

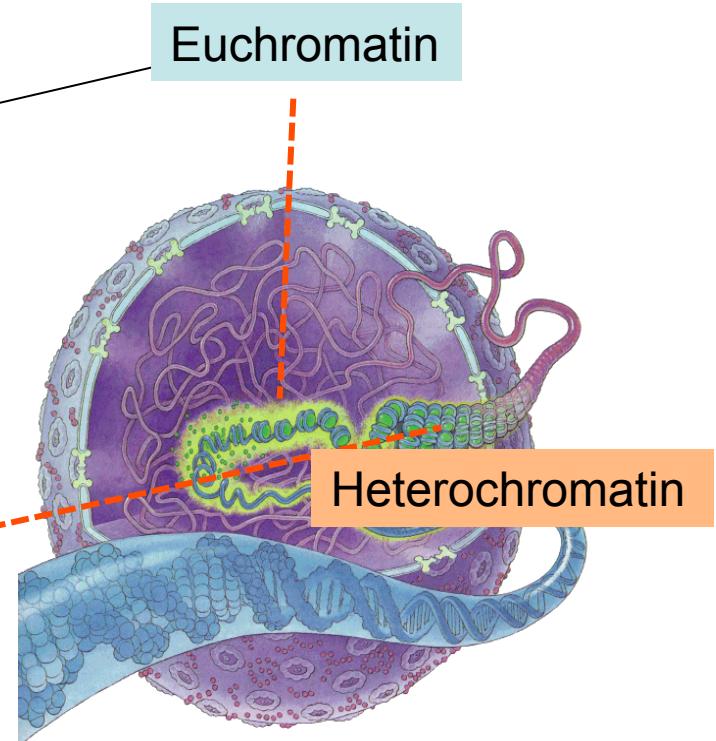
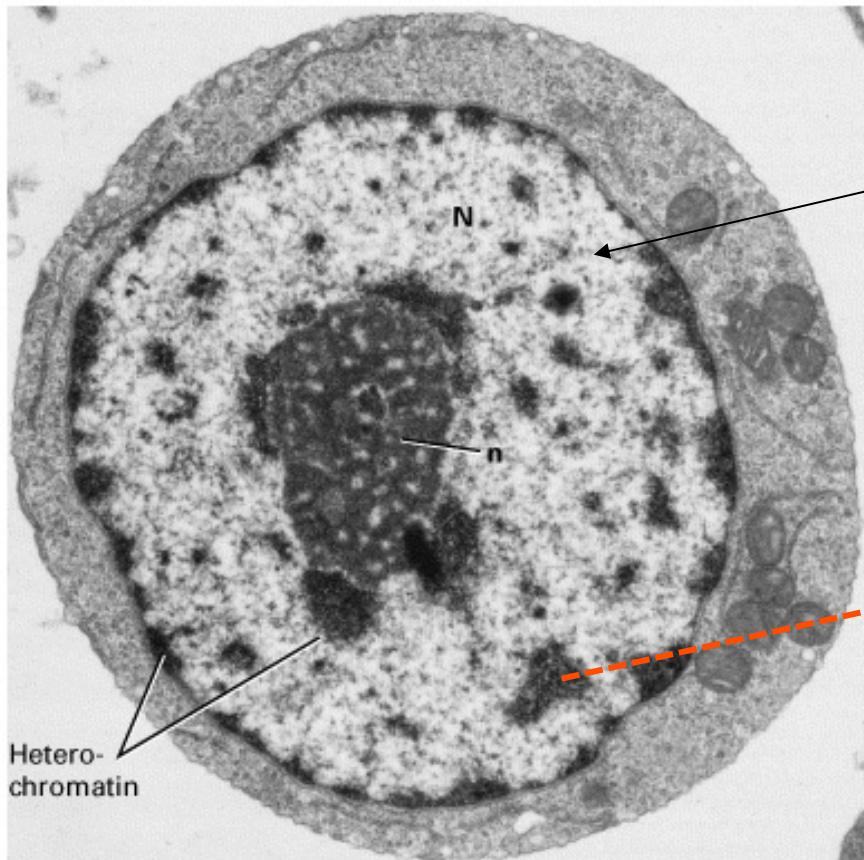
Lecture I-2_2018

Chromatin organisation and histone modifications:
setting, removing, reading and inheritance

Overview

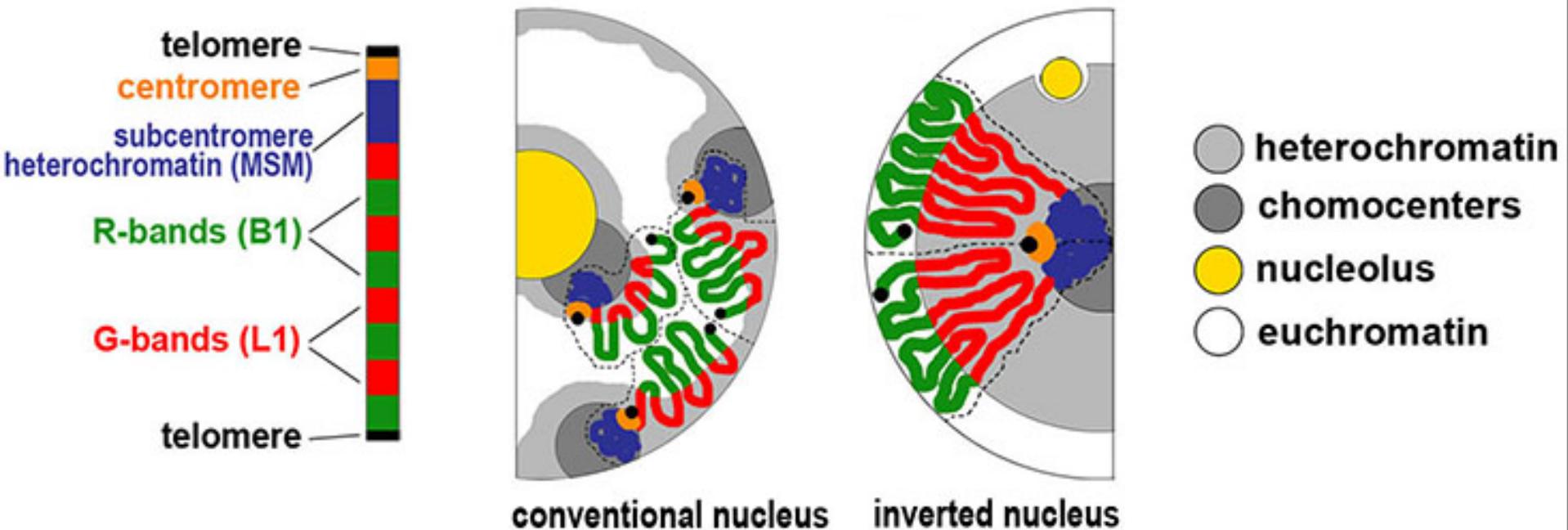
- Chromatin and chromosomes, chromatin dynamics during cell replication & cell division.
- Chromosome folding and looping. Approaches to analyse chromatin structures and states „*in vivo*“.
- Chromatin organisation: Nucleosomes, histones and histone variants.
- Introduction into chromatin modifications and their nomenclature.
- Introduction into the reactions of chromatin modifying enzymes,
- Concept of epigenetic reversibility and molecular function: introduction into writers (establishing enzymes), readers (binders) and erasers (demodifying enzymes).
- Model for the mechanisms of “epigenetic inheritance”,

Chromatin in the nucleus: hetero- and euchromatin



Electron micrograph of a thin section of a bone-marrow stem-cell
Lodish, 4th ed.

Chromatin in the nucleus: distribution of hetero- and euchromatin



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SFB1064

Chromosomes are attached to the nuclear envelope (lamina) via specific regions mediated by lamines (proteins).

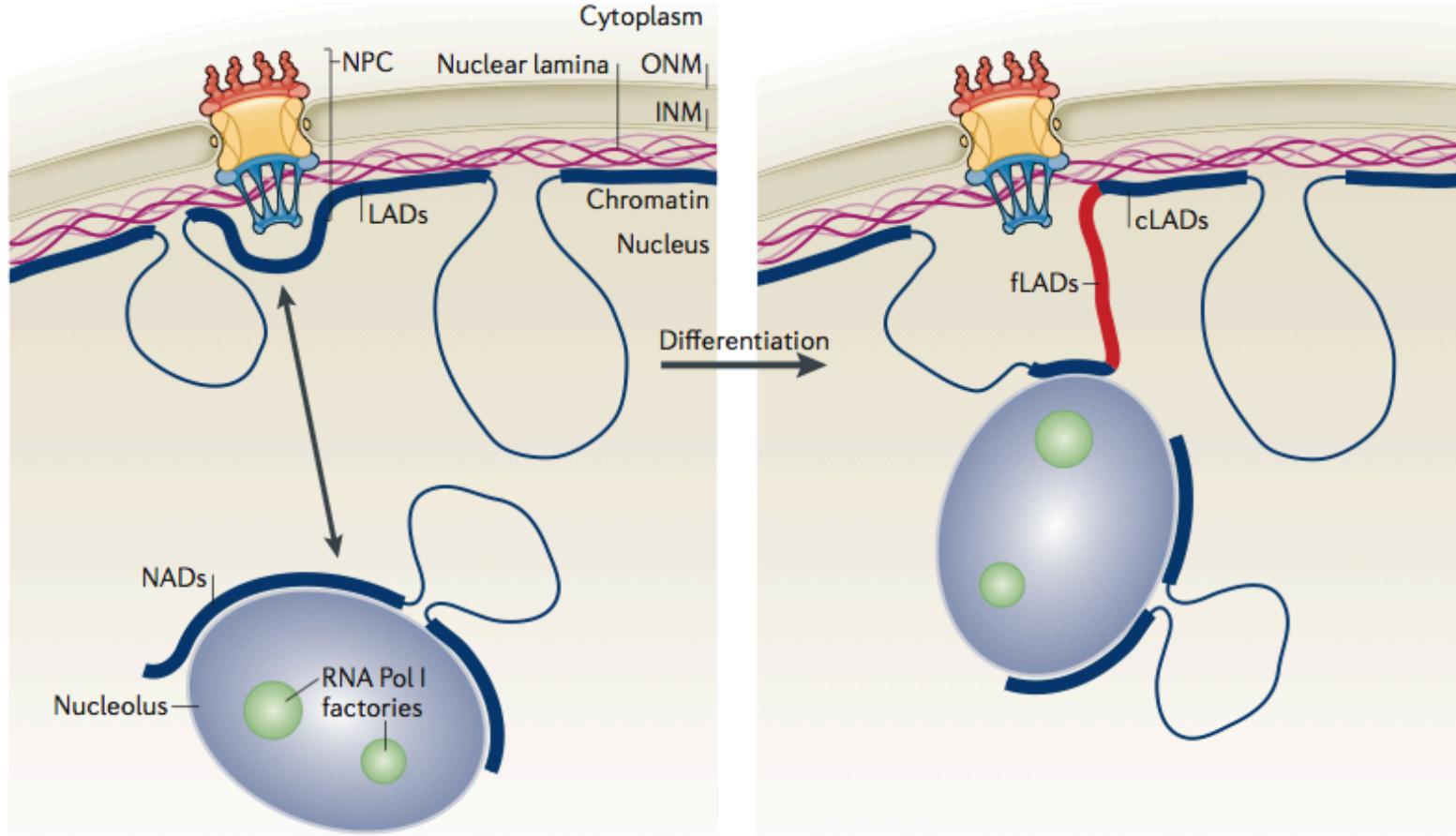


Figure 3 | The nuclear envelope affects genome organization and function. Lamina-associated domains (LADs) are regions of condensed chromatin that are bound by the nuclear lamina. LADs are also enriched for marks such as histone 3 Lys9 dimethylation (H3K9me2), which is a mark of heterochromatin and silent genes. Regions of open chromatin, in which genes are actively transcribed, loop out into the interior of the nucleus. As cells differentiate, constitutive LADs (cLADs) remain associated with the lamina, whereas facultative LADs (fLADs) become detached as the genes that they contain become active. After mitosis, some LADs relocate to the periphery of the nucleolus in the following G1 phase of the cell cycle, although the mechanism underlying this remains unclear. Sequences preferentially located at the nucleolar periphery (nucleolus-associated chromatin domains; NADs) have been identified independently by high-throughput sequencing of DNA associated with biochemically isolated nucleoli. NADs are enriched for pericentric satellite repeats, A- and T-rich sequences and gene-poor regions. INM, inner nuclear membrane; NPC, nuclear pore complexes; ONM, outer nuclear membrane.

Chromosome territories

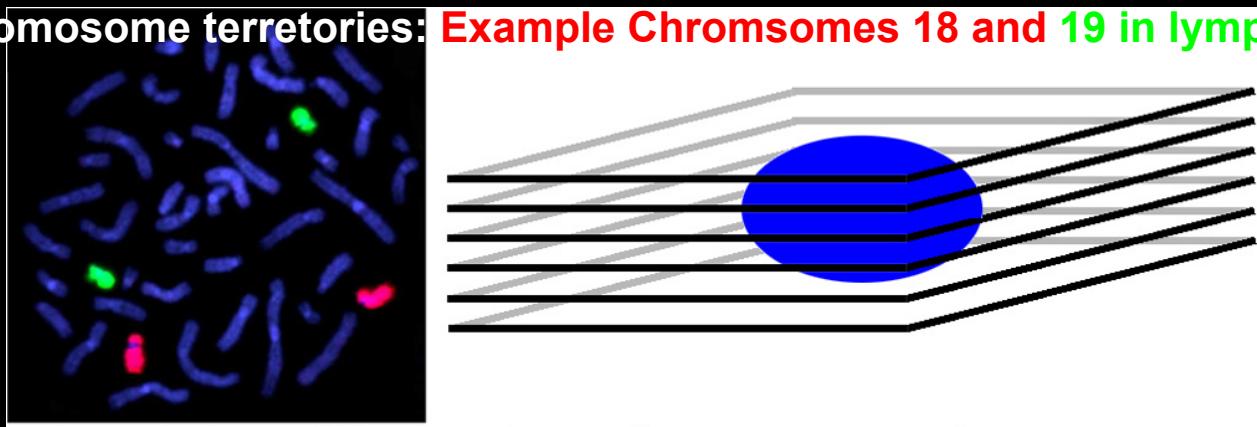
Chromosomes occupy distinct „territories“ in interphase (transcriptionally active) nuclei.

The conformation of chromosomes in these territories is determined by organisational features:

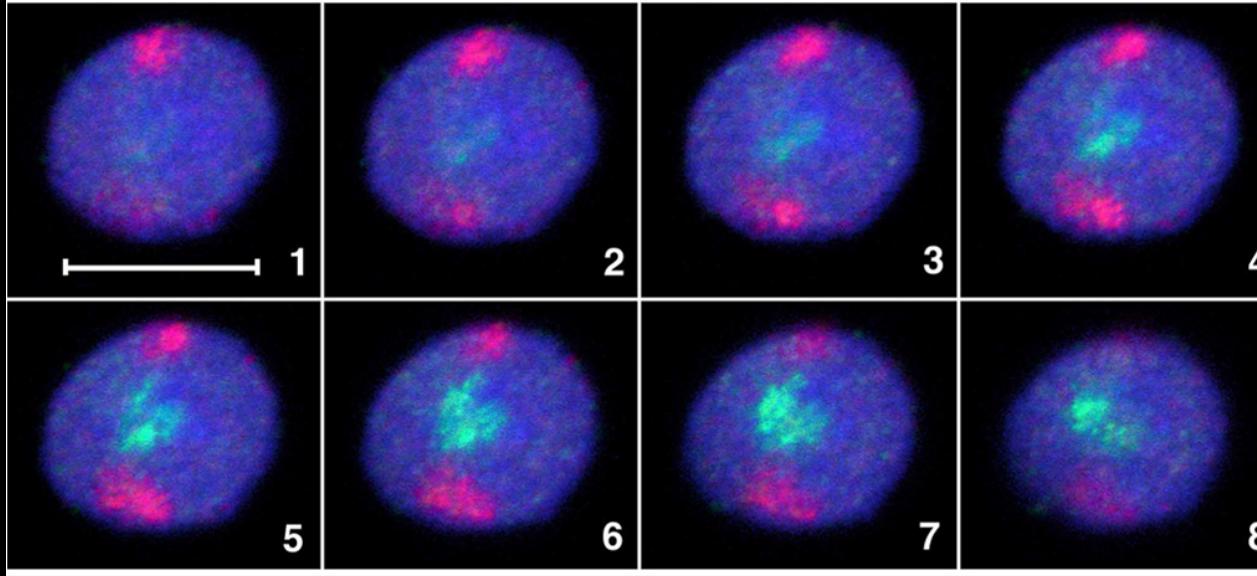
Some parts of the chromosomes attach to the nuclear lamina/envelope (through lamina attachment sites = LADs).

Each chromosome is further organized in functional domains (topology associated domains = TAD's).

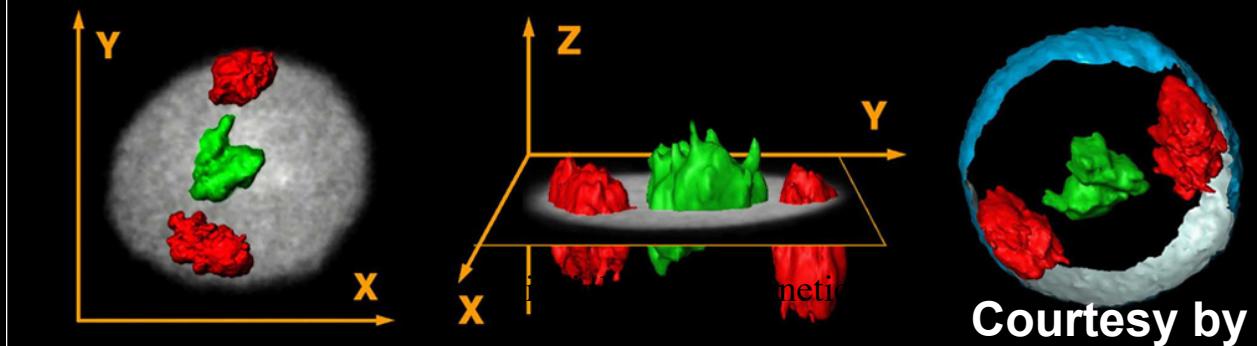
Chromosome territories: Example Chromosomes 18 and 19 in lymphocytes



light optical
serial sections



human
lymphocyte
nucleus

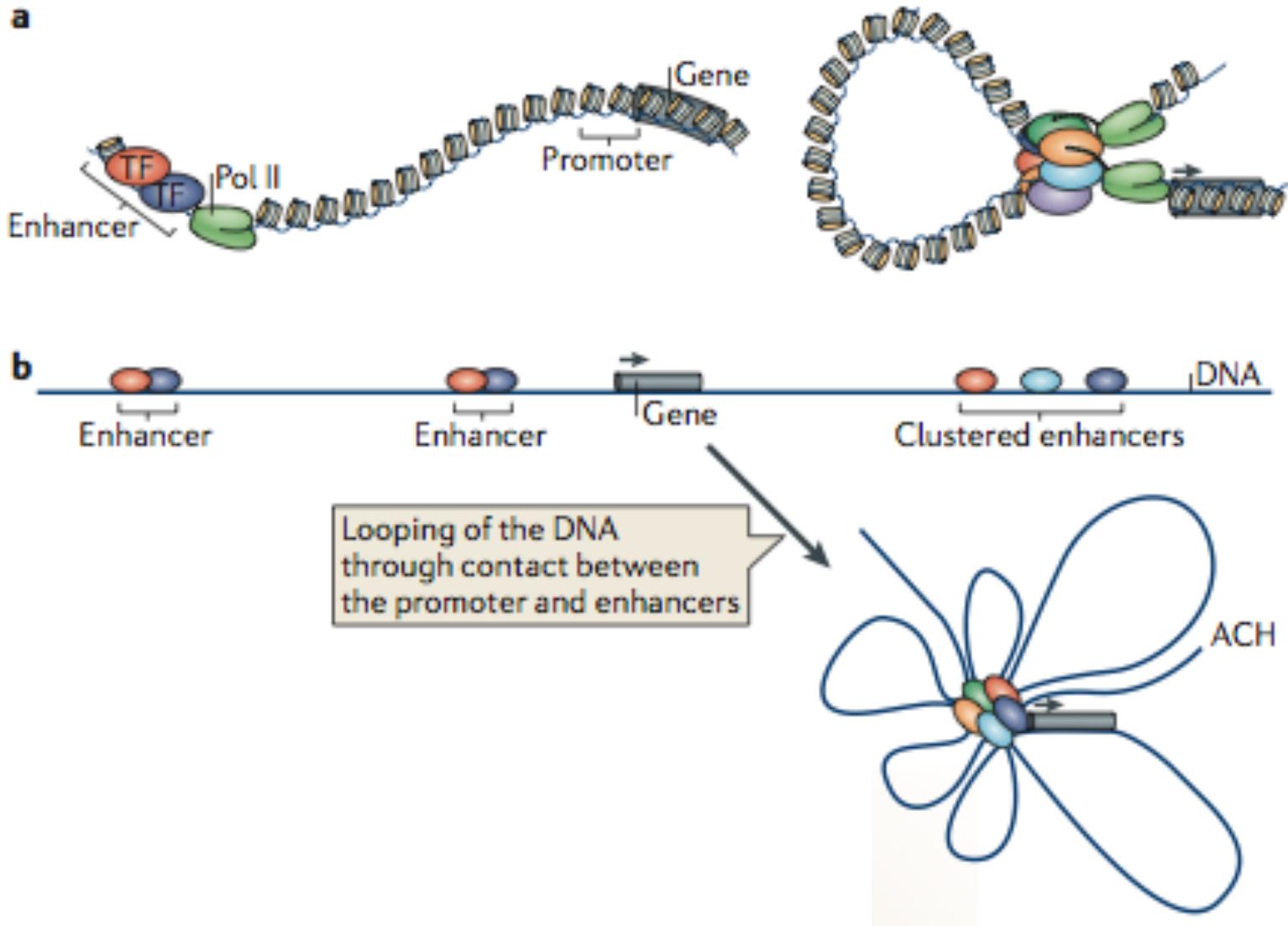


3D-computer
reconstruction

Courtesy by Thomas Cremers

The chromosomes themselves are organized in smaller loops containing genes that are „co-regulated“. These functional domains are named topology associated domains („TAD“s).

The linear sequence of genes and their regulatory elements is folded into 3D loops in the nucleus



3D loops are formed in larger domains of the nucleus - the so called topology associated domains (TADs)

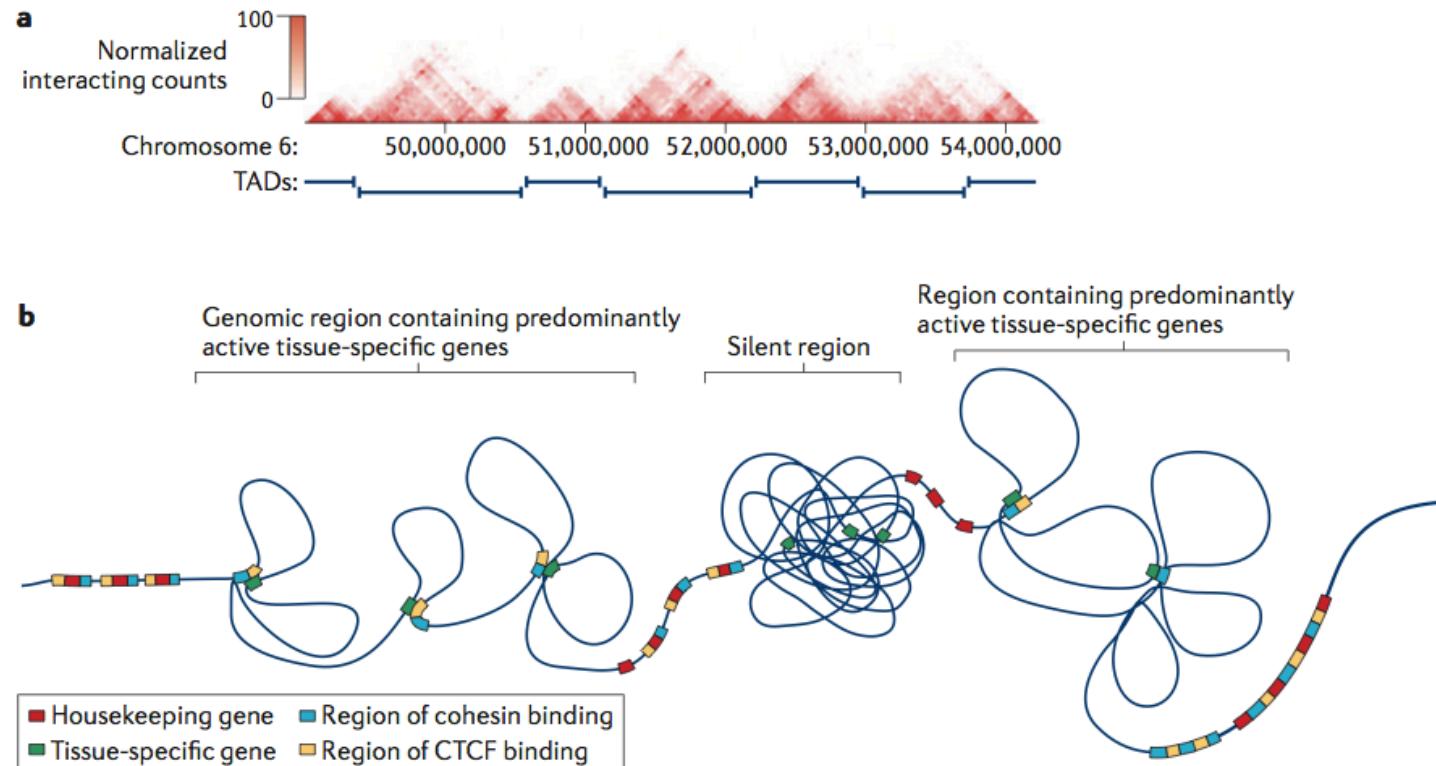


Figure 2 | Topologically associating domains. **a** | Hi-C profiles reveal that the mammalian genome is organized into topologically associating domains (TADs): regions that show high levels of interaction within the region and little or no interaction with neighbouring regions. The heat map represents normalized Hi-C interaction frequencies. **b** | Schematic of putative TAD structures. The central regions of TADs show high levels of chromatin interaction and coincide with the presence of tissue-specific genes and their associated enhancers, the interactions of which with their cognate promoters are facilitated by the presence of cohesin and CCCTC-binding factor (CTCF). The border regions between TADs are enriched for housekeeping genes, which are often clustered together and generally lack the widely dispersed distal enhancers that are found around tissue-specific genes. The border regions show high levels of CTCF and cohesin binding, although only CTCF seems to prevent interactions between TADs. Figure, part **a**, is reprinted from REF. 38, Nature Publishing Group.

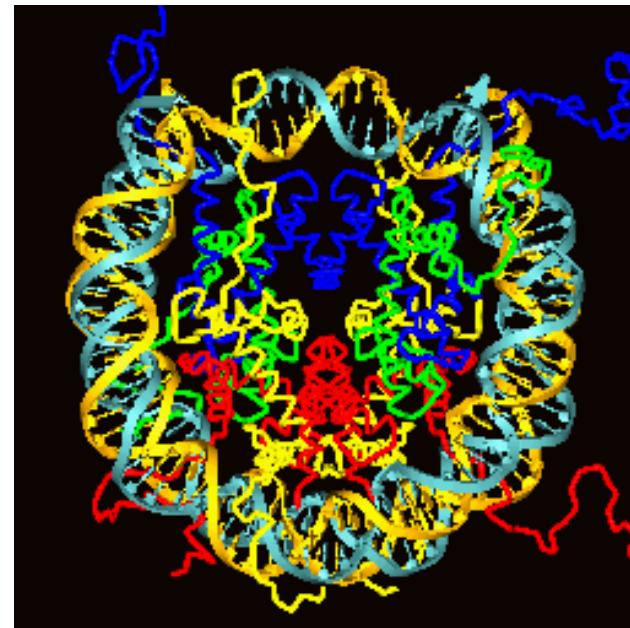
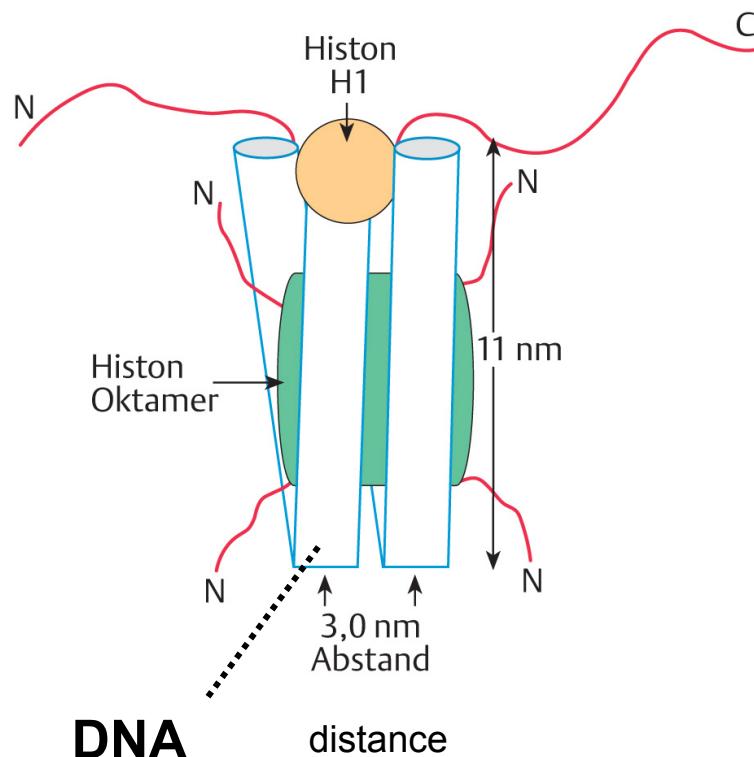
Question:

How is this topology of chromosomes organized at the domain wide and the local gene specific level?

Answer:

Both the local (gene specific) and the domain specific (many genes in TAD's and LAD's) organisation is linked to specific epigenetic modifications and the recruitment of reader proteins that allow looping and gene activation or gene silencing.

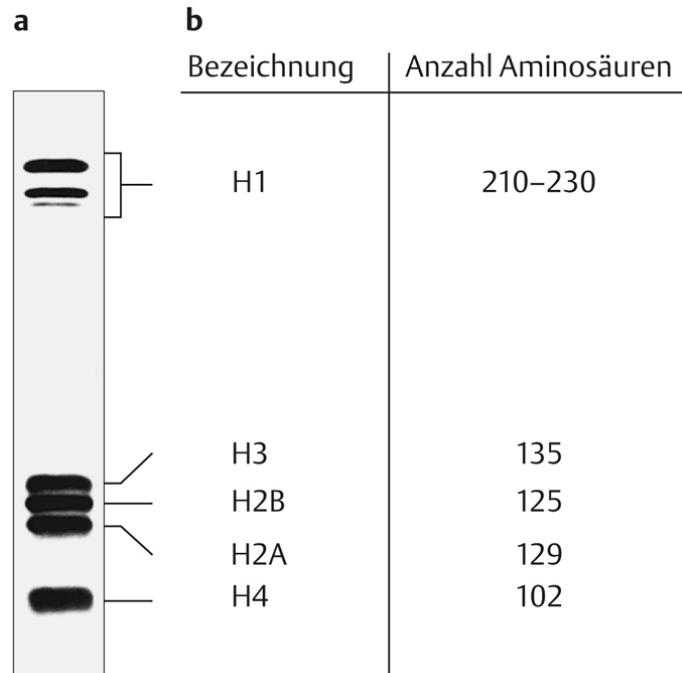
From the higher order to the local organization of gene regulation: the role of histone modifications



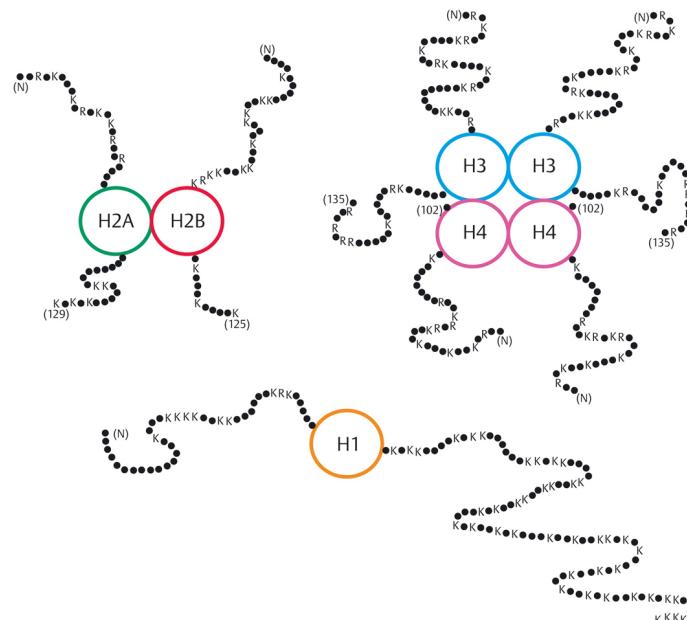
The nucleosome consisting of a histone octamer (2 identical tetramers composed of 4 different histone protein types H2A, H2B, H3 and H4) and DNA wrapped around the histone octamer. A linker histone (Histone H1) can be bound at the entry/exit of the DNA in the nucleosome fixing the nucleosomal structure.

Histone „tails“ shown as red lines protrude out of the core octamer and are modified to modulate the interaction of histone with the DNA and the recruiting of reader proteins.

Histone - the core proteins of the nucleosome



gelectrophoretic separation of Histones



Schematic representation of the inner globular structure of histones and The protruding N- and C-terminal regions (tails) which have unordered flexible structures and many + charged amino acids

Histones are the most abundant proteins in the nucleus. They are small (102- 135 amino acids only) very basic proteins with enriched numbers of arginines(R) and lysines (K). They are evolutionary well conserved..

The role of Histone Variants in chromatin

Histones are very highly conserved proteins, but in human/mammals many variant forms of histones exist with few amino acid changes (mostly) at the c-terminus

For example:

Histone H3 has four main variants:

H3.1, H3.2, H3.3, CenpA

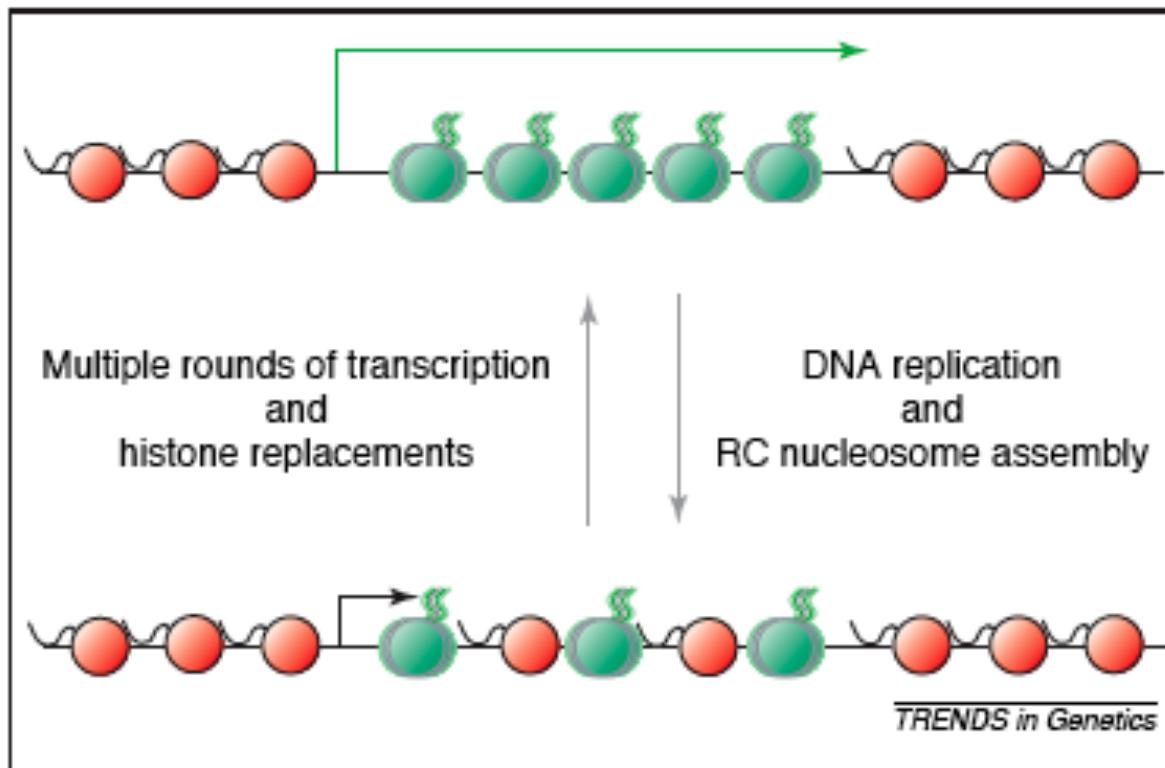
Histone H2A has three main variants:

H2A, H2AZ, H2AX

These variants are enriched in specific regions of the genome (chromatin) and are linked to specific regulatory functions.

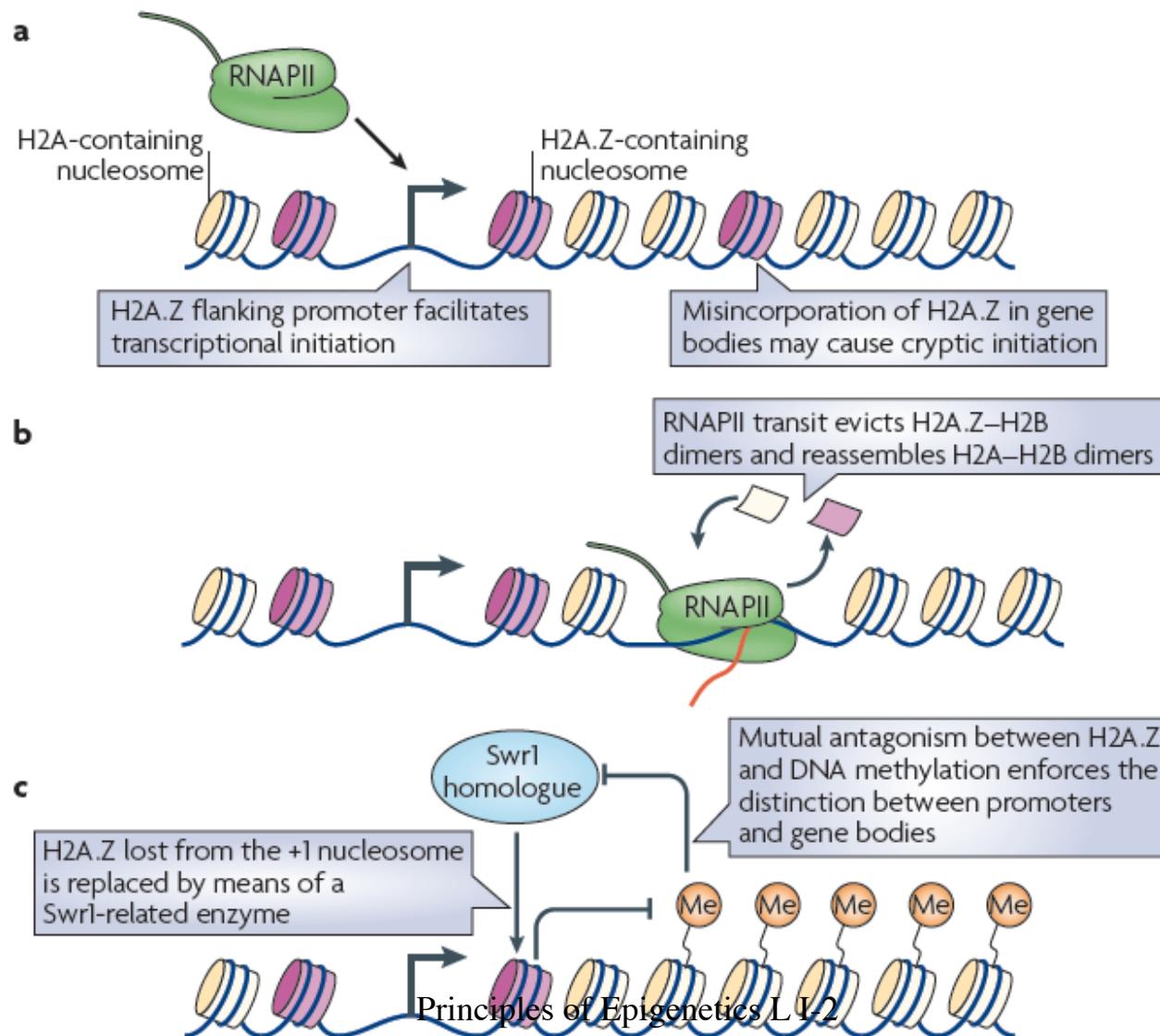
Histone Variants:

Nucleosomes in the body of strongly transcribed genes carry the histone variant H3.3 instead of the „canonical“ variant H3.1

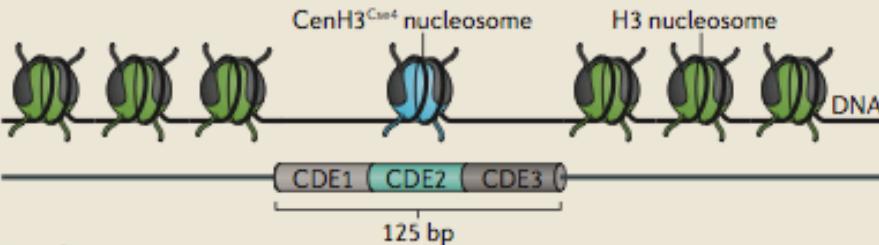


Histone variant H3.3 containing nucleosomes
Histone variant H3.1 containing nucleosomes

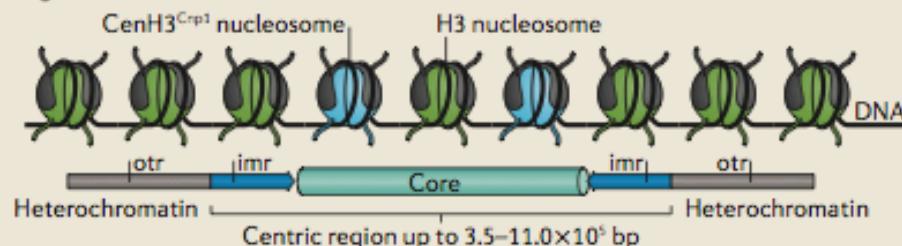
Nucleosome around active promoters are locally containing the histone variant H2AZ instead of the canonical H2A



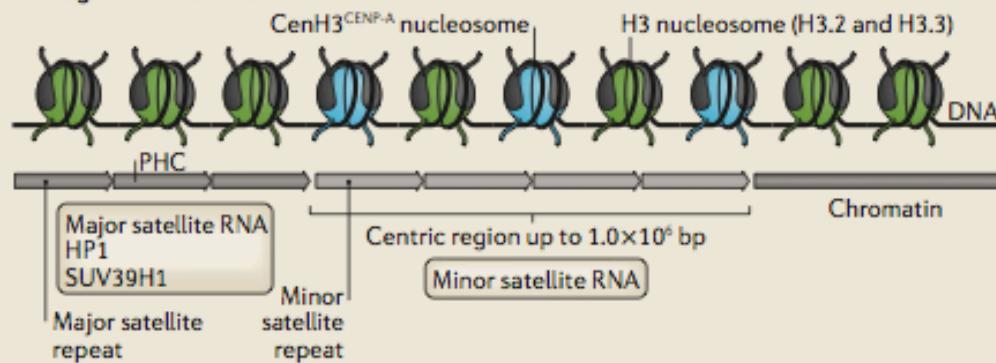
a *S. cerevisiae*
Regional centromere



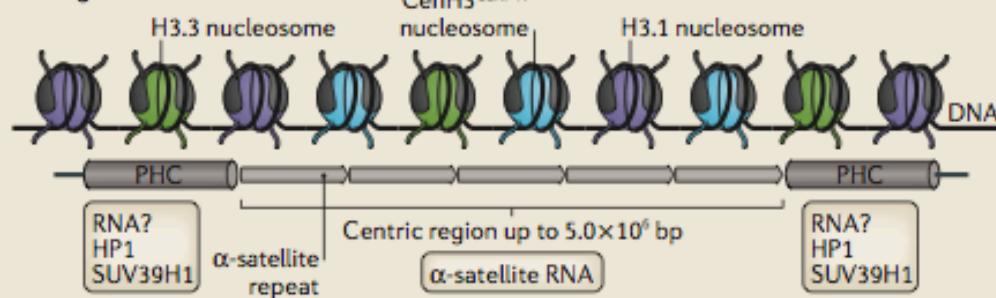
b *S. pombe*
Regional centromere



c *M. musculus*
Regional centromere



d *H. sapiens*
Regional centromere

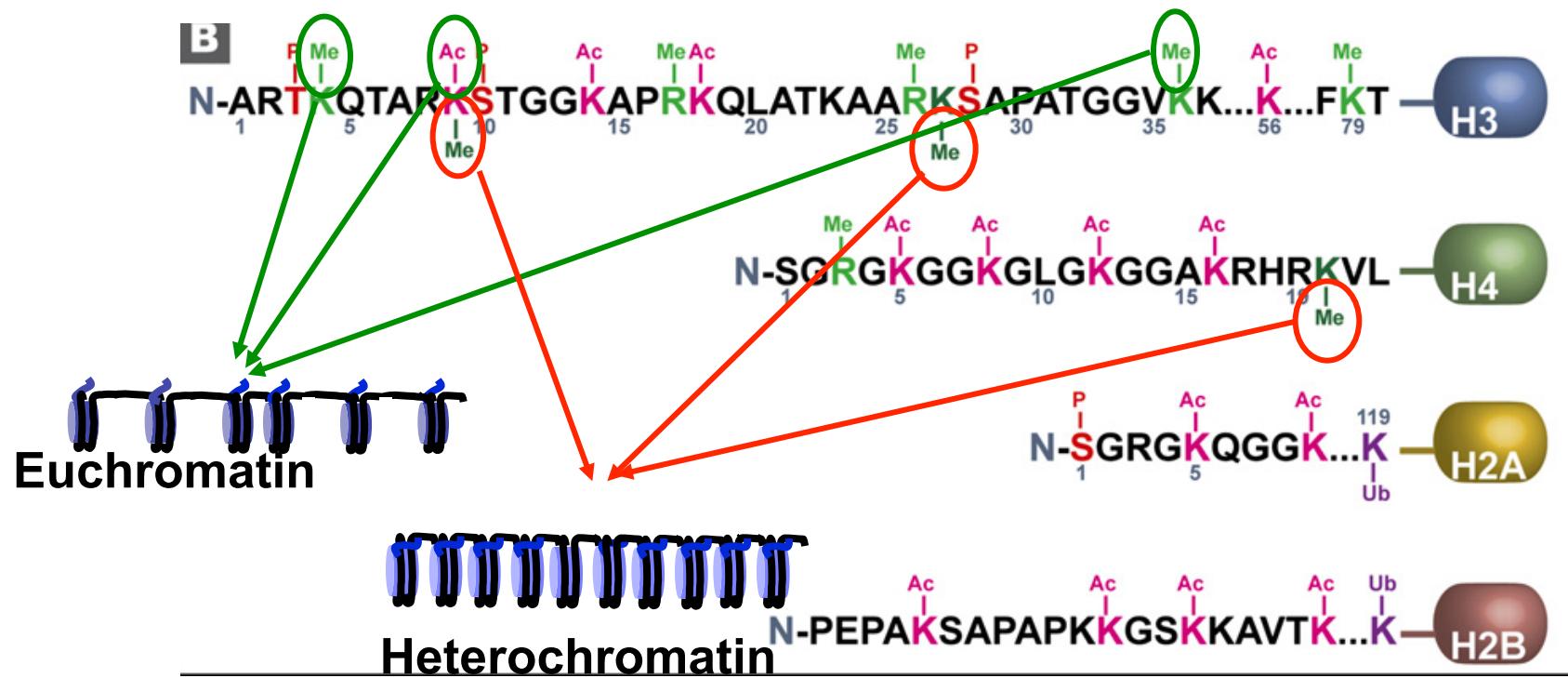


Histone-modifications

General rules:

- Histone modifications are established/removed when histones are in nucleosomes („in place“).
- Histone modifying enzymes have a specificity for certain amino acids. They only catalyse one specific reaction with the „help“ of co-factors.
- Histone modifications can be enzymatically removed – an alternative mode is that the modified histone is exchanged for non-modified histone in the nucleosome.

Histone modifications are specific for open and closed chromatin structures



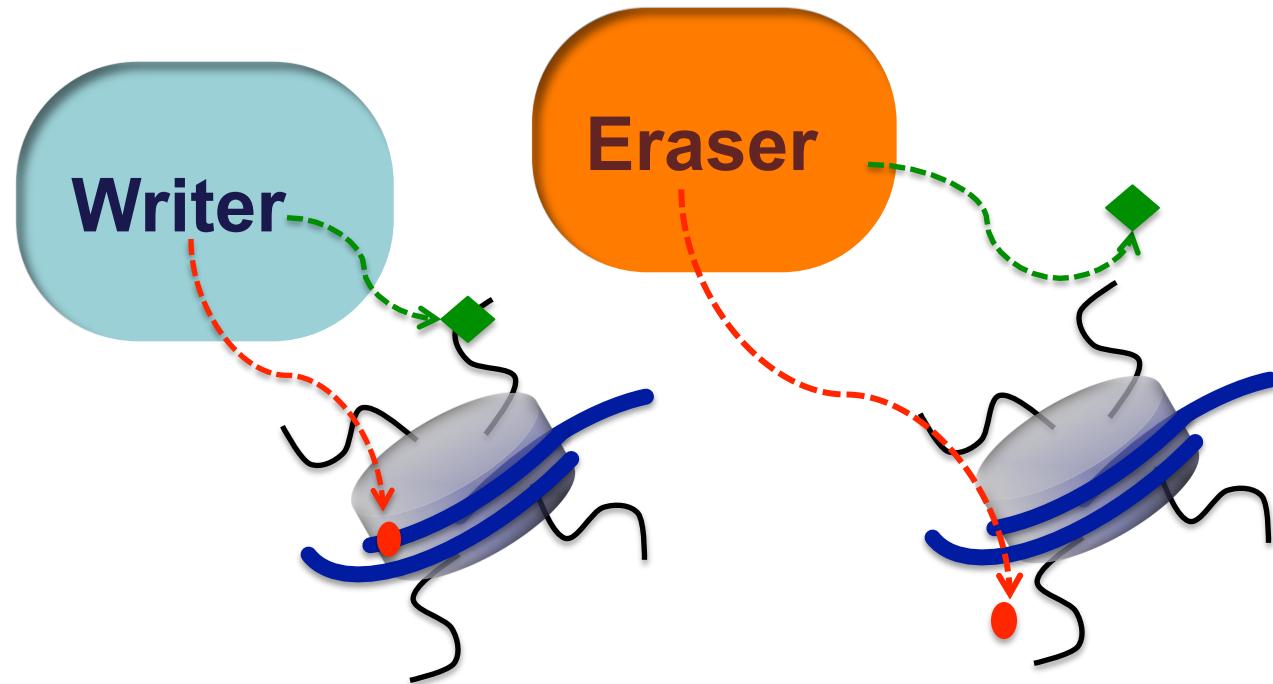
Histone modification classes: many positions and forms (total number > 140)

Table 1. Different Classes of Modifications Identified on Histones

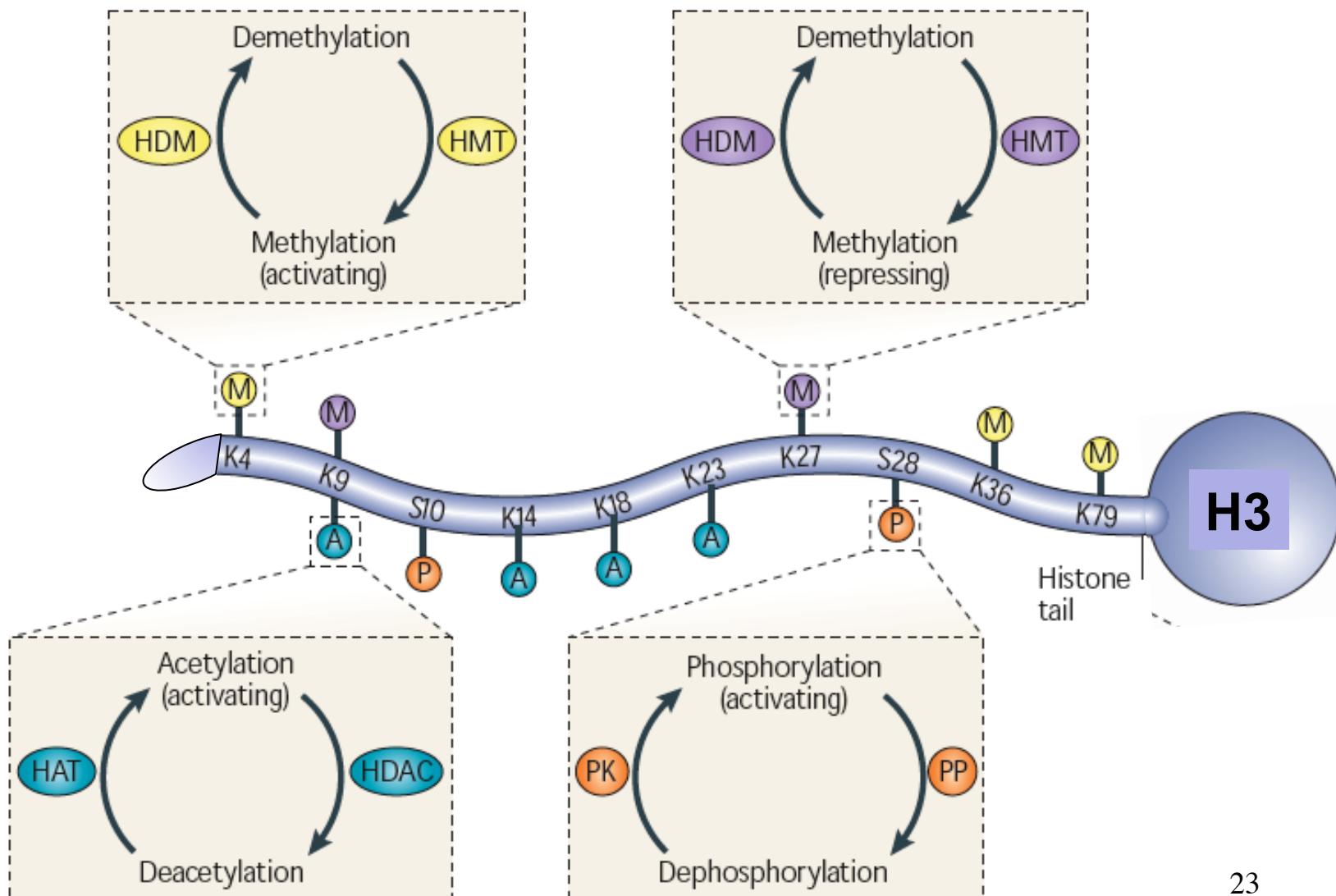
Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	K-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	K-me1 K-me2 K-me3	Transcription, Repair
Methylation (arginines)	R-me1 R-me2a R-me2s	Transcription
Phosphorylation	S-ph T-ph	Transcription, Repair, Condensation
Ubiquitylation	K-ub	Transcription, Repair
Sumoylation	K-su	Transcription
ADP ribosylation	E-ar	Transcription
Deimination	R > Cit	Transcription
Proline Isomerization	P-cis > P-trans	Transcription

Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

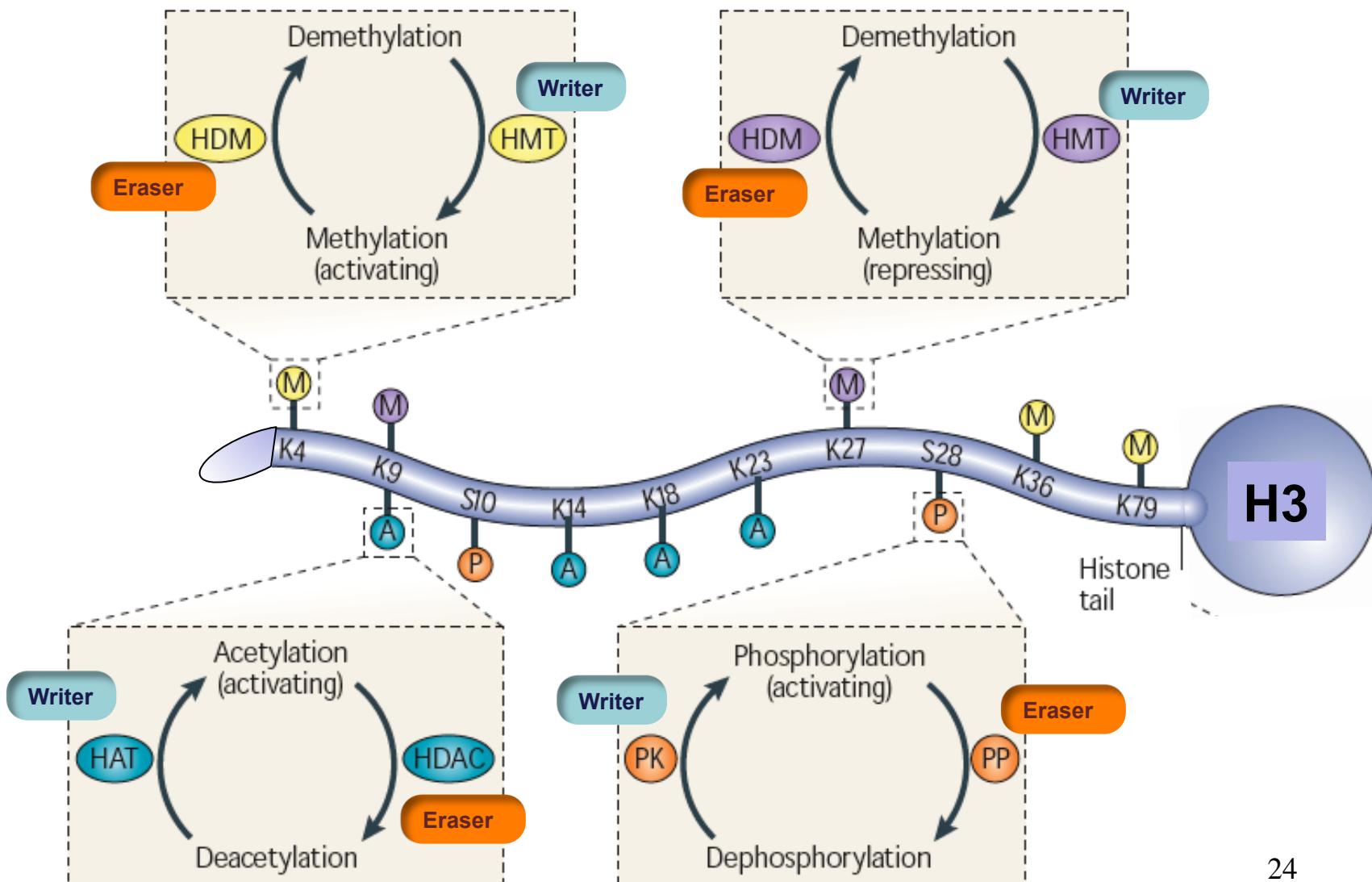
Histone modifications are set and erased by specific enzymes with antagonistic enzymatic reactions



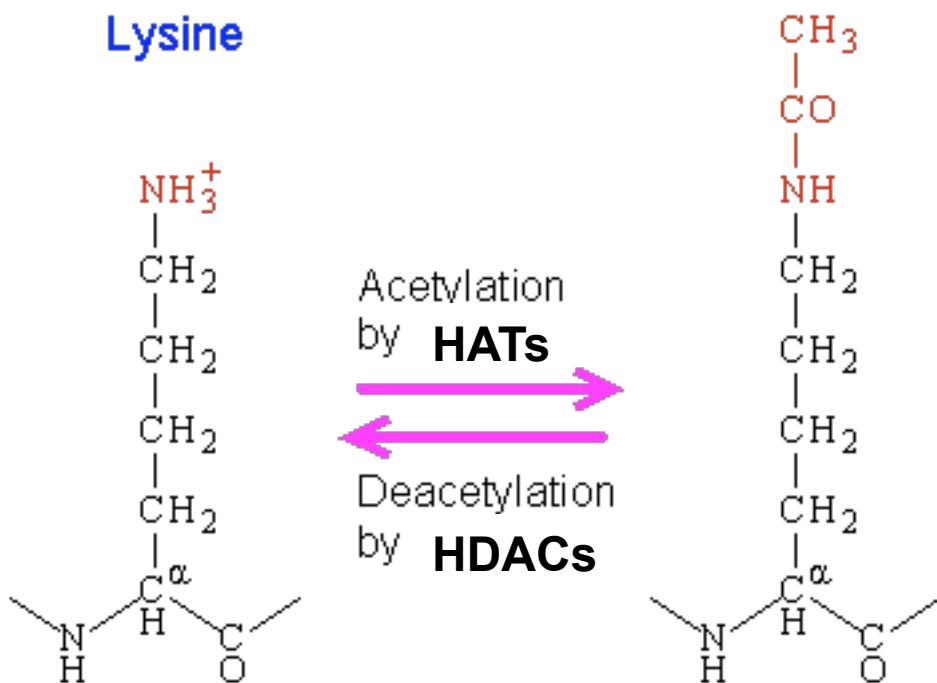
The three major types of histone-modifications are controlled by specific enzymes in forward and reverse reactions



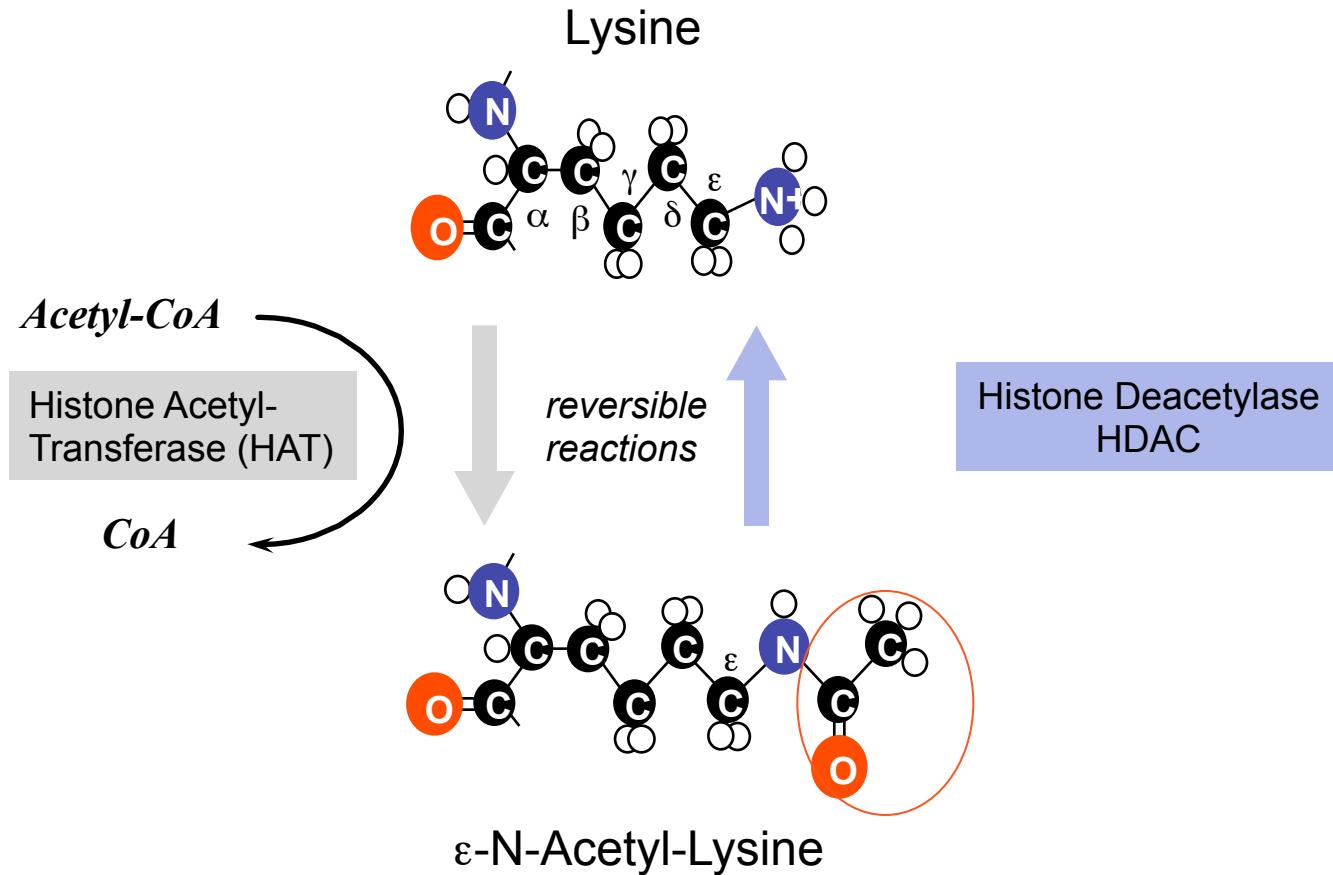
The three major types of histone-modifications are controlled by specific enzymes in forward and reverse reactions



Acetylation of histones



Acetylation of histones



Important positions for lysine acetylation in the NH_2 tails of histones
H3 and H4 : Histone H3: K9, K14,K18, 23 and Histone H4: K5, K8, K12, K16

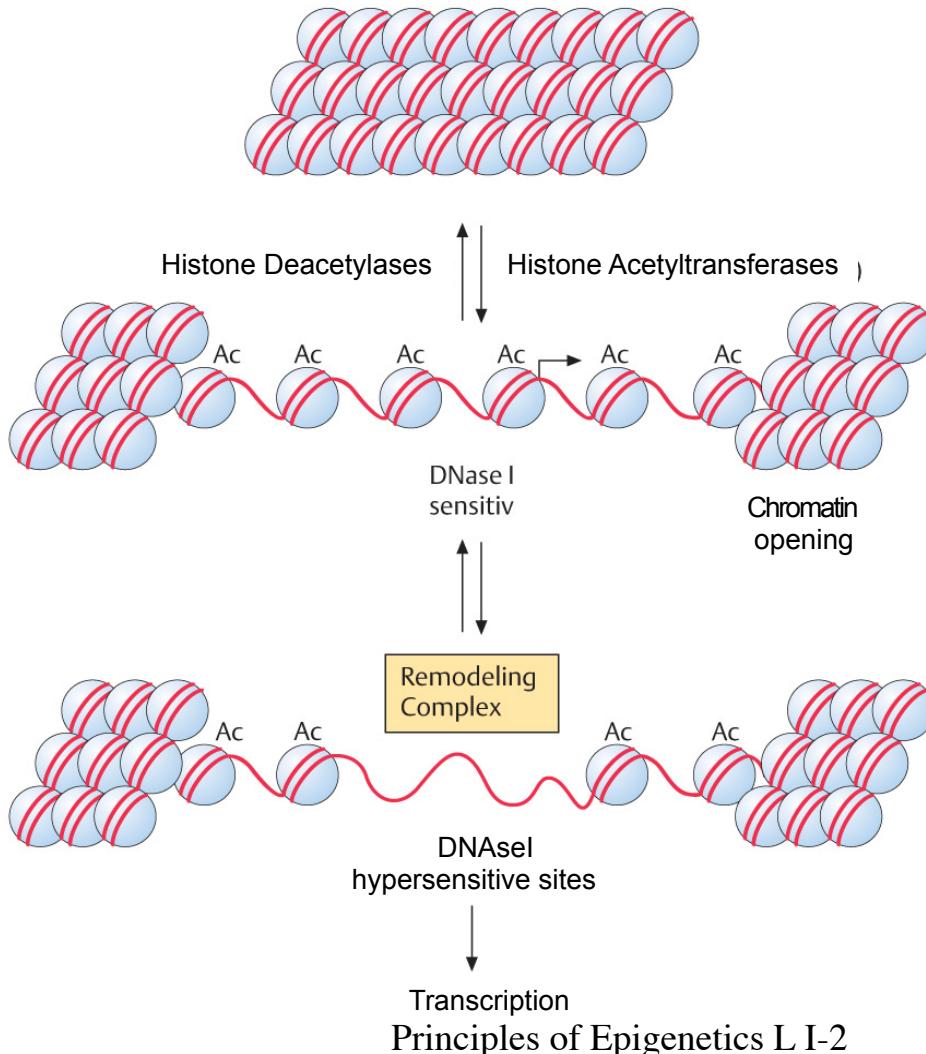
Acetylation is catalysed by HATs = Histone-Acetyl-Transferases

- HATs transfer acetyl-groups from Acetyl-Co-A to Lysine
- HATs are frequently bound to (associated with) transcription-activating complexes (e.g. CBP/P300)
- Lysine-acetylation in histones H3 and H4 facilitates the opening of chromatin by reducing the contacts of histones with DNA.
- The acetylation weakens the interaction of nucleosomes with DNA – nucleosomes can be moved-shifted along the DNA.

Histone acetylation is removed by HDACs = Histone-deacetylases

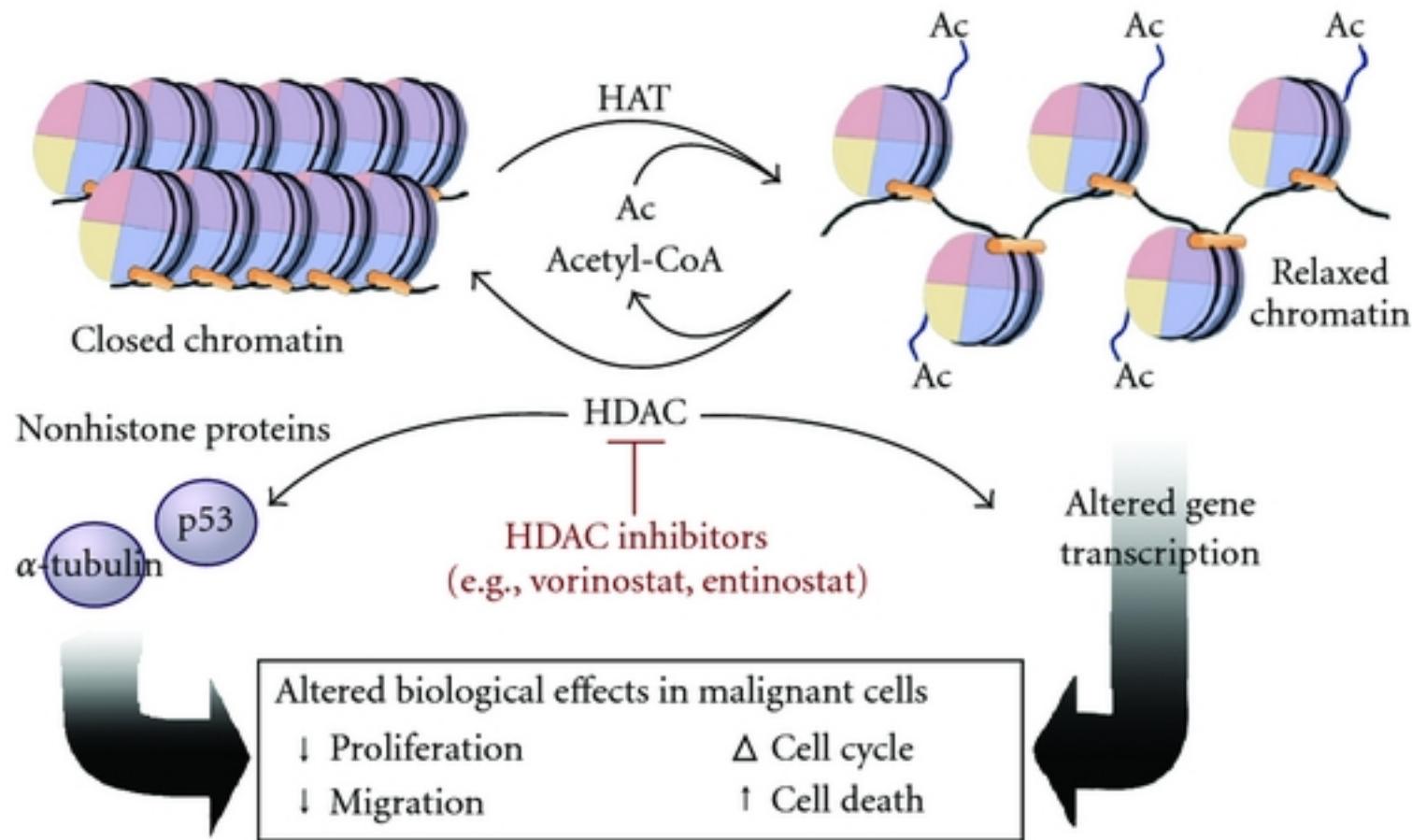
- HDACs remove the ϵ -N-acetylgroups from lysine residues in nucleosomes.
- HDACs constitute several classes of enzymes, some are also known as Sirtuines.
- HDACs catalyse the deacetylation with two several distinct reaction types.
- HDACs are often associated with repressor complexes or heterochromatising factors.
- Transkription poor regions are usually deacetylated (an example is the inactive X-Chromosome in female mammals).

Epigenetic modifications and chromatin condensation: the role of acetylation



Open regions are highly accessible for cleavage by DNA-cutting enzymes such as DNasel or Tn5 (ATAC) or MNase

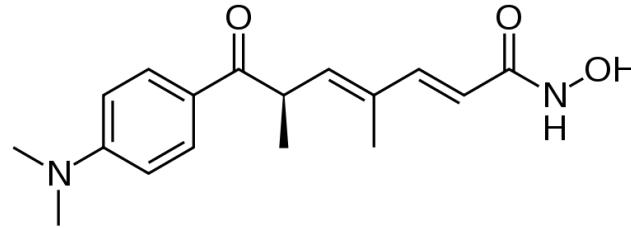
Acetylation of histones “opens” or “relaxes” the chromatin



Histone deacetylases can be inhibited by small molecules (competitive inhibitors)

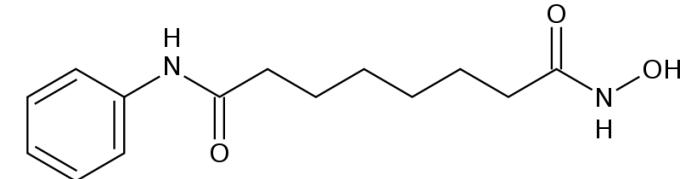
Trichostatin A = TSA

inhibits HDACs 1, 3, 4, 6 and 10

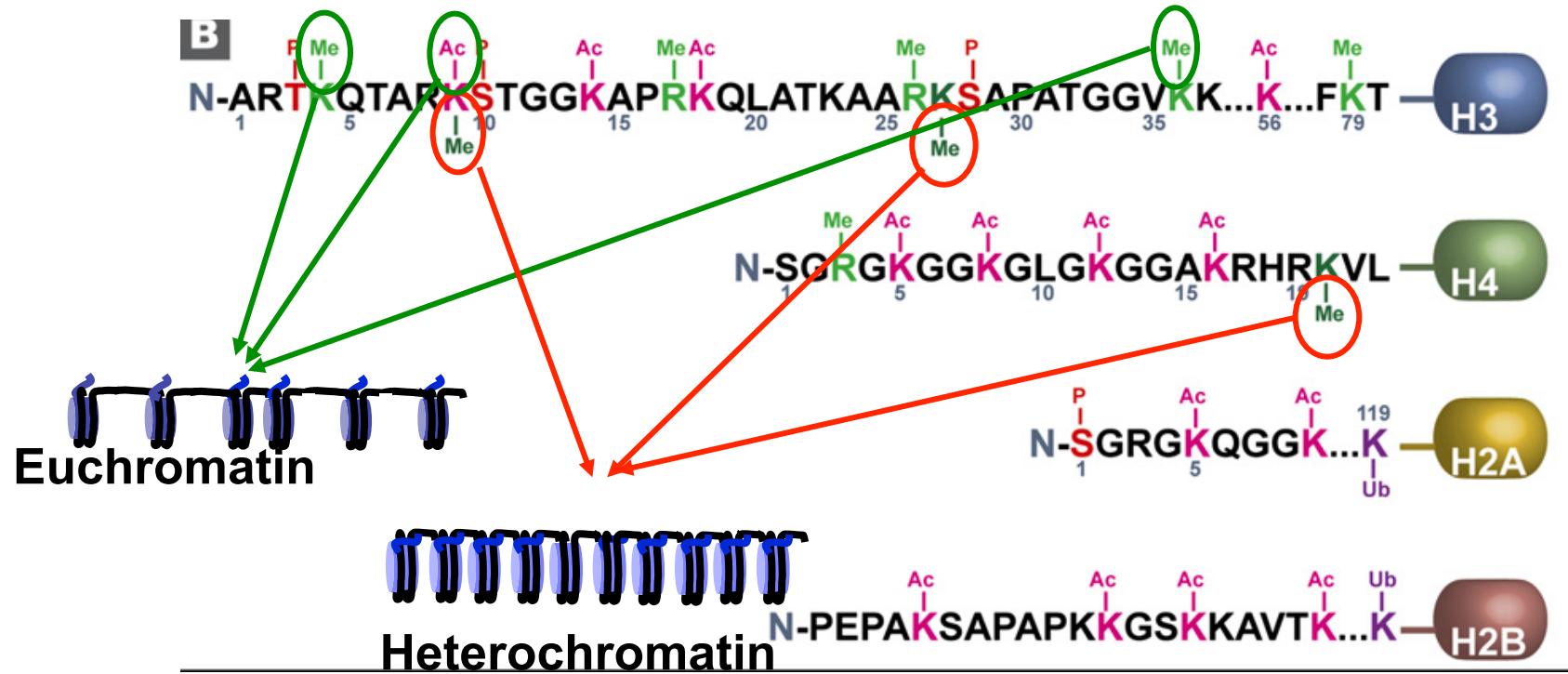


Vorinostat/suberoylanilide hydroxamic acid =

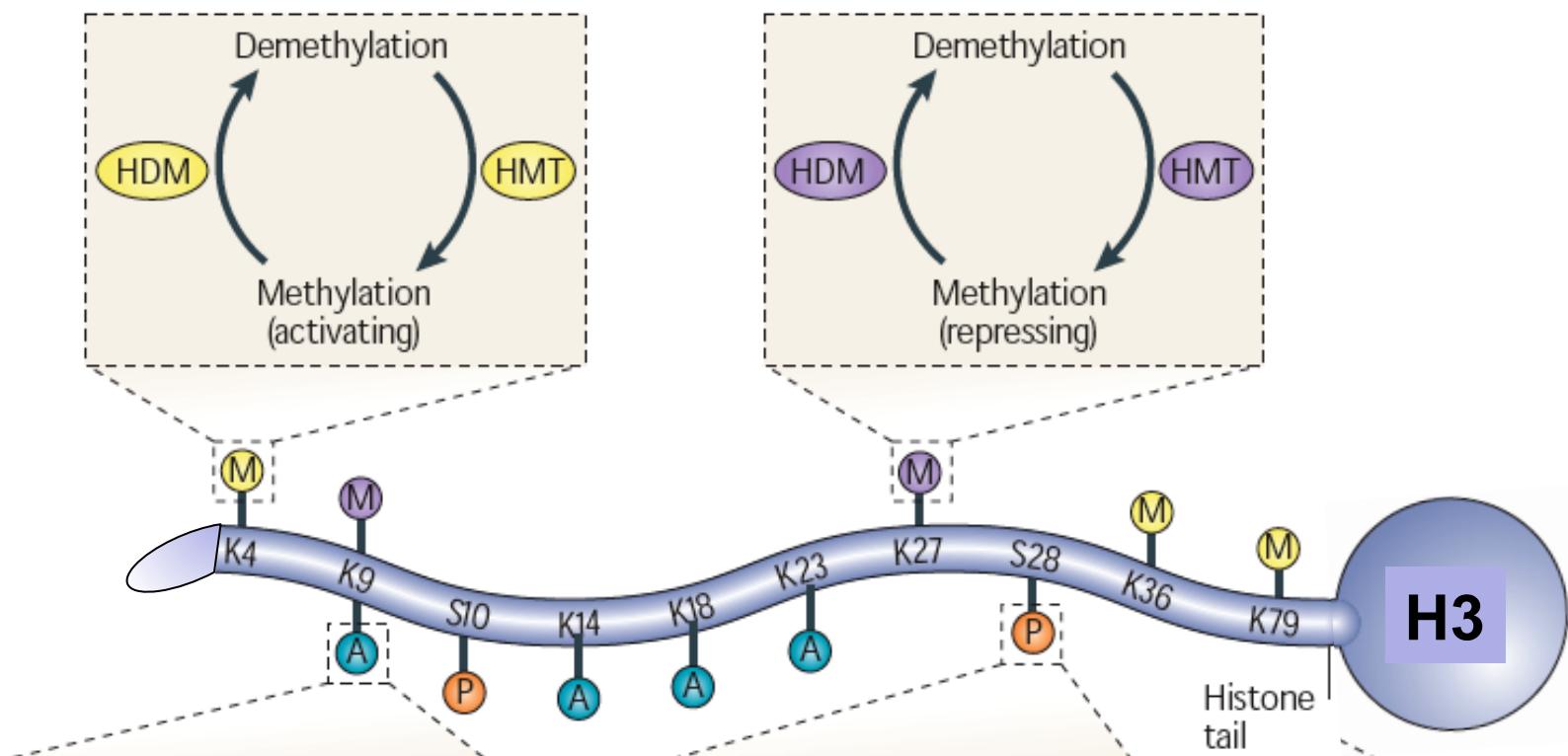
SAHA inhibits HDACs (no clear specificity)



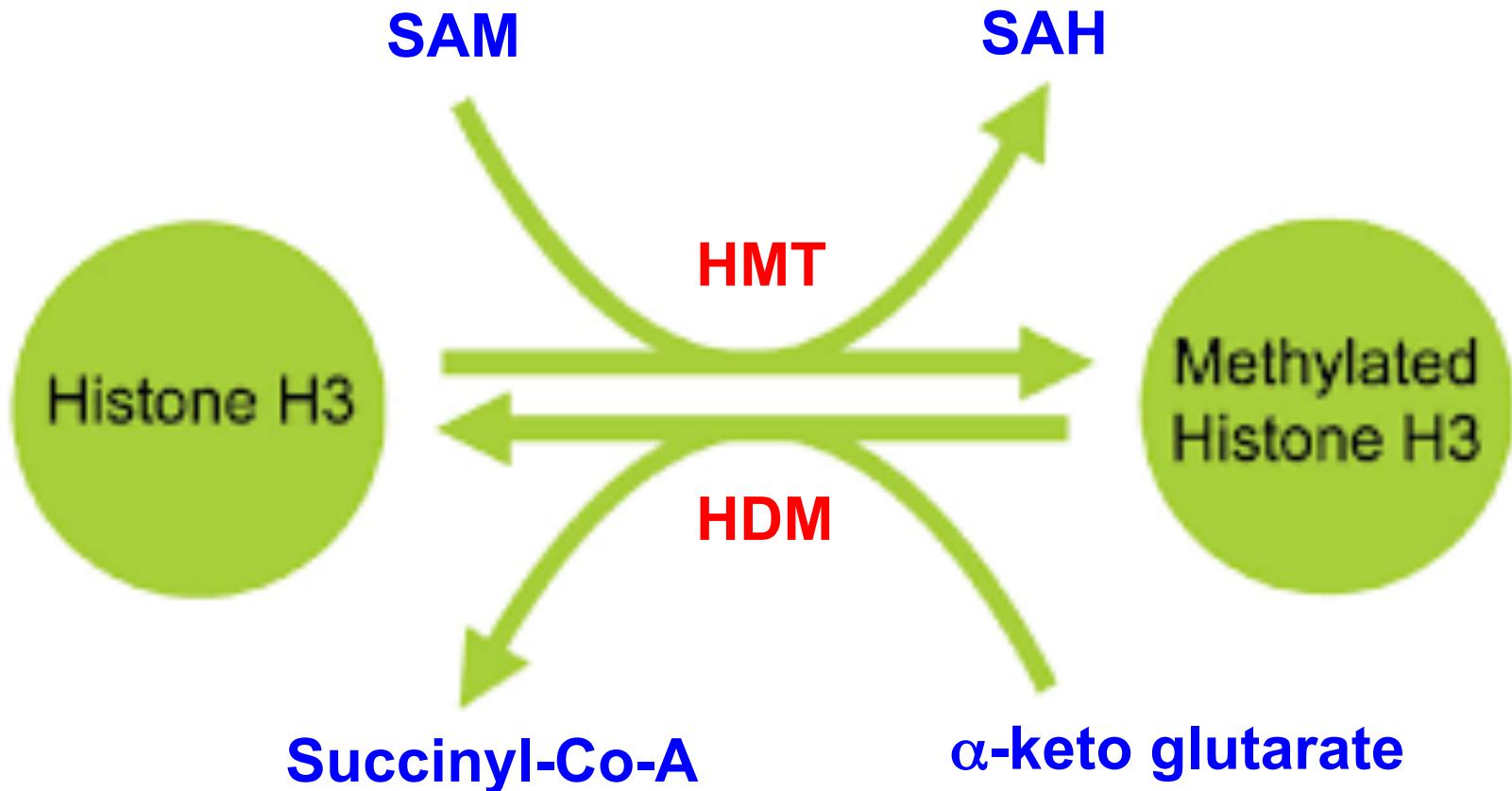
Histone methylation and open and closed chromatin structures



2. Histone-methylation: setting & removal (writing & erasing)

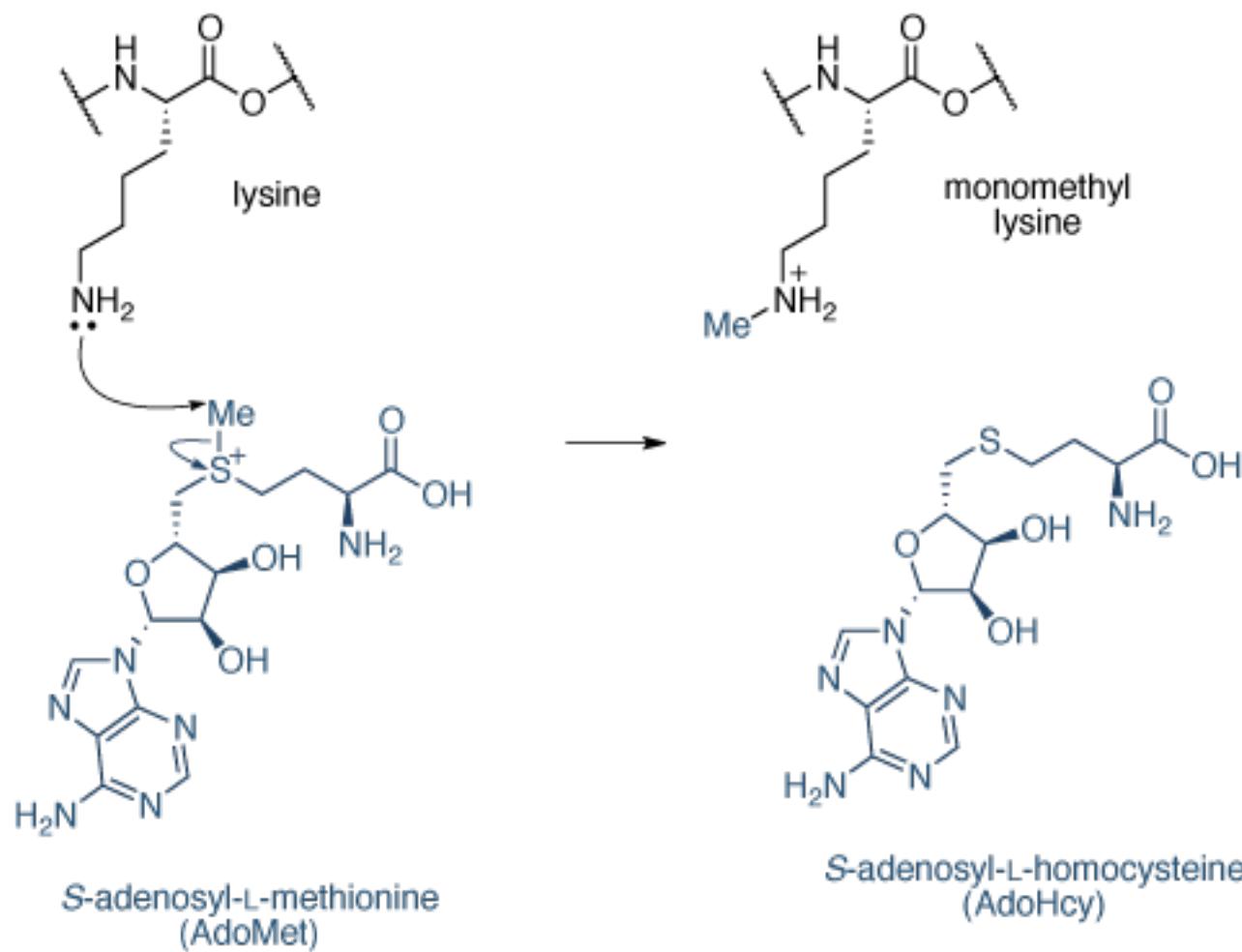


The general cofactors for histone modifying and demodifying enzymes (S-adenosyl-methionine, α -ketoglutarate)



HMT = Histone methyltransferase

HDM = Histone demethylase



Histone Methyltransferases (HMTs)

Methylation of histones at the ε N-position was first described in 1964.

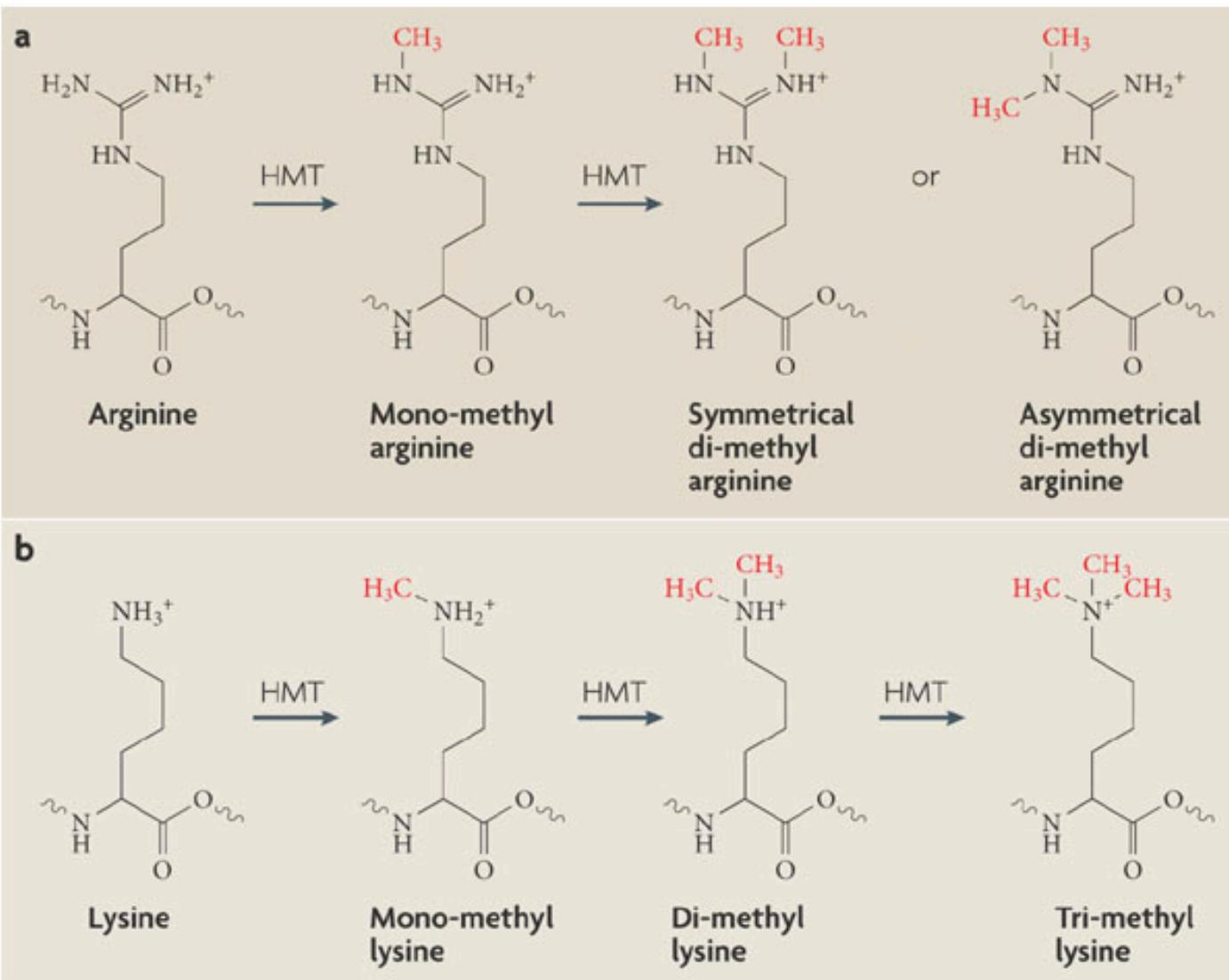
The first enzymes catalysing this modification (HMTs) were identified in mammals and Drosophila in the early 1990s (SuVAR39, MLL1,2,3)

HMTs contain a catalytic domain and additional regulatory domains (SET domain and or chromo- or bromo-domains)

All HMTs use the co-factor S-adenosyl-methionine (SAM) for the methylation reaction

Arginine specific HMTs are called PRMT's

The various forms of Histone methylation at arginines and lysines



Local setting of histone modifications

How is the gene (locus) specific local modification established?

Which mechanisms target the enzymes (writers) to these specific regions?

How are modifications recognized - which proteins interact with modified histones (readers)?

How are modifications inherited/maintained over replication?

How are modifications removed (erasers) from histones?

Local/regional activity of HMTs

HMTs

Specificity

SUV39H1	H3K9
SUV39H2	H3K9
G9a	H3K9
ESET/SETDB1	H3K9
SUV4 20H1	H4K20
SUV420H2	H4K20
SpSet 9	H4K20
EZH2	H3K27
RIZ1	H3K9

Localisation

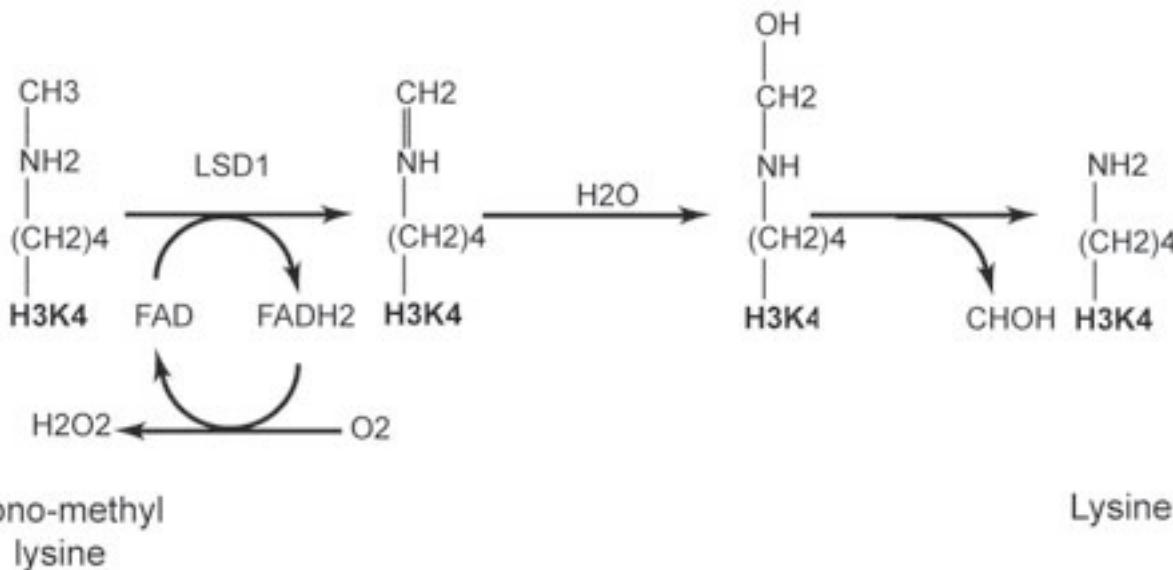
Closed
Hetero-
chromatin

MLL3	H3K4
MLL4	H3K4
MLL5	H3K4
SET1A	H3K4
SET1B	H3K4
NSD1	H3K36
SYMD2	H3K36
DOT1	H3K79

Open
Euchromatin

Removal of Histone Methylation by Lysine Demethylases: Two type and cofactors

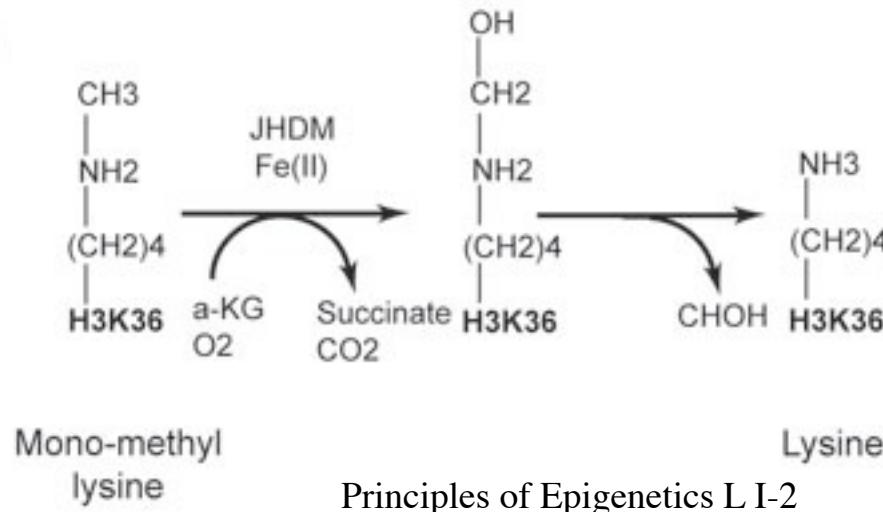
(a)



Mono-methyl
lysine

Lysine

(b)

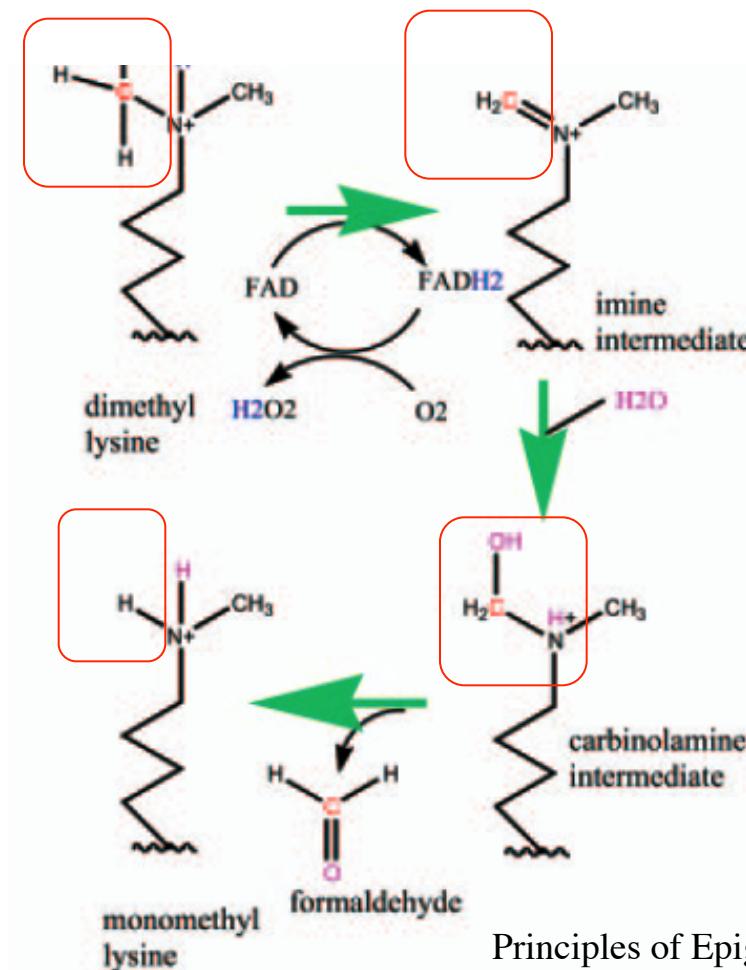


Mono-methyl
lysine

Lysine

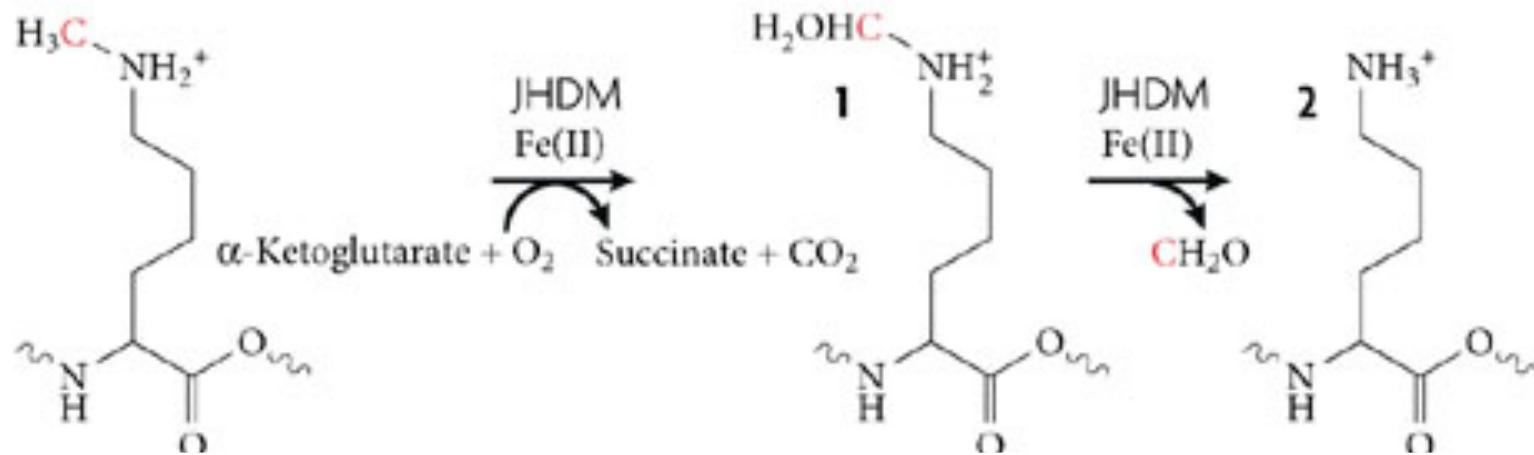
Removal of Lysine Methylation by Demethylases

A demethylase (e.g. LSD1) is recruited to chromatin by repressor complexes to locally demethylate H3K4me3



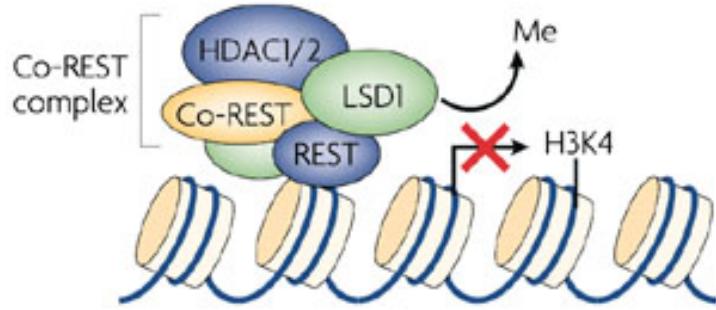
LSD1 uses FAD as a cofactor to covert and cleave formaldehyde from the histone flavin adenine dinucleotide (FAD) is a redox cofactor

2. Type of Lysine Demethylase (Jumonji-type)



Jmj1 uses **α -ketoglutarate as a cofactor**. The cofactor is converted to succinate to finally oxidise CH_3 and to release formaldehyde from the histone

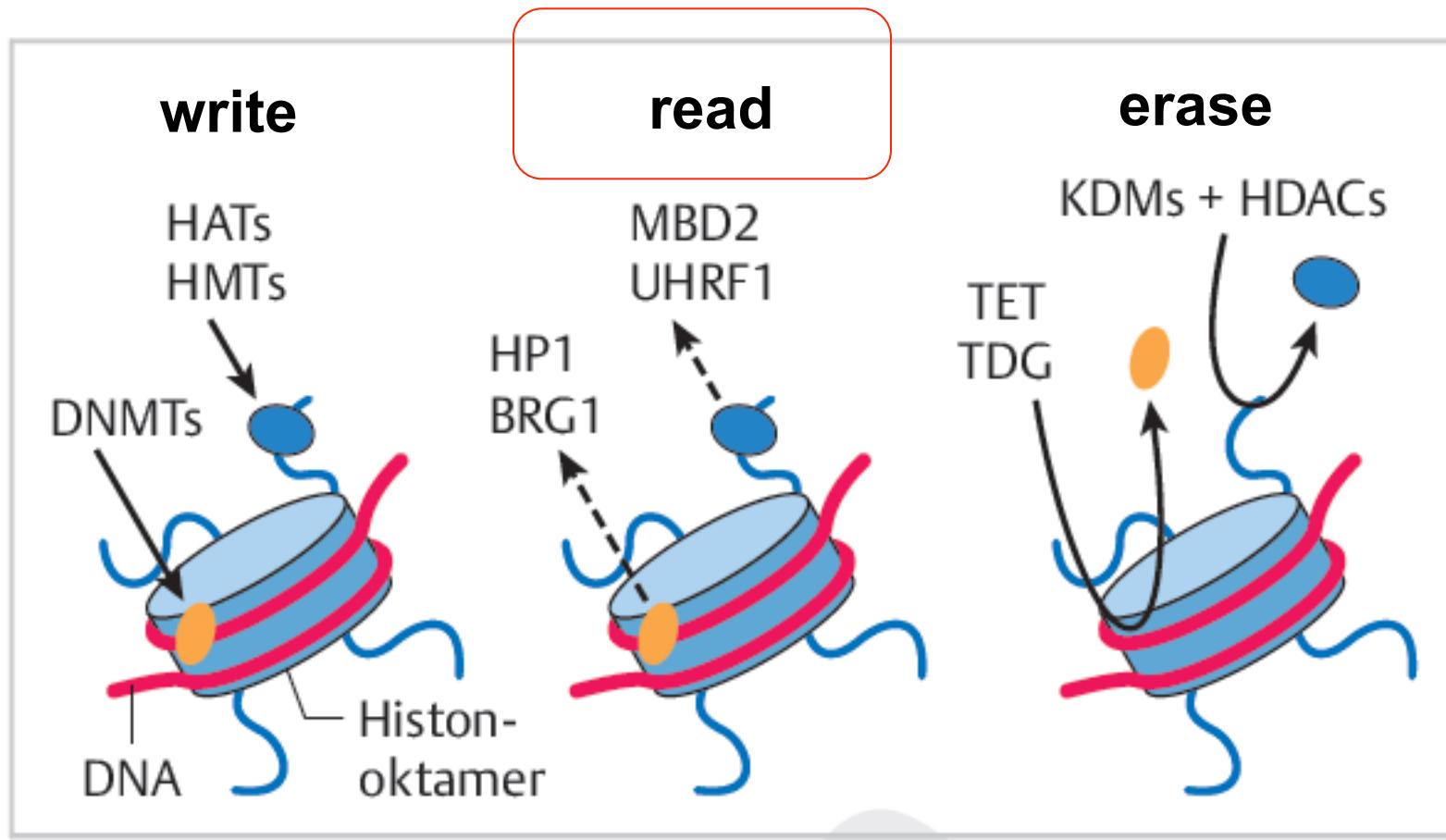
Lysine Demethylase (LSD1) can be part of repressor complexes



REST is a neuronal specific transcription factor. It forms together with Co-REST, HDAC1/2 and LSD1 a repressor complex binding to promoters of genes which have to be silenced upon development, i.e. neuron specific genes in non-neuronal cell types.

Histone H3 becomes demethylated at K4 and deacetylated = transcriptional silent (repressed).

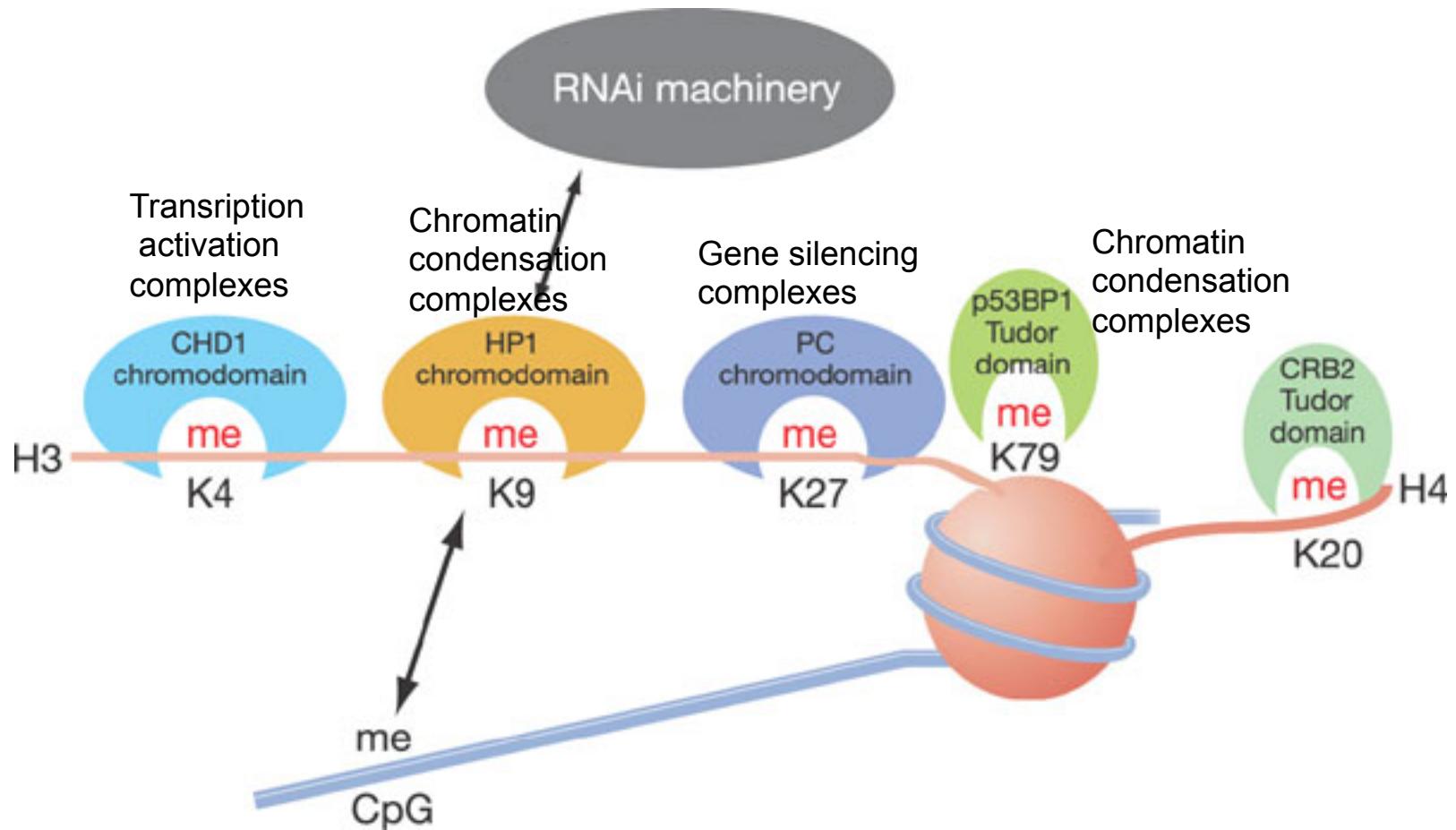
Histone modifications can be „read“ by specific proteins to convey local specific functions



Readers of histone modifications

Readers of histone modifications use a set of similar protein domains to execute their specific binding

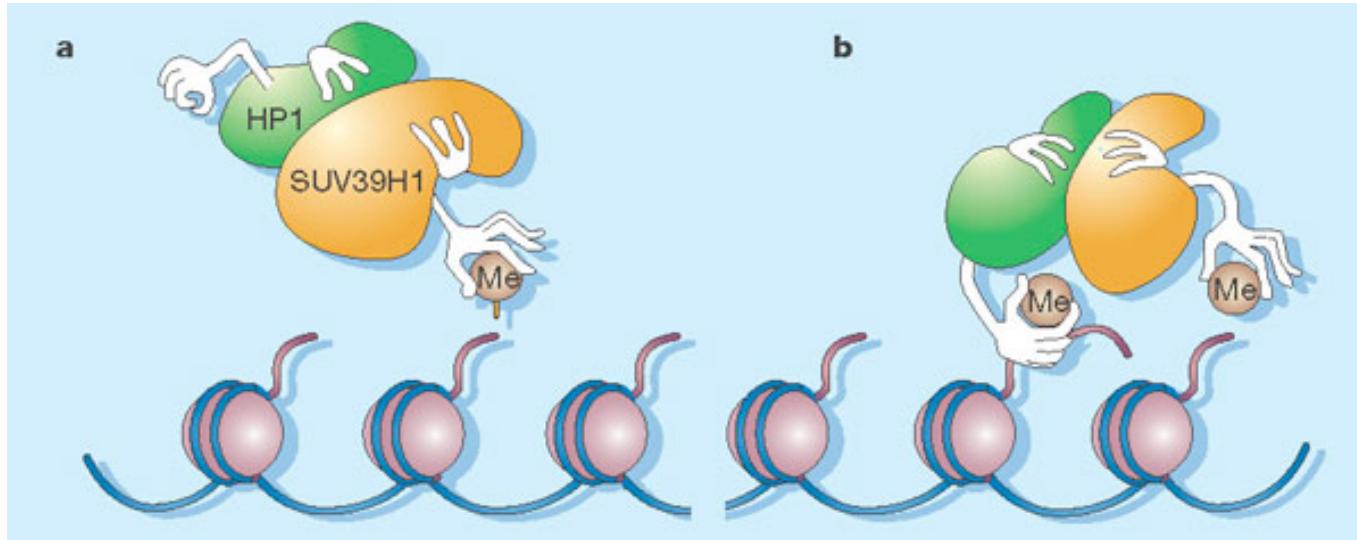
Reader domains recognizing distinct histone modifications



Reading histone modifications to form heterochromatin

HP1 = heterochromatin protein 1

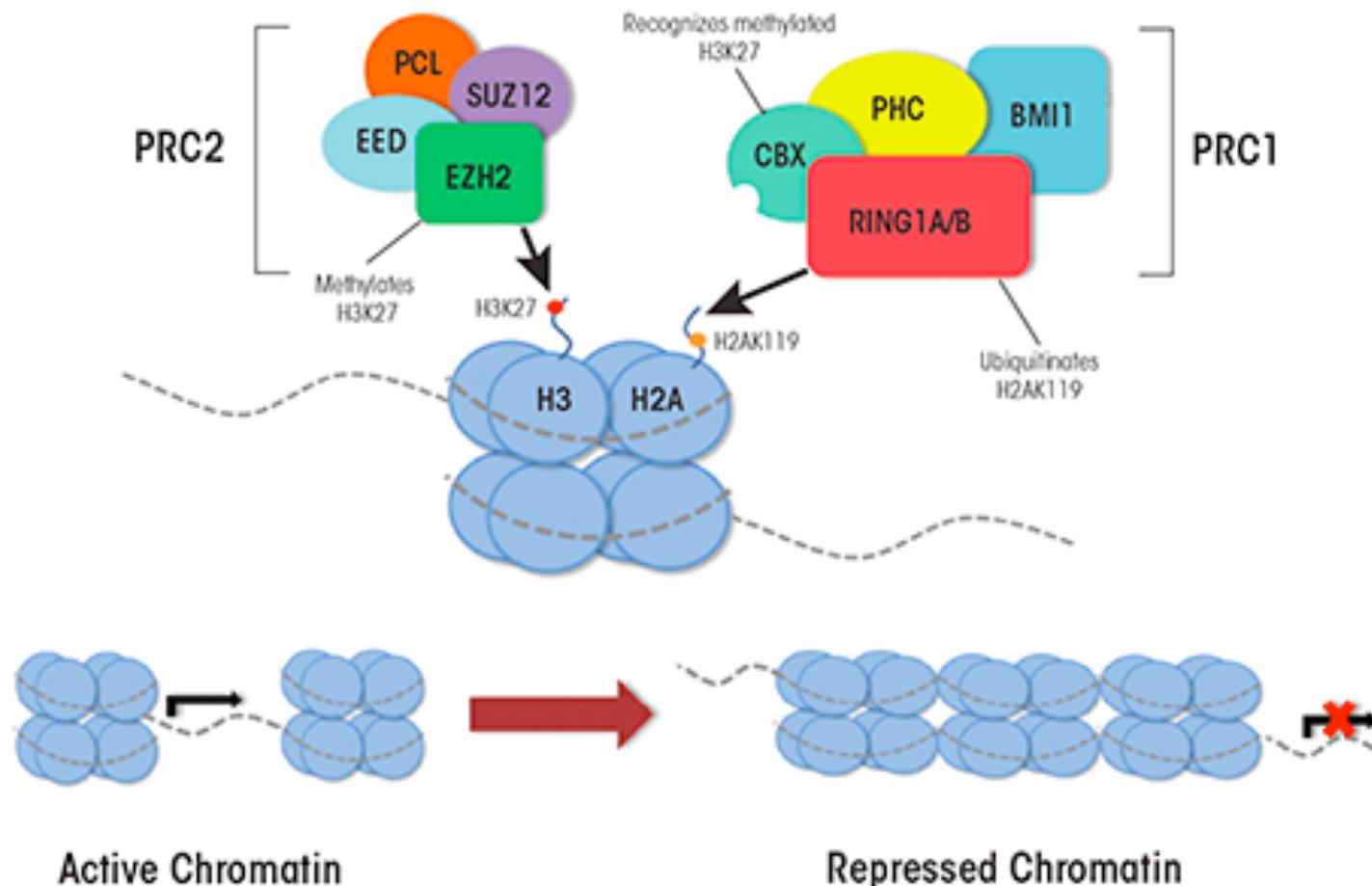
- Recognizes and binds to H3K9me2/3.
- It helps to propagate (spread) heterochromatic histone modifications by recruiting the Histone methyltransferase SUV39H1.



Silencing of genes during development: PcG group proteins = negative transcriptional regulators/silencers

- **PcG** = Polycomgroup-Protein proteins are key proteins of large multiprotein **PRC** complexes.
- **PRC** complexes bind to specific recognition elements = PREs.
- **PRC1** complex contains an enzyme that ubiquitinates histone H2B (H2AUb119).
- **PRC2** complex contains the histone methylating enzymes such as EzH to modify H3K27me3.
- Both complexes form and stabilize local (facultative) heterochromatin at promoters or enhancers of developmentally regulated genes – i.e. they „silence“ those genes in cells where they are not supposed to be expressed

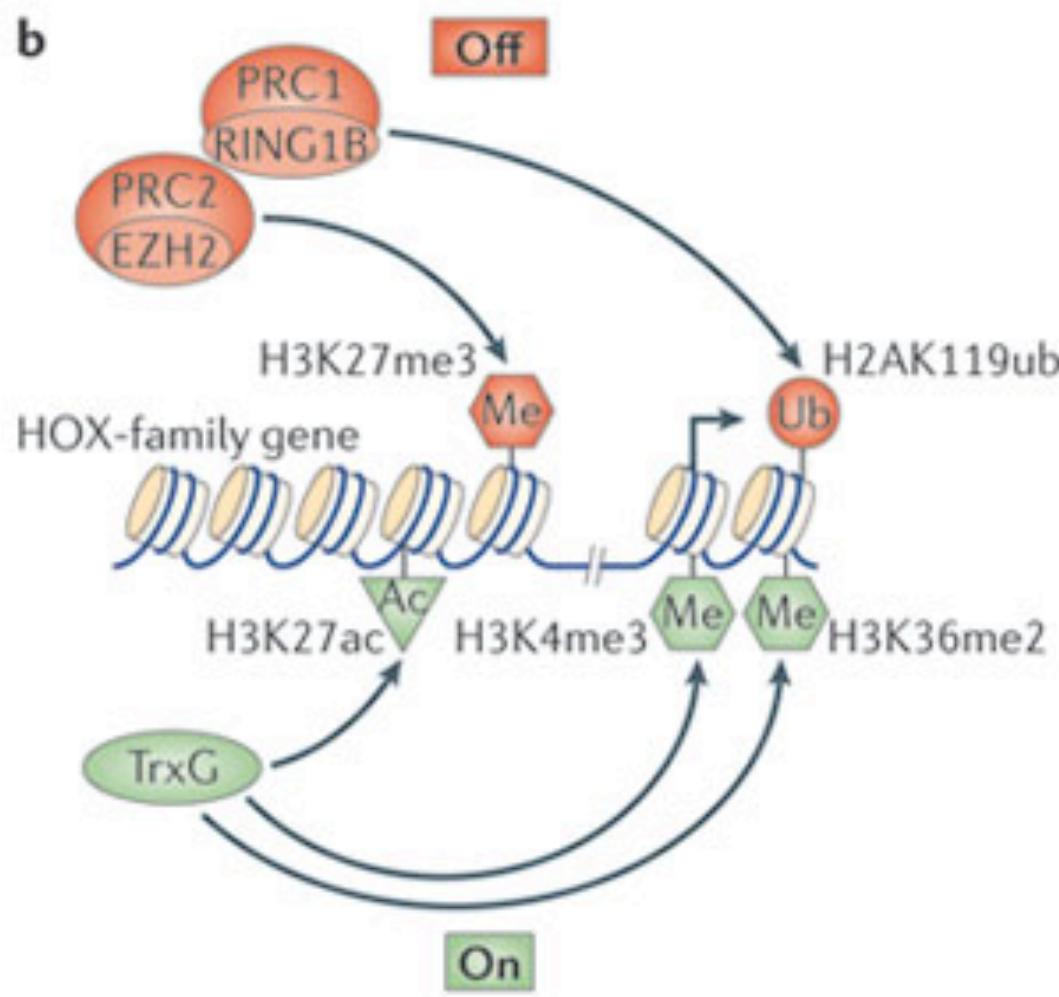
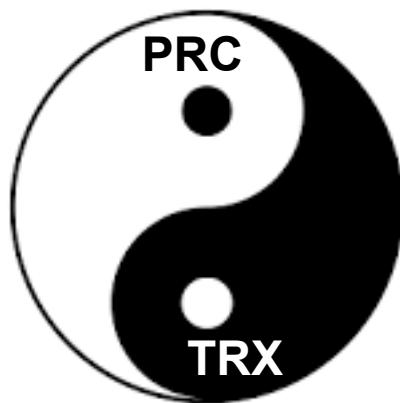
Silencing of genes during development: PcG group proteins = negative transcriptional regulators/silencers



Activation of transcription through chromatin opening: The trithorax complex proteins

- Trithorax-group-proteins (**TRX**) are transcriptional activators and the antagonists of PRC complexes – they keep genes active in cells.
- **TRX** proteins usually contain a characteristic „BROMO“ domain through which they attract transcriptional regulators/activators.
- **TRX** are often in complex with euchromatic HMTs (H3K4me3, H3K36me3) and HATs (H3K9/H4K12/14) to locally establish and spread open chromatin around promoters/regulatory regions.

The developmental „Ying-Yang“ of gene regulation by histone modifying complexes



Propagation of chromatin states (Histone Modifications) through cell division

Histone modifications are established on Histones not on the DNA!

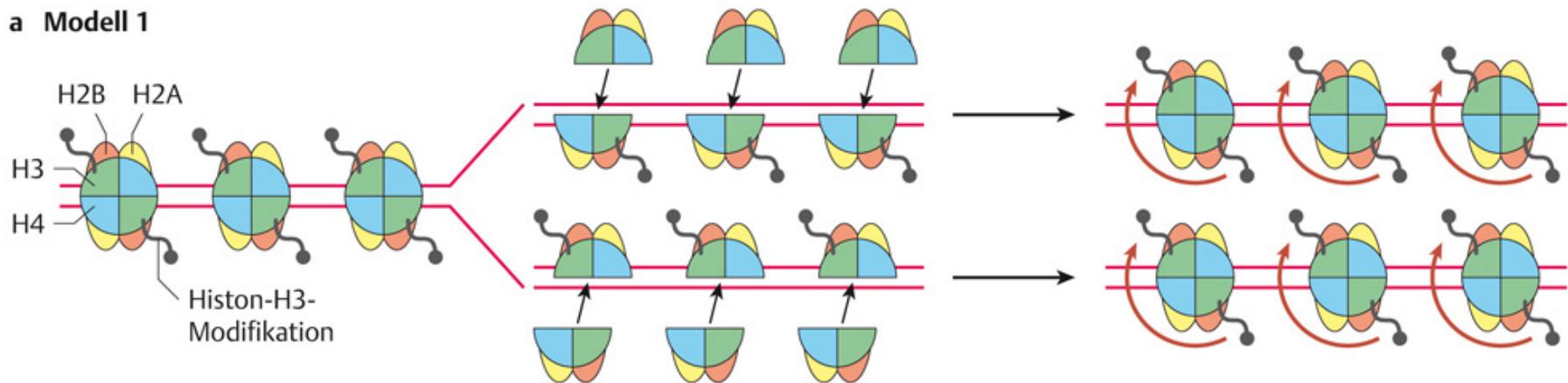
One of the key questions in epigenetics is therefore how specific states of modifications can be propagated = inherited through cell division (across DNA/chromosome replication).

Remember: During replication chromosomes and nucleosomes of the chromosomes are duplicated.

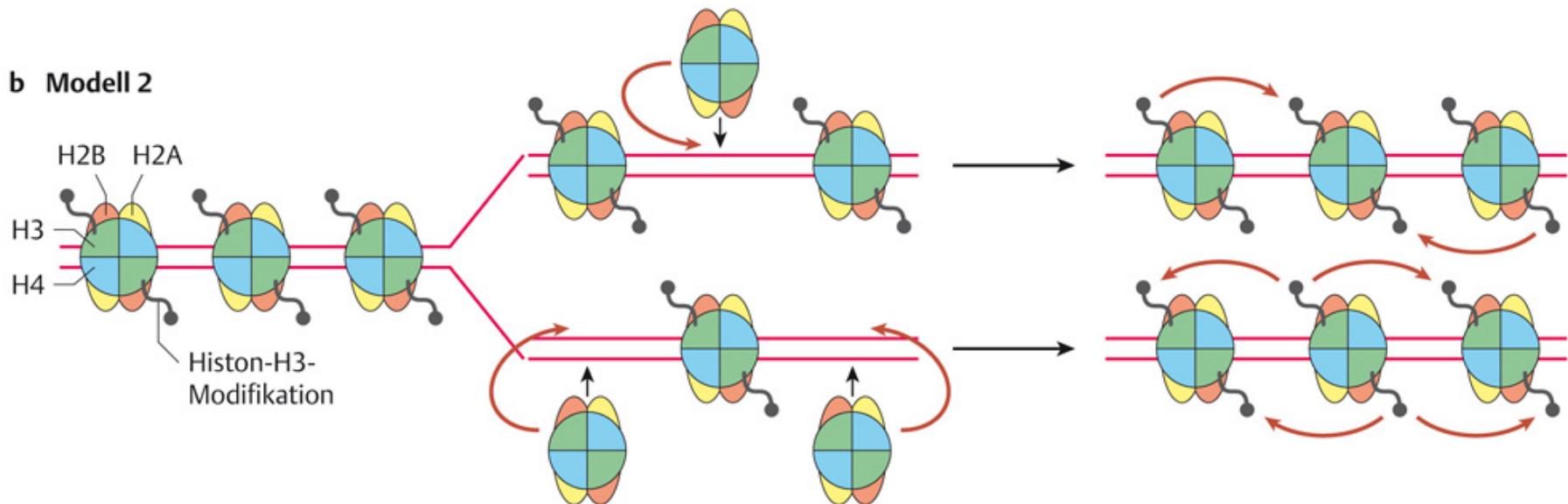
How do the new nucleosomes „inherit“ the status of the parental chromosome (active or inactive modifications)?

Models for the inheritance of chromatin modifications

a Modell 1



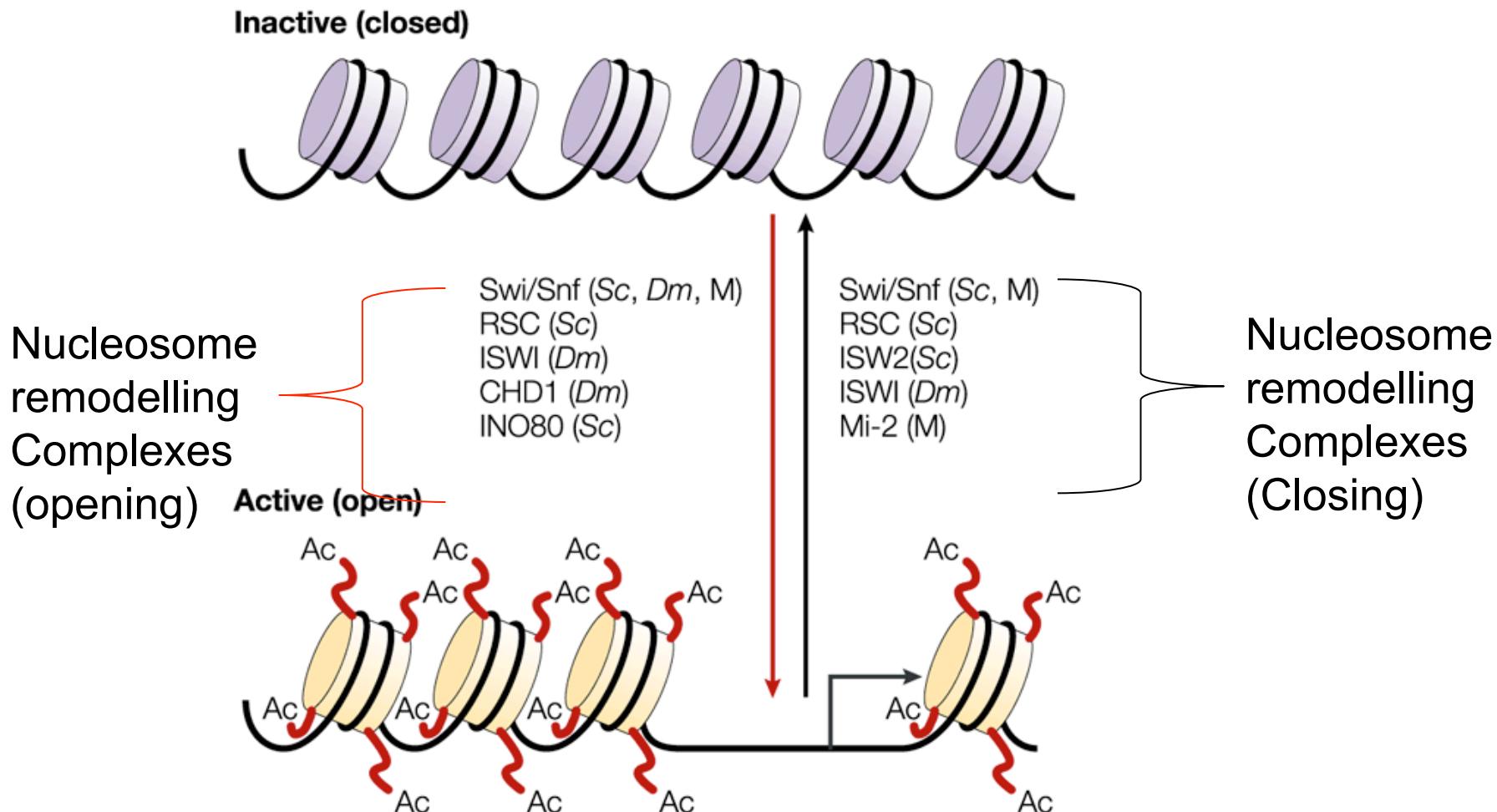
b Modell 2



Nucleosome remodelers

Nucleosome remodelers are complexes of many proteins
With a core unit of an ATP driven remodeler that
Actively moves DNA-along the nucleosomes

Nucleosomes can be actively „moved“ in Chromatin by nucleosome remodeling complexes

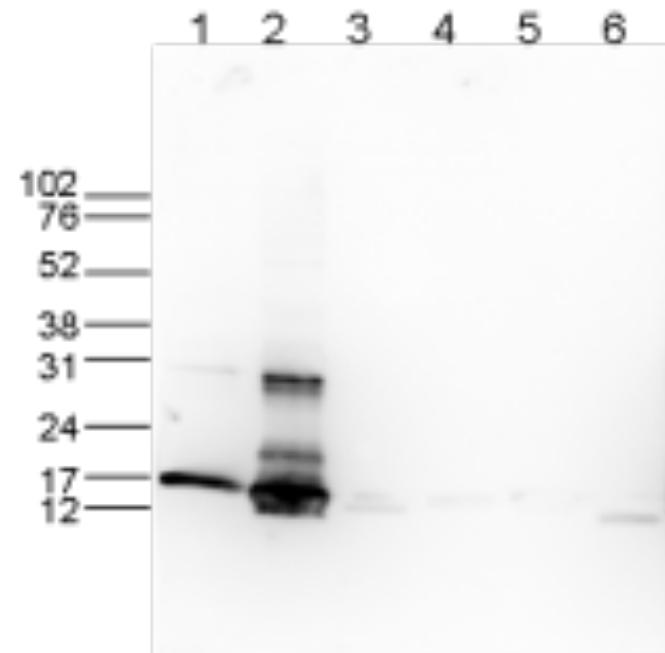
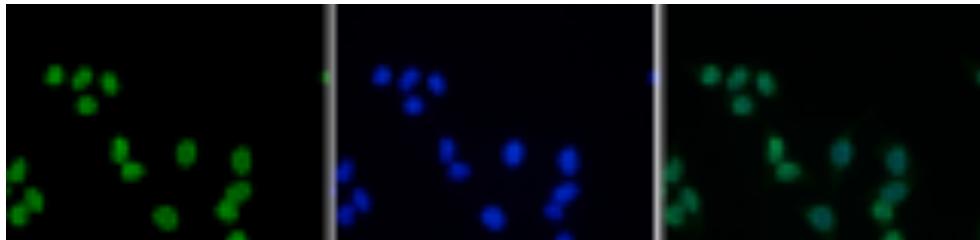


Principles of Epigenetics L I-2

Method for the analysis of histone modifications

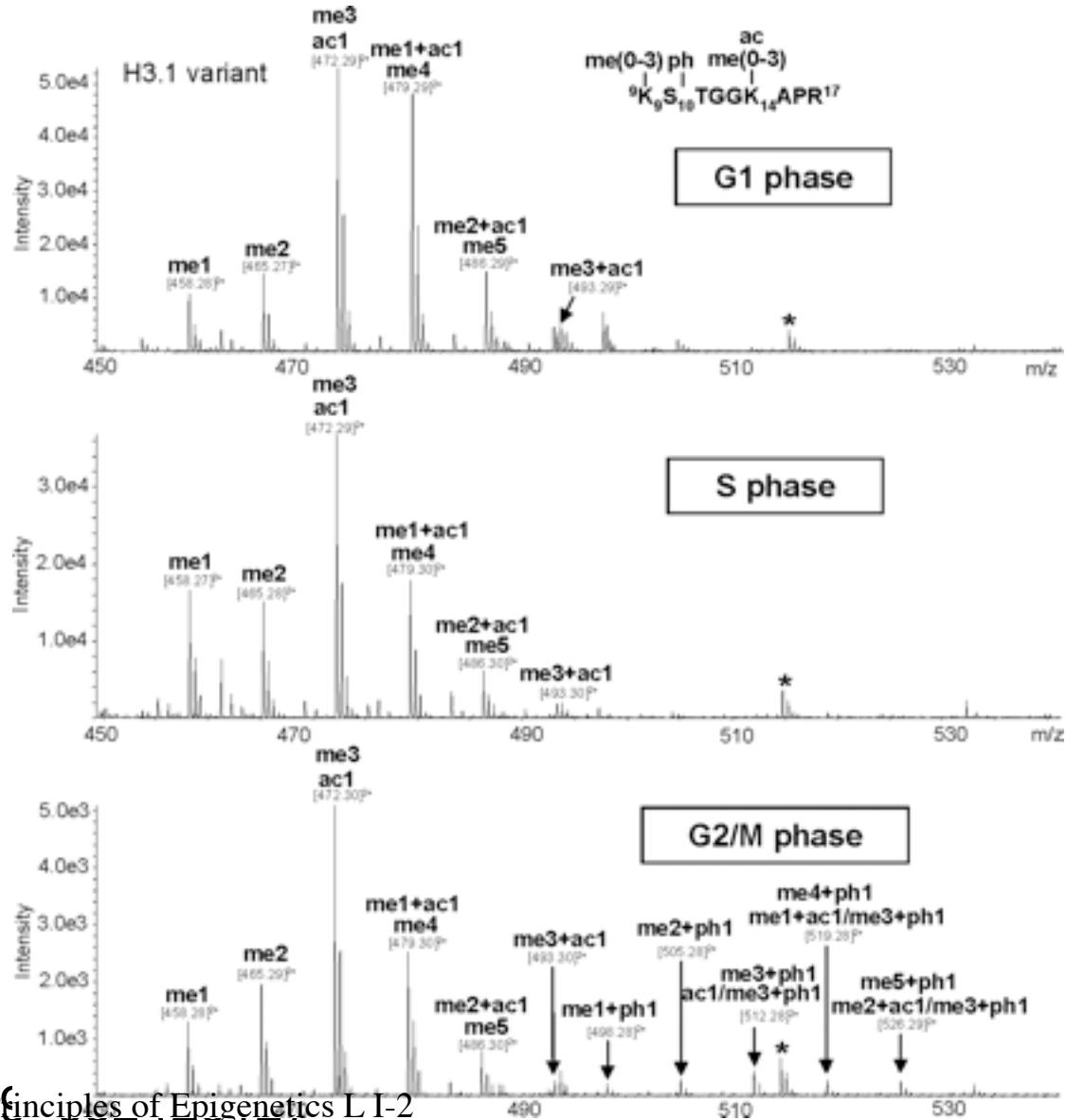
Analysis of the total content of modified histones of a cell or tissue: **Use of modifications specific antibodies**

- Immuno-fluorescence
- Western-blot

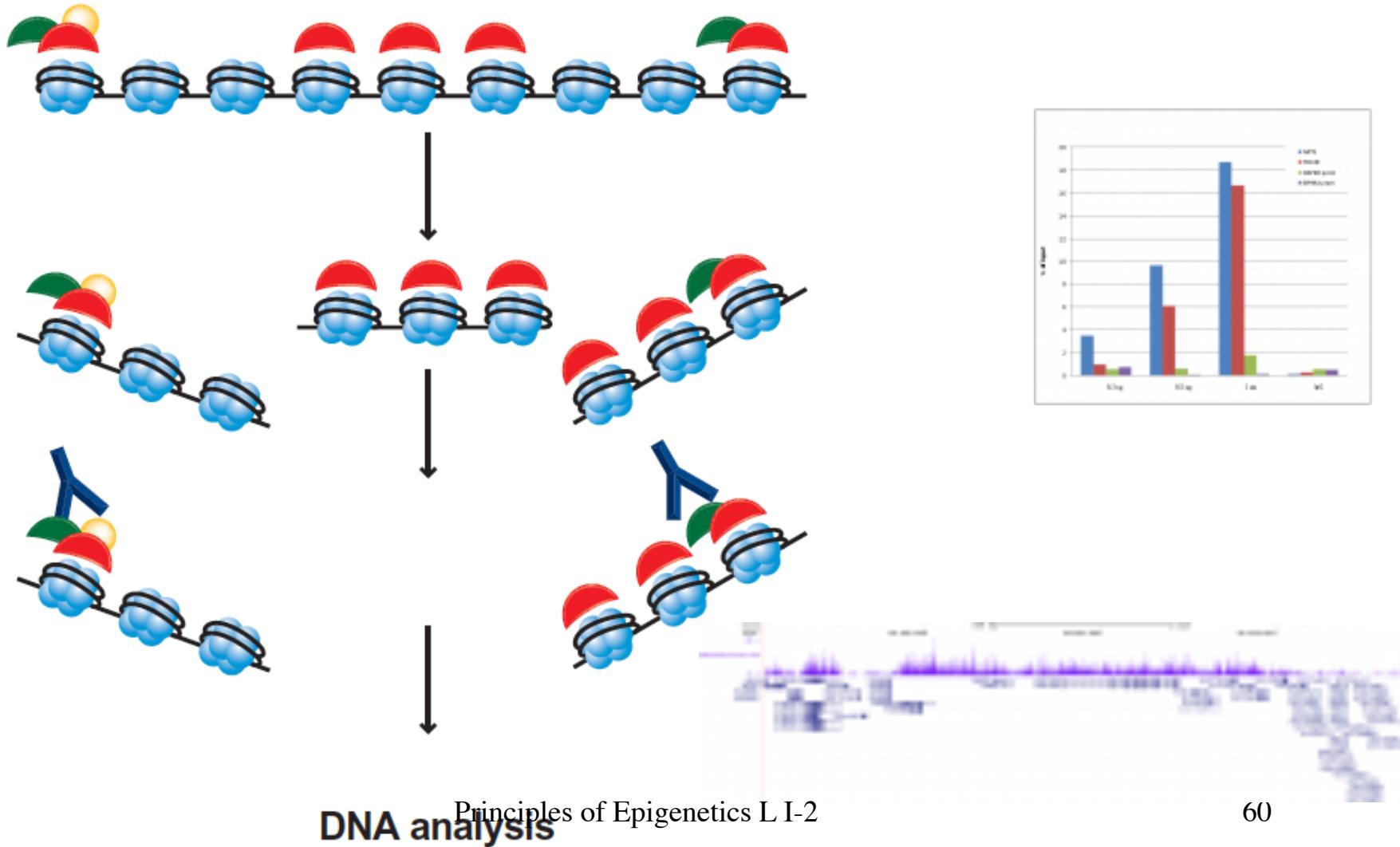


Method for the analysis of histone modifications

Determination of the total content of modified histones using Mass-Spectrometry:

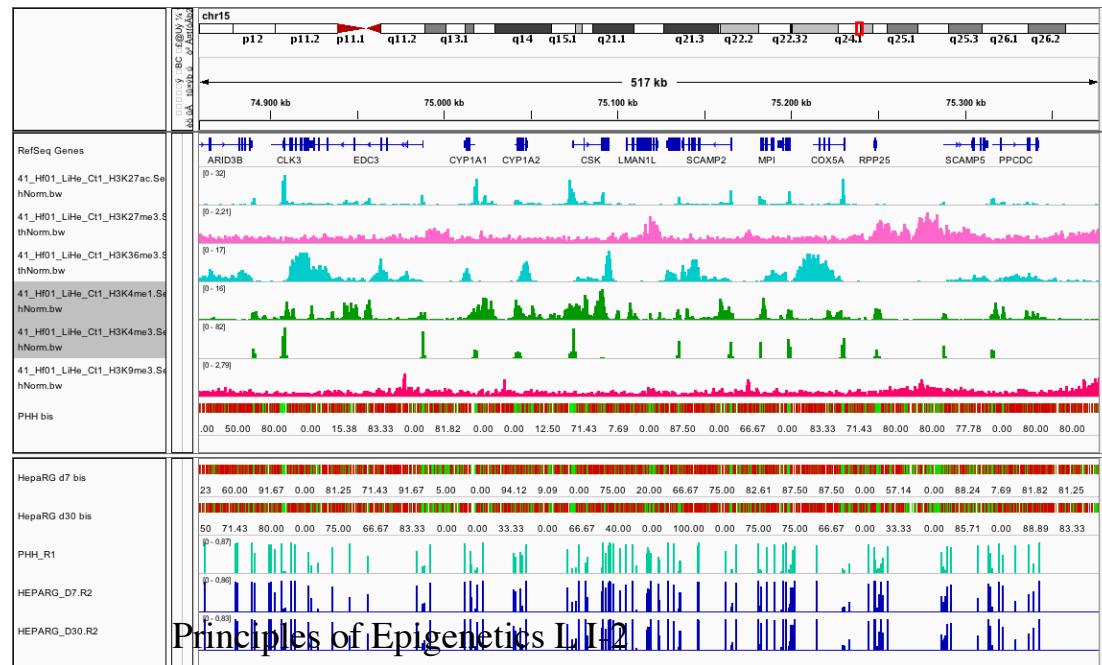
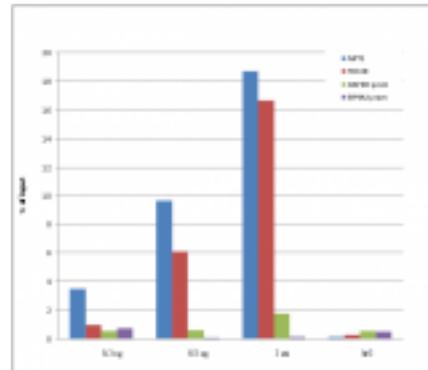


Localisation of Histone modifications at specific genes/promoters etc. using Chromatin-Immunoprecipitation = ChIP



ChIP-Seq – mapping of enrichment using NGS methods

Analysis at the locus via PCR



Analysis genome wide by NGS