MPMI Vol. 17, No. 7, 2004, pp. 711–719. Publication no. M-2004-0421-01R.

The Arabidopsis TIR-NB-LRR Gene *RAC1* Confers Resistance to *Albugo candida* (White Rust) and Is Dependent on *EDS1* but not *PAD4*

Mohammad H. Borhan,[1] Eric B. Holub,[2] Jim L. Beynon,[2] Kevin Rozwadowski,[1] and S. Roger Rimmer[1]

[1]Agriculture and Agri-Food Canada, Saskatoon Research Center, Saskatoon, SK., S7N 0X2, Canada; [2]Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF, U.K.

Submitted 30 June 2003. Accepted 25 January 2004.

Resistance to *Albugo candida* isolate Acem1 is conferred by a dominant gene, *RAC1*, in accession Ksk-1 of *Arabidopsis thaliana*. This gene was isolated by positional cloning and is a member of the Drosophila toll and mammalian interleukin-1 receptor (TIR) nucleotide-binding site leucine-rich repeat (NB-LRR) class of plant resistance genes. Strong identity of the TIR and NB domains was observed between the predicted proteins encoded by the Ksk-1 allele and the allele from an Acem1-susceptible accession Columbia (Col) (99 and 98%, respectively). However, major differences between the two predicted proteins occur within the LRR domain and mainly are confined to the β-strand/β-turn structure of the LRR. Both proteins contain 14 imperfect repeats. *RAC1*-mediated resistance was analyzed further using mutations in defense regulation, including: *pad4-1*, *eds1-1,* and *NahG,* in the presence of the *RAC1* allele from Ksk-1. White rust resistance was completely abolished by *eds1-1* but was not affected by either *pad4-1* or *NahG*.

Plant defense against colonization by a biotrophic pathogen is thought to be triggered by either direct or indirect interaction between the pathogen ligand (product of the pathogen avirulence gene) and a corresponding plant resistance protein (product of a plant resistance gene). Several resistance genes (*R* genes) against bacteria, fungi, viruses, and nematodes have been cloned from *Arabidopsis thaliana* and various crop species (Ellis et al. 2000; Holub 2001; Hulbert et al. 2001; Takken and Joosten 2000). The majority of plant *R* genes encode nucleotide-binding site leucine-rich repeat (NB-LRR)-type proteins. NB-LRR proteins can be further grouped into two subclasses based on their N-terminal sequence: those containing a coiled-coil (CC) domain (CC-NB-LRR), or those containing a domain with similarity to Drosophila toll and mammalian interleukin-1 receptor (TIR) (TIR-NB-LRR) (Hammond-Kosack and Jones 1997; Jones and Jones 1997; Young 2000).

LRR are involved in protein–protein interactions and occur in a number of proteins with different function (Kobe and Deisenhofer 1994, 1995). Domain exchange between LRR of closely related *R* genes supports their role in pathogen recognition (Ellis et al. 1999; Wulff et al. 2001). Variation among *R* genes occurs mainly in their LRR domain, typically in the solvent exposed β-strand/β-turn structure within the LRR domain. Comparison of this motif among *R* gene homologs suggests that the β-strand/β-turn structure has been under diversifying selection (Bittner-Eddy et al. 2000; Botella et al. 1998; McDowell et al. 1998; Meyers et al. 1998; Parniske et al. 1997). Evidence for this is based on the ratio of nonsynonymous (K_a) to synonymous (K_s) nucleotide substitution at the β-strand/β-turn motif. A K_a/K_s ratio >1 indicates that diversification has occurred under positive selection pressure from the evolving pathogen. This suggests that the β-strand/β-turn motif may be involved in ligand binding as has been shown for a polygalacturonase-inhibiting protein with its polygalacturonase ligand (using site-directed mutagenesis of the β-strand/β-turn motif) (Leckie et al. 1999). A chimeric gene constructed from flax rust resistance genes *P* and *P2* showed that amino acid changes in the β-strand/β-turn motif are sufficient to alter *P2* to *P* specificity (Dodds et al. 2001).

The TIR domains of plant R proteins are thought to have a similar function to the homolog domains from Drosophila toll and human interleukin-1 receptor and act as a signaling domain (Hammond-Kosack and Jones 1997). However, analysis of recombinant alleles of *L* genes from flax indicates that the TIR domain also may play a role in pathogen specificity (Ellis et al. 1999; Luck et al. 2000).

R proteins may interact indirectly with pathogen effector proteins, which target regulators of plant innate immunity. This idea, developed as the "guard hypothesis" (Dangl and Jones 2001), reasonably explains the interactions shown to be required for resistance mediated by RPM1 and RPS2 in *A. thaliana* (Axtell and Staskawicz 2003; Mackey et al. 2002, 2003), where RIN4 may be targeted by AvrRpm1, AvrB, or AvrRpt2. The R proteins monitor the state of RIN4 and induce the resistance response when the bacterial Avr proteins interact with RIN4. However, evidence for the guard hypothesis with respect to the TIR-NB-LRR class of *R* genes has as yet not been obtained.

Recognition of a pathogen by a plant initiates a rapid response localized to the infection site and manifested by changes in ion flux and production of reactive oxygen species that lead to induction of downstream signals and defense genes (Kombrink and Schmelzer 2001; Morel and Dangl 1997). Initiation of local defense also results in signals that induce systemic acquired resistance (SAR) in uninfected distal parts of the plant, resulting in broad-spectrum resistance (Dong 2001; Shah and Klessig 1999). The role of salicylic acid (SA) in plant defense and induction of SAR has been shown by treatment of plants with SA or its synthetic analogs

Corresponding author: S. R. Rimmer; Telephone: (306) 956-2831; Fax: (306) 956-7247; E-mail: rimmerr@agr.gc.ca.

such as 2, 6-dichloroisonicotinic acid (INA) and benzothiadiazole (Klessig et al. 1994). Furthermore, transgenic plants expressing the bacterial SA-degrading enzyme, NahG, are unable to induce SAR (Delaney et al. 1995).

Several mutants in *A. thaliana* have been identified that affect disease resistance responses associated with defense regulatory genes such as: *AtSGT1b* (homolog of the yeast gene SGT1) (Austin et al. 2002; Tör et al. 2002), *EDS1* (enhanced disease susceptibility) (Parker et al. 1996), *NDR1* (non-race-specific disease resistance) (Century et al. 1997), *PAD4* (phytoalexin deficient) (Glazebrook et al. 1997), and *RAR1* (homolog of a barley gene required for Mla powdery mildew resistance) (Muskett et al. 2002; Tornero et al. 2002). Resistance specified by the *RPS4* gene to the bacterial pathogen *Pseudomonas syringae* expressing avr*Rps4* (Gassmann et al. 1999) and the oomycete *Peronospora parasitica* specified by *RPP1*, *RPP2*, *RPP4,* and *RPP5,* which all encode TIR-NB-LRR proteins, is abolished by *eds1* (Aarts et al. 1998; Parker et al. 1996; Rusterucci et al. 2001). In contrast, many CC-NB-LRR resistance genes are independent of *EDS1* but dependent on *NDR1* (Aarts et al. 1998; Century et al. 1997). Both *EDS1* and *PAD4* encode lipase-like proteins (Falk et al. 1999; Jirage et al. 1999) and function within the same defense pathways that regulate SA accumulation (Feys et al. 2001; Zhou et al. 1998).

White rust disease caused by the oomycete pathogen *Albugo candida* (Pers. ex Fr.) O. Kuntze, naturally occurs on *Arabidopsis thaliana* (Holub et al. 1995) and three resistance genes to *Albugo candida* (*RAC*) isolate Acem1 were identified (Borhan et al. 2001). Here we report cloning of the first white rust resistance gene to isolate Acem1 of *A. candida* (*RAC1*) from Ksk-1 accession of *Arabidopsis thaliana*. We also describe the effect on *RAC*-mediated resistance of standard mutations that previously were used to characterize defense signaling in downy mildew resistance.

RESULTS

Isolation of *RAC1*.

The *A. thaliana* accession, Keswick-1 (Ksk-1), is resistant to isolate Acem1 of *A. candida* and shows flecking necrosis surrounded by yellowing with no blisters (FYN phenotype) in infected cotyledons 3 to 5 days after inoculation (dai). Weiningen (Wei-1), Wassilewskija (Ws-0), and Columbia (Col-0) are three Acem1-susceptible accessions that allow unrestricted growth and reproduction of the pathogen in cotyledons and leaves, with no obvious symptoms on the upper surfaces but emergence of the characteristic white blisters (sporulating infections) on the lower surface (green with blisters; GB phenotype) (Holub et al. 1995). Resistance of Ksk-1 to Acem1 is due to the presence of a dominant gene, *RAC1*, which was mapped on chromosome 1 to an interval of approximately 280 kb between restriction fragment length polymorphism markers m254 (telomeric) and m253 (centromeric) using a population of 124 F_6 Wei-1 × Ksk-1 inbred lines (Borhan et al. 2001). The Col bacterial artificial chromosome (BAC) libraries, TAMU and IGF, (Choi et al. 1995; Mozo et al. 1998) were used for physical mapping and a BAC contig was made for the interval (Fig. 1). The sequence of BAC F27M3 from the *RAC1* interval (Fig. 1A) contained a putative resistance gene (At1g31540) with homology to the *P. parasitica* resistance gene *RPP5* (Parker et al. 1997). This gene also was present on the overlapping BAC, T8E3. The presence of At1g31540 on BAC F24B2 was confirmed using polymerase chain reaction (PCR) primers flanking At1g31540 (Fig. 1A). No other sequence with homology to *R* genes could be identified within the *RAC1* interval; therefore, At1g31540 was considered a likely candidate gene for *RAC1*.

A binary cosmid library was constructed from Ksk-1 genomic DNA to enable us to isolate the corresponding At1g31540 allele from Ksk-1. PCR products flanking At1g31540 were used to screen this library. Four clones (K8, K25, B31, and M27) were identified and positioned along the *RAC1* interval using their terminal sequences. All four clones overlapped in a region encompassing At1g31540 (Fig. 1B). Each of the four clones was used to transform the Acem1-susceptible accession Ws-0. Transgenic plants (T_1) were selected for their resistance to DL-phosphinothricin (PPT) and self pollinated to produce a T_2 generation. One-week-old seedlings from each T_2 line were inoculated with Acem1 and interaction phenotypes were recorded at 3 to 5 dai (for scoring the FYN interaction phenotype) and at 7 to 14 dai (for scoring the GB interaction phenotype). Transgenic lines containing any of the four cosmid clones were resistant to Acem1 and showed the FYN interaction phenotype. After pathology scoring, seedlings were sprayed with PPT. A 3:1 ratio for resistance to Acem1 and also PPT ensured the presence of a single insertion in transgenic lines. Resistance or susceptibility to Acem1 also correlated with resistance or susceptibility to PPT.

Based on the terminal sequence of Ksk-1 DNA inserts, cosmid clones were positioned along T8E3 and F27M3 and an approximate 10-kb overlap among the four clones was determined. All four cosmid clones restored the resistance phenotype in transgenic lines; therefore, we concluded that *RAC1* was delimited to the 10-kb fragment common to the four cosmids (position 47,700 to 57,900 bp on BAC T8E3, which is delimited by T7 ends of cosmid B31 and K8 respectively) (Fig 1B). The cosmid clone K25 was chosen for sequencing of the 10-kb overlap. Analysis of this sequence showed that Ksk-1 contained an At1g31540 homolog (hereafter called *RAC1*), and two intergenic regions of approximately 1.7 and 3.3 kb that flank the predicted start and stop codon of the gene, respectively. A 428-bp duplication present at approximately 3.5 kb after the translation stop codon and 2 kb before the translation start codon of *rac1* allele in Col-0 also was present at the same location in Ksk-1. Search of DNA and protein databases showed that the 428-bp fragment has no homolog in other parts of the *A. thaliana* genome and does not encode any protein.

Sequence of *RAC1* gene and structure of the predicted protein.

The sequence of *RAC1* and its flanking sequences from Ksk-1 showed more than 95% identity to *rac1* from Col-0. The Ksk-1 allele is 4,981 bp (GenBank accession no. AY522496) and the Col allele is 4,450 bp. The major difference between *RAC1* and its flanking sequence in Ksk-1 and Col was the insertion of two transposons, 524 bp in Ksk-1 and 1,030 bp in Col-0. The 1,030-bp insertion in Col that was not present in Ksk-1 was located 1.3 kb after the predicted *rac1* translation stop codon. We determined the end of the *RAC1* transcript in Ksk-1 and Col by 3′ RACE (rapid amplification of cDNA ends), which identified a 684-bp untranslated region (UTR) (Fig. 2), confirming that the 1,030-bp transposon is located outside the *rac1* transcript. Alignment of the *RAC1* genomic sequence with its predicted cDNA showed that the 524-bp transposon is present only in *RAC1* Ksk-1 and is inserted in an intronic region. We used gene-specific primers and determined by reverse transcriptase (RT)-PCR the location of six introns, and the sequence of *RAC1* Ksk-1 cDNA (Fig. 2). We also identified a 74-bp 5′ UTR of *RAC1* Ksk-1 using gene-specific primers. DNA analysis software (Vector NTI Suite 7; Invitrogen Life Technologies, Carlsbad, CA, U.S.A.) predicted an open reading frame (ORF) of 3,480 bp for the *RAC1* gene. An alternative transcript for *RAC1* was

identified by RT-PCR in which intron 1 was retained. This causes a shift in the start codon, resulting in loss of the TIR domain from the predicted RAC1 protein (Fig. 2).

RAC1 encodes a predicted cytoplasmic protein of 1,160 amino acids (aa) and molecular weight of 133 kDa, which belongs to the TIR-NB-LRR class of *R* genes. The predicted TIR domain starts at amino acid 14 and ends at amino acid 142 and the NB domain starts at amino acid 167 and ends at amino acid 470. The NB domain contains possible p-loop (GSSGIGK), kinase 2 (LIIID), and kinase3a (FGYSGR) motifs at positions 211 to 217, 296 to 301, and 318 to 323, respectively. TIR and NB domains of RAC1 in Ksk-1 are more than 98% identical to their counterparts in rac1 Col. The C-terminal half of the RAC1 protein that contains the LRR domain shows 90% identity to rac1 Col LRR. Based on the consensus for plant proteins with cytoplasmic LRR (Jones and Jones 1997), we identified 14 imperfect LRRs in both alleles of *RAC1* (Fig. 2). The last 252 aa of RAC1 proteins from both Ksk-1 and Col were defined as a non-LRR-segment (NLS), because it lacks the consensus structure for a LRR domain.

A blast search identified several putative *R* genes on chromosome 5 of *A. thaliana* with more than 60% identity and more than 65% similarity (At5g46260, At5g46270, and At5g46520) to RAC1 protein. Among previously described plant resistance proteins, RPP5 and RPP1-WS-A (Botella et al. 1998; Parker et al. 1997) were found to be the most similar to the RAC1 protein, with 56 and 52% similarity, respectively. Alignment of RAC1-predicted proteins from Ksk-1 and Col-0 showed that their TIR domains were highly conserved, with only a single amino acid difference of isoleucine (I) in Ksk-1 for lysine (K) in Col-0 at amino acid position 74. The NB domains of the two proteins also were highly similar, with only 6 aa different along the 304 aa of this domain. The number of K_a versus K_s amino acid substitutions at the NB domain were equal (6 aa each), indicating no preference for sequence diversification of this domain of the RAC1 protein. Comparison of K_a versus K_s nucleotide substitution between *RAC1* alleles from Ksk-1 and Col showed a higher number of K_a over K_s at the LRR and NLS sequences of the gene. A majority of the substitution in LRR domain of RAC1 occurred within the solvent exposed β sheet of this domain (Fig. 3), which also has been reported to be hypervariable among alleles of other plant *R* genes (Bittner-Eddy et al. 2000; Botella et al. 1998; McDowell et al. 1998; Meyers et al. 1998; Parniske et al. 1997).

Resistance conferred by *RAC1* is independent of PAD4 and NahG.

The SA-deficient transgenic line, Col-NahG, was used to determine whether SA may play a role in *RAC1*-mediated resistance to Acem1. We crossed Col-NahG with Ksk-1, and observed the FYN phenotype (similar to Ksk-1) in F_1 seedlings inoculated with Acem1. F_2 seedlings segregated in a 3:1 ratio (325:119, resistant/susceptible; $\chi^2 = 0.77$, $P < 0.05$) following inoculation with Acem1, indicating that NahG had no effect on the ratio expected for segregation of *RAC1* resistance. Gene-specific primers were used to identify 52 F_2 lines (from a total of 93) that had inherited both *RAC1*-Ksk1 and *NahG*; all of these plants exhibited the FYN resistance phenotype. No difference in resistance was observed micro-

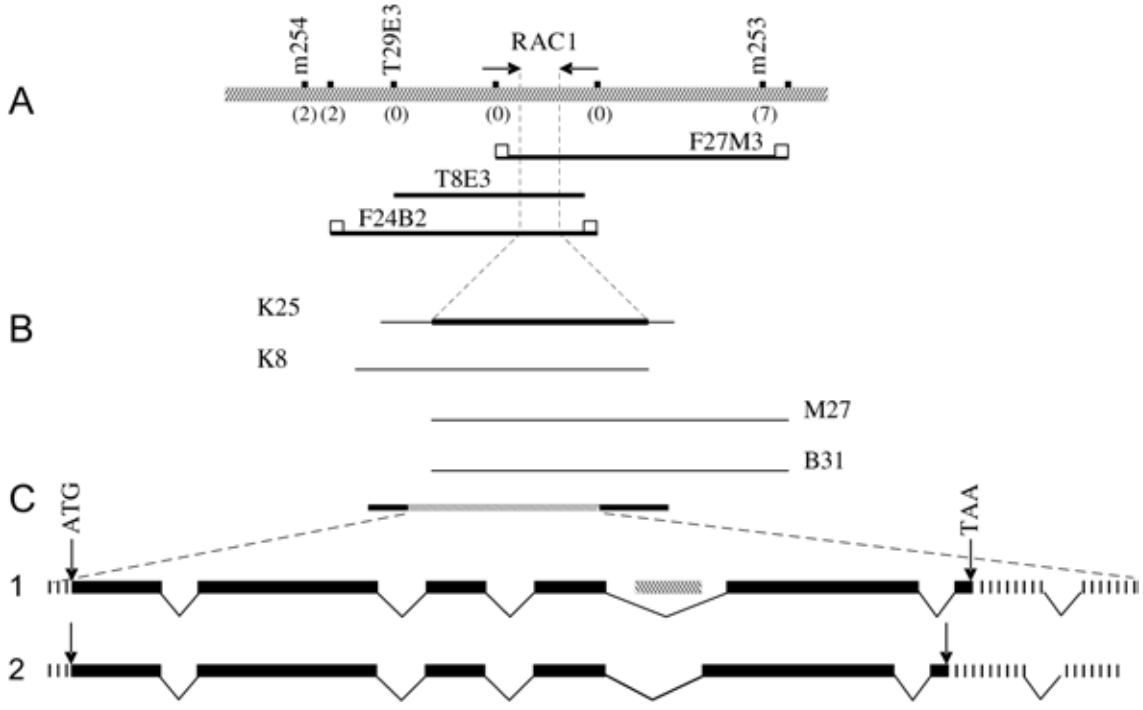

Fig. 1. Genetic and physical map of *RAC1* on chromosome 1 of *Arabidopsis thaliana*. **A,** Location of *RAC1* and the bacterial artificial chromosome (BAC) contig spanning this locus on chromosome 1 of *A. thaliana*. The hatched bar indicates the position on chromosome 1 relative to molecular markers. The number of recombinants is indicated in brackets for regions between a marker and *RAC1* using a mapping population of 124 F6 lines (Borhan et al. 2001). Solid bars are the BACs that contain *RAC1*. Open squares on the BACs represent end sequences used for contigging. **B,** Position of four cosmid clones from the accession Ksk-1, relative to the BAC contig. The solid bar on cosmid K25 represents a 10-kb overlap delimited by the T7 (right) end of K8 and the T7 (left) end of B31. **C,** *RAC1* transcripts for Ksk-1(1) and Col (2). *RAC1* locus (4,981 bp long showed by hatched line) that encodes an open reading frame of 3,480 bp (3,483 in Col) (solid bars between the ATG start and TAA stop codons) interrupted by five introns (solid lines). Intron 4 (1,105 bp) contains a transposon (524 bp) shown by the hatched bar that is absent from *rac1* allele in Col. The size of exons (5′ to 3′) for *RAC1* allele in Ksk-1 are 461, 1,117, 297, 411, 1,179, and 15 and for *rac1* allele in Col are 461, 1,117, 297, 411, 1,182, and 15. Intron sizes (5′ to 3′) for *RAC1* allele in Ksk-1 are 80, 127, 105, 1,105, and 84 and for *rac1* allele in Col are 79, 127, 110, 567, and 84. Bars with vertical lines are untranslated regions (UTRs). Seventy-four base pairs of 5′ UTR was amplified by reverse-transcriptase polymerase chain reaction using gene-specific primers. The 3′ UTR in both alleles contains an intron of 95 bp. The 3′ UTR for Ksk-1 allele of *RAC1* is 687 bp and for *rac1* allele in Col is 694 bp.

scopically between *NahG/RAC1* plants and Ksk-1 wild-type plants following inoculation with Acem1.

Similarly, we tested the effect of two defense mutants on *RAC1*-mediated resistance. The mutations *eds1* and *pad4* were chosen as examples that previously were described as essential regulators of resistance conferred by other TIR-NB-LRR genes. We crossed Ksk-1 with Ws-*eds1-1* and Col-*pad4-1*. If either mutation abolishes *RAC1* resistance, then a 9:7 ratio (resistant/susceptible) was expected for segregation of a dominant *R* gene and a recessive defense mutation. A ratio of 9:7 (173:107, resistant/susceptible; $\chi^2 = 3.48$, $P > 0.05$) was observed for the F_2 population of Ws-*eds1-1* × Ksk-1, indicating that *eds1* inhibits *RAC1* resistance. In contrast, the F_2 population of Ksk-1 × Col-*pad4-1* segregated 3:1 (225:60, resistant/susceptible, $\chi^2 = 2.36$, $P > 0.05$), indicating that *pad4* had no effect on *RAC1*-mediated resistance.

F_2 plants that were homozygous for either defense mutation were identified using marker-assisted selection. Homozygous *eds1-1* plants (29 lines from 93 F_2 individuals showing the GB phenotype), which contained one or two alleles of *RAC1*-Ksk1, all were susceptible to Acem1, whereas homozygous *pad4-1* plants (4 lines from 54 F_2 individuals showing the FYN phenotype) exhibited a resistance phenotype similar to the wild-type Ksk-1. Genotypes and phenotypes of homozygous *eds1-1*/*RAC1* and *pad4-1*/*RAC1* lines were confirmed by further progeny testing of F_3 families derived from the selected F_2 lines (data not shown). With light microscopy, no difference was observed between cotyledons of *pad4-1/RAC1* homozygous plants and Ksk-1 plants inoculated with Acem1, both exhibiting the Ksk-1 characteristic hypersensitive response (HR) and restriction of hyphae to the site of infection. In contrast, eds1-1/RAC1 homozygous lines permitted pathogen growth that was unrestricted, similar to that observed on the Ws-1 susceptible accession (Fig. 4).

DISCUSSION

We isolated *RAC1*, a resistance gene to isolate Acem1 of *Albugo candida* in *Arabidopsis thaliana* accession Ksk-1. The

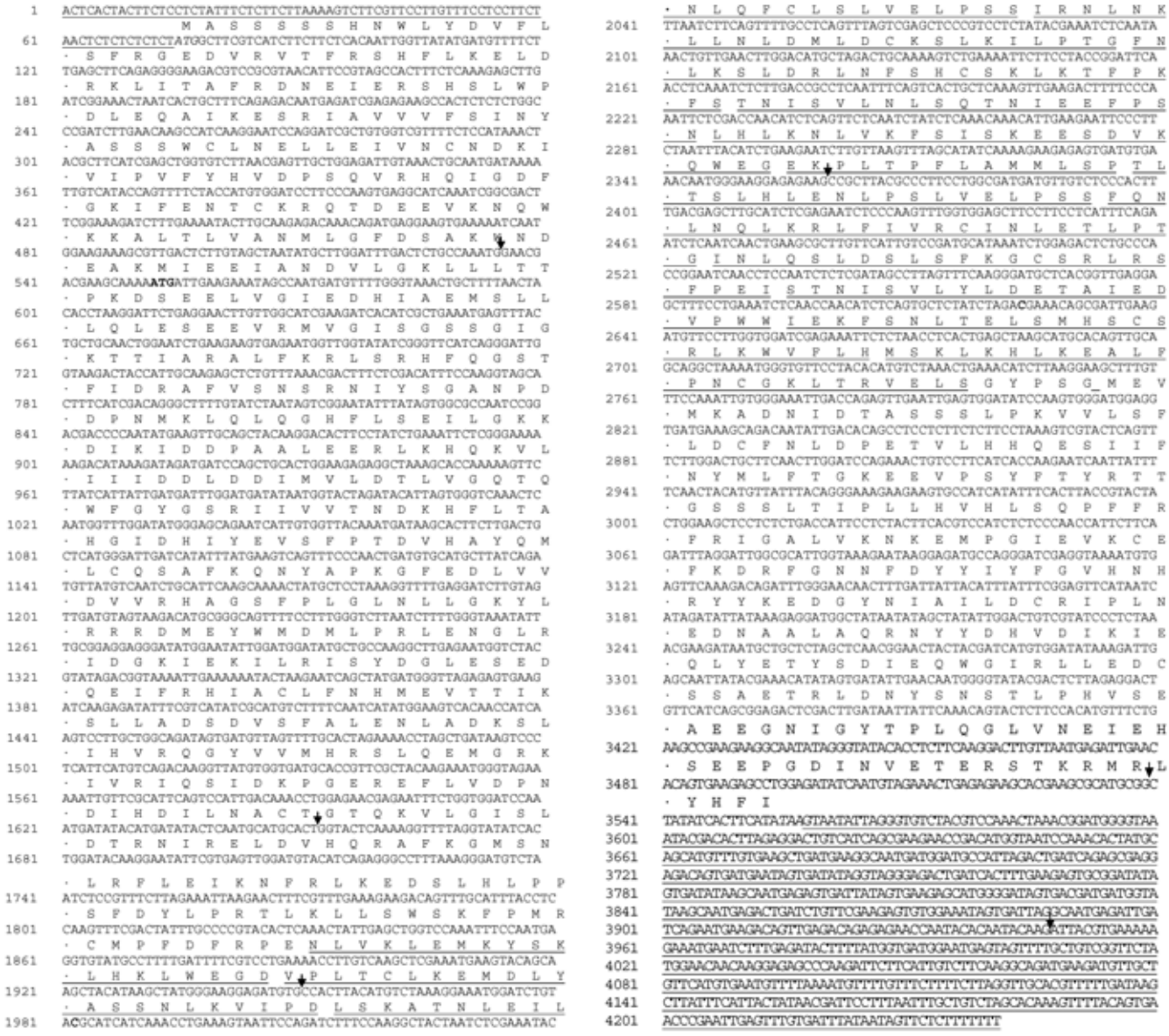

Fig. 2. Sequence of *RAC1* cDNA (GenBank accession number AY522496) and its predicted protein. Translation start and stop codons are italicized. Underlined sequences are 5′ and 3′ untranslated region. Introns are indicated by arrows. Shadowing indicates possible Kinase 1a (P-loop), Kinase 2, and Kinase 3a consensus of the nucleotide binding domain. Predicted leucine-rich repeats for the cytoplasmic R proteins (Jones and Jones 1997) are underlined. ATG in bold shows the translation start site for an alternate cDNA of *RAC1* when intron 1 is retained.

overall structure (number and length of introns and exons) of the *RAC1* gene in Ksk-1 is similar to the susceptible Col allele, except for intron 4 in Ksk-1, which contains a transposon of 524 bp. This makes it nearly twice the size of intron 4 in Col-0. We compared an approximately 5-kb sequence of the intergenic regions flanking *RAC1* in Ksk-1 and Col-0. A 1,042-bp-long transposon occurs in the 3′ intergenic region of *rac1* Col-0 that is absent from Ksk-1. Analysis of the *RAC1* transcript showed that these transposons do not affect the *RAC1* gene product.

More than 150 *R* gene homologs with NB-LRR structure have been identified in Col-0 (The Arabidopsis Genome Initiative 2000), with nearly half of them located in clusters containing three or more genes (Holub 2001). However, like *RPM1* and *RPS2*, *RAC1* occurs as a singlet gene locus. The most similar sequences to *RAC1* based on the full sequence using BLASTP is a cluster of putative TIR-NB-LRR genes on a region between 18,850 and 18,900 kb on chromosome 5. Alignment of RAC1 with predicted proteins from this cluster showed that AT5g46520 has the highest homology (67%) to RAC1. Search of the *A. thaliana* genome with the *RAC1* sequence and its flanking intergenic region did not show any segmental duplication for the *RAC1* interval. Comparison of RAC1 protein with predicted proteins of TIR-NB-LRR genes from other plants showed that N (for resistance to *Tobacco mosaic virus*) (Whitham et al. 1994) is more similar than others

Fig. 3. C-terminal half of the RAC1 protein containing 14 leucine-rich repeats (LRRs), followed by 252 amino acids that do not have an LRR consensus structure (non-LRR segment). **A,** Alignment of 14 LRRs of RAC1 protein. Nonsynonymous amino acid substitutions in the RAC1 predicted protein from Columbia are shown above each line, whereas synonymous amino acids are underlined. The consensus sequence for predicted cytoplasmic LRR of plant *R* genes (Jones and Jones 1997) is given below the alignment. In the consensus, X is any amino acid and "a" indicates any one of the six hydrophobic amino acids (A, F, L, I, M, or V). Amino acids that are repeated more than 50% in a specific position are included in the consensus. The sequences between the two dotted lines correspond to the β-strand/β-turn motif conserved in all LRR proteins and thought to be involved in ligand binding (Jones and Jones 1997). **B,** The non-LRR segment of RAC1 protein from Ksk-1. Amino acid substitutions in the RAC1 predicted protein from Col are indicated above each line.

to RAC1 (24.4%). *RAC1* encodes a predicted cytoplasmic protein (1,160 aa) of TIR-NB-LRR. The LRR domain of plant R proteins is thought to detect an invading pathogen by direct or indirect interaction with proteins produced by pathogen *Avr* genes. This proposed function has been substantiated by the diversity in LRR domains caused by amino acid substitution and difference in number or length of the repeats. Particularly interesting is the higher rate of substitution of K_a to K_s amino acids at the solvent-exposed face of the repeat (Caicedo et al. 1999; McDowell et al. 1998; Parniske et al. 1997), which is the binding surface of the LRR in ribonuclease inhibitor (Kobe 2001). Comparison between the predicted LRR from RAC1 Ksk-1 with the similar domain in rac1 Col shows they have the same number of LRRs of similar length and the sequence of the two proteins is more diverse at the LRR domains compared with the N terminal TIR and NB domains. Of the nucleotide substitutions in the LRR domain of *RAC1,* 79% are nonsynonymous, with 69% of them occurring at the solvent-exposed β sheet (-L-L-) structure of the repeat. Most of the nucleotide substitutions in the NLS part of *RAC1* alleles were nonsynonymous, indicating that this region probably has been under positive selection and might play a role in pathogen recognition. Deletion of the NLS from the flax rust resistance gene, *P2*, abolished its function (Dodds et al. 2001).

The most striking similarity between the two RAC1 proteins from Ksk-1 and Col-0 occurs in the TIR domain. Along the 139 aa of TIR domain (amino acid 14 to amino acid 142) there is only one amino acid change in position 74: isoleucine (I) in Ksk1 to lysine (K) in Col-0. This suggests that the TIR domain of RAC1 may not affect recognition specificity, contrary to the *L6* and *L7* alleles from flax that confer different specificities to flax rust and only differ in DNA sequence in the TIR domain (Ellis et al. 1999).

Alternative splicing for TIR-NB-LRR genes, *N*, *L6*, *RPS4,* and *Y-1*, a gene for resistance to potato virus Y, has been reported (Ayliffe et al. 1999; Dinesh-Kumar and Baker 2000; Jordan et al. 2002; Lawrence et al. 1995; Vidal et al. 2002). In the case of *RAC1*, amplification of the transcript by gene-specific primers revealed a copy of *RAC1* transcript that retained intron 1. Retention of the *RAC1* intron aborts translation of the TIR domain and the predicted protein contains only NB and LRR domains. *N* and *L6* have a truncated form that, in the case of the *N* gene, results from alternative splicing of 70 bp within intron 3; and, in the case of *L6*, is caused when intron 3 is unspliced. The *Y-1* gene has three alternative transcripts, two of them with C-terminal truncation that causes deletion of most of the LRR region, and one transcript identical to the full-length gene except for the lack of a 75-bp putative alterna-

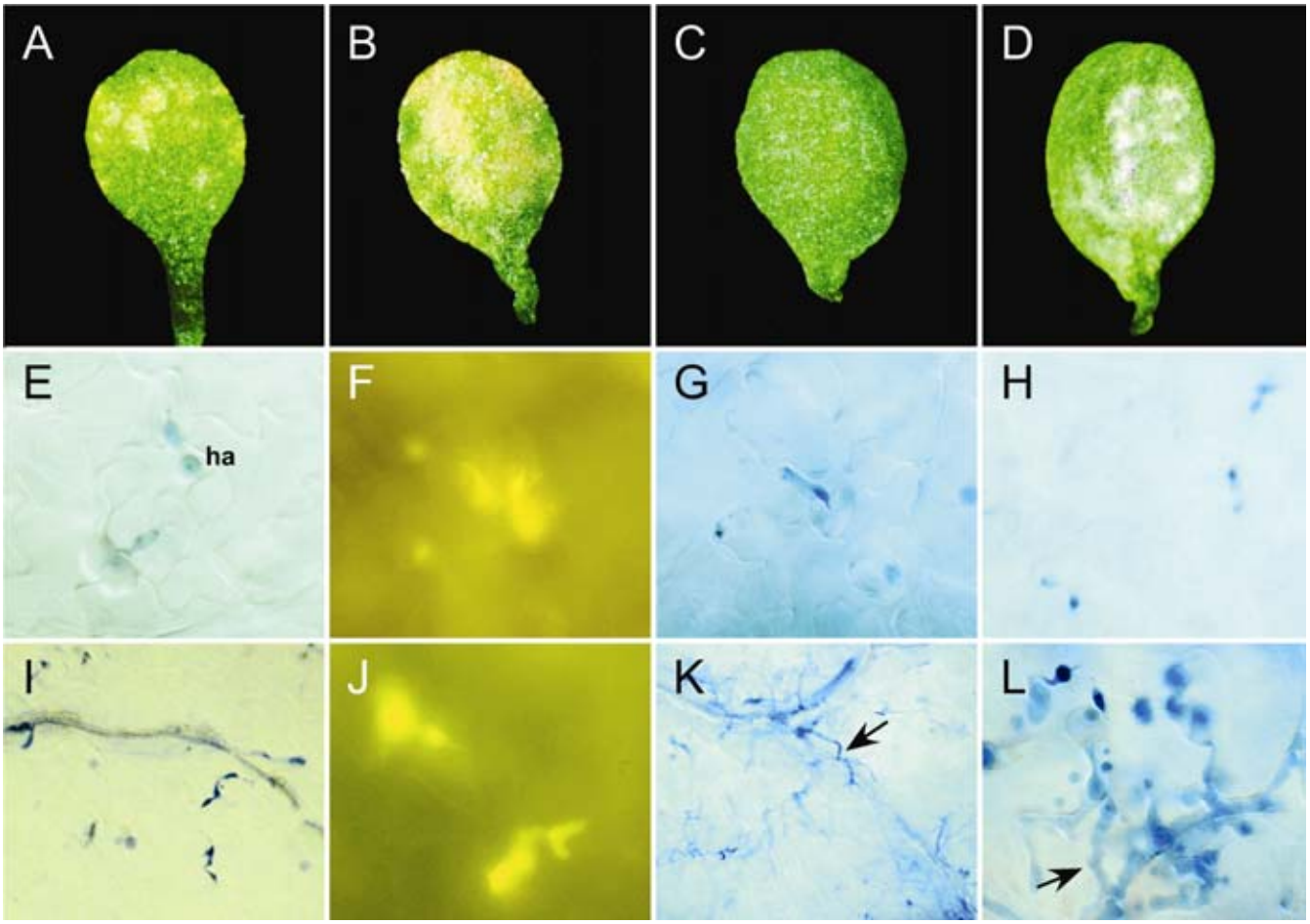

Fig. 4. Interaction phenotypes of wild-type, transgenic, and mutant lines of *Arabidopsis thaliana* following inoculation with *Albugo candida* isolate Acem1. **A,** Cotyledon of the resistant accession Ksk-1 at 5 days after infection (dai) showing flecking necrosis surrounded by yellowing with no blisters (FYN) resistance phenotype described by Holub and associates (1995). **B,** Ws transgenic line containing *RAC1* gene from Ksk-1 at 5 dai showing FYN phenotype. **C,** Upper surface of a cotyledon of Ws-0 at 10 dai. **D,** Pustules of *A. candida* on the underside of the same cotyledon as **C**. **E,** Cotyledon of Ksk-1 wild type at 6 dai; haustorium (ha) formation in the infected cell. **F,** Hypersensitive response (HR) of Ksk-1 at 6 dai under UV excitation. **G,** Ws transformed with *RAC1* at 6 dai. **H,** *RAC1/NahG* plant at 6 dai. **I,** *RAC1/pad4.1* plant at 6 dai. **J,** HR of infected cells in *RAC1/pad4.1* plant at 6 dai viewed under UV excitation. **K,** Unrestricted growth of *Albugo* sp. (arrow) in *RAC1/eds1.1* plant at 6 dai. **L,** Ws-0 at 6 dai showing unrestricted growth of hyphae (arrow). **E**, **G**, **H**, **I**, **K,** and **L** are infected tissues stained by trypan blue and viewed by differential interference contrast microscopy.

tively spliced intron 2 located within the TIR domain of *Y-1* gene. The alternative transcript might be important for complete resistance as for the *N* gene (Dinesh-Kumar and Baker 2000) or regulating the extent of the response to pathogen infection similar to a proposed role for the alternative transcripts of *TLR4* in mouse (Jordan et al. 2002). Zhang and Gassmann (2003) have shown that, for the *RPS4* gene in *A. thaliana*, alternative splicing is required for resistance and deletion of introns 2 and 3 abolishes resistance conferred by *RPS4*.

A. thaliana disease resistance mutants identify an essential role for SA in defense against biotrophic pathogens. The role of SA in resistance against Acem1 was tested using NahG. Heterozygous or homozygous *RAC1-NahG* plants were not compromised in their resistance to Acem1 and showed symptoms identical to the wild-type resistant parent, Ksk-1. Two mutants, *eds1* and *pad4*, that affect resistance initiated by TIR-NB-LRR-type proteins were tested in the Ksk-1 background. *RAC1* resistance was abolished completely in homozygous *RAC1*/*eds1-1* plants but was unaffected in homozygous lines with the *RAC1/pad4* genotype. Unlike *NahG* and *pad4,* which show reduction in SA, production of SA is almost completely abolished in *eds1* plants (Feys et al. 2001). Therefore, it seems likely that a basal level of SA in *pad4* and NahG plants is sufficient for *RAC1*-induced resistance to Acem1. Alternatively, it could be that SA simply is not required. Feys and associates (2001) showed that the EDS1 and PAD4 proteins interact directly with each other and both affect the resistance response mediated by the same spectrum of *R* genes.

Our data show that *RAC1,* unlike other previously described *A. thaliana* TIR-NB-LRR resistance genes, does not require *PAD4* for induction of defense. Exclusive requirement of EDS1 for the *RAC1* defense pathway is evidence of a dual role for EDS1 in induction of defense as proposed by Feys and associates (2001). One suggested role is through interaction with PAD4 and potentiation of defense signals, which seem to be dispensable for RAC1, and the other role is independent of PAD4 (either EDS1 alone or in combination with other partners yet to be identified). Rusterucci and associates (2001) crossed the *A. thaliana* mutant Ws-*lsd* (lesion-simulating disease resistance) (Dietrich et al. 1997) with *eds1* or *pad4* and obtained double-mutant lines *eds1*/*lsd1* and *pad4/lsd1* to study the role of EDS1 and PAD4 in regulation of cell death. Their finding reinforces the observations reported by Feys and associates (2001) that EDS1 has a dual function in the SA defense pathway. One role is in initiation of localized HR, independent of PAD4 that causes HR at the site of infection, and the other role is in combination with PAD4 in potentiation of early defense signals. Microscopy observations of *RAC1*/*pad4* lines infected with Acem1 and detection of HR restricted to the infection site show that potentiation of defense signals by PAD4 is not required for complete resistance to Acem1 infection, and that basal level of SA is enough for the induction of defense initiated by RAC1.

MATERIALS AND METHODS

Plant materials and pathology test.

Plant growth, pathogen storage, and inoculation have been described in detail by Holub and associates (1995) and Borhan and associates (2001). *A. thaliana* seedlings were grown under a 16-h photoperiod and temperature of 16 to 20°C. One-week-old seedlings were drop inoculated using a suspension of Acem1 sporangia (2×10^4 per milliliter). The sporangia suspension was incubated at 14 to 16°C for 2 to 4 h prior to inoculation to ensure the release of zoospores. Disease was evaluated at 3 to 5 dai for the FYN interaction phenotype and at 10 to 14 dai for the GB interaction phenotype.

Microscopy observations.

Inoculated seedlings (three to five) were fixed and cleared by placing in absolute methanol for 1 day, then transferred to chloral hydrate (3 g ml^{-1}) for 2 h to become transparent. Seedlings were placed on a glass slide in a drop of 50% glycerol and covered with a cover slip. *Albugo candida* inside infected cotyledons was detected by staining with lactophenol-trypan blue as described by Koch and Slusarenko (1990). Microscopy observations were done using differential interference contrast (DIC) with a Zeiss microscope.

Construction of Ksk-1 cosmid library.

DNA was prepared from 4- to 6-week-old leaves of Ksk-1 by CsCl method (Ausubel et al. 1994). DNA was partially digested with *Sau*3AI and ligated into the binary cosmid vector pSLJ75515 digested with *Bam*H1. Clones were packaged using the Gigapack Gold III XL extract (Stratagene, La Jolla, CA, U.S.A.) and transferred into *Escherichia coli* strain XL1-blue MRA (Stratagene). Bacterial clones were sown on LB plates containing tetracycline at 12.5 μg ml^{-1}, IPTG at 20 μg ml^{-1}, and X-gal at 50 μg ml^{-1}. Approximately 40,000 clones were duplicated on nylon membranes by overlaying Hybond-N+ membrane (Amersham Pharmacia Biotech Inc., Sunnyvale, CA, U.S.A.) according to the manufacturer's instructions. Screening the library was carried out according to standard techniques (Ausubel et al. 1994) using radiolabeled PCR-amplified fragments flanking the putative *RAC1* gene. Forward and reverse primers for amplification of a 694-bp 3′ and a 1,446-bp 5′ fragment were cgaagcgtgttcaagaagg, cttactttgtagccacacctgc, ctcacgggcaacatgagtttac, and ggaggactactgacattgcca, respectively. DNA from cosmid clone was prepared using standard alkaline lysis procedure.

Sequencing *RAC1* and analysis of its sequence.

DNA extracted from the cosmid clone K25 using a Qiagen midi-prep kit (Qiagen Inc., Chatsworth, CA, U.S.A) was used as the template for sequencing. The sequence of *rac1* allele from Col was used to design primers from both strands of *rac1* gene on intervals of approximately 500 bp. Some of the primers used for sequencing the *RAC1* genomic clone also were used to sequence the cloned cDNA fragment amplified by RT-PCR. Contiging DNA sequences, prediction and translation of ORFs, and alignment of DNA or protein molecules were carried out using Vector NTI Suite 7 computer program. Domains of RAC1 protein were predicted by Pfam. Programs signalP (version 1.1) and TMpred were used to search for signal peptide and transmembrane regions, respectively.

Generation of lines containing *RAC1* with *eds1-1* or *pad4-1*.

Ksk-1 plants were crossed to Col::*NahG*, Ws-*eds1-1,* and Col-*pad4-1*. F_1 plants were grown and tested for the presence of both *RAC1* alleles from Ksk-1 and either Col-0 or Ws-0 to ensure the success of the cross. F_2 seed were harvested from self-pollinated F_1 plants. After pathology scoring, F_2 seedlings were transplanted into individual pots and grown to mature plants. The genotype of F_2 plants for *eds1-1* allele was determined as described by Rustérucci and associates (2001). The *pad4-1* mutation was detected in an F_2 population of Col-*pad4-1* × Ksk-1 using primers gtgataaccggccattcaac and cgcgtatctgcttctcacac. The PCR product of 992 bp was digested with *Bsm*FI (New England Biolabs Inc., Ontario, CA, U.S.A.), which does not digest the *pad4-1* mutant but cuts the wild-type allele into two fragments of approximately 700 and 300 bp. Digested PCR products were resolved on a 1.5% agarose gel. The primer pairs for determining the *RAC1* genotype were atgaggaagtaagtctccac and cattgctcaaagttgaa gac, which detect the presence or absence of the transposon

insertion in the intron 4 of *RAC1* allele. The *NahG* allele was detected using PCR primers gcgaagtgcaagtgctgttca and tcgcgcaactcgtataactc.

Agrobacterium-mediated transformation of *Arabidopsis* spp.

Cosmid clones were transferred into *Agrobacterium tumefaciens* GV3101 by conjugation and grown on LB plates containing tetracycline (12.5 μg ml^{-1}) and gentamycin (25 μg ml^{-1}). Presence of inserts of cosmid clones were confirmed by PCR on a single clone of transformed *A. tumefaciens* using the same primers as were used for selection of cosmid clones from the Ksk-1 library. Ws-0 plants were transformed using the floral dip method (Clough and Bent 1998). Transformants were selected by spraying 7- to 10-day-old seedlings with DL-phosphinothricin at 100 μg ml^{-1}.

RNA preparation and RT-PCR.

RNA was extracted from 2-week-old seedlings of Ksk-1 and Col-0 using Trizol (Invitrogen Life Technologies). Tissue (1 g) was ground in a mortar and pestle in the presence of liquid nitrogen, suspended in 10 ml of Trizol, and left at room temperature for 5 min. After two rounds of purification by chloroform, the supernatant was transferred to a new tube and RNA was precipitated by adding 0.6 volume of isopropanol and leaving the mixture at room temperature for 20 min. RNA (1 mg) was used to isolate mRNA using an oligo-dT column (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.) according to the manufacturer's procedures. First-strand cDNA was prepared at 50°C from 500 ng of mRNA using a thermoscript RT-PCR kit (Invitrogen Life Technologies) and oligo-dT primer. Second-strand cDNA was synthesized using gene-specific primers and performing PCR on 2 μl of products of first-strand cDNA with a PCR program of 94°C for 4 min, 94°C for 1 min, 55°C for 1 min, and 72°C for 4 min for 35 cycle; and finally 72°C for 7 min. Gene-specific primers used for synthesizing RAC1 cDNA were as follows: RAC1-RT1, aaggcttga gaatagtctac; RAC1-RT2, agagtgaaagatcaagagata, RAC1-RT3: cttatgtagcttgctgtact; RAC1-RT4, aaggcatacacctcattgga; RAC1 5′ UTR-A, tccttctaactctctctctct; RAC1 Seq. 5A, tgtaaaaactttgtg ctagac; and RAC1 Seq. 15-AS, actcactacttctcctctat.

ACKNOWLEDGMENTS

We thank S. Ersali for his excellent technical help and R. Underwood for preparing the figures.

LITERATURE CITED

Aarts, N., Metz, M., Holub, E., Staskawicz, B. J., Daniels, M. J., and Parker, J. E. 1998. Different requirements for EDS1 and NDR1 by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 95:10306-10311.

Austin, M. J., Muskett, P., Kahn, K., Feys, B. L. Jones, J. D., and Parker, J. E. 2002. Regulatory role of SGT1 in early *R* gene-mediated plant defenses. Science 295:2077-2080.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. 1994. Current Protocols in Molecular Biology. John Wiley and Sons Inc., New York

Axtell, M. J., and Staskawicz, B. J. 2003. Initiation of *RPS2*-specified disease resistance in *Arabidopsis* is coupled to the *AvrRpt2*-directed elimination of RIN4. Cell 112:369-377.

Ayliffe, M. A., Frost, D. V., Finnegan, E. J., Lawrence, G. J., Anderson, P. A., and Ellis, J. G. 1999. Analysis of alternative transcripts of the flax *L6* rust resistance gene. Plant J. 17:287-292.

Bittner-Eddy, P. D., Crute, I. R., Holub, E. B., and Beynon, J. L. 2000. *RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. Plant J. 21:177-188.

Borhan, M. H., Brose, E., Beynon, J .L., and Holub, E. B. 2001. White rust (*Albugo candida*) resistance loci on three *Arabidopsis* chromosomes are closely linked to downy mildew (*Peronospora parasitica*) resistance loci. Mol. Plant Pathol. 2:87-95.

Botella, M. A., Parker, J. E., Frost, L. N., Bittner-Eddy, P. D., Beynon, J. L., Daniels, M. J., Holub, E. B., and Jones, J. D. 1998. Three genes of the Arabidopsis *RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. Plant Cell 10:1847-1860.

Caicedo, A. L., Schaal, B. A., and Kunkel, B. N. 1999. Diversity and molecular evolution of the *RPS2* resistance gene in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U.S.A. 96:302-306.

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

Choi, S., Creelman, R. A., Mullet, J. E., and Wing, R. A.1995. Construction and characterisation of a bacterial artificial chromosome library of *Arabidopsis thaliana*. Weeds World 2:17-20.

Clough, S. J., and Bent, A. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. 16:735-743.

Dangl, J. L., and Jones, J. D. G. 2001. Plant pathogens and integrated defence responses to infection. Nature 411:826-833.

Delaney, T. P., Friedrich, L., and Ryals, J. A. 1995. *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. U.S.A. 92:6602-6606.

Dietrich, R. A., Richberg, M. H., Schmidt, R., Dean, C., and Dangl, J. L. 1997. A novel zinc-finger protein is encoded by the *Arabidopsis LSD1* gene and functions as a negative regulator of plant cell death. Cell 88:685-694.

Dinesh-Kumar, S. P., and Baker B. J. 2000. Alternatively spliced *N* resistance gene transcripts: their possible role in tobacco mosaic virus resistance. Proc. Natl. Acad. Sci. U.S.A. 97:1908-1913.

Dodds, P., Lawrence, G., and Ellis, J. 2001. Six amino acid changes confined to the Leucine-Rich Repeat beta-Strand/beta-Turn motif determine the difference between the P and P2 rust resistance specificities in Flax. Plant Cell 13:163-178.

Dong, X. 2001. Genetic dissection of systemic acquired resistance. Curr. Opin. Plant Biol. 4:309-314.

Ellis, J., Dodds, P., and Pryor, T. 2000. Structure, function and evolution of plant disease resistance genes. Curr. Opin. Plant Biol. 3:278-284.

Ellis, J. G., Lawrence, G. J., Luck, J. E., and Dodds, P. N. 1999. Identification of regions in alleles of the flax rust resistance gene *L* that determines differences in gene-for-gene specificity. Plant Cell 11:495-506.

Falk, A., Feys, B. J., Frost, L. N., Jones, J. D., 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. U.S.A. 96:3292-3297.

Feys, B. J., Moisan, L. J., Newman, M. A., and Parker, J. E. 2001. Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. EMBO (Eur. Mol. Biol. Organ.) J. 20:5400-5411.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E. E., Crute, I. R., Holub, E. B., Hammerschmidt, R., and Ausubel, F. M. 1997. Phytoalexin-deficient mutants of *Arabidopsis* reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. Genetics 146:381-392.

Gassmann, W., Hinsch, M. E., and Staskawicz, B. J. 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.

Hammond-Kosack, K. E., and Jones, J. D. G. 1997. Plant disease resistance genes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:575-607.

Holub, E. B. 2001. The arms race is ancient history in *Arabidopsis*, the wildflower. Nat. Rev. Genet. 2:516-527.

Holub, E. B., Brose, E., Tör, M., Clay, C., Crute, I. R., and Beynon, J. L. 1995. Phenotypic and genotypic variation in the interaction between *Arabidopsis thaliana* and *Albugo candida*. Mol. Plant-Microbe Interact. 8:916-928.

Hulbert, S. H., Webb, C. A., Smith, S. M., and Sun, Q. 2001. Resistance gene complexes: Evolution and utilization. Annu. Rev. Phytopathol. 39:285-312.

Jirage, D., Tootle, T. L., Reuber, T. L., Frost, L. N., Feys, B. J., Parker, J. E., Ausubel, F. M., and Glazebrook, J. 1999. *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signaling. Proc. Natl. Acad. Sci. U.S.A. 96:13583-13588.

Jones, D. A., and Jones, J. D. G. 1997. The role of leucine-rich repeat proteins in plant defenses. Adv. Bot. Res. 24:89-167.

Jordan, T., Schornack, S., and Lahaye, T. 2002. Alternative splicing of transcripts encoding Toll-like plant resistance proteins-what's the functional relevance to innate immunity. Trends Plant Sci. 7:392-398.

Klessig, D. F., Malamy, J., Hennig, J., Sanchez-Casas, P., Indulski, J., Grynkiewicz, G., and Chen, Z. 1994. Induction, modification, and perception of the salicylic acid signal in plant defense. Biochem. Soc. Symp. 60:219-229.

Kobe, B. 2001. Crystallization and crystal structure determination of ribonuclease A-ribonuclease inhibitor protein complex. Methods Mol. Biol. 160:201-211.
Kobe, B., and Deisenhofer, J. 1994. The leucine-rich repeat: a versatile binding motif. Trends Biochem. Sci. 19:415-421.
Kobe, B., and Deisenhofer, J. 1995. Proteins with leucine-rich repeats. Curr. Opin. Struct. Biol. 5:409-416.
Koch, E., and Slusarenko, A. 1990. *Arabidopsis* is susceptible to infection by a downy mildew fungus. Plant Cell 2:437-445
Kombrink, E., and Schmelzer, E. 2001. The hypersensitive response and its role in local and systemic disease resistance. Eur. J. Plant Pathol. 107:69-78.
Lawrence, G. J., Finnegan, E. J., Ayliffe, M. A., and Ellis, J. G. 1995. The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. Plant Cell 7:1195-1206.
Leckie, F., Mattei, B., Capodicasa, C., Hemmings, A., Nuss, L., Aracri, B., De Lorenzo, G., and Cervone, F. 1999. The specificity of polygalacturonase-inhibiting protein (PGIP): a single amino acid substitution in the solvent-exposed beta-strand/beta-turn region of the leucine-rich repeats (LRRs) confers a new recognition capability. EMBO (Eur. Mol. Biol. Organ.) J. 18:2352-2363.
Luck, J. E., Lawrence, G. J., Dodds, P. N., Shepherd, K. W., and Ellis, J. G. 2000. Regions outside of the leucine-rich repeats of flax rust resistance proteins play a role in specificity determination. Plant Cell 12:1367-1377.
Mackey, D., Belkhadir, Y., Alonso, J. M., Ecker, J. R., and Dangl, J. L.. 2003. *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates *RPS2*-mediated resistance. Cell 112:379-389.
Mackey, D., Holt III, B. F., Wiig, A., and Dangl, J. L. 2002. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for *RPM1*-mediated resistance in *Arabidopsis*. Cell 108:743-754.
McDowell, J. M., Dhandaydham, M., Long, T. A., Aarts, M. G., Goff, S., Holub, E. B., and Dangl, J. L. 1998. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of *Arabidopsis*. Plant Cell 10:1861-1874.
Meyers, B. C., Shen, K. A., Rohani, P., Gaut, B. S., and Michelmore, R. W. 1998. Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 10:1833-1846.
Morel, J., and Dangl, J. L. 1997. The hypersensitive response and the induction of cell death in plants. Cell Death Differ. 4:671-683.
Mozo T., Fischer S., Shizuya H., and Altmann, T. 1998. Construction and characterization of the IGF Arabidopsis BAC library. Mol. Gen. Genet. 258:562-570.
Muskett, P. R., 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 defenses against multiple pathogens. Plant Cell 14:979-992.
Parker, J. E., Coleman, M. J., Szabo, V., Frost, L. N., Schmidt, R., van der Biezen, E. A., Moores, T., Dean, C., Daniels, M. J., and Jones, J. D. 1997. The *Arabidopsis* downy mildew resistance gene *RPP5* shares similarity to the toll and interleukin-1 receptors with *N* and *L6*. Plant Cell 9:879-894.
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.
Parniske, M., Hammond-Kosack, K. E., Golstein, C., Thomas, C. M., Jones, D. A., Harrison, K., Wulff, B. B., and Jones, J. D. 1997. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. Cell 91:821-832.
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.
Shah, J., and Klessig, D. F. 1999. Salicylic acid: signal perception and transduction. Pages 513-541 in: Biochemistry and Molecular Biology of Plant Hormones. M. A. Hooykaas and K. R. Libbenga, eds. Elsevier Science B.V., New York.
Takken, F. L., and Joosten, M. H. 2000. Plant resistance genes: their structure, function and evolution. Eur. J. Plant Pathol. 106:699-713.
Tör, M., Gordon, P., Cuzick, A., Eulgem, T., Sinapidou, E., Mert-Turk, F., Can, C., Dangl, J. L., and Holub, E. B. 2002. *Arabidopsis* SGT1b is required for defense signaling conferred by several downy mildew resistance genes. Plant Cell 14:993-1003.
Tornero P., Merit, P., Sadanandom, A., Shirasu, K., Innes, R. W., and Dangl, J. L. 2002. *RAR1* and *NDR1* contribute quantitatively to the function of *Arabidopsis* disease resistance genes in both simple and non-linear pathways. Plant Cell 14:1005-1015.
The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796-815.
Vidal, S., Cabrera, H., Andersson, R. A., Fredriksson, A., and Valkonen J. P. T. 2002. Potato gene *Y-1* is an *N* gene homolog that confers cell death upon infection with potato virus Y. Mol. Plant-Microbe Interact. 15:717-727.
Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R. Corr, C., and Baker, B. 1994. The product of the tobacco mosaic virus resistance gene *N*: Similarity to Toll and the interleukin-1 receptor. Cell 78:1101-1115.
Wulff, B. B., Thomas, C. M., Smoker, M., Grant, M., and Jones, J. D. 2001. Domain swapping and gene shuffling identify sequences required for induction of an Avr-dependent hypersensitive response by the tomato Cf- 4 and Cf-9 proteins. Plant Cell 13:255-272.
Young, N. D. 2000. The genetic architecture of resistance. Curr. Opin. Plant Biol. 3:285-290.
Zhang, X., and Gassmann, W. 2003. RPS4-mediated disease resistance requires the combined presence of RPS4 transcripts with full-length and truncated open reading frames. Plant Cell 15:2333-2342.
Zhou, N., Tootle, T. L., Tsui, F., Klessig, D. F., and Glazebrook, J. 1998. PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. Plant Cell 10:1021-1030.

AUTHOR-RECOMMENDED INTERNET RESOURCES

The Arabidopsis Information Resources database: www.arabidopsis.org
Center for Biological Sequence Analysis world wide web prediction server: www.cbs.dtu.dk/services/SignalP
The Sanger Institute Protein families database of alignments and HMMs: www.sanger.ac.uk/Software/Pfam/index.shtml
John Innes Center Sainsbury laboratory molecular plant genetics group plasmid list: www.sainsbury-laboratory.ac.uk/jonathan-jones/plasmid-list/plasmid.htm