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Review

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Host-Microbe Interactions: Shaping the Evolution of the Plant Immune Response

Stephen T. Chisholm,[1,2,3] Gitta Coaker,[1,2] Brad Day,[1,2,4] and Brian J. Staskawicz[1,*]
[1] Department of Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, Berkeley, CA 94720, USA
[2] These authors contributed equally to this work.
[3] Present address: Colorado State University, Bioagricultural Sciences and Pest Management, C129 Plant Science Building, Fort Collins, CO 80523, USA.
[4] Present address: Michigan State University, Department of Plant Pathology, 107 Center for Integrated Plant Systems, East Lansing, MI 48824, USA.
*Contact: stask@nature.berkeley.edu
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The evolution of the plant immune response has culminated in a highly effective defense system that is able to resist potential attack by microbial pathogens. The primary immune response is referred to as PAMP-triggered immunity (PTI) and has evolved to recognize common features of microbial pathogens. In the coevolution of host-microbe interactions, pathogens acquired the ability to deliver effector proteins to the plant cell to suppress PTI, allowing pathogen growth and disease. In response to the delivery of pathogen effector proteins, plants acquired surveillance proteins (R proteins) to either directly or indirectly monitor the presence of the pathogen effector proteins. In this review, taking an evolutionary perspective, we highlight important discoveries over the last decade about the plant immune response.

The ability to detect and mount a defense response to potential pathogenic microorganisms has been paramount to the evolution and developmental success of modern-day plants. According to fossil records, the establishment of the first land plants occurred approximately 480 million years ago. However, molecular-clock estimates suggest that land plants evolved more than 700 million years ago (Heckman et al., 2001). More interestingly, the establishment of early land plants was facilitated by the interaction with symbiotic fungal associations, suggesting that plants have coevolved with microbes since their first appearance on land (Gehrig et al., 1996). Although one can only speculate about subsequent events, the evolution of land plants has been shaped by molecular interactions with epiphytic, symbiotic, and pathogenic microbes.

Plants are constantly exposed to microbes. To be pathogenic, most microbes must access the plant interior, either by penetrating the leaf or root surface directly or by entering through wounds or natural openings such as stomata, pores in the underside of the leaf used for gas exchange. Once the plant interior has been breached, microbes are faced with another obstacle: the plant cell wall, a rigid, cellulose-based support surrounding every cell. Penetration of the cell wall exposes the host plasma membrane to the microbe, where they encounter extracellular surface receptors that recognize pathogen-associated molecular patterns (PAMPs) (Figure 1). Perception of a microorganism at the cell surface initiates PAMP-triggered immunity (PTI), which usually halts infection before the microbe gains a hold in the plant. However, pathogenic microbes have evolved the means to suppress PTI by interfering with recognition at the plasma membrane or by secreting effector proteins into the plant cell cytosol that presumably alter resistance signaling or manifestation of resistance responses (Figure 1). Interestingly, the ability to deliver pathogen proteins directly into plant host cells to alter plant defense has become a unifying theme among plant pathogens (phytopathogens). Once pathogens acquired the capacity to suppress primary defenses, plants developed a more specialized mechanism to detect microbes, referred to in this review as effector-triggered immunity (ETI). Effector-triggered immunity involves the direct or indirect recognition of the very microbial proteins used to subvert PTI by plant resistance (R) proteins. Activation of R protein-mediated resistance also suppresses microbial growth, but not before the invader has had an opportunity for limited proliferation (Figure 1). Not surprisingly, pathogens seem to have adapted effectors to interfere with ETI.

PAMP Recognition and PAMP-Triggered Immunity

PAMP-triggered immunity may be the plant's first active response to microbial perception. As will be outlined herein, PTI is initiated upon recognition of conserved microbial features by plant cell-surface receptors, and its induction is associated with MAP kinase signaling, transcriptional induction of pathogen-responsive genes, production of reactive oxygen species, and deposition of callose to reinforce the cell wall at sites of infection, all of which contribute to prevention of microbial growth (Nurnberger et al., 2004). Though the molecular mechanisms underlying PTI are not completely elucidated, much

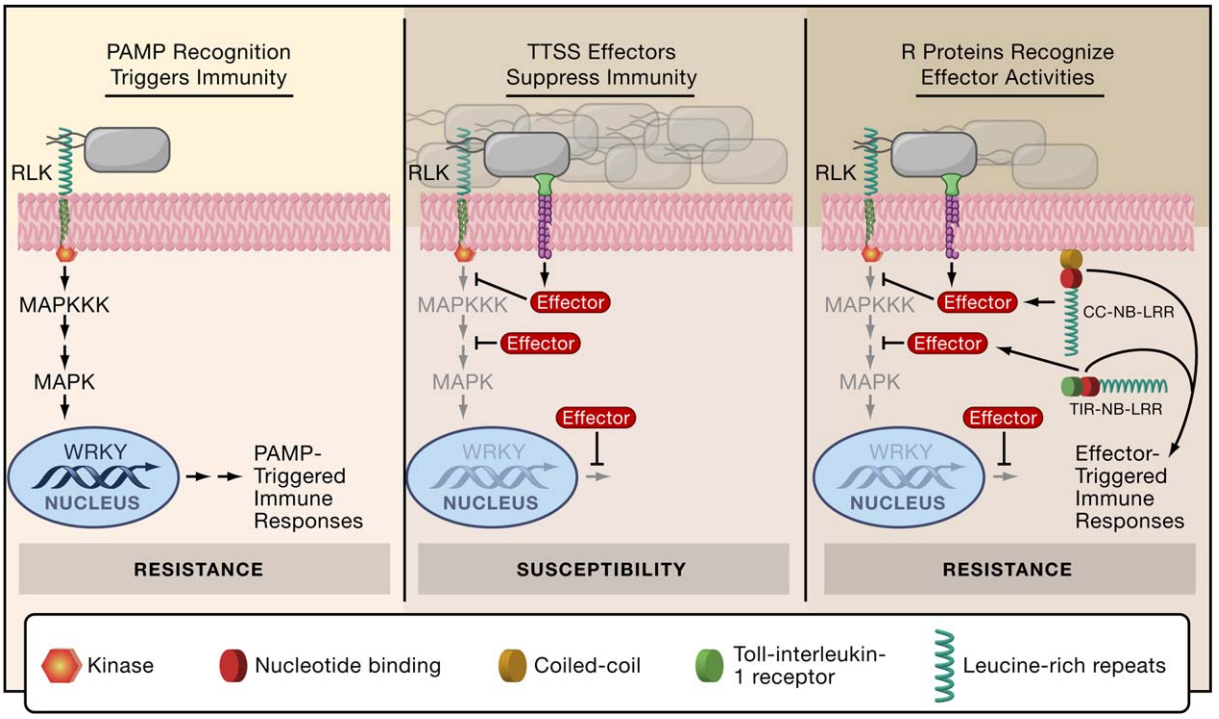

Figure 1. Model for the Evolution of Bacterial Resistance in Plants

Left to right, recognition of pathogen-associated molecular patterns (such as bacterial flagellin) by extracellular receptor-like kinases (RLKs) promptly triggers basal immunity, which requires signaling through MAP kinase cascades and transcriptional reprogramming mediated by plant WRKY transcription factors. Pathogenic bacteria use the type III secretion system to deliver effector proteins that target multiple host proteins to suppress basal immune responses, allowing significant accumulation of bacteria in the plant apoplast. Plant resistance proteins (represented by CC-NB-LRR and TIR-NB-LRR; see text) recognize effector activity and restore resistance through effector-triggered immune responses. Limited accumulation of bacteria occurs prior to effective initiation of effector-triggered immune responses.

work has been done cataloguing microbial features that trigger PTI. PAMPs fulfill a function critical to the lifestyle of the organism, are highly structurally conserved across a wide range of microbes, and are not normally present in the host (Nurnberger et al., 2004). For example, plants recognize multiple cell-surface components of Gram-negative bacteria, including lipopolysaccharide, a major constituent of the outer membrane, and flagellin, the protein subunit of the flagellum. Similarly, plants respond to chitin and ergosterol, major constituents of the cell wall of higher fungi. Several excellent reviews of phytopathogen PAMP biology have been recently published, and readers are directed to Nurnberger et al. (2004) as well as Zipfel and Felix (2005) for a thorough discussion of this topic.

Our most complete understanding of the plant response to PAMPs relates to perception of flagellin, the protein subunit of flagella (Gomez-Gomez and Boller, 2002). Flagella are required for bacterial motility, and, while the central region of flagellin is variable, the N- and C-terminal portions are highly conserved across eubacteria, making this an excellent PAMP. Flagellin is recognized as a PAMP by many plant species (Felix et al., 1999) as well as by mammalian innate immunity receptors (Underhill and Ozinsky, 2002). In *Arabidopsis* plants, a 22 amino acid peptide (flg22) corresponding to the highly conserved flagellin amino terminus is sufficient for host receptor activation (Felix et al., 1999). Identification of this potent peptide elicitor facilitated detailed analysis of flagellin responses using whole plant and protoplast systems. In a thorough examination of signaling components required for defense responses triggered by flg22 peptide in *Arabidopsis* protoplasts, Sheen and colleagues identified a complete MAP kinase cascade and WRKY transcription factors that function downstream of flg22 perception (Asai et al., 2002). Though this signaling machinery was identified based on involvement in response to a bacterial PAMP, activation of defenses by WRKY overexpression decreased symptoms caused by both bacteria and fungi, indicating that the resistance mechanisms induced following flagellin perception are not specific to bacteria. Alternatively, as the authors suggest, multiple PAMP signaling pathways may converge and activate defenses via overlapping MAP kinase cascades and transcription factors (Asai et al., 2002).

The *Arabidopsis* flagellin receptor FLS2 is a receptor-like kinase (RLK) consisting of extracellular leucine-rich repeats (LRRs) and an intracellular serine/threonine kinase domain (Gomez-Gomez and Boller, 2000). Mutant plants lacking FLS2 are insensitive to flagellin (Gomez-Gomez and Boller, 2000), and a link between flagellin perception

and restriction of pathogen growth has been established (Zipfel et al., 2004). Boller and colleagues demonstrated that FLS2 recognition of flg22 induces defenses that restrict bacterial growth, and plants lacking FLS2 are more susceptible to a bacterial pathogen. Furthermore, *fls2* mutant plants were treated with various bacterial extracts presumably containing PAMPs in addition to flagellin. Growth of bacteria subsequently inoculated on the PAMP-treated *fls2* mutants was reduced even though these plants lack the flagellin receptor. This indicates additional PAMPs present in the extracts were recognized by host cells through receptors other than FLS2 (Zipfel et al., 2004). Therefore, the action of multiple PAMP receptors function to restrict microbial growth.

Work related to flagellin perception has set the standard for laboratories studying PAMP perception and PTI. Currently, the major foci of this field are isolation of amino acid motifs responsible for receptor activation, identification of PAMP receptors, quantification of the response mediated by perception of individual PAMPs, and determination of the overlap among host responses to various PAMPs and other resistance determinants. One of the most abundant bacterial proteins, elongation factor Tu (EF-Tu), is actively recognized as a PAMP by *Arabidopsis* plants (Kunze et al., 2004). The first 18 amino acids of EF-Tu are sufficient to induce plant defense responses and are recognized by a host cell-surface receptor, recently identified as an RLK (C. Zipfel, personal communication). It is interesting to note there are over 200 RLKs encoded by the *Arabidopsis* genome. It is logical to speculate that additional PAMP receptors may also be RLKs.

Pathogen Effectors

If PAMP receptors recognize such universal features of microbes and initiate defense responses, how do pathogenic microorganisms successfully infect a plant? Once plants evolved a PAMP-triggered immune system enabling the detection of PAMPS, some pathogens evolved the ability to evade this type of resistance. There is emerging evidence that, during infection, pathogens actively suppress the plant's PAMP-triggered defenses. It is likely that all pathogenic microbes encode effectors that suppress PAMP-triggered defenses, but the best characterized come from phytopathogenic bacteria. Gram-negative bacterial pathogens acquired a type III secretion system (TTSS) through either horizontal gene transfer or adaptation of the flagellar apparatus. The evolution of the TTSS enabled bacteria to directly deliver effector proteins into plant cells, suppressing PAMP defense responses (Figure 1).

Bacterial-Pathogen Effectors

Bacterial pathogens of animals are known to secrete only a limited number of effectors into host cells. However, plant pathogens such as *P. syringae* can secrete approximately 20 to 30 effectors during infection (Chang et al., 2005). Effectors promote pathogenicity, and the TTSS is essential for the development of disease symptoms and bacterial multiplication (Staskawicz et al., 2001). By their collective action, effectors are hypothesized to alter plant physiology in susceptible hosts to sustain pathogen growth. Both fungal and bacterial effector proteins that are delivered to plants can possess enzyme activity (Table 1). These enzymes are responsible for modifying host proteins to enhance pathogen virulence and evade detection. Pathogens must protect themselves from these potentially detrimental effector enzymatic activities. Recent experimental evidence in mammalian pathogenesis demonstrates that effector unfolding is required for TTSS secretion (Akeda and Galan, 2005). Effectors may have prokaryotic chaperones keeping them unfolded prior to secretion, or effectors may possess eukaryotic activators. For instance, *P. syringae* AvrRpt2 is delivered to plant cells as an inactive enzyme, whereupon it is activated by eukaryotic cyclophilins such as *Arabidopsis* ROC1 (see below) (Coaker et al., 2005).

The *P. syringae* effectors AvrPto, AvrRpt2 and AvrRpm1 inhibit defense responses elicited by PAMP recognition (Hauck et al., 2003; Kim et al., 2005b). Furthermore, wild-type *Xanthomonas campestris* effectors suppress the plant defense response to the bacterial PAMP lipopolysaccharide. *X. campestris* TTSS-deficient bacteria have no effect on PAMP perception (Keshavarzi et al., 2004). These results support the hypothesis that bacterial effectors delivered by the TTSS may have a crucial role in suppressing PAMP-triggered defense responses elicited by PAMPs.

Bacterial effector proteins have also been implicated in activating plant transcription. Members of the *Xanthomonas* AvrBs3 effector family (e.g., AvrBs3, AvrXa10, and AvrXa7) contain a C-terminal nuclear localization signal (NLS) and an acidic transcriptional activation domain (AAD). These features imply that this family of effectors function in the plant nucleus to alter transcription during infection. In fact, the NLS of AvrBs3 is functional, and the AAD of AvrXa10 is capable of transcriptional activation of reporter genes in *Arabidopsis* and yeast (Zhu et al., 1998). Furthermore, AvrXa7 binds to dA/dT double-stranded DNA sequences (Yang et al., 2000). In summary, these data suggest that the AvrBs3 effector family alters plant nuclear gene transcription during pathogen infection, likely as a means to downregulate host defenses.

Three plant signaling molecules regulate plant defense against microbial attack: salicylic acid (SA), jasmonic acid (JA), and ethylene (Thomma et al., 2001). Ethylene-dependent signaling is important for the plant's response to pathogens, mechanical wounding, and wounding induced by herbivores. SA-dependent signaling is critical in establishing local and systemic bacterial resistance, while JA-dependent signaling is induced in response to mechanical wounding and herbivore predation. The SA and JA defense pathways are mutually antagonistic, and bacterial pathogens have evolved to exploit this fact to overcome SA-mediated defense responses (Kunkel and Brooks, 2002). During infection, *Pseudomonas* bacteria produce coronatine, a JA mimic that contributes to virulence by suppressing SA-mediated host responses (He et al., 2004; Reymond and Farmer, 1998). Coronatine is not the only bacterial factor that interferes with

Table 1. Enzymatic Activity of Biochemically Characterized Effectors and Selected Elicitors

Effector	Organism	Biochemical Function	Plant Target(s)	*R* Gene	Phenotype	Reference
AvrRpt2	*Pseudomonas syringae*	Protease[a]	RIN4	RPS2	Cleaves RIN4, Interferes with *R* gene-mediated defense, inhibits basal defense, and manipulates JA pathway	Reviewed by Mudgett (2005)
AvrB	*Pseudomonas syringae*		RIN4	RPM1	RIN4 phosphorylation, manipulates JA pathway	Reviewed by Mudgett (2005)
AvrRpm1	*Pseudomonas syringae*		RIN4	RPM1	RIN4 phosphorylation, inhibits basal defense.	Reviewed by Mudgett (2005)
HopPtoD2	*Pseudomonas syringae*	Protein phosphatase[a]			Suppresses programmed cell death and PR expression	Reviewed by Mudgett (2005)
AvrPphB	*Pseudomonas syringae*	Protease[a]	PBS1	RPS5	Cleaves PBS1, manipulates JA pathway	Reviewed by Mudgett (2005)
AvrPtoB	*Pseudomonas syringae*	E3 ligase,[a] ubiquitin-conjugating enzyme		Pto		Janjusevic et al., 2005
XopD	*Xanthomonas campestris*	Cysteine protease[a]	SUMO			Reviewed by Mudgett (2005)
AvrXv4	*Xanthomonas campestris*	Cysteine protease	SUMO	XV4		Reviewed by Mudgett (2005)
AvrBsT	*Xanthomonas campestris*	Cysteine protease	SUMO			Reviewed by Mudgett (2005)
Avr2	*Cladosporium fulvum*	Protease inhibitor	Rcr3	Cf-2	Inhibits RCR3 activity	Rooney et al., 2005
Avr4	*Cladosporium fulvum*	Chitin binding[a]	Chitinase	Cf-4		van den Burg et al., 2003
Avr-Pita	*Magnaporthe grisea*	Metalloprotease		Pi-ta		Jia et al., 2000
Pep-13	*Phytophthora sojae*	Calcium-dependent cell wall transglutaminase[a]		Elicitor	Activates plant defense responses	Brunner et al., 2002
EPI10	*Phytophthora infestans*	Kazal-like protease inhibitor[a]	Subtilisin A, P69B subtilase	Elicitor	Interacts and interferes with tomato PR-related protein P69B and subtilisin A	Tian et al., 2005
EPI1	*Phytophthora infestans*	Kazal-like protease inhibitor[a]	P69B subtilase	Elicitor	Interacts and interferes with tomato PR-related protein P69B	Tian et al., 2004

PR, pathogenesis-related.
[a] Biochemical function has been demonstrated in vitro.

SA-mediated defense responses. Multiple effector proteins have been shown to manipulate the JA pathway in concert, such as AvrB, AvrRpt2, AvrPphB, HopPtoK, and AvrPphE$_{pto}$ (He et al., 2004).

To cause disease, pathogens need to overcome multiple layers of defense responses. Cell wall fortification during infection, achieved by callose deposition in cell wall appositions (papillae), just below penetration sites, is a common defense response. Three *P. syringae* effectors prevent plant cells from establishing cell wall-based defenses (DebRoy et al., 2004; Hauck et al., 2003). AvrPto suppresses papillae formation, while AvrE and HopPtoM

suppress callose deposition during infection (DebRoy et al., 2004; Hauck et al., 2003).

A central component of plant resistance responses is the hypersensitive response (HR), a form of programmed cell death localized to infection sites. Several effector proteins from *P. syringae* pathovars are known to inhibit the HR (reviewed in Nomura et al., 2005), though in most cases the molecular basis of this inhibition is as yet unclear. A recent report identified several *P. syringae* effectors that suppress the HR (Jamir et al., 2004). The authors further demonstrated that these effectors could also inhibit cell death triggered by the proapoptotic protein Bax in yeast as well as plants. This result suggests that certain bacterial effectors have evolved the ability to suppress programmed cell-death responses. Rather than actively suppress the HR, additional effectors seem to interfere with recognition events that trigger an HR. For instance, AvrRpt2 interferes with the HR triggered by AvrRpm1 (Ritter and Dangl, 1996), but AvrRpt2 is not known to suppress cell death in general.

Of the vast number of bacterial effector proteins that have been cloned, only a few have been biochemically characterized (Table 1). Characterized effectors possess enzyme activity and modify host proteins to promote bacterial virulence. Effectors may have evolved to target key components of PAMP-triggered defense, or they may target a variety of different host proteins to promote pathogenicity. Thus, a major challenge in this field is to elucidate the biochemical functions and targets of these effectors. While pairwise amino acid comparisons provide little information as to possible enzymatic functions of effectors, insights into their enzymatic function have emerged by analyzing protein structure prediction programs to uncover conserved catalytic residues and protein folds.

The *P. syringae* effector AvrPtoB inhibits ETI-associated programmed cell death in susceptible tomato plants, although this phenotype did not enable prediction of enzymatic activity (Abramovitch et al., 2003). Crystallization of the C-terminal domain of AvrPtoB, which is responsible for inhibiting programmed cell death, revealed homology to components of eukaryotic E3 ubiquitin ligases (Janjusevic et al., 2005). Furthermore, AvrPtoB was demonstrated to possess ubiquitin ligase activity in vitro. Mutation of key residues eliminated the ubiquitin ligase activity of AvrPtoB in vitro and its ability to inhibit cell death in vivo (Janjusevic et al., 2005). These results suggest that AvrPtoB acts as a mimic of host ubiquitin ligases, transferring ubiquitin to plant proteins involved in regulating programmed cell death.

The *Xanthomonas* effectors XopD, AvrXv4, and AvrBsT are cysteine proteases that interfere with the plant SUMO protein conjugation pathway (Roden et al., 2004) (Table 1). SUMO is posttranslationally linked to proteins in a way analogous to the ubiquitin conjugation system. In contrast to ubiquitination, sumoylation does not promote protein degradation but instead alters a number of different functional parameters, depending on the protein substrate. Sumoylation alters protein properties such as subcellular localization, partnering, DNA binding, and activation of transcription factors (Hilgarth et al., 2004). In the presence of these effectors, host proteins are desumoylated.

The *P. syringae* effector AvrRpt2 is also a cysteine protease that cleaves the peptide sequence VPxFGxW (Chisholm et al., 2005; Jones and Takemoto, 2004). AvrRpt2's protease activity is essential for its self-processing activity and virulence function within the plant cell. During infection, AvrRpt2 cleaves the *Arabidopsis* resistance regulator RIN4. Genetic evidence demonstrates that AvrRpt2 targets additional proteins to promote pathogenesis, and several *Arabidopsis* proteins that contain variations of the peptide sequence VPxFGxW were cleaved by AvrRpt2 in a transient expression system (Chisholm et al., 2005; Kim et al., 2005a). Thus, AvrRpt2 likely eliminates multiple proteins during infection, and these are virulence targets of this protease.

Fungal- and Oomycete-Pathogen Effectors

A characteristic feature of most biotrophic fungi is their ability to form the haustorium, a specialized infection structure. Unlike Gram-negative bacteria, no TTSS has been discovered for fungal pathogens, and their effectors may be delivered from haustoria into the plant intercellular space (apoplast). While enzyme activity has been demonstrated for a few fungal effectors, the activity of most remains elusive, and little evidence exists for their role in virulence or PTI suppression. Additionally, many fungal effectors are small peptides, but it remains unclear how these elicitors gain entry into the plant cell and contribute to fungal pathology. Most cloned fungal effectors are small proteins of unknown function containing a signal for secretion into the apoplast (Table 2).

A large-scale analysis of haustorially secreted proteins from the flax rust fungi, *Melampsora lini*, was recently conducted that identified 21 secreted proteins (Table 2) (Catanzariti et al., 2005). Interestingly, the effector AvrP123 was identified and contains homology to a Kazal serine protease inhibitor (Table 2). Two secreted effector proteins, AvrP4 and AvrM, were able to induce ETI-programmed cell death when expressed inside the plant cell, suggesting that secreted AvrP4 and AvrM are translocated inside plant cells during infection (Catanzariti et al., 2005).

Two effector proteins, Avr2 and Avr4, have been characterized from the leaf-mold fungus *Cladosporium fulvum* (Table 1 and Table 2). Avr2 encodes a cysteine-rich protein that binds and inhibits the secreted tomato cysteine protease Rcr3 (Rooney et al., 2005). The Avr4 effector contains a chitin binding domain that binds chitin (van den Burg et al., 2003), a major component of fungal cell walls. As a mechanism for perceiving chitin as a PAMP, plants likely evolved chitinases to release the active polymers from the cell walls of invading pathogens, thereby triggering defense responses. To counter perception and activation of specific chitin-induced defense responses in plants, *C. fulvum* Avr4 is thought to shield the fungal cell wall from plant chitinases.

Oomycetes are pathogenic eukaryotes that are more closely related to brown algae than fungi. Recently, an

Table 2. Summary of Cloned Fungal and Oomycete Effectors

Effector	Organism	Characteristics	*R* Gene	References
Avr2	*Cladosporium fulvum*	58 aa, cysteine-rich protein, protease inhibitor	Cf-2	Reviewed in Rivas and Thomas (2005)
Avr4	*Cladosporium fulvum*	104 aa, cysteine-rich protein, chitin binding domain	Cf-4	Reviewed in Rivas and Thomas (2005)
Avr9	*Cladosporium fulvum*	63 aa, cysteine knot motif	Cf-9	Reviewed in Rivas and Thomas (2005)
Ecp2	*Cladosporium fulvum*	165 aa, cysteine-rich protein	Cf-ECP2	Reviewed in Rivas and Thomas (2005)
Avr-Pita	*Magnaporthe grisea*	233 aa, metalloprotease, protease motif required for Pi-ta recognition	Pi-ta	Orbach et al., 2000
PWL1, PWL2	*Magnaporthe grisae*	145 aa, glycine-rich hydrophilic proteins		Reviewed in Lauge and De Wit (1998)
AVR2-YAMO	*Magnaporthe grisae*	223 aa, homology to neutral Zn^{2+} proteases		Reviewed in Lauge and De Wit (1998)
AvrM	*Melampsora lini*	343–377 aa, multiple homologs, no cysteine residues	M	Catanzariti et al., 2005
AvrP4	*Melampsora lini*	95 aa, cysteine-rich protein	P4	Catanzariti et al., 2005
AvrP123	*Melampsora lini*	117 aa, cysteine-rich protein, homology to Kazal ser protease inhibitor	P1, P2, P3	Catanzariti et al., 2005
Nip1	*Rhynchosporium secalis*	82 aa, cysteine-rich protein, stimulates plasma-membrane H^+ ATPase, toxin	Rrs1	Reviewed in Lauge and De Wit (1998)
AvrL567	*Melampsora lini*	150 aa, 285 aa, 225 aa, polymorphic	L5, L6, L7	Dodds et al., 2004
$ATR1^{NdWsB}$	*Hyaloperonospora parasitica*	310 aa, Conserved RXLR aa motif, polymorphic	RPP1	Rehmany et al., 2005
ATR13	*Hyaloperonospora parasitica*	153 aa, polymorphic, heptad leucine/isoleucine repeat motif		Allen et al., 2004
Avr3a	*Phytophthora infestans*	147 aa, synteny with ATR1 locus	R3a	Armstrong et al., 2005
Avr1b	*Phytophthora sojae*	138 aa	Rps1b	Shan et al., 2004

All cloned effectors contain secretion-signal peptide enabling secretion into the plant apoplast. Size of proteins in amino acids (aa) is given for preproteins.

oomycete effector, $ATR1^{NdWsB}$, was cloned (Rehmany et al., 2005) (Table 2). In addition to a signal peptide for secretion into the plant apoplast, $ATR1^{NdWsB}$ contains the amino acid motif RXLR, which is highly conserved among three different oomycete effectors, additional secreted oomycete proteins, and malarial parasites. The RXLR motif is similar to a host-targeting signal required for translocation of malarial proteins into host cells (Hiller et al., 2004). Because $ATR1^{NdWsB}$ can be detected inside the plant cytosol, this motif may be required for translocating secreted oomycete proteins from the apoplast to the cytosol.

Viral-Pathogen Effectors

A primary means by which plants defend against viral infection is RNA silencing, which regulates accumulation of endogenous and foreign RNA molecules. A potent trigger of RNA silencing is double-stranded RNA. The majority of plant viruses have RNA genomes, and double-stranded viral secondary structures or replication intermediates may trigger host silencing of the entire viral genome, preventing systemic viral spread. Viral virulence determinants—which may be considered effectors—suppress the host RNA silencing response. Just as the ability of bacterial and fungal effectors to suppress PAMP-triggered defenses limits the host range of those pathogens, the success of a virus' silencing suppressor may be a major factor determining the host range of that virus. Suppressors have been identified in many plant viruses, and the molecular functions of several have been characterized in detail (Soosaar et al., 2005). These studies determined that different suppressors interfere with unique components of the host-silencing machinery, suggesting that many viruses independently developed the means to suppress silencing. Not surprisingly, plants use a second defense mechanism to recognize and

restrict virus movement. Specific R proteins recognize viral components–either silencing suppressors or other proteins–that accumulate following successful viral replication and translation. The same effector-triggered immune responses against bacteria and fungi also function to restrict viruses. In fact, a recombinant virus that expressed the bacterial effector protein AvrPto triggered resistance and restricted virus spread on tomato plants containing components of the Pto-Prf surveillance machinery (Tobias et al., 1999).

Gene-for-Gene Resistance

As described above, the evolution of secreted effector proteins by plant pathogens ultimately led to the acquisition of plant proteins that specifically recognize these bacterial, fungal, and viral effectors. This pairwise association describing the recognition of effectors within the plant cell has been characterized genetically as gene-for-gene resistance (Flor, 1971). In the presence of a cognate R effector association, resistance is activated, resulting in the initiation of defense signaling and host resistance. Resistance is manifested as localized cell death at the site of infection and inhibition of pathogen growth. Conversely, in the absence of this paired interaction, the pathogen eludes detection by the host plant, resulting in pathogen proliferation within the plant cell and the onset of disease.

Plants have evolved systematic defense mechanisms capable of both recognizing and responding to a myriad of bacterial, fungal, oomycete, and viral pathogens as well as resistance against nematodes and insects. To date, numerous *R* genes have been cloned from a wide range of plant species. Despite the broad spectrum of resistance imparted by R proteins, these gene products can be categorized into two main classes based on domain organization (Figure 2) (Dangl and Jones, 2001).

Resistance-Protein Domain Architecture

The largest class of resistance genes cloned to date is represented by a family of proteins containing a nucleotide binding (NB) site and leucine-rich repeat (LRR) domains. Nucleotide binding motifs share sequence similarities with the NB regions of apoptosis regulators such as *CED4* from *Caenorhabditis elegans* and *Apaf-1* from humans (Dangl and Jones, 2001). This would suggest that R protein activity may require, at least in part, the activity associated with ATP binding and/or hydrolysis (Tameling et al., 2002). The LRR is typically 20–30 amino acids in length, and these motifs have been identified in proteins ranging from viruses to eukaryotes. These proteins participate in a range of processes from development to disease resistance. Collectively, LRRs appear to be involved in formation of protein-protein interactions. The NB-LRR class of *R* genes can be further divided into coiled-coil (CC) NB-LRR and Toll-interleukin-1 receptor (TIR) NB-LRR according to their N-terminal domain (Figure 2). The N terminus influences the requirement for downstream defense-response components (Feys and Parker, 2000). Within the NB-LRR class, the best characterized members

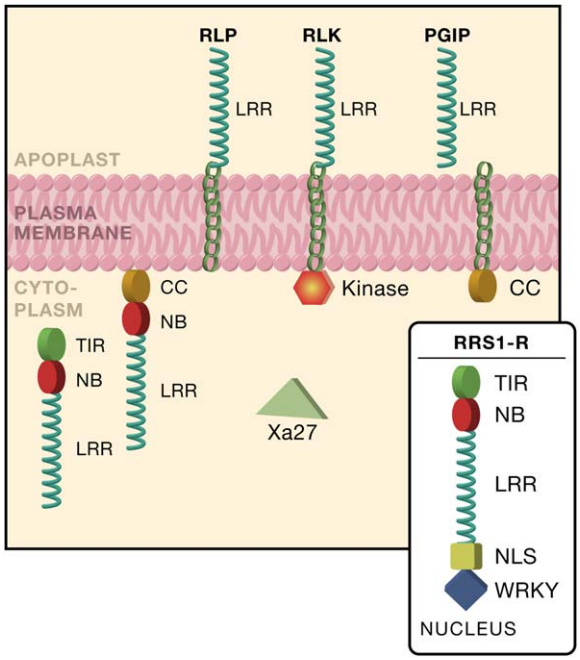

Figure 2. Classes of Resistance Proteins

Resistance (R) proteins are classified according to their domain organization. The two main classes of R proteins are the nucleotide binding leucine-rich repeat (NB-LRR) and the extracellular LRR (eLRR) resistance proteins. The NB-LRR class is the most abundant, and members can possess amino-terminal coiled-coil (CC) or Toll-interleukin-1 receptor (TIR) domains. The RRS1-R protein is a novel member of the NB-LRR class containing a carboxy-terminal nuclear localization signal (NLS) and a domain with homology to WRKY transcription factors. RRS1-R is nuclear localized after interacting with the pathogen effector PopP2. Three subclasses of eLRRs have been classified according to their domain structure: RLPs, RLKs, and PGIPs. Recently, a novel R protein, Xa27, was identified. Xa27 possesses no discernable amino acid sequence similarities to proteins of known functions and has no homologs outside of rice.

include RPS2, RPM1, and RPS5, *Arabidopsis* R proteins specifying resistance to *P. syringae* carrying the bacterial effectors AvrRpt2, AvrRpm1/AvrB, and AvrPphB, respectively. In *Arabidopsis* alone, over 150 proteins are predicted to be NB-LRR proteins. Collectively, this class of R proteins determines resistance to bacterial, viral, fungal, and oomycete pathogens (Dangl and Jones, 2001).

A second major class of *R* genes encodes extracellular LRR (eLRR) proteins. Three subclasses of eLRRs have been classified according to their domain structures (Fritz-Laylin et al., 2005). These subclasses include RLP (receptor-like proteins; extracellular LRR and transmembrane [TM] domain), RLK (extracellular LRR, TM domain, and cytoplasmic kinase) and PGIP (polygalacturonase-inhibiting protein; cell wall LRR) (Figure 2). The best characterized RLPs are represented by the tomato *Cf* genes, which confer resistance to infection by the biotrophic leaf-mold pathogen *C. fulvum* (Jones et al., 1994). Biochemical analysis of proteins secreted by *C. fulvum* during its growth within the apoplast of tomato leaves has led to

the identification of the race-specific elicitors Avr2, Avr4, and Avr9 (de Wit, 1995; Joosten et al., 1994). Independently, these elicitors govern recognition of the fungus by resistant plants carrying the resistance genes *Cf-2*, *Cf-4*, and *Cf-9*, respectively. Although Cf proteins lack an obvious signaling domain, it is believed that defense signaling is mediated through interactions with other proteins. Xa21, an RLK present in rice, responds to effector molecules secreted from the Gram-negative pathogen *Xanthomonas oryzae* (Ronald et al., 1992; Shen and Ronald, 2002; Song et al., 1995) and provides resistance to a broad range of *Xanthomonas* pathogens (Wang et al., 1996).

While the majority of characterized R proteins fall into the above classes, there are examples of R proteins with novel domain architecture. For instance, RRS1-R that recognizes *Ralstonia solanacearum* is a TIR-NB-LRR protein that also contains an carboxy-terminal nuclear localization signal and WRKY transcriptional activation domain (Deslandes et al., 2003). More recently, the Xa27 R protein from rice was cloned (Gu et al., 2005). Xa27 is a novel protein that does not share homology with other R proteins. Interestingly, expression of the resistant Xa27 allele occurs only in the vicinity of tissue infected by *Xanthomonas oryzae* pv. *oryzae* expressing the cognate effector protein a*vrXa27*. The identification of *Xa27* marks the first example of a differentially expressed R protein whose induction specificity dictates resistance.

Surveillance

Research in the past 5 years has led to a better understanding of the complex surveillance mechanisms that coordinate resistance responses in *Arabidopsis*. Although many *R* genes and their corresponding pathogen effectors have been cloned, direct binding between them has rarely been demonstrated. Contrary to predicted models, it is now clear that bacterial effector recognition and signaling has likely evolved as an indirect mechanism. This seemingly limited repertoire of plant resistance receptors begs the question of how an effector-triggered immune response in plants coordinates resistance to a broad range of pathogens and their corresponding effectors. The majority of characterized bacterial effectors possess enzyme activity (Table 1) and modify plant proteins. Evidence is emerging that the enzymatic functions of multiple effectors target the same host proteins. Rather than develop receptors for every possible effector, host plants have evolved mechanisms to monitor common host targets. By monitoring for perturbations, R proteins indirectly detect the enzymatic activity of multiple effectors (Van der Biezen and Jones, 1998).

Molecular evidence for indirect pathogen recognition has come from work studying resistance responses in *Arabidopsis* plants following infection with *P. syringae* expressing the effector AvrPphB, a cysteine protease (Figure 3A) (Shao et al., 2003). The activity of AvrPphB is indirectly detected by the R protein RPS5. This work demonstrated that perception and subsequent resistance signaling is initiated not by the direct perception and association of R protein-effector molecule pairing but by an indirect mechanism. During infection, AvrPphB cleaves the host protein PBS1. AvrPphB cleavage of PBS1 is then perceived by the R protein RPS5, which in turn activates resistance signaling (Figure 3A).

Additional studies have also revealed similar, indirect mechanisms for resistance signaling (Axtell and Staskawicz, 2003; Mackey et al., 2002, 2003). The best characterized example of the activation of resistance by way of monitoring bacterial effector activity is that of the *Arabidopsis* protein RIN4. RIN4 is monitored by at least two R proteins, RPM1 and RPS2 (Figures 3B and 3C). RPM1 and RPS2 have each been shown to physically associate with RIN4 in planta (Axtell and Staskawicz, 2003; Mackey et al., 2002). The *Arabidopsis* protein RPM1 recognizes two unrelated *P. syringae* effector proteins, AvrRpm1 and AvrB (Bisgrove et al., 1994). Interestingly, the soybean RPG1 R protein recognizes AvrB but not AvrRpm1. Although RPM1 and RPG1 are both NB-LRR proteins, they show limited sequence homology, suggesting that they evolved independently to detect AvrB (Ashfield et al., 2004). When AvrRpm1 or AvrB is delivered to the plant cell, RIN4 is hyperphosphorylated, which in turn leads to the activation of RPM1-mediated resistance (Figure 3B). Thus, although RPM1 resistance is activated in the presence of either AvrB or AvrRpm1, it is activated through an indirect mechanism (i.e., detection of the modified state of RIN4). It has recently been shown that AvrRpm1 inhibits PAMP-triggered defense responses, presumably through its modification of RIN4 and other host targets (Kim et al., 2005b).

As discussed previously, numerous effectors suppress PAMP-triggered immunity. In addition to inhibiting PAMP-triggered defense responses, multiple effectors inhibit localized programmed cell death, a hallmark of *R* gene defense. A third *P. syringae* effector, AvrRpt2, also targets RIN4 during infection (Axtell and Staskawicz, 2003; Mackey et al., 2003). AvrRpt2 is a protease that directly cleaves RIN4 (Coaker et al., 2005). RPM1 does not detect cleavage of RIN4; in fact, in the presence of AvrRpt2, RPM1 is not able to detect the presence of AvrRpm1 or AvrB (Ritter and Dangl, 1996). However, the resistance protein RPS2 is activated following RIN4 cleavage, thereby recognizing AvrRpt2 (Figure 3C) (Axtell et al., 2003; Day et al., 2005). AvrRpt2 activity also inhibits PAMP-triggered defense responses, possibly by RIN4 cleavage (Kim et al., 2005b). RIN4 is therefore a convergence point for two resistance signaling pathways, involving at least two R proteins and three effectors.

It is reasonable to hypothesize that AvrB and AvrRpm1 evolved to suppress PAMP-triggered defense functions mediated by RIN4. Subsequently, the plant developed RPM1 to detect these perturbations. The AvrRpt2 effector may have later evolved as a mechanism to interfere with the RPM1 disease-resistance pathway and restore pathogen virulence. Finally, RPS2 evolved to recognize the protease activity of AvrRpt2, which in turn restored resistance in the host plant. We hypothesize that the above example

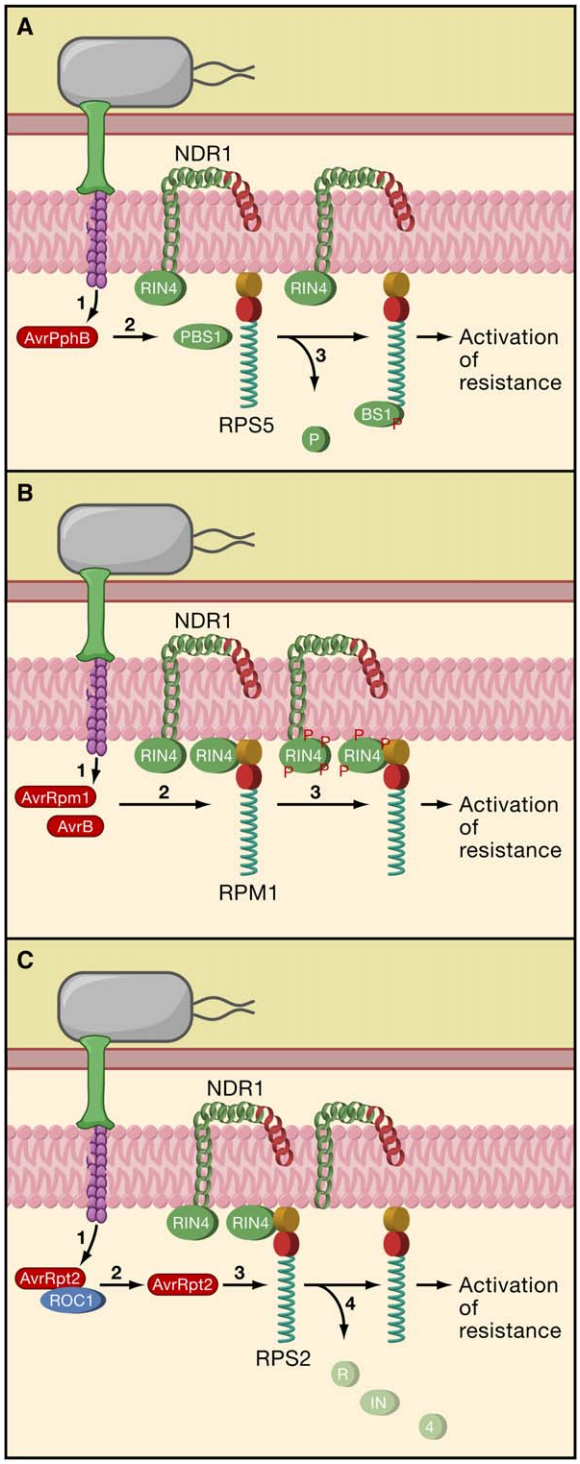

Figure 3. Indirect Pathogen Recognition through Host Surveillance of Effector Targets

Numbering reflects proposed sequence of events. NDR1 is required for the activation of bacterial resistance mediated by all three members of the CC-NB-LRR class of resistance proteins described in this figure (reviewed in Dangl and Jones, 2001).

(A) Resistance to *Pseudomonas syringae* expressing the effector AvrPphB, a cysteine protease, is mediated by the *Arabidopsis* R protein RPS5, a CC-NB-LRR protein. AvrPphB cleaves the *Arabidopsis* protein PBS1. RPS5 indirectly detects the AvrPphB effector through PBS1 cleavage.

(B and C) One of the best characterized examples of indirect recognition of pathogens involves the *Arabidopsis* protein RIN4.

(B) In the presence of the *P. syringae* effectors AvrB or AvrRpm1, RIN4 is hyperphosphorylated (indicated by "P"). The *Arabidopsis* CC-NB-LRR R protein RPM1 monitors RIN4 and is activated following this phosphorylation.

(C) RIN4 is a negative regulator of a second CC-NB-LRR, RPS2, which detects the activity of *P. syringae* AvrRpt2, a cysteine protease. AvrRpt2 is delivered into *Arabidopsis* as an inactive molecule, and association with the host protein ROC1 (a folding catalyst) induces AvrRpt2 protease activity. Through a direct targeting mechanism, AvrRpt2 cleaves RIN4 at two sites, resulting in the dissociation of RIN4 from RPS2 and subsequent activation of effector-triggered immunity.

reflects a common evolutionary struggle between host resistance mechanisms and pathogen effectors. The suppression of PAMP-triggered defense responses through the acquisition of effector enzymes has led to the development of ETI in plants. Rather than developing a new form of resistance, the pathogen and plant are locked in a coevolutionary conflict between effectors and R proteins.

Although there is evidence in support of the indirect-recognition model for bacterial effector recognition, plants may employ alternate detection mechanisms for other pathogens. It is still unclear whether fungal and oomycete pathogens are perceived directly or indirectly by host *R* genes. One example of direct recognition of a fungal effector is that of AvrPita, which is recognized by the rice resistance gene Pi-ta. AvrPita has been shown to directly bind to Pi-ta by yeast two-hybrid and in vitro binding assays (Jia et al., 2000). There is also a well-characterized example of indirect recognition in fungal pathogenesis. The tomato Cf-2 R protein recognizes the *C. fulvum* effector Avr2. During infection, Avr2 binds to and inhibits the secreted tomato protease Rcr3, which in turn is responsible for Cf-2 activation (Rooney et al., 2005). Whether R proteins recognize most pathogen effectors directly or indirectly is a question that remains to be elucidated. While indirect mechanisms of pathogen recognition permit the detection of multiple unrelated effectors by a single R protein, a direct interaction between pathogen effectors and R proteins would allow for the detection of structurally conserved effector molecules. Direct detection would only be efficient against multiple effectors containing common structural motifs. Therefore, indirect recognition likely evolved following direct recognition as a means to detect emerging effector diversity.

One example of *R* gene and effector gene coevolution has been described between the *Arabidopsis R* gene RPP13 and an oomycete effector, ATR13 (Allen et al., 2004). Due to the extreme diversity of both ATR13 and RPP13, both genes are hypothesized to be under balancing selection, where a diverse array of alleles is stably maintained. Analysis of 24 *Arabidopsis* accessions demonstrated that the RPP13 locus exhibits high levels of polymorphism. Amino acid variation in LRR domains of RPP13 were predominant (Rose et al., 2004). The

evidence of balancing selection of *R* genes and effector genes suggests that both host and pathogen are locked in a coevolutionary conflict, where efforts to escape resistance by the pathogen are matched by new host recognition capacities. This rapid evolution of both the R protein and the effector may reflect a direct interaction between the two.

Future Directions and Major Unanswered Questions

Plants possess multiple cell-surface receptors that recognize PAMPs common to the majority of microbes. In addition to isolating bacterial PAMP receptors, the field is rapidly moving toward identification of fungal PAMPS and their cognate receptors. There have already been more than a dozen PAMPs identified, presumably each with its own receptor (Nurnberger et al., 2004). Any given microbe will potentially be recognized by more than one PAMP receptor. Does activation of multiple PAMP receptors increase the amplitude of the PTI response or do multiple RLKs act in concert to tailor their response to the detected microbe? Once PTI is initiated, what distinguishes PAMP-triggered responses from ETI responses? Though the mechanisms of pathogen perception are unique, PTI and ETI may use similar mechanisms to limit pathogen growth. Future studies employing genomic and proteomic technologies will allow detailed global comparison of these pathways.

Numerous laboratories are determining the enzymatic functions of effectors as well as identifying their host targets. If a primary function of bacterial effectors is suppression of PTI, then identification of effector targets may well elucidate the molecular basis of PTI. In addition to suppressing PTI, pathogen effectors may also possess additional virulence components necessary to cause disease. We are only beginning to identify and decipher the role of fungal and oomycete effector proteins in plant disease and resistance. Among the more interesting and important questions are how are secreted fungal and oomycete effectors delivered inside the plant cell and what are their cellular targets? It is hypothesized that effectors are secreted from haustoria, an elaborate fungal and oomycete structure that is contiguous with the plant plasma membrane. Endocytosis of these effectors may occur through specialized host cell receptors.

The discovery that the activation of ETI can be negatively regulated and involves an indirect-recognition mechanism has forced the field to reevaluate previous paradigms. How does release of negative regulation of R proteins induce downstream signaling leading to transcriptional reprogramming and manifestation of resistance?

Elucidation of mechanisms controlling the evolution of plant-microbial interactions will be greatly impacted by new technologies that include rapid genome sequencing and the development of computational methods to analyze the wealth of genomic information. Concomitant studies that employ postgenomic technologies that include systems biology approaches will ultimately allow us to understand the expression of all genes and proteins in a plant that are simultaneously expressed during the expression of resistance. These technologies will allow us to understand the complex interactions that occur between multiple pathways that are expressed during resistance. Ultimately, a complete understanding of the molecular basis of plant disease resistance will allow the application of these discoveries to construct plants that contain novel combinations of disease-resistance pathways that are durable and recognize a wide spectrum of pathogens.

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