damine and 5-carboxyfluorescein absorb) were fit to the weighted sum of standard spectrum of a duplex labeled only with donor $F^D(\lambda_{em},490)$ and the fluorescence signal of the sample $F(\lambda_{em},560)$ excited at 560 nm (where only 5-carboxytetramethylrhodamine absorbs)

$$F(\lambda_{em},490) = c \cdot F^D(\lambda_{em},490) + (ratio)_A \cdot F(\lambda_{em},560) \quad (2)$$

c and $(ratio)_A$ are the fitted weighting factors of the two spectral components. The fit was made in the range λ_{em} = 500 to 540 nm (where only D emits) and λ_{em} = 570 to 650 nm (where both D and A emit). $(ratio)_A$ is the acceptor fluorescence signal of the FRET measurement normalized by $F(\lambda_{em},560)$ as shown in Eq. 4 (*34*).

$$(ratio)_A = \frac{F(\lambda_{em},490) - c \cdot F^D(\lambda_{em},490)}{F(\lambda_{em},560)} = E \cdot \frac{\varepsilon^D(490)}{\varepsilon^A(560)} + \frac{\varepsilon^A(490)}{\varepsilon^A(560)} \quad (3)$$

$(ratio)_A$ is linearly dependent on the efficiency of transfer E; it normalizes the sensitized FRET signal for the quantum yield of 5-carboxytetramethylrhodamine, for the concentration of the duplex molecule, and for any error in percentage of acceptor labeling. ε^D and ε^A are the molar absorption coefficient of D and A at the given wavelength. The ratio of absorption coefficient $\varepsilon^D(490)/\varepsilon^A(560)$ = 0.43 was determined from the absorbance spectra of the doubly labeled molecules and $\varepsilon^A(490)/\varepsilon^A(560)$ = 0.08 was determined from the excitation or absorbance spectrum of a singly 5-carboxytetramethylrhodamine-labeled molecule.

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21. Sequences of hammerhead riboxymes and substrates. S and R represent complementary sets of oligoribonucleotides where either R or S carries both of the dyes. See also reference for RNA duplexes (*15*).

Construct **A**: SA11, RA11, 5′-GGG CUC UGA UGA GCG CAA GCG AAA CUC C; SA12, RA12, 5′-GGG CCU CUG AUG AGC GCA AGC GAA ACU CC; SA13, RA13, 5′-GGG UCC UCU GAU GAG CGC AAG CGA AAC UCC; SA14, RA14, 5′-GGG CUC CUC UGA UGA GCG CAA GCG AAA CUC C; SA15, RA15, 5′-GGG UCU CCU CUG AUG AGC GCA AGC GAA ACU CC; SA16, RA16, 5′-GGG CUC UCC UCU GAU GAG CGC AAG CGA AAC UCC; SA17, RA17, 5′-GGG UCU CUC CUC UGA UGA GCG CAA GCG AAA CUC C. **B**: RB13, 5′-Fl-GGA CCG AAA CCC C-Rh, SB13, 5′-GGG GUdC AGG ACC GCA AGG UCC UCU GAU GAG GUC C; RB14, 5′-Fl-GGA CCG AAA CUC CC-Rh, SB14, 5′-GGG AGU dCAG GAC CGC AAG GUC CUC UGA UGA GGU CC; RB15, 5′-Fl-GGA CCG AAA CUG CCC-Rh, SB15, 5′-GGG CAG UdCA GGA CCG CAA GGU CCU CUG AUG AGG UCC; RB17, 5′-Fl-GGA CCG AAA CUG UGC CC-Rh, SB17, 5′-GGG CAC AGU dCAG GAC CGC AAG GUC CUC UGA UGA GGU CC. **C**: RC18, SC18, 5′-GGG CCG AAA CUG CCG CAA GGC AGU dCAC CUC C. **D**: RD20, 5′-Fl-GGA GCG UCU GAU GAG GGC CC-Rh, SD20, 5′-GGG CCC GAA ACU GCC GCA AGG CAG UdCA CGC UCC.

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35. We thank R. Clegg and G. Vámosi, Göttingen, for discussions and advice, U. Kutzke, G. Heim, and A. Zechel, Göttingen, for technical assistance, A. Heaton, B. Patel, and C. S. Vörtler, Göttingen, for critical reading of the manuscript. Supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie (F.E.) and an EEC grant (E.W.). Coordinates of the P atoms have been deposited at the Brookhaven Protein Data Bank.

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Isolation of the Tomato *Cf-9* Gene for Resistance to *Cladosporium fulvum* by Transposon Tagging

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The tomato *Cf-9* gene confers resistance to infection by races of the fungus *Cladosporium fulvum* that carry the avirulence gene *Avr9*. The *Cf-9* gene was isolated by transposon tagging with the maize transposable element *Dissociation*. The DNA sequence of *Cf-9* encodes a putative membrane-anchored extracytoplasmic glycoprotein. The predicted protein shows homology to the receptor domain of several receptor-like protein kinases in *Arabidopsis*, to antifungal polygalacturonase-inhibiting proteins in plants, and to other members of the leucine-rich repeat family of proteins. This structure is consistent with that of a receptor that could bind Avr9 peptide and activate plant defense.

Plants can defend themselves against infection by viruses, bacteria, fungi, nematodes, insects, and even other plants. Plant defenses are often activated by specific interaction between the product of a disease resistance (*R*) gene in the plant and the product of a corresponding avirulence (*Avr*) gene in the pathogen (*1*). Without either of these genes, plant defenses are not activated and infection by the pathogen is permitted. To understand how specific plant defense is regulated, it is necessary to learn the nature of the *R* and *Avr* gene products, the way they interact, and the chain of events that results.

In the interaction between tomato (*Lycopersicon esculentum*) and the leaf mould fungus *Cladosporium fulvum*, the avirulence gene *Avr9* has been characterized (*2*). *Avr9* specifies a 28–amino acid secreted peptide that elicits a necrotic response when injected into tomato plants carrying the *Cf-9* resistance gene. We have now isolated *Cf-9* by transposon tagging, using a maize *Activator-Dissociation* element (*Ac-Ds*)–based system to target a specific gene from tomato. To tag *Cf-9*, we used a transgenic tomato line (*3*) carrying a *Ds* element located 3 centimorgans (*4*) from the *Cf-9* locus, which had previously been mapped to the short arm of chromosome 1 (*5*). To activate this *Ds* element, we used a genetically unlinked stabilized *Ac* (*sAc*), itself incapable of transposition (*6*). Appropriate crosses and selections were carried out (Fig. 1A) to produce plants heterozygous for *Ds* and *sAc* and homozygous for *Cf-9*. These plants were crossed to plants lacking *Cf-9* but homozygous for an *Avr9* transgene (*7*, *8*) (Fig. 1A). The progeny of this cross, which were heterozygous for *Cf-9* and *Avr9*, became necrotic and died shortly after seed germination, but those mutant for *Cf-9* survived (Fig. 1B).

Approximately 160,000 progeny were germinated (Fig. 1B) and 118 survivors were recovered. Of these, 65 arose by clonal propagation of 10 independent mutations (*8*). The remaining 53 arose independently, giving a total of 63 independent mutations. Of these, 21 were variegated for necrosis (Fig. 1C) and carried both *Ds* and *sAc*, 33 were stable and carried *Ds*, and 9 were stable but did not carry *Ds*. In addition to the 21 variegated mutations that were inferred to carry *Ds* insertions in *Cf-9*, 16 more were identified among the stable mutants by activation with *sAc*, which suggests a total of at least 37 independent *Ds* inser-

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tions into *Cf-9*. Of these, 28 have been mapped to the same 3-kb region of the tomato genome (Fig. 2). All stable mutants tested were susceptible to race 5 of *C. fulvum*, which indicates concordance between the loss of response to the *Avr9* transgene and loss of resistance to a race of the fungus carrying *Avr9*. The correlation between multiple independent mutations of *Cf-9* and multiple independent *Ds* insertions in a defined region, together with the *sAc*-dependent instability of these mutations, provides strong evidence that the *Cf-9* gene has been tagged.

Mutant 18 (Fig. 2), carrying a single stable *Ds*, was used to isolate *Cf-9* by plasmid rescue (*9*). Analysis of the flanking genomic sequence suggested that *Cf-9* contains an uninterrupted open reading frame (ORF) encoding a protein of 863 amino acids. Five complementary DNA (cDNA) clones with sequences identical to the *Cf-9* genomic sequence were identified from a Cf9 cDNA library (*10*). The predicted protein (Fig. 3) contains seven structural domains. The NH_2-terminal domain A (23 amino acids) is consistent with a signal peptide (*11*). Domain B, the presumed NH_2-terminus of the mature protein, contains several cysteines. Domain C consists of 28 imperfect copies of a 24–amino acid leucine-rich repeat (LRR) that has been shown in many organisms to be important in protein-protein interaction. Domain D (28 amino acids) has no conspicuous features. Domain E (18 amino acids) is very acidic, with 10 negatively charged residues and no positively charged residues. The hydrophobic domain F (37 amino acids) is consistent with a transmembrane domain. The COOH-terminal domain G (21 amino acids) is very basic, with eight positively charged residues and only two negatively charged residues. The COOH-terminus concludes with the residues KKRY (*12*). In animals and yeast, the COOH-terminating motif KKXX (where X is any amino acid) (*12*) functions as a signal for retrieval of

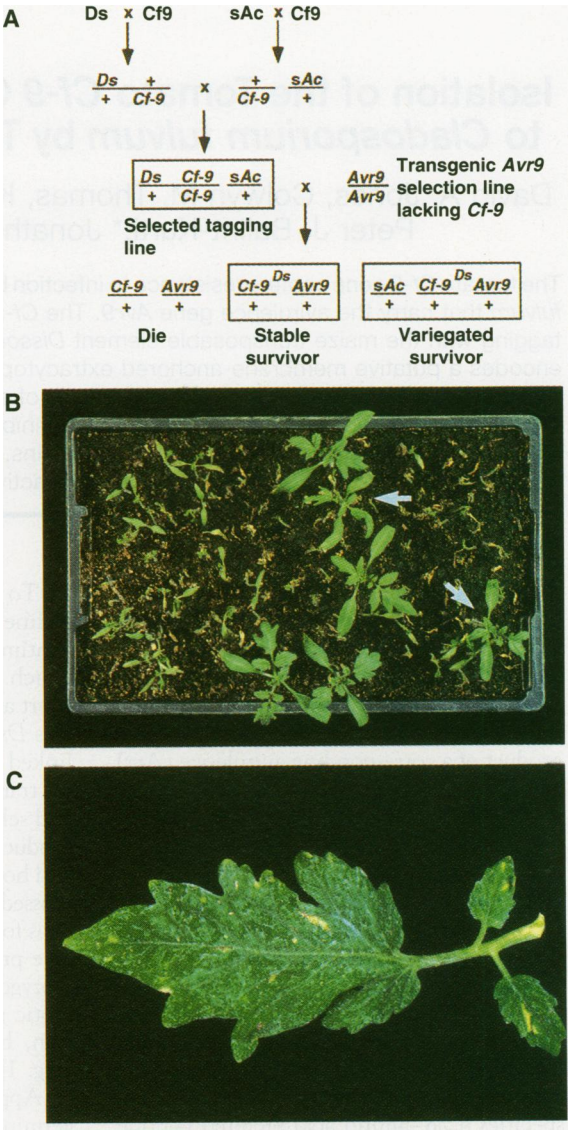

Fig. 1. *Cf-9* tagging experiments. (**A**) Breeding experiments to obtain tagging parents heterozygous for *Ds* and *sAc* and homozygous for *Cf-9* were initiated with the use of a *Ds* line, AAT6514-33 (*3*); a *sAc* line, SLJ10512A (*6*); and Cf9, a line homozygous for *Cf-9*. To tag *Cf-9*, the tagging parents were crossed as female parents to the tomato line SLJ6201B (*7*), which is homozygous for the *Avr9* transgene and lacks *Cf-9*. Some of the predicted outcomes of the tagging experiment are indicated. (**B**) Germinating seedlings from a single fruit generated in experiment one (*8*) show six survivors, two of which are variegated (arrow), on a background of dead or dying seedlings. DNA analysis showed the six mutants to have been derived from the same *Ds* insertion in *Cf-9* (*4*), one of 10 clonally represented mutation events recovered. (**C**) Leaflet of a mutant plant variegating for necrosis, which is consistent with restoration of *Cf-9* function due to *sAc*-induced somatic excision of *Ds* from *Cf-9* and provides evidence for the cell-autonomous expression of *Cf-9*.

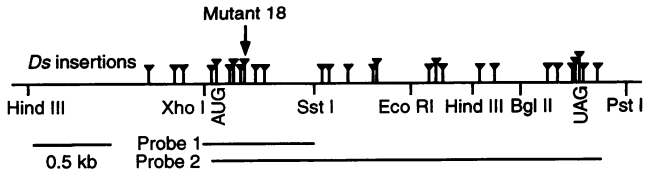

Fig. 2. PCR-generated map of *Ds* insertions in *Cf-9*. Specific *Cf-9* primers were used in conjunction with *Ds* primers to map the *Ds* insertions on the basis of PCR product size. Positions of *Ds* insertions (above the line) are shown relative to the restriction map encompassing *Cf-9* (below the line). Positions of the translation start (AUG) and stop (UAG) codons are indicated. The extents of *Cf-9* probe DNAs (*9, 10*) used in hybridization analyses are also indicated.

Fig. 3. Primary structure of the Cf-9 protein. The amino acid sequence (*12*) predicted from the DNA sequence of *Cf-9* (GenBank accession number U15936) has been divided into seven domains (A to G) as described in the text. In domain C, the conserved L of the LRRs is often replaced by I, F, or V, and occasionally by M (particularly the first L of each repeat), and the conserved I by L, F, or V. These are highlighted in blue and other conserved amino acids in red. In domain E, the acidic amino acids are highlighted in green. In domain G, the basic amino acids are highlighted in purple. N-glycosylation sites are underlined.

membrane-bound proteins from the Golgi apparatus to the endoplasmic reticulum (*13*). Domains E, F, and G are consistent with a transmembrane domain flanked by charged anchoring domains—positively charged on the cytoplasmic side and negatively charged on the extracytoplasmic side (*14*). Twenty-two N-glycosylation sites are distributed among domains B, C, and D. The Cf-9 protein therefore appears to be an extracytoplasmic glycoprotein anchored to a cell membrane, with the majority of the extracytoplasmic domain made up of LRRs.

Cf-9 was introgressed into tomato from *L. pimpinellifolium*. *Cf-9* probe 1 (Fig. 2) was hybridized to DNA gel blots of genomic DNA from the tomato cultivar Moneymaker (line Cf0), from a near-isogenic line carrying *Cf-9* (line Cf9), and from *L. pennellii* (Fig. 4A). There are at least 11 major hybridizing bands in Cf9, several of them unique, which suggests that they are on the introgressed segment of DNA that carries *Cf-9*. This has been confirmed by the crossing of Cf9 with *L. pennellii* and examination of the segregation of *Cf-9*–homologous bands among the F_2 progeny. Three bands, including the 6.7 kb Bgl II *Cf-9* band (*9*), cosegregate with *Cf-9*, whereas others were linked either distally or proximally (*4*). Therefore, *Cf-9* appears to be a member of a small, clustered multigene family, which is consistent with the genetic complexity proposed for this locus (*5*) and observed for resistance loci in other plant species (*15, 16*). The disease-sensitive Cf0 and *L. pennellii* lines show no more than five or six major hybridizing bands. Differences in copy number could be a consequence of gene duplication and unequal crossing-over, which is consistent with proposed models for the evolution of complex resistance loci (*1, 15*).

Cf-9 probe 2 (Fig. 2) was hybridized to RNA gel blots of mRNA from lines Cf0 and Cf9 (Fig. 4B). A single band of about 3 kb, a size consistent with the size of the *Cf-9* cDNA clones, was observed in both lines. The similar hybridization intensity, broadness of the bands, and a slight but consistently reduced mobility in Cf9 suggest similar families of transcripts with only minor differences in size both within and between the two lines.

Cf-9 was found to be homologous to many members of the LRR superfamily of proteins (*17*). Two classes of plant proteins carrying LRRs were the most homologous to Cf-9: (i) the receptor-like protein kinases (RLPKs) RLK5, TMK1, and a kinase-defective homolog TMKL1 from *Arabidopsis* (*18*); and (ii) antifungal polygalacturonase-inhibiting proteins (PGIPs) from several plants (*19*) (Table 1). Although the homology detected in these proteins is due mainly to the LRRs, there is also homology with domains B and D outside the LRRs of Cf-9 (Fig. 5).

The 24–amino acid LRRs of these extracellular proteins differ from the predominantly 23–amino acid LRRs of intracellular proteins (*4*) by the insertion of a glycine in the consensus sequence LXXLXXLXXLX-LXXNXLXXIPXX (where X is any amino acid) (*12*), to produce the consensus sequence LXXLXXLXXLXXNXLXGXIP-XX in plants, or by the insertion of a leucine or alanine to produce the consensus sequence (L/A)LXXLXXLXXLXXNXLX-XIPXX in other species (Table 1). Apart from some N-glycosylation sites, there are few conserved amino acids interstitial to the LRR backbone, except among the PGIPs (*4*). Complete sequence alignments are possible for the PGIPs, because the number of LRRs, the location of several N-glycosylation sites, and some of the sequence that is interstitial to the LRRs is conserved, but this is not so for Cf-9 and the RLPKs. Clearly, Cf-9 shares an evolutionary relation with the PGIPs and RLPKs but has diverged considerably with respect to (i) the number and interstitial sequences of its LRRs, (ii) its attachment to a cell membrane as compared with the PGIPs' lack of attachment, and (iii) its lack of a cytoplasmic serine-threonine protein kinase domain as compared with the RLPKs, which suggests that it may also have diverged functionally.

Recently, the *Arabidopsis RPS2* gene for

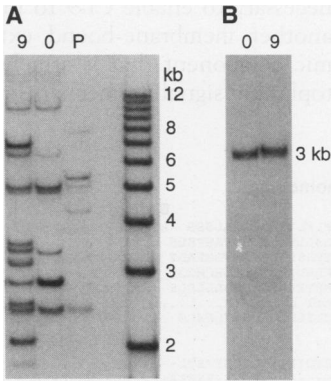

Fig. 4. DNA and RNA gel blots probed with *Cf-9*. (**A**) Genomic DNAs (3 μg) from the tomato cultivars Moneymaker (0), the Moneymaker near-isogenic line Cf9 (9) carrying *Cf-9*, and *L. pennellii* (P) were digested with Bgl II, electrophoretically separated on a vertical 1% agarose gel, and capillary-blotted onto a Hybond N filter. The filter was hybridized with the [^{32}P]dCTP-labeled probe 1 (Fig. 2) and washed with high stringency at 65°C in 0.2 × standard saline citrate and 0.1% SDS. (**B**) Polyadenylated RNAs (10 μg) from mature unchallenged leaves of the tomato cultivars Moneymaker (0) and Cf9 (9) were electrophoretically separated on a 1.4% agarose gel and capillary-blotted onto a Hybond N filter. The filter was probed with [^{32}P]dCTP-labeled probe 2 (Fig. 2) and washed with high stringency at 65°C in 0.1× saline sodium phosphate EDTA and 0.1% SDS.

Table 1. Comparison of the extracytoplasmic LRRs of Cf-9, PGIPs, and several membrane-spanning LRR proteins (*12*). The consensus LRR is shown for each protein, with frequent amino acid substitutions indicated below (dashes indicate any amino acid). Abbreviations: GP, platelet glycoprotein; LH, leutenizing hormone; CG, choriogonadotropin; MA, membrane-anchored transmembrane proteins with only a very short cytoplasmic domain; S, secreted; TM, transmembrane proteins with substantial cytoplasmic domains; 7M, seven membrane-spanning domains; PGs, polygalacturonases; vWf, von Willebrand factor; ser-thr PK, serine-threonine protein kinase; IL-1R, interleukin-1 receptor; and ABP, actin-binding protein.

Protein	LRR consensus	Repeat number	Membrane association	Ligand	Signaling mechanism	Reference
Cf-9	L--L--L--LDLSSNNL-G-IPS- F N F	28	MA	Avr9?	Unknown	This work
PGIPs	L--L--L--L-LS-N-L-G-IP--	10	S	Fungal PGs	–	(*19*)
RLK5	L--L--L--L-L--N-LSG-IP--	21	TM	Unknown	Ser-thr PK	(*18*)
TMK1	L--L--L--L-L--N-L-G-IP--	13	TM	Unknown	Ser-thr PK	(*18*)
TMKL1	L--L--L-SL-L--N-LSG-LP--	7	TM	Unknown	Unknown	(*18*)
Toll (*Drosophila*)	LF-H--NL--L-L--N-L--LP--	15	TM	Activated spätzle protein	IL-1R–like	(*25*)
GP 1bα (mammal)	LL--LP-L--L-LS-N-LTTLP-G	7	TM	Activated vWf	ABP binding	(*26*)
LH-CG receptor (mammal)	AF--L-----L-IS---L--LP-- L I	12	7M	LH,CG	G-protein–coupled	(*27*)

resistance to *Pseudomonas syringae* and the tobacco *N* gene for resistance to tobacco mosaic virus have been reported to carry LRRs (*20*). However, these LRRs are not well conserved, are quite variable in length, and lack the conserved glycine of plant extracytoplasmic LRRs. N is suggested to be a cytoplasmic protein and RPS2 may also be cytoplasmic. N and RPS2 are more homologous to one another than to Cf-9. Conceivably, there are two distinct classes of *R* genes with LRRs; those like *Cf-9*, which have extracellular ligands, and those like *N* and possibly *RPS2*, which have intracellular ligands.

The LRR domains of a number of receptors have been shown to bind protein ligands (*21*) (Table 1). The presence of LRRs in Cf-9 is therefore consistent with the idea that Cf-9 acts as a receptor for a specific protein ligand, most likely the Avr9 peptide. However, the LRR region of Cf-9 seems excessively large for binding of the 28–amino acid Avr9 peptide to be its sole function. An Avr9-binding domain may occupy only a small portion of the LRR region, with the remaining LRRs providing structure to the protein. Alternatively, the large LRR domain could reflect interaction with other Cf-9 molecules or with another plant protein, perhaps one binding Avr9.

The LRR motif is a receptor module that has been combined with several different cytoplasmic signaling mechanisms (Table 1). Unlike these receptors, Cf-9 does not have any obvious cytoplasmic signaling capacity. However, Cf-9 could generate a cytoplasmic signal by interaction between its transmembrane domain or cytoplasmic tail and a cytoplasmic signaling mechanism. Structurally, Cf-9 resembles the membrane-bound receptor domain of RLPKs but lacks the protein kinase domain. Conversely, the tomato *Pto* gene for resistance to *Pseudomonas syringae* pv. *tomato* encodes a protein kinase (*16*) resembling the membrane-bound kinase domain of RLPKs but lacking the receptor domain. Cf-9 and Pto might represent components of receptor and signaling mechanisms that are analogous to T cell activation by CD4, which has an external receptor domain, a transmembrane domain, and a 38–amino acid cytoplasmic domain (*22*). This cytoplasmic domain interacts with a tyrosine protein kinase, $p56^{lck}$, that is attached to the membrane by an NH_2-terminal myristoylation site (*23*), as postulated for the Pto kinase (*16*). Alternatively, the cytoplasmic tail of Cf-9 may have no interactive function at all but may be simply an anchoring domain required for attachment of an extracytoplasmic protein to the membrane. Membrane attachment may be necessary to enable Cf-9 to interact with another membrane-bound extracytoplasmic component that is attached to a cytoplasmic signaling mechanism.

Additional genes, *Rcr-1* and *Rcr-2*, are needed for full Cf-9 function (*24*) and might encode proteins that cooperate with Cf-9 in the production of a defense activation signal. The next challenge is to discover the nature of the proteins that cooperate with Cf-9 to activate plant defenses in response to Avr9.

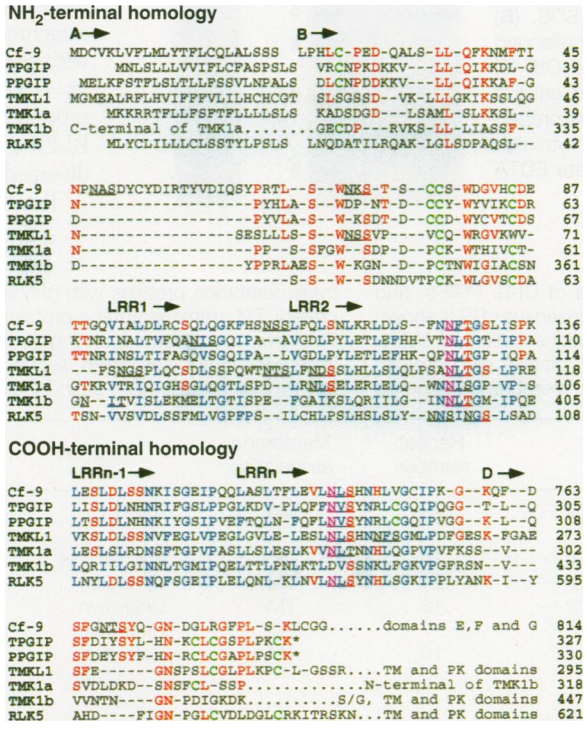

Fig. 5. Sequence alignments between Cf-9 and homologous proteins. Alignments for the NH_2- (domains A, B, and part of C) and COOH-termini (domain D and part of C) of Cf-9 and the PGIPs (*19*), represented by tomato (T) and pear (P) PGIPs and the RLPKs (*18*). TMK1 has two regions of homology with the NH_2-terminus of Cf-9, which is consistent with an NH_2-terminal duplication. The first, located at the NH_2-terminus, is designated TMK1a, and the second, located between two blocks of LRRs and preceded by a short stretch of hydrophobic residues, is designated TMK1b. The site of signal peptide cleavage (indicated by a gap) is similar throughout. Cysteines conserved at the NH_2-terminus but not at the COOH-terminus of Cf-9 are highlighted in green. The conserved LRR1 is truncated, begins with V or I rather than L, and lacks the characteristic N as compared with the other LRRs. The LRR backbone, LXXLXXLXXLXLXXNXLXGXIPXX (*12*), in which L is often replaced by I, F, or V, and I by L, F, or V, is highlighted in blue. Other sequence identities are highlighted in red. N-glycosylation sites are underlined and those conserved in LRR2 and LRRn are highlighted in purple. Abbreviations: Tm, transmembrane: PK, protein kinase; S/G, serine-glycine–rich.

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8. Two tagging experiments were done. In experiment one, two female tagging parents were increased to 19 by propagation of cuttings. Fruits from crosses done on up to 10 fruiting trusses per plant were harvested, and seed from each fruit was kept and sown separately to enable detection of clonally represented mutation events. In experiment two, 140 tagging parents were used. Fruits from crosses done on up to four fruiting trusses per plant were harvested, and seed from each fruit truss was kept and sown separately as above.
9. To isolate *Cf-9*, genomic DNA from mutant 18 was cut with Bgl II, which does not cut within the *Ds* element, then recircularized with T4 DNA ligase and used to transform *Escherichia coli* by electroporation. The *Ds* element contained an origin of DNA replication and a chloramphenicol resistance gene, both functional in *E. coli* (*3*). The plasmid recovered, pM18B, carried 6.7 kb of tomato DNA, including 2066 base pairs (bp) of the 5' end of an ORF truncated at the 3' end by the Bgl II site used for plasmid rescue and interrupted by the *Ds* element 93 bp 3' to the start codon. The remainder of the gene was recovered by plasmid rescue with the use of Xba I, which cuts within the *Ds* element but still allowed rescue of one end of the *Ds* and its flanking tomato DNA, up to an Xba I site about 14.5 kb 3' of the Bgl II site. The plasmid recovered, pM18X, carried an additional 522 bp of ORF 3' of the Bgl II site. The two regions of tomato DNA flanking the *Ds* in pM18B were subcloned in both orientations as Bam HI–Bgl II fragments, with the use of Bam HI sites just inside either end of the *Ds*, into the Bam HI site of a pBluescript polylinker. Deletion derivatives were created by restriction endonuclease digestion and religation with the use of sites within the pBluescript polylinker and the cloned DNA. These were sequenced with pBluescript or *Ds* primers. Sequencing over the restriction sites used for making deletions, over sequence gaps, and over the 3' end of the ORF contained in pM18X was completed with specific primers. The region spanning the site of the *Ds* insertion was polymerase chain reaction (PCR) amplified from *Cf-9* by means of flanking primers—one 5' of the Xho I site and the other 3' of the Sst I site. The PCR product was digested with Xho I and Sst I and cloned into pBluescript to generate pCf9XS, which contained the insert used as probe 1 (Fig. 2 and Fig. 4A). DNA sequencing confirmed the inferred target sequence.
10. RNA was prepared (*6*) from the cotyledons and first leaves of 400 14-day-old, glasshouse-grown Cf9 seedlings that had been injected with a concentration of crude Avr9 peptide that was one-quarter of the minimum concentration required to induce ne-

crosis within 24 hours. Eighty seedlings were harvested 0, 3, 6, 12, and 24 hours after injection. An equal mass of total RNA taken at each time point was pooled, and polyadenylated [poly(A)$^+$]RNA was prepared by oligo(dT) cellulose chromatography. An Amersham cDNA cloning kit was used to prepare and clone cDNA as described by the manufacturer. Eco RI adapters were ligated to double-stranded cDNA and subsequently ligated to Eco RI–digested arms of the phage vector λgt10. The phage was packaged and used to infect *E. coli* C600 cells. Approximately 5×10^5 recombinant plaques were transferred to Hybond N$^+$ membranes and screened with probe 1 (*9*). Five *Cf-9* cDNA clones were recovered, and the full-length inserts were cloned as Bam HI fragments into pUC119 and sequenced with primers derived from the genomic sequence of *Cf-9* and with primers flanking the polylinker cloning site of pUC119. The full-length insert of one of these clones was used as probe 2 (Fig. 2 and Fig. 4B).

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Interaction of a Protein Phosphatase with an *Arabidopsis* Serine-Threonine Receptor Kinase

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A protein phosphatase was cloned that interacts with a serine-threonine receptor–like kinase, RLK5, from *Arabidopsis thaliana*. The phosphatase, designated KAPP (kinase-associated protein phosphatase), is composed of three domains: an amino-terminal signal anchor, a kinase interaction (KI) domain, and a type 2C protein phosphatase catalytic region. Association of RLK5 with the KI domain is dependent on phosphorylation of RLK5 and can be abolished by dephosphorylation. KAPP may function as a signaling component in a pathway involving RLK5.

Many signal transduction pathways involved in the control of cell proliferation and differentiation originate with transmembrane receptors containing cytoplasmic protein kinase domains. Although much of the research done has focused on receptor tyrosine kinases (RTKs) (*1*), receptor serine-threonine kinases have been identified as well. These include the transforming growth factor β and activin receptor superfamily (*2*) and all known receptor-like kinases from higher plants (*3*, *4*). We report here the identification of a protein phosphatase that interacts with the phosphorylated form of a putative plant receptor serine-threonine kinase. This interaction is reminiscent of the mechanisms by which RTKs activate cellular signaling events. The signaling process of RTKs includes recognition of a polypeptide ligand, dimerization of the receptor, and autophosphorylation of tyrosine residues in the cytoplasmic portion of the molecule. These phosphorylated tyrosines with their flanking amino acids serve as high-affinity binding sites for cellular proteins containing Src homology 2 (SH2) domains (*5*). Therefore, activation of RTKs leads to the formation of protein complexes at the plasma membrane that are capable of transmitting signals to the next molecule in the signal cascade.

The *RLK5* gene from *Arabidopsis thaliana* encodes a protein with features characteristic of the polypeptide growth factor receptor kinases: a large NH_2-terminal extracellular domain, a single transmembrane domain, and a COOH-terminal protein kinase catalytic domain (*3*). The protein kinase domain of *RLK5*, when expressed as a fusion protein in *Escherichia coli*, autophosphorylates exclusively on serine and threonine residues (*6*).

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To identify components of a signal transduction pathway involving RLK5, interaction cloning (*7*, *8*) was used. An *Arabidopsis* complementary DNA (cDNA) expression library was screened for proteins that interact with the protein kinase catalytic domain of RLK5 (RLK5CAT). The probe used in this filter-binding assay was a glutathione-S-transferase (GST)–RLK5CAT fusion protein labeled with ^{32}P at a protein kinase A recognition site at the junction of the fusion (*8*). A positive clone was purified, subcloned, and sequenced (*9*). The cDNA insert encodes a 239–amino acid domain referred to as the KI domain. Sequence comparison has not revealed any strong homologies with previously reported sequences (*10*).

The possibility that interaction between the KI domain and RLK5 is phosphorylation-dependent was explored by in vitro binding studies. Analyses of protein-protein interaction on membrane filters (*11*) demonstrated that the KI domain is capable of binding to RLK5CAT, which is autophosphorylated in *E. coli* (*6*). Treatment with a type 1 serine-threonine protein phosphatase, ZmPP1 (*12*), abolished the interaction, whereas phosphatase treatment in the presence of okadaic acid, an inhibitor of type 1 protein phosphatases, did not interfere with the interaction. Furthermore, the KI domain does not bind a mutant form of RLK5 that is incapable of autophosphorylation (Fig. 1). These results show that association of the KI domain with RLK5 requires phosphorylation. Furthermore, the KI domain does not indiscriminately bind phosphoproteins, including ^{32}P-GST, or an autophosphorylated receptor-like kinase from *Zea mays* (*13*).

Full-length cDNA clones were identified by screening of an *Arabidopsis* cDNA library with a nucleic acid probe corresponding to the KI domain. The full-length cDNA encodes a 582–amino acid protein with a predicted molecular weight of 65 kD (Fig. 2A). The NH_2-terminus