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The histone methyltransferase SDG8 regulates shoot branching in *Arabidopsis*

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ABSTRACT

Histone lysine methylation is an evolutionally conserved modification involved in determining chromatin states associated with gene activation or repression. Here we report that the *Arabidopsis* SET domain group 8 (SDG8) protein is a histone H3 methyltransferase involved in regulating shoot branching. Knockout mutations of the *SDG8* gene markedly reduce the global levels of histone H3 trimethylation at lysines 9 and 36 as well as dimethylation at lysine 36. The *sdg8* mutants produce more shoot branches than wild-type plants. The expression of *SPS/BUS* (supershoot/bushy), a repressor of shoot branching, is decreased in *sdg8* mutants, while *UGT74E2* (UDP-glycosyltransferase 74E2), a gene associated with increased shoot branching, is up-regulated in *sdg8* mutants. The altered expression of *SPS/BUS* and *UGT74E2* correlates with changed histone H3 methylation at these loci. These results suggest that SDG8 regulates shoot branching via controlling the methylation states of its target genes.

Histone lysine methylation is critical to many important biological processes such as transcriptional regulation, heterochromatin formation, stem cell maintenance and differentiation, and genome integrity [1–3]. Histone H3 can be methylated at lysine residues 4, 9, 27, 36, and 79 and histone H4 can be methylated at lysine 20 [1,2]. Further, lysine methylation can exist in three distinct states: monomethylated, dimethylated, and trimethylated. Methylation at different lysine residues and different methylation states at the same lysine residue differentially impact chromatin structure and transcription [1,2].

All known lysine methylation of histones, with the exception of H3 lysine 79 methylation, is carried out by methyltransferases that contain the SET-domain. This is an evolutionary conserved domain named after three *Drosophila* proteins; suppressor of variegation 3–9, enhancer of zeste, and trithorax [4]. The *Arabidopsis* genome encodes at least 30 SET-domain proteins [5,6], and some of these proteins have been shown to be histone methyltransferases. For instance, the *Arabidopsis* trithorax-like protein 1 (ATX1) and ATX2 can trimethylate and dimethylate lysine 4 of histone H3, respectively [7]. In addition, several *Arabidopsis* SET-domain proteins have been shown to mono- and/or dimethylate H3 lysine 9 [8–11]. However, methyltransferases responsible for trimethylation of H3 lysine 9 remains to be identified in plants. The SET-domain group protein 8 (SDG8), also known as EFS (Early Flowering in Short days), is thought to be an H3 lysine 4 methyltransferase or an H3 lysine 36 methyltransferase [12–14]. SDG8 has been shown to regulate flowering time [12–14] and may play an important role in regulating shoot branching (this paper).

Shoot branching enhances vegetative growth and generates multiple sites for seed production, thus serves as a major determinant of plant biomass and seed yield. In flowering plants, shoot branches originate from undifferentiated stem cells within leaf axils called axillary meristems [15]. After initiation, axillary meristems develop into axillary buds. The buds may grow out to become a branch or remain dormant in the axils of leaves until outgrowth is promoted. In *Arabidopsis*, a number of genes have been found to regulate shoot branching. These genes control shoot branching through affecting axillary meristem initiation and/or axillary bud outgrowth [15]. For example, the *LATERAL SUPPRESSOR* (*LAS*) gene is required for the initiation of axillary meristems [16], while *MAX* (more axillary growth) and *BRC1/TBL1* (branched1/teosinte branched1-like 1) genes are involved in the regulation of axillary bud outgrowth [15,17,18]. The *SPS/BUS* (supershoot/bushy, hereafter referred to as SPS) gene, encoding a cytochrome P450, represses both axillary meristem initiation and axillary buds outgrowth [19,20]. In addition, increased expression of *UGT74E2*, a UDP-glucosyltransferase gene potentially involved in auxin catabolism, correlates with increased shoot branching [21,22].

In the present study, we describe the involvement of SDG8 in the regulation of shoot branching. SDG8 knockout mutants exhibited increased shoot branching, repression of *SPS* transcript, and a significant increase in *UGT74E2* transcript. The altered expression of *SPS* and *UGT74E2* correlates with changed H3 methylation patterns at both loci. Thus, these data suggest SDG8 plays an important role in regulating the expression of genes controlling shoot branching in *Arabidopsis*.

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Materials and methods

Plant growth conditions. Arabidopsis wild-type (Columbia-0) and *sdg8* mutant plants were grown under compact fluorescent lights (100 μmol m^{-2} s^{-1}, 14 h-light/10 h-dark cycle) in Schultz potting soil at 22 °C in a growth room.

Isolation of sdg8 T-DNA insertion mutants. Two T-DNA insertion lines for *SDG8* (SALK_014569 and SALK_026442) were obtained from the SALK collection [23] at the Arabidopsis Biological Resource Center. T-DNA insertions were verified by PCR genotyping using T-DNA left border and SDG8 gene-specific primers. Homozygotes of each line were identified by PCR from F2 generation plants. Segregation analysis of kanamycin-resistant marker indicates that there is only one T-DNA insertion in each of the mutant lines. The homozygous T-DNA insertion mutant isolated from SALK_014569 is designated *sdg8-4*. The mutant isolated from SALK_026442 has been designated *sdg8-2* [12].

Preparation of histone extracts and immunoblot analysis. Histone-enriched protein extracts were prepared as described [8]. Histone-enriched proteins were separated on 15% SDS–polyacrylamide gels and subsequently transferred to Immobilon P membrane (Millipore) using an electrophoretic blotting system at 400 mA for 90 min. Membranes were blocked with 5% (w/v) bovine serum albumin followed by incubation with primary antibody (listed in Supplementary Table 1) and then a secondary antibody conjugated to horseradish peroxidase. The antibody complexes were detected by enhanced chemiluminescence (Pierce).

Expression and purification of GST fusion proteins. The open reading frame of *SDG8* encompassing the SET domain (amino acids 801–1290) was amplified by RT-PCR and subcloned into the EcoRI and NotI sites of the pGEX-4T-1 vector (GE Healthcare). Recombinant GST and GST-SDG8 (801–1290) fusion proteins were purified using glutathione–Sepharose beads (GE Healthcare) according to the manufacturer's instructions.

Histone methyltransferase assay. In vitro histone methyltransferase assay was performed as described [24] with modifications. Briefly, purified recombinant GST-SDG8 (801–1290) or GST (as a negative control) was incubated with 10 μg of chicken core histones (Upstate) and 2 μCi of *S*-adenosyl-[methyl-^{3}H]-L-methionine (GE Healthcare) in 50 μl of methyltransferase buffer (50 mM Tris–HCl, pH 8.5, 20 mM KCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM 2-mercaptoethanol, 1 mM dithiothreitol, and 250 mM sucrose) for 2 h at 30 °C. Proteins were resolved on a 15% SDS–polyacrylamide gel and visualized by Coomassie staining and fluorography.

RT-PCR and real-time PCR. Total RNA was isolated from leaves of 4-week-old Arabidopsis plants using Trizol reagent (Invitrogen). cDNA was synthesized from the total RNA samples using the SuperScript First-Strand Synthesis System with an oligo(dT) primer (Invitrogen). PCR amplification of the cDNA was performed using gene-specific primers as listed in Supplementary Table 2. Quantitative real-time PCR analysis was performed on a LightCycler 2.0 instrument (Roche) as described [25].

Chromatin immunoprecipitation assay. Chromatin samples were prepared from rosette leaves of 4-week-old *sdg8* mutant and wild-type plants as described [26]. Immunoprecipitations were performed using the EZ-ChIP kit (Upstate) according to the manufacturer's protocol. The antibodies used are provided in Supplementary Table 1. The DNA isolated from immunoprecipitated chromatin was analyzed by PCR using gene-specific primers (Supplementary Table 3) targeted to multiple regions of the *SPS* and *UGT74E2* genes. The amount of immunoprecipitated DNA between samples was normalized by amplifying *Actin-2* as described [27].

Results and discussion

SDG8 is required for trimethylation of lysines 9 and 36 in histone H3 in vivo

The *Arabidopsis* SDG8 protein shows homology with both the yeast Set2 methyltransferase, which exclusively methylates H3 lysine 36 [28], and with the *Drosophila* Ash1 methyltransferase, which can methylate lysines 4 and 9 in H3 ([29]; Supplementary Figure 1). To determine whether SDG8 is required for H3 methylation in vivo, we isolated two *SDG8* T-DNA insertion mutant alleles, *sdg8-2* and *sdg8-4* (see Materials and methods). RT-PCR analysis showed that the two mutants had no detectable *SDG8* transcripts (Fig. 1). This indicates that the T-DNA insertion in *sdg8-2* and *sdg8-4* completely knocked out the expression of *SDG8*. Thus, both *sdg8-2* and *sdg8*-4 are loss-of- function mutants of the *SDG8* gene. Histone extracts from the wild-type and *sdg8* mutants were probed with antibodies specific for methylated lysines in H3. As shown in Fig. 1, mono-, di- and trimethylation of H3 lysine 4 were unaffected in the *sdg8-2* and *sdg8-4* mutants. Similarly, mono-, di- and trimethylation of H3 lysine 27 were unaffected in *sdg8* mutants (data not shown). Although monomethylation and dimethylation of lysine 9 were unchanged, trimethylation of lysine 9 was significantly reduced in the *sdg8* mutants (Fig. 1). Furthermore, dimethylation and trimethylation of lysine 36 were dramatically decreased in the *sdg8* mutants (Fig. 1).

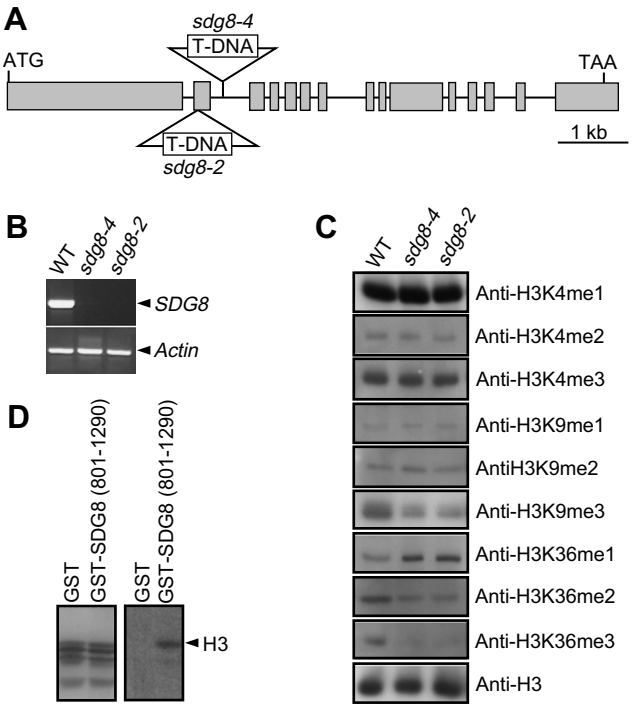

Fig. 1. Knockout mutations of *sdg8* affect histone H3 lysine 36 and lysine 9 methylation. (A) T-DNA insertion sites in *SDG8*. Exons and introns are represented by filled boxes and lines, respectively. (B) RT-PCR analysis of *SDG8* expression in *sdg8* mutants and wild-type (WT) plants. Both *sdg8-2* and *sdg8-4* contain no detectable *SDG8* transcript. Actin serves as an internal control for the RT-PCR. (C) Immunoblotting analysis of methylation state of histone H3 from WT and *sdg8* mutants. Histones were probed with antibodies against mono-, di-, or tri-methylation of H3 lysine 4, 9, or 36 (K4, K9, K36). Histone H3 antibody (anti-H3) was used to monitor equal loading. (D) SDG8 has intrinsic H3 methyltransferase activity. Chicken core histones were incubated with [^{3}H]SAM and GST-SDG8 (amino acids 801–1290) or GST (as a negative control). [^{3}H]methylated histones were resolved by SDS–PAGE, stained with Coomassie (left panel), and visualized by fluorography (right panel).

We determined whether SDG8 has histone methyltransferase activity in vitro. As shown in Fig. 1, recombinant SDG8 exhibited weak methyltransferase activity towards H3 of native core histones. This result indicates that SDG is a histone H3 methyltransferase. However, SDG8 could not methylate recombinant H3 or synthetic H3 peptides, which precluded us from defining the lysine residues in H3 methylated by SDG8 in vitro.

Methyltransferases that can mono- and/or dimethylate H3 lysine 9 have been reported in *Arabidopsis*. However, the enzymes responsible for trimethylation of H3 lysine 9 are still unidentified in plants. Structural studies indicate that trimethyltransferases usually contain a phenylalanine (F) while mono-or dimethyltransferases have a tyrosine (Y) in a conserved site known as F/Y switch [4]. In *Arabidopsis*, none of the proteins homologous to SUV39H1 (a mammalian H3 lysine 9 trimethyltransferase), named SUVH1–SUVH10, are likely to be trimethyltransferases because they do not have a phenylalanine at the F/Y switch site [5,10]. SDG8 carries a phenylalanine at the F/Y switch site (Supplementary Figure 1). Furthermore, knockout mutations of the SDG8 gene resulted in strong reduction in trimethylation of H3 lysine 9 (Fig. 1C) and SDG8 had H3 methyltransferase activity in vitro (Fig. 1D). Our data suggest that SDG8 might be a methyltransferase involved in H3 lysine 9 trimethylation in *Arabidopsis*, but we cannot exclude the possibility that SDG8 affects H3 lysine 9 trimethylation through an indirect mechanism.

In agreement with a recent report [14], we found that loss-of-function mutations of *SDG8* resulted in a significant reduction of di- and trimethylation of H3 lysine 36 and an increase in monomethylation of H3 lysine 36 (Fig. 1C). In addition, we found that *sdg8* mutations caused a marked reduction in the global levels of lysine 9 trimethylation (Fig. 1C), which has not been reported previously. Taken together, our data suggest that SDG8 is required for di- and trimethylation of H3 lysine 36 and trimethylation of H3 lysine 9. Although several histone methyltransferases have been reported to target more than one lysine residue [9,28,30], to our knowledge, SDG8 is the first reported methyltransferase that affects both histone H3 lysine 9 and lysine 36 methylation. Interestingly, recent identification of histone demethylases that can demethylate both trimethylated lysine 9 and lysine 36 has revealed a potential link between these two modifications [3].

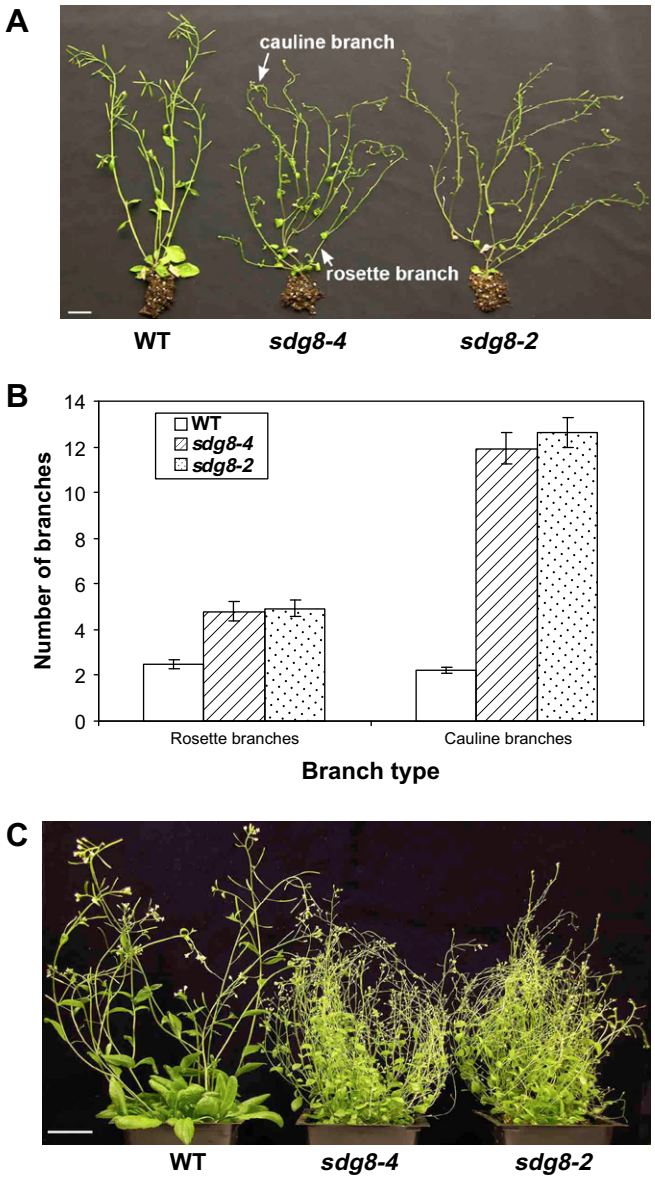

Fig. 2. The increased shoot branching phenotype of *sdg8* mutants. (A) *sdg8* mutants showing increased shoot branching. WT, *sdg8-2*, and *sdg8-4* plants were grown under a 14-h light photoperiod for 50 days. Scale bar = 2 cm. (B) Quantitative analysis of shoot branching. The number of branches was counted after 53 days of growth. Error bars represent the standard errors of the means; $n = 13–28$. (C) Phenotype of *sdg8* mutants (9 week-old) showing increased branching. There were 3 plants in each pot. Scale bar = 3 cm.

sdg8 mutants exhibit increased shoot branching

Both *sdg8-2* and *sdg8-4* mutants have a bushy appearance, are 80% wild-type height, and the cauline and rosette leaves are smaller than those of wild-type plants (Fig. 2). The bushy phenotype of

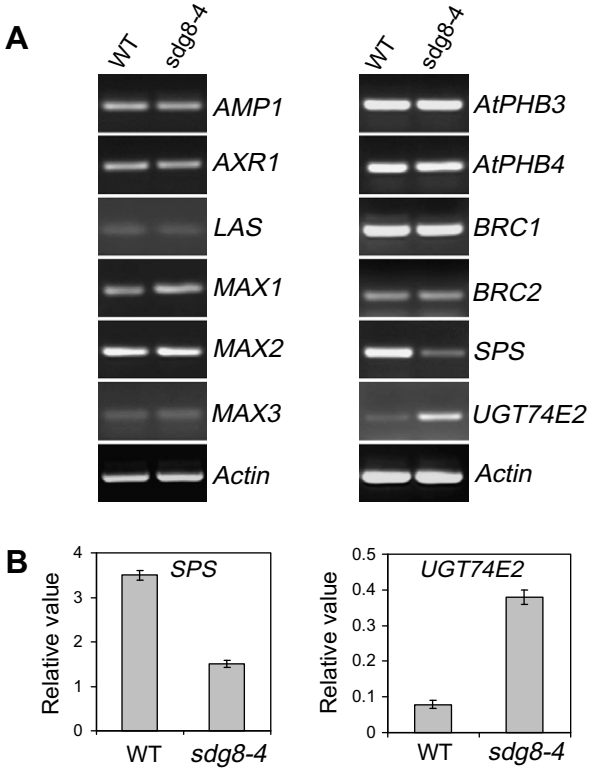

Fig. 3. Effect of *sdg8* mutation on the expression of shoot branching regulatory genes. (A) RT-PCR analysis of gene expression in rosette leaves of WT and *sdg8-4* plants. Actin as used as a control for equal cDNA input. (B) Quantitative real-time PCR. RNA levels for each gene were expressed relative to the amount of ubiquitin mRNA. Error bars represent standard deviation; $n = 9$.

the *sdg8* mutants appears to be due to increased shoot branches. Shoot branches are derived from axillary meristems in the axils of leaves [15]. According to their origin, *Arabidopsis* shoot branches can be grouped into two types: rosette and cauline branches [15]. Rosette branches are the branches originating from the axils of rosette leaves (basal leaves) and cauline branches are those originating from the axils of cauline leaves (non-basal leaves). As shown in Fig. 2B, *sdg8* mutants had a significantly higher number of cauline branches than wild-type plants. The number of rosette branches was also increased in the *sdg8* mutants. The increased shoot branching in the loss-of-function *sdg8* mutants suggests that the histone methyltransferase SDG8 represses shoot branching in *Arabidopsis*.

sdg8 mutations affect the expression of SPS and UGT74E2 genes

The prominent branching phenotype of *sdg8* mutants led us to investigate whether the expression of genes controlling shoot branching is affected by the *SDG8* mutations. Using RT-PCR analysis, we examined the expression in the *sdg8* mutants of a number of genes known to be critical for shoot branching: *AMP1* (altered meristems program 1, [31]), *AtPHB3* and *AtPHB4* (*Arabidopsis* prohibitin 3 and prohibitin 4, [21,22]), *AXR1* (auxin resistant 1; [15]), *BRC1* (branched1, [17,18]), *BRC2* [17,18], *LAS* (lateral suppressor, [16]), *MAX* (more axillary growth, [15]), *SPS* (supershoot, [19,20]), and *UGT74E2* (UDP-glycosyltransferase 74E2, [21,22]).

As shown in Fig. 3A, the transcript levels of all the examined genes except *SPS* and *UGT74E2* in the *sdg8-4* mutant were similar to that in wild-type plants. The transcript level of *SPS* was decreased while the expression of *UGT74E2* was increased in the *sdg8-4* mutant relative to wild-type (Fig. 3). Similar results were observed for the *sdg8-2* mutant (data not shown).

SPS encodes a cytochrome P450 and loss-of-function *sps* mutants exhibit massive proliferation of shoots and confer a bushy phenotype [19,20]. We found that the expression of *SPS* was decreased in the *sdg8* mutants (Fig. 3). These results suggest that SDG8 is important for maintaining SPS gene expression. The expression of the UDP-glucosyltransferase *UGT74E2* is markedly increased in *Arabidopsis* plants that exhibit increased shoot branching [21,22]. Consistent with this observation, we found that *UGT74E2* expression was increased in *sdg8* mutants (Fig. 3). Together, these data indicate that transcriptional downregulation of *SPS* and upregulation of *UGT74E2* are associated with the increased shoot branching in *sdg8* mutants.

H3 methylation patterns at the SPS and UGT74E2 genes are altered in sdg8 mutants

To determine whether the altered expression of *SPS* and *UGT74E2* in *sdg8* mutants correlates with changed histone H3 methylation, we examined the methylation status of the chromatin at the *SPS* and *UGT74E2* genes by chromatin immunoprecipitation assay. Since SDG8 globally affects trimethylation of H3 lysine 9 and di-and trimethylation of H3 lysine 36 (Fig. 1C), we analyzed the methylation of lysines 9 and 36 of the chromatins associated with the *SPS* and *UGT74E2* genes. As shown in Fig. 4, the wild-type and *sdg8-4* mutant had similar levels of mono-, di-, and trimethylation of H3 lysine 9 at the promoter and the transcribed region of the *SPS* gene. Compared to the wild-type plants, the levels of di- and trimethylation of H3 lysine 36 at the promoter and the

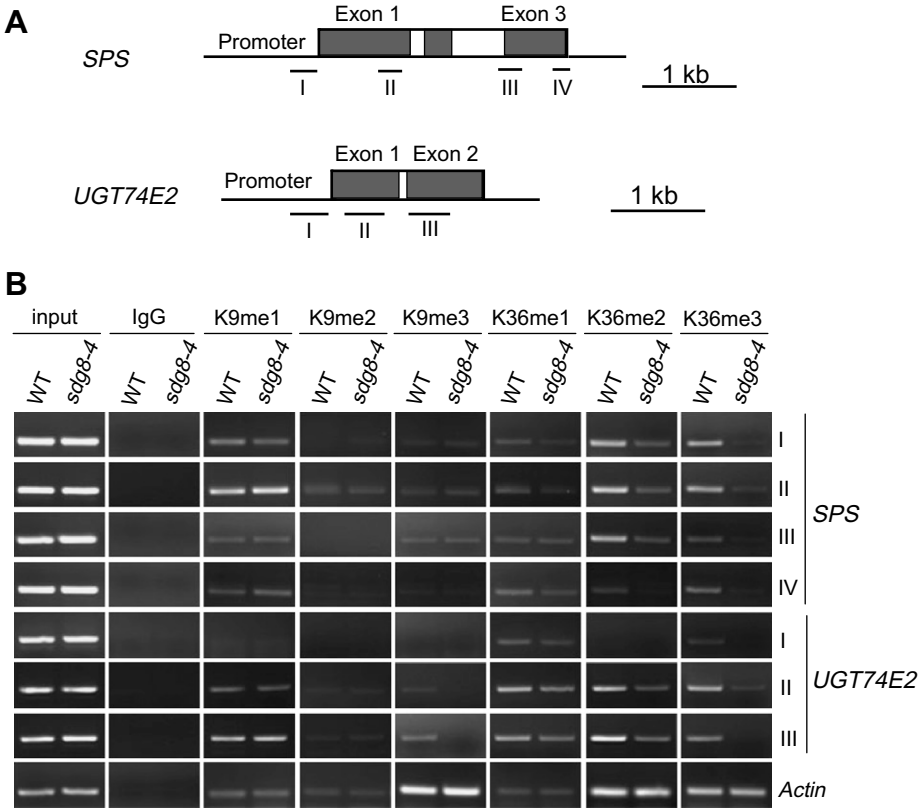

Fig. 4. Analysis of histone H3 methylation on lysines 9 and 36 at the *SPS* and *UGT74E2* loci. (A) Schematic structures of the *SPS* and *UGT74E2* genes. The filled and open boxes represent exons and introns, respectively. I to IV represent the regions examined by chromatin immunoprecipitation-PCR. (B) H3 methylation at lysines 9 and 36 in *SPS* and *UGT74E2* chromatin. Chromatin from WT or *sdg8-4* mutants was immunoprecipitated with methylation state-specific antibodies. Immunoprecipitated DNA was analyzed by PCR. Chromatin isolated before immunoprecipitation (input) served as positive control. Chromatin precipitated with normal immunoglobulin G (IgG) served as negative control. Actin was used as an internal control. The results were reproducible in two independent experiments.

transcribed region of the *SPS* gene were reduced in *sdg8-4* mutant while the level of monomethylation of H3 lysine 36 was unchanged (Fig. 4). These results suggest that SDG8 is involved in H3 lysine 36 di- and trimethylation in *SPS* chromatin. Knockout of SDG8 caused significant reduction but not abolishment of global trimethylation of H3 lysine 9 (Fig. 1C), suggesting that other unidentified methyltransferases contributing to H3 lysine 9 trimethylation may exist. Because the wild-type and *sdg8-4* mutant showed the same level of H3 lysine 9 trimethylation at the *SPS* gene (Fig. 4), one or more unidentified methyltransferases would likely be responsible for trimethylation of H3 lysine 9 in *SPS* chromatin. For the *UGT74E2* gene, the levels of di- and trimethylation of H3 lysine 36 and trimethylation of lysine 9 at the transcribed region were decreased in *sdg8-4* mutant (Fig. 4), which is consistent with the results obtained in the histone extracts (Fig. 1C). These data suggest that SDG8 is involved in H3 lysine 36 di- and trimethylation and lysine 9 trimethylation in *UGT74E2* chromatin.

It is known that H3 lysine 9 trimethylation is associated with repression of flowering genes in *Arabidopsis* [32]. Di- and trimethylation of H3 lysine 36 can be linked to transcription activation or repression under different conditions [1,33]. Repressed *SPS* expression in *sdg8* mutants correlates with decreased di- and trimethylation of H3 lysine 36 in *SPS* chromatin (Figs. 3 and 4), suggesting that SDG8-mediated di- and trimethylation of H3 lysine 36 at the *SPS* locus is required for the expression of the *SPS* gene. Activated *UGT74E2* expression in *sdg8* mutants inversely correlates with decreased trimethylation of lysine 9 and di- and trimethylation of lysine 36 in *UGT74E2* chromatin (Figs. 3 and 4). These data suggest that SDG8 in wild-type *Arabidopsis* plants may repress *UGT74E2* expression by maintaining these methylation marks in *UGT74E2* chromatin.

In addition to its involvement in regulation of flowering time ([12–14]; data not shown), we show here that the histone methyltransferase SDG8 is involved in the regulation of shoot branching in *Arabidopsis*. Our results suggest that SDG8 regulates shoot branching through methylation and regulation of genes that are crucial for branching. Future studies that combine microarray, chromatin immunoprecipitation, and new DNA sequencing technology are likely to reveal the full complement of genes regulated by SDG.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.06.096.

References

[1] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[2] B. Li, M. Carey, J.L. Workman, The role of chromatin during transcription, Cell 128 (2007) 707–719.
[3] Y. Shi, J.R. Whetstine, Dynamic regulation of histone lysine methylation by demethylases, Mol. Cell 25 (2007) 1–14.
[4] S.C. Dillon, X. Zhang, R.C. Trievel, X. Cheng, The SET-domain protein superfamily: protein lysine methyltransferases, Genome Biol. 6 (2005) 227.
[5] L.O. Baumbusch, T. Thorstensen, V. Krauss, A. Fischer, K. Naumann, R. Assalkhou, I. Schulz, G. Reuter, R.B. Aalen, The *Arabidopsis thaliana* genome contains at least 29 active genes encoding SET domain proteins that can be assigned to four evolutionarily conserved classes, Nucleic Acids Res. 29 (2001) 4319–4333.
[6] N.M. Springer, C.A. Napoli, D.A. Selinger, R. Pandey, K.C. Cone, V.L. Chandler, H.F. Kaeppler, S.M. Kaeppler, Comparative analysis of SET domain proteins in maize and Arabidopsis reveals multiple duplications preceding the divergence of monocots and dicots, Plant Physiol. 132 (2003) 907–925.
[7] A. Saleh, R. Alvarez-Venegas, M. Yilmaz, O. Le, G. Hou, M. Sadder, A. Al-Abdallat, Y. Xia, G. Lu, I. Ladunga, Z. Avramova, The highly similar Arabidopsis homologs of trithorax ATX1 and ATX2 encode proteins with divergent biochemical functions, Plant Cell 20 (2008) 568–579.
[8] J.P. Jackson, L. Johnson, Z. Jasencakova, X. Zhang, L. PerezBurgos, P.B. Singh, X. Cheng, I. Schubert, T. Jenuwein, S.E. Jacobsen, Dimethylation of histone H3 lysine 9 is a critical mark for DNA methylation and gene silencing in *Arabidopsis thaliana*, Chromosoma 112 (2004) 308–315.
[9] K. Naumann, A. Fischer, I. Hofmann, V. Krauss, S. Phalke, K. Irmler, G. Hause, A.C. Aurich, R. Dorn, T. Jenuwein, G. Reuter, Pivotal role of AtSUVH2 in heterochromatic histone methylation and gene silencing in Arabidopsis, EMBO J. 24 (2005) 1418–1429.
[10] M.L. Ebbs, J. Bender, Locus-specific control of DNA methylation by the Arabidopsis SUVH5 histone methyltransferase, Plant Cell 18 (2006) 1166–1176.
[11] T. Thorstensen, A. Fischer, S.V. Sandvik, S.S. Johnsen, P.E. Grini, G. Reuter, R.B. Aalen, The Arabidopsis SUVR4 protein is a nucleolar histone methyltransferase with preference for monomethylated H3K9, Nucleic Acids Res. 34 (2006) 5461–5470.
[12] Z. Zhao, Y. Yu, D. Meyer, C. Wu, W.H. Shen, Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36, Nat. Cell Biol. 7 (2005) 1156–1160.
[13] S.Y. Kim, Y. He, Y. Jacob, Y.S. Noh, S. Michaels, R. Amasino, Establishment of the vernalization-responsive, winter-annual habit in Arabidopsis requires a putative histone H3 methyltransferase, Plant Cell 17 (2005) 3110–3301.
[14] L. Xu, Z. Zhao, A. Dong, L. Soubigou-Taconnat, J.P. Renou, A. Steinmetz, W.H. Shen, Di- and tri- but not monomethylation on histone H3 lysine 36 marks active transcription of genes involved in flowering time regulation and other processes in *Arabidopsis thaliana*, Mol. Cell Biol. 28 (2008) 1348–1360.
[15] T. Bennett, O. Leyser, Something on the side: axillary meristems and plant development, Plant Mol. Biol. 60 (2006) 843–854.
[16] T. Greb, O. Clarenz, E. Schafer, D. Muller, R. Herrero, G. Schmitz, K. Theres, Molecular analysis of the LATERAL SUPPRESSOR gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation, Genes Dev. 17 (2003) 1175–1187.
[17] J.A. Aguilar-Martínez, C. Poza-Carrión, P. Cubas, Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds, Plant Cell 19 (2007) 458–472.
[18] S.A. Finlayson, Arabidopsis Teosinte Branched1-like 1 regulates axillary bud outgrowth and is homologous to monocot Teosinte Branched1, Plant Cell Physiol. 48 (2007) 667–677.
[19] T. Tantikanjana, J.W. Yong, D.S. Letham, M. Griffith, M. Hussain, K. Ljung, G. Sandberg, V. Sundaresan, Control of axillary bud initiation and shoot architecture in Arabidopsis through the SUPERSHOOT gene, Genes Dev. 15 (2001) 1577–1588.
[20] B. Reintanz, M. Lehnen, M. Reichelt, J. Gershenzon, M. Kowalczyk, G. Sandberg, M. Godde, R. Uhl, K. Palme, Bus, a bushy Arabidopsis CYP79F1 knockout mutant with abolished synthesis of short-chain aliphatic glucosinolates, Plant Cell 13 (2001) 351–367.
[21] O. van Aken, K Morreel, B. van de Cotte, H. Fromm, W. Boerjan, D. Inzé, F. Van Breusegem, *Arabidopsis* mitochondrial prohibitin AtPHB4 regulates a stress regulon involved in auxin homeostasis, in: 14th International Conference on Arabidopsis Research, Abstract No. 258 (2006).
[22] O. van Aken, T. Pecenková, B. van de Cotte, R. De Rycke, D. Eeckhout, H. Fromm, G. De Jaeger, E. Witters, G.T. Beemster, D. Inzé, F. van Breusegem, Mitochondrial type-I prohibitins of Arabidopsis thaliana are required for supporting proficient meristem development, Plant J. 52 (2007) 850–864.
[23] J.M. Alonso, A.N. Stepanova, T.J. Leisse, et al., Genome-wide insertional mutagenesis of *Arabidopsis thaliana*, Science 301 (2003) 653–657.
[24] J. Li, D. Moazed, S.P. Gygi, Association of the histone methyltransferase Set2 with RNA polymerase II regulates transcription elongation, J. Biol. Chem. 277 (2002) 49383–49388.
[25] X.B. Li, X.P. Fan, X.L. Wang, L. Cai, W.C. Yang, The cotton ACTIN1 gene is functionally expressed in fibers and participates in fiber elongation, Plant Cell 17 (2005) 859–875.
[26] A.V. Gendrel, Z. Lippman, R. Martiessen, V. Colot, Profiling histone modification patterns in plants using genomic tiling microarrays, Nat. Methods 2 (2005) 213–218.
[27] L. Johnson, X. Cao, S. Jacobsen, Interplay between two epigenetic marks. DNA methylation and histone H3 lysine 9 methylation, Curr. Biol. 12 (2002) 1360–1367.
[28] C. Beisel, A. Imhof, J. Greene, E. Kremmer, F. Sauer, Histone methylation by the Drosophila epigenetic transcriptional regulator Ash1, Nature 419 (2002) 857–862.
[29] B.D. Strahl, S.D. Briggs, C.J. Brame, J.A. Caldwell, S.S. Koh, H. Ma, R.G. Cook, J. Shabanowitz, D.F. Hunt, M.R. Stallcup, C.D. Allis, Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression, Mol. Cell. Biol. 22 (2002) 1298–1306.
[30] J.A. Cartagena, S. Matsunaga, M. Seki, D. Kurihara, M. Yokoyama, K. Shinozaki, S. Fujimoto, Y. Azumi, S. Uchiyama, K. Fukui, The Arabidopsis SDG4 contributes

to the regulation of pollen tube growth by methylation of histone H3 lysines 4 and 36 in mature pollen, Dev. Biol. 315 (2008) 355–368.
[31] A.M. Chaudhury, S. Letham, S. Craig, E.S. Dennis, *amp1*: a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering, Plant J. 4 (1993) 907–916.
[32] S. Sung, R.J. Schmitz, R.M. Amasino, A PHD finger protein involved in both the vernalization and photoperiod pathways in Arabidopsis, Genes Dev. 20 (2006) 3244–3248.
[33] D.Y. Lee, C. Teyssier, S.D. Strahl, M. Stallcup, Role of protein methylation in regulation of transcription, Endocr. Rev. 26 (2005) 147–170.