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## A Histone Acetyltransferase Regulates Active DNA Demethylation in *Arabidopsis*

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### Abstract

Active DNA demethylation is an important part of epigenetic regulation in plants and animals. How active DNA demethylation is regulated and its relationship with histone modification patterns are unclear. Here, we report the discovery of IDM1, a regulator of DNA demethylation in *Arabidopsis*. IDM1 is required for preventing DNA hypermethylation of highly homologous multicopy genes and other repetitive sequences that are normally targeted for active DNA demethylation by Repressor of Silencing 1 and related 5-methylcytosine DNA glycosylases. IDM1 binds methylated DNA at chromatin sites lacking histone H3K4 di- or trimethylation and acetylates H3 to create a chromatin environment permissible for 5-methylcytosine DNA glycosylases to function. Our study reveals how some genes are indicated by multiple epigenetic marks for active DNA demethylation and protection from silencing.

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**Supplementary Materials** [www.sciencemag.org/cgi/content/full/336/6087/1445/DC1](http://www.sciencemag.org/cgi/content/full/336/6087/1445/DC1) Materials and Methods Figs. S1 to S17 Tables S1 to S7 References (12–20)

Epigenetic control of gene expression involves dynamic regulation of DNA methylation and histone modification marks (1, 2). A DNA repair–based mechanism functions in active DNA demethylation in plants and possibly in animals as well (3). In plants, active DNA demethylation is initiated by a family of 5-methylcytosine DNA glycosylases that includes ROS1, DME, DML2, and DML3 (3). Although a large body of literature concerns the regulation of DNA methylation by histone modification patterns (1, 2), little is known about the regulation of active DNA demethylation.

We developed a polymerase chain reaction (PCR)–based marker for reporting DNA methylation status at the 3' region of *At1g26400* (also referred to as *DT-77*)(4). This marker suggests that the *At1g26400* region is hypermethylated in *ros1* mutant alleles (Fig. 1A). We screened a population of homozygous transferred DNA (T-DNA) insertion lines of *Arabidopsis* for DNA hypermethylation mutants. Two of the identified mutants (Fig. 1A), referred to as *idm1* (for increased DNA methylation 1), have T-DNA insertions in the gene *At3g14980* (fig. S1A). Bisulfite sequencing showed that, compared to the Col wild-type control, the *idm1* and *ros1-4* mutants have higher levels of DNA methylation in all sequence contexts at *DT-77* (Fig. 1B). There is also increased CG and CHG (where H is C, A, or T) methylation at the *At4g18650* gene promoter, which was previously shown to be targeted for demethylation by ROS1 (5) (Fig. 1C). ROS1 was originally identified because it is required for preventing the *RD29A-LUC* and *35S-NPTII* trans-genes from transcriptional silencing (6). From an independent genetic screen for mutants impaired in the prevention of silencing of the *35S-NPTII* transgene, we found the *idm1-3* mutation that changes glutamic acid-451 to a premature stop codon and causes the silencing of *35S-NPTII* but not the *RD29A-LUC* transgene (fig. S2).

Double-mutant analysis indicated that the DNA hypermethylation phenotypes of *ros1-4* and *idm1-1* are not additive (Fig. 1, B and C, and fig. S3A). These results suggest that *IDM1* and ROS1 may function in the same genetic pathway to prevent DNA hypermethylation. Quantitative reverse transcription PCR analysis showed that *ROS1* or *ROS3* expression is not reduced in the *idm1* mutants (fig. S4A). In addition, like *ROS1* (3), *IDM1* expression level was reduced in RNA-directed DNA methylation (RdDM) pathway mutants (fig. S4B), which suggests that *IDM1* expression is sensitive to non-CG methylation. The hypermethylation in *idm1-1* at CHG and CHH sequence contexts could be suppressed by the *npr1-3* mutation (7), suggesting that the methylation at both *DT-77* and *At4g18650* promoter is caused by RdDM (Fig. 1, B and C).

Southern blot analysis showed that *idm1* does not affect the DNA methylation at 5S or 45S ribosomal DNA or at the 180–base pair centromeric repeat (fig. S5). We compared the genome-wide DNA methylation profiles of *idm1-1* and wild-type plants. There was no significant difference between *idm1-1*, *ros1-4*, or *ros1-3dml2-1dml3-1* (*rdd*) (8) and wild-type plants in their overall genome methylation patterns (fig. S6). We identified 1098, 4991, and 9290 loci where DNA methylation levels are increased significantly in *idm1-1*, *ros1-4*, and *rdd*, respectively (tables S1 to S3). In contrast, only 75, 106, and 1052 loci were found to have a decreased DNA methylation in *idm1-1*, *ros1-4*, and *rdd*, respectively (tables S4 to S6). Out of the 1098 hypermethylated loci in *idm1-1* (referred herein as DT loci for demethylation target loci), seven were selected for validation by individual locus bisulfite sequencing and were all confirmed to be hypermethylated in *idm1-1* as well as *ros1-4* or *rdd* (fig. S3). The DT loci are distributed across the five chromosomes (fig. S7A), are enriched in small RNAs (fig. S7B), and the majority of them overlap with repeats (table S1 and fig. S8). Genic loci account for ~80% of the DT loci in *idm1-1* (fig. S7C). The hypermethylation in genic regions in *idm1-1* mutant plants is not restricted to CGs (Fig. 1, B and C, and fig. S3). More than half of these hypermethylated genic loci in *idm1-1* are in multigene families where the member genes are highly homologous.

About 52% and 63% of the 1098 hyper-methylated loci in *idm1-1* are also hypermethylated in the *ros1-4* and *rdd* mutants, respectively, based on the methylome analysis (table S1 and fig. S7D). As a comparison, the same analysis showed that ~81% (as opposed to a theoretical 100%) of the hypermethylated loci in *ros1-4* overlap with those in *rdd* (table S2 and fig. S7D). Three loci that were identified as hypermethylated in *idm1-1* but not *ros1-4* or *rdd* were analyzed by individual locus bisulfite sequencing (fig. S3B). The analysis shows that all of them are hypermethylated in *ros1* or *rdd* as well as in *idm1*. In addition, four other loci that were identified as hypermethylated in the *rdd* mutant but not in *idm1-1* in the DNA methylome study were tested by individual locus bisulfite sequencing and found hypermethylated in *idm1-1* (Fig. 1C and fig. S3). These results suggest that the actual number of hypermethylated loci in *idm1-1* is larger and the percentage of overlap with the hypermethylated loci in the *rdd* mutant is likely higher. It thus appears that the majority of the loci hypermethylated in *idm1* are also hypermethylated in the *rdd* mutant. This is consistent with the *idm1ros1* double-mutant analysis that indicated that IDM1 and ROS1 (and DML2 and DML3) function in the same DNA demethylation pathway.

The expression levels of 15 genes that have increased DNA methylation in or near the genes in *idm1-1* and *ros1-4* mutants were examined. We found that seven of the tested genes show a substantial reduction in their transcript levels in the mutants (fig. S8). Our results suggest that, like ROS1, IDM1 is critical for preventing the transcriptional silencing of some transgenes and endogenous genes.

The tissue pattern of *IDM1* expression is similar to those of *DML2* and *ROS1* (fig. S9). IDM1 is predicted to have a MBD domain, a PHD finger domain and an N-acetyltransferase domain (fig. S1D). We found that the PHD finger of IDM1 specifically binds the N-terminal tail of histone H3 and that the binding is inhibited by H3K4 di- or trimethylation (fig. S10A). H3R2 methylation also appears to inhibit the binding (fig. S10A). In vitro affinity pull-down assays confirmed that the PHD finger of IDM1 recognizes H3 N-terminal tail and that the recognition is prevented by H3K4 di- or trimethylation (Fig. 2A). In contrast, H3K9 methylation does not inhibit the interaction (Fig. 2A and fig. S10A). C732W or C740W mutation (fig. S11A) abolishes the binding of IDM1 to histone H3 (Fig. 2A). Expression of the wild-type but not the C732W or C740W mutant version of *IDM1* under its native promoter could complement the DNA hypermethylation phenotype of the *idm1-1* mutant (Fig. 2B and fig. S10B). The results suggest that histone H3 binding is important for IDM1 function in vivo. IDM1 binds to the DNA containing CG methylation, and the binding is competitively blocked by unlabeled methylated, but not unmethylated, DNA of the same sequence (Fig. 2C and fig. S12). We also tested DNA with CG methylation corresponding to the *DT-77* locus and found that IDM1 binds specifically to methylated *DT-77* DNA (Fig. 2D).

We expressed and purified IDM1 fragments containing the putative HAT and PHD domains (IDM1-C1) or the HAT domain only (IDM1-C2) (fig. S13, A and B) from insect cells. Using core histones, histone H3 (Fig. 3A) and oligonucleosome (fig. S13C) as substrates, we found that IDM1-C1, but not IDM1-C2, has an acetyltransferase activity on histone H3. K14, K18, and K23 of H3 are acetylated by IDM1-C1 in vitro (Fig. 3B). Mass spectrometry analysis of the in vitro acetylated histone H3 confirmed that K18 and K23, as well as K14, are acetylated and K18 and K23 are the main targets of acetylation by IDM1-C1 (fig. S13, D and E). D924A, E941Q, or M942A mutation in conserved residues in the HAT domain abolished or diminished the in vitro acetyltransferase activity of IDM1-C1 (Fig. 3C). Expression of the D924A or M942A mutated *IDM1* under its native promoter failed to complement the DNA hypermethylation phenotypes of the *idm1-1* mutant (Fig. 3D and fig. S10B). The results suggest that the histone H3 acetylation activity is necessary for IDM1 function in preventing DNA hypermethylation in plants. The C732W, but not the C740W,

mutation in the PHD domain required for H3 interaction also abolished the acetyltransferase activity of IDM1-C1 (Fig. 3C), suggesting that the PHD domain can affect the acetyltransferase activity of IDM1 independently of its role in binding histone H3. Chromatin immunoprecipitation (ChIP) assays showed that acetylated histone H3K18 and H3K23 marks are reduced considerably in *idm1-1* mutant plants specifically at the DT loci (Fig. 4A), suggesting that IDM1 is critical for H3K18 and H3K23 acetylation in vivo.

ChIP assays also indicated that IDM1 is enriched at all of the DT loci tested (Fig. 4B). The DT loci have CG methylation (Fig. 1, B and C, fig. S3, and table S1), and several of them were found to have low levels of H3K4 dimethylation by ChIP assays (fig. S14). In contrast, IDM1 is not enriched in the highly expressed *At1g01260*, *At1g10950*, and *At1g30440* genes (Fig. 4B) that have abundant CG methylation (9) and a high level of H3K4 dimethylation. DT loci in general correspond to sequences with low H3K4 mono-, di-, and trimethylation, relative to a comparison group of CG methylated and expressed genes (10) (fig. S15). The results are consistent with the notion that IDM1 binds to methylated DNA through its MBD domain but, because of its PHD finger domain, the binding is prevented at loci where there is a high level of H3K4 di- or trimethylation. Transposons are characterized by high CG methylation and low H3K4 di- or trimethylation (10). However, IDM1 does not affect the methylation status of most transposons, which are associated with heterochromatin (fig. S7C). This discrimination against heterochromatic regions could potentially be explained by the inhibition of IDM1 interaction with histone H3 by H3R2 methylation (fig. S10A), which is known to be associated with heterochromatin in yeast (11). The DNA hypermethylation phenotypes of *idm1* mutants and the HAT activity and DNA- and histone-binding characteristics suggest that IDM1 may be important in selectively binding to the DT loci and creating a permissive chromatin environment for DNA demethylation enzymes. Consistent with this notion, ChIP results show that ROS1 protein is enriched at the DT loci in wild-type plants but that the enrichment is reduced to basal levels of nonspecific binding in *idm1* mutant plants (Fig. 4C).

Active DNA demethylation in plants is achieved through a base excision repair pathway that is initiated by the ROS1 subfamily of 5-methylcytosine DNA glycosylases (3). Our results here suggest that IDM1 controls the DNA methylation levels of a subset of loci targeted by the 5-methylcytosine DNA glycosylases. It is critical for preventing DNA hypermethylation and transcriptional silencing of some transgenes and endogenous genes with repetitive sequences or highly homologous genes in multi-gene families. We showed that IDM1 is a histone H3 acetyltransferase that is capable of recognizing methylated DNA through its MBD domain and recognizing unmethylated histone H3K4 through its PHD domain. Thus, IDM1 can recognize multiple epigenetic features at some demethylation target loci and create acetylated H3K18 and H3K23 marks, which then may be recognized by DNA demethylation enzymes or their interacting partner proteins (fig. S16) because IDM1 does not appear to colocalize with ROS1 (fig. S17).

## Supplementary Material

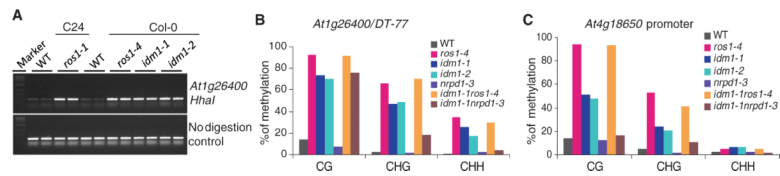
Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

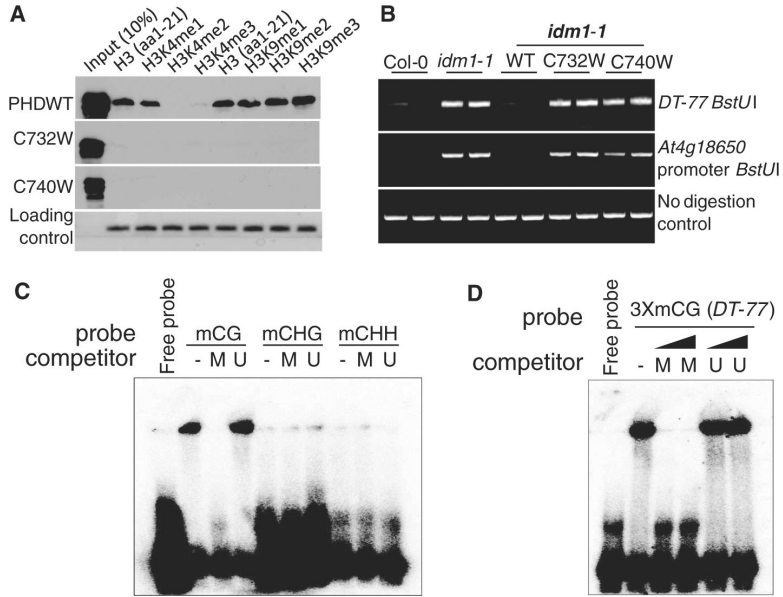
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## References and Notes

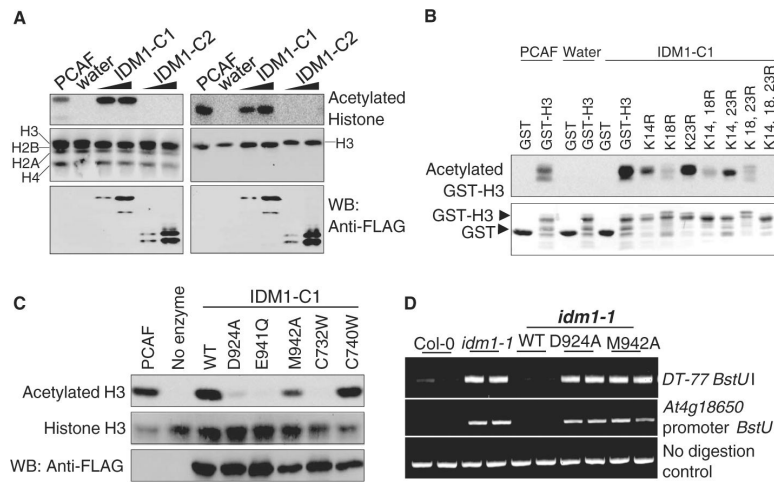
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**Fig. 1.** Identification and characterization of the *idm1* mutants. **(A)** Analysis showing the effects of *idm1* mutations on *At1g26400* **(B)** and *At4g18650* **(C)** of DNA methylation level at the *At1g26400* locus by chop-PCR (4). Un-DNA methylation in different sequence contexts and genetic interactions digested DNA is shown as a control. **(B and C)** Bisulfite sequencing data with *ros1* or *nrdp1*. WT, wild type.

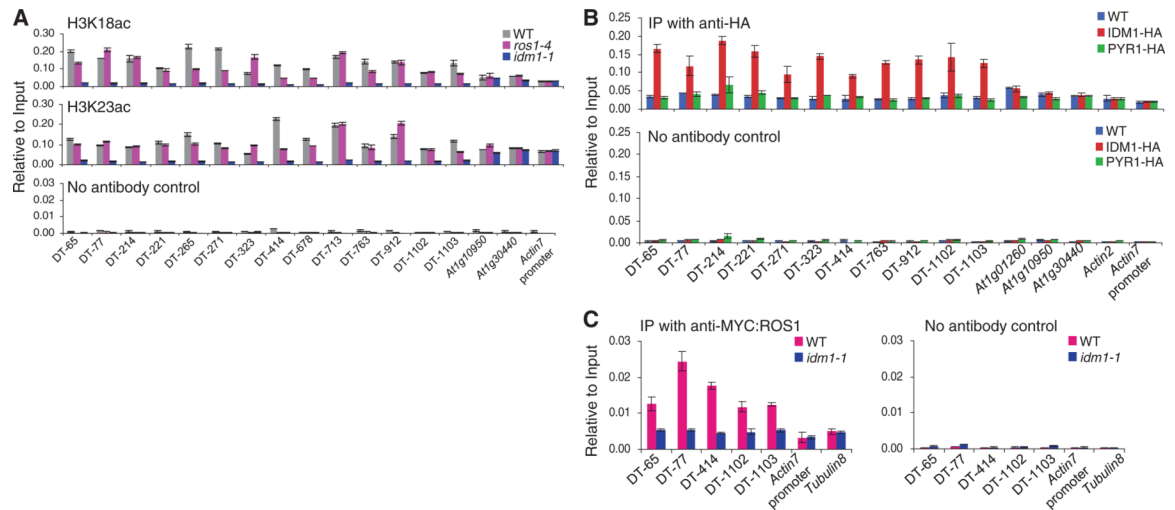


**Fig. 2.** Functional analysis of the PHD finger and MBD domains of IDM1. **(A)** Western blot analysis of the PHD finger of IDM1 in a peptide pull-down assay. Coomassie blue–stained biotinylated histone peptides are shown as a loading control. **(B)** DNA hypermethylation phenotypes of *idm1-1* plants transformed with WT or mutant forms of *IDM1*. **(C)** Electrophoretic mobility shift assay (EMSA) of IDM1-N (amino acids 1 to 400) binding to methylated oligonucleotides (fig. S12). -, no competitor; M, methylated; U, unmethylated. **(D)** EMSA showing IDM1-N (amino acids 1 to 400) binding to a methylated oligonucleotide probe corresponding to the *DT-77* locus.



**Fig. 3.** Histone acetyltransferase activity of IDM1. **(A)** HAT assays. (Top) Autoradiograph for acetylated histone. (Middle) Coomassie blue–stained membrane picture. (Bottom) Western blot detection of IDM1 with antibody to FLAG. p300-CBP-associated factor (PCAF) was used as a positive control. **(B)** HAT assay results using recombinant histone H3 mutated at different lysine positions as substrates. **(C)** HAT assay results using histone H3 as substrate and using WT and mutated forms of IDM1-C1 proteins as enzymes. **(D)** DNA hypermethylation phenotypes of *idm1-1* plants transformed with WT or mutant forms of IDM1.



**Fig. 4.**

Histone acetylation marks and IDM1 and ROS1 association with chromatin. **(A)** H3K18 acetylation (ac) and H3K23ac levels at the DT loci and control regions. ChIP was performed with antibodies to H3K18ac and H3K23ac. **(B)** Association of IDM1 protein with DT loci. ChIP was performed in WT, PYR1:3HA, and IDM1:3HA:YFP transgenic plants with antibody to hemagglutinin. **(C)** Effect of *idm1* on ROS1 protein association with DT loci. ChIP was performed in MYC:ROS1/*ros1-4* (WT) and MYC:ROS1/*idm1-1* plants using antibody to MYC. The ChIP signal was quantified as relative to input DNA. The no-antibody precipitates served as a negative control. Standard errors were calculated from three technical repeats.