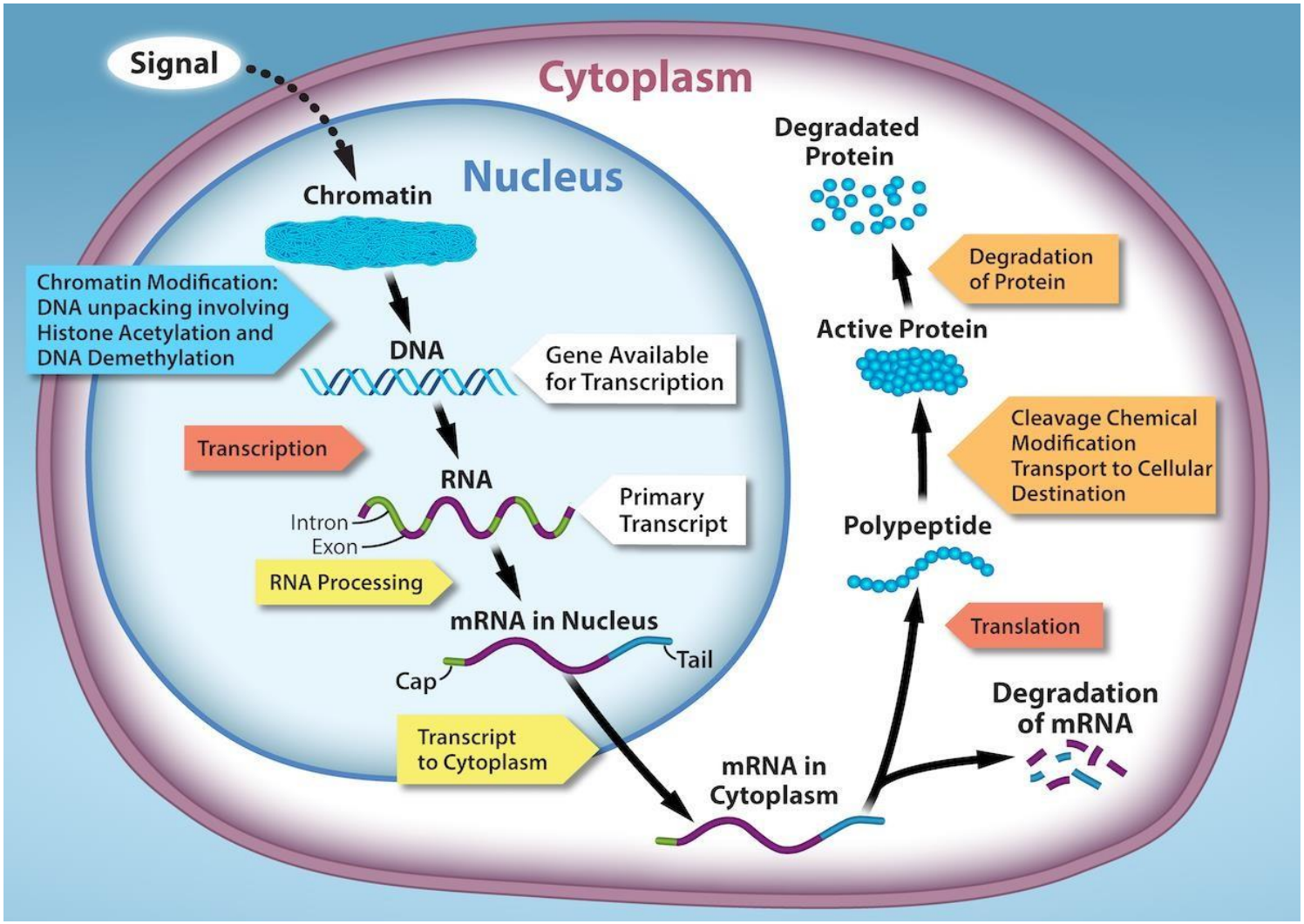
Regulation of Gene Expression

Lecture 4

Locations of gene regulation



- For a cell to function properly, necessary proteins must be synthesized at the proper time and place.
- The process of turning on a gene to produce RNA and protein is called **gene expression**.
- The regulation of gene expression conserves energy and space.
- The control of gene expression is extremely complex.
- Malfunctions in this process can lead to the development of many diseases, **including cancer.**

Outline

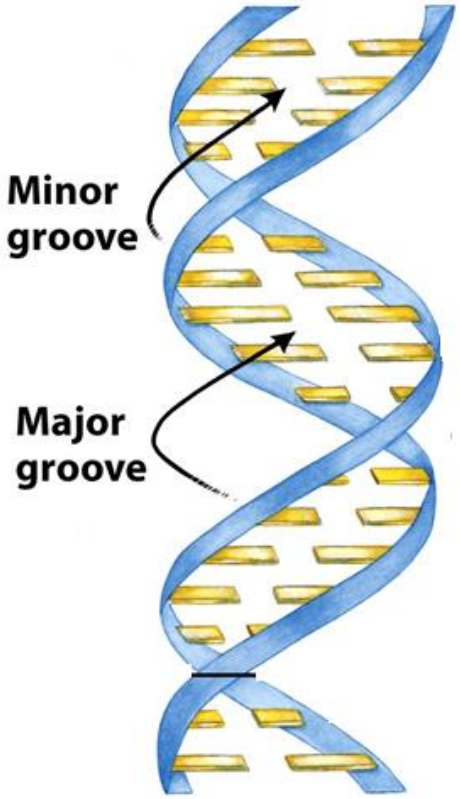
- Molecular components involved in transcriptional regulation, and how they can contribute to the processes underpinning cancer.
- DNA–protein interactions in transcriptional regulation.
- Role of miRNAs in the regulation of post-transcriptional gene expression is presented.

- Cancer is a disease of the genome at the cellular level that may be manifested by alterations in gene expression.
- Gene expression may be modulated in various ways, particularly through the regulation of transcription.
- Mutations in the promoter region of genes can alter the regulation of gene expression and lead to carcinogenesis.

- **Transcription factors** are proteins that bind to enhancer regions of the genome and recruit the transcription machinery to the promoter DNA regions, which then initiate the genes' transcription.

- Binding of regulatory proteins to specific DNA sequences results in controlling gene expression.

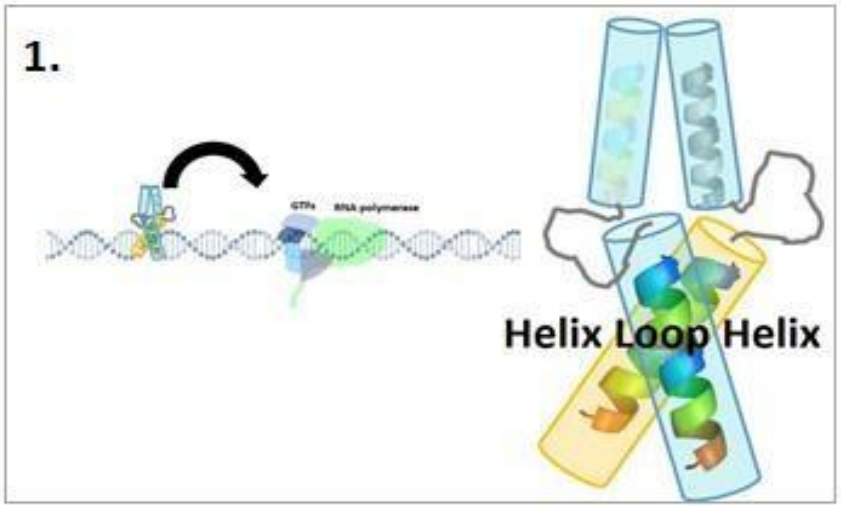
- Regulatory proteins gain access to the bases of DNA at the major groove via their DNA-binding motifs.

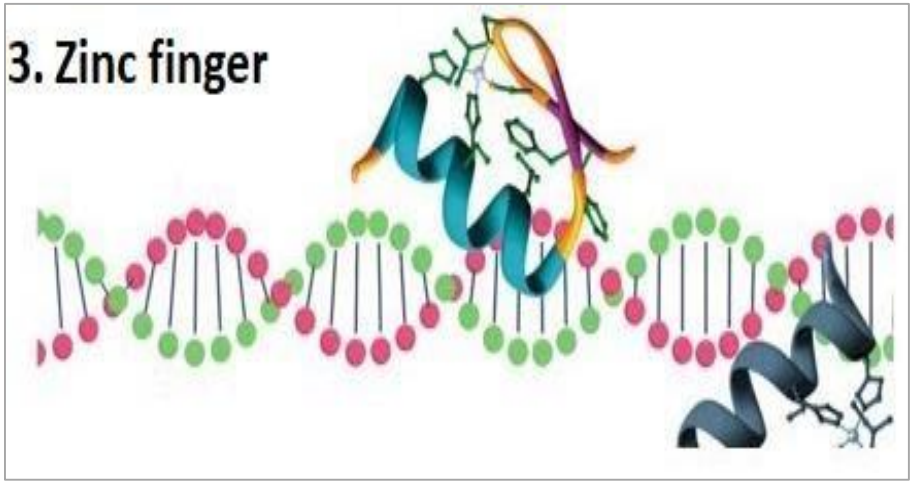


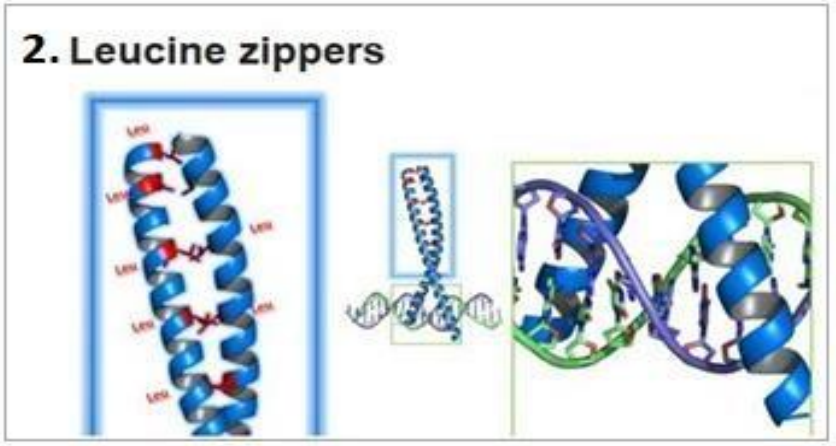
- Transcription factors contain a set of independent protein modules or domains include:
  - DNA-binding domains (necessary to recognize and bind to the DNA strand),
  - transcriptional activation domains (interacts with other proteins)
  - dimerization domains, and
  - ligand-binding domains.

Common types of DNA-binding domains

- These domains are characteristic proteins conformations that enable a transcription factors to bind DNA







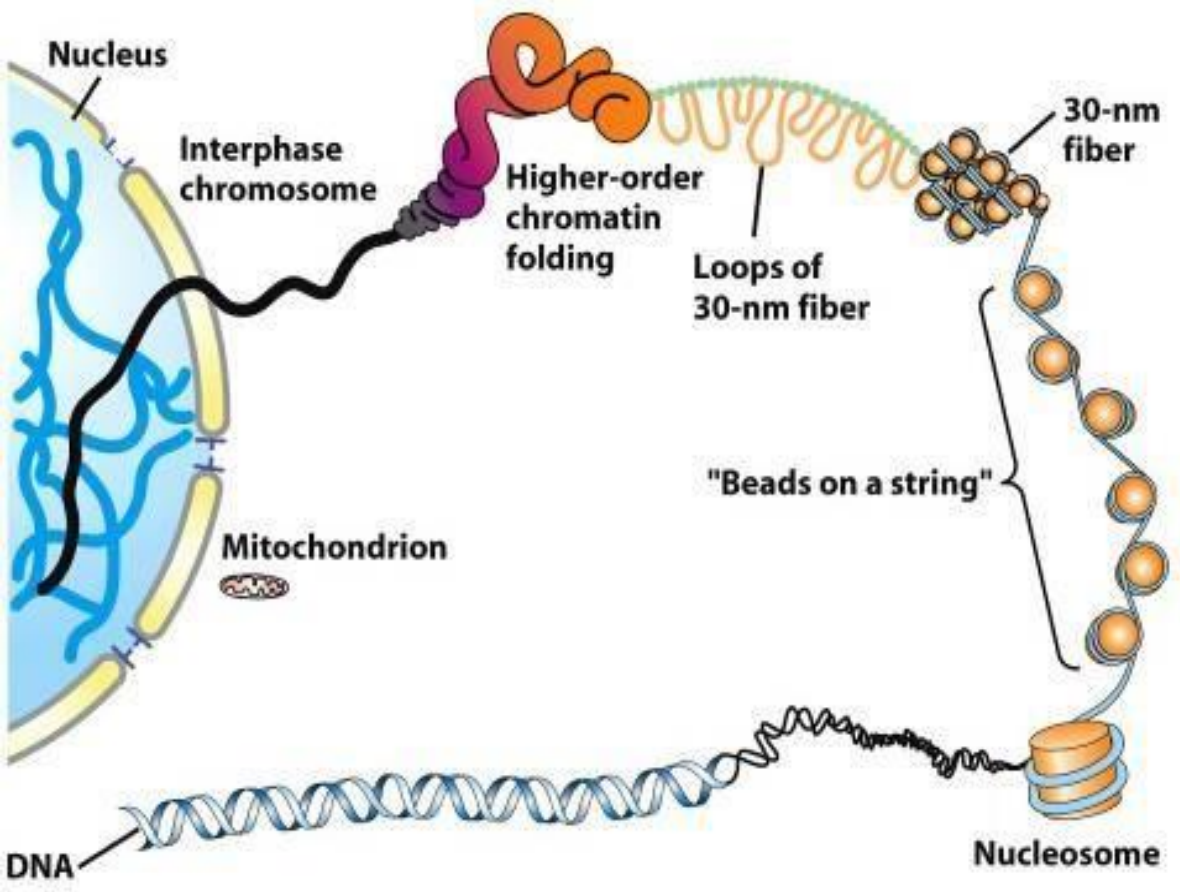
- Some transcription factors work in pairs (a “dimer”) and require a dimerization domain which facilitates protein–protein interactions between the two molecules.
- Interactions between transcription factors are a common theme in transcriptional regulation.
- Some transcription factors only function upon binding of a ligand and therefore require a ligand-binding domain.

- **The activity of a transcription factor can be regulated by several means:**
  - Synthesis in particular cell types only,
  - Covalent modification such as phosphorylation, ligand binding and cell localization.
- **Oncogenic signaling pathways could make harmful effects like:**
  - uncontrolled growth,
  - evasion of apoptosis, or
  - aberrant differentiation converge on a single transcription factor that regulates a set of genes to produce a transformed phenotype.

Thus, misregulation of a single transcription factor can cause cancer

Organization of DNA Within a Cell

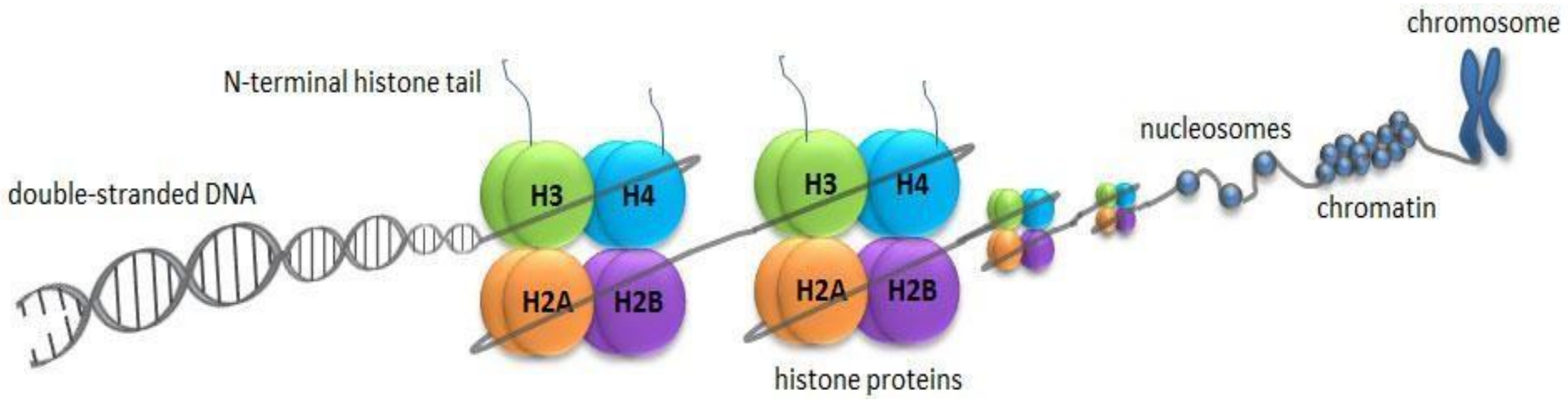
- 2 meters of DNA is packed into a 10 mm diameter cell



from Lodish *et al.*, Molecular Cell Biology, 6th ed. Fig 6-1

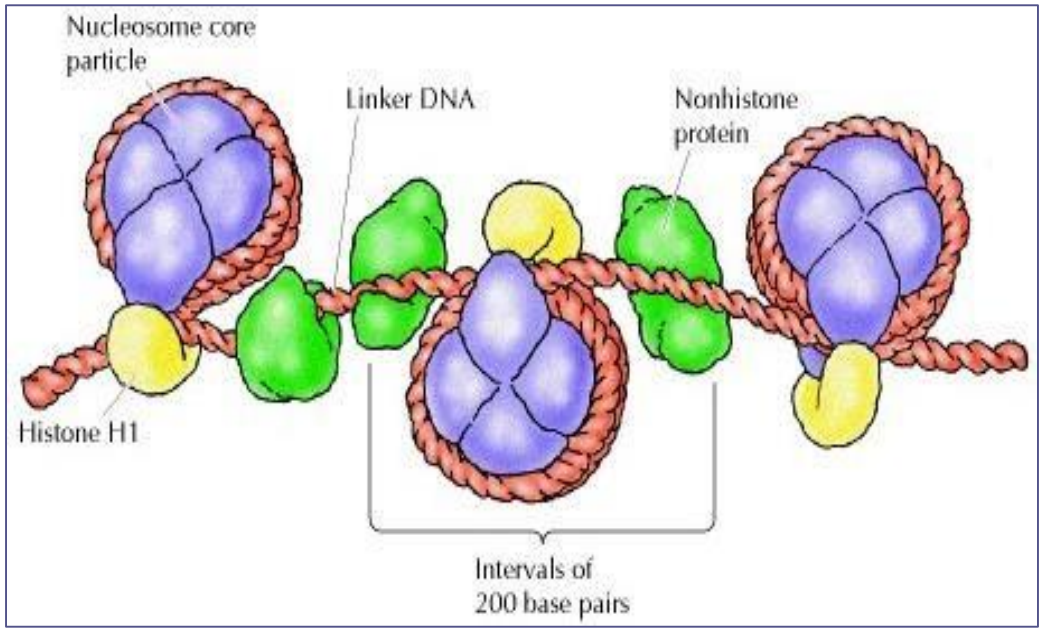
Chromatin Structure

- Human DNA is present in the nucleus of cells in the form of 46 chromosomes.
- Chromosomes are made of chromatin: a thread of DNA (60%) and protein (40%).
- The structure of chromatin—from compacted to relaxed—can change, and it is this feature that enables it to have a regulatory role in transcription.

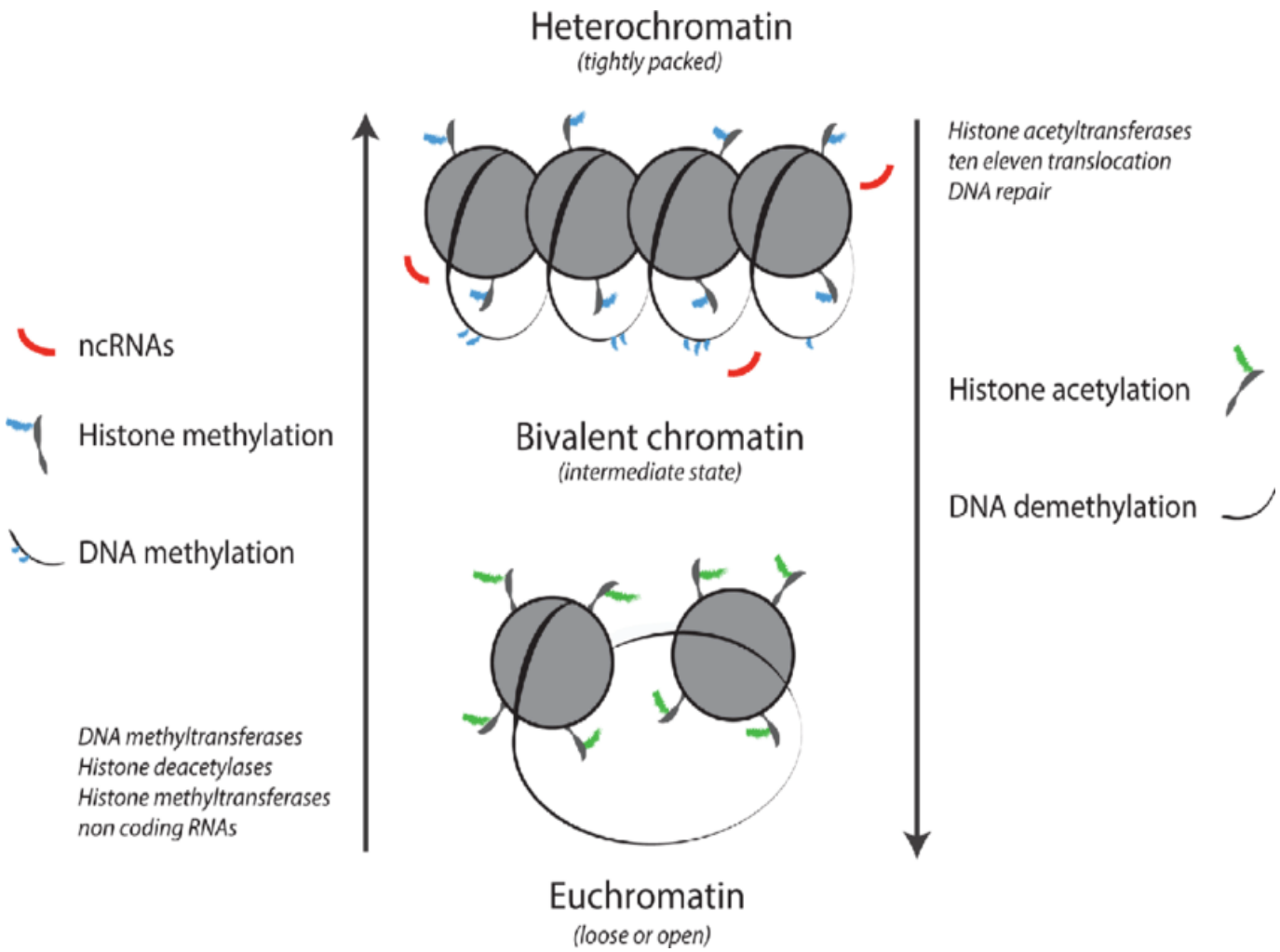


Important Information

- Chromatin has an important role beyond being a structural scaffold.
- The degree of compaction or relaxation of chromatin determines how readily the DNA in a portion of chromatin can be transcribed. How?



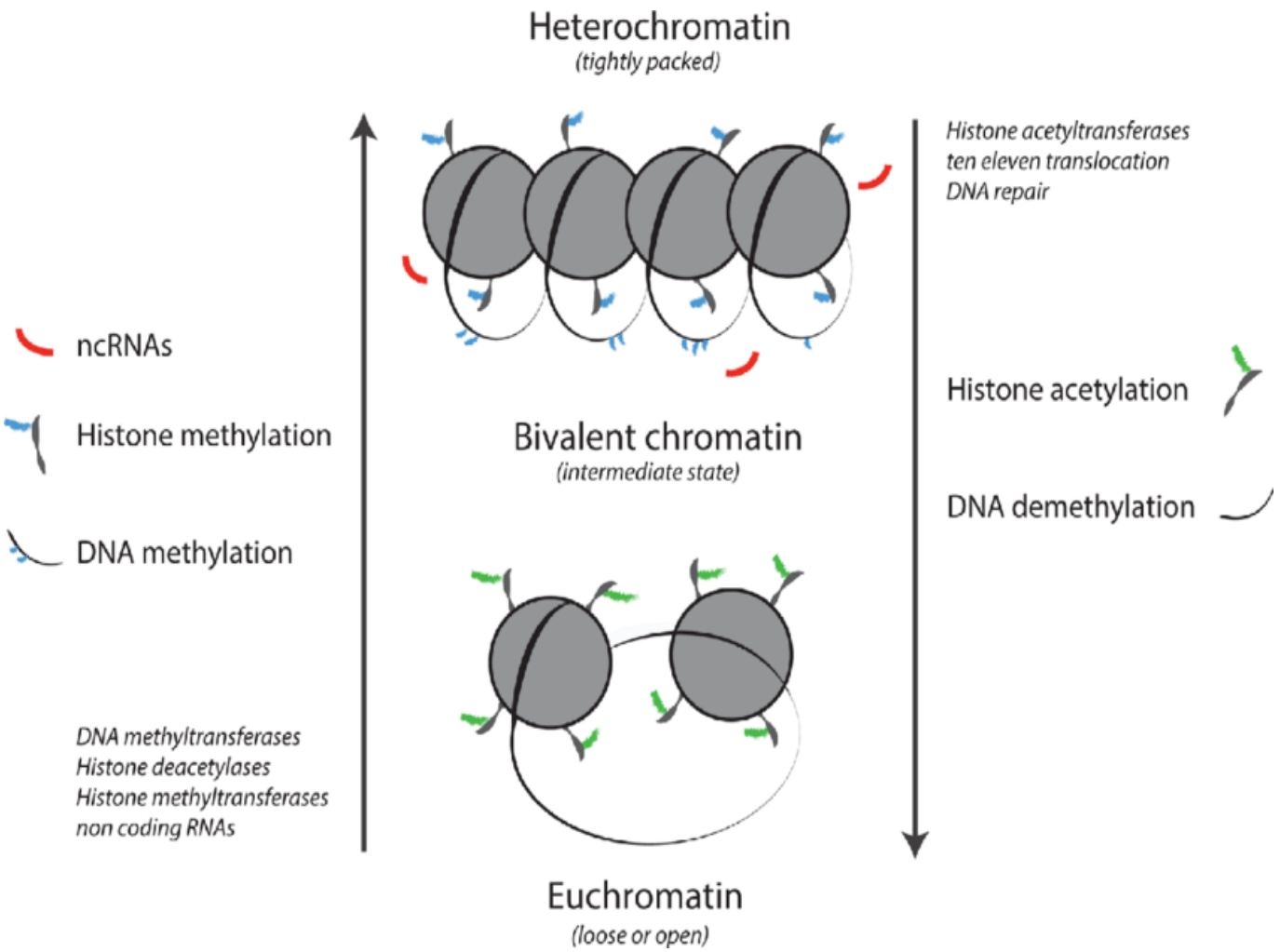
The DNA is wrapped around histones in nucleosome core particles and sealed by histone H1. Nonhistone proteins bind to the linker DNA between nucleosome core particles.



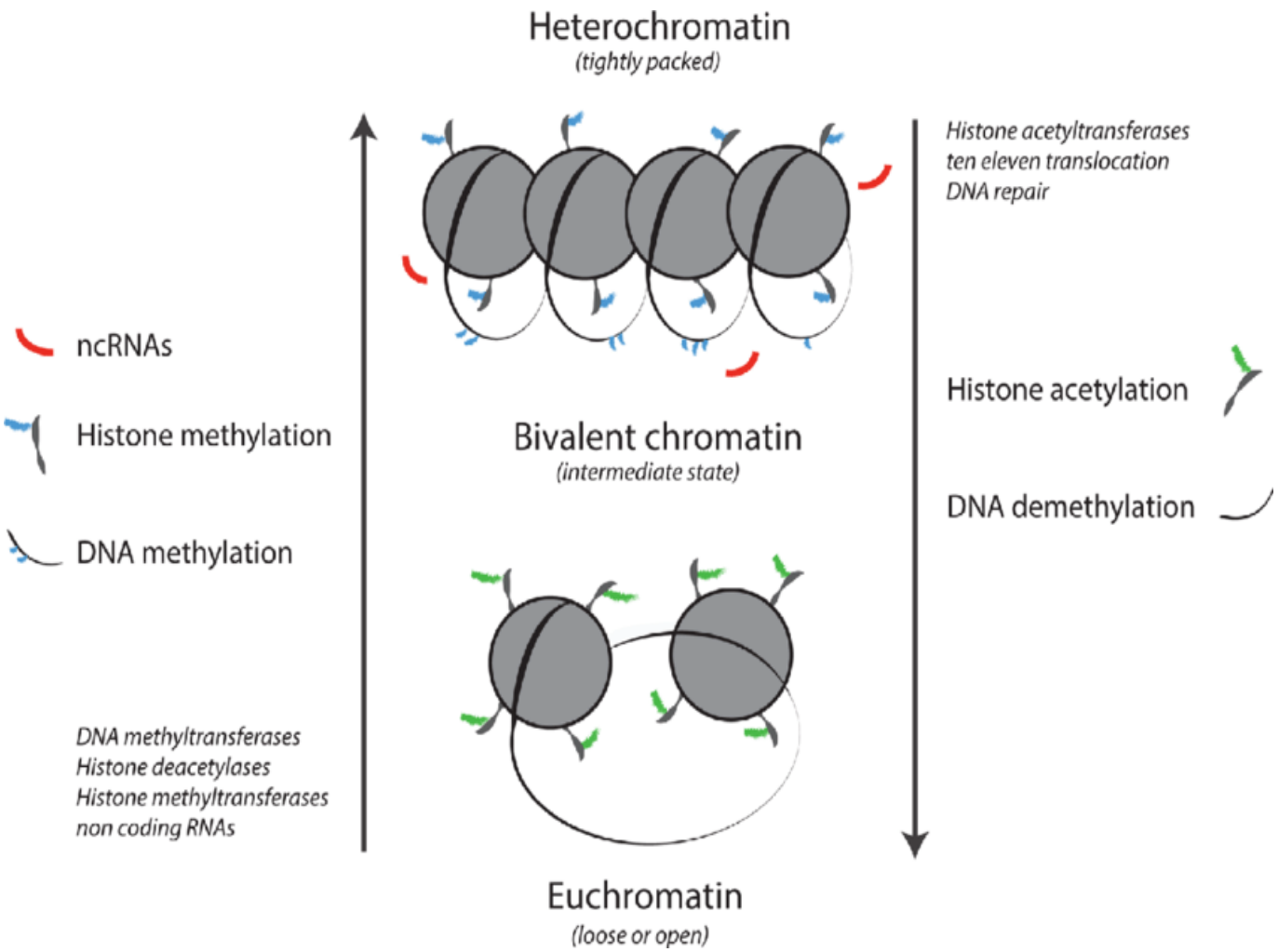
Chromatin exists as euchromatin or heterochromatin, with an intermediate bivalent state.

Loosely wound euchromatin is accessible by RNA polymerase II and transcription factors.

The genes contained within heterochromatin generally cannot be expressed.



DNA methyltransferases, histone deacetylases, and methyltransferases produce a **heterochromatic state**,
whereas histone acetyltransferases and ten-eleven translocation (TET), along with some types of DNA repair, contribute to a euchromatic state.



Evidence suggests that noncoding RNAs, DNA methylation, and histone acetylation and histone methylation act together to influence chromatin structure and the expression of associated genes.

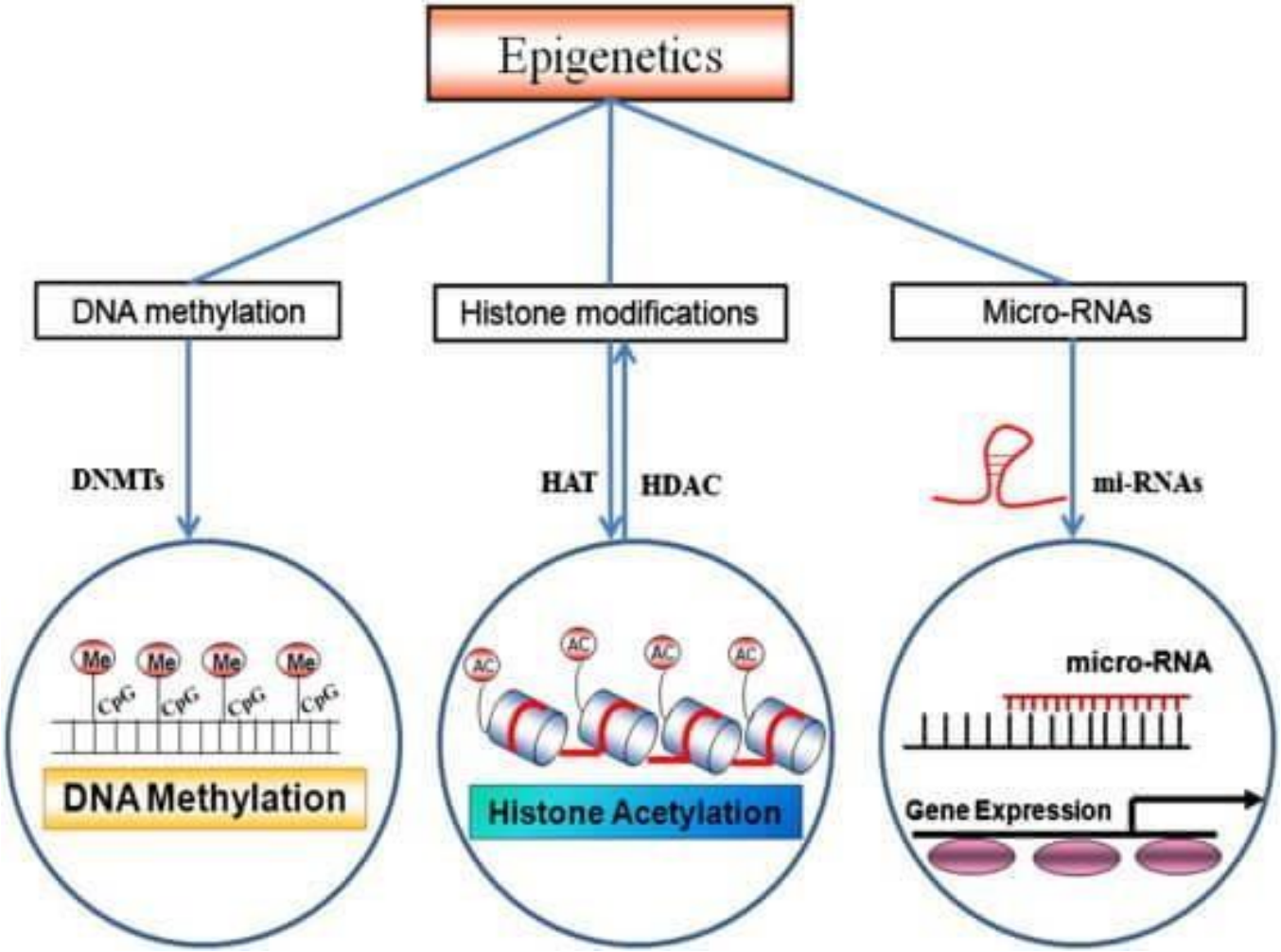
Epigenetics

- Epigenetics is defined as the study of heritable changes of DNA, not involving changes in a DNA sequence, that regulate gene expression (Dunn et al., 2003; Jain, 2003).

OR....

- Epigenetics is the study of heritable changes in gene expression that do not require, or do not generally involve, changes in genomic DNA sequence.

Type of Epigenetics

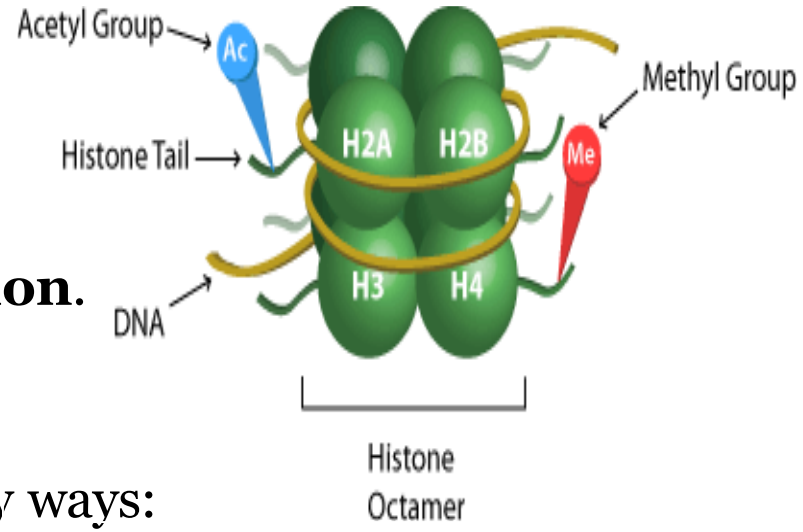


Epigenetic regulation of transcription

- These modifications affect the structure and conformation of chromatin and, consequently, transcriptional regulation.
- Epigenetic alterations in gene expression do not cause a change in the nucleotide sequence of the DNA and therefore are not mutations.
- Two types of epigenetic mechanisms will be discussed: histone modifications and DNA methylation

Histones

- Histones are subject to diverse post- translational modifications such as:
  - acetylation, - methylation,
  - Phosphorylation - ubiquination.
- The addition of **ubiquitin** to a substrate protein is called **ubiquitination**.

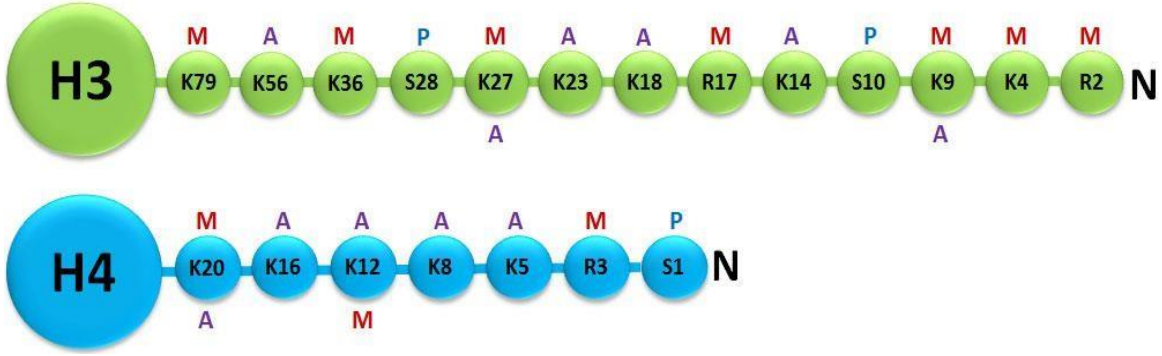


- **Ubiquitination** affects proteins in many ways:
  - it can mark them for degradation via the proteasome,
  - alter their cellular location,
  - affect their activity, and
  - promote or prevent protein interactions

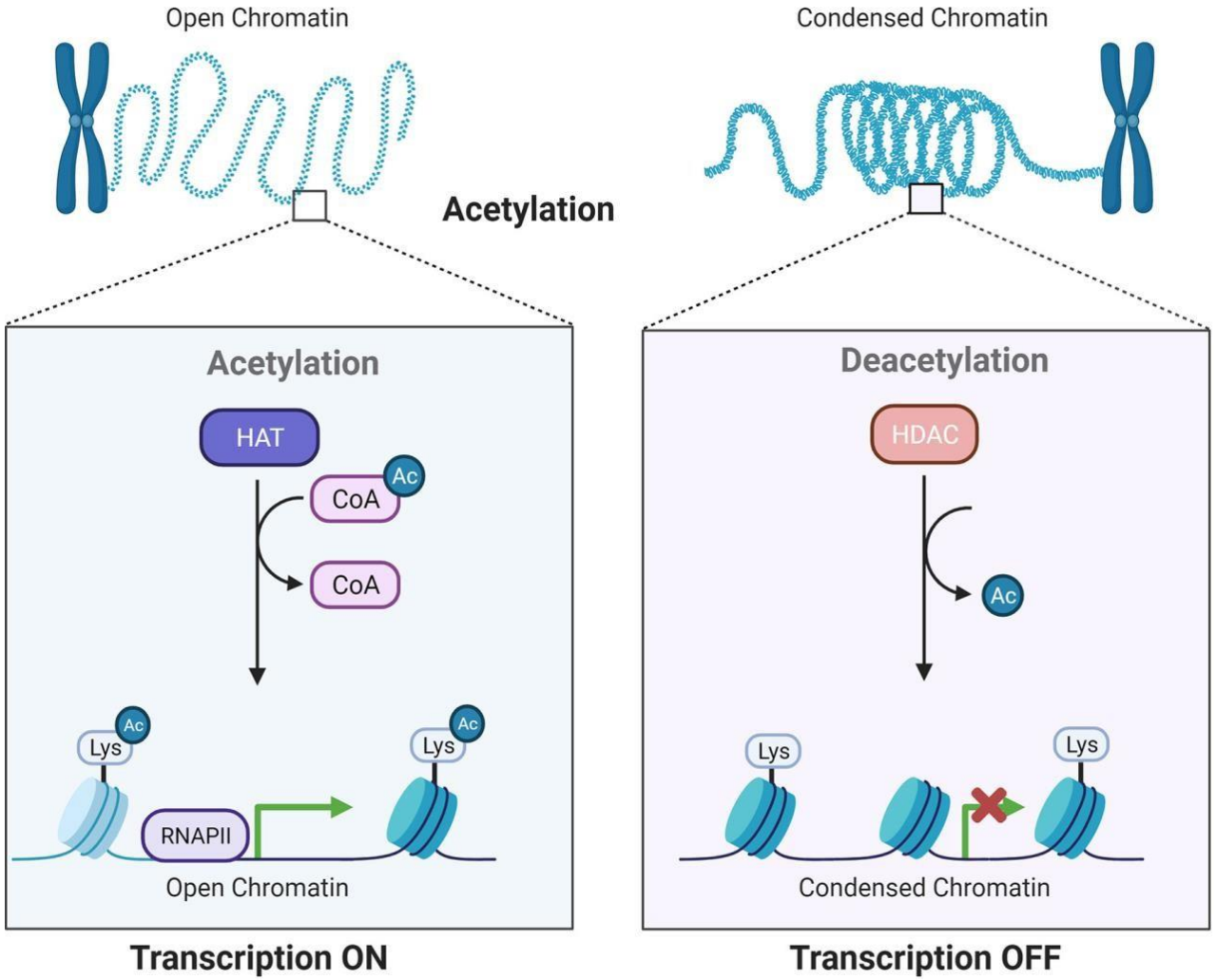
Histone modification

1- Histone acetylation/ deacetylation

- The mechanism for acetylation and deacetylation takes place on the NH_3^+ groups of Lysine amino acid residues.
- These residues are located on the tails of histones that make up the nucleosome of packaged dsDNA.



N-terminal tail modifications of H3 and H4. M=methylated, A=acetylated, P=phosphorylated.

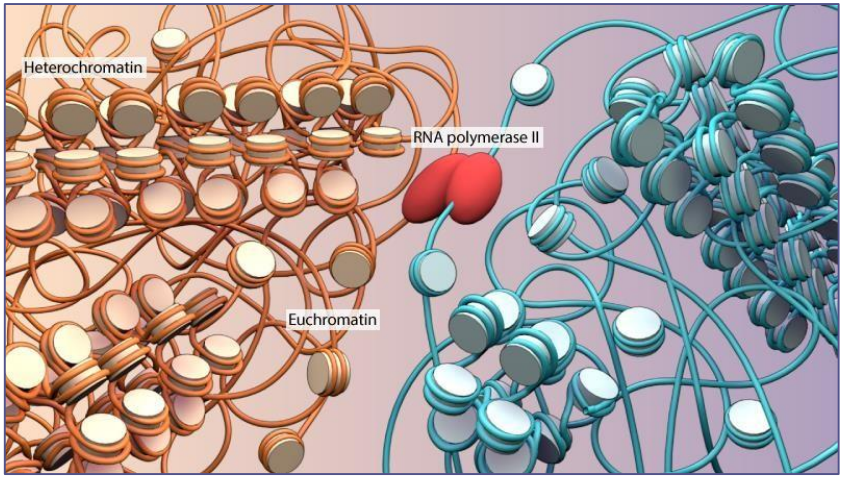


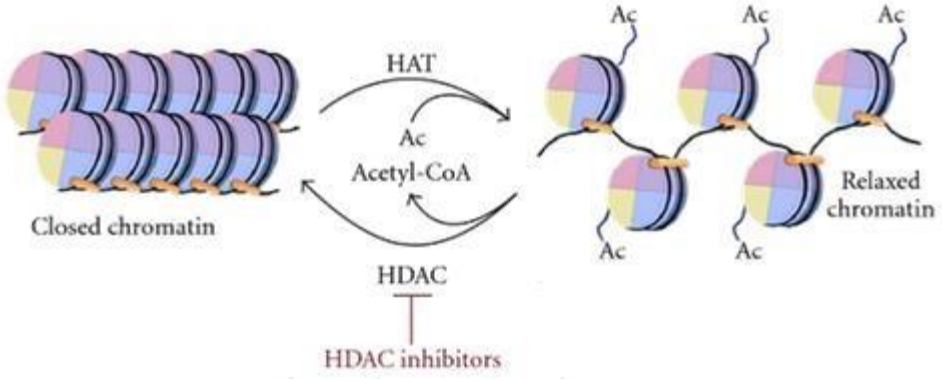
Acetylation by HATs and deacetylation by HDACs influence gene transcriptional activity. HATs and HDACs add or remove acetyl groups at the N–terminus lysine, which leads to an open or condensed state of the chromatin.

Mechanism of action

- The acetylation pattern of histones alters chromatin structure and affects gene expression.
- Acetylation has the effect of changing the overall charge of the histone tailfrom positive to neutral.
- Nucleosome formation is dependent on the electrostatic interactions between negatively charged DNA and positively charged histones.
- **Acetylation of the histone tails** disrupts this association, leading to weaker binding of the nucleosomal components.
- By this way, the DNA is more accessible and leads to more transcription factors being able to reach the DNA.
- Thus, acetylation of histones is known to increase the expression of genes through transcription activation.
- An imbalance in the equilibrium of histone acetylation has been associated with tumorigenesis and cancer progression.

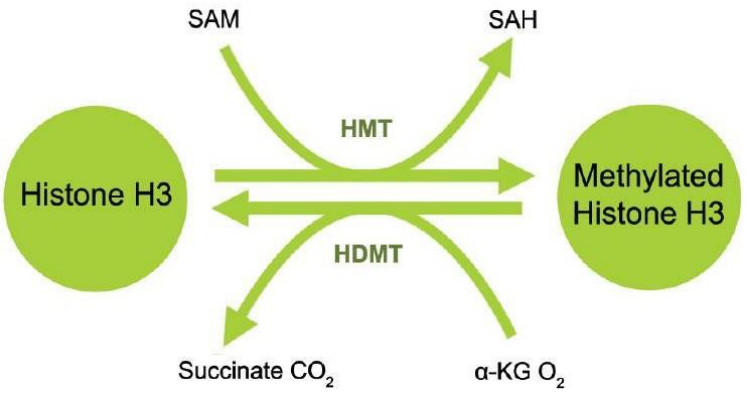
- Histone acetyltransferases (HATs; add acetyl groups) and histone deacetylases (HDACs; remove acetyl groups) are two families of enzymes that produce the pattern.
- Acetylation of histones relaxes chromatin folding and this correlates with enhanced transcriptional elongation by RNA polymerase II.
- HDACs remove acetyl groups and restore a positive charge to lysine residues of the histone tails which stabilize chromatin compaction and higher-level packaging.





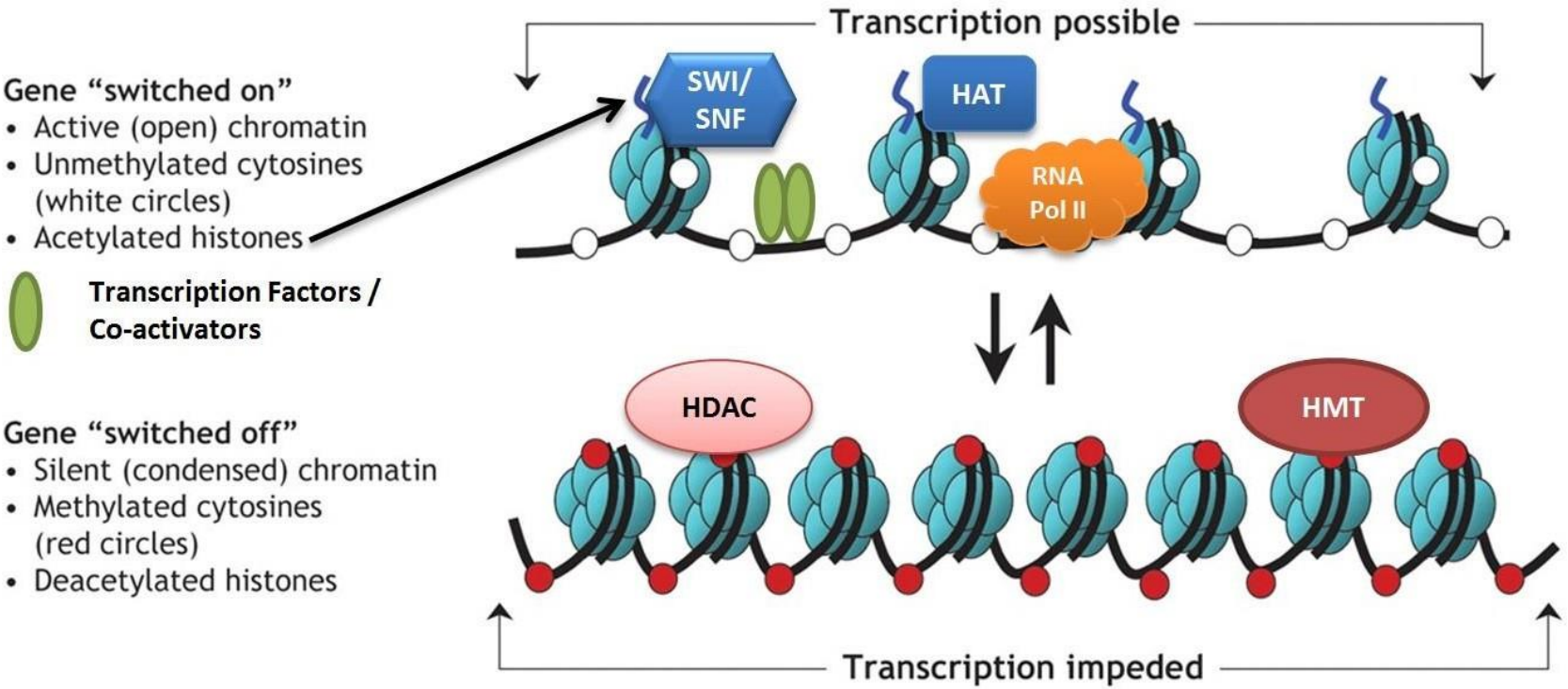
2- Histone Methylation/Demethylation

- Histone methylation is defined as the transfer of one, two, or three methyl groups from S-adenosyl-L-methionine (SAM) to lysine or arginine residues of histone proteins by histone methyltransferases (HMTs).
- Depending on which residual to catalyze on, there are two types of HMTs: histone lysine N-methyltransferase (KMT) and histone arginine N- methyltransferase.
- Methylation (off) and demethylation (on) of histones turns the genes in DNA "off" and "on," respectively, either by loosening their tails, thereby allowing transcription factors and other proteins to access the DNA, or by encompassing their tails around the DNA, thereby restricting access to the DNA.



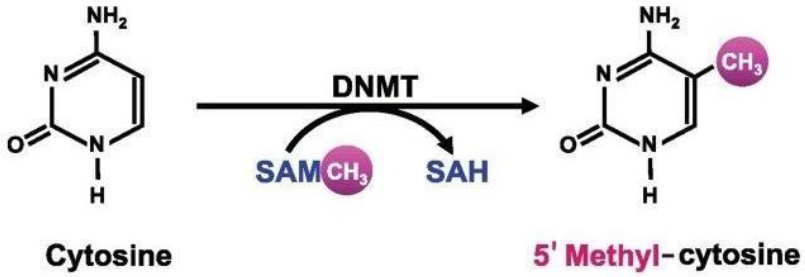
- In the cell nucleus, when histone methylation occurs, specific genes within the DNA complexed with the histone may be silenced.

- Histone demethylation is the removal of methyl groups in modified histone proteins via histone demethylases.
  These demethylases have been found to have potential oncogenic functions and involvement in other pathological processes.



DNA methylation

- Another epigenetic process that affects transcriptional regulation is DNA methylation. DNA methylation is the addition of a methyl group to position 5 of cytosine.
- DNA methylation involves transfer of a methyl group from SAM to cytosine.

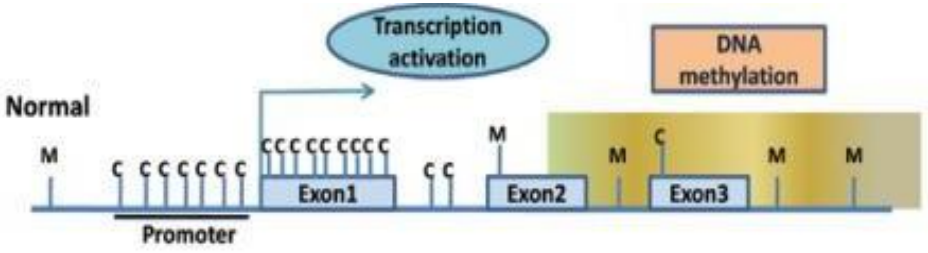


- Due to its significance, DNA cytosine methylation is considered by many as a fifth, 'forgotten' base of DNA.
- Only 3–4% of all cytosines in DNA are methylated.

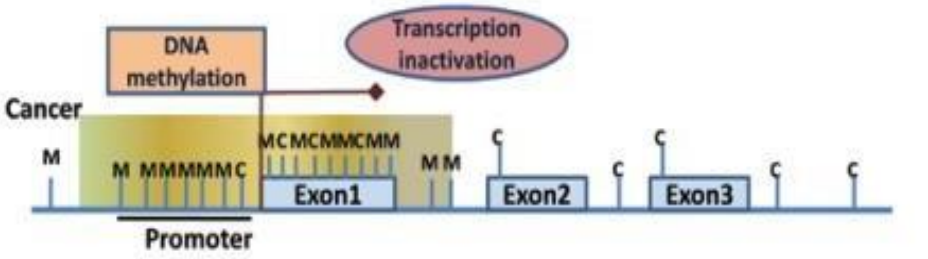
- DNA methyltransferases or DNMTs are the enzymes involved in DNA methylation .
- There are 4 DNMTs (DNMT1, 2, 3A, 3B).
  - DNMT1 ensures the methyl mark is added to the newly synthesized DNA.
  - DNMT2 methylates tRNA.
  - DNMT3a and DNMT3b are the enzymes responsible for establishing tissue-specific cytosine methylation *de novo* during embryonic development; such methylation is a decisive event in cell differentiation and development.
- The methylation of the DNA is thought to lead to transcriptional repression by inhibiting transcription factor binding to regulatory sequences,

CpG islands

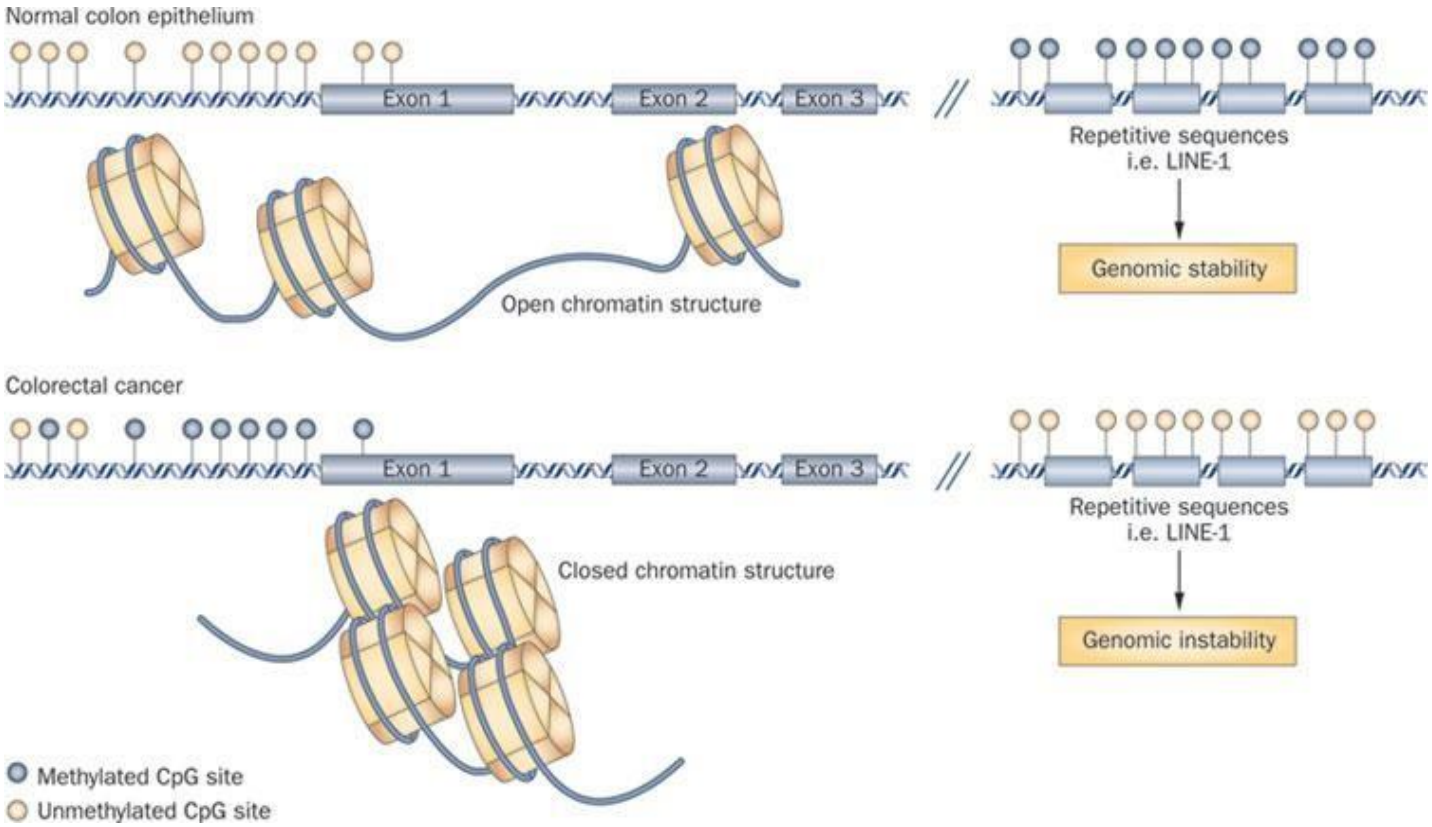
- CpG islands, are located in the promoter region of 50% of human genes.



- In general, the CpG islands found in gene promoter regions are not methylated in normal tissues and transcription may occur.



In normal cells, CpG islands in active promoters are not methylated, thus allowing transcriptional activation.
CpG islands within coding regions are often methylated. Reverse patterns are observed in cancer cells.

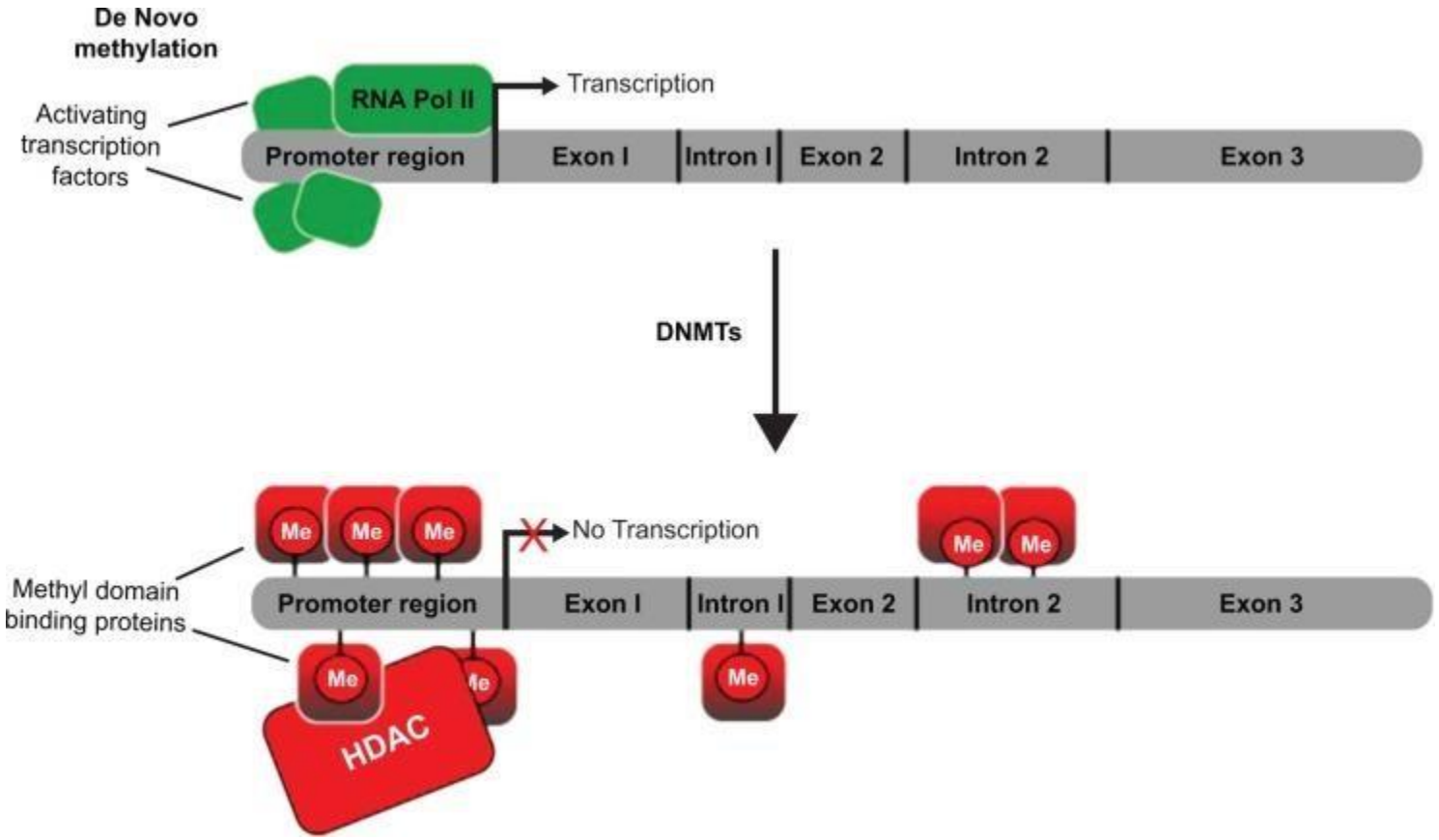


CpG island DNA hypermethylation and global DNA hypomethylation in colorectal cancer as compared with normal colonic epithelium.

Unmethylated CpG islands within the promoter region of genes are correlated with an open chromatin structure (euchromatin),

Methylated CpG islands are correlated with a condensed, closed chromatin structure (heterochromatin) and transcriptional silencing.

Normal colonic epithelium generally has unmethylated CpG islands in the promoter regions of genes, whereas aberrant hypermethylation of promoter associated CpG islands is a hallmark of neoplasms.



RNA polymerase II (RNA Pol II) and transcription factors bind to unmethylated (shown in green) upstream promoter region of a gene and transcription proceeds. Methylation (shown in red) of specific CpG sites near the promoter regions allow binding of histone deacetylases (HDACs) and other methyl domain-binding proteins that prevents RNA Pol II binding and inhibits transcription.

DNA methyl transferases (DNMTs) methylate CpG sites (not shown) within the promoter region. Methylation is also observed in the intragenic region, the function of which is still not well understood.

The Epigenetic Modifications and cancer (methylation/demethylation-Acetylation/ deacetylation in DNA and histones):

The chromatin consists of the nucleosome, which comprises a sequence of DNA wrapped around core histones. The cis elements existed in DNA sequences such as promoters are abnormally modified including promoter hypermethylation of tumor suppressor genes, promoter hypomethylation of oncogenes.

On the other hand, the aberrant core histone modifications such as deacetylation or site-specific methylations of histones on tumor suppressor genes, demethylation or acetylations of histone on oncogenes are co-operationally interactions with DNA alterations to interfere the gene expression and promote the carcinogenesis of endometrial cancer.