Sheet no.11

MOLECULAR BIOLOGY

Writer: Noor Abu Hantash
Corrector: Toleen Haddad
Doctor: Mamoun Ahram

Regulation of transcription in eukaryotes

Regulation is more complex in eukaryotes ,
We have more elements, more proteins and more genes. Everything is more complex except they have the same principle (noncovalent interactions, activators, inhibitors, DNA binding site elements for specific proteins)

Regulatory mechanisms

Although the control of gene expression is far more complex in eukaryotes than in bacteria,the same basic principles apply.(higher levels of control in eukaryotes)

Transcription in eukaryotic cells is controlled by:

1)Cis-acting elements:

Promoters, promoter proximal elements, enhancers, and silencers

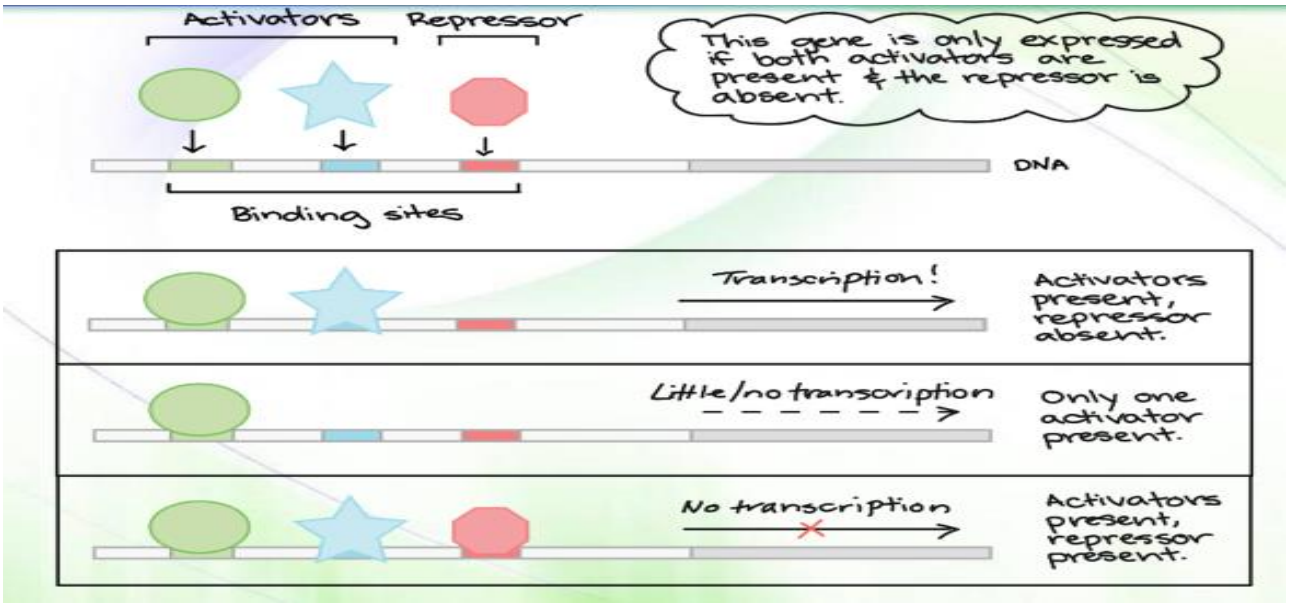
2)Trans-acting factors:

transcriptional regulatory proteins (activators, repressors):they do modification for:

a) DNA and chromatin structural modification (they can modify DNA & histons(nucleosome in other words) or DNA itself as a structure.

b) DNA chemical modification (example: methylation of cytosine)

3)Noncoding RNA molecules(does not exist in bacteria like cis and trans, they play important role in regulation)



Here there are different types of activators : they bind to different elements controlling the tuning of transcription either high or low transcription depending on the type and number of activators.
And there is a repressor that causes inhibition or blocking of transcription.

How do TFs regulate gene expression?

Genetic changes: are changes in sequence of DNA (nucleotides) like changing C→ G

Epigenetic changes: are the structural changes of DNA or chromatin without affecting the sequence.

Transcription factors(TFs) cause epigenetic/epigenomic changes in DNA and chromatin.

What is epigenetics?

Epi: “above” or “in addition to”(in addition to changing in sequence there are changes in structure)

It indicates genetic alterations in gene expression without a change in the DNA sequence.

the changes happen by :

- Chromatin packaging
- Chemical modification of histones
- Chemical modification of DNA

General structure of TFs

Positive transcription factors have **at least two domains**: (activators)

1)DNA-binding domain

2)Activation 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.

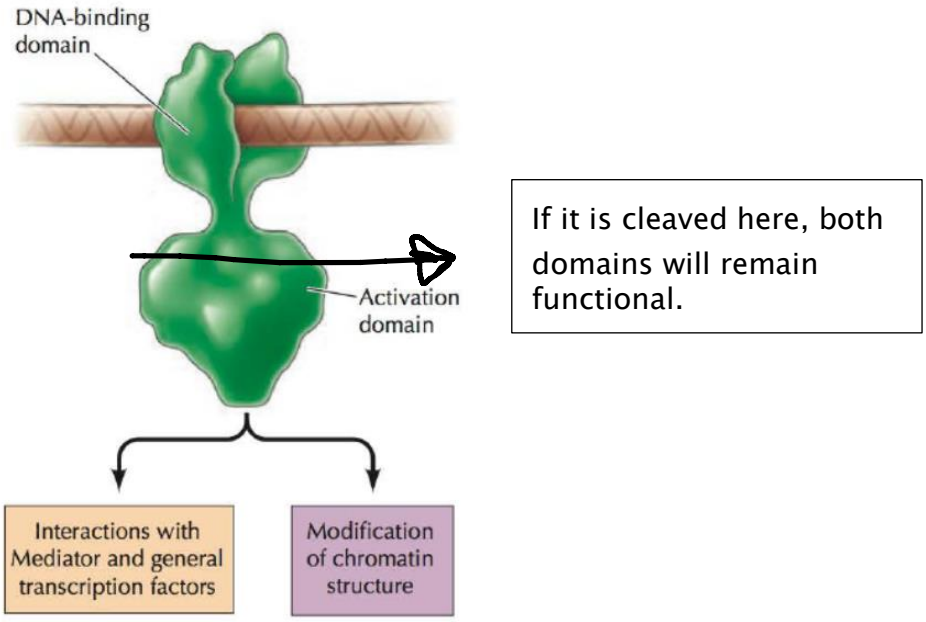
Imagine that the protein has 2 hands; the first hand is DNA binding domain that binds to DNA while the other hand is the activation domain.

Both are different in structure; they look different

If we cleave them they will stay functional , they are independent on each other.

The activation domains

Activation domains(part of protein that activates,stimulates the transcription) stimulate transcription by : interacting with general transcription factors,special transcription factors, mediators that bind enhancers, DNA modifiers facilitating the assemble of a transcription complex on the promoter, modifying the chromatin.



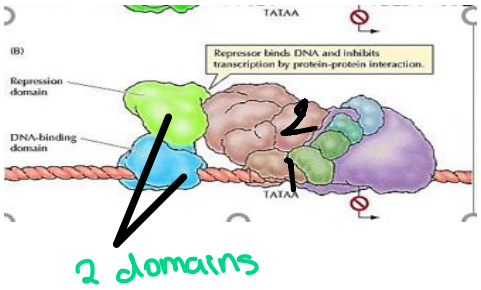
If it is cleaved here, both domains will remain functional.

Eukaryotic repressors

Repressors bind to specific DNA sequences and inhibit transcription.

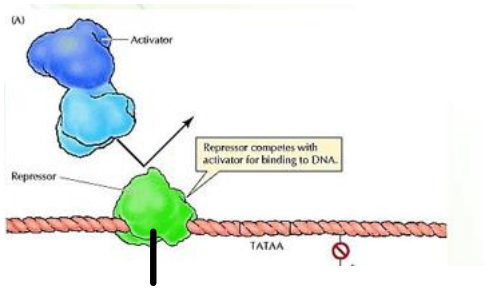
Repressors may have (2 types)

1)both DNA-binding and protein-binding domains



Here repressor has 2 domains
1)DNA binding domain
2)repressor domain that binds to general transcription factors, mediators, preinitiating factors, RNA pol…preventing and blocking transcription.

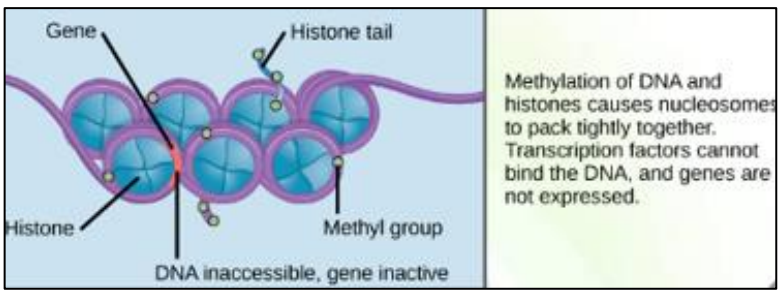
2)DNA-binding domains, but not protein-interaction domains



It has 1 single domain (DNA binding domain)
Prevents activators from binding DNA so there is competition between activator and repressor to bind DNA

Modulation of chromosomal structure

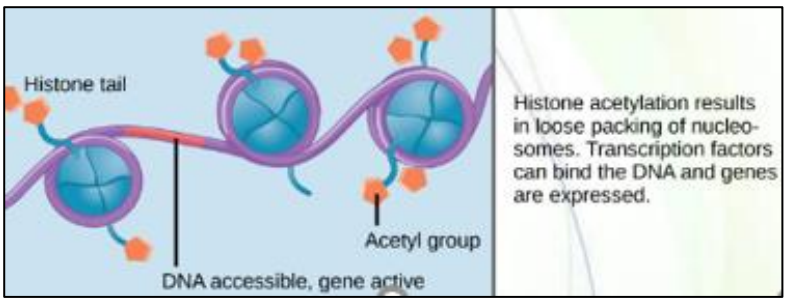
The packaging of eukaryotic DNA in chromatin has important consequences in terms of its availability as a template for transcription
Actively transcribed genes are found in loose chromatin (euchromatin)
Inactive genes are located in highly packed heterochromatin.
Regulatory proteins switch between the two structures of chromatin.
Activators bind to DNA elements either promoter, PPE ,...
Cause modification in the chromosomal structure
There are 2 types of chromosomal structure :
1)heterochromatin☹packed, inactive gene



Highly packed, no access for protein to bind to DNA , all DNA sequences are hidden and wrapped around histone core.

2)euchromatin☺: actively transcribed gene

Loose DNA , accessible DNA to bind with transcriptional factors, Activators , stimulators and inducers.



All cells have the same DNA , same genes ,but they express genes differently , because:

1) They produce different transcription factors
2) DNA is packed differently in these different cells

Within same cell :

Regulation proteins (transcriptional factors) can switch genes between both structures:

Euchromatin→ (loosen DNA , accessible to transcriptional factors)

Heterochromatin→ (block ,scilence , not accessible to transcriptional factors)

How does packing on and packing off happen?

Next page...

Chromatin remodeling factors

Remodeling means altering the shape and structure of DNA.

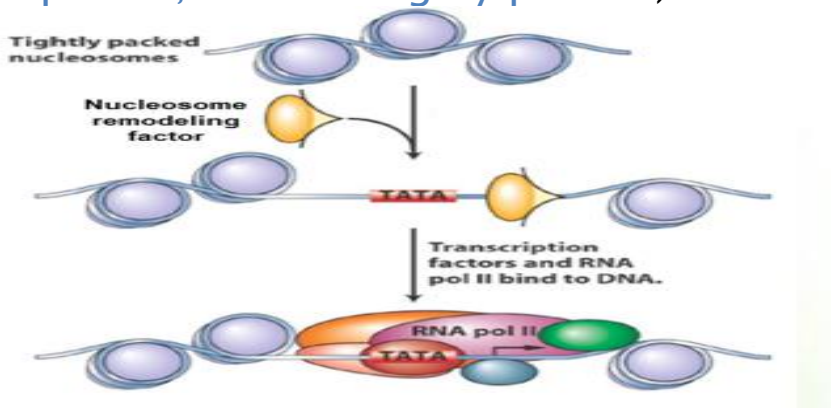
They facilitate the binding of transcription factors by

1) Removing histones from DNA(DNA is free and accessible)

2)Repositioning nucleosomes making DNA accessible(remodeling factors bind to DNA pushing histones a bit further freeing certain DNA sequence like TATA box) see the pic below

3)Altering nucleosome structure allowing protein binding to DNA(change the shape of DNA exposing certain sequence to transcriptional factors)

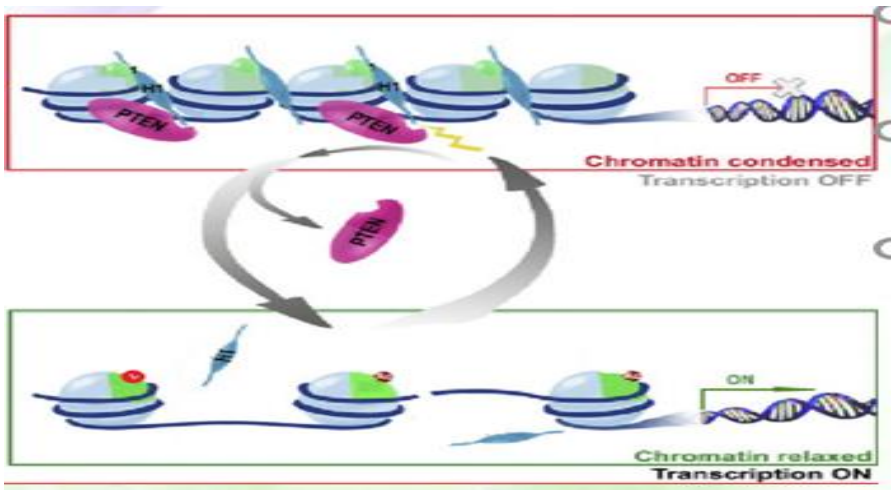
Chromatin remodeling factors can be associated with transcriptional activators(make DNA accessible, free of Histones) and repressors(hidden sequence, DNA is highly packed).



One mechanism of changing structure of chromosome is:

Changing nucleosome structure by histone 1

Transcriptional regulatory proteins either release Histone 1 (H1) from DNA or facilitate its binding (modifying H1 by allowing it to bind (packed DNA)or these proteins can remove H1 freeing DNA sequences)



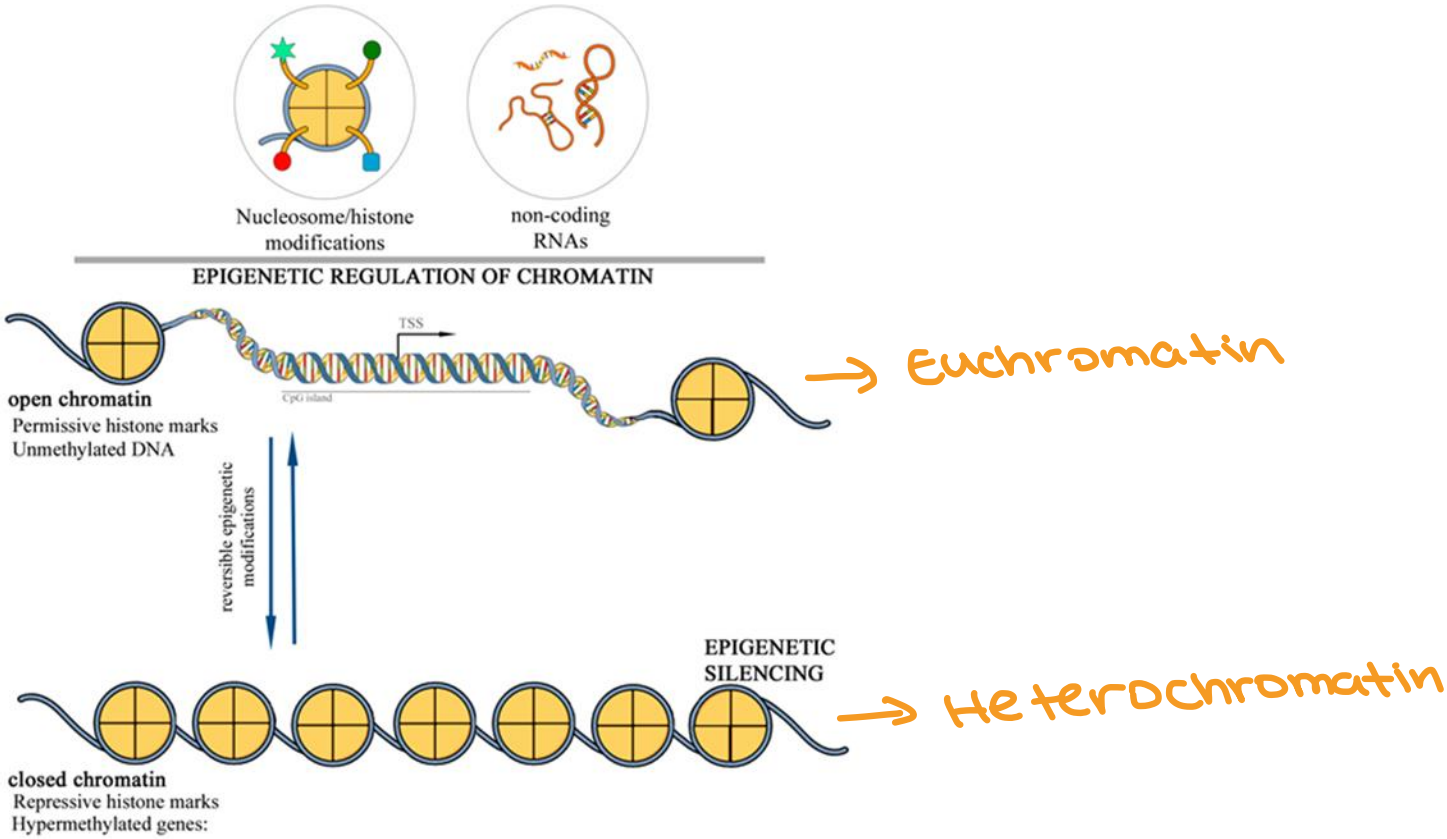
How else are chromosomal structures altered

Change of compactness of the chromatin by:

1)Chemical modification of histones:

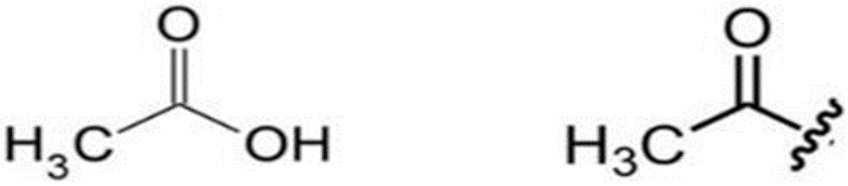
Acetylation, methylation, and phosphorylation

2)Binding of noncoding RNAs to DNA



Histone acetylation

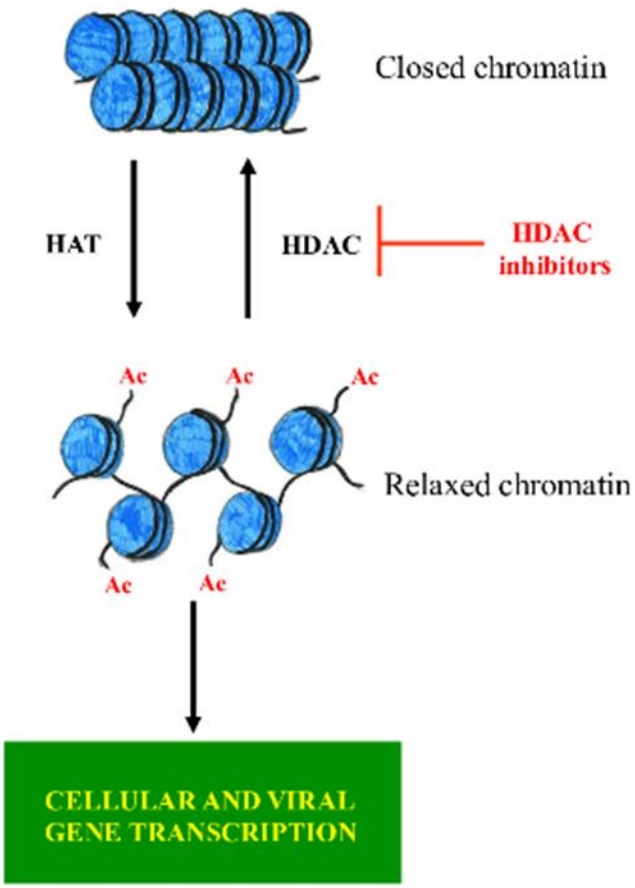
Acetylation: addition of acetyl group

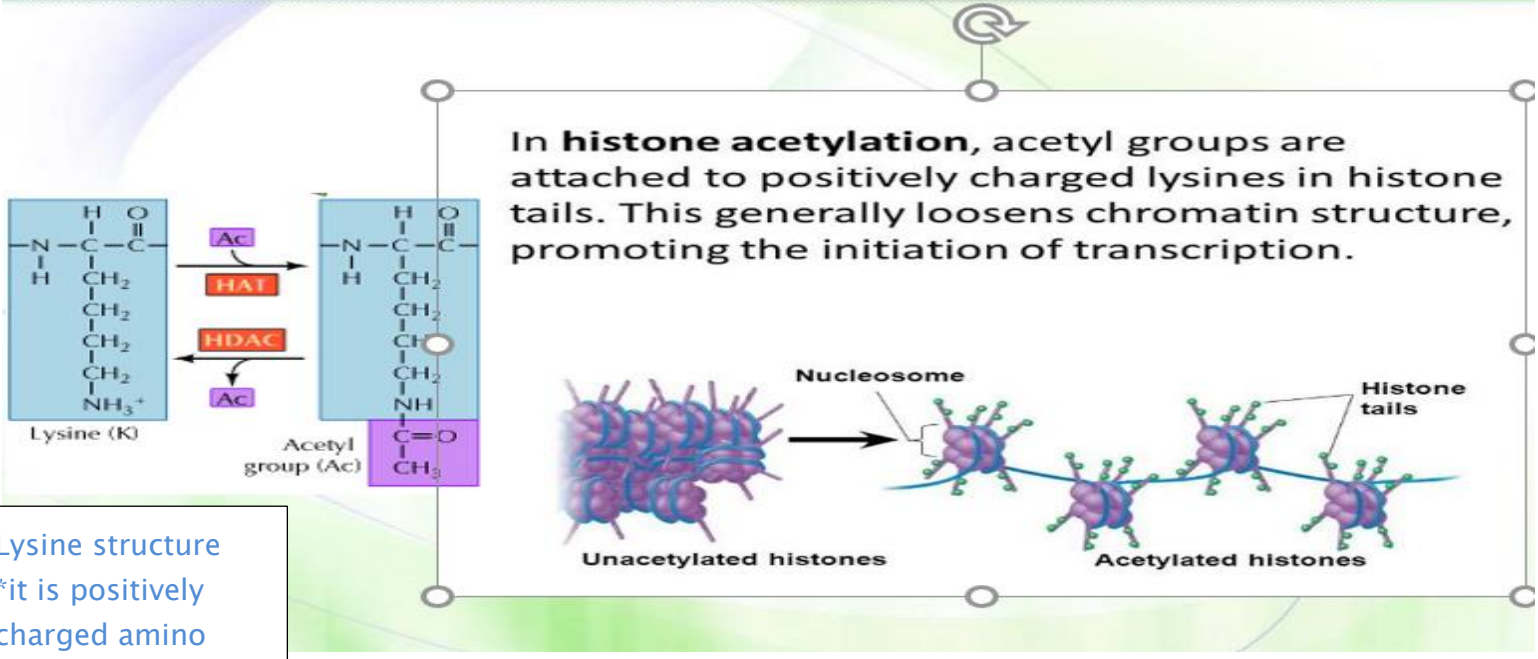


The core histones (H2A, H2B, H3, and H4) have two domains (internal 3-dimensional structures):

1)A histone-fold(fold=domain),internal structure which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle facilitating the formation of nucleosome core particle.

2)An amino-terminal tail, which extends outside of the nucleosome, and is rich in lysine(a.a)





Lysine structure *it is positively charged amino acid, facilitates interaction between histones and DNA that is negatively

There are many lysines in histone, so the interaction between histone and DNA is highly tight.

Enzymatic association

Transcriptional activators and repressors are associated with histone acetyltransferases and deacetylases, respectively

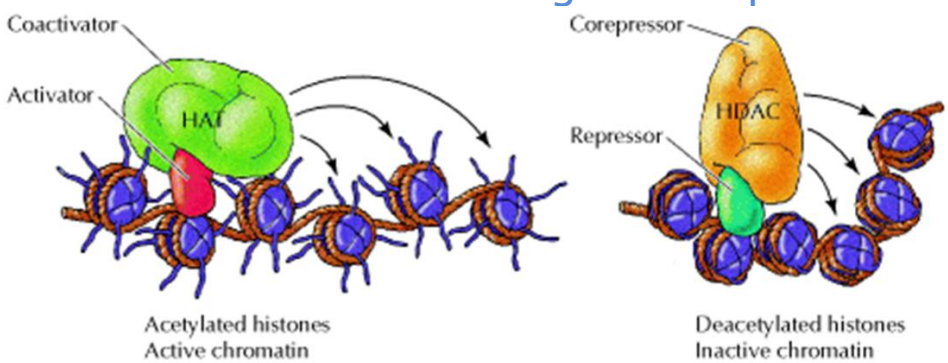
Lysine can be acytelated by acetyl transferase enzyme

So the +ve charge will be masked and removed; as a result, the interaction between DNA and histone will be weaker and DNA will be loose allowing the formation of euchromatin→facilitating and activating transcription.

On other hand, deacytelase enzyme removes acetyl group from lysine, so the +ve charge will be apparent→ strong interaction will occur and highly packed heterochromatin will form.

TFIID associates with histone acetyltransferases

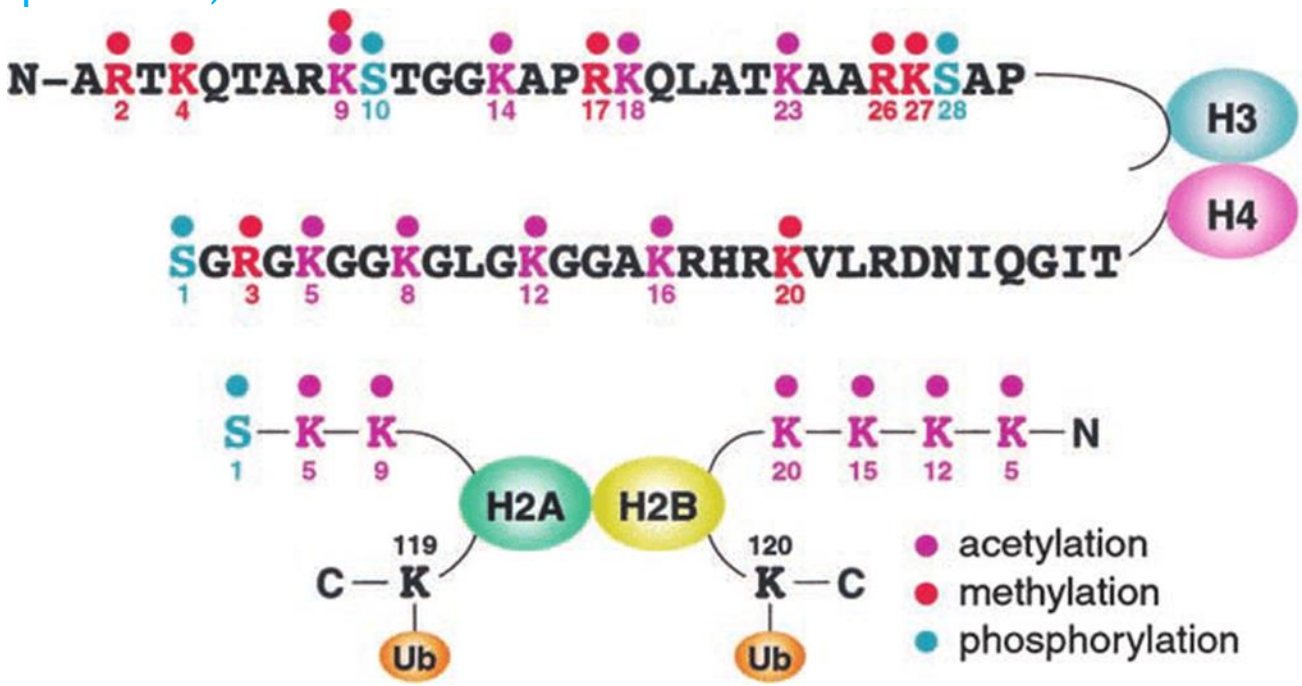
TFIID is the first protein that binds to promoter region, modifies DNA making it loose and accessible to other proteins allowing them to bind to different sites on DNA inducing transcription.



Other modifications of histones

Histone can also be methylated or phosphorylated.

Effect is dependent on sites of modification(to which lysine are these groups added)



These modifications are a bit of a source of complex because of large quantities of lys residues, in the pic above it is represented with K symbol, so depending on which Lys is modified, we can have activation, inhibition, repression of transcription→ but the overall that acytelation does is activating transcription.

Again, the challenge of chromatin

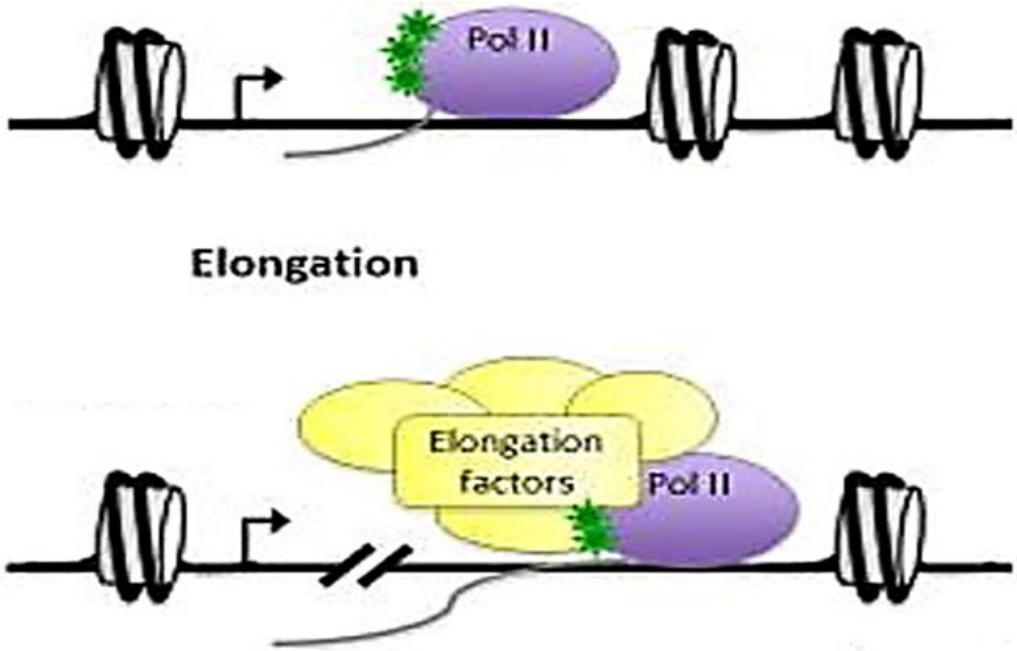
RNA pol II has same problem as DNA pol in reading and synthesis. Challenge in presence of nucleosome, nucleosome core complex and histone, so histone must be removed from DNA, allowing RNA pol to transcribe DNA and synthesize RNA. This is done by using elongation factors.

Elongation factors are associated with RNA pol specifically the tail of RNA pol that is phosphorylated and often associates with (capping factors, splicing factors and polyadenylation factors) but here it is binding to elongation factors, causing removing of histones.

Chromatin is still a challenge to RNA polymerase II during transcription.

Elongation factors associate with the phosphorylated C-terminal tail of RNA polymerase II when RNA elongation is initiated.

These elongation factors include both histone modifying enzymes (e.g., histone acetyl transferases)so it acetylates histones and the interaction becomes weaker and chromatin remodeling factors that transiently displace nucleosomes during transcription, removes histones and dismantles nucleosome structure.



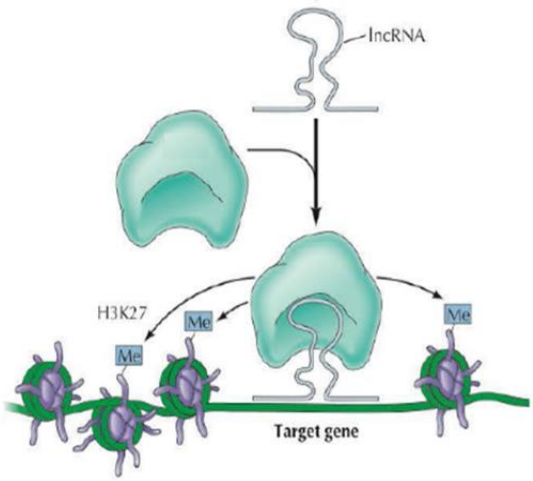
Role of noncoding RNAs

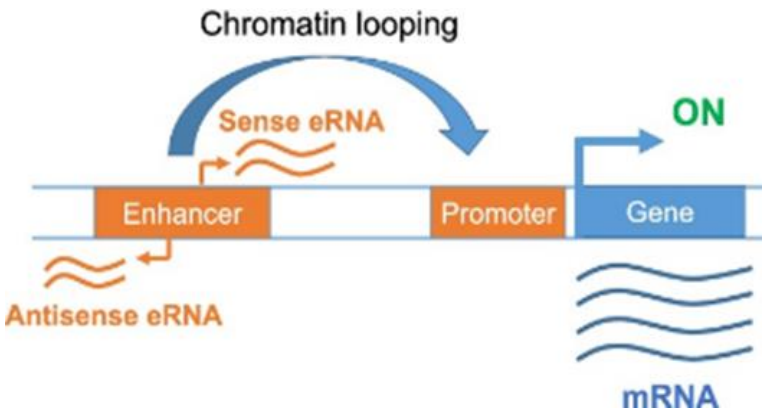
We will talk about Noncoding RNA especially the long ones (they are long relatively comparing to smaller RNA like microRNA, circular RNA and piRNA).

More than 50,000 long noncoding RNAs (lncRNA), which are >200 nucleotides long, are encoded by the human genome(these 50,000 lnRNAs were discovered last 5 years ,imagine how many intense researches were done!)

LncRNAs can be homologous to certain DNA sequences and form complexes with chromatin and DNA modifiers to repress gene expression via chromatin condensation and histone methylation(they can bind,

hybridize and anneal certain sequences of DNA and once they do that, many things could happen, they can associate with different proteins and enzymes that can modify chromatin, chemically modify histones via methylation resulting in regulation condensation(packaging) or relaxation.
Other lncRNAs can complex with general or specialized transcription factors (e.g. TFIIB), mediator(blocking interaction between promoter's proteins and enhancer), or RNA processing proteins. We don't know every thing they do yet.
Some enhancers can be **transcribed** into eRNA (e : stands for enhancers) that can regulate transcription of adjacent genes and other functions that aren't discovered yet.





X chromosome inactivation

LncRNA can act in cis or trans.(cis means in same level , like eRNA that affects nearby genes and trans mean travelling to other chromosomes influencing transcription in it).

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

As known females have 2 X and males have 1 X

So to be fair, one of these X in females randomly becomes inactivated (dosage compensation)

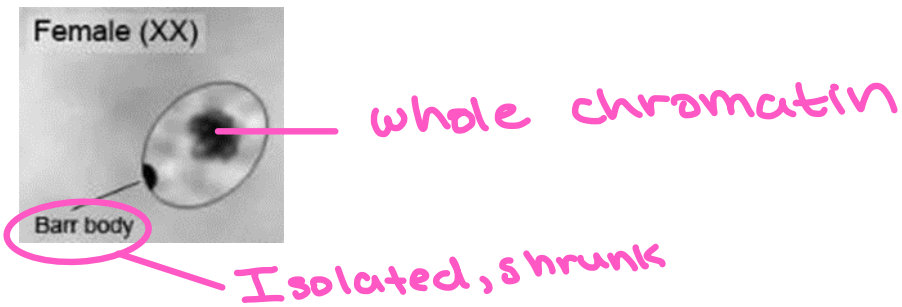
So we get 1 active x in males and 1 active x in females

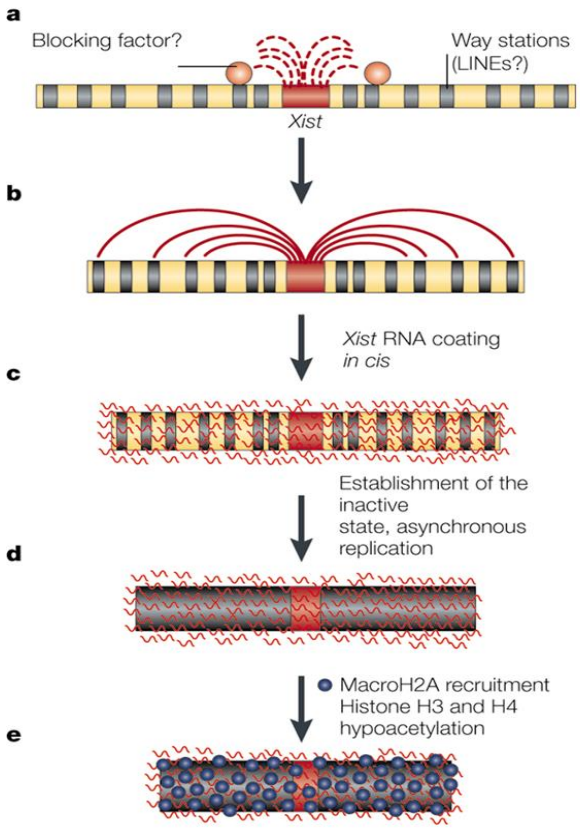
Inactivation of 1 x is random , in 1 cell the 1st X is active in another the 2nd X is the active

main principle:

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 number (and activity) of X chromosomes between males and females.





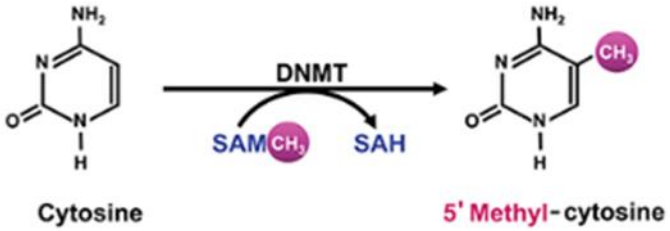
Inactivation occurs:

a) Transcription of xist RNA from X-RNA to be inactivated
b) lncRNA is produced , coating the X chromosome that produces it
c) recruiting number of proteins that methylate H3 cause chromosomal condensation
d) the chromosome gets shrunkبكَرمِش forming barr body
e) it may cause other modification like hypoacetylation: removal of acetyl group so interaction becomes stronger and tightly packed.

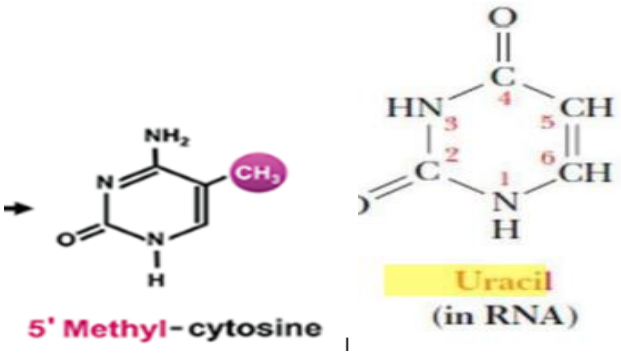
DNA methylation

Cytosine residues can be methylated groups at the 5'-carbon position specifically at CG sequences (called CpG isalnds near promoters).

Cytosine can be methylated and forms 5`-methyl cytosine

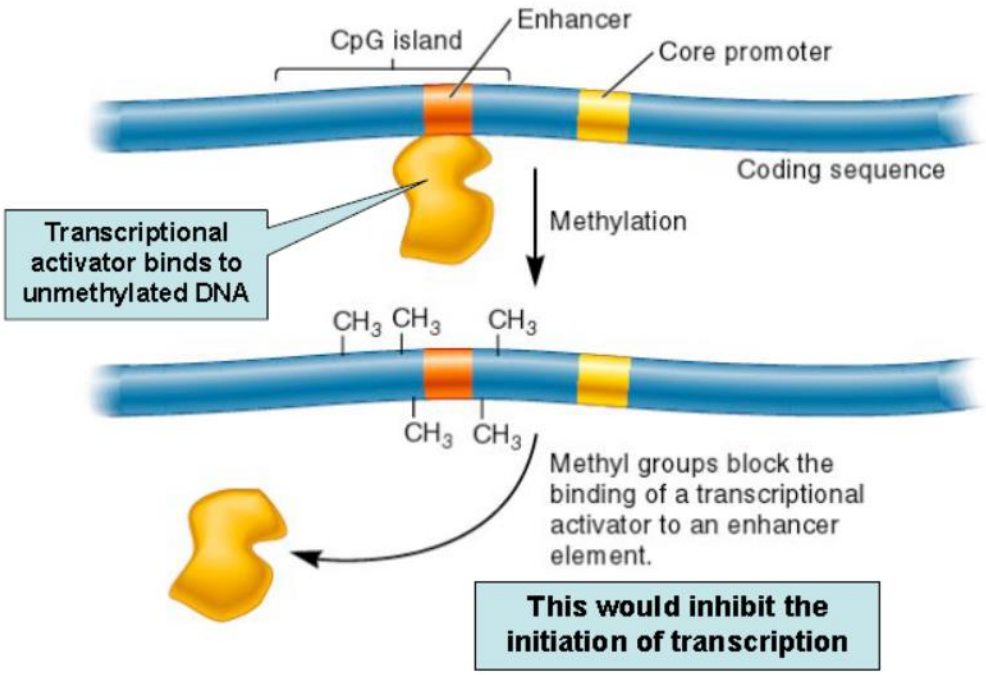


The doctor asked to go to first lecture and compare between U and 5`methyl cytosine And here is the difference:



Promoters of some genes are rich in CG sequences so we call them CpG islands.

DNA methylation reduces gene transcription by blocking of activator binding to DNA and inducing heterochromatin formation cytosine methylation is common in cancer cells causing disturbance leading to activation and inactivation genes.



Genetic imprinting

A phenomenon related to DNA methylation known as controlling maternal and paternal gene expression

Some genes have to be transcribed paternally only or maternally only

Or from both parents, any change can cause diseases.

Methylation is maintained following replication and is inherited.

Methylation is a mechanism of genomic imprinting (either the paternal gene or the maternal

gene is active).

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

40

The power of epigenetics

- Non-sequence dependent inheritance

These mice have different colours even though they have the same genes but different epigenome.

Epigenome is really an important phenomenon in regulating gene expression.
It is complex
Scientists still try to understand how it works exactly.
In the figures, there are identical twins have the same exact DNA sequence but with different pattern of acetylation and methylation of histone molecules and DNA
resulting in different gene expression that causes differences between them.
Causes:
Lifestyle and even stress
One of them may play sport and eat healthy food while the other doesn't and that will cause differences in epigenome.

Epigenetics is significant and heritable

Having a certain DNA sequence doesn't mean having a specific phenotype; you can control your own destiny and don't blame your failures on your DNA. The article above tells that we can control our DNA modification and that stress can change epigenome and DNA modification and of course they can be inherited.

الدكتور حكى عنده نكتة من 15 سنة تدريس بحكيها لازم نحكيلكم إياها 😋

من المعروف إنه الأخوة بالرضاعة حرام يتزوجوا لأنه حليب الأم يُؤثر على

Epigenome

2 babies have different genes but same epigenetics

فهل الأطفال اللي برضعوا الحليب الصناعي يُعتبروا إخوان بالرضاعة !

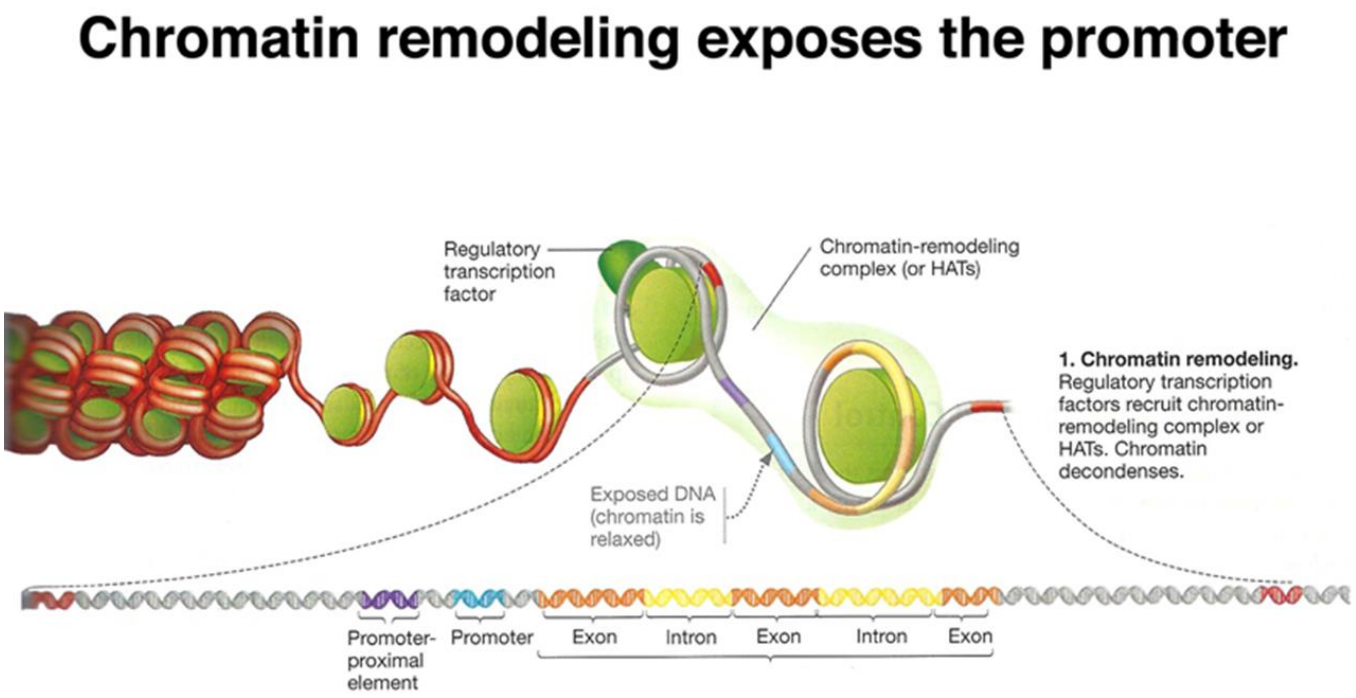
لسّا العلماء بدرسوا الموضوع

أوك نكمل🥲

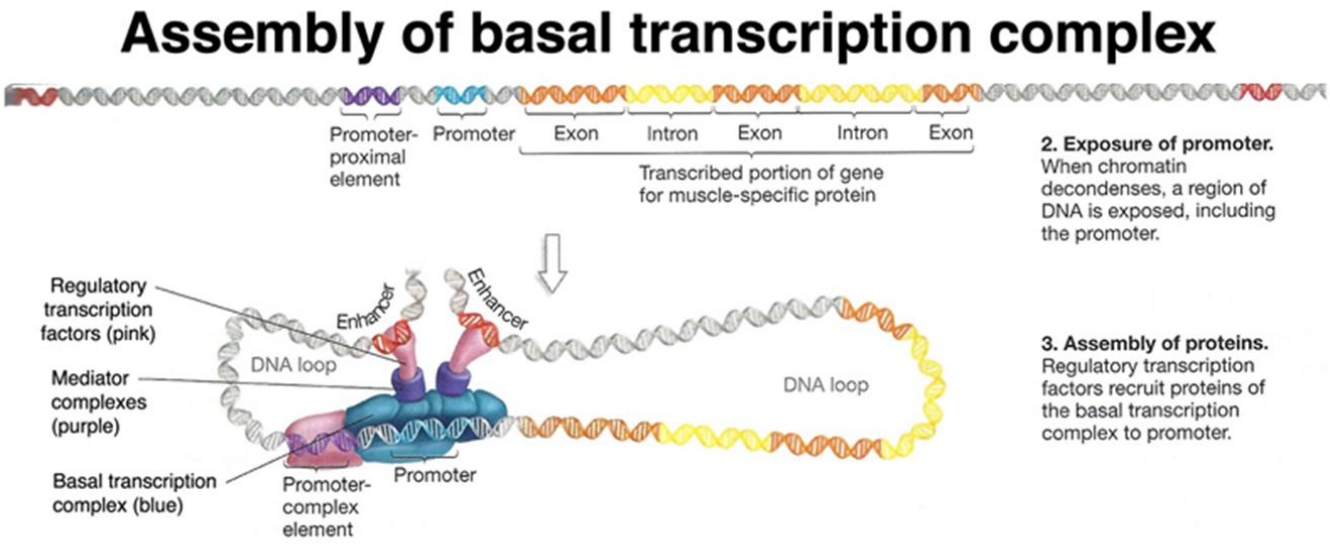
Let's catch up with the whole topic:

A scenario

A little more detailed process

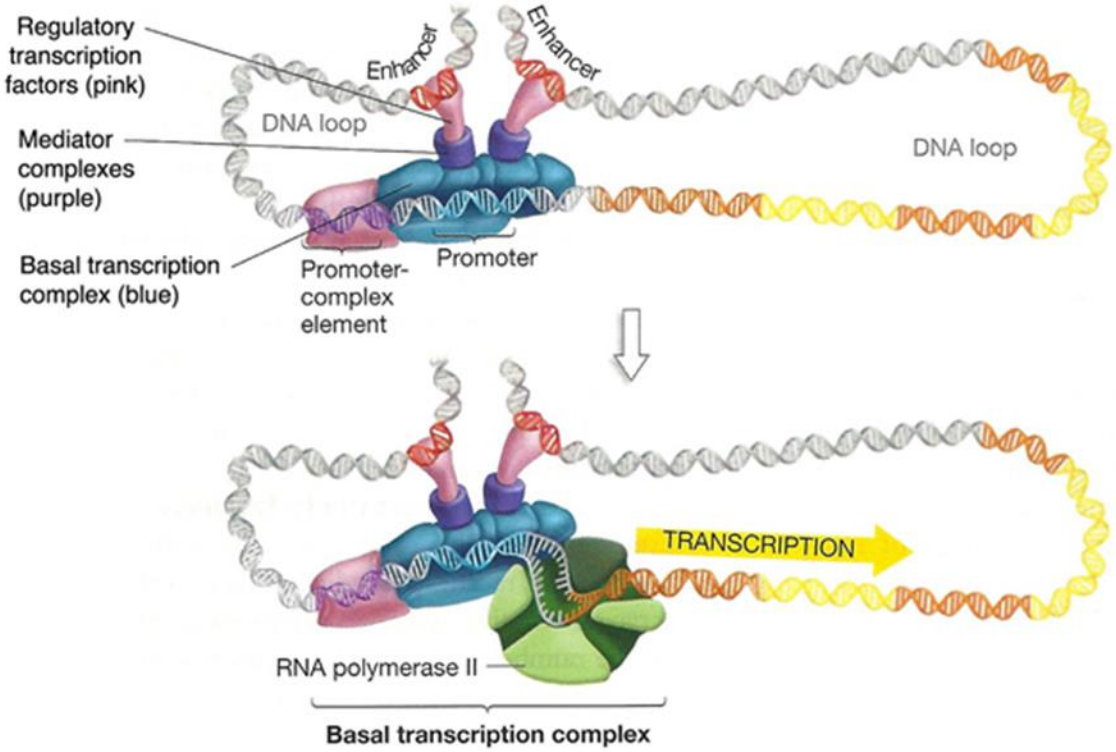


Step1: enzymes bind to DNA modifying it chemically and structurally Important sequence like TATA box are hidden so these remodelling factors and proteins modify DNA so these sequences get exposed.



Step 2: the promoter is now accessible by preinitiation complex, it is exposed not packed any more causing assembly of preinitiation complex on promoter that can interact with other regulatory proteins that are bound with PPE, enhancers…

RNA polymerase joins transcription complex



3. Assembly of proteins. Regulatory transcription factors recruit proteins of the basal transcription complex to promoter.

4. Attachment of RNA polymerase. RNA polymerase II completes the basal transcription complex; transcription begins.

Step 3: attachment of RNA pol and starts transcribing genes

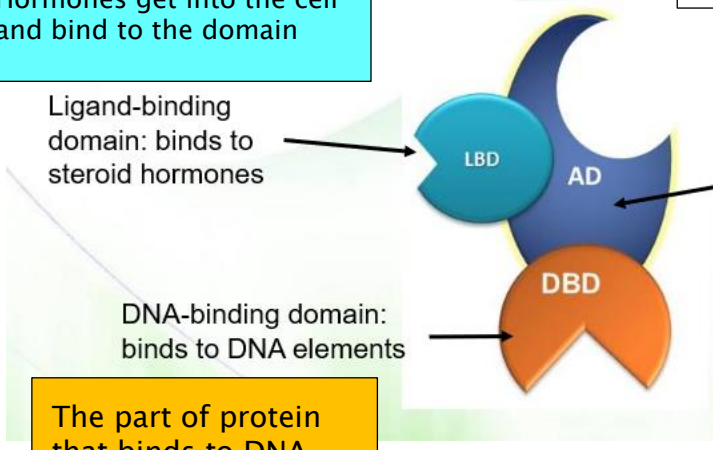
Example:

Nuclear steroid receptor SNR

General structure of SNRs

Ligand: small molecule like hormone.
Hormones get into the cell and bind to the domain

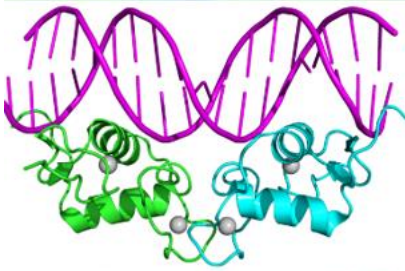
It has 3 domains (internal 3D structure functioning independently)



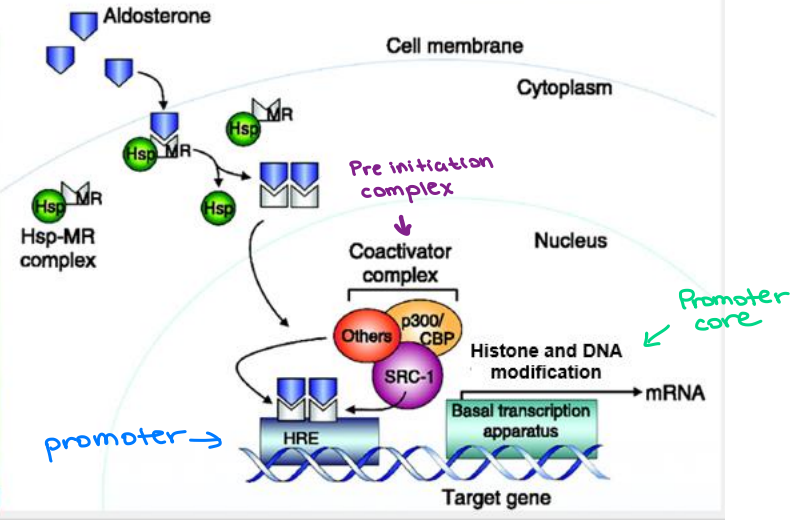
Part of protein that binds to coactivator or corepressor (activates transcription or represses it)

The part of protein that binds to DNA

Steroid hormone receptors

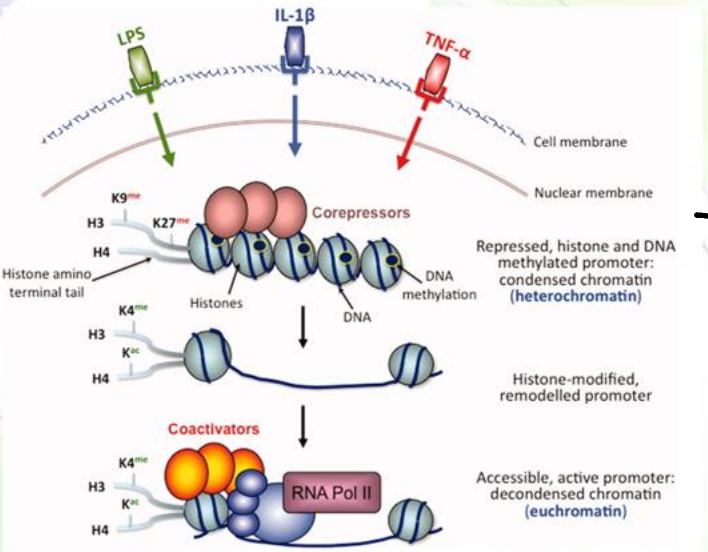


Receptors bind steroid hormones at *ligand-binding domain*, then translocate into the nucleus where they bind specific DNA sequences called *hormone response element (PPE)* via *their DNA-binding domain*, and recruit and bind transcriptional regulatory proteins using their *activation domain*.



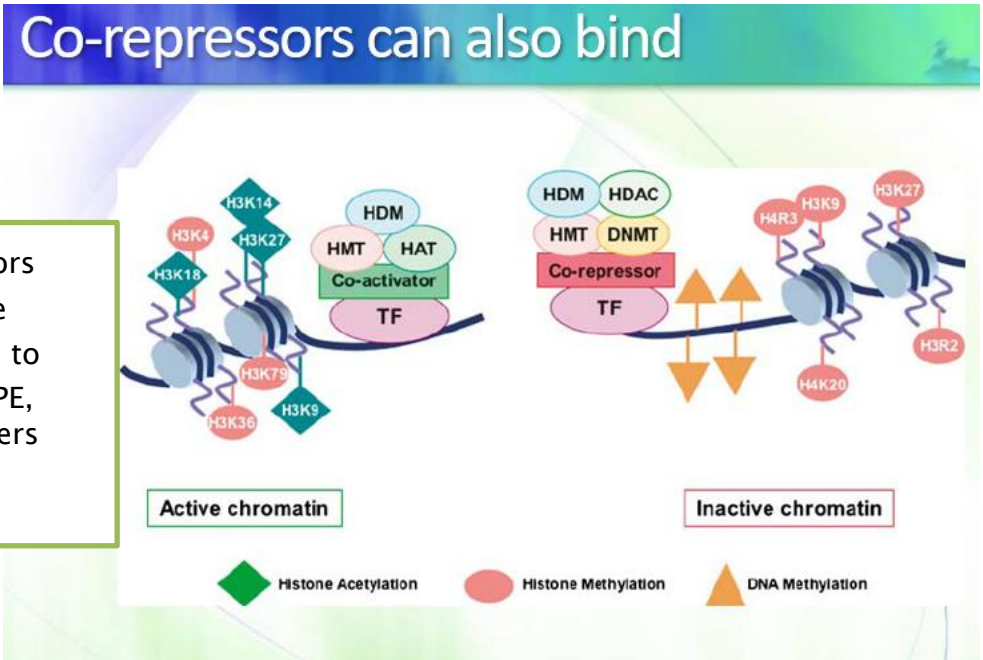
How does it function?
Steroid hormone (aldosterone that is important in regulation kidney function and amount of water), oestrogen, progesterone, androgen, female and male sex hormones, cortisol or cortison they are hydrophobic =lipid-like
They diffuse through plasma membrane and bind to the receptors at LBD (ligand binding domain) then 2 receptors dimerise (form dimer) then the dimer will get into nucleus binding to hormone response element (DNA specific element) this element will bind to DBD (DNA binding domain) recruiting many proteins including coactivators.
Hormone response element is a promoter proximal element that is close to core promoter so it interacts with preinitiation complex activating RNA pol 2 to start transcription.
AD: is responsible to interact with all these proteins, forming whole proteins complex.

Also, linking outside to inside



Same thing with other receptors
Cell surface receptors bind to their ligands causing signal transduction and activating different proteins, eventually signal is relayed inside nucleus and activate corepressor and coactivator changing the structure of chromatin (loosening it so they can bind to regulatory protein) or compacting it so it can't be accessed to proteins.

Activators
Activate binding to DNA, PPE, enhancers



Repressors
Influence gene expression, blocking transcription and inactivating gene expression

Past paper

58. Deacetylation of histones has which of the following effects?

A) Uncoiling of histone structure, preventing it from being accessed by transcriptional machinery.
B) Uncoiling of histone structure, allowing it to be accessed by transcriptional machinery.
C) Coiling of histone structure, preventing it from being accessed by transcriptional machinery.
D) Coiling of histone structure, allowing it to be accessed by transcriptional Machinery.

Answer: C

59. One of the following is NOT a regulation by epigenetics:

A) Methylation of histones
B) A point mutation of the promoter regions
C) Methylation of cytosines within promoter regions
D) Binding of noncoding RNAs to promoters regions
E) Conversion of heterochromatin to euchromatin

Answer: B

التعلم من النجاح أمر مهم، لكنك لن تصل للنجاح دون التعلم من الفشل. 😊

دعواتكم