Genetics & molecular biology

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Lecture 28/6

- Peroxisomes
  - Membrane enclosed the spherical organelle
  - Variable in size

They have collection of proteins called peroxins that can be abbreviated ⟶ pex 1, 2, …

These proteins are responsible for the peroxisomes function

Function of peroxisomes:-

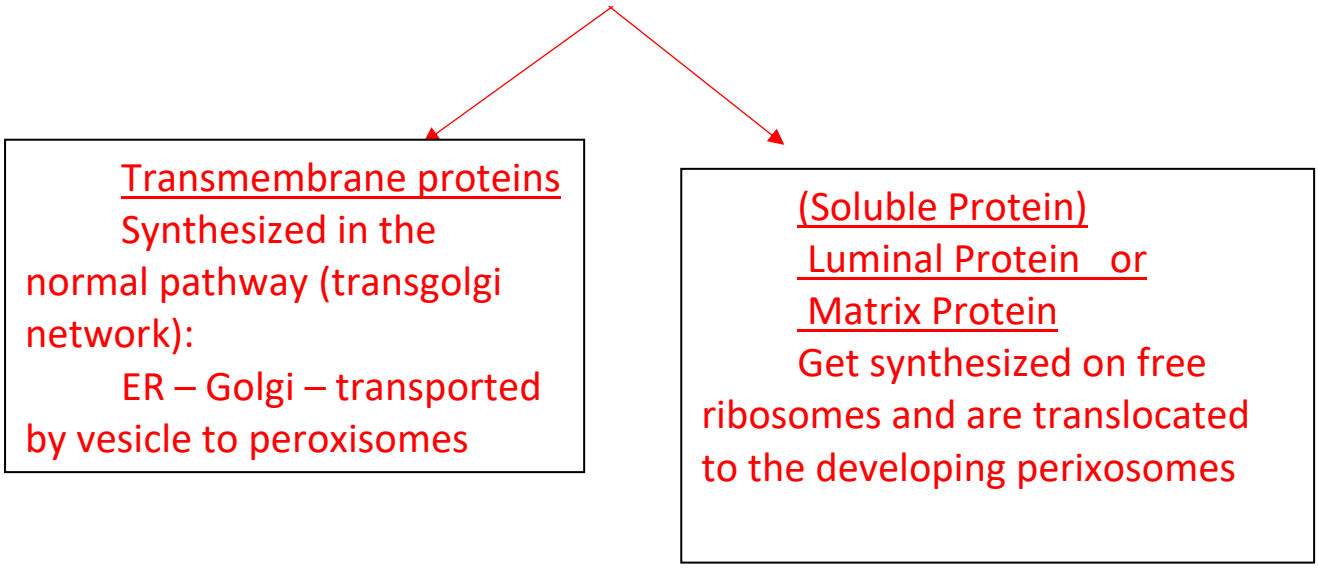
1. Get rid from oxidative stress. (that happen due to metabolic reactions or the condition surround the cell such as hypoxia)
2. Degradation from some molecules not for the purpose of getting rid of them but for the purpose of harnessing the energy of the molecules (by oxidative phosphorylation) .
3. Synthesis of some molecules.

- On average human cells contain 500 peroxisomes.
- This number is variable because Peroxisomes (just like mitochondria) can divide by fission and also grow by fusion (they are dynamic structures)

For example: if we have high oxidative stress, I will expect to have high no. of peroxisomes to get rid from the Ros.

Peroxins

- They are encoded by 85 genes, so around 85 or more pex's are available
- most peroxins are metabolic enzymes (reduction ,oxidation)



- The most important function for the peroxisomes:

Get rid of the oxidative stress that is catalysed by special enzyme called **catalase**.

$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$ remove O_2

$H_2O_2 + AH_2 \xrightarrow{\text{Catalase}} A + 2\ H_2O$

Adding O_2

Next, synthesis of the molecule inside the peroxisomes are various ,but mostly lipid s

1. Lysine amino acid (non-essential Amino. Acid)
2. Lipids:

Cholesterol, Dolichol (synthesized from farnesyl), Bile Acids (product of cholesterol), Plasmalogen (phospholipids or Glycerophospholipid with one ether bond, ROR bond ,important in membranes of the brain and heart

How does a peroxisome form?

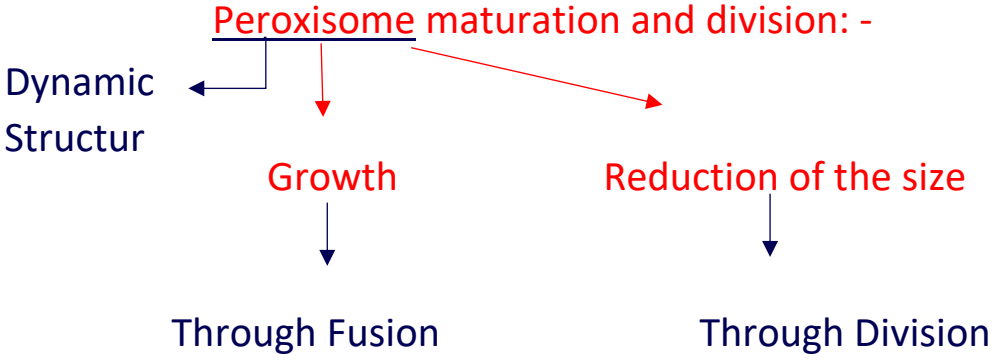
Synthesis of their transmembrane proteins as we have mentioned earlier happens in the trans golgi network, from where they bud just like in lysosome formation.

There are many Peroxisomal transmembrane proteins that help form the bud , Pex3, Pex17…….
But the most important one is Pex3

Because it acts as site of interaction for some peroxisome proteins that come from the free ribosomes, such as Pex19 that will bind the cytosolic side of Pex3.
Vesicles will bud , having transmembrane pex 3 and pex 19 bound to each ,more than one of these vesicles will fuse to form a developing peroxisome,
to finally become a mature peroxisome the pex from the free ribosomes have to be translocated into the developing peroxisomes by a channel protein called (importomer) into the lumen of peroxisomes.

As a mature organelle, it can now divide and undergo fusion as it is variable in size.

What signals the luminal pex's synthesized on free ribosomes to be translocated into developing peroxisomes ?
they have sequence called peroxisome targeting signal 1 (PTS1) or (PTS2)-peroxisomal targeting signals



Ex. Studying the histology of brain tissue after a Stroke

- The tissue in which infarction has happened appears white in colour and normal tissue appears grey in colour.
- Tissue that has undergone infarction is dead tissue
- Tissue around the area of infarct was viewed and was found to have high number of peroxisomes
- Why? a rescue reaction to reduce oxidative stress

This experiment was be done on the mice by inducing thrombosis and viewing the white area this rescue reaction May aid in brain cell regeneration in small amount and that's why the manifestations of the stroke do slowly subside with time(like paralysis) .

Peroxisomal disease

Losing of the enzymes that are normally found in the peroxisomes and participate in their function means losing the function of peroxisomes.

1. **Loss of one enzyme and its function is less severe than losing much more than one enzyme.**

2. **Peroxisomal biogenesis disorder that involve multiple peroxisomal enzyme deficiencies due to failure of import, e.g. Zellwegar syndrome:**

Due to mutation in at least 10 genes ,ex.mutation in Pex's that have roles in the formation(biogenesis)of Pex such as Pex3, Pex19,PTS1

Very high oxidative stress that is lethal either during fetal development or just after birth .

3. **X-Linked adrenoleukodystrophy (XALD**)

Defect in transport of very long chain fatty acid (VL CFA) across the peroxisomal membrane for the oxidation process.

More than one tissue is affected.

Nucleus

× The nucleus distinguishes eukaryotic from prokaryotic cells
× Houses the cell's genome and reserves the genetic information
× Acts as a cell's control centre
× Separation of the genome from the cytoplasm to allow gene expression regulation
× Limits the access of selected proteins to the genetic material
× Separates genome from the site of mRNA translation
× A double membrane envelope
× Prevents the free passage of molecules between the nucleus and the cytoplasm
× Makes the nucleus a distinct biochemical compartment × Contains nuclear pore complexes that allow regulated exchange of molecules (selective proteins and RNAs) between the nucleus and the cytoplasm

The Nuclear Envelope Structure

Components:

1. Two membranes(are continuous with each other)

A. **Outer**: continuous with the ER and ribosomes are bound to Its cytoplasmic surface. Has proteins that bind the cytoskeleton but not those that give the tubular ER structure

B. **Inner**

2. An underlying nuclear lamina underneath nuclear membrane that provide support, a type of intermediate filament a fibrous structural meshwork composed of lamins, nuclear lamina is attached to the inner surface of nuclear envelope by interaction with protein's on it .
   Example of such proteins are emerin LBR, LINC complexes, histones and other chromosomal proteins)

3. Nuclear pore complexes where the inner and outer nuclear membranes join together they are Interruptions(openings) in

the nuclear membrane, they are very complicated large proteins that allow molecules to move in and out of the nucleus, and are under very high regulation to maintain integrity of DNA inside .

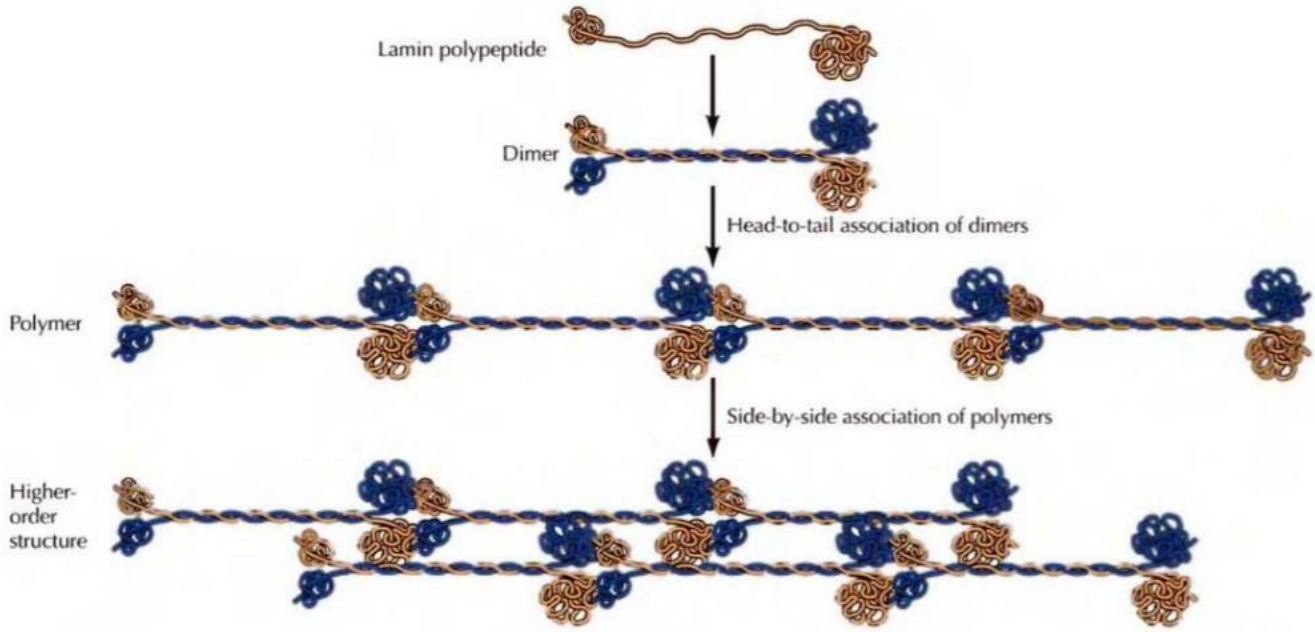


FIGURE 9.4 Model of lamin assembly The lamin polypeptides form dimers in which the central α-helical regions of two polypeptide chains are wound around each other. Further assembly may involve the head-to-tail association of dimers to form linear polymers and the side-by-side association of polymers to form higher order structures.

Description of structure of nuclear lamina (diagram above):

1. Made from a set of lamins (Lamins have a head and tail region)
2. The lamin polypeptides dimerise (head to head and tail to tail orientation)
3. Dimers Aligned side by side, head to tail association of dimers (head of one dimer next to tail of the other) to form linear polymers
4. Side by side association of polymers to form higher order structure ,with areas of over lap and areas of no overlap .

Molecular medicine application: Nuclear lamina diseases or laminopathies

× X-linked Emery-Dreifuss muscular dystrophy

× Stiff elbows, neck and heels

× Conduction block in the heart thus, they may need a pacemaker

× Wasting and weakening of the muscles

× Emerin is mutated

Emerin is absent , nuclear lamina is present ,so lamin is present but no emerin to bind lamnin to inner nuvlear membrane .

× Can also be inherited in non-sex-linked manner if nuclear lamins A and C (LMNA) are mutated

× LMN mutations cal also cause Dunnigan-type partial lipodystrophy, Charcot-MarieTooth disorder type 2B1, Hutchinson-Gliford progeria

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The nuclear pore complex

- Diameter ~120 nm
- 125 million Dalton size
- Contain 30 different pore proteins called nucleoporins
- Function: transport of small polar molecules, ions and macromolecules (proteins such as, transcription factors, and RNAs)
- Structure:

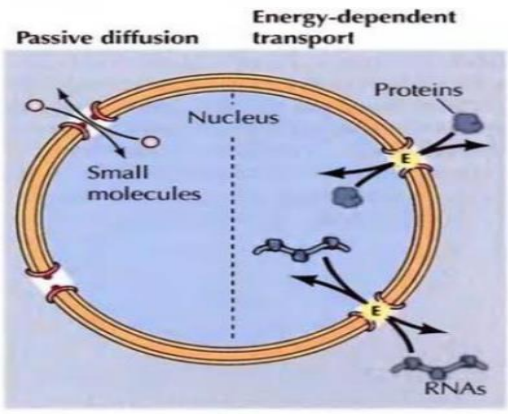


FIGURE 9.6 Molecular traffic through nuclear pore complexes Small molecules are able to pass rapidly through open channels in the nuclear pore complex by passive diffusion. In contrast, macromolecules (proteins and RNAs) are transported by a selective, energy-dependent mechanism.

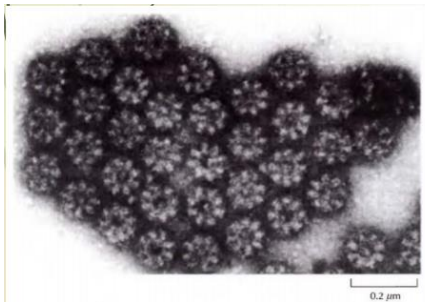
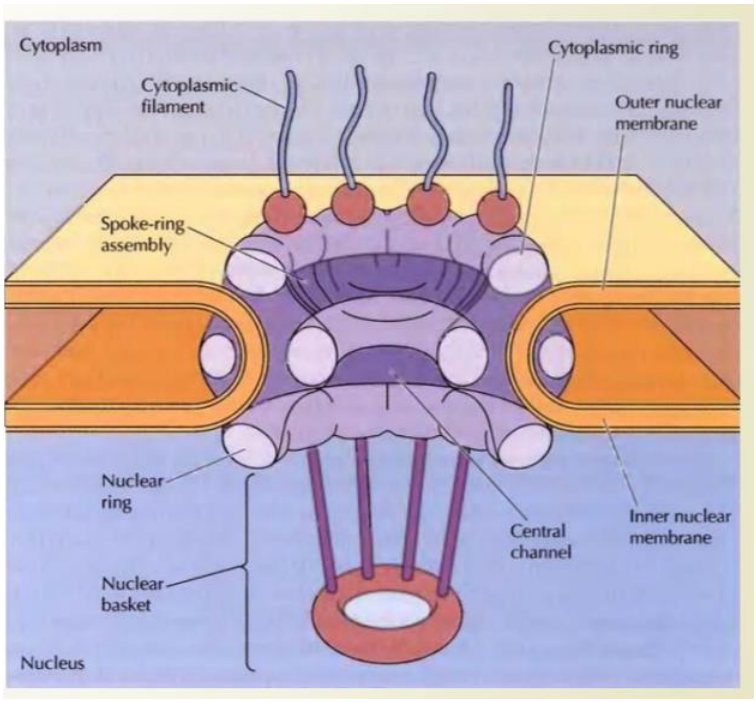


FIGURE 9.8 Model of the nuclear pore complex The complex consists of an assembly of eight spokes attached to rings on the cytoplasmic and nuclear sides of the nuclear envelope. The spoke-ring assembly surrounds a central channel. Cytoplasmic filaments extend from the cytoplasmic ring, and filaments forming the nuclear basket extend from the nuclear ring.

If we take cross section of the nuclear membrane and put it under the microscope, we're going to see flower shapes, each flower has 8 petals

- It is an eightfold symmetry , formed by spokes with a large central channel (pore)
- It has three rings,(nuclear cytosolic and a ring with the membrane)
- The eightfold spokes are anchored within the nuclear envelope at the sites of fusion between the inner and outer nuclear membranes are attached to the nuclear and cytosolic rings
- The spokes are made of Cytoplasmic and nuclear filaments
- On the cytosolic part of nuclear envelop the spokes are cytoplasmic filaments (spikes)
- In the nuclear side spokes form the nuclear basket.

4 spokes shown in a longitudinal section



* What directs a protein to be inserted into the nucleus ?
  By a signal sequence called Nuclear Localization Signal (NLS) ⟶ can be of different types like

1. Stretch of AAs
2. Bipartite NLS are more common:
   Two groups of AAs are separated into 2 groups on 2 different regions , the two amino acid groups are relatively close to each other.
3. Basic or classical NLS
4. Some NLS are far apart and depend on protein folding

* how can molecule be directed out of the nucleus?
  By another signal sequence called nuclear exit signals (NES)

*Directionality

The regulation of entering and exiting through the pore complexes
Example: mRNA (is need it to be outside the nucleus to bind to the ribosome and there is no purpose for it inside the nucleus.
This regulation will be achieved by protein called Ran (gtp binding protein, binding both GTP and GDP each time with a different conformation)
Inside the nucleus RAN changes conformation by exchange from GDP to GTP catalysed Ran GEF, so RAN inside the nucleus is mostly RAN-GTP

Outside the nucleus RAN mostly bound to the GDP,

so there will be hydrolysis of phosphate bond so that GTP becomes GDP. (which releases energy ,favourable process)

The 2 different ways that facilitate the exchange between the two conformations of RAN controls the directionality of movement from inside to outside of the nucleus and vice versa.(if you don't understand this part it will be made clear by reading the examples bellow)

Protein import through the nuclear pore complex

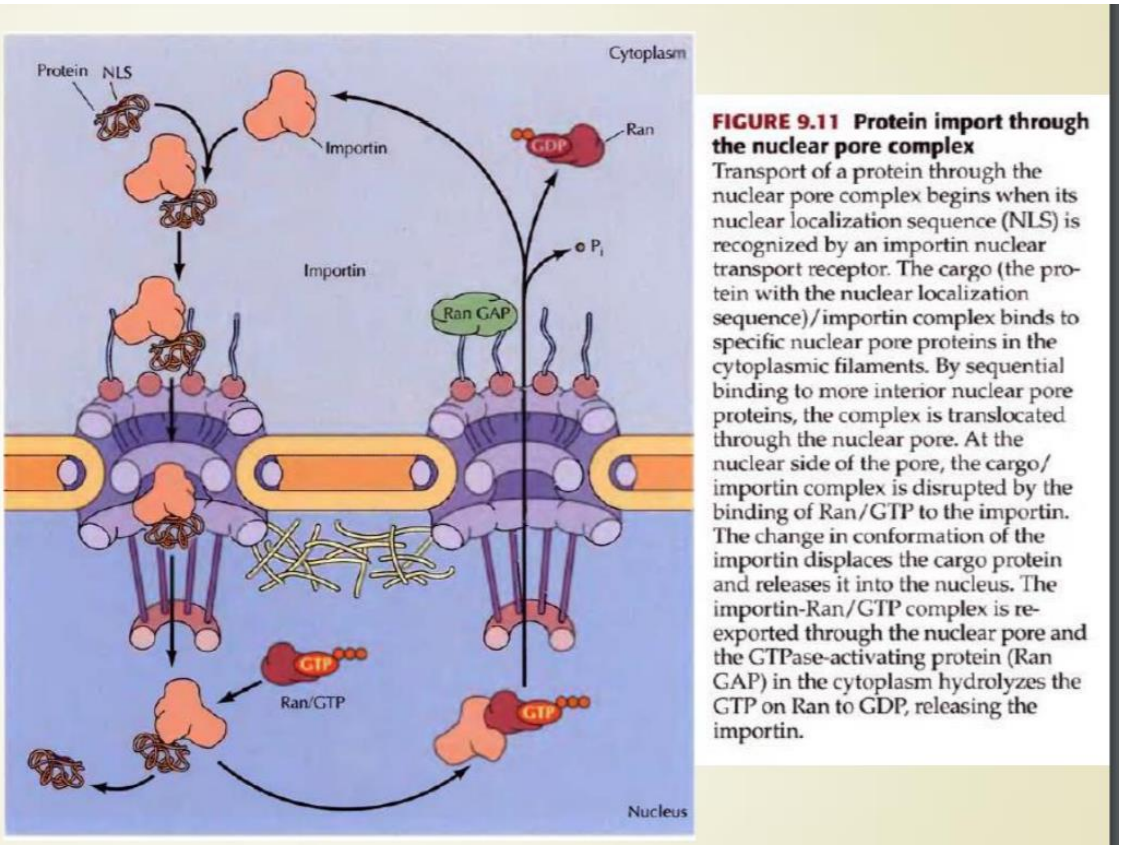


FIGURE 9.11 Protein import through the nuclear pore complex
Transport of a protein through the nuclear pore complex begins when its nuclear localization sequence (NLS) is recognized by an importin nuclear transport receptor. The cargo (the protein with the nuclear localization sequence)/importin complex binds to specific nuclear pore proteins in the cytoplasmic filaments. By sequential binding to more interior nuclear pore proteins, the complex is translocated through the nuclear pore. At the nuclear side of the pore, the cargo/importin complex is disrupted by the binding of Ran/GTP to the importin. The change in conformation of the importin displaces the cargo protein and releases it into the nucleus. The importin-Ran/GTP complex is re-exported through the nuclear pore and the GTPase-activating protein (Ran GAP) in the cytoplasm hydrolyzes the GTP on Ran to GDP, releasing the importin.

Protein export through the nuclear pore complex

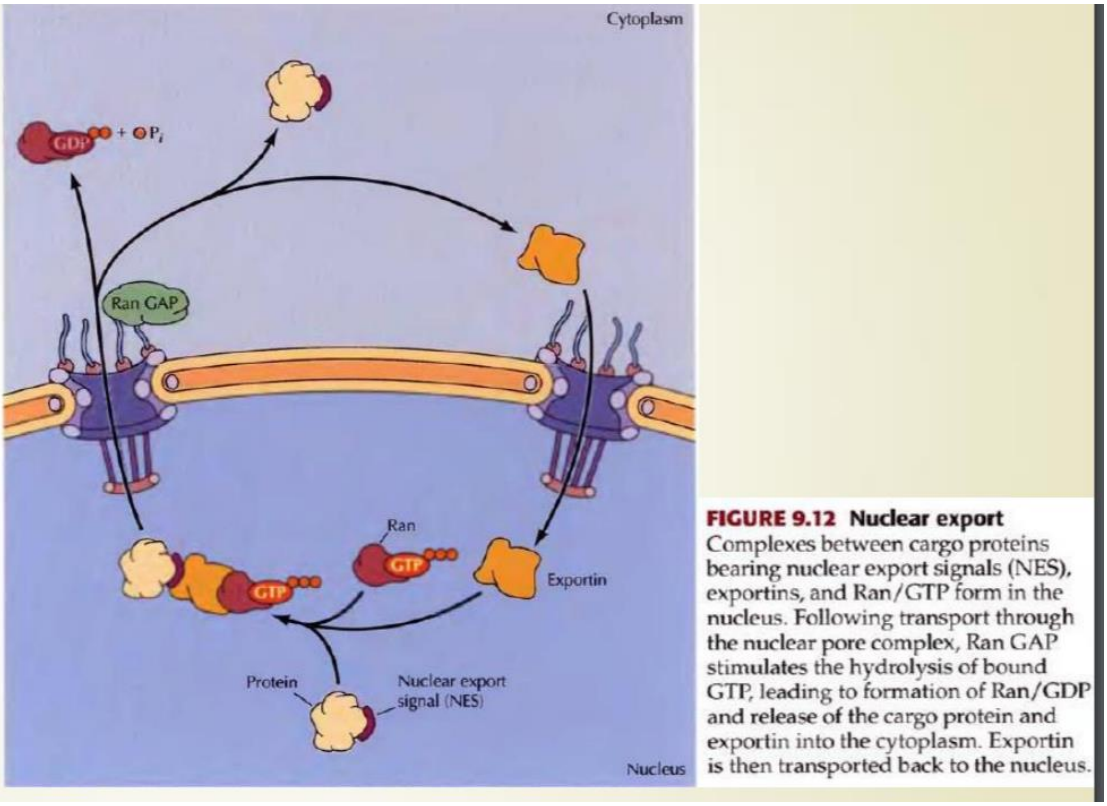


FIGURE 9.12 Nuclear export
Complexes between cargo proteins bearing nuclear export signals (NES), exportins, and Ran/GTP form in the nucleus. Following transport through the nuclear pore complex, Ran GAP stimulates the hydrolysis of bound GTP, leading to formation of Ran/GDP and release of the cargo protein and exportin into the cytoplasm. Exportin is then transported back to the nucleus.

Mechanisms of nuclear protein import regulation:

1- Cytoplasmic proteins may bind and mask the cargo's nuclear localization signals NLS.

2- Phosphorylation.

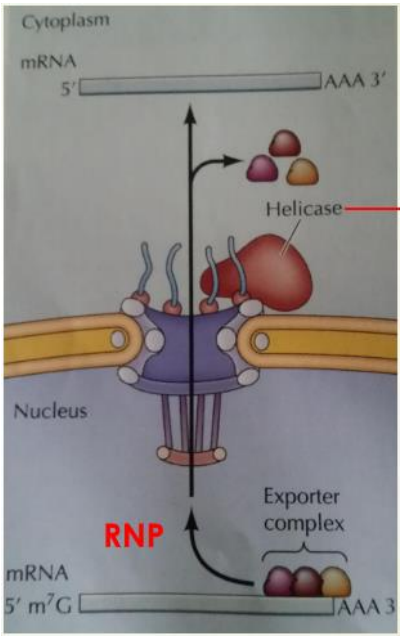
mRNA export from nucleus:

1- It is first synthesized as a pre-mature mRNA in the nucleus, requiring splicing, capping and poly-adenylation. This is important in stabilizing mRNA to escape degradation.

2- Exporter complex *(proteins complex)* is then recruited to pre-mRNA forming ribonucleoprotein (mRNP).

3- The mRNPs are translocated through nuclear pore complexes that are embedded in the nuclear envelope

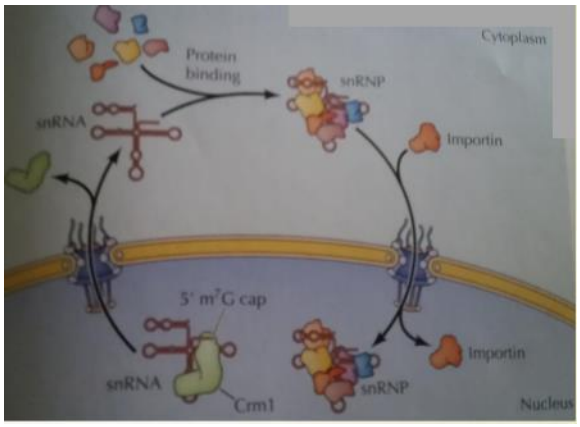
4- Proteins dissociate from the mRNA upon reaching the cytoplasm by a **Helicase**. The presence of this helicase on the cytoplasmic side preserves directionality of mRNA transport preventing it from re-entering the nucleus.



snRNA transport between nucleus and cytoplasm

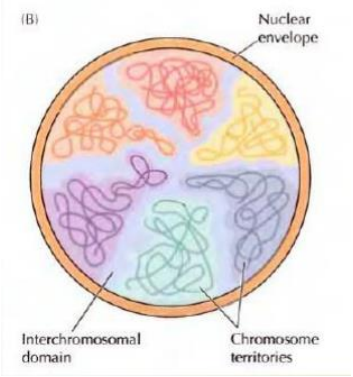
Small nuclear RNA (snRNA) is a class of RNA molecules that are also synthesized inside the nucleus. However, to become functional, certain proteins must bind to it in the cytosol.

They leave the nucleus → Bind to proteins → snRNP → Imported back into the nucleus.



Chromosome organization

Chromosomes do not randomly wind around one another, but they occupy discrete territories within the nucleus called the 'Chromosome Territories'.



For a gene to be active, in a specific region on the chromosome, it localizes on the periphery of the territory becoming closer to the 'Interchromosomal domain', where RNA processing and transport also occurs. These regions on the chromosome are called 'Euchromatin'.

When being inactive, it is present within the territory. These regions are called 'Heterochromatin'.

Heterochromatin	Euchromatin
Highly condensed	Decondensed
Transcriptionally **inactive**	Transcriptionally **active**
Includes **non-transcriptional** DNA sequences such as telomeres and centromeres	Contains **transcriptional** DNA regions
Located close to the nuclear envelope and around the nucleolus and binds to lamins and proteins of the inner nuclear membrane	Localized to the periphery of chromosome teritorries adjacent to channels between the chromosomes

There are more **sub-compartments** *(regions)* within the nucleus in which distinct nuclear processes occur.

Replication factories are clustered sites of DNA replication where replication of multiple DNA molecules takes place. There are multiple replication forks per factory.

Nuclear bodies are nuclear organelles that compartmentalize the nucleus and concentrate proteins and RNAs that function in specific nuclear processes.

TABLE 9.2 Nuclear Bodies

Nuclear body	Number per nucleus	Function
Cajal body	0–10	snRNP assembly
Clastosome	0–3	Proteasomal proteolysis
Histone locus body	2–4	Transcription and processing of histone pre-mRNAs
Nuclear speckle	20–50	Storage of pre-mRNA splicing factors
Nuclear stress body	2–10	Response to stress
Paraspeckle	10–20	Some A-to-I RNA editing
PML body	10–30	Transcriptional regulation, DNA repair
Polycomb body	10–20	Gene silencing

Note 1: PML bodies are especially important in X-inactivation processes causing gene silencing.

Note 2: Paraspeckle bodies function in Adenosine-to-inosine (A-to-I) RNA editing. Which is an important post-transcriptional modification that affects the information encoded from DNA to RNA to protein.