Involvement of Arabidopsis HOS15 in histone deacetylation and cold tolerance

Jianhua Zhu*,†1, Jae Cheol Jeong1, Yanmei Zhu1, Irina Sokolchik1, Saori Miyazaki1, Jian-Kang Zhu2, Paul M. Hasegawa3, Hans J. Bohnert1,‡, Huazhong Shi*,‡‡, Dae-Jin Yun*,‡‡, and Ray A. Bressan†1

*State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100094, China; 
1Department of Horticulture and Landscape Architecture, Center for Plant Environmental Stress Physiology, Purdue University, West Lafayette, IN 47907-2010; 2Department of Botany and Plant Sciences, University of California, Riverside, CA 92521; 3Environmental Biotechnology National Core Research Center and Division of Applied Life Science, BK21 Program, Graduate School of Gyeongsang National University, Jinju 660-701, Korea; 
4Departments of Plant Biology and of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801; and 5Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409

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Histone modification in chromatin is one of the key control points in gene regulation in eukaryotic cells. Protein complexes composed of histone acetyltransferase or deacetylase, WD40 repeat protein, and many other components have been implicated in this process. Here, we report the identification and functional characterization of HOS15, a WD40-repeat protein crucial for repression of genes associated with abiotic stress tolerance through histone deacetylation in Arabidopsis. HOS15 shares high sequence similarity with human transducin-beta like protein (TBL), a component of a repressor protein complex involved in histone deacetylation. Mutations of the HOS15 gene renders mutant plants hypersensitive to freezing temperatures. HOS15 is localized in the nucleus and specifically interacts with histone H4. The level of acetylated histone H4 is higher in the hos15 mutant than in WT plants. Moreover, the stress inducible RD29A promoter is hyperinduced and associated with a substantially higher level of acetylated histone H4 in the hos15 mutant under cold stress conditions. Our results suggest a critical role for gene activation/repression by histone acetylation/deacetylation in plant acclimation and tolerance to cold stress.

Plants respond to environmental stresses by altering the expression of many genes. The altered status of gene expression is closely associated with an acclimated condition that renders the plant more stress tolerant. Among the genetic mechanisms leading to these gene expression changes, chromatin remodeling has until recently received little attention. In eukaryotes, transcriptional control of gene expression occurs within the context of chromatin that is composed of nucleosomal subunits. Each nucleosome consists of ~146 bp of DNA wrapped twice around an octamer of core histones (H2A, H2B, H3, and H4). Individual nucleosomes and more compacted structures in chromatin are refractory to transcription. Chromatin remodeling, which alters accessibility of genes to transcriptional regulatory proteins, is now recognized as a central component of gene regulation associated with a metastable (epigenetic) genetic status.

Two general types of chromatin-modifying complexes have been described. One type covalently modifies histone N-terminal tails protruding from the nucleosome core (e.g., by acetylation, methylation, or phosphorylation) (1, 2). The second class controls the ATP-dependent nucleosome-remodeling complexes that noncovalently modify and reposition nucleosomes in the chromatin structure (3, 4). Acetylation and deacetylation of lysine residues in the N termini of histones represent extensively studied types of chromatin modifications that have been shown to play fundamental roles in diverse chromatin-based processes. Hyperacetylation of histones H2B, H3, and H4 has been generally associated with transcriptionally active chromatin (5), whereas the chromatin of inactive regions is enriched in deacetylated histones (6). Additional support for the existence of a direct molecular link between histone acetylation status and transcription regulation (7–9) is provided by the finding that many transcriptional coactivators, including GCN5, PCAF, CBP/p300, and SRC-1/ACTR, possess intrinsic histone acetyltransferase activity. Moreover, transcriptional repressors such as NuRD, SIN3, Groucho/Tup1, and SMRT/N-CoR associate with histone deacetylases (7–9). In plants, histone modification has been shown to be involved in metastable changes required to maintain altered cellular and tissue properties after several rounds of mitosis (10). For example, repression of FLC gene expression during vernalization in Arabidopsis was reported to be associated with histone deacetylation and methylation (11, 12).

Because plants must produce new cells and continue growth during and after adaptation (acclimation) to new environments, it could be expected that chromatin-mediated metastable genetic changes are involved in the process. However, evidence for the involvement of chromatin remodeling in gene expression and adaptation responses to the environment remains elusive. Here, we report that HOS15, a WD40-repeat protein, functions to control gene expression through histone deacetylation in chromatin. HOS15 was identified in a forward genetic screen for mutations that alter abiotic stress signaling (13, 14). The hos15 mutant plants accumulate higher levels of transcripts of many stress-regulated genes and are hypersensitive to freezing temperature. HOS15 interacts specifically with and promotes deacetylation of histone H4, indicating that chromatin remodeling plays an important role in gene regulation in plant responses and tolerance to abiotic stresses.

Results

Isolation of the hos15 Mutant. A large population of Arabidopsis plants expressing a RD29A::LUC fusion gene (13) were mutagenized with T-DNA, and the T2 progeny were screened for altered expression of RD29A::LUC in response to low temperature, ABA, or osmotic treatment. The hos15 mutant showed a substantially higher level of RD29A::LUC expression in response to all treatments (Fig. 1). All of 67 F1 plants from a hos15 x WT cross exhibited a WT phenotype. The selfed F2 progeny segregated...
was used as the loading control.

stress-responsive genes, COR15A
light (16 h of light; 8 h of darkness) for indicated time points. (Fig. 2B). This suggests that HOS15 affects the expression of CBF transcription.

To identify other targets of HOS15 gene regulation, comprehensive analysis of gene expression profiles in the hos15 plants under cold stress was achieved through the use of Affymetrix microarrays. The microarray data were analyzed with affylinui software included in the R package (15–17). A total of 136 genes showed higher expression levels by at least 2-fold in hos15 plants compared with WT when exposed to cold [supporting information (SI) Dataset S1]. These genes did not appear to be restricted to specific functional categories because they are annotated to encode proteins with diverse cellular functions. The expression of five genes (At5g64000, At5g61900, At2g18660, At1g35230, and At4g36110) randomly selected from the 136 was validated by real-time RT-PCR analyses (Fig. S1).

By comparing our results with the published microarray data, we found that, among the 136 genes that are more expressed in hos15 compared with WT plants after cold treatment, 39 were identified as cold-induced in WT plants (Dataset S2 and refs. 18–22). The proportion of cold-induced genes that are hyperinduced in hos15 (28.7%) is much higher than that for genes in general, which range from 2.3% to 10.8% (18–22). Interestingly, database comparisons also revealed that among the 136 genes, 8 whose transcripts are more abundant in cold-treated hos15 plants compared with WT plants are actually down-regulated in WT plants by cold (Dataset S3 and refs. 18–22). Real-time RT-PCR analysis of four of these genes confirmed that their transcripts were more abundant in hos15 plants with or without cold treatment but were reduced in WT after cold stress (Fig. S2). All of these except PR5 have unknown functions (23, 24). Statistical analysis and database comparisons also revealed that expression of 27 genes with various functions was reduced in hos15 plants after cold treatment compared with WT (Dataset S4). None of these are cold-inducible and only four of them (At5g24780, At2g3770, At3g28270, and At1g62510) are down-regulated by cold in WT (18–22). Real-time RT-PCR analysis of four of these genes confirmed that their transcripts were reduced in hos15 plants under cold treatment (Fig. S3).

HOS15 is a Negative Regulator of Stress-Regulated Gene Expression. Consistent with the RD29A::LUC expression patterns, induction of the luciferase transcript and the endogenous RD29A gene transcript was substantially higher in the hos15 mutant than WT after stress treatments (Fig. 2A). The expression of two other stress-responsive genes, COR15A and ADH1, was also more induced in hos15 plants in response to stress treatments (Fig. 2A). We also examined whether the expression levels of the CBF transcription factors are altered in the hos15 mutant plants. There was only a slight reduction in transcript levels of CBF1, CBF2, and CBF3 in the hos15 mutant plants 2 h after cold stress (Fig. 2B).

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Forty-five out of 52 homozygous hos15 plants transformed with the WT K8K14.4 gene in the T2 generation displayed WT phenotypes (Fig. 4B and C). Thus, disruption of At5g67320 in hos15 plants appears to be responsible for the observed altered phenotypes.

Analyses of two HOS15 full-length cDNA sequences of G4GST7 and M66M07STM (GenBank accession no. BE526642) indicated that the HOS15 gene contains 14 exons and 13 introns (Fig. 4A). Real-time RT-PCR analysis revealed that the HOS15 gene is slightly up-regulated by cold, NaCl and ABA treatments (Fig. 4D). Amino acid sequence analysis suggested that HOS15 encodes a protein containing a LisH motif and eight WD40-repeats (Fig. 4A). HOS15 shares high similarity with human transducin beta-like 1 (TBL1) proteins (26–28) (Fig. 4E).

**HOS15 is Important for Histone Deacetylation.** In humans, TBL1 interacts with histones and functions in a repressor protein complex to regulate gene activity. Fig. 5A shows that HOS15 can interact with histone H4 but not histone H2B, which appears to be different from human TBL1 that can interact with both histone H4 and H2B (27). Differences in N-terminal sequences between TBL1 and HOS15 may explain the different histone interaction preferences of these two proteins (29). However, an interaction of HOS15 with H2B may not easily be observed in the yeast nucleus, as used in our assay. Confocal microscopy revealed that a HOS15-GFP fusion protein is predominantly localized in the nucleus as expected for a repressor complex protein (Fig. 5B).

hos15 mutant plants accumulated a higher level of nuclear tetra-acetylated histone H4 compared with WT plants (Fig. 5C) after cold treatment. Thus, HOS15 not only interacts with histone H4 but also regulates the acetylation status of histone H4. Moreover, using a chromatin immunoprecipitation (ChIP) assay, we analyzed acetylation of histone H4 associated with the RD29A promoter, one of the hyperinduced genes in the hos15 mutant. Chromatin of WT and hos15 plants was immunoprecipitated by using antibody against tetra-acetylated H4. As shown in Fig. 5D, RD29A promoter DNA was more abundant in precipitated chromatin from the hos15 mutant than from WT plants. Thus, HOS15 appears to function in the repression of RD29A expression by facilitating deacetylation of histone H4 associated with the RD29A promoter.

**HOS15 Has Repressor Activity.** Because HOS15 appears to function as a part of a transcriptional corepressor complex, we used a transient expression assay to test the potential gene repression function of HOS15. HOS15 fused to the yeast Gal4 BD effector and a constitutively expressed reporter gene containing four upstream Gal4 binding sites (Gal4 (4X)-D1–3(4X)-GUS) was cotransfected into Arabidopsis proplasts (Fig. 5E) (30). As expected, HOS15 caused repression of the reporter gene expression by a similar amount (≈60%) conferred by a known repressor protein, ARF1M (Fig. 5E). This result provides direct evidence that HOS15 has a repression function in vivo.
Discussion

Our results suggest that HOS15 functions as a repressor to control gene expression important to cold tolerance through chromatin modification. Sequence analysis indicates that HOS15 protein shares highest similarity with the human protein TBL1, i.e., NCoR/SMART corepressor, suggesting that these proteins interact with TBL1, N-CoR, and N-CoR exists as large protein complexes with an estimated size of 1.5–2.0 MDa and are primarily associated with TBL1 and HDAC (26, 27, 29, 33). Even though HOS15 resembles TBL1 and appears to function as a component in a protein repressor complex, BLAST searches against Arabidopsis databases failed to identify any significant homologs of proteins that interact with TBL1, i.e., NCoR/SMART corepressor, suggesting that these components in a repressor complex involving HOS15 do not share a high level of sequence identity with those in animal cells.

The involvement of the chromatin acetylation/deacetylation process in cold tolerance (34) suggests that before and during cold acclimation, histone acetylation (reduced corepressor activity) of positive effector genes of cold tolerance, and deacetylation (increased corepressor activity) of negative effector genes of cold tolerance help determine the cold tolerance status of the plant. Genes that fail to become repressed in the hos15 mutant background (Fig. S2) are candidates for such negative effectors that could contribute to the cold sensitivity of the hos15 mutant, even though it exhibits hyperinduction of many cold regulator genes. There were a number of genes with lower levels of expression in hos15, and some of these could be key positive effectors, so their reduced expression may contribute to the freezing sensitivity of the mutant. The precise negative and positive effectors influenced by the hos15 mutation that may explain the observed cold sensitivity of the mutant are unknown. However, it is clear that modification of these targets impact cold tolerance more than the up-regulated COR genes, because the hos15 mutation suppresses any influence of their overexpression and causes loss of cold tolerance. As we have indicated for other cold sensitive mutants (14), hyperexpression of COR genes also may result from an increased signal resulting from hypersensitivity to cold.

Chromatin remodeling is associated with the control of gene expression to establish different epigenetic states of the cell. Epigenetic changes are normally considered to be the main basis of cell type identity and organization (e.g., production of petal cells versus leaf cells during the change from vegetative to floral status). In fact, histone modification has been shown to control the cold-induced (vernalization) flowering response (35). From our results, it is now apparent that altered epigenetic status also is involved in the establishment of a condition of tolerance to different environments as suggested (36).

It is hard to conceive of very specific gene targets for hos15, considering the known general function of the repressor complex, unless a large number of different complexes form from various combinations of different family members of the components, including the HOS15/TBL1-like protein family. Alternatively, partial loss of function of the repressor complex could simply shift its activity to a target discrimination status, influenced by environmental factors such as cold. Several other gene products with traditionally understood “housekeeping” functions also present specific phenotypes upon mutation (37). Identification of other components of the repressor protein complex involving HOS15 and its direct target genes and their functions will certainly reveal more about the role of chromatin structure dynamics in plant cold tolerance.
Materials and Methods

Isolation of the hos15 Mutant. A T-DNA insertional population of Arabidopsis thaliana plants (ecotype C24) expressing the homozygous transgene R2D29A::LUC (13), referred to as WT) was generated by using Agrobacterium tumefaciens-mediated transformation. T2 seeds of transgenic plants were used to screen for mutants exhibiting altered expression of R2D29A::LUC in response to cold, ABA, and/or osmotic stress by using a luminescence imaging system as described in ref. 13. The hos15 mutant was identified as showing higher expression of R2D29A::LUC gene in response to cold, salt, and ABA treatments.

Freezing Tolerance. Cold-acclimated and nonacclimated hos15 and WT plants grown in soil at the rosette stage were used for a whole plant freezing tolerance tests and an electrolyte leakage assay as described in refs. 14, 38, and 39.

Northern Blot Analysis. For gene regulation studies, 2-week-old WT and hos15 seedlings grown on germination medium [1× Murashige and Skoog salts, 2% sucrose (pH 5.7)] were untreated or treated with either low temperature, ABA, or NaCl. Total RNA extraction and subsequent RNA gel analysis were performed as described in ref. 40. The gene specific probes were amplified by PCR with primers as listed in Dataset S5.

Cloning of HOS15 and Construction of Plasmids. DNA flanking the left border of the inserted T-DNA in hos15 plants was isolated by thermal asymmetric interlaced (TAIL) PCR (40). The HOS15 gene was amplified by PCR, using primers 15A and 15B. The resulting PCR fragment was cloned into the binary vector pCAMBIA1200 between the KpnI and XbaI sites and the identity of the insert was confirmed by sequencing. The resulting construct was then introduced into hos15 mutant plants through Agrobacterium tumefaciens-mediated (strain GV3101) T-DNA transformation. Primary transformants were isolated on MS medium containing 50 mg/liter hygromycin (Invitrogen). Progenies of the transformants were evaluated for R2D29A::LUC expression and freezing tolerance.

The coding region of HOS15 was amplified from the full-length cDNA clone GSG47T (accession no. N96838) by PCR with the primers 15C and 15D. The PCR fragment was fused in-frame at the c-terminus of GFP in the pEGAD vector. The resulting construct was introduced into Arabidopsis WT plants through Agrobacterium tumefaciens-mediated (strain GV3101) T-DNA transformation. The analysis of GFP expression of the transgenic plants that were resistant to 5 mg/ml gatifloxacin (Inviron). Progenies of the transformants were evaluated for R2D29A::LUC expression and freezing tolerance.

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Real-Time PCR Analysis. Real-time RT-PCR analysis was performed as described in ref. 42. Primers used for real-time RT-PCR analysis are listed in Dataset S5.

Yeast Two-Hybrid Assays. The HOS15 coding region was amplified by PCR and cloned in-frame between the NcoI and SmaI sites of pAS2, resulting in the bait plasmid pAS2-HOS15. HT81 (histone H2B (A1g07790) and HFO1 [histone H4 (A13g6320)] coding regions were amplified by PCR and in-frame cloned into pACT2 between the BamHI and XhoI sites to generate prey constructs, pACT2-HT81 and pACT2-HFO1. Plasmid DNA of bait and prey constructs was transformed into the Saccharomyces cerevisiae strain Y190. Individual colonies of transformants were streaked on agar plates containing complete (SC) media lacking tryptophan and leucine and grown for 24 h. Yeast cells were transferred onto a nitrocellulose transfer membrane (NT BioTrace, 0.45 μm), and β-galactosidase (β-gal) filter assays were performed as described in ref. 43.

Determination of Histone Acetylation. Nuclei were isolated from 500 mg of Arabidopsis seedling tissues by using Honda’s buffer as described in ref. 44. Purified nuclei were resuspended and briefly sonicated in PBS buffer. Twenty micrograms of nuclear protein and 1 μg of purified core histones from chicken (Upstate Biotechnology) were separated by SDS/PAGE and blotted onto a PVDF membrane (Milligore). Anti-tetra-acetylated-histone H4 (1:5,000) or anti-histone H4 (1:2,000) (Upstate Biotechnology) primary antibodies, anti-rabbit horseradish peroxidase coupled antibody, and the ECL system (Amer sham) were used to detect acetylated and unacetylated histone H4.

ChIP Assay. The ChIP assay was performed following the protocol established in ref. 45. With 300 mg of 2-week-old plants, and the same anti-tetra-acetylated-histone H4 antibody that was used for Western blot analysis. Precipitated DNA was dissolved in 75 μl of TE and 2 μl of was used for PCR. Quantitative PCR was used to determine the amounts of genomic DNA immunoprecipitated in the ChIP experiments. The primers used in ChIP assays are listed in Dataset S5.

Gene Repression Assay. The reporter construct containing the GUS reporter gene used in this study was described in ref. 30. Effector genes were under the control of the CaMV 35S double enhancer promoter followed by a transla tional enhancer from the Tobacco mosaic virus 5′ leader sequence (46) and a 3′ nopaline synthase untranslated region. The fusion constructs were introduced into Arabidopsis leaf protoplasts from 2-week-old seedlings by PEG-mediated transformation as described in ref. 47. The fluorometric GUS activity assay was performed as described in ref. 48. The control plasmid carrying the luciferase gene under the control of the 35S promoter was used as an internal control to normalize the data for eliminating variations in the experimental conditions (49).

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