CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

18

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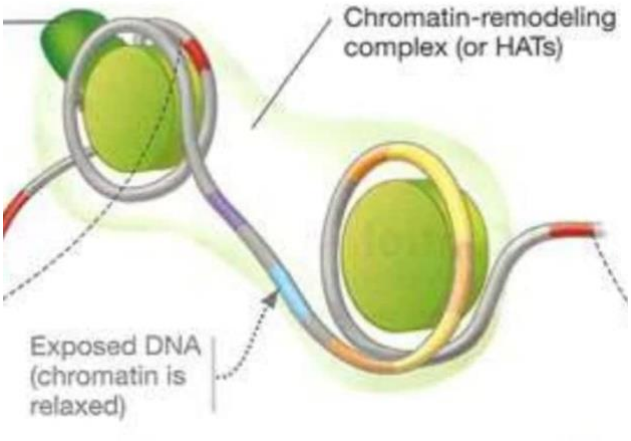
A short revision about the last lecture:

Transcription factors: a set of proteins that have a role in transcription.



Some of their functions:

1-changing the position of nucleosomes.

2-acetylate or deacetylate histones; which results in loosing chromatin structure (more active form).

3-methylation of cytosine (in DNA). or sometimes methylation of the histone its self; which results in more condensed form of chromatin.

4- they help in the production of long noncoding RNA (**lncRNA**).>>as discussed in the last lecture, lncRNA is a result of gene called **xist** which is located in the inactive X-chromosome.

once lncRNA is formed, it recruits other proteins to methylate HISTONEs, this results in formation of **Barr body** (condensed X-chromosome).

**Note that changing position of nucleosome exposes the promoter region.

*Promoter region contains proximal promoter element or a classical core promoter (TATA box).

****

The scenario of transcription process:

in the last lecture we knew that some genes are can't be expressed as their promoters are wrapped by nucleosomes.

So, there is a set of proteins called nuclear regulation factors (**NRFs**), they bind with the DNA in order to expose promoter region by:

-pushing histones away from promoter region

-remove histones

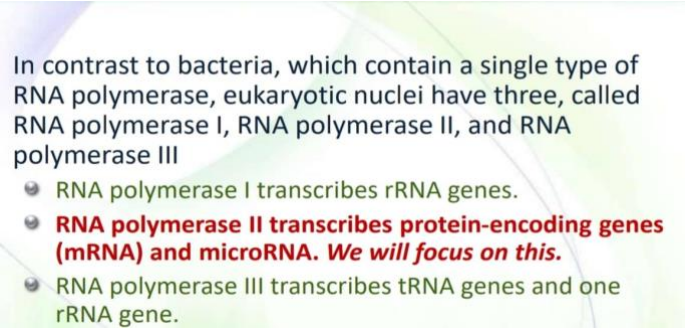
-altering nucleosomes structures – in this case the gene is wrapped around the nucleosome. But due to the conformational changes, polymerase II can transcript it-.

**

Now the **RNA polymerase II** -a polymerase responsible for transcription in **eukaryotes**- can bind to the promoter region

**this picture below is a revision for the types of polymerases in eukaryotes from transcription slides.

Note: the doctor said that we will focus on RNA polymerase II.



** You may ask your self

What stimulate the cell to do transcription for a gene or stop it?

-there are many signals that stimulate cells for such cases, steroid hormone is one of them.

-as we know, steroid hormones are lipophilic molecules which cross plasma membrane. So, their receptors are found in the cytosolic side of the cell.

* once steroid hormone crosses plasma membrane, it binds with its receptor (ligand binding domain of this receptor (LBD)) in the cytosolic side.

Note that there is extra information in the first three pages (everything before northern blotting) that I added for clarification. if you don't understand, you can watch just the first 6 minutes of doctor Maamoun's video.

* In order to bring a stimulation. This complex (hormone receptor complex) must activate transcription of a certain gene. So, it enters the nucleus and bind to a sequence known **as Hormone Response Element** (HRE) via its **DNA binding domain (DBD)**.

** remember that receptors have these domains

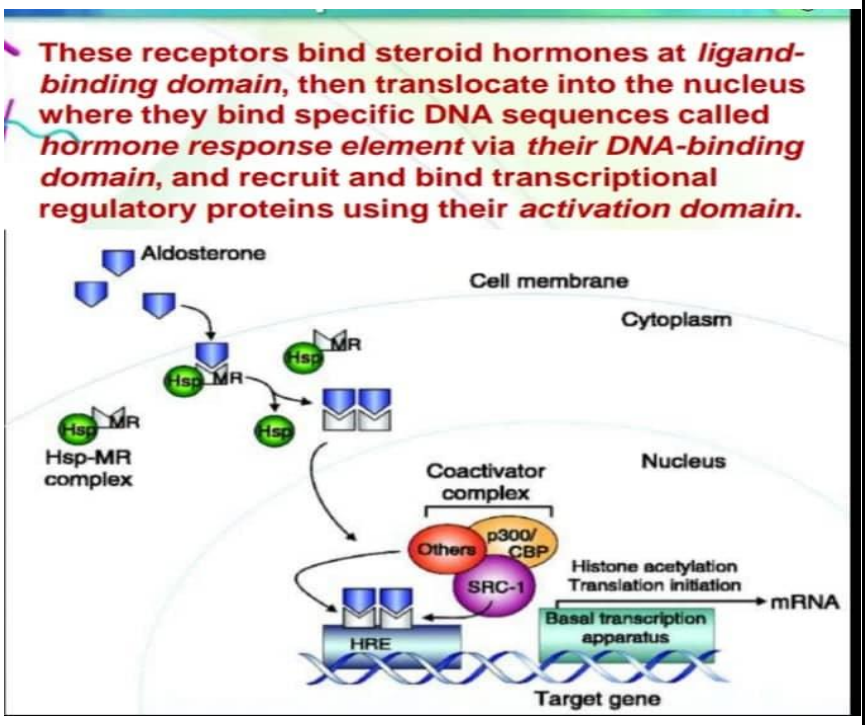
-**DNA binding domain DBD**

-**ligand binding domain LBD**

-**activator domain**: which can bind with **activators** or **repressors**

For knowledge

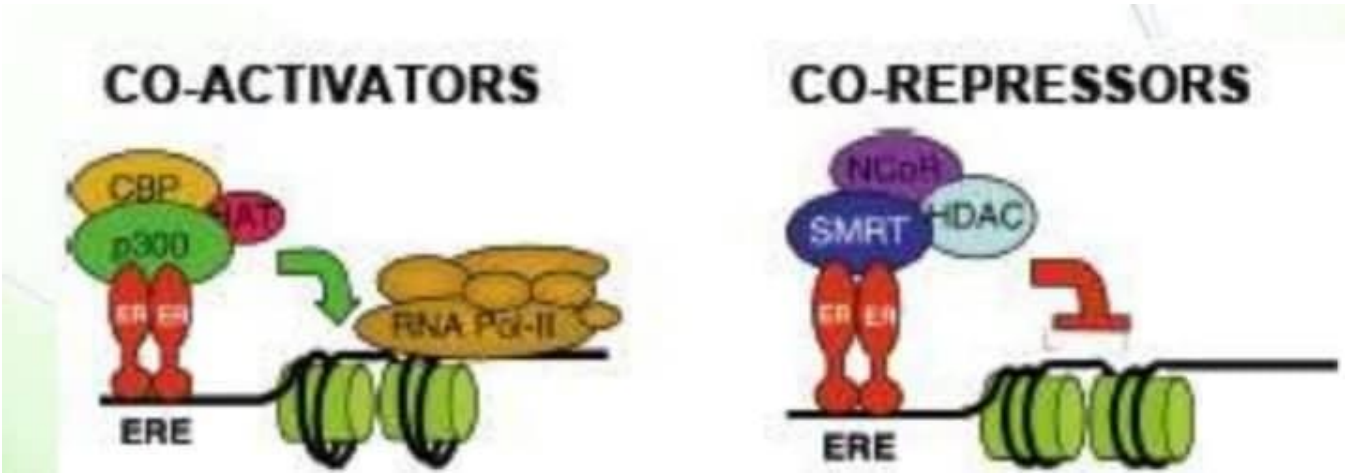
** HRE is a consensus sequence, which means that it is a common sequence (we can distinguish it once we see it), in which there's probably a gene (we study it further to know if there's or not.



*once **hormone-receptor-complex binds** to **HRE** it requires other proteins to bind with its **Activator domain**

-as we said they may be repressors >>to prevent transcription of this gene, or they may be activators-.

Note that activators that bind with activation domain are associated with **Hormone Acetyl Transferases HATs** which acetylate the lysine of HISTONES. Also, the repressors are associated with **Hormone Deacetylases HDAC** which do the opposite of **HATs.**



Remember that histone is a protein core consists of 8 subunits, two molecules of

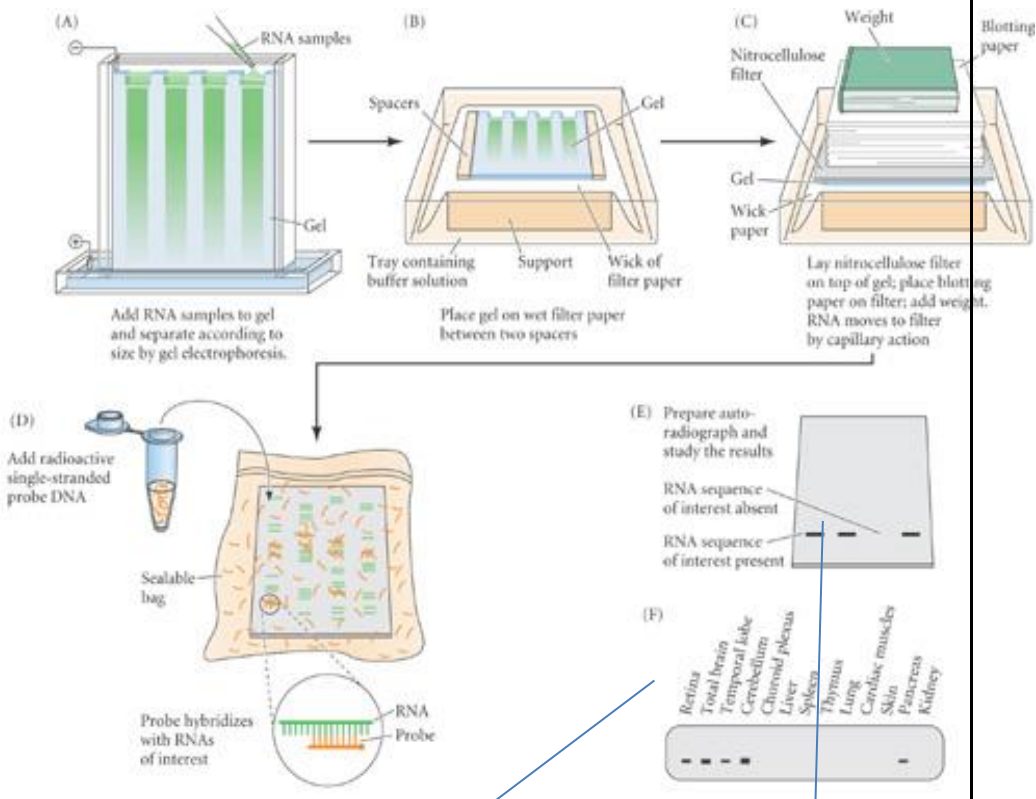
H2A, H2B, H3, H4

If this nucleosome is associated with a protein called H1 then it's chromatosome.

How can we measure RNA levels and place of expression?

Northern blotting

- This is done exactly like Southern blotting except that RNA from cells is isolated instead of DNA.
- RNA molecules are fractionated based on size by gel electrophoresis. You will have large range of sizes.
- The fractionated RNA molecules are transferred onto a membrane.
- RNA molecules are targeted by a labeled DNA probe with sequence that is complementary to a specific RNA molecule.
- What information can you deduce from it? U can know <u>if this gene is expressed or not</u> <u>and the degree of its expression in different conditions</u> (This is why we use it).



-The thicker the sample, the more transcribed the gene.

What can u know here?

-We can obtain what tissues expressed that gene and how much.

What can u know from these samples?

-They have the same length

-The third sample doesn't express that particular gene.

Note: *when u r looking at southern blotting, u r looking at the mature RNA.*

Why? Splicing, capping, and polyadenylation processes are quick so it is hard to get a pre-mRNA.

Examples: What can u tell about each sample?

We have mRNA molecules with different sizes. Why?

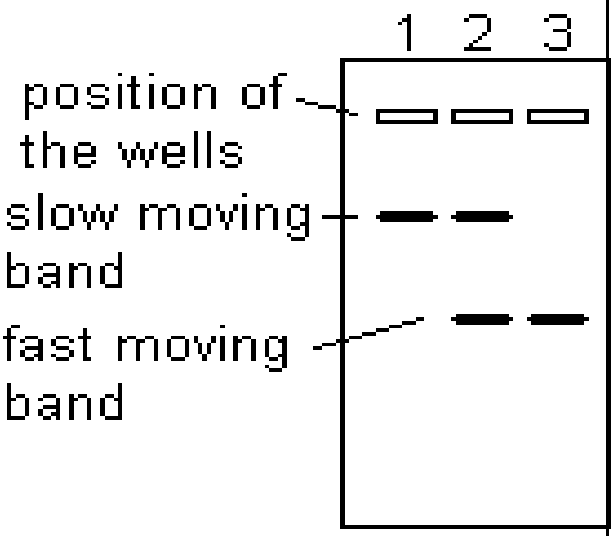
-Alternative splicing, note that the second sample has got the two molecules.

-It could be a mutation, removal of the termination sequence in the DNA so the synthesis of the RNA doesn't stop until it hits another subsequent or get tired etc.

In the other example, we are gonna study the effect of a certain drug on the expression of a certain gene.

Notice that the drug **facilitates sample 3, inhibits sample 1, and has no effect on sample 2.**

So, the more drug you add, the higher the expression.



- What prove that I didn't change <u>the volumes</u> of the samples (by adding more RNA for sample 3 than for sample 1) and that the drug works well (by increasing the expression of AMG gene)?
- The housekeeping gene which doesn't get affected by any outer condition has the same amount for the three samples.

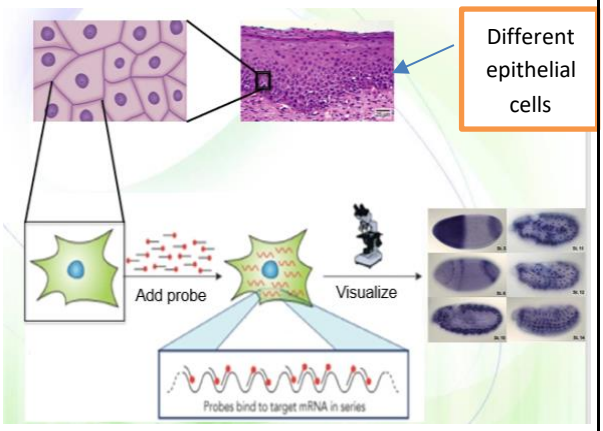
E.g. actin and tubulin.

If the housekeeping gene concentration was different, it wouldn't be true that the drug has succeeded.

A gene with constant expression (examples: actin, tubulin)

In situ hybridization

- In situ hybridization method reveals the distribution of specific RNA molecules in cells in tissues.
- RNA molecules can be hybridized when the tissue is incubated with a complementary DNA or RNA probe.
- I add a probe. The probe will bind to the RNA exactly where it exists. (If it exists in certain cells, they will emit a signal).



✓ In this way the patterns of differential gene expression can be observed in tissues, and the location of specific RNAs can be determined in cells.

✓ I know what cells express what gene and the location exactly.

If I have two tissue sections, one of them contains cancer and the other doesn't. I take all of the RNAs from each one and do northern blotting for each one. Can I conclude that the more intense band gene is overexpressed by the cancer cells? No, it could be that the cancer cells affect the fibroblast cells or any other cells and the gene is overexpressed in these cells not in the cancer cells. (some cancer types cause inflammation)

What do we need to do?

We do in situ hybridization and look at hybridization at a specific location in tissue section.

This is true for the protein that exist here.

- By doing immunohistochemistry (on the right), we know that the protein is found in the basement membrane but don't know its source exactly!
- By doing in situ hybridization (the picture on the left) we know that the source is undifferentiated epithelial cells (which emit the signal).
- They express the gene into RNA and produce proteins that r released and get deposited in the basement membrane (so we know what cells expressed the particular protein).