Genetics
& Cell biology

◉ Sheet ○ Slides

Number

7

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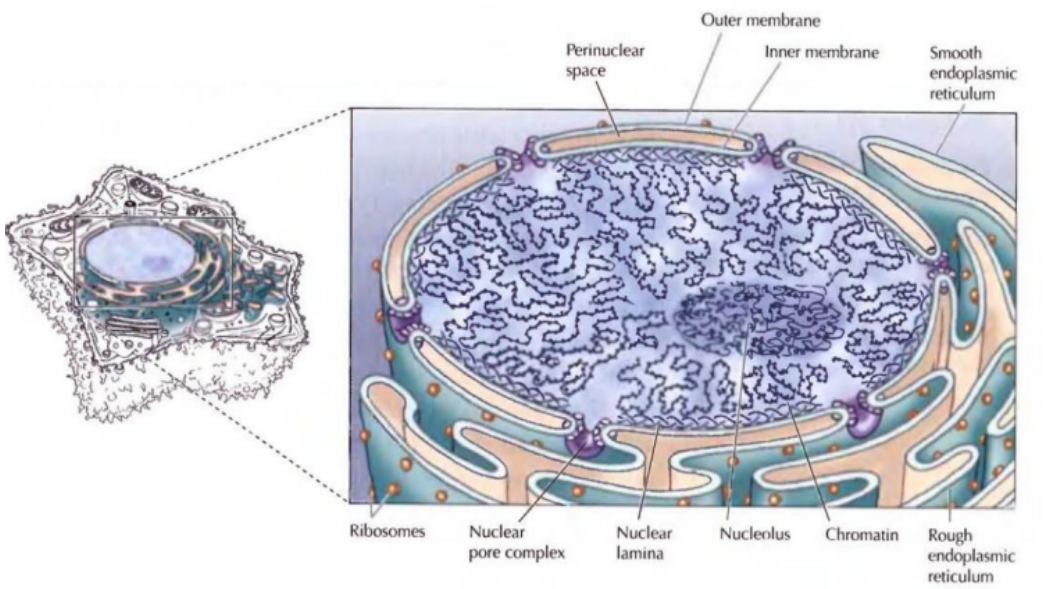
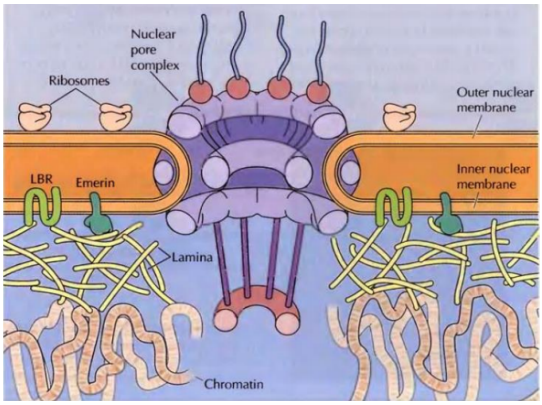
The nucleus

Nucleus: it's an organelle; it functions as the brain of the cell; it contains all the genetic information (DNA) needed to maintain the normal function of the cell; it is organized very well so that the function of the cell will not be affected.

1-The nucleus is surrounded by two membranes: **outer** and **inner membranes** (flattened sacs); they surround the nucleus and form the **nuclear envelope**. The outer membrane is continuous with the endoplasmic reticulum and ribosomes are bound to its cytoplasmic surface; it also has proteins that bind the cytoskeleton but not those that give the tubular ER structure. The 2 membranes prevent the free passage of molecules between the nucleus and the cytoplasm and make the nucleus a distinct biochemical compartment.



2-The nucleus needs communication with the cytosol to exchange materials, and such communication is done by **nuclear pores** (small openings between non-continuous parts of the envelope). Nuclear pores are large complex proteins; they allow organized movement of molecules (proteins, mRNA, rRNA, …. etc) to or from the nucleus. A nuclear pore is composed of complete circles; in the middle, there is a space, so the circles can constrict allowing materials to enter or get out from the nucleus.



-Nuclear pore complexes are where the inner and outer nuclear membranes join together.

Nuclear lamina

Nuclear lamina is composed of filaments that are anchored to the inner membrane to give mechanical strength and support to the inner membrane and to maintain the shape and the maximum size of the nucleus. Nuclear lamina is a fibrous structural meshwork composed of lamins and other associated proteins such as, emerin, LBR, LINC complexes, histones and other chromosomal proteins. Let's discuss the arrangement of these proteins to give such a function :

1- The **lamin** polypeptide with all its types is the basic unit for nuclear lamina.

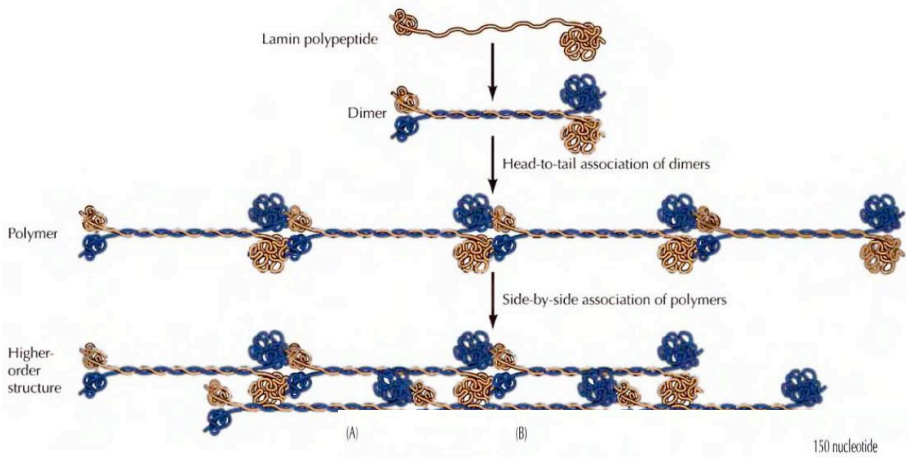
2- Dimerization (head to tail).

3-Polymerization of dimers from head to tail.

4- Side by side association of polymers to give higher order structures.

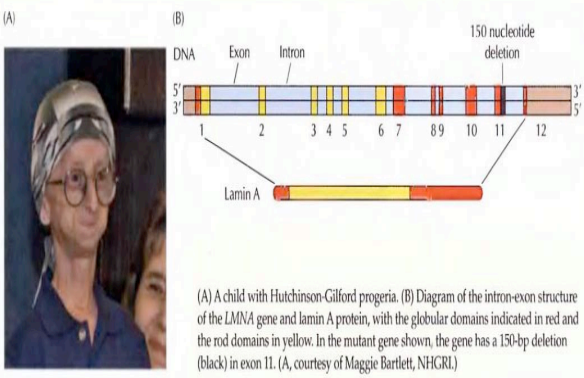
X-linked Emery-Dreifuss muscular dystrophy: -

It's an uncommon muscular dystrophy; this disease occurs due to mutations in emerin.



-Symptoms:-

1. Stiff elbows, neck and heels.
2. Conduction block in the heart; thus, patients may need a pacemaker.
3. Wasting and weakening of the muscles.



(A) A child with Hutchinson-Gilford progeria. (B) Diagram of the intron-exon structure of the *LMNA* gene and lamin A protein, with the globular domains indicated in red and the rod domains in yellow. In the mutant gene shown, the gene has a 150-bp deletion (black) in exon 11. (A, courtesy of Maggie Bartlett, NHGRI.)

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

-LMN mutations can also cause **Dunnigan-type partial lipodystrophy**, **Charcot-Marie-Tooth disorder type 2B1, and Hutchinson-Gliford progeria**.

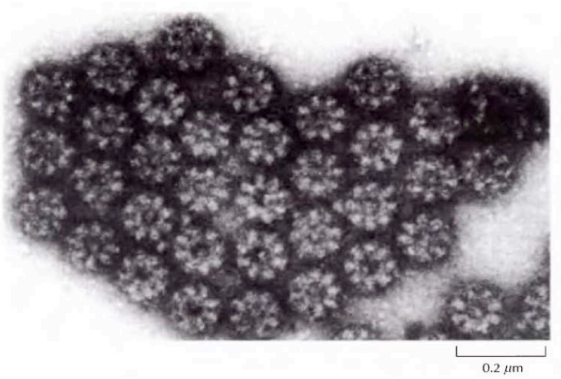
Nuclear pore complex

-Contains a lot of proteins.
-The nuclear pore complex has a diameter of 120nm. It is very large and because of its diameter, molecules with diameters less than 120nm (125 million Dalton) can pass through it.
-There are 30 different pore proteins called **nucleoporins**; therefore, multiple molecules can pass through the nuclear pores. However, keep in mind that **not all pores can allow the passage of all molecules**.

Structure: eightfold symmetry spokes with a large central channel.

Function: transports small polar molecules, ions and macromolecules (proteins such as transcription factors and RNAs).

Keep in mind that when we first talked about the shape of the pore, we said it's composed of circles, which are 3D in shape (ball like structure nearly), but the shape appears as a 2-dimensional structure, so if we take a cross section of the membrane, the pores will look like the picture on the right, like arranged roses.

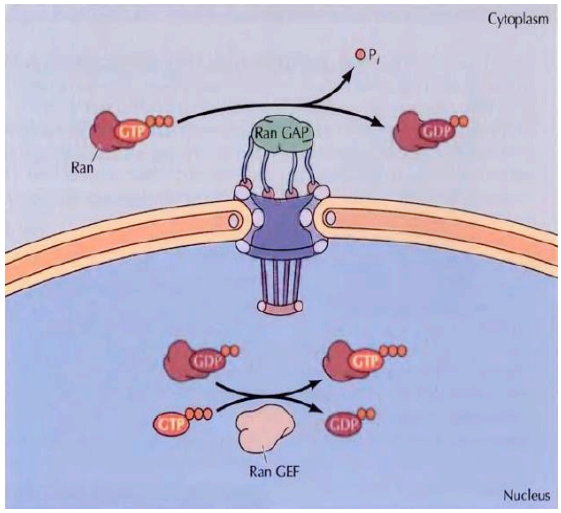


What makes different molecules or proteins enter the nucleus (be imported)? There are some signals, called **nuclear localization signals (NLS)** which have many types; they contain specific amino acid sequences which target the molecules to the nuclear membrane. NLS are recognized by nuclear transport receptors that direct cargo proteins to the nuclear pore complex. Some NLSs are far apart and **depend on protein folding**.

-How these molecules will move across the membrane from outside to inside or from inside to outside?

The amount or concentration of the molecules are almost equal on both sides of the membrane, so there is **no concentration gradient** that allows them to move. Another problem is there are also active and inactive forms of the molecules, so which molecules will be moved? We have other molecules than NLS; they control the direction of the crossing molecules, for example, GTP binding proteins like **RAN-GTP**; let's explain it:

-RAN exists in the cell in two forms: GDP-bound and GTP-bound.



-Its conformational change depends on binding GDP or GTP (that does not mean that GTP is always found outside or GDP inside, as both (GTP and GDP) can be found outside and inside).

-There are some factors that control RAN: -
1-RAN GTPase activating protein **(RAN GAP)** in the **cytosol** which hydrolyzes the binding GTP.
2-Guanine nucleotide exchange factor **(RAN GEF)** in the **nucleus**, which causes the conversion of RAN GDP to RAN GTP and vice versa.
So the mechanism of conversion in the outside is different from that in the inside, and this shows that entry into the nucleus is under control.

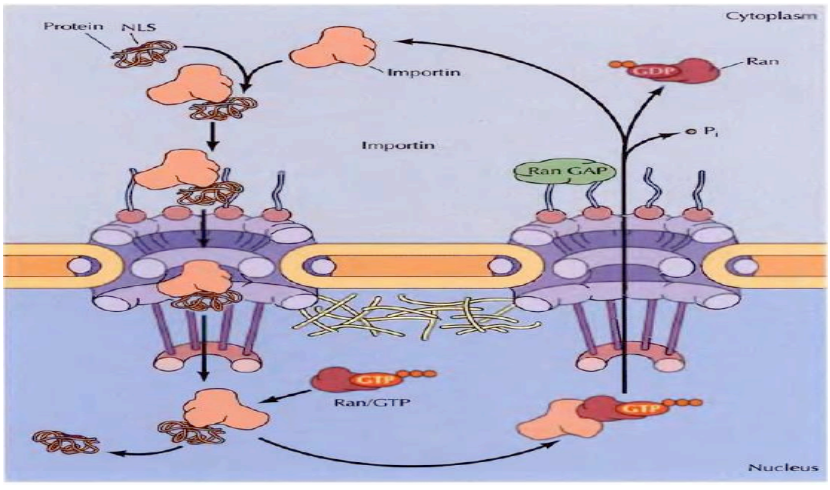
-There is no molecule that can move through the membrane without binding RAN (especially RAN-GTP).

Protein importing:

Let's assume that we have to import a protein to the nucleus:
1-Simply the protein will bind to NLS.
2-This NLS will be recognized by **importin protein** (its name tells the function of it).
3-Importin protein will carry the protein bound NLS to the nuclear complex pore and then to the nucleus.

4-This complex enters the nucleus (protein-NLS bound to importin protein complex).

5-RAN-GTP binds importin protein and causes conformational changes to the complex leading to displacement of the protein and its release.



6-Now, RAN-GTP and importin protein bind each other making a complex, so they leave through the nuclear pore.
7-Hydrolysis by the RAN-GTPase in the cytosol. Now, we have

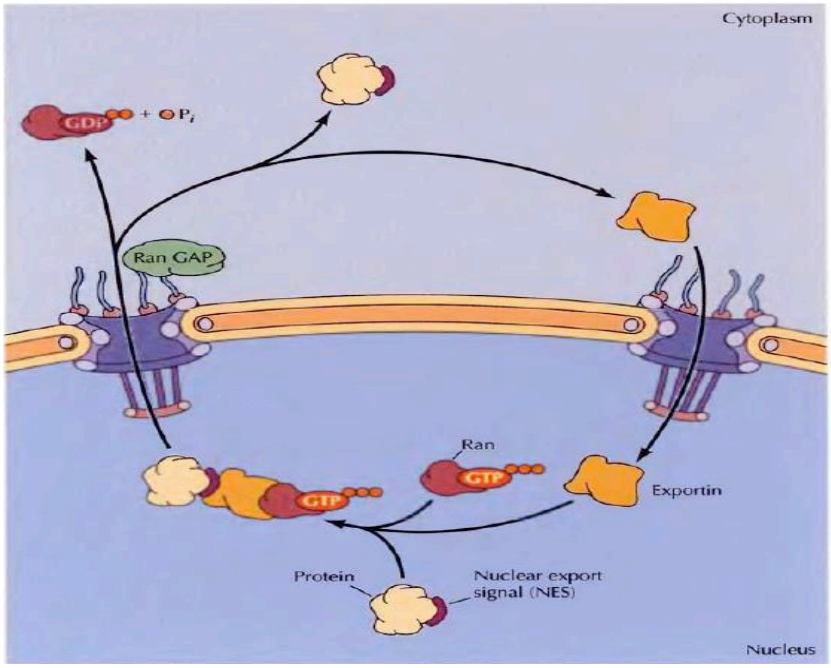
recycled importin protein and RAN-GDP.

Mechanisms of nuclear protein import regulation:

1-Cytoplasmic protein binding and masking of the cargo's NLS

2-Phosphorylation

Protein exporting:



Transcription factors are good examples for exported proteins (after finishing their function).

1-Exported protein has a different signal from the imported protein which is **nuclear export signal NES.**
2-This NES will be recognized through exportin protein.
3-This exportin protein can't bind NES alone and get out, as it needs the help from RAN-GTP, which -along with exportin- binds to the protein-bound NES. The complex (exportin protein, the protein with NES and the RAN-GTP) exit through the nuclear pore.

4-Now, this complex is separated, so RAN-GTPase hydrolyzes it and we end up with the exported protein, RAN-GDP and recycled exportin protein which targets another NES and exports another molecule.

-RAN-GTP is hydrolyzed after both exporting and importing.

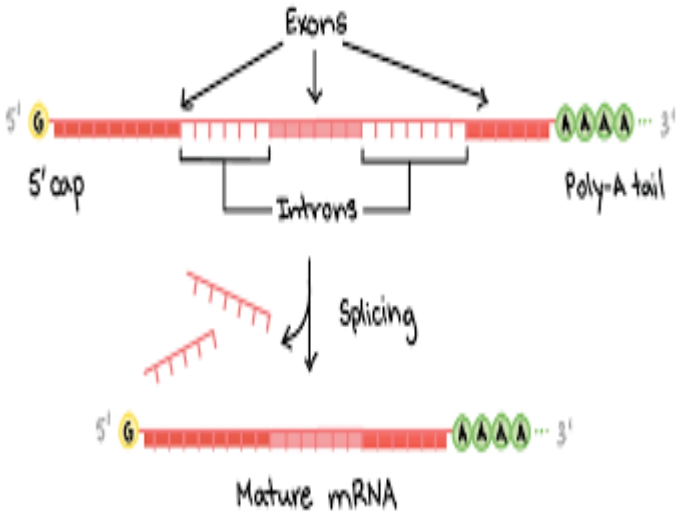
-So, we talked about the movement of proteins and how it is controlled through **localization signals NLS/NES, exportin / importin proteins and RAN-GTP**. In contrast there are high amounts of transcriptional factors which go under covalent modification (most of them go under phosphorylation, which could be activation or inhibition depending on the pathway. Phosphorylation as covalent modification may also mean signaling) for activation and we will talk about that in details when we take cell signaling.

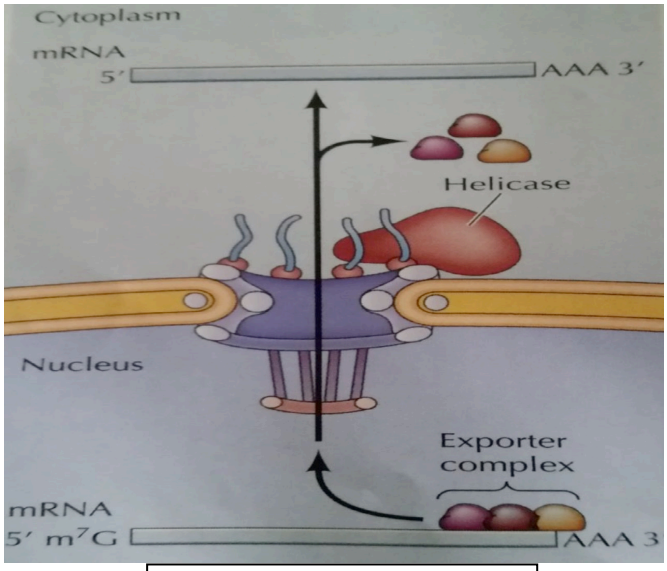
Transporting mRNA

After finishing transcription, primary mRNA goes through modification, splicing (removing introns and joining exons together) and maintaining its structure (mRNA is a very unstable molecule; it is degraded rapidly so it needs some protection because any lost part of its sequence means producing mutated proteins which cause diseases).

Poly A tail (other name is **polyadenylation**, a sequence of non-coding adenine nucleotides which will not be translated; this sequence is not related to the sequence of mRNA) will be added on 3' end of the mRNA (its end) to protect it. mRNA as a molecule can't be transported by itself; therefore, it needs binding proteins, so it will cross the pore as a complex (mRNA and proteins) to the outside (cytosol).

mRNA must be free to bind a ribosome and complete its function (translation), so an enzyme called **Helicase** completes the separation of mRNA from the binding protein. Helicase is found on the cytosolic side of nuclear pores. It will displace the mRNA from the binding protein and make it free.





The direction is from 5' to 3'

-We have talked previously about the directional controls of the crossing proteins (RAN-GTP/ RAN-GDP) so what are the directional controls of mRNA?

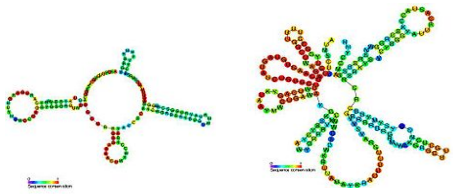
1-It is helicase, because it is found only on the cytosolic face. Helicase will direct the mRNA to go from the nucleus to the cytosol.

2-The presence of the ribosome outside the nucleus prevents mRNA to go inside the nucleus.

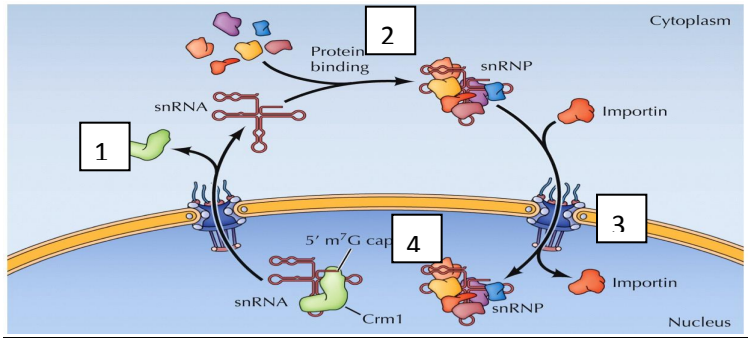
3-mRNA binds a protein and forms a complex, so even if there were ribosomes inside the nucleus, the protein-mRNA complex will not bind to the ribosomes because it can't recognize the ribosome as a complex, and keep in mind that this complex always disassociates in the cytosol where it binds to a ribosome.

Small nuclear RNA (snRNA):

-It's the same as mRNA, it needs binding proteins to be transported. It's a very short sequence that has a hairpin like structure. It makes several dimerizations within itself to form different shapes.

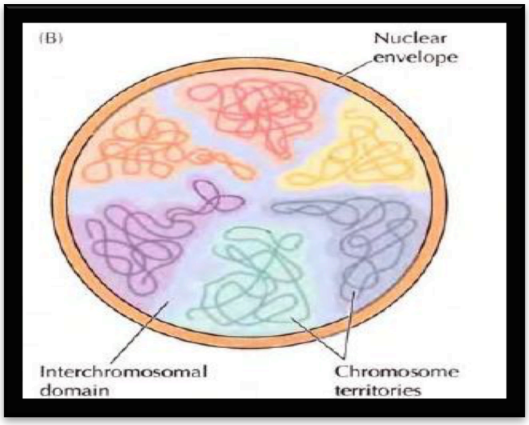


-snRNA is a small molecule and from its name (nuclear) it functions in the nucleus, but it must be part of a protein complex in order to function, so it is transported to the outside of the nucleus to produce the protein complex because proteins are produced in the cytosol, then the snRNA goes back to the nucleus as a part of a protein complex.



1-Free snRNA in the cytosol.
2-Binds to a protein for transportation and the complex is named snRNP (small nuclear ribonucleo proteins)
3-Then it binds importin protein to enter the nucleus through the pore
4-The snRNA reaches the nucleus as a complex so it now becomes useful to use, then it is released to the cytosol again.

Chromosome organization:-



We have 46 chromosomes. They do not wind around each other randomly, but they occupy discrete territories within the nucleus, like a pizza!

-Why it is active (where there is transcription, making RNA…) in the interchromosomal domains and almost inactive (where there is no binding to transcription factors) in the center? Let's imagine that the nucleus is a house, when you are on the door, you can take whatever you want from the outside (transcription factors, proteins,..) and use them for transcription, making proteins, RNA,…. But if you

were inside the house at the middle, you can't reach the proteins and molecules to be active, and that's why there is difference in the activity.

-**Chromatin** is composed of the 46 chromosomes with **histones** and proteins that perform folding and packaging inside the nucleus.

-Every chromosome is located in its sector.

-Between every two territories, there is an **interchromosomal domain**.
-In each territory, the chromosomes are moving according to the active gene.
-RNA processing and transport occur in interchromosomal domains.

-There are two types of chromatin according to cellular activity:-

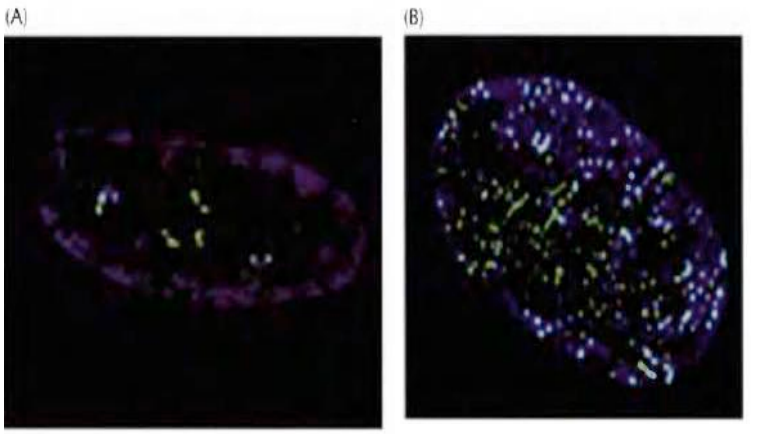
Heterochromatin	Euchromatin
Highly condensed	**Decondensed, loose, open structure**
Transcriptionally inactive	**Transcriptionally active**
Includes non-transcriptional DNA sequences such as telomeres and centromeres	**Contains transcriptional DNA regions**
Located close to the nuclear envelope and around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

The functional internal organization of the nucleus from a functional point of view:

The nucleus will be divided according to the site where it's active and functioning.

Different processes occur in different regions so the nucleus is divided according to the function (structurally, they are not divided).

–The pictures at the right show a cell dyed with Fluorescence to study and show the replication factories; notice their activity
A: The cell before dividing
B: The cell after dividing (increased number of factories).



-**Replication factories** are clustered sites of DNA where replication of **multiple DNA molecules** takes place.

-There are multiple replication forks per **one DNA molecule** in order to finish replication quickly, so the factory is bigger than the fork.

Nuclear bodies

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

-Nucleolus is considered as one of the nuclear bodies.

-This table shows many examples on nuclear bodies, don't memorize numbers in the slides.

Nuclear body	Function
Cajal body	**snRNP assembly**
Clastosome	**Proteasomal proteolysis for protein degradation**
Histone locus body	**Transcription and processing of histone pre-mRNAs**
Nuclear speckle	**Storage of pre-mRNA splicing factors**
Nuclear stress body	**Responds to stress that may occur during transcription if there were some errors, so during stress, these bodies will take place altering the correction and repair mechanisms**
Paraspeckle	**Some active to inactive (a to i) RNA editing, still under research**
PML-body	**Transcriptional regulation and DNA repair**
Polycomb body	**Gene silencing, brings methyl group and adds it to some regions and makes them silent.**

((وصفة الإنجاز الناجح مكونة من أربعة مُكوّنات أساسية فحسب: اختر مهمّة تُحبها، امنحها أفضل ما لديك، اغتنم الفرص التي تلوح لك، وكن أحد أفراد الفريق))