MYB96 recruits the HDA15 protein to suppress negative regulators of ABA signaling in Arabidopsis

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Unlike activation of target genes in response to abscisic acid (ABA), how MYB96 transcription factor represses ABA-repressible genes to further enhance ABA responses remains unknown. Here, we show MYB96 interacts with the histone modifier HDA15 to suppress negative regulators of early ABA signaling. The MYB96-HDA15 complex co-binds to the promoters of a subset of RHO GTPASE OF PLANTS (ROP) genes, ROP6, ROP10, and ROP11, and represses their expression by removing acetyl groups of histone H3 and H4 from the cognate regions, particularly in the presence of ABA. In support, HDA15-deficient mutants display reduced ABA sensitivity and are susceptible to drought stress with derepression of the ROP genes, as observed in the myb96-1 mutant. Biochemical and genetic analyses show that MYB96 and HDA15 are interdependent in the regulation of ROP suppression. Thus, MYB96 confers maximal ABA sensitivity by regulating both positive and negative regulators of ABA signaling through distinctive molecular mechanisms.
Essential plants have evolved a remarkable ability to adapt to the environment, which is largely mediated by abscisic acid (ABA)-dependent signaling pathways. ABA is recognized by plasma membrane-localized cell receptors, including PYRABACTIN RESISTANCE/PYRABACTIN RESISTANCE-LIKE/REGULATORY COMPONENT OF ABSCISIC ACID RECEPTOR (PYR/PYL/RCAR) family proteins. These receptors constitute early ABA signaling modules, together with PROTEIN PHOSPHATASE 2Cs (PP2Cs) and subfamily 2 SNF1-RELATED PROTEIN KINASEs (SnRK2s). In the presence of ABA, the PYR receptors inhibit PP2C activity and thereby derepress the catalytic functions of SnRK2s. A number of transcription factors from bZIP, NAC, MYC, and MYB families are then activated to stimulate downstream ABA responses.

The R2R3-type MYB96 transcription factor is a master transcriptional regulator that mediates a variety of plant responses to ABA, including seed germination, drought tolerance, stomatal conductance, lateral root development, hormone biosynthesis, anthocyanin accumulation, and cuticular wax biosynthesis. The ABA-inducible MYB96 transcription factor binds to the promoters of many ABA-responsive genes and usually activates expression to optimize plant growth and fitness under unfavorable conditions. Notably, like many plant transcription factors, MYB96 has also been suspected to bind to the promoters of ABA-repressed genes, such as negative regulators of ABA signaling, and sometimes functions as a transcriptional repressor to further enhance plant responses to environmental stresses. However, it remains unclear how MYB96 represses gene expression and what trans-factor mediates MYB96-dependent gene repression.

Two classes of signaling G proteins exist in eukaryotes: heterotrimeric G proteins and monomeric Ras/Ras-like small GTPases. In particular, Rho family GTPases, which belong to the Ras superfamily, are likely major molecular switches that mediate diverse cellular responses to multiple extracellular signals. A subset of ROP GTPases plays negative roles in ABA responses in Arabidopsis. For instance, overexpression of ROP2 and ROP6 reduces ABA sensitivity during seed germination and stomatal closure. The ROP10 mutation enhances ABA-induced suppression of seed germination, primary root growth, and lateral root formation with increased expression of ABA-responsive genes. ROP11 is also a negative regulator of multiple ABA responses, including seed germination, early seedling growth, stomatal closure, and drought responses. Further, ROP11 acts at an early step of ABA signaling by interacting with ABI1, a receptor component, at the plasma membrane and protecting ABI1 phosphatase activity from inhibition by the ABA receptor complex. The ROP signaling networks coordinate many downstream pathways to ensure large repertoires of output responses.

In this study, we find that MYB96 negatively regulates a subset of ROP genes, which repress ABA signaling at the early stages of signal transduction. Repression of ROP genes by MYB96 requires the histone modifier HDA15. The MYB96 transcription factor specifically binds to promoters of the ROP6, ROP10, and ROP11 genes and recruits the HDA15 protein to catalyze H3 and H4 deacetylation at cognate sites. While MYB96 alone acts as a transcriptional activator, the MYB96–HDA15 complex can perform repressive functions in gene regulation. This results indicate that MYB96 acts as both a transcriptional activator and repressor to enhance ABA signaling.

Results

MYB96 interacts with HDA15. MYB96 is a pivotal regulator of ABA signaling and mediates a variety of physiological responses required for environmental stress adaptation in plants. Consistent with MYB96 being a transcriptional activator, a number of ABA-inducible genes are primary targets of MYB96. However, accumulating evidence suggests that many negative regulators of ABA signaling may also be repressed in the myb96-ox overexpression mutant, suggesting that MYB96 may further enhance ABA responses by directly repressing ABA-repressed genes.

We postulated that MYB96 may recruit additional trans-factor(s) to repress gene expression. To obtain clues to the identities of potential interacting factors, we performed yeast-two-hybrid (Y2H) screening. Preliminary analysis identified the HDA15 protein as an interaction partner of MYB96. To validate this observation, the MYB96 protein fused with the GAL4 activation domain (AD) was coexpressed in yeast cells with the full-length HDA15 protein fused with the GAL4 DNA binding domain (BD). Growth on selective medium showed that MYB96 interacts with HDA15 (Fig. 1a). Deletion constructs further revealed that an N-terminal fragment of MYB96 containing the R2R3-MYB DNA binding domain was responsible for interaction with the N-terminal fragment of HDA15 (Fig. 1b, c). A zinc-finger motif in HDA15 might contribute to mediating the protein–protein interactions. Interactions of MYB96 and HDA15 were mutually specific. No other HDAC protein associated with MYB96 (Supplementary Figure 1). Likewise, other MYB transcription factors involved in ABA responses did not physically associate with HDA15 (Supplementary Figure 2).

To further support the in vivo interactions between MYB96 and HDA15, we conducted bimolecular fluorescence complementation (BiFC) analysis. The HDA15 sequence was translationally fused in-frame to the 5′-end of a gene sequence encoding the N-terminal half of yellow fluorescent protein (YFP), and the MYB96 gene was fused in-frame to the 5′-end of a gene encoding the C-terminal half of YFP (cYFP). The fusion constructs were then transiently coexpressed in Arabidopsis protoplasts. As a result, strong yellow fluorescence was detected in the nucleus for the MYB96–HDA15 combination, while no fluorescence signal was observed when empty vectors were coexpressed (Fig. 1d and Supplementary Figure 3).

We confirmed in planta interactions of MYB96 and HDA15 by coimmunoprecipitation (Co-IP). 35S:MYB96-MYC and 35S:HDA15-GFP constructs were coexpressed in leaf cells of Nicotiana benthamiana by Agrobacterium infiltration. Total protein extracts were immunoprecipitated with an anti-MYC antibody, which mainly pulls down the MYB96-MYC protein. Then immunoblot analysis was performed with an anti-green fluorescent protein (anti-GFP) antibody and the HDA15 fusion protein was detected (Fig. 1e), indicating in planta interactions between MYB96 and HDA15.

HDA15 promotes ABA signaling. Our results raised the possibility that HDA15 is involved in ABA signaling, possibly in connection with MYB96-regulated signaling networks. Thus we obtained T-DNA insertional mutants, hda15-1 and hda15-2, and produced HDA15-overexpressing HDA15-ox transgenic plants (Supplementary Figure 4) to examine ABA sensitivity in germination rate. In the absence of ABA, the germination rate of hda15-deficient mutant and HDA15-ox transgenic seeds was indistinguishable from wild-type seeds (Fig. 2a, b). However, in the presence of ABA, hda15 mutant seeds exhibited reduced sensitivity to ABA (Fig. 2a, b), similar to myb96-1 mutant seeds. In contrast, higher ABA sensitivity was observed in...
**HDA15-ox** and **myb96-ox** transgenic seeds during germination (Fig. 2a, b). The differential sensitivity was more pronounced at higher concentrations of ABA (Supplementary Figure 5). Moreover, plant tolerance to drought stress, which is mainly conferred by ABA32, was also influenced by HDA15 (Fig. 2c). The hda15 mutant plants exhibited higher susceptibility to water deficit, whereas HDA15-ox transgenic plants were more tolerant to drought stress than wild-type plants (Fig. 2c, d), similar to MYB96-misexpressing plants (Supplementary Figure 6), suggesting that HDA15 positively regulates ABA responses.

Consistent with the positive role of HDA15 in ABA responses, the HDA15 gene was induced by ABA treatment (Fig. 2e and Supplementary Figure 7). Abiotic stress factors, including osmotic and cold stress, also promoted HDA15 expression (Supplementary Figure 7). In addition, the expression of some ABA-responsive genes was influenced by HDA15 activity. Even in the absence of exogenous ABA application, HDA15-ox transgenic plants exhibited increased transcript accumulation of ABA-responsive marker genes such as **RESPONSIVE TO DESSICATION 22** (RD22), **RD29A**, and **COLD-REGULATED 47** (COR47)33, whereas the hda15-1 mutant showed reduced transcript accumulation of these genes (Fig. 2f). Furthermore, ABA induction of the **RD** and **COR** genes was also impaired in the hda15-1 and myb96-1 mutants, regardless of plant ages (Fig. 2g and Supplementary Figure 8). These results indicate that HDA15 is an ABA signaling component that promotes ABA responses.

**MYB96 and HDA15 act synergistically in ABA signaling.** Considering that MYB96 interacts with HDA15 and that they have overlapping functions in ABA signaling, MYB96 and HDA15 may act together in the control of ABA sensitivity. We
first examined whether the physical interaction between MYB96 and HDA15 is affected by ABA. pMYB96:MYB96-MYC x pHDA15:HDA15-GFP transgenic Arabidopsis plants were generated and used for Co-IP assays. Co-IP followed by immunoblot analysis revealed that MYB96 and HDA15 form a complex, and interaction was further enhanced as ABA concentration increases (Fig. 3a), suggesting that they work together especially at high concentrations of ABA.

We then analyzed the genetic relationship between MYB96 and HDA15. To this end, the myb96-1hda15-1 double mutant was generated to analyze ABA responses including seed germination and drought tolerance. In the presence of ABA, seed germination...
rate was significantly delayed in wild type, whereas *myb96-1* and hda15-1 mutant seeds exhibited reduced sensitivity to ABA (Fig. 3b). Notably, the *myb96-1hda15-1* double mutant showed further reduction in ABA sensitivity compared to *myb96-1* and hda15-1 seeds (Fig. 3b), particularly at a high concentration of ABA (Fig. 3b). Similarly, the *myb96-1hda15-1* double mutant also exhibited a higher susceptibility to drought stress than *myb96-1* and hda15-1 single mutants (Fig. 3c). The reduced sensitivity to ABA in *myb96-1hda15-1* was supported by expression of the RD and COR genes. ABA induction of RD and COR genes was compromised in *myb96-1* and hda15-1 mutants and more profoundly in the *myb96-1hda15-1* double mutant (Fig. 3d and Supplementary Figure 8). These results indicate that MYB96 and HDA15 act synergistically to confer ABA sensitivity at least in the processes of seed germination and drought tolerance.

**MYB96 recruits HDA15 to the ROP promoters.** Previous microarray data assumed that MYB96 represses some negative regulators of ABA signaling. To test this possibility, we examined transcript accumulation of negative regulators of ABA signaling in wild-type, *myb96-ox*, and *myb96-1* seedlings, including ABI1, HIGHLY ABA-INDUCED PP2C GENE 2 (HA12), HYPERSENSITIVE TO ABA 1 (HAB1), ABI3-INTERACTING PROTEIN 2 (AIP2), PLANT U-BOX 9 (PUB9), MORE AXILLARY GROWTH 2 (MAX2), DWD HYPERSENSITIVE TOABA 1 (DWA1), DWA2, and ROP genes. These genes are known as negative regulators of ABA signaling, and their genetic mutants display enhanced ABA responses, such as delayed germination, reduced lateral root length, and increased drought tolerance. Quantitative real-time reverse transcriptase-PCR (RT-qPCR) analysis showed that most of the genes were unaffected by MYB96, except for a subset of ROP genes, ROP6, ROP10, and ROP11 (Fig. 4a; Supplementary Figure 9), which are known to negatively regulate early ABA signaling. These ROP genes were specifically and significantly activated in *myb96-1* but reduced in *myb96-ox* (Fig. 4a).

Based on the mutual interaction of MYB96 and HDA15 and the transcriptional repressor activity of HDA15, we hypothesized that MYB96 works together with HDA15 to repress the ROP genes. Although HDA15 broadly influenced the expression of ROP genes, the three ROP genes were specifically upregulated in the hda15-1 mutant (Fig. 4b). To further estimate the relevance of MYB96 and HDA15 in ABA-dependent ROP suppression, we tested transcript levels of ROP6, ROP10, and ROP11 in *myb96-1*, hda15-1, and *myb96-1hda15-1* mutant seedlings treated with ABA. The ROP genes were repressed in response to ABA in wild type, but ABA repression of ROP genes was impaired, and higher expression of ROP genes was observed in the *myb96-1*, hda15-1, and *myb96-1hda15-1* mutants (Fig. 4c). Although MYB96 and HDA15 were synergistic in the control of ABA responses (Fig. 3a–d), they both were required at least for the suppression of ROP expression in response to ABA (Fig. 4c).

We then asked whether the ROP6, ROP10, and ROP11 genes are direct regulatory targets of MYB96 and HDA15. To answer this question, we employed 35S:MYB96-MYC and 35S:HDA15-GFP transgenic plants and performed chromatin immunoprecipitation (ChIP) using the corresponding antibodies. ChIP-qPCR analysis showed that MYB96 binds to specific regions of the ROP promoters (Fig. 5a), and consistently, HDA15 bound to a similar region at each gene promoter (Fig. 5b). The binding regions of MYB96 and HDA15 at the ROP chromatin were maintained in transgenic plants expressing native promoter-driven constructs (Supplementary Figure 10).

These observations suggest that MYB96 most likely binds to specific promoter regions and recruits HDA15 that has no DNA-binding specificity. In support, while the hda15-1 mutation did not influence MYB96 binding to the ROP loci (Fig. 5a), binding of HDA15 to the ROP promoters was markedly reduced in the *myb96-1* background (Fig. 5b). These results indicate that HDA15 requires MYB96 to associate with the ROP promoters and repress expression.

**MYB96–HDA15 complex catalyzes histone deacetylation at ROP genes.** Since HDA15 represses gene expression by promoting H3 and H4 deacetylation, we asked whether the MYB96–HDA15 complex catalyzes removal of H3 and H4 acetylation (H3ac and H4ac) at the ROP promoters. To this end, ChIP assays using anti-H3ac and anti-H4ac antibodies were performed. In the presence of ABA, acetylation levels of H3 and H4 promoters were significantly reduced in the wild-type background (Fig. 6a, b). However, ABA repression of H3ac and H4ac accumulation at the ROP promoters was significantly reduced in the wild-type background (Fig. 6a, b). As a negative control, we also analyzed accumulation of H3ac and H4ac at the gene body regions of the ROP genes (Fig. 5). As expected, histone acetylation levels were unchanged in *myb96-1*, hda15-1, and *myb96-1hda15-1* mutants, regardless of ABA treatment (Fig. 6a,
b). These results indicate that MYB96 binds to specific regions of the ROP promoters and recruits HDA15 to remove the acetyl groups of H3 and H4 at the promoter regions.

Considering the requirement of MYB96 for HDA15 recruitment (Fig. 5), we wanted to know whether MYB96 is also required for histone deacetylation at the ROP promoters. We performed ChIP assays with anti-H3ac antibody using 35S:HDA15-GFP/myb96-1 plants. While H3ac levels at the ROP promoters were significantly reduced in 35S:HDA15-GFP transgenic plants compared with wild-type plants even in the absence...
of ABA, suppression was diminished in 35S:HDA15-GFP/myb96-1 (Fig. 6c). Consistently, in the absence of ABA, theROPgenes were repressed in 35S:HDA15-GFP transgenic plants, but this transcriptional repression was restored in 35S:HDA15-GFP/myb96-1 plants (Fig. 6d). These results indicate that the HDA15 protein binds to the ROM promoters and catalyzes histone deacetylation in a MYB96-dependent manner.

**MYB96 represses ROM genes in an HDA15-dependent manner.**

We next asked whether MYB96 repression of ROM genes also depends on HDA15. To answer this question, we carried out transient expression assays using Arabidopsis protoplasts. Reporter constructs, in which the ROM and KETOACYL-COA SYNTHASE (KCS) promoter sequences were fused with the minimal 35S promoter, were coexpressed with an effector construct overexpressing MYB96 in Arabidopsis mesophyll protoplasts isolated from wild-type and hda15-1 leaves (Fig. 7a). Reporter GUS activity measurement revealed that MYB96 significantly repressed ROM promoter activity in the wild-type background, but the repressive function was impaired in the hda15-1 background (Fig. 7b). The impaired MYB96 function in hda15-1 was recovered by complementation of HDA15 (Supplementary Figure 12).

In addition, as a control, we also examined whether HDA15 affects MYB96-dependent ABA-inducible genes. Expression of MYB96 alone was sufficient to activate the KCS genes, which are the primary target genes directly activated by MYB9612, and the hda15-1 mutation did not influence MYB96 regulation of ABA-inducible genes (Fig. 7b). Furthermore, coexpression of MYB96 and HDA15 in wild-type protoplasts also did not influence KCS1 expression (Supplementary Figure 12), suggesting that HDA15-selectively inhibits MYB96-repressed genes.

We also measured H3ac accumulation at the ROM promoters in 35S:MYB96-MYC and 35S:MYB96-MYC/hda15-1 plants. As expected, H3ac levels at the ROM promoters were reduced in 35S:MYB96-MYC even in the absence of ABA, but reduction in H3ac levels was impaired in the hda15-1 background (Fig. 7c). Transcript accumulation of the ROM genes further supported these observations. Reduction of the ROM genes in 35S:MYB96-MYC was restored in 35S:MYB96-MYC/hda15-1 (Fig. 7d). These observations indicate that the role of MYB96 in ROM repression depends on HDA15. Overall, the MYB96–HDA15 complex represses the ROM genes, which are negative regulators of ABA signaling, through histone deacetylation to strongly promote ABA responses (Fig. 8).

**Discussion**

Transcriptional responses to environmental challenges are usually linked to chromatin regulation44,45. Accumulating evidence has shown that genome-wide chromatin modification/remodeling accompanies water-deficit stress46–49. For instance, global changes in histone variant H2A.Z deposition occur especially in stress-responsive genes, and thearp6 mutant exhibits higher sensitivity to osmotic stress with hyperacetylation of stress-responsive genes50.

Consistently, a significant number of chromatin modifiers/remodelers are differentially expressed in response to ABA and/or drought stress to confer appropriate responses to environmental disadvantages50–54. As a supporting evidence, the Arabidopsis SWI/SNF chromatin-remodeling ATPase BRAHMA (BRM) represses ABA responses by directly promoting occupancy of the +1 nucleosome at the transcription start site of the ABI5 locus in the absence of ABA55. ABI5 expression is derepressed in brm mutants with reduction of the +1 nucleosome occupancy55, and consistently, brm mutants exhibit ABA hypersensitivity55. Moreover, BRM interacts with SnRK2s and clade A PP2Cs, and BRM function is further shaped by its phosphorylation state56. When ABA levels increase, SnRK2s are activated and clade A PP2Cs are inhibited by PYR/PYL ABA receptors. ABA-activated SnRK2s phosphorylate BRM, which leads to inhibition of BRM activity and thereby ABI5 induction. Under normal growth conditions with low ABA levels, dephosphorylation of BRM by PP2Cs restores BRM activity and represses ABI5 expression56.

Several histone modifiers are also involved in abiotic stress responses. Two evolutionarily conserved Arabidopsis Trithorax Group proteins (TrxGs), ARABIDOPSIS TRITHORAX 4 (ATX4) and ATX5, directly bind to the ABA-HYPERSENSITIVE GENE 3 (AHG3) locus, which acts as a negative regulator in ABA signaling, and regulate H3K4me3 deposition to activate expression57. Accordingly, theatx4andatx5single and theatx4atx5double mutants exhibit higher drought tolerance and ABA-hypersensitive phenotypes57. The RPD3-type histone deacetylases HDA6 and HDA19 also regulate the expression of ABA and abiotic stress-responsive genes by forming huge transcriptional repressor complexes, together with HISTONE DEACETYLATION COMPLEX 1 (HDC1), MULTICOPY SUPPRESSOR OF IRA 1 (MSI1), ETHYLENE RESPONSE FACTOR 7 (ERF7), HISTONE DEACETYLASE 2 C (HD2C), and SWI-INDEPENDENT 3 (SIN3) in Arabidopsis19,38–61. These complexes usually repress expression of ABA-related genes by promoting histone deacetylation at cognate loci. Mutations of the components result in hyperactivation of ABA-responsive genes and thus hypersensitivity to ABA and salt stress and increased tolerance of osmotic stress19,38–60,62,63.

In this study, we found that HDA15 acts as a positive regulator of ABA signaling by repressing ROM genes, which are pivotal repressors of early ABA signaling26,27,29,41. Binding selectivity of HDA15 to the ROM loci is defined by the MYB96 transcription factor. In the presence of ABA, the MYB96–HDA15 complex co-binds to the ROM promoters and removes acetyl groups of histone H3 and H4 to stimulate ABA responses. Consistently, hda15andmyb96mutants exhibit reduced ABA sensitivity, whereas transgenic plants overexpressing HDA15 and MYB96 are hypersensitive to ABA. Overall, ABA signaling is shaped by multiple layers of regulation54, and epigenetic mechanisms are a key regulatory scheme in plant stress responses.
MYB96 regulates early signaling components of ABA pathways, including the ROP genes. Consistently, MYB96 has a strong impact on plant tolerance to abiotic stresses and regulates a variety of ABA responses, including lateral root development, stomatal movement, hormone biosynthesis, and cuticular wax biosynthesis. Like many plant transcription factors, MYB96 functions as a transcriptional repressor for some promoters but an activator for others. Our preliminary genome-wide identification of direct binding sites of MYB96 provides support that MYB96 binds to promoters of both ABA-repressed and ABA-induced genes. MYB96 transcriptional activity would be determined in part by trans-factors, such as histone-modifying enzymes. In this
Fig. 4 MYB96 and HDA15 regulate expression of Rho of Plants (ROP) genes. 

**a** Transcript accumulation of ROP genes in wild type, myb96-ox, and myb96-1. 

**b** Transcript accumulation of ROP genes in wild type, 35S:HDA15-GFP, and hda15-1. In **a, b**, 2-week-old seedlings grown under long days (LDs) were harvested for total RNA isolation. Statistically significant differences between the wild-type and transgenic or mutant plants (Student’s t test, \( *P < 0.05 \)) are indicated by asterisks. 

**c** Effects of abscisic acid (ABA) on the expression of ROP genes in myb96-1, hda15-1, and myb96-1hda15-1. Two-week-old seedlings grown under LDs were transferred to MS-liquid medium supplemented with 20 μM ABA and incubated for the indicated time period (h). Transcript accumulation was analyzed by quantitative real-time reverse transcriptase-PCR. Three independent biological replicates were averaged, and statistical significance of the measurements was analyzed by analysis of variance (ANOVA) (one-way ANOVA with Fisher’s post hoc test, \( *P < 0.05 \)). M mock, A ABA. Different letters represent a significant difference at \( P < 0.05 \). Source data are provided as a Source Data file.

Fig. 5 MYB96 and HDA15 co-bind to the ROP promoters. In **a, b**, putative MYB-binding sites are indicated by arrowheads. Black underbars indicate the regions of PCR amplification after chromatin immunoprecipitation (ChIP). ChIP-quantitative PCR assays were conducted to examine enrichment of MYB96 and HDA15 at ROP loci. Three independent biological replicates were averaged, and statistical significance of the measurements was analyzed by Student’s t test (\( *P < 0.05 \)). Bars indicate the standard error of the mean. 

**a** Binding of MYB96 to ROP loci. 

**b** Binding of HDA15 to ROP loci in a MYB96-dependent manner. Source data are provided as a Source Data file.
study, we found that, while MYB96 alone is sufficient to activate ABA-inducible genes such as RD and KCS genes, MYB96 requires HDA15 to inhibit the expression of ABA-repressible genes. In particular, the MYB96–HDA15 complex selectively represses the ROP genes, which are negative regulators of ABA signaling.

Given the synergistic interactions between MYB96 and HDA15, the HDA15 protein most likely has regulatory targets non-overlapping with MYB96 in the control of ABA responses, although HDA15 target genes are elusive so far. HDA15 alone can confer ABA sensitivity independently of MYB96. Probably, each individual member deals with mild ABA responses, but the physical interaction between MYB96 and HDA15 enables to induce strong and robust ABA responses by regulating upstream core components.
One remaining question is how HDA15 selectively represses the ROP genes without affecting MYB96-activated genes. Physical interactions with additional ABA signaling components or trans-factors might be involved. Moreover, the strength of MYB96 activation may also be involved. MYB96 activation above the low threshold allows it to mainly act as a transcriptional activator to promote genes contributing to stress tolerance in plants. At a high level of MYB96 activation, a significant portion of MYB96 proteins above a high threshold may get a chance to interact with HDA15, which is also significantly activated under harsh stress conditions, to additionally repress negative regulators of ABA signaling, further enhancing ABA responses. In support, MYB96–HDA15 interactions are profoundly enhanced at high concentrations of ABA. Taken together, MYB96 is most likely a master regulator of ABA signaling, ensuring full activation of ABA responses.

HDA15 function is shaped by light conditions. HDA15 interacts with PHYTOCHROME INTERACTING FACTOR 1 (PIF1) and PIF3, which act as negative regulators in light responses and repress light-mediated cotyledon development, chlorophyll biosynthesis, and hormonal signaling in the dark. The HDA15–PIF complexes co-bind to the target genes and repress histone H3 acetylation levels, repressing expression in the dark.

Active roles of HDA15 in light have also been demonstrated. NF-YC1, NF-YC3, NF-YC4, and NF-YC9 interact with HDA15 in the light, and they co-target the promoters of a set of hypocotyl
hypocotyl elongation is suppressed in light\textsuperscript{71,72}. However, in the acetylation levels at the associated regions. As a consequence, photosynthesis\textsuperscript{75,76}. The point of crosstalk between light and increased H4ac levels and thereby etiolated growth\textsuperscript{71}.

**Methods**

**Plant materials and growth conditions.** Arabidopsis thaliana (Columbia-0 ecotype) was used for all experiments unless otherwise specified. Plants were grown under long-day conditions (LDs: 16 h light/8 h dark cycles) with cool white fluorescent light (120 μmol photons/m²/s) at 22–23 °C. 

**Seed germination assays.** All seeds were harvested at the same time and dried for 2 days at 4 °C in darkness. Stratified seeds were transferred to a culture room at 23 °C under LDs and germinated on MS medium (half-strength MS salts, 0.05% MES, pH 5.7 and 1% agar) supplemented with various concentrations of ABA. To measure seed germination percentage, radicle emergence from the seed coat and endosperm was used as a phenotypic marker and counted at regular intervals. Each germination assay was performed in biological triplicates.

**ABA treatment and drought.** For ABA treatment, 2-week-old seedlings grown under LDs were transferred to half-strength MS-medium supplemented with 20 μM (+)-ABA (Sigma-Aldrich, D.O.S2768) (Alfa Aesar, Ward Hill, MA, USA). Drought stress was induced in 2-week-old plants grown in soil under LD conditions by halting watering. To prevent direct air-drying of seedlings, small pores were made in the plastic cover 7 days after the start of drought conditions, and the cover was removed 7 days later. Survival rates were measured for each group of plants. Three biologically independent measurements of at least 30 plants were averaged.

**RT-qPCR analysis.** Total RNA samples were extracted from whole seedlings using TRI reagent (TAKARA Bio, Singa, Japan) according to the manufacturer’s recommendations. Total RNA samples were pretreated with an RNase-free DNase to eliminate genomic DNA contamination. The first-strand cDNA was synthesized from 2 μg of total RNA by reverse transcription using Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Dr. Protein, Seoul, Korea) with oligo[d(T)]\textsubscript{18}.

**RT-qPCR experiments were performed on the Step-One Plus Real-Time PCR System (Applied Biosystems).** The PCR primers used are listed in Supplementary Table 1. The comparative C\textsubscript{T} method was used to determine the relative gene expression with the expression of the EUKARYOTIC TRANSLATION INITIATION FACTOR 4A1 (eIF4A1) gene (Atg31920) as an internal control. All RT-qPCR reactions were performed using three independent replicate samples. The specificity of RT-qPCR results was determined by melt curve analysis of the amplified products using the standard method.

**Y2H assays.** For Y2H assays, the expression vectors and yeast strains used were supplied by the BD Matchmaker system (Clontech, Mountain View, CA, USA). The yeast strain AH109 harboring the LexA and His reporter genes was used. P-galactosidase activity was measured using o-nitrophenyl-β-d-galactopyranoside (ONPG) as a substrate.

**BIFC assays.** The full-size MYB96 and HDA15 coding sequence were fused in-frame to the 5′-end of a gene sequence encoding the C-terminal half of EYFP in the p5SAT–eYFP-C1 vector (E3082) or the N-terminal half of EYFP in the p5SAT-N-C1 vector (E0801). The JDD14 RFP construct was visualized as a nucleus localization marker\textsuperscript{79}. The recombinant constructs were co-transformed into Arabidopsis mesophyll protoplasts by the PEG-calcium transfection method. Transformed protoplasts were incubated at 23 °C for 12–16 h in darkness. Emitted fluorescence was monitored by Zeiss LSM510 confocal microscope (Carl Zeiss, Jena, Germany).

**ChiP assays.** Harvested plant materials were cross-linked with 1% formaldehyde, ground in liquid nitrogen, and solubilized in NLB buffer (50 mM HEPE, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 μg/mL pepstatin A, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Chromatin was fragmented on ice with a probe sonicator to obtain approximately 200–800 bp fragments. After sonication, the suspension was centrifuged twice for 20 min at 13,200 x g. The extract was precleared with 100 μL of salmon sperm DNA/protein A agarose beads (16–157, Millipore, Billerica, USA) for 1 h. The preclarified supernatant was incubated overnight with 10 μL of corresponding antibodies, including anti-MYC (05-724, Millipore; 1:500 dilution), anti-H3ac (06-599, Millipore; 1:500 dilution), anti-H4ac (06-866, Millipore; 1:500 dilution), anti-GFP (ab290, Abcam, Cambridge, USA: 1:500 dilution), or no antibody (control). Then the supernatant was mixed with 100 μL of salmon sperm DNA/protein A agarose beads and incubated for 3 h on a rotating wheel. The beads were washed with 10 mL of NLB buffer, 1 mL of low-salt buffer (20 mM Tris–Cl, pH 8, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, and 0.1% SDS), 1 mL of high-salt buffer (20 mM Tris–Cl, pH 8, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, and 0.1% SDS), and 1 mL of LiCl buffer (10 mM Tris–Cl, pH 8, 0.25 M LiCl, 1 mM EDTA, 1% sodium deoxycholate, and 1% Nonidet P-40). DNA was purified using phenol/chloroform/isoamyl alcohol and sodium acetate (pH 5.2). The levels of precipitated DNA fragments were quantified using qPCR using specific primer sets (Supplementary Table 2). Values were normalized according to input DNA levels. Values for control plants were set to 1 after normalization against eIF4A for qPCR analysis.

**Transient expression assays.** For transient expression assays using Arabidopsis protoplasts, reporter and effector plasmids were constructed. The reporter plasmid (modified pMBA1305 vector) contains a minimal 35S promoter sequence and the GUS-coding gene\textsuperscript{80}. The core cis-elements on the KCS and ROP promoters were inserted into the reporter plasmid. pmKCS and pmROP mutant constructs were generated with mutations within core cis-elements (Supplementary Table 3). To construct effector plasmids, MYB96 and HDA15 cDNAs were inserted into the effector plasmid pBA002 containing the CaMV 35S promoter by using EcoRI and XhoI restriction enzymes.

Five leaves were cut into 0.5-mm strips with a fresh razor blade and digested with gentle shaking in an enzyme solution containing 1% (w/v) cellulose RS, 0.1%
Supplementary Information

4a

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ARTICLE

10 mM MES (pH 5.7), 1 mM CaCl₂, 1 mM MgCl₂, 0.1% bovine serum albumin (w/v) for 3 h at room temperature. The enzyme solution was sieved through a 70-μm nylon mesh (Carolina Biologicals, Burlington, NC) and spun at 45 × g for 5 min. The pellet was washed twice and re-suspended in wash solution (0.6 M mannitol, 4 mM MES, pH 5.7). Recombinant reporter and effector plasmids were cotransformed into Arabidopsis protoplasts by polyethylene glycol-mediated transformation. After 16-h incubation in the dark at 23°C, transformed protoplasts were processed for quantifying β-glucuronidase (GUS) activity. A CaMV 35S promoter-luciferase construct was also cotransformed as an internal control. The luciferase assay was performed using the Luciferase Assay System Kit (Promega, Madison, WI).

Co-IP assays. A. tumefaciens cells containing 35S:MYB96-MYC and 35S:HA15-GFP constructs were infiltrated into 3-week-old N. benthamiana leaves. Tobacco leaves were homogenized in liquid nitrogen, and total proteins were extracted in protein extraction buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% glycerol, 0.05% Nonidet P-40, 2.5 mM EDTA, 1 mM phenylmethylsulfonfluoride, and 1X complete cocktail of protease inhibitors). After protein extraction, 5% of the extracts were used as input control. The protein extracts were mixed with anti-MYC antibodies (05-724, Millipore, Billerica, MA, USA; 1:500 dilution) coupled to Protein-A sepharose beads (Sigma-Aldrich, St Louis, MO, USA) and incubated for 4h at 4°C. The precipitated samples were washed at least four times with the protein precipitation buffer and then eluted by 1X SDS-polyacrylamide gel electrophoresis (PAGE) loading buffer to subject to SDS-PAGE with anti-MYC (1:2000 dilution; Millipore) or anti-GFP antibodies (1:500 dilution; sc-9996, Santa Cruz Biotech., Dallas, TX, USA). Similar protocols were used for Co-IP assays for Arabidopsis plants.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Data supporting the findings of this work are available within the paper and its Supplementary Information files. A reporting summary for this article is available as a Supplementary Information file. The source data underlying Fig. 1a, c–e, 2a–g, 3a–d, 4a–c, 5a, b, 6a–d, and 7b–d, as well as Supplementary Figures 1–12 are provided as a Source Data file. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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P.J.S. conceived and designed the experiments and wrote the paper. H.G.L. conducted experiments and contributed to the study design under the supervision of P.J.S.

Additional information
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