Mediator subunit MED25 links the jasmonate receptor to transcriptionally active chromatin

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Jasmonoyl-isoleucine (JA-Ile), the active form of the plant hormone jasmonate (JA), is sensed by the F-box protein CORONATINE INSENSITIVE 1 (COI1), a component of a functional Skp–Cullin–F-box E3 ubiquitin ligase complex. Sensing of JA-Ile by COI1 rapidly triggers genome-wide transcriptional changes that are largely regulated by the basic helix–loop–helix transcription factor MYC2. However, it remains unclear how the JA-Ile receptor protein COI1 relays hormone-specific regulatory signals to the RNA polymerase II general transcriptional machinery. Here, we report that the plant transcriptional coactivator complex Mediator directly links COI1 to the promoters of MYC2 target genes. MED25, a subunit of the Mediator complex, brings COI1 to MYC2 target promoters and facilitates COI1-dependent degradation of jasmonate–ZIM domain (JAZ) transcriptional repressors. MED25 and COI1 influence each other's enrichment on MYC2 target promoters. Furthermore, MED25 physically and functionally interacts with HISTONE ACETYLTRANSFERASE1 (HAC1), which plays an important role in JA signaling by selectively regulating histone (H) 3 lysine (K) 9 (H3K9) acetylation of MYC2 target promoters. Moreover, the enrichment and function of HAC1 on MYC2 target promoters depend on COI1 and MED25. Therefore, the MED25 interface of Mediator links COI1 with HAC1-dependent H3K9 acetylation to activate MYC2-regulated transcription of JA-responsive genes. This study exemplifies how a single Mediator subunit integrates the actions of both genetic and epigenetic regulators into a concerted transcriptional program.

jasmonate | nuclear hormone receptor | MED25 | COI1 | MYC2

Jasmonate (JA) is an oxylipin-derived plant hormone that regulates diverse aspects of plant immunity and development (1, 2). Decades of studies in the model plant *Arabidopsis thaliana* have revealed a core JA signaling module consisting of the F-box protein CORONATINE INSENSITIVE 1 (COI1) (3), a group of jasmonate–ZIM domain (JAZ) proteins (4–6), and the basic helix–loop–helix transcription factor MYC2 (7, 8). COI1 forms a functional Skp–Cullin–F-box (SCF) E3 ubiquitin ligase SCF^{COI1} along with Cullin1 and Skp1-like1 (ASK1) (9, 10), MYC2 acts as a master transcription factor that differentially regulates diverse aspects of JA responses (11–13), and the JAZ proteins are substrates of SCF^{COI1} and serve as transcriptional repressors of MYC2 (4, 5, 14).

The identification of jasmonoyl-isoleucine (JA-Ile) as the receptor-active form of the hormone, along with the discovery that sensing of JA-Ile involves formation of the SCF^{COI1}–JAZs coreceptor complex (4, 15–17), represented a breakthrough in our mechanistic understanding of JA signaling. In the absence of the hormone, JAZ repressors interact with and repress the activity of MYC2. In response to internal or external cues that trigger JA-Ile synthesis, elevated JA-Ile levels promote SCF^{COI1}-dependent degradation of JAZ repressors, and thereby activate (de-repress) the MYC2-directed transcriptional program. These discoveries imply that sensing of the active hormone is tightly linked to transcription of JA-responsive genes throughout the genome. In this context, an important challenge in the study of JA signaling is unraveling the molecular determinants that enable the JA-Ile receptor to transmit hormone-specific regulatory signals to the RNA polymerase II (Pol II) general transcription machinery, which transcribes most protein-coding genes in eukaryotic cells (18).

The intimate association between sensing of JA-Ile and genome-wide transcriptional reprogramming implies that coordinated epigenetic regulatory events, such as histone modifications and chromatin remodeling, are an integral part of JA signaling. However, it remains unclear how plants integrate the actions of multiple epigenetic regulators and the aforementioned genetic regulators (i.e., COI1, MYC2, JAZs, etc.) into a concerted transcriptional program.

To investigate these closely related issues, we sought to identify COI1-interacting proteins, reasoning that the molecular determinants that bridge COI1 with the general transcription machinery and chromatin must interact physically with COI1. Among the COI1-interacting proteins we identified was the MED25 subunit of *Arabidopsis* Mediator (19–22), an evolutionarily conserved multisubunit coregulatory complex whose activity is essential for Pol II-dependent transcription in eukaryotic cells (23–29).

Significance

Sensing of the plant hormone jasmonate (JA) by the F-box protein CORONATINE INSENSITIVE 1 (COI1) triggers profound transcriptional changes that are regulated by the master regulator MYC2. However, it remains unclear how COI1 communicates with the general transcription machinery and chromatin. Here, we show that MED25, a subunit of the Mediator coactivator complex, physically and functionally interacts with COI1 on the promoters of MYC2 targets. MED25 also physically and functionally interacts with HISTONE ACETYLTRANSFERASE1 (HAC1), which selectively regulates histone (H) 3 lysine (K) 9 acetylation of MYC2 targets. Therefore, MED25 integrates regulatory signals that converge on the promoters of MYC2 targets. Our results reveal a fundamental mechanism by which Mediator coordinates the actions of both genetic and epigenetic regulators into a concerted transcriptional program.

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Here, we report that MED25 bridges COI1 to Pol II and chromatin during JA signaling. We found that MED25 physically interacts with COI1 on MYC2 target promoters and facilitates COI1-dependent degradation of JAZ proteins. MED25 also physically and functionally interacts with HISTONE ACETYLTRANSFERASE1 (HAC1), a histone modification enzyme that selectively regulates histone (H) 3 lysine (K) 9 acetylation (H3K9ac) of MYC2 target promoters during JA signaling. Moreover, MED25 cooperates with both COI1 and HAC1 on MYC2 target promoters. Therefore, MED25 directly links the JA-Ile receptor to transcriptionally active chromatin during hormone-elicited activation of MYC2.

Results

COI1 Is Enriched on the Promoters of *JAZ8* and *ERF1*. Given that COI1-dependent JA-Ile perception triggers rapid degradation of JAZ proteins, which interact with and repress MYC2 in the resting stage, we hypothesized that COI1 associates with promoter regions bound by MYC2. To test this possibility, we performed chromatin immunoprecipitation (ChIP)-quantitative PCR (qPCR) assays to measure the enrichment of COI1 on the chromatin of *JAZ8* and *ERF1*, which are direct transcriptional targets of MYC2 (5, 11, 21) (Fig. 1*A* and Fig. S1). ChIP-qPCR assays of wild-type (WT) seedlings using an anti-COI1 antibody revealed that, without JA-Ile stimulation, COI1 was much more highly enriched on the G-box regions and transcription start sites (TSSs) of these genes than on the upstream promoter regions and gene bodies (Fig. 1 *A* and *B*), indicating that COI1 preferentially associates with the G-box and TSS regions of *JAZ8* and *ERF1*.

To determine whether and how the promoter association of COI1 is regulated by JA signaling, we examined the JA-Ile–induced pattern of enrichment of COI1 on the TSSs of *JAZ8* and *ERF1* by ChIP-qPCR. In the absence of JA-Ile, COI1 enrichment levels on the TSSs of *JAZ8* and *ERF1* were relatively high, whereas following JA-Ile treatment, COI1 enrichment levels were reduced within 15 min and continued to decrease throughout the

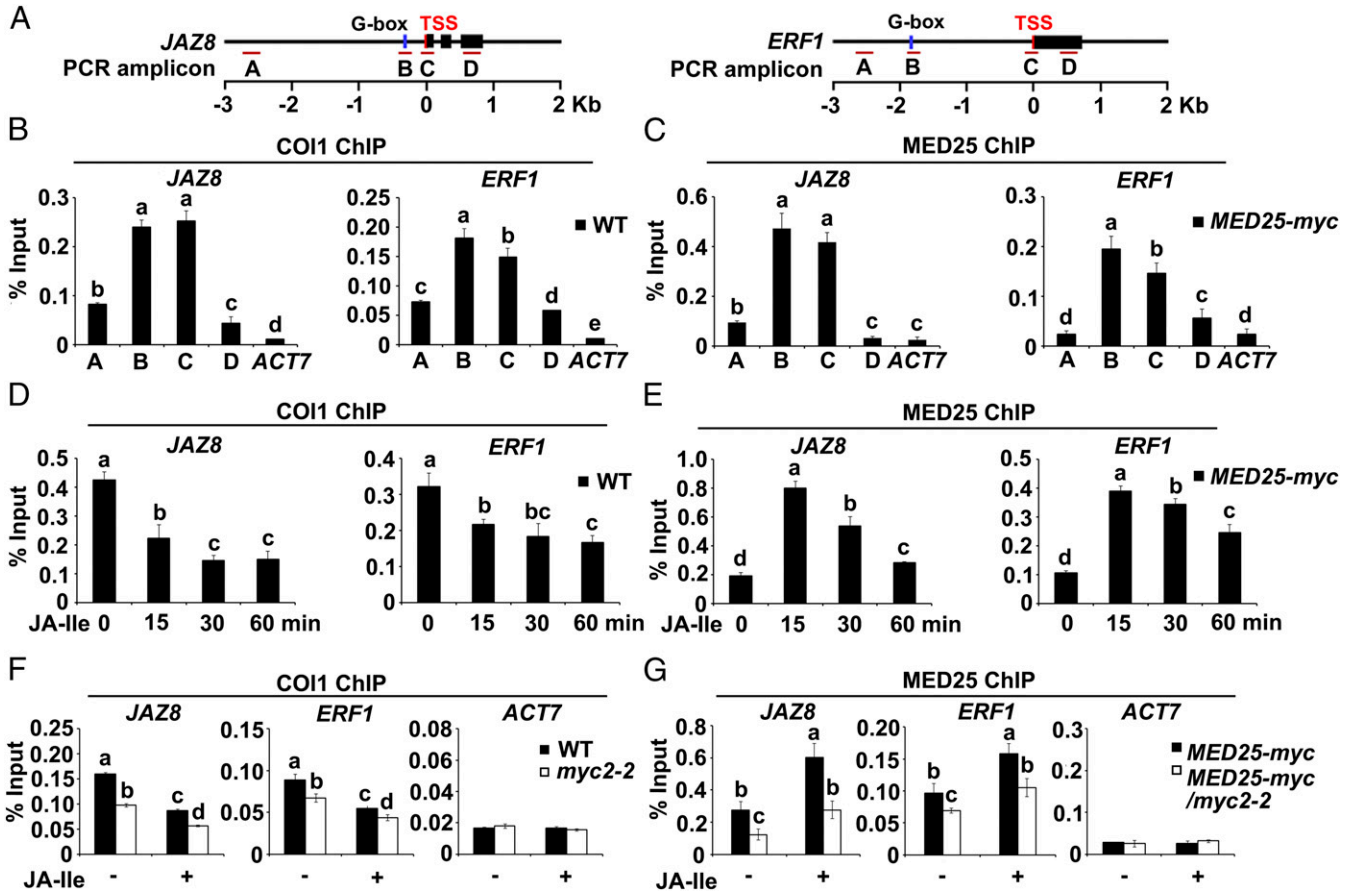

Fig. 1. Enrichment of COI1 and MED25 on the promoters of *JAZ8* and *ERF1*. (*A*) Schematic diagrams of *JAZ8*, *ERF1*, and PCR amplicons indicated as letters A–D used for ChIP-qPCR. (*B*) ChIP-qPCR showing the enrichment of COI1 on the chromatin of *JAZ8* and *ERF1*. Chromatin of WT plants was immunoprecipitated using anti-COI1 antibody. (*C*) ChIP-qPCR showing enrichment of MED25 on the chromatin of *JAZ8* and *ERF1*. Chromatin of *MED25-myc* plants was immunoprecipitated using anti-myc antibody. (*D*) ChIP-qPCR showing enrichment of COI1 on the TSS regions of *JAZ8* and *ERF1* upon JA-Ile stimulation. WT plants were treated with 30 μM JA-Ile for the indicated times before cross-linking, and chromatin from each sample was immunoprecipitated using anti-COI1 antibody. (*E*) ChIP-qPCR showing enrichment of MED25 on the TSS regions of *JAZ8* and *ERF1* upon JA-Ile stimulation. *MED25-myc* plants were treated with 30 μM JA-Ile for the indicated times before cross-linking. Chromatin of each sample was immunoprecipitated using anti-myc antibody. (*F*) ChIP-qPCR assays showing that *myc2-2* impairs the enrichment of COI1 on the TSSs of *JAZ8* and *ERF1* before and after JA-Ile stimulation. WT and *myc2-2* plants were treated with or without 30 μM JA-Ile for 15 min before cross-linking, and chromatin of each sample was immunoprecipitated using anti-COI1 antibody. (*G*) ChIP-qPCR assays showing that *myc2-2* impairs the enrichment of MED25 on the TSSs of *JAZ8* and *ERF1* before and after JA-Ile stimulation. *MED25-myc* and *MED25-myc/myc2-2* plants were treated with or without 30 μM JA-Ile for 15 min before cross-linking, and chromatin of each sample was immunoprecipitated using anti-myc antibody. For *B*–*G*, precipitated DNA was quantified by qPCR, and the DNA enrichment is shown as a percentage of input DNA. *ACTIN7* (*ACT7*) was used as a nonspecific binding site. Error bars indicate SD of three independent experiments ($n = 3$). ANOVA was performed for statistical analysis; bars with different letters are significantly different from each other ($P < 0.01$).

remainder of the experiment (Fig. 1*D*). These results reveal that COI1 is enriched on MYC2 target promoters and that this enrichment is down-regulated by hormone stimulation.

Further, we examined whether depletion of MYC2 affects the enrichment of COI1 on the promoters of *JAZ8* and *ERF1*. In the absence of JA-Ile, COI1 enrichment levels on the TSSs of *JAZ8* and *ERF1* were significantly reduced in *myc2-2* (8) in comparison to the WT (Fig. 1*F*). JA-Ile treatment decreased COI1 enrichment in both *myc2-2* and the WT, and the level of COI1 enrichment was lower in *myc2-2* than in the WT (Fig. 1*F*). These results indicate that the COI1 enrichment on MYC2 target promoters depends on the function of MYC2.

MED25 Interacts with COI1 and Facilitates COI1-Dependent Degradation of JAZ1. To identify the molecular determinants that link COI1 to MYC2 target promoters, we performed yeast two-hybrid (Y2H) assays to identify COI1-interacting proteins. Significantly, one of the COI1-interacting proteins we identified was the MED25 subunit of the plant Mediator coactivator complex (Fig. 2*A*). To confirm the physical interaction between MED25 and COI1, we performed in vitro pull-down experiments using a purified maltose-binding protein (MBP)–tagged MED25 fragment ($MED25^{551-836}$-MBP) and histidine (His)-tagged COI1 (COI1-His). $MED25^{551-836}$ could pull down COI1 (Fig. 2*B*), indicating that MED25 interacts with COI1 in vitro.

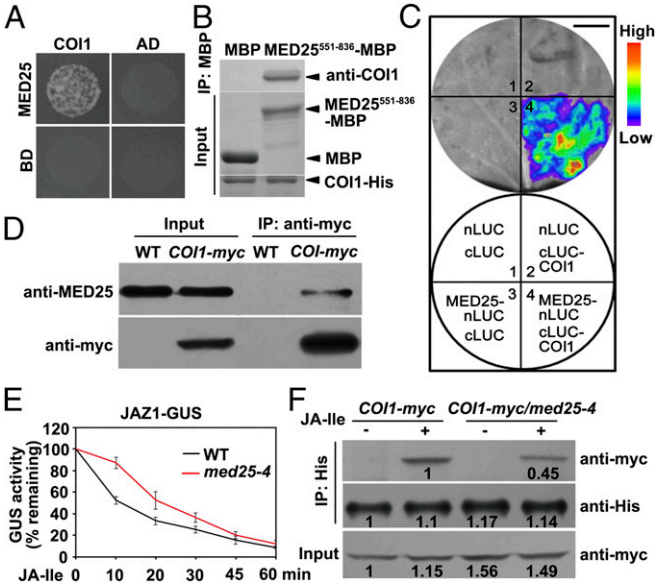

Fig. 2. MED25 interacts with COI1 and facilitates COI1-dependent degradation of JAZ1. (*A*) Y2H assays showing that MED25 interacts with COI1. Transformed yeast strain was plated on SD medium lacking His, Leu, and Trp (SD/-3). (*B*) In vitro pull-down assays between $MED25^{551-836}$-MBP and COI1-His. COI1-His was pulled down by $MED25^{551-836}$-MBP immobilized on amylose resin. Protein bound to amylose resin was eluted and analyzed by immunoblotting using anti-COI1 antibody. (*C*) MED25 associates with COI1 in LCI assays. (*Top*) LUC images of *N. benthamiana* leaves coinfiltrated with various constructs are shown in the lower quadrant of the circle. The pseudocolor bar shows the range of luminescence intensity. (Scale bar, 1 cm.) (*D*) Co-IP assay between MED25 and COI1. Proteins extracted from WT and *COI1-myc* plants were immunoprecipitated using anti-myc antibody and immunoblotted using anti-MED25 antibody. (*E*) JA-Ile–triggered degradation of the *JAZ1-GUS* reporter in WT and *med25-4* backgrounds. Seven-day-old seedlings were treated with 30 μM JA-Ile for the indicated durations before quantification of GUS activity. Error bars indicate SD of three independent experiments ($n = 3$). (*F*) Pull-down assays between JAZ1-His and COI1. Protein extracts from *COI1-myc* and *COI1-myc/med25-4* seedlings were incubated with recombinant JAZ1-His protein in the presence or absence of 30 μM JA-Ile. COI1 was pulled down by JAZ1-His immobilized on nickel-nitrilotriacetic acid (Ni-NTA; Novagen) resin and eluted and analyzed by immunoblotting using anti-myc antibody. Bands were quantified using ImageJ.

To determine whether MED25 interacts with COI1 *in planta*, we conducted firefly luciferase (LUC) complementation imaging (LCI) assays in *Nicotiana benthamiana* leaves (30). In these experiments, MED25 was fused to the N-terminal half of LUC (nLUC) to produce MED25-nLUC, whereas COI1 was fused to the C-terminal half of LUC (cLUC) to produce cLUC-COI1. *N. benthamiana* cells coexpressing MED25-nLUC and cLUC-COI1 displayed strong fluorescence signals, whereas those coexpressing nLUC and cLUC-COI1 or MED25-nLUC and cLUC displayed no signal (Fig. 2*C*), confirming that the MED25–COI1 interaction occurs in vivo. Domain mapping with LCI assays revealed that the MED25–COI1 interaction requires the glutamine-rich domain of MED25 and the F-box domain of COI1 (Fig. S2).

Next, we performed coimmunoprecipitation (co-IP) experiments using *COI1-myc* plants (9) and anti-MED25 antibody (21), and found that COI1-myc could pull down endogenous MED25 (Fig. 2*D*), corroborating that MED25 interacts with COI1 *in planta*.

To understand the functional relevance of MED25–COI1 interaction in JA signaling, we first investigated whether depletion of MED25 would affect the COI1-dependent degradation of JAZ proteins, a convenient reporter for assessing the in vivo JA response (4, 5). For this purpose, we introduced the JAZ1–β-glucuronidase (GUS) fusion protein (4) into the *med25-4* mutant (21) background. As shown in Fig. 2*E*, JA-Ile–triggered degradation of JAZ1-GUS was considerably slower in *med25-4* than in the WT, confirming that MED25 plays an important role in COI1-dependent degradation of JAZ1-GUS. We then performed an in vitro pull-down assay to test whether MED25 plays a role in JA-Ile–mediated promotion of COI1–JAZ1 interaction. In these experiments, protein extracts from COI1-myc/WT and COI1-myc/*med25-4* seedlings were incubated with recombinant His-tagged JAZ1 protein (JAZ1-His) in the presence of JA-Ile, and protein bound to JAZ1-His was detected with an anti-myc antibody. The ability of JA-Ile to promote the COI1–JAZ1 interaction was reduced in *med25-4* relative to the WT (Fig. 2*F*), suggesting that MED25 contributes to JA-Ile–mediated promotion of the COI1–JAZ1 interaction.

MED25 and COI1 Reciprocally Affect Each Other's Enrichment on the Promoters of *JAZ8* and *ERF1*. Our observations that COI1 is enriched on MYC2 target promoter, and that MED25 interacts with COI1 *in planta*, suggested that the MED25–COI1 interaction occurs on the promoters of MYC2 targets. To test this possibility, we compared the JA-Ile–induced pattern of MED25 enrichment with that of COI1 on the chromatin of *JAZ8* and *ERF1*. ChIP-qPCR assays using MED25-myc plants showed that, in the absence of JA-Ile treatment, MED25 was significantly more enriched on the G-box and TSS regions of *JAZ8* and *ERF1* than on the upstream promoter regions and gene bodies (Fig. 1 *A* and *C*), indicating that in the resting stage, MED25 exhibits an enrichment pattern similar to that of COI1 on MYC2 target promoters (Fig. 1 *C* vs. *B*). In response to JA-Ile treatment, however, MED25 enrichment levels on the TSSs of *JAZ8* and *ERF1* rapidly increased, reaching a maximum after 15 min (Fig. 1*E*), indicating that the JA-Ile–induced enrichment pattern of MED25 is distinct from that of COI1 (Fig. 1 *E* vs. *D*). Not surprisingly, MED25 enrichment on the TSS regions of *JAZ8* and *ERF1* was decreased in *myc2-2* in comparison to the WT before and after JA-Ile treatment (Fig. 1*G*), indicating that the enrichment of MED25 on MYC2 target promoters depends on the function of MYC2.

The rapid reduction in enrichment of COI1 on MYC2 target promoters upon JA-Ile treatment, in contrast to the rapid increase in enrichment of MED25, prompted us to ask whether COI1 and MED25 are simultaneously associated with the promoters of MYC2 targets. To address this question, we performed sequential ChIP-qPCR assays using chromatin from *MED25-myc* seedlings, which was sequentially immunoprecipitated with anti-COI1 and anti-myc antibodies. The results revealed high enrichment of the TSSs of *JAZ8*

and *ERF1* (Fig. 3 *A* and *B*), indicating that COI1 and MED25 are indeed enriched on the promoters of these genes at the same time.

To further understand the significance of the MED25–COI1 interaction on MYC2 target promoters, we assessed whether depletion of MED25 affects the enrichment of COI1 on the TSSs of *JAZ8* and *ERF1*. In the absence of JA-Ile, COI1 enrichment levels on the TSSs of *JAZ8* and *ERF1* were substantially reduced in *med25-4* in comparison to the WT (Fig. 3*C*). JA-Ile treatment decreased COI1 enrichment in both *med25-4* and the WT, and the level of COI1 enrichment was lower in *med25-4* than in the WT (Fig. 3*C*). These results, together with the finding that the *med25-4* mutation showed a negligible effect on COI1 protein levels before and after JA-Ile treatment (Fig. S3*A*), indicate that MED25 mainly affects the enrichment of COI1 on the promoters of *JAZ8* and *ERF1* before JA-Ile stimulation.

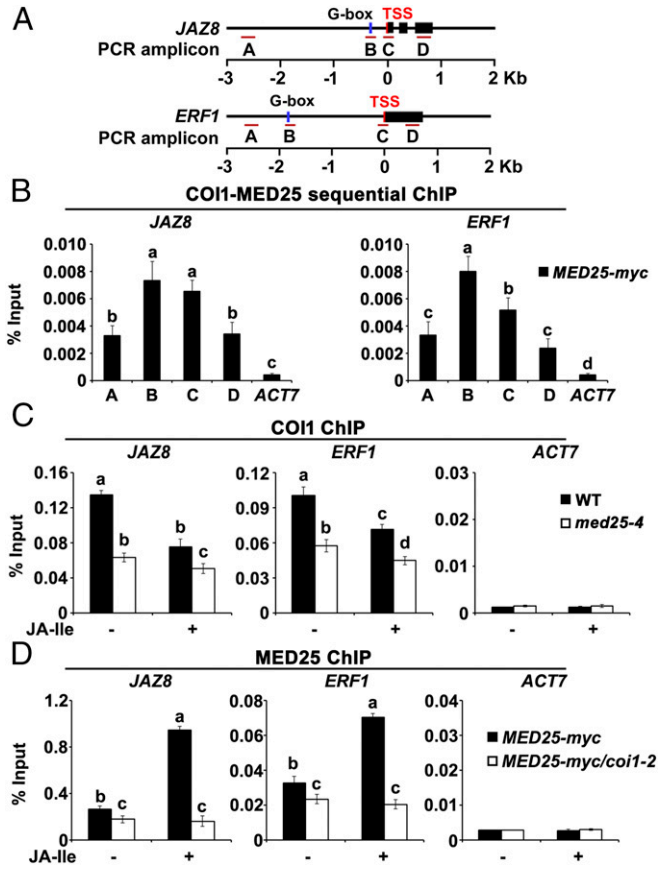

Fig. 3. COI1 and MED25 affect each other's enrichment on the promoters of *JAZ8* and *ERF1*. (*A*) Schematic diagrams of *JAZ8*, *ERF1*, and the PCR amplicons indicated as letters A–D used for ChIP-qPCR. (*B*) Sequential ChIP analysis showing that COI1 and MED25 co-occupy the promoters of *JAZ8* and *ERF1*. Chromatin of *MED25-myc* plants was immunoprecipitated with anti-COI1 antibody, and then with anti-myc antibody. (*C*) ChIP-qPCR assays showing that *med25-4* impairs the enrichment of COI1 on the TSSs of *JAZ8* and *ERF1* upon JA-Ile stimulation. WT and *med25-4* plants were treated with or without 30 μM JA-Ile for 15 min before cross-linking, and chromatin of each sample was immunoprecipitated using anti-COI1 antibody. (*D*) ChIP-qPCR assays showing that *coi1-2* impairs the enrichment of MED25 on the TSSs of *JAZ8* and *ERF1* upon JA-Ile stimulation. *MED25-myc* and *MED25-myc/coi1-2* plants were treated with or without 30 μM JA-Ile for 15 min before cross-linking, and chromatin of each sample was immunoprecipitated using anti-myc antibody. For *B–D*, the precipitated DNA was quantified by qPCR, and DNA enrichment is displayed as a percentage of input DNA. *ACT7* was used as a nonspecific binding site. Error bars indicate SD of three independent experiments ($n = 3$). ANOVA was performed for statistical analysis; bars with different letters are significantly different from each other ($P < 0.01$).

In parallel, we performed ChIP-qPCR to investigate whether depletion of COI1 affects enrichment of MED25 on the TSSs of *JAZ8* and *ERF1*. In the absence of JA-Ile, MED25 enrichment levels on the TSSs of *JAZ8* and *ERF1* were slightly reduced in *coi1-2* in comparison to the WT (Fig. 3*D*). By contrast, in the presence of JA-Ile, MED25 enrichment levels on the TSSs of *JAZ8* and *ERF1* were greatly reduced in *coi1-2* (Fig. 3*D*). These results, together with the finding that the *coi1-2* mutation showed negligible effect on MED25 protein levels (Fig. S3*B*), indicate an important role for COI1 in JA-Ile–induced recruitment of MED25 to MYC2 target promoters.

Collectively, the above results reveal important functions of MED25 for linking COI1 to MYC2 target promoters in the resting stage, and thereby facilitating COI1-dependent degradation of JAZ repressors in response to hormone elicitation. These two aspects of mechanistically related functions favor the hormone-induced activation of MYC2.

MED25 Interacts with HAC1, Which Is also Enriched on the Promoters of *JAZ8* and *ERF1*. To further explore the significance of the MED25–COI1 interaction on MYC2 target promoters, we tested the possible interaction of MED25 with histone modification enzymes whose activities are associated with active transcription. Among the three *Arabidopsis* CREB-binding protein (CBP)–like proteins that exhibit histone acetyltransferase activity (31–33), MED25 preferentially interacted with HAC1, but not with HAC5 and HAC12, in Y2H assays (Fig. 4*A*). Domain mapping with Y2H assays indicated that the middle domain (MD), together with the activator-interacting domain (ACID) of MED25 (21) and the HAC1 fragment containing the transcription adaptor putative Zinc finger (TAZ)-type Zinc finger (Znf-TAZ) domain (31, 33), is involved in the MED25–HAC1 interaction (Fig. S4).

In co-IP assays using *MED25-myc* plants and an anti-HAC1 antibody, endogenous HAC1 could be pulled down by MED25-myc (Fig. 4*B*). In LCI assays, *N. benthamiana* cells cotransformed with MED25 fused to nLUC (MED25-nLUC) and HAC1 fused to cLUC (cLUC-HAC1) displayed strong fluorescence signals, whereas those cotransformed with MED25-nLUC and cLUC or cLUC-HAC1 and nLUC displayed no signal (Fig. 4*C*), confirming that the MED25–HAC1 interaction occurred in vivo.

To determine whether HAC1 is also recruited to the promoters of *JAZ8* and *ERF1*, we performed ChIP-qPCR experiments with transgenic plants expressing functional *HAC1-GFP* fusion (Fig. S5) and an anti-GFP antibody. In the absence of JA-Ile treatment, HAC1 was mainly enriched on the TSSs of *JAZ8* and *ERF1* (Fig. 4 *D* and *E*). In response to JA-Ile treatment, HAC1 enrichment on the TSS of *JAZ8* was obviously elevated at 15 min, reaching a peak at 30 min. By contrast, JA-Ile–triggered induction of HAC1 enrichment on the TSS of *ERF1* occurred later (Fig. 4 *D* and *F*). These results indicate that, similar to MED25 and COI1, HAC1 is also enriched on the promoters of *JAZ8* and *ERF1*, and this enrichment is regulated by JA-Ile stimulation.

Depletion of HAC1 Affects JA-Responsive Gene Expression and Impairs H3K9ac Levels of *JAZ8* and *ERF1* Promoters. To determine the biological significance of the MED25–HAC1 interaction, we compared JA-responsive gene expression between the WT and the *hac1-4* mutant (34). JA-Ile–induced expression of *VEGETATIVE STORAGE PROTEIN 1* (*VSP1*), a marker gene for the JA-regulated wound response (35) (Fig. S1), exhibited a slight yet significant reduction in *hac1-4* expression (Fig. 5*A*). By contrast, the JA-Ile–induced expression of the plant defensin gene *PDF1.2*, a marker gene for the JA-regulated pathogen response (36) (Fig. S1), was markedly reduced in this mutant (Fig. 5*A*). These results suggest that HAC1 preferentially affects pathogen-responsive

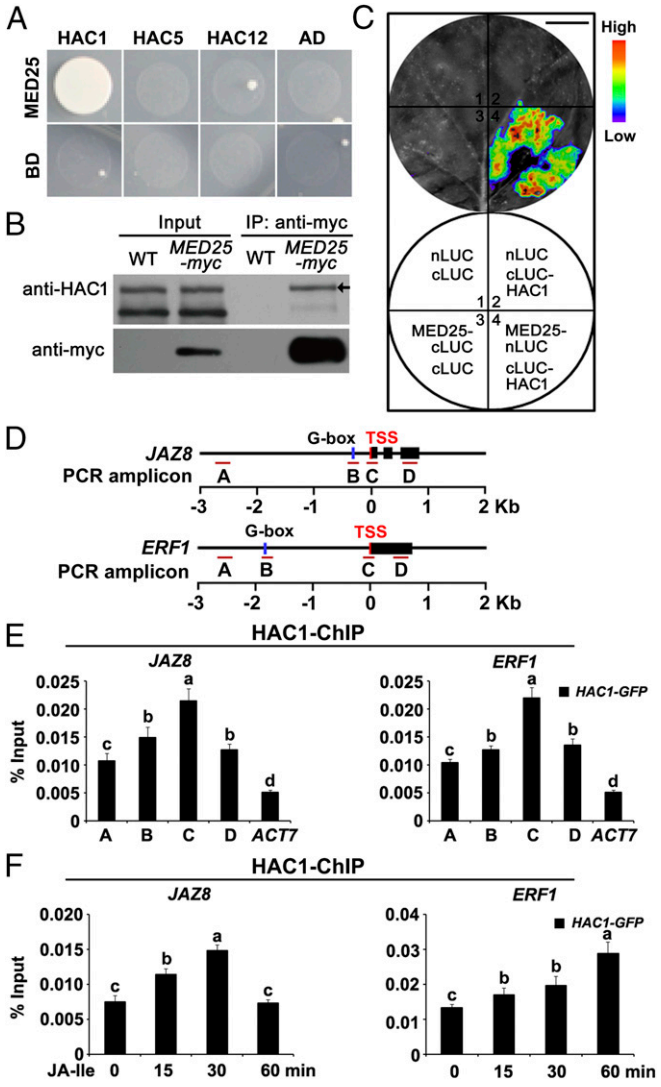

Fig. 4. MED25 interacts with HAC1, which is recruited to the promoters of *JAZ8* and *ERF1*. (*A*) Y2H assays showing that MED25 interacts with HAC1 but not HAC5 and HAC12. Transformed yeast strains were plated on SD medium lacking His, Ade, Leu, and Trp (SD/-4). (*B*) Co-IP assay of MED25 with HAC1. Proteins extracted from WT and *MED25-myc* plants were immunoprecipitated using anti-myc antibody and immunoblotted using anti-HAC1 antibody. The arrow indicates the position of HAC1. (*C*) LCI assays showing that MED25 interacts with HAC1. (*Top*) LUC images of *N. benthamiana* leaves coinfiltrated with the different construct combinations are shown in the lower quadrant of the circle. The pseudocolor bar shows the range of luminescence intensity. (Scale bar, 1 cm.) (*D*) Schematic diagrams of *JAZ8*, *ERF1*, and PCR amplicons indicated as letters A–D used for ChIP-qPCR. (*E*) ChIP-qPCR showing enrichment of HAC1 on the chromatin of *JAZ8* and *ERF1*. Chromatin of *HAC1-GFP* plants was immunoprecipitated using anti-GFP antibody. (*F*) ChIP-qPCR showing enrichment of HAC1 on the TSSs of *JAZ8* and *ERF1* upon JA-Ile stimulation. *HAC1-GFP* plants were treated with 30 μM JA-Ile for the indicated durations before cross-linking, and the chromatin of each sample was immunoprecipitated using anti-GFP antibody. For *E* and *F*, the precipitated DNA was quantified by qPCR, and DNA enrichment is displayed as a percentage of input DNA. *ACT7* was used as a nonspecific binding site. Error bars indicate the SD of three independent experiments ($n = 3$). ANOVA was performed for statistical analysis; bars with different letters are significantly different from each other ($P < 0.01$).

genes. Not surprisingly, JA-Ile–induced expression of MYC2 direct target genes, including *JAZ8* and *ERF1* (Fig. S1), was significantly reduced in *hac1-4* (Fig. 5*A*).

To evaluate the impact of HAC1 on JA-regulated gene expression on a genome-wide scale, we performed RNA-sequencing (RNA-seq) experiments to compare the transcriptome profiles between WT and *hac1-4* seedlings treated with or without JA-Ile (*Materials and Methods*). Quality assessment of the RNA-seq data is shown in Fig. S6 *A* and *B*. We identified 3,354 genes that were up-regulated by JA-Ile at any time point (1 or 24 h) in WT [fold change > 1.5, false discovery rate (FDR)-adjusted $P <$ 0.05). We also identified 2,010 genes whose expression was significantly reduced in *hac1-4* compared with the WT at any time point after JA-Ile treatment (fold change > 1.5, FDR-adjusted $P <$ 0.05) (Fig. S6*C* and Dataset S1). Comparison of these two sets of genes led to the identification of 890 genes showing significantly reduced expression in JA-Ile–treated *hac1-4* seedlings compared with JA-Ile–treated WT seedlings (fold change > 1.5, FDR-adjusted $P < 0.05$) (Fig. S6*C* and Dataset S2). Thus, HAC1 is involved in the activation of around 26.5% (i.e., 890 of 3,354) of the JA-Ile–up-regulated genes. These 890 genes were defined as HAC1– and JA-Ile–co–up-regulated genes. Gene ontology (GO) analysis indicated that these genes are enriched in pathways related to JA response, wounding response, and other defense responses (Dataset S2). The top 10 enriched biological processes are shown in Fig. S6*E*. Not surprisingly, many well-characterized JA-inducible genes were identified as HAC1– and JA-Ile–co–up-regulated genes (Fig. 5*B* and Dataset S2). This list includes genes involved in JA metabolism, JA signaling, and JA-induced defense responses (Fig. 5*B*).

In parallel, we identified 3,305 genes that were down-regulated by JA-Ile in WT (fold change > 1.5, FDR-adjusted $P < 0.05$). We also identified 535 genes whose expression was significantly higher in *hac1-4* compared with the WT at any time point after JA-Ile treatment (fold change > 1.5, FDR-adjusted $P < 0.05$) (Fig. S6*D* and Dataset S3). Comparison of these two sets of genes led to the identification of 153 genes that were less repressed in JA-Ile–treated *hac1-4* seedlings compared with JA-Ile–treated WT seedlings (fold change > 1.5, FDR-adjusted $P < 0.05$) (Fig. S6*D* and Dataset S4). Thus, HAC1 is involved in the repression of around 4.6% (i.e., 153 of 3,305) of the JA-Ile–down-regulated genes. These 153 genes were defined as HAC1– and JA-Ile–co–down-regulated genes. GO analysis indicated that this group of genes does not show significant enrichment in any biological processes (Dataset S4). Taken together, our RNA-seq experiments indicated that HAC1 mainly acts as a coactivator of JA-induced gene expression.

To determine the substrate specificity of HAC1 for histone modifications, we compared the histone acetylation profiles of the WT and *hac1-4*. In response to JA-Ile treatment, global levels of H3ac, but not H4ac, were reduced in *hac1-4* to a greater extent than in the WT (Fig. 5*C*), indicating that HAC1 mainly affects H3ac. Next, we examined the site specificity of HAC1 for H3ac and found that, in response to JA-Ile treatment, global H3K9ac levels were reduced in *hac1-4* to a greater extent than in the WT (Fig. 5*C*), indicating that HAC1 mainly affects H3K9ac, a chromatin mark that is typically associated with actively transcribed genes (32). ChIP-qPCR assays revealed that, in WT plants, H3K9ac was primarily enriched on the TSSs of *JAZ8* and *ERF1* (Fig. S7 *A* and *B*), and that H3K9ac enrichment on these TSSs was increased by JA-Ile treatment (Fig. S7 *A* and *C*), indicating that the H3K9ac enrichment pattern on MYC2 target promoters is similar to that of HAC1 and MED25. In *hac1-4* mutants, JA-Ile–induced H3K9ac levels on the TSSs of *JAZ8* and *ERF1* were dramatically reduced in comparison to the WT (Fig. 5 *D* and *E*), confirming that the defective JA-responsive gene expression in *hac1-4* is linked to impaired H3K9ac of these MYC2 target promoters. Consistently, JA-Ile–induced enrichment of the conserved C-terminal domain of Pol II on the TSSs of *JAZ8* and *ERF1* was obviously reduced in *hac1-4* in comparison to the WT (Fig. 5 *D* and *F*), indicating that HAC1 function is required for the recruitment of Pol II to MYC2 target promoters.

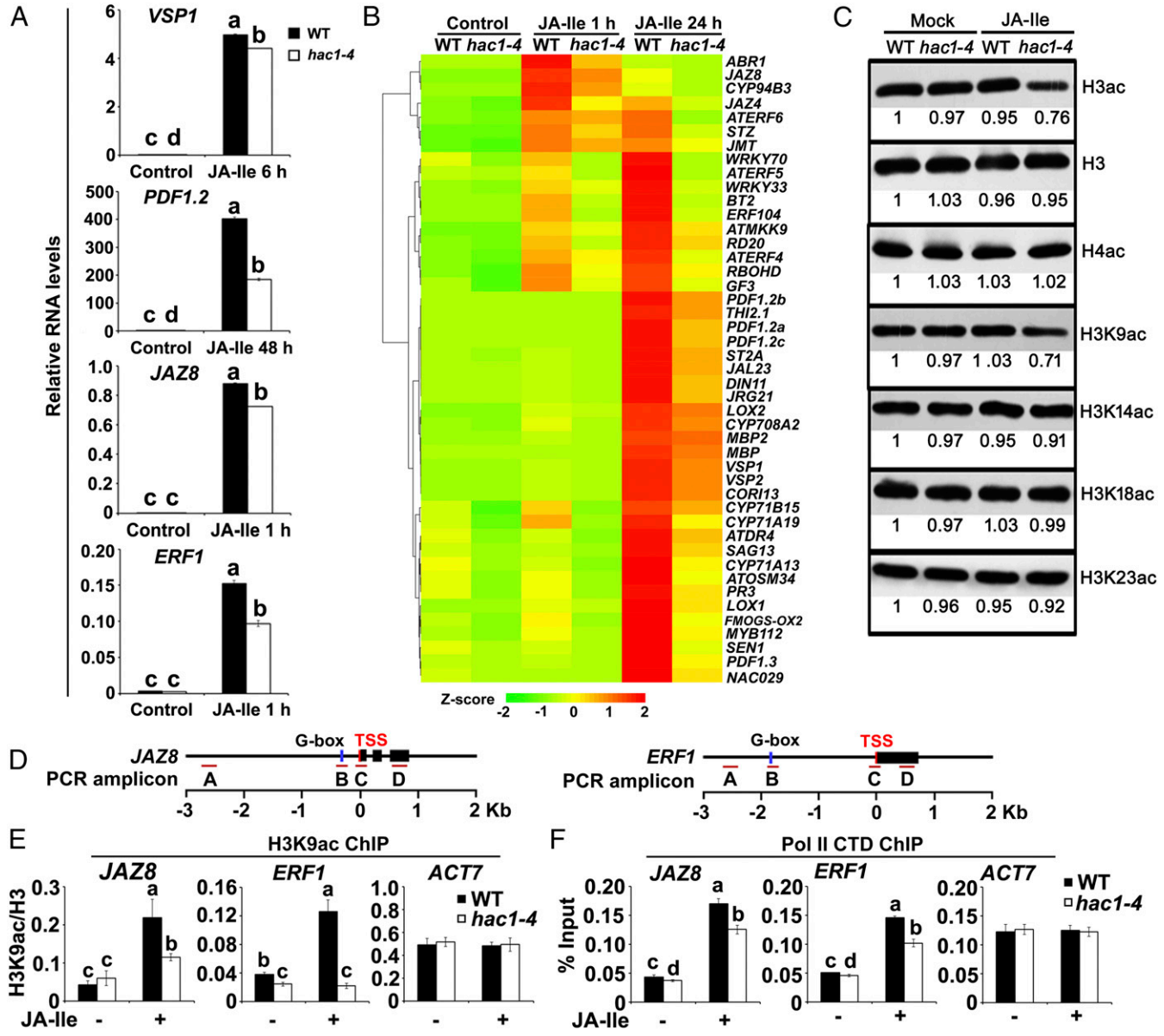

Fig. 5. Depletion of HAC1 impairs JA-responsive gene expression and reduces H3K9ac accumulation on the promoters of *JAZ8* and *ERF1*. (*A*) qRT-PCR showing JA-Ile–induced expression of indicated genes in WT and *hac1-4*. WT and *hac1-4* plants were treated with or without 30 μM JA-Ile for the indicated durations. Error bars indicate SD of three independent experiments ($n = 3$). ANOVA was performed for statistical analysis; bars with different letters are significantly different from each other ($P < 0.01$). (*B*) Hierarchical clustering of the selected JA-Ile–responsive genes showing reduced expression in *hac1-4* plants at the indicated time points. (*C*) Protein gel analyses showing global H3K9ac levels in the WT and *hac1-4* in response to JA-Ile. WT and *hac1-4* plants were treated with or without 30 μM JA-Ile for 30 min before extraction of nuclear proteins for immunoblotting using the indicated antibodies. Bands were quantified using ImageJ. (*D*) Schematic diagrams of *JAZ8*, *ERF1*, and PCR amplicons indicated as letters A–D used for ChIP-qPCR. (*E*) ChIP-qPCR assays showing that *hac1-4* impairs the enrichment of H3K9ac on the TSS regions of *JAZ8* and *ERF1* in response to JA-Ile. WT and *hac1-4* plants were treated with or without 30 μM JA-Ile for 30 min before cross-linking, and chromatin of each sample was immunoprecipitated using anti-H3 and anti-H3K9ac antibodies. Precipitated DNA was quantified by qPCR, and H3K9ac levels are normalized to H3. (*F*) ChIP-qPCR assays showing that *hac1-4* impairs the enrichment of Pol II C-terminal domain (CTD) on the TSS regions of *JAZ8* and *ERF1* in response to JA-Ile stimulation. WT and *hac1-4* plants were treated with or without 30 μM JA-Ile for 30 min before cross-linking, and chromatin of each sample was then immunoprecipitated using anti-Pol II CTD antibody. Precipitated DNA was quantified by qPCR, and DNA enrichment is displayed as a percentage of input DNA. For *E* and *F*, *ACT7* was used as a nonspecific binding site. Error bars indicate SD of three independent experiments ($n = 3$). ANOVA was performed for statistical analysis; bars with different letters are significantly different from each other ($P < 0.01$).

Enrichment and Function of HAC1 on the Promoters of *JAZ8* and *ERF1* Depend on the Function of COI1 and MED25. To understand how HAC1, a general histone modification enzyme, selectively regulates H3K9ac of JA-responsive genes, we asked whether depletion of COI1 affects the enrichment levels of HAC1 itself and HAC-dependent H3K9ac on the promoters of *JAZ8* and *ERF1*. ChIP-qPCR assays revealed that, in *coi1-2*, JA-Ile–induced enrichment of HAC1-GFP on the TSSs of *JAZ8* and *ERF1* was substantially lower than in the WT (Fig. 6 *A* and *B*). Consistent with this, H3K9ac levels on these TSSs were also dramatically reduced in *coi1-2* (Fig. 6 *A* and *C*). Given that COI1 did not affect HAC1 protein levels (Fig. S8), these results demonstrate that the enrichment and function of HAC1 on MYC2 target promoters depend on COI1.

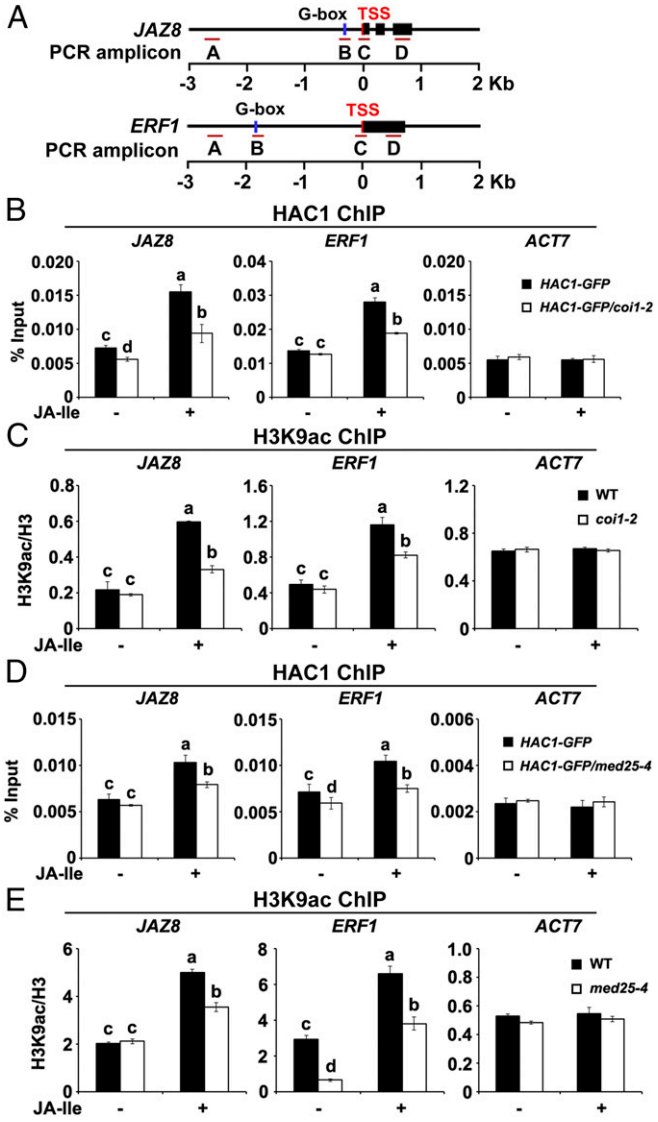

Fig. 6. Depletion of COI1 or MED25 impairs the function of HAC1 on the promoters of *JAZ8* and *ERF1*. (*A*) Schematic diagrams of *JAZ8*, *ERF1*, and PCR amplicons indicated as letters A–D used for ChIP-qPCR. (*B*) ChIP-qPCR assays showing that *coi1-2* impairs the enrichment of HAC1 on the TSS regions of *JAZ8* and *ERF1* in response to JA-Ile. *HAC1-GFP* and *HAC1-GFP/coi1-2* plants were treated with or without 30 μM JA-Ile for 30 min before cross-linking, and chromatin of each sample was then immunoprecipitated using anti-GFP antibody. Precipitated DNA was quantified by qPCR, and DNA enrichment is displayed as a percentage of input DNA. (*C*) ChIP-qPCR assays showing that *coi1-2* impairs the enrichment of H3K9ac on the TSS regions of *JAZ8* and *ERF1* in response to JA-Ile. WT and *coi1-2* plants were treated with or without 30 μM JA-Ile for 30 min before cross-linking, and chromatin of each sample was then immunoprecipitated using anti-H3 and anti-H3K9ac antibodies. Precipitated DNA was quantified by qPCR, and H3K9ac levels are normalized to H3. (*D*) ChIP-qPCR assays showing that *med25-4* impairs the enrichment of HAC1 on the TSS regions of *JAZ8* and *ERF1* in response to JA-Ile. *HAC1-GFP* and *HAC1-GFP/med25-4* plants were treated with or without 30 μM JA-Ile for 30 min before cross-linking, and chromatin of each sample was then immunoprecipitated using anti-GFP antibody. Precipitated DNA was quantified by qPCR, and DNA enrichment is displayed as a percentage of input DNA. (*E*) ChIP-qPCR assays showing that *med25-4* impairs the enrichment of H3K9ac on the TSS regions of *JAZ8* and *ERF1* in response to JA-Ile. WT and *med25-4* plants were treated with or without 30 μM JA-Ile for 30 min before cross-linking, and chromatin of each sample was then immunoprecipitated using anti-H3 and anti-H3K9ac antibodies. Precipitated DNA was quantified by qPCR, and H3K9ac levels are normalized to H3. For *B*–*E*, *ACT7* was used as a nonspecific binding site. Error bars indicate SD of three independent experiments ($n = 3$). ANOVA was performed for statistical analysis; bars with different letters are significantly different from each other ($P < 0.01$).

Parallel ChIP-qPCR assays revealed that, in *med25-4*, JA-Ile–induced enrichment of HAC1-GFP (Fig. 6 *A* and *D*) and HAC-dependent H3K9ac (Fig. 6 *A* and *E*) on the TSSs of *JAZ8* and *ERF1* was greatly reduced in comparison to the WT. Considering that MED25 does not affect the protein levels of HAC1 (Fig. S8), our results demonstrate that the enrichment and function of HAC1 on MYC2 target promoters also depend on MED25.

MED25 and JAZ1 Simultaneously Interact with MYC2 in the Resting Stage, and Hormone Elicitation Shows Differential Effects on the MED25–COI1 Interaction and the MED25–MYC2 Interaction. The above results, together with our recent finding that MED25 physically and functionally interacts with MYC2 (21), raise a possibility that MED25 and JAZ proteins could be bound simultaneously to MYC2 in the resting stage. To test this, we performed co-IP experiments by transiently expressing combinations of MYC2-myc, Flag epitope (Flag)-tagged MED25 (MED25-Flag), and JAZ1-GFP in *N. benthamiana* leaves. As expected, both MED25-Flag and MYC2-myc were coimmunoprecipitated by JAZ1-GFP (Fig. 7*A*), indicating that MED25-Flag and JAZ1-GFP could be bound simultaneously to MYC2-myc in the resting stage.

To substantiate these observations, we conducted co-IP experiments with transgenic *Arabidopsis* plants expressing JAZ1-GFP (Fig. S9) and anti-MYC2 or anti-MED25 (21) antibody. Again, endogenous MYC2 and MED25 could be coimmunoprecipitated by JAZ1-GFP (Fig. 7*B*). Together, these results demonstrate that the MED25 coactivator and JAZ repressors could be bound simultaneously to the master transcription factor MYC2 and suggest the existence of a JAZ–MYC2–MED25 ternary complex. In this context, our study reveals two aspects of closely related functions for MED25 in regulating JA signaling. First, in the resting stage, it brings COI1 to MYC2 target promoters through physical interaction. Second, upon JA-Ile elicitation, it cooperates with both genetic and epigenetic regulators to activate MYC2-dependent gene transcription. In this perspective, we speculate that JA-Ile elicitation might affect the MED25–COI1 interaction as well as the MED25–MYC2 interaction.

To determine whether JA-Ile treatment affects the MED25–COI1 interaction *in planta*, we treated *COI1-myc* plants with JA-Ile and examined the ability of COI1-myc to pull down native MED25. Upon JA-Ile treatment, the ability of COI1-myc to pull down MED25 was slightly reduced at 15 min and dramatically reduced at 60 min (Fig. 7*C*), suggesting that the MED25–COI1 interaction is weakened upon JA-Ile elicitation. This observation is consistent with the above results that the enrichment of COI1 on MYC2 target promoters is decreased upon JA-Ile elicitation (Fig. 1*D*).

Similarly, we treated *MYC2-myc* plants (37) with JA-Ile and examined the ability of MYC2-myc to pull down native MED25. Upon JA-Ile treatment, the ability of MYC2-myc to pull down MED25 was slightly increased at 15 min and obviously increased at 60 min (Fig. 7*D*), suggesting that, in contrast to the above MED25–COI1 interaction case, the MED25–MYC2 interaction is enhanced upon JA-Ile elicitation. This observation is consistent with the above results that the enrichment of MED25 on MYC2 target promoters is increased upon JA-Ile elicitation (Fig. 1*E*) and the recent finding that JAZ repressors compete with MED25 for interaction with MYC transcription factors (22).

In summary, we propose a working model (Fig. 7*E*). In the absence of JA-Ile, MED25 and JAZ proteins could be bound to MYC2, and therefore form a JAZ–MYC2–MED25 ternary complex. At this stage, JAZ proteins do not interact with COI1 but interact with MYC2, and thereby function as transcription repressors. It is noteworthy that, at this stage, the MED25–COI1 interaction is relatively strong, whereas the MED25–MYC2 interaction is relatively weak because JAZ

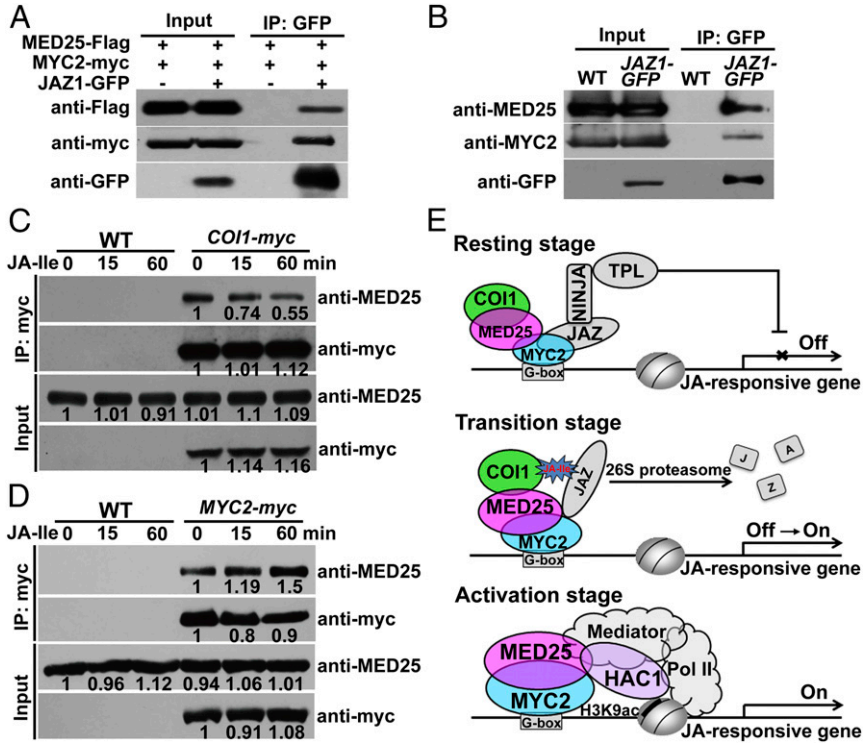

Fig. 7. MED25 cooperates with both genetic and epigenetic regulators in regulating hormone-induced activation of MYC2. (*A*) Co-IP assays of MED25, MYC2, and JAZ1 in *N. benthamiana*. MED25-Flag and MYC2-myc were transiently coexpressed with or without JAZ1-GFP in *N. benthamiana* leaves. Protein extracts were immunoprecipitated using anti-GFP antibody and analyzed by immunoblotting with anti-Flag, anti-myc, and anti-GFP antibodies. (*B*) Co-IP assay of MED25, MYC2, and JAZ1 in *Arabidopsis*. Proteins extracted from WT and *JAZ1-GFP* plants were immunoprecipitated using anti-GFP antibody and immunoblotted using anti-MED25 and anti-MYC2 antibodies. (*C*) Co-IP assay between MED25 and COI1. WT and *COI1-myc* plants were treated with or without 30 μM JA-Ile for the indicated times. Protein from each sample was immunoprecipitated using anti-myc antibody and immunoblotted using anti-MED25 antibody. Bands were quantified using ImageJ. (*D*) Co-IP assay between MED25 and MYC2. WT and *MYC2-myc* plants were treated with or without 30 μM JA-Ile for the indicated times. Protein from each sample was immunoprecipitated using anti-myc antibody and immunoblotted using anti-MED25 antibody. Bands were quantified using ImageJ. (*E*) Proposed working model for the mechanistic roles of MED25 in regulating JA-Ile–induced activation of MYC2. In the resting stage, the MED25–COI1 interaction is relatively strong, whereas the MED25–MYC2 interaction is relatively weak because JAZ repressors compete with MED25 for interaction with MYC transcription factors. Basal levels of MED25 bring COI1 to MYC2 target promoters through physical interaction. In the hormone-mediated transition stage, JA-Ile acts as molecular glue to promote the formation of the COI1–JAZ coreceptor complex, which leads to proteasome-dependent degradation of JAZ repressors. During this stage, the MED25–COI1 interaction is weakened in a hormone-dependent manner, whereas the MED25–MYC2 interaction is enhanced in a hormone-dependent manner. Upon degradation of JAZ repressors, MED25 interacts with MYC2 and recruits HAC1 as well as Pol II to the promoters of MYC2 target genes, and thereby activate their expression. NINJA, Novel Interactor of JAZ; TPL, TOPLESS.

repressors interfere with the interaction of MED25 with MYC transcription factors (22). Basal levels of MED25 bring COI1 to MYC2 target promoters through physical interaction. In response to stress or developmental cues, plants produce JA-Ile, which acts as molecular glue to promote the formation of the COI1–JAZ coreceptor complex (17). During this stage, JAZ proteins transiently switch the repressor function into a coreceptor function in a JA-Ile–dependent manner (22). Coincidently, the MED25–COI1 interaction is weakened, whereas the MED25–MYC2 interaction is enhanced. The formation of the COI1–JAZ coreceptor complex will eventually lead to proteasome-dependent degradation of JAZ proteins. Upon JAZ degradation, MED25 shows enhanced interaction with MYC2 and recruits HAC1 as well as Pol II to the promoters of MYC2 target genes, and thereby activates their expression. This model highlights the mechanistic function of MED25 in transmitting the hormone-specific signals from the JA-Ile receptor protein COI1 to activate MYC2-regulated gene transcription.

Discussion

The recent biochemical isolation of the plant Mediator complex from *Arabidopsis* (19) significantly facilitated the characterization of the diverse functions of individual plant Mediator subunits (38–40). However, a thorough mechanistic understanding of plant Mediator function remains elusive. In particular, it is unclear how specific Mediator subunits integrate regulatory signals from diverse internal or external cues, as well as how they convey these signals to the Pol II general transcription machinery to regulate the expression of specific target genes.

In this work, we show that MYC2 forms a ternary complex together with the MED25 coactivator and the JAZ repressors in the resting stage and reveal functions of MED25 in regulating JA signaling. First, in the resting stage, MED25 brings COI1 to MYC2 target promoters through physical interaction, and thereby facilitates COI1-dependent degradation of JAZ repressors. Second, upon hormone elicitation, MED25 brings the coactivator HAC1 to MYC2 target promoters and thereby regulates H3K9ac, which favors gene activation. These findings, together with our previous observation that MED25 bridges MYC2 and Pol II for preinitiation complex assembly during JA-regulated gene transcription (21), advance the overall understanding about how the JA signals are transmitted to activate gene expression.

However, several aspects of the molecular details by which the multitalented MED25 executes these versatile functions remain to be further explored. An important aspect concerns the functional significance of MED25–COI1 interaction for JA signaling. The COI1 enrichment on MYC2 target promoters is relatively

high in the resting stage and exhibits a rapid reduction upon JA-Ile treatment (Fig. 1*D*). The findings that the MED25 enrichment on MYC2 target promoters is relatively low in the resting stage and exhibits a rapid induction upon JA-Ile treatment (Fig. 1*E*) and that depletion of MED25 reduces COI1 enrichment on MYC2 target promoters in the resting stage and impairs COI1-dependent degradation of JAZ proteins in response to JA-Ile treatment (Figs. 2*E* and 3*C*) support the notion that the major functional relevance of MED25–COI1 interaction in JA signaling is to bring COI1 to MYC2 target promoters in the resting stage and to facilitate COI1-dependent degradation of JAZ proteins upon JA-Ile elicitation. We reasoned that JA-Ile–triggered reduction of the COI1 enrichment on MYC2 target promoters could be due to the finding that JA-Ile stimulates the formation of the COI1–JAZ coreceptor complex, and thereby promotes JAZ degradation, since JAZ degradation might lead to disassociation of COI1 from MYC2 target promoters.

Recent structural studies of the COI1–JAZ coreceptor complex (17) and the MYC–JAZ repression complex (22) have revealed that hormone-mediated protein interaction is a major strategy governing JA-Ile perception and JAZ repression of MYC transcription factors. Here, we show that, in the resting stage, there exists a JAZ–MYC2–MED25 ternary complex and that hormone elicitation triggers extensive changes of protein interactions involving MED25 and the major components of the core JA signaling module. Specifically, the MED25–COI1 interaction is relatively strong in the resting stage and tends to be weakened upon JA-Ile elicitation (Fig. 7*C*). In contrast, the MED25–MYC2 interaction is relatively weak in the resting stage and tends to be enhanced upon JA-Ile elicitation (Fig. 7*D*). These results suggest that hormone elicitation exerts differential effects on the MED25–COI1 interaction and the MED25–MYC2 interaction. In light of the observations that JAZ proteins undergo pronounced conformational changes (through the Jas motif) before and after hormone elicitation, and therefore switch their interactions with MYC transcription factors (in the absence of JA-Ile) or with COI1 (in the presence of JA-Ile) (17, 22), it is reasonable to speculate that the JA-Ile–dependent alterations of the MED25–COI1 interaction or the MED25–MYC2 interaction may be coupled with conformational changes of the COI1-JAZ coreceptor and/or their interacting MYC2 and MED25. Future structural studies should provide insight into the mechanism by which MED25 changes its interaction with the hormone receptor COI1 or with the master transcription factor MYC2. Similarly, structural studies promise to provide insight into the mechanism by which MED25 facilitates JA-Ile–induced degradation of JAZ proteins by enhancing COI1–JAZ interaction.

In addition to the JA-Ile receptor protein COI1, we found that MED25 physically and functionally interacts with the evolutionarily conserved coactivator HAC1 (31–33). Our results support a scenario by which, in response to JA-Ile elicitation, MED25 recruits HAC1 to MYC2 target promoters, and thereby regulates H3K9ac. Notably, we found that JA-Ile treatment led to obvious reduction of H3ac and H3K9ac levels in *hac1-4* but not in the WT (Fig. 5*C*). The distinct action modes of JA-Ile on H3ac and H3K9ac levels between *hac1-4* and the WT can be explained by previous observations that JA could activate the expression of *HISTONE DEACETYLASE6* (*HDA6*) and *HDA19*, two histone deacetylase genes that affect H3ac levels (41, 42). Considering that histone acetylation and deacetylation play an important role in the regulation of gene expression, it is reasonable to speculate that plants have evolved a mechanism to keep a dynamic balance between histone acetyltransferase and histone deacetylase activities. Under this scenario, JA-Ile may simultaneously up-regulate the activities of histone acetyltransferases and histone deacetylases, thereby maintaining a steady-state level of H3ac and H3K9ac in the WT. In *hac1-4* mutants, the JA-Ile–induced up-regulation of H3ac or H3K9ac through HAC1 is blocked, but JA-Ile still reduces H3ac and H3K9ac through activating *HDA6* and *HDA19*. These effects likely lead to reduced H3ac and H3K9ac levels of *hac1-4* in response to JA-Ile treatment.

Our study suggests two related directions for future exploration. First, recent advances have led to the surprising discovery that, in addition to JA, the main receptors for several other plant hormones, including auxin (43), gibberellin (44), abscisic acid (45), and salicylic acid (46), are localized in the nucleus and directly linked to hormone-regulated gene transcription. An important future direction will be to determine whether the signaling paradigm described herein can be extended to other hormones whose receptors are localized in the nucleus. If this is the case, it will be critical to study the principles that govern the general and/or context-specific functions of Mediator, and especially to determine how a single multiprotein complex can perform so many diverse tasks.

Moreover, this study reveals several aspects of striking analogy between plant and animal nuclear hormone receptor (NR) systems. First, the overall protein domain composition of the plant MED25 is largely similar to that of its animal counterpart (19, 21). Second, in addition to interacting with MYC2, the plant MED25 interacts with the JA-Ile receptor protein COI1; in an analogous manner, the human MED25 engages in a ligand-dependent interaction with retinoic acid receptor (RAR) and several other NRs, which are themselves transcription factors (26, 28, 29, 47). Thus, in term of their interaction with MED25, the JA-Ile coreceptor complex (SCF^{COI1}–JAZs), together with the master transcription factor MYC2, resembles the NR system of metazoans (26, 28, 29, 47). Third, we show here that the plant MED25 cooperates with HAC1, an *Arabidopsis* ortholog of the well-studied animal histone acetyltransferase CBP, for JA-Ile–triggered activation of MYC2; in a similar manner, the animal MED25 also cooperates with CBP for RAR activation (48). Thus, the plant MED25 and its animal counterpart cooperate with similar epigenetic regulators in distinct signaling pathways. Animal Mediator was first biochemically isolated as a thyroid hormone receptor-associated protein (TRAP) complex (47), and has been shown to be an indispensable NR-interacting coactivator (26, 28, 29, 47). These previous observations, together with our findings, support a scenario in which plants and animals have evolved distinct, but nonetheless largely similar, mechanisms for NR activation at the level of transcriptional regulation. Future studies aimed at elucidating the roles of plant Mediator in integrating different plant hormone responses should provide deeper insight into these mechanisms.

Materials and Methods

Plant Materials and Growth Conditions. *A. thaliana* ecotype Columbia was used as the WT. The following plant materials used in this study were previously described: *coi1-2* (9), *med25-4* (21), *hac1-4* (34), *COI1-myc* (9), *MED25-myc* (21), *MYC2-myc* (37), and *JAZ1-GUS* (4). Further details can be found in *SI Materials and Methods*.

Plasmid Construction and Plant Transformation. To construct *pHAC1:HAC1-GFP* (*HAC1-GFP*), the coding sequence of *GFP* was amplified and cloned into pCAMBIA1300 to obtain *pCAMBIA1300-GFP*, and the promoter and coding sequence of *HAC1* were amplified and subsequently cloned into *pCAMBIA1300-GFP* to obtain *pHAC1:HAC1-GFP*. Primers used for plasmid construction are listed in Table S1. Further details can be found in *SI Materials and Methods*.

Details of additional experimental procedures, such as Y2H assay, antibody generation, protein expression and in vitro pull-down assays, co-IP assays, nuclear protein extraction and global histone acetylation analysis, ChIP-qPCR assays, sequential ChIP, qRT-PCR assay, RNA-seq and data analysis, LCI assay, and quantitative GUS activity assay, can be found in *SI Materials and Methods*.

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