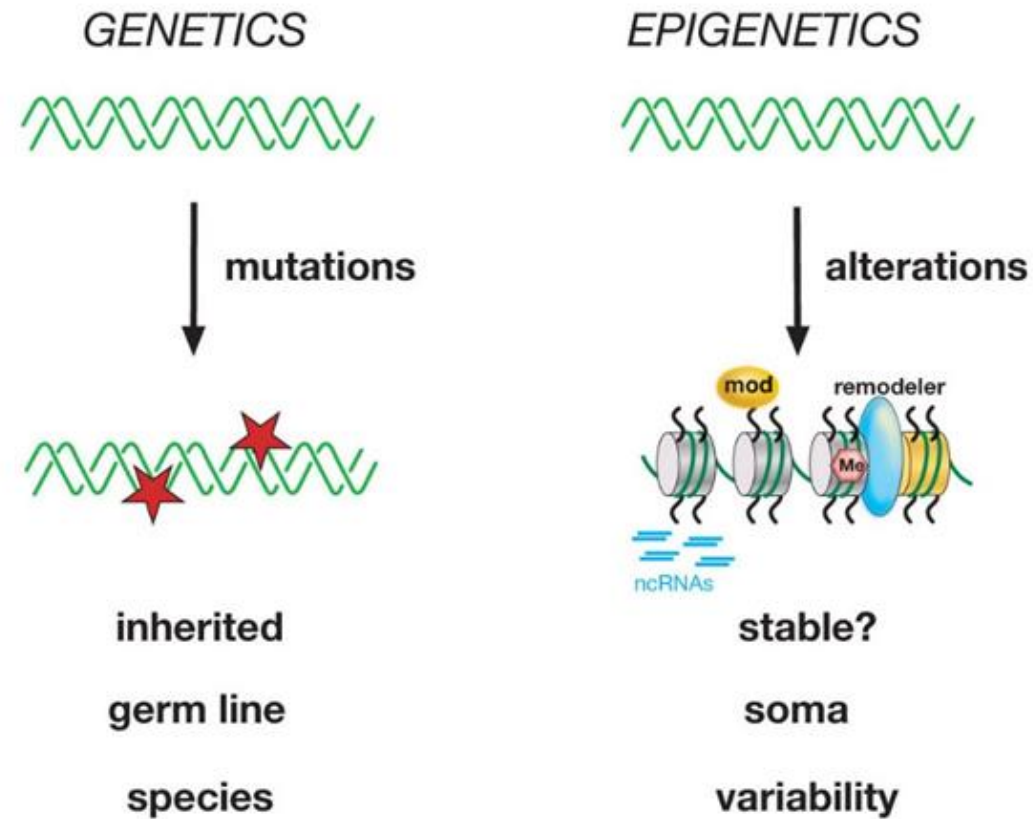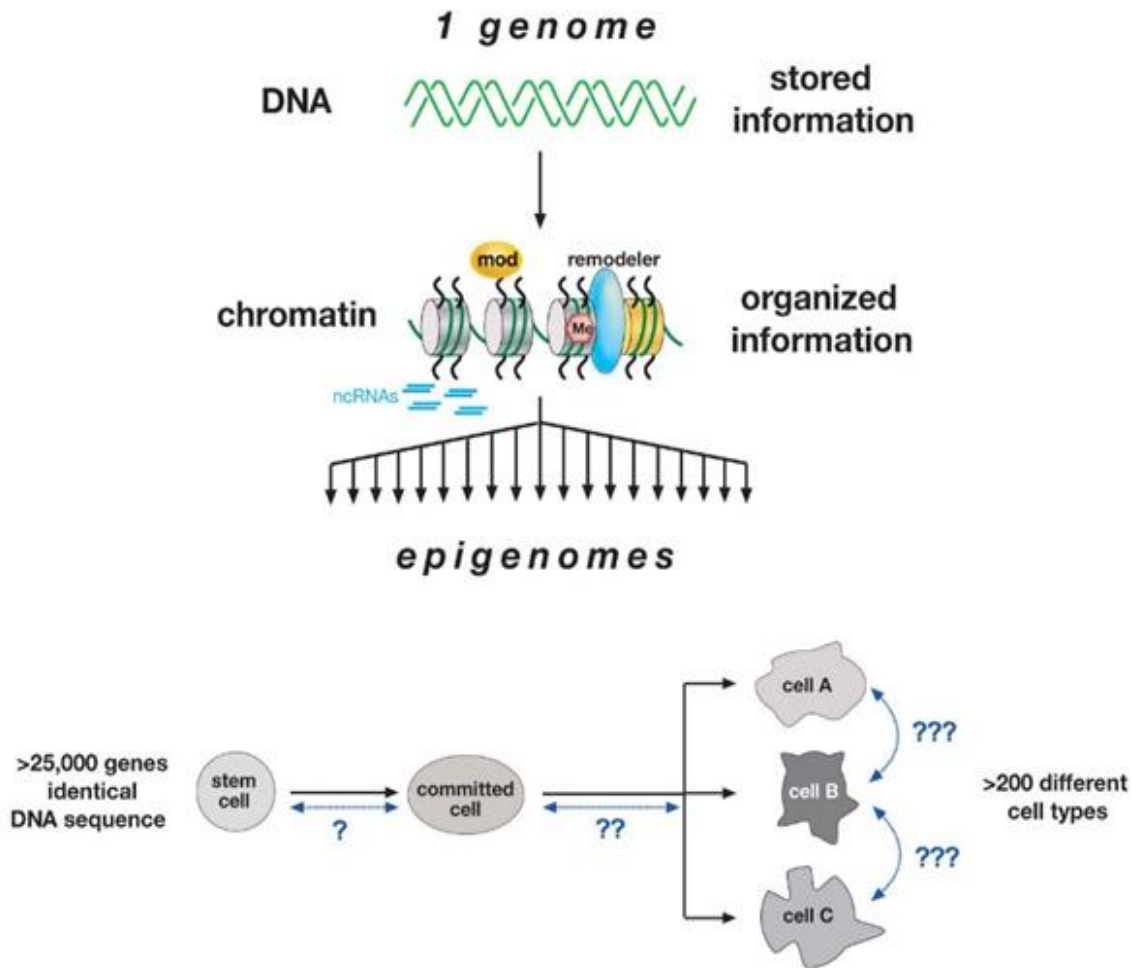# PR11_Epigenomika/Epigenomics

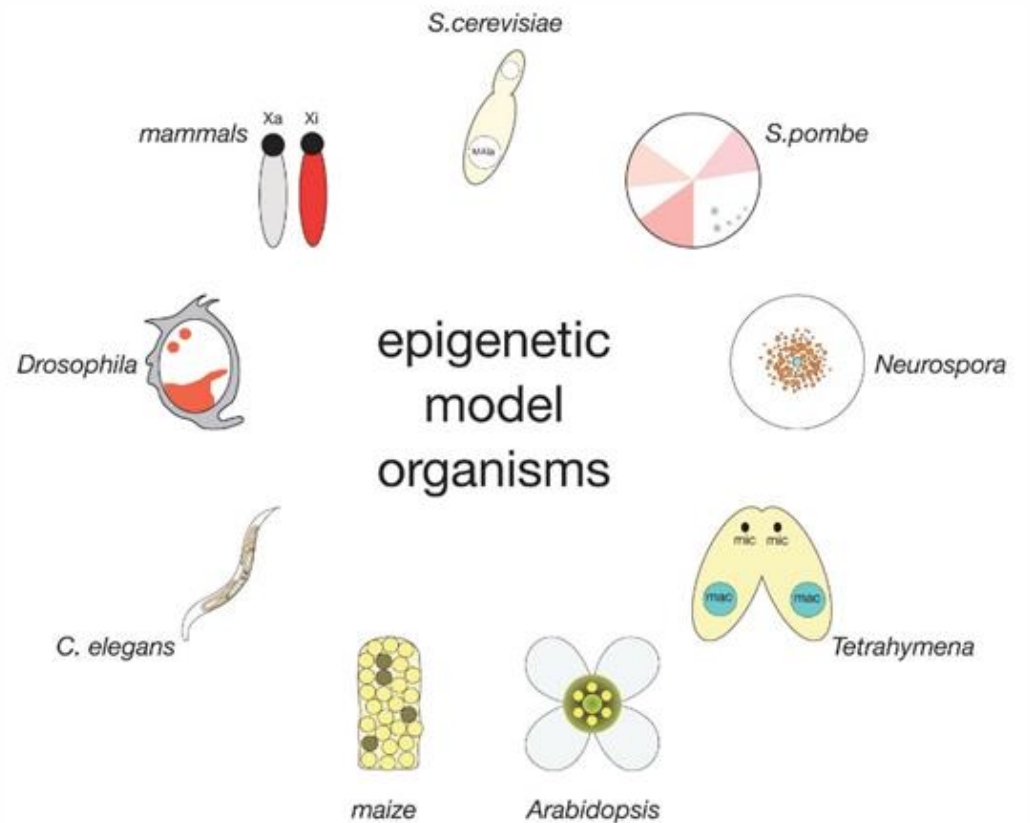# Introduction to the study of epigenetics/ Uvod v epigenetiko

Figure 3. Genetics Versus Epigenetics

GENETICS: Mutations (*red stars*) of the DNA template (*green helix*) are heritable somatically and through the germ line. *EPIGENETICS:* Variations in chromatin structure modulate the use of the genome by (1) histone modifications (mod), (2) chromatin remodeling (*remodeler*), (3) histone variant composition (*yellow nucleosome*), (4) DNA methylation (*Me*), and (5) noncoding RNAs. Marks on the chromatin template may be heritable through cell division and collectively contribute to determining cellular phenotype.

1 genome

DNA — stored information

chromatin — organized information

ncRNAs

epigenomes

>25,000 genes identical DNA sequence

stem cell → committed cell → cell A / cell B / cell C

>200 different cell types

Figure 4. DNA Versus Chromatin

The genome: Invariant DNA sequence (*green double helix*) of an individual. The epigenome: The overall chromatin composition, which indexes the entire genome in any given cell. It varies according to cell type, and response to internal and external signals it receives. (*Lower panel*) Epigenome diversification occurs during development in multicellular organisms as differentiation proceeds from a single stem cell (the fertilized embryo) to more committed cells. Reversal of differentiation or transdifferentiation (*blue lines*) requires the reprogramming of the cell's epigenome.

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mammals — Xa Xi

S.cerevisiae

S.pombe

Drosophila

Neurospora

epigenetic model organisms

C. elegans

maize

Arabidopsis

Tetrahymena

Figure 2. Model Organisms Used in Epigenetic Research
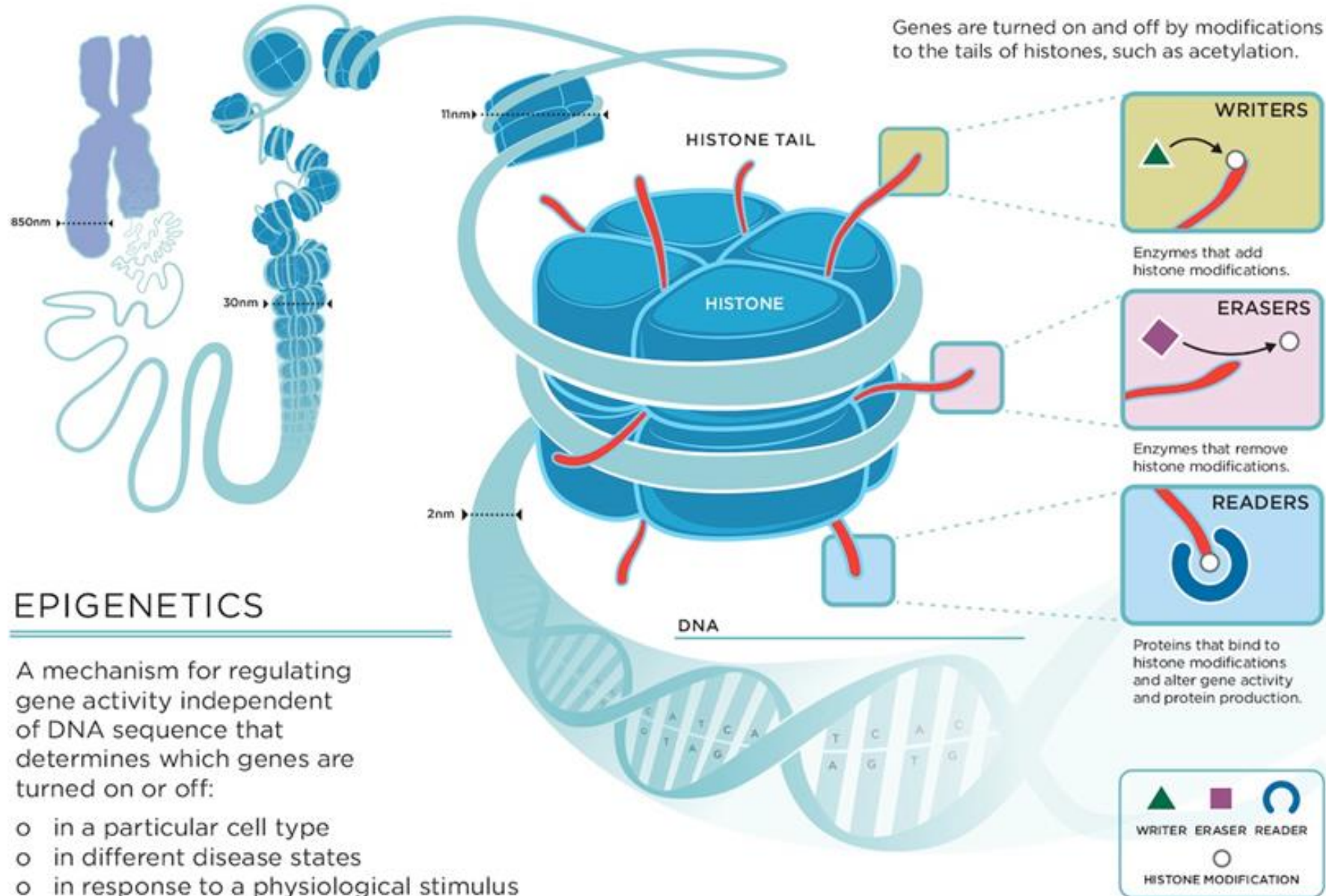
Schematic representation of model organisms used in epigenetic research. *S. cerevisiae*: Mating-type switching to study epigenetic chromatin control. *S. pombe*: Variegated gene silencing manifests as colony sectoring. *Neurospora crassa*: Epigenetic genome defense systems include repeat-induced point mutation, quelling, and meiotic silencing of unpaired DNA, revealing an interplay between RNAi pathways, DNA and histone methylation. *Tetrahymena*: Chromatin in somatic and germ-line nuclei are distinguished by epigenetically regulated mechanisms. *Arabidopsis*: Model for repression by DNA, histone, and RNA-guided silencing mechanisms. *Maize*: Model for imprinting, paramutation, and transposon-induced gene silencing. *C. elegans*: Epigenetic regulation in the germ line. *Drosophila*: Position-effect variegation (PEV) manifest by clonal patches of expression and silencing of the white gene in the eye. *Mammals*: X-chromosome inactivation.

Epigenetics © 2006 Cold Spring Harbor Laboratory Press
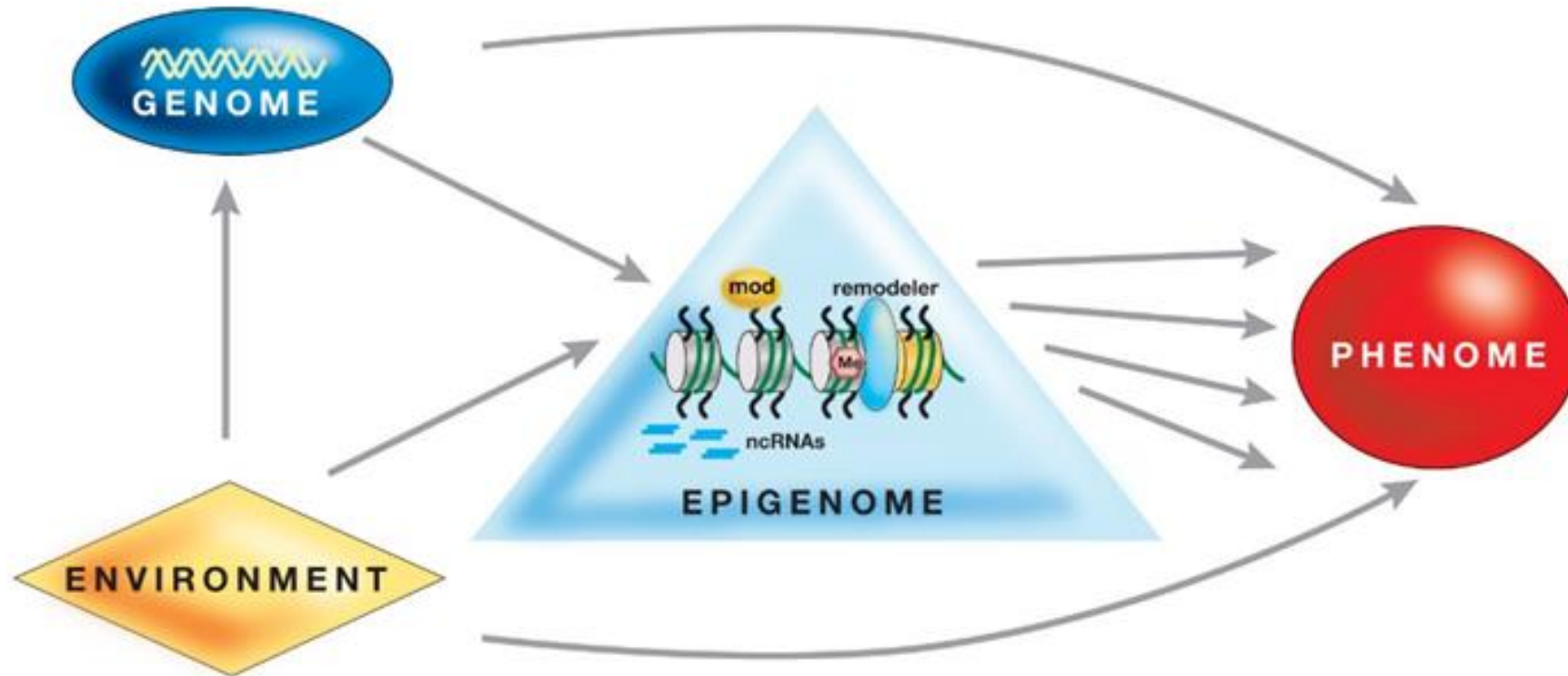
Figure 7. The Epigenotype Plays a Critical Role along with the Genotype and Environmental Factors in Determining Phenotypes

Known epigenetic factors affecting gene expression and genome stability include DNA methylation, chromatin-remodeling complexes, covalent histone modifications, the presence of histone variants, or noncoding regulatory RNAs (ncRNA).
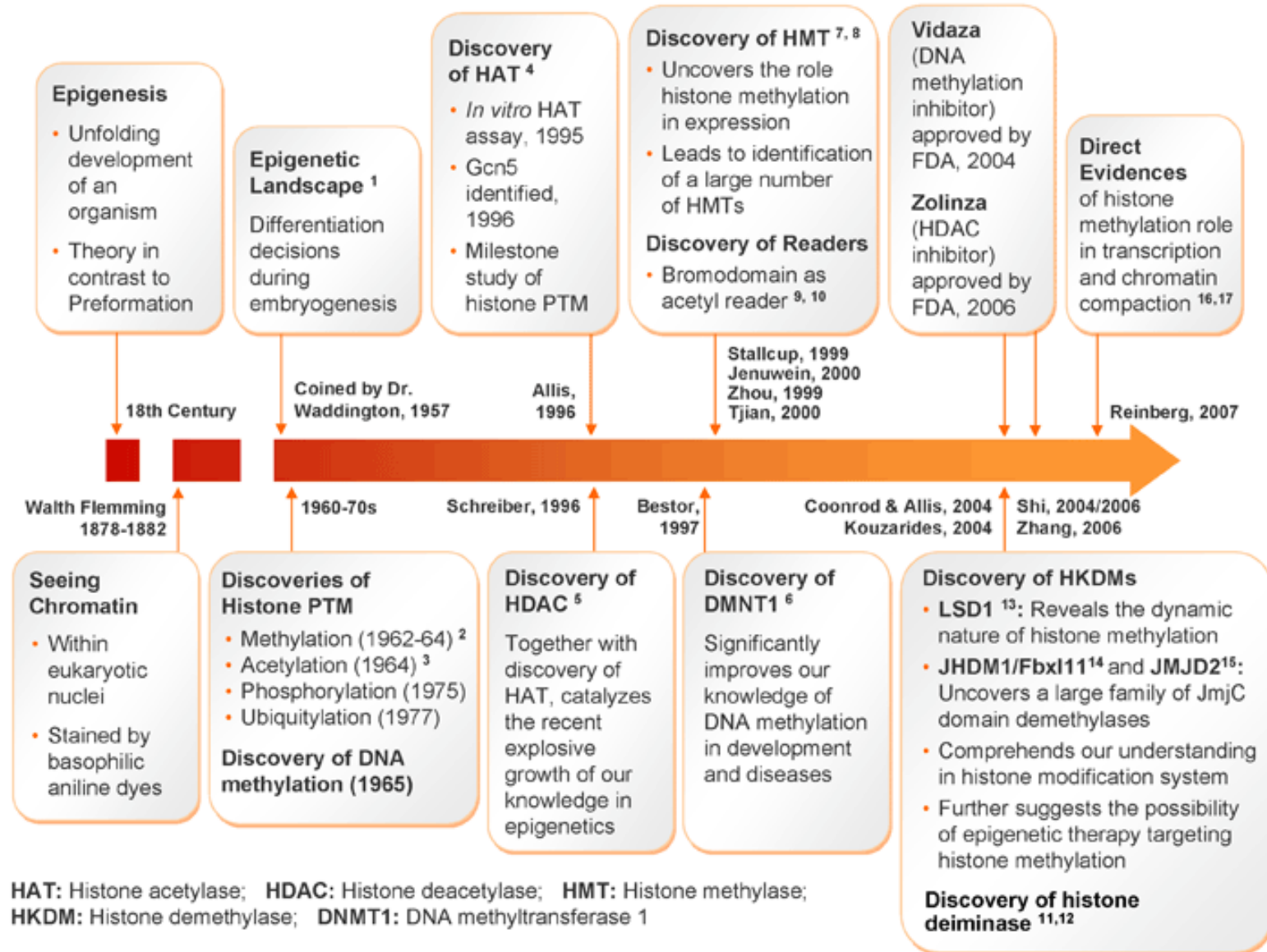
CHROMOSOME CHROMATIN FIBRE NUCLEOSOME

Genes are turned on and off by modifications to the tails of histones, such as acetylation.

11nm

850nm

30nm

2nm

HISTONE TAIL

HISTONE

DNA

WRITERS

Enzymes that add histone modifications.

ERASERS

Enzymes that remove histone modifications.

READERS

Proteins that bind to histone modifications and alter gene activity and protein production.

EPIGENETICS

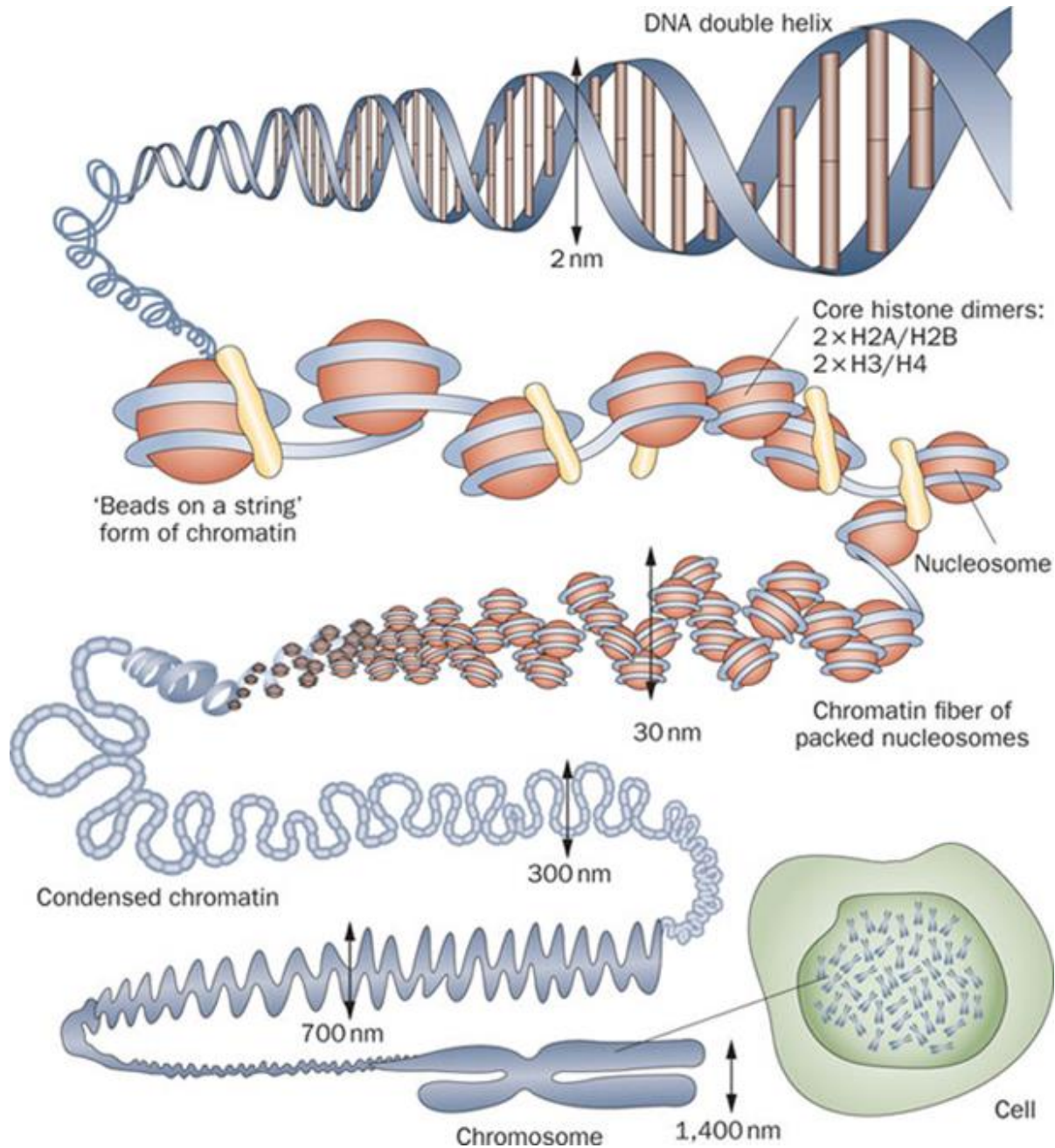A mechanism for regulating gene activity independent of DNA sequence that determines which genes are turned on or off:

o in a particular cell type
o in different disease states
o in response to a physiological stimulus

WRITER ERASER READER

HISTONE MODIFICATION

Brief history of epigenetics

Epigenesis
- Unfolding development of an organism
- Theory in contrast to Preformation

Epigenetic Landscape [1]

Differentiation decisions during embryogenesis

Discovery of HAT [4]
- *In vitro* HAT assay, 1995
- Gcn5 identified, 1996
- Milestone study of histone PTM

Discovery of HMT [7, 8]
- Uncovers the role histone methylation in expression
- Leads to identification of a large number of HMTs

Discovery of Readers
- Bromodomain as acetyl reader [9, 10]

Vidaza (DNA methylation inhibitor) approved by FDA, 2004

Zolinza (HDAC inhibitor) approved by FDA, 2006

Direct Evidences of histone methylation role in transcription and chromatin compaction [16,17]

18th Century

Coined by Dr. Waddington, 1957

Allis, 1996

Stallcup, 1999
Jenuwein, 2000
Zhou, 1999
Tjian, 2000

Reinberg, 2007

Walth Flemming 1878-1882

1960-70s

Schreiber, 1996

Bestor, 1997

Coonrod & Allis, 2004
Kouzarides, 2004

Shi, 2004/2006
Zhang, 2006

Seeing Chromatin
- Within eukaryotic nuclei
- Stained by basophilic aniline dyes

Discoveries of Histone PTM
- Methylation (1962-64) [2]
- Acetylation (1964) [3]
- Phosphorylation (1975)
- Ubiquitylation (1977)

Discovery of DNA methylation (1965)

Discovery of HDAC [5]

Together with discovery of HAT, catalyzes the recent explosive growth of our knowledge in epigenetics

Discovery of DMNT1 [6]

Significantly improves our knowledge of DNA methylation in development and diseases

Discovery of HKDMs
- LSD1 [13]: Reveals the dynamic nature of histone methylation
- JHDM1/Fbxl11[14] and JMJD2[15]: Uncovers a large family of JmjC domain demethylases
- Comprehends our understanding in histone modification system
- Further suggests the possibility of epigenetic therapy targeting histone methylation

Discovery of histone deiminase [11,12]

HAT: Histone acetylase;   HDAC: Histone deacetylase;   HMT: Histone methylase;
HKDM: Histone demethylase;   DNMT1: DNA methyltransferase 1

DNA packaging and chromatin architecture/ Pakiranje DNA in kromatinska arhitektura

DNA double helix

2 nm

Core histone dimers:
2 × H2A/H2B
2 × H3/H4

'Beads on a string' form of chromatin

Nucleosome

Chromatin fiber of packed nucleosomes

30 nm

Condensed chromatin

300 nm

700 nm

Chromosome

1,400 nm

Cell

Organizational network of chromatin in the cell

Chromatin is DNA that is complexed to histone and nonhistone nuclear proteins and condenses to form a chromosome (approximately 1,400 nm in width). The condensed chromatin (approximately 700 nm in diameter) is composed of much finer chromatin (300 nm in diameter) and also nucleosomes (30 nm in diameter) that are used to package the genome into the cell nucleus. The core particle of the nucleosomes is composed of 147 bp of genomic DNA (2 nm in diameter) wrapped around a histone octamer that consists of two copies of the major types of histones (H2A, H2B, H3 and H4), which have varying functions.

Histone variants
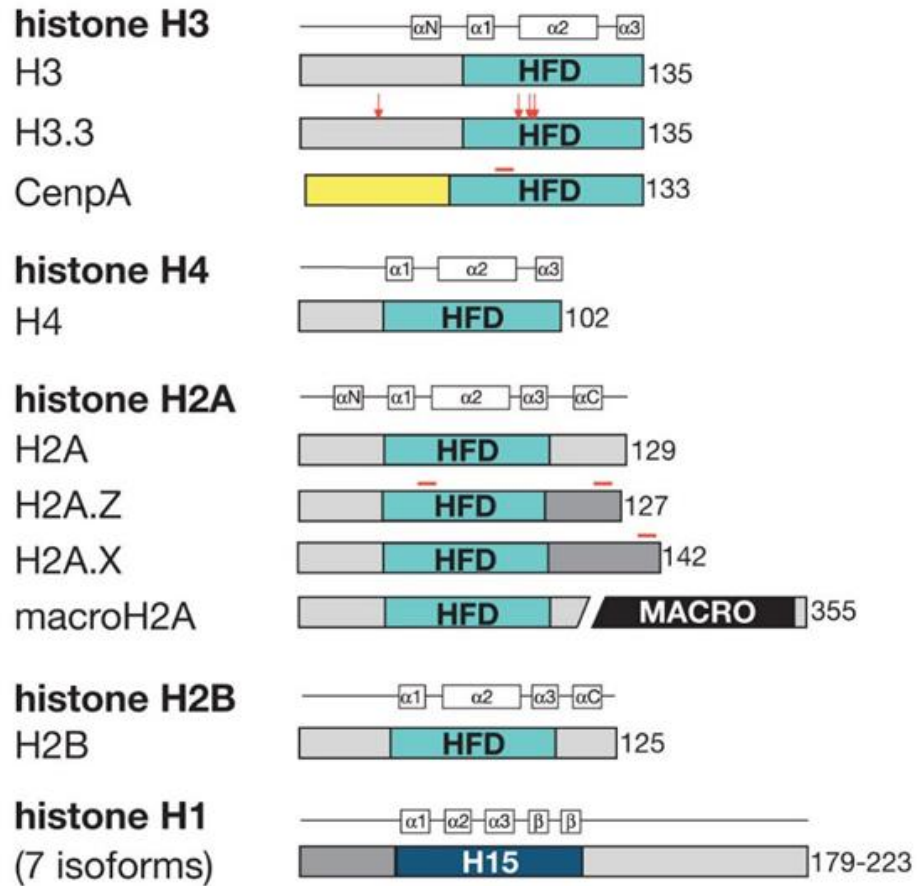
Figure 12. Histone Variants

Protein domain structure for the core histones (H3, H4, H2A, H2B), linker histone H1, and variants of histones H3 and H2A. The histone fold domain (HFD) where histone dimerization occurs, and regions of the protein that differ in histone variants (shown in *red*) are indicated.

Several variant histones are deposited independently of replication at particular regions of chromosomes. Such histone variants include cenH3, which forms the nucleosomal foundation for the centromere, and H3.3, which replaces histones that are lost during dynamic processes that disrupt nucleosomes. Furthermore, various H2A variants participate in DNA repair, gene regulation and other processes.

The incorporation of histone variants is particularly important to confer variations in chromatin structure to ensure dynamic patterns of transcriptional regulation in eukaryotes. Recent knockdown and knockout studies in various cellular systems, as well as direct mutational evidence from human cancers, now suggest a crucial role for histone variant regulation in processes as diverse as differentiation and proliferation, meiosis and nuclear reprogramming.

Nucleosome with histone posttranslational modifications
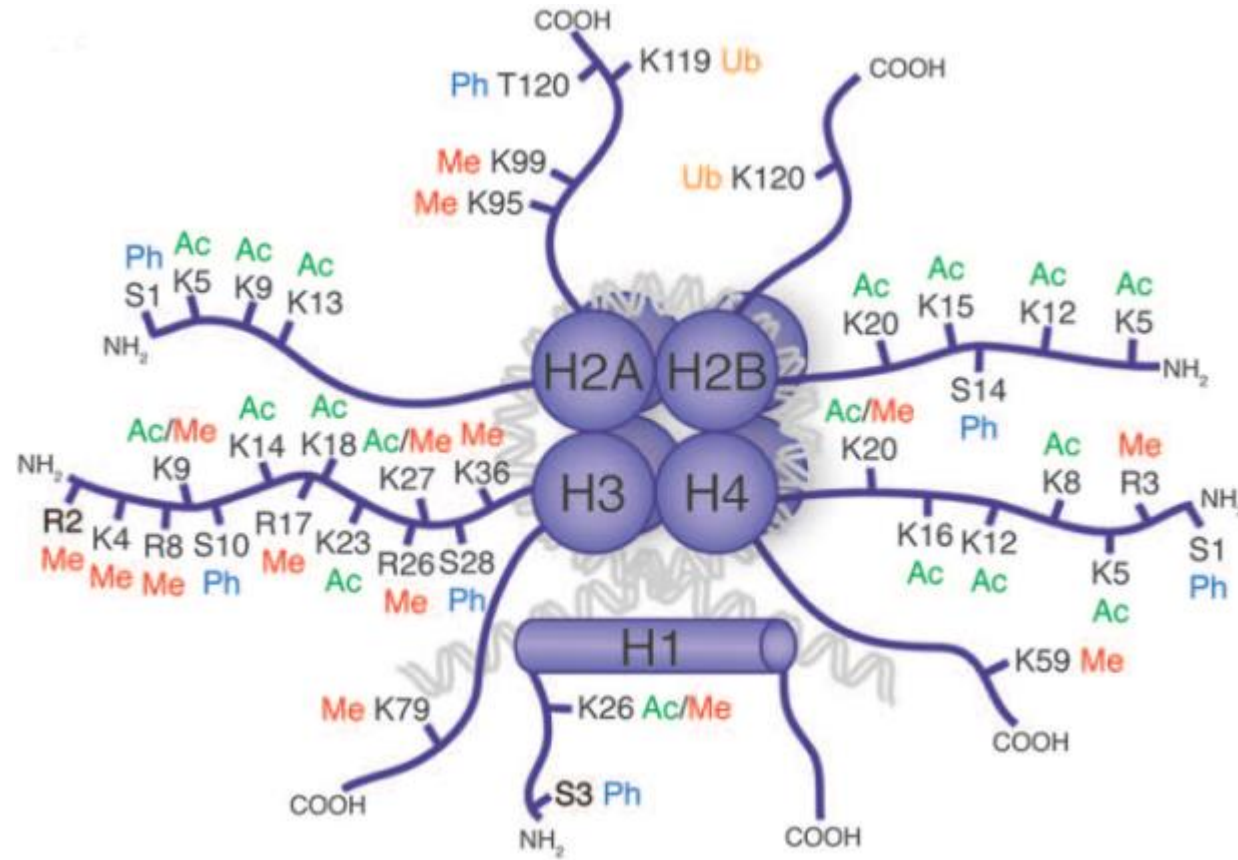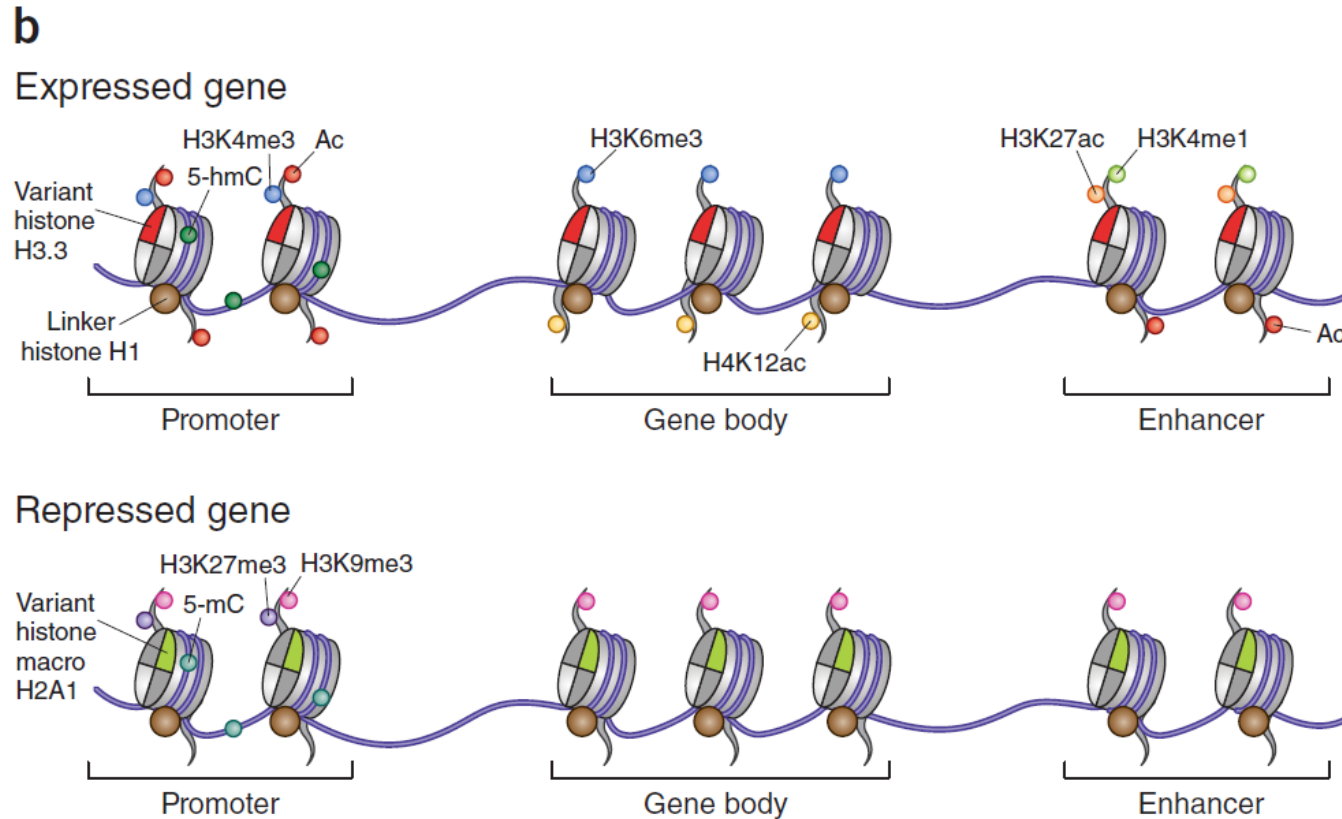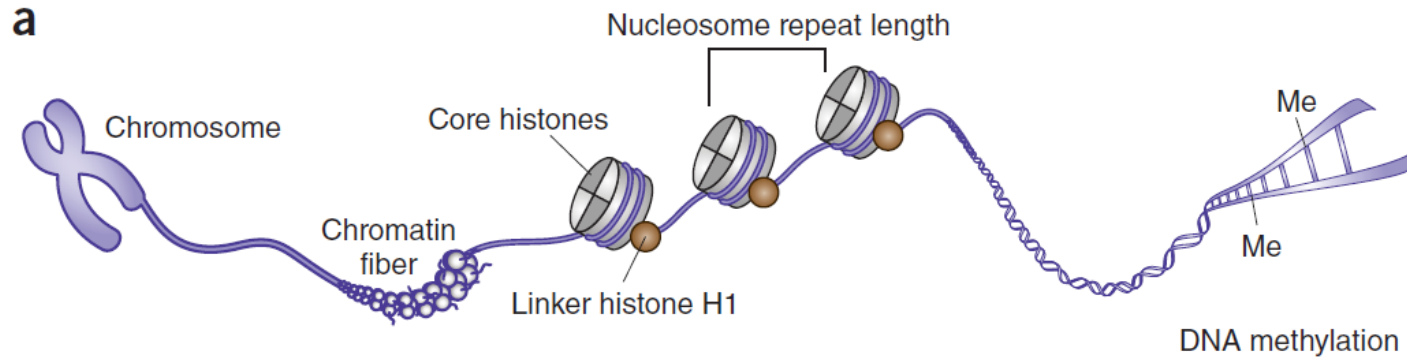
The epigenome and chromatin organization
(a) Chromosomes are organized into domains of loose (euchromatin) or highly condensed (heterochromatin) chromatin and other loosely defined higher-order structures (such as 'globules'), some of which are tethered to the nuclear membrane. An 11-nanometer 'beads-on-a-string' chromatin fiber is comprised of nucleosomal arrays connected by linker DNA and *linker histones, which function as major regulators of nucleosomal repeat length*. Me, methyl group.
(b) The distribution of DNA methylation and a small subset of ~130 post-translational histone markings, linker histones and core histone variants represents different regulation at active promoters, gene bodies and enhancers (top) as compared to silenced and repressed chromatin (bottom).
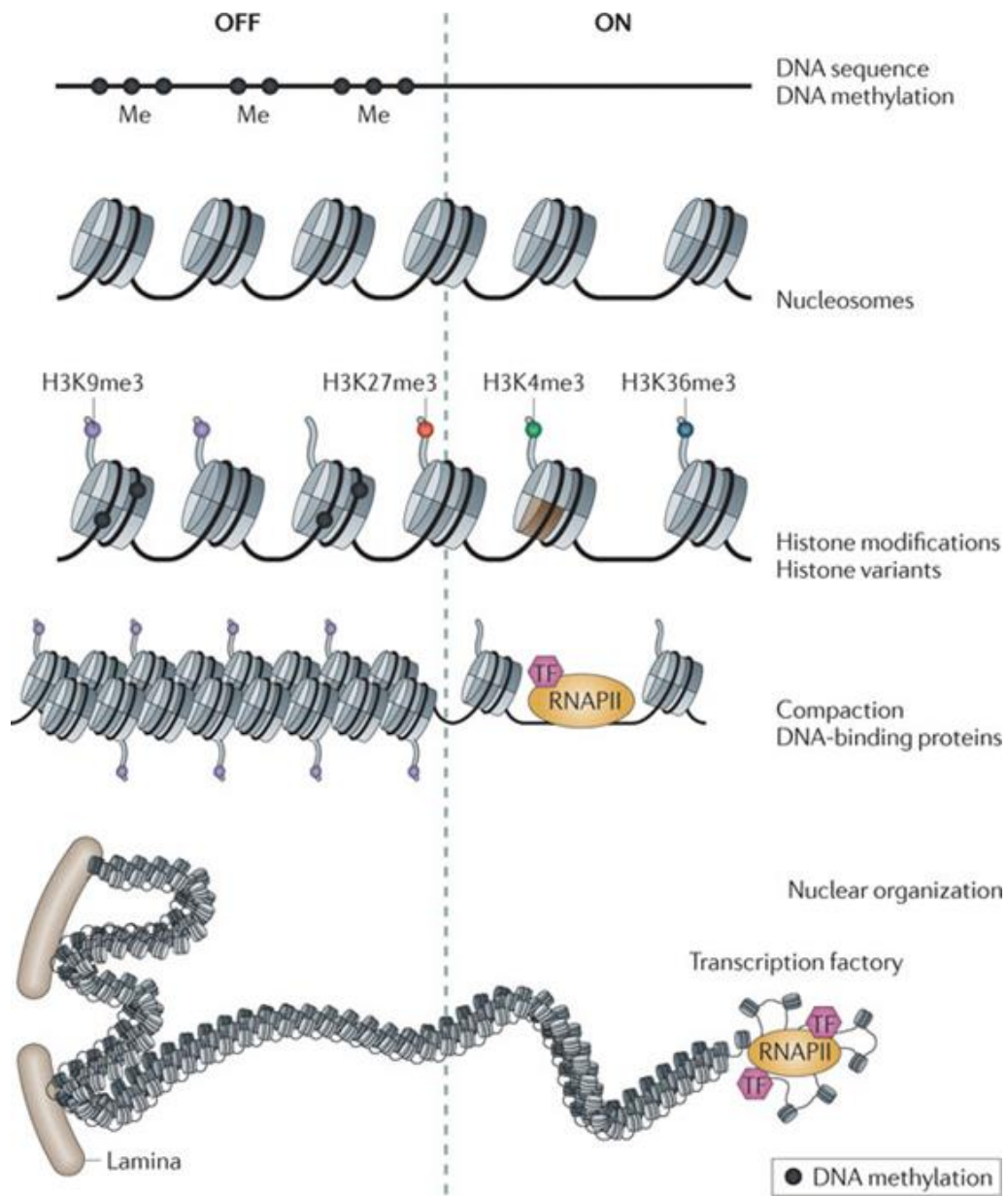
Layers of chromatin organization in the mammalian cell nucleus

Broadly, *features at different levels of chromatin organization are generally associated with inactive (off) or active (on) transcription*. From the top, genomic DNA is methylated (Me) on cytosine bases in specific contexts and is packaged into nucleosomes, which vary in histone composition and histone modifications (for example, histone H3 lysine 9 trimethylation (H3K9me3)); **these features constitute the primary layer of chromatin structure**. Here, different histone modifications are indicated by coloured dots and histone variants such as H2A.Z are brown. **DNA in chromatin may remain accessible to DNA-binding proteins such as transcription factors (TFs) and RNA polymerase II (RNAPII) or may be further compacted**. Chromatin can also organize into **higher-order structures** such as nuclear lamina-associated domains and transcription factories. *Each layer of organization reflects aspects of gene and genome regulation*.

a Chromatin features at a representative transcribed gene
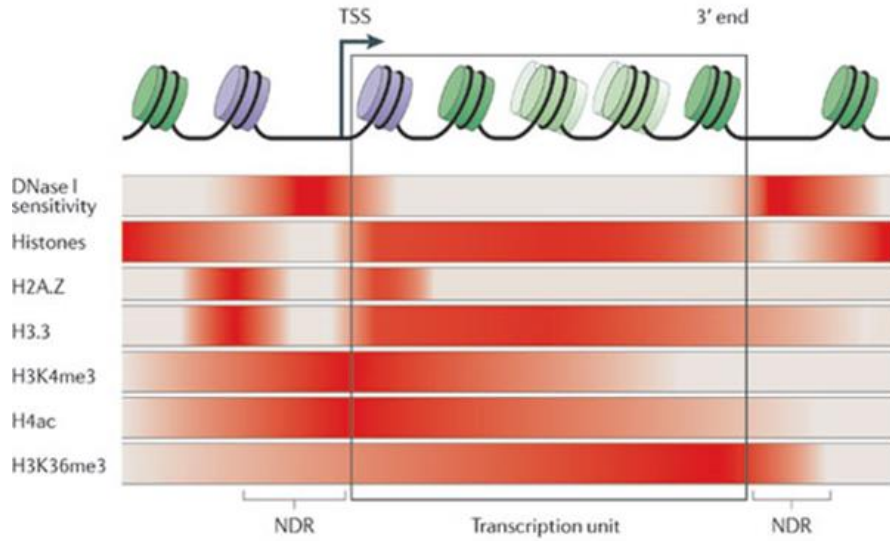
b Active gene

c Inducible gene

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Chromatin structure and DNA accessibility at genes
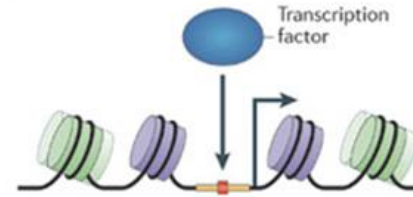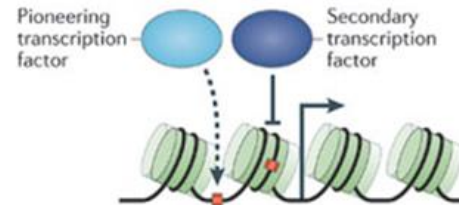
a| Schematic view of primary chromatin features at a hypothetical transcribed gene. Heat maps (high = red, low = beige) indicate the density and location of the chromatin features listed on the left. *Active genes are typically characterized by regions of low nucleosome occupancy (nucleosome depleted regions (NDRs)), which coincide with DNase I hypersensitive sites and are located upstream and downstream of the transcription unit (boxed area).* Flanking the NDR at the promoter (which is indicated by the right-angled arrow, labelled TSS for 'transcription start site') are two highly positioned nucleosomes, which exhibit histone H4 acetylation (H4ac) and H3 trimethylation at lysine 4 (H3K4me3) and frequently contain histone variants H3.3 and H2A.Z. H3.3 is also deposited downstream in the gene body. H3K36me3 accumulates in the gene body towards the 3′ end.

b| Nucleosome occupancy at a hypothetical constitutively active promoter. An A/T-rich tract disfavours stable nucleosome formation and enhances exposure of the binding site to facilitate transcription-factor interaction within the NDR.

c| Nucleosome occupancy at a hypothetical repressed promoter. Transcription-factor binding sites are typically embedded within nucleosomes that occlude interaction. Nucleosome mobilization is required for transcriptional activation and involves initial binding of the pioneering transcription factor within the nucleosome linker region, which, in turn, recruits histone modifiers and ATP-dependent chromatin remodellers to expose additional binding sites for a secondary transcription factor.

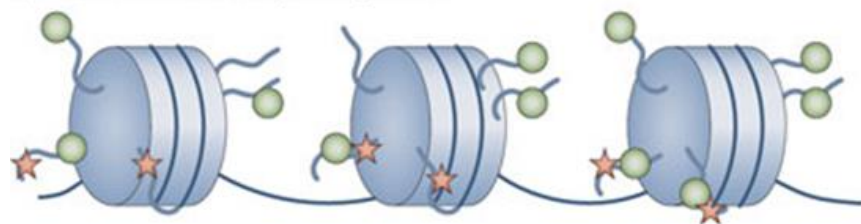Condensed chromatin: transcriptionally inert
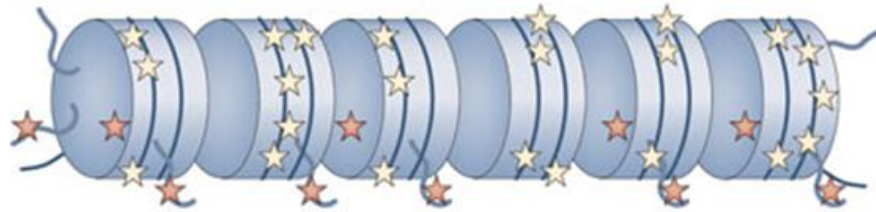
HATs
HMTs
DMs

HDACs
DNMTs
HMTs
DMs

Open chromatin: transcriptionally active

Epigenetic marks:

DNA: ☆ Hypermethylation at CpG sites

Histones: Hypoacetylation
★ Methylation H3K9, H3K27, H4K20

Epigenetic marks:

DNA: Hypomethylation at CpG sites

Histones: ◯ Hyperacetylation
★ Methylation H3K4, H3K36, H4K79

Epigenetic markers of open and condensed chromatin

Specific epigenetic modifications are characteristic of different chromatin structures. *Transcriptionally inert chromatin consists of tightly condensed, methylated DNA coiled around histone octamers that are deacetylated and methylated at particular lysine residues.* Conversely, *expanded chromatin is characterized by nonmethylated DNA loosely coiled around acetylated nucleosome complexes. This structure makes gene promoters accessible to the transcriptional apparatus*.

Abbreviations: DM, demethylase; DNMT, DNA methyltransferase; HAT, histone acetyl transferase; HDAC, histone deacetylase; HMT, histone methyltransferase.

Chemical modifications of DNA and histones and their association with chromatin and transcription states

Target	Modification	Nucleotide or amino acid	Residue position	Chromatin state	Transcription state
DNA	Methylation	Cytosine (C)	CpG islands	Closed	Repressed
Histones	Acetylation	Lysine (K)	H2AK5, H2BK12, H2BK15, H3K9, H3K14, H3K18, H3K56, H4K5, H4K8, H4K13, H4K16	Open	Active
	Methylation	Arginine (R)	H3R17, H3R23, H4R3	Open	Active
	Methylation	Lysine (K)	H3K4, H3K36, H3K79	Open	Active
			H3K9, H3K27, H4K20	Closed	Repressed
	Phosphorylation	Serine (S) or threonine (T)	H3T3, H3S10, H3S28, H2BS14	Open	Active
	Sumoylation	Lysine (K)	H2AK126, H2BK6, H2BK7	Closed	Repressed
	Ubiquitylation	Lysine (K)	H2AK119	Closed	Repressed
			H2BK120	Open	Active
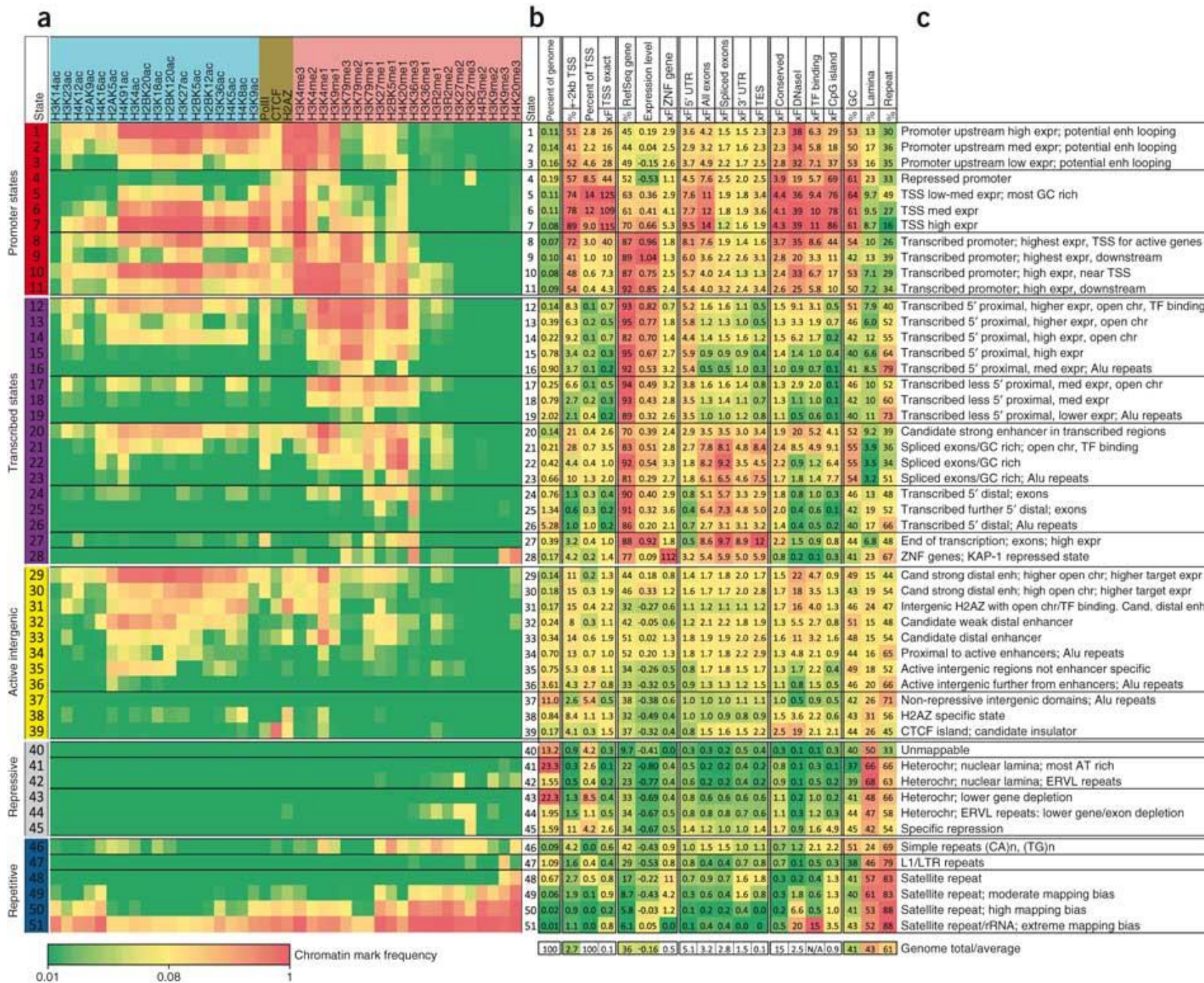
Chromatin state definition and functional interpretation

(a) Chromatin mark combinations associated with each state. Each row shows the specific combination of marks associated with each chromatin state and the frequencies between 0 and 1 with which they occur (color scale). These correspond to the emission probability parameters of the Hidden Markov Model (HMM) learned across the genome during model training.

(b) Genomic and functional enrichments of chromatin states. %, percentage; xF, fold enrichment.

(c) Brief description of biological state function and interpretation (chr, chromatin; enh, enhancer).

Chromatin state maps

Genome-wide maps of chromatin state can now be readily acquired using microarrays or next generation sequencing technologies. These datasets reveal local and long range chromatin patterns that offer insight into the locations and functions of underlying regulatory elements and genes. These patterns are dynamic across developmental stages and lineages.

Modifying the structure of chromatin/ Modifikacije kromatinske strukture

SIGNALS

H2BK123ub

H3K79me

H3K4me ⊣ H3K9me

HP1 ⊢ H3K9ac

H3R17me

H4K16ac ⊣ H4K20me

H3K9ac ⊢ H3K9me

HP1 ⊣ H3S10ph

H4K16ac ⊢ H4K20me

DNA methylation
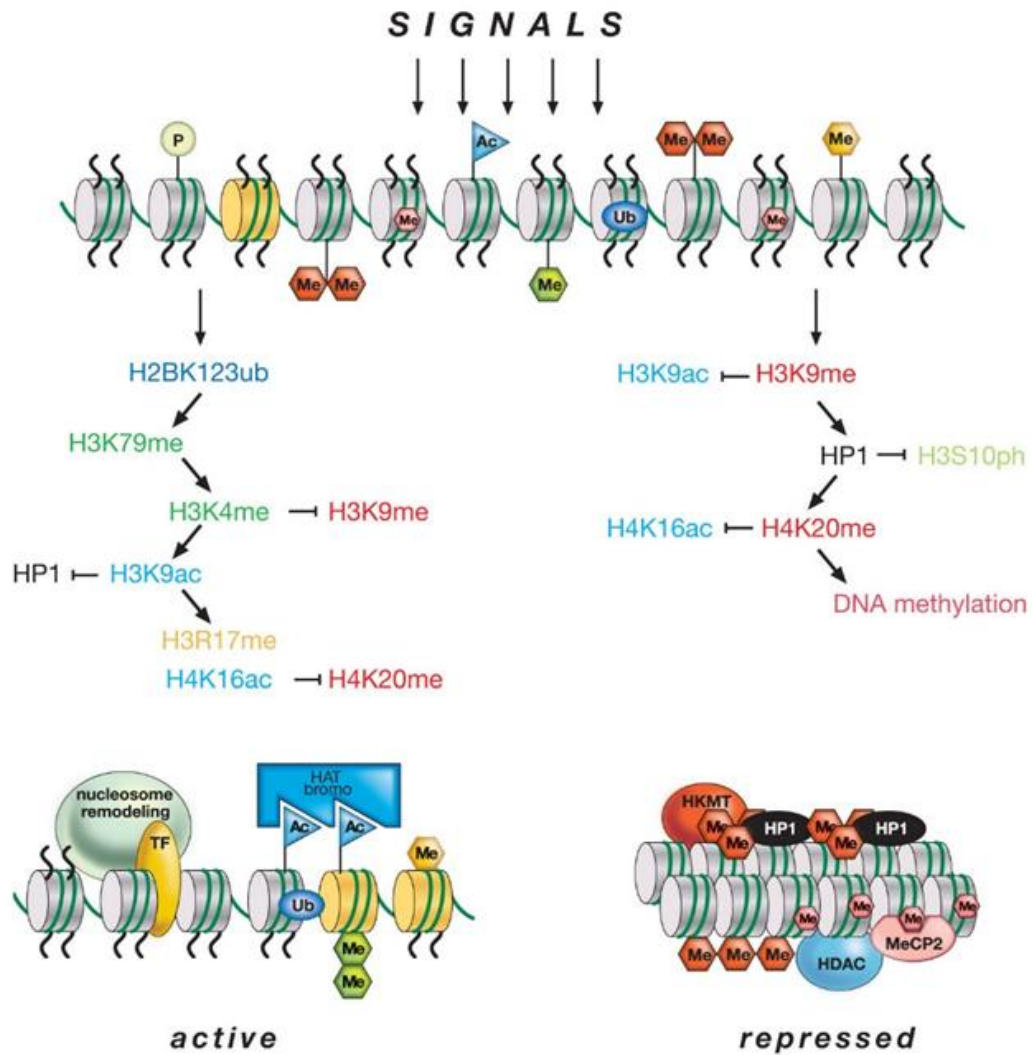
active

repressed

Figure 11. Coordinated Modification of Chromatin

The transition of a naïve chromatin template to active euchromatin (*left*) or the establishment of repressive heterochromatin (*right*), involving a series of coordinated chromatin modifications. In the case of transcriptional activation, this is accompanied by the action of nucleosome-remodeling complexes and the replacement of core histones with histone variants (*yellow*, namely H3.3).

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signals

cell membrane

transducers

nuclear membrane

naïve template

HAT + remodelers | HDAC + DNA methylation | ?? | Polycomb

gene ON | gene OFF | nuclear relocation | locus architecture
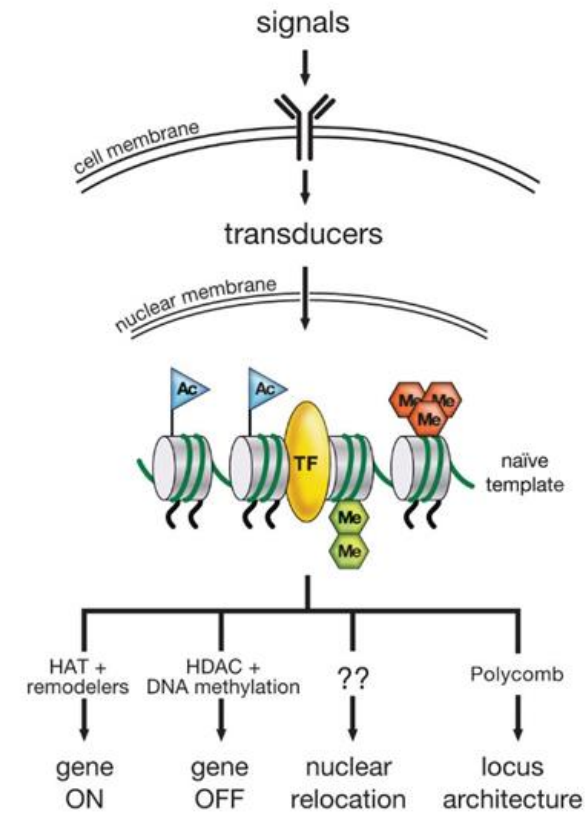
Figure 2. Central Role of Transcription Factors in Epigenetic Gene Control

Transcription factors (TF), which are frequently regulated in response to extracellular signals, are responsible for gene activation, transcriptional repression, gene relocation within nuclear compartments, and chromatin architecture of gene loci. The transcription factors fulfill these diverse functions by interacting with coactivators (including histone acetyltransferases [HATs]), corepressors (including histone deacetylases [HDACs]), chromatin-remodeling machines, and Polycomb protein complexes.

Epigenetics © 2006 Cold Spring Harbor Laboratory Press
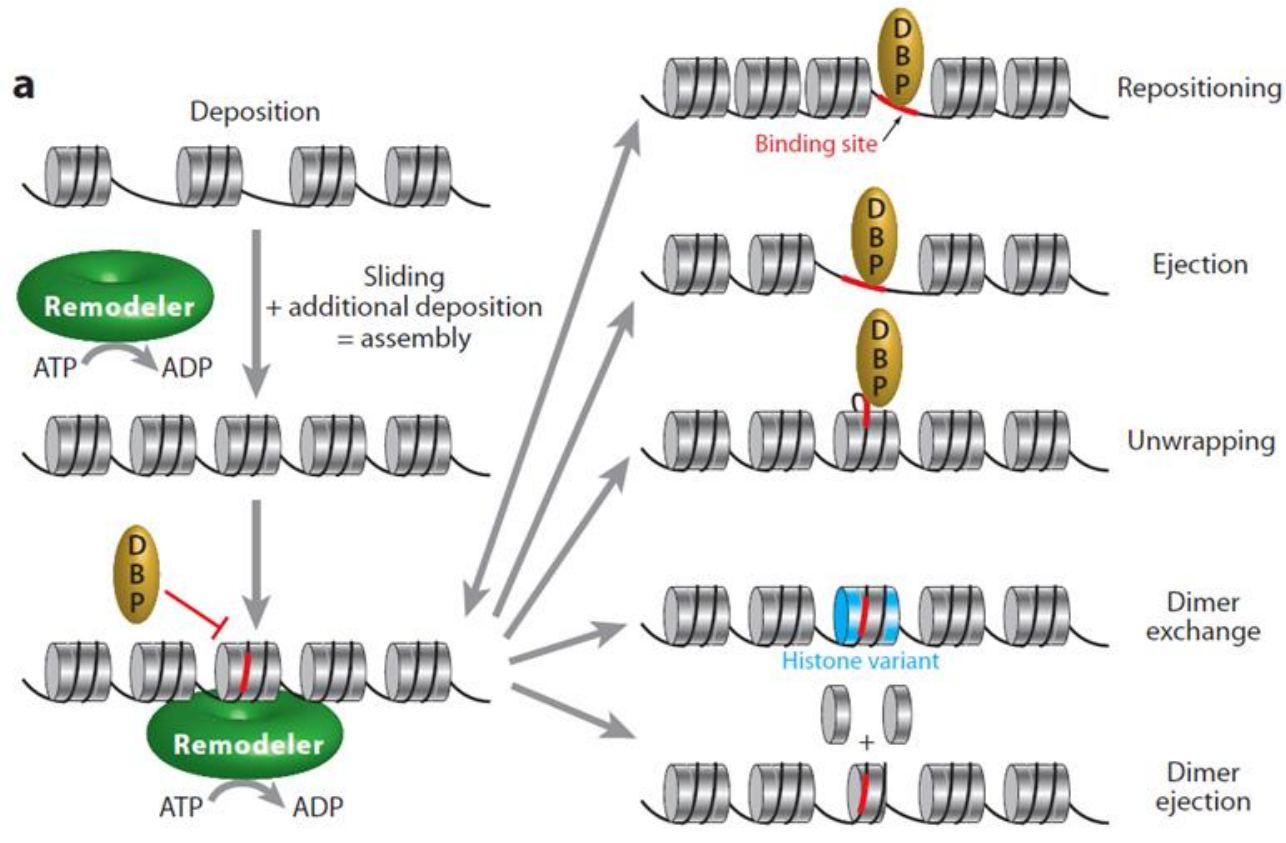
The different outcomes of chromatin remodeling

Remodelers (green) can assist in chromatin assembly by moving already deposited histone octamers, generating room for additional deposition

(a). Remodeler action on a nucleosome array results in various products that can be classified in two categories:
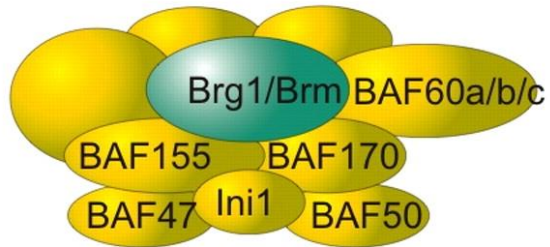
(b) **site exposure**, in which *a site (red) for a DNA-binding protein (DBP), initially occluded by the histone octamer, becomes accessible by nucleosomal sliding (repositioning), or nucleosomal eviction (ejection), or localized unwrapping*, and

(c) **altered composition**, in which the *nucleosome content is modified by dimer replacement* [exchange of H2A-H2B dimer with an alternative dimer containing a histone variant (blue)] *or through dimer ejection*.

SWI/SNF
(SWI2/SNF2-type ATPase)

Brg1/Brm BAF60a/b/c
BAF155 BAF170
BAF47 Ini1 BAF50

NURF
(ISWI-type ATPase)

BPTF
ISWI
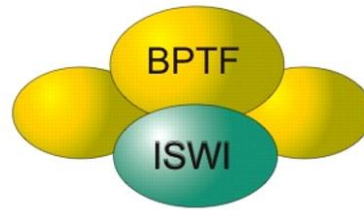
NuRD
(Mi-2-type ATPase, HDAC)

HDAC1 MBD3
Mi-2 MTA1/2/3
HDAC2 RbA p48

INO80
(INO80-type ATPase)

RUVBL1/2 Ies2/6
BAF53a INO80 Arp5/8
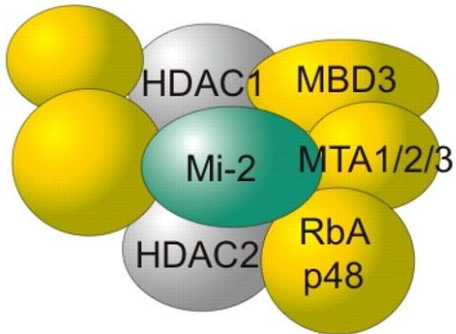
ATP-dependent chromatin remodeling complexes and their function in chromatin organization

The **remodeler complexes are classified into four groups** *based on their specific ATPase catalytic subunits*. **Chromatin remodeling is associated with both chromatin activation and repression**.

SWI/SNF: switching defective/sucrose nonfermenting; BRM: Brahma; BRG1: Brahma-related gene 1; NURF: nucleosome remodeling factor; ISWI: imitation switch; NuRD: nucleosome remodeling and deacetylase; INO80: inositol requiring 80.
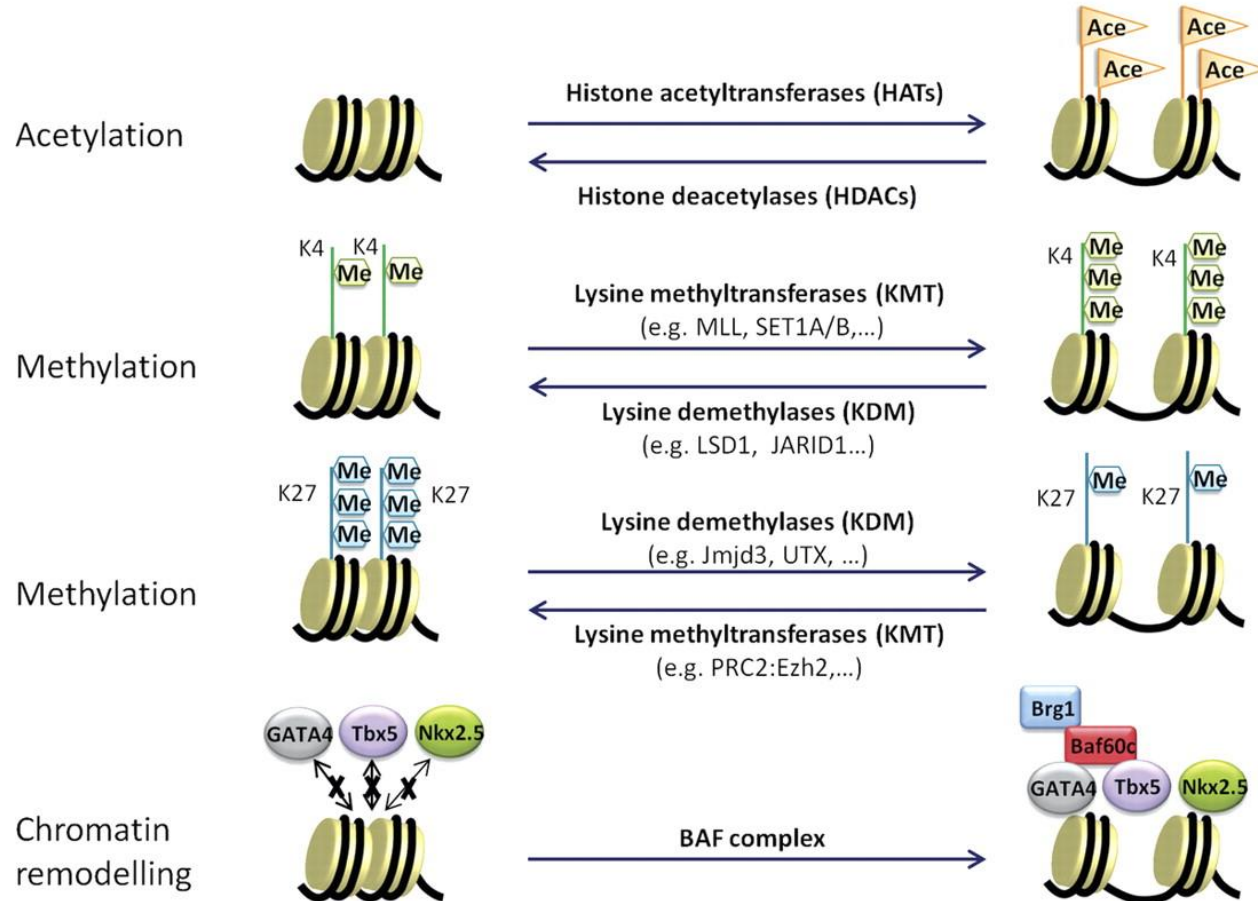
Schematic illustration of the regulation of chromatin structure
The **assembly of epigenetic modulating enzymes controls chromatin structure**. **Heterochromatin** prevents the access of transcription factors (TF), whereas **euchromatin** is accessible for transcriptional activation. These structures are **controlled by histone modifications and chromatin remodelling factors**.
K, Lysine; Me, Methyl group; Ace, Acetylation; PRC, Polycomb repressor complex.

The **eukaryotic chromatin structure can be remodeled by at least five mechanisms**:
(1) the nucleosome formation, entailing tendency of nucleosome formation, nuclesome occupation levels, and arrangement of nucleosomes;
(2) adding covalent modifications to histones;
(3) replacing histones with histone variants;
(4) methylations at DNA cytosine; and
(5) small and long non-coding RNAs.
All of these **chromatin remodeling processes** change constituent, condensation, accessibility, and interacting proteins of the chromatin structures. Therefore, chromatin remodeling marks reflecting chromatin structural modification status include DNA methylations, nucleosome formation, proportion of nucleosomes containing histone variants, and histone modifications such as histone methylations, acetylations, ubiquitylations, and phosphorylations. By the action of ATP-dependent chromatin remodelers, the combination of specific chromatin modification marks are arranged and interact with specific proteins involved in transcription, DNA replications, and DNA repair. Therefore, distinct arrangement of chromatin remodeling marks may play roles as **chromatin remodeling code**.

## Model of the overall structure of the epigenome in normal human cells

This diagram shows the **balanced state of chromatin, nucleosome positioning and DNA methylation, which maintains the normal packaging state of DNA**. A silenced gene (indicated by a red X over the transcription start site designated by the arrow) at the top of the figure has its promoter CpG island occupied by a Polycomb group (PcG) complex (indicated by a red shaded area) that mediates chromatin changes that include the repressive histone modification trimethylation of lysine 27 on histone 3 (H3K27me3). There is no CpG DNA methylation within the gene promoter CpG island (shown by pale blue circles) and nucleosomes are positioned over the transcription start site. Sites upstream from the promoter are heavily DNA methylated (shown by red circles). The gene promoter illustrated below the silenced gene has been signalled to adopt a fully active transcription state and retains the active H3K4me3 marks at the promoter. It also has acetylation of key H3 and H4 lysines, the presence of the variant histone, H2A.Z (not shown) and H3K36me3 in the gene body to facilitate transcriptional elongation. The transcription start region (indicated by an arrow) is not occupied by nucleosomes. Just below, a distal enhancer is shown for this gene with an active nucleosome configuration, and the signature histone modification for enhancers, H3K4me1, is present. Finally, towards the bottom of the figure, the packaging of the majority of the cellular DNA into a transcriptionally repressed configuration is depicted, with compacted nucleosomes, the presence of H3K9me2 and H2K9me3, which are signature repressive marks for constitutive heterochromatin, the presence of heterochromatin protein 1 (HP1; also known as CBX5) and extensive DNA methylation. The folding of the heterochromatin into chromosomal locations in the nucleus is shown.
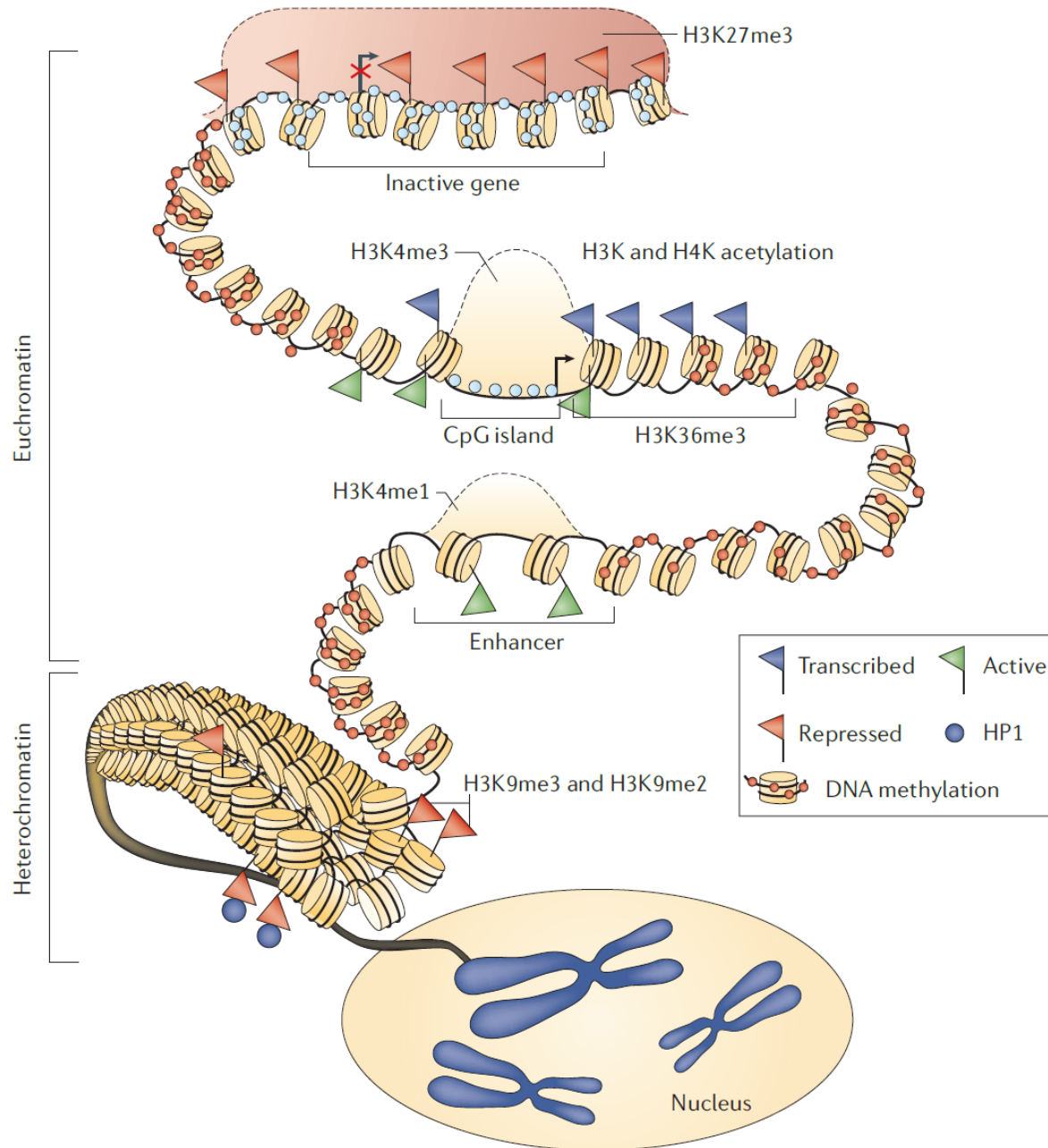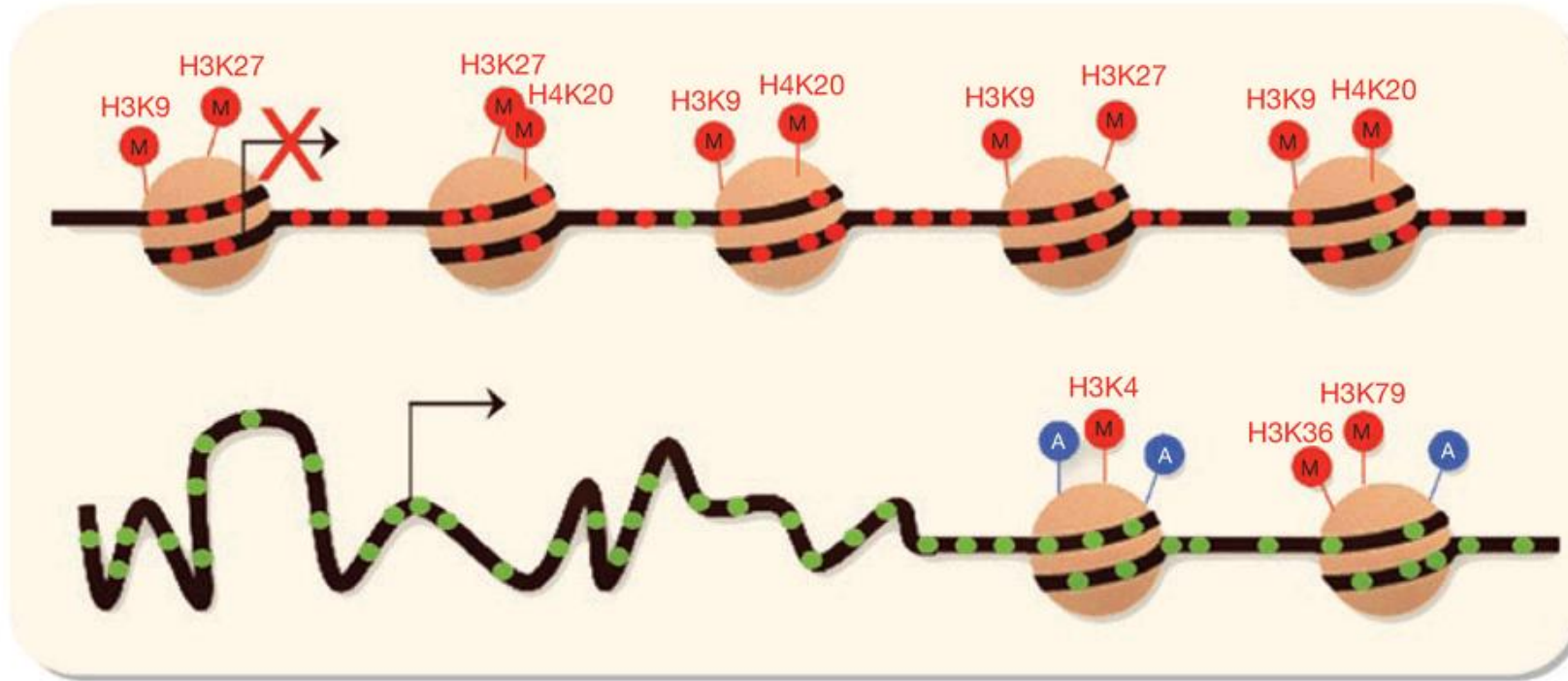
Nucleosome positioning patterns

Nucleosome positioning plays an important role in transcriptional regulation. *Transcriptionally active gene promoters possess a nucleosome-free region at the 5' and 3' untranslated region, providing space for the assembly and disassembly of the transcription machinery. The loss of a nucleosome directly upstream of the TSS is also necessary for gene activation*, whereas the *occlusion of this position leads to transcription repression*. DNA methylation regulates transcription, and thus interferes with nucleosome positioning. **Methylated DNA seems to be associated with 'closed' chromatin domains**, where DNA is condensed into strictly positioned nucleosomes, thereby impeding transcription. Conversely, **unmethylated DNA is associated with 'opened' chromatin domains, which allow transcription**.
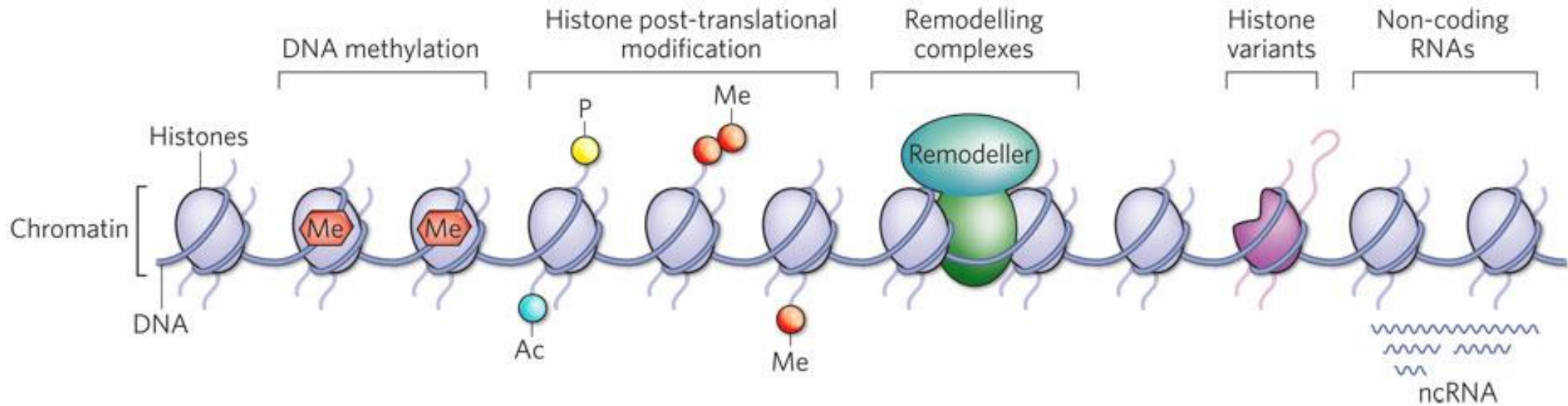
Mechanisms involved in chromatin modifications

Five broad and interrelated mechanisms are known to **affect chromatin structure**: **DNA methylation, histone modification, remodelling by chromatin-remodelling complexes, insertion of histone variants, and the effects of non-coding RNAs (ncRNAs)**. All five have been shown to be **essential contributors to the development and cell-fate determination of tissues.**

Ac, acetyl; Me, methyl; P, phosphate.

DNA methylation/
DNA metilacija
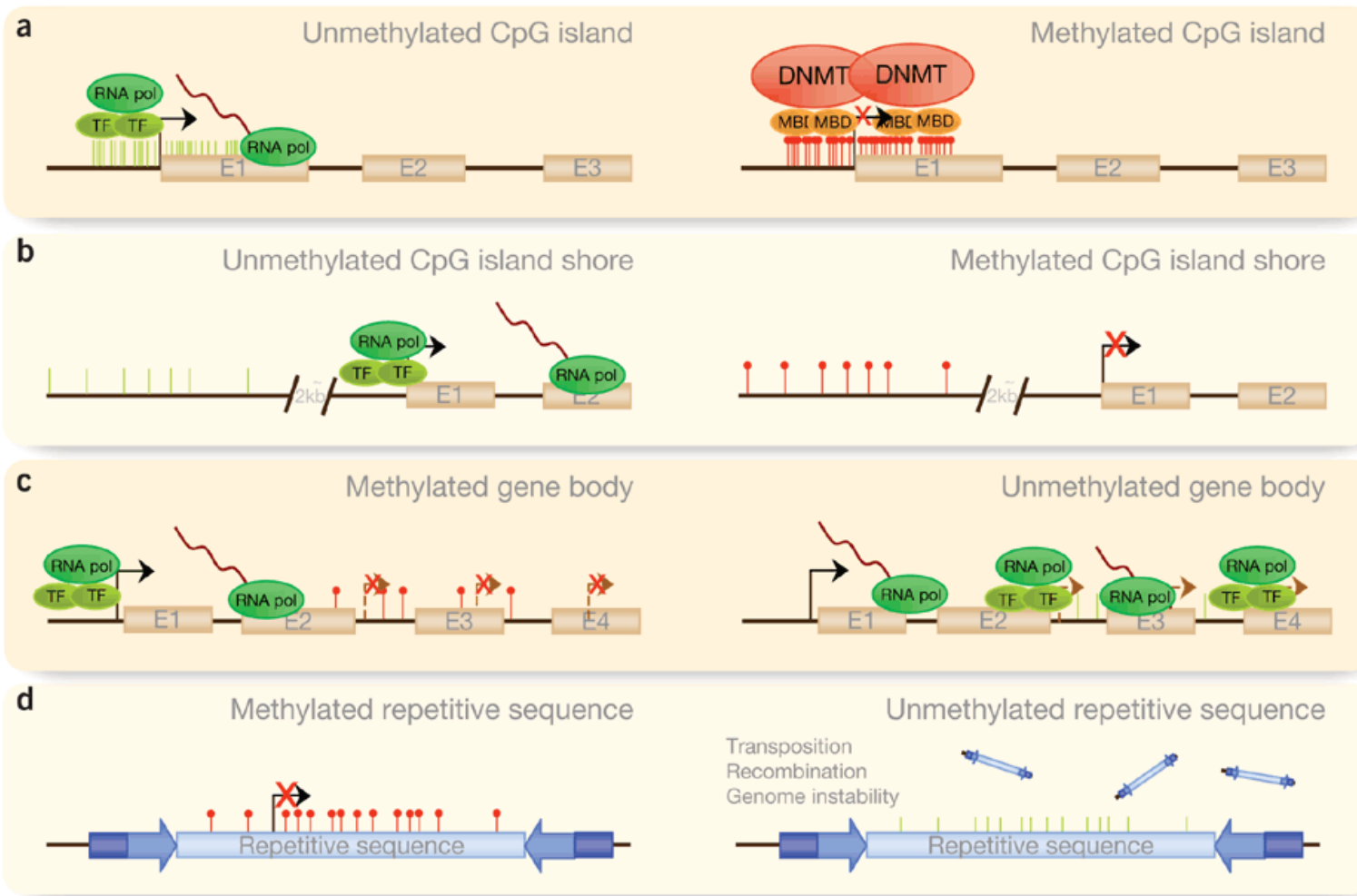
DNA methylation patterns

DNA methylation can occur in different regions of the genome. The alteration of these patterns leads to disease in the cells. The normal scenario is depicted in the left column and alterations of this pattern are shown on the right.

(a) CpG islands at promoters of genes are normally unmethylated, allowing transcription. Aberrant hypermethylation leads to transcriptional inactivation. (b) The same pattern is observed when studying island shores, which are located up to 2 kb upstream of the CpG island.

(c) However, when methylation occurs at the gene body, it facilitates transcription, preventing spurious transcription initiations. In disease, the gene body tends to demethylate, allowing transcription to be initiated at several incorrect sites.

(d) Finally, repetitive sequences appear to be hypermethylated, preventing chromosomal instability, translocations and gene disruption through the reactivation of endoparasitic sequences. This pattern is also altered in disease.

DNA methylation

CpG island — TSS →

---TCGACGATCGAATTTCGGC---
---AGCTGCTAGCTTAAAGCCG---
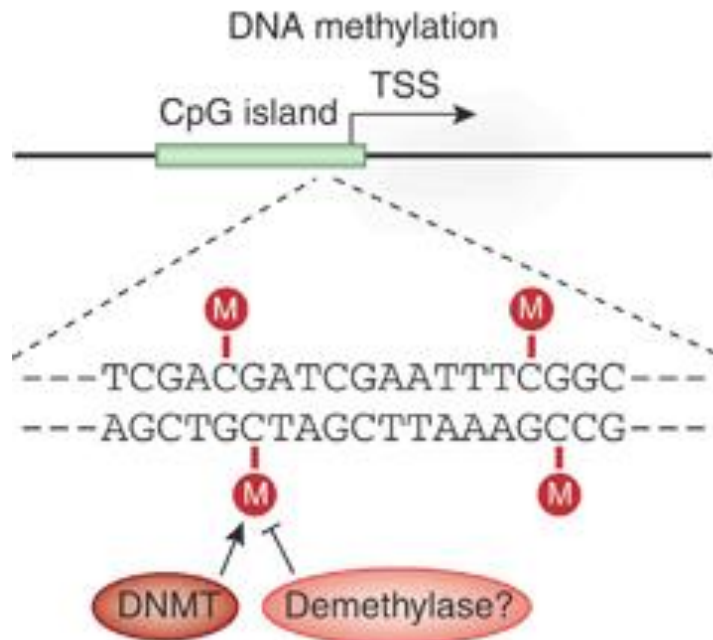
DNMT   Demethylase?

DNA methyltransferases
    DNMT1 (maintenance)
    DNMT3a (de novo)
   DNMT3b (de novo)

DNA demethylase?
    Base excision repair
    GADD45b pathway
    MBD proteins
    TET1 pathway

DNA methylation readers
    MeCP2
    MBD1-4
    Kaiso protein family

DNA methylation and demethylation

A majority of mammalian gene promoters contain dense clusters of cytosine-guanine dinucleotides called **CpG islands**, at which methylation can occur to dramatically influence gene transcription. In this example, the CpG island (green bar) overlaps the transcription start site. *At CpG dinucleotides, methylation is catalyzed by DNA methyltransferases (DNMTs)*. De novo DNMTs direct the methylation of unmethylated CpGs, whereas maintenance DNMTs recognize hemi-methylated DNA and methylate the complementary strand. The existence of a direct demethylase is controversial, but a number of different mechanisms have been proposed to regulate removal of the methyl moiety, including excision and replacement of the entire base pair. **DNA methylation marks are read by a family of proteins with methyl binding domains (MBD proteins), which includes MeCP2. Each of these targets may represent candidates for therapeutic treatments of disorders characterized by aberrant DNA methylation.**
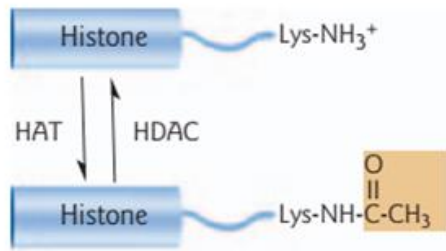
Post-translational modifications of histones/
Posttranslacijske modifikacije histonov
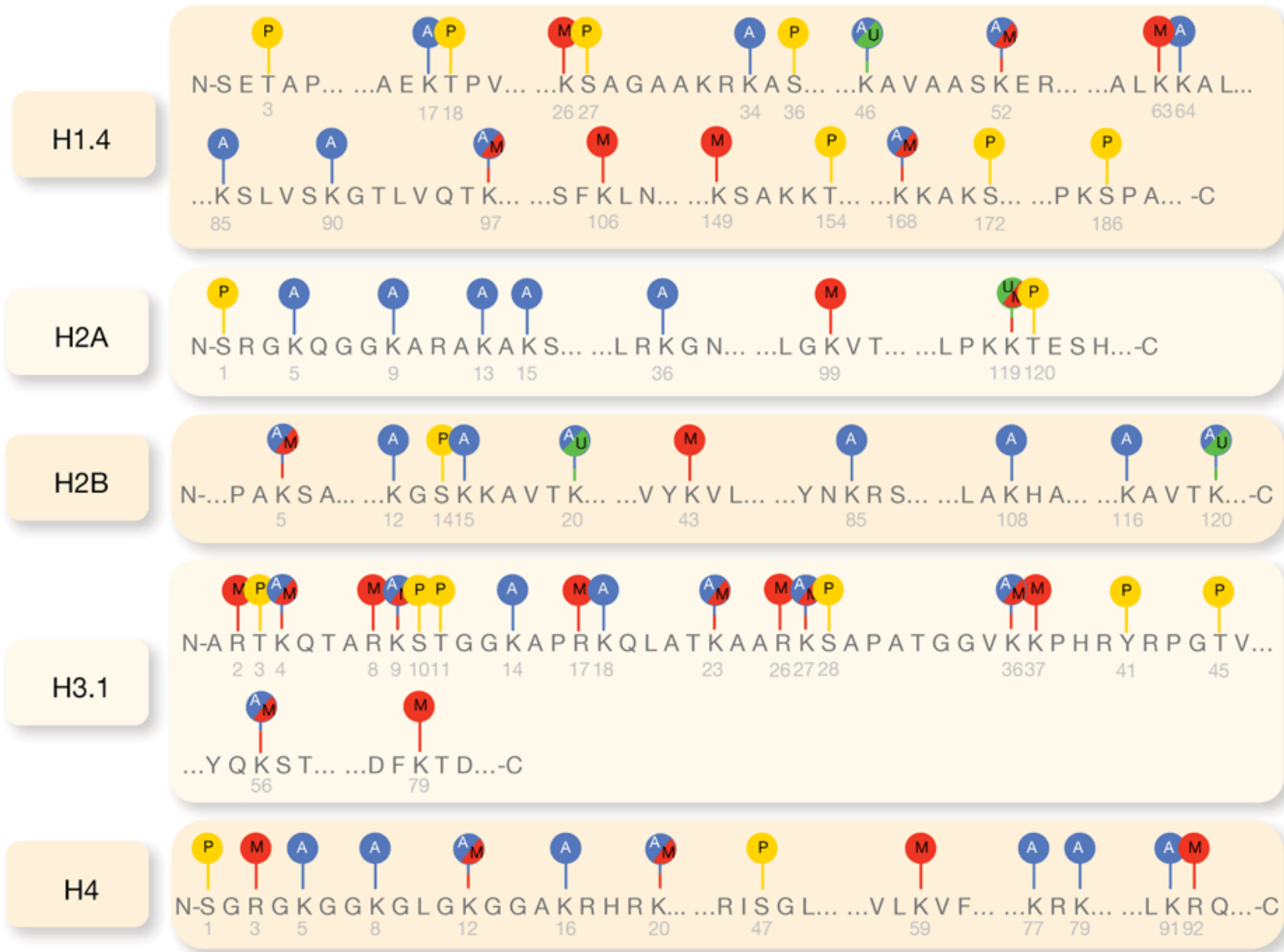
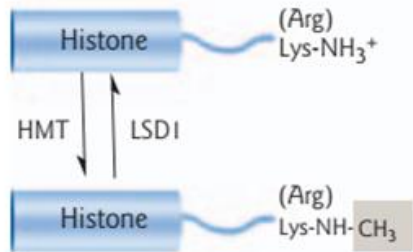**Histone modifications**

All histones are subject to **post-transcriptional modifications**, which mainly occur in histone tails. The main post-transcriptional modifications are depicted in this figure: **acetylation (blue), methylation (red), phosphorylation (yellow) and ubiquitination (green).** The number in gray under each amino acid represents its **position in the sequence**.
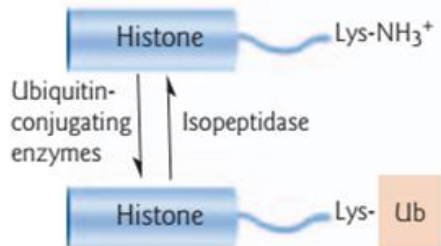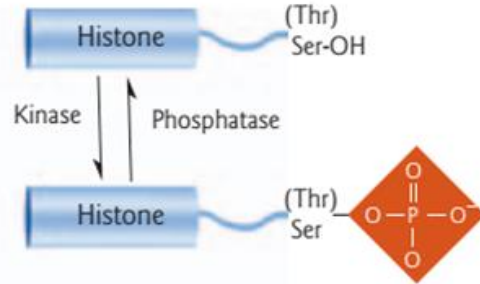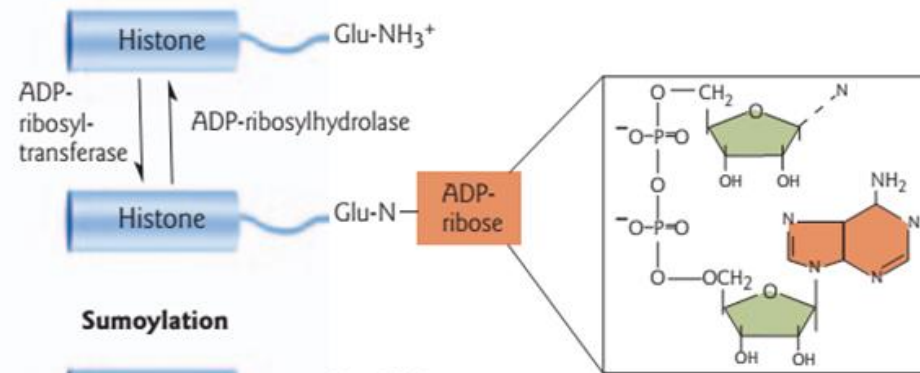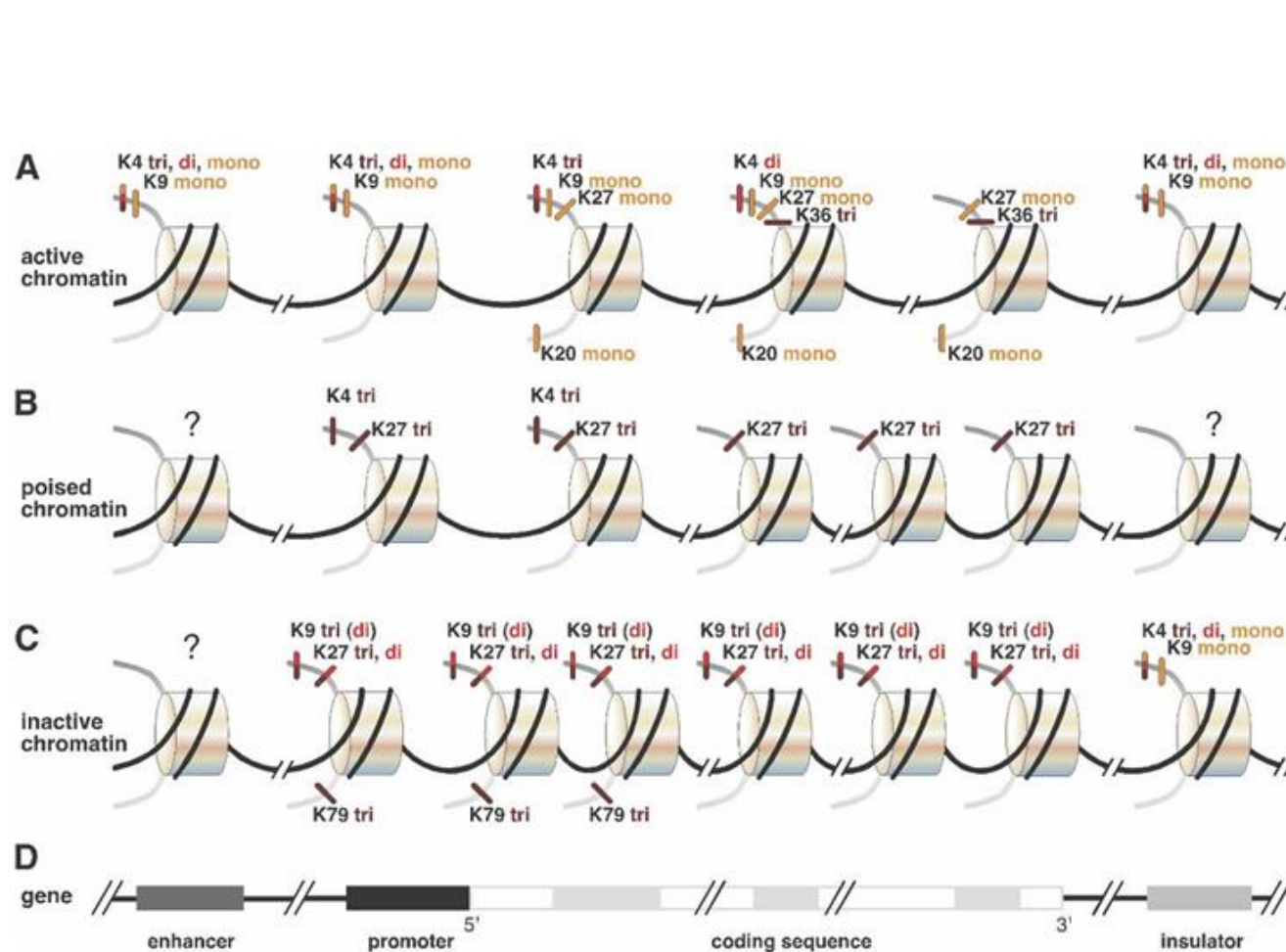
Post-translational modification of histone N-terminal tails

Six types of modifications to histone tails (wavy lines) known to play a role in gene regulation are depicted: acetylation, methylation, ubiquitinylation, phosphorylation, ADP-ribosylation, and sumoylation.

Schematic overview of histone modifications

(A) Active chromatin marks. Nucleosomes encompassing the transcribed region of a gene—a promoter, enhancer, and insulator, respectively—are shown (structure of gene and regulatory elements are represented below). The N-terminal "tails" of histone H3 are shown in dark gray, and the tails of H4 are in light gray. *H3/K4 methylation and H3/K9 monomethylation are enriched at the enhancer, the promoter, and the 5'end of the active gene. H3/K27 and H4/K20 monomethylation is enriched over the transcribed region, whereas H3/K36 trimethylation peaks at the 5' of the active gene.* Note that **active genes are also enriched in H3, H4, and H2A acetylation**.

(B) Poised chromatin marks. Four nucleosomes encompassing the transcribed region of a gene poised for transcription and one nucleosome each on a promoter element, an enhancer element, and an insulator are shown. The *promoter and the transcribed region are enriched in the repressive mark H3/K27 trimethylation, whereas the region around the transcription start is also enriched in the active mark H3/K4 trimethylation.* This **combination of active and repressive marks** can poise genes for activation and forms a so-called "bivalent domain".

(C) Inactive chromatin marks. Modifications of histones H3 and H4 in nucleosomes encompassing a repressed or silenced gene are shown. *The coding sequence and promoter of the inactive gene are enriched in H3/K9 and H3/K27 di- and trimethylation. The 5'end of the gene and the promoter region are marked by H3/K79 trimethylation*, whereas the insulator element carries activating marks.

(D) Scheme of a protein-coding gene with exons shown as light-gray boxes and introns as white boxes. Cis-acting regulatory sequences (enhancer, promoter, and insulator) are represented by black and dark-gray boxes.
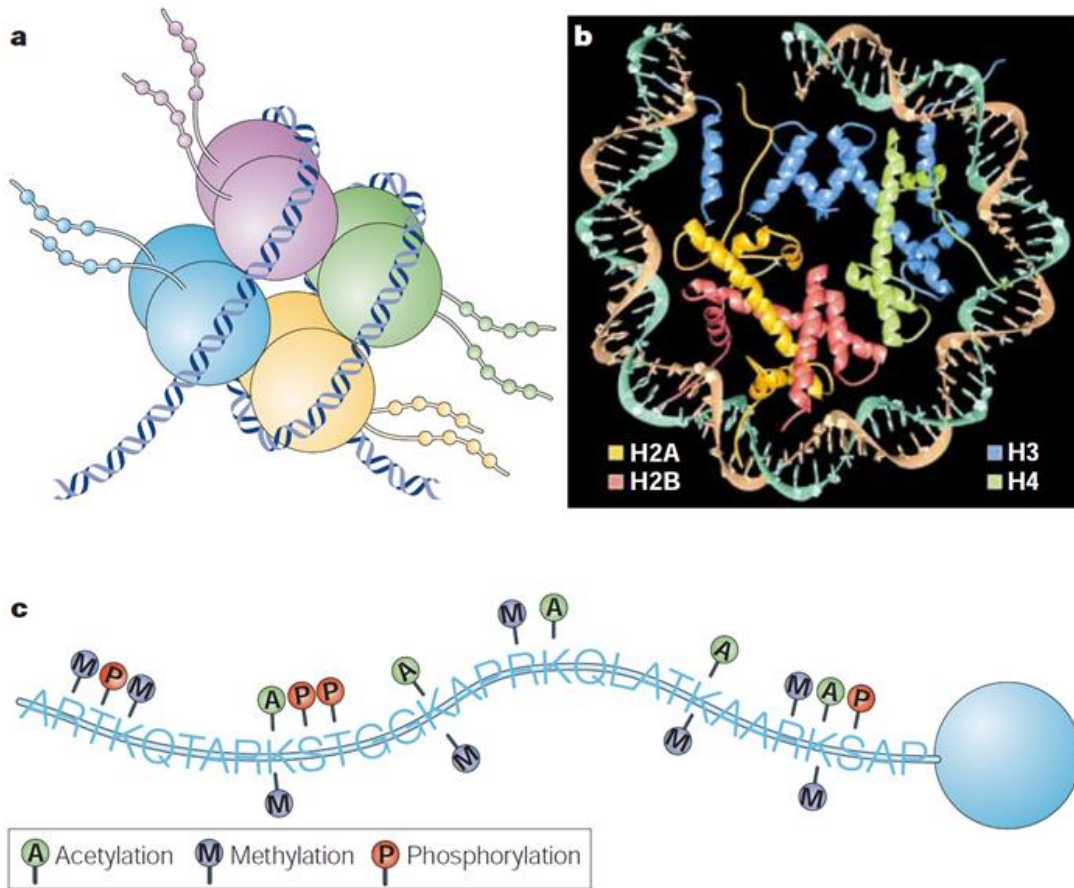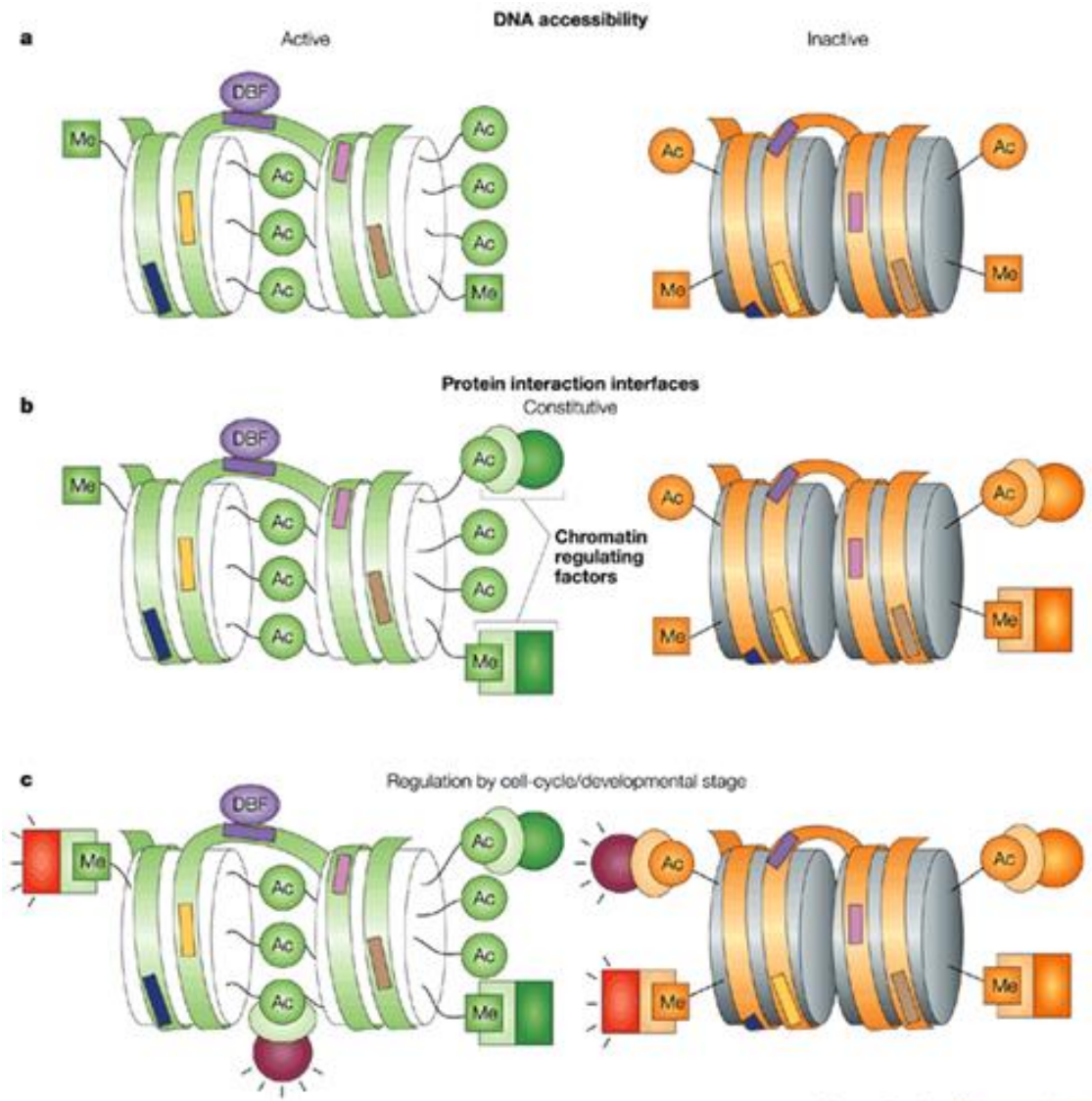
The nucleosome and the histone code

a| Each **nucleosome** comprises an **octamer of histone molecules**, which consists of an **H32–H42 tetramer and two H2A–H2B dimers**. The amino (N) termini of histones project out of the nucleosome core and interact with DNA. These **histone tails can be epigenetically modified, and function as signal integration platforms**.

b| Crystal structure of the nucleosome depicting the interaction of DNA with histones.

c| The **first 30 amino acids in the N terminus of the human histone H3 are illustrated**. *Many sites in the N terminus can be targets for epigenetic tagging* by, for example, acetylation, phosphorylation and methylation. Regulation of each site is independent, and the integration of epigenetic tags elicits a finely tuned transcriptional response. **The integration of signalling at the level of epigenetics is commonly referred to as the histone code.**

Based on the **combinatorial nature of these tail modifications**, it has been suggested that they **constitute a code**. This *code is thought to dictate dynamic transitions between transcriptionally active and transcriptionally silent chromatin states by creating binding platforms for specific chromatin remodelling factors, while preventing others from binding.* However, although it is well established that covalent histone modifications, alone or in combination, can influence nucleosome mobility and function as a scaffold for the recruitment of regulatory proteins, the *universality of the histone code remains controversial*.

Deciphering the histone code

a| **Histone codes can control DNA accessibility and the ability of DNA-binding factors (DBFs; purple oval) to target their cognate sites on nucleosomes** (coloured rectangles on DNA ribbon).

b| **Histone codes can also provide interaction interfaces for protein modules (chromo- and bromodomains) that are present in functionally diverse chromatin-regulating factors and environments.** Chromatin codes and interacting factors in heterochromatin and euchromatin are colour coded (orange and green) to distinguish between the two environments. Constitutive interactions between chromatin code and nuclear factors have been shown to exist.

c| There might be instances in which **these interactions are regulated by cell-cycle or developmental stage** (highlighted interaction modules). This hypothetical model enables a chromatin code to act at a point in development that is distinct to that of its generation.

Ac, acetylation; Me, methylation.

Nature Reviews | Immunology

Histone modification machinery/
**Proteini, ki so vključeni v modifikacije DNA in histonov**
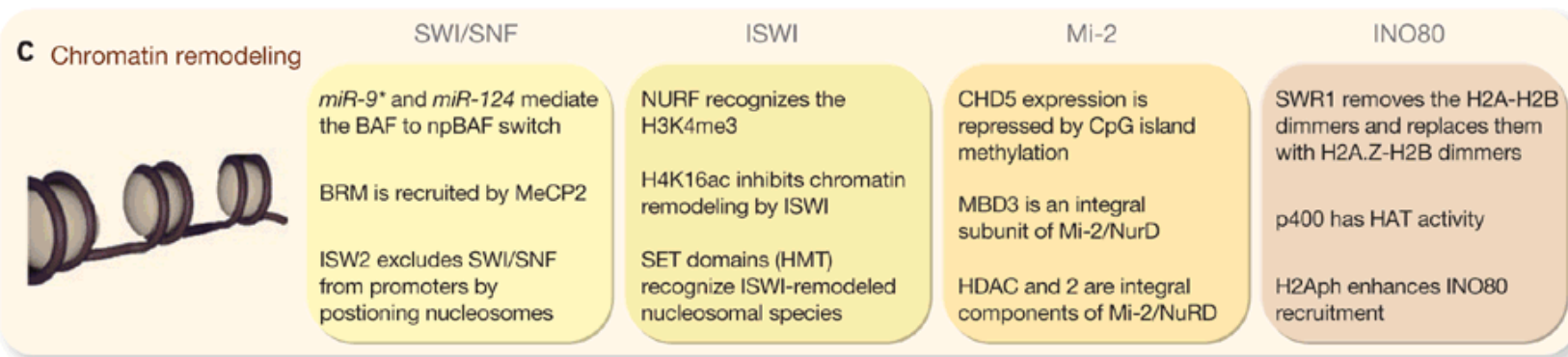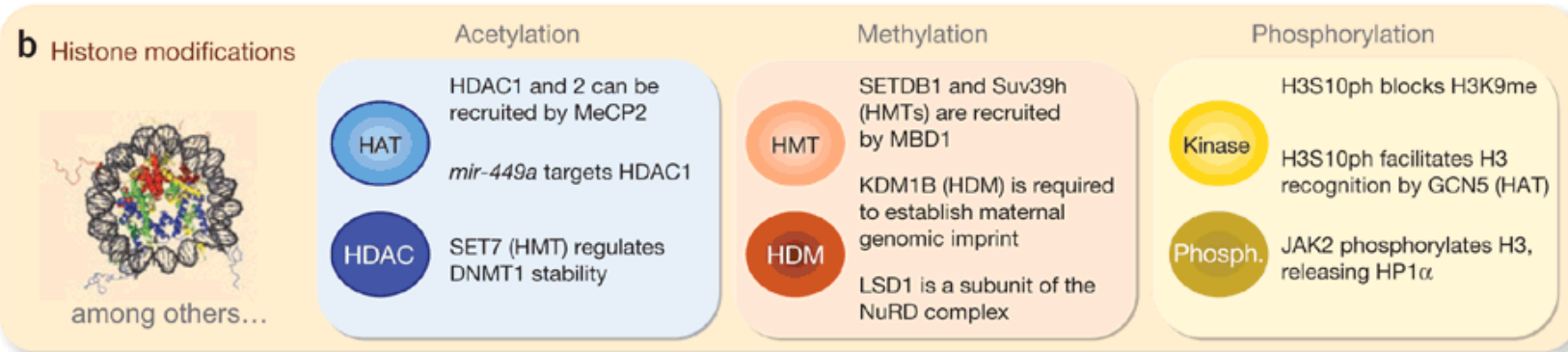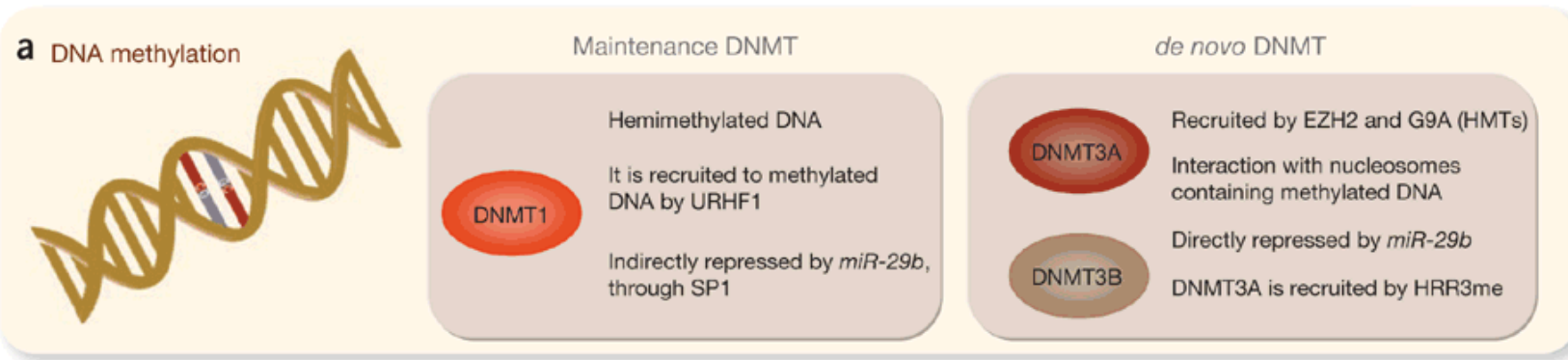
Epigenetic machinery and interplay among epigenetic factors

Epigenetic marks are catalyzed by different epigenetic complexes, whose principal families are illustrated here. (**a–c**) Epigenetic regulation depends on the interplay among the different players: DNA methylation (**a**), histone marks (**b**) and nucleosome positioning (**c**). The interaction among the different factors brings about the final outcome. This figure illustrates selected examples of the possible interrelations among the various epigenetic players.

Epigenetic Control by Chromatin Remodeling
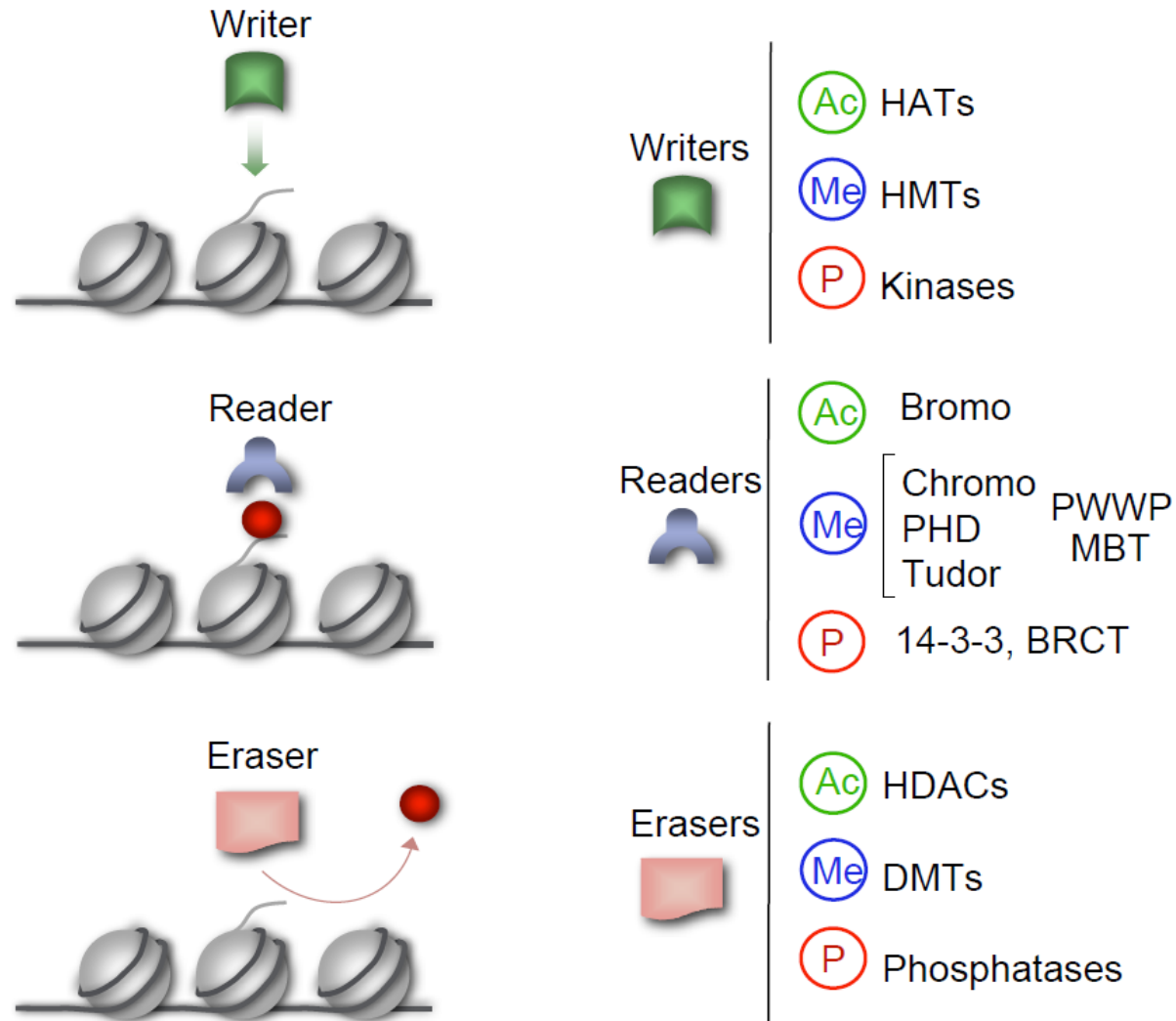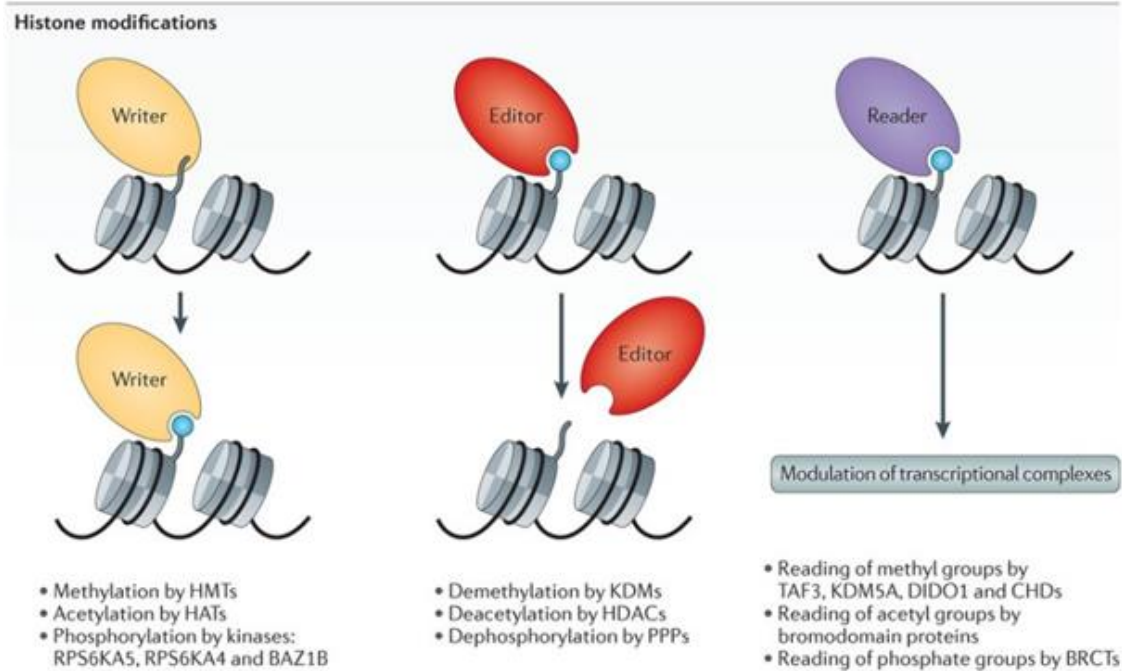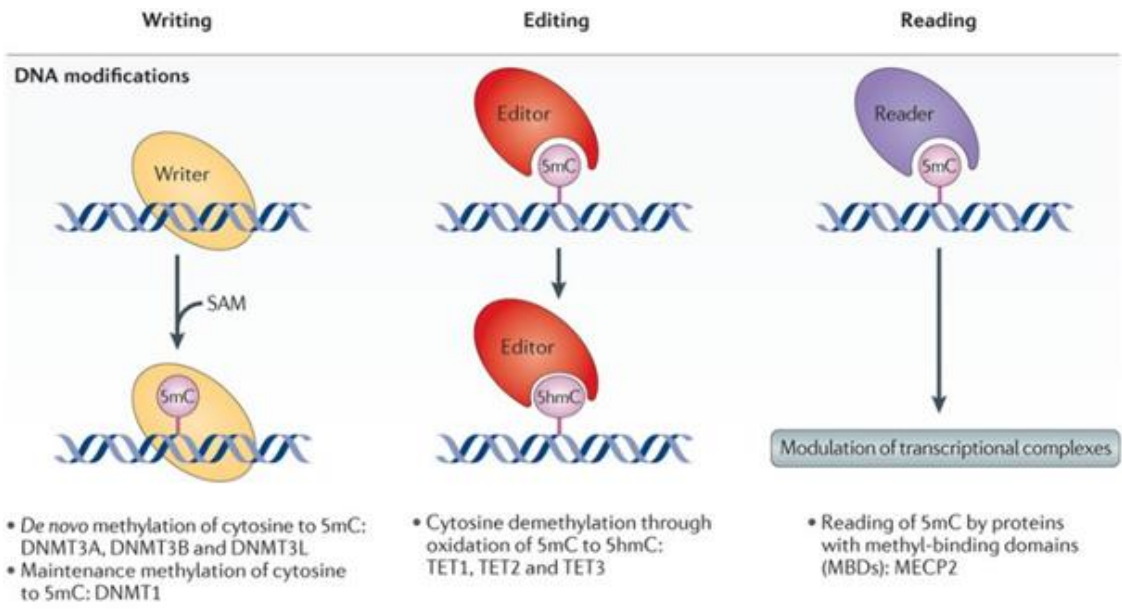
Enzymes involved in DNA and histone modification pathways

Enzymes that establish a mark on either DNA or the histone tail are termed 'writers'. These modifications can be removed or modified by 'editing' enzymes. The third class of enzymes includes the 'readers' of an epigenetic mark, which mediate the interaction of the mark with a protein complex to exert effects on transcription. The **top** panel depicts **DNA modifications, such as DNA methylation and demethylation**, and the enzymes involved; the **bottom** panel shows **histone modifications and the enzymes involved**. Examples for each class of enzyme are given.

5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; BAZ1B, tyrosine protein kinase BAZ1B; BRCT, BRCT domain-containing protein; CHD, chromodomain helicase DNA-binding protein; DIDO1, death-inducer obliterator 1; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; KDM, lysine-specific histone demethylase; MECP2, methyl-CpG-binding protein 2; PPP, serine/threonine protein phosphatase; RPS6K, ribosomal protein S6 kinase; SAM, S-adenosyl-L-methionine, TAF3, transcription initiation factor TFIID subunit 3; TET, TET 5mC hydroxylase.
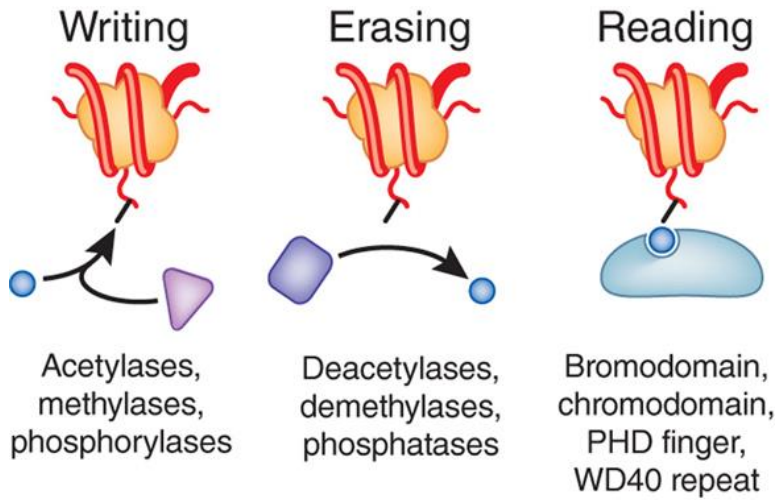
'Writers' introduce histone marks (circles), **'erasers'** take them out and **'readers'** can recognize a particular form of histone modification.
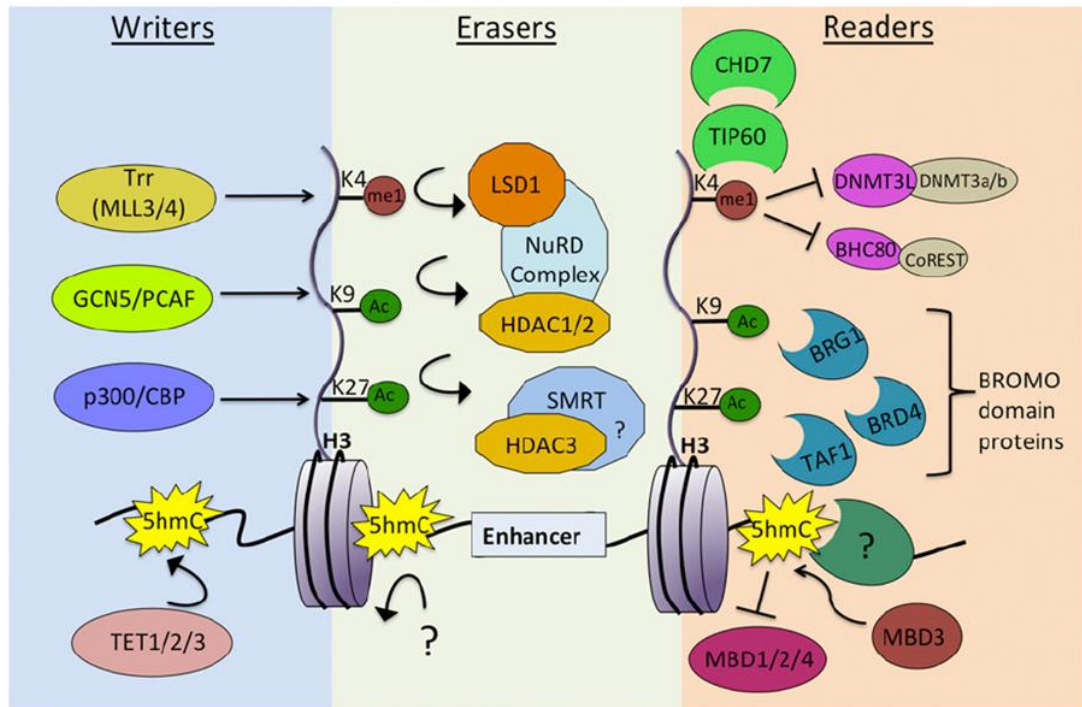
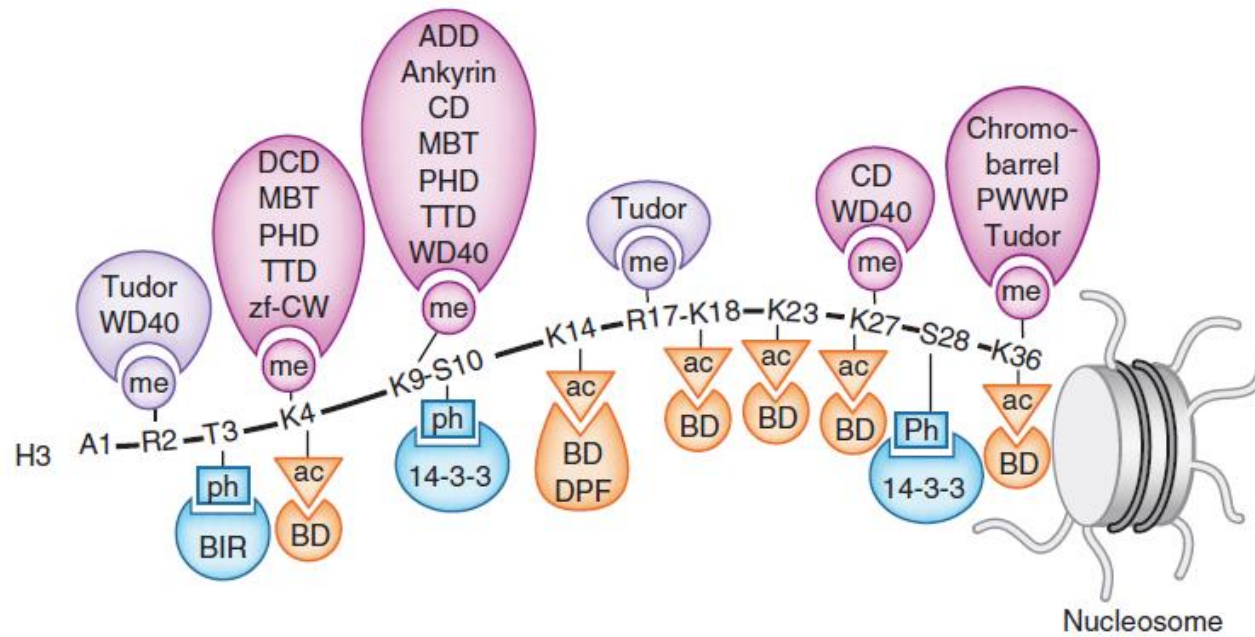**Writers, Erasers, and Readers of Major Enhancers Marks**

Proteins capable of adding (writers), removing (erasers), and recognizing (readers) major enhancer-associated chromatin modifications, including H3K4me1, H3K9ac, H3K27ac, and 5hmC, are shown.

Polycomb chromodomain	HP1 chromodomain	CHD1 chromodomain	BPTF/ING2 PHD finger	Bromodomain
H3K27me3	H3K9me3	H3K4me3	H3K4me3	H3/4Kac

Examples of reading histones in which particular **domains** occurring in many of the non-histone-associated proteins are known to **bind specific modifications of chromatin**.

a Effect of multiple PTMs

b Combinatorial readout in *cis*

c Combinatorial readout in *trans*
Intranucleosomal
Internucleosomal

d Multisubunit complex

Combinatorial readout of PTMs

(a–c) Recognition of a target PTM is influenced by adjacent PTMs on the same histone tail (a) and the combined action of multiple readers within the same protein (b,c). (d) Multivalent engagement of readers within individual subunits of the complex. The **reader-harboring proteins can also contain the catalytic domains (which act as writers and erasers) or scaffolding domains that bridge their host proteins with other subunits of the complex. Readers can recognize PTMs on a single histone tail (cis mechanism) or different histone tails (trans mechanism).**

Readers of histone PTMs

Recognition of the methylated (me) lysine, methylated (me) arginine, acetylated (ac) lysine and phosphorylated (ph) serine and threonine residues of the N-terminal histone H3 tail by indicated readers.

Table 1 Histone readers and their target PTMs

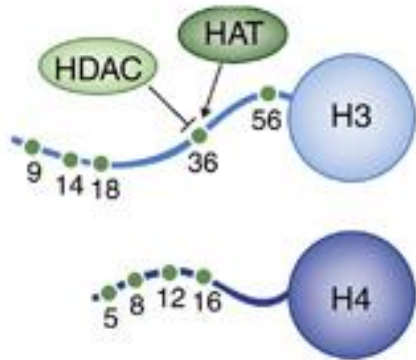| Recognition of	Reader	Histone PTM
Methyllysine	ADD	H3K9me3
	Ankyrin	H3K9me2, H3K9me1
	BAH	H4K20me2
	Chromo-barrel	H3K36me3, H3K36me2, H4K20me1, H3K4me1
	Chromodomain	H3K9me3, H3K9me2, H3K27me3, H3K27me2
	DCD	H3K4me3, H3K4me2, H3K4me1
	MBT	H3Kme1, H3Kme2, H4Kme1, H4Kme2
	PHD	H3K4me3, H3K4me2, H3K9me3
	PWWP	H3K36me3, H4K20me1, H4K20me3, H3K79me3
	TTD	H3K4me3, H3K9me3, H4K20me2
	Tudor	H3K36me3
	WD40	H3K27me3, H3K9me3
	zf-CW	H3K4me3
Methylarginine	ADD	H4R3me2s
	Tudor	H3Rme2, H4Rme2
	WD40	H3R2me2
Acetyllysine	Bromodomain	H3Kac, H4Kac, H2AKac, H2BKac
	DBD	H3KacKac, H4KacKac
	DPF	H3Kac
	Double PH	H3K56ac
Phosphoserine or	14-3-3	H3S10ph, H3S28ph
phosphothreonine	BIR	H3T3ph
	Tandem BRCT	H2AXS139ph
Unmodified histone	ADD	H3un
	PHD	H3un
	WD40	H3un

Histone-Modifying Enzymes
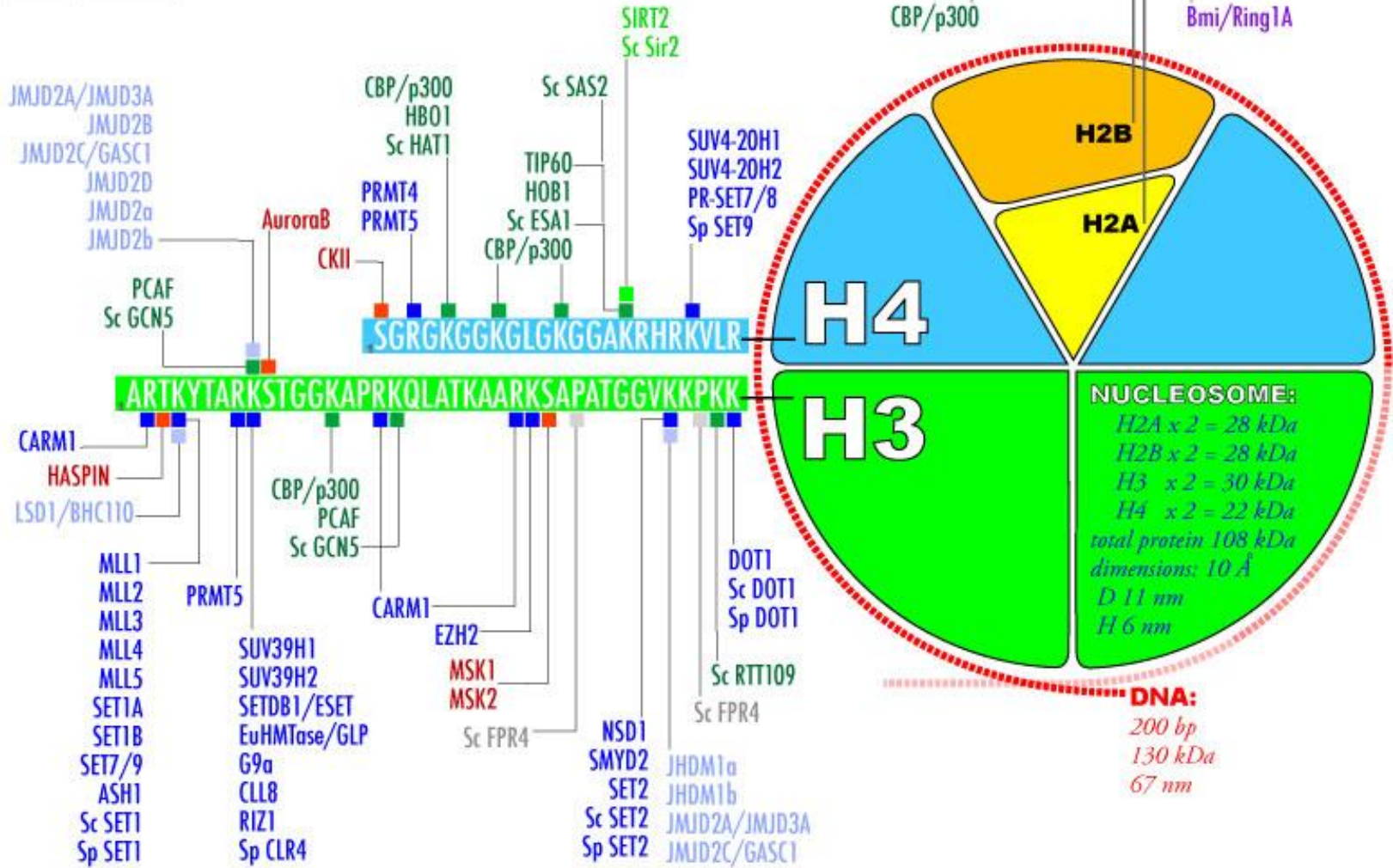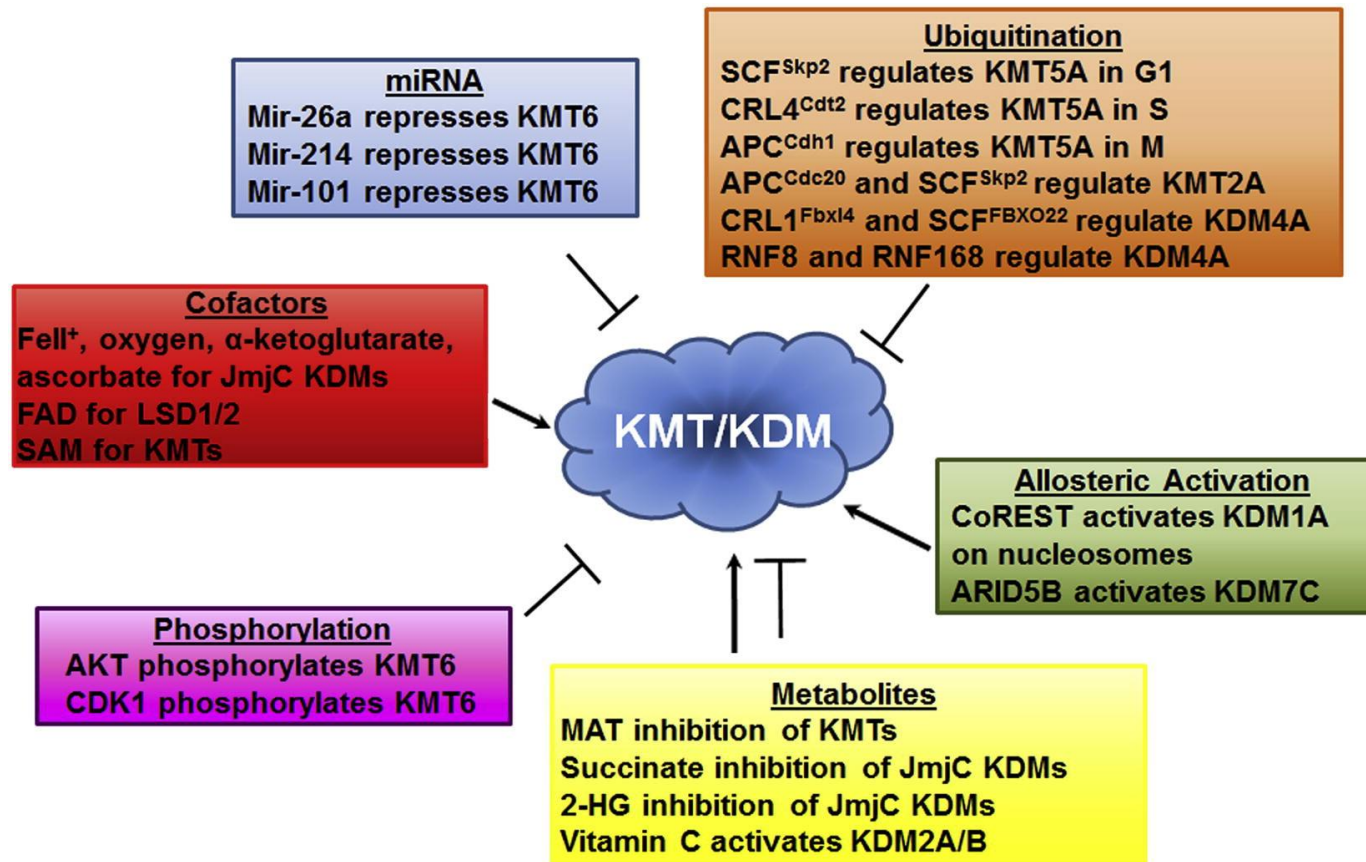
KMTs and KDMs Are Dynamically Regulated through Multiple Mechanisms
KMTs and KDMs are subject to inhibition or activation through the regulatory mechanisms that are indicated.
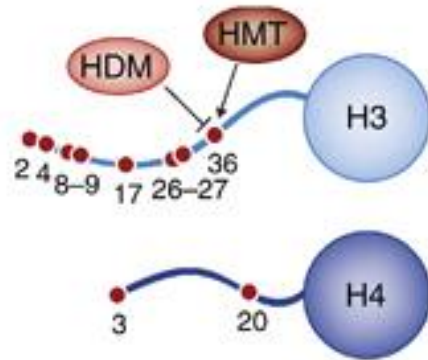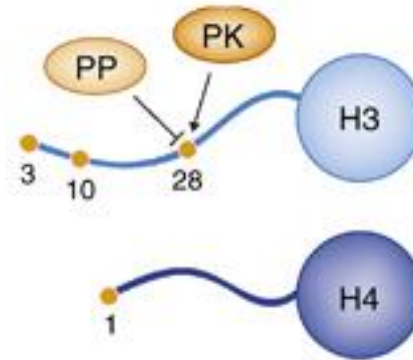**KMTs: lysine methyltransferases; KDMs: lysine demethylases**

Summary of well-understood histone modifications and histone-modifying enzymes

(a) **Histone acetylation at numerous lysine residues** on histone tails is **catalyzed by histone acetyltransferases (HATs) and removed by histone deacetlyases (HDACs).** Histone acetylation is generally a transcriptionally permissive mark. Different HAT and HDAC enzymes are listed below. Importantly, specific HDACs isoforms are differentially expressed across brain structures and appear to uniquely regulate different aspects of cognition.

(b) **Histone methylation at lysine and arginine residues** on histone tails is **catalyzed by histone methyltransferases (HMTs) and removed by histone demethylases (HDMs).** Histone methylation at different amino acid residues has been linked to both transcriptional activation and transcriptional repression. Methylation can occur in mono-, di-, or even tri-methylated states. Many HDMs and HMTs are specific for modifications at individual amino acids on histone tails or even a specific number of methyl groups.

(c) **Histone phosphorylation at serine residues** is **catalyzed by protein kinases (PKs) such as mitogen- and stress-activated protein kinase 1 (MSK1), whereas phosphorylation marks are removed by protein phosphatases such as protein phosphatase 1 (PP1).** Histone phosphorylation is generally linked to transcriptional activation.

Table 1. Chromatin Modifications, Readers, and Their Function

| Chromatin Modification	Nomenclature	Chromatin-Reader Motif	Attributed Function
DNA Modifications			
5-methylcytosine	5mC	MBD domain	transcription
5-hydroxymethylcytosine	5hmC	unknown	transcription
5-formylcytosine	5fC	unknown	unknown
5-carboxylcytosine	5caC	unknown	unknown
Histone Modifications			
Acetylation	K-ac	BromodomainTandem, PHD fingers	transcription, repair, replication, and condensation
Methylation (lysine)	K-me1, K-me2, K-me3	Chromodomain, Tudor domain, MBT domain, PWWP domain, PHD fingers, WD40/β propeller	transcription and repair
Methylation (arginine)	R-me1, R-me2s, R-me2a	Tudor domain	transcription
Phosphorylation (serine and threonine)	S-ph, T-ph	14-3-3, BRCT	transcription, repair, and condensation
Phosphorylation (tyrosine)	Y-ph	SH2[a]	transcription and repair
Ubiquitylation	K-ub	UIM, IUIM	transcription and repair
Sumoylation	K-su	SIM[a]	transcription and repair
ADP ribosylation	E-ar	Macro domain, PBZ domain	transcription and repair
Deimination	R→Cit	unknown	transcription and decondensation
Proline isomerisation	P-cis⇔P-trans	unknown	transcription
Crotonylation	K-cr	unknown	transcription
Propionylation	K-pr	unknown	unknown
Butyrylation	K-bu	unknown	unknown
Formylation	K-fo	unknown	unknown
Hyroxylation	Y-oh	unknown	unknown
O-GlcNAcylation (serine and threonine)	S-GlcNAc; T-GlcNAc	unknown	transcription

Modifications: me1, monomethylation; me2, dimethylation; me3, trimethylation; me2s, symmetrical dimethylation; me2a, asymmetrical dimethylation; and Cit, citrulline. Reader domains: MBD, methyl-CpG-binding domain; PHD, plant homeodomain; MBT, malignant brain tumor domain; PWWP, proline-tryptophan-tryptophan-proline domain; BRCT, BRCA1 C terminus domain; UIM, ubiquitin interaction motif; IUIM, inverted ubiquitin interaction motif; SIM, sumo interaction motif; and PBZ, poly ADP-ribose binding zinc finger.

[a]These are established binding modules for the posttranslational modification; however, binding to modified histones has not been firmly established.

TABLE 1 Some important enzymes involved in epigenetic modifications

Enzyme	Type of modification	Effect on gene expression
DNMT1, DNMT3L, DNMT3A, DNMT3B	Maintenance and de novo DNA methylation	Gene expression suppression
TET family	DNA demethylation	Induction of gene expression
IDH family	DNA demethylation	Induction of gene expression
HMTs	Methylation of lysine in histone protein	H3K4me3 → transcription activation; H3K9me or H3K27me → transcription repression
HDMs	Demethylation of lysine in histone protein	Transcription activation or repression based on the lysine residue
HATs	Histone acetylation	Transcription activation
HDACs classes I-IV	Histone deacetylation	Transcription repression
SIRT1-7	Histone deacetylation	Transcription repression
MSK1 (RPS6KA5)	Histone phosphorylation	Transcription activation

DNMT, DNA methyltransferase; TET, ten eleven translocation; IDH, isocitrate dehydrogenase; HMT, histone methyltransferase; HDM, histone demethylase; HAT, histone acetyltransferase; HDAC, histone deacetylase; SIRT, sirtuin; MSK1, mitogen- and stress-activated protein kinase-1; RPS6KA5, ribosomal protein S6 kinase, 90kDa, polypeptide 5.
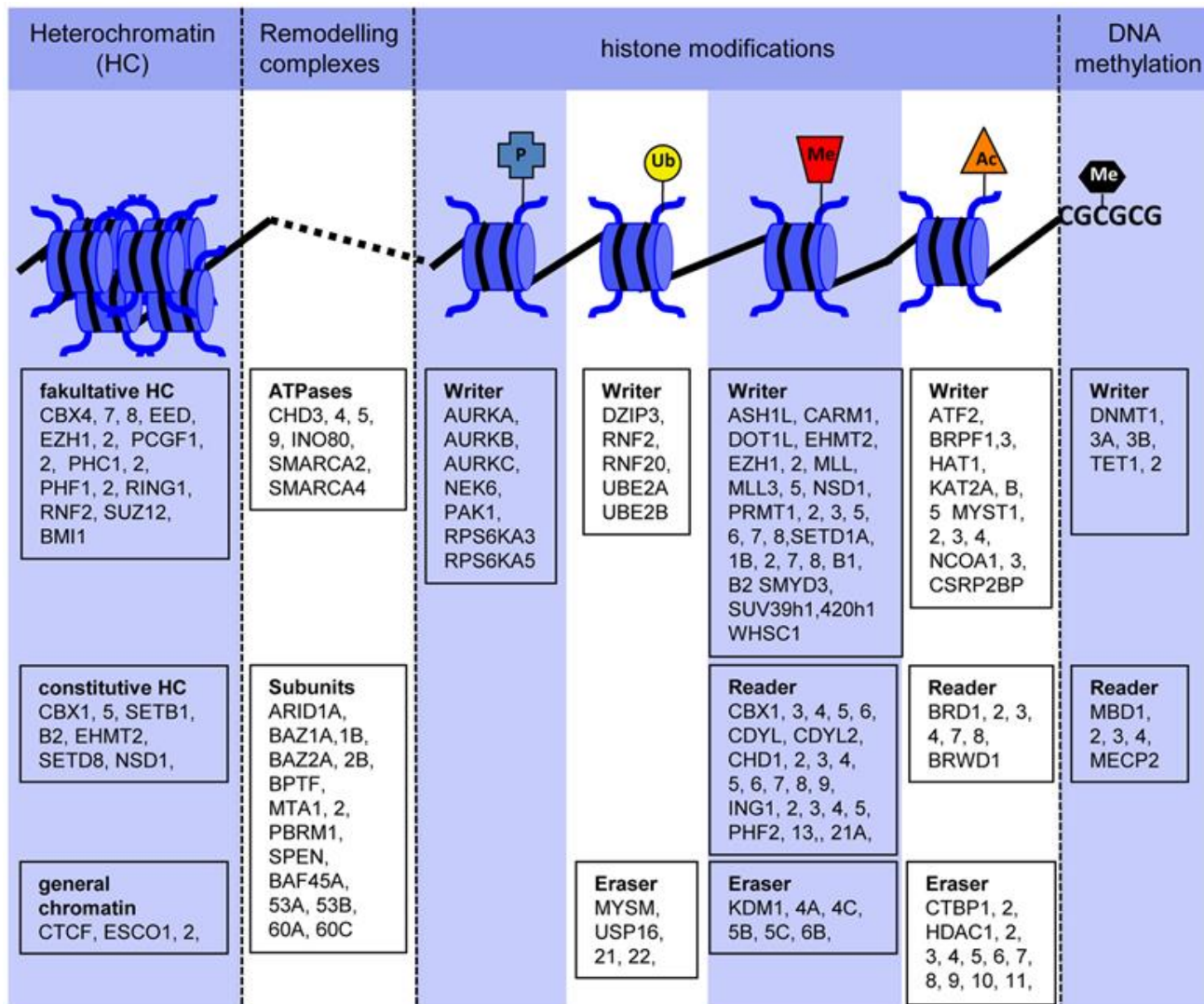
Compilation of 150 representative human genes involved in epigenetic regulation

The genes were identified, selected, and classified according to their function based on published literature and database searches. In a further step, the genes related to histone modifications were sub-classified as writers (coding for modifying enzymes such as methyltransferases), as erasers (coding for de-modifying enzymes such as histone deacetylases) and as readers (coding for proteins that bind to the respective modification).

Gene activator recruits histone acetyltransferase
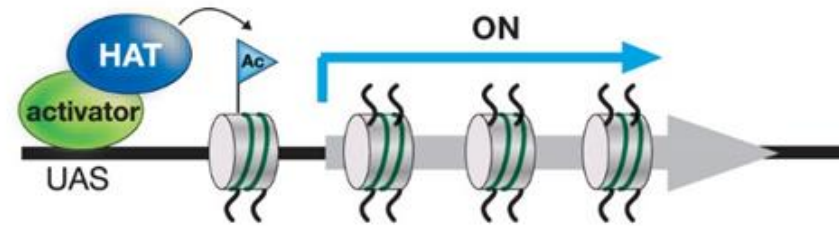
Gene repressor recruits histone deacetylase
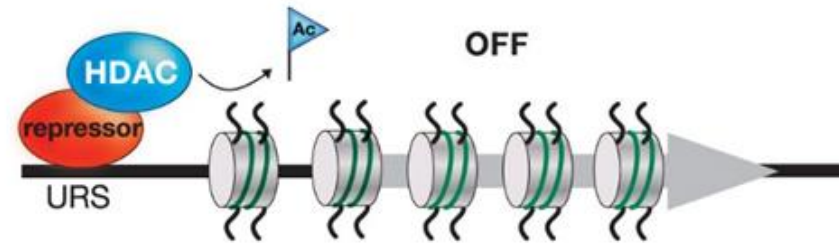
Figure 2. Histone-modifying Enzymes Are Recruited to Promoters by DNA-binding Transcription Factors

Histone acetyltransferases (HAT) are recruited by activators that bind to specific upstream activating sequences (UAS). This enzyme catalyzes the acetylation of local histones, known to contribute to transcriptional activation. Histone deacetylases (HDAC) are recruited by repressors of transcription that bind to upstream repressive sequences (URS) and deacetylate local histones. This contributes to transcriptional repression.
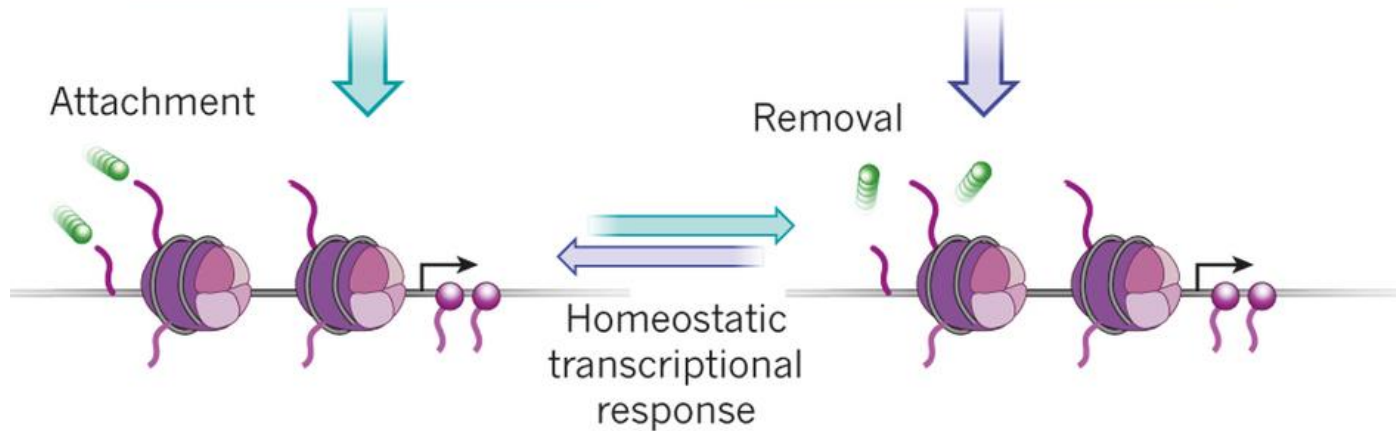
DNA methylation and post-translational modifications of histones link metabolites and transcription

Changes in nutrition or fluctuations in metabolism induce homeostatic transcriptional responses. **Several intermediary metabolism products change enzymatic activity of chromatin-associated proteins in a dose-dependent manner. 'Writer' enzymes that attach marks covalently to chromatin or DNA and 'erasers' that remove these modifications act as metabolic sensors.** Chromatin modifications remodel DNA–histone interactions and help to regulate the recruitment of transcriptional complexes to genes that control cellular function and survival.
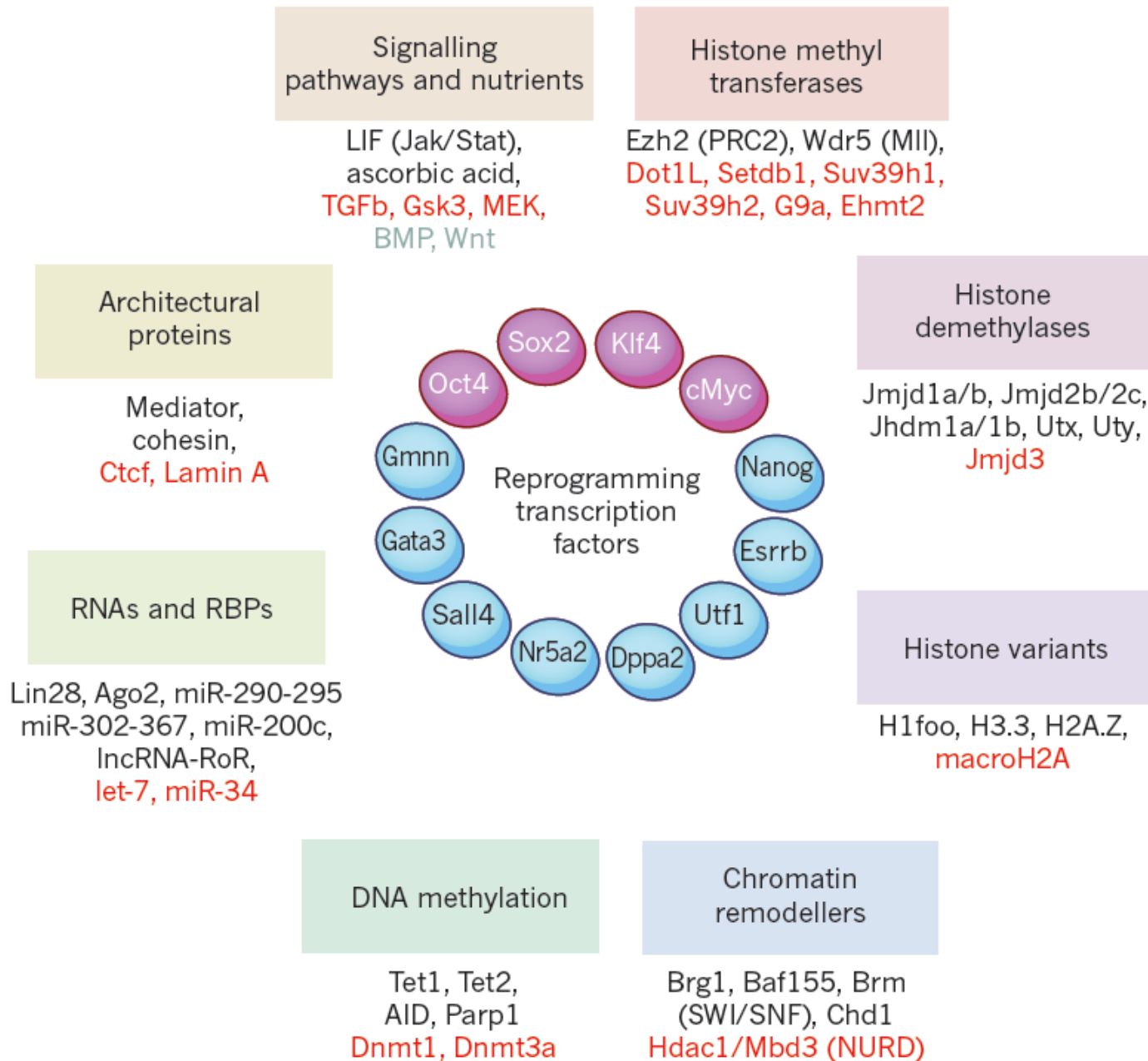
DNMT, DNA methyltransferases; FAD, flavin adenine dinucleotide; HDACs, histone deacetylases; HMTs, histone methyltransferases; KATs, lysine acetyltransferases; KDMs, lysine demethylases; O-GlcNAc, O-linked *N*-acetylglucosamine; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; β-OHB, β-hydroxybutyrate; SAM, *S*-adenosylmethionine; TET, ten-eleven translocation protein.

Signalling pathways and nutrients

LIF (Jak/Stat), ascorbic acid, TGFb, Gsk3, MEK, BMP, Wnt

Histone methyl transferases

Ezh2 (PRC2), Wdr5 (Mll), Dot1L, Setdb1, Suv39h1, Suv39h2, G9a, Ehmt2

Architectural proteins

Mediator, cohesin, Ctcf, Lamin A

Histone demethylases

Jmjd1a/b, Jmjd2b/2c, Jhdm1a/1b, Utx, Uty, Jmjd3

RNAs and RBPs

Lin28, Ago2, miR-290-295 miR-302-367, miR-200c, lncRNA-RoR, let-7, miR-34

Histone variants

H1foo, H3.3, H2A.Z, macroH2A

DNA methylation

Tet1, Tet2, AID, Parp1 Dnmt1, Dnmt3a

Chromatin remodellers

Brg1, Baf155, Brm (SWI/SNF), Chd1 Hdac1/Mbd3 (NURD)

Reprogramming transcription factors: Sox2, Klf4, cMyc, Oct4, Nanog, Gmnn, Esrrb, Gata3, Utf1, Sall4, Nr5a2, Dppa2

Interplay between reprogramming factors and molecules influencing chromatin state

Different **transcription factors** that have been shown to **trigger induced pluripotency, with the most broadly used combination (Oct4, Klf4, Sox2, c-Myc) highlighted**. Some molecules have been shown to **facilitate (black) or inhibit (red) reprogramming**. Bone morphogenetic proteins (BMPs) and Wnts have stage-dependent enhancing or suppressive roles during iPSC formation.
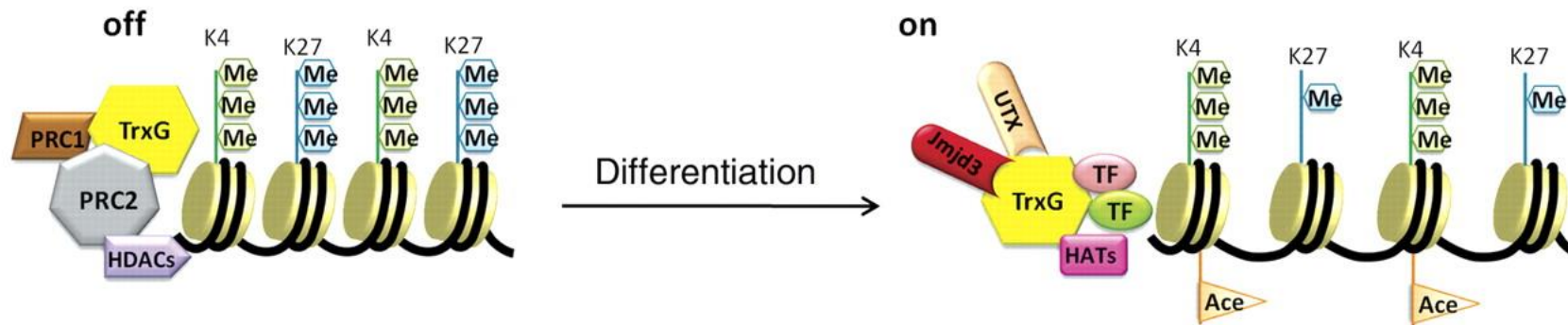
RBPs: RNA binding proteins; iPSC: induced pluripotent stem cell.

Epigenetic control of cellular differentiation/
Epigenetska kontrola celične diferenciacije

A Pluripotency genes
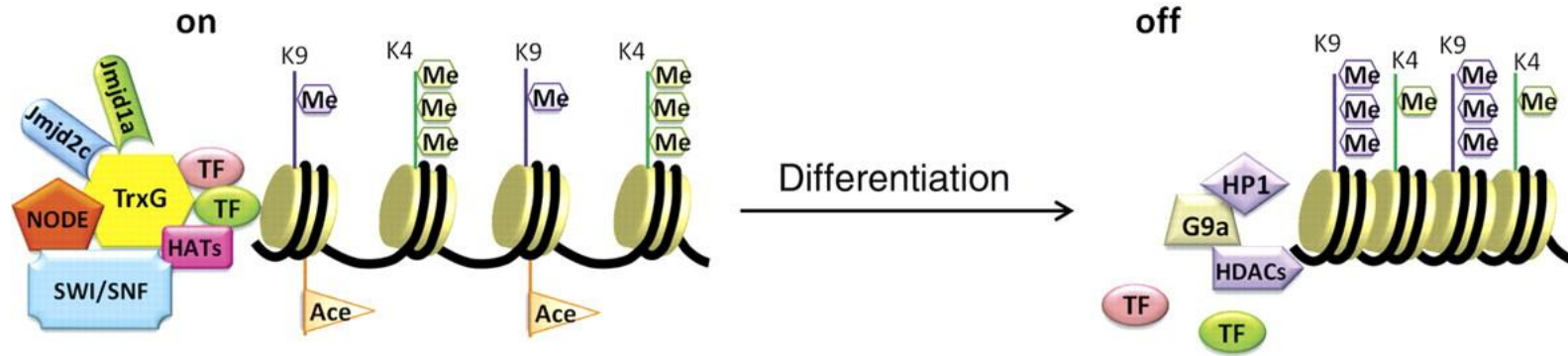
B Lineage-committed genes

Epigenetic control of stem cell differentiation

Appropriate differentiation requires a differential regulation of pluripotency genes and lineage-committed genes.

(A) Pluripotency genes are silenced during differentiation.

(B) *Lineage-committed genes often show bivalent modification in embryonic stem cells. Upon differentiation the lineage-committed gene needs to be derepressed*, whereas other lineages need to be suppressed. However, some genes that are required during later time points may remain in a bivalent state, allowing activation at the appropriate time point.

Current Opinion in Cell Biology
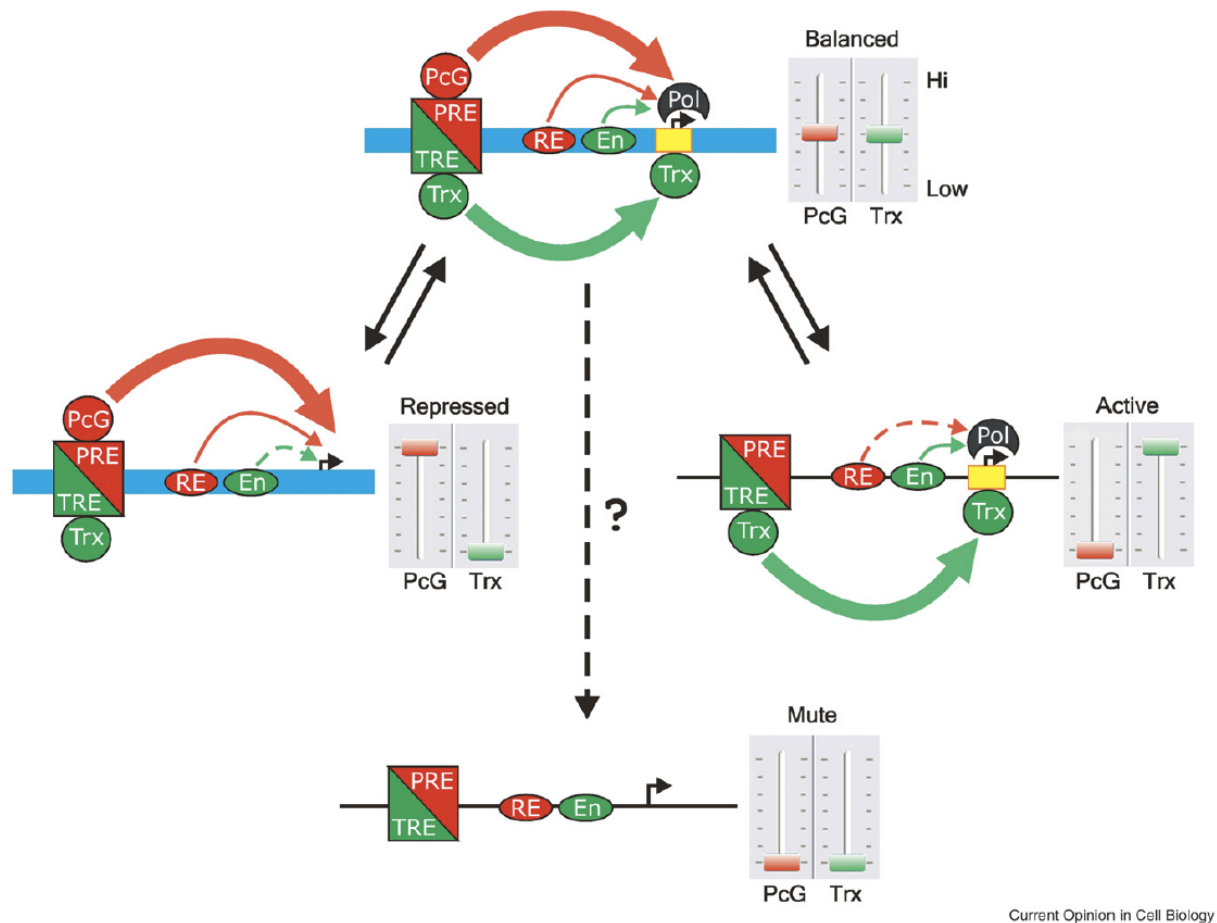
Switching epigenetic states with PcG/trxG regulatory system

Accumulated evidence suggests that the **PcG/Trx regulatory system is better viewed as a pair of interdependent, gradually adjustable regulators (shown here as sliders) rather then a binary ON/OFF switch.** Although we expect that PcG/Trx regulation can assume a continuum of states characterized by different relative levels of PcG and Trx function exerted upon a target gene (displayed as a position of corresponding sliders between 'High' and 'Low' settings) the **figure illustrates four basic states defined as 'Balanced', 'Repressed', 'Active' and 'Mute'.** In the 'balanced' state the target gene is embedded in broad domain of H3K27me3 (indicated in blue), yet its promoter region is marked at the same time with H3K4me3 (yellow rectangle) and binds RNA pol II (black cap). This state results from the balanced action of PcG/Trx complexes bound at the **PRE/TRE** as well as an input from other **repressor (RE) and enhancer (En) elements**. Upon differentiation, the 'Balanced' state frequently transits into fully repressed or fully active states. Later, this transition can be reversed and the states re-programed. The 'Repressed' state is characterized by broad H3K27 trimethylation of target genes and absence of RNA pol II, COMPASS, and H3K4me3 from the promoter. Note that in this state, Trx can still bind at the PRE/TRE. The 'Active' state is marked by the presence of abundant activators, strong transcriptional activity, and binding of the COMPASS complex with H3 K4 trimethylation in the promoter region. In the 'Mute' state neither regulators nor RNA pol II associate with the gene, which correlates with the absence of both H3K27me3 and H3K4me3. How this state is achieved is not known.

PcG: Polycomb; trxG: Trithorax

Occurrence of TF binding sites can determine chromatin dynamics

Sequence-specific DNA-binding proteins including transcription factors (TFs) are key determinants of gene regulation and chromatin architecture.
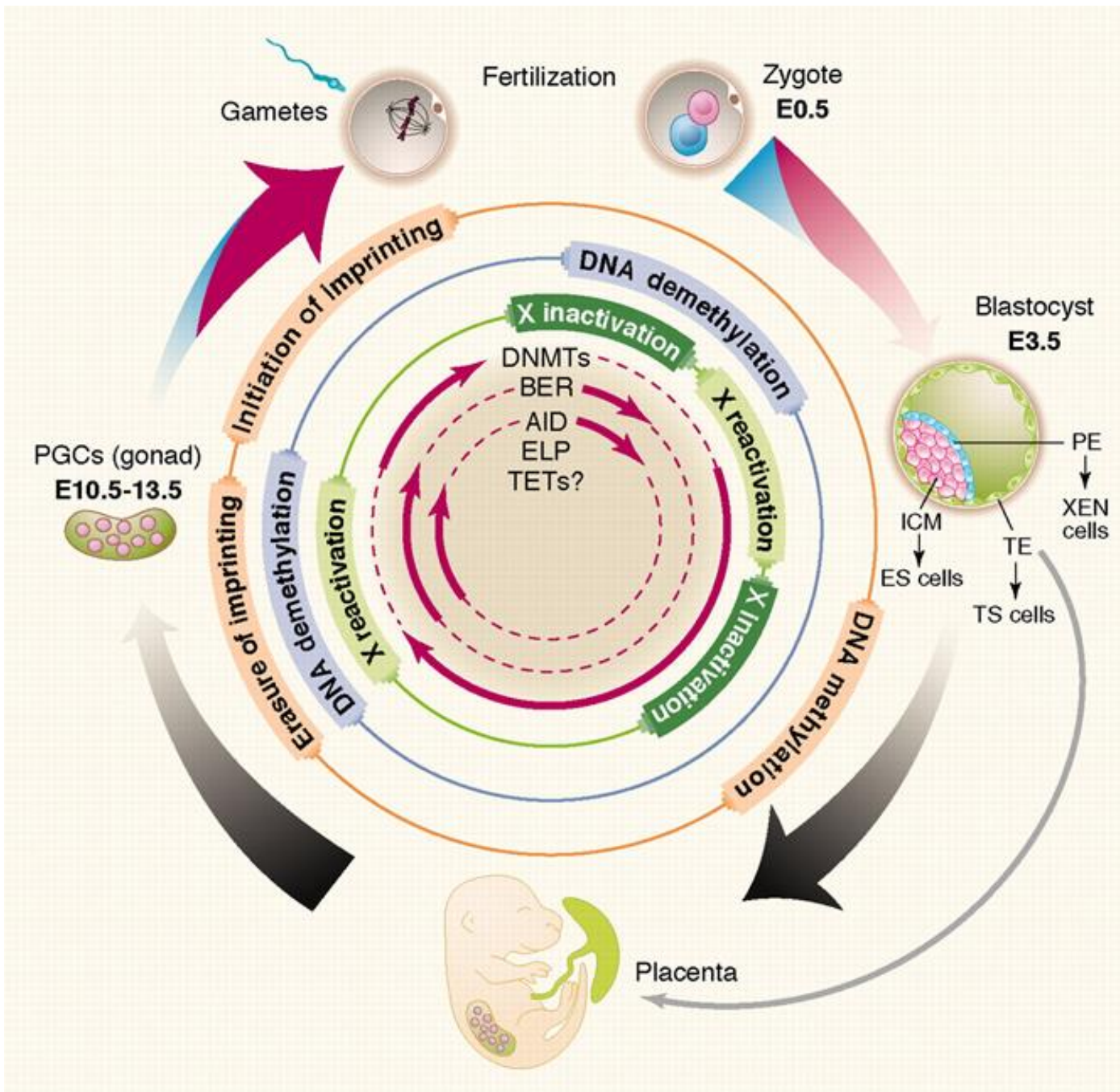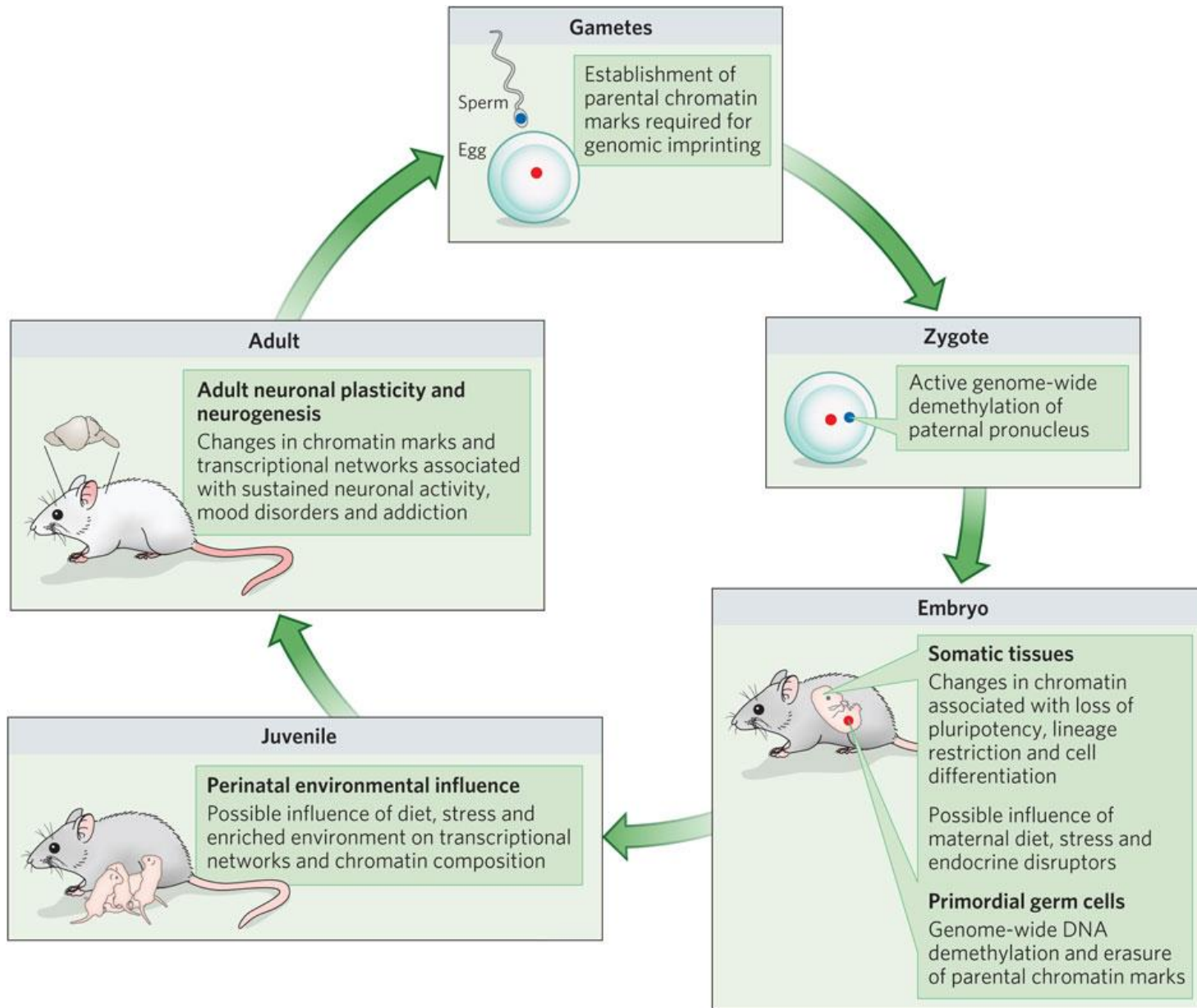
Epigenetic Reprogramming in Animal Development
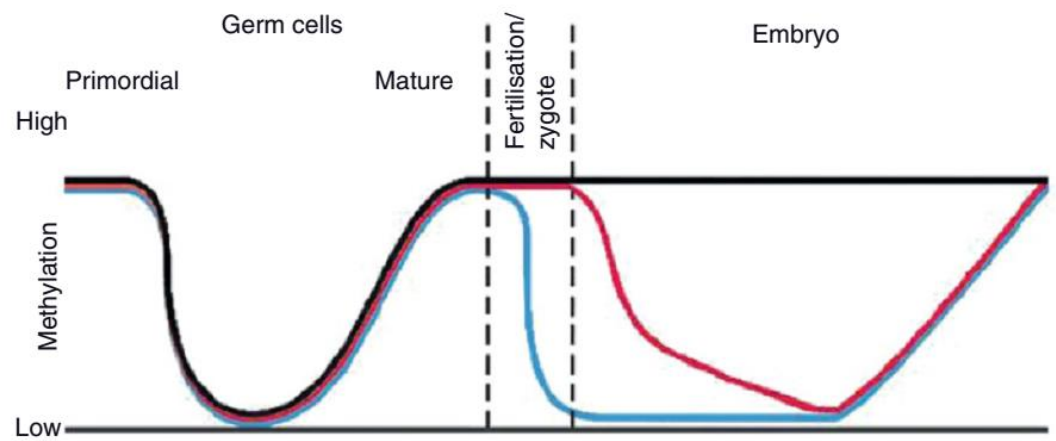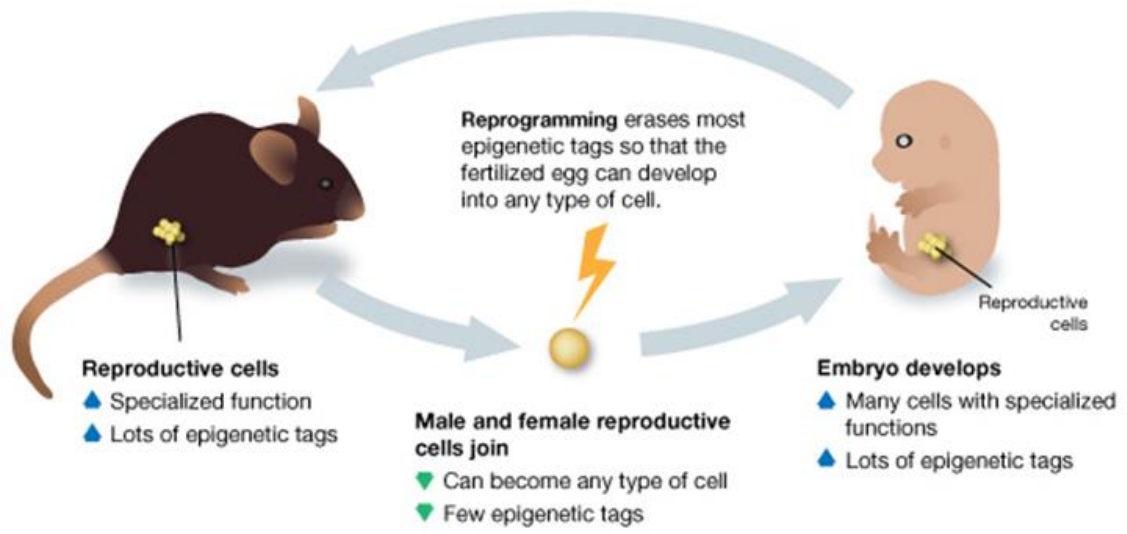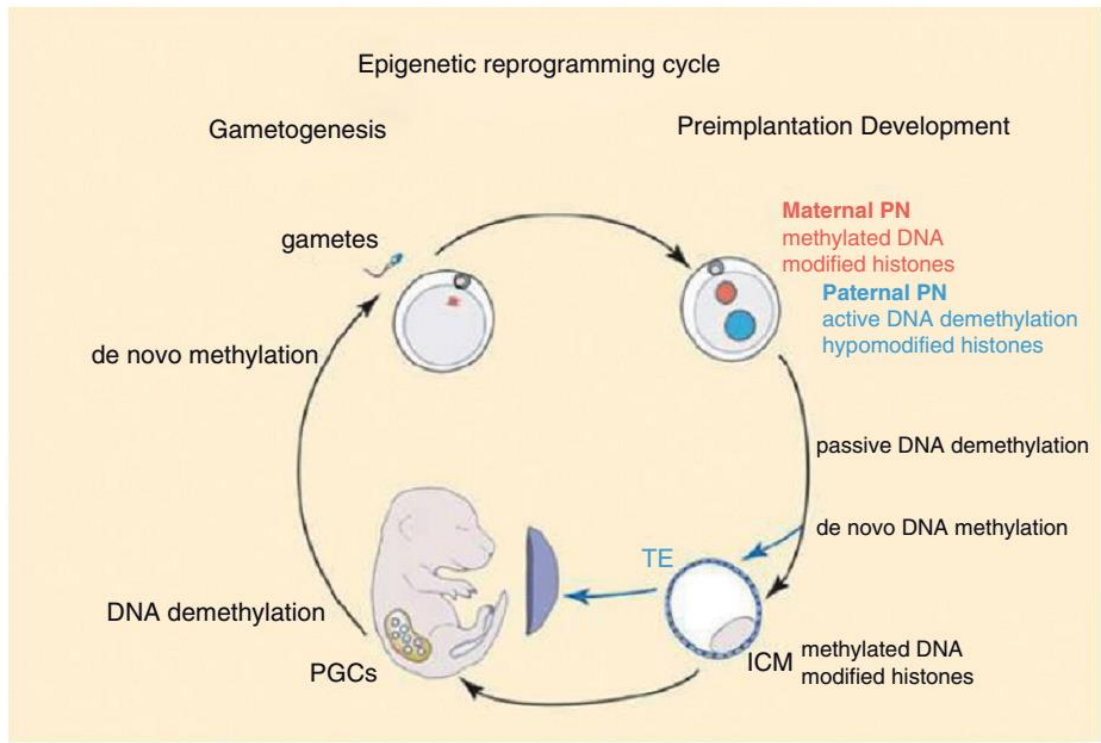
Figure 4.2 Epigenetic reprogramming cycle. Methylation on maternal and paternal DNA during gametogenesis and first steps of embryogenesis.
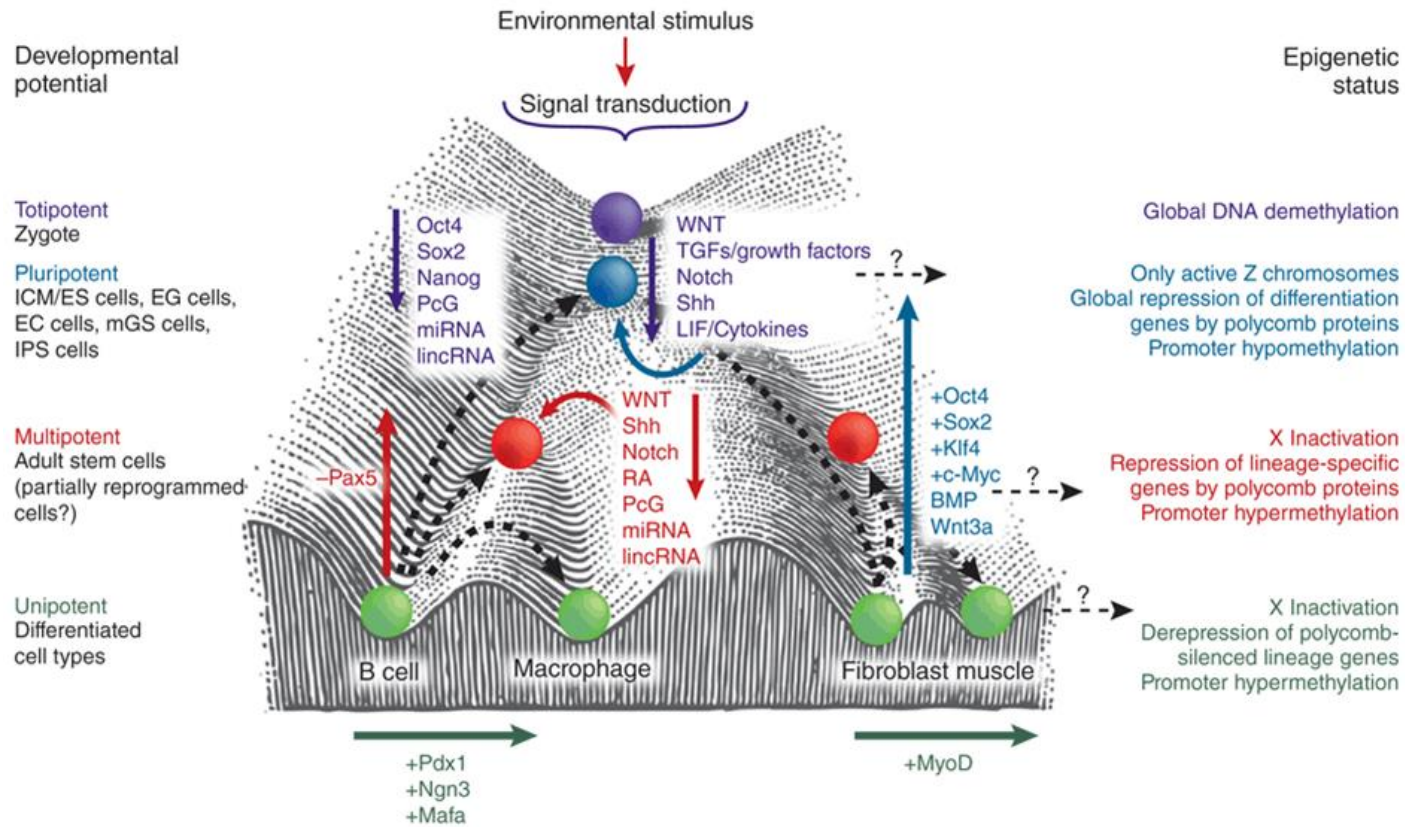
Gametes

Establishment of parental chromatin marks required for genomic imprinting

Sperm

Egg

Zygote

Active genome-wide demethylation of paternal pronucleus

Embryo

Somatic tissues

Changes in chromatin associated with loss of pluripotency, lineage restriction and cell differentiation

Possible influence of maternal diet, stress and endocrine disruptors

Primordial germ cells

Genome-wide DNA demethylation and erasure of parental chromatin marks

Juvenile

Perinatal environmental influence

Possible influence of diet, stress and enriched environment on transcriptional networks and chromatin composition

Adult

Adult neuronal plasticity and neurogenesis

Changes in chromatin marks and transcriptional networks associated with sustained neuronal activity, mood disorders and addiction

Contribution of various chromatin-remodelling events throughout the life of an organism

Chromatin modifications occurring at different time points during the life of an organism have been associated with various short- to long-lasting regulatory events that affect the development and the function of the brain and other tissues.

Developmental potential

Totipotent
Zygote

Pluripotent
ICM/ES cells, EG cells,
EC cells, mGS cells,
IPS cells

Multipotent
Adult stem cells
(partially reprogrammed
cells?)

Unipotent
Differentiated
cell types

Environmental stimulus

Signal transduction

Oct4
Sox2
Nanog
PcG
miRNA
lincRNA

WNT
TGFs/growth factors
Notch
Shh
LIF/Cytokines

WNT
Shh
Notch
RA
PcG
miRNA
lincRNA

−Pax5

+Oct4
+Sox2
+Klf4
+c-Myc
BMP
Wnt3a

B cell   Macrophage   Fibroblast muscle

+Pdx1
+Ngn3
+Mafa

+MyoD

Epigenetic status

Global DNA demethylation

Only active Z chromosomes
Global repression of differentiation
genes by polycomb proteins
Promoter hypomethylation

X Inactivation
Repression of lineage-specific
genes by polycomb proteins
Promoter hypermethylation

X Inactivation
Derepression of polycomb-
silenced lineage genes
Promoter hypermethylation

Waddington's epigenetic landscape is a metaphor for how gene regulation determines development.

Depiction of potential cell signaling in Waddington's model of epigenetic determination of development

Colored marbles correspond to differentiation states. Arrows represent the directionality of factor influence for development with '+' indicating addition and '–' indicating removal of a given factor or signal. The downward blue arrow at the top left of the 'hill' reflects direction of normal development, whereas the upward blue arrow at the bottom right of the hill depicts the direction of cellular reprogramming during generation of iPSCs. Coloring of text for names of factors and signaling pathways correspond to their function within the given developmental stage.
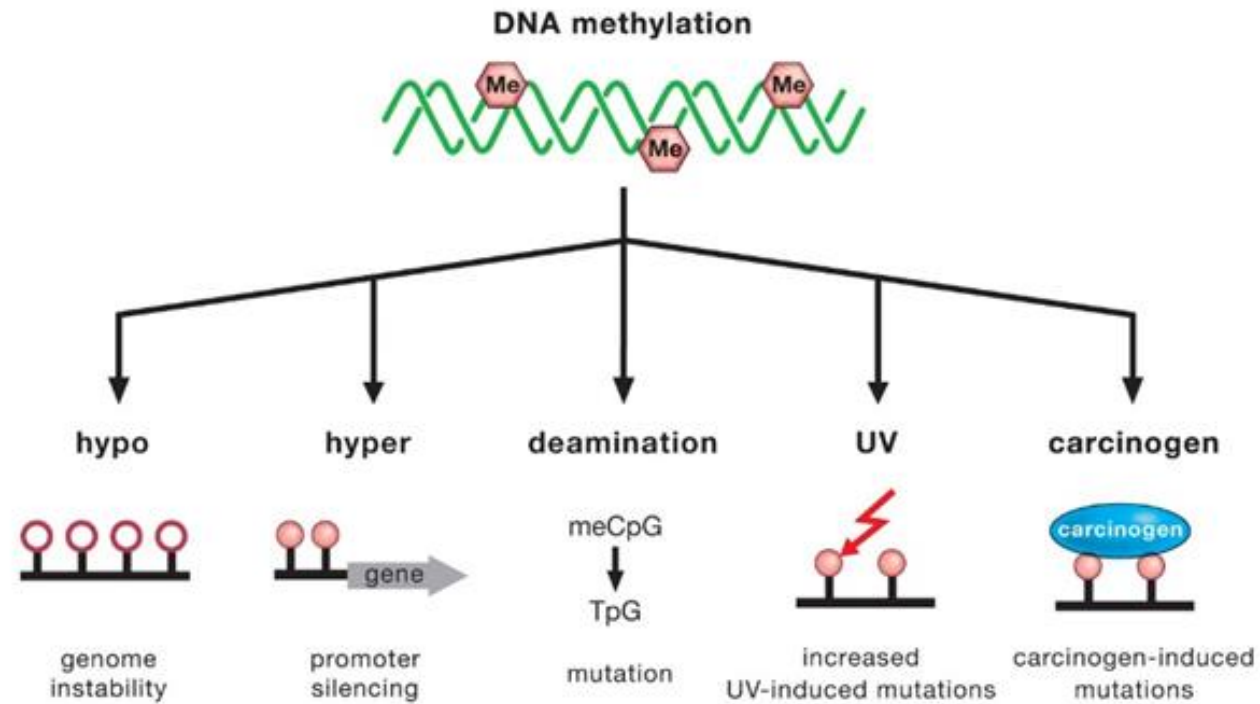
Epigenetske spremembe in bolezni

CHROMATIN RELATED DISEASES
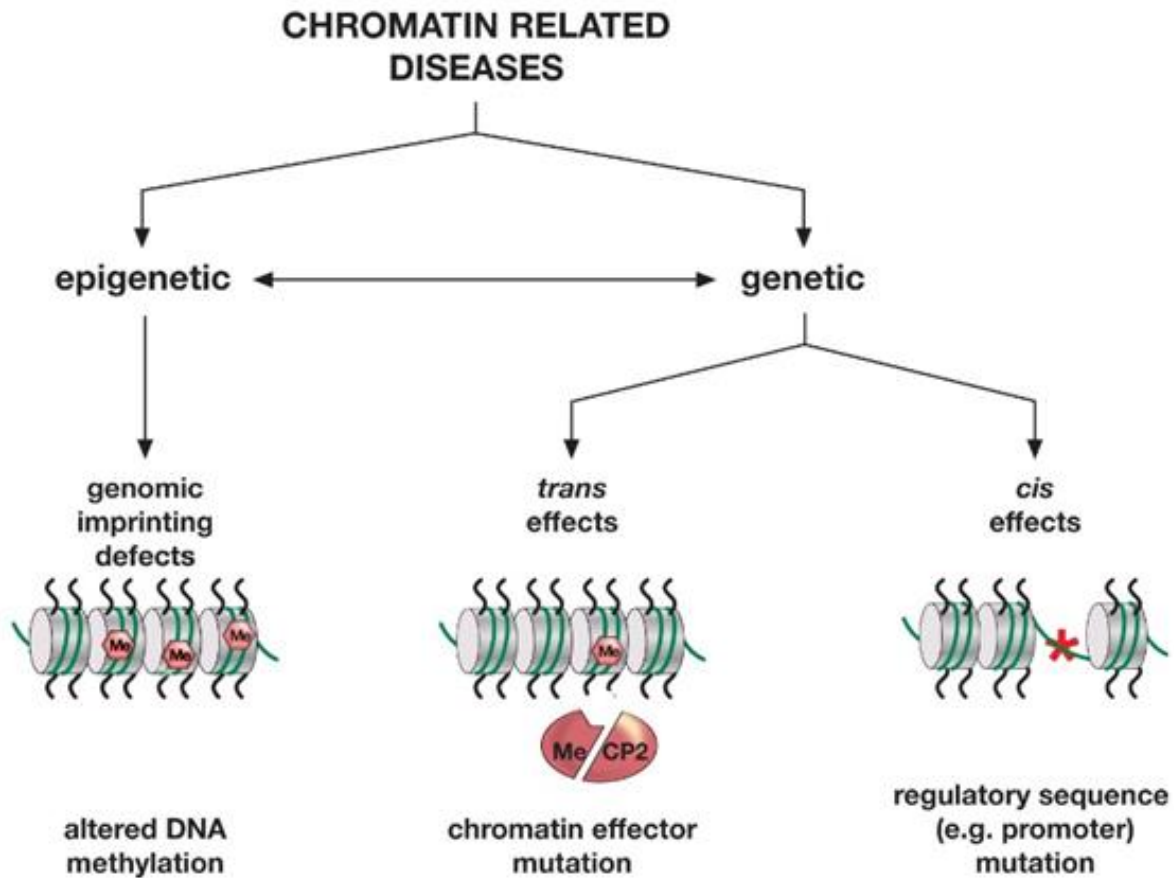
Figure 1. Genetic and Epigenetic Mechanisms Underlying Chromatin-related Disorders
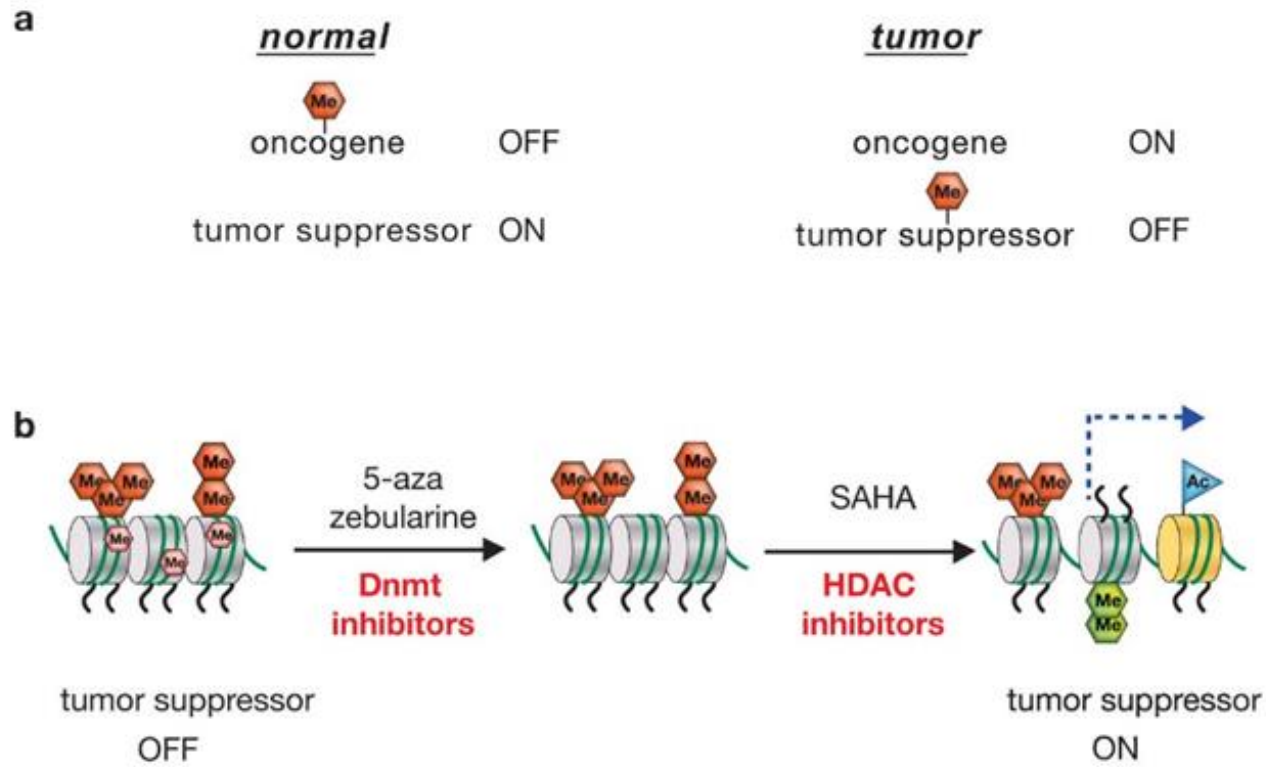
Epigenetic mechanisms typically involve the alteration of DNA methylation or chromatin at imprinted loci, so disrupting monoallelic expression. Genetic mechanisms can be categorized into two classes. *trans* effects include the loss or dysfunction of chromatin-associated factors which can in turn alter chromatin structure and gene expression at certain genomic regions. *cis* effects represent mutations in noncoding regions that may be necessary for regulation. These mutations, which may include the expansion of DNA repeats, can lead to chromatin alterations which affect genome stability and gene expression.

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DNA methylation

hypo   hyper   deamination   UV   carcinogen

genome instability   promoter silencing   meCpG → TpG mutation   increased UV-induced mutations   carcinogen-induced mutations

Figure 1. Epigenetic Alterations
**Involving DNA Methylation Can Lead to Cancer by Various Mechanisms**

Loss of DNA cytosine methylation (hypo) results in genome instability. Focal hypermethylation in gene promoters (hyper) causes heritable silencing and therefore inactivation of tumor suppressor genes. Additionally, methylated CpG sites are hotspots for C→T transition mutations caused by spontaneous hydrolytic deamination. Methylation of CpG sites also increases the binding of some chemical carcinogens to DNA and increases the rate of UV-induced mutations.

Epigenetics © 2006 Cold Spring Harbor Laboratory Press

Figure 19. Epigenetic Modifications in Cancer

(a) Aberrant epigenetic marks at cancer-causing loci typically involve the derepression of onco-genes or silencing of tumor suppressor genes. Epigenetic marks known to alter a normal cell include DNA methylation, repressive histone methylation, and histone deacetylation. (b) The use of epigenetic therapeutic agents for the treatment of cancer has consequences on the chromatin template, illustrated for a tumor suppressor locus. Exposure to Dnmt inhibitors results in a loss of DNA methylation, and exposure to HDAC inhibitors results in the acquisi-tion of histone acetyl marks and subsequent downstream modifications, including active his-tone methyl marks and the incorporation of histone variants. The cumulative chromatin changes lead to gene re-expression.

Current Opinion in Cell Biology
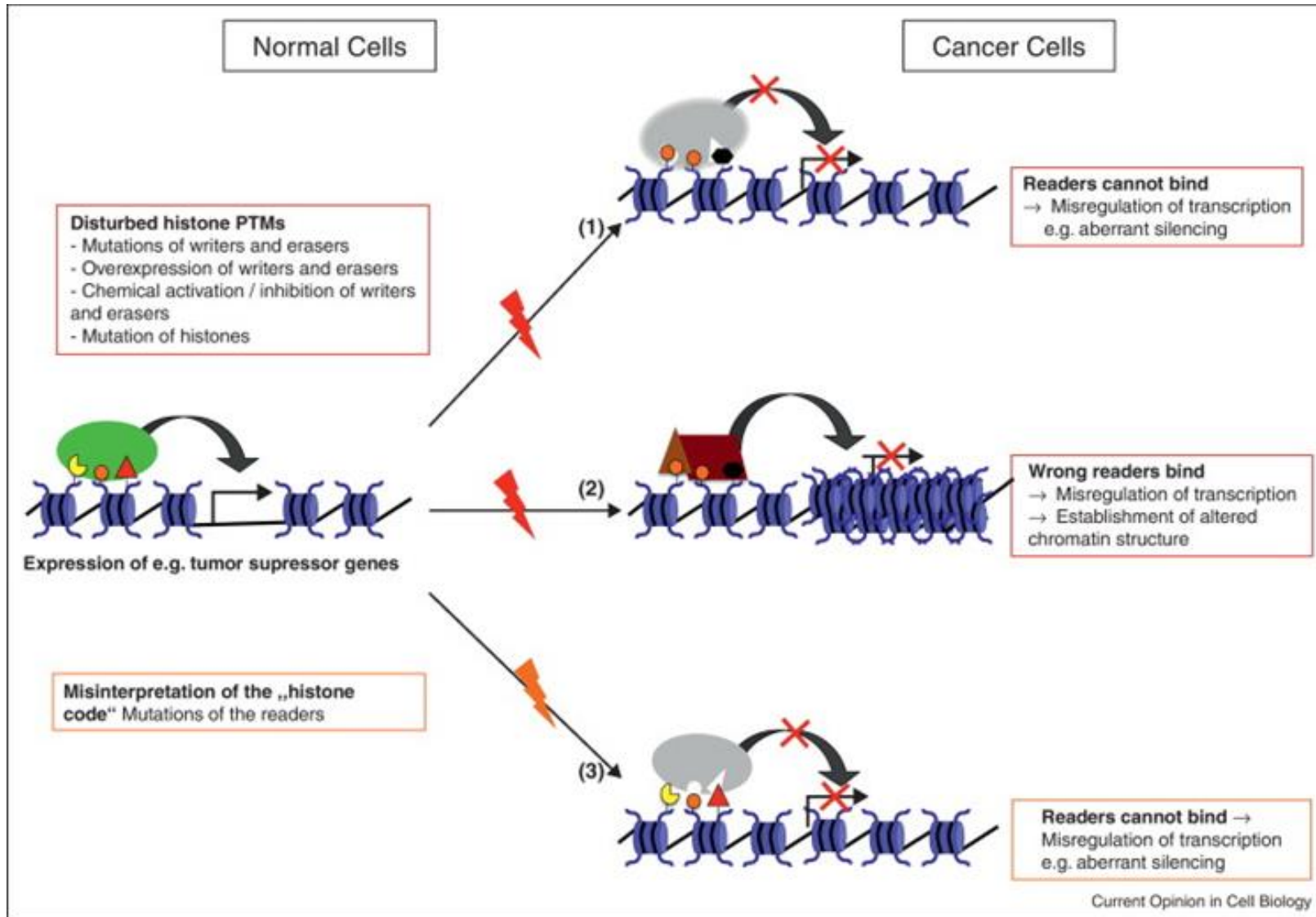
Genes can be miss-regulated by several pathways

Histone PTMs can be changed by for example genetic mutation, overexpression, chemical inhibition or activation of an epigenetic regulator (readers/writers). The consequence is that the 'histone code' at specific genomic regions (such as promoters) is altered and the appropriate reader cannot bind anymore (1), or a wrong reader is recruited, both resulting in disturbance of chromatin structure (e.g. establishment of heterochromatin causing e.g. alterations of mutation rate in the affected regions) or misregulation of genes (2). Additionally mutations of reader itself could result in a wrong interpretation of the 'histone code' and misregulation of gene expression (3). These mechanisms can, as depicted here, silence gene (e.g. tumour suppressors) but also lead to gene activation (e.g. oncogenes).

Table 1 Epigenetic modifications in human diseases

Aberrant epigenetic mark	Alteration	Consequences	Examples of genes affected and/or resulting disease
Cancer			
DNA methylation	CpG island hypermethylation	Transcription repression	*MLH1* (colon, endometrium, stomach[11]), *BRCA1* (breast, ovary[11]), *MGMT* (several tumor types[11]), *p16INK4a* (colon[11])
	CpG island hypomethylation	Transcription activation	*MASPIN* (pancreas[92]), *S100P* (pancreas[92]), *SNCG* (breast and ovary[92]), *MAGE* (melanomas[92])
	CpG island shore hypermethylation	Transcription repression	*HOXA2* (colon[20]), *GATA2* (colon[20])
	Repetitive sequences hypomethylation	Transposition, recombination genomic instability	*L1* (ref. 11), *IAP*[11], *Sat2* (ref. 107)
Histone modification	Loss of H3 and H4 acetylation	Transcription repression	*p21WAF1* (also known as *CDKN1A*)[11]
	Loss of H3K4me3	Transcription repression	*HOX* genes
	Loss of H4K20me3	Loss of heterochromatic structure	*Sat2, D4Z4* (ref. 107)
	Gain of H3K9me and H3K27me3	Transcription repression	*CDKN2A, RASSF1* (refs. 115–116)
Nucleosome positioning	Silencing and/or mutation of remodeler subunits	Diverse, leading to oncogenic transformation	*BRG1, CHD5* (refs. 127–131)
	Aberrant recruitment of remodelers	Transcription repression	*PLM-RARa*[103] recruits NuRD
	Histone variants replacement	Diverse (promotion cell cycle/destabilization of chromosomal boundaries)	H2A.Z overexpression/loss
Neurological disorders			
DNA methylation	CpG island hypermethylation	Transcription repression	Alzheimer's disease (*NEP*)[135]
	CpG island hypomethylation	Transcription activation	Multiple sclerosis (*PADI2*)[135]
	Repetitive sequences aberrant methylation	Transposition, recombination genomic instability	ATRX syndrome (subtelomeric repeats)[135,143]
Histone modification	Aberrant acetylation	Diverse	Parkinson's and Huntington's diseases[135]
	Aberrant methylation	Diverse	Huntington's disease and Friedreich's ataxia[135]
	Aberrant phosphorylation	Diverse	Alzheimer's disease[135]
Nucleosome positioning	Misposition in trinucleotide repeats	Creation of a 'closed' chromatin domain	Congenital myotonic dystrophy[151]
Autoimmune diseases			
DNA methylation	CpG island hypermethylation	Transcription repression	Rheumatoid arthritis (*DR3*)[154,155]
	CpG island hypomethylation	Transcription activation	SLE (*PRF1, CD70, CD154, AIM2*)[6]
	Repetitive sequences aberrant methylation	Transposition, recombination genomic instability	ICF (*Sat2, Sat3*), rheumatoid arthritis (*L1*)[152,155]
Histone modification	Aberrant acetylation	Diverse	SLE (*CD154, IL10*, IFN-γ)[6]
	Aberrant methylation	Diverse	Diabetes type 1 (*CLTA4, IL6*)[159]
	Aberrant phosphorylation	Diverse	SLE (NF-κB targets)
Nucleosome positioning	SNPs in the 17q12-q21 region	Allele-specific differences in nucleosome distribution	Diabetes type 1 (*CLTA4, IL6*)
	Histone variants replacement	Interferes with proper remodeling	Rheumatoid arthritis (histone variant macroH2A at NF-κB targets)[157]

TABLE 3 Epigenetic aberrations reported in psychiatric disease

Histone modification	Direction of change	Affected gene or histone codes	Morbidity
Acetylation	▲	HDAC1	Schizophrenia
	▲	HDAC2, HDAC5	MDD (phase)
	▲	HDAC4	Depressive phase of bipolar disorder
	▼	HDAC6, HDAC8	Depressive and remission phase of bipolar disorder
Methylation	▲	H3K9me2	Schizophrenia
	▼	H3K4me of *GAD1* promoter	Schizophrenia

DNA modification	Direction of change	Affected gene	Morbidity
DNA methylation	▲	*RELN, SOX10*	Schizophrenia
	▲	*WDR18*	Schizophrenia (male)
	▲	*GABAA*	Suicide
	▲	*NR3C1*	Child abuse
	▲	*DAT1, HERP*	Alcoholism
	▼	Atrial natriuretic peptide	Alcoholism
	▲	*5-HTT*	MDD
	▲	*HTR2A*	Schizophrenia and bipolar disorder
	▲	*DAT1, DRD2*	Anorexia nervosa
	▲	*PER1, CRY1, SORBS3, S100A2*	Alzheimer disease
	▼	*MB-COMT*	Schizophrenia and bipolar disorder
	▼	*RPL39*	Bipolar disorder (females)
	▼	Genome-wide DNA	Autism
	▼	Genome-wide DNA	Schizophrenia
	▼	*PPIEL*	Bipolar II
	▼	*MAOA*	Smoking (tobacco)

RNA modification	Direction of expression change	Affected miRNA	Morbidity
miRNAs	▲	miR-15a, miR-15b, miR-195, miR-107, miR-181b; exosomal miR-497, miR-29c	Schizophrenia
	▼	miR-24, miR-26b, miR-30e, miR-92, miR-346	Schizophrenia and schizoaffective disorder
	▼	Circulating miR-134	Manic phase of bipolar disorder

HDAC, histone deacetylase; miRNA, microRNA.
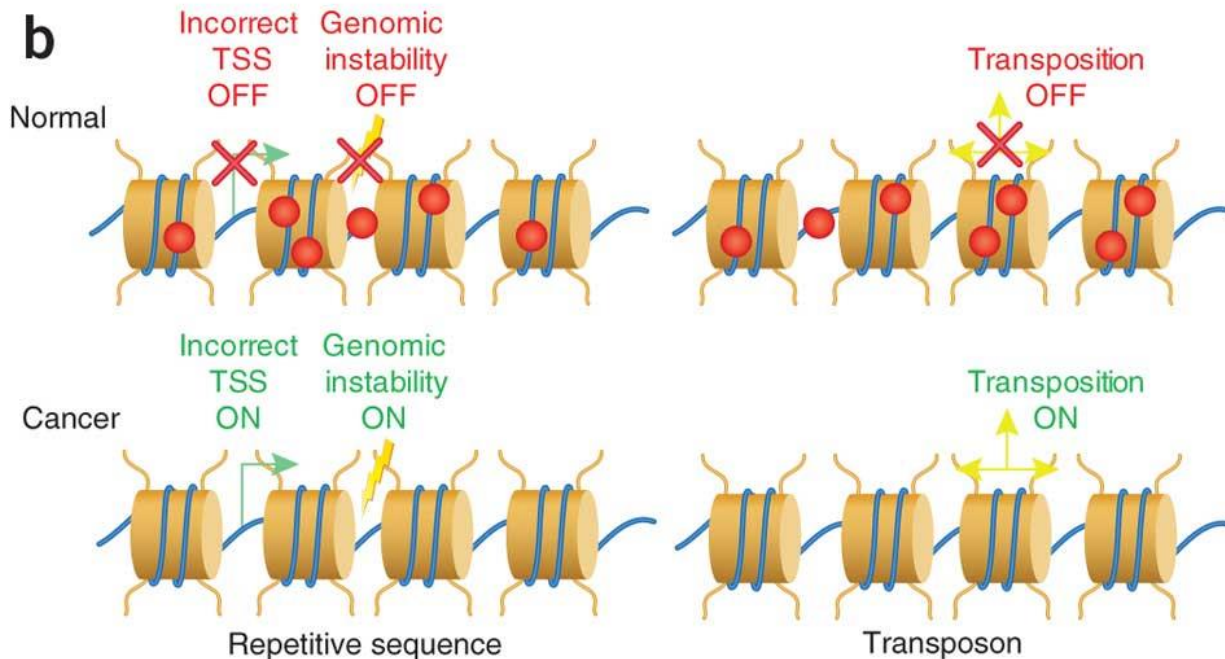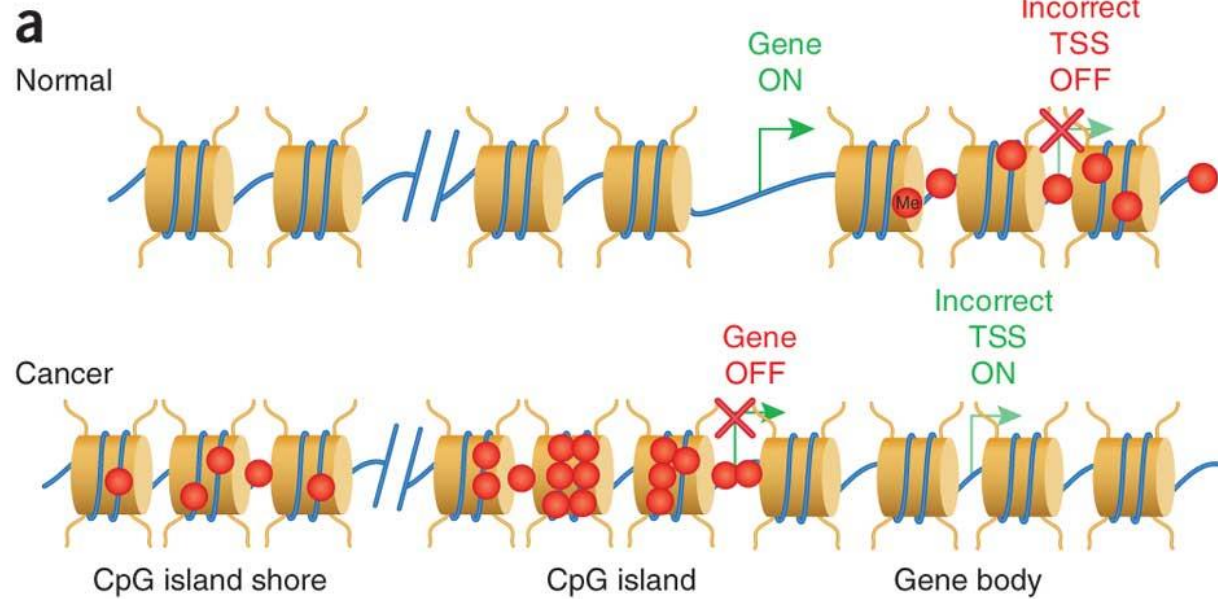
DNA methylation patterns in normal and cancer cells

DNA methylation takes place along the whole genome, and its disruption is a typical hallmark of cancer.

(a) In **normal cells** (top), *CpG islands and CpG island shores usually remain unmethylated*, allowing gene transcription. Additionally, *DNA methylation within the gene bodies* avoids spurious transcription initiations. In **cancer cells** (bottom), by contrast, although *both CpG islands and CpG island shores may be strongly methylated, gene bodies lack this modification*. As a result, transcription of many genes gets blocked, and aberrant transcription may occur from incorrect transcription start sites (TSSs).

(b) In *normal cells* (top), *methylation of repetitive sequences prevents genomic instability* and, again, spurious transcription initiations. Moreover, *transposable elements cannot be activated in a methylated environment*. In **cancer cells** (bottom), *global hypomethylation triggers genomic instability* and aberrant transcription initiations. *Concomitant activation of transposons may lead to gene disruption*.
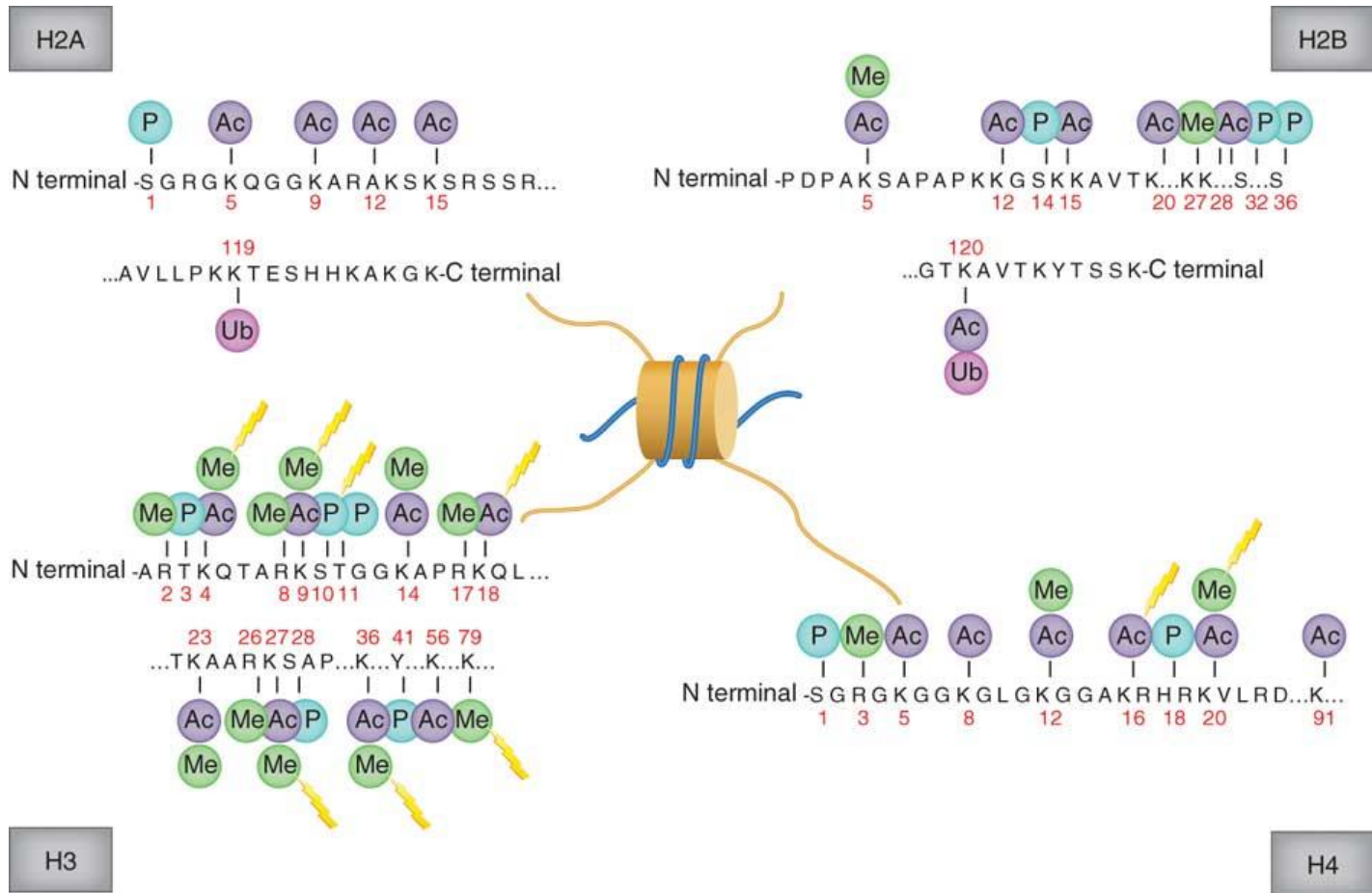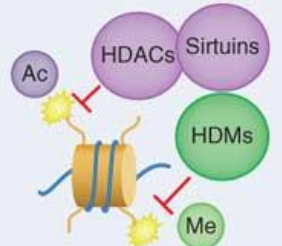
Histone modification patterns in normal and cancer cells

Mainly along their protruding N-terminal tails, but also within their C-terminal regions, histones can undergo diverse post-translational modifications. In the right combination and translated by the appropriate effectors, these modifications contribute to establishing the global and local condensed or decondensed chromatin states that eventually determine gene expression. This figure depicts the *main modifications of the four core histones in normal cells* (type and position in the amino acid sequence). Furthermore, and because *disruption of their normal patterns is related to cancer, histone modifications typically associated with the disease have also been highlighted.*

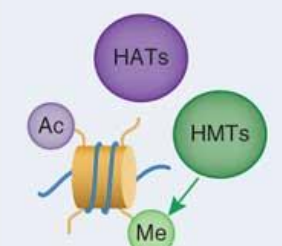Ac, acetylation; Me, methylation; P, phosphorylation; Ub, ubiquitination.

Disruption of normal patterns of covalent histone modifications is another hallmark of cancer. One of the most characteristic examples is the **global reduction of the trimethylation of H4K20 (H4K20me3) and acetylation of H4K16 (H4K16Ac)**, along with DNA hypomethylation, at repeat sequences in many primary tumors

Category	Gene
HDACs	HDAC1
	HDAC2
	HDAC6
Sirtuins	SIRT1
	SIRT2
	SIRT3
	SIRT7
HDMs	KDM1A
	KDM2B
	KDM4C
	KDM5A
	KDM5B
	KDM5C
	KDM6A
	KDM6B
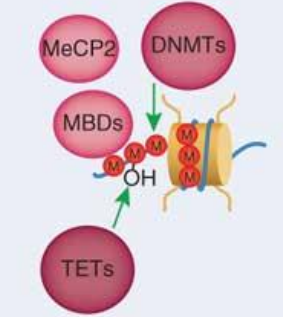
Category	Gene
HATs	CREBBP
	EP300
	MYST3
	MYST4
	KAT5
HMTs	MLL
	EZH2
	NSD1
	PRDM2
	SMYD3
	WHSC1
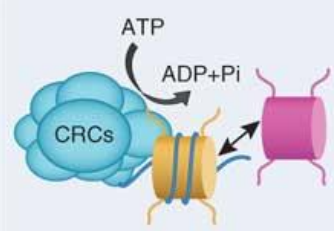
Category	Gene
DNMTs	DNMT1
	DNMT3A
	DNMT3B
(2OG)-Fe(II)–dependent oxygenases	TET1
	TET2
Methyl-CpG binding proteins	MBD1
	MBD2
	MBD3
	MBD4
	MECP2

Category	Gene
Histone variants	H2AFZ
Chromatin remodeling factors	ARID1A
	CHD5
	CHD7
	MTA1
	MTA2
	MTA3
	SMARCA2
	SMARCA4
	SNF5

3CACC6

Category	Gene
Histone modification readers	ING1
	ING2
	ING3
	ING4
	ING5

Selection of epigenetic genes disrupted in human tumors
Mutation, deletion and/or altered expression of genes encoding components of the various epigenetic machineries are typically observed in human tumors. The figure shows a selection of genes encoding enzymes that add, remove and recognize histone modifications, as well as members of the DNA methylation machinery, whose deregulation is connected to cancer.
CRCs, chromatin remodeling complexes; Ac, acetylation; Me, methylation.
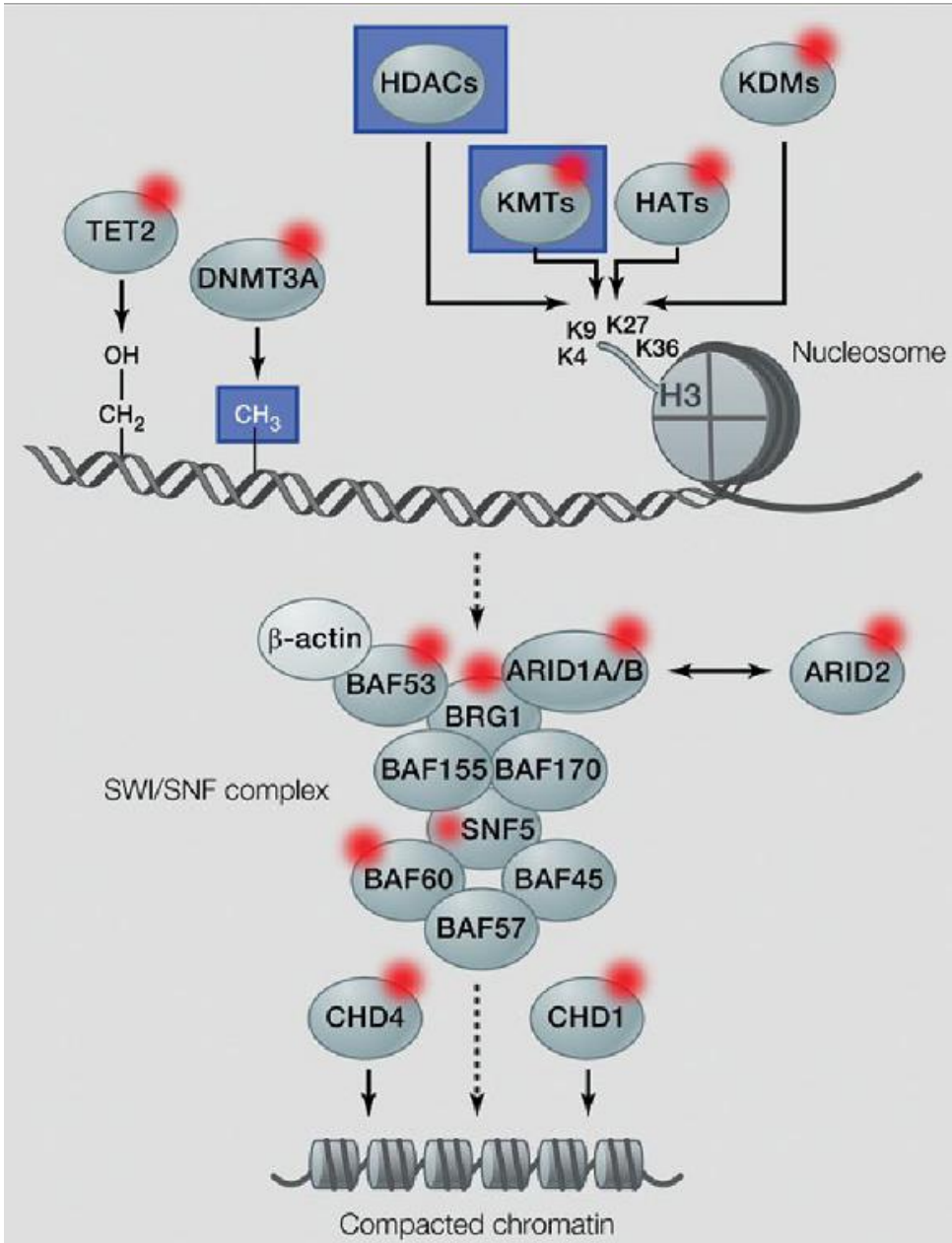
Genes Encoding Epigenetic and Chromatin Regulators Are Frequent Targets of Mutations in Cancer

The enzymes DNMT3A and TET2 regulate 5-methylcytosine and 5-hydroxymethylcytosine production in genomic DNA; the genes encoding these enzymes are frequently mutated in leukemias. The histone H3 component of the nucleosome undergoes extensive modifications involving its lysine (K)-rich tail. Genes encoding enzymes that read, produce, or interpret these modifications are frequently mutated in cancer. Examples include histone lysine methyltransferases (KMTs), histone lysine demethylases (KDMs), and histone acetyltransferases (HATs). Genes encoding components of the SWI/SNF chromatin-remodeling complex are also recurrently mutated in cancer. Novel therapeutics targeting chromatin and epigenetic mechanisms have entered clinical use or are in development (boxed).
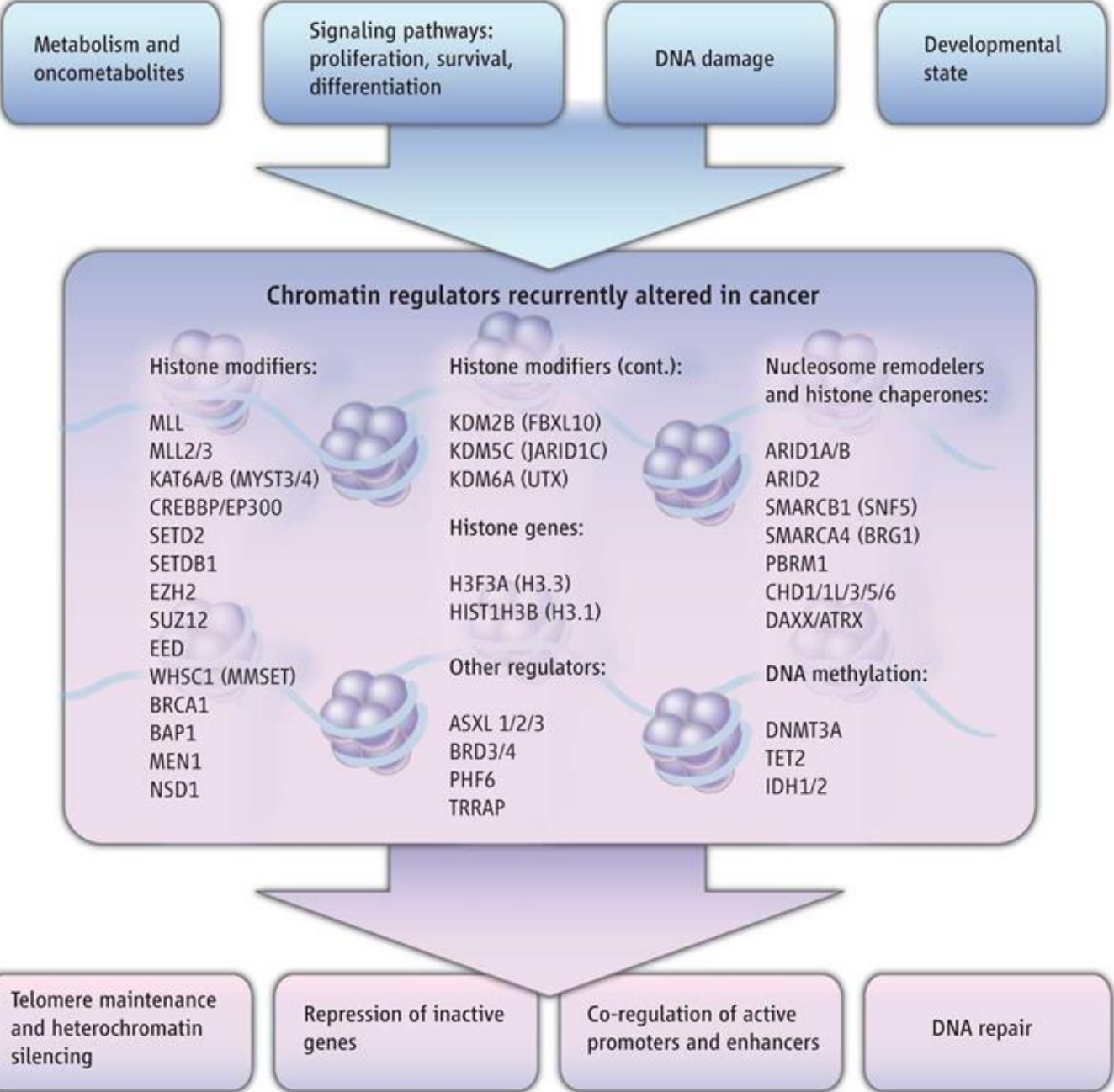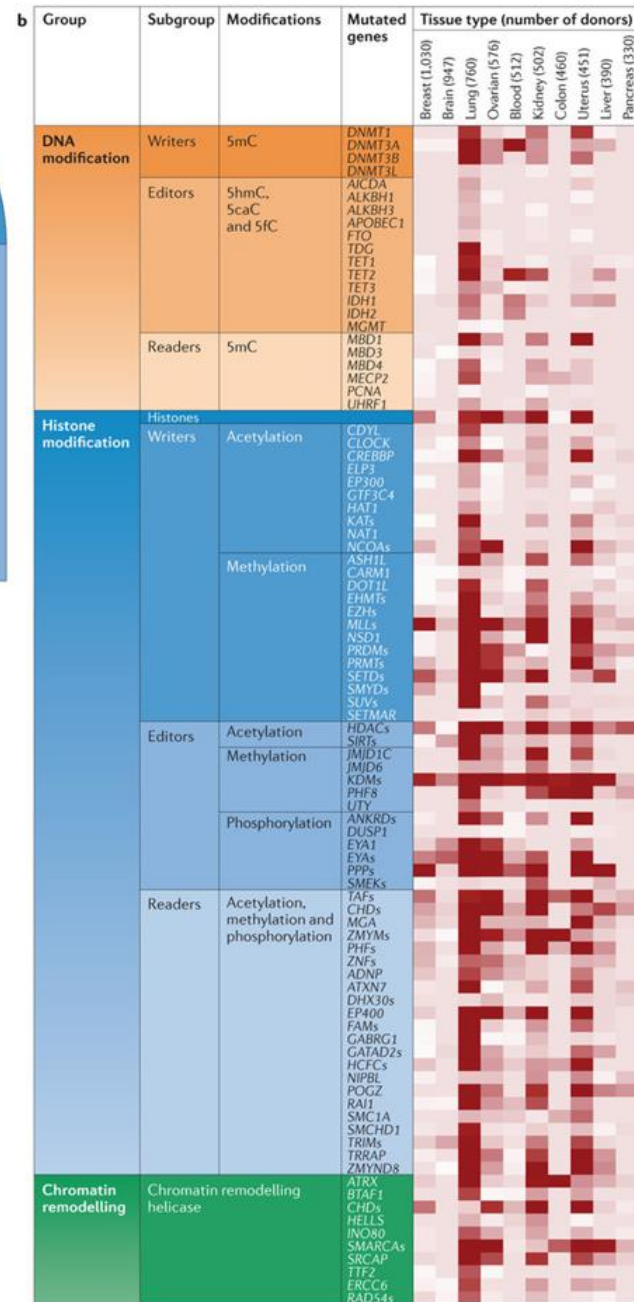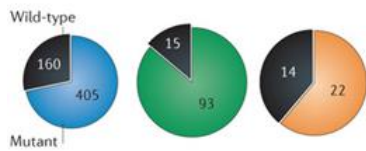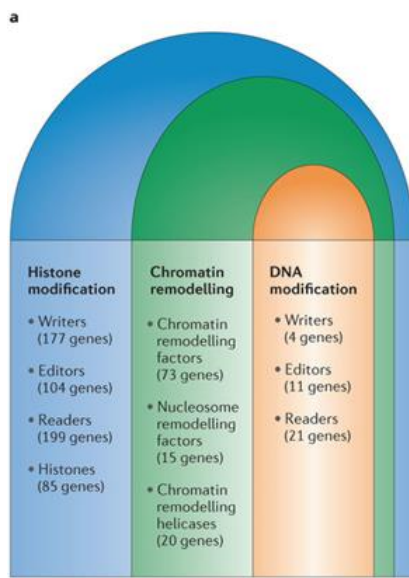
Mutations in regulators of the epigenome identified in cancer

a| Using several data browsers, ~709 epigenetic enzymes that were identified can be grouped into three major categories: histone modification, chromatin remodelling and DNA modification. Each group is divided into subgroups based on their functions. The total number of genes in each subgroup is given in brackets. Each pie chart lists the number of genes that were found to be mutated in at least two tumour samples (indicated in blue, green or orange) and the number of unmutated genes (black) for each group of epigenetic enzymes.

b| Examples of mutated genes in the groups of DNA modification, histone modification and chromatin remodelling enzymes, based on data as of January 2013 in the International Cancer Genome Consortium (ICGC) data set. The number of analysed tumour tissues is given. Several tumour entities have high frequencies of mutations in epigenetic enzymes. Note that these data are not adjusted for chromosomal instability or mutator phenotypes, hence the frequencies reflect a combination of probable driver mutations in epigenetic regulators, in addition to the background mutation rate for the tumour type.

5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine.
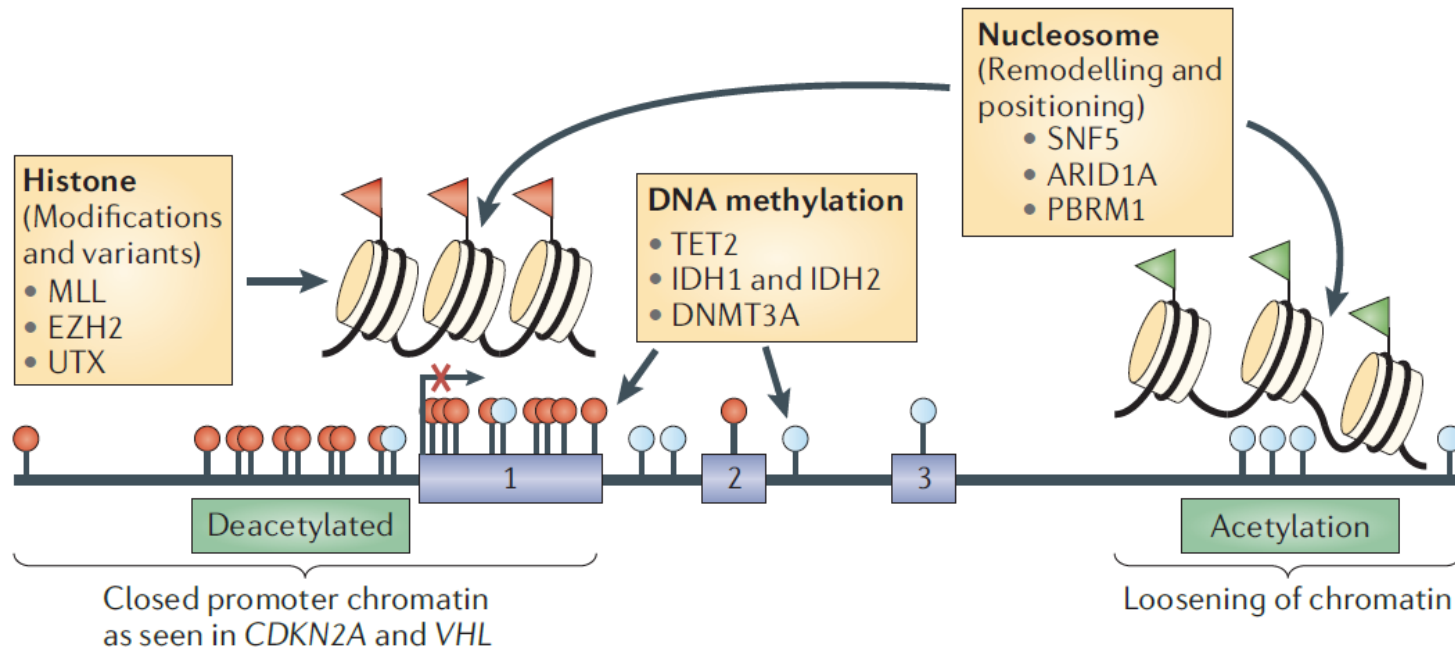
The cancer epigenome and relevant gene mutations

The **cancer epigenome is characterized by simultaneous global losses in DNA methylation** (indicated by pale blue circles) **with hundreds of genes that have abnormal gains of DNA methylation** (indicated by red circles) **and repressive histone modifications** (indicated by red flags) **in promoter region CpG islands**. The hypomethylated regions have an abnormally open nucleosome configuration and abnormally acetylated histone lysines (indicated by green flags). Conversely, abnormal DNA hypermethylation in promoter CpG islands is associated with nucleosomes positioned over the transcription start sites of the associated silenced genes (indicated by an arrow with a red X). Recent whole-exon sequencing of human cancers has shown a high proportion of mutations in genes in leukaemias, lymphomas, and ovarian, renal and pancreatic cancers, and rhabdomyosarcoma (indicated in yellow boxes), which are depicted as helping to mediate either abnormal DNA methylation, histone modifications and/or nucleosome remodelling.

ARID1A, AT-rich interactive domain-containing protein 1A; DNMT3A, DNA methyltransferase 3A; EZH2, ehancer of zeste 2; IDH1, isocitrate dehydrogenase 1; MLL, mixed lineage leukaemia; PBRM1, protein polybromo 1; SNF5, SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin, subfamily B, member 1; VHL, Von Hippel–Lindau.
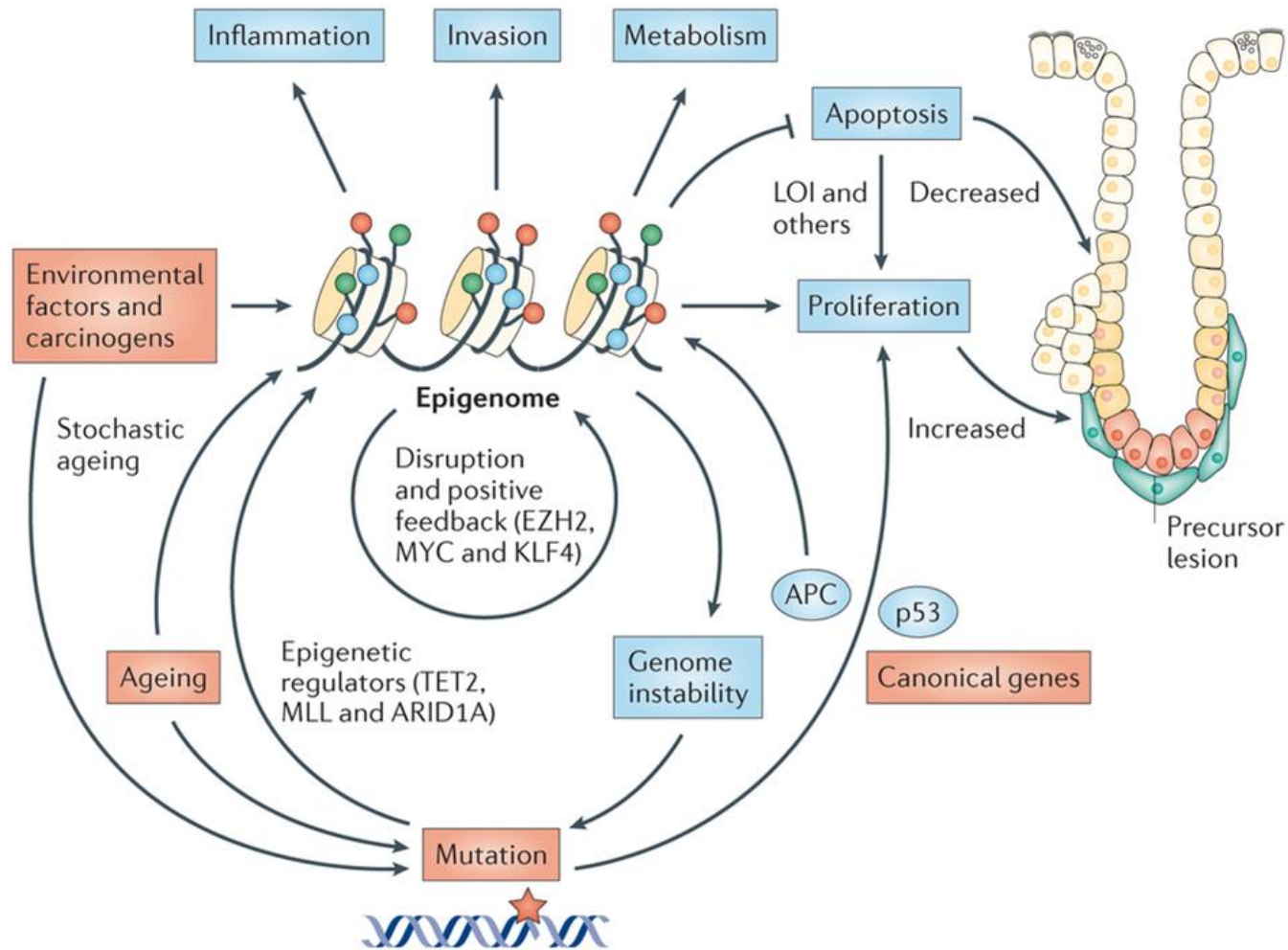
Collaboration of epigenetic modification and mutation in the hallmarks of cancer

The *epigenome sits at the intersection of the environment, genetic mutation and tumour cell growth*. *Environmental factors, such as carcinogens or diet, as well as injury and inflammation, cause epigenetic reprogramming. The epigenome also accumulates damage stochastically and through ageing. The machinery for maintaining epigenetic integrity can be stably disrupted in either of two ways: by mutation or by epigenetic change itself with positive feedback.*

Some epigenetic modifications, such as shifting methylation boundaries at CpG islands and shores, lead to metabolic change and enhanced proliferation. Instability of CpG island methylation boundaries also contributes to epigenetic dysregulation, allowing for selection in response to the cellular environment for cellular growth advantage at the expense of the host. ARID1A, AT-rich interactive domain-containing protein 1A; KLF4, Krüppel-like factor 4; MLL, mixed lineage leukaemia; TET2, tet methylcytosine dioxygenase 2.

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Histone-modification maps for a typical chromosome in normal and cancer cells
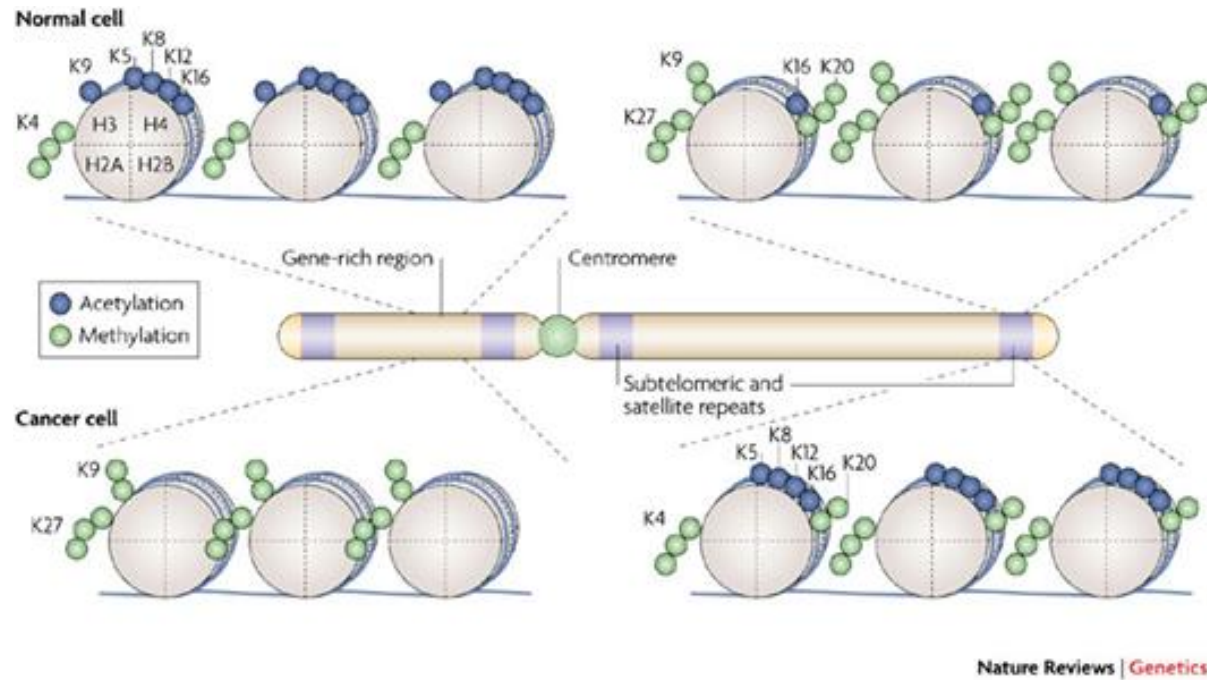
Nucleosomal arrays are shown in the context of chromosomal location and transcriptional activity. Octamers consisting of histones H2A, H2B, H3 and H4 are represented as grey cylinders. Histone acetylation and methylation (di- and tri-) are shown. In 'normal' cells, genomic regions that include the promoters of tumour-suppressor genes are enriched in histone-modification marks associated with active transcription, such as acetylation of H3 and H4 lysine residues (for instance K5, K8, K9, K12 and K16) and trimethylation of K4 of H3. In the same cells, DNA repeats and other heterochromatic regions are characterized by trimethylation of K27 and dimethylation of K9 of H3, and trimethylation of K20 of H4, which function as repressive marks. **In transformed cells, this scenario is disrupted by the loss of the 'active' histone-marks on tumour-suppressor gene promoters, and by the loss of repressive marks such as the trimethylation of K20 of H4 or trimethylation of K27 of histone H3 at subtelomeric DNA and other DNA repeats. This leads to a more 'relaxed' chromatin conformation in these regions.**

HATi
Garcinol
Curcumin
Anacardic acid

HDACi
NaB
Phenylacetate
Phenylbutirate
VPA
TSA
Vorinostat
Panobinostat
Belinostat
Romidepsin
MS-275
MGCD-0103
(1H) Pyrroles

DNMTi
Vidaza
Decitabine
FCDR
Zebularine
S110
Procaine
Procainamide
Hydralazine
EGCG
RG108

HATs

HDACs

Sirtuins

Ac

DNMTs

Me

HMTs

HDMs

SIRTi
Sirtinol
EX-527
Splitomicin
Cambinol
Salermide
Salermide
Tenovin-1
Tenovin-6

HMTi
Chaetocin
DZNep
BIX-01294
AMI-1
AMI-5
Allantodapsone
RM-65

HDMi
Pargyline
Phenelzine
Tranylcypromine

Epigenetic drugs for cancer therapy
Numerous compounds have been reported to be effective against cancer cells by inhibiting components of the epigenetic machineries. This figure shows the most important epigenetic drugs classified depending on their particular epigenetic targets.

TABLE 2 — Examples of known epigenetic modifiers with therapeutic implications or health effects

Class	Modifier	Type of epigenetic modification
Prescribed drugs	Valproate	HDAC inhibitor (increases H3 and H4 acetylation)
	Lithium	HDAC inhibitor (increases H3 acetylation and phosphoacetylation)
	Imipramine	Inhibition of HDAC5 (increases histone acetylation of BDNF III and IV promoters)
	Amitriptyline	Inhibition of HDACs, H3 acetylation, and DNA demethylation by the inhibition of DNMT activity
	Fluoxetine	Reverses the stress-induced decreases in H3K9me3 as well as H3 acetylation in dentate gyrus and hippocampus, respectively
	Escitalopram	Reduces the mRNA levels of *DNMT1* and *DNMT3A*, and DNA methylation of *S100A10* gene promoter
	Tranylcypromine (MAOI)	Inhibition of *LSD1* (lysine-specific demethylase 1 that demethylates H3K4me1, H3K4me2, H3K9me1, and H3K9me2) inducing transcription
	Haloperidol	H3 phosphorylation at serine 10, H3K14 phosphoacetylation
	Clozapine	Increases H3K4me3 and DNA demethylation of *RELN* and *GAD67* promoters
	Sulpiride, amisulpiride (and MS-275)	H3K9 and H3K14 acetylation and DNA demethylation of *RELN* and *GAD67* promoters
	Lurasidone	Increases HDAC1, HDAC2, and HDAC5 expression
Research drugs	Butyrate, trichostatin A	Histone acetylation
	RG108, 5-azacytidine	*DNMT* inhibitor
Nutrients and vitamins	Choline	The main source of methyl groups
	S-adenosylmethionine	The main methyl donor
	Folic acid	DNA methylation
	Vitamin B12	DNA methylation
	Selenium	*DNMT* inhibitor
	EGCG (tea)	*DNMT* inhibitor
Contaminants and abused drugs	Bisphenol A	Global DNA hypomethylation
	Arsenic	Global DNA hypomethylation
	Alcohol	DNA hypermethylation of several genes
	Oxidative stress	DNA hypomethylation and increase in 5-hydroxymethylcytosine level
	Opioids/nicotine and cocaine	DNA hypermethylation/histone acetylation
Physical therapies	ECT	H3 and H4 acetylation, DNA demethylation due to *GADD45b* induction

HDAC, histone deacetylase; BDNF, brain-derived neurotrophic factor; DNMT, DNA methyltransferase; mRNA, messenger RNA; EGCG, epigallocatechin gallate; ECT, electroconvulsive therapy.

Table 1 — Selected Epigenetic Drugs

Drug	Compound	Study Phase
DNMT inhibitors	Azacitidine (Vidaza)	US FDA–approved in MDS
	Decitabine (Dacogen)	US FDA–approved in MDS
	S110	Phase I
	CP-4200 (elaidic azacytidine)	Preclinical
	Nanaomycin A	Preclinical
HDAC inhibitors	Vorinostat (Zolinza)	US FDA–approved in CTCL
	Romidepsin (Istodax)	US FDA–approved in CTCL
	Panobinostat	Phase II
	Belinostat	Phase I/II
	Valproic acid	Phase II
	Belinostat	Phase II/III
HMT inhibitors	Deazaneoplanocin A (DZNep)	Preclinical
	Quinazoline derivatives	Preclinical
	Ellagic Acid	Preclinical
Histone demethylase inhibitors	Polyamine analogues	Preclinical
	Hydroxamate analogues	Preclinical
HAT inhibitors	Spermidinyl-CoA derivatives	Preclinical
	Hydrazinocurcumin	Preclinical
	Pyrazolone-containing small molecules	Preclinical

CoA = coenzyme A; CTCL = cutaneous T-cell lymphoma; DNMT = DNA methyltransferase; HAT = histone acetyltransferase; HDAC = histone deacetylase; HMT = histone methyltransferase; MDS = myelodysplastic syndrome.

| Disorder/disease	Epigenetic dysregulation	Potential treatments
Rett syndrome	Mutation in *MeCP2* gene	HDAC inhibitors HDAC inhibitors
Age-associated cognitive decline	Impaired H4K12 acetylation in response to learning event	
Schizophrenia	Hypermethylation of *reelin* gene, decreased H3K4me3, and increased H3K27me3 at GAD 67 promoter	DNMT inhibitors, HDAC inhibitors
Rubenstein–Taybi syndrome	Mutation of *CBP* gene	HDAC inhibitors
Drug addiction	Multiple changes in DNA methylation and histone modifications at striatal plasticity genes, increased MeCP2	HDAC inhibitors specifically during extinction training
Coffin–Lowry syndrome	Mutation in *RSK2* gene	PP1 inhibitor
Alzheimer's disease	Aberrant histone acetylation and phosphorylation, DNA hypomethylation at several genes	HDAC inhibitors, S-adenosylmethionine, methyl-donor rich diets
Depression	Increased H3K27me2 at specific promoters of *BDNF* gene, downregulation of HDAC5 in hippocampus, DNA methylation differences in catecholamine-signaling genes	HDAC inhibitors, specific HMTs or HDM inhibitors
Angelman syndrome	Abnormal DNA methylation-related imprinting of maternal alleles	–
Prader–Willi syndrome	Abnormal DNA methylation-related imprinting of paternal alleles	–
Fragile-X syndrome	Hypermethylation of DNA at *FMR* genes	–

Selected List of Psychiatric Disorders and Syndromes with Epigenetic Origins or Treatments
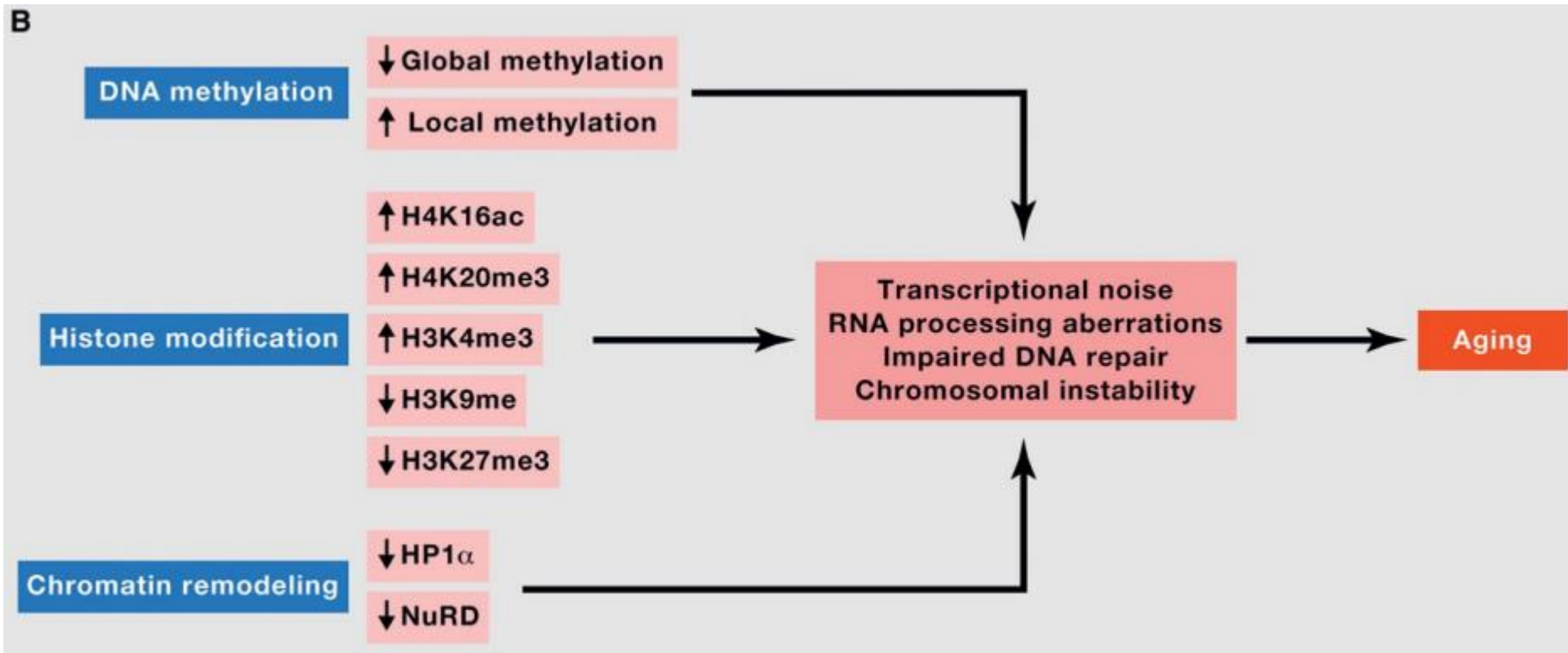
Epigenetic alterations in aging

Alterations in the methylation of DNA or acetylation and methylation of histones, as well as of other chromatin-associated proteins, can induce epigenetic changes that contribute to the aging process.
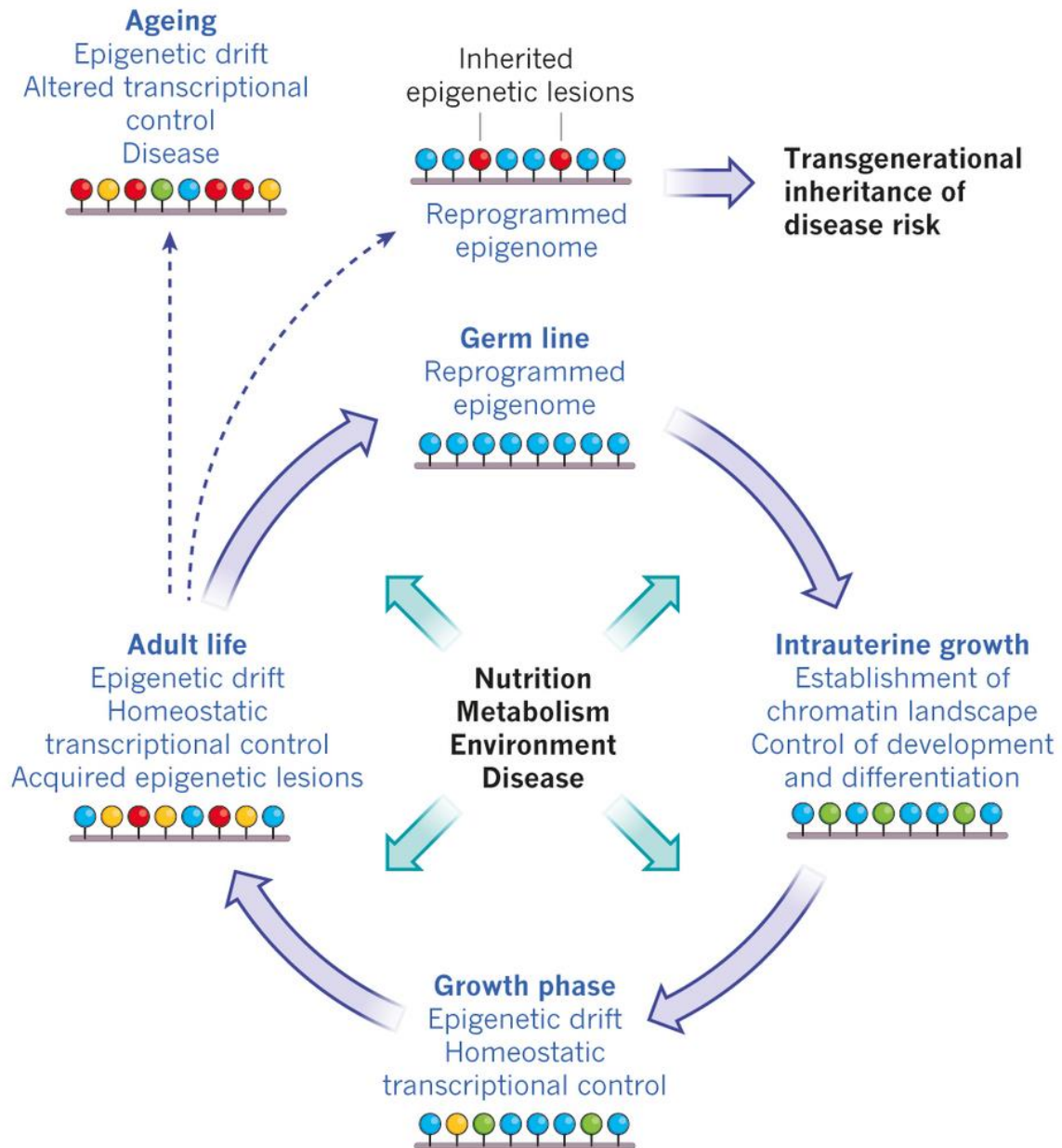
Epigenetic drift and transgenerational inheritance of disease risks

DNA methylation and histone marks are established during embryonic development to maintain cell lineage commitment. After birth the chromatin landscape retains a dynamic configuration throughout life. Changes of chromatin marks within a gene locus, termed epigenetic drift, occur in response to nutritional, metabolic, environmental or pathological signals and are part of homeostatic adaptations. When adverse epigenetic drift compromises a cell's capacity to adequately respond to challenges, disease susceptibility increases stochastically. Under some circumstances epigenetic marks may escape epigenetic reprogramming during gametogenesis and be inherited by subsequent generations. *Transgenerational inheritance of epigenetic regulation would contribute to disease susceptibility by transmitting an acquired epigenetic predisposition to the next generation independently of genetically inherited risk factors.*
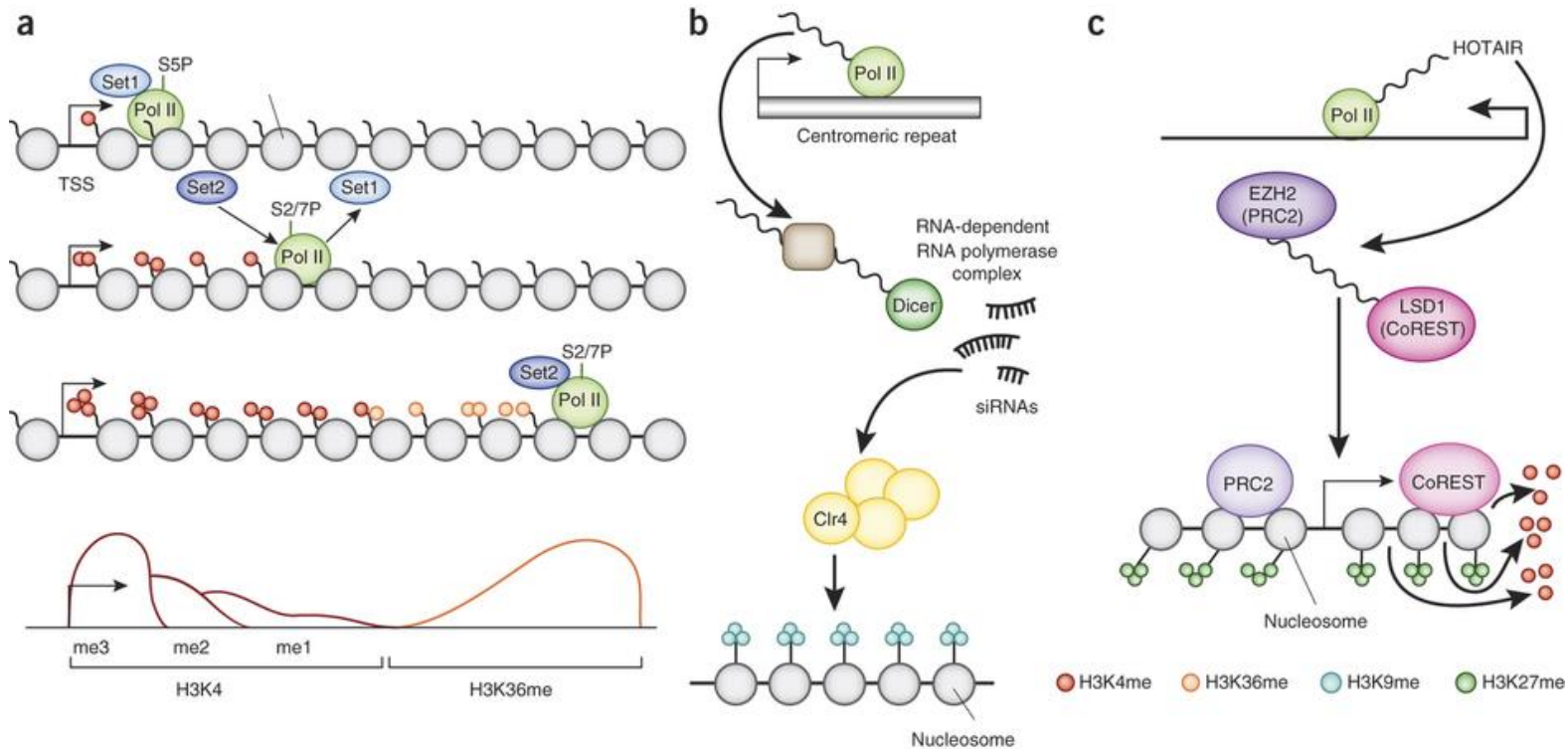
Histone modification: cause or cog?
Steven Henikoff & Ali Shilatifard
Trends Genet. 2011 27(10):389-396.

Describing histone modifications in terms of information or language suggests overwhelming complexity and leaves mechanistic questions unaddressed. By contrast, **DNA accessibility provides a simple testable paradigm for understanding the role of nucleosomes in gene regulatory processes**. The view that emerges is that **dynamic processes affecting nucleosomes result in patterns of histone modifications**, which in turn affect the physical properties of nucleosomes and help to maintain the active or silent state of chromatin. **Such dynamic processes include not only transcription factor binding, RNA polymerase elongation and nucleosome remodeling, but also the targeting action of long non-coding RNAs**. A fuller understanding of how these dynamic processes result in histone modification patterns will be needed to evaluate the functional significance of histone modifications in gene expression and other chromatin-associated processes.

Key histone modifications are better understood as cogs in the machinery that regulates transcriptional elongation, heterochromatic silencing and other processes. We find that the considerably older **DNA accessibility paradigm** [H. Weintraub, M. Groudine. Chromosomal subunits in active genes have an altered conformation. Science, 193 (1976), pp. 848–856] provides a more useful description of the dynamic chromatin landscape and is supported by recent findings.

Histone modifications as consequences of dynamic chromatin processes.

(a) **During transcription**, Set1 interacts with initiating, Ser5-phosphorylated (S5P) Pol II and methylates H3K4 proximal to promoters (top). With the onset of transcriptional elongation, Pol II is predominantly Ser2/7-phosphorylated (S2/7P), leading to dissociation of Set1 (middle). Set2 associates with Ser2/7-phosphorylated Pol II, which then deposits H3K36me throughout the gene body concurrent to transcriptional elongation (bottom schematic). Below the schematics is a diagram of the distributions of H3K4me and H3K36me in genes.

(b) In **siRNA-dependent targeting**, Pol II transcribes centromeric repeats, which are transcribed by RNA-dependent RNA polymerase and processed by Dicer to generate siRNAs, which target the Clr4 methyltransferase complex to sites of future heterochromatin to establish H3K9me.

(c) In **lincRNA-dependent targeting**, Pol II transcribes a lincRNA (HOTAIR), which associates with components of the PRC2 H3K27 trimethyltransferase complex (EZH2) and the CoREST H3K4 demethylase complex (LSD1). HOTAIR then targets these complexes to promoters to remove H3K4me3 and establish H3K27me3, contributing to a repressive chromatin state.

Histone modifications are cogs in dynamic chromatin processes, wherein histone modifications reinforce changes in nucleosome occupancy, positioning or composition mediated by processes such as transcriptional elongation, chromatin remodeling and the targeting actions of noncoding RNAs.

Box 1. What is epigenetics?

Epigenetics: long-lived and reversible modifications to nucleotides or chromosomes that do not change the sequence but can alter gene expression and phenotype.

Epigenetic layers: DNA methylation; stably maintained histone modifications (e.g., H3K27me3, H3K9me3); chromatin loops; chromosomal organization and location within the nucleus; noncoding RNAs (bound to DNA).

Epigenetic regulators or players: chromatin-binding factors; chromatin remodeling proteins; noncoding RNAs; DNA methyl-transferases; histone-modifying enzymes.

What is epigenetic? Allelic differences in transcription not determined by sequence; cell lineage inheritance; long-lived modifications from past environmental exposures.

What is not epigenetic? Regulation of transcription without accompanying long-lived modifications; transcription factors (although they may direct epigenetic states).

Metastable: a physics term describing a state of precarious stability that is also a useful term for labeling the long-lived but reversible characteristics of epigenetics [102].

Common epigenetic myths

(i) DNA sequence does not matter for epigenetic modifications.
   Although this statement may be true for allelic states such as imprinting and X chromosome inactivation, sequence features such as promoter CpG content or other nucleotide bias (such as G-skew on one DNA strand) can be very important in determining the position of DNA methylation as an epigenetic modification [103,104,85] and methylation patterns are heritable with genotype [105].

(ii) Epigenetic marks determine transcription (but not vice versa).
   Although this was the historical thinking in the field, many recent examples demonstrate that transcription is required for establishment of some epigenetic modifications. Epigenetic modifications may actually be historical marks of past transcriptional events that influence later transcriptional responsiveness.

(iii) Transcriptional differences between genetically identical individuals must be epigenetic and environmentally driven.
   Although an attractive idea, not all transcriptional differences are epigenetic and many epigenetic differences are stochastic rather than environmentally determined.

(iv) DNA methylation patterns, once established in early development, are stably maintained.
   Although methylation patterns appear to be much less variable overall between individuals or cell types than transcriptional patterns, there are some concrete examples of changes to methylation levels in neurons following activity-dependent responses [1,2,106].

(v) DNA methylation is always associated with transcriptional silencing.
   Although this is true for CpG-rich promoters that are subject to imprinting, X chromosome inactivation, or cell fate determination (Table 1), outside of these situations the rules change, and higher DNA methylation levels are generally associated with higher expression [16,17].