Cell, Vol. 109, 537–540, May 31, 2002, Copyright ©2002 by Cell Press

Plant Immunity and Film Noir: What Gumshoe Detectives Can Teach Us about Plant-Pathogen Interactions

Minireview

David S. Schneider[1]
Department of Microbiology and Immunology
Stanford Medical School
Stanford, California 94303

Summary

Plant cells practice constant vigilance using resistance (R) proteins to monitor pathogenic processes. Three papers published recently in *Cell* and one in *Science* provide support for a model in which plant cells set up surveillance of signal transduction pathways, preparing to destroy the cell if any untoward fiddling with cellular physiology is detected. The demonstration of three separate examples of such a system suggests that it is broadly used and should provoke a reexamination of microbial pathogenesis in animal cells to look for similar mechanisms.

Introduction

When a plant is infected by a microorganism to which it is immune, R gene function in the infected cell produces, among other responses, a hypersensitive response (HR). The HR is rapid death of the infected and neighboring cells and may block the spread of infection. Plants also have basal immune responses that can function in the absence of R-mediated recognition, leading to the expression of so-called pathogenesis-related genes. The papers discussed in this review all concern the regulation of R-mediated recognition in response to bacterial pathogens. Plants, of course, can respond to a much wider range of insults, and R genes have been identified that are required for reactions to challenges as diverse as viral infections and insect feeding. The papers discussed here support a model that could be used to describe these other systems as well.

A common theme among bacterial pathogens of all organisms is the use of type III secretion apparatuses (TTSA), a sort of syringe that injects proteins into the cytoplasm of the host. The bacteria use the TTSA to secrete effector proteins into the host cells (Staskawicz et al., 2001). These effectors alter the physiology of the host in a variety of ways and act to make the host a more hospitable place for the pathogen. In a heist film, this would be the point where the bank robbers cut the power to open the safe. Plants have developed an immune response to this gambit. Plant cells synthesize R proteins that counteract the effects of TTSA effectors, presumably by detecting the presence of the effector protein, thus triggering an HR. These R proteins are called resistance proteins because their function is required for plant resistance to the microbes secreting the TTSA effectors. A bacterial mutant lacking the TTSA effector will not induce an HR in a plant possessing the cognate R protein. Instead, the bacteria are virulent and cause disease. The effectors are thus called avirulence proteins because their presence results in an avirulent infection in a resistant plant. This seems like an odd thing for the bacteria to do. Current thinking suggests that the avirulence proteins are not produced to cause the bacteria to reveal their presence, like a robber singing in the vault. Rather, bacteria synthesize these proteins to increase virulence when infecting r^- plants.

The differences in nomenclature in plant and animal pathogenesis can be confusing as we give similar phenotypes opposite names. How we name things is important, as it guides our thinking about future experiments. Typically, when referring to host-pathogen interactions, we rely on war imagery. To translate between terminologies used to describe plant and animal host-pathogen interactions, detective fiction may be provide a better metaphor. Dangl and Jones (2001) proposed a model in which R proteins act as guards to monitor the behavior of molecules that are targets of TTSA effectors. Perhaps a more illustrative example is to consider the R proteins to be private eyes in a film noir. The detectives are hired to observe the behavior of a "mark" to determine if he is interacting with criminals (TTSA effectors). As anyone who has watched these films knows, if the detective observes funny business, it isn't going to end well and the movie is likely to conclude in a bloodbath. So it is in the plant, where an R protein induces an HR that kills the infected cell.

Identification of a Missing Link

As the plant R gene-bacterial avr gene story developed, it appeared that there was one-to-one R gene-avr gene correspondence. R proteins were suggested to be direct receptors for Avr proteins. As molecular details were discovered, this model became difficult to support in all cases. For example, strains of the bacterial pathogen *Pseudomonas syringae* carrying AvrRpm1 induce an HR when infecting RPM1-expressing *Arabidopsis*. However, the sequence-unrelated AvrB can also trigger RPM1-dependent HR. Genetic analysis of the system failed to reveal a molecule that could connect the Avr proteins with RPM1, and there was no evidence for a direct interaction between RPM1 and either effector.

The paper by Mackey et al. (2002) reveals the missing link. The authors identified a protein, RIN4, which acts as a bridge from AvrB or AvrRpm1 to RPM1. These interactions were first identified using a two-hybrid approach in which RIN4 was identified as a protein that bound both bacterial AvrB and RPM1 baits. These data were corroborated with in planta immunoprecipitations. In addition, AvrB and AvrRpm1 were shown to target RIN4 for phosphorylation by an unknown mechanism.

The model presented to explain the function of these proteins suggests that RPM1 acts as a guard whose job is to provide surveillance of RIN4 (Figures 1 and 2). Binding or modification of RIN4 by Avr proteins leads to the activation of RPM1 through some disturbance in the RIN4-RPM1 interaction. This in turn leads to an HR and resistance to infections.

Why do bacteria target RIN4? When the authors reduced RIN4 activity by making transgenic plants that

[1]Correspondence: dschneider@stanford.edu

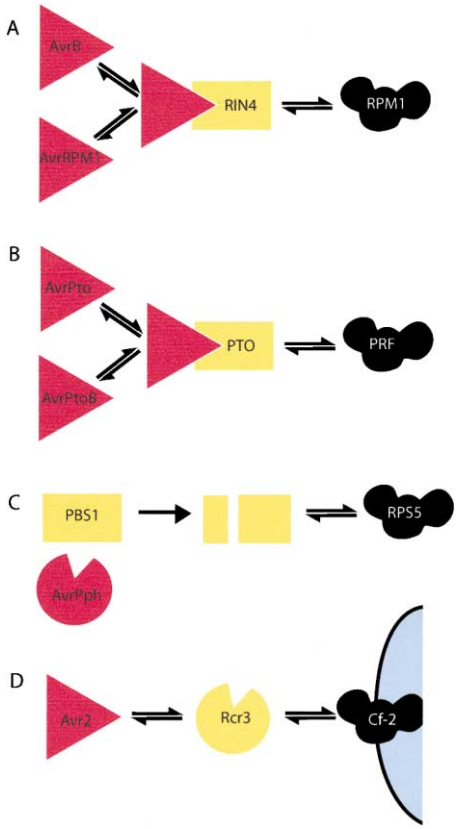

Figure 1. Model for Plant-Pathogen Interactions

(A) Either AvrB or AvrRpm1 bind RIN4, resulting in phosphorylation of RIN4. RPM1 detects this change and induces an HR.
(B) AvrPto or Avr PtoB bind to PTO activating PRF.
(C) The bacterial protease AvrPphB cuts PBS1. This is monitored by RPS5 and leads to its activation.
(D) Interaction between bacterial avirulence protein Avr2 and host protease RCR3 is detected by Cf-2, leading to an HR.

expressed antisense RIN4 RNA, the ability of RPM1 to raise an RPM1-dependent HR response was reduced, as anticipated, but the ability of a related R gene (RPS2) to produce an HR response was not affected. RIN4, therefore, does not affect HR responses in general, but affects RPM1 specifically. Unexpectedly, however, the transgenes with reduced RIN4 function also increased resistance to a *P. syringae* strain lacking AvrB and AvrRpm1, as well as an oomycete pathogen, *Peronospora parasitica*. Further, these plants had increased constitutive expression of one immune-responsive transcript. Taken together, these data suggest that RIN4 is a negative regulator of the basal immune response of the plant. The authors, therefore, hypothesize that the function of AvrB and AvrRpm1 may be to increase the activity of RIN4 and thus suppress the basal immune response. This would result in better conditions for growth of *P. syringae*.

The reason behind the inability to identify RIN4 as an R gene in genetic studies became apparent during this analysis. The phenotype of a strong antisense allele of RIN4 suggested that complete loss of RIN4 function is lethal. Severe loss of RIN4 function resulted in dwarf

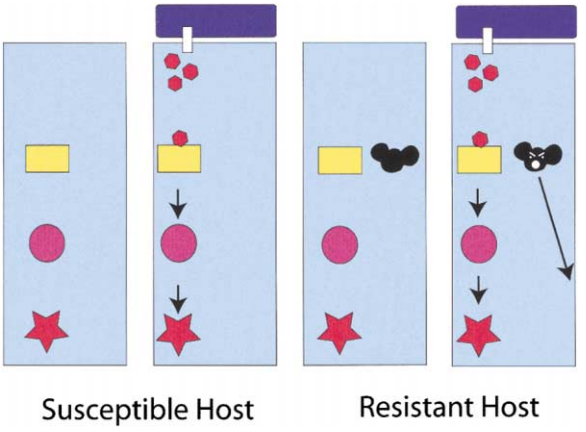

Figure 2. Guard Proteins Raise an Alarm in Resistant Hosts

Bacteria secrete TTSA effectors (red hexagons) into the cell. In a susceptible host, the effect is increased virulence. In a resistant host, the guard protein detects a change in the yellow host protein.

plants, suggesting that the gene serves a developmental role as well as an immune role in the plant.

Replacing the One-to-One R Gene-avr Gene Hypothesis

The one-to-one R gene-avr gene hypothesis is clearly unraveling as AvrRpm1 and AvrB are unrelated by sequence but target the same molecule in the plant cell. A similar story is told in the paper by Kim et al. (2002 [this issue of *Cell*]). The tomato kinase PTO has been shown to physically interact in a two-hybrid setting with *P. syringae* AvrPto, so it was puzzling that deletion of the AvrPto gene from some *P. syringae* strains did not eliminate PTO-specific avirulence activity. This result suggested that these strains might carry another effector acting through PTO in planta. The authors used PTO as bait in a two-hybrid screen to identify AvrPtoB from *P. syringae*. AvrPto and AvrPtoB share limited sequence similarity but both bind PTO. AvrPtoB is secreted through the TTSA and induces an HR when expressed in PTO plant cells—all characteristics of a bona fide Avr protein.

AvrPtoB helps define a veritable crime family of avr genes spread across three genera. The authors identified homologs in *Xanthomonas* and *Erwinia* strains as well as a variety of *Pseudomonas* pathovars. This is somewhat unusual, as most avr genes are carried only by a limited range of bacterial strains.

The HR response to AvrPto requires not only PTO but the gene PRF, which shares sequence similarity with RPM1. PTO, like RIN4, is a member of a signal transduction cascade activating the basal immune response in the plant. In contrast to RIN4, however, PTO mutants are viable and have an R phenotype on their own. PTO mutants do not raise an HR in response to *P. syringae* carrying AvrPto. In many respects, the PTO system looks like the RIN4 system described above (Figure 1). In fact, the guard model was first used to describe PRF function (Van der Biezen and Jones, 1998; Dangl and Jones, 2001). This model suggests that one of the two bacterial Avr proteins, AvrPto or AvrPtoB, binds PTO. This interaction is monitored by PRF, which in turn activates an HR when it detects a change in PTO.

There Are Many Ways to Tip off the Detectives

The paper by Shao et al. (2002 [this issue of *Cell*]) adds to this model by expanding our understanding of the type of events that may be monitored by detectives. These authors show that a *Yersinia* TTSA effector, YopT, and a *Pseudomonas* avirulence protein, AvrPphB, define a family of cysteine proteases. Like AvrPtoB, this family contains 19 members, some of which are involved in bacterial pathogenesis in plants and animals. In vertebrate cells, YopT is shown to cleave Rho GTPases, releasing them from the membrane and thus disrupting the actin cytoskeleton. In plants, AvrPphB proteolytic activity is shown to be essential for eliciting the HR response in plants. Just like the examples above, two plant genes, PBS1 (a kinase) and RPS5 (an R protein) are required for resistance to AvrPphB. The substrate of the AvrPphB protease in the plant is unknown. The authors predict AvrPphB cleaves PBS1. This change in state would activate RPS5 that in turn would induce an HR (Figure 1).

Kruger et al. (2002) report on a system in which an extracellular signal is monitored by a transmembrane R protein, Cf-2, in cultivated tomatoes. In contrast to the intracellular R proteins discussed above, this R protein has extracellular LRRs and a short intracellular domain. Cf-2 is required to induce an HR in response to the fungal pathogen *Cladosporium fulvum* expressing an Avr2 gene. The authors demonstrate that the tomato gene RCR3 is required for the function of Cf-2, much as RIN4, PTO, and PBS1 are required by their own cognate R genes. RCR3 is a secreted papain-like cysteine endoprotease, and although its interactions with Avr2 have yet to be worked out, this system can be formally described using a guard hypothesis. In this case, the R protein Cf-2 would monitor RCR3 in the extracellular space and trigger an HR in response to a change in state.

Taken together, these four papers show us a number of variations on the guard hypothesis. A given R protein may recognize the activities of multiple effectors. This is accomplished not by binding to the effector but by detecting physiological changes in the cell. These changes may take many forms; they could involve the binding of an effector protein to its target, the detection of covalent modifications, or proteolytic cleavage both inside and outside the cell (Figures 1, 2, and 3).

Plants Build a Network of Stakeouts in the Cell

Three of the guard proteins (RPM1, PRF, RPS5) described in these papers fall into the class of molecules having a TIR (or CC)-NBS-LRR structure (TIR, Toll/interleukin receptor; CC, coiled coil; NBS, nucleotide recognition; LRR, leucine-rich repeat) (Dangl and Jones, 2001). This class of genes forms a large family of which there are approximately 150 members in *Arabidopsis* (Initiative, 2000). What is the plant doing with so many R genes? These R genes could be used as individual sentinels to monitor the entire cellular metropolis (Figure 3). Alternatively, many R proteins could be used to man stakeouts of a small number of important targets.

Perhaps the best use of the film noir metaphor is as a predictive tool to reveal how bacteria counter a plant's defenses. If bacteria watched film noir they would learn that there are ways to avoid surveillance. First, detectives can be gagged. This approach is best demonstrated in vertebrate cells during *Yersinia* pathogenesis where the TTSA effector YOPJ has been found to inhibit NFkB signaling in animal cells and presumably blocks the innate immune response to this infection. Homologs of these effectors are found in plant pathogens and could serve an analagous function by blocking plant immune responses (Orth et al., 2000). Another technique is to raise a smoke screen to camouflage pathogenic interactions. Bacteria produce a number of TTSA effector proteins containing LRRs with as yet unassigned functions. It is possible that bacteria have coevolved molecules that act as competitive inhibitors by binding targets of R proteins, thus preventing guard protein binding (Staskawicz et al., 2001). A final trick is to perform the old switch-a-roo. The way this works in a movie is the detective is fooled into following a guy in a trenchcoat while the real mark is hauled into a van. In this case bacteria might synthesize molecules that mimic an unperturbed effector target and fool the R protein into thinking that nothing has changed when in reality the TTSA effector has completed its work. The human cytomegalovirus (CMV) uses such a trick. Normally, virus-infected cells can reduce surface expression of MHC to signal natural killer (NK) cells that the host cell is suffering from an infection. Cytomegalovirus expresses the protein UL18, which mimics MHC, tricking NK cells into acting as if all is clear (Reyburn et al., 1997). This blocks natural killer cell-mediated lysis of CMV-infected cells.

The recent data in plants demonstrate that there is probably not a gene-for-gene correspondence between avr and resistance genes. Rather, it is likely that structurally similar families of pathogen proteins can act through a given R protein. Stated this way, the plant immune surveillance system begins to sound like the "pattern recognition" model proposed for animal cells (Janeway and Medzhitov, 2002), whereby the animal cell uses receptors for common essential microbial products such

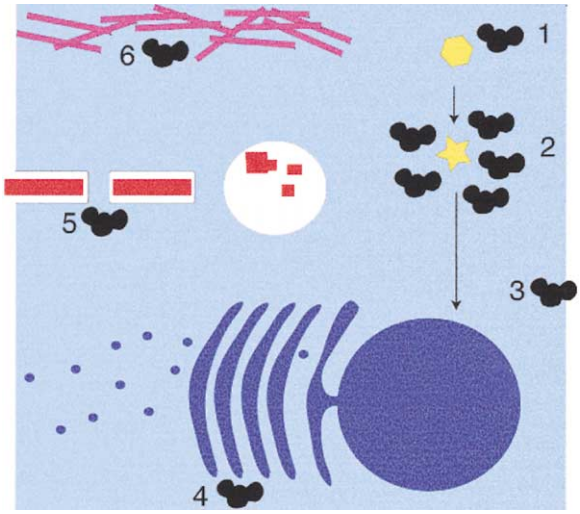

Figure 3. Guard Posts May Monitor Physiology throughout the Cell

(1) A single guard molecule monitoring a single signaling molecule. (2) A garrison of different guards monitoring a critical signaling point. (3) Monitoring the extracellular space. (4) Guarding the secretory apparatus. (5) Guarding the phagocytic apparatus. (6) Guarding the cytoskeleton.

as lipopolysaccharide (LPS) to detect signs of an infection. All bacteria are considered dangerous, whether or not they are causing disease. The plant, too, is detecting a pattern caused by a variety of microorganisms. Rather than monitor a microbial product, plant cells watch for alterations in their physiology. Described in this way, the HR response seems to be acting according to the danger hypothesis, "where there's smoke, there's fire" immune response. In this model, immune activation occurs when a cell detects a danger signal (Gallucci and Matzinger, 2001). These danger signals are molecules that are released by a pathogenic event such as cell lysis. This can be detected, for example, by measuring the extracellular concentration of heat shock proteins, which should not be found in an extracellular milieu.

The guard model fits aspects of two competing immune response models in animals. By providing another example of immune activation, plants remind us that there is likely a continuum of activation mechanisms. At one end, there are molecules that detect bacterial products. At the other end, a cell can look for gross signs of tissue damage, and in the middle lies a system that monitors bacterial products by looking at their effect on cellular physiology.

Do Animal Cell Detectives Monitor Infections?

Genome projects reveal that the R proteins form large families that are found not only in plant genomes but the human genome as well. Where are the corresponding animal R-like genes? Why haven't we seen gumshoe detectives on guard in vertebrates? Perhaps we already have evidence for the existence of these plant-like responses but we don't describe them in an appropriate manner to make them obvious.

In looking for similarities between immune responses in plant and animal cells, we might concentrate on infectious processes that resemble HR-producing infections in plants. This should include infections that result in apoptosis. Programmed cell death is a common outcome of bacterial infections in animal cells (Monack and Falkow, 2000). For example, secretion of TTSA effectors through the spi2 apparatus during a *Salmonella* infection both reorganizes endosome sorting in an infected macrophage and leads to death. Maybe these two events are linked because the host monitors endosome traffic and programmed cell death is activated if traffic is disrupted. Of course, the pathogen may evolve to double-cross the host and turn this response to its own advantage. The important point is that the type of response seen in the animal cells is similar to that seen in plants.

Toll plays an important role in immunity as a probable receptor in the innate immune system. Perhaps the most completely described system exists in the fly, in which the protein semmelweis (sem) is thought to be a direct receptor for gram-positive bacterial products, and this results in the activation of a Toll-mediated signal transduction cascade (Michel et al., 2001). Toll signaling can be described in terms of a guard system in which Toll monitors the state of sem. This description can be useful from an evolutionary perspective. Tolls are structurally related to R proteins and can be described as membrane-spanning R proteins with their binding domains in the extracellular space and their effector domains located intracellularly. Toll may have evolved from detectives monitoring physiological changes in the extracellular space. This description of Toll as a detective is useful for another reason. It is apparent that there is a continuum of mechanisms involved in the recognition of infections. This may also apply to the role of Toll proteins in immune function. We should be aware of such a possibility so that we do not accidentally label everything Toll does as a pattern recognition event. This same caveat applies to the Nod proteins, a group of mammalian intracellular proteins with structures similar to plant TIR-NBS-LRR R proteins.

Key questions remain in this who-dunnit. Who hires the detectives? Recent work suggests that R proteins signal through a ubiquitination-protein degradation pathway (Austin et al., 2002; Azevedo et al., 2002). At last, one of the signal transduction mechanisms of R proteins has been revealed. Now the targets of this degradation response must be identified. How do the detectives watch their marks? The actual surveillance mechanisms used by R proteins must be determined. The available data suggest that R proteins monitor changes in their targets, but we must determine whether they do this by monitoring wild-type proteins, modified proteins, or both. Last, if animal cells do use guard mechanisms, we must determine how these work. Cytoplasmic R-like proteins have been identified in humans, but surprisingly these have not been reported in *Drosophila*. Have flies recruited other proteins to their detective agency, or do insects lack this particular immune response?

Selected Reading

Austin, M.J., Muskett, P., Kahn, K., Feys, B.J., Jones, J.D., and Parker, J.E. (2002). Science *295*, 2077–2080.

Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K., and Schulze-Lefert, P. (2002). Science *295*, 2073–2076.

Dangl, J.L., and Jones, J.D. (2001). Nature *411*, 826–833.

Gallucci, S., and Matzinger, P. (2001). Curr. Opin. Immunol. *13*, 114–119.

Initiative, T.A.G. (2000). Nature *408*, 796–815.

Janeway, C.A., Jr., and Medzhitov, R. (2002). Annu. Rev. Immunol. *20*, 197–216.

Kim, Y.J., Lin, N.-C., and Martin, G.B. (2002). Cell *109*, 589–598.

Kruger, J., Thomas, C.M., Golstein, C., Dixon, M.S., Smoker, M., Tang, S., Mulder, L., and Jones, J.D. (2002). Science *296*, 744–747.

Mackey, D., Holt, B.F., Wiig, A., and Dangl, J.L. (2002). Cell *108*, 743–754.

Michel, T., Reichhart, J.M., Hoffmann, J.A., and Royet, J. (2001). Nature *414*, 756–759.

Monack, D., and Falkow, S. (2000). Int. J. Med. Microbiol. *290*, 7–13.

Orth, K., Xu, Z., Mudgett, M.B., Bao, Z.Q., Palmer, L.E., Bliska, J.B., Mangel, W.F., Staskawicz, B., and Dixon, J.E. (2000). Science *290*, 1594–1597.

Reyburn, H.T., Mandelboim, O., Vales-Gomez, M., Davis, D.M., Pazmany, L., and Strominger, J.L. (1997). Nature *386*, 514–517.

Shao, F., Merrit, P.M., Bao, Z.Q., Innes, R.W., and Dixon, J.E. (2002). Cell *109*, 575–588.

Staskawicz, B.J., Mudgett, M.B., Dangl, J.L., and Galan, J.E. (2001). Science *292*, 2285–2289.

Van der Biezen, E.A., and Jones, J.D. (1998). Trends Biochem. Sci. *23*, 454–456.