Plant Innate Immunity: Perception of Conserved Microbial Signatures

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Keywords

Abstract

Plants and animals sense conserved microbial signatures through re-
ceptors localized to the plasma membrane and cytoplasm. These recep-
tors typically carry or associate with non-arginine-aspartate (non-RD)
kinases that initiate complex signaling networks cumulating in robust
defense responses. In plants, coregulatory receptor kinases have been
identified that not only are critical for the innate immune response but
also serve an essential function in other regulatory signaling pathways.

Contents

PROLOGUE

Here we provide a brief historical perspective on the discoveries of plant receptors that perceive conserved microbial signatures. We review major concepts and definitions of resistance genes and conserved microbial signatures; discuss the functions, characteristics, and identities of plant receptors involved in the perception of conserved microbial signatures; examine how this knowledge might

guide the discovery of novel receptors; and assess the current state of the field, with a focus on the emerging knowledge regarding signaling events occurring at the plasma membrane. We conclude with a look at how systems-biological analyses are providing new insights into the interactions of plants with pathogens and describe emerging applications for crop improvement.

MOVING FORWARD BUT FORGETTING NOTHING

The ability to recognize patterns encountered in the past and respond to their reoccurrence through specific signaling pathways is one of the most fundamental principles of information processing in biological organisms. During the course of evolution, plants and animals have acquired the capability to perceive endogenous and microbially derived compounds and respond with robust defense responses.

In the early 1970s, scientists discovered that plants possess perception systems for microbially derived compounds and that triggering these systems induces the production of antimicrobial metabolites termed phytoalexins (7, 110, 112). These initial studies involved the use of fungal and oomycete pathogens and their perception in a wide range of plant species (9, 50, 110). Several years later, Albersheim and colleagues (81) demonstrated that endogenous plant cell wall polysaccharides produced during pathogen infection are also able to elicit similar defense responses. Collectively, these endogenous or microbially derived molecules were referred to as elicitors.

Many of these elicitors were assumed to be pathogen cell wall–derived glucans, and in the mid-1980s it was demonstrated that a glucan heptamer from cell wall preparations of the oomycete *Phytophthora sojae* pv. *glycinea* could serve as an elicitor (190, 191). Soybean or pea plants treated with this and other elicitors derived from oomycete or fungal cell walls become resistant to subsequent infection with a pathogen strain that can normally cause disease (called a virulent strain) (9, 79).

These observations gave rise to the concept that elicitor-induced resistance was likely to be broad spectrum—that is, that a particular elicitor is common to a wide range of pathogens—and further suggested a central role for elicitor recognition in plant immunity.

An intense hunt began in the late 1980s to identify more elicitors, their corresponding receptors, and the genes encoding them. Progress was driven by innovative biochemists that used cell culture bioassays (e.g., parsley, soybean, tomato, rice) to monitor early responses of plant cells (e.g., ion fluxes, medium alkalization, reactive oxygen species production, protein phosphorylation) to diverse microbial signals (e.g., oligosaccharides, peptides, lipids) (19, 61, 80, 121). Although this approach led to the discovery of many more elicitors, it was far more challenging to identify the corresponding receptors. Biochemists were able to demonstrate the presence of specific high-affinity elicitor-binding sites on intact plant cells and isolated plasma membranes (13, 44, 160, 193), but attempts to purify these receptors were mostly unsuccessful. Although the pure biochemical approach led to the identification of the soluble glucan heptamer elicitor-binding protein from soybean [glucan-binding protein (GBP)] (148, 213), the lack of available genetic tools made it difficult to test its requirement for glucan perception and binding. Proof that the soybean protein is a receptor is still lacking.

Concurrent but independent efforts were launched by animal biologists to identify receptors for microbial inducers of immunity, equivalent to the elicitors of the plant world. The groundwork for receptor discovery was laid as early as the 1890s, when heat-stable molecules of microbial origin were shown to induce fever and shock in a mammalian host. Foremost among the inducers was endotoxin [lipopolysaccharide (LPS)], represented in most gram-negative bacteria (17). Widely known for its ability to induce septic shock, LPS is perhaps the most powerful elicitor of inflammation known in mammals, but it is not unique in a qualitative sense. Lipopeptides, double-stranded RNA, microbial DNA, flagellin, and

Broad-spectrum resistance: immunity to a wide range of pathogens (e.g., all species within a genus or several pathogen genera); not restricted to a single specific strain

other molecules of microbial origin elicit inflammatory responses similar to those provoked by LPS. The identification of the receptors for these molecules was a central challenge in the field of animal and plant immunity (178).

SOME PLANT RESISTANCE GENES RECOGNIZE CONSERVED MICROBIAL SIGNATURES

In 1942, Flor, working with the rust disease of flax, proposed the gene-for-gene hypothesis based on genetic analyses of the variation within host and pathogen populations. He used the terms host resistance genes (*R* genes) and pathogen avirulence genes (*avr* genes) (66, 67). The presence of corresponding *avr-R* genes in each organism leads to recognition and the activation of defense responses, limiting infection. Flor's hypothesis suggested that specific sensors for microbial molecules were present in their hosts. Many of these *R* genes were highly variable, being present in only a few plant varieties, and many *R* genes did not confer broad-spectrum resistance, specifying resistance to only some races of a particular pathogen species.

Because both virulent and avirulent pathogens often carry elicitors, these molecules were not considered to be the determinants of race-specific resistance (2). These observations led to a long debate among plant biologists. Many believed that gene-for-gene resistance had little to do with elicitor perception and that R proteins were not receptors for elicitors (111). Still, some scientists predicted that products of certain *R* genes might in fact recognize conserved microbial signatures (19, 177). The isolation of diverse classes of *R* genes allowed for direct testing of these disparate views.

In the 1990s, an avalanche of genetic experiments in many labs led to the isolation of the first *R* genes from multiple plant species. These discoveries established that diverse molecules and mechanisms govern the resistance phenotypes described by Flor and Albersheim. Many of the first *R* genes isolated encoded cytoplasmic NLRs [nucleotide-binding site domain (NBS), leucine-rich repeat (LRR)–containing intracellular proteins]: These include *Arabidopsis RPS2* (*resistance to Pseudomonas syringae 2*), which was isolated using a map-based cloning approach (147), as well as flax *L6* and tobacco *N*, which were isolated by transposon tagging (126, 222). Both N and L6 carry a TOLL-interleukin receptor (TIR) domain. RPS2 recognizes the *P. syringae avr* gene product avrRpt2 (8, 16, 139, 140, 147). L6 recognizes specific variants of the flax rust protein AVRL567 in a sequence-specific manner (53, 54). N recognizes the helicase domain of TMV (tobacco mosaic virus) replicase proteins (25, 162). Many additional NLR proteins were later shown to directly or indirectly perceive highly variable *avr* gene products, now called effector proteins. Pathogens secrete effectors into the plant apoplast or, in the case of bacteria, directly into the plant cell using type III secretion.

Other *R* genes isolated at this time included the tomato PTO kinase, the rice XA21 receptor kinase (RK), and the tomato receptor-like protein (RLP) CF9, which lacks a kinase domain (101, 141). As *Arabidopsis* became established as a highly tractable genetic system, many laboratories interested in the plant innate immune response shifted their focus to studies of *Arabidopsis* NLRs, which are abundant in dicot genomes and give rise to easily distinguished phenotypes.

Of the plethora of *R* genes isolated in the 1990s, only rice *Xa21*, which encodes an RK with predicted extracellular LRR, transmembrane (TM), juxtamembrane (JM), and intracellular kinase domains, was hypothesized to recognize a conserved microbial signature, a property not previously noted in studies of most other *R* genes (96, 116, 177, 198). ~~Indeed, we now know that XA21 binds a type I secreted, sulfated peptide called AxYs22, derived from the Xanthomonas oryzae pv. oryzae (Xoo) Ax21 protein, which is highly conserved in all Xanthomonas species as well as closely related genera (82, 133).~~

Resistance gene (*R* gene): the single polymorphic locus in the host that confers resistance to a particular strain or class of microbes

Avirulence gene (*avr* gene): a microbial gene that encodes a protein (including modifying enzymes, scavenging molecules, and effectors) that determines the specificity of the interaction with the host

Conserved microbial signature: a widely distributed and conserved microbial molecule that is required for basic microbial fitness; host perception triggers an innate immune response

Toll-like receptors (TLRs): one class of receptors involved in conserved microbial signature perception in animals

Erratum

Soon after the isolation of the plant R proteins, the fly *Toll* and mouse Toll-like receptor *Tlr4* genes were isolated and shown to encode membrane-anchored receptors that are also involved in microbial recognition and defense. TOLL and TLR4 carry striking structural similarities to XA21 as well as the TIR motif found in L6 and N. Like XA21, TLR4 recognizes a conserved microbial signature, LPS. TOLL recognition of a cleaved endogenous peptide triggers the production of antimicrobial peptides. In contrast to XA21, which carries a non-arginine-aspartate (non-RD) kinase integral to the receptor, TLR4 and TOLL associate with non-RD kinases [e.g., IRAK1 (interleukin-1 receptor-associated kinase 1) and RIP1 (receptor-interacting protein 1)] through adaptor proteins (178).

In plants, interest in recognition of conserved microbial signatures was rekindled five years after the isolation of XA21 by the identification of flagellin—or peptides spanning the conserved flg22 peptide present in its N-terminal region—as a strong elicitor of the *Arabidopsis* immune response (60) and the isolation of the corresponding receptor, *FLS2*, in 2000 by positional cloning and transgenic complementation of a null genetic background. The observation that XA21, TLR4, and FLS2 have similar domain structures and that they all recognize conserved microbial signatures suggested that they might function in a similar manner (**Figure 1**).

The subsequent demonstration that FLS2 binds to flagellin provided the first molecular evidence that plant receptors can physically bind conserved microbial signatures and that this binding contributes to the defense response (35, 228). A few years later, *Arabidopsis* EFR (EF-Tu receptor) was identified as the receptor for the conserved microbial signature EF-Tu (elongation factor thermo-unstable) (227). EFR is also an LRR-RK and was identified based on its homology with FLS2. It belongs to the same RK subfamily as FLS2 and XA21 (subfamily XII) (196).

Just as it has taken a long time for the plant biology community to accept that some

classically defined *R* genes, such as *XA21*, actually encode for receptors for conserved microbial signatures, it took a long time for some to accept that FLS2 plays an important role in conferring host resistance during infection. Opinion began to shift with the discovery that a mutation in *FLS2* left *Arabidopsis* susceptible to the bacterial pathogen *P. syringae* (228) and with the demonstration that XA21 could bind a highly conserved *Xoo*-derived peptide (133). In addition to the well-characterized XA21, FLS2, and EFR RKs, several RLPs and RKs have been shown or hypothesized to be involved in recognition of other conserved microbial signatures (**Table 1**) (20, 32, 178, 211). Conversely, a number of conserved microbial signature molecules, including proteins, fatty acids, and oligosaccharides, have been isolated from bacteria, fungi, and oomycetes, but their receptors have not been identified (20).
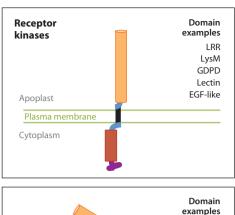
A DISCURSIVE SNAPSHOT OF THE PLANT INNATE IMMUNE SYSTEM
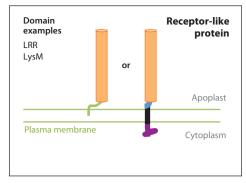
Knowledge about the molecular structures of elicitors and their cognate receptors provided a critical framework for understanding plant response to infection (20, 102, 159). Researchers are now in a position to advance some general principles on their nature and function.
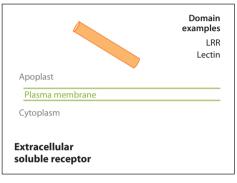
Conserved microbial signatures (exogenous elicitors) are now generally considered to be equivalent to animal pathogen-associated molecular patterns (PAMPs) (159). As these conserved molecules also occur in non-pathogenic bacteria, several researchers prefer the term microbe-associated molecular patterns (MAMPs). One key aspect of the definition of PAMPs and MAMPs is that they are conserved and widely distributed within a class of microbes (144). For this reason, we use the term conserved microbial signatures throughout this review. Endogenous elicitors that are released from the host by enzymatic or mechanical processes controlled by the pathogen are now widely referred to as danger-associated molecular patterns (DAMPs) (20, 142).

Non-RD kinases: kinases that lack the highly conserved arginine (R) that precedes the catalytic aspartate (D) typical of most kinases
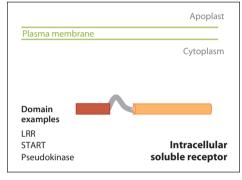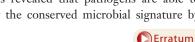
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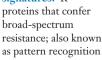
Structure of known and predicted receptors of conserved microbial signatures. Plant receptors of conserved microbial signatures contain four principal structural subfamilies, shown here schematically along with examples of known or potential ligand-binding domains for each. (For details on specific examples for each subgroup, see **Table 1**.) Structural domains are marked according to the key. To date, all confirmed plant receptor kinases recognizing a conserved microbial signature carry a non-arginine-aspartate (non-RD) kinase domain. Abbreviations: EGF, epidermal growth factor; GDPD, glycerophosphoryldiester phosphodiesterase; GPI, glycophosphatidylinositol; LRR, leucine-rich repeat; START, StAR-related lipid transfer.

Highly Conserved Microbial Signatures Are Not Invariant

Owing to the explosion of studies on perception of conserved microbial signatures, it has become apparent that some of these molecules are distributed quite widely across genera (e.g., flagellin) or more narrowly within a genus (e.g., Pep13) (23). Researchers have also confirmed that plants carry a genetically diverse repertoire of receptors of conserved microbial signatures, with differences between and within species. For example, rice XA21 has so far been identified only in the wild species *Oryza longistaminata*, even though the ligand Ax21 is highly conserved in all *Xanthomonas* species and related genera. Recognition of the conserved microbial signature EF-Tu or the glucan heptamer is restricted to Brassicaceae or Fabaceae, respectively (64, 123).

Recent research from several laboratories has revealed that pathogens are able to modify the conserved microbial signature by

Receptors of conserved microbial signatures: R proteins that confer broad-spectrum resistance; also known as pattern recognition receptors (PRRs)

Erratum

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Table 1 Table of confirmed and predicted plant receptors of conserved microbial signatures

Name	Plant[a]	Overall structure	Ligand–binding domain	Ligand/epitope	Pathogen	Function	Criteria	Reference(s)
Confirmed receptors of conserved microbial signatures								
Receptor kinases								
XA21	Rice	Non–RD RK	LRR	~~Ax21/AxYˢ22~~ Unknown	*Xoo*	~~Quorum-sensing~~ Unknown	Required for broad-spectrum resistance to *Xoo*, ~~required for AX21 perception in vivo, directly binds conserved microbial signature~~	134, 199, 229–232
FLS2	*Arabidopsis*	Non–RD RK	LRR	Flagellin/flg22	*Pseudomonas tabaci*	Mobility	Required for broad-spectrum resistance to bacteria, required for flagellin perception in vivo, directly binds conserved microbial signature	35, 78
		Potential additional ligands		axYˢ22-A1	*Xoo*	Unknown	Required for A1 peptide perception in vivo, biological relevance unknown	47
				Clavata 3/CLV3 peptide	*Arabidopsis*	Stem cell regulator	Required for defense-related CLV3 perception in mesophyll protoplasts and the shoot apical meristem, potentially binds CLV3 peptide with low affinity	128
EFR	*Arabidopsis*	Non–RD RK	LRR	Elongation factor TU/elf18	*Escherichia coli*	Protein translation	Required for broad-spectrum resistance to bacteria, required for EF-Tu perception in vivo, directly binds conserved microbial signature	228
WAK1[a]	*Arabidopsis*	RD RK	EGF-like	OG	Lytic fragments of plant cell wall	Plant cell wall	Required for OG perception in vivo, binds conserved microbial signature	24
Receptor-like proteins								
CEBiP	Rice	RLP/GPI-anchored membrane protein	LysM	Chitin	Fungi	Cell wall component	Required for chitin perception in vivo, required for resistance against fungi, mediates chitin binding, likely directly binds conserved microbial signature	105
LYM1 and LYM3	*Arabidopsis*	RLP/GPI-anchored membrane protein	LysM	PGN	Bacteria	Cell wall component	Required for PGN perception in vivo, required for resistance against bacterial pathogens, directly binds conserved microbial signature	223
EIX	Tomato	RLP	LRR	EIX	Yeast	Cell wall-degrading enzyme	Required for EIX perception in vivo, mediates EIX binding, likely binds conserved microbial signature	177

(*Continued*)

Table 1 (Continued)

Name	Plant[a]	Overall structure	Ligand-binding domain	Ligand/epitope	Pathogen	Function	Criteria	Reference(s)
					Predicted receptors of conserved microbial signatures			
					Receptor kinases			
Pi-D2	Rice	Non-RD RK	Lectin	Unknown	*Magnaporthe grisea*	Unknown	Non-RD kinase required for broad-spectrum resistance against *Magnaporthe grisea*	33
XA26	Rice	Non-RD RK	LRR	Unknown	*Xoo*	Unknown	Non-RD kinase required for broad-spectrum resistance against *Xoo*	202
RLK1	Tobacco	Non-RD RK	Lectin	Potentially CAP-Pa28	*Phytophthora capsici*	Unknown	Non-RD kinase, potentially binds conserved microbial signature	119
SNC4	Arabidopsis	Non-RD RK	GDPD	Unknown	Unknown	Unknown	Non-RD kinase, auto-activated protein variant causing resistance response	18
CERK1	Arabidopsis	RD RK	LysM	Chitin, PGN	Fungi, Bacteria	Cell wall component	Required for chitin and PGN perception in vivo, required for resistance against fungal and bacterial pathogens, directly binds conserved microbial signatures with a low affinity	150, 216, 223
		Potential additional ligands		Unknown but likely PGN	*Pst*	Unknown	Required for basal resistance to *Pst*, required for responses triggered by an unknown bacterial conserved microbial signature	76, 77
					Receptor-like proteins			
CF-4	Tomato	RLP	LRR	Avr4	*Cladosporium fulvum*	Chitin binding, protection from plant chitinases	Required for Avr4 perception in vivo, required for resistance to *Cladosporium fulvum*, potentially binds widely distributed ligand	200
SNC2	Arabidopsis	RLP	LRR	Unknown	*Pst*	Unknown	Required for resistance to *Pst*	227
RLP30	Arabidopsis	RLP	LRR	Unknown	*Psp*	Unknown	Required for resistance to *Psp*	217
RLP52	Arabidopsis	RLP	LRR	Unknown	*Erysiphe cichoracearum*	Unknown	Required for resistance to *Erysiphe cichoracearum*	170
VE1	Tomato	RLP	LRR	Unknown	*Verticillium dahliae race 1*	Unknown	Required for resistance to several *Verticillium dahliae* and *Verticillium albo-atrum* race 1 isolates	110

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Extracellular soluble receptors								
MBL1	Pepper	Soluble predicted plasma membrane–localized protein	Lectin	D-Mannose containing molecules	Xcv	Potential cell wall component	Required for resistance to Xcv	95
GBP	Soybean	Soluble predicted plasma membrane–localized protein	Glucan binding	Beta-glucan heptamer	Phytophthora sojae	Cell wall component	Directly binds conserved molecular signature	149, 214
XA21D	Rice	Soluble predicted plasma membrane–localized protein	LRR	~~Ax21/AxYS22~~ Unknown	Xoo	~~Quorum sensing~~ Unknown	Required for broad-spectrum resistance to Xoo	218, 229–232
Cytoplasmic receptors								
RPG1	Barley	Cytoplasmic plasma membrane–localized non-RD kinase	Pseudokinase	Unknown	Puccinia graminis	Unknown	Non-RD kinase required for broad-spectrum resistance to Puccinia graminis, directly binds conserved hypersensitive response–inducing ligands isolated from urediniospores	22, 153
RPG5	Barley	Cytoplasmic non-RD kinase	NLR	Unknown	Puccinia graminis	Unknown	Non-RD kinase required for broad-spectrum resistance to Puccinia graminis	21
WKS1	Wheat	Cytoplasmic non-RD kinase	START	Unknown	Puccinia striiformis	Unknown	Non-RD kinase required for broad-spectrum resistance to Puccinia striiformis	71

Abbreviations: EGF, epidermal growth factor; EIX, ethylene-inducing xylanase; GDPD, glycerophosphoryldiester phosphodiesterase; GPI, glycophosphatidylinositol; LRR, leucine-rich repeat; NLR, nucleotide-binding site domain, leucine-rich repeat–containing; OG, oligogalacturonide; PGN, peptidoglycan; Pop, Pseudomonas syringae pv. phaseolicola 1448A; Pst, Pseudomonas syringae pv. tomato DC3000; RD, arginine-aspartate; RK, receptor kinase; RLP, receptor-like protein; START, StAR-related lipid transfer; Xcv, Xanthomonas campestris pv. vesicatoria; Xoo, Xanthomonas oryzae pv. oryzae.

[a]WAK1 is the only plant receptor involved in innate immune signaling identified so far that recognizes an endogenous danger–associated molecular pattern (DAMP) signal.

Hypersensitive response (HR): a form of programmed cell death induced after pathogen recognition and believed to contribute to the restriction of pathogen growth

▶ Erratum

mutations or posttranslational modifications to mask their recognition. This may represent an active virulence strategy of the pathogen to evade recognition by a given set of receptors. For example, flagellins from distinct *Acidovorax avenae* isolates with the same primary amino acid sequence are differentially recognized in rice depending on the presence or absence of glycosylation (30, 91, 207). Glycosylated *P. syringae* pv. *glycinea* flagellin, mediated by the putative glycosyltransferase ORF1, is no longer recognized by its host, soybean (205). Similarly, several isolates of *P. syringae* pv. *tomato* or of *Xanthomonas campestris* pv. *campestris* display amino acid polymorphisms within flagellins that mask their recognition in tomato or *Arabidopsis*, respectively (27, 200). In the case of rice, tyrosine sulfation is required for the XA21-mediated immune response (46, 233). Pathogens also employ scavenging proteins, which bind conserved microbial signatures, to evade recognition. The fungal plant pathogen *Cladosporium fulvum*, for example, secretes molecules (called ECP6s) that are able to bind chitin, making it inaccessible for plant receptors (49).

The Plant Response to Conserved Microbial Signatures Can Be Weak or Robust

The recognition of conserved microbial signatures and DAMPs by plant receptors—also called pattern recognition receptors (PRRs)—represents the first layer of the plant immune system (38, 102). Plant biologists often call this immune response pattern- or PAMP-triggered immunity (PTI). Virulent pathogens are able to suppress PTI by employing effectors that target signaling components. Plants, in turn, have evolved specific recognition machinery to detect such effectors. These plant molecules belong to several classes of proteins, the most abundant being the NLRs (55, 59, 140). The activation of these effector receptors, either directly or indirectly, leads to effector-triggered immunity (ETI) and is often associated with a hypersensitive response (HR) (20, 102).

PTI does not always fully restrict pathogen proliferation; it sometimes leads to a qualitatively weak defense response. However, PTI can also result in a very robust resistance response, as observed for XA21- and FLS2-mediated immunity in rice (198, 207). In fact, in monocots, most of the predicted receptors of conserved microbial signatures confer robust resistance (**Table 1**). Similarly, the observations that *Arabidopsis* mutants impaired in their response to multiple conserved microbial signatures are hypersusceptible to a wide range of pathogens indicate the importance of PTI in resistance (179, 187). Conversely, ETI can also be weak or strong, depending on the allele (58, 102). Thus the perception of conserved microbial signatures can be weak or robust depending on the ETI or PTI system being studied.

Plant recognition of effectors often triggers an HR, whereas an HR is not always present during recognition of conserved microbial signatures. Still, this distinction is not clear-cut, as some conserved microbial signatures are also known to elicit HR-like symptoms in their hosts. For example, flagellin from an avirulent strain of *A. avenae* and LPS triggers an HR in rice (52, 207). Similarly, several conserved microbial signatures from oomycetes, called elicitins, induce an HR-like response in tobacco (105, 224).

RECEPTORS OF CONSERVED MICROBIAL SIGNATURES AND BEYOND

An Attempt to Define and Predict Receptors of Conserved Microbial Signatures

As indicated above, receptors of conserved microbial signatures typically confer broad-spectrum resistance. Microbes have evolved changes in the amino acid sequence and posttranslational modification of the ligands that can influence detection and secrete effectors to suppress PTI.

Most plant receptors of conserved microbial signatures identified so far are plasma membrane–localized RKs, RLPs, and extracellular soluble proteins that recognize their ligands in the apoplastic space (**Table 1**). In addition, several structurally related proteins are predicted to be receptors of conserved microbial signatures as they have a confirmed role in (broad-spectrum) disease resistance or are known to bind a conserved microbial signature (**Table 1**). The recognition of conserved microbial signatures is most likely not restricted to the apoplastic space because several cytoplasmic non-RD kinases were recently identified as conferring broad-spectrum disease resistance (21, 22, 71). Clearly, as more genomes (especially monocot genomes) are explored, new receptor variants will be found (**Figure 1**, **Table 1**).

To date, all characterized plant RKs that carry the non-RD kinase motif are involved in the recognition of conserved microbial signatures. Furthermore, such proteins typically confer the broad-spectrum resistance characteristic of receptors of conserved microbial signatures. Thus the non-RD motif might be diagnostic of a role in innate immune signaling. However, the converse is not true: Not all kinases involved in immune responses belong to the non-RD subclass as, for example, many receptors of conserved microbial signatures associate with coregulatory RKs that belong to the RD kinase subclass. Therefore an RD kinase may still be involved in the defense response, but it is typically not the initial kinase involved in signal initiation. To date, all receptors involved in DAMP perception belong to the RD subclass. Similarly, RKs governing developmental responses are typically RD kinases.

RKs, and in particular RKs from the non-RD kinase subclass, underwent a huge expansion in plants as compared with animals (48, 196). Whereas humans have 10 predicted TM receptors of conserved microbial signatures that associate with non-RD kinases, plants have many times more. It is tempting to speculate that plants compensate for the lack of an adaptive immune system with an increased reliance on recognition of conserved microbial signatures. Furthermore, there are vast differences within the plant kingdom. For example, genome analyses have revealed that rice has approximately 10 times as many non-RD RKs (328 non-RD RKs) as *Arabidopsis* (35 non-RD RKs) (31, 48, 178). It may be that the expansion of non-RD kinases in rice is representative of a divide between monocots and dicots.

Innate Immunity Mediated by XA21 and XA21D

As described above, the rice *Xa21* gene confers broad-spectrum resistance to *Xoo* and was isolated by positional cloning from the wild species of rice, *O. longistaminata* (198). ~~XA21-mediated immunity is triggered by the type I secreted, sulfated peptide AxYs22. Isolated field strains that lack the predicted secretion or sulfation components are only weakly virulent in plants carrying *XA21* (40, 46). This suggests that the sulfated Ax21 protein is required for bacterial fitness under field infection conditions.~~ Activation of XA21 mediated immunity requires the sulfotransferase encoded by *raxST* (S.W. Han & P.C. Ronald, 2012 submitted). Similarly to *Pseudomonas* ORF1, which is critical for glycosylation and determines host specificity, *raxST* can be considered an avirulence determinant because strains lacking *raxST* are not recognized by XA21 and are virulent in rice greenhouse tests.

~~The conservation of Ax21 in all sequenced *Xanthomonas* species and closely related genera indicates that Ax21 serves a key biological function.~~ Eight *rax* required for activation of Xa21-mediated immunity genes have been isolated so far: *raxA*, *raxB*, and *raxC* encode components of a predicted type I secretion system; *raxST*, *raxP*, and *raxQ* encode enzymes involved in sulfation; and *raxH* and *raxR* encode a predicted histidine kinase and cognate response regulator, respectively (26, 192, 197). ~~This, together with the finding that the expression of all eight *rax* genes is density dependent and inducible at low densities by the~~

Non-RD kinase motif: a hallmark of kinases involved in innate immune signaling in plants and animals

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exogenous application of Ax21, suggested that ~~Ax21 is a quorum sensing (QS) factor (132).~~

~~QS is a process where small molecules serve as signals to recognize cell population size, leading to changes in expression of specific genes when the QS factor has accumulated to a certain threshold concentration (132). We have now shown that Ax21-mediated QS controls motility, biofilm formation, and virulence (83, 176). Genetic evidence suggests that *Xoo* RaxH is the receptor for Ax21 (83). These studies point to a model where XA21 intercepts Ax21/RaxH-mediated bacterial communication and uses this information to mount a potent immune response.~~

~~These studies establish a critical role for a conserved microbial signature both in QS of a gram-negative bacterium and in activation of the host immune response. Because analysis of the genome sequences of gram-negative bacteria reveals an abundance of predicted type-I-secretion-system secreted peptides or small proteins (83, 146), it may be that other gram-negative bacteria also use small proteins for QS. Furthermore, it is possible that some of the hundreds of predicted orphan receptors of conserved microbial signatures in rice and other plant species—for which no corresponding conserved microbial signature has been identified—will detect such molecules (48).~~

An *Xa21* family member called *Xa21D* is able to confer partial resistance to *Xoo* and confers the same broad-spectrum resistance, ~~suggesting that recognition of Ax21 also triggers XA21D-mediated resistance.~~ *Xa21D* encodes a predicted secreted extracellular soluble protein with an LRR domain 98% identical to that of XA21 (217). In contrast to XA21, XA21D lacks both TM and intracellular signaling relay domains. How does an exclusively extracellular-localized receptor induce an intracellular signaling cascade? XA21D potentially works analogously to MD2, an extracellular soluble protein necessary for LPS perception in mammals. LPS binding to MD2 induces a heterocomplex formation with TLR4 and subsequent intracellular signaling (1).

Innate Immunity Mediated by FLS2, a Receptor with Multiple Ligands

Flagellin, the building block of the eubacterial flagellum, is recognized by its cognate receptor FLS2 in nearly all plant species tested. The recognized domain within flagellin is not necessarily the same for all plant species. For example, in addition to the classical flg22 peptide, tomato is able to recognize a shorter version (called flg15) and a second, newly identified, conserved 28-amino-acid region just C-terminal of flg22 (called flgII-28) (14, 27, 172). Similarly, rice is able to recognize flg22 but is more responsive to full-length flagellin protein (204). This difference in recognition specificity is an intrinsic property of the respective receptors of conserved microbial signatures and is most likely caused by changes in the extracellular LRR ligand-binding domain.

In recent years orthologous FLS2 receptors have been isolated from tomato, tobacco, and rice (84, 172, 204). All of these receptors display high levels of identity to *Arabidopsis* FLS2 at the amino acid level and also mediate flagellin perception. It is not known how these orthologous receptors recognize their ligands, because computational and phylogenetic approaches suggested different amino acid residues as important for ligand binding (3, 20, 56). Crystallographic studies of the extracellular LRR domains in complex with the cognate ligands are needed to reconcile these disparate views.

Two recent studies suggested that AtFLS2 has a broader recognition capacity than initially anticipated. First, Ax21-derived peptides (in particular the axY^{s}22-A1 peptide, which does not naturally occur in *Xoo*) activate FLS2-mediated *Arabidopsis* immunity. Genetic studies suggest that the A1 peptide occupies the same binding site as flg22 (47). This result is surprising because rice FLS2 does not appear to sense Ax21 or Ax21-derived peptides; that is, rice plants lacking XA21 (that still carry OsFLS2) do not respond to Ax21, AxY^{s}22, or the A1 peptide with measurable resistance (133, 198). It is not known how AtFLS2 retained or acquired its ability to recognize the A1 peptide in the course

of evolution or whether FLS2 from other dicots or monocots also recognizes similar peptides.

AtFLS2 has also been suggested to mediate stem cell–specific immunity through the recognition of Clavata 3-peptide (CLV3-p) (127). The CLV3-p receptor regulates stem cell homeostasis in the shoot apical meristem (12). CLV3-p triggers responses reminiscent of those elicited by flg22 in mesophyll protoplasts. However, the ligand-binding affinity of AtFLS2 for CLV3-p is low compared to flg22 (12, 14). Nonetheless, in this study, both *clv3* and *fls2* mutants showed a reduced defense gene expression and increased susceptibility to virulent bacterial pathogens in the shoot apical meristem (127). It will be interesting to test whether orthologous CLV3 peptides in other plant species also contribute to immunity mediated by FLS2.

Future studies will help elucidate whether FLS2 recognition of Ax21-derived and CLV3-related peptides is relevant outside the laboratory and whether heterodimerization between different *Arabidopsis* receptors of conserved microbial signatures increases the recognition capacity, as has recently been shown for mammalian TLRs (108). It is also worthwhile to consider whether the relaxed specificity observed for FLS2 allows the efficient use of a limited number of receptors for the detection of multiple conserved microbial signatures.

Innate Immunity Mediated by EFR

The recognition of EF-Tu or its fully active elicitor peptide (elf18) is restricted to the family Brassicaceae (123). The extracellular LRR of EFR is highly glycosylated, which seems to be important for ligand binding as mutation of a single predicted glycosylation site compromises elf18 binding despite correct localization of the mutated protein to the plasma membrane (85). Elf18 most likely binds to the concave surface of the horseshoe-like LRR as mutations of predicted ligand-binding sites identified by several computational methods compromise EFR-mediated immune responses (89).

Innate Immunity Mediated by CEBiP, CERK1, LYM1, and LYM3: LysM Domain–Containing Receptors Recognizing Glucan-Based Conserved Microbial Signatures

Chitin (a polymer of *N*-acetyl-D-glucosamine) is a major component of fungal cell walls and is recognized by animals and plants. Rice and tomato cell cultures respond to chitin fragments by membrane depolarization or medium alkalization, respectively (63, 121). The cognate receptor CEBiP (chitin oligosaccharide elicitor-binding protein) in rice was identified by biochemical binding assays, peptide sequencing, subsequent cloning, and silencing of the coding gene (104). CEBiP is an RLP with extracellular LysM domains involved in ligand binding, a single TM domain, and a cytoplasmic C-terminal tail without a kinase domain. It is predicted to be GPI (glycophosphatidylinositol)–anchored to the membrane. Thus, CEBiP differs from EFR, FLS2, and XA21 in that it lacks a predicted signaling module integral to the receptor. It is therefore tempting to speculate that CEBiP recruits a non-RD kinase for signal initiation, reminiscent of the association of the non-RD kinases IRAK1 and RIP1 with animal receptors of conserved microbial signatures such as NLRs and TLRs (108). However, no such protein has been identified. Instead, CERK1 (chitin elicitor receptor kinase 1), a LysM-containing RD RK, is required for full chitin responsiveness in rice and directly interacts with CEBiP, forming ligand-induced heteromeric complexes in vivo (194).

CEBiP orthologs are also involved in chitin perception in other plant species. Silencing of CEBiP in barley leads to enhanced susceptibility to the fungal pathogen *Magnaporthe oryzae* (208). Similarly, in the legume *Medicago truncatula* LYM2, a GPI-anchored protein orthologous to CEBiP is able to bind chitin fragments via its LysM domains (65).

In *Arabidopsis*, mutations in *AtCERK1* abolish sensitivity to chitin fragments (149, 215). In a forward biochemical study aimed at

isolating chitin-binding proteins, AtCERK1 was identified as one of the major chitin-binding complex components (164). However, because AtCERK1 displays a relatively low chitin-binding affinity in vitro (95), it is not clear what the actual receptor protein is. Another predicted component of this complex is one of the three CEBiP homologs encoded in the *Arabidopsis* genome. One of these AtCEBiP proteins is present in the chitin-binding complex, but at low stoichiometry (164). It is not known whether any of these three CEBiP homologs is required for chitin perception in *Arabidopsis*.

AtCERK1 is not only involved in chitin perception, because an *Arabidopsis* line carrying a mutation in *AtCERK1* is also impaired in the perception of an additional distinct conserved microbial signature and is more susceptible to bacterial pathogens (75, 76, 223). Peptidoglucans (PGNs), major components of bacterial cell walls, were recently identified to be the sought-after conserved microbial signatures (223). The two LysM domain–containing predicted GPI-anchored proteins AtLYM1 and AtLYM3 bind directly to PGN in vitro at a physiologically relevant concentration. AtCERK1 also binds to PGN, albeit with much lower affinity. All three proteins are nonredundantly required for PGN perception in vivo and for resistance to bacterial pathogens (223). These results suggest that CERK1 serves a role as coreceptor or coregulatory RK in at least two independent conserved microbial signature binding complexes, namely chitin and PGNs. So far no non-RD kinase has been identified as required for chitin and PGN perception. If no such non-RD kinase partners are found, LysM domain–containing receptors recognizing glucan-based conserved microbial signatures would be the first receptors of conserved microbial signatures that lack a non-RD signaling domain.

Innate Immunity Mediated by LeEIX1 and LeEIX2

Tobacco and tomato recognition of a 22-kDa fungal protein, β-1-4-endoxylanase—also referred to as ethylene-inducing xylanase (EIX)—triggers ethylene production. Recognition of EIX is independent of its enzymatic activity (72). A map-based cloning approach in tomato isolated two RLPs, LeEIX1 and LeEIX2, as potential receptors of conserved microbial signatures (175). Both RLPs can bind EIX, but only LeEIX2 confers EIX responsiveness. LeEIX1 is a negative regulator of EIX perception as it attenuates EIX-induced ethylene production when overexpressed in tobacco (11). Surprisingly, LeEIX1 requires the tobacco ortholog of the coregulatory RK AtBAK1 (BRI1 associated kinase 1)—which is known to positively contribute to flagellin perception in tobacco (88)—to exert its negative function (11).

Innate Immunity Mediated by the Cytoplasmic Non-RD Kinases RPG1 and WKS1

Stem rust on barley is caused by *Puccinia graminis* f. sp. *tritici*, which was once a devastating disease in North America. The introduction of *RPG1* (*reaction to Puccinia graminis 1*) in commercial variants has conferred a durable broad-spectrum resistance to many isolates since the mid-1940s. The *R* gene corresponding to *RPG1* was identified by map-based cloning and found to encode a dual protein kinase (22, 92). Both kinases of RPG1 carry the non-RD motif and are required for disease resistance, yet only protein kinase 2 has catalytic activity in vitro (152). RPG1 localizes to the plasma membrane and cytosol, where it is phosphorylated within minutes after urediniospore attachment to the leaf surface (154). Phosphorylation is required for RPG1 protein degradation via the proteasome pathway after pathogen perception, and both kinases are required for disease resistance (153, 154).

Two potential ligands were recently isolated from urediniospores of *P. graminis* by affinity chromatography. Only the simultaneous application of both potential ligands induced RPG1-dependent HR-like response in barley and RPG1 phosphorylation and degradation (155). Both proteins independently bind

directly to RPG1 in the yeast two-hybrid assay, yet it is not clear how these proteins can enter into the plant cytoplasm or whether they form heteromeric complexes *in planta*. Importantly, the predicted functional domains of both potential ligands are highly conserved in many fungal plant pathogens. Based on these results, we hypothesize that RPG1 is a cytoplasmic receptor of conserved microbial signatures or is part of a receptor complex that initiates downstream signaling. Similarly, in wheat the intracellular non-RD kinase WKS1 (wheat kinase start 1) confers broad-spectrum disease resistance to many different races of the strip rust fungus *Puccinia striiformis* f. sp. *tritici* (71).

Surveillance of Plant Cell Wall Integrity Mediated by WAK1

Plants sense microbial intrusion by recognizing DAMPs such as lytic plant cell wall fragments, e.g., oligogalacturonides (OGs) (19, 158). Through use of a domain swap approach between EFR and WAK1 (wall-associated kinase 1), it was demonstrated that fusion of the WAK ectodomain with the EFR TM and intracellular kinase domain is able to perceive OGs and induce typical EFR-mediated responses, such as ethylene production and defense gene expression (24). Similarly, the TM and cytoplasmic kinase domains of WAK1 fused with the ectodomain of EFR are able to perceive elf18, triggering an oxidative burst (24).

Because WAK1 is an RD kinase that recognizes an endogenous ligand produced during infection rather than a receptor for conserved microbial signatures (which generally fall into the non-RD kinase subclass) it is tempting to speculate that WAK1 is functionally distinct from receptors of conserved microbial signatures. Instead, it may fall into the subclass of RKs that are involved in development. These include many RD RKs, such as BRI1 (brassinosteroid insensitive 1). In support of this hypothesis, OGs are potentially perceived not only during pathogen infection but also during normal growth (184). These

intriguing studies further support a clear connection between defense and developmental response.

A PREDICTED FUNCTIONAL IMPLICATION FOR THE NON-RD KINASE MOTIF IN INNATE IMMUNE SIGNAL INITIATION

The activation mechanism of RD kinases, which carry a conserved arginine immediately preceding the catalytic aspartate in subdomain VIb (100, 156), is well studied. Most require phosphorylation of the activation segment for full kinase activity (156). The phospho-group in the activation segment coordinates the positively charged amino group of the arginine, leading to stabilization of the otherwise highly flexible activation segment and thereby enhancing enzymatic activity.

In non-RD kinases an uncharged amino acid, usually a cysteine or glycine, replaces the arginine of RD kinases, suggesting a different mechanism of activation (48). Several different regulatory mechanisms have been observed for mammalian non-RD kinases, such as relief of autoinhibition by C-terminal extension (120, 195) or tyrosine phosphorylation in the P+1 loop immediately downstream of the activation segment (143). The crystal structures of several non-RD kinases not involved in innate immune signaling reveal a highly ordered conformation of the activation segment in the absence of phosphorylation (157, 185, 195, 210). These results suggest that non-RD kinases are constitutively active, which might represent a general theme of non-RD kinase regulation. Support for this hypothesis comes from recent results showing that the ATPase XB24 promotes XA21 autophosphorylation, holding it in a biologically inactive state. Only upon ligand binding does the ATPase disassociate, triggering XA21 activation (31).

Arabidopsis FLS2 and EFR and rice XA21 display only a relatively weak kinase activity in vitro compared with their coregulatory RD kinase counterparts and with RD kinases involved in development (187; X. Chen, S. Zuo,

Activation segment: a positional conserved region of protein kinases that regulates their catalytic activity

B. Schwessinger & P.C. Ronald, unpublished data). In addition, the kinase activity of the non-RD kinases IRAK1, RIP1, RIP2, RIP4, and XA21 is at least partially dispensable for their function in immunity (6, 178, 217). In contrast, the catalytic activity of several coregulatory RD kinases—such as IRAK4 (99), RIP3 (39, 87), and AtBAK1 (179, 187)—seems to be crucial for their function. Together, these observations suggest that at least part of the function of non-RD kinases is to serve as phosphorylation-dependent scaffold proteins (48, 78).

SIGNAL TRANSDUCTION MEDIATING THE PERCEPTION OF CONSERVED MICROBIAL SIGNATURES AT THE PLASMA MEMBRANE

Studies of animal TM receptors indicate that the first event after ligand binding is often a ligand-induced conformational change (134). This conformational change leads to homo- and heteromerization, kinase activation by repositioning of the intracellular domain, and interaction with downstream signaling components (103). Even though a ligand-induced conformational change has not been demonstrated for plant receptors of conserved microbial signatures, it is assumed that such a change constitutes a likely first downstream signaling event.

Phosphorylation is the earliest measurable response upon perception of conserved microbial signatures (80, 186). For example, conserved microbial signature treatment of tomato, pine, parsley, and bean cells leads to phosphorylation of specific proteins (28, 61, 80, 181). The importance of these phosphorylation events was revealed by demonstrating that kinase inhibitors block all other known early signaling events, such as Ca^{2+} uptake and membrane depolarization (43, 61). Interestingly, PTI signaling seems to require constant dephosphorylation to stay turned off, as phosphatase inhibitors induce responses reminiscent of conserved microbial signature treatments (62, 202) and silencing of phosphatases leads to cell death (163).

PHOSPHATASES TUNE DOWN THE SIGNAL

Phosphatases directly target the kinases of receptors of conserved microbial signatures. The general kinase-associated protein phosphatase (KAPP) interacts with the AtFLS2 kinase domain and negatively regulates AtFLS2 signaling (77). In rice, XA21 does not bind KAPP (214). Instead, XA21-mediated immunity is down-regulated by the type 2C phosphatase XB15 (XA21-binding protein 15) (163). The reduction in XB15 expression leads to spontaneous cell death, suggesting an additional important role of XB15 in the constitutive regulation of XA21 and potentially other receptors of conserved microbial signatures in the absence of pathogens. The interaction of XB15 requires XA21 kinase activity. Phosphorylation of specific amino acids in the XA21 JM domain is critical for binding of XB15 and other binding proteins, suggesting that the JM domain serves as a key scaffolding domain. XB15 likely targets phosphorylation sites important for XA21 activation (34, 163). To gain insight into the regulation of non-RD receptors of conserved microbial signatures such as XA21, it will be important to identify the full complement of auto- and transphosphorylation sites and investigate their functional role in PTI signaling.

COREGULATORY RECEPTOR KINASES: BAK1, SERKs, AND EVER MORE RECEPTOR KINASES

AtBAK1, an LRR-RK of the SERK (somatic embryogenesis receptor kinase) family, forms ligand-dependent heteromeric complexes with several receptors of conserved microbial signatures, such as AtFLS2, AtEFR, and other RKs such as AtPEPR1 and -2 (AtPEP1 receptor 1 and 2), which are involved in endogenous peptide-mediated secondary defense signaling (37, 88, 166, 179, 187). AtBAK1 was initially identified as a positive regulator of brassino-steroid (BR) responses (an important pathway regulating plant growth and development), forming a ligand-dependent complex in vivo

with the LRR-RK AtBRI1, the main BR receptor (42, 117). In addition, *AtBAK1* and its closest homolog *AtBKK1* (*BAK1-like 1*) are genetically partially redundant negative regulators of cell death (86, 114, 187). AtBAK1 also interacts with the LRR-RK AtBIR1 (BAK1 interacting receptor 1) and the copine protein AtBON1 (BONZAI 1) (73, 221). Both *atbon1* and *atbir1* display constitutive cell death that is partially reversible by high temperatures and mutations in genes typically associated with ETI signaling (73, 136, 221). Because one of these genes encodes the NBS-LRR protein SNC1 (suppressor of npr1-1, constitutive 1), it is possible that SNC1 guards the integrity and/or activity of a multimeric complex containing AtBAK1, AtBKK1, AtBIR1, and AtBON1.

Atbak1 mutants are impaired in the perception of a plethora of fungal-, bacterial-, and oomycete-derived conserved microbial signatures (36, 70, 179). Yet only the identification of the novel mutant allele *atbak1-5*, which is specifically impaired in PTI signaling without displaying any other pleiotropic defects in development or cell-death control, revealed that AtBAK1 contributes directly significantly to disease resistance against biotrophic and hemibiotrophic pathogens (179, 187).

AtBAK1 has a specific mechanistic requirement for the different signaling pathways. AtBAK1-dependent cell-death control, PTI, and BR signaling require the kinase activity of AtBAK1 but in a differential manner. The phosphorylation site T450 in the activation segment of AtBAK1 is not required for its role in cell-death control but is essential for its function in PTI and BR signaling (219). In the BR signaling pathway, AtBAK1 accomplishes the function of a signal enhancer via bidirectional transphosphorylation and activation between AtBAK1 and AtBRI1 (219). This role as signal enhancer can be uncoupled from its role in PTI signaling, as a single amino acid change close to the active site of AtBAK1 changes its phosphorylation capacity and renders it unfit specifically for innate immune signaling (187). This suggests that AtBAK1 differentially regulates the three signaling pathways by

discriminative auto- and transphosphorylation events (187). This hypothesis is further supported by the identification of a tyrosine phosphorylation site in the C-terminal tail of AtBAK1 specifically required for BR signaling (161). These results indicate that intracellular domains of different ligand-binding receptors display distinct activation mechanisms. In the case of *Arabidopsis* FLS2 and EFR, phosphorylation of the receptor and coregulatory RKs is a consequence of rather than a requirement for the near-instantaneous complex formation with AtBAK1 (186, 187). This is in contrast to the requirement of AtBRI1's kinase activity for the ligand-induced complex formation with AtBAK1 and its phosphorylation in vivo (219). The differential mechanistic requirement is not restricted to the intracellular domain, because a single amino acid change in the extracellular LRR domain of AtBAK1 specifically enhances the complex formation with AtBRI1 and BR signaling but blocks its interaction with AtFLS2 (98).

AtBAK1 is not the only AtSERK family member found to be involved in PTI signaling. Its closest homolog, AtBKK1, plays a partially redundant role and forms ligand-dependent complexes with at least two receptors of conserved microbial signatures in vivo (179). Similar partially redundant roles among other AtSERK family members were observed for BR signaling (36).

The requirement of coregulatory RKs is conserved in both dicots and monocots. For example, in *Nicotiana benthamiana*, plants silenced for *NbBAK1* are less sensitive to several conserved microbial signatures and are more susceptible to oomycete pathogens (29, 88). The suppression of expression of a *SERK* homolog in lettuce renders plants more susceptible to the fungal pathogen *Sclerotinia* (183); similarly, the suppression of *LeSERK1* or *LeBAK1* expression in tomato compromises the LeVe1-mediated resistance to *Verticillium dahliae* and *Verticillium albo-atrum* race 1 (68, 69). In rice, the overexpression of *OsSERK1* confers increased resistance to *M. oryzae* (93). Rice lines carrying mutations in *XAK1* (*XA21*

The ability to recognize flagellin and BR, which both rely on coregulatory RKs from the SERK family, seems to have arisen with the acquisition of seed development, because predicted FLS2 and BRI1 orthologs are absent from the genome of the lycophyte *Selaginella moellendorffii* (3, 51). In contrast, *SERK* homologs can be found in the genomes of all land plants—including *S. moellendorffii* and the moss *Physcomitrella patens*—but are absent from green algae. Thus it appears that FLS2 (and possibly other receptors of conserved microbial signatures and BR receptors) has potentially co-opted the same evolutionarily more conserved signaling module involved in plant endogenous peptide signaling already present in mosses (107). Similar LysM domain–containing proteins, which are involved in glucan-based conserved microbial signature perception, first appeared in vascular plants and are absent from mosses and algae. Does this mean that nonvascular plants do not recognize microbial signatures? No. *P. patens* and brown algae are also responsive to crude elicitor preparations of fungal pathogens or cell wall components released during the infection process (165, 167). Very little is known about the immune system of green algae, the closest ancestors of land plants.

associated kinase 1), an *OsSERK1* ortholog, are incapable of mounting an XA21-mediated immune response (X. Chen & P.C. Ronald, unpublished data). The function of SERKs also seems to be conserved in BR signaling because rice plants silenced for *XAK1* are partially insensitive to BR (X. Chen & P.C. Ronald, unpublished data) (see sidebar, A Brief Excursion into the Evolutionary Origin of Conserved Microbial Signature Perception in Plants).

Several additional coregulatory RKs have been implicated in the perception of conserved microbial signatures. These include AtFER (FERONIA), AtCRK20a (cysteine rich kinase 20a), HvCRK1 (cysteine rich kinase 1), and many others (57, 113, 115, 171). An important future research focus will be to elucidate the explicit role of these predicted coregulatory RKs in PTI.

MEMBRANE-ASSOCIATED CYTOPLASMIC KINASES

Several cytoplasmic membrane–localized kinases, such as AtBIK1 (*Botrytis* induced kinase 1), AtPBS1 (AvrPphB susceptible 1), and AtPBLs (PBS1-like proteins), are partially redundant in the signal transduction immediately downstream of the recognition of conserved microbial signatures, including flg22, elf18, and chitin (137, 225). AtBIK1 constitutively interacts with AtFLS2 and AtEFR, and possibly with AtBAK1. After ligand recognition, AtBIK1 is released from the receptor complex (137, 225). The release of AtBIK1 from the multimeric RK complex requires AtBAK1, suggesting that AtBAK1 activates AtBIK1 via transphosphorylation. The only identified phosphorylation sites on AtBIK1 are in the activation segment of residues that are known to be required for full enzymatic activation of RD kinases. Mutation of these conserved sites compromises AtBIK1 kinase activity and its function in PTI signaling. It is currently unknown whether these sites are auto- or transphosphorylation sites and whether AtBIK1 requires phosphorylation on additional regulatory sites for full activation. AtFLS2 and AtBAK1 kinase domains are transphosphorylated by AtBIK1 in vitro. In the future it will be interesting to identify the corresponding phosphorylation sites and test their functions in immune signaling. How AtBIK1 mediates AtBAK1-independent signaling downstream of AtCERK1 and its functional relationship with Ca^{2+}-dependent signaling and MAPK (mitogen-activated protein kinase) cascades remain important research areas.

RECEPTOR ENDOCYTOSIS

It is known that in animals receptor endocytosis extends beyond signal attenuation by depleting ligand-binding sites at the plasma membrane (151). Endocytosed receptors signal from endosomes and inside the nucleus, triggering different physiological responses owing to differential complex composition and regulation (151, 220). Several plant receptors of conserved microbial signatures, such as AtFLS2 and LeEIX2, undergo ligand-induced

endocytosis (10, 173). Coregulatory RKs of the SERK family are also internalized (124, 180), but it is unknown whether this occurs while in complex with the ligand-binding receptor of conserved microbial signatures. Although the exact roles and molecular mechanisms of plant receptor endocytosis are still poorly understood, pharmacological studies suggest that it represents an important step in signal transduction (74, 97, 189).

Recognition of conserved microbial signatures by membrane bound receptors leads to rapid depolarization of the plasma membrane potential, a rapid oxidative burst, and activation of intracellular kinase cascades. A brief review of these signal transduction events is provided in the **Supplemental Appendix** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). This appendix also includes a brief description of the role of E3 ligases in PTI signaling and the importance of receptor biogenesis in the endoplasmic reticulum.

Many additional signaling modules that we are unable to discuss in detail, such as Ca^{2+} signature decoding proteins and kinases (122), MAPK cascades (209), extracellular ATP (206), and small RNAs (106), also participate in a complex spatial-temporal signaling network that includes PTI signaling (**Figure 2**).

HOW PERCEPTION OF CONSERVED MICROBIAL SIGNATURES LEADS TO RESISTANCE

The final aim of conserved microbial signature perception is the induction of efficient defense responses that limit pathogen growth. This involves a massive transcriptional reprogramming via the activation of transcription factors and chromatin remodeling (138). The resulting protein synthesis leads to production of PR (pathogenesis-related) and other defense-related proteins that contribute to pathogen restriction mostly via unknown mechanisms. PTI also alters metabolite composition (182), and in Brassicaceae glucosinolates are one of the major groups of secondary defense com-

pounds (15, 41). In addition, plants reinforce physical barriers by closing their stomata and enhancing cell wall fortification (90, 145).

Plant defenses are also influenced by many abiotic and biotic factors. These factors include hormone levels, the circadian clock, development, abiotic stress, and environmental conditions such as temperature (**Figure 2**) (5, 71, 174, 218).

SYSTEMS ANALYSES OF THE INNATE IMMUNE RESPONSE

The burgeoning field of systems biology provides new methodologies to make sense of plant stress responses, which are often controlled by highly complex signal transduction pathways that may involve tens or even thousands of proteins (131). Several recent studies have expanded our knowledge of the plant immune system and led to the identification of new components.

A recent yeast two-hybrid study tested the interaction of more than 8,000 *Arabidopsis* proteins, including all known core plant innate immune components, with potential effector proteins from the two divergent pathogens *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis*. Effectors from both pathogens did not specifically target immune receptors but rather converged on highly interconnected signaling hubs to disturb the plant innate immune system (150).

To elucidate stress response signaling networks in rice, Seo and colleagues (188) constructed an interactome of 100 proteins by yeast two-hybrid assays around key regulators of the rice biotic and abiotic stress responses. The interactome was validated using protein-protein interaction assays, coexpression of transcripts, and phenotypic analyses. Using this interactome-guided prediction and phenotype validation, the authors identified 10 novel regulators of stress tolerance, including 2 from protein classes not previously known to function in stress responses.

Advances in plant genomics, transcriptomics, and proteomics have led to the accumulation of sufficient public data to
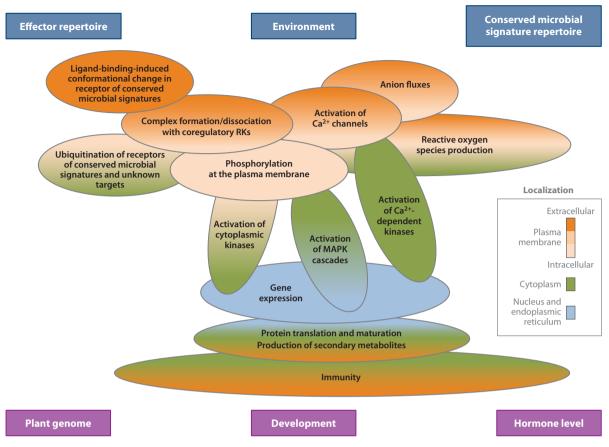
Figure 2

Schematic representation of the conserved signaling network triggered by recognition of conserved microbial signatures. The perception of conserved microbial signatures activates a complex signaling network from the plasma membrane to the nucleus, leading to protein translation and secretion that culminates in immunity. The plant immune system is modified by multiple exogenous and endogenous factors (*boxes*). Individual signaling modules are depicted schematically as ovals, with the upstream modules on top. Each module's localization is color-coded according to the key. Abbreviations: MAPK, mitogen-activated protein kinase; RK, receptor kinase.

construct systems-level models of plant gene interactions. Such models allow for the prediction and systematic discovery of genes and associated pathways that control diverse phenotypes (129, 130). The construction of AraNet and RiceNet—experimentally tested, genome-scale gene networks for *Arabidopsis* and rice, respectively—provides another strategy to explore the plant response to conserved microbial signatures (128, 131). For example, using a RiceNet guilt-by-association approach followed by focused protein-protein interaction assays, Lee et al. (131) identified and validated novel regulators of XA21-mediated immunity.

UTILIZING KNOWLEDGE OF PERCEPTION OF CONSERVED MICROBIAL SIGNATURES FOR AGRONOMIC IMPROVEMENT

Research into the innate immune response has advanced many areas of biology. Because pests and disease take an estimated 40% bite out of potential global yield, knowledge of the plant immune response is important for agricultural production. The development of crop varieties with enhanced resistance to disease remains an important goal in the field. Recent studies demonstrate that the transfer

of receptors of conserved microbial signatures between plant species and families confers broad-spectrum resistance to pathogens that were not previously controlled or recognized (68, 125, 198). These studies indicate that the identification of novel receptors combined with their integration into crop genomes by breeding or modern molecular tools can have important consequences for agricultural improvements. Engineered strategies, such as generation of new chimeric receptors, have also proven useful. For example, expression of a chimeric receptor generated by fusing the rice CEBiP ectodomain to the TM and kinase domains of XA21 increased chitin responsiveness and enhanced resistance to *M. oryzae* (119). Similarly, fusion proteins between FLS2 or WAK1 and EFR are also functional, indicating the broad applicability of this approach (4,

24). Future goals include engineering of receptors with broadened and novel recognition specificities.

A complementary strategy is to use knowledge of the conserved microbial signatures to develop reagents that can immunize hosts against infection or antagonists that disrupt QS-mediated virulence activities and biofilm formation (203), a process thought to be involved in 65%–80% of bacterial infections (45). For example, the production of inexpensive, nontoxic, synthetic structural analogs to Ax21, which can be readily applied in the field, can be used to induce XA21-mediated immunity. Conversely, antagonists that can disrupt critical virulence functions of the pathogen—possibly by binding the putative Ax21 receptor, RaxH—would cripple its ability to form biofilms (83, 176).

▶ Erratum

SUMMARY POINTS

1. In plants, receptors of conserved microbial signatures are widespread and diverse and share structural and functional similarities with animal Toll-like receptors. Predicted plant receptors include soluble extracellular proteins, receptor kinases, receptor-like proteins, and intracellular kinases.

2. Recent studies suggest that a single receptor can be involved in the perception of multiple conserved microbial signatures.

3. Currently known plasma membrane–localized receptors of conserved microbial signatures require coregulatory receptor kinases that are shared with developmentally regulated signaling pathways. Signaling specificity is likely achieved through distinct complex formations and phosphorylation events.

4. The non-RD kinase motif is a hallmark of kinases associated with receptors of conserved microbial signatures. This conservation suggests yet-to-be-discovered mechanistic properties that are advantageous for signal initiation.

5. Many of the signaling modules and their temporal activation following perception of conserved microbial signature are conserved among monocotyledonous and dicotyledonous plant species; however, copy number and important mechanistic attributes vary between these two main classes of flowering plants.

FUTURE ISSUES

1. Many predicted plant receptors of conserved microbial signatures have not yet been demonstrated to bind a cognate (microbially-derived) ligand. Many more receptors remain to be characterized, and exploiting the huge genomic diversity of plants will advance this goal.

2. Structural studies are required to identify surface interactions between ligands and their cognate receptors.

3. There is a paucity of knowledge on the mechanisms regulating activation of non-RD kinases and their interactions with coregulatory proteins.

4. The knowledge gained about receptors of conserved microbial signatures can be used to improve crop species using existing and engineered receptors.

5. The plant immune response is complex and is intimately connected to the response to abiotic and developmental factors.

DISCLOSURE STATEMENT

ACKNOWLEDGMENTS

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▶ Erratum

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▶ Erratum

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RELATED RESOURCES

Arabidopsis 2010 project: **http://plantsp.genomics.purdue.edu/projects/lrr/Clouse2010.htm**

Arabidopsis Interactome Network Map: **http://interactome.dfci.harvard.edu/A_thaliana**

Arabidopsis Membrane Interactome: **http://www.associomics.org**

AraNet: **http://www.functionalnet.org/aranet**

Kinase.com: **http://www.kinase.com**

PhosPhAt: **http://phosphat.mpimp-golm.mpg.de**

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