A family of conserved bacterial effectors inhibits salicylic acid-mediated basal immunity and promotes disease necrosis in plants

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Salicylic acid (SA)-mediated host immunity plays a central role in combating microbial pathogens in plants. Inactivation of SA-mediated immunity, therefore, would be a critical step in the evolution of a successful plant pathogen. It is known that mutations in conserved effector loci (CEL) in the plant pathogens *Pseudomonas syringae* (the ΔCEL mutation), *Erwinia amylovora* (the *dspA/E* mutation), and *Pantoea stewartii* subsp. *stewartii* (the *wtsE* mutation) exert particularly strong negative effects on bacterial virulence in their host plants by unknown mechanisms. We found that the loss of virulence in ΔCEL and *dspA/E* mutants was linked to their inability to suppress cell wall-based defenses and to cause normal disease necrosis in *Arabidopsis* and apple host plants. The ΔCEL mutant activated SA-dependent callose deposition in wild-type *Arabidopsis* but failed to elicit high levels of callose-associated defense in *Arabidopsis* plants blocked in SA accumulation or synthesis. This mutant also multiplied more aggressively in SA-deficient plants than in wild-type plants. The *hopPtoM* and *avrE* genes in the CEL of *P. syringae* were found to encode suppressors of this SA-dependent basal defense. The widespread conservation of the HopPtoM and AvrE families of effectors in various bacteria suggests that suppression of SA-dependent basal immunity and promotion of host cell death are important virulence strategies for bacterial infection of plants.

Plant immunity to pathogen infections is believed to have contributed to the remarkable success of land plants on earth. Accumulation of the signaling molecule salicylic acid (SA) is associated with many immune responses in plants, such as systemic acquired resistance, basal resistance, and even gene-for-gene resistance, playing a central role in plant defense to many microbial pathogens (1–4). SA-mediated immunity involves increased expression of a suite of responsive genes and subsequent, largely undefined, cellular changes that collectively inhibit pathogen multiplication. Despite the presence of powerful plant defense responses, numerous microbes have evolved the ability to colonize plants and often cause devastating diseases. Given the importance of SA-mediated plant immunity, successful pathogens are expected to have evolved virulence mechanisms to evade or suppress SA-mediated host immunity. To date, however, no known pathogen virulence mutants have been shown to be defective in evading or suppressing this important host defense.

Many Gram-negative plant and mammalian bacteria evolved an essential virulence system, the conserved type III secretion system (TTSS), to inject virulence effector proteins into the host cell, promoting disease development (5–8). TTSS genes are often clustered in a large pathogenicity island (PAI). Although direct evidence is lacking, acquisition of a TTSS PAI through horizontal gene transfer is believed to have played a particularly important role in the evolution of bacteria as aggressive pathogens of plants and animals.

Erwinia amylovora and *Pseudomonas syringae* are important necrosis-causing bacterial pathogens of plants. *E. amylovora* causes severe systemic infection in rosaceous plants, killing leaves, stems, and roots, whereas *P. syringae* infects a wide spectrum of plant species, most strains causing local necrotic lesions in infected tissues. Despite the differences, the two bacteria contain similar TTSS PAIs, including a partially overlapping conserved effector locus (CEL) (9, 10). The CEL of *P. syringae* pv. *tomato* (*Pst*) strain DC3000 contains at least four characterized effector genes [*avrE*, *hopPtoM*, *hrpW*, and *hopPtoA1* (formerly CEL *ORF5*)] that are conserved among diverse *P. syringae* pathovars (11). Individual mutations in these effector genes do not significantly affect bacterial multiplication (12–15). Yet, deletion of these four effector genes as well as *shcM* (the chaperone gene for *hopPtoM*; ref. 12) and *ORF2* (a putative chaperone gene for *avrE*) in the ΔCEL mutant results in drastically reduced virulence in tomato (11). The *E. amylovora* CEL contains *dspA/E* (an orthologue of *avrE*) and *hrpW* (9, 10). Mutations in the *dspA/E* gene abolish the pathogenicity of *E. amylovora* in apple (9, 10). Similarly, mutations in the *dspE* gene of *Pantoea agglomerans* pv. *gypsophilae* and the *wtsE* gene (homologous to *avrE*) of *Pantoea stewartii* subsp. *stewartii* result in great reductions of bacterial virulence in gypsophila and corn, respectively (16, 17). The molecular basis of the loss of virulence in these bacterial mutants is not understood.

In this study, we used a combination of cytological examination, characterization of host and bacterial mutants, and host gene expression analysis to study the functions of AvrE, HopPtoM, and DspA/E in *Arabidopsis–Pst* DC3000 and apple–*E. amylovora* interactions. We show that these conserved bacterial effectors play a dual role in the inhibition of SA-mediated basal immunity and promotion of disease necrosis.

Materials and Methods

Plant Growth and Bacteria Enumeration. Methods for plant growth and bacteria enumeration were described previously (18, 19). Unless specified, all experiments reported in this paper were performed at least three times with similar results.

Callose Staining. Callose staining was performed 7–9 h after bacterial inoculation as described previously, except that no dexamethasone was used (18, 20). Leaves were examined with a Zeiss Axiophot D-7082 Photomicroscope with an A3 fluorescence cube. The number of callose depositions was determined with IMAGEPRO PLUS software (Media Cybernetics, Silver Spring, MD). The values provided are the averages and standard deviations of more than four independent leaves for each treatment.

Bacterial Mutants and Recombinant Plasmids. The following wild-type (WT) and mutant bacterial strains were used: *Pst* DC3000 (WT; provided by Diane Cuppels, Southern Crop Protection and

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Abbreviations: SA, salicylic acid; CEL, conserved effector locus; *Pst*, *Pseudomonas syringae* pv. *tomato*; HR, hypersensitive response; cfu, colony-forming units.

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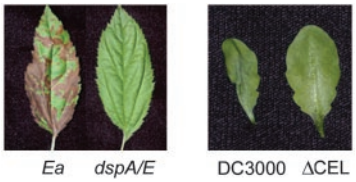

Fig. 1. Responses of apple (cultivar Jonathan, *Left*) and *Arabidopsis thaliana* (ecotype Col-0 *gl1*, *Right*) to infiltration of high concentrations of bacteria suspensions (OD_{600} of 0.2). Apple leaves inoculated with WT *E. amylovora* (*Ea*) showed disease necrosis (brown color) 17–18 h after inoculation, whereas apple leaves inoculated with the *dspA/E* mutant did not show any necrosis in two experiments and a delayed, very spotty necrosis at 24 h in one experiment. *Arabidopsis* leaves inoculated with DC3000 collapsed (shrunken appearance in this picture) 22–24 h after inoculation, whereas *Arabidopsis* leaves inoculated with the ΔCEL mutant showed delayed disease necrosis at 28–30 h after inoculation. Pictures were taken at 24 h after inoculation.

Food Research Centre, London ON, Canada), *E. amylovora* CFBP1430 (WT; ref. 10), *Pst* DC3000 ΔCEL mutant (11), *Pst* DC3000 *hrpA*$^-$ mutant (21), and *E. amylovora dspA/E*$^-$ mutant (10). Construction of pORF43, which carries *hopPtoM–shcM* in pUCP19, was described previously (12). pEF carries a 6,329-bp *Afl*II(blunted)–*Kpn*I fragment, which contains *avrE–ORF2*, in the *Bam*HI(blunted)–*Kpn*I sites of pDSK519-P_{avrPto}. pDSK519-P_{avrPto} is a pDSK519 (22) derivative containing the promoter region of $avrPto_{DC3000}$. The AvrPto promoter was amplified by using *Pst* DC3000 genomic DNA, primer A (5′-CTGCATGC-(*Sph*I site)GGTCCAGGAAATGAATACCCAG-3′), and primer B (5-ATGGATCC(*Bam*HI)CATATG(*Nde*I)TATAC-CCTCTTTAGTACAG-3′), and was cloned into the *Sph*I–*Bam*HI sites of pDSK519. In pEF, *avrE* expression was controlled by its native promoter, and *ORF2* expression was controlled by the heterologous *avrPto* promoter. pW5 carries a 4,294-bp *Stu*I(blunted)–*Bam*HI fragment, which contains *hrpW–hopPtoA1*, in the *Pst*I(blunted)–*Bam*HI sites of pDSK519.

RNA Blot Analysis. Total RNA was extracted from leaves by using the RNAgents isolation kit and following the manufacturer's instructions (Promega). Total RNA was fractionated on formaldehyde gels, blotted onto nylon membranes, and hybridized with radioactively labeled probes as previously described (23).

Results

CEL-Encoded Effectors and DspA/E Are Required for Disease Necrosis in Compatible *Arabidopsis–Pst* DC3000 and Apple–*E. amylovora* Interactions. Recent studies suggested that several type III effectors promote disease by suppressing the hypersensitive response (HR), a rapid cell death response mainly associated with host-range restriction of pathogens (24–31). HR cell death occurs much faster than disease-associated cell death in the same plant. If DspA/E and CEL-encoded effectors functioned as HR suppressors, we would expect to observe a more rapid, HR-like cell death response in host leaves infiltrated with a high concentration (OD_{600} = 0.2) of the *dspA/E* or ΔCEL mutant compared with leaves infiltrated with the same concentration of the WT bacteria. However, neither the *dspA/E* mutant nor the *Pst* DC3000 CEL mutant elicited an accelerated cell death in leaves of apple or *Arabidopsis*, respectively. On the contrary, both mutants caused much-delayed cell death, as indicated by slower tissue necrosis, compared with their parental WT bacteria, even at this high bacterial concentration (Fig. 1). This result shows that CEL-encoded effectors and DspA/E are not involved in the suppression of an HR. On the contrary, DspA/E and CEL-encoded effectors are required for the development of host cell death that is associated with disease necrosis.

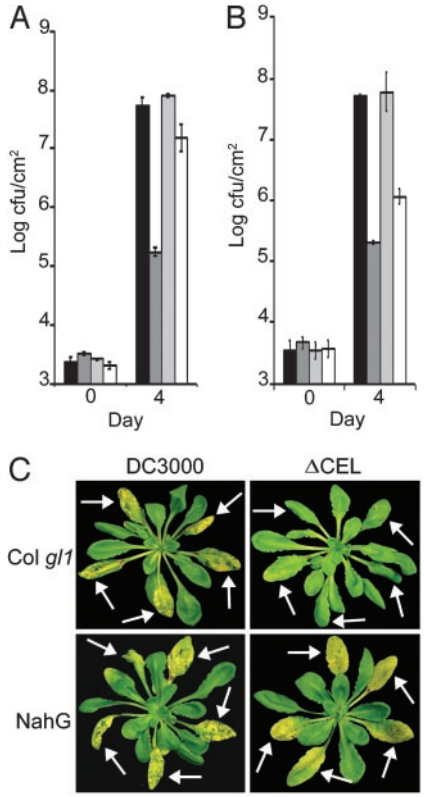

Fig. 2. Enhanced growth of the ΔCEL mutant in SA-impaired plants. (*A*) DC3000 growth in Col-0 (black bars) and NahG (light gray bars) plants. ΔCEL mutant growth in Col-0 (dark gray bars) and NahG (white bars) plants. cfu, Colony-forming units. (*B*) DC3000 growth in WT (black bars) and *eds5* (light gray bars) plants. ΔCEL mutant growth in WT (dark gray bars) and *eds5* (white bars) plants. (*C*) Disease symptoms of Col-0 and NahG leaves infected by DC3000 or the ΔCEL mutant (OD_{600} = 0.002). Arrows indicate inoculated leaves.

Increased Multiplication of the ΔCEL Mutant in *Arabidopsis* Plants Affected in SA Accumulation or Synthesis. We next examined the growth of the ΔCEL mutant in several *Arabidopsis* genotypes that are defective in host-defense pathways. We found that the ΔCEL mutant consistently multiplied to a higher level and caused more pronounced chlorosis and necrosis in NahG plants than in WT Col-0 plants (Fig. 2 *A* and *C*). NahG plants cannot accumulate SA, owing to the degradation of SA by *nahG*-encoded SA hydroxylase (32). The ΔCEL mutant also multiplied more efficiently in the *eds5* mutant, which is defective in the synthesis of SA upon pathogen infection (33), but the increase was modest compared with that in NahG plants (Fig. 2*B*). Recent studies suggest that *eds5* and NahG plants may be affected in overlapping but distinct pools of SA (34) and that catechol, the product of NahG-mediated degradation of SA, may partially contribute to the loss of nonhost plant resistance (35). These observations may explain the more significant increase of ΔCEL mutant growth in NahG plants. In contrast, no significant increase in ΔCEL mutant growth was detected in *ein2* plants, which are defective in ethylene-mediated host defenses (36), compared with that in Col-0 plants (data not shown). This result suggests that, unlike the SA pathway, the ethylene-response pathway is not involved in CEL effector-mediated suppression of host defenses.

ΔCEL and *dspA/E* Mutants Activate Host Cell Wall-Based Defense. Even though *Arabidopsis* leaves inoculated with the ΔCEL mutant bore few macroscopic disease symptoms, we found, by cytological examination, that these leaves had a much higher

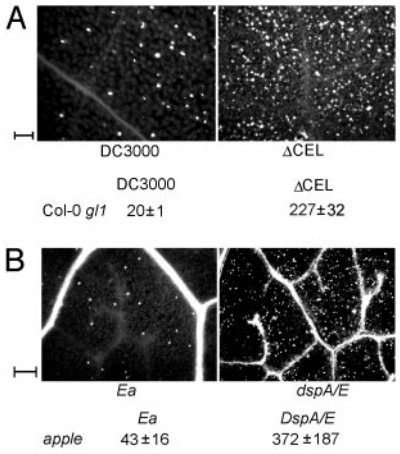

Fig. 3. Callose deposition in *Arabidopsis* and apple leaves. (*A*) Portions of *Arabidopsis* Col-0 *gl1* leaves stained for callose deposits (white dots in these images) after inoculation with WT DC3000 or the ΔCEL mutant. (*B*) Portions of WT apple leaves stained to show callose deposition after inoculation with WT *Erwinia amylovora* (*Ea*) or the *dspA/E* mutant. Below leaf images, average numbers of callose deposits per field of view (0.9 mm^2) are displayed with standard deviations as errors. (Scale bar, 100 μm.)

level (≈10-fold) of defense-associated callose deposition in the host cell wall than did leaves inoculated with *Pst* DC3000 (Fig. 3*A*). Similarly, we found that apple leaves inoculated with the *dspA/E* mutant had a much higher level (≈10-fold) of callose deposition in the host cell wall than did those inoculated with the WT bacteria (Fig. 3*B*). Thus, mutations in effector genes within the CELs of two different pathogens, *Pst* DC3000 and *E. amylovora,* result in greatly reduced virulence. This reduction in virulence correlated with a severe impairment in their ability to suppress host cell wall-based defense and to promote disease necrosis.

The ΔCEL Mutant Is Unable to Suppress SA-Dependent Basal Immunity. A previous study showed that the *Pst* DC3000 *hrcC* mutant, which cannot secrete any of the >40 effectors, activated cell wall-based callose deposition in *Arabidopsis* (18). Furthermore, this cell wall defense was effectively suppressed by transgenic expression of the DC3000 effector AvrPto. Global *Arabidopsis* gene expression analysis suggested that the *hrcC* mutant-induced and AvrPto-suppressed cell wall defense was SA-independent (18).

To characterize the nature of cell wall-based basal immunity elicited by the ΔCEL mutant, we examined the SA-dependence of cell wall defenses activated by *hrp/hrc* and ΔCEL mutants in *Arabidopsis* plants. We found that the *hrpA* mutant elicited very high levels of callose deposition in Col-0, NahG, and *eds5* leaves (Fig. 4 *A* and *D*), confirming the SA-independent nature of cell wall-based immunity elicited by *hrp/hrc* mutants. In contrast, the ΔCEL mutant elicited strong callose deposition only in Col-0 leaves, not in NahG (Fig. 4*B*) or *eds5* mutant (Fig. 4*E*) plants. It is interesting to note that in Col-0 leaves, the ΔCEL mutant elicited consistently less callose deposition than the *hrpA* mutant (Fig. 4 *G* and *H*), suggesting that AvrPto and other effectors in the ΔCEL mutant may still be partially repressing callose-associated defense. As a control, WT *Pst* DC3000 suppressed callose deposition in the host cell wall irrespective of host genotypes, giving similarly low levels of callose deposition in leaves of Col-0, NahG, and *eds5* (Fig. 4 *C*, *F*, *G*, and *H*). Taken together, these results clearly demonstrate that the ΔCEL mutant is not able to suppress SA-mediated cell wall defense, which is distinct from *hrp/hrc* mutant-activated and AvrPto-suppressed SA-independent cell wall defense.

CEL-Encoded Effectors Suppress SA-Dependent Basal Immunity Without Affecting the Expression of Known SA-Responsive Defense Genes. Genome-wide microarray analysis was conducted to further characterize SA-dependent activation and inactivation of cell wall-based immunity in *Arabidopsis* by WT *Pst* DC3000 and the ΔCEL mutant (see *Supporting Materials and Methods*, which is

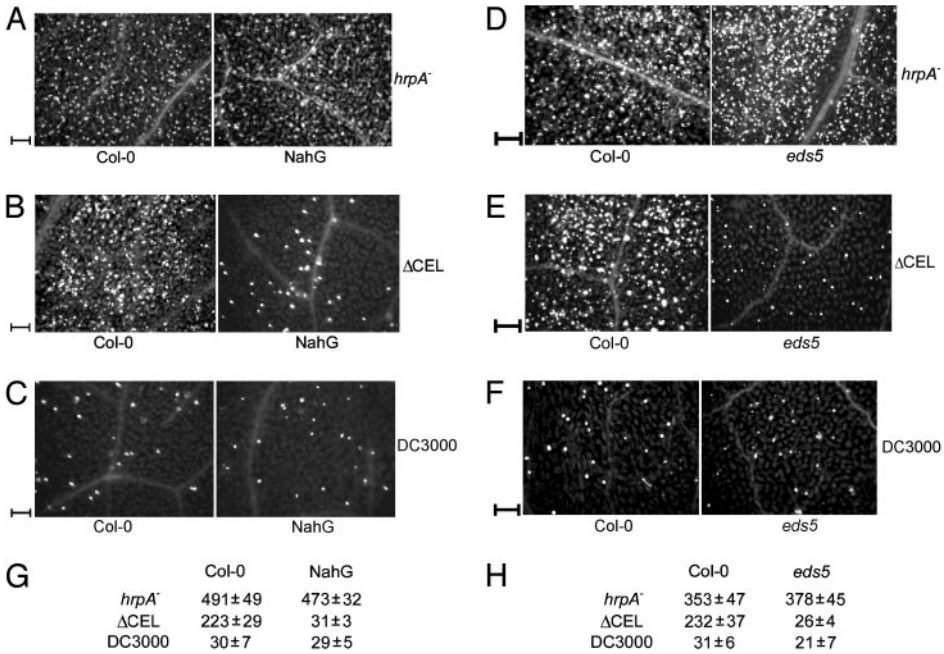

Fig. 4. Callose deposition in *Arabidopsis* leaves. (*A–C*) Portions of *Arabidopsis* Col-0 and NahG leaves stained to show callose deposition after inoculation with the *hrpA* mutant (*A*), the ΔCEL mutant (*B*), and WT DC3000 (*C*). (*D–F*) Portions of *Arabidopsis* Col-0 and *eds5* leaves stained for callose after inoculation with the *hrpA* mutant (*D*), the ΔCEL mutant (*E*), and WT DC3000 (*F*). (*G* and *H*) Average numbers of callose depositions per field of view (0.9 mm^2) with standard deviations displayed as errors. (Scale bar, 100 μm.)

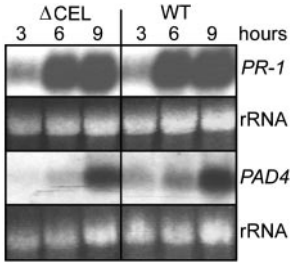

Fig. 5. RNA gel blot analysis of *PR-1* and *PAD4* expression 3, 6, and 9 h after infiltration of 1×10^8 cfu/ml ΔCEL mutant (ΔCEL) and DC3000 (WT). Below each autoradiogram is a picture of the ethidium bromide-stained 28S ribosomal RNA.

published as supporting information on the PNAS web site). We used the 22,500-element ATH1 *Arabidopsis* GeneChip (Affymetrix, Santa Clara, CA), which contains nearly all of the *Arabidopsis* genes in the genome. Remarkably, the strong activation and repression of SA-dependent cell wall immunity by the ΔCEL mutant and *Pst* DC3000, respectively, were not accompanied by strong activation and repression of well-known SA-responsive genes, such as *PR* (pathogenesis-related) genes. None of the known SA-responsive genes was expressed at a significantly higher level in ΔCEL mutant-inoculated leaves than in *Pst* DC3000-inoculated leaves (Fig. 8, which is published as supporting information on the PNAS web site). We also conducted Northern blot analysis of leaves inoculated with 10^8 cfu/ml bacteria (100 times higher than concentrations used for the microarray experiments to minimize the bacterial population differences). Again, no significant difference in *PR-1* or *PAD4* gene expression was observed (Fig. 5). Thus, CEL effector-mediated inactivation of the SA-dependent cell wall-based immunity does not involve changes of classical SA-responsive gene expression. However, because the entire SA regulon in *Arabidopsis* has not been defined yet, we cannot exclude the possibility that CEL-encoded effectors modulate expression of some yet-to-be-identified SA-regulated genes.

HopPtoM and AvrE Are Suppressors of SA-Dependent Basal Immunity. In contrast to the single effector mutation in the *E. amylovora dspA/E* mutant (10), the *Pst* DC3000 ΔCEL mutant lacks four known effector genes, *avrE*, *hopPtoM*, *hrpW,* and *hopPtoA1* (11). To identify the specific effector(s) involved in the suppression of SA-dependent cell wall defense in *Arabidopsis*, we carried out subclone-based complementation experiments. Two independent subclones, pEF, which contains *avrE-ORF2*, and pORF43, which contains *hopPtoM-shcM*, partially or completely restored the ability of the ΔCEL mutant to multiply in WT Col-0 *gl1* plants (Fig. 6 *A* and *B*). pORF43 also fully restored the ability of the ΔCEL mutant to cause disease necrosis and chlorosis. In contrast, a subclone containing the *hrpW* and *hopPtoA1* genes neither increased the multiplication nor restored the ability to cause disease symptoms of the ΔCEL mutant in Col-0 *gl1* plants (data not shown).

pEF and pORF43 also partially or completely complemented the ΔCEL mutant for suppression of callose-associated host cell wall defense (Fig. 6 *C–F*). In the chromosome, *avrE* and *ORF2* are encoded by two different, but adjacent, operons with opposite transcriptional directions (11). We had to use the heterologous *avrPto* promoter to drive the expression of *ORF2* from one direction and the native *avrE* promoter to drive the expression of *avrE* from the other direction in pEF (see *Materials and Methods*). The incomplete complementation of the CEL mutation by the *avrE–ORF2* subclone may therefore be attributed to the use of the heterologous *avrPto* promoter. In contrast, the

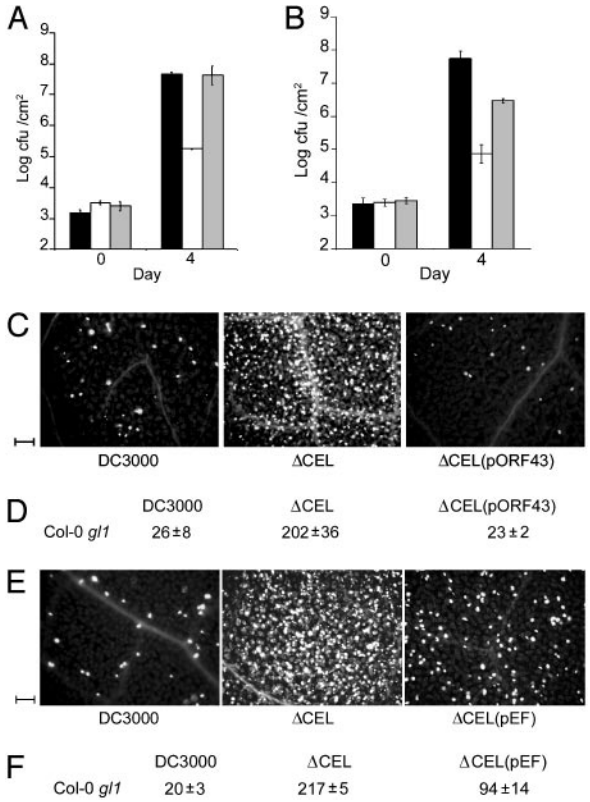

Fig. 6. Complementation of the ΔCEL mutant. (*A*) Bacterial populations in Col-0 *gl1* plants inoculated with DC3000 (black bars), the ΔCEL mutant (white bars), and the ΔCEL mutant complemented with pORF43 (light gray bars). (*B*) Bacterial populations in young leaves of Col-0 *gl1* plants inoculated with DC3000 (black bars), the ΔCEL mutant (white bars), and the ΔCEL mutant complemented with pEF (light gray bars). (*C*) Callose deposits in portions of *Arabidopsis* Col-0 *gl1* leaves inoculated with DC3000, the ΔCEL mutant, and the ΔCEL mutant containing pORF43. (*D*) Average numbers of callose deposits per field of view with standard deviations displayed as errors. (*E*) Callose deposits in portions of *Arabidopsis* Col-0 *gl1* leaves inoculated with DC3000, the ΔCEL mutant, and the ΔCEL mutant containing pEF. (*F*) Average numbers of callose deposits per field of view with standard deviations displayed as errors.

hopPto–shcM genes are encoded by the same operon and we used their native promoter to drive the expression of both genes.

Discussion

Type III effectors secreted through the type III secretion system are now considered to be the primary virulence factors in many plant pathogenic bacteria. Genomic analysis shows that *P. syringae* carries a remarkable repertoire of type III effectors, with the current estimate of at least 40 effectors in strain DC3000 alone (37, 38). However, mutations in most effector genes do not show a large effect on bacterial virulence, presumably because of the functional subtlety and redundancy of most effectors when delivered by bacteria. In contrast to most effectors, mutations in the HopPtoM/AvrE families of effector genes often give a drastic virulence effect (9–11, 16, 17, 39), strongly suggesting that the functions of these particular effectors are not only important but also distinct from the majority of other effectors produced by the same bacterium. The strong virulence loss of the bacterial mutants defective in these effectors also provides a rare opportunity for studies using appropriate bacterial and host mutants in the context of native host–bacteria interactions. We show here that the severe virulence defect of the *Pst* DC3000 ΔCEL mutant is caused by the inability of this mutant to promote

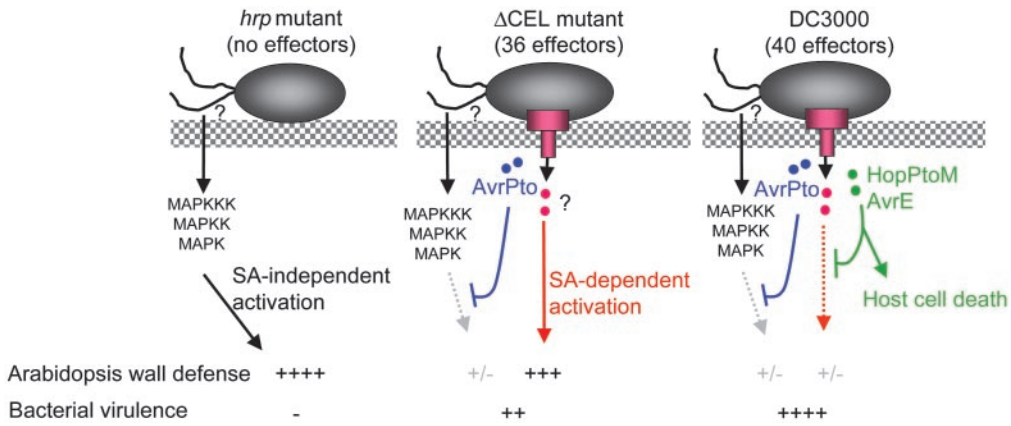

Fig. 7. A hypothetical model for the activation and inactivation of cell wall-based basal immunity during *Pst* DC3000 infection of susceptible *Arabidopsis*. MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase.

disease necrosis and to suppress the SA-dependent host cell wall defense in *Arabidopsis*. We show that the *dspA/E* mutant of *E. amylovora*, like the ΔCEL mutant of *Pst* DC3000, activates a strong cell wall-based response in host leaves, whereas the WT bacteria effectively suppress this defense. Even though we could not determine whether DspA/E-suppressed cell wall defense in apple leaves is also dependent on SA because of the inherent experimental limitations in the apple–*E. amylovora* system, the previously demonstrated functional cross-complementation of DspA/E and AvrE (9) strongly suggests that DspA/E also targets SA-dependent cell wall defense in apple.

We found that both *avrE–ORF2* and *hopPtoM–shcM* can partially or completely complement the growth defect of the ΔCEL mutant in *Arabidopsis*. This result suggests that these two effector–chaperone pairs (AvrE–ORF2 and HopPtoM–ShcM), which do not share any sequence similarities, are at least partially functionally redundant in the *Arabidopsis–Pst* DC3000 interaction. This functional redundancy in *Pst* DC3000 may explain the previously observed lack of a strong bacterial growth phenotype in tomato plants of either the *avrE* mutant or the *hopPtoM* mutant alone (12, 13), although there may also be tomato- or *Arabidopsis*-specific reactions. For example, even though the *hopPtoM* mutant is not affected in bacterial multiplication in tomato, it is reduced in causing disease symptoms in this host (12). On the other hand, the drastic effect of the *dspA/E* mutation in *E. amylovora* can now be explained by the apparent lack of a functionally redundant effector, *hopPtoM*, within the CEL of *E. amylovora*.

A very recent study suggests that AvrRpt2 from *Pst* strain JL1065, which presumably also carries a CEL similar to that of *Pst* DC3000, might function downstream or independently of SA to promote virulence in the *Arabidopsis–Pst* DC3000 interaction (40). We therefore tested the ability of AvrRpt2 to complement the *Pst* DC3000 ΔCEL mutant in *rps2*$^{-}$ *Arabidopsis* plants (41). However, AvrRpt2 did not increase the ability of the ΔCEL mutant to multiply or to cause disease symptoms in *rps2*$^{-}$ *Arabidopsis* plants (data not shown), suggesting that the host target(s) of AvrRpt2 is different from those of HopPtoM and AvrE. Interestingly, the general virulence function of AvrRpt2 appears to be independent of the ability of AvrRtp2 to suppress the RPM1-dependent HR in *Arabidopsis* and may involve modulation of the auxin-response pathway (40).

Our demonstration of the HopPtoM/AvrE-mediated suppression of the SA-dependent basal immunity and promotion of disease necrosis likely has broad implications for our understanding of bacterial pathogenesis in diverse plants. This is because effectors of the HopPtoM/AvrE family appear to be among the most widespread in plant-pathogenic bacteria. For example, *avrE*-homologous genes are found in several plant pathogenic bacteria (Table 1, which is published as supporting information on the PNAS web site), whereas *hopPtoM* is apparently found in all examined *P. syringae* pathovars (11). The widespread presence of the HopPtoM and AvrE families of effectors in many pathogens therefore suggests a common need of plant-pathogenic bacteria to target conserved host pathways. Results presented here suggest that SA-dependent basal immunity and disease necrosis are two conserved host pathways targeted by potentially all members of the HopPtoM and AvrE families in different bacteria.

The requirement of the HopPtoM and AvrE families of effectors for promoting disease necrosis during pathogenesis (Fig. 1) is worthy of further discussion. Two previous studies show that inhibiting host cell death negatively affects the pathogenesis of *Pseudomonas syringae* pv. *maculicola* ES4326 and *Pst* T1 in *Arabidopsis* and tomato, respectively, suggesting that *P. syringae* might be partly a necrotrophic pathogen (42, 43). As a necrotrophic pathogen, *Pst* DC3000 would benefit from nutrient release associated with host cell death. It is therefore possible that the high multiplication ability of WT CEL^{+} bacteria in plants results from the additive activities of CEL-encoded effectors in suppressing host defense and promoting nutrient leakage associated with host cell death. Such a dual virulence function could result from these effectors having multiple targets in the host or a single host target that is involved in both host cellular immunity and host cell survival.

To date, the most extensively characterized aspect of SA-mediated immunity is probably the expression of SA-responsive genes. However, yet to be elucidated are the entire SA-controlled defense network (including basal and inducible) and, more importantly, the specific host cellular modifications during SA-mediated immunity that ultimately inhibit bacterial pathogens. Study of HopPtoM/AvrE/DspA/E-mediated virulence functions may provide critical information about those particular SA-dependent host cellular changes that are actually responsible for the elusive bacterial resistance mechanism in plants. We show that CEL-mediated suppression of the SA-dependent basal immunity is not associated with a significant down-regulation of the expression of classical *PR* genes. Thus, HopPtoM and AvrE may act on SA-dependent basal immunity at a cellular process either downstream of or parallel to the expression of these known *PR* genes.

Results from our previous work on AvrPto (18) and the current work on AvrE and HopPtoM in the *Arabidopsis–Pst* DC3000 pathosystem illustrate that plant cell wall-based basal

immunity is a key battlefield that has been subjected to repeated activation and inactivation by bacterial pathogens via SA-independent and SA-dependent plant-signaling pathways (Fig. 7). *hrp* mutants activate cell wall-based basal defense in an SA-independent manner, likely via the MAPK3/6 pathway, through perception of flagellin and other pathogen-associated molecular patterns (44). In the ΔCEL mutant, the AvrPto class of effectors inactivates the SA-independent basal defense. However, the host cell partially overcomes the AvrPto-mediated inactivation of SA-independent basal defense and activates an SA-dependent basal defense pathway. The bacterial factors involved in this SA-dependent activation are not known, but they may be type III effectors or the type III secretion process *per se*. In DC3000, HopPtoM and AvrE inactivate the SA-dependent basal defense and promote disease-associated host cell death.

In conclusion, we have identified a key group of conserved type III effectors in plant-pathogenic bacteria that target SA-dependent basal immunity and promote disease necrosis in plants. This finding provides an attractive explanation for the strict requirement of the HopPtoM and AvrE families of effectors for bacterial virulence in multiple pathosystems. Our results suggest that a synergistic combination of suppression of SA-dependent basal immunity and promotion of disease necrosis may be key to the aggressive colonization of plants by *P. syringae*, *E. amylovora*, and perhaps other necrosis-causing plant-pathogenic bacteria.

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1. Dangl, J. L. & Jones, J. D. (2001) *Nature* **411,** 826–833.
2. Dong, X. (2001) *Curr. Opin. Plant Biol.* **4,** 309–314.
3. Glazebrook, J. (2001) *Curr. Opin. Plant Biol.* **4,** 301–308.
4. Kunkel, B. N. & Brooks, D. M. (2002) *Curr. Opin. Plant Biol.* **4,** 325–331.
5. He, S. Y. (1998) *Annu. Rev. Phytopathol.* **36,** 363–392.
6. Galan, J. E. & Collmer, A. (1999) *Science* **284,** 1322–1328.
7. Cornelis, G. R. & Van Gijsegem, F. (2000) *Annu. Rev. Microbiol.* **54,** 735–774.
8. Buttner, D. & Bonas, U. (2002) *EMBO J.* **21,** 5313–5322.
9. Bogdanove, A. J., Kim, J. F., Wei, Z., Kolchinsky, P., Charkowski, A. O., Conlin, A. K., Collmer, A. & Beer, S. V. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 1325–1330.
10. Gaudriault, S., Malandrin, L., Paulin, J. P. & Barny, M. A. (1997) *Mol. Microbiol.* **5,** 1057–1069.
11. Alfano, J. R., Charkowski, A. O., Deng, W. L., Badel, J. L., Petnicki-Ocwieja, T., van Dijk, K. & Collmer, A. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 4856–4861.
12. Badel, J. L., Nomura, K., Bandyopadhyay, S., Shimizu, R., Collmer, A. & He, S. Y. (2003) *Mol. Microbiol.* **49,** 1239–1251.
13. Lorang, J. M. & Keen, N. T. (1995) *Mol. Plant-Microbe Interact.* **1,** 49–57.
14. Charkowski, A. O., Alfano, J. R., Preston, G., Yuan, J., He, S. Y. & Collmer, A. (1998) *J. Bacteriol.* **180,** 5211–5217.
15. Badel, J, L., Charkowski, A. O., Deng, W. L. & Collmer, A. (2002) *Mol. Plant–Microbe Interact.* **15,** 1014–1024.
16. Mor, H., Manulis, S., Zuck, M., Nizan, R., Coplin, D. L. & Barash, I. (2001) *Mol. Plant–Microbe Interact.* **3,** 431–436.
17. Frederick, R. D., Ahmad, M., Majerczak, D. R., Arroyo-Rodriguez, A. S., Manulis, S. & Coplin, D. L. (2001) *Mol. Plant–Microbe Interact.* **10,** 1213–1222.
18. Hauck, P., Thilmony, R. & He, S. Y. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 8577–8582.
19. Katagiri, F., Thilmony, R. & He, S. Y. (Sept. 30, 2002) in *The Arabidopsis Book*, eds. Somerville, C. R. & Meyerowitz, E. M. (Am. Soc. Plant Biologists, Rockville, MD), 10.1199/tab.0039, www.aspb.org/publications/arabidopsis/.
20. Adam, L. & Somerville, S. C. (1996) *Plant J.* **9,** 341–356.
21. Wei, W., Plovanich-Jones, A., Deng, W.-L., Collmer, A., Huang, H.-C. & He, S. Y. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 2247–2252.
22. Keen, N. T., Tamaki. S., Kobayashi, D., Trollinger, D. (1988) *Gene* **70,** 191–197.
23. Zhao, Y., Thilmony, R., Bender, C.L., Schaller, A., He, S. Y. & Howe, G. A. (2003) *Plant J.* **36,** 485–499.
24. Jackson, R. W., Athanassopoulos, E., Tsiamis, G., Mansfield, J. W., Sesma, A., Arnold, D. L., Gibbon, M. J., Murillo, J., Taylor, J. D. & Vivian, A. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 10875–10880.
25. Tsiamis, G., Mansfield, J. W., Hockenhull, R., Jackson, R. W., Sesma, A., Athanassopoulos, E., Bennett, M. A., Stevens, C., Vivian, A., Taylor, J. D. & Murillo, J. (2000) *EMBO J.* **19,** 3204–3214.
26. Mackey, D., Belkhadir, Y., Alonso, J. M., Ecker, J. R. & Dangl, J. L. (2003) *Cell* **112,** 379–389.
27. Axtell, M. J. & Staskawicz, B. J. (2003) *Cell* **112,** 369–377.
28. Abramovitch, R. B., Kim, Y. J., Chen, S., Dickman, M. B. & Martin, G. B. (2003) *EMBO J.* **22,** 60–69.
29. Jamir, Y., Guo, M., Oh, H. C., Petnicki-Ocwieja, T., Chen, S., Tang, X., Dickman, M. B., Collmer, A. & Alfano, J. R. (2004) *Plant J.* **37,** 554–565.
30. Espinosa, A., Guo, M., Tam, V. C., Fu, Z. Q. & Alfano, J. R. (2003) *Mol. Microbiol.* **49,** 377–387.
31. Bretz, J. R., Mock, N. M., Charity, J. C., Zeyad, S., Baker, C. J. & Hutcheson, S. W. (2003) *Mol. Microbiol.* **49,** 389–400.
32. Delaney, T. P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gur-Rella, M., Kessmann, H., Ward, E. & Ryals, J. (1994) *Science* **266,** 1247–1250.
33. Nawrath, C., Heck, S., Parinthawong, N. & Metraux, J. P. (2002) *Plant Cell* **14,** 275–286.
34. Ferrari, S., Plotnikova, J. M., De Lorenzo, G. & Ausubel, F. M. (2003) *Plant J.* **2,** 193–205.
35. van Wees, S. C. & Glazebrook, J. (2003) *Plant J.* **4,** 733–742.
36. Alonso, J. M., Hirayama, T., Roman, G., Nourizadeh, S. & Ecker, J. R. (1999) *Science* **5423,** 2148–2152.
37. Collmer, A., Lindeberg, M., Petnicki-Ocwieja, T., Schneider, D. J. & Alfano, J. R. (2002) *Trends Microbiol.* **10,** 462–469.
38. Greenberg, J. T. & Vinatzer, B. A. (2003) *Curr. Opin. Microbiol.* **1,** 20–28.
39. Lorang, J. M., Shen, H., Kobayashi, D., Cooksey, D. & Keen, N.T. (1994) *Mol. Plant–Microbe Interact.* **7,** 508–515.
40. Chen, Z., Kloek, A. P., Cuzick, A., Moeder, W., Tang, D., Innes, R. W., Klessig, D. F., McDowell, J. M. & Kunkel, B. N. (2004) *Plant J.* **4,** 494–504.
41. Kunkel, B. N., Bent, A. F., Dahlbeck, D., Innes, R. W. & Staskawicz, B. J. (1993) *Plant Cell* **15,** 865–875.
42. Stone, J. M., Heard, J. E., Asai, T. & Ausubel, F. M. (2000) *Plant Cell* **10,** 1811–1822.
43. Lincoln, J. E., Richael, C., Overduin, B., Smith, K., Bostock, R. & Gilchrist, D. G. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 15217–15221.
44. Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., Boller, T., Ausubel, F. M. & Sheen, J. (2002) *Nature* **415,** 977–983.