (97). In addition, flg22-activated MPK3 and MPK6 phosphorylate tandem zinc finger protein 9 (TZF9), residing in cytoplasmic processing (P) bodies, the sites for mRNA storage and decay, for full PTI responses (107). Flg22-activated MPK4 phosphorylates PAT1, a key component in mRNA decay, leading to its accumulation in P-bodies and contributing to posttranscriptional regulation of gene expression (132). MAPKs also regulate the activation of specific transcription factors in PTI signaling (see below). Compared to MAPKs, the CDPK substrates controlling defense gene expression are less understood.

Transcriptional Reprograming Leads to Pattern-Triggered Immunity–Associated Gene Expression

PTI elicitation induces rapid, dynamic, and global transcriptional changes of plant genes involved in a broad range of biological functions (82). Specific transcription factors directly phosphorylated by MAPKs or acting downstream of Ca^{2+} signaling have been shown to play a role in regulating MAMP-responsive gene expression. The *Arabidopsis* transcription factors ERF104 and BES1 phosphorylated by MPK6 play positive roles in MAMP-responsive gene expression (10, 70), whereas the trihelix transcription factor ASR3 phosphorylated by MPK4 negatively regulates expression of a subset of MAMP-responsive genes (81). In other cases, transcription factors are not the direct targets of MAPKs, but instead their activation is monitored by MAPK phosphorylation substrates. MPK3 and MPK6 phosphorylate a subset of MPK3/MPK6-targeted VQ-motif-containing proteins (MVQs), each of which interacts with a specific subclass of WRKYs via the VQ motif (119). Phosphorylation by MPK3/MPK6 destabilizes MVQ1 and subsequently activates WRKYs (119). The transcription factors CaM-binding protein 60g (CBP60g) and its homolog SARD1 bind to the promoter of the SA biosynthetic gene *ICS1* and regulate MAMP-induced SA accumulation (159, 173). Interestingly, CBP60g and SARD1 directly bind to the promoters of genes encoding PRR complexes or signaling components, including *BAK1*, *BIK1*, and *MPK3*, and modulate their expression (147).

Specific modulation of the general transcription machinery also plays an essential role in warranting rapid activation of MAMP-responsive genes. MAMP treatments induce rapid and transient phosphorylation of RNA polymerase II (RNAPII) C-terminal domain (CTD) via cyclin-dependent kinase Cs (CDKCs), which are phosphorylated and activated by MPK3 and MPK6(83). CTD phosphatase-like 3 (CPL3) dephosphorylates MAMP-activated CTD phosphorylation and negatively regulates expression of a large portion of MAMP-responsive genes (83). The Mediator complex is also implicated in plant defense, yet its precise connection with PTI signaling is not established (82). Using a forward genetic screen based on flg22-induced gene transcriptional change, protein poly(ADP-ribosyl)ation (PARylation), which is reversibly regulated by poly(ADP-ribose) polymerases (PARPs) and poly(ADP-ribose) glycohydrolases (PARGs), was indicated to play a role in regulating MAMP-responsive gene expression (41). flg22 treatment induces the PARylation of the forkhead-associated domain protein DAWDLE, a substrate of *Arabidopsis* PARP2, to regulate some of late PTI responses (42). It is postulated that PARylation of DDL may induce chromatin remodeling that creates a permissive chromatin environment for RNAPII-mediated transcription of MAMP-responsive genes. Thus, the coordinated actions of the general transcriptional machinery, gene-specific transcription factors, and chromatin remodeling contribute to the rapid and accurate transcriptional reprogramming of MAMP-responsive genes.

CONCLUSIONS AND PERSPECTIVE

Tremendous progress has been achieved over the past decades in the identification of microbial patterns, characterization of pattern-triggered plant responses, and elucidation of plant PRR receptors and signaling networks. Despite differences in certain details, a

common theme is that conserved microbial patterns are perceived by plant PRRs and trigger a series of physiological and cellular responses and transcriptional reprogramming, collectively contributing to plant defense against a broad spectrum of pathogens. Activation of PRRs, which are often cell surface–resident RLKs or RLPs, is regulated by dynamic association with coreceptors and various regulatory components as well as by the posttranslational modifications of the receptor complexes. The activated receptor complexes further relay the signaling to different intracellular modules, including RLCKs, G proteins, MAPKs, and CDPKs, leading to complex and intertwined physiological and cellular responses and transcriptional reprogramming to mount effective PTI.

Perception of MAMPs or DAMPs by PRRs instantaneously emanates layered and intertwined molecular, cellular, and physiological responses. Although the relationship and epistasis of these responses remain ambiguous in most cases, increase of $[Ca^{2+}]_{cyt}$ appears to be a prerequisite for other responses to occur, such as the ROS burst and PM depolarization. Considering the difference between cytosolic and extracellular Ca^{2+} concentrations in plant cells, a rapid $[Ca^{2+}]_{cyt}$ burst likely ensures the sensitivity of defense elicitation. The mechanism by which $[Ca^{2+}]_{cyt}$ increase is sensed to exert multifaceted cellular functions remains elusive. A ROS burst is a robust and common response upon MAMP perception, but it is a potential double-edged sword for plants. Not surprisingly, NADPH oxidases, the key enzymes mediating a ROS burst, are regulated at multiple levels, including phosphorylation by BIK1 and CDPKs, G proteins, and signaling molecules, such as PA, with both positive and negative effects. It will be interesting to dissect how NADPH oxidase activity is orchestrated by these components upon MAMP perception. MAPK activation is another robust and universal PTI response. In chitin-triggered signaling, MAPK cascades are directly activated by the upstream RLCK in the PRR complex (162). How MAPKs are activated remains enigmatic in flg22 and elf18 signaling. Different PRRs perceive distinct MAMPs/DAMPs but recruit the same group of coreceptors, such as BAK1. Notably, BAK1 and related RLKs are also coreceptors of LRR-RLKs involved in plant growth and cell differentiation and elongation (88, 103). How these coreceptors function as shared signaling modules yet maintain a high degree of signaling specificity in different biological processes remains an open question.

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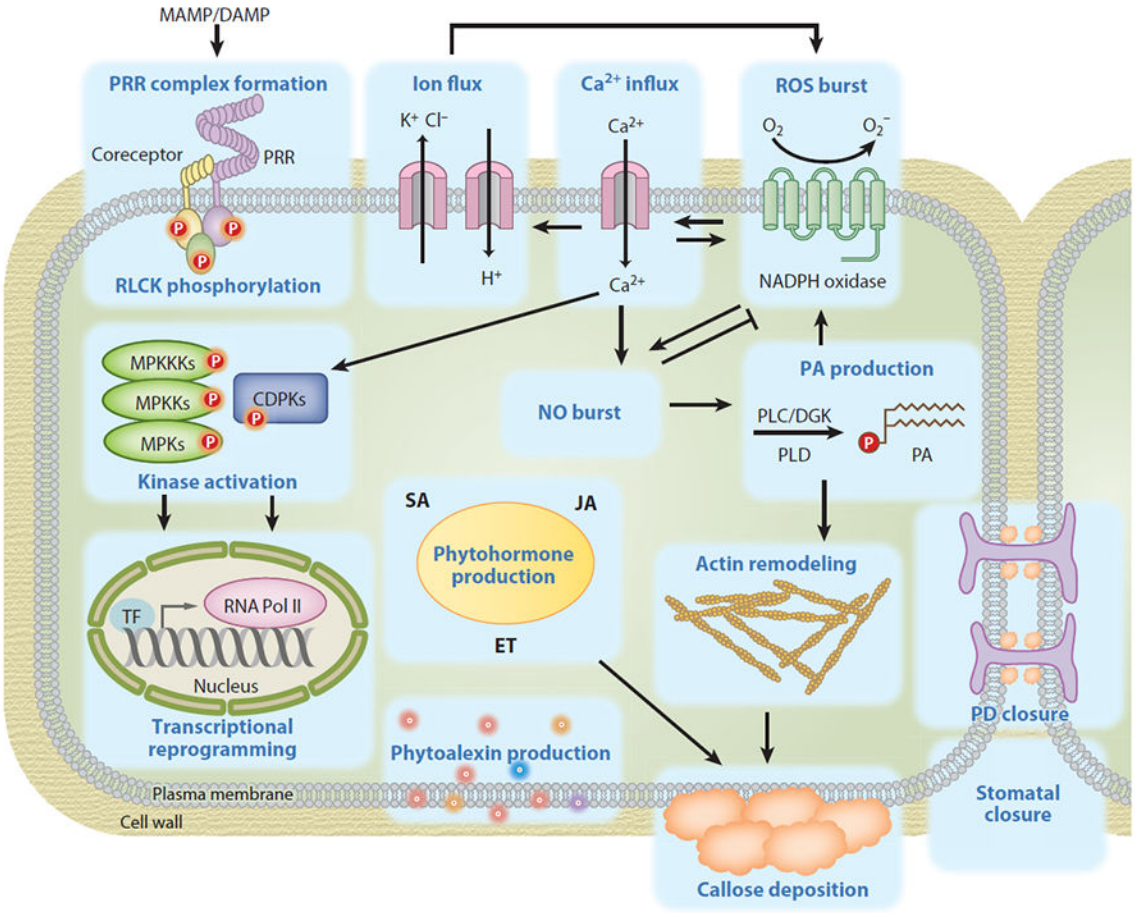

Figure 1.

Cellular and physiological responses triggered by patterns in plants. Plant cell surface–resident pattern recognition receptors (PRRs) perceive microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs) and recruit the coreceptors, leading to a series of intertwined cellular and physiological responses. PRR complex formation is accompanied by rapid transphosphorylation in the complex and phosphorylation of receptor-like cytoplasmic kinases (RLCKs). Activation of PRR complexes activates mitogen-activated protein kinase (MAPK) cascades and calcium-dependent protein kinases (CDPKs), which regulate gene transcriptional changes and other cellular responses. The hallmarks of pattern-triggered immunity (PTI) responses include calcium influx, ion efflux, actin filament remodeling, plasmodesmata (PD) and stomatal closure, callose deposition, and production of reactive oxygen species (ROS), nitride oxide (NO), phosphatidic acid (PA), phytoalexins, and phytohormones. Collectively, these responses contribute to plant resistance against a variety of pathogens. The potential connections among different responses, which were mainly derived from the studies using inhibitors, are indicated with an arrowed line for positive regulation and a T-shaped line for negative regulation. Abbreviations: DGK, diacylglycerol kinase; ET, ethylene; JA, jasmonic acid; PLC, phospholipase C; PLD, phospholipase D; SA, salicylic acid; TF, transcription factor.

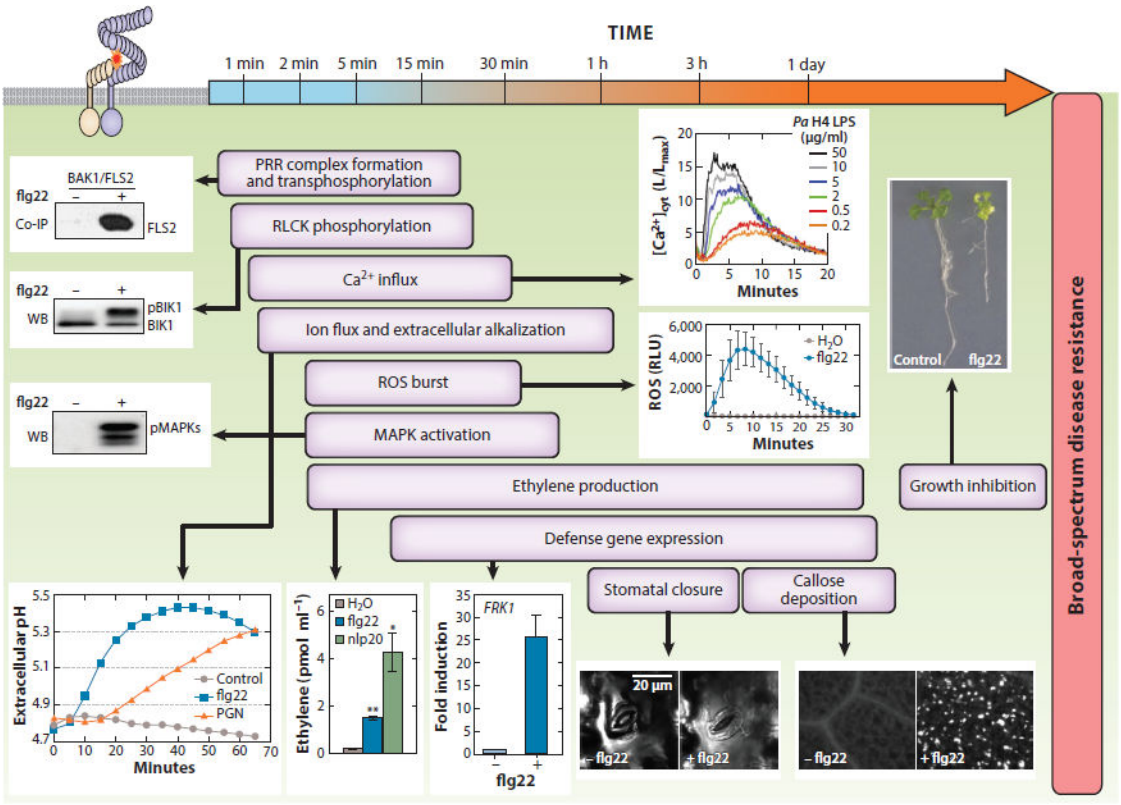

Figure 2.

Temporal dynamics of hallmark responses upon pattern perception. Pattern perception by pattern recognition receptors (PRRs) activates complex signaling events, culminating in a series of cellular and physiological responses with spatial and temporal dynamics. Some of the responses occur as early as seconds to minutes or as long as hours to days. Some responses are temporarily induced, whereas some are long lasting. The exemplary hallmark responses are shown with a timescale on the top. Images modified with permission from References 52 (extracellular alkalization) and 53 (stomatal closure), and adapted by permission from Macmillan Publishers Ltd: Reference 3, copyright 2015 (ethylene production), Reference 23, copyright 2007 (growth inhibition), and Reference 128, copyright 2015 (Ca^{2+} influx). Abbreviations: Co-IP, co-immunoprecipitation; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; PGN, peptidoglycan; RLCK, receptor-like cytoplasmic kinase; RLU, relative light unit; ROS, reactive oxygen species; WB, Western blot.

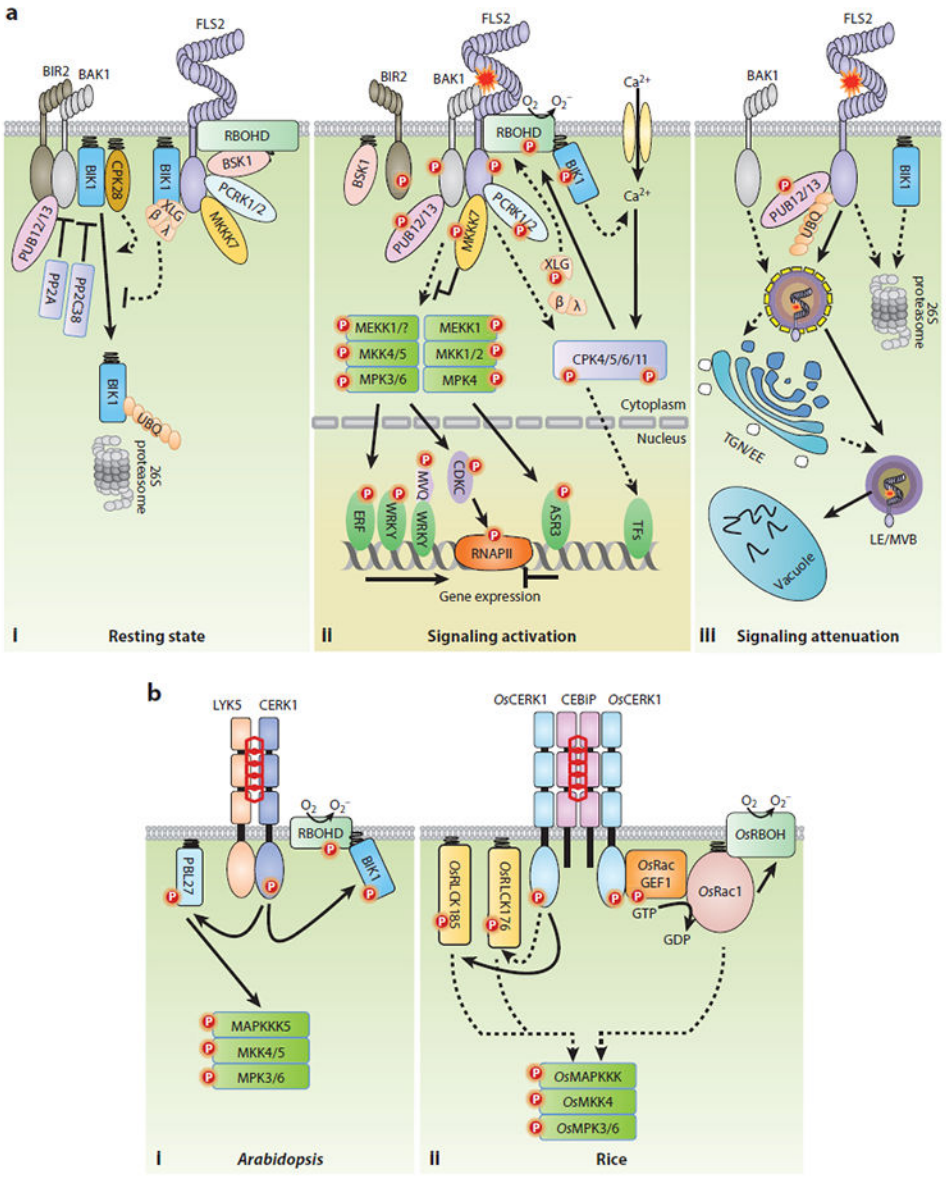

Figure 3.

Elicitation of early pattern recognition receptor (PRR) signaling in *Arabidopsis* and rice. (*a*) flg22-induced early signaling in *Arabidopsis*. (*i*) In the resting state, FLS2 does not form a stable complex with BAK1 but interacts with several receptor-like cytoplasmic kinases (RLCKs) (including BIK1, PCRK1/2, and BSK1), NADPH oxidase RBOHD, and heterotrimeric G proteins (XLG2/Gβ/Gγ). BAK1 interacts with the pseudokinase BIR2 to block the interaction between BAK1 and FLS2, the phosphatase PP2A to reduce its kinase activity, and the E3 ligases PUB12 and PUB13. BIK1 also associates with BAK1, the heterotrimeric G proteins, the phosphatase PP2C38, and CPK28. CPK28 phosphorylates BIK1, likely promoting BIK1 degradation through the 26S proteasome, whereas the heterotrimeric G proteins may suppress BIK1 degradation. (*ii*) Upon flg22 perception, FLS2 associates and transphosphorylates with BAK1, subsequently releasing BIK1, BSK1, BIR2,

and the heterotrimeric G proteins from the FLS2-BAK1 complex. BIK1 phosphorylates RBOHD to regulate reactive oxygen species (ROS) production. RBOHD activity is also regulated by CDPKs and XLG2. The activated PRR complex further activates two mitogen-activated protein kinase (MAPK) cascades (MEKK1-MKK1/2-MPK4 and MEKK-MKK4/5-MPK3/6) and CPK4/5/6/11, which regulate gene transcriptional changes through phosphoregulation of transcription factors and the general transcription machinery. The FLS2 signaling is also positively regulated by PCRK1/2 and negatively regulated by MKKK7. (*iii*) To attenuate the activated PRRs, the plant E3 ubiquitin ligases PUB12 and PUB13 are phosphorylated by BAK1 and recruited to FLS2 for FLS2 ubiquitination and degradation in the vacuole or 26S proteasome. flg22 perception also induces FLS2 endocytosis and intracellular trafficking, which may lead to FLS2 degradation or full activation of defense responses. Other PRR complex components, such as BAK1 and BIK1, may also undergo endocytosis and degradation. Similar signaling mechanisms also apply to other PRRs, in particular, leucine-rich repeat (LRR)–receptor-like kinase (RLK)-encoded PRRs. Abbreviations: LE/MVB, late endosome/multivesicular body; TGN/EE, trans-Golgi network/early endosome. (*b*) Chitin-induced early signaling in *Arabidopsis* and rice. (*i*) In *Arabidopsis*, LYK5 heterodimerizes with CERK1 upon chitin perception. Phosphorylation of CERK1 further phosphorylates and activates PBL27, which directly interacts with MAPKKK5 and phosphorylates and activates the MAPK cascade (MAPKKK5-MKK4/5-MPK3/6). CERK1, to a lesser extent, also phosphorylates BIK1, which contributes to a ROS burst. (*ii*) In rice, *Os*CERK1 is recruited to chitin elicitor-binding protein (CEBiP) upon chitin perception. Activated *Os*CERK1 phosphorylates *Os*RLCK185 and likely *Os*RLCK176, which are required for the activation of chitin-induced MAPK cascades consisting of *Os*MAPKKK-*Os*MKK4-*Os*MPK3/6. *Os*CERK1 also phosphorylates *Os*RacGEF1, leading to the activation of *Os*Rac1. *Os*Rac1 is required for the chitin-induced MAPK activation and regulates ROS burst by interacting with *Os*RBOH.

Table 1

The summary of identified microbe-associated molecular patterns (MAMPs)/damage-associated molecular patterns (DAMPs) and corresponding receptors

Category of origins	Molecules/fragments	Sources	PRRs	PRR types	References
Bacteria	flg22	Bacterial flagellin	FLS2 (*Arabidopsis* and other plants)	LRR-RLK	23, 51
	flgII-28	Flagellin from *Pseudomonas syringae* pathovars	FLS3 (*Solanaceae*)	LRR-RLK	61
	elf18	Bacterial elongation factor Tu (EF-Tu)	EFR (*Brassicaceae* species)	LRR-RLK	177
	Peptidoglycan (PGN)	Bacterial cell wall component	LYM1/LYM3 (*Arabidopsis*)	LysM-RLP	160
			*Os*LYP4/*Os*LYP6 (Rice)	LysM-RLP	91
	Lipopolysaccharide (LPS)	Bacterial cell wall component	LORE (putative) (*Arabidopsis*)	Lectin-RLK	128
	RaxX	Secreted protein from *Xanthomonas oryzae* pv. *oryzae*	XA21 (putative) (Rice)	LRR-RLK	124, 144
	csp22	Bacterial cold shock protein	*Nb*CSPR (putative) (tobacco)	LRR-RLP	134
	eMAX	A proteinaceous component from *Xanthomonas axonopodis* pv. *citri*	ReMAX (RLP1) (putative) (*Brassicaceae*)	LRR-RLP	65
	PrpL	Serine protease from *Pseudomonas aeruginosa* PA14	Unknown	Unknown	22
Fungi	Chitin	Fungal cell wall	LYK5/CERK1 (*Arabidopsis*)	LysM-RLK	20, 95
			*Os*CEBiP (Rice)	LysM-RLP	57, 69
	EIX	Fungal xylanase	*Le*Eix1/*Le*Eix2 (Tomato)	LRR-RLP	65, 131
	Endopolygalacturonases (PGs)	*Botrytis cinerea* pectinases	RLP42 (putative) (*Arabidopsis*)	LRR-RLP	169
	SCFE1	Proteinaceous component from *Sclerotinia sclerotiorum*	RLP30 (putative) (*Arabidopsis*)	LRR-RLP	170
	β-Glucan	Fungal cell wall polysaccharide	Unknown	Unknown	129
	Ergosterol	Sterol in fungal cell membrane	Unknown	Unknown	72
	BcSpl1	*B. cinerea* cerato-platanin family protein	Unknown	Unknown	47
Oomycetes	nlp20	Necrosis- and ethylene-inducing peptide from oomycetes and other microbes	RLP23 (*Arabidopsis*)	LRR-RLP	3
	INF1	Oomycete elicitin	ELR (*Sm*RLP85) (putative) (potato)	LRR-RLP	34
	Pep-13	Oomycete transglutaminase	Unknown	Unknown	17
	XEG1	Secreted glycoside hydrolase family 12 protein	Unknown	Unknown	106
	Hepta-β-glucoside	β-Glucan from oomycete cell wall	Unknown	Unknown	44
Plants	*At*PEPs	Secreted peptides from precursor PROPEPs	PEPR1/PEPR2 (*Arabidopsis*)	LRR-RLK	75, 166

Category of origins	Molecules/fragments	Sources	PRRs	PRR types	References
	PIP1/PIP2	PAMP-induced secreted peptides	RLK7 (*Arabidopsis*)	LRR-RLK	62
	High mobility group box 3 (HMGB3)	Secreted plant proteins	Unknown	Unknown	25
	Oligogalacturonides (OGs)	Plant cell wall component	WAK1 (putative) (*Arabidopsis*)	EGF-RLK	18
	eATP	Extracellular ATP	DORN1 (*Arabidopsis*)	Lectin-RLK	26

Abbreviations: *At*PEPs, plant elicitor peptides from *Arabidopsis*; EGF, epidermal growth factor; LRR, leucine-rich repeat; LysM, lysine motif; PAMP, pathogen-associated molecular pattern; PROPEPs, precursor proteins of *At*PEPs; PRR, pattern recognition receptor; RLK, receptor-like kinase; RLP, receptor-like protein.