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# Map-Based Cloning of a Protein Kinase Gene Conferring Disease Resistance in Tomato

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**The *Pto* gene in tomato confers resistance to races of *Pseudomonas syringae* pv. *tomato* that carry the avirulence gene *avrPto*. A yeast artificial chromosome clone that spans the *Pto* region was identified and used to probe a leaf complementary DNA (cDNA) library. A cDNA clone was isolated that represents a gene family, at least six members of which genetically cosegregate with *Pto*. When susceptible tomato plants were transformed with a cDNA from this family, they were resistant to the pathogen. Analysis of the amino acid sequence revealed similarity to serine-threonine protein kinases, suggesting a role for *Pto* in a signal transduction pathway.**

Gene-for-gene interactions, in which plant disease resistance involves a single resistance (*R*) gene in the plant that responds specifically to a single avirulence gene in the pathogen, have been described for numerous plant-pathogen pairs (*1*). Disease susceptibility results if either the plant *R* gene or the pathogen avirulence gene is absent from the interacting organisms. Avirulence genes corresponding to specific *R* genes have been cloned from bacterial and fungal pathogens (*2*). However, a lack of knowledge about the products of *R* genes has made their isolation difficult and has hindered efforts to examine their role in the recognition and defense against specific pathogens.

Tomato, *Lycopersicon esculentum*, offers many advantages for the cloning of *R* genes solely on the basis of their position on a genetic linkage map. Because tomato has been the subject of more than 50 years of plant breeding, over 27 loci have been identified that confer resistance to many agriculturally important fungi, nematodes, viruses, and bacteria (*3*). Many of these loci have been located to small intervals on genetic linkage maps (*4*). A high-density, restriction fragment length polymorphism (RFLP) map (*5*) and a yeast artificial chromosome (YAC) library have been developed for tomato (*6*). These resources, combined with the relatively small genome size of tomato (950 megabases) and low level of repeated sequences, have expedited map-

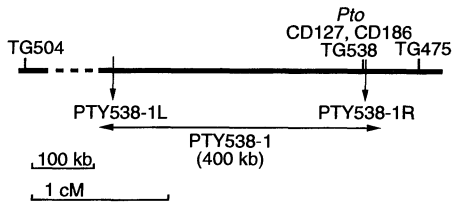


**Fig. 1.** Genetic map of the *Pto* region on chromosome 5 (bold line). The locations are indicated of tomato genomic RFLP markers (TG), the YAC end clones PTY538-1L and PTY538-1R, cDNA clones CD127 and CD186, and *Pto*. The YAC, PTY538-1, that spans this region is shown below. Genetic distance, in centimorgans (cM), is based on linkage analysis of 251 $F_2$ plants (*10*). The size of the YAC in kilobases was determined by pulsed-field gel electrophoresis with yeast strain AB972 as a standard (*31*).

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based cloning in this species (*7*).

We used the interaction of tomato and *Pseudomonas syringae* pv. *tomato*, the causative agent of bacterial speck, to isolate a plant *R* gene by map-based cloning. Resistance to *P. syringae* pv. *tomato* in tomato is conferred by the *Pto* locus on chromosome 5 (*8*). An avirulence gene (*avrPto*) from *P. syringae* pv. *tomato* induces resistance specifically in tomato cultivars containing *Pto* (*9*). Thus, the interaction between tomato and *P. syringae* pv. *tomato* involves a gene-for-gene system. With the use of a high-resolution linkage map of the *Pto* region and closely linked RFLP markers (*10*), we now have identified a YAC spanning the locus and isolated a cDNA clone of the *Pto* gene.

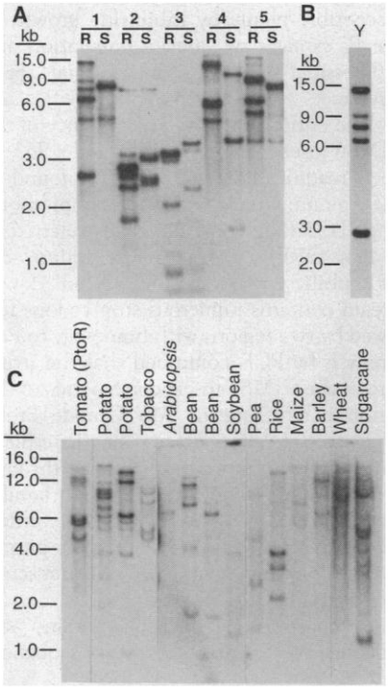


**Fig. 2.** (**A**) DNA blot analysis of the *Pto* gene family in tomato. Genomic DNA from near isogenic tomato lines Rio Grande–PtoR (R) and Rio Grande (S) was isolated from leaves (*32*), and 3-μg aliquots were digested with Bst NI (lanes 1R and 1S), Dra I (lanes 2R and 2S), Hae III (lanes 3R and 3S), Eco RI (lanes 4R and 4S), and Eco RV (lanes 5R and 5S). Samples were separated by electrophoresis on a 1% agarose gel, blotted onto Hybond $N^+$ membrane (Amersham), and then hybridized to $^{32}$P-labeled CD127 insert (*33*). (**B**) DNA blot analysis of YAC PTY538-1 (lane Y). Total yeast DNA from PTY538-1 was digested with Bst NI, separated on a 1% agarose gel, and blotted onto Hybond $N^+$ membrane. The membrane was probed with $^{32}$P-labeled CD127 insert (*32*). (**C**) DNA blot analysis of *Pto* gene homologs in other species. Genomic DNA (*34*) was digested with Eco RI and separated by electrophoresis on a 1% agarose gel. The gel was blotted onto Hybond $N^+$ membrane, which was hybridized with $^{32}$P-labeled CD127 (*32*). Hybridization conditions were as described in (*32*). Filter was washed to 0.5× SSC at 65°C and exposed to film for 24 hours for the Solanaceous species (first four lanes) and 7 days for the remaining species.

The *Pto* gene was genetically mapped by the scoring of RFLPs and resistance reactions to bacterial inoculation of 251 $F_2$ progeny derived from a cross between a plant homozygous for the R allele (*Pto/Pto*; Rio Grande–PtoR) and a near isogenic susceptible line [*pto/pto*; Rio Grande (*10*)]. One RFLP marker, TG538, cosegregated with the *Pto* locus (*10*) and was used to screen a tomato YAC library (6). A 400-kb clone, PTY538-1, was identified that hybridized to this marker (Fig. 1). End-specific probes corresponding to the right (PTY538-1R) and left (PTY538-1L) arms of PTY538-1 were isolated by inverse polymerase chain reaction (PCR) (*11*) and placed on the high-resolution linkage map of the region (*10*). The PTY538-1L arm mapped 1.8 centimorgans from *Pto*, whereas PTY538-1R cosegregated with *Pto* (Fig. 1). To confirm that PTY538-1 encompassed *Pto*, it was necessary to identify a plant with a recombination event between PTY538-1R and *Pto*. We therefore used markers TG538 and PTY538-1R to analyze a total of 1300 plants from various $F_2$ populations, $F_3$ families, and over 50 cultivars. One plant was identified that was homozygous for the TG538 allele associated with *Pto* but was also homozygous for the PTY538-1R allele associated with *pto* (susceptible allele) (*12*). All progeny from this plant were resistant to *P. syringae* pv. *tomato*, indicating that the plant was homozygous *Pto/Pto*. This result indicates that PTY538-1 spans the *Pto* locus.

A sample of DNA from PTY538-1 was isolated from agarose after separation on a clamped homogeneous electric-field gel and used to probe approximately 920,000 plaque-forming units of a leaf cDNA library (*13*). Of approximately 200 hybridizing plaques, 30 were investigated further. The cDNA inserts were amplified by PCR and used to probe a tomato mapping population consisting of 85 plants with recombination events in the *Pto* region (*10*). Two of the clones, CD127 and CD146 (both 1.2 kb), contained sequences that cross-hybridized with each other. When CD127 was mapped, it cosegregated with *Pto* (Fig. 1). The genetic cosegregation of CD127 with *Pto* and the fact that the clone was isolated from a leaf-tissue library made the cDNA a strong candidate for the *Pto* gene.

The CD127 clone detected numerous polymorphic fragments when hybridized with blots of genomic DNA from Rio Grande–PtoR and Rio Grande plants (Fig. 2A). This detection indicated that the clone might contain exons spanning a large region or that it represents a family of related genes. To distinguish between these possibilities, we probed the leaf cDNA library with CD127 insert and isolated an additional 14 cross-hybridizing clones, ranging from 0.6 to 2.4 kb. Analysis of these clones indicated that at least six different expressed genes with homology to CD127 exist in Rio Grande–PtoR (*14*). To investigate the genome location of the family members, total DNA from the YAC transformant PTY538-1 was digested with Bst NI and analyzed by DNA blot hybridization (Fig. 2B). The YAC contained all of the CD127-hybridizing fragments, with the exception of a 5-kb band that is common to both Rio Grande–PtoR and Rio Grande (Fig. 2B). Therefore, CD127 represents a gene family that is clustered primarily at the *Pto* locus.

To determine if homologs of the CD127 gene family are present in other plant species, we performed Southern blot analysis on genomic DNA isolated from six dicotyledonous species and five monocotyledonous species. Homologs of CD127 were identified in all species examined (Fig. 2C). Multiple bands were detected in many of the species, indicating the possible presence of a gene family similar to that in tomato. This sequence conservation raises the possibility that other plant species contain genes with structural, and perhaps functional, similarity to the CD127 gene family.

Analysis of RNA blots was used to determine if there were differences in transcript size or abundance produced by the CD127 family members among resistant or susceptible tomato lines (Fig. 3). A prominent 1.3-kb band was observed in resistant and susceptible lines (Fig. 3). A fainter band of 2.5 kb may indicate the presence of less abundant, longer transcripts in the CD127 family. No obvious induction of gene expression was observed upon infection with *P. syringae* pv. *tomato* (*12*).

Genetic complementation tests were

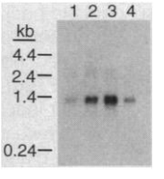


**Fig. 3.** RNA blot analysis of *Pto* gene family transcripts. Poly(A)$^+$ RNA of Rio Grande (*pto/pto*, lane 1), Rio Grande–PtoR (*Pto/Pto*, lane 2), Spectrum 151 (*Pto/pto*, lane 3), and Moneymaker (*pto/pto*, lane 4) was isolated from leaf tissue of plants 5 weeks after germination, separated on a 1.4% agarose-formaldehyde gel, and blotted onto nitrocellulose (*33*). The blot was hybridized with $^{32}$P-labeled CD127 insert as described in (*32*). The difference in signal among samples is due to unequal loading of RNA as indicated by the hybridization of the identical filter with a probe for ribulose bisphosphate carboxylase transcript (*12*). Sizes of markers (RNA ladder, GIBCO BRL) are indicated in kilobases.

conducted to determine if a member of the CD127 gene family confers resistance to *P. syringae* pv. *tomato*. Two cDNA clones, representative of the two size classes of transcripts, were initially chosen for this analysis [CD127 (1.2 kb) and CD186 (2.4 kb)]. The cDNA inserts were placed in the sense orientation into the binary $T_i$ vector pBI121 (Clontech Laboratories, Palo Alto, California) under transcriptional control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter. The resulting plasmids were designated pPTC5 (CD127) and pPTC8 (CD186). After electroporation of the constructs into *Agrobacterium tumefaciens* LBA4404, the bacteria were used to transform cotyledon explants of a susceptible tomato cultivar, Moneymaker (*15*). Transformants containing integrated copies of each construct were identified by kanamycin selection and DNA blot analysis (*15*).

Five weeks after the transformed plants were transferred to soil, single leaves were inoculated with *P. syringae* pv. *tomato* strain T1(pPTE6) carrying *avrPto* (*9*, *16*). As controls, 4-week-old seedlings of Rio Grande–PtoR, Moneymaker, and Moneymaker transformed with pBI121 alone were also inoculated. Of two plants that were confirmed to contain the integrated transgene from pPTC8, both were resistant to *P. syringae* pv. *tomato* strain T1(pPTE6) (*17*). None of the nine transformants containing integrated copies of pPTC5 exhibited resistant phenotypes (*17*).

To confirm that *P. syringae* pv. *tomato* resistance was due to the introduction of the CD186 cDNA insert, a resistant R0 transgenic plant (PTC8/39) was crossed to a susceptible control plant (cv. Rio Grande). Of 22 backcross progeny examined, nine inherited the CD186 transgene. This result closely fit a 1:1 segregation ratio and indicated that the original integration of pPTC8 sequences in PTC8/39 occurred at a single locus. The same nine backcross-resistant (BC-R) plants containing the CD186 transgene displayed no disease symptoms upon inoculation with T1(pPTE6) (*17*) (Fig. 4A). The remaining 13 plants lacked the CD186 transgene and displayed typical symptoms of bacterial speck (*17*) (Fig. 4A). All 22 progeny plants were susceptible to *P. syringae* pv. *tomato* strain T1, which lacks *avrPto* (*9*, *12*).

Because plants showing no disease symptoms after inoculation with T1(pPTE6) might still harbor a large population of *P. syringae* pv. *tomato*, we monitored the colony-forming units of bacteria in the progeny plants and in control plants over a period of 4 days after inoculation (Fig. 4B). The nine BC-R plants exhibiting no disease symptoms contained $10^3$-fold fewer bacteria per square centimeter of leaf area than the backcross susceptible plants at the end of this time period. Lower bacterial populations in BC-R plants than in Rio Grande–PtoR plants were observed and may have been the result of a higher abundance of *Pto* protein in BC-R plants that resulted from the constitutive 35S promoter. Thus, CD186 functionally complements *pto* in susceptible plants by inhibiting growth of the *P. syringae* pv. *tomato* population and suppressing symptoms of bacterial speck disease.

The entire 2.4-kb DNA sequence of the CD186 insert was determined and a 963-bp open reading frame (ORF1) was found in the region nearest the 35S CaMV promoter in pPTC8 (Fig. 5A). Hereafter referred to as *Pto*, ORF1 encodes a 321–amino acid hydrophilic protein. The region downstream contains numerous stop codons followed by two regions with homology to *Pto*. Analysis by PCR confirmed that the structure of the CD186 insert corresponds to the genomic structure in Rio Grande–PtoR. Thus, the CD186 insert may be the result of an infrequent transcriptional read-through event into a pseudogene of the *Pto* family. Analysis of RNA blots with the entire CD186 insert as a probe detected a prominent 1.3-kb and faint 2.5-kb transcript identical to CD127 (*12*).

Comparison of the deduced amino acid sequence of *Pto* with the protein sequences

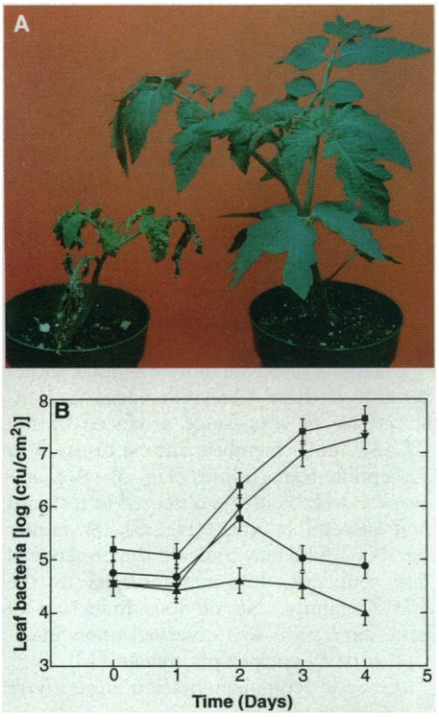


**Fig. 4.** (**A**) Representative plants of (left) the susceptible backcross progeny and (right) the resistant backcross progeny. Plants were inoculated as described with *P. syringae* pv. *tomato* strain T1(pPTE6) (*16*). Photographs were taken 5 days after inoculation. (**B**) Growth of *P. syringae* pv. *tomato* in leaves of tomato. Leaves of 7-week-old plants were inoculated with *P. syringae* pv. *tomato* strain T1(pPtE6) and bacterial populations were determined at the specified time points (*35*). Tomato lines examined were Rio Grande–PtoR (circles), Moneymaker (squares), backcross progeny with (triangles), or without pPTC8 (inverted triangles). Values are the means of three samples, each consisting of three leaf disks from three different plants. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap.

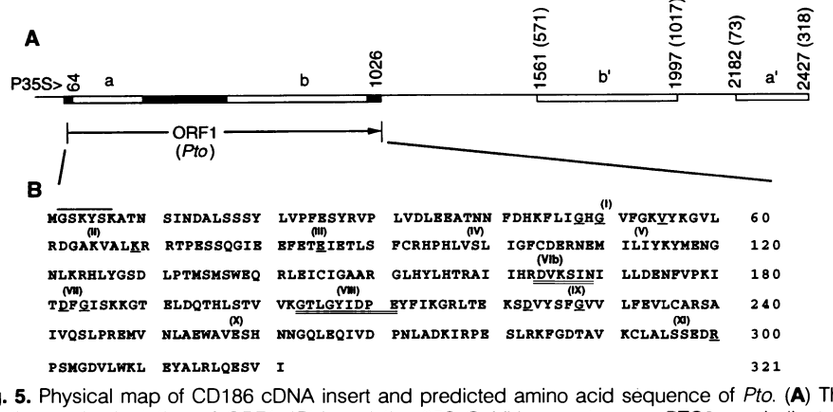


**Fig. 5.** Physical map of CD186 cDNA insert and predicted amino acid sequence of *Pto*. (**A**) The position and orientation of ORF1 (*Pto*) and the 35S CaMV promoter on pPTC8 are indicated. Regions designated a′ and b′ are 87% and 82% homologous, respectively, to a and b in ORF1. DNA base pairs corresponding to ORF1 a′ and b′ are shown above the line. Numbers in parentheses refer to positions in ORF1 corresponding to a′ and b′. (**B**) Deduced amino acid sequence of ORF1 (*Pto*) shown in one-letter code (*36*). The positions of subdomains characteristic of protein kinases (*19*) are indicated in parentheses above the sequence. Amino acids that are highly conserved among protein kinases are underlined (*19*). Residues that indicate serine-threonine specificity are double-underlined (*19*). A potential myristoylation site (*26*) at the $NH_2$ terminus is overlined. The nucleotide sequence has been deposited in GenBank (accession number U02271).

contained in GenBank release 77 (*18*) uncovered similarities with the catalytic domains of many plant, mammalian, and lower eukaryote serine-threonine protein kinases. Eleven subdomains, including 15 invariant amino acids characteristic of protein kinases, are present in the expected locations (Fig. 5B). Sequences indicative of serine-threonine kinase activity occur in subdomains VI (consensus DLKPEN) and VIII [consensus G(T/S)XX(Y/F)XAPE (*19*)]. To examine further its relation to the protein kinases, the *Pto* sequence was compared to the Protein Kinase Catalytic Domain Database (*19*). The five most similar matches were to other putative serine-threonine protein kinase genes of plant origin, including three with unknown function from *Arabidopsis* [TMK1, GenBank accession number L00670; ARK1, number M80238; and RLPK, number M84659 (*20*)], one of unknown function from maize [ZMPK1, number X52384 (*21*)], and one from *Brassica* that is believed to be involved in the recognition of self-pollen by stigma cells [SRK6, number M76647 (*22*)]. The similarity between *Pto* and SRK6 is particularly interesting because SRK6 appears to be involved in a specific cell-cell interaction (pollen cell–stigma papillar cell) like that between tomato and *P. syringae* pv. *tomato* (*22*). Other than plants, the closest matches in the database were to mammalian serine-threonine kinases of the *Raf* family (*23*).

The observation that transcripts of similar size and abundance are produced from the *Pto* gene family in both resistant and susceptible tomato lines raises the question of what molecular differences account for the different responses to the pathogen. Two possibilities are that the expression levels differ for specific members of the *Pto* gene family that confer resistance or that resistant plants contain a gene with a minor sequence difference resulting in a *Pto* protein of unique activity. Sequence comparisons of DNA among *Pto* gene family members in resistant and susceptible lines and the development of gene-specific probes and *Pto* antibodies should help to distinguish between these possibilities.

The presence of short *Pto*-homologous segments in CD186 in close proximity to *Pto* raises the possibility that recombination events have occurred in this region, shuffling domains among gene family members. Such intralocus recombination, perhaps by unequal crossing-over, would be increased by the clustering of family members at the *Pto* locus and could provide a mechanism for generating resistance specific for new races of *P. syringae* pv. *tomato* and perhaps to other phytopathogens. Possible occurrence of clustered *R* gene families in other plant species may explain why several disease-resistance loci (especially those corresponding to different races of the same pathogen) often map within small genetic intervals (*24*).

The elicitor-receptor model of gene-for-gene interactions suggests that the products of *R* genes function as specific receptors for pathogen elicitors (*25*). The *Pto* protein contains no obvious membrane-spanning or extracellular domain that would suggest function as an external receptor for a pathogen signal. A potential myristoylation site occurs at the $NH_2$ terminus; the protein might therefore be membrane-associated in a manner similar to that of the myristoylated tyrosine protein kinase $p60^{src}$ (*26*) (Fig. 5B). Association with the plasma membrane could place *Pto* in proximity to an extracellular receptor having a cytoplasmic domain. Analogous signaling complexes are known in mammals. The elucidation of the *Ras* pathway suggests that these systems are highly conserved among eukaryotes (*27*). Alternatively, *Pto* may exist in the cytoplasmic milieu and participate at a later step of a phosphorylation cascade. By analogy to *Ras* signaling, it is possible that the activation of *Pto* leads, by multiple steps, to the activation of defense gene expression. Finally, because there are several members of the *Pto* gene family in both resistant and susceptible lines and because gene expression does not require *P. syringae* pv. *tomato* infection, it seems possible that *Pto*-like protein kinases may also participate in other cellular activities.

Some elicitors of plant defense responses induce phosphorylation and dephosphorylation of specific plant proteins, and inhibitors of mammalian protein kinases inhibit the expression of certain plant defense genes (*28*). Thus, kinase-phosphatase activities appeared to be involved in signaling between plants and pathogens. The discovery that a disease-resistance gene encodes a serine-threonine protein kinase establishes a phosphorylation cascade as part of the recognition and response phases of plant defense.

The role of AvrPto protein in eliciting a resistance reaction is unknown. This protein may act by directly or indirectly modulating kinase activity encoded by *Pto*. In this regard, it is notable that several hypersensitive response and pathogenicity (Hrp) proteins isolated from phytopathogenic *Pseudomonas* species share homology with proteins from the human pathogen *Yersinia* that are involved in the secretion of virulence determinants (*29*). Two of these determinants have serine-threonine protein kinase and phosphatase activities (*30*). Thus, mechanisms of pathogen recognition in plants and of signal-transduction modulation by bacterial phytopathogens may share features in common with mammalian bacterial pathogenesis.

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13. Six-week-old plants of Rio Grande–PtoR and TA208 (*Pto*/*Pto*) were inoculated by dipping into a solution of avirulent *P. syringae* pv. *tomato* strain PT11 ($4 \times 10^7$ colony forming-units (CFU)/ml), 10 mM $MgCl_2$, and 0.05% L-77 Silwet (Union Carbide, Southbury, CT) dispersed in distilled water. Leaf tissue was harvested at 2, 6, 22, 48, and 72 hours after inoculation, polyadenylated [poly(A)$^+$] RNA was prepared, and equal amounts of each sample were pooled before library construction. The cDNA library was constructed in vector λgt10 with the use of a mixture of random and oligo(dT) primers (Stratagene).
14. Primers were designed with the use of partial sequence data from both ends of CD127 and used with PCR to amplify a product from the insert of the cDNA clones. PCR products were digested with restriction enzymes recognizing sites with four base pairs (for example, Hae III, Hin fI, Taq I), and the fragments were separated in a gel composed of 3% Nusieve GTG agarose (FMC Bioproducts) and 1% ultrapure agarose. Six different cDNA types were identified on the basis of their patterns of restriction fragments (J. Chunwongse, G. B. Martin, S. D. Tanksley, unpublished results).
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16. Leaflets were inoculated as in (*13*) but with $2 \times 10^7$ CFU/ml of the *P. syringae* pv. *tomato* strain. Under these conditions, symptoms of bacterial speck appeared after 5 to 7 days on susceptible plants.
17. A resistant reaction was indicated by the absence of necrotic specks on the inoculated leaves. A susceptible reaction was indicated by numerous necrotic specks surrounded by chlorotic halos. Reactions were scored 8 to 10 days after inoculation.
18. Comparison was made with the program BLAST, from S. F. Altschul, W. Gish, W. Miller, E. W. Meyers, and D. J. Lipman [*J. Mol. Biol.* **215**, 403 (1990)].
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32. Genomic DNA was isolated as described in (*8*). Hybridization was as described in [R. Bernatzky and S. D. Tanksley, *Genetics* **112**, 887 (1986)] with the use of random-hexamer $^{32}$P-labeled [A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983)] PCR product ($1 \times 10^6$ to $2 \times 10^6$ cpm/ml of buffer) amplified from the cDNA clone. The filter was washed to 0.5× standard saline citrate at 65°C and exposed to x-ray film for 24 hours.
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34. Genomic DNA was isolated from the following plants and digested with Eco RI (amount of DNA loaded on gel is indicated): tomato (Rio Grande–PtoR, 3 μg); potato (M200, hybrid: *Solanum tuberosum*/*S. berthaultii*, 5 μg); potato (B11B, *S. berthaultii*, 5 μg); tobacco (Samsun, 5 μg); *Arabidopsis thaliana* (Col-0, 1 μg); bean (*Phaseolus acutifolius*, G40178, 3 μg); bean (*P. acutifolius*, PI310800, 3 μg); soybean (*Glycine max*, Centennial, 4 μg); pea (*Pisum sativum*, Sparkle, 10 μg); rice (*Oryza sativa*, IRAT, 3 μg); maize (*Zea mays*, RI24, 15 μg); barley (*Hordeum vulgare*, SE16, 15 μg); wheat (*Triticum aestivum*, R-4, 15 μg); sugarcane (*Saccharum spontaneum*, SES208, 10 μg).
35. Leaves were inoculated as in (*16*). At each time point, nine 0.63-cm$^2$ leaf disks were taken from Rio Grande–PtoR, Moneymaker, and backcross progeny plants with or without pPTC8. The nine disks were randomly divided into sets of three and the disks were macerated in 10 mM $MgCl_2$. The density of bacterial populations was determined by the plating of serial dilutions and the counting of colony-forming units on King's medium B supplemented with rifampicin (20 μg/ml) and kanamycin (25 μg/ml) [E. O. King, N. K. Ward, D. E. Raney, *J. Lab. Clin. Med.* **44**, 301 (1954)].
36. Abbreviations for the amino acids are: 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.
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# Analysis of CD36 Binding Domains: Ligand Specificity Controlled by Dephosphorylation of an Ectodomain

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**The protein CD36 is a membrane receptor for thrombospondin (TSP), malaria-infected erythrocytes, and collagen. Three functional sequences were identified within a single disulfide loop of CD36: one that mediates TSP binding (amino acids 87 to 99) and two that support malarial cytoadhesion (amino acids 8 to 21 and 97 to 110). One of these peptides (p87-99) is a consensus protein kinase C (PKC) phosphorylation site. Dephosphorylation of constitutively phosphorylated CD36 in resting platelets and a megakaryocytic cell line led to the loss of collagen adhesion and platelet reactivity to collagen, with a reciprocal increase in TSP binding. PKC-mediated phosphorylation of this ectodomain resulted in a loss of TSP binding and the reciprocal acquisition of collagen binding. In site-directed mutagenesis studies, when the threonine phosphorylation site was changed to alanine, CD36 was expressed in a dephosphorylated state and bound to TSP constitutively.**

Regulation of membrane receptor function may be mediated by translocation of the receptor to the cell surface or by association or dissociation of the receptor from regulatory molecules. Although posttranslational modifications such as glycosylation or phosphorylation regulate many protein interactions, the rapid regulation of these characteristics has been confined to events that occur within the membrane or on the cytoplasmic side of the receptor. CD36 is an 88-kD membrane glycoprotein present on platelets, monocytes, erythroid precursors, endothelial cells, and several tumor cell lines and is a receptor for the adhesive platelet and extracellular matrix protein TSP (*1*–*6*), *Plasmodium falciparum*–infected erythrocytes (*7*–*9*), and collagen (*10*). CD36 is one of several TSP binding sites on platelets, and purified CD36 binds TSP (*1*–*6*). In addition, CD36 acts as a receptor that is critical for the cytoadherence of *P. falciparum*–infected erythrocytes to endothelium (*7*–*9*). Both TSP binding and malarial cytoadhesion are inhibited by the same monoclonal antibody (*1*, *11*), suggesting that the domains that mediate these events may be related. CD36 is also one of several reported collagen receptors (*12*).

Random CD36 domains were expressed in a recombinant expression system, and we screened the library by probing with biotinylated purified TSP, a biotinylated peptide (CSVTCG) (*13*) corresponding to a CD36 binding domain within TSP (*2*), and $^{51}$Cr-labeled malaria-infected erythrocytes (*14*). Positively hybridizing clones were subjected to secondary or tertiary rounds of screening and then sequenced. Analysis of the clones identified overlapping consensus sequences as candidate binding domains. Peptides corresponding to the predicted amino acid sequence of these regions were synthesized and examined for TSP binding and malarial cytoadhesion (*15*).

The domains of CD36 that are responsible for TSP binding and for the cytoadhesion of *P. falciparum*–infected erythrocytes were identified by random domain library screening (RANDOLS) (Fig. 1A) and confirmed by means of synthetic peptides (Fig. 1B). Three functional sequences were identified within a single putative disulfide loop of CD36 defined by Cys$^7$ and Cys$^{243}$: one that mediates TSP binding (amino acids 87 to 99) and two that support malarial cytoadhesion (amino acids 8 to 21 and 97 to 110). The malarial binding domain, residues 97 to 110 that we have identified, overlaps p93-110 identified as a binding site

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