Current Biology, Vol. 13, R400–R402, May 13, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00321-X

Plant Pathology: Monitoring a Pathogen-Targeted Host Protein

Dispatch

Jeff Ellis and Peter Dodds

A plant protein RIN4 is targeted and modified by bacterial pathogens as part of the disease process. At least two host resistance proteins monitor this pathogen interference and trigger the plant's defence responses.

The way in which pathogens cause disease in plants, and how plants defend themselves against pathogens, are crucial research issues for global food security. The model plant *Arabidopsis thaliana* is playing an important part in unravelling these problems. The best way to control crop diseases is to use naturally occurring genetic mechanisms that have evolved in plants to counter pathogen attack. Plant disease resistance genes that detect infecting pathogens and trigger defence responses control the first step in the chain of defence. The products of disease resistance genes have long been considered to encode a pathogen-surveillance system. Recent discoveries [1–3] have shed important new light on just how this system functions.

The plant bacterial pathogen *Pseudomonas syringae* delivers effector proteins, often also called 'avirulence' (Avr) proteins, to plant cells via the type III secretion process. These proteins can enhance virulence in the absence of plant resistance genes [4,5]; but in the presence of host resistance genes, the same proteins can invoke strong defence responses leading to elimination of the pathogen. AvrB, AvrRpm1 and AvrRpt2 are three well-studied pathogen effector proteins. These three proteins exhibit no obvious sequence similarity, either to each other or to other proteins that suggest function but, in the absence of corresponding host resistance proteins, they do enhance pathogenicity [4,5]. It has now been shown that these proteins modify or eliminate the host protein RIN4. Two different host resistance proteins, RPM1 and RPS2, detect these modifications and trigger a reaction in the infected plant that involves expression of defence proteins and localized host cell death (Figure 1).

RIN4 is a 200 residue protein the sequence of which provides no clear clue to its function. Mutation of RIN4 causes —with one exception described below – no clear visual phenotype in *Arabidopsis*. RIN4, AvrB and AvrRpm1 co-immunoprecipitate and probably form part of a complex in infected plants (Figure 1). AvrB and AvrRpm1 induce modification of RIN4, revealed by a distinct shift in the protein's electrophoretic mobility [1]. This modification, which can be reversed by calf alkaline phosphatase treatment, is consistent with RIN4 phosphorylation. How AvrB and AvrRpm1, which have no protein kinase signatures, bring about this modification is yet to be determined. The delivery of the *Pseudomonas* effector protein AvrRpt2 to plant cells has a different effect — the post-translational elimination of RIN4 [2,3].

It is now proposed, but not yet proven, that RIN4 is an activator of the basal plant defence response. This is a low background level of defence, in the absence of which a diseased plant is even 'sicker' than normal. The modification or elimination of RIN4 is postulated to be a bacterial virulence mechanism that leads to reduced basal defence. There is indirect, but strong, evidence for this. Expression of a *AvrRpt2* transgene in *Arabidopsis* was found to cause a 10–50-fold increase in intercellular numbers of *Pseudomonas syringae* compared to non-transgenic controls [5]. Because AvrRpt2 causes elimination of RIN4, it is likely that the increase in bacterial titre in the diseased transgenic plants is a direct consequence of RIN4 elimination.

An alternative to the basal defence hypothesis is that the elimination of RIN4 leads to an increased nutritional status of the infected plant [5] — note that plant pathogenic bacteria are confined to the extracellular 'apoplast' compartment, which is nutritionally poor relative to the cytoplasm. Measuring the induced exression levels of the many known *Arabidopsis* defence genes — some of which have products that are secreted into the apoplast — during compatible plant–pathogen interactions in wild-type and *rin4* mutant plants should provide more evidence for or against the basal defence reduction model.

The Role of Resistance Proteins

In contrast to basal broad-spectrum defence, plant disease resistance (*R*) genes control high levels of disease resistance, commonly specific to particular pathogen strains. The *Arabidopsis* resistance protein RPS2 confers resistance to *Ps. syringae* strains that produce AvrRpt2, while the resistance protein RPM1 gives resistance to strains producing either AvrRpm1 or AvrB. Both these resistance proteins are members of the nucleotide binding–leucine-rich repeat (NBS-LRR) resistance protein family, and confer resistance to *Ps. syringae* by activating defence responses including plant cell death during early infection.

RPM1 and RPS2 both occur in complexes with RIN4 (Figure 1) and probably monitor pathogen-induced changes of RIN4. Even in the absence of pathogens, loss-of-function *rin4* mutations are lethal in *RPS2* plants but have no visible phenotype in *rps2* mutants [2,3]. These results imply RIN4 is a negative regulator of the death-inducing activity of RPS2, and that RPS2 monitors pathogen–induced degradation of RIN4 and triggers resistance when RIN4 levels drop.

RPM1 is only about 25% identical in sequence to RPS2, but it also activates cell death in response to *Ps. syringae* (*avrRPM1* or *avrB*). RPM1 functions differently to RPS2, however, in that it is probably activated by the AvrB/AvrRpm1-induced phosphorylation

CSIRO Plant Industry, GPO BOX 1600 Canberra ACT 2601, Australia. E-mail Jeff.Ellis@csiro.au

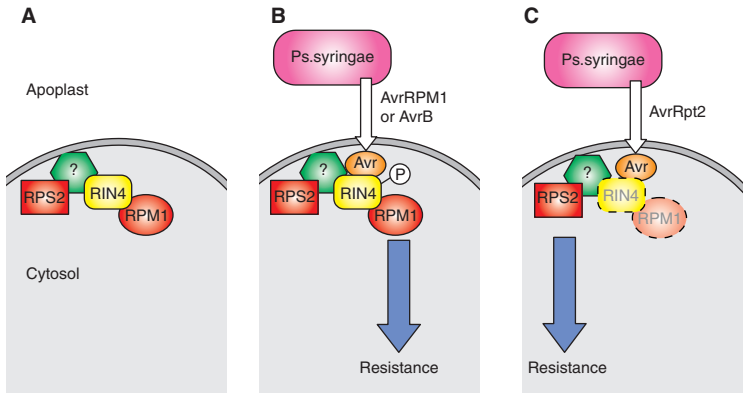

Figure 1. Indirect detection of *Ps. syringae* effector proteins in *Arabidopsis*.

(A) An R protein complex in *Arabidopsis*. Recent evidence [1–3] indicates that RPM1, RIN4 and RPS2 are components of a multiprotein complex (probably with other unidentified proteins). It is not yet known whether RPS2 interacts directly with RIN4, or is associated via intermediary proteins. RPS2 and RPM1 are both peripherally associated with the plasma membrane [3,9], although neither they nor RIN4 are predicted to be membrane bound; so some other component of the complex may be an integral plasma membrane protein. (B) AvrRPM1 and AvrB induction of RPM1 resistance. The *Ps. syringae* effectors AvrRpm1 and AvrB appear to interact directly with RIN4, leading to its phosphorylation and triggering signaling by the RPM1 which initiates a resistance response. The RIN4–AvrB interaction apparently does not affect RPS2, which can be co-immunoprecipitated with AvrB [10]. (C) AvrRpt2 induction of RPS2 resistance. The AvrRpt2 effector protein causes degradation of RIN4 and triggers RPS2-mediated resistance. It was suggested [1] that the RPM1 protein is degraded when RIN4 levels decline, but this was based on observations in plants containing *RPS2*, which mount a resistance response when RIN4 is reduced. As RPM1 is degraded early during *R* gene mediated-resistance responses [9], this response rather than the loss of RIN4 may be the cause of RPM1 degradation.

of RIN4, and not by the absence of RIN4. In fact, RPM1 resistance does not work in transgenic plants that make AvrRpt2, and so are predicted to be deficient in RIN4 as a result of AvrRpt2-directed elimination of the protein [5]. It would thus appear that RIN4 is a negative regulator of RPS2, but that phosphorylated RIN4 positively regulates RPM1's resistance activity. The involvement of RIN4 in resistance mediated by NBS-LRR proteins is however not universal. In transgenic plants that make AvrRpt2, where by inference RIN4 is degraded, resistance conferred by two other *R* genes, *RPS4* and *RPS5*, is not affected [5].

Evolutionary Implications

A logical evolutionary chain of events in the interaction between *Arabidopsis* and *Ps. syringae* is as follows. First, the host plant evolved a basal defense response involving RIN4; second, the pathogen evolved a means of subterfuge, for example modification of RIN4, that increases its virulence; and third, the host plant countered by evolving a RIN4 monitoring system, involving the *R* genes, allowing it to deploy active defense mechanisms. At this point, the pathogen appears to have adopted alternative strategies to alter the RIN4 target, degradation or phosphorylation, in an effort to circumvent the host's recognition mechanism. Many virulent strains have discarded both types of effector protein and rely on other effectors, the functions of which have not yet been elucidated but may also involve weakening host basal defense.

The observation that RPM1 and RPS2 detect pathogen effector-induced changes in a host protein, rather than the effector products themselves, means that the pathogen cannot easily escape being recognised by these resistance proteins simply by altering sequence features of its effector proteins. Any pathogen that delivers an effector protein variant that maintains its primary function of modifying RIN4 would still be recognized. Indeed, there is no sequence relationship between AvrB and AvrRpm1, yet both trigger RPM1-mediated resistance.

As resistance involves the recognition of effector function, and not effector sequence, there would be little selective pressure for *RPM1* to accumulate sequence variation to respond to alterations in the effector proteins it indirectly recognizes. Indeed, the *RPM1* gene sequence has remained constant over a long period of time, and the only polymorphism known at this locus is an alternative allele in which this gene has been deleted entirely [6]. These two alleles have been maintained by balancing selection in diverse *Arabidopsis* populations, and the allele frequencies have been proposed to fluctuate in response to pathogen pressure. The high frequency of the deletion allele in some populations also implies a fitness cost of the active allele in the absence of pathogens, possibly because occasional modification of RIN4 in healthy plants triggers unnecessary defense responses. The natural level of variation among *RPS2* alleles is also low and balanced polymorphisms between functional and non-functional alleles appear to occur [7]. Balanced polymorphisms between highly conserved functional resistance gene alleles and non-functional alleles may be indicative of resistance gene systems that involve detection of pathogen effector function via the monitoring of altered host proteins.

In contrast to *RPM1* and *RPS2*, many other plant *R* genes are characterized by high levels of variation between alleles or paralogs with different recognition specificities. In these cases, evidence for diversifying selection implies a relatively rapid evolution of new *R* gene variants. It may be that these signatures are indicative of R proteins that interact directly with effector proteins, as this type of interaction could lead to a situation where pathogen genes for effector proteins accumulate alterations that allow them to retain function yet escape detection. Here the corresponding resistance genes would then be selected to detect

the altered effectors. Of course, such direct R protein–effector protein interaction would not preclude the involvement of other host proteins in a recognition complex.

Finally, an interesting observation made in one of the recent papers [2] is that the genome sequence of *Arabidopsis* reveals about 200 potential resistance genes, which seems inadequate to counter a large number of potential pathogens each expressing large numbers of potential effectors — the *Ps. syringae* strain DC3000 genome alone encodes more than 30 predicted effectors [8]. The argument is that the relatively small number of *R* genes is sufficient to monitor a limited number of host targets of the diverse pathogen effectors. This may be the case, but considering the allelic diversity at many R gene loci, the approximately 200 potential *R* genes in the genome of the single sequenced individual genotype must greatly underestimate the level of variation in the *Arabidopsis* gene pool.

References

1. Mackey, D., Holt, B.F.,Wiig, A. and Dangl, J.L. (2002). RIN4 interacts with Pseudomonas syringae type III efector molecules and is required for RPM1-mediated resistance in A*rabidopisis*. Cell *108*, 743–754.
2. Mackey, D., Belkhadir, Y., Alonsos, J.M., Ecker, J.R. and Dangl, J.L. (2003). *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell *112*, 379–389.
3. Axtell, M.J. and Staskawicz B.J. (2003). Initiation of RPS2-specified disease resistance is coupled to AvrRpt2-directed elimination of RIN4. Cell *112*, 369–377.
4. Ritter, C. and Dangl, J.L. (1995). The *avrRpm*1 gene of *Pseudomonas syringae* pv *maculicola* is required for virulence on Arabidopsis. Mol. Plant Microbe Interact. *8*, 444–453.
5. Chen, Z., Kloek, A.P., Boch, J., Katagiri, F. and Kunkel, B. (2000). The Ps. syringae avrRpt2 gene product promotes pathogen virulence from inside plant cells. Mol. Plant Microbe Interact. *13*, 1312–1321.
6. Stahl, E.A., Dwyer, G., Maurico, R., Kreitman, M. and Bergelson, J. (1999). Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis.* Nature *400*, 667–670.
7. Mauricio, R., Stahl, E.A., Korves, T., Tian, D., Kreitman, M. and Bergelson, J. (2003) Natural selection for polymorphism in the disease resistance gene RPS2 of *Arabidopsis thaliana*. Genetics, *163*, 735–746.
8. Collmer, A., Lindeberg, M., Petnicki-Ocwieja, T., Schneider, D.J. and Alfano, J.R. (2002). Genomic mining type III secretion system effectors in Pseudomonas syringae yields new picks for all TTSS prospectors. Trends Microbiol. *10*, 462–469.
9. Boyes, D.C., Nam, J. and Dangl, J.L. (1998). The *Arabidopsis thaliana RPM1* disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensistive response. Proc. Natl. Acad. Sci. U.S.A. *95*, 15849–15854.
10. Leister, R.T. and Katagiri, F. (2000). A resistance gene product of a nucleotide binding site-leucine rich repeat class can form a complex with bacterial avirulence proteins *in vivo*. Plant J. *22*, 345–354.