

Resistance gene signaling in plants – complex similarities to animal innate immunity

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During the past year several important publications have significantly enhanced our current understanding of plant disease resistance. Among the most important discoveries are the role of SGT1 in resistance (*R*) gene mediated defenses, mounting support for the so-called ‘guard hypothesis’ of *R* gene function, and providing evidence for intramolecular interactions within *R* proteins as a mode of signaling control. There are many emerging parallels between the plant *R* genes and animal innate immunity receptor complexes. Plant SGT1 shows similarity to co-chaperones of the animal Hsp90 complex, and many receptor-like *R* gene products appear to interact indirectly with their pathogen-derived signal. Considering these and other similarities, researchers from both fields should be looking carefully over each other’s shoulders.

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Abbreviations

Avr	avirulence protein
CC	coiled-coil
CP	coat protein
EDS	enhanced disease susceptibility
HR	hypersensitive response
Hsp	heat shock protein
LPS	lipopolysaccharide
LRR	leucine-rich repeat
NB	nucleotide-binding
NDR	non-race-specific disease resistance
<i>R</i> gene	resistance gene
<i>R</i> protein	resistance protein
RAR1	required for <i>Mla</i> resistance 1
SGT1	suppressor of <i>G₂</i> allele of SKP1
SKP1	s-phase kinase associated protein
TIR	Toll and interleukin-1 receptor
TLR	Toll-like receptor

Introduction

Similar to animals, plants offer a bountiful and concentrated supply of resources for would-be pathogens. Until

recently, research on animal immunity has focused mainly on so-called ‘acquired immunity’. Acquired immunity is the process by which the circulating immune system of animals somatically generates new resistance specificities against non-self invaders. Underlying this level of resistance in animals is the more evolutionarily ancient innate immune system, which relies on pre-formed receptors to detect conserved microbial-specific patterns and trigger downstream defense responses [1]. Plants, however, lack the ability to somatically generate new resistance specificities, and rely instead on meiotically formed pathogen-recognition mechanisms. This does not mean that plants have a less active or less effective response to pathogens. The triggering of resistance (*R*) gene pathways gives rise to massive cellular ion influxes, an oxidative burst leading to the accumulation of superoxide and hydrogen peroxide, nitric oxide production, the hypersensitive response (HR; a form of programmed cell death thought to limit pathogen ingress), and the production of toxic antimicrobial metabolites [2]. We are just beginning to understand the elegant and varied defensive mechanisms that plants utilize to get around their two main limitations — lack of a circulating immune system and immobility.

In this review, we hope to give the reader a brief insight into some of the most interesting recent discoveries concerning *R* gene-mediated defense signaling. Additionally, we provide historical context for these discoveries and comparisons to the animal innate immune response. For those interested in further reading on broader topics in plant disease resistance, we recommend the yearly ‘Biotic interactions’ section in the journal *Current Opinion in Plant Biology*.

Resistance genes — sentries at the gate

For plant pathogens to utilize host plant resources they must first circumvent preformed defense mechanisms, such as the dense epidermal layer and waxy coverings on most leaves. Successful pathogens avoid these early obstacles by stealth (e.g. slipping in through stomatal openings on leaves and stems) and brute force (e.g. fungal and oomycete ‘penetration pegs’, which literally drive a growing spike through the epidermis). Having found their way into the plant apoplast (the space between cells), pathogens must interact directly with individual plant cells to gain access to nutrients necessary for growth and reproduction. This is achieved by the production of virulence factors (also called effector proteins) that are released into the apoplast or injected directly into plant cells. Bacterial plant pathogens use a type III secretion

system (a hypodermic needle-like pilus similar to those of *Salmonella*, *Shigella*, *Yersinia* and pathogenic *Escherichia coli*) to deliver effector proteins directly into the host cell [3]. Examples of this would include the avirulence (Avr) proteins discussed below. Because plants lack a circulating immune system, most plant cells must be individually capable of recognizing pathogens and activating an effective defense system.

The sentries at the gate are plant resistance (R) proteins, which recognize pathogen-encoded effectors either directly or indirectly (see guard hypothesis below). In this role, pathogen-encoded effectors are called avirulence (avr) proteins. *Avr* genes are structurally diverse and are theoretically maintained in their respective genomes by virtue of virulence roles advantageous to the pathogen. Evidence for virulence function has been demonstrated for several, but not all, Avr proteins [3]. Recognition is typically 'race specific', meaning that a given R protein recognizes the Avr proteins from one or very few pathogen isolates. This *R-avr* genetic interaction initiates what is referred to as gene-for-gene resistance [4].

Five classes of highly polymorphic, but mostly structurally conserved, R proteins have been identified and these can be broadly grouped into those with extracellular or intracellular leucine-rich repeat (LRR) regions [2]. Thus far, the putative intracellular LRR class is the largest. The model plant species *Arabidopsis thaliana* is predicted to encode approximately 150 related *R*-like genes with central nucleotide binding domains (NBs) and carboxy-terminal LRRs. The putative encoded proteins differ structurally in their amino termini, where they have either a region of 'Toll and IL-1 receptor' (TIR) homology or a predicted coiled-coil motif (CC). Loss-of-function mutations in genes of this NB-LRR class only result in the loss of disease-resistance phenotypes, suggesting that the NB-LRR class is a gene family dedicated to *R* function. By contrast, there is another class of extracellular LRR proteins that are multifunctional [2].

Although no plant resistance gene has been crystallized, the three dimensional structure has been solved for LRR-containing proteins from numerous other species [5]. LRRs are fairly uniform in structure across kingdoms and typically consist of a ligand-binding face composed of repeating β strands backed by α helices. The LRR is under diversifying selection in plant *R* genes [6], and potentially provides an evolutionarily flexible interface for ligand binding. In mammalian ribonuclease inhibitor, which is essentially one large LRR, the β strands combine flexibly to efficiently bind and inhibit a wide variety of ribonucleases with sequence identities as low as 24% [7]. *In vitro* generated [8] and naturally occurring [9] recombination events between the LRRs of highly related *R* genes can result in reversals of *Avr* recognition specifi-

cities. These data strongly implicate the LRR as the pathogen-recognition specificity determinant, but what do R proteins recognize?

Guard hypothesis — gone fishing

Almost every laboratory studying *R-avr* genetic interactions has tried their hand at proving a physical interaction between their favorite R and Avr proteins. With the exception of the *in vitro* interaction between Pi-ta (a CC-NB-LRR R protein) from rice and its corresponding avirulence protein AvrPita [10*] from the fungal pathogen *Magnaporthe grisea*, no direct interaction between a pathogen avirulence product and an NB-LRR R protein has been demonstrated.

Pto from tomato encodes a serine/threonine kinase that can phosphorylate a variety of targets, some of which have proven defense functions. It is probably an atypical R gene. Pto requires the NB-LRR type protein Prf to trigger defense responses against *Pseudomonas syringae* bacterial strains expressing *avrPto* [11]. Yeast two-hybrid and genetic data strongly suggest a physical interaction between Pto and AvrPto, but AvrPto does not appear to interact with Prf; thus, the Prf/Pto-*avrPto* genetic and Pto-AvrPto physical interactions may have been the Rosetta Stone for *R-avr* interactions all along.

The 'guard hypothesis' suggests that the NB-LRR protein Prf detects and potentially 'guards', or monitors, the Pto-AvrPto physical interaction [2,12]. If AvrPto functions as a virulence factor targeting the Pto kinase and altering its ability to activate defenses, then Prf might be activated as a consequence of this interaction. So, Prf may act similar to a fishing pole with Pto as the bait and AvrPto as the trophy catch. Additionally, evidence is mounting that this model could be generalized to other *R-avr* interactions, suggesting that many of us have been fishing without bait for years. The explicit assumptions of the guard hypothesis are: first, there is specificity in the interaction of R protein-host target (or 'guardee') pairings; and second, that the Avr-guardee interaction in the absence of an R protein is a positive virulence mechanism for the pathogen.

Arabidopsis plants with the functional *R* gene *RPM1* (for 'resistance to *Pseudomonas syringae* pv *maculicola*') are resistant to *Pseudomonas syringae* pathogens expressing *avrRpm1*. Despite considerable investigator effort, there is no evidence for a direct RPM1-AvrRpm1 physical interaction. The recently described RIN4 (for 'Rpm1 interactor 4') protein, which has no known motifs, interacts in yeast two-hybrid assays and *in vivo* with both proteins and is necessary for the *RPM1-avrRpm1*-mediated HR [13**]. All three of these proteins localize to the cell membrane fraction and mutations in AvrRpm1 that inhibit proper localization also strongly reduce the associated HR. RIN4 is not necessary for the function of

the closely related *RPS2* gene, demonstrating the expected specificity for a putatively guarded host target of AvrRpm1 virulence function. Nevertheless, RIN4 and RPS2 do interact *in vivo*, although they do not interact by yeast two-hybrid analysis. This suggests that RPM1 and RPS2 may be found within conserved protein complexes, although their specific binding to individual components differ (D Mackey, Y Belkadir, JL Dangl, unpublished data). Similar observations have been made for the RAR1 (for 'required for *Mla* resistance') and SGT1b proteins (see below).

The tomato *Cf2* gene conditions resistance to the fungal pathogen *Cladosporium fulvum* expressing *Avr2* and also requires the recently cloned *RCR3* gene for its resistance function [14•]. *RCR3* (for 'required for *Cladosporium* resistance') encodes a functional cysteine protease, suggesting interesting parallels with the *Drosophila* Toll receptor. Toll requires a ligand processed by a cysteine protease for downstream signaling [15] and, similar to *Cf2*, is required for innate immunity to fungal pathogens. Because *RCR3* is not required for other highly related *Cf* resistance genes, it is also a good candidate for a guarded protein. Nevertheless, no direct physical contacts have been demonstrated for this three-way genetic interaction. Several other candidate host genes for the role of 'guardee' exist for various *R-avr* pairs and have been summarized in a review by Van der Hoorn *et al.* [16•]. To date, none of the guardee candidate proteins have a clear function as the target of a pathogen virulence factor, although RIN4 does have a deduced function in plant defense [13••]. Therefore, although evidence is mounting in its favor, the jury is still out on the guard hypothesis.

Resistance gene signaling – a little self control

When the *R-avr* interaction is triggered, how does signal transduction proceed? Circumstantial evidence has accumulated that R proteins have intramolecular interactions that might affect their ability to signal downstream and bind other molecules. The tomato *Mi* gene, a CC–NB–LRR protein, is required for resistance to potato aphids and root-knot nematodes [17]. Amino-terminal domain swapping experiments between the functional *Mi-1.1* allele and the non-functional *Mi-1.2* allele generated lethal phenotypes [18]. When these same constructs were transiently expressed, pathogen-free cell death phenotypes similar to those seen following the HR were observed and the cell death phenotype could be suppressed by coexpression of the amino-terminal domain from the parent allele. These data suggest that the amino-terminal domain of Mi regulates signaling from the LRR, leading to cell death. RIN4 and AtTIP49a, a recently described negative regulator of some *R* genes [19], each interact by yeast two-hybrid assay with the amino-terminal portion of RPM1; they also demonstrate a marked

reduction in their interaction with RPM1 when the two-hybrid bait includes the NB domain, suggesting that this domain may inhibit some RPM1 interactions. The most convincing, but still incomplete, evidence for the importance of intramolecular interactions as regulators of R protein function has only just emerged.

Moffett *et al.* [20••] worked with potato Rx, a CC–NB–LRR protein necessary for recognition of potato virus X (PVX) via its coat protein (CP). They demonstrated that functional Rx could be reconstituted by transiently coexpressing separate portions of the protein. Functional Rx was generated *in trans* using combinations of both CC–NB and LRR or CC and NB–LRR. Additionally, they utilized immunoprecipitation experiments to show that these molecules physically interact *in vivo* when transiently coexpressed. Whether they interact directly or are brought together by shared interactions with other proteins is unknown because *in vitro* interaction experiments were not reported. Importantly, the expression of biologically active CP triggered a normal HR and eliminated the interaction between the separately expressed Rx peptides. Taken together, these data suggest that Rx intramolecular interactions are modified as a result of Avr protein perception, and that this renders Rx competent for further downstream signaling. CP did not immunoprecipitate with Rx, suggesting either that Rx is in complex with, or rapidly recruits, other proteins following PVX infection (the guard hypothesis) or that the interaction of Rx with CP is only transient and, therefore, not detectable by immunoprecipitation.

Interestingly, two recently identified *R* genes, RPW8.1 and RPW8.2, which confer resistance to numerous powdery mildew isolates, consist of only a CC motif and a single transmembrane domain [21]. Because these proteins, similar to many NB–LRR type R proteins, require *EDS1* (enhanced disease susceptibility) for their function, it will be interesting to see if they additionally require and interact with an LRR type protein, thus naturally recapitulating the *Rx* findings.

RAR1 and SGT1b – complex arrangements

Most NB–LRR resistance genes have been demonstrated to signal predominately through one of two pathways [22]. These two pathways were initially defined by mutations in the *EDS1* [23] and *NDR1* (non-race-specific disease resistance; [24]) genes; CC–NB–LRRs signal through NDR1, whereas TIR–NB–LRRs signal through *EDS1* [22], although there are exceptions [25]. When either *NDR1* or *EDS1* are non-functional, *R* gene signaling through these pathways is abolished and the result is complete susceptibility. PAD4 (phytoalexin deficient 4), which interacts *in vivo* with *EDS1* [26], is also required for the function of TIR–NB–LRRs. *EDS1* and *PAD4* encode proteins that have homology to catalytic lipases and may be involved in lipid signaling [27,28]. The involvement of

lipid signals in both animal [29] and plant [30] cell death has been previously documented. Nevertheless, no catalytic function has been demonstrated for either EDS1 or PAD4. *NDR1* encodes a putative glycosylphosphatidylinositol (GPI)-anchored protein (B Staskawicz, personal communication), although nothing is known about its biochemical function.

Adding significantly to our knowledge of *R-avr* signal transduction are numerous recent publications concerning the *RAR1* and *SGT1* genes. *RAR1* was initially identified in barley and acts as a non-redundant convergence point for race-specific disease resistance to numerous powdery mildew isolates [31]. The predicted *RAR1* protein has two novel 60 amino acid zinc-binding domains (designated CHORD for cysteine- and histidine-rich domain) and a plant-specific carboxy-terminal extension. Animal proteins sharing this CHORD domain all have a carboxy-terminal domain not found in plant *RAR1* proteins, a region of so-called *SGT1* homology. The *SGT1* protein in yeast is a component of the SCF (SKP cullin F-box) complex, which is an integral component in protein ubiquitylation [32].

There are two *SGT1* genes in *Arabidopsis*, designated *SGT1a* and *SGT1b*. Two papers have now revealed the importance of the *Arabidopsis SGT1b* gene in the resistance response mediated by numerous *R* genes, including both CC–NB–LRR and TIR–NB–LRR pathways [33*,34*]. Additionally, *SGT1* has a role in non-host resistance, which refers to a presumably non-specific class of resistance when a plant species is resistant to all known isolates of a given pathogen. For example, *Nicotiana benthamiana* plants silenced for *SGT1* become susceptible to bacterial pathogens normally pathogenic to members of the Brassicaceae [35*]. These data strongly suggest that *SGT1*, similar to *RAR1*, serves as a convergence point for numerous defense-related pathways. Not all non-host resistance is compromised in *SGT1*-silenced *N. benthamiana* plants, and there are *Arabidopsis R* genes that do not require *SGT1b* [33*]. In some cases these *R* genes do require *RAR1*. For other *R* genes, either both genes (e.g. *RPP5*) or neither (e.g. *RPP1A*) gene are required. These results are presently puzzling, especially considering that *RAR1* and *SGT1b* interact *in vivo* in *Arabidopsis*. One possible explanation for these observations is that *SGT1a* and *b* act together as a convergence point for these pathways. This is unlikely, however, as *sgt1a* loss-of-function mutants do not alter the response of the *SGT1b*-independent *R* genes (K Shirasu, personal communication). Unfortunately, an *sgt1a/sgt1b* double mutant is lethal (K Shirasu, personal communication).

SGT1b also interacts *in vivo* with two E3 ubiquitin ligase subunits, SKP1 (s-phase kinase associated protein 1) and CUL1 (cullin 1) [36**,37**]. SCF complexes have E3 ligase activity and define substrate specificity for ubiqui-

tylation [38]. These interactions prompted Azevedo *et al.* [36**] to test *SGT1b* for interaction with CSN4 and CSN5, two components of the COP9 (constitutive photomorphogenesis 9) signalosome. The COP9 signalosome resembles the lid portion of the 19S regulatory subunit of the 26S proteasome, and was originally identified for its role in photomorphogenesis [39*]. CSN4 and CSN5 interact with *SGT1b* and, although *RAR1* additionally interacts with these components, *RAR1* is not required for the *SGT1*–CSN4/5 interactions. Furthermore, silencing of CSN3 and CSN8, two additional components of the COP9 signalosome, inhibits *N* gene-mediated resistance to tobacco mosaic virus in *N. benthamiana* [37**].

To use an analogy, auxin (a phytohormone involved in multiple developmental processes) induces the expression of the *AUX/IAA* (auxin/indole acetic acid inducible) genes [40]. *AUX* proteins can negatively regulate their own expression, and auxin relieves this negative regulation by inducing the degradation of *AUX* repressor proteins at the COP9 signalosome [39*]. It is not yet clear if the COP9 signalosome might mechanistically regulate defense responses in a similar fashion. There is evidence that at least one *R* protein, *RPM1*, is degraded following elicitor perception just before onset of the HR [41]. Intriguingly, *RPM1* does not accumulate in an *Arabidopsis rar1* mutant [42*], suggesting that *RAR1* is required for either *RPM1* stability or accumulation. Presently, no data concerning the degradation of other resistance proteins is available and potential targets of regulation by the COP9 signalosome are unknown. Evidence is also rapidly growing that ubiquitylation controls much more than protein turnover. For example, ubiquitylation of VP16 in yeast simultaneously activates its transcriptional activities and drives its future degradation [43].

Are there functional similarities between these *R* gene signaling molecules and the animal innate immune response? Actually, there are quite a few. For example, bacterial lipopolysaccharide (LPS) is a potent activator of animal innate immune responses. LPS associates with the LPS-binding protein and CD14 in the plasma membrane [44]. Perhaps the putatively GPI-linked *NDR1* is acting in a similar way to the known GPI-linked CD14. CD14 is associated with triton insoluble, heterogeneous regions of the plasma membrane called lipid rafts, and is not competent to transduce the LPS signal on its own [45,46]. Numerous other signaling proteins are also constitutively localized with CD14 or rapidly recruited into the CD14 lipid raft, including heat shock protein (Hsp) 70, Hsp90, and the Toll-like receptor (TLR) 4 [47,48]. Co-localization of these proteins in lipid rafts appears to be necessary for full cellular LPS stimulation [46]. TLR4 has homology to the amino-terminal regions of plant TIR–NB–LRRs and it will be interesting to see whether other molecules in the CD14 signalosome function in *R*-dependent responses in plants.

As with TLR4, steroid hormone receptors, such as the progesterone receptor, form mature complexes with Hsp70 and Hsp90, and these complexes require the proteins Hop (hsp-organizing protein) and p23 [49,50]. Human and *Arabidopsis* SGT1, similar to Hop and p23, have tetratricopeptide repeats (TPR), a degenerate 34 amino acid sequence involved in protein–protein interactions [51]. Strengthening the links that tie these numerous proteins together, *Arabidopsis* Hsp90 physically interacts with human p23 via the TPR [52]. Excitingly, Hsp90 and SGT1-like proteins were recently isolated in a screen for RAR1 interactors from *N. benthamiana* [37**]. Potentially coming full circle to the SGT1–COP9 interaction, the mature progesterone receptor in complex with Hsp90 and p23 binds ligand and is subsequently phosphorylated, ubiquitinated and degraded by the 26S proteasome [50].

Final thoughts

Although some of our comparisons to the innate mammalian immune response are presently circumstantial, the inclusion of *SGT1* and *RAR1* in the stable of *R* gene-mediated signaling components is exciting and full of potential; the breadth and quality of biochemical data in animal literature should supply the plant disease resistance field with an abundance of launching points for future experiments. Because of the many unique advantages of *Arabidopsis* as a genetic model, such as rapidly progressing efforts to identify loss-of-function (T-DNA) alleles for every gene and the relative ease of working with whole organisms, the animal innate and acquired immune response community should also keep a watchful eye on molecular plant pathologists.

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