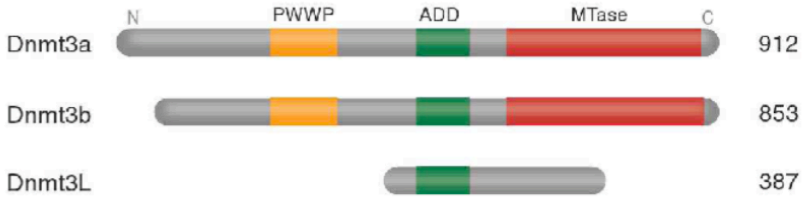
LECTURE 13

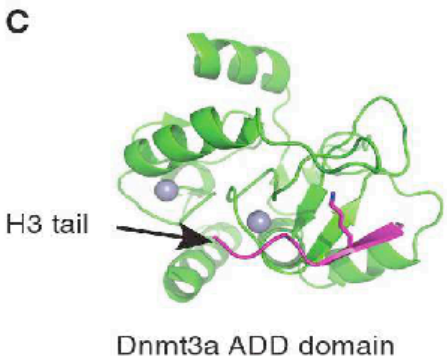
COORDINATION OF HISTONE AND DNA METHYLATION

Linking de-novo DNA methylation to histone methylation (DNMT3a, DNMT3b)



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

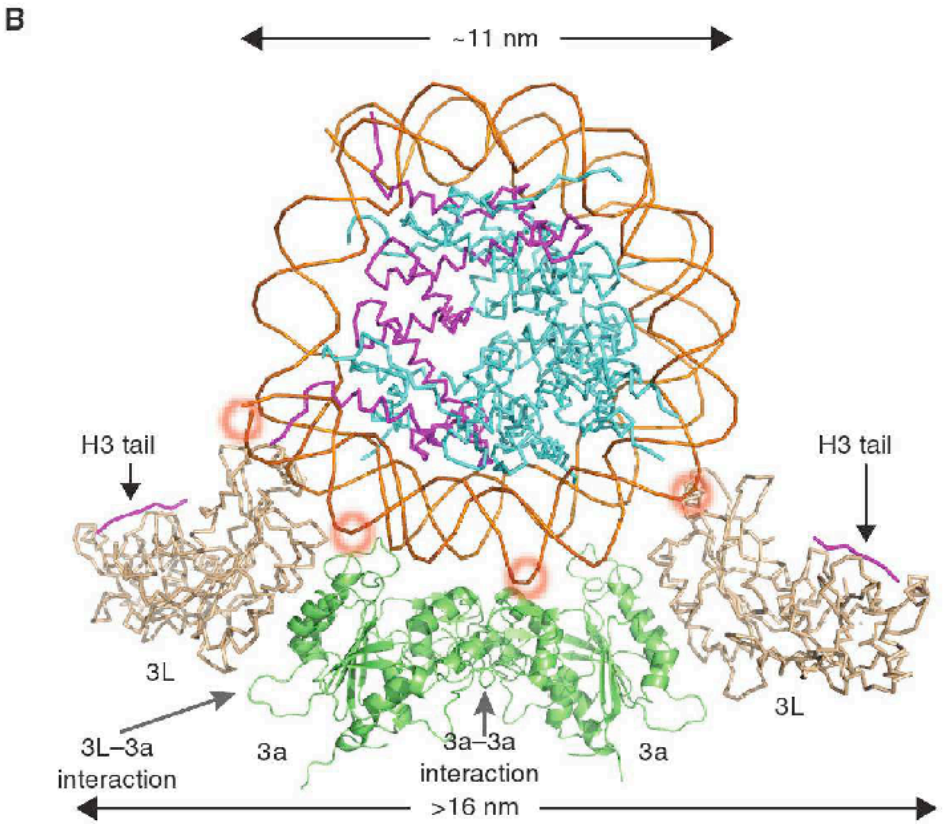


Dnmt3a ADD domain

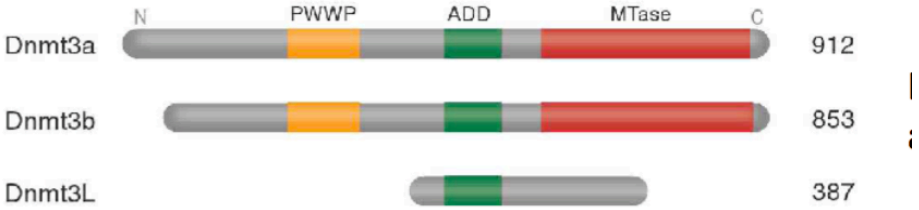
ADD domain of DNMT3a
*Interacts with the **unmethylated** Histone H3 tails*

→ *a link of de-novo DNMTs with histone methylation*

Linking de-novo DNA methylation to histone methylation



- DNMT3L forms a complex with DNMT3a → 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



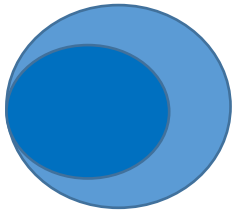
Linking de-novo DNA methylation to histone methylation

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

Linking de-novo DNA methylation to histone methylation

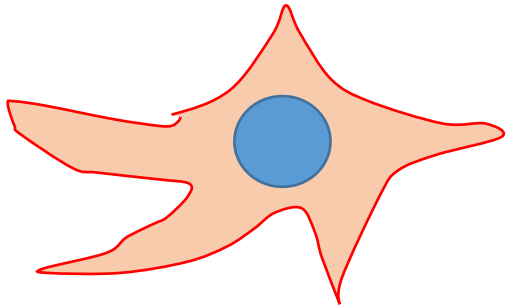
Embryonic stem cells

Olig1/2
Neuronal transcription factor: OFF



DIFFERENTIATION

Neuronal progenitor cells



Olig1/2
Neuronal transcription factor: ON

REDUCED REPRESENTATION BISULFITE SEQEUNCING (RRBS)

ChIP seq for active histone modification (H3K4me1,2,3)

ChIP seq for inactive histone modification (H3K27me3)

RNA-seq

Which genes are on/off?

Confront with bivalent/monovalent status (chromatin structure!!!)

Relate to DNA methylation at CpG islands ?

REDUCED REPRESENTATION BISULFITE SEQEUNCING (RRBS)

ChIP seq for active histone modification (H3K4me1,2,3)

ChIP seq for inactive histone modification (H3K27me3)

RNA-seq

Which genes are on/off?

Confront with bivalent/monovalent status (chromatin structure!!!)

Relate to DNA methylation at CpG islands?

DNMT3L links histone methylation to DNA methylation

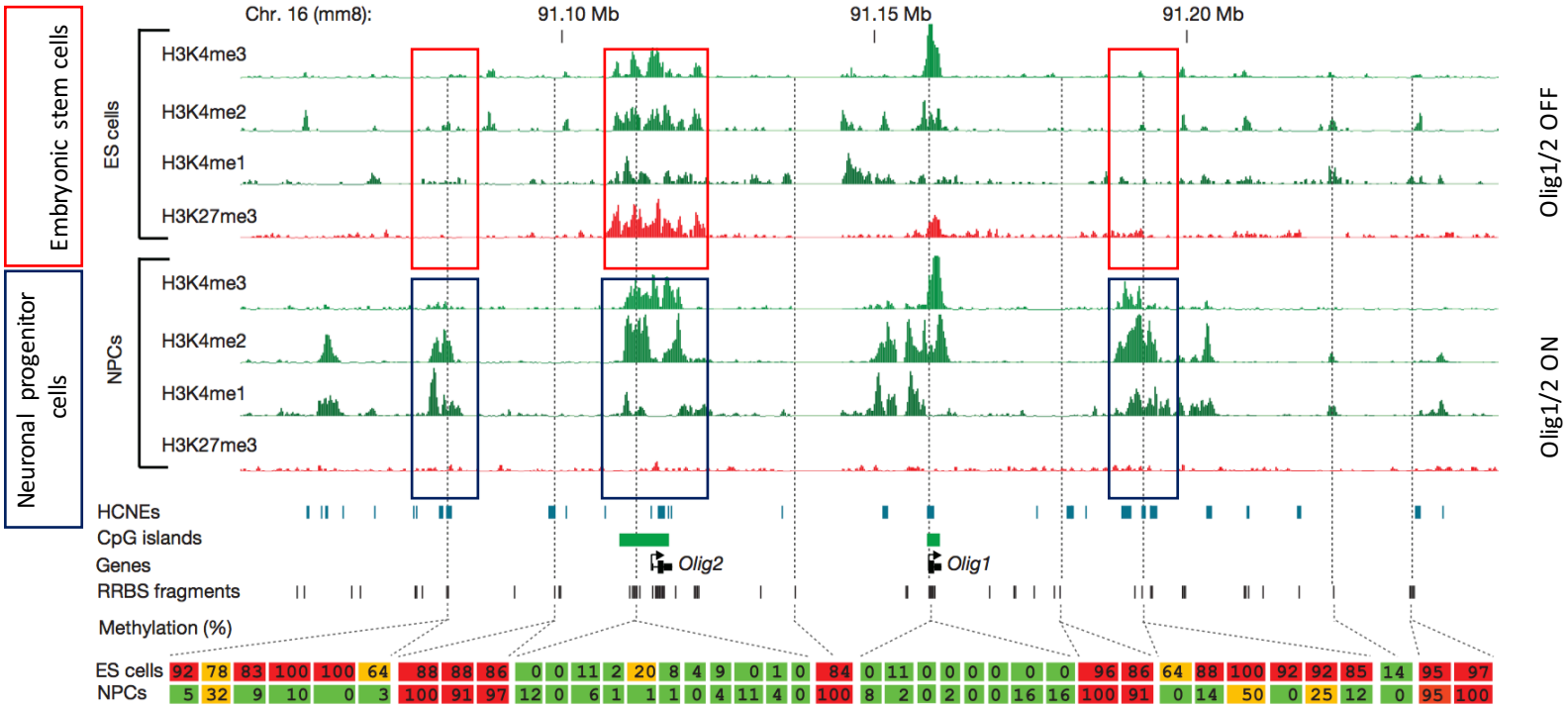


Figure 3 | Developmentally regulated de-methylation of highly conserved non-coding elements. Comparison of histone and DNA methylation levels across the *Olig1*/*Olig2* neural-lineage transcription factor locus. ChIP-Seq tracks for H3K4me1/2/3 and H3K27me3 in ES cells and NPCs are shown. The unmethylated 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%).

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.

Meissner et al. 2008 Nature

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

DNMT3L links histone methylation to DNA methylation

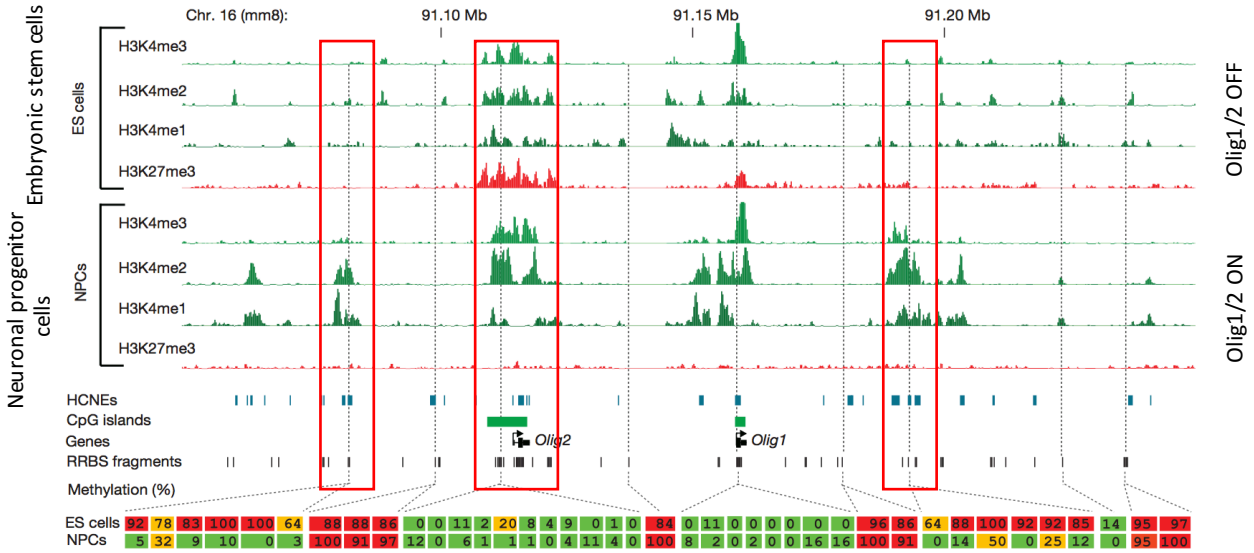


Figure 3 | Developmentally regulated de-methylation of highly conserved non-coding elements. Comparison of histone and DNA methylation levels across the *Olig1*/*Olig2* neural-lineage transcription factor locus. ChIP-Seq tracks for H3K4me1/2/3 and H3K27me3 in ES cells and NPCs are shown. The unmethylated 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%).

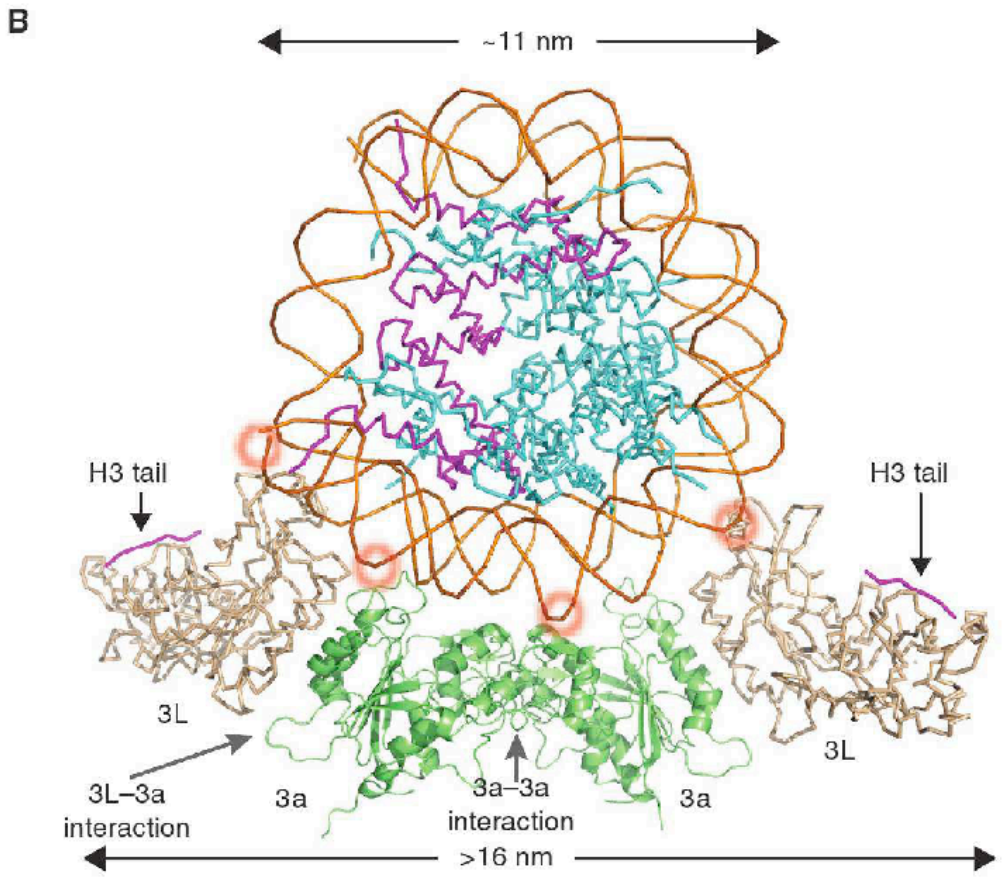
H3K4me0: DNA METHYLATION IN CpG ISLANDS

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



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

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

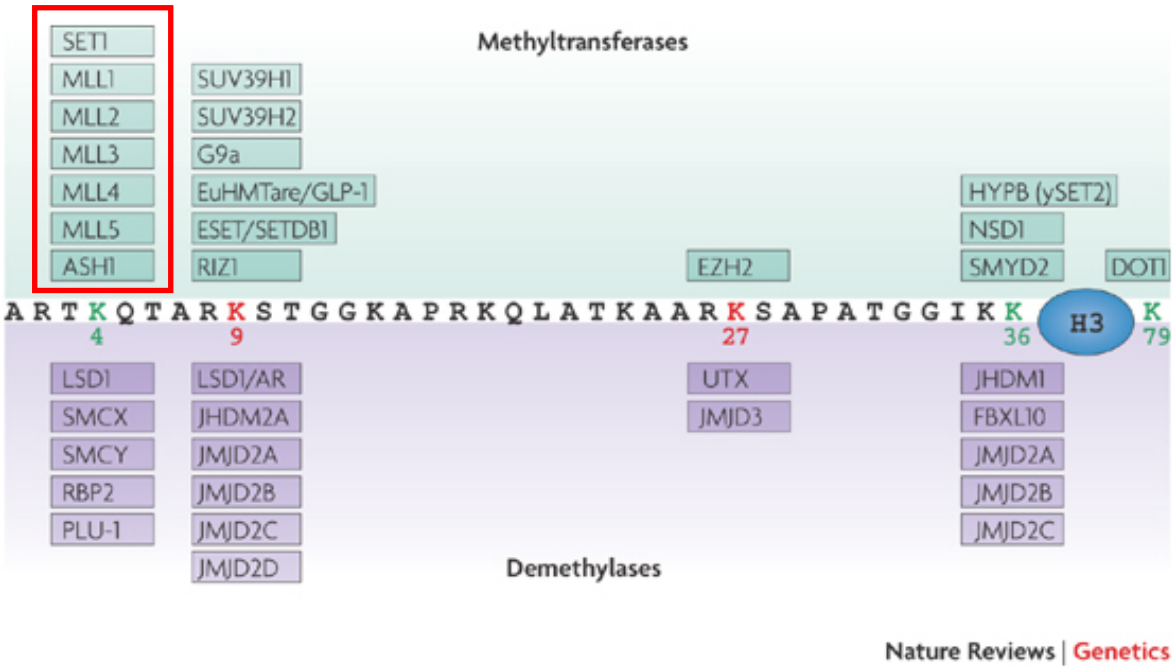
Mutated DNMT3L does not bind to unmethylated H3K4→ no DNA methyaltion at CpG islands!!

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

H3K4 HKMTs have an important role in defining CpG methylation levels

H3K4 methylation and CpG island methylation

H3K4 HKMTs and CpG methylation



MLL1 and SET1 HKMTs are most relevant

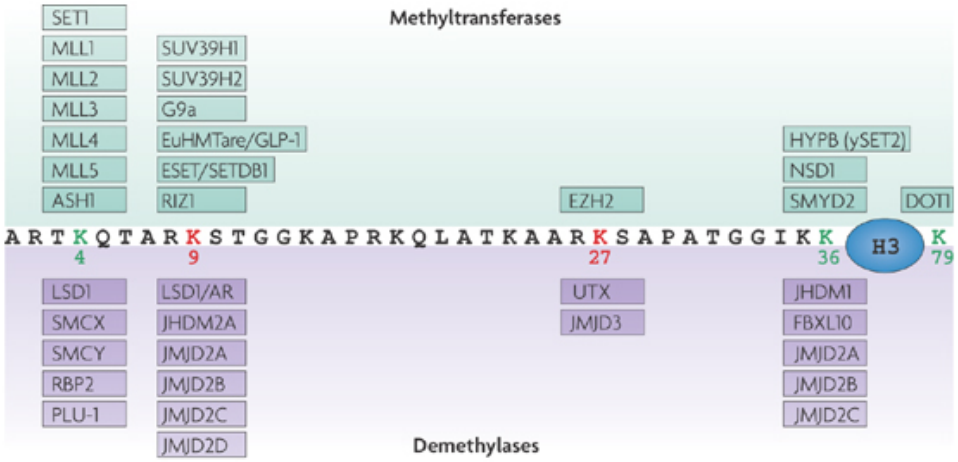
H3K4 specific HKMTs are important for the activation of gene expression

→ MLL are conserved and central for gene activation

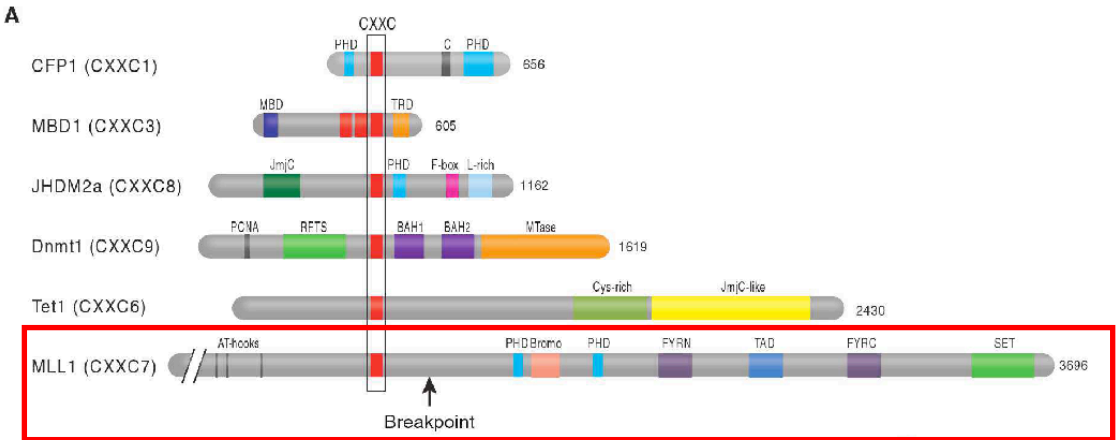
→ MLL proteins are required to activate Hox gene during embryonic development

→ 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



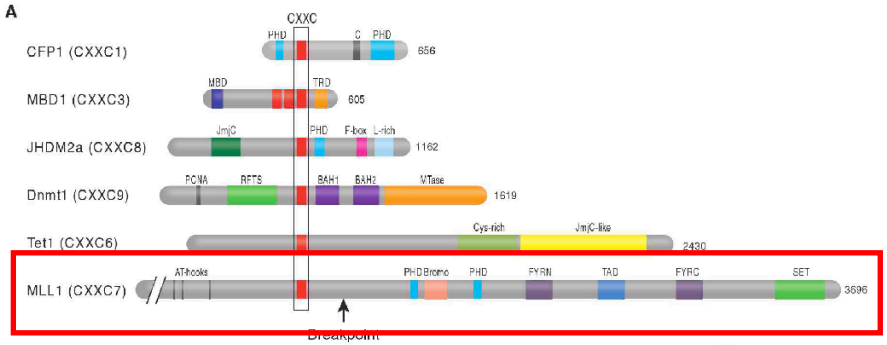
Nature Reviews | Genetics

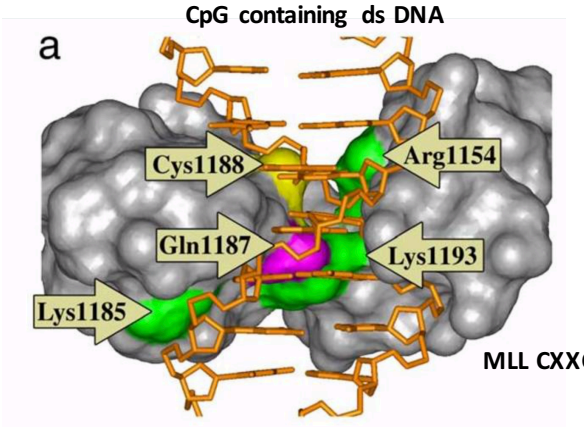


The CXXC domain binds un-methylated CpG islands

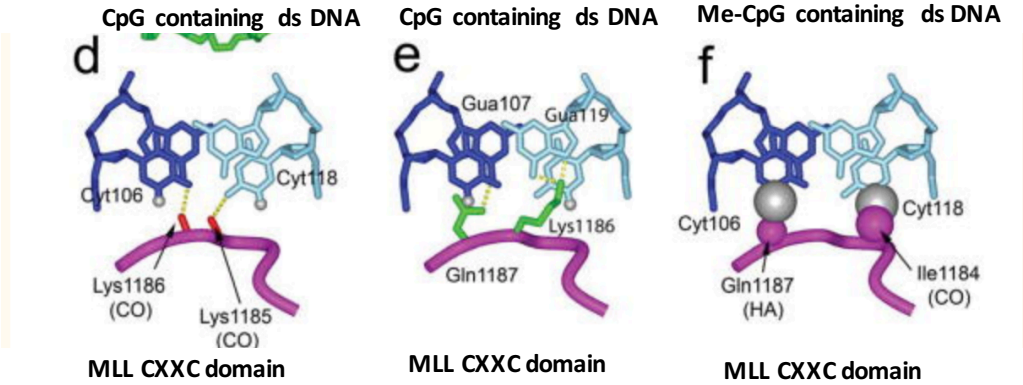
MLL1 binds to UNMETHYLATED CpGs

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





The CXXC domain binds un-methylated CpG islands



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

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

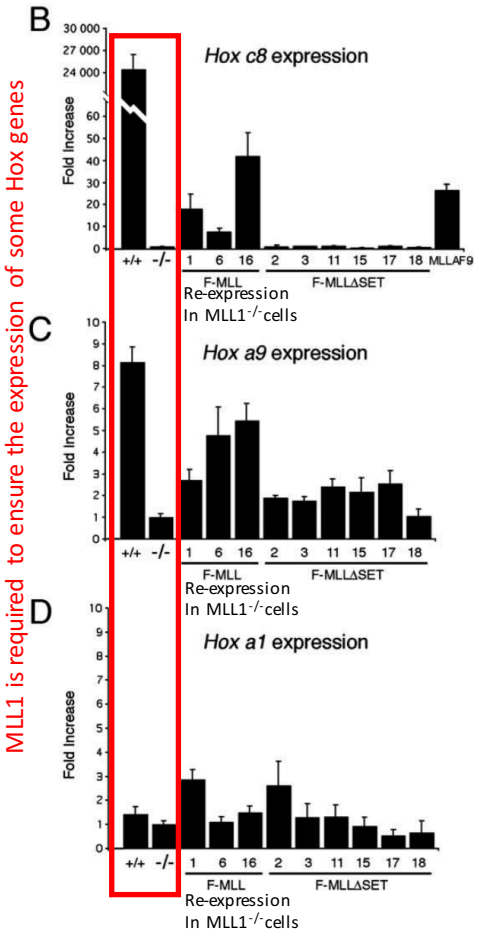
Me-CpG: f) The positions of H5 protons that are substituted by CH3 groups in methylated DNA are shown as **gray spheres**
Methylation of either of these cytosines result in a steric clash with the protein backbone, thus precluding binding of methylated DNA.

MLL1 binds to UNMETHYLATED CpGs

Cierpicki et al. Nat Struct Mol Biol. 2010, Structure of the MLL CXXC domain – DNA complex and its functional role in MLL-AF9 leukemia

MLL HKMTs mediate H3K4methyaltion and prevent CpG methylation

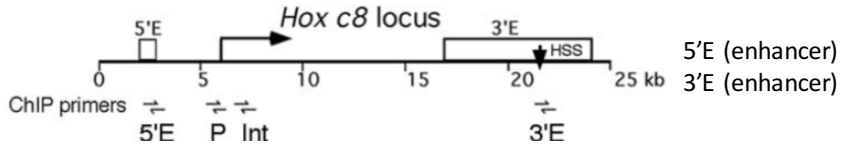
MLL1 is essential to activate the expression of Hox genes; Hox genes are essential for embryoid developments
This Study: MLL1 knock-out mice → use primary mouse embryonic fibroblasts to study Hox gene expression

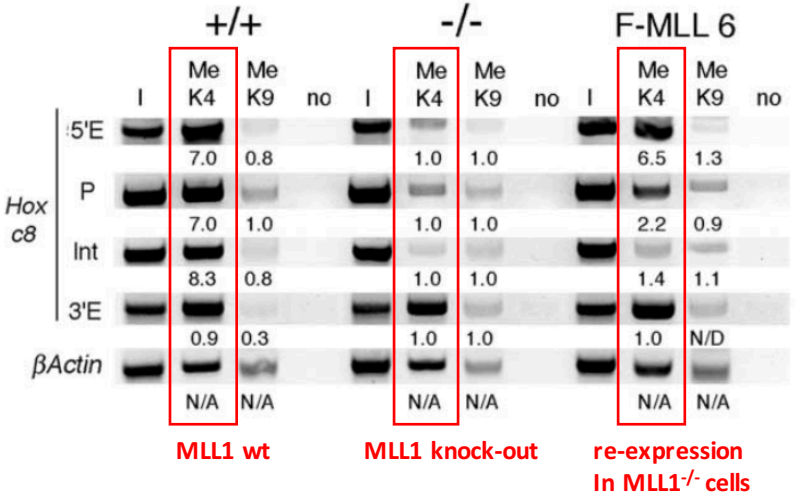


MLL1 is required to ensure the expression of some Hox genes

Milne et al. Molecular Cell 2002

H3K4me ChIP at the Hox c8 locus
Analysis by PCR





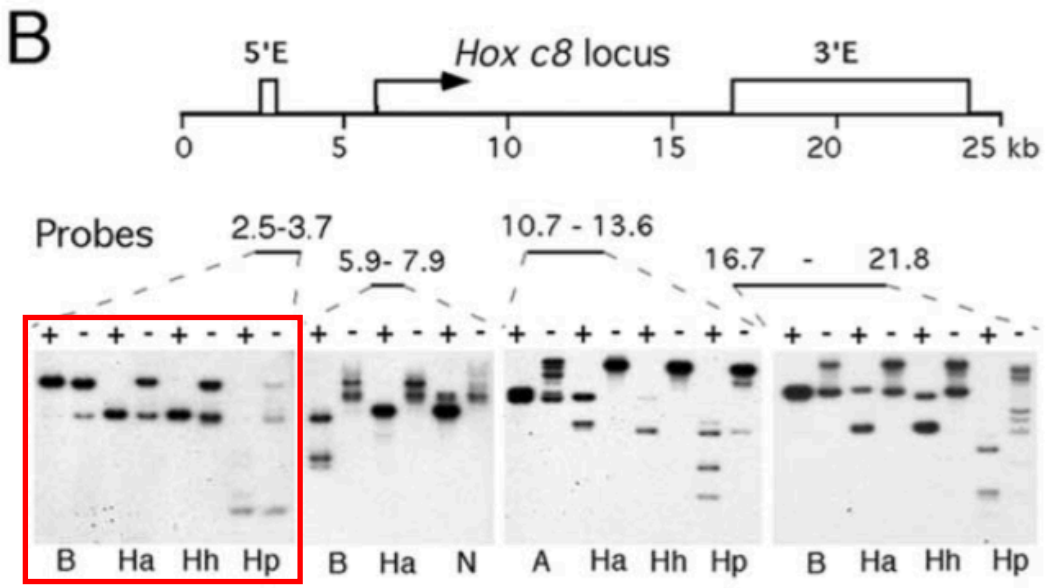
MLL1 is central for H3K4 methylation at Hox genes

MLL1 is essential to Impose H3K4 methylation

(control: H3K9me: MLL1 does not change H3K9me)

Note: Stduy was performed before stricte antibody specificity validation was indroduced:
ChIP does not discriminate Between H3K4me1, H3K4me1, H3K4me3

H3K4 specific MLL HKMTs prevent CpG methylation



+: wild-type; -: MLL1 null
DNA methylation sensitive restriction enzymes:
B: BstUI:
Ha:HaeII
Hh:HhaI
Hp:AvaI
Cut when CpG unmethylated; do not cut when CpG methylated

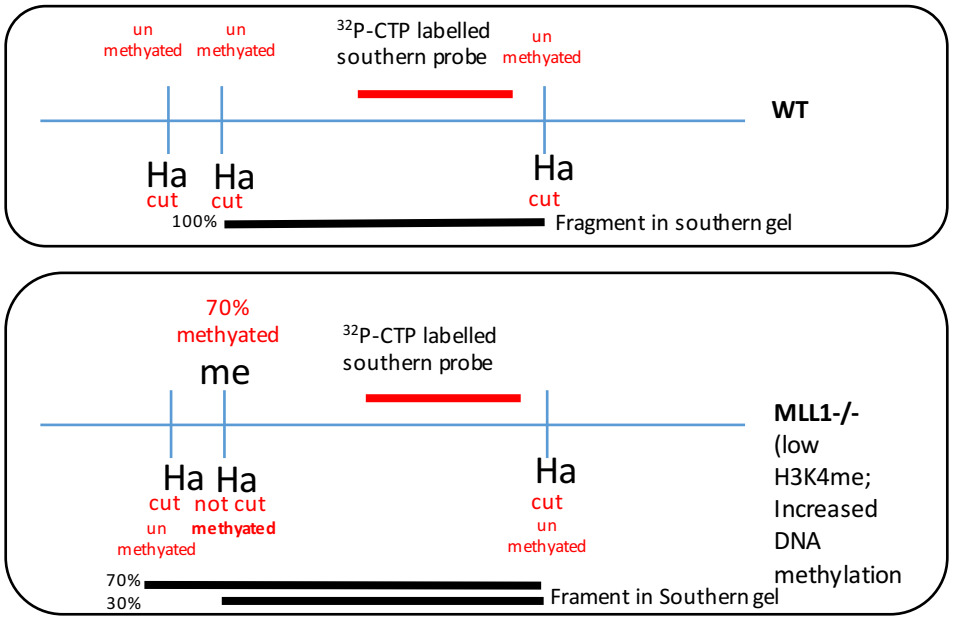
HaeII

Prepare genomic DNA from MLL1 wt and MLL1 knock-out cells

Digest with DNA methylation sensitive restriction enzymes

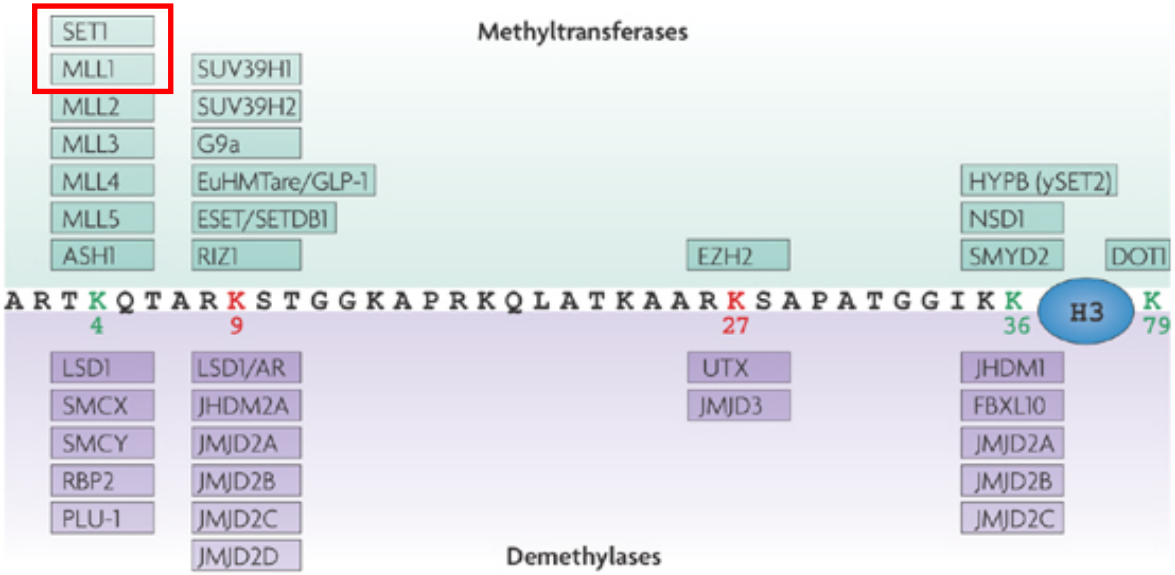
Make southern blot and hybridize with probes that recognize segments of the the Hox c8 locus that host methylation sensitive restriction sites

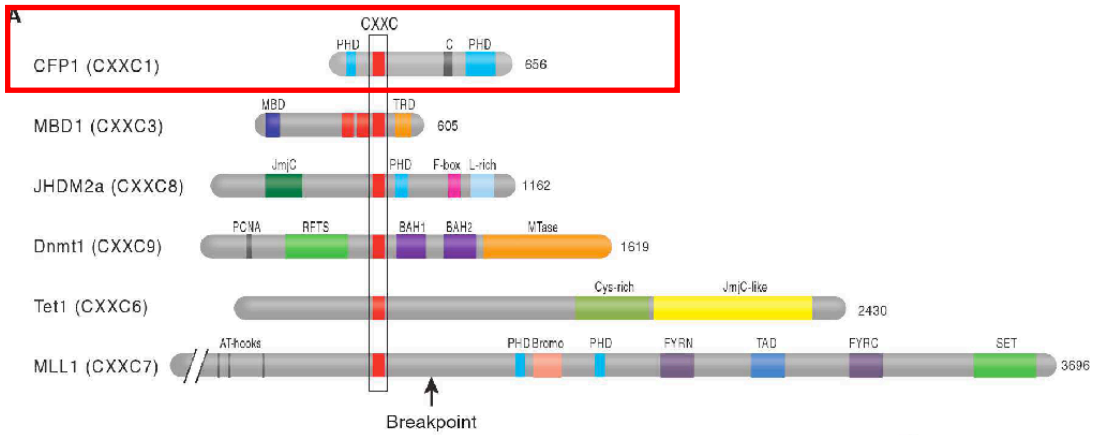
General result: in MLL1-/- cells there is 1 band more (red box)



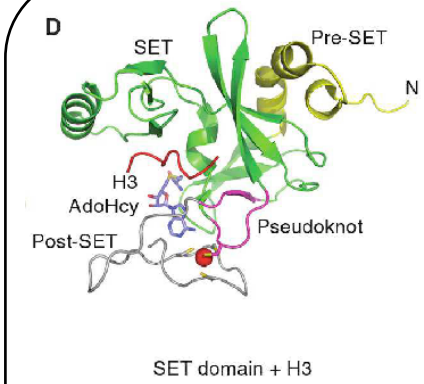
Milne et al. Molecular Cell 2002

SET1 AND CFP1 LINK H3K4 methylation and DNA methylation





The CXXC domain binds un-methylated CpG islands



SET domain + H3

SET1 H3K4 HKMT
Figure: SET1 in vicinity to histone H3 tail.

Note: CFP1 is an interacting partner of SET1.
CFP1 has CXXC domain that binds unmethylated CpG

Scenario: unmethylated CpG; CFP1 binds and recruits SET1 → H3K4me increases

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

Un methylated CpG islands (CGI)

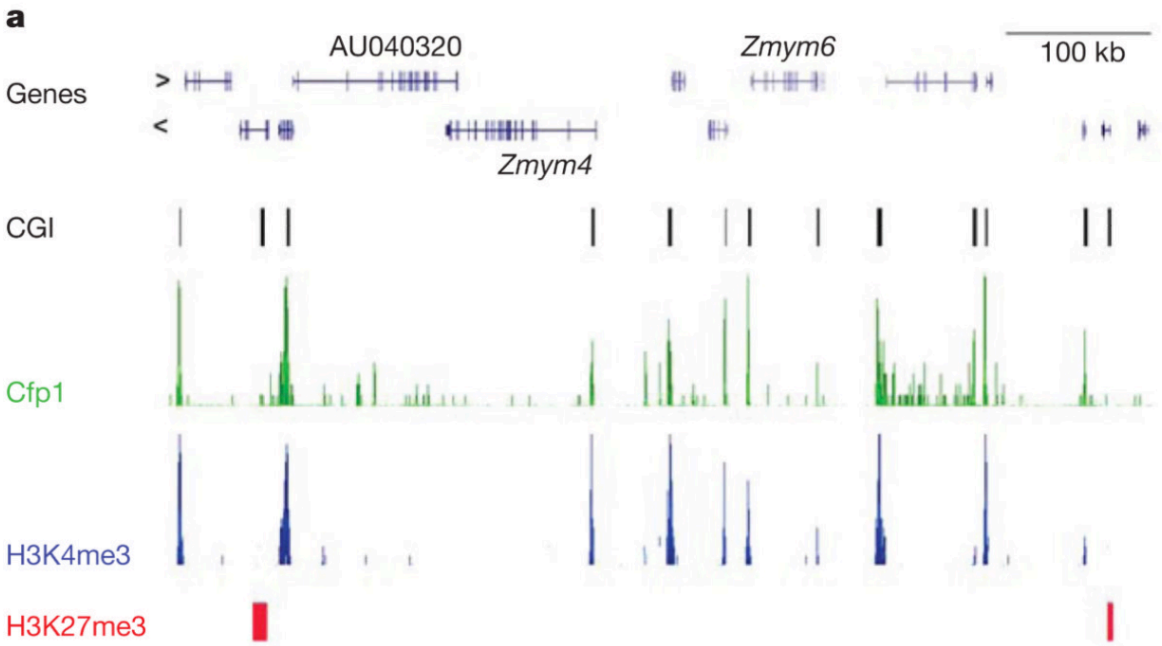


Figure 2. Genome-wide ChIP sequencing shows a tight association between Cfp1 and H3K4me3 at CGIs

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 column[29]. 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

Thomson et al. Nature 2010

ChIP seq on brain cells:
CpG islands that show high H3K4me3 but are unmethylated (see earlier slides) and are enriched for CFP1 (interacts with SET1 H3K4 HKMT)

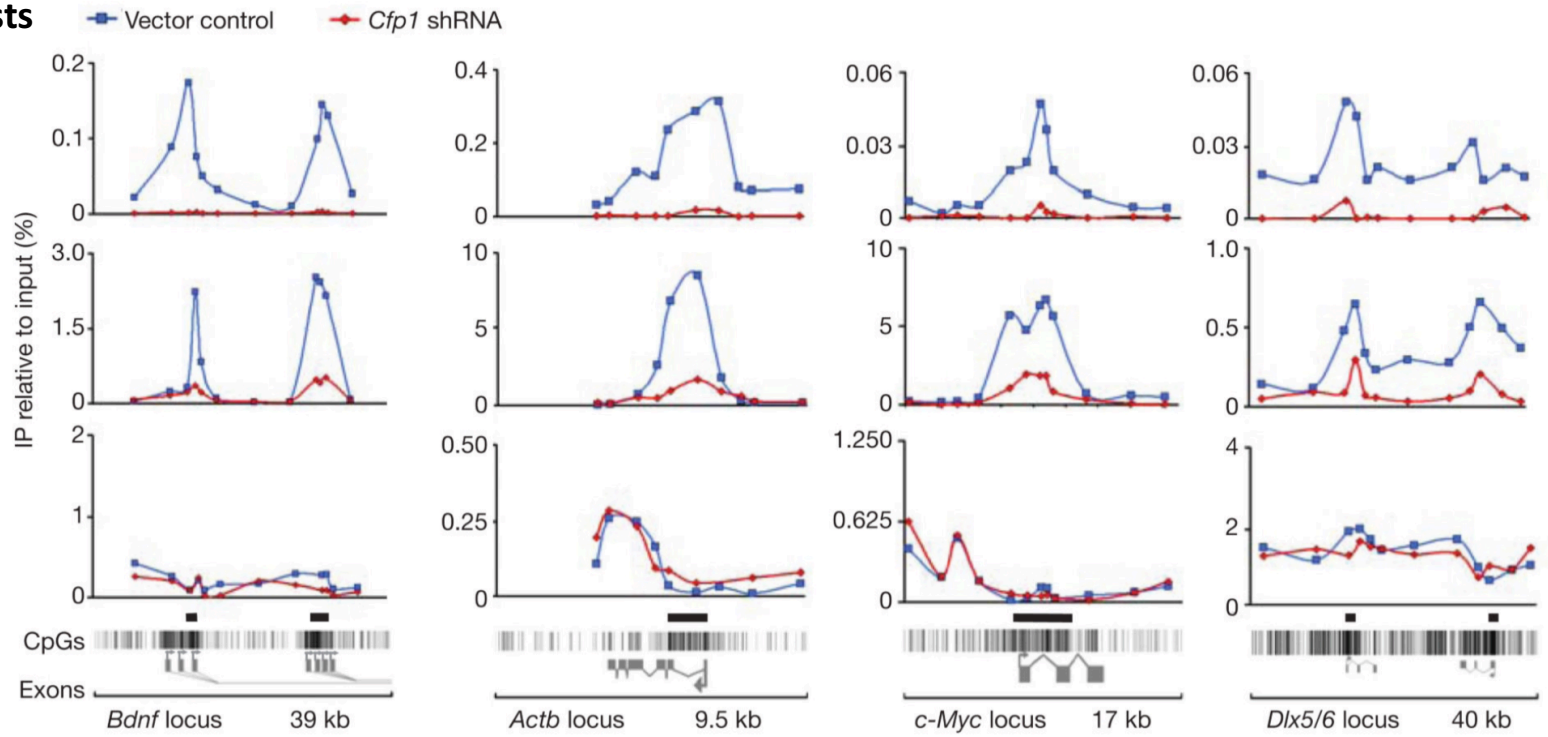
CFP1 CXXC domain is required to bind to unmethylated CpG islands

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



Control modification (does not change after siCFP1)

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 3×105 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

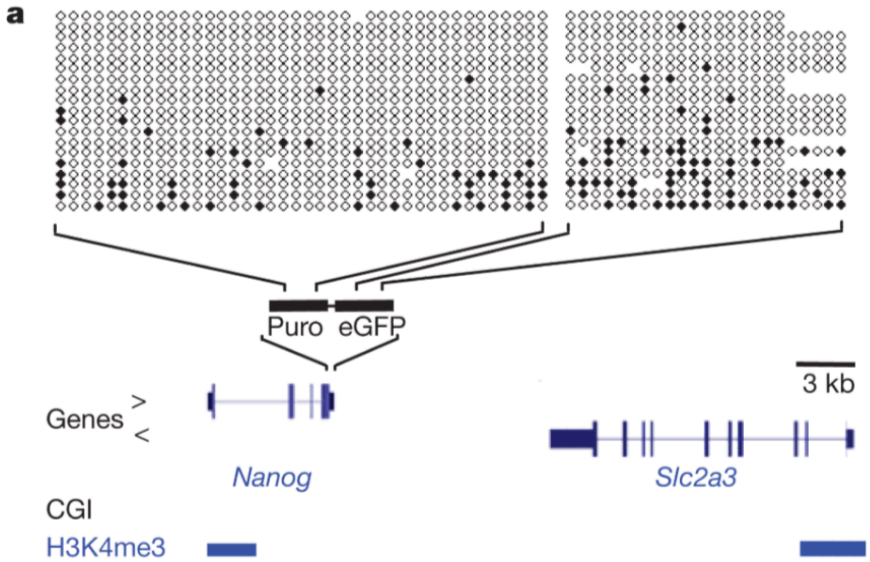
Experimental model system:

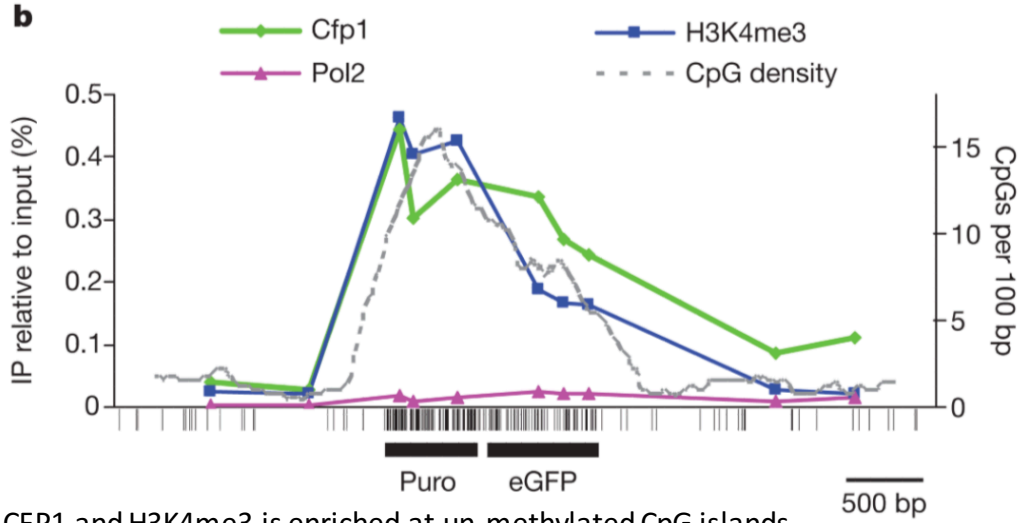
→ Embryonic stem cells are stably transfected with a DNA fragment that contains puromycin and EGFP: both sequences are enriched in extremely CG rich (but are protein coding)

→ The fragment does NOT contain a promoter

→ A) bisulfite seqeuncing: the inserted CpG rich DNA sequence is NOT METHYLATED

→ B) ChIP seq 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.

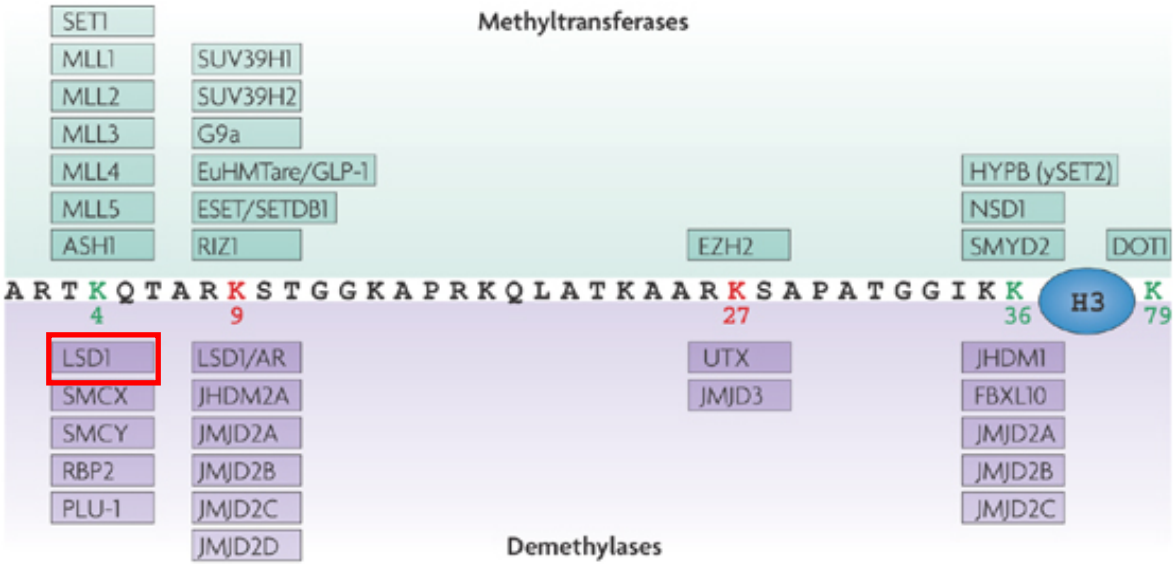
RESULT: un-mentylated CpG are sufficient to recruit CFP1 + SET1

To increase H3K4me3, also in the absence of transcription

THAT MEANS THAT THE **UNMETHYLATED CpG SEQUENCE IS SUFFICENT TO DIRECT H3K4me3**

H3K4 de-methylation and CpG island methylation

Lecture 5: Coordination of histone and DNA methylatiON

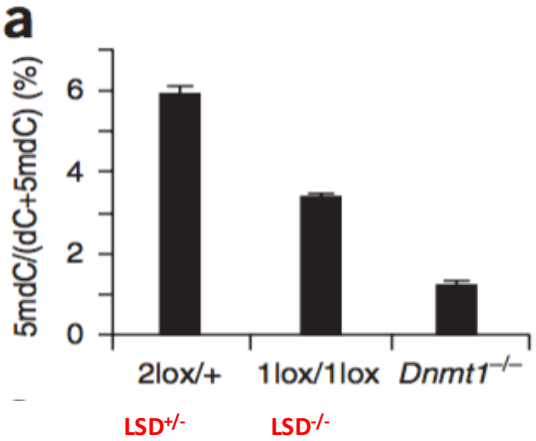


Nature Reviews | Genetics

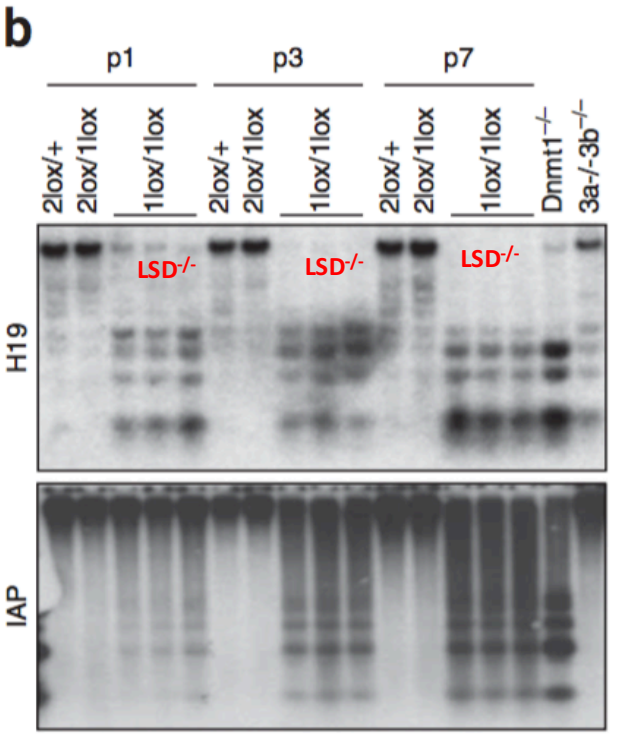
The H3K4 de-methylase LSD1 (KDM1A) is essential for establishing DNA methylation

LSD1 is a H3K4 specific demethylase: oxidizes H3K4me2,1 → H3K4me0

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



Loss of LSD1 results in
Reduced DNA methylation



Southern blotting using CH3 sensitive restriction enzymes: a probe for the H19 and IAP imprinted gene locus are used. These are classic loci are controlled by DNA methylation (high % of CpG methylation frequency)

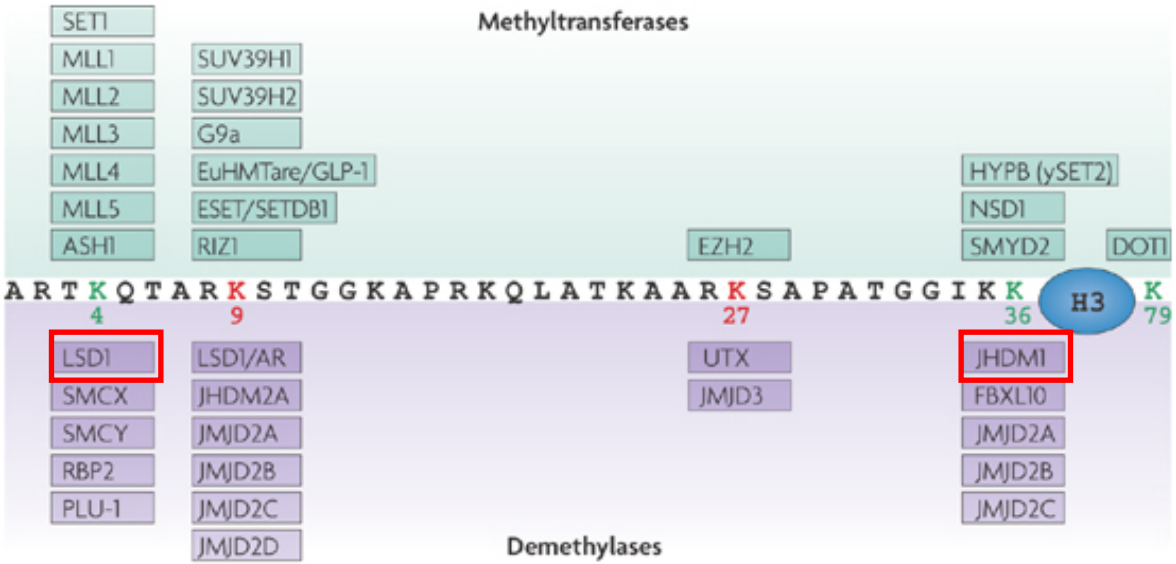
Note: Loss of DNA methylation results in Efficient restriction digest (more small fragment). This means that DNA methylation is strongly reduced
Situation is similar to DNMT1 knock-out cells

Recruitment of LSD1 eliminates H3K4me1,2 resulting in H3K4me0
This creates a binding site for DNMT3L → thus recruiting the DNMT3L-DNMT3a tetramer

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

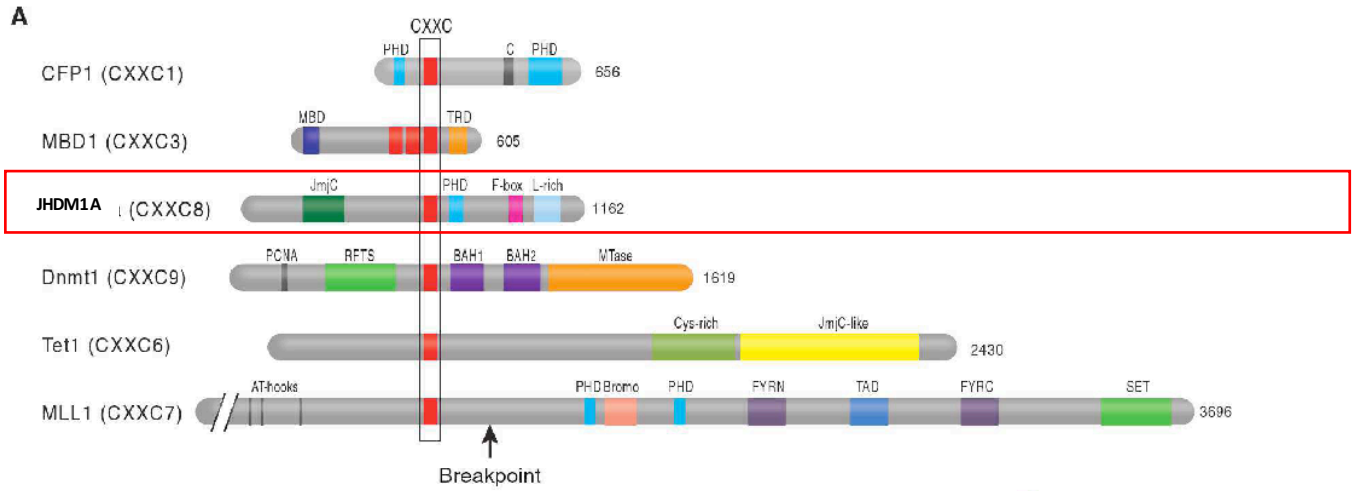
H3K36 de-methylation and CpG island methylation

Lecture 5: Coordination of histone and DNA methylatiON

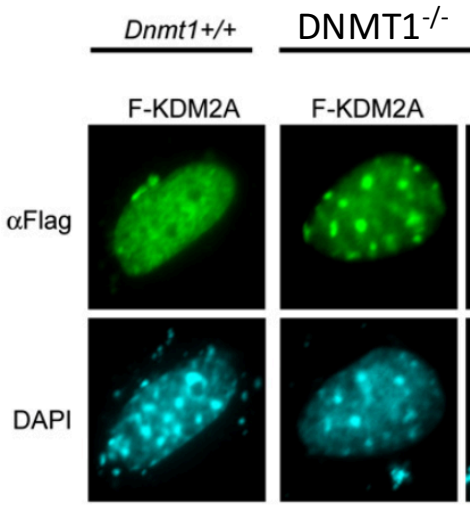


Nature Reviews | Genetics

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



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**

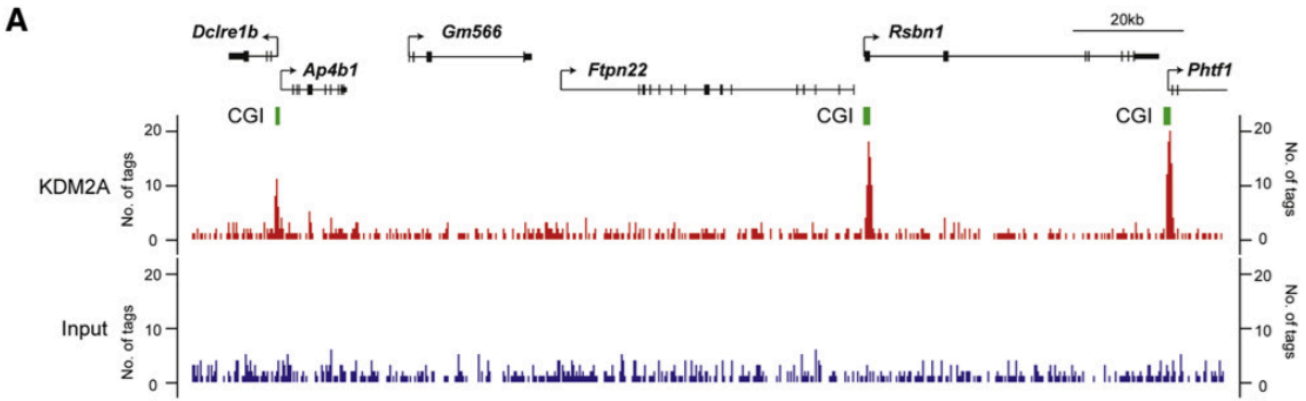
JHDM1A/KDM2A is a histone de-methylase that ensures low H3K36me2/me1 levels at CpG islands

CXXC domain binds un-methylated CpG islands

Tet1 has CXXC zinc finger domain. However, the CXXC domain of TET1 has no DNA binding activity and is dispensable for its catalytic activity in vivo. Other interacting proteins recruit Tet1 to DNA

Blackledge et al. Molecular Cell 2010

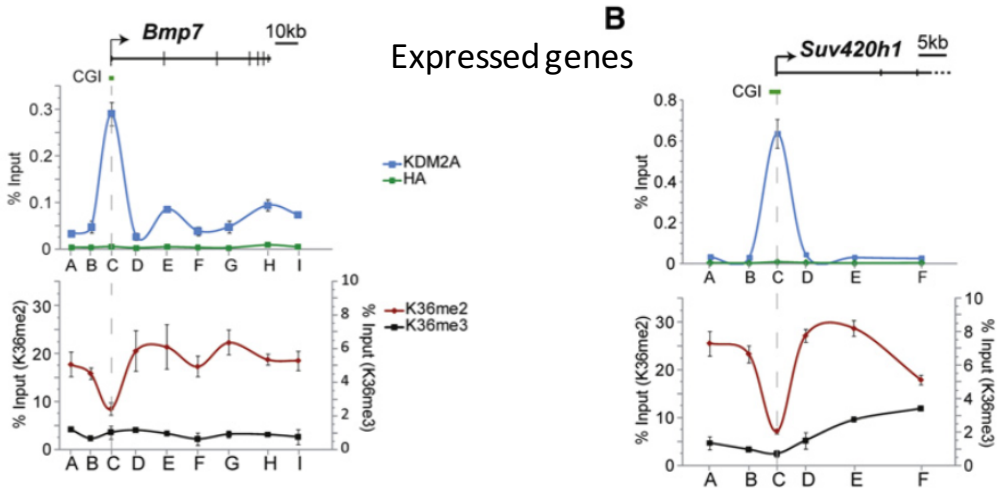
CXXC domains mediate binding to unmethylated CpGs: H3K36 specific KDM2A/JHDM1A (K-Histone De-methylase)



unmethylated CpG

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

Interaction is dependent on the CXXC domain



H3K36me2 is high in gene body but low at promoter
High levels of KDMA2 at unmethylated CpG islands is paralleled by low H3K36me2 levels

Blackledge et al. Molecular Cell 2010

H3K9 methylation and CpG island methylation

The relation of H3K9me and DNA methylation

SET1
MLL fam.

H3K4me high → *H3K9me low* → Gene ON

G9a/GLP
Suv39H1/2

H3K9me high → *H3K4me low* → Gene OFF

SET1
MLL fam.

H3K4me high → *CpG methylation low* → Gene ON

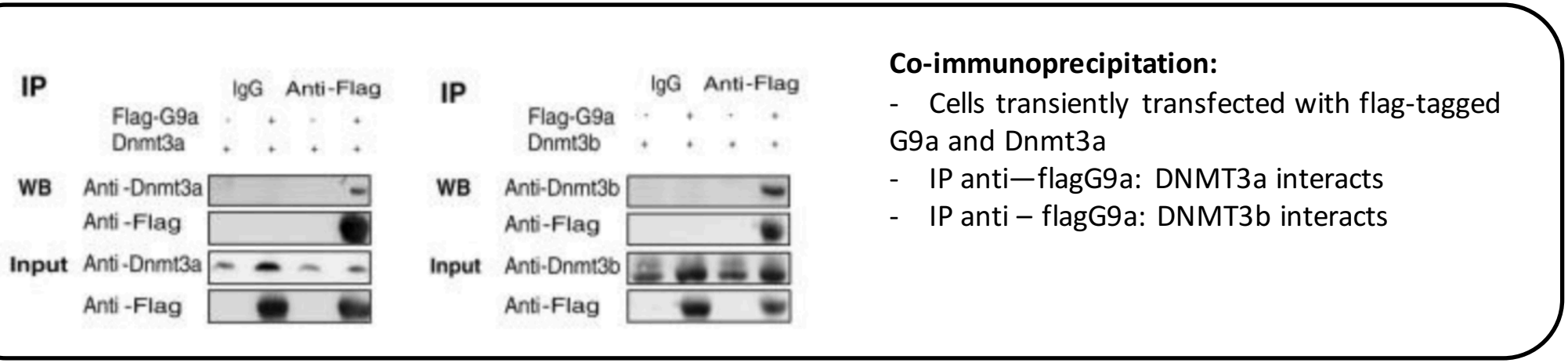
G9a/GLP
Suv39H1/2

H3K9me high → *CpG methylation ???* → Gene OFF

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



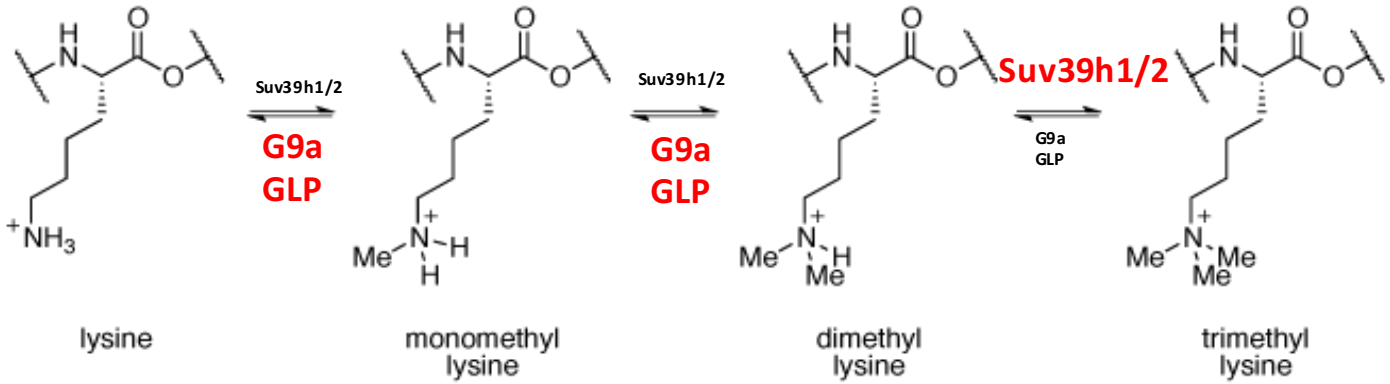
Co-immunoprecipitation:

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

Epsztejn-Litman Nat. Struct. Mol. Biol. 2008

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

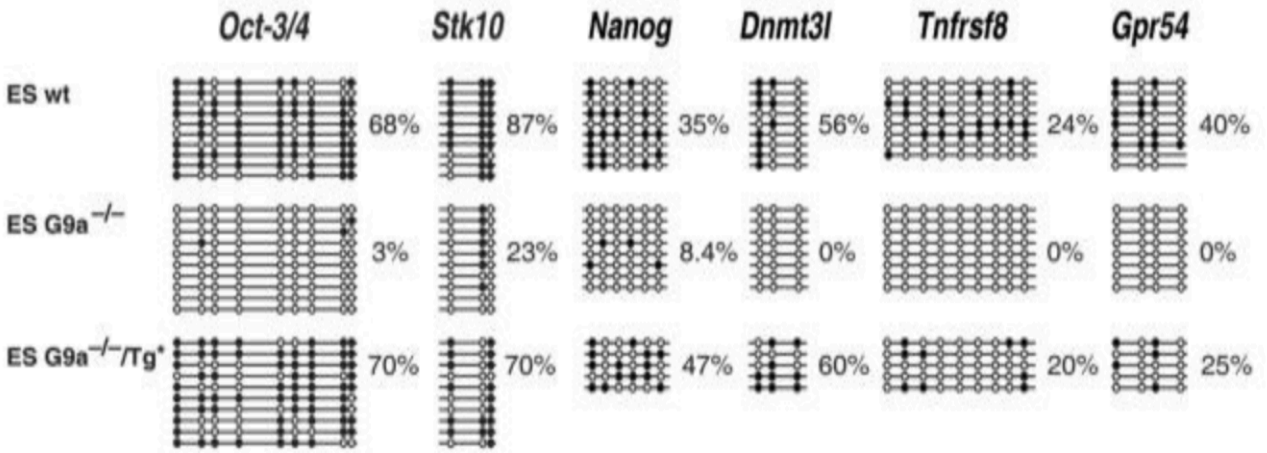
Oct4, Stk10, Gpr54
Nanog, Dnmt3L, Tnfrsf8
OFF
DNA METHYLATION

G9a – knock-out cells in differentiation

Show a loss of DNA methylation

As detected by bisulfite sequencing

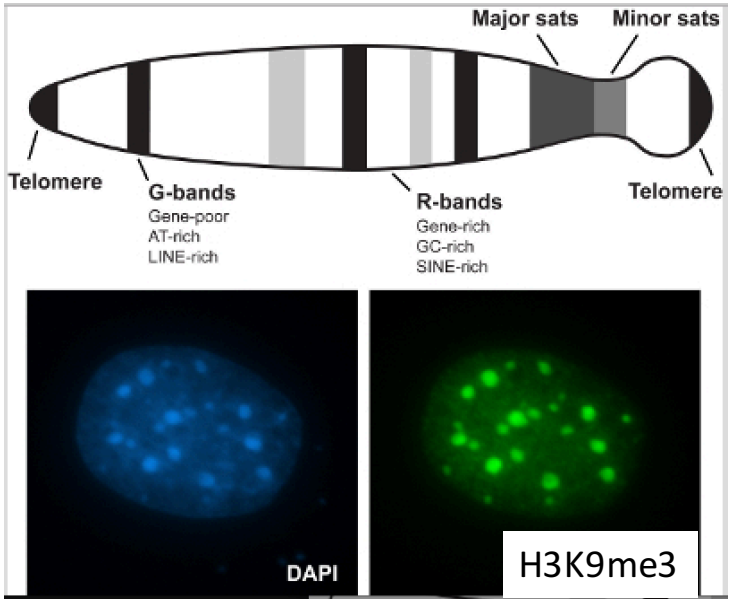
In CpG islands of several genes

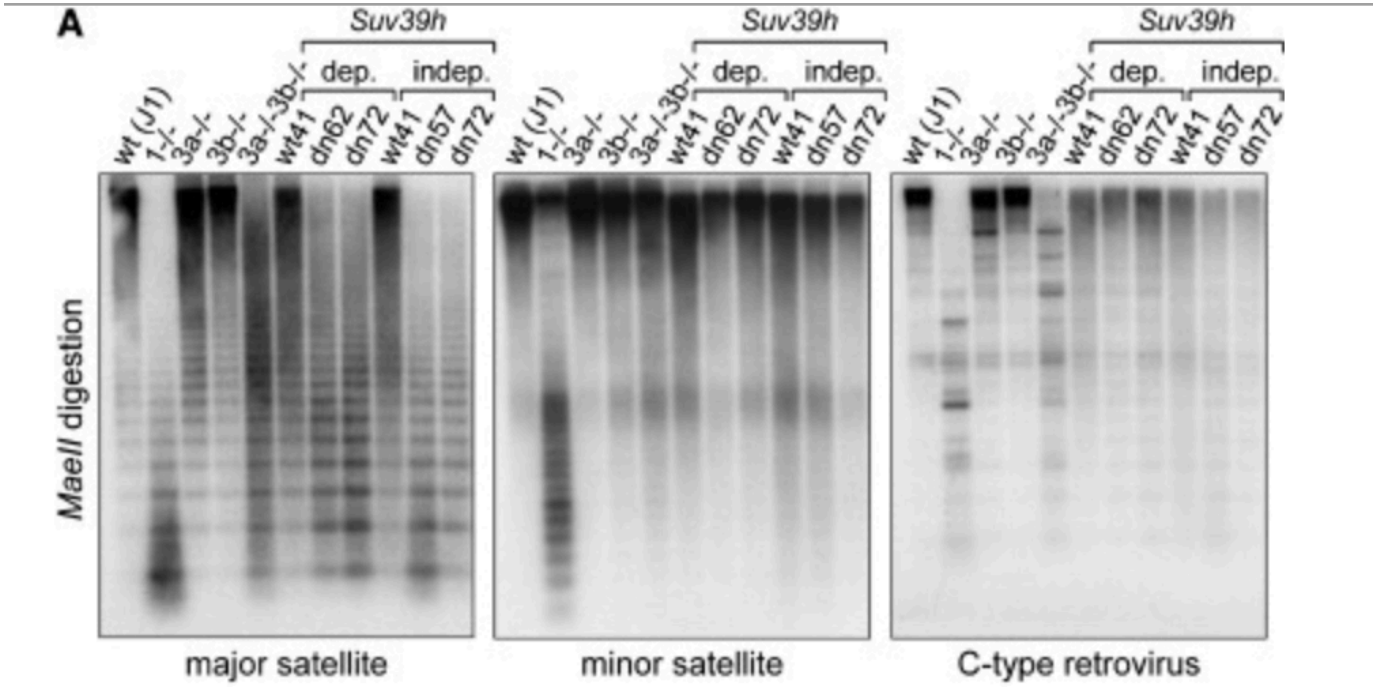


G9a: Required for silencing of transposable elements, repeat elements, retroviral insertions, imprinting centers but also in gene expression control

Lehnertz el al. 2009

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



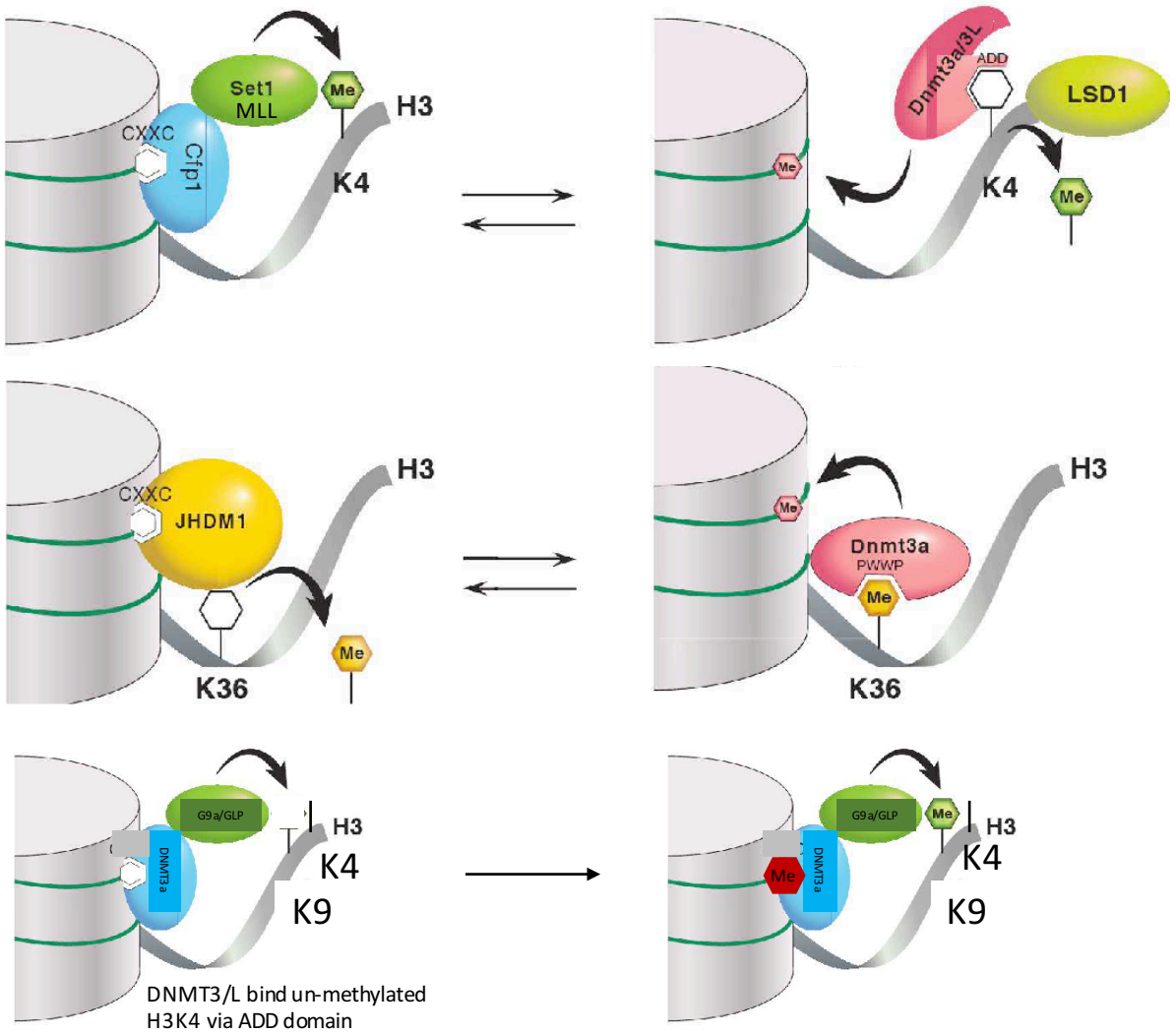


Loss of Suv39h1 HMTases is linked to reduced DNA methylation at pericentric repeats

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

Lehnertz el al. 2003

Histone methylation and DNA methylation



DNMT3/L bind un-methylated H3K4 via ADD domain