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Report

AvrPtoB Targets the LysM Receptor Kinase CERK1 to Promote Bacterial Virulence on Plants

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Summary

Plant innate immunity relies on a set of pattern recognition receptors (PRRs) that respond to ligands known as pathogen-associated molecular patterns (PAMPs) [1–3]. To overcome such immunity, phytopathogenic bacteria deliver virulence molecules called effector proteins into the plant cell that collectively promote pathogenesis [4–7]. The vast majority of PRRs controlling PAMP-triggered immunity (PTI) and the mechanisms used by specific effectors to suppress these pathways are mostly unknown. Here, we show that the *Arabidopsis* LysM receptor kinase CERK1 [8, 9], which is critical for chitin elicitor signaling and resistance to fungal pathogens, plays an essential role in restricting bacterial growth on plants. This is supported by the fact that CERK1 is a target of the bacterial type III effector protein AvrPtoB, which blocks all defense responses through this receptor. AvrPtoB ubiquitinates the CERK1 kinase domain in vitro and targets CERK1 for degradation in vivo. We show that CERK1 is a determinant of bacterial immunity, but its contribution is overcome by bacteria expressing AvrPtoB. Our results reveal a new pathway for plant immunity against bacteria and a role for AvrPtoB E3-ligase activity in suppressing PTI.

Results and Discussion

The first event in active recognition of plant pathogens by their hosts is elicitation of host pattern recognition receptors (PRRs) by conserved pathogen-derived molecules called pathogen-associated molecular patterns (PAMPs). Elicitation of PRRs induces a variety of host responses, including activation of mitogen-activated protein kinases (MAPKs), evolution of a burst of reactive oxygen species (ROS), activation of defense gene induction, deposition of callose into cell walls, and functional immunity through undefined mechanisms [1–3]. The paradigm for PAMP-triggered immunity (PTI) is perception of bacterial flagellin protein through an induced receptor complex composed of the leucine-rich repeat (LRR) receptor kinases FLAGELLIN-SENSING 2 (FLS2) and BRI1-ASSOCIATED KINASE 1 (BAK1) [10, 11]. Pathogens secrete effector proteins to inhibit signaling events and enhance virulence; for example, the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) secretes some 30 effectors into the cell via a specialized type III secretion apparatus [5]. FLS2 and BAK1 contribute to PTI and congruently are targeted by the bacterial effectors AvrPto and AvrPtoB [12, 13]. However, whereas BAK1 is a common component of multiple PRR pathways [10, 11], FLS2 functions specifically as the flagellin receptor and, hence, comprises a minor component of PTI against bacteria. Thus, there is an important need to identify BAK1-independent PTI pathways and the effectors that target them.

Recognition of the fungal PAMP chitin by the *Arabidopsis* receptor kinase CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) [8, 9] is independent of BAK1 or related molecules (Figure S1 available online). In a screen to find bacterial effectors capable of suppressing PAMP signaling, we identified several that are capable of suppressing chitin responses, including AvrPtoB of *Pto* DC3000 (Figure S2). Despite the fact that chitin is a component of fungi, related molecules such as the cell wall component peptidoglycan are known in bacteria, and we hypothesize that some of these may act as PAMPs on plant cells. Suppression of this pathway by several effectors implies that it is an important target of pathogenic bacteria. To test the range of defenses suppressed by AvrPtoB, we examined PAMP-triggered defense responses in transgenic *Arabidopsis* plants expressing *avrPtoB* from an inducible promoter (line JR13). Treatment of these plants with flg22 peptide (containing the active epitope of flagellin) or chitin elicited the generation of reactive oxygen species (ROS), induction of defense gene expression, and deposition of callose into cell walls (Figures 1A, 1B, 1C, and S3). Typically, chitin elicitation induced weaker activation of defense responses compared to flg22 treatment. All of these responses were suppressed efficiently by prior induction of the *avrPtoB* transgene with dexamethasone, but not by treatments of the leaves with a mock solution (Figures 1A, 1B, and 1C). Furthermore, chitin-induced activation of MAPKs, one of the earliest signaling events upon PAMP treatment, was also abolished by AvrPtoB (Figure S4). Thus, AvrPtoB abrogates a broad range of BAK1-independent defenses at a very early point after chitin perception.

The receptor kinase CERK1 is essential for chitin signaling in *Arabidopsis* and contains three extracellular LysM carbohydrate-binding motifs and an intracellular protein kinase domain [8, 9]. We reasoned that broad suppression of defenses implies that AvrPtoB might target CERK1 directly. To test this, we examined the ability of AvrPtoB to interact with the CERK1 kinase domain in a yeast two-hybrid assay. As controls for specificity, we included the kinase domains of three receptor kinases involved in PTI (EF-Tu receptor [EFR] [14], FLS2, and BAK1) and two with roles in plant development (BRASSINOSTEROID-INSENSITIVE 1 [BRI1] [15] and CLAVATA 1 [CLV1] [16]). As a positive control, we included the Pto kinase, which is a known AvrPtoB interactor [17]. Only CERK1 and Pto interacted with AvrPtoB in these experiments (Figure S5). In addition, the unrelated effector AvrPto did not interact with CERK1 (Figure 2A). We did not detect AvrPtoB interaction in yeast with the BAK1 and FLS2 kinase domains, respectively, although it was recently reported that both full-length proteins coimmunoprecipitate with AvrPtoB [13, 18]. It is possible that additional regions of the BAK1 and FLS2 cytoplasmic domains are required for the AvrPtoB

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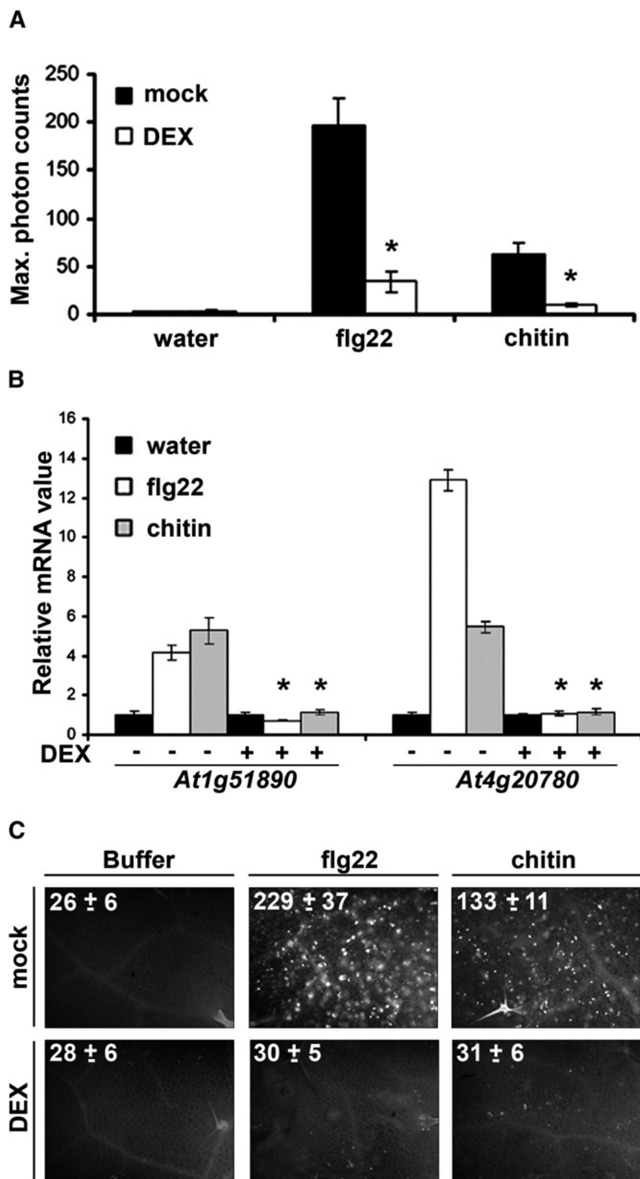

Figure 1. Suppression of Chitin-Induced Defenses by AvrPtoB

(A) ROS burst in transgenic *Arabidopsis* JR13 (Dex:*avrPtoB*) plants upon water, 100 nM flg22, or 100 μg/ml chitin treatment. Plants were treated with dexamethasone to induce the transgene or in a mock solution. Error bars represent SEM. Statistical significance compared to mock-induced plants ($p \leq 0.01$) is indicated by asterisks.

(B) Quantitative RT-PCR analysis of defense gene expression 60 min after treatment with water, 100 nM flg22, or 100 μg/ml chitin in transgenic *Arabidopsis* JR13 (Dex:*avrPtoB*) plants, with or without *avrPtoB* induction. Error bars represent SEM. Statistical significance compared to mock plants ($p \leq 0.01$) is indicated by asterisks.

(C) Callose deposition in transgenic *Arabidopsis* JR13 (Dex:*avrPtoB*) plants treated with buffer, 2 μM flg22, or 100 μg/ml chitin, with or without *avrPtoB* induction. Numbers show quantification of callose deposits (callose dots/cm^2) with PDQuest. Means ± SEM are shown.

The results shown in (A)–(C) are representative of at least three independent experiments.

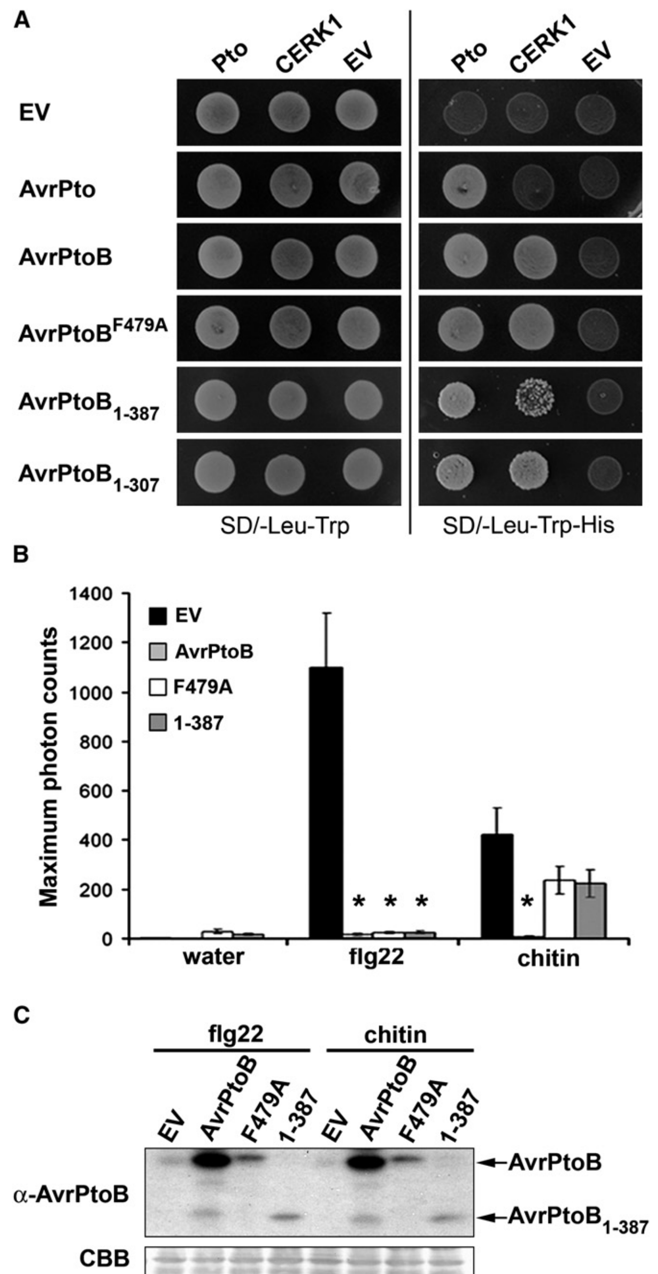

Figure 2. AvrPtoB Interacts with the LysM-Receptor Kinase CERK1

(A) Yeast two-hybrid assays showing CERK1 and Pto interaction with AvrPto, AvrPtoB, $AvrPtoB^{F479A}$, $AvrPtoB_{1-387}$, and $AvrPtoB_{1-307}$ as indicated. Empty vectors (EV) were included as negative controls. Yeast growth on SD/-Leu/-Trp media confirms the presence of both vectors for protein expression. Growth on SD/-Leu/-Trp/-His indicates protein-protein interaction.

(B) AvrPtoB E3-ligase mutants do not suppress chitin responses. *AvrPtoB* and derivatives or empty vector *(EV)* were expressed transiently in transgenic 11C *N. benthamiana* leaves and assayed for the ROS burst after treatment with water, 100 nM flg22, or 100 μg/ml chitin. Error bars represent SEM. Statistical significance compared to EV ($p \leq 0.01$) is indicated by asterisks. The results shown are representative of four independent experiments.

(C) Western blot showing accumulation of AvrPtoB protein forms in (B).

interaction. These results indicate CERK1 as a likely target of AvrPtoB for suppression of chitin perception.

AvrPtoB is a modular protein with separable subdomains [19, 20]. In tomato, residues 1–307 confer virulence to *Pto* DC3000 infections, whereas the N-terminal 307 and 387 residues are sufficient for interaction with the Pto and Fen kinases, respectively [21]. The C-terminal region (residues 400–550)

encodes a ubiquitin E3-ligase domain that promotes Fen degradation. In *Arabidopsis*, residues 1–387 are sufficient to coimmunoprecipitate with and inhibit BAK1 [13]. To determine the requirements for AvrPtoB interaction with CERK1, we introduced constructs encoding AvrPtoB$_{1-307}$, AvrPtoB$_{1-387}$, and the E3-ligase-deficient mutant AvrPtoB^{F479A} into yeast. All forms interacted with CERK1, indicating that the E3-ligase domain was dispensable for the interaction. In addition, AvrPtoB$_{1-307}$ bound to CERK1 (Figure 2A). Despite the different techniques used, this may suggest a structural difference in the interactions between AvrPtoB and BAK1 or CERK1, respectively (Figure 2A) [13]. To extend these observations, we examined whether the ability of AvrPtoB fragments to bind CERK1 was correlated with suppression of chitin-induced defense responses. We first investigated the molecular basis of chitin perception in *Nicotiana benthamiana*, a species that allows facile transient gene expression assays [22]. We identified a tomato cDNA (TC177084) from the TIGR database (http://biocomp.dfci.harvard.edu/tgi/) with 70% nucleotide identity to *AtCERK1* within a region encoding the kinase domain. Primers designed from this sequence were used to amplify a homologous cDNA from *N. benthamiana* that we designated *NbCerk1* (Figure S6A). Silencing of this gene in *N. benthamiana* plants with a tobacco rattle virus (TRV) vector [23] specifically reduced *NbCerk1* mRNA levels, but not those of *NbBak1* or *NbFls2*, and abolished chitin-induced ROS production (Figures S6B and S6C). Chitin responses were restored in the silenced plants by transient expression of *AtCERK1*, demonstrating that the loss of chitin responsiveness in the silenced plants was due to the loss of the orthologous *NbCerk1* gene (Figure S7). *AtCERK1* was not silenced by VIGS with *NbCerk1* because the genes lack regions of 21 contiguous identical nucleotides [24]. We transiently expressed AvrPtoB, AvrPtoB^{F479A}, AvrPtoB$_{1-387}$, or an empty vector control in transgenic *N. benthamiana* plants expressing the tomato *Prf* gene (line 11C [25]) because, unlike wild-type [21], it does not trigger cell death in response to AvrPtoB^{F479A} or AvrPtoB$_{1-387}$ (data not shown). Expression of AvrPtoB or any of its derivatives completely suppressed flg22-induced ROS, as reported by others [13, 21]. Interestingly, only full-length AvrPtoB, but not the 1–387 or F479A derivatives, fully suppressed chitin-induced ROS (Figures 2B and 2C). However, some reduction of chitin-induced ROS in the presence of AvrPtoB^{F479A} or AvrPtoB$_{1-387}$ was observed, compared to the empty vector treatment. It is possible that AvrPtoB N-terminal residues may partially inhibit CERK1 kinase activity. These data show that interaction with CERK1 is not sufficient for full suppression of signaling and reveal a novel role for the AvrPtoB E3-ligase domain in suppression of PTI.

We hypothesized that AvrPtoB might ubiquitinate CERK1 to promote its degradation and suppress host immunity. Posttranslational modification of proteins with ubiquitin is frequently a signal for degradation of the modified protein within the cell. Ubiquitination assays that use purified recombinant proteins confirmed that AvrPtoB ubiquitinated Fen kinase, but not Pto kinase, as expected [21]. The CERK1 kinase domain was also ubiquitinated by AvrPtoB in these experiments (Figure 3A). To test whether AvrPtoB targets CERK1 for degradation in vivo, we analyzed CERK1 protein levels in transgenic JR13 *Arabidopsis* plants expressing *avrPtoB* (Figure 3B). CERK1 protein diminished after 12 hr of AvrPtoB expression and was undetectable by 48 hr, whereas levels were unchanged in mock-induced controls. The flagellin receptor FLS2 was not degraded in these experiments. To test whether CERK1 disappearance required AvrPtoB E3 ligase activity, we coexpressed AtCERK1 with AvrPtoB or AvrPtoB^{F479A} in *N. benthamiana* leaves. CERK1 protein was detectable in the presence of AvrPtoB^{F479A}, but not AvrPtoB, indicating that degradation occurs in vivo in an E3-ligase dependent manner (Figure 3C). Degradation mediated by AvrPtoB was specific because levels of Fen kinase, but not the receptor kinase BAK1, were reduced in its presence (Figure S8). A small AvrPtoB-dependent reduction in FLS2 levels could be consistently observed in this experiment. Overall, the data indicate that AvrPtoB degrades CERK1 in an E3-ligase-dependent manner.

Ubiquitinated cytosolic proteins are often degraded by the 26S proteasome, whereas membrane-bound proteins may be degraded through the lysosome in mammals, and vacuoles may be degraded in yeast and plants [26]. To identify the major CERK1 degradation pathway, we blocked protein degradation by using the proteasomal inhibitor MG132 or the vacuolar-type H^+-ATPase inhibitor Bafilomycin A1 [27]. In AvrPtoB coexpression experiments in *N. benthamiana*, CERK1 was detected only in the presence of Bafilomycin A1, indicating that degradation may occur in the vacuole (Figure 3D). In contrast, Fen protein was detected only in the presence of MG132, consistent with proteasomal degradation as previously described [21] (data not shown). Targeting of host membrane proteins to the vacuole for degradation is a common strategy used by animal viruses [28] but is novel for type III effectors of phytopathogenic bacteria.

The data above suggest a novel role for CERK1 in bacterial immunity. To test this, we assayed bacterial growth on two *Arabidopsis* backgrounds containing T-DNA insertions in the *CERK1* gene. These mutants, designated Col-0 *cerk1-2* and Ws-4 *cerk1-3*, did not accumulate CERK1 protein (Figures 4A and 4B). We infected *Arabidopsis* plants by spray inoculation of *Pto* DC3000 bacteria onto leaves. This mimics natural infection conditions and is one of the most sensitive techniques to assess plant susceptibility to bacterial pathogens [2, 29]. The Ws-4 line is a natural *fls2* mutant, so a Col-0 *fls2* T-DNA insertion mutant was added to the study for direct comparison. Infections with *Pto* DC3000 showed greater symptom development on both Col-0 *cerk1-2* and Ws-4 *cerk1-3* plants compared to the respective wild-type lines (Figure 4C). Disease symptoms due to *CERK1* mutation were particularly severe in the Ws-4 background and pronounced on younger leaves. We measured bacterial growth on each line 2 days after inoculation (Figure 4D). The data indicate approximately equal contributions of FLS2 and CERK1 to immunity. Growth of this strain was further enhanced on Ws-4 *cerk1-3* relative to Ws-4, consistent with the absence of both FLS2 and CERK1 receptors in this line. Accordingly, a nonpathogenic *Pto* DC3000 strain containing a mutation in the *hrcC* gene required for effector secretion (*Pto* DC3000 *hrcC*) showed enhanced growth on both Col-0 *cerk1-2* and Ws-4 *cerk1-3* plants, demonstrating a role for CERK1 in PAMP perception (Figure 4D). Overall, we show an important role for CERK1 in restricting bacterial growth, similar in magnitude to the contribution of FLS2.

We next analyzed the ability of AvrPtoB to counter CERK1-mediated defense during bacterial infections. We compared bacterial replication on Ws-4 or Ws-4 *cerk1-3* plants infected with *Pto* DC3000 or on an isogenic strain lacking the *avrPtoB* gene (*Pto* DC3000Δ*avrPtoB*) [30]. Whereas, in Ws-4 plants, *Pto* DC3000Δ*avrPtoB* growth was restricted by one log (cfu/cm^2) compared to *Pto* DC3000, both strains grew to similar levels on Ws-4 *cerk1-3* plants (Figure 4E). The data show that AvrPtoB overcomes CERK1-mediated resistance and that

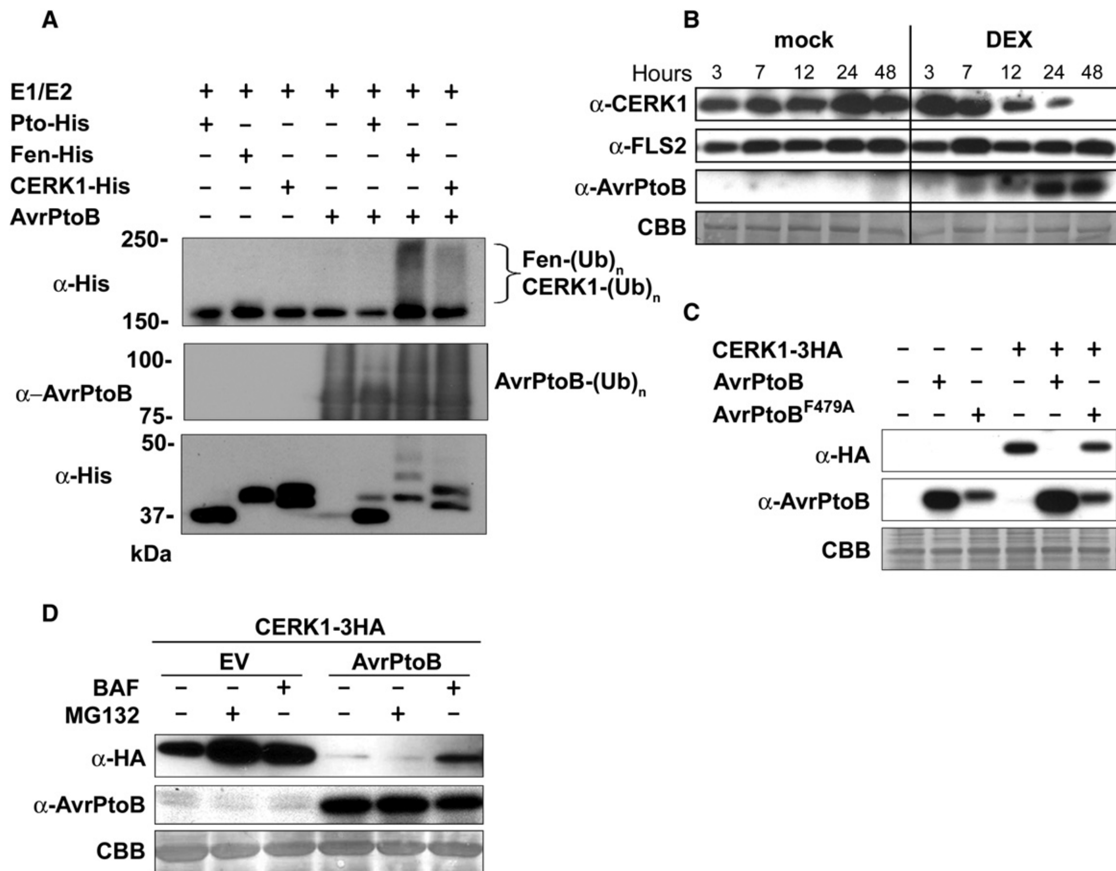

Figure 3. AvrPtoB Ubiquitinates and Degrades CERK1 in a Vacuole-Dependent Manner

(A) In vitro ubiquitination assays with recombinant E1 ubiquitin-activating and E2 ubiquitin-conjugating enzymes, ubiquitin (Ub), GST-AvrPtoB, and His-tagged fusion proteins as indicated. CERK1-His, Fen-His, and AvrPtoB were detected as polyubiquitinated [−(Ub)$_n$] proteins with α-His and α-AvrPtoB antibodies.

(B) Expression of AvrPtoB in *Arabidopsis* triggers a reduction in CERK1 levels. Immunoblots to detect CERK1, FLS2, and AvrPtoB accumulation were performed in transgenic *Arabidopsis* JR13 (Dex:*avrPtoB*) plants after mock or DEX treatment.

(C) CERK1 degradation is dependent on AvrPtoB E3-ligase activity. Immunoblots showing AtCERK1 accumulation in the presence of AvrPtoB or AvrPtoB^{F479A} when coexpressed transiently in *N. benthamiana*.

(D) AvrPtoB degrades CERK1 in a vacuole-dependent manner. As in (C), after treatment with 100 μM MG132 or 300 nM Bafilomycin A1 (BAF).

The results shown in (A)–(D) are representative of at least three independent experiments.

CERK1 is an important AvrPtoB target in vivo. We further studied whether CERK1 contributes to nonhost resistance to *P. syringae* pv *phaseolicola* (*Psp*) RW60. This strain was previously shown to overcome nonhost resistance on *Arabidopsis* Ws-3 plants only when expressing the *avrPtoB* gene [31]. *Psp* RW60 grew poorly on Ws-4 plants, but growth was increased by about one log (cfu/cm^2) when expressing *avrPtoB* compared to the same strain containing an empty vector construct (Figure 4F). Strikingly, *Psp* RW60 growth returned to wild-type levels when *avrPtoB* carried the *F479A* mutation, consistent with a key role for E3-ligase activity in suppression of CERK1. Growth promotion due to *avrPtoB* was abolished on Ws-4 *cerk1-3* plants, where *Psp* RW60 expressing *EV*, *avrPtoB*, or *avrPtoB^{F479A}* grew to similar levels (Figure 4F). Finally, we examined whether CERK1 was degraded by AvrPtoB in vivo after bacterial infections on Col-0 plants. CERK1 levels were strongly reduced in total plant extracts upon *Pto* DC3000 infiltration after 24 hr and absent after 36 hr, whereas infiltration of buffer or *Pto* DC3000 *hrcC* did not cause a major change. Interestingly, CERK1 levels were also reduced after challenge with *Pto* DC3000Δ*avrPtoB*, but this was delayed relative to *Pto* DC3000 infections (Figure 4G). We also observed a slight but consistent reduction in FLS2 levels in leaves infiltrated with *Pto* DC3000 after 36 hr as reported recently [18]. The results are consistent with a role for the AvrPtoB E3-ligase domain in pathogenesis but suggest that AvrPtoB is not the only *Pto* DC3000 effector that targets CERK1.

Our data reveal that, in addition to its demonstrated role in innate immunity to fungal pathogens, CERK1 acts as a BAK1-independent receptor kinase controlling bacterial immunity in *Arabidopsis*. Consistent with a fundamental role in plant innate immunity, CERK1 is targeted by AvrPtoB for degradation and likely by other bacterial type III effectors as well. AvrPtoB has emerged as a kinase suppressor with broad roles in repression of innate immunity [13, 17, 18, 21]. AvrPtoB directly targets the kinase domains of FLS2, BAK1, CERK1, Fen, Pto, and probably others [13, 18] by using a bipartite strategy. The N-terminal region specifies a kinase interaction domain that may target the catalytic cleft directly [13, 32]. This is true for all known kinase-AvrPtoB interactions, with possible roles in inhibition of kinase activity. Second, as shown here and elsewhere, the E3 ligase domain is of variable

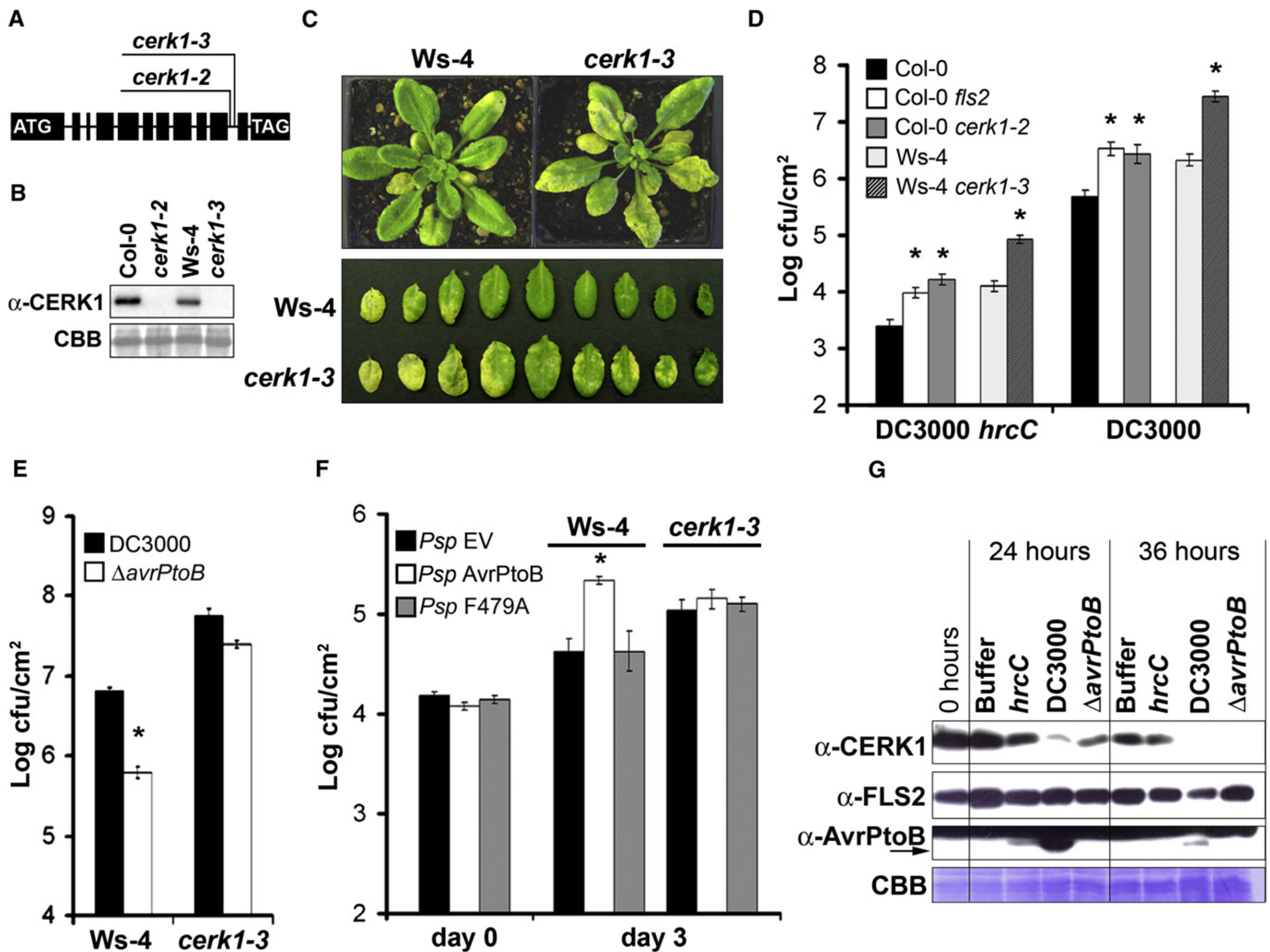

Figure 4. CERK1 Is a Determinant of Bacterial Immunity

(A) *CERK1* gene model showing *cerk1-2* and *cerk1-3* T-DNA insertion sites.

(B) Immunoblot to detect CERK1 protein in wild-type and mutant *Arabidopsis* lines.

(C) Disease symptoms on Ws-4 and Ws-4 *cerk1-3* plants after spray inoculation with *Pto* DC3000 bacteria at 10^8 colony forming units ml^{-1} (cfu/ml). Pictures were taken 3 days after inoculation. Plants show representative symptoms of five independent experiments.

(D) Growth of *Pto* DC3000 and *Pto* DC3000 *hrcC* on wild-type and mutant *Arabidopsis* plants 2 days after spray inoculation as in (C). Error bars indicate SEM. Statistical significance compared to wild-type ($p \leq 0.05$) is indicated by asterisks. The results are representative of five independent experiments.

(E) AvrPtoB overcomes *CERK1*-mediated resistance. Growth of *Pto* DC3000 and *Pto* DC3000Δ*avrPtoB* on Ws-4 and Ws-4 *cerk1-3* spray inoculated as in (C). Error bars indicate SEM. Statistical significance compared to *Pto* DC3000 ($p \leq 0.01$) is indicated by asterisks. The results are representative of five independent experiments.

(F) AvrPtoB E3-ligase activity is required to enhance virulence in *Arabidopsis*. Growth of *Psp* RW60 carrying an empty vector (EV), *avrPtoB*, or $avrPtoB^{F479A}$ on Ws-4 and Ws-4 *cerk1-3* plants 3 days after syringe inoculation with 2×10^7 cfu/ml bacteria. Error bars represent SEM. Statistical significance compared to *Psp* RW60 EV infection ($p \leq 0.05$) is indicated by asterisks. The results are representative of four independent experiments.

(G) *Pto* DC3000 infection triggers CERK1 degradation. Immunoblots showing CERK1, FLS2, and AvrPtoB accumulation in Col-0 leaves after buffer, *Pto* DC3000 *hrcC*, *Pto* DC3000, or *Pto* DC3000Δ*avrPtoB* infiltration. This experiment was repeated three times with similar results.

importance in physical removal of the targeted kinase. Together, these mechanisms provide a strategy for kinase deactivation with inbuilt redundancy. Importantly, all eukaryotic Ser-Thr and Tyr protein kinases have a generalized fold and are monophyletic [33]. Within plants, the RLK/Pelle gene family, which includes the known targets of AvrPtoB, makes up 60% of kinases and is the predominant form of receptor kinases in the *Arabidopsis* genome [34]. Thus, there are many more potential targets of AvrPtoB within plants. In addition, the previous model, in which recruitment of the E3 ligase domain as a specific evolutionary response to recognition of the AvrPtoB N-terminal domain by tomato Fen [21], does not hold, given an expanded view of possible AvrPtoB targets in all facets of immunity. The example of AvrPtoB may hint at a bacterial strategy for pathogenicity in which the host plasma membrane proteome is targeted somewhat unspecifically by type III effectors, but this remains to be investigated.

Interestingly, AvrPtoB deletion mutants that bind FLS2 but cannot associate with BAK1 do not suppress flg22 responses [13]. This suggests that suppression of flg22 recognition by truncated AvrPtoB forms occurs through BAK1 inhibition rather than via the FLS2 receptor itself. Suppression of BAK1 by AvrPtoB probably occurs by inhibition of kinase activity and may have broad effects through the multiple BAK1-dependent PAMP-signaling pathways [13]. Supporting the role for AvrPtoB E3-ligase activity on PTI suppression, FLS2 was

recently shown to be targeted for ubiquitination and degradation by AvrPtoB [18]. This mechanism is apparently redundant with BAK1 inhibition for suppression of flagellin responses. Because of this redundancy, it is so far unclear whether FLS2 degradation is necessary for inhibition of the recognition complex by AvrPtoB. Conversely, degradation was clearly required for CERK1 inhibition. Interestingly, although only full-length AvrPtoB could suppress chitin-induced ROS completely, some reduction could be observed in the presence of the F479A and 1–387 derivatives. This is suggestive of inhibition of CERK1 kinase activity, at least in these effector overexpression experiments.

Although bacteria do not contain chitin per se, similar carbohydrate-based structures are present in periplasms, biofilms, and secretomes of Pseudomonad bacteria [35–37] and constitute potential ligands for the three modular LysM domains of CERK1. Fascinatingly, CERK1 is highly related to Nod factor receptors from *Lotus* and *Medicago* [38], which function as entry receptors in symbiotic interactions with rhizobial bacteria [39, 40]. This evolutionary relatedness suggests a legume-specific recruitment and functional diversification of CERK1-like PRRs for fundamentally opposed plant-bacterial interactions [38], which may have originated from related bacterial ligands. Alternatively, CERK1 may act as an auxiliary of multiple PRRs, analogous to the requirement for BAK1 for signaling by FLS2 and other receptors [10, 11]. Indeed, direct binding of chitin to CERK1 has not yet been demonstrated. Identification of the PAMP conferring CERK1-mediated resistance will provide conclusive insights.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and seven figures and can be found with this article online at http://www.current-biology.com/supplemental/S0960-9822(09)00626-5.

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References

1. Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., and Felix, G. (2006). The Arabidopsis receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. Plant Cell *18*, 465–476.
2. Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D., Felix, G., and Boller, T. (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. Nature *428*, 764–767.
3. Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., and Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. Plant Cell *16*, 3496–3507.
4. Hann, D.R., and Rathjen, J.P. (2007). Early events in the pathogenicity of Pseudomonas syringae on Nicotiana benthamiana. Plant J. *49*, 607–618.
5. Schechter, L.M., Vencato, M., Jordan, K.L., Schneider, S.E., Schneider, D.J., and Collmer, A. (2006). Multiple approaches to a complete inventory of Pseudomonas syringae pv. tomato DC3000 type III secretion system effector proteins. Mol. Plant Microbe Interact. *19*, 1180–1192.
6. Gohre, V., and Robatzek, S. (2008). Breaking the barriers: Microbial effector molecules subvert plant immunity. Annu. Rev. Phytopathol. *46*, 189–215.
7. He, P., Shan, L., Lin, N.C., Martin, G.B., Kemmerling, B., Nurnberger, T., and Sheen, J. (2006). Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. Cell *125*, 563–575.
8. Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., and Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc. Natl. Acad. Sci. USA *104*, 19613–19618.
9. Wan, J., Zhang, X.C., Neece, D., Ramonell, K.M., Clough, S., Kim, S.Y., Stacey, M.G., and Stacey, G. (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell *20*, 471–481.
10. Heese, A., Hann, D.R., Gimenez-Ibanez, S., Jones, A.M., He, K., Li, J., Schroeder, J.I., Peck, S.C., and Rathjen, J.P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc. Natl. Acad. Sci. USA *104*, 12217–12222.
11. Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J.D., Felix, G., and Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature *448*, 497–500.
12. Xiang, T., Zong, N., Zou, Y., Wu, Y., Zhang, J., Xing, W., Li, Y., Tang, X., Zhu, L., Chai, J., et al. (2008). Pseudomonas syringae effector AvrPto blocks innate immunity by targeting receptor kinases. Curr. Biol. *18*, 74–80.
13. Shan, L., He, P., Li, J., Heese, A., Peck, S.C., Nurnberger, T., Martin, G.B., and Sheen, J. (2008). Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. Cell Host Microbe *4*, 17–27.
14. Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J., Boller, T., and Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell *125*, 749–760.
15. Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell *90*, 929–938.
16. Clark, S.E., Williams, R.W., and Meyerowitz, E.M. (1997). The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. Cell *89*, 575–585.
17. Kim, Y.J., Lin, N.C., and Martin, G.B. (2002). Two distinct Pseudomonas effector proteins interact with the Pto kinase and activate plant immunity. Cell *109*, 589–598.
18. Gohre, V., Spallek, T., Haweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J.W., and Robatzek, S. (2008). Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. Curr. Biol. *18*, 1824–1832.
19. Abramovitch, R.B., Kim, Y.-J., Chen, S., Dickman, M.B., and Martin, G.B. (2003). *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J. *22*, 60–69.
20. Xiao, F., He, P., Abramovitch, R.B., Dawson, J.E., Nicholson, L.K., Sheen, J., and Martin, G.B. (2007). The N-terminal region of Pseudomonas type III effector AvrPtoB elicits Pto-dependent immunity and has two distinct virulence determinants. Plant J. *52*, 595–614.
21. Rosebrock, T.R., Zeng, L., Brady, J.J., Abramovitch, R.B., Xiao, F., and Martin, G.B. (2007). A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity. Nature *448*, 370–374.
22. Goodin, M.M., Zaitlin, D., Naidu, R.A., and Lommel, S.A. (2008). Nicotiana benthamiana: Its history and future as a model for plant-pathogen interactions. Mol. Plant Microbe Interact. *21*, 1015–1026.
23. Liu, Y., Schiff, M., and Dinesh-Kumar, S.P. (2002). Virus-induced gene silencing in tomato. Plant J. *31*, 777–786.
24. Thomas, C.L., Jones, L., Baulcombe, D.C., and Maule, A.J. (2001). Size constraints for targeting post-transcriptional gene silencing and for RNA-directed methylation in Nicotiana benthamiana using a potato virus X vector. Plant J. *25*, 417–425.
25. Balmuth, A., and Rathjen, J.P. (2007). Genetic and molecular requirements for function of the Pto/Prf effector recognition complex in tomato and Nicotiana benthamiana. Plant J. *51*, 978–990.
26. Malik, B., Price, S.R., Mitch, W.E., Yue, Q., and Eaton, D.C. (2006). Regulation of epithelial sodium channels by the ubiquitin-proteasome proteolytic pathway. Am. J. Physiol. Renal Physiol. *290*, F1285–F1294.
27. Bowman, E.J., Siebers, A., and Altendorf, K. (1988). Bafilomycins: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc. Natl. Acad. Sci. USA *85*, 7972–7976.

28. Lehner, P.J., Hoer, S., Dodd, R., and Duncan, L.M. (2005). Downregulation of cell surface receptors by the K3 family of viral and cellular ubiquitin E3 ligases. Immunol. Rev. *207*, 112–125.
29. Mittal, S., and Davis, K.R. (1995). Role of the phytotoxin coronatine in the infection of Arabidopsis thaliana by Pseudomonas syringae pv. tomato. Mol. Plant Microbe Interact. *8*, 165–171.
30. Lin, N.C., and Martin, G.B. (2005). An avrPto/avrPtoB mutant of Pseudomonas syringae pv. tomato DC3000 does not elicit Pto-mediated resistance and is less virulent on tomato. Mol. Plant Microbe Interact. *18*, 43–51.
31. de Torres, M., Mansfield, J.W., Grabov, N., Brown, I.R., Ammouneh, H., Tsiamis, G., Forsyth, A., Robatzek, S., Grant, M., and Boch, J. (2006). Pseudomonas syringae effector AvrPtoB suppresses basal defence in Arabidopsis. Plant J. *47*, 368–382.
32. Wu, A.-J., Andriotis, V.M.E., Durrant, M.C., and Rathjen, J.P. (2004). A patch of surface-exposed residues mediates negative regulation of immune signaling by tomato pto kinase. Plant Cell *16*, 2809–2821.
33. Taylor, S.S., Radzio-Andzelm, E., and Hunter, T. (1995). How do protein kinases discriminate between serine/threonine and tyrosine? Structural insights from the insulin receptor protein- tyrosine kinase. FASEB J. *9*, 1255–1266.
34. Shiu, S.H., and Bleecker, A.B. (2003). Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. Plant Physiol. *132*, 530–543.
35. Erbs, G., Silipo, A., Aslam, S., De Castro, C., Liparoti, V., Flagiello, A., Pucci, P., Lanzetta, R., Parrilli, M., Molinaro, A., et al. (2008). Peptidoglycan and muropeptides from pathogens Agrobacterium and Xanthomonas elicit plant innate immunity: Structure and activity. Chem. Biol. *15*, 438–448.
36. Talaga, P., Fournet, B., and Bohin, J.P. (1994). Periplasmic glucans of Pseudomonas syringae pv. syringae. J. Bacteriol. *176*, 6538–6544.
37. Dunne, W.M., Jr. (2002). Bacterial adhesion: Seen any good biofilms lately? Clin. Microbiol. Rev. *15*, 155–166.
38. Zhang, X.C., Wu, X., Findley, S., Wan, J., Libault, M., Nguyen, H.T., Cannon, S.B., and Stacey, G. (2007). Molecular evolution of lysin motif-type receptor-like kinases in plants. Plant Physiol. *144*, 623–636.
39. Radutoiu, S., Madsen, L.H., Madsen, E.B., Felle, H.H., Umehara, Y., Gronlund, M., Sato, S., Nakamura, Y., Tabata, S., Sandal, N., et al. (2003). Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature *425*, 585–592.
40. Limpens, E., Franken, C., Smit, P., Willemse, J., Bisseling, T., and Geurts, R. (2003). LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science *302*, 630–633.