The Leucine-Rich Repeat Domain Can Determine Effective Interaction Between *RPS2* and Other Host Factors in Arabidopsis *RPS2*-Mediated Disease Resistance

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ABSTRACT

Like many other plant disease resistance genes, *Arabidopsis thaliana RPS2* encodes a product with nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains. This study explored the hypothesized interaction of *RPS2* with other host factors that may be required for perception of *Pseudomonas syringae* pathogens that express *avrRpt2* and/or for the subsequent induction of plant defense responses. Crosses between Arabidopsis ecotypes Col-0 (resistant) and Po-1 (susceptible) revealed segregation of more than one gene that controls resistance to *P. syringae* that express *avrRpt2*. Many F_2 and F_3 progeny exhibited intermediate resistance phenotypes. In addition to *RPS2*, at least one additional genetic interval associated with this defense response was identified and mapped using quantitative genetic methods. Further genetic and molecular genetic complementation experiments with cloned *RPS2* alleles revealed that the Po-1 allele of *RPS2* can function in a Col-0 genetic background, but not in a Po-1 background. The other resistance-determining genes of Po-1 can function, however, as they successfully conferred resistance in combination with the Col-0 allele of *RPS2*. Domain-swap experiments revealed that in *RPS2*, a polymorphism at six amino acids in the LRR region is responsible for this allele-specific ability to function with other host factors.

PLANT disease resistance is often controlled by gene-for-gene interaction between plant resistance (*R*) genes and pathogen avirulence (*avr*) genes (Crute and Pink 1996; Hammond-Kosack and Jones 1997). When *R* and *avr* alleles of matched specificity are present, the plant induces strong defense responses that restrict pathogen growth. This defense-inducing capacity is likely to require the action of many host factors in addition to the *R* gene product.

The interaction between *R* and *avr* gene products has often been modeled as a receptor-ligand interaction, and a small number of examples provide support for direct physical interaction (Scofield *et al.* 1996; Tang *et al.* 1996; Jia *et al.* 2000; Leister and Katagiri 2000). To date, new pathogen recognition specificities have most often been traced to variation within the leucine-rich repeat (LRR)-encoding domain of *R* genes, reinforcing the concept that the LRR is primarily a pathogen recognition domain (Parniske *et al.* 1997; Thomas *et al.* 1997; McDowell *et al.* 1998; Meyers *et al.* 1998; Ellis *et al.* 1999; Noel *et al.* 1999; Bittner-Eddy *et al.* 2000; Luck *et al.* 2000). A similar paradigm is well developed for LRR receptor proteins from mammals and other organisms (*e.g.*, Braun *et al.* 1991; Kobe and Deisenhofer 1994; Marino *et al.* 2000). Individual plants carry hundreds of apparent *R* genes and substantial allelic diversity can exist among the LRR-encoding domains of *R* genes, giving rise to a wide array of pathogen recognition specificities (Ellis *et al.* 2000; Young 2000). Structural variation within other *R* gene domains and within pathogen *avr* alleles can also contribute to new pathogen recognition specificities (Herbers *et al.* 1992; Ellis *et al.* 2000; White *et al.* 2000).

A simple receptor-ligand model for the interaction of *R* and *avr* gene products does not preclude a requirement for additional host factors in defense signaling. These other host factors may act upstream, downstream, in parallel, or in concert with an interaction between *R* and *avr* gene products. In one example, the *Rar1* gene is required for the function of some *Mla R* gene alleles in barley (Shirasu *et al.* 1999). Two tomato *R* gene products, the *Pto* kinase and the *Prf* nucleotide-binding site (NBS)-LRR protein, are both required for the resistance response against *P. syringae* pathogens that express *avrPto* (Martin *et al.* 1993; Salmeron *et al.* 1996), but physical interaction between the Pto and Prf proteins has not been reported. The presence of a high-affinity binding site for Avr9 peptide in both *Cf-9*$^+$ and *Cf-9*$^-$ tomato cell extracts suggests that other gene products are required for a defense-inducing interaction to take place between *Cf-9* and *Avr9* (Kooman-Gersmann *et al.* 1996).

In some cases, genes have been identified that contribute to defense signaling in multiple *R*/*avr* gene pathways. *Prf* of tomato is required for the function of both

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Pto and the *Pto* homolog *Fen*, and thus is shared between two separate pathways that mediate responses to different ligands (Salmeron *et al.* 1994). *EDS1*, *NDR1*, *PBS2*, and *PBS3* provide examples of Arabidopsis genes for which mutations disrupt multiple, but not all, gene-for-gene interactions (Innes 1998). *Rcr* loci are required for the function of tomato *Cf-9* and *Cf-2 R* genes (Hammond-Kosack *et al.* 1994). The literature on classical resistance genetics and breeding contains many additional examples of "modifier" loci that alter the activity or quantitative strength of one or more resistance loci (Michelmore 1995; Crute and Pink 1996; Hammond-Kosack and Jones 1997). Hence the presence and strength of the defense response in a given gene-for-gene resistance pathway can be modulated by variation at *avr* genes, *R* genes, or accessory plant loci. However, the molecular basis of these defense-determining interactions remains poorly understood.

The disease resistance gene *RPS2* of *Arabidopsis thaliana* blocks infection by *Pseudomonas syringae* pathogens that express the avirulence gene *avrRpt2* (Kunkel *et al.* 1993; Yu *et al.* 1993). As part of this response, resistant plants develop the hypersensitive response, (HR), a programmed cell death process that arises within hours at and around the site of infection. The HR is associated with disease resistance in many gene-for-gene systems (Goodman and Novacky 1994; Greenberg 1997). Like many other *R* genes, *RPS2* encodes an NBS-LRR protein (Bent *et al.* 1994; Mindrinos *et al.* 1994; Young 2000). The present study was initially designed to identify additional host genes that function with *RPS2* in defense activation. Ecotype Col-0 is *RPS2/RPS2* and responds to *P. syringae* expressing *avrRpt2* by inducing defense responses and limiting bacterial growth (Kunkel *et al.* 1993; Yu *et al.* 1993). The Arabidopsis ecotype Po-1 was previously identified as susceptible to *P. syringae* expressing *avrRpt2* (Whalen *et al.* 1991), but the cause of susceptibility was not determined. Here we use genetic and molecular genetic analysis of Col-0 and Po-1 to show the involvement of one or more loci other than *RPS2* in controlling the *avrRpt2*-specific resistance response. Allele-specific interactions were observed. We discovered that the LRR-encoding domain is the RPS2 determinant of allele-specific interactions between *RPS2* and one or more of the other loci that participate in *RPS2*-mediated resistance.

MATERIALS AND METHODS

Plant and bacterial strains: growth, inoculation, and transformation procedures: *P. syringae* pv. *tomato* (Pst) DC3000 and *P. syringae* pv. *glycinea* Race 4 (Psg) carrying pVSP61 (empty vector, no *avr* gene) or pV288 (pVSP61 + *avrRpt2*) were constructed and used as described (Kunkel *et al.* 1993). Arabidopsis ecotype Col-0 was originally obtained from S. Somerville (Stanford University, Stanford, CA) and Po-1 was obtained from the former *Arabidopsis* Information Service seed bank (now available from ABRC, Columbus, OH; http://www.aims.cps.msu.edu/aims/). The Po-1 lines used in this study were derived from a line produced by two generations of single-seed descent. Arabidopsis plants were grown from seed in growth chambers under a 9-hr photoperiod at 22° and were moved after inoculation and scoring to a 24-hr photoperiod for flowering and seed production.

To assay for the HR, bacterial suspensions of $\sim 2 \times 10^8$ cfu/ml of Psg strains carrying pVSP61 or pV288 were infiltrated into leaf mesophyll tissue by vacuum infiltration, with a disposable plastic Pasteur pipette, or with a 1.0-ml syringe applied to the undersurface of healthy, fully expanded Arabidopsis leaves (Kunkel *et al.* 1993; Yu *et al.* 1993). Leaves were scored for HR symptoms at 24–48 hr after inoculation. To assay for disease, Pst bacterial suspensions of 5×10^5 or 1×10^6 cfu/ml in 10 mm $MgCl_2$ were inoculated into plant leaves as described for the HR assay above (Whalen *et al.* 1991). The inoculated leaves were scored for disease symptoms (necrosis and yellowing) 4 days after inoculation. To determine levels of bacterial growth in the leaves of Arabidopsis, leaves of at least six plants per bacterial strain were vacuum infiltrated with bacterial suspensions of 2×10^4 cfu/ml or 5×10^4 cfu/ml. Bacterial growth was monitored by dilution plating of leaf samples at various time points between days 0 and 4 after inoculation as described previously (Whalen *et al.* 1991).

A modified vacuum infiltration procedure was used for transformation of Arabidopsis with constructs delivered by *Agrobacterium tumefaciens* strain GV3101(pMP90) (Bechtold *et al.* 1993; Clough and Bent 1998). Controls for experiments with transgenic plants included Po-1 and Col-0 ecotypes either grown on 0.5× MS/0.8% agarose media without antibiotics and transplanted to soil or transformed with the parent binary cosmid pCLD04541 (Bancroft *et al.* 1997), selected on antibiotic media, and transplanted to soil.

Genetic linkage analysis: Genetic mapping with Po-1 × Col-0 F_2 individuals and F_3 families was performed using the indicated cleaved amplified polymorphic sequence (CAPS), simple sequence length polymorphism (SSLP) markers (Research Genetics, Huntsville, AL), and restriction fragment length polymorphism (RFLP) markers (ABRC) that map throughout the Arabidopsis genome (Nam *et al.* 1989; Konieczny and Ausubel 1993; Bell and Ecker 1994; Rhee *et al.* 1998; http://www.arabidopsis.org/). For plant genomic DNA, one to two inner rosette leaves from F_2 plants, or ~1 g fresh weight of leaves from ≥30 F_3 plants, were collected after testing plants for the HR phenotype, immediately frozen in liquid N_2, and stored at −70°. Genomic DNA was isolated using a CTAB-based protocol (Rogers and Bendich 1988). PCR for genetic mapping was essentially as described (Konieczny and Ausubel 1993; Bell and Ecker 1994). For the *RPS2* CAPS, a portion of *RPS2* was amplified using primers 53 (5′-CAG AGC TTT GAG ACA G-3′) and 54 (5′-GTA CTC CAA GTC ATG-3′), and an aliquot of the PCR product was digested with restriction enzyme *Eco*RI and resolved by agarose gel electrophoresis. The 16 individuals mentioned as the "biased mapping set" were selected by screening a total of 785 Po-1 × Col-0 F_2 individuals by hand inoculation with Psg *avrRpt2*$^+$ to test for the HR and by genotyping at *RPS2* using the *Eco*RI-based CAPS marker. Unless otherwise noted, molecular biological methods used in these and other experiments were essentially as described (Ausubel *et al.* 1997).

In initial mapping studies, significant associations between marker and defense phenotype were assessed using 57 susceptible F_2 individuals using the chi-square statistic to test for deviation from a 3:1 or 1:2:1 ratio ($P < 0.05$). Statistically significant associations were observed between the resistance phenotype and the three markers nga8, RPS2, and DHS1A.

More detailed genetic mapping was performed using "set I" (131 F_3 families derived by self-fertilization from randomly

chosen Po-1 × Col-0 F_2 individuals from 5 different F_1 plants) separately or with "set II" (16 F_3 lines from the biased mapping set described above and 53 F_3 families derived from other Po-1 × Col-0 F_2 individuals homozygous at *RPS2*). Phenotypes of the F_3 families were determined using at least two separate pots, each containing ≥9 and typically 14 or more plants from each F_3 family. Plants were inoculated with Psg *avrRpt2*$^+$ by vacuum infiltration and before viewing of labels the set of F_3 plants in a pot were assigned a single group score for severity of the HR on a scale of 0–4. Each infiltration set included one pot each of Col-0 and Po-1 as controls. The following categories were used: (1) no HR, all leaves on all plants show no HR or at most HR1; (2) rare and/or weak HR, most leaves do not show extensive tissue collapse, a few leaves may show HR3, with most leaves showing HR1–2; (3) intermediate HR, most leaves on all plants show an intermediate HR2 or HR3, with some leaves showing HR4; (4) full HR, all leaves on all plants show extensive tissue collapse (HR4–5); (segregating) majority of plants show HR4–5 but some plants show no HR or intermediate HR (HR0–3). After being placed into these categories without reference to labels, variation of HR within an F_3 family was evaluated by comparing the response of the plants between duplicate pots of the same F_3 family. As a check, independent scoring of selected experiments by other laboratory personnel produced consistent categorization of F_3 families.

F_3 mapping data were analyzed using QGene v3.06 (Nelson 1997), with map distances for molecular marker maps obtained from the Lister and Dean RI map (Rhee *et al.* 1998; http://www.arabidopsis.org). Single interval mapping protocols were used and significance of association between marker and phenotype was determined using a cutoff LOD value of 3.0.

DNA sequencing: The DNA sequence of Po-1 *RPS2* was determined for both strands using dideoxy sequencing methods and *RPS2* internal primers. One PCR product amplified from genomic Po-1 DNA and cloned into pBluescript II SK(+) was used for initial sequencing. This PCR product was generated using the primers aa#1 (5′-CGGGATCCATGGATTTCAT CTCATCTCTT-3′) and 46S (5′-ACAGAGTGCTCTTAGC-3′). Any deviations from the known Col-0 *RPS2* sequence were then checked using independent Po-1 *RPS2* PCR products. Note that no introns are present in Col-0 or Po-1 *RPS2*. The promoter region of Col-0 *RPS2* was cloned from a genomic subclone (Bent *et al.* 1994) as a 1.3-kb *Sal*I-*Bam*HI fragment into pBluescript II SK(+); the promoter region of Po-1 *RPS2* was cloned from a PCR product generated using RPS2-P1K-Cla (5′-CGGCATCGATAGACAGGTCCCCCTTTTA-3′) and RPS2#60 (5′-CTCCGTTACTTGCAC-3′), and multiple cloned independent PCR products were pooled for sequencing. Sequence comparisons were made using SeqApp v1.9a169 (D. Gilbert, Bloomington, IN; http://www.ftp.bio.indiana.edu).

Construction of *RPS2* + 1.0-kb native promoter constructs: For complementation experiments, Col-0 and Po-1 alleles of *RPS2* were cloned together with their native promoter sequences into the binary vector pCLD04541 (Bancroft *et al.* 1997). PCR products were amplified from genomic DNA using high-fidelity *Pfu* DNA polymerase (Stratagene, La Jolla, CA) and the primers *RPS2*-P1k-Cla (see above) and *RPS2*-Sal46 (5′-GGAATTC**GTCGAC**ACAGAGTGCTCTTAGCTC-3′), giving a product spanning from −980 bp upstream from the start of the *RPS2* open reading frame to +30 bp downstream from the stop codon. Products from at least two independent PCR reactions were separately cloned into the relevant vectors and tested in plants. Products were restricted with *Cla*I and *Sal*I and cloned into pBluescript II SK(+) and then into *Cla*I/*Xho*I-restricted pCLD04541. Constructs were then transferred into the Agrobacterium strain GV3101 (pMP90) (Koncz and Schell 1986) by triparental mating.

Generation of *RPS2* promoter-swap and LRR-swap constructs: The 980-bp segments of the *RPS2* promoter immediately upstream of the *RPS2* open reading frame (ORF) were amplified by PCR from Po-1 and Col-0 genomic DNA using high-fidelity *Pfu* DNA polymerase and the primers *RPS2*-P1k-Sac (5′-GCAC**GAGCTC**AGACAGGTCCCCCTTTTA-3′) and *RPS2*-1Cla-R (5′-AATCCAT**A**T**CGAT**GATTTCTCGCTC-3′). *RPS2*-1Cla-R incorporates a single base change (underlined) 1 bp upstream from the ATG start codon that creates a *Cla*I restriction site (boldface letters). Products were restricted with *Sac*I and *Cla*I and cloned into *Sac*I/*Cla*I-restricted pBluescript II SK(+). The *RPS2* open reading frame was similarly amplified and cloned using *RPS2*-1Cla-F2 (5′-CGGC**ATCGA**T**T**ATG GATTTCATCTCATCTCTT-3′) and *RPS2*-Sal46 (described above). *RPS2*-1Cla-F2 also creates a *Cla*I restriction site (boldface letters) one base upstream of the ATG start codon (underlined). Purified PCR products were digested with *Cla*I, blunted with mung bean nuclease (New England Biolabs, Beverly, MA), digested with *Sal*I, ligated with the pBluescript II SK(+)/*RPS2* promoter constructs (described above) that had been digested with *Eco*RI, and then blunt ended and digested with *Sal*I. Each type of *RPS2* promoter construct was ligated with each of two *RPS2* ORF sequences (from the same *RPS2* allele) that were products of separate PCR reactions. The resulting *RPS2* promoter + ORF constructs were restricted out of pBluescript using *Sac*I and *Sal*I restriction enzymes, ligated into *Sac*I/*Xho*I-digested pCLD04541, transformed into *Escherichia coli*, and then transferred into Agrobacterium for plant transformation as described above. Products from at least two independent PCR reactions were used to create separate constructs that were independently tested in plants.

To generate the *RPS2* LRR-swap constructs, the pBluescript *RPS2* + 1.0-kb native promoter constructs described in the previous paragraph were used. A 1.35-kb *Hin*dIII fragment encoding the LRR domain from the Po-1 construct was replaced with the corresponding fragment from the Col-0 construct and vice versa. Products from at least two independent PCR reactions were used to create separate constructs. *RPS2* LRR-swap constructs were transferred into pCLD04541 as *Cla*I/*Sal*I fragments and used as described above.

RESULTS

Response of Po-1 to *P. syringae* expressing *avrRpt2*: To investigate the response of Po-1 to infection by *P. syringae* expressing *avrRpt2*, leaves of Po-1 and Col-0 were inoculated by syringe or by vacuum infiltration with the virulent Pst strain DC3000 or with Pst DC3000 expressing *avrRpt2* (DC3000*avrRpt2*$^+$). Wild-type Col-0, which is resistant to *avrRpt2*, developed few or no disease symptoms when inoculated with Pst DC3000*avrRpt2*$^+$ at a titer of 10^6 colony-forming units (cfu)/ml (Table 1). In confirmation of previous work (Whalen *et al.* 1991), Po-1 plants developed necrotic lesions and pronounced chlorosis 4 days after inoculation with DC3000*avrRpt2*$^+$, which are similar to the symptoms observed on susceptible Col-0 *rps2*/*rps2* mutants or on wild-type Col-0 inoculated with DC3000 (Table 1).

The lack of a resistance response in Po-1 was quantified by measuring the extent of pathogen growth within the plant. In Po-1 inoculated with either DC3000 or DC3000*avrRpt2*$^+$, bacteria grew to high levels (Figure 1). These levels were similar to those attained by

TABLE 1

Response of *Arabidopsis* ecotypes Po-1, Col-0, and Col-0 *rps2/rps2* to *P. syringae* that express *avrRpt2*

Plant line (genotype)	HR score at 24 hr: Psg R4*avrRpt2*$^+$	Disease score at 96 hr	
		Pst DC3000	Pst DC3000*avrRpt2*$^+$
Col-0 (*RPS2/RPS2*)	HR+ 4.0–5.0	Disease 3.5–5.0	No disease 0.0–1.0
D203 (Col-0 *rps2/rps2*)	No HR 0.0–1.0	Disease 4.0–5.0	Disease 4.0–5.0
Po-1 (*?/?*)	No HR 0.0–1.0	Disease 3.5–5.0	Disease 4.0–5.0

Each entry reports results from at least three experiments with five or more plants per ecotype per experiment; numbers are the range of mean scores for independent experiments. For the HR assay, bacteria were inoculated at 2×10^8 cfu/ml. Hypersensitive response was scored on a scale of 0–5. HR score ≤1.5: little or no HR, no visible tissue collapse. ≥3.5: HR+, extensive cell death, obvious collapse of inoculated tissue. >1.5 and <3.5: intermediate HR. For disease assay, bacteria were inoculated at 2×10^6 cfu/ml. Disease was evaluated by symptoms of chlorosis and small necrotic lesions, and scored on a scale of 0–5. Disease score ≤1.5: little or no disease. 1.5–2.5: mild disease. 3.0–4.0: moderate disease. ≥4.0: severe disease.

DC3000*avrRpt2*$^+$ in susceptible *rps2* mutants of Col-0 or by DC3000 (no *avr*) in wild-type Col-0. In contrast, growth of DC3000*avrRpt2*$^+$ in wild-type Col-0 plants was restricted, reaching maximum levels of 10^4–10^5 cfu/cm^2 (Figure 1).

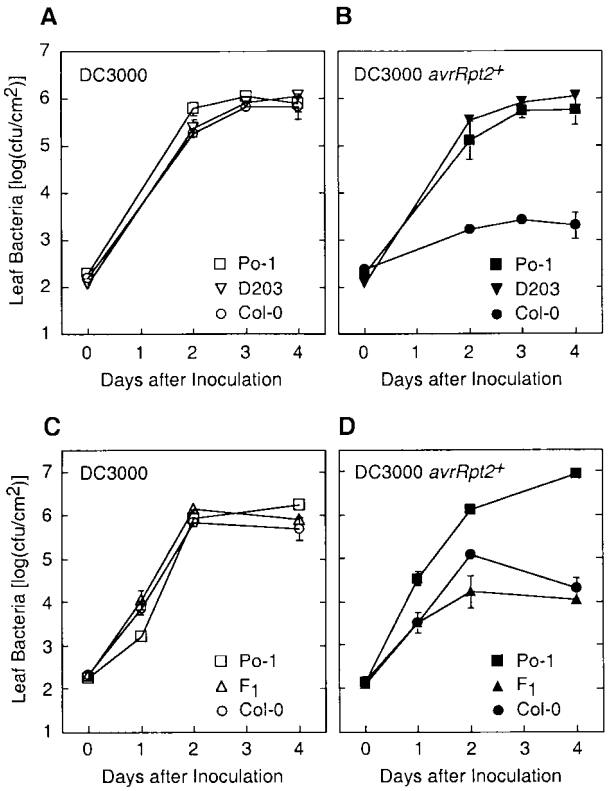

FIGURE 1.—Growth of virulent and avirulent *P. syringae* pv. *tomato* within Arabidopsis leaves. A and B are from the same experiment; C and D are from a separate single experiment. Plants were all inoculated with the indicated bacterial strain. D203, Col-0 *rps2-201/rps2-201*; F_1, F_1 progeny from Po-1 × Col-0. Two leaves from each of six plants were sampled for each data point; values shown are mean ± SE.

The hypersensitive response (HR) is a programmed cell death response that develops within hours at and around the site of infection. The ability of Po-1 to develop an HR in response to *P. syringae* pv. *glycinea* (Psg) expressing *avrRpt2* was tested by syringe or vacuum infiltration with a high titer of bacteria (10^8 cfu/ml; KLEMENT *et al.* 1964). While Col-0 plants exhibited a strong, visible HR within 24 hr of inoculation, Po-1 plants did not manifest an HR at the macroscopic level in response to Psg *avrRpt2*$^+$ (Table 1). Po-1 plants do have the capacity to induce gene-for-gene defenses and the HR in response to *P. syringae* pathogens, however, as Po-1 activates these responses when inoculated with *P. syringae* that express *avrRps4* (HINSCH and STASKAWICZ 1996; data not shown). Although the HR is not always required for an effective resistance response (YU *et al.* 1998; BENDAHMANE *et al.* 1999), it is closely associated with the disease resistance response mediated by *RPS2* and most other *R* genes (KUNKEL *et al.* 1993; YU *et al.* 1993; GOODMAN and NOVACKY 1994; GREENBERG 1997). In this study the level of the HR was frequently used as an indicator of the *avrRpt2-RPS2* resistance response of the plant.

To summarize, Col-0 plants inoculated with *P. syringae* expressing *avrRpt2* developed an HR, restricted pathogen growth, and did not develop disease. In response to the same bacteria Po-1 plants did not manifest an HR, limited pathogen growth poorly, and developed disease. The simplest explanation for Po-1 susceptibility to *P. syringae* that express *avrRpt2* would be that Po-1 carries a nonfunctional allele of *RPS2*.

Multigenic control of *RPS2*-mediated defense: The genetic basis of susceptibility in Po-1 was investigated by crossing ecotypes Po-1 and Col-0. Po-1 × Col-0 F_1

TABLE 2

HR in response to Psg R4 *avrRpt*$^+$ in F_1 from crosses involving Po-1

Cross (including reciprocal)	No. of F_1 tested	Mean HR score (and phenotype)
Po-1 × Col-0	$n = 18$	4.68 ± 0.16 (HR+)
Po-1 × No-0	$n = 26$	4.74 ± 0.15 (HR+)
Po-1 × D203	$n = 27$	0.15 ± 0.14 (HR+)
Po-1 × 101C	$n = 29$	0.60 ± 0.16 (HR+)

D203, Col-0 *rps2-203/rp2-203;* 101C, Col-0 *rps2-101/rp2-101.* For the HR assay, bacteria were inoculated at 2×10^8 cfu/ml. Hypersensitive response was scored on a scale of 0–5 ± SE. HR score ≤1.5: little or no HR, no visible tissue collapse. ≥3.5: HR+, extensive cell death, obvious collapse of inoculated tissue. >1.5 and <3.5: intermediate HR. For each entry, data were pooled for multiple crosses including reciprocal crosses.

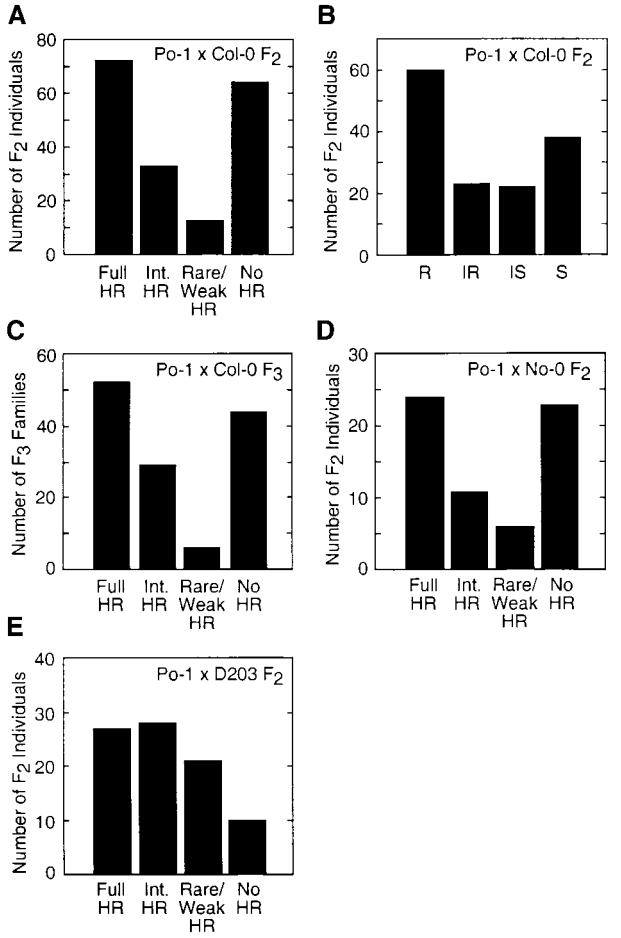

FIGURE 2.—Phenotypic segregation in F_2 and F_3 progeny of Po-1 crossed to various genotypes, as indicated. A and B represent different F_2 populations, with A subjected to the HR assay and B subjected to the disease assay (see MATERIALS AND METHODS). C–E also report results of HR assays; C reports data for F_3 families rather than for F_2 individuals.

individuals and those from reciprocal crosses exhibited a strong disease-resistant phenotype and a full HR in response to *avrRpt2* infection, indicating dominance of the Col-0 genotype in determining resistance (Figure 1b; Table 2). However, in the F_2 of reciprocal crosses, intermediate phenotypes were consistently observed in addition to the two parental phenotypes. These were grouped into intermediate-resistant (moderate HR) and intermediate-susceptible (rare and/or weak HR) classes (Figure 2). The presence of the intermediate phenotypes was also observed using disease assays rather than HR assays (Figure 2B), was confirmed in separate HR and disease assays with other F_2 populations (data not shown), and was confirmed with F_3 families derived from individual F_2 plants (Figure 2C). If all but the most disease-susceptible or HR$^-$ class of F_2 individuals were grouped together as "resistant," F_2 segregation ratios were in some cases consistent with a 3:1 ratio. However, grouping individuals with such different phenotypes into a single class seemed inappropriate, especially given the much clearer bimodal phenotypic groupings obtained in other studies with the same pathosystem but with different parents (*e.g.*, KUNKEL *et al.* 1993). F_2 and F_3 data also did not fit a 1:2:1 ratio for segregation of a single gene with incomplete dominance.

To test whether susceptibility in Po-1 segregated as a multigenic trait in combination with genetic backgrounds other than Col-0, Po-1 was crossed to the ecotype No-0, which like Col-0 is resistant to *P. syringae* that express *avrRpt2.* The Po-1 × No-0 F_1 were resistant (Table 2), indicating dominance, but as was the case in the Po-1 × Col-0 populations, F_2 phenotype distribution revealed intermediate phenotypes in addition to the parental phenotypes (Figure 2D) and F_2 segregation patterns did not fit single-gene models. These findings again suggested the involvement of multiple genes in specifying *avrRpt2*-specific resistance.

While the *avrRpt2*-specific resistance response data were not consistent with the segregation of a single dominant *R* gene or with standard ratios for digenic inheritance, such as 9:7 or 9:3:4, the data also did not resemble the bell-shaped curves that are often observed in F_2 populations segregating for a quantitative trait controlled by a large number of genes displaying small additive effects (FALCONER and MACKAY 1996). Instead, the bimodal distribution of Po-1 × Col-0 and Po-1 × No-0 F_2 and F_3 phenotypes indicated that resistance segregates as a multigenic trait controlled by a small number of major-effect genes or by a single dominant gene and a small number of "modifier" genes.

Genetic evidence for Po-1 *RPS2* functionality: To determine whether the *RPS2* allele of Po-1 is compromised for response to *avrRpt2*, the *RPS2* genotype was determined for F_2 lines that were also scored for resistance phenotype (Table 3). A single-base pair *Eco*RI CAPS within *RPS2* was identified that differentiates the *RPS2* alleles of Col-0 and Po-1. F_2 individuals were identified that are homozygous for the Po-1 *RPS2* allele, yet they

TABLE 3

Distribution of *avrRpt2*-specific defense phenotype according to *RPS2* genotype in Po-1 × Col-0 F_2

		Resistance phenotype			
RPS2 genotype	Total F_2	Full HR	Intermediate HR	Weak/rare HR	No HR
Po/Po	36	8	3	2	23
Po/Col	40	25	8	2	5
Col/Col	42	26	12	1	3
Total	118	59	23	5	31

Numbers shown are the number of randomly chosen F_2 individuals in each phenotypic/genotypic class. HR assay and scoring are as described in MATERIALS AND METHODS. Underlined numbers represent particularly informative classes (see text).

showed a partially or fully disease-resistant phenotype (Table 3). These F_2 individuals suggested that, despite the lack of *avrRpt2*-specific resistance in wild-type Po-1, the Po-1 *RPS2* allele can function in a partial Col-0 background. Another class of F_2 individuals was homozygous for the Col-0 *RPS2* allele but showed little or no disease resistance (Table 3). These individuals indicated that other Po-1 loci can cause functional *RPS2* alleles to be ineffective for resistance signaling in response to *avrRpt2*. Results consistent with these F_2 data were obtained in repeat assays with F_3 families derived from the key F_2 lines and in 16 additional F_2 individuals identified among 785 Po-1 × Col-0 F_2 (see MATERIALS AND METHODS). To reiterate, these classes of F_2 *RPS2* homozygotes indicated that the Po-1 allele of *RPS2* can be functional and/or that the progeny of Po-1 × Col-0 crosses segregate for genes other than *RPS2* that control disease resistance.

In Po-1 × Col-0 F_2 populations, the defense phenotype did not segregate independently of the *RPS2* genotype (Table 3). F_2 plants homozygous for the Col-0 *RPS2* allele were most frequently resistant and F_2 plants homozygous for the Po-1 *RPS2* allele were most frequently susceptible. Because resistance/susceptibility did not segregate entirely independently of the *RPS2* genotype, we hypothesized that one or more of the other resistance-modifying genes is linked to *RPS2*. A separate but not mutually exclusive hypothesis was that *RPS2* is one of the genes contributing to the *avrRpt2*-specific resistance response, with allele-specific interactions causing the presence or absence of resistance.

Mapping of *RPS2*-pathway loci: An approximate map position for one or more other *RPS2*-pathway loci that alter the defense response against *P. syringae* that express *avrRpt2* was determined using a population of 131 random F_2-derived F_3 families from crosses between Po-1 and Col-0. A second population of 69 F_3 families contained a small biased population of 16 lines in which the resistance phenotype was the opposite of that predicted by the *RPS2* genotype (*e.g.*, underlined classes in Table 3), as well as 53 other F_3 families not from set I and chosen due to homozygosity at *RPS2*. Plants were inoculated with Psg *avrRpt2*$^+$ by vacuum infiltration and scored for the HR. Previously mapped CAPS or RFLP markers were used to determine genotype across the Arabidopsis genome with genetic intervals of 50 cM or less.

Analysis of the initial marker data set revealed linkage of the *avrRpt2*-specific response to at least two regions on chromosome 4, near markers nga8, RPS2, and DHS1A, and detected no linkage to chromosomes 1–3 or 5 (data not shown). The additional F_3 lines and additional chromosome 4 markers were subsequently used for higher resolution mapping. Quantitative trait statistical analysis of the marker data, using single-interval mapping methods, localized genetic determination of the *avrRpt2*-specific response to two discrete genetic intervals (Figure 3). The strongest effect was at the *RPS2* locus. A second locus that contributed to the *avrRpt2*-specific response was linked to marker DHS1, roughly 33 cM away from RPS2. The possibility that additional loci linked to RPS2 on chromosome 4 also contribute to this phenotype cannot be excluded. No linkage association was detected between the defense trait and any markers on chromosomes 1–3 or 5 (Figure 3).

Allele-specific functionality of *RPS2*: The discovery of Po-1 × Col-0 F_2 individuals that are homozygous for the Po-1 allele of the *RPS2* allele but that show a resistant phenotype suggested that the Po-1 *RPS2* allele can be functional when it is in a partially Col-0 background. Functionality of Po-1 *RPS2* was investigated further by testing for the resistance response of plants carrying the Po-1 *RPS2* allele in a Col-0 *rps2/rps2* background. In a genetic approach, Po-1 was reciprocally crossed with Col-0 mutants *rps2-201/rps2-201* (D203) and *rps2-101C/rps2-101C* (101C). The *rps2-201* allele carries a point mutation that causes a single-amino-acid change in the LRR and creates a nonfunctional RPS2 protein, while the *rps2-101C* allele contains a frame-shift mutation that causes a premature stop codon at the front of RPS2 (BENT *et al.* 1994; MINDRINOS *et al.* 1994). In the progeny of D203 or 101C crosses to Po-1, all F_1 were HR$^-$ (Table 2). However, ~70% of the F_2 showed an intermediate or strong HR (Figure 2E; data not shown). These results again suggested (see also Table 3) that the Po-1 allele of *RPS2* is functional when moved into a partially Col-0 genetic background but cannot signal for resistance in conjunction with Po-1 alleles of these resistance-modifying loci.

An alternative hypothesis to explain these results was that Po-1 genes other than *RPS2* are capable of mediating the HR in conjunction with Col-0 genes other than *RPS2*. To test this hypothesis, we investigated whether any HR$^+$ individuals were homozygous for the nonfunctional Col-0 *rps2-201* or *rps2-101C* mutant alleles of *RPS2*. The *RPS2* genotype was determined for all F_2 progeny

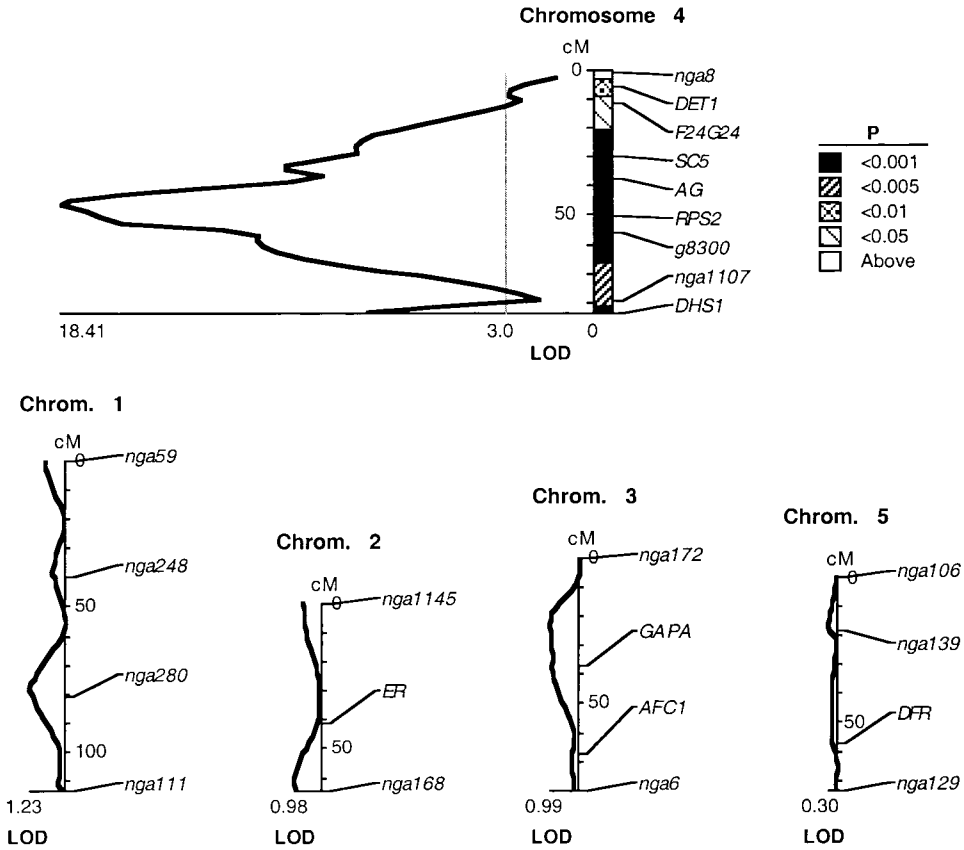

FIGURE 3.—Significance of association between genetic intervals and phenotype (response to *P. syringae* that express *avrRpt2*). Output from single-interval mapping performed using the QGene computer program is shown with HR scores and genotypic data for 131 randomly chosen Po-1 × Col-0 F_3 families as input (see MATERIALS AND METHODS). Trace shows LOD score; maximum LOD score for a given chromosome is noted on the *x*-axis. Centimorgan scale (*y*-axis) shows relative map position along chromosome of molecular markers. Patterned bar represents significance scores as *P* values. Note that no significant associations were observed for markers on chromosomes 1–3 or 5 (data not shown).

that showed an intermediate or strong HR, and all 56 HR^+ F_2 individuals carried at least one copy of the Po-1 *RPS2* allele (data not shown). This suggested that Po-1 *RPS2* is the cause of *avrRpt2*-specific resistance signaling in these lines. However, because of possible contributions from loci tightly linked to *RPS2*, this result still did not conclusively rule out the possibility that resistance is mediated by interaction among genes other than *RPS2*.

Functionality of Po-1 *RPS2* was investigated more precisely by molecular complementation. The Po-1 *RPS2* allele under ~1.0 kb of native Po-1 *RPS2* promoter was cloned into a binary cosmid and transferred by Agrobacterium-mediated transformation into the Col-0 *rps2/rps2* mutants D203 and 101C. Transformants were found to produce a resistance response upon challenge with Psg *avrRpt2*, indicating that the Po-1 *RPS2* allele can be functional in a Col-0 genetic background (Figure 4a). It was noted, however, that the HR in these lines was intermediate in intensity.

In a reciprocal experiment, the Col-0 *RPS2* allele under 1.6 or 1.0 kb of native promoter was transformed into Po-1 plants. The Col-0 *RPS2* allele complemented Po-1 to resistance in response to Psg $avrRpt2^+$ (Figure 4A). This complementation result was significant, as it indicated that the absence of *avrRpt2*-specific resistance in Po-1 is due not only to defects at other loci, but also to the Po-1 allele of *RPS2*.

To summarize the above genetic and molecular genetic complementation experiments, allele-specific interactions were observed between *RPS2* and one or more other loci. Col-0 *RPS2* could function with the Po-1 allele of one or more genes other than *RPS2* that control *avrRpt2*-specific disease resistance, while *RPS2* from Po-1 did not function with the Po-1 alleles of these other genes. Po-1 *RPS2* did function with the Col-0 alleles of these other genes, as did Col-0 *RPS2*. The Po-1 alleles of *RPS2* and this other gene or genes are each capable of disease resistance function, but they cannot function with each other.

Sequence of Po-1 *RPS2* allele: To investigate possible structural differences between the Po-1 and Col-0 *RPS2* alleles that might account for their differences in resistance signaling, the Po-1 allele of *RPS2* was cloned and sequenced (GenBank accession no. AF368301). The derived amino acid sequence revealed a substantial number of differences—11 amino acid changes—between the Po-1 and Col-0 *RPS2* alleles (Figure 5). Many of the nonconservative amino acid changes are located in the leucine-rich repeat (LRR) region, but residue changes are scattered over much of the RPS2 ORF. The derived amino acid sequence did not reveal obvious structural features that might suggest that the Po-1 allele of *RPS2* is nonfunctional.

No transcriptional differences between Col-0 and Po-1 *RPS2* alleles: Previous Northern analysis of *RPS2* mRNA from noninoculated Po-1 and Col-0 leaf tissue

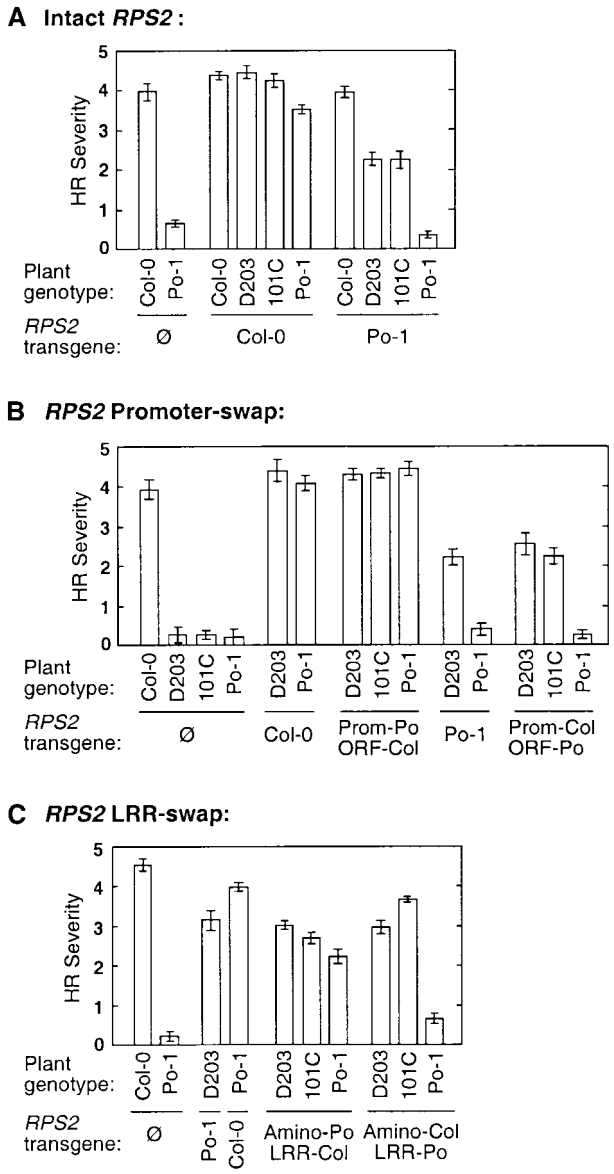

FIGURE 4.—Molecular complementation experiments using cloned *RPS2* constructs. Values shown are mean ± SE for severity of HR in multiple T_1 transformants tested for their response to Psg R4 *avrRpt2*$^+$. Plants were transformed with the following: (A) an intact *RPS2* gene driven by 1.0 kb of native RPS2 promoter from the genotype indicated; (B) an intact RPS2 open reading frame driven by 1.0 kb of native promoter or by heterologous RPS2 promoter from a different genotype, as indicated; (C) *RPS2* LRR-swap constructs fusing promoter and amino-terminus-encoding region from one *RPS2* allele with the LRR-encoding region from a heterologous *RPS2* allele, as indicated. Ø, plants transformed with vector (no *RPS2* insert), or, in some cases, nontransformed plants carried through growth and transplanting in parallel with transformants but on nonselective media.

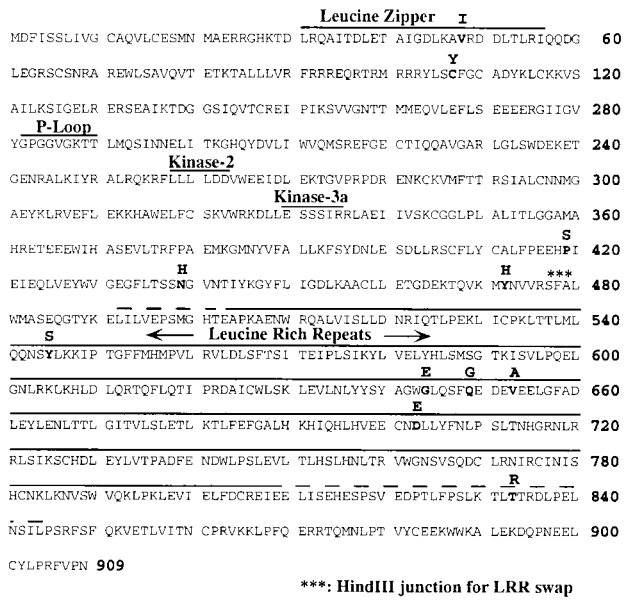

FIGURE 5.—Derived amino acid sequence encoded by *RPS2* of Po-1. Differences with Col-0 RPS2 are in boldface type and the Col-0 amino acid is shown directly above. Lines indicate the approximate extent of putative functional domains; broken lines for the leucine-rich repeat reflect the imperfect nature of the LRR in the *RPS2* gene product. ***, *Hin*dIII site that formed junction for LRR-swap alleles (see Figure 4).

did not show a discernible difference in expression between the Po-1 and Col-0 *RPS2* transcripts (BENT *et al.* 1994), suggesting that differences in the level of transcription do not account for the difference in resistance signaling activity between Po-1 and Col-0 *RPS2* alleles.

To further investigate whether transcriptional differences between Po-1 and Col-0 *RPS2* transcripts are responsible for the difference in defense signaling activity of the two alleles, the *RPS2* promoter sequences were investigated. Approximately 1.0 kb of genomic DNA immediately upstream of the Col-0 and Po-1 *RPS2* open reading frames was cloned, sequenced, and compared. Across this 986-bp sequence, the Po-1 *RPS2* promoter differed from the Col-0 *RPS2* promoter at only 7 bp positions, none obviously disrupting a promoter motif (see GenBank accession nos. AL049483 and AF368301).

To directly test for differences in the Po-1 and Col-0 *RPS2* promoters that might effect disease resistance, a "promoter-swap" molecular complementation strategy was pursued. PCR primers at −1 and −986 relative to the ATG start of *RPS2* were used to amplify and clone the native *RPS2* promoters of the Po-1 and Col-0 alleles. Heterologous combinations of promoter and *RPS2* alleles in the binary vector pCLD04541 were used to transform Po-1 and *rps2/rps2* mutants of Col-0. The ability of the chimeric transgenes to signal for resistance in response to Psg *avrRpt2*$^+$ was assayed by inoculating leaves of T_1 transformant plants and monitoring the HR. In both Col-0 and Po-1 genetic backgrounds, the resistance response to *avrRpt2* by the Col-0 *RPS2* transgene driven by the Po-1 *RPS2* promoter was indistinguishable from the resistance response of the Col-0 *RPS2* transgene under its native promoter (Figure 4B). The Po-1 *RPS2* transgene driven by the Col-0 *RPS2* promoter behaved like the Po-1 *RPS2* transgene under its own

promoter in the Col-0 or Po-1 backgrounds (Figure 4B). These results provided functional evidence that Po-1 and Col-0 *RPS2* promoters do not differ in any appreciable manner that might account for differences in the phenotypic expression of *RPS2*-mediated defense responses.

Differences responsible for allele-specific interaction are in the LRR domain: *R* gene products contain identifiable motifs such as a coiled-coil domain, NBS, and LRR (Hammond-Kosack and Jones 1997; Young 2000). We pursued further domain-swap experiments to determine if functional differences betweeen the Po-1 and Col-0 alleles of *RPS2* could be assigned to amino acid differences in a given domain.

The Po-1 and Col-0 alleles of *RPS2* under the control of 1.0 kb of native promoter in a binary vector were used as the parent constructs. From the parent constructs, the 3′ 1.35-kb fragment of Po-1 *RPS2* encoding the LRR was cloned out and replaced with the 3′ 1.35-kb fragment of Col-0 *RPS2* and vice versa. The chimeric LRR-swap constructs were transformed into Po-1 and into the Col-0 *rps2/rps2* mutants D203 and 101C by Agrobacterium-mediated transformation, and transformants were tested for their HR in response to Psg *avrRpt2*. We found that the Po-1 amino terminus + Col-0 LRR constructs could mediate an intermediate level of HR in Po-1 and in Col-0 *rps2/rps2* genetic backgrounds, indicating that the amino terminus of Po-1 RPS2 can function even in a Po-1 genetic background (Figure 4C). The Col-0 amino terminus + Po-1 LRR constructs, on the other hand, mimicked the results obtained with intact Po-1 *RPS2*: an intermediate HR was observed in Col-0 *rps2/rps2* genetic backgrounds, and no HR was conferred in a Po-1 genetic background (Figure 4C). The Col-0 RPS2 LRR domain corrected the nonfunctional Po-1 RPS2 LRR domain for resistance in a Po-1 genetic background. This indicated that the LRR domain is the key structural determinant for allele-specific interactions between *RPS2* and other host loci that modify the *avrRpt2/RPS2* pathway in this Col-0/Po-1 system.

DISCUSSION

This study explored the interaction of *RPS2* with other hypothesized host factors required for the perception of *P. syringae* pathogens that express *avrRpt2* and/or for the subsequent induction of plant defense responses. Progeny of crosses between a resistant and a susceptible ecotype of Arabidopsis revealed segregation of more than one gene controlling this defense response. Polymorphism between the Po-1 and Col-0 alleles of *RPS2* was a major factor determining the strength of the *avrRpt2*-specific resistance response, but it was not the only factor. At least one additional genetic interval that contributes to this phenotype was identified and mapped. We discovered that Po-1 *RPS2* can function in a Col-0 genetic background, but not in Po-1. In RPS2, the LRR domain was responsible for ineffective interaction between Po-1 *RPS2* and one or more of the other Po-1 loci.

Roles of the LRR: LRR domains are found in a wide array of proteins from all taxa and are present in almost all structural classes of plant *R* genes that mediate gene-for-gene disease resistance (Kobe and Deisenhofer 1994; Hammond-Kosack and Jones 1997; Marino *et al.* 2000). LRRs are involved in the perception of protein or peptide ligands in a number of systems, including interactions between the Drosophila Toll receptor and the dorsal/ventral patterning factor Spatzle; human follicle stimulating hormone and its receptor; and among plant development proteins such as CLAVATA1, 2, and 3 (Kobe and Deisenhofer 1994; Fletcher *et al.* 1999; Marino *et al.* 2000). However, LRRs have also been shown to mediate intracellular interactions among proteins not thought of as "receptors" and "ligands," such as yeast adenylate cyclase and Ras (*e.g.*, Suzuki *et al.* 1990).

In plant *R* gene products, studies suggest that the LRR domains are major determinants of recognitional specificity for Avr factors (Ellis *et al.* 2000). Evolution of new pathogen specificity has been traced to shifts in solvent-exposed LRR residues that are caused by single-base changes, insertion or deletion events, and by equal- or unequal-exchange meiotic recombination events within *R* genes or between closely linked homologous *R* genes in a cluster (Ellis *et al.* 2000).

Roles other than pathogen recognition have also been hypothesized for the LRR of *R* gene products, but these have been less clearly demonstrated. In this study we obtained evidence that the LRR region can influence effective interaction with host factors. Consistent with our results, a study with the Arabidopsis *R* gene *RPS5* also suggested a role for the LRR domain in interaction with other host factors (Warren *et al.* 1998). A nonfunctional *RPS5* allele containing a mutation in the third repeat of the LRR blocked the resistance conferred by other *R* genes, and overexpression of wild-type *RPS5* did not suppress the dominant-negative phenotype of the mutant allele (Warren *et al.* 1998). This *RPS5* mutation of the third LRR might have caused increased binding to a pathway component(s) shared by multiple *R* genes and thereby interfered with essential downstream signaling. In our study, the difference in interaction between Col-0 and Po-1 *RPS2* and other host loci was attributed to six amino acid differences between the RPS2 LRR domains. In the future, it will be interesting to see whether amino acid polymorphisms within the LRR of *RPS2* alleles from other ecotypes correlate with the level of the resistance response.

The *RPS2* and *RPS5* examples fit into a generalized model proposed by Grant and Mansfield (1999) to account for the involvement of additional loci in *R-Avr* interactions. In their model, the LRR protein only indirectly matches the *Avr* protein and is involved in inter-

preting signals generated from other cellular proteins, designated signaling linker proteins (SLIKs), which directly interface with the *Avr* peptide. The presence of the elicitor or *Avr* factor, or its activity, may alter the normal configuration of the SLIK or SLIK complex, leading to functional interaction with the *R* gene product and subsequent resistance pathway activation (Grant and Mansfield 1999). The interactions that we observed involving *avrRpt2*, *RPS2*, and other host factors may, upon further investigation, form one example of this type of SLIK interaction.

***RPS2*-interacting loci:** As an initial step toward isolation of the *RPS2*-interacting host factors predicted by our genetic studies, quantitative trait methods were used to map genetic intervals associated with the *avrRpt2*-specific response. The bimodal distribution of resistance phenotypes among Po-1 × Col-0 and Po-1 × No-0 F_2 (Figure 2) classically would indicate that the phenotype, in this case resistance in response to *avrRpt2*, is controlled by a small number of major-effect genes or a single dominant gene and a small number of "modifier" genes. The observed bias toward defense phenotypes that correlated with the *RPS2* genotype (HR^+ if homozygous for Col-0 *RPS2*, HR^- if homozygous for Po-1 *RPS2*; see Table 3) had suggested that *RPS2* would have a significant phenotypic effect and/or that other relevant loci would be linked to *RPS2*. Mapping supported both hypotheses. The defense phenotype associated most strongly with the *RPS2* locus, which was also shown by other means to have a major effect on resistance phenotypes (Figure 3). The other genetic interval associated with the response to *P. syringae* that express *avrRpt2* also mapped to chromosome 4, ~33 cM away from *RPS2*. As mentioned previously, the possibility that additional loci linked to *RPS2* on chromosome 4 also contribute to this phenotype could not be excluded.

Reports of multigenic control of resistance are gaining relevance in research on the molecular basis of defense signal transduction as resources improve for the mapping and cloning genes known only by phenotype. A number of other Arabidopsis genes have been identified for which mutant alleles disrupt defense pathways (Glazebrook 1999). None of the well-studied genes (such as *NDR1*, *EDS1*, *PAD4*, *DND1*, *LSD1*, and *PBS2*) map to the intervals on chromosome 4 identified in this study. Further experimental effort will be required to isolate and characterize the *RPS2*-interacting host factor(s) described in this study.

Direct protein associations among host factors known to be required for the *R-avr* signaling complex have yet to be demonstrated. In the closest example to date, Pto kinase has been shown to directly phosphorylate Pti1 (Zhou *et al.* 1995). In a more immediate example, Leister and Katagiri (2000) used AvrRpt2 to coprecipitate RPS2 and another unidentified protein in antibody pull-down experiments. Interestingly, RPS2 could also be precipitated by AvrB despite the fact that RPS2 does not confer resistance to *P. syringae* that express *avrB* (Leister and Katagiri 2000). This result is consistent with genetic evidence for interference between *RPS2* and *RPM1* resistance signaling pathways when pathogens that express *avrB* or *avrRpm1* and *avrRpt2* are coinoculated (Reuber and Ausubel 1996; Ritter and Dangl 1996).

Although the interacting loci found in this study are characterized as defense pathway loci, it is also possible that these loci are active in disease susceptibility. *avrRpt2* has been shown to promote virulence in the absence of *RPS2* (Chen *et al.* 2000), and one or more of the loci identified in this study may encode a protein that is a target for the virulence activity of AvrRpt2.

Allele-specific interactions: The strong resistance response of ecotype Col-0 to *P. syringae* that express *avrRpt2* is known to be dependent on *RPS2* (Kunkel *et al.* 1993; Yu *et al.* 1993). The lack of an effective response in Po-1 initially suggested that Po-1 does not carry a functional *RPS2* allele. We discovered that Po-1 carries an allele of *RPS2* that confers *avrRpt2*-specific resistance in other genetic backgrounds, implying that defects in other Po-1 loci cause loss of *RPS2* function. Intriguingly, the Col-0 *RPS2* allele under native *RPS2* promoter complemented Po-1 for resistance when introduced by transformation, suggesting that Po-1 *RPS2* is also partly responsible for the nonfunctional resistance in Po-1. As noted above, we found that the LRR is the domain responsible for the *RPS2* component of these allele-specific interactions.

Allele-specific interactions were not confined to the Po-1 allele of *RPS2*. The discovery of Po-1 × Col-0 F_2 individuals and F_3 families that were homozygous for Col-0 *RPS2* but disease susceptible indicated that, in certain mixed Po-1/Col-0 genetic backgrounds, allele-specific interactions among resistance-modulating loci could also prevent resistance signaling through the otherwise functional Col-0 *RPS2*. The fully resistant phenotype of the Po-1 × Col-0 F_1 indicated that the nonproductive interaction between alleles that prevent Col-0 *RPS2* function is recessive.

In contrast to the above, nonproductive interactions were dominant when we monitored interaction between Po-1 *RPS2* and the *RPS2*-interacting loci. The F_1 of Po-1 × Col-0 *rps2/rps2* mutants were HR^- (Table 2). Po-1 *RPS2* could function in concert with the Col-0 alleles at these other loci (Figure 4A), but could not function in the heterozygous background of these F_1.

As a separate matter, we were intrigued that complementation experiments involving all or part of Po-1 *RPS2* often produced a weak or intermediate HR (Figure 3). Our interpretation of this result is that Po-1 RPS2 (including the Po-1 amino terminus/Col-0 LRR fusion), even when functional, cannot interact with other host factors as effectively as Col-0 RPS2. It may also be the case that Po-1 RPS2 does not recognize the *avrRpt2* ligand as effectively. Although some quantitative

reduction in responsiveness to the *avrRpt2* ligand cannot be excluded, the constructs containing domains from Po-1 *RPS2* could clearly mediate responses to *P. syringae* that express *avrRpt*2. In contrast, the host genotype at loci other than *RPS2* had a pronounced effect, correlating with the presence or near-complete absence of a response to pathogen (Figure 4).

A separate example of allele-specific interactions that affect expression of resistance was recently provided by the demonstration of monogenic and novel digenic resistance mediated by three *RXC* loci in the Arabidopsis ecotypes Col-0 and Landsberg erecta (Ler) in response to the bacterial pathogen *Xanthomonas campestris* (Buell and Somerville 1997). In the *RXC* defense system, monogenic resistance is determined by the presence of the Col-0 allele of *RXC2* while in its absence, digenic resistance is specified by the presence of the Col-0 allele of *RXC4* in conjunction with the Ler allele of *RXC3*. Numerous combinations of the six *RXC* alleles were shown to confer intermediate levels of resistance (Buell and Somerville 1997). The lack of resistance in Po-1 carrying Po-1 *RPS2*, and in some mixed Col-0/Po-1 backgrounds carrying Col-0 *RPS2*, may or may not have a similar molecular basis as the allele-specific interactions observed for *RXC* loci.

In studies on the lesion mimic Arabidopsis mutant *cep*, mapping crosses between genetically heterogeneous ecotypes showed that expression of the mutant phenotype was conditioned not only by the *cep* locus but also by two other loci that were designated *CPR20* and *CPR21* (Silva *et al.* 1999). *CPR20* mapped to the lower arm of chromosome 4 and was required for the *cep* phenotype, while *CPR21* of chromosome 1 was often but not always required for the *cep* phenotype (Silva *et al.* 1999). The genetic interval encompassing *CPR20* does not overlap with the genetic intervals on chromosome 4 that were found to contribute to the *avrRpt2*-resistance phenotype.

Sequence differences among *R* gene alleles have been shown to cause quantitative variation in the defense response in many systems (reviewed in Ellis *et al.* 2000). The general finding of quantitative variation in defense responses has been observed in many additional disease resistance systems (Michelmore 1995; Crute and Pink 1996). Our study highlights the fact that this variation can be due as much to altered interaction among host factors as to altered interaction between *R* gene product and pathogen-derived elicitors. Responsiveness to *P. syringae* that express *avrRpt2* could be observed with all natural and synthetic alleles of *RPS2* that were studied. Allele-specific interaction between other host factors and the LRR domain of RPS2 played the primary role in determining whether or not gene-for-gene defense responses were triggered.

In the future, it should be particularly informative to isolate and characterize the *RPS2/avrRpt2*-pathway gene(s) implicated by this study, and to determine the precise structural determinants that control effective interaction between the RPS2 protein and its interacting host factors.

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