Nucleus Structure and Traffic between the Nucleus and Cytoplasm

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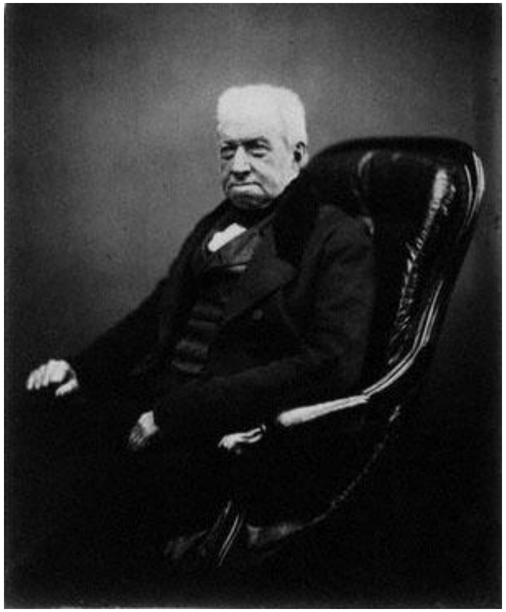
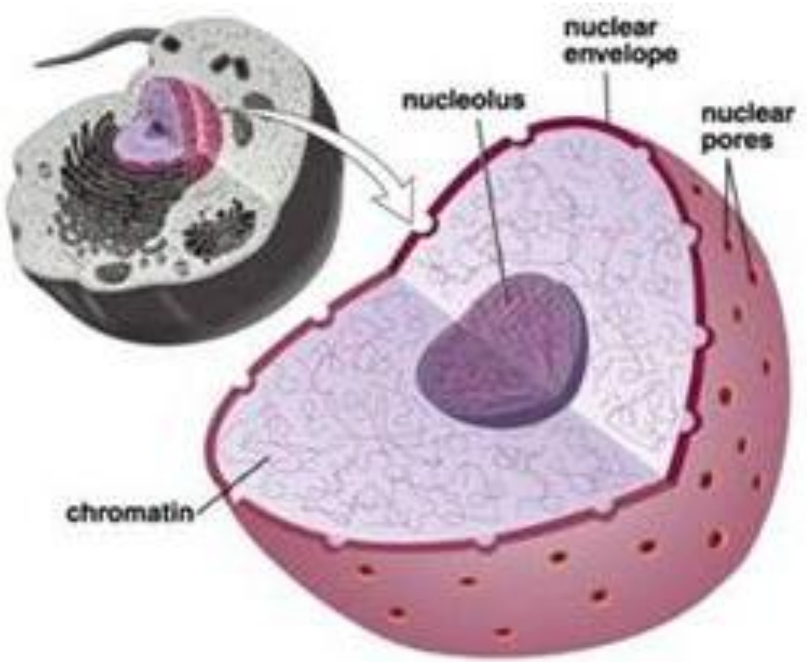
History

- Discovered in 1831 by Scottish botanist Robert Brown
- Suggested the nucleus played a key role in fertilization and development of the embryo in plants
- The name nucleus was derived from the Latin word



Robert Brown
1773-1858

Introduction



- Membrane-enclosed organelle found in eukaryotic cells
- Generally found in the central region of the cell (in animal cells)
- Largest and most easily seen organelle

Introduction

- The cell nucleus appears as a rounded or elongated structure located near the centre of the cell.

- Nuclei range in size from about 1 µm to more than 10 µm in diameter.

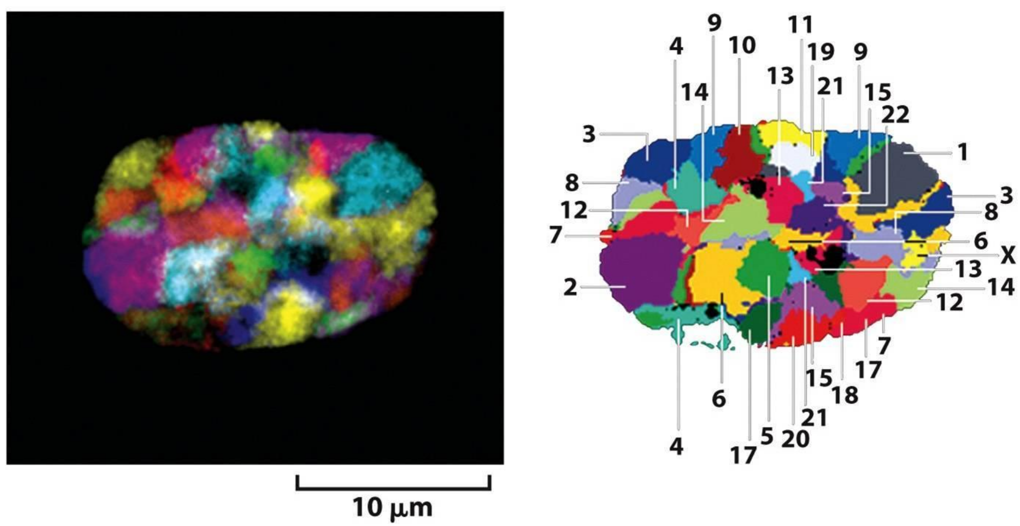
Introduction

•The nucleus contains all the cell's chromosomes, allowing regulation of gene expression

•Although the nucleus lacks internal membranes, nuclei are highly organized and contain many subcompartments.

•Each chromosome occupies a distinct region or territory.

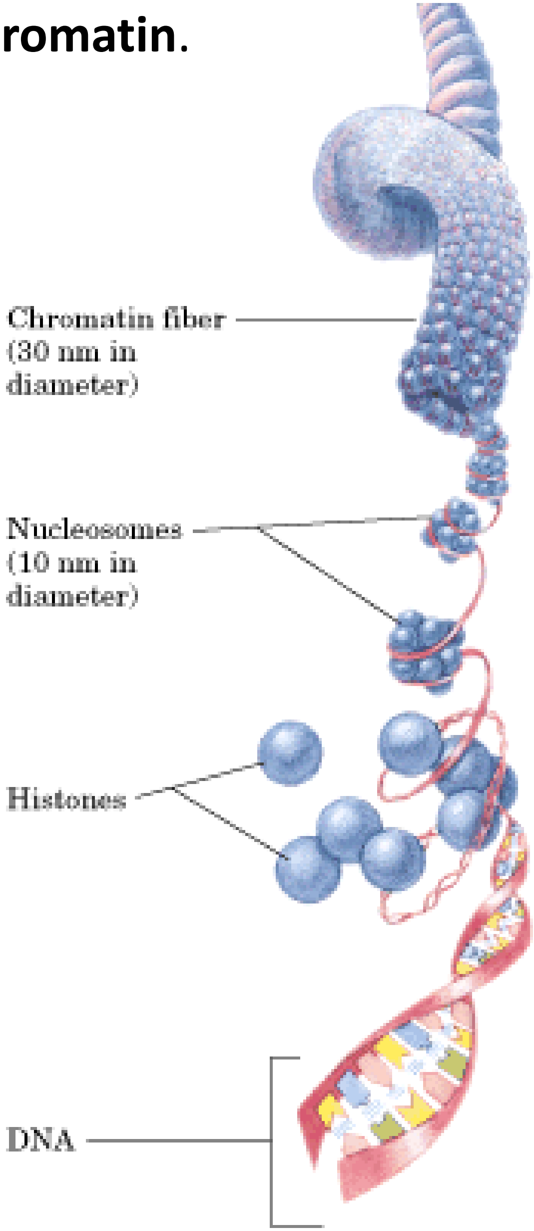
•This prevents chromosomes from becoming entangled with one another.

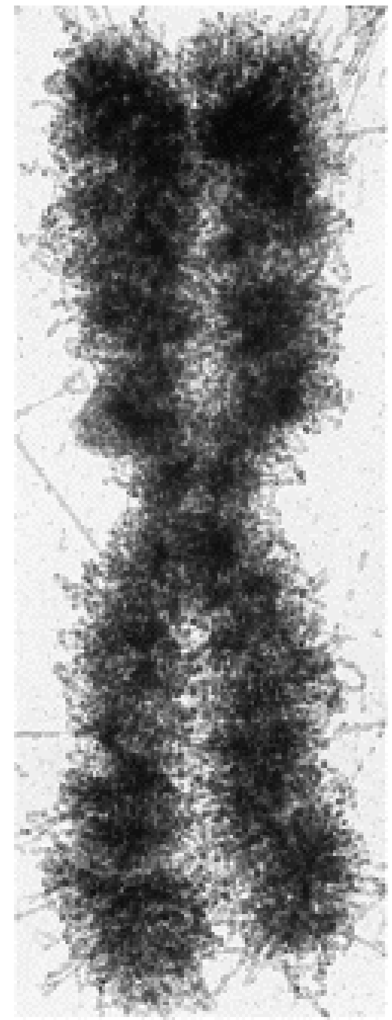


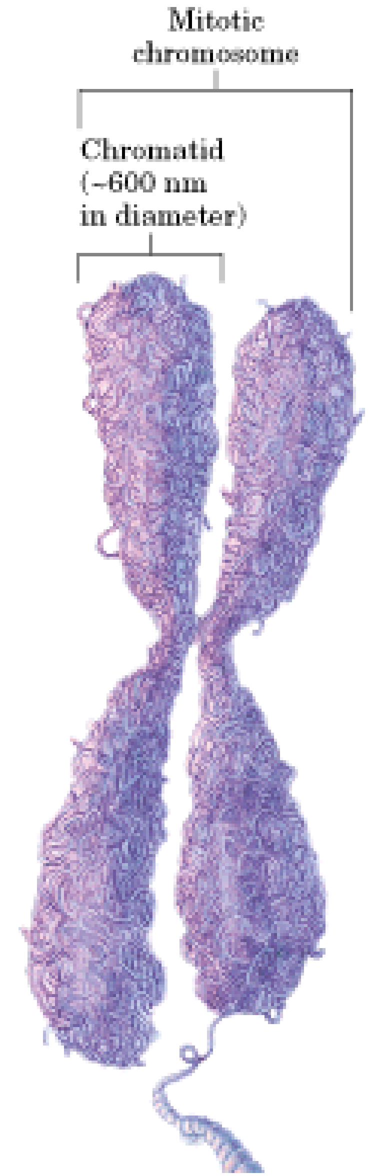
All the chromosome territories (domains) that make up the human genome can be visualised simultaneously in intact interphase nuclei, each in a different colour.

Introduction

•The nucleus harbours the cell's genetic material (DNA) packaged in the form of **chromatin**.

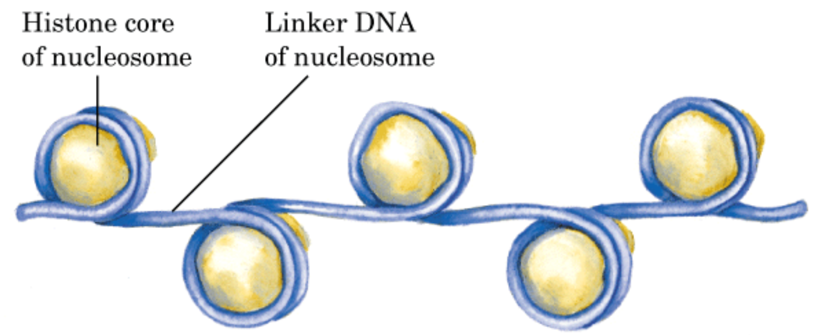




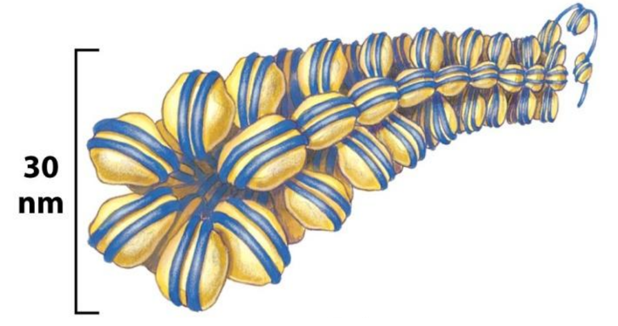


Introduction

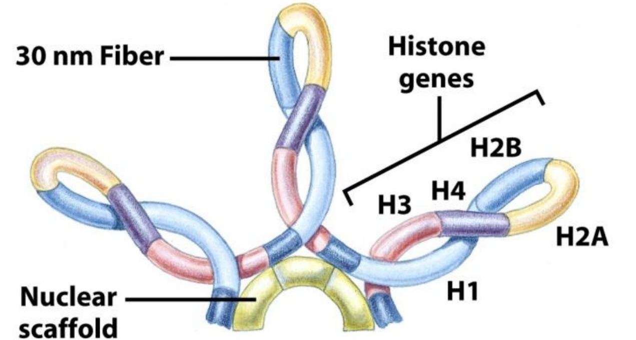
- DNA in eukaryotic cells is packaged into nucleosomes, which contain proteins called histones



- Nucleosomes are packaged to form 30 nm fibers



- Compaction of 30 nm fibers uses nuclear scaffolds



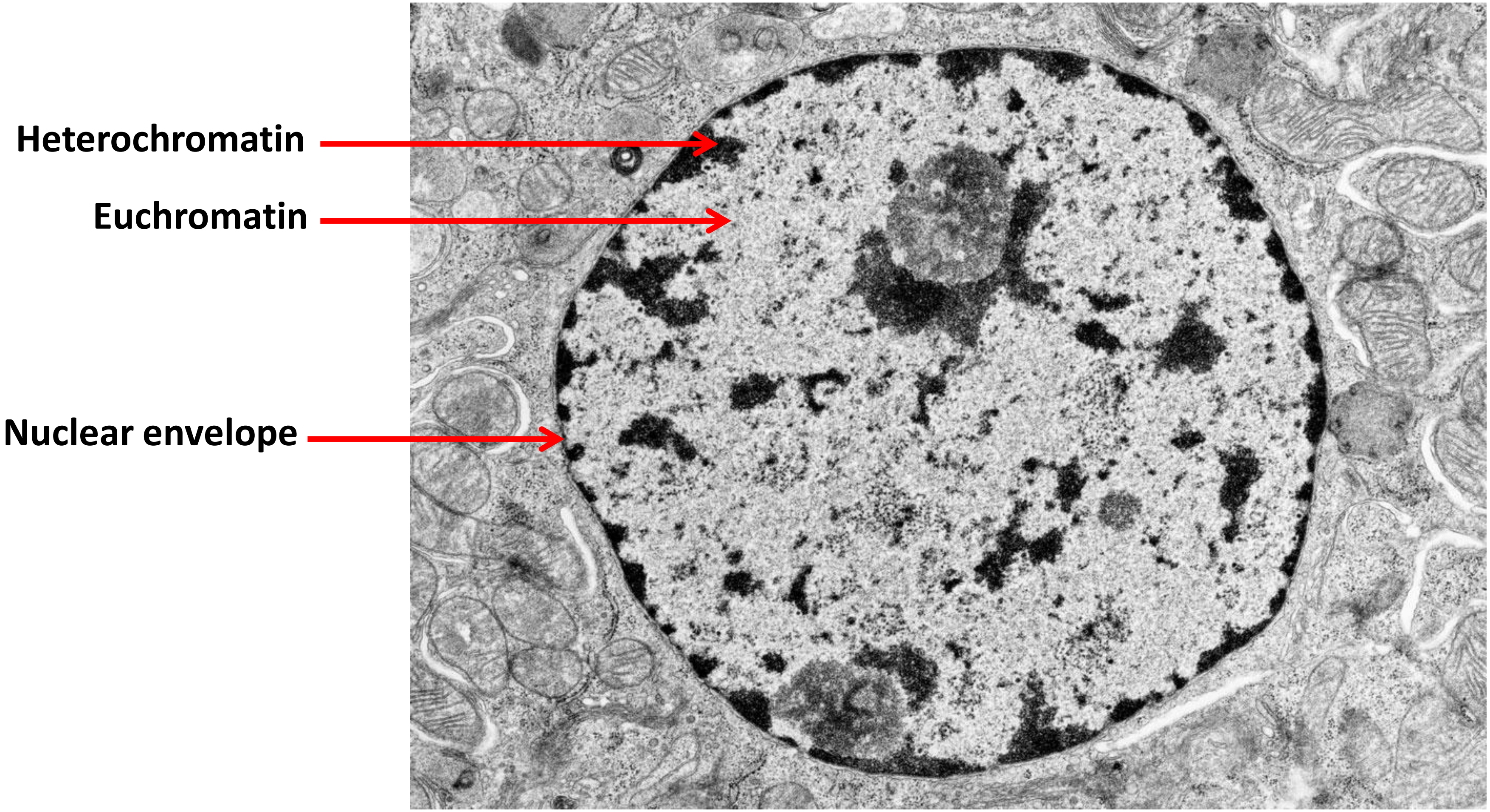
Chromatin

- The DNA in the nucleus is found in different configurations.
- In electron micrographs, some of the DNA appears darkly stained because it is relatively highly-compacted.
- This DNA is called **heterochromatin** and is not actively copied into mRNA carrying the information on how to build proteins.
- Much of the heterochromatin is found near the nuclear envelope.

Chromatin

- Most of the remaining DNA is less densely compacted and is called **euchromatin**.
- Genes that are actively expressed are found in this fraction.
- In most cells, much more of the DNA is euchromatic rather than heterochromatic.
- Collectively, heterochromatin and euchromatin are known as **chromatin** (from the Greek word *chroma* for colour).
- Chromatin is made of nuclear DNA and its associated proteins (mainly histones) and is in fact the chromosomal material.

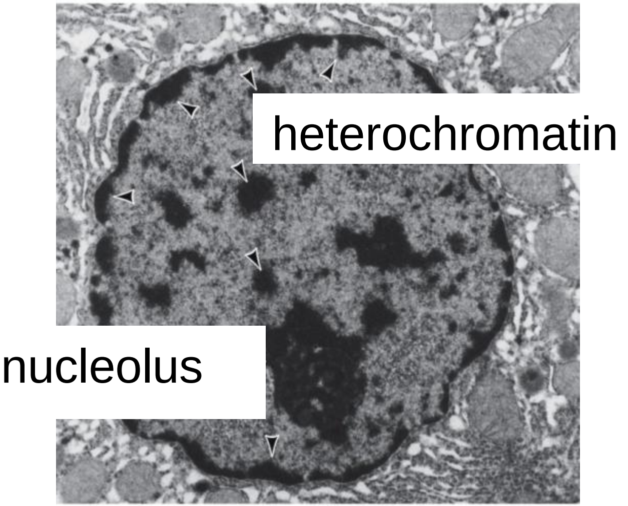
Chromatin

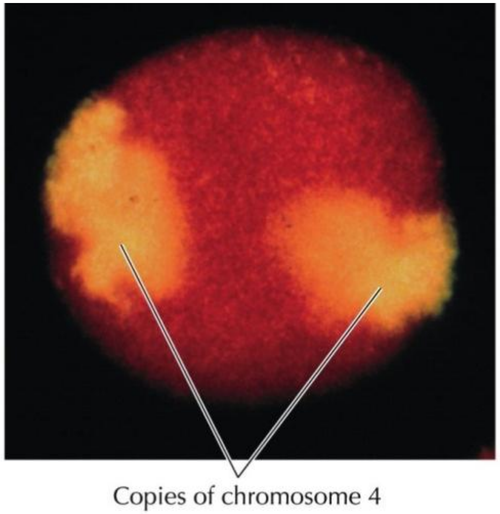


The DNA in the nucleus exists in two forms that reflect the level of activity of the cell. These two forms are referred to as heterochromatin and euchromatin.

Introduction

- Chromatin compaction influences activity of DNA in transcription
- Heterochromatin is highly condensed transcriptionally inactive
- Euchromatin is decondensed and transcriptionally active





Introduction

- Although nucleus lacks membrane-bound subcompartments, the nucleus contains distinct **domains** where specific functions are carried out.
- Examples of nuclear domains include:
  - the **nucleolus** (where rRNA is synthesised and ribosomal subunits are assembled);
  - **chromosomal domains** (which prevent a particular chromosome from becoming entangled with others);
  - the **nucleoskeleton** or the **nuclear matrix** (which provides structural support to the nucleus and helps to organise nuclear functions).

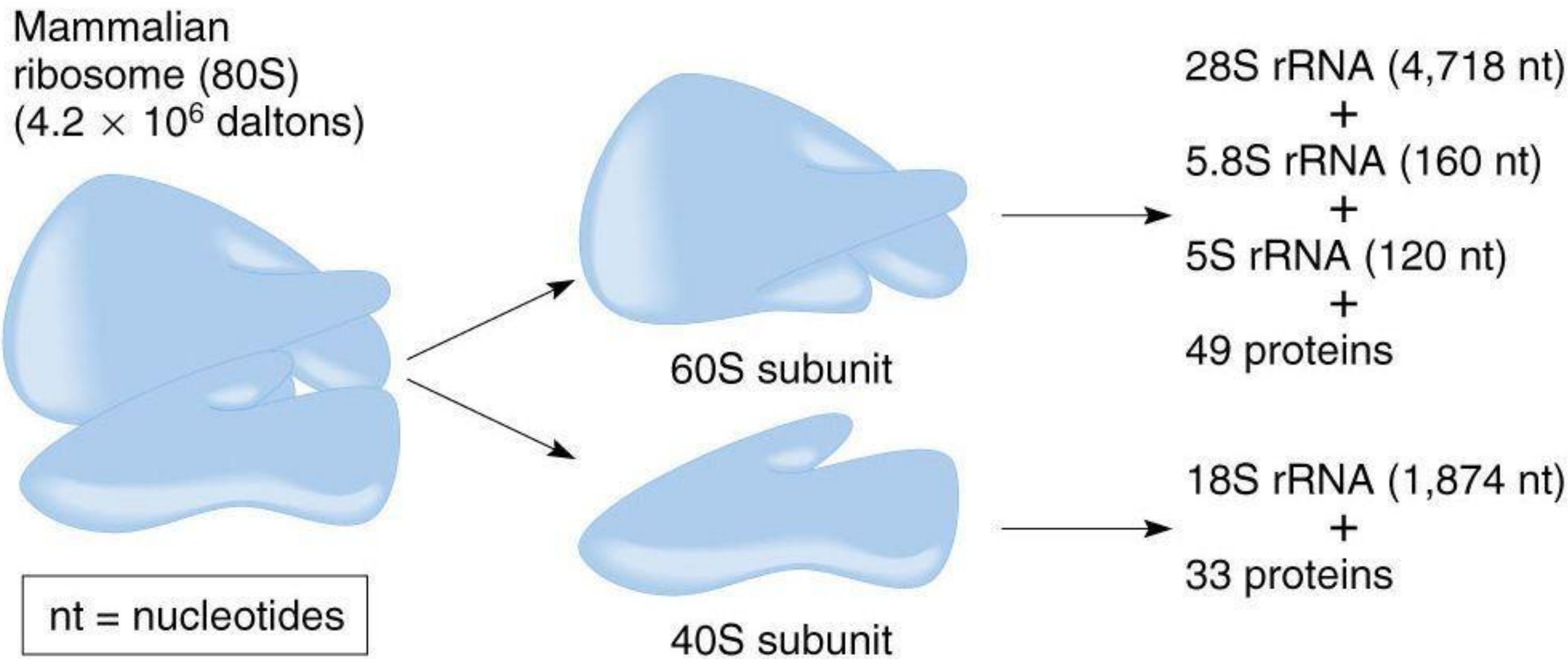
The Nucleolus

- The most noticeable domain of the nucleus is the **nucleolus**.
- Site of rRNA transcription, processing, some aspects of ribosome assembly.
- Most normal cells have a single nucleolus, but multiple nucleoli are sometimes found in cells.
- Ribosomal subunits are assembled in the nucleolus where rRNA is made.
- The size of the nucleolus varies, depending on the amount of ribosome biogenesis in a given cell.

The Nucleolus

- The nucleolus contains all the components needed for the assembly of ribosomal subunits.
- These components include the rRNA genes from multiple chromosomes, rRNAs, enzymes for the synthesis and processing of rRNAs, and ribosomal proteins.
- The ribosomal proteins are imported from the cytoplasm for assembly into the ribosomal subunits.

The Nucleolus

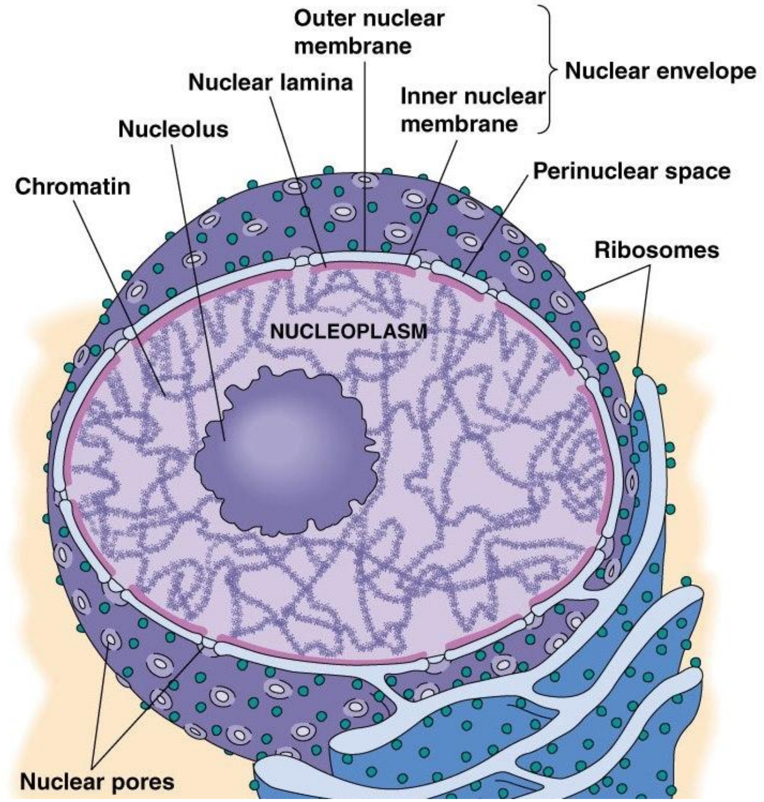


The Nucleolus

- The ribosomal subunits produced in the nucleus are exported through nuclear pore complexes to function during protein synthesis in the cytoplasm.
- The export of the ribosomal subunits is carrier-mediated and requires the Ran GTPase.

Nuclear Compartment

- The **nuclear envelope** is a highly regulated membrane barrier that separates the **nucleoplasm** from the cytoplasm.
- There are three structural components of the nuclear envelope:
  - **outer** and **inner nuclear membranes**
  - **nuclear pores**
  - **nuclear lamina**.
- The **nucleolus**, whose primary function is to assemble ribosomal subunits, is the largest structure in the cell nucleus.



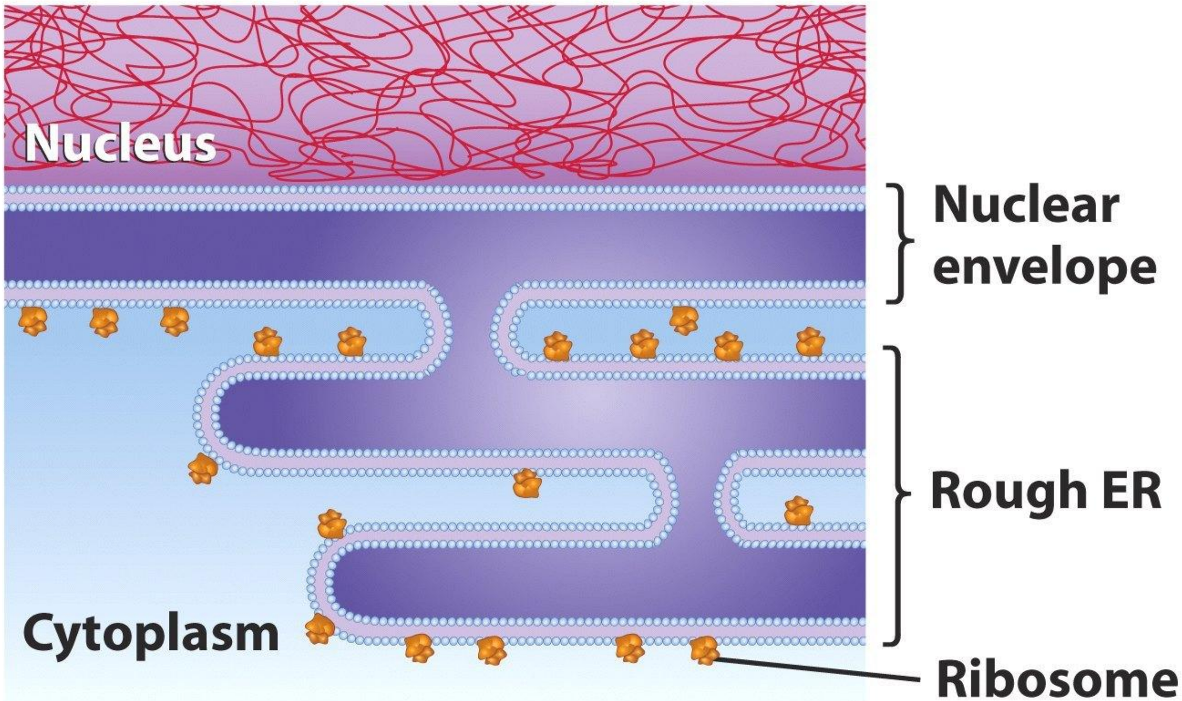
The Nuclear Envelope

- The nucleus is bounded by a special membrane system, the **nuclear envelope**, which defines the nuclear compartment.
- The nuclear envelope is formed from two distinct lipid bilayers: the **outer nuclear membrane** and the **inner nuclear membrane**.
- Like other biological membranes, the nuclear membranes are permeable only to small nonpolar molecules.
- The bilayers of the nucleus are impermeable to larger polar molecules (such as glucose and amino acids), ions, and macromolecules.

The Nuclear Envelope

•The outer nuclear membrane connects the nuclear membranes to the rest of the ER membranes.

•It exhibits the characteristics of the rough ER and is involved in the synthesis of membrane and secreted proteins.



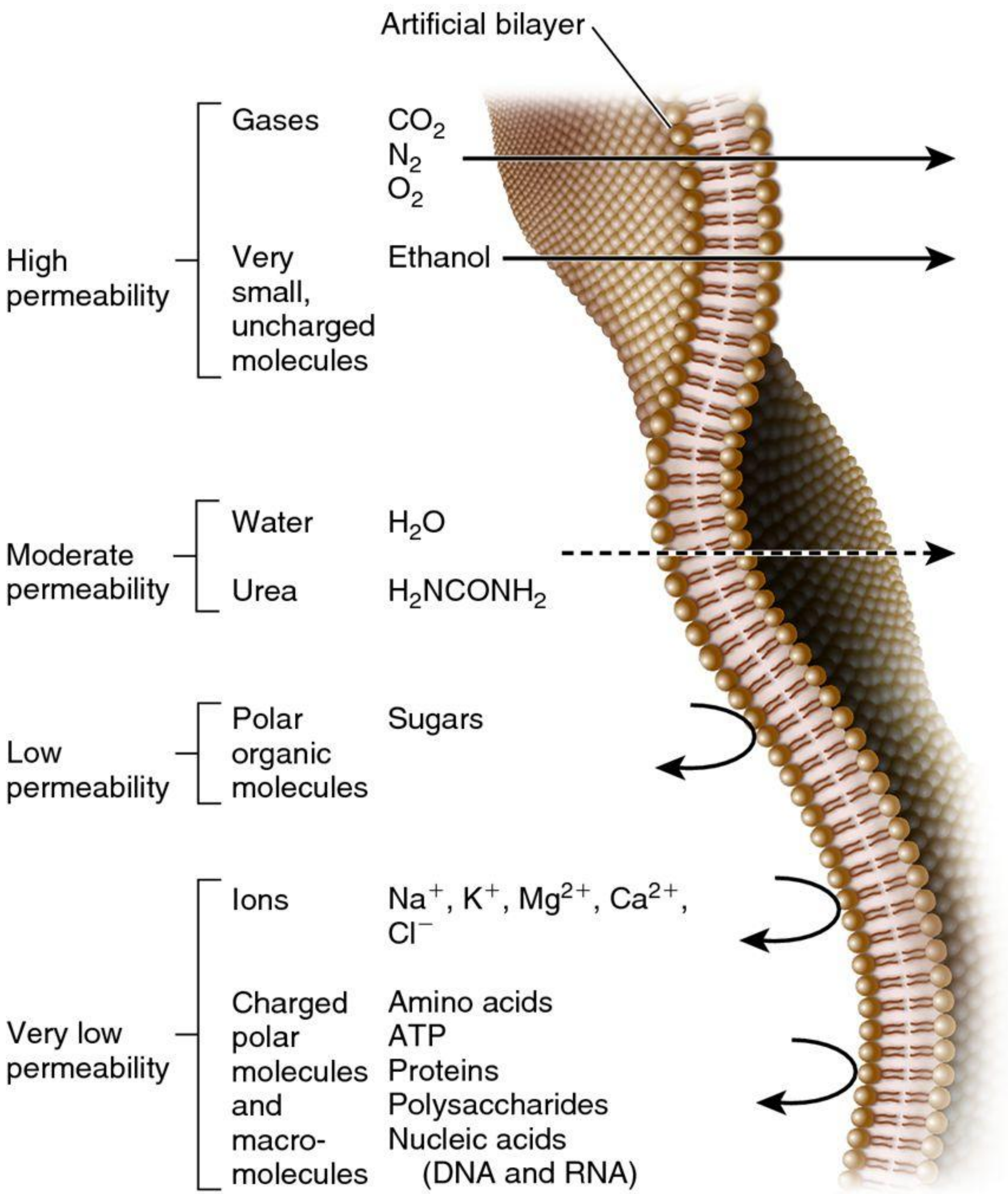
Structure of the Nuclear Membrane

- **Two lipid bilayers:** outer nuclear membrane and inner nuclear membrane.
- The **outer nuclear membrane** is continuous with the membrane of the ER.
- Therefore, the space between the inner and outer nuclear membranes, the **perinuclear space**, is directly connected with the lumen of the ER.
- The outer nuclear membrane has ribosomes bound to its cytoplasmic surface.
- The proteins made on these ribosomes are transported into the perinuclear space.

Remember This?

•Like other cell membranes, the nuclear membranes are phospholipid bilayers, which are permeable only to small nonpolar molecules.

•Other molecules are unable to diffuse through the phospholipid bilayer.



Structure of the Nuclear Membrane

- Different composition of outer nuclear and inner nuclear membrane, with outer nuclear membrane similar to ER membrane.
- The inner nuclear membrane carries special nucleus-specific proteins that provide binding sites for chromatin.
- In contrast to the outer nuclear membrane, the inner nuclear membrane is a domain distinct from ER with a unique set of membrane proteins attaching the nuclear membranes to the intermediate filaments of the lamina and to chromatin.

Structure of the Nuclear Membrane

- The inner nuclear membrane is also in close contact with the **nuclear lamina** that are formed by proteins called **lamins**.
- Lamins are 80 kD proteins related to intermediate filaments of cytoskeleton
- The nuclear lamins provide structural support to the nucleus and participate in the breakdown and reformation of the nuclear envelope during the cell cycle.
- Outer nuclear and inner nuclear membrane are joined at the nuclear pore complex.

Nuclear pore complexes

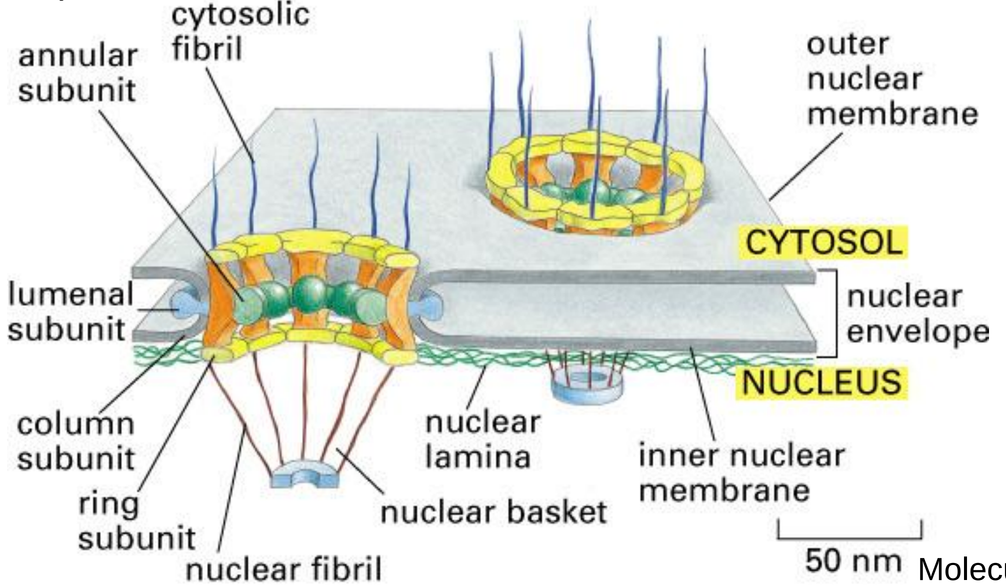
- **nuclear pore complexes** occupy the nuclear pores and they are integrated into the nuclear membranes and the nuclear lamina.
- The nuclear pore complexes are constructed from multiple copies of about 30 protein subunits called **nucleoporins**.
- The nucleoporins make a symmetric structure with an outside diameter of 120 nm and an internal channel 9 nm in diameter.
- The nuclear pore complex controls the passage of molecules into and out of the nucleus.

Nuclear pore complexes

- Number: Average cell has over 4000 NPCs.

- **Channel**: Pathway for free diffusion is 9 nm x 15 nm. Can open to a diameter of more than 25 nm allowing passage of large complexes.

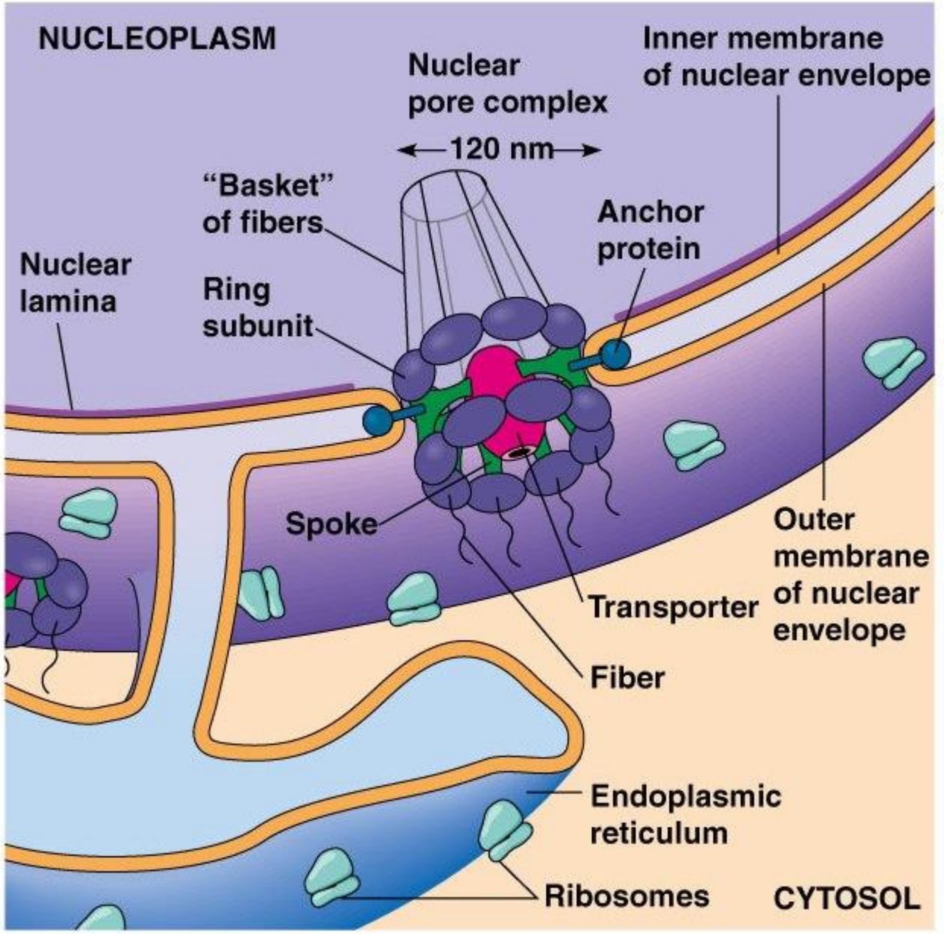
- **Passage across the NPC:**
  - Proteins < 40 kD pass through the NPC by diffusion, but this is often inefficient.
  - Larger proteins and RNA pass through NPC only selectively (Gated Transport).



Molecular Biology of the Cell, Fig. 12-10 1

Nuclear pore complexes

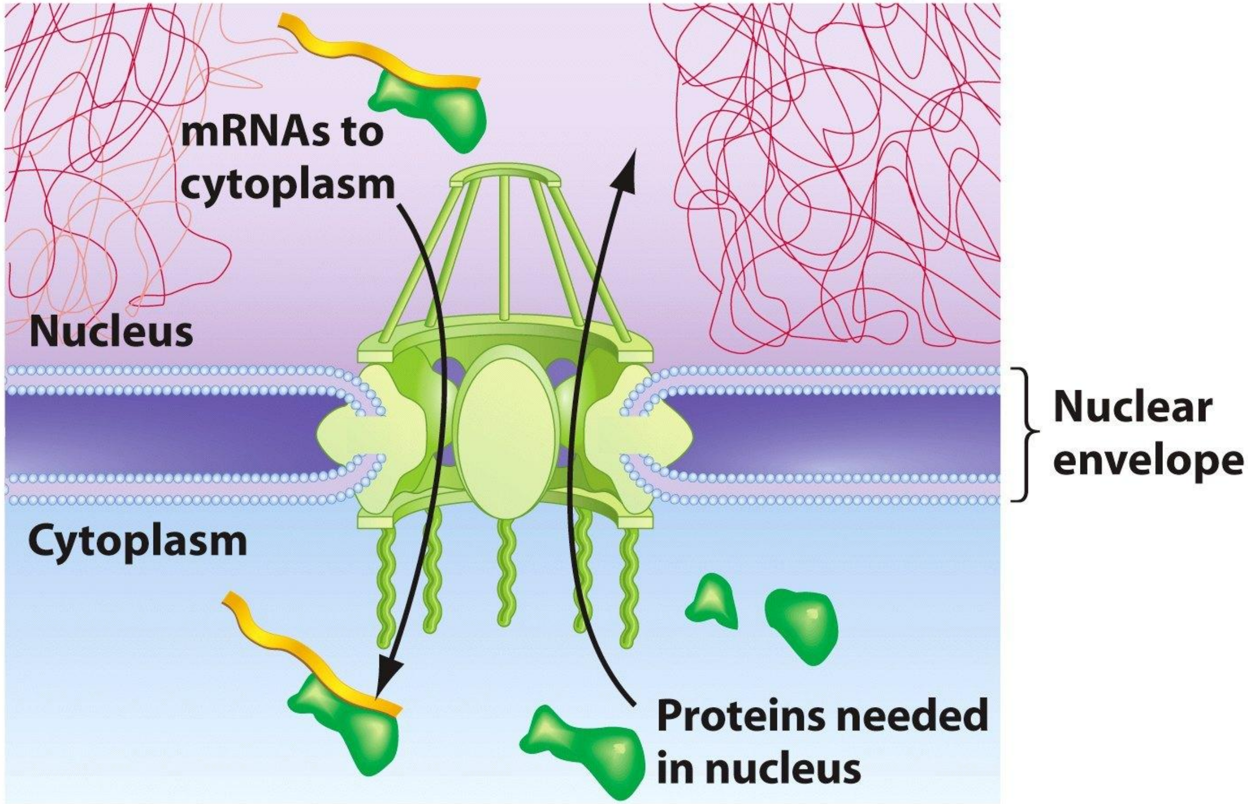
- The nuclear pore complex is a large open octagonal assembly of different proteins (**nucleoporins**).
- The nuclear pore complex is anchored to the outer and inner nuclear membranes by the anchor proteins and the spoke proteins.
- The transporter is the aqueous channel of the nuclear pore complex.
- Adjacent to these structures are the ring subunits, which form the cytoplasmic and nucleoplasmic faces of the complex.
- In addition, fibers protrude from both the cytoplasmic and the nucleoplasmic sides of the complex.
- On the nucleoplasmic side, the fibers meet at a particular point to form a basket-like structure.



Nuclear–Cytoplasmic Traffic

- The nuclear pore complexes are the only channels through which small polar molecules, ions, and macromolecules are able to travel between the nucleus and the cytoplasm.
- Small polar molecules, ions, and proteins below 60 kDa (corresponding to proteins below 9 nm in diameter) are able to pass rapidly through open channels in the nuclear pore complex by **passive diffusion**.
- Larger macromolecules, however, are transported by a **receptor-mediated**, **energy-dependent** mechanism that acts mainly to import proteins above 60 kDa to the nucleus and export mRNAs to the cytoplasm.

Nuclear–Cytoplasmic Traffic



The nuclear proteins include:

(i) **structural proteins** of the nuclear lamina, **nucleoskeleton**, and **chromatin**

(ii) **DNA and RNA polymerases**;

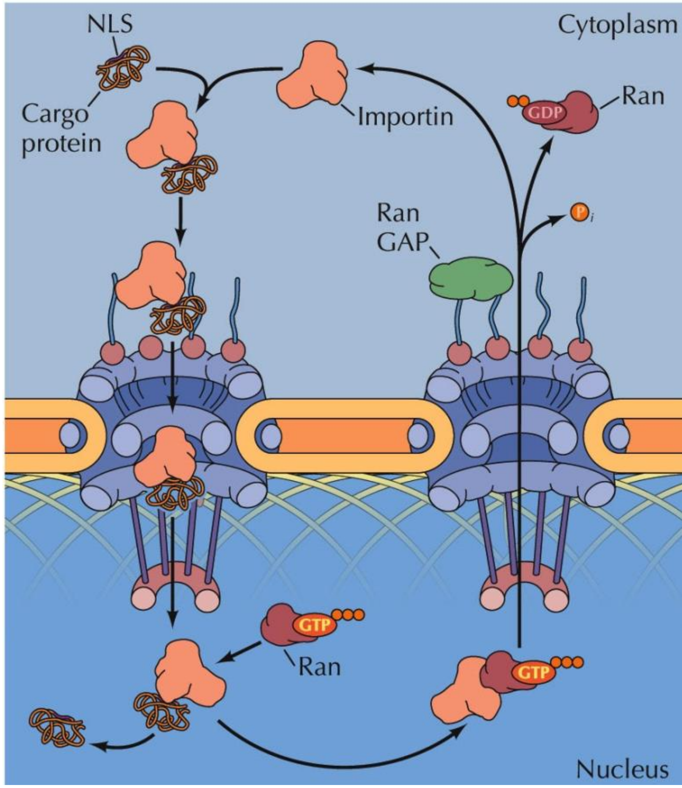
(iii) gene regulatory proteins.

Nuclear–Cytoplasmic Traffic

- Nuclear proteins contain sequence information required for their import into the nucleus.
- A **nuclear localisation signal** (**NLS**) is often a short stretch of **amino acids**.
- Receptors for nuclear import (also referred to as **importins**) are soluble, cytoplasmic proteins that bind to the NLS of cargo proteins.
- Interactions between nuclear import receptors and nucleoporins are critical for protein entry into the nucleus through the nuclear pore.

Nuclear–Cytoplasmic Traffic

- **Import of proteins to nucleus**:
- NLS recognized by **nuclear transport receptors – importins**
- Activity of nuclear transport receptors regulated by **Ran**, a GTP-binding protein
- Importins bind cargo at NLS sequence
- Move through nuclear pore
- Ran-GTP unloads and takes importin out.

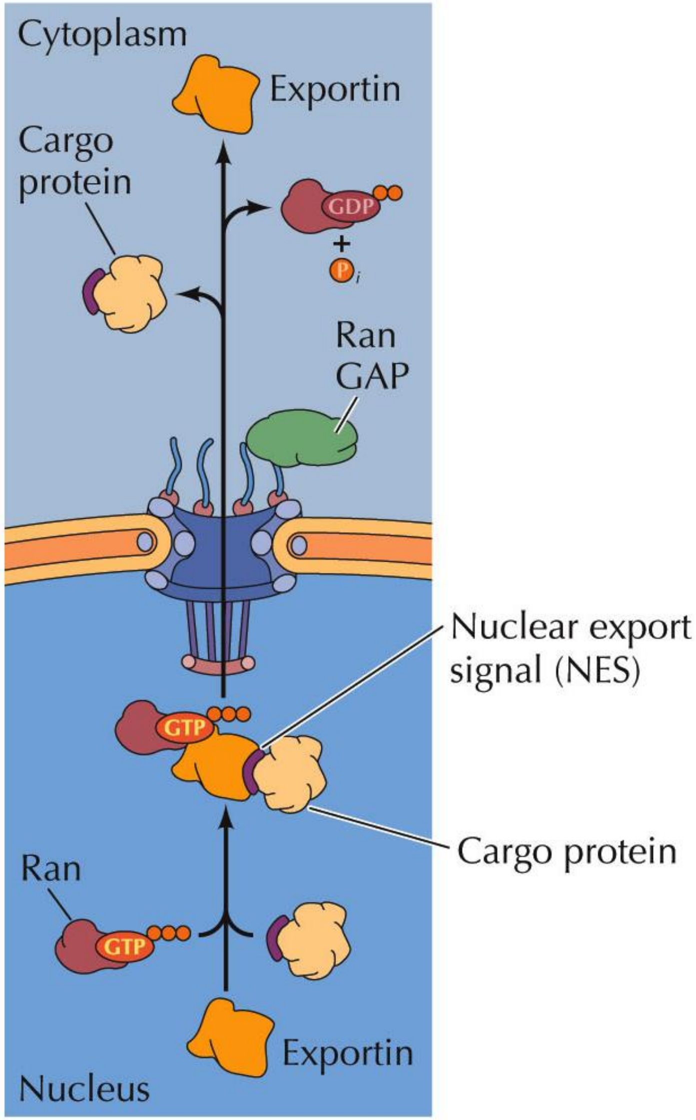


Nuclear–Cytoplasmic Traffic

- Short stretches of amino acids rich in leucine act as the most common **nuclear export signals (NESs)**
- Proteins that shuttle between the nucleus and the cytoplasm often contain both NLSs and NESs.
- A nuclar export receptor (**exportin**) binds protein cargoes that contain NESs in the nucleus and transports them to the cytoplasm.
- Nuclear export receptors also interact with the proteins of nuclear pore complexes.

Nuclear–Cytoplasmic Traffic

- **Nuclear export signals (NES):**
- Required for proteins targeted for export
- Signals recognized by **exportins** to direct transport to cytoplasm
- Less well characterized than NLS
- Ran also required for nuclear export
- Ran/GTP promotes binding of export and their cargo proteins,
- Ran/GTP dissociates complexes between importins and cargos



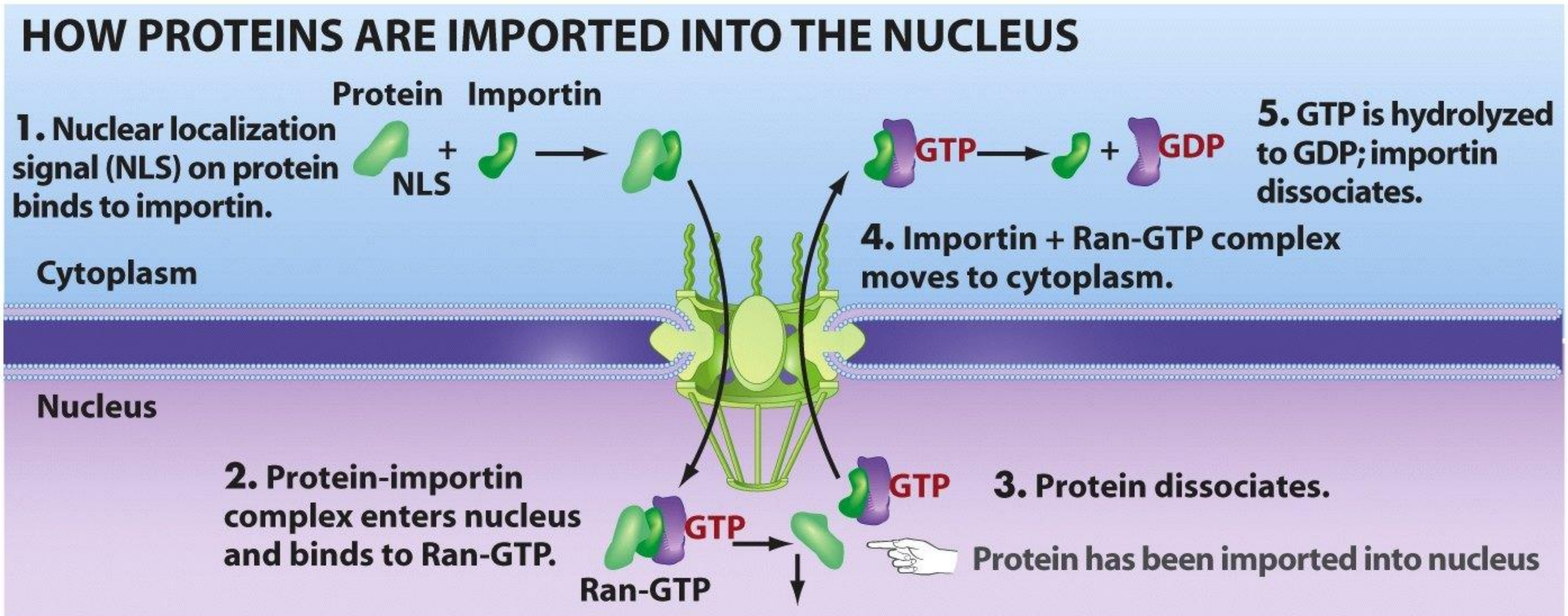
Nuclear–Cytoplasmic Traffic

- **Ran** is a small GTPase (*i.e.* an enzyme that can bind and hydrolyse GTP) that is common to all eukaryotes.
- Ran controls nuclear transport by binding nuclear import and export receptors and affecting their ability to bind their protein cargos.
- Ran is found both in the nucleus (as bound to GTP) and in the cytoplasm (as bound to GDP).
- The asymmetric distribution of Ran–GDP in the cytoplasm and Ran–GTP in the nucleus ensures that proteins are transported only in the proper **direction**.

Nuclear–Cytoplasmic Traffic

- **Ran–GTP** interacts with an importin carrying its cargo to cause the dissociation of the protein cargo from the importin in the nucleus.
- In contrast, **Ran–GTP** interacts cooperatively with an exportin and its cargo to mediate the formation of an export complex in the nucleus.
- The nuclear transport receptors return to their compartment of origin after delivering the cargoes.

Nuclear–Cytoplasmic Traffic



Nuclear–Cytoplasmic Traffic

Protein Import Through the Nuclear Pore Complex

1. A protein destined for import (the cargo) carries a nuclear localisation signal (NLS) which is recognised and bound by a cytoplasmic receptor protein (importin).
2. The soluble importin/cargo complex makes multiple contacts with nucleoporins which facilitate the transport of the complex through the nuclear pore.
3. The imported cargo is released in the nucleus after binding Ran–GTP.

Protein Export Through the Nuclear Pore Complex

1. A protein destined for export (the cargo) carries a nuclear export signal (NES) which is recognised and bound by a nuclear receptor protein (exportin).
2. The soluble exportin/cargo complex also binds Ran–GTP in the nucleus before interacting with nucleporins.
3. The exported cargo is released in the cytoplasm when the GTP is hydrolysed to GDP.

Nuclear–Cytoplasmic Traffic of RNAs

Messenger RNAs are exported from the nucleus as RNA-protein complexes

- All mRNAs produced in the nucleus function in the cytoplasm and must be exported.
- The same nuclear pore complexes used for protein transport are also used for mRNA export.
- Like protein transport, mRNA export is also receptor-mediated and energy-dependent.
- Unlike proteins, mRNAs are exported as RNA–protein complexes and do not require exportins or the Ran GTPase.
- mRNA export is dependent on the action of several novel transport factors.

Ribosomal subunits are assembled in the nucleolus and exported by exportin 1

- Ribosomal subunits are assembled in the nucleolus where rRNA is made.

- Ribosomal proteins are imported from the cytoplasm for assembly into the ribosomal subunits.

- Export of the ribosomal subunits is carrier-mediated and requires Ran.

tRNAs are exported by a dedicated exportin

- Exportin-t is the transport receptor for tRNAs.
- tRNA export requires Ran.
- tRNA export may be affected by modifications of the tRNAs.
- tRNAs may be re-imported into the nucleus.

Summary

- The nucleus is a defining feature of eukaryotic cells and contains all the cell's chromosomes. Chromosomes occupy distinct territories in the nucleus.
- Nuclei vary in appearance according to cell type and organism.
- The nucleus contains subcompartments called domains that are not membrane-bounded.
- The nucleus is bounded by a double membrane that is perforated by NPCs, the only channels for molecular trafficking into and out of the nucleus.
- Small molecules and macromolecules smaller than 60 kDa can diffuse through the NPCs, but transport of larger macromolecules requires specific signals.
- Most nuclear transport is mediated by importins and exportins, which recognise the specific signals and also interact with the NPCs.
- Many proteins are imported into the nucleus, and those that shuttle are also exported.
- Ran GTPase controls the directionality and irreversibility of nuclear transport.
- mRNAs are exported from the nucleus as RNA–protein complexes. mRNA export requires several novel factors.
- Ribosomal subunits are assembled in the nucleolus and exported by multiple recepors.

Extra reading:

p704-712

Chapter 12

*Figures are mainly obtained from Molecular Biology of the Cell, 5th Ed.