

mediates post-segregational killing²⁷. Gerdes and co-workers²⁷ have shown that Sok (antisense) RNA binds to Hok (target) RNA and inhibits its translation, and that Sok does not initiate binding through loop-loop contact, but through its 5' tail (T. Franch, pers. commun.). When the Sok stem-loop is swapped for one derived from the antisense RNA of a different killer system, Sok is not inactivated. Therefore, only the pairing of the 5' tail is required.

As yet, no results from eukaryotic systems, natural or artificial, address the extent (and biological relevance) of antisense-sense RNA pairing. Even though the complexity of large folded RNAs makes an experimental approach to this question a formidable problem, artificial-antisense-RNA strategies would benefit from our finding out whether extended duplexes are formed. Maybe the key to success is not contiguous sequence complementarity but, rather, an emphasis on finding sequence windows in the target that are topologically and kinetically favored²⁸.

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References

- 1 Wagner, E. G. H. and Simons, R. W. (1994) *Annu. Rev. Microbiol.* 48, 713–742
- 2 Eguchi, Y., Itoh, T. and Tomizawa, J. (1991) *Annu. Rev. Biochem.* 60, 631–652
- 3 Nordström, K. (1990) *Cell* 63, 1121–1124
- 4 Austin, S. and Nordström, K. (1990) *Cell* 60, 351–354
- 5 Uhlin, B.-E. and Nordström, K. (1978) *Mol. Gen. Genet.* 165, 167–179
- 6 Pritchard, R. H., Barth, P. T. and Collins, J. (1969) *Symp. Soc. Gen. Microbiol.* 19, 263–297
- 7 Nordström, K., Molin, S. and Light, J. (1984) *Plasmid* 12, 71–90
- 8 Tomizawa, J. and Itoh, T. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 6096–6100
- 9 Stougaard, P., Molin, S. and Nordström, K. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 6008–6012
- 10 Brenner, M. and Tomizawa, J. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 405–409
- 11 Söderbom, F., Binnie, U., Masters, M. and Wagner, E. G. H. (1997) *Mol. Microbiol.* 26, 493–504
- 12 Brantl, S. and Wagner, E. G. H. (1996) *J. Mol. Biol.* 255, 275–288
- 13 Brantl, S. (1994) *Mol. Microbiol.* 14, 473–483
- 14 Brantl, S. and Wagner, E. G. H. (1997) *J. Bacteriol.* 179, 7016–7024
- 15 Zeiler, B. and Simons, R. W. (1998) in *RNA Structure and Function*, pp. 437–464, CSH Laboratory Press
- 16 Nordström, K. and Wagner, E. G. H. (1994) *Trends Biochem. Sci.* 19, 294–300
- 17 Tomizawa, J. (1984) *Cell* 38, 861–870
- 18 Tomizawa, J. (1990) *J. Mol. Biol.* 212, 683–694
- 19 Malmgren, C. et al. (1997) *J. Biol. Chem.* 272, 12508–12512
- 20 Malmgren, C., Engdahl, H. M., Romby, P. and Wagner, E. G. H. (1996) *RNA* 2, 1022–1032

- 21 Siemering, K. R., Praszquier, J. and Pittard, A. J. (1994) *J. Bacteriol.* 176, 2677–2688
- 22 Brantl, S. and Wagner, E. G. H. (1994) *EMBO J.* 13, 3599–3607
- 23 Novick, R. P. et al. (1989) *Cell* 59, 395–404
- 24 Blomberg, P., Wagner, E. G. H. and Nordström, K. (1990) *EMBO J.* 9, 2331–2340
- 25 Hjalt, T. Å. H. and Wagner, E. G. H. (1995) *Nucleic Acids Res.* 23, 580–587
- 26 Pörschke, D. and Eigen, M. (1971) *J. Mol. Biol.* 62, 361–381
- 27 Gerdes, K. et al. (1997) *Annu. Rev. Genet.* 31, 1–31
- 28 Patzel, V. and Sczakiel, G. (1998) *Nat. Biotechnol.* 16, 64–68
- 29 Wagner, E. G. H., Blomberg, P. and Nordström, K. (1992) *EMBO J.* 11, 1195–1203
- 30 Lin-Chao, S. and Cohen, S. N. (1991) *Cell* 65, 1233–1242
- 31 Delihias, N. (1995) *Mol. Microbiol.* 15, 411–414
- 32 Persson, C., Wagner, E. G. H. and Nordström, K. (1990) *EMBO J.* 9, 3777–3785
- 33 Wilson, I. W., Praszquier, J. and Pittard, A. J. (1994) *J. Bacteriol.* 176, 6497–6508

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Plant disease-resistance proteins and the gene-for-gene concept

More than 50 years ago, Harold Flor, working with flax and the flax rust fungus, defined plant-pathogen interactions genetically, producing the gene-for-gene hypothesis¹. This classic concept is based on the observation that disease resistance in plants commonly requires two complementary genes: an avirulence (*Avr*) gene in the pathogen and a matching, resistance (*R*) gene in the host. The biochemical interpretation of this hypothesis is a receptor-ligand model in which plants activate defence mechanisms upon R-protein-mediated recognition of pathogen-derived *Avr* products.

During pathogen infections of plants that lack corresponding R proteins, *Avr* products might function as virulence factors, subverting host cellular functions through interactions with plant-encoded pathogenicity targets². In order to combat infection, plants produce R proteins that specifically detect the appearance

of *Avr* products. For example, resistance against viruses involves recognition of the viral coat protein or the viral replicase¹. R-protein-mediated recognition of *Avr* products causes activation of host defences, which commonly are associated with calcium fluxes, generation of superoxide and nitric oxide, and localized plant cell death^{3–5}. Pathogens are able to evade recognition when the *Avr* proteins are lost or mutated¹. Both pathogen and plant have therefore developed specialized strategies to secure their survival and propagation.

Nucleotide-binding leucine-rich-repeat proteins are widespread

Jeff Ellis and co-workers have cloned the flax *L* alleles originally used by Flor to establish the gene-for-gene concept (Ref. 6 and J. Ellis, pers. commun.). *L* genes encode nucleotide-binding leucine-rich-repeat (NB-LRR) proteins

that constitute the major R-protein class in gene-for-gene plant resistance. More than 30 NB-LRR-encoding genes, often members of closely linked multigene families, have now been isolated from several plant species; these proteins provide resistance to pathogens as diverse as insects, nematodes, fungi, oomycetes, bacteria and viruses (Table I).

Hundreds of NB-LRR proteins are likely to be present in the model plant species *Arabidopsis thaliana*. The prevalence of NB-LRR proteins in various plant species is consistent with their proposed function as adaptable surveillance molecules for rapidly evolving pathogens¹. Thus, Flor's gene-for-gene model for R-*Avr* interaction conceptually parallels the vertebrate antibody-antigen interaction. The lack of a circulatory system in plants might be compensated for by a capacity to express each R protein constitutively in every cell that potentially could be attacked.

A biochemical model for nucleotide-binding leucine-rich-repeat proteins

NB-LRR proteins are predicted to be modular cytoplasmic molecules that consist of a C-terminal leucine-rich repeat (LRR) recognition domain connected,

Table I. Recognition of Avr proteins from diverse pathogens by nucleotide-binding leucine-rich-repeat (NB-LRR) proteins from several plant species

Pathogen	Species	Avr protein	Plant	NB-LRR protein	Refs
Aphid	<i>Macrosiphum euphorbiae</i>	?	Tomato	Mi-1	7 and P. Vos, pers. commun.
Nematodes	<i>Meloidogyne</i> sp.	?	Tomato	Mi-1	8 and P. Vos, pers. commun.
	<i>Globodera pallida</i>	?	Potato	Gpa2	W. Stiekema, pers. commun.
	<i>Heterodera avenae</i>	?	Wheat	Cre3	9
Fungi	<i>Melampsori lini</i>	?	Flax	L, L ¹⁻¹²	6 and J. Ellis, pers. commun.
	<i>M. lini</i>	?	Flax	M	6
	<i>Fusarium oxysporum</i>	?	Tomato	I ₂	10
	<i>Magnaporthe grisea</i>	Avr2-YAMO	Rice	Pi-ta	B. Valent, pers. commun.
Oomycetes	<i>Peronospora parasitica</i>	?	<i>Arabidopsis thaliana</i>	RPP1, RPP10, RPP14	11
	<i>P. parasitica</i>	?	<i>Arabidopsis</i>	RPP2	J. Beynon, pers. commun.
	<i>P. parasitica</i>	?	<i>Arabidopsis</i>	RPP5	1
	<i>P. parasitica</i>	?	<i>Arabidopsis</i>	RPP8	12
	<i>Bremia lactucae</i>	?	Lettuce	Dm3	13, 14
Bacteria	<i>Pseudomonas syringae</i>	AvrPto	Tomato	Prf	15
	<i>Ps. syringae</i>	AvrRpt2	<i>Arabidopsis</i>	RPS2	1
	<i>Ps. syringae</i>	AvrB/AvrRpm1	<i>Arabidopsis</i>	RPM1	1
	<i>Ps. syringae</i>	AvrRps4	<i>Arabidopsis</i>	RPS4	B. Staskawicz, pers. commun.
	<i>Ps. syringae</i>	AvrPphb	<i>Arabidopsis</i>	RPS5	16
	<i>Xanthomonas campestris</i>	?	Rice	Xa1	17
	<i>X. campestris</i>	AvrBs2	Pepper	Bs2	B. Staskawicz, pers. commun.
Viruses	Tobacco mosaic virus	Replicase	Tobacco	N	1
	Potato virus X	Coat protein	Potato	Rx	D. Baulcombe, pers. commun.

by a central nucleotide-binding adaptor, to various N-terminal effectors^{1,18}. Despite recent efforts, direct evidence for physical interaction between plant LRRs and Avr proteins is not yet available. However, LRR domains could create fast-adapting recognition surfaces for Avr signals¹⁹. In R proteins that possess extracellular LRRs, a predicted parallel β -sheet could function as a ligand-binding surface^{20,21}. Comparative analysis of NB-LRR proteins also shows that the predicted solvent-exposed residues of the β -sheets are hypervariable and subject to diversifying selection pressures. This indicates that recognition specificity resides in this part of the LRRs¹¹⁻¹³. By analysis of *in vivo*- and *in vitro*-generated recombinants between different flax *L* alleles, Ellis and colleagues confirmed experimentally that the LRRs constitute the principal determinant of specificity for Avr products⁶. Differential specificities of R proteins are often associated with duplications, deletions and sequence exchanges within the regions that encode the LRRs^{1,6,10-12,14}. These rearrangements are probably caused by unequal recombination and/or gene-conversion events involving allelic and linked homologues – processes that create variation in the β -sheet of the LRR domain²⁰.

The N-termini of the flax *L* proteins, like those of some other NB-LRR proteins, are similar to the cytoplasmic

effector domains of the *Drosophila melanogaster* and human TOLL and interleukin-1 receptors (the TIR domain)¹. Other NB-LRR proteins have different N-terminal domains, which often contain putative leucine-zipper (LZ) motifs. Mutational analysis in *Arabidopsis* revealed that TIR-NB-LRR and LZ-NB-LRR proteins employ distinct signalling pathways²². NB-LRR proteins that have a TIR effector domain signal through EDS1, a predicted lipase (J. Parker, pers. commun.) whereas most LZ-NB-LRR proteins examined employ the membrane-associated NDR1 protein^{22,23}. Both NB-LRR protein subclasses specify resistance to more than one pathogen type. This suggests that there is no relationship between a particular protein structure and a pathogen class, and that plants can employ different resistance pathways against the same pathogen²².

The central NB domain comprises three motifs predicted to bind ATP or GTP, and several conserved motifs whose functions are not known¹. This ~320-residue region has homology to two activators of apoptosis in animal cells: APAF-1 and CED-4. By analogy to these well-characterized regulators of programmed cell death, the corresponding domain in NB-LRR proteins might operate as an intramolecular signal transducer¹⁸. Domain swaps involving several flax *L* alleles reveal a requirement

for intramolecular interactions⁶ and, thus, NB-LRR proteins might serve as adaptor molecules that link recognition and signal delivery. For example, Avr signals perceived by the LRRs might initiate nucleotide hydrolysis at the NB domain. This might provide the energy necessary for a conformational change in the NB-LRR protein, exposing its N-terminal effector portion, to trigger a defence response.

The role of Prf in the Pto signalling pathway

Currently, one of the most thought-provoking examples of gene-for-gene pathogen recognition is tomato resistance to bacterial speck disease (Fig. 1). Resistance involves recognition of an 18.3-kDa bacterial protein, AvrPto, by two host proteins, Pto and Prf^{15,24}. Pto is a Ser/Thr kinase that interacts physically with the AvrPto protein, can interact with and phosphorylate a second Ser/Thr kinase, Pti1 and can bind to several defence-related transcription factors, Pti4, Pti5 and Pti6. Within the *Pto* gene cluster lies *Fen*, a *Pto* homologue that confers sensitivity to the cell-death-inducing organophosphorous insecticide, fenthion. Prf is an NB-LRR protein required for both Pto and Fen function¹⁵. Current models state that resistance to bacterial speck disease is mediated by a phosphorylation cascade triggered by the Pto kinase after

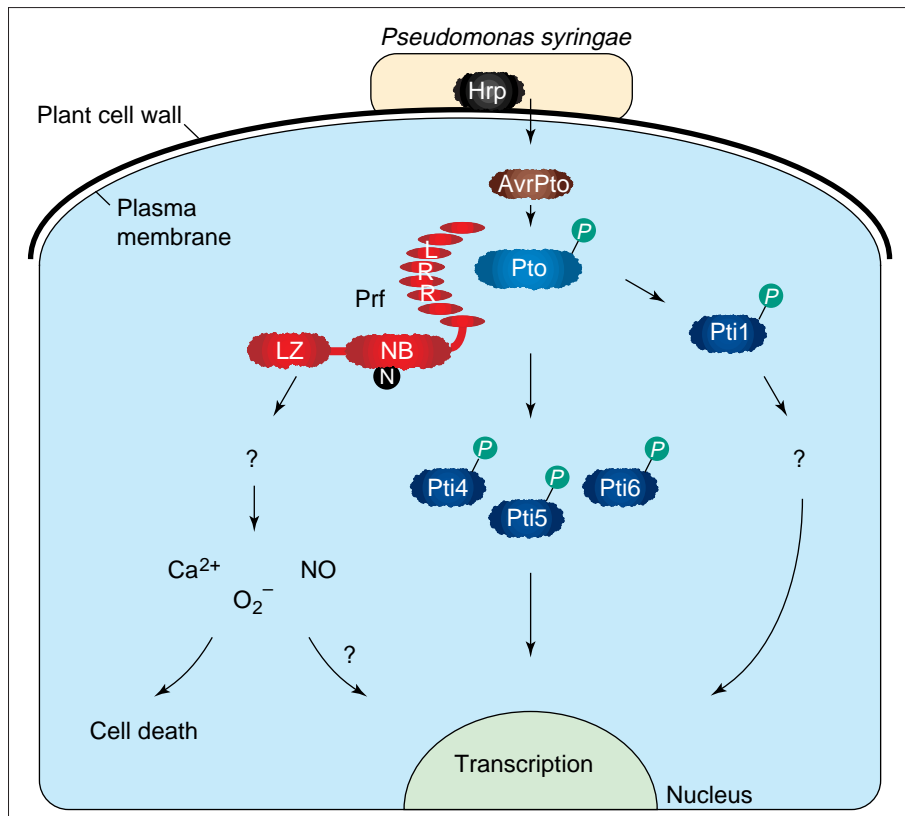


Figure 1

Model for the function of the tomato nucleotide-binding leucine-rich-repeat (NB-LRR) protein Prf in Pto-mediated recognition of the bacterial AvrPto protein. Hrp, contact-dependent bacterial (type III) secretion system; LRR, leucine-rich repeat domain; LZ, leucine-zipper motif; N, NTP; NB, nucleotide-binding site; NO, nitric oxide; P, phosphate.

specific recognition of the bacterial AvrPto protein²⁴. The function of Prf in this proposed signal transduction pathway is still unknown.

The prevailing role of the NB-LRR proteins in gene-for-gene interactions suggests that Prf is the key recognition component in Pto-mediated resistance. The Pto kinase does not require AvrPto for interaction with the Ptis in the yeast two-hybrid system, or for *in vitro* phosphorylation of Pti1. Binding of AvrPto might therefore abolish the interaction between Pto and the Ptis and, thereby, negate the basal resistance conferred by Pto. Thus, AvrPto could be a virulence factor that has found its pathogenicity target in the Pto kinase (Fig. 1). Prf could have evolved subsequently in order to recognize an AvrPto-activated molecule, such as the AvrPto-Pto complex, and thereby initiate the defence response. Similarly, Prf could induce cell death upon recognition of a fenthion-activated molecule, such as a fenthion-Fen complex. Dual recognition specificity has indeed been observed for other NB-LRR proteins^{1,7,8}. In the above scenario, the Prf-AvrPto-Pto recognition complex has evolved from an AvrPto-Pto pathogenicity complex.

Conclusion

In plants, the NB-LRR proteins specify gene-for-gene resistance to animal, fungal, bacterial and viral pathogens, and collectively constitute a comprehensive pathogen-detection system. This innate, genetic recognition-response apparatus resembles the animal immune system. R proteins might detect the association of plant pathogenicity targets with pathogen virulence factors that are then destined to become Avr products. Modification of host metabolism or suppression of basal resistance mechanisms through Avr interaction with pathogenicity targets would result in enhanced virulence. Indeed, many *Arabidopsis* genes, when mutated, confer enhanced disease susceptibility²⁵. Conceivably, one particular Avr product could correspond to one specific pathogenicity target, which, in turn, could be safeguarded by one matching R protein. In order to adapt rapidly to pathogen Avr modification or loss, novel recognition specificities in R proteins are created through the generation of sequence variation in the β -sheet of the LRR domain.

Much needs to be learned about the biochemistry of NB-LRR proteins.

How is a pathogen signal perceived by the LRR domain and transduced intramolecularly to activate defence signalling? So far, little is understood about the nature of the Avr proteins and their possible virulence function. We keenly anticipate the cloning of Avr proteins from insects, nematodes and fungi, and the identification of their direct host targets.

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References

- 1 Hammond-Kosack, K. E. and Jones, J. D. G. (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 575–607
- 2 Collmer, A. (1998) *Curr. Opin. Plant Biol.* 1, 329–335
- 3 Hammond-Kosack, K. E. and Jones, J. D. G. (1996) *Plant Cell* 8, 1773–1791
- 4 Delledone, M. *et al.* (1998) *Nature* 394, 585–588
- 5 Durner, J. *et al.* (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 10328–10333
- 6 Ellis, J. *et al.* (1997) *Annu. Rev. Phytopathol.* 35, 271–291
- 7 Rossi, M. *et al.* (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 1663–1668
- 8 Milligan, S. B. *et al.* (1998) *Plant Cell* 10, 1307–1319
- 9 Lagudah, E. S., Moullet, O. and Appels, R. (1997) *Genome* 40, 659–665
- 10 Simons, G. *et al.* (1998) *Plant Cell* 10, 1055–1068
- 11 Botella, M. A. *et al.* *Plant Cell* (in press)
- 12 McDowell, J. M. *et al.* *Plant Cell* (in press)
- 13 Meyers, B. C. *et al.* *Plant Cell* (in press)
- 14 Meyers, B. C. *et al.* *Plant Cell* (in press)
- 15 Oldroyd, G. E. D. and Staskawicz, B. J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 10300–10305
- 16 Warren, R. F. *et al.* (1998) *Plant Cell* 10, 1439–1452
- 17 Yoshimura, S. *et al.* (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 1663–1668
- 18 Van der Biezen, E. A. and Jones, J. D. G. (1998) *Curr. Biol.* 8, R226–R227
- 19 Jones, D. A. and Jones, J. D. G. (1996) *Adv. Bot. Res.* 24, 89–167
- 20 Parniske, M. *et al.* (1997) *Cell* 91, 821–832
- 21 Thomas, C. M. *et al.* (1997) *Plant Cell* 9, 2209–2224
- 22 Aarts, N. *et al.* (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 10306–10311
- 23 Century, K. S. *et al.* (1997) *Science* 278, 1963–1965
- 24 Frederick, R. D. *et al.* (1998) *Mol. Cell* 2, 241–245
- 25 Rogers, E. E. and Ausubel, F. M. (1997) *Plant Cell* 9, 305–316

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