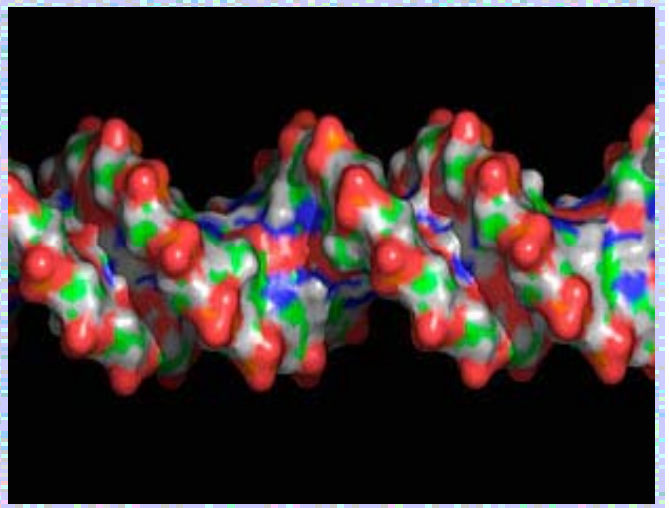
Epigenome: The Unseen Genome



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Epigenome

Genome wide study of methylation and other epigenetic markers and their role

Epigenetics

Study of the changes in the gene expression that are mitotically and/or meiotically heritable and do not involve a change in the DNA sequence

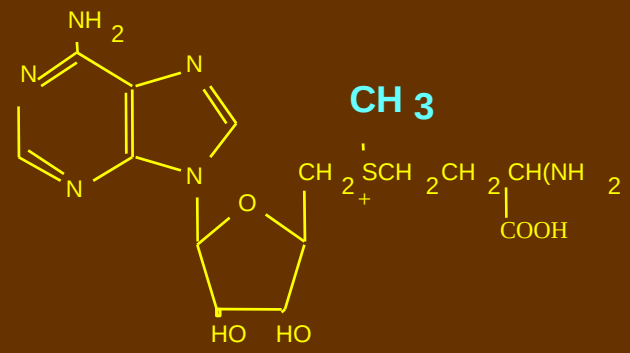
Biochemical Reactions in Epigenetics

1. DNA methylation

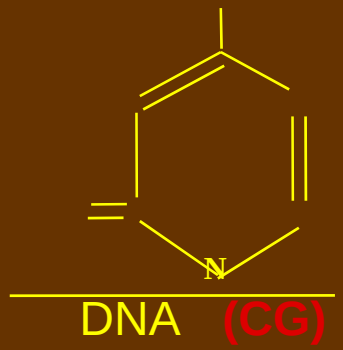
2. Histone modifications

DNA Methylation

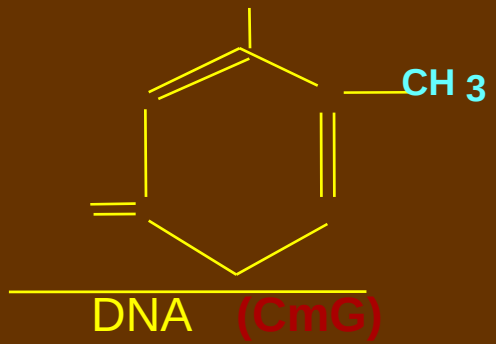
- Covalent addition of methyl group to 5^{th} position of cystosine within CpG di-nucleotides
- CpG dinucleotides are non-randomly distributed across the genome as per their methylation status
- It is a complex process catalyzed by DNA methyl transferase
- Methyl group donor is s-adenosyl L -methionine

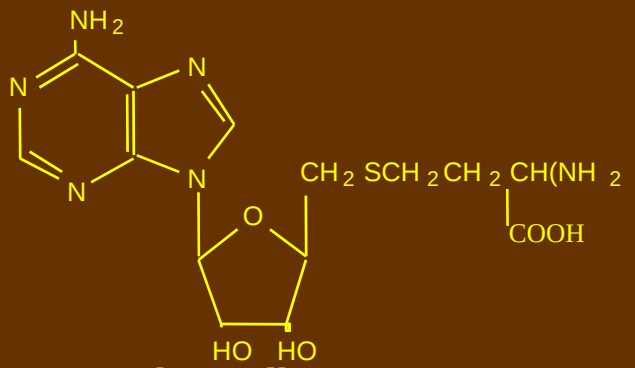


S-Adenosylmethionine
(Adomet or SAM)



DNA methyltransferase





S-Adenosylhomocysteine

(AdoHcy or SAH)

Major Targets For Cytosine Methylation

- CpG islands
- G+C isochores
- CpG hotspots

CpG Islands

- Clusters of CpG usually at promoter or at early exons
- 0.5 - 5 kb in length and occurring on average every 100 kb
- Normally unmethylated but methylation increases during ageing and diseases like cancer
- GC rich (60%-70%) & ratio of CpG to GpC of at least 0.6
- Chromatin containing CpG islands is generally heavily acetylated, lacks histone H1, and includes a %nucleosome-free region and called as open chromatin

G+C Isochores

- Large (>300kb) and homogenous groups of varying G+C content in human genome
- Five isochore families:

Family	G+C content
L_1 & L_2	~40%
H_1 & H_2	45% & 50%
H_3	53%

- Correlation between DNA replication timing and different isochore families - G+C rich regions replicate early and condense late

CpG Hotspots

- CpG sites act as hotspots for mutations
- Upto 30% of point mutations in germ line result from MeCpG $\rightarrow$TpG transitions by the spontaneous deamination of 5-MeC
- MeC directs some carcinogens to CpG dinucleotides

DNA Methylating Enzymes

- **Four mammalian DNA methyltransferases (DNMTs)**

1. **DNMT1**
2. **DNMT2**
3. **DNMT3A**
4. **DNMT3B**

DNMT1

- Global maintenance methylase as affinity for hemimethylated DNA that arise from DNA replication
- Maitenance of methylation signal and de novo methylation
- Overexpression leads to de novo methylation of endogenous CpG islands in cancer cell lines

DNMT2

- Not yet fully understood
- May function in controlling centromeric regions

DNMT3A and DNMT3B

- de novo methylase
- Major role during early embryogenesis
- Genome wide de novo remethylation at the time of implantation
- Essential for normal mammalian development

The Bilateral Interrelationship Of Chromatin And DNA Methylation

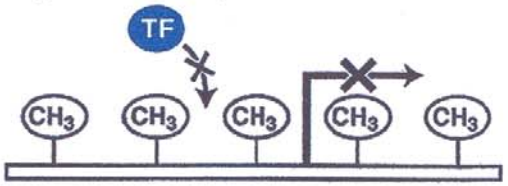
- **DNA methylation is a reversible reaction - the DNA methylation pattern is a balance of methylation and demethylation**
- **Active demethylation is directed by chromatin structure**
- **Proteins that inhibit histone acetylation inhibit demethylation - a mechanism for regional hypermethylation in cancer**

DNA Methylation and Transcriptional Repression

1. Direct interference with transcription activator factor binding

a. Active transcription

b. Repression by inhibition of TF binding

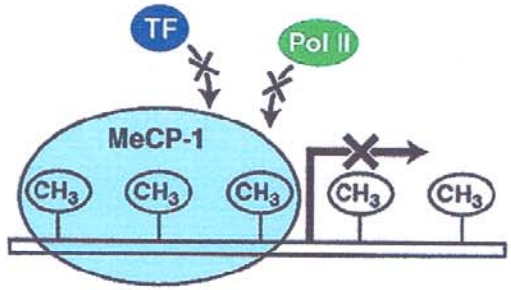


Examples: Methylation sensitive TF: *AP-2, E2F, NFkB*
Methylation insensitive TF: *Sp1*

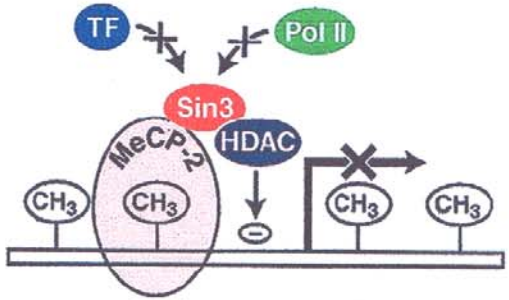
2. Specific transcriptional repressors

a. Active transcription

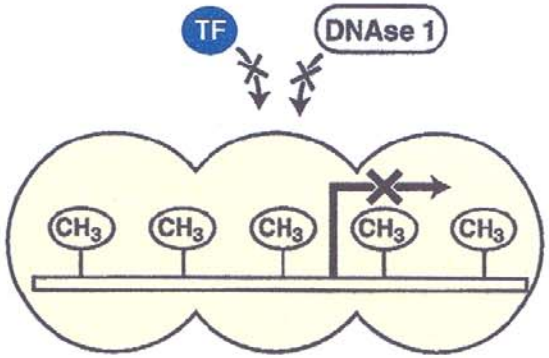
b. Repression by MeCP-1



c. Repression by MeCP-2



3. Inactive chromatin structure formation



methylated CpG: 

unmethylated CpG: 

Histone Modifications

1. Acetylation & deactylation

2. Methylation & demethylation

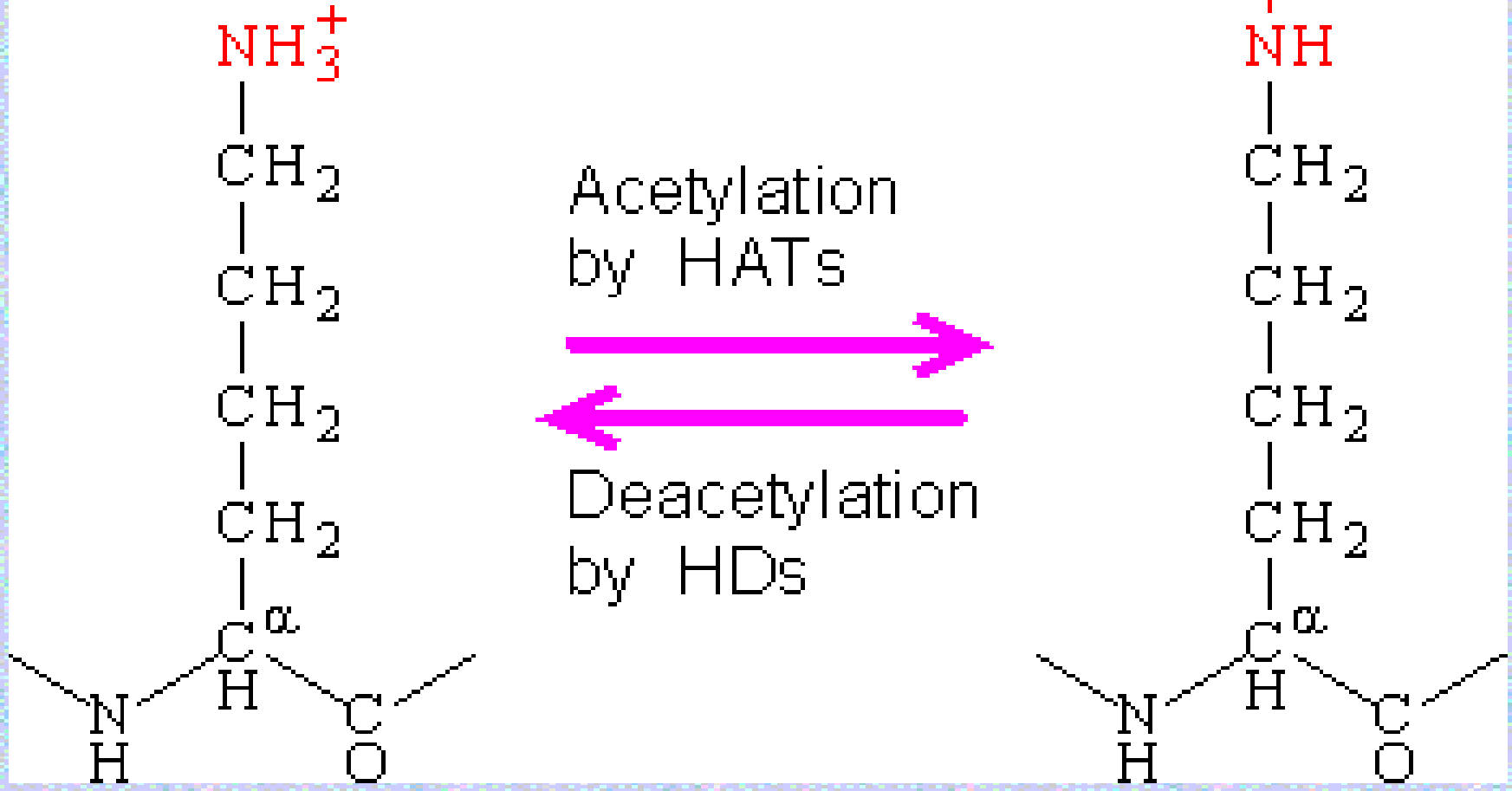
Histone Acetylation & Deacetylation

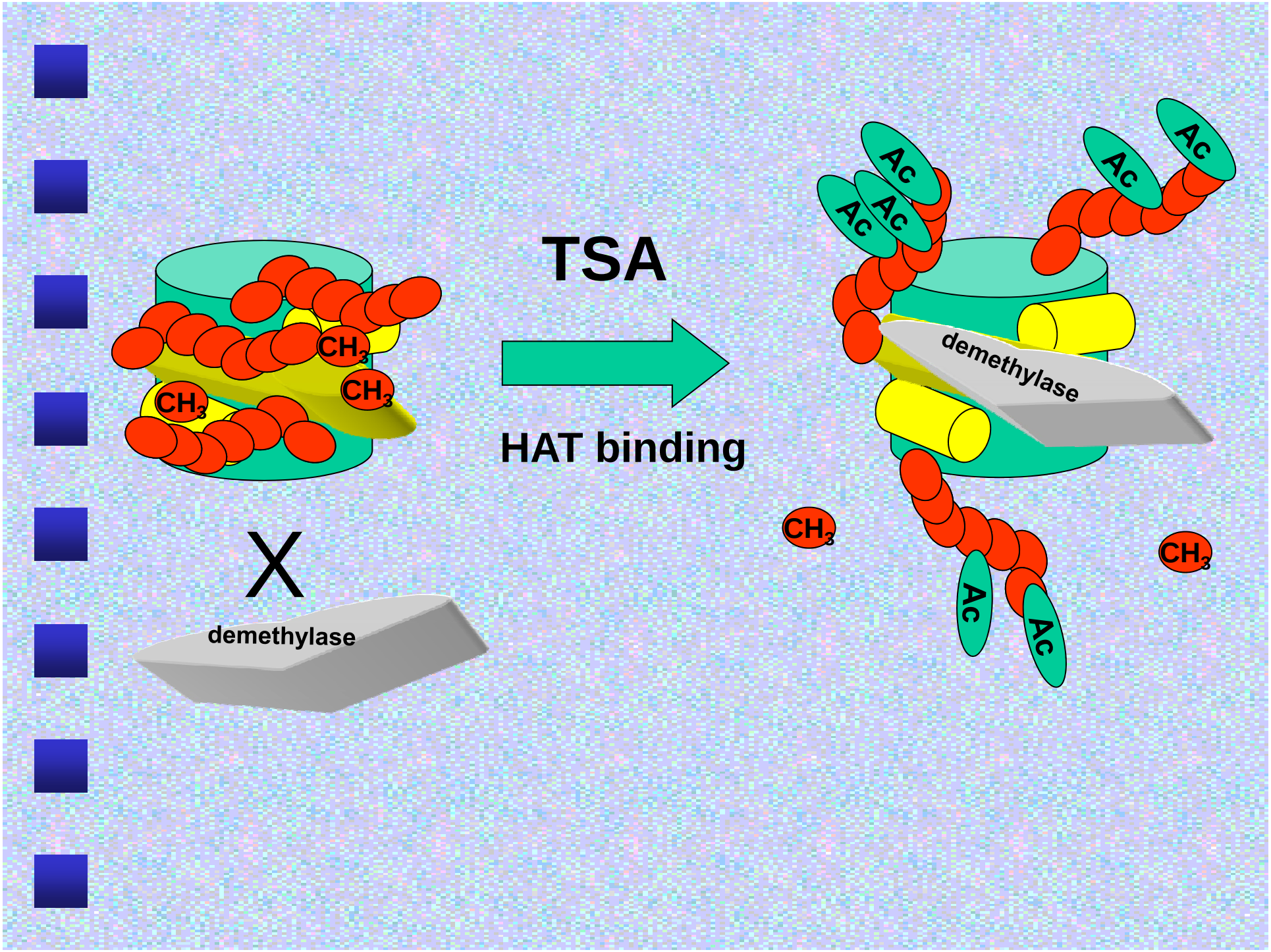
- **The acetylation of histones (H) mainly H_3 and H_4 is done at lysine 9 & 14 and lysine 5, 8, 12, 16 respectively by enzyme systems histone acetyltransferases**

- **Transfer of acetyl co-enzyme A to the lysine residue leads to neutralizing the positive charge. Histone acetylation loosens chromatin packaging and correlates with transcriptional activation**

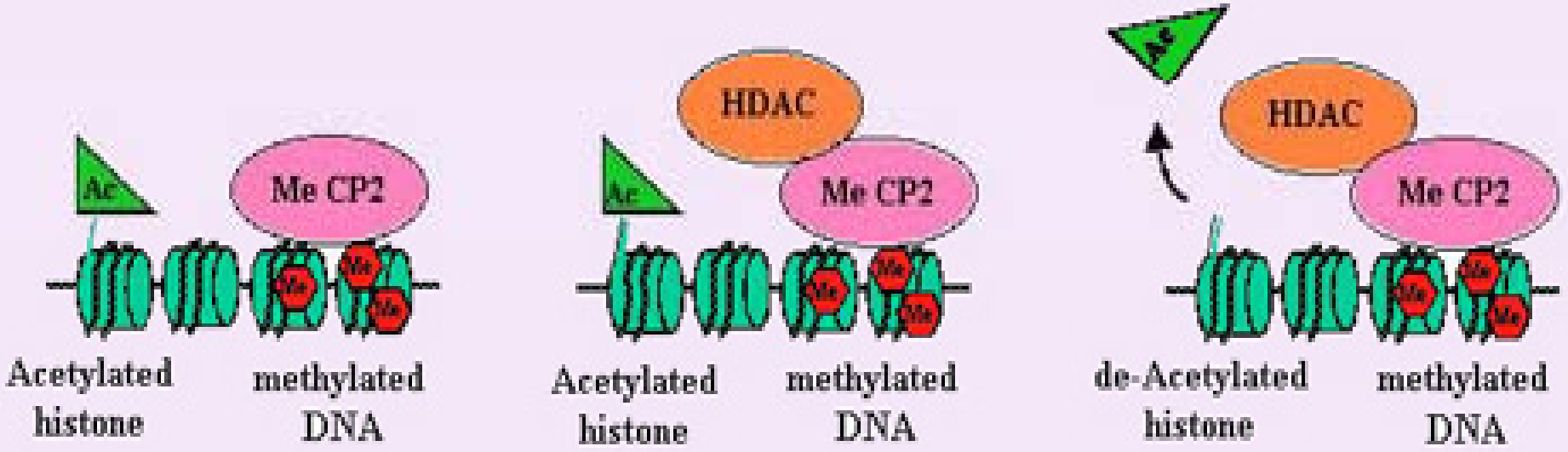
- **Histone deacetylases remove the acetyl groups re-establishing the positive charge in the histones which are associated with repression of transcription**

Lysine




TSA
HAT binding
CH3
CH3
CH3
X
demethylase
Ac
Ac
Ac
Ac
Ac
Ac
demethylase
CH3
CH3
Ac
Ac

DNA methylation induces Histone de-acetylation



Me CP2

binds methylated DNA

→

then recruits

HDAC

→

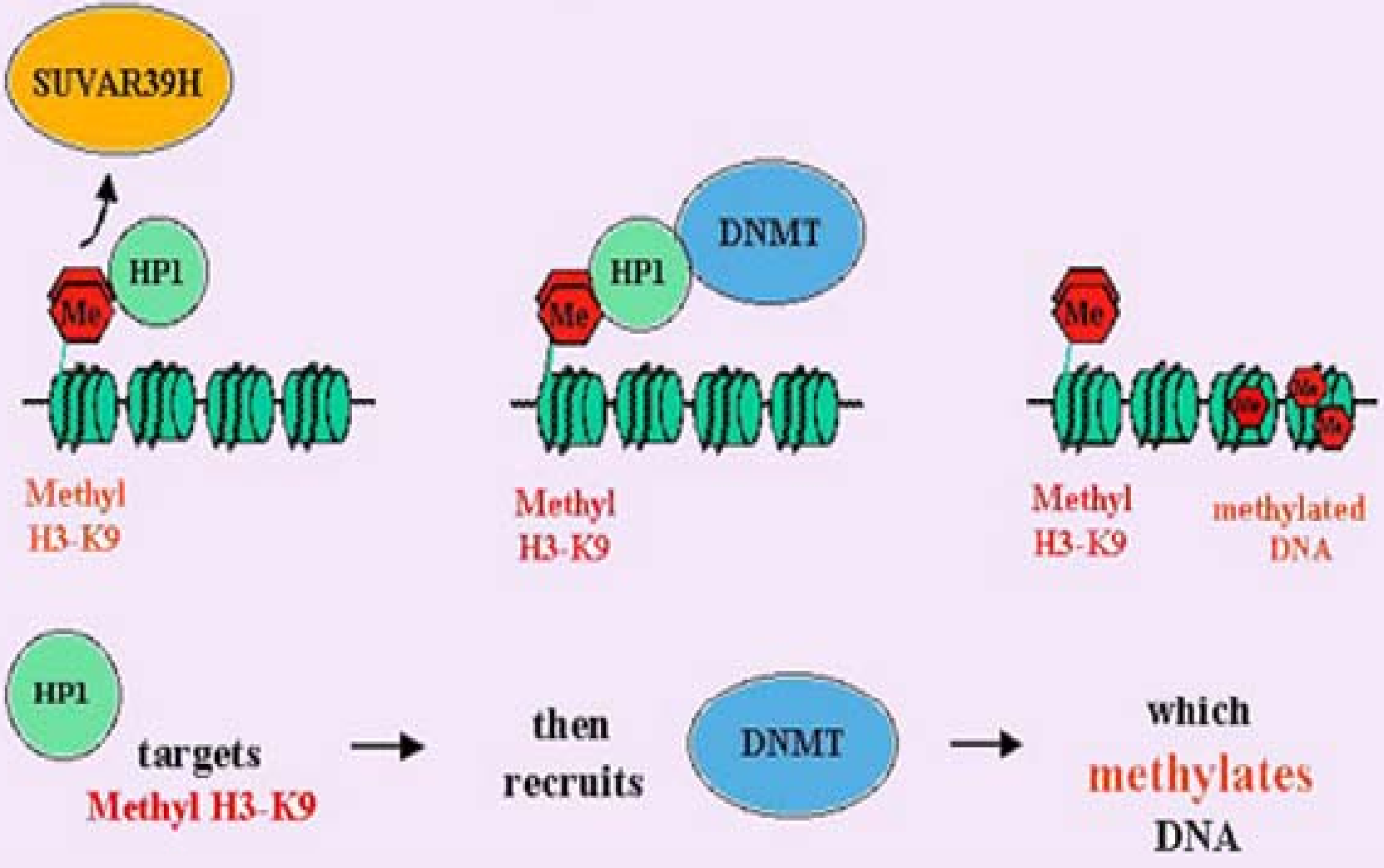
which de-Acetylates Histones

Histone Methylation

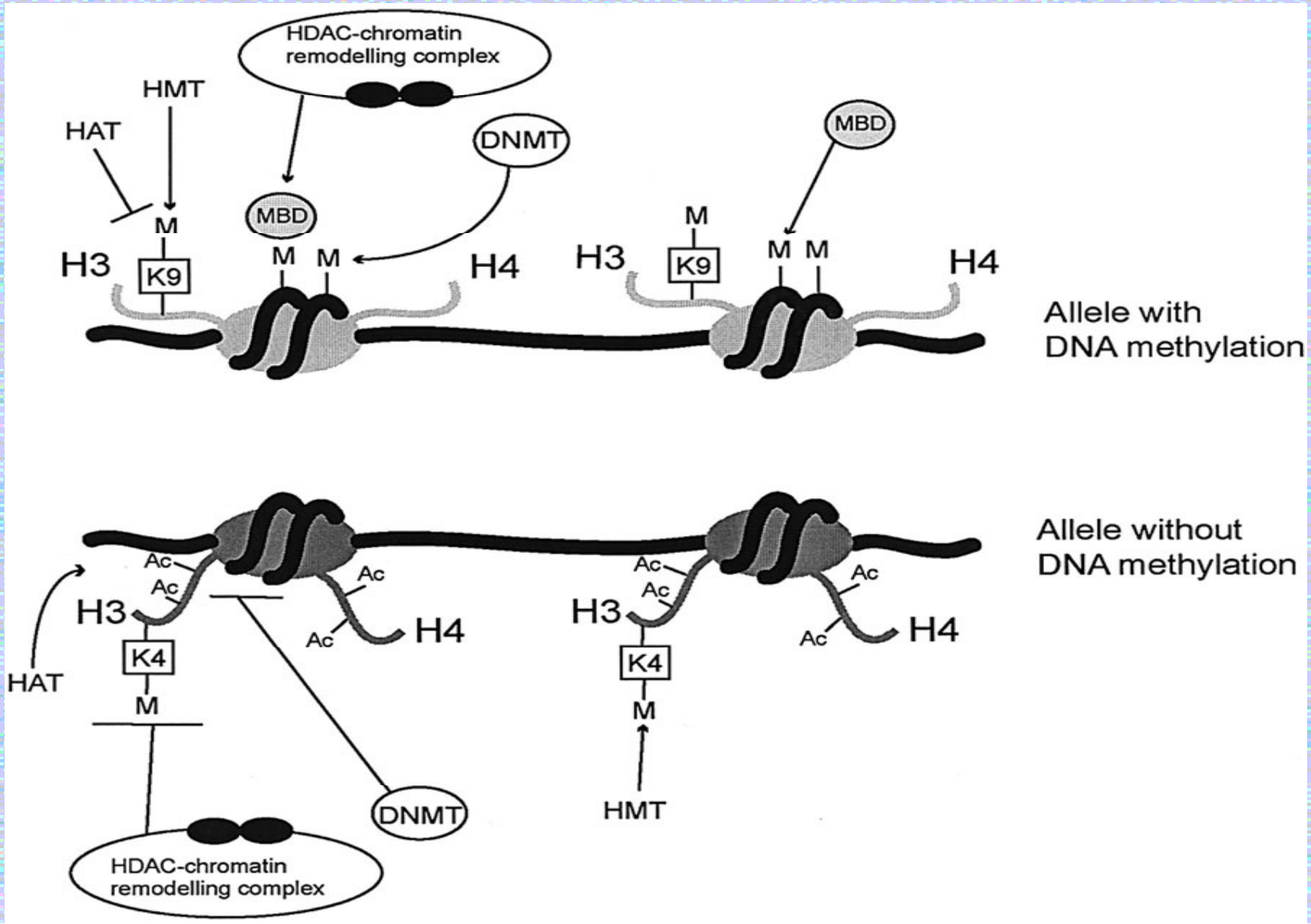
- Histone methyl transferase directs site-specific methylation of amino-acid residues such as lysine 4 & 9 in the tail of the histone H3

- Methylation of lysine 9 in histone H3 directs the binding of non-coding RNA, histone deacetylase to control chromatin structure and gene expression

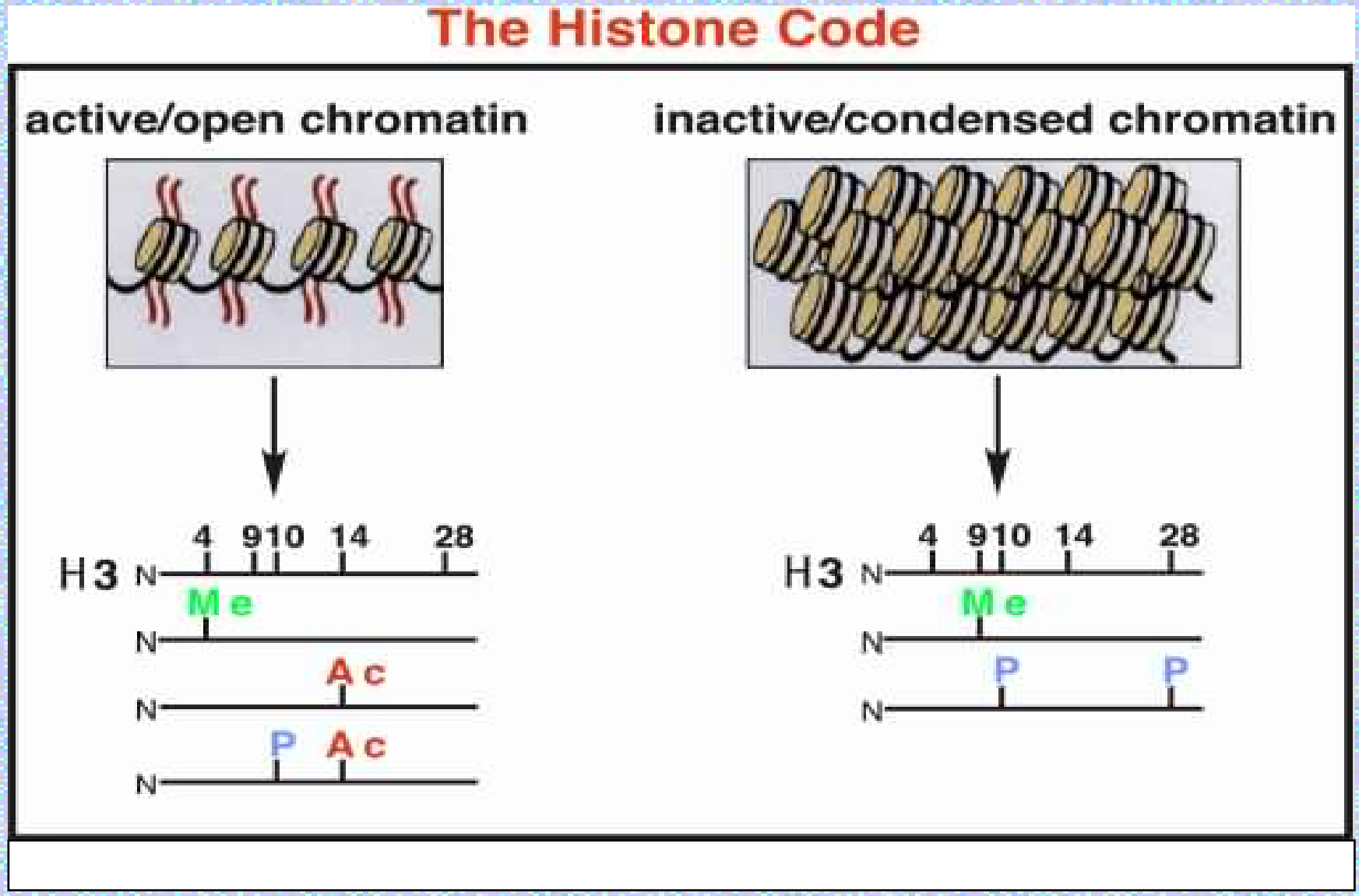
Histone H3-K9 methylation induces DNA methylation

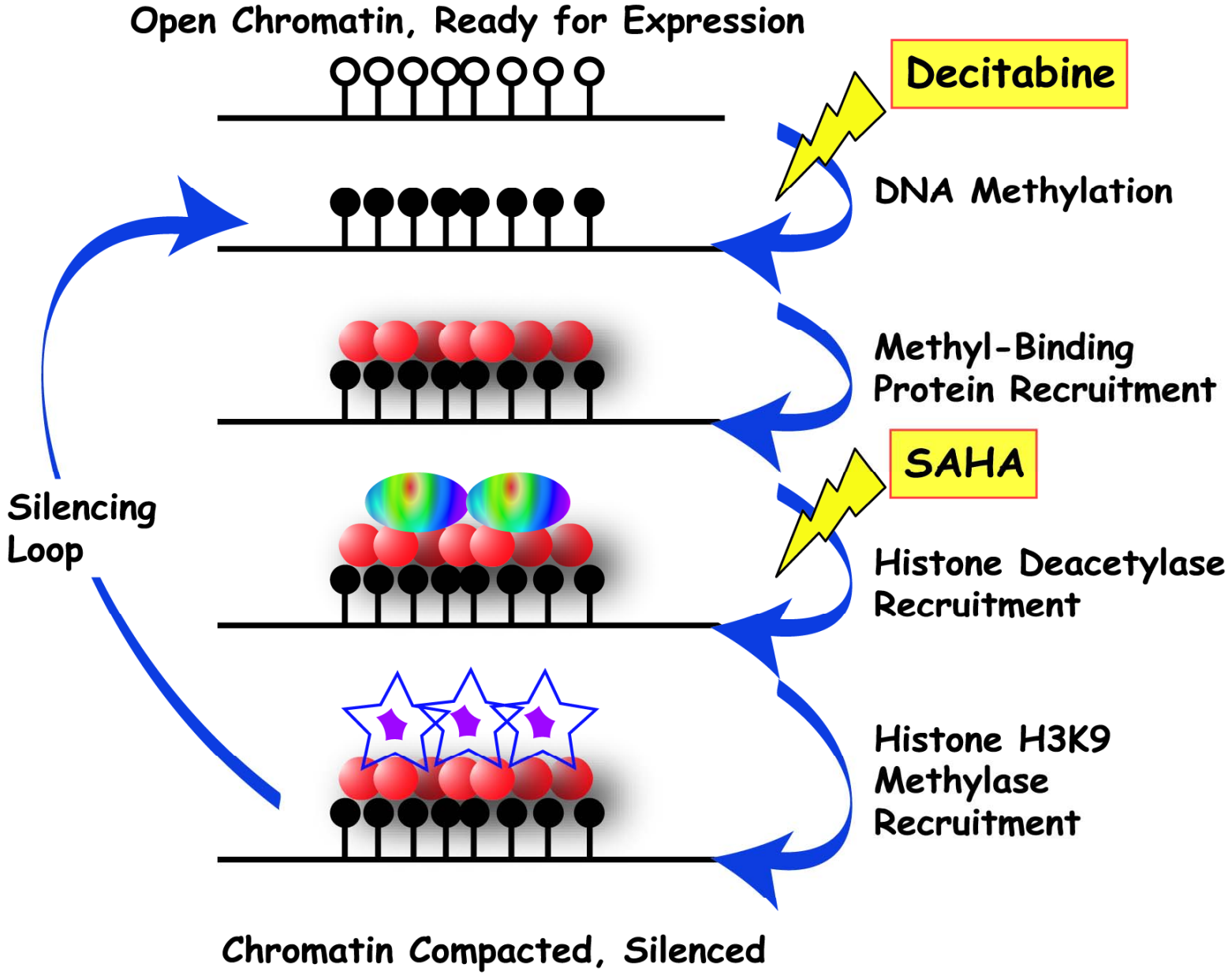


Relationship Between DNA Methylation & Histone Modification



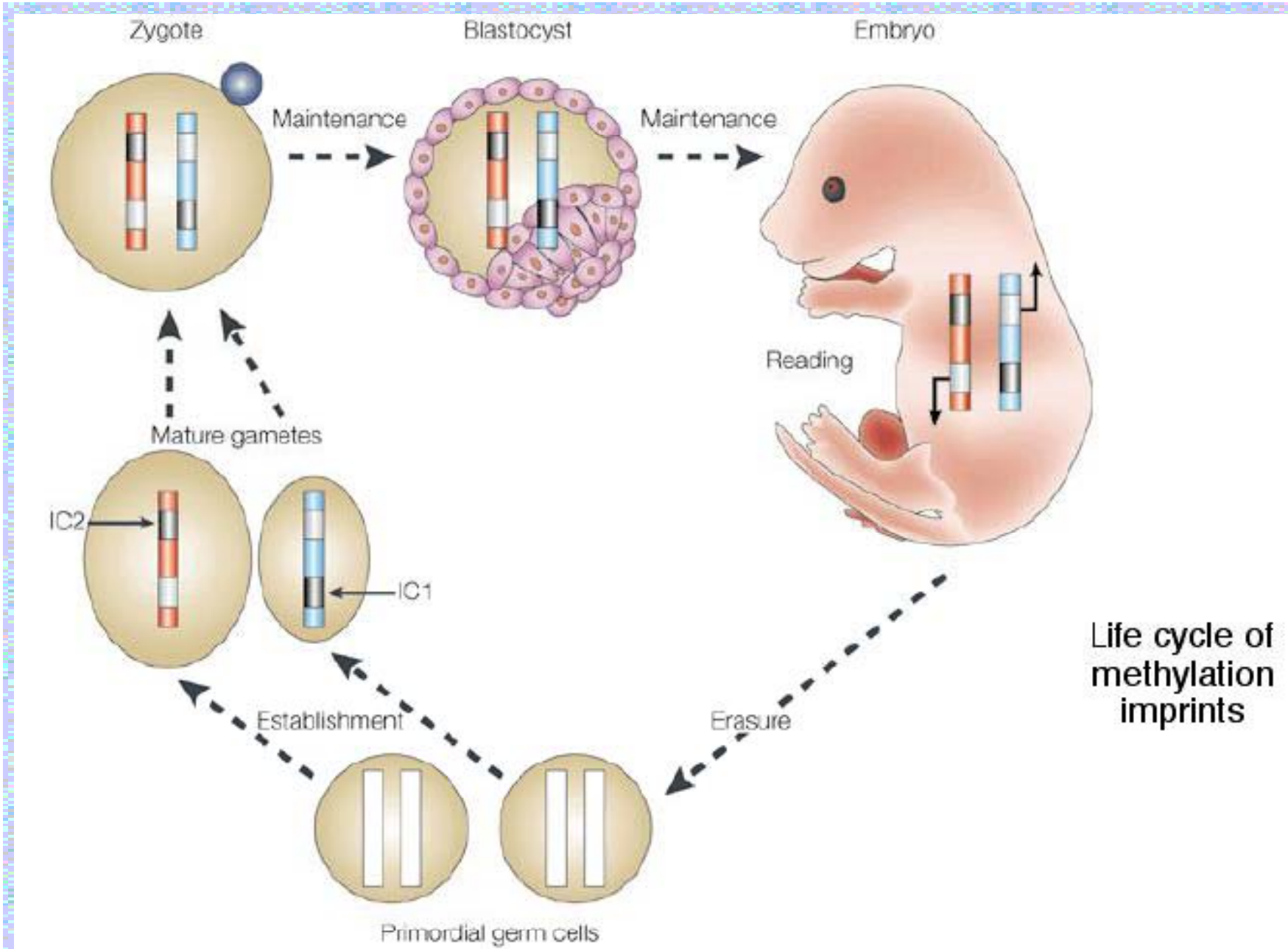
Relationship Between Histone Modification & Chromatin Structure



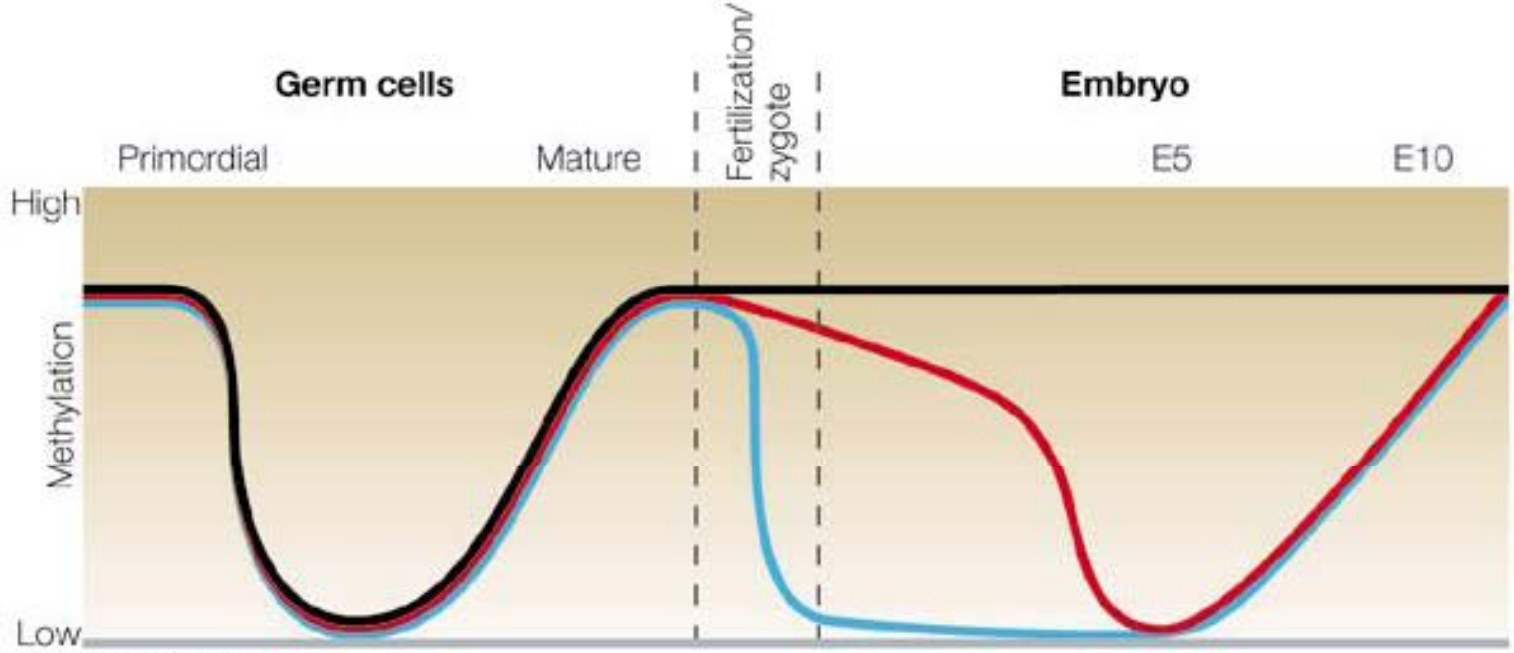

Open Chromatin, Ready for Expression
Decitabine
DNA Methylation
Methyl-Binding
Protein Recruitment
SAHA
Histone Deacetylase
Recruitment
Histone H3K9
Methylase
Recruitment
Chromatin Compacted, Silenced
Silencing
Loop

Genome Imprinting

- Parents have enzymes which add methyl groups to gamete's genes
- Methylation of DNA messes up the grooves to which the regulatory proteins bind
- Regulatory proteins usually have domains which fit into the smooth double-helix grooves. But adding methyl (CH3) puts bumps in the grooves
- So imprinting affects gene expression during embryo development, may be even for many generations


Zygote
Blastocyst
Embryo
Maintenance
Mairtenance
Reading
Mature gametes
IC2
IC1
Establishment
Erasure
Primordial germ cells
Life cycle of methylation imprints
Nature Reviews | Genetics

Methylation reprogramming in the germline and embryo



Black - methylated

Gray - non-methylated

Red - maternal

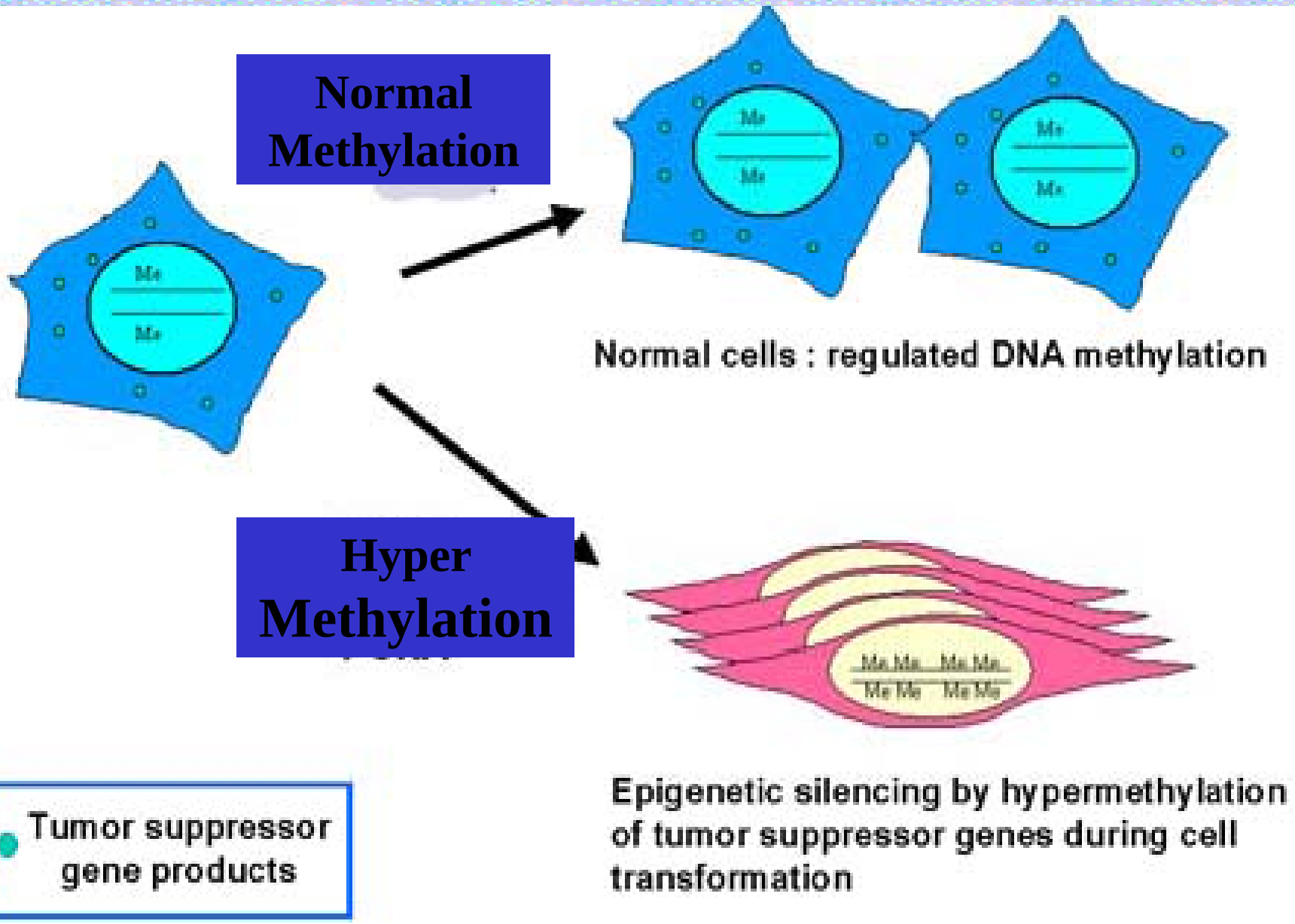
Blue - paternal

Nature Reviews | Genetics

Reprogramming & Cloning

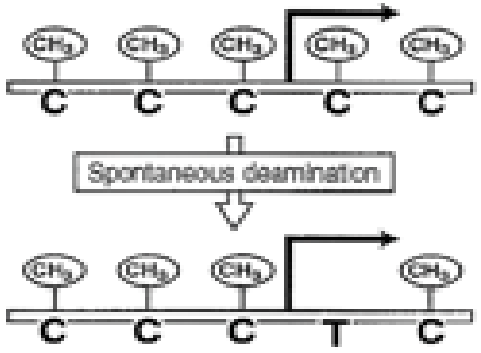
- Imprinting methylation are reprogrammed during germ cell development
- Methylation patterns in imprinted genes are unstable in embryonic stem cells and instability retained in cloned embryos leading to developmental problems
- In cloned morulae and blastocytes, levels of DNA methylation is too high (like in somatic cells) particularly in trophectopderm cells (normally undermethylated)
- High level of downregulation of gene expression in placentae

Hypermethylation & Cancer



Hypermethylation & Cancer

1. Oncogenic point mutations



Example: *p 53* gene

2. Activation of proto-oncogenes

a. Silencing by methylation

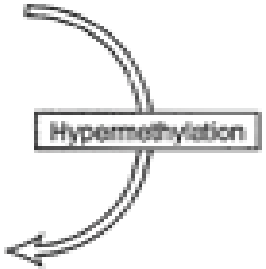
b. Active transcription

Examples: *bcl-2* in CLL, *K-ras* in lung and colon cancer

3. Inactivation of tumor-suppresor genes

a. Normal active transcription of tumor suppressor

b. Silencing of transcription by methylation



Examples: *Rb* in retinoblastoma, *VHL* in renal carcinoma, *p16* in many solid tumors, *p15* in acute leukemia, myeloma

4. Chromosomal instability due to failure of DNA methylation

a. Normal methylation

b. Loss of *de novo* methylase activity

methylated CpG: 

unmethylated CpG: 

The Future of Epigenomics

- Epigenetic therapy
  - Reactivation of methylated or silenced tumor suppressor or mutator genes
- Application in cloning and stem cell therapy in manipulating reprogramming processes


THANK YOU