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# Innate Immunity in Plants: An Arms Race Between Pattern Recognition Receptors in Plants and Effectors in Microbial Pathogens

Thomas Boller<sup>1\*</sup> and Sheng Yang He<sup>2\*</sup>

For many years, research on a suite of plant defense responses that begin when plants are exposed to general microbial elicitors was underappreciated, for a good reason: There has been no critical experimental demonstration of their importance in mediating plant resistance during pathogen infection. Today, these microbial elicitors are named pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) and the plant responses are known as PAMP-triggered immunity (PTI). Recent studies provide an elegant explanation for the difficulty of demonstrating the role of PTI in plant disease resistance. It turns out that the important contribution of PTI to disease resistance is masked by pathogen virulence effectors that have evolved to suppress it.

Plants are exposed to myriads of potential microbial pathogens, but the world is still green. Why? Plants possess an innate immune system that efficiently detects and wards off potentially dangerous microbes (1–3). A first layer of this system is based on the amazingly sensitive perception of pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) through pattern recognition receptors (PRRs) at the plant's cell surface (Fig. 1). For example, plants perceive bacterial flagellin through a PRR known as FLS2 (flagellin sensitive 2), a leucine-rich repeat receptor kinase (LRR-RK) located in the plasma membrane. Similarly, mammals use the Toll-like receptor TLR5 to perceive bacterial flagellin and mount multifaceted downstream immune responses (1, 4). The responses to flagellin and other MAMPs have been called PAMP-triggered immunity (PTI). Successful pathogens produce effectors to inhibit PTI, but plants, in turn, can perceive such effectors through additional receptors—typically nucleotide-binding leucine-rich repeat (NB-LRR) proteins—to mount a second layer of defense called effector-triggered immunity (ETI). Although the importance of ETI (formerly known as gene-for-gene resistance) in plant immunity is well established, only recently have we begun to appreciate a fundamental role of PTI in mediating plant-microbe interactions. Here, we highlight recent literature on PRR signaling and the ability of microbial pathogens to suppress PTI as a key virulence strategy.

## Perception of Microbes Through Pattern Recognition Receptors

A hallmark of PRRs is their sensitivity and specificity: Plants possessing the appropriate PRRs

perceive a specific MAMP at subnanomolar concentrations, whereas plants lacking the PRRs are completely blind to it. A given MAMP is recognized through a specific conserved epitope, such as the stretch of 22 amino acids (flg22) in the N terminus of flagellin. Can pathogens avoid perception by the PRRs? It appears they can, but at a cost. Introduction of mutations into flagellin that make the molecule unrecognizable by FLS2 also render the microbe motionless and reduce its virulence (5). Thus, the specificity of the PRR appears to be focused exactly on a highly conserved domain of the MAMP that is functionally important to the microbe.

FLS2 homologs exist in all higher plants for which genomic information is available (1), and the rice homolog is functionally active as a flagellin receptor (6). Hence, flagellin perception through FLS2 homologs is evolutionarily old and conserved. Another well-characterized PRR of *Arabidopsis*, EFR (EF-Tu receptor), perceives bacterial EF-Tu (elongation factor Tu). Perception of this MAMP seems to be confined to the Brassicaceae and is not found in other dicots or monocots, which suggests that EF-Tu perception is evolutionarily young. However, all plant genomes so far sequenced contain homologs of the EFR-encoding gene with a comparable LRR structure. The rice genome encodes about 40 such homologs (1). One of them, found in some rice cultivars, is the disease resistance gene *Xa21*, the protein product of which appears to recognize a quorum-sensing molecule of the rice pathogen *Xanthomonas oryzae*; thus, although the EFR-type PRRs show elements of conserved sequences among plants, they appear to recognize different MAMPs in different plant families (1).

One of the first steps in signaling of the FLS2 receptor is its interaction with BRI1-associated kinase (BAK1), an LRR-RK (7, 8). This comes as a surprise, because BAK1 has previously been known as a co-receptor of the plant hormone

brassinosteroid receptor BRI1, as its name indicates (9). How can the same co-receptor function both in defense signaling and hormonal signaling? BRI1 and BAK1 are phosphorylated upon activation in hormonal signaling (9). Does this also occur in the FLS2-BAK1 interaction, and does differential phosphorylation contribute to the specificity of downstream responses? Is there competition between PTI and the brassinosteroid response for the co-receptor? What is the function of the four BAK1 homologs, the somatic embryogenesis-related kinases (SERKs), in *Arabidopsis*? Dysfunction of two of these, bak1 and serk4, sends *Arabidopsis* seedlings to death with symptoms of the hypersensitive response, a hallmark immune response of ETI (7, 9, 10).

Despite the specificity and sensitivity of MAMP perception by PRRs, it has taken the scientific community a long time to accept that such systems could support plant disease resistance during pathogen infection. Opinion began to shift with the discovery that mutations in the fls2 receptor left *Arabidopsis* plants unusually susceptible to the bacterial pathogen *Pseudomonas syringae* (11). Even more convincing were observations that pathogens actively deploy virulence factors as a virulence strategy to suppress PTI.

## Suppression of PTI by Pathogen Effectors

A variety of bacterial virulence factors, including the phytotoxin coronatine, extracellular polysaccharides, and proteinaceous effectors secreted through the type III secretion system (TTSS), suppress PTI (1–3). Most spectacularly, two secreted effectors, AvrPto and AvrPtoB (from *P. syringae* strain DC3000), physically interact with the kinase domains of FLS2, EFR, or BAK1 (12–14). Such physical interactions inhibit the kinase activity of PRRs (12) or interfere with the formation of FLS2-BAK1 complexes (13). Whereas AvrPto seems to be a novel protein, AvrPtoB contains a C-terminal domain that resembles E3 ubiquitin ligase; ubiquitination by this domain initiates degradation of a tomato kinase (Fen) that is part of a unique and presumably ancient ETI pathway (15). The same domain also initiates degradation of PRRs, and thus its more important role may be in defeating PTI (14, 16). The ability of AvrPto and AvrPtoB to derail PRRs provides a satisfying explanation for previous discoveries that these effectors could suppress a variety of responses of PTI, including callose deposition, activation of kinase cascades, and expression of MAMP-responsive proteins and small RNAs (17–19). Not all bacteria—indeed, not even all strains of *P. syringae*—express AvrPto and AvrPtoB, which suggests that other strategies exist to inhibit PRR signaling. Indeed, the effector HopA11, present in many but not all *P. syringae* strains, is a phosphothreonine lyase that dephosphorylates mitogen-activated protein kinases (MAPKs) MPK3 and MPK6 to terminate

<sup>1</sup>Zürich-Basel Plant Science Center, Botanical Institute, University of Basel, Hebelstrasse 1, 4056 Basel, Switzerland. <sup>2</sup>Department of Energy Plant Research Laboratory, Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA.

\*To whom correspondence should be addressed. E-mail: thomas.boller@unibas.ch (T.B.); hes@msu.edu (S.H.E.)

PRR signaling (20). Interestingly, another member of this effector family dephosphorylates kinases involved in mammalian innate immunity (21), showing that pathogens can apply the same mechanism of host immune modulation to both plants and mammals.

Pathogen effectors target more than PRRs or the MAPK cascade to suppress PTI. These effectors also attack processes directly downstream of PRR signaling and other consequent events (Fig. 1) (22). For example, the *P. syringae* effector HopU1 modifies several *Arabidopsis* RNA-binding proteins, including GRP7, by adenosine diphosphate ribosylation. HopM1, another *P. syringae* effector, triggers degradation of the *Arabidopsis* MIN7 protein, which is a member of the ARF family of guanine nucleotide exchange factors involved in vesicle trafficking. Plants lacking either *grp7* or *min7* are abnormally susceptible to bacterial infection, implicating RNA metabolism and vesicle trafficking as part of the plant's immune response to pathogens (22). The *P. syringae* effector HopI1 resides in the chloroplast, where its action (presumably through interaction with Hsp70 chaperones) suppresses accumulation of salicylic acid, a plant hormone key to defense responses; three other *P. syringae* effectors—AvrRpm1, AvrB, and AvrRpt2—interact with or modify proteins such as RIN4 and RAR1 that regulate pathogen- and effector-triggered immunity (22).

The many examples of physical associations between pathogen effectors and regulators of host immune responses have spurred a notion that pathogen effectors can be used as molecular probes to identify unknown components of the plant innate immune system, including those involved in PTI. This is an exciting time for researchers in this area, especially because recent functional genomics studies suggest that bacteria, fungi, oomycetes, and nematodes that are pathogenic to plants could collectively deliver hundreds of virulence effectors into host cells. Identification of the plant targets of this vast repertoire of pathogen effectors will likely yield many new discoveries that could have a great impact on our understanding of plant immunity, pathogenesis, and plant biology for years to come.

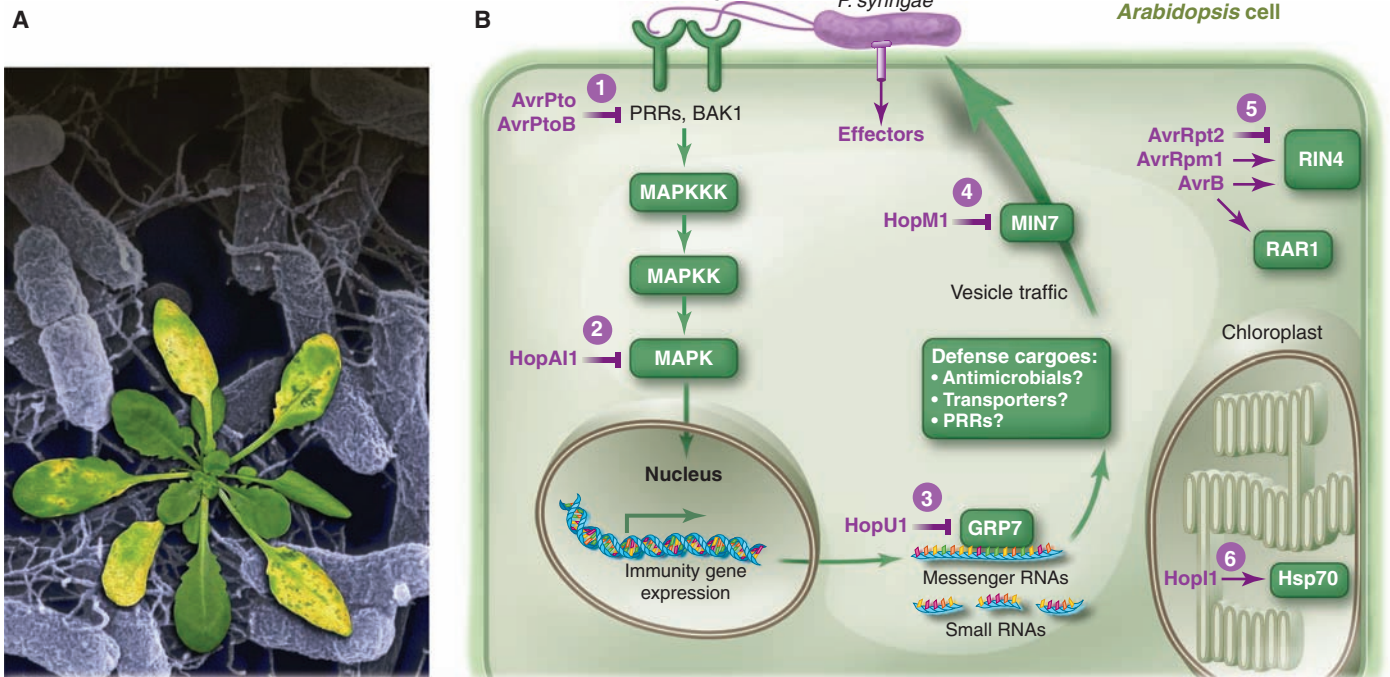
### Concluding Remarks

The past few years have witnessed paradigm-shifting advances in the field of plant-microbe interactions. Contributing to these advances are experimental demonstrations of a functional role of PRRs in plant disease resistance and the discovery that many bacterial virulence factors are involved in suppressing PRR signaling and PTI-associated immune responses. Nonetheless, current research is limited by heavy reliance on information derived from essentially a single pathosystem: the interaction between the plant *Arabidopsis* and the bacterium *P. syringae* (Fig.

1). Thus, our current understanding of plant-pathogen interactions is of a pioneering but preliminary nature. It remains to be seen whether the conceptual framework emerging from the study of this pathosystem will translate to other plant-microbe interactions. The incredibly diverse interactions between plants and microbes suggest that other systems will involve many novel mechanisms, which are likely to refine or even challenge the current models. However, because all pathogens carry MAMPs that may be recognized by plants, yet all plants are still susceptible to virulent pathogens, it is certain that activation and suppression of PTI is a general principle underpinning plant-microbe interactions.

### References and Notes

1. T. Boller, G. Felix, *Annu. Rev. Plant Biol.* **60**, 379 (2009).
2. S. T. Chisholm, G. Coaker, B. Day, B. J. Staskawicz, *Cell* **124**, 803 (2006).
3. J. D. G. Jones, J. L. Dangl, *Nature* **444**, 323 (2006).
4. N. K. Clay, A. M. Adio, C. Denoux, G. Jander, F. M. Ausubel, *Science* **323**, 95 (2009); published online 18 December 2008 (10.1126/science.1164627).
5. K. Naito *et al.*, *Mol. Plant Microbe Interact.* **21**, 1165 (2008).
6. R. Takai *et al.*, *Mol. Plant Microbe Interact.* **21**, 1635 (2008).
7. D. Chinchilla *et al.*, *Nature* **448**, 497 (2007).
8. A. Heese *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 12217 (2007).
9. X. Wang *et al.*, *Dev. Cell* **15**, 220 (2008).
10. K. He *et al.*, *Curr. Biol.* **17**, 1109 (2007).
11. C. Zipfel *et al.*, *Nature* **428**, 764 (2004).



**Fig. 1.** Concept of activation and suppression of PTI during pathogen infection. **(A)** An *Arabidopsis* plant showing disease symptoms (in the foreground; natural size) after infection by *P. syringae* bacteria (electron microscopy image in the background; magnification, 10,000 $\times$ ). **(B)** A conceptual diagram of PRR signaling and action of several *P. syringae* effectors for which the plant targets and

immune suppression function have been characterized. Green and purple colors indicate plant targets and *P. syringae* effectors, respectively. Numbers in circles denote six steps targeted by effectors: MAMP perception (PRRs), the MAPK cascade (MPK3 and MPK6), RNA metabolism (GRP7), vesicle traffic (MIN7), regulators of PTI (RIN4 and RAR1), and chloroplast function (Hsp70) (22).

12. T. Xiang *et al.*, *Curr. Biol.* **18**, 74 (2008).
13. L. Shan *et al.*, *Cell Host Microbe* **4**, 17 (2008).
14. V. Göhre *et al.*, *Curr. Biol.* **23**, 1824 (2008).
15. T. R. Rosebrock *et al.*, *Nature* **448**, 370 (2007).
16. S. Gimenez-Ibanez, *Curr. Biol.* **19**, 423 (2009).
17. P. Hauck, R. Thilmony, S. Y. He, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8577 (2003).
18. P. He *et al.*, *Cell* **125**, 563 (2006).
19. L. Navarro, F. Jay, K. Nomura, S. Y. He, O. Voinnet, *Science* **321**, 964 (2008).
20. J. Zhang *et al.*, *Cell Host Microbe* **1**, 175 (2007).
21. H. Li *et al.*, *Science* **315**, 1000 (2007).
22. A. Block, G. Li, Z. Q. Fu, J. R. Alfano, *Curr. Opin. Plant Biol.* **11**, 396 (2008).
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## PERSPECTIVE

# To Nibble at Plant Resistance Proteins

F. L. W. Takken<sup>1\*</sup> and W. I. L. Tameling<sup>2\*</sup>

To intercept invading microbes that threaten growth and reproduction, plants evolved a sophisticated innate immune system. Recognition of specialized pathogens is mediated by resistance proteins that function as molecular switches. Pathogen perception by these multidomain proteins seems to trigger a series of conformational changes dependent on nucleotide exchange. The activated resistance protein switches on host defenses, often culminating in the death of infected cells. Given their control over life and death, activity of these proteins requires tight regulation that involves intramolecular interactions between the various domains.

**D**iscrimination between self and non-self is a fundamental ability of immune systems. Vertebrates rely on both an innate and an adaptive immune system of which the last is based on immunological memory. In contrast, plants primarily rely on their innate immune system in which each individual plant cell can autonomously mount a defense response (*1*). Two layers can be distinguished in the plant immune system. One is based on extracellular trans-membrane receptors that recognize conserved microbe-associated molecules and induce a relatively weak immune response that, nevertheless, effectively halts colonization by most microbes. The second layer is effective against specialized pathogens that can successfully break through the first layer and is based on highly polymorphic resistance (R) proteins. R proteins act mainly (but not exclusively) intracellularly and confer protection against (hemi-) biotrophic pathogens that need living host tissues for their proliferation. During infection, these pathogens (which include many viruses, bacteria, fungi, oomycetes, and nematodes) produce virulence factors (effectors), of which several suppress the first layer of the plant's immune system (*1*), clearing the way for infection. Some effectors, or the perturbations they cause in the plant, are perceived by R proteins, which consequently set off strong defense responses in the plant that often leads to suicide of the infected cells (*1*).

Most R proteins are multidomain NB-LRRs ("nibblers"), named after their central nucleotide-binding (NB) and leucine-rich repeat (LRR) domains. The NB domain is part of a larger domain, the so-called NB-ARC domain, which consists of three subdomains: NB, ARC1, and ARC2 (*2*). The N termini of these R proteins are structurally diverse; some have homology to the Toll and human interleukin 1 receptor (TIR) and are called TIR-NB-LRRs. Others are commonly referred to as CC-NB-LRRs, because most carry predicted coiled-coil (CC) regions (*1, 2*). Plant NB-LRRs, together with the metazoan cell death regulators Apaf-1 and CED-4, form the NB-ARC family within the class of STAND [signal transduction adenosine triphosphatases (ATPases) with numerous domains] proteins (*3*). The NACHT sister family within this class encompasses the animal NLR (NACHT-LRR/NOD-LRR) innate immune receptors, where the NB domain is also fused to an LRR domain (*4*). STAND proteins are proposed to function as molecular switches, regulating cellular responses through nucleotide-dependent conformational changes (*2, 3*). Here, we discuss and evaluate the R protein-switch model in the context of other STAND proteins.

Because R proteins have the potential to trigger host cell death, their activity needs to be tightly regulated. They should be strongly inhibited in the absence of a pathogen, but rapidly activated upon attack. How is this process controlled? It appears that inappropriate activation is prevented by autoinhibition, which seems to be mainly accomplished by intramolecular interactions between the various domains. Interaction and mutagenesis studies with various NB-ARC and NLR proteins, including R proteins, identified both the N-terminal part of the repeat do-

main and the ARC2 subdomain to be essential for this autoinhibition (*5, 6*) (Fig. 1). Disturbance of the interaction between these two subdomains, by mutations or domain swaps, diminishes autoinhibition and results in constitutive R protein activation (*5, 7*).

The LRR domain is not only involved in negative regulation, but provides positive control as well. Expression of truncated R proteins that lack the LRR domain and carry autoactivating mutations in the NB-ARC domain generally does not induce full host defenses unless the corresponding LRR domain is coexpressed (*7*). Furthermore, various studies have shown that the C-terminal part of the LRR domain provides pathogen recognition specificity (*2, 7*). Hence, the LRR domain has a dual function; it provides autoinhibition and it translates pathogen recognition into activation. How exactly the LRR recognizes a pathogen is unclear. Whereas some R proteins bind effectors directly, others require an intermediary host factor. This factor often interacts with the N-terminal domain of the R protein and could represent either the virulence target (thereby acting as a guard) or a target mimic (thereby acting as a decoy) (*8, 9*). In this situation, the LRR is likely involved in sensing the effector-induced perturbations of the target. Either way, effector recognition evokes R-protein activation, a process that, as with other STAND proteins, requires the R protein to bind nucleotides [adenosine diphosphate or adenosine triphosphate (ADP/ATP)] (*2, 10–15*).

Biochemical studies on the tomato R protein I-2 revealed that it tightly binds ADP *in vitro* and that mutations reducing its ATP-hydrolysis rate result in constitutive defense activation. On the basis of these data, it was proposed that R proteins function as nucleotide-controlled molecular switches (*10*). In this model, the ADP-bound state represents the "OFF" state and the ATP-bound state the "ON" state of the protein (Fig. 1). Recognition of an effector triggers a conformational change that results in an "intermediate" open state, which enables ADP to be exchanged for ATP. Upon ATP-binding, the R protein adopts its active conformation ("ON" state) that subsequently unchains, in a still unknown way, host defenses. ATP hydrolysis eventually returns the protein to its autoinhibited "OFF" state.

Recently, this model gained support by the observation that related STAND proteins also tightly bind ADP in their autoinhibited state (*16–18*). Furthermore, the hypothesis that effector-binding sets the stage for nucleotide-exchange was substantiated by data on two STAND proteins: the *Escherichia coli* transcriptional regu-

<sup>1</sup>Plant Pathology, Swammerdam Institute for Life Sciences (SILS), University of Amsterdam, Post Office Box 94215, 1090 GE Amsterdam, the Netherlands. <sup>2</sup>Laboratory of Phytopathology, Wageningen University, Post Office Box 8025, 6700 EE Wageningen, the Netherlands.

\*To whom correspondence should be addressed. E-mail: F.L.W.Takken@uva.nl (F.L.W.T.); wladimir.tameling@wur.nl (W.I.L.T.)