University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 4

Done By: Ahmad Fo2ad

Corrected By: Alma Jarkas

Subject: Cell Biology

Doctor: Diala Abu-Hassan

Price:

Date:

Cell biology Science:

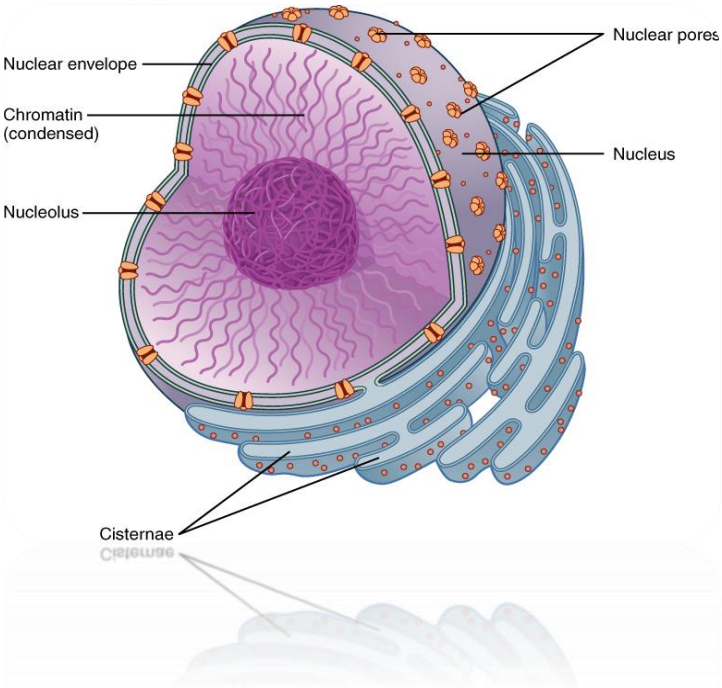
The science that concerns about the structure, function and organization of the cellular components and the cell as the basic unit of life.

It takes care of different pathways; like, signalling pathways, transport of molecules and many other topics as well.

Studying these topics can be either at **the microscopic level**, using microscopes like; the focal microscope, florescent microscope and the electron microscope, or at **the molecular level**, where we observe and study the molecules that mediate these pathways.

Nucleus is like the brain in the cell, it is **the control centre** of the cell, it control all these processes that happen inside the cell, and keeps the genetic information coded by the nucleic acids inside the nucleus, since this genetic material is very important. But still, some molecules need to move in and out, so there are some ways (nuclear pore complexes) to transport these molecules, these ways are very regulated, to keep the integrity of the cell, because if the genetic material is disrupted in some way it will be reflected on the health and well-being of the cell.

There are some proteins that regulate the expression of the genetic material such as the **Transcription factors**.



The nucleus is surrounded by an envelope that separates it from the cytoplasm**, making the nucleus a distinct biochemical compartment.** When we talked previously about the ribosomes we said that they have a membrane to separate their

environment (which is acidic) from the cytosol, and they have hydrolases, so if this membrane is disrupted these hydrolases will digest the components of the cytosol, that's why the nucleus has a membrane for a good protection and regulation of the movement of the molecules in and out.

The Structure of Nuclear Envelope

Nuclear envelope is a **bilayer membrane** consisting of two parts, an **outer membrane** and **inner membrane**. The outer membrane of the nuclear envelope is **continuous with the membrane of the endoplasmic reticulum**. {Note: the foldings of the ER are called **trabeculae**}. The inner membrane is separate from the ER.

The inner and outer membranes are kind of connected in a **turned area**. The outer membrane of the nucleus also has some ribosomes attached to it **at the cytoplasmic side**.

Beneath the inner membrane, there is a meshwork of fibrous proteins called **nuclear lamina**. It gives **support to the inner membrane** and **maintains the shape of the nucleus**.

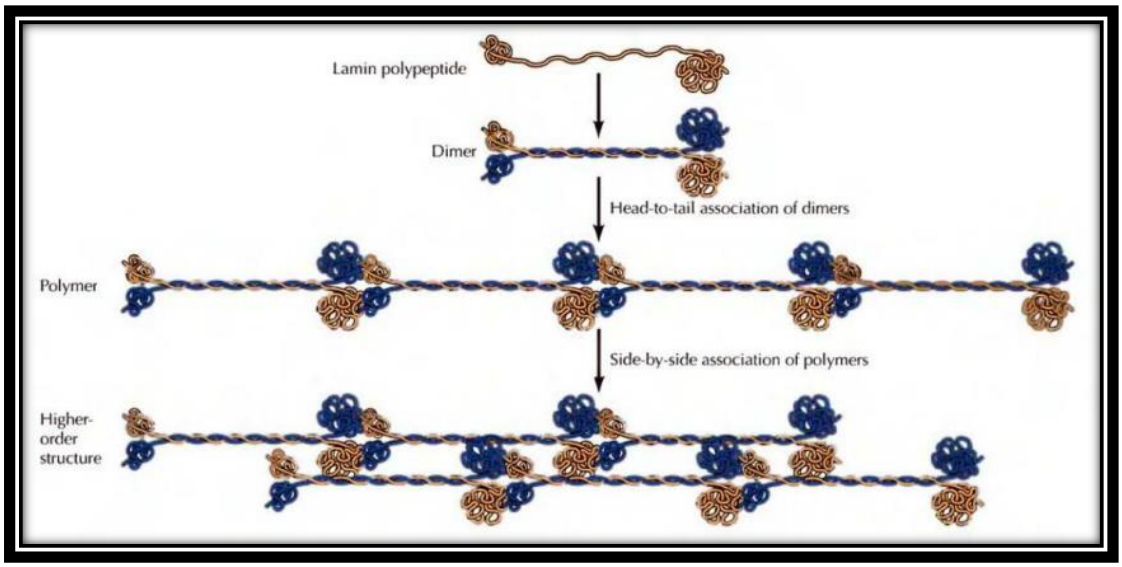
The nuclear lamina **attaches to proteins** found in the inner membrane and to proteins found inside the nucleus.

Some of the proteins that are found in the inner membrane of the nuclear envelope to which the nuclear lamina binds include:

1- **Emerin**
2- **Laminin binding protein, also called Laminin B Receptor(LBR)**
3- **LINC complexes**
4- **Other proteins**

The nuclear lamina also interact with proteins inside the nucleus like

1- **Histones**
2- **Other chromosomal proteins**



The last component of the nuclear membrane is the nuclear pore complexes, where the inner and outer membranes are connected.

Before talking about the nuclear pores, we need to talk about the fibrous proteins making up the nuclear lamina. These proteins are called lamin proteins, and there are different types of lamins→lamin A,B,C….etc.

Lamin proteins fold in a certain way, also they form **helices of two polypeptide chains that wind around each** other forming dimeric structure. Then these dimers bind to each other's **head to tail** forming -more complicated structure- polymer structure. For further mechanical strength, these polymer structures interact with each other's **in an alternating fashion** forming more complex structure of these fibrous proteins.

Refer To Pic above

Applications or examples on **diseases** that might affect the protein components of nuclear lamina and result in diseases, these diseases are called (nuclear lamina diseases) or **laminopathy**.

Example on one of these diseases, there is an X-linked mutation in one of the lamin proteins, resulting in one type of muscular dystrophy (ضمور العضلات).We talked previously about proteins in the inner membrane that interact with the nuclear lamina, one of these proteins is called emerin, a mutation in emerin specifically, is an X-linked mutation related with "**Emery-Dreifuss muscular dystrophy**"→which is weakness

in different types of muscles like the cardiac and skeletal muscles. And since it affects the cardiac muscles, there will be problems in the conduction by the pacemaker {Note: pacemaker cells are those which initiate the action potential and transfer it to other cells of the heart}. So it will lead to problems in the conduction of action potential resulting in heart failure. These patients require artificial pacemakers to generate action potential.

There are also other problems not related to sex-linked inheritance, like the mutation in nuclear lamin A and C results in symptoms similar to the symptoms of Emery-Dreifuss muscular dystrophy.

Mutations in other types of lamin results in different types of diseases, mutations in the lamins result in Dunnigan-type partial lipodystrophy, Charcot-Marie-Tooth disorder type 2B1, Hutchinson-Gliford progeria.

The person to the right suffers Hutchinson-Gliford progeria characterized by small face. This is due to mutation in lamins, specially lamin A.

Nuclear pore complex

Is the only way the molecules can move into and out of the nucleus.

- Diameter ~120 nm.
- Size ~125 million Dalton size.
- 30 different pore proteins called **nucleoporin.**

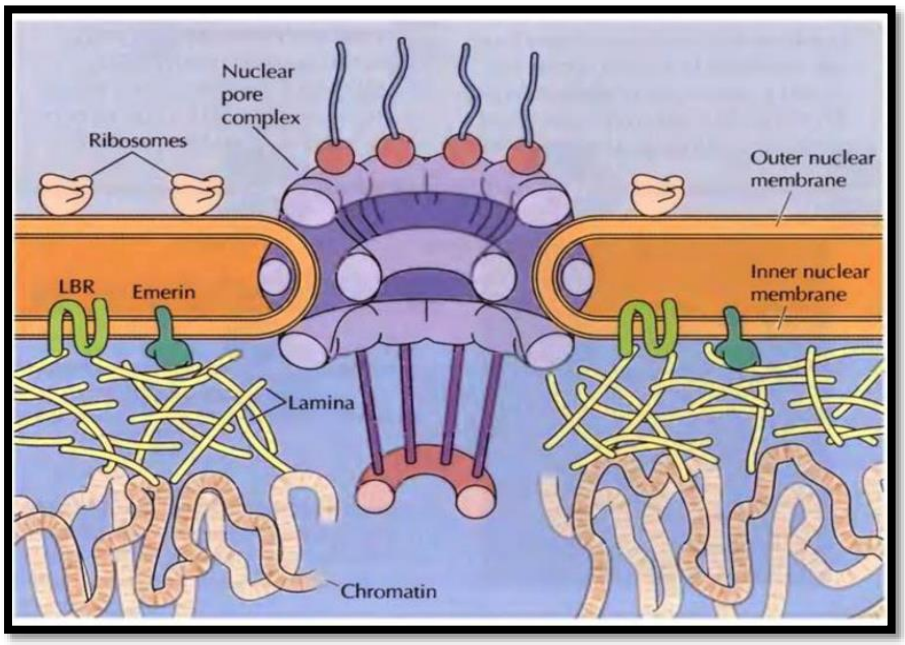
There are few molecules that can pass through the nuclear envelope such as the gases and few small molecules. But the small polar molecules need to pass through a channel, since they can't pass through the envelope. The small and polar molecules like ions, polypeptides and proteins move through these pores. The ions can move by facilitated diffusion, according to concentration gradient, proteins as well can move by facilitated diffusion through these pores. Simple diffusion happens only with small non-polar molecules like gases.

Each nuclear pore complex looks like a flower made up of 8 cylinders, that's why it's called eightfold symmetry spokes. If we take a

longitudinal section, these eight spokes will appear at the middle of two rings. Meaning that the pore is made of **nuclear ring** made of proteins towards the nucleus, and a **cytoplasmic ring** made of another set of proteins towards the cytoplasm, and in between are the eight spokes. These eight spokes form a circle between the two rings. There are filaments →**cytoplasmic filaments** projecting outside.
. →**Nuclear filaments** projecting inside.

Nuclear filaments projecting inside form structure called the **nuclear basket**.

What is the function of cytoplasmic filaments and the nuclear filaments? They aid in the regulation of the movement of molecules, they open and close allowing molecules to pass or not.



How to move a molecule to the nucleus?

How do the **carrier molecules** know that a certain molecule has to be transported to the nucleus, not to any other compartment?

There is a signal, **this signal is built in the primary sequence of the polypeptide**, not added to the molecule like ubiquitin, but it is part of the original structure of the protein. This signal is called **nuclear localization signals (NLS)**. There are different types of nuclear

localization signals, one of them is found in the T antigen and it is composed of 7 amino acids Pro-Lys-Lys-Lys-Arg-Lys-Val. So **if this signal is found in the primary sequence of a protein, it will be identified and transported to the nucleus.** This sequence **doesn't have to be continuous sequence**, the 7 amino acids can be separated as illustrated in the 2nd example"nucleoplasmin" that is going to be moved into the nucleus, since it has NLS, this NLS is made of 6 amino acids Lys-Arg separated by 10 amino acids then we have 4 other Lys residues.

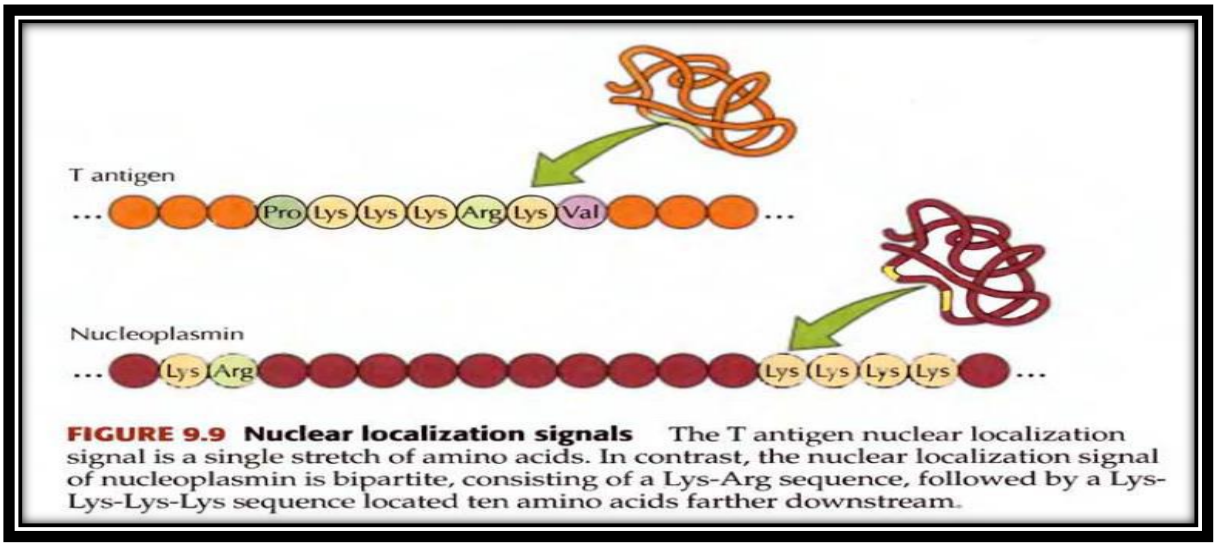


FIGURE 9.9 Nuclear localization signals The T antigen nuclear localization signal is a single stretch of amino acids. In contrast, the nuclear localization signal of nucleoplasmin is bipartite, consisting of a Lys-Arg sequence, followed by a Lys-Lys-Lys-Lys sequence located ten amino acids farther downstream.

Although they are far apart, they can be recognized as NLS.{Remember: these residues are in the primary sequence, this polypeptide will be folded, so these amino acids may be far apart in the primary structure, **but in the tertiary structure they might be close**, so they can be identified}.

- **NLS contains a lot of Lysine.**

Carrier proteins:

Their function is to **transport the molecules in and out of the nucleus**. If the molecule contains NLS, it can't be transported across the nucleus unless the carrier proteins are present. Example; if I have a transcription factor in the cytoplasm that is activated by a certain signaling pathway that lead to the phosphorylation of this transcription factor and the phosphorylation leads to its activation. The active transcription factor needs to be transported into the nucleus through the pores in order to do its function, then in the nucleus it binds to a specific region on the DNA called **regulatory region**, then **affecting gene expression** by either

activation or inhibition of gene expression. Whenever the transcription factor finishes its job it leaves the nucleus, and it is ready to do its function again.

What determines to bring the transcription factor in or out of the nucleus?

Phosphorylation induces conformational changes that open the structure of the transcription factor **showing the NLS signal**; therefore it will be **identified by the carrier molecule,** then transfer the transcription factor inside the nucleus.

There is another signal called **nuclear export signal (NES)** that is recognized by another carrier protein, then **transport the transcription factor outside**.

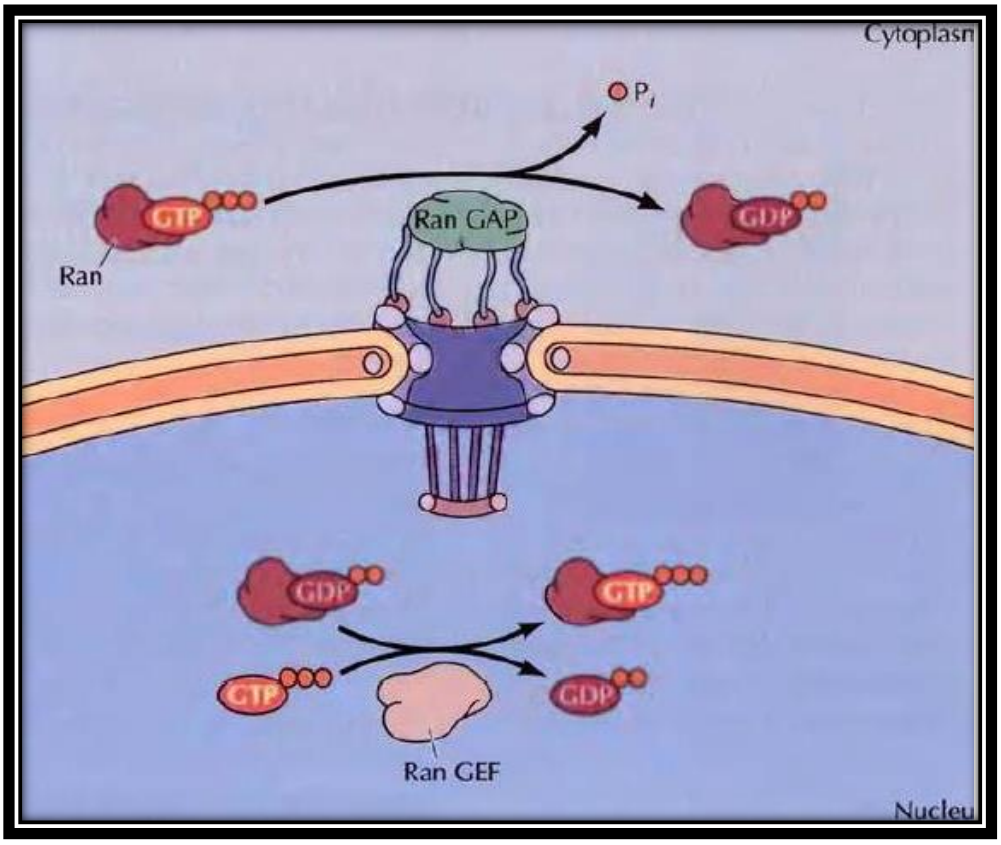
- Carrier proteins are known as **importins** and **exportins.**

Directional movement through the nuclear pore complex (Ran molecules)

There are also other molecules that r**egulate and affect the directionality of movement**, an example on these molecules is called **Ran molecule.** Ran molecule **is a GTP/GDP binding protein**, meaning that it can bind to GTP or GDP.

Ran with GTP can hydrolyze one phosphate becoming Ran-GDP, **this happens only on the outer surface of the envelope** (inside the cytosol). Once Ran-GDP is formed it can be transported into the nucleus through the pore. Once I have it **inside**, there is another molecule called **GEF** (GTP Exchange factor), this protein is different from the molecules that hydrolyze GTP to GDP, this **GEF removes the GDP and exchange it with GTP reforming Ran-GTP**, causing **conformational changes** in the Ran molecule so it can interact with the proteins in different way (its function was mentioned and will be discussed later in this lecture). GEF is **only found in the nucleus**; thereby the activation of Ran to interact

with other proteins only happens in the nucleus.



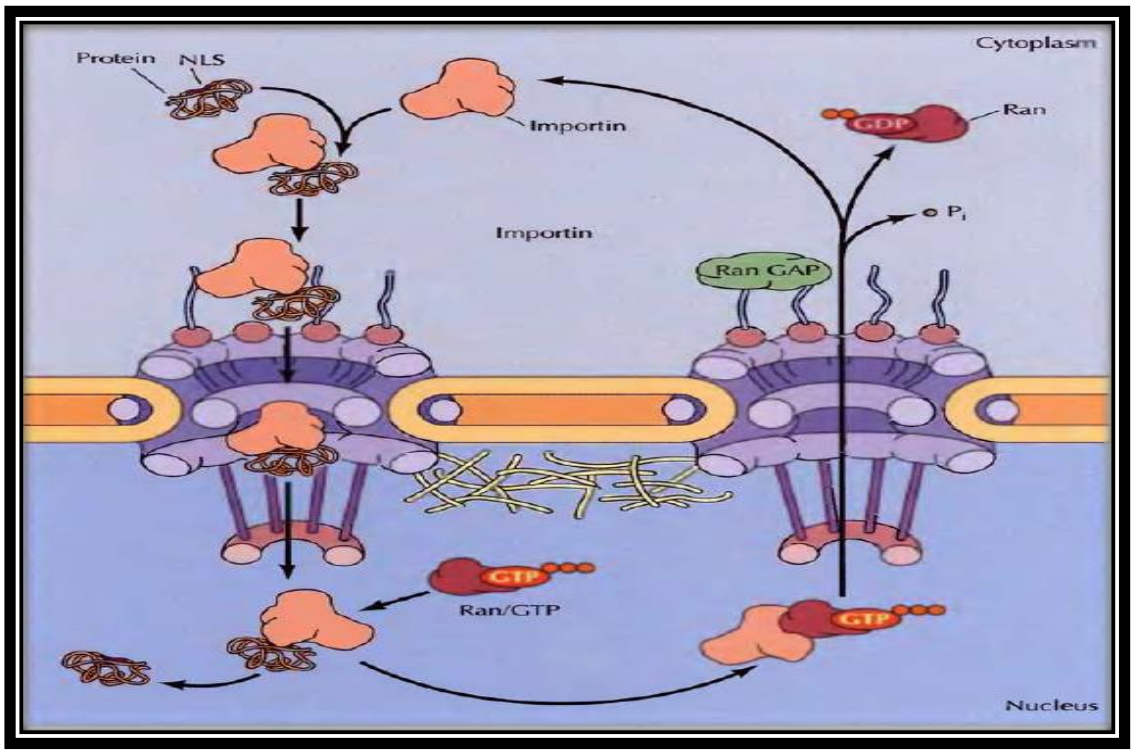
The following subjects will be discussed now

Transport of proteins
- **Protein import through the nuclear pores**
- **Protein export through the nuclear pores**

Protein import through the nuclear pore complex

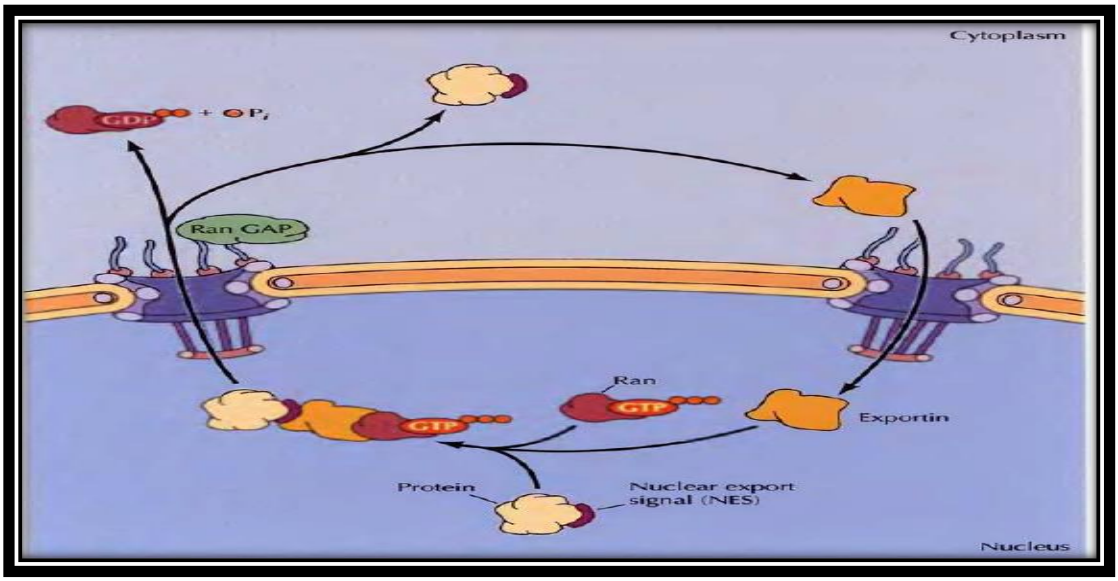
Suppose that we have a protein that needs to be transported into the nucleus, **it has to have NLS**, this NLS is going to be **recognized by carrier** proteins, in this case the carrier proteins are called **importins**. Once the importin binds to NLS of the protein to be transported, **they become a single unit** that travels through the pore. **Once it is inside they need to be separated from each other** in order for the **protein to perform its function**, and in order for the **carrier to be recycled**. Remember that Ran-GTP is found inside the nucleus, and it can bind to the carrier protein "the importin". {Note: Ran-GDP can't bind to the importin}. Once Ran-GTP binds, **it induces conformational changes in the importin**, so **the importin releases the transported protein.**

Now we have the Ran-GTP bound to the free carrier that can leave the nucleus through the pores to the cytoplasm. **Outside Ran-GTP is hydrolyzed to Ran-GDP and P**, this induces **conformational changes in Ran molecule** leading to **release the importin** in the cytoplasm. Now the importin is recycled and it can bind another protein with NLS.



Protein export through the nuclear pore complex

Suppose a protein that needs to be transported from the nucleus to the cytoplasm. **It has to have a signal**, this signal is different signal than that recognized by the importins, this signal is called **nuclear export signal (NES),** this NES is going to be **recognized by exportins**, but it can't be recognized by exportins until the exportins are bounded to Ran-GTP, once exportins are bound to Ran-GTP, this induces **conformational changes** in the exportins, so the exportins can bind to the NES of the protein to be transported. The formed complex crosses the pore to the cytoplasm. In the cytoplasm, the phosphate of GTP is hydrolysed forming Ran-GDP, **Ran-GDP induces conformational changes, releasing the components of this complex** * Ran-GDP * exportin *the transported protein.



- *Conclusion*:
- **the function of Ran→Regulation the directionality of movement**
- **Ran-GTP: binds the importin to release the transported protein inside**
- **Ran-GTP: binds the exportin to activate the binding of the**

Mechanisms of nuclear protein import regulation:

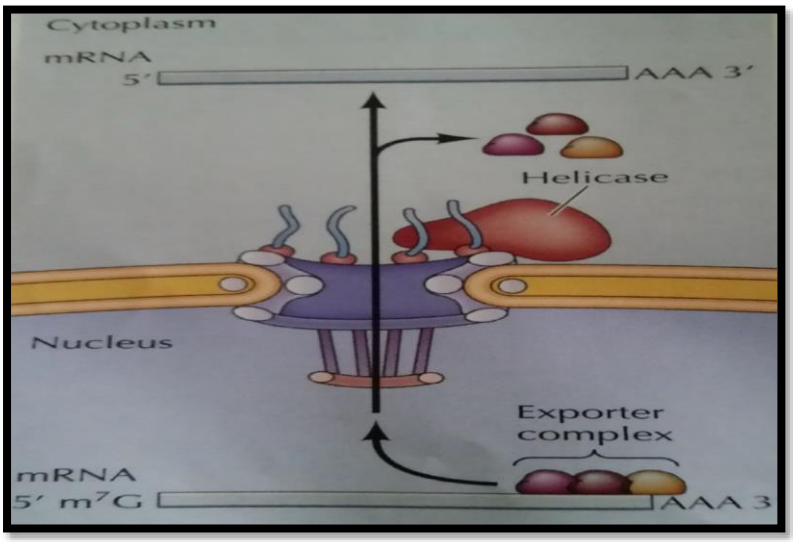
Nuclear localization signal (NLS) and nuclear export signal(NES) → these signals are part of the primary sequence of the protein, but they are **hidden as a result of the folding of the protein.** The protein that needs to be transported can be **activated by** either **phosphorylation** or **release of certain proteins that are bound to the original protein**, resulting in conformational changes in the transported protein that finally **will expose these signals** to either the importins or the exportins.

Conclusion: activation of a certain protein to be transported is achieved by showing NLS or NES that can be done by one of two ways:

1- **Phosphorylation→ conformational changes→ exposure of NLS or NES.**
2- **Release of certain proteins that are bound to the protein that needs to be transported→conformational changes→ exposure of NLS or NES**

RNA transport across the nucleus

Proteins are only one type of molecules that can pass through the nuclear pores, there are also many other molecules like RNA molecules can be transported through the pores.



1-mRNA transport

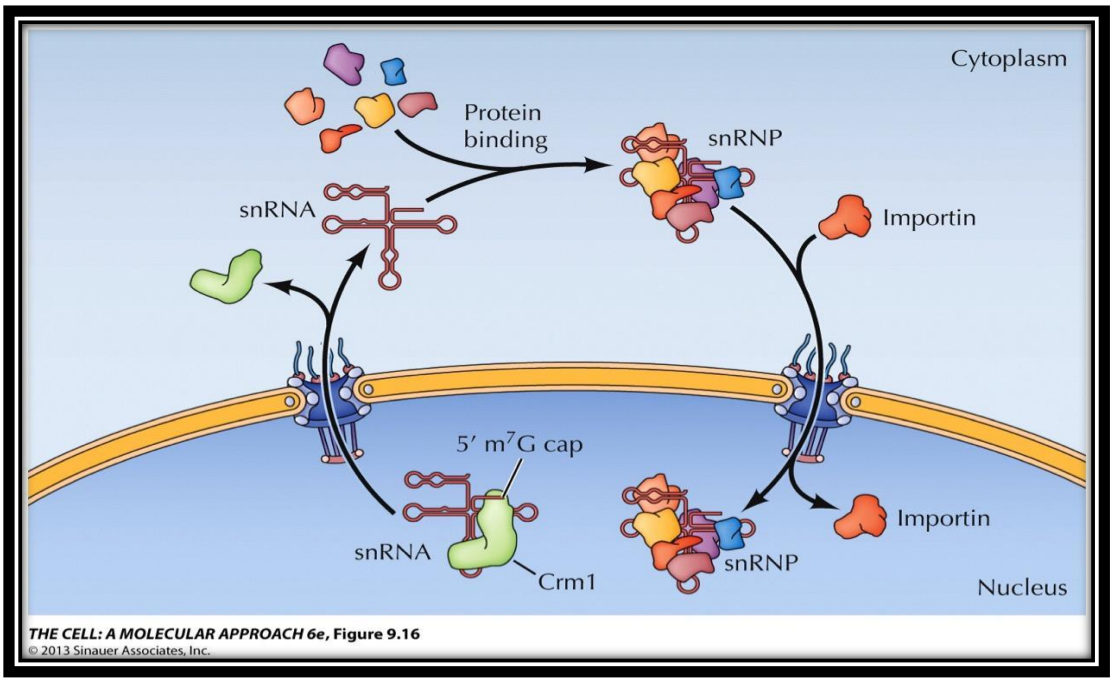
mRNA needs to be transferred from the nucleus to the cytoplasm. What happens is that **some modifications**

are added to the mRNA molecule **to make it recognizable by the exportin**.

These modifications are specifically **mythelation** on the guanine **(5'm7G) cap** that is going to be recognized by the exportin, the exportin then carries mRNA to the cytoplasm. Once mRNA is outside, it can't directly undergo translation because of the presence of the exportin connected to it.

There is an enzyme right after the pore called **helicase**, this enzyme removes the exportin that is connected to mRNA molecule, **making the mRNA free and ready for translation**.

2-snRNA "small nuclear RNA"



snRNA molecules are part of the RNA processing machinery, which means that **they make some modifications on the RNA molecules**. snRNA is going to be moved outside the nucleus, **methylation happens here again (5'm 7G) cap**, this 5'm 7G cap that is found on snRNA is going to be **recognized by a carrier called Crm1** resulting in binding of Crm1 to snRNA, only then it can be transferred through the pore. Once it is outside, snRNA is still bound to Crm1, so they need to be separated. When snRNA is liberated, **it binds with some proteins** forming **snRNP** "small nuclear RNA binding protein". snRNP can be transported to the

nucleus through the pores by binding to importin. **In the nucleus snRNP separates from the importin**, and snRNP is going to regulate the processes of RNA molecules.

The question is why did the snRNA was transported to the cytoplasm then brought again to the nucleus?

- Because the main function of snRNA is **to bind to protein components** that are basically synthesized in the cytoplasm, **then transport these proteins to the nucleus** in order **to modify the RNA molecules** that are synthesized inside the nucleus with these proteins.

I apologize for any mistake