The *Pseudomonas syringae* type III effector HopBB1 fine tunes pathogen virulence by gluing together host transcriptional regulators for degradation

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Running Title:

HopBB1 manipulates TCP14 and JAZ proteins to enhance bacterial virulence

Highlights

- TCP14 contributes to disease resistance by negatively regulating JA response.
- HopBB1 interacts with TCP14 and JAZ3.
- HopBB1 activates a subset of JA response genes and promotes bacterial virulence.
- HopBB1 recruits TCP14 and JAZ3 for SCF^{COI1}-dependent degradation.

eTOC blurb

The *Pseudomonas syringae* type III effector HopBB1 modulates two negative regulators of plant Jasmonic Acid 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.

Summary

Plants integrate phytohormone signals to balance growth and defense. Pathogen virulence effectors can physically interact with, and modulate, host immune system components. Independently evolved pathogen effectors from three branches of life (ascomycete, eubacteria and oomycete) converge onto the Arabidopsis TCP14 transcription factor. We demonstrate that TCP14 regulates plant immune system outputs by transcriptionally repressing the Jasmonic Acid (JA) hormone signaling pathway. A previously unstudied *Pseudomonas syringae* (*Psy*) type III effector, HopBB1 interacts with TCP14, connects it physically to JAZ3, a JA response repressor, and activates SCF^{COI1}-dependent degradation of the complex. HopBB1 thus disrupts the inhibitory association between JAZ3 and MYC2, a transcriptional activator of JA response. We provide a mechanistic understanding of how HopBB1 fine-tunes host phytohormone crosstalk by precisely manipulating subsets of both the TCP14 and MYC regulons to avoid pleiotropic host responses, while still promoting pathogen proliferation.

Introduction

A robust immune system defends plants against most microbes. Plants deploy surface-localized pattern recognition receptors (PRR) to detect conserved microbial associated molecular patterns (MAMP), 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 intracellular receptors with 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 by

either direct interactions with ligand, including effector proteins, or by recognition of effector-modified host cellular machines that are the nominal effector target, or decoys of these (Maekawa et al., 2011); (Jones and Dangl, 2006);(van der Hoorn and Kamoun, 2008); (Keestra et al., 2013); (Xu et al., 2014); (Boyer et al., 2011). NLR activation initiates effector-triggered immunity (ETI). Interactions between pathogen effectors and host immune receptors drive co-evolution of plants and their pathogens. Deciphering the mechanisms by which the effector repertoires from divergent pathogens act will provide a more comprehensive view of the host cellular machinery responsible for plant immune system function.

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 life styles (Robert-Seilaniantz et al., 2011). In Arabidopsis, activation of SA-dependent responses limit the growth of biotrophic or hemibiotrophic pathogens. On the other hand, JA-dependent responses limit the growth of necrotrophic pathogens and herbivorous insects. 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).

At steady state, the JA response transcriptional output is 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 (MYC2/3/4) that are repressed by JAZ proteins are the

positive regulators of JA-mediated responses in Arabidopsis (Fernandez-Calvo et al., 2011). JAZ 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 MAMP treatment or *Psy* 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 an SCF-(Skip-cullin-F-box)-type E3 ubiquitin ligase. This results in proteasome-mediated degradation of JAZ proteins, thus allowing MYC-dependent activation of JA response genes (Sheard et al., 2010; 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). Hence, biotrophic or hemibiotrophic pathogens that are resisted by SA-mediated immune responses will benefit from activation of JA-dependent responses (Browse, 2009; He et al., 2004; Zheng et al., 2012). Consistent with this model, Arabidopsis *coi1* or *myc* mutants exhibit enhanced disease resistance against the hemibiotrophic bacterial pathogen *Pseudomonas syringae* (*Psy*) (Fernandez-Calvo et al., 2011)

Two *Psy* type III effectors, HopX1 and HopZ1a, and a phytotoxin called coronatine are able to activate the JA pathway. Coronatine is a structural mimic of JA-Ile that promotes COI1-dependent JAZ degradation (Katsir et al., 2008). HopX1 is a cysteine protease that cleaves the central ZIM domain shared by JAZ proteins, resulting in COI1-independent JAZ degradation (Gimenez-Ibanez et al., 2014). HopZ1a is an acetyltransferase that acetylates soybean and Arabidopsis JAZ proteins and promotes COI1-dependent JAZ turnover (Jiang et al., 2013). Both HopX1 and HopZ1a were identified from *Psy* strains deficient in coronatine biosynthesis and each can rescue the growth defects of a *Psy* mutant unable to synthesize coronatine (Gimenez-Ibanez et al., 2014) (Jiang et al., 2013). Effects of

HopX1 or HopZ1a action on the global MYC-dependent transcriptional are not known, though each causes de-repression of several JA response genes (Gimenez-Ibanez et al., 2014; Jiang et al., 2013).

Interactome studies revealed that candidate effector repertoires from three evolutionarily diverse pathogens [*P. syringae* (*Psy;* eubacteria), *H. arabidopsidis* (*Hpa*; oomycete) and *Golovinomyces orontii* (*Go*; ascomycete)] converge onto a limited set of interconnected Arabidopsis proteins (Mukhtar et al., 2011; Wessling et al., 2014) (Dreze et al., 2011). These three pathogens express different infection strategies and are separated by 2.5 billion years of evolution. Each of them harbors a group of independently evolved effectors lacking sequence homology. Three of the nine convergent host targets, TCP13, TCP14 and TCP15, belong to the conserved TCP (teosinte branched1 from *Zea mays*, CYCLOIDEA from *Antirrhinum majus*, and the PROLIFERATING CELL FACTORS 1 and 2 from *Oryza sativa*) transcription factor family (Mukhtar et al., 2011; Wessling et al., 2014) (Cubas et al., 1999; Martin-Trillo and Cubas, 2010). The reference Arabidopsis Col-0 genome encodes 24 TCP family members that share a bHLH domain (the TCP domain) and are versatile regulators of plant development and hormone signaling (Cubas et al., 1999; Martin-Trillo and Cubas, 2010) (Lopez et al., 2015). TCP14 is localized to sub-nuclear foci and its co-expression 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 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).

Here, we significantly extend these observations and provide a mechanistic model for how one of the TCP14-targeting effectors suppresses defense and promotes *Psy* virulence. Our data demonstrate that the previously unstudied bacterial type III effector, HopBB1, alters transcription from sub-sets of

two transcriptional regulons, TCP14 and MYC to de-repress a subset of JA responses and promote virulence while avoiding pleiotropic effects associated with full elimination of either regulon.

Results

TCP14 is a negative regulator of JA signaling

Our previous work demonstrated that a *tcp14* mutation enhanced susceptibility to oomycete pathogen, *Hpa* isolate Emwa1 (Mukhtar et al., 2011; Wessling et al., 2014). We confirmed and extended this result using a second *tcp14* allele (Figure S1A). We generated transgenic Arabidopsis over-expressing YFP-TCP14 from the *UBQ10* promoter (Grefen et al., 2010) (Figure S1H). These plants are modestly smaller at the same developmental stage (Figure 1A and 1B) and displayed enhanced disease resistance when challenged by the virulent *Hpa* isolate Noco2, consistent with function of the YFP-TCP14 protein *in vivo* (Figure S1B). We examined the *in planta* growth of *P. syringae* pv. *tomato* strain DC3000 (*Pto* DC3000) and a coronatine-deficient mutant, *Pto* DC3000 *cor*$^{-}$. Plants overexpressing TCP14 expressed enhanced resistance to *Pto* DC3000 to the same levels as *coi1* mutants (Figure 1C, left). This effect required coronatine, as it was not observed following infection with *Pto* DC3000 *cor*$^{-}$, suggesting that the enhanced resistance is mainly due to an attenuated JA response (Figure 1C, right). *tcp14* mutants were unaltered in their response to *Pto* DC3000 (Figure 1C, left), but rescued the growth defects of *Pto* DC3000 *cor*$^{-}$. 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 analyzed the transcriptomes of wild type Col-0, *tcp14* mutants and transgenic plants overexpressing TCP14 in two-week old seedlings; the time point where altered *Hpa* infection phenotypes were observed (Mukhtar

et al., 2011; Wessling et al., 2014). We first defined the JA and SA response regulons in a time course treatment of Col-0 with 50 μM MeJA (a precursor of JA-Ile) and 300 μM BTH (a functional analogue of SA) (Figure S1C-S1G and Table S1). 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 S1E; Table S1). As expected, these sets of genes were strongly enriched for biological processes related to JA and SA responses (Figure S1F and S1G). 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.

No significant transcriptome changes were observed in the *tcp14* mutants relative to Col-0, probably due to redundancy between TCP family members (Kieffer et al., 2011; Steiner et al., 2012). A total of 203 genes were differentially expressed in TCP14-overexpressing plants (FDR≤0.01; 1.5 fold-change difference relative to Col-0; Figure 1D and Table S2). Genes down-regulated by TCP14 overexpression were significantly enriched for JA response markers (26/102; p=1.50e-20, hypergeometric test; cluster 1, Figures 1D and 1E). Indeed, many of these down-regulated genes were also weakly expressed in the *coi1-16* mutant, which is blind to JA (Figure 1D). These genes included established JA markers (*VSP2*, *LOX2* and *JAZ3*) and genes required for anthocyanin biosynthesis (i.e., *PAP1*, *DFR*, *AT5MAT*, *LDOX* and *UF3GT*) (Table S2). Gene ontology enrichment analyses also indicated that biological processes related to the JA output are repressed in plants that overexpress TCP14 (Figure S1I). In contrast, only 6 of the 101 genes that were up-regulated in the *UBQ10::YFP-TCP14-3* line are markers of the SA response (Figure 1D and 1E), suggesting that TCP14-driven repression of the JA pathway was not an indirect consequence of activated SA

response. This transcriptional profile support the conclusion that TCP14 contributes to plant immunity as a negative regulator of JA response.

The P. syringae *effector HopBB1 interacts with TCP14 and JAZ3 in vivo*

We focused on the TCP14-interacting *Psy* type III effector HopBB1 because it also interacted with JAZ3 (Mukhtar et al., 2011). We validated the interactions between HopBB1-TCP14 and HopBB1-JAZ3. We infected YFP-TCP14 overexpressing plants with *Pto* DC3000 *cor*$^{-}$ expressing HopBB1-HA at native levels. We observed that HopBB1-HA was pulled down following immunoprecipitation of YFP-TCP14, indicating that these two proteins associate *in vivo* during *Psy* infection. Similarly, conditionally-overexpressed HopBB1 co-immunoprecipitated JAZ3 in either Arabidopsis or *N. benthamiana* overexpressing JAZ3 (Figure 2A, 2E and S2A).

We mapped the domains required for the HopBB1-TCP14 and HopBB1-JAZ3 interactions using both Y2H and co-immunoprecipitation assays following transient co-expression in *N. benthamiana.* The N-terminus of HopBB1 (aa1-110), which is homologous to the N-terminus of the *Psy* type III effector HopF2, was dispensable for interaction with either TCP14 or JAZ3 (Figure 2B and 2F). The remainder of HopBB1 (aa111-283) has no annotated function, but was sufficient to mediate association with either TCP14 or JAZ3 (Figure 2B and 2F). The C-terminal TCP14 sequence (aa179-489) downstream of the conserved TCP DNA binding domain was sufficient for interaction with HopBB1 (Figure 2C and 2D and S2B). Deletion of either the JAZ3 N-terminus (aa1-207) or C-terminus (aa298-352) did not eliminate its association with HopBB1 in yeast (Figure 2G and S2B). We thus deduced that the overlapping region (aa208-297) is required for HopBB1-interacting, which is supported by their association in *N. benthamiana.* (Figure 2H). We extended our Y2H analysis to include 18 of the 24 Arabidopsis TCP family members and all 12 members of the JAZ family in Y2H

assays. HopBB1 selectively interacted with subsets of each family (Figure S2C and S2D). Collectively, these data are consistent with the hypothesis that HopBB1 associates with two negative regulators of the JA signaling pathway in vivo.

HopBB1 de-represses JA response

We tested the physiological relevance of HopBB1-myc expression using two independent transgenic lines (Figure 3A and 3B; Methods). As predicted, these plants were hypersensitive to JA-mediated inhibition of root elongation (Figure S3A). We then measured the growth of *Pto* DC3000 and *Pto* DC3000 *cor*$^{-}$ on the HopBB1 expressing plants. We found that *Pto* DC3000 *cor*$^{-}$ is more virulent on HopBB1 transgenic plants than on wild type Col-0, demonstrating that HopBB1 complements this strain's coronatine deficiency (Figure 3C), analogous to overexpression of TCP14 (Figure 1C). As expected, neither strain can grow on the *coi1-16* mutant, which is blind to JA and thus ectopically active for SA-mediated immune responses (Figure 3C) (He et al., 2012).

We compared the transcriptome of HopBB1-myc expressing plants to Col-0 at steady state and identified 628 differentially expressed genes (593 up- and 35 down-regulated; FDR ≤ 0.01; 1.5 fold-change relative to Col-0) (Table S3). Many of our JA response genes (93/672) were also up-regulated in the HopBB1 expressing line (Table S3), and the average expression of all 672 JA-responsive genes was higher in these transgenic plants (Figure 3D). Notably, some marker genes of JA biosynthesis, signaling and JA-SA crosstalk were dramatically activated in HopBB1 transgenic plants, such as *JAZ1*, *LOX2*, *NAC019* and *BSMT1* (Figure 3E). Remarkably, JA response genes are enriched in the overlap between HopBB1-upregulated and TCP14-suppressed genes: out of the 102 genes that were down-regulated by TCP14 overexpression (Figure 1D), 12 were up-regulated in HopBB1 transgenic plants and 10 of these are JA markers ($p = 2.26e-17$; hypergeometric test) (Table

S4). Genes specific to our BTH/SA response were also enriched in the HopBB1 up-regulated genes (139/2357; p=6.49e-34; hypergeometric test). However, genes that are strongly associated with SA-mediated defense responses (e.g., *PR-1*, *PR-5*, *ICS1*, *WRKYs*) were not differentially expressed, suggesting that the SA response activated in HopBB1-expressing plants is likely to be insufficient for robust defense.

As expected, the JA response genes defined in our study were enriched for MYC2 binding motifs in their promoters (Figure S3B). Importantly, these genes were also 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 up-regulated by HopBB1 (Figure S3B and S3C). Interestingly, overexpression of HopBB1 by constitutive or inducible promoters did not cause the chlorotic leaf phenotype observed previously after either coronatine/JA treatment or HopX1 expression (Figure S3D) (Gimenez-Ibanez et al., 2014; Kloek et al., 2001). Consistent with this observation, the expression of MYC-dependent and JA-responsive photosynthetic genes (Qi et al., 2015) are not altered in HopBB1 expressing plants (Figure S3E). In sum, our transcriptome data are consistent with our pathology data and support the contention 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 162 sequenced *Psy* genomes (Markowitz et al., 2012). Nearly 60% (94) of *Psy* genomes carry one, and only one, functional version of these four JA-activating virulence factors (Figure S3E and Table S5). Strikingly, in the few cases where genes encoding two of these JA-modulating virulence factors

exist in a single strain, one of them is invariably either truncated or has mutations in functionally essential residues (defined for HopX; (Nimchuk et al., 2007)). The phylogeny of the 94 *Psy* isolates containing JA-activating factors suggests that independent gene gain/loss occurred in each lineage (Figure S3F and 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 (McCann et al., 2013).

HopBB1 disassociates JAZ3 from MYC2

We investigated the biochemical mechanism by which HopBB1 de-represses JA response. Upon JA-Ile perception by COI1, JAZ proteins are degraded via the SCF^{COI1} complex (Sheard et al., 2010; Thines et al., 2007). HopBB1 expression was not sufficient to reduce JAZ3 accumulation (Figure 4A and S4A), in contrast to HopX1 and HopZ1a (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-IP assay in *N. benthamiana*, indicating that HopBB1 interferes with the interaction of MYC2 and JAZ3 (Figure 4B). We developed a bimolecular fluorescence complementation (BiFC)-based assay to examine this disassociation *in vivo*. The BiFC construct expressed JAZ3-nYFP, cYFP-MYC2 and mRFP1 as a co-expression reporter; a second construct carried an estradiol inducible HopBB1-CFP (Grefen and Blatt, 2012). Agrobacteria carrying these constructs were co-infiltrated to test the ability of HopBB1 co-expression to block the reconstitution of YFP signal dependent on JAZ3-MYC2 interaction (Figure 4C and S4B). In the absence of HopBB1, we found that 80% of the RFP-expressing cells had YFP signals in nuclei (Figure 4D). Since all three proteins were engineered on the same vector, this represents the combined efficiency of transformation and BiFC. When the JAZ3-MYC2 BiFC vector was co-expressed with vectors expressing either free CFP or CFP tagged with HaRxL45, an *Hpa* effector that interacts with TCP14 but not JAZ3 (Wessling et al., 2014) (Figure S4C), the efficiency of BiFC was unaltered (Figure 4D). However, co-expression with

HopBB1-CFP dramatically reduced the percentage of re-constituted YFP signal in CFP-and RFP-positive nuclei, supporting our contention that HopBB1 interferes with the interaction between JAZ3 and MYC2 in vivo (Figure 4D).

TCP14 is subject to JA-mediated degradation in the presence of HopBB1 and JAZ3.

Transient co-expression of HopBB1 with TCP14 in *N. benthamiana* did not alter the level of TCP14 protein (Figure 5A). Because JA triggers the degradation of JAZs, we tested the potential role of JA in regulating TCP14 accumulation. We found that TCP14 was not subject to JA-mediated protein degradation by itself or when co-expressed with either HopBB1 or JAZ3 (Figure 5B). In this assay, 50μM of MeJA was sufficient to trigger degradation of JAZ3 by 3 hrs post-infiltration (Figure 5B and 5C). Importantly, when TCP14, JAZ3, MYC2 and HopBB1 were co-expressed, both TCP14 and HopBB1 accumulation were dramatically reduced upon MeJA treatment, as was JAZ3. As anticipated, MYC2 levels were not altered in these experiments (Figure 5C). These results suggested that HopBB1 mediates TCP14 turnover in the presence JA and JAZ3 (Figure 5B). TCP14 turnover was not mediated by HaRxL45 co-expression in the presence of JAZ3 (Figure 5D), implying that effectors interacting with TCP14 modulate its activity by at least two different mechanisms.

We isolated a HopBB1 mutant, $HopBB1_{G126D}$, via its loss of interaction with TCP14 and retention of its ability to associate with JAZ3 (Figure 5E-5G and Figure 5S). This mutation occurs in the C-terminal HopBB1 region that was sufficient to bind either TCP14 or JAZ3 (Figure 2B and 2C). Remarkably, both $HopBB1_{G126D}$ failed to mediate TCP14 turnover, but was still degraded with JAZ3 in the presence of MeJA (Figure 5H).

HopBB1-mediated degradation of TCP14 requires SCF^{COI1}

JA-dependent, HopBB1-mediated degradation of TCP14 and JAZ3 suggested a requirement for SCF^{COI1}. 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 6A). A COI1-JAZ interacting surface in the C-terminal Jas domain is required for JA-triggered degradation of JAZ proteins (Sheard et al., 2010). Based on structural information (Sheard et al., 2010) and conservation, we generated $JAZ3_{P302A\ R305A}$, an allele that cannot interact with COI1 and is thus MeJA resistant (Figure S6). Importantly, $JAZ3_{P302A\ R305A}$ still interacted with HopBB1 in yeast, suggesting that its overall structure is not altered (Figure S6D). When we co-expressed $JAZ3_{P302A\ R305A}$ with TCP14 and HopBB1, MeJA-induced degradation of HopBB1 and TCP14 was blocked (Figure 6B). This observation indicated that SCF^{COI1}-dependent degradation of JAZ3 is required for HopBB1-mediated, JA-dependent turnover of TCP14.

We addressed whether HopBB1 promoted TCP14 turnover through SCF^{COI1} during bacterial infection. JA treatment of transgenic Arabidopsis carrying *UBQ10::YFP-TCP14* did not alter the accumulation of TCP14, despite the presence of wild type JAZ3 in these plants (Figure 6C). We then monitored TCP14 levels following inoculation with *Pto* DC3000 *cor*$^-$ (EV) or *Pto* DC3000 *cor*$^-$ (HopBB1). Delivery of native levels of HopBB1 led to reduced TCP14 protein accumulation, while transcript levels were unchanged (Figure 6D). TCP14 degradation in these experiments required the SCF^{COI1} complex, since it was blocked in *coi1-1* plants expressing *UBQ10::YFP-TCP14* (Figure 6D). Thus, HopBB1 promotes the degradation of TCP14 by connecting it to the SCF^{COI1} degradation pathway, ultimately facilitating the activation of JA responses.

HopBB1 recruits TCP14 into a JAZ3-containing sub-nuclear structure.

We previously demonstrated that TCP14 re-locates HopBB1 into an uncharacterized sub-nuclear structure (Figure 7A and 7B and S7A) (Wessling et al., 2014). Similarly, we noted that JAZ3 also

forms sub-nuclear foci, as known for JAZ1 and JAZ9 (Figure 7A) (Withers et al., 2012). Here, we found that HopBB1 can also be re-localized into JAZ3-foci (Figure 7B and S7B). Strikingly, JAZ3-foci and TCP14-foci did not overlap when these two proteins were co-expressed, implying that they represent different functional structures (Figure 7B and S7C). We then co-expressed a CFP-tagged HopBB1 together with TCP14 and JAZ3. In nuclei with HopBB1-CFP, but not CFP alone, the CFP, YFP and RFP signals co-localized in the same foci (Figure 7C and S7D), suggesting that HopBB1 connected TCP14 and JAZ3 from separate sub-nuclear foci into the same structure.

HopBB1 recruits TCP14 to SCF^{COI1} and drives co-localization of TCP14 and JAZ3.

We utilized TCP14 and JAZ3 mutants to examine requirements for the formation of sub-nuclear foci. The TCP domain contains a bHLH structure that is sufficient for DNA binding and dimerization (Kosugi and Ohashi, 2002). Amino acids H121, R130 and L161 in the TCP domain are conserved in all 24 Arabidopsis TCP proteins. Single mutation in any of these three amino acids abolished TCP4 DNA binding (Aggarwal et al., 2010). We generated a $TCP14_{H121Q\ R130K\ L161N}$ mutant and found that it 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 (Figure 7D, and S7E-7I). Thus, formation of the TCP14 nuclear foci is dependent on its ability to bind DNA.

We tested whether the JAZ3 nuclear foci represent a structure for its degradation by examining the localization of JAZ3 in *coi1-1* and the localization of $JAZ3_{P302A\ R305A}$, which has lost the ability to interact with COI1 (Figure S6). We observed that JAZ3-RFP formed subnuclear foci in Col-0, but not in *coi1-1*, a COI1 allele without detectable protein accumulation (He et al., 2012); $JAZ3_{P302A\ R305A}$ was unable to form nuclear foci (Figure 7E and 7F). Thus, formation of JAZ3 nuclear foci requires COI1. Importantly, $JAZ3_{P302A\ R305A}$ was re-localized into TCP14 subnuclear foci only in the presence of

HopBB1 (Figure 7G and 7H). In sum, these data are consistent with a model where HopBB1 links template DNA-bound TCP14 to a degradation complex containing JAZ3.

Discussion

We demonstrate that the HopBB1 type III effector protein modulates subsets of two Arabidopsis transcriptional 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 in Arabidopsis rescues the virulence defect of a pathogenic *Psy* strain lacking the JA structural mimic, coronatine, suggesting its role as a regulator of host JA response (Figure 3). HopBB1 has dual functions in de-repressing the JA signaling pathway: HopBB1 facilitates TCP14 degradation through SCF^{COI1} by connecting it to JAZ3 (Figure 5); HopBB1 disrupts the inhibitory association between JAZ3 and MYC2, leading to MYC2-dependent transcriptional activation of JA responses (Figure 4). However, only subsets of either the TCP14 or MYC regulons are perturbed (Figures 1D, 1E and S3C). 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 by the *coi1* null phenotype or coronatine treatment), while maintaining the ability to modulate defense hormone signaling to the pathogen's advantage.

JA regulates a variety of developmental programs and defense responses (Browse, 2009);(Robert-Seilaniantz et al., 2011);(Wasternack and Hause, 2013). The specificity and diversity of JA-mediated responses is partially achieved through the diverse action of JAZ repressors with their interacting transcription factors (Kazan and Manners, 2012). The JAZ-interacting MYC2/3/4 TFs are largely responsible for activating JA-mediated defense responses against bacterial pathogen *Psy* and a generalist herbivore *Spodoptera littoralis* (Fernandez-Calvo et al., 2011). Consistent with this, the

immune system phenotypes of a *myc2/3/4* triple mutant is comparable to that of a pleiotropic *coi1* null allele (Fernandez-Calvo et al., 2011; Lorenzo et al., 2004). 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 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-Ibanez et al., 2014);(Kloek et al., 2001). In contrast, we conclude that HopBB1 expression specifically activates a subset of JA-mediated responses (Figure 3D and 3E). This conclusion is supported by several observations. First, expressing HopBB1 in Arabidopsis activates only about 18% (168 of 933) of JA responsive genes (Figure S3C). Second, HopBB1 manipulates JA response by dissociating the JAZ3-MYC2 complex, leading to SCF^{COI1}dependent degradation of the JAZ3-HopBB1-TCP14 complex (Figure 6 and 7). Third, HopBB1 is apparently more selective than HopX1 or the action of coronatine, since it only interacts with a small subset of JAZ proteins (Figure S2C). Fourth, the chlorotic phenotypes observed in HopX1 transgenic plants and coronatine treated plants are not visible following either constitutive or conditional expression of HopBB1 (Figure 3A and S3D). Importantly, coronatine-induced chlorosis can be decoupled 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) (Uppalapati et al., 2007). The evolutionary mechanism driving the mutual exclusiveness of JA-modulating virulence factors in *Psy* genomes (Figure S3E 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., 2010) (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). Such JAZ proteins might antagonize coronatine function. Consistent with this suggestion, the MiSSP7 effector from the biotrophic mutualistic fungus *Laccaria bicolor* represses JA responsive genes by stabilizing the *Populus* JAZ6 protein (Plett et al., 2014). 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 decoy 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 *Psy* as a negative regulator of JA signal pathways (Figure 1). JA responsive genes are repressed in plants overexpressing TCP14 (Figure 1D) and *tcp14* mutants rescue the growth defects of *Pto* DC3000 *cor-* (Figure 1C). However, TCP14 may regulate other defense pathways against different pathogens. The modest dwarf phenotype caused by overexpression of TCP14 was not observed in mutants deficient in JA biosynthesis (*aos*) or signaling (*coi1*), but is reminiscent of mutants with activated NLR-mediated defense (Figure 1A) (Park et al., 2002) (Zhang et al., 2003). TCP14 physically interacts with SRFR1 and contributes to the effector-triggered immunity (Kim et al., 2014). Furthermore, in various tissues, TCP14 promotes cytokinin and gibberellic acid growth hormone responses (Kieffer et al., 2011; Resentini et al., 2015);(Steiner et al., 2012), which are required for intact immune response against oomycete and fungal pathogens (Choi et al., 2010) (Argueso et al., 2012) (Navarro et al.,

2008). Thus, we speculate that the TCP14-interacting effectors identified from *Psy*, *Hpa* and *Go* will manipulate TCP14 via different mechanisms to facilitate proliferation of pathogens with different life cycles and infection strategies. It is thus 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 than HopBB1.

Other TCP family proteins also regulate hormone homeostasis in defense. TCP4, together with other members in the CIN TCP subclass, can activate Arabidopsis *LOX2* (*LIPOXYGENASE 2*), encoding a JA biosynthesis enzyme (Schommer et al., 2008). TCP20 also associates with the *LOX2* promoter, but instead represses its expression (Danisman et al., 2012). On the other hand, TCP8 and TCP9 act redundantly to activate *ICS1* (*ISOCHORISMATE SYNTHASE 1*), a key enzyme in pathogen-induced SA biosynthesis (Wang et al., 2015). TCP21 is a component of the Arabidopsis circadian clock that controls circadian-dependent SA accumulation, probably through its activation of two positive regulators of *ICS1* (Zheng et al., 2015). Notably, TCP9, which is directly regulated by TCP20 (Danisman et al., 2012), is up-regulated by TCP14 overexpression in our data, implying a complex hierarchical TCP network regulating JA and SA outputs. Importantly, effectors potentially differentiate TCP family members for selective manipulation of hormone homeostasis and subsequent defense response. HopBB1 interacts with the C-terminus of TCP14, which is not conserved across TCP proteins (Figure 2C and 2D; (Martin-Trillo and Cubas, 2010)). The HopBB1-interacting TCPs lack a significant shared primary sequence other than the TCP domain, suggesting that the effector recognizes the TCP domain or a higher order structure including it (Figure S2D). As noted, the SAP11 effector from phytoplasma specifically degrades other TCP proteins to prevent the activation of JA biosynthesis upon insect feeding (Sugio et al., 2011). Collectively, these data support the hypothesis that divergent effectors can modulate TCP-controlled hormone homeostasis by differentially targeting TCP family members or by differentially manipulating a convergent TCP, like

TCP14. Assigning precise functions to individual TCP family members that are effector targets will illuminate the breadth of mechanisms available to pathogens to tune host immune responses to their benefit.

Experimental Procedures

Transient protein expression in N. benthamiana

N. benthamiana plants were grown at 24°C (day) / 20°C (night) under a 16-h light / 8-h dark cycle. The bacteria were collected and re-suspended in 2 ml resuspension buffer (10 mM MES pH5.6, 10 mM MgCl2 and 200 μM acetosyringone) to a final concentration of the OD600=0.2. GV3101 carrying 35S 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 (Lindbo, 2007). *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 hours after infiltration unless otherwise indicated.

Immunoblot and co-immunoprecipitation analyses

Leaf tissues were ground in liquid nitrogen, and extracted with 150 μL of grinding buffer (50mM Tris pH8.0, 1% SDS, 1mM EDTA) also containing 1ul/ml β-mercaptoethanol and 1x protease inhibitor (Sigma-Aldrich). The lysates were centrifuged at 12,000 rpm for 10 min at 4ºC. 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 1x 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. Immunoblots 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).

HopBB1 mutagenesis

$JAZ3_{P302A\ R305A}$ and $TCP14_{H121Q\ R130K\ L161N}$ were generated using QuikChange 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 manufacture'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.

Author Contributions

LY, PJPLT, PE and JD designed the study. LY, PJPLT, PE, SB, YH, OF, MEE and PM performed experiments. LY, PJPLT and JD wrote the paper.

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Figure and Table Legends

Figure 1. TCP14 represses JA response and promotes disease resistance.

(A) Three week old plants of Col-0, *UBQ10::YFP-TCP14-3* and *UBQ10::YFP-TCP14-4*. Bar=5mm

(B) Accumulation of YFP-TCP14 protein in transgenic plants.

(C) Mutation in *TCP14* enhances the virulence of *Pto* DC3000 *cor*$^-$. Two-week-old Col-0, homozygous *tcp14* mutants (*tcp14-6*, *tcp14-7*) and transgenic plants overexpressing TCP14 were dip inoculated with either *Pto* DC3000 or *Pto* DC3000 *cor*$^-$ at OD_{600}=0.05. Bacterial colony formation units (CFU) were measured after three days. *tcp14* mutants rescued the growth defects of *Pto* DC3000 *cor*$^-$, while overexpression of TCP14 limited *Pto* DC3000 growth. Dashed line indicates the CFU at day 0. Error bars represent ±SD. Statistics were performed using one-way ANOVA

test with Tukey-Kramer HSD with 95% confidence. The result displayed is one of three independent analyses giving similar results.

(D) JA-responsive genes are repressed in TCP14-overexpressing plants. Hierarchical clustering of the 203 genes identified as differentially expressed in the pairwise comparison between Col-0 and *UBQ::YFP-TCP14-3* (FDR≤0.01; 1.5 fold-change difference). An additional TCP14-overexpressing line (*UBQ::YFP-TCP14-4*) and the *coi1-16* mutant were also included in the clustering analysis. Genes that are responsive to MeJA or BTH/SA in our assay (Figure S1) are indicated on the right. Cluster 1: genes repressed in *UBQ::YFP-TCP14* that are very weakly expressed in *coi1-16*; Cluster 2: genes repressed in *UBQ::YFP-TCP14* but not in *coi1-16*; Cluster 3: genes upregulated in *UBQ::YFP-TCP14*. Table S2 shows the complete RNA-seq results for all Arabidopsis genes.

(E) Genes down-regulated in *UBQ::YFP-TCP14-3* are enriched for MeJA markers. Dashed lines represent the proportion of genes that belong to each group in the Arabidopsis genome. Enrichment of MeJA or BTH/SA markers in the sets of genes up- or down-regulated by the overexpression of TCP14 was tested using the hypergeometric test. Marker genes were defined as genes that were specifically up-regulated by each hormone in Col-0 in our experimental set-up (Figure S1). Genome: Expected proportion of MeJA (or BTH/SA) markers in Arabidopsis genome. Up-regulated: Proportion of MeJA (or BTH/SA) markers in genes up-regulated by *UBQ::YFP-TCP14*. Down-regulated: Proportion of MeJA (or BTH/SA) markers in genes down-regulated by *UBQ::YFP-TCP14*.

Figure S1 (Related to Figure 1)

(A) *tcp14* mutants are more susceptible to *Hpa* Emwa1 than Col-0.

(B) Overexpressing TCP14 in *Arabidopsis* enhances disease resistance against *Hpa Noco2*.

(C)–(G) Defining JA and SA responsive genes in Col-0. (C) Overview of the experimental conditions used to define genes responsive to MeJA and BTH by RNA-seq. Two-week-old seedlings were

sprayed with either hormone or a mock solution and harvested at three times after the treatment (1 h, 5 hrs and 8 hrs). This experiment includes two completely independent biological replicates of each condition. (D) 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. (E) 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. (F) Overview of the genes up-regulated by BTH at each time-point. The table shows Gene Ontology terms (biological processes) enriched in this set of genes. (G) 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.

(H) *TCP14* transcripts are over-accumulated in UBQ10::TCP14 plants.

(I) 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. Gene Ontology enrichment analyses were performed with the PlantGSEA toolkit (Yi et al., 2013).

Figure 2. HopBB1 interacts with TCP14 and JAZ3 *in planta*.

(A) *Pto*-delivered HopBB1 associates with TCP14 in Arabidopsis. *Pto* DC3000 *cor*$^{-}$ with empty vector (EV) or HA-tagged HopBB1 (BB1-HA) were hand-infiltrated at OD_{600}=0.05 into leaves of transgenic Arabidopsis expressing YFP-TCP14. Leaves were harvested 24 hrs after inoculation.

(B) The C-terminus (111-283) of HopBB1 is sufficient to associate with TCP14 *in N. benthamiana*. Proteins were transiently expressed in *N. benthamiana* from a 35S promoter. Samples were harvested 20-24hrs after inoculation.

(C) HopBB1 interacts with the C-terminus (180-489) of TCP14 in yeast.

(D) HopBB1 associates with the C-terminus (180-489) of TCP14 in *N. benthamiana*.

(E) HopBB1 interacts with JAZ3 in Arabidopsis. HopBB1-myc conditionally expressed from an estradiol-inducible promoter was transformed into Arabidopsis constitutively expressing JAZ3-HA from a 35S promoter. Three-week-old seedlings were induced with 50μM estradiol and sampled 6 hrs after induction.

(F) The C-terminus (111-283) of HopBB1 is sufficient to associate with JAZ3 in *N. benthamiana*.

(G) HopBB1 interacts with an uncharacterized JAZ3 domain (206-302) in yeast.

(H) HopBB1 associates with an uncharacterized JAZ3 domain (206-302) in *N. benthamiana*.

Figure S2 (Related to Figure 2)

(A) JAZ3 interacts with HopBB1 in *N. benthamiana*. JAZ3-GFP and HopBB1-myc were transiently expressed in *N. benthamiana* leaves from a 35S promoter.

(B) Protein accumulation in Figure 2C and 2G.

(C) Protein interaction between HopBB1 and TCP family members.

(D) Protein interaction between HopBB1 and JAZ family proteins.

Figure 3. HopBB1 activates JA response.

(A) Transgenic Arabidopsis plants expressing HopBB1 are morphologically indistinguishable from Col-0 wild type. Bar=5mm

(B) Protein accumulation in transgenic plants.

(C) Plants expressing HopBB1 complement the growth defects of *Pto* DC3000 *cor*$^{-}$. The growth of *Pto* DC3000 was not affected by pre-existing HopBB1. *Pto* DC3000 *cor*$^{-}$ is more virulent on HopBB1 transgenic plants than on wild type. Bacterial colony formation units (CFU) were measured after three days. Dashed line indicates the CFU at day 0. Error bars represent ±SD. Statistics were performed using a one-way ANOVA test with Tukey-Kramer HSD with 95% confidence. The result displayed is one of three independent analyses giving similar results.

(D) JA-responsive genes are activated in transgenic plants expressing HopBB1-myc. The z-score transformed expression of 672 JA responsive marker genes (Figure S1) is shown for three biological replicates of Col-0 and transgenic plants expressing HopBB1-myc.

(E) Expression of selected JA markers. The average expression of well-recognized JA markers are shown based on three replicates from Col-0 and HopBB1-myc plants. Error bars represent ±SD. Gene expression was defined by RNA-seq and is shown as RPKM (Reads Per Kilobase of transcript per Million reads mapped). Table S3 shows the complete RNA-seq results for all Arabidopsis genes.

Figure S3 (Related to Figure 3)

(A) 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.

(B) Co-occurrence of consensus TCP (GGNCCC) and MYC (CACGTG) binding sites is enriched (p=2.41e-13; hypergeometric test) in the promoters of 88 MeJA-regulated genes from Figure S1. 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.

(C) 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.

(D) 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 sampled for photograph. Bar=5mm. The protein accumulation of conditionally expressed HopBB1 is shown in the right panel.

(E) The expression of photosynthesis genes is not altered in *35S::HopBB1-myc* expressing plants .

(F) The distribution of HopBB1, HopX1, HopZ1a and *coronatine biosynthesis* pathway in 153 sequenced *Pseudomonas syringae* genomes (IMG/ER). The HopBB1 (IMG Gene ID: 2508791705), HopX1 (IMG Gene ID: 646026307), HopZ1a (IMG Gene ID: 641210484) and the coronatine *biosynthesis* pathway genes (IMG Gene IDs: 2508870783, 2508870788, 2508870791, 2508870760, 2508870761, 2508870763, 2508870764, 2508870765,

2508870767, 2508870768) were used as query to search for homologous proteins in 153 *P. syringae* genomes using the "IMG homologous hit" web-tool. A hit with over 80% protein sequence identity was considered positive. Each homologue was manually checked for the integrity of reading frame. Numbers in the cells of the table are the JGI-IMG Gene IDs for each gene.

Δ: Truncation due to a premature stop codon. N-Y: An Asn to Tyr mutation in the conserved A domain of putative HopX protein. ? = incomplete sequence information.

Figure 4. HopBB1 disrupts the association between JAZ3 and MYC2.

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

(B) HopBB1 reduces the association between JAZ3 and MYC2 *in planta*. Proteins were transiently co-expressed in *N. benthamiana*. HopBB1 was induced using a gradient of estradiol (0.01μM, 1μM, 10μM) 6 hrs after Agrobacteria infiltration. Samples were harvested 18 hrs after induction.

(C) Design of the bimolecular fluorescence complementation (BiFC) assay.

(D) HopBB1 disrupts the BiFC signal generated from the association between JAZ3 and MYC2. Proteins were transiently co-expressed in *N. benthamiana*. HaRxL45-CFP or HopBB1-CFP was induced 6 hrs after Agrobacteria infiltration. Microscopy was conducted 18 hrs after induction. Eight to ten confocal images with 1 mm^2 field of view were taken from four randomly sampled leaf discs on each leaf. RFP-positive cells were examined for the presence of CFP and YFP signal in nuclei. The yellow bars represented the percentage of YFP positive nuclei in CFP/RFP expressing cells. Neither CFP alone nor HaRxL45-CFP reduced the efficiency of reconstructed YFP signal

from JAZ3 and MYC2 association. Co-expressing HopBB1 reduces the efficiency from 80% to 20%.

Figure S4 (Related to Figure 4)

(A) HopBB1 does not trigger JAZ3 degradation in *N. benthamiana*. HopBB1-myc was induced using a gradient of estradiol (0.01μM, 1μM, 10μM, 20 μM)

(B) Quantification of the HopBB1-mediated disruption of MYC2-JAZ3 association. 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. 12-15 images from three independent experiments were pooled for the summary presented in Figure 4D.

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

Figure 5. TCP14 is subject to JA-mediated degradation in the presence of HopBB1 and JAZ3

(A) HopBB1 alone does not trigger the TCP14 degradation. Leaves were co-infiltrated with Agrobacteria delivering 35S::TCP14-myc or EST::HopBB1-YFP-HA genes. Expression of HopBB1 was induced 18 hrs later and samples harvested after 4 hrs post-induction with indicated concentrations of estradiol.

(B) TCP14 is subject to JA-mediated degradation in the presence of HopBB1. Agrobacteria carrying vectors expressing each protein under 35S constitutive promoter were co-infiltrated into *N. benthamiana* leaves. 50μM of MeJA was hand-infiltrated into leaves 24 hours post inoculation. Eight leaf discs per sample were harvested at time 0 (-) or 3 (+) hrs after infiltration. Numbers

below western signal indicate the relative signal intensity. The same method was applied to (C), (D) and (H).

(C) MYC2 is not co-degraded with MeJA treatment.

(D) HaRxL45 cannot mediate TCP14 degradation with the presence of JAZ3 and MeJA. HaRxL45, an effector from *Hpa*, can interact with TCP14 (Wessling et al., 2014), but not JAZ3 (Figure S4C).

(E) The HopBB1$_{G126D}$ mutant loses interaction with TCP14, but not JAZ3 in yeast. Also see Figure S5.

(F) The HopBB1$_{G126D}$ mutant loses association with TCP14 in *N. benthamiana.*

(G) The HopBB1$_{G126D}$ mutant associated with JAZ3 in *N. benthamiana.*

(H) HopBB1$_{G126D}$ cannot mediate TCP14 degradation in the presence of JAZ3 and MeJA.

Figure S5 (Related to Figure 5)

(A) Protein accumulation data for Figure 5E.

Figure 6. HopBB1-mediated degradation of TCP14 requires SCF^{COI1} pathway

(A) HopBB1-mediated degradation of TCP14 is blocked by 26S proteasome inhibitor, MG132. Agrobacteria carrying vectors expressing each protein under 35S constitutive promoter were co-infiltrated into *N. benthamiana* leaves. 50μM MeJA and 50μM MG132 were co-infiltrated 24 hours post inoculation. Eight leaf discs per sample were harvested at the indicated time after infiltration.

(B) The recruitment of JAZ3 to SCF^{COI1} is required for HopBB1-mediated degradation of TCP14. The JAZ3$_{P302A\ R305A}$ allele is resistant to SCF^{COI1}-mediated degradation; neither HopBB1 nor TCP14 were degraded upon MeJA treatment in the presence of JAZ3$_{P302A\ R305A}$. Agrobacteria carrying

each gene were co-inoculated 20 hrs before treated with 50μM MeJA treatment. Samples were harvested at the indicated time.

(C) YFP-TCP14 is not subject to JA-mediated degradation in the absence of HopBB1 in Arabidopsis. Two-week old Arabidopsis seedlings expressing *UBQ10::YFP-TCP14* were sprayed with mock (0.1% ethanol and 0.01% Silwet) or 50μM MeJA solution, and sampled at the indicated time. Each sample was pooled from eight seedlings.

(D) *Pto*-delivered HopBB1 reduces TCP14 protein level in wild type Col-0, but not *coi1-1* mutant. Arabidopsis plants expressing *UBQ10::YFP-TCP14* were inoculated with *Pto* DC3000 *cor*$^-$ carrying empty vector (EV) or *Pto* DC3000 (HopBB1-HA) at an OD_{600}=0.05. Samples were harvested 24 hrs after inoculation. *YFP-TCP14* transcripts from samples with the same treatment were quantified using real-time PCR. Expression levels were normalized to multiple endogenous controls including UBQ5 (AT3G62250), TUB (AT5G62690) and SAND (AT2G28390).

Figure S6 (Related to Figure 6)

(A) Alignment of the conserved Jas motifs from 12 Arabidopsis JAZ proteins. The P302 and R305 were highlighted in red boxes. These two residues are important for the interaction between COI1 and a JAZ1-derived Jas peptide (Sheard et al., 2010).

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

(C) $JAZ3_{P302A\ R305A}$ is resistant to MeJA-triggered degradation. Agrobacteria expressing each protein from a 35S promoter were co-infiltrated 20 hrs before MeJA treatment. Samples were harvested at the indicated time.

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

Figure 7. HopBB1 recruits TCP14 to a JAZ3-containing degradation site

(A) Localization of HopBB1, TCP14 and JAZ3 in nuclei. HopBB1 is evenly distributed in nuclei, while TCP14 and JAZ3 form subnuclear foci. Bar=5μM Proteins were transiently expressed in *N. benthamiana*.

(B) TCP14 and JAZ3 re-localize HopBB1 to nuclear foci. HopBB1 was re-localized to subnuclear foci by TCP14 (top) and JAZ3 (middle). However, TCP14 and JAZ3 localize in distinct nuclear foci (bottom). Histograms represent the intensity of fluorescent signal on the pathway of the lines in the "Merged" panel.

(C) HopBB1 (top), but not CFP (bottom), drives TCP14 and JAZ3 into the same sub-nuclear foci. JAZ3-RFP and TCP14-YFP were driven under 35S promoter, HopBB1-CFP was driven by estradiol-inducible promoter.

(D) The formation of TCP14 foci depends on its binding DNA ability. YFP-TCP14 expressed under UBQ10 promoter forms subnuclear foci in transgenic Arabidopsis. $TCP14_{H121Q\ R130K\ L161N}$ expressed under the same promoter fails to form subnuclear foci –although it retains its localization in nuclei. Images were taken from cotyledon epidermal cells in transgenic Arabidopsis.

(E) The formation of JAZ3 foci depends on its ability to associate with COI1. JAZ3-RFP expressed from a constitutive 35S promoter forms subnuclear foci in transgenic Arabidopsis Col-0, but not *coi1-1* mutant. *coi1-1* is a null allele of *COI1* that does not make detectable COI1 protein (He et al., 2012). Images were taken from cotyledon epidermal cells in transgenic Arabidopsis.

(F) $JAZ3_{P302A\ R305A}$ cannot form subnuclei foci in *N. benthamiana*.

(G) TCP14 cannot re-localize $JAZ3_{P302A\ R305A}$.

(H) TCP14 re-located $JAZ3_{P302AR305A}$ to nuclear foci in the presence of HopBB1.

A-C, F-H: proteins were transiently expressed under 35S promoter in *N. benthamiana*.

Figure S7 (related to Figure 7)

(A) Orthogonal slices view of TCP14-RFP and HopBB1-YFP co-localization in sub-nuclear foci. Bar=5 μM

(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.

Middle panel is the xy plane, left 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.

(E) Alignment of the TCP domain from 24 Arabidopsis TCP family members. The mutated H121 R130 and L161 were highlighted in red boxes. 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).

(F) The $TCP14_{H121Q\ R130K\ L161N}$ retains the ability to interact with HopBB1 and TCP14 in yeast.

(G) The $TCP14_{H121Q\ R130K\ L161N}$ retains the ability to interact with HopBB1 in *N. benthamiana*.

(H) The $TCP14_{H121Q\ R130K\ L161N}$ homo-dimerizes.

(I) The $TCP14_{H121Q\ R130K\ L161N}$ associates with wild type TCP14.

A-D, G-I: Proteins were transiently expressed under 35S promoter in *N. benthamiana*.

Extended Experimental Procedures

Plants

Arabidopsis Col-0, *tcp14-6* (cs108688, backcrossed to Col-0 4 times) (Mukhtar et al., 2011), *tcp14-7* (SAIL_1145_H03, 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 S1C). 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 using UBQ10::YFP-TCP14-3 (4 replicates), UBQ10::YFP-TCP14-4 (1 replicate), 35S::HopBB1-myc-10 (3 replicates) and *coi1-16* (2 replicates) were performed using steady-state seedlings grown under the same conditions as the ones used in the hormone treatment

experiment. In all experiments, each biological replicate corresponds to approximately 30 seedlings grown on the same pot.

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 50bp single-end reads. Raw sequencing data are available at the NCBI Sequence Read Archive under accession number SRAXXXXXX.

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 27,208 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. The scripts used to process the data and generate the figures (Heatmap, Boxplot) were deposited in GitHub.

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 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.

For the HopBB1-mediated disruption of JAZ3-MYC2 interaction, rBiFC (JAZ3+MYC2) and EST::HopBB1-CER-HA were co-inoculated at OD600=0.1 and OD600=0.2, respectively. Six hours after inoculation, 20μM estradiol was infiltrated. Samples were collected 20-24 hours after inoculation. 12-15 images of 50-100 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) and *Pto* DC3000 *cor*$^-$ (*HopBB1*) were generated by transforming *Pto* DC3000 *cor*$^-$ with either pJC531 (empty vector) or pJC531 (HopBB1). HopBB1 is expressed under its native promoter. Plant inoculations and bacterial growth assays were performed as described (He et al., 2012). All assays were performed independently a minimum of 3 times with similar results.

Phylogenetic Analysis

Phylogenetic history of *Pseudomonas* was inferred by multi-locus alignment using MUSCLE (Edgar, 2004) to align 20 single copy conserved COGs (Table S6) from all 850 *Pseudomonas* genomes that included a complete set of these genes, extracted from the *Integrated Microbial Genomes* (*IMG*) system http://img.jgi.doe.gov/; (Markowitz et al., 2012). A phylogenetic tree was constructed using the Maximum Likelihood method (Jones et al., 1992) implemented in MEGA6 (Tamura et al., 2013). The branch that included all *P. syringae* was extracted from this tree, including its nearest outgroup. This branch included 162 genomes, 143 of which were annotated as *P. syringae.* There were no genomes annotated as *P. syringae* in other branches of the larger tree. The tree was visualized using iTOL (http://itol.embl.de/) (Letunic and Bork, 2007).

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