Molecular Cell Biology

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Module – 2

Nuclear Structure, Chromatin Packing and Transport, Transport of Biomolecules

Lecture - 1

Nuclear Structure

Introduction

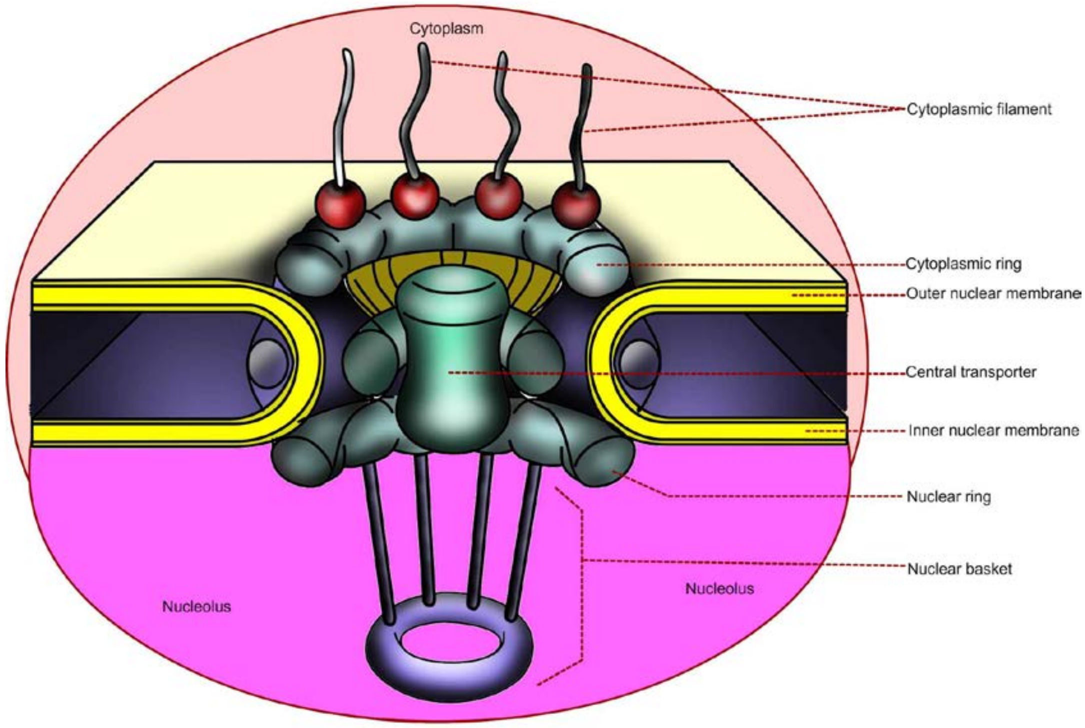
- Flemming in 1879 used the word “chromatin”
- He referred it to the substance intensely stained with basic dyes in the nucleus
- Nuclein, earlier isolated by Meischer in 1871, was thought to be responsible for this staining due to its high affinity to basic dyes
- The term ‘‘nucleolus’’ was coined by Valentin (1839) referring to a secondary nucleus or a ‘‘nucleus within a nucleus.’’
- In 1888 Waldeyer used the term “chromosome”.
- Large amounts of DNA are packed into chromosomes and separated from the cytoplasm where protein synthesis takes place.
- After transcription, mRNA molecules exit the nucleus through the nuclear envelope into the cytoplasm.
- In the interphase nuclei, chromatin is present in the condensed form called heterochromatin or in a dispersed form called euchromatin.
- Condensed chromatin attaches itself to the internal side of the nuclear envelope.
- One or many large bodies visible in the nuclei are called nucleoli.
- Nucleoli contain RNA and proteins without any membrane and are the sites for the transcription of ribosomal RNAs

Nuclear envelope

- The nuclear envelope structure is better visualized through electron microscope.
- It has two membranes separated by **perinuclear space** of about 10-15 nm in width.
- The membranes have lipid bilayer structure like any other membrane including the plasma membrane.
- The outer surface contains ribosomes and the nuclear membrane directly continues as ER membrane especially the one with ribosomes.
- Nuclear envelope is interrupted with many nuclear pores.
- A fibrous lamina of proteins (50-80 nm thick) attach with the inner membrane but not with the pores.

- The pores are enclosed by circular structures called annuli.

Nuclear pore complex



- The NPC with 100-nm diameter is a hollow cylinder embedded in the nuclear envelope.
- It has about 30 different proteins termed nucleoporins (Nups).
- The NPC acts as the gate between the nucleus and the cytoplasm.
- Macromolecules carrying specific import and export signals are only allowed to pass through water and metabolites can freely pass through.
- The central channel is filled and surrounded with FG Nups having many large domains rich in phenylalanine and glycine repeats.
- The nuclear basket has eight filaments that reach into the nucleoplasm, attached to each other by a ring at the end.
- Electron microscopy tomographs have shown that filaments extend from this basket into the nucleus
- The cytoplasmic filaments form highly mobile molecular rods projecting into the cytoplasm.

- These filaments help NPCs to reach about 100 nm into the nucleus and cytoplasm.

NLS and NES

- The transport of molecules through the NPC is restricted by size; below a mass of approximately 60 kDa, macromolecules can passively diffuse across the NPC.
- Such small macromolecules below this cut-off also often contain a nuclear localization signal.
- Nuclear localization sequences (NLSs) for import into the nucleus and nuclear export sequences (NESs) for export are required.
- These signals are recognized by transport factors, each with specific signal preferences.

Transport receptors

- Transport receptors are usually from the karyopherin (importin and exportin) families, with a shared α-super helical structure.
- Karyopherins can bind to the NLSs or NESs of their cognate cargoes, to the FG Nups and to the GTPase Ran.
- Import cycle starts with the formation of the cargo–karyopherin complex in the cytoplasm, which is the rate-limiting step *in vivo*, and translocates through the NPC.Disassembling the complex occurs on the nuclear side by the binding of Ran-GTP to the karyopherin.
- Ran cofactors are present in both the nucleoplasm and cytoplasm and a Ran-specific nuclear transport factor (NTF2) maintains a high concentration of nuclear Ran-GTP and of cytoplasmic Ran-GDP.
- NES on a cargo is recognized by a cognate karyopherin–Ran-GTP dimer in the nucleus and, after translocation across the NPC, the NES-cargo–karyopherin–Ran-GTP complex is disassembled on the cytoplasmic side, through activation of Ran GTPase activity by cytoplasmic RanGAP, achieving directionality.
- Not all transport factors require Ran, nor belong to the karyopherin family; however, notably, all transport receptors can interact directly with FG Nups

Cargoes

- NPC can be considered an enzyme for transport, in which only the correct substrates (such as transport factors and their cargoes) can bind to the active site and so pass across the nuclear envelope.
- In living cells, this high transport rate is represented by several transport factors carrying many importing and exporting cargoes, including ribonucleoproteins (RNPs).
- **Import cargoes** are mostly proteins that have been synthesized in the cytoplasm and are needed in the nucleus. There are also proteins that, once they reach the nucleoplasm, are exported out again by karyopherins such as XPO1 (also known as CRM1).
- **Export cargoes** are usually RNAs, as complexes made of RNA and proteins.
- The ribosomal subunits and messenger RNPs (mRNPs) are the most abundant of these export cargoes.
- Protein cargoes (60kDa) are smaller than mRNP cargoes (100 MDa)

FG Nups

- FG Nups contain the docking sites for most NTF–cargo complexes en route through the NPC.
- FG Nups are characterized by regions of multiple Phe-Gly repeats separated by hydrophilic spacer sequences of 5–30 amino acids24.
- FG Nups can be classified into two groups: symmetric, which are found on both sides of the NPC (for example NuP62), and asymmetric, which have a clear nuclear or cytoplasmic location
- NPC is not a physical gate that opens and shuts but is constructed to behave like one.
- Both the actual transport step through the NPC and the exclusion of nonspecific macromolecules from this step are largely mediated by the milieu formed by the FG Nups.

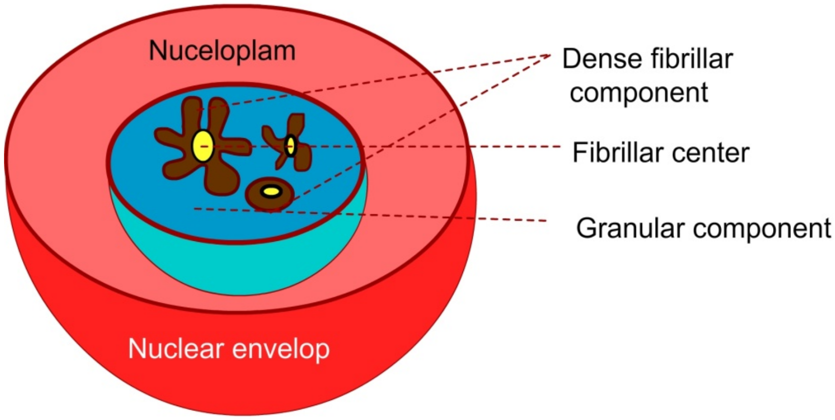
- In and around the central tube of the NPC there is a dense network of intertwined Phe-Gly repeat filaments that form an obstacle to the passive diffusion of most macromolecules, leading to their exclusion from the NPC.
- Transport factors overcome this exclusion through their capacity to bind Phe-Gly repeats, moving from binding site to binding site across the NPC.
- The FG Nups may exhibit a polymer brush-like behavior, pushing away nonspecific macromolecules, or they may collapse on binding, providing temporary passage through the meshwork.
- In contrast, FG Nups may be cross linked by amyloid-like interactions to form “a hydrogel-based, sieve-like permeability barrier that allows rapid entry of nuclear transport receptors”; the Phe-Gly repeats may form a lining to the central tube, which NTFs can access but other macromolecules cannot.
- A combination of these behaviors may be at work

Nucleolus

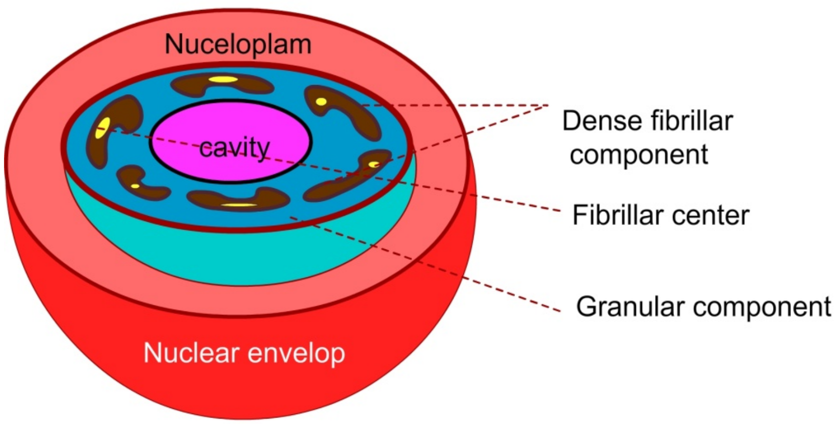
- The nucleolus is involved in the synthesis and processing of ribosomal RNAs (rRNAs) and their assembly into ribosomal subunits
- It is the largest nuclear domain.
- Nucleoli show lightly staining fibrillarcentres (FCs) of about 0.1**–**1.0 **μ**m in size, surrounded by dense fibrillar component (DFC), which is usually more heavily stained than the rest of the nucleolus.
- Fibrillarcentres are the interphase equivalents of the nucleolus organiser regions.
- The other part of the nucleolus is filled with granules (called the granular component**—**GC).
- The granules probably represent pre-ribosomal particles in various stages of maturation.

Substructure of nucleoli

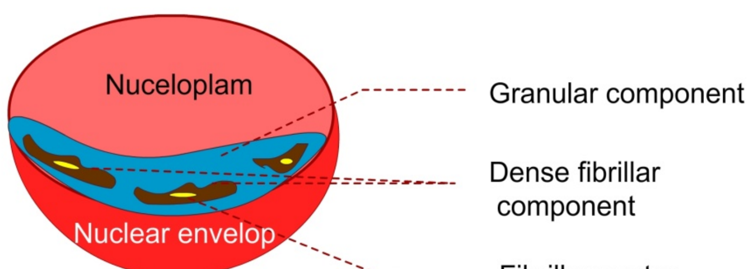
Animal nucleolus



Plant nucleolus



Yeast nucleolus



Nucleolus structure is evolved

- The complex structural organization of the nucleolus differs between anamniotes to amniotes.
- Anamniotes are vertebrates not having an amnion; They lay eggs in water.
- Amniotes are the organisms (reptiles, birds, etc) laying eggs in the environment.
- In this transition the rDNAintergenic region increases.
- Amnion is a protective membrane which covers the embryo in mammals, birds and reptiles.
- It contains the amniotic fluid. This fluid provides the embryo with the required nutrients.

Nucleolus organizer region (NOR)

- Nucleolus is formed in the Nucleolus Organizer Region.
- It is surrounded by filaments called the pars fibrosa (PF).
- The filaments are formed of ribosomal RNA that are newly transcribed.
- After the division of nucleus, this region gets associated with the nucleus. Several copies of genes of ribosomal RNA are present in this region.
- Many tandem copies of the genes of these ribosomal RNA are found in the NOR.
- Karyotype analysis by means of silver nitrate staining can be used to identify NOR.
- About 10 NORs found in a cell.

Functions of nucleolus

- The major function of nucleolus is the production and assembling of ribosomal subunits.
- 50% of the total production of RNA in cells occurs in the nucleolus.
- Nucleolus is the site for the transcription of rRNA precursor molecule from DNA.
- These RNAs are packaged with certain specific forms of proteins to produce ribosomal units of varying size.
- The ribosomal units are then transported into the cytoplasm for translation and protein synthesis.

Non-ribosomal functions of nucleolus

- Nucleoli have nonstandard functions apart from being ribosome factories
- Nucleolus is also involved in mRNA export or degradation,
- Biosynthesis of the signal recognition particle (SRP),
- biogenesis of some snRNAs and tRNAs,
- the sequestration of regulatory proteins,
- control of the cell cycle,
- Aging, and stress sensing.

Study Questions

1. What is the difference between euchromatin and heterochromatin?
2. What are importins and exportins?
3. The two nuclear membranes are separated by

   a) Nucleolus b) Perinuclear space c) Mitochondria d) Endoplasmic reticulum
4. Match the following

Nuclear pore complex	Eggs
Amniotes	snRNA
Nucleolus	Flemming
Chromatin	Nucleoporins

5. The term “nucleolus” was coined by -----------------.