بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 14

Transcription Regulations pt.2

Written by : DST
Leen Alzoubi

Reviewed by : Leen Alzoubi

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الذي انتهى علمه إلى الإحاطة ببواطن الأشياء وخفاياها كما أحاط بظواهرها.

الورود: ورد في القرآن (٤٥) مرة.

الشاهد: ﴿ قَالَ نَبَّأَنِيَ ٱلْعَلِيمُ ٱلْخَبِيرُ ﴾ [التحريم:٣].

اضغط هنا لشرحٍ أكثر تفصيلًا

How are chromosomal structures altered?

The doctor start the lecture with some information about:
All things related to eukaryotic cells like human cells are more complex than bacteria because in Their genome they don't have histones or nucleosomes like human genomic structure.

- Change of compactness of the chromatin by:
  - Change the structure and position of nucleosomes
  - Chemically modify histones
    Acetylation, methylation, and phosphorylation
  - Chemically modify cytosine
  - Binding of noncoding RNAs to DNA

In eukaryotic cells, DNA does not exist as naked DNA; instead, it exists in the form of **chromatin**. Therefore, gene expression is controlled by modifying, modulating, changing, or altering the chromatin structure. And this is done by:

1. Chromatin remodeling factors

- These factors change the position of histones. Histones are organized as octamers within nucleosomes

2. Modifying enzymes of histones by acetylating histone molecules

3. Modifying cytosines

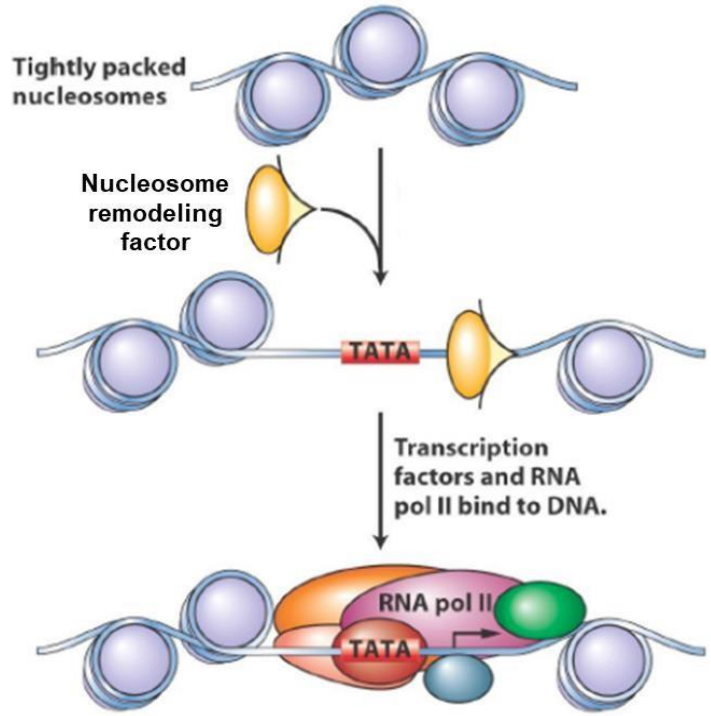
4. Non-coding RNA molecules

Change the structure and position of nucleosomes

Chromatin remodeling factors

- They facilitate the binding of transcription factors by
  - Removing histones from DNA
  - Repositioning nucleosomes making DNA sequences accessible
  - Altering nucleosome structure allowing protein binding to DNA

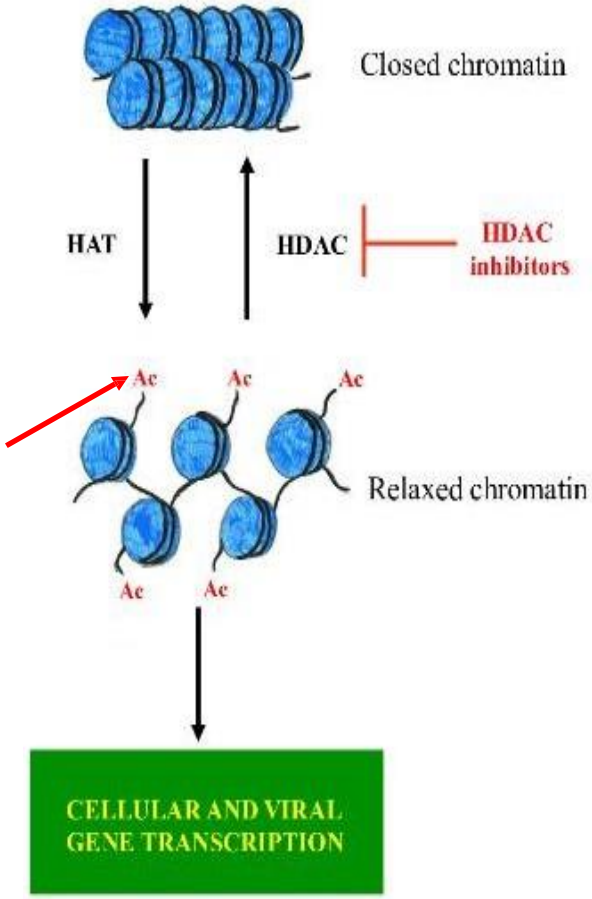
- Chromatin remodeling factors can be associated with transcriptional



Chemical modification of histones

Histone acetylation

- Histones bind non-specifically to DNA due to its negative charge.
- The components that are repeated in all nucleotides are the negatively charged phosphate groups. You can expect that histones have positively charged amino acids (lysine and arginine).



- The core histones (H2A, H2B, H3, and H4) have two domains (internal 3-dimensional structures):
  - A histone-fold, which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle.
  - An amino-terminal tail (indicated by a red arrow), which extends outside of the nucleosome, and is rich in lysine

Histone Acetylation

Basic concept

The tails of histone molecules contain a large number of lysine residues. Although histone proteins as a whole are rich in lysine and arginine residues, we are more concerned with lysine residues because they are the primary sites that undergo regulation and modification.

Type of modification: Acetylation

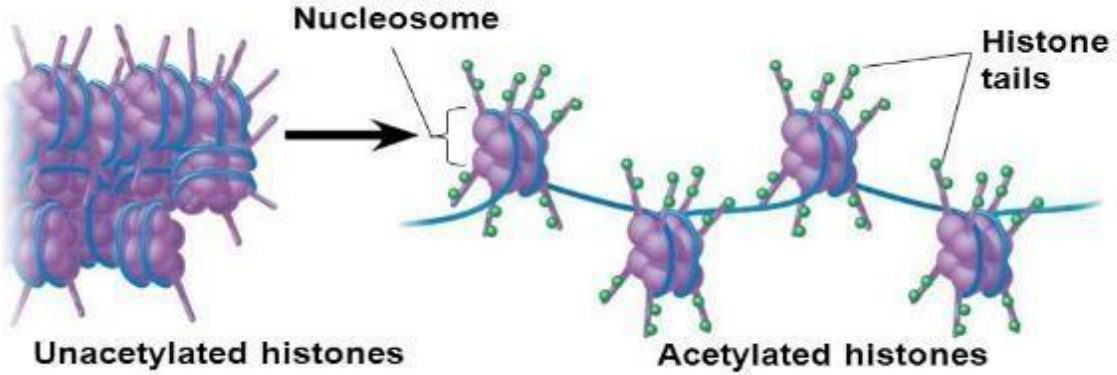
- Acetylation refers to the addition of an acetyl group to lysine residues on histone tails.
- Normally, lysine carries a positive charge. When lysine is acetylated, the positive charge disappears (lysine becomes neutral). Neutralization of lysine's positive charge weakens the histone–DNA interaction and leads to relaxation of DNA around the histones. Relaxation of DNA causes a structural change: DNA is transformed from heterochromatin (condensed, transcriptionally inactive) to euchromatin (open, transcriptionally active)

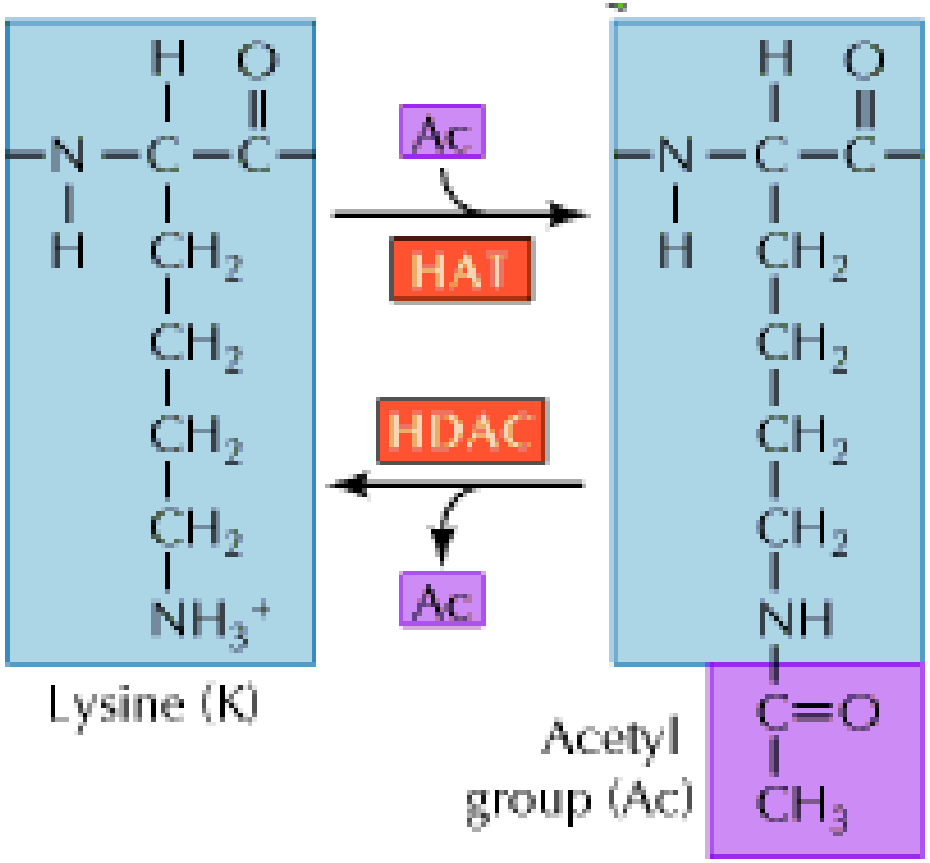
Effect on DNA accessibility

- As chromatin becomes relaxed:
  - DNA exposure increases and important DNA sequences become accessible, including Promoter regions, Promoter-proximal elements, Enhancer regions. Because these regulatory DNA sequences are exposed transcription factors (General transcription factors, Gene-specific (regulatory) transcription factors) can bind to DNA, inducing transcription

Acetylation of lysine

In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails. This generally loosens chromatin structure, promoting the initiation of transcription.

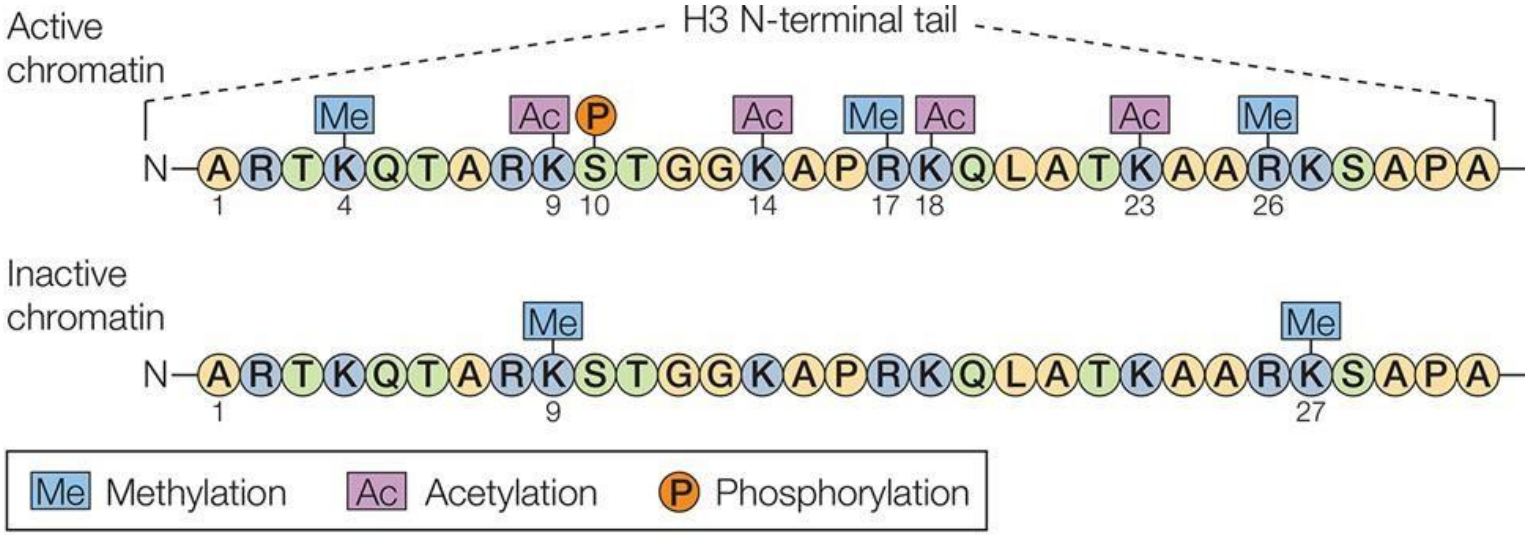




Other modifications of histones

- Histone can also be methylated or phosphorylated.
- The effect, whether transcriptional activation or repression, depends on the modification sites.
- Histone modifications can: (1) alter chromatin structure and (2) provide binding sites for other proteins that can either activate or repress transcription.

- The tails of histone proteins contain specific amino acid residues that can be modified.
- These residues can undergo several types of modifications, including Acetylation, Methylation, Phosphorylation
- Acetylation of lysine residues Causes relaxation of chromatin and is generally associated with increased gene expression
- The effects of phosphorylation and methylation are not fixed.
- Their outcome Depends on the specific gene and DNA sequence. These modifications may lead to either activation of gene expression or suppression of gene expression depending on the transcription factor that will bind (it may be a depressor or an activator).



- The symbol of lysine is K

General structure of TFs

- There are about 2000 transcription factors encoded in the human genome, that is 10% of protein-coding genes.
- Positive transcription factors have at least two independent domains:
  - DNA-binding domain
  - Activation domain or functional domain

- What is a domain?
  - A three-dimensional structure that is part of a protein's structure. It forms independently of the rest of the protein and usually has a function.
  - In other words, it can be separated from the protein and still be functional.

Characteristics of Protein Domains

1)Definition of a domain

- A domain is a super-secondary structure (It is made up of multiple secondary structural elements, such as α-helices and β-strands. Domains are often rich in α-helical structures)

2)Functional nature of domains

- Domains are usually functional units. Each domain typically performs a specific function within the protein

3)Independent folding of domains

- Domains fold independently of the rest of the protein. If a domain is separated from the protein it can fold on its own maintaining its function

Activating Transcription Factors: Activating transcription factors consist of two main domains:

1. Activation domain

- Contains the enzymatic activity
- Interacts with other transcription factors

2. DNA-binding domain

- Responsible for binding to DNA

DNA binding domain

DNA-binding domain:

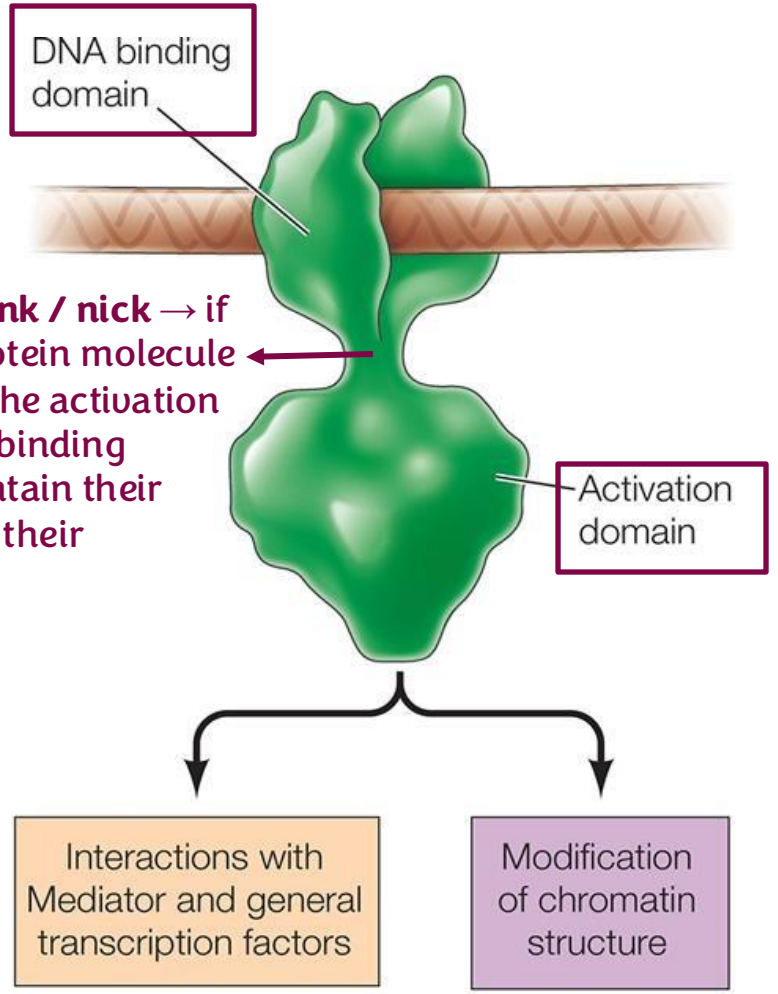
- It binds directly to DNA.
- The amino acid sequence of the DNA-binding domain specifies and determines the exact DNA sequence where the interaction between the transcription factor and DNA occurs.

Transcription factors and sequence specificity:

- Each transcription factor binds to specific DNA sequences with specific promoters.
- Because each transcription factor recognizes its own DNA sequence, this results in gene-specific regulation of transcription.
- Each gene has its own promoter proximal elements.
- These elements are essential sites through which regulation of gene expression occurs.

The activation domains

- Activation domains stimulate transcription by
  - interacting with Mediator proteins and general transcription factors, such as TFIID, to recruit the RNA polymerase and facilitate the assembly of a transcription complex on the promoter
  - Modifying chromatin



Extension / link / nick → if we cut the protein molecule right here → the activation and the DNA-binding domains maintain their structure and their function.

Activation Domain

- The activation domain interacts with other transcription factors (activators) and these protein-protein interactions help stimulate transcription.
- The activation domain is associated with enzymatic activities that cause modification of DNA (such as cytosine modification, histones modification, and it also participates in chromatin remodeling activity that changes the position of histone molecules within nucleosomes, making DNA more accessible for transcription.)

The previous slides illustrate the function of transcription factors. We have two significant transcription factors:

1. Transcription factor 2D **(TFIID)**
2. Transcription factor 2H **(TFIIH)**: It has kinase activity, which phosphorylates the C-terminal domain (CTD) of RNA polymerase, enhancing its activity.

- The second enzymatic activity is helicase activity, which unwinds the DNA, creating the open promoter complex and exposing single-stranded DNA.

Each cell responds to insulin in its own specific way. The reason for this is that different cells contain different transcription factors, which determine which genes will be expressed in response to insulin signaling.

Insulin Signaling Pathway and Regulation of Gene Expression

What is the story?

- Insulin binds to its cell-surface receptor and activates it --> Activation occurs at the cytosolic portion of the receptor, which contains a kinase domain --> This kinase domain phosphorylates certain intracellular proteins --> From these proteins, some will activate RAS --> RAS activates RAF --> RAF activates MEK --> MEK activates ERK --> ERK enters the nucleus --> Inside the nucleus, ERK phosphorylates transcription factors (TFs) --> These activated transcription factors bind to DNA --> Transcription factors bind DNA at specific sequences (promoter region or promoter proximal elements) which leads to the expression of specific genes.

Tissue-Specific Gene Expression in Response to Insulin

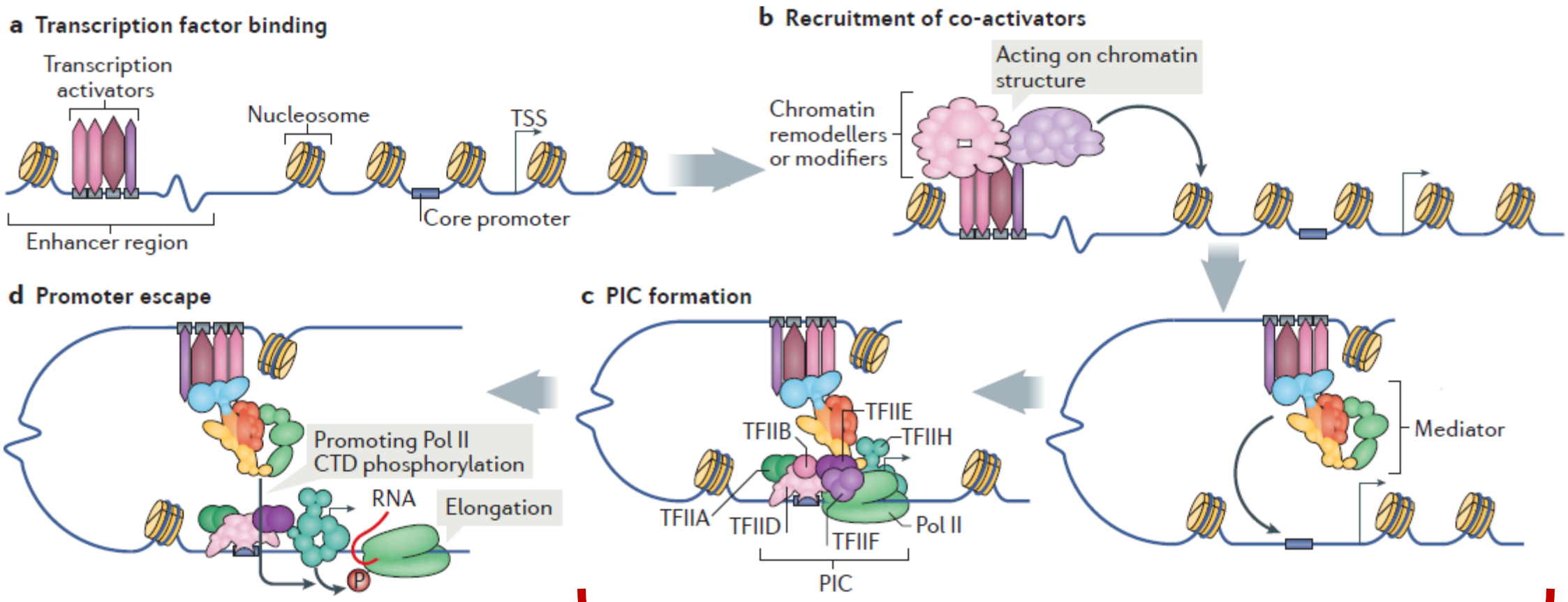
Although the signaling pathway is similar, the genes expressed differ by tissue:

- **In the liver:**
  - The expressed genes are those that activate glycogen synthesis (Glucose → Glycogen).
  - There is also expression of glucose transporters, which transport glucose into the cell.
- **In muscle:**
  - Some genes promote conversion of a small amount of glucose to glycogen.
  - However, most expressed genes are those that produce energy, including genes that stimulate: Glycolysis, Krebs cycle, Electron transport chain (ETC), ATP production (overall energy production)
- **In fat cells:**
  - The expressed genes are genes that store fat.

A model of transcriptional activation

1) Binding of transcriptional activators to the transcriptional regulatory regions(s) (e.g. enhancer or promotor proximal element)

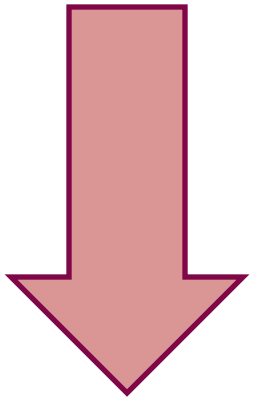
2) Recruitment of chromatin remodeling factors



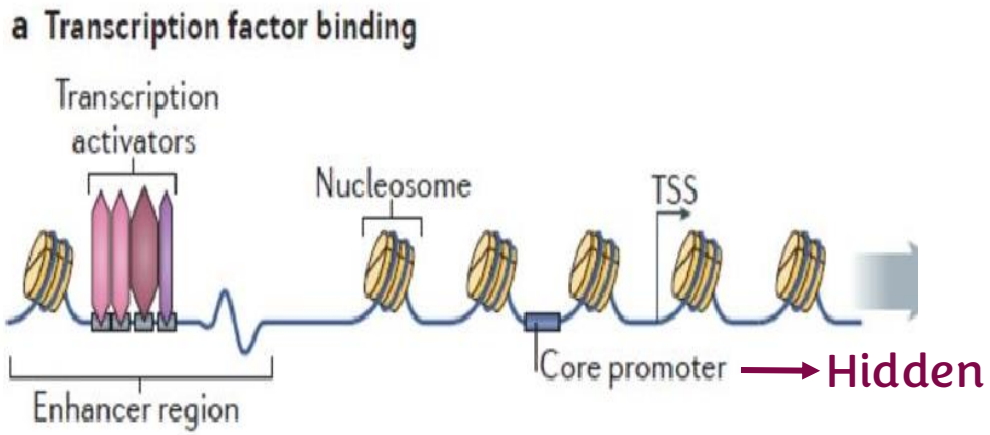
4) Phosphorylation of RNA polymerase II

3) Formation of pre-initiation complex

This is how gene regulation occurs in eukaryotic cells by modifying DNA's chromatin structure :



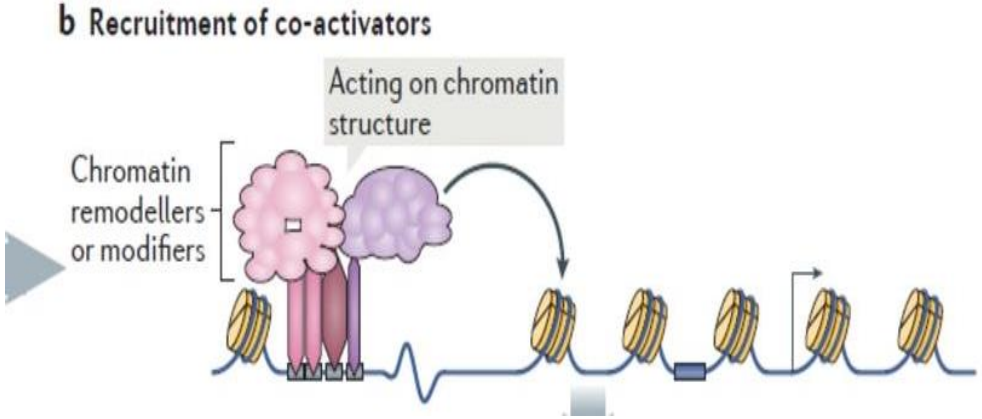
1) Binding of transcriptional activators to the enhancer region



1)Initial state (inactive gene)

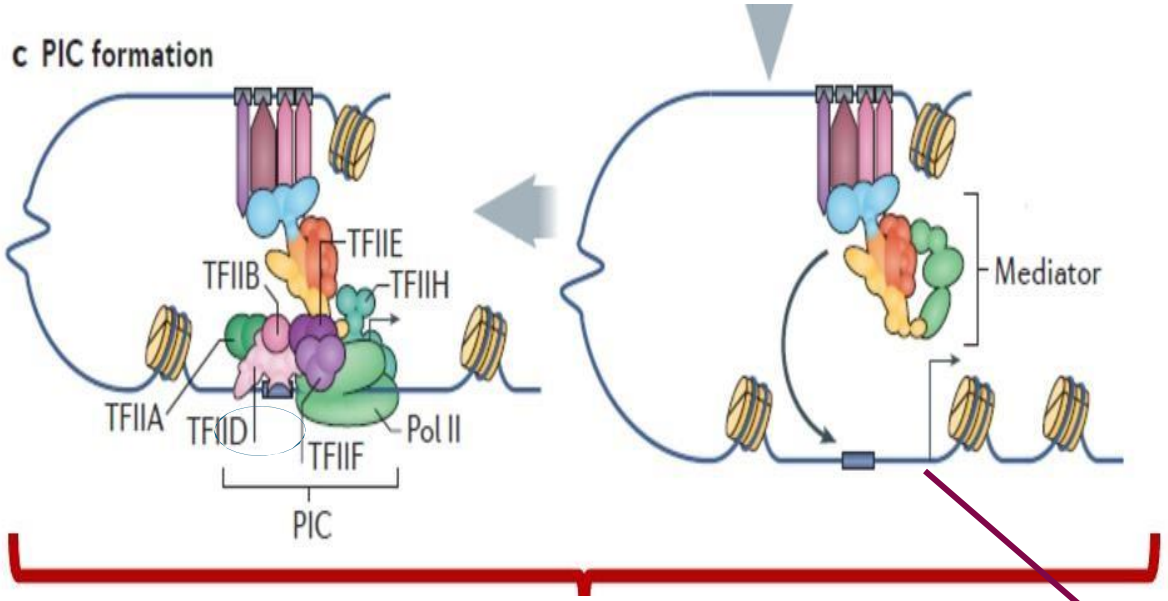
- Proteins may be bound to the enhancer region, but they are not active and therefore are not doing anything.
- The transcription start site (TSS) and the promoter region are hidden within nucleosomes, hidden by histone proteins

2) Recruitment of chromatin remodeling factors



2)When cell signaling occurs, it leads to:

- Activation of chromatin remodeling factors, or
- Activation of transcription factors that possess enzymatic activity. These transcription factors may have acetyltransferase activity, leading to acetylation of histones other modifications can also cause phosphorylation or methylation of histones
- As a result, the chromatin structure changes from heterochromatin to euchromatin, which exposes the promoter region.

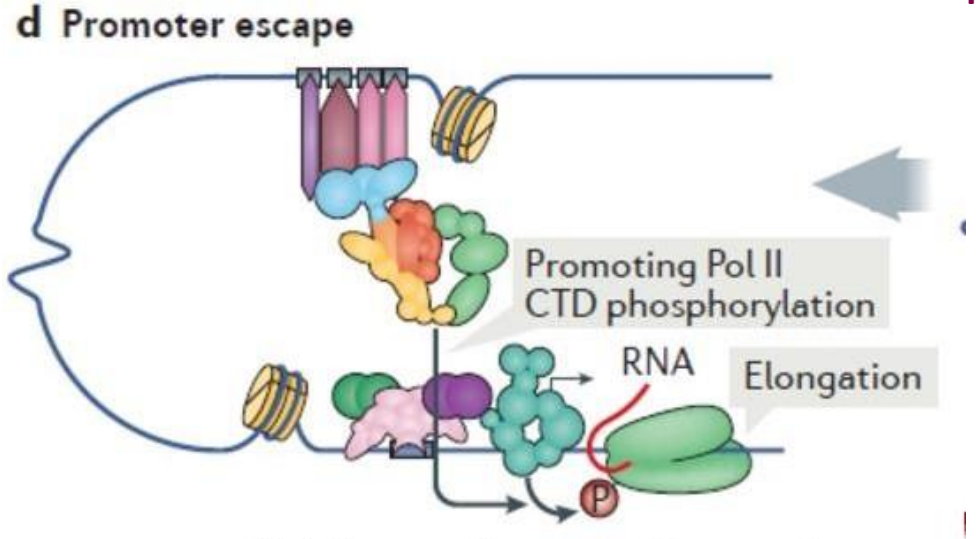


3) Formation of pre-initiation complex

3) Binding of transcription factors and complex formation

- Transcription factors bind to the enhancer region. The enhancer region forms a DNA loop, bringing it into proximity with the promoter region allowing these transcription factors to bind to the promoter region. This leads to the formation of the pre-initiation complex

Exposed promotor region



4) Phosphorylation of RNA polymerase II

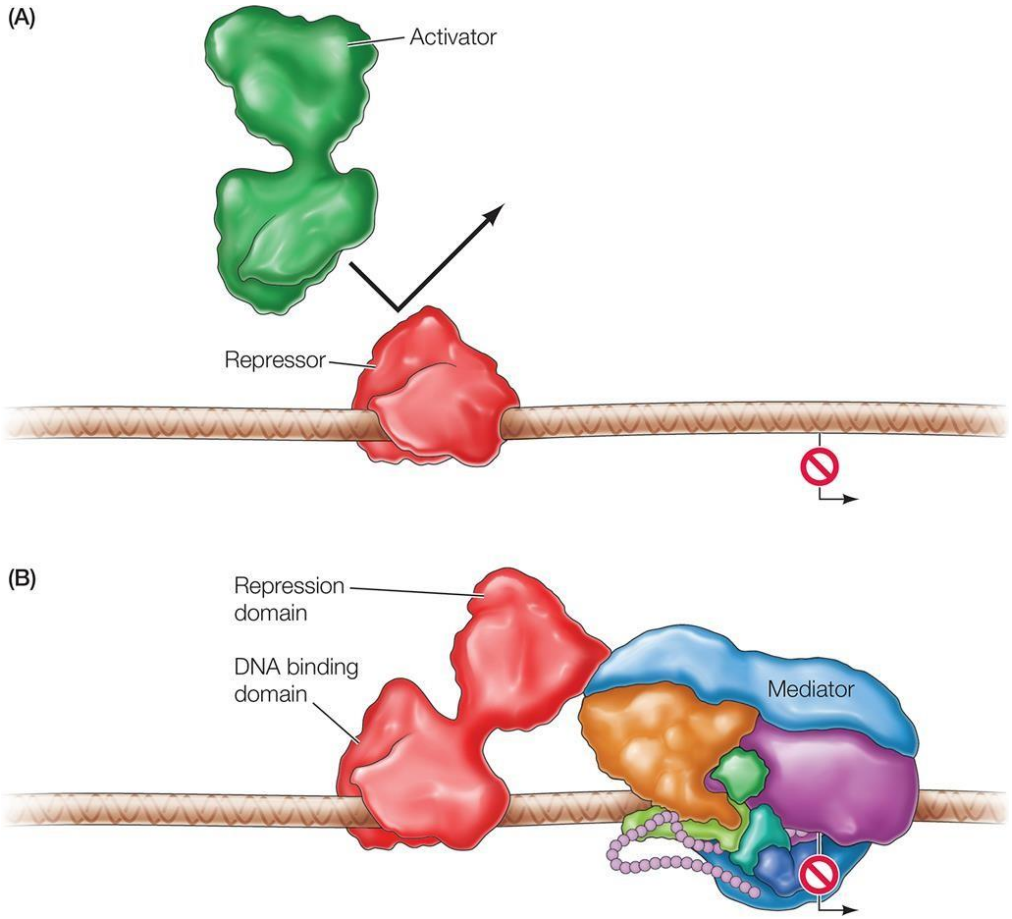
4) Activation of gene expression

- Formation of the pre-initiation complex results in activation of gene expression.

Eukaryotic repressors

- (A) Some repressors block the binding of activators to regulatory sequences

- (B) Other repressors have active repression domains that inhibit transcription by interactions with Mediator proteins or general transcription factors, as well as with corepressors that act to modify chromatin structure.



Eukaryotic Repressors (Two Types)

A) Repressors with only a DNA-binding domain

- These repressors have only a DNA-binding domain. They bind directly to DNA and prevent activators from binding to the same DNA sequences. In this way, they function as competitive inhibitors, blocking transcriptional activation without modifying chromatin.

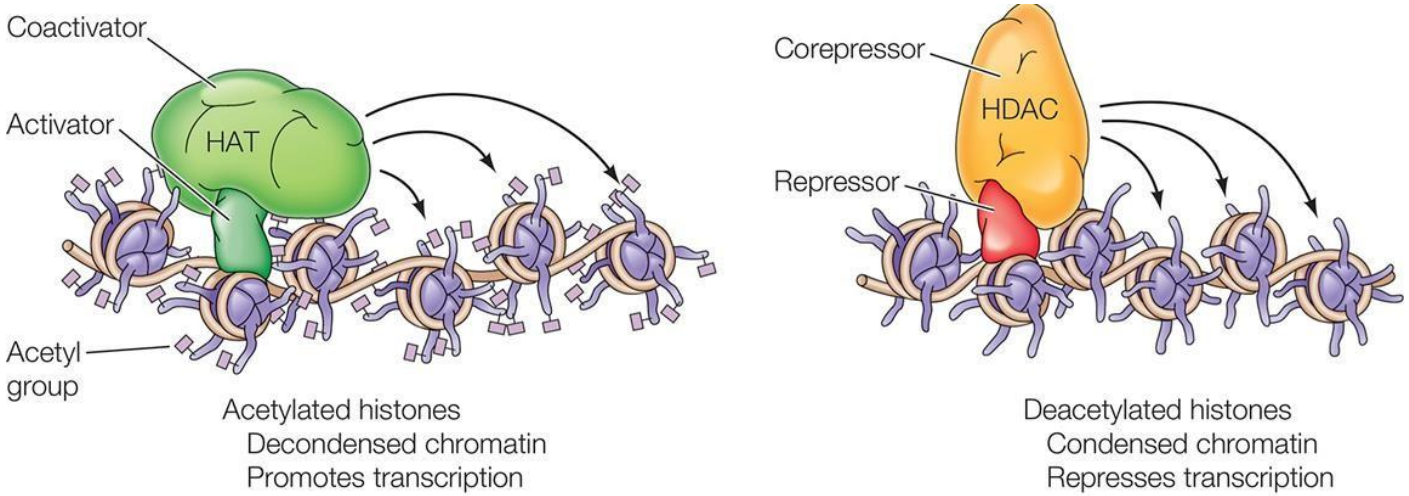
B) Repressors with an additional repressor domain

- These repressors have:
  - A DNA-binding domain, and
  - A repressor domain.
- The repressor domain causes deacetylation of histones, which restores the positive charge on lysine residues strengthens histone-DNA interactions in this way it converts chromatin from euchromatin to heterochromatin. This chromatin condensation leads to inactivation of gene expression.
- Repression can also involve other modifications such as Methylation, Phosphorylation, or any modification that results in suppression of gene expression.

Enzymatic association

- Transcriptional activators and repressors are associated with coactivators and corepressors, which have histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities, respectively.
  - Histone acetylation is characteristic of actively transcribed chromatin.
  - **TFIID associates with histone acetyltransferases.**

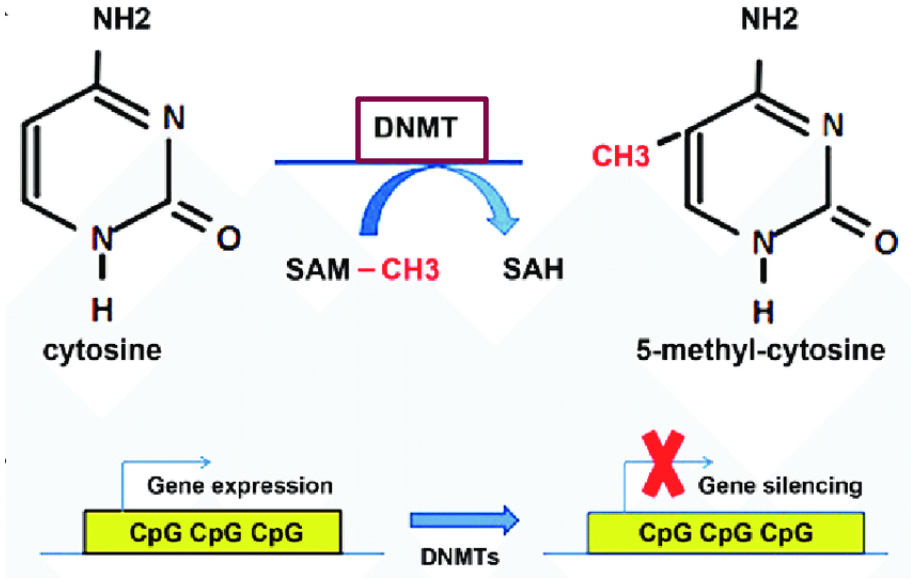
The first transcription factor that is recruited is **TFIID**; when it binds to the promoter region, it has histone acetyltransferase activity, leading to acetylation of histones and causing relaxation of the promoter region. As a result of this chromatin relaxation, other transcription factors along with RNA polymerase are subsequently recruited, and transcription begins.



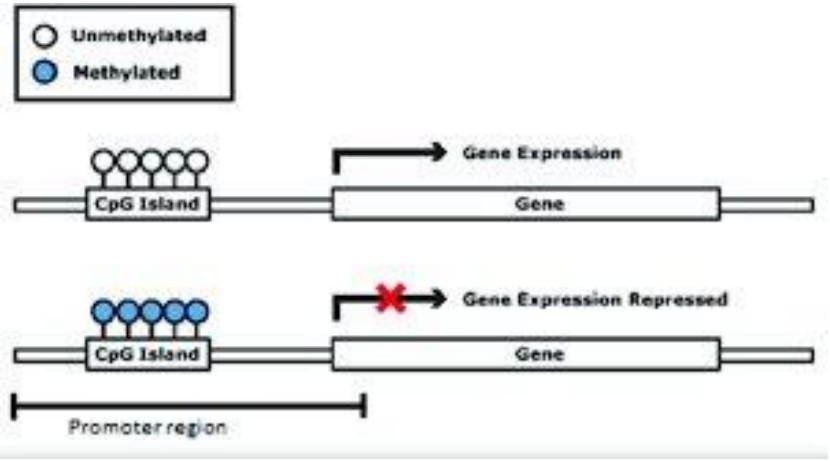
Chemical modification of cytosine

DNA methylation

- Cytosine residues can be methylated at the 5'-carbon position specifically at CG sequences (called CpG islands near promoters).
- DNA methylation reduces gene transcription by blocking of activator binding to DNA and inducing heterochromatin formation.



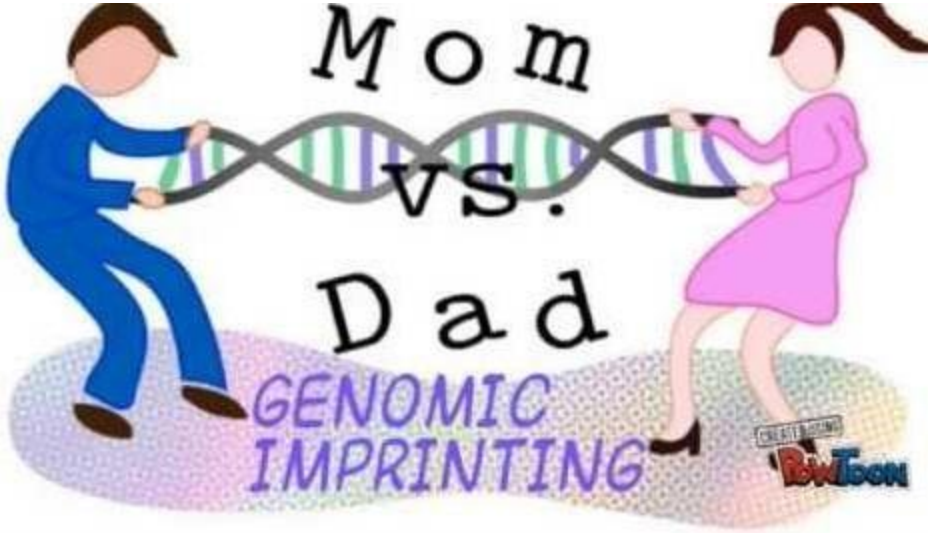
Cytosine can be methylated in the promoter region, which contains CpG islands—these are DNA sequences rich in cytosine (C) and guanine (G); in this region, cytosine can be methylated by a DNA methyltransferase, leading to promoter silencing so that transcription factors cannot bind to it. **In cancer,** this regulation can occur either through cytosine methylation of tumor suppressor gene promoters, resulting in no expression of tumor suppressor genes, or through demethylation of oncogenes, which causes them to be overexpressed and leads to cancer development without the presence of actual mutations in the DNA.



Genetic imprinting

- Methylation is a mechanism of genomic imprinting (either the paternal genes or the maternal genes are active).
  - This is the case for 75 genes.
- Methylation is inherited following DNA replication.

Genetic imprinting refers to the situation in which, for some genes (approximately 75 genes), either the paternal gene or the maternal gene must be active, but not both. This program is established very early, at fertilization. The choice of which allele is active is not random; the paternal gene and the maternal gene are not equivalent, although the exact reason for this difference is still unknown. If the activity of the paternal and maternal genes were interchanged, this could lead to a genetic disease, and if both genes were active, this would also result in a genetic disease. This phenomenon is known to involve **cytosine methylation** in some way.

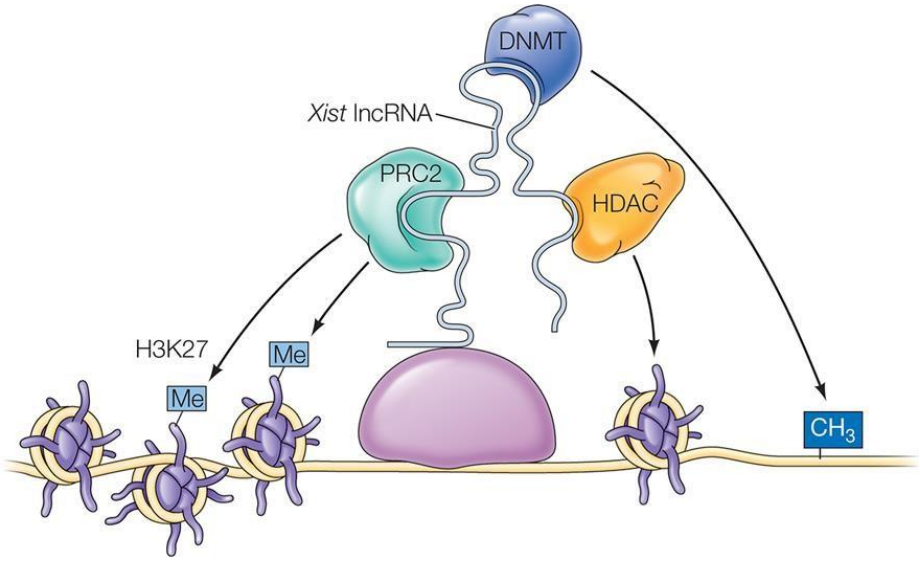


Binding of noncoding RNAs to DNA

Remember: **75%** of human DNA is transcribed. Only about **2%** of this transcribed DNA is protein-coding, while the remaining portion is transcribed into non-coding RNAs

Role of noncoding RNAs

- More than 50,000 long noncoding RNAs (lncRNA), which are >200 nucleotides long, are encoded by the human genome.
- LncRNAs can be homologous to certain DNA sequences and form complexes with chromatin and DNA modifiers to activate or repress gene expression via chromatin modification and histone methylation.
- LncRNAs can complex with general or specialized transcription factors (e.g. TFIIB), Mediator, or RNA processing proteins
- LncRNA can act in cis or trans



Non-coding RNA Molecules

Non-coding RNA molecules are classified as follows:

- Long non-coding RNAs (lncRNAs): more than 200 nucleotides in length.
- Short non-coding RNAs: less than 200 nucleotides in length.

Long Non-coding RNAs (lncRNAs): What Do They Do?

Our knowledge about lncRNAs is still limited.

- lncRNAs can be homologous to certain DNA sequences or complementary to them. This means they can bind to DNA, particularly at promoter regions or enhancers, and therefore regulate gene expression.
- RNA molecules do not have one universal or predefined structure because RNA is single-stranded and can base-pair with itself. Each RNA molecule has its own structure determined by its own sequence. However, each individual RNA molecule adopts one specific structure, similar to proteins.
- Because of this defined structure, lncRNAs can interact with proteins. These proteins may include histone acetyltransferases, histone deacetylases, methyltransferases, and others.
- Through these interactions, lncRNAs can bind to and modify DNA, leading to either activation or inhibition of gene transcription.

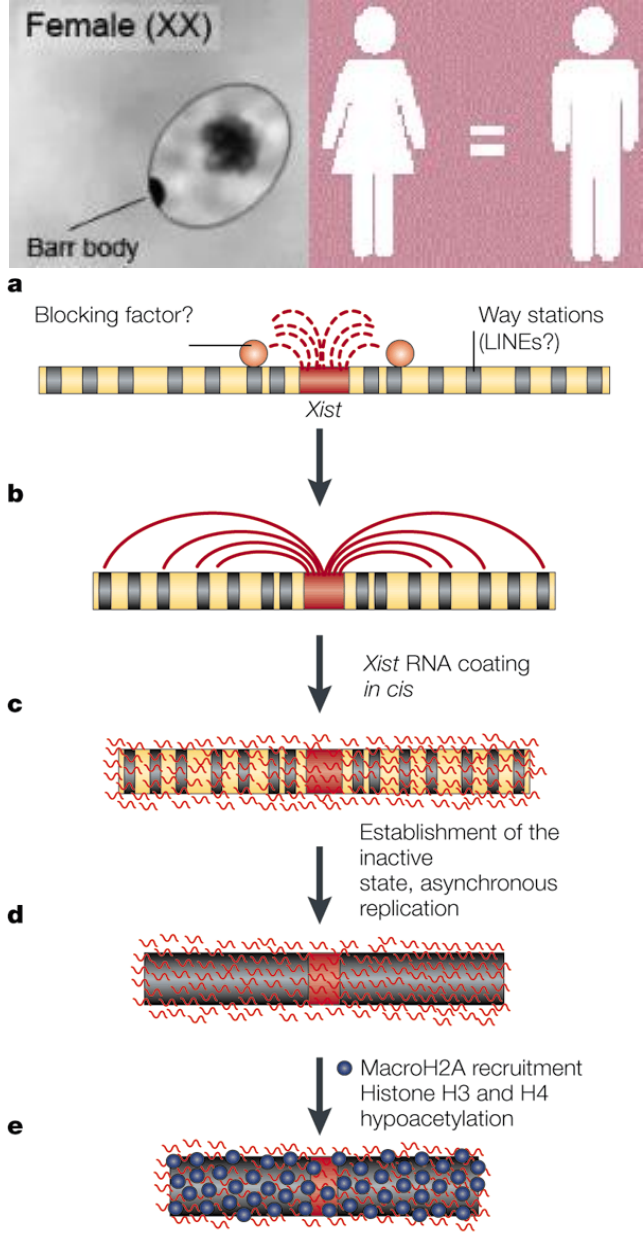
Cis and Trans Action of lncRNAs

- lncRNAs can act in cis or in trans.
  - Cis: lncRNAs act on the same chromosome from which they are produced.
  - Trans: lncRNAs are produced from one chromosome and act on another chromosome.

Example on the previous concept:

X chromosome inactivation

- A long noncoding RNA (lncRNA) is transcribed from Xist gene located on one of the two X chromosomes in females.
- The Xist RNA coats the X chromosome and promotes the recruitment of a protein complex that methylates histone 3 leading to chromosomal condensation.
- This results in X-chromosome inactivation in a phenomenon called **dosage compensation** to equate the number (and activity) of X chromosomes between males and females.



Nature Reviews | Genetics

X-Chromosome Inactivation

In females, there are two X chromosomes, whereas in males there is only one X chromosome. During development in all females, one of the X chromosomes becomes inactivated, so eventually each female cell has one active X chromosome. This process happens randomly and early in development.

What Does “Randomly” Mean?

If we look at an organ such as the liver, which is made up of billions of cells, and examine their X chromosomes, we will notice that in one patch, group, or cluster of cells, the first X chromosome is active, and the other is inactive. In another cluster, the opposite is observed. This means that X-chromosome inactivation is random.

This randomness has consequences: females can sometimes escape disease (dominant), or they can be affected by diseases (recessive).

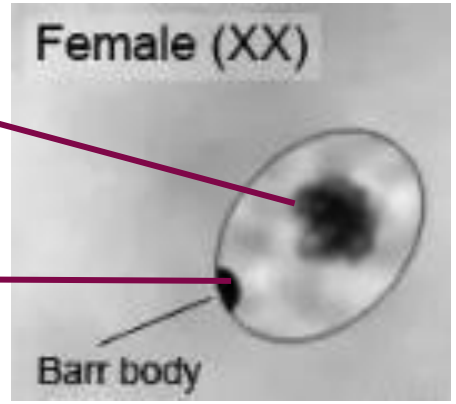
How Is the X Chromosome Inactivated?

There is a gene on the X chromosome called Xist. This gene is transcribed into a long non-coding RNA (lncRNA). The RNA does not go anywhere else; it stays in the same region and does not move to other chromosomes, meaning it acts in a cis-acting manner.

The Xist RNA coats the X chromosome, and this coating leads to methylation, phosphorylation, and deacetylation. As a result, the X chromosome shrinks and becomes inactivated.

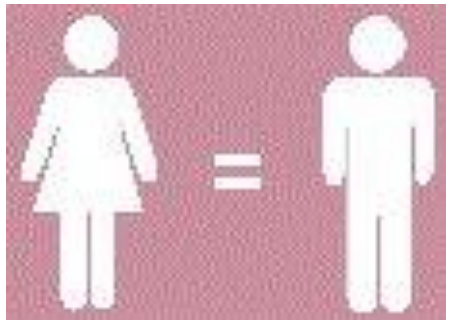
The Active X chromosome

The inactive X chromosome
also called Barr body



Dosage Compensation

This phenomenon is called dosage compensation. As a result, both males and females have one active X chromosome.

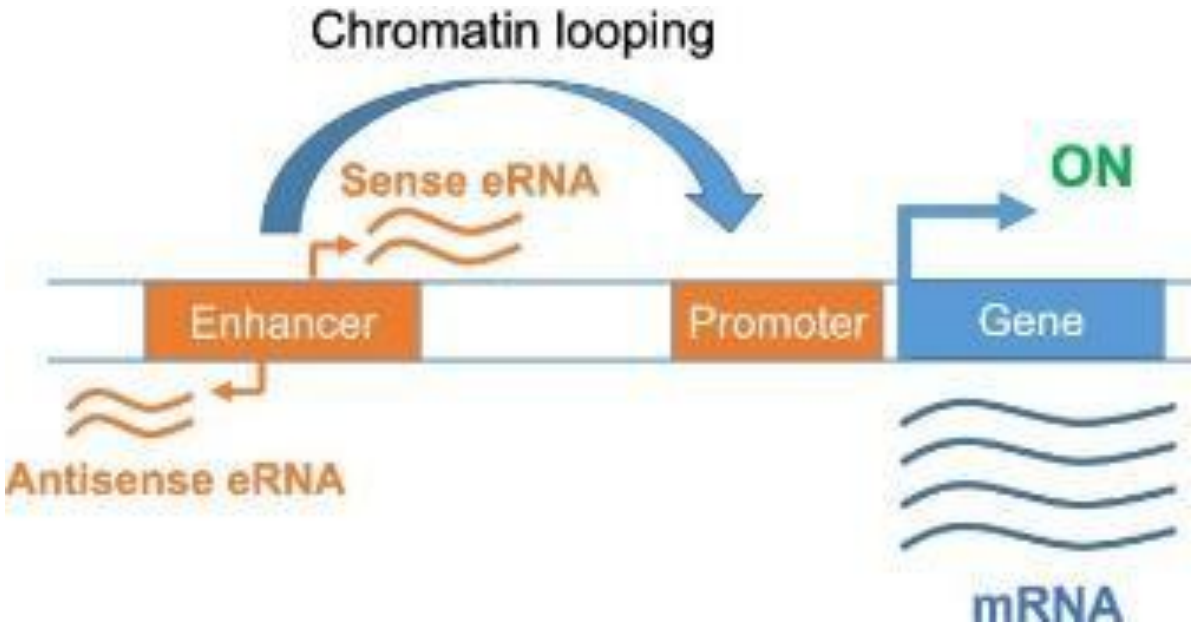


Enhancer RNA (eRNA)

- Some enhancers can be transcribed into RNA, hence called eRNA, that can regulate transcription of adjacent genes.

Enhancer RNA (eRNA)

- Sometimes, enhancers can be transcribed. How this happens is not yet known, and in some cases, promoters can also be transcribed.
- The resulting enhancer RNA (eRNA) can be complementary to the enhancer, and therefore it can regulate enhancers and promoters as well.
- Whether this transcription represents noise (a mistake) or has a functional role is still debatable



Identical twins have the exact same genetic information

But their epigenomes become increasingly different over time

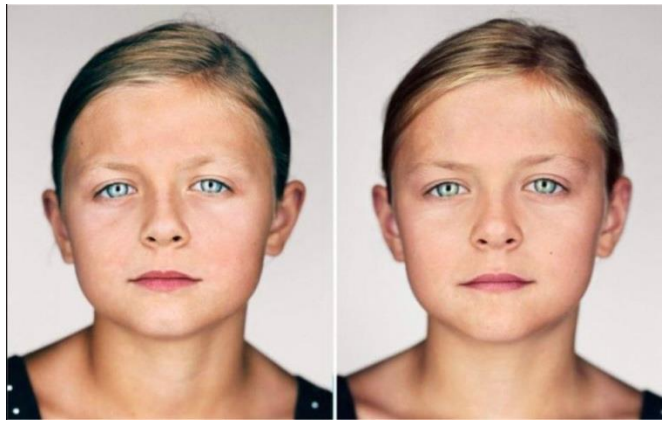
- Epigenetic changes can cause dramatic differences between twins, including many cases where one twin develops a disease and the other does not.

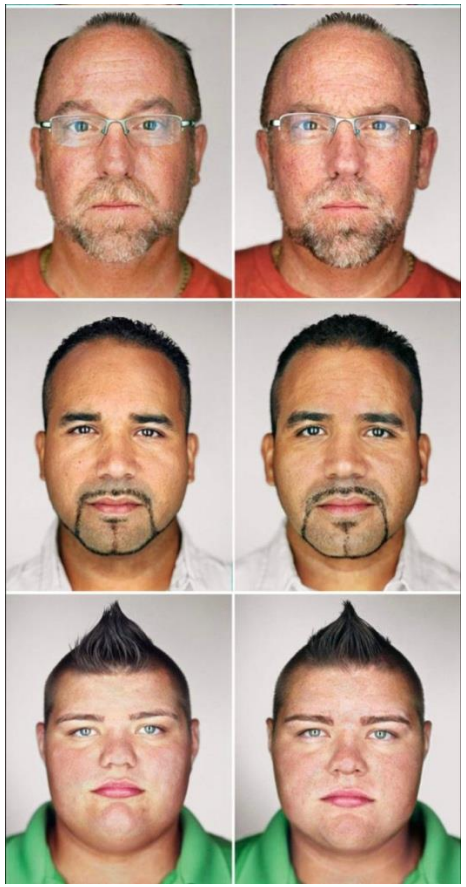
National Human Genome Research Institute

The power of epigenetics

Identical genetics but different colors ---->DNA modifications (epigenetics)

- Non-sequence dependent inheritance





Identical twins can show subtle differences. This happens even though they have the same DNA and are genetically identical, because they can be **epigenetically different**. These epigenetic differences depend on several factors, including:

- Environmental factors
- Lifestyle
- Stress

Epigenetic changes can be heritable or acquired.

Epigenetics is significant and heritable

PNAS

Stress-induced gene expression and behavior are controlled by DNA methylation and methyl donor availability in the dentate gyrus

Emily A. Saunderson[a,1], Helen Spiers[b], Karen R. Mifsud[a], Maria Gutierrez-Mecinas[a,2], Alexandra F. Trollope[a,3], Abeera Shaikh[a], Jonathan Mill[b,c], and Johannes M. H. M. Reul[a,4]

[a]Neuro-Epigenetics Research Group, University of Bristol, Bristol BS1 3NY, United Kingdom; [b]Institute of Psychiatry, King's College London, London SE5 8AF, United Kingdom; and [c]University of Exeter Medical School, University of Exeter, Exeter EX2 5DW, United Kingdom

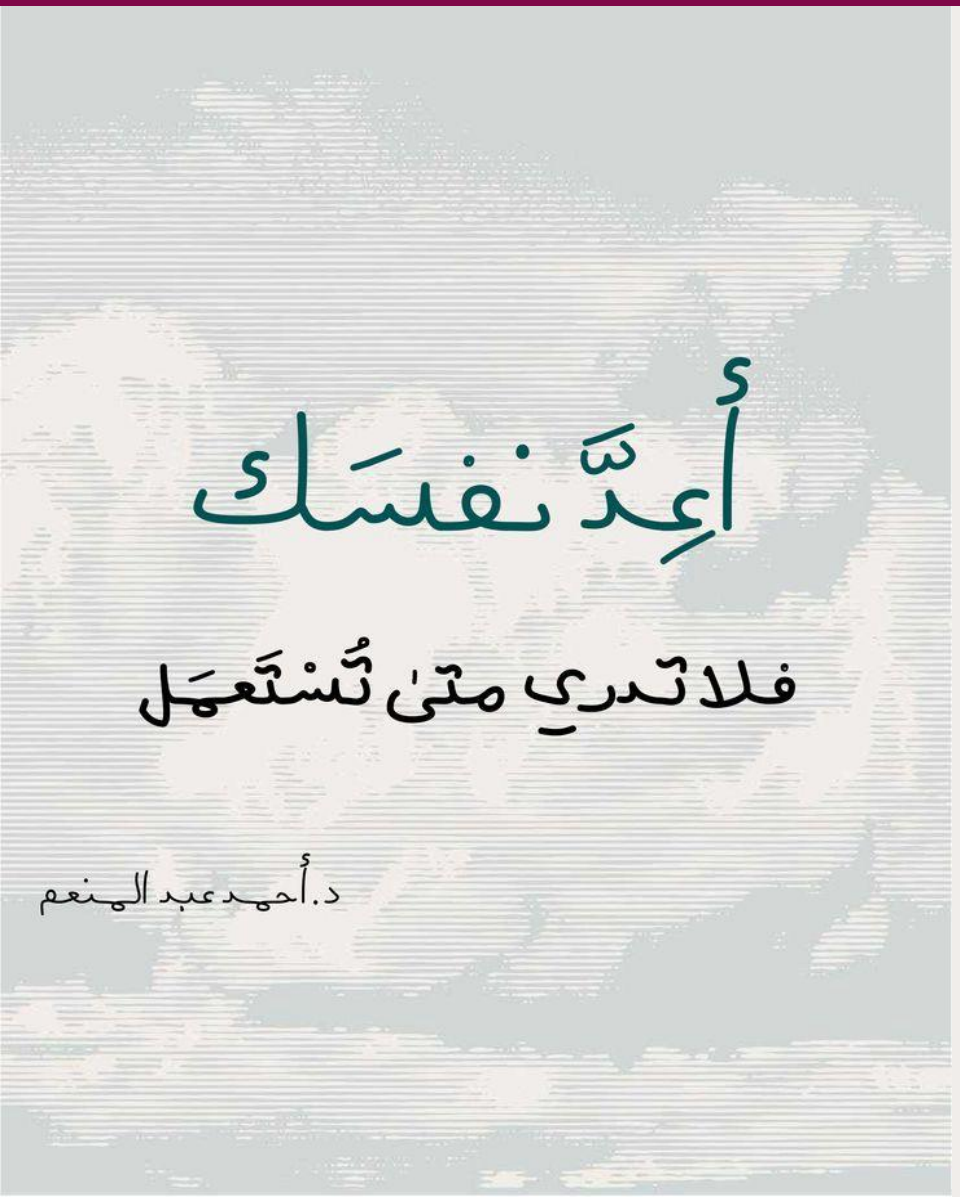
Your DNA Is Not Your Destiny

Having a mutation in DNA does not necessarily mean that you will develop the disease.

Take-home message

- Gene expression is regulated by regulatory proteins that would ultimately:
  - Guide the RNA polymerase (or other regulatory proteins) to the promoter
  - Strengthen/stabilize the RNA polymerase (or other regulatory proteins) binding to the promoter
  - Activate the RNA polymerase (or other regulatory proteins)
  - Create the open promoter complex for the RNA polymerase (or other regulatory proteins)
  - OR the opposite of the above in case of repressors.
  - All of the above effects are mediated via modulating non-covalent interactions between the amino acids of proteins and specific sequences of DNA.

رسالة من الفريق العلمي:



فالله إذا استعمل عبدًا هيّأه قبلها، وربّى فيه
ما يصلح به موضعُ الحاجة

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			