

# **Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail**

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## **Supplemental Information**

### **Materials and methods**

#### **Recombinant chromatin preparation**

Expression and purification of wt *Xenopus laevis* histones was performed as described (1). H3K4me3 and H3K9me3 were generated by native protein ligation (2). The coding sequence for *X.laevis* H3 $\Delta$ 1-20 C21A was amplified by PCR and cloned into the pET3d expression vector. The truncated H3 protein was expressed and purified like the wt histones. The H3 N-terminal peptide containing residues 1-20 and tri-methylated lysines 4 or 9 were synthesized using Fmoc-based solid-phase synthesis and activated at the C-terminus by thioesterification. Ligation of the activated H3 peptide to the truncated H3 histone and purification of the ligation product was performed as described (2). Identity and purity of histones was verified by SDS-PAGE as well as by mass spectrometry.

Assembly of histone octamers containing H3unmod, H3K4me3, and H3K9me3 as well as reconstitution of recombinant oligonucleosomes was performed by salt dialysis as described using the 12 $\times$ 200 $\times$ 601 template (1,3). Briefly, octamers were reconstituted using H3unmod, H3K4me3, or H3K9me3 and purified by gel filtration on Superdex 200 (GE Healthcare). Scavenger DNA corresponding to a fragment of 148 bp length PCR amplified from pUC18 was used in all reconstitutions. Assembly reactions were titrated at different octamer:DNA ratios. Reproducibly, a octamer:DNA

ratio of 1.1:1 resulted in saturated nucleosomal arrays. Reconstituted material was used for all assays without further purification after extensive dialysis against 10 mM TEA, 0.1 mM EDTA, pH 7.5.

### **Binding of ADD domains to peptides arrays**

CelluSpots arrays were provided by Intavis AG (Köln, Germany). The array was blocked by incubation in TTBS buffer (10 mM Tris/HCl pH 8.3, 0.05% Tween-20 and 150 mM NaCl) containing 5% non-fat dried milk at 4 °C overnight, then washed three times with TTBS buffer, and incubated with purified GST-tagged ADD domain of Dnmt3a or 3b (1  $\mu$ M) at room temperature for 2 hours in interaction buffer (100 mM KCl, 20 mM HEPES, pH 7.5, 1 mM EDTA, 0.1 mM DTT and 10% glycerol). After washing with TTBS buffer three times, the array was incubated with goat anti-GST antibody (GE Healthcare #27- 4577-01) 1:5000 dilution in TTBS buffer for 1 h at room temperature. Then, the array was washed three times with TTBS and incubated with horseradish peroxidase conjugated anti-Goat antibody (Invitrogen #81-1620) 1:12000 in TTBS for 1 h at room temperature. Finally, the array was washed three times with TTBS and submerged in ECL developing solution (Thermo Fisher Scientific) and image was captured in X-ray film.

### **Binding of ADD domains to native histones**

Native histones were isolated from HEK293 cell line by acid extraction method as described (4). For the binding specificity analysis of ADD domains, 7.5  $\mu$ g of native histones were separated in 16% SDS-PAGE and the histones transferred to the Nitrocellulose membrane by western blotting. After staining with Ponceau S, the Nitrocellulose membrane was blocked by incubation in TTBS buffer (10 mM Tris/HCl pH 8.3, 0.05% Tween-20 and 150 mM NaCl) containing 5% non-fat dried milk at 4 °C overnight. The membrane was then washed once with TTBS buffer, and incubated with purified GST-tagged ADD domain of Dnmt3a or Dnmt3b (1  $\mu$ M) at room temperature for 90 min in interaction buffer (100 mM KCl, 20 mM HEPES pH

7.5, 1 mM EDTA, 0.1 mM DTT and 10% glycerol). After washing in TTBS buffer, the membrane was incubated with goat anti-GST antibody (GE Healthcare #27-4577-01 at 1:3000 dilution) in TTBS buffer for 1 h at room temperature. Then, the membrane was washed three times with TTBS and incubated with horseradish peroxidase conjugated anti-Goat antibody (Invitrogen #81-1620 1:3000) in TTBS for 1 h at room temperature. Finally, the membrane was submerged in ECL developing solution (Thermo Scientific) and image was captured in X-ray film.

### **Methylation of the DNA in recombinant chromatin**

16 nM chromatin (unmodified and modified with H3K4me3, and H3K9me3) were methylated using 2  $\mu$ M Dnmt3a2 or Dnmt3a-C, in reaction buffer (20 mM Hepes pH 7.2, 1 mM EDTA, 100 mM KCl) in the presence of S-adenosyl-L-methionine (0.32 mM) for 2 h at 37°C. Naked DNA was methylated as a control. Dnmt3a2/3L complex was formed by pre-incubation of equimolar amounts of both proteins at room temperature for 30 min, then used to methylate the chromatin. Duplicate reactions were set up in parallel for each sample type. The reaction was stopped by freezing in liquid nitrogen. Afterwards, proteinase K digestion was performed, followed by clean-up of DNA using DNA purification Kit (Macherey Nagel, Düren, Germany).

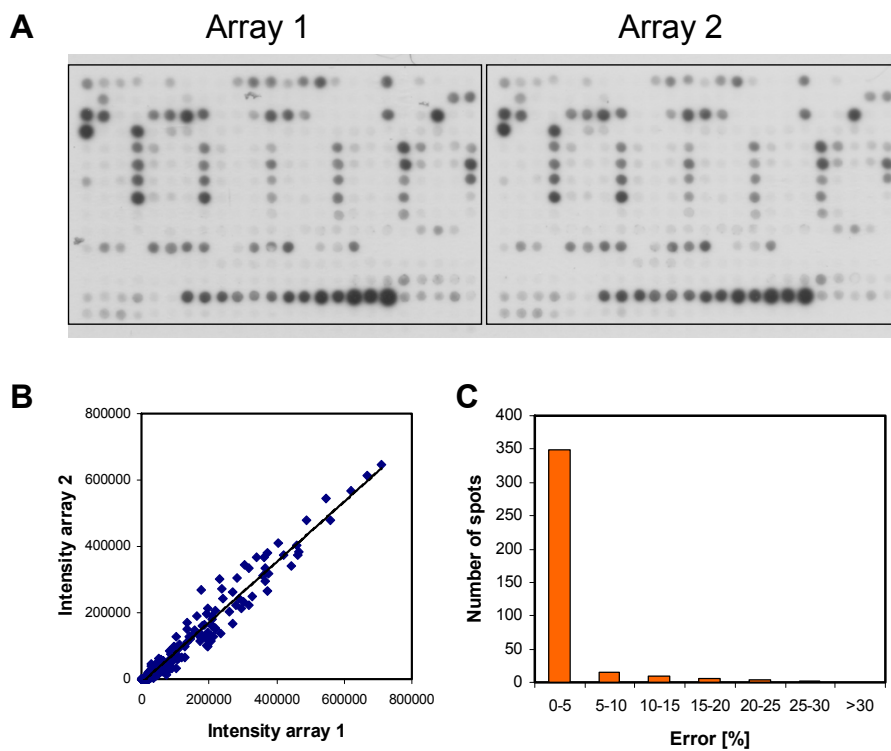
### **Bisulfite conversion, subcloning and sequencing**

The purified DNA was treated with bisulfite as described (5). The bisulfite converted DNA was used as template for PCR using primers designed for the single nucleosome (Top strand: forward GGT TAT GTG ATG GAT TTT ATA and reverse CTA TTC AAY ACA TAC ACA AAA TATA; bottom strand: forward TAA TAT ATG TAT AGG ATG TAT ATA TTT GA and reverse TCR AAT TAT ATA ATA AAC CCT ATAC; with Y representing C or T and R representing A or G). The PCR program was: 95°C, 3 min; (94°C 45 s , 43°C 35 s, 72 °C 20 s) 35 cycles, 72°C 5 min. The PCR products were purified by ChargeSwitch® PCR Clean-Up Kit (Invitrogen) and subcloned using the StrataClone™ Kit (Stratagene). Around 100 clones for each PCR product were picked for each individual experiment and sequenced using ABI BigDye Terminator

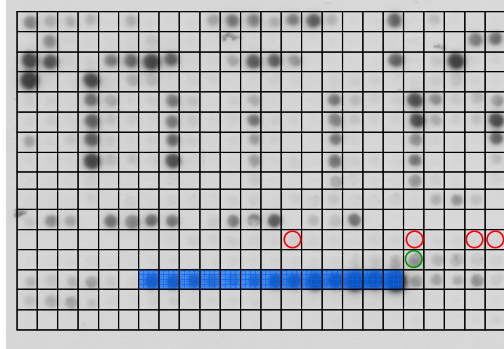
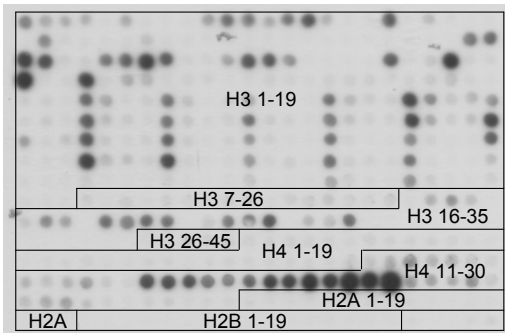
chemistry (BigDye Terminator v3.1 K) using 3730xl ABI 96-capillary sequencer systems equipped with capillaries of 50 cm separation length. BISMA and BDPC was used to perform quality control, derive DNA methylation patterns from the sequencing results, calculate the average methylation level of single CpG sites and present the DNA methylation pattern (6-8).

## Supplemental Figures

Suppl. Fig. 1: Reproducibility of ADD binding to peptide arrays. A) The arrays contain two identical sets of peptides. B) Plots of the binding intensities to the two corresponding spots in the two replicates of the array. C) The relative deviation of intensities to the two corresponding spots after normalization of activity to the strongest spot on the array.



Suppl. Fig. 2: Binding of the Dnmt3a ADD domain to non-H3 1-19 spots. The left part shows the binding pattern of the ADD domain on the histone tail array. On the right side some H4 tail spots are annotated.



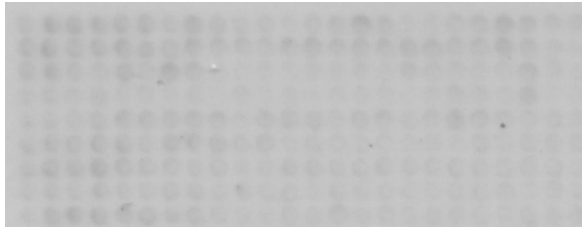
H4R3me2s

H4 11-30 K16ac

H4 11-30 K16ac + additional modifications

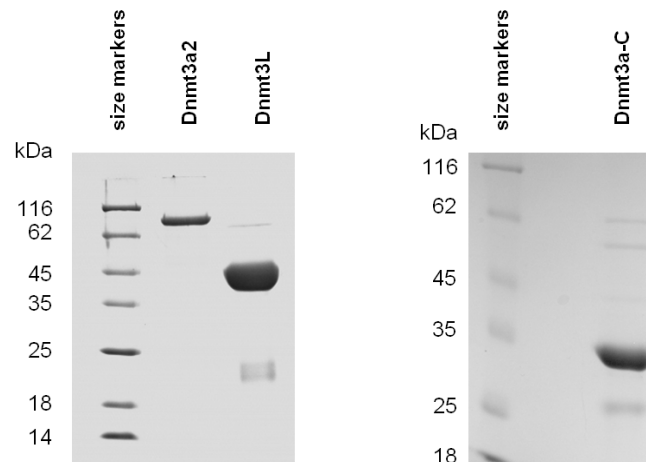
- K12ac K16ac
- K16ac R17me2s
- K16ac R17me2a
- K16ac R19me2s
- K16ac R19me2a
- K16ac K20me1
- K16ac K20me2
- K16ac K20me3
- K16ac K20ac
- K12ac K16ac K20me1
- K12ac K16ac K20me2
- K12ac K16ac K20me3
- K12ac K16ac K20ac

Suppl. Fig. 3: The Dnmt3a ADD domain did not bind to H3 1-19 peptides, if they were methylated at the N-terminus. The picture shows the same array design as in all other figures, with the only difference that the N-termini of all peptides were acetylated. We confirmed by antibody binding, that the peptides spotted in this array are fully functional (data not shown).

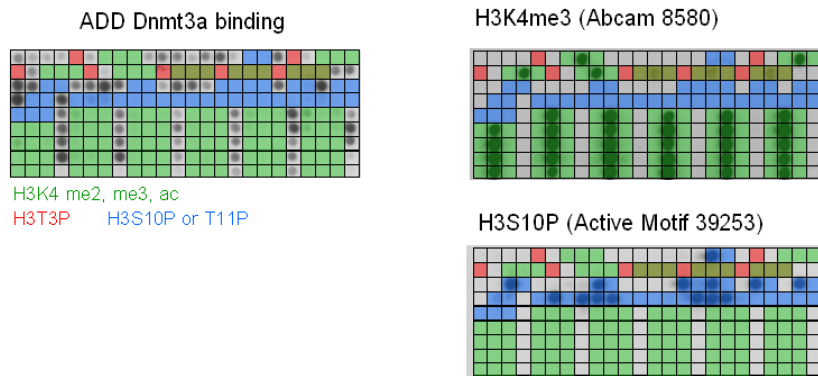


N-terminus acetylated

Suppl. Fig 4: Examples of Coomassie stained SDS polyacrylamide gel pictures of the protein preparations used in this study.



Suppl. Fig. 5: Binding of control antibodies to the peptide arrays used in this study. In the left panel, binding of the Dnmt3a ADD domain is shown. Peptides which carry a modification that prevents binding are shaded in green red and blue. On the right side, the binding of two different antibodies to the arrays is shown, with shading same as for the ADD domain. These results clearly illustrate that the spots containing the H3K4me3 or H3S10P modifications are present on the array.





## Supplemental References

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