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Report

Pseudomonas syringae Effector AvrPto Blocks Innate Immunity by Targeting Receptor Kinases

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Summary

Plants use receptor kinases, such as FLS2 and EFR, to perceive bacterial pathogens and initiate innate immunity. This immunity is often suppressed by bacterial effectors, allowing pathogen propagation. To counteract, plants have evolved disease resistance genes that detect the bacterial effectors and reinstate resistance. The *Pseudomonas syringae* effector AvrPto promotes infection in susceptible plants but triggers resistance in plants carrying the protein kinase Pto and the associated resistance protein Prf. Here we show that AvrPto binds receptor kinases, including *Arabidopsis* FLS2 and EFR and tomato LeFLS2, to block plant immune responses in the plant cell. The ability to target receptor kinases is required for the virulence function of AvrPto in plants. The FLS2-AvrPto interaction and Pto-AvrPto interaction appear to share similar sequence requirements, and Pto competes with FLS2 for AvrPto binding. The results suggest that the mechanism by which AvrPto recognizes virulence targets is linked to the evolution of Pto, which, in association with Prf, recognizes the bacterium and triggers strong resistance.

Results

Plants use plasma-membrane-localized pattern-recognition receptors (PRRs), which contain an extracellular leucine-rich-repeat domain and a cytoplasmic serine/threonine kinase domain, to detect pathogen-associated molecular patterns (PAMPs) and trigger innate immunity [1, 2]. Phytopathogenic bacteria have evolved effector proteins that are delivered into plant cells through the type III secretion system to inhibit the PAMP-triggered immunity (PTI) [3]. Plants, in turn, have adapted to effector-mediated virulence by evolving resistance proteins that directly or indirectly detect the effectors inside the plant cell and trigger strong immune responses (effector-triggered immunity; ETI) [3] that often give rise to complete resistance to the invading pathogen. However, little is known about virulence targets of the effectors and how effector virulence activities have shaped the evolution of resistance proteins. In tomato plants, the resistance protein Pto directly interacts with the *Pseudomonas syringae* effector AvrPto inside the plant cell to activate ETI [4]. Interestingly, Pto resembles the cytoplasmic kinase domain of receptor-like kinases but lacks the extracellular leucine-rich-repeat domain [5]. Pto constitutively interacts with Prf, a typical resistance protein that contains a nucleotide-binding domain and a leucine-rich-repeat domain and is crucial for the activation of ETI [6]. In plants lacking Pto or Prf, AvrPto inhibits PTI and enhances bacterial virulence [7]. A recent report showed that AvrPto acts upstream of the mitogen-activated protein kinase (MAPK) cascade to inhibit the PTI signaling pathway, but the target(s) remains to be identified [8]. AvrPto is plasma-membrane-localized through myristic-acid modification at the N terminus [9], but its biochemical function is unknown.

To understand the biochemical function of AvrPto, we have solved the crystal structure of the Pto-AvrPto complex [10]. Structural and biochemical analyses demonstrated that AvrPto is an inhibitor of the Pto kinase. AvrPto occupies the P+1 loop and blocks the access of Pto to its substrates. We hypothesize that the kinase-inhibition ability is intrinsic to the virulence function of AvrPto, namely the interference with PTI by blocking PRRs. Two well-known PRRs in plants are FLS2 and EFR, which perceive bacterial flagellar peptide flg22 and EF-Tu peptide efl26, respectively [11, 12]. To determine whether FLS2 and EFR kinases might be structurally related to Pto, we searched Protein Data Bank (PDB) with the primary sequences. Among the known structures of serine/threonine kinases, IRAK-4 is most closely related to Pto, FLS2, and EFR kinases, particularly in the P+1 loop (Figure S1 available online). This similarity permitted the use of IRAK-4 structure as a template for FLS2 and EFR that was compared with the Pto structure. Figures S1B and S1C show that the two kinases are highly similar in overall structure and P+1 loop, with a root-mean-square deviation of 1.285 Å over 241 Ca atoms. We reasoned that AvrPto might similarly bind the kinase domain of receptor kinases. To test this, we coexpressed His-tagged FLS2 or EFR kinase domains with GST-AvrPto in *E. coli* and assayed them for AvrPto-FLS2 and AvrPto-EFR interactions in vitro. Pull-down assays showed that the FLS2 and EFR kinases were, indeed, copurified with GST-AvrPto (Figures 1A and 1B). GST alone did not bind FLS2 and

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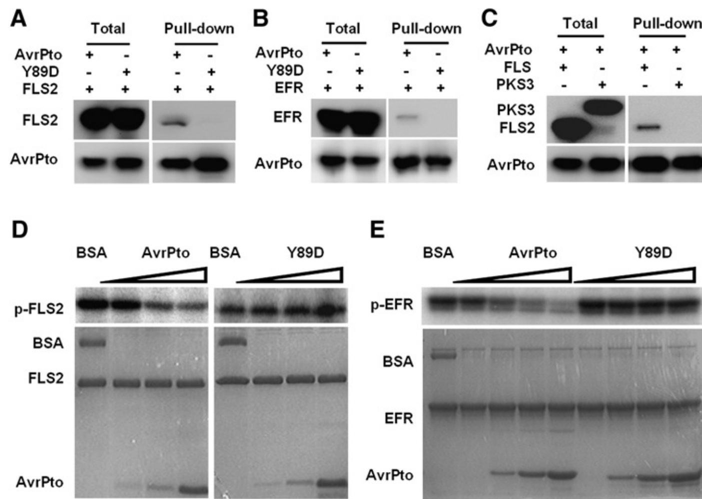

Figure 1. AvrPto Binds and Inhibits Receptor Kinases FLS2 and EFR In Vitro

(A–C) AvrPto binds the kinase domain of FLS2 (A) and EFR (B), but not PKS3 (C). Recombinant GST-AvrPto (AvrPto) or GST-AvrPto^{Y89D} mutant (Y89D) protein was coexpressed with the His-tagged FLS2 or EFR kinase domain or PKS3 in *E. coli*, and protein complex was affinity-purified with glutathione-conjugated agarose beads. The presence of His-FLS2, His-EFR, His-PKS3, and GST-AvrPto in the protein complex was detected by western blots with anti-His and anti-AvrPto antibodies.

(D and E) AvrPto inhibits the FLS2 (D) and EFR (E) autophosphorylation in a Y89-dependent manner. The recombinant FLS2 or EFR kinase was incubated with increasing amounts of AvrPto, AvrPto^{Y89D}, or BSA in the kinase assay buffer, and the autophosphorylation of the kinases was determined by autoradiography. The results shown are a representative of four independent experiments.

EFR. AvrPto Y89 plays a crucial role in Pto interaction by maintaining a proper conformation of GINP motif and making direct contact with Pto, and the AvrPto^{Y89D} mutation abolishes the binding and inhibition of Pto kinase [10]. The AvrPto^{Y89D} mutation also significantly diminished its binding to the FLS2 and EFR kinases (Figures 1A and 1B). We asked whether AvrPto nonspecifically binds serine/threonine kinases. GST pull-down assay showed that PKS3, a serine/threonine protein kinase involved in salinity responses [13], did not interact with AvrPto (Figure 1C), suggesting that AvrPto is specific to the receptor kinases. BIAcore surface-plasmon-resonance analysis further demonstrated that FLS2 binds AvrPto at an equilibrium constant of 0.19 μM, but not the control protein BSA (Figure S2). The wild-type (WT) AvrPto significantly inhibited EFR autophosphorylation and notably reduced FLS2 autophosphorylation in vitro, whereas the AvrPto^{Y89D} mutant was greatly compromised in its ability to inhibit FLS2 and EFR kinases (Figures 1D and 1E), indicating that AvrPto inhibits FLS2 and EFR kinase activities in a Y89-dependent manner.

To test whether FLS2 and AvrPto can interact in vivo, we coexpressed 35S::AvrPto-3FLAG and the full-length FLS2-HA fusion protein under the control of native *FLS2* promoter in protoplasts and carried out coimmunoprecipitation assays. As shown in Figure 2A, FLS2-HA is present in the AvrPto-3FLAG immune complex, and its presence is severely reduced in the AvrPto^{Y89D}-3FLAG mutant immune complex. FLS2 is known to associate with its coreceptor BAK1 only when induced by flg22

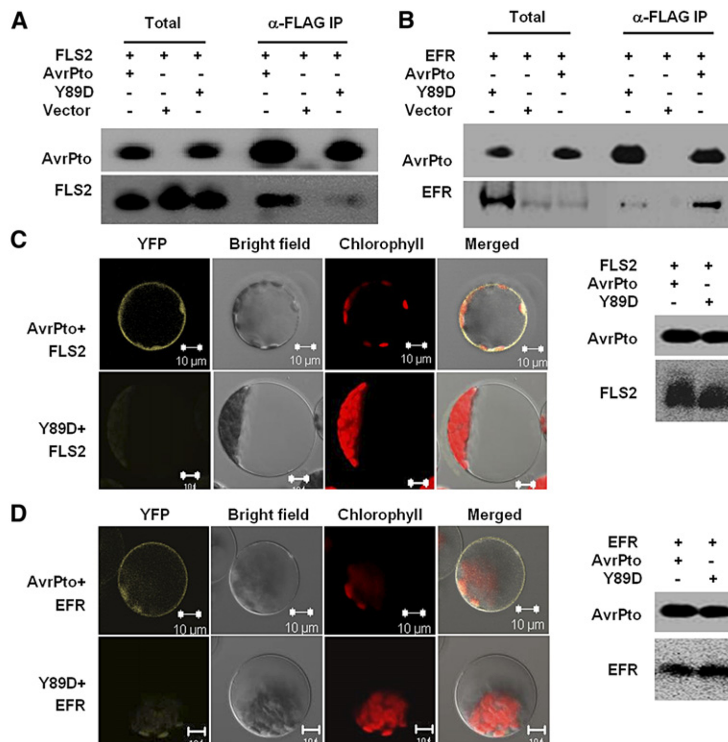

Figure 2. AvrPto Can Interact with FLS2 and EFR In Vivo

(A and B) Coimmunoprecipitation assays. The full-length FLS2-HA (A) or EFR-HA (B) construct was cotransfected with AvrPto-3FLAG or AvrPto^{Y89D}-3FLAG into protoplasts, and total protein was incubated with an agarose-conjugated anti-FLAG monoclonal antibody. The presence of FLS2-HA, EFR-HA, and AvrPto-3FLAG in the immune complex was detected by western blot with anti-HA or anti-FLAG antibodies. The results shown are a representative of five independent experiments.

(C and D) Bimolecular fluorescence complementation (BiFC) assay for FLS2-AvrPto and EFR-AvrPto interactions. Full-length FLS2-cYFP (C) or full-length EFR-cYFP (D) was cotransfected with AvrPto-nYFP or AvrPto^{Y89D}-nYFP into protoplasts, and the protoplasts were visualized under a confocal microscope. The images shown are representative of multiple protoplasts (FLS2+AvrPto, 23; FLS2+AvrPto^{Y89D}, 25; EFR+AvrPto, 21; EFR+AvrPto^{Y89D}, 43). Protein blots on the right show protein levels as detected with anti-AvrPto (for AvrPto-nYFP) and anti-HA (for FLS2-cYFP and EFR-cYFP, which contain a HA tag) antibodies. The results shown are a representative of three 3 independent experiments.

[14, 15]. We tested whether the FLS2-AvrPto interaction is indirectly mediated by BAK1. AvrPto and FLS2 interacted similarly in the presence or absence of flg22 in the WT protoplasts (Figure S3A) and in *bak1-1* mutant protoplasts (Figure S3B) [16], demonstrating that the FLS2-AvrPto interaction is independent of BAK1. Coimmunoprecipitation experiments also detected the Y89-dependent interaction between AvrPto-3FLAG and EFR-HA in protoplasts (Figure 2B). Bimolecular fluorescence complementation (BiFC) assay was used to further test whether AvrPto interacts with FLS2 and EFR in vivo. The C-terminal half of YFP (cYFP) was fused to the C terminus of full-length FLS2, and the N-terminal half of YFP (nYFP) was fused to the C terminus of AvrPto. The FLS2-cYFP and AvrPto-nYFP fusion proteins were examined for their ability to regulate *FRK1-LUC* expression in response to the flg22 peptide. Whereas the *fls2* mutant protoplasts transfected with an empty vector did not respond to the flg22 peptide, transfection of the full-length FLS2-cYFP fully restored the flg22-induced expression of *FRK1-LUC*, similar to when protoplasts were transfected with the full-length FLS2-HA construct, indicating that the fusion protein was fully functional in the plant cell (Figure S4A). Conversely, transfection of AvrPto-nYFP into protoplasts inhibited the flg22-induced expression of *FRK1-LUC* (Figure S4B), indicating that the nYFP fusion did not affect the immune-suppressing function of AvrPto. We then examined whether these two fusion proteins interacted in protoplasts. Figure 2C shows that protoplasts cotransfected with AvrPto-nYFP and FLS2-cYFP displayed strong fluorescence, whereas those cotransfected with the AvrPto^{Y89D}-nYFP and FLS2-cYFP showed only marginal fluorescence, indicating that AvrPto interacts with FLS2 in the plant cell in a Y89-dependent manner. The difference in florescence was not caused by different amounts of YFP fusion proteins, because similar levels of the nYFP- and cYFP-fusion proteins were detected in protoplasts transfected with AvrPto-nYFP and AvrPto^{Y89D}-nYFP (Figure 2C). Protoplasts cotransfected with AvrPto-nYFP and the empty cYFP plasmid did not show florescence complementation (Figure S5). Similarly, coexpression of EFR-cYFP and AvrPto-nYFP resulted in YFP complementation, whereas coexpression of EFR-cYFP and AvrPto^{Y89D}-nYFP did not (Figure 2D). Together, these experiments support the hypothesis that AvrPto targets receptor kinases FLS2 and EFR in the plant cell.

Because the kinase activity is required for the function of FLS2 although the mechanism remains unknown [17], the inhibition of FLS2 by AvrPto is expected to globally block all downstream immune responses initiated by FLS2, including induced gene expression [8, 18, 19], a transient accumulation of H_2O_2 in plants, and callose deposition at the cell wall [20]. To test this, we generated transgenic plants expressing AvrPto under the control of an estrogen-inducible promoter and examined flg22-induced responses. Figure S6 shows that AvrPto inhibited all flg22-induced responses tested. Thus, the results are consistent with the AvrPto inhibition of FLS2 and EFR kinase activity in vitro.

We next determined whether the FLS2-AvrPto and EFR-AvrPto interactions are required for the inhibition of MAPK cascade and downstream responses by AvrPto [8]. Treatment of wild-type protoplasts with flg22 and elf26 stimulated the MAPK activity, and the expression of the wild-type AvrPto protein reduced the MAPK activation in response to flg22 or elf26 treatment (Figures S7A and S7B). Equal amounts of MAPK protein were detected in various samples, indicating that AvrPto expression did not reduce MAPK protein accumulation. This inhibition was diminished when the AvrPto^{Y89D} was used, indicating that the FLS2-AvrPto and EFR-AvrPto interactions are required for AvrPto to inhibit the MAPK cascade. We also tested whether the interaction is required for blocking downstream signaling by measuring the flg22- and elf26-induced expression of *FRK1-LUC.* Consistent with a poor interaction with FLS2 and EFR, the AvrPto^{Y89D} mutant was significantly compromised in its ability to inhibit the *FRK1-LUC* expression, indicating that the ability to interact with FLS2 and EFR is required for the immune-suppressing activity of AvrPto (Figures S7C and S7D). Together, these data supported the notion that AvrPto blocks PAMP-induced immune responses by targeting PRRs.

Because Pto and receptor kinases appear to be structurally similar, it is possible that Pto and receptor kinases bind to AvrPto in a similar manner. This is supported by the fact that Y89 is required for interaction with both Pto [10] and receptor kinases. We performed additional experiments to further determine the similarity between Pto-AvrPto and FLS2-AvrPto interactions. The kinase activity of Pto is required for AvrPto binding, and the mutation at the ATP-binding site of Pto abolishes its interaction with AvrPto [4, 10]. To test whether phosphorylation of FLS2 is also required for AvrPto interaction, we mutated the FLS2 kinase ATP-binding site (K898) and examined the binding with AvrPto. Indeed, the mutation compromised the FLS2-AvrPto interaction in vitro (Figure 3A). Coimmunoprecipitation assay showed that this mutation significantly reduced FLS2-AvrPto interaction in vivo (Figure 3B). The AvrPto GINP loop interacts with Pto through main-chain hydrogen bonding and van der Waals contacts mediated by I96 side chain [10], and mutations of I96 abolish the interaction [9]. Coimmunoprecipitation assay showed that the AvrPto^{I96A} mutation reduces, but does not abolish, AvrPto-FLS2 interaction in vivo (Figure 3C). To further test whether Pto-AvrPto and FLS2-AvrPto interactions are mechanistically similar, we determined whether Pto competes with FLS2 for AvrPto binding in vitro by using GST pull-down assay. In the presence of Pto, AvrPto binds Pto, and the FLS2-AvrPto binding is greatly diminished (Figure 3D), indicating that Pto indeed competes with FLS2 for AvrPto binding. FLS2-HA, AvrPto-3FLAG, and Pto were coexpressed in protoplasts to test whether the competition occurs in vivo. Coimmunoprecipitation assays indicated that the coexpression of Pto significantly reduced FLS2-AvrPto interaction (Figure 3E). Residues His49 and Val51 in Pto are required for interaction with AvrPto, and the Pto$^{H49D/V51D}$ mutations diminish the interaction with AvrPto in vitro [10]. The Pto$^{H49D/V51D}$ mutant was unable to compete for AvrPto (Figure 3E). This explains a previous report that overexpression of Pto partially relieves the inhibition of *FRK1-LUC* expression by AvrPto [8]. Together, these results illustrated that AvrPto-Pto and AvrPto-FLS2 interactions involve at least partially overlapping interfaces.

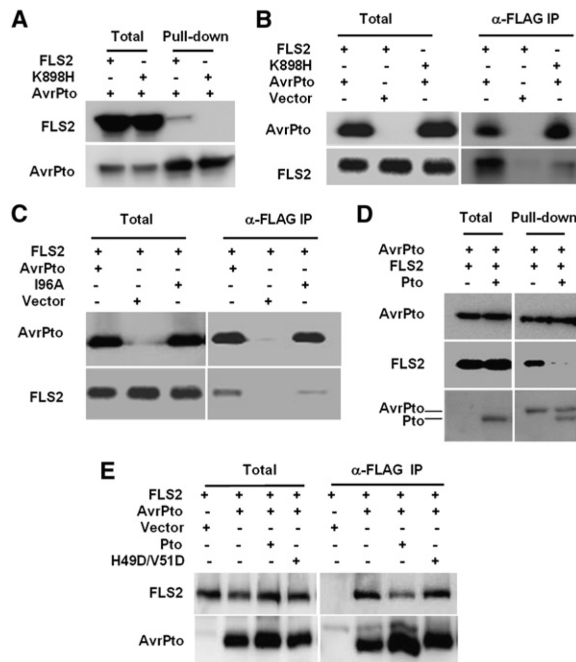

Figure 3. Similarity of Pto-AvrPto and FLS2-AvrPto Interactions

(A) The ATP-binding site of FLS2 is required for AvrPto interaction in vitro. Recombinant GST-AvrPto protein was coexpressed with the His-tagged kinase domain of WT FLS2 or FLS2^{K898H} in *E. coli*, and the protein complex was purified with glutathione-conjugated agarose beads. The presence of His-FLS2 in the protein complex was detected by western blot with anti-His antibodies. The amount of GST-AvrPto was indicated by western blot with anti-AvrPto antibodies.

(B) The ATP-binding site of FLS2 is required for interaction with AvrPto in vivo. Protoplasts were cotransfected with AvrPto-3FLAG and full-length wild-type FLS2-HA or FLS2^{K898H}-HA constructs. Coimmunoprecipitation assay was used to detect the presence of FLS2-HA protein in the AvrPto-3FLAG immune complex.

(C) The AvrPto^{I96A} mutation reduces FLS2-AvrPto interaction in vivo. Protoplasts were cotransfected with WT AvrPto-3FLAG or AvrPto^{I96A}-3FLAG and full-length FLS2-HA constructs, and coimmunoprecipitation assay was used to detect the presence of FLS2-HA in the AvrPto-3FLAG immune complex.

(D) Pto competes with FLS2 for AvrPto binding in vitro. Purified His-FLS2 was incubated with GST-AvrPto in the presence or absence of Pto and was affinity-purified with glutathione-conjugated agarose, and the presence of His-FLS2 and GST-AvrPto in the bound fraction was detected by western blot with anti-His and anti-AvrPto antibodies, respectively. The presence of bound Pto is detected by Ponceau S staining of protein gel blot.

(E) Pto interferes with the FLS2-AvrPto interaction in vivo. Protoplasts were cotransfected with the full-length FLS2-HA and AvrPto-3FLAG constructs along with the 35S::Pto or 35S::Pto$^{H49D/V51D}$ construct. Protein extract was precipitated with agarose-bead-conjugated anti-FLAG antibody. The bound protein complex was subjected to western blot with anti-HA or anti-AvrPto antibodies.

The results shown are a representative of two independent experiments.

AvrPto delivered by *P. syringae* enhances virulence in both *Arabidopsis* and susceptible tomato plants [8, 21]. To determine whether FLS2-AvrPto interaction is important for AvrPto virulence, we first tested whether the virulence function of AvrPto in *Arabidopsis* plants depended on FLS2 (Figure 4A). As reported before [22], the *fls2* mutant plants were significantly more susceptible than were the wild-type (WT) plants to strain DC3000, which contains *avrPto* (t test, $p < 0.05$). In WT *Arabidopsis* plants, the *P. syringae* strain lacking *avrPto* (Δ*avrPto*) grew to a significantly lower level than did DC3000 (Figure 4A; t test, $p < 0.05$), confirming a role of AvrPto in virulence on *Arabidopsis* plants. However, both the DC3000 and Δ*avrPto* strains grew to similarly high levels on the *fls2* mutant plants, indicating that AvrPto plays an important role in overcoming FLS2-mediated resistance.

To determine whether the AvrPto virulence in tomato plants is associated with its ability to interact with the flg22 receptor LeFLS2, which is orthologous to *Arabidopsis* FLS2 [23], we sought to test whether LeFLS2 is similarly targeted by AvrPto and whether this targeting is relevant to the virulence function in tomato plants. Because the *Arabidopsis FLS2* promoter-*LeFLS2* transgene does not result in detectable LeFLS2 protein accumulation in *Arabidopsis* [23], we expressed LeFLS2-HA under the control of 35S promoter. Figure 4B shows that LeFLS2-HA restored the flg22-induced *FRK1-LUC* expression in *Arabidopsis fls2* mutant protoplasts. Protoplasts coexpressing AvrPto completely lost the LeFLS2-HA-mediated *FRK1-LUC* induction, whereas protoplasts coexpressing AvrPto^{Y89D} retained > 60% of the response (Figure 4B). The effect of AvrPto^{I96A} was between the WT AvrPto and AvrPto^{Y89D}. Coimmunoprecipitation indicated that LeFLS2 indeed interacted with AvrPto in a Y89-dependent manner (Figure 4C). To determine whether Y89 is required for the virulence function in tomato plants when AvrPto is delivered by the bacterium, we introduced the *avrPto*^{Y89D} mutant into *P. syringae* strain T1 and tested its ability to enhance bacterial growth in susceptible tomato plants. As expected, whereas the WT *avrPto* enhanced bacterial growth in tomato plants, the *avrPto*^{Y89D} mutant was impaired in virulence activity and unable to enhance bacterial growth (Figure 4D), indicating that the ability of AvrPto to bind receptor kinases is correlated with its virulence function in tomato plants.

Discussion

The results described here demonstrate that AvrPto is capable of interacting with FLS2, EFR, and LeFLS2 in vivo, and this interaction is correlated with its ability to block PTI and virulence function in susceptible tomato plants. Furthermore, AvrPto enhances virulence on WT but not *fls2* mutant *Arabidopsis* plants, indicating that AvrPto is required for overcoming the FLS2 resistance. AvrPto is likely to target and inhibit additional receptor kinases. Indeed, yeast two-hybrid assays on selected *Arabidopsis* receptor kinases that are most similar to Pto showed that AvrPto interacted with and inhibited the autophosphorylation of the receptor-like kinase At2g23200 (Figure S8). Although the biological function of At2g23200 is not known, the gene is induced by PAMPs (data not shown). This could help explain the fact that AvrPto overexpression inhibits defenses induced by at least three different PAMPs [8]. The ability of AvrPto to target multiple PRRs has an apparent advantage because plants use multiple PRRs to perceive pathogenic bacteria [2]. AvrPto delivered by the bacterium is likely to exist at a relatively low level in the plant cell, a level that might or might not be sufficient to bind

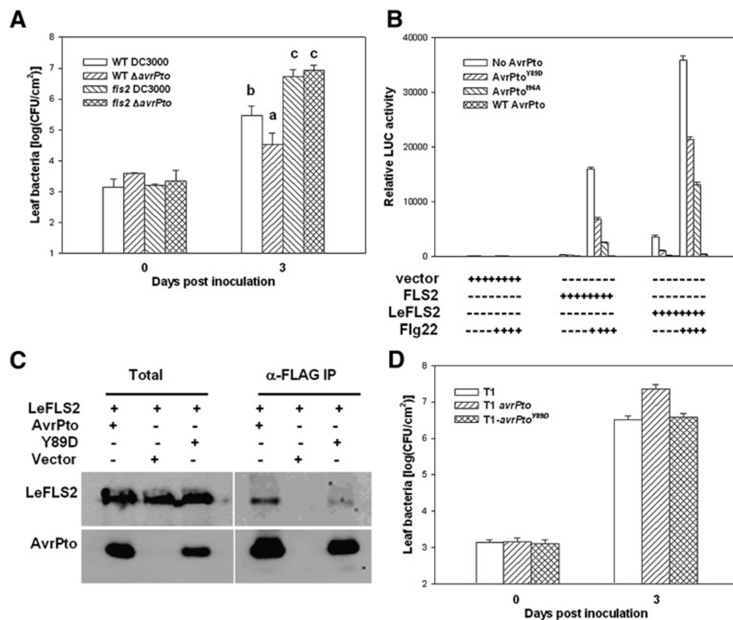

Figure 4. Association of AvrPto Virulence Function with Receptor Kinases

(A) *avrPto* enhances *P. syringae* virulence on WT but not *fls2* mutant plants. Six-week-old WT (Col-0 ecotype) or *fls2* mutant plants were spray-inoculated with the indicated bacterial strains. Strain DC3000 contains *avrPto* and is virulent on *Arabidopsis*. The Δ*avrPto* strain is a derivative of DC3000 with *avrPto* deleted. Leaf bacterial population was determined at the indicated days after inoculation. Each data point consisted of four replicates. Error bars indicate standard deviation (SD). The experiment was repeated four times with similar results. Different letters denote significant difference at $p < 0.05$ (Student's t test).

(B) LeFLS2-3FLAG complements the Arabidopsis *fls2* mutant, and its signaling is blocked by AvrPto. Arabidosis *fls2* mutant protoplasts were cotransfected with *FRK1-LUC* and FLS2-HA, LeFLS2-HA, or an empty vector in the presence or absence of AvrPto^{I96A}-3FLAG, AvrPto^{Y89D}-3FLAG, AvrPto-3FLAG, or an empty vector and induced with 100 nM flg22 for 3 hr, and the luciferase activity was determined. Each data point consisted of three replicates. Error bars indicate SD.

(C) AvrPto interacts with LeFLS2 in a Y89-dependent manner. *Arabidopsis* protoplasts were transfected with LeFLS2-HA along with an empty vector, AvrPto-3FLAG, or AvrPto^{Y89D}-3FLAG and immunoprecipitated with agarose-conjugated anti-FLAG antibody, and the immune complex was subjected to western-blot analyses with anti-FLAG or anti-HA monoclonal antibodies.

(D) AvrPto Y89 is required for virulence in susceptible tomato plants. The *P. syringae* pv. *tomato* strains carrying a plasmid-borne WT *avrPto* gene, the *avrPto^{Y89D}* mutant, and the strain lacking the *avrPto* gene (T1) were infiltrated into susceptible tomato plants, and leaf bacterial population was determined at the indicated times. Each data point consists of three replicates. Error bars represent SD. The data shown are a representative of three independent experiments.

all PRRs that also exist at low levels. However, it is conceivable that not all PRRs are activated by PAMPs when a plant cell encounters a bacterium and that bacteria might only need to inhibit PRRs in close contact with the bacterium to block immune responses. PRR-mediated perception of PAMPs plays a critical role in the adaptation of land plants to an environment surrounded by a large number of potential pathogens [3]. An increasing number of bacterial effectors are found to suppress PAMP-mediated signaling pathways [9, 24–26]. The findings that AvrPto targets multiple PRRs to block immune responses illustrate a novel virulence mechanism in *P. syringae* and highlight an important battleground in plant-microbe coevolution.

The fact that Pto and related PRR kinases interact with AvrPto sheds light on the process of plant-microbe coevolution. Receptor kinases are ancient PRRs for pathogen recognition [3]. A recent work shows that AvrPto is present in a number of *P. syringae* isolates infecting a wide range of host plants including cucurbit, mulberry, bean, tomato, and tobacco [27], suggesting an important role of AvrPto in the adaptation of *P. syringae* to these plants. Thus it is likely that AvrPto is an ancient effector evolved to inhibit receptor kinases in these plants. In contrast, Pto is present only in a few wild tomato species [28], and Pto family proteins appear to be limited to *Solanaceae* plants, suggesting recent evolution of this family. The closest homlogs outside *Solanum* are mostly receptor kinases. Both the AvrPto-Pto and AvrPto-FLS2 interactions require an ATP-binding site in the kinase and AvrPto Y89, which makes direct contact with the kinase [10]. Moreover, Pto and FLS2 interact with AvrPto in a competitive manner, suggesting that at least some interaction interfaces are similar. It is possible that the mechanism by which Pto recognizes AvrPto is mechanistically linked to the virulence function of AvrPto toward receptor kinases.

Like AvrPto, many pathogen effectors interact with multiple host proteins, only one of which is critical in mediating the recognition of effector by the corresponding disease resistance protein. For example, the *Cladosporium fulvum* effector Avr2 is a cysteine protease inhibitor that interacts with Rcr3 and other tomato cysteine proteases. The interaction of Avr2 with Rcr3, but not other cysteine proteases, triggers resistance through tomato resistance protein Cf2 [29]. Interestingly, the inhibition of Rcr3 by a synthetic protease inhibitor does not trigger the resistance. This is analogous to the case of AvrPto-mediated resistance, where the AvrPto-Pto interaction, but not the inhibition of the Pto kinase activity per se, triggers the Prf resistance [10]. Another well-known case is the *Arabidopsis* RPM1-interacting protein RIN4, a negative regulator of PTI [24]. This protein [30–32], as well as probably its homologous proteins [33], interacts with *P. syringae* effectors AvrRpt2, AvrRpm1, and AvrB. The interaction of RIN4, but not other RIN4 homologs, with the three effectors triggers strong resistance when the cognate resistance proteins RPM1 and RPS2 are present. The virulence function of AvrRpt2, however, does not appear to be mediated by its association with RIN4, because AvrRpt2 is a cysteine protease that cleaves the negative PTI regulator RIN4, an action counteractive for the virulence function [24]. Similarly, the AvrRpm1-RIN4 association is not required for AvrRpm1 virulence [34]. The third example is the *Arabidopsis* protein kinase PBS1, which mediates the

recognition between *P. syringae* effector AvrPphB and the resistance protein RPS5. AvrPphB is a cysteine protease that cleaves PBS1, and this cleavage triggers the RPS5-mediated resistance [35]. *Arabidopsis* carries additional protein kinases containing AvrPphB cleavage sites. It will be important to determine whether any of the homologs of Rcr3, RIN4, or PBS1 are targeted by the above effectors for virulence.

Supplemental Data

Experimental procedures and eight figures are available at http://www.current-biology.com/cgi/content/full/18/1/74/DC1/.

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