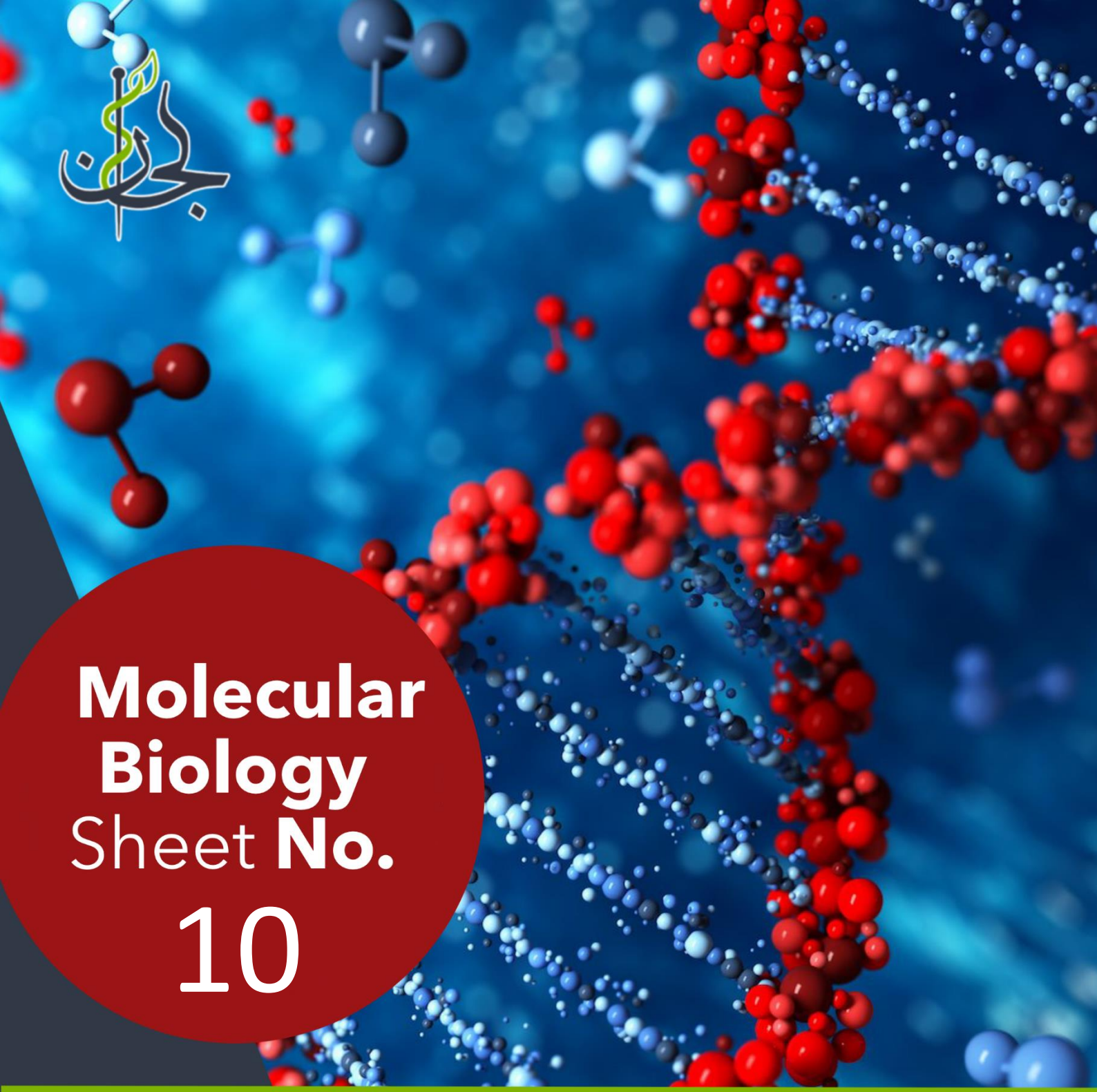

Molecular Biology Sheet No. 10

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Grammatical correction Baker Shiha

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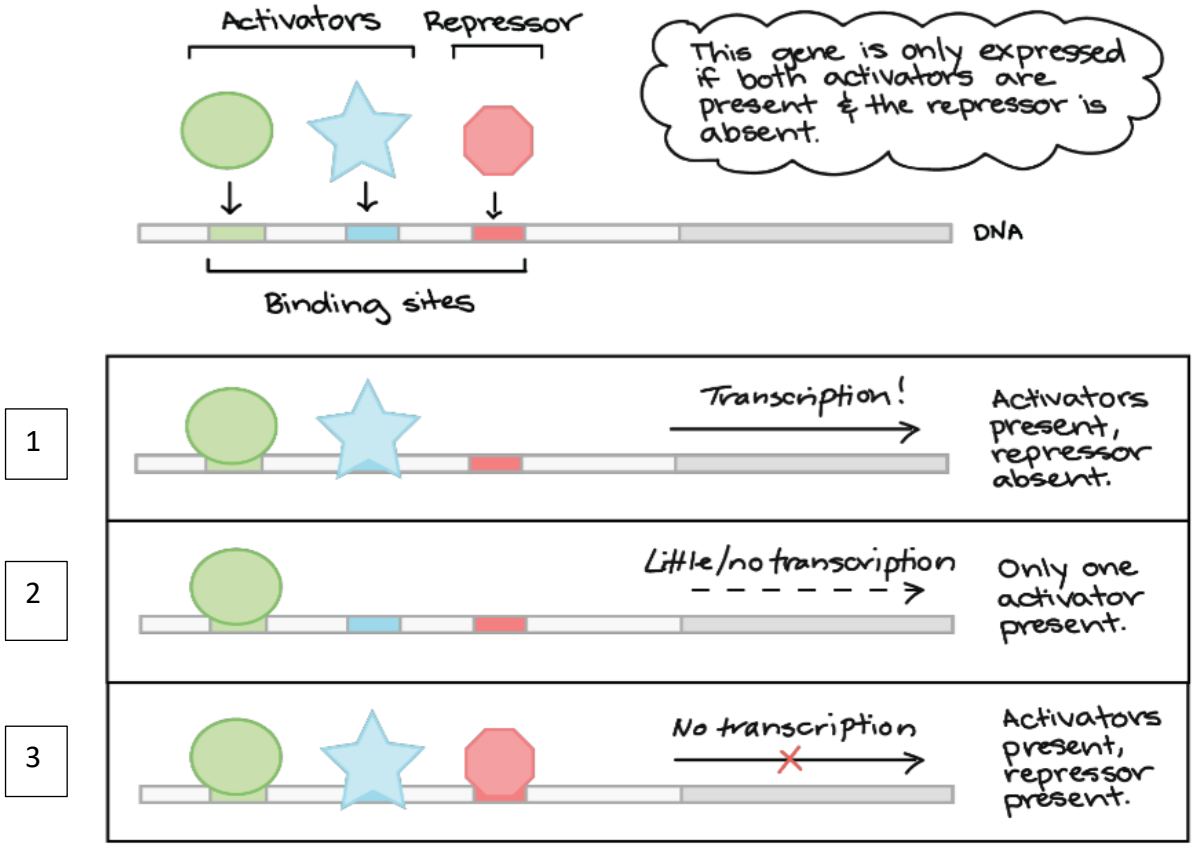
REGULATION OF TRANSCRIPTION IN EUKARYOTIC

- In eukaryotic cell things are more complex ; there are more elements, proteins and genes.
- Eukaryotic cells are more complex except they have the same basic principles. That you have noncovalent interaction, activators, inhibiters and DNA binding site element specific for certain protein .
- For **regulation of transcription** , there are more levels of control in eukaryotic . they are controlled by :-

- **CIS – ACTING ELEMENTS :-** " like bacteria "
  Promoters, proximal promoter elements, enhancers, and silencers.
- **TRANS – ACTING FACTORS :-** " proteins "
  These proteins can be Transcriptional regulatory proteins (activators, repressors, coactivators, corepressor).These are involved in a lot of regulation processes:
  1, DNA and chromatin structural modification
  2, chemical modifications on histones
  3, The DNA itself can be modified (example: methylation of cytosine)

eukaryotic have also something that bacteria does not have which is :

- **NONCODING RNA MOLECULES :-**
  There are many noncoding RNA molecules that play role in regulating transcription. We will talk about all of these levels of regulation in eukaryotic cell .



- What we have here is same concept again , we can have different types of activators , you can have repressors as well and there are elements on the DNA .
- According to the type of the activator & to the number of activators transcription rate is determined (tunning up the level of transcription) .

- As we see in the first example, transcription can be very effective transcription (strong transcriptional activity). When more than one activator is present. (Without a repressor)
- Also, it can have very little or no transcription with the presence of only one activator.
- The presence of a repressor will inhibits transcription (no transcription) even though activators are present.

In conclusion, transcriptional activity depends on what binds to these elements in DNA sequence.

How do transcription factors regulate gene expressions ?

- Transcription factor do something called **epigenetic/epigenomic changes in DNA and chromatin**.

  (Epigenetics is totally different than genetic change or genetic control)

- genetic change or genetic control is a change in the sequence of DNA, (nucleotides) for example: changing a cytosine to guanine which is a genetic change.

- However, epigenetic or epigenomic is a higher level of regulation ("epi": "Above" or "in addition to". Which is changing the structure of DNA or/and structure of chromatin itself
- It indicates genetic alternations in gene expression without a change in DNA sequence . HOW??

- chromatin packaging .
- Chemical modification of histones.
- Chemical modification of DNA .

= TRANSCRIPTION FACTORS AND HOW THEY LOOK LIKE =

- Basically, these positive (activators) transcription factors have at least two domains :

- DNA binding domains (binds to DNA)
- Activation domain (domain that is responsible for doing the function)

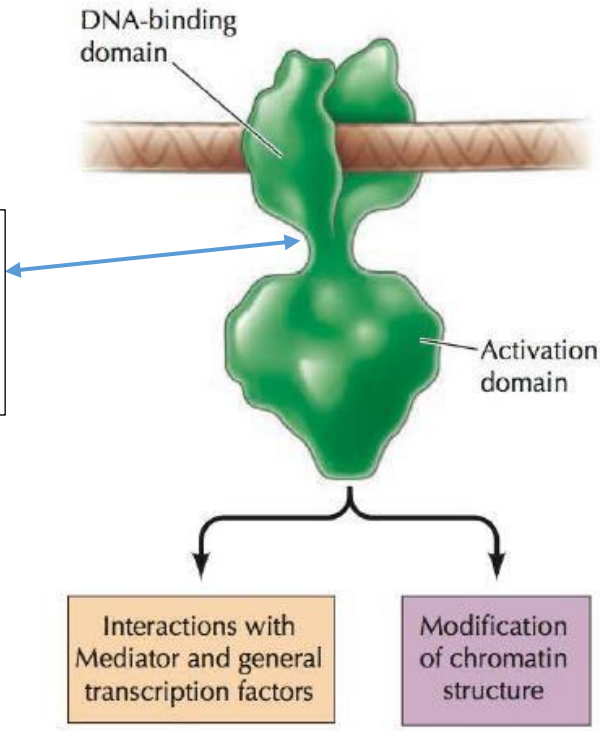
- **What do we mean by domains ?**
- Domain is basically three-dimensional structure that's part of protein's structure .
- So, you can imagine a protein having two arms or two hands, each with a certain different structure than the other, working independently of the rest of the proteins .

- **domain biochemically**

This means that domains will still be functioning even if we separate these domains from the rest of the protein. That's the idea of a

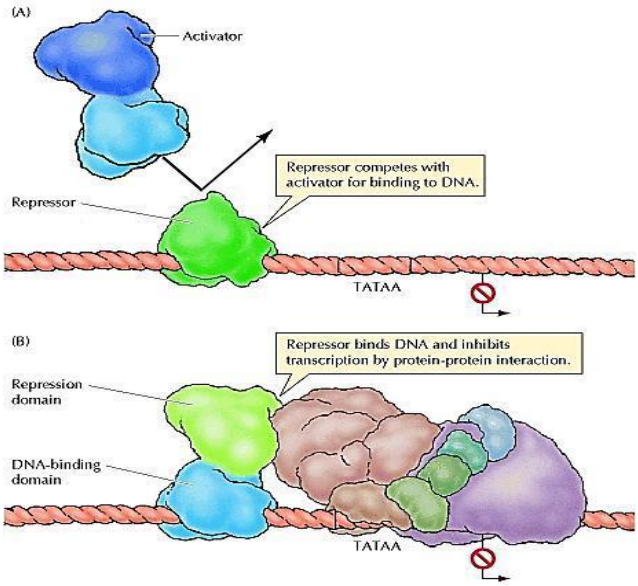
= THE ACTIVATION DOMAINS =

So the idea here is that if we cleave the protein here, both domains will still be functional



- The activation domain stimulates transcription by interacting with other proteins .
- these other proteins can be general transcription factors , specific transcription factors , mediator transcription Factors that binds the enhancer and DNA modifiers…… (and this binding will facilitates the assembly of transcription complex on the promoter)
- or what the activation domain can do is it can modify the structure of chromatin.

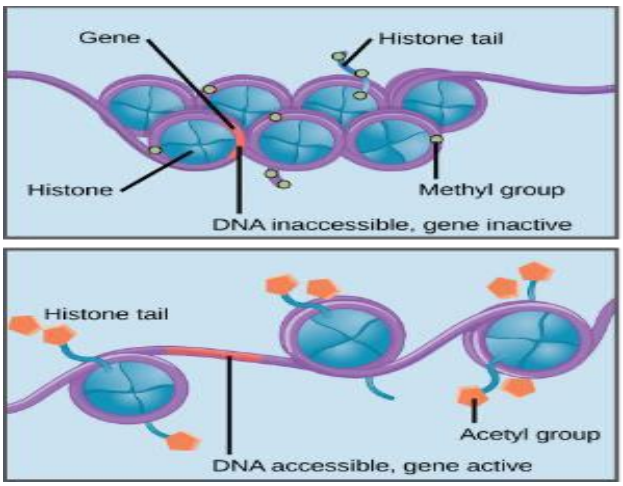
= EUKARYOTIC REPRERSSORS =



- There are two types of repressors :

- a repressor having both a DNA binding domain and repressor domain. The repressors domain will bind to general transcription factors , mediators, RNA polymerase, pre-initiation complex or any other protein .what they do is that They prevent and block transcription .
- other repressors have one DNA binding domain only, which binds to DNA preventing activators from binding to the DNA. (There is a competition between the repressors and activators in binding to DNA) .

= Modulation of chromosomal structure =



Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleo-somes. Transcription factors can bind the DNA and genes are expressed.

Some activators bind to DNA elements(to promoter or to promoter proximal element) and modify chromosomal structure (heterochromatin to euchromatin or vice versa).But how is this related to transcription regulation ???

The packaging of eukaryotic DNA in chromatin has important consequences in terms of its availability as a template for transcription . (This means that the structure of the chromatin (heterochromatin or euchromatin) plays an important role in determining which genes are available for transcription and which are not)

- actively transcribed genes are found in euchromatin. Where DNA structure is loose making genes accessible to transcriptional factors, inducers stimulators , activators….,thus actively transcribed
- Inactive genes are located in highly packed heterochromatin. This is where DNA is highly packed and there is no access for proteins to bind to DNA because all DNA sequence are hidden and wrapped around the histones core, thus inactively transcribed

As we said before, all cells have the same DNA and genes but they express genes differently and one reason that they express genes differently is that **they produce different transcription factors** . Another factor is that **DNA is packed differently in different cells .**

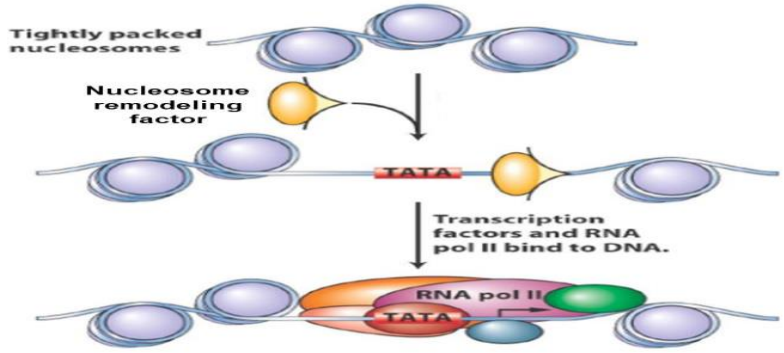
Cells that need this gene to be transcribed have the gene in a euchromatin, in other cell where transcription of this gene is not required have same gene but exist in a heterochromatin .

Going back to regulation of transcription

- Even within same cell, transcription factor or regulatory protein can switch genes between both structures euchromatin and heterochromatin.
- Whenever cells want to activate a certain genes , regulatory protein loosen the chromatin making DNA accessible to transcription factors
- Whenever they want to silence genes and block transcription regulatory proteins pack chromatin where the gene exist so it cannot be accessed by transcription factors

<u>How does the packing and unpacking of DNA happen ?</u>

= CHROMATIN REMODELING FACTORS =



- One mechanism via chromatin remodeling factors . From the name you can tell what they do exactly ; they remodel and change the shape and structure of chromatin by one of three mechanism :-

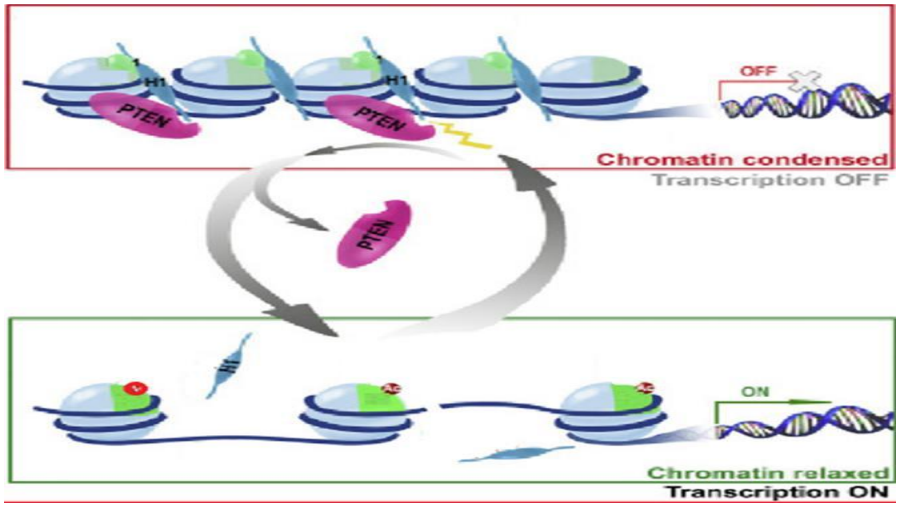
- removing histones from DNA making DNA accessible again.
- Repositioning nucleosomes making DNA accessible again.

  What's happening here is remodeling factors bind to DNA and it push histones a bit further, and freeze a certain DNA sequence like TATA box that attracts transcription factors to the DNA binding site and the RNA polymerase and so on .
- Alternating nucleosomes structure allowing protein binding to DNA .

it's different from repositioning nucleosomes . what it do is same that they change shape of DNA and exposing a certain sequence to regulatory or transcriptional protein .

Chromatin remodeling factors can be associated with activators and repressors; However, repressors do the opposite , they hide these sequences and make the DNA highly packed .

= *CHANGING HISTONES STRUCTURE BY HISTONE 1* =

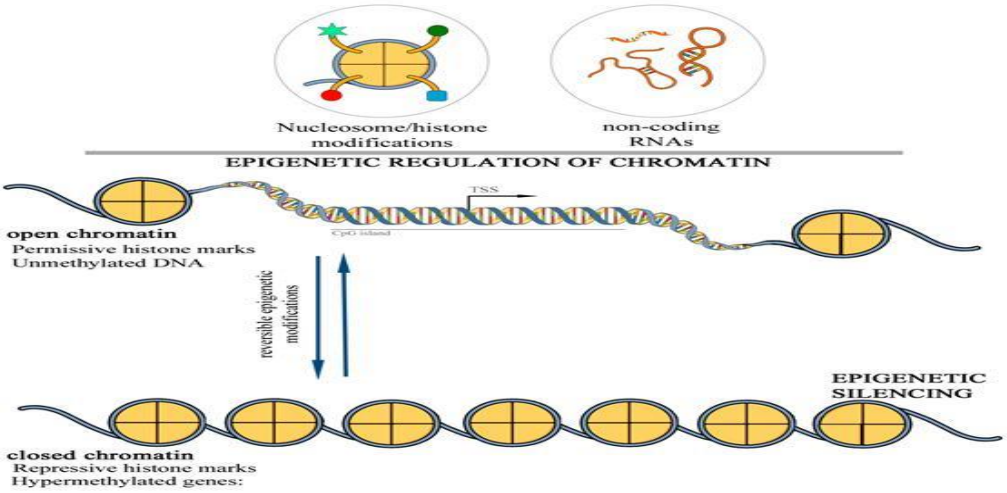


One mechanism of changing structure of nucleosomes or switching DNA from euchromatin to the heterochromatin structure by modifying histone 1 and effecting there binding abilities.

Recall: Histone 1 is the linker which holds nucleosomes together, making the chromatin more dense

certain proteins bind to histones, preventing them from binding to DNA making chromatin loosely packed and more accessible by transcription factors.

= HOW ELSE ARE CHROMOSOMAL STRUCTURE ALTERED ?

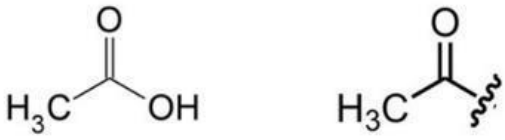


- There are two mechanisms in addition to remodeling chromatin . these mechanisms include :-

- Chemical modification of histones
- Binding of noncoding RNAs to DNA

= HISTONE ACETYLATION =

- Is one mechanism of histone chemical modification .
- What we mean by acetylation is the addition of acetyl to another group so the OH is replaced by another group .



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Histones are composed of two domains: (p.4 to know what is meant by domain)

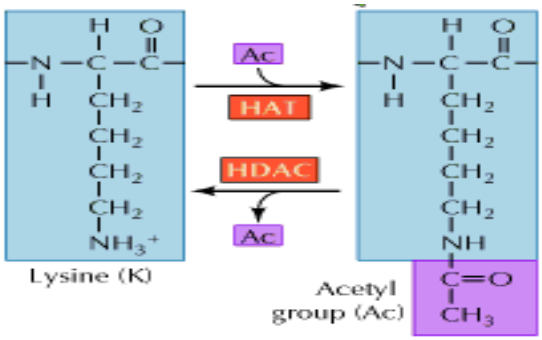
Histone-fold (the internal structure of histones),

Which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle.

Amino-terminal tail, (exists at the amino terminus of these proteins).

- The tail is rich with lysine . what is lysine ?
- It's an amino acid that makeup histones molecules.

This is the structure of lysine

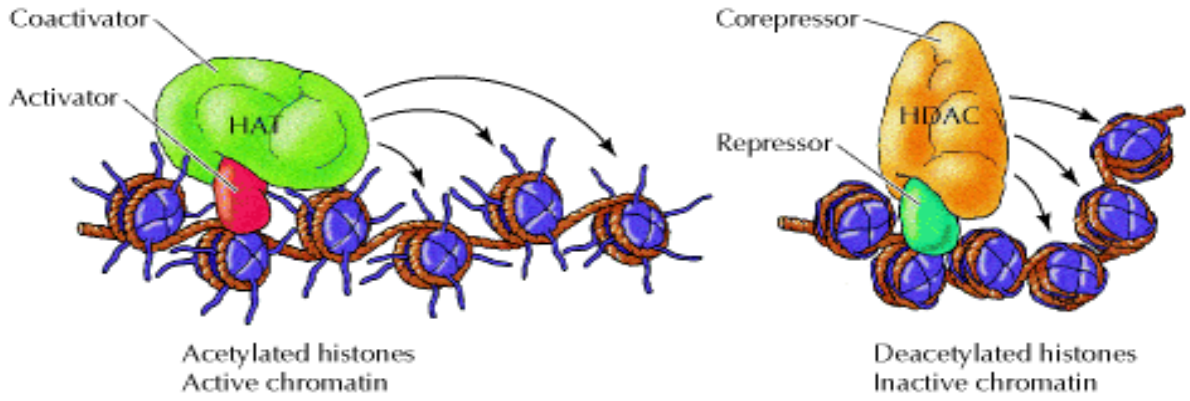


- It's a positively charged amino acid so it has positive charge that facilitates the interaction between histones and DNA because DNA is negatively charge .
- Whenever the lysine has positive charge ; the interaction between the DNA and proteins are very tight .
-

In **histone acetylation**, acetyl groups are attached to positively charged lysines in histones.(This is done by acetyltransferase)

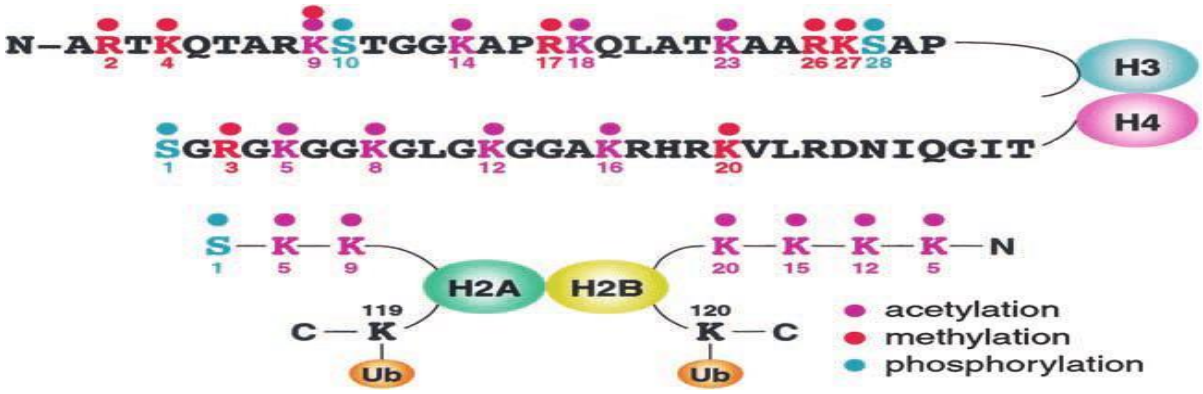
When lysine is acylated the positive charge is masked and removed . So, the interaction between histones and DNA become weaker and chromatin become loose because of this week interaction between the histones and DNA, facilitating or activating transcription .

= ENZYMATIC ASSOCIATION =



- The way that activators and repressors function is that they are associated with **histone acetyl transferases** or **deacetylases enzyme**(inhibitors)
- Deacetylases remove acetyl group from lysine exposing the positive charge, thus interaction between the DNA and histones becomes highly packed .
- It turns out that transcription factor TFIID specifically has a histone acetyl transferase .
- TFIID is the first protein that binds to the promoter region , it modifies DNA so it become loose and accessible to other proteins , allowing other proteins to binds to different sites on DNA and inducing transcription .

= other modification of histones

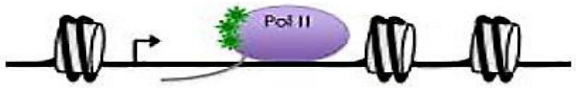


- There are other modifications of histones so they can be methylated or phosphorylated .
- The effect depends on the site of modification (to which lysine these groups added) .
- It's a little complex because there are many lysine residues represented as " K "

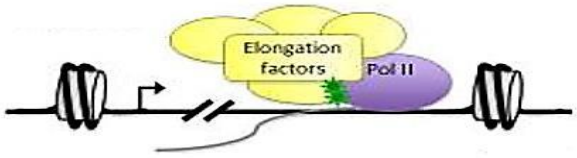
- There are many histones molecules and depending on which lysine is modified you can have activation induction of transcription or repression of transcription

-

- ***= AGAIN , THE CHALLENGE OF CHROMATIN =***



Elongation



- Just like DNA polymerases having a problem with replicating DNA In presence of histone complex & nucleosomes, RNA polymerase reading DNA also has the same problem .

- So histones must be removed from DNA in order for RNA polymerase to transcribe DNA and synthesis RNA .
- This is done by using elongation factors that are associated with RNA polymerase and they specifically binds to tail of the RNA polymerase .

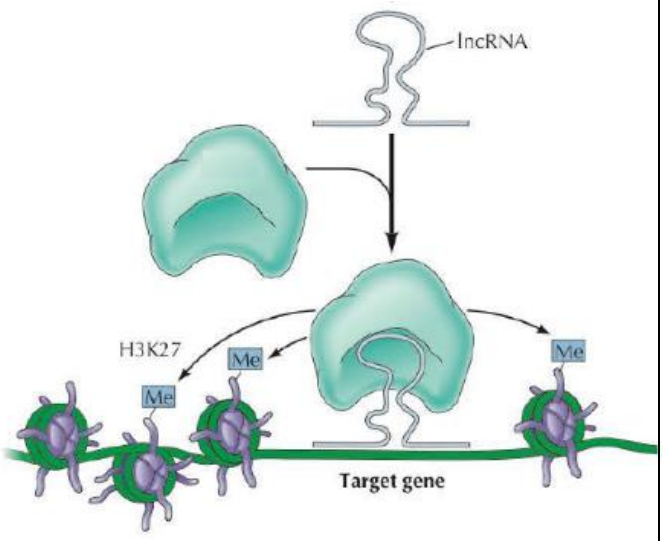
(Remember the tail which is connected to capping factors , splicing factors or polyadenylation factors) ,same thing with elongation factors they binds to phosphorylated tail of RNA polymerase 2 and as soon as RNA synthesis is initiated .

- The elongation factors remove histones. They have histone modifying enzyme (histone acetylases or acetyl transferases to acetylates histones and their interaction with DNA become weaker).
- **They also contain chromatin remodeling factor that remove histones and dismantled nucleosome structure .**

Role of noncoding RNAs in changing the compactness of chromatin:

- More than 50,000 **long noncoding RNAs** (lncRNA) have been discovered in the past 5 years
- -They are >200 (more than 200) nucleotides long and encoded by the human genome.

  -lncRNAs are considered relatively long to differentiate them from smaller RNA molecules which are micro-RNA (miRNA) as well as other types like circular RNA, Piwi-Interacting RNA (piRNA).



- LncRNAs can be homologous to certain DNA and form complexes with chromatin and DNA modifiers to repress gene expression via chromatin condensation and histone methylation.

  -meaning they can bind/hybridize/anneal to these certain sequences. Once they do they become associated with different proteins and different enzymes that can modify the chromatin; chemically modify the histones molecules via methylation or regulate chromatin condensation.

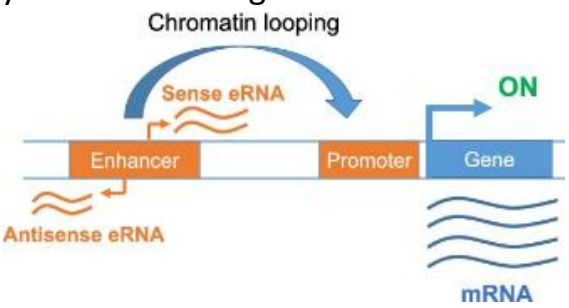
  -regulation of chromatin <u>condensation</u> includes <u>relaxing</u> or <u>packing</u> of DNA molecules.

- Other lncRNAs can bind and complex with general or specialized transcription factors (e.g. TFIIB~ general transcription factor 2B), mediator, or RNA processing proteins.
- Some enhancers can be transcribed into **e**RNA (**e** stands for enhancer) that can regulate transcription of adjacent genes. eRNA influence transcription of nearby genes.

  -keep in mind that we don't know much about these different types of RNA (lncRNA& eRNA) and what they <u>exactly</u> do but they do a lot of things.

X chromosome inactivation

- LncRNA can act in cis or trans, it acts in cis just like eRNA that can influence nearby genes in a cis manner (cis means same level), and we can



also have the production of long non-coding RNA molecules that can travel to other chromosomes influencing the transcription of other genes on these other chromosomes, in other words they can act in trans as well.

- one type of lnRNA molecule we'll talk about is Xist RNA (pronounced zest):

- A long noncoding RNA (lncRNA) is transcribed from *Xist* gene located on **one** of the two X chromosomes in females.

  - Xist RNA acts in a cis manner.

  -females have two X chromosomes whereas males have one X chromosome. So, females have more X chromosomes than men.

  but in females it is pathological to have two active X chromosomes, because the amount of genes would be doubled, so what happens is one of the X chromosomes in cells gets inactivated randomly, this phenomenon is called **dosage compensation**.

  -the idea here is at the end you have one active X chromosome in females and one active X chromosome in males.

  -the inactivation in X chromosome in females is random so some cells have on X chromosome active and in other cells the <u>other</u> X chromosome would be active that's why it's called mosaicism.

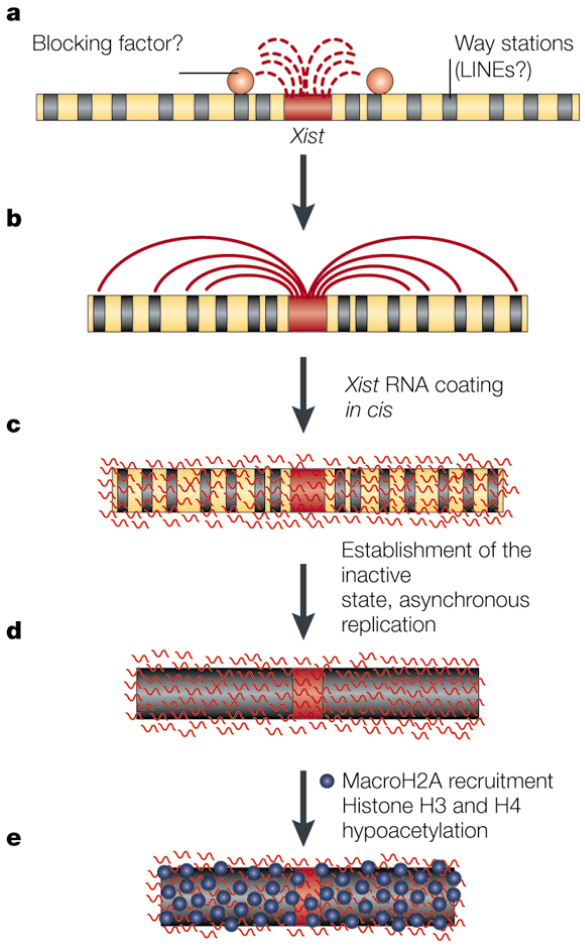
  the figure shows the way the inactivation occurs in:

  A. first thing that happens is transcription of the Xist RNA from the X chromosome to be inactivated.

  B. the long non-coding RNA is produced which is the Xist RNA.

  C. Xist RNA coats the X chromosome that produced it in a cis manner, in order to inactivate the chromosome.

  D. this promotes the recruitment of several proteins that methylate histone 3 and that leads to chromosomal condensation.

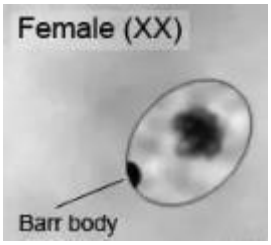


E. the chromosome gets shrunk and becomes what is known as a **Barr body**.

-other modifications take place as well such as hypoacetylation; that is removal of the acetyl group so that the interaction becomes really strong between histones and DNA which results in tight packing of DNA.

- in a female cell nucleus, you will find the whole chromatin (structure located at the middle of nucleus in the picture)

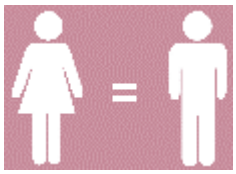
and the inactivated X chromosome isolated and shrunk represented as the Barr body.



In summary:

- 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 a random X-chromosome inactivation in a phenomenon called dosage compensation to **equate** number (and activity) of X chromosomes between males and females.

DNA methylation

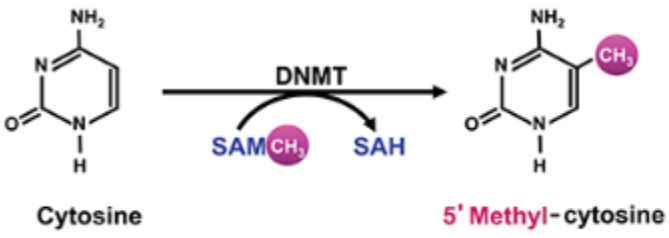


another modification that occurs to DNA itself not histones.

- Some of Cytosine residues can be methylated groups at the 5'-carbon position.

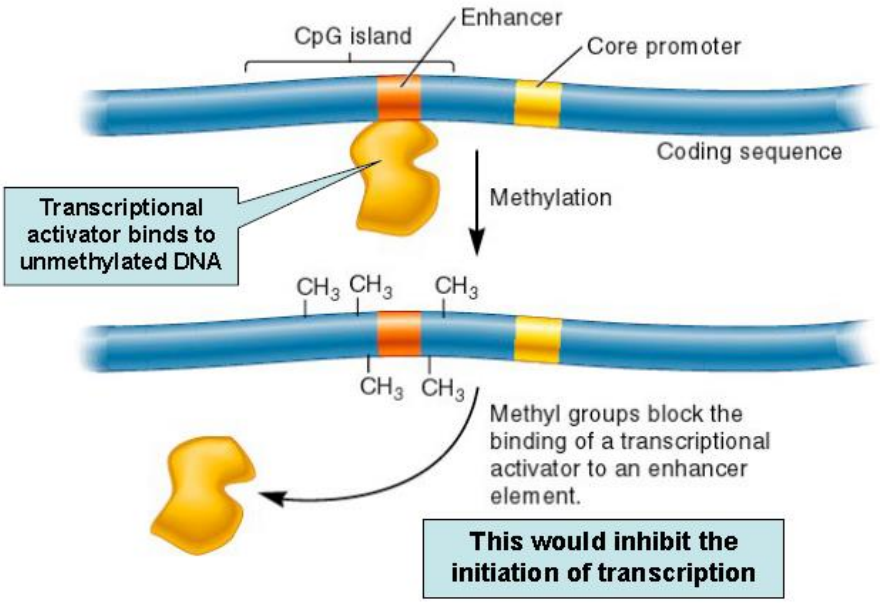
- pay attention to the difference between the structure of cytosine, methyl-cytosine, and uracil.

What exactly happens?



- Regions near the core of promoters of some genes can have a rich sequence of CGs and this is called CpG islands.
- the cytosines in the CpG islands get methylated.
- the methylation occurs specifically at CG sequences (CpG islands near promoters), producing 5-methyl cytosine.
- DNA methylation reduces gene transcription (inactivation of genes)

  by blocking of activator binding to DNA and inducing heterochromatin formation.



it's common in cancer cells that there is disturbance in cytosine methylation, leading to activation and inactivation of certain genes.

DNA methylation is important because it's related to a phenomenon known as:

Genetic imprinting

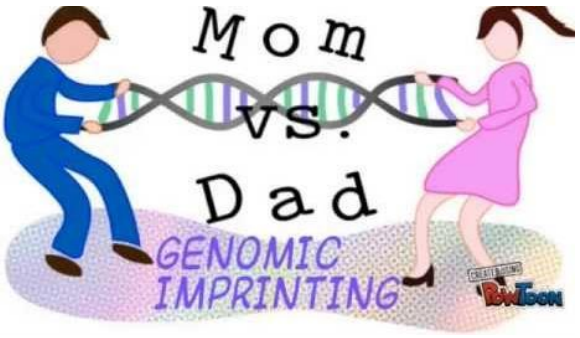
Usually both genes (paternal and maternal)are active; However, there are certain genes that must be transcribed specifically, this means that for this particular gene the paternal gene should be active, in other genes maternal genes should be active

.

if there is inactivation of one of these genes, production of only the maternal gene or the paternal gene certain diseases can result.

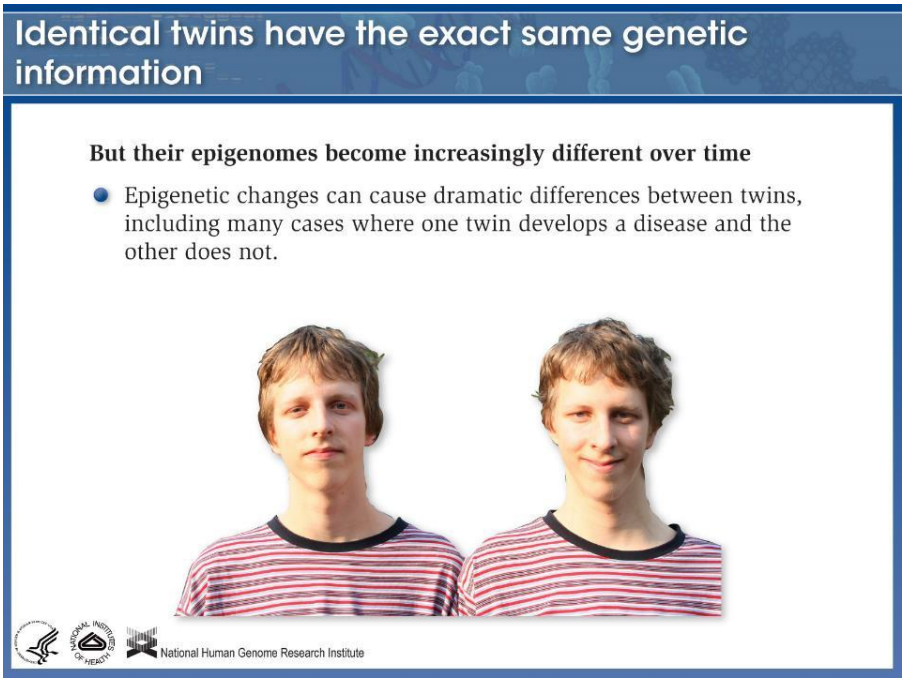
- Genetic imprinting is controlling maternal and paternal gene expression via methylation.

- Methylation is maintained following replication and is inherited..



Epigenetics is an important phenomenon in regulating gene, expression its very complex and we're still trying to understand how it works and what it does exactly.

- identical twins have the same exact DNA sequence, yet if you look at their DNA and their genome you can find different pattern of methylation and acetylation of histone molecules and DNA as well.



- that results in differences in gene expression, that's why they look different somehow, and you'll find differences between them; one of them can lead a healthy lifestyle, taking vitamins and playing sports, and the other person could be a menace, leading unhealthy lifestyle, drinking carbonated drinks like Pepsi or Coca-Cola, eating candies and chocolate…etc. that leads to this person having (unhealthy) a different epigenome.

The power of epigenetics

- Non-sequence dependent inheritance

Again, twins can have the same DNA sequence but they have different epigenome, and that leads to settle differences in their looks and maybe their behavior as well.

so, these mice have different colors even though they have the same genome, the same DNA sequence because of having different epigenome.

Epigenetics is significant and heritable

- having the same DNA sequence does not mean that you will have certain phenotype.
- This is because we can control our epigenome, Our DNA modification.(below is a headline of an article that shows that stress can change DNA modification, thus DNA expression.

DNA modification and epigenetics can be inherited as well.

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], Abeera Shaikh[a], Jonathan Mill[b,c], and Johannes M. H. M. Reul[a,4]

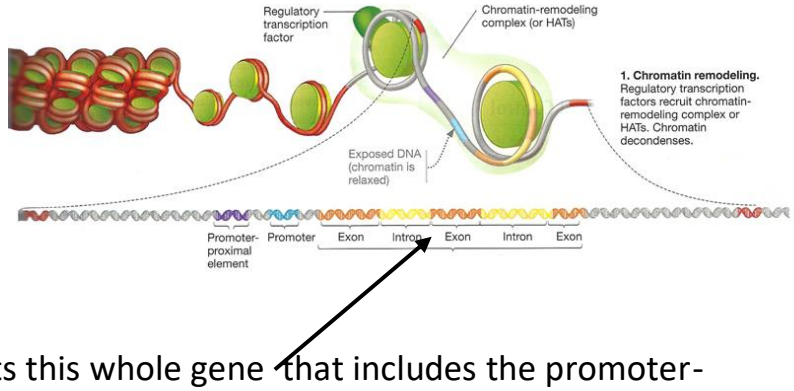
[a]Neuro-Epigenetics Research Group, University of Bristol, Bristol BS1 3NY, United Kingdom; [b]Institute of Psyc United Kingdom; and [c]University of Exeter Medical School, University of Exeter, Exeter EX2 5DW, United

A scenario of how things work inside the cell

- A little more detailed process:

the first thing that happens is that you have chromatin remodeling factors, all the other enzymes that bind to the DNA and chemically modify the chromatin or structurally modify the chromatin. the important sequences like the TATA box are hidden, these remodeling factors and proteins modify the DNA so that these sequences get exposed.

Chromatin remodeling exposes the promoter



the exposed sequence represents this whole gene that includes the promoter-proximal element, promoter and exons ….etc.

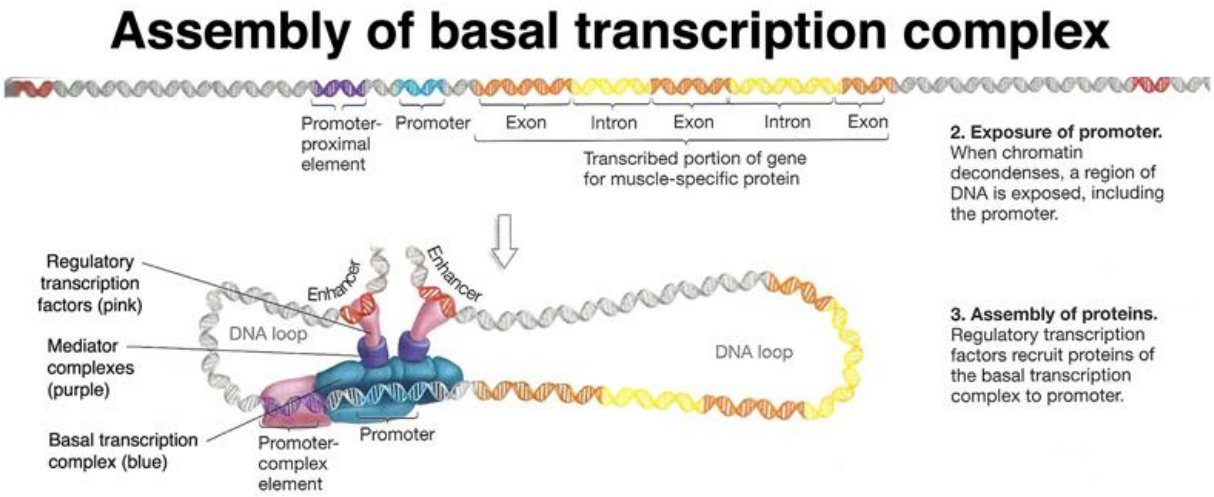
so, step no.1 is: **Chromatin remodeling exposes the promoter.**

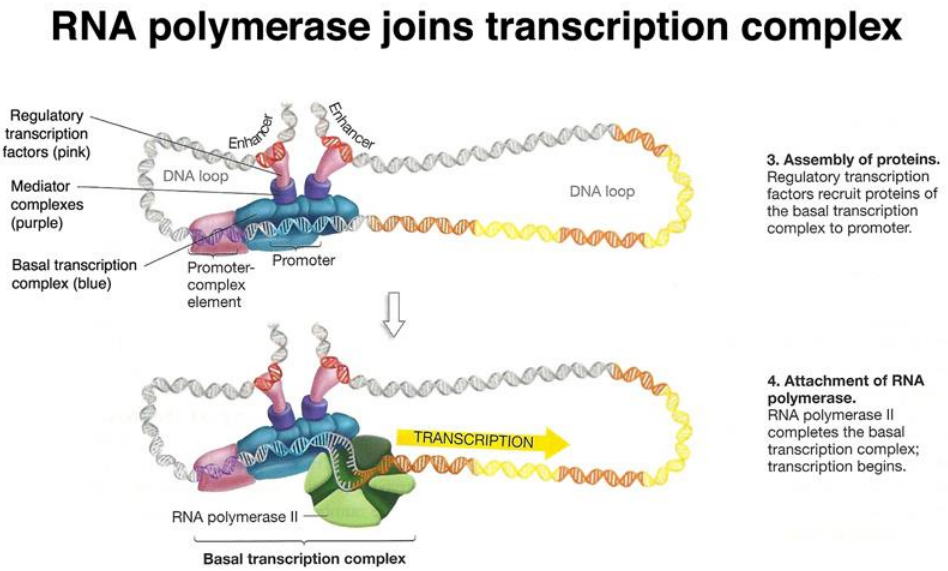
the exposure of the promoter means it's not packed anymore, it is exposed and can be accessed by the preinitiation complex. after the exposure comes the formation of the preinitiation complex on the promoter.

so, following the exposure of the promoter, we have assembly of the preinitiation complex which can also interact with the other regulatory proteins that are bound

to the promoter proximal element as well as the enhancers.

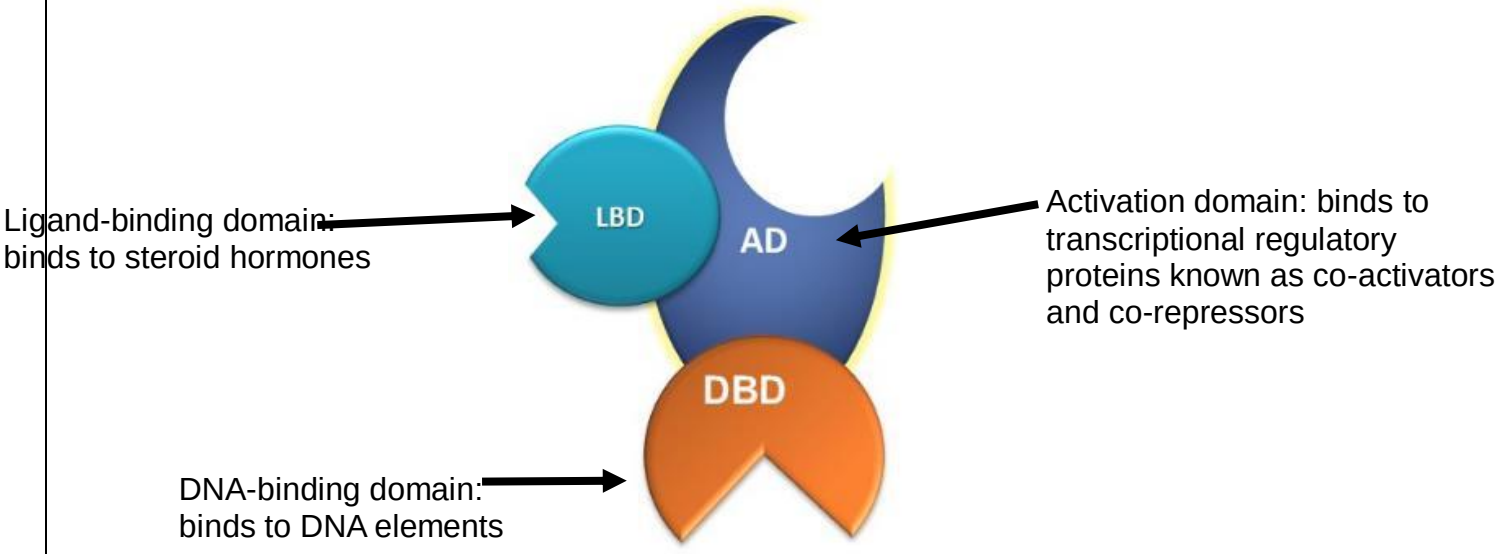
then afterwards attachment of the RNA polymerase happens, and it starts to transcribe genes.





Example on a transcription factor involving nuclear steroid receptor

General structure of SNRs -steroid nuclear receptors



remember domains are internal 3-dimensional structures that function independently of each other.

These receptors have three domains:

1- ligand-binding domain:

a ligand is a small molecule like a hormone for example.

hormones get into the cells and it binds to the ligand -binding domain.

2-DNA-binding domain:

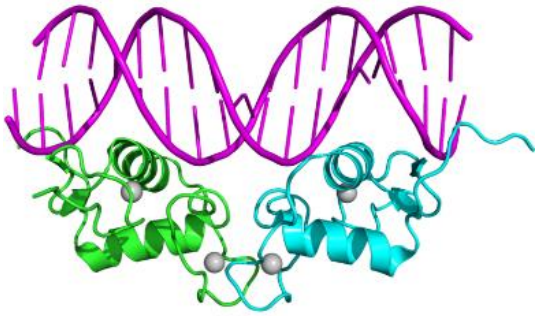
the part of the protein that binds to the DNA.

3-Activation domain:

the part of the protein that can bind to co-activators or co-repressors.

it's the part that would activate transcription or repress transcription of certain genes.

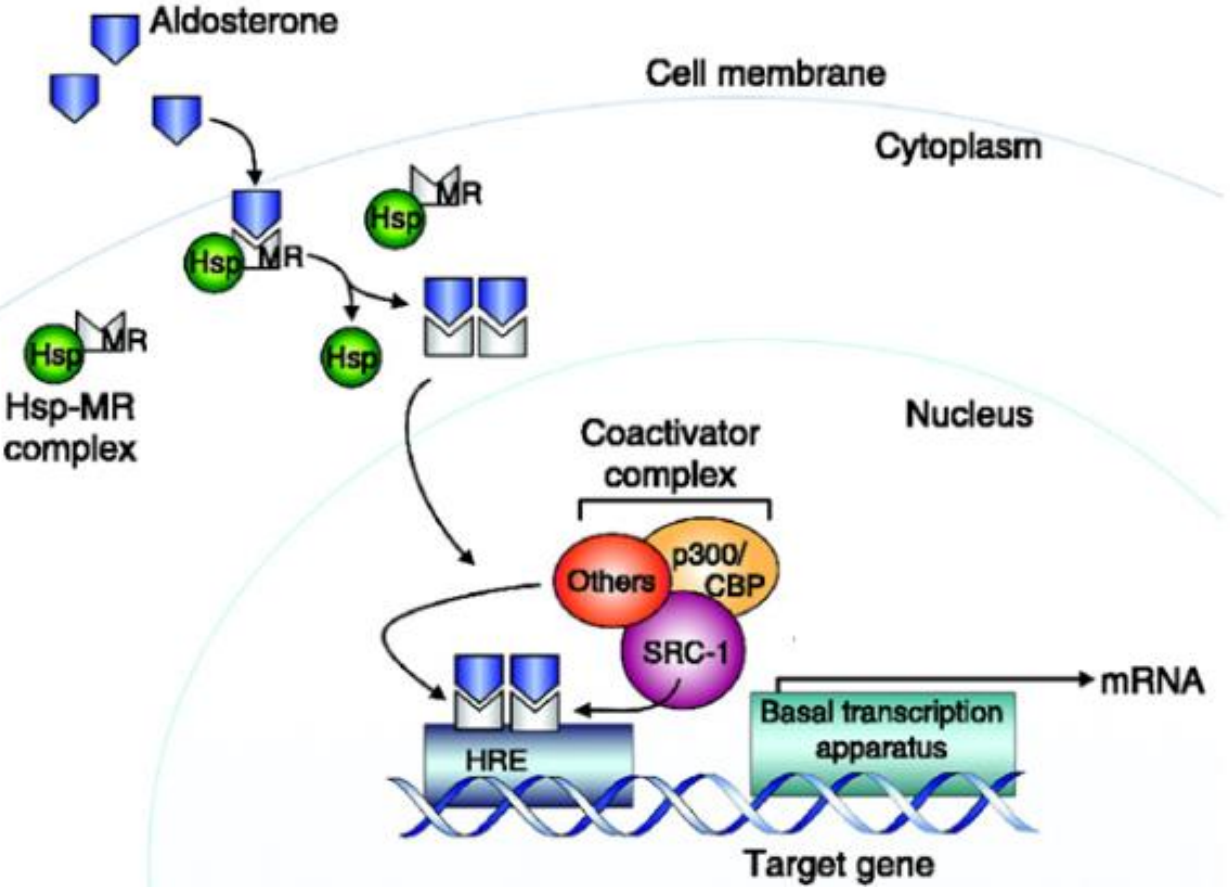
Steroid hormone receptors



-a steroid includes things like hormones, such as androgens -the male sex hormones, estrogens, progesterone -the female sex hormones, cortisol or cortisone, as well as aldosterone.

-aldosterone can regulate kidney function: it regulates the amount of water inside our body.

<u>Function of steroid hormone receptors:</u>



-these hormones are small, and hydrophobic meaning that they are lipid-like, so they can diffuse through the plasma membrane inside the cell.

once they're inside the cell they bind to their receptor,

- the ligand (the hormone) binds to the ligand-binding domain, which leads to dimerization of the receptor, meaning that the receptor forms a dimer with another receptor.

-the dimer gets inside the nucleus and then it can bind to a hormone response element.

- hormone response element (HRE) is a specific sequence that binds to the DNA-binding domain (DBD).

-**REMEMBER** an element is a specific sequence in the DNA.

- **<u>the hormone response element is a promoter proximal element(PPE), it's close to the core promoter.</u>**

- once the receptor-ligand complex binds to the hormone response element, it recruits many proteins that include co-activators or co-repressors, using their *activation domain.*

- and interaction takes place between these proteins (coactivator complex) with the pre-initiation complex that binds to the promotor.

pre-initiation complex → basal transcription apparatus in the picture.

-this interaction activates the RNA polymerase to start transcription, and deactivates in case of co-repressors.

Also, linking outside to inside

the same thing goes with other receptors – the cell surface receptors that each one of them can bind to its own ligand e.g.: hormones, growth factors...etc.

activation of the receptor occurs once the ligand binds to it, then signal transduction gets activated which is activating one protein

which activates another

protein which activates a third protein n and so on

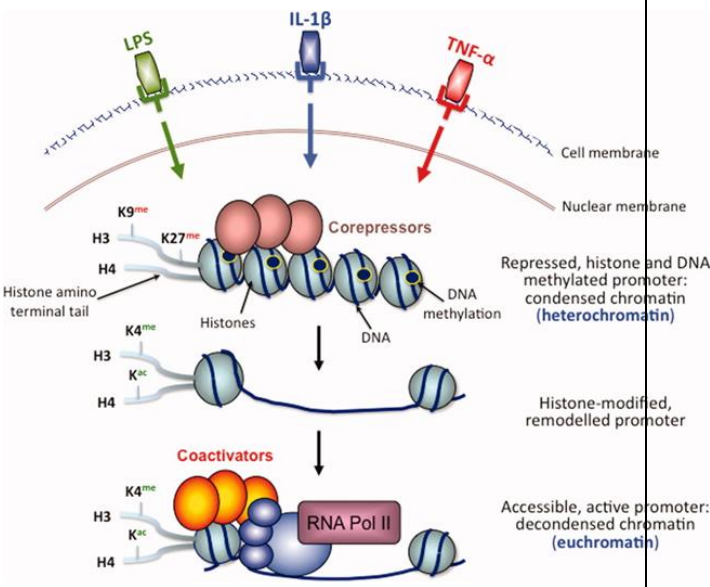
eventually the signal is relayed inside the nucleus,

activation of corepressors or

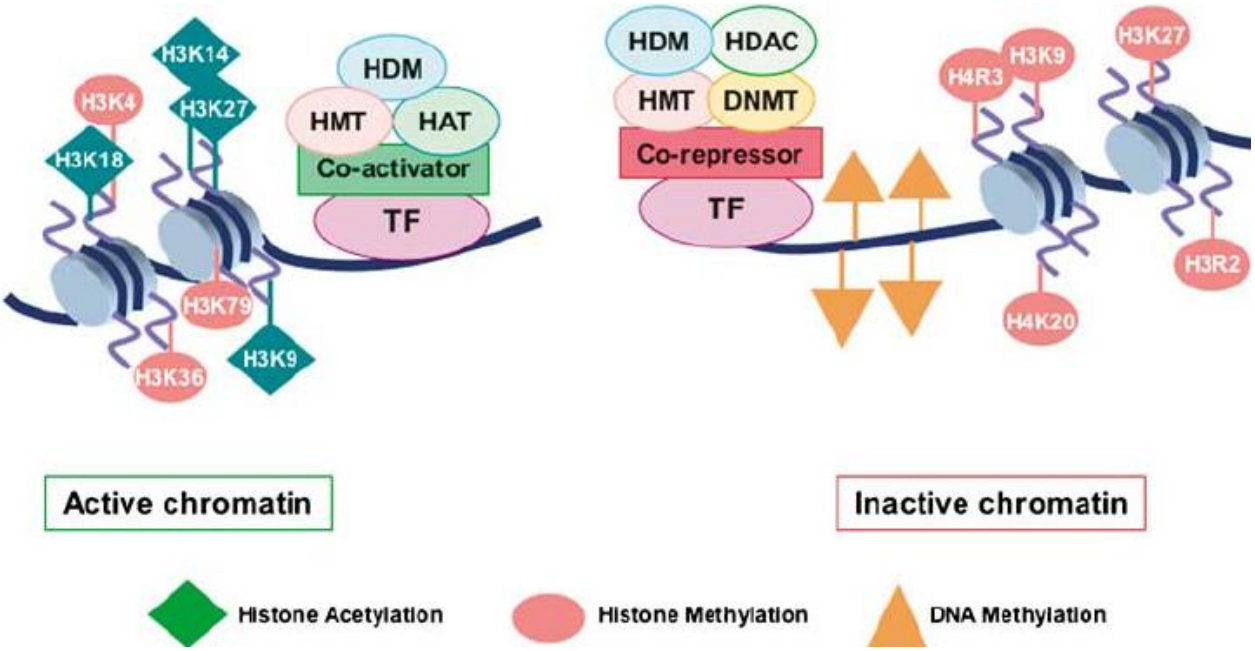
coactivators happen, and they bind to promoter

proximal elements or enhancers,

changing the structure of the chromatin either



decondensing it, making DNA more accessible by proteins(coactivators, repressors, and regulatory proteins). Or **compacting** it, packing the DNA so that it cannot be accessed by all of these proteins.



you can either have activators binding to the DNA promotor proximal elements which in turn activates transcription.

or you can have repressors influencing gene expression by inactivating it and blocking transcription.

THANK YOU 😊