SET DOMAIN GROUP25 Encodes a Histone Methyltransferase and Is Involved in FLOWERING LOCUS C Activation and Repression of Flowering

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Covalent modifications of histone lysine residues by methylation play key roles in the regulation of chromatin structure and function. In contrast to H3K9 and H3K27 methylations that mark repressive states of transcription and are absent in some lower eukaryotes, H3K4 and H3K36 methylations are considered as active marks of transcription and are highly conserved in all eukaryotes from yeast (Saccharomyces cerevisiae) to Homo sapiens. Paradoxically, protein complexes catalyzing H3K4 and H3K36 methylations are less-extensively characterized in higher eukaryotes, particularly in plants. Arabidopsis (Arabidopsis thaliana) contains 12 SET DOMAIN GROUP (SDG) proteins phylogenetic classified to Trithorax Group (TrxG) and thus potentially involved in H3K4 and H3K36 methylations. So far only some genes of this family had been functionally characterized. Here we report on the genetic and molecular characterization of SDG25, a previously uncharacterized member of the Arabidopsis TrxG family. We show that the loss-of-function mutant sdg25-1 has an early flowering phenotype associated with suppression of FLOWERING LOCUS C (FLC) expression. Recombinant SDG25 proteins could methylate histone H3 from oligonucleosomes and mutant sdg25-1 plants showed weakly reduced levels of H3K36 dimethylation at FLC chromatin. Interestingly, sdg25-1 transcriptome shared a highly significant number of differentially expressed genes with that of sdg26-1, a previously characterized mutant exhibiting late-flowering phenotype and elevated FLC expression. Taken together, our results provide, to our knowledge, the first demonstration for a biological function of SDG25 and reveal additional layers of complexity of overlap and nonoverlap functions of the TrxG family genes in Arabidopsis.

As a sessile organism, plants adopted during evolution specific developmental processes to endure unfavorable conditions. Plants develop in three phases, which are embryonic, vegetative, and reproductive. Transition from one phase to another is precisely regulated, involving the perception and integration of a variety of endogenous signals and environmental cues. Flowering represents the transition from the vegetative to reproductive development, which in Arabidopsis (Arabidopsis thaliana) is mainly controlled by four pathways: the autonomous pathway that constitutively activates flowering, the gibberellin pathway that promotes flowering when plants are grown under noninductive conditions, the vernalization pathway that promotes flowering after prolonged cold exposure, and the photoperiod pathway that induces flowering in response to increasing day length (Komeda, 2004). FLOWERING LOCUS C (FLC), which encodes a transcription factor belonging to the MADS-box family, is a key component in the flowering time control. It represses flowering in a dosage-dependent way and its expression level is fine tuned by multiple regulators (for review, see He, 2009).

Chromatin carries genetic and epigenetic information in all eukaryotes. Chromatin remodeling through histone exchange and/or histone covalent modifications is necessary for the correct deployment of developmental programs, for the maintenance of cell fates, and for the adaptation to environmental changes. Despite the difference in sequence and structure, many plant MADS-box genes including FLC, like animal homeotic (HOX) genes, are controlled by fac-
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SDG25 Is Involved in FLC Activation and Flowering Repression

In Arabidopsis, the role of TrxG proteins in the regulation of gene expression and development is not fully understood.

RESULTS

Relationship of the Arabidopsis TrxG Family Members and Structure of SDG25

Initial phylogenetic analysis of SET-domain proteins in Arabidopsis identified 19 proteins as homologs of TRX and ASH1, both belonging to the TrxG family in animals (Baumbusch et al., 2001). Subsequent analysis of this group and expanded the phylogenetic analysis including proteins from Arabidopsis and rice (Oryza sativa) as well as proteins from nonplant species that have been demonstrated as H3K4 and/or H3K36 methyltransferases.

1. The SDG25 protein promotes flowering through reduction of FLC expression. The SDG25 protein shows histone methyltransferase activity in vitro when oligonucleosomes were used as substrate. In the sdg25-1 mutant plants, weakly decreased levels of H3K36 dimethylation were detected at some regions of FLC. Transcriptomic analysis revealed 43 downregulated and 122 up-regulated genes in the sdg25-1 mutant. Despite the down-regulation of FLC in both sdg25-1 and sdg8 but up-regulation of FLC in sdg26, the number of perturbed genes found in sdg25-1 significantly overlapped with that previously reported in sdg26 but not in sdg8. To our knowledge, our results provide the first demonstration for biological function of SDG25 and reveal additional layers of complexity of the TrxG family members in the regulation of expression of common and specific genes.

In comparison, the function of TrxG in Arabidopsis is less-extensively characterized. Sequence analysis initially identified five TRX homologs (ATX1/SET, ATX2/SDG30, ATX3/SDG14, ATX4/SDG16, and ATX5/SDG29), seven TRX related (ATXR1/SDG35, ATXR2/SDG36, ATXR3/SDG2, ATXR4/SDG38, ATXR5/SDG15, ATXR6/SDG34, and ATXR7/SDG25), four ASH1 homologs (ASHH1/SDG26, ASHH2/SDG8, ASHH3/SDG7, and ASHH4/SDG24), and three ASH1 related (ASHR1/SDG37, ASHR2/SDG39, and ASHR3/SDG4), as members of the TrxG family in Arabidopsis (Baumbusch et al., 2001). To date, only some of these genes are functionally characterized. Disruption of ATX1 causes pleiotropic phenotypes including homeotic transformation, root and leaf defects, and early flowering, whereas the atx2 mutants do not have any obvious phenotype (Alvarez-Venegas et al., 2003; Pien et al., 2008; Saleh et al., 2008). The sdg26 mutants show late flowering and elevated levels of FLC expression (Xu et al., 2008). The sdg8 (alleles also known as efs and ccr1) mutants show an early flowering and other pleiotropic phenotypes (Kim et al., 2005; Zhao et al., 2005; Dong et al., 2008; Xu et al., 2008; Cazzonelli et al., 2009). Loss or gain of function of ASH3/SDG4 causes male sterility (Cartagena et al., 2008; Thorstensen et al., 2008). Perturbed levels of histone methylation in planta have been reported for some mutants, including reduced H3K4 methylation in atx1 and atx2 (Alvarez-Venegas et al., 2003; Pien et al., 2008; Saleh et al., 2008), reduced H3K4 and H3K36 methylation in sdg8/efs/ccr1 (Kim et al., 2005; Zhao et al., 2005; Dong et al., 2008; Xu et al., 2008; Cazzonelli et al., 2009) and in sdg4 (Cartagena et al., 2008), and histone methyltransferase activity has been demonstrated in vitro for ATX1 (Alvarez-Venegas et al., 2003), SDG26 (Xu et al., 2008), and SDG8 (Dong et al., 2008; Xu et al., 2008).
Isolation and Characterization of the Early Flowering Phenotype of the \textit{sdg25-1} Mutant

To investigate the biological function of SDG25, we obtained a T-DNA insertion line of the SALK collection (Alonso et al., 2003), SALK_149692, from the Arabidopsis Biological Resource Center (ABRC). Genomic PCR analysis confirmed that this line contains a T-DNA insertion in the second exon of \textit{SDG25} (Fig. 2A; data not shown). Homozygous (called hereinafter mutant \textit{sdg25-1}) plants were obtained by self-pollination. Reverse transcription (RT)-PCR analysis showed that the full-length \textit{SDG25} transcripts were absent in the mutant plants (Fig. 2B), indicating that \textit{sdg25-1} is a loss-of-function mutant.

Compared to wild-type \textit{Columbia} (Col), the \textit{sdg25-1} mutant showed an early flowering phenotype (Fig. 2C). Flowering time is temporally controlled by the developmental state (for example, plant size and age) and regulated by environmental cues, such as light and temperature. To further investigate the flowering phenotype of the mutant, we compared the flowering time of \textit{sdg25-1} with \textit{Col} at different photoperiods and with or without vernalization treatment. As shown in Figure 2D, flowering of the \textit{sdg25-1} mutant plants, like that of wild type, was promoted by long-day photoperiods as well as by vernalization treatment. In all tested conditions, the \textit{sdg25-1} mutant plants flowered earlier than wild type. Flowering time measured from a developmental perspective, such as the total number of rosette leaves, further confirmed the early flowering phenotype of the \textit{sdg25-1} mutant (Fig. 2E). In the same experiments, the \textit{sdg25-1} mutant and wild-type \textit{Col} were compared for the number of juvenile leaves, which are characterized by the absence of abaxial trichomes. The mutant plants showed a reduced number of juvenile leaves (Fig. 2F), indicating that the juvenile vegetative phase has been shortened. The adult phase also has been shortened since the mutant plants also showed a reduced number of adult leaves with abaxial trichomes (=total rosette leaf number in Fig. 2E minus juvenile leaf number in Fig. 2F).

To confirm that the mutant phenotype is indeed caused by the loss of function of SDG25, the \textit{sdg25-1} mutant was transformed with a genomic fragment containing the SDG25 gene (Fig. 3A). As shown in

![Figure 1: SDG25 is a member of the TrxG family. A, Phylogenic analysis of 12 Arabidopsis SDG proteins (At, Arabidopsis), nine rice SDG proteins (Os, rice), together with H3K4 and H3K36 methyltransferases so far found in fungi (Sp, Schizosaccharomyces pombe; Sc, yeast; Nc, Neurospora crassa; Dm, Drosophila melanogaster; Hs, Homo sapiens). The plant proteins fall in two groups: Group 1 contains proteins of other organisms involved primarily in H3K36 methylation whereas group 2 contains proteins of other organisms involved primarily in H3K4 methylation. B, Comparison of structural domains of ATX1, ATX2, and SDG25. The SET domain is the most highly conserved, and the post-SET Cys-rich region C is also well conserved in all three proteins. The other domains (PWWP, FYR, and PHD) found in ATX1 and ATX2 are not conserved in SDG25. Instead, SDG25 contains a GYF domain. C, Sequence alignment of the C-terminal last 133 amino acids from SDG25, SpSET1, and HsSET1. This highly conserved region contains the SET domain and the post-SET Cys-rich region.]

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Figure 3B, the transgene SDG25 rescued the sdg25-1 mutant, resulting in flowering time similar to wild type. Examination of two other T-DNA insertion lines within the SDG25 gene, GK-630A05-022799 and SAIL_446_F12 (http://www.arabidopsis.org), revealed that these mutants have an early flowering phenotype similar to sdg25-1. Taken together, our data establish SDG25 as an important gene involved in repression of flowering.

Expression of FLC Is Down-Regulated in the sdg25-1 Mutant

Both the autonomous and vernalization pathways act to repress FLC expression to induce flowering (Baurle and Dean, 2006). To investigate the molecular mechanism underlying the early flowering phenotype of sdg25-1, we compared the expression level of FLC between the mutant and wild-type plants. As shown in Figure 4, FLC expression was down-regulated in the sdg25-1 mutant compared to wild-type plants. The MADS AFFECTING FLOWERING1 (MAF1) to MAF5 genes encode MADS-box proteins closely related in sequence to FLC and could repress flowering when ectopically overexpressed (Ratcliffe et al., 2003). Expression of these MAF genes was, however, unchanged in the sdg25-1 mutant (Fig. 4), indicating that the early flowering phenotype of sdg25-1 is specifically associated with down-regulation of FLC expression.

H3K36 Dimethylation Is Slightly Down-Regulated at Some Regions of FLC in the sdg25-1 Mutant

Down-regulation of FLC expression in the sdg25-1 mutant is consistent with the expected role of SDG25 as an active TrxG member in transcriptional activation. To investigate histone methylation status in the mutant, we utilized antibodies specifically recognizing dimethyl-H3K4, trimethyl-H3K4, or dimethyl-H3K36. Western-blot analysis revealed similar levels of histone methylations between sdg25-1 and wild type (data not shown), indicating that SDG25 is not a major enzyme for global methylation on H3K4 or H3K36. We investigated H3K4 and H3K36 methylation at several regions of FLC (Fig. 5A) by chromatin immunoprecipitation (ChIP) analysis. While sdg25-1 barely affected levels of H3K4 dimethylation and trimethylation, reduced levels of H3K36 dimethylation were observed at some regions (c–g) of FLC in sdg25-1 compared to wild type (Fig. 5B). These reductions are weak but were reproducibly observed in two independent experiments. Real-time PCR analysis confirmed that compared with wild type the sdg25-1 mutant contained reduced levels of H3K36 dimethylation at FLC locus (see Supplemental Fig. S1).
SDG25 Is Ubiquitously Expressed in Different Organs, and the SDG25 Protein Is Localized in the Cytoplasm and the Nucleus and Can Methylate Oligonucleosomes in Vitro

Besides early flowering, sdg25-1 did not show any additional visible phenotype. To better understand SDG25 function, we investigated its expression pattern and protein activities. We found that SDG25 is expressed ubiquitously in seedling, leaf, inflorescence, and root (Fig. 6A). The GFP-SDG25 fusion protein was localized in the cytoplasm and the nucleus (Fig. 6B). To investigate enzyme activity of SDG25, we expressed two fragments of 320 (SDG25S) and 699 (SDG25L) amino acids in length of the C terminus of the protein. Both SDG25S and SDG25L showed histone H3 methyltransferase activity when oligonucleosomes were used as substrates (Fig. 6C), whereas such activity was not detectable when free core histones (not shown) or mononucleosomes (Fig. 6C) were used as substrates. Similar substrate preference for oligonucleosomes with higher-order chromatin structure had also been previously observed for SDG8 and SDG26 proteins (Xu et al., 2008). Taken together, our data provide experimental demonstration of SDG25 as a histone H3 methyltransferase and suggest that SDG25 could have a broad function.

Transcriptome Analysis Reveals Overrepresentation of Common Genes Perturbed in the sdg25-1 and sdg26-1 Mutants

We investigated the global genome expression pattern in sdg25-1 through transcriptome profiling analysis. As performed previously for the sdg8 and sdg26 mutants (Xu et al., 2008), 6-d-old seedlings of wild type and sdg25-1 mutant were used in this analysis. We found that 43 genes were down-regulated and 122 genes up-regulated to more than 1.5-fold in the sdg25-1 mutant seedlings compared to the wild type (see Supplemental Table S1). These differentially expressed genes are involved in several processes including transcription, phytohormone and external stimuli responses, metabolism, and transport. Comparison of these differentially expressed genes with those previously described in atx1-1 (Pien et al., 2008), atx2-2 (Saleh et al., 2008), sdg8-1, and sdg26-1 (Xu et al., 2008) revealed that a significantly overrepresented number of genes are in common and are misregulated in the same direction between the sdg25-1 and sdg26-1 mutants whereas only a few genes are in common between the sdg25-1 and sdg8-1 mutants (Fig. 7A). We also did not detect a signifi-
Together, our data indicate that SDG25 is closely related in regulation of genome transcription. A similar behavior of flowering phenotype was previously reported for the sdg8/efs mutants (Soppe et al., 1999; Kim et al., 2005; Zhao et al., 2005). The early flowering phenotype of sdg25-1 compared to sdg8/efs is weaker. This is likely associated, at least in part, with the down-regulation of MAF1, MAF4, and MAF5 expression specifically observed in the sdg8/efs mutants (Kim et al., 2005; Zhao et al., 2005), but not in the sdg25-1 mutant. Besides early flowering no other obvious phenotypes were observed in the sdg25-1 mutant. This is in contrast to the pleiotropic phenotype of the sdg8/efs mutants, which includes reduced organ size, increased shoot branching, altered carotenoid composition, and low fertility (Soppe et al., 1999; Zhao et al., 2005; Dong et al., 2008; Xu et al., 2008; Cazzonelli et al., 2009).

Transcriptome analysis also indicated that few genes overlapped between the lists of genes differentially expressed in the sdg8/efs and sdg25 mutants. It appears clear that SDG25 and SDG8/EF5 essentially regulate different target genes. While a global reduction of H3K36 di- and trimethylation had been observed in the sdg8/efs mutants (Zhao et al., 2005), but not in the sdg25-1 mutant. This is in contrast to the pleiotropic phenotype of the sdg8/efs mutants, which includes reduced organ size, increased shoot branching, altered carotenoid composition, and low fertility (Soppe et al., 1999; Dong et al., 2008; Xu et al., 2008; Cazzonelli et al., 2009).

DISCUSSION

Our study identified SDG25 as a positive regulator of FLC expression, which inhibits flowering. Both the juvenile and adult phases of sdg25-1 mutant plants are shortened, resulting in an early transition to reproductive development. The early flowering phenotype of sdg25-1 compared to wild type was observed under different photoperiods and with or without vernalization. Nonetheless, the sdg25-1 mutant still responded to induction by long-day photoperiods and vernalization, indicating that SDG25 acts downstream of the latter two signaling pathways. A similar behavior of flowering phenotype was previously reported for the sdg8/efs mutants (Soppe et al., 1999; Kim et al., 2005; Zhao et al., 2005). The early flowering phenotype of sdg25-1 compared to sdg8/efs is weaker. This is likely associated, at least in part, with the down-regulation of MAF1, MAF4, and MAF5 expression specifically observed in the sdg8/efs mutants (Kim et al., 2005; Zhao et al., 2005), but not in the sdg25-1 mutant. Besides early flowering no other obvious phenotypes were observed in the sdg25-1 mutant. This is in contrast to the pleiotropic phenotype of the sdg8/efs mutants, which includes reduced organ size, increased shoot branching, altered carotenoid composition, and low fertility (Soppe et al., 1999; Zhao et al., 2005; Dong et al., 2008; Xu et al., 2008; Cazzonelli et al., 2009).

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and ATX1 is partially responsible for H3K4 trimethylation at *FLC* (Pien et al., 2008). Our study indicates that *sdg25-1* may have affected *FLC* expression through defects on H3K36 dimethylation. At this stage, however, we cannot exclude the possibility that *sdg25-1* may affect H3K4 methylation at some unknown genome regions. Recombinant SDG25 proteins only showed modest methyltransferase activity on oligonucleosomes, making it impractical to analyze enzyme specificity by in vitro biochemical approaches. H3K4 methylation was found in more than two-thirds of Arabidopsis genes (Zhang et al., 2009). From currently characterized mutants of the Arabidopsis TrxG family genes, only *atx1* showed a mild reduction in the global level of H3K4 trimethylation (Alvarez-Venegas and Avramova, 2005) and *sdg4* showed reduction of H3K4 di- and trimethylation in specific floral tissues (Cartagena et al., 2008). Locus-specific reduction of H3K4 methylation was observed in *atx1* (Alvarez-Venegas and Avramova, 2005; Pien et al., 2008), *atx2* (Saleh et al., 2008), and *sdg8/efs* (Kim et al., 2005; Cazzonelli et al., 2009). Our study here eliminates SDG25 being responsible for the genome-wide H3K4 methylation. Thus the major enzyme(s) involved in the H3K4 methylation in Arabidopsis remain to be identified and characterized.

The transcriptome of *sdg25-1* showed little overlap with that of *sdg8-1*, *atx1*, or *atx2*, but showed a significant overlap with that of *sdg26-1* (Fig. 7). Recombinant SDG26 protein could methylate oligonucleosomes in vitro but perturbation of histone methylation in *sdg26-1* had not been detected (Xu et al., 2008). In contrast to *sdg25-1*, *sdg26-1* showed elevated expression of *FLC* and a late-flowering phenotype. Because histone methylation remained unchanged at *FLC* in mutant plants (Xu et al., 2008), *sdg26-1* might have affected *FLC* expression indirectly. Our finding of a great similarity between the transcriptomes of *sdg25-1* and *sdg26-1* mutants indicates that perturbed expression of the shared genes in the two mutants are independent from modifications of *FLC* expression and flowering time. Molecular mechanisms underlying regulation of shared target genes by SDG25 and SDG26 will require more investigations; however, our finding of regulation of transcription of a common set of genes by SDG25 and SDG26 constitutes an important step toward understanding the relationship and biological functions of the TrxG family genes. Finally, it is interesting to note that SDG25 and SDG26 also have unique (nonoverlapping) target genes; an important number of genes are perturbed specifically in *sdg25-1* or *sdg26-1* mutant plants (Fig. 7). It thus seems evident that SDG25 and SDG26 have both redundant and nonredundant functions.

Although we cannot rule out the possibility that some of the genes identified in transcriptome analysis might represent secondary rather than primary targets of SDG25, the great number of up-regulated genes found in *sdg25-1* mutant plants suggests that SDG25 may also act as a repressor of transcription. TrxG proteins are generally involved in transcriptional activation. Nevertheless, it is feasible that canonical positive regulators such as SDG25 may also play a role as negative regulators in certain contexts, and finding so many up-regulated genes in *sdg25-1* mutant plants makes this idea attractive. Several possibilities
exist to explain a repressor role of TrxG proteins in transcription. First, in yeast (*Saccharomyces cerevisiae*) H3K36 methylation is known to negatively influence transcription of some genes through, at least in part, recruitment of histone deacetylase activity (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005). Second, the genic patterns of distribution of histone methylations are important for transcriptional activity; H3K4 trimethylation occurring predominantly near the 5′ transcription start site marks transcriptional engagement whereas H3K36 dimethylation occurring predominantly in the coding and 3′ end of genes marks transcriptional elongation in all studied eukaryotes including Arabidopsis (Oh et al., 2008; Zhang et al., 2009). Enhancement of H3K36 dimethylation within the 5′ transcription start site may interrupt initiation and is associated with reduction of gene expression (Biswas et al., 2006; Oh et al., 2008). Last but not least, some TrxG proteins are known to be involved in methylation of diverse sites of histones, e.g. ASH1 can methylate H3K4, H3K9, and H4K20 (Beisel et al., 2002); NSD1 can methylate both H3K36 and H4K20 in vitro (Rayasam et al., 2003); and sdg8 mutant plants exhibit reduced levels of H3K36 dimethylation, H3K36, and H3K9 trimethylation (Dong et al., 2008). H3K9 and H4K20 methylations are generally recognized as repressive marks of transcription. More investigations will be necessary to examine these above listed possibilities in SDG25-mediated repression of gene expression.

### MATERIALS AND METHODS

#### Sequence Analysis

For phylogenetic analysis, the entire protein sequences were aligned using ClustalW multiple sequence alignment program and then optimized manually by removing poorly aligning regions, which leaves only the SET domain. The finalized file was subjected to phylogenetic analysis using MEGA3.0 with bootstrapping set at 500 replicates. The resulting consensus tree was displayed using the TreeView program. Protein structure analysis for conserved domains was performed using ScanProsite program (http://www.expasy.org/prosite/).

#### Plant Materials and Growth Conditions

The *sdg25-1* mutant corresponds to SALK_149692 (Col) of T-DNA insertion strains from the ABRC. Mutant and wild-type Col plants were grown either on plates containing Murashige and Skoog media or in pots with soil. Growth conditions were as previously described (Zhao et al., 2005). Flowering time was measured as days to bolting as well as from a developmental perspective, as the total number of rosette leaves (Michaels and Amasino, 1999).

#### Complementation of Mutant Plants

A fragment of about 10 kb in length that covers the whole SDG25 gene with promoter and terminator was isolated by restriction enzyme digestion (*BamHI*-*ClaI) from bacterial artificial chromosome MDH9. This fragment was then cloned in the *BamHI*-*ClaI* sites of *BlueScript II* SK vector (Stratagene, www.stratagene.com), resulting in pBSK-SDG25G. Finally the SDG25-containing *BamHI* fragment from pBSK-SDG25G was cloned into the *BamHI*-*BamHI* sites of pCAMBIA1300 vector (CAMBIA; http://www.cambia.org) resulting in pCAMBIA1300-SDG25G. The pCAMBIA1300-SDK25G plasmid was introduced into *Agrobacterium tumefaciens* for plant transformation. The *sdg25-1* mutant plants were transformed using the floral-dip method (Clough and Bent, 1998).

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**Figure 7.** Comparison of differentially expressed genes in *sdg25-1, sdg26-1,* and *sdg8-1* mutants. A, The circles represent the genes that were down-regulated (left) or up-regulated (right) in *sdg25-1* mutant upon transcriptome analysis. The total number of genes is shown in brackets. The percentages of these genes that were also down- or up-regulated in *sdg26-1*, in both *sdg26-1* and *sdg8-1*, or in *sdg8-1* are shown. * *Statistically significant overlap: *P* = 3.9 × 10^{-69} (left) and *P* = 7.1 × 10^{-81} (right). B, RT-PCR analysis of expression of some genes perturbed in both *sdg25-1* and *sdg26-1* mutants. *ACTIN* serves as an internal control.
Isolation of SDG25 cDNA

The entire coding region of SDG25 cDNA was PCR amplified from Col inflorescence cDNA using the forward primer 5'-gagtagctagATGGTTGCGGT-GATTCAC-3' and the reverse primer 5'-ccgctagacTGATTCCTGTTCT-GAAACACAG-3' (lowercase letters represent non-SDG25 nucleotides added to introduce restriction enzyme sites). The PCR product was digested and cloned into BamHI-XhoI sites of pCR-H-TOPo vector (Invitrogen; http://www.invitrogen.com), resulting in pCR-SDG25. Sequencing analysis revealed that the cDNA fragment was 4,167 bp in length and encoded a protein of 1,388 amino acids (database accession no. EU014691). This slightly differs from the predicted At5g42401.1 sequence in the Arabidopsis (Arabidopsis thaliana) database because of some alternative intron splicing. Alignment of amino acid sequences between SDG25 and At5g42401.1 was shown in Supplemental Figure S2. We further confirmed the 3' end region of SDG25 by 3'-RACE analysis.

Protein Subcellular Localization

The SDG25 cDNA from pCR-SDG25 was amplified by PCR and cloned via a BP clonase reaction in the Gateway donor vector pDONR201 (Invitrogen, http://www.invitrogen.com). They were recombined by Gateway technology into pK7WG2.0 vector (Karimi et al., 2002), resulting in GFP::SDG25 fusion gene under the control of the 3SS promoter. The fusion gene was introduced into Arabidopsis plants by Agrobacterium tumefaciens-mediated transformation. The epifluorescence and differential interference contrast images were taken using a confocal laser-scanning microscope, Zeiss model LSM510 (Carl Zeiss). Essential results were reproducibly obtained from three independent transgenic lines.

Recombinant Protein Production and Enzyme Activity Assay

Two fragments SDG25L and SDG25S encoding the amino acid residues 690 to 1,388 and 1,069 to 1,388 of the SDG25 protein were PCR amplified from Arabidopsis cDNA using the forward primer 5'-atgtgatccGGATGTGAAAGCAAT-3' and reverse 5'-TTATCGCCGGAGATTTATGTTGGAG-3', respectively. They were subsequently cloned into BamHI-XhoI sites of pGEX-4T-1 (Amersham-Pharmacia Biotech; http://www.amersham.com), resulting in pGEX-SDG25L and pGEX-SDG25S. Expression and purification of GST-fused proteins from bacteria transformed with pGEX-SDG25L or pGEX-SDG25S were performed according to the previously described procedure (Dong et al., 2005). Histone methyltransferase assays were performed as previously described (Xu et al., 2008). The recombinant SDG714 protein that methylates H3K9 (Ding et al., 2007) was used as a control in this study.

Analysis of Histone Methylation in Plants

Specific antibodies used in this study are: antidimethyl H3K4 (Upstate catalog no. 07–473; http://www.millipore.com), antitrimethyl-H3K4 (Upstate catalog no. 07–473; http://www.millipore.com), antitrimethyl-H3K4 (Upstate catalog no. 07–473; http://www.millipore.com), antitrimethyl-H3K4 (Upstate catalog no. 07–473; http://www.millipore.com). Western analysis of Arabidopsis histones, ChIP, and semiquantitative PCR assays were performed as previously described (Zhao et al., 2005; Xu et al., 2008). Real-time PCR analysis was performed using specific sets of primers: 5'-GCTGCATAAA-GCTGCATAAA-3' and reverse 5'-AAAGTCTGTTCTGCTGCTGCT-3', with the reverse primer 5'-TGTGATCGAATATGTTGGAG-3' and reverse 5'-TGTGATCGAATATGTTGGAG-3' and reverse 5'-GACCCATGCAATCGTAGTTA-3'. Western analysis of Arabidopsis histones, ChIP, and semiquantitative PCR assays were performed as previously described (Zhao et al., 2005; Xu et al., 2008). Real-time PCR analysis was performed using specific sets of primers: 5'-GCTGCATAAA-GCTGCATAAA-3' and reverse 5'-AAAGTCTGTTCTGCTGCTGCT-3', with the reverse primer 5'-TGTGATCGAATATGTTGGAG-3' and reverse 5'-TGTGATCGAATATGTTGGAG-3' and reverse 5'-GACCCATGCAATCGTAGTTA-3'.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number EU014691.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Real-time PCR on ChIP samples showing levels of H3K4 and H3K36 dimethylation at FLC in wild type and sdm2-1 mutant.

Supplemental Figure S2. Alignment of amino acid sequences of SDG25 and At5g42400.1.

Supplemental Table S1. List of differentially expressed genes found in the sdm2-1 mutant.

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