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Research article

The *AtMYB44* promoter is accessible to signals that induce different chromatin modifications for gene transcription

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ABSTRACT

AtMYB44 transcripts accumulate non-specifically under diverse stress conditions and with various phytohormone treatments in *Arabidopsis thaliana*. We investigated the chromatin modifications caused by various signals to uncover the induction mechanism of *AtMYB44* transcription. Bisulfite sequencing confirmed a previous database illustrating that the *AtMYB44* promoter and gene-body regions are completely DNA methylation-free. Chromatin immunoprecipitation (ChIP) assays revealed that the nucleosome density is remarkably low at the *AtMYB44* promoter region. Thus, the promoter region appears to be highly accessible for various *trans*-acting factors. ChIP assays revealed that osmotic stress (mannitol treatment) lowered the nucleosome density at the gene-body regions, while abscisic acid (ABA) or jasmonic acid (JA) treatment did so at the proximal transcription start site (TSS) region. In response to mannitol treatment, histone H3 lysine 4 trimethylation (H3K4me3) and H3 acetylation (H3ac) levels within the promoter, TSS, and gene-body regions of *AtMYB44* were significantly increased. However, occupancy of histone variant H2A.Z was not affected by the mannitol treatment. We previously reported that salt stress triggered a significant decrease in H2A.Z occupation without affecting the H3K4me3 and H3ac levels. In combination, our data suggest that each signal transduced to the highly accessible promoter induces a different chromatin modification for *AtMYB44* transcription.

1. Introduction

During their life cycle, plants are exposed to diverse developmental and environmental signals. Phytohormones such as abscisic acid (ABA) and jasmonates (JAs) are important signal transducers that mediate gene activation for proper cellular responses. In the early stage of cellular responses, the expression of a variety of genes encoding transcription factors that activate or repress the transcription of other genes involved in the response is induced. For instance, under osmotic stress conditions caused by drought, salinity, or mannitol treatment (Zhu et al., 1997; Osakabe et al., 2014), the expression of numerous transcription factor genes belonging to the MYB, bZIP, AP2/ERF, and NAC families is induced in an ABA-dependent manner (Fujita et al., 2011; Yoshida et al., 2014).

The Arabidopsis transcription factor AtMYB44 is a component of various signaling pathways. Several lines of independent study have reported that AtMYB44 regulates diverse cellular processes, including ABA-mediated tolerance to osmotic stress (Jaradat et al., 2013; Jung et al., 2008; Persak and Pitzschke, 2014), ethylene-modulating insect-defense (Liu et al., 2011; Lü et al., 2013), and disease resistance (Shim et al., 2013; Zou et al., 2013). AtMYB44 also functions in many physiological programs, including flowering time (Jung et al., 2008), leaf senescence (Jaradat et al., 2013), systemic resistance induced by plant growth-promoting fungus (Hieno et al., 2016), and primary root elongation (Zhao et al., 2016). Reflecting its diverse biological roles, transcripts of the *AtMYB44* gene (At5g67300) are upregulated by a variety of environmental stresses, hormone treatments, and microbial infections (Kranz et al., 1998; Yanhui et al., 2006 Jung et al., 2010; Jaradat et al., 2013).

Gene transcription is mediated by RNA polymerase II (RNAPII) and is associated with changes in chromatin architecture and remodeling at the promoter, TSS, and gene-body regions (Cairns, 2009; Yamamuro et al., 2016). Most of all, DNA methylation of a promoter results in the suppression of gene transcription, and can be altered when plants are exposed to abiotic stresses, which in turn directly or indirectly affect transcription of the corresponding gene (Chinnusamy and Zhu, 2009; Kim et al., 2015). In the chromatin of eukaryotic cells, genomic DNA wraps around a histone core consisting of H2A, H2B, H3, and H4 to constitute a nucleosome. Thus, activation of a gene promoter involves dynamic competition between *trans*-acting factors and nucleosomes

Abbreviations: ABA, abscisic acid; GUS, β-glucuronidase; JA, jasmonic acid; MS, Murashige and Skoog; qRT-PCR, quantitative reverse-transcription PCR; RNAPII, RNA polymerase II; TSS, transcription start site

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(Struhl and Segal, 2013; Zhang et al., 2015). During gene transcription, histones are post-translationally modified by (de)acetylation and (de) methylation, or replaced by their structural variants (Lai and Pugh, 2017).

It is of interest to determine how diverse signals upregulate *AtMYB44* gene expression non-specifically. We initially postulated the presence of a common message generated from diverse signals, which would ultimately affect chromatin modification at the *AtMYB44* locus. Our data show that the *AtMYB44* promoter region is cytosine methylation-free and has low nucleosome density, making it accessible to various *trans*-acting factors. In addition, osmotic stress generated by mannitol treatment induced histone modifications that differ from those induced by ABA, JA, or salt treatment. These results indicate that each signal employs specific transcription factors on the *AtMYB44* promoter and recruits signal-specific histone-modifying enzymes to evoke distinct chromatin modifications.

2. Methods

2.1. Plant materials and treatments

Arabidopsis seeds of *histone acetyltransferase* (*hac*) mutants, *hac1-3* (*SALK*-080380C), *hac4-1* (*SALK*-051750C), and *hac5-3* (*SALK*-075639C), were obtained from The Arabidopsis Information Resource (TAIR). Seeds were surface sterilized and stored at 4 °C for 3 days for stratification. Half-strength Murashige and Skoog (MS) medium containing 2% sucrose (hereafter referred to as MS medium) was used as basic medium. The seeds were sown on solid MS medium and grown in a growth chamber with a 16 h light/8 h dark cycle at 23 ± 1 °C and ~100 μmol photons $m^{-2} s^{-1}$. Two-week-old plants were transferred to MS liquid medium supplemented with 300 mM mannitol and grown for an additional 6 h, to mimic osmotic stress (Zhu et al., 1997). For phytohormone treatments, 10 μM ABA or 10 μM JA was added to the medium.

2.2. DNA methylation detection (bisulfite sequencing)

Genomic DNA was isolated from 2-week-old plants using the Quick-DNA™ Universal kit (Zymo Research), following the manufacturer's instructions. Then, 500 ng of DNA was used for bisulfite conversion using the EZ DNA Methylation-Lightning™ kit (Zymo Research) and particular DNA regions were amplified by PCR using specific primers (Table S1). The PCR products were purified and analyzed by sequencing.

2.3. Histochemical β-glucuronidase (GUS) assay

Two-week-old *AtMYB44pro*::*GUS* #22 (Jung et al., 2008) seedlings treated with mannitol were transferred to GUS buffer containing 1.0 mM X-Gluc (Jefferson et al., 1987) and incubated at 37 °C for 3 h. Then, chlorophylls in the plant tissues were washed out with 70% ethanol, and the GUS activity was visualized under a digital microscope (Leica EZ4D).

2.4. Quantitative reverse-transcription PCR

Total RNAs were extracted using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich), and used for first-strand cDNA synthesis by the SuperScript® III First-Strand Synthesis SuperMix (Invitrogen). Quantitative PCR (qPCR) was carried out using the SolGent™ 2 × Real-Time Smart Mix (SolGent) and specific primers (Table S1). Thermocycling and fluorescence detection were performed using the Mx3005P qPCR system (Agilent Technologies). The PCR reactions were initiated at 95 °C for 15 min, and followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. *AtACTIN2* (At3g18780) was used as an internal control. The relative transcript level was calculated by the $2^{-\triangle\triangle Ct}$ method, where $\triangle Ct = Ct^{AtMYB44} - Ct^{AtACTIN2}$ and $\triangle\triangle Ct = \triangle Ct^{Mannitol} - \triangle Ct^{Control}$ (Livak and Schmittgen, 2001).

2.5. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed using the EpiQuik™ Plant ChIP kit (EpiGentek), according to the manufacturer's instructions. For immunoprecipitation, the following antibodies were purchased: anti-RNAPII CTD repeat YSPTSPS 4H8 (Abcam; ab5408), anti-histone H3 (Abcam; ab1791), anti-acetyl-histone H3 (H3ac; Merck Millipore; 06–599), anti-acetyl-histone H4 (H4ac; Merck Millipore; 06–866), anti-histone H3 acetyl K9 (H3K9ac; Abcam; ab10812), anti-histone H3 trimethyl K4 (H3K4me3; Abcam; ab8580), anti-histone H3 trimethyl K27 (H3K27me3; Merck Millipore; 07–449), and anti-histone H2A.Z (Abcam; ab4174). Normal rabbit IgG (Merck Millipore; 12–370) was used as a negative control in the ChIP assays. Immunoprecipitated DNA fragments were used for qPCR with specific primers (Table S1) designed from the nucleotide sequences of the *AtMYB44* promoter, TSS, and gene-body regions.

2.6. Statistical analysis

The drought stress treatment experiments were repeated independently two or three times, conducting the qRT-PCR and ChIP-qPCR analyses with triplicate samples. The statistical analysis was performed using Duncan's test (Duncan, 1955) at a 95% confidence level.

3. Results and discussion

3.1. DNA methylation and nucleosome density on AtMYB44

The genome-wide analysis data (http://epigenomics.mcdb.ucla.edu) disclosed that DNA methylation is very rarely detected within the *AtMYB44* gene-coding regions and a long (~3 kilobases [kb]) upstream region, and that the nucleosome density on the *AtMYB44* promoter (~3 kb upstream from the TSS) is relatively low, and there are two nucleosome-rich regions on the *AtMYB44* gene-coding region (Casper et al., 2018). In plant genomes, cytosine methylation occurs at three sequence sites: CG, CHG, and CHH, where H represents A, T, or C (Saze et al., 2012; Vanyushin and Ashapkin, 2011). Sodium bisulfite converts unmethylated cytosine, but not 5-methylcytosine, into uracil, which is converted into thymine via PCR amplification of the corresponding DNA fragments (Chinnusamy and Zhu, 2009). To confirm the database information, we performed bisulfite sequencing assays of *AtMYB44*, specifically regions C (promoter) and E (gene-body region) (Fig. 1A), which have higher CG contents than other regions. After sodium bisulfite treatment of the genomic DNA extracted from 2-week-old Arabidopsis seedlings, all of the cytosine residues in these regions were converted into thymine, as revealed in the subsequent sequencing analysis indicating that *AtMYB44* gene region is methylation-free (Fig. 1B).

In Arabidopsis, changes in DNA methylation status modulate gene expression in response to environmental stresses (Tricker et al., 2012; Xu et al., 2015). In general, DNA methylation is linked to gene repression. However, previous studies have shown that DNA methylation may also occur during gene activation (Halpern et al., 2014; Siegfried and Simon, 2010; Zilberman et al., 2007). Thus, we examined whether osmotic stress influences the transcription of *AtMYB44* by altering its DNA methylation status; however, mannitol treatment did not affect DNA methylation in either the promoter or gene body regions of *AtMYB44* (Fig. 1B).

The genome-wide analysis disclosed that the nucleosome density on the *AtMYB44* promoter (~3 kb upstream from the TSS) is relatively low, and there are two nucleosome-rich regions on the *AtMYB44* gene-coding region (Casper et al., 2018). We performed ChIP assays with

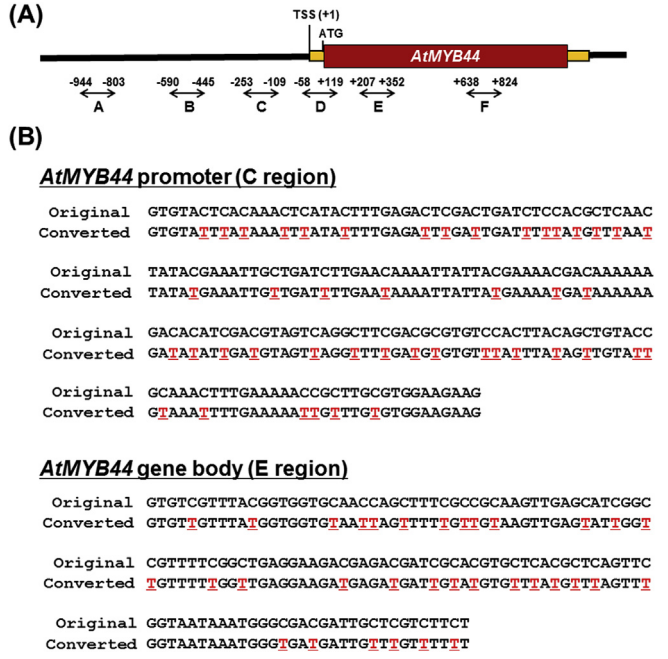

Fig. 1. DNA methylation on *AtMYB44* promoter and gene-body regions. (A) Schematic diagram of the *AtMYB44* locus (At5g67300). TSS (+1), transcription start site; ATG, translation initiation site. 5′- and 3′-UTR (untranslated) regions are depicted in yellow bars. Primers specific to each region (A through F) were designed from the nucleotide sequences (Table S1) and used in the qPCR assays. (B) DNA methylation status within the *AtMYB44* regions. Two-week-old wild-type (Col-0) Arabidopsis plants were used for genomic DNA extraction. The extracted genomic DNA was treated without (original sequences) or with (converted sequences) sodium bisulfite. Converted DNA samples were amplified by PCR using specific primers (Table S1) for two fragments including the C (promoter) and E (gene-body) regions, as indicated in Fig. 1A. The PCR products were purified and their nucleotide sequences were analyzed. The experiments were performed in duplicate. Nucleotide residues converted by the sodium bisulfite treatment are indicated in red and underlined. The bisulfite sequencing assay performed under osmotic stress (300 mM mannitol for 6 h) yielded a result identical to those under normal conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

anti-histone H3 antibody and confirmed that the nucleosome density was markedly lower at the *AtMYB44* promoter (regions A, B, and C) than at the proximal TSS (region D) and gene-body regions (E and F) (Fig. 2).

In plants, nucleosome occupancy influences the transcription of numerous genes (Kim et al., 2008; Kumar and Wigge, 2010). In response to mannitol treatment, the nucleosome density was reduced in the *AtMYB44* gene-coding region (Fig. 2), indicating that nucleosome eviction correlates to gene expression. In addition, ABA or JA treatment decreased the nucleosome density in the proximal TSS region. These results suggest that each signal can freely access the *AtMYB44* promoter, but causes nucleosome eviction at different regions of the *AtMYB44* gene.

Our analyses (PlantPAN 2.0, http://plantpan2.itps.ncku.edu.tw/) indicate that the *AtMYB44* promoter (~1.5 kb) contains putative binding sites for many transcription factors, including MYB, NAC, bHLH, bZIP, WRKY, and ARF, which are related to various signaling pathways (Fig. S1). Number of conserved sequences for drought/osmotic stress signals, DRE (dehydration-responsive element, TAACCGacct) and ABRE (abscisic acid-responsive element, cACGTGgc), are also located on this promoter region.

There is a positive correlation between DNA methylation and nucleosome occupancy (Collings et al., 2013), which can be depleted in highly accessible regions, such as active promoters and enhancers

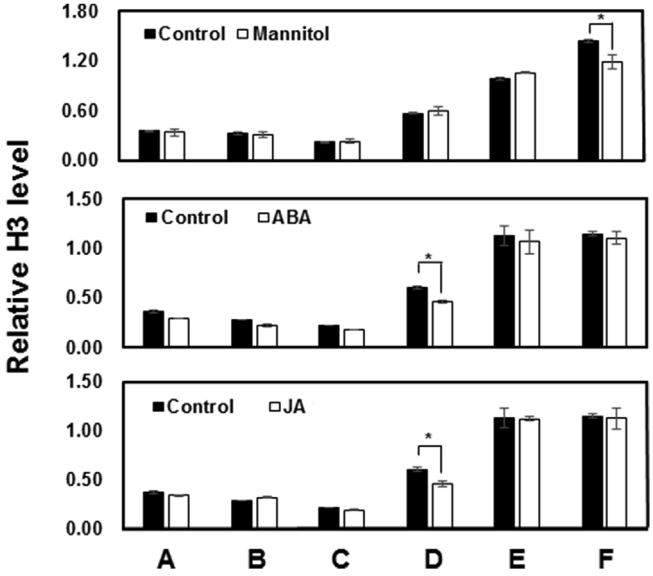

Fig. 2. Alterations in nucleosome density at *AtMYB44* genomic regions in response to abiotic stresses and phytohormone treatments. Two-week-old wild-type (Col-0) Arabidopsis plants were transferred to 1/2 MS liquid medium (2% sucrose) supplemented without (control) or with 300 mM mannitol, 10 μM ABA, or 10 μM JA, grown for a further 6 h, and used for ChIP assays with anti-histone H3 antibodies. qPCR was performed using specific primers (Table S1), and the ChIP signals were normalized to the input DNA and an internal control (*AtACTIN2*). The experiments were performed in duplicate. Columns marked with an asterisk differ significantly ($P < 0.05$). Bars represent the standard error.

(Chodavarapu et al., 2010; Collings and Anderson, 2017). The open promoters which are the promoters of constitutively expressed genes usually have methylation- and nucleosome-free sites (Cairns, 2009). Thus, AtMYB44 promoter is highly accessible to diverse *trans*-acting factors via the presence of several binding sites and it has a relatively low nucleosome density coupled with the lack of DNA methylation which all indicate the role for triggering differential transcription.

3.2. *AtMYB44* transcription in response to mannitol treatment

The transcript accumulation of a particular gene is regulated at the transcriptional and post-transcriptional levels, and the activation of transcription is associated with changes in chromatin architecture and remodeling at the corresponding promoters (Cairns, 2009). The qRT-PCR assays showed that mannitol treatment increased the transcript level of *AtMYB44* (Fig. 3A), which confirmed previous observations (Jung et al., 2010), as well as those of the control stress marker genes *RD29*A and *COR15*A (Fig. S2). In addition, *GUS* expression was markedly increased when the *AtMYB44pro*::*GUS* transgenic plants (Jung et al., 2008) were grown under osmotic stress (Fig. 3B). In this *AtMYB44pro*::*GUS* transgenic plants, ~3 kb DNA fragment upstream of *AtMYB44* TSS containing all present tested regions (A, B, and C) (Fig. 1A) was cloned to drive the expression of *GUS* gene (Jung et al., 2008). This confirms the important role of the promoter in the regulation of *AtMYB44* in response to stressful conditions.

Next, we confirmed that the increased transcript level of *AtMYB44* is caused by an increase in transcription and not the suppression of mRNA degradation in response to mannitol treatment. ChIP assays with the anti-RNAPII antibody revealed that RNA polymerases are enriched throughout the *AtMYB44* genomic region in response to mannitol treatment, especially around the TSS (Fig. 4A). In the control experiments, similar results were obtained in the *RD29*A and *COR15*A gene regions (Fig. S2), which is consistent with the results of Kim et al. (2008). Our results show that the *AtMYB44* transcript is upregulated via

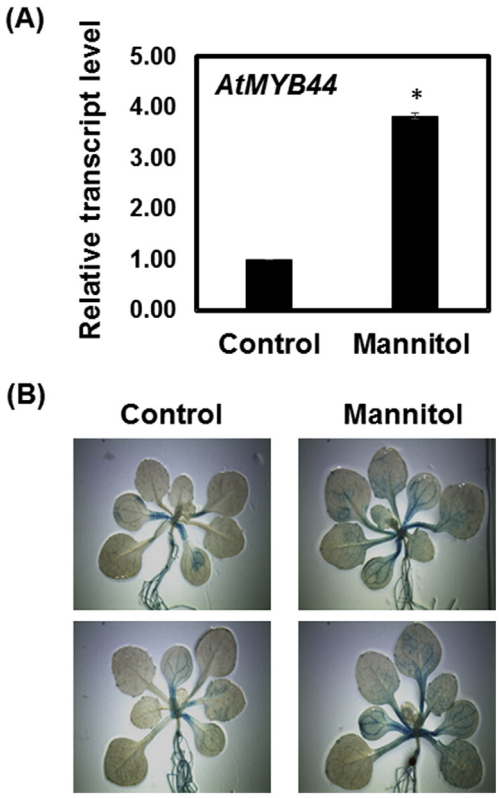

Fig. 3. Transcript levels of *AtMYB44* gene under osmotic conditions. (A) Accumulation of gene transcripts by osmotic stress (mannitol treatment). Two-week-old wild-type (Col-0) Arabidopsis plants were transferred to MS liquid medium (2% sucrose) supplemented without (control) or with 300 mM mannitol. The plants were grown for an additional 6 h, and used for total RNA extraction. Quantitative reverse-transcription PCR (qRT-PCR) was performed using specific primers (Table S1). *AtACTIN2* was used as an internal control. The relative transcript level was calculated by formula $2^{[-\ (Ct.AtMYB44\ -\ Ct.AtACTIN2)]}$. Bars represent the standard error. (B) Activation of the *AtMYB44* promoter in response to mannitol treatment. Two-week-old *AtMYB44pro*::*GUS* #22 (Jung et al., 2008) plants were treated with 300 mM mannitol for 6 h, and the GUS (β-glucuronidase) activity was visualized using a digital microscope (Leica EZ4D).

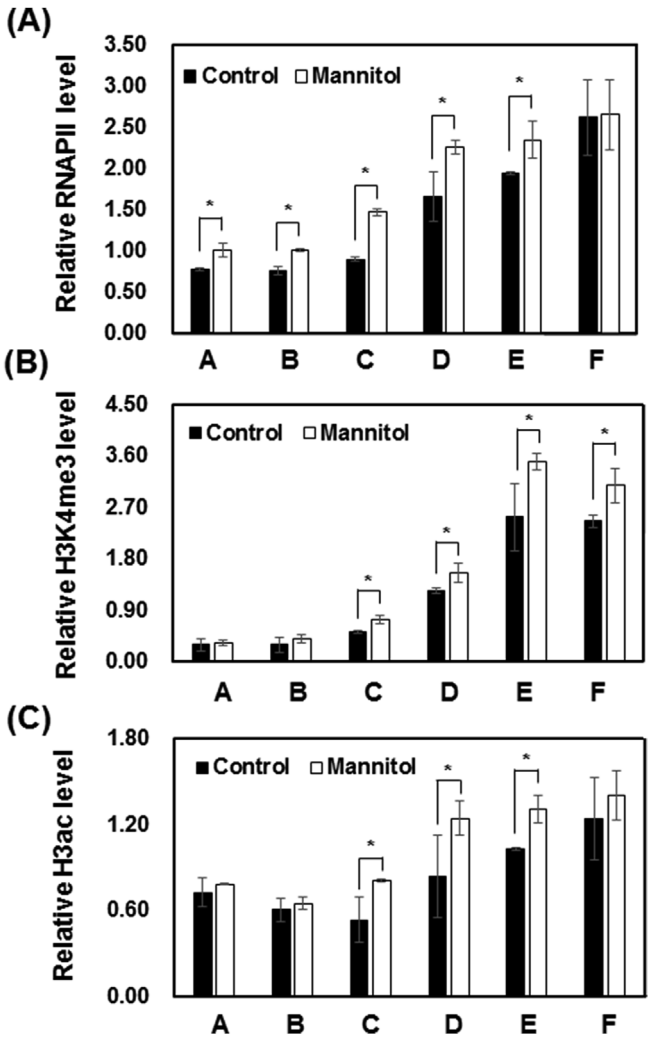

Fig. 4. RNA polymerase occupancy and histone modifications at the *AtMYB44* locus in response to mannitol treatment. Two-week-old wild-type (Col-0) Arabidopsis plants were treated without (control) or with 300 mM mannitol for 6 h, and used for ChIP-qPCR assays with (A) anti-RNAPII, (B) anti-H3K4me3, and (C) anti-H3ac antibodies. qPCR was performed using specific primers (Table S1) for fragments A through F as indicated in Fig. 1A. The ChIP signal was normalized to the input DNA and an internal control (*AtACTIN2*). The experiments were performed in duplicate. Columns marked with an asterisk differ significantly ($P < 0.05$). Bars represent the standard error.

RNA polymerase-mediated transcription, although additional regulation at the post-transcriptional level cannot be ruled out.

3.3. Chromatin modifications on AtMYB44 in response to osmotic stress

ChIP-qPCR assays revealed that mannitol treatment significantly increased histone H3 lysine 4 trimethylation (H3K4me3) and acetylation (H3ac) levels within the *AtMYB44* TSS and gene-body regions (Fig. 4B and C) while slightly increased levels of H3 acetylation of lysine 9 (H3K9ac) and H4 acetylation (H4ac) (Fig. S3). In a control experiment with *RD29*A, same stress conditions increased the levels of H3 acetylation (H3ac) and H4ac, as well as H3K9ac and H3K4me3 (Fig. S4), which is consistent with the results of Kim et al. (2008). Supporting the observation, *AtMYB44* transcript levels were lower in the *hac* mutants including *hac1-3*, *hac4-1*, and *hac5-3* in response to mannitol treatment (Fig. 5). The minor reduction of *AtMYB44* transcript levels in these single mutants under osmotic stress may be due to functional redundancy between more than 10 *HAC* genes in the Arabidopsis genome (Boycheva et al., 2014).

In response to osmotic stress (drought or dehydration), a number of genes are upregulated in association with an increase in histone modifications such as H3K4me3, H3K9ac, and H3 acetylation of lysine 27 (H3K27ac) (Kim et al., 2008, 2012). In this study, we observed that osmotic stress increased *AtMYB44* transcription in accordance with H3ac and H3K4me3 levels at the gene locus (Fig. 4B and C). It is not unusual for a signal to induce both H3 acetylation and methylation, which are both gene activation markers. In a previous study, the H3K4me3 levels at some stress-responsive genes were decreased in the *hda6* mutant, although HDA6 is involved in histone deacetylation (Chen et al., 2010). In addition, H3K4me3 promotes histone acetylation at the proximal promoter region of yeast genes, whereas H3K4me2 reduces histone acetylation near the 5′ ends of genes (Kim and Buratowski, 2009). This implies a close association between histone acetylation and methylation.

The levels of H3 lysine 27 trimethylation (H3K27me3), a histone modification indicative of gene repression (Kim et al., 2015; Kwon et al., 2009), were too low to be detected in the *AtMYB44* promoter and gene-body regions in untreated and mannitol-treated plants (Fig. S5). This result is consistent with genome-wide analysis data (http://epigenomics.mcdb.ucla.edu). Previously, H3K27me3 was found to accumulate at two cold-responsive genes, namely *COR15*A and *AtGOLS3*, and the H3K27me3 enrichment on these genes was reduced in response to cold exposure in association with an increase in their transcript levels

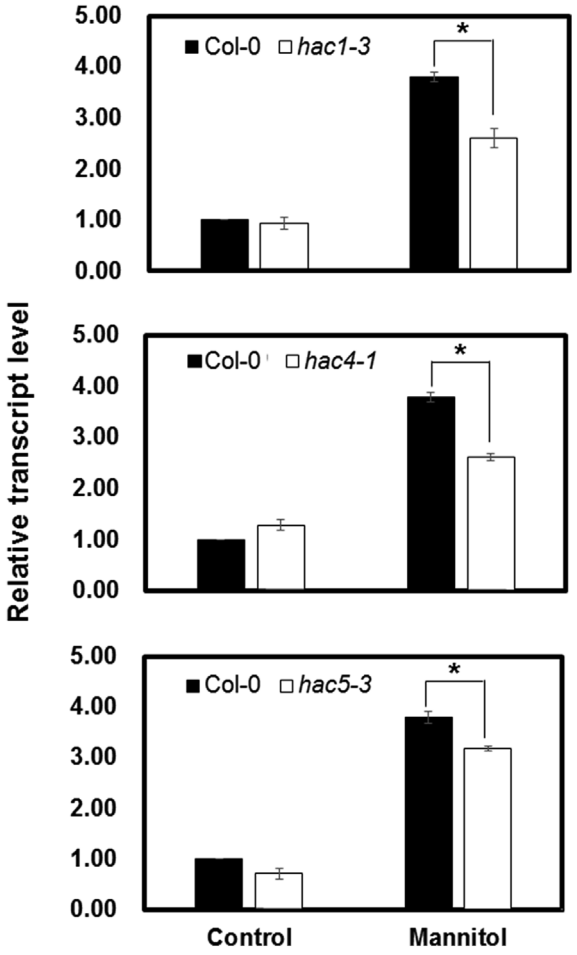

Fig. 5. *AtMYB44* transcript levels in *histone acetyltransferase (hac)* mutants under osmotic stress. Two-week-old wild-type (Col-0) and *hac* mutant plants growing on MS medium (2% sucrose) were transferred to MS liquid medium (2% sucrose) supplemented without (control) or with 300 mM mannitol. The plants were grown for an additional 6 h, and used for total RNA extraction. Quantitative reverse-transcription PCR (qRT-PCR) was performed using specific primers (Table S1). *AtACTIN2* was used as an internal control. The relative transcript level was calculated by formula $2^{[-\,(\mathrm{Ct}.AtMYB44\,-\,\mathrm{Ct}.AtACTIN2)]}$. Columns marked with an asterisk differ significantly ($P < 0.05$). Bars represent the standard error.

(Kwon et al., 2009). Although the expression level is determined not only by H3K27me3 deposition as proposed by Kwon et al. (2009), the H3K27me3-free status of the *AtMYB44* locus could also contribute to the rapid responsiveness of this gene to stress signal(s).

Recent studies have suggested that the replacement of H2A by histone variant H2A.Z promotes variability in gene expression (Deal and Henikoff, 2011; To and Kim, 2014). In general, H2A.Z-containing nucleosomes wrap DNA more tightly and thus their enrichment across a gene body is correlated with a low transcription level (Coleman-Derr and Zilberman, 2012). In a study of thermosensory response in Arabidopsis, Kumar and Wigge (2010) proposed that the occupancy of H2A.Z-containing nucleosomes can physically disturb the binding of RNAPII, which can suppress expression of their target genes. Sura et al. (2017) reported that upon drought stress, H2A.Z was removed from induced genes. More recently, we observed that H2A.Z occupation was significantly decreased at the *AtMYB44* promoter, transcription start site (TSS), and gene-body regions in response to salt stress (Nguyen and Cheong, 2018). Histone modifications including H3K4me3 and H3ac as well as H4ac were not affected under the salt stress conditions. In the present study, however, osmotic stress did not significantly alter the occupancy of the histone variant H2A.Z on the *AtMYB44* promoter, TSS, and gene-body regions (Fig. S6).

Henikoff and Shilatifard (2011) suggested that histone modification is a consequence, rather than a cause, of transcription and nucleosome remodeling. Each stress or phytohormone signal transduced via different signaling pathways may employ specific transcription factors (Knight and Knight, 2001; Depuydt and Hardtke, 2011). Benveniste et al. (2014) performed computational analyses of human cell lines and found that transcription factor binding at promoters predicts histone modification within a gene, which can be caused by interactions between transcription factors and their specific histone-modifying complexes. Weiste and Dröge-Laser (2014) observed that the Arabidopsis transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. Song et al. (2015) also showed that sequence-specific transcription factors interact with a COMPASS-like complex that activates histone methyltransferases to generate H3K4me3 for specific gene expression in Arabidopsis.

We observed that treatment with ABA, JA, mannitol, or salt resulted in different types of chromatin modification at the *AtMYB44* locus. Thus, it is unlikely that these signals generate a common message to cause identical chromatin modifications at the *AtMYB44* locus. Instead, the signals tested in this study may employ specific transcription factors that target the *AtMYB44* promoter and consequently recruit signal-specific histone-modifying enzymes to induce distinct chromatin modifications.

4. Conclusions

The low nucleosome density, lack of DNA methylation, and presence of different putative transcription factor-binding sites in the region suggest that the *AtMYB44* promoter is open and highly accessible to various *trans*-acting factors involving diverse signals. This explains why *AtMYB44* transcripts accumulate non-specifically in response to different stressful conditions and phytohormone treatments. We observed that treatment with ABA, JA, mannitol, or salt resulted in different chromatin modifications at the *AtMYB44* gene locus. Thus, it is unlikely that these signals generate a common message to cause identical chromatin modifications at the *AtMYB44* locus. Instead, each signal may employ specific transcription factors that target the *AtMYB44* promoter to recruit signal-specific histone-modifying enzymes and induce distinct chromatin modifications.

Conflicts of interest

The authors declare no conflicts of interest.

Author's contributions

NHN conducted the experiments and wrote the manuscript. JJC designed the research and edited the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2018.06.030.

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