The Xanthomonas Type III Effector XopD Targets the Arabidopsis Transcription Factor MYB30 to Suppress Plant Defense

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INTRODUCTION

Plant and animal pathogens inject type III effectors (T3Es) into host cells to suppress host immunity and promote successful infection. XopD, a T3E from Xanthomonas campestris pv vesicatoria, has been proposed to promote bacterial growth by targeting plant transcription factors and/or regulators. Here, we show that XopD from the B100 strain of X. campestris pv campestris is able to target MYB30, a transcription factor that positively regulates Arabidopsis thaliana defense and associated cell death responses to bacteria through transcriptional activation of genes related to very-long-chain fatty acid (VLCFA) metabolism. XopD specifically interacts with MYB30, resulting in inhibition of the transcriptional activation of MYB30 VLCFA-related target genes and suppression of Arabidopsis defense. The helix-loop-helix domain of XopD is necessary and sufficient to mediate these effects. These results illustrate an original strategy developed by Xanthomonas to subvert plant defense and promote development of disease.

Pathogen-induced transcriptional regulation in host cells plays a crucial role in the establishment of plant defense and associated cell death responses. Indeed, members of various TF families have been reported to be involved in defense gene regulation in Arabidopsis thaliana (Eulgem, 2005; Dubos et al., 2010). Among them, the MYB gene MYB30 was previously identified as being activated in Arabidopsis plants inoculated with the strain 147 of X. campestris pv. campestris (Xcc) (Daniel et al., 1999). Overexpression of MYB30 led to accelerated and intensification of HR in response to avirulent pathogens and to the induction of an HR-like response upon treatment with virulent strains. Enhanced resistance to pathogens and accumulation of HR molecular markers in these plants indicated that MYB30 is a positive regulator of the signaling pathway controlling the establishment of cell death in response to pathogens (Vailleau et al., 2002). Putative MYB30 target genes are involved in the lipid biosynthesis pathway that leads to the production of very-long-chain fatty acids (VLCFAs), suggesting a role of this pathway in the control of HR and plant defense responses (Raffaele et al., 2008).

Although a considerable number of bacterial T3Es have been identified during the last few years, few data on their host targets have been reported. Here, we identify MYB30 as a plant target of XopD, which induces MYB30 relocation to nuclear foci. Furthermore, the specific interaction between the two proteins results in repression of MYB30 transcriptional activity and reduced defense and cell death–associated responses. Together, our data suggest that targeting MYB30 and blocking its activity may represent a strategy developed by Xanthomonas to suppress plant defense and promote disease development.

**RESULTS**

**XopD<sub>Xcv</sub> Recruits MYB30 to Nuclear Bodies Where Both Proteins Interact**

TFs and/or regulators of transcription have been proposed to be direct targets of the SUMO protease XopD from the Xcv strain 85-10 (XopD<sub>Xcv</sub>) (Kim et al., 2008). Since MYB30 is a TF identified during Xcc infection, which is SUMOylated in vitro (Okada et al., 2009), we sought out to investigate whether XopD may target MYB30. Arabidopsis MYB30 was previously shown to be homogenously localized in the nucleus, both in Nicotiana benthamiana leaves and Arabidopsis seedlings (Froidure et al., 2010) (Figure 1A), whereas XopD<sub>Xcv</sub> is localized in subnuclear structures, previously referred to as nuclear foci or nuclear bodies (Hotson et al., 2003; Canonne et al., 2010) (Figure 1A). Surprisingly, coexpression of both proteins in N. benthamiana induced MYB30 relocation to XopD<sub>Xcv</sub>-containing nuclear foci (Figure 1A). Two additional Arabidopsis MYB TFs, homogenously distributed within the nucleus when expressed alone (MYB96, which belongs to the MYB30 subgroup in the classification of Arabidopsis MYB TFs, and MYB123, which is an unrelated MYB protein involved in biosynthesis of proanthocyanidins in the seed coat [Dubos et al., 2010]) were also detected in nuclear foci after coexpression with XopD<sub>Xcv</sub> (Figure 1B; see Supplemental Figure 1 online).

Next, we tested whether there is a physical interaction between XopD<sub>Xcv</sub> and MYB30 using Förster resonance energy transfer–fluorescence lifetime imaging microscopy (FRET-FLIM). A significant reduction of the average cyan fluorescent protein (CFP) lifetime was measured in nuclei coexpressing MYB30-CFP and XopD<sub>Xcv</sub>-yellow fluorescent protein venus (YFPv) compared with nuclei expressing MYB30-CFP alone (Table 1). Importantly, this protein interaction was demonstrated not only in nuclear foci but also in the nucleoplasm (outside nuclear foci), where much weaker YFPv and CFP fluorescence was detected. By contrast, despite their colocalization, no protein interaction could be detected between XopD<sub>Xcv</sub>-YFPv and either CFP-tagged MYB96 or MYB123 in or outside nuclear foci (Table 1). The specificity of the XopD<sub>Xcv</sub>/MYB30 interaction was additionally demonstrated using PopP2, a nuclear-localized T3E secreted by Ralstonia solanacearum that, as XopD, belongs to the CE clan of Cysteine proteases (Deslandes et al., 2003). No interaction between the two proteins was detected despite the nuclear colocalization of PopP2 and MYB30 (Figure 1A, Table 1).

These data demonstrate (1) that MYB30 is relocalized to nuclear bodies by XopD<sub>Xcv</sub>, and (2) that the in planta interaction between MYB30 and XopD<sub>Xcv</sub> is specific.

**XopD<sub>Xcv</sub> Negatively Regulates MYB30 Transcriptional Activity**

MYB30 promotes activation of the promoter of the KCS1 gene, involved in the biosynthesis of VLCFAs (Raffaele et al., 2008). Fusion of the KCS1 promoter to the β-glucuronidase (GUS) reporter gene allows quantification of its activation by MYB30 in N. benthamiana. Coexpression of XopD<sub>Xcv</sub> repressed MYB30-mediated transcriptional activation of the KCS1p, whereas coexpression of PopP2 had no effect on MYB30 transcriptional activity (Figure 1C). In an additional specificity test, we used MYB96, which is also able to activate the KCS1p (Seo et al., 2011). Consistent with the lack of protein interaction between XopD<sub>Xcv</sub> and MYB96, coexpression of XopD<sub>Xcv</sub> had no effect on MYB96-mediated activation of the KCS1p (Figure 1E). Finally, immunoblot analysis showed that MYB30 (but not MYB96) accumulation was enhanced in the presence of XopD<sub>Xcv</sub> (Figures 1D and 1F). Consistent with this observation, detection of CFP-tagged MYB30 by confocal microscopy was facilitated by its coexpression with XopD<sub>Xcv</sub>. These results demonstrate the specific effect of XopD<sub>Xcv</sub> on the control of MYB30 transcriptional activity and protein accumulation.

**Structure-Function Analysis of the Interaction between XopD<sub>Xcv</sub> and MYB30**

Since XopD<sub>Xcv</sub> is a modular protein, to determine if a particular XopD domain or activity is necessary for the observed effects on MYB30, the following XopD<sub>Xcv</sub> mutant versions were used: (1) a previously characterized XopD version, deleted from the recently described N-terminal domain (XopD<sub>Xcv</sub>216-760), (2) a point mutant in the HLH domain (XopD<sub>Xcv</sub>216-760-V333P) that is not able to bind DNA, (3) a catalytic mutant in the Cys protease domain (XopD<sub>Xcv</sub>216-760-C685A), and (4) a deleted version lacking the two EAR motifs (XopD<sub>Xcv</sub>216-760 ΔEAR) (Kim et al., 2008) (Figure 2A).
YFP-tagged versions of the four XopD mutant proteins localized to nuclear foci and, when coexpressed with MYB30-CFP, induced MYB30 relocalization to these subnuclear structures (Figure 2D). In addition, all XopD mutants were able to interact with MYB30 in the nucleus (Table 1) and repressed MYB30 transcriptional activation of \textit{KCS1p}, although this effect was weaker than that observed for the XopD\textsubscript{Xcv} full-length protein (Figure 2B). Likewise, weaker repression of salicylic acid (SA)-induced activation of \textit{PR1p} by XopD\textsubscript{Xcv} was previously reported (Canonne et al., 2010). Finally, the four XopD mutants induced stabilization of MYB30 protein expression (Figure 2C).

Together, these data show that XopD-mediated effects on MYB30 are not dependent on either XopD deSUMOylation or DNA binding activities or on the presence of its two EAR-type motifs. It is therefore more likely that the physical interaction between MYB30 and XopD prevents MYB30 from activating its targets.

**Analysis of the Effects of XopD from Strain Xcc8004 on MYB30**

Since \textit{Xcv} is not able to infect \textit{Arabidopsis} (Jones et al., 1998), we next looked for XopD proteins encoded by \textit{X. campestris} strains for which \textit{Arabidopsis} is host. BLASTP analysis using the XopD\textsubscript{Xcv} sequence identified among other hits a virulence protein from the \textit{Xcc} strain 8004 that corresponds to a shorter version of XopD deleted in the N-terminal region (Figure 3B) (Qian et al., 2005; Canonne et al., 2010). XopD\textsubscript{Xcc8004} expression and secretion from its native promoter was shown by HA tagging the 3’ end...
of the xopD coding sequence in the genome of Xcc8004* (carrying an hrgG* mutation to allow constitutive expression of all hrg genes [Wengelnik et al., 1999]) and Xcc8004*ΔhrcV (carrying a deletion in a conserved component of the T3SS [Rossier et al., 2000]) (Figure 3A). No HA-tagged protein was detectable in the untransformed Xcc8004* strain. hrg-dependent secretion of XopDxcc8004 was demonstrated by detection of the protein in culture supernatants of Xcc8004*, but not Xcc8004*ΔhrcV (Figure 3A). Absence of the intracellular chaperone GroEL in the supernatants demonstrated that protein detection in those fractions was not due to bacterial lysis (Figure 3A).

We next investigated the effect of XopDxcc8004 on MYB30. In contrast with XopDxcc, XopDxcc8004 fused to YFPv presented a nearly homogenous nuclear localization in N. benthamiana cells (some cytoplasmic fluorescence is also detected) and did not alter MYB30 homogenous nuclear distribution (Figure 3E). Despite their nuclear colocalization, no interaction between MYB30 and XopDxcc8004 could be detected, either using N- or C-terminally tagged XopDxcc8004 versions (Table 1), which rules out the possibility that the position of the YFPv tag impedes detection of the interaction of XopDxcc8004 with MYB30. Finally, XopDxcc8004 did not significantly affect MYB30-mediated transcriptional activation of KCS1p or MYB30 protein accumulation (Figures 3C and 3D). Together, these data suggest that XopDxcc8004 is not able to target MYB30.

### Table 1. FLIM Measurements Show That MYB30 Physically Interacts with XopD in Nuclear Bodies of N. benthamiana and Arabidopsis Epidermal Cells

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aMean lifetime in nanoseconds.

bTotal number of measured nuclei.

cPercentage of FRET efficiency (E = 1 – tDA/tD) calculated by comparing the lifetime of the donor in the presence of the acceptor (tDA) with its lifetime in the absence of the acceptor (tD).

dP value of the difference between the donor lifetimes in the presence and in the absence of the acceptor (t test).

*Measurements were performed in nuclear areas outside nuclear foci.
MYB30 nuclear localization or interact with MYB30 (Figure 3E, Table 1). Furthermore, as XopD<sub>Xcc8004</sub>, XopD<sub>Xcv</sub><sup>416-760</sup> did not significantly affect MYB30-mediated transcriptional activation of the KSC1<sub>p</sub> or accumulation of the MYB30 protein (Figures 3C and 3D). Second, a chimeric protein was generated by fusing the HLH domain of XopD<sub>Xcv</sub> (XopD<sub>Xcv</sub><sup>216-415</sup>) to the N terminus of XopD<sub>Xcc8004</sub> (XopD<sub>Xcv</sub><sup>216-415</sup>-XopD<sub>Xcc8004</sub>). Third, XopD<sub>Xcv</sub><sup>216-415</sup> was expressed alone. Similarly to XopD<sub>Xcv</sub>, YFP-tagged XopD<sub>Xcv</sub><sup>216-415</sup>-XopD<sub>Xcc8004</sub> and XopD<sub>Xcv</sub><sup>216-415</sup> were localized in nuclear foci. Coexpression of these two proteins with MYB30 induced MYB30 relocation to nuclear foci (Figure 3E). In addition, the protein interaction between MYB30 and XopD<sub>Xcv</sub><sup>216-415</sup>-XopD<sub>Xcc8004</sub> and XopD<sub>Xcv</sub><sup>216-415</sup> was confirmed (Table 1). Finally, both XopD<sub>Xcv</sub><sup>216-415</sup>-XopD<sub>Xcc8004</sub> and XopD<sub>Xcv</sub><sup>216-415</sup> induced repression of MYB30 transcriptional activity and accumulation of MYB30 (Figures 3C and 3D). Together, these data show that the HLH domain of XopD<sub>Xcv</sub> is necessary and sufficient to localize XopD to nuclear bodies and induce XopD interaction with MYB30 leading to repression of its transcriptional activity.

Analysis of the Effects of XopD from Strain XccB100 on MYB30

Since the HLH domain is absent from XopD<sub>Xcc8004</sub>, we continued our analysis with strain B100 of Xcc<sub>B100</sub> (Vorho¨lter et al., 2003), which infects Arabidopsis and is predicted to encode a protein very similar to XopD<sub>Xcv</sub> (i.e., containing an HLH domain) (Canonne et al., 2010) (Figure 3B). As for Xcc8004, we demonstrated that XccB100 was able to produce HA-tagged XopD expressed from its native promoter and secrete the protein in an hrp<sup>-</sup>-dependent manner (Figure 3A).

YFP-v-tagged XopD<sub>XccB100</sub> was localized to nuclear bodies and induced MYB30 nuclear relocalization (Figure 3E). In addition, using FRET-FLIM assays, we showed that XopD<sub>XccB100</sub> interacted with MYB30 (Table 1). Unfortunately, nonspecific binding to beads of both MYB30 and XopD<sub>Xcc8004</sub> prevented us from confirming the specificity of XopD<sub>XccB100</sub>/MYB30 protein interaction using coimmunoprecipitation as an alternative technique to FRET-FLIM (see Methods). However, using an improved YFP-based bimolecular fluorescence complementation system, the specific interaction between AtMYB30 and XopD<sub>XccB100</sub> was confirmed (see Supplemental Figure 2 online).

XopD<sub>XccB100</sub> negatively regulated MYB30-mediated transcriptional activation of KCS1<sub>p</sub> at a similar rate to XopD<sub>Xcv</sub> and induced enhanced MYB30 protein accumulation (Figures 3C and 3D). Interestingly, the nuclear localization of a XopD<sub>XccB100</sub> derivative lacking its HLH domain (XopD<sub>XccB100</sub><sup>437-802</sup>) was similar to that of XopD<sub>Xcc8004</sub> and XopD<sub>Xcv</sub><sup>416-760</sup>. In addition, expression of XopD<sub>XccB100</sub><sup>437-802</sup> did not change MYB30 nuclear localization and failed to interact with MYB30 (Figure 3E, Table 1). Furthermore, XopD<sub>Xcc8004</sub>, XopD<sub>Xcc8004</sub><sup>416-760</sup>, and XopD<sub>XccB100</sub><sup>437-802</sup> did together with the YFP-v-tagged XopD constructs as indicated in (A). The interaction (+) between MYB30-CFP and YFP-v-tagged XopD constructs as determined by FRET-FLIM is indicated. Bars = 15 μm.
Figure 3. Analysis of XopD<sub>Xcc8004</sub> and XopD<sub>XccB100</sub> and Their Effects on MYB30.

(A) Expression analysis of XopD<sub>Xcc8004</sub> (left) and XopD<sub>XccB100</sub> (right). Strains Xcc8004* or XccB100* (1), Xcc8004* (XopD-HA) or XccB100* (XopD-HA) (2), and Xcc8004* ΔhrcV (XopD-HA) or XccB100* ΔhrcV (XopD-HA) (3) were incubated in MOKA rich medium (total extract) or secretion medium (supernatant). Total protein extracts (10-fold concentrated) and trichloroacetic acid–precipitated filtered supernatants (200-fold concentrated) were analyzed by immunoblotting using anti-HA antibodies to detect XopD or anti-GroEL antibodies to show that bacterial lysis had not occurred.

(B) Schematic representation of constructs. N-terminal extension, essential V and L residues in the HLH domain, tandemly repeated EAR motifs, conserved catalytic residues in the Cys protease (prot) domain, and NLS motif are shown.

(C) Fluorimetric GUS assays in leaf discs 36 h after <i>N. benthamiana</i> agroinfiltration of KCS1p::GUS alone (white bar) or coexpressed with MYB30 either
not have a significant effect on the MYB30-mediated transcriptional activation of KSC1p or on the accumulation of the MYB30 protein (Figures 3C and 3D), underlying the importance of the HLH domain in XopD-mediated effects on MYB30.

**XopD<sub>ccB100</sub> and Not XopD<sub>ccB8004</sub> Targets MYB30 to Suppress Arabidopsis Defense during Xcc Infection**

The data described above suggest that XopD<sub>ccB100</sub> may target MYB30 during *Arabidopsis* infection by *Xcc*. Indeed, the protein interaction between MYB30 and XopD<sub>ccB100</sub> was confirmed in *Arabidopsis*, whereas XopD<sub>ccB8004</sub> did not interact (Figure 4A, Table 1).

To test the hypothesis that XopD<sub>ccB100</sub> but not XopD<sub>ccB8004</sub> is able to target MYB30 in *Arabidopsis*, a *xopD* null mutant XccB100<sub>ΔxopD</sub> was generated and then compared with either XopD<sub>ccB100</sub>- XopD<sub>ccB8004</sub>, or a GUS control, expressed from a constitutive promoter to ensure similar protein expression levels (see Supplemental Figure 3 online). As a control, an XccB100 strain expressing the GUS construct was also generated. We then investigated whether XopD<sub>ccB100</sub> can repress MYB30 transcriptional targets in *Arabidopsis* during Xcc infection, as suggested by the experiments in *N. benthamiana*. Expression of *KCS1, FDH*, and *HCD1*, which belong to the VLCFA pathway and have previously been described as being regulated by MYB30 to induce plant defense (Raffaele et al., 2008), was significantly weaker in Columbia-0 (Col-0) plants inoculated with XccB100 (+GUS) or XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB100</sub>) compared with XccB100<sub>ΔxopD</sub> (+GUS)- or XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB8004</sub>)-inoculated plants (Figure 4B). In addition, the difference in MYB30 target gene expression after inoculation with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB100</sub>) and XccB100 (+GUS) suggested that stronger expression of XopD<sub>ccB100</sub> under a constitutive promoter led to more efficient suppression of MYB30 activity. Together, these data suggest that XopD<sub>ccB100</sub> suppresses *Arabidopsis* defense in a XopD<sub>ccB100</sub>-dose-dependent manner.

No difference in bacterial growth was detected in Col-0 wild-type plants inoculated with XccB100 (+GUS) or XccB100<sub>ΔxopD</sub> (+GUS), when using a bacterial inoculum of either 5 × 10<sup>4</sup> or 5 × 10<sup>5</sup> colony-forming units (cfu/mL) (Figure 4C; see Supplemental Figure 4 online). By contrast, significantly different bacterial growth rates were detected after inoculation of these two strains using 10<sup>5</sup> cfu/mL, suggesting that a certain XopD dose may be required to detect XopD contribution to promotion of bacterial growth in Col-0 plants (Figure 4D). It is important to note that, in contrast with the more widely reported *Arabidopsis* inoculation assays with *Pseudomonas syringae*, inoculations with *X. campestris* using bacterial densities of 10<sup>9</sup> (and even 10<sup>10</sup>) cfu/mL are frequently reported in the literature (Buell and Somerville, 1997). Consistent with the observed dose-dependent XopD-mediated effects, Col-0 plants inoculated with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB100</sub>) were more susceptible than those inoculated with XccB100 (+GUS) using 5 × 10<sup>5</sup> cfu/mL (Figure 4C). This can be explained by the use of a constitutive promoter that might lead to higher XopD protein expression and, therefore, more efficient suppression of MYB30 activity. Inoculation with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB8004</sub>) led to plant resistance levels similar to those obtained after XccB100<sub>ΔxopD</sub> (+GUS) inoculation. Importantly, the difference in bacterial growth displayed by a previously described MYB30ko mutant line (Raffaele et al., 2006) after inoculation with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB100</sub>) and XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB8004</sub>) was significantly reduced (0.26 ± 0.001 log<sub>10</sub> (cfu/cm<sup>2</sup>)) compared with the difference shown by Col-0 wild-type plants (0.52 ± 0.005 log<sub>10</sub> (cfu/cm<sup>2</sup>)), further confirming that MYB30 is targeted by XopD<sub>ccB100</sub> (and not XopD<sub>ccB8004</sub>) in *Arabidopsis* (Figure 4C). Furthermore, Col-0 plants inoculated with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB100</sub>) and XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB8004</sub>), which expresses a XopD version containing the N-terminal and the HLH domains of XopD<sub>ccB100</sub>, displayed significantly higher bacterial growth rates than plants inoculated with XopD<sub>ccB8004</sub> (Figure 4C). This finding confirms that the HLH domain of XopD is sufficient to suppress MYB30-mediated resistance.

Our data show that XopD<sub>ccB100</sub> but not XopD<sub>ccB8004</sub> is able to target MYB30 in *Arabidopsis*. To obtain further proof that XopD mediates suppression of *Arabidopsis* defense through its action on MYB30, we next took advantage of the previously reported observation that MYB30 overexpression in *Arabidopsis* induces the appearance of HR-like symptoms after inoculation with XccB8004 (Vailleau et al., 2002) (Figure 5A). Considering our results, we predicted that this HR-like response reflects the lack of protein interaction between MYB30 and XopD<sub>ccB8004</sub>, which would leave MYB30 free to activate its target genes and trigger HR. By contrast, inoculation of TAP-tagged MYB30-overexpressing plants (MYB30<sub>OE</sub>) with the Xcc strain B100 led to leaf chlorosis (Figure 5A), consistent with the protein interaction between XopD<sub>ccB100</sub> and MYB30 that would hijack MYB30, thus preventing the activation of the HR response. This phenotypic difference is dependent on XopD<sub>ccB100</sub> since MYB30<sub>OE</sub> *Arabidopsis* plants inoculated with XccB100<sub>ΔxopD</sub> (+GUS) developed HR-like symptoms similar to those observed after inoculation with XccB8004 (Figure 5A). Moreover, inoculation of MYB30<sub>OE</sub> plants with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB100</sub>) induced the appearance of chlorotic symptoms, whereas inoculation with XccB100<sub>ΔxopD</sub> (+XopD<sub>ccB8004</sub>) led to the development of an HR-like response (Figure 5A). These visual observations were...
confirmed by ion leakage measurements in leaf disk assays after plant inoculation with the different strains (Figure 5B).

Interestingly, development of cell death correlated with improved plant resistance. Indeed, MYB30OE plants inoculated (5 \times 10^5 cfu/mL) with \textit{Xcc} B100 (+GUS) or \textit{Xcc} B100 \Delta xopD (+XopD:\textit{Xcc} B100) were more susceptible than \textit{Xcc} B100 \Delta xopD (+XopD:\textit{Xcc} 8004)- or \textit{Xcc} B100 \Delta xopD (+GUS)-inoculated plants (Figure 5C). Moreover, inoculation with \textit{Xcc} B100 \Delta xopD (+XopD:\textit{Xcc} B100) induced higher bacterial growth than wild-type \textit{Xcc} B100-inoculation, reflecting the fact that higher XopD:\textit{Xcc} B100 protein expression results in more efficient suppression of MYB30 activity. Similar to Col-0 wild-type plants, MYB30OE plants inoculated with \textit{Xcc} B100 \Delta xopD (+XopD:\textit{Xcc} B100) displayed significantly higher bacterial growth rates compared with XopD:\textit{Xcc} B100-inoculated plants, confirming the role of the HLH domain in repressing MYB30-mediated resistance.

Finally, expression of \textit{KCS1}, \textit{FDH}, and \textit{HCD1} was significantly lower in plants inoculated with \textit{Xcc} B100 (+GUS) or \textit{Xcc} B100 \Delta xopD (+XopD:\textit{Xcc} B100) than after inoculation with \textit{Xcc} B100 \Delta xopD (+GUS) or \textit{Xcc} B100 \Delta xopD (+XopD:\textit{Xcc} 8004) (Figure 5D), confirming that XopD:\textit{Xcc} B100 mediates suppression of \textit{Arabidopsis} defense through repression of MYB30 transcriptional activation.

**DISCUSSION**

The identification of host targets of T3Es is a fundamental question in plant pathology. Indeed, despite active research and the significant number of bacterial T3Es identified, information about their host molecular targets remains limited. The growing number of reports about T3Es delivered into the plant cell nucleus suggests that effector proteins may target nuclear host components (or functions) essential for the establishment of plant immune responses (Deslandes and Rivas, 2011). For example, the Xcv T3E AvrBs3, a member of the transcription activator-like effector family, acts as a TF and induces developmental reprogramming in plant cells (Kay et al., 2007). Indeed, manipulation of host transcription appears to be a strategy conserved between plant and animal pathogens to subvert host immunity (Mukherjee et al., 2006; Zhang et al., 2007).
XopD expression appears to induce a reorganization of the plant nuclear structure that leads to nonspecific modification of the distribution of nuclear proteins. Indeed, XopD induced relocalization of all tested proteins in nuclear foci, including GFP fused to a nuclear localization signal (GFP-NLS; see Supplemental Figure 5 online). In an attempt to investigate the precise nature of the subnuclear structures in which XopD is localized, we coexpressed XopD with different subnuclear markers. However, XopD expression induced nonspecific relocalization of all tested markers, rendering these studies inconclusive. XopD HLH domain is necessary and sufficient to induce nuclear foci formation, suggesting that the DNA binding capacity of XopD may be required for this process. Along these lines, it was previously proposed that XopD may function at the level of chromatin remodeling, perhaps through its DNA binding domain (Kim et al., 2008). The 4',6-diamidino-2-phenylindole staining showed that DNA accumulation is weaker in nuclear bodies where XopD is expressed compared with the nucleoplasm where DNA nuclear distribution remains otherwise unaltered (see Supplemental Figure 6 online).

Here, we identified Arabidopsis MYB30 as a first target of XopD. XopD-specific interaction with MYB30 resulted in transcriptional repression of its VLCFA-related target genes. Importantly, this effect appears to be specific to MYB30 since the transcriptional activity of MYB96, also recruited to nuclear bodies in the presence of XopD, was not affected in the same conditions. Nuclear bodies have been proposed to serve as protein storage and/or recycling sites, since protein sequestration in nuclear bodies regulates the concentration of active TFs and cofactors in the nucleoplasm, where transcription takes place (Zhong et al., 2000). An additional role for nuclear bodies in repression of TF activity has been previously reported (Li et al., 2000; Tashiro et al., 2004). However, XopD targeting of MYB30 appears to be independent of nuclear foci formation since (1) no protein interaction was detected between XopD and MYB96 or MYB123, which were also present in nuclear foci when plants 48 h after infection with the indicated strains (10^7 colony-forming units/mL). Three independent experiments with five plants (four leaves/plant) were performed.

(B) Quantification of cell death by measuring electrolyte leakage before (white bars) and 24 h after inoculation (gray bars) of MYB30 OE Arabidopsis plants with the indicated strains (10^7 colony-forming units/mL). Statistical differences using multiple-factor analysis of variance (P value < 10^-4) are indicated by letters.

(C) MYB30 OE Arabidopsis plants were inoculated with the indicated strains (5 × 10^5 colony-forming units [CFU/mL]) and bacterial growth measured 0 (white bars) and 3 d after inoculation (gray bars). Data were collected from four independent experiments with six individual plants (four leaves/plant). Statistical differences using multiple-factor analysis of variance (P value < 10^-4) are indicated by letters.

(D) Expression analysis of the MYB30 target genes KCS1, FDH, and HCD1 in MYB30 OE Arabidopsis plants after inoculation with the indicated strains (10^7 cfu/mL). Expression values of the individual genes were normalized using SAND family and β-tubulin4 as internal standards. Mean and SE values were calculated from the results of four independent experiments with three individual plants (four leaves/plant). Statistical differences according to a Student’s t test P value < 0.05 are indicated by letters.
coexpressed with XopD, and (2) the physical interaction between XopD and MYB30 also occurred in the soluble nuclear fraction, despite the very weak fluorescence detected outside nuclear foci.

Considering XopD modular structure and varied biochemical activities, it was previously suggested that XopD may mediate multiple protein–DNA and protein–protein interactions to alter host transcription (Kim et al., 2008). Our results provide a first confirmation of this idea. Indeed, our work suggests that XopD-mediated modification of the nuclear structure may be part of a general virulence strategy, which allows Xanthomonas to perturb plant cell responses to bacterial infection. Additionally, XopD-specific targeting and suppression of MYB30 activity, which appears to be independent of nuclear foci formation, highlights an original bacterial strategy to suppress a host component essential for the establishment of plant defense. Future studies should (1) determine the role of nuclear foci formation and (2) uncover additional XopD-related molecular interactions during Xanthomonas infection.

XopD expression led to MYB30 protein accumulation. These results are reminiscent of those obtained for PSEUDO-RESPONSE REGULATOR5 (PRR5), a regulatory component of circadian cycling that promotes relocation and accumulation in nuclear foci of an additional circadian clock regulator, TOC1/PRR1 (Wang et al., 2010). In addition, XopD has been proposed to modulate host transcript levels of defense-associated genes (Kim et al., 2008). However, (1) XopD-induced accumulation of MYB30 expressed from a constitutive 35S promoter, (2) coexpression of XopD with a fusion of the MYB30 promoter to the reporter GUS gene (MYB30p:GUS) led to GUS activity values undistinguishable from those detected when MYB30p:GUS was expressed alone (see Supplemental Figure 7A online), and (3) no significant difference in MYB30 transcript levels was detected in Arabidopsis plants inoculated with XccB100 or XccB100xopD strains (see Supplemental Figure 7B online), indicating that XopD-mediated accumulation of MYB30 is postranslationally (not transcriptionally) controlled.

Beyond its role as transcriptional activator of VLCFA-related genes, MYB30 is also involved in an amplification loop that modulates SA biosynthesis that, in turn, regulates plant cell death responses (Raffaele et al., 2006). By contrast, XopD was previously reported to reduce SA levels in tomato after Xcv infection (Kim et al., 2008). Interestingly, expression of the SA biosynthesis marker gene IC51 was significantly reduced in plants inoculated with Xcc strains expressing XopDXccB100, but not XopDXccB004 (see Supplemental Figure 8 online). Therefore, downregulation of the SA pathway correlates with repression of MYB30 activity by XopDXccB100, which may additionally contribute to bacterial virulence. Importantly, XopDXccB100 was able to promote virulence in Arabidopsis in a dose-dependent manner, since higher XopDXccB100 expression, when using a constitutive promoter, led to increased plant susceptibility. Likewise, detection of XopDXccB100-mediated promotion of bacterial growth in Col-0 wild-type plants required increasing the bacterial inoculum and would have been missed using lower bacterial doses for bacterial growth measurements. These results reflect the fact that, in most cases, removing a T3E does not significantly affect virulence, and determining the contribution to virulence of a given T3E is not always straightforward (Cunnac et al., 2011). In this sense, MYB30KO lines have been a useful tool to confirm XopD as a repressor of MYB30-mediated defense during Xcc infection. Moreover, the difference in bacterial growth rates displayed by MYB30ko mutant plants after inoculation with XccB100xopD (+XopDXccB100) and XccB100ΔxopD (+XopDXccB004) was significantly compromised compared with Col-0 wild-type plants. These data provide further confirmation that MYB30 is targeted by XopDXccB100 (and not XopDXccB004) in Arabidopsis. Interestingly, the remaining difference in bacterial growth detected in MYB30ko plants after inoculation with XccB100ΔxopD (+XopDXccB100) and XccB100ΔxopD (+XopDXccB004) likely mirrors the presence of additional XopD targets (other than MYB30) contributing to the outcome of the Xcc–Arabidopsis interaction.

Consistent with the modular structure of XopD and based on our results, it is tempting to speculate that XopD-dependent bacterial strategies used to subvert plant resistance may vary depending on the Xanthomonas/host plant interaction. Previous work showed that XopDXcc, is involved in promoting virulence in tomato and that XopD SUMO-protease, EAR transcription repressor, and DNA binding activities are at least partially involved in this process (Kim et al., 2008). Significantly, XopDXccB004 carrying three EAR motifs and a functional Cys protease domain (see Supplemental Figure 9 online), was not able to suppress MYB30-mediated defense, suggesting that these domains are not sufficient for repression of MYB30 by XopD in Arabidopsis. By contrast, the HLH domain, present in XopDXccB100 and absent in XopDXccB004, was essential to repress MYB30-mediated Arabidopsis defense. Indeed, in agreement with the N. benthamiana data showing that XopD HLH motif is sufficient to suppress MYB30 transcriptional activation, Col-0 plants inoculated with XccB100ΔxopD (+XopDXccB100) displayed significantly higher bacterial growth rates than those inoculated with XccB100ΔxopD (+XopDXccB004)1-437 displayed significantly higher bacterial growth rates than those inoculated with XopDXccB004. It is thus tempting to speculate that the EAR and the Cys protease domains in XopD are likely involved in targeting host defense-related components other than MYB30. Confirmation of this idea was obtained by the finding that the level of bacterial growth detected after inoculation with XccB100ΔxopD (+XopDXccB100)1-437 was intermediate between that displayed by plants inoculated with XccB100ΔxopD (+XopDXccB100) and XccB100ΔxopD (+XopDXccB004), strongly suggesting that additional XopD host targets are involved in the outcome of the interaction between Xcc and Arabidopsis, as additionally indicated by the results obtained with MYB30ko plants. Future work will determine whether the “Swiss army knife” XopD protein structure correlates with the existence of multiple host targets and strategies to promote virulence.

METHODS

Constructs

Primers used in this study are shown in Supplemental Table 1 online. Unless otherwise indicated, plasmids used in this study were constructed by Gateway technology (GW; Invitrogen) following the instructions of the manufacturer. PCR products flanked by the attB sites were recombined into the pDONR207 vector (Invitrogen) via a BP reaction to create the corresponding entry clones with attl sites. Inserts cloned into the entry...
clones were subsequently recombined into the destination vectors via an LR reaction to obtain the expression constructs. MYB96 entry clone was obtained after amplification of the AtMYB96 gene from Col-0 genomic DNA, followed by ligation into pK207 (Froidevaux et al., 2010). Similarly, MYB30 promoter was amplified from Col-0 genomic DNA and cloned into pK207. XopD\textsuperscript{216-760} \textsuperscript{V333P} and XopD\textsuperscript{216-760} \textsuperscript{EAR} were amplified from pEZRK-LCY vectors kindly provided by Mary Beth Mugdett (Standford University) (Kim et al., 2008), XopD\textsubscript{Xcc8004} and XopD\textsubscript{Xcc8100} were amplified from Xcc8004 and XccB100 genomic DNA, respectively. XopD\textsubscript{XCC8004} \textsuperscript{416-760} and XopD\textsubscript{XCC8100} \textsuperscript{415-415} were amplified from genomic DNA from Xcc. XopD\textsubscript{Xcc8100} \textsuperscript{537-802} was amplified from XccB100 genomic DNA. The chimeric construct containing the sequence encoding the HLH domain from XopD\textsubscript{Xcc} fused to XopD\textsubscript{Xcc8004} \textsuperscript{416-415} was engineered using chimeric PCR.

TAP- and YFPv-tagged constructs were generated by recombination of the corresponding entry vectors with pBin19-3SS-GW-TAP and pBin19-3SS-GW-YFPv destination vectors, respectively (YFPv for YFP-venus, an enhanced form of the YFP [Froidevaux et al., 2010]). A pBin19-3SS-YFPv-GW destination vector was used to generate fusion proteins with the YFPv tag at their N terminus. The MYB30 promoter was recombined into the pKGWFS7 destination vector (Canonne et al., 2010), resulting in a plant expression vector that contains a transcriptional fusion between the MYB30p and the GUS reporter gene.

**Bacterial Strains**

Both XccB100\textsuperscript{*} and XccB1100\textsuperscript{*} \textit{hcrV} strains were obtained using the sacB system (Schara et al., 1994). Briefly, the XccB1100\textsuperscript{*} strain was generated by triparesarial mating using plasmid pKi18 (hrpG\textsuperscript{2}), which contains a mutation in \textit{hrpG} that allows constitutive expression of all \textit{hrp} genes (Wengelnik et al., 1999) and pRK2073 as helper plasmid (Finan et al., 1986). To generate the XccB1100\textsuperscript{*} \textit{hcrV} strain, a GoldenGate-compatible plasmid pKi18 \textit{hcrV} was introduced in XccB1100\textsuperscript{*} using the same protocol. GoldenGate is a cloning method based on the use of Type II restriction enzymes (88al in our study) (Engler et al., 2008). Both pKi18 plasmids were kindly provided by Laurent Noël (Laboratoire des Interactions Plantes-Microorganismes, Castanet-Tolosan, France) who also provided XccB1100\textsuperscript{*} \textit{hcrV} strains.

The XccB100 xopD deletion mutant was also generated using the sacB system. The 699-bp upstream and 685-bp downstream regions of full-length xopD were amplified using Xcc B100 genomic DNA as template. PCR products were subsequently cloned in a pK18 plasmid, which was verified by PCR.

For construction of the XccB100 \textit{\Delta xopD} mutant strain, plAFR6-GW-3\textsuperscript{X}HA vectors (Canonne et al., 2010) were used via an HA-tagged GUS vector, XopD\textsubscript{Xcc8100}XopD\textsubscript{Xcc8100} \textsuperscript{415-437} or XopD\textsubscript{Xcc8004} were introduced into XccB100 \textit{\Delta xopD} by triparesarial mating to allow expression of HA-tagged proteins under the control of a constitutive lac promoter.

For epitope tagging of XopD for secretion assays, the C-terminal end of XopD\textsubscript{Xcc8100} and XopD\textsubscript{Xcc8004} fused to an HA epitope were amplified by PCR as 343- and 321-bp fragments, respectively. The amplified fragments were digested with BamHI and XbaI and cloned into the suicide plasmid pVO155 (Oke and Long, 1999). These constructs were introduced into XccB1100 and XccB100 \textit{\Delta hcrV} strains, and into Xcc8004\textsuperscript{*} and Xcc8004\textsuperscript{*} \textit{\Delta hcrV} strains, respectively.

Xcc8004\textsuperscript{*}, B100\textsuperscript{*}, 8004\textsuperscript{*} \textit{\Delta hcrV}, and B100\textsuperscript{*} \textit{\Delta hcrV} strains expressing HA-tagged XopD were cultivated overnight at 28°C in MOKA rich medium (Blanvillain et al., 2007) or in secretion medium (Rossier et al., 1999). Secretion experiments were performed as described previously (Rossier et al., 1999) and XopD proteins detected by immunoblot.

**Fluorescence Microscopy and FRET-FLIM and Data Analysis**

CFP and YFPv fluorescence in \textit{N. benthamiana} leaves was analyzed by confocal laser scanning microscopy as previously described (Canonne et al., 2010). FRET-FLIM experiments were performed according to Froidevaux et al. (2010). Statistical comparisons between control (donor alone) and assay (donor + acceptor) lifetime values were performed using Student’s \textit{t} test.

**Coimmunoprecipitation Assays**

As an alternative to FRET-FLIM assays, coimmunoprecipitation experiments were performed in an attempt to demonstrate the specificity of the XopD\textsubscript{Xcc8100/MYB30} protein interaction. In these experiments, \textit{Nicotiana benthamiana} leaf tissue transiently expressing the different protein combinations (MYB30-TAP, MYB96-TAP, XopD\textsubscript{Xcc8100} HA, XopD\textsubscript{Xcc8004} HA, MYB30-TAP + XopD\textsubscript{Xcc8100} HA, MYB96-TAP + XopD\textsubscript{Xcc8100} HA, MYB30-TAP + XopD\textsubscript{Xcc8004} HA, or MYB96-TAP + XopD\textsubscript{Xcc8004} HA) was harvested 36 h after agroinfiltration and ground in liquid nitrogen. Ground tissue was resuspended in extraction buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% [v/v] glycerol, 1 mM PMSF, and 1% plant protease inhibitor cocktail [Sigma-Aldrich]) and centrifuged at 14,000 g for 10 min at 4°C. Protein concentration in the supernatant was determined with the Bradford protein assay kit (Bio-Rad) using BSA as a standard. The supernatant was split into two fresh tubes and used as input for the anti-HA and anti-TAP immunoprecipitations using beads prewashed in extraction buffer. All steps during immunoprecipitations were performed at 4°C. Immunoprecipitated proteins were washed with extraction buffer and eluted by boiling for 5 min in Laemmli buffer. However, nonspecific binding of both MYB and XopD proteins to beads was observed in all cases, rendering these experiments inconclusive. This problem of nonspecific protein binding was systematically observed despite all attempted modifications to the protocol, such as extensive washing after immunoprecipitation (up to 10 washing steps), modification of the composition of the extraction buffer to get more stringent conditions (adding detergent from 0.1 to 1% Nonidet P-40, or a combination of Nonidet P-40 and Triton X-100) or increasing salt concentration up to 300 mM), blocking the beads with 1% BSA prior to immunoprecipitation, or changing the epitope used for tagging the protein (GFP, myc, etc.).

**Bimolecular Fluorescence Complementation**

A mutated (152L) YFPv moiety (kindly provided by Laurent Deslandes, Laboratoire des Interactions Plantes-Microorganismes, Castanet-Tolosan, France) was used to reduce self-assembly between the YFPv N- and C-terminal fragments, thus enabling visualization of protein–protein interactions with a high signal-to-noise ratio (Kodama and Hu, 2010). Fluorescence in \textit{N. benthamiana} leaf samples was monitored 36 h after agroinfiltration of the induced constructs with a fluorescence microscope (DMIRBE Leica) using a \times 20 long working distance objective lens (numerical aperture = 0.4). Images were acquired with a CCD camera (Color Coolview; Photonic Science). An identical setup was used for all samples. Briefly, 32 images per sample were acquired for a total leaf area of 8 mm\textsuperscript{2} (n = 32 images). For each image, fluorescent nuclei were counted and the mean fluorescence intensity per nucleus was measured using image analysis (Image Pro-Plus Software; Media Cybernetics).

**Protein Gel Blot Analysis**

For detection of TAP-, HA-, and YFPv-tagged proteins, blots were respectively incubated with rabbit PAP soluble complex (Sigma-Aldrich), rat monoclonal anti-HA (clone EF10 [Roche]; 1:5000), and mouse
monoclonal anti-GFP IgG1 K (clones 7.1 and13.1 [Roche]; 1:10,000) antibodies, linked to horseradish peroxidase. Anti-GroEL rabbit polyclonal (Stressgen Biotechnologies) was used at 1:10,000. Proteins were visualized using the Immobilon kit (Millipore) following the manufacturer’s instructions.

Plant and Bacterial Materials

All Arabidopsis thaliana lines used in this study were in the Col-0 background. Plants were grown in Jiffy pots under controlled conditions, as previously described (Froidure et al., 2010). The MYB30ko and TAP-tagged MYB30OE lines were described before (Froidure et al., 2010).

Agrobacterium tumefaciens–mediated transient expression in N. benthamiana leaves and Arabidopsis seedlings was performed as described (Froidure et al., 2010).

For plant inoculations, leaves of 4-week-old plants were syringe-infiltrated using the indicated strains at the specified bacterial densities. In planta bacterial growth analysis was performed as described previously (Froidure et al., 2010). Data were subsequently submitted to a statistical analysis using Statgraphics Centurion XV.II professional software (Statpoint Technologies). Normality of residues was verified by the Kolmogorov-Smirnov test. The effect of the tested strains was analyzed by multiple-factor analysis of variance (P < 0.05).

RNA Extraction and Quantitative RT-PCR Analysis

Material for RNA analysis was ground in liquid nitrogen, and total RNA was isolated using the Nucleospin RNA plant kit (Macherey-Nagel) according to the manufacturer’s recommendations. Reverse transcription was performed using 1 μg of total RNA.

Real-time quantitative PCR was performed on a Light Cycler 480 II machine (Roche Diagnostics) using Roche reagents. Relative expression was calculated as the ΔCp between each gene and the average of internal controls (SAND family [At2g28390] and β-tubulin 4 [At5g44340]). Average ΔCp was calculated from four independent experiments with three individual plants (four leaves/plant). Primers used for quantitative RT-PCR analysis are described by Froidure et al. (2010) or Raffaele et al. (2008) or in Supplemental Table 1 online.

Fluorimetric GUS Assays

For GUS reporter assays, the indicated constructs were transiently expressed in N. benthamiana leaves using Agrobacterium. Leaf discs were collected 36 h after agroinoculation, frozen in liquid nitrogen, and stored at −80°C until processing. GUS activity was measured as described (Froidure et al., 2010).

Quantification of Cell Death Using Electrolyte Leakage

For electrolyte leakage measurements, eight Arabidopsis leaf discs (5 mm diameter) were harvested 24 h after inoculation with the indicated strains, washed, and incubated at room temperature in 10 mL of distilled water before measuring conductivity. Three independent experiments were performed with three plants (four leaves/plant).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: MYB30 (NM_113812), MYB96 (NM_125641), MYB123 (NM_122946), XopD (BKO07963), XopDXccB100 (xccb100_1256), and XopDXccB8004 (XC_1213).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. MYB123 Is Relocalized to Nuclear Foci in the Presence of XopD.

Supplemental Figure 2. Specific Interaction between MYB30 and XopDXccB100 by Bimolecular Fluorescence Complementation.

Supplemental Figure 3. Expression of XopD Proteins in Engineered Xcc Strains.

Supplemental Figure 4. Bacterial Growth Rates Displayed by Col-0 Plants after Inoculation with a Bacterial Density of 5 x 10^6 colony-forming units/mL.

Supplemental Figure 5. A GFP-NLS Fusion Protein Is Relocalized to Nuclear Foci When Coexpressed with XopD.

Supplemental Figure 6. Nuclear Reorganization Induced by XopD Expression in Plant Cells.

Supplemental Figure 7. XopD Is Not Able to Regulate MYB30 Gene Expression.

Supplemental Figure 8. XopDXccB100, but Not XopDXccB8004, Suppresses ICS1 Transcript Accumulation.

Supplemental Figure 9. XopDXccB100 and XopDXccB8004 Display SUMO Protease Activity in N. benthamiana.

Supplemental Table 1. Primers Used in This Study.

Supplemental References.

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AUTHOR CONTRIBUTIONS

J.C., D.M., and S.R. designed the research, performed experiments, analyzed data, and wrote the article. D.R. analyzed data. A.J., C.P., and C.B. performed and analyzed FRET-FLIM experiments.

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REFERENCES


Supplemental Table 1. Primers Used in This Study.


Correction


The amino acid protein XopD_{XccB100}^{437-801} was incorrectly listed throughout the article as XopD_{XccB100}^{437-802}.
Supplemental Figure 1. MYB123 is relocalized to nuclear foci in the presence of XopD.

(A) Confocal images of epidermal cells of *N. benthamiana* leaves 36 hours after *Agrobacterium*-mediated transient expression of MYB123-CFP expressed alone (top) or in the presence of XopD<sub>Xcv</sub>-YFP<sub>v</sub> (bottom). Bright field images are shown on the right. Bar = 15 µm. (B) Immunoblot analysis using an anti-GFP antibody, 36 hours after agroinfiltration of MYB123-CFP alone (1) or together with XopD<sub>Xcv</sub>-YFP<sub>v</sub> (2). Ponceau S staining confirms equal loading (bottom). The lack of interaction between MYB123-CFP and XopD<sub>Xcv</sub>-YFP<sub>v</sub> as determined by FRET-FLIM is indicated (-).
Supplemental Figure 2. Expression of XopD proteins in engineered Xcc strains. Total protein extracts from the indicated strains (10-fold concentrated) were analysed by immunoblotting using anti-HA antibodies to detect expression of GUS (1,2), XopD<sub>Xcc</sub>B100 (3) XopD<sub>Xcc</sub>8004 (4) and XopD<sub>Xcc</sub>B100<sup>1-437</sup> (5).
Supplemental Figure 3. Bacterial growth rates displayed by Col-0 plants after inoculation with a bacterial density of $5 \times 10^6$ cfu/ml. Col-0 Arabidopsis plants were inoculated with the indicated strains ($5 \times 10^6$ cfu/ml). Bacterial growth was measured 0 (white bars) and 3 dpi (gray bars). Data were collected from 4 independent experiments with 6 individual plants (4 leaves/plant). Statistical differences using Multiple Factor ANOVA (p-value < $10^{-5}$) are indicated by letters.
Supplemental Figure 4. A GFP-NLS fusion protein is relocalized to nuclear foci when co-expressed with XopD. Confocal images of epidermal cells of *N. benthamiana* leaves 36 hours after *Agrobacterium*-mediated transient expression of GFP-NLS expressed alone (top) or in the presence of XopD<sub>Xcv</sub>-CFP (bottom). Bright field images are shown on the right. Bar = 15 µm. Similar results were obtained when GFP-NLS was expressed in the presence of XopD<sub>XccB100</sub>-CFP.
Supplemental Figure 5. Nuclear reorganization induced by XopD expression in plant cells. Confocal images of epidermal cells of *N. benthamiana* leaves 36 hours after *Agrobacterium*-mediated transient expression of XopD<sub>XccB100</sub>-YFPv (A). Nuclear DNA was stained using DAPI (B). Merged image shows that DAPI staining is weaker in nuclear bodies where XopD<sub>XccB100</sub> accumulates (C). Bright field image (D). Bar = 15 µm.
Supplemental Figure 6. Specific interaction between MYB30 and XopD\textsubscript{XccB100} demonstrated by Bimolecular Fluorescence Complementation. Fewer and less fluorescent nuclei were detected when expressing MYB30-YFP\textsubscript{C} and XopD\textsubscript{Xcc-8004-YFP\textsubscript{N(I152L)}} or MYB96-YFP\textsubscript{C} and XopD\textsubscript{XccB100-YFP\textsubscript{N(I152L)}} as compared to leaves samples expressing MYB30-YFP\textsubscript{C} and XopD\textsubscript{XccB100-YFP\textsubscript{N(I152L)}}. (A) MYB30-YFP\textsubscript{C} and XopD\textsubscript{XccB100-YFP\textsubscript{N(I152L)}} (left), MYB30-YFP\textsubscript{C} and XopD\textsubscript{Xcc-8004-YFP\textsubscript{N(I152L)}} (middle) and MYB96-YFP\textsubscript{C} and XopD\textsubscript{XccB100-YFP\textsubscript{N(I152L)}} (right) were transiently co-expressed in \textit{N. benthamiana} leaves. Fluorescence was monitored 36 hpi using a YFP filter. Bars = 15µm. (B) Mean fluorescence intensity per nucleus is shown for each protein combination. (C) Immunoblot analysis using an anti-GFP antibody shows equal expression of all proteins 36 hours after agroinfiltration. MYB30-YFP\textsubscript{C} and XopD\textsubscript{XccB100-YFP\textsubscript{N(I152L)}} (1), MYB30-YFP\textsubscript{C} and XopD\textsubscript{Xcc-8004-YFP\textsubscript{N(I152L)}} (2) and MYB96-YFP\textsubscript{C} and XopD\textsubscript{XccB100-YFP\textsubscript{N(I152L)}} (3). Ponceau S staining confirms equal loading (bottom).
Supplemental Figure 7. XopD is not able to regulate MYB30 gene expression. (A) Fluorimetric GUS assays in leaf discs 36 hours after N. benthamiana agroinfiltration of MYB30p:GUS alone (1) or co-expressed with XopD_{Xcc:B100}-YFPv (2) or XopD_{Xcc:8004}-YFPv (3). Mean values and SEM values were calculated from the results of four independent experiments, with two to four replicates per experiment. MU, methylumbelliferone. Immunoblot analysis showing expression of YFPv-tagged constructs is shown at the bottom. Ponceau S staining confirms equal loading. (B) Expression analysis of MYB30 in Col-0 Arabidopsis plants after inoculation with the indicated strains (10^7 cfu/ml). Expression values of MYB30 were normalized using SAND family and $\beta$-tubulin4 as internal standards. Mean values and SEM values were calculated from the results of 4 independent experiments with 3 individual plants (4 leaves/plant). Statistical differences according to a Student’s t test P value < 0.005 are indicated by stars.
Supplemental Figure 8. XopD_{XccB100}, but not XopD_{Xcc8004}, suppresses *ICS1* transcript accumulation. Expression analysis of *ICS1* in MYB30_{OE} and Col-0 Arabidopsis plants after inoculation with the indicated strains (10^7 cfu/ml). The expression value of *ICS1* was normalized using the expression level of using SAND family and β-tubulin4 as internal standards. Mean values were calculated from 4 independent experiments with 3 individual plants per experiment. Statistical differences according to a Student’s *t* test P value < 0.05 are indicated by letters.
**Supplemental Figure 9.** XopD<sub>XccB100</sub> and XopD<sub>Xcc8004</sub> display SUMO protease activity in *N. benthamiana*. Significant reduction in the detection of SUMO-modified proteins 36 hours after agroinfiltration of YFP-tagged XopD<sub>XccB100</sub> and XopD<sub>Xcc8004</sub> with an HA-tagged Le SUMO construct (Hotson et al., 2003) is shown by immunoblot analysis. As previously described, XopD<sup>216-760-C685A</sup>, mutated in the conserved Cys residue in XopD catalytic core, is not able to hydrolyze the SUMO substrates (Hotson et al., 2003). Expression of XopD proteins was revealed using an anti-GFP antibody. Ponceau S staining of the membrane illustrates equal loading.
**Table 1. Oligonucleotide Primers Used in This Study**

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SUPPLEMENTAL REFERENCES