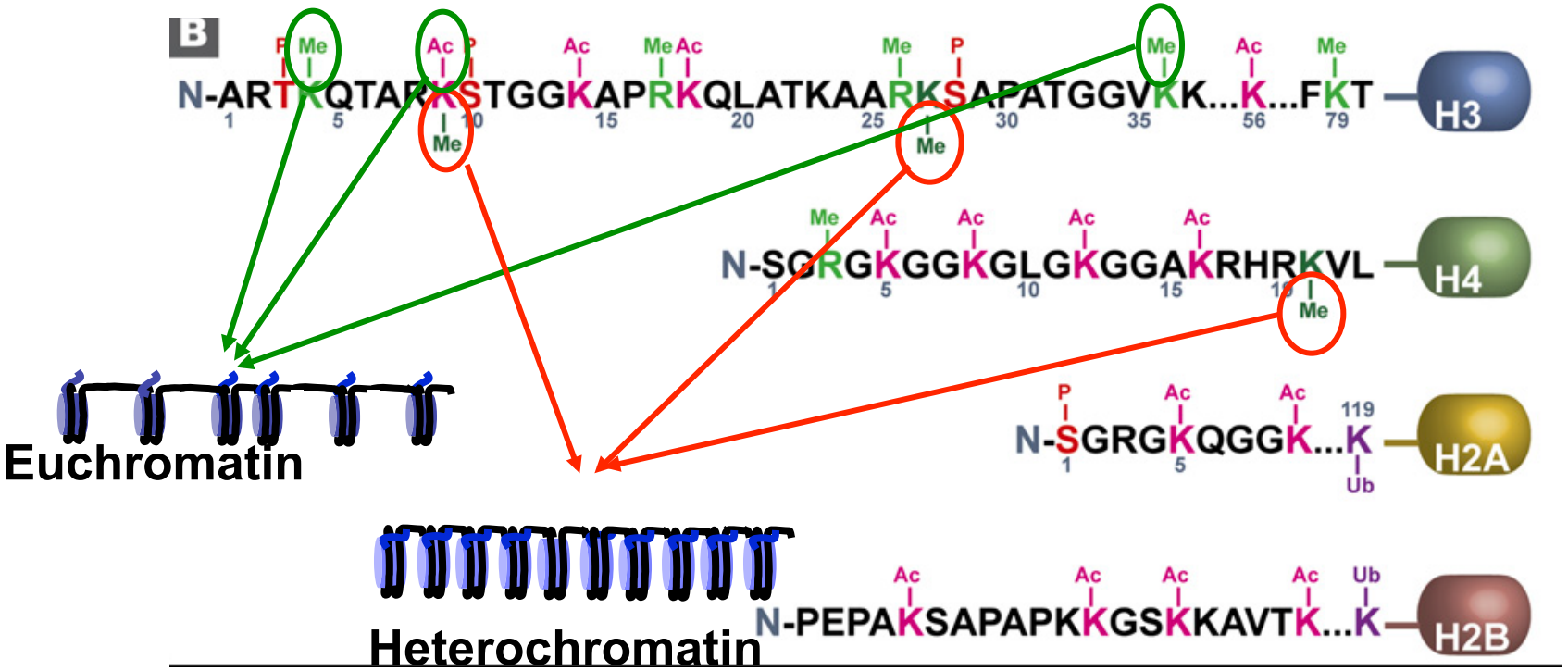
Lecture I-3_2018

Chromatin based epigenetic control II

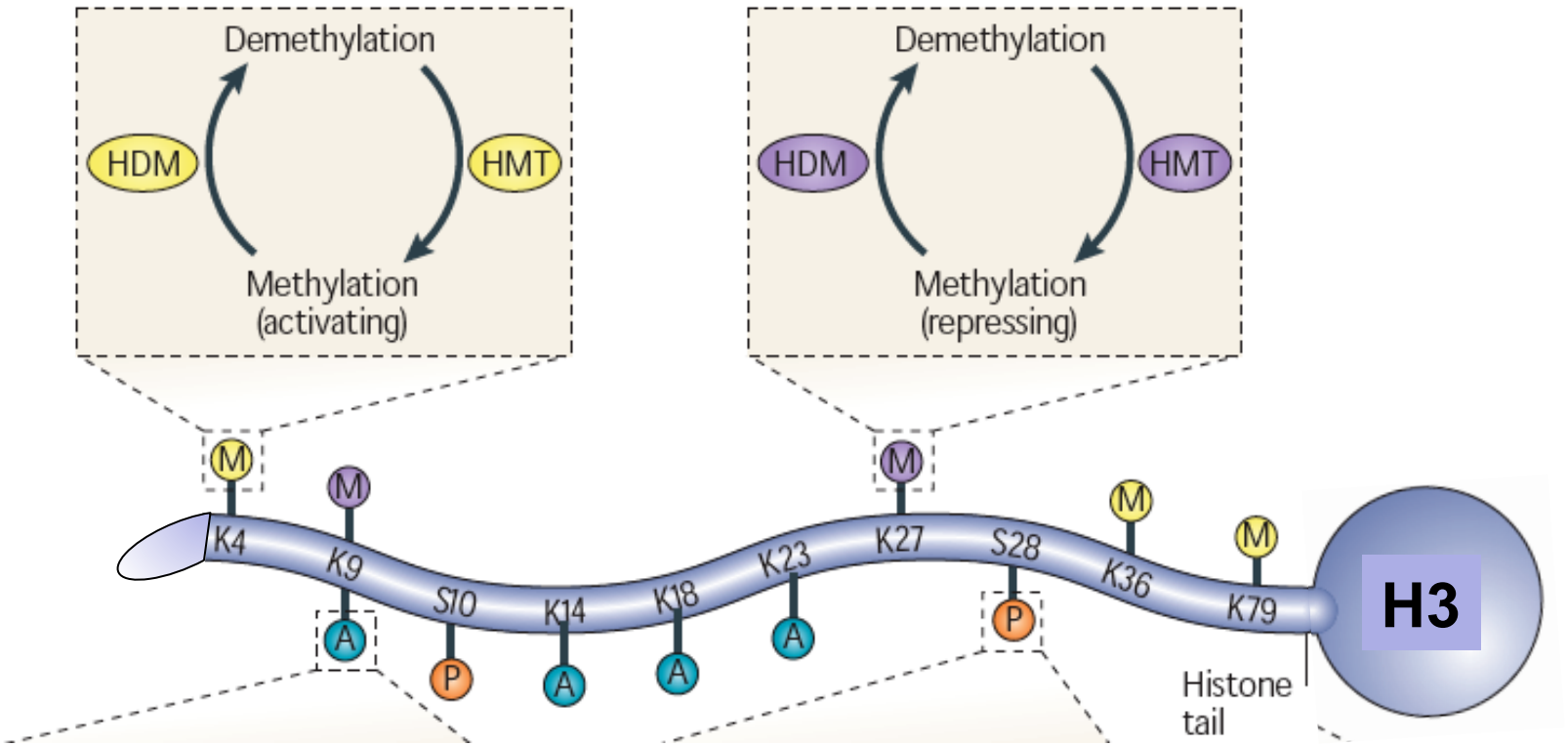
Overview

- Enzymology of histone methylation
- Role and function of key histone modifications
- Methods to analyse histone modifications in chromatin (ChIP, IF, MassSpec),
- Histone modifications and gene regulation: classification of regulatory regions, link to silent “heterochromatin”.
- Model for the mechanisms of “epigenetic inheritance”,

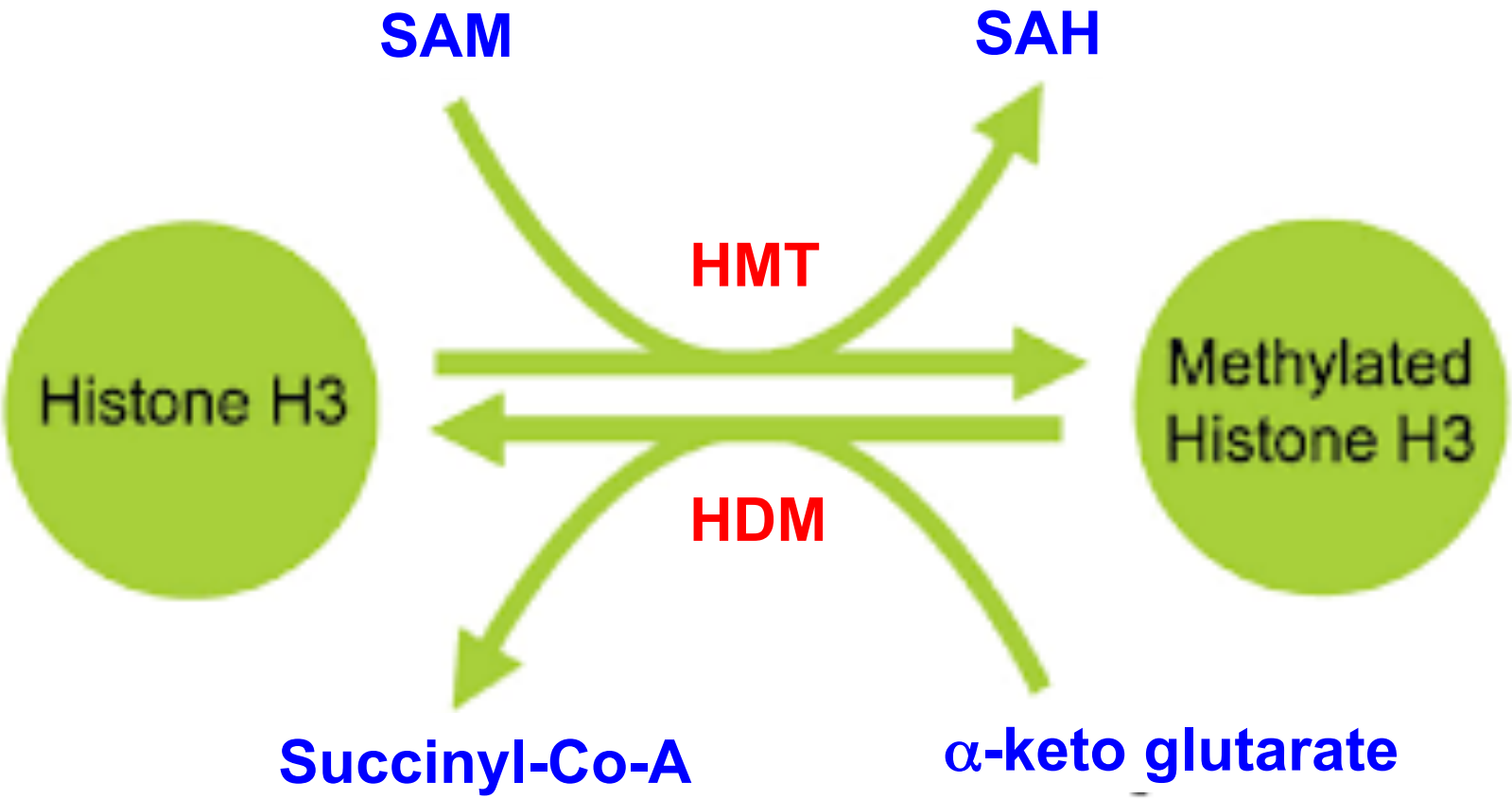
Histone methylation and open and closed chromatin structures



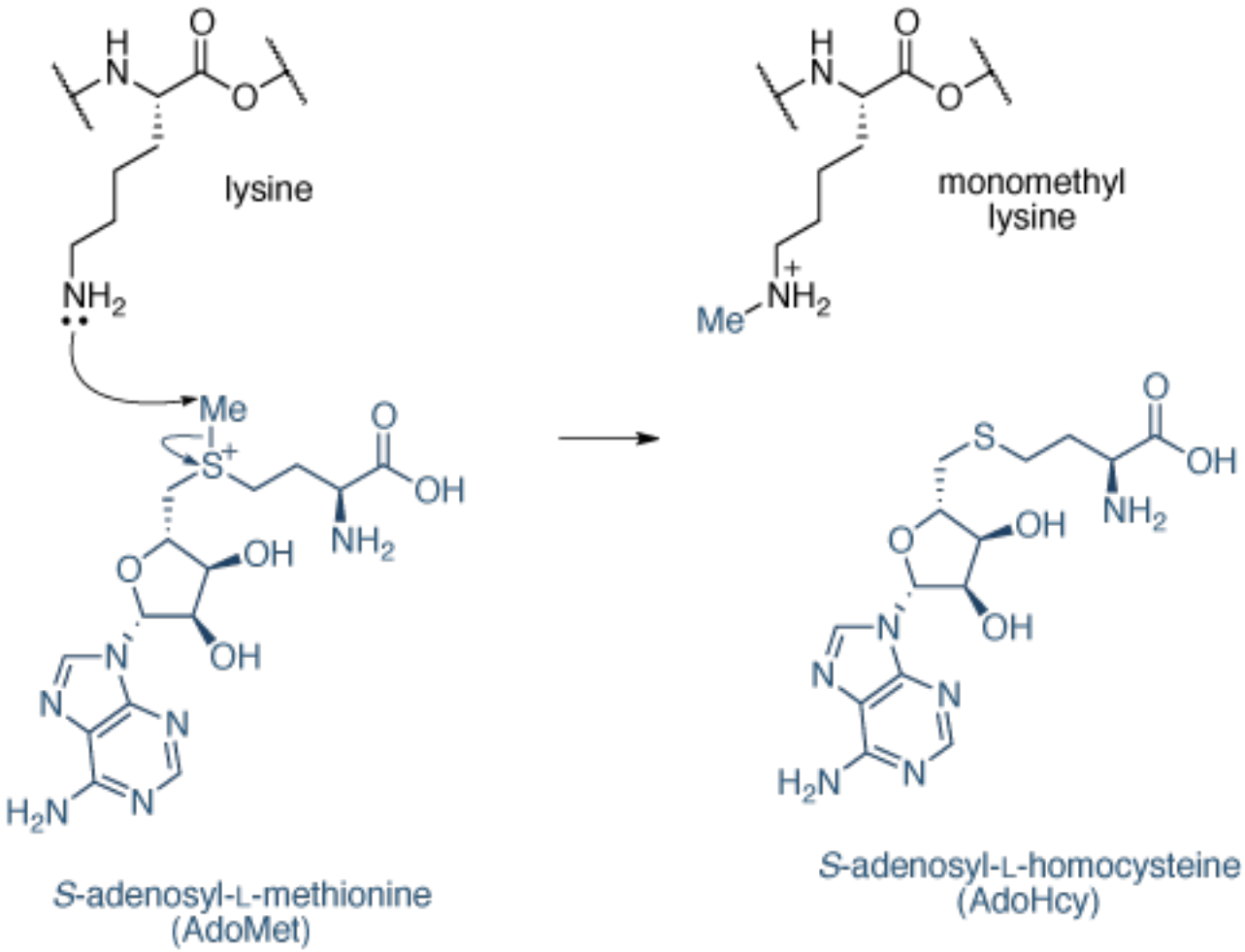
2. Histone-methylation: setting & removal (writing & erasing)



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



HMT = Histone methyltransferase
HDM = Histone demethylase


lysine
monomethyl lysine
S-adenosyl-L-methionine (AdoMet)
S-adenosyl-L-homocysteine (AdoHcy)

Histone Methyltransferases (HMTs)

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

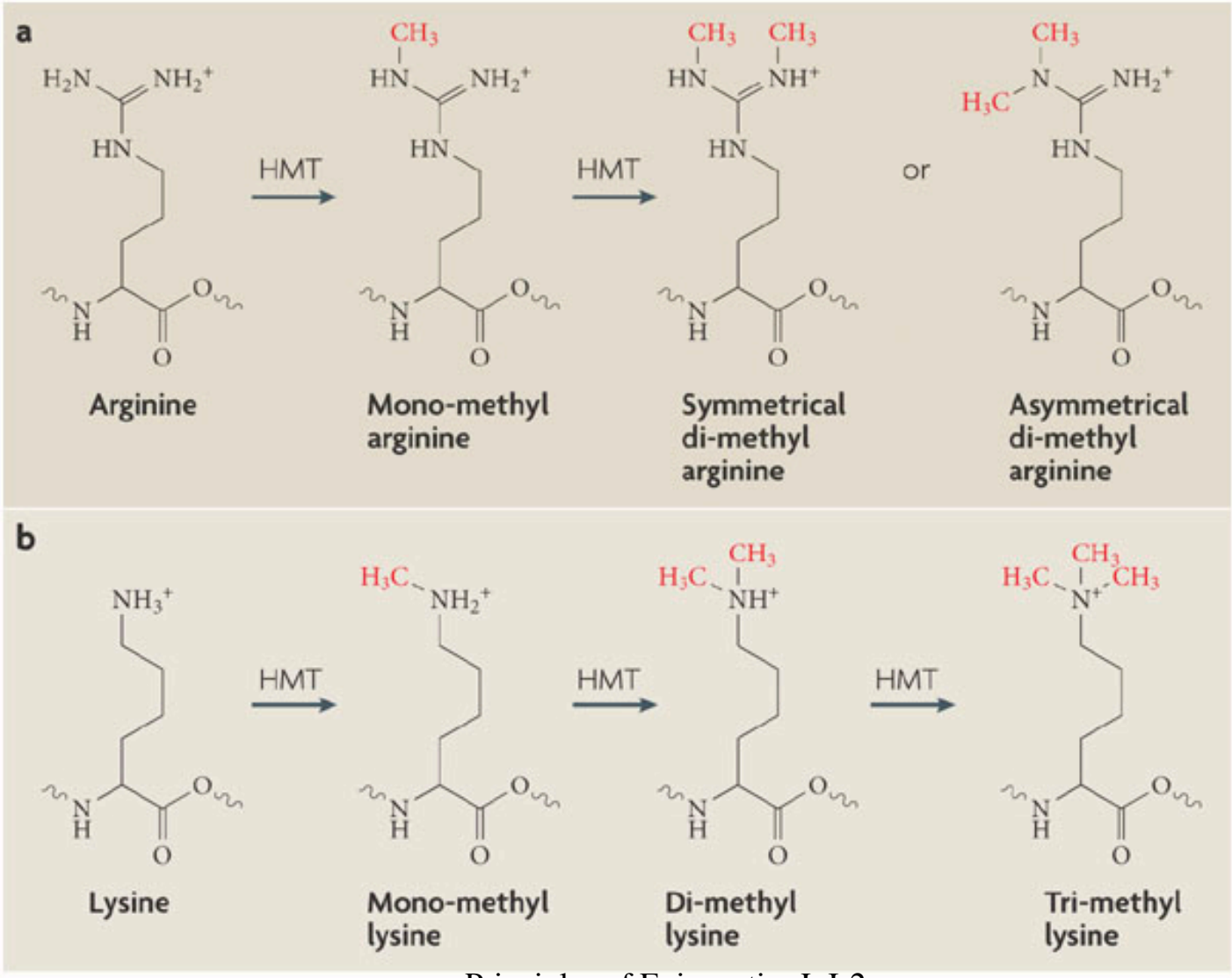
The first enzymes catalysing this modification (HMTs) were identified in mammals and Drosophila in the early 1990s (SuVAR39, MLL1,2,3)

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



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Local setting of histone modifcations

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

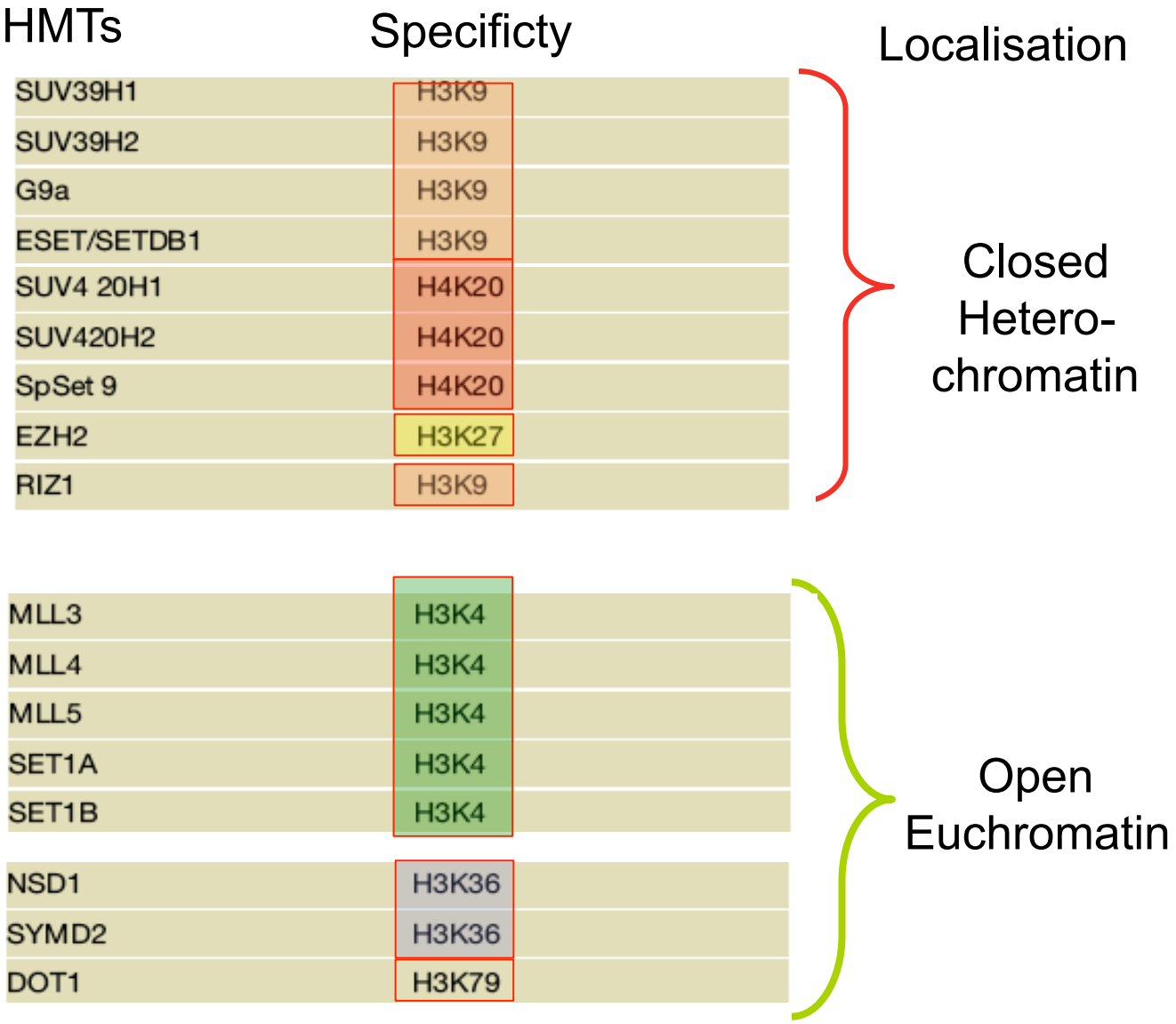
Which mechanisms target the enzymes (writers) to these specific regions?

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

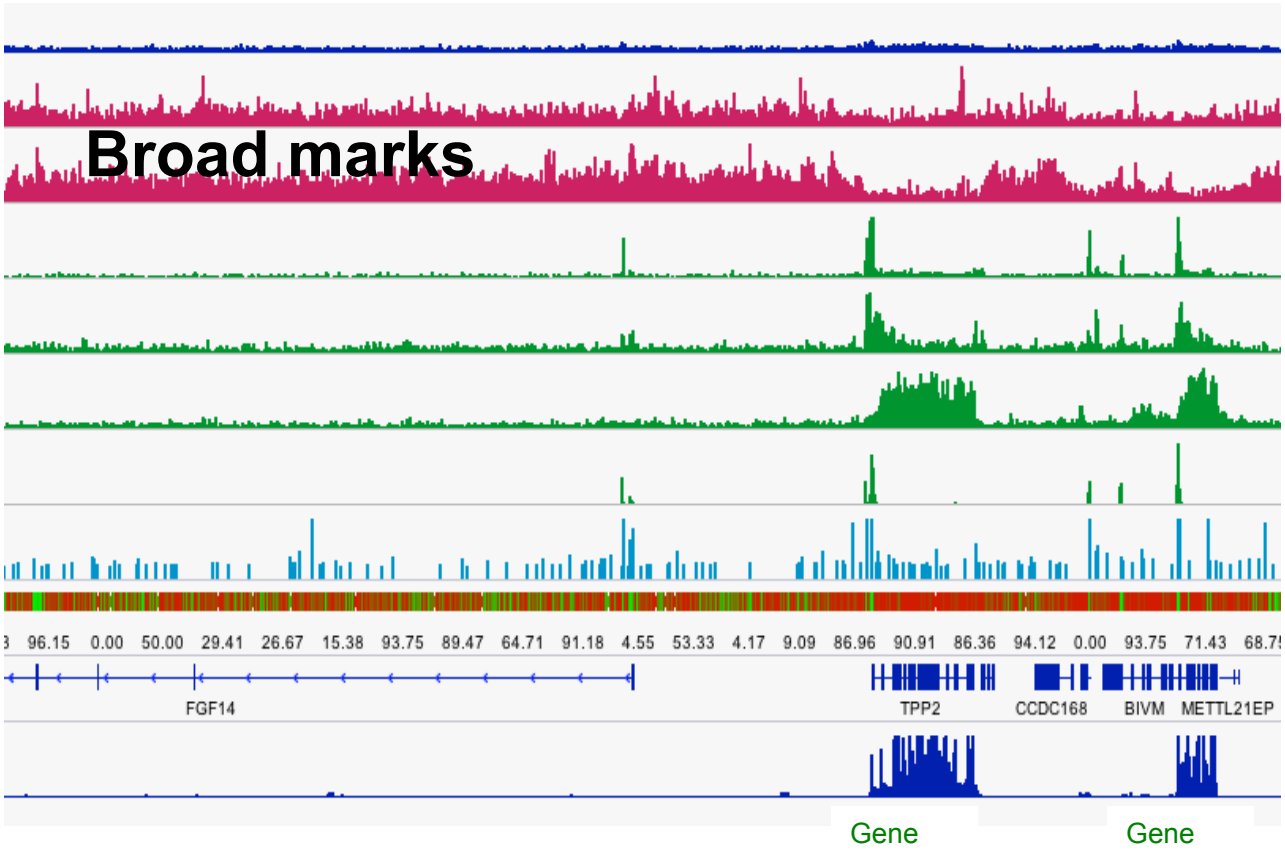
How are modifications inherited/maintained over replication?

How are modifications removed (erasers) from histones?

Local/regional activity of HMTs



Where are histone marks located



H3K27me3

H3K9me3

H3K4me1

H3K4me3

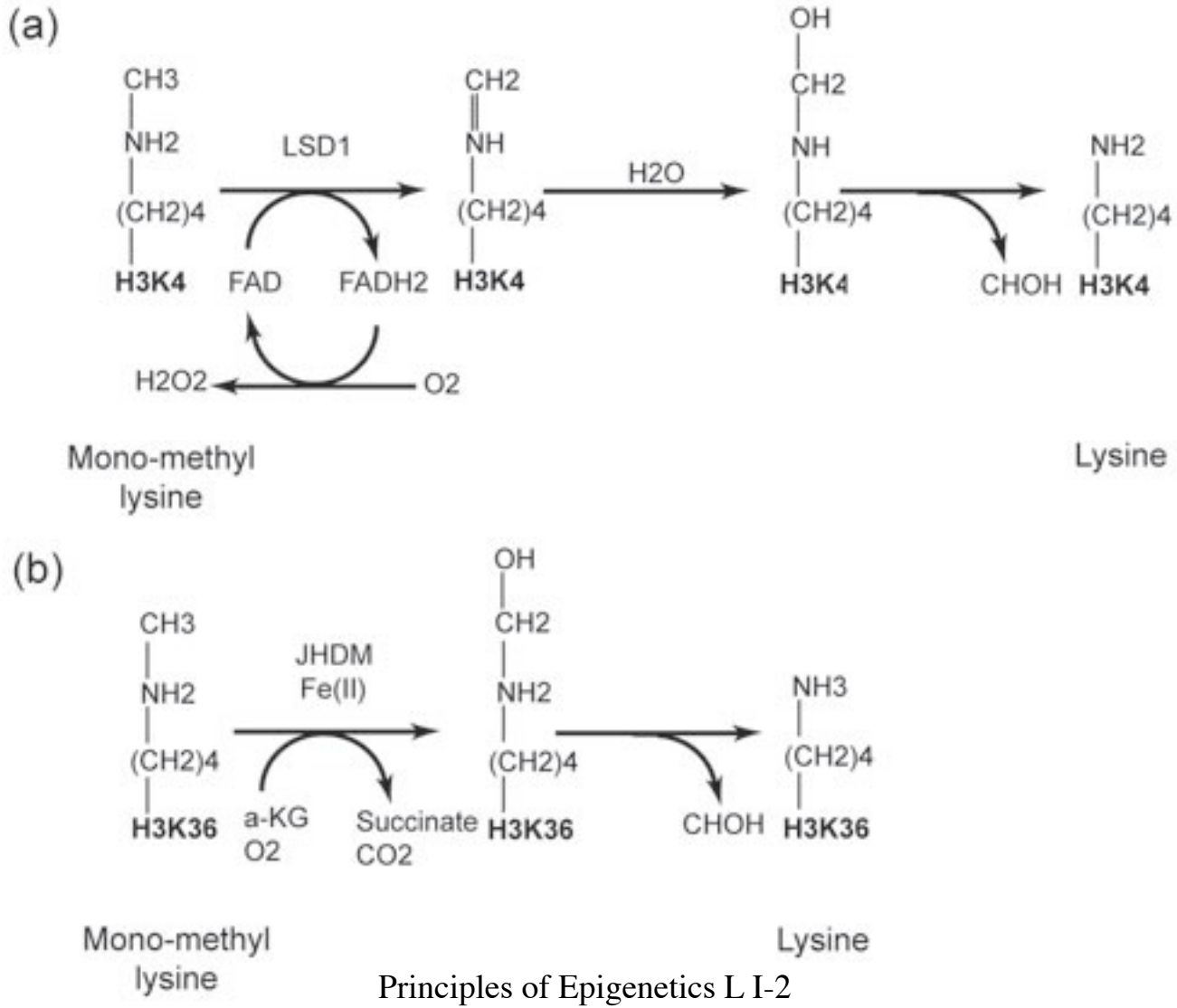
H3K36me3

DNAseI open chromatin

mRNA-Seq

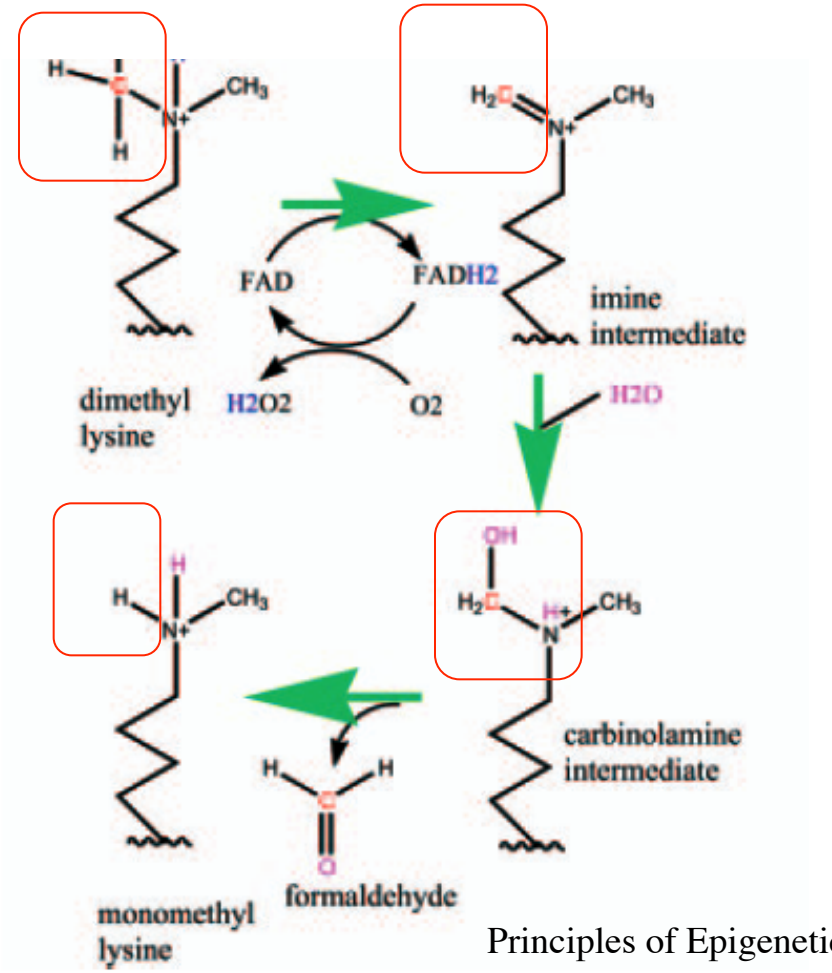
Local marks

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



Removal of Lysine Methylation by Demethylases

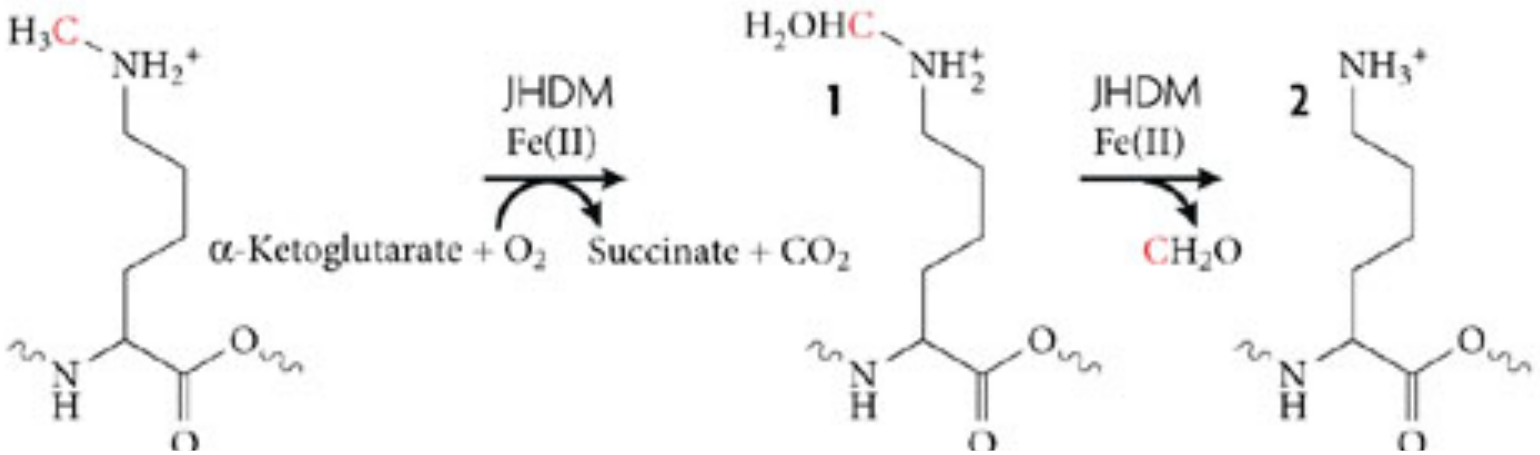
A demethylase (e.g. LSD1) is recruited to chromatin by repressor complexes to locally demethylate H3K4me3



LSD1 uses FAD as a cofactor to convert and release formaldehyde from the histone

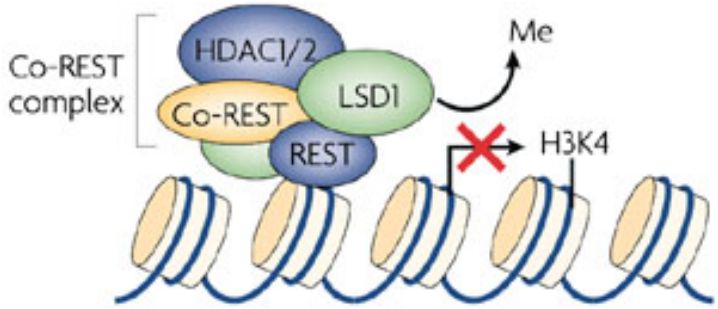
flavin adenine dinucleotide (*FAD*) is a redox *cofactor*

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

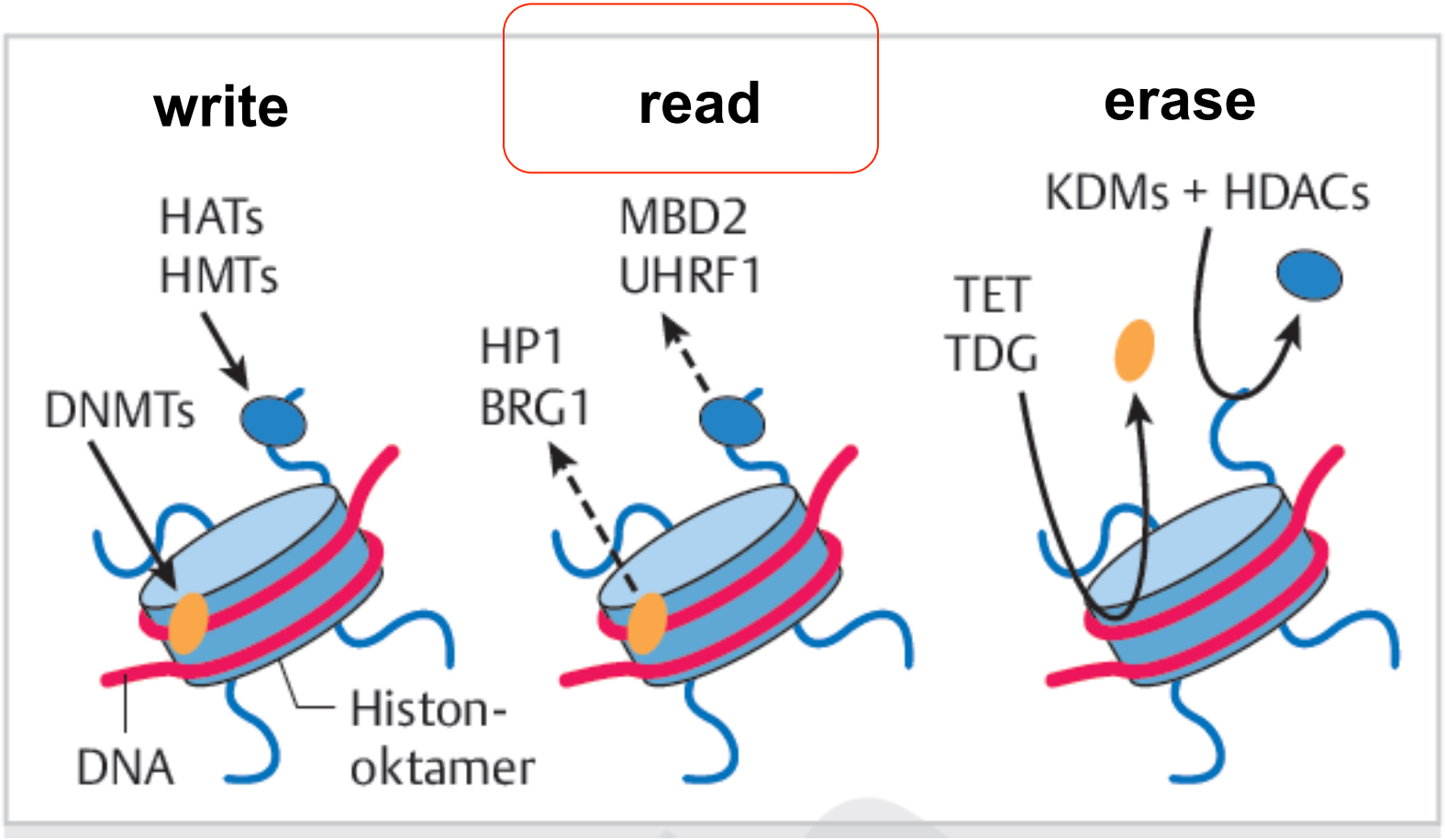
Lysine Demethylase (LSD1) can be part of repressor complexes



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).

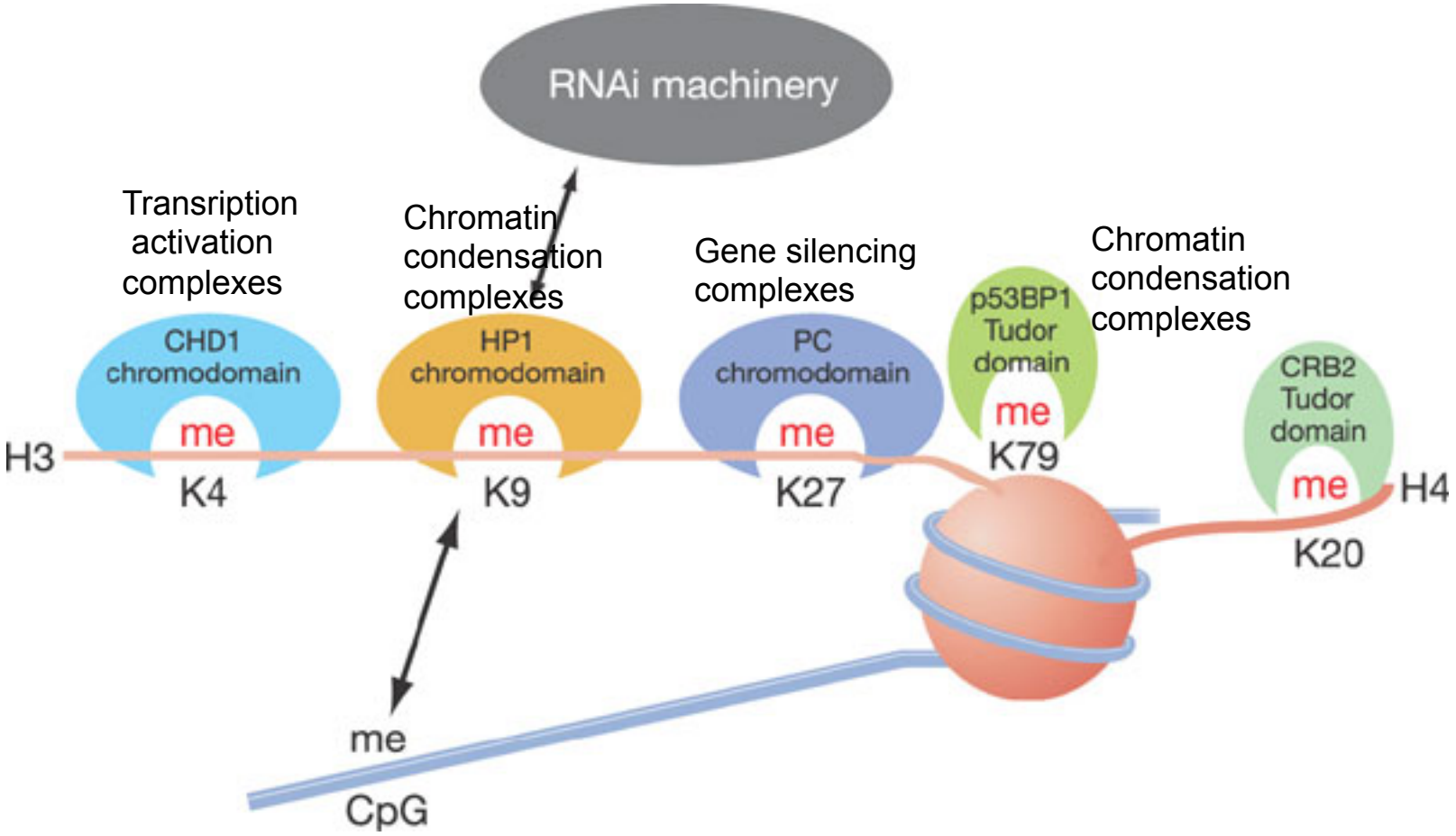
Histone modifications can be „read“ by specific proteins to convey local specific functions



Readers of histone modifications

Readers of histone modifications use a set of similar protein domains fo excute their specific binding

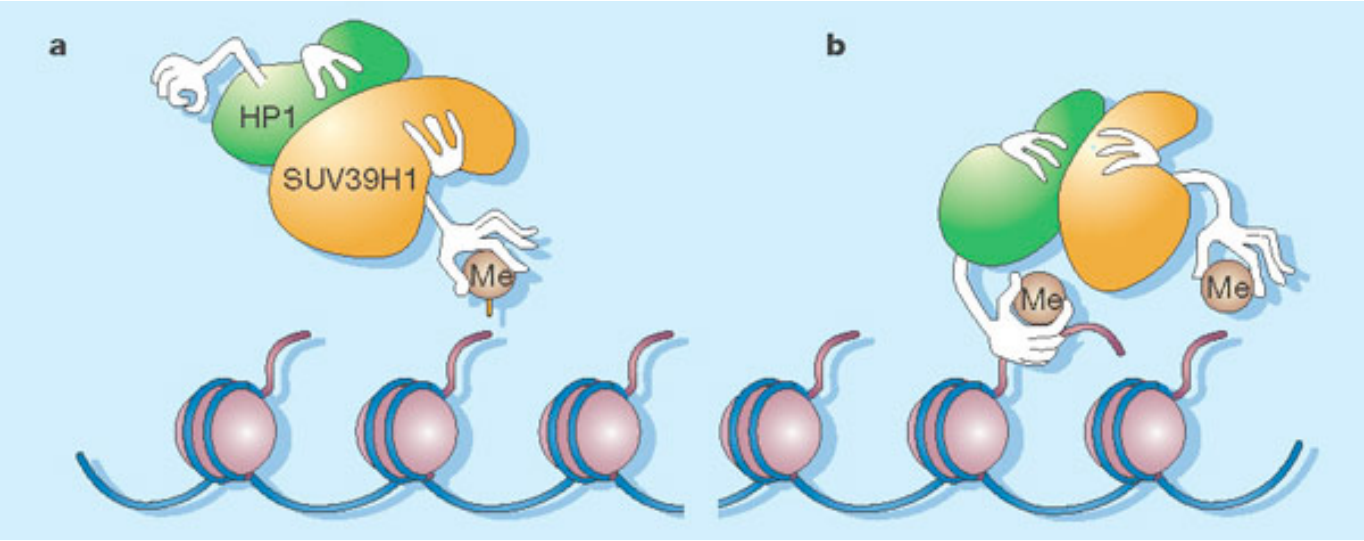
Reader domains recognizing distinct histone modifications



Reading histone modifications to form heterochromatin

HP1 = heterochromatin protein 1

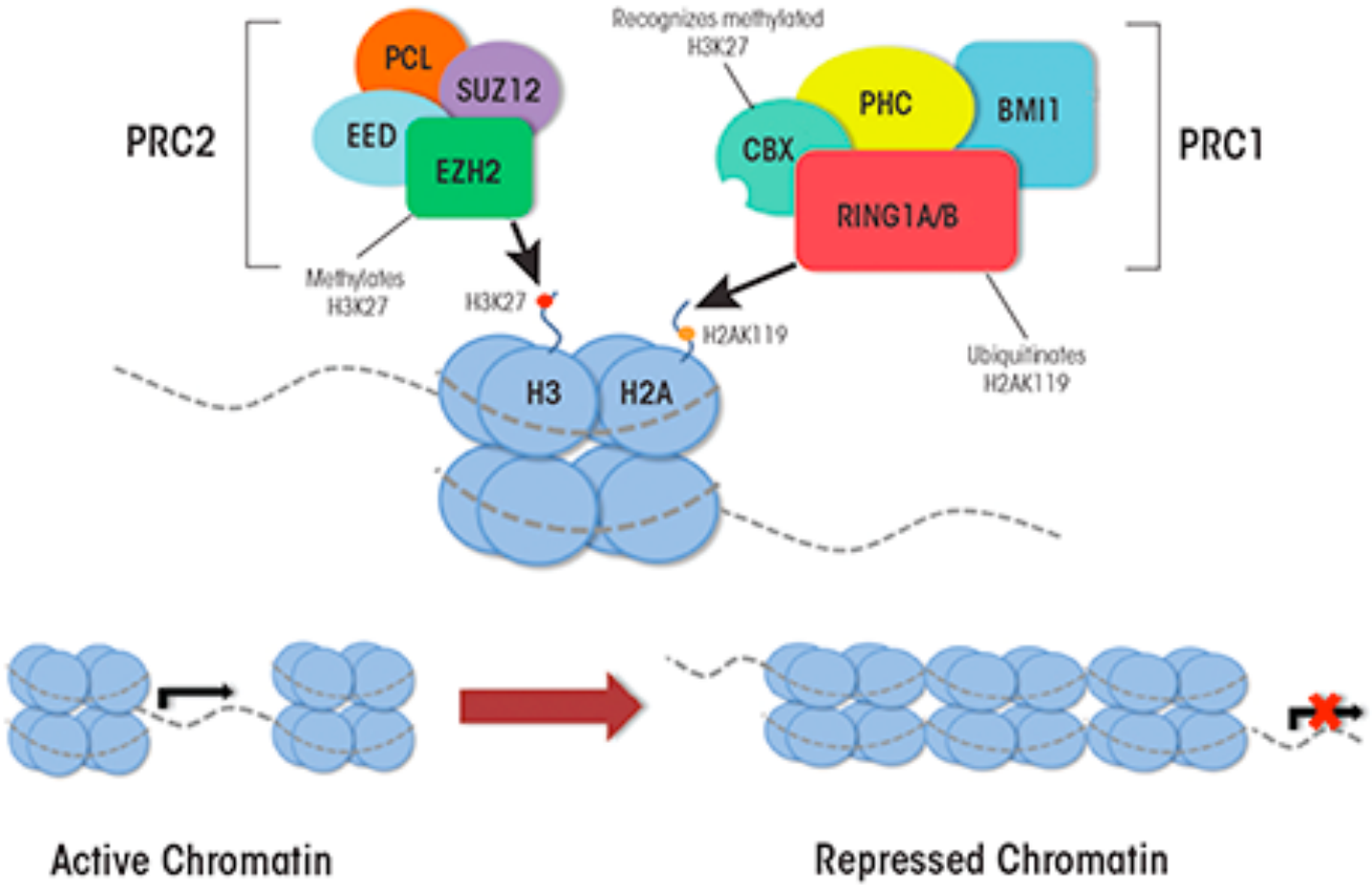
- Recognizes and binds to H3K9me2/3.
- It helps to propagate (spread) heterochromatic histone modifications by recruiting the Histone methyltransferase SUV39H1.



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

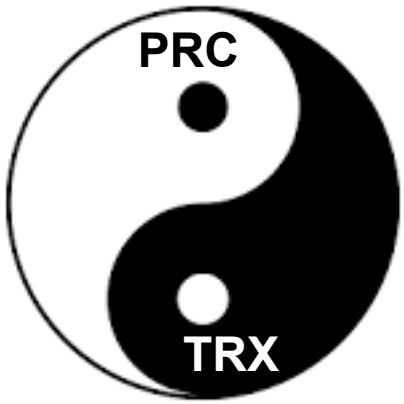
Silencing of genes during development: PcG group proteins = negative transcriptional regulators/silencers

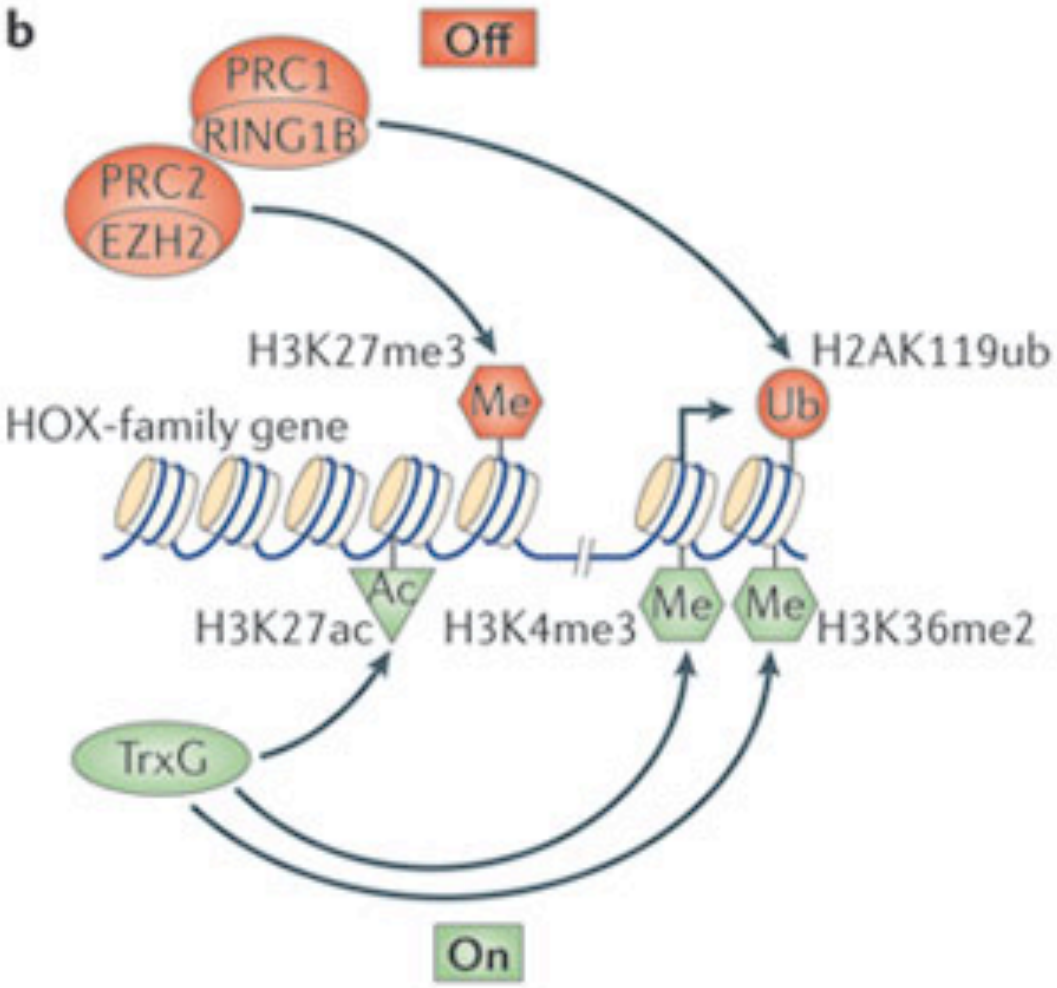


Activation of transcription through chromatin opening: The trithorax complex proteins

- Trithorax-group-proteins (TRX) are transciptional 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





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Propagation of chromatin states (Histone Modifications) through cell division

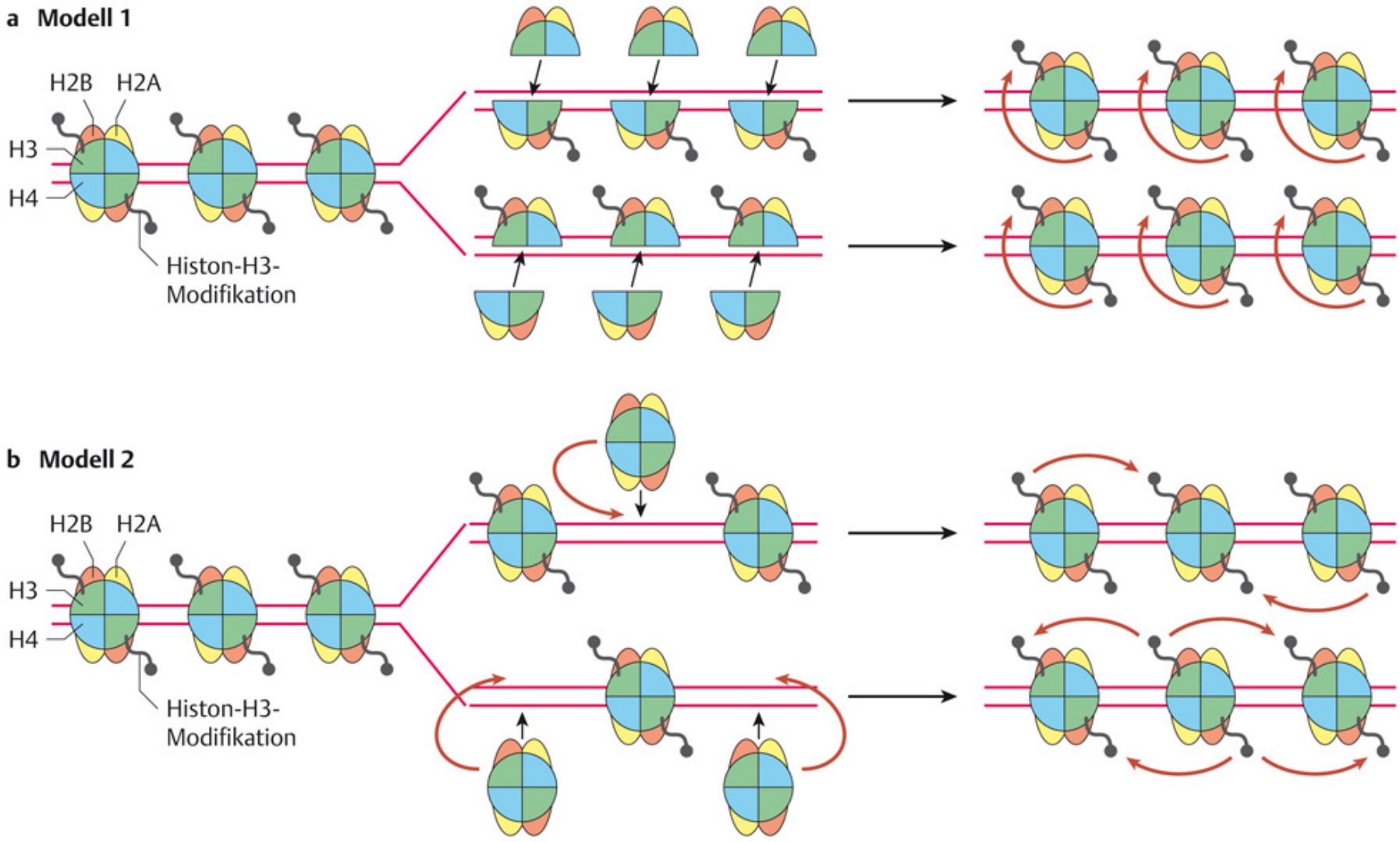
Histone modifications are established on Histones not on the DNA!

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).

Remeber: 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)?

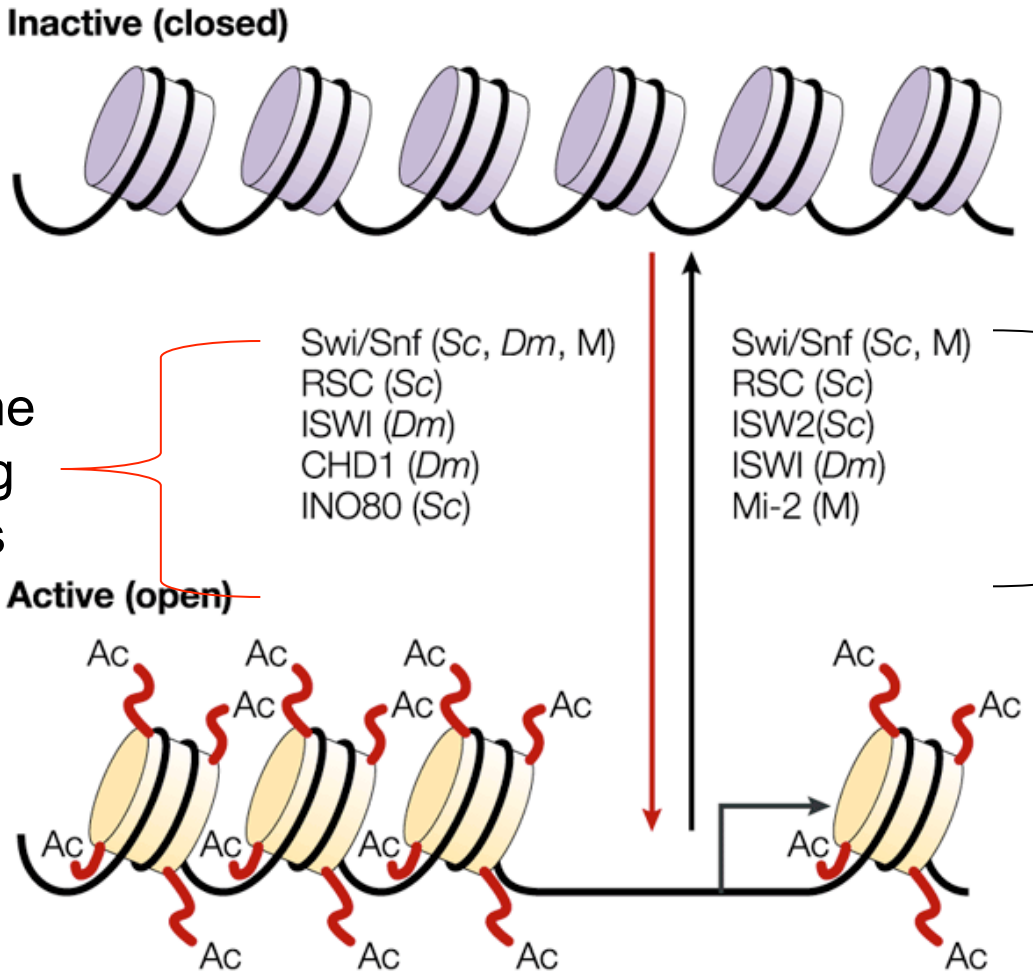
Models for the inheritance of chromatin modifications



Nucleosome remodellers

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

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



Nucleosome remodelling Complexes (opening)

Nucleosome remodelling Complexes (Closing)

Principles of Epigenetics L I-2

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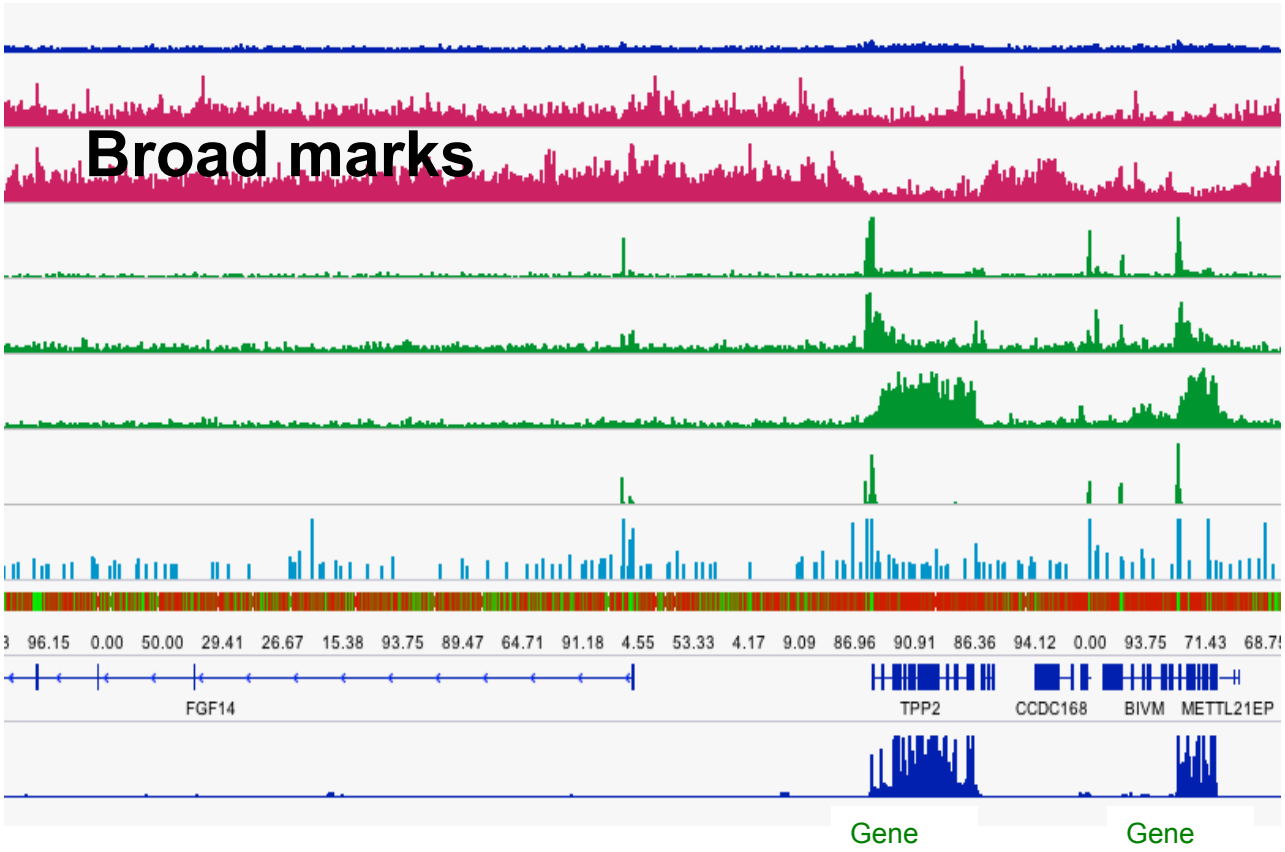
Where are histone marks located



H3K27me3

H3K9me3

H3K4me1

H3K4me3

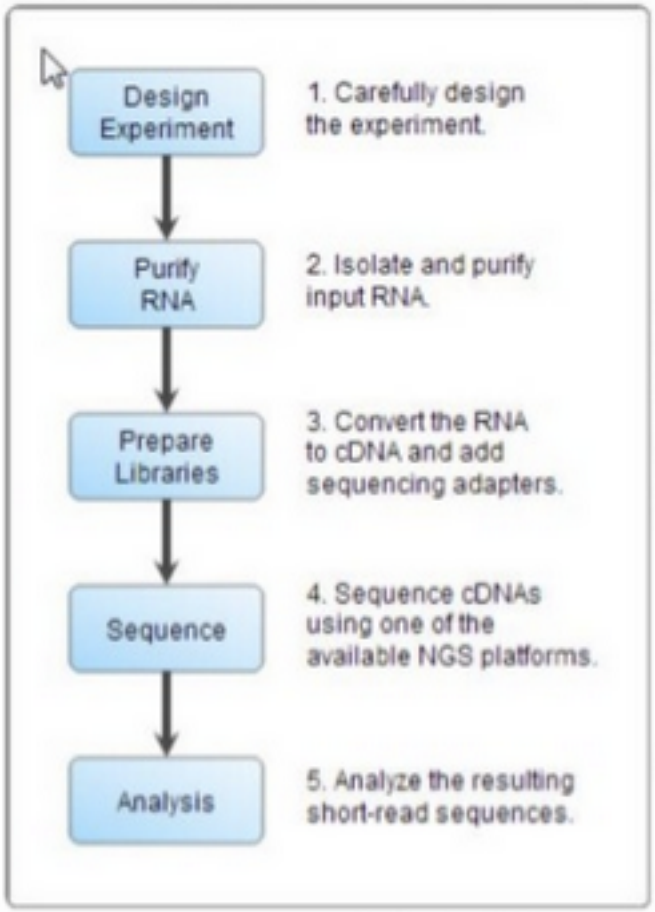
H3K36me3

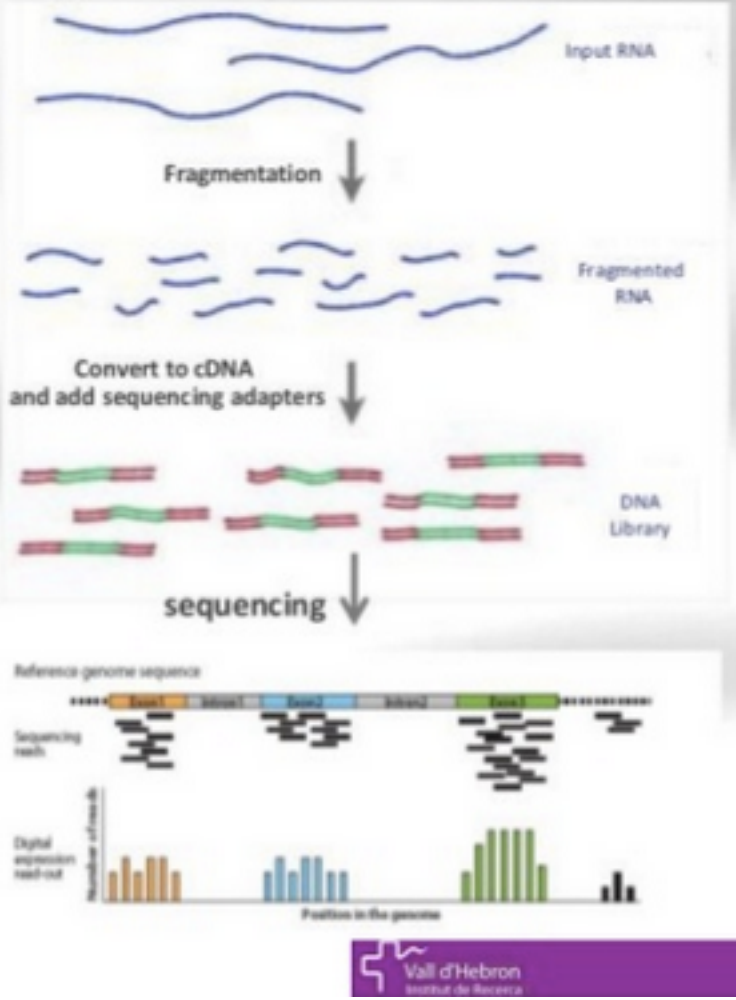
DNAseI open chromatin

mRNA-Seq

Local marks

RNA-seq analysis workflow





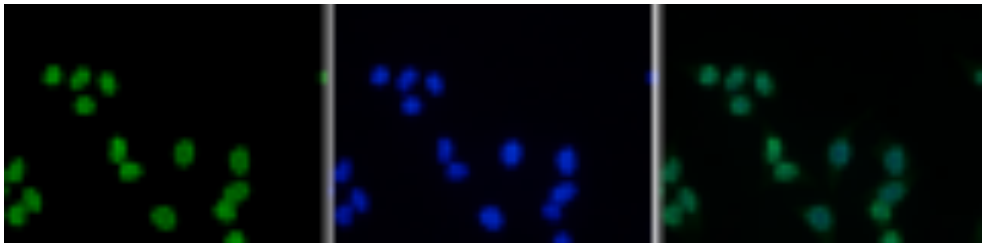
Method for the analysis of histone modifications

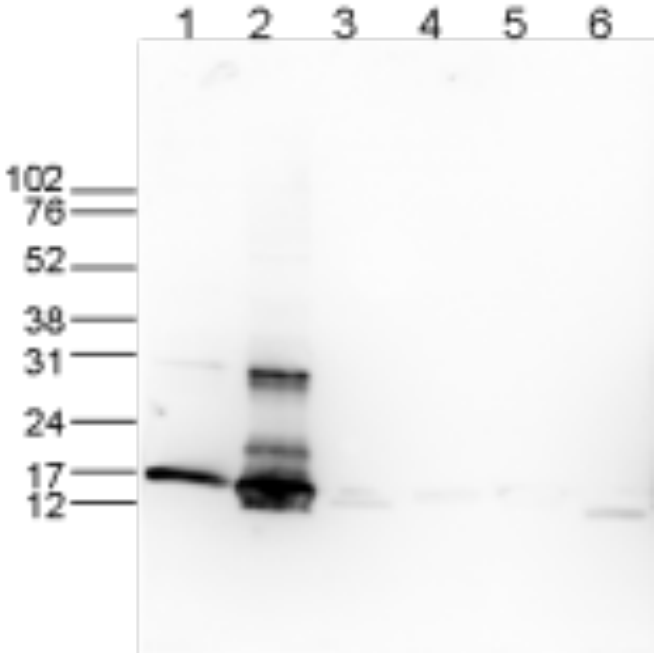
http://antibody.uni-saarland.de/

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

- Western-blot

- Immuno-fluorescence

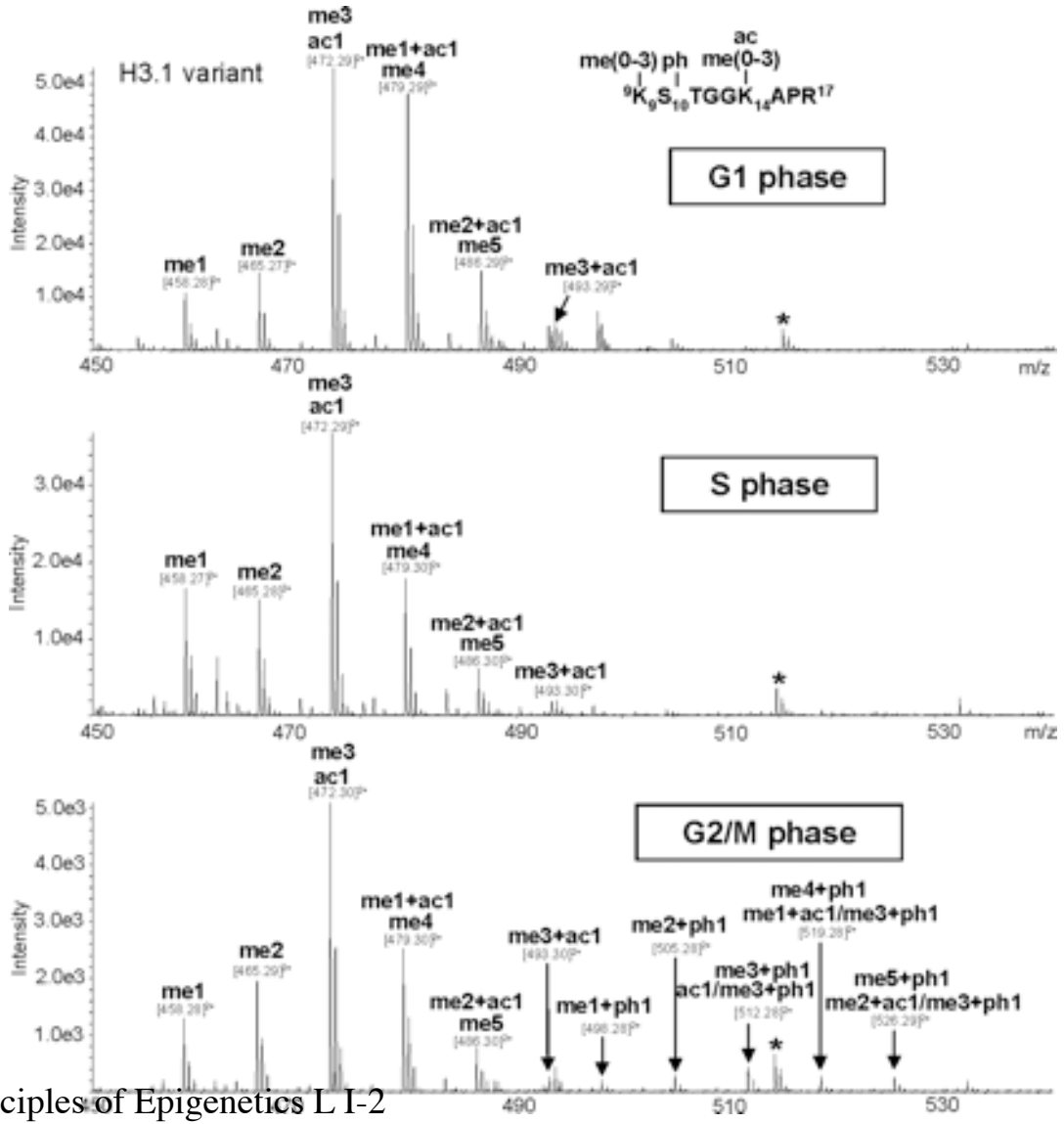




Mass Spectrometry for the analysis of histone modifications

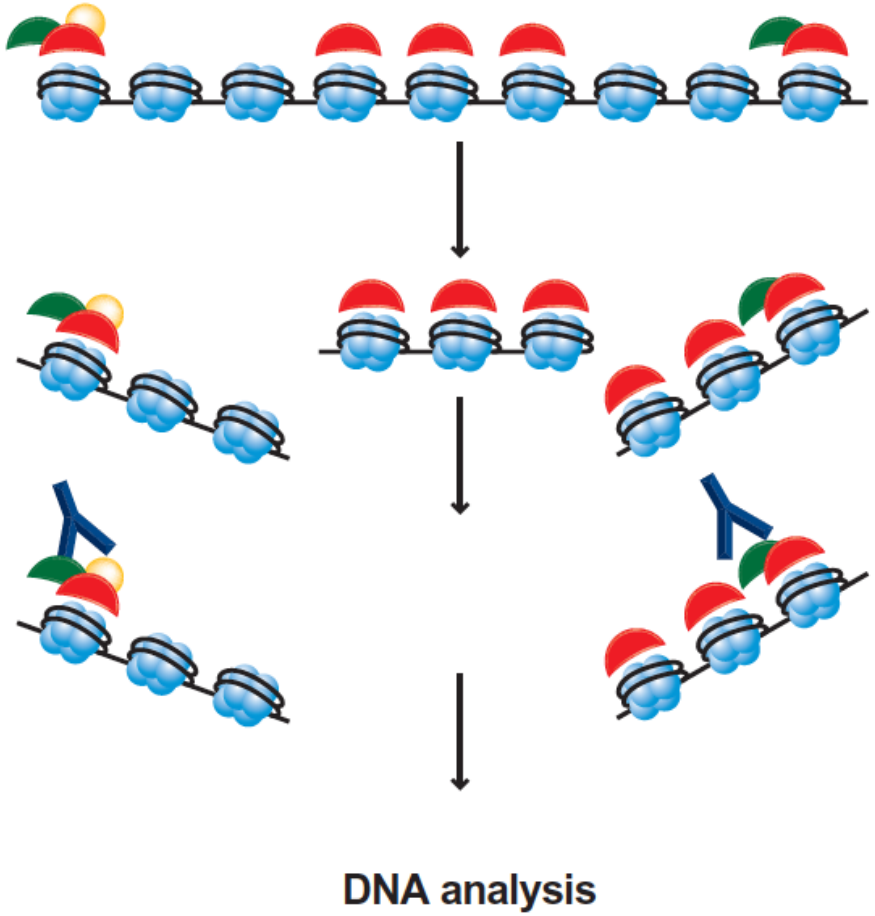
Determination of the total content of modified histones using Mass-Spectrometry:

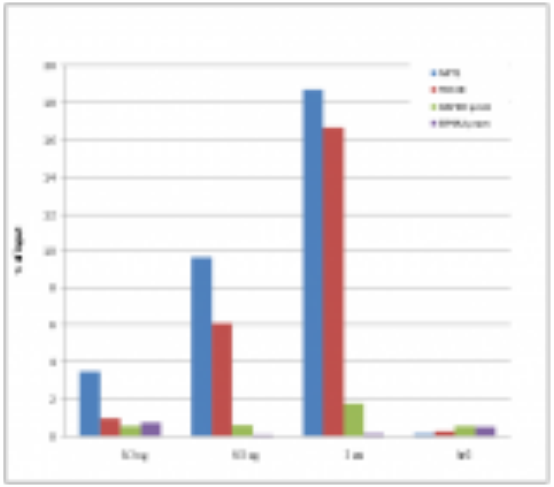
Example dynamics during cell cycle



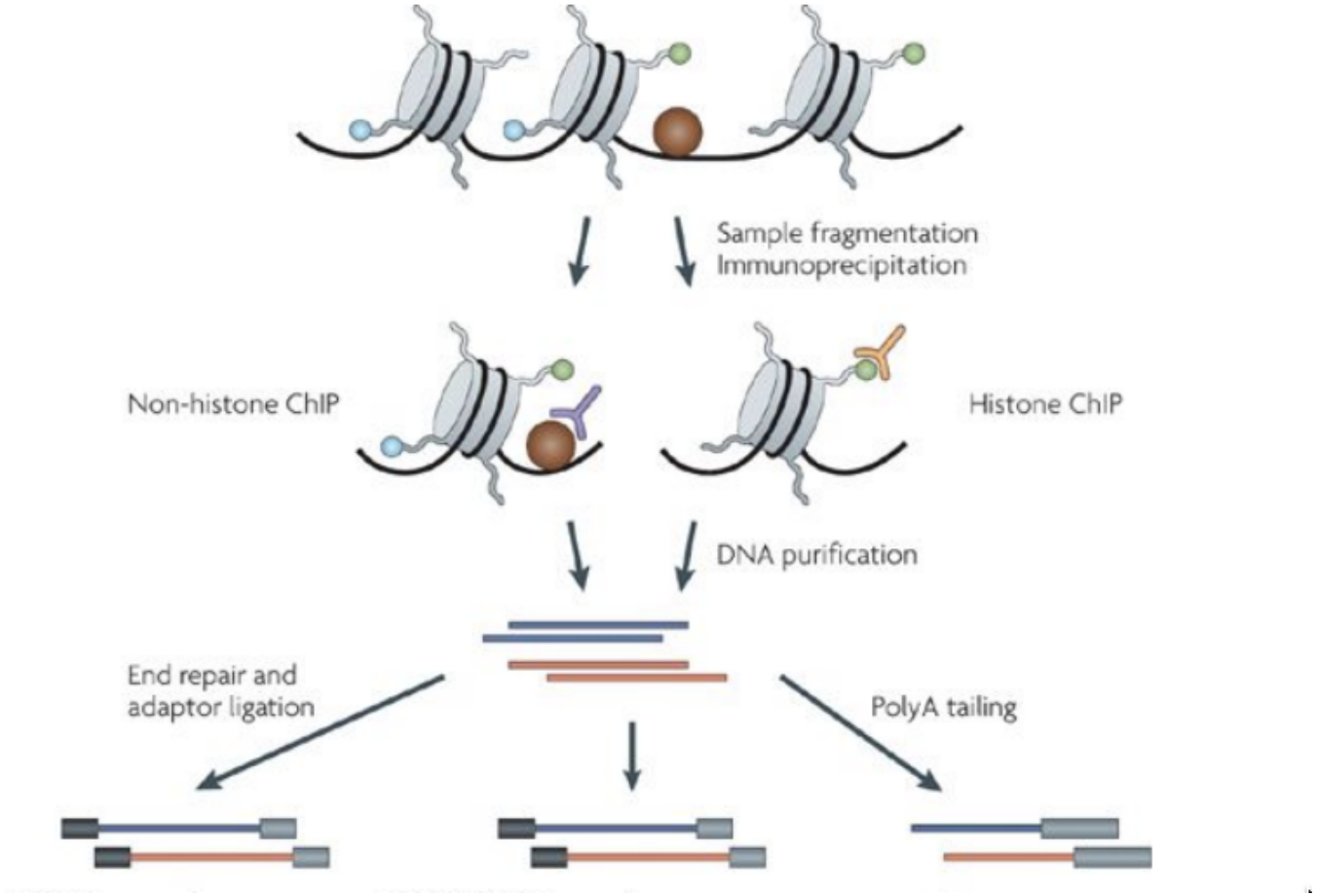
***Nature Methods* 9**, 649–652 (2012)

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

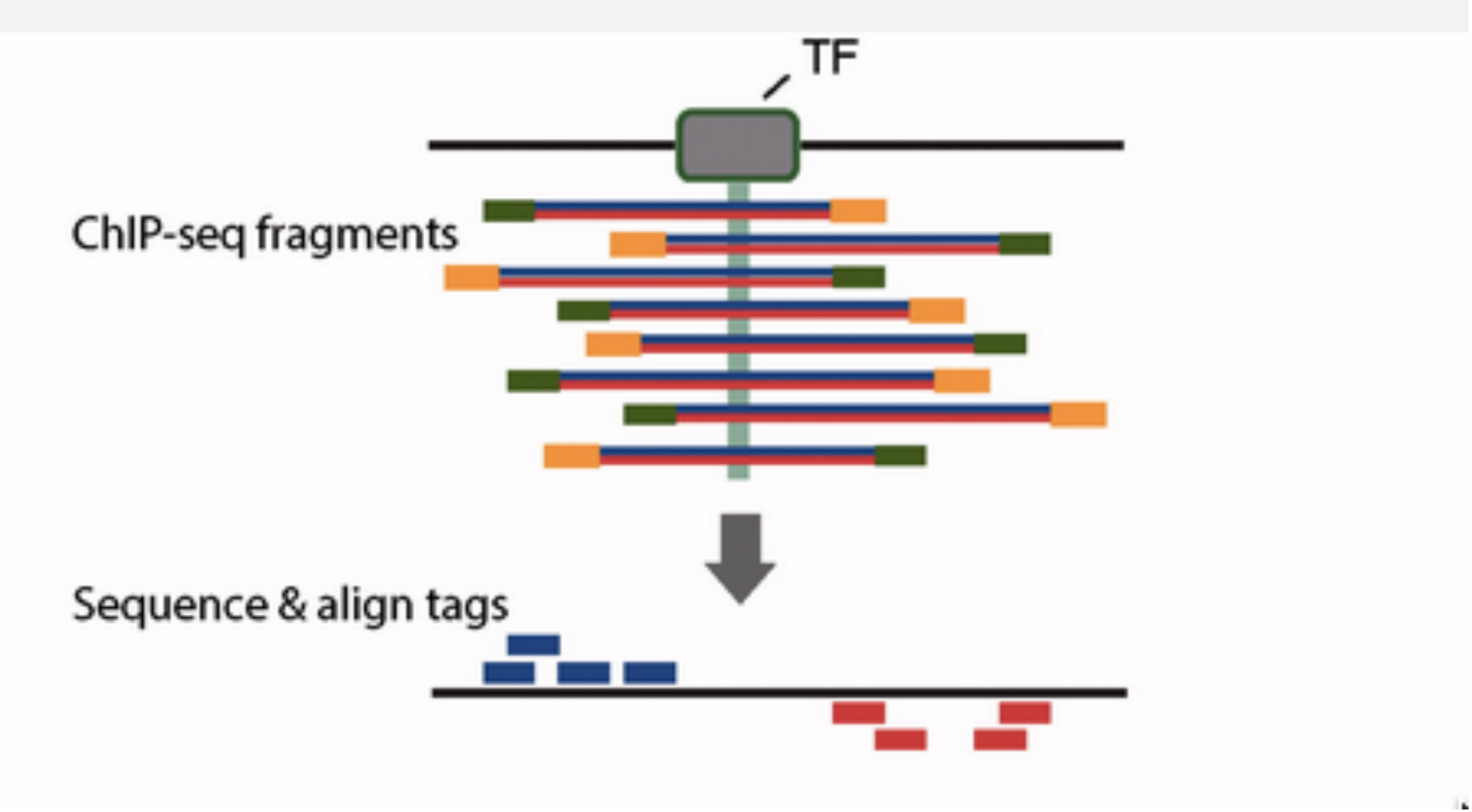




Localisation of Histone modifications at specific genes/promoters etc. using Chromatin-Immunoprecipitation = ChIP

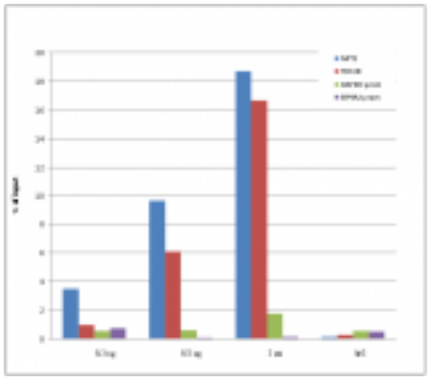


Localisation of Histone modifications at specific genes/promoters etc. using Chromatin-Immunoprecipitation = ChIP



ChIP-Seq – mapping of enrichment using NGS methods

Analysis at the locus via PCR



Analysis genome wide by NGS