Genetics
& Cell biology

◉ Sheet ○ Slides

Number

12

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At the beginning of the lecture the doctor started talking about a jelly fish which can have green, blue, red or yellow florescent protein.

Scientists injected the sperm and the egg with different florescent proteins, and in this example it was green and blue, and then fertilization happens, so the nerve cells of the offspring either have a blue protein or a green protein, so this is a way to study how these jelly fishes are connected to each other.

In another experiments, they managed to make only a certain regions florescent according to the promoter they used and where they used it .

Also , they once tried to plant an ear pinna (auricle) on the back of a mouse as a part of organ transplantation experiment

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Transcriptional regulatory proteins

We have already talked about transcriptional regulatory proteins

Remember : Domains : a 3D structure in a protein that folds independently from the rest of the protein, which means that if I cut out this domain , its structure remains unchanged no matter what.

In this type of proteins (with domains) they found that in the transcriptional regulatory protein we have at least 2 types of domains; **DNA binding domain** and an **activation domain** (protein binding domain)

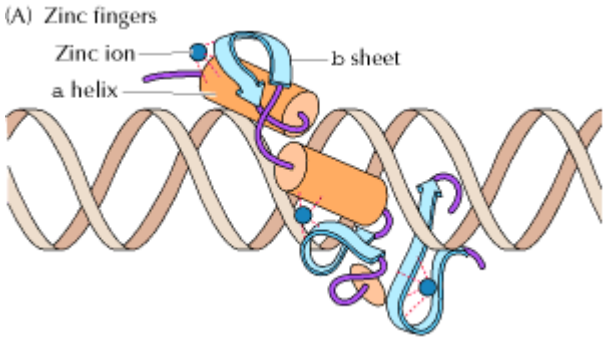
So what the protein does is that it binds to the DNA by the DNA binding domain, and the other domain interacts with other proteins (e.g. : regulatory proteins , RNA polymerase , transcription factor, etc.)

So if I take out DNA binding domain from the protein, it still have the ability to bind to DNA molecules (still functional), same for protein binding domain; if you take it out it still can bind to other proteins, and that`s what genetic engineering does

In genetic engineering , we play around by taking the DNA binding domain from Protein#1 add it to activation domain of Protein#2 , so this complex will target the DNA sequence that is recognized by protein#1 but it interacts with proteins of protein#2

Looking at the DNA binding domains we find different structures in different receptors, e.g.

A- **the zinc finger domain** found in steroid receptors, it looks like a finger and has zinc ions in between



How steroids function? 1. They are small hormones, they can diffuse through the plasma membrane (don`t need a transporter or a carrier because they are hydrophobic)

2. then they bind to a receptor found in the cytosol or in the nucleus (it depends), note : receptors in cytosol eventually goes inside the nucleus

3. in the nucleus they bind to certain regions in the DNA -regulatory regions- called **Enhancers** regulating Gene Expression, so they **don`t** bind to promoters instead they bind to enhancers, note: steroids are specific to certain genes

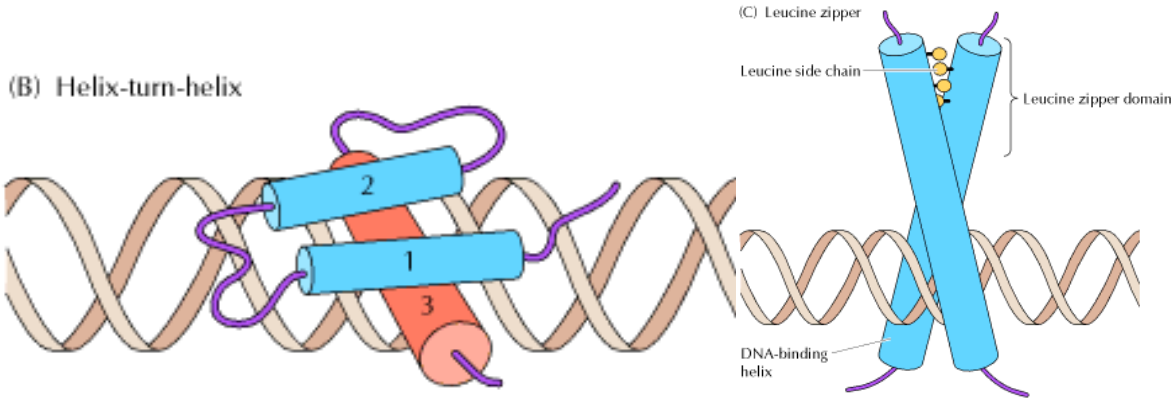
Steroids can regulate gene expression by binding specifically to Enhancers of these genes, and that`s how gene expression occurs.

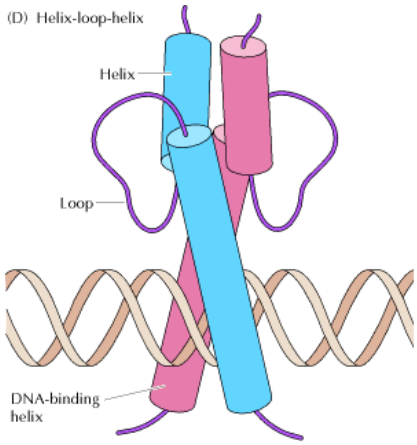
B- Helix-turn-helix motif

C- Leucine zipper (CREP) , found in cAMP-response element binding protein (CREB)

D- Helix-loop-helix

** different names and structures but they all share 2ry & 3ry structures





Now, talking about the **Activation Domain:**

The activation domain is not well studied as the DNA binding domain, but we have different structures with different compositions; you might have

- acidic domains that contains glutamate (a lot of glutamate) and aspartate
- glutamine rich domains
- Proline rich domains

  and a lot of other different domains that can interact with certain regulatory proteins

And the way they regulate gene expression is by 1, 2 OR 3

1. Stimulating transcription by interacting with certain proteins (transcription proteins)

2. They can recruit and facilitate the interaction with other proteins (facilitate the assembly of transcription complex on the promoter)

3. By modifying chromatin

e.g. **c**AMP-**R**esponse **E**lement (CRE) **B**inding protein (**CREB**), which contains Leucine zipper as mentioned before

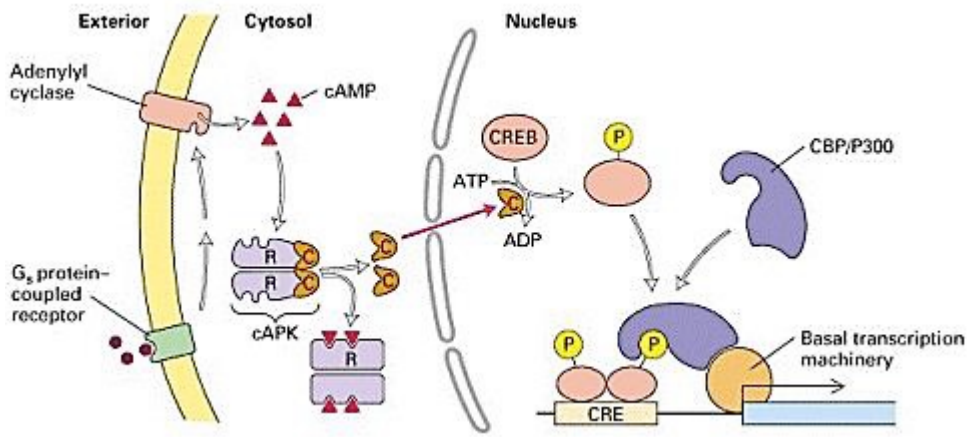
the way it functions : it`s regulated by protein kinase A, pk-A is stimulated by certain receptors on the cell surface, so it`s regulated by cAMP then induction for adenylate cyclase (mentioned in previous lecture)

cAMP binds to pk-A , so pk-A is activated , it goes to the nucleus and phosphorylates CREB , so CREB is active , it can then interact with other transcription factors by CREB-binding protein (CBP) , it can then interact with CRE (cAMP response element) which is found on the DNA , facilitating interaction with either a mediator or RNA polymerase.

In case it interacts with RNA polymerase; induction of gene expression of certain genes that has the CRE

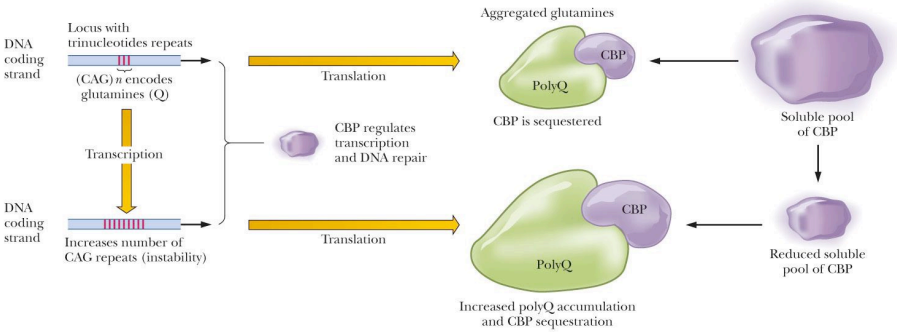
Mins 00:00 - 10:00

If I take the CRE and put it upstream of another gene, this gene will be regulated by CRE as well (genetic manipulation).
If I put it upstream the luciferase gene; what would happen? Florescence.



Huntington's disease: is a neurological progressive disease, symptoms usually appear around 30s, it`s fatal, it results from a mutation in a transcription factor named Huntington.
Huntington: is a transcription protein that has a CAG repeats, those CAG repeats found in the protein coding region, and it codes for glutamine, so the protein has many glutamine molecules, probably 10-15 glutamines, they don`t affect the protein function, but there is a possibility that the DNA polymerase mistakes while replicating DNA, so instead of 15 CAGs we will have 100 or more, which will affect the protein structure, and the protein is dysfunctional, (note : glutamine is a binding site for CREB-binding protein (CBP)), so what happens is that those poly-glutamine products bind to a lot of CBPs, and a lot of those CBPs bind to ONE Huntington TP, so it will be sequestered and there will be no CBPs to bind to CREB, so no gene transcription what so ever, and that what makes this disease so progressive and can`t be repaired.

Briefly: Huntington is a TP that binds to CBPs → poly-glutamine residues increase in number → they bind to CBPs hiding it → no enough CBPs to bind to CREB → Huntington disease.



So those are the activators, now talking about the repressors.

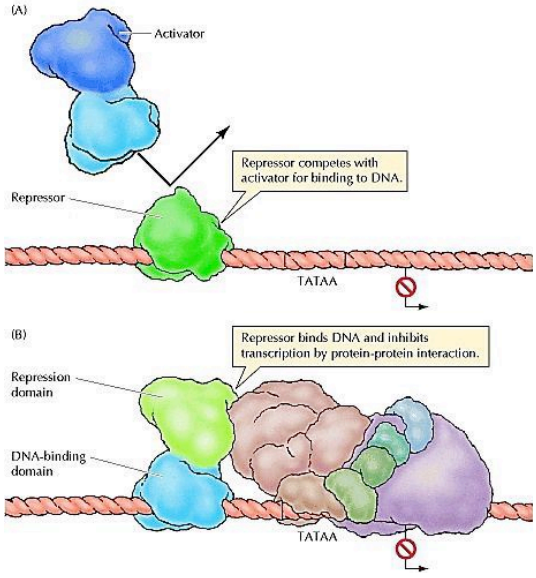
Repressors:

Repressors can bind to regulatory sequences found in the promoter region, they can suppress/ block/ inhibit transcription.

Mechanism of action:

Either they have both DNA binding domain and protein binding domain, so the bind to DNA and the protein binding domain binds to the **basal transcriptional complex** and hold it so blocking transcription, no phosphorylation of RNA polymerase and they cannot move forward

OR they only have a DNA binding domain, which means they work as competitive inhibitors (activator and repressor compete on binding to DNA)



Another level of regulation:

All things mentioned above are talking about the regulation of initiation, but another level of regulation is what happens in the elongation step.

Scientists found out that for certain genes transcription starts, and we have elongation of the DNA, the mRNA is being produced or synthesized and coming

out of the RNA polymerase, but shortly after initiation we have 2 proteins : **Elongation regulatory proteins : DSIF , NELF** which are negative regulatory proteins, they bind to RNA polymerase and stop it from moving forward so there is no elongation until another protein **(P-TEFb)** induces phosphorylation of these proteins and when phosphorylated

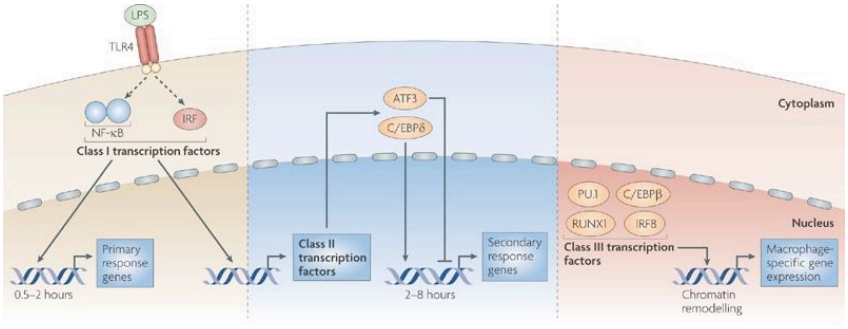
- One of them is released and can`t bind to the complex anymore : NELF
- The other one still attached but can`t inhibit the RNA polymerase anymore: DSIF

This way of regulation allows **productive elongation** (productive elongation: means that the regulation is producing the final product from mRNA so the gene and the transcription process are productive)

So regulation is either for initiation or elongation after the transcription starts. (Please refer to slide#27 for the picture).

(Mins 10:00 – 19:11)

Transcription is divided into many parts forming a transcriptional network: Primary, Secondary, Tertiary transcription, etc.
During primary transcription, we produce transcription factors, those which are produced through 1ry transcription induces the 2ry transcription.
2ry transcription also produces other TFs that induces the 3ry transcription and so on, so it`s a network of transcription.
e.g. during an experiment dihydrotestosterone (an androgen) was added to cells after 24hrs → nothing changed in the cell`s morphology, cells started to change only on day3, day6 a very noticeable change in morphology.
Notice that it takes less than 24hrs to produce a protein, so after 24hrs only 1ry transcription happened and produced some transcriptional factors but still some genes weren`t transcribed yet, but on day3 there was a time for 2ry transcription to start so you can notice some change in the cells.



Names mentioned in picture not included

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Epigenetics:

Epi- means above which indicates a higher level of regulation, regulation mentioned before was basic regulation, in the basic regulation if a mutation occurs to a part of the DNA sequence, a defect in gene expression results (stimulation or inhibition).

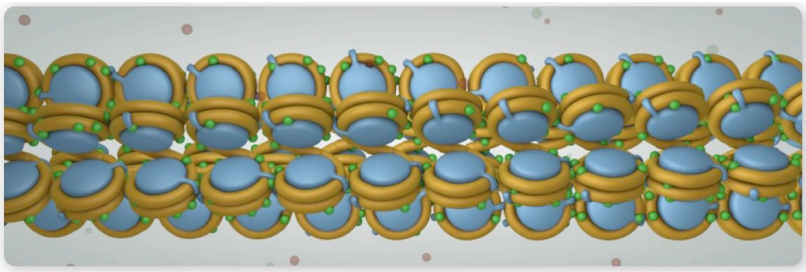
Epigenetics is a higher level of regulation without changing the DNA sequence, this happens by changing the way the DNA is packaged (the structure of the DNA itself).

What is the effect of the change in DNA structure?

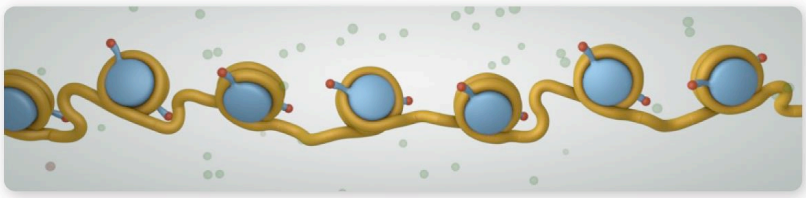
One of the examples is that DNA is either heterochromatin (condensed\ packaged around the histones) or euchromatin (loose\ relaxed).

In loose chromatin there is an access to genes, so TFs can bind to DNA sequence and start transcription, but in packaged chromatin, TFs can`t reach the DNA because it`s hidden, so even if I have nucleosomes and histones I still need to release histones from DNA so RNA polymerase can read DNA and start transcription.

So usually the inactive genes are condensed\ packed, and active genes are euchromatin.

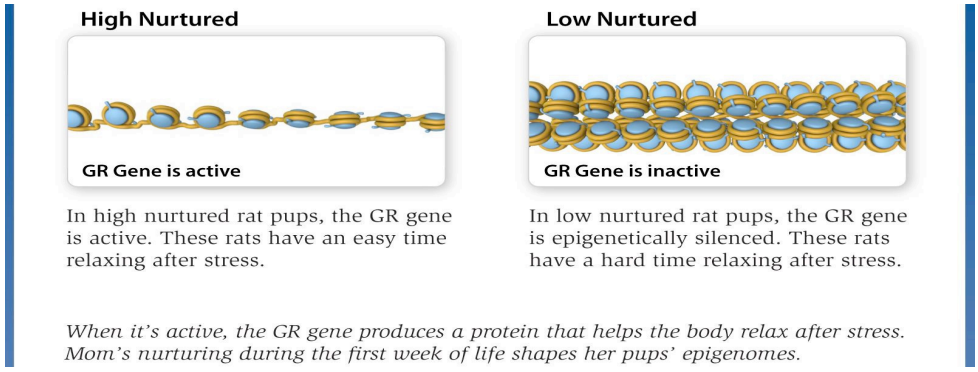


The epigenome tightly wraps **inactive** genes, making them unreadable.



The epigenome relaxes **active** genes, making them easily accessible.

According to that, identical twins have the same exact DNA sequence but the way the DNA is packed is different, so the response to everything of both twins is different completely as a result of having different chromatin structures.



This is a funny experiment on mice:

Glucocorticoid receptor gene (GR) is either packed or loose.

Scientist found out that if the mother nurturers her rat pups very well, the pups grow to be happy, less tense and relaxed rats, and their GR is found to be loosely packaged (active), yet when the mother doesn`t provide good care for the pups they grow to be stressed out and really tense and their GR is found to be condensed.

Conclusion: behavior affects packaging of DNA.

Now, how are chromosomal structures altered?

1- Chemical modification of histones : acetylation, methylation, phosphorylation ana SUMO (small ubiquitin-related modifier: small peptides added to histones – new field of research)
   Also, methylation of cytosine residues can happen.
   All are part of epigenetics
2- Complexing of TFs with nucleosomes remodeling factors: modification of formation of the nucleosome itself (DNA packaging)
3- Binding of non-coding RNAs to DNA

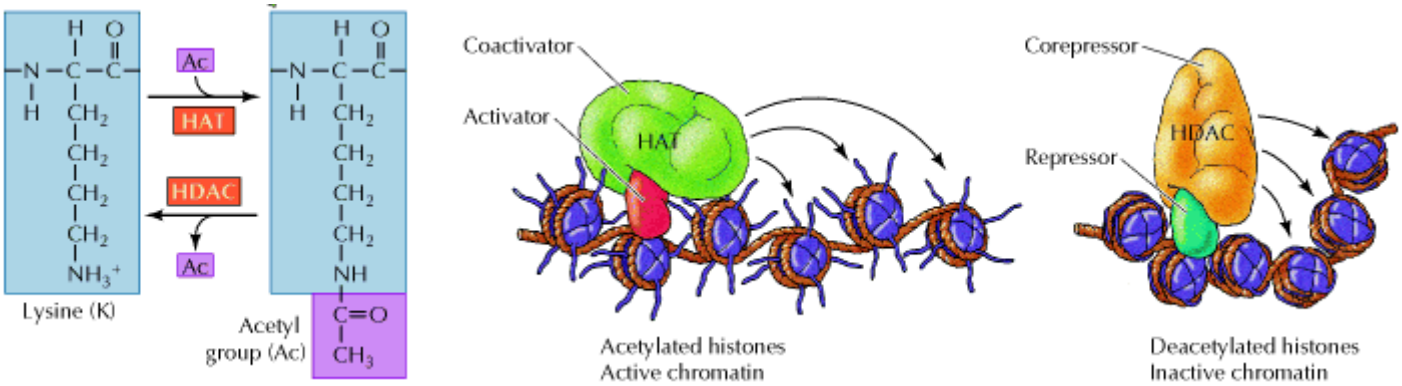
Histones acetylation:

Histones are proteins rich with positively charged amino acids (lysine, arginine).

Here we concentrate on lysine found in histones not bound\free\ +vely charged so it can strongly interact with the DNA which is –vely charged → DNA packaged.

But when histones are acetyalated by adding an acetyl group to lysine the +veld charge is neutralized and interaction of histones with DNA becomes weaker → relaxed DNA\ active.

- Acetylation is a process of converting heterochromatin to euchromatin (inactive to active), with no change in DNA sequence, which results in activating gene expression.
- Acetylation is done by histone acetyltransferase (HAT) enzyme, while deacetylation is catalyzed by acetylase enzyme.
- 2 enzymes simply change the gene expression by changing chromatin structure.
- A transcription factor which is TFIID (TF2D) is associated with HAT, how? TFIID wants to activate the gene expression , it binds to the promoter region, induces the HAT which acetylates the histones relaxing the DNA, and it becomes easier for other TFs to come and bind to DNA → gene expression ON.



(Mins 19:11 – 30:00)

- Another example: We have 2 nuclear receptors which are RAR (retinoid acid receptor) and RXR (retinoid X receptor), 2 different receptors which bind to retinoid forming a dimer, this dimer binds to certain regulatory genes in DNA suppressing gene expression→ they bind to a promoter region; specifically to HRE (hormone response element), which is associated with HAD (histone deacetylase), removing the acetyl group and the DNA becomes dense\ packed\ inactive → gene expression OFF. (refer to slide#37 for picture)

  ** same thing happens for histones phosphorylation and methylation
  - Phosphorylation of histones : **activation** of gene expression
  - Methylation of histones : **inactivation** of gene expression

    ** notice that all these modification mechanisms are related; phosphorylation leads to histones acetylation activating gene expression and at the same time inhibition of methylation.

    Students` questions:

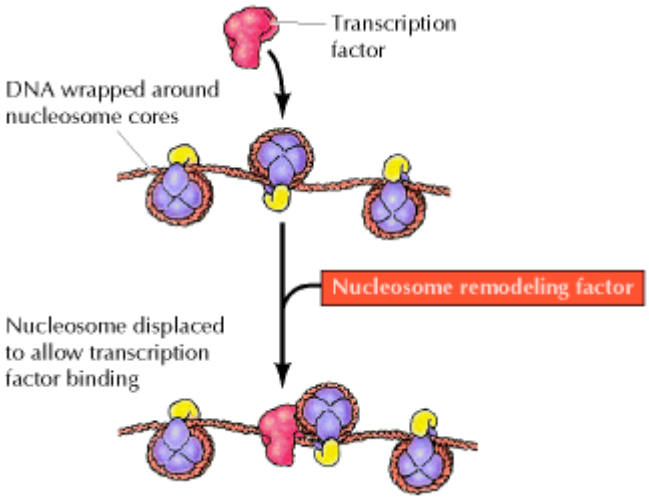
- activation of HAT causes acetylation for all DNA or a certain region? Only a certain region.
- How phosphorylation inhibits methylation? Interaction with certain enzymes related to methylation.

Nuclear remodeling factors:

They remove histones allowing DNA replication to take place (reposition nucleosomes) by facilitating the binding of TFs by:

1- Repositioning nucleosomes making DNA accessible (pushing nucleosomes aside)
2- Altering necleosome structure allowing protein to bind to DNA
3- Totally remove histones from DNA.

** remodeling factors can be associated with transcriptional activators and repressors. E.g. TFIID that has enzymatic activity.



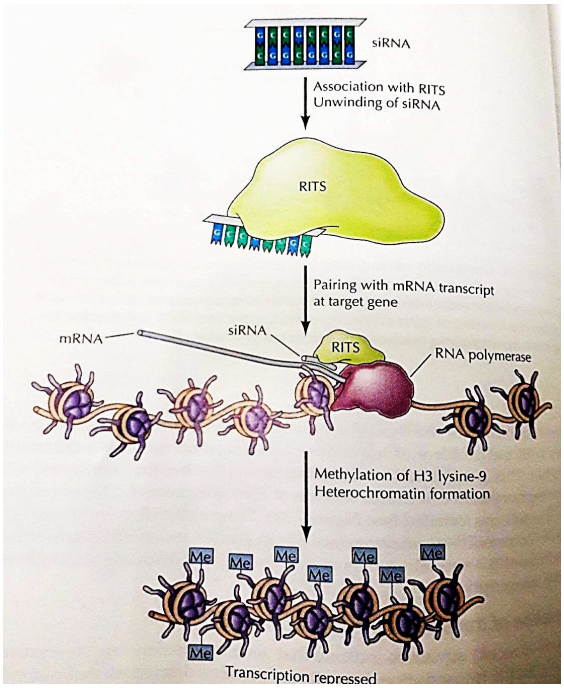
(Mins 30:00 – 38:23)

Non-coding RNAs:

At old times we only had 3 types of RNA molecules: mRNa, tRNA and rRNa, then we discovered that we have way too more than 3 types like microRNA, long non-coding RNA,..,etc.

We`ll talk about microRNA in the last lecture Inshallah.

Non-coding RNAs are molecules that can bind to mRNA, so when mRNa is synthesized they come and bind to the complementary sequence on mRNA, they bind to it and hybridize to it preventing elongation of mRNA causing inhibition of transcription and maybe induction of methylation.



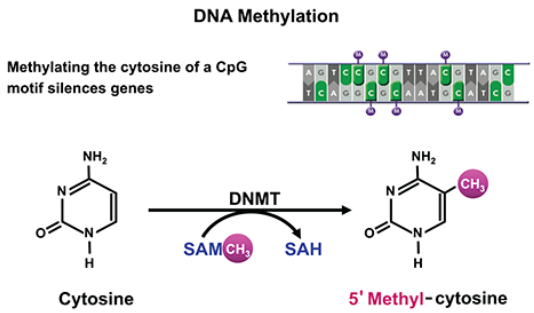
DNA methylation:

Not methylation of histones, methylation of DNA itself.

We have once talked about DNA methylation (adenine) in DNA repair but here we`re talking about methylation of cytosine residues.

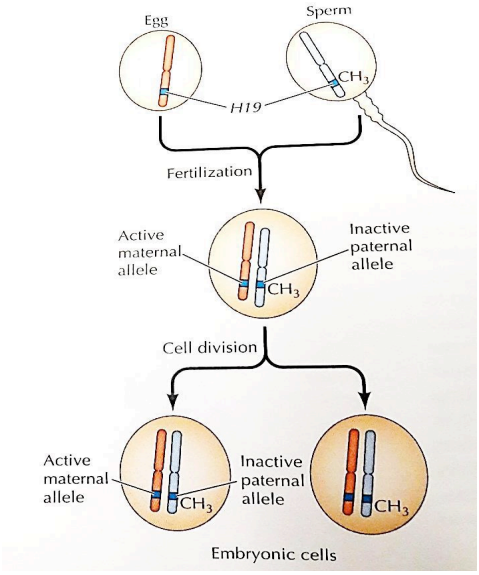
Scientists have noticed that some genes have a lot of CGs (beware CGs not GCs), in those regions which are named CPG islands, when the cytosine is methylated, suppression of transcription happens.

And what`s really amazing about this is that methylation is maintained during replication, meaning that during replication when complementary strands are binding to each other, BOTH strands should be methylated (methylated cytosine will bind to Guanine, and the neighboring Guanine will bind to methylated cytosine on the other strand)



- Methylation is a mechanism of genomic imprinting, which means that only one allele of the gene will be active, it`s ALWAYS the paternal OR the maternal allele that is active and NEVER both.
  And it depends on the gene itself which one should be active, the maternal or the paternal.

- The maternal allele is always the active one in certain genes, and the paternal are the active ones in other genes, if not, certain diseases develop.
- We have 70 genes that are regulated by genomic imprinting, either paternal or maternal and that`s regulated by methylation.



Finally talking about **X chromosome inactivation:**

As we all know females have 2X chromosomes while males have one, so the 2nd X chromosome must be inactive in females and that`s why it`s methylated.

- Methylation of X is random, any of the 2 \ not specific.
- On the inactive X chromosome there is a gene named **xist** which is the only region that is active, this gene produces a long non-coding RNA molecules, they are not translated\not producing proteins, a lot of non-coding RNA molecules are produced, they coat\cover the X chromosome, then methylation of all histones of the inactive X, forming a condensed chromosome named the **bar body.**
  (refer to slide#42 for the picture)

(Mins 38:00 – 45:00)

In the end the doctor started talking about an experiment done on three groups

The first experiment : they extracted a DNA and put it in a test tube then exposed it to an emotional atmosphere and they looked at the DNA, and noticed that there is a difference in DNA packaging.

The second experiment : they took white blood cells from a person and they extracted the DNA and put them in a test tube and they exposed the test tubes to different emotional atmosphere (anger,silence..) , they found that there is similar epigenetic changes happened to the persons's DNA in the same person and in the test tubes , then they separated the person and the test tube in a separate room , and the epigenetic changes remained similar .

The third experiment: they took photons and put the extracted DNA with it , they noticed that the photons , instead of being scattered they became in harmony.

The conclusion : the surrounding environment affects our DNA and our DNA affects the photons and the surrounding environment as well .

Sorry for any mistakes :)

I was once really convinced that the (white or black) question isn`t fair, but now I know that even if it seems that there`s a little grey color in between, it`s you the one who`s waiting to be pushed to one of the sides.

" TO BE OR NOT TO BE …"