a minimal effect on the steady-state concentration of *axi1* transcripts.

Thus, at low concentrations, LCOs containing *trans*–fatty acyl substituents activate the expression of *axi1* in protoplasts of the nonlegume tobacco, conferring on these cells the ability to grow in the absence of auxin. As LCOs mimic auxin in activating the expression of a tobacco gene that apparently mediates auxin action, our data suggest that mitosis of protoplasts triggered by auxin shares steps in signal transduction with LCO-stimulated cell division. At least part of the mechanism necessary for nodule formation in legumes is therefore also present in nonleguminous plants. Thus, LCOs may be considered as plant growth regulators and may be related to as yet unidentified endogenous plant signals.

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5. Radioisotopically labeled and nonlabeled β-1,4-tri-*N*-acetyl-D-GlcN tetrasaccharides were prepared and purified as described (*8*). Tri-*N*-GlcNAc tetrasaccharide was *N*-acylated with *cis*-9- or *cis*-11-octadecenoic anhydrides (Nu Check Prep, Elysion, MN) as follows. Anhydrides (40 μl) were dissolved in 1.44 ml of 2-propanol. The solution was stirred at 37°C, and 2 mg of tetrasaccharide dissolved in 320 μl of 10% (v/v) acetic acid was slowly added. The mixture was stirred under argon at 37°C for 16 hours and subsequently dried. To remove excess anhydrides, we suspended the residue in 0.5 ml of water and extracted it once with ethyl acetate. The aqueous phase was dried, and the residue was extracted three times with 1-ml portions of 60% (v/v) acetonitrile at 40°C. The combined supernatants were applied to a preparative reversed-phase HPLC column. For the synthesis of *N*-octadecanoyl-tri-*N*-GlcNAc tetrasaccharide, 25 mg of stearic anhydride (Sigma) and 2 mg of tetrasaccharide were dissolved in 3.5 ml of chloroform:2-propanol [1:1 (v/v)] at 37°C. The mixture was stirred under argon at 37°C overnight. After the solvents were evaporated with nitrogen, acylated chitooligosaccharides were extracted with 50% (v/v) acetonitrile at 56°C and subsequently subjected to preparative HPLC. To attach elaidic acid to the free amino group of the tetrasaccharide, we dissolved 2 mg of tri-*N*-GlcNAc tetrasaccharide in 640 μl of 10% (v/v) acetic acid and added the solution to a vial containing 25 mg of *trans*-9-octadecenoic anhydride (Sigma) in 2.9 ml of 2-propanol. The mixture was stirred under argon for 16 hours at 37°C and dried. The residue was extracted with 50% (v/v) acetonitrile at 56°C, and 1-ml portions were applied to a C_{18} column. In the case of *trans*-11-octadecenoic acid, we attached the free acid (Sigma) to the primary amine through the action of 2-chloro-1-methylpyridinium iodide (Sigma) in acetonitrile as described (*3*).
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10. Fatty acids were released from LCOs by saponification [5% (w/v) KOH, 18 hours, 80°C]. After acidification, fatty acids were extracted with chloroform and analyzed by GLC on a Nukol column (15 m by 0.53 mm, 0.5-μm film thickness; Supelco). After split injection (ratio, 10:1), the column temperature was programmed from 100° to 220°C at a rate of 10°C/min. Helium was the carrier gas, at a flow rate of 30 ml/min.
11. The incubation mixture contained 10 mM sodium phosphate (pH 6.8), *N*-acylated tri-*N*-[^{14}C]acetyl-β-1,4-D-GlcN tetrasaccharide (10 nCi), and 40 μg of recombinant *Serratia marcescens* chitinase (*4*), in a final volume of 20 μl. After incubation at 37°C for 16 hours, the reaction mixture was inactivated by heating. Degradation products were separated by thin-layer chromatography and subjected to autoradiography (*8*).
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Structure of the *Arabidopsis RPM1* Gene Enabling Dual Specificity Disease Resistance

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Plants can recognize pathogens through the action of disease resistance (*R*) genes, which confer resistance to pathogens expressing unique corresponding avirulence (*avr*) genes. The molecular basis of this gene-for-gene specificity is unknown. The *Arabidopsis thaliana RPM1* gene enables dual specificity to pathogens expressing either of two unrelated *Pseudomonas syringae avr* genes. Despite this function, *RPM1* encodes a protein sharing molecular features with recently described single-specificity *R* genes. Surprisingly, *RPM1* is lacking from naturally occurring, disease-susceptible *Arabidopsis* accessions.

Plants express sophisticated genetic systems to recognize pathogens. Complex *R*-gene loci have been defined in a variety of plant-pathogen interactions. Genetic analyses suggest that each *R*-encoded specificity responds to a single *avr*-dependent pathogen signal (*1*). The recent cloning of four *R* genes (*2–7*) and accumulation of cloned bacterial and fungal *avr* genes (*8*) provide limited insight into the structural basis of specificity, although most models predict that *R* gene products interact with specific pathogen signal molecules produced in an *avr*-dependent manner (*9*).

RPM1 was identified in *A. thaliana* accession Col-0 as conferring resistance to *P. syringae* isolates expressing the *avrRpm1* gene (*10, 11*). Functional homologs of *RPM1* exist in pea, bean, and soybean (*12*). Resistance in *A. thaliana* to the *P. syringae avrB* gene also mapped to the *RPM1* interval (initially termed *RPS3*) (*13*), and genetic analyses of *A. thaliana* mutants have suggested that *RPM1* conferred resistance to *P. syringae* expressing either *avrRpm1* or *avrB* (*14*). Because the sequences of *avrB* and *avrRpm1* are unrelated (*12, 15*), *RPM1* appears to determine a dual specificity.

RPM1 was mapped by restriction fragment length polymorphism (RFLP) analysis

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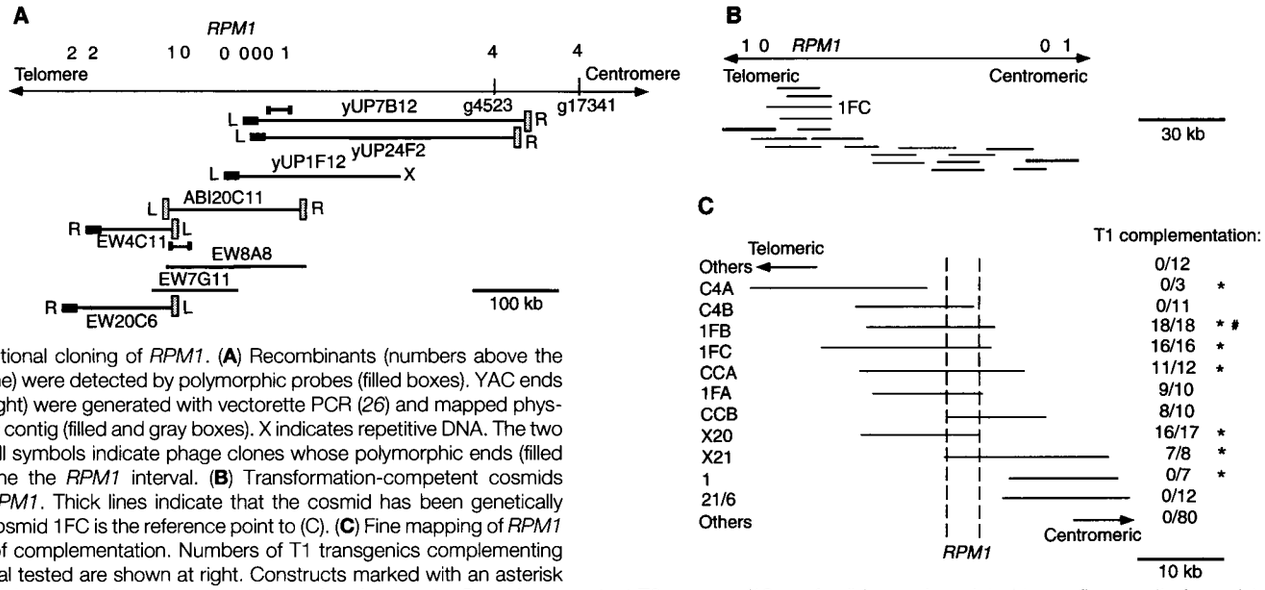

Fig. 1. Positional cloning of *RPM1*. (**A**) Recombinants (numbers above the chromosome) were detected by polymorphic probes (filled boxes). YAC ends (L, left; R, right) were generated with vectorette PCR (*26*) and mapped physically on this contig (filled and gray boxes). X indicates repetitive DNA. The two small barbell symbols indicate phage clones whose polymorphic ends (filled boxes) define the *RPM1* interval. (**B**) Transformation-competent cosmids spanning *RPM1*. Thick lines indicate that the cosmid has been genetically mapped. Cosmid 1FC is the reference point to (C). (**C**) Fine mapping of *RPM1* by means of complementation. Numbers of T1 transgenics complementing over the total tested are shown at right. Constructs marked with an asterisk were tested in parallel for complementation of resistance to *P. syringae* DC3000 expressing either *avrRpm1* or *avrB*. Also, between two and six T1 individuals from this group were allowed to self-pollinate, and antibiotic-resistant T2 progeny (18 per family) were inoculated to confirm results for resistance to both *avr* genes. The number symbol indicates a cosmid that was also used to complement the *rpm1* accession Nd-0.

(*11*). We assembled an overlapping set of yeast artificial chromosome (YAC) clones covering approximately 800 kb and identified two clones, W8A8 and W7G11 (DNA inserts from accession Col-0 and thus containing *RPM1*), encompassing the *RPM1*-proximal recombination breakpoints. Plant transformation-competent cosmids were isolated from these YACs (*16*) and organized into a contig (Fig. 1B).

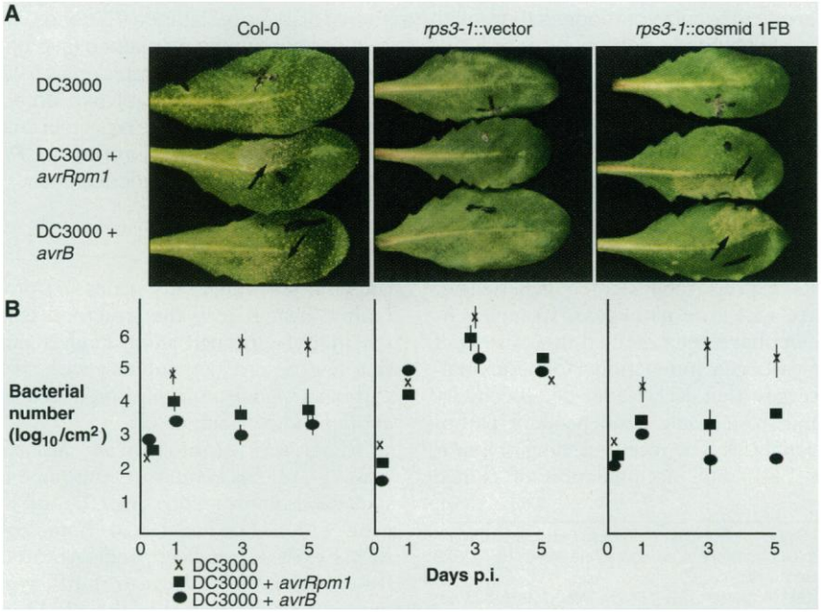

Fig. 2. *RPM1* complementation. (**A**) Restoration of HR. T1 transgenics containing an empty vector or cosmid 1FB, and matched Col-0 controls, were inoculated (arrows) opposite black ink marks with *P. syringae* DC3000 or *avr*-expressing derivatives (listed at left) at 5×10^7 colony-forming units (CFU) per milliliter; leaves were photographed 12 hours post-inoculation (p.i.) (*11*, *12*). (**B**) Complementation inhibits growth of *P. syringae* DC3000 expressing *avrRpm1* or *avrB*. Wild-type Col-0 and *rps3-1* T2 plants transgenic for empty vector or cosmid 1FB were inoculated with *P. syringae* DC3000 or *avr*-expressing derivatives at 10^5 CFU/ml. Bacterial growth was monitored over 5 days by collection of leaf punches and titration of bacteria on antibiotic plates selective for markers on the DC3000 chromosome and the plasmid carrying either *avrRpm1* or *avrB* (*11*, *12*). Data points are the means and SD from triplicate determinations. Where no SD is shown, it was smaller than the symbol.

We transformed two loss-of-function *rpm1* mutants (*rpm1-1* and *rps3-1*) (*14*) and the naturally occurring *rpm1* accession Nd-0 with these cosmids (*17*). We assessed complementation by inoculating T1 and T2 transgenics with *P. syringae* strain DC3000 [virulent on all *A. thaliana* accessions (*18*)] expressing *avrRpm1*. For complementation, we expected a hypersensitive response (HR) indicative of *RPM1* action (Fig. 2A) (*11*, *12*). Cosmids CCB and X20 defined a minimal region of 5 kb containing *RPM1* (Fig. 1C). Complementation also rendered plants resistant to DC3000 expressing *avrB* (asterisk, Fig. 1C; Fig. 2A) (*11*, *12*). We quantitated disease resistance by measuring the growth of bacteria in the plants (Fig. 2B). Transformants complementing *rpm1* alleles for triggering of an HR also restricted growth (100-fold) of DC3000 expressing either *avrRpm1* or *avrB* (*11*, *12*). These data show that *RPM1* is confined to a 5-kb region and that this region allows recognition of *P. syringae* expressing either *avrRpm1* or *avrB*.

We identified *RPM1* within the minimum complementing region by sequencing genomic DNA from Col-0 DNA and four mutant *rpm1* alleles (Fig. 3) (*19*). In 4.5 kb of wild-type DNA, we identified only one large, intronless open reading frame (ORF) of 2778 base pairs (bp) encoding a predicted protein product of 926 amino acids. This predicted mRNA size is in agreement with data from RNA blots (Fig. 4B). A sequence in the expressed sequence tag (EST) database (database accession number T44885) is identical to a

A

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1   MASATVDFGI GRILSVLENE TLLLSGVHGE IDKMKKELLI MKSFLEDTHK
51  HGGNGSTTTT TQLFQTFVAN TRDLAYQIED ILDEFGYHIH GYRSCAKIWR
101 AFHFPRYMWA RHSIAQKLGM VNVMIQSISD SMKRYYHSEN YQAALLPPID
151 DGDAKWVNNI SESSLFFSEN SLVGIDAPKG KLIGRLLSPE PQRIVVAVVG
201 MGGSGKTTLS ANIFKSQSVR RHFESYAWVT ISKSYVIEDV FRTMIKEFYK
251 EADTQIPAEL YSLGYRELVE KLVEYLQSKR YIVVLDDVWT TGLWREISIA
301 LPDGIYGSRV MMTTRDMNVA SFPYGIGSTK HEIELLKEDE AWVLFSNKAF
351 PASLEQCRTQ NLEPIARKLV ERCQGLPLAI ASLGSMMSTK KFESEWKKVY
401 STLNWELNNN HELKIVRSIM FLSFNDLPYP LKRCFLYCSL FPVNYRMKRK
451 RLIRMWMAQR FVEPIRGVKA EEVADSYLNE LVYRNMLQVI LWNPFGRPKA
501 FKMHDVIWEI ALSVSKLERF CDVYNDDSDG DDAAETMENY GSRHLCIQKE
551 MTPDSIRATN LHSLLVCSSA KHKMELLPSL NLLRALDLED SSISKLPDCL
601 VTMFNLKYLN LSKTQVKELP KNFHKLVNLE TLNTKHSKIE ELPLGMWKLK
651 KLRYLITFRR NEGHDSNWNY VLGTRVVPKI WQLKDLQVMD CFNAEDELIK
701 NLGCMTQLTR ISLVMVRREH GRDLCDSLNK IKRIRFLSLT SIDEEEPLEI
751 DDLIATASIE KLFLAGKLER VPSWFNTLQN LTYLGLRGSQ LQENAILSIQ
801 TLPRLVWLSF YNAYMGPRLR FAQGFQNLKI LEIVQMKHLT EVVIEDGAMF
851 ELQKLYVRAC RGLEYVPRGI ENLINLQELH LIHVSNQLVE RIRGEGSVDR
901 SRVKHIPAIK HYFRTDNGSF YVSLSS*
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B

rps3-1: ΔA, frameshift at R818 to GCVLHKDFRI*
rps3-2: A to T; L301F
rps3-3: T to A; Y87 stop
rps3-4: G to A; G766E

C

	P-loop:		Kinase 2a:		Conserved domain 1 (kinase 3a?):
RPM1	200 GMGGSGKTT	279	KRYIVVLDDV	307	GSRVMMTTRDMNV
RPS2	182 GPGGVGKTT	255	KRFLLLLDDV	283	KCKVMFTTRSIAL
N	216 GMGGVGKTT	294	KKVLIVLDDI	323	GSRIIITTRDKHL
L^6	266 GMGGIGKTT	342	FKILVVLDDV	297	QSRFIITSRSMRV

	Conserved domain 2:		Conserved domain 3:
RPM1	373 CQGLPLAIASLGS	431	LKRCFLYC
RPS2	345 CGGLPLALITLGG	404	LRSCFLYC
N	372 AKGLPLALKVWGS	440	DIACFLRG
L^6	387 TAGLPLTLKVIGS	446	DIACFFIG

Fig. 3. (**A**) Deduced RPM1 protein sequence (*27*). Leucine and isoleucines in the putative leucine zipper are in bold on line 1; five other domains discussed in the text are bold and underlined. The LRR region is in italics; proline residues useful in defining LRR units are in bold. Open circles above amino acid residues indicate positions of mutations in the loss-of-function alleles detailed in (**B**) (*27*, *28*). (**C**) Alignment of conserved domains among four *R* genes. Numbers refer to NH_2-terminal residue position for the respective sequences (*27*).

portion of the *RPM1* genomic sequence. We obtained this clone from the Ohio State Arabidopsis Stock Center and found its full sequence to be colinear with the Col-0 genomic sequence, with a polyadenylation sequence beginning 364 bases 3′ of *RPM1*.

The *RPM1* ORF (Fig. 3A) contains features found in the predicted polypeptide sequences of other *R* genes (*2–5*, *7*, *9*): a potential six-heptad amphipathic leucine zipper (positions 10 to 51), two motifs of a nucleotide binding site (NBS; positions 200 to 208 and 279 to 288) (*20*), and 14 imperfect leucine-rich repeats (LRRs) from position 553 (*21*). These features most closely resemble those of the *A. thaliana RPS2* gene (23% identity and 51% similarity), which confers resistance to *P. syringae* expressing *avrRpt2* (*3*, *4*). In addition to the NBS and LRRs, three other sequence blocks are shared with *RPS2*, the tobacco *N* gene, and the flax L^6 gene: a potential kinase 3a site of the NBS (positions 307 to 319), a hydrophobic stretch (positions 373 to 385) potentially involved in membrane association, and a short domain from positions 431 to 438 (aligned in Fig. 3C). Three potential N-glycosylation sites were found at positions 54, 610, and 917.

We identified mutations in four *rpm1* loss-of-function alleles (Fig. 3B) (*14*). A nucleotide deletion in codon 818 causes a frame shift and termination after 11 amino acids in *rps3-1*, which suggests a functional requirement for the COOH-terminus of *RPM1*, including the final three LRR repeat units. The leucine to phenylalanine change in *rps3-2* introduces a bulky aromatic side chain that may alter juxtaposition of the kinase 2 and kinase 3a domains in the NBS. A glycine to glutamic acid exchange in *rps3-4* introduces a charge and may disrupt the α-helical structure of an LRR unit (*21*). Finally, *rps3-3* is a nonsense mutation resulting in termination at codon 87.

Probes internal to the *RPM1* ORF (probes 1 and 2, Fig. 4A) detect neither alterations in expression level or message size nor structural changes in DNA from the mutant *rpm1* alleles. RNA blots reveal a rare mRNA of roughly 3.0 kb, which is consistent with the *RPM1* ORF size (Fig.

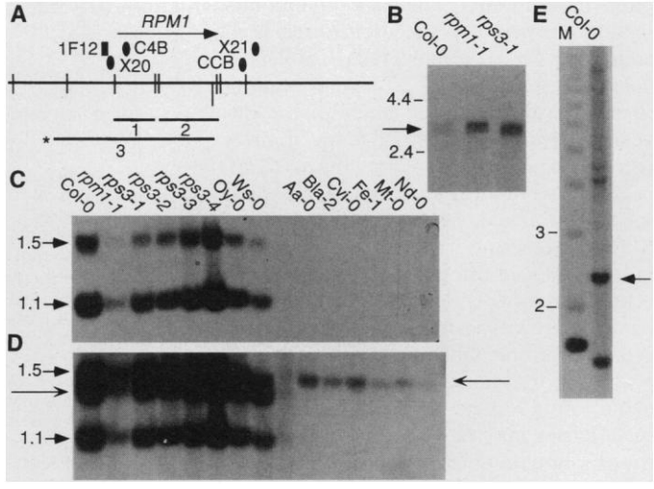

Fig. 4. Expression and genomic organization of *RPM1*. (**A**) Schematic fine map of *RPM1* showing the ORF (arrow), end points of critical cosmids (solid ovals), and the left end of YAC yUP1F12 (solid rectangle) (Fig. 1, A and C). Cosmid names are inside of end-points, and orientation is reversed with respect to Fig. 1. Restriction sites indicated are either Hind III or Xba I (double digestion releases inserts from pCLD05451). Probes 1 and 2 are 1.1-kb and 1.5-kb *RPM1* ORF-internal Hind III fragments. The Xho I site (asterisk) of the 4.5-kb Xho I–Xba I probe 3 is derived from the 1FC cosmid cloning site. (**B**) RNA blot analysis of *RPM1* mRNA (arrow). Polyadenylated RNA was isolated from leaves of wild-type accession Col-0 (2 μg) and two mutants (4 μg), and blots were hybridized with pooled probes 1 and 2, washed at high stringency (*29*), and exposed to Kodak X-AR film for 7 days. RNA size standards (in kilobases) were from Gibco-BRL. (**C** and **D**) Structure of the *RPM1* locus. Blots from Hind III–digested genomic DNA were hybridized with pooled probes 1 and 2 (*29*); exposure lasted for 30 hours (C). Hybridization was done with probe 3 (D) at high stringency; exposure lasted for 30 hours. The *rpm1-1* and Nd-0 lanes are underloaded. Accessions Col-0, Oy-0, and Ws-0 are *RPM1*, and all induced mutant alleles are Col-0. Aa-0, Bla-2, Cvi-0, Fe-1, Mt-0, and Nd-0 are all naturally occurring *rpm1* accessions (*18*, *20*). Thick arrowheads indicate *RPM1* gene fragments; thin arrowheads indicate a novel fragment present in natural *rpm1* accessions. (**E**) Low-stringency blot of Eco RI–digested Col-0 genomic DNA probed with pooled probes 1 and 2 under low-stringency conditions (*29*) with a 4-hour exposure. Arrow indicates a fragment containing the *RPM1* ORF. M is a 1-kb ladder (Gibco-BRL).

4B). Two similarly sized transcripts are produced, as observed for *RPS2* (*4*) and the tomato *Cf-9 R* gene (*6*). In contrast, six naturally occurring accessions of *A. thaliana* that are susceptible to infection with *P. syringae* expressing *avrRpm1* or *avrB* lack *RPM1* (Fig. 4C). This is unusual because common features of the *R* locus structure are multigene families and the presence of a homolog or homologs at the corresponding position in susceptible plants (*1–7*, 9). *RPM1* may be recently evolved or may have been lost in *A. thaliana* accessions through genomic instability at *RPM1*. A DNA probe extending upstream from the *RPM1* ORF (probe 3, Fig. 4A) detected a band of weaker relative intensity in the *rpm1*-null accessions that was roughly 200 bp larger than the corresponding band in *RPM1* accessions (Fig. 4D). All six *rpm1*-null accessions contained the same size band, which suggests that a single event has introduced or deleted *RPM1* from this locus. Low-stringency hybridization revealed one strongly hybridizing and several weakly hybridizing bands that may represent *RPM1*-related sequences elsewhere in the *A. thaliana* genome (Fig. 4E).

Whether *RPM1*, or any *R* gene product, directly interacts with the corresponding *avr*-dependent signal is unknown. The signal produced by *P. syringae* strains expressing either *avrRpm1* or *avrB* could be structurally similar, and the dual specificity of *RPM1* could reflect a single, or overlapping, binding site. The lack of mutants separating these two specificities seems to argue that the *A. thaliana* RPM1 molecule does not possess two *avr*-signal binding sites. However, genetic analyses of soybean cultivars reveal allelic *R* specificities recognizing *P. syringae* expressing *avrRpm1* or *avrB* or both (*22*). Alternatively, dual specificity could be a consequence of *RPM1* interacting in a pathway with signals transduced through a promiscuous receptor of *avr*-dependent signals. Plants defend themselves against a variety of pathogens and presumably maintain a large repertoire of functional *R* specificities. The evolution of *R* genes determining multiple specificities may be one way of reducing the absolute number of *R* genes required to meet these demands.

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16. J. Ecker, *Methods* **1**, 186 (1990); E. Ward and G. Jen, *Plant Mol. Biol.* **14**, 561 (1990); E. Grill and C. Somerville, *Mol. Gen. Genet.* **226**, 484 (1991). Maintenance of YAC libraries on selective medium, preparation of DNA and yeast plugs, and colony screening were done according to (*23*); CHEF gels were done according to (*24*). We probed four *A. thaliana* libraries in YACs using the closest RFLP markers, converted YAC ends to RFLP markers, and mapped recombination breakpoints among 220 informative meioses (*11*). Monomorphic YAC ends were used to isolate phage clones from a Col-0 genomic library, and these were used to define polymorphisms. Genomic DNA of yeast containing YAC clones W8A8 and W7G11 was size-fractionated (to a size of 15 to 20 kb), partially digested with Sau 3AI, and cloned into the Bam HI site of pCLD04547. W8A8 YAC DNA isolated from low melting point agarose CHEF gels by agarase digestion (New England Biolabs) was digested with Hinf I, labeled, and used to select cosmid clones containing *Arabidopsis* inserts by colony hybridization. Cosmids were ordered with the use of various end probes and by labeling of cosmids themselves after Hind III–Xba I digestion.
17. N. Bechtold, J. Ellis, G. Pelletier, *C. R. Acad. Sci. Paris* **316**, 1194 (1993). Cosmids transformed into *Agrobacterium tumafaciens* strain GV3101 carrying helper plasmid pMP90 (*25*) were streaked onto selective yeast extract peptide plates for 2 days at 28°C. Selective Luria broth (LB) medium (500 ml) was inoculated with 3 ml of single-clone cultures grown for 18 hours in LB under antibiotic selection. Cells were harvested after 18 hours at 28°C and resuspended in infiltration medium [0.5x Murashige-Skoog salts; 1x B5 vitamins; 5.0% sucrose; 0.044 μM benzylaminopurine (10 ml of 1 mg/ml stock in dimethyl sulfoxide)] to an optical density at 600 nm of 0.8 to 1.2. *Agrobacterium* strains were DNA blotted and analyzed for lack of cosmid rearrangement before transformation. Vacuum infiltration modified from (*3*) is available on request. After transformation, T1 seeds (10% of total) were surface-sterilized and transformants were selected on MS medium containing kanamycin at 50 μg/ml, Cefotaxime (200 μg/ml), and Rovral (Rhone-Poulenc; 20 mg/ml). Transformed plants (four to five Kan^R transformants per infiltrated plant) were selected for 10 days on MS-kanamycin (50 μg/ml) plates, transferred to potting mix, and inoculated after 5 weeks of growth in 9-hour days.
18. M. Whalen, R. Innes, A. Bent, B. Staskawicz, *Plant Cell* **3**, 49 (1991). Strains, plasmids carrying *avr* genes, and techniques are described in (*11*, *12*).
19. Nested deletions (Erase-a-base; Promega) from 1.1- and 1.5-kb Hind III fragment subclones (cosmid X20) and a 4.5-kb Xho I–Xba I subclone (cosmid 1FC) in pBS-SK were sequenced on both strands with the use of T7 DNA polymerase and α-^{35}S-labeled deoxyadenosine triphosphate. The 3′ sequence was determined from a 7.5-kb Xho I–Xho I clone (cosmid CCB) using new primers to extend the existing sequence (primer walking). We amplified *rpm1* alleles as five separate, overlapping fragments ranging in length from 669 to 953 bp from genomic *rps3-1*, *rps3-2*, *rps3-3*, and *rps3-4* DNA (*14*) by means of the polymerase chain reaction (PCR). Primer pairs included a T3 RNA-polymerase promoter sequence (5′-AATTAACCCTCACTAAG-3′) at the 5′ end of one primer and a T7 RNA-polymerase promoter sequence (5′-TAATACGACTCACTATAGGG-3′) at the 5′ end of the other, which allowed direct sequencing with T3 and T7 sequencing primers. Pooled products of four independent PCR reactions were purified by filtration (Millipore Ultrafree-MC filter unit; cutoff, 30,000 daltons), and 250 ng was used as a template (SequiTherm Long Read Cycle Sequencing Kit, Epicentre Technologies, Madison, WI) with IRD41 end-labeled T3 or T7 sequencing primers (LiCor, Lincoln, NE) on a LiCor 4000L DNA sequencer. Mutations were confirmed on both strands. The GenBank accession number of the nucleotide sequence of *RPM1* is X87851.
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27. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
28. Four new alleles have been sequenced since submission of this paper: *rps3-5* (EMS-B38) [Gly^{384} → Arg^{384} (GGA to AGA)]; *rps3-6* (EMS-B61) [Leu^{186} → Phe^{186} (CTT to TTT)]; *rps3-7* (ENU-B7) [Pro^{498} → Ser^{498} (CCC to TCC)]; and *rps-3-8* (ENU-B8) [(Asn^{812} → Ile^{812} (AAT to ATT)] (P. Mowery and R. W. Innes, unpublished data). An asterisk in the sequence is a stop.
29. Hybridization was done in 5x standard saline citrate (SSC), 5x Denhardt's solution, 0.1% SDS, and herring sperm DNA (100 μg/ml) unless otherwise stated. Membranes (HyBond N, Amersham) were washed in 2x SSC and 0.1% SDS at 65°C for 30 min, then in 1x SSC and 0.1% SDS at 65°C for 30 min. Low-stringency blots were prehybridized and hybridized in 0.5 M Na_2HPO_4 buffer (pH 7.2), 1% bovine serum albumin, 1 mM EDTA, and 7% SDS for 16 hours at 55°C. Washings were done twice for 20 min at 50°C in 40 mM Na_2HPO_4 buffer (pH 7.2), 1 mM EDTA, and 5% SDS, followed by two more washes under the same conditions but with only 1% SDS. All other standard techniques were as described in F. Ausubel *et al.*, *Current Protocols in Molecular Biology* (Wiley, New York, 1987) and in J. Sambrook, S. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), vol. 2.

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