The Nucleus

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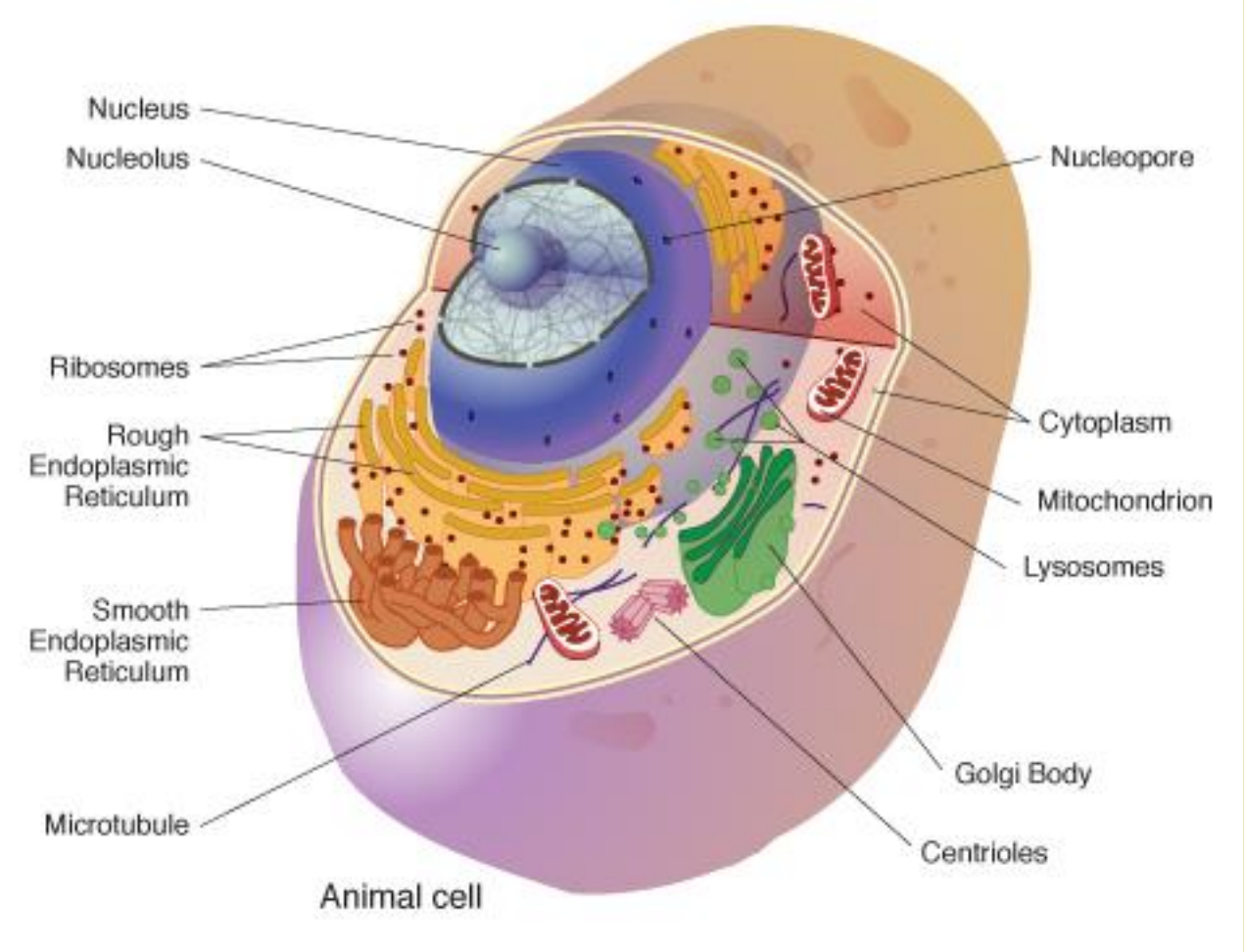
Cell biology

- A branch of biology that studies the different structures and functions of the cell and focuses on the cell as the basic unit of life.
- Cell biology explains the structure and organization of the organelles, their physiological properties, metabolic processes, signalling pathways, cell cycle, and interactions with their environment.
- Cell biology is studied at both the microscopic and molecular level



geneed.nlm.nih.gov

ILOs and Textbook

- Understand the structure of the nucleus and the nuclear membrane.
- Know the nuclear lamina diseases
- Textbook:

The Cell: A Molecular Approach by Cooper, 6th edition

- Chapter 9

Introduction

- The nucleus distinguishes eukaryotic from prokaryotic cells
- Houses the cell's genome and reserves the genetic information
- Acts as a cell's control center
- 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

The Nuclear Envelope and Traffic between the Nucleus and the Cytoplasm

- 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

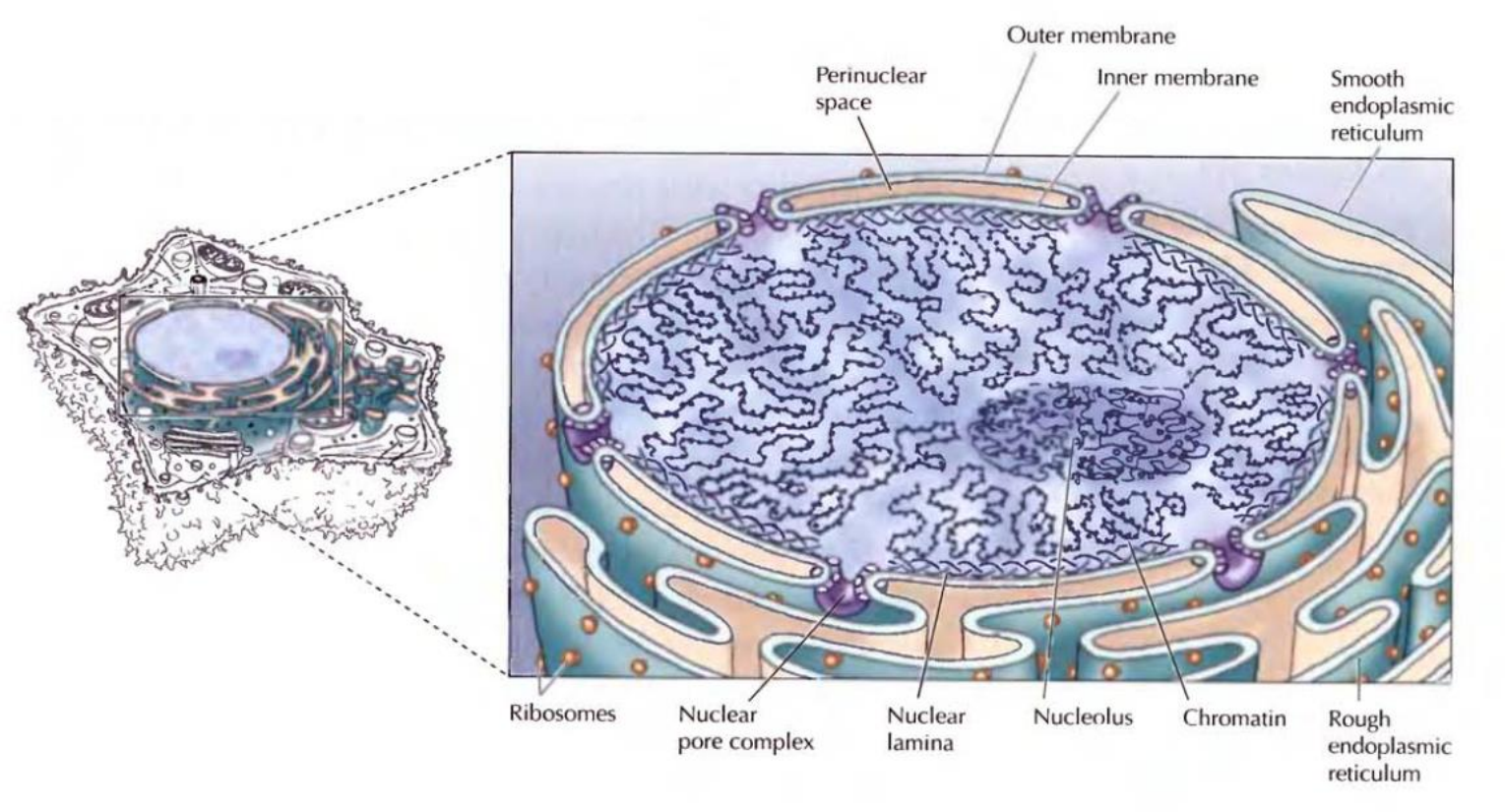
- Components:

1. Two membranes

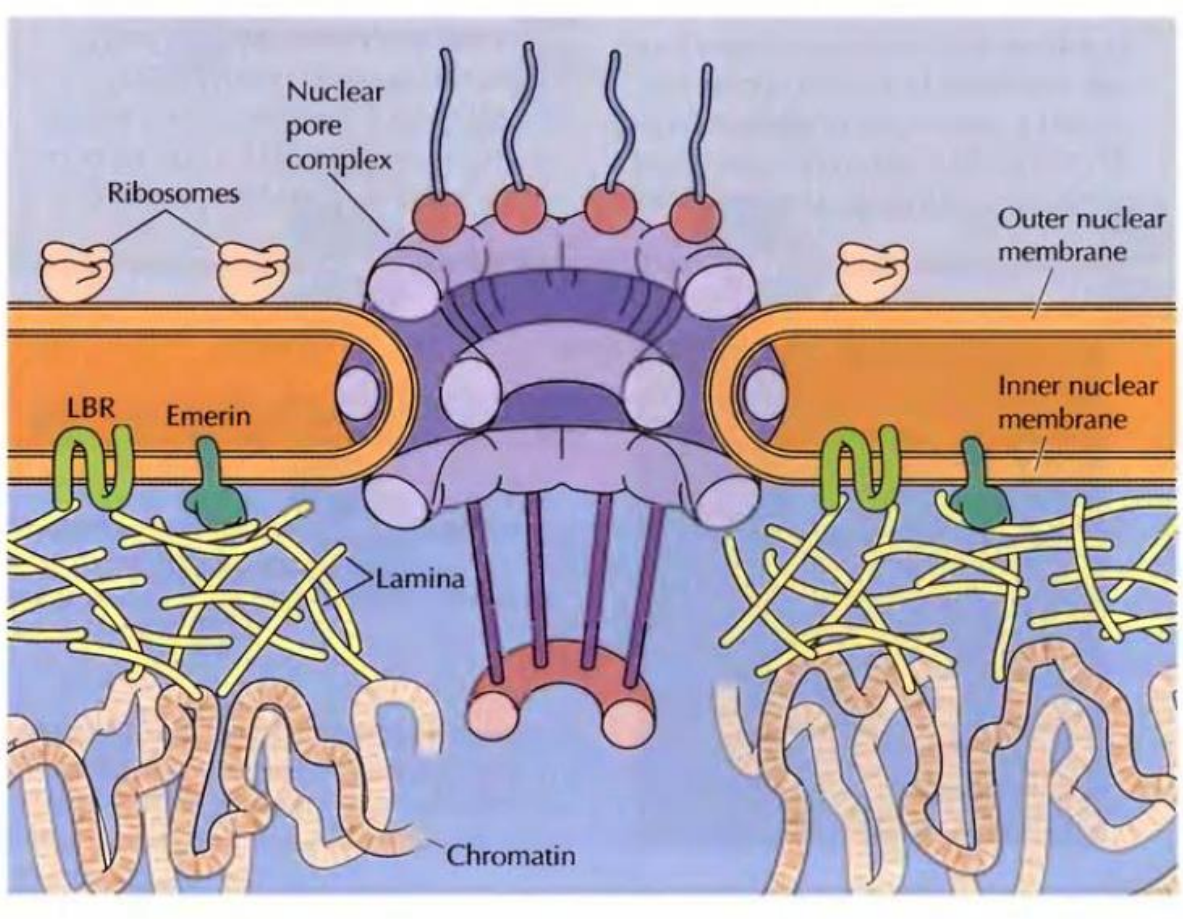
A. **Outer**: continuous with the ER and ribosomes are bound to Its cytoplasmic surface.

Has proteins that bind the cytoskeleton but not those that give the tubular ER structure

B. **Inner**



The Nuclear Envelope Structure



- Components:

2. An underlying **nuclear lamina** (a fibrous structural meshwork composed of lamins and other associated proteins such as, emerin, LBR, LINC complexes, histones and other chromosomal proteins)

3. **Nuclear pore complexes** where the inner and outer nuclear membranes join together

FIGURE 9.5 The nuclear lamina
The inner nuclear membrane contains several integral proteins, such as emerin and the lamin B receptor (LBR) that interact with nuclear lamins. The lamins also interact with chromatin.

Nuclear Lamina structure and organization

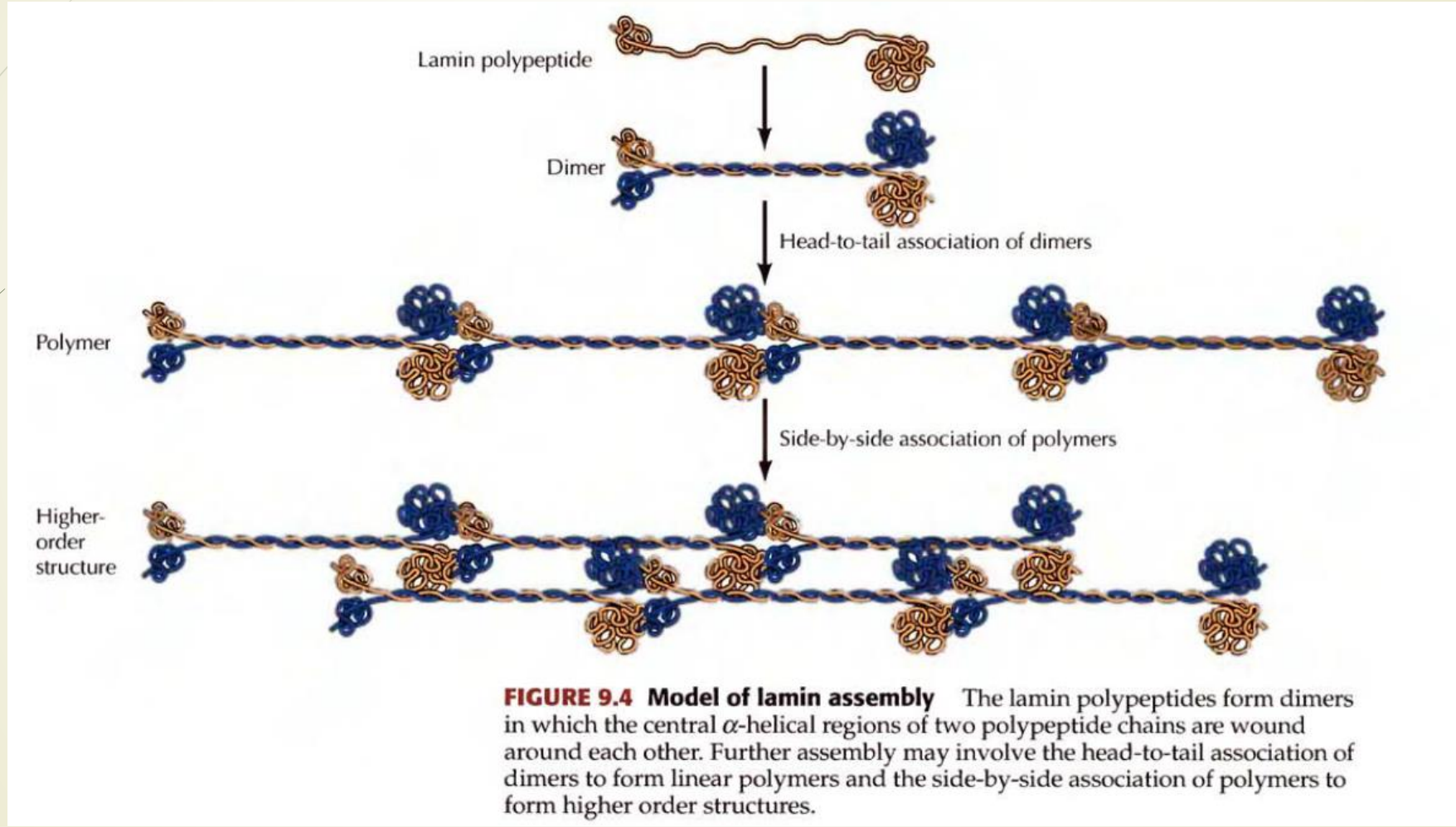
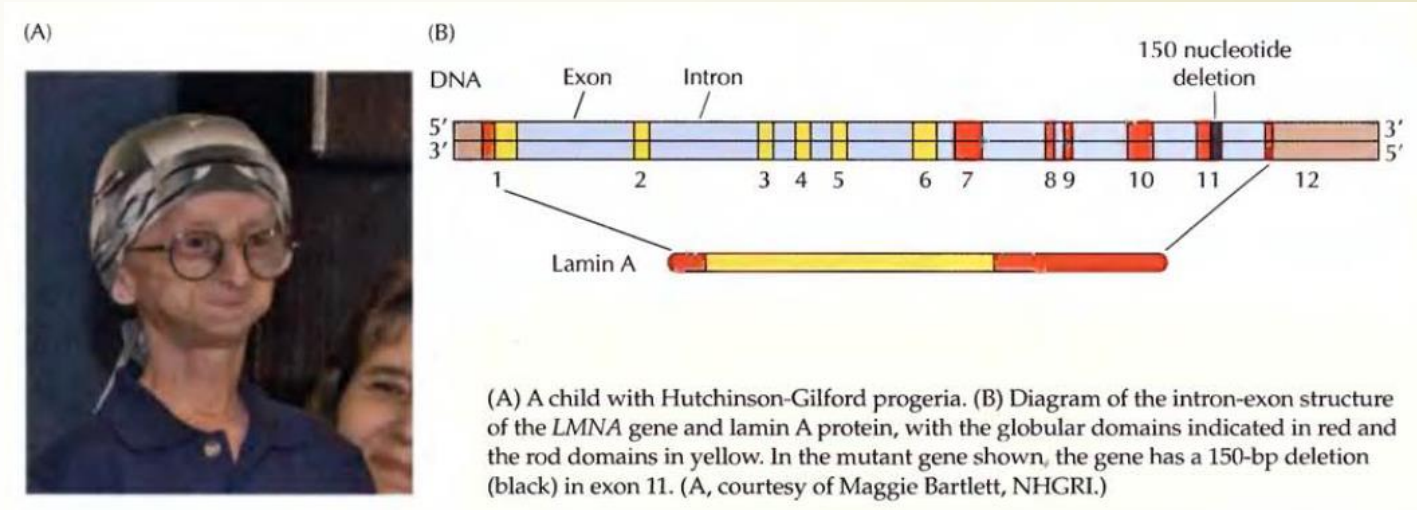


FIGURE 9.4 Model of lamin assembly The lamin polypeptides form dimers in which the central α-helical regions of two polypeptide chains are wound around each other. Further assembly may involve the head-to-tail association of dimers to form linear polymers and the side-by-side association of polymers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

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
- 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-Marie-Tooth disorder type 2B1, Hutchinson-Gliford progeria



(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.)

The nuclear pore complex

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

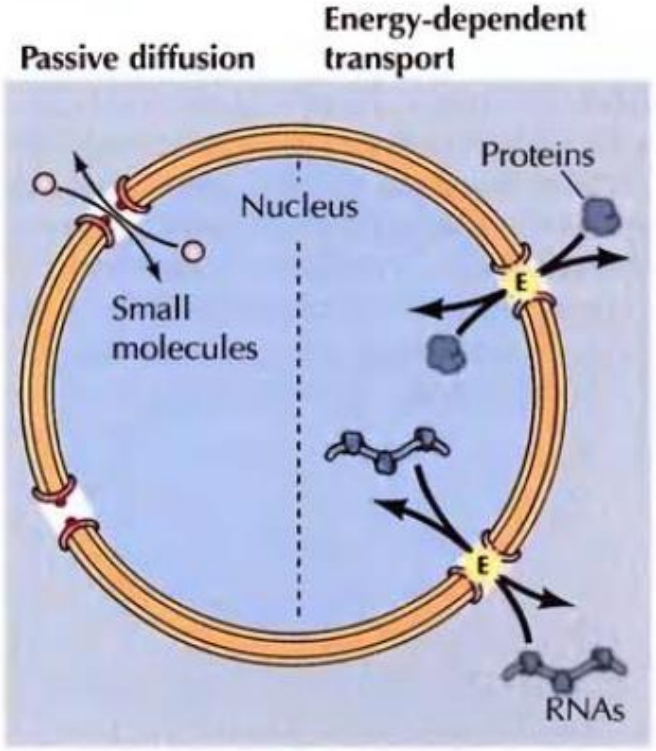


FIGURE 9.6 Molecular traffic through nuclear pore complexes
Small molecules are able to pass rapidly through open channels in the nuclear pore complex by passive diffusion. In contrast, macromolecules (proteins and RNAs) are transported by a selective, energy-dependent mechanism.

The nuclear pore complex

- **Structure**: eightfold symmetry spokes with a large central channel
- Cytoplasmic and nuclear filaments
- The eightfold spokes are anchored within the nuclear envelope at the sites of fusion between the inner and outer nuclear membranes

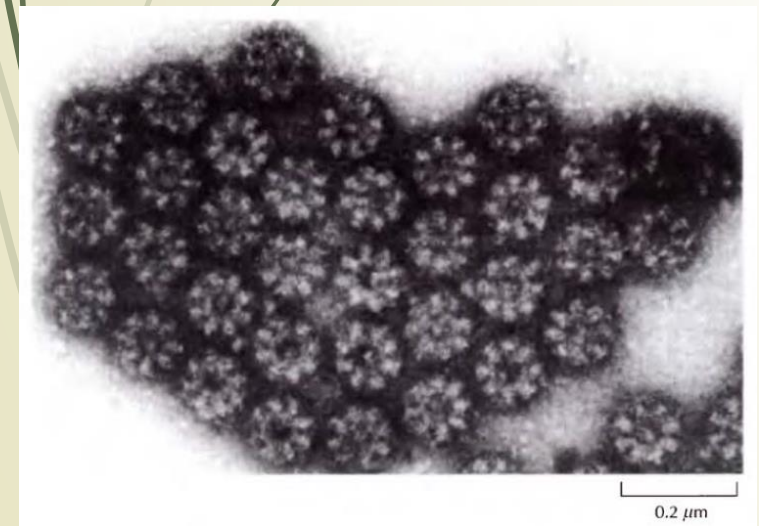
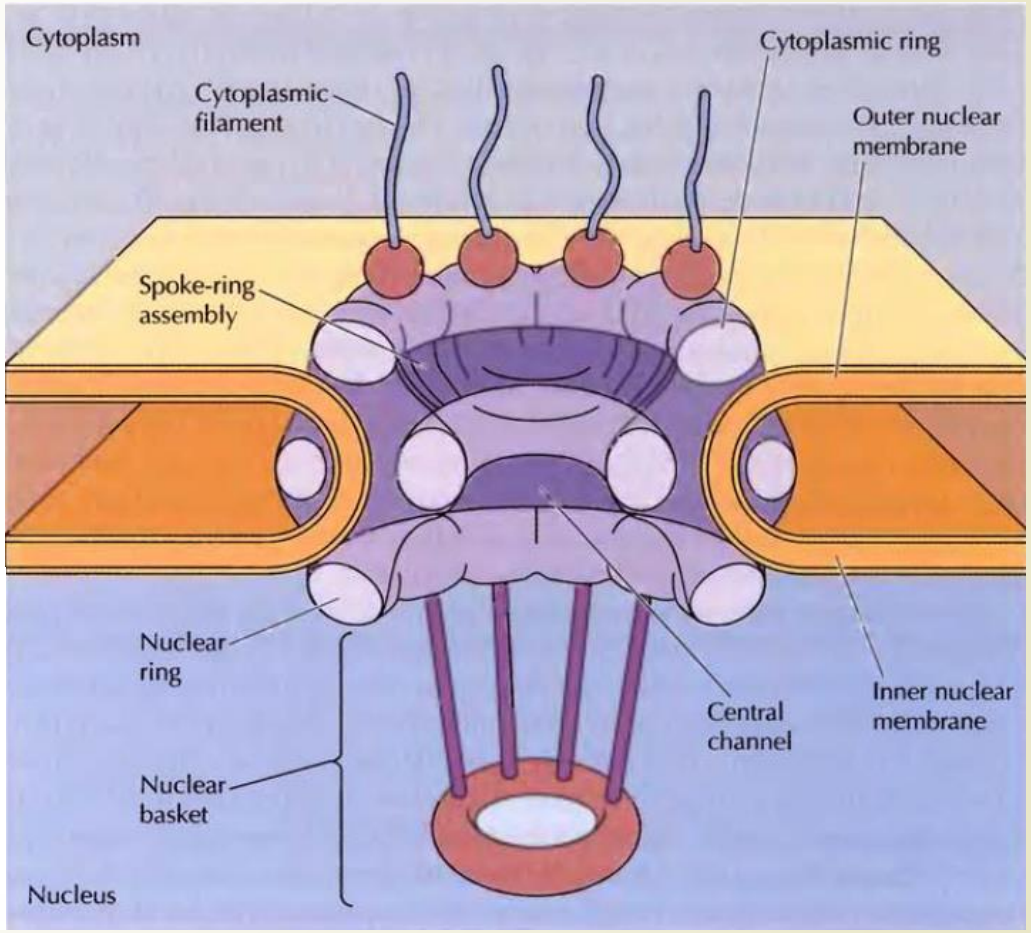


FIGURE 9.8 Model of the nuclear pore complex The complex consists of an assembly of eight spokes attached to rings on the cytoplasmic and nuclear sides of the nuclear envelope. The spoke-ring assembly surrounds a central channel. Cytoplasmic filaments extend from the cytoplasmic ring, and filaments forming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



Selective transport of proteins to and from the nucleus

- Nuclear localization signals **(NLS)**
- NLS are recognized by nuclear transport receptors that direct cargo proteins to the nuclear pore complex
- Bipartite NLS are more common
- Basic or classical NLS
- Some NLS are far apart and depend on protein folding

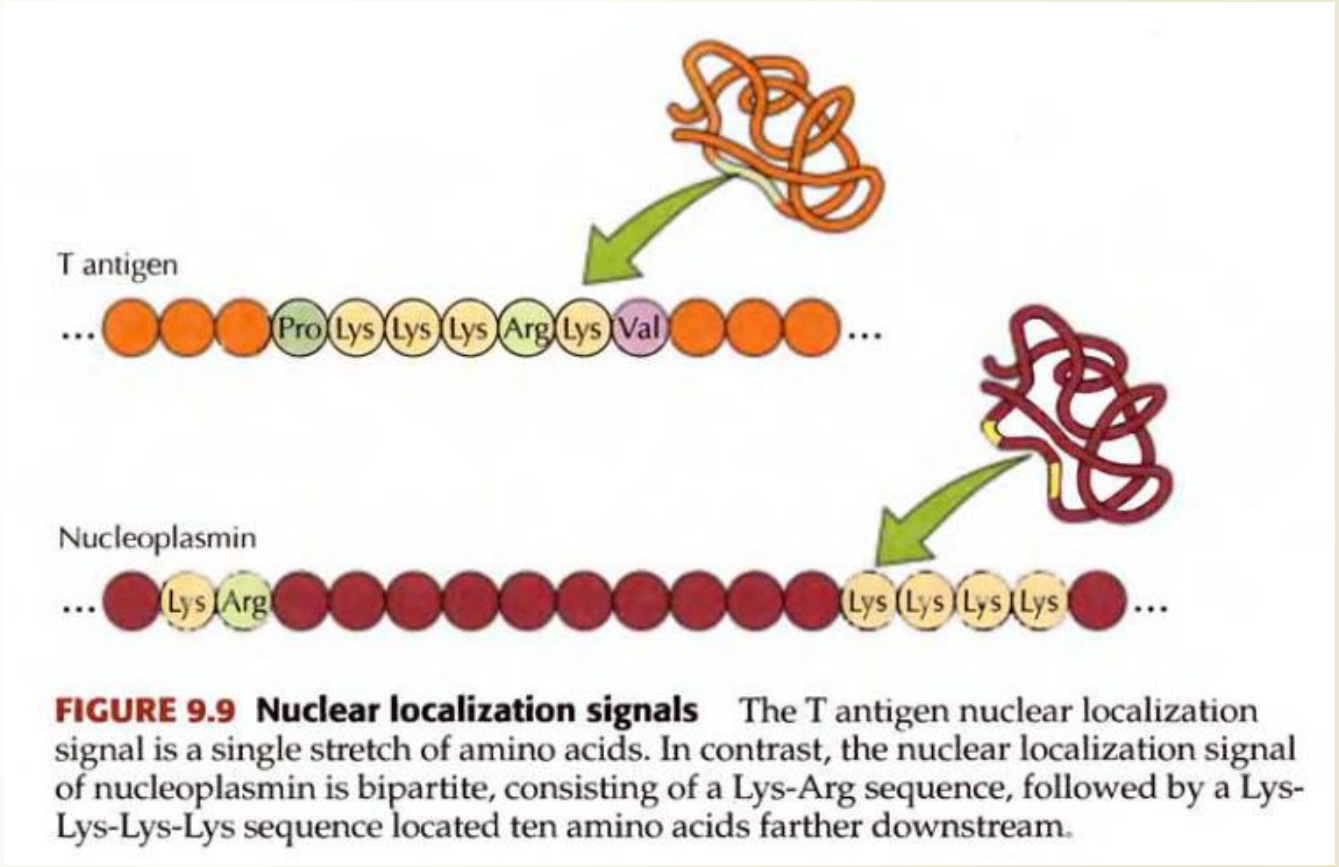


FIGURE 9.9 Nuclear localization signals The T antigen nuclear localization signal is a single stretch of amino acids. In contrast, the nuclear localization signal of nucleoplasmin is bipartite, consisting of a Lys-Arg sequence, followed by a Lys-Lys-Lys-Lys sequence located ten amino acids farther downstream.

Directional movement through the nuclear pore complex

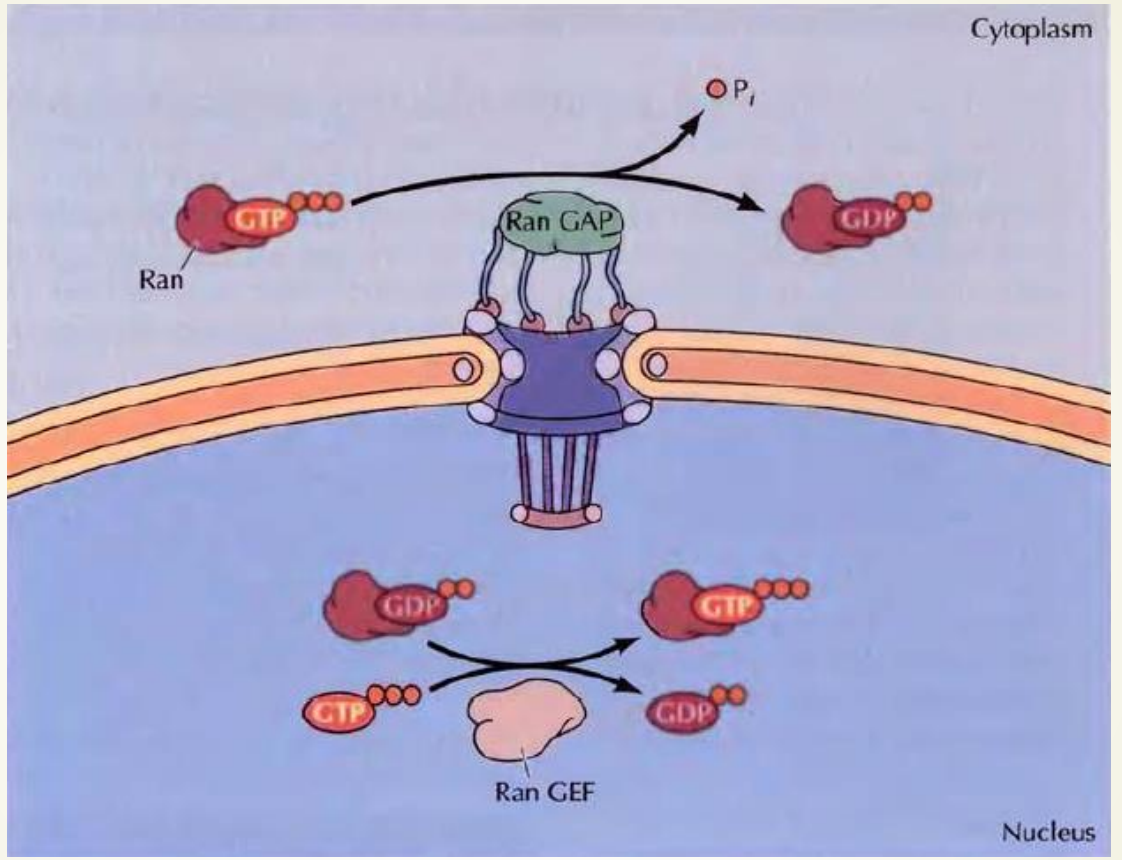


FIGURE 9.10 Distribution of Ran/GTP across the nuclear envelope An unequal distribution of Ran/GTP across the nuclear envelope is maintained by the localization of Ran GTPase-activating protein (Ran GAP) in the cytoplasm and Ran guanine nucleotide exchange factor (Ran GEF) in the nucleus. In the cytoplasm, Ran GAP (which is bound to the cytoplasmic filaments of the nuclear pore complex) stimulates the hydrolysis of GTP bound to Ran, leading to the conversion of Ran/GTP to Ran/GDP. In the nucleus, Ran GEF stimulates the exchange of GDP bound to Ran for GTP, leading to the conversion of Ran/GDP to Ran/GTP. Consequently, a high concentration of Ran/GTP is maintained within the nucleus.

Resources

- http://sites.sinauer.com/cooper6e/animation0901.html

Protein import through the nuclear pore complex

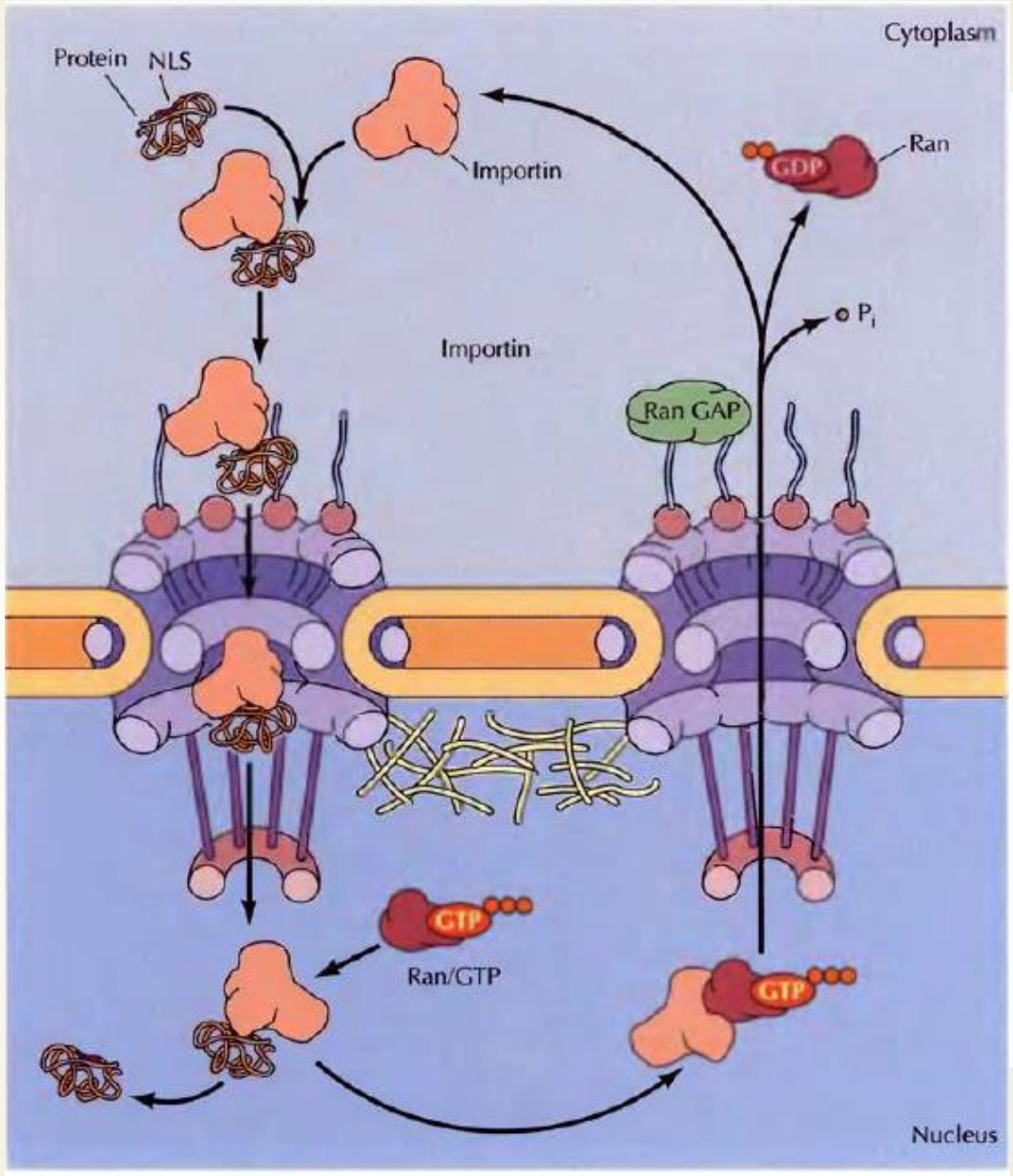


FIGURE 9.11 Protein import through the nuclear pore complex
Transport of a protein through the nuclear pore complex begins when its nuclear localization sequence (NLS) is recognized by an importin nuclear transport receptor. The cargo (the protein with the nuclear localization sequence)/importin complex 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

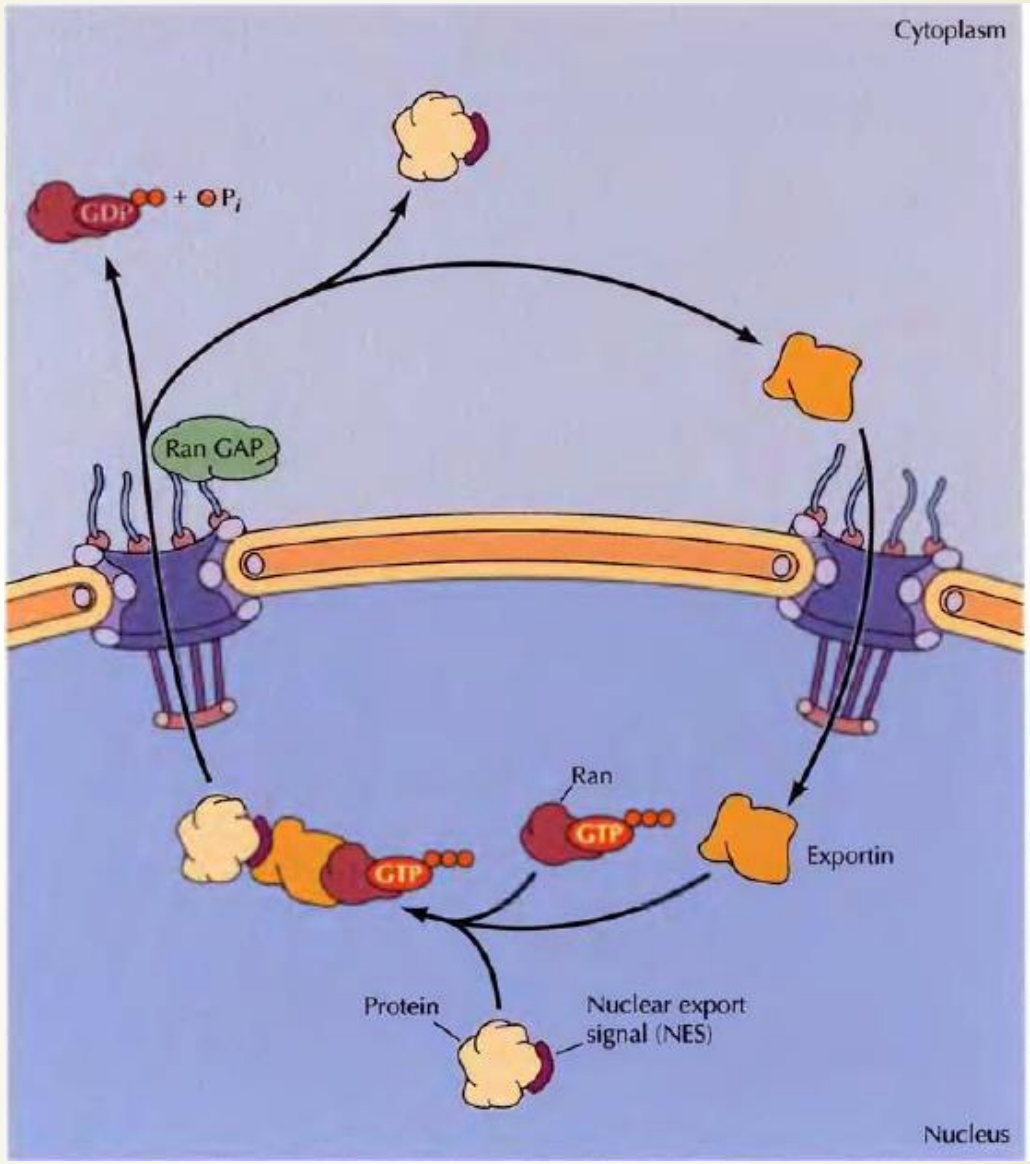


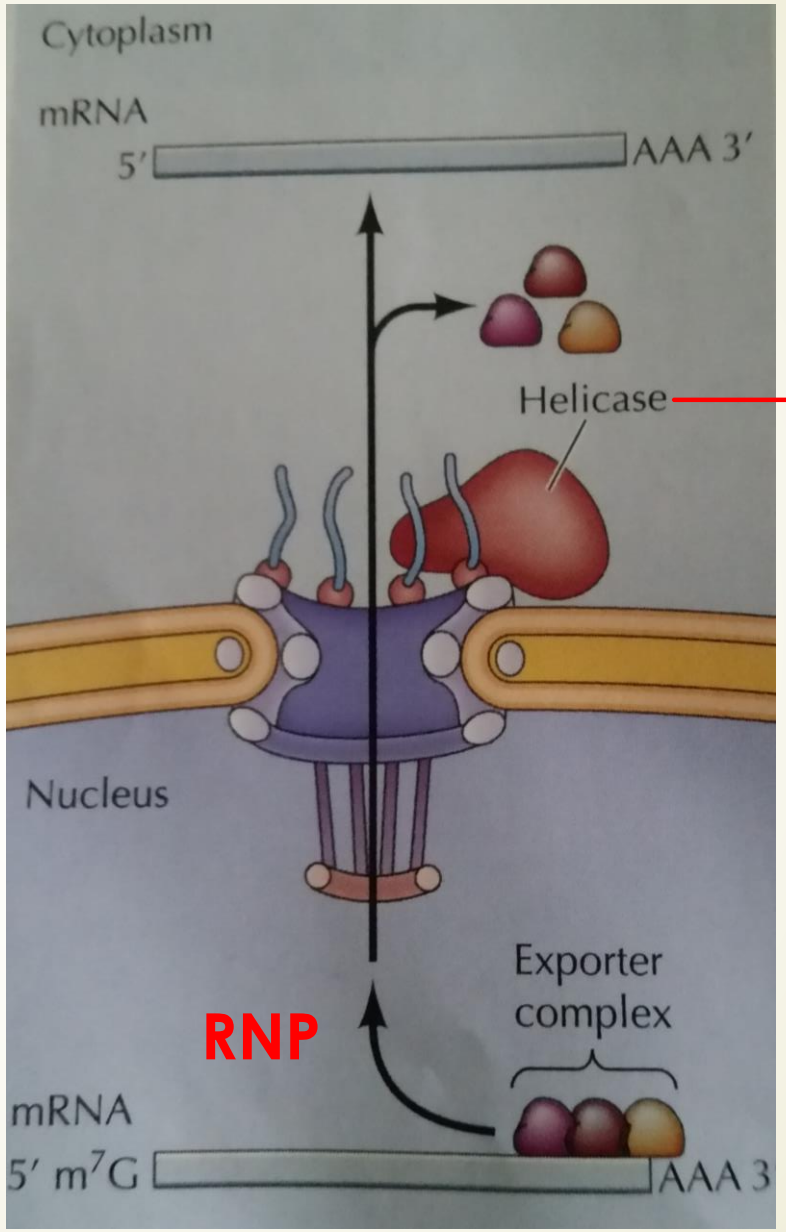
FIGURE 9.12 Nuclear export Complexes between cargo proteins bearing nuclear export signals (NES), exportins, and Ran/GTP form in the nucleus. Following transport through the nuclear pore complex, Ran GAP stimulates the hydrolysis of bound GTP, leading to formation of Ran/GDP and release of the cargo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

Regulation of nuclear protein import

Mechanisms of nuclear protein import regulation:

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

mRNA Export

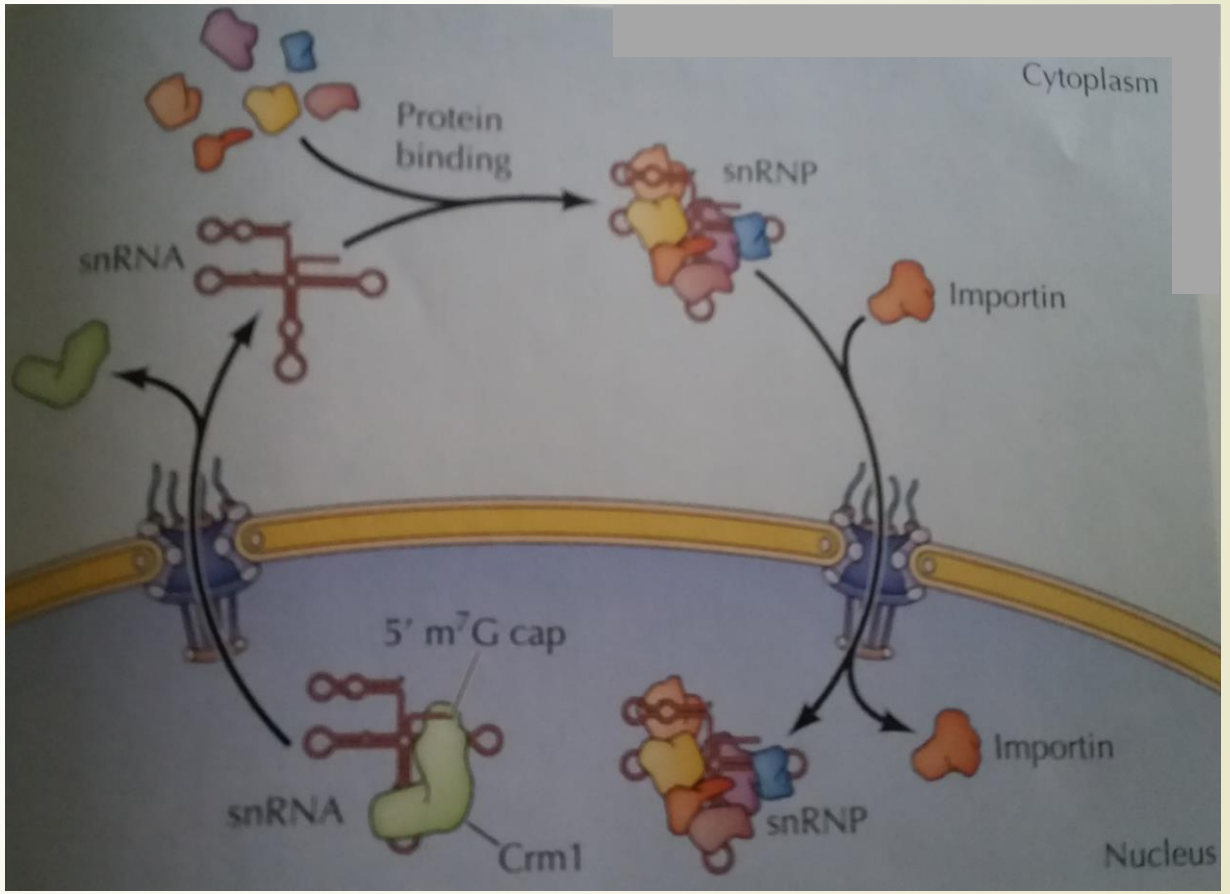


Directionality

Exporter complex is recruited to pre-mRNA

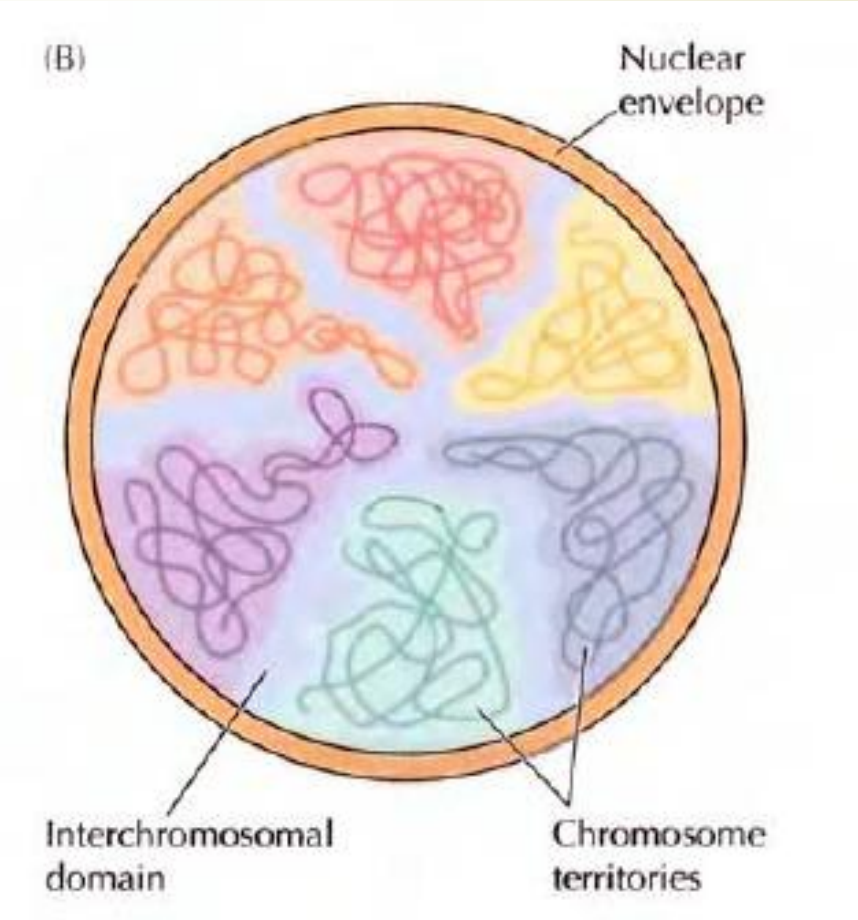
snRNA transport between nucleus and cytoplasm

- Components of RNA processing machinery



Chromosome organization

- Chromosomes do not randomly wind around one another but they occupy discrete territories within the nucleus
- RNA processing and transport occur in interchromosomal domains

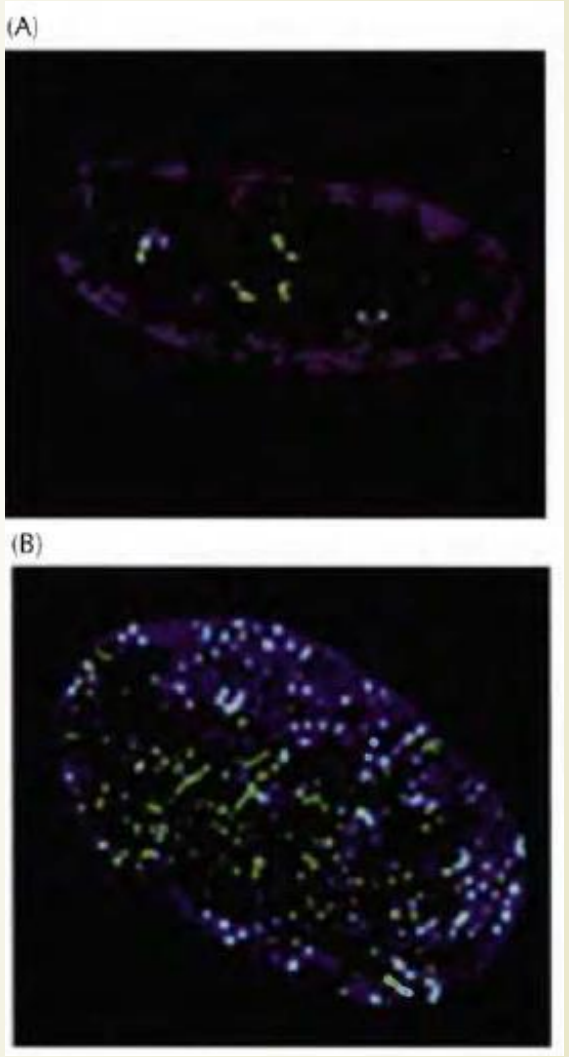


Chromosome organization and gene expression

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

The functional internal organization of the nucleus

- Sub-compartments or 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.



Examples on nuclear bodies

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

Nucleolus is a nuclear body

- Has no surrounding membrane
- Is associated with chromosomal regions that contain about 200 copies of the genes for 5.8S, 18S and 28S rRNAs to synthesize large amounts of ribosomes.
- 5.8S, 18S and 28S rRNAs genes transcribed as a single unit by polymerase I in the nucleolus.
- **Functions**:

1. rRNA synthesis

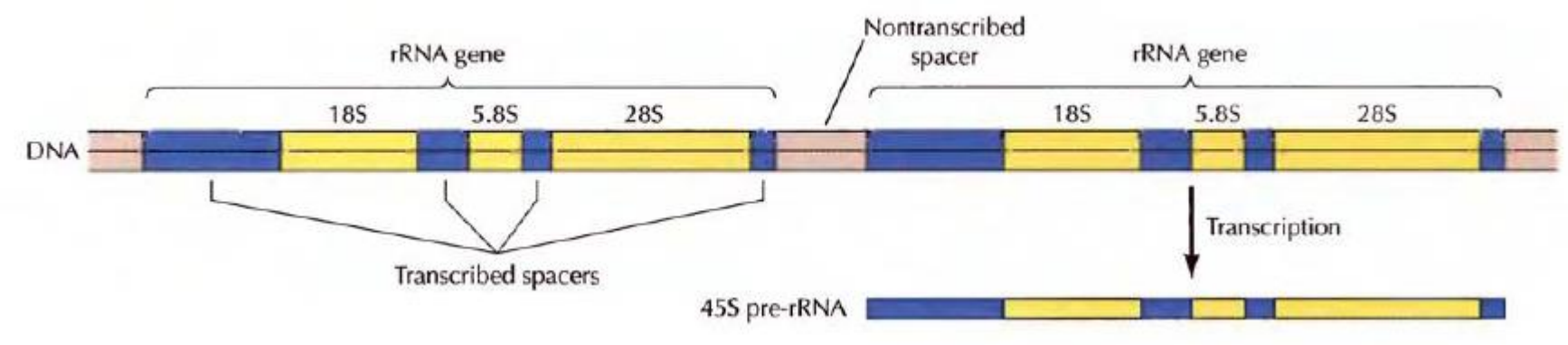


FIGURE 9.25 Ribosomal RNA genes Each rRNA gene is a single transcription unit containing the 18S, 5.8S, and 28S rRNAs as well as transcribed spacer sequences. The rRNA genes are organized in tandem arrays, separated by nontranscribed spacer DNA.

Nucleolus is a nuclear body

- **Functions**:

1. rRNA synthesis
2. Ribosome production
3. RNA modification and assembly of ribonucleoprotein particles
4. Small RNA production such as tRNA, snRNA, RNase P RNA (the catalytic part of the tRNA processing enzyme), SRP (targets proteins to ER)
5. Cell division and response to stress

snoRNAs

- Localized RNAs to the nucleolus
- They complex with proteins to form snoRNPs
- Function:

1. Pre-rRNA processing like splicesomes of pre-mRNA by cleavage of pre-rRNA into 5.8S, 18S and 28S products.
2. Base pairing with pre-rRNA (they contain ~15 nucleotides complementary to pre-rRNA) to target it by the enzymes that catalyse base modification

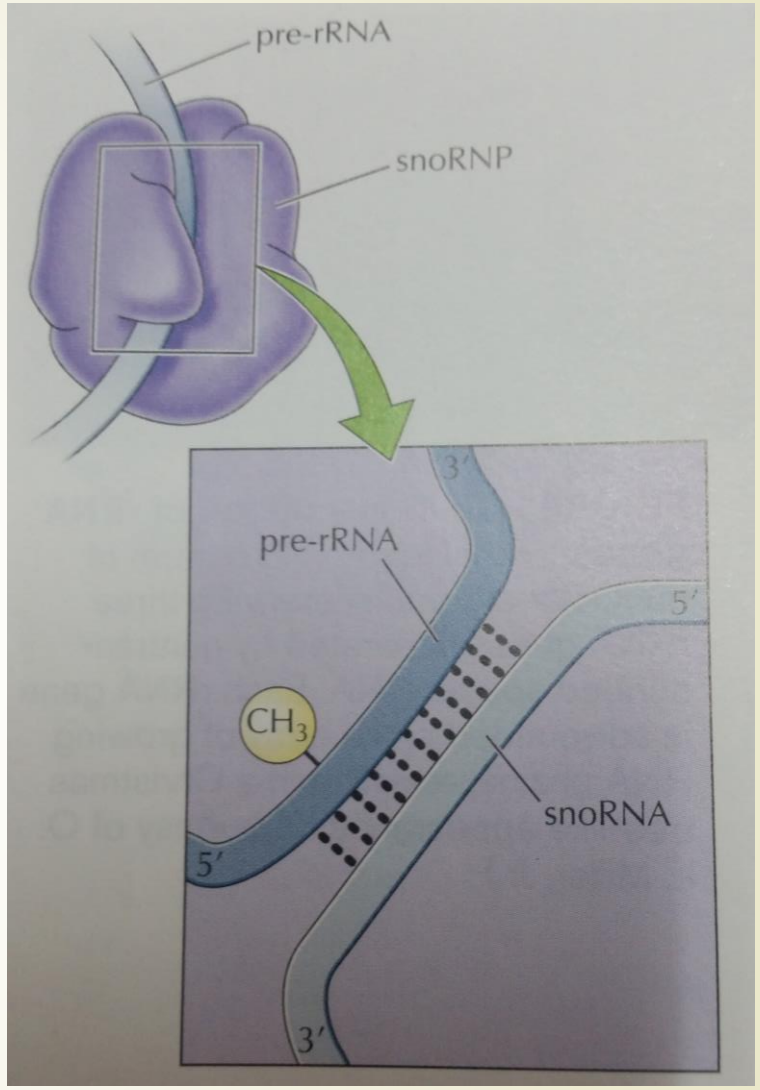


FIGURE 9.30 Role of snoRNAs in base modification of pre-rRNA The snoRNAs contain short sequences complementary to rRNA. Base pairing between snoRNAs and pre-rRNA targets the enzymes that catalyze base modification (e.g., methylation) to the appropriate sites on pre-rRNA.

Transcription and processing of rRNA

- Processing of pre-rRNA involves:

1. Cleavage within the external transcribed spacer (ETS) near the 5'end
2. Removal of the ETS at the 3'end
3. Base modification (methylation of ribose and some bases)

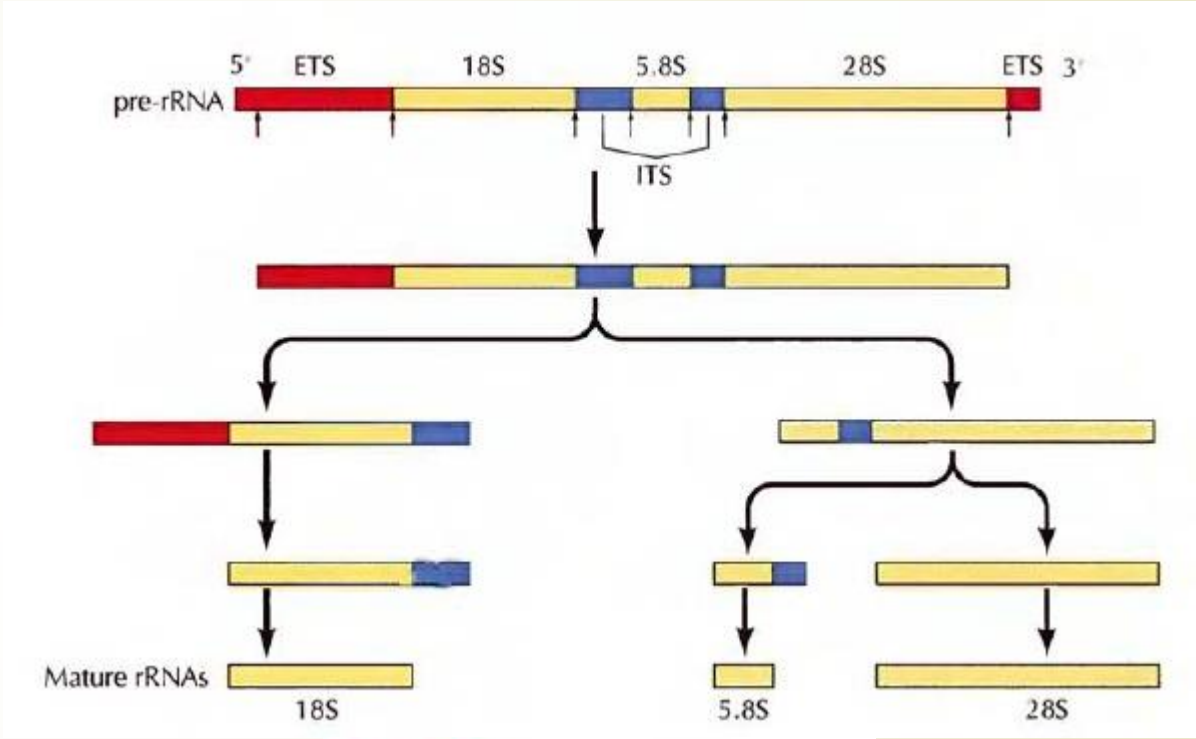


FIGURE 9.29 Processing of pre-rRNA The higher eukaryote 45S pre-rRNA transcript contains external transcribed spacers (ETS) at both ends and internal transcribed spacers (ITS) between the sequences of 18S, 5.8S, and 28S rRNAs. The pre-rRNA is processed via a series of cleavages to yield the mature rRNA species.

Ribosome assembly

- Assembly of precursor RNA, both ribosomal proteins and 5S rRNA
- Steps:
- Ribosomal proteins are transcribed outside the nucleolus by polymerase II then translated in the cytoplasm.
- 5S rRNA is transcribed outside the nucleolus by polymerase III
- Ribosomal proteins associate with rRNA while pre-rRNA is being synthesized

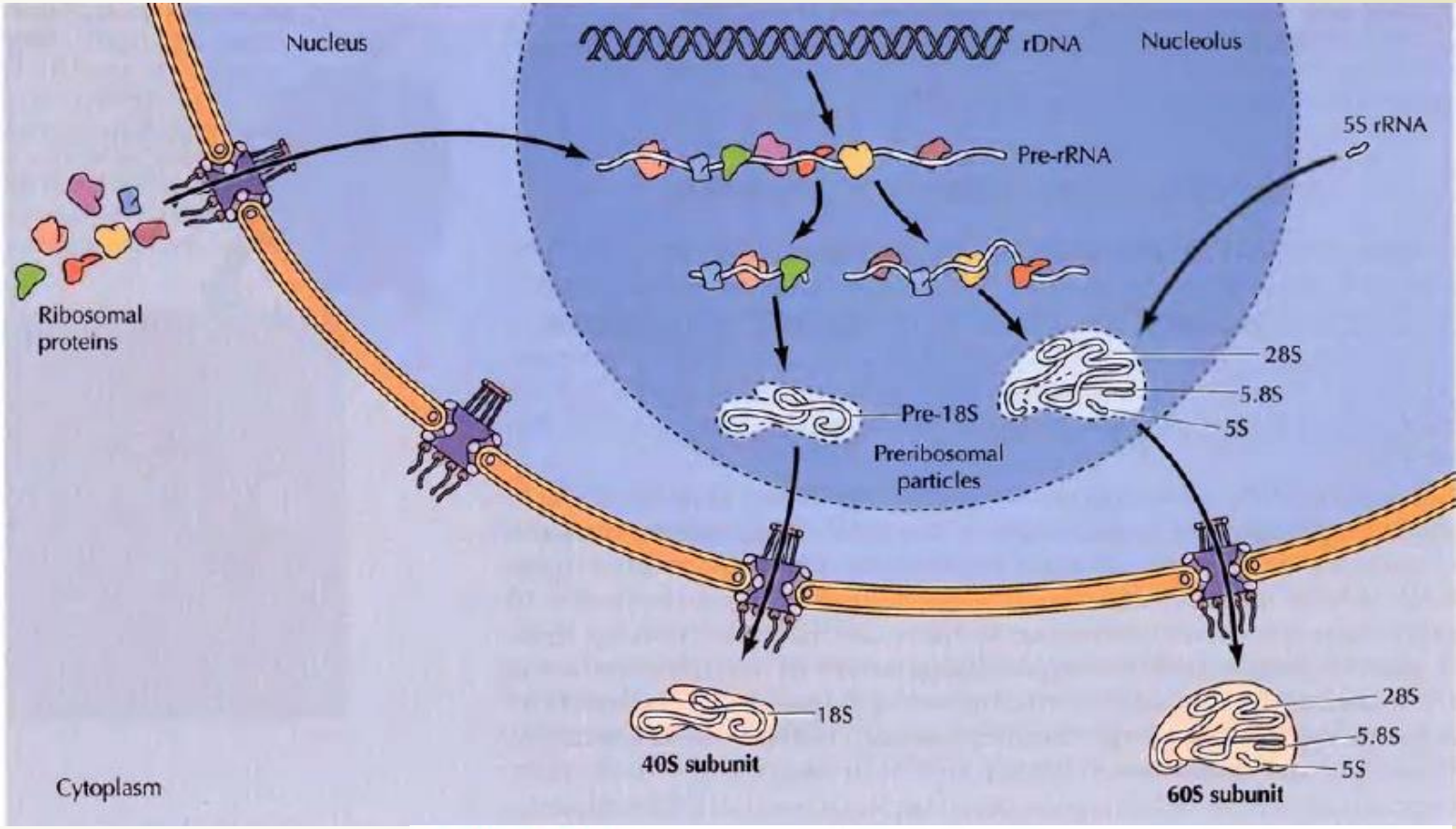


FIGURE 9.31 Ribosome assembly Ribosomal proteins are imported to the nucleolus from the cytoplasm and begin to assemble on pre-rRNA prior to its cleavage. As the pre-rRNA is processed, additional ribosomal proteins and the 5S rRNA (which is synthesized elsewhere in the nucleus) assemble to form preribosomal particles. The final steps of maturation follow the export of preribosomal particles to the cytoplasm, yielding the 40S and 60S ribosomal subunits.

Ribosome assembly

- Steps continued:
- Small subunit processing is simpler by 4 endonuclease cleavages in the nucleus
- Large subunit (28S, 5.8S and 5 rRNA) processing is more complex with extensive nuclease cleavage in the nucleolus
- **Ribosomal subunit maturation**: pre-ribosomal particles are exported to the cytoplasm to form the active 40S and 60S subunits of ribosomes