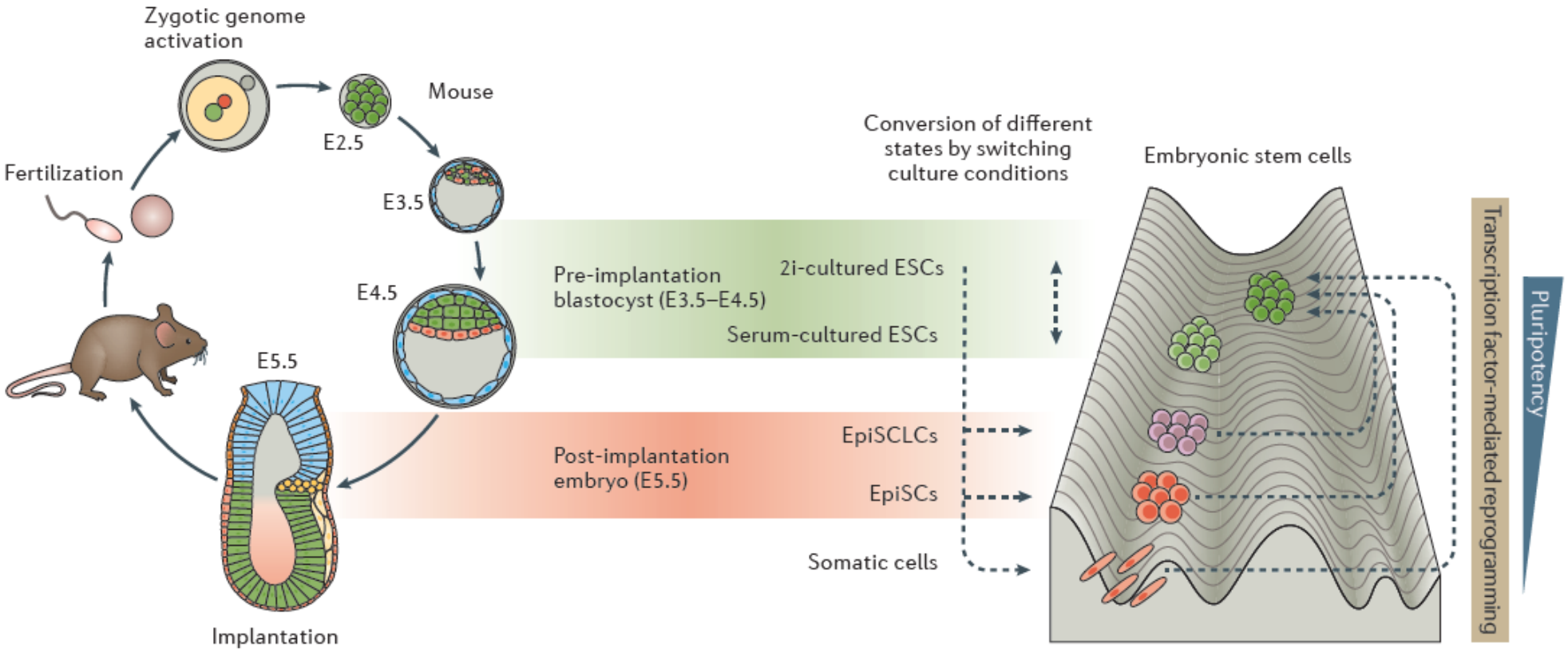
V10: Cellular differentiation - Epigenetics

E4.5 epiblast cells: represent ground-state pluripotency

Implantation: stage of pregnancy at which the blastocyst adheres to the wall of the **uterus**.

After implantation (E5.5): **epiblast cells** undergo a strong wave of epigenetic



Atlasi & Stunnenberg, *Nature Rev Genet* **18**, 643–658 (2017)

Epigenetic mechanisms

Epigenetics refers to **alternate phenotypic states** that are

not based on **differences in genotype**, and are potentially reversible,

but are generally stably maintained during cell division.

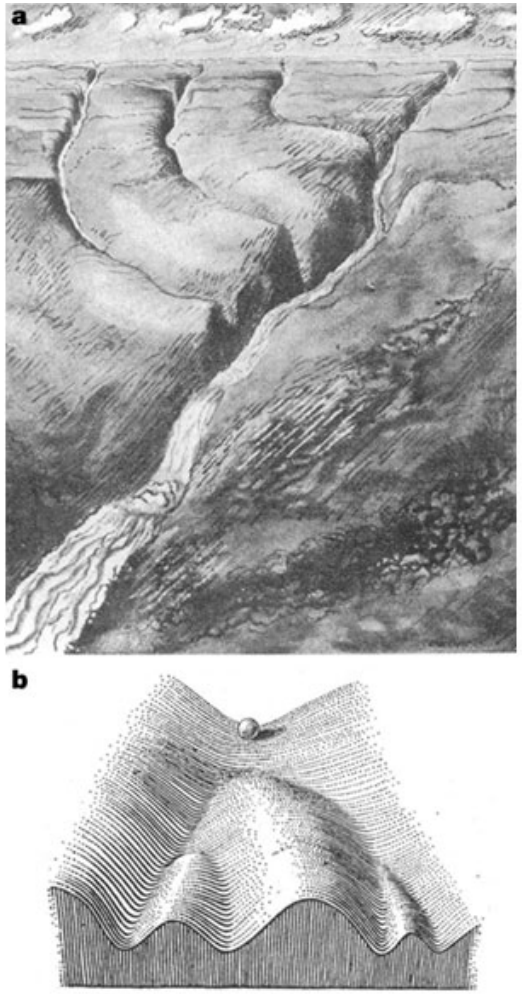
Examples: imprinting, twins, cancer vs. normal cells, differentiation, ...

Multiple mechanisms interact to collectively establish

- alternate states of chromatin structure (open – packed/condensed),
- **histone modifications**,
- composition of associated proteins (e.g. histones),
- transcriptional activity,
- activity of microRNAs, and
- in mammals, **cytosine-5 DNA methylation** at CpG dinucleotides.

Laird, Hum Mol Gen 14, R65 (2005)

Waddington's epigenetic landscape for embryology



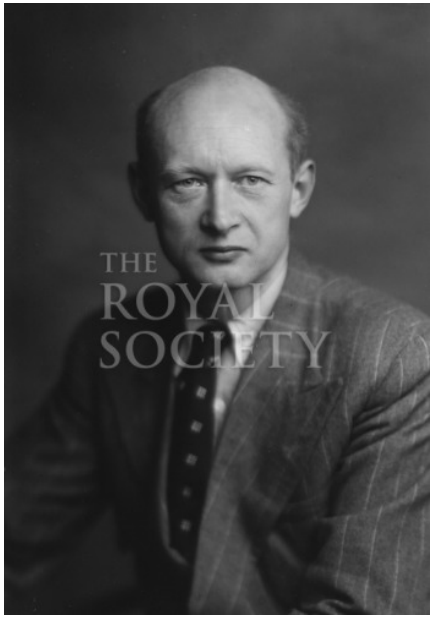
Slack, Nature Rev Genet 3, 889-895 (2002)

Waddington worked in **embryology**

a) is a painting by John Piper that was used as the frontispiece for Waddington's book *Organisers and Genes*.
It represents an epigenetic landscape.

Developmental pathways that could be taken by each cell of the embryo are metaphorically represented by the path taken by water as it flows down the valleys.

b) Later depiction of the epigenetic landscape. The ball represents a cell, and the bifurcating system of valleys represents bundles of trajectories in state space.

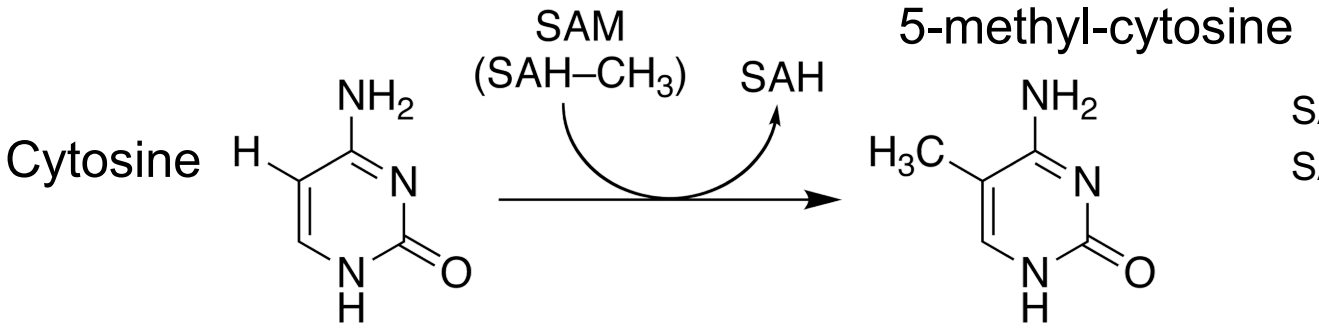


Conrad Hal Waddington (1905 – 1975)
pictures.royalsociety.org

Cytosine methylation

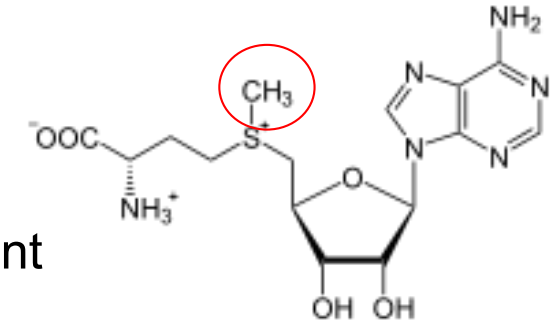
Observation: 3-6 % of all cytosines are methylated in human DNA.

This methylation occurs (almost) exclusively when cytosine is followed by a guanine base -> **CpG dinucleotide**.



SAM: S-adenosyl-methionine

SAH: S-adenosyl-homocysteine



Mammalian genomes contain much fewer (only 20-25 %) of the CpG dinucleotide than is expected by the G+C content (we expect 1/16 ≈ 6% for any random dinucleotide).

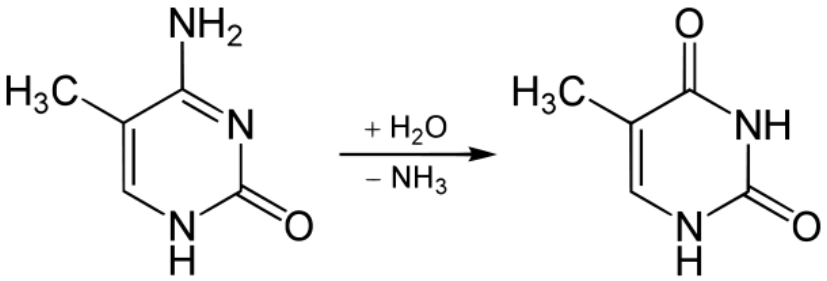
This is typically explained in the following way:

As most CpGs serve as targets of DNA methyltransferases, they are usually methylated …. (see following page)

Esteller, Nat. Rev. Gen. 8, 286 (2007)
www.wikipedia.org

Cytosine methylation

But 5-Methylcytosine can easily **deaminate** to **thymine**.

5-methyl-cytosine 

thymine

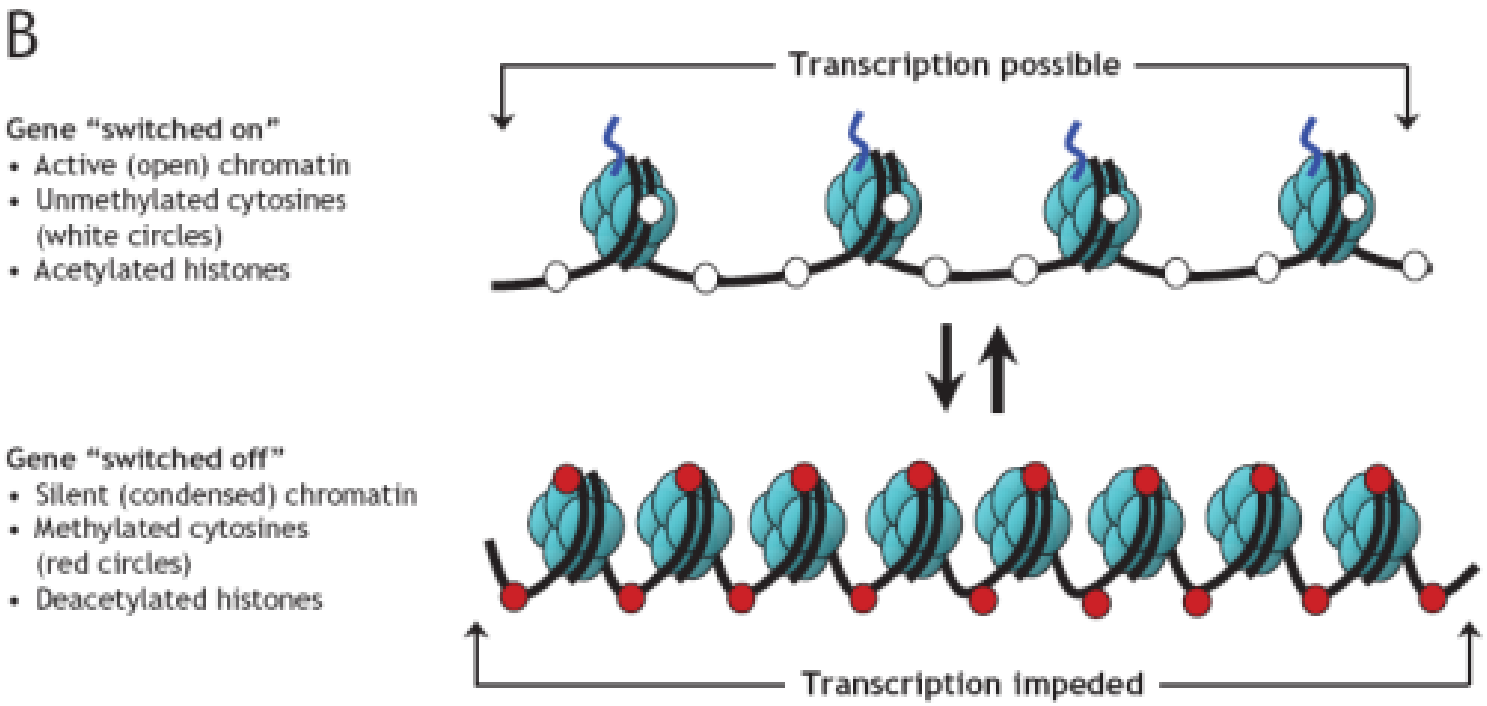
If this mutation is not repaired, the affected CpG is permanently converted to TpG (or CpA if the transition occurs on the reverse DNA strand).

Hence, methylCpGs represent **mutational hot spots** in the genome.
If such mutations occur in the germ line, they become heritable.

A constant loss of CpGs over thousands of generations
can explain the low frequency of this
special dinucleotide in the genomes of human and mouse.

Esteller, Nat. Rev. Gen. 8, 286 (2007)
www.wikipedia.org

chromatin organization affects gene expression



Schematic of the reversible changes in chromatin organization that influence gene expression:

genes are expressed (switched on) when the chromatin is **open** (active), and they are inactivated (switched off) when the chromatin is **condensed** (silent).

White circles = unmethylated cytosines;

red circles = methylated cytosines.

Rodenhiser, Mann, CMAJ 174, 341 (2006)

Altered DNA methylation upon cancerogenesis

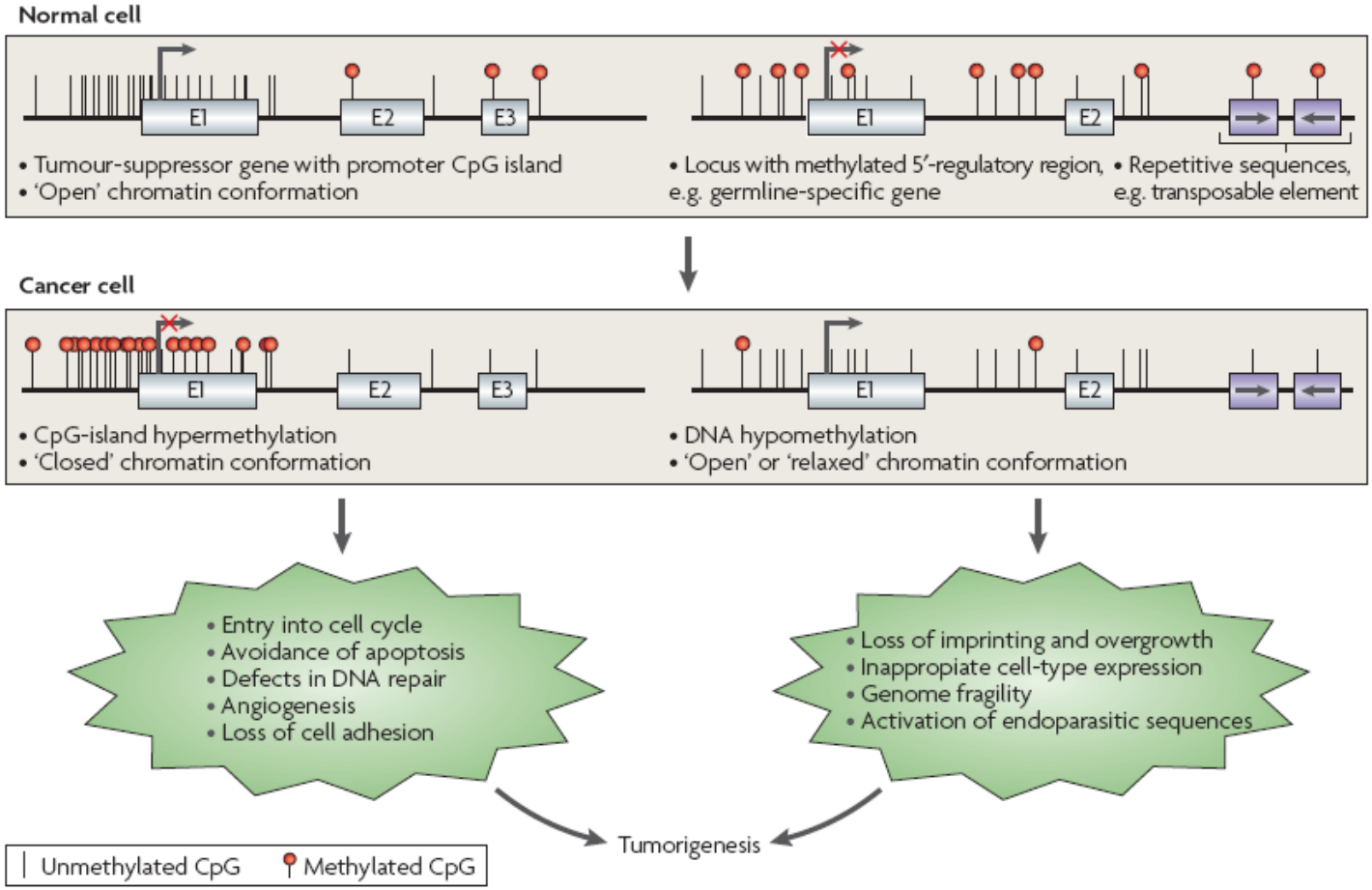


Figure 1 | **Altered DNA-methylation patterns in tumorigenesis.** The hypermethylation of CpG islands of tumour-suppressor genes is a common alteration in cancer cells, and leads to the transcriptional inactivation of these genes and the loss of their normal cellular functions. This contributes to many of the hallmarks of cancer cells. At the same time, the genome of the cancer cell undergoes global hypomethylation at repetitive sequences, and tissue-specific and imprinted genes can also show loss of DNA methylation. In some cases, this hypomethylation is known to contribute to cancer cell phenotypes, causing changes such as loss of imprinting, and might also contribute to the genomic instability that characterizes tumours. E, exon.

Esteller, Nat. Rev. Gen. 8, 286 (2007)

Genomic Imprinting: Mono-allelic expression; one allele (either from the mother or the father) is silenced.

Typically, this is implemented by methylating the silenced allele.

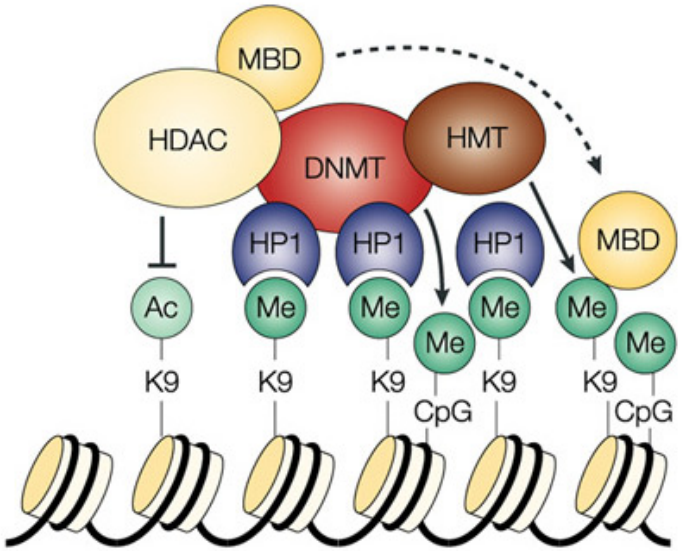
The human genome contains ca. 8% of **retroviral sequences**. Typically, these are also silenced by DNA methylation.

Enzymes that control DNA methylation and histone modfications

These dynamic chromatin states are controlled by reversible epigenetic patterns of **DNA methylation** and **histone modifications**.

Enzymes involved in this process include
- DNA methyltransferases (DNMTs),
- histone deacetylases (HDACs),
- histone acetylases,
- histone methyltransferases (HMT) and the
- methyl-binding domain protein MECP2

with its methyl-binding domain (MBD) that binds specifically to me-cytosine.



HP1: heterochromatin protein 1

Rodenhiser, Mann, CMAJ 174, 341 (2006)
Feinberg AP & Tycko P (2004) Nature Reviews: 143-153

DNA methylation

Typically, unmethylated clusters of CpG pairs are located in
tissue-specific genes and in essential **housekeeping genes.**

(House-keeping genes are involved in routine maintenance roles and are expressed in most tissues.)

These clusters, or **CpG islands**, are targets for proteins
that bind to unmethylated CpGs and initiate gene transcription.

In contrast, **methylated CpGs** are generally associated with silent DNA,
can block methylation-sensitive proteins and can be easily mutated.

The **loss** of normal DNA methylation patterns is the
best understood epigenetic cause of **disease.**

In animal experiments, the removal of genes that encode DNMTs is lethal;
in humans, overexpression of these enzymes has been linked
to a variety of cancers.

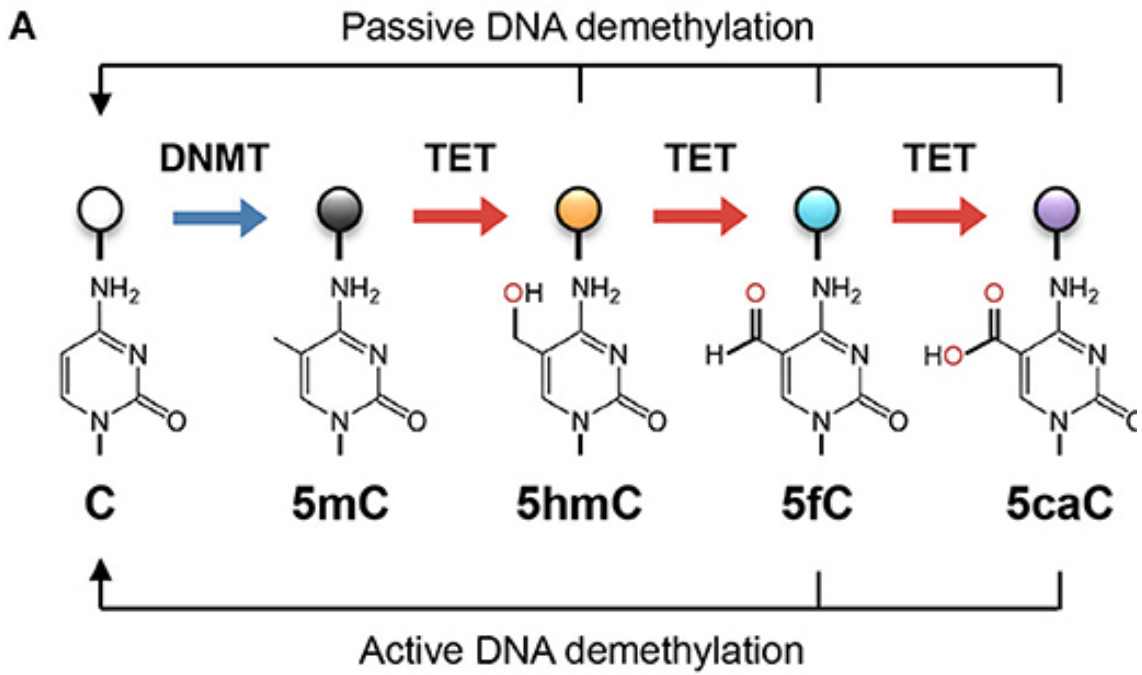
Rodenhiser, Mann, CMAJ 174, 341 (2006)

Higher forms of methylation – Tet enzymes

Unmodified cytosine (C) is methylated by DNA methyltransferases (DNMTs) at the 5 position to become 5-methylcytosine (5mC).

TET proteins oxidize 5mC into 5-hydroxymethylcytosine (5hmC), a stable epigenetic mark, and subsequently to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).

TET can demethylate DNA via replication-dependent (passive) or replication-independent (active) mechanisms.



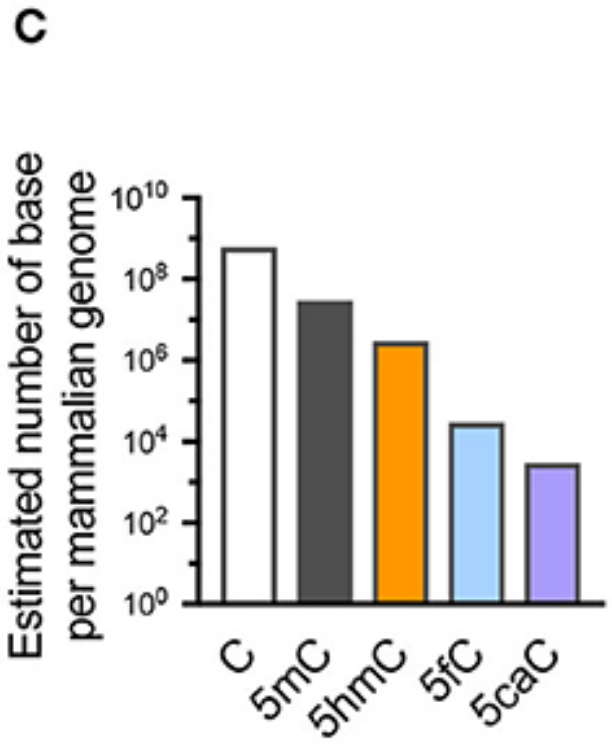
Lio & Rao, Front. Immunol. (2019)

Higher forms of methylation – abundance

The approximate abundance of unmodified and modified cytosines in the haploid human/mouse genome.

About 5% of cytosine is methylated (5mC); in most cells, the vast majority of 5mC is present at CG dinucleotides although it is low at CpG islands.

5hmC amounts to about 1-10% of 5mC (estimated at 10% here as in embryonic stem cells),
while the levels of 5fC and 5caC are each about an order of magnitude lower than the previous oxidative modification.



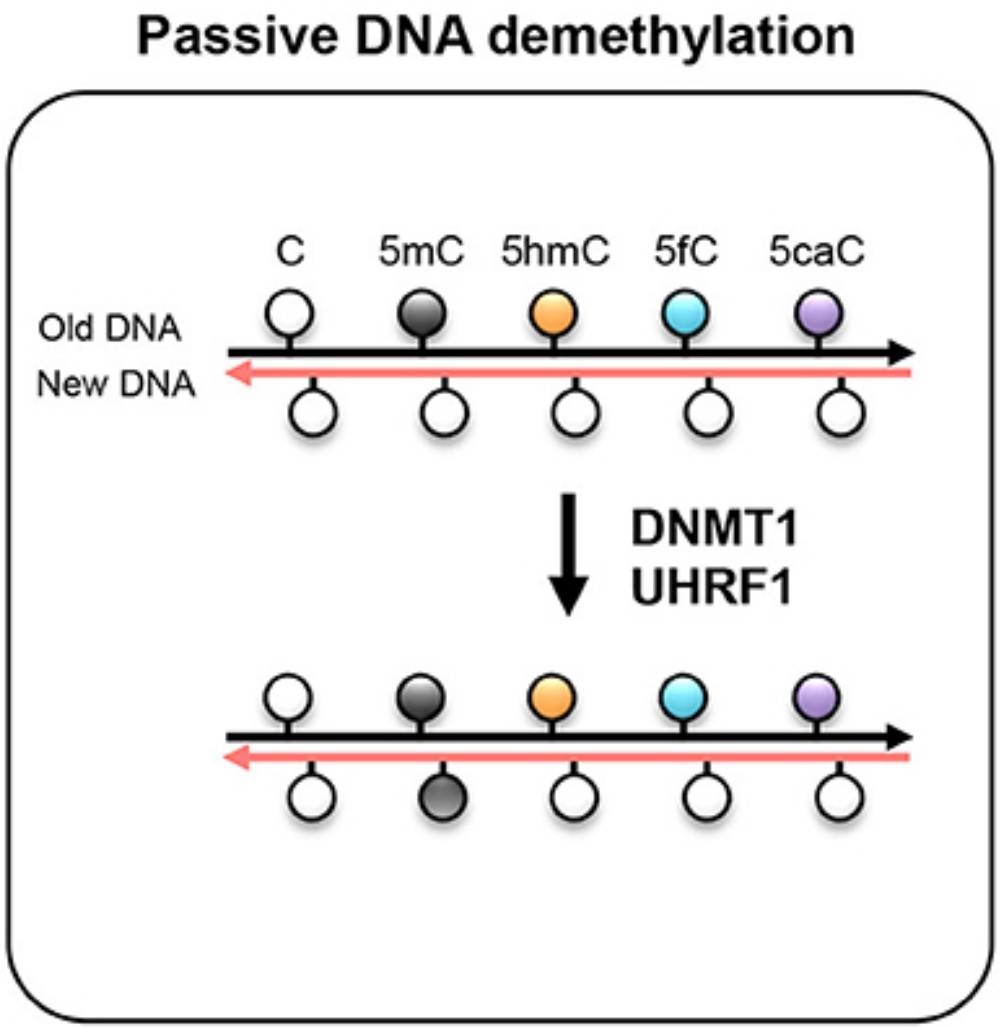
Lio & Rao, Front. Immunol. (2019)

Passive DNA methylation

The DNMT1/UHRF1 complex recognizes 5mC at the hemi-methylated CpG motif during DNA replication and methylates the unmodified cytosine on the newly synthesized DNA strand.

However, the oxidized methylcytosines 5hmC, 5fC, and 5caC are not recognized by DNMT1/UHRF1, resulting in unmodified cytosine on the new DNA strand.

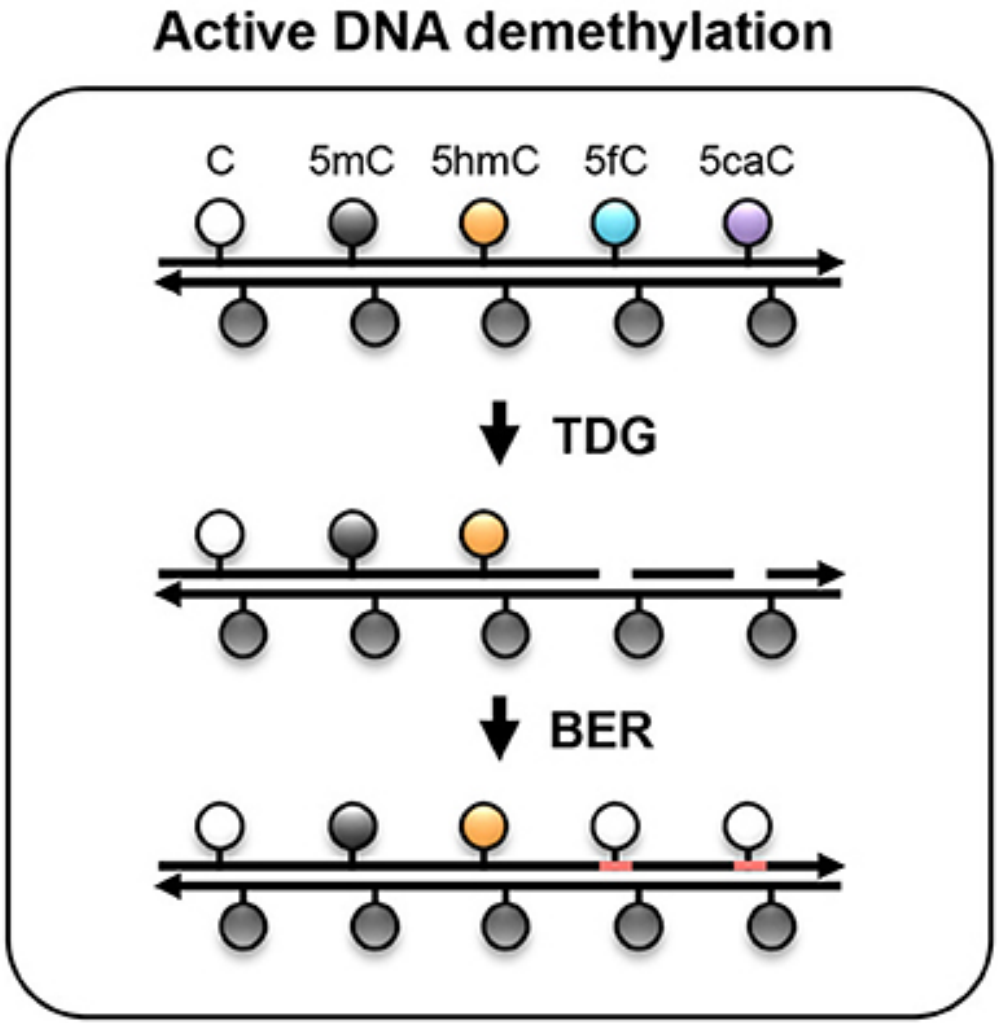
Further DNA replication in the presence of continuing TET activity will result in progressive dilution of 5mC in the daughter cells.



Lio & Rao, Front. Immunol. (2019)

Active DNA methylation

While 5hmC is stable and persists in the genome, 5fC and 5caC can be recognized and **excised** by thymine DNA glycosylase (TDG),
and the resulting abasic sites are repaired as unmodified C by base excision repair (BER).



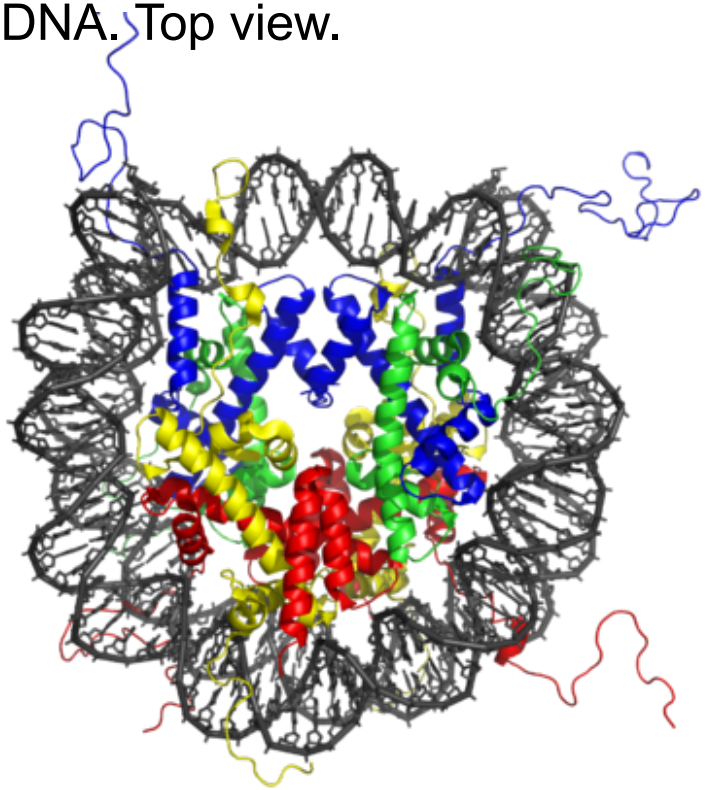
Lio & Rao, Front. Immunol. (2019)

(review V2) The histone code

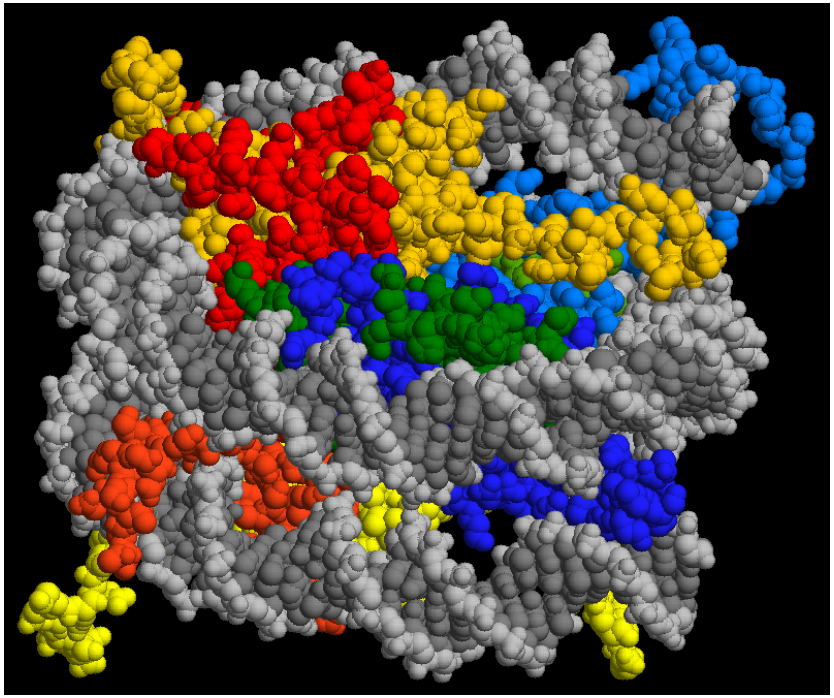
The DNA of eukaryotic organisms is packaged into chromatin, whose basic repeating unit is the **nucleosome**.

A nucleosome is formed by wrapping 147 base pairs of DNA twice around an octamer of four core histones, **H2A** , **H2B** , **H3** and **H4** (2 copies of each one).

X-ray structure of the nucleosome core particle consisting of core histones, and DNA. Top view.



Side view shows two windings of DNA and two histone layers



www.wikipedia.org

(review V2) Post-translational modifications of histone tails

The disordered histone tails comprise 25-30% of the histone mass.

They extend from the compact histone multimer to provide a platform for various **post-translational modifications (PTMs)**.

These modifications affect the histones' ability to bind DNA and to other histones.

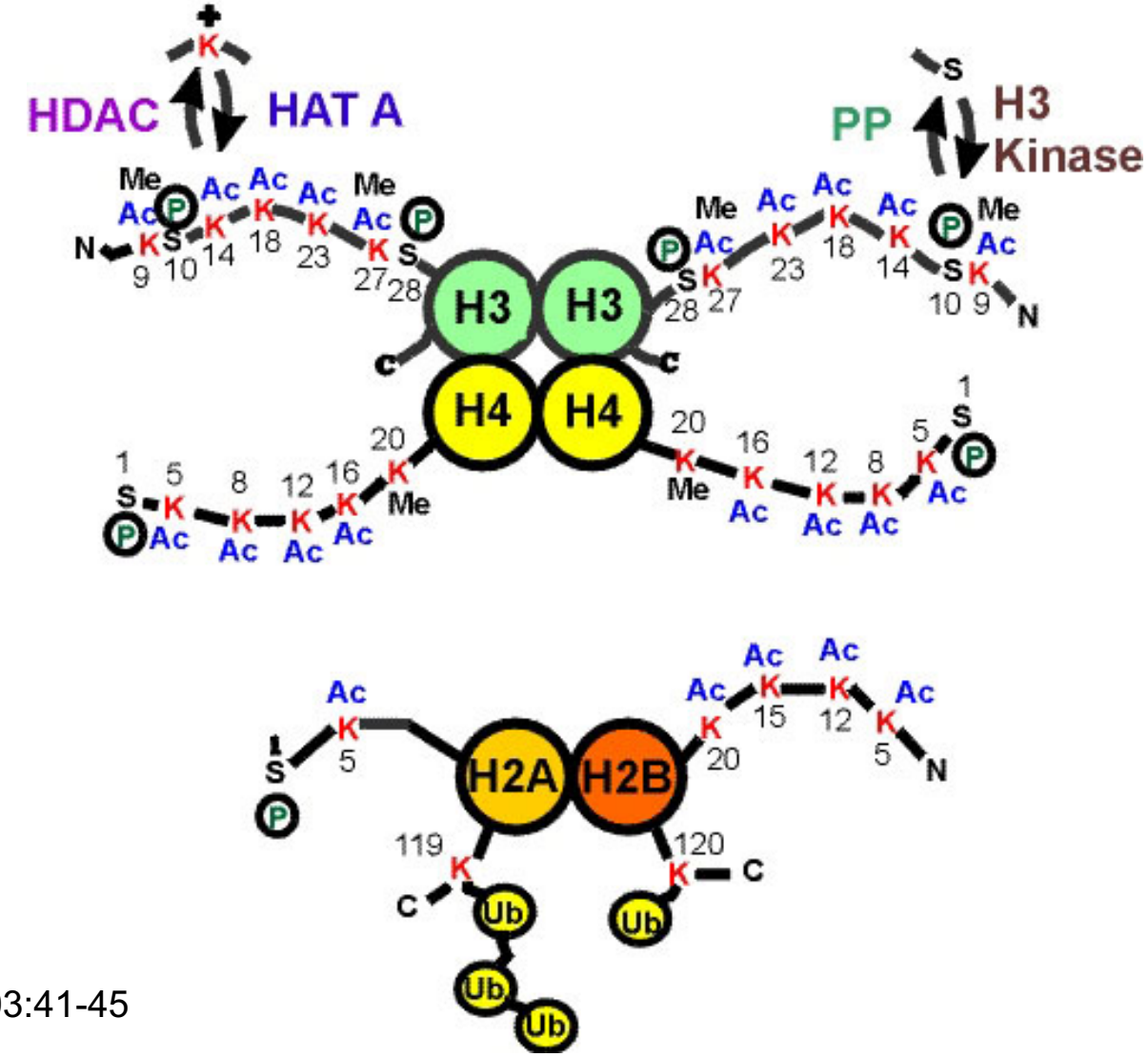
This, in turn, affects **gene expression.**

*ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS**

By V. G. Allfrey, R. Faulkner, and A. E. Mirsky

THE ROCKEFELLER INSTITUTE

PNAS 1964;51:786
First report on PTMs of histones



Strahl BD and Allis CD, 2000. Nature 403:41-45

Mode of action of histone PTMs

Histone PTMs exert their effects via two main mechanisms.

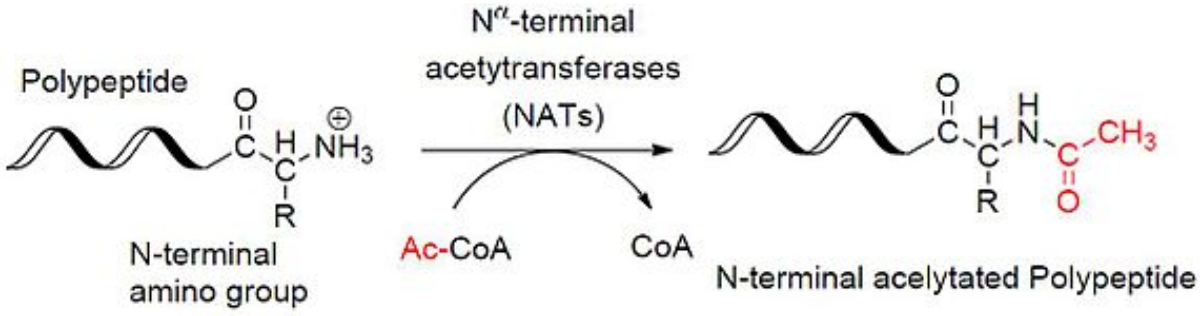
(1) PTMs directly influence the overall structure of chromatin, either over short or long distances.

(2) PTMs regulate (either positively or negatively) the binding of effector molecules.

Bannister, Kouzarides, Cell Res. (2011) 21: 381–395.

PTMs of histone tails

Histone **acetylation** and **phosphorylation** effectively reduce the positive charge of histones.



This potentially disrupts electrostatic interactions between histones and DNA.

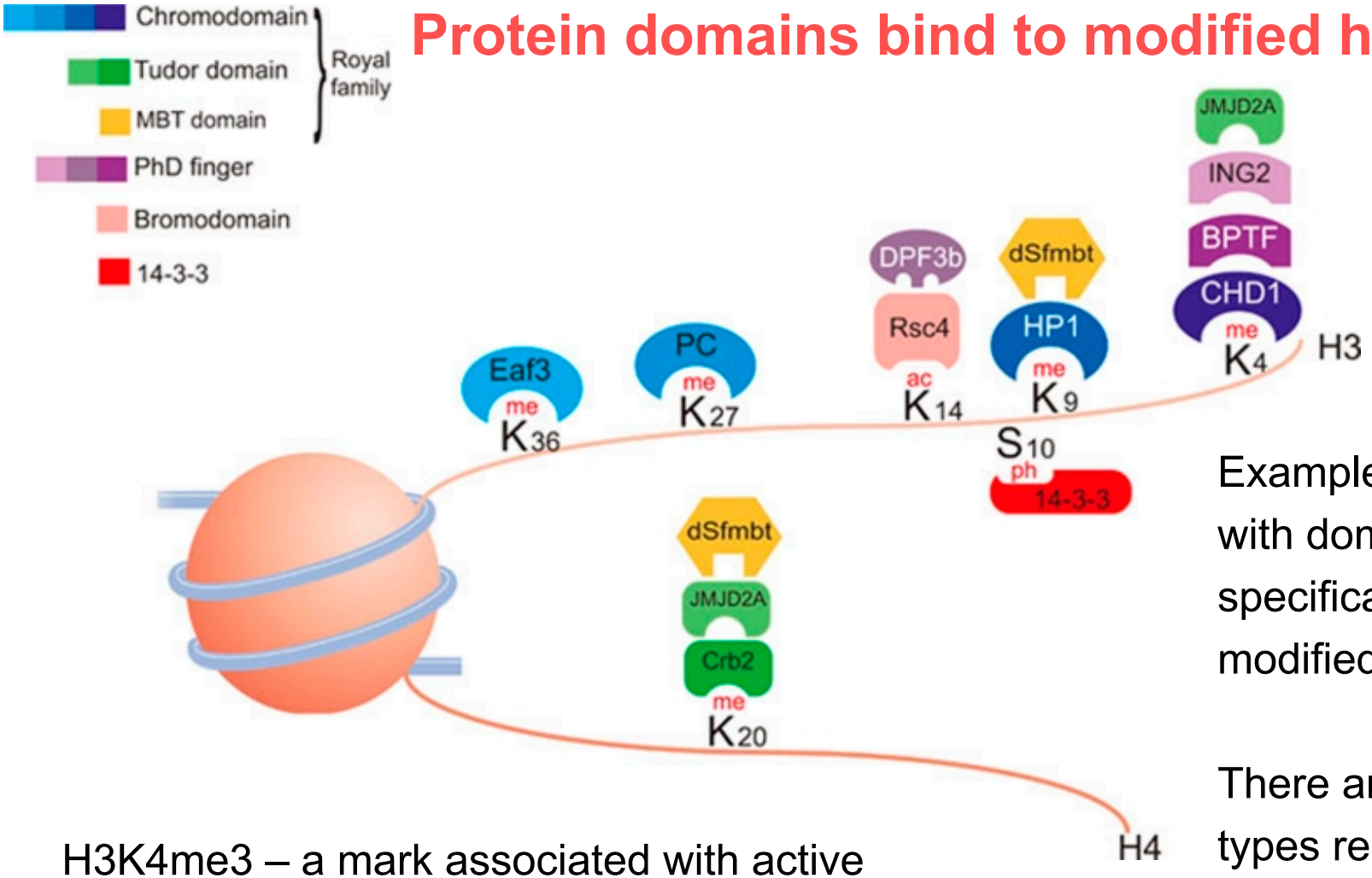
This presumably leads to a less compact chromatin structure, thereby facilitating DNA access by protein machineries such as those involved in transcription.

Histone **methylation** mainly occurs on the side chains of lysines and arginines.

Unlike acetylation and phosphorylation, however, histone methylation does not alter the charge of the histone protein.

Bannister, Kouzarides, Cell Res. (2011) 21: 381–395.
By Ybs.Umich - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=31240656

Protein domains bind to modified histones



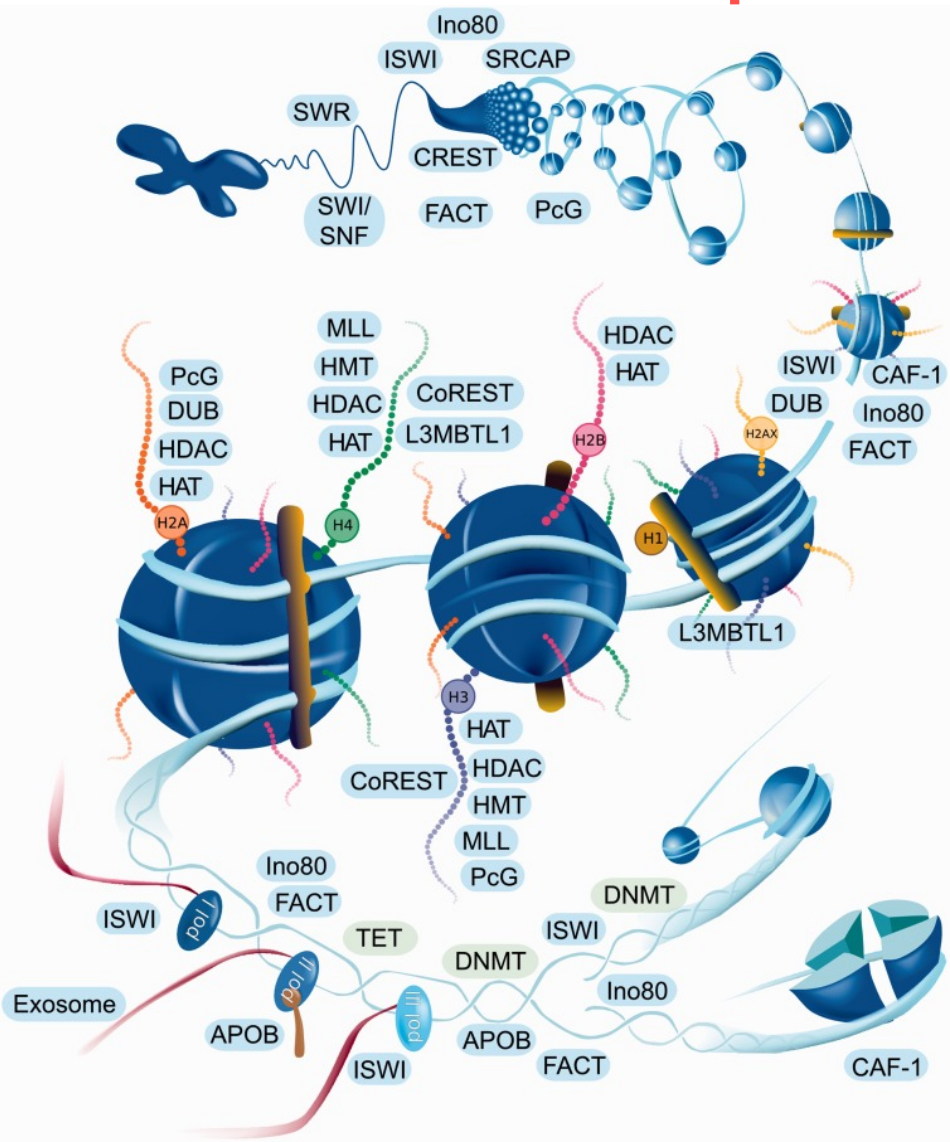
H3K4me3 – a mark associated with active transcription – is recognized by a PHD finger within the ING family of proteins (ING1-5). The ING proteins in turn recruit additional chromatin modifiers such as HATs and HDACs.

Examples of proteins with domains that specifically bind to modified histones.

There are more domain types recognizing lysine methylation than any other PTM.

Bannister, Kouzarides
Cell Res. (2011) 21: 381–395.

Epifactors database



Side view shows two windings of DNA and two histone layers

The database EpiFactors stores detailed and curated information about 815 proteins and 69 complexes involved in epigenetic regulation.

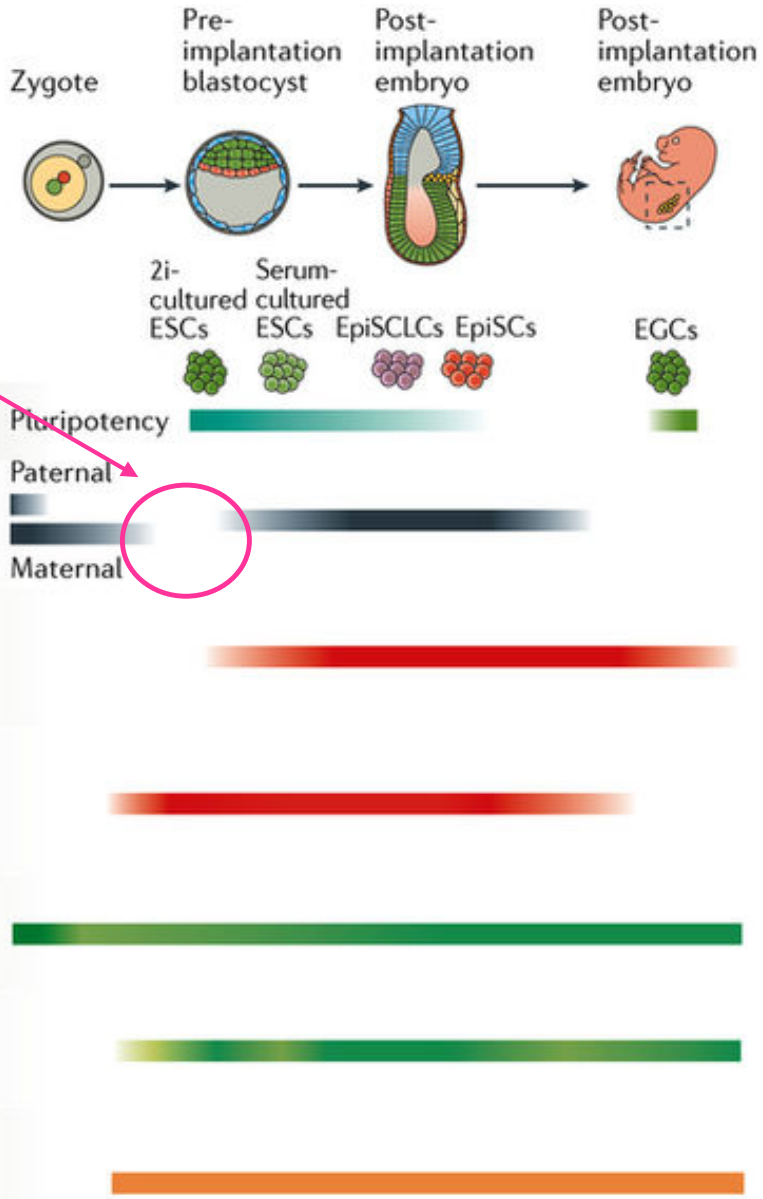
http://epifactors.autosome.ru/protein_complexes

Database (Oxford). 2015; 2015: bav067.

Dynamics of epigenetic modifications

DNA methylation is erased in the paternal and maternal genomes after fertilization and is put back on at later developmental stages.

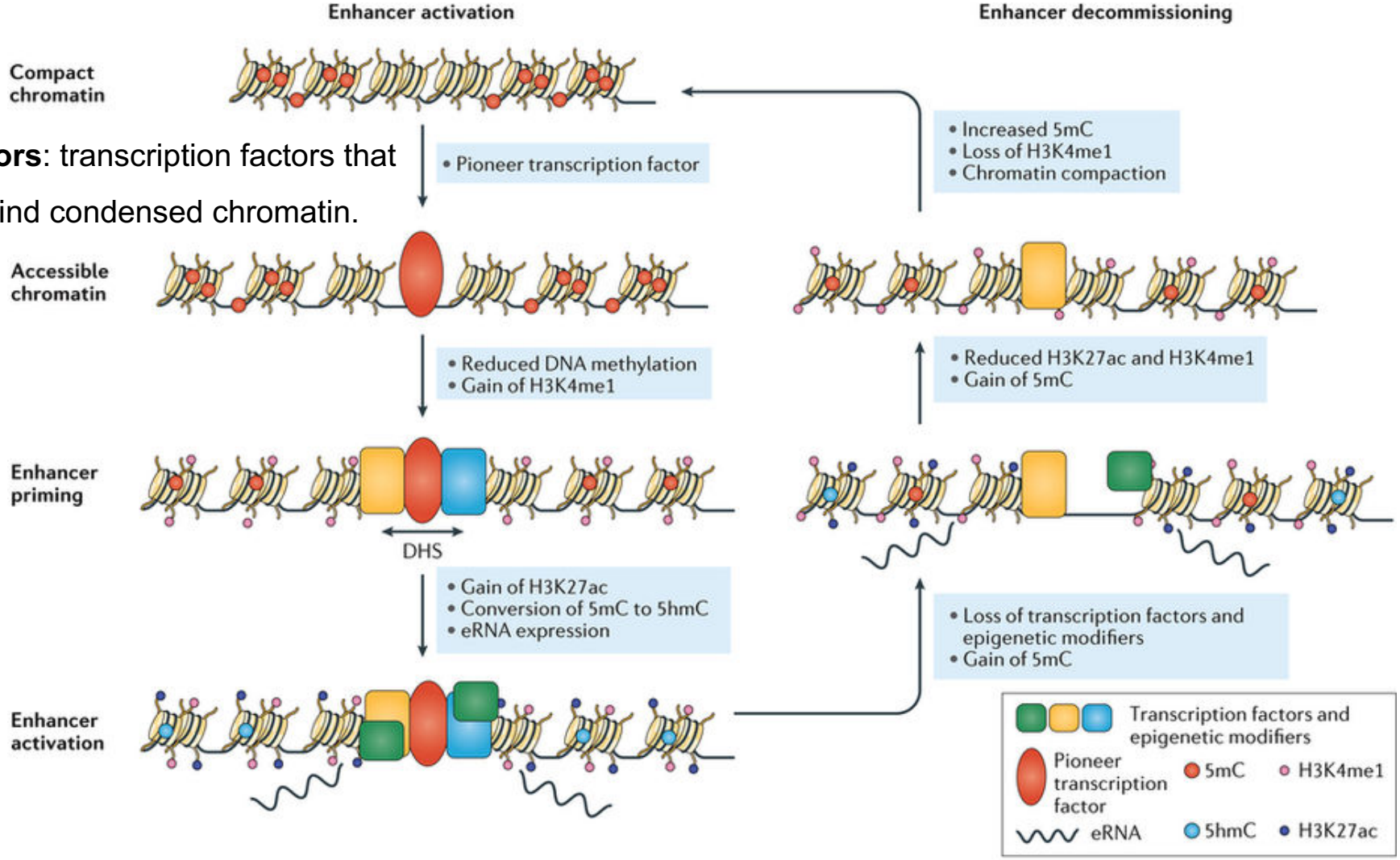
Chromatin modification	Writers	Erasers	Location	Function
DNA methylation	DNMT1, DNMT3A and DNMT3B	TET1, TET2 and TET3	CpG dinucleotides	Silencing and others
H3K27me3	PRC2	• UTX1 • JMJD3	CpG-rich promoters and intergenic regions	Silencing
H3K9me2	G9A and GLP	• JMJD2A, JMJD2B, JMJD2C and JMJD2D • JMJD1A, JMJD1B and JMJD1C	Gene bodies, intergenic regions and enhancers	Silencing
H3K4me3	COMPASS-like proteins (SET1, MLL1–MLL2)	• JARID1A, JARID1B, JARID1C and JARID1D • KDM2B	Mainly promoters	Possibly activating
H3K27ac	HATs (including CBP/p300, GNATs and MYSTs)	HDACs and sirtuins	Promoters and enhancers	Activating
H3K4me1	COMPASS-like proteins (MLL3–MLL4)	LSD1 and LSD2	Promoters, enhancers and intergenic regions	Priming and/or activating



Atlasi & Stunnenberg, *Nature Rev Genet* **18**, 643–658 (2017)

Events during enhancer activation / decommissioning

Pioneer factors: transcription factors that can directly bind condensed chromatin.



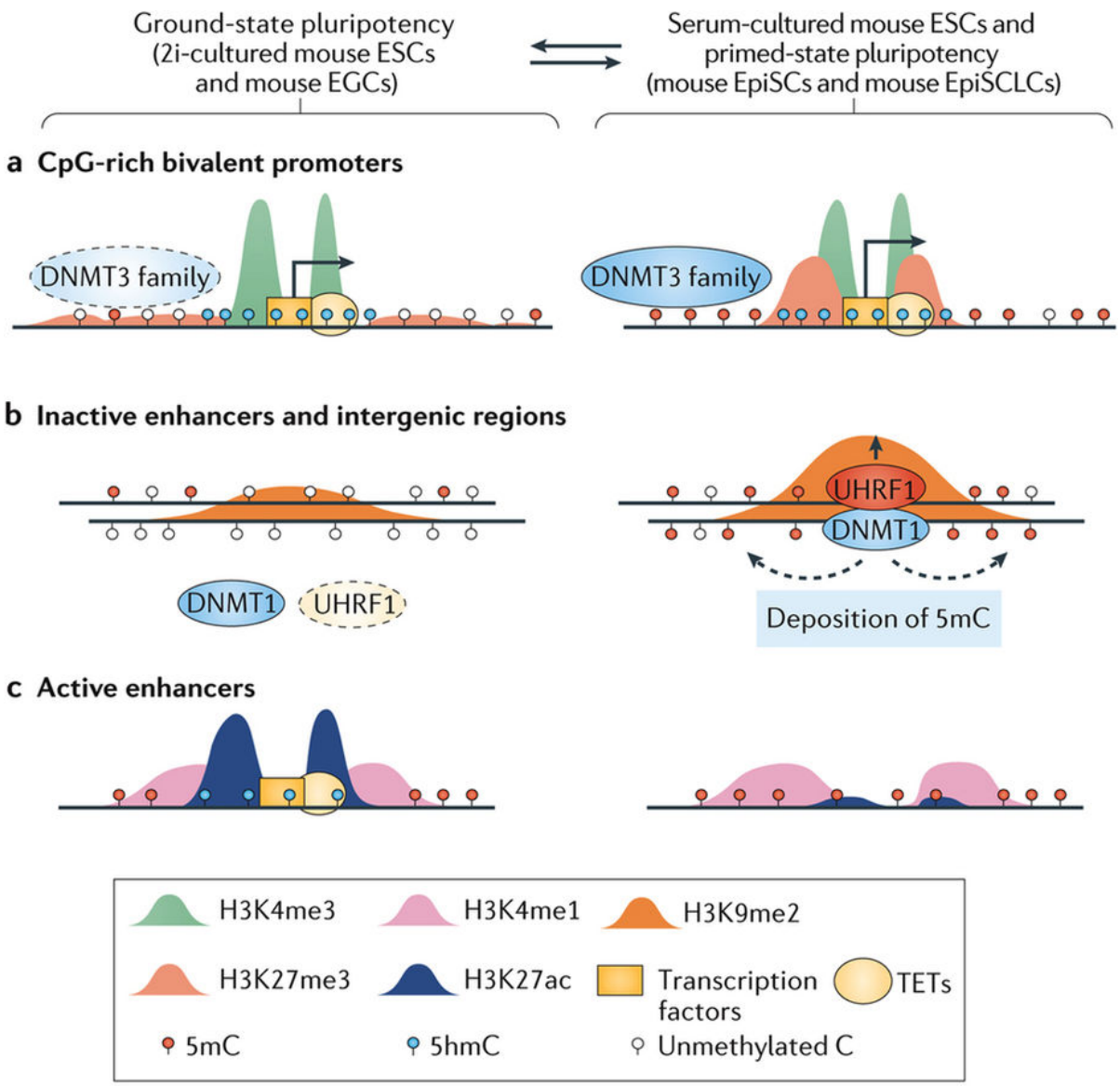
5mC: 5-methyl-cytosine

5hmC: 5-hydroxy-methyl-cytosine

Nature Reviews | Genetics

Atlasi & Stunnenberg, *Nature Rev Genet* **18**, 643–658 (2017)

Interplay between DNA methylation and histone modifications



Bivalent chromatin are segments of DNA, bound to histone proteins, that have both repressing and activating epigenetic regulators in the same region. These regulators work to enhance or silence the expression of genes. Since these regulators work in opposition to each other, they normally interact with chromatin at different times. However, in bivalent chromatin, both types of regulators are interacting with the same domain at the same time. Bivalent chromatin domains are normally associated with promoters of transcription factor genes that are expressed at low levels. Bivalent domains have also been found to play a role in developmental regulation in pluripotent embryonic stems cells, as well as gene imprinting.

Atlasi & Stunnenberg, *Nature Rev Genet* **18**, 643–658 (2017)

www.wikipedia.org

Paper #8

An Intrinsic Epigenetic Barrier for Functional Axon Regeneration

Yi Lan Weng, Ran An, Jessica Cassin, Jessica Joseph, Ruifa Mi, Chen Wang, Chun Zhong, Seung-Gi Jin, Gerd P. Pfeifer, Alfonso Bellacosa, Xinzhong Dong, Ahmet Hoke, Zhigang He, Hongjun Song, Guo-li Ming*

Neuron 94, 337-346.e6 (2017)

Paper presentation June 25, 2019

see also
Scarlett J. Barker, Li-Huei Tsai
MethyLock: DNA Demethylation Is the Epigenetic Key to Axon Regeneration
Neuron, 94, 221-223 (2017)