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H2A.Z-containing nucleosomes are evicted to activate *AtMYB44* transcription in response to salt stress

Nguyen Hoai Nguyen, Jong-Joo Cheong*

Center for Food and Bioconvergence, Seoul National University, Seoul 08826, Republic of Korea

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ABSTRACT

Transcripts of the Arabidopsis transcription factor gene, *AtMYB44*, accumulate rapidly to mediate a tolerance mechanism in response to salt stress. The *AtMYB44* promoter is activated by salt stress, as illustrated in *AtMYB44pro::GUS* transgenic plants. Chromatin immunoprecipitation (ChIP) assays revealed that RNA polymerases were enriched on the *AtMYB44* gene, especially on TSS-proximal regions, and nucleosome density was markedly reduced in the *AtMYB44* gene-body region in response to salt stress. In addition, H2A.Z occupation was significantly decreased at the *AtMYB44* promoter, transcription start site (TSS), and gene-body regions. Histone modifications including histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 and H4 acetylation (H3ac and H4ac) were not affected under the same stress conditions. We found a decrease in the number of AtMYB44 proteins bound to their own gene promoters in response to salt stress. These results suggest that salt stress induces the eviction of H2A.Z-containing nucleosomes from the *AtMYB44* promoter region, which may weaken its affinity for binding AtMYB44 protein that acts as a repressor for *AtMYB44* gene transcription under salt stress-free conditions.

1. Introduction

Under drought conditions, the salt concentration in the soil increases as the moisture content decreases, and this can inflict serious stresses on growing plants. To overcome cellular ion imbalance under salt stress conditions, plants express a group of transcription factor genes at an early stage of the stress. Expression of the Arabidopsis transcription factor gene *AtMYB44* increases in leaf epidermal guard cells in response to salt stress [1]. Transgenic Arabidopsis [1] and soybean [2] overexpressing this gene exhibit enhanced tolerance to a sudden increase in the salt concentration in the soil.

In the chromatin of eukaryotic cells, genomic DNA is wrapped around a histone octamer consisting of H2A, H2B, H3, and H4, and together these components form a nucleosome. In gene transcription mediated by RNA polymerase, promoter activity is modulated by dynamic competition between nucleosomes and transcription factors. Thus, gene transcription is accompanied by nucleosome eviction or replacement with other nucleosomes with different compositions [3,4]. For instance, replacement of H2A with histone variant H2A.Z promotes variable gene expression without affecting gene DNA methylation [5,6]. In yeast, the chromatin remodeling SWR1 complex replaces H2A/H2B with H2A.Z/H2B dimers in nucleosomes, while the INO80 complex reverses this process [7]. H2A.Z-related nucleosomal reorganizations can change nucleosome structure, stability, and dynamics, leading to alterations in gene expression. In addition to alterations in nucleosome density and composition, gene transcription is also associated with other chromatin remodeling activities such as histone modification, DNA methylation, and small RNA-based chromatin modifications that can take place at the promoter, transcription start site (TSS), and gene-body regions [8,9]. In general, histone modifications including H3 lysine 4 trimethylation (H3K4me3) and H3 acetylation of lysine 9 (H3K9ac) or lysine 27 (H3K27ac) around the TSS are strongly associated with gene expression [10,11].

In the present study, we investigated the chromatin modifications that result in a significant increase in the number of *AtMYB44* transcripts in response to salt stress. We observed that H2A.Z occupation at the *AtMYB44* gene was significantly decreased at the promoter region. Furthermore, the binding of AtMYB44 proteins to their own gene promoters was clearly diminished under salt stress conditions. This suggests that salt stress induces the eviction of H2A.Z-containing nucleosome from the promoter region. This, in turn, alters the binding affinity of AtMYB44 protein, which acts as a repressor of *AtMYB44* gene transcription.

* Corresponding author.
E-mail address: cheongjj@snu.ac.kr (J.-J. Cheong).

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2. Materials and methods

2.1. Plant growth and treatment

Arabidopsis thaliana (ecotype Columbia) plants were grown on half-strength Murashige and Skoog (1/2 × MS) medium supplemented with 2% sucrose (w/v) and 1.2% phyto agar (w/v). The growth chamber conditions included a light cycle of 16-h on/8-h off, a light intensity of ~100 μmol photons m^{-2} s^{-1}, and a temperature of 23 ± 1 °C. To test the salt stress responses, two-week-old plants were transferred to liquid 1/2 × MS medium without NaCl (control) or supplemented with 250 mM NaCl, and grown for a further 6 h in the same growth chamber.

2.2. Histochemical β-glucuronidase (GUS) assay

Two-week-old transgenic Arabidopsis plants, *AtMYB44pro::GUS* #22 [1], were treated and used for GUS assays following procedures reported previously [1]. GUS activity was visualized as the presence of a blue precipitate in the plant tissue and photographed under a microscope (DE/Axio Imager A1, Carl Zeiss).

2.3. Quantitative reverse-transcription PCR (qRT-PCR)

Total plant RNA was prepared using a Spectrum Plant Total RNA kit (Sigma-Aldrich), and used for first-strand cDNA synthesis using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). For quantitative PCR (qPCR), SolGent 2 × Real-Time Smart Mix (SolGent) was used with specific DNA-primers (Supplementary Table 1). The Mx3005P qPCR system (Agilent Technologies) was used for qPCR and the thermocycling conditions were as follows: 95 °C for 15 min (1 cycle), 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s (40 cycles). The qPCR internal control was *AtACTIN2* (At3g18780).

2.4. Chromatin immunoprecipitation (ChIP) assay

Two-week-old Arabidopsis plants were used for cross-linking in 1% formaldehyde buffer, and then for the ChIP assay using an Epi-Quik Plant ChIP kit (Epigentek). Antibodies to RNA polymerase II (RNAPII) CTD repeat YSPTSPS (4H8), histone H3, histone H3 acetyl K9 (H3K9ac), histone H3 trimethyl K4 (H3K4me3), histone H2A.Z, and green fluorescent protein (GFP) were purchased from Abcam Co. Antibodies to acetyl-histone H3 (H3ac) and acetyl-histone H4 (H4ac) were from Merck Millipore. Normal rabbit IgG (Merck Millipore) was included as a negative control in the ChIP assay. The ChIP-qPCR experiments were repeated independently two or three times. Statistical analysis was performed using Duncan's test [12] at a 95% confidence level.

3. Results and discussion

3.1. Activation of the AtMYB44 promoter in response to salt stress

The qRT-PCR assays showed that treatment of two-week-old Arabidopsis with 250 mM NaCl strongly increased the level of *AtMYB44* transcripts, as well as those of the stress marker genes *RD29A* and *COR15A* (Fig. 1A). This was consistent with previous northern blot results [1]. In addition, *GUS* expression in the leaf epidermal guard cells of the *AtMYB44pro::GUS* transgenic plants was greatly enhanced when grown under salt stress conditions (Fig. 1B). These results indicate that the *AtMYB44* promoter is activated to induce the accumulation of gene transcripts in response to salt stress.

Genome-wide analysis (http://epigenomics.mcdb.ucla.edu) has

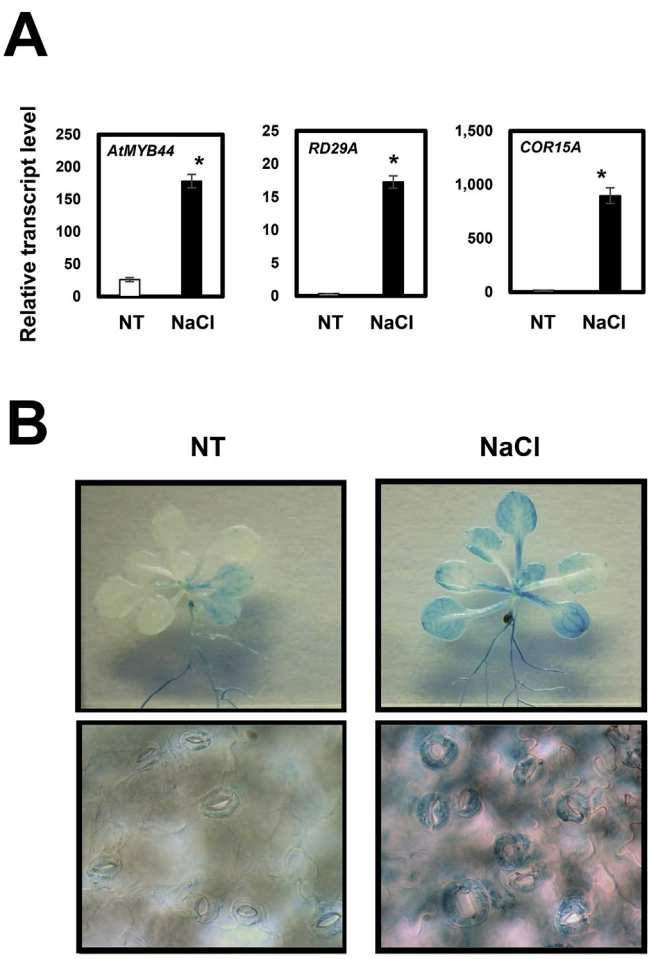

Fig. 1. Expression of *AtMYB44* gene in response to salt stress. A, Accumulation of *AtMYB44* gene transcripts under abiotic stress. Two-week-old wild-type (Col-0) plants growing on 1/2 × MS medium (2% sucrose) were carefully transferred to 1/2 × MS liquid medium (2% sucrose) without NaCl (NT) or supplemented with 250 mM NaCl (NaCl) for 6 h and used for total RNA extraction. qRT-PCR was performed using specific primers (Supplementary Table 1). *AtACTIN2* was used as an internal control. The experiments were performed three independent times and in triplicate for each. Columns marked with an asterisk indicate significant difference ($P < 0.05$). Bars represent standard error. B, Activation of the *AtMYB44* promoter in response to salt stress. Two-week-old *AtMYB44pro::GUS* #22 (Jung et al., 2008) transgenic Arabidopsis plants were used for histochemical β-glucuronidase (GUS) assays. GUS activity was visualized following the protocol described by Jung et al. (2008) using a microscope (DE/Axio Imager A1, Carl Zeiss).

shown that the *AtMYB44* promoter region is methylation-free and contains two nucleosome-rich regions on the *AtMYB44* gene-body [13,14]. Different primer sets (Supplementary Table 1) were designed to detect the *AtMYB44* promoter, TSS, and gene-body regions (as depicted in Fig. 2) and used in ChIP-qPCR assays. ChIP-qPCR with anti-RNAPII antibody revealed that RNA polymerases were enriched on the *AtMYB44* gene, especially on TSS-proximal regions, under salt stress conditions (Fig. 2A). In the control experiments, RNA polymerases were also enriched on two stress marker genes, *RD29A* and *COR15A* (Supplementary Fig. 1). These results indicate that the *AtMYB44* transcript is upregulated in response to salt stress via RNA polymerase-mediated transcription, and not by reducing the degradation of constitutively-expressed gene transcripts. Together, the results show that salt stress upregulates *AtMYB44* expression by enhancing its own promoter activity, leading to the recruitment of RNA polymerases to facilitate gene transcription.

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