Arabidopsis Downy Mildew Resistance Gene RPP27 Encodes a Receptor-Like Protein Similar to CLAVATA2 and Tomato Cf-9

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The Arabidopsis Ler-RPP27 gene confers AtSgt1b-independent resistance to downy mildew (Peronospora parasitica) isolate Hiks1. The RPP27 locus was mapped to a four-bacterial artificial chromosome interval on chromosome 1 from genetic analysis of a cross between the enhanced susceptibility mutant Col-0 and transformed into mutant Col-0 avirulent Columbia-0 (Col-0) virulent isolates of downy mildew pathogen. A full-length RPP27 cDNA was isolated, and analysis of the deduced amino acid sequences showed that the gene encodes a receptor-like protein (RLP) with a distinct domain structure, composed of a signal peptide followed by extracellular Leu-rich repeats, a membrane spanning region, and a short cytoplasmic carboxyl domain. RPP27 is the first RLP-encoding gene to be implicated in disease resistance in Arabidopsis, enabling the deployment of Arabidopsis techniques to investigate the mechanisms of RLP function. Homology searches of the Arabidopsis genome, using the RPP27, Cf-9, and Cf-2 protein sequences as a starting point, identify 59 RLPs, including the already known CLAVATA2 and TOO MANY MOUTHS genes. A combination of sequence and phylogenetic analysis of these predicted RLPs reveals conserved structural features of the family.

A wide range of parasites, including viruses, bacteria, fungi, nematodes, and insects, exploit plants as a source of food and shelter. Plants have evolved mechanisms to recognize the potential colonists and defend themselves. The defense is often activated by the direct or indirect interaction of the disease resistance (R) gene in the plant and the avirulence (Avr) gene in the pathogen (Dangl and Jones, 2001; Holub, 2001). The absence of either of these genes results in infection by the pathogen.

To date, numerous R genes have been cloned from a wide range of plant species, including Arabidopsis, flax (Linum usitatissimum), tomato (Lycopersicon esculentum), tobacco (Nicotiana tabacum), sugar beet (Beta vulgaris), apple (Malus domestica), rice (Oryza sativa), barley (Hordeum vulgare), and maize (Zea mays). Their structural and functional comparisons have been well documented, revealing several different classes (Hammond-Kosack and Jones, 1997; Hammond-Kosack and Parker, 2003; Tör et al., 2003). The largest group of R genes encodes cytoplasmically localized proteins that contain a central nucleotide binding (NB) site and a carboxyl Leu-rich repeat (LRR) domain (NB-LRR genes). This group can be further subdivided into two major subclasses: those having an amino-terminal coiled-coil (CC) domain (CC-NB-LRR) and those containing an amino-terminal domain resembling the cytoplasmic signaling domain of the Toll and Interleukin-1 (TIR) transmembrane receptors (TIR-NB-LRR). The CC-NB-LRR subclass includes examples such as the Arabidopsis RPS2 (Mindrinos et al., 1994) and RPM1 (Grant et al., 1995) genes conferring bacterial resistance, RPP13 (Bittner-Eddy et al., 2000) and RPP8 (McDowell et al., 1998) conferring downy mildew (Peronospora parasitica) resistance, and HRT (Cooley et al., 2000) conferring viral resistance from the same locus as RPP8. The TIR-NB-LRR subclass includes genes such as the tobacco N (Whitham et al., 1994) gene for viral resistance, the flax L6 (Lawrence et al., 1995) gene for rust resistance, and the Arabidopsis RPP5 (Parker et al., 1997) and RPP1 (Botella et al., 1998) genes for downy mildew resistance. Sequencing of the complete genome of Arabidopsis has revealed approximately 149 NB-LRR genes (Meyers et al., 2003).

The second group contains the cytoplasmic Ser/Thr kinase and has been represented by PTO (Martin et al., 1993), which confers resistance to the bacterial pathogen Pseudomonas syringae pv tomato.

The third group of R genes encodes the receptor-like kinases (RLKs). The characteristic features of these
proteins are an extracellular LRR domain with a single transmembrane spanning region and a cytoplasmic kinase domain. This group contains the rice Xa21 gene (Song et al., 1995), which confers resistance to bacterial pathogen Xanthomonas oryzae pv oryzae.

Receptor-like proteins (RLPs) comprise the fourth group of R genes. These are similar to RLK genes in that they encode extracellular LRRs and a C-terminal membrane anchor but lack the cytoplasmic kinase domain. Members of this group include the tomato Cf-2, Cf-4, Cf-5, and Cf-9 genes conferring resistance to the fungal pathogen Cladosporium fulvum (Jones et al., 1994; Dixon et al., 1996), the tomato Ve genes for Verticillium resistance (Kawchuk et al., 2001), and the apple HcrVf2 gene for resistance to Venturia inaequalis (Belfanti et al., 2004).

To date, all cloned Arabidopsis R genes conferring resistance to the oomycete downy mildew pathogen belong to the NB-LRR class. Here, we describe the molecular cloning of the Arabidopsis RPP27 gene that confers resistance to several isolates of P. parasitica and encodes an RLP. The coding sequence of this gene predicts a protein with topological features similar to Arabidopsis CLAVATA2 (CLV2) and tomato Cf-9.

RESULTS
Identification and Isolation of the RPP27 Gene

RPP27 was previously identified as a gene in the accession Landsberg erecta (Ler-0) that functions independently of AtSGT1b and confers resistance to Peronospora isolate Hiks1. Generation of mapping material and segregation data from the cross between a 35-kb deletion mutant Col-sgt1b and Ler-0 were described previously (Tör et al., 2002). Linkage of RPP27 to the molecular marker g4026 on chromosome 1 was determined in this previous work. Using 410 Hiks1 susceptible F2 families, RPP27 was mapped further between two PCR markers, Nga280 and F12M16 (Fig. 1A). Two new markers, AC1 and AC2, were generated from the sequence information of two bacterial artificial chromosomes (BACs), T18A20 and T22H22, and used to identify 14 key recombinant individuals. Four overlapping BAC clones, T18A20, F15I1, F20D21, and T22H22, span the RPP27 interval (Fig. 1B). The sequence information and annotations of these BAC clones were examined in detail and a Cf-like gene on the BAC clone F20D21 (F20D21.29) was identified. Another PCR marker, CFL, was generated...
from the sequence of this gene and used for mapping. This marker cosegregated with the RPP27 phenotypic data (Fig. 1B), indicating that this gene was a strong candidate for RPP27. A PCR-cloning approach was then taken to clone the corresponding region of this Cf-like gene from Ler-0. Using the available sequence information of BAC clones and accommodating possible misannotation of the gene, a fragment of 6,393 bp (encompassing a 2,034-bp promoter region, 3,366-bp coding region, and 993 bp beyond the stop codon) was targeted to be cloned from Ler-0. This region was PCR amplified using a proofreading DNA polymerase and cloned into a binary vector to produce the construct MT27 (Fig. 1C). The cloned insert and the corresponding genomic region from the Ler-0 RPP27 locus were verified by DNA sequencing.

Transgenic Complementation of RPP27 Function

The wild-type Columbia-0 (Col-0) carries the RPP7 gene that recognizes the downy mildew isolate Hiks1 (Holub et al., 1994; McDowell et al., 2000). Therefore, the construct MT27 was transformed into Col-rpp7.1 mutant plants to confirm that the putative Cf-like gene corresponds to the RPP27 gene. Fifteen independent transgenic T2 seedlings were assessed for resistance against Hiks1. All the lines segregated for the RPP27 and rpp27 phenotype (mostly 3:1), correlating with basta resistance and sensitivity, respectively, indicating that the cloned DNA fragment carries the RPP27 gene.

Homozygous T3 families were then obtained from these transgenic lines and examined for their capacity to generate H2O2 24 h after inoculation with Hiks1 using 3,3′-diaminobenzidine (DAB). This compound captures H2O2 and forms a reddish brown polymer at sites of peroxidase activity (Thordal-Christensen et al., 1997), thus providing a means for detecting an oxidative burst in host cells surrounding penetrating hyphae. More than 100 cotyledons from nontransgenic Col-rpp7.1 and from lines transformed with RPP27 were examined. No DAB staining was observed around the infection sites of cotyledons from nontransformed plants. Instead, normal pathogen growth was observed (Fig. 2A). In the cotyledons of RPP27-transformed plants, DAB staining was observed around the infection site mainly in one or two cells per infection site (Fig. 2B). However, the DAB staining observed in the transgenic cotyledons was not as strong as we reported previously for RPP7-mediated resistance (Tor et al., 2002), suggesting that Ler-RPP27 confers partial resistance to Hiks1.

We examined the pathogen development and interaction phenotype in detail. Three days after inoculation, normal pathogen development was observed in the cotyledons of control nontransformed seedlings, whereas extensive mycelial growth and trailing necrosis with a conidiophore development, which was observed occasionally 7 d after inoculation.

Figure 2. Pathogen development and interaction phenotypes of transformed and nontransformed plants inoculated with downy mildew isolate Hiks1. Cotyledons stained with DAB 1 d after inoculation and examined under a light microscope for H2O2 accumulation are shown in A and B (bar = 50 μm). A, Normal pathogen development and no H2O2 detection was observed in Col-rpp7.1. B, Accumulation of H2O2 was detected with DAB staining around the Hiks1 penetration sites in Col-rpp7 transformed with RPP27. Cotyledons stained with lactophenol-trypsin blue 3 d and 7 d after inoculation and viewed under a light microscope to reveal pathogen mycelium and necrotic plant cells are shown in C and E (bar = 10 μm). C, Col-rpp7.1, shown with normal pathogen development, fully susceptible to Hiks1 3 d after inoculation. D, Col-rpp7.1::Ler-RPP27, showing mycelium growth beyond the penetration site but surrounded by a trail of necrotic plant cells 3 d after inoculation. E, Col-rpp7.1::Ler-RPP27, showing extensive mycelial growth and trailing necrosis with a conidiophore development, which was observed occasionally 7 d after inoculation.
and host cells appeared to be intact (Fig. 2C). However, less pathogen growth and trailing necrosis was observed in colonized areas of transgenic seedlings (Fig. 2D). Trailing necrosis was more extensive in transgenic seedlings 7 d after inoculation, and occasionally the pathogen produced conidiophores (Fig. 2E).

**RPP27 Recognizes More Than One Isolate of Downy Mildew**

We extended the analysis to see whether RPP27 confers resistance to any isolate of downy mildew other than Hiks1. Col-0 compatible isolates but Ler-0 incompatible isolates, including Aswa1, Edco1, Emco2, Emco5, Emwa2, Goco1, Gowa1, Maks9, Noco2, and Noks1, were used to inoculate homozygous transgenic Col-0 and nontransgenic mutant Col-0. Asexual sporulation was measured by quantifying sporangiophore production as described previously (Tör et al., 2002) and summarized as follows: N, no sporulation; L, low sporulation (1–20 sporangiophores); medium sporulation (21–100 sporangiophores); and H, heavy sporulation (mean > 20 sporangiophores). R genes that have been molecularly characterized in the wild type accessions are indicated in parentheses (see Holub, 2001). n.d., not determined.

### Table 1. Phenotypes of Four Arabidopsis Accessions used to Determine Whether the RPP27 Allele from Ler Confers Resistance to Downy Mildew Isolate Hiks1 and to 10 Other Isolates that are Virulent in Col and Avirulent in Ler-0

<table>
<thead>
<tr>
<th>Downy Mildew Isolate</th>
<th>Arabidopsis Accession</th>
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<tr>
<td></td>
<td>Col-0</td>
</tr>
<tr>
<td>Hiks1</td>
<td>N (RPP7)</td>
</tr>
<tr>
<td>Aswa1</td>
<td>H</td>
</tr>
<tr>
<td>Edco1</td>
<td>H</td>
</tr>
<tr>
<td>Emwa2</td>
<td>H</td>
</tr>
<tr>
<td>Gowa1</td>
<td>H</td>
</tr>
<tr>
<td>Maks9</td>
<td>H</td>
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<td>H</td>
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<td>Emco5</td>
<td>H</td>
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<tr>
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<td>H</td>
</tr>
<tr>
<td>Noco2</td>
<td>H</td>
</tr>
<tr>
<td>Noks1</td>
<td>H</td>
</tr>
</tbody>
</table>

Approximately 50 7-d-old seedlings were spray inoculated with downy mildew conidiophores. Asexual sporulation was quantified by counting sporangiophores 7 d after inoculation as described previously (Tör et al., 2002) and summarized as follows: N, no sporulation; L, low sporulation (1–20 sporangiophores per cotyledon; the mean is indicated by a number); medium sporulation (11–20 sporangiophores); and H, heavy sporulation (mean > 20 sporangiophores). R genes that have been molecularly characterized in the wild type accessions are indicated in parentheses (see Holub, 2001). n.d., not determined.

Sequence Analysis of the RPP27 Gene and Transcript

The construct MT27 that carries the Ler-0 genomic DNA fragment was sequenced using a primer walking strategy and shown to contain a 6,461-bp insert. This DNA fragment was sequenced using a primer walking approach previously (Tör et al., 2002) and was compared with the controls. Control seedlings Col-0 and Col-0pp7.1 were susceptible to all the isolates tested. However, RPP27 transgenic Col-0pp7.1 was susceptible to Aswa1, Edco1, Emwa2, Gowa1, and Maks9 but resistant to Emco2, Goco1, Noco2, and Noks1. Interestingly, RPP27 transgenic Col-0pp7.1 inoculated with Emco5 showed the phenotype of low sporulation (L3). The results are summarized in Table 1.
in Col-0) was in the largest exon; and one came just after the 3' untranslated region. The large INDEL caused a frameshift in the largest exon resulting in a premature stop codon. These polymorphisms therefore have a significant effect on the predicted amino acid sequences of RPP27 protein from Col-0 accession and may also have played a role in the misannotation of the region described above.

**Predicted RPP27 Protein Structure**

The open reading frame of the RPP27 gene encodes a predicted protein of 1,044 amino acids (molecular mass of 116.9 kD) with structural similarity to other RLPs, including Arabidopsis CLV2 (Jeong et al., 1999) and TOO MANY MOUTHS (TMM; Nadeau and Sack, 2002), and tomato Cf-9 (Jones et al., 1994) and Cf-2 (Dixon et al., 1996). A variety of bioinformatics approaches were used to predict the structure of RPP27 (see “Materials and Methods”). As with the tomato Cf-9 and Arabidopsis CLV2 protein, RPP27 can be divided into seven domains (Fig. 3): an initial signal peptide (domain A, M1-S20), followed by an LRR (domain B, Q21-S143), an LRR (domain C, I144-G946), a variable region (domain D, residues R947-C972), an acidic region (domain E, D973-L997), a predicted transmembrane domain (domain F, A998-F1020), and a short cytoplasmic tail (domain G, D1021-P1044).

Domain C constitutes the majority of the predicted RPP27 protein and consists of 30 imperfect copies of extracellular LRRs with a consensus sequence of LxxLxxLxxLxxNxxLxxGxPx. This region has an island of variable and mostly hydrophobic sequences between positions 789F and 855D that matches similar regions in other RLPs; the exact structure of this region cannot be specified using sequence analysis methods. It is possible that this region provides a flexible hinge to the two flanking LRR domains (C1 and C3), allowing them to articulate relative to each other.

Table II. **Sequence polymorphisms in the RPP27 region between Col-0 and Ler-0**

<table>
<thead>
<tr>
<th>Nucleotide Position*</th>
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<th>Ler-0</th>
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<tr>
<td>−1,044</td>
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<td>G</td>
</tr>
<tr>
<td>−1,024</td>
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<tr>
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<td>G</td>
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<tr>
<td>−412</td>
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<td>C</td>
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<td>412b</td>
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<td>G</td>
</tr>
<tr>
<td>1,799b</td>
<td>T</td>
<td>C</td>
</tr>
</tbody>
</table>
| 2,574c               | −     | CGAGTACCAGAAAAACTCGGCTCTAGT-TGATCTATCCAACAACAGATTATG-
|                     |       | GAAACCTACCTACAT |
| 4,576                | −     | T     |

*Nucleotide positions are numbered with respect to translational start site. **Polymorphism is in the intron. ***Polymorphism is in the exon. Deletion.

**Figure 3.** Predicted domain structure of RPP27. Domains A to G correspond with previous diagrams of Cf-9 (Jones et al., 1994; see text for descriptions). The B region is divided into B1, B2, and B3 to show the presence of its predicted TM region (B2, underlined). The site of the large deletion relative to Ler-0 is marked with an asterisk. The C region is shown divided into C1 (main LRR block), C2 (non-LRR island), and C3. The presence of the island of non-LRR sequence before the final four LRRs is a common element in RLPs and may be a structural hinge that allows the C1 block to adopt its correct conformation. The C3 region is highly conserved within the family and may be required for multiprotein complex formation.

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**Legend for Figures:**

- **A**: MKNVFIULLLLCVFAISPS
- **B1**: QREEINQHNGYHQLKYKQQ
- **B2**: GVLYFLAALISSACG1GSGFLFI
- **B3**: PTTTVLPSKLTDKLRFLQYLWILLLGQ
- **C1**: ITQESLDLYVFVPWDMDDDDOCCQWESIMC
- **C2**: LPPFeWFLWLVAGSLRNESLILN
- **C3**: LKRLKIAALDNLSNPLGSLMEQV
- **D**: LGKNNHLVDSLQNLQNLGQ
- **E**: CNLNLGVYFLSGLGLYQKQ
- **F**: VGAACCTACCTACAT
- **G**: LXXLXXLXNLXXNLXXLXLSGXIPXX
Despite similarities between RPP27 and the product of Cf genes (particularly Cf-2 and Cf-9), there are two striking differences. First, relative to the tomato Cf proteins and the vast majority of other Arabidopsis RLPs, RPP27 has a highly divergent amino-terminal B domain, with an initial region of approximately 120 amino acids preceding a region of significant homology with Cf-2 and Cf-9.

Second, in contrast to all other plant RLPs identified thus far, which are predicted to have a single transmembrane domain at the C terminus, transmembrane prediction using the TMHMM server identifies two putative transmembrane (TM) domains in RPP27, one at the C terminus and one in the variable N-terminal domain (66G–88I). However, since transmembrane prediction algorithms are known to be misled by hydrophobic stretches (Chen et al., 2002), we examined the N-terminal region in depth. In contrast to the C-terminal transmembrane domains, which share a recognizable sequence signature and conserved residues, the N-terminal predicted transmembrane domain of RPP27 does not match any characterized transmembrane domains in any protein. In addition, analyses of other LRR proteins show both a high degree of sequence divergence across LRRs, and some regions of LRR proteins contain hypervariable stretches. These points, in combination with the lack of identified proteins with what would represent an entirely novel fold, suggest to us that this region of RPP27 encodes a receptor-like protein and confers disease resistance.

**RLP Family Analysis**

The consensus RLP fold consists of multiple LRRs, followed by a transmembrane domain and a short cytoplasmic tail. Because LRRs are often found in proteins with non-RLP folds, sequence-based methods of homolog detection can inadvertently include many non-RLPs in database searches. Similarly, transmembrane prediction tools can overpredict TM domains in hydrophobic stretches. To discriminate true RLPs from sequences with different overall folds, we employed a multistep analysis. First, we used RPP27, Cf-2, and Cf-9 as BLAST queries against Arabidopsis. We then scored Arabidopsis proteins with a hidden Markov model (HMM; Krogh et al., 1994) that we constructed to specifically match the RLP topology. Sequences with significant BLAST e-values or strong scores against our HMM were examined and found to include many RLKs and other non-RLP proteins. We scored these proteins against PFAM HMMs and removed any proteins with e-values less than 0.5 to any non-LRR PFAM HMMs. Remaining proteins were submitted to the TMHMM transmembrane prediction server (Krogh et al., 2001). Proteins with no predicted transmembrane domains at the C terminus (or no very close homologs with a predicted transmembrane domain) were removed from the set, and the remainder was multiple aligned. Examination of the multiple sequence alignment revealed a conserved and almost entirely ungapped region of approximately 100 amino acids in domain C. Sequences not matching this region or the consensus sequence signature at the predicted transmembrane domain were removed. This procedure was intentionally stringent and identified a conservative set of 58 sequences in addition to RPP27. A more detailed analysis of the RLP family in plants is in progress.

We then constructed a series of multiple sequence alignments for these 59 sequences, as well as for the tomato Cf-2 and Cf-9 proteins. These alignments were examined and the highest quality alignment selected. From this alignment, we constructed two separate alignments, one that was essentially global (removing columns with mostly gaps) and one restricted to the C-terminal conserved domains C3 to F (Fig. 4). Phylogenetic trees were estimated using parsimony, neighbor-joining, and maximum likelihood methods, with bootstrap analysis to identify subtrees with high bootstrap support. Tree topologies were examined and found to be fairly consistent both across methods and across the two alignments; most differences were restricted to the coarse branching order in the trees. A consensus tree topology was inferred using bootstrap analysis and the PHYLIP consense software (Fig. 5).

The Arabidopsis RLP family contains 59 genes, including RPP27, CLV2, TMM, and 56 previously unidentified family members. These are distributed throughout the genome as follows: 17 genes, including CLV2, TMM, and RPP27, on chromosome 1; 12 on chromosome 2; 16 on chromosome 3; 8 on chromosome 4; and 6 on chromosome 5. The distribution is similar to NB-LRR genes, with several complex loci containing 3 or more RLPs and others that are singlets or doublets (Fig. 6). Further details are available at http://phylogenomics.berkeley.edu/PlantResistanceGene/ArabRLPs.html.

**DISCUSSION**

The predicted polypeptide encoded by the RPP27 gene has structural features that indicate a receptor-like function. The signal peptide targeting the membrane at the amino terminus, the putative extracytoplasmic protein-protein interaction domain (LRR), the single transmembrane domain, and the short cytoplasmic tail present an overall structure of the large class of RLPs found in many species across the kingdom. To date, only two functional RLPs have been identified in Arabidopsis: CLV2 and TMM. CLV2 (Jeong et al., 1999) is involved in meristem and organ development and is required for the accumulation and stability of the receptor kinase CLV1 (Clark et al., 1997).
TMM (Nadeau and Sack, 2002) is involved in stomatal and epidermal development. Functional RLPs that are involved in disease resistance have been described in other plant species, including the tomato Cf genes (Hammond-Kosack and Jones, 1997; Joosten and De Wit, 1999) for leaf mold resistance and the Ve genes for Verticillium resistance (Kawchuk et al., 2001). RPP27 provides the first example of an RLP in Arabidopsis that is associated with disease resistance. As discussed below, this discovery is particularly interesting because the pathogen downy mildew is an obligate biotrophic parasite that produces haustorial feeding structures in host cells. We have identified 59 RLPs in Arabidopsis using RPP27, Cf-9, and Cf-2. In a recent study, Shiu and Bleecker (2003) identified a superfamily of putative RLPs from the Arabidopsis genome. Their aim was to identify essentially all Arabidopsis proteins in Col-0 that share any recognizable sequence similarity with the extracellular portion of the RLKs. They used the extracellular domains of 35 representative RLKs to conduct BLAST searches of the Arabidopsis genome, with a cutoff value of 10 e–10. Shiu and Bleecker (2003) identified a superfamily of putative RLPs from the Arabidopsis genome. Their aim was to identify essentially all Arabidopsis proteins in Col-0 that share any recognizable sequence similarity with the extracellular portion of the RLKs. They used the extracellular domains of 35 representative RLKs to conduct BLAST searches of the Arabidopsis genome, with a cutoff value of 10 e–10. Shiu and Bleecker (2003) selected a final set of 173 proteins for cluster analysis, using the unweighted pair group method with arithmetic mean algorithm based on pairwise BLAST e-values, and produced a hierarchical tree shown in their paper. Examination of these clusters reveals a significant fraction of proteins that do not match the canonical RLP topology (transmembrane region and extracellular LRR). These proteins may well be related in some way to RLPs but are likely to have different functions. By contrast, our approach was deliberately conservative and designed to produce a set of proteins that matched the canonical RLP structure; we excluded any proteins containing structural domains not found in the RLPs and required a single transmembrane domain at the C terminus. Examination of the two sets of proteins shows that all but one of the proteins we identify as RLPs are also found by Shiu and Bleecker (2003). The exception, At1g58190, is clearly a member of the RLP class, which contains 49 LRRs, a transmembrane domain (identified by TMHMM), and a short cytoplasmic tail. Of the 117 proteins included by Shiu and Bleecker (2003) but not by us, all but four contain additional domains not found in RLPs (e.g. GDPD, B-Lectin, etc.) or are missing identifiable transmembrane segments at the C terminus. However, none of these four contain recognizable LRRs and therefore do not match the canonical RLP structure.

Phylogenetic tree estimation of these RLPs (along with the tomato Cf genes) place RPP27, CLV2, TMM, Cf-2, and Cf-9 on the same branch of the evolutionary tree, suggesting that proteins involved in both disease resistance and development may have evolved from a common origin (Fig. 5). We expect that RPP27 is not the sole example of disease resistance among these RLPs in Arabidopsis. Putative T-DNA mutants in Col-0 background exist for at least 31 of these RLPs. Challenging these mutants with Col-avirulent isolates of bacterial and filamentous pathogens such as P. syringae and downy mildew, respectively, may yield more information on the involvement of these RLPs in disease resistance.

The RPP27 protein has an overall topology consistent with the canonical RLP fold (Fig. 3): an amino-terminal...
signal peptide (M1-S20), followed by Leu-rich (Q21-S143) and LRR domains (I144-G946), an acidic region (D973-L997), a transmembrane domain (A998-F1020), and a short cytoplasmic tail (D1021-P1044). Analysis of the family of Arabidopsis RLPs reveals a conserved region of approximately 150 amino acids immediately preceding the transmembrane domain (Fig. 4). The number of LRR motifs is extremely variable across Arabidopsis RLPs; some members of the family have as few as four apparent repeats, while others have as

Figure 5. Phylogenetic tree of the RLP family in Arabidopsis. Amino acid sequences of 59 RLPs from Arabidopsis, as well as Cf-2 and Cf-9, were aligned with MAFFT. The tree was generated from a truncated alignment consisting of the conserved C3-F domains. A total of 100 bootstrap replicates of this alignment were made. PHYLIP’s Neighbor program was used to build the trees, and the Consense program generated the consensus tree and bootstrap values. Other bootstrapped trees (not shown) were built with parsimony and maximum likelihood methods from the full and truncated alignments. All trees were similar to this one, in that certain subfamilies appeared in every tree with high bootstrap values. The joining of these subfamilies in the higher nodes was inconsistent and invariably gave low bootstrap values. Note that At1g54480 is the Col-0 allele of RPP27.
many as 49 repeats. The LRR motif itself varies in form across family members and at positions; some are extremely hard to detect using sequence-based methods. A hypervariable region of approximately 50 to 75 amino acids is found nested between detectable LRR motifs in many RLPs; the precise role played by this region is not known. In RPP27, this hypervariable region is found between residues 789 and 855.

The majority of the RPP27 protein comprises extracellular LRR. However, there is no signal transduction domain, suggesting that additional proteins are required to facilitate the transmission of an Avr-induced conformational change from the extracytoplasmic to cytoplasmic domain and subsequently to activate the defense response. A possible function for the RPP27 protein can be proposed based on the hypothesized model of the CLV family in Arabidopsis. According to this model, the RLP encoded by CLV2 (Jeong et al., 1999) and an extracellular LRR RLK encoded by CLV1 (Clark et al., 1997) form heterodimers and potentially act as a receptor for CLV3, a small secreted ligand (Fletcher et al., 1999), to activate the signal transduction cascade with the involvement of the kinase-associated protein phosphatase KAPP (Stone et al., 1998). Involvement of heterodimerization has also been suggested for other receptor-mediated signaling pathways in plants. For example, the S locus receptor kinase (SRK; Stein et al., 1996), and the S locus glycoprotein (SLG; Stein et al., 1991) genes are required for self-incompatibility in Brassica. It has been proposed that the S domains of SRK and SLG form a heterodimer after binding the pollen-derived ligand and activate the signaling pathway through the kinase domain of SRK (Stein et al., 1996).

Similar modes of action for some of the R genes are also proposed. The rice gene Xa21 encodes an RLK and confers resistance to the bacterial pathogen X. oryzae pv oryzae (Song et al., 1995). However, Xa21D (Wang et al., 1998), a natural variant of Xa21, lacks the kinase and the membrane domain and still confers resistance to the same isolates of the pathogen. Wang et al. (1998) hypothesized that Xa21D forms a heterodimer with an endogenous RLK and activates the signaling cascade. Similar mechanisms for cytoplasmic signaling have been investigated for the Cf-9 protein (Rivas et al., 2002) and proposed for the tomato Ve genes (Kawchuk et al., 2001).

To date, information on the RLP-mediated defense responses have come mainly from the studies with the Cf genes. Early Cf-mediated responses, including the

Figure 6. Distribution of RLPs in Arabidopsis relative to two subclasses of NB-LRR genes.
production of active oxygen species (Piedras et al., 1998), involvements of mitogen-activated protein kinases (Romeis et al., 1999), calcium-dependent protein kinases (Romeis et al., 2002), and ACRE genes (Durrant et al., 2000), have been reported. In addition, Rcr3, a secreted Cys protease (Krüger et al., 2002), has been cloned and shown to be a positive regulator of Cf-2-dependent resistance and autonecrosis. However, studying the signaling components of tomato Cf genes has been hindered by the lack of large-scale mutagenesis and microarray analysis because of its large genome size. We have reported previously (Tor et al., 2002) that RPP27-mediated resistance functions independently of AtSGT1b. With the identification of RPP27, we can take advantage of techniques and tools developed for Arabidopsis to understand the mechanism of the RLP-mediated resistance. As a complementary approach, we are conducting large-scale mutant screens to identify genes that are involved in RPP27-mediated resistance.

Inoculation of RPP27 transgenic Col-rpp7.1 plants with different Ler-0 incompatible isolates of downy mildew showed that RPP27 confers full resistance to four isolates and partial resistance to Emco5 (Table I). Results with the isolate Noco2 are intriguing because this isolate was used to clone RPP5 from Ler-0, and there was no indication from genetic analysis for an additional R-gene specificity on chromosome 1 (Parker et al., 1997). There may be two possible explanations for this finding. First, although the RPP27 gene was cloned by PCR using a proofreading enzyme, a PCR error may have been introduced, creating a new specificity. However, we found no differences between sequence of the PCR insert and the corresponding genomic region from the Ler-0. In addition, sequencing the cDNA clones and RACE products revealed no sequence difference. Alternatively, a second gene in Col-0 that is tightly linked in repulsion to the RPP27 gene in Ler-0 may be playing a role in conferring resistance to Noco2. This second gene (designated RPP29) is currently being investigated.

In a plant-pathogen interaction, the products of R genes recognize the effector molecules of the pathogen either directly (Bryan et al., 2000) or through an interacting partner (Mackey et al., 2002) and trigger the downstream signaling pathways. In the case of RPP27, it is tempting to speculate that recognition occurs in the intercellular space through the participation of the LRR region of RPP27 directly binding the effector molecules of the pathogen. Rethage et al. (2000) reported that intercellular wash fluids from infected Arabidopsis leaves have race-specific elicitor activity, indicating that effector molecules may be secreted from Peronospora into the intercellular space. However, they have yet to identify a peptide with potential avirulence activity. This method (De Wit and Spiksman, 1982) has been successfully used to study tomato/Cladosporium interaction and to clone Avr genes from C. fulvum (Van Kan et al., 1991; Van Den Ackerveken et al., 1992). However, binding studies with AVR9 peptide suggest that the Cf-9 protein is not the primary receptor of AVR9 (Kooman-Gersmann et al., 1996), and no AVR9/Cf-9 affinity could be detected (Luderer et al., 2001). It is important to note here that although C. fulvum is a biotrophic pathogen of tomato, it grows exclusively in the intercellular space and it does not produce haustoria. By contrast, downy mildew produces haustoria into the cells of the host plant (Fig. 1), and RPP27-mediated resistance appears to be posthaustorial. Therefore, it may be that the recognition between RPP27 protein and the corresponding Avr gene product occurs in the extrahustorial matrix, the sealed area between the host and the haustorial cytoplasm (Manners and Gay, 1983). Alternatively, this haustorial parasite may also secrete proteins along its hyphae in intercellular spaces of the host similar to C. fulvum.

Downy mildew genes that elicit a defense response in Arabidopsis have been described as Arabidopsis thaliana-recognized (ATR) avirulence determinants, including the genetic identification of ATR1-mediated resistance and the corresponding Avr gene product occurs in the extrahustorial matrix, the sealed area between the host and the haustorial cytoplasm (Manners and Gay, 1983). Alternatively, this haustorial parasite may also secrete proteins along its hyphae in intercellular spaces of the host similar to C. fulvum.

Materials and Methods

Plant Lines

Arabidopsis Col-0 and Ler-0 were used in this study. The mutants Col-sgt1b and Col-rpp7.1 were described elsewhere (Tor et al., 2002). All seeds were vernalized at 4°C for 3 to 4 d before germination. Growth conditions for the plants were as described previously (Holub et al., 1994).

Growth of Downy Mildew Isolates

All isolates of downy mildew (Peronospora parasitica) were maintained on Wisslewijska-icsl1 (Parker et al., 1996). Preparation of inoculum for experiments and the assessment of sporulation were as described (Tor et al., 2002).

Light Microscopy

Seedlings of infected and noninoculated controls were stained with DAB to detect H2O2, as described previously (Thordal-Christensen et al., 1997). To visualize development of Peronospora and microscopic lesions, cotyledons were stained with a solution of phenol, lactic acid, glycerol, and water (1:1:1:1) supplemented with 1 mg/ml of trypan blue according to a previously described method (Koch and苏联ırıko, 1990).

Map-Based Cloning of RPP27

The linkage of RPP27 locus to the molecular marker g4026 on chromosome 1 was described previously (Tor et al., 2002). In addition to 310 Hiks1 susceptible F1, Col-cbl1 X Ler-0 families used for cloning AtSGT1b (Tor et al., 2002), an additional 100 Hiks1 susceptible F1 families were identified from the same cross and included for fine mapping of RPP27. Several PCR-based markers from either side of g4026 were generated using the sequences of BAC clones and information from the Cereon Genomics SNP/INDEL database (Cambridge, MA). Initially, RPP27 was mapped to an interval spanned by NGA280 and the F2M16 (generated from the BAC clone F2M16). Then, the locus was fine

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mapped between the markers AC1 and AC2. AC1 was generated using primers 5′-CCCCATTTAAAGGCCAACATC-3′ and 5′-TGGTTTCGGAATCATTACAT-3′, and the polymorphism between Col-0 and Ler-0 was detected by the presence of a deletion of 26 bp. AC2 was amplified with the primers 5′-CCGATTTCGCAATTTTAC-3′ and 5′-CGCCATTTGCAAAATGACAT-3′. The product was cleaved with MboII to reveal the polymorphism. Four overlapping BAC clones (Tl82A0, F151, F20D21, and T22H22) span the RPP27 interval. The marker CFL was generated from the sequence information of the Cf-like gene on the BAC clone F20D21 using primers 5′-TGTAAATAAGGTTTGGACAGTC-3′ and 5′-5′-CATGCACTATGTTGACGG-3′, and the polymorphism between Col-0 and Ler-0 was detected by the presence of a 68-bp deletion. The RPP27 locus cosegregated with the marker CFL. The Cf-like gene on the BAC clone F20D21 was targeted for cloning. The candidate gene was amplified from L. Homozygous T3 plants were then obtained and used for the subsequent experiments.

**RACE and Sequence Analysis**

Total RNA was isolated from infected and noninfected cotyledons and flowers using the RNAeasy plant mini kit (Qiagen USA, Valencia, CA) according to the manufacturer’s instructions. RACE PCR was performed as described (Tor et al., 2002) using the RACE primer specific primers 5′-TACACCGCTTCTCCTATGTTGCA-3′ and 5′-CTTCTCCATPTGCTCTTCAA-3′ using a proofreading enzyme, Elongase (Invitrogen, Glasgow, UK), and blunt-end cloned into Smal digested binary vector pCAMBIA3300 (http://www.cambia.org), which carries the BAR gene. The construct was designated as MT27 and used for transformation.

**Agrobacterium-Mediated Transformation**

The construct MT27 was electroporated into Escherichia coli strain DH10B, and the construct was then introduced into Agrobacterium tumefaciens strain GV3101 by electroporation and the Col-rpp7.1 plants were transformed by the floral dip method (Clough and Bent, 1998). Transformants were selected by spraying 7-d-old plants grown in soil with 0.04% Basta (AgrEvo, Norfolk, UK). Selected plants were self-pollinated to produce T2 seeds and tested with Hiks1 isolate of Peronospora. Homozygous T2 plants were then obtained and used for the experiments.

**Sequence data from this article have been deposited with the EMBL/GenBank data libraries under accession numbers AC005287, AJ585978, and AJ585979, respectively. The accession numbers for CLV2, TMM, CF-9, and CF are NP_17617, Q63892,CAA55274, and T10504, respectively.**

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**LITERATURE CITED**


RPP27 Encodes a Receptor-Like Protein and Confers Disease Resistance


conferred by several downy mildew (Peronospora parasitica) resistance genes. Plant Cell 14: 993–1003


RETRACTION

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Törm., Brown D., Cooper A., Woods-Tör A., Sjölander K., Jones J.D.G., and Holub E.B.

Arabidopsis Downy Mildew Resistance Gene RPP27 Encodes a Receptor-Like Protein Similar to CLAVATA2 and Tomato Cf-9.

The authors wish to communicate the following retraction. This article reported that the allele of gene At1g54480 from Landsberg erecta (Ler)-0 encodes a receptor-like protein that confers resistance to Hiks1 and at least four Ler-0 avirulent/Columbia (Col)-0 virulent isolates of downy mildew (Peronospora parasitica) pathogen in an rpp7.1 mutant background of Col-gf1. Since the publication of the article, the authors have been unable to repeat the results in the original Col-rpp7.1 background as well as in other Col-rpp7 mutants, including Col-rpp7.6, Col-rpp7.7, Col-rpp7.8, Col-rpp7.9, Col-rpp7.10, and Col-rpp7.11. The observed disease resistance in the putative transformants was likely due to seed contamination of the plant lines used for transformation. For this reason, and because the authors are no longer confident in the data in Figure 2 and Table I, they hereby retract their Plant Physiology article. Figure 1 showing the map location of the RPP27 locus (for SGT1b-independent Hiks1 resistance) is not in question. However, the gene within this mapping interval from Ler-0 that confers RPP27 function has yet to be determined. The authors deeply regret this error and sincerely apologize for the inconvenience and confusion this mistake caused Plant Physiology and its readership.
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