**Cell Host & Microbe**

*Pseudomonas syringae* Type III Effector HopBB1 Promotes Host Transcriptional Repressor Degradation to Regulate Phytohormone Responses and Virulence

**Graphical Abstract**

**Highlights**
- The transcriptional regulator TCP14 represses JA response to promote disease resistance
- The *Pseudomonas syringae* type III effector HopBB1 interacts with TCP14
- HopBB1 activates TCP14-repressed JA response genes and promotes bacterial virulence
- HopBB1 targets TCP14 for SCF\(^{COII}\)-dependent degradation by connecting it to JAZ3

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**In Brief**
Yang et al. demonstrate that the *Pseudomonas syringae* type III effector HopBB1 modulates two negative regulators of plant jasmonic acid (JA) signaling, TCP14 and JAZ3, and “glues” them together for degradation, resulting in precise activation of a subset of JA output responses that promote bacterial virulence.

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Pseudomonas syringae Type III Effector HopBB1 Promotes Host Transcriptional Repressor Degradation to Regulate Phytohormone Responses and Virulence

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SUMMARY

Independently evolved pathogen effectors from three branches of life (ascomycete, eubacteria, and oomycete) converge onto the Arabidopsis TCP14 transcription factor to manipulate host defense. However, the mechanistic basis for defense control via TCP14 regulation is unknown. We demonstrate that TCP14 regulates the plant immune system by transcriptionally repressing a subset of the jasmonic acid (JA) hormone signaling outputs. A previously unstudied Pseudomonas syringae (Ps) type III effector, HopBB1, interacts with TCP14 and targets it to the SCFCoII degradation complex by connecting it to the JA signaling repressor JAZ3. Consequently, HopBB1 de-represses the TCP14-regulated subset of JA response genes and promotes pathogen virulence. Thus, HopBB1 fine-tunes host phytohormone crosstalk by precisely manipulating part of the JA regulon to avoid pleiotropic host responses while promoting pathogen proliferation.

INTRODUCTION

A robust immune system defends plants against most microbes. Plants deploy surface-localized pattern recognition receptors to detect conserved microbe-associated molecular patterns (MAMPs), which leads to the activation of MAMP-triggered immunity (MTI). To counteract MTI, pathogenic microbes deploy virulence factors, often termed effector proteins, into plant cells, where they interact with host factors to subvert defense responses or to alter nutrition distribution. To counteract effector protein action, plants evolved a large, polymorphic family of intra-cellular receptors with a nucleotide-binding domain and leucine-rich repeats, termed NLRs. Plant NLR receptors are analogous to animal NLR innate immune receptors. NLR receptors in both kingdoms are activated either by direct interactions with ligands, including effector proteins, or by recognition of effector-modified host cellular machines that are the nominal effector targets or decoys of those targets (Bentham et al., 2016; Jones and Dangl, 2006; van der Hoorn and Kamoun, 2008). NLR activation initiates effector-triggered immunity (ETI). Deciphering the mechanisms by which effector repertoires from divergent pathogens act will provide a more comprehensive view of the host cellular machinery responsible for plant immune system function.

Interactome studies revealed that candidate effector repertoires from three evolutionarily diverse pathogens—P. syringae (Psy; eubacteria), H. arabidopsidis (Hp; oomycete), and Golovinomyces orontii (Go; ascomycete)—converge onto a limited set of interconnected Arabidopsis proteins (Dreze et al., 2011; Mukhtar et al., 2011; Weßling et al., 2014). TCP14, a transcription factor belonging to the conserved TCP (teosinte branched1, CYCLOIDEA, PROLIFERATING CELL FACTORS 1 and 2) family, is one of the convergent host targets. The reference Arabidopsis Col-0 genome encodes 24 TCP family members that share a basic helix-loop-helix (bHLH) domain (the TCP domain) and are versatile regulators of plant development and hormone signaling (Lopez et al., 2015). TCP14 physically interacts with SRFR1 and contributes to effector-triggered immunity (Kim et al., 2014). In various tissues, TCP14 promotes cytokinin and gibberellic acid growth hormone responses (Kieffer et al., 2011; Resentini et al., 2015; Steiner et al., 2012). TCP14 is localized to sub-nuclear foci and its coexpression resulted in the re-localization of 22/33 tested nuclear-localized effectors from the three pathogens noted above (Weßling et al., 2014). Additionally, the phytoplasma SAP11 effector associates with...
Figure 1. TCP14 Represses JA Response and Promotes Disease Resistance

(A) Shown are 3-week-old plants of Col-0, tcp14-6, UBQ::YFP-TCP14-3 and UBQ::YFP-TCP14-4. Scale bar represents 5 mm.

(B) tcp14 mutants are more susceptible to Hpa Emwa1 than Col-0. Overexpressing TCP14 in Arabidopsis enhances disease resistance against Hpa Noco2. The means represent the numbers of sporangiophores (sp) on each cotyledon (n > 50).

(C) Mutation in TCP14 enhances the virulence of DC3000 cor-

(legend continued on next page)
other members of the TCP family to repress JA biosynthesis, which ultimately enhances the feeding behavior of its insect vector, the leaf hopper (Sugio et al., 2011).

Plant cells integrate growth and division cues with defense cues via phytohormone signaling interactions (Belkhadir et al., 2014; Robert-Seilaniantz et al., 2011). The antagonistic regulatory relationship between the defense hormones jasmonic acid (JA) and salicylic acid (SA) endows a plant with the flexibility to prioritize defense responses against pathogens with diverse lifestyles (Robert-Seilaniantz et al., 2011). In Arabidopsis, activation of SA-dependent responses limits the growth of biotrophic or hemibiotrophic pathogens. On the other hand, JA-dependent responses limit the growth of necrotrophic pathogens and herbivorous insects. Hence, biotrophic or hemibiotrophic pathogens inhibited by SA-mediated immune responses will benefit from activation of JA-dependent responses (Browse, 2009; He et al., 2004; Zheng et al., 2012). Host cellular machines regulating the SA-JA balance are therefore attractive targets for effectors that mimic the action of either hormone, misdirect the defense response, and thus facilitate pathogen or pest proliferation (Kazan and Lyons, 2014).

The transcriptional outputs of JA response are repressed by a group of JASMONATE ZIM DOMAIN (JAZ) proteins through their association with transcription factors (Chini et al., 2007; Zhang et al., 2015). Three MYC transcription factors (MYC2, MYC3, and MYC4) repressed by JAZ proteins are positive regulators of JA-mediated responses in Arabidopsis (Kazan and Manners, 2013). JA proteins directly or indirectly recruit a transcription co-repressor complex containing Topless (TPL) and histone deacetylase to repress MYC activity (Pauwels et al., 2010). JA biosynthesis is induced during normal development or following either MAMP treatment or Pseudomonas syringae infection (Lewis et al., 2015; Schmelz et al., 2003). JAZ proteins bind isoleucine-conjugated JA, which facilitates their physical interaction with the CORONATINE-INSENSITIVE 1 (COI1) F-box component of a Skip-cullin-F-box (SCF)-type E3 ubiquitin ligase (Sheard et al., 2010). This results in proteasome-mediated degradation of JAZ proteins and allows MYC-dependent activation of JA response genes (Thines et al., 2007; Wasternack and Hause, 2013). The MYC regulon controls a pleiotropic physiological and developmental response including the repression of SA-dependent transcriptional output (Kazan and Manners, 2013).

At least two Psyr type III effectors, HopX1 and HopZ1a, and the phytotoxin coronatine can activate the JA pathway. Coronatine is a structural mimic of JA-Ile (Katsir et al., 2008). HopX1 is a cysteine protease that eliminates JAZ proteins by cleaving their central ZIM domain (Gimenez-Ibanez et al., 2014). HopZ1a is an acetyltransferase that acetylates soybean and Arabidopsis JAZ proteins, promoting COI1-dependent JAZ turnover (Jiang et al., 2013). Both HopX1 and HopZ1a were identified from Psyr strains deficient in coronatine biosynthesis, and each can rescue the growth defects of a Psyr mutant unable to synthesize coronatine (Gimenez-Ibanez et al., 2014; Jiang et al., 2013). Effects of HopX1 or HopZ1a action on the global, JA-activated transcriptional landscape have not been defined, though each causes de-repression of a few tested JA response genes (Gimenez-Ibanez et al., 2014; Jiang et al., 2013).

Here, we provide a mechanistic model for how one of the many TCP14-targeting effectors suppresses defense by manipulating the host defense hormone network to promote Psyr virulence. Our data demonstrate that the previously unstudied bacterial type III effector, HopBB1, alters subsets of targets from two heretofore unlinked transcriptional regulons, TCP14 and MYC, to de-repress a subset of JA responses and promote virulence while avoiding pleiotropic effects associated with full misregulation of either regulon.

**RESULTS**

TCP14 Is a Negative Regulator of JA Signaling

We showed that a tcp14 mutant enhanced susceptibility to the avirulent Emwa1 isolate of the oomycete pathogen, Hpa (Mukhtar et al., 2011; Weßling et al., 2014). We confirmed and extended this result using a second tcp14 allele and transgenic Arabidopsis overexpressing YFP-TCP14 from the UBQ promoter (Figures 1A and 1B). These plants were modestly smaller than wild-type plants at the same developmental stage (Figures 1A and S1A) and displayed enhanced disease resistance when challenged with the virulent Hpa isolate Noco2 (Figure 1B). We examined the in planta growth of P. syringae pv. tomato strain DC3000 (Pto DC3000, hereafter DC3000) and a coronatine-deficient mutant, Pto DC3000 cor− (hereafter DC3000 cor−), on tcp14 mutants and TCP14 overexpression lines. Plants overexpressing TCP14 displayed enhanced resistance to DC3000 at the same levels as coi1 mutants (Figure 1C). The growth of DC3000 cor− was the same on Col-0 and plants overexpressing TCP14 (Figure 1C). tcp14 mutants were unaltered in their response to DC3000 but rescued the growth defects of DC3000.
cor- (Figure 1C). These disease phenotypes suggest that TCP14 regulates immune system output by suppressing the JA response.

To test the hypothesis that TCP14 is a negative regulator of the JA pathway, we defined a comprehensive set of marker genes for the JA and SA responses (Figures S1B–S1F; Table S1) and analyzed the transcriptomes of Col-0, tcp14 mutants, and transgenic plants overexpressing TCP14 in 2-week-old seedlings; the time point when altered infection phenotypes were observed. A total of 203 genes were differentially expressed in TCP14-overexpressing seedlings compared to Col-0 (Figure 1D; Table S2). Genes downregulated by TCP14 overexpression were significantly enriched for genes that are activated by JA treatment (26/102; p = 2.19e-19, hypergeometric test; cluster 1, Figures 1D and 1E). Indeed, many of these downregulated genes were also weakly expressed in the coi1-16 mutant (Figure 1D; Table S2). In contrast, only 6 of the 101 genes that were upregulated in the UBQ::YFP-TCP14-3 line are markers of the SA response (Figure 1D), suggesting that TCP14-driven repression of the JA pathway was not a consequence of activated SA response. No global transcriptome changes were observed in tcp14 mutants relative to Col-0 in non-infected plants (Table S2). However, some JA-responsive genes, including VSP2 and those required for anthocyanin biosynthesis, were upregulated in the mutants (Figure 1F).

We also examined transcriptional alterations in these plants 24 hr after infection with DC3000 cor-.. When compared to infected Col-0, both coi1-16 and the UBQ::YFP-TCP14-4 line showed weaker activation of JA-responsive genes (Figures 1G and 1H; Table S2). Although the suppression of the JA response is an obvious transcriptional alteration in either wild-type or infected UBQ::YFP-TCP14 plants (Figures 1D and 1H), TCP14 may also participate in the regulation of other sectors of the plant immune system. Indeed, the enhanced disease phenotype in UBQ::YFP-TCP14 also correlates with suppression of ABA-responsive genes and responses related to ABA signaling (Figure 1I, Table S2). Moreover, TCP14-overexpressing lines displayed enhanced activation of SA-responsive genes after infection (Figure 1I; Table S2), consistent with their enhanced resistance to both DC3000 and Hpa isolate Noco2 (Figures 1B and 1C). However, only 43 genes were differentially expressed in the infected tcp14-6 mutant relative to wild-type plants (Figure 1G; Table S2). We do not know if the effects of tcp14 mutation would be more dramatic at different time points in the response. Overall, this transcriptional profile supports the conclusion that TCP14 contributes to plant immunity as a negative regulator of subsets of the JA response.

**The *P. syringae* Effector HopBB1 Interacts with TCP14 In Vivo**

To demonstrate how effectors modulate TCP14 function, we focused first on an uncharacterized TCP14-interacting *Psy* type III effector, HopBB1 (Mukhtar et al., 2011). In yeast, HopBB1 selectively interacts with a subset of 24 *Arabidopsis* TCP family members (Figure S2A). We validated the interactions between HopBB1 and TCP14 in planta by inoculating YFP-TCP14 overexpressing *Arabidopsis* with DC3000 cor- expressing HopBB1-HA at native levels. We observed that HopBB1-HA co-immunoprecipitated with YFP-TCP14 (Figure 2A), demonstrating that these two proteins associate in vivo during *Psy* infection. We used random mutagenesis to isolate a HopBB1 mutant, HopBB1G126D that lost interaction with TCP14 in yeast-two-hybridization (Y2H) and failed to associate with TCP14 in planta (Figures 2A, 2B, and S2B). HopBB1111-283 that contains G126, but has no annotated function, was sufficient for association with TCP14 (Figure 2C). TCP14180-489 downstream of the conserved TCP DNA binding domain was sufficient for interaction with HopBB1 in yeast and in planta (Figures 2D, 2E, and S2C). TCP14180-216 co-immunoprecipitated with HopBB1 (Figure 2E). We replaced every six amino acids in this region with a structurally flexible sequence (NAIRS; Wilson et al., 1985), and revealed that the TCP14 sequence motif 204-RSAAST-209 is necessary for interaction between full length TCP14 and HopBB1 (Figures 2F, 2G, and S2D). Collectively, these data are consistent with the hypothesis that HopBB1 associates with TCP14 in vivo.

**HopBB1 De-represses JA Response**

We tested the hypothesis that HopBB1 targets TCP14 to manipulate plant JA response. Following delivery of native levels via type III secretion, HopBB1, but not HopBB1G126D, partially rescued the growth defects of DC3000 cor- on Col-0 plants (Figures 3A and S3A). Growth promotion of DC3000 cor-, contributed by HopBB1 was suppressed by overexpression of TCP14 and in coi1 (Figure 3A). These observations indicate that HopBB1 partially complements the defects of coronatine deficiency, that this can be modulated by TCP14, and that it requires COI1.

We then investigated the effect of HopBB1 on the transcriptome of wild-type plants 24 hr after the infection. As expected, the transcriptome of plants infected with DC3000 was significantly different from those sprayed with either a mock or DC3000 cor- (EV) (Figures 3B and 3C). The set of 697 genes that were more strongly induced by DC3000 than by DC3000 cor- (EV) was enriched in biological processes related to JA and ABA responses (Table S3). Remarkably, infection with DC3000 cor- (HopBB1) resulted in a global transcriptional signature that resembled infection with DC3000 (Figure 3C), supporting our conclusion that HopBB1 can rescue the impaired ability of DC3000 cor- to establish infection. A total of 129 of the 672 (19%) JA-responsive genes were expressed to higher levels in plants infected with DC3000 or DC3000 cor- (HopBB1) than in plants with DC3000 cor- (EV) treatment. Although the transcriptional changes induced by DC3000 cor- (HopBB1G126D) qualitatively resembled those induced by DC3000 (Figure 3C), these JA-responsive genes were less activated (p value = 2.2e-16, Student’s t test), indicating that interaction with TCP14 is required for the full virulence function of HopBB1 (Figure 3D).

To exclude the possibility that the transcriptome change induced by bacteria-delivered HopBB1 may be confounded by other effectors that may influence JA signaling, we defined the transcriptome of transgenic plants expressing only HopBB1. As expected if HopBB1 potentiates JA responses, these plants were hypersensitive to JA-mediated inhibition of root elongation (Figure S3C). In addition, DC3000 cor- is more virulent on HopBB1 transgenic plants than on wild-type Col-0, demonstrating that heterologous HopBB1 complements this strain’s coronatine deficiency (Figure 3F), analogous to tcp14 (Figure 1C). We compared the transcriptome of HopBB1-expressing plants to Col-0 at steady state and identified 628 differentially
expressed genes (593 upregulated and 35 downregulated) (Table S3). Many of our JA response marker genes (93/672; p = 3.41e−47; hypergeometric test) were upregulated in the HopBB1 expressing plants (Table S3), and the average expression of all 672 JA-responsive genes was higher in these transgenic plants (Figure 3G). JA response genes were enriched in the overlap between HopBB1-upregulated and TCP14-suppressed genes: out of the 102 genes that were downregulated by steady-state TCP14 overexpression (Figure 1D; Table S4), 12 were upregulated in HopBB1 transgenic plants and 10 of these are JA markers (p = 2.26e−17; hypergeometric test) (Table S4). Genes specific to BTH/SA response were also enriched by HopBB1 overexpression (Figure 3D; Table S4). Interestingly, neither constitutive nor conditional overexpression of HopBB1 caused the chlorotic leaf phenotype observed previously after either coronatine treatment or HopX1 expression (Figure S3F; Gimenez-Ibanez et al., 2014; Kloek et al., 2001). Consistent with this observation, the expression of MYC2-dependent and JA-responsive photosynthetic genes (Qi et al., 2015) was not altered in HopBB1-expressing plants (Figure S3G). In sum, our transcriptome data are consistent with our pathology data and support the hypothesis that HopBB1 activates a sector of the overall JA response that is co-regulated by TCPs and MYC.

We surveyed the genomic distribution of HopBB1, coronatine biosynthetic genes, HopX1, and HopZ1a in 287 Psy genomes. Only four (1.3%) genomes contain two presumably functional JA-activating virulence factors (Figure 3H; Table S5). Nearly 50% (141) of Psy genomes carry one, and only one, functional version of these four JA-activating virulence factors (Figure 3H; Table S5). Strikingly, in a few additional cases where HopX1 and coronatine biosynthetic genes co-exist in a single strain, the HopX1 alleles have mutations in functionally essential residues (Nimchuk et al., 2007). The phylogeny of the 141 Psy isolates suggests that independent gene gain and/or loss occurred.

As expected, the JA response genes defined in our study were enriched for MYC2 binding motifs in their promoters (Figure S3D). In fact, these genes were enriched for co-occurrence of MYC2 and TCP binding sites (Franco-Zorrilla et al., 2014; Kosugi and Ohashi, 2002). Out of the 88 JA response genes that contain consensus MYC and TCP motifs in their promoters, 22 (25%) were also upregulated by HopBB1 expression (Figure S3E). Interestingly, neither constitutive nor conditional overexpression of HopBB1 caused the chlorotic leaf phenotype observed previously after either coronatine treatment or HopX1 expression (Figure S3F; Gimenez-Ibanez et al., 2014; Kloek et al., 2001). Consistent with this observation, the expression of MYC2-dependent and JA-responsive photosynthetic genes (Qi et al., 2015) was not altered in HopBB1-expressing plants (Figure S3G). In sum, our transcriptome data are consistent with our pathology data and support the hypothesis that HopBB1 activates a sector of the overall JA response that is co-regulated by TCPs and MYC.

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in each lineage (Figure 3H; Table S5). This is particularly true across otherwise very closely related strains from the *Psy* pathovar actinidae, currently responsible for epidemic disease outbreaks that threaten the kiwi industries of New Zealand and Italy (Table S5; McCann et al., 2013).

**HopBB1-Mediated Degradation of TCP14 Requires SCFCOI1**

Given that HopBB1 interacts with TCP14 and de-represses JA responses during infection, we investigated how HopBB1 disrupts TCP14 function. Delivery of native levels of HopBB1, but not HopBB1<sub>G126D</sub>, led to reduced TCP14 protein accumulation (Figure 4A). MeJA treatment alone did not alter the accumulation of TCP14, indicating that the HopBB1-induced turnover of TCP14 was not a consequence of activated JA response (Figure 4B). TCP14 degradation in these experiments required the SCFCOI1 complex, since it was blocked in *coi1-1* plants expressing UB-Q::YFP-TCP14 (Figure 4C). Thus, HopBB1 promotes the degradation of TCP14 via the SCFCOI1 degradation pathway during infection, ultimately facilitating the activation of JA responses.
transcripts were quantified using real-time PCR. Error bars indicate ± SD.

(A) Bacterial-delivered HopBB1, but not HopBB1G126D, induced turnover of TCP14 during infection on Arabidopsis. Bacteria were hand-inoculated into leaves of 4-week-old plants at an OD600 = 0.05. Samples were harvested 24 hr after inoculation.

(B) YFP-TCP14 is not subject to JA-mediated degradation in the absence of HopBB1 in Arabidopsis. The 2-week-old seedlings expressing UBQ::YFP-TCP14 were sprayed with mock or 50 μM MeJA solution and sampled at the indicated time. Each sample was pooled from eight seedlings.

(C) Pto-delivered HopBB1 reduces TCP14 protein level in wild-type Col-0, but not in col1-1 mutant. Experiments were performed as Figure 4A. YFP-TCP14 transcripts were quantified using real-time PCR. Error bars indicate ± SD.

**HopBB1 Interacts with JAZ3**

We sought to define which component(s) of the SCFCOI1 pathway mediated TCP14 degradation. We confirmed the finding that JAZ3 interacted with HopBB1 (Mukhtar et al., 2011; Weßling et al., 2014; Figure S4A). Conditionally expressed HopBB1 co-immunoprecipitated JAZ3 in both transgenic Arabidopsis and transiently expressing *N. benthamiana* leaves (Figures 5A and S4D). HopBB1111-283 was also sufficient for association with JAZ3 (Figure S5B). However, HopBB1G126D retained interaction with JAZ3 (Figures S4C and S4D), suggesting that a different association surface within the HopBB1 C terminus is required. JAZ3P302A R305A is required for HopBB1 interaction, which is supported by association analyses in yeast and *N. benthamiana* (Figures 5C and 5D).

In contrast to HopX1 and HopZ1a, HopBB1 expression was not sufficient to alter JAZ3 accumulation (Figure S4E; Gimenez-Ibanez et al., 2014; Jiang et al., 2013). However, increasing HopBB1 levels did reduce the amount of MYC2 associated with JAZ3 in a competitive co-immunoprecipitation assay in *N. benthamiana*, indicating that HopBB1 interferes with the interaction of MYC2 and JAZ3 (Figure 5E). We developed a bimolecular fluorescence complementation (BiFC)-based assay to examine this disassociation in vivo. We co-infiltrated Agrobacterium strains carrying either a BiFC construct expressing JAZ3-nYFP, cYFP-MYC2, and mRFP as a co-expression reporter or a second construct carrying an estradiol-inducible HopBB1-CFP to test the ability of HopBB1 co-expression to block JA3-MYC2-interaction-mediated YFP reconstruction (Figures 5F, S4F, and S4G). Co-expression of HopBB1-CFP or HopBB1G126D-CFP dramatically reduced the percentage of reconstituted YFP signal in CFP- and RFP-positive nuclei. Neither CFP nor CFP tagged with HaRxL45 (an Ha effector that interacts with TCP14, but not JAZ3) (Weßling et al., 2014) altered the BiFC efficiency (Figures 5F and S4H). These observations support our contention that HopBB1-JAZ3 association interferes with the interaction between JAZ3 and MYC2 in vivo.

**TCP14 Is Subject to JA-Mediated Degradation in the Presence of HopBB1 and JAZ3**

Since JAZ proteins are subject to SCFCOI1-mediated degradation, we tested how HopBB1 and JAZ3 influence the degradation of TCP14 in a reconstructed degradation system in *N. benthamiana*. TCP14 was not subject to JA-mediated protein degradation when transiently expressed (Figures 4B and 6B) or when co-expressed with either HopBB1 or JAZ3 (Figures 6A and 6B). Importantly, when TCP14, JAZ3, and HopBB1 were co-expressed, levels of all three proteins were dramatically reduced upon MeJA treatment (Figure 6B). As anticipated, MYC2 levels were not reduced in these experiments (Figure 6C). Thus, HopBB1-mediated TCP14 turnover requires JA and JAZ3.

The protease inhibitor MG132 can block JA-triggered degradation of JAZ proteins (Chini et al., 2007; Thines et al., 2007); it also blocked HopBB1-mediated degradation of TCP14 (Figure 6D). We generated JAZ3P302A R305A, an allele that cannot interact with COI1 and is thus MeJA resistant (Figures 6E, S5A, and S5B). Importantly, JAZ3P302A R305A still interacted with HopBB1 in yeast, suggesting that its overall structure is not altered (Figure S5C). When we co-expressed JAZ3P302A R305A with TCP14 and HopBB1, MeJA-induced degradation of HopBB1 and TCP14 was blocked (Figure 6E). This observation suggested that SCFCOI1-dependent degradation of JAZ3 is required for HopBB1-mediated, JA-dependent turnover of TCP14. TCP14 turnover was not driven by HaRxL45 co-expression in the presence of JAZ3 (Figure 6F), implying that effectors interacting with TCP14 modulate its activity by at least two different mechanisms. Remarkably, HopBB1G126D failed to mediate TCP14 turnover but was still degraded with JAZ3 in the presence of MeJA (Figure 6G). Conversely, the TCP14RSAAST/NAAIRS mutant that fails to interact with HopBB1 was also resistant to HopBB1-mediated degradation (Figure 6H). Thus, HopBB1-mediated degradation of...
TCP14 requires its interactions with TCP14 and the degradation of JAZ3 through SCFCOI1.

**HopBB1 Recruits TCP14 into a JAZ3-Containing Sub-nuclear Structure**

We previously demonstrated that TCP14 re-locates HopBB1 into an uncharacterized sub-nuclear structure (Figures 7A and 7B, top, and S6A; Weßling et al., 2014). Similarly, we noted that JAZ3 also forms sub-nuclear foci (Figure 7A). We found that HopBB1 can also be re-localized into JAZ3 foci (Figures 7B, middle, and S6B). Strikingly, JAZ3 foci and TCP14 foci did not overlap when co-expressed, implying that they represent different structures (Figures 7B, bottom, and S6C). We then co-expressed a CFP-tagged HopBB1 together with TCP14 and JAZ3. In nuclei with HopBB1-CFP (but not with CFP alone), the CFP, YFP, and RFP signals co-localized in the same foci (Figures 7C and S6D), suggesting that HopBB1 re-distributed TCP14 and JAZ3 from separate sub-nuclear foci into the same structure. Although HopBB1_{G126D} failed to associate with TCP14 in both Y2H and co-IP assay, it was still re-localized by TCP14, suggesting that these assays report different interaction affinities (Figure S6E). For this reason, we could not specifically test the requirement of HopBB1-TCP14 interaction in re-distributing JAZ3 and TCP14. However, TCP14_{RSAAST/NAIRS}, which still forms nuclear foci but cannot interact with HopBB1, also cannot be fully re-distributed to the JAZ3-containing foci in the presence of HopBB1 (Figure 7D). This result confirms that re-distribution of TCP14 to JAZ3-containing foci requires its association with HopBB1.

We utilized TCP14 and JAZ3 mutants to examine functional requirements for the formation of sub-nuclear foci. Amino acids H121, R130, and L161 in the TCP domain are conserved in all 24 Arabidopsis TCP proteins (Figure S6F). Single mutation in any of these abolished TCP4 binding to DNA (Aggarwal et al., 2014).
TCP14H121Q R130K L161N almost completely abolished the formation of sub-nuclear foci in transgenic Arabidopsis, although it was still exclusively localized in nuclei and retained its ability to homodimerize and associate with HopBB1 (Figures 7E and S6G–S6I). Thus, the formation of the TCP14 nuclear foci is dependent on TCP14 DNA binding activity.

We tested whether the JAZ3 nuclear foci represent a structure for its degradation. We observed that JAZ3-RFP formed sub-nuclear foci in Col-0, but not in coi1-1 (Figure 7F), a COI1 allele without detectable protein accumulation (He et al., 2012). JAZ3P302A R305A was unable to form nuclear foci (Figure 7G). Thus, formation of JAZ3 nuclear foci requires COI1. Importantly, JAZ3P302A R305A was re-localized to TCP14 sub-nuclear foci only in the presence of HopBB1 (Figures 7H and 7I). In sum, these data are consistent with a model wherein HopBB1 links to SCF COI1 signaling to the pathogen’s advantage.

DISCUSSION

We demonstrate that the HopBB1 type III effector protein modulates subsets of two Arabidopsis regulons—those negatively regulated by TCP14 and activated by MYC2—leading to a fine-tuned perturbation in plant defense output that facilitates bacterial pathogen proliferation. Expressing HopBB1 from bacteria or in Arabidopsis rescues the virulence defect of a pathogenic Psy strain lacking the JA-Ile structural mimic, coronatine, suggesting its role as a regulator of host defense (Figure 3). HopBB1 has dual functions in de-repressing the JA signaling pathway: it facilitates the degradation of TCP14 and possibly other TCPs through SCF COI1 by connecting JAZ3 to it (Figures 4, 6, and 7), and it disrupts the inhibitory association between JAZ3 and MYC2, leading to MYC2-dependent transcriptional activation of JA responses (Figure 5), which may contribute to the residual function of HopBB1G126D in activating JA response (Figure 5D). However, only subsets of either the TCP14 or MYC regulons are transcriptionally perturbed in the presence of HopBB1 (Figures 1D and S3E). Thus, we propose that HopBB1 has evolved to minimize pleiotropic negative effects on host physiology generated by wholesale de-repression of the JA response output (defined here by MeJA treatment) while maintaining the ability to modulate defense hormone signaling to the pathogen’s advantage.

Four Psy virulence factors—coronatine, HopX1, HopZ1a and HopBB1—activate the JA response at different steps in the signaling pathway. Coronatine and HopX1 stimulate an overlapping spectrum of JA-related phenotypes, including activation of a few tested JA-responsive genes, promotion of stomatal opening, and induction of chlorotic symptoms in infected plants. This pleiotropy is likely attributable to the ability of HopX1 to directly cleave almost all members of the JAZ family; this is functionally analogous to coronatine action (Gimenez-Ibáñez et al., 2014; Kloek et al., 2001). In contrast, we conclude that HopBB1 expression specifically activates a subset of JA-mediated responses (Figures 3D and S3E). This conclusion is supported by several observations. First, expressing HopBB1 in Arabidopsis activates only about 18% (168 of 933) of JA-responsive genes (Figures 3D and S3E). This conclusion is supported by several observations. First, expressing HopBB1 in Arabidopsis activates only about 18% (168 of 933) of JA-responsive genes (Figures 3D and S3E). This conclusion is supported by several observations. First, expressing HopBB1 in Arabidopsis activates only about 18% (168 of 933) of JA-responsive genes (Figures 3D and S3E). This conclusion is supported by several observations. First, expressing HopBB1 in Arabidopsis activates only about 18% (168 of 933) of JA-responsive genes (Figures 3D and S3E). This conclusion is supported by several observations. First, expressing HopBB1 in Arabidopsis activated only about 18% (168 of 933) of JA-responsive genes (Figures 3D and S3E).
in HopX1 transgenic plants and coronatine-treated plants are not visible following either constitutive or conditional expression of HopBB1 (Figures 3E and S3F). Importantly, coronatine-induced chlorosis can be de-coupled from bacterial growth promotion and repression of SA-dependent responses (Kloek et al., 2001). We suggest that HopBB1 fine-tunes JA response by targeting a sub-group of JAZ proteins, leading to transcriptional activation of genes enriched in those co-regulated by TCP and MYC.

Modulation of plant JA responses is an important virulence strategy for phytopathogenic bacteria (Gimenez-Ibanez et al., 2014; Jiang et al., 2013; Zheng et al., 2012). The evolutionary mechanism driving the mutual exclusivity of JA-modulating virulence factors in Psy genomes (Figure 3H and Table S5) is unknown, but is consistent with negative frequency-dependent selection driven by the centrality of JA response manipulation to Psy virulence, balanced by host immune recognition. This particular arms race is evident in various plants. The ZAR1 NLR innate immune receptor in Arabidopsis recognizes the acetylation activity of HopZ1a on the ZED1 pseudokinase (Lewis et al., 2013). Alleles of HopX are recognized by the as-yet-uncloned R2 disease-resistance gene in beans (Mansfield et al., 1994). Additionally, plants can evolve JAZ proteins that are resistant to COI1-mediated degradation (Chung and Howe, 2009; Shyu et al., 2012). These JAZ proteins might antagonize coronatine function. Although a host surveillance mechanism recognizing
HopBB1 has not been discovered, it could be achieved by monitoring an as-yet-unknown activity on TCP14 or JAZ3, or on the relevant interacting domains integrated into recently described decay fusion NLR proteins (Cesari et al., 2014).

TCP14 is targeted by effectors from three evolutionarily divergent pathogens (Weßling et al., 2014). Our results demonstrated that TCP14 contributes to disease resistance against Psyr as a negative regulator of JA signaling (Figure 1). JA-responsive genes are repressed in seedlings overexpressing TCP14 (Figure 1D), and tcp14 mutants rescue the growth defects of DC3000 cor− (Figure 1C). However, TCP14 may regulate other defense pathways against different pathogens at different developmental stages or in different tissues. Indeed, Kim et al. (2014) suggested that TCP8, TCP14 and TCP15 are part of a transcriptional complex involved in NLR-mediated immune system signaling. Thus, we speculate that the TCP14-interacting effectors identified from Psyr, Hpa, and Go will manipulate TCP14 via different mechanisms to facilitate proliferation of pathogens with different life cycles and infection strategies. It is therefore noteworthy that an Hpa-derived TCP14-interacting effector, HaRxL45, fails to activate degradation of TCP14 in the presence of JA, indicating that this effector modulates TCP14 in a manner mechanistically different from that of HopBB1. Future studies will explore the different sectors of the immune response that are under direct control of TCP14.

**EXPERIMENTAL PROCEDURES**

**Transient Protein Expression in N. benthamiana**

N. benthamiana plants were grown at 24°C (day) and 20°C (night) under a 16-hr light and 8-hr dark cycle. Agrobacteria were collected and re-suspended in 2 mL re-suspension buffer (10 mM MES [pH 5.6], 10 mM MgCl2, and 200 μM acetoxyserine) to a final concentration of OD600 = 0.2. To reach equal protein accumulation, the final concentration of agrobacteria expressing HopBB1 and HopBB1-T208D was OD600 (0.20) and OD600 (0.2), respectively. GV3101 carrying 3SS promoter-driven p19 protein was co-infiltrated at OD600 = 0.05 in each experiment to prevent the onset of post-transcriptional gene silencing and improve the efficiency of transient expression. Agrobacterium tumefaciens GV3101 (pMP90) transformed with mixtures of binary vector constructs were infiltrated into N. benthamiana leaves using a needleless syringe. Samples were harvested 24 hr after infiltration unless otherwise indicated.

**Immunoblot and Co-immunoprecipitation Analyses**

Leaf tissues were ground in liquid nitrogen and extracted with 150–200 μL of grinding buffer (50 mM Tris [pH8.0], 1% SDS, 1 mM EDTA) also containing 1 μL/mL β-mercaptoethanol and 1 x protease inhibitor (Sigma-Aldrich). The lysates were centrifuged at 12,000 rpm for 10 min at 4°C. Supernatants were collected, and the protein concentration was determined with the BioRad Bradford quantification method (BioRad).

For co-immunoprecipitation analyses, proteins were extracted from 0.5 g of fresh tissue using 2 mL extraction buffer (50 mM HEPES [pH 7.5], 50 mM NaCl, 10 mM EDTA [pH 8.0], 0.5% Triton X-100, 5 mM DTT, and 1 x Plant protease inhibitor cocktail from Sigma-Aldrich). Magnetic labeling and separation of tagged proteins was performed using mMACS Epitope Tag Protein Isolation Kit (Miltenyi Biotec). Protein samples were separated by SDS-PAGE. Immuno-bLOTS were performed with a 1:1,000 dilution of α-HA (Roche), 1:1,000 dilution of α-GFP (Roche), 1:1,000 dilution of α-myc, and 1:1,000 dilution of α-FLAG. Blots were detected by ECL prime (GE Healthcare).

**Mutagenesis**

JAZ3P302A R305A and TCP14H121Q R130K L161N were generated using the Quik-Change Lightning Site-Directed Mutagenesis Kit (Agilent). Random mutagenesis of HopBB1 was performed using the GeneMorph II EZClone Domain Mutagenesis Kit. A pJG4-5-HopBB1 construct was mutagenized according to the manufacturer’s protocol. The library was transformed into yeast strain RFY206. Each RFY206 (pJG4-5-HopBB1) clone was mated with yeast EGY48 strain carrying pEG202-TCP14 or pEG202-JAZ3. HopBB1 clones that lost interaction with either TCP14 or JAZ3, but not both, were sequenced. If multiple mutations were present in one clone, single mutations were introduced into wild-type HopBB1 and confirmed by re-testing.

**Real-Time PCR**

Total RNAs were extracted with the RNeasy Plant Mini kit (QIAGEN). cDNAs were synthesized using SuperScript III Reverse Transcriptase (Invitrogen). qPCR was performed using SYBR green master mix (Applied Biosystems) with the following cycle: 95°C for 3 min, 40 cycles of 95°C for 15 s, and 72°C for 20 s. Expression levels were normalized to multiple endogenous controls including UBQ5 (AT3G62250), TUB (AT5G62690), and SAND (AT2G28390).

**Statistics for In Plant Bacterial Growth**

In Figures 1C, 3A, and IF, error bars represent ± SD. Statistics were performed using one-way ANOVA test with Tukey-Kramer HSD with 95% confidence. In each case, the result displayed is one of three independent analyses giving similar results.

**ACCESSION NUMBERS**

The NCBI Gene Expression Omnibus accession number for the RNA-seq data reported in this paper is GEO: GSE90606.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, six figures, and six tables and can be found with this article online at http://dx.doi.org/10.1016/j.cjhm.2017.01.003.


Supplemental Information

_Pseudomonas syringae_ Type III Effector HopBB1
Promotes Host Transcriptional Repressor Degradation
to Regulate Phytohormone Responses and Virulence

Li Yang, Paulo José Pereira Lima Teixeira, Surojit Biswas, Omri M. Finkel, Yijian He, Isai Salas-Gonzalez, Marie E. English, Petra Epple, Piotr Mieczkowski, and Jeffery L. Dangl
sFigure 1

A) α-GFP, RuBisCo, Col-0, UBQ::YFP-TCP14, TCP14

B) Gene expression levels for TCP14

C) PCA analysis showing different treatments: MeJA, BTH, Mock, T0h, T1h, T5h, T8h

D) Up-regulated and Down-regulated genes

E) BTH: 2357 unique genes

F) MeJA: 933 unique genes

G) Top10 enriched GO terms for MeJA and BTH clusters
sFigure 2

A

B

C

D

TCP14: 180-PANFTSLNISLRSSGSSMSLPSHFRSAASTFSPNNI-216
TCP14-PANFTS: 180-NNAIRSLSNISLRSSGSSMSLPSHFRSAASTFSPNNI-216
TCP14-LNISLR: 180-PANFTSNAAIRSSGSSMSLPSHFRSAASTFSPNNI-216
TCP14-SSGSSM: 180-PANFTSLNISLRNAAIRSSGSSMSLPSHFRSAASTFSPNNI-216
TCP14-SLPSHF: 180-PANFTSLNISLRSSGSSMNAAIRSRSAASTFSPNNI-216
TCP14-RSAAST: 180-PANFTSLNISLRSSGSSMSLPSHFNAAIRSFSPNNI-216
TCP14-FSPNNI: 180-PANFTSLNISLRSSGSSMSLPSHFRSAASTNAAIRS-216
sFigure 3

A. Pto DC3000 cor- (-AvrRpt2101-255)

EV      BB1      BB1G126D

1/12  12/12  11/12

B. 35S::BB1-myc

α-myc  Rubisco

Col-0  -4  -10

36

C. Root Length (mm)

Mock  10uM

Col-0  35S::BB1-4  35S::BB1-10  col1-16

D. Expected vs. Observed

Number of genes

p = 2.60e-34

p = 2.41e-13

D\(\text{MYC (CACGTG) (GGNCCC)}\)

D\(\text{TCP}\)

D\(\text{MYC+TCP}\)

E. MYC+TCP promoter (1129)

Up-regulated by MeJA (933)

Response to water deprivation:
NCED3 (AT3G14440)
ERD14 (AT1G76180)
LEA4-5 (AT5G06760)

JA biosynthesis/signaling:
ACX1 (AT4G16760)
AOX2 (AT3G25770)
AOX3 (AT3G25780)
JAZ1 (AT1G19180)
NAC019 (AT3G15500)

F. Col-0  Col-0  EST::HopBB1-YFP-HA

COR  estradiol  estradiol

20uM estradiol

hr 0  4  8  18

IB:αHA

G. Gene expression (RPKM)

CAB1  RBCS1A
sFigure 5

A

JAZ12 ELPIARRASLQFLKPRDKEVKNKPT
JAZ2 ELPIARRASLQFLKPRDKEVKNKPT
JAZ4 GILQTKASLQFLKPRDEIVNSPIY
JAZ9 SVFQKASLQFLKPRDEIVSMTYK
JAZ5 VERTARRASLQFLKPRDEIVMYPY
JAZ6 VERTARRASLQFLKPRDEIVMYPY
JAZ7 QHASMKEFLHSLQKRSLIKEH
JAZ8 PKASMKEFLQSLQKRSLIKEH

B

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α-LexA

α-HA
Figure S1 (Related to Figure 1)

(A) Accumulation of YFP-TCP14 protein (left) and transcripts (right) in transgenic plants. Error bars represent ±SD.

(B)–(F) Defining JA and SA responsive genes in Col-0. (B) Overview of the experimental conditions used to define genes responsive to MeJA and BTH by RNA-seq. (C) Principal Component Analysis (PCA) showing the overall relationship of the RNA-seq libraries used to define MeJA and BTH/SA markers. Colors represent different time-points and symbols represent treatments. Biological replicates are labeled r1 and r2. (D) Number of genes up- and down-regulated by each treatment defined using the edgeR package (FDR ≤ 0.01; 1.5 fold-change difference). While 261 genes were up-regulated by both hormones, 672 and 2096 genes were up-regulated specifically by MeJA and BTH, respectively, which define our set of marker genes. Table S1 shows the complete RNA-seq results for all Arabidopsis genes. (E) Overview of genes up-regulated by BTH at each time-point. The table shows Gene Ontology terms (biological processes) enriched in this set of genes. (F) Overview of the genes up-regulated by MeJA at each time-point. The table shows Gene Ontology terms (biological processes) enriched in this set of genes.

(G) Gene Ontology terms (biological processes) enriched in the set of genes identified as differentially expressed in the UBQ::YFP-TCP14-3 line. The three clusters correspond to the hierarchical clustering analysis presented in Figure 1D.
**Figure S2 (Related to Figure 2)**

(A) Protein interaction between HopBB1 and 18 of 24 Arabidopsis TCP family members in yeast.

(B) Protein accumulation in Figure 2B.

(C) Protein accumulation in Figure 2D.

(D) NAAIRS-scanning mutagenesis in the TCP14_{180-216} region.

TCP14_{RSAAST/NAAIRS} cannot interact with HopBB1.

**Figure S3 (Related to Figure 3)**

(A) HopBB1 and HopBB1_{G126D} were delivered into plant cells when expressed in *Pto DC3000 cor*-. HopBB1 or HopBB1_{G126D} were cloned upstream of *AvrRpt2*^{101-255}. The chimeric proteins were expressed under the control of NPTII promoter (Vinatzer et al., 2005).

(B) Protein accumulation in transgenic plants expressing HopBB1-myc.

(C) Plants expressing HopBB1 are hypersensitive to JA-mediated inhibition of root elongation. One-week-old seedlings grown on vertical plates were transferred to mock plates or plates with 10µM MeJA. Root length was measured one week after transfer. Error bars indicate ±SD. Statistics were performed using one-way ANOVA test with Tukey-Kramer HSD with 95% confidence. Similar results were obtained from two independent experiments.

(D) Co-occurrence of consensus MYC (CACGTG) and TCP (GGNCCC) binding sites is enriched in the promoters of MeJA-regulated genes from Figure S1F,
Table S1 (88/933, p=2.41e-13; hypergeometric test). We searched for these motifs in the 1kb upstream region relative to the start codon of 27206 nuclear protein-coding genes (TAIR10). The observed number of promoters containing each or both motifs was contrasted to the expected number in each category, given the motif’s frequency in the entire genome and tested for over-representation using the hypergeometric test.

(E) The overlap between genes up-regulated by MeJA and HopBB1, and with the co-occurrence of TCP14 and MYC binding sites in their promoters. We verified that 22 (25%) of the 88 JA-responsive genes containing both MYC and TCP binding sites in their promoters are also up-regulated by HopBB1, which is more than expected by random sampling (2.18%; p=1.49e-16; hypergeometric test). Importantly, this list includes genes required for JA biosynthesis and signaling.

(F) Induced expression of HopBB1 does not trigger chlorosis (left). Four-week old plants were either treated with 50µM of coronatine or 20µM of estradiol for five days. The fifth leaves from three representative plants were photographed. Bar=5mm. The protein accumulation of conditionally expressed HopBB1 is shown in the right panel.

(G) The expression of representative photosynthesis genes is not altered in HopBB1-myc expressing plants.

Figure S4 (Related to Figure 5)
(A) Protein interaction between HopBB1 and 12 Arabidopsis JAZ family members in yeast. HopBB1 interacts with a subset of JAZ proteins.

(B) Protein accumulation for Figure 5C.

(C) HopBB1 G126D retains interaction with JAZ3 in yeast.

(D) HopBB1 G126D retains interaction with JAZ3 in N. benthamiana.

(E) HopBB1 does not promote JAZ3 degradation in Arabidopsis. Conditional expression of HopBB1-myc in transgenic plants expressing 35S::JAZ3-HA does not alter the accumulation of JAZ3.

(F) Quantification of the HopBB1-mediated disruption of MYC2-JAZ3 association. Proteins were transiently co-expressed in N. benthamiana. HaRxL45-CFP, HopBB1-CFP or HopBB1 G126D-CFP was induced 6 hrs after Agrobacteria infiltration. Microscopy was conducted 18 hrs after induction. Eight to ten confocal images with 1 mm² field of view were taken from four randomly sampled leaf discs on each leaf. Images were taken from YFP, CFP and RFP channels. Nuclei were traced only in the RFP channel. Following that, the nucleus signal peaks in each individual channel were counted, and the degree of overlap was compared. Four independent experiments were pooled for the summary presented in Figure 5F.

(G) HopBB1 does not alter MYC2 level in N. benthamiana. Proteins were transiently co-expressed in N. benthamiana.

(H) JAZ3 does not associate with HaRxL45 in planta. Proteins were transiently co-expressed in N. benthamiana from a constitutive 35S promoter.

Figure S5 (Related to Figure 6)
(A) Alignment of the conserved Jas motifs from 12 Arabidopsis JAZ proteins. The P302 and R305 are highlighted with a red arrow.

(B) JAZ3_{P302A\ R305A} cannot interact with COI1 in the presence of coronatine. 50µM of coronatine was added to yeast medium. Protein accumulation is shown in the right panel.

(C) JAZ3_{P302A\ R305A} interacts with HopBB1 in yeast.

Figure S6 (related to Figure 7)

(A) Orthogonal slices of TCP14-RFP and HopBB1-YFP co-localization in sub-nuclear foci. Bar=5 µM. For all orthogonal slices in (A)-(D), the “Merge” panel is the xy plane, right panel (red) is the yz plane, and top panel (green) is the xz plane. The crosshairs indicate the location of the yz and xz planes.

(B) Orthogonal slices view of JAZ3-RFP and HopBB1-YFP co-localization in sub-nuclear foci.

(C) Orthogonal slices view of the distinct sub-nuclear localization of TCP14-YFP and JAZ3-RFP in a nucleus.

(D) Orthogonal slices view of the co-localization of TCP14-YFP, HopBB1-CFP and JAZ3-RFP in sub-nuclear foci.

(E) HopBB1_{G126D} co-localizes with TCP14 when transiently co-expressed in N.benthamiana.

(F) Alignment of the TCP domain from 24 Arabidopsis TCP family members. The mutated H121, R130 and L161 were highlighted with a red arrow. These
residues are conserved in TCP14. Mutation in each individual residue significantly reduced the ability of TCP4 protein to bind DNA (Kosugi and Ohashi, 2002).

(G) TCP14<sub>H121Q R130K L161N</sub> retains the ability to interact with HopBB1 and TCP14 in yeast.

(H) TCP14<sub>H121Q R130K L161N</sub> retains the ability to interact with HopBB1 in <i>N. benthamiana</i>.

(I) TCP14<sub>H121Q R130K L161N</sub> homo-dimerizes.

A-E, H-I: Proteins were transiently expressed from the 35S promoter in <i>N. benthamiana</i>.

**Table S1 (related to Figure 1):** Transcriptional response to MeJA or BTH in Col-0.

**Table S2 (related to Figure 1):** Transcriptional changes induced by TCP14 mutation or overexpression.

**Table S3 (related to Figure 3):** Transcriptional changes induced by bacteria delivered HopBB1 or heterologous HopBB1 expression in Arabidopsis.

**Table S4 (related to Figure 3):** Comparison between genes expression altered by HopBB1 and TCP14 expression.

**Table S5 (related to Figure 3):** Distribution of JA-activating virulence factors in 287 Psy genomes.

**Table S6 (related to experimental procedures):** Primers, seed stocks, constructs, sequences and core genes for phylogenetic analysis.
Extended Experimental Procedures

Plants

Arabidopsis Col-0, tcp14-6 (SAIL_1145_H03, backcrossed to Col-0 four times) (Mukhtar et al., 2011), tcp14-7 (cs108688, backcrossed to Col-0 twice) (Wessling et al., 2014), coi1-16 (Ellis and Turner, 2002; He et al., 2012), and all transgenics were sown and grown as described (Boyes et al., 1998). Primers for genotyping and constructs for generating transgenic Arabidopsis were listed in Table S6.

Yeast two hybridization

HopBB1, JAZ3, TCP14, COI1 and mutant derivatives were cloned into gateway-compatible pJG4-5 (-Trp) or pEG202 (-His) vectors. pJG4-5 and pEG202 constructs were transformed into competent yeast strains EGY48 and RFY206, respectively following manufacturer’s protocol (Frozen-EZ Yeast Transformation II™, Zymo Research) and selected on plates with dropout media. Each strain also carries the GAL4 reporter on psH18-34 (-Ura). Positive colonies were verified by yeast colony PCR. After mating the strain EGY48 and RFY206, diploid yeasts were plated on selective medium (-H-W-U) supplied with 100µM X-Gal for developing blue color from 2-6 days. To measure protein accumulation, yeast colonies were suspended in 50 µl 0.2N NaOH for 10 minutes. Cells were then collected by centrifugation and re-suspended in 1 x loading buffer. Protein levels were examined by western blotting.

RNA sequencing
In order to define a comprehensive set of marker genes for the JA and SA responses, we used RNA-seq to assess the transcriptome of the Arabidopsis Col-0 ecotype over a time-course hormone treatment (Figure S1B). Two-week-old seedlings were sprayed with 50 µM MeJA (Sigma), 300 µM BTH (Actigard 50WG) or a mock solution (0.02% Silwet, 0.1% ethanol). Samples were harvested 1h, 5h and 8h after spraying. This experiment was repeated twice. The experiments shown in Figures 1D, 1E and 1F were performed using steady-state seedlings grown under the same conditions as the ones used in the hormone treatment experiment. Lines Col-0 (4 replicates), UBQ10::YFP-TCP14-3 (4 replicates), UBQ10::YFP-TCP14-4 (1 replicate), and coi1-16 (2 replicates) were used in the experiment presented in Figures 1D and E; whereas Col-0 (3 replicates), tcp14-6 (3 replicates) and tcp14-7 (3 replicates) were used in the experiment shown in Figure 1F. Bacteria-infected plants were used in the experiments shown in Figures 1G, 1H and 1I. For this, the strain Pto DC3000 cor- (at OD<sub>600</sub>=0.2 with 10mM MgCl<sub>2</sub> and 0.04% Silwet L-77) was sprayed onto Col-0 (3 replicates), tcp14-6 (3 replicates), UBQ10::YFP-TCP14-4 (3 replicates) and coi1-16 (3 replicates). Samples were harvested for RNA preparation 24 hours post infection.

We also evaluated the effect of bacteria-delivered HopBB1 on the transcriptome of wild-type plants (shown in Figures 3B, 3C and 3D). For this, two-week-old Col-0 seedlings were sprayed with a mock solution (10 mM MgCl<sub>2</sub>) or bacteria [Pto DC3000 (EV), Pto DC3000 cor- (EV); Pto DC3000 cor- (HopBB1); Pto DC3000 cor- (HopBB1<sub>G126D</sub>)] at OD<sub>600</sub>=0.2 with 10mM MgCl<sub>2</sub> and 0.04% Silwet L-77.
Samples were harvested 24 hours after infection. This experiment included three biological replicates. The transcriptome of the transgenic line HopBB1-myc-10 (3 replicates) was also compared to the one of Col-0 seedlings at steady-state conditions (shown in Figure 3G). In all experiments, each biological replicate corresponds to approximately 30 seedlings grown on the same pot.

For RNA isolation, plant tissue was ground to a fine powder using the Qiashredder tissue homogenizer (Qiagen) and total RNA was extracted using the RNeasy Plant Mini kit (Qiagen). Illumina-based RNA-seq libraries were prepared from 1000ng total RNA. Library quality control and quantification were performed using a 2100 Bioanalyzer instrument (Agilent) and the Quant-iT PicoGreen dsDNA Reagent (Invitrogen), respectively. The Illumina HiSeq2500 sequencer was used to generate single-end reads. Raw sequencing data are available at the NCBI Gene Expression Omnibus GEO:GSE90606.

RNA-seq reads were mapped against the TAIR10 reference genome using Tophat (Trapnell et al., 2009). Alignment parameters were set to allow only one mismatch and to discard reads mapping to multiple positions in the reference. HTSeq (Anders et al., 2015) was then used to count reads mapping to each one of the 27,206 nuclear protein-coding genes. Differential gene expression analyses were performed with the edgeR package (Robinson et al., 2010) using the False Discovery Rate (FDR) method for correction of multiple comparisons (Benjamini and Hochberg, 1995). Genes with FDR below 0.01 and a fold-change variation greater than 1.5X were considered differentially expressed between conditions. Gene Ontology enrichment analyses were performed with the
PlantGSEA toolkit (Yi et al., 2013) and with the Cytoscape plugin ClueGO (Bindea et al., 2009).

We identified a total of 933 and 2357 genes that were significantly up-regulated (FDR≤0.01; 1.5 fold-change difference relative to the mock control) in at least one of the three time-points analyzed after treatment with MeJA or BTH, respectively (Figure S1D; Table S1). As expected, these sets of genes were strongly enriched for biological processes related to JA and SA responses (Figures S1E and S1F). After filtering out the 261 genes upregulated by both hormones, we defined a set of 672 and 2096 markers of the JA and SA responses, respectively.

Confocal microscopy

Microscopy was conducted 16-24 hours after infiltration using a LSM 7 DUO (Carl Zeiss). Leaf disc samples were imaged with a 40x water objective. Between 5 and 15 nuclei were observed in each repetition. The confocal images were edited with Zen 2009 (Zeiss) and Adobe Photoshop CS2. Zen 2009 (Zeiss) and Excel (Microsoft) were used to create histograms. For the HopBB1-TCP14-JAZ3 co-localization assay, JAZ3-RFP and TCP14-YFP were driven under 35S promoter, HopBB1-CFP or HopBB1G126D-CFP was driven by estradiol-inducible promoter. Estradiol was applied 6 hours after the co-infiltration of Agrobacteria. The primers and constructs used for confocal analysis are listed in Table S6. GV3101 carrying 35S promoter-driven p19 protein was co-infiltrated at
OD

600 = 0.05 in each experiment to prevent the onset of post-transcriptional gene silencing and improve the efficiency of transient expression (Lindbo, 2007).

For transient protein expression in *N. benthamiana*, Agrobacteria at annotated concentration were suspended in 10mM MgCl₂, 10mM MES and 100μM acetosyringone, and hand infiltrated into *N. benthamiana* leaves. For the HopBB1-mediated disruption of the JAZ3-MYC2 interaction, rBiFC (JAZ3+MYC2) and EST::HopBB1-CFP-HA, EST::HopBB1\textsubscript{G126D}-CFP-HA or EST::HaRxL45-CFP-HA were co-inoculated at OD\textsubscript{600}=0.1 and OD\textsubscript{600}=0.2, respectively (Grefen and Blatt, 2012). Six hours after inoculation, 20μM estradiol was infiltrated. Samples were collected 20-24 hours after inoculation. 4-6 images of 50-80 cells/per field were taken in each repetition.

**Disease resistance assay**

*Pto* DC3000 and *Pto* DC3000 cor- were described in (He et al., 2012). *Pto* DC3000 cor- (EV), *Pto* DC3000 cor- (HopBB1) and *Pto* DC3000 cor- (HopBB1\textsubscript{G126D}) were generated by transforming *Pto* DC3000 cor- with either pJC531 (empty vector), pJC531 (HopBB1) or pJC531 (HopBB1\textsubscript{G126D}). HopBB1 or HopBB1\textsubscript{G126D} were expressed from the native promoter. Bacterial growth assays in *Arabidopsis* were performed by spray or dipping inoculation as described. Bacterial cultures were resuspended in 10 mM MgCl₂ with 0.04% Silwet L-77. Plants were sprayed or dipped with a bacterial suspension at OD\textsubscript{600}=0.2. Results displayed in Figures 1C, 3A and 3F were performed independently a minimum of
3 times with similar results. Hpa infection was performed as described in (Mukhtar et al., 2011).

Bacterial colony formation units (CFU) were measured after three days. Dashed line indicates the CFU at day 0. Statistics in Figures 1C, 3A and 3F were performed using one-way ANOVA test with Tukey-Kramer HSD with 95% confidence.

**Effector Delivery Assay**

The coding regions of HopBB1 or HopBB1\textsubscript{G126D} were cloned into pBAV178 (proNPTII:: Gateway cassette-AvrRpt\textsubscript{2}\textsuperscript{101-255}) (Vinatzer et al., 2005). pBAV178 (HopBB1) and pBAV178 (HopBB1\textsubscript{G126D}) were transformed into Pto DC3000 cor-. Bacteria were infiltrated into leaves of four-week-old Col-0 plants at OD\textsubscript{600}=0.01 with 10mM MgCl\textsubscript{2}, and cell death was scored after 16-20 hours.

**Phylogenetic Analysis**

The phylogenetic history of *Pseudomonas* was inferred by multi-locus alignment using MUSCLE (Edgar, 2004) to align amino acid sequences of 31 single copy core genes from the 2681 *Pseudomonas* genomes available for download on the PATRIC database (Wattam et al., 2014). Using the resulting tree, *P. syringae* genomes were identified by selecting the smallest monophyletic group containing all genomes annotated as *P. syringae*. This analysis resulted in a dataset 287 *P. syringae* genomes (Table S5). Pan genome analysis was performed on this subset using Roary (Page et al., 2015) with default parameters. The nucleotide
sequences of the resulting 84 core genes (Table S6) were used to construct a new phylogenetic tree. Trees were constructed using a Maximum Likelihood method (Jones et al., 1992) implemented in MEGA7 (Tamura et al., 2013) with 100 bootstrap iterations. The HopBB1 (from *Pseudomonas syringae pv. mori str. 301020*), HopX1 (from *Pseudomonas syringae pv. tabaci str. ATCC 11528*), HopZ1a (from *Pseudomonas syringae pv. syringae* strain A2) and the coronatine biosynthesis pathway genes (from *Pseudomonas syringae pv. tomato str. DC3000*) were used as blast queries to search for homologous proteins in these 287 *P. syringae* genomes. A hit with over 80% protein sequence identity was considered positive. For HopZ1a, the difference between HopZ1a and HopZ1b annotated in (Ma et al., 2006) was used as guideline. Each homologue was manually checked for the integrity of reading frame. The tree was visualized using iTOL (http://itol.embl.de/) (Letunic and Bork, 2007). A newick file is available for download and interactive viewing at http://itol.embl.de/shared/HopBB1.
References


