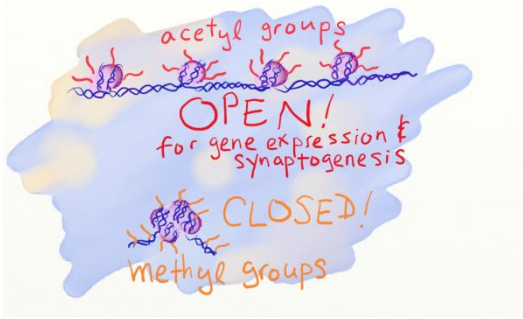
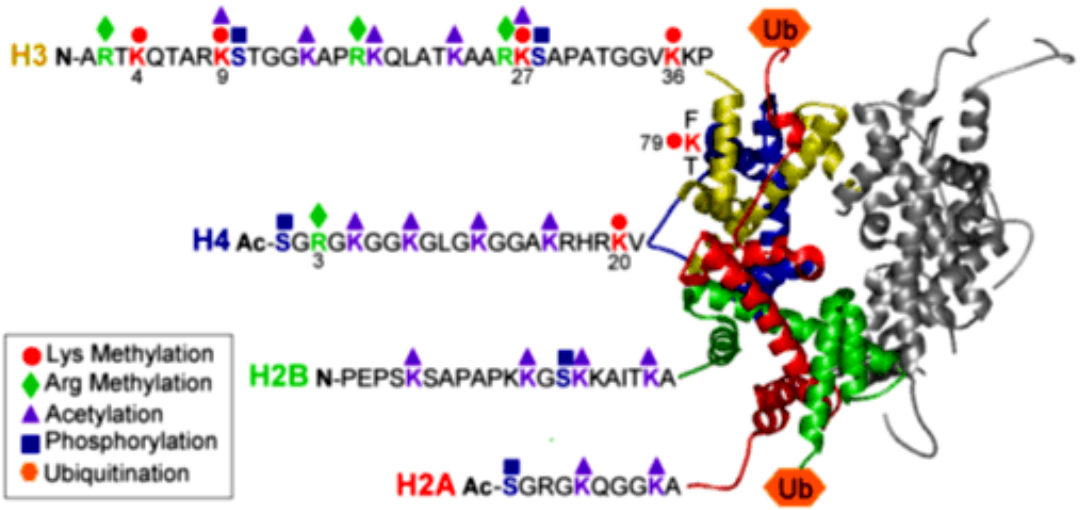


Histone modifications, DNA methylation and chromosome condensation



Dr.Rasime Kalkan

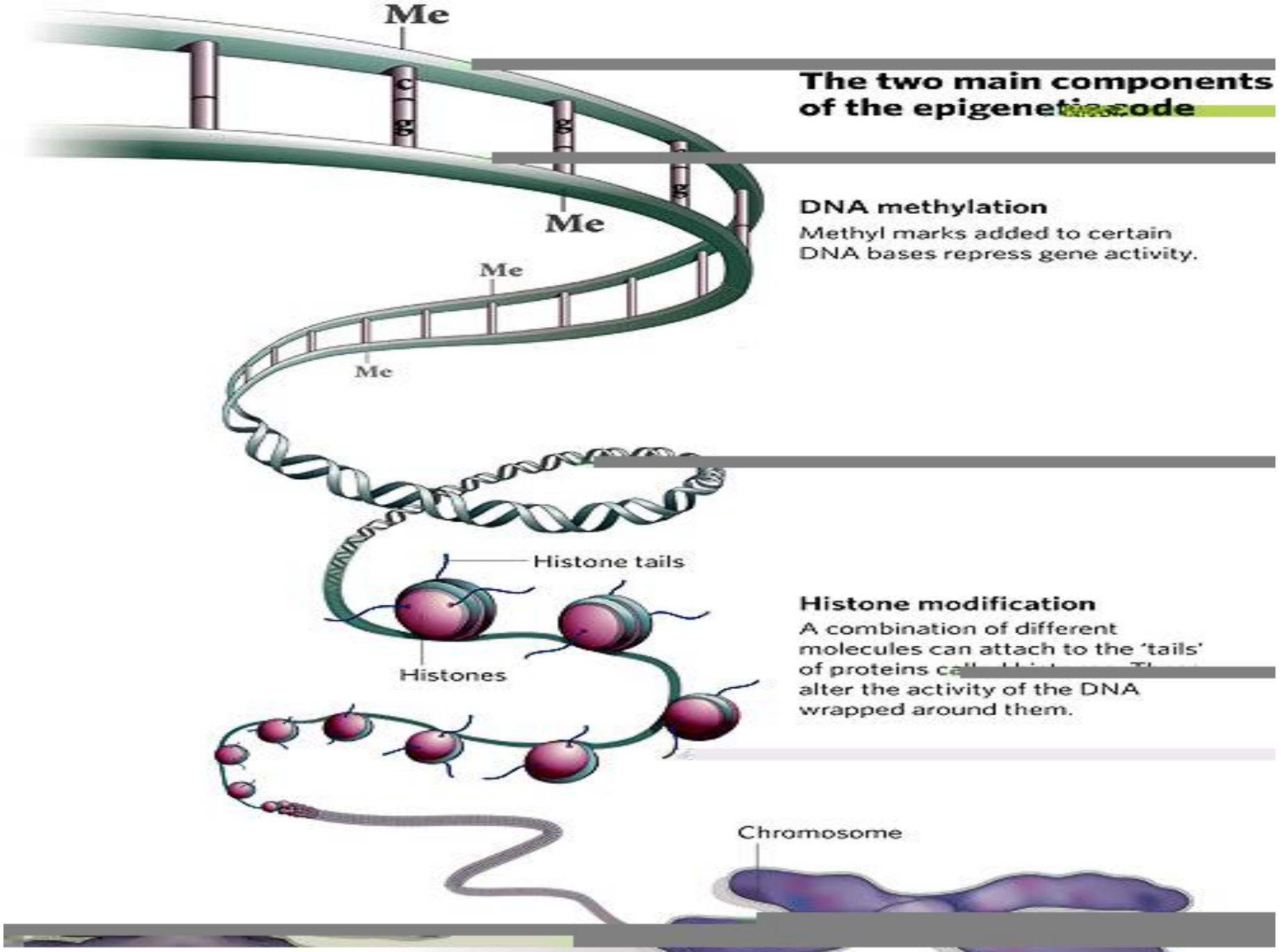
Outline

1. **Overview of histone modifications:**
    a. Types of modifications and modifiers
    b. General roles of modifications

2. **Specific modifications (acetylation, methylation, etc):**
    a. Residues/positions that are frequently modified
    b. Enzymes that add/remove the modification
    c. Biological roles
3. **Chromatin Modification - DNA Methylation**

4. **Epigenetics**

5. **Summary**



The two main components of the epigenetic code

DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

Histone modification

A combination of different molecules can attach to the 'tails' of proteins ca… alter the activity of the DNA wrapped around them.

Cowperthwaite MC, Economo EP, Harcombe WR, Miller EL, Meyers LA (2008) The Ascent of the Abundant: How Mutational Networks Constrain Evolution. PLoS Comput Biol 4(7): e1000110. doi:10.1371/journal.pcbi.1000110

Types of histone modifications

Chromatin Modifications	Functions Regulated
Acetylation	Transcription, Repair, Replication, Condensation
Methylation (lysines)	Transcription, Repair
Methylation (arginines)	Transcription
Phosphorylation	Transcription, Repair, Condensation
Ubiquitylation	Transcription, Repair
Sumoylation	Transcription
ADP ribosylation	Transcription
Deimination	Transcription
Proline Isomerization	Transcription

Post-translational modifications on histone proteins alter chromatin structure and, consequently, chromatin function

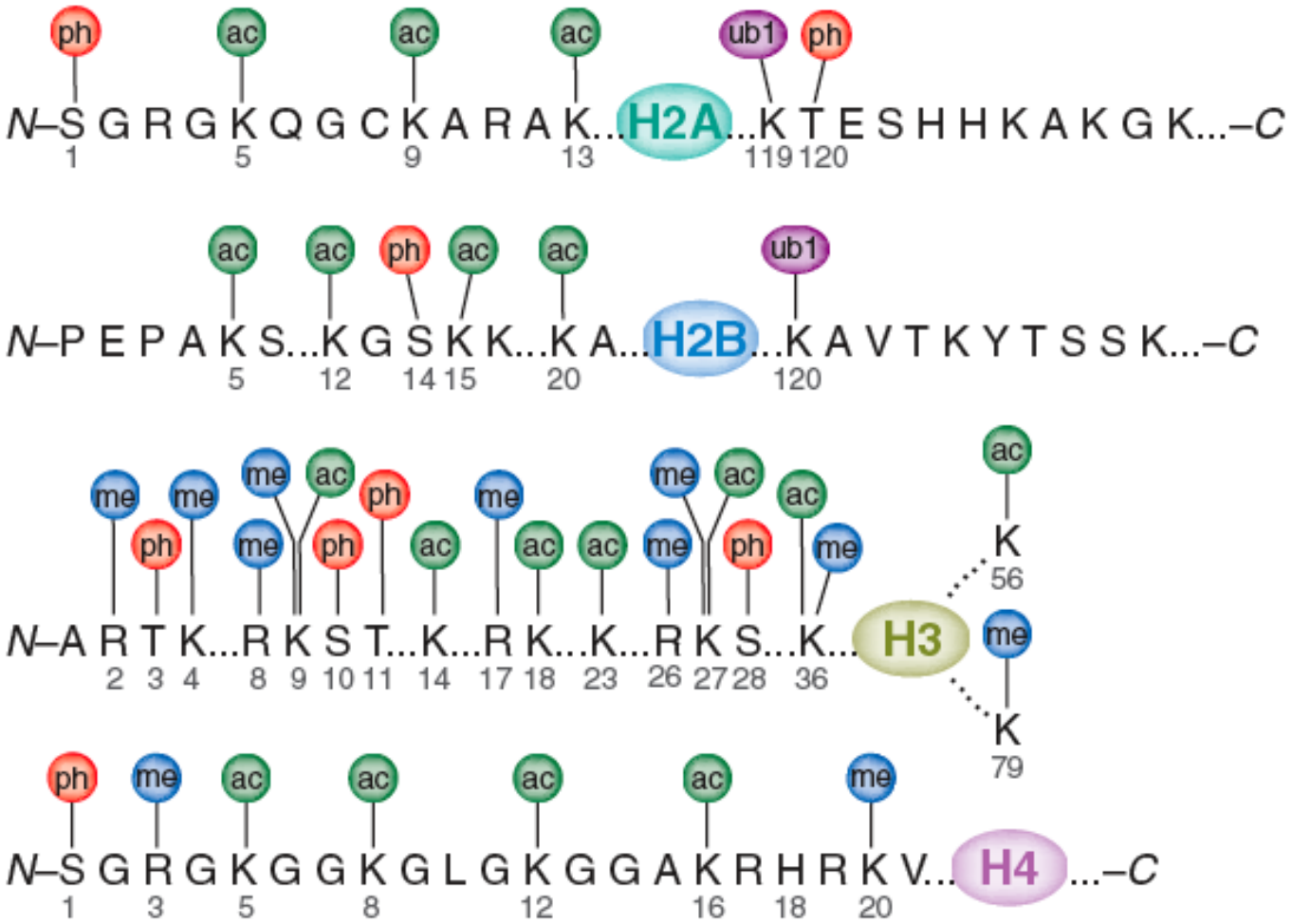
Histone Modifications

- De/Acetylation
- Methylation
- Phosphorylation
- Ubiquitination
- ADP-Rybosilation
- Swi/Snf complex, which, *in vitro*, uses the energy of ATP hydrolysis to disrupt histone-DNA interactions

Histone Modifications - Role

- Transcription – Acetylation/Methylation
- DNA repair – H2A -Phosphorilation
- Mitosis – chromosomal arrangement
- Chromatin assembly – DNA replication

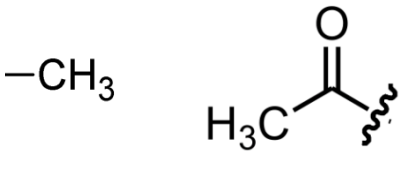
Types of Histone Modifications



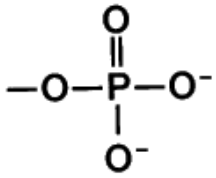
Bhaumik, Smith, and Shilatifard, 2007.

Features of Histone Modifications

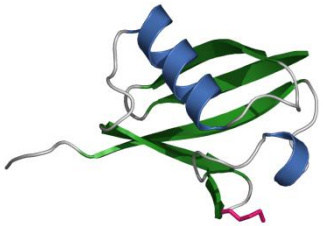
- Covalently attached groups (usually to histone tails)



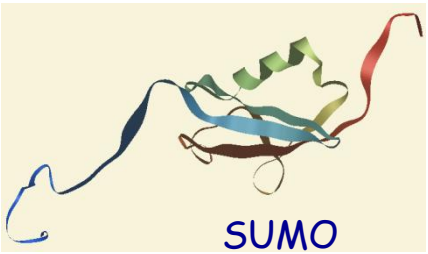
Methyl Acetyl



Phospho



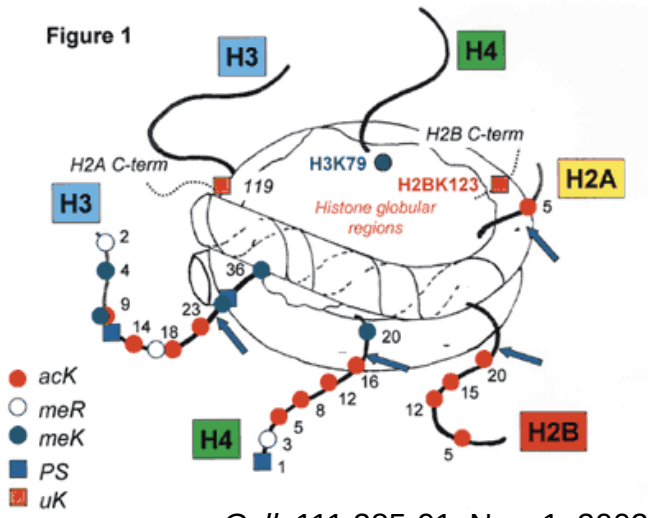
Ubiquitin



SUMO

- Reversible and Dynamic
  - Enzymes that add/remove modification

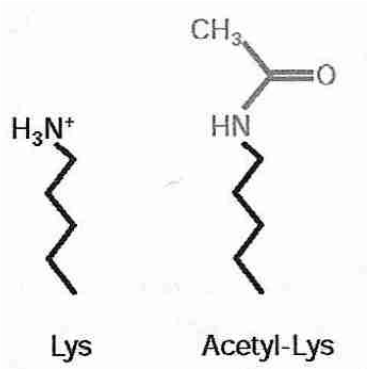
- Have diverse biological functions

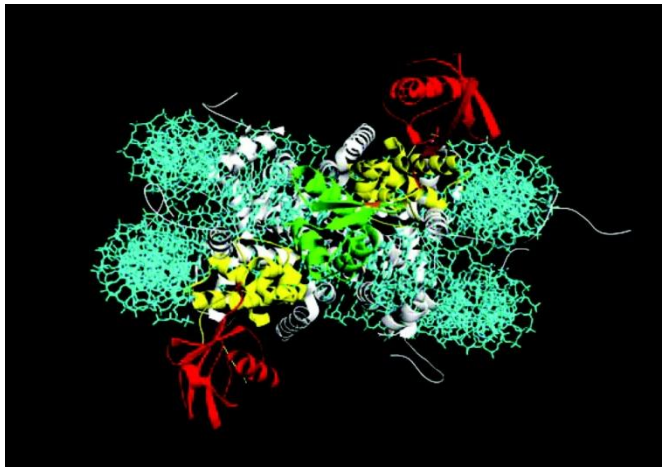


Cell, 111:285-91, Nov. 1, 2002

Features of Histone Modifications

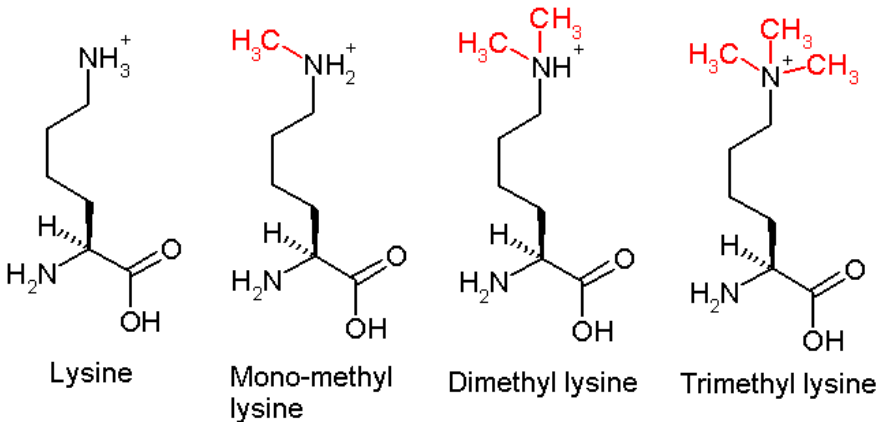
- Small vs. Large groups





Ub = ~8.5 kDa
H4 = 14 kDa

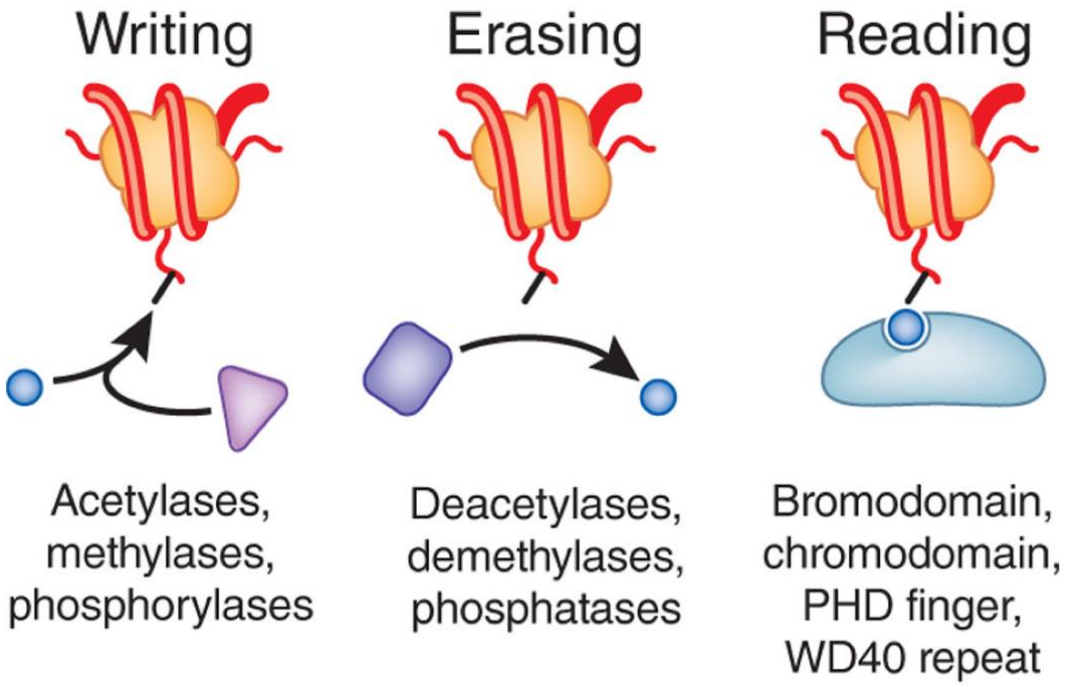
- One or up to three groups per residue



Jason L J M et al. J. Biol. Chem. 2005

Histone Modifications and Modifers

- **Writers**: enzymes that add a mark
- **Readers**: proteins that bind to and "interpret" the mark
- **Erasers**: enzymes that remove a mark



Tarakhovsky, A., Nature Immunology, 2010.

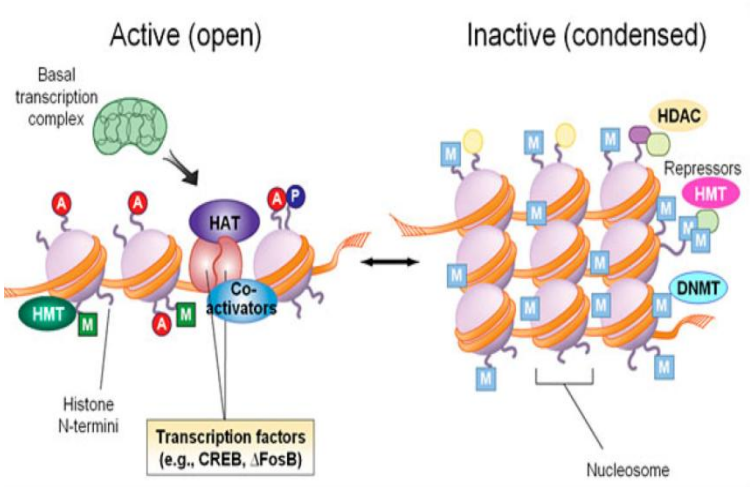
Histone Modifications and Modifers

Residue	Modification	Modiying Enzyme
Lysine	Acetylation Deacetylation	HAT HDAC
Lysine	Methylation Demethylation	HMT HDM
Lysine	Ubiquitylation Deubiquitylation	Ub ligase Ub protease
Serine/Threonine	Phosphorylation Dephosphorylation	Kinase Phosphatase
Arginine	Methylation Demethylation	PRMT Deiminase/Demethylase

- Others: Sumoylation (Lysine), ADP Ribosylation (Glutamate)

Histone Modifiers

- **Do not bind to DNA themselves**
  - Can be recruited by:
    - Histone modifications (through chromodomains, bromodomains, etc.)
    - Transcription factors
    - RNA (fission yeast, mammals, plants)
    - DNA damage
- **Act as transcriptional co-regulators**
- **Enhance activities of transcriptional repressors or activators**
  - Co-repressor: ex. HDACs
  - Co-activator: ex. HATs



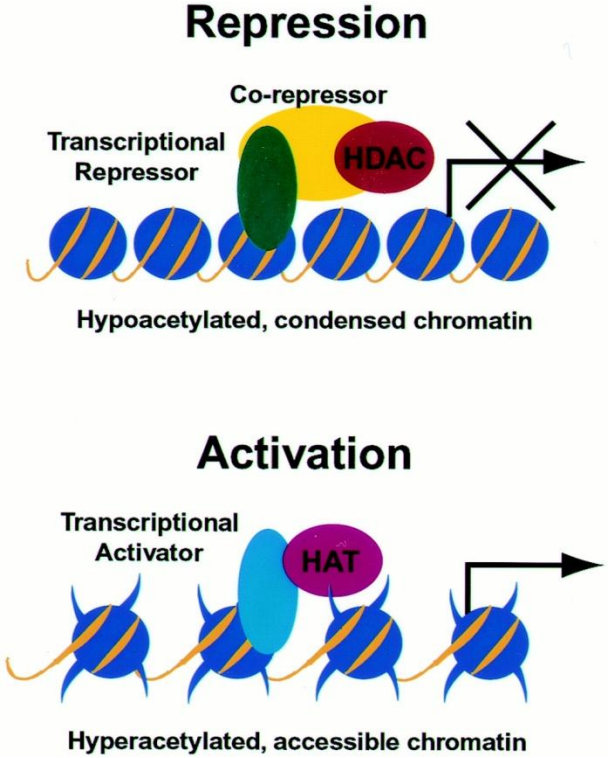
General Roles of Histone Modifications

- **Intrinsic**
  - Single nucleosome changes
- **Extrinsic**
  - Chromatin organization: nucleosome/nucleosome interactions
  - Alter chromatin packaging, electrostatic charge

Kouzarides, Cell 2007

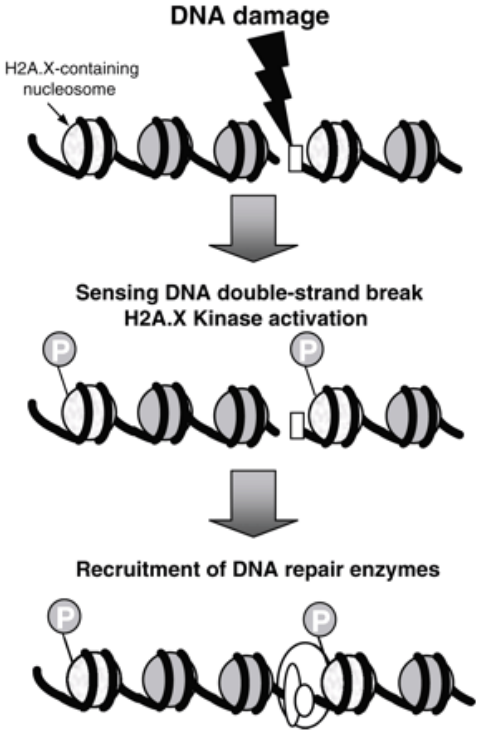
General Roles of Histone Modifications

Gene Regulation



Wade P A Hum. Mol. Genet. 2001.

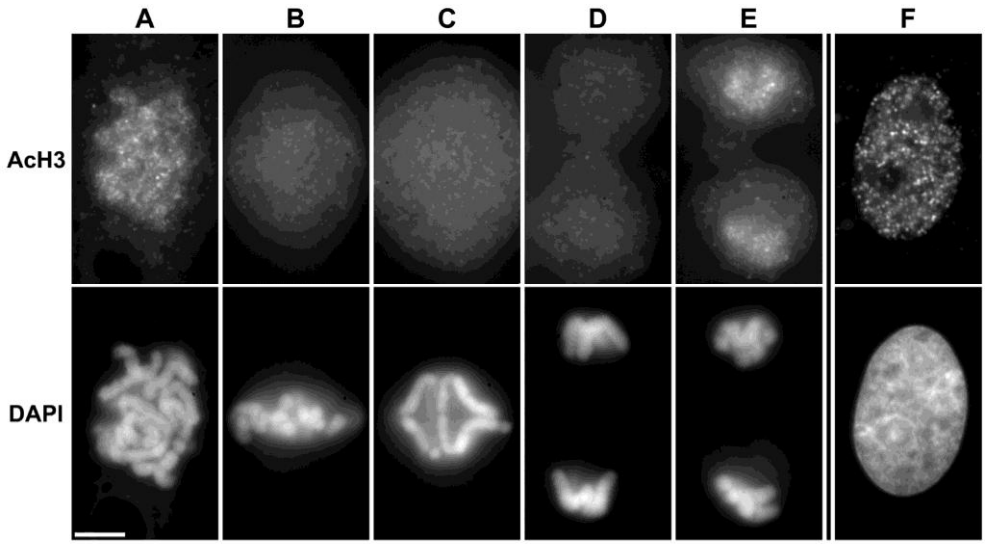
DNA Damage



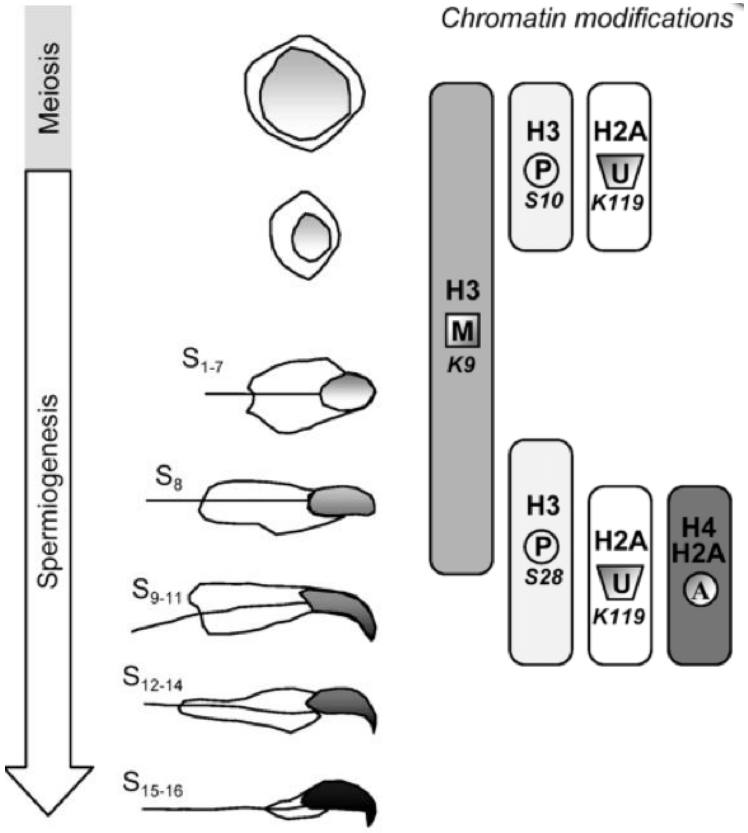
Moggs and Orphanides, Toxicological Sciences, 2004.

General Roles of Histone Modifications

Chromatin Condensation



Spermatogenesis



Kruhlak M J et al. J. Biol. Chem. 2001.

HATs vs HDACs

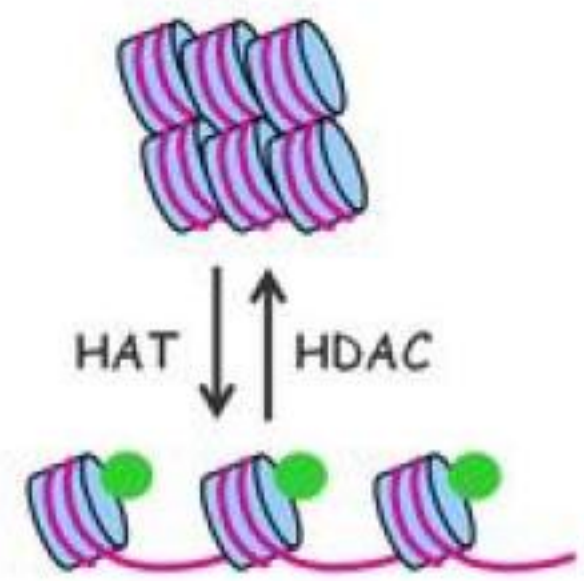
- Histone acetyl transferases (HATs)
- Histone deacetylase complexes (HDACs).

➢ methyl groups are ***added*** to lysine side chains by a set of different ***histone methyl transferases*** and removed by a set of histone demethylases

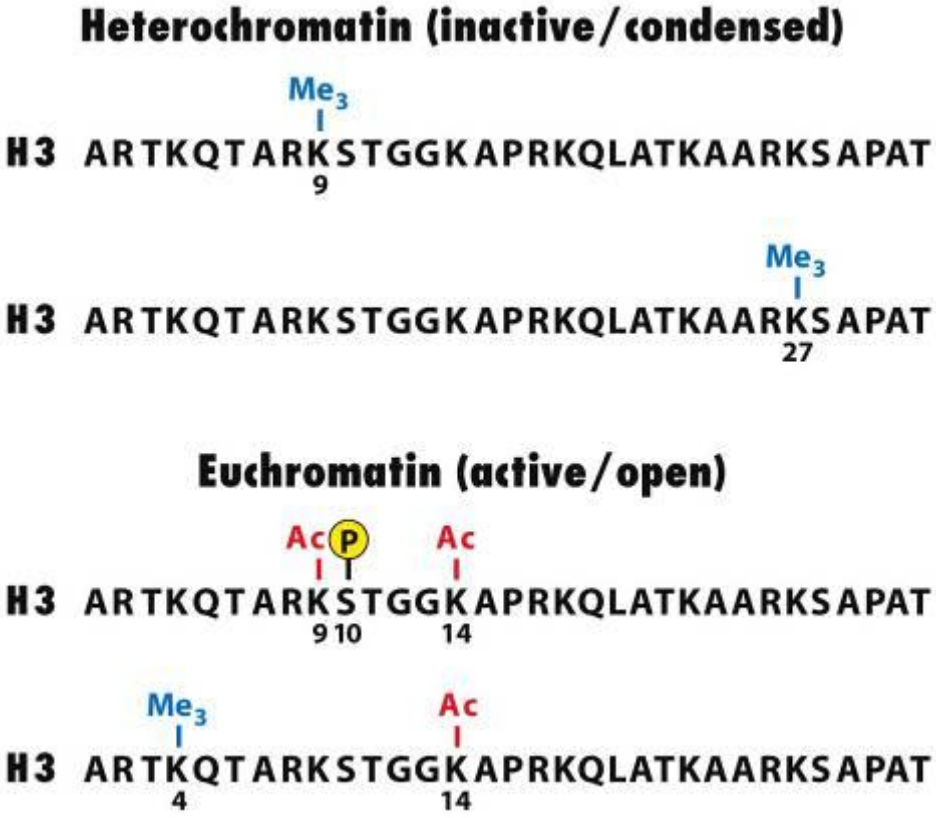
- **Hyperacetylation (high) → open nucleosome and chromatin structure → transcription activation;**
- **Hypoacetylation (low) → tight nucleosome and chromatin structure → transcription repression.**
- **A balanced acetylation level of the genome is critical to the normal function of the cell and organism**

Histone Deacetylases (HDACs)

- Multi-enzyme complexes
- Targeted by transcriptional repressors
- Deactylate histone tails



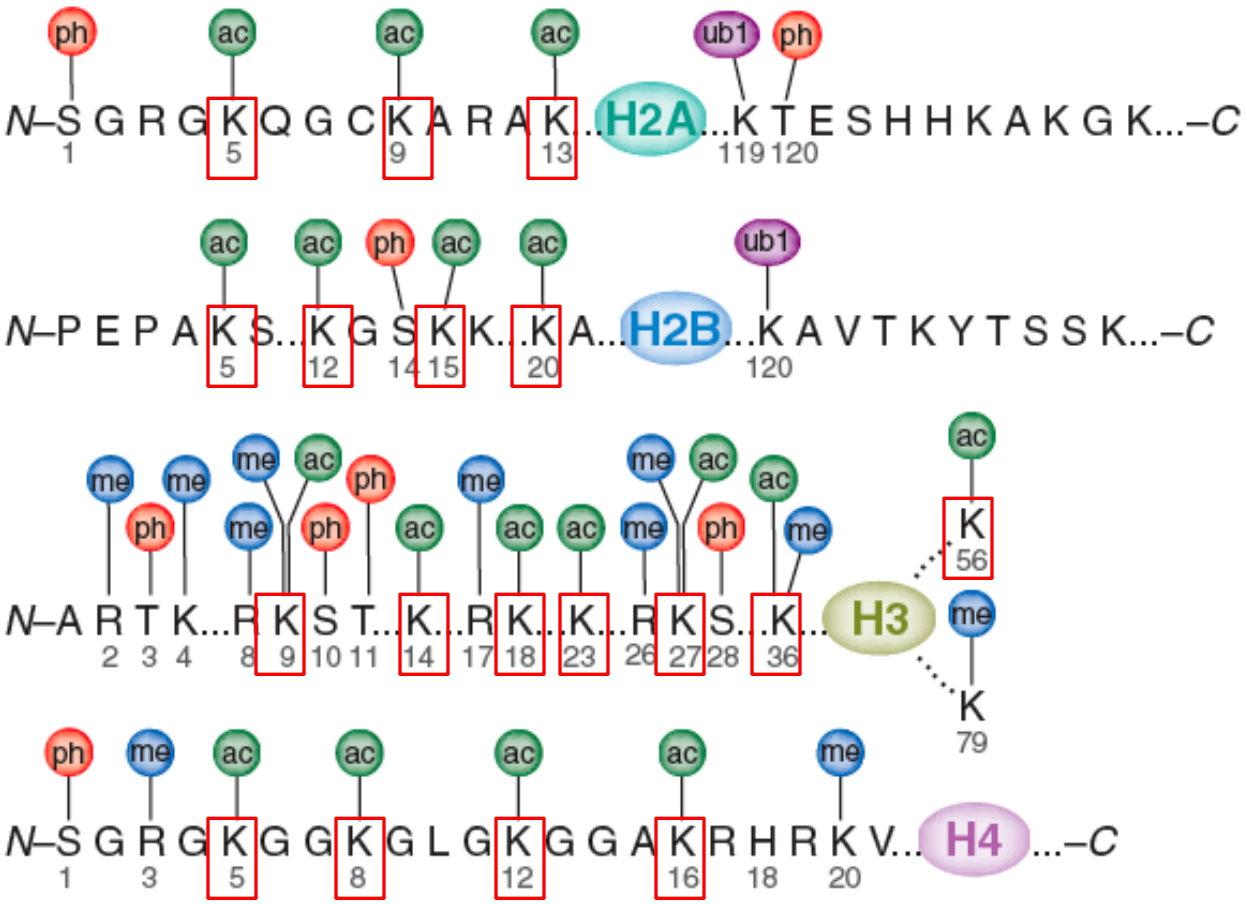
Histone Modifications Associated with Heterochromatin and Euchromatin



Bhaumik, Smith, and Shilatifard, 2007.

Specific Histone Modifications

Lysine Acetylation

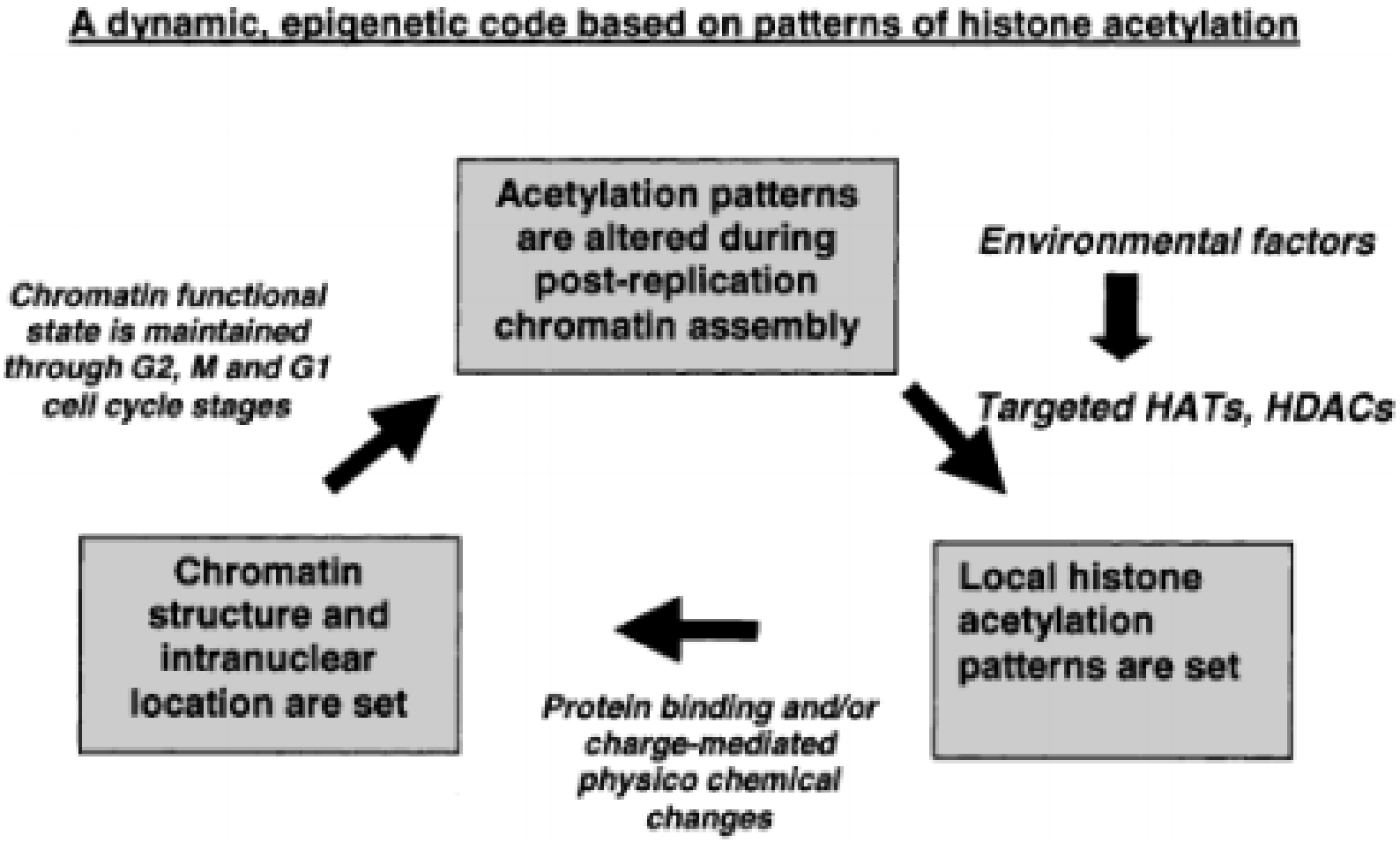


Bhaumik, Smith, and Shilatifard, 2007

Acetylation

- **Many lysine residues can be acetylated**
  - mainly on histone tails (sometimes in core)
- **Can be part of large acetylation domains**
- **Modifying enzymes:**
  - often multi-enzyme complexes
  - can modify multiple residues
- **Well correlated with transcriptional activation**
- **Other roles (chromatin assembly, DNA repair, etc.)**
- HATs catalyze the transfer of an acetyş group to the amino group of lysine. Lysine's positive charge and the this action has the potential to weaken the interactions between histones and DNA.

A simple model summarising how patterns of histone acetylation may be involved in the regulation of chromatin structure and function through the cell cycle



Histone acetylation and an epigenetic code Bryan M. Turner, BioEssays 22:836±845, ß 2000 John Wiley & Sons, Inc

Acetylation mechanism

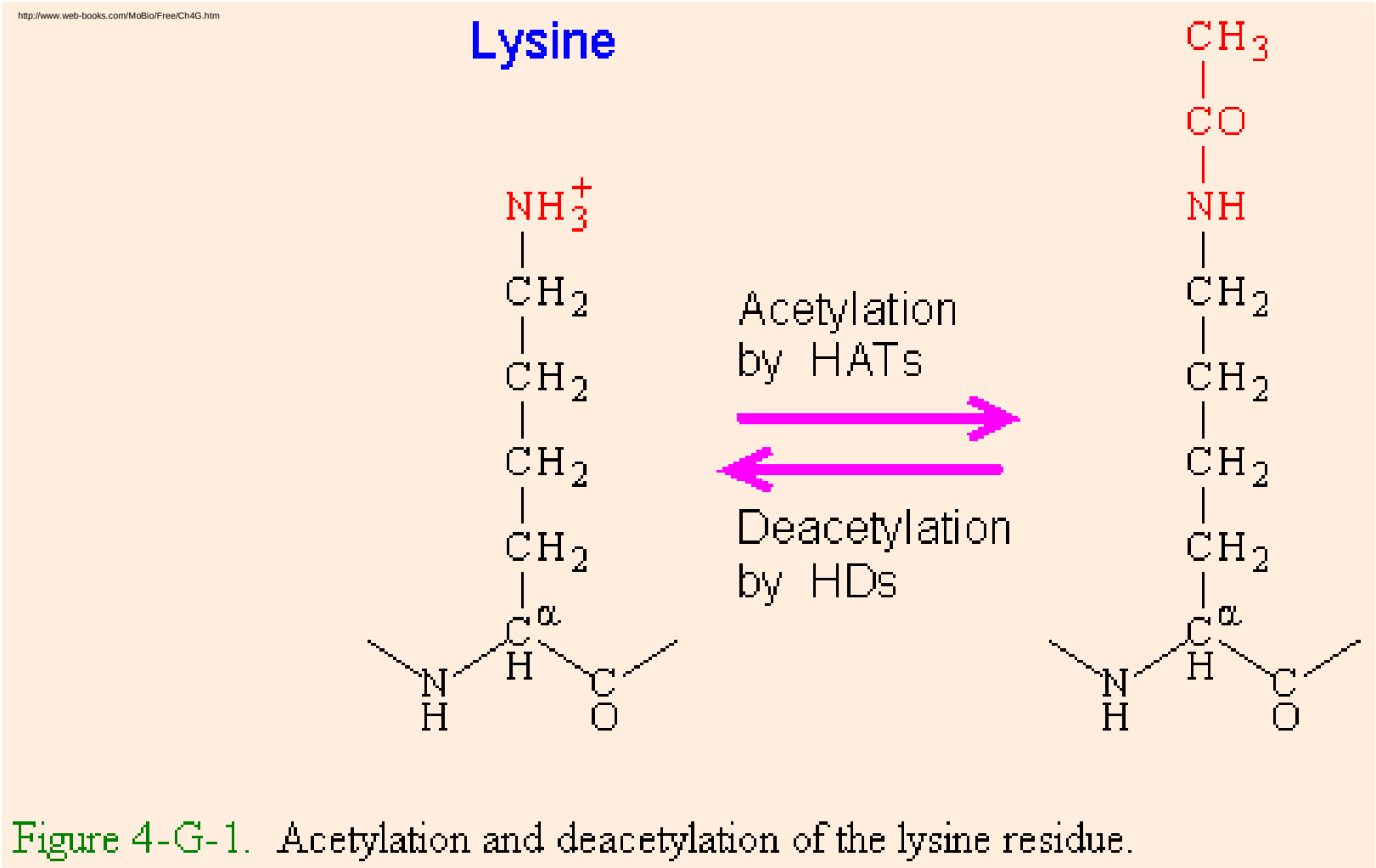
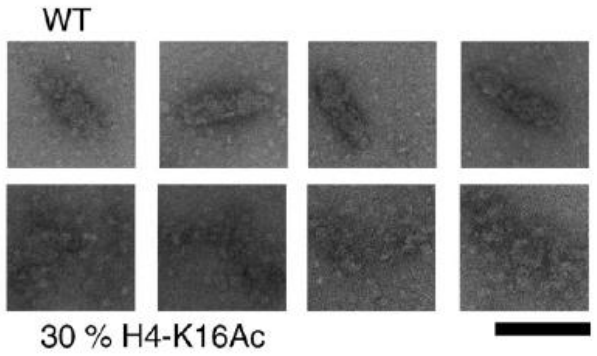


Figure 4-G-1. Acetylation and deacetylation of the lysine residue.

Histone acetylation reduce the positive charge of histones and disrupt electorstatic interactions between histones and DNA.

Roles of Acetylation

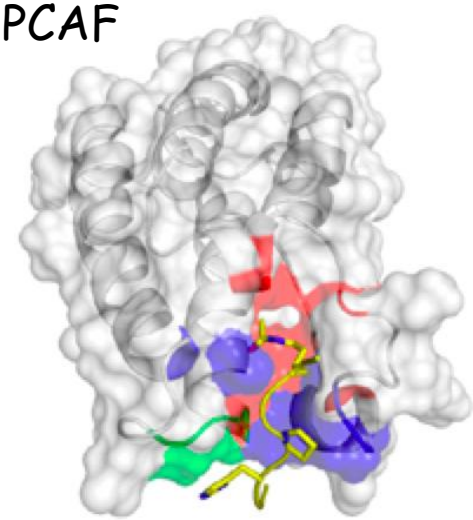
1. **Opens up chromatin:**
   - Reduces charge interactions of histones with DNA (K has a positive charge)
   - Prevents chromatin compaction (H4K16ac prevents 30nm fiber formation)
   - Causes less compact chromatin structure, facilitating DNA access by protein machinery such as transcription..



Robinson et al., J. Mol. Biol., 2008.

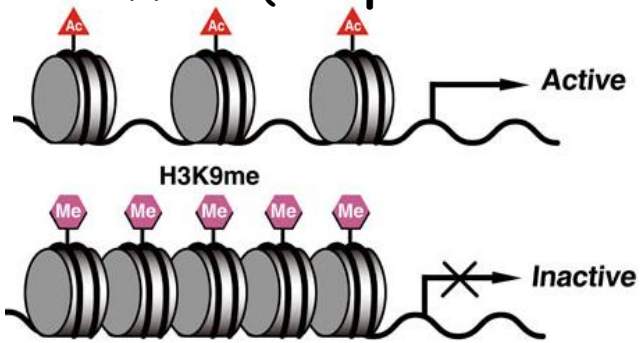
2. **Recruits chromatin proteins with bromodomains**

PCAF



Mujtaba et al., Oncogene, 2007.

3. May occur at same residues as methylation with repressive effect (**competitive antagonism**)

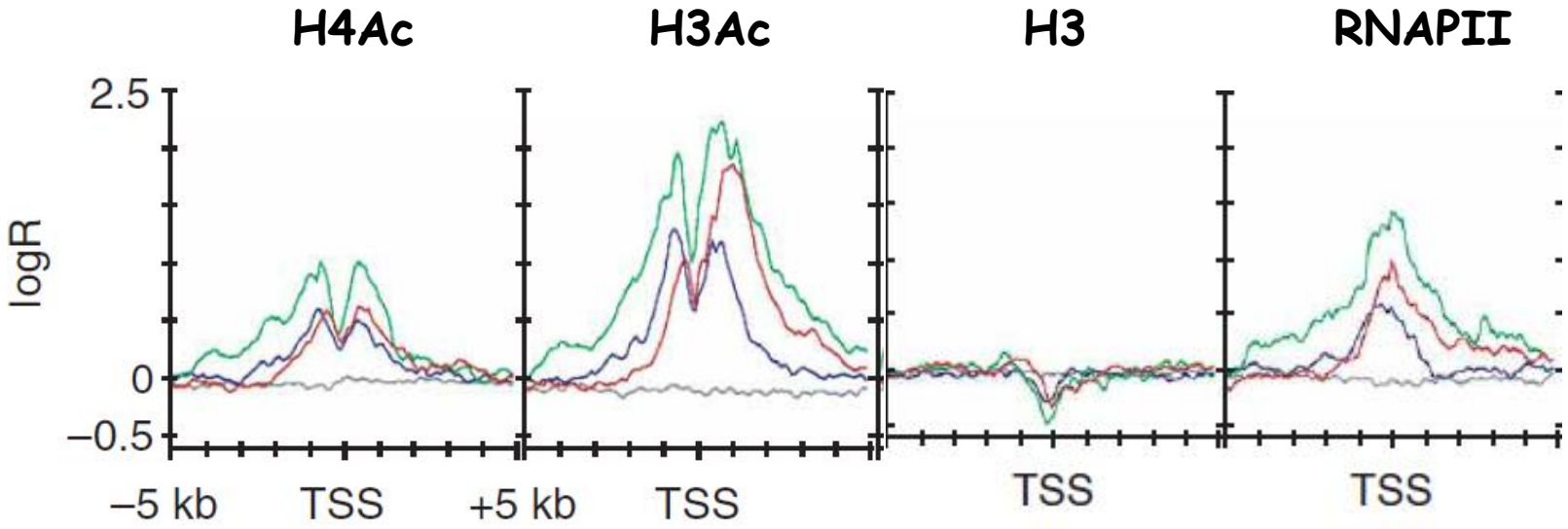


Yang and Chen, Cell Research, 2011.

Roles of Acetylation

4. **Highly correlated with active transcription**

i.e. enriched at TSS of actively transcribed genes



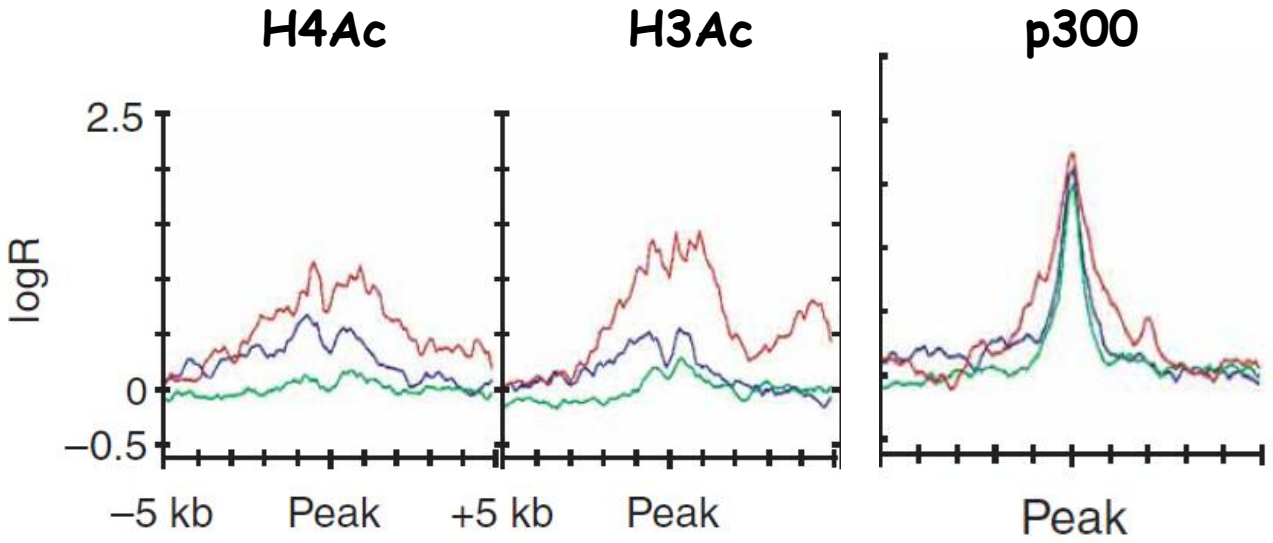
Expression:
P1<P2<P3<P4

208 TSS investigated

Heintzman N et al., Nature Genetics, 2

Roles of Acetylation

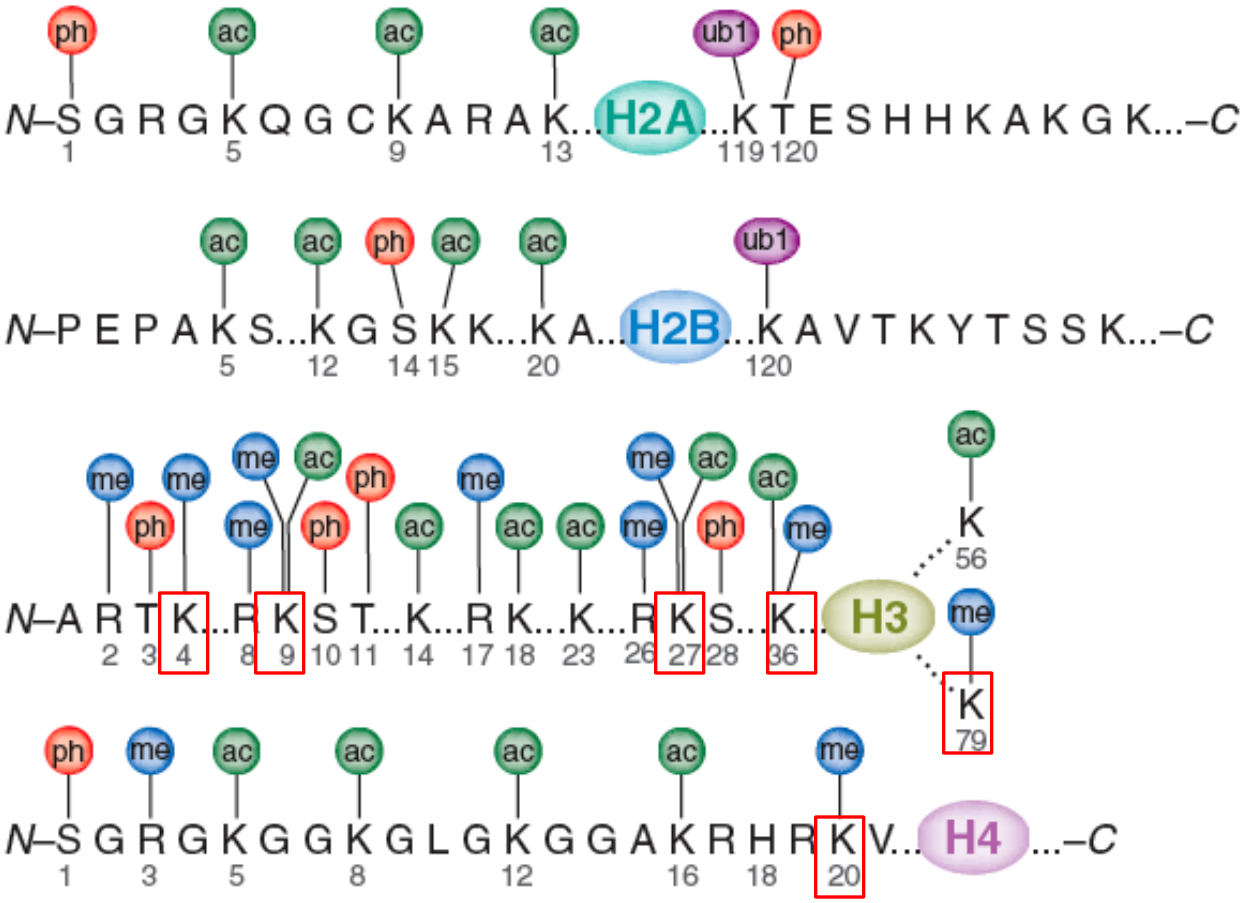
5. **Correlated with binding of activating transcription factors**
   i.e. enriched at promoters and enhancers



Expression:
E1<E2<E3
74 enhancers
(distal p300 binding sites)

Heintzman N et al., Nature Genetics, 2

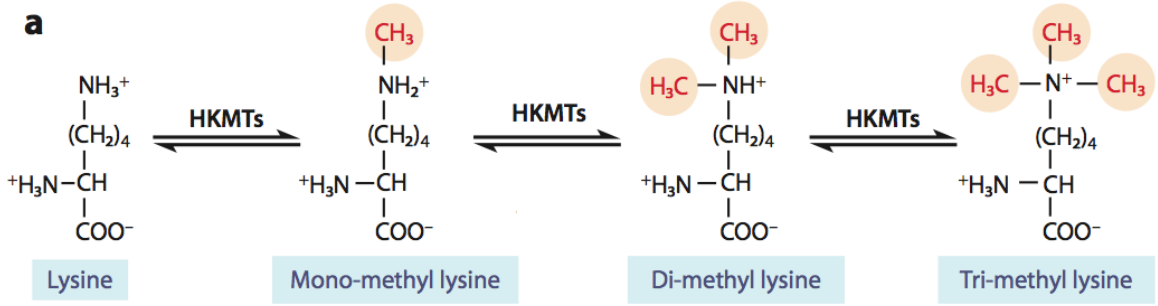
Lysine Methylation



Bhaumik, Smith, and Shilatifard, 2007.

Lysine Methylation

- **Many lysine residues can be methylated**
  - Mainly on histone tails (sometimes in core)
  - Can be mono-, di-, or tri-methylated



- **Depending on residue and number of methyl groups, can be associated with active or repressive transcription**
- **Unlike acetylation and phosphorilation, histone methlation does not alter the charge of the histone protein.**
- Other roles
  - Transcriptional elongation
  - Pericentromeric heterochromatin
  - X chromosome inactivation

Liu et. al, Annu. Rev. Plant Biol., 2010

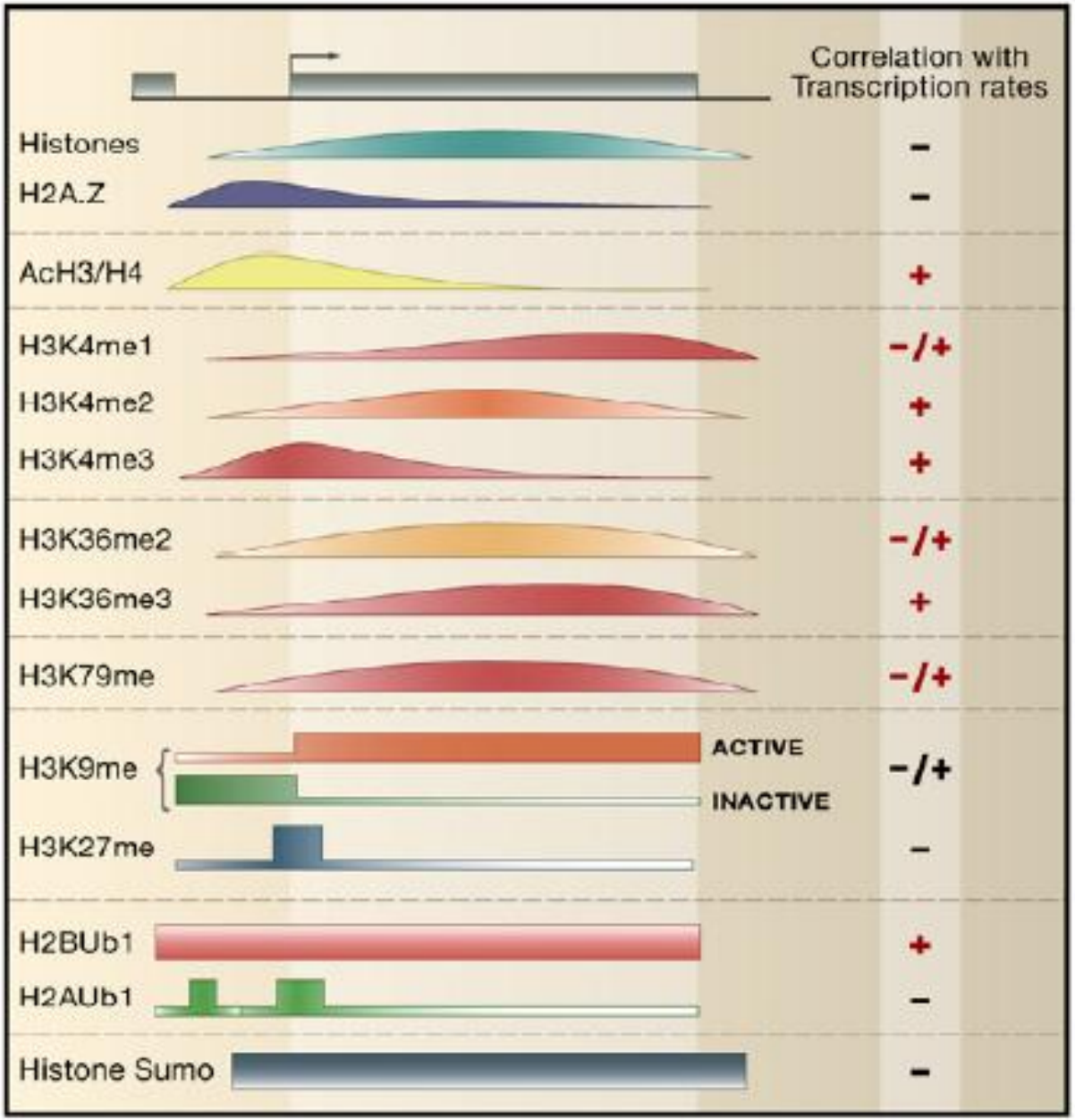
Other Histone Modifications

Table 1. Different Classes of Modifications Identified on Histones

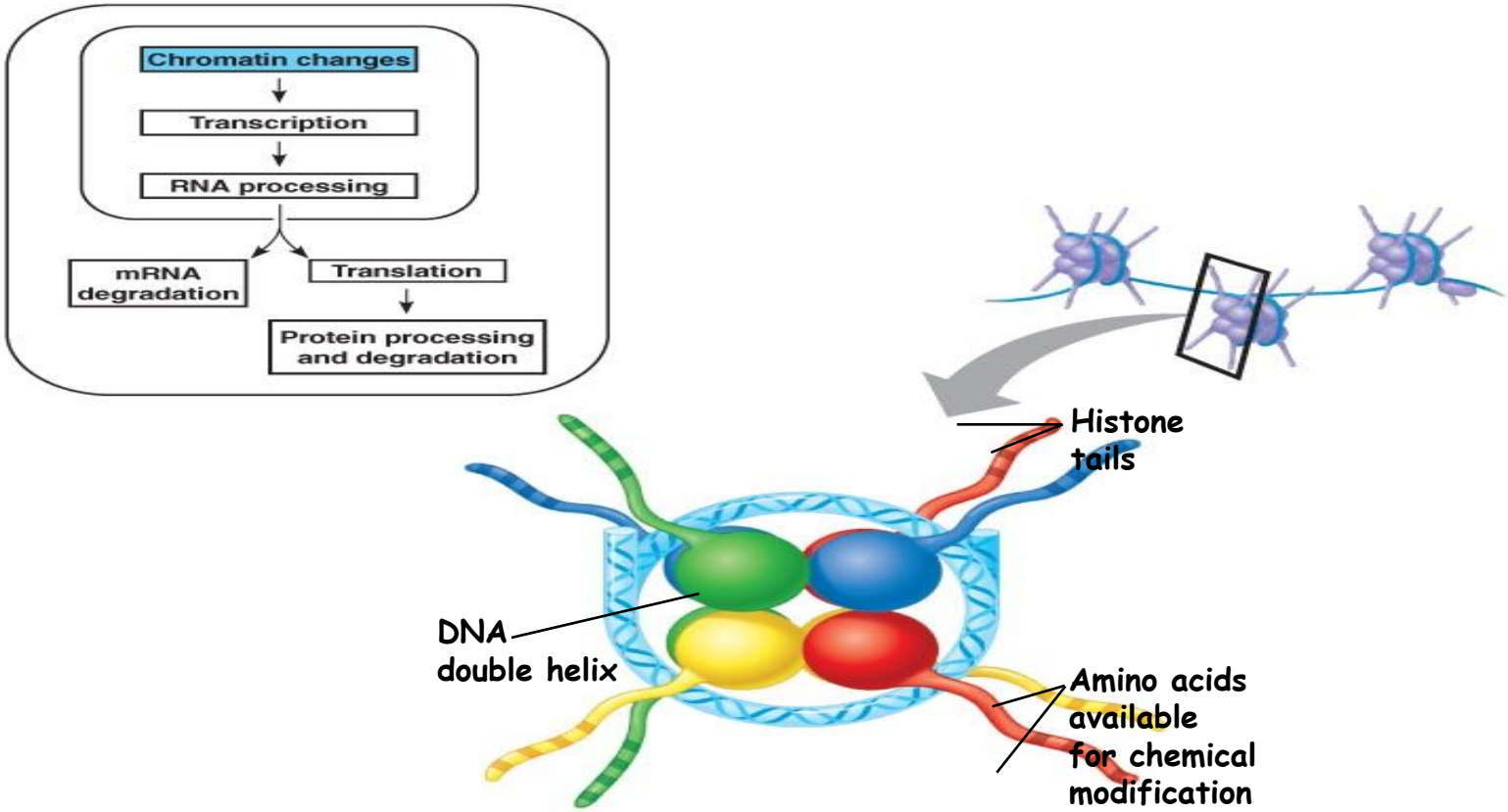
Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **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

Kouzarides, Cell, 200

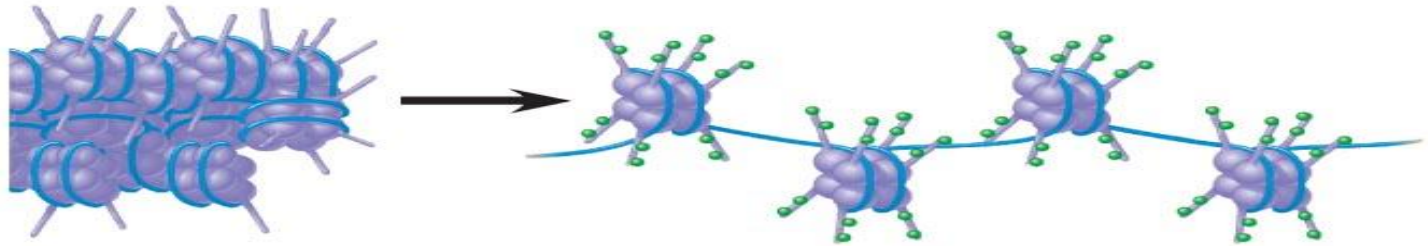
Histone Modifications in Relation to Gene Transcription



Li e. al. (2007)
Cell 128, 707



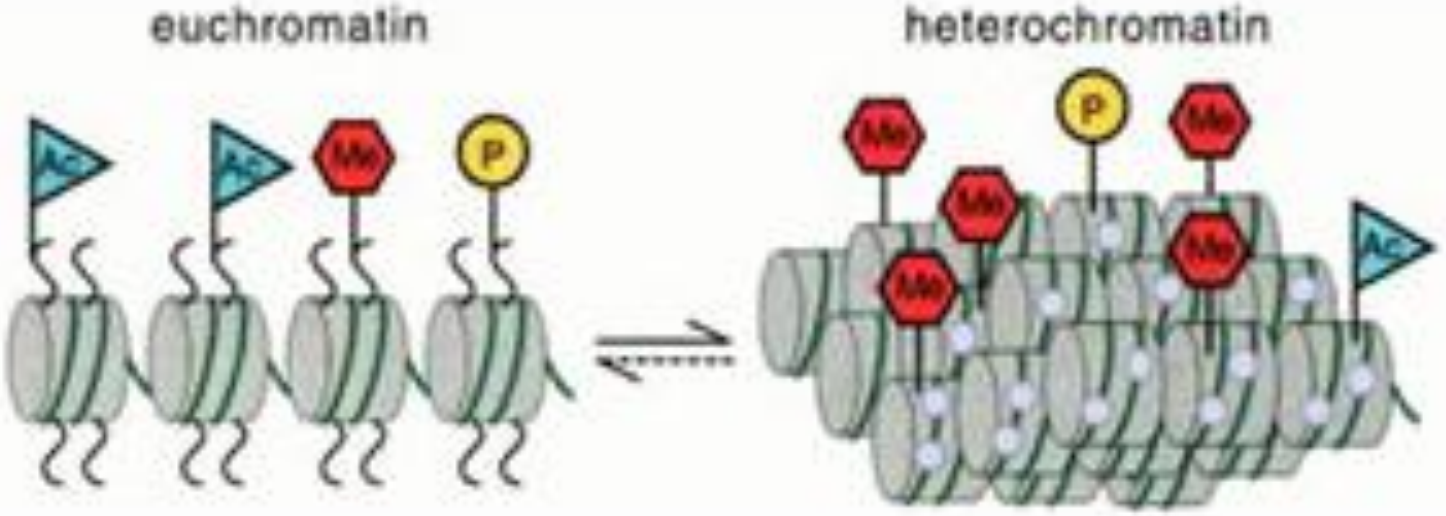
(a) Histone tails protrude outward from a nucleosome



Unacetylated histones Acetylated histones

(b) Acetylation of histone tails promotes loose chromatin structure that permits transcription

DNA Methylation and Histone Modifications help to compartmentalize the genome into domains of different transcriptional potentials



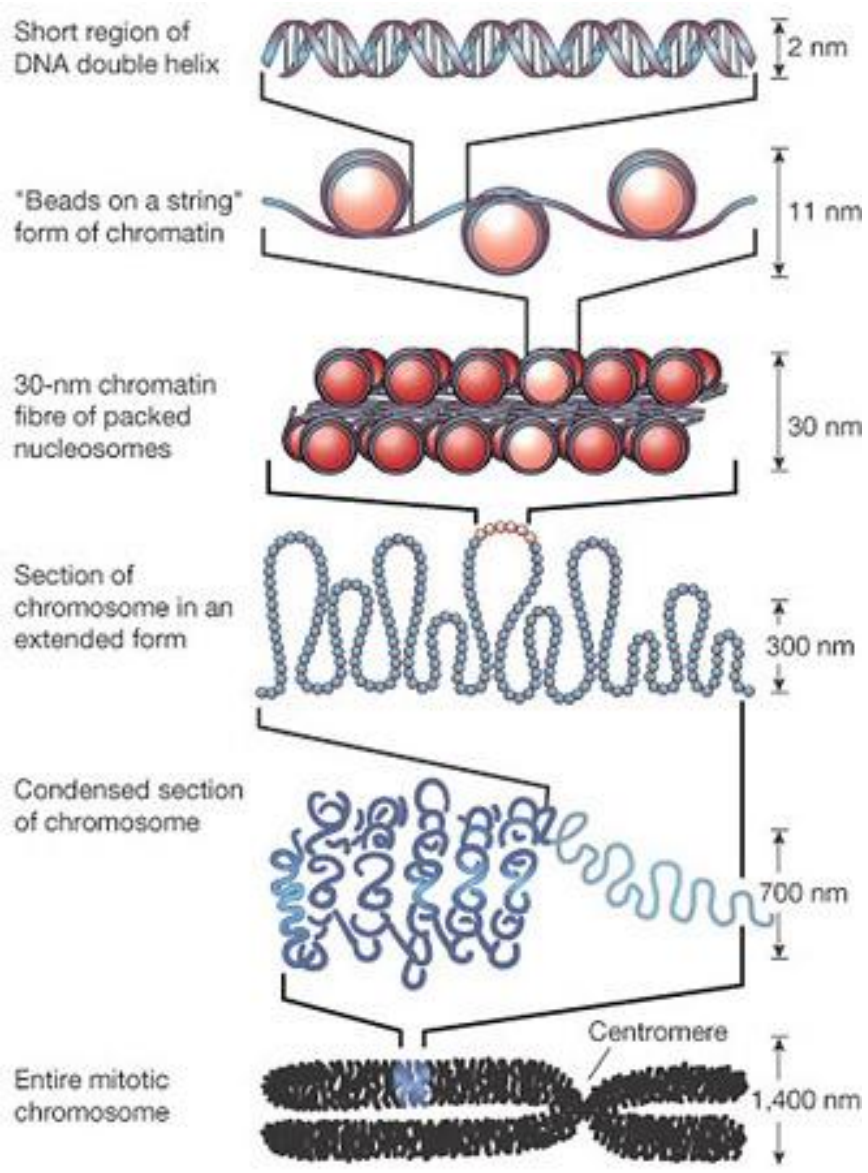
hyperacetylated histones
Low DNA methylation
H3-K4 methylation
H4-K16 acetylation

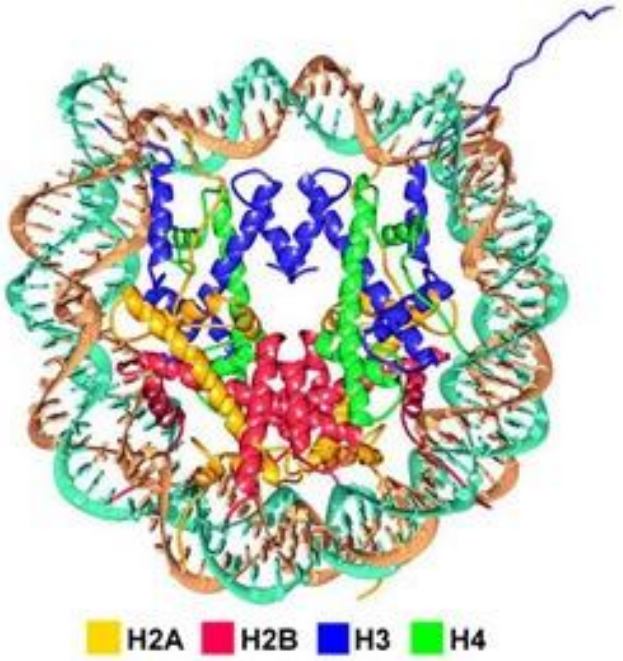
hypoacetylated histones
Dense DNA methylation
H3-K9 methylation
H4-K20 methylation

Slide courtesy of Trevor Archer adapted from Jenuwein & Allis, Science 2001)

Chromatin Modification

Chromatin Structure





Nucleosome (histone octamer wrapped with 146 bp of DNA).

ttp://beyondthedish.wordpress.com/tag/chromatin-fiber/

Chromatin Roles

- Compactization
  - Packing ~2 meters of DNA in to a ~10 micron diameter nucleus.
  - Different compactization levels: euchromatin and heterochromatin.
- Regulation of gene expression
  - Influencing DNA accessibility through Nucleosome Occupancy.
  - Histone variants.
  - Nucleosome tail modifications.
  - **Co-regulation**: Effect common chromatin regions.
- Regulation of DNA replication, repair, recombination and more…

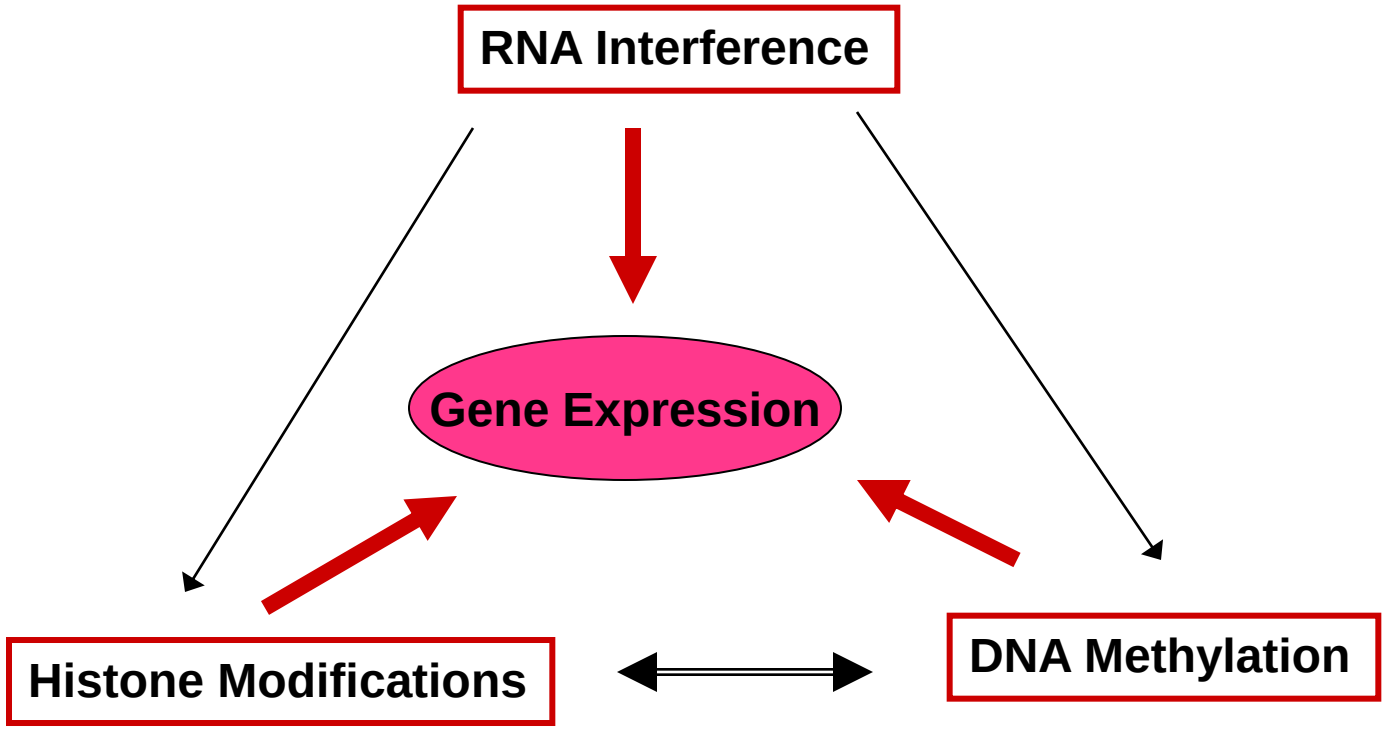
Mechanism of Chromatin Remodeling

- Mechanism of chromatin remodeling involves:
  - Mobilization of nucleosomes
  - Loosening of association between DNA and core histones
- Catalyzed remodeling of nucleosomes involves formation of distinct conformations of nucleosomal DNA/core histones when contrasted with:
  - Uncatalyzed DNA exposure in nucleosomes
  - Simple nucleosome sliding along a DNA stretch

Epigenetics

Heritable and/or acquired changes in gene expression that occur without changes in DNA sequence.

Epigenetics Mechanisms

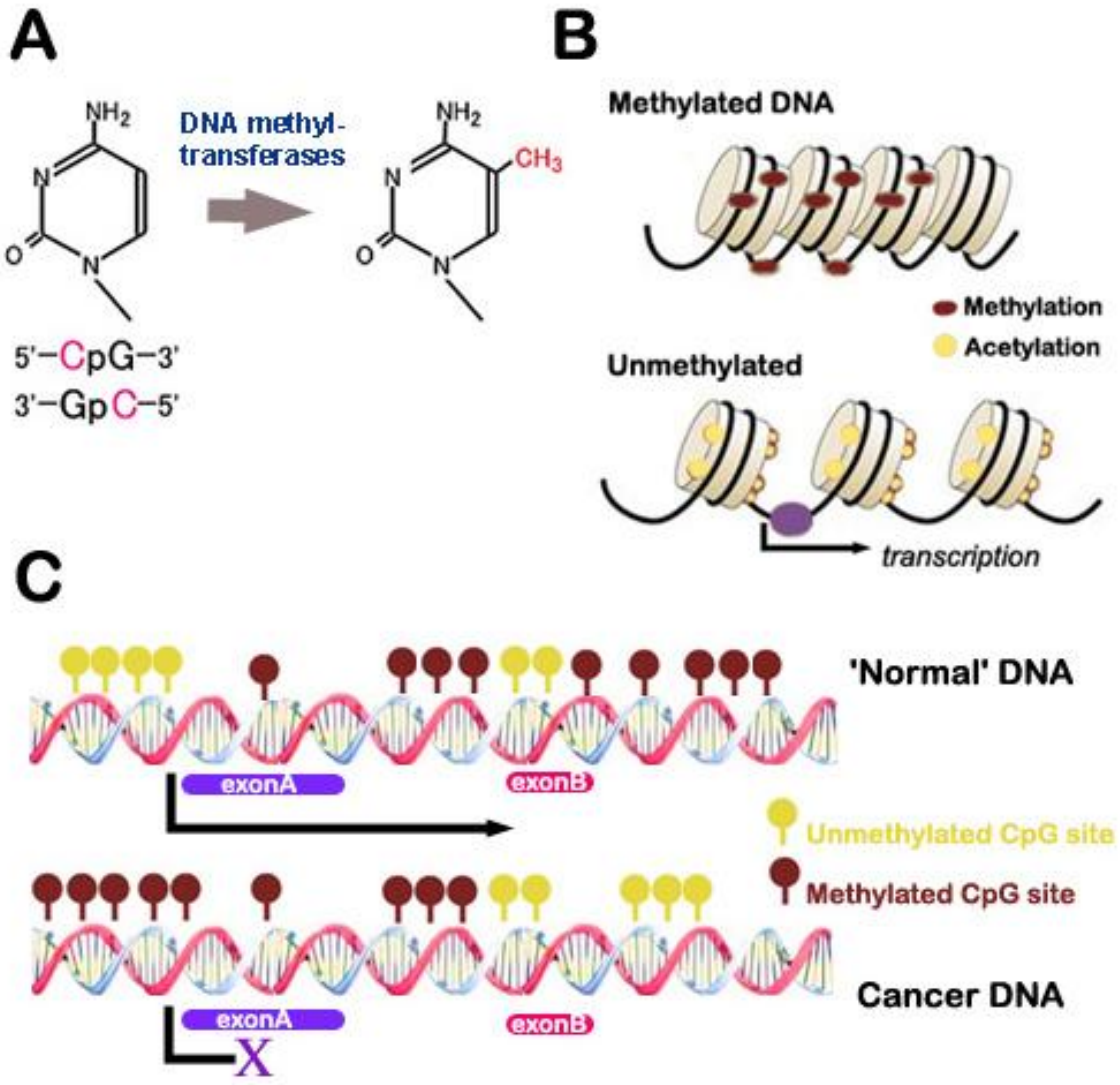


Epigenetic Inheritance

- Although the chromatin modifications just discussed do not alter DNA sequence, they may be passed to future generations of cells

- The inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called epigenetic inheritance

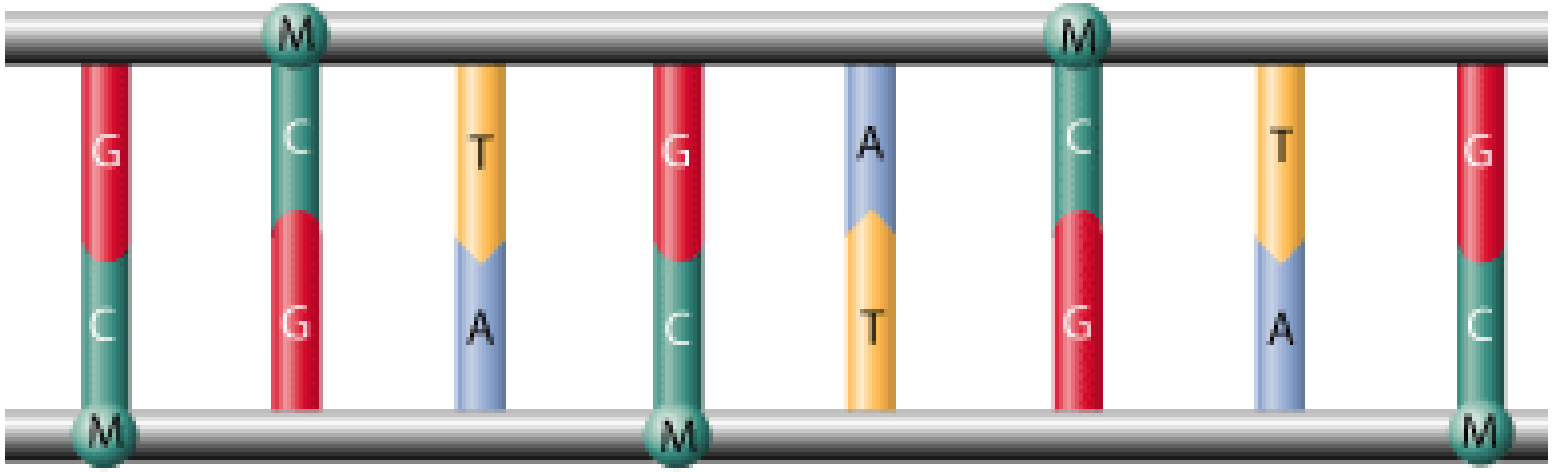
DNA Methylation

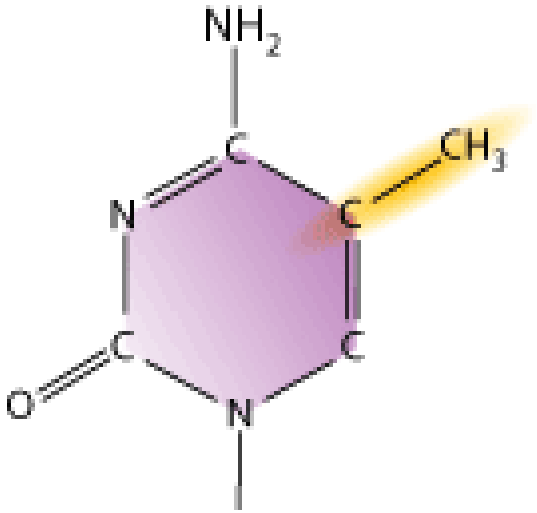


Hypomethylation
Hypermethylation

http://www.cellscience.com/reviews7/Taylor1.jpg

DNA methylation is the addition of a methyl group to the carbon-5 position of cytosine residues.





DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).

Natural Roles of DNA Methylation in Mammalian System

- Imprinting
- X chromosome inactivation
- Heterochromatin maintenance
- Developmental controls
- Tissue specific expression controls

- DNA methylation usually inhibits the transcription of eukaryotic genes
  - Especially when it occurs in the vicinity of the promoter

- In vertebrates and plants, many genes contain CpG islands near their promoters
  - These CpG islands are 1,000 to 2,000 nucleotides long

  - In housekeeping genes
    - The CpG islands are unmethylated
    - Genes tend to be expressed in most cell types

  - In tissue-specific genes
    - The expression of these genes may be silenced by the methylation of CpG islands

What protects CpG islands from DNA methylation?

(1) CpG islands are unmethylatable by the existing de novo methytransferases. However, this is unlikely because they become densely methylated on the inactive X chromosome and in cancer cells.

(2) CpG islands are protected from methylation by the binding of factors which exclude Dnmts.

(3) CpG islands are maintained in a methylation-free state with the aid of DNA demethylase that actively remove methyl-CpGs.

(4) The atypical base composition and lack of methylation reflect abnormal DNA metabolism at these CpG islands. For example, recombination and/or repair may be concentrated at these sites, which may result in high level of DNA turnover.

(5) Early embryonic transcription from a CpG island promoter is required to ensure that DNA methylation is excluded. However, there is no evidence that transcription excludes CpG methylation.

(6) A complex relationship between DNA methylation and chromatin structures in some eukaryotes, including plants.

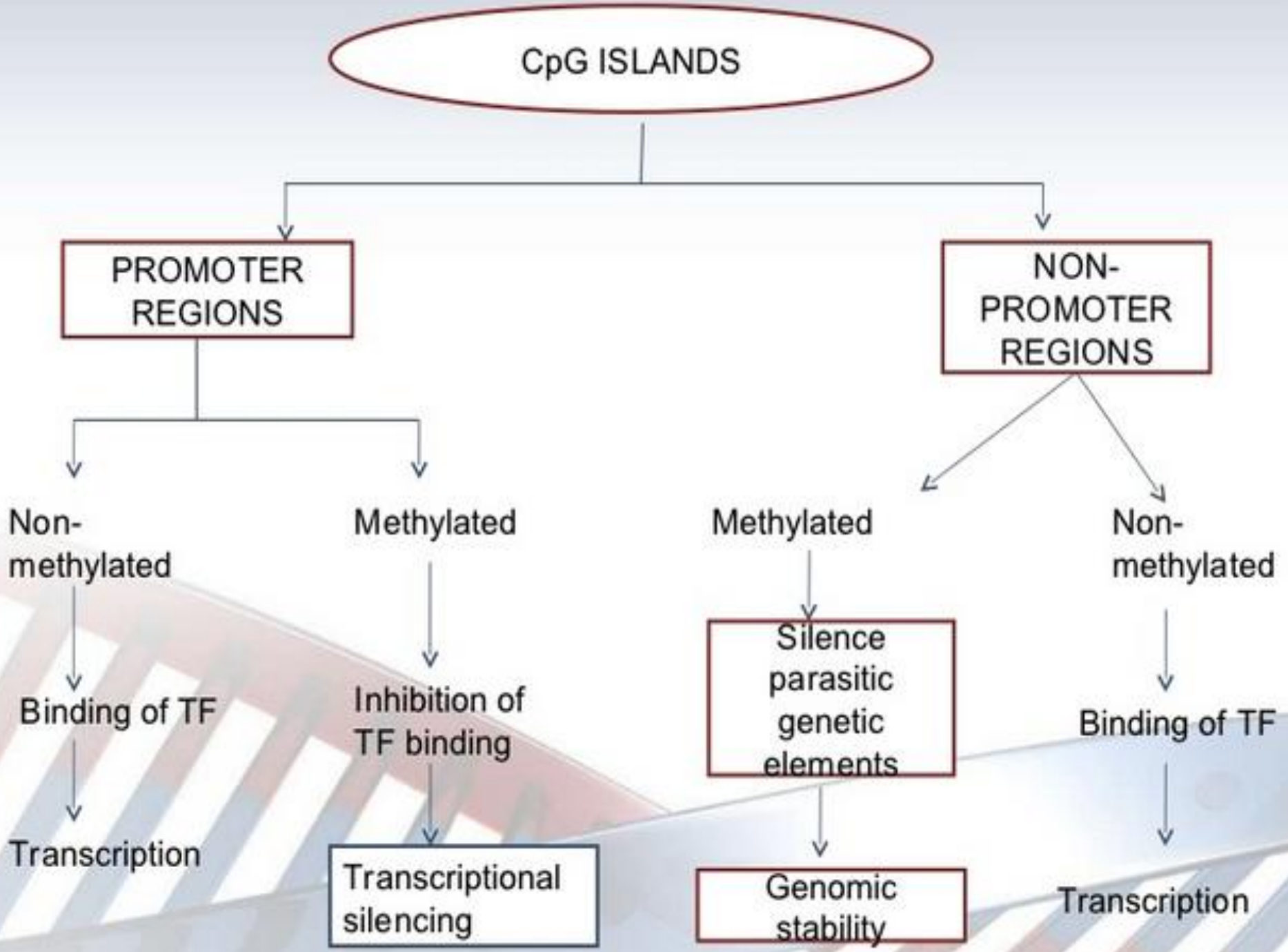
Regulation of gene expression by DNA methylation

(1) Several studies in early 1980s showed that genes can be silenced by artificial methylation of CpG sites and silenced genes can be activated by treatment with 5-azacytidine, which inhibits DNA methylation in living cells.

(2) Interference with transcription factor binding: Transcription factors that recognize GC-rich sequence motifs can be interfered by the presence of the methyl groups in the methylated CpGs.

(3) Attraction of methyl-CpG-binding proteins: methyl-CpG-binding proteins (MeCP1 and MeCP2), methyl-CpG-binding domain (MBD) proteins (MBD1, MBD2, MBD3, MBD4), another unrelated protein, Kaiso. These proteins recruit repressory protein complexes that in turn interact with histone deacetylases (HDAC).

(4) Complex interrelationship between DNA methylation and histone modification, which result in heterochromatin formation and gene silencing.



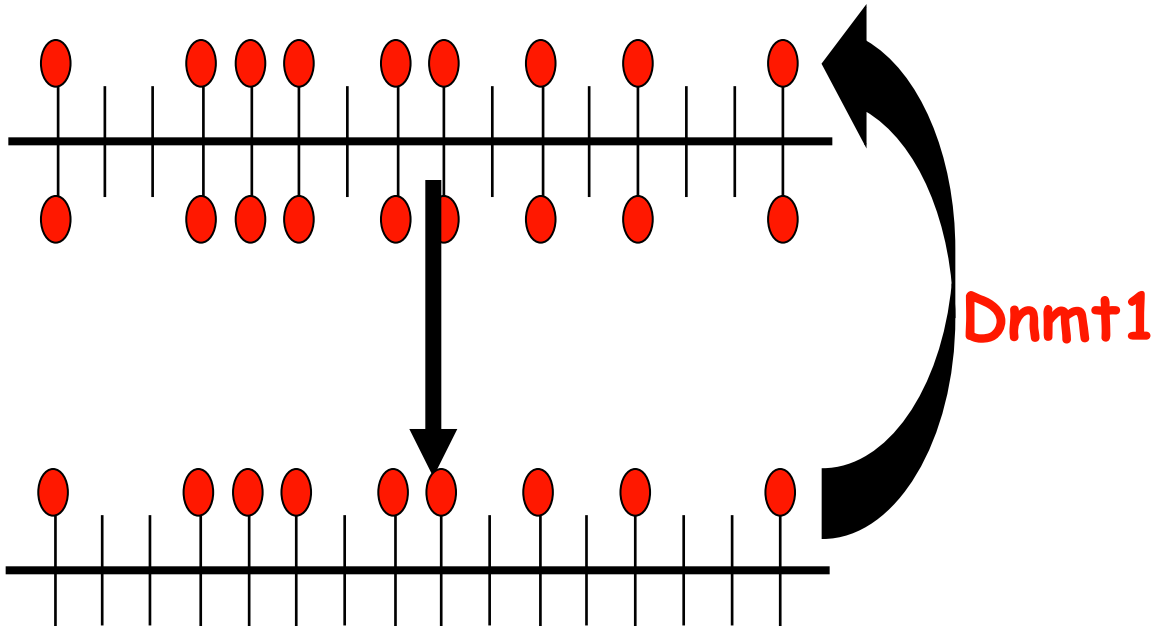
Manel Esteller, Human Molecular Genetics, 2007, Vol. 16, Review Issue 1 R50–R59.

Methylation patterns are heritable

The fact that methylation patterns are heritable was initially established using DNA-methylation-sensitive restriction enzymes (Bird and Southern 1978). The early studies also showed that either both CpGs in a complementary pair were methylated, or neither was methylated, which fitted well with the predictions of the maintenance model.

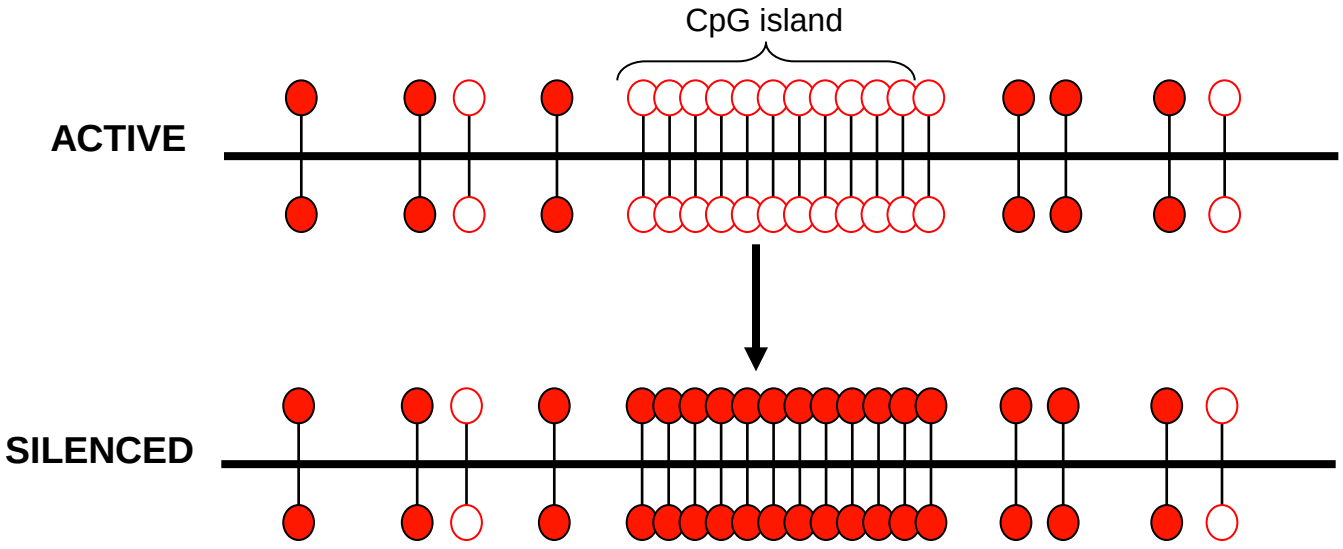
The mammalian maintenance DNA methytransferase

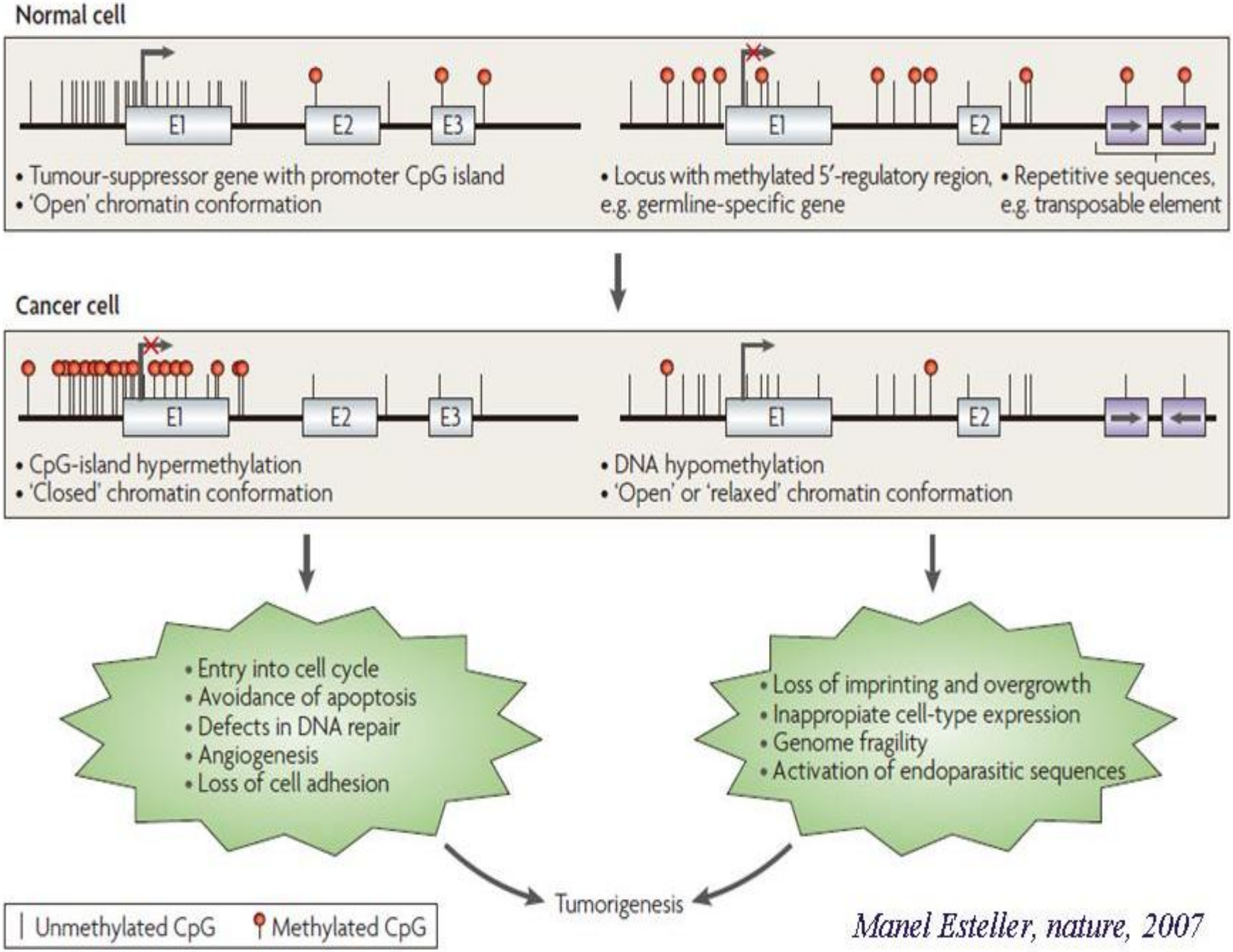
DNA methyltransferase was first purified in mammalian species in 1983 (Bestor & Ingram, 1983 PNAS 80: 5559-63). The preferred DNA substrate of this enzyme, **Dnmt1**, is DNA methylated at CpG on one strand only (hemimetylated DNA). Thus, this enzyme seemed to be a **maintenance DNA methytransferase**.



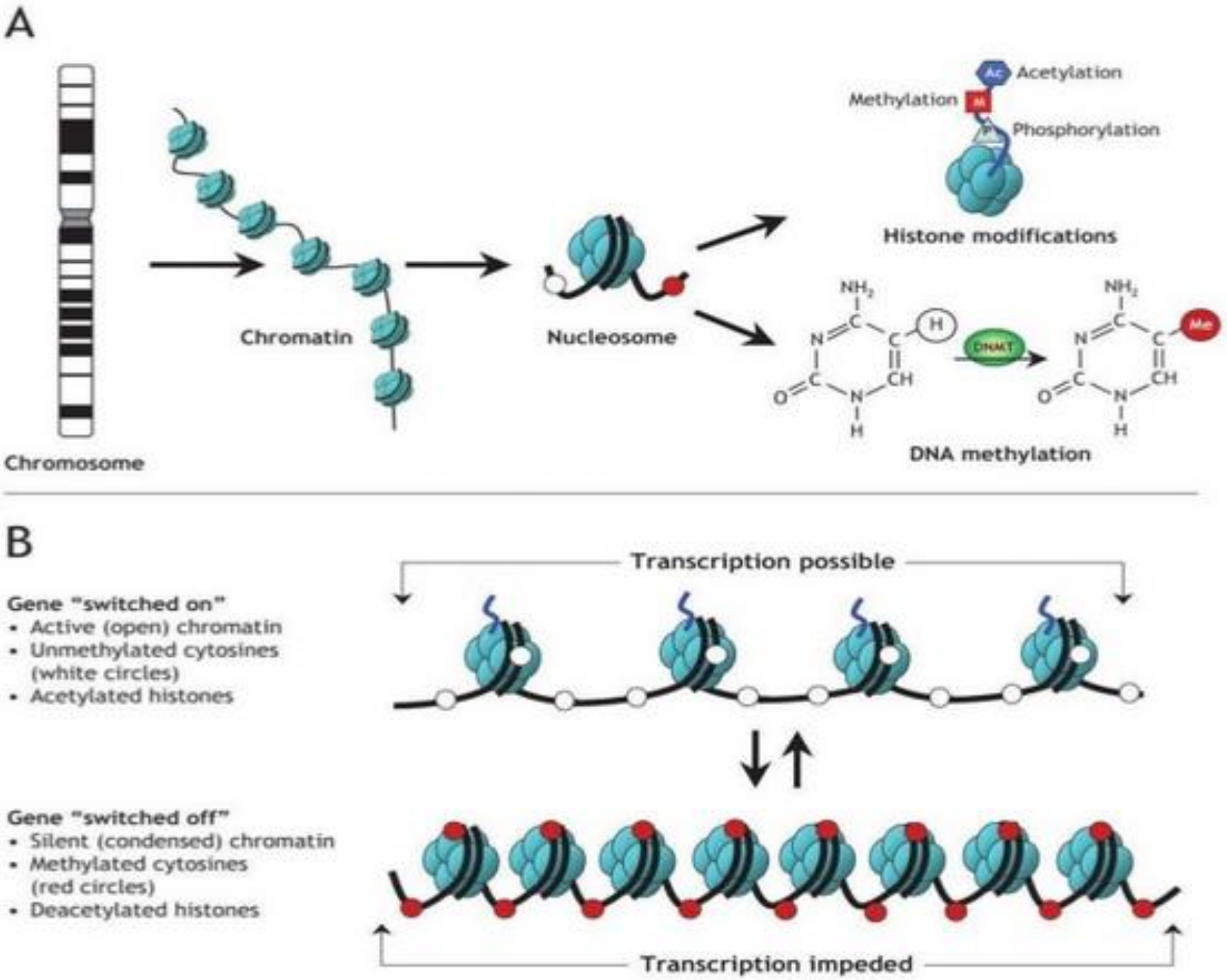
What sequences are methylated in our genome?

- DNA from mammalian somatic tissues is methylated at 70% of all CpG sites.
- Highly methylated sequences include satellite DNAs, repetitive elements including transposons, nonrepetitive intergeneic DNA, and exons of genes.
- Key exceptions of this global methylation of the mammalian genomes are the CpG islands (regions with high CpG density). Most CpG islands marks the promoters and 5′ domains of genes. Approximately 60% of human genes have CpG island promoters.

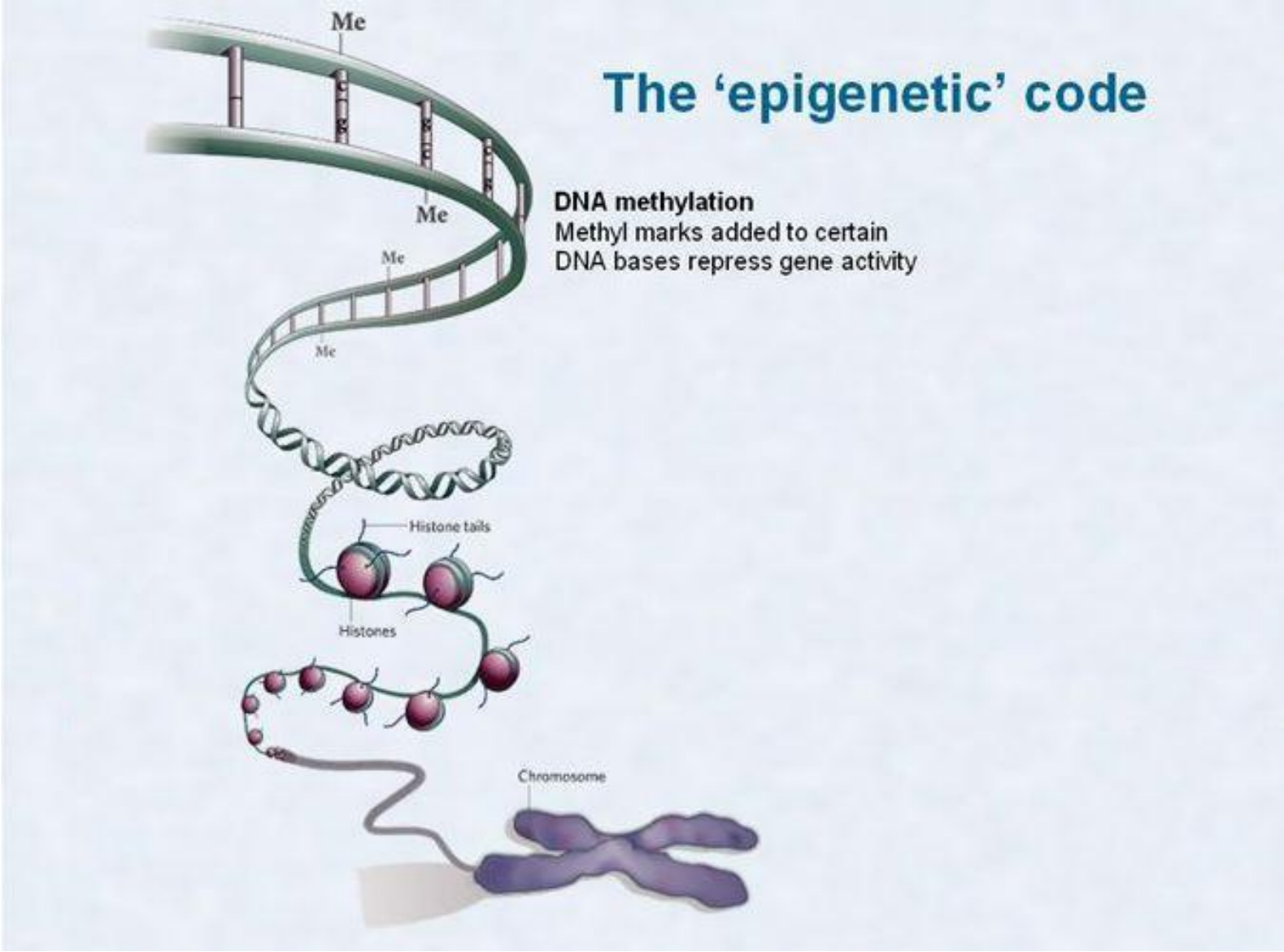




Manel Esteller, nature, 2007


A
Chromosome
Chromatin
Nucleosome
Acetylation
Methylation
Phosphorylation
Histone modifications
DNMT
DNA methylation
B
Gene "switched on"
• Active (open) chromatin
• Unmethylated cytosines (white circles)
• Acetylated histones
Transcription possible
Gene "switched off"
• Silent (condensed) chromatin
• Methylated cytosines (red circles)
• Deacetylated histones
Transcription impeded

DNA Methylation & the Epigenetic Code

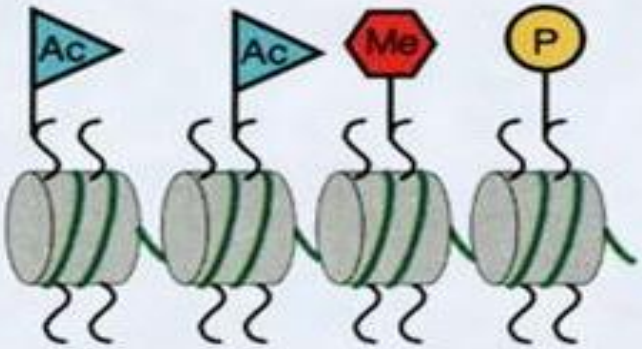


Cowperthwaite MC, Economo EP, Harcombe WR, Miller EL, Meyers LA (2008) The Ascent of the Abundant: How Mutational Networks Constrain Evolution. PLoS Comput Biol 4(7): e1000110. doi:10.1371/journal.pcbi.1000110

Structure & Epigenetics of Euchromatin versus Heterochromatin

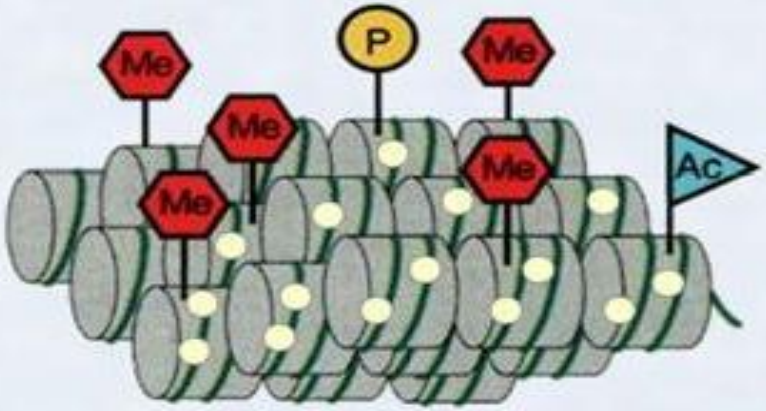
DNA methylation and histone modifications help to compartmentalize the genome into domains of different transcriptional potentials

Euchromatin



- High histone acetylation
- Low DNA methylation
- H3-K4 methylation

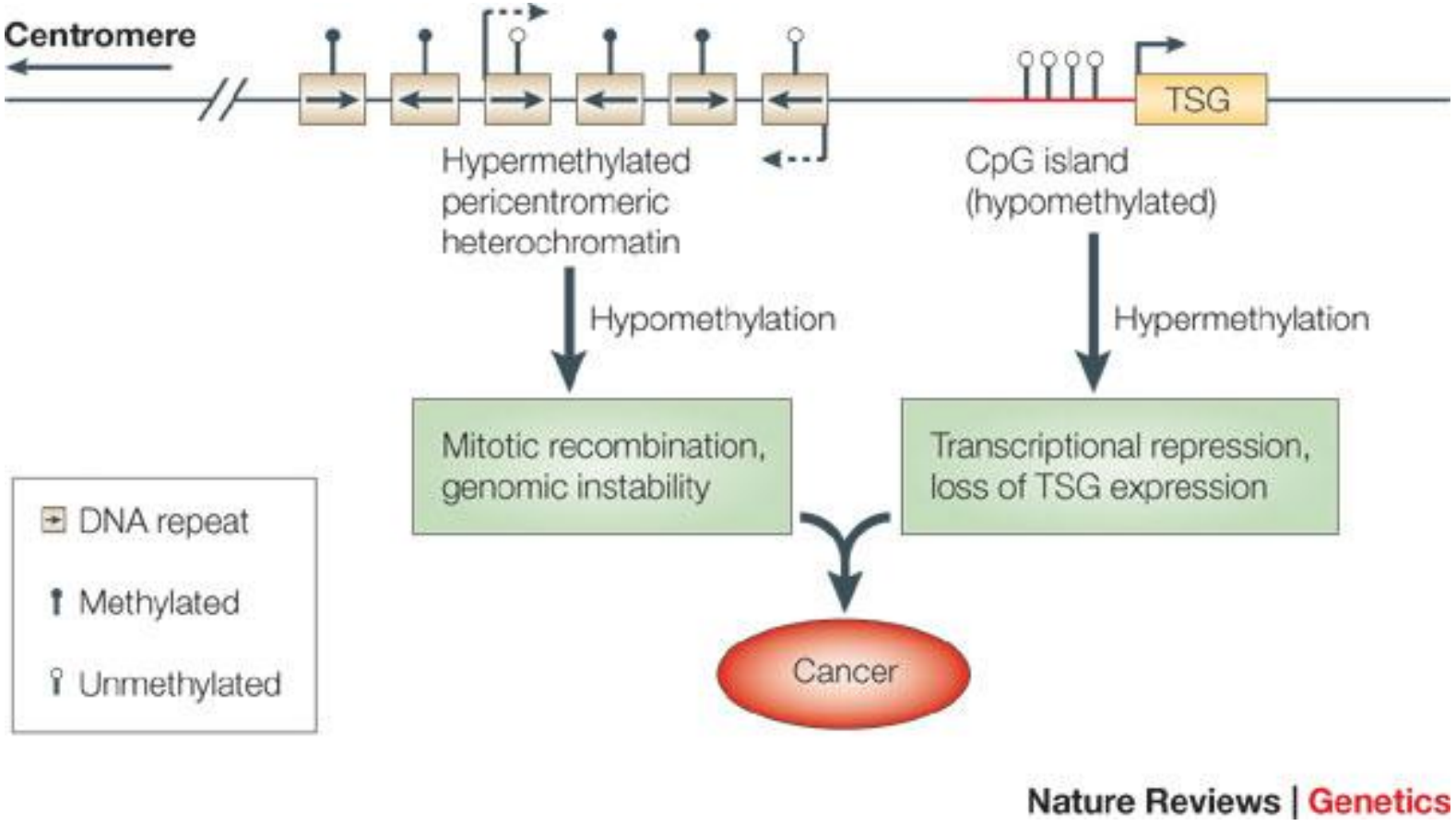
Heterochromatin



- Low histone acetylation
- Dense DNA methylation
- H3-K9 methylation

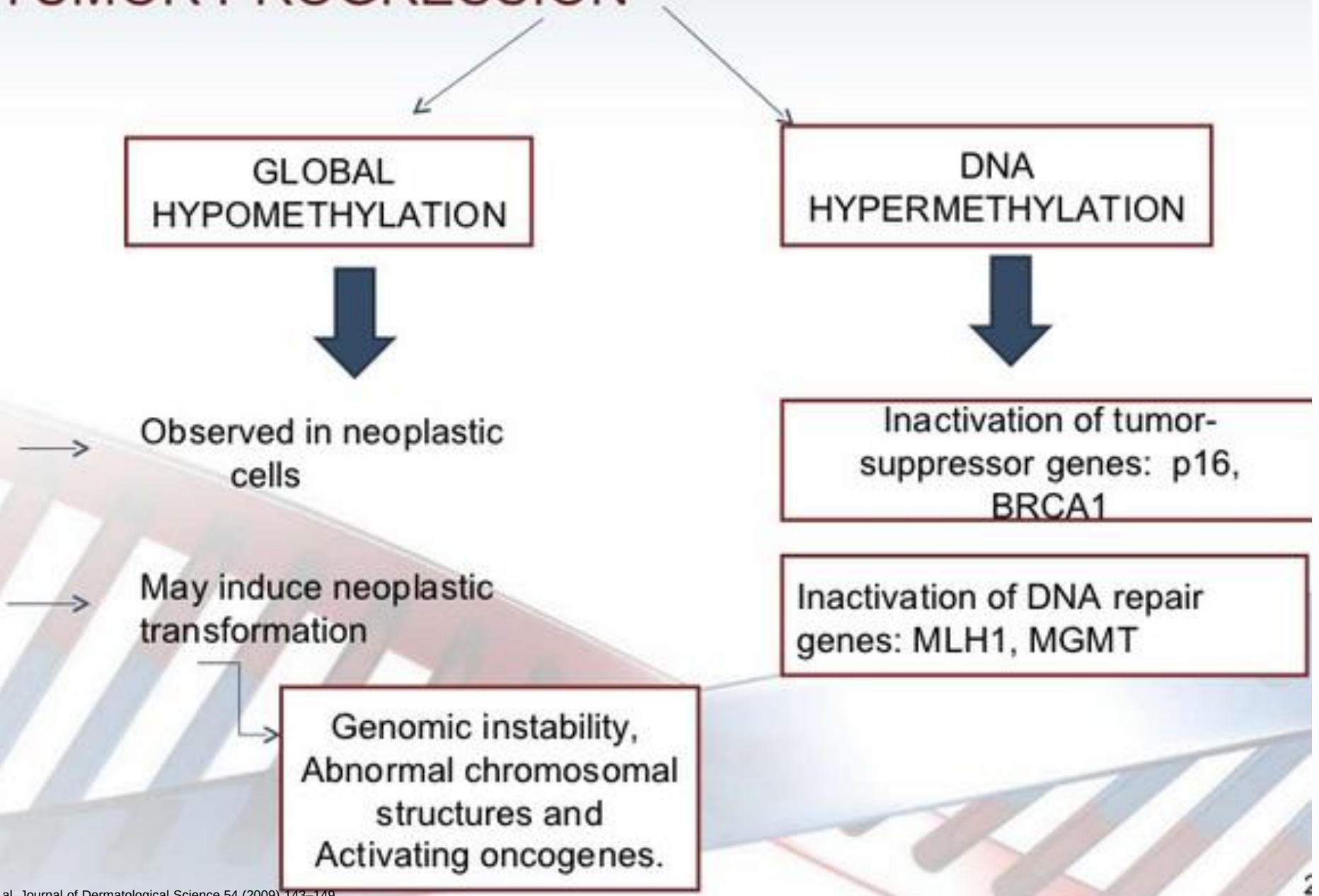
Slide courtesy of Trevor Archer adapted from Jenuwein & Allis, Science 2001)

DNA Methylation and Cancer



Robertson, Nature Reviews Genetics, Vol6, 597

METHYLATION IMBALANCE may contribute to TUMOR PROGRESSION



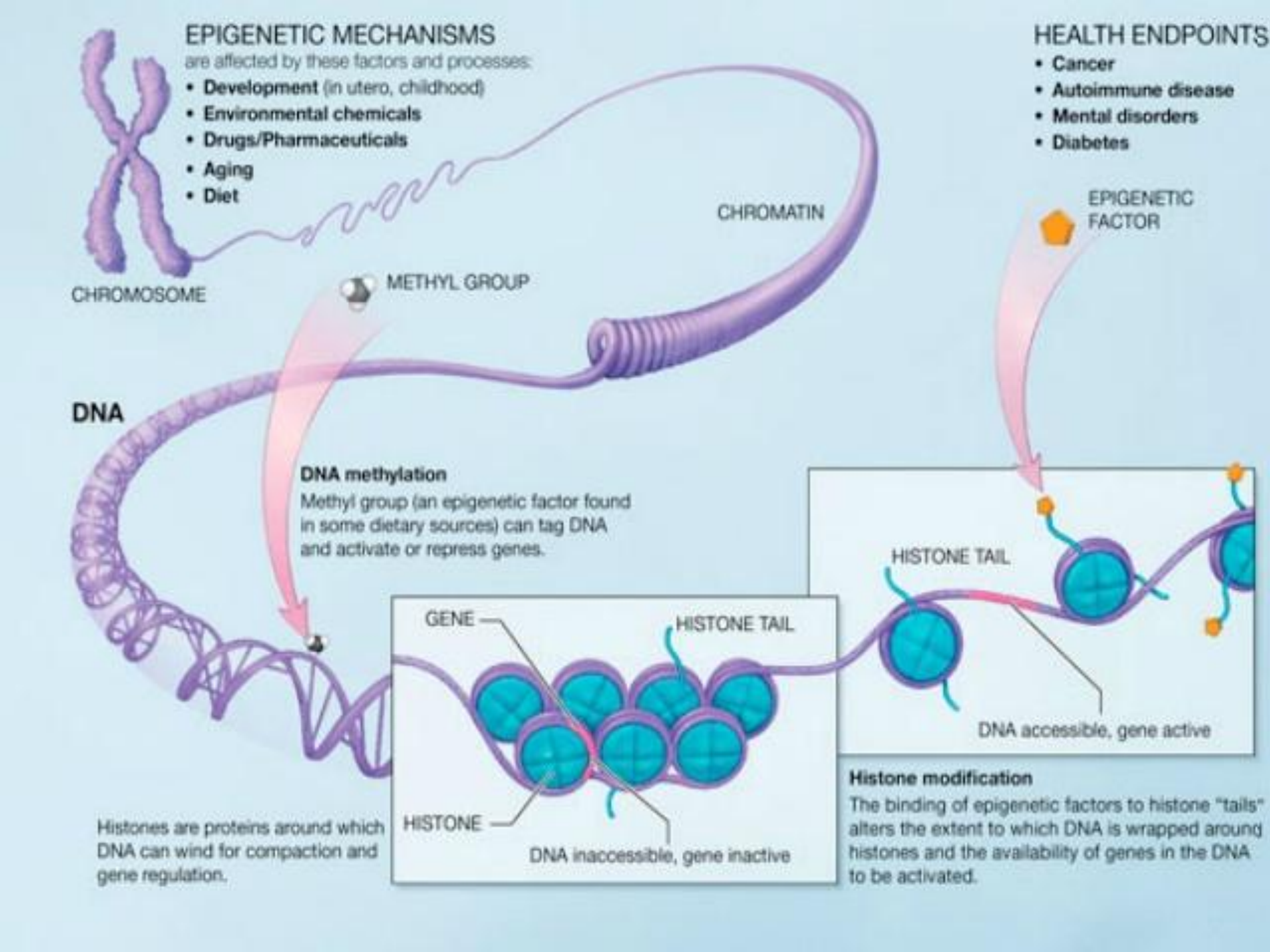
Li et al, Journal of Dermatological Science 54 (2009) 143–149

DNA Methylation and Other Human Diseases

-- **Imprinting Disorder:**
- Beckwith-Wiedemann syndrom (BWS)
- Prader-Willi syndrome (PWS)
- Transient neonatal diabetes mellitus (TNDM)

-- **Repeat-instability diseases**
- Fragile X syndrome (FRAXA)
- Facioscapulohumeral muscular dystroph

-- **Defects of the methylation machinery**
- Systemic lupus erythemtosus (SLE)
- Immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome


EPIGENETIC MECHANISMS
are affected by these factors and processes:
• Development (in utero, childhood)
• Environmental chemicals
• Drugs/Pharmaceuticals
• Aging
• Diet
HEALTH ENDPOINTS
• Cancer
• Autoimmune disease
• Mental disorders
• Diabetes
CHROMOSOME
CHROMATIN
METHYL GROUP
EPIGENETIC FACTOR
DNA
DNA methylation
Methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes.
HISTONE TAIL
GENE
HISTONE TAIL
HISTONE
DNA inaccessible, gene inactive
DNA accessible, gene active
Histones are proteins around which DNA can wind for compaction and gene regulation.
Histone modification
The binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.