Systemic acquired resistance (SAR) is a long-lasting plant immunity against a broad spectrum of pathogens. Biological induction of SAR requires the signal molecule salicylic acid (SA) and involves profound transcriptional changes that are largely controlled by the transcription coactivator NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1). However, it is unclear how SAR signals are transduced from the NPR1 signaling node to the general transcription machinery. Here, we report that the Arabidopsis thaliana Mediator subunit16 (MED16) is an essential positive regulator of SAR. Mutations in MED16 reduced NPR1 protein levels and completely compromised biological induction of SAR. These mutations also significantly suppressed SA-induced defense responses, altered the transcriptional changes induced by the avirulent bacterial pathogen Pseudomonas syringae pv tomato (Pst) DC3000/avrRpt2, and rendered plants susceptible to both Pst DC3000/avrRpt2 and Pst DC3000. In addition, mutations in MED16 blocked the induction of several jasmonic acid (JA)/ethylene (ET)–responsive genes and compromised resistance to the necrotrophic fungal pathogens Botrytis cinerea and Alternaria brassicicola. The Mediator complex acts as a bridge between specific transcriptional activators and the RNA polymerase II transcription machinery; therefore, our data suggest that MED16 may be a signaling component in the gap between the NPR1 signaling node and the general transcription machinery and may relay signals from both the SA and the JA/ET pathways.

INTRODUCTION

Plants, like animals, have evolved sophisticated innate immune systems to protect themselves against microbial invasion and colonization (Jones and Takemoto, 2004). Systemic acquired resistance (SAR) is a plant-specific immunity that develops throughout a plant after localized foliar infection by a pathogen (Ryals et al., 1996; Durrant and Dong, 2004). SAR provides long-lasting protection against subsequent infections by a broad spectrum of pathogens; therefore, the identification of key signaling components of SAR has been one of the major focuses in the field.

The signal molecule salicylic acid (SA) and the transcription coactivator NONEXPRESSOR OF PATHOGENESIS-RELATED (PR) GENES1/NON-INDUCIBLE IMMUNITY1/SA-INSENSITIVE1 (NPR1/NIM1/SA1I) have been identified as the key regulators of SAR (Gaffney et al., 1993; Cao et al., 1994; Delaney et al., 1995; Shah et al., 1997). Exogenous application of SA induces NPR1-dependent gene transcription and disease resistance, whereas mutations in NPR1 block SA- and pathogen-induced transcriptional reprogramming and compromise both basal immunity and SAR (Cao et al., 1997; Ryals et al., 1997; Shah et al., 1997). NPR1 is thus a master transcriptional regulator functioning downstream of SA. NPR1 contains two protein–protein interaction domains (an ankyrin-repeat and a Broad-complex, Tramtrack, and Bric-a-brac/Pox virus and Zinc domain) and interacts with seven TGA transcription factors and three structurally related NIM1-INTERACTING (NIMIN) proteins (Zhang et al., 1999; Després et al., 2000; Zhou et al., 2000; Weigel et al., 2001). On the PR1 gene promoter, interaction of NPR1 with TGA transcription factors helps recruit SUPPRESSOR OF SN1, 2 (SSN2) to counteract SUPPRESSOR OF NPR1, INDUCIBLE1 (SN1) repression (Li et al., 1999; Song et al., 2011). The NIMIN proteins appear to negatively regulate SA/NPR1 signaling, preventing detrimental hyperactivation of immune responses (Weigel et al., 2005). The NPR1 signaling node thus plays a critical role in transducing the SAR signal. However, it is not known how the SAR signal is relayed from the NPR1 signaling node to the general transcription machinery.

SA-mediated immunity is generally effective against biotrophs, whereas jasmonic acid (JA)/ethylene (ET)–mediated signaling is central for resistance against necrotrophs (Glazebrook, 2005). SA and JA signaling mostly antagonize each other, although they may function synergistically under certain conditions (Pena-Cortés et al., 1993; Doares et al., 1995; Schenk et al., 2000; van Wees et al., 2000; Spoel et al., 2003; Mur et al., 2006). Proteins regulating SA/JA/ET crosstalk have been identified in Arabidopsis thaliana (Petersen et al., 2000; Kachroo et al., 2001; Li et al., 2004; Brodersen et al., 2006; Ndamukong et al., 2007). The SA signal transducer NPR1 acts as a crucial modulator in SA-mediated suppression of JA signaling and regulates SA-mediated
expression of several genes encoding transcription (co)factors that suppress JA-dependent gene expression (Spoel et al., 2003; Li et al., 2004; Ndamukong et al., 2007). Whereas the NPR1-interacting TGA transcription factors participate in both SA- and JA/ET-induced defense signaling, their positive role in JA/ET signaling seems to be abolished in the presence of SA (Ndamukong et al., 2007; Zander et al., 2010). Crosstalk between SA and JA/ET signaling is believed to ensure efficient prioritization of immune responses against biotrophs and necrotrophs (Spoel et al., 2007); however, molecular mechanisms underlying the crosstalk remain elusive.

In eukaryotic cells, RNA polymerase II (RNAPII) catalyzes the transcription of all protein-encoding genes (Woycik and Hampsey, 2002). The transcription process is regulated by a collection of countless transcriptional regulatory proteins (Kadonaga, 2004). A multiprotein complex named Mediator has attracted considerable attention in recent years, because of its essential role in transcription (Kim et al., 1994; Kornberg, 2005; Takagi and Kornberg, 2006; Conaway and Conaway, 2011a). Mediator exists in the cell in multiple functionally distinct forms and serves as either a transcriptional activator or a repressor, depending on its associated protein components (Conaway and Conaway, 2011b). The Mediator core is composed of more than 20 subunits organized into three modules named head, middle, and tail (Guglielm et al., 2004; Chadick and Asturias, 2005). The Mediator core can associate with the RNAPII complex to form the holoenzyme, which stimulates basal transcription and supports activation of transcription by specific transcription activators (Mittler et al., 2001; Baek et al., 2002; Zhu et al., 2006; Ansari et al., 2009). By interacting with a particular transcription activator or a class of transcription activators, individual Mediator subunits relay diverse signals to the general transcription machinery leading to pathway-specific gene transcription (Balamotis et al., 2009; Kagey et al., 2010; Takahashi et al., 2011). The Mediator core can also interact with a kinase module, which precludes its binding to the RNAPII complex, resulting in transcriptional repression (Holstege et al., 1998; Akoulitch et al., 2000; Knuesel et al., 2009). The functionally distinct forms of Mediator thus provide various regulatory mechanisms to fine-tune gene-specific and pathway-specific transcriptional reprogramming (Balamotis et al., 2009).

The Mediator complex is highly conserved in eukaryotes ranging from yeast to humans (Boué et al., 2002; Bourbon, 2008). The Arabidopsis Mediator complex, recently purified to near homogeneity, contains 21 conserved and six putative Arabidopsis-specific Mediator subunits (Bäckström et al., 2007). Mediator functions as a bridge between the RNAPII complex and specific transcription activators; therefore, it is expected that individual Mediator subunits interact with and receive signals from a subset of the ~1500 transcription factors encoded by the Arabidopsis genome (Riechmann et al., 2000). Indeed, several Arabidopsis Mediator subunits have been implicated in specific signaling pathways. Arabidopsis Mediator subunit25 (MED25)/PHOTOCROME AND FLOWERING TIME1 (PFT1) and MED14/STRUWWELPETER (SWP) were first identified as key regulators of flowering and cell proliferation, respectively (Autran et al., 2002; Cerdán and Chory, 2003). Recently, MED25/PFT1 and two other Arabidopsis Mediator subunits, MED8 and MED21, were found to regulate JA-dependent defense responses (Dhawan et al., 2009; Kidd et al., 2009). In addition, Arabidopsis MED17, MED18, and MED20a have been shown to play a role in small and long noncoding RNA production (Kim et al., 2011).

MED16 is required for lipopolysaccharide-induced gene expression in Drosophila melanogaster (Kim et al., 2004). MED16 appears to be a specific binding partner of the differentiation-inducing factor, relaying the activation signal from the differentiation-inducing factor to the basal transcription machinery during lipopolysaccharide-induced innate immune response. In plants, Arabidopsis MED16 was first reported as a positive regulator of acclimation to freezing temperatures after exposure to low temperatures and was named SENSITIVE TO FREEZING6 (SFR6) (Knight et al., 2009). Knight et al. speculated that MED16/SFR6 might modulate the activity of C-box binding factor (CBF) transcription factors or help recruit CBF proteins into the nucleus in regulating cold on-regulated (COR) gene expression. It is likely that MED16/SFR6 is the target in Mediator for CBF-dependent pathway activation of COR gene transcription.

In a genetic screen for mutants insensitive to exogenous NAD⁺ (ien) (Zhang and Mou, 2009), we identified the ien1/med16-1 mutant, in which exogenous NAD⁺-induced PR1 gene expression was significantly inhibited. Characterization of ien1/med16/sfr6 in this current study revealed that MED16 is not only a key positive regulator of SAR, but also a convergence point of SA- and JA/ET-mediated defense pathways.

**RESULTS**

**An Arabidopsis Mutant Insensitive to Exogenous NAD⁺ Exhibits Increased Susceptibility to the Avirulent Bacterial Pathogen Pseudomonas syringae pv tomato DC3000/avrRpt2**

We previously reported that exogenous NAD(P) induces PR gene expression and disease resistance (Zhang and Mou, 2009), suggesting that extracellular NAD(P) [eNAD(P)] is likely an elicitor of plant immune responses. To identify new components in the eNAD(P)-activated signaling pathway, we performed a genetic screen designed to isolate ien mutants. Seeds of a PR1 LUCIFERASE transgenic line were mutagenized with ethyl methanesulfonate, and the M2 seedlings grown on one-half-strength Murashige and Skoog (MS) medium were treated with 5 mM of exogenous NAD⁺ and screened. One mutant, ien1, exhibited decreased PR1 expression after NAD⁺ treatment (Figures 1A and 1B). NAD (P) leaking into the extracellular compartment during pathogen infection might contribute to defense gene induction (Zhang and Mou, 2009); therefore, we asked whether the ien1 mutation affects pathogen-induced defense gene expression. To this end, we inoculated ien1 plants with the avirulent bacterial pathogen Pseudomonas syringae pv tomato (Pst) DC3000/avrRpt2. After 24 h, the inoculated leaves were collected for PR gene analysis. Compared with the wild type, Pst DC3000/avrRpt2–induced expression of PR1, PR2, and PR5 was drastically decreased in the ien1 plants (Figures 2A to 2C). Interestingly, Pst DC3000/avrRpt2–induced lesion formation, which is a visual defense phenotype (Dangl et al., 1996), was alleviated in ien1 (Figure 2D). Consistently,
zygous

The phenotype cosegregates with the wild type (see Supplemental Figure 1 online). This suggests that the mutant phenotypes were caused by a mutation in *SFR6*. To further confirm that the mutant phenotypes were caused by a mutation in *SFR6*, we attempted to complement the mutant with the full-length *SFR6* genomic coding sequence driven by the 3SS constitutive promoter. The transgenic plants exhibited wild-type morphology, strong induction of *PR1* gene by NAD+, and wild-type levels of resistance to *Pst DC3000/avrRpt2* (see Supplemental Figure 2 online), supporting that *IEN1* is *SFR6*. To identify the *ien1* mutation, a set of overlapping PCR fragments covering *At4g04920* was

**Figure 1.** Isolation of the *ien1* Mutant.

**A** Exogenous NAD+-induced *PR1* gene expression in *ien1* and wild-type (WT) seedlings. Seedlings grown on one-half-strength MS medium were treated with sprays of 1 mM of NAD+ solution. Total RNA was extracted from plant tissues collected 24 h later and subjected to RNA gel blot analysis. The UBQ5 gene was used as a loading control.

**B** Exogenous NAD+-induced *PR1* gene expression in *ien1* and wild-type soil-grown plants. Leaves of 4-week-old soil-grown plants were infiltrated with 1 mM of NAD+ solution. Total RNA was extracted from leaf tissues collected 24 h later and subjected to real-time qPCR analysis. Expression was normalized against constitutively expressed UBQ5. Data represent the mean of three independent samples with SD. Different letters above the bars indicate significant differences (P < 0.05, Student’s t test).

*Pst DC3000/avrRpt2* grew significantly more in *ien1* than in the wild type (Figure 2E). Thus, *IEN1* is a positive regulator of *Pst DC3000/avrRpt2*-induced defense responses.

**IEN1 Encodes MED16**

Morphologically, the *ien1* plants were a paler shade of green than the wild type (see Supplemental Figure 1 online). This phenotype cosegregates with the *ien1* defense phenotype and was therefore used to map the *IEN1* locus. We crossed homozygous *ien1*, which is in the Columbia background, to the polymorphic ecotype Landsberg erecta. Linkage analysis of 118 F2 plants with *ien1* morphology placed the *IEN1* locus between the molecular markers CIW5 and CIW6 on chromosome 4. Recombination analysis of 1214 F2 mutant plants was only able to include the *IEN1* locus in an interval of ~3.1 Mbp between markers m228 and m268, because of the low frequency of recombination in the centromeric region (Figure 3A). This low frequency of recombination was also encountered when the *sfr6-1* mutation was cloned in the same interval (Knight et al., 2009). Similar to *ien1*, *sfr6* mutants are also a paler shade of green than the wild type, indicating that *ien1* might be allelic to *sfr6*. To test this, we obtained the T-DNA insertion line SALK_048091 (*sfr6-2*) carrying a T-DNA insertion in *At4g04920* from the ABRC and crossed it with *ien1*. All *F1* plants were paler than the wild type and similar to the parental *ien1* and *sfr6* plants (see Supplemental Figure 1 online). Moreover, SALK_048091 showed significantly decreased induction of *PR1* by exogenous NAD+ and enhanced susceptibility to *Pst DC3000/avrRpt2* (Figures 3B and 3C). These results indicate that *ien1* is allelic to *sfr6*. To further confirm that the *ien1* mutant phenotypes were caused by a mutation in *SFR6*, we attempted to complement the *ien1* mutant with the full-length *SFR6* genomic coding sequence driven by the 3SS constitutive promoter. The transgenic plants exhibited wild-type morphology, strong induction of *PR1* gene by NAD+, and wild-type levels of resistance to *Pst DC3000/avrRpt2* (see Supplemental Figure 2 online), supporting that *IEN1* is *SFR6*. To identify the *ien1* mutation, a set of overlapping PCR fragments covering *At4g04920* was

**Figure 2.** Defense Phenotypes of the *ien1* Mutant.

(A) to (C) *Pst DC3000/avrRpt2*-induced *PR1* (A), *PR2* (B), and *PR5* (C) gene expression in *ien1* and wild-type (WT) plants. Four-week-old soil-grown plants were inoculated with *Pst DC3000/avrRpt2* (OD<sub>600</sub> = 0.001). Total RNA was extracted from the inoculated leaves collected 24 h later and subjected to real-time qPCR analysis. Data represent the mean of three independent samples with SD. All experiments were repeated three times with similar results. cfu, colony-forming units.

(D) Visual phenotype of *Pst DC3000/avrRpt2*-infected *ien1* and wild-type leaves. Four-week-old soil-grown plants were inoculated with *Pst DC3000/avrRpt2* (OD<sub>600</sub> = 0.001) or infiltrated with 10 mM of MgCl<sub>2</sub> as a control. Photos were taken 4 d after inoculation.

(E) Growth of *Pst DC3000/avrRpt2* in *ien1* and wild-type plants. Four-week-old soil-grown plants were inoculated with *Pst DC3000/avrRpt2* (OD<sub>600</sub> = 0.001). The *in planta* bacterial titers were determined immediately and 2 and 4 days postinoculation (dpi). Data represent the mean of eight independent samples with SD. Different letters above the bars in (A), (B), and (C) or on the right side of the lines in (E) indicate significant differences (P < 0.05, Student’s t test). All experiments were repeated three times with similar results. cfu, colony-forming units.
amplified from *ien1* and sequenced. A single base substitution of A for G was detected at the nucleotide 4396 (counting from the A in the ATG start codon). This mutation introduced a stop codon in the 11th exon (Figure 3E). We then successfully developed a cleaved amplified polymorphic sequence (CAPS) marker based on the mutation to genetically distinguish the *ien1* mutant from the wild type (Figure 3D). At4g04920 was recently shown to encode *Arabidopsis* MED16; therefore, *ien1* was renamed *med16-1* (Bourbon et al., 2004; Bäckström et al., 2007).

**MED16 Suppresses SAR-Negative Regulators and Promotes SAR-Positive Regulators**

Mediator is a transcription coregulator, and mutations in the *MED16* gene compromise resistance to *Pst* DC3000/avrRpt2, suggesting that the *med16/sfr6* mutation may alter *Pst* DC3000/avrRpt2–induced gene expression. To identify potential candidate genes that are regulated by MED16, we performed a microarray experiment to compare *Pst* DC3000/avrRpt2–induced transcriptome changes in *med16-1* and the wild type. We examined genes that showed a twofold or larger difference in their expression levels between *med16-1* and the wild type (National Center for Biotechnology Information Gene Expression Omnibus series number GSE38999). As shown in Supplemental Table 1 online, after *Pst* DC3000/avrRpt2 infection, a large number of defense genes, including *ENHANCED DISEASE SUSCEPTIBILITY5 (EDS5), SA INDUCTION DEFICIENT1 (SID1), AVRPPH3 SUSCEPTIBILITY3 (PBS3), HOPLIST1-INTERACTING3 (WIN5), GH3-LIKE DEFENSE GEN1 (GDS1), AGD2-LIKE DEFENSE RESPONSE PROTEIN1 (ALD1), FLAVIN-DEPENDENT MONOXYGENASE1 (FMO1), ACCELERATED CELL DEATH6 (ACD6), AZELAIC ACID INDUCED1 (AZ1),* and *SUPPRESSOR OF FATTY ACID DESATURATION DEFICIENCY1 (SFD1), GLY1*, and many NPR1 target genes, such as *PR1, PR2, PR5, VACUOLAR SORTING RECEPTOR6 (VSR6), WRKY30, WRKY35, WRKY54, WRKY59, WRKY66, and WRKY70*, were potentially all upregulated or downregulated in *med16-1*. Interestingly, a group of SAR-negative regulators, including *SN1, NIMIN1, NIMIN2, NIMIN3, WRKY38, WRKY58, and WRKY62*, were potentially all upregulated in *med16-1* (see Supplemental Table 1 online). The numbers of differentially expressed genes were based on comparisons of single arrays with no replication; therefore, statistical significance could not be assessed. However, this microarray data provided a set of candidate genes for further analysis. To confirm and extend the microarray results for the selected candidate genes, we used real-time quantitative PCR (qPCR) to monitor the induction kinetics of *SN1, NIMIN1, NIMIN2, NIMIN3, WRKY38, WRKY58, WRKY62, DECREASE IN INDUCED RESISTANCE1 (DIR1), AZ1, PR1, PR2, and PR5* in *med16-1, sfr6-2*, and the wild type after *Pst* DC3000/avrRpt2 infection. Consistent with the microarray results, the
induction of two SAR-negative regulators (WRKY38 and WRKY62) in med16/sfr6 was faster and stronger than in the wild type (Kim et al., 2008), whereas the induction of the SAR-positive regulator AZI1 in med16/sfr6 was inhibited (Figure 4) (Jung et al., 2009). In addition, the expression of the SAR-positive regulator DIR1 was decreased in med16/sfr6 plants (Maldonado et al., 2002). As a late response gene, PR1 was upregulated at 8 and 16 h after infection, but its expression was significantly decreased at 24 h in med16/sfr6 mutants (Figure 4). Interestingly, PR5 was only marginally induced in med16/sfr6 (Figure 4). Taken together, these results indicate that MED16 may regulate plant immune responses by suppressing SAR-negative regulators and promoting SAR-positive regulators.

MED16 Functions Downstream of SA

To test whether pathogen-induced SA accumulation is altered in med16/sfr6 plants, we measured SA levels in Pst DC3000/avrRpt2-infected med16/sfr6 and wild-type plants. As shown in Figures 5A and 5B, although free SA levels in the med16/sfr6 mutant plants at 12 h after Pst DC3000/avrRpt2 infection were lower than in the wild type, they were not significantly different from those in the wild type, suggesting that MED16 is not a major contributor to SA biosynthesis. The med16/sfr6 mutation inhibits Pst DC3000/avrRpt2-induced PR gene expression; therefore, MED16 may function downstream of SA to regulate immune responses. To test this, PR gene expression in med16/sfr6 plants treated with soil drenches and foliar sprays of the SA biologically active analog benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) was examined. We used BTH instead of SA, because BTH is a stronger inducer of defense genes than SA (Friedrich et al., 1996). In agreement with an earlier report (Wathugala et al., 2012), BTH-induced expression of PR1, PR2, and PR5 was dramatically decreased in the med16/sfr6 mutant plants (Figures 5C to 5E). Thus, MED16 plays a role downstream of SA in regulating defense gene expression, and mutations in MED16 significantly compromise SA responsiveness.

We also examined BTH-induced pathogen resistance in med16/sfr6 plants. The growth of the virulent bacterial pathogen Pst DC3000 in BTH-treated med16/sfr6 plants was significantly higher than in BTH-treated wild-type plants (Figure 5F). This result is consistent with the conclusion that MED16 functions downstream of SA as a positive regulator of plant immune responses.

**Figure 4.** Pst DC3000/avrRpt2–Induced Kinetic Expression of 12 Defense Genes in med16/sfr6 Mutants.

Plants were inoculated with the avirulent bacterial pathogen Pst DC3000/avrRpt2 (OD600 = 0.001). Leaf tissues were collected at the indicated time points. Total RNA was extracted from the inoculated leaves and analyzed for the expression of indicated genes using real-time qPCR. Expression was normalized against constitutively expressed UBQ5. Data represent the mean of three independent samples with SD. An asterisk (*) indicates that the expression level of the gene in the wild type (WT) was either significantly lower (WRKY38 and WRKY62) or significantly higher (DIR1, AZI1, PR1, and PR5) than in both med16-1 and sfr6-2 (P < 0.05, Student’s t test). The experiment was repeated with similar results.
defense responses, we examined the expression of NPR1, PR1, PR2, and PR5 in med16-1, sfr6-2, npr1, med16-1 npr1, sfr6-2 npr1, and wild-type plants during the infection of the virulent bacterial pathogen Pst DC3000. The background expression levels of NPR1 were lower in med16-1/sfr6 than in the wild type, but after Pst DC3000 infection, NPR1 was induced to a level just slightly lower than that in the wild type (Figure 6A). Note that the expression levels of NPR1 were significantly decreased in the npr1-3 mutant, probably because of mRNA decay caused by the premature stop codon in the mutant (Cao et al., 1997; Chang et al., 2007). Compared with the wild type, both med16/sfr6 and npr1 compromised Pst DC3000–induced PR gene expression (Figures 6B to 6D), which is consistent with the previous report (Wathugala et al., 2012). The med16/sfr6 mutations showed a stronger effect on PR5, whereas npr1 had a stronger effect on PR1, and med16/sfr6 and npr1 displayed a comparable effect on the expression of PR2. In the med16/sfr6 npr1 double mutants, expression of PR2 and PR5 was further decreased to a level lower than that in either single mutant (Figures 6B to 6D). These results suggest that MED16, like NPR1, plays a positive role in Pst DC3000–induced PR gene expression.

We also tested Pst DC3000 growth in med16/sfr6, npr1, and the double mutant med16/sfr6 npr1. Pst DC3000 grew significantly more in med16/sfr6 and npr1 than in the wild type, and the growth of Pst DC3000 in med16/sfr6 and npr1 was comparable, suggesting that MED16 also plays a role in basal resistance (Figure 6E). Interestingly, Pst DC3000 growth increased about sevenfold in the med16/sfr6 npr1 double mutant plants compared with that in either single mutant (Figure 6E). Because med16/sfr6 did not significantly affect Pst DC3000–induced SA accumulation (Figures 6F and 6G), these results support the conclusion that MED16 functions downstream of SA in plant immunity (Wathugala et al., 2012).

MED16 Is Required for the Establishment of SAR

Mutations in the MED16 gene compromise SA responsiveness; therefore, MED16 may play a role in SAR. To test this hypothesis, we analyzed the biological induction of SAR in med16/sfr6 plants. Three lower leaves on each plant were infiltrated with either 10 mM of MgCl2 (mock treatment) or P. syringae pv maculicola (Psm) ES4326 (Psm treatment). After 2 d, we measured SAR treatment–induced SA accumulation and gene expression in the upper, untreated systemic leaves. As shown in Figures 7A and 7B, free SA and total SA levels in the systemic leaves of the med16/sfr6 plants were comparable with those in the wild type. However, the expression of six genes (PR1, PR2, PR5, GST11, EDR11, and SAG21), which are induced in systemic leaves during SAR (Maleck et al., 2000), was significantly decreased in the med16/sfr6 plants (Figures 7C to 7H), suggesting that MED16 is required for SAR treatment–induced defense gene expression in systemic leaves.

To test whether MED16 plays a role in the execution of SAR on secondary infection, we challenge-inoculated the upper, untreated systemic leaves with Psm ES4326 2 d after the primary infection and monitored SA accumulation. At 12 h after the challenge inoculation, med16/sfr6 plants accumulated significantly less free SA and total SA than wild-type plants (Figures 7I and 7J), indicating that MED16 is required for full accumulation of

**MED16 Plays a Positive Role in Basal Immunity**

Because med16 influences the expression of many NPR1 target genes (see Supplemental Table 1 online), Arabidopsis MED16 may positively contribute to basal immunity like NPR1 does (Wathugala et al., 2012). To compare the roles of MED16 and NPR1 in basal

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Figure 5. Pst DC3000/avrRpt2–Induced SA Accumulation and BTH–Induced Defense Responses in med16/sfr6 Mutants.

(A) and (B) Free SA (A) and total (B) levels in Pst DC3000/avrRpt2–infected med16/sfr6 and wild-type (WT) plants. Four-week-old soil-grown plants were inoculated with Pst DC3000/avrRpt2 (OD600 = 0.002). The inoculated leaves were collected at the indicated time points for SA measurement. Data represent the mean of four independent samples with SD. FW, fresh weight; SAG, SA–2–O–β–glucoside.

(C) to (E) BTH–induced PR1 (C), PR2 (D), and PR5 (E) gene expression in med16/sfr6 and wild-type plants. Four-week-old soil-grown plants were treated with soil drenches plus foliar sprays of 0.3 mM of BTH solution. Leaf tissues were collected at the indicated time points and subjected to total RNA extraction and real-time qPCR analysis. Data represent the mean of three independent samples with SD.

(F) BTH–induced resistance to Pst DC3000 in med16/sfr6 and wild-type plants. Four-week-old soil-grown plants were treated with soil drenches plus foliar sprays of 0.3 mM of BTH solution (+BTH) or water (−BTH). After 24 h, the plants were inoculated with Pst DC3000 (OD600 = 0.001). The in planta bacterial titers were determined immediately and 4 d after inoculation. Data represent the mean of eight independent samples with SD. Different letters above the bars in (A), (B), and (F) indicate significant differences (P < 0.05, Student’s t test), and an asterisk (*) in (C), (D), and (E) indicates that the expression level of the gene in the wild type was significantly higher than in both med16-1 and sfr6-2 (P < 0.05, Student’s t test). Note that the comparison was made separately among the wild type, med16-1, and sfr6-2 for each time point. All experiments were repeated three times with similar results. cfu, colony–forming units.
SA in systemic leaves after challenge inoculation. To test whether SAR induction in med16/sfr6 plants is effective for limiting pathogen growth, bacterial titers were determined 3 d after the challenge inoculation. As shown in Figure 7K, SAR activation induced strong resistance in the wild-type plants. By contrast, SAR treatment did not induce any resistance in the systemic leaves of med16/sfr6 plants, as illustrated by the similar levels of Psm ES4326 growth in both the mock-treated and the SAR-treated med16/sfr6 plants. These results demonstrate that MED16 is a key positive regulator of SAR.

MED16 Functions Downstream of JA and ET and Is Required for Resistance to Necrotrophic Fungal Pathogens

Arabidopsis MED8 and MED25 have been implicated in JA-mediated defense against necrotrophic pathogens in Arabidopsis (Kidd et al., 2009). Induction of the JA/ET-responsive gene PDF1.2 by methyl jasmonate (MeJA) is inhibited in med25/pft1 mutants and further decreased in the med8 med25 double mutant. Both med8 and med25 are more susceptible to the leaf-infecting necrotrophic pathogens Botrytis cinerea and Alternaria brassicicola (Kidd et al., 2009). We reasoned that MED16 might also play a role in JA/ET-mediated antimicrobial signaling. To this end, we analyzed the induction of several JA/ET-responsive genes by 1-aminocyclopropane-1-carboxylic acid (ACC; the immediate precursor of ET), MeJA, or a combination of ACC and MeJA. Consistent with the previous reports (Penninckx et al., 1998; Norman-Setterblad et al., 2000), the JA/ET-responsive genes PDF1.2, CHIB/PR3, and HEL/PR4 were induced by both ACC and MeJA, and the induction was synergistically enhanced by the combination of ACC and MeJA (Figures 8A to 8C). Interestingly, the induction of PDF1.2 and CHIB by ACC, MeJA,
and their combination was almost completely blocked in med16, (Wathugala et al., 2012) and the induction of HEL was also significantly suppressed. To test whether MED16 is required for the induction of PDF1.2, CHIB, and HEL during pathogen infection, we inoculated med16-1, sfr6-2, and wild-type plants with B. cinerea, a necrotrophic pathogen activating JA and ET responses. As shown in Figures 8D to 8F, whereas all three genes were significantly induced by B. cinerea in the wild-type plants, the induction of PDF1.2 and CHIB was completely blocked, and that of HEL was also dramatically decreased in the med16/sfr6 plants. Taken together, these results indicate that MED16 is required for JA/ET-mediated defense gene expression.

To determine whether MED16 plays a role in resistance to necrotrophic pathogens, we inoculated med16-1, sfr6-2, and...
wild-type plants with *B. cinerea* and *A. brassicicola*. The *med8*, *med25-1*, and *med25-2* plants were included as controls in the experiments. Results showed that all five mutants exhibited enhanced susceptibility to both *B. cinerea* and *A. brassicicola* (Figures 8G to 8L). In the experiment for *B. cinerea* infection, 0% and ~17% of the inoculated leaves from *med16/sfr6* and wild-type plants, respectively, showed no necrosis, whereas ~56% and 0% of the inoculated leaves from *med16/sfr6* and wild-type plants, respectively, were dead or decayed (Figure 8G). The average lesion sizes (diameter) on *med16/sfr6* plants were ~10.4 and ~3.4 mm, respectively (Figure 8L). BTH treatment made both *med16/sfr6* and wild-type plants more susceptible to *B. cinerea* infection, as indicated by the increased lesion sizes (see Supplemental Figure 3 online). In the test for *A. brassicicola* infection, 0% and ~8% of the inoculated leaves from *med16/sfr6* and wild-type plants, respectively, showed no necrosis, whereas ~17% and 0% of the inoculated leaves from *med16/sfr6* and wild-type plants, respectively, were dead or decayed (Figure 8H). The average lesion sizes on *med16/sfr6* plants were ~10.3 and ~3.3 mm, respectively (Figure 8J). These results together demonstrate that MED16 is another Mediator subunit playing an important role in basal resistance against necrotrophic fungal pathogens.

**MED16 Modulates NPR1 Protein Accumulation**

To explore the possible mechanism of action of MED16 in SAR, we first examined whether BTH treatment alters the subcellular localization of the MED16 protein. A green fluorescent protein (GFP)-MED16 fusion has been shown to be localized predominantly in the nucleus (Knight et al., 2009). BTH treatment did not change the subcellular localization of the GFP-MED16 fusion and a MED16-GFP fusion (see Supplemental Figure 4 online). Both MED16 and NPR1 positively contribute to SAR; therefore, MED16 might transcriptionally and/or posttranscriptionally regulate NPR1. To test this possibility, we generated transgenic *npr1-3* plants expressing a Myc-NPR1 fusion driven by its endogenous promoter. The *NPR1:Myc-NPR1* transgene complemented all of the *npr1-3* mutant phenotypes, including reduced tolerance to SA toxicity, lack of inducible *PR* gene expression, and enhanced pathogen susceptibility (see Supplemental Figure 5 online). The transgene was crossed into the *med16 npr1-3* double mutant. Pathogen infection does not induce *NPR1* transcript accumulation in *npr1-3* (Figure 6A); therefore, these plants allowed us to monitor the induction of the transgene. As shown in Figure 9A, whereas BTH treatment induced the expression of the transgene in both genetic backgrounds, the induction level was lower in *NPR1:Myc-NPR1 med16* plants than in *NPR1:Myc-NPR1* plants. Although we found that *NPR1:Myc-NPR1 med16* plants accumulated less Myc-NPR1 protein than *NPR1:Myc-NPR1* plants (Figures 9B and 9C), it is not clear whether the reduction in Myc-NPR1 protein levels was caused by the decreased transcript level. To unambiguously address whether MED16 posttranscriptionally regulates NPR1, we crossed the previously characterized 35S:NPR1-GFP transgene into the *med16 npr1-3* double mutant (Kinkema et al., 2000). The *med16* mutation did not alter the expression of the transgene no matter whether the plants were treated with or without BTH (Figure 9A), which allowed us to determine whether *med16* modulates NPR1-GFP nuclear localization and/or total protein accumulation. The mutation *med16* did not block BTH-induced NPR1-GFP nuclear localization (see Supplemental Figure 6 online). However, total NPR1-GFP protein levels decreased dramatically in the *med16* genetic background (Figure 9D), indicating that MED16 is required for NPR1 protein accumulation.

**DISCUSSION**

The understanding that establishment of SAR involves profound transcriptional changes has been well documented (Maleck et al., 2000). Although the transcription coactivator NPR1 and its interacting TGA transcription factors have been shown to mediate these transcriptional changes (Cao et al., 1994; Zhang et al., 2003), it is unclear how SAR signals are transduced from the NPR1 signaling node to the RNAPII transcription machinery. In a genetic screen for *Arabidopsis* mutants insensitive to exogenous NAD+, which activates defense responses in plants, we identified MED16 as a key regulator functioning downstream of eNAD+. Results from characterization of the *med16/sfr6* mutant plants suggest that MED16, a subunit in the tail module of the *Arabidopsis* Mediator complex, may be a signaling component in the gap between SAR-specific transcription activators and the general transcription machinery.

In animal cells, eNAD(P) is a well established signal molecule that activates intracellular signaling events, including immune responses (Billington et al., 2006). However, it is not clear whether plants and animals use similar mechanisms to process or perceive eNAD(P) and to transduce eNAD(P)-activated signals. The animal NAD(P)-metabolizing ectoenzyme CD38 converts eNAD(P) into the secondary messengers cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate, which in turn activates intracellular signaling (Ceni et al., 2003; Partida-Sánchez et al., 2003; Krebs et al., 2005). We recently showed that expression of the human CD38 compromises SAR in *Arabidopsis* (Zhang and Mou, 2012), suggesting that plants may use different mechanisms to perceive eNAD(P). Here we found that mutations in *MED16* block exogenous NAD+-induced *PR1* gene expression and compromise SAR (Figures 3B and 7K), indicating that MED16 is a downstream regulator that transduces eNAD+-activated signals to the RNAPII transcription machinery in *Arabidopsis*. MED16 and the Mediator complex are highly conserved in plants and animals (Bäckström et al., 2007); therefore, it may be possible that MED16 is also the Mediator subunit transferring eNAD(P)-activated signals to the transcriptional machinery in animal cells.

Biological induction of SAR was abolished completely in *med16/sfr6* plants (Figure 7D), similar to that in *npr1* and the *tgα6 tga2 tga5* triple knockout mutant (Cao et al., 1994; Zhang et al., 2003), suggesting that MED16, like NPR1 and the TGA factors (TGA2, TGA5, and TGA6), is an essential positive regulator of SAR. We found that expression of the SAR marker genes *PR1*, *PR2*, *PR5*, *GST11*, *EDR11*, and *SAG21* was significantly decreased in *med16/sfr6* (Figures 7C to 7H), indicating that MED16, like NPR1, is required for the transcriptional changes occurring in systemic leaves during the establishment of SAR (Maleck et al., 2000). Therefore, NPR1, the TGA factors, and MED16 may
Figure 8. Induction of JA/ET-Responsive Genes and Resistance to Necrotrophic Pathogens in med16/sfr6 Mutants.

(A) to (C) ACC- and MeJA-induced expression of PDF1.2, CHIB, and HEL in med16/sfr6 and wild-type (WT) plants. Ten-d-old seedlings grown on one-half-strength MS medium were transplanted onto one-half-strength MS medium (−) or one-half-strength MS medium supplemented with 0.1 mM of ACC, 0.1 mM of MeJA, or both (A+M). Total RNA was extracted from plant tissues except roots collected 24 h later and subjected to real-time qPCR analysis. The UBQ5 gene was used as a loading control.
constitute a signaling pathway operating during biological induction of SAR.

In contrast with biological induction of SAR, induction of SAR by chemical inducers revealed differences among med16/sfr6, npr1, and tga6 tga2 tga5 plants. In npr1 plants, SA and its analog 2,6-dichloroisonicotinic acid did not induce PR gene expression and disease resistance (Cao et al., 1994). In tga6 tga2 tga5 plants, although one report showed that induction of PR1 by SA was only slightly delayed (Blanco et al., 2009), 2,6-dichloroisonicotinic acid did not induce resistance (Zhang et al., 2003). However, BTH induced low levels of PR gene expression and a considerable level of disease resistance in med16/sfr6 plants (Figures 5C to 5F) (Wathugala et al., 2012). These results suggest that MED16, NPR1, and the TGA factors may use different mechanisms in regulating SA-activated defense responses, and that BTH treatment activates both MED16-dependent and MED16-independent defense responses.

NPR1 and TGA factors are pathway-specific transcription activators, whereas MED16 is a subunit of Mediator, a protein complex in the general transcription machinery; therefore, MED16 likely functions downstream of NPR1 and the TGA factors to relay signals from the NPR1 signaling node to the general transcription machinery. Mediator is well known to transfer signals from pathway-specific transcription activators (Balamotis et al., 2009; Kagey et al., 2010; Takahashi et al., 2011); however, it is unclear whether Mediator also influences the homeostasis of the transcription activators. Here we show that NPR1 protein levels were decreased in med16 plants (Figures 9B and 9D), suggesting that MED16 may regulate SA responsiveness and basal immunity partially through modulating NPR1 protein accumulation (Figures 5C to 5F and 6A to 6E). Although the mechanism underlying the regulation is unclear, this result indicates that Mediator not only perceives signals from specific transcription activators but also actively regulates the homeostasis of the transcription activators. Whether NPR1 or TGA factors are physically associated with MED16 during biological induction of SAR needs to be addressed in future research.

MED16 negatively regulates SA biosynthesis by suppressing the SA biosynthesis gene ICS1/SID2 (Nawrath and Métraux, 1999; Wildermuth et al., 2001). In npr1 plants, ICS1 transcripts and SA accumulate to much higher levels than in the wild type. We found that, on pathogen infection, SA levels in med16/sfr6 and wild-type plants were not significantly different (Figures 5A, 5B, 6F, and 6G), suggesting that MED16 may not participate in the NPR1-mediated feedback inhibition of SA biosynthesis. High levels of SA cause toxicity to plants; therefore, both npr1 and tga6 tga2 tga5 exhibited hypersensitivity to high concentrations of SA (Kinkema et al., 2000; Zhang et al., 2003). However, med16/sfr6 seedlings were as tolerant as the wild type to SA toxicity, suggesting that MED16 is not involved in NPR1- and TGA factor-mediated SA tolerance. These MED16-independent pathways mediated by NPR1 and the TGA factors also deserve future investigation.

Mutations in the MED16 gene suppressed JA/ET- and the necrotrophic pathogen B. cinerea–induced expression of several JA/ET-responsive genes (Figures 8A to 8F) (Wathugala et al., 2012), and med16/sfr6 mutants exhibited enhanced susceptibility to B. cinerea and A. brassicicola (Figures 8G to 8L), suggesting that MED16 functions downstream of JA/ET in defense signaling pathway. Recently, Arabidopsis Med8, Med21, and Med25 have been shown to regulate resistance to necrotrophic pathogens (Dhawan et al., 2009; Kidd et al., 2009). Med25 seems to be required for both background and JA-induced expression of JA-responsive genes, whereas Med8 seems to play a minor role in regulating PDF1.2 expression. The effect of the med8 mutation on JA-induced expression of PDF1.2 could only be readily detected in the med8 med25 double mutant (Kidd et al., 2009). Although MED21 RNA interference lines showed increased susceptibility to B. cinerea and A. brassicicola (Dhawan et al., 2009), its function in regulating defense gene expression has not been reported. These results together indicate that Mediator is a pivotal player in plant immunity, with four subunits having been shown to be required for resistance to necrotrophic fungal pathogens in Arabidopsis.

Our data show that MED16 is required for induction of both SA- and JA/ET-responsive genes, indicating that MED16 is a positive regulator of the SA and the JA/ET signaling pathway. Although Kidd et al. (2009) reported that the med25 mutation also decreased SA-induced defense gene expression, we did not detect any enhanced susceptibility to the biotrophic bacterial pathogen Pst DC3000 in med25 mutant plants (see Supplemental Figure 7A online). Consistent with this, biological induction of SA was not significantly altered in med25 plants (see Supplemental Figure 7B online). The med8 mutant displayed enhanced...
susceptibility to Pst DC3000 (see Supplemental Figure 7A online) but did not show significant defects in biological induction of SAR (see Supplemental Figure 7B online). Therefore, the MED16 Mediator subunit is required for both biological induction of SAR and JA/ET-mediated resistance to necrotrophs.

It is generally accepted that SA and JA signaling pathways antagonize each other (Kunkel and Brooks, 2002; Pieterse et al., 2009). In recent years, a number of proteins regulating SA-JA/ET crossstalk have been identified in Arabidopsis (Petersen et al., 2000; Kachroo et al., 2001; Spoel et al., 2003; Li et al., 2004; Brodersen et al., 2006; Ndamukong et al., 2007). Whereas most of these regulators, including NPR1, inversely regulate SA and JA signaling pathways, the TGA factors seem to positively contribute to both SA and JA/ET signaling (Zander et al., 2010). The TGA factors are required for antagonizing the negative effect of MYC2/JIN1 (a negative regulator of PDF1.2) in the JA/ET pathway, and this positive function might be abolished in the presence of SA (Zander et al., 2010). Interestingly, MED16 suppresses several negative regulators of the SA signaling pathway (Figure 4). Whether this function of MED16 is involved in crosstalk between SA and JA/ET is currently unknown. MED16 positively regulates both SA and JA/ET signaling; therefore, it might function as a convergence point in Mediator conveying signals from both SA and JA/ET pathways.

The multiprotein Mediator complex functions as a bridge between specific transcription activators and the general transcription
machinery. Individual Mediator subunits interact with pathway-specific activators to coordinate and transfer pathway-specific signals to the transcription machinery. *Arabidopsis* MED21 has been shown to interact with a RING E3 ligase histone mono-ubiquitination1 (HUB1), which positively regulates resistance to necrotrophs (Dhawan et al., 2009). Although several other Mediator subunits, including MED8, MED16, and MED25, have been implicated in SAR, basal resistance, and/or resistance to necrotrophs, their interacting partners in defense signaling pathways are unknown. It is also unknown whether the remaining subunits function in plant immunity. Future research focusing on these questions would shed new light on the molecular mechanisms by which Mediator regulates gene transcription in plant immune responses.

**METHODS**

**Plant Materials and Growth Conditions**

The wild-type Columbia ecotype of *Arabidopsis thaliana* was used. Transgenic plants and homozygous T-DNA insertion lines were generated or identified as described in the Supplemental Methods 1 online using gene-specific primers (see Supplemental Table 2 online). The med16/sfr6 npr1 double mutants were generated by crossing med16/sfr6 with npr1-3 and identified in the segregating F2 populations based on their morphology and confirmed by the med16-1 CAPS marker (see Supplemental Table 3 online) or by PCR using the primers flanking the T-DNA insertion in sfr6-2, and the npr1-3 mutation was confirmed by a derived CAPS marker (De Fraia et al., 2010). *Arabidopsis* seeds were sown on autoclaved soil (Metro-Mix 200; Grace-Sierra) and vernalized at 4°C for 3 d. Plants were germinated and grown at 23 to 25°C under a 16-h light/8-h dark regime.

**Pathogen Infection**

Inoculation of plants with *Pseudomonas syringae pv tomato* (Pst) DC3000/avrRpt2 and Pst DC3000 was performed by pressure-infiltration with a 1-mL needleless syringe as described previously (Clarke et al., 1998).

For *Botrytis cinerea* and *Alternaria brassicicola* inoculation, pathogens were grown on BD Difco Potato Dextrose Agar (Becton, Dickinson and Company) for ~10 d at 25°C. Spores were harvested, resuspended in BD Difco Potato Dextrose Broth (Becton, Dickinson and Company) at a density of 1 to 5 x 10^5 spores/mL, and incubated for 2 h before inoculation. Then, 5-µL spore suspensions were dropped on the adaxial surface of rosette leaves, where the leaves were gently wounded with a needle. Symptoms were monitored for 3 to 4 d, and infection ratings from 0 to 3 were assigned to the inoculated leaves (0, no infection/necrosis; 1, leaves showing some necrosis; 2, leaves showing severe necrosis; 3, dead/decayed leaves). In each experiment, 16 to 20 plants per genotype were inoculated, and three to four leaves from each plant were scored after 4 d for symptom development.

**SA Measurement**

SA measurement was done by HPLC as described by Verberne et al. (2002).

**Chemical Treatment**

Exogenous NAD^+ and BTH treatment were performed as previously described (Zhang and Mou, 2009). For ACC and MeJA treatment, 10-d-old seedlings grown on one-half-strength MS medium were transplanted onto one-half-strength MS medium supplemented with either 0.1 mM of ACC, 0.1 mM of MeJA, or both. Seedlings for the negative control were also transplanted onto one-half-strength MS medium. Plant tissues except roots were collected and subjected to total RNA extraction.

**RNA and Protein Analysis**

RNA extraction and RNA gel blot analysis were performed as described by Cao et al. (1997). Reverse transcription and real-time qPCR were performed as previously described using gene-specific primers (see Supplemental Table 4 online; De Fraia et al., 2010). The relative quantity of a gene is expressed in relation to ubiquitin5 (UBQ5) using the equation 2^\(^{−}\)\(\Delta\)Ct[UBQ5] − Ct[GENE], where 2 represents perfect PCR efficiency and Ct stands for cycle threshold. Total protein extraction and protein gel blot analysis were conducted as described by Mou et al. (2003).

**Microarray Analysis**

Three biological replicates with leaves from eight plants per sample were collected at 0, 4, 8, and 24 h after inoculation. RNA concentration was determined on a NanoDrop Spectrophotometer (ThermoFisher Scientific), and sample quality was assessed using the 2100 Bioanalyzer (Agilent Technologies). Equal quantities of RNA from the three biological replicates were pooled. cDNA was synthesized from 200 ng of total RNA and used as a template for in vitro transcription in the presence of T7 RNA Polymerase and cyanine-labeled CTPs using the Quick Amp Labeling kit (Agilent Technologies) according to the manufacturer’s protocol. The amplified, labeled complementary RNA was purified with the RNeasy Mini kit (Qiagen). For each array, 1.65 µg of Cy 3-labeled complementary RNA was fragmented and hybridized with rotation at 65°C for 17 h. Samples were hybridized to *Arabidopsis* 4 × 44k arrays (Agilent Technologies). The arrays were washed according to the manufacturer’s protocol and then scanned on a G2505B scanner (Agilent Technologies). Data were extracted using Feature Extraction 10.1.1.1 software (Agilent Technologies). Microarray experiments were performed in the microarray core laboratory of the Interdisciplinary Center for Biotechnology Research at the University of Florida.

Before comparative analysis, the individual signal intensity values obtained from the eight microarray probes were log transformed (using 2 as the base) and normalized to ensure that meaningful biological comparisons can be made. More specifically, we first estimated the lower quartile, median, and upper quartile values by pooling all samples and then scaling and shifting the individual log-transformed signal intensities using the estimated quartile values as the references. As shown in Supplemental Figure 8 online, the normalized signal intensity data sets have similar median intensities and dynamic ranges. After normalization, a probe-by-probe comparison was performed between different time points of the same genotype using the 0-h sample as the reference and between med16-1 and the wild type at the same time point. In each comparison, fold change values were computed for each gene. The gene expression fold changes were computed based on the normalized log-transformed signal intensity data. The comparison results were further explored to obtain numbers of overlapped genes between med16-1 and the wild type.

**Statistical Methods**

All statistical analyses were performed with the data analysis tools (Student’s t test: Two Sample Assuming Unequal Variances) in Microsoft Excel (Microsoft Office 2004 for Macintosh).

**Accession Numbers**

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession...
Supplemental Data

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

**Supplemental Figure 1.** Morphology of *ien1*, SALK_048091 (sfr6-2), and Their F1 Progeny.

**Supplemental Figure 2.** Genetic Complementation of the *ien1* Mutant.

**Supplemental Figure 3.** Effect of BTH Treatment on Resistance to *B. cinerea* in *med16* and *med25* Mutants.

**Supplemental Figure 4.** Effect of BTH Treatment on the Subcellular Localization of the MED16 Protein.

**Supplemental Figure 5.** Characterization of the NPR1:Myc–NPR1 Transgene.

**Supplemental Figure 6.** Subcellular Localization of NPR1-GFP in *med16* Plants.

**Supplemental Figure 7.** Basal Resistance and SAR Induction in *med8* and *med25* Mutants.

**Supplemental Figure 8.** Normalization of the Microarray Data Sets Obtained from the Eight Microarray Probes.

**Supplemental Table 1.** Defense Genes Differentially Expressed in the Eight Microarray Probes.

**Supplemental Table 2.** Primers for Identification of Homozygous T-DNA Insertion Lines.

**Supplemental Table 3.** The CAPS Marker for the *med16*-1 Mutation.

**Supplemental Table 4.** Primers Used for qPCR in This Study.

**Supplemental Methods 1.** Supplemental Methods for the Supplemental Data.

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AUTHOR CONTRIBUTIONS

X.Z., C.W., and Z.M. designed the research; X.Z. and C.W. performed research and analyzed data; Y.Z. performed the microarray experiment; Y.S. analyzed the microarray data; Z.M. wrote the article.

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REFERENCES


