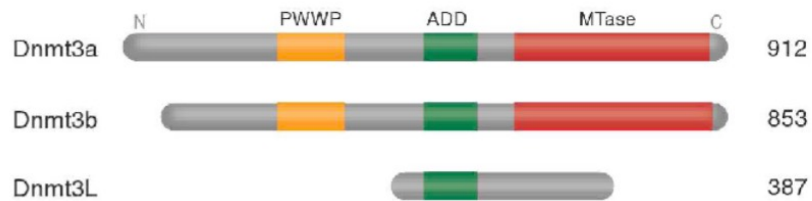


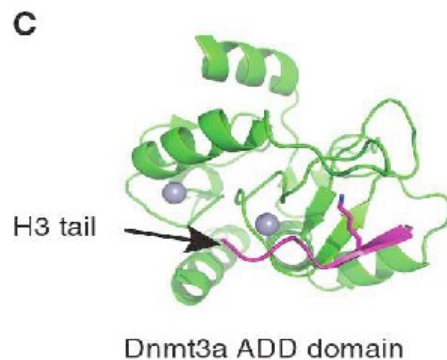
COORDINATION OF HISTONE AND DNA METHYLATION

The ADD domain in DNMT proteins binds to histone tails



De-novo DNMT family has 2 enzymatic active members (DNMT3a, b) and one regulatory factor DNMT3L

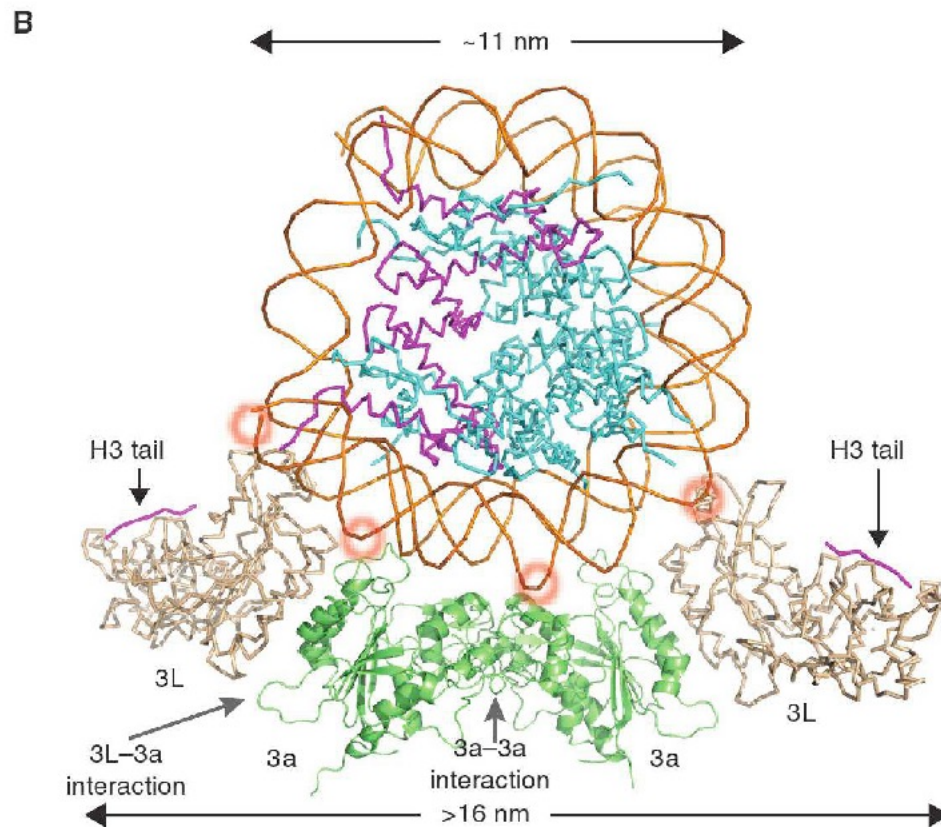
- PWWP (Proline-Tryptophane-Tryptophane-Proline) domain: protein or DNA interaction domain
- ADD (ATRX-DNMT3-DNMT3L) domain: highly similar between DNMT proteins: **CAN INTERACT WITH HISTONE TAILS**



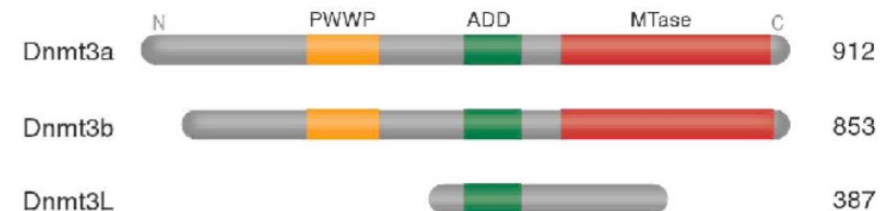
***ADD domain of DNMT3a
Interacts with the unmethylated Histone H3 tails***

→ a link of de-novo DNMTs with histone methylation

Linking de-novo DNA methylation to histone methylation



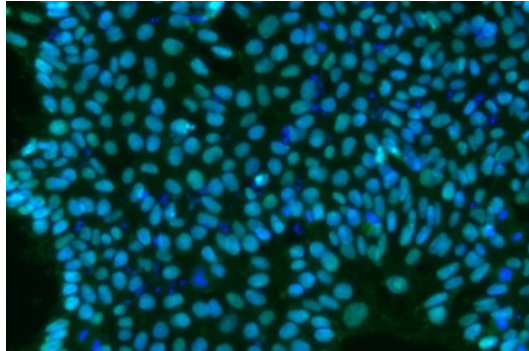
- DNMT3L forms a heterodimer with DNMT3a → dimers form tetramer: 2x DNMT3L; 2x DNMT3a (best studied); DNMT3L also interacts with DNMT3b
- **Phenotype of DNMT3L knock-out = phenotype of DNMT3a = DNMT3a and DNMT3L are functionally linked**
- Deletion of interaction domains that link DNMT3a to DNMT3L results in enzymatic inactivation = DNMT3a function depends on tetramer formation and DNMT3L!!
- Histone H3 tails interact with ADD domains of DNMT3a/b and DNMT3L (only DNMT3L shown); red circles: interaction with DNA



Linking de-novo DNA methylation to histone methylation

How can we find out whether there is a functional link between histone modifications and DNA methylation???

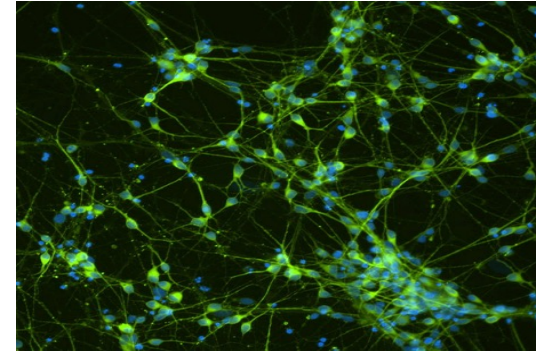
Follow the kinetics of histone and DNA methylation at genes in a relevant biological context



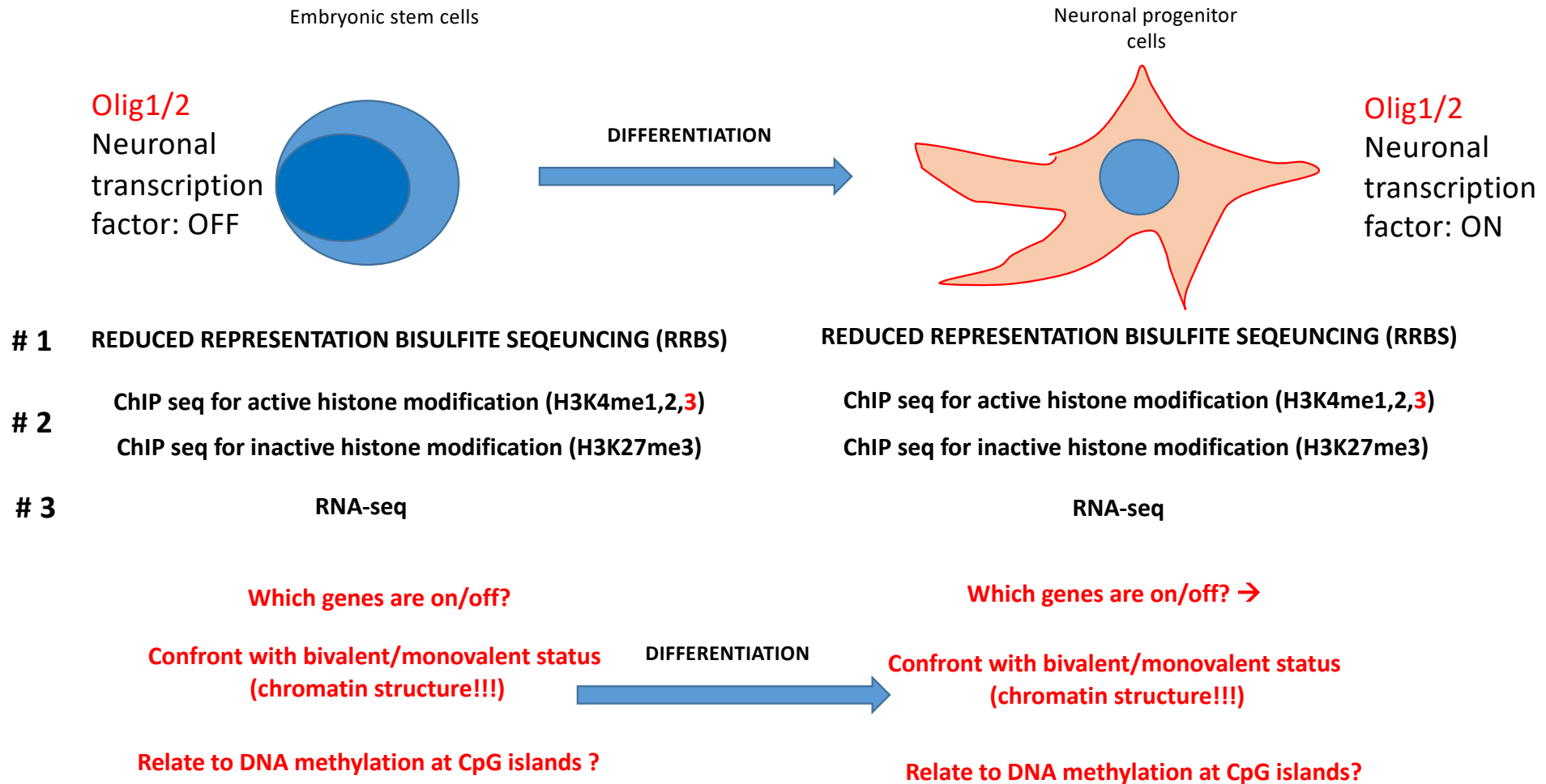
DIFFERENTIATION
IN VITRO



Change in gene expression
Change in epigenome
(histones and DNA)

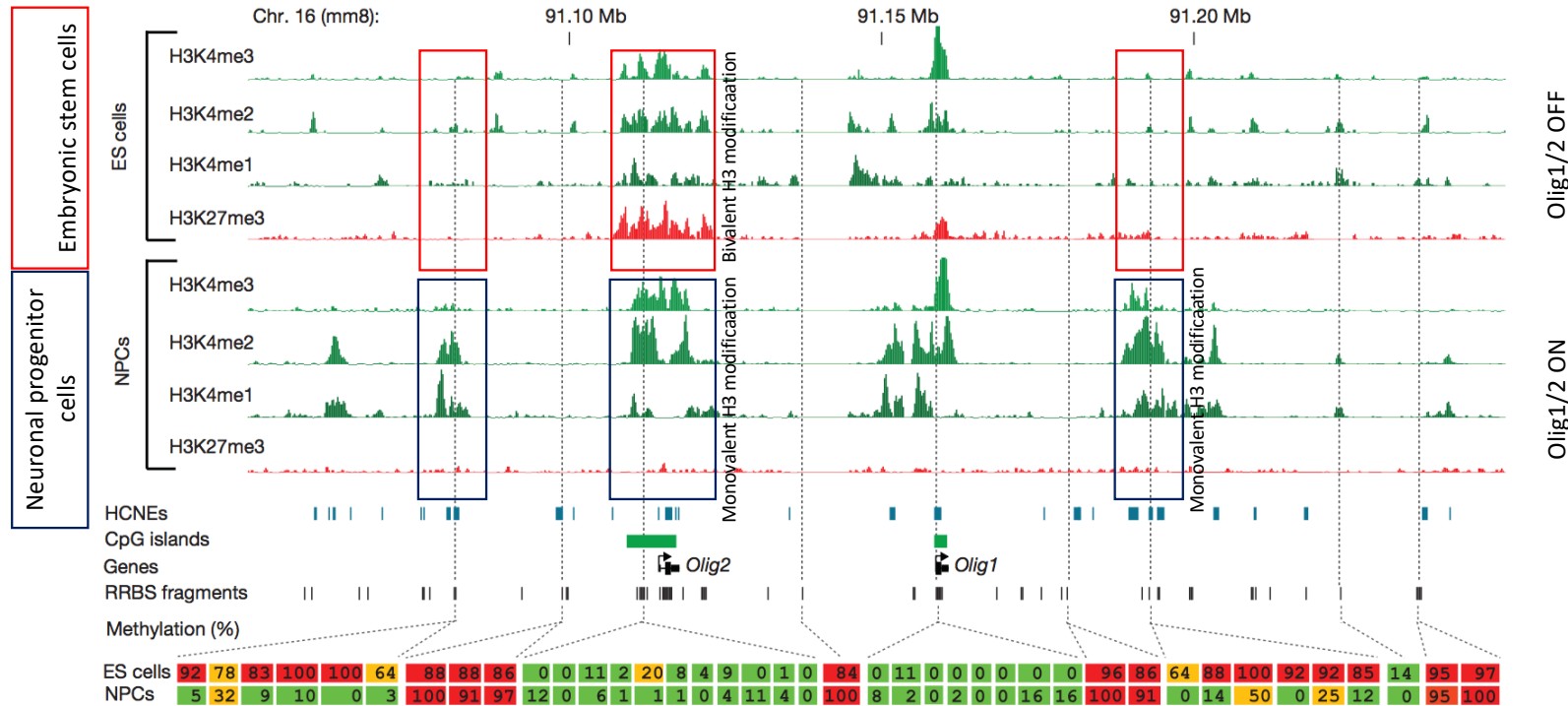


Linking de-novo DNA methylation to histone methylation



A link between H3K4 and CpG island methylation at the entire genome level

DNMT3L links histone methylation to DNA methylation



Olig1/2 OFF
Olig1/2 ON

Note:
- Olig1/2 have a bivalent status in mouse embryonic stem (ES) cells → **bivalent** (H3K27me3/H3K4me3) → not expressed

- Olig1/2 are **monovalent** active: no H3K27me3 but H3K4me3 → expression in NPCs

Figure 3 | Developmentally regulated de-methylation of highly conserved non-coding elements. Comparison of histone and DNA methylation levels across the *Olig1/Olig2* neural-lineage transcription factor locus. ChIP-Seq tracks for H3K4me1/2/3 and H3K27me3 in ES cells and NPCs are shown. The unmethylated CpG-rich promoters are bivalent and inactive in ES cells and resolve to univalent H3K4me3 on activation in NPCs. H3K4me2

enrichment appears over HCNEs distal to the two genes, and this correlates with CpG de-methylation. Inferred methylation levels for 40 out of 215 sampled CpGs are shown and colour-coded. Red indicates largely methylated (>80%); green indicates largely unmethylated (<20%), and orange indicates intermediate levels (≥20% and ≤80%).

HCNE: A conserved non-coding sequence (CNS) is a DNA sequence of noncoding DNA that is evolutionarily conserved. HCNEs can be important sites of evolutionary divergence as mutations in these regions may alter the regulation of conserved genes, producing species-specific patterns of gene expression.

DNMT3L links histone methylation to DNA methylation

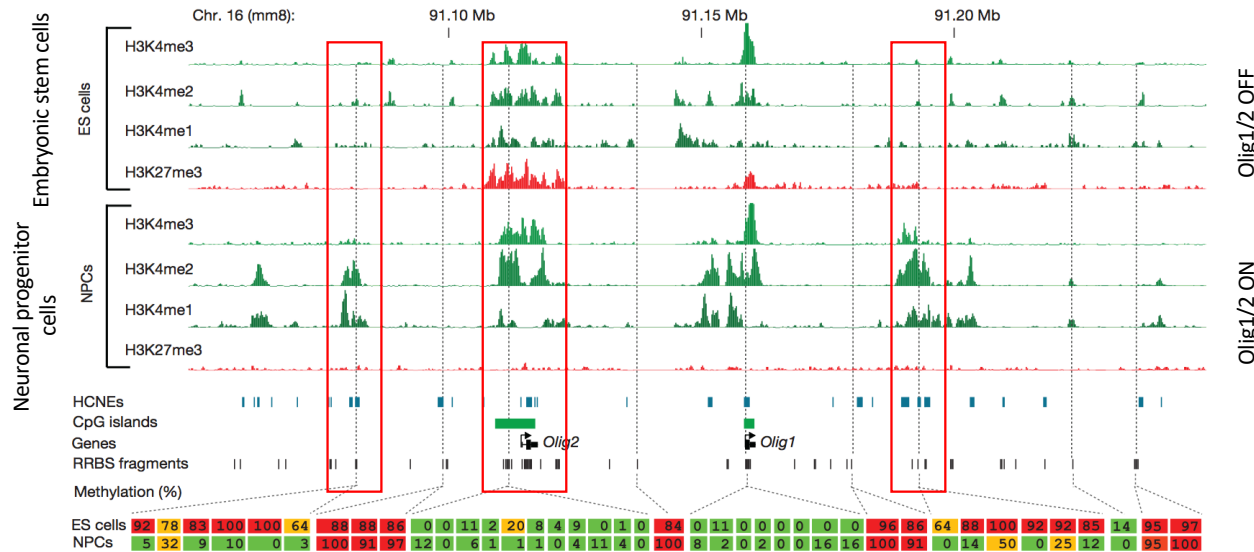


Figure 3 | Developmentally regulated de-methylation of highly conserved non-coding elements. Comparison of histone and DNA methylation levels across the *Olig1/Olig2* neural-lineage transcription factor locus. ChIP-Seq tracks for H3K4me1/2/3 and H3K27me3 in ES cells and NPCs are shown. The unmethylated CpG-rich promoters are bivalent and inactive in ES cells and resolve to univalent H3K4me3 on activation in NPCs. H3K4me2

enrichment appears over HCNEs distal to the two genes, and this correlates with CpG de-methylation. Inferred methylation levels for 40 out of 215 sampled CpGs are shown and colour-coded. Red indicates largely methylated (>80%); green indicates largely unmethylated (<20%), and orange indicates intermediate levels (≥20% and ≤80%).

H3K4me0: DNA METHYLATION IN CpG ISLANDS
H3K4me1,2,3: NO DNA METHYLATION IN CpG ISLANDS

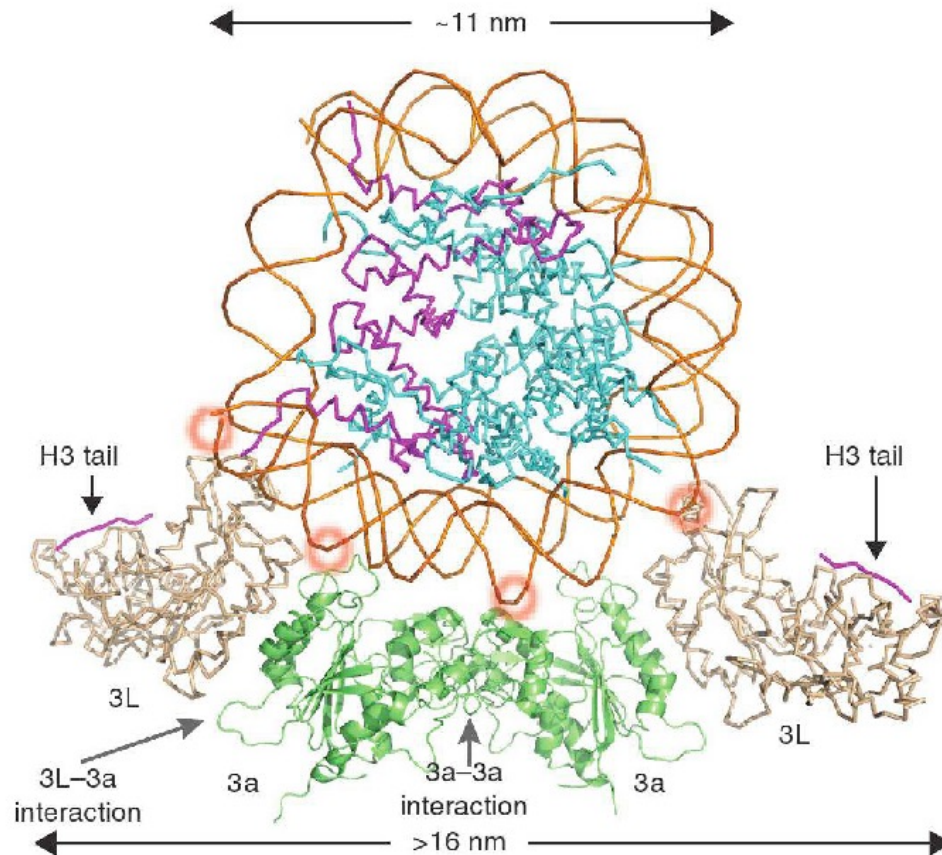


De novo DNA methyl-
transferases
translate patterns of
H3K4methylation
into heritable patterns of
gene expression

HOW???

DNMT3L links histone H3K4 methylation to DNA methylation

B



DNMT3L/ab ADD domain binds with high affinity to un-methylated Histone H3 tails

DNMT3L/ab in tetramer binds unmethylated histone H3 → CpG methylation by DNMT3a/DNMT3b

Mutated DNMT3L does not bind to unmethylated H3K4 → no DNA methylation at CpG islands!!

De novo DNA methyl-transferases translate patterns of H3K4 methylation into heritable patterns of gene expression

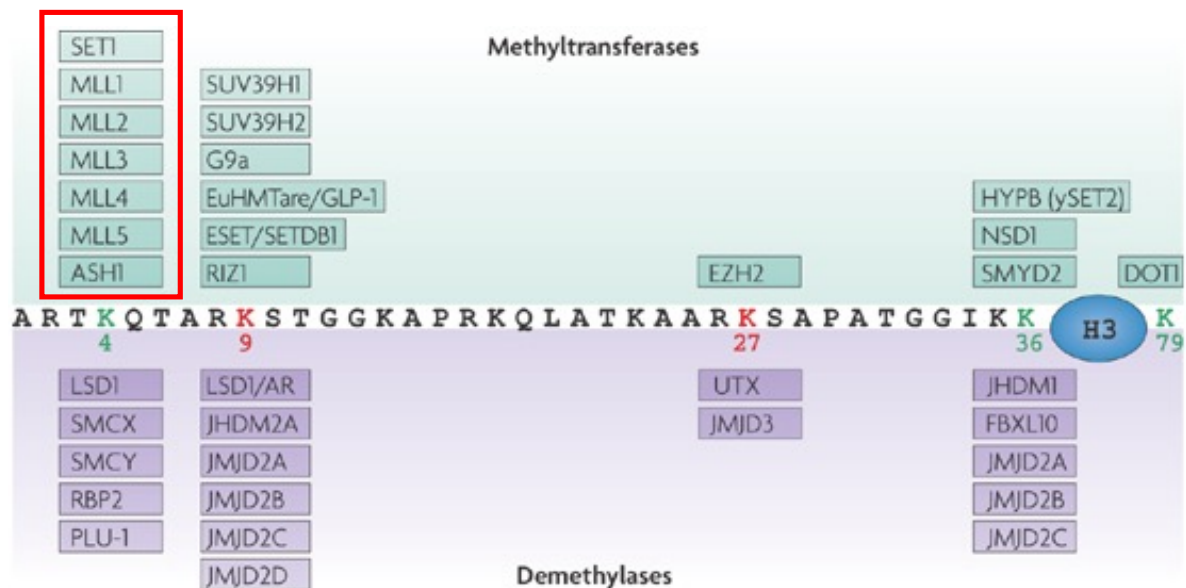
H3K4 HKMTs have an important role in defining CpG methylation levels

Overall epigenetic code that links H3K4 methylation to DNA methylation

Can the epigenetic code between DNA methylation and Histone methylation be validated by studying H3K4 HMTases?

MLL1: H3K4 methylation and CpG island methylation

H3K4 HKMTs and CpG methylation



Nature Reviews | Genetics

MLL1 and SET1 HKMTs are most relevant

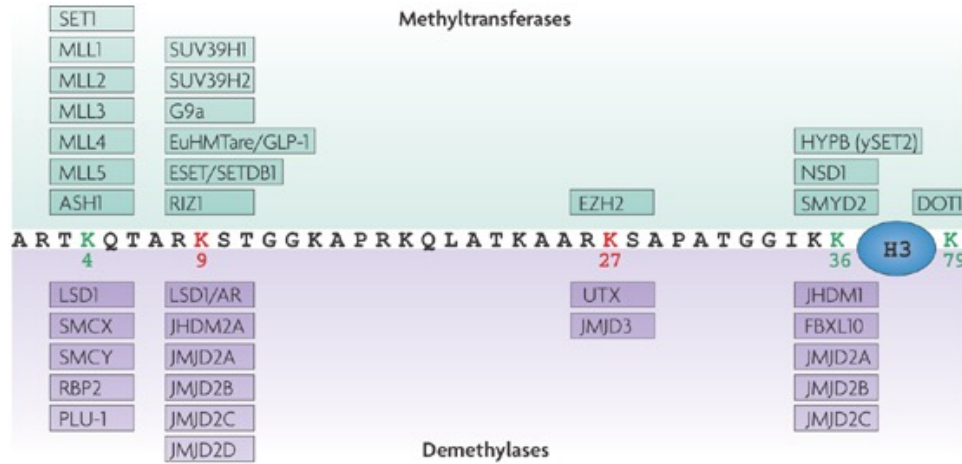
H3K4 specific HKMTs are important for the activation of gene expression

→ MLL are **conserved** and central for gene activation

→ For example: MLL proteins are required to activate Hox gene during embryonic development

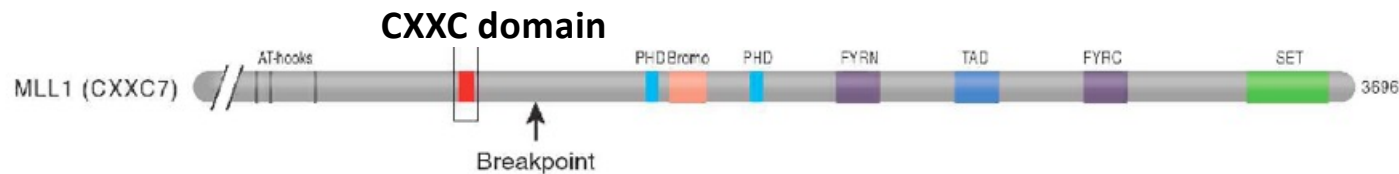
→ MLL proteins are often involved in translocations in myeloid and lymphoid leukemias (→ MLL hybrid gene results HKMTase action at inappropriate genes)

Is there a link between H3K4me and DNA methylation to coordinate gene expression



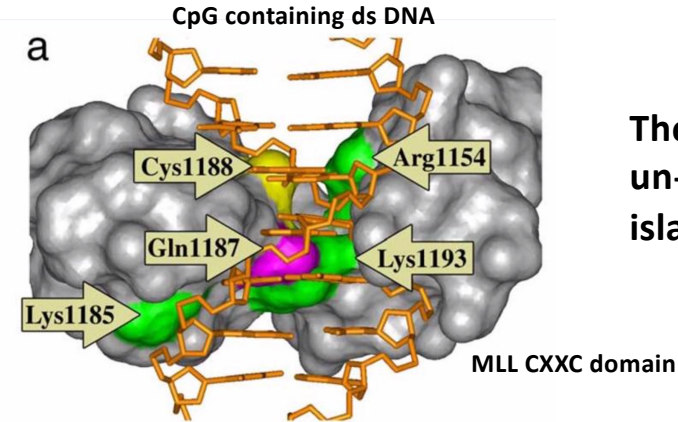
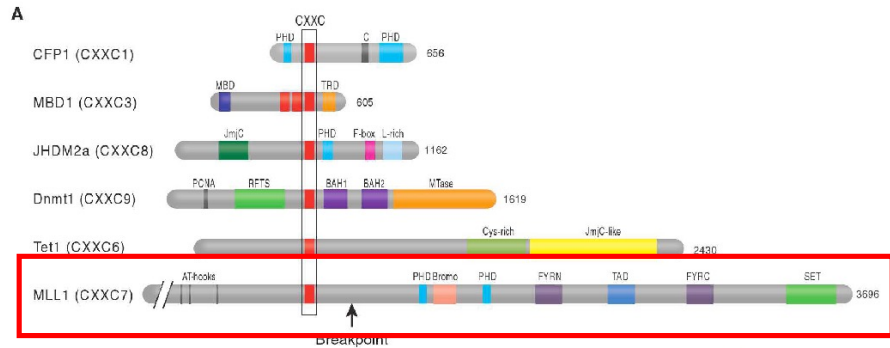
The CXXC domain binds un-methylated CpG islands

Nature Reviews | Genetics

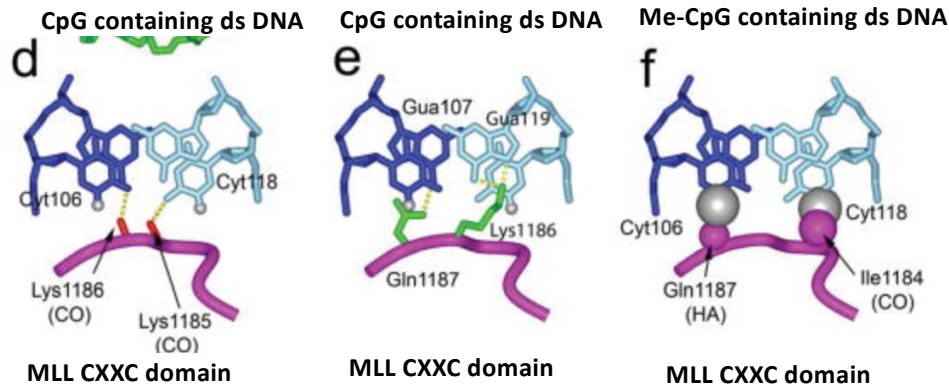


MLL1 binds to UNMETHYLATED CpGs

Is there a link between H3K4me and DNA methylation to coordinate gene expression? -- Molecular data: the CXXC domain binds unmethylated CpG islands --



The CXXC domain binds un-methylated CpG islands



MLL1 binds to UNMETHYLATED CpGs

CpG:

d) Hydrogen bonds involving the carbonyls of Lys1185 and Lys1186 and N4-amine groups of Cyt106 and Cyt118.

e) Hydrogen bonds formed between sidechains of Gln1187, Lys1186 and Gua107, Gua119, respectively.

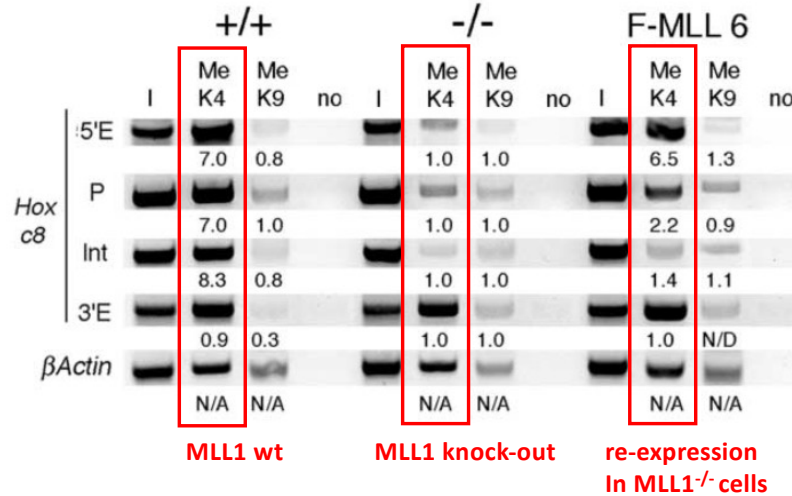
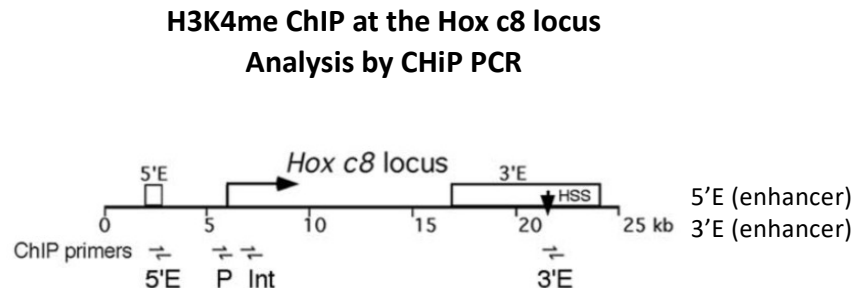
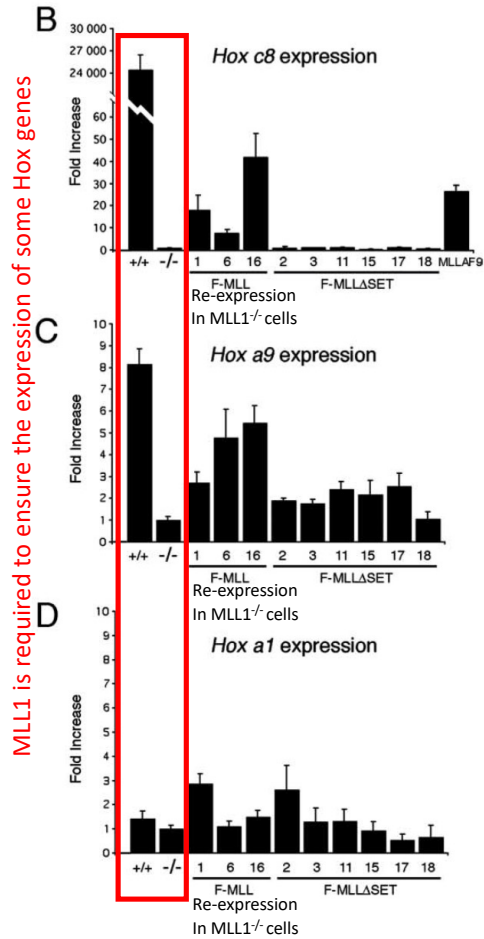
Me-CpG:

f) The positions of H5 protons that are substituted by CH3 groups in methylated DNA are shown as **gray spheres**

Methylation of either of these cytosines result in a steric clash with the protein backbone, thus precluding binding of methylated DNA.

MLL HKMTs mediate H3K4methylation and prevent CpG methylation -- FUNCTIONAL RELEVANCE FOR GENE EXPRESSION??--

MLL1 is essential to activate the expression of Hox genes; Hox genes are essential for embryo development
This Study: MLL1 knock-out mice → use primary mouse embryonic fibroblasts to study Hox gene expression



MLL1 is essential to impose H3K4 methylation

(control: H3K9me: MLL1 does not change H3K9me)

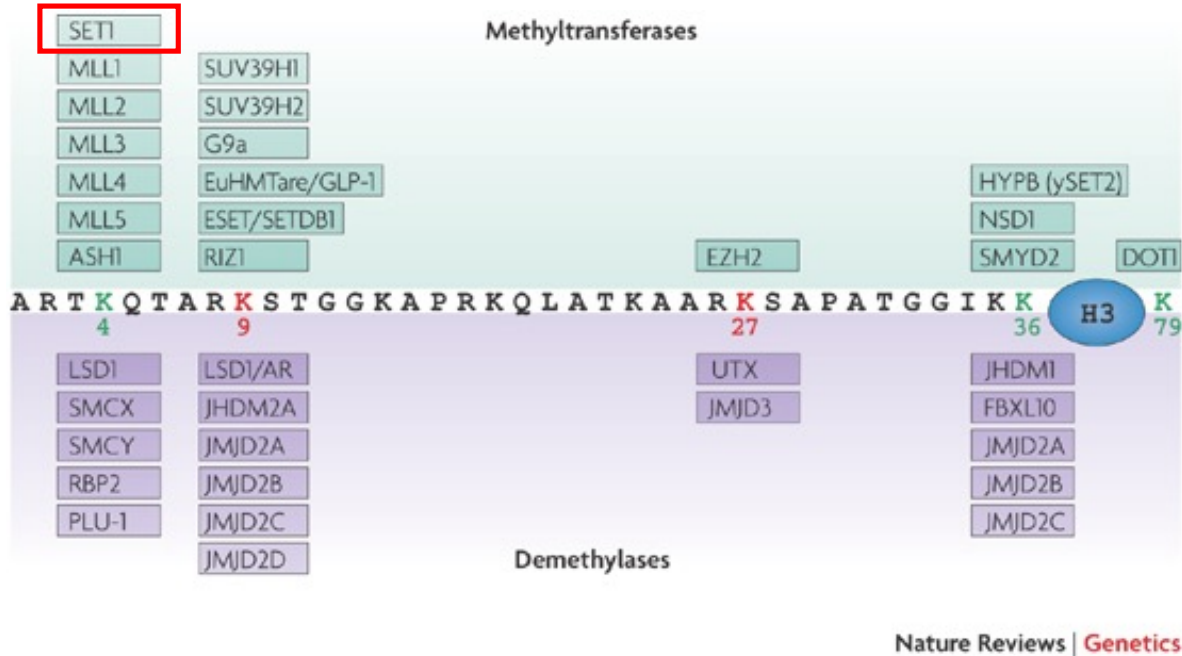
Note: Study was performed before strict antibody specificity validation was introduced:

ChIP does not discriminate between H3K4me1, H3K4me2, H3K4me3

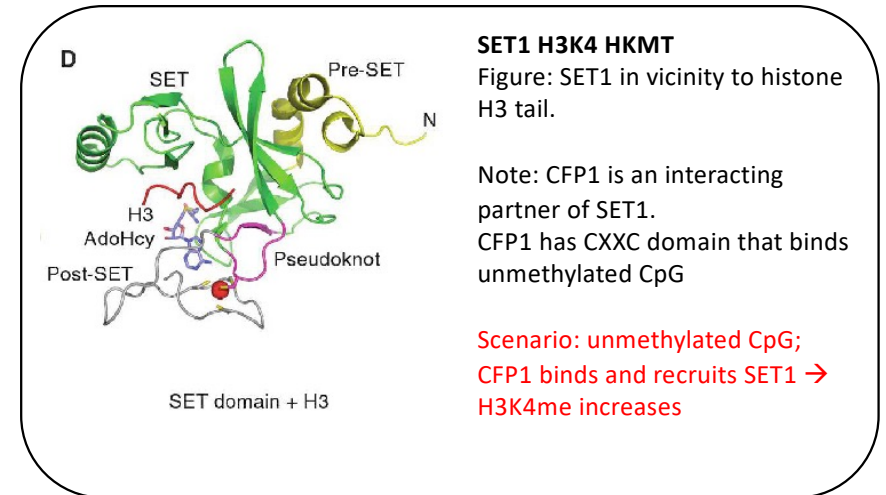
MLL1 is central for H3K4 methylation at Hox genes

SET1, CPF1: Recruitment of H3K4 methylation to un-methylated CpG

SET1 AND CFP1 LINK H3K4 methylation and DNA methylation



The CXXC domain binds un-methylated CpG islands

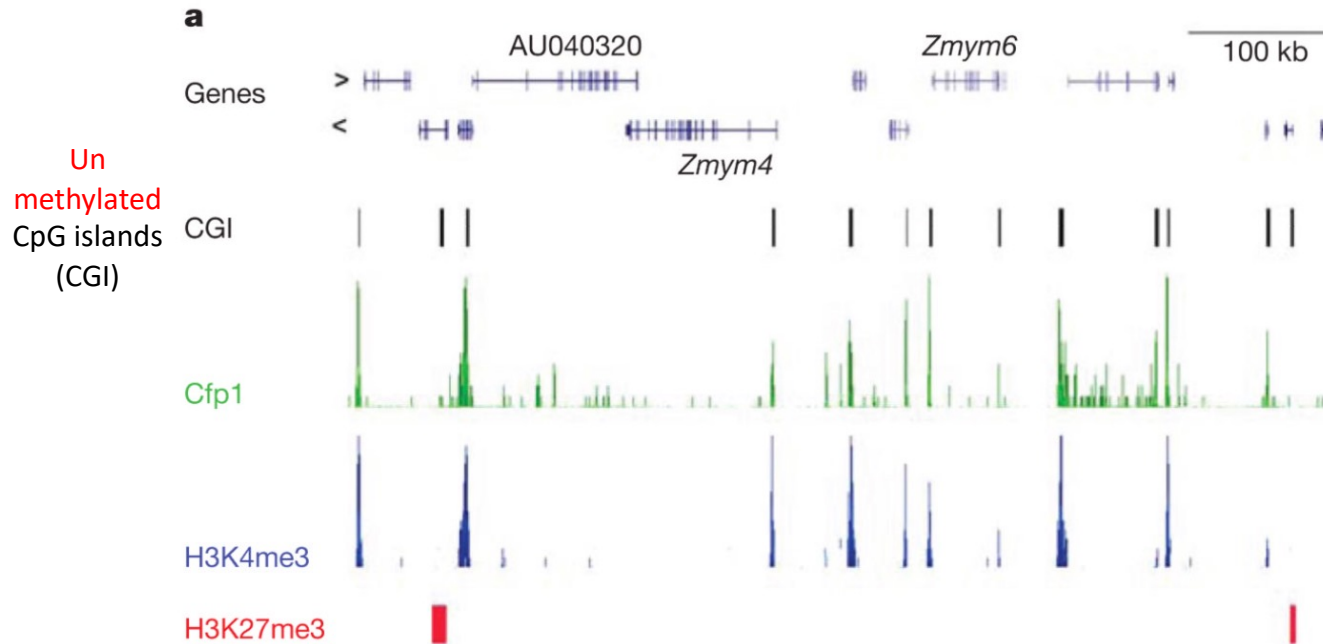


SET1 does not have CXXC domain

SET1 interacts with CFP1

SET1 H3K4 HKMT binds CFP1 and is recruited by CFP1 to un-methylated CpG islands

CFP1 is enriched at peaks of H3K4me3 that overlap with CpG islands



Strategy: ChIP seq on brain cells:

1. Pull out un-methylated CpG islands at promoters
2. Check presence of H3K4me3
3. Map Cfp1 occupancy

→ CpG islands that show high H3K4me3 but are unmethylated

(see earlier slides) and are enriched for CFP1 (interacts with SET1 H3K4 HKMT)

CFP1 CXXC domain is required to bind to unmethylated CpG islands

Figure 2. Genome-wide ChIP sequencing shows a tight association between Cfp1 and H3K4me3 at CGIs

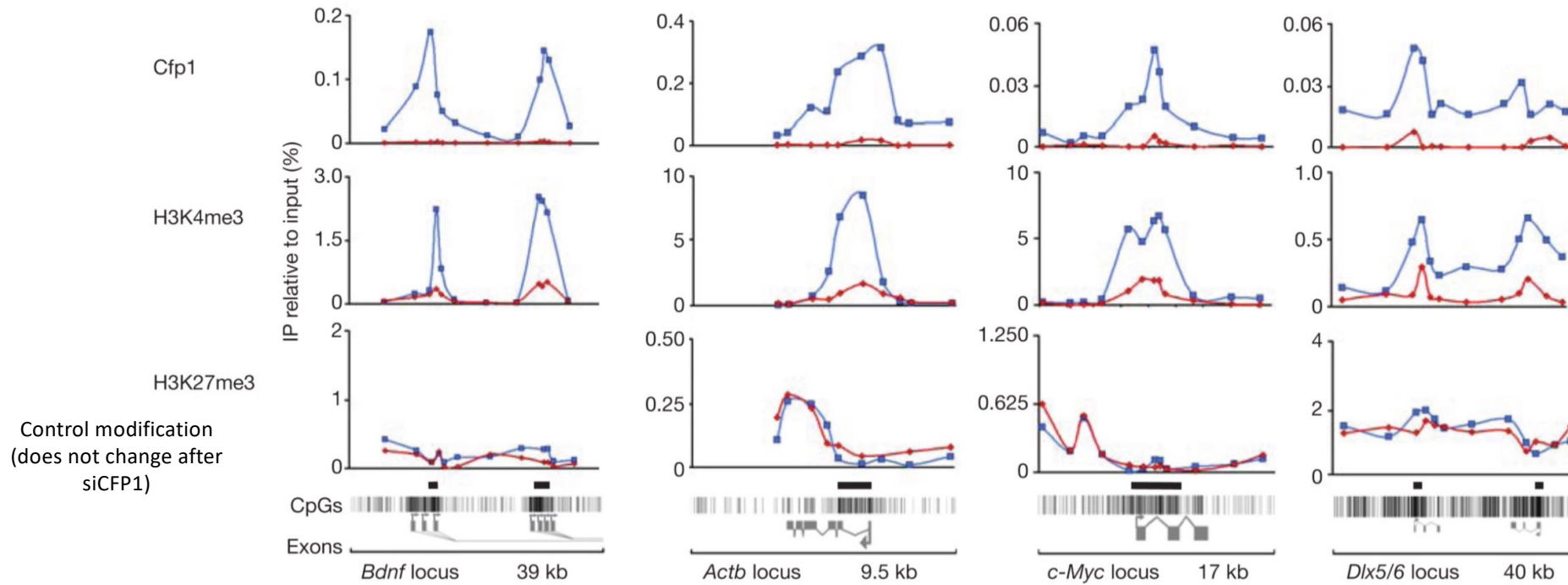
a, Typical Cfp1 ChIP-Seq profiles from whole mouse brain. For comparison, we also carried out H3K4me3 ChIP-Seq. The data were aligned with non-methylated CGIs mapped in mouse brain using a CXXC affinity column²⁹. The panel shows a typical region of the genome from chromosome 4 (nucleotides 126,333,759–127,054,849) demonstrating the coincidence of Cfp1 and H3K4me3 peaks with CGIs. A subset of genes is labelled (RefSeq). Two CGIs that lack H3K4me3 and Cfp1 coincide with sites of H3K27me3 binding (red rectangles; data of ref. 30 for mouse brain). **b**, Venn diagram showing strong overlap

CFP1 is enriched at peaks of H3K4me3 that overlap with CpG islands

LOSS OF CFP1 RESULTS IN A REDUCED H3K4me3 at unmethylated CpG ISLANDS → LOSS OF SET1 RECRUITMENT!!!!
= CFP1 is essential to recruit SET1 to CpG islands

NIH3T3 fibroblasts

— Vector control — *Cfp1* shRNA



ChIP qPCR using *Cfp1*, H3K4me3 and H3K27me3 antibodies at selected loci in vector-only control and *Cfp1*-depleted NIH3T3 cells. The results were replicated with an independent clone expressing the same shRNA combination (data not shown) and with each of two individual shRNA constructs (see Supplementary Fig. 3).

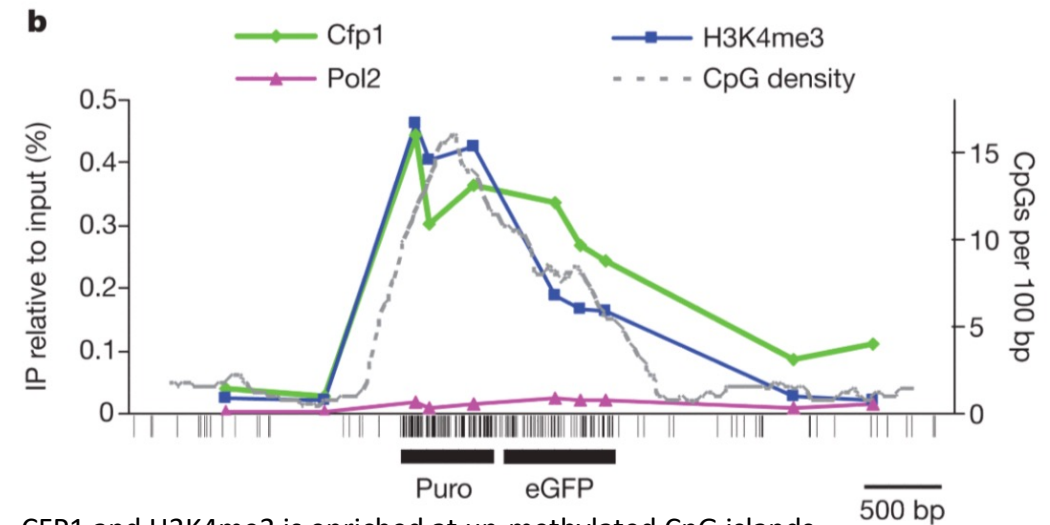
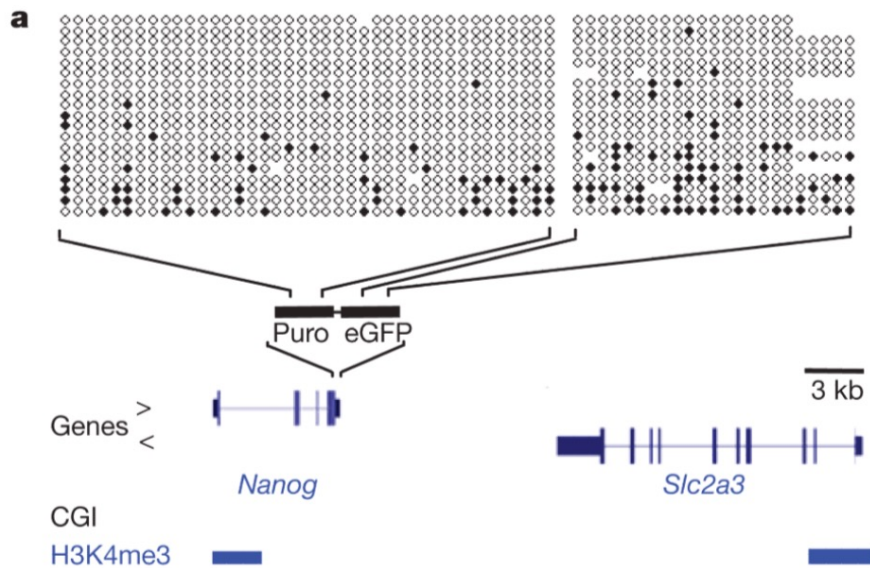
Thomson et al. Nature 2010

3T3 fibroblasts: The '3T3' designation refers to the abbreviation of "3-day transfer, inoculum 3×10^5 cells." This cell line was originally established from the primary mouse embryonic fibroblast cells that were cultured by the designated protocol, so-called '3T3 protocol'. The primary mouse embryonic fibroblast cells were transferred (the "T") every 3 days (the first "3"), and inoculated at the rigid density of 3×10^5 cells per 20 cm² dish (the second "3") continuously. The spontaneously immortalized cells with stable growth rate were established after 20 to 30 generations in culture, and then named '3T3' cells. Specifically, "3T3-L1" is one of the current lines.

CFP1 is enriched at peaks of H3K4me3 that overlap with CpG islands

Experimental model system: Question: does the system also work on an “engineered genome setup”?

- A DNA fragment that contains puromycin and EGFP (without promoter) was inserted into the Nanog locus (3' end) in undifferentiated mESCs: both sequences are enriched in extremely CG rich (but are protein coding); Nanog is expressed in undifferentiated mESCs = active region
- The fragment does NOT contain a promoter
- A) bisulfite sequencing: the inserted CpG rich DNA sequence is NOT METHYLATED
- B) ChIP - PCR using CFP1, H3K4me3 and RNA PolII

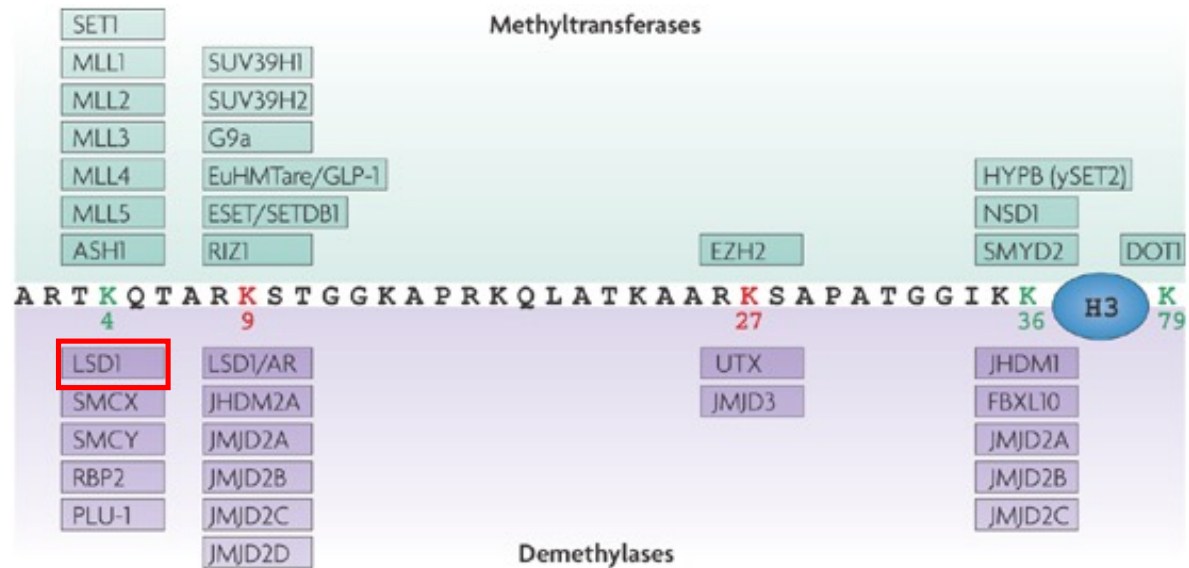


CFP1 and H3K4me3 is enriched at un-methylated CpG islands
 BUT: RNA Pol II is not recruited; why? → fragment does not contain promoter.
 RESULT: un-methylated CpG are sufficient to recruit CFP1 + SET1
 To increase H3K4me3, also in the absence of transcription

THAT MEANS THAT THE UNMETHYLATED CpG SEQUENCE IS SUFFICIENT TO DIRECT H3K4me3

LSD1: H3K4 de-methylation and CpG island methylation

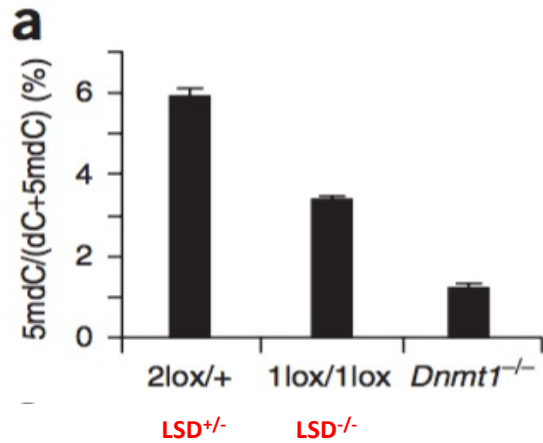
The H3K4 de-methylase LSD1 (KDM1A) is essential for establishing DNA methylation



The H3K4 de-methylase LSD1 (KDM1A) is essential for establishing DNA methylation

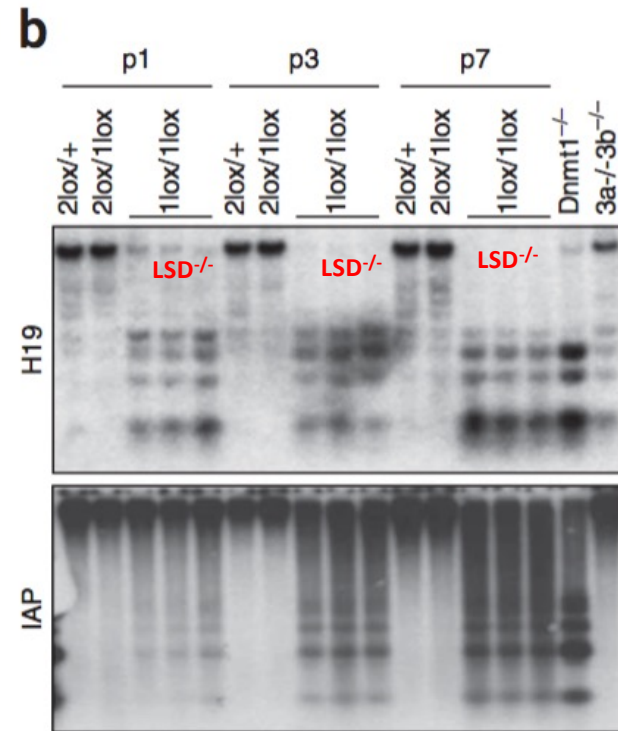
LSD1 is a H3K4 specific demethylase: oxidizes H3K4me_{2,1} → H3K4me₀

LSD1 conditional knock-out mice die early in embryogenesis (E5.5) and show strongly reduced DNA methylation



Loss of LSD1 results in
Reduced DNA methylation

**Recruitment of LSD1 eliminates H3K4me_{1,2} resulting in H3K4me₀
This creates a binding site for DNMT3L → thus recruiting the
DNMT3L-DNMT3a tetramer**



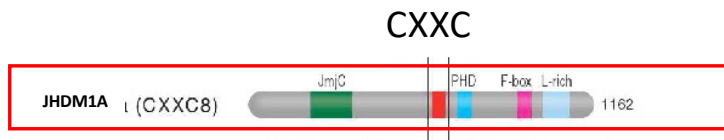
Southern blotting using CH3 sensitive restriction enzymes: a probe for the H19 and IAP **imprinted** gene locus are used. These are classic loci that are controlled by DNA methylation (high % of CpG methylation frequency) in one of the 2 alleles (paternal or maternal)

Note: Loss of DNA methylation results in Efficient restriction digest (more small fragment). This means that DNA methylation is strongly reduced
Situation is similar to DNMT1 knock-out cells

**DNA methylation sensitive restriction enzyme –
does not cut when CpG is methylated**

KDNM2A/JHDM1: H3K36 de-methylation and CpG island methylation

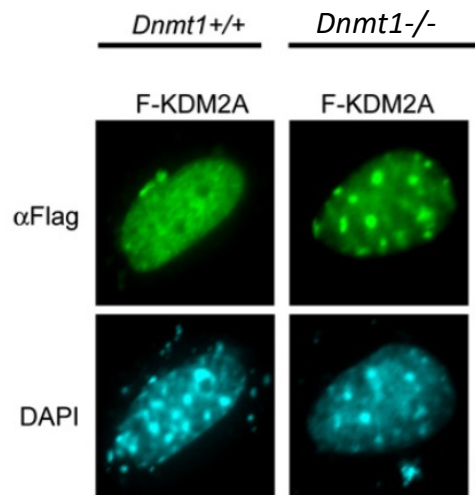
CXXC domains mediate binding to unmethylated CpGs: H3K36 specific KDM2A/JHDM1A (K-Histone De-methylase)



JHDM1A/KDM2A is a histone de-methylase that ensures low H3K36me2 levels at CpG islands

CXXC domain binds un-methylated CpG islands

PRIMARY OBSERVATION THAT JHDM1A IS LINKED TO DNA METHYLATION

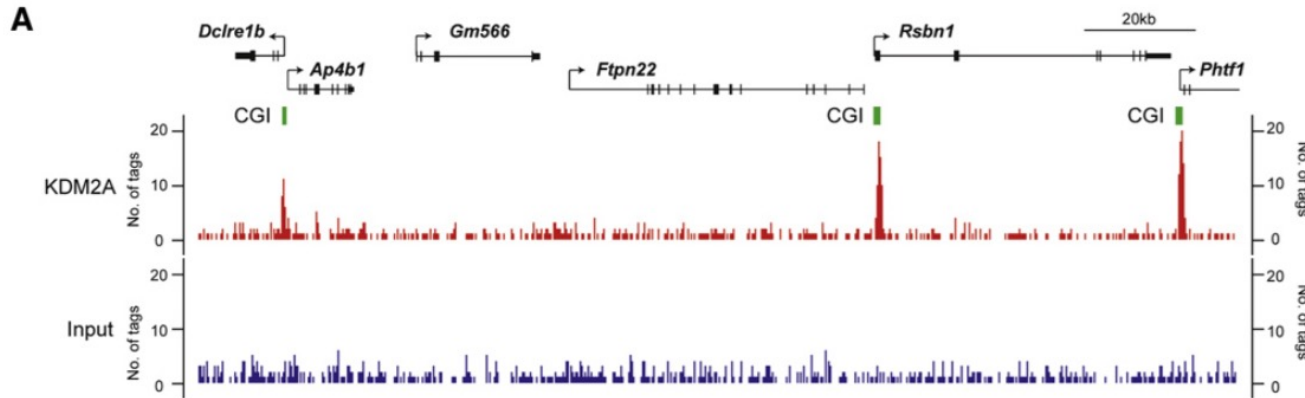


WT ES cells: **chromocenters are DNA methylated (a large block of methylated DNA)**: Flag-tagged JHDM1a is randomly distributed in the nucleus

DNMT1^{-/-} ES cells:

No DNA methylation at chromocenters = a large block of DNA are without DNA methylation. In this case KDM2A/JHDM1a localizes to chromocenters = **is attracted by unmethylated CpGs**

CXXC domains mediate binding to unmethylated CpGs: H3K36 specific KDM2A/JHDM1A (K-Histone De-methylase)

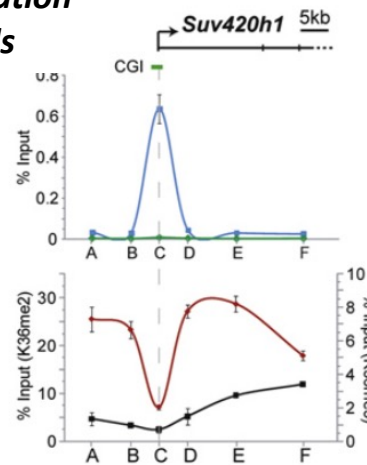
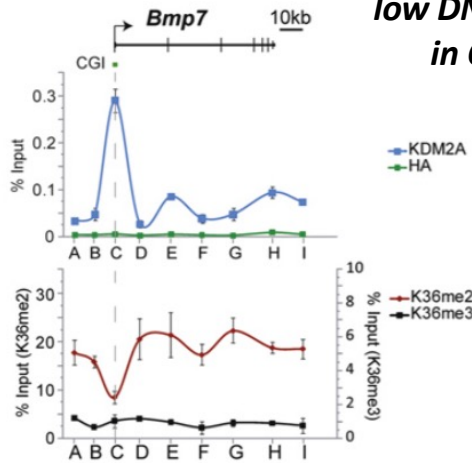


unmethylated CpG

ChIP seq on ES cells: JHDM1a/KDM2A
Concentrate on un-methylated CpG islands

Interaction is dependent on the CXXC domain

*Expressed genes =
low DNA methylation
in CpG islands*

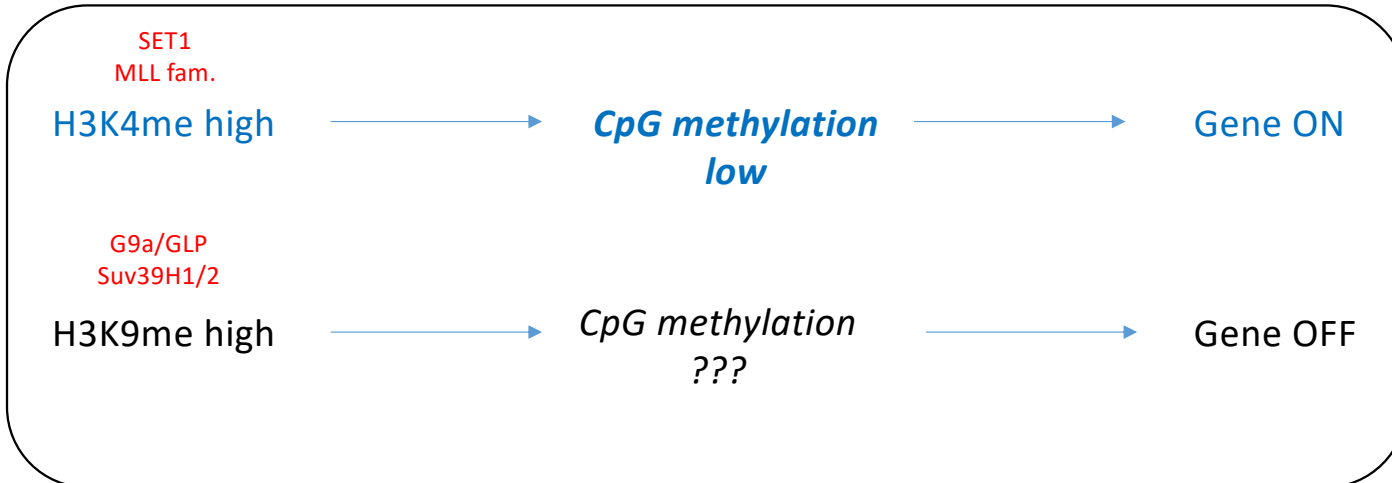
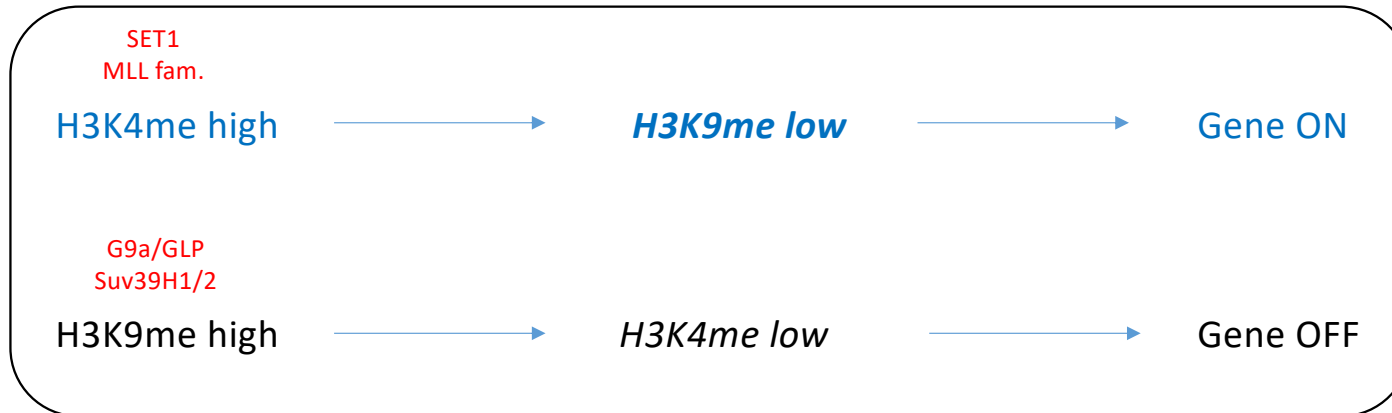


H3K36me2 is high in gene body but low at promoter

High levels of KDM2A at unmethylated CpG islands is paralleled by low **H3K36me2** levels

H3K9 methylation and CpG island methylation

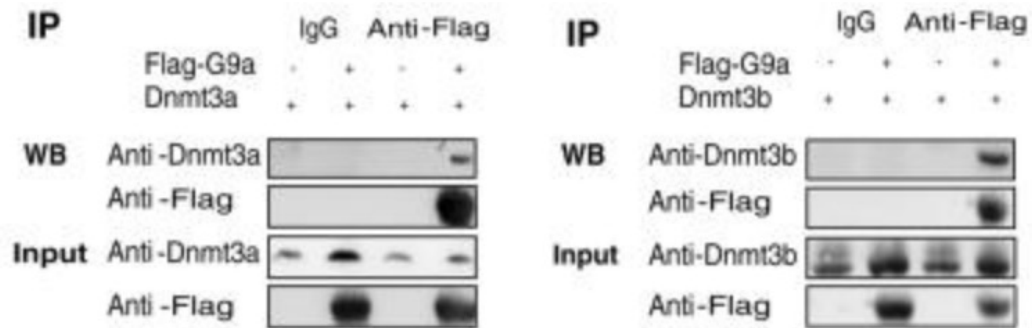
The relation of H3K9me and DNA methylation



Is there a direct link between H3K9me and DNA methylation?

The role of the G9a/GLP heterodimer in controlling DNA methylation

G9a HMTase and GLP HMTase form dimer and methylate H3K9m1; H3K9me2

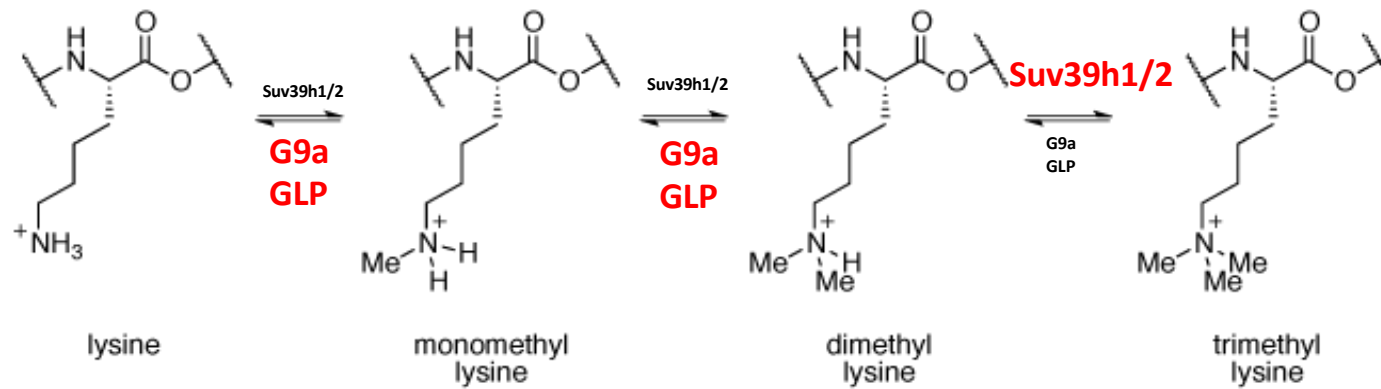


Co-immunoprecipitation:

- Cells transiently transfected with flag-tagged G9a and Dnmt3a
- IP anti-flagG9a: DNMT3a interacts
- IP anti-flagG9a: DNMT3b interacts

The role of the G9a/GLP heterodimer in controlling DNA methylation

H3K9 methylation



The role of the G9a/GLP heterodimer in controlling DNA methylation

Embryonic stem cells:

Self-renewing mESCs
(pluripotent)

Oct4, Stk10, Gpr54
Nanog, Dnmt3L, Tnfrsf8: **ON**
NO DNA METHYLATION

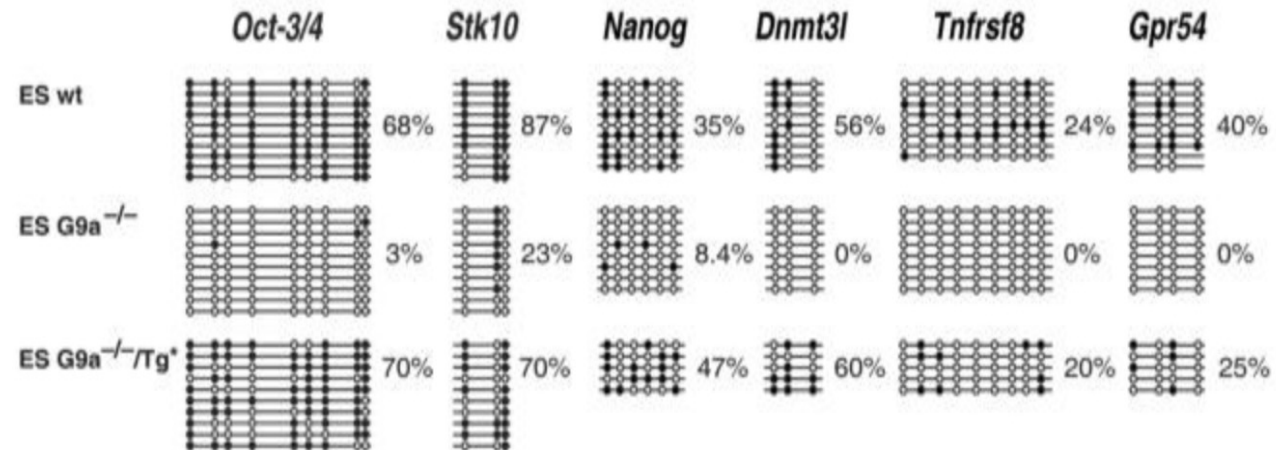
retinoic
acid

Differentiated mESCs

Oct4, Stk10, Gpr54
Nanog, Dnmt3L, Tnfrsf8: **OFF**
DNA METHYLATION

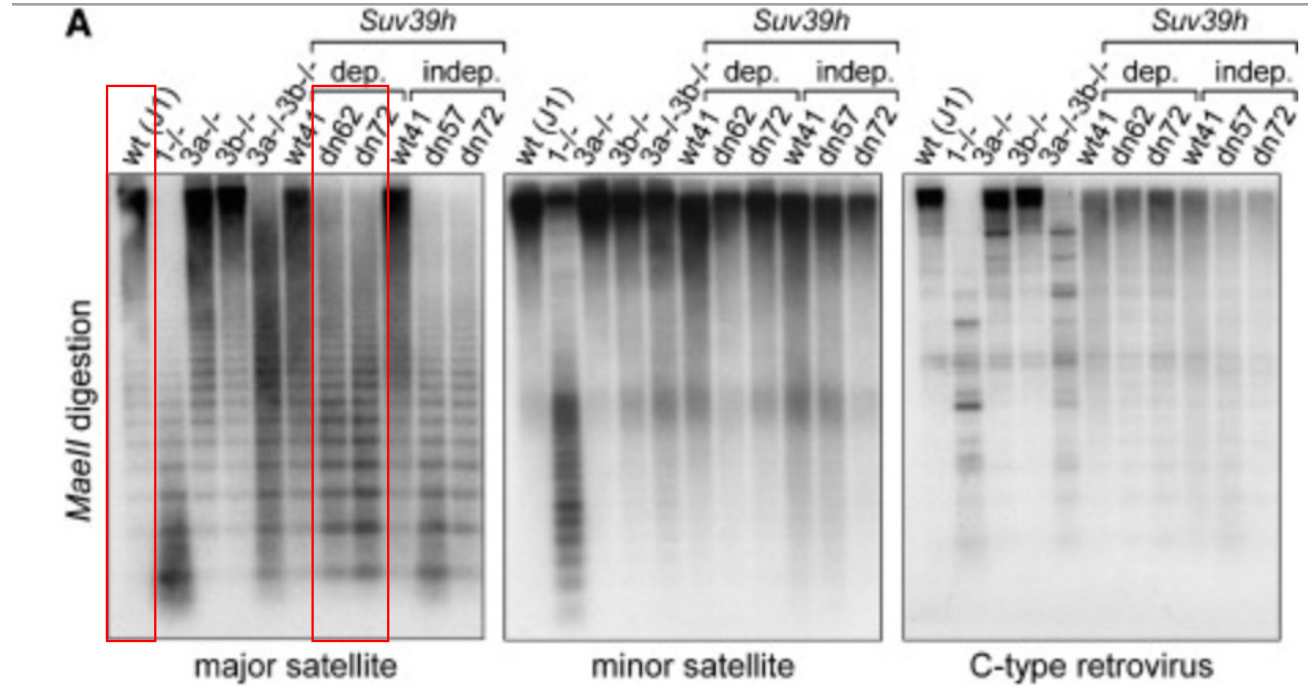
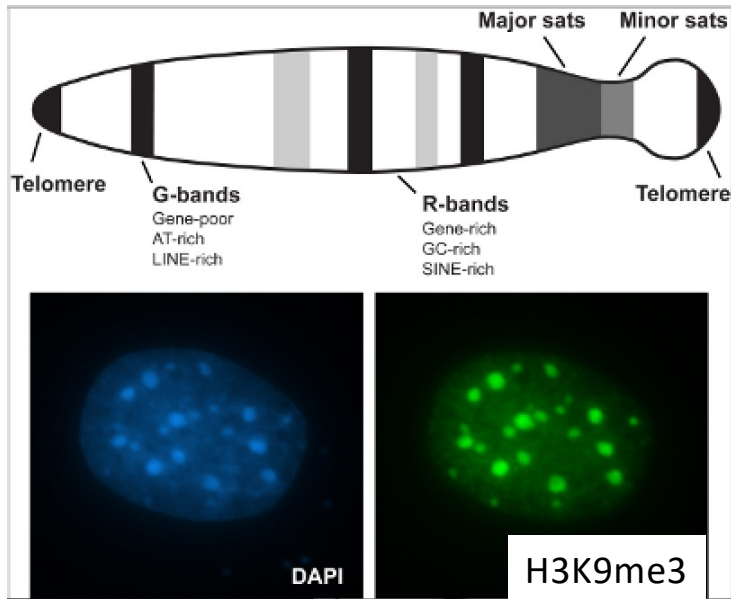
differentiated G9a –
knock-out ES cells in
differentiation

Show a loss of DNA
methylation
As detected by bisulfite
sequencing
In CpG islands in
promoter regions of
several genes



G9a: Required for silencing of transposable elements, repeat elements, retroviral insertions, imprinting centers but also in **gene expression control**

The role of SUV39h1/2 in controlling DNA methylation



Loss of *Suv39h1* HMTases is linked to reduced DNA methylation at pericentric major satellite repeats

Southern blot using genomic DNA that was digested with methylation sensitive restriction enzyme. DNA on blot was hybridized using probes for minor satellite, major satellite and C-type retroviral DNA

Histone methylation and DNA methylation

