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The SWI/SNF subunit SWI3B regulates IAMT1 expression via chromatin remodeling in Arabidopsis leaf development

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ABSTRACT

The SWI/SNF complex is crucial to chromatin remodeling in various biological processes in different species, but the distinct functions of its components in plant development remain unclear. Here we uncovered the role of SWI3B, a subunit of the *Arabidopsis thaliana* SWI/SNF complex, via RNA interference. Knockdown of *SWI3 B* resulted in an upward-curling leaf phenotype. Further investigation showed that the RNA level of *IAA carboxyl methyltransferase 1* (*IAMT1*), encoding an enzyme involved in auxin metabolism, was dramatically elevated in the knockdown (*SWI3B-RNAi*) plants. In addition, activation of *IAMT1* produced a leaf-curling phenotype similar to that of the *SWI3B-RNAi* lines. Database analysis suggested that the last intron of *IAMT* contains a site of polymerase V (Pol V) stabilized nucleosome, which may be associated with SWI3B. Data from a micrococcal nuclease (MNase) digestion assay showed that nucleosome occupancy around this site was downregulated in the leaves of *SWI3B-RNAi* plants. In addition, knockdown of *IAMT1* in the *SWI3B-RNAi* background repressed the abnormal leaf development. Thus, SWI3B-mediated chromatin remodeling is critical in regulating the expression of *IAMT1* in leaf development.

1. Introduction

Establishment and regulation of chromatin structure is critical for many processes, such as DNA replication and gene transcription, that are essential for normal development of all organisms. The accessibility of chromatin to protein factors is regulated by nucleosome sliding or disruption of histone–DNA interactions mediated by ATP-dependent chromatin remodeling complexes, including the SWI2/SNF2, ISWI, Mi-2 (CHD1) and INO80 subfamilies [1,2]. The functional core of the SWI/SNF complex includes a SWI2/SNF2 ATPase, SNF5, SWP73, and a pair of SWI3 subunits, conserved in different organisms [3]. The SWI/SNF complex may target promoters and other regulatory elements to act as activators or repressors of gene expression by changing the stability of nucleosomes [4]. The complex is important for hormone crosstalk and has roles in development and stress responses in plants [5]. For instance, Brahma (BRM), the central DNA-dependent ATPase in the complex, is involved in the embryonic, vegetative and reproductive development of plants [6–9].

Arabidopsis thaliana encodes four SWI3 proteins with functional variations [10] that may be dependent on their different combinations in SWI/SNF complexes in plant cells. For instance, mutation of *SWI3A* or *SWI3 B* results in disruption of embryo development, while mutation of *SWI3C* or *SWI3D* causes dwarfism, abnormal vegetative development and reduced fertility [10]. SWI3 B contains a SWIRM domain, a SANT domain and a leucine zipper, and can form either homodimers or heterodimers with other SWI3 subunits. A previous study showed that SWI3 B interacts with HYPERSENSITIVE TO ABA1 (HAB1) and is involved in ABA signal transduction [11]. A long noncoding RNA (lncRNA)-binding protein, IDN2, has also been found to interact with SWI3 B in guiding the SWI/SNF complex and establishing nucleosomes in specific genomic regions for generation of lncRNA [12], providing evidence for the association between chromatin remodeling and transcriptional silencing in plant cells.

Because T-DNA insertions in *SWI3 B* result in arrest of embryo development at the globular stage and severe repression of *SWI3 B* leads to variations of abnormal pleiotropic development [10,13], we tried to

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specifically knock down the expression of this gene by RNA interference to characterize its function in the vegetative stage. Surprisingly, knockdown of *SWI3 B* resulted in an upward leaf-curling phenotype. Previous studies have shown that mutant plants deficient in cell division, polarity, and auxin response exhibit abnormal leaf curvature [14,15]. We found that the RNA level of *IAA carboxyl methyltransferase 1* (*IAMT1*), encoding a protein involved in the conversion of IAA to methyl IAA ester (MeIAA) [16,17], was much higher in the knockdown (*SWI3B-RNAi*) plants than in wild-type plants. Overexpression of *IAMT1* dramatically altered the leaf curvature of Arabidopsis, producing a phenotype similar to that of *SWI3 B* knockdown plants. Micrococcal nuclease (MNase) digestion analysis indicated that nucleosome occupancies in the *IAMT1* genomic region differ between the wild-type and *SWI3B-RNAi* leaves. Knockdown of *IAMT1* in the *SWI3B-RNAi* plants repressed the leaf-curling phenotype, suggesting that *IAMT1* is an important target of SWI3 B in the regulation of leaf development.

2. Materials and methods

2.1. Plant materials and growth conditions

The RNAi transgenic lines used in this study were generated in the *Arabidopsis thaliana* Columbia-0 (WT) background. The *IAMT1-D* [16] seeds were obtained from the Qin laboratory. Seeds were surface sterilized for 2 min in 75% ethanol followed by 5 min in 1% NaClO solution, rinsed five times with sterile water, plated on Murashige and Skoog (MS) medium with 1.5% sucrose and 0.8% agar, and stratified at 4 °C in the dark for 2 d. Plants were grown under long-day conditions (16 h of light/8 h of dark, 80 $\mu E\, s^{-1}\, m^{-2}$ light intensity) at 22 °C.

2.2. Generation of transgenic plants

To generate the constructs for RNAi, the fragment with an intron and multiple cloning sites was cut from *pYLRNAi.5* by *Bam*HI and *Pst*I and ligated into *pCambia1302* to generate the *pCambia1302-RNAi* vector. For the *SWI3B-RNAi* construct, a specific fragment of *SWI3 B* was obtained by RT-PCR amplification and cloned into the *pCambia1302-RNAi* vector in sense and antisense orientations adjacent to an intron. The fragment including both orientations and the intron was subcloned into the *pFGC5941* vector with *Xho*I/*Spe*I. For the *IAMT1-RNAi* construct, a fragment of *IAMT1* was amplified and cloned into the *pCambia1302-RNAi* vector in sense and antisense orientations adjacent to an intron, and then subcloned into the *pCanG-myc* vector with *Bam*HI/*Spe*I. The constructs were transformed into Agrobacterium *EHA105*, which was then used to transform *Arabidopsis thaliana* (Columbia) by the floral-dip method [18]. Homozygous lines of transgenic plants were used in this study. The sequence information of the primers used in this study was listed in Table S1.

2.3. Leaf section analysis

The paraffin section analysis was performed as previously described [19] with modifications. The mature rosette eighth leaves were collected and fixed in FAA (formaldehyde/acetic acid/ethanol) for 12 h. Samples were vacuumed for 1 h and kept at room temperature for 2 days, and then dehydrated with a graded ethanol series and embedded in paraffin. Sections (around 8–10 mm thick) were cut with a microtome (Leica), stained with 1% (w/v) safranin O and 1% (w/v) fast green FCF, detected with a microscope and photographed.

2.4. Real time PCR analysis for gene expression

Total RNA was extracted using the Plant RNAprep Pure Kit (TIANGEN) with DNase I treatment following the manufacturer's instructions. Reverse transcription was performed using a PrimeScript RT Reagent Kit (Takara). The complementary DNA was subjected to PCR using SYBR Premix Ex Taq (Takara) in a Bio-Rad CFX 96 system (C1000 Thermal Cycler) and detected with Bio-Rad CFX Manager software (Bio-Rad, Hercules, CA). Real time RT-PCR was performed with three replicates and *ACTIN2* as a reference gene.

2.5. Nuclear extraction, MNase treatment and DNA purification

Two grams of 3.5-week-old plants without roots were ground in liquid nitrogen and resuspended in 15 mL lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol, 5 mM β-mercaptoethanol and 1 × protease inhibitors) for 1 h at 4 °C. After filtration through two layers of Miracloth, the combined filtrates were centrifuged (3200*g*, 20 min at 4 °C). The pellet was washed once with HBB buffer (25 mM Tris-HCl pH 7.6, 0.44 M sucrose, 10 mM $MgCl_2$, 0.1% Triton X-100 and 10 mM β-mercaptoethanol) and once with HBC buffer (20 mM Tris-HCl, pH 7.5, 352 mM sucrose, 8 mM $MgCl_2$, 0.08% Triton X-100, 8 mM β-mercaptoethanol and 20% glycerol) [20]. The obtained nuclei were resuspended with 650 µL (100 µL input, 550 µL treated sample) of MNase reaction buffer (10 mM Tris-HCl, pH8, 15 mM NaCl, 60 mM KCl, 1 mM $CaCl_2$), and the isolated chromatin was digested with 0.1 units/µL–0.2 units/µL (final concentration) of Micrococcal Nucleases (Takara) for 13 min in reaction buffer at 37 °C. To terminate the reaction, 50 µL of stop buffer (100 mM EDTA, 100 mM EGTA), 50 µL 10% SDS, and 40 µg proteinase K (Roche) was added and samples were incubated at 60 °C for 1 h. Samples were incubated with 1 U RNase (Ambio) at 37 °C for 1 h and then at 4 °C overnight [12]. DNA was purified with phenol/chloroform extraction and precipitated with salt and ethanol. Purified DNA was run on a 1.5% agarose gel, and the 150-bp bands corresponding to the mononucleosomal fraction were excised [21] and extracted with the Gel Extraction Kit. The quantitative PCR results were calculated as percentage of input DNA followed by normalization over the *gypsy-like retrotransposon* (*AT4G07700*) +1 loci for each sample [22].

2.6. Accession numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative and GenBank/EMBL databases under the accession codes given in parentheses: *SWI3 B* (AT2G33610); *IAMT1* (AT5G55250); *YUC1* (*AT4G32540*); *YUC6* (*AT5G25620*); *IAA3* (*AT1G04240*); *IAA17* (*AT1G04250*); *AXR6* (*AT4G02570*); *HASTY* (*AT3G05040*).

3. Results

3.1. The SWI3B-RNAi plants display an upward-curling leaf phenotype

Because the T-DNA mutants of *SWI3 B* are embryo lethal [10], to characterize the functions of this gene in vegetable development, we knocked down *SWI3 B* by targeting a specific region (a 253 bp fragment from positions 508–761 of the coding sequence) via RNA interference. As a result, the RNA level of *SWI3B*, but not those of the other SWI3 subunits, was dramatically decreased in these transgenic lines (Fig. S1). The *SWI3B-RNAi* plants displayed normal leaf development in the first three weeks after germination. Surprisingly, some leaves started to curl upward in the fourth week (Fig. 1A). More than ten independent transgenic lines were obtained, and all of them had similar phenotypes, suggesting SWI3 B is important for leaf curvature. Although the *SWI3B-RNAi* plants also displayed other phenotypes, such as shorter primary roots and altered responses to ABA and IAA (Fig. S2), we focused on the protein's regulatory mechanism in leaf development. The data from leaf sections supported the possibility that the leaf structure was altered in the absence of *SWI3 B* (Fig. 1B). Two representative lines (Fig. 1A, C) were chosen for further analysis.