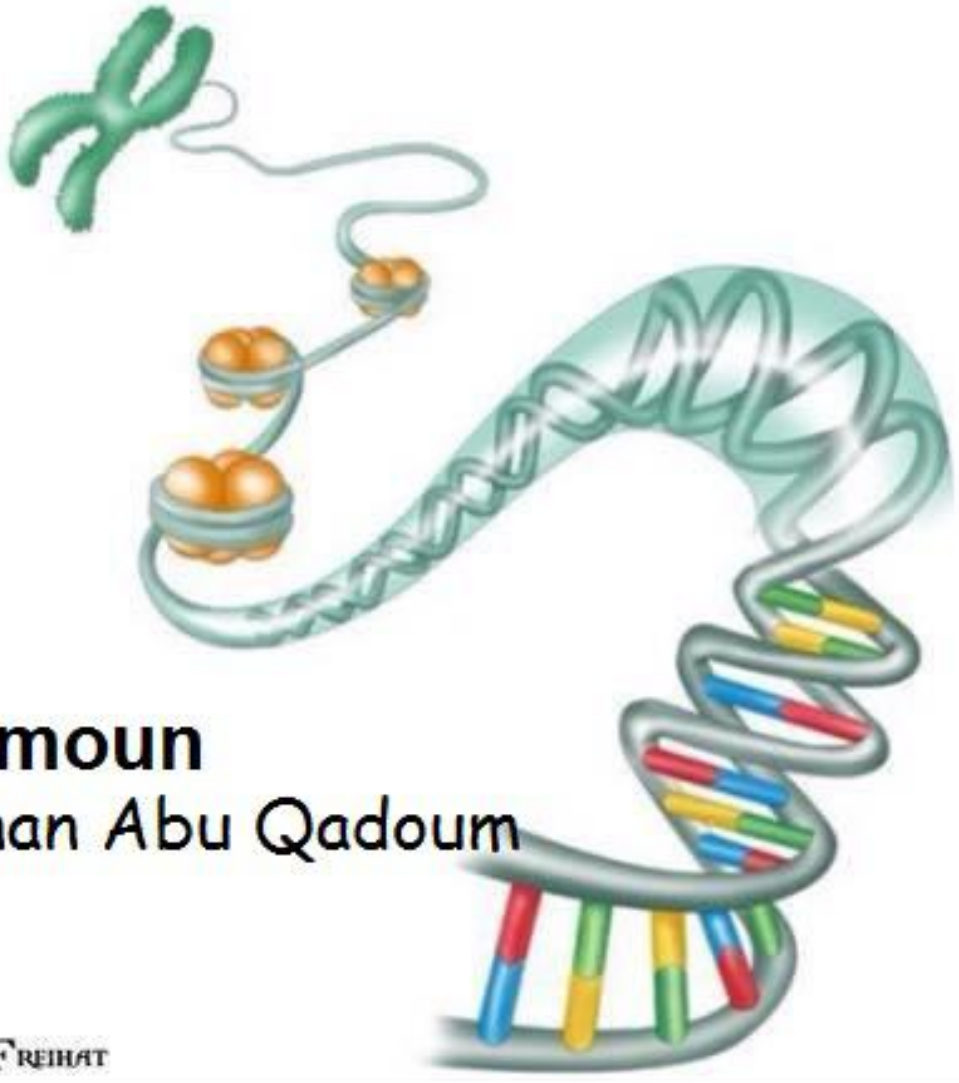
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Lecture # 7

Title:

Dr. Dr. Mamoun

Done By: Afnan Abu Qadoum

Date:

Price:

Designed by Nadeen Al-Freihat

Peroxisomes and The Nucleus

Salam everyone ☺ today we will talk about Peroxisomes and the Nucleus , this sheet contains most of the things written in the slides .

peroxisomes :

Peroxisomes are small membrane -enclosed organelles, basically they contain enzymes that carry out mainly **oxidation reactions** and just like mitochondria ,they replicate by division and they can also fuse.

-Most human cells contain about(500) peroxisomes , number varies according to the type and the function of the cell.

-There are about 85 genes that encode peroxisomal proteins ,collectively these proteins are known as **peroxins** .

There are two types of these proteins :

☝: membrane proteins . ✌: matrix proteins (internal).

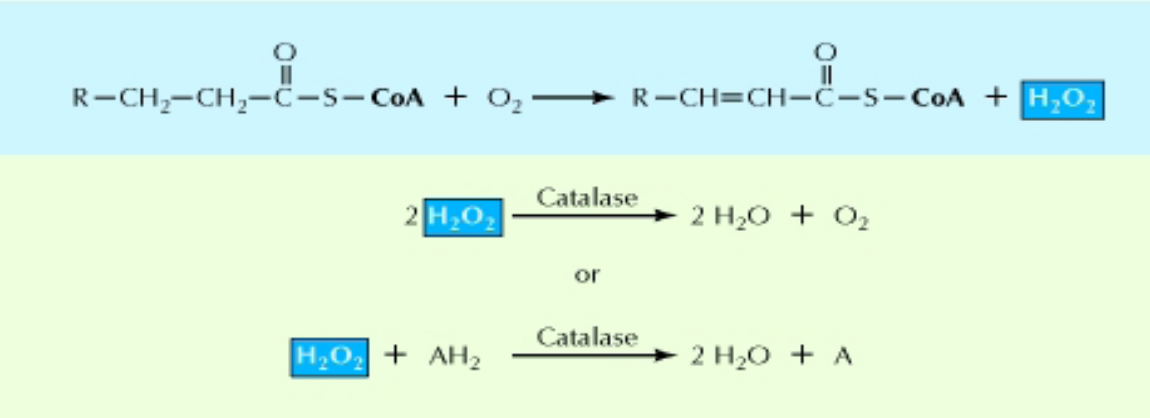
The way they are synthesized and transported is different .For **the internal proteins**, they are synthesized in the cytosol on free ribosomes then they are imported and carried into peroxisomes .

On the other hand ; **membrane proteins** come from the **ER** and act as receptors and channels for the import of these internal proteins .

So, before talking about how proteins are synthesized and imported into peroxisomes , let's talk about the function of these organelles :

_They carry out oxidation reactions ,a byproduct of these oxidation reactions is **hydrogen peroxide** .Hydrogen peroxide is harmful to the cell, so as a protective mechanism ,peroxisomes have the enzyme (**catalase**) that

can convert hydrogen peroxide into water .



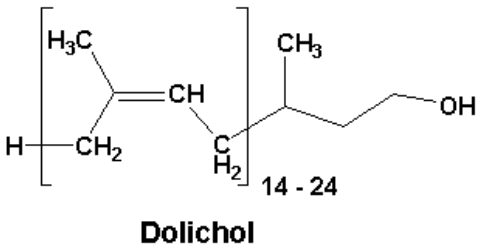
_There are many substrates that are broken down inside peroxisomes by oxidation ,like : (uric acid , fatty acid and some amino acids) .

{ Remember that **fatty acids** are oxidized in both **peroxisomes** as well as the **mitochondria** in *eukaryotic* cells } .

_ Peroxisomes are also responsible for the **synthesis** of other molecules such as : (cholesterol, Dolichol, bile acids as well as Plasmalogens) ,so U expect that cells specialized in synthesizing bile acids or cholesterol to have a large number of peroxisomes .

So let's talk about some of these molecules :

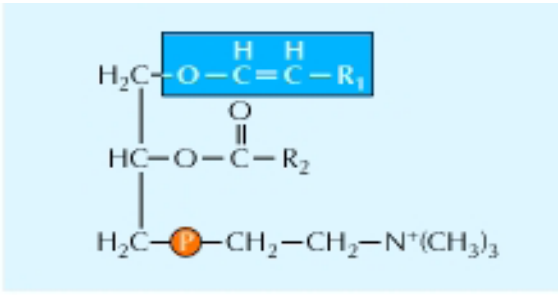
Dolichol is made from **farnesyl** .we talked about Dolichol when we talked about the addition of sugar molecules on asparagine residues of the proteins synthesized in the ER to make them glycoproteins (i.e. during glycosylation , when we attach sugars to proteins ; these sugars are first synthesized on **dolichol** that is anchored in the ER membrane , then they are transferred to the protein).



Dolichol

Plasmalogen : is a phospholipid (one of the unusual

phospholipids in having rather than an ester group , it has an **ether** group on **carbon number 1**), **plasmalogens** are important in membrane structure of heart and brain .



✍. How are proteins transported into peroxisomes ?

Basically , it starts with two proteins known as : peroxin 3 (**Pex *3***) and peroxin 19 (**Pex <u>19</u>**) .

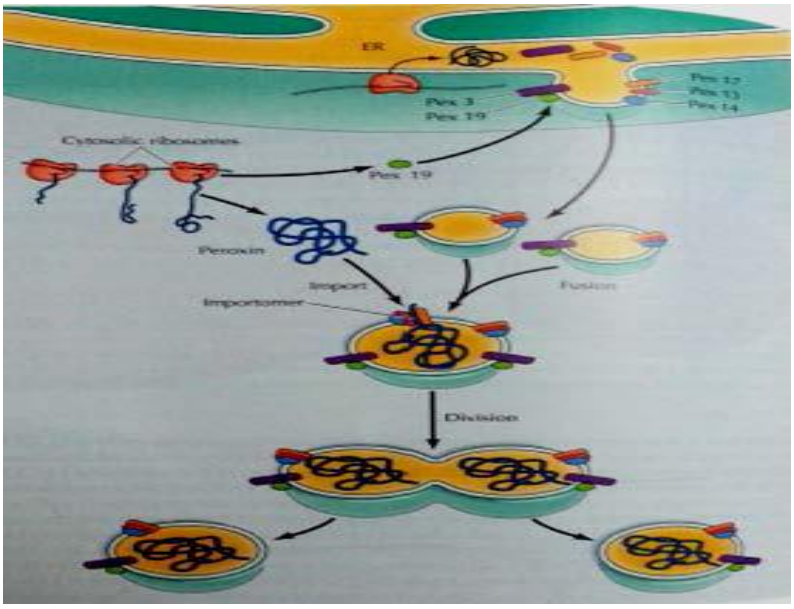
Note : in the slides it's written (pex **<u>9</u>**) …-it should be (pex **<u>19</u>**) , so correct it ☺ .

Pex 19 is a **cytosolic** protein that has a **farnesyl** group and it is recruited into the membrane of the ER , then it interacts with pex3. what happens is that these two can facilitate the formation of peroxisome out of the ER (Pex 3 recruits Pex19 to initiate budding of peroxisome from ER).

Eventually ; the peroxisome that is formed (the new peroxisome) can generate by itself a new peroxisomes or it can fuse with an older one.

<u>Notice</u> that the peroxisome can be formed either by the division of a preexisting one or by budding from the ER .So if you (for example) eradicate (remove) all the peroxisomes in a cell , this cell can generate new peroxisomes with no problems(i.e without the need of a preexisting one) ☺ . It is not the case with mitochondria , as there must be a preexisting mitochondrion to make new ones ; there are some prominent and important differences between these two organelles (so pay attention to these differences in terms of structure and function as we're describing this or that ☺).

{ The complex of Pex3 and Pex19 is the main mechanism by which you have the formation of a new peroxisome } .



- so recap the story , we have a portion of the ER membrane that has Pex 3 and other membrane peroxins that were synthesized in the ER and inserted in the membrane , this part of the membrane will be eventually the peroxisomal membrane . Pex 3 has recruited pex19 from the cytosol and the peroxisome has bud off . Now ,we want to import the matrix proteins that are synthesized in the cytosol , so we need transporters and channels , the transporter are receptors found in the cytosol , and for the channels, some of those membrane peroxins are channels and are called importomers; as they import the internal proteins from the cytosol .

-The reason why a cell knows that this is a peroxisomal protein is that these proteins have a **targeting sequence** known as (**peroxisome targeting signal1** (PTS1) **or** (PTS2)) **.T**hese signals are recognized by cytosolic **transporters** (or receptors) that bind to them and carry them to the surface of the peroxisome, they make a complex with the membrane peroxins { so these membrane proteins ,that also form channels, interact with the imported peroxisomal proteins that will eventually get inside the peroxisome } , then you have the channel opened up and you have the entry of peroxisomal proteins inside .

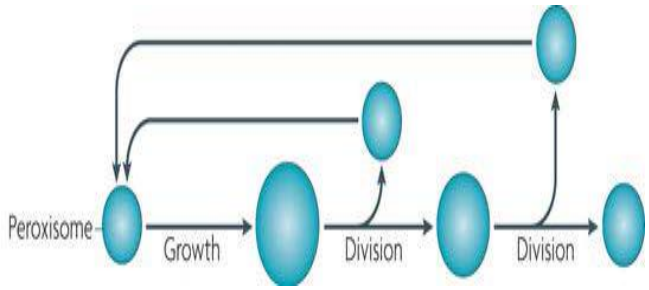
-The channel is known as (importomer) , so the importomer is a complex of peroxisomal membrane proteins that facilitate the entry of internal peroxisomal proteins from cytosol to the inside of the peroxisome .

-Again , we know that these are peroxisomal proteins because they have (PTS 1 or PTS2) ☺ .

-Peroxisomes exist everywhere in all eukaryotic cells (all cells except the red blood cells ☺), but that doesn't mean that all peroxisomes are equal like the mitochondria , they can be different (they can be heterogeneous , they can contain different enzymes , different distribution of theses enzymes and proteins in general and different functions) depending on the **function** needed for a certain type of cells .

So sometimes you can find the peroxisome in a certain cell with a lot of **catalase** because that what it needs, or you can have a lot of fatty acid metabolizing and oxidizing enzymes according to the cell function and so on**. In liver cel**l –for example- you have the enzymes necessary for the synthesis of **bile acids** (one of the functions of liver cells is to produce bile acids) , but you don't have that in other cells .

-The peroxisomes are dynamic (they undergo growth , division ,….) and again they contain different enzymes and proteins depending on what the cell needs .



Different proteins are added at different times producing different peroxisomes

Disorders related to peroxisomes :

in terms of disorders related to peroxisomes , they can be different , they can result from : a **single gene** disorder(one gene is mutated) : in this type , *the function of the peroxisome* is affected , or it can be due to a problem in the **biogenesis** of the peroxisome itself (peroxisomal biogenesis disorders

PBDs), this means that if you have a mutation in Pex genes (Pex 3 or 19 or other pexes) which are necessary for biogenesis of the peroxisome , that will affect the synthesis , generation and formation of peroxisomes as a whole .

- let's say you have a mutation in PTS , it means that impotomer or the proteins that recognize peroxisomal proteins will not be able to recognize them , so they will not be imported to peroxisomes , and multiple peroxisomal enzymes will be *defective* .

* Zellweger syndrome :

It is a PBD , it is lethal and it happens due to mutations in any one of 10 genes that affect receptors that bind the PTS1 . So , let's say if there is a mutation in receptor X ,these proteins will not be imported into the peroxisome resulting in **Zellweger syndrome** , if there is a mutation in receptor Y it will be the same condition …

-X-linked adrenoleukodystrophy (XALD):

It is also a PBD and It has defective transport of very long chain fatty acid (VLCFA) across the **peroxisomal membrane** ,so there is defective metabolism of very long chain fatty acid, and there will be accumulation of these VLCFA , and this is very bad especially for the CNS.

{You would expect that depending on the cell , not every cell will be affected by this condition (certain cells that depend on the metabolism of these fatty acids in **peroxisomes ,** since metabolism of fatty acids can also exist in the mitochondria) }.

Congrats , The first part of the sheet is done :D :D

****

The nucleus

The main function of the nucleus is being a **repository/storage** of the genetic material (DNA and pre- mRNA (**immature**)).

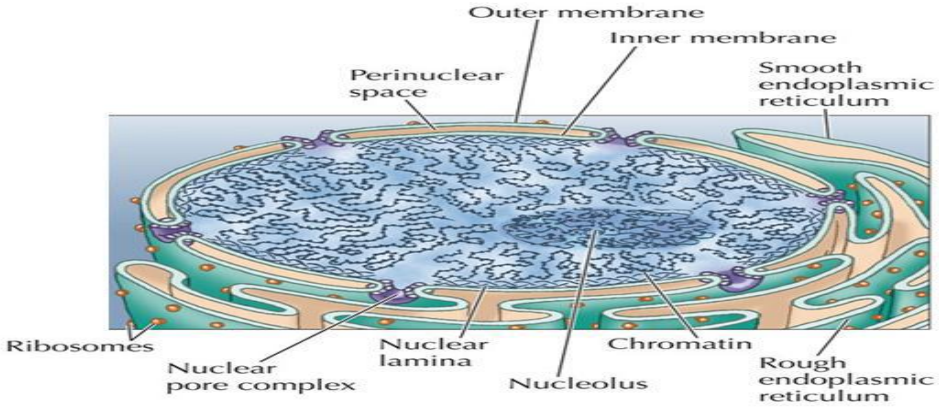
-The nucleus is surrounded by a two membrane system known as (**The nuclear envelop)** that adds an additional level of gene regulation post-transcriptionally.

-This envelop is permeable only to selective molecules , so **RNA** molecules that are made inside the nucleus can be transported outside , and you have also the entry of some molecules to the nucleus such as (transcription factors) through the envelop .

-If you look at the structure of the nucleus , inside you have the nucleus itself with the DNA , and the DNA (in eukaryotic cells) is bound to proteins , and this complex of DNA and protein is known as **Chromatin** .

So, **Chromatin** is the molecule that is composed of DNA and DNA-binding proteins , as simple as that ☺ .

These DNA-binding protein can be **general** ; like histones that wrap DNA) ,some of these proteins can be **Very** specific ;like transcription factors that bind to certain sequences in DNA .



Chromatin is **not** homogeneous (it isn't like that this part of DNA is similar to that part and so on ☺) but there are certain specialized regions inside and they have different functions .

- As we said, the nucleus is surrounded by an envelope and this envelop is a two membrane system (the inner membrane and the outer membrane) .

-The outer membrane is **a continuation of the ER membrane** , and there are some **shared** proteins between the ER membrane and the outer membrane of the nuclear envelop , but there are also some **specialized proteins** that exist on the ER membrane , and other specialized proteins on the outer membrane (so each membrane has specialized proteins as well as some general proteins) . Also , there are some ribosomes associated with the outer membrane of the nuclear envelop .

The inner membrane :

The inner membrane is very **specific** to the nucleus, it contains specialized proteins and channel proteins (that allow for the export and import of molecules in and out of the nucleus) .

☆There is a space (**perinuclear space**) between the inner membrane and the outer membrane of the nucleus , and this space is very similar to the ER lumen (the environment of ER) since it is a continuation of the ER itself ☺ .

The nucleus itself has a cytoskeleton , it has a network of proteins that support the structure of the nucleus and this is known as the **Matrix** (matrix proteins) , similar to the cytoplasmic cytoskeleton .

- The cytoskeleton of the nucleus is known as (**the nuclear lamina**) , this lamina is composed of proteins known as (**Lamins**) , and there are 3 genes that code for 7 of these proteins that can form higher order structure.

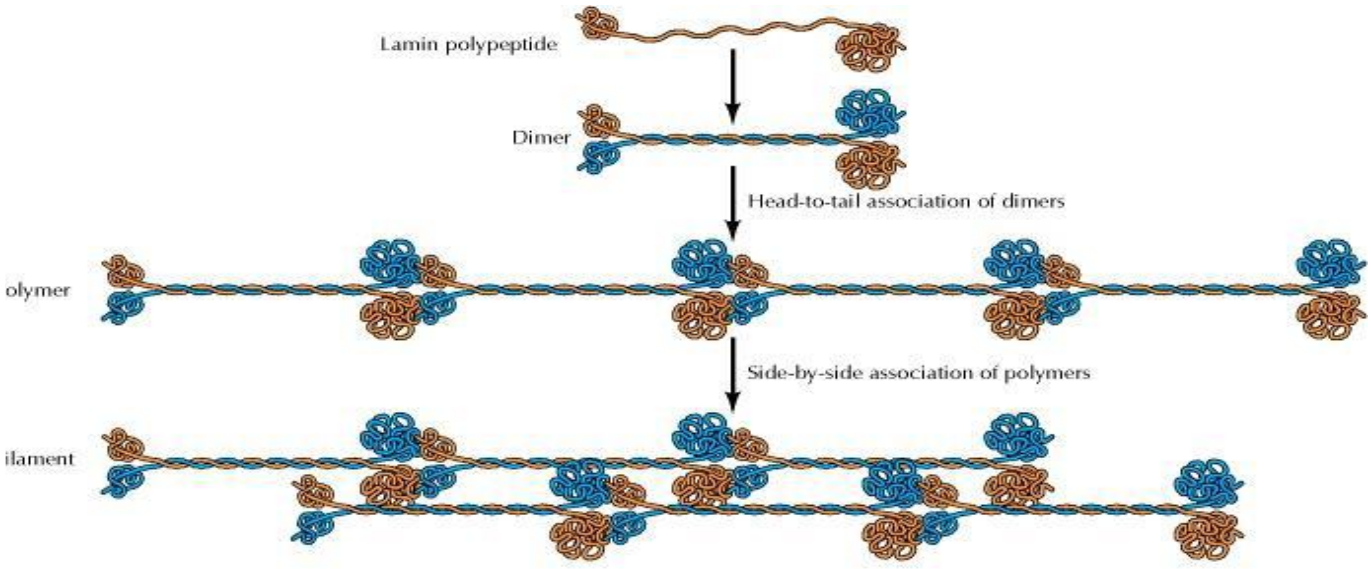
The (Lamins) are members of a family of filaments called **(intermediate filaments)** .Remember that we have three types of cytoskeletal proteins :

☝. Microtubules.

✌.Intermediate filaments.

👌.Actin microfilaments.

The Lamins are an example of intermediate filaments , the nuclear lamina is composed of this Lamin protein , two of them will form a **dimer** , then dimers will form higher structures (more complex structures) , eventually you will have a network of these proteins .



Notice that the ends of these proteins are exactly the same (the way they interact with each other is **Antiparallel** manner **,** meaning that you will have both ends of the intermediate filaments being the same) .

The importance of this nuclear lamina is that it is not really separated from the surrounding molecules , so it can interact with DNA, Chromatin and the inner membrane .

This interaction is mediated by **three proteins** :

☝. Lamin binding protein receptor (LBR) ---> a protein of the *inner* membrane .

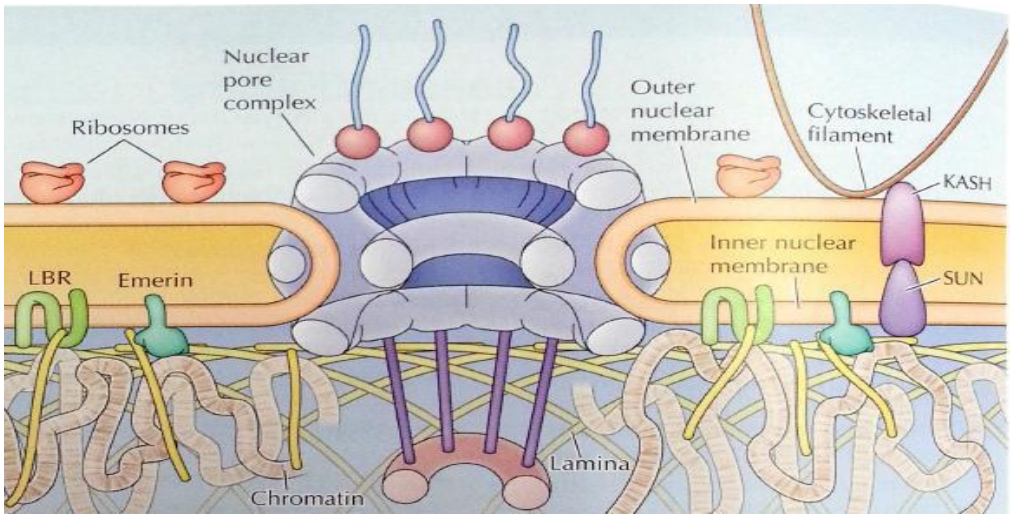
✌.Emerin --->part of the *inner* membrane .

👌.SUN--->it is an *inner* membrane protein that interacts with another protein which is part of the *outer* membrane known as $ **KASH** $:P

What is important about the interactions between SUN and KASH is that SUN interacts with Chromatin , and KASH interacts with the cytoskeletal

These cytocolic cytoskeletal filaments can interact with the cell membrane, the cell membrane will interact with proteins **outside the cell** , so any movement outside the cell will be sensed by the DNA

filaments , so the whole cell components are connected to each other (the DNA is not separated from what's happening outside), so if a cytoplasmic filament moves, the DNA will sense that there is a movement ☺ .



the interaction between the (SUN , LBR , Emerin) and the chromatin takes place on regions in the DNA known as (**heterochromatin**) .

Heterochromatin represents the areas of DNA that are not actively transcriped (no active genes in these regions) , which is different than **Euchromatin** (which is actively transcriped).

Nuclear lamina diseases :

There are certain diseases related to deficiency or mutation in **Lamin** protein , for example :

- X-linked Emery-dreifuss muscular dystrophy (what a name ! -_-) ,caused by a mutation in Emerin . Another type of this disease ,which is autosomal dominant is caused by mutations in Lamins A and C .

☆ inherited disorders caused by mutations in A-type lamin :

☝**.Marie-charcot-tooth disease type** 2B1 (MCT disease)

✌**.Hutchinson-gilford progeria** --- > people with this syndrome have (Premature aging) even though they are young , they look very old.

👌**. Dunnigan-type partial lipodystrophy.**

_____ .

✍**. Why** do these syndromes develop when you have a mutation in lamin proteins ,especially that these diseases are :

1) heterogeneous (not exactly the same) .

2) tissue specific (even though Lamin B or A ,for example, are common in all cells , only certain cells that are affected) .**?**

☆ there are two hypothesis to explain this :

First one : "gene expression " hypothesis ---> proteins like (SUN, Lamin ,LRB) are connected to the chromatin , this interaction can affect gene expression , so if there is a deficiency in this interaction ,there will be disturbance in gene expression (because there is no strong interaction) so that **heterochromatin** becomes **euchromatin (actively transcribed)** , so the cell starts to express certain genes (they become active) .

Second one : " Mechanical stress " ----- > if you have a deficiency in lamin protein , since these proteins interact indirectly with the cytoskeletal proteins , this interaction will be disturbed . Now , what cell depends more on cytoskeletal proteins (especially the microfilaments /Actin filaments) ?? it is the MUSCLE CELL ☺

So muscles are affected by these diseases , as a result of this disturbance , muscle cells can't function well in contraction and relaxation (like in **MCT** disease the patient will have muscle wasting) .

Export and import through the nucleus :

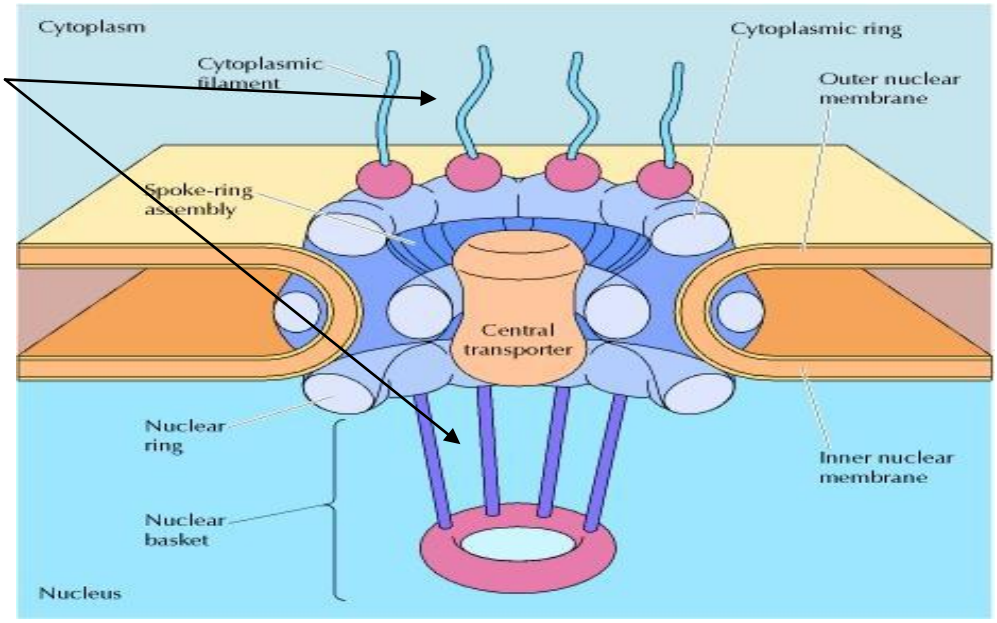
There is specific import and export in and out of the nucleus , and it is mediated by a **nuclear pore complex** on the inner membrane that extends to the outer membrane as well, so there is selective transport of molecules .

The nuclear pore complex is composed of proteins known as (**Nucleoporins**), and they allow for the export of RNA molecules (for example) outside the nucleus , and the import of specific proteins inside (like transcription factors)

The complex is composed of what is known as (**Spokes (ropes)**), there are eight of them outside and eight inside.

The nuclear (internal) spokes also form what is known as (**the nuclear basket**) .

spokes The internal and external spokes interact with ring structures that form internally the **central channel that allows for the entry and exit of molecules**



✍.How are nuclear proteins synthesized ??

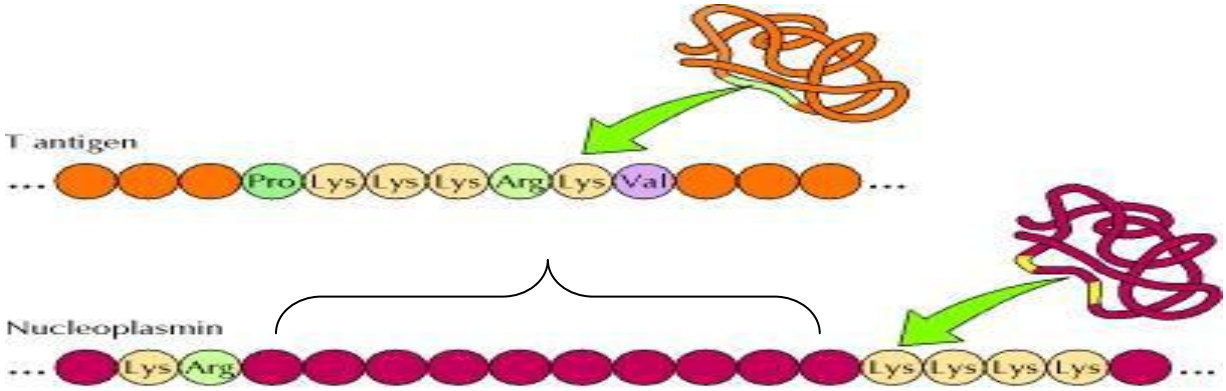
They are not synthesized in the ER , they are synthesized in the cytosol on free ribosomes , and these have (**nuclear localization sequence**) NLS .

There are *two* sequences that can determine that this protein is nuclear or not both contain basic positively charged amino acids.

One of them is a continuous sequence of Lysine and Arginine (at the N terminus) , the other sequence is known as (**Bipartite**) or **(Signal patch sequence)** where you have Lys and Arg (two of them) , and they can be separated by a number of amino acids that are not related , and then a sequence of Lysine .

since the sequences are divided by a sequence (<u>any sequence</u>) of amino acids ; they are known as a **Bipartite** ☺ .

This forms a signal patch (like the story of lysosomal enzymes) .



If you mutate Lys or Arg , then the transport of these proteins *will be affected* , but if you mutate any of the amino acids in the separating seguence (marked in the figure) nothing will happen (i.e. if you delete any of them , or change any of them into something else , still the protein will be transported into the nucleus ☺) .

-The transport of nuclear proteins depends on the function of a protein known as **(Ran) ,** which is a GTP binding protein (like : RAS , ARF, Rab) .

Ran is responsible for regulating the import and export of proteins through the nucleus , and Ran is regulated by binding to GTP or GDP ,(just like any

other GTP binding protein , the proteins that are responsible for regulating the function of Ran are known as (Ran GEFs --- > GTP Exchange Factors , and Ran GAPs --- > GTPase Activating Proteins .)

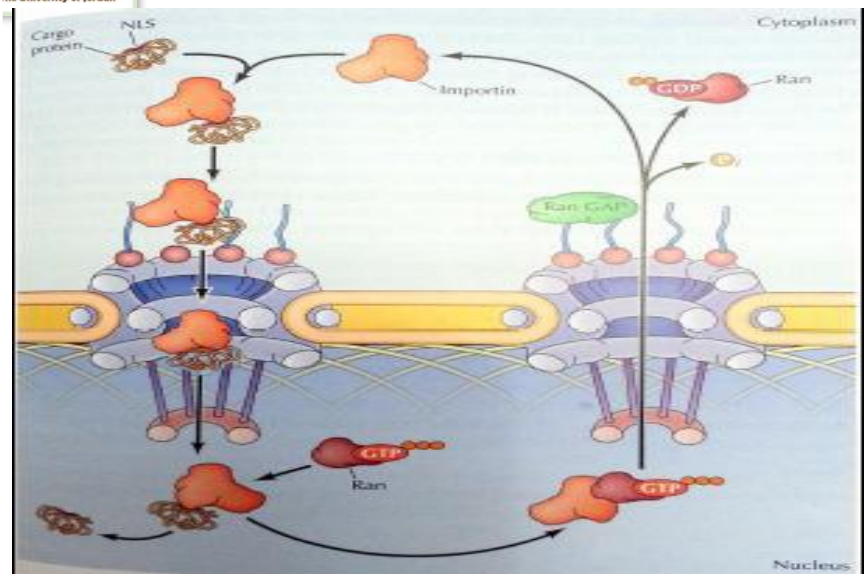
In the nucleus, there is a high proportion of (Ran –GEFs) , while in the cytosol there is (Ran-GAPs) , and that's why outside (in the cytoplasm) you can find high peroportion of Ran bound to GDP while in the nucleus it is bound to GTP ☺ .

Now , the way they function is the following :

There are certain proteins known as **Importins** , and these proteins bind to the nuclear protein in the cytosol as the nuclear protein has a *nuclear localization signal* , so you have binding of the importin to the protein and they are transported via the pore complex to the nucleus .

Once they are inside , Ran (which is now bound to GTP) recognizes the complex (recognizes that there is an importin protein that is bound to nuclear protein) ,so what it does is that it interacts with the importin , then the importin will release the nuclear protein , then the complex of importin and Ran is transported outside of the nucleus to the cytosol. Once it goes outside the nucleus ; Ran-GAP (the inactivating protein) hydrolyses GTP into GDP) then Ran-GDP dissociates from the importin , now importin is free again to bind to another protein and so on …

Ran-GDP returns back to the nucleus by a specific pore complex, and there it is activated by GEFs to work again .



.{ I know it is a long sheet >_< but please …be strong …please :D }.

Exporting proteins outside the nucleus :

These proteins contain a sequence known as (**nuclear export signal NES**) which is recognized by certain proteins known as (**Exportins**) , these Exportins bind to the protein which has NES , and Ran-GTP as well recognizes the complex , so it binds to the complex and it takes the exportin with the protein outside the nucleus , once it goes outside , Ran is inactivated , releasing the whole complex , so the protein is released outside the nucleus to the cytoplasm , and then Ran as well as the exportin can be imported again to the nucleus .

Impotins ------- > take proteins from cytoplasm to nucleus .

Exportins--------> take proteins from the nucleus to the cytoplasm (outside) .

And both of them are regulated by Ran proteins .

****

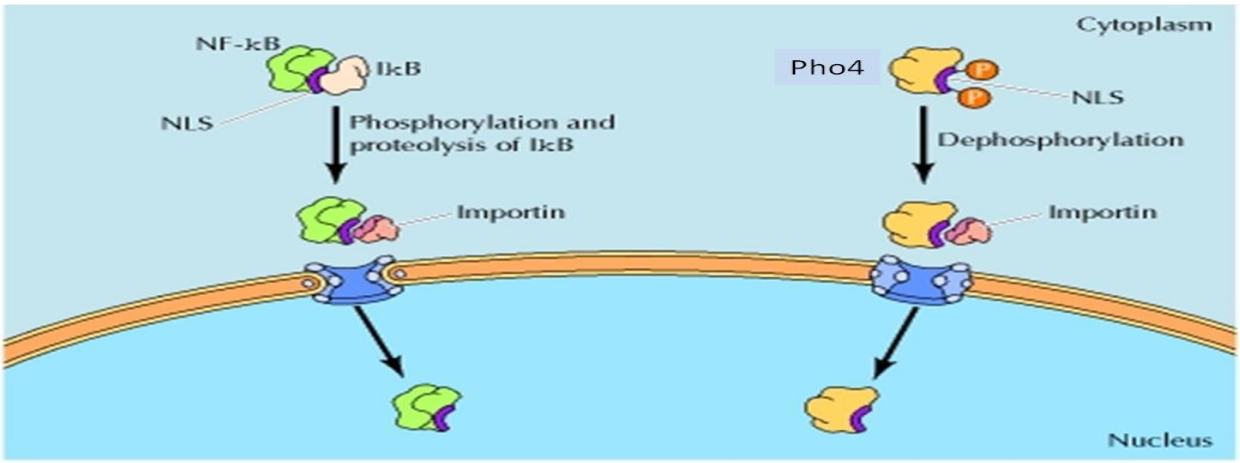
The import of proteins can be regulated ,carried and transported to the inside of the nucleus by mechanisms other than Ran and nuclear localization signal , and this regulation can take place as a result of **phosphorylation** .

There are two examples (both of them are transcription factors) and that is what we need (we want transcription factors that would stay in the cytosol until the cell has the right signal ,so that the transcription factor goes to the nucleus where it regulates gene expression) and this regulation is via **phosphorylation**.

**There are two proteins that are regulated by phosphorylation , but in different mechanisms .

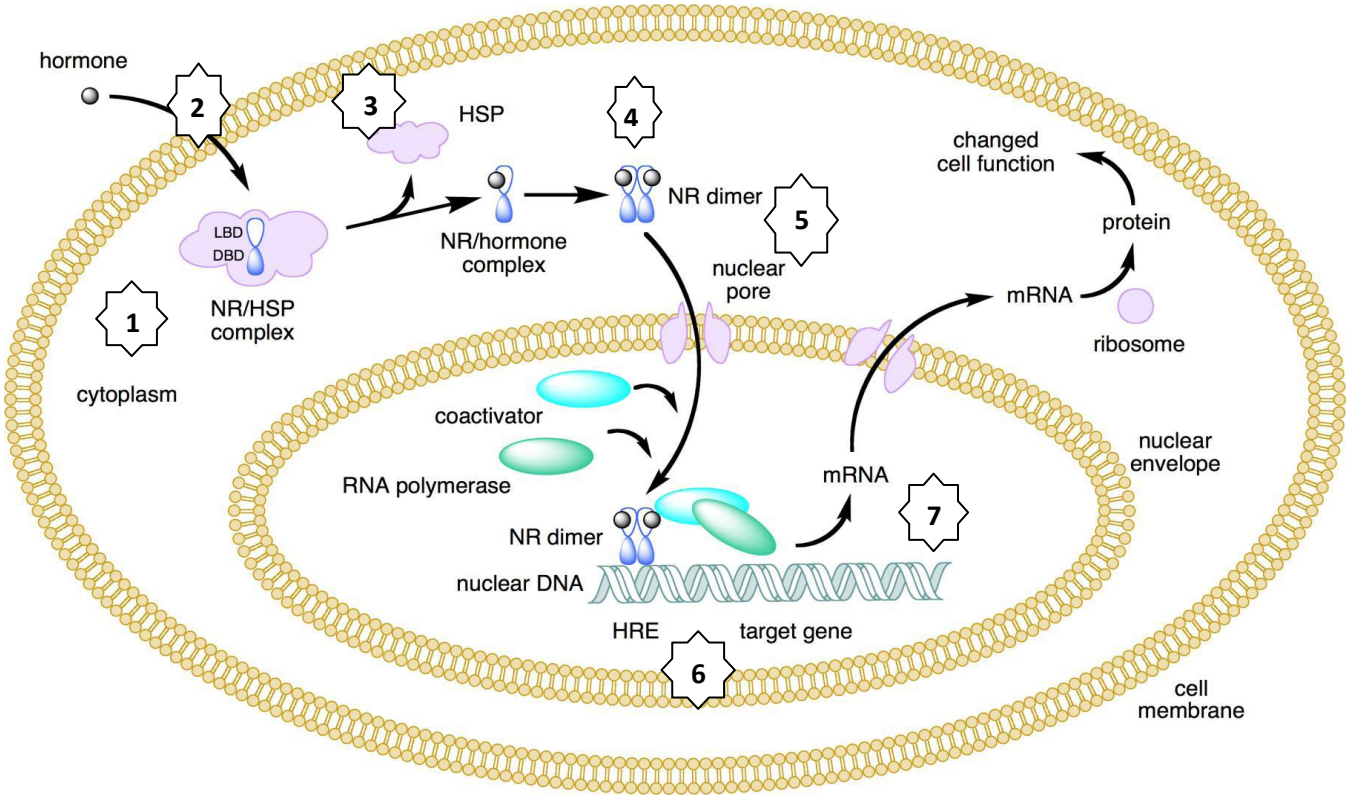
1- In one of them you have the (**NF-Kappa B**) which is a transcription factor , in the cytosol you find this protein bound to an inhibitor (inhibitor to Kappa B protein **IKB**) , this interaction is mediated by NLS, so IKB masks the NLS (nuclear localization signal) . When the right signal exists (when the cell really needs this protein(KB) to go into the nucleus) ,the inhibitor is phosphorylated , once it is phosphorylated , it will be released from KB protein , importins can then bind to NLS which is now exposed , and the protein goes in .

2- The other protein is known as (**Pho4)** which has a NLS , and the amino acids surrounding the signal are **phosphorylated** , so importins in this case can't bind to this protein , but when the right signal exist ,you have **De-phosphorylation** of the transcription factor , importins can then bind to NLS and we will have the entry (import) of **Pho4 .**



Regulation of steroid receptors : (important and not in the book)

-Steroid hormones like : Estrogens , Androgens and progesterones , these hormones are lipophilic , and they are small , so they can go into cells without any carrier or transporter (they can diffuse through the plasma membrane) .
- Once they go into the cytosol , they can bind to a receptor , so they have the **estrogen receptor , Androgen receptor and the progesterone receptor** , and they all exist in the *cytosol* , but not by themselves , they are bound to a **(Heat shock proteins)** .So these heat shock proteins surround these receptors , once you have the entry of the hormones into the cytosol , they will bind to their receptors , heat shock proteins are released , allowing for the formation of dimers , { so you have an estrogen receptor making a dimer with other estrogen receptor and each one of them is bound to a hormone } once you have this dimer formation in addition to the hormone , the whole complex goes into the nucleus to regulate gene expression*(activation or inhibition)*



From the figure above : 1) no ligand , NR (nuclear receptor) is cytosolic .

2) hormone binding .

3)dissociation of HSP .

4) dimerization .

5)Translocation .

6) specific NR-DNA binding .

7) gene expression .

If we look at the nuclear receptors , we will find that they contain certain domains , for example :

☝. Ligand binding domain (LBD) , this is where the hormone will bind .

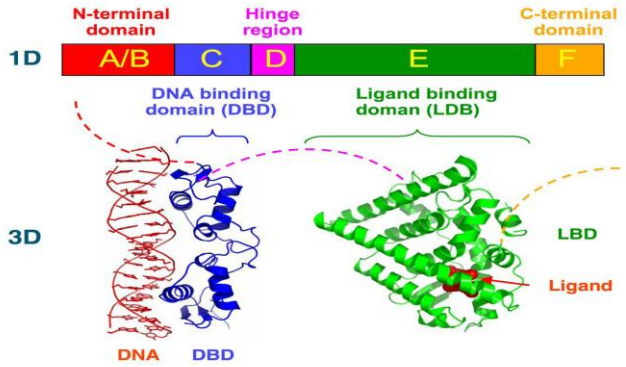
✌. DNA binding domain (DBD) , this where you have the formation of a receptor-DNA complex .

👌. Activation function domain (AF) that regulates transcription .

These domains are **independen**t on each other .

so let's say that we take the DNA binding domain of the estrogen receptor , and we put it next to the ligand binding domain of the Androgen receptor , in this case it is the Androgen that will induce binding of estrogen receptor to the DNA and not the estrogen receptor !

So these receptors are different from each other , remember when we talked about the green fluorescent protein (where we have a vesicular protein that is conjugated to green fluorescent protein) so we can follow the protein in a cell because it gives a color . Same thing here, we can manufacture a protein that is composed of different domains .



Finally , the sheet is done ☺ ☺ ☺

Your colleague : Afnan Abu Qadoum . ☺

☆special thanks to my brother Owais for his support (yalla 3eeeeesh :3).

I f you run into a wall, don't turn around and give up.

Figure out how to climb it, go through it, or work around it…