

Supplemental methods

Evaluation of DYNAMET cassettes

The MBD domain of MBD6 (Zemach and Grafi 2003), the SRA domain of SUVH4 and of SUVH9 (Johnson et al. 2007, 2008) were fused to a nuclear localization signal (NLS) in frame with a fluorescent protein (Supplemental Fig. S1A). The three cassettes are driven by the HTR5 promoter. All three DYNAMET fusion proteins were detected in transient assays in tobacco cells (Supplemental Fig. S1B,C), but fluorescence was not detected in stably transformed *Arabidopsis* plants carrying either *pHTR5:SUVH4Δ-SRA-EGFP* or *pHTR5:SUVH9Δ-SRA-Venus*. SUVH4 and SUVH9 crystal structures revealed extensive inter-domain interactions (Du et al. 2014; Johnson et al. 2014). The inability to detect SRA domain-based DYNAMET in stable transgenic plants could result from destabilized SRA domain molecules. To help stabilize SRA-based DYNAMETs, we used the entire SUVH4 and SUVH9 proteins that both contain a SET domain C-terminally to the SRA domain. The histone methyltransferase (HMT) activity of SUVH4 SET domain can be completely abolished by conversions of two tyrosines to phenylalanines (Tyr475/493Phe). The SET domain of SUVH9 has no detectable HMT activity and is unable to bind methyl donor cofactor (Johnson et al. 2008, 2014). Accordingly, we generated two new DYNAMETs consisting on a nuclear encoded SUVH9 or SUVH4 with a mutated SET domain at Tyr475/493Phe (SUVH4-mSET) fused respectively to a fluorescent protein driven by the HTR5 promoter (Supplemental Fig. S1A). The SUVH4-mSET-EGFP DYNAMET was linked to a second cassette expressing a histone H2B fused to a red fluorescent protein mCherry (H2B-mCherry) also under the control of the *HTR5* promoter. Fluorescence of both DYNAMET fusions was similarly detectable in transient assays in tobacco cells (Supplemental Fig. S1D,E) but only the SUVH9-Venus fusion protein could be detected in stable transgenic plants (Fig. 1C). In SUVH4-mSET-EGFP transgenic plants, fluorescence was never detected in the transgenic lines. Only the linked H2B-mCherry chromatin reporter could be detected (Supplemental Fig. S2E). However, transcripts of SUVH4-mSET-EGFP were detected by RT-PCR in transgenic lines (data not shown), suggesting that SUVH4-mSET-EGFP is destabilized when mutated at any of these residues.

Chromatin Immunoprecipitation

500 µg of inflorescence tissues were collected for each genotype and cross-linked with 1 % formaldehyde for 10 min under vacuum. Cross-linking was stopped by adding glycine to a final concentration of 0.125 M. Liquid nitrogen was used to grind the tissues to a fine powder, which was mixed to 30 ml of Extraction Buffer 1 (0.4 M sucrose, 10 mM Tris-HCl pH 8, 10 mM MgCl₂, 5 mM β-mercapto-ethanol, 0.1 mM PMSF, protease inhibitor cocktail (Complete™, Roche), mixed gently and let on ice for 15 min. The solution was filtered twice with Miracloth into a fresh 50 ml falcon tube, and spinned for 20 min at 4000 rpm at 4°C. The pellet was resuspended in 1 ml of Extraction Buffer 2 (0.25 M sucrose, 10 mM Tris-HCl pH 8, 10 mM MgCl₂, 1 % Triton X-100, 5 mM β-mercapto-ethanol, 0.1 mM PMSF, protease inhibitor cocktail (Complete™, Roche) and transferred to 1.5 ml Eppendorf tube, followed by 10 min centrifugation (12000 rpm, 4°C). The resulting pellet was resuspended in 300 µl of Extraction Buffer 3 (1.7 M sucrose, 10 mM Tris-HCl pH 8, 0.15 % Triton X-100, 2 mM MgCl₂, 5 mM β-mercapto-ethanol, 0.1 mM PMSF, protease inhibitor cocktail (Complete™, Roche), layered on top of 300 µl of Extraction Buffer 3 in a clean tube and spinned for 1 h (14000 rpm, 4°C). The chromatin pellet was then resuspended in 300 µl of Nuclei Lysis Buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, 1 % SDS, protease inhibitor cocktail (Complete™, Roche) and sonicated with a Diagenode Bioruptor machine (10 cycles, ON 30 s/ OFF 1 min), spinned at full speed for 5 min at 4°C to pellet debris. The supernatant was transferred to a fresh tube, and the chromatin solution brought to 3 ml with ChIP Dilution Buffer (1.1 % Triton X-100,

1.2 mM EDTA, 16.7 mM Tris-HCl pH 8, 167 mM NaCl). 1 ml of chromatin was added to new low-binding tubes and pre-cleared with 50 μ l of agarose beads for 1 h on a rotating wheel. 1 ml of pre-cleared chromatin was mixed with 80 μ l of equilibrated agarose beads and 3 μ g of anti-GFP antibody (Chromotek), and incubated overnight at 4°C on a rotating wheel. Beads were washed with the following sequence: twice 5 min with Low Salt Wash Buffer (150 mM NaCl, 0.1 % SDS, 1 % TritonX-100, 2 mM EDTA, 20 mM Tris-HCl pH8.1), twice 5 min with High Salt Wash Buffer (500 mM NaCl, 0.1 % SDS, 1 % TritonX-100, 2 mM EDTA, 20 mM Tris-HCl pH8.1), twice 5 min with LiCl Wash Buffer (0.25 mM LiCl, 1 % NP40, 1 % sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH 8.1), and twice 5 min with TE Buffer (10 mM Tris-HCl pH 8, 1 mM EDTA). Immune complexes were eluted twice with 250 μ l of Elution Buffer (1 % SDS, 0.1 M NaHCO₃) by incubating at 65°C for 15 min with gentle agitation, and combining both eluates. 20 μ l NaCl (5M) was added to the combined eluates, which were reverse-cross linked at 65°C overnight, and treated with 2 μ l proteinase K (10 mg/ml) for one hour at 45°C. Enzymatic activity was stopped by adding 500 μ l of Elution buffer and 20 μ l 5 M NaCl. The resulting DNA fragments were purified using Diagenode ChIP purification kit, and resuspended in 50 μ l of distilled water. Indexed libraries were generated using Illumina's TruSeq ChIP Sample Prep Kit and sequenced on either an Illumina HiSeq 2500 sequencer by FASTERIS SA, following the manufacturer's instructions, or on a NextSeq machine at Cold Spring Harbor Genomic Core Facility. The outputs were as follows:

Library	#reads (millions)
CHH-Venus rep1	30.4 m
CHH-Venus rep2	27.6 m
Input CHH rep1	21.8 m
Input CHH rep2	25.2 m
CG-Venus rep1	29.5 m
CG-Venus rep2	29.7 m
Input CG rep1	21.1 m
Input CG rep2	18.9 m
CHH-Venus, <i>cmt2</i> rep1	24.3 m
CHH-Venus, <i>cmt2</i> rep2	35.3 m
Input CHH-Venus, <i>cmt2</i> rep1	19.2 m
Input CHH-Venus, <i>cmt2</i> rep2	21.6 m
CHH-Venus, <i>drm1drm2cmt2cmt3</i> rep1	13.1 m
CHH-Venus, <i>drm1drm2cmt2cmt3</i> rep2	21.3 m
Input CHH-Venus, <i>drm1drm2cmt2cmt3</i> rep1+2	15.3 m
CHH-Venus, <i>drm2</i> rep1	18.5 m
CHH-Venus, <i>drm2</i> rep2	20.6 m
Input CHH-Venus, <i>drm2</i> rep1+2	17.2 m

Supplemental references

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