# WARNING
# This is a summary not a total overview of what comes in the test!!!

Keep in mind that you have to carefully look at the slides of Lectures 1 to 3 (***all*** of them)

Lecture 4 (partially - e.g. look at the general slides for imprinting and X-inactivation

Lecture 5 (only slide 18 as an addition to lecture 2)

Lecture 6 : slides 38, 50, 51 and 53

# Epigenetics: Genetic Definition

Study of (heritable) phenotypic changes that are caused modifications of DNA and chromatin and not by altered composition of the DNA (base changes)

# Epigenetic modifications

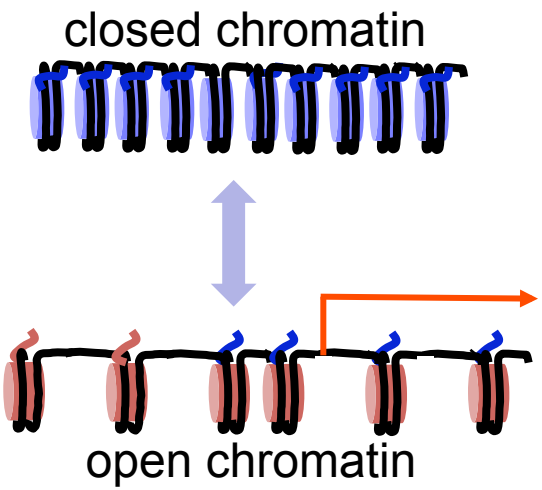
**DNA-methylation**
($5^{me}$Cytosine, $6^{me}$adenine)

Lecture 2

**RNAs**
(mi-, pi-, si- and
long non-coding RNAs)

**Histone modifications**
(Methylation, Acetylation,
Phosphorylation,...)

Lecture 3




**DNA-sequence & structure**
gene structure, length,
base content, variation...)

**Histone variants**
(H1.1, H2AX, H2AZ,H3.3,
CenpA..)

Lecture 3

**None-histone proteins**
Transcriptional control proteins
Chromatin-Remodelling proteins,
Structural proteins

# Organisation of chromatin in the nucleus:

## The two states of chromatin

- Euchromatin:
  Less densely packed (Feulgenreaction) or less electron dense (EM) domains in the interhase nucleus.
  Histones are marked by euchromatic modifications.

- Heterochromatin:
  Dark „matter“/areas in the nucleus, more densely packed, electron dense and intensively stained by feulgen in the interphase nucleus.
  Histones contain specific heterochromatic modifications

# Components of chromatin

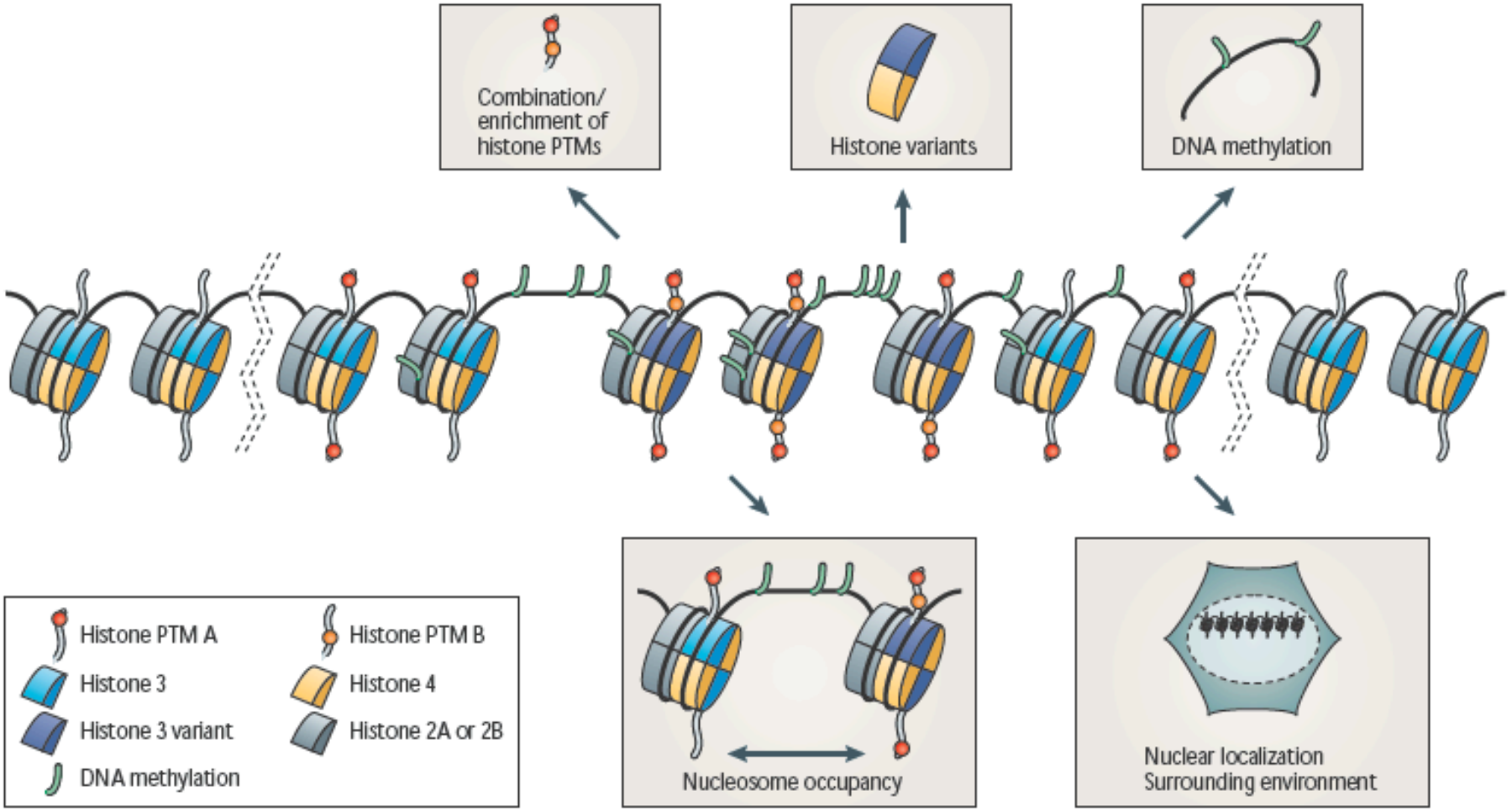
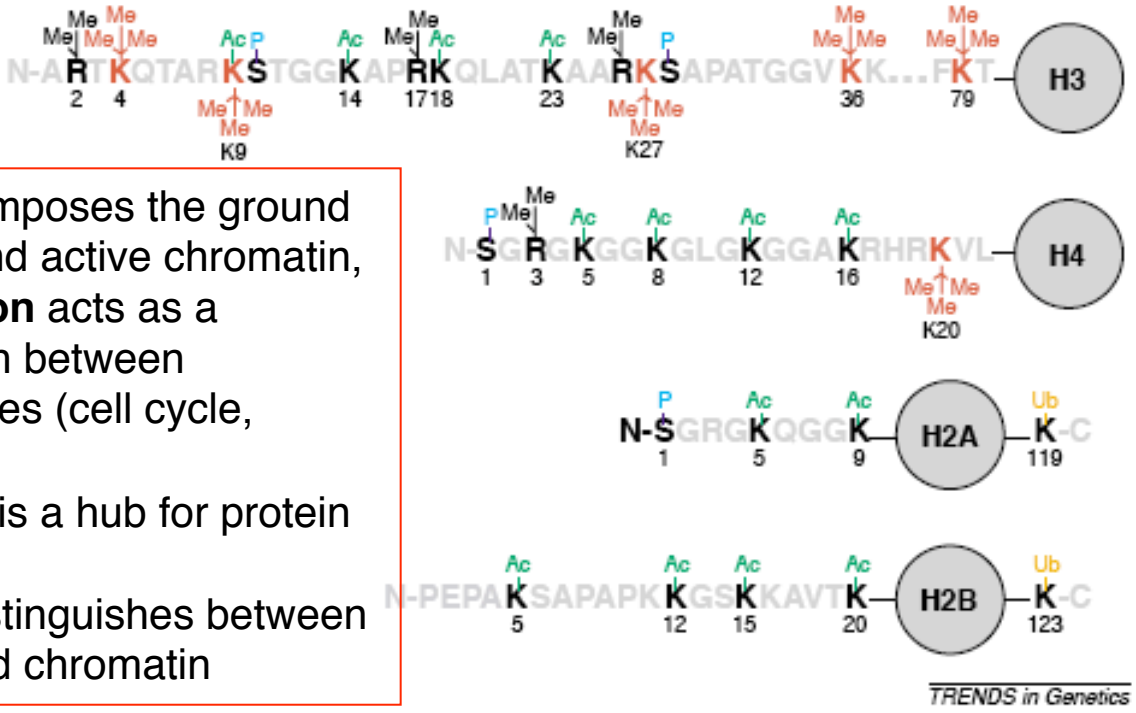



Figure 1 | **Characteristics of a chromatin domain**. Schematic depicting modifications that define different chromatin domains. The range of factors that can contribute to the characteristics of a domain are shown in the shaded boxes. The dashed lines represent the separation between two adjacent domains. PTM, post-translational modification.

# Specificity of histone modifications: amino acid, position and extend




**Acetylation** composes the ground state of open and active chromatin,
**Phosphorylation** acts as a functional switch between methylation states (cell cycle, repair),
**Ubiquitination** is a hub for protein docking,
**Methylation** distinguishes between open and closed chromatin

**Figure 1.** The known post-translational covalent modifications of histones (H2A, H2B H3 and H4). Lysine (K) methylation (Me) is represented in red. Acetylation (Ac), phosphorylation (P) and ubiquitination (Ub) are indicated in green, blue and orange, respectively. Arginine (R) methylation is represented in black. Modifications shown above each amino acid correlate with activation, whereas the lysine methylation shown below correlates with repression. The highest degree of methylation possible for each residue is displayed. Note that in H4, K20 methylation might also participate in activation.

# Histone-modifications

- Histones can be modified at various amino acids.
- Modification are enzymatically catalized (mostly) when histones are „in place“, i.e. within nucleosomes.
- The modifications occur in a sequential manner.
- The concert of histone modifications „codes“ the nucleosomes for structural or functional aspects.

# Histone modifications are associated with specific chromatin structures

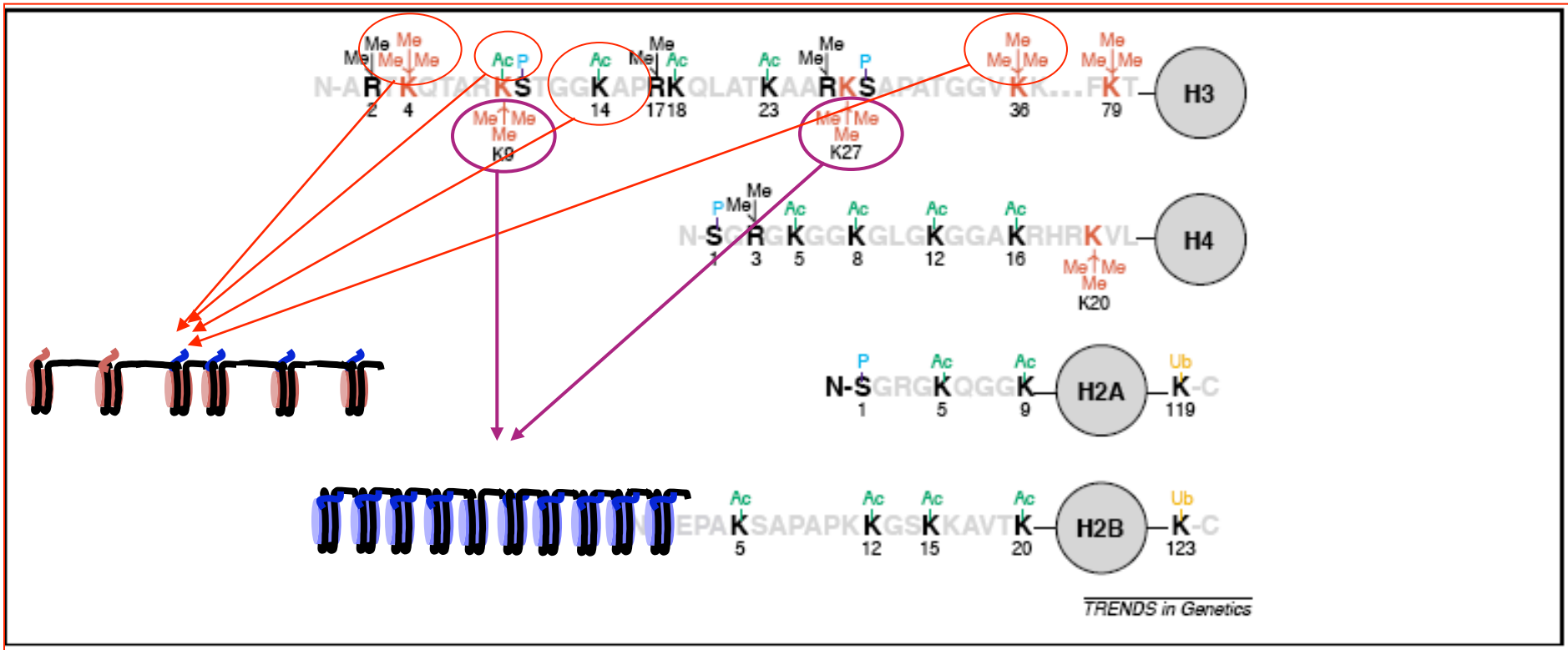



**Figure 1.** The known post-translational covalent modifications of histones (H2A, H2B H3 and H4). Lysine (K) methylation (Me) is represented in red. Acetylation (Ac), phosphorylation (P) and ubiquitination (Ub) are indicated in green, blue and orange, respectively. Arginine (R) methylation is represented in black. Modifications shown above each amino acid correlate with activation, whereas the lysine methylation shown below correlates with repression. The highest degree of methylation possible for each residue is displayed. Note that in H4, K20 methylation might also participate in activation.

# Histone-modifications

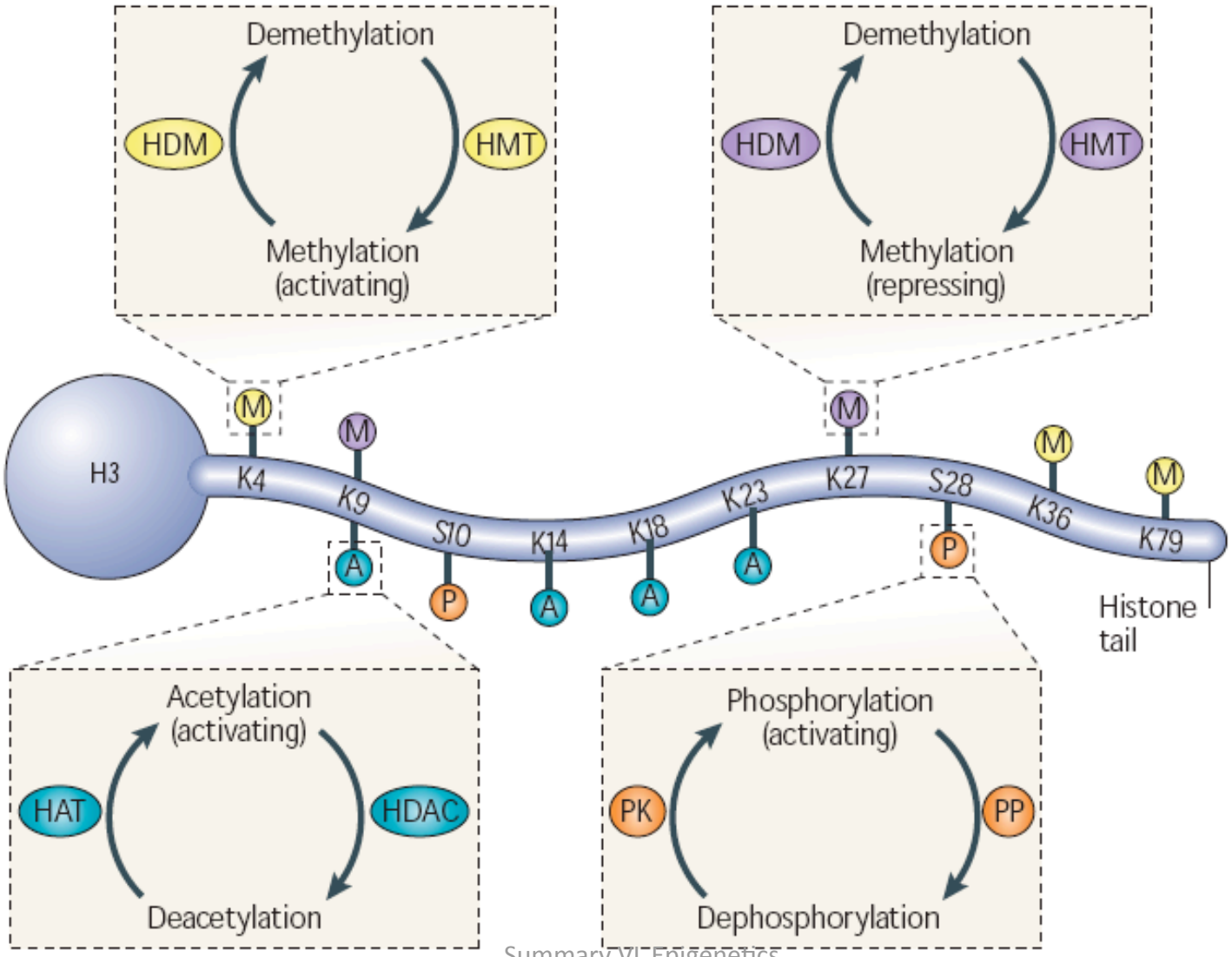
- General notions:

- Most modifications are established/removed when histones are in nucleosomes

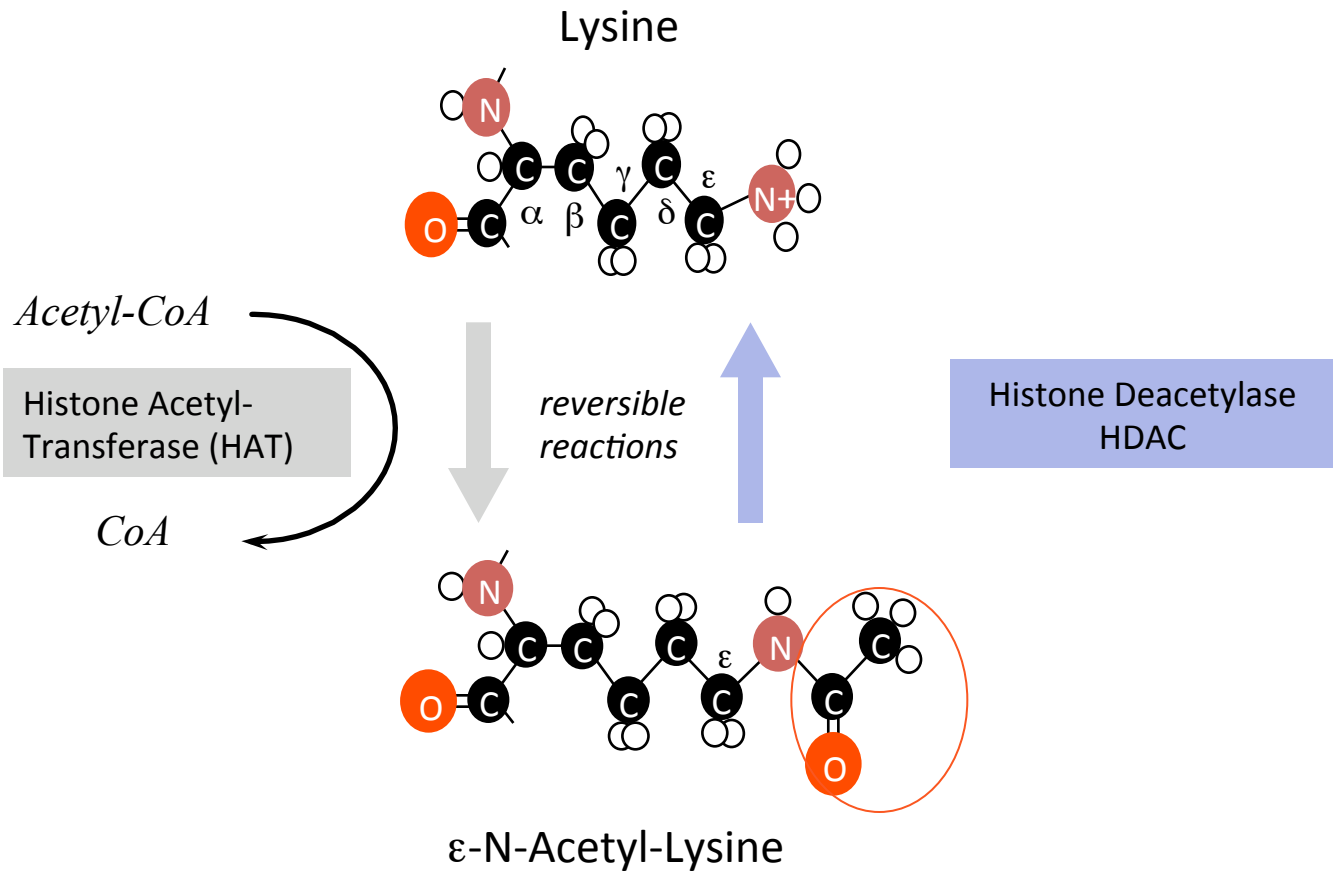
- The recognition depends on the amino-acid neighborhood and the general protein structure context.

- Removal of a modifications can either occur directly or by„exchanging“ a modified histone by a non-modified histone.

# Histone-modification are controlled by specific enzymes in forward and reverse reactions



# Acetylation of histones




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

# Lysine methylation in histones is removed by specific Demethylases (HDMs)

Two types of demethylases : Jumonji or LSD1

Both remove methyl groups from Lysine residues in a sequential and substrate specific way.

Jmj use $\alpha$-ketoglutarate as a cofactor which is converted to succinate to finally oxidise $CH_3$ and to release formaldehyde from the histone. Found in activator (e.g. JmjD2) and repressor (e.g. Jmj1a) complexes.

LSD1 uses FAD as a cofactor to covert and cleave formaldehyde from the histone

LSD1 is targeted by repressor complexes to demethylate H3K4me3.

# Reading of histone modifications:

Histone modifications function as anchors to recruit proteins to chromatin

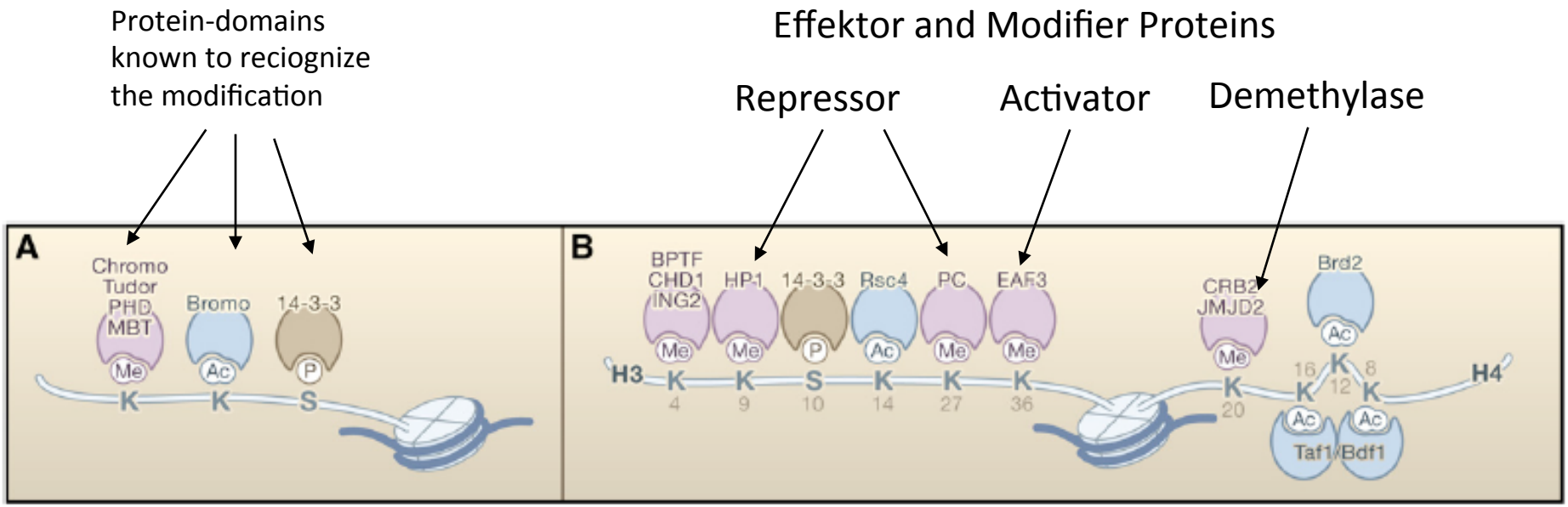



**Figure 1. Recruitment of Proteins to Histones**

(A) Domains used for the recognition of methylated lysines, acetylated lysines, or phosphorylated serines. (B) Proteins found that associate preferentially with modified versions of histone H3 and histone H4.

# Important histone modification reader and modulator proteins

- HP1 = heterochromatin protein 1 binds to H3K9me2/3. HP1 stabilizes heterochromatin structures and interact with other heterochromatin forming proteins. HP1 contains a CHROMO domain found in several hetero-chromatin associated proteins.

- PcG = Polycomgroup-Protein proteins form large complexes with other proteins. PcG complexes bind to polycomb response elements = PREs. PcG complexes contain histone modifying enzymes such as EzH (H3K27me3) or Ring1b (H2AUb119) to form heterochromatin at promoters of developmentally expressed genes (e.g. Hox genes) in non expressing cells.

- Trx= Trithorax-group-proteins. Trx proteins are transciptional co-activators and contain characteristic *BROMO* domains. TRX are often in complex with HMTs (H3K4me3) and HATs (H3K9/H4K12/14) acetylation to locally establish open chromatin at promoters.

# Methods for mapping of epigenetic modifications

**ChIP =** Chromatin Immunoprecipitation is a universal method to map proteins or protein modifications in chromatin.

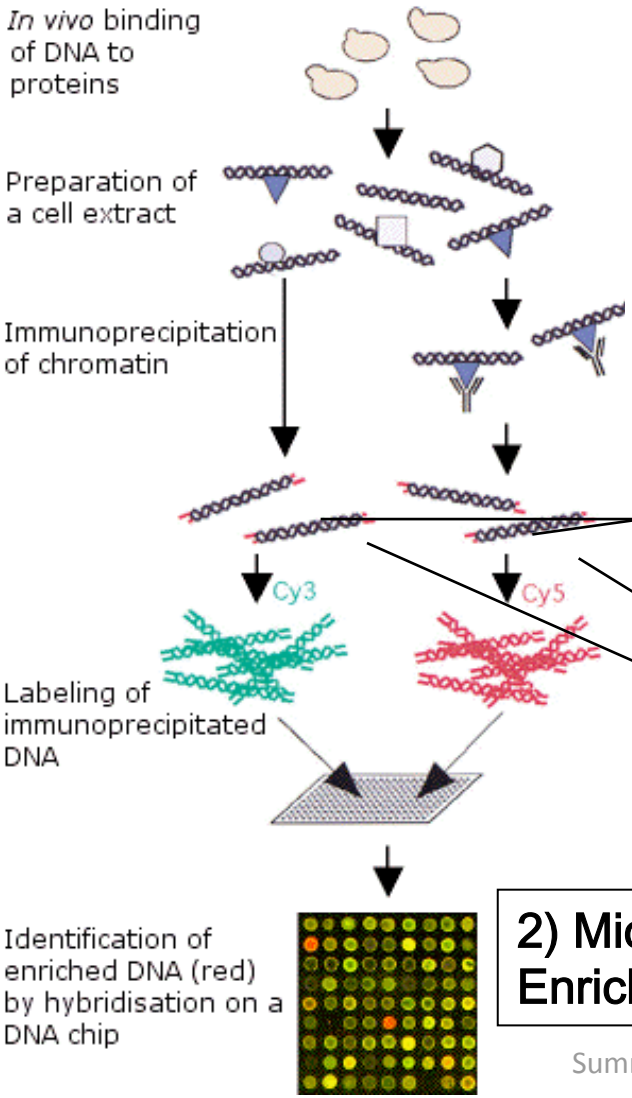
The method depends on the availability of antibodies which bind specifically to the protein (such as transcriptional factors, RNA-Polymerase, repressors) or to the modified histones (e.g. H3K4me3) at certain locations in chromatin.

To capture the bound antibody the chromatin is crosslinked (by formaldehyde) after binding. Then the crosslinked chromatin/DNA complexes fraction is extracted by "fishing" out the antibody (e.g. by a secondary anti-antibody which is linked to magnetic beads).

The "fished" chromatin fraction is "de"-crosslinked and the DNA released and extracted. To determine which regions show an enrichment for the modifications in the genome (i.e. the region where the protein/modification was bound) the following down stream processing can be performed:

1) Region specific Q-PCR reactions e.g. at known gene promoters
2) Hybridization of the released DNA to micro-arrays containing specific oligonucleotides covering all gene promoters
3) Massive parallel sequencing of the released DNA by next generation sequencing and computational alignment across the genome and "read counting".

# Principle of ChIP




1) Selected Q-PCR at specific regions (bound vs input)

3) Next generation sequencing of bound Material versus unbound control (input)

2) Micro array analysis
Enriched vs input material (DNA)

# Nucleosome dynamics and changes in chromatin structure

Histones in nucleosomes are not static:

i) Histones can be assembled by chaperones into newly formed nucleosomes.

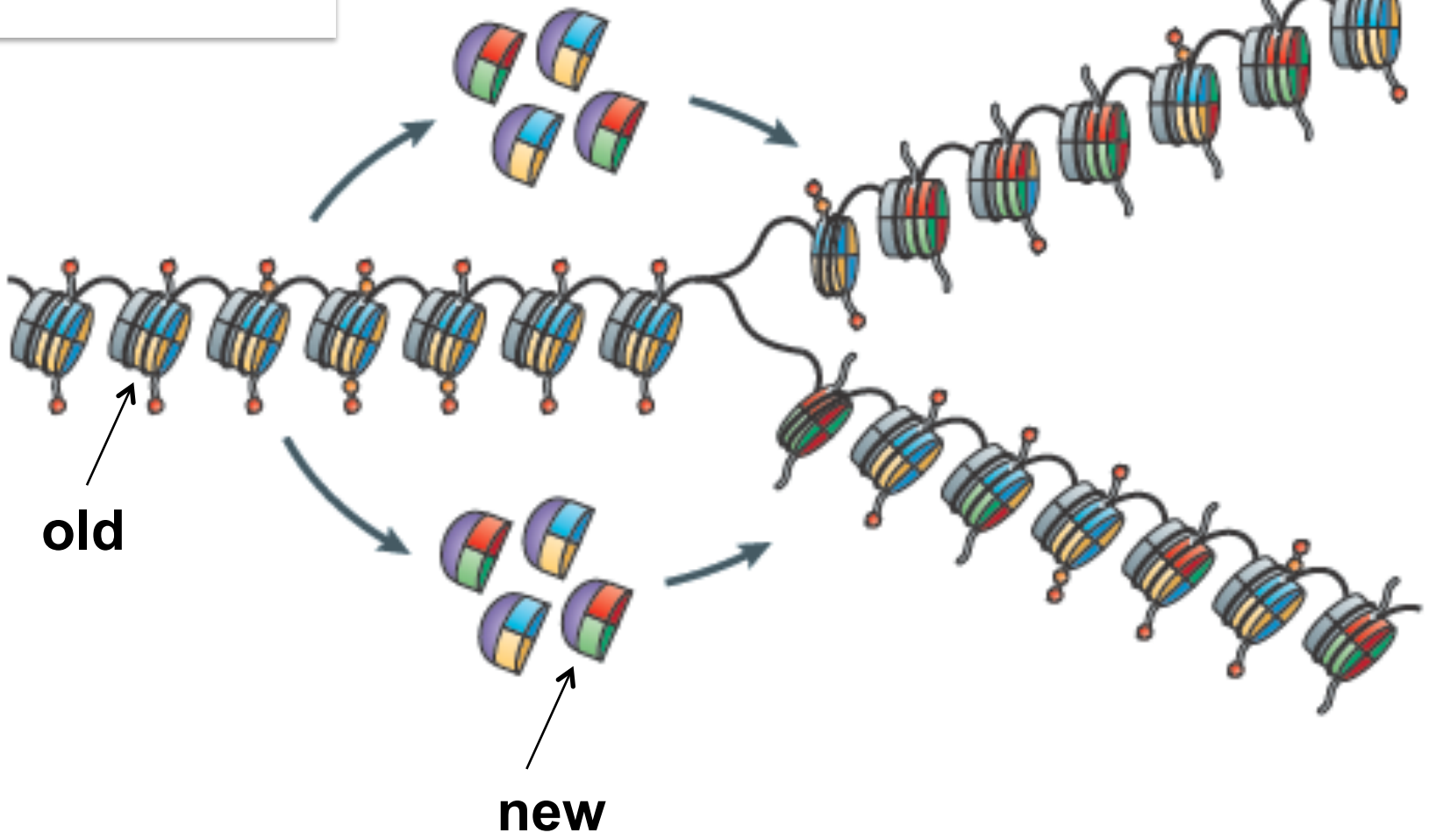
ii) Single modified histone of dimers/tetramers can be exchanged by specific chaperones against unmodified histones or histone variants.

iii) Nucleosomes can be moved along the DNA (or the DNA along the nucleosomes) to release or hide certain DNA sequences for recognition.

All processes are regulated and catalysed by enzymes.

# New nucleosomes are assembled in newly replicated DNA:

## Histone modifications are maintained in the old nucleosomes



# Nucleosome remodelling

The density and presence (distribution and spacing) of nucleosomes in chromatin (on the DNA) can be altered by enzymes which are components of **chromatin remodelling complexes (CRCs)**

CRCs are important to locally open the chromatin to induce transcription. The central “moving” enzymatic unit in CRCs are ISWI or SNF like proteins.

# DNA-methylation specificity

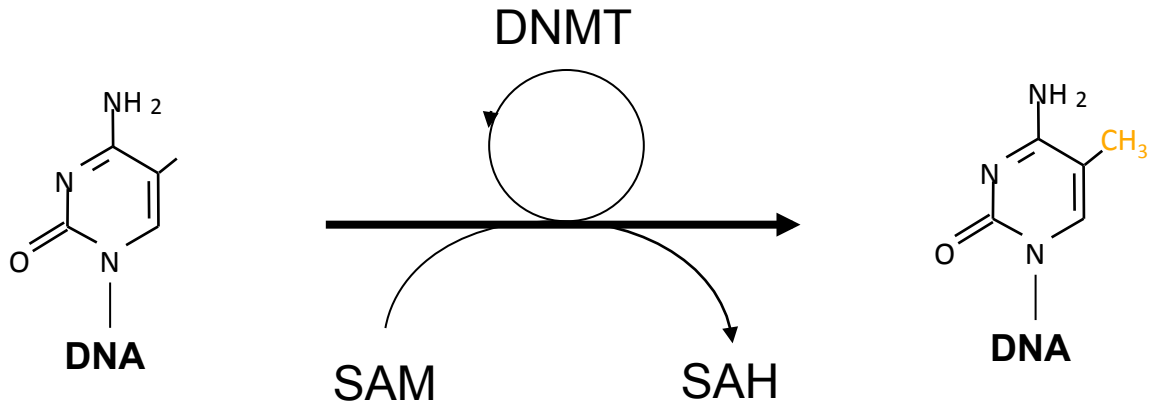
- DNA of most organisms contains modified bases which can be: C5-Methyl-Cytosine, N4-Methyl-Cytosine, N6-Methyl-Adenine.

- DNA-methylation occurs after replication and is enzymatically catalized by DNA-methyltransferases (DNMTs).

- DNA-methylation is universially catalysed by enzymes which transfer a methylgroup ($CH_3$) from the methylgroup donor S-Adenosyl-Methionin (SAM = S-adenosyl-methionine) to the DNA base.

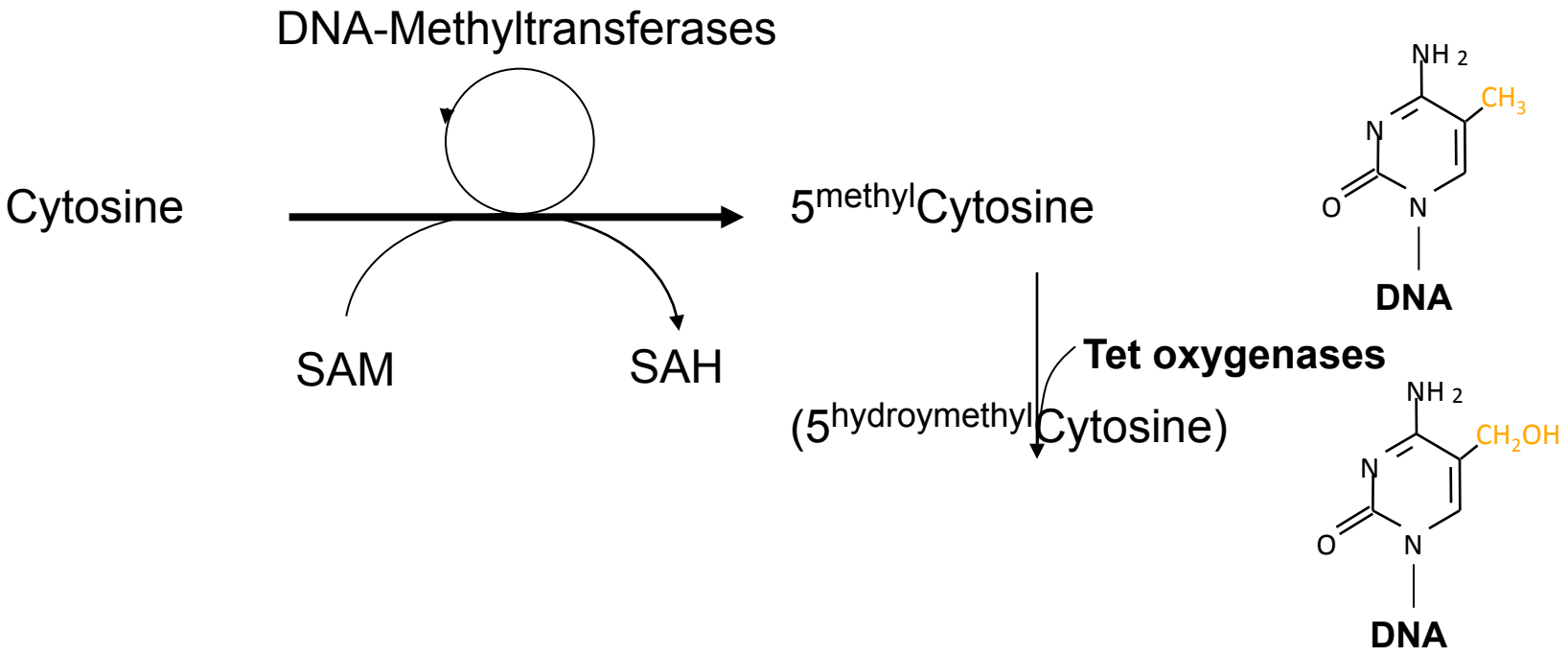
# DNA-methylation:

## general mechanism

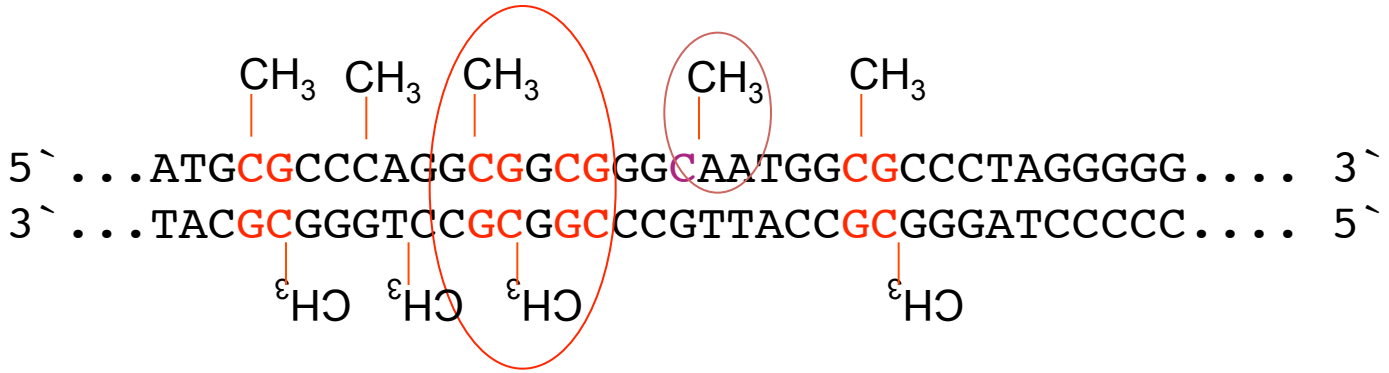



A 5‘CpG3‘ dinucleotide in the DNA of mammals is recognized by the DNA-methyltransfrease (DNMT) and a methyl group is transferred from the methylgroup donor S-adenosyl-methionine (SAM) to the carbon 5 of the cytosine ring. SAM is converted in S-adenosyl-homocysteine (SAH).

# In human/mammals methylated cytosines can be hydroxylated by oxigenases (Tet 1,2,3)



# Cytosine methylation in mammals/human



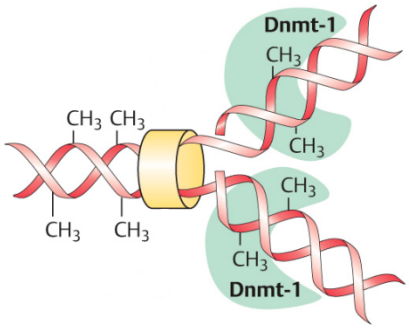

DNA methylation in mammals occurs almost exclusively at CpG dinucleotides. In stem cells cytosines can be methylated at non-CpG positions such as CNG sequence context or any CA(N)

# DNA-methylation in mammals

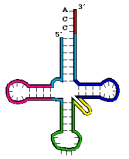
- The CpG dinucleotide is relatively rare in the DNA.
- Less than 2% of all nucleotides are CpG's and their genomic distribution is non-random.
- Most CpGs are found in clusters, so called CpG islands which are mostly not methylated.
- CpG islands are found in promoters/ 5' end of about 50% of all genes.
- An exception are CpG islands on the Xi and some imprinted genes which are methylated in one of the alleles.

# DNA-Methyltransferasen (DNMT ‘s)

## ‚Maintenance‘ Methyltransferase

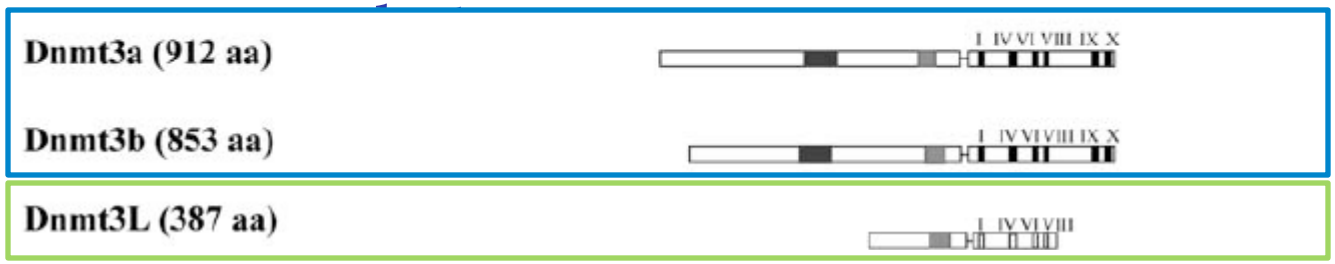




## t-RNA methylatransferase‘

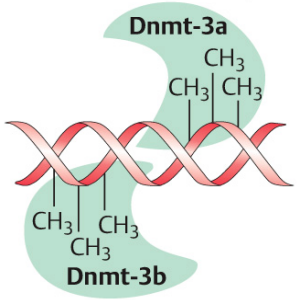



tRNA

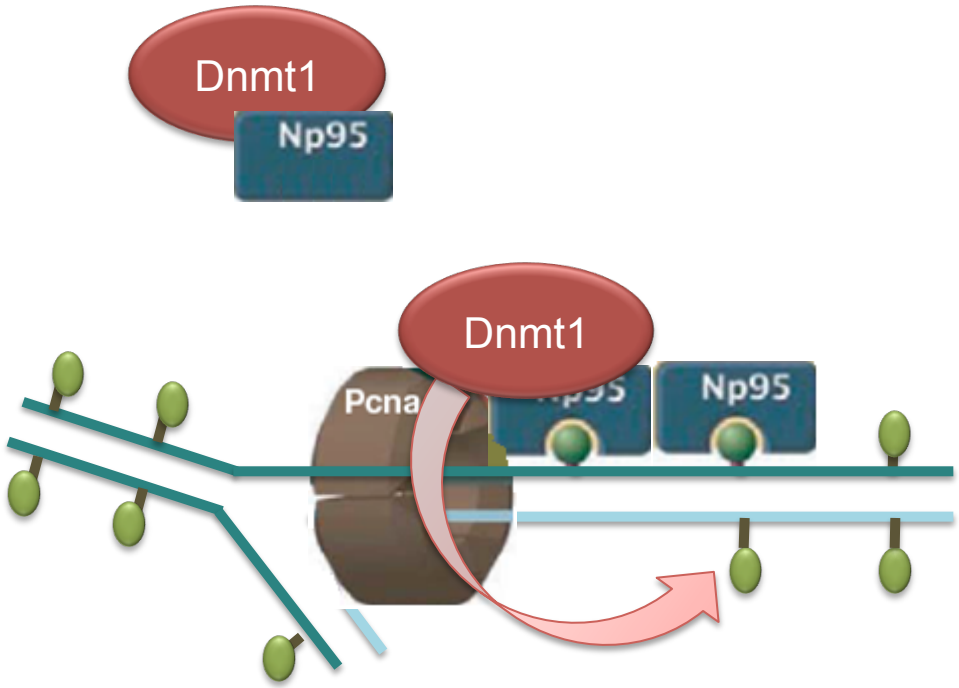
## ‚De novo methylatransferase‘




reg.



# Regulation of DNMT1 by NP95




- Reading of $^{me}$CpG **and** localisation of Dnmt1 at replication forks by NP95
- NP95 is animportant regulator for DNA-methylation maintenance at most genomic regions

# DNA-methylation: regulatory functions

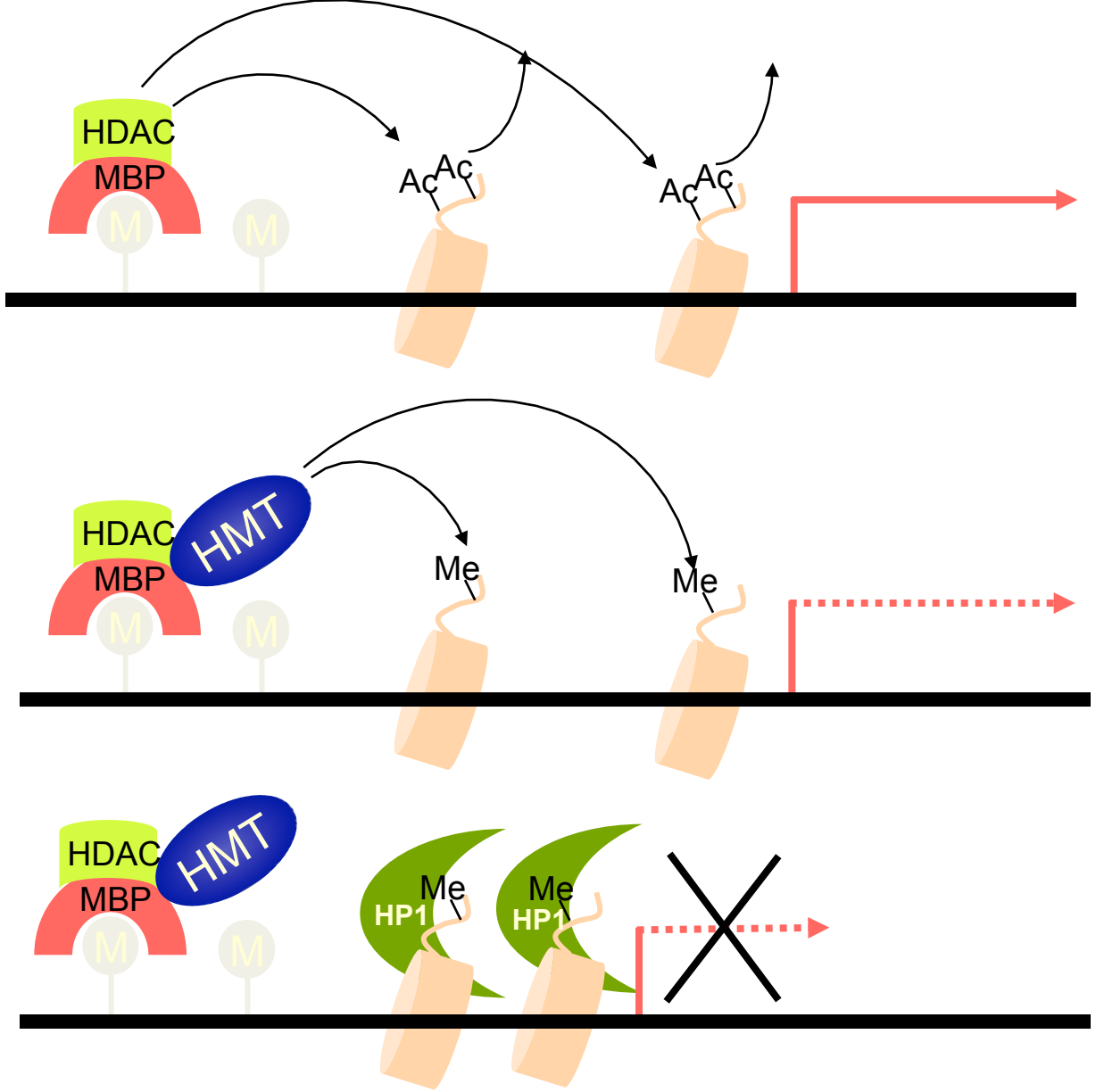
DNA-methylation:

- Is essential for cellular survival and normal embryonic development but is not essential in embryonic stem cells
- Controls  tissues specific gene expression
- Controls X-chromosome inactivation and Genomic Imprinting
- Silences expression of endogenous retroviruses, pseudogenes and transposable elements
- Is important to maintain heterochromatin

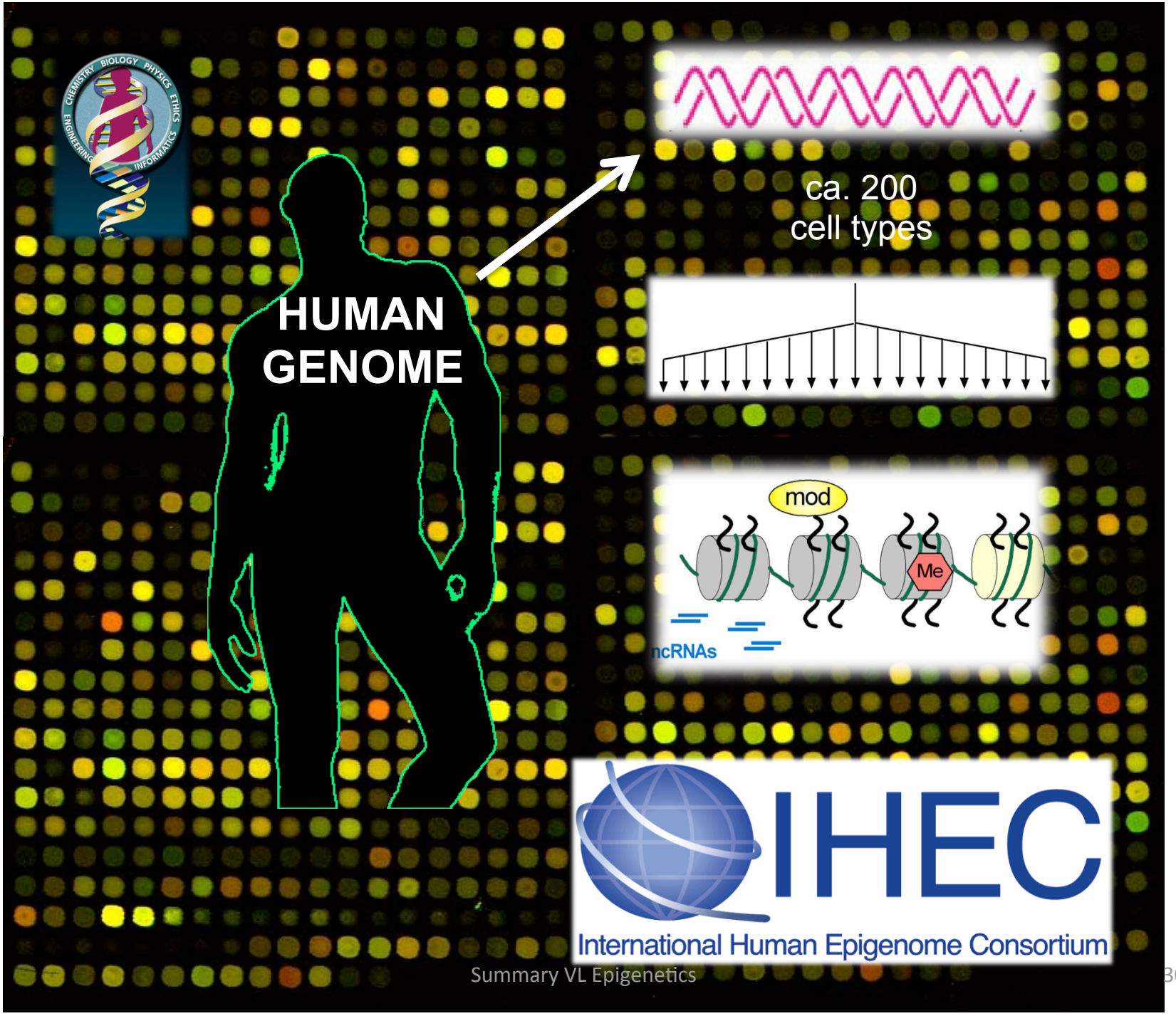
# DNA-methylation: communication/interaction with histone modifications

DNA-methylation is recognized by DNA-methylation binding proteins such as MBD1,2,3,4 or MeCP2

Some MBD proteins are present in chromatin modifying (repressor) complexes (such as NuRD) and mediate the crosstalk between heterochromatic histone- and DNA-modifications.




Methylated DNA is recognized by MBD proteins which recruit HDAC inducing deacetylation and chromatin compaction. The complex contains additional HMTs (such as Suv39H or G9A) which methylated H3K9me2/3 to generate templates for HP1 binding and stable heterochromatin formation (at permanent heterochromatic sites such as centromers)


HUMAN
GENOME
ca. 200
cell types
mod
Me
ncRNAs
IHEC
International Human Epigenome Consortium

# Epigenomics

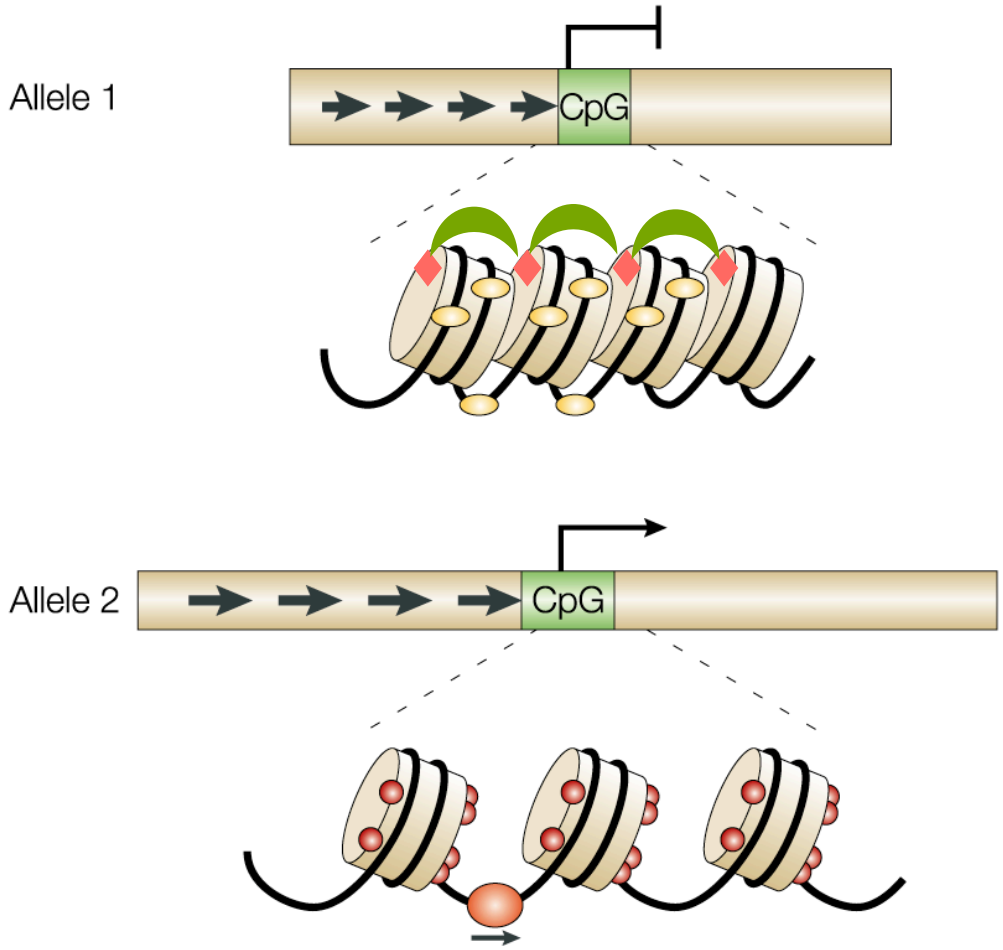
1. New technologies (particularly next generation sequencing allow to determine the modificytion along genomes in each cell, i.e. to determine epigenomes.
2. Large parts of the non-protein coding genome are transcribed and have an important role in the regulation of the genome.The precise function of these regions is not yet know.
3. All genes have characteristic histone modifications. DNA-methylation plays an important role to silence large parts of the genome but genes with CpG island promoters (50% of all genes).
4. Histone modifications correlate very well with the transcription status of the respective genes.
5. Retrotransposable elements, imprinted genes, X-chromosomes and repeated DNA elements are silenced by DNA methylation helps to inactive pseudogenes

# Genomic Imprinting: Definition

A germ line derived epigenetic marking results in a parental-specific expression of autosomal genes in somatic cells.

Epigenetic marks (imprints) control the monoallelic expression of genes.

# Genomic Imprinting - molecular mechanisms



# Genomic Imprinting: Control of genes by epigenetic modifications

- Imprints are based on reversible epigenetic modifications

- Imprints are established in the germ line
  - By first erasing the somatic marks in early germ cells followed by a « parent of origin » specific « de novo » establishement of sex specific imprints

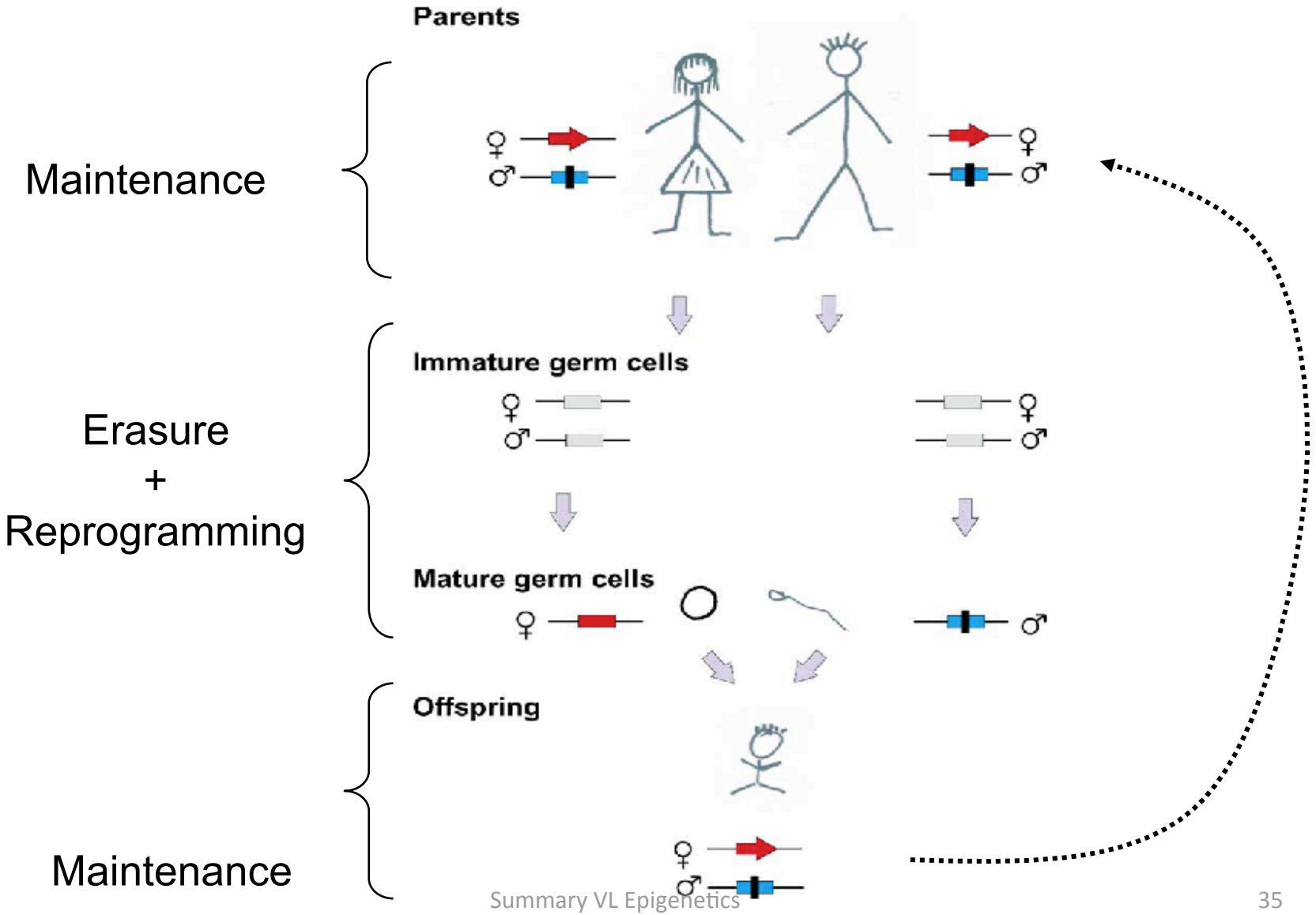
- After fertilizaion imprints are maintained (inherited over somatic cell divisions during development into adulthood.

- DNA methylation constitutes an essential imprinting modification

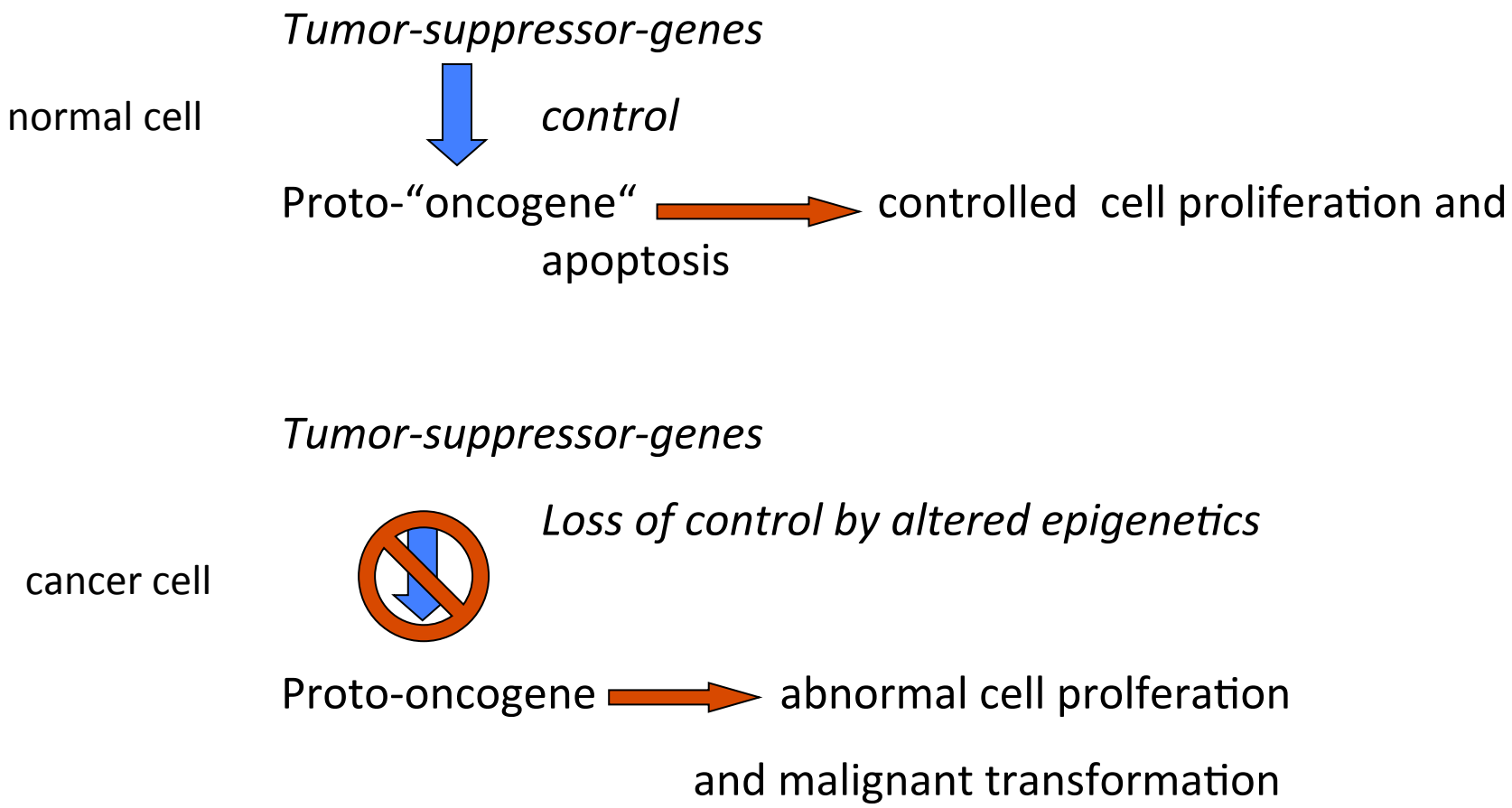
- Histone modifications are important co-players and « translators » of imprinted expression

- At some imprinted genes non-coding macro RNAs are involved in setting/maintenance of imprinting

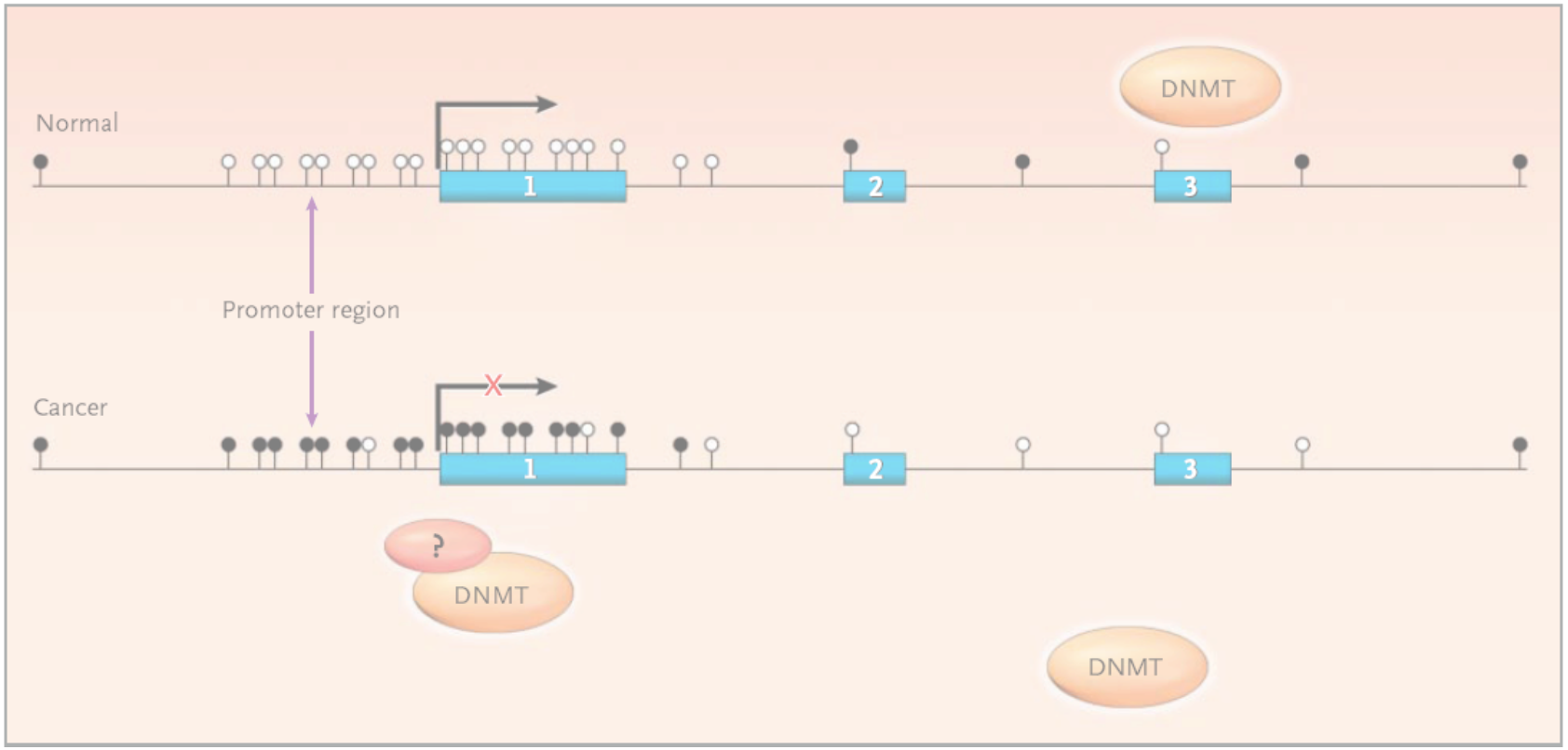
# Life cycle of Genomic Imprints



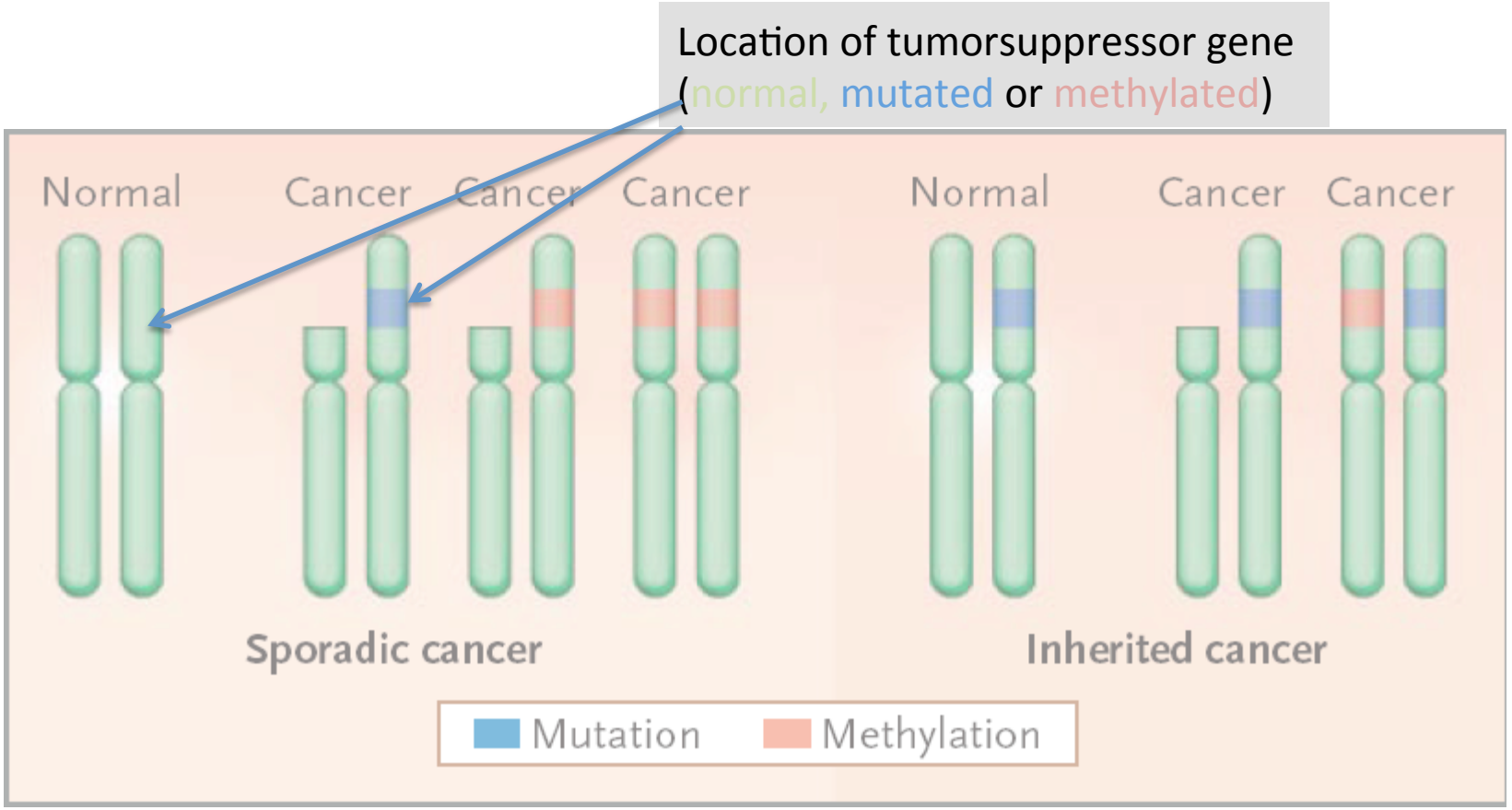
# Development of cancer is caused by a loss of tumor suppressor genes – epigenetics contributes to this



# Cancer cells show a genome wide loss of DNA methylation and a gain of methylation at gene promoters of tumor suppressor genes

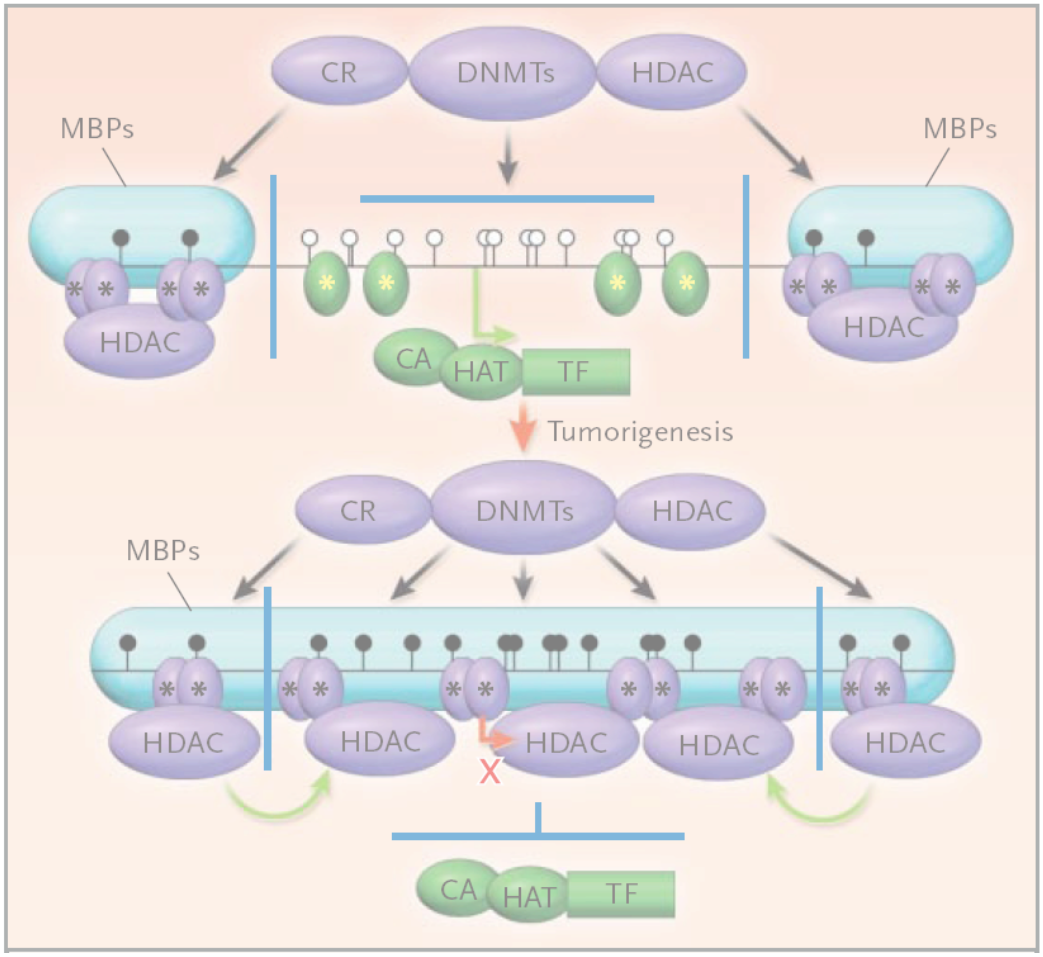


# Epigenetic extension of Knudson‘s „Two hit hypothesis“




Epigenetic changes such as DNA methylation of promoters lead to the inactivation of One or both alleles – as a consequence the tumor suppressor gene is completely inactive

# Epigenetic „Silencing“ of CpG islands in cancer




Histone H3 Acetyliert
H3K4 Methylierung
Histone H3 Deacetyliert
H3K9 Methylierung

CA: Transkriptional Co-Aktivatoren
HAT: Histone Acetyltransferasen
HDAC: Histone Deacetylasen

MBP: Methyl Bindig Protein
TF: Transkriptionsfaktoren
CR: Transkritionale Co-Repressoren

# Epigenetics and cancer: summary

1. Cancer is a multi-factorial, progressive disease, the balance of e.g. oncogenes/tumor suppressor genes is disturbed.

2. Epigenetic alteration have an important role in cancer development.

3. In most cancer types, both alleles of a gene are mutated/activated.

4. The abnormal DNA methylation pattern in cancer can used for cancer diagnostics.

5. Several inhibitors of DNMTs and HDACs are currently in clinical evaluation.