# Structure, function and evolution of plant disease resistance genes

Jeff Ellis*, Peter Dodds and Tony Pryor

Gene-for-gene plant disease resistance involves two basic processes: perception of pathogen attack, followed by responses to limit disease. Perception involves receptors with high degrees of specificity for pathogen strains, which are encoded by disease resistance genes. Large repertoires of distantly related resistance (R) genes with diverse recognitional specificities are found within a single plant species. The generation of R-gene polymorphism involves gene duplication, followed by DNA-sequence divergence by point mutation, and by deletion and duplication of intragenic DNA repeats encoding blocks of leucine-rich elements. Recombination between related genes reassorts this variation to further diversify gene sequences. Pathogen pressure selects functional resistance specificities and results in the maintenance of R-gene diversity. Recent genome-sequence data reveal that the NBS-LRR (i.e. nucleotide-binding site-leucine-rich repeat) class of R genes represents as much as 1% of the *Arabidopsis* genome. Experimental data have shown that the LRR has a role in determination of specificity. Mutation experiments, in which R-gene signaling has been dissociated from specificity in constitutive signal mutants, have provided the potential for non-specific resistance to be expressed from pathogen-infection-induced promoters in transgenic plants.

**Addresses**
CSIRO-Plant Industry, GPO Box 1600, Canberra, Australian Capital Territory 2601, Australia
*e-mail: ellisj@pi.csiro.au

**Current Opinion in Plant Biology** 2000, **3**:278–284

1369-5266/00/$ – see front matter


**Abbreviations**

| | |
|---|---|
| ***avr*** | *avirulence* |
| **CLV** | CLAVATA |
| ***Dm3*** | *downy mildew 3* |
| **LRR** | leucine-rich repeat |
| **NBS** | nucleotide-binding site |
| **PK** | protein kinase |
| ***Prf*** | *Pseudomonas resistance and fenthion sensitivity* |
| ***Pto*** | *Pseudomonas tomato resistance* |
| **R** | resistance |
| ***RPP5*** | *resistance to Peronospora parasitica 5* |
| ***RPS2*** | *resistance to Pseudomonas syringae 2* |
| **TIR** | Toll/interleukin-1-receptor homology region |
| **TMV** | tobacco mosaic virus |

## Introduction

Five classes of gene-for-gene disease resistance (R) genes have been defined according to the structural characteristics of their predicted protein products (see [1,2] for recent reviews published in this series). Data from the genetic analysis of plant–pathogen interactions and more recent, but limited, data from molecular analysis support the model in which the products of R genes act as receptors for the direct or indirect products (i.e. ligands) of pathogen *avirulence* (*avr*) genes. The receptor–ligand interactions are very specific and mutations that modify or inactivate *avr* genes allow pathogens to avoid recognition. Thus, two pertinent evolutionary questions are what is the molecular basis of R-gene specificity and how do new resistance specificities evolve?

## NBS-LRR genes

The majority of R genes cloned so far encode proteins with a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region. Several NBS-LRR-containing R genes have been cloned for the first time in the past year [3•–5•,6,7•,8•]. Although extremely divergent in DNA sequence, the gene products of the NBS-LRR class are readily recognised by several distinctive motifs in their amino-terminal half, which are conserved in amino-acid sequence and order, and by carboxy-terminal LRRs [9•]. The NBS-LRR class of genes is abundant in plant species. For example, in *Arabidopsis*, it is estimated that at least 200 different NBS-LRR genes exist comprising up to 1% of the genome [9•]. Two major subclasses exist [1,9•]: one with an amino-terminal Toll/interleukin-1-receptor homology region (TIR) and another without the TIR region. Some surprising observations have come from recent genomic analysis. First, the TIR-NBS-LRR subclass has not been recognised in the grasses in spite of the fact that this subclass is predominant (i.e. comprises 75% of all NBS-LRR genes) in *Arabidopsis* and is known in at least one gymnosperm [9•]. Whether the absence of the TIR class is a feature of all monocots or just grasses is unknown. Second, in *Arabidopsis*, a number of genes that are predicted to encode only the TIR region were detected [9•]. Further analysis will be needed to confirm the status of these genes.

Two complete haplotypes (i.e. sets of genes in a complex locus) of the *RPP5* (*resistance to Peronospora parasitica*) locus containing TIR-NBS-LRR genes in *Arabidopsis* have recently been completely sequenced and analysed [10••]. Nine paralogues (i.e. adjacent related genes that have arisen by duplication) are found in the Landsberg *erecta* (L*er*) haplotype and seven paralogues are found in the Columbia ecotype (Col-0). Only one gene in L*er* (the *RPP5* resistance specificity) and two in Col-0 are predicted to encode a full-length TIR-NBS-LRR gene. The others contain premature stop codons or retrotransposon insertions. The two haplotypes are distinguished from the genes flanking the locus by their high level of polymorphism. This polymorphism includes the position and sequence of retrotransposon insertions in the two loci.

The Mla locus (which encodes powdery mildew resistance) in barley provides a further indication of the sequence complexity of NBS-LRR loci [11]. Here, three distinct families of NBS-LRR genes are found (with inter-family amino-acid similarities of 46–51% and no overall

significant DNA similarity) within a 240 kilobase interval in which family members are interspersed.

## The extracellular LRR class

The extracellular LRR class of R genes includes the rice *Xa21* gene for resistance against bacterial blight (*Xanthomonas* resistance) and the *Cf* genes of tomato for resistance against the fungal pathogen *Cladosporium fulvum*. The *Xa21* product has the classic receptor-kinase format — an extracellular LRR, a membrane-spanning region and an intracellular protein-kinase domain (see [1] for references of the earlier literature). The *Cf* gene products contain extracellular LRRs and a transmembrane domain, but lack a significant intracellular region that could constitute a signalling component (e.g. a protein kinase domain [1]). How the proposed Cf receptors transduce signals across the cell membrane remains unknown. The molecular analysis of the CLAVATA (CLV) system in *Arabidopsis,* which is involved in the development of the shoot meristem, has, however, recently provided clues to the nature of this signal [12•].

Three components of the CLV system have been identified: first, CLV1, a transmembrane LRR receptor kinase, which is analogous to Xa21; second, CLV2, an extracellular LRR protein with a membrane-spanning anchor, which is structurally analogous to Cf proteins; and third, CLV3, a small extracellular protein that is the potential ligand that interacts with and cements an activated signalling complex involving CLV1 and CLV2. Whereas the *Cladosporium*-encoded small avirulence proteins (e.g. Avr9) may be analogous to CLV3, the predicted transmembrane receptor-kinase component of the tomato Cf system remains to be identified. Additional support for this model is provided by the observation that one member of the *Xa21*-complex locus in rice, *Xa21D* [13], encodes a truncated homologue of *Xa21* that is structurally analogous to Cf proteins, having only LRR and transmembrane domains. *Xa21D* confers partial resistance to bacterial blight in transgenic rice. The extracellular LRR class of disease resistance genes may well have evolved by recruitment of genes that were initially responsible for developmental processes in multicellular organisms. It is interesting that the Toll signalling pathway in flies and mammals also has a dual role in development and pathogen resistance [14].

## The *Pseudomonas tomato resistance* (*Pto*) gene

The *Pto* gene for bacterial speck resistance in tomato, which encodes a serine/threonine protein kinase (PK) with no LRR region, requires the presence of the linked NBS-LRR gene *Prf* (*Pseudomonas resistance and fenthion sensitivity*) for activity (see [1,2] for earlier original references). No other R genes in the PK class have been identified to date. An extensive mutational analysis has been reported recently that confirms that *Pto* is the receptor for the corresponding ligand encoded by the bacterial *avr* gene *Avr–Pto*, and that the *Prf* gene does not act upstream of *Pto* in signalling the resistance response [15••]. The molecular analysis of the *Pto–Prf* system is among the most elegant and detailed studies of R-gene function.

## The molecular basis of R-gene specificity

The flax *L* gene, a member of the TIR-NBS-LRR subclass, has provided an excellent system in which to analyse the molecular basis of R-gene specificity. Eleven alleles of the flax *L* gene, ten of which encode different flax rust resistance specificities, have been sequenced [16••]. The comparison of the allele sequences revealed that most alleles contain polymorphic bases spread across the whole coding region, with the largest variation in the LRR-coding region. Comparison of the predicted amino-acid sequences encoded by the most closely related pairs of alleles provides information concerning regions of the polypeptide that are important for the differences in gene-for-gene specificity. For example, the L6 and L11 proteins, which are identical in the TIR and NBS regions, differ by 33 amino-acid substitutions in the LRR. This indicates that the differences between *L6* and *L11* resistance specificities are caused by differences in their LRR regions. *In vitro* exchanges between alleles and analysis of transgenic plants into which the resulting hybrid *L* genes have been introduced also indicate the importance of LRR variation in specificity differences. Nevertheless, *L6* and *L7*, which have different specificities, differ only in the sequences encoding the amino-terminal TIR region, which indicates that polymorphism in this region can also affect resistance specificity.

## Evolution of R genes and specificities

For an increasing number of R genes, including the NBS-LRR genes, evidence of the selection for diversity of codons encoding residues in the LRR region that are predicted to be solvent exposed, and hence may constitute ligand contact points, has been observed [6,10••,13,17–20]. Like the initial analysis of *Cf* genes in tomato [17], subsequent comparison of DNA sequences within NBS-LRR gene loci has revealed evidence of past exchanges of blocks of sequence by recombination [6,10••,16••]. Whether such exchanges occur by sequential crossing over or gene conversion has not been determined. DNA-sequence analysis also provides evidence for recombinational events that increase and decrease the size of the LRR region.

Modification of the length of the LRR appears to be an important contributor to R-gene diversification. For example, whereas the genes at the *Cf4/9* locus of tomato vary principally because of multiple nucleotide substitutions, the related genes at the unlinked *Cf2/5* locus have additionally undergone deletion/expansion events involving individual LRR-repeat units [21]. Furthermore, these events have been restricted to the amino-terminal LRR region of the protein, a region of Cf proteins that determines specificity differences between paralogues [22]. In flax *L* alleles, the LRR repeats are more degenerate and the DNA sequences encoding the repeats are probably not sufficiently related for inter-repeat recombination. Nevertheless, examples occur in which blocks of sequence

encoding LRR units within flax and *Arabidopsis* NBS-LRR R genes have undergone duplication [10••,16••]. These direct repeats then are able to undergo unequal exchange events that can give rise to cycles of repeat expansion and reduction. For example, although most *L* alleles in flax contain two direct repeats of 450 base pairs comprising six individual LRR repeat units, functional alleles with either one or four copies of the 450-base-pair repeat occur [16••]. Another example is provided by the *RPP5* locus where more complex arrangements of direct repeats consisting of sets of four individual LRR units exist [10••]. Exchange events giving rise to paralogues with 8, 13, 21 and 25 LRR units have taken place during the evolution of the locus.

Mutant R genes resulting from expansion and contraction of LRR-region repeats have been recovered from genetic experiments in both flax and *Arabidopsis* [23,24]. Thus, whereas point mutation can alter specificity by varying the identity of potential ligand contact points in the LRR, recombination/gene conversion can play a dual role in reassorting variation in alleles and paralogues, and also by reduction and expansion of the number of LRR units. The reduction/expansion events could change the spatial distribution of ligand contact points and adjust either affinity or specificity for different ligands.

## The role of unequal exchange events at complex R loci

Re-assortment of sequence polymorphism by meiotic recombination is a principal factor in R-gene evolution. Where R genes exist as complexes of directly repeated genes that are related in sequence, two alternatives for sequence exchange are possible. First, 'equal exchange' in which the first gene in the complex may only recombine with the first gene in the homologous complex, the second gene with the second homologue, and so on. Second, 'unequal exchange' in which each gene in the sequence may recombine with any other gene in the homologous complex. Sequential unequal exchange between ribosomal RNA genes, for example, either by crossing-over or gene conversion, results in rapid homogenization of sequence differences so that paralogues tend to be nearly identical. Because homogenization is not observed for genes at the *Dm3* (*downy mildew 3*) and *Pto* loci, where individual genes in the complexes have more similarity with orthologues in related species than with paralogues [19,25], Michelmore and Meyers [25] argue that unequal exchanges have been less important than equal exchanges in the evolution of R genes. These arguments ignore the role of selection. For ribosomal RNA genes, purifying selection acts to maintain homogeneity in sequence. In contrast, R genes are subjected to diversifying selection.

Furthermore, the simple notion of tandem R genes, in which the first gene in one haplotype is most related to the first in a homologous haplotype, is not borne out by the complete sequencing of the *RPP5* haplotype from two ecotypes of *Arabidopsis* [10••]. Haplotypes can contain different numbers of genes and, as observed for the *RPP5* locus, the degree of sequence similarity between genes from different haplotypes does not necessarily reflect their position in the cluster [10••]. The probability of paralogues pairing during meiosis is likely to be proportional to the level of sequence similarity between the interacting gene sequences and is also likely to be influenced by intergenic sequences. Recombination between highly diverged sequences at complex loci is probably rare compared to recombination between closely related genes. In loci with highly diverged paralogues, such as Dm3, recombination may therefore be limited to closely related members, thus preserving sequence relationships between orthologues in related species. Sequence exchange between paralogues may, however, be more common where greater sequence similarity exists.

Importantly in plant populations, the frequency of chimeric alleles resulting from equal versus unequal exchange is probably not determined solely by the frequency of the exchange event but also by selection for novel resistance specificities that arise from these processes. Thus, unequal recombination events, which give rise to chimeric genes ([4•]; Q Sun, N Collins, T Pryor, S Hulbert, unpublished data), are observed experimentally in homozygotes for the Rp1D rust resistance locus in maize and inferred from sequence analysis of the *RPP8* locus in *Arabidopsis* [6]. Furthermore, the patchwork of sequence variation shared between paralogues of *Cf* genes [17], *RPP5* [10••], *RPP1* [20] and *RPP8* [6] also indicates that exchange occurs between paralogues. There is also initial evidence for exchange of information between distinct *Cf* loci (i.e. 'ectopic exchange' [26•]).

## Molecular population genetics of R genes

Population genetic analysis of wild plant species can provide information concerning the frequencies and diversity of resistance alleles in nature, and on the selection forces maintaining resistance and leading to the evolution of new specificities in natural populations. The high level of genomic/molecular biological information that is accumulating on *Arabidopsis* and *Arabidopsis*–pathogen interactions is stimulating the increased use of this wild plant in population analysis of host–pathogen interactions [27•,28•].

The data on *Arabidopsis* genes that are known to have a function in resistance are beginning to indicate some differences in the nature of certain NBS-LRR resistance loci. *RPP1*, *RPP5* and *RPP8* are found in complex loci with from two to nine paralogues, and each locus contains two or more identified resistance specificities (e.g. the virus resistance gene *HRT* is a paralogue of *RPP8* [29•]). The genes at each locus are highly polymorphic, have been subjected to diversifying selection and their sequences provide evidence for recombination. These features are shared by the 'classical' resistance genes that have been identified in crop plants, such as the *Cf* genes of tomato [17,21,22], the *L* and *M* rust-resistance genes of flax [16••,23], the *Rp1* rust-resistance genes of maize [4•], the tobacco mosaic virus (TMV)-resistance gene *N* (see [1] for original references), the potato

virus X (PVX)-resistance locus *Rx* [3•], and the *Dm3* downy mildew resistance locus of lettuce [19].

In contrast, *RPS2* (*resistance to Pseudomonas syringae 2*) [27•], *RPM1* (a gene conferring the ability to recognize *Pseudomonas* pathogens carrying *AvrRpm1* or *AvrB*) [28•] and *RPS4* [5•] are simple loci containing a single gene with only minor allelic variation in DNA sequence. There are no indications or reports of these genes having undergone diversifying selection, and they each have only a single identified resistance specificity. (*RPS4* and its homologues are somewhat unusual in that each gene is found in close proximity to a second divergently transcribed NBS-LRR gene with which it shares only 35% nucleotide identity [5•].) Some of these features are similar to the tomato NBS-LRR gene *PRF* (see [2] for references), the sequence of which is highly conserved within tomato.

Genes in the first (more complex) class have been identified using an oomycete pathogen species, *Peronsospora parasitica*, which occurs in natural field infections of *Arabidopsis*. Genes in the second (less complex) group have been identified in the laboratory using bacterial pathogens isolated from non-*Arabidopsis* hosts, and represent *Arabidopsis*–pathogen interactions that, to our knowledge, have not been described in the field. Whether these distinctions will be maintained after more in-depth investigation remains to be seen. Nevertheless, two interesting questions remain unanswered. First, do the apparently contrasting characteristics of the two classes of *Arabidopsis* genes reflect the different nature of the pathogens, that is, oomycetes versus bacteria? Second, and more interestingly, do the different characteristics of the two groups result from the fact that the first class is subject to co-evolutionary pressures from a field pathogen and the second class is not? An open mind needs to be maintained on whether some of the second class of genes are primarily involved in an as yet unidentified function other than classical gene-for-gene or race-specific disease resistance. Perhaps these genes are involved in non-host resistance, that is, resistance to pathogen species that are adapted to another host species. In the absence of co-evolution of host and pathogen, diversifying selection would not affect the second class. These considerations may be important in the choice of pathosystems for study and the interpretation of results from population-genetic analyses in *Arabidopsis*.

A popular metaphor in the plant R-gene evolutionary/population biology literature sees the co-evolution of R genes and pathogen avirulence as an 'arms race'. This metaphor has provided a useful conceptual framework for the consideration of the evolution of multiple disease resistance specificities. In this scenario, the effectiveness of an R-gene specificity in the host is lost (defeated) as a result of mutation of the corresponding pathogen Avr gene so that the pathogen avoids recognition. This, in turn, imposes selection pressure on the host for new resistance specificities, which may arise at the 'defeated' locus or elsewhere in the genome, and the cycle continues. There is accumulating evidence (see [30]) that Avr genes have a positive function in virulence and hence confer a selective advantage to pathogens in the absence of a corresponding R gene. Thus, a newly 'defeated' (but still functional) R gene and its cognate Avr gene may be maintained in the host and pathogen for long periods by balancing selection, and fluctuate in frequency. Furthermore, selection in the pathogen for novel Avr genes is also predicted by this model. These predictions are in agreement with the observations of high levels of polymorphism for resistance and avirulence in natural host/pathogen populations [31,32].

One recent evolutionary study provides a detailed examination of the *RPM1* locus (*P. syringae* resistance in the laboratory) of *Arabidopsis* [28•]. Using a collection of 26 ecotypes of diverse provenance, the authors confirm an earlier observation, which was based on fewer ecotypes, that the resistance is associated with a single NBS-LRR gene whereas susceptibility is always associated with a large deletion covering *RPM1*. Both alleles are found widely in *Arabidopsis* throughout its natural distribution. From a molecular evolutionary analysis of DNA-sequence polymorphisms flanking the locus, Stahl *et al.* [28•] establish that the two alleles are ancient ($10^6$ years old) have been maintained by balancing selection and have fluctuated in frequency. In the absence of an identified field pathogen that interacts in a gene-for-gene sense with *RPM1*, the nature of this selection for active *RPM1* is speculative. Interestingly, the maintenance of the deletion allele suggests that under certain conditions, the active allele imposes a genetic load on the host. What this fitness cost may be is an interesting question. So is the question of why the null allele is only represented by a deletion and not by insertion or point mutations, which frequently inactivate other NBS-LRR R genes in *Arabidopsis* [10••]. Stahl *et al.* [28•] also make a critical analysis of the 'arms race' model, which they propose predicts that 'variation for disease resistance will be transient, and that host populations generally will be monomorphic at disease-resistance loci'. The model is correctly rejected on the basis that, first, resistance and susceptibility alleles have existed at *RPM1* for $10^6$ years, and second, plant populations in general show considerable variation at R-gene loci. Rejection of the model is, however, contingent on this over-restrictive interpretation of the 'arms race' metaphor.

## Downstream resistance signalling components

Although not the topic of this review, one recent report is relevant to R-gene evolution. The authors cloned the *Bs2* gene (for *Xanthomonas* blight resistance) from pepper and demonstrated that it functions in several Solanaceous species but not in species outside of the Solanceae [7•]. One interpretation of this observation is that downstream components of R-gene signalling pathways are co-adapted within species to particular R-gene products; a phenomenon referred to as 'restricted taxonomic functionality' [7•]. This is somewhat surprising considering the ubiquity of NBS-LRR genes in all plant species and extensive

variation of NBS-LRR family members within species. From the practical standpoint, this observation suggests that successful trans-species transfer of R genes by genetic engineering may be limited to closely related genera unless the downstream components of resistance signalling are also transferred and are able to engage with the more terminal signalling components in the new species. Experiments already in progress will no doubt provide further insights.

## Conclusions and future directions

Significant progress has been made during the past year in understanding the determinants of R-gene specificity and how these specificities evolve. In particular, mutational analysis of *Pto* in tomato and recombinational analysis of *L* alleles in flax have identified features of the two distinct classes of proteins encoded by these genes that are involved in recognition and signalling processes. In addition, the large-scale sequence analysis of complex R-gene haplotypes has shed light on the processes of diversifying selection, sequence exchange, and expansion/contraction of LRRs that underlie the evolution of new resistance specificities. Important questions that remain include the nature of the interaction between R-gene products (other than Pto) and their cognate avirulence proteins. An intriguing and plausible model has emerged for Cf-9–Avr-9 interaction and requires testing. It will be interesting to see whether other R–Avr interactions also involve a ternary (or higher order) complex. We also look forward to the molecular analysis of population genetics of R genes from multi-allelic series or complex loci in wild-plant–pathogen ecosystems to shed further light on the nature of the selection processes acting on these loci.

## Update

Since the submission of this review several new publications relevant to this topic have appeared. The cloning of a further *Peronospora* resistance gene from *Arabidopsis*, *RPP13*, has been reported [33]. Three specificities have been identified at the locus, which appears to be a single gene with highly variable multiple alleles that are subject to diversifying selection in the LRR region.

The first analysis demonstrating a biological role of alternative products, a feature shared by all TIR-NBS-LRR resistance genes, has been carried out for the N gene of tobacco (which provides resistance to TMV) [34]. *In vitro*-constructed variants of *N*, which in transgenic plants encode only the major of the two alternatively spliced messages associated with wild-type gene transcripts, retain the capacity to detect TMV and induce HR. However, the R gene is 'weakened' in these variants in which, unlike in the wild-type, TMV escapes the HR lesion, systemically infects the host plant, then induces HR at locations distant to the infection site. Interestingly, the ratio between the two alternative mRNA products of the wild-type gene inverts during the first seven hours after the infection of *NN* plants and then returns to the pre-infection state. The full implications of these observations to *N* gene and other TIR-NBS-LRR gene functions are awaited.

The first report of a plant gene isolated in a yeast two-hybrid screen using a TIR-NBS-LRR gene has appeared [35]. The NBS domain (now named the NB-ARC domain to highlight the shared similarities of this region with human Apaf-1, plant R-proteins and nematode CED-4 proteins) of RPP5 interacts with a plant protein that is similar to the bacterial proteins RelA/SpoT, which are involved in signaling during the synthesis/degradation of guanosine phosphates, (p)ppGpp. Such proteins were hitherto unknown in eukaryotes. A biological role for these proteins in plant disease resistance is yet to be demonstrated.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest


1. Ellis J, Jones D: **Structure and function of proteins controlling strain-specific pathogen resistance in plants.** *Curr Opin Plant Biol* 1998, **1**:288-293.

2. Martin GB: **Functional analysis of plant disease resistance genes and their downstream effectors.** *Curr Opin Plant Biol* 1999, **2**:273-279.

3. • Bendahmane A, Kanyuka K, Baulcombe D: **The *Rx* gene from potato controls separate virus resistance and cell death responses.** *Plant Cell* 1999, **11**:781-791.

The authors studied an NBS-LRR (non-TIR class) gene for resistance to potato virus X (PVX). The corresponding *avr* gene in the virus encodes the viral coat protein. The reaction of resistant plants to the virus is described as 'extreme resistance' and does not involve HR. Thus, HR is not required for resistance, at least in this instance. It is shown that lack of HR is not the property of the *Rx* gene itself, but is caused by a particular anti-viral defense response that is triggered in the *Rx*–PVX interaction. This response, controlled by unidentified genes, prevents viral replication and gene expression with the consequence that viral coat protein does not accumulate. However, when coat protein is expressed in transgenic *Rx* plants using a constitutive promoter, HR does occur. The authors conclude that expression of the coat protein from the constitutive promoter is not affected by the anti-viral response and that coat protein accumulates to a threshold level needed for HR induction.

4. • Collins N, Drake J, Ayliffe M, Sun Q, Ellis J, Hulbert S, Pryor T: **Molecular characterization of the maize Rp1-D rust resistance haplotype and its mutants.** *Plant Cell* 1999, **11**:1365-1376.

The NBS-LRR gene *Rp1-D* for maize rust resistance occurs in a complex locus of approximately eight paralogues. Considerable haplotypic variation among maize lines carrying different rp1 resistance specificities is detected by DNA gel-blot analysis. Analysis of 27 mutants recovered from *Rp1-D* homozygotes shows that nine different deletion events have occurred by unequal crossing-over events that involve several different pairing alternatives. Initial evidence is provided that the recombination occurs in the NBS-LRR coding region and produces chimeric genes.

5. • Gassmann W, Hinsch ME, Staskawicz BJ: **The *Arabidopsis* RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes.** *Plant J* 1999, **20**:265-277.

The authors identified the first TIR-NBS-LRR R gene, *RPS4* from *Arabidopsis*, that confers resistance to a bacterial pathogen (*Pseudomonas syringe*). This finding indicates that particular NBS-LRR R-gene classes are not matched to specific pathogen species.

6. McDowell JM, Dhandaydham M, Long TA, Aarts MGM, Goff S, Holub EB, Dangl JL: **Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of *Arabidopsis*.** *Plant Cell* 1998, **10**:1861-1874.

7. Tai TH, Dahlbeck D, Clark ET, Gajiwala P, Pasion R, Whalen MC,
• Stall RE, Staskawicz BJ: **Expression of the Bs2 pepper gene confers resistance to bacterial spot disease in tomato.** *Proc Natl Acad Sci USA* 1999, **96**:14153-14158.

A description of a non-TIR class NBS-LRR R gene from *Capsicum* that is notable for an enormous intron (27 kb) that made isolation and identification of the gene difficult. This gene, which corresponds to the *avrBs2* avirulence gene in the bacterial pathogen *Xanthomonas campestris*, has been 'durable' in agriculture. That is, no pathogenic strains of the pathogen lacking *avrBs2* have emerged in spite of strong selection pressure imposed by the use of this gene in commercial pepper cultivars. Mutation of the *avrBs2* gene seems to reduce fitness of the pathogen. This observation underlines the potential role for 'defeated' R genes in natural populations. Such host genes prevent the increase in frequency of the corresponding *avr* genes in the pathogen, thus potentially decreasing the overall fitness of the pathogen population.

8. Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L,
• Hayasaka H, Katayose Y, Sasaki T: **The Pib gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes.** *Plant J* 1999, **19**:55-64.

Pib is the first R gene described that controls resistance to the very important rice blast pathogen *Magnaporthe grisea*. The authors underline the caution needed in interpreting data on whether R-gene mRNA levels are influenced by pathogen infection.

9. Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S,
• Sobral BW, Young ND: **Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily.** *Plant J* 1999, **20**:317-332.

An extensive phylogenetic analysis of over 400 NBS-LRR related sequences from several plant species that are represented in several public and private DNA databases. The *Arabidopsis thaliana* database is the most extensive followed by the rice database.

10. Noel L, Moores TL, van Der Biezen EA, Parniske M, Daniels MJ,
•• Parker JE, Jones JDG: **Pronounced intraspecific haplotype divergence at the *RPP5* complex disease resistance locus of *Arabidopsis*.** *Plant Cell* 1999, **11**:2099-2112.

This paper describes the first analysis of the complete sequence of a complex R-gene locus. Sequence information from two haplotypes, one from Landsberg *erecta* (95 kb) and one from Columbia ecotypes (90 kb), is analysed and molecular events, such as point mutations causing premature stop codons, transposon insertion and diversifying selection, acting on the LRR region are uncovered.

11. Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP: **The Mla (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240 kb DNA interval on chromosome 5S (1HS) of barley.** *Genetics* 1999, **153**:1929-1948.

12. Jeong S, Trotochaud AE, Clark SE: **The *Arabidopsis CLAVATA2***
• **gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase.** *Plant Cell* 1999, **11**:1925-1934.

The CLV system in *Arabidopsis* provides a model for *Cf* R-gene function. It is interesting to note that the *CLV1* coding region (encoding an LRR protein) is highly polymorphic with respect to other single copy genes in *Arabidopsis.* Nevertheless, in contrast to LRR regions of R genes, *CLV1* is not under diversifying selection and the variation is not concentrated in the DNA-sequence encoding the xxLxLxx structural motif of the LRR, which is the variable region in R genes.

13. Wang G-L, Raun D-L, Song W-Y, Sideris S, Chen L, Pi L-Y, Zhang S, Zhang Z, Fauquet C, Gaut BS *et al.*: **Xa21D encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution.** *Plant Cell* 1998, **10**:765-779.

14. Underhill DM, Ozinsky A, Smith KD, Aderen A: **Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages.** *Proc Natl Acad Sci USA* 1999, **96**:14459-14463.

15. Rathjen JP, Chang JH, Staskawicz BJ, Michelmore RW:
•• **Constitutively active Pto induces a Prf-dependent hypersensitive response in the absence of avrPto.** *EMBO J* 1999, **18**:3232-3240.

The most thorough mutational analysis reported so far for an R gene. Mutant forms of *Pto* were tested in a yeast two-hybrid system for *avr–Pto* encoded protein interaction and in a transient assay system in plants for their ability to induce HR, either dependently or independently of the avr protein. Several mutants that were constitutive for HR (i.e. no *avr* gene product required to induce HR) were isolated among mutants in the P+1 region, which is conserved in genes encoding protein kinases. The use of these sorts of constitutive resistance mutants for pathogen-inducible expression and synthetic resistance is discussed.

16. Ellis JG, Lawrence GJ, Luck JE, Dodds PN: **Identification of regions**
•• **in alleles of the flax rust resistance gene L that determine differences in gene-for-gene specificity.** *Plant Cell* 1999, **11**:495-506.

DNA sequence variation between alleles at the *L* rust-resistance locus of flax, and amino-acid sequence variation between their products, is described. Ten of the eleven alleles express different rust resistance specificities, providing an unprecedented opportunity to investigate the basis of specificity differences between alleles. The greatest variation is found in the LRR sequence and most, but not all, of the variation is associated with the predicted solvent exposed xxLxLxx motifs. Evidence was found for specificity differences caused by either TIR- or LRR-region polymorphisms. Chimeric *L* alleles, constructed *in vitro* and tested in transgenic plants, also provided evidence for the involvement of the LRR region in specificity determination. Several of these chimeras, particularly those with exchanges in the LRR region, no longer express the resistance specificities of the parental alleles and are either non-functional or express novel specificities that are undetected by the rust strains used in resistance tests. One chimeric gene with the TIR and most of the NBS from L2 and the LRR from L10 did express a novel specificity, providing resistance to only one of five rusts recognised by L10.

17. Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BBH, Jones JDG: **Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato.** *Cell* 1997, **91**:821-832.

18. Dodds PN, Lawrence G, Pryor T, Ellis J: **Genetic analysis and evolution of plant disease resistance genes.** In *Molecular Plant Pathology*. Edited by Dickinson M, Beynon J. Sheffield: Sheffield Academic Press; 2000:in press.

19. Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore RW: **Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection.** *Plant Cell* 1998, **10**:1833-1846.

20. Botella MA, Parker JE, Frost LN, Bittner-Eddy PD, Beynon JL, Daniels MJ, Holub EB, Jones JDG: **Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants.** *Plant Cell* 1998, **10**:1847-1860.

21. Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD: **The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number.** *Plant Cell* 1998, **10**:1915-1925.

22. Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti P, Hatzixanthis K, Jones JDG: **Characterisation of the tomato Cf-4 gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in Cf-4 and Cf-9.** *Plant Cell* 1997, **9**:2209-2224.

23. Anderson PA, Lawrence GJ, Morrish BC, Ayliffe MA, Finnegan EJ, Ellis JG: **Inactivation of the flax rust resistance gene M associated with loss of a repeated unit within the leucine-rich repeat coding region.** *Plant Cell* 1997, **9**:641-651.

24. Parker JE, Coleman MJ, Szabo V, Frost LN, Schmidt R, van der Biezen EA, Moores T, Dean C, Daniels MJ, Jones JDG: **The *Arabidopsis* downy mildew resistance gene *RPP5* shares similarity to the toll and interleukin-1 receptors with N and L6.** *Plant Cell* 1997, **9**:879-894.

25. Michelmore RW, Meyers BC: **Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process.** *Genome Res* 1998, **8**:1113-1130.

26. Parniske M, Jones JD: **Recombination between diverged clusters of**
• **the tomato Cf-9 plant disease resistance gene family.** *Proc Natl Acad Sci USA* 1999, **96**:5850-5855.

The authors continue the analysis of sequences related to the *Cf4/9* genes that occur at three loci on chromosome 1 of tomato and document a potential inter-locus gene transfer event. This finding raises the possibility that promiscuous 'sequence sampling' between different R-gene loci is involved in R-gene evolution. For example, is there transfer of information (ectopic recombination) between the closely related but unlinked *L* and *M* loci in flax? This paper provides useful and thoughtful discussion of these matters.

27. Caicedo AL, Schaal BA, Kunkel BN: **Diversity and molecular**
• **evolution of the *RPS2* resistance gene in *Arabidopsis thaliana*.** *Proc Natl Acad Sci USA* 1999, **96**:302-306.

A description of allelic variation at the *Rps2* locus in a collection of *Arabidopsis thaliana* ecotypes.

28. Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J: **Dynamics**
• **of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*.** *Nature* 1999, **400**:667-671.
A description of variation flanking the *Rpm1* locus of *Arabidopsis*. The earlier observation that susceptible strains carry the same deletion of the complete *Rpm1* gene is extended to 27 ecotypes.

29. Cooleya MB, Pathiranaa S, Wua H, Kachrooa P, Klessig DF:
• **Members of the *Arabidopsis* HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens.** *Plant Cell* 2000, **12**:663-676.
A paralogue of the *Peronsospora* resistance gene *RPP8* encodes resistance to turnip crinkle virus (TCM). Like *Rx/Gpa2* (virus/nematode resistance [3•]), *HRT/RPP8* are paralogues that confer resistance to 'radically different' pathogens. The avirulence factor detected by *HRT* is the viral coat protein.

30. Collmer A: **Determinants of pathogenicity and avirulence in plant pathogenic bacteria.** *Curr Opin Plant Biol* 1998, **1**:329-335.

31. Frank SA: **Models of plant–pathogen coevolution.** *Trends Genet* 1992, **8**:213-219.

32. Bevan JR, Crute IR, Clarke DD: **Variation for virulence in *Erysiphe fischeri* from *Senecio vulgaris*.** *Plant Pathol* 1993, **42**:622-635.

33. Bittner-Eddy PD, Crute IR, Holub EB, Beynon JL: ***RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*.** *Plant J* 2000, **21**:177-188.

34. Dinesh-Kumar SP, Baker BJ: **Alternatively spliced N resistance gene transcripts: their possible role in tobacco mosaic virus resistance.** *Proc Natl Acad Sci USA* 2000, **15**:1908-1913.

35. van der Biezen EA, Sun J, Coleman MJ, Bibb MJ, Jones JD: ***Arabidopsis* RelA/SpoT homologs implicate (p)ppGpp in plant signaling.** *Proc Natl Acad Sci USA* 2000, **97**:3747-3752.