

Elicitors, Effectors, and *R* Genes: The New Paradigm and a Lifetime Supply of Questions

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Annu. Rev. Phytopathol. 2007. 45:399–436

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

This article's doi:
10.1146/annurev.phyto.45.062806.094427

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0066-4286/07/0908-0399\$20.00

Key Words

PAMP, MAMP, avirulence, gene-for-gene, leucine-rich repeat

Abstract

The plant basal immune system can detect broadly present microbe-associated molecular patterns (MAMPs, also called PAMPs) and induce defenses, but adapted microbes express a suite of effector proteins that often act to suppress these defenses. Plants have evolved other receptors (R proteins) that detect these pathogen effectors and activate strong defenses. Pathogens can subsequently alter or delete their recognized effectors to avoid defense elicitation, at risk of a fitness cost associated with loss of those effectors. Significant research progress is revealing, among other things, mechanisms of MAMP perception, the host defense processes and specific host proteins that pathogen effectors target, the mechanisms of R protein activation, and the ways in which pathogen effector suites and *R* genes evolve. These findings carry practical ramifications for resistance durability and for future resistance engineering. The present review uses numerous questions to help clarify what we know and to identify areas that are ripe for further investigation.

NB: nucleotide binding

LRR: leucine-rich repeat

INTRODUCTION

In the middle of the previous century, landmark findings were made regarding gene-for-gene plant disease resistance, infection-induced synthesis of antimicrobial phytoalexins and PR proteins, and pathogen virulence through production of toxins and hydrolytic enzymes (108). Subsequently, when scientists met 10–20 years ago to discuss pathogen virulence and plant disease resistance mechanisms, key questions emerged. These included:

Do plants have a different *R* gene for almost every strain of every potential pathogen? If not, what do they have that makes them resistant to so many potential pathogens?

Why do pathogens have *avr* genes if, unlike toxins or hydrolytic enzymes, these *avr* genes just hurt the pathogen's chances for success?

How relevant are defense activation by chitin or plant cell wall fragments, or defense responses such as phytoalexin or PR (pathogenesis-related) protein production? Because, in contrast to gene-for-gene systems, these have not been shown to play a causal role in disease resistance.

How does the “lock-and-key” interaction between *R* and *avr* gene products work?

On the heels of impressive research progress, a revised four-part model for plant disease resistance has emerged that provides some answers to these questions. This four-

part model has been nicknamed, only partially in jest, the new “Central Dogma” of plant pathology. This important model describes an evolutionary process. Plants, in addition to their preformed physical and chemical barriers, first have an immune system that can detect generic conserved components of most microorganisms. In part two, certain microbes become adapted pathogens of certain plant species by evolving virulence factors that actively suppress parts of the general defense response in these hosts. In part three, adapted pathogens are repelled when the host species evolves specific *R* genes, whose products indirectly detect the defense-suppressing virulence factors by detecting their effect on specific host proteins. Finally, the pathogen evolves further and escapes detection by the *R* gene product by eliminating the detected virulence factor or suppressing the defenses induced by *R* gene products. **Figure 1** illustrates this model. Similar models have also been described elsewhere (31, 44, 88).

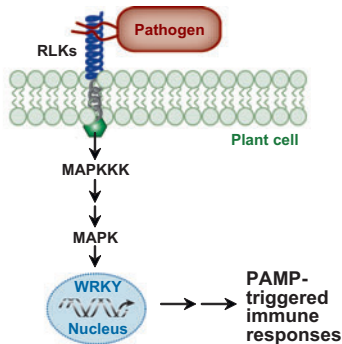
The model of **Figure 1** is an important and successful crystallization of many findings. In this review we describe a subset of the recent discoveries about virulence and resistance that expand or solidify the “take-home” generalizations of this new model. However, this new paradigm has brought into focus a new set of questions, exceptions, and unexplained findings. The questions from 10–20 years ago that have been answered in part are being revisited, and the new Central Dogma is already undergoing revision. We present a useful collection of questions

Figure 1

Model for the evolution of bacterial resistance in plants. (a) Recognition of pathogen-associated molecular patterns (such as bacterial flagellin) by extracellular receptor-like kinases (RLKs) promptly triggers basal immunity, which includes signaling through MAP kinase cascades and transcriptional reprogramming mediated by plant WRKY transcription factors. (b) Pathogenic bacteria use the type III secretion system to deliver multiple effector proteins that target host proteins and suppress basal immune responses, allowing significant accumulation of bacteria in the plant apoplast. (c) Plant resistance proteins (*R* gene products, such as a TIR-NB-LRR protein) recognize effector activity and restore resistance through strong effector-triggered immune responses. (d) Pathogen avoids *R* gene-mediated defenses by modifying or eliminating the effector(s) that triggers those defenses. This state resembles that shown in (b) except the pathogen has had to alter or lose an effector protein, or deploy an additional effector protein. Similar models can be drawn for other plant pathogens. Figure redrawn from (31).

a

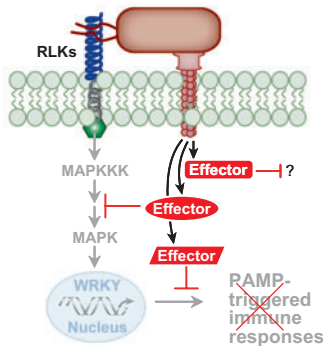
PAMP recognition triggers immunity



Resistance

b

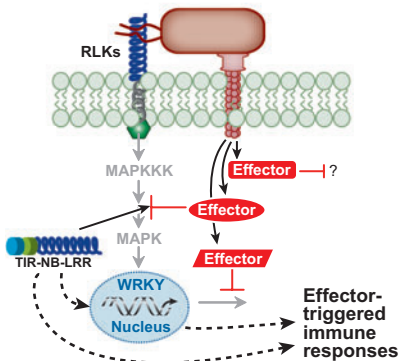
TTSS effectors suppress immunity



Susceptibility

c

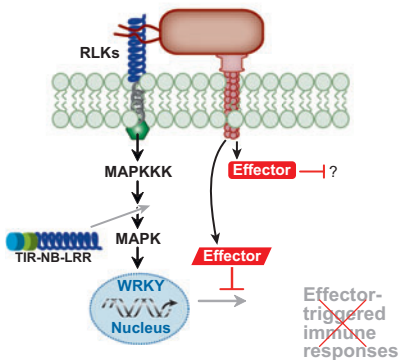
R proteins recognize effector activities







Resistance

d

Effector recognized by R protein is lost or modified



Susceptibility (but less virulence)

-  Kinase
-  Nucleotide-binding
-  Toll-interleukin 1 receptor
-  Leucine-rich repeats

MAMP:

microbe-associated
molecular pattern

PAMP:

pathogen-associated
molecular pattern

and briefly summarize alternative models, modified terminology, and/or opportunities for important future research. Areas with potential for future disease resistance engineering are also highlighted.

MAMPS, MAMP RECEPTORS, AND BASAL IMMUNITY (AND NOMENCLATURE!)

The first panel of **Figure 1** alludes to a system often referred to as the basal immune system, which induces responses referred to as basal defenses. It was discovered over 30 years ago (4, 22, 67) that plant defense responses can be activated by relatively generic signals of pathogen presence, which were often called elicitors [for recent reviews see (31, 88, 130, 196)].

One key concept surrounding basal immune systems is that they recognize certain broadly conserved molecules associated with a wide range of pathogens. The term pathogen-associated molecular pattern (PAMP) was developed by researchers of the mammalian innate immune system to describe this type of defense-activating compound. The term MAMP (for microbe-associated molecular pattern) is gaining favor because nonpathogenic microorganisms also possess PAMPs. Well-developed examples of MAMPs that are detected by plants include bacterial flagellins, lipopolysaccharides or elongation factor-Tu, fungal chitin, or oomycete Pep-13 or heptaglucoisides (87, 196). A related concept from both plant and animal research is that the genes for host MAMP receptors are relatively stable and heritable, allowing the capacity for early detection of microbial infections to be preserved and passed from generation to generation (81, 130). This is in contrast to mammalian adaptive immune systems that “reinvent the wheel” of recognition specificity in each new individual. A third concept is the perception that basal immunity has a relatively primitive and inferior immune capacity relative to adap-

tive immunity. This idea derives in part from the observation that basal defenses are only partially effective at restricting pathogens. It also derives from the concept that basal defenses are relatively static, i.e., capable of evolving to recognize novel infection threats only over many generations, whereas plant disease resistance mediated by *R* genes is sometimes portrayed as the plant adaptive immune system. Each of these ideas requires clarification and revision, especially when the goal is to accurately describe plant immune systems. It is important to disentangle the terminology and paradigms used to describe plants from those borrowed from animal research with only partial success. For example, some *R* genes compose a more rapidly evolving component of the plant basal immune system than MAMP receptors, but they are not an “adaptive immune system” in that they do not regularly undergo useful diversification and selection in the somatic cells of individuals.

Most readers will already be familiar with the concept of *R* (resistance) and *Avr* (avirulence) genes. Gene-for-gene disease resistance is economically important—it is used in numerous crops to confer highly effective disease resistance (108, 148, 158). Plants have many *R* genes and pathogens have many *Avr* genes. Simply described, disease resistance is observed if the product of any particular *R* gene has recognition specificity for a compound produced due to a particular pathogen *Avr* gene. We will see below that many *Avr* gene products contribute to pathogen virulence.

What Is the Difference between a MAMP and an Avr Gene Product?

The difference is becoming less clearly defined. Formally, the latter are named avirulence genes because they cause avirulence in the presence of *R* genes. In the absence of a cognate *R* gene, *Avr* genes often make a quantitative contribution to virulence yet are not

essential for pathogen viability, although these are not defining features (think, for example, of an essential viral replicase that is shown to be an *Avr* gene). Some *Avr* proteins can evolve substantially or may be entirely absent from certain strains of a pathogen, whereas MAMPs are defense elicitors that are evolutionarily stable, forming a core component of the microorganism that cannot be sacrificed or even altered much without seriously impairing viability. These traditional definitions still have utility, but exceptions are now known and new classifications for these defense elicitors and their counterparts in the host are being actively considered. This review is organized around the traditional definitions, but also highlights their shortcomings and considers some alternatives.

The term MAMP (microbe-associated molecular pattern) is increasingly used in place of PAMP because it lends greater accuracy to our thinking. As noted above, many microorganisms carry these defense-eliciting molecules yet are not pathogens, or are not pathogens of many of the hosts that can detect their MAMPs (12). A plant normally grows in the presence of hundreds of microbial species, including many nonpathogenic microorganisms that it would seemingly be counter-productive to defend against. This raises a challenging question: **In the biologically realistic setting of an intact plant infested with living microorganisms, how much MAMP needs to be present, and in what plant tissues, for defenses to be triggered?** One can postulate that microorganisms must reach a critical mass in the plant interior before the basal immune system is strongly activated; for example, smaller or primarily external/epiphytic microbial populations are usually less potent at inducing *PR* gene expression and other active defenses. Further tissue specificity was suggested by a recent study in which stomate closure was discovered as a plant defense against bacterial infections (117). Purified MAMPs triggered stomate closure and bacteria did as well, but only when they swarmed around the stom-

atal opening. Apparently, a threshold level of MAMP must be present before the response is activated.

There is a more basic question to ask: **Has MAMP perception ever been shown to significantly improve plant disease resistance?** The plant pathology literature carries numerous examples where purified pathogen-derived compounds caused elevated plant disease resistance. Defense pathways have been turned on, *PR* proteins expressed, and intact plants may even have been shown to allow less pathogen growth. However, in these experiments the compounds usually have been applied to plants by humans, in doses and/or locations that may not mimic natural infections. One might pursue experiments in which expression of a MAMP is knocked out in the pathogen, but these strains will generally show reduced rather than enhanced virulence due to the central contribution of most MAMPs to pathogen viability. The first experiments to convincingly show a contribution of MAMP perception to whole-plant disease resistance took a different approach, mutation of the host receptor (198). *Arabidopsis* plants lacking the flagellin receptor *FLS2*, a transmembrane protein with extracellular leucine-rich repeats (LRR) and an intracellular protein kinase, showed increased susceptibility to infection by *Pseudomonas syringae* pv. *tomato* strain DC3000. Note that DC3000 does cause disease on plants that carry a functional *FLS2*. Absence of *FLS2* makes the plants *more* susceptible to this pathogen. The contribution of flagellin perception to resistance was detected only when the bacteria were sprayed onto the leaf exterior and not when they were introduced directly into the apoplast (198). *FLS2*-activated responses are known to arise quickly, within minutes (56). Perhaps early detection, as the first few pathogen individuals are entering the plant, is required to allow sufficient defense activation (including stomatal closure) before the pathogen can build appreciable interior populations and more effectively counter host defenses.

VIGS:
virus-induced gene
silencing

amiRNA: artificial
micro-RNAs

The MAMP receptor knock-out approach was subsequently used to show an apparent contribution to resistance by *Arabidopsis* EFR1 (197). Like FLS2, EFR1 is a transmembrane LRR-kinase. EFR1 controls responsiveness to bacterial EF-Tu, an abundant protein that is a highly conserved component of the protein synthesis apparatus. Detection of EF-Tu has been found only in plants of the Brassicaceae, but there is practical relevance to this finding: transient *in planta* expression of *Agrobacterium*-delivered T-DNA was significantly improved in plants lacking EFR1. *Arabidopsis* researchers may now be able to use the *Agrobacterium*-mediated transient gene expression method, which has fostered impressive research progress in the less tractable *Nicotiana benthamiana* model system. Identification and knock-out of MAMP receptors are not simple processes, especially as one moves beyond *Arabidopsis* to other plants, but they may be the best approaches to demonstrate the relevance of any particular MAMP receptor in plant disease resistance. Virus-induced gene silencing (VIGS), artificial micro-RNAs (amiRNA), and mutation TILLING offer approaches to knock down gene expression in plants of economic interest, beyond *Arabidopsis* or *Nicotiana benthamiana* (34, 71, 142, 151), but the candidate receptor gene must first be identified.

The terminology shift from PAMP to MAMP highlights a key question: **Across the range of plant-microbe associations, how widely is it the case that potential pathogens are nonpathogens primarily because of basal defenses activated via MAMP detection?** Another way of phrasing this question is, How often would potential pathogens actually be pathogens on a given host if not for MAMP-activated basal immunity? This is another question for which we still need answers, and the difficulty is not solely due to the need for research on a wide range of pathosystems. Mutational or expression-knock-down strategies might be applied to address this question, disrupting specific MAMP receptors, but if a pathogen

expresses multiple MAMPs that engage multiple MAMP receptors, the contribution of any single receptor may be quantitative and difficult to detect.

How Can Successful Pathogens Grow in the Host Despite Presenting Increasingly More MAMPs as They Reach Higher Population Levels?

As discussed below, pathogens deploy effectors that suppress the basal immune system. The plethora of pathogen effectors that are devoted to suppressing basal defenses can be construed as evidence that basal defenses are indeed effective against potential pathogens that fail to suppress them. The ability of effectors to suppress basal defenses is host specific, which likely contributes to the ability of a microorganism to be a “pathogen” only on a subset of hosts.

How Stable Are MAMPs?

In addition to defense-suppressing capacities (see below), pathogens have evolved other defense-minimizing strategies. Some pathogens carry versions of a MAMP that are not detected by their host. It is logical that the best-characterized flagellin perception systems of plants (FLS2) and animals (TLR5) both recognize flagellin domains that are highly conserved. These domains of flagellin protein are constrained by requirements for precise intra- and intermolecular contacts to form the functional flagellin polymers that compose the bulk of a flagellum (e.g., 9). Yet a small number of pathogen species have been identified that carry sufficiently different amino acid sequences in these flagellin domains to escape detection by the host (9, 56, 137, 164). Variability in the conserved/recognized flagellin domain has even been detected among different strains within a single species and pathovar, *Xanthomonas campestris* pv. *campestris* (164). This type of variability is often observed among *Acr* genes rather than MAMPs. MAMPs are generally

portrayed as broadly conserved and essential proteins that are stable targets for recognition by host immune systems, and this concept remains valid. But the adaptability of natural organisms furnishes exceptions to most rules, and the MAMP definition requires an equally adaptable mindset.

As additional defense-avoiding strategies, some microorganisms may shed or mask at least some of their MAMPs when they infect a host. For example, it has been suggested that some bacteria shed their flagella upon entering the host, and it has been demonstrated that some flagellin-knock-out bacteria can retain virulence if introduced directly onto the host (50, 121, 137, 168). Exopolysaccharides contribute to pathogen virulence or to the infectivity of plant symbionts such as nitrogen-fixing rhizobium bacteria (60, 63). Proposed molecular roles for exopolysaccharides include not only action as low MW signaling molecules or as protectants against antimicrobial compounds or osmotic stress, but also the masking of bacterial epitopes that might otherwise trigger host defense reactions. Bacterial lipopolysaccharides are MAMPs, with somewhat variable structures and hence variable defense-eliciting activities, but are also possible protectants that enhance virulence by excluding plant antimicrobial compounds (49).

What Do MAMP Receptors Look Like?

There are very few answers to this question, but a trend has been established. FLS2 and EFR1, the *Arabidopsis* receptors for bacterial flagellin and EF-Tu, respectively, both encode transmembrane proteins with an extracellular LRR and an intracellular protein kinase (62, 197). **Figure 2** provides more detail regarding the structure of LRR domains. The first LRR-kinase found to be involved in plant defense was an *R* gene product, Xa21 of rice, and other plant *R* proteins also have this structure (159). In the appropriate experimental context FLS2 itself functions in ways that resemble an *R* pro-

tein (41, 198), making it reasonable to anticipate that further overlap between *R* proteins and MAMP receptors will be uncovered in the future. A separate LRR receptor protein has been identified that detects ethylene-inducing xylanase (144). Importantly, the extracellular portion of FLS2 was recently shown to directly bind a peptide that matches the eliciting flagellin sequence (30). This direct binding is in keeping with past predictions but it is a departure from the “guard” mechanisms described at greater length below, in which some *R* proteins do not directly bind pathogen effectors, but rather detect them indirectly via their perturbation of host proteins. FLS2 and EFR1 respond to entirely different MAMPs yet both activate very similar plant defense responses (197). The defenses downstream of FLS2 have been studied in detail, revealing a number of interesting features [e.g., (95, 117, 119, 125, 126, 140, 153, 163)].

Beyond MAMP detection, plants carry systems that allow detection of wounding or herbivory, and it is intriguing that the receptors for at least two of these signals are also transmembrane LRR-kinases. It is also intriguing that the eliciting signals for these receptors are plant-derived compounds. Systemins are peptides produced from a prosystemin protein upon herbivore attack, and the systemin receptor of Solanaceae is an LRR-kinase (150). In the other example, the *Arabidopsis* PROPEP1 gene is inducible by wounding, methyl jasmonate, or ethylene, and a peptide derived from this protein directly binds to and activates the PEPR1 LRR-kinase, which in turn activates defense-associated gene expression (194). Constitutive activation of this system caused elevated resistance to *Pythium irregulare*, a type of broad host-range fungal pathogen for which effective plant *R* genes are not known. The PEPR1 system may act to minimize opportunistic infection of wounded tissues by amplifying innate immune responses.

The similarity of MAMP receptors between plants and animals has frequently been

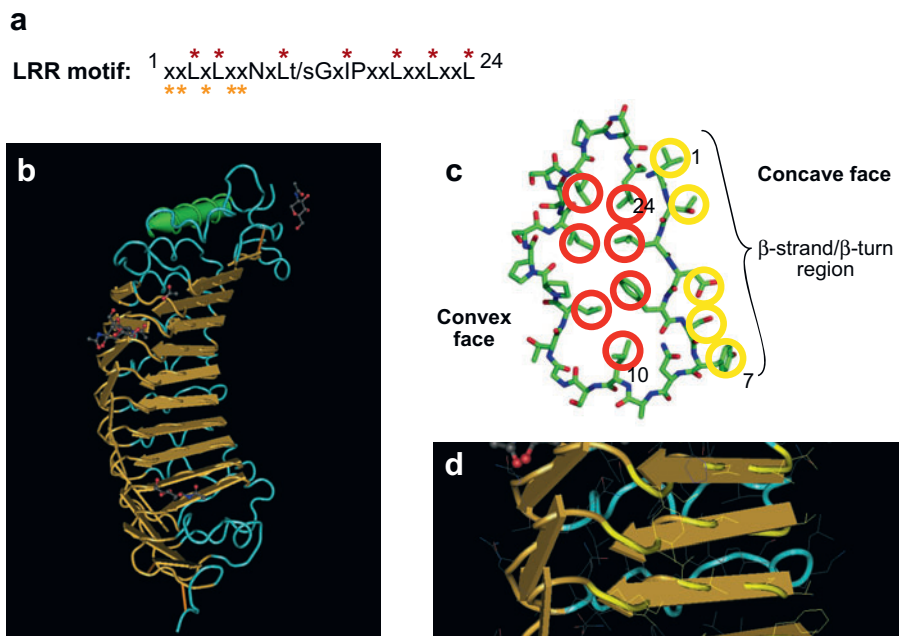


Figure 2

Leucine-rich repeat (LRR) structure. (a) Consensus amino-acid motif for a plant extracellular LRR. (b) An LRR protein; polygalacturonase inhibiting protein (1OGQ) of *Phaseolus vulgaris*. A typical LRR domain carries 21–25 amino acids per repeat and forms a large helix of multiple such repeats. The entire LRR domain is curved and the concave surface carries a β -sheet (β -strand/ β -turn region, yellow and gold highlighting). (c) A single LRR (transverse section through the structure in b). The leucines and other hydrophobic residues that occur at regular intervals are driven to the protein interior in an aqueous environment (red highlight in c; asterisks in a), leaving the more variable “x” residues exposed on the protein surface. (d) Close-up of the β -strand/ β -turn region, where 5 solvent exposed residues per repeat (yellow highlighted in a, c, and d) are primary candidates for determining pathogen specificity. Figures redrawn from (45, 46). Other LRR types have slightly different structures; many R protein LRR domains carry degenerate (nonconsensus) subsegments that will adopt other 3D shapes.

noted. FLS2 and human TLR5, for example, are both receptors with extracellular LRRs that perceive flagellin and activate innate immune responses (12). However, the two proteins recognize different flagellin domains and the LRRs do not exhibit common derivation. Although the two proteins arose independently, these and other mechanistic parallels between the immune systems of plants and animals remain a fascinating area for further research (12). For example, some plant R gene products carry domains with similarity to the intracellular domains of Toll and human interleukin receptors.

An intriguing aspect of some MAMP receptors is their multifunctionality. The sys-

temin receptor of tomato has the identical amino acid sequence as the *Arabidopsis* BRI1 receptor for brassinolide hormone. In *Drosophila*, the Toll receptor controls embryonic development and then later controls innate immunity responses in the same animal (134). *Arabidopsis* ERECTA is a transmembrane LRR-kinase that controls developmental traits such as inflorescence configuration but also mediates resistance against *Ralstonia solanacearum* (vascular wilt bacteria) and *Plectosphaerella cucumerina* (necrotrophic fungi) (61, 107). It is not yet clear how this multifunctionality arises. Some transmembrane LRR-kinases are known to homodimerize but also to require heteromeric

“coreceptor” proteins for function (e.g., 16, 91, 189). Multifunctionality may indicate that the protein is a coreceptor and not the primary ligand receptor. Alternatively, it may derive from a capacity to directly bind more than one type of ligand with high specificity, possibly depending on the coreceptors present in the complex. LRRs produce a broad interaction surface that is well suited to interact with multiple ligands. Multifunctionality also may derive from the downstream signaling components that are available to the receptor-kinase in different cell types, subcellular locations, or developmental stages.

The completely sequenced *Arabidopsis*, rice, and poplar genomes show that individual plants carry a few hundred different LRR-kinase proteins, an additional few hundred predicted transmembrane kinases with non-LRR extracellular domains, and a further set of predicted extracellular LRR proteins with a transmembrane C terminus and little or no cytoplasmic domain (58, 157, 178). Some of these proteins participate in plant development and other processes but many of these receptors may recognize MAMPs or effectors, either exclusively or as one of their multiple ligands. Thus, they remain a likely target group from which to identify additional MAMP-receptors and R proteins.

The paradigm noted above for systemin, PROPEP1, and some *R* genes—that pathogen action on the host can cause release of host-derived compounds that have defense-inducing activity—was demonstrated over 30 years ago by Albersheim and colleagues (4). Many pathogens secrete plant cell wall-degrading enzymes such as xylanases, pectate lyases, and polygalacturonases. Oligogalacturonides of intermediate chain lengths (i.e., 5–15 hexose units), derived from plant cell wall polysaccharides, were shown in many studies to induce defense-associated responses (e.g., 40, 68). In a striking example that again involves plant LRR proteins, it has more recently been shown that pea plants secrete a polygalacturonase-inhibiting protein (PGIP)

that is essentially a large LRR with specificity for certain fungal polygalacturonases (45). This protein enhances plant disease resistance, and its effectiveness may be due only in part to limitation of polygalacturonase degradation of host cell walls. By delaying rather than entirely blocking the activity of pathogen polygalacturonases, PGIP may also cause more defense-eliciting oligogalacturonide intermediates and fewer monosaccharides to be present. PGIP is the first plant LRR protein for which a crystal structure was established (46).

What Should We Call the Defense-Eliciting Molecular Patterns that are Produced from Host Compounds Rather than from Pathogen-Derived Compounds?

The proposal has been made to call these compounds MIMPs (microbe-induced molecular patterns) (112). MIMPs compose a very significant class of elicitors, as is described below when indirect recognition of Avr/virulence effector proteins is discussed. In the case of wound/herbivory-induced molecular patterns such as those perceived by PEPR1 receptors, the accurate acronym would be WHIMPs (112). There are very important evolutionary implications to the distinction between MIMPs (or WHIMPs) as opposed to MAMPs and those Avr proteins that are directly bound by host receptors. Directly bound compounds might escape detection due to small changes in structure, thereby conferring advantage to the pathogen. But a pathogen will be detected regardless of the structure of the molecules it produces as long as it causes a host compound to be altered into a detectable MIMP or WHIMP (183). Furthermore, many different species of defense-suppressing pathogens and herbivores might cause production of the same MIMP or WHIMP, making detection of this compound a broadly effective element of the plant immune system. We return to these subjects at multiple junctures below.

HOW DOES MAMP BIOLOGY RELATE TO PRACTICAL DISEASE CONTROL?

Many farmers can name useful *R* genes but few have heard of PAMP receptors or MAMP receptors. Are those priorities misplaced? Any answer will relate in part to the following question: **How many different MAMPs does a single host typically detect from a single potential pathogen, and to what extent can an effective basal immune response be activated by a single type of MAMP as opposed to an array of MAMPs?** The growing assumption is that there are multiple MAMP receptors that each make additive contributions to the overall disease resistance of a plant, as one aspect of quantitative and multigenically controlled basal resistance. Many other types of traits also contribute quantitatively to resistance (such as leaf canopy architecture and proclivity to retain leaf surface moisture) (128).

Which MAMPs and MAMP Receptors Make Significant Contributions to Resistance?

Forward genetic screens are needed to direct attention toward phenotypically significant molecular mechanisms, and should help to identify MAMP receptors (or other traits) that make the most significant contributions to quantitative resistance. The answer will, in many cases, be pathosystem specific, suggesting that at least some MAMP research should focus on major diseases of major crops. Resistance can be dissected by QTL analysis to identify the candidate genes at primary contributing loci, but this only identifies polymorphic traits. Mutational studies may be needed to identify nonpolymorphic traits that are significant contributors to resistance. As an alternative, expression of genes that encode LRR-kinases might be knocked-down by reverse-genetic methods, followed by testing with pathogens or elicitors.

Regardless of their present contribution to resistance, transgenic introduction of novel MAMP receptors may in the future allow researchers to engineer plants with enhanced disease resistance. Native receptors might be “souped up” to recognize other MAMPs or to enhance the defenses they activate. MAMP receptors might be moved across species to expand the basal immune system of the recipient. MIMPs or WHIMPs may also serve as targets for engineering. For example, orthologs of the *Arabidopsis* WHIMP precursor PROPEP1 are already present in many agriculturally important plant families, and this type of compound may offer substantial opportunities for crop improvement (194). There are also many MAMP-independent approaches to the enhancement of plant disease resistance, but MAMPs and MAMP receptors deserve consideration. MAMPs that are relatively constant (immutable) are particularly attractive targets for detection by immune systems. However, any efforts to improve upon the MAMP detection systems of plants must account for a key observation: MAMP-activated defenses are frequently blocked by pathogens.

PATHOGEN EFFECTORS HAVE DUAL FUNCTIONS IN VIRULENCE AND AVIRULENCE

Defense-suppressing effectors appear in part two of the four-part model described above and in **Figure 1**. Effectors, such as toxins and effector proteins, can be defined as pathogen-derived molecules intended to promote pathogen virulence by interacting with the host. In other words, effectors are virulence factors that usually do not have a “house-keeping” function in microbial growth and development outside of the host. Of course, when an effector is recognized by a host defense receptor, the intended virulence function is often overshadowed by a dominant avirulence function. In this section, we focus on the impressive progress in deciphering effector biology.

Why Are *avr* Genes Maintained by Pathogens?

Many effectors were first identified on the basis of their avirulence activity. These were appropriately called *Avr* genes since their *R* gene-mediated activity induces defenses that prevent virulence (108). However, it was widely assumed that they must contribute in some way to pathogen fitness, for example, by contributing to virulence on a susceptible host. Earlier views of this and related subjects can be very stimulating to read [see (52, 57, 93, 187)]. Today, the virulence role of many effectors is well established.

Effector genes were first isolated as avirulence genes, by screening bacterial genomic libraries for genes that convert virulent bacteria to avirulence (162; isogenic pairs of virulent and avirulent pathogen strains remain a powerful tool for many types of studies). A powerful clue to effector biology emerged when the first *R* genes were cloned and found to encode cytoplasmically localized proteins (discussed below). This raised the question: **How do intracellular R proteins perceive the presence of extracellular pathogens?** The identification of bacterial *hrp* mutants helped provide answers (6, 104). The hypersensitive response (HR) is a robust defense response frequently associated with *R* gene-mediated resistance, and includes the death of plant cells local to the site of infection (75). The *hrp* mutations disrupted the ability of phytopathogenic bacteria to cause the HR on resistant hosts and pathogenesis on susceptible hosts, providing evidence that the avirulence and virulence activities of effectors are fundamentally related. These findings paralleled work on viral *avr* genes demonstrating that they also provide functions essential for virulence (72, 149).

Two major breakthroughs led to an appreciation that bacterial effectors are active inside the cells of the host: effector proteins expressed directly inside host cells (e.g., via transiently or stably delivered DNA) frequently possessed avirulence activity similar to that observed when they are expressed by

the pathogen (6, 64), and some of the proteins encoded by *hrp* genes form a pilus capable of secreting bacterially encoded proteins into the extracellular milieu (6, 85, 99). The *hrp*-pilus is now called the type-three secretion system (TTSS), and is known to be central to the virulence of numerous bacterial pathogens of plants and animals. Together, these results led to the hypothesis and subsequent confirmation that type III effector proteins, as they are now called, can be delivered via the TTSS from the bacteria into the cytosol of plant cells where they contribute to virulence (6, 28).

How Many Effectors Does a Pathogen Have?

Individual pathogens deliver dozens of effectors into cells of the host. Bacteria deliver many type III effectors beyond those known to be Avr proteins, and newly identified type III effectors currently are called *Hop* genes (for *hrp* outer proteins; note that some of these may have Avr-activity in some hosts) (6). The number of type III effectors that bacteria introduce into hosts is large; individual strains express anywhere from 20 to nearly 100 (38, 103). Recent work has demonstrated that oomycetes also secrete effectors into both the extracellular space and the inside of host cells (89). Based on a motif that predicts which oomycete proteins will be secreted, the oomycetes are likely to secrete many more effectors than do bacteria (89, 179). Many of the effectors that are secreted into the extracellular space by oomycete pathogens carry an RXLR amino acid motif that targets the proteins for endocytosis by host cells, as is also seen in effectors from malaria parasites of humans (21, 89). Thus, pathogens can deliver a potpourri of effectors into cells of the host, by a variety of mechanisms.

How Do R Proteins Recognize the Presence of Effectors?

This is one of the most prominent questions in plant pathology research. As was discussed

HR: hypersensitive response

TTSS: type-three secretion system

above, MAMPs can be recognized by direct interaction with a defense receptor. Similarly, it was widely hypothesized that cytosolic R proteins would serve as receptors that directly interact with intracellular effectors. Indeed, this appears to be true in a number of cases [(43, 48, 84, 152, 167); see below]. However, in many cases, direct interaction between effector and R protein does not explain effector detection. An alternative model came with formulation of the “guard hypothesis,” which postulated that R proteins recognize effectors indirectly (183). It was proposed that effectors target host proteins other than R proteins, and that perturbation of those host targets is the trigger that leads to R protein activation. The *P. syringae* effector protein AvrPphB provides a straightforward example: This protease cleaves a host protein kinase, and the cognate R protein (RPS5) detects such cleavage (3, 154). Thus, these types of R proteins “guard” the targets of effectors and induce defense responses when those targets are perturbed. Numerous effectors are now known to be recognized indirectly [e.g., (13, 110, 111, 145, 154)]. So perception of pathogen effectors by R proteins occurs in one of two ways: either directly, analogous to recognition of MAMPs by MAMP-receptors, or indirectly via their perturbation of “guarded” host targets. The two types of recognition have important ramifications with respect to the durability of resistance conferred by a particular R gene.

How Do Effectors Avoid Recognition by R Proteins?

The evolution of effectors is influenced by how they are perceived by R proteins. An effector contributes to virulence only if recognition by R proteins is avoided. Mutation to avoid recognition is a viable option for effectors that are directly recognized. Changes in effector protein sequence can potentially disrupt the physical interaction with an R protein. A key question is whether those changes are compatible with the virulence activity of

the effector. If the effector can maintain its activity in the context of such mutations, it will escape recognition while maintaining virulence function. There is interesting evidence concerning proteins from virus, fungus, and oomycete plant pathogens that apparently have evolved to escape host detection [e.g., (7, 10, 37, 47, 72, 106)]. However, for effectors that are recognized indirectly it may be much more rare to evolve forms that escape recognition while maintaining virulence activity. The effector would generally have to stop perturbing the host target to avoid detection, but the virulence contribution of such effectors will usually be dependent on perturbing the host target. Exceptions may arise [see for example (32, 101)]. The effector may attack more than one different host target and avoid detection by ceasing to attack the guarded host target while continuing to impact other targets. But a trend seems to be emerging that directly recognized effectors often undergo diversification while indirectly recognized effectors are either present or deleted [(112); see discussion below].

PATHOGEN EFFECTORS PROMOTE VIRULENCE BY SUPPRESSING HOST DEFENSES

How Do Defense-Suppressing Effectors Enhance Pathogen Virulence?

Recent work shows that effectors have highly adapted virulence functions. They perturb specific host targets in order to disrupt specific host processes—often host defenses. Thus the main question can be rephrased as two questions: **What defense processes are suppressed by effectors? What host targets are perturbed by effectors?** Understanding the host targets is key to understanding, mechanistically, how the effectors function. The most information to date about the virulence activity of pathogen-encoded effectors has come from studies of type III effectors from bacterial pathogens.

Inhibition of MAMP-activated signaling is critical for bacterial virulence. For a long time the role of MAMPs in resistance was unclear. Unlike classical R proteins, postulated MAMP receptors had not been demonstrated to have strong roles in resistance. However, it is now apparent that at least one MAMP receptor, FLS2, can make significant contributions to resistance (41, 71, 74, 95, 164, 197, 198). Many of the cases in which MAMP perception makes an obvious contribution to resistance are nonhost interactions (41, 71, 74) or interaction of a TTSS-mutant with a host plant (73, 95). Thus, bacteria that express non-adapted effectors or are unable to deliver effectors lack pathogenicity, at least in part, because they fail to inhibit MAMP-signaling. Indeed, numerous effectors have now been directly demonstrated to suppress MAMP-induced defense responses.

MAMPs, either purified or presented by TTSS-deficient bacteria, induce numerous defense readouts that reflect the diversity of defense responses induced by MAMP receptors. One way of viewing the response induced by MAMP-signaling is as a global transcriptional response (126, 169). Both individual gene transcription and global changes have been used to demonstrate suppression of MAMP-signaling by numerous bacterial type III effectors. Hauck et al. used transcriptome analysis to show that AvrPto suppresses a broad spectrum of transcription induced by TTSS-deficient bacteria (73). He et al. demonstrated that AvrPto and AvrPtoB act upstream of MAP kinase signaling to suppress transcription of a few transcripts induced by flagellin (74). Li et al. showed that a large number of effectors are able to inhibit flagellin-induced transcription of the *NHO1* transcript (100).

A landmark 1995 paper (23) anticipated the widely documented fact that MAMPs induce cell wall-based responses that can be inhibited by numerous bacterial type III effectors (41, 42, 71, 73, 94, 95, 160). Papillae (also known as cell wall appositions) are localized cell wall thickenings induced by bacteria and a variety

of other pathogens. Callose has been widely used as a marker for papillae. Numerous effectors suppress MAMP-induced callose deposition. Key to the production of papillae is polarized vesicle traffic that delivers cell-wall reinforcing and antimicrobial components to the site of interaction with the pathogen (141). The example of HopM1 shows how a bacterial effector can inhibit pathogen-induced callose deposition, in this case by inactivating a host small G protein that positively regulates vesicle traffic (129). HopM1 induces proteasome-dependent degradation of multiple *Arabidopsis* proteins, including a G protein activating guanine exchange factor (GEF). Notably, knock-out of the gene encoding this GEF phenocopies the contribution of HopM1 to bacterial virulence, indicating that this protein and presumably the vesicle traffic that it helps to regulate are an important virulence target for the bacteria (129). Vesicle traffic and cell wall-based defenses are also critical for resistance of plants against nonhost powdery mildew fungi (33, 105). By preventing penetration of the fungus into the interior of the leaf, these defenses prevent further development of the infection. The role of MAMPs in penetration-based resistance is unknown. However, because mutations that block penetration-resistance can cause powdery mildew susceptibility in non-host plants, it is reasonable to speculate that host-adapted powdery mildews encode effectors that inhibit cell wall-based defenses in that pathogen's host plant.

Basal resistance can involve reduced vascular flow into minor veins of leaves, and this process also can be inhibited by defense-suppressing effectors (132).

Plant invasion is a crucial stage of pathogenesis for which multiple effector biology stories are likely to emerge. The role of MAMP detection in stomatal closure that reduces entry of *P. syringae* bacteria was noted in a previous section (117). *P. syringae* is proposed to use the phytotoxin coronatine (a jasmonic acid mimic) to overcome this defense by suppressing the salicylic acid and abscisic acid

GEF: guanine exchange factor

EPI: extracellular protease inhibitor

signaling required to keep the stomata closed. Many eukaryotic microorganisms enter the plant by hyphal growth through stomata. **Are there MAMP-associated defenses that reduce stomatal entry of eukaryotic pathogens? Are there MAMP detection systems that work especially well in other entry routes?** This might include the plant vasculature, or root or shoot epidermal cells after the cuticle has been breached. Animal innate immunity must be down-regulated at topologically exterior surfaces including the lung and gut epithelia (90). **Do plants express reduced MAMP-sensitivity in their epidermal cells?**

Eukaryotic microorganisms produce large suites of effectors that are only beginning to be understood, and which represent another area very ripe for future study (89). Different trends are emerging from study of these pathogens; for example, some effectors block the delivery of antimicrobial enzymes or compounds by preventing their secretion. Additionally, a number of fungal and oomycete effectors can disarm these molecules after they are secreted. It has long been appreciated that plants secrete antimicrobial enzymes such as glucanases and chitinases that degrade the cell walls of invading fungi (108). Degradation of pathogen cell walls can directly inhibit pathogen growth and/or release defense-eliciting compounds from the pathogen. But pathogens secrete molecules capable of inhibiting antimicrobial compounds (5). In a new and more explicit example, it was found that *C. fulvum* Avr4 inhibits tomato chitinases (181). As is often the case with bacterial effectors, Avr4 was originally known as an avirulence gene product. Oomycete pathogens such as *Phytophthora infestans* are now known to carry a wide suite of secreted extracellular protease inhibitors (EPIs) that inhibit plant-encoded apoplastic proteases (172–175). Thus, as opposed to suppressing defense response activation, these virulence effectors deactivate components of a deployed defense response.

The HR is a robust defense response that is associated with resistance to a variety of biotrophic and hemibiotrophic pathogens. Numerous type III effectors have been shown to inhibit HR cell death. Some of these are generalized inhibitors of PCD whose effects are observed in yeast cells (2, 80). One such effector, AvrPtoB, mimics a host E3 ubiquitin ligase (1, 82). Other type III effectors inhibit the HR induced by a specific R protein. For example, AvrPphC prevents an HR induced by AvrPphF in certain bean cultivars (79). Similarly, AvrRpt2 specifically blocks the HR induced by the R protein RPM1 in *Arabidopsis* (139). This function of AvrRpt2 results from its degradation of RIN4, which is required for accumulation and function of RPM1 (13, 95, 110). Thus type III effectors inhibit the HR by a variety of strategies, both specific and general.

Pathogen host range is now believed to be strongly influenced by the possession of appropriate effectors that are adapted to suppression of defense in particular hosts. The host range of a pathogen could be enhanced by carrying many effectors with quantitatively overlapping activities, because only a subset of effectors may function in a given host. Yet R gene-mediated detection of effectors represents a flip side to this strategy, as even a single effector that is detected by many potential host plants may significantly limit a pathogen's host range (190).

FUTURE CONSIDERATIONS REGARDING DEFENSE SUPPRESSING EFFECTORS

We now know that defense-suppressing effectors target a multitude of host processes. Further studies of defense suppression will likely reveal more mechanisms of effector function, and significantly, will reveal the host processes that they target.

Space constraints allow only brief comment on a number of other outstanding questions for future research:

- **What are the roles of other effectors, in bacteria and in other pathogens?** Loss of single effectors can cause subtle effects; disruption of TTSS dramatically reduces virulence. **In what way are all of the other effectors contributing to pathogenesis, and what host processes are they targeting?**
- **How many host processes need to be inhibited?** An effector that blocks defense activation may be more effective than many effectors that block individual defense responses.
- **Are multiple effectors frequently needed to inhibit single host processes?** Effectors that act strongly when artificially overexpressed prior to infection may not be sufficient in normal infections because of lower expression or delayed delivery. Pathogens may need to deliver effectors that each partially suppress the same pathway (e.g., 74).
- **Will effector targets correspond with quantitative trait loci that influence disease resistance?** As mentioned previously, polymorphism is needed before differences in impact can be detected.
- **Are important broad host-range necrotrophic pathogens such as *Rhizoctonia* or *Botrytis* successful because they carry a few particularly effective effectors, and/or an unusually broad suite of effectors?**
- **How do defense-suppressing effectors contribute to the virulence of pathogens with different lifestyles, such as necrotrophic bacteria or rust fungi?** Commonalities exist in the host defense-signaling responses to distinct pathogens. **Do effectors from distinct pathogens suppress the same host pathways?**
- **Do effectors from one type of pathogen frequently suppress defenses induced by other types of pathogens, and allow disease causation by microorganisms that would**

not on their own cause disease? Such phenomena have been noted in the synergy of, for example, Potato virus X and Potato virus Y, which is due largely to suppression of gene silencing by one of the pathogens (8). Other examples may emerge with important implications for the ecology of mixed infections, including opportunistic secondary infections.

R PROTEINS

Although *R* genes and their products do not appear until part three of the four-part model outlined above, they have long been prominent in the minds of the plant breeders and farmers who rely heavily on *R* genes for practical disease control. The initial cloning and molecular characterization of *Avr* genes and then *R* genes (19) were major landmarks for the same reason that study of *R* genes remains highly relevant: These genes do an amazing job of stopping plant diseases.

The shortcomings of *R* genes are also well known (19, 88). They are not available for all diseases, especially for necrotrophic pathogens (**why no *R* genes against necrotrophs?**). More prominently, *R* genes are famous for working but then failing as pathogen populations evolve (see **Figure 1**, part 4) (88, 115). Yet some *R* genes remain effective for decades, and for numerous plant diseases their utility is unquestioned. Study of *R* genes should yield knowledge with many practical applications, and the past few years have yielded important discoveries.

Earlier findings about *R* genes have been widely reviewed (19, 39, 53, 69, 88). The vast majority of *R* genes encode proteins that carry a leucine-rich repeat (LRR) domain, either as part of intracellular NB-LRR proteins that also carry a nucleotide binding (NB) site and other conserved domains, as an extracellular LRR in transmembrane receptor-kinase proteins, or in “receptor-like proteins” that have an extracellular LRR and a transmembrane domain but then very little at the intracellular C terminus of the protein. **Figure 2**

describes the appearance of an LRR. LRR domains are also found in many other proteins in the biological kingdom, and often control ligand binding or protein-protein interaction (17, 96). One particularly interesting example of LRR function comes in the adaptive immune system of jawless vertebrates such as eels, which lack T cell receptors and other adaptive immunity elements typical of more recent vertebrates. The jawless vertebrates instead shuffle a small number of progenitor LRR-encoding genes to generate a wide array of immune specificities (133). This drives home the idea that LRRs are a highly adaptable structural platform on which very different binding specificities can evolve.

Studies of a number of plant *R* gene families have shown that LRR domains can be under diversifying selection (53, 88). In most other proteins the key domains are conserved across taxa, presumably due to natural selection to maintain function. But in some *R* gene families the predicted solvent-exposed residues along the concave face of the LRR (the β -strand/ β -turn region; see **Figure 2**) not only lack conservation, but are significantly more diverse than expected from random drift. This suggests selective pressure to adopt new function in this part of these *R* proteins. The prediction has been that the diversifying sites encode the pathogen-specificity domains of *R* proteins, and that their evolution permits recognition of different pathogen Avr proteins. But, at what site do *R* proteins recognize pathogens? This important question is taken up below, after a few other aspects of disease resistance biology are introduced.

Do *R* Genes Really Act by Themselves, While Horizontal Resistance Is Multigenically Controlled?

Most of the genes required for an *R* gene's phenotype are not detectably polymorphic within a plant species, resulting in observable segregation only of single *R* loci that control a particular gene-for-gene resistance

trait. It is now clear that many genes contribute to *R* gene phenotypes. These genes may not be polymorphic because they contribute to the phenotype of many *R* genes, or are otherwise essential for plant fitness. Other genes that contribute to an *R* gene's phenotype may be difficult to detect because they make small contributions to overall disease resistance, or because the plant carries genes with sufficient functional overlap to mask polymorphism at any single locus. PR protein or phytoalexin biosynthesis genes may represent this class. This review does not cover our growing knowledge of genes that function "downstream" of *R* genes, but many are now known (31, 55, 70, 88, 185). These genes are often discovered by mutational analysis, by their induced expression during infections, or due to physical interaction of the protein product with a protein known to be involved in defense. As one nice example, a set of E3 ubiquitin ligases was recently discovered by expression profiling and subsequent phenotypic testing of RNAi-silenced plants (195). The proteins were shown to make significant contributions to *R* gene-mediated defense in Solanaceae and Brassicaceae, through a presently unknown mechanism.

Turning to horizontal resistance [resistance that is apparently not race specific; (184)], a few points deserve brief mention. First, the stereotypical horizontal resistance that is only partially effective is in many cases controlled by multiple polymorphic loci (minor genes) that each make small contributions, as anticipated by Vanderplank and others. But in some instances, horizontal resistance can be controlled by a single gene of major effect, such as barley *mlo* (27). Second, the basal MAMP-based resistance systems discussed at length above are likely contributors to nonrace-specific resistance, but this has never been demonstrated for an agricultural crop. Third, partial/quantitative resistance is not "useless" or "too hard to work with;" it has undergone significant improvement by plant breeders through large multiyear phenotype-based selection programs,

and plays a significant economic role in disease control (128, 148, 158, 184). For some diseases it is sought because *R* genes are not available, but for others it is used because *R* genes have proven to be unreliable (non-durable) and are overtly being avoided (128, 148, 158, 184). *R* genes are practically important, but horizontal resistance traits are also important.

R PROTEINS DETECT *Avr* PROTEINS: DIRECT OR INDIRECT INTERACTION?

It is significant that many *R* genes are polymorphic—entirely absent in some accessions, or readily mutable to evolve new pathogen specificities. They represent a flexible component of the plant immune system. Dodds, Ellis, Lawrence, and colleagues have put together a very important body of work on this topic, working with the flax/flax-rust pathosystem that was also used by Flor (47, 48, 53, 54, 57). The *L* genes, for example, are an *R* gene family from flax that occurs as a tightly linked multigenic cluster of related NB-LRR-encoding genes. They evolve at a faster rate than most genes, with polymorphism generated not only by single base mutations or small insertion/deletions but also by intragenic recombination with equal exchange, by intragenic recombination with unequal exchange to make longer/shorter LRRs in the resulting products, and/or by equal or unequal extragenic recombination to generate more/fewer *R* genes at the locus (39, 53). The flax rust *AvrL567* gene family that allows some of these *R* genes to confer resistance is also highly diversified (47). The evidence suggests a gene-for-gene arms race in which *Avr* alleles emerge that escape host detection yet retain virulence function, with new plant specificities also emerging that detect these variant *Avr* proteins.

A recent landmark paper used yeast two-hybrid assays to show direct interaction between the *Avr* and *R* proteins, with specificity for physical interaction matching the *R/Avr*

resistance specificity observed in the plant-pathogen interactions (48). A similar coevolutionary story, with substantial divergence in both the *R* and *Avr* gene families, has been described for the Rpp13/Atr13 interaction involving *Arabidopsis* and *Hyaloperonospora* (7, 146). Separately, direct interaction between NB-LRR protein and *Avr* protein has been shown in other examples (43, 84). Given that many other *R* genes or *R* gene analogs exist in linked clusters that exhibit substantial allelic divergence across accessions within the species, additional examples of direct interaction between *R* protein and *Avr* protein seem likely to emerge.

R gene diversification is only one part of the story; other *R* genes appear to be under conservative rather than divergent selection (88, 98). As was mentioned above in the section on effectors, some *R* proteins respond to the perturbation of a host protein rather than detecting pathogen *Avr* proteins by direct binding. In most cases known to date, the *R* proteins that act via an indirect “guard” mechanism turn out to be conserved, nondiverging *R* proteins. The *Arabidopsis* RPS5 example was noted above: This NB-LRR *R* protein activates defense if the *Arabidopsis* protein kinase PBS1 is proteolytically cleaved (154). The corresponding *P. syringae* *Avr* gene, *avrPphB*, encodes a protease that is delivered into host cells via type III secretion, and *AvrPphB* specifically cleaves PBS1 (154). In the presence of RPS5 a slight shift in *AvrPphB* structure would not be sufficient to escape detection; the pathogen must instead cease proteolytic cleavage of the guarded host protein PBS1 if it is to escape detection by RPS5. RPS5, which occurs as a single-copy gene in *Arabidopsis*, shows minimal allelic variability (171). It makes sense that such an *R* protein would be conserved: PBS1 structure is unlikely to evolve at an accelerated rate and, accordingly, the *R* protein, RPS5, should be under selection for conservation rather than divergence.

The above represents a major new piece of the plant pathology dogma, but the dogma

can already be questioned. **Is it accurate to stereotype plant R genes as falling into one of two classes: those that evolve more rapidly and directly bind pathogen Avr proteins, and those that evolve very slowly and detect Avr perturbation of host proteins?** Current findings suggest the validity of this concept, but work in additional pathosystems is needed to fully substantiate these categories. It can be predicted that there will be cases of direct R/Avr interaction that have been quite stable over time (for example, if the recognized Avr sites are not readily alterable without significant loss of virulence function), and of indirect detection systems that nevertheless have diversified substantially [as has been suggested for the Cf loci of tomato; (145)]. In their landmark paper, Dodds and colleagues speculate that R gene defenses against biotrophic pathogens may have a greater tendency to be forced toward diversification, as the pathogens have been purged of effectors that attack the host in ways that can be recognized by “guard” R proteins (48).

FUNCTIONAL MATTERS: HOW DO NB-LRR PROTEINS CARRY OUT THEIR TASKS?

NB-LRR proteins are the most common type of R protein. We have only partial knowledge of how these proteins convert pathogen

recognition into defense activation, but significant insights have recently been achieved. One of the defining domains of these proteins is the nucleotide binding site, which must be functional for NB-LRR proteins to confer disease resistance. Takken and colleagues have now shown that the I-2 NB-LRR protein of tomato binds and hydrolyzes ATP (166). Mutant forms of I-2 were generated that are impaired in ATP hydrolysis but not in ATP binding, and their function suggests that the ATP- rather than the ADP-bound state of I-2 is the active form that triggers defense signaling (166). The similar findings made with *Arabidopsis* RPS5 (3) and with certain animal proteins (176) suggest that this is a general mechanism for NB-LRR proteins. Upon ADP binding the I-2 protein displayed an increased affinity for ADP, suggestive of a change of conformation. In light of these and related findings, Takken and colleagues suggest a functional model in which the LRRs control the molecular state of the NB-ARC domain (165). Pathogen recognition triggers nucleotide-dependent conformational changes that might induce oligomerization or otherwise provide a scaffold for activation of downstream signaling components. Parts of this model are shown in **Figure 3**.

Defining domains of NB-LRR proteins include not only the nucleotide binding

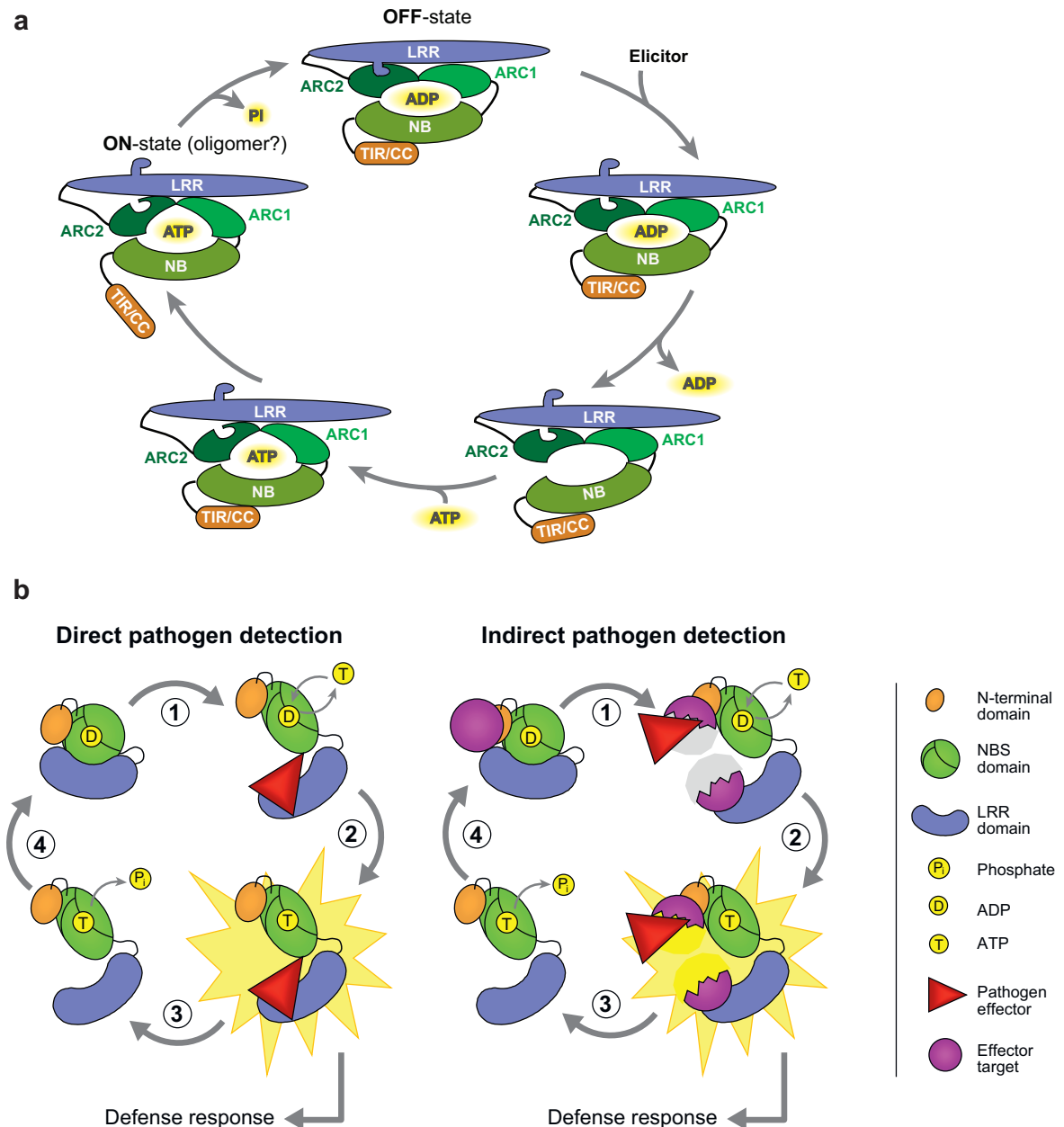
Figure 3

Two related models for NB-LRR R protein activation. (a) Switch function of ATP/ADP binding (165). In the absence of stimulation, an NBS-LRR protein is in the OFF-state (resting state), in which the LRR exerts its negative regulatory role by stabilizing the ADP-bound state. The presence of an elicitor (effector, Avr) affects the LRR domain, which induces a conformational change in the NB-ARC domain that allows the release of ADP. ATP binding subsequently triggers a second conformational change in the N-terminal effector domain, releasing its signaling potential. The ATPase activity of the protein attenuates the signaling response and returns the protein to its resting state. Figure is courtesy of (165). (b) Signaling is activated in a similar way for both direct (*left*) and indirect (*right*) modes of pathogen detection (44). Presence of the pathogen effector (1) alters the structure of the NBS-LRR protein through direct binding (*left*) or modification of additional plant proteins (*right*), allowing exchange of ADP for ATP. Note that the eliciting protein may initially interact both with the N-terminal domain and the LRR. Binding of ATP to the NBS domain (2) results in activation of signal transduction through the creation of binding sites for downstream signaling molecules and/or the formation of NBS-LRR protein multimers. Dissociation of the pathogen effector and modified effector targets (if present) (3), along with hydrolysis of ATP (4), returns the NBS-LRR protein to its inactive state. Figure is courtesy of (44).

site and LRRs, but also some small conserved regions between these motifs that (together with the NB) have been called the NB-ARC domain (88, 182). These sites share homology and likely share mechanisms with animal proteins such as Apaf-1 and CED-4 and the more recently discov-

ered NOD/NACHT/CATERPILLAR proteins that play roles in animal inflammation, cell death, and immunity (176). It will be important for plant immunologists to monitor future research on these animal proteins.

Aspects of the models in **Figure 3** derive from an extremely influential paper in



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which Moffett and colleagues showed that domains of the potato CC-NB-LRR protein Rx physically interact, and that stimulation by the pathogen Avr protein (PVX coat protein) disrupts some of these interactions (122). They proposed a model, sometimes called the “jackknife” model, in which pathogen elicitor causes disruption of intramolecular associations, freeing the CC, NB, and/or LRR domains for interaction with other proteins (122).

Dodds and colleagues, working with flax L6, present additional evidence that the presence of a bound nucleotide is required for the NB-LRR protein to adopt a recognition-competent conformation (48). Parts of the ARC domain of these proteins likely fold together with the P-loop to form a functional nucleotide-binding site, while other portions apparently interact with LRR domains (**Figure 3a**). Rairdan & Moffett further support this model (138). Both groups also used domain swaps to localize pathogen recognition specificity to the C-terminal half of the LRR domain (48, 138). The experiments with potato Rx suggest that the ARC region, through its interaction with the LRR, translates elicitor-induced modulations of the LRR into a signal initiation event. However, it was shown that physical disruption of the LRR-ARC interaction is not required for signal initiation. The authors propose instead that this activity can lead to multiple rounds of elicitor recognition, providing a means of signal amplification (138). Ade et al. working with *Arabidopsis* RPS5, provide further evidence that the LRR-ARC interaction suppresses R protein activation and that exposure to the Avr-derived structure (cleaved PBS1) alters this interaction, stimulating ADP release and ATP uptake at the nucleotide binding site that places the protein in an active conformation for defense signaling (**Figure 3**) (3).

Some models from both plant and animal research also suggest that NB-LRR proteins act as scaffolds that oligomerize upon activation and bring physically associated signaling

proteins into proximity (78, 83, 165). Mestre & Baulcombe have shown that tobacco N (an NB-LRR protein) oligomerizes upon recognition of pathogen, and that this oligomerization requires a functional nucleotide-binding site (118). Oligomerization could still occur in loss-of-function N proteins that carry mutations in the conserved TIR or RNBS-A motifs, suggesting that oligomerization is an early event in the activation of NB-LRR proteins for defense signaling.

The above models for NB-LRR protein function do not exclude other means for modulation of the defense signaling activity of NB-LRR proteins. Peart et al. have recently discovered a CC-NB-LRR protein that functions together with N (a TIR-NB-LRR protein) to confer resistance against *Tobacco mosaic virus* (135). Meyers et al. noted that the *Arabidopsis* genome, in addition to over 130 genes encoding TIR-NB-LRR or CC-NB-LRR proteins, also carries over 50 genes for apparent “shorter” R-like proteins lacking an LRR (e.g., TIR-NB, or TIR, or CC-NB) (120). The possibility that interaction among heterologous NB-LRR-like proteins modulates defense signaling is only starting to be explored. As another theme, it was recently shown that R gene specificity toward pathogens can be mediated by differential expression of an R gene in the presence of the Avr effector. Resistant and susceptible alleles of rice Xa27 encode identical proteins, but expression of only the resistant allele is induced upon challenge with *Xanthomonas* expressing AvrXa27, a type III-secreted effector that localizes to the plant nucleus (65). Ectopic expression of Xa27 was shown to induce resistance.

At What Site Do R Proteins Recognize Pathogens? Is It the LRR of NB-LRR Proteins that Is Recognizing Guarded Host Targets?

LRR domains have been implicated in determining pathogen specificity. However, instances of specificity contributions from

N-terminal regions of NB-LRR proteins have also been reported (77, 109, 123). Tomato Prf is an NB-LRR protein required for resistance against *P. syringae* that express AvrPto or AvrPtoB, two effector proteins that target the tomato protein kinase Pto. Mucyn et al. comment: "Pto and Prf associate in a coregulatory interaction that requires Pto kinase activity and N-myristoylation for signaling. Pto interacts with a unique Prf N-terminal domain outside of the NBARC-LRR domain and resides in a high molecular weight recognition complex dependent on the presence of Prf. In this complex, both Pto and Prf contribute to specific recognition of AvrPtoB. The data suggest that the role of Pto is confined to the regulation of Prf and that the bacterial effectors have evolved to target this coregulatory molecular switch" (123). Note also that the N terminus of RPS5 interacts with the eliciting PBS1 protein, and the N terminus of tobacco N interacts with the eliciting TMV p50 helicase (3, 25). It is proposed that the LRR does play a key role in elicitor recognition, but that initial binding of elicitor to R protein may often be mediated by N-terminal domains (**Figure 3**).

How Do R Proteins with Extracellular LRRs Work?

Multiple *R* genes have been identified that encode transmembrane receptor-kinase or receptor-like proteins with an extracellular LRR (70). Some functional mechanisms of these proteins were discussed earlier in this review while describing MAMP receptors. As for NB-LRR proteins, those mechanisms are only partially understood. The paradigm of direct interaction between ligand and LRR is well established for transmembrane LRR receptors and is the likely mechanism by which *Arabidopsis* FLS2 binds flagellin (30). However, tomato Cf-2 carries an extracellular LRR but has been implicated as recognizing the corresponding pathogen Avr2 peptide indirectly, when Avr2 physically interacts with and inhibits the tomato cysteine protease Rcr3

(145). CITRX thioredoxin interacts with the short cytoplasmic tail of Cf-9, and is a putative adaptor protein that may connect Cf-9 and the ACIK1 protein kinase during the Cf-9/Avr9-induced defense response (127). FLS2 undergoes ligand-induced endocytosis when exposed to flagellin-like peptides, but other aspects of how FLS2 itself accomplishes ligand and specificity or activates downstream signaling remain to be discovered (141). Transmembrane receptor kinase proteins often autophosphorylate their homodimer partners, and Song and colleagues have shown for rice Xa21 that this autophosphorylation is involved in protein stabilization (192). They suggest that this stabilizes Xa21 against a developmentally controlled proteolytic activity that affects disease resistance (188, 192).

The proteins with which R proteins (or MAMP receptors) physically interact are logical focal points for further research. The pathogen Avr proteins that directly interact with an NB-LRR protein, or the host targets of pathogen effectors whose alteration is detected by NB-LRR proteins, are of course highly relevant NB-LRR-interacting proteins. Proteins such as Hsp90, Rar1, and Sgt1, which carry chaperone and proteasome functions, also play significant roles in enabling the function of NB-LRR proteins, both by controlling preactivation levels of R proteins and in contributing to defense signaling (76, 88). However, there is a glaring gap in our current knowledge.

What Proteins Are Directly "Downstream" of and Acted Upon by NB-LRR Proteins as a Causal Step in the Activation of Defense Responses?

For most R proteins this simply is not known, and it is an obvious area for future research. R proteins rapidly activate defense responses that include an oxidative burst, protein kinase activity, ion channel gating, and defense-associated gene expression, making these processes a logical place to expect mechanistic linkages with R proteins (131). However,

direct linkages were absent until very recent work with barley MLA10, an NB-LRR *R* gene product that acts against the powdery mildew pathogen *Blumeria graminis* (formerly *Erysiphe graminis*) when those strains express Avr_{A10}.

Well-controlled studies of MLA10, albeit with overexpressed MLA10, have now shown that this protein exists not only in the cytoplasm but also in the nucleus (156). A yeast two-hybrid screen revealed interaction of MLA10 with certain WRKY transcription factors. WRKY proteins have been known for many years to play both activating and repressing roles in the expression of defense-associated genes (180). It was observed that recognition of Avr_{A10} induces MLA10 association with specific WRKY factors that otherwise repress defense expression (156). Importantly, a mechanistic linkage with MAMP-elicited defenses is proposed (156): Defenses are elicited by pathogen-derived MAMPs, certain WRKY proteins limit the extent of MAMP-induced defenses, then Avr detection by MLA10 is thought to remove this WRKY-mediated check on MAMP-induced defenses, which are thereby expressed more strongly and rapidly, leading to highly effective defense and an HR. The question immediately arises as to how widely this mechanism applies to other NB-LRR proteins. Many effectors induce an HR when they are inducibly expressed inside plant cells without expression of other MAMPs, indicating that activated MAMP-signaling is not always required.

Separate studies have also suggested this theme of nuclear activity of NB-LRR proteins. These include the function of a fused NB-LRR-WRKY protein, the nuclear presence of the tobacco N protein, and the nuclear activity of AvrBs3-family pathogen effector proteins (25, 43, 66).

A dozen years after *R* genes were first isolated, the signaling proteins that act immediately downstream of *R* proteins are still largely unknown. Numerous forward genetic mutant screens have been conducted but these generally have not revealed the immediate down-

stream targets, possibly because of their functional redundancy and/or their lethality when mutated. Yeast two-hybrid methods have been in use for many years with some notable successes but with relatively low efficiency. Isolation of NB-LRR protein complexes, followed by MALDI-TOF or other mass spectrometric identification of interacting proteins, is presently being pursued. If NB-LRR proteins do not interact tightly or stably with their downstream targets, other approaches may be needed. Identification of direct targets of activated NB-LRR proteins or other *R* proteins is a key goal for future research on plant disease resistance.

ARE THERE OTHER TYPES OF *R* GENES? WHAT DEFINES AN *R* GENE?

The structural range of *R* gene types was expanded in a significant way with the cloning of rice *Pi-d2*, a gene that confers gene-for-gene resistance against the fungal pathogen *Magnaporthe grisea*. *Pi-d2* encodes a transmembrane receptor-kinase with a B-lectin extracellular domain rather than an LRR (29). Barley Rpg1, which has been effective against barley stem rust for over 60 years, encodes a protein kinase with similarity to transmembrane LRR-kinases, yet is not an LRR protein (24). These are but two examples of the mechanistic divergence that is increasingly being discovered among *R* proteins, a situation that raises the above two questions.

Since the work of Harold Flor, *R* genes have been defined in various ways as the polymorphic plant genes that control gene-for-gene disease resistance (specificity for some but not all races of a pathogen species). However, this definition may not be entirely useful. For example, if a gene conferring obvious disease resistance and encoding an NB-LRR or LRR-kinase protein is found for which pathogen race-specificity has not yet been observed, it remains appropriate to call this an *R* gene (and it does not justify premature characterization of the gene as universally effective

and non-race specific!). The resistance-conferring gene that is polymorphic between plant accessions also may not be the LRR-encoding gene in that defense pathway, as first demonstrated for the resistance of tomato against *P. syringae* expressing *avrPto*, which was generated by introgression of the *Pto* kinase from a wild *Lycopersicon* species (136). The necessary NB-LRR protein for *AvrPto* detection, *Prf*, was apparently present in tomato but was nonfunctional in the absence of *Pto*. Does this make *Pto* an *R* gene? Yes. Does this mean *Prf* is not an *R* gene? No. As a third example, *Hm1* of maize determines race-specific disease resistance but does not do so through “recognition” of a dominant *Avr* gene. Instead, virulence is dominant in the pathogen due to toxin production, and *Hm1* encodes an NADPH-dependent toxin reductase that inactivates the pathogen toxin (86).

There are additional examples that stretch the possible definitions of *R* genes. The HR is a common aspect of *R* gene-mediated defense, yet, for example, naturally occurring alleles of potato *Rx* or *Arabidopsis* *RPS4* or *RPS6* confer resistance against virus or bacteria without development of an HR (18, 59). Thus, the induction of an HR is not a requirement of an *R* gene. *R* genes are sometimes thought of as antimicrobial but genes such as tomato *Mi-1* or wheat *H6* confer race-specific resistance against nematodes and insects rather than microorganisms (51, 147). Single *R* genes are generally thought to function against a single type of pathogen, but *Mi-1* genes function against multiple species of root-knot nematode and aphids. Thus causation of HR, specificity for microorganisms, and action against a single pathogen species are not defining hallmarks of *R* genes.

The product of *Arabidopsis* *FLS2* is an LRR-kinase like the product of rice *Xa21* (a definite *R* gene). Some researchers have not included *FLS2* as an *R* gene because it does not confer strong resistance or an HR (62). It is now understood that *FLS2* fails to induce resistance because bacterial effectors suppress *FLS2*-induced defense signaling. *FLS2* also

is now known to reduce infection by at least one virulent bacterial pathogen (198), to contribute to resistance against a pathogen not adapted to infect *Arabidopsis* (41), and to exhibit specificity for the flagella of some but not all strains of a single pathogen species (164). Should *FLS2* now be considered an *R* gene? And what about other *R* gene-like MAMP receptors?

Numerous genes have been identified for which mutation causes plants to be broadly disease resistant. These include non-*R* genes such as *Arabidopsis* *CPR1*, *DND1*, or *LSD1*, for which mutation causes resistance against many pathogen species and constitutive expression of defenses such as *PR-1* (70). Yet these also include genes such as *Arabidopsis* *RPW8.1*, *RPW8.2*, *PMR5* or *EDR1*, or barley *MLO* that, when mutated, cause pathogen-specific resistance but not constitutive broad-spectrum resistance, and in some cases control HR-like necrotic responses to the cognate pathogen (70, 191). It may be increasingly unproductive to insist on a strict definition of *R* gene. In most instances, these genes confer very strong disease resistance, confer HR cell death in response to infection, act at the earliest stages of pathogen detection, exhibit pathogen race-specificity, and encode some type of LRR-containing protein or require an LRR protein for their function. These types of *R* proteins are by far the most common and have received the most study, but the diversity of other resistance-associated protein structures and functional mechanisms present a complex (and intriguing) challenge for future research.

Additional questions about *R* genes have received partial answers but need further examples to more clearly establish mechanistic trends.

Can the Same *R* Gene Act Against More than One *Avr* Gene?

Arabidopsis *RPM1* acts against two sequence-unrelated effector proteins, *AvrRpm1* and *AvrB* (31). In this case, both effector proteins

affect the RIN4 protein “guarded” by RPM1. Tomato *Mi1* genes and members of the *Arabidopsis* *HRT/RPP8* family can act against entirely distinct pathogen taxa—nematodes and insects in the one case, oomycetes and viruses in the other (35, 147). These disparate pathogens may be attacking the same host target, or the R proteins may be guarding multiple targets, or different alleles of these *R* genes may encode receptors for distinct pathogen-derived ligands.

Can the Same Host Target Come Under Attack by More than One Different Pathogen Effector?

Multiple effectors (AvrRpm1, AvrB, and AvrRpt2) target RIN4. In turn, *Arabidopsis* has evolved multiple R proteins (RPM1 and RPS2) that recognize these distinct modifications of RIN4 (31).

Is the Same Avr Gene Product Ever Recognized by More than One Mechanism?

Yes: *Arabidopsis* and soybean both recognize *P. syringae* that express *avrB*. Ashfield et al. showed that the soybean *R* gene is not an ortholog of *Arabidopsis* RPM1 (11). In another example, the *P. syringae* effector AvrPtoB can be recognized by tomato expressing Pto/Prf, and a mutant form of AvrPtoB can be recognized by Rsb/Prf (2). Thus, it appears that Prf may “guard” two different targets of AvrPtoB. The first example demonstrates convergent evolution of two independently derived NB-LRR genes toward recognition of the same pathogen effector protein. The second example demonstrates evolution of a single NB-LRR protein to recognize distinct targets of a single effector. Some pathogen effectors have more than one target in the host. For example, AvrRpt2 cleaves multiple *Arabidopsis* proteins in addition to RIN4 (32). Similarly, it would not be surprising if the E3 ligase activity of AvrPtoB caused ubiquitylation of multiple plant proteins (82). Hence, plants may use

a variety of strategies to recognize individual effectors.

How Fully Conserved Are the “Conserved” Types of R Proteins?

Citing only two of the available examples, both *RPS2* and *RPM1* show substantial conservation across *Arabidopsis* accessions, as is now predicted when an *R* gene product is guarding a conserved host protein. But *Arabidopsis* carries two allele classes of *RPS2*, and some *Arabidopsis* accessions entirely lack *RPM1* (114, 155, 161). Both alleles of *RPS2* confer recognition of AvrRpt2, but slight variations in the LRR permit one allele to function only in certain *Arabidopsis* genetic backgrounds (15). What might the function of *RPS2* be in backgrounds in which it does not recognize AvrRpt2? For both *RPM1* and *RPS2* there is evidence that the genes cause a fitness penalty to the host in the absence of the cognate pathogen (97, 170). This and other evidence (e.g., 171) suggests that in wild plant populations that have not undergone deliberate breeding by humans, there can be balancing selection for both the presence and absence of “conserved” *R* genes.

How Has *R* Gene Diversity and Immune System Function Been Shaped at the Population and Species Level for Optimized Species Fitness Across Hundreds of Generations and Diverse Environments?

Population-level considerations are a fascinating area for disease resistance research [see e.g., (20, 26, 113, 155, 193)]. Two concepts deserve brief mention. The concept of frequency-dependent selection suggests that more common *R* alleles present stronger selection pressure on the pathogen population to shed the corresponding *Avr* gene, which will in turn reduce positive selection for presence of that *R* allele—resulting in some degree of balance (or oscillation) of the frequency of different *R* alleles in the host population. The

second concept, that of heterozygote advantage, suggests that the recognition capacity of an individual plant's immune system can be enhanced by heterozygosity at *R* gene loci, and also that host populations will benefit from *R* gene diversity.

RESISTANCE DURABILITY AND BREAKDOWN

The fourth part of the model from **Figure 1** shows the *R* gene failing as pathogen populations evolve to escape detection. This might seem to be an inevitable event, but many *R* genes have remained functionally effective despite decades of intensive use (93, 115). Nevertheless, *R* gene durability remains a significant practical issue, and one that merits substantial attention in future research.

Why Are Some *R* Genes Durable Whereas Others Are Not?

The above sections have already touched upon two key areas that influence *R* gene durability: the relative importance of the recognized pathogen effector protein to overall pathogen fitness, and the extent to which a directly recognized effector can undergo subtle structural changes and retain function. Especially in non-virus pathogens, resistance-breaking pathogen isolates often entirely lose the relevant avirulence gene from their genome. The fitness penalty that this loss of a MAMP or effector causes, either in virulence or in overall pathogen fitness, should be a strong factor in determining the durability of resistance that functions through recognition of that MAMP or effector (92). Bacteria and oomycete pathogens are now known to have evolved large suites of virulence-enhancing proteins, many of which can be sacrificed with only minor losses of virulence (6, 89). The activity of the effector may be dispensable (at least on that particular host), or the pathogen may have another effector that provides a redundant virulence activity. From this an important idea emerges: the relative durability of

resistance among *R* genes might be predicted in advance by assessing the relative fitness contributions of the corresponding pathogen effectors (186). This may become a very useful tool if sufficiently inexpensive means become available to identify and assess the relevant pathogen effectors.

A second factor will influence the durability of resistance: the ability of the effector to avoid recognition while maintaining its virulence activity. As was discussed above, an effector that is recognized directly may be able to mutate to prevent activation of resistance while still contributing to virulence. Thus, indirectly recognized effectors that make a major contribution to pathogen fitness would seemingly provide the best recognition targets for durable resistance. MAMPs that are widely conserved and essential might seem to be more ideal targets for recognition than dispensable effectors. MAMPs are indeed a primary target of plant immune systems, but their widespread presence on nonthreatening microorganisms, together with the ability of pathogens to suppress MAMP-elicited defenses, has tempered their value as elicitors of strong defense responses. However, re-engineering of MAMP recognition may be an important area for future creativity in molecular plant breeding.

The broader evolutionary capacity of the pathogen is another trait that strongly influences resistance durability (115). Populations of some pathogens evolve slowly while other species exhibit sexual reproduction (increasing genetic variation among progeny), many generations per season (allowing greater selection on the relevant host genotypes), and/or long-distance dispersal via wind currents, animals or mechanical transport (speeding genotype spread across large areas). Every pathogen species is different. Viruses often have limited genome complexity but nevertheless have tremendous evolutionary capacity due to their very large population sizes and short generation times. The rust fungi, an enduring plague of humankind, seem ideally adapted to evolve resistance-breaking

isolates because they couple complex genomes and annual sexual reproduction with multiple asexual generations that select and amplify adapted genotypes, and they spread adapted genotypes widely via windborne urediospores. Assessment of a pathogen's broader evolutionary potential can help predict the likely durability of *R* gene-based disease control methods (115).

How Can We Make or Use *R* Genes in Ways that Confer Durable Resistance?

Additional approaches exist to enhance the durability of genetic resistance, beyond assessing the fitness contribution of the recognized effector, the capacity for structural variation within directly recognized effectors, or the overall evolutionary potential of the pathogen. Two practical case studies from wheat and lettuce (36, 116) provide excellent examples of how *R* genes can be more effective when they are used in rotation, with monitoring of the current races of pathogen in a region to allow pre-emptive breeding and release of varieties with appropriate *R* genes. Equally important is the idea of using *R* genes only as needed, and removing *R* genes from use before they become widely ineffective. Careful coordination among pathologists, plant breeders, and growers is needed to fully exploit such a system; in some instances this cooperation has been legally mandated.

A related and very important concept is that of stacking *R* genes. For a given pathogen, the goal is to keep more than one effective *R* gene present in every individual plant so that pathogen reproduction will be restricted even if individuals are present that have lost avirulence for one of the *R* genes. Stacking has been achieved through traditional breeding and now is possible by plant transformation. Transformation technologies should allow identification and deployment of multiple *R* genes from wild germplasm and sexually incompatible relatives, generating previously

unattainable combinations of stacked *R* genes while avoiding introduction of undesirable alleles at other loci of an elite genotype (143). Pursuit of this approach is constrained by the fact that some *R* genes do not function properly in heterologous systems, possibly because the guarded host protein is absent, or because of pathogen-independent *R* protein activation in the heterologous system. Investment in transgenic stacking of *R* genes also has been limited by fear that sufficient resistance durability will not be achieved, and by the opposition in some quarters against any transgenic crops (70).

Mixed host approaches, in which lines with different resistance genotypes are cultivated in single field, have also been shown to provide benefits in some settings. This is a complex undertaking that defies oversimplification (124). Combining (or alternating) use of pesticides and *R* genes, both of which can select for insensitive pathogen strains, has also been explored to increase the durability of disease control (e.g., 36).

Will Molecular Knowledge of Elicitors, Effectors, and *R* Proteins Ever Be Useful?

The answer is already "yes." Numerous commercial and public plant breeding programs use molecular markers for *R* gene alleles to guide progeny selection while reducing the need for more expensive disease tests. Use of *R* genes from heterologous species and the stacking of multiple *R* genes that act against a single pathogen species are often suggested, and the molecular tools for this type of effort are in increasingly common use (e.g., 14, 102). A larger challenge will be to use our growing understanding of MAMPs, pathogen effectors, and *R* protein mechanisms to improve plant disease resistance. There are many possible avenues. The work of Shen et al. suggests that in at least some systems, MAMP perception can activate very strong plant defenses that are held in check until activated *R* proteins remove that negative

regulation (156). This delicately balanced system presents many opportunities for engineering. As a separate approach, identification of effectors that make a major virulence contribution may allow identification of the best *R* genes to utilize. Directly recognized effector proteins might be used to screen for improved *R* genes that recognize effector domains that can tolerate little or no change. The understanding that effectors often attack host targets may allow placement of those host targets and their guardian *R* proteins into heterologous plant species, thereby converting

adapted pathogens into “nonhost” pathogens. Effectors can allow identification of host processes perturbed to promote disease, possibly allowing modification of those targets toward insensitivity, or use of the genes for those host targets as QTL markers for plant breeding. These are just a few possible ideas. As our understanding of the molecular mechanisms of pathogen virulence and plant immunity continues to grow, it will remain important that the brightest minds and ample funding be attracted both to basic and applied research goals.

SUMMARY POINTS

1. A new paradigm for defense activation has emerged in which plants recognize microbe-associated molecular patterns (MAMPs) and thereby activate basal defenses, pathogens express effectors that suppress basal defenses, some plants express *R* proteins that directly or indirectly recognize effectors and activate strong defenses, and some pathogens modify or eliminate the effectors that the host can recognize so that the pathogen regains at least some virulence on hosts that express these *R* proteins.
2. A limited number of MAMPs have been defined. The receptors for these MAMPs have so far tended to be transmembrane proteins that have an extracellular leucine-rich repeat (LRR) domain.
3. Because pathogen effector proteins often contribute to virulence by suppressing or disrupting host defense responses, the study of effectors is revealing fascinating pathogen adaptation to host biology and identifying specific plant processes that contribute to disease resistance.
4. *R* proteins are most commonly intracellular NB-LRR proteins or extracellular LRR-carrying receptors, but other proteins types can also be classified as *R* proteins.
5. *R* proteins may recognize pathogen effectors by direct physical interaction, or they may recognize them indirectly by sensing the host proteins upon which effectors have acted.
6. Directly recognized effectors may escape detection by altering their shape while retaining virulence function, but indirectly recognized effectors in many cases can escape detection only by ceasing virulence activity.
7. Many NB-LRR *R* proteins are apparently maintained in an ADP-bound “off” state by interactions of LRR and NB-ARC domains. Elicitation disrupts these interactions and allows ADP release/ATP binding, opening the protein for defense-signaling protein-protein interactions. Some effectors or effector products bind initially to N-terminal domains of the NB-LRR protein, but apparently are then detected by the LRR domain. Some NB-LRR proteins are present in both the cytoplasm and the nucleus.

8. The durability of the disease resistance encoded by a particular R protein is strongly influenced by whether the R protein directly or indirectly recognizes the effector, by the extent to which the pathogen can retain virulence after altering or eliminating the recognized effector, and by the overall capacity of the pathogen for rapid evolution.

FUTURE ISSUES

1. What are the MAMPs and MAMP receptors that most strongly affect defense activation, especially for the most destructive diseases of valuable crop species?
2. What are the host defense processes that effectors target and disrupt, especially for fungal pathogens where there has been minimal research to date?
3. What are the immediate downstream targets that R proteins directly modulate in order to activate strong defense responses?
4. How can knowledge of elicitors, effectors and R genes be translated into practical disease control measures that confer durable disease resistance?

ACKNOWLEDGMENTS

We apologize to the many colleagues who have influenced our thinking through work that was not explicitly cited.

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Errata

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