The Plant Cell, Vol. 14, 1005–1015, May 2002, www.plantcell.org © 2002 American Society of Plant Biologists

RAR1 and *NDR1* Contribute Quantitatively to Disease Resistance in Arabidopsis, and Their Relative Contributions Are Dependent on the *R* Gene Assayed

Pablo Tornero,[a] Peter Merritt,[b] Ari Sadanandom,[c] Ken Shirasu,[c] Roger W. Innes,[b] and Jeffery L. Dangl[a,d,1]

[a] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599
[b] Department of Biology, Indiana University, Bloomington, Indiana 47405
[c] Sainsbury Laboratory, John Innes Centre, Colney, Norwich NR4 7UH, United Kingdom
[d] Department of Microbiology and Immunology and Curriculum in Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Plant disease resistance (*R*) genes mediate specific pathogen recognition, leading to a successful immune response. Downstream responses include ion fluxes, an oxidative burst, transcriptional reprogramming, and, in many cases, hypersensitive cell death at the infection site. We used a transgenic Arabidopsis line carrying the bacterial avirulence gene *avrRpm1* under the control of a steroid-inducible promoter to select for mutations in genes required for *RPM1*-mediated recognition and signal transduction. We identified an allelic series of eight mutants that also were allelic to the previously identified *pbs2* mutation. Positional cloning revealed this gene to be *AtRAR1*, the Arabidopsis ortholog of barley *RAR1*, a known mediator of *R* function. *AtRAR1* is required for both full hypersensitive cell death and complete disease resistance mediated by many, but not all, tested *R* genes. Double mutant analysis of *Atrar1* in combination with the *R* signal intermediate *ndr1* suggests that AtRAR1 and NDR1 can operate in both linear and parallel signaling events, depending on the *R* gene function triggered. In *Atrar1* null plants, the levels of RPM1-myc are reduced severely, suggesting that AtRAR1 may regulate R protein stability or accumulation.

INTRODUCTION

Plant recognition of pathogens is mediated by large families of highly polymorphic disease resistance (*R*) genes (Dangl and Jones, 2001; Jones, 2001). The products of these genes function to recognize, directly or indirectly, the products of pathogen-encoded avirulence (*Avr*) genes (Nimchuk et al., 2001). Recognition stimulates a signal transduction cascade leading to the activation of multiple defense responses, including, in many cases, hypersensitive plant cell death (HR) at the site of infection (reviewed by Heath, 2000). Most *R* products contain a central nucleotide binding site and C-terminal Leucine-rich repeat domains (NB-LRR). There are ~150 NB-LRR proteins encoded in the complete Arabidopsis genome (Arabidopsis Genome Initiative, 2000). The N termini of these proteins contain either potential coiled-coil (CC) or Toll–Interleukin 1 receptor homology (TIR) domains.

Genetic screens in Arabidopsis have defined several loci required for *R* function. There is evidence from these studies that the NB-LRR class of R proteins trigger multiple signaling pathways. Many, but not all, CC-NB-LRR proteins require NDR1, a protein of undefined biochemical function (Century et al., 1995, 1997). In contrast, all tested members of the TIR class require the EDS1 protein (Parker et al., 1996). EDS1 encodes a protein of unknown function, although it has homology with lipases (Falk et al., 1999). Whether or not these pathways converge into a simple linear signal transduction cascade is unknown, but it is unlikely given the fact that no locus defined by mutant phenotype is required for the function of all NB-LRR proteins.

RPM1 conditions resistance to *Pseudomonas syringae* strains expressing either *avrRpm1* or the sequence-unrelated *avrB* (Bisgrove et al., 1994; Grant et al., 1995). Thus, RPM1 recognizes one of two different Avr proteins. This recognition occurs inside the plant cell, because *P. syringae* uses the evolutionarily conserved type III secretion system to deliver disease effector proteins, including AvrRpm1 and AvrB, into the host cell (He, 1998). RPM1 is a peripheral plasma membrane protein (Boyes et al., 1998), and both AvrRpm1 and AvrB are among a class of *P. syringae* disease effector proteins that are myristoylated when expressed in the plant cell and are targeted to the plant plasma membrane (Nimchuk et al., 2000).

[1] To whom the correspondence should be addressed. E-mail dangl@email.unc.edu; fax 919-962-1625.
Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.001032.

We recently described a conditional screen for mutants affecting the function of the Arabidopsis *RPM1* gene (Tornero et al., 2002). Expression of transgenic *avrRpm1* or *avrB* (Gopalan et al., 1996; Tornero et al., 2002) in an *RPM1* background leads to a whole seedling cell death response similar to the HR, enabling facile isolation of mutants unable to activate the *RPM1* pathway. We isolated mutations in five loci required for *RPM1*-mediated resistance after the conditional expression of *avrRpm1* in mutagenized seedlings, in addition to a large number of *rpm1* alleles (Tornero et al., 2002). One of these signaling loci, originally termed *lra1* (for loss of recognition of *avrRpm1*), was defined by eight loss-of-function alleles. Here, we demonstrate that these *lra1* mutations are allelic to the previously described *pbs2-1* mutation (Warren et al., 1999) and define the Arabidopsis ortholog of barley *RAR1*. The barley *RAR1* gene was identified originally by means of genetic screens targeting the barley powdery mildew disease resistance gene *Mla12* (Freialdenhoven et al., 1994; Shirasu et al., 1999).

Barley *RAR1* is required for full HR and complete resistance mediated by many, but not all, highly related *Mla R* alleles. Interestingly, the amino acid differences between RAR1-dependent and RAR1-independent *Mla* alleles can be as little as 5% (Halterman et al., 2001; Zhou et al., 2001). We detail the effects of null *Atrar1* alleles in signaling mediated by the CC-NB-LRR proteins RPM1, RPS2, and RPS5 and the TIR-NB-LRR protein RPS4. Each recognizes *P. syringae* expressing the appropriate *avr* gene (RPM1, Grant et al., 1995; RPS2, Bent et al., 1994; Mindrinos et al., 1994; RPS5, Warren et al., 1998; RPS4, Gassmann et al., 1999).

We also address the effects of *Atrar1* mutations on the presumed CC-NB-LRR gene, *RPP7*, that conditions recognition of the *Peronospora parasitica* (*Pp*) isolate Hiks1 (Holub et al., 1994) and present double mutant analysis using *Atrar1* in combination with *ndr1*. *Atrar1* and *ndr1* null mutations have additive effects on resistance mediated by some *R* genes, indicating that AtRAR1 and NDR1 can function in separate signal transduction pathways. Our data additionally support the notion that *RAR1* and *NDR1* can act in a single pathway. Finally, we provide evidence that the levels of an epitope-tagged RPM1 protein are reduced considerably in plants with an *Atrar1* mutation. We conclude that the relative importance of *Atrar1* and *ndr1* in CC-NB-LRR *R* gene function in the Arabidopsis accession Columbia (Col-0) is dependent on the *R* gene in question.

RESULTS

Using a recently described conditional expression system (Tornero et al., 2002), we isolated eight mutants that exhibited severely attenuated *avrRpm1*-induced cell death (Figure 1). These were all alleles of one gene, which we originally named *LRA1*. All eight mutants were susceptible to *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000(*avrRpm1*) (see below), confirming that the loss of *avrRpm1*-transgene responsiveness reflected an inability to respond to AvrRpm1, regardless of the delivery system.

We next asked whether the spectrum of *R* functions altered by *lra1* was related to any of the known loci required for *R*-dependent responses. Therefore, we tested all *lra1* alleles for resistance to isogenic *Pst* DC3000 strains expressing *avrRpt2*, *avrRps4*, or *avrPphB*. Recognition of these *Avr* genes is mediated *RPS2*, *RPS4*, and *RPS5*, respectively (Bent et al., 1994; Mindrinos et al., 1994; Grant et al., 1995; Warren et al., 1998). Recognition of *Pst* DC3000(*avrRpt2*) and *Pst* DC3000(*avrPphB*) was compromised, but recognition of *Pst* DC3000(*avrRps4*) was affected only negligibly (see below). Similarly, we inoculated all of the *lra1* alleles with *Pp* isolates Cala2, Hiks1, and Emwa1 to assess the function of the *R* genes *RPP2*, *RPP7*, and *RPP4*, respectively. Only *RPP4* function was compromised (data not shown). Genetic analysis indicated that *lra1* is allelic to *pbs2-1* (Warren et al., 1999) and maps to chromosome V.

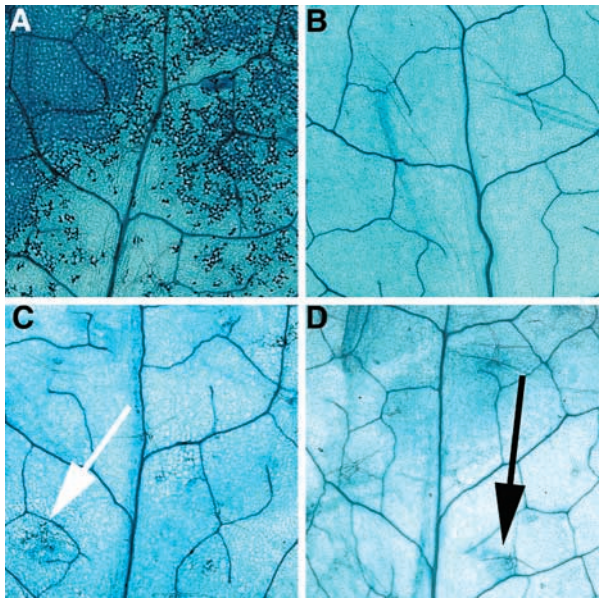

Figure 1. *lra1* Mutants Do Not Respond to Inducible Expression of *avrRpm1*.

Three-week-old plants containing an inducible *avrRpm1* expression system were sprayed with estradiol and stained with trypan blue 24 hr later. Trypan blue stains leaf veins and dead cells, revealing the region undergoing HR.

(A) Line a11 (*RPM1*, *LRA1*). Note the extent of cell death.

(B) Line a11r (*rpm1-1*, *LRA1*) contains the transgene from a11 crossed into the isogenic *rpm1-1* allele. Note the lack of cell death.

(C) and **(D)** *lra1-1* (*RPM1*, *lra1-1*) **(C)** and *lra1-2* (*RPM1*, *lra1-2*) **(D)**. The arrows point to areas of faint staining. Note that the *lra1-1* mutant is *Atrar1-21* and the *lra1-2* mutant is *Atrar1-22*.

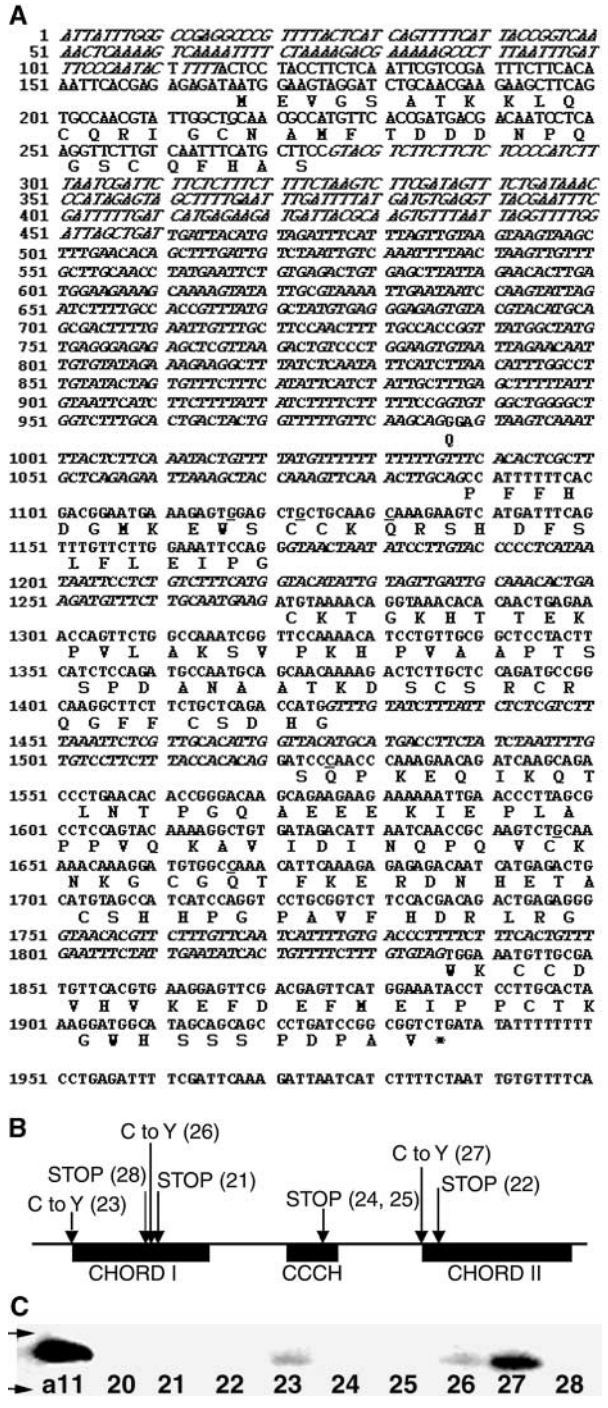

Figure 2. *LRA1/PBS2* Is *AtRAR1*.

(A) Structure of the *AtRAR1* gene. The predicted protein is portrayed in single-letter amino acid abbreviations. The mutations found in *Atrar1-21* to *Atrar1-28* are underlined. Nucleotides not present in the mRNA are shown in italic type.

(B) Relationship of *Atrar1* mutations to the RAR1 protein domain structure. "C to Y" indicates a missense mutation resulting in a Cys-to-Tyr substitution. "STOP" indicates mutations that produce a stop codon. The allele number follows each mutation.

(C) AtRAR1 protein expression in the allelic series. Total proteins were extracted from each of the mutants and analyzed by protein gel blot analysis using anti-RAR1 serum. Equal loading was ensured by Ponceau staining. The top arrow indicates a molecular mass marker of 33 kD, and the bottom arrow indicates a molecular mass marker of 25 kD. Note that there is no detectable protein in the *Atrar1-20* mutant or in the mutants produced by stop codons (*Atrar1-21*, *-22*, *-24*, *-25*, and *-28*), and there is less detectable protein where a mutation from Cys to Tyr has occurred (*Atrar1-23*, *-26*, and *-27*).

The previous linkage of *pbs2-1* to markers on chromosome I (Warren et al., 1999) may be attributable to a γ-ray–induced translocation involved in the induction of this mutant (P. Merritt and R.W. Innes, unpublished data).

Using 120 F2 plants, we were able to localize *lra1-1* to several overlapping bacterial artificial chromosome and P1 clones on the bottom arm of chromosome V (see Methods). An ortholog of the barley *RAR1* gene was contained in this genetic interval (Shirasu et al., 1999). Attempts at polymerase chain reaction amplification of the *AtRAR1* open reading frame from the *pbs2-1* mutant failed, suggesting that *AtRAR1* was disrupted in this mutant. Therefore, we amplified and sequenced *AtRAR1* from all eight *lra1* mutants. This analysis revealed a single nucleotide substitution in each mutant (see below). In agreement with the Shirasu and Parker groups, we renamed the Col-0 alleles (starting with *pbs2-1*) *Atrar1-20* to *Atrar1-28*.

As shown in Figure 2, the predicted AtRAR1 protein includes the zinc-coordinating CHORD I and CHORD II domains and the central CCCH domain described previously for barley *RAR1* (Shirasu et al., 1999). The deduced barley and Arabidopsis proteins are 60% identical. The point mutations in the ethyl methanesulfonate–derived *Atrar1-21* to *Atrar1-28* alleles produce either premature stop codons or Cys-to-Tyr exchanges (Figure 2B) that are expected to disrupt the demonstrated zinc binding coordinated by these residues (Shirasu et al., 1999). Because both early stops and disruption of zinc binding could destabilize the AtRAR1 protein, we analyzed the levels of AtRAR1 protein in all eight *lra1* mutants and in the original *pbs2-1* mutant.

Protein gel blot analysis using a polyclonal antibody raised against the full AtRAR1 protein revealed no detectable RAR1 protein in *Atrar1-20*. Neither did we identify this protein in the mutants produced by a stop codon (*Atrar1-21*, *-22*, *-24*, *-25*, and *-28*). We detected AtRAR1 protein in the mutants produced by a missense mutation (Cys to Tyr) in all cases. Note that the levels of protein are lower where mutations in the CHORD I domain have occurred (*Atrar1-23* and *-26*), compared with a mutation in the CHORD II domain (*Atrar1-27*). In the case of the CHORD I domain, it is possible that the Cys residues are required for proper folding and

Table 1. HR in *rar1* Mutant Plants

	No. of Leaves Exhibiting Visible HR[a]	
Bacterial Genotype[b]	Col-0	rar1-20
avrB::Ω[c]	1 of 53	2 of 45
avrRpt2	55 of 61	24 of 55
avrRpm1	59 of 59	12 of 60
avrPphB	65 of 68	17 of 60

[a] Number of leaves showing visible tissue collapse out of the total number of leaves injected at 18 to 24 hr after injection.
[b] *Pst* strains expressing the indicated avirulence genes were injected at an OD_{600} of 0.075 ($\sim 3.75 \times 10^7$ colony-forming units/mL).
[c] This strain expresses a nonfunctional *avr* gene and is unable to induce a visible HR in Col-0 plants.

stability, possibly also for function. The levels in *Atrar1-27* strongly suggest that the Cys residues in CHORD II are required for proper folding and function.

We investigated in detail the effects of *Atrar1* alleles on signaling by various *R* genes. In preliminary tests, all alleles behaved essentially the same (data not shown); thus, we focused on the null alleles *Atrar1-20* and *Atrar1-21*. The *Atrar1-20* allele severely attenuated, but did not eliminate, the induction of HR mediated by RPM1, RPS2, and *RPS5* (Table 1). The *RPM1*-mediated HR, typically visible 5 to 8 hr after inoculation, was delayed by several hours. Moreover, only 20% of inoculated leaves had collapsed by 18 to 22 hr after inoculation (tissue collapse caused by the susceptible response becomes visible beginning ~24 hr after inoculation at the inoculum dose used).

The HR mediated by *RPS2* and *RPS5* typically occurs 16 to 20 hr after inoculation, but these too were attenuated severely in the *Atrar1-20* mutant (Table 1). We observed identi-

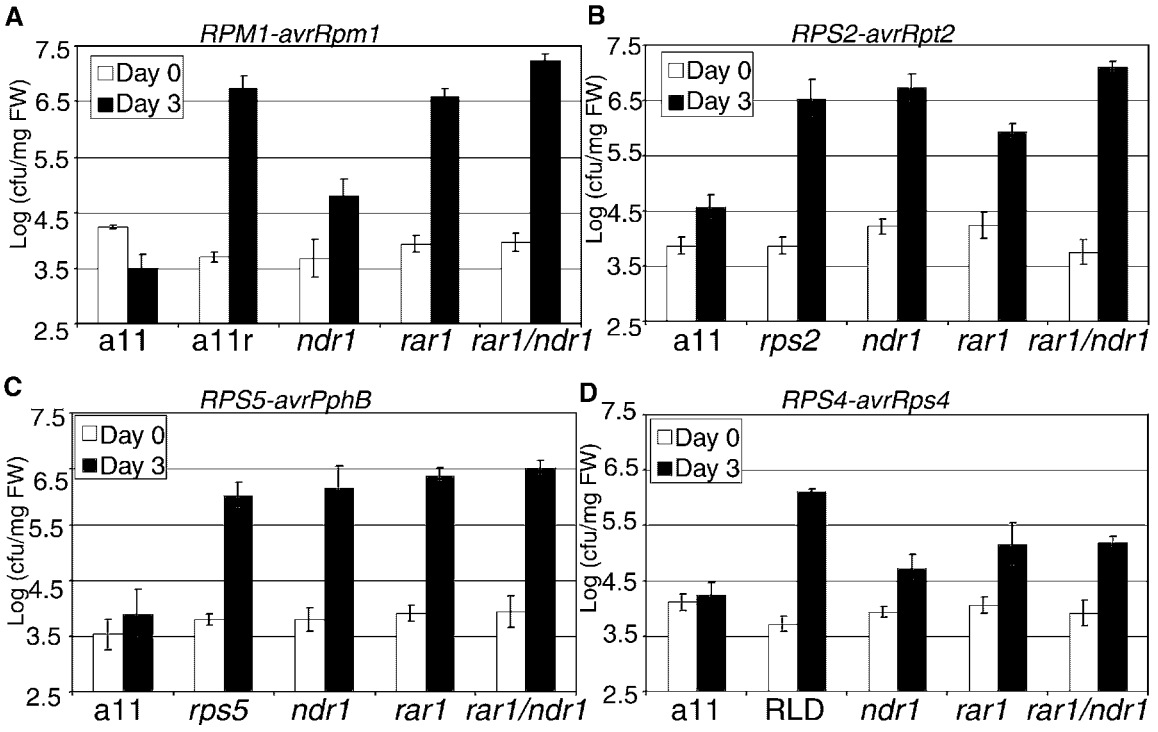

Figure 3. *AtRAR1* and *NDR1* Act Differently to Control *R* Function.

Wild-type and mutant Arabidopsis lines were inoculated with *Pst* DC3000 strains containing the indicated *avr* genes. On day 0 (white columns) and day 3 (black columns), bacteria were extracted from the plants and enumerated. Bacterial numbers are expressed as the logarithm of colony-forming units per milligram of fresh weight (cfu/mg FW). The average and SE of four independent replicates are shown. These experiments were performed three times with similar results. *ndr1-1* was described previously (Century et al., 1995).
(A) The susceptible control is a11r, an isogenic a11 derivative but in a *rpm1-1* background (Grant et al., 1995).
(B) The susceptible control is the isogenic *rps2-101C* allele (Mindrinos et al., 1994).
(C) The susceptible control is the isogenic *rps5-2* allele (Warren et al., 1998).
(D) The susceptible control is accession RLD (Hinsch and Staskawicz, 1996).

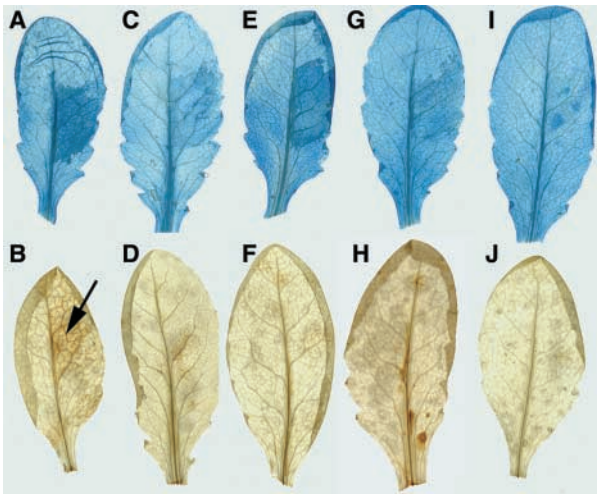

Figure 4. Staining of HR and Reactive Oxygen Intermediates in *Atrar1* and Related Mutants upon *Pst* DC3000(*avrRpm1*) Inoculation.

Plants were inoculated as described in Table 1 on the right side of the leaf and stained with trypan blue 20 hr after inoculation (**[A]**, **[C]**, **[E]**, **[G]**, and **[I]**) or with DAB 1.5 hr after inoculation (**[B]**, **[D]**, **[F]**, **[H]**, and **[J]**). Trypan blue stains veins and dead cells dark blue, and DAB stains total peroxides as a brown precipitate, as indicated by the arrow in panel **(B)**.
(A) and **(B)** a11.
(C) and **(D)** a11r.
(E) and **(F)** *ndr1-1*.
(G) and **(H)** *Atrar1-21*.
(I) and **(J)** *Atrar1-21 ndr1-1*.

cal results for *Atrar1-21* (data not shown). The inherent weakness of the HR induced by *RPS4* in the Col-0 genetic background (Gassmann et al., 1999; our unpublished data) made it impossible to test in this assay. In summary, for all three *R* genes tested, the HR was reduced dramatically, but not eliminated entirely, indicating that RAR1 contributes significantly to the HR.

Next, we quantified the effect of *Atrar1-21* (and of several other alleles; data not shown) on *R* function by measuring bacterial growth after dip inoculation of seedlings (Tornero and Dangl, 2001). Col-0 expresses all of the relevant *R* genes, and we used null or strong loss-of-function *rpm1*, *rps2*, *rps4*, and *rps5* alleles as fully susceptible controls. As shown in Figure 3, disease resistance controlled by *RPM1* and *RPS5* was eliminated in *Atrar1-21*. *RPS2* function was nearly, but not completely, eliminated. We reproducibly observed ~10 to 20% residual *RPS2* function. *Atrar1-21* had only a very minor effect on *RPS4* function in this assay. These data extend those reported previously for the *Atrar1-20* allele (Warren et al., 1999).

We also compared the effect of the *ndr1-1* mutation on the function of these *R* genes, because our original genetic analysis (see above) suggested that the Col-0 *Atrar1* alleles we isolated affected the same spectrum of *R* functions as *ndr1-1* (Century et al., 1995). *RPM1* function was diminished only partially in an *ndr1-1* background, whereas *RPS2* and *RPS5* functions were eliminated (Figure 3). The level of *RPS4* function in Col-0 retained in the *Atrar1-21* allele was statistically the same as that retained in the *ndr1-1* mutant and the parental line a11 (Student's *t* test; $\alpha = 0.05$) (Figure 3D). Interestingly, this is not the case for *RPS4* function measured in Landsberg *erecta*–derived *Atrar1* alleles (Muskett et al., 2002). These changes in disease resistance were mirrored by the appearance of visible disease symptoms in all susceptible interactions (data not shown). In addition, there was no effect of the *Atrar1* mutation on the growth of virulent *Pst* DC3000 or virulent *Pp* (Noco2 isolate), so the *Atrar1* mutants do not express enhanced disease susceptibility phenotypes.

We examined cell death and hydrogen peroxide production at the cellular level in *ndr1* and *Atrar1* mutants inoculated with *Pst* DC3000(*avrRpm1*). As reported previously by Century et al. (1995) and as illustrated in Figure 4, *ndr1-1* retained the ability to induce an *RPM1*-dependent HR (cf. Figures 4A and 4E). Interestingly, the *ndr1-1* mutant does not support an obvious *RPM1*-dependent oxidative burst, as indicated by greatly reduced staining of the inoculated zone with 3,3′-diaminobenzidine (DAB; Figure 4F) (Shapiro and Zhang, 2001). Consistent with previous analyses of barley *rar1* mutants, we found no DAB staining in *Atrar1-21* (Figure 4H).

To address further the relative contributions of *RAR1* and *NDR1* to HR and disease resistance, we constructed an *Atrar1-21 ndr1-1* double null mutant. The double mutant expressed the *Atrar1* phenotype for severe attenuation of *RPM1*-dependent HR, as shown in Figure 4I. Additionally, the double mutant also resembled the fully susceptible *Atrar1* single mutant in bacterial growth assays (Figure 3).

Thus, *AtRAR1* appears to act in the same pathway as *NDR1* during *RPM1*-dependent responses, and its activity is required for both the residual *RPM1*-dependent HR and the residual disease resistance observed in *ndr1-1* mutants. *RPS5* function is eliminated fully in either single mutant, and the double mutant phenotype resembles either single mutant phenotype (Figure 3). This is consistent with *AtRAR1* and *NDR1* acting in the same pathway, although no relative order can be implied. In contrast, *RPS2* function in the *Atrar1 ndr1-1* double mutant resembles that of the fully susceptible *ndr1-1* single mutant. Thus, we conclude that *AtRAR1* is more important than *NDR1* in the transduction of *RPM1* function, that the reverse is true for *RPS2* function, and that both are essential for *RPS5* function. These data suggest that the relative contributions of *AtRAR1* and *NDR1* to resistance mediated by a given *R* gene can vary.

The apparently complex functional relationship between *RAR1* and *NDR1* prompted us to analyze another *R* function measurable in the Col-0 background. We chose the *RPP7* gene, which conditions resistance to the Hiks1 isolate of the obligate biotrophic oomycete parasite *Pp* (Holub et al.,

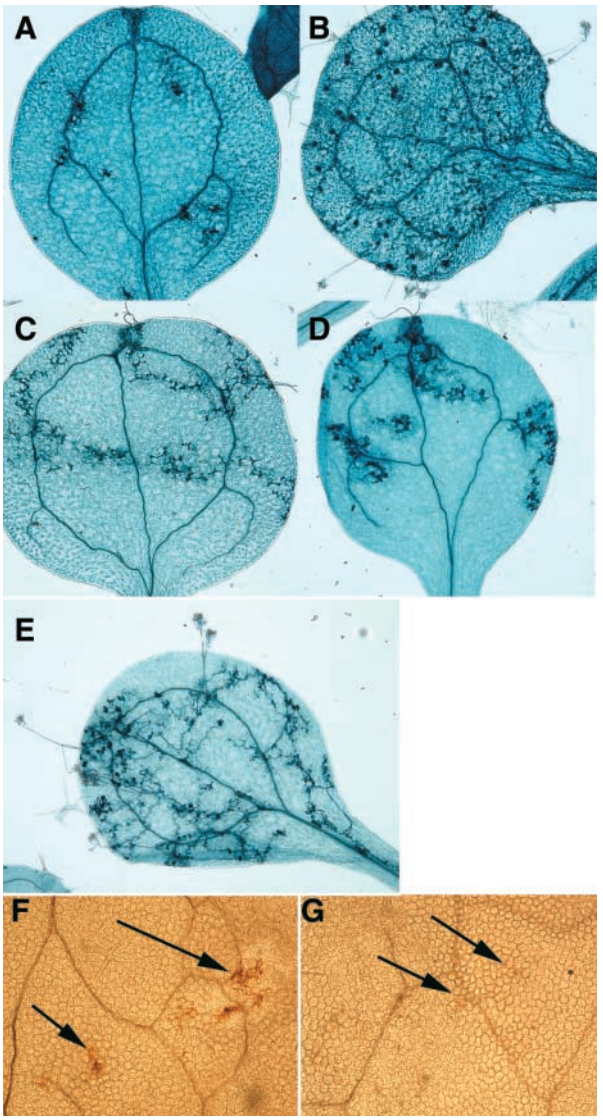

Figure 5. Staining of HR Sites and Production of Reactive Oxygen Intermediates Is Altered during *RPP7*-Mediated Responses.

Plants were inoculated with *Pp* strain Hiks1 by spraying with an aqueous suspension containing 5×10^4 oospores/mL and then stained with trypan blue 5 days after inoculation (**[A]** to **[E]**) or with DAB 2 days after inoculation (**[F]** and **[G]**). The arrows in panels **(F)** and **(G)** point to sites of hyphae penetration.
(A) and **(F)** a11.
(B) *rpp7*.
(C) *ndr1-1*.
(D) and **(G)** *Atrar1-21*.
(E) *Atrar1-21 ndr1-1*.

1994). *RPP7* function normally is associated with a small HR at the attempted infection site, as shown in Figure 5A using trypan blue staining. In contrast, a lack of host cell response and full sporulation were observed in an *rpp7* mutant (Figure 5B). As noted previously (McDowell et al., 2000), *RPP7* function was altered only slightly by *ndr1-1*, in that fungal hyphae elongated and the host responded with HR in cells surrounding those hyphae (Figure 5C). This phenotype is termed trailing necrosis (Morel and Dangl, 1998). We also observed trailing necrosis in *Atrar1-21* mutants after *Pp* Hiks1 infection (Figure 5D) and no sporulation, as described previously (Warren et al., 1999).

The *Atrar1-21 ndr1-1* double mutant exhibited markedly reduced *RPP7* function, resulting in low but reproducible production of sporangiophores and a reduction in trailing necrosis compared with either single mutant (Figure 5E). To quantitate these interactions, we compiled infection sites into three phenotypic classes (Figure 6) (Morel and Dangl, 1998): HR, trailing necrosis, and free hyphae without associated host cell response. The *Atrar1-21 ndr1-1* double mutant compromised *RPP7* function much more than either single mutant. We conclude from these data that *RAR1* and *NDR1* act in separable pathways to partially mediate *RPP7* function. Yet, even the *Atrar1-21 ndr1-1* double mutant retained significant *RPP7* function. Thus, although both *RAR1* and *NDR1* are necessary for complete *RPP7* function, they are not sufficient for it.

Shirasu and colleagues (1999) suggested that RAR1 functions as part of a protein complex that regulates protein degradation. We reported previously that an RPM1-myc protein expressed by the native *RPM1* promoter disappears just before the onset of HR triggered by each of the bacterial *avr-R* combinations assayed here (Boyes et al., 1998). Therefore, we crossed the *RPM1-myc* transgene used in that study to *Atrar1-21* and selected F2 progeny that carried the null mutant allele (*Atrar1-21*/*Atrar1-21*, *RPM1*/*RPM1*, and *RPM1-myc*/*RPM1-myc*). We also created the corresponding controls (*AtRAR1*/*AtRAR1*, *RPM1*/*RPM1*, and *RPM1-myc*/*RPM1-myc*) by introgression. Each line carries the conditional *avrRpm1* expression system and the same RPM1-myc transgene. Surprisingly, the levels of RPM1-myc that we detected in the *Atrar1-21* mutant were reproducibly much lower than the levels in *AtRAR1* plants (Figure 7). This striking result strongly suggests that RAR1 function is required for the accumulation and/or stability of RPM1-myc. The very low levels of RPM1-myc that we observed in *Atrar1-21* precluded the determination of its disappearance after pathogen inoculation.

DISCUSSION

The signaling pathways that lead to disease resistance are complex. Previous work had established that some Arabidopsis *R* genes require the function of *NDR1* and others re-

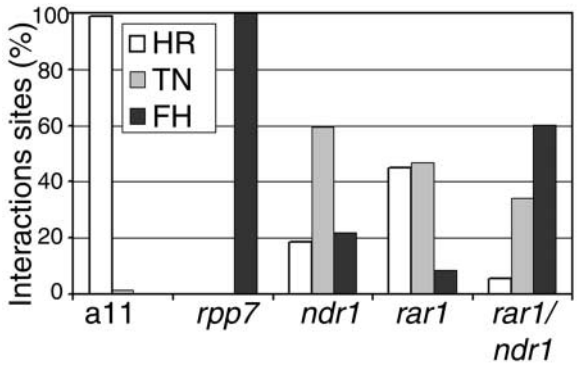

Figure 6. *Atrar1* and *ndr1-1* Affect *RPP7* Function Additively.

Plants were inoculated with *Pp* strain Hiks1 as described in Figure 5. Seven days after inoculation, the plants were stained with trypan blue, and the interactions sites were classified as HR, trailing necrosis (TN), and free hyphae (FH). A minimum of 591 interactions per genotype from three independent experiments are represented.

quire *EDS1*. Additionally, some but not all *R* genes require salicylic acid accumulation and *NPR1*/*NIM1* function (reviewed by Glazebrook, 2001). At least one *R* gene, RPP7, appears to use *NDR1* and *EDS1* in combination to mediate some or all of its function (McDowell et al., 2000; but see below). Finally, ethylene- and jasmonic acid–dependent signals also can influence *R* function (Clarke et al., 2000). Here, we provide compelling evidence that the Arabidopsis ortholog of barley *RAR1* also is required for the action of several, but not all, tested *R* genes. Thus, a key step in *R* signaling is conserved evolutionarily. We further demonstrate that *AtRAR1* and *NDR1* contribute differently to the overall efficiency of the defense response, depending on the *R* function being assayed. This relative contribution can be simple and linear or quantitative and separable.

AtRAR1 is a single-copy gene. Perhaps surprisingly, all of the mutations were either stop codons or changes in the very conserved Cys residues (Shirasu et al., 1999). This may reflect a screening bias or may imply that weak loss-of-function *Atrar1* alleles have only weak effects on *R* function and would be missed in screens relying on strong loss-of-function phenotypes for mutant detection. Alternatively, there may be some internal functional redundancy in the RAR1 protein. For example, the CHORD I and CHORD II domains could act independently in *R* signaling. If this is the case, it follows that only strong loss-of-function or null phenotypes affecting both CHORD I and CHORD II function would be isolated. These results are in contrast to the original definition of *RAR1* in barley, for which two partial loss-of-function alleles were described and about which it was speculated that *RAR1* might be essential (Shirasu et al., 1999).

We further establish that the functions of some Col-0 *R* genes are not altered grossly by the *Atrar1* mutation. *RPS4*, *RPP7*, and *RPP2* are not totally compromised in the Col-0 *Atrar1-20* and *Atrar1-21* backgrounds (Warren et al., 1999; but see Muskett et al., 2002, for analysis of *RPS4* function in Landsberg *erecta*). The deduced RPS4 protein is of the TIR-NB-LRR subclass, as is RPP2 (Holub, 2001). RPP7 has not been isolated, but it is defined by several allelic loss-of-function ethyl methanesulfonate alleles (A. Cuzick and E. Holub, unpublished data). It has been mapped to a 100-kb region that is rich in CC-NB-LRR genes (Holub, 2001; A. Cuzick and E. Holub, unpublished data). There are no TIR-NB-LRR genes in this region. If one of the CC-NB-LRR genes proves to encode RPP7, then examples of both R protein structural subclasses will exist that do not require RAR1 for signaling. Conversely, *RPP4*, which requires *AtRAR1*, was shown recently to belong to the TIR-NB-LRR class (van der Biezen et al., 2002). Thus, the requirement for RAR1 function in Col-0 does not correlate with the structural subclass of the R protein in question.

Several aspects of our data strongly support the conclusion that *AtRAR1* quantitatively contributes to *R* signaling in conjunction with *EDS1* and *NDR1*:

(1) At least three *R* gene functions in Col-0 (*RPM1*, *RPS2*, and *RPS5*) are altered significantly in *Atrar1* mutants. Each of these requires *NDR1* to function, as measured by the restriction of bacterial growth, but this effect is quantitative. Furthermore, we can differentiate *Atrar1* from *ndr1-1* based

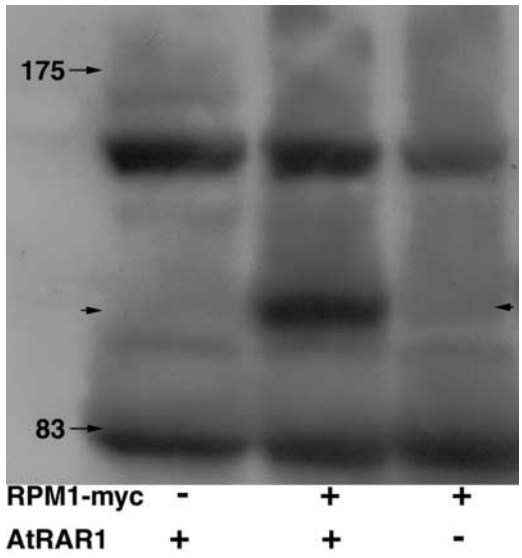

Figure 7. RPM1-myc in *Atrar1* Mutants.

Plants were harvested at 5 weeks of age, and total extracts were prepared as described by Boyes et al. (1998). Arrows with numbers indicate the positions of molecular mass markers (kD). The arrows at both sides of the figure indicate the position of RPM1-myc. Three independent experiments were performed, and each gave similar results.

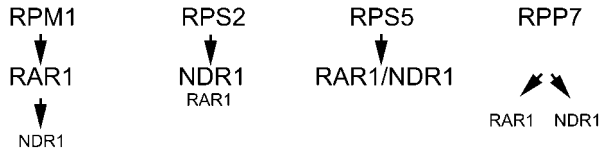

Figure 8. Genetic Requirements for the *R* Gene Functions Tested.

The font size reflects the relative contribution of each locus to the function of each R protein listed at top. A larger font implies that the null mutant compromises *R* function severely, and a smaller font implies a moderate effect. A locus placed in the same vertical orientation implies a single pathway. Note that the order of action can be inferred clearly only in the case of *RPM1*. Loci side by side imply that no relationship was determined, and split arrows represent parallel pathways.

on their effects on HR induction: the *ndr1-1* null mutation still produces an essentially wild-type *RPM1*-dependent HR, whereas *Atrar1* mutations greatly attenuate, but do not abolish, this response. We also observed stochastic reduction in the HR mediated by RPM1 and RPS2 in *Atrar1* null mutants. Some leaves in each experiment consistently expressed a seemingly wild-type response. Therefore, *AtRAR1* is required for the restriction of pathogen growth, but it contributes differentially to the HR triggered by any given *R* gene.

(2) At least three R gene functions in Col-0 (*RPS4*, *RPP2*, and *RPP7*) are not compromised obviously in *Atrar1* mutants (Warren et al., 1999; see above). RPS4 requires EDS1 for signaling (Gassmann et al., 1999). RPP2, a TIR-NB-LRR protein active against *Pp* isolate Cala2, also is largely *EDS1* dependent (Parker et al., 1996).

(3) *AtRAR1* can act in combination with *NDR1*. We demonstrated that *Atrar1-21 ndr1-1* double mutants expressed the *Atrar1-21* phenotype with respect to *RPM1* function, the *ndr1-1* phenotype with respect to *RPS2* function, the phenotype of either single mutant with respect to *RPS5* function, and an additive phenotype with respect to *RPP7* function. Figure 8 presents an interpretation of these results in which the size of the font represents the relative contribution of each gene to the particular *R* function tested. Thus, the relative contributions of *AtRAR1* and *NDR1* to resistance are dependent on which *R* function is assayed. Both *RPP2* and *RPP4* are suppressed very modestly in *Atrar1-20*, *Atrar1-21*, and *ndr1-1* single mutants (Warren et al., 1999; our unpublished data). Moreover, the *Atrar1-21 ndr1-1* double mutant does not enhance the suppression of these *R* functions significantly (data not shown), as it did for *RPP7*. Thus, there is a level of specificity achieved in the quantitative level of resistance that is determined by a combination of particular R proteins with NDR1, AtRAR1, and EDS1. This conclusion is puzzling at first because the specificity of *R* genes is assumed to lie exclusively in the R protein itself. Nevertheless, the genetically defined requirements for *R* gene function produce a "fingerprint" that informs the eventual phenotypic output. This information might allow the plant to use the same *R* genes in different contexts, multiplying the effectiveness of the NB-LRR system as a whole.

Recent work by Shirasu and colleagues (Azevedo et al., 2002) has shown that the AtRAR1 protein interacts physically with a plant ortholog of the yeast SGT1 protein (Kitigawa et al., 1999). SGT1 is a regulatory component of the SCF complex (Skp1, Cullin, F-box) (reviewed by Deshaies, 1999; Bachmair et al., 2001) that acts as an E3 ligase in ubiquitination of target proteins. Modification of target proteins by ubiquitin, or ubiquitin-like molecules, can lead to their degradation via the proteasome or can serve to regulate function directly (Hicke, 2001). The simplest model for the role of RAR1 in *R* function is that it directs either the removal of a negative regulator (Gray et al., 1999) or the activation of a positive regulator (Wang et al., 2001) by recruitment of that factor to the SCF via SGT1 and subsequent ubiquitination.

Our finding that RPM1-myc levels are reduced severely in the *Atrar1-21* background supports this general model but does not address whether AtRAR1 acts positively and directly on RPM1-myc stability or whether it acts by removing a negative regulator of RPM1-myc stability. It is possible that the main function of AtRAR1 is to regulate the steady state levels of some, but not all, R proteins present in the plant cell. However, this model is difficult to reconcile with the finding that R proteins that differ by as little as 5% differ in their requirements for barley RAR1 (Zhou et al., 2001).

The evidence presented by our colleagues (Azevedo et al., 2002; Muskett et al., 2002; Tör et al., 2002) and in this report suggests that, in addition to the response specificity encoded in the R protein, additional specificity operates at the level of post-translational regulation. The fact that RAR1-dependent and RAR1-independent barley R proteins differ by $<5\%$ (Zhou et al., 2001) focuses attention on how those amino acid differences mediate the subsequent signaling or regulation of R protein stability and/or accumulation.

METHODS

Plant Lines

The transgenic *Arabidopsis thaliana* Columbia lines a11 and a11r have been described (Tornero et al., 2002). Briefly, these lines allow the conditional expression of avrRpm1 upon the application of estradiol. The background of a11 is Columbia (RPM1), and that of a11r is rpm1-1 (Grant et al., 1995). The Atrar1-21 line used for all described experiments was backcrossed twice to its parental line, a11. Plants were grown in a short day regimen, as described by Ritter and Dangl (1996). Mutant lines used were *ndr1-1* (a null allele; Century et al., 1997), *pbs2-1* (Warren et al., 1999), *rps2-101C* (Mindrinos et al., 1994), *rps5-2* (Warren et al., 1998), and a strong loss-of-function allele of *rpp7* (McDowell et al., 2000). We constructed double mutant lines by crossing *Atrar1-21* with *ndr1-1*, selecting F2 plants that were

rar1 based on phenotype against *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000(*avrRpm1*) and confirmed by sequencing of the mutation and F2 plants that were *ndr1* based on polymerase chain reaction (PCR)–based molecular markers as described by Rusterucci et al. (2001).

Pathogen Strains and Quantitation of Bacterial Growth in Leaves

Pst DC3000 derivatives containing pVSP61 (empty vector), a*vrRpm1*, *avrB*, *avrB*::Ω (a disrupted nonfunctional version of *avrB*), *avrRpt2*, *avrPphB*, or *avrRps4* were maintained as described (Ritter and Dangl, 1996). Plant inoculations and counting of the bacteria were performed as described (Tornero and Dangl, 2001). Where indicated, high concentrations of bacteria (OD_{600} = 0.075, 3.75 × 10^7 colony-forming units/mL) were infiltrated into the bottom part of the leaf with a blunt syringe to test for the induction of hypersensitive plant cell death.

Peronospora parasitica isolate Hiks1 was maintained and inoculated on 10-day-old plants as described (Holub et al., 1994). Inoculated plants were kept under a sealed propagator lid to achieve high RH in a growth chamber at 19°C under an 8-hr light period (100 to 160 $\mu E \cdot m^{-2} \cdot sec^{-1}$). To evaluate the infection, plants were stained with trypan blue (Koch and Slusarenko, 1990) 5 days after inoculation and observed with a microscope. Infection sites were scored as "free hyphae," "trailing necrosis," or "hypersensitive response" (Morel and Dangl, 1998). Free hyphae was defined as hyphal growth without detectable plant cell death. Trailing necrosis was defined as hyphal growth with surrounding plant cell death. Hypersensitive response was defined as plant cell death at the infection site with no hyphal growth beyond the infection site.

Genetic Analysis

Allelism between *lra1* mutants and *pbs2-1* (Warren et al., 1999) was determined by standard genetic crosses followed by analysis of F1 and F2 progeny for resistance to estradiol treatment (induction of the *avrRpm1* transgene) as described (Tornero et al., 2002) or by hand inoculations of *Pst* DC3000(*avrRpm1*). Mapping populations were established by crossing Landsberg *erecta* with *lra1-1* (*Atrar1-21*) and *lra1-8* (*Atrar1-28*) and analyzing the response of F2 individuals to infection by *Pst* DC3000(*avrRpm1*). Plants that were susceptible to these bacteria were allowed to self and were retested in the F3 generation, and a sample of their genomic DNA was extracted by conventional methods (Ausubel et al., 1987). DNA from 35 of these plants was used in PCR amplification of known PCR-based molecular markers (www.arabidopsis.org) to obtain approximate mapping positions.

Subsequently, we refined this interval using newly developed molecular markers (available upon request) and a total of 120 F2 individuals, localizing *lra1* to several overlapping bacterial artificial chromosome (BAC) and P1 clones. The genetic interval containing *lra1* was defined by a single recombinant event between a marker derived from nucleotides 50,446 to 50,174 on P1 clone MIO24 and *lra1* and by three recombinant events between a marker derived from nucleotides 16,930 to 17,292 on BAC K2OJ1 and *lra1*. Note that the first marker is 43,400 nucleotides 3′ of the *AtRAR1* open reading frame, because the nucleotide numbering of this BAC is inverted relative to that of BAC K2OJ1. Attempts to amplify the *AtRAR1* open reading frame from the *pbs2-1* allele failed, consistent with a deletion or rearrangement of *AtRAR1* in the *pbs2-1* mutant. Therefore, we sequenced the *AtRAR1* gene (nucleotides 5276 to 7046 of BAC MIO24; primers available upon request) from all eight *lra1* mutants and found the mutations described.

Analysis of Protein Levels

Generation of the anti-RAR1 antiserum is described by Azevedo et al. (2002). For protein gel blot analysis, tissue from 3-week-old plants was extracted with a buffer containing 50 mM Tris-HCl, pH 7.5, 10% glycerol, 50 mM NaCl, 10 mM $MgCl_2$, 5 mM EDTA, 1 mM DTT, 5 mM 2-mercaptoethanol, 1 × proteinase inhibitor (Sigma), and 0.3% insoluble polyvinylpyrrolidone. Protein concentrations were determined by Bradford (1976) assay (Bio-Rad). For immunodetection, 40-μg protein samples were electrophoresed on 15% polyacrylamide gels and run in the presence of 0.38 M Tris and 0.1% SDS. Proteins were transferred from the gels to nitrocellulose filters by electroblotting, incubated with primary anti-RAR1 antibody and horseradish peroxidase–conjugated secondary antibody, and detected with enhanced chemiluminescence (ECL+; Amersham).

For detection of RPM1-myc, we used plants that carry a transgenic *RPM1-myc* expressed from the native *RPM1* promoter (Boyes et al., 1998). This line was introgressed into the a11 line. The resulting line has two functional *RPM1* genes. Similarly, we introgressed *Atrar1-21* into this line. Protein extraction and protein gel blot analysis were performed as described (Boyes et al., 1998).

Microscopy

Trypan blue staining was performed according to Koch and Slusarenko (1990). Stained leaves were observed with an Eclipse E800 microscope (Nikon, Tokyo, Japan) equipped with a Spot charge-coupled device camera (Diagnostic Instruments, Sterling Heights, MI). The images shown were taken with a ×4 objective using transmitted light. Images were processed using Spot software (version 2.1; Diagnostic Instruments) and Photoshop (version 5.5; Adobe Systems, Mountain View, CA). Images from whole leaves (Figure 4) were taken by directly scanning the mounted tissue with a Microtek 8700 color scanner (Redondo Beach, CA). Hydrogen peroxide was detected by staining with 3,3′-diaminobenzidine using a modification of the protocol described by Thordal-Christensen et al. (1997). In brief, leaves were excised 90 min after inoculation and vacuum infiltrated with a solution (1 mg/mL) of 3,3′-diaminobenzidine. Subsequently, the leaves were kept under high humidity and darkness for 5 hr, cleared with ethanol, and mounted on slides.

ACKNOWLEDGMENTS

We thank Swapna Putcha for her help with genetic mapping and Dr. Jane Parker for sharing map information before publication. This work was funded by National Science Foundation Grant IBN-9724075, renewed under the Arabidopsis 2010 project as IBN-0114795, and by U.S. Department of Agriculture National Research Initiative Competitive Grants Program Grant 99-35301-7848 to J.L.D. and National Institutes of Health Grant R01GM46451 to R.W.I. We gratefully acknowledge a Syngenta Collaborative Research Grant to J.L.D., a postdoctoral fellowship from the Spanish Ministry of

Science and Education to P.T., and a U.S. Department of Agriculture National Needs predoctoral fellowship to P.M. A.S. and K.S. are supported by grants from The Gatsby Charitable Foundation.

Received December 7, 2001; accepted February 22, 2002.

REFERENCES

Arabidopsis Genome Initiative (2000). Analysis of the genome of the flowering plant *Arabidopsis thaliana*. Nature **408,** 796–815.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1987). Current Protocols in Molecular Biology. (New York: John Wiley & Sons).

Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K., and Schulze-Lefert, P. (2002). The RAR1 interactor SGT1 is an essential component of R-gene triggered disease resistance. Science, in press.

Bachmair, A., Novatchkova, M., Potuschak, T., and Eisenhaber, F. (2001). Ubiquitination in plants: A post-genomic look at a post-translational modification. Trends Plant Sci. **6,** 463–470.

Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B.J. (1994). *RPS2* of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. Science **265,** 1856–1860.

Bisgrove, S.R., Simonich, M.T., Smith, N.M., Sattler, N.M., and Innes, R.W. (1994). A disease resistance gene in Arabidopsis with specificity for two different pathogen avirulence genes. Plant Cell **6,** 927–933.

Boyes, D.C., Nam, J., and Dangl, J.L. (1998). The *Arabidopsis thaliana RPM1* disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. Proc. Natl. Acad. Sci. USA **95,** 15849–15854.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248–254.

Century, K.S., Holub, E.B., and Staskawicz, B.J. (1995). *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. Proc. Natl. Acad. Sci. USA **92,** 6597–6601.

Century, K.S., Shapiro, A.D., Repetti, P.P., Dahlbeck, D., Holub, E., and Staskawicz, B.J. (1997). NDR1, a pathogen-induced component required for Arabidopsis disease resistance. Science **278,** 1963–1965.

Clarke, J.D., Volko, S.M., Ledford, H., Ausubel, F.M., and Dong, X. (2000). Roles of salicylic acid, jasmonic acid, and ethylene in *cpr*-induced resistance in Arabidopsis. Plant Cell **12,** 2175–2190.

Dangl, J.L., and Jones, J.D.G. (2001). Plant pathogens and integrated defence responses to infection. Nature **411,** 826–833.

Deshaies, R.J. (1999). SCF and cullin/RING H2-based ubiquitin ligases. Annu. Rev. Cell Dev. Biol. **15,** 435–467.

Falk, A., Feys, B., Frost, L.N., Jones, J.D.G., Daniels, M.J., and Parker, J.E. (1999). *EDS1*, an essential component of *R* gene-mediated disease resistance in Arabidopsis has homology to eukaryotic lipases. Proc. Natl. Acad. Sci. USA **96,** 3292–3297.

Freialdenhoven, A., Scherag, B., Hollricher, K., Collinge, D.B., Thordal-Christensen, H., and Schulze-Lefert, P. (1994). Nar-1 and Nar-2, two loci required for Mla_{12}-specified race-specific resistance to powdery mildew in barley. Plant Cell **6,** 983–994.

Gassmann, W., Hinsch, M., and Staskawicz, B. (1999). The Arabidopsis RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. Plant J. **20,** 265–277.

Glazebrook, J. (2001). Genes controlling expression of defense responses in *Arabidopsis*: 2001 status. Curr. Opin. Plant Biol. **4,** 301–308.

Gopalan, S., Bauer, D.W., Alfano, J.R., Lonlello, A.O., He, S.Y., and Collmer, A. (1996). Expression of the *Pseudomonas syringae* avirulence protein AvrB in plant cells alleviates its dependence on the hypersensitive response and pathogenicity (Hrp) secretion system in eliciting genotype-specific hypersensitive cell death. Plant Cell **8,** 1095–1105.

Grant, M.R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R.W., and Dangl, J.L. (1995). Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. Science **269,** 843–846.

Gray, W.M., del Pozo, J.C., Walker, L., Hobbie, L., Risseeuw, E., Banks, T., Crosby, W.L., Yang, M., Ma, H., and Estelle, M. (1999). Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. Genes Dev. **13,** 1678–1691.

Halterman, D., Zhou, F., Wei, F., Wise, R.P., and Schulze-Lefert, P. (2001). The MLA6 coiled-coil, NBS-LRR protein confers AvrMla6-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. Plant J. **25,** 335–348.

He, S.-Y. (1998). Type III secretion systems in plant and animal pathogenic bacteria. Annu. Rev. Phytopathol. **36,** 363–392.

Heath, M.C. (2000). Hypersensitive response-related death. Plant Mol. Biol. **44,** 321–334.

Hicke, L. (2001). Protein regulation by monoubiquitin. Nat. Rev. Mol. Cell. Biol. **2,** 195–201.

Hinsch, M., and Staskawicz, B.J. (1996). Identification of a new *Arabidopsis* disease resistance locus *RPS4*, and cloning of the corresponding avirulence gene, *avrRps4*, from *Pseudomonas syringae* pv. *pisi*. Mol. Plant-Microbe Interact. **9,** 55–61.

Holub, E. (2001). The arms race is ancient history in *Arabidopsis*, the wildflower. Nat. Rev. Genet. **2,** 516–527.

Holub, E.B., Beynon, J.L., and Crute, I.R. (1994). Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. **7,** 223–239.

Jones, J.D.G. (2001). Putting knowledge of plant disease resistance genes to work. Curr. Opin. Plant Biol. **4,** 281–287.

Kitigawa, K., Skowyra, D., Elledge, S.J., Harper, J.W., and Hieter, P. (1999). *SGT1* encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin complex. Mol. Cell **4,** 21–33.

Koch, E., and Slusarenko, A.J. (1990). *Arabidopsis* is susceptible to infection by a downy mildew fungus. Plant Cell **2,** 437–445.

McDowell, J.M., Cuzick, A., Can, C., Benyon, J., Dangl, J.L., and Holub, E.B. (2000). Downy mildew (*Peronospora parasitica*) resis-

tance genes in Arabidopsis vary in functional requirements for *NDR1*, *EDS1*, *NPR1*, and salicylic acid accumulation. Plant J. **22,** 523–530.

Mindrinos, M., Katagiri, F., Yu, G.-L., and Ausubel, F.M. (1994). The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell **78,** 1089–1099.

Morel, J.-B., and Dangl, J.L. (1998). Suppressors of the Arabidopsis *lsd5* cell death mutation identify genes involved in regulating disease resistance responses. Genetics **151,** 305–319.

Muskett, P.J., Kahn, K., Austin, M.J., Moisan, L.J., Sadanandom, A., Shirasu, K., Jones, J.D.G., and Parker, J.E. (2002). Arabidopsis *RAR1* exerts rate-limiting control of *R* gene–mediated defense against multiple pathogens. Plant Cell **14,** 979–992.

Nimchuk, Z., Marois, E., Kjemtrup, S., Leister, R.T., Katagiri, F., and Dangl, J.L. (2000). Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. Cell **101,** 353–363.

Nimchuk, Z., Rohmer, L., Chang, J.H., and Dangl, J.L. (2001). Knowing the dancer from the dance: R gene products and their interactions with other proteins from host and pathogen. Curr. Opin. Plant Biol. **4**, 288–294.

Parker, J.E., Holub, E.B., Frost, L.N., Falk, A., Gunn, N.D., and Daniels, M.J. (1996). Characterization of *eds1*, a mutation in Arabidopsis suppressing resistance to *Peronospora parasitica* specified by several different *RPP* genes. Plant Cell **8,** 2033–2046.

Ritter, C., and Dangl, J.L. (1996). Interference between two specific pathogen recognition events mediated by distinct plant disease resistance genes. Plant Cell **8,** 251–257.

Rusterucci, C., Aviv, D.H., Holt, B.F., Dangl, J.L., and Parker, J.E. (2001). The disease resistance signaling components *EDS1* and *PAD4* are essential regulators of the cell death pathway controlled by *LSD1* in Arabidopsis. Plant Cell **13,** 2211–2224.

Shapiro, A.D., and Zhang, C. (2001). The role of NDR1 in avirulence gene-directed signaling and control of programmed cell death in Arabidopsis. Plant Physiol. **127,** 1089–1101.

Shirasu, K., Lahaye, T., Tan, M.-W., Zhou, F., Azevedo, C., and Schulze-Lefert, P. (1999). A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. Cell **99,** 355–366.

Thordal-Christensen, H., Zhang, Z., Wei, Y., and Collinge, D.B. (1997). Subcellular localization of H_2O_2 in plants: H_2O_2 accumulation in papillae and hypersensitive response during barley-powdery mildew interaction. Plant J. **11,** 1187–1194.

Tör, M., et al. (2002). An *SGT1*-like gene in Arabidopsis is essential for defense signalling conferred by several downy mildew (*Peronospora parasitica*) resistance genes including *RPP7*. Plant Cell **14,** 993–1003.

Tornero, P., and Dangl, J.L. (2001). A high throughput method for quantifying growth of phytopathogenic bacteria in *Arabidopsis thaliana*. Plant J. **28,** 475–481.

Tornero, P., Chao, R.A., Luthin, W.N., Goff, S.A., and Dangl, J.L. (2002). Large-scale structure-function analysis of the Arabidopsis RPM1 disease resistance protein. Plant Cell **14,** 435–450.

van der Biezen, E.A., Freddie, C.T., Kahn, K., Parker, J.E., and Jones, J.D.G. (2002). Arabidopsis RPP4 is a member of the RPP5 multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signaling components. Plant J. **29,** 439–451.

Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J., and Chen, Z.J. (2001). TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature **412,** 346–351.

Warren, R.F., Henk, A., Mowery, P., Holub, E., and Innes, R.W. (1998). A mutation within the leucine-rich repeat domain of the Arabidopsis disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. Plant Cell **10,** 1439–1452.

Warren, R.F., Merritt, P.M., Holub, E.B., and Innes, R.W. (1999). Identification of three putative signal transduction genes involved in R gene-specified disease resistance in Arabidopsis. Genetics **152,** 401–412.

Zhou, F., Kurth, J., Wei, F., Elliot, C., Vale, G., Yahiaoui, N., Keller, B., Somerville, S., Wise, R.P., and Schulze-Lefert, P. (2001). Cell-autonomous expression of barley *Mla1* confers race-specific resistance to the powdery mildew fungus via a *Rar1*-independent signaling pathway. Plant Cell **13,** 337–350.