بسم الله الرحمن الرحيم

MOLECULAR BIOLOGY

FINAL – Lecture # 13

# Regulation of transcription pt.2

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا


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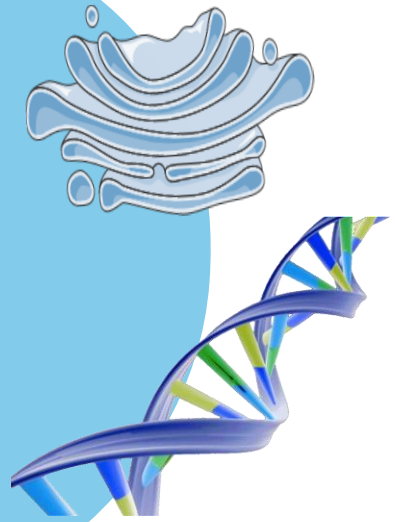
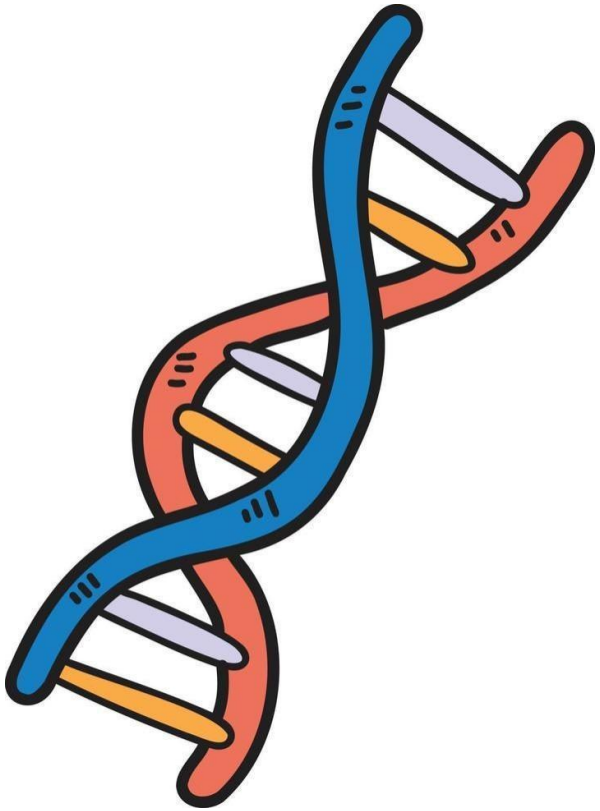


# Short Quiz on the previous lecture



# How are chromosomal structures altered?

The doctor start the lecture with some informations about:
All things related to eukaryotic cells like humans are more complex than bacteria because in there genome they don't have histones or nucleosomes like human genomic structure.
In eukaryotic cells they use multiple steps and multiple mechanisms for transcription and translation
Which are these :

- Change of compactness of the chromatin by:
  - Change the structure and position of nucleosomes
  - Chemically modify histones and DNA
  - And long non coding RNA that they discovered newly
  - Acetylation, methylation, and phosphorylation
  - Chemically modify cytosine
  - Binding of noncoding RNAs to DNA

Change the structure and position of nucleosomes

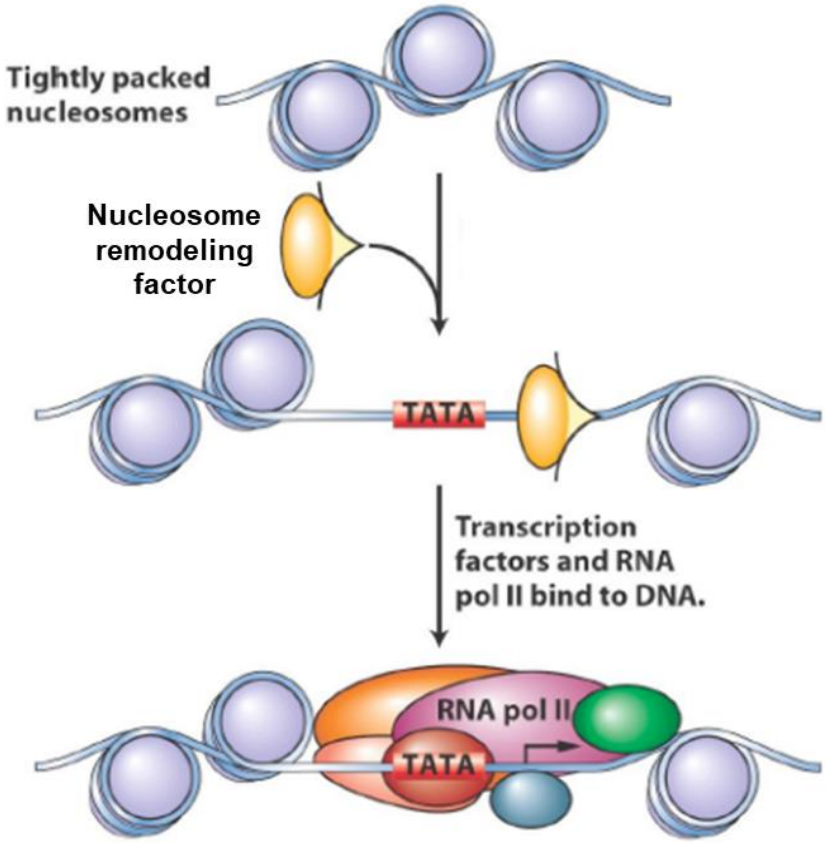
# Chromatin remodeling factors

Cells can reposition nucleosomes to expose DNA wrapped around histones. This can reveal regulatory sequences in the DNA, like promoters, proximal elements, and enhancers. They can also remove histones, making the DNA accessible. Alternatively, they can loosen DNA-histone interactions, reducing the number of DNA turns around histones, from two turns to one.

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

  – The proteins that are responsible for these changes are called:

- Chromatin (nucleosome, protein) remodeling factors can be associated with transcriptional activators and repressors. These aren't found in bacteria

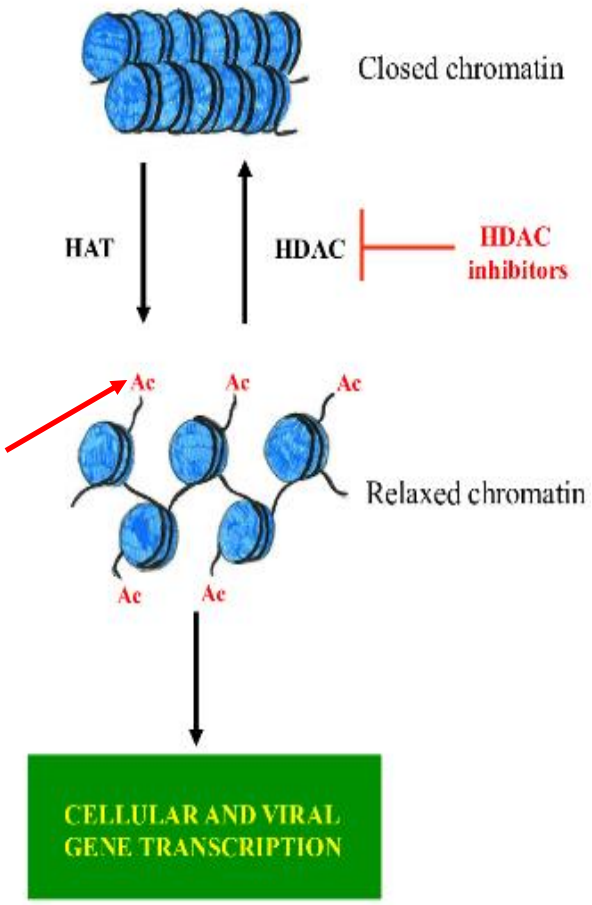


# Chemical modification of histones

## The well Studied modification

# Histone acetylation

- Every regulatory proteins can bind to DNA at a specific sequences.
- 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).

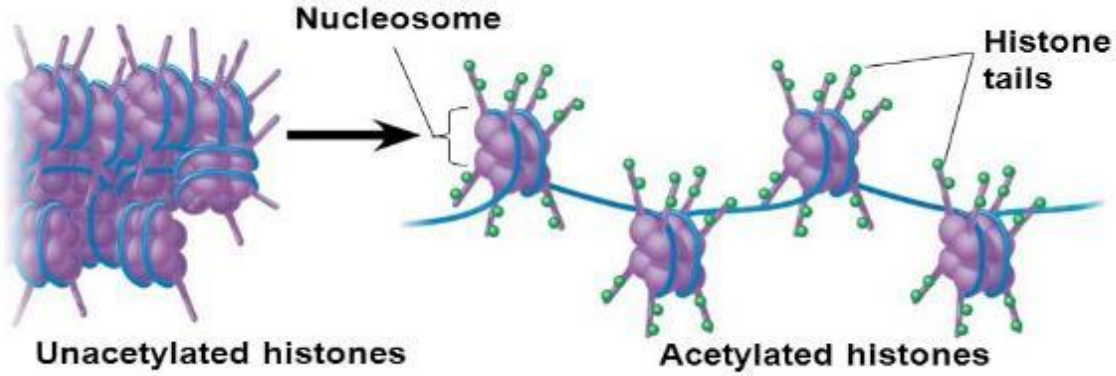


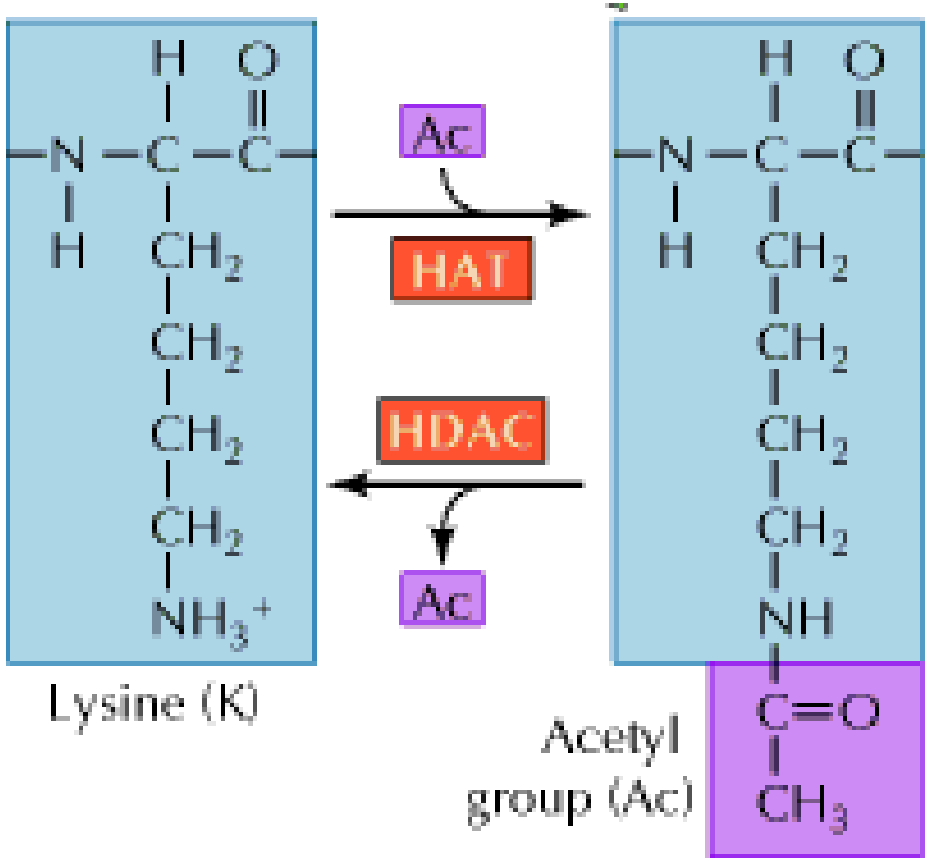

- he core histones (H2A, H2B, H3, and H4) have two domains (internal 3-dimensional structures):
  - A histone-fold, which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle.
  - An amino-terminal tail (indicated by a red arrow), which extends outside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

One of the most well studied Mechanisms

# 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.



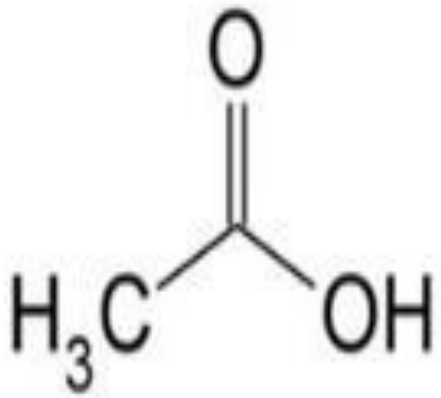
Histones have two domains:

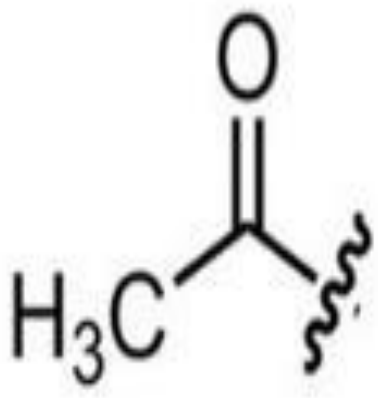
1. histone fold which makes the interaction with the DNA
2. amino terminus which is rich in lysine

These lysine groups will be acetylated → Acetyl group will bind to the positive R group of lysine

-The function of the positive charge in the R group is to make a further interaction with the DNA.

After acetylation, the positive charge will gone making the interaction weaker and the DNA become loose.



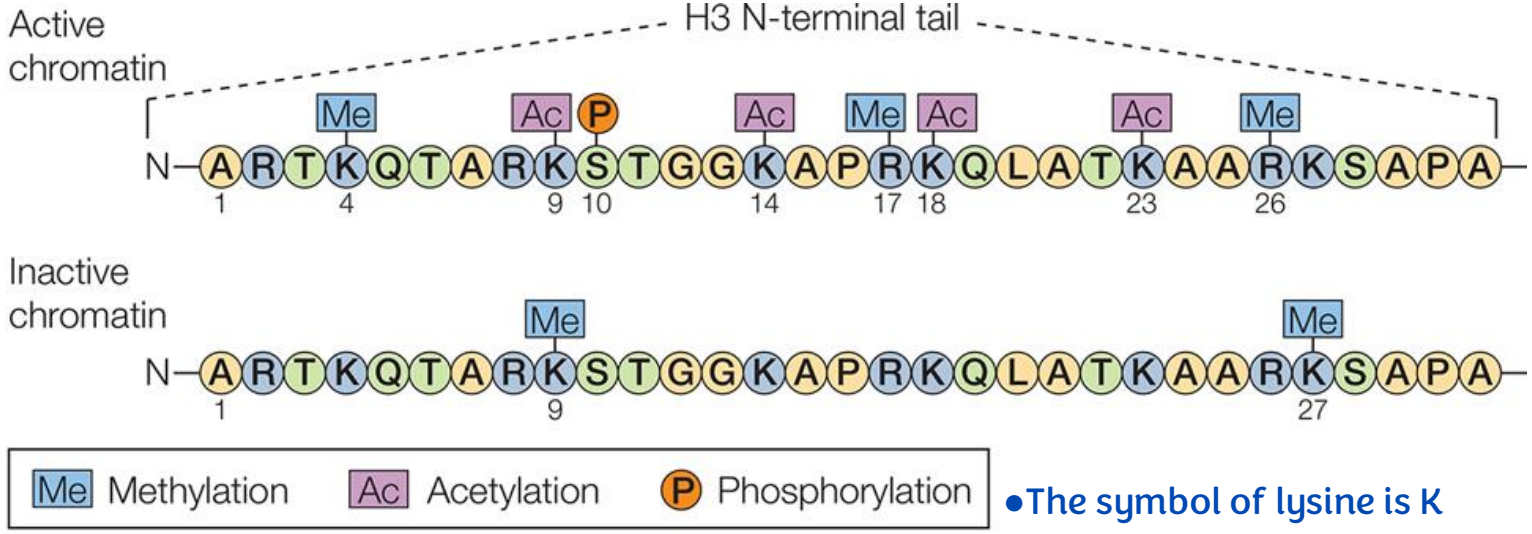



# Other modifications of histones

- Histone can also be methylated (it is like putting a mask on the positive charge), which modulates interactions with DNA, or phosphorylated, adding a negative charge that repels DNA due to the negative phosphate groups.
- 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.

– this modification can change chromatin structure by loosening or tightening the wrapping between DNA and histones.

–As we said, phosphorylating the cytosolic domain of the receptor creates additional binding sites for other molecules (docking sites).




●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 which is a good percentage.
- Positive transcription factors have at least two independent domains:
  - DNA-binding domain
  - Activation domain or functional domain that induced enzymatic activity

- 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.

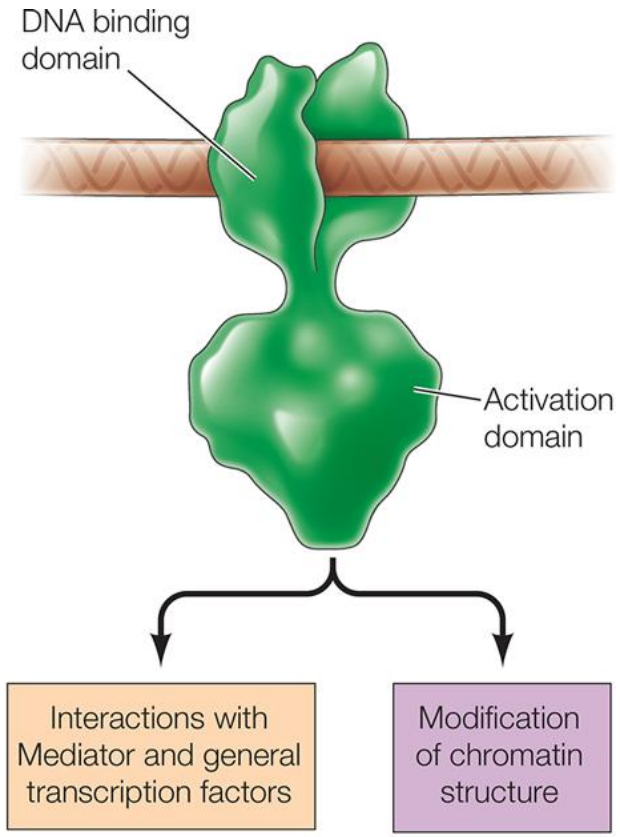
# The activation domains

أستغفرك وأتوب إليك ..سبحانك اللهم وبحمدك

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

Another function of TFIID is to interact with histone acetyltransferase enzymes, which transfer an acetyl group to histones, neutralizing their positive charge.

  - Modifying chromatin involves enzymatic activities that lead to phosphorylation, methylation, or acetylation of histones. Additionally, chromatin remodeling factors can loosen the DNA and expose the sequences.



The previous slide illustrates the functions of transcription factors

We have two 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.

Remember the doctor when he mimics the sound of a Porsche.

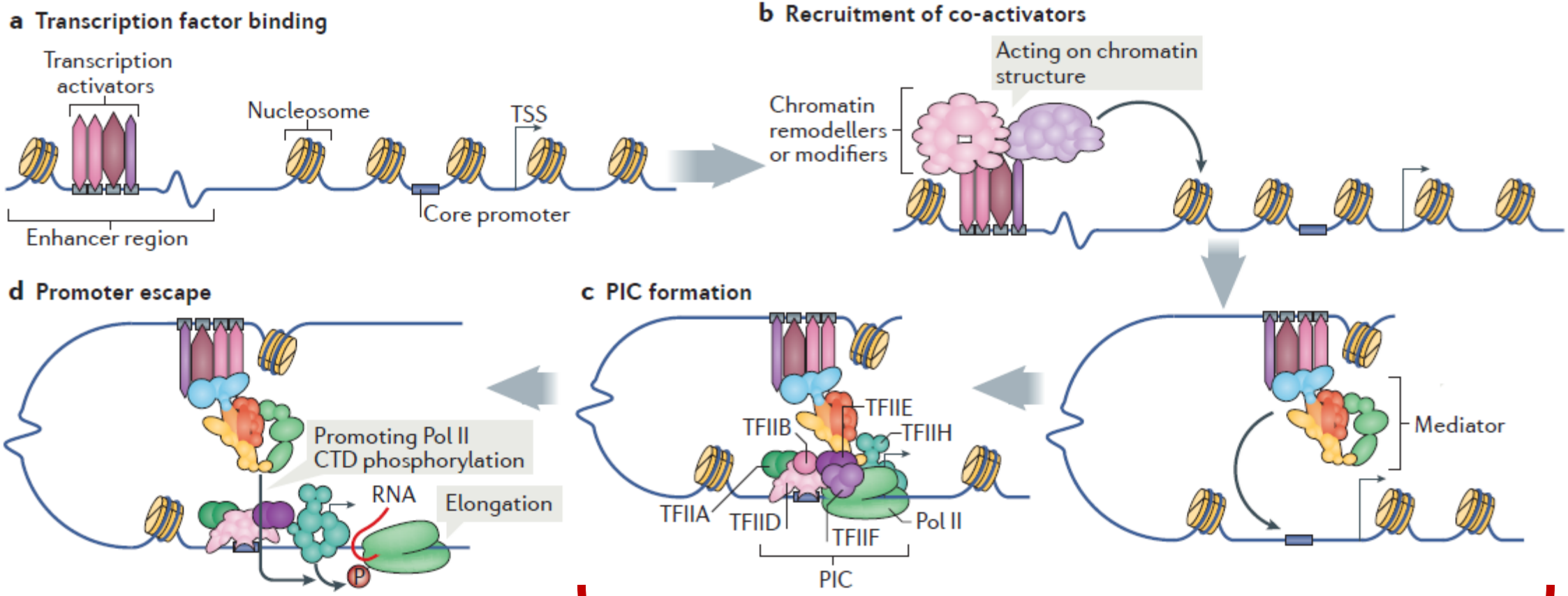
- The second enzymatic activity is helicase activity, which unwinds the DNA, creating the open promoter complex and exposing single-stranded DNA.

# A model of transcriptional activation

How the gene become activated ?

**1) Binding of transcriptional activators to the enhancer region**

**2) Recruitment of chromatin remodeling factors**

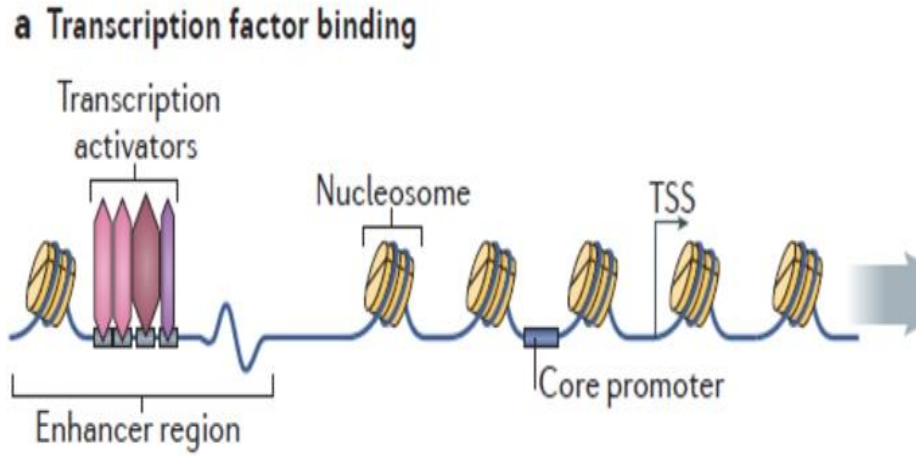



**3) Recruitment of pre-initiation complex**

**4) Phosphorylation of RNA polymerase II**

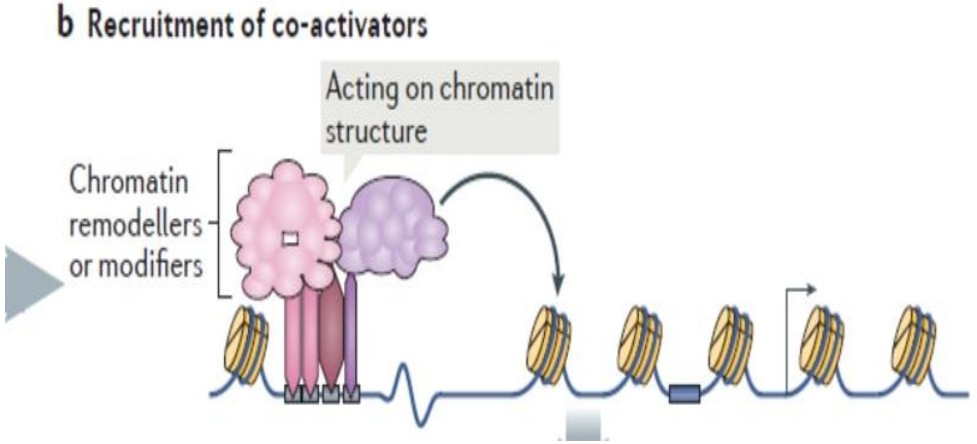
Explanation in the next slide

## 1) Binding of transcriptional activators to the enhancer region

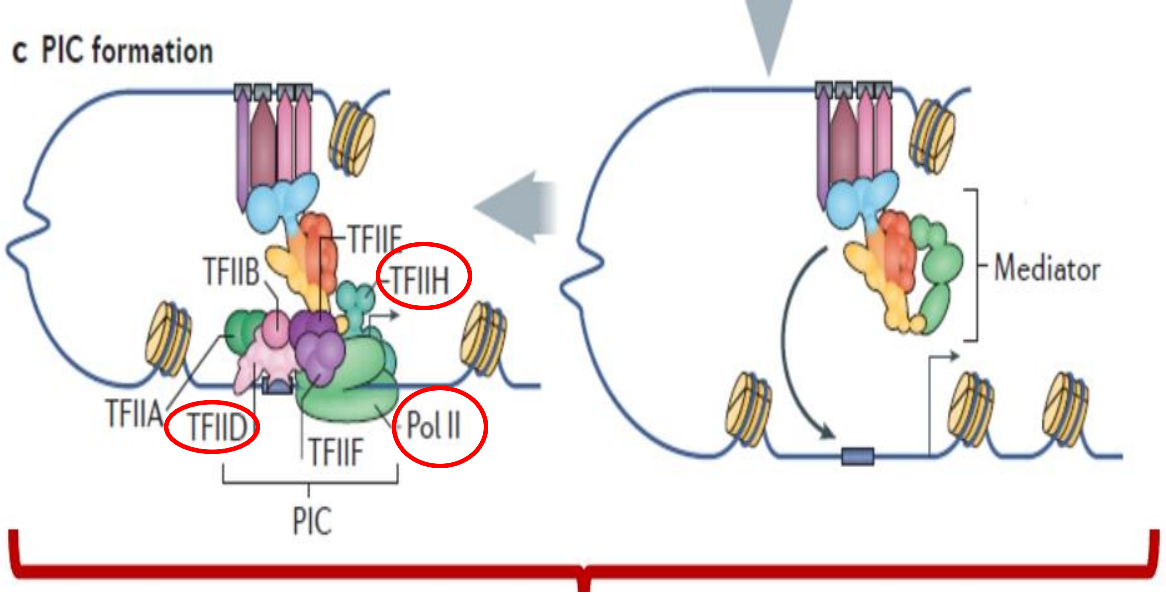



1. Certain transcription factors, such as TFIID, are among the first to bind to the promoter region, specifically recognizing the TATA box or other core promoter elements. Other transcription factors may interact with enhancers or promoter-proximal elements to regulate gene expression.

## 2) Recruitment of chromatin remodeling factors

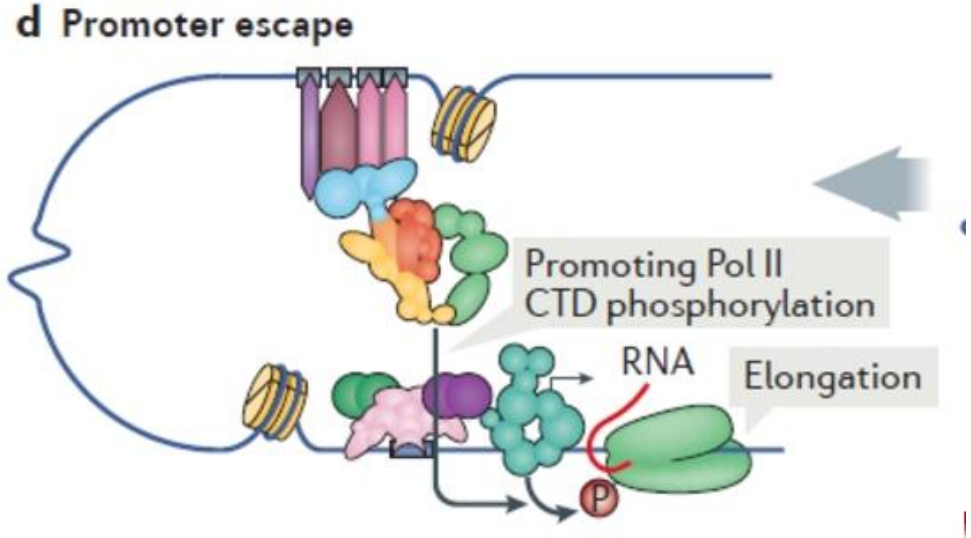



2. The initial transcription factor binds to the enhancer region, facilitating the recruitment of additional transcription factors and coactivators to form a functional regulatory complex.




**3) Recruitment of pre-initiation complex**

3. The enhancer forms a DNA loop, bringing it into proximity with the promoter region. This interaction facilitates the recruitment of transcription factors and coactivators, which in turn promote the assembly of the preinitiation complex ( the circled one).




**4) Phosphorylation of RNA polymerase II**

4. phosphorylation of RNA polymerase, activation of transcription by transcription factors.

- Each gene has unique regulatory elements and mechanisms. For example, in some genes, regulatory proteins first bind to the promoter-proximal element, followed by the enhancer, and finally the promoter region. In other cases, the preinitiation complex (PIC) assembles directly on the promoter, with regulatory proteins subsequently interacting with the promoter-proximal element and enhancer. Additionally, some genes can be transcribed without an enhancer.

# The doctor mentioned Examples of Pathways Activating Transcription Factors, such as:

**1. RAS Pathway and Transcription Factor Activation:**

○ Growth factor binds to a receptor on the cell surface, activating the RAS protein.

○ RAS triggers a cascade (RAF → MEK → ERK), and ERK enters the nucleus.

○ ERK phosphorylates transcription factors like ELK1, activating them and allowing them to bind to specific DNA regions, leading to gene expression.

**2. Protein Kinase A (PKA) and Transcription Factor Activation:**

○ A hormone (e.g., epinephrine) binds to a receptor, producing cAMP.

○ cAMP activates PKA, which moves into the nucleus.

○ PKA phosphorylates CREB (cAMP Response Element-Binding Protein), activating it to bind to specific DNA sequences (CRE regions) and promote trantranscription.

**3. In cell cycle,** cyclin D helps activate the transcription factor E2F by freeing it from Rb, allowing it to promote genes for the cell cycle.

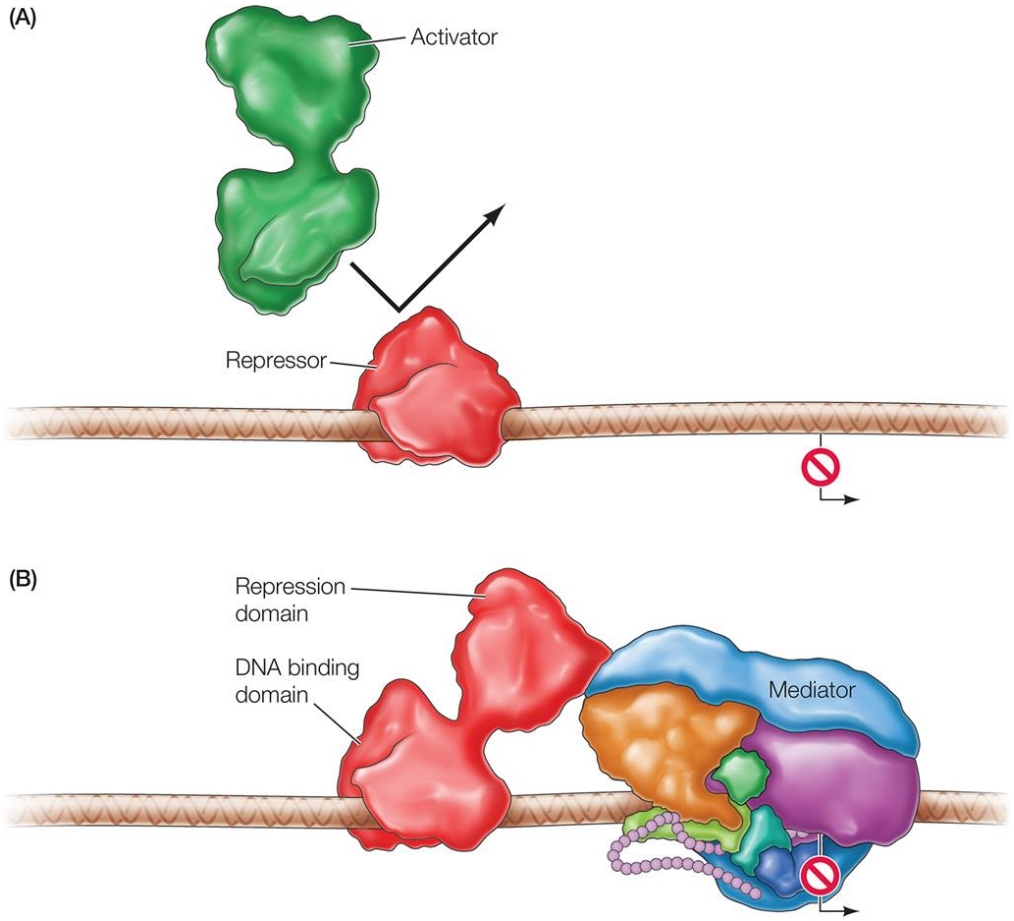
# Eukaryotic repressors

They might have a DNA-binding domain that allows the protein to bind to specific DNA sequences, potentially preventing activators from interacting with the DNA and thereby inhibiting gene expression.

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

Others have two domains, DNA binding domain and repressor domain instead of activation domain.

- (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.

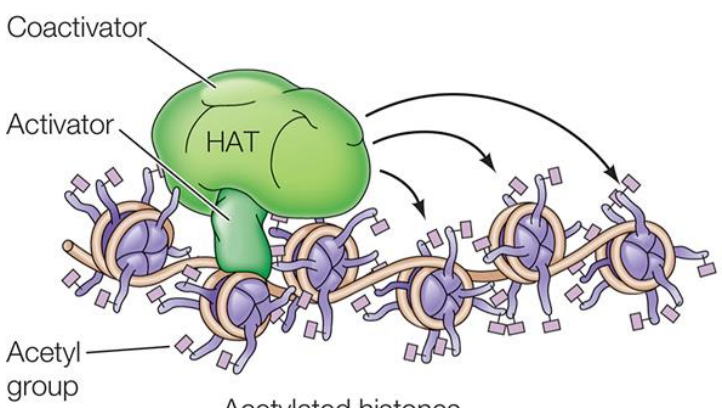


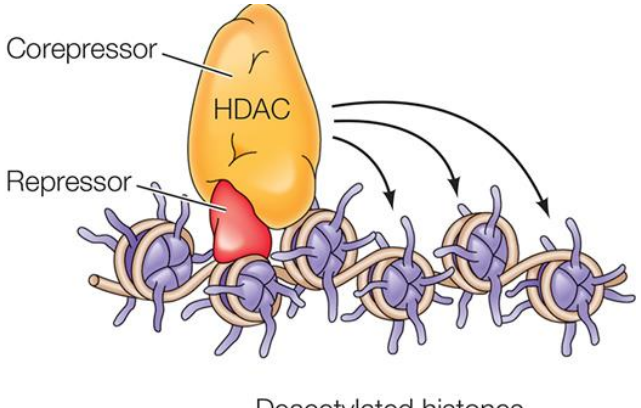

They are very important because some genes have to be active and some they have to be inhibited

# Enzymatic association

- Transcriptional activators and repressors are associated with coactivators and corepressors, which have histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities, respectively.

- Transcriptional activators and repressors work with coactivators and corepressors. Coactivators have histone acetyltransferase (HAT) activity, which adds acetyl groups to histones, loosening their interaction with DNA and activating transcription.
- Corepressors have histone deacetylase (HDAC) activity, which removes acetyl groups, strengthening histone–DNA interaction and forming heterochromatin.
  - Histone acetylation is characteristic of actively transcribed chromatin.
  - **TFIID associates with histone acetyltransferases.**

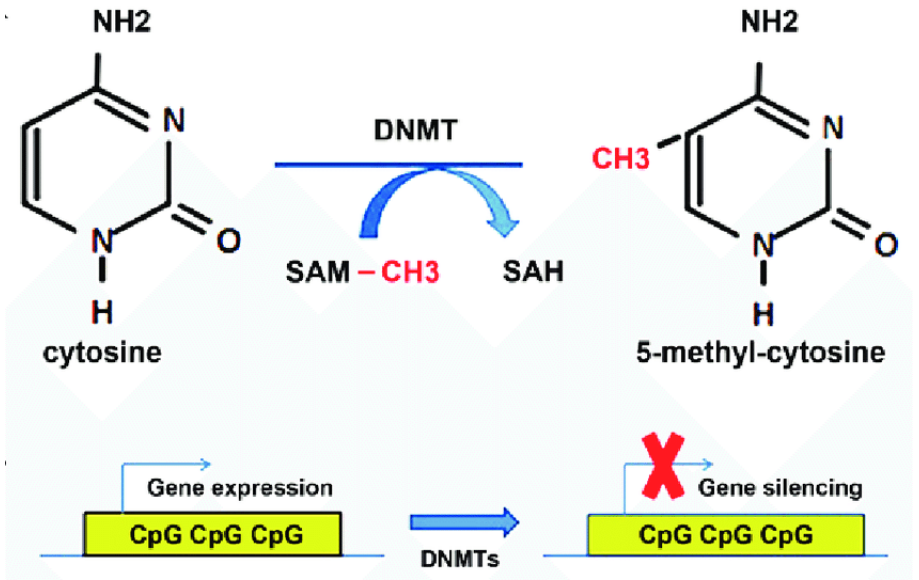


# Chemical modification of cytosine

# 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.




○ When cytosine in the promoter region is methylated, **gene expression is repressed.**

- Promoter regions often contain CpG islands, where cytosines and guanines are linked by phosphate groups.

- Methylation of cytosines in these regions recruits proteins that compact chromatin, leading to repression and heterochromatin formation.

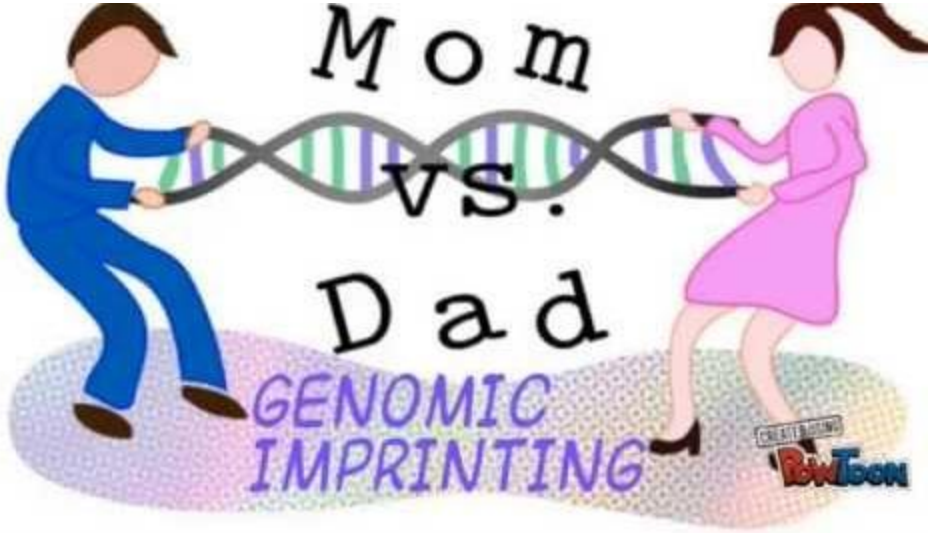
- Methylation of cytosine creates binding sites for specific proteins that aid in repression.

The doctor explained that in cancer cells, some genes are hypermethylated (less activated) or hypomethylated (more activated).

# 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.

Cytosine methylation plays a key role in genetic imprinting. Normally, we inherit two copies of each gene (maternal and paternal), and if one is inactivated, the other can often **compensate**.
However, in about 75 imprinted genes, only one copy is active while the other is silenced based on its parent of origin (maternal or paternal).
-This imprinting is established during gamete formation and is highly specific.
-If the imprinting pattern is disrupted or reversed, it can lead to diseases.




We will study a gene where both maternal and paternal genes must be expressed; if one is not, it leads to disease.

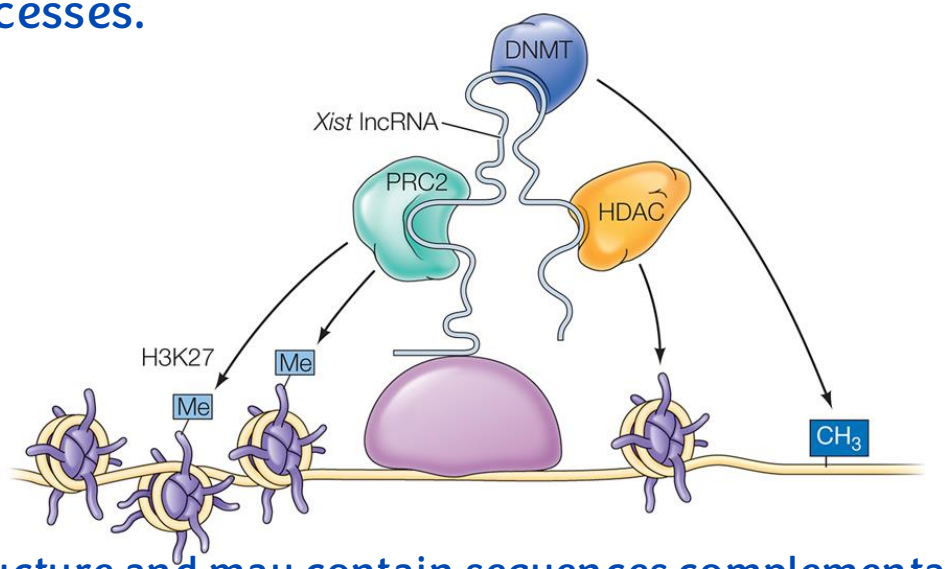
# Binding of noncoding RNAs to DNA

# 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.

Cis: works on the same chromosome it was transcribed from
Trans: works on another chromosome

There are 50,000 genes that are transcribed but not translated= non coding RNA (they don't code for proteins)
Shorter than 200 nucleotides (miRNA)
Longer than 200 nucleotides (lncRNA)
lncRNA They regulate DNA structure, transcription, and the stability of both DNA and RNA. They can also interact with proteins to influence various cellular processes.

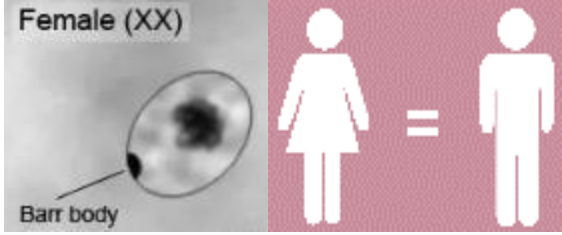



RNA has a 3D structure and may contain sequences complementary to DNA, allowing it to bind to DNA and form complexes. This binding can create sites for protein recruitment, acting as a DNA-binding domain or a scaffold for protein interactions.
**Examples of recruited proteins:**
- histone acetyl transferases, histone deacetylases, methyl transferases & demethylases (activation & inhibition).

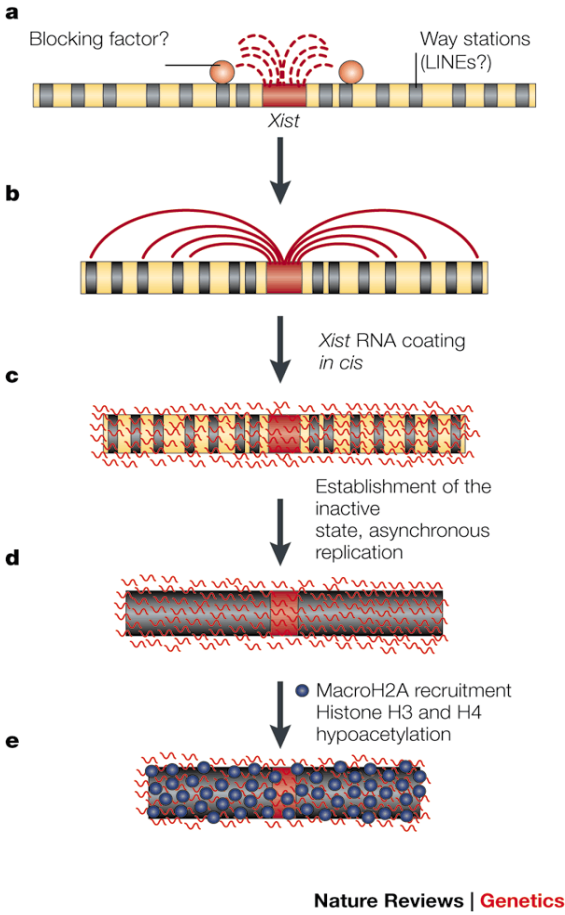
Example on the previous concept

# X chromosome inactivation

The inactivated X chromosome is called Barr body & is crumbled




- 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.




**How is the X chromosome inactivated?**

There is a gene on the X chromosome called **Xist**, which is transcribed into a lncRNA. This lncRNA coats the X chromosome in cis and triggers its inactivation. (Deacetylation & cytosine methylation)

- 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.

Same amount of proteins is produced in both genders

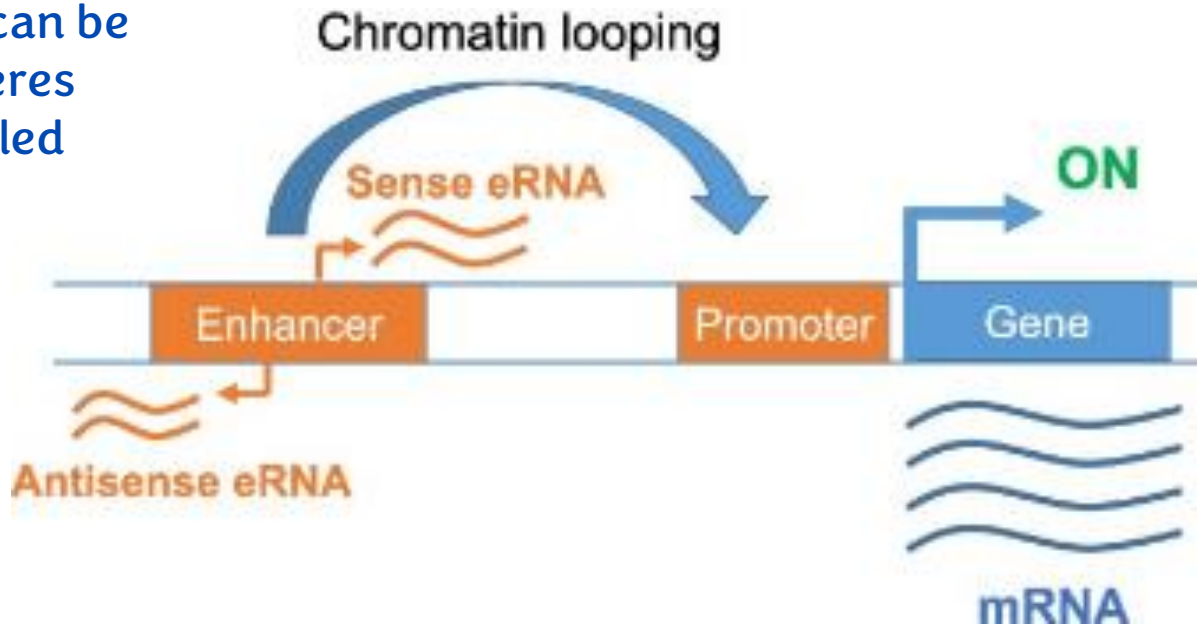
Females do not have more genes than males due to X chromosome inactivation. The inactivated X chromosome is chosen randomly, which gives females a chance to escape certain diseases based on which X is inactivated and in which tissue. This results in a mosaic pattern.

# Enhancer RNA (eRNA)

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

eRNA can bind to enhancers since they're complementary

- Promoters & telomeres can be also transcribed (telomeres can produce lncRNA called TERRA)



# 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.

Identical twins have little differences due to epigenetics.

National Human Genome Research Institute

# The power of epigenetics

- Non-sequence dependent inheritance

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

# 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

Epigenetics can be heritable


سبحانك اللهم وبحمدك،
نشهد أنّ لا إله إلّا
أنت، نستغفرك ونتوب
إليك.

# 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.

# For any feedback, scan the code or click on

Corrections from previous versions:

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

# Additional Resources:

# رسالة من الفريق العلمي:

إن الانسان بقدر معاناتهِ في ترتيب نفسه وضبط خطاه، بقدر الرّاحة النّفسيّة التي ينالها جزاء هذا الجهد والتعب!
لذلك؛ كان لزامًا أن تتدرب على 《الإلزام》 أن تُلزِمَ نفسَك التعب، وتُلزمَ جسدك العمل، وتُلزمَ قلبك الاستقامة، وتُلزمَ وقتكَ الدقة، وتلزمَ روحَكَ التسليم، وأن حياتُكَ لله.

بودكاست لازم تسمعوه وقت البريك :)
https://youtu.be/3gX4kZkcNf0?si=7gTlmECCvM0s0kIw