Supplemental Table

Table S1. Immunoprecipitation-Mass Spectrometry Data for Flag-KYP (related to Figure 2).

The next most abundant proteins were 8-fold less abundant and were not reproducible in multiple replicates.

		Spectra		Unique Peptides		NSAF		% Coverage	
Protein	**Accession**	**Rep1**	**Rep2**	**Rep1**	**Rep2**	**Rep1**	**Rep2**	**Rep1**	**Rep2**
KYP	AT5G13960	432	142	26	20	19399.26	4519.88	39.30%	25.60%

Extended Experimental Procedures

Protein and DNA preparation

The N-terminal truncated KYP (residues 93 - 624) was cloned into a self-modified vector, which fuses a hexa-histidine tag plus a yeast sumo tag to the N-terminus of the target gene. The plasmid was transformed into *E. coli* strain BL21(DE3) RIL (Stratagene). The transformed cell was cultured at 37 °C until OD_{600} reached 0.8, after which the cell was cooled to 17 °C and Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.25 mM to induce protein expression overnight. The recombinant protein was first purified using nickel affinity chromatography column (GE Healthcare). The hexa-histidine tag was cleaved by ulp1 protease and further removed by a second step nickel affinity chromatography column (GE Healthcare). The target protein was further purified using a Heparin column and a Superdex G200 gel filtration column (GE Healthcare). Normally, around 0.1 mg protein can be obtained from 1 L bacterial culture. The purified protein was concentrated to 15 mg/ml and stored at -80 °C. All the mutants were constructed using a QuikChange Mutagenesis Kit (Stratagene) and purified with the same protocol as the wild-type protein.

DNA oligos of 15-nt length containing a single central mCHH site (forward strand: 5’-GGTACTXATCAGTAT-3’, X = 5mC; reverse strand: 5’-ACTGATGAGTACCAT-3’) or a single central mCHG site (forward strand: 5’-GGTACTXAGCAGTAT-3’, X = 5mC; reverse strand: 5’-ACTGCTGAGTACCAT-3’) were annealed together to generate 13-bp DNA duplexes with a central single methylation site and two-nucleotide AT overhangs at the 3’-ends. The modified oligonucleotides were purchased from Keck Oligonucleotide Synthesis

Facility at Yale University and the unmodified oligonucleotides were purchased from Invitrogen Inc.

Crystallization

Before crystal screening, the purified KYP protein was diluted to 9 mg/ml and then mixed with mCHH-containing DNA duplex, the cofactor S-adenosyl-L-homocysteine (SAH), and unmodified histone H3(1-15) peptide at a molar ratio of 1 : 1.5 : 3 : 3 at 4 ℃ for 2 hours. Crystallization was carried out at 20 ℃ using the hanging drop vapor diffusion method by mixing 1 μl protein sample and 1 μl reservoir solution and equilibrating against 500 μl reservoir solution. The complex was crystallized under the condition of 30% PEG200, 5% PEG3000, and 0.1 M MES, pH 6.0. Square shaped crystals appeared in 2 weeks. The crystals of KYP in complex with mCHH DNA and SAH or KYP in complex with mCHG DNA and SAH were obtained by the same protocol and under the same conditions as mentioned above for KYP-mCHH DNA-SAH-H3(1-15) peptide complex. The crystals were directly mounted on a nylon loop and flash-cooled into liquid nitrogen for diffraction data collection. The diffraction data of KYP-mCHH DNA-SAH complex and KYP-mCHH DNA-SAH-H3(1-15) peptide complex were collected at beamline X29A, National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL), New York. The diffraction data of KYP-mCHG DNA-SAH complex were collected at beamline 24ID-E, Advanced Photon Source (APS) at Argonne National Laboratory (ANL), Chicago. All the data were indexed, integrated, and further scaled with the program HKL2000 (Otwinowski and Minor, 1997). The statistics of the diffraction data are summarized in **Table 1**.

Structure determination and refinement

The structure of KYP-mCHH DNA-SAH complex was solved using single-wavelength anomalous dispersion method with zinc anomalous signal implemented in the program Phenix (Adams et al., 2010). The model building was carried out using the program Coot (Emsley et al., 2010) and structural refinement using the program Phenix (Adams et al., 2010). Throughout the refinement, a free *R* factor was calculated using 5% random chosen reflections. The stereo chemistry of the structural models were analyzed using the program Procheck (Laskowski et al., 1993). The structures of KYP-mCHH DNA-SAH-H3(1-15) peptide complex and KYP-mCHG DNA-SAH complex were solved using the molecular replacement method implemented in the program Phenix (Adams et al., 2010) using the structure of KYP-mCHH DNA-SAH complex as a model. The structural refinement was carried out using the same protocol. The statistics of the refinement are shown in **Table 1**. All molecular graphics were generated with the program Pymol (DeLano Scientific LLC, http://www.pymol.org/).

Plant material

All plants used in this study were of the *Arabidopsis thaliana* Columbia-0 (Col-0) accession, with WT referring to the parental strain. The KYP knock-out line used was *kyp-6*, which contains a T-DNA insertion (SALK_041474) (Johnson et al., 2007). 100 mg of leaves were collected from 3-week-old plants for western analysis and DNA isolation for whole-genome bisulfite sequencing.

KYP constructs

A 7 kb fragment containing the KYP gene (including 1.6 kb upstream of the ATG) was amplified and cloned into pENTR. A Kas I restriction site was introduced at the ATG and a biotin ligase recognition peptide (BLRP) followed by 3x FLAG epitope tag was introduced. All mutants were made using QuikChange (Stratagene) in this pENTR vector and then the tagged constructs were recombined into JP746 (Johnson et al., 2008) and introduced into Agrobacterium strain AGL0.

Western blots

Western blots were done by grinding 0.1 g of leaves in liquid nitrogen and resuspending in 100 ul lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM $MgCl_2$, 10% glycerol, 0.1% NP40, 5 mM β-mercaptoethanol, plus protease inhibitors). After a 5 minute centrifuge, Flag-KYP was purified by incubation with 50 ul Flag-magnetic beads and bound proteins were eluted. Western analysis was performed using ECL prime western blotting kit (GE Healthcare).

EMSA

Electrophoretic mobility-shift assays were done on samples that had been incubated at room temperature for 30 minutes and contained either 50, 100, or 200 ng of purified KYP or mutant KYP, in 25 mM Tris pH 6.8, 50 mM NaCl, 10 mM $MgCl_2$, 5% glycerol, 0.4 mg/ml BSA, 5 mM β-mercaptoethanol, 0.5 mg/ml polyglutamate (as non-specific competitor), and ^{32}P-labeled

probe as described previously (Johnson et al., 2007). Complexes were separated using 8% polyacrylamide gel electrophoresis and visualized by autoradiography.

Histone methyltransferase assays

700 ng of purified KYP protein (equals 0.5 µM final concentration) were pre-incubated with 1 µM S-adenosyl methionine in 20 µl of reaction buffer (20 mM KCl, 8 mM $MgCl_2$, 50 mM Tris-Cl pH 8, 5 % glycerol, 7 mM β-mercaptoethanol) for 5 minutes at 25 ºC. For radioactivity assays Adenosyl-L-methionine, S-[methyl-3H] (PerkinElmer) was used and 5 µM biotin-conjugated H3 peptides (residues 1 – 21, un-, mono- or di-methylated K9, Millipore) were added to the pre-incubated samples. After 1 min at 25 ºC the methyltransferase reaction was stopped and the biotinylated peptides were purified with 140 µl stop/bind buffer (0.5 M NaCl, 10 mM EDTA, 50 mM Tris-Cl pH 8) containing 20 µl streptavidin-coupled magnetic beads (Dynabeads MyOne Streptavidin C1, Life Technologies). Bound peptides were washed four times with 180 µl Tris-buffered saline + 0.1 % Tween-20, resuspended in 100 µl water and radioactivity was measured using liquid scintillation counting. For western blot analysis of histone methyltransferase activity, 5 µM recombinant human histone H3.1 (NEW ENGLAND BioLabs) was added to the pre-incubated samples and reactions were incubated for 10 min at 25 ºC. Proteins were separated using SDS-PAGE and silver-stained or transferred onto nitrocellulose membrane for immunoblot analysis with the Odyssey infrared imaging system (LI-COR). Methylation states and levels were measured and normalized against H3 levels using primary antibodies against H3 (ab1791 or ab10799, Abcam), H3K9me1 (07-450,

Millipore), H3K9me2 (abcam1220, Abcam) and H3K9me3 (ab8898, Abcam) and secondary infrared antibodies (LI-COR).

Whole-genome bisulfite sequencing

Whole-genome bisulfite sequencing (BS-Seq) libraries for initial tests of KYP-FLAG complementation were generated as previously reported (Stroud et al., 2013), while BS-seq libraries for mutant versions of the KYP-FLAG transgene (as well as parallel control libraries) were generated using TruSeq DNA multiplexing kit (Illumina). All libraries were sequenced using the HiSeq 2000 platform following manufacturer instructions (Illumina) at a read length of 50 bp. Methylation over *kyp* CHG DMRs for various libraries sequenced was calculated as previously described (Stroud et al., 2013) and *kyp* DMRs themselves were previously described (Stroud et al., 2013). Complementing CHG DMRs were defined as DMRs that returned to WT levels of percent methylation +/- 10% of the WT methylation level in a KYP-FLAG transgenic line.

Supplemental References

Adams, P. D., Afonine, P. V., Bunkoczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., *et al.* (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr D Biol Crystallogr *66*, 213-221.

Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010). Features and development of Coot. Acta Crystallogr D Biol Crystallogr *66*, 486-501.

Johnson, L. M., Bostick, M., Zhang, X., Kraft, E., Henderson, I., Callis, J., and Jacobsen, S. E. (2007). The SRA methyl-cytosine-binding domain links DNA and histone methylation. Curr Biol *17*, 379-384.

Johnson, L. M., Law, J. A., Khattar, A., Henderson, I. R., and Jacobsen, S. E. (2008). SRA-domain proteins required for DRM2-mediated de novo DNA methylation. PLoS Genet *4*, e1000280.

Laskowski, R. A., Macarthur, M. W., Moss, D. S., and Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr *26*, 283-291.

Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol *276*, 307-326.

Stroud, H., Greenberg, M. V., Feng, S., Bernatavichute, Y. V., and Jacobsen, S. E. (2013). Comprehensive analysis of silencing mutants reveals complex regulation of the Arabidopsis methylome. Cell *152*, 352-364.