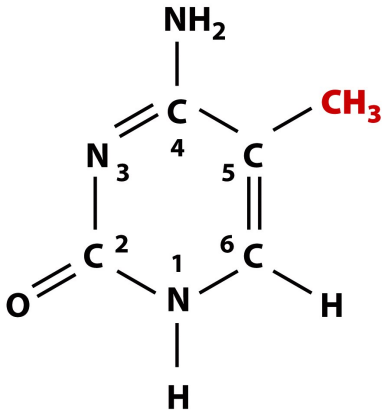
Epigenetics – DNA methylation

Biosciences 741: Genomics

Fall, 2013

Week 13

DNA Methylation



- Most methylated cytosines are found in the dinucleotide sequence CG, denoted mCpG.
- The restriction enzyme *Hpa*II recognizes and cleaves the sequence CCGG, but cannot cleave the sequence when the second cytosine is methylated.

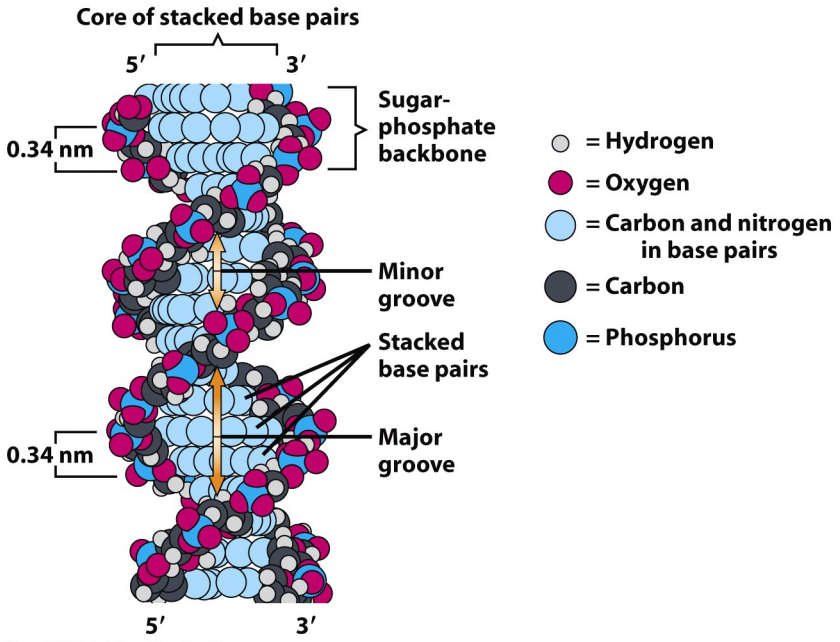
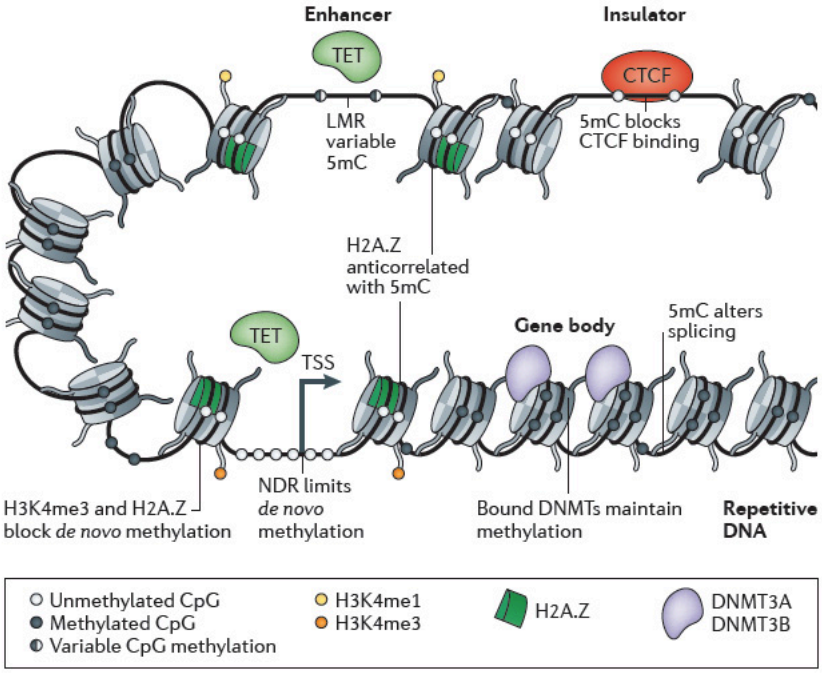


Figure 9-12 Principles of Genetics, 4/e

DNA methylation and gene regulation

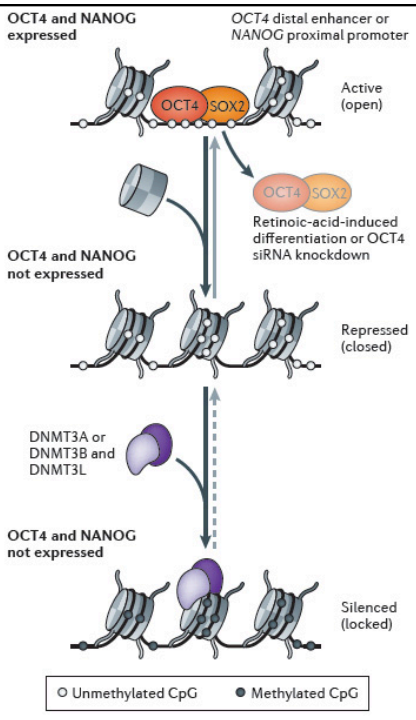
- Actively expressed genes, and particularly their promoters, are generally under-methylated in the specific tissues in which they are expressed.
- This has been implicated as being both cause and effect - in other words, methylation interferes with expression, and expression interferes with methylation.
- Methylation may help to keep inappropriate genes (and transposable elements) turned off.
- DNA methylation helps to regulate X chromosome inactivation in female mammals (myoD, azaC, etc).



Jones (2012) *Nat. Rev. Genet.* 13, 484-492.

Gene silencing often precedes DNA methylation of promoters.

However, methylation of the gene "body" increases transcription!



Jones (2012) *Nat. Rev. Genet.* 13, 484-492.

Maintenance of DNA methylation in plants & animals

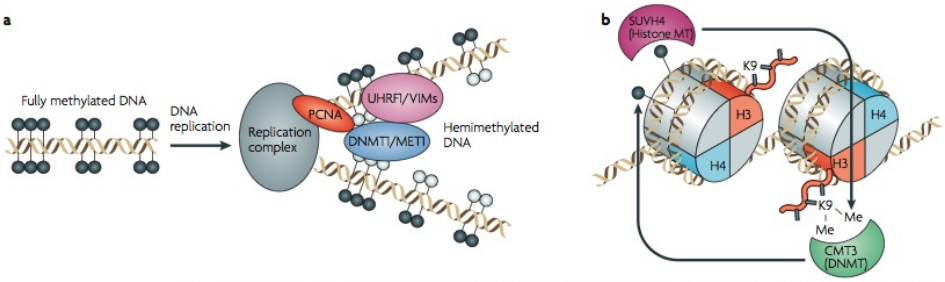
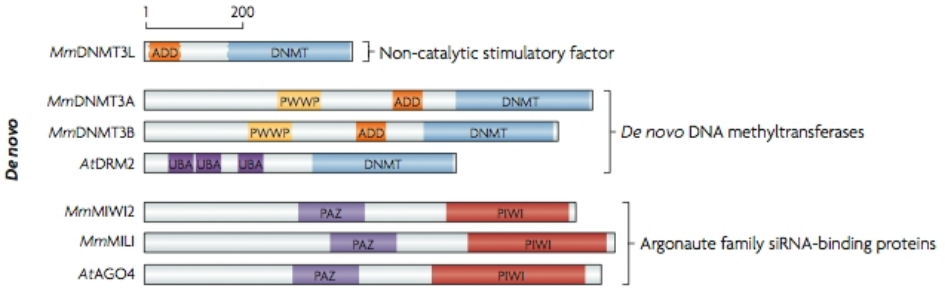


Figure 6 | **Maintenance of DNA methylation in plants and mammals. a** | Model depicting the maintenance of CG methylation during replication. DNA methyltransferase 1 (DNMT1) is proposed to be recruited to replication foci through interactions with ubiquitin-like plant homeodomain and RING finger domain 1 (UHRF1) — a SET- or RING-associated (SRA) domain protein that specifically interacts with hemimethylated DNA — and with proliferating cell nuclear antigen (PCNA). After being recruited, DNMT1 functions to maintain methylation patterns by restoring the hemimethylated DNA to a fully methylated state. In plants, DNA METHYLTRANSFERASE 1 (MET1, also known as DMT1) and the VARIANT IN METHYLATION (VIM, also known as ORTHRUS) family of SRA domain proteins, which are homologues of DNMT1 and UHRF1, respectively, are likely to function in a similar manner to maintain CG methylation patterns. Black and white circles represent methylated and unmethylated cytosines, respectively. **b** | Model depicting the maintenance of CHG methylation in plants. A reinforcing loop of DNA and histone methylation is proposed to maintain CHG methylation in plants. The CHROMOMETHYLASE 3 (CMT3) DNA methyltransferase maintains methylation in the CHG context, which is recognized by the SRA domain of the SUPPRESSOR OF VARIEGATION 3-9 HOMOLOGUE 4 (SUVH4, also known as KYP) histone methyltransferase (histone MT). SUVH4 catalyses histone 3 lysine 9 dimethylation (H3K9me2), a modification that is required for the maintenance of CHG methylation, and the chromodomain of CMT3 binds methylated H3 tails.

Law & Jacobsen (2010) Nat. Genet. 11, 204-220.

De novo DNA methylases



Law & Jacobsen (2010) Nat. Genet. 11, 204-220.

Maintenance vs. *de novo* DNA methylases

- DNMT1 is considered to be a "maintenance" DNA methylase because it has low affinity for unmethylated (vs. hemimethylated) DNA, and also because it is (usually) part of the DNA replication complex.
- DNMT3A and DNMT3B are considered to be *de novo* methylases because they are recruited by chromatin-binding proteins and can methylate unmethylated DNA.
- Nevertheless, mouse knockout experiments have shown that DNMT3s do have a small but significant role in the maintenance of DNA methylation (how? why?).
- Likewise, DNMT1 may also have a small but significant role in *de novo* methylation (how? why?).

Regulation of DNA methylation

- Unmethylated CpG islands are bound by the CXXC zinc finger protein CFP1, which recruits H3K4 methylases (me3) and is sufficient to maintain the unmethylated state.
- Although *Cfp1* knockout cells lose H3K4me3 at CpG islands, nevertheless if the gene continues to be expressed then the promoter will also continue to be unmethylated.
- CpG island promoters can be silenced by H3K27 methylation, in which case they may remain unmethylated.
- Repressed promoters - H3K9 dimethylases are recruited in complexes with DNMT3A or DNMT3B for complete, stable promoter silencing.
- DNMT3b is retained at the centromere!

De novo DNA methylation in plants & animals

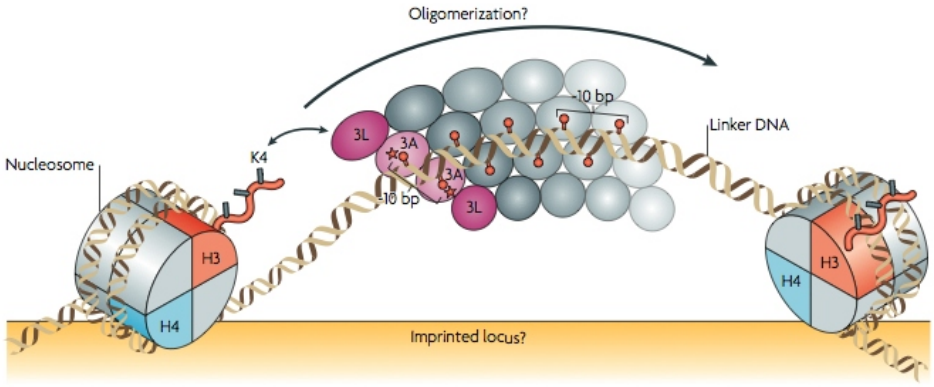
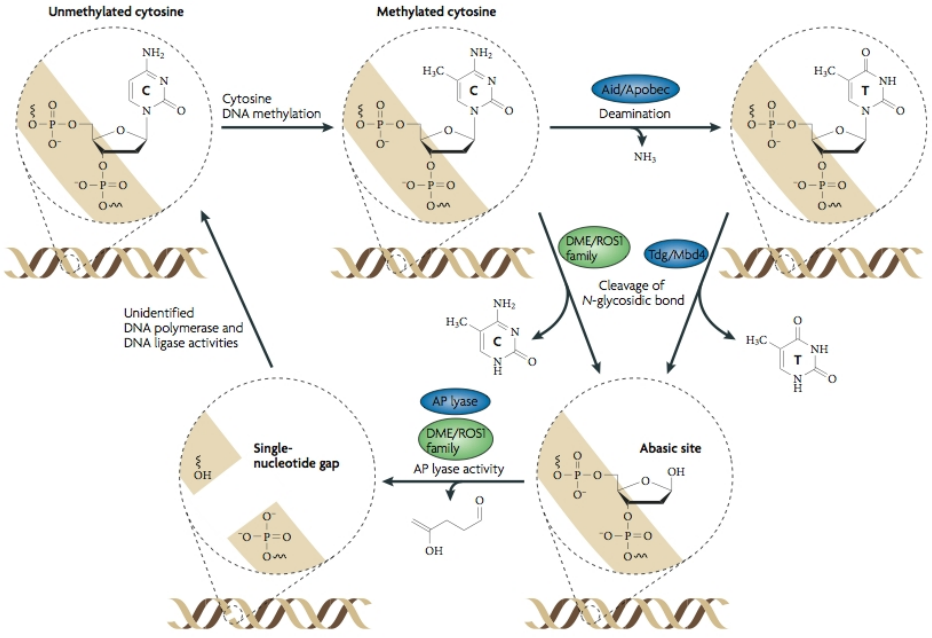


Figure 3 | **Model of recruitment of the *de novo* methylation machinery by unmethylated histone 3 lysine 4 tails.** The amino-terminal domain of DNA methyltransferase 3-like (DNMT3L, shown as 3L) possesses a cysteine-rich domain that interacts with unmethylated histone 3 lysine 4 (H3K4) tails, and this interaction is proposed to recruit or activate the DNMT3A2 isoform. The carboxy-terminal domains of DNMT3L and DNMT3A (shown as 3A) form a tetrameric complex in which two DNMT3A proteins interact with each other and are flanked by two DNMT3L proteins. The DNMT3A active sites (red stars) are thought to be separated by approximately one helical turn and therefore could catalyse methylation (red circles) on opposite DNA strands ~10 bp apart. After being recruited to a specific locus, the DNMT3L–DNMT3A tetramer might be able to oligomerize, which could result in an ~10 bp periodic pattern of DNA methylation along the same DNA strand.

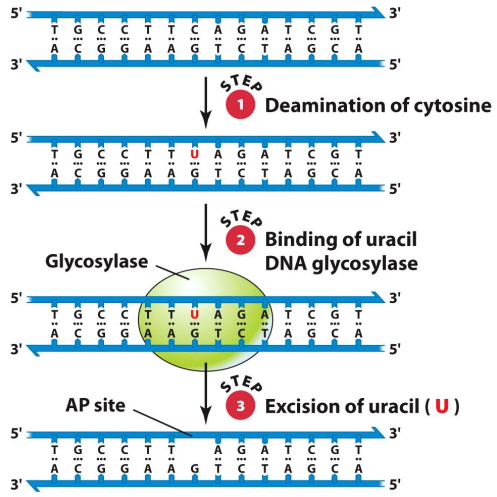
Law & Jacobsen (2010) Nat. Genet. 11, 204-220.

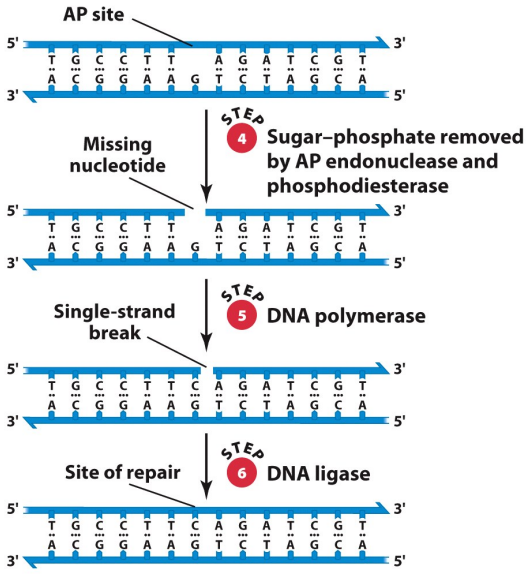
Active demethylation of DNA

Law & Jacobsen (2010) Nat. Genet. 11, 204-220.



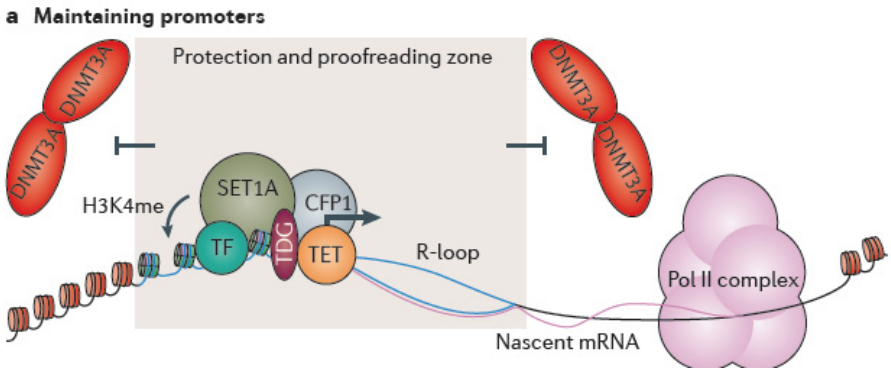
Base Excision Repair of cytosine deamination





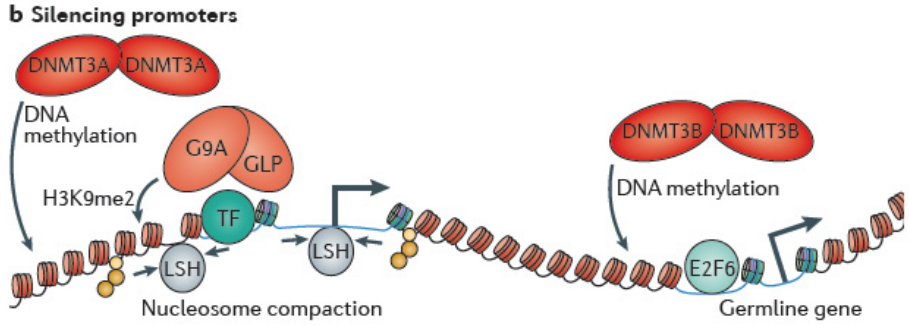
Mechanisms of demethylation

- AID/APOBEC contributes to DNA demethylation in primordial germ cells (PGCs).
- TET1 can oxidize 5mC to 5hmC. It is highly expressed in PGCs. Loss of 5mC from the paternal genome in the fertilized egg correlates with an increase in 5hmC that is specific to the male pronucleus.
- TET3-depleted zygotes fail to demethylate the male pronucleus.
- On the one hand, both the SMUG1 and TDG glycosylases have strong activity towards 5hmU:G mismatches.
- On the other hand, some 5hmC may be removed passively, as it fails to be replicated by DNMT1.



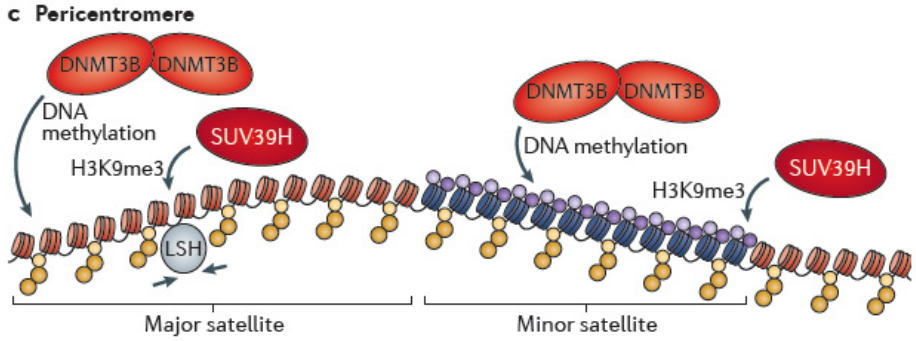
DNA methylation and its targets in mouse embryonic stem cells.
a | Most transcription start site (TSS)-associated CpG islands are protected from DNA methylation Components that confer this protection include: transcription factor ('TF' in the figure) binding; nucleosome exclusion; and histone H3 lysine 4 (H3K4) methyltransferases, such as SET domain containing 1A (SETD1A; recruitment of which is directed by CXXC finger protein 1 (CFP1)) or MLL proteins. Active transcription may also inhibit DNA methylation by forming DNA–nascent RNA helices, which induce R-loops of single-strand DNA (ssDNA) that exclude *de novo* methylation. The presence of catalytic enzymes associated with DNA demethylation, such as the TET enzymes or thymidine DNA glycosylase (TDG), may prevent aberrant methylation.

Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.



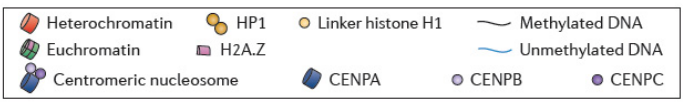
b | Stable silencing of promoter regions requires recruitment of repressive transcription factors, which direct the recruitment of the chromatin remodeller LSH, linker histone H1, heterochromatin protein 1 (HP1), H3K9 methyltransferases (the G9A–GLP complex is shown) and *de novo* DNA methyltransferases, frequently in that order. At germline gene promoters, DNA methyltransferase 3B (DNMT3B) is more directly involved in gene silencing than at other genes and acts downstream of transcriptional repressors (E2F6 is shown).

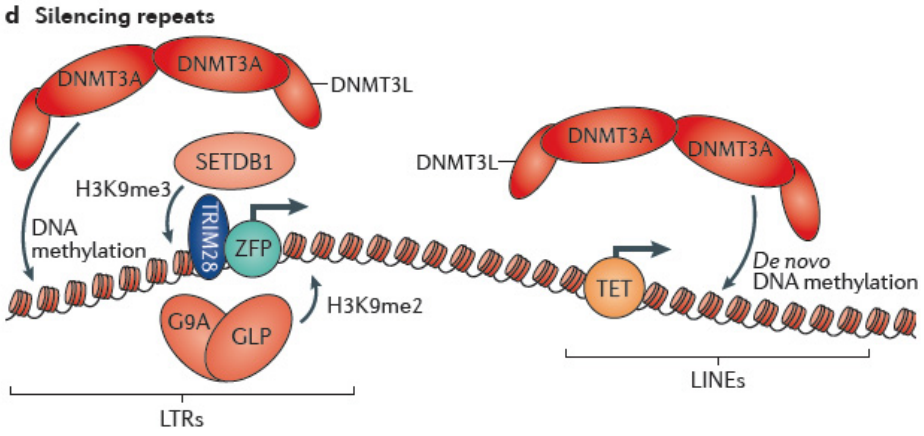
Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.



c | Pericentromeric repeats are predominantly targeted by DNMT3B. At major satellites, DNMT3B is secondary to the activity of H3K9 methyltransferases SUV39H1 and SUV39H2 (shown as SUV39H). DNMT3B may be more immediately involved in minor satellite silencing by directly interacting with the centromeric nucleosome.

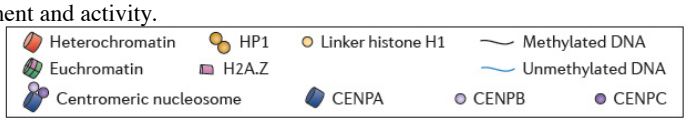
Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.

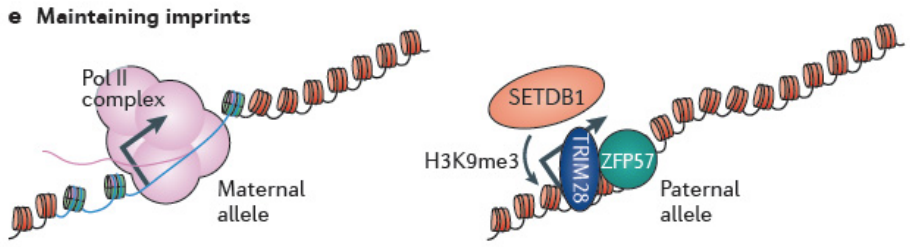




d | Repetitive elements such as LTR-containing retroelements and LINEs are also silenced by DNA methylation, although for LTRs, DNA methylation acts downstream of zinc finger protein (ZFP)-based recruitment of TRIM28 and the H3K9 methyltransferase SETDB1. LINE elements exhibit enriched hemimethylation and hydroxymethylation, which may be countered by *de novo* methyltransferase recruitment and activity.

Smith et al. (2013)
Nat. Rev. Genet.
14, 204-220.





e | In somatic cells, maintenance methylation is frequently sufficient to propagate parent-specific imprints. However, several imprints may have strong activation potential and are continuously silenced through ZFP57 binding to methylated DNA, which in turn recruits TRIM28 and the H3K9 methyltransferase SETDB1. CENPA, centromere protein A; Pol II, RNA polymerase II.

Smith et al. (2013)
Nat. Rev. Genet.
14, 204-220.

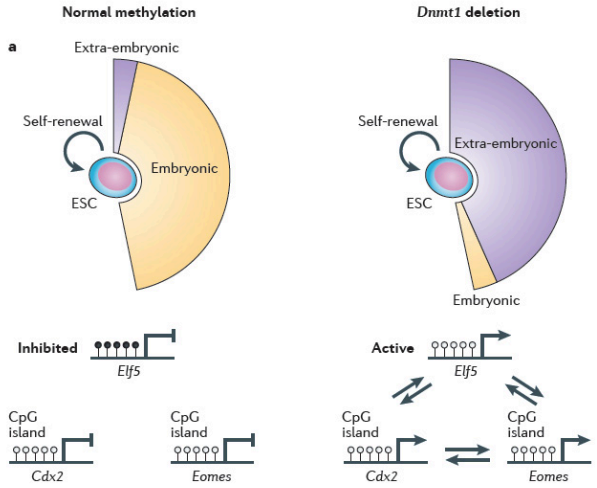
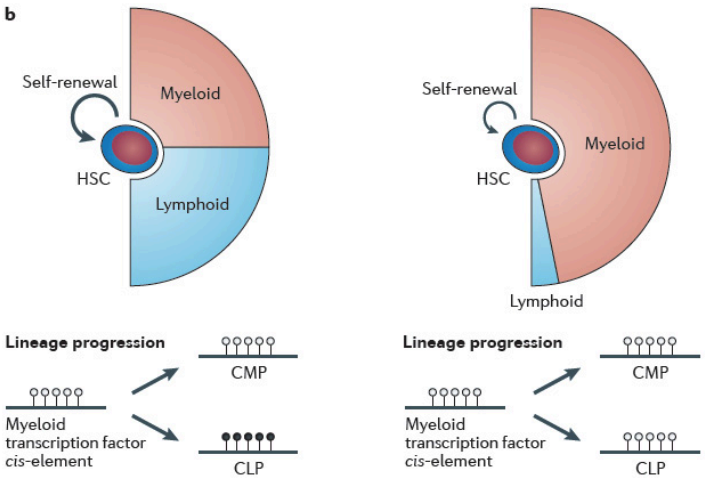


Figure 2 | **Lineage restriction and renewal of embryonic and adult stem cells. a** | In wild-type mouse embryonic stem cells (ESCs), extra-embryonic potential is restricted to a small population of cells that show retroelement expression; most cells are self-renewing and are unable to commit to extra-embryonic lineages. The maintenance of embryonic potential is in part conferred by hypermethylation and silencing of *Elf5*. In *Dnmt1*-knockout cells, *Elf5* is unmethylated and expressed, along with *Cdx2* and *Eomes*, which permits extra-embryonic differentiation.

Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.



b | DNA methylation balances myeloid versus lymphoid commitment in haematopoietic stem cells (HSCs) as they exit from self-renewal to become restricted progenitors. Unlike ESCs, HSC self-renewal is inhibited in the absence of DNMT1, perhaps due to cell-cycle checkpoints. However, in *Dnmt1* hypomorphic HSCs, commitment to the myeloid lineage is favoured, possibly because hypermethylation of myeloid-associated transcription factor *cis*-regulatory elements is impaired, stabilizing myeloid precursor (CMP) and destabilizing lymphoid precursor (CLP) fates.

Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.

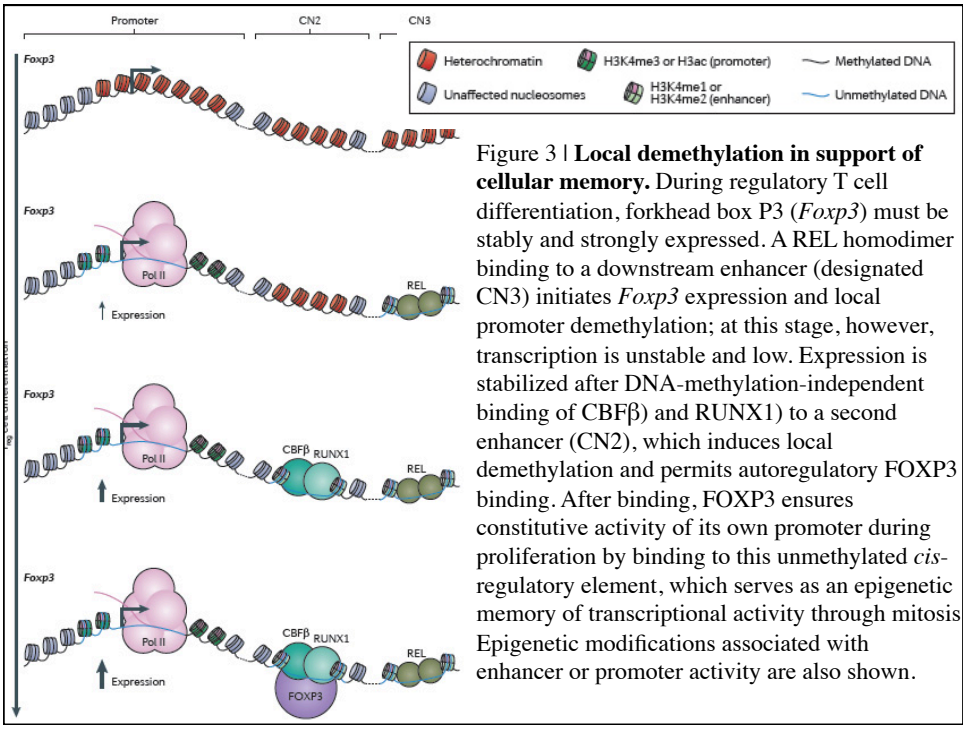


Figure 3 | **Local demethylation in support of cellular memory.** During regulatory T cell differentiation, forkhead box P3 (*Foxp3*) must be stably and strongly expressed. A REL homodimer binding to a downstream enhancer (designated CN3) initiates *Foxp3* expression and local promoter demethylation; at this stage, however, transcription is unstable and low. Expression is stabilized after DNA-methylation-independent binding of CBFβ) and RUNX1) to a second enhancer (CN2), which induces local demethylation and permits autoregulatory FOXP3 binding. After binding, FOXP3 ensures constitutive activity of its own promoter during proliferation by binding to this unmethylated *cis*-regulatory element, which serves as an epigenetic memory of transcriptional activity through mitosis Epigenetic modifications associated with enhancer or promoter activity are also shown.

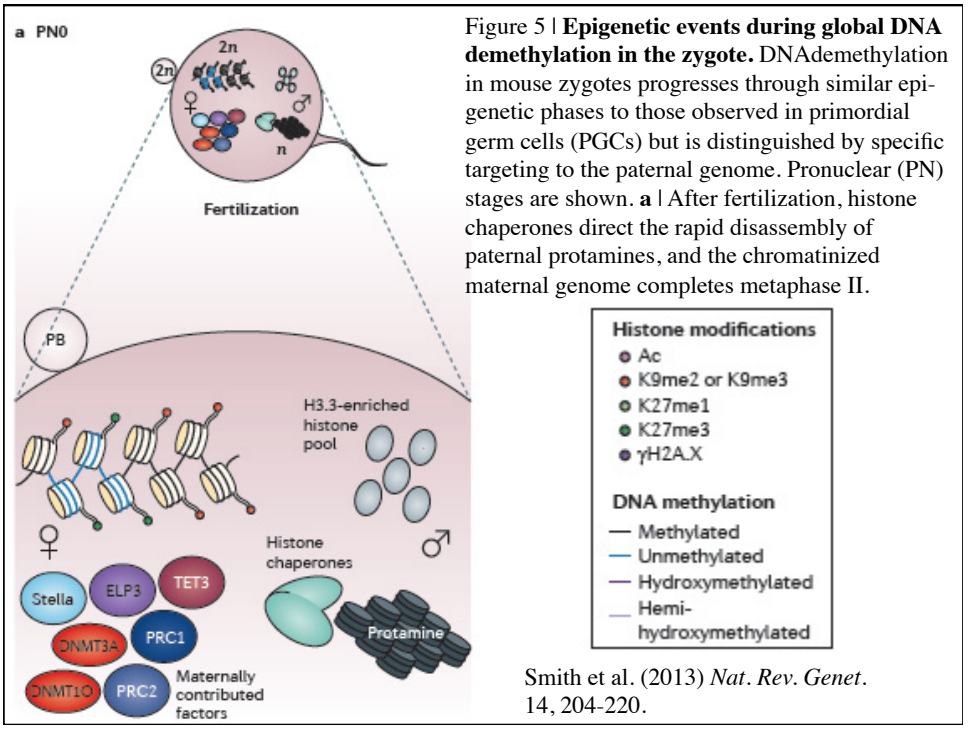
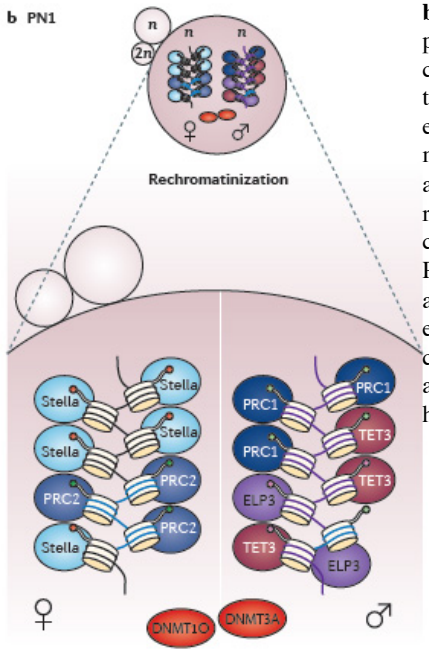


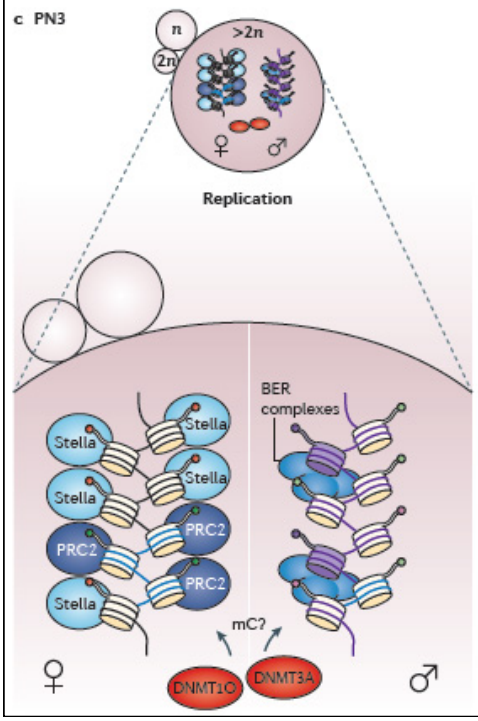
Figure 5 | **Epigenetic events during global DNA demethylation in the zygote.** DNAdemethylation in mouse zygotes progresses through similar epi-genetic phases to those observed in primordial germ cells (PGCs) but is distinguished by specific targeting to the paternal genome. Pronuclear (PN) stages are shown. **a** | After fertilization, histone chaperones direct the rapid disassembly of paternal protamines, and the chromatinized maternal genome completes metaphase II.

Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.

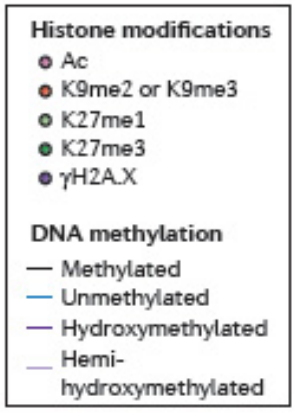


b | During rechromatinization of the paternal pronucleus, the incorporation of histone H3.3-containing nucleosomes co-occurs with rapid acetylation (possibly assisted by histone acetyltransferease elongator complexprotein 3 (ELP3)), hydroxymethylation mediated by TET3, H3K27 methylation and Polycomb repressive complex 1 (PRC1) recruitment. The maternal genome, which is already chromatinized, recruits epigenetic silencers such as PRC2 and Stella. Stella recognizes H3K9 methylation and protects against paternally targeted remodelling events. DNA demethylation as measured by immunochemistry is most strongly observed during this phase as a consequence of the global conversion to hydroxymethylcytosine (hmC) by TET3.

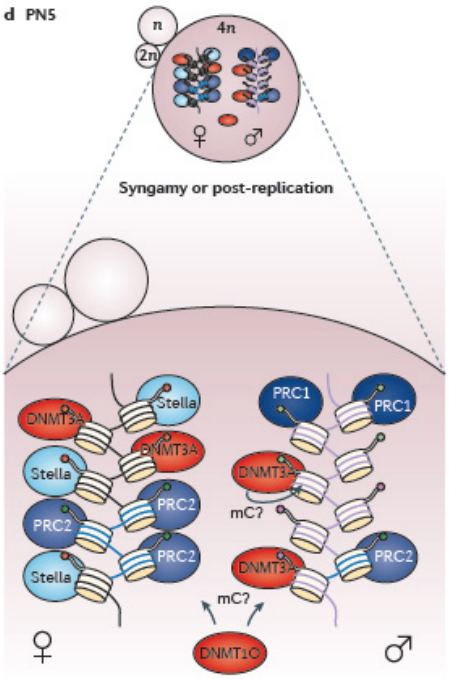
Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.



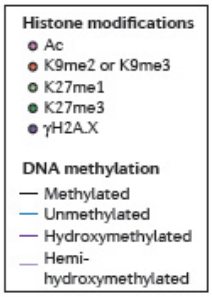
c | Global demethylation to unmodified cytosine is not robustly observed until DNA replication. This stage includes a biased enrichment for base excision repair (BER) complexes and γH2A.X in the paternal genome. At this point, paternal hyperacetylation is dampened, presumably through histone deacetylase activity (not shown) while repressive modifications, such as H3K27 trimethylation by PRC2, emerge.



Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.



d | After the completion of DNA synthesis (syngamy), DNA methylation is retargeted to many regions, such as the long terminal repeat (LTR) promoters of endogenous retroelements. Remethylation may be directed by DNMT3A or DNMT1O. Hemimethylated and hemihydroxymethylated sequences are presumably directed towards a completely unmethylated state after subsequent cleavage divisions. Genome content (*n*) is highlighted in each panel to indicate cell-cycle stage. The question marks in the lower part of the figure Indicate uncertainty regarding which DNMT participates in remethylation.

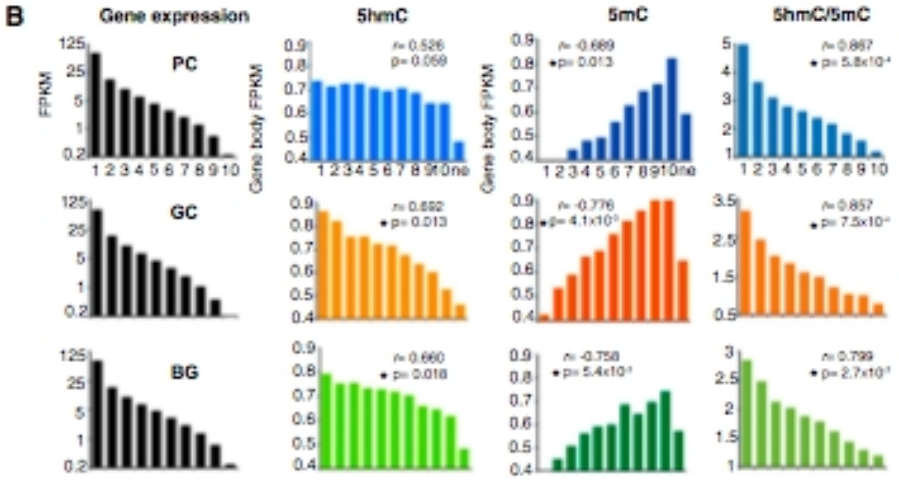


Smith et al. (2013) *Nat. Rev. Genet.* 14, 204-220.

5-hydroxy methyl cytosine

- 5hmC is more than 10-fold enriched in cells in the nervous system, in comparison to somatic cells. In the nervous system, 5hmC marks active genes.
- 5hmC is also used as a chromatin mark in embryonic stem cells, where it is specifically present in promoters, and/or protein coding sequences, of specific subsets of active genes.
- MeCP2 binds to both 5mC (enriched in nonexpressed genes) and 5hmC. Other methyl-binding proteins reportedly are all specific for 5mC (SFN 2013).
- However, the Rett syndrome causing mutation R133C preferentially affects 5hmC binding!

5-hydroxy methyl cytosine is associated with the protein coding Sequence ("body") of actively-expressed genes in the nervous system



FPKM = fragments per kilobase, per million DNA fragments.

Mellen et al. (2012) Cell 151, 1417-1430.

Discussion Questions

- Discuss the regulation of DNA methylation at CpG islands by factors such as H2A.Z, transcription factor binding, and CFP1.
- Discuss the silencing of promoters by transcription factors, the H3K9 dimethylase G9a, and DNMT3A and DNMT3B. How is this similar or different from the silencing of transposable elements?
- Discuss how local demethylation can produce a cellular "memory" in dividing and non-dividing cells? How does this relate to the role(s) of 5hmC in the nervous system?
- Discuss the evidence for paternal genome specific hydroxy-methylation and demethylation of the paternal genome in the zygote.