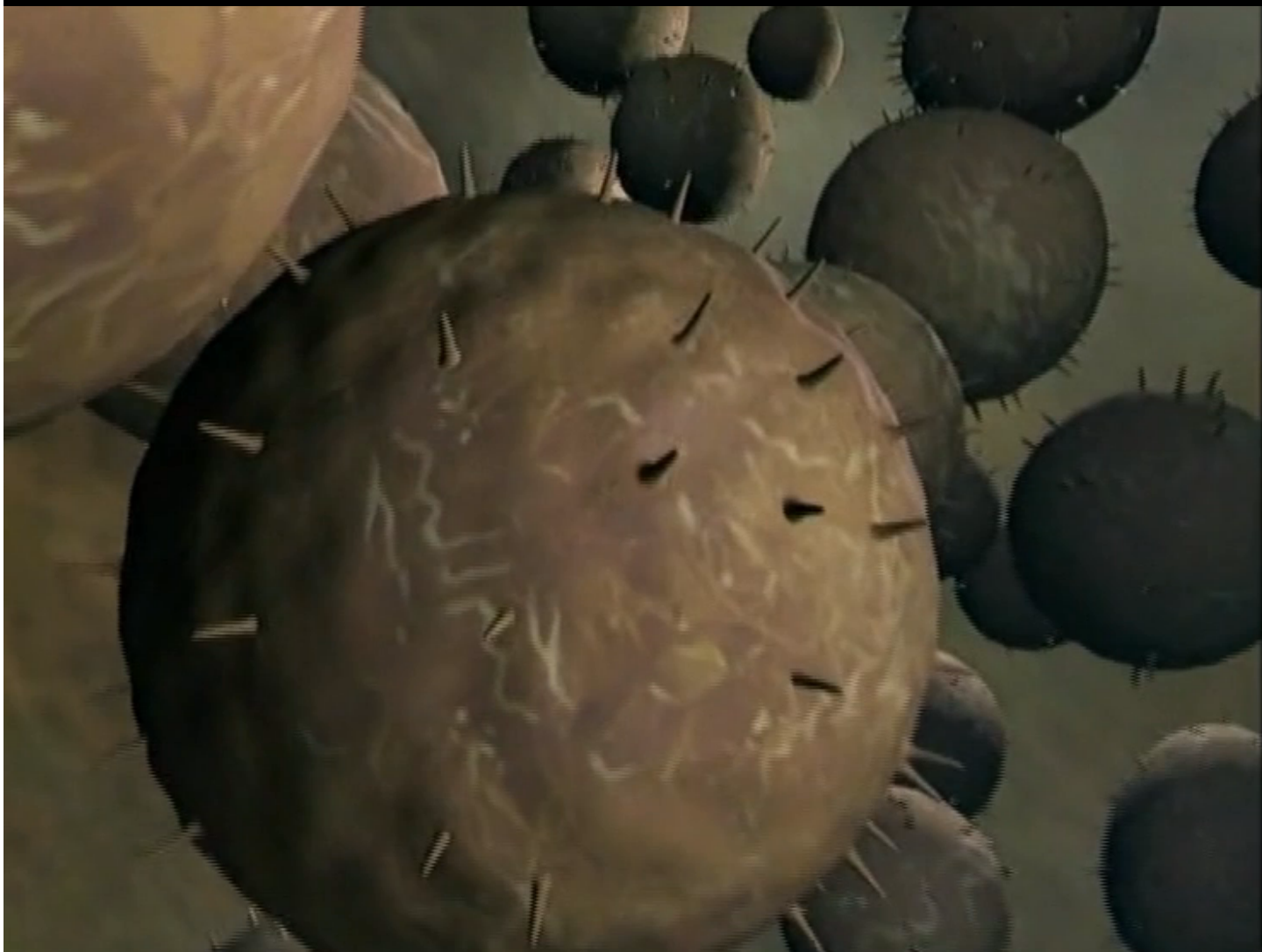


# **Reticolo Endoplasmatico (ER)**

*e*

*dintorni*

**Dove si trova?**



# HISTORY

- ❑ Discovered in 1902 by Italian Scientist Emilio Verratti.
- ❑ He was student of Golgi and used Golgi's staining procedures and found a new subcellular structure.
- ❑ Despite of his careful observation he was not able to convince the scientific community, that such an organelle existed.
- ❑ Therefore his work was disregarded and put away, while research on golgi apparatus, other organelles went dashing forward, ER was left behind.
- ❑ In retrospect, it is difficult to explain how in an era of excitement and interest in sub cellular structure this organelle was slow to be acknowledged.

## Discoverers



EMIL VERATTI

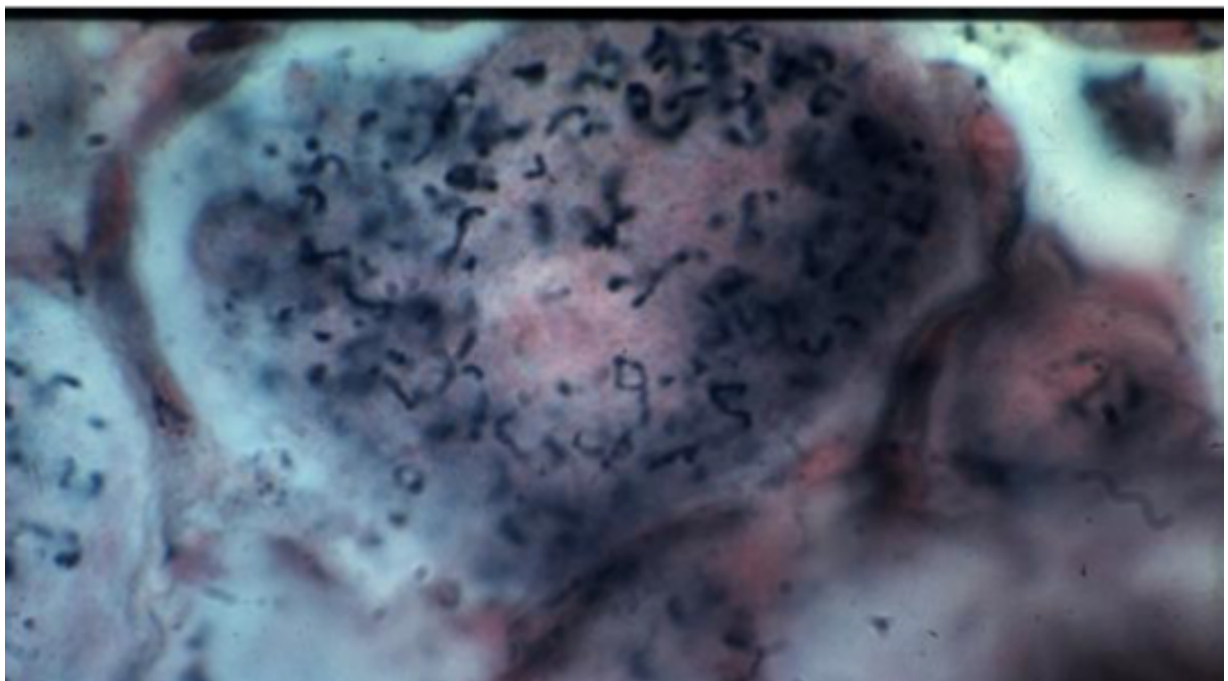
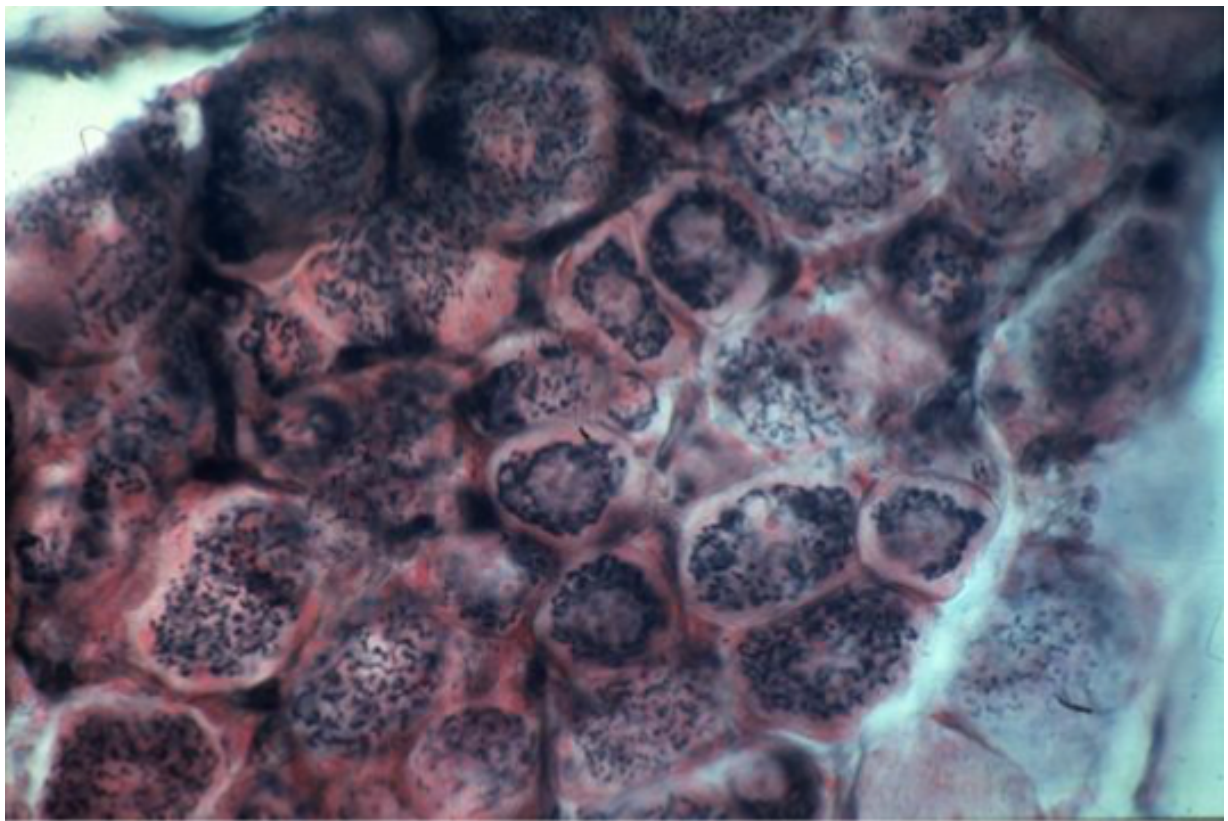


KEITH PORTER



GEORGE PALADE





# Rediscovery

- Technological advancement enabled for the rediscovery of ER.
- 1953- Keith Porter developed electron microscopy techniques that allowed him to observe net like (reticulum) structure within (endo) the cytoplasm (plastic).
- Hence named Endoplasmic Reticulum.
- In 1954 he teamed up with father of modern cell biology, George Palade and together they obtained high resolution images and finally proved the existence of this organelle.
- Over 50 years after its discovery the ER stepped into the limelight and, was accepted as a bonafide organelle attracting much curiosity and becoming the object of many investigation.

## Discoverers



EMIL VERATTI

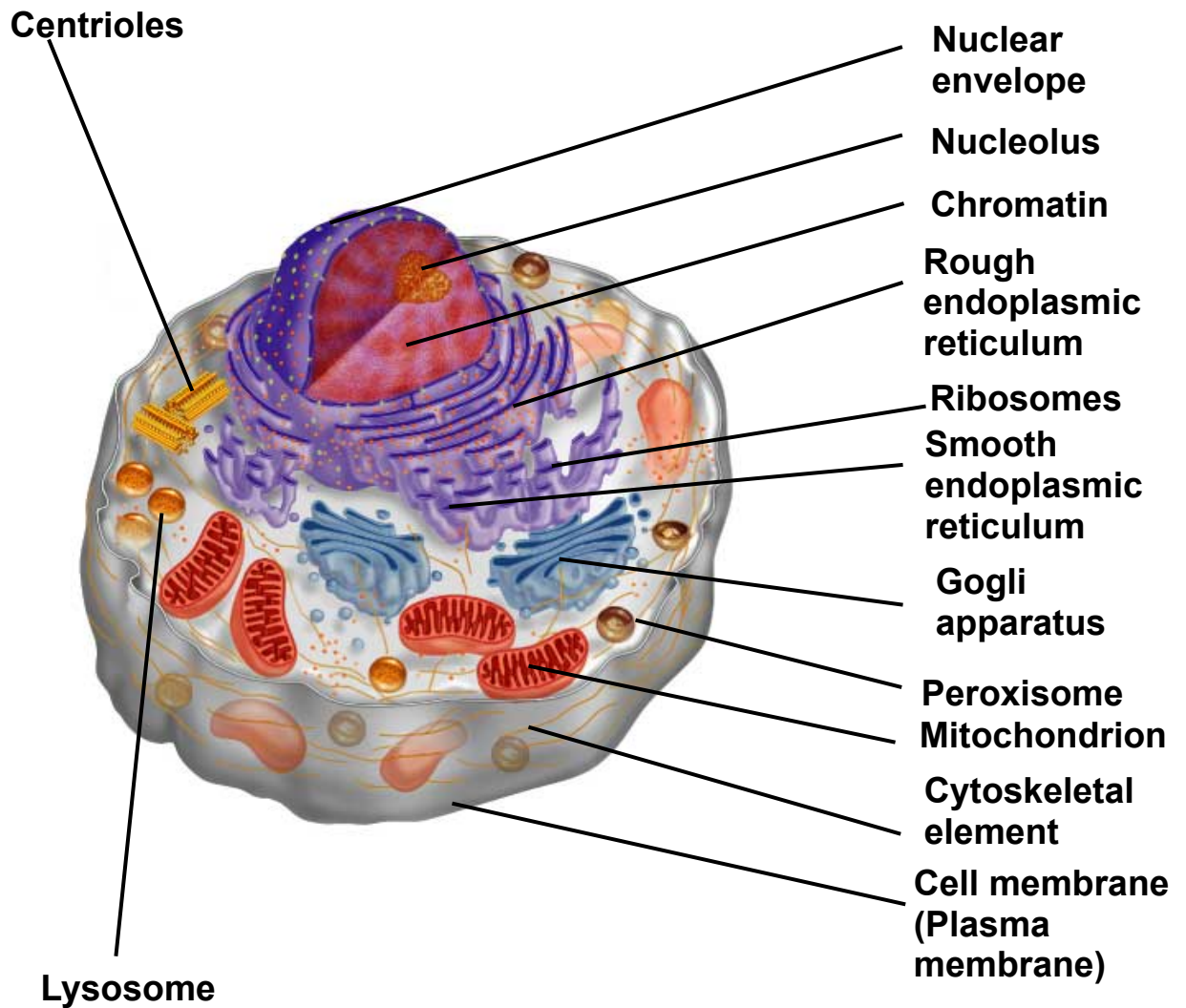


KEITH PORTER



GEORGE PALADE

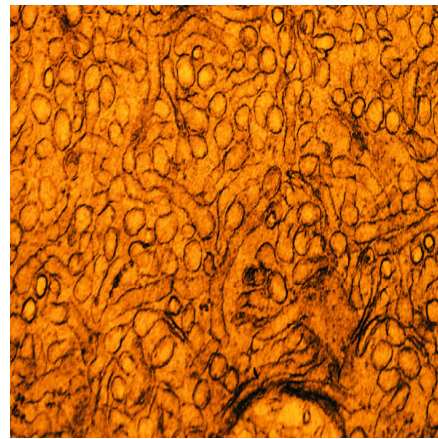




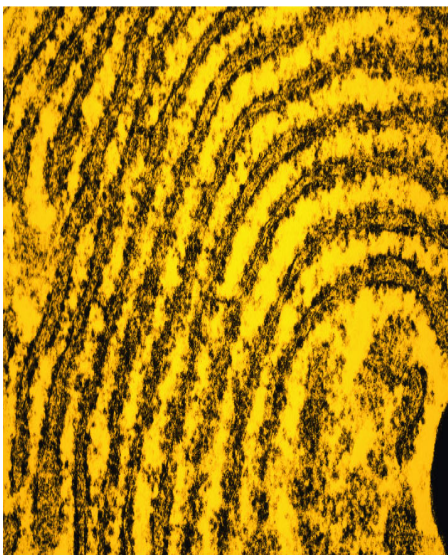
*The organelles within eukaryotic cells  
**subdivide**  
the cell into functional compartments*

# The organelles within eukaryotic cells subdivide the cell into functional compartments

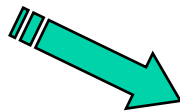
- The endomembrane system is the site of biomolecule synthesis and considerable molecular movement



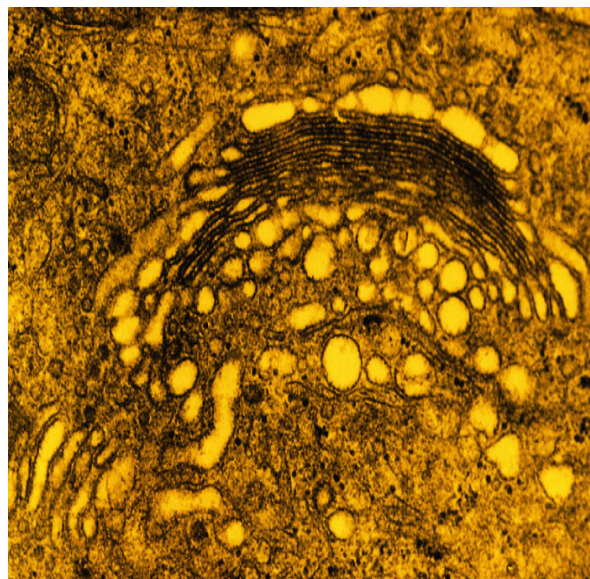
**Smooth ER**  
(no ribosomes attached)



**Rough ER**  
(with ribosomes attached)



**Golgi apparatus**

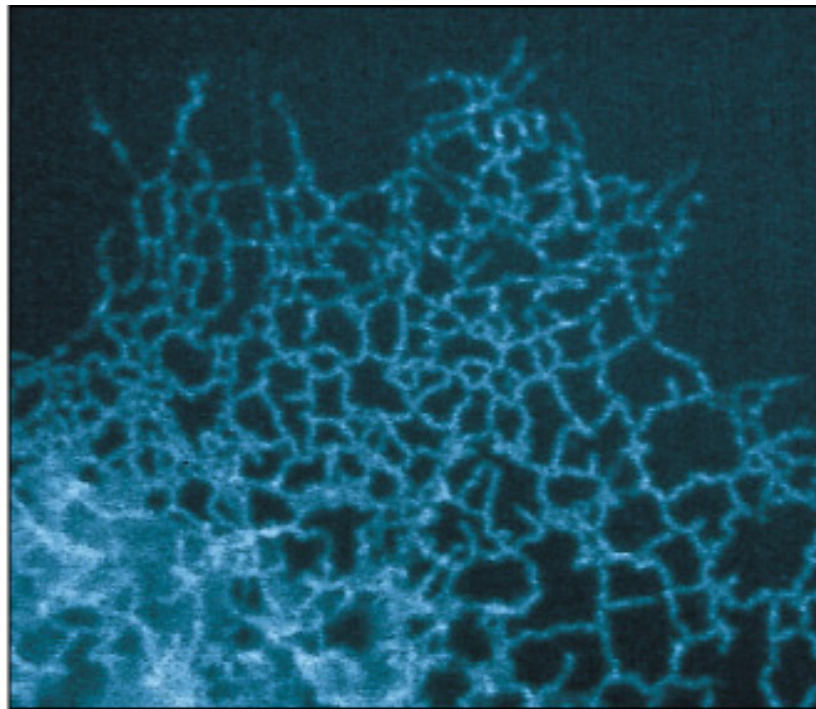


Intracellular compartment	Percentage total cell volume
Cytosol	54
Mitochondria	22
Rough ER	9
Smooth ER + Golgi	6
Nucleus	6
Peroxisomes	1
Lysosomes	1
Endosomes	1



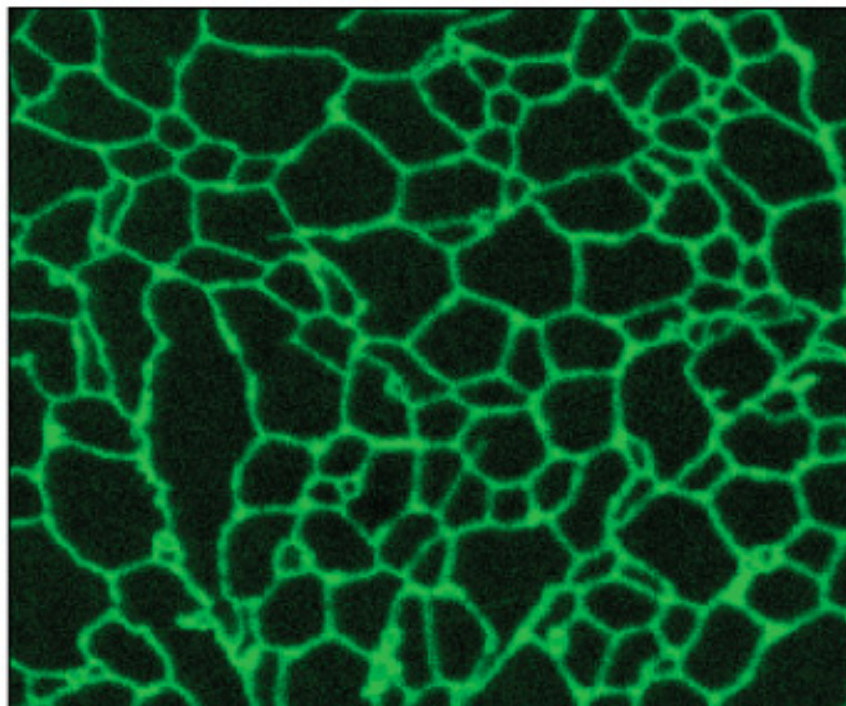
# THE ENDOPLASMIC RETICULUM

is a mesh of interconnected membranes that serves a function involving protein synthesis and transport



(A)

2  $\mu\text{m}$



(B)

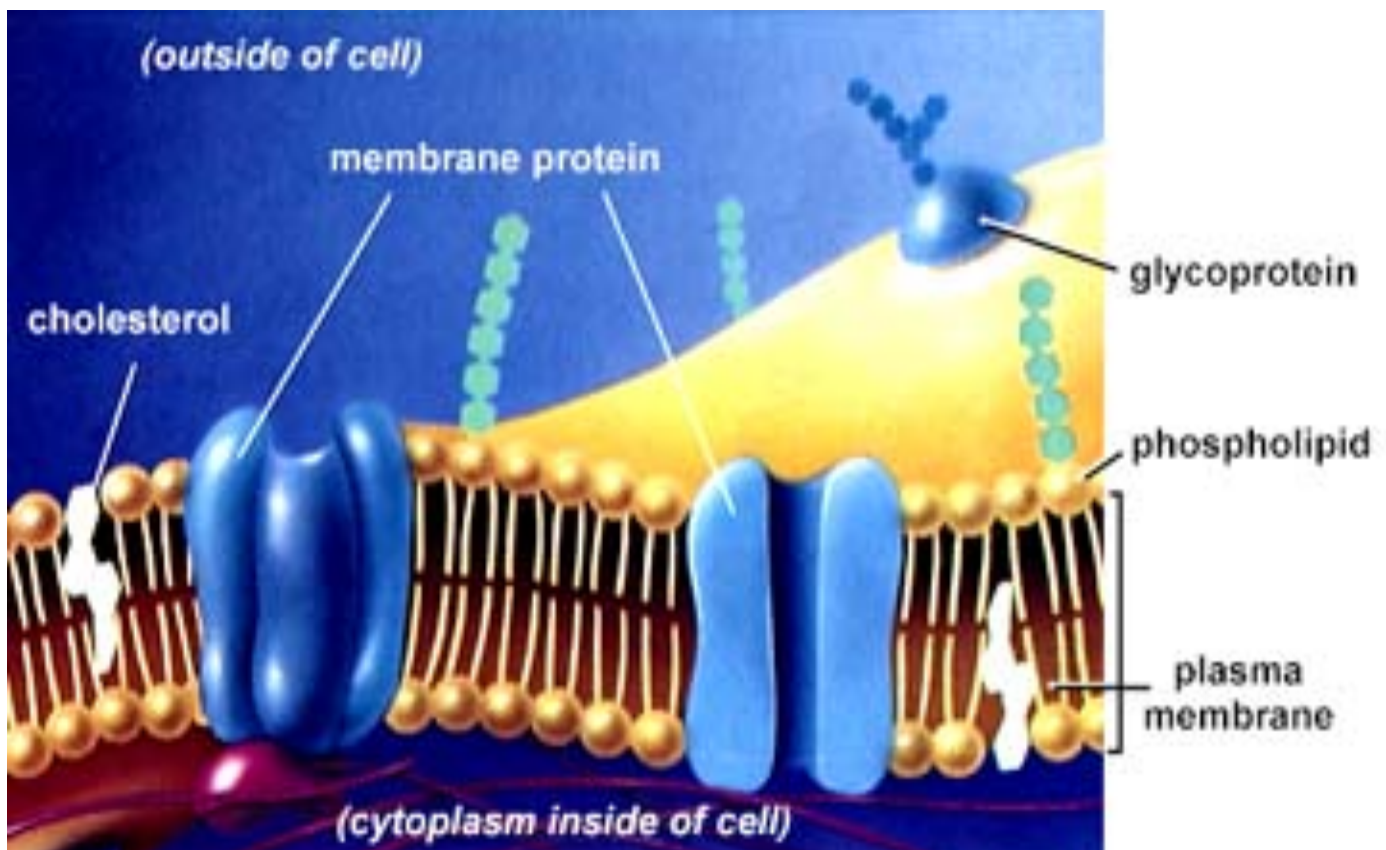
10  $\mu\text{m}$

tion.

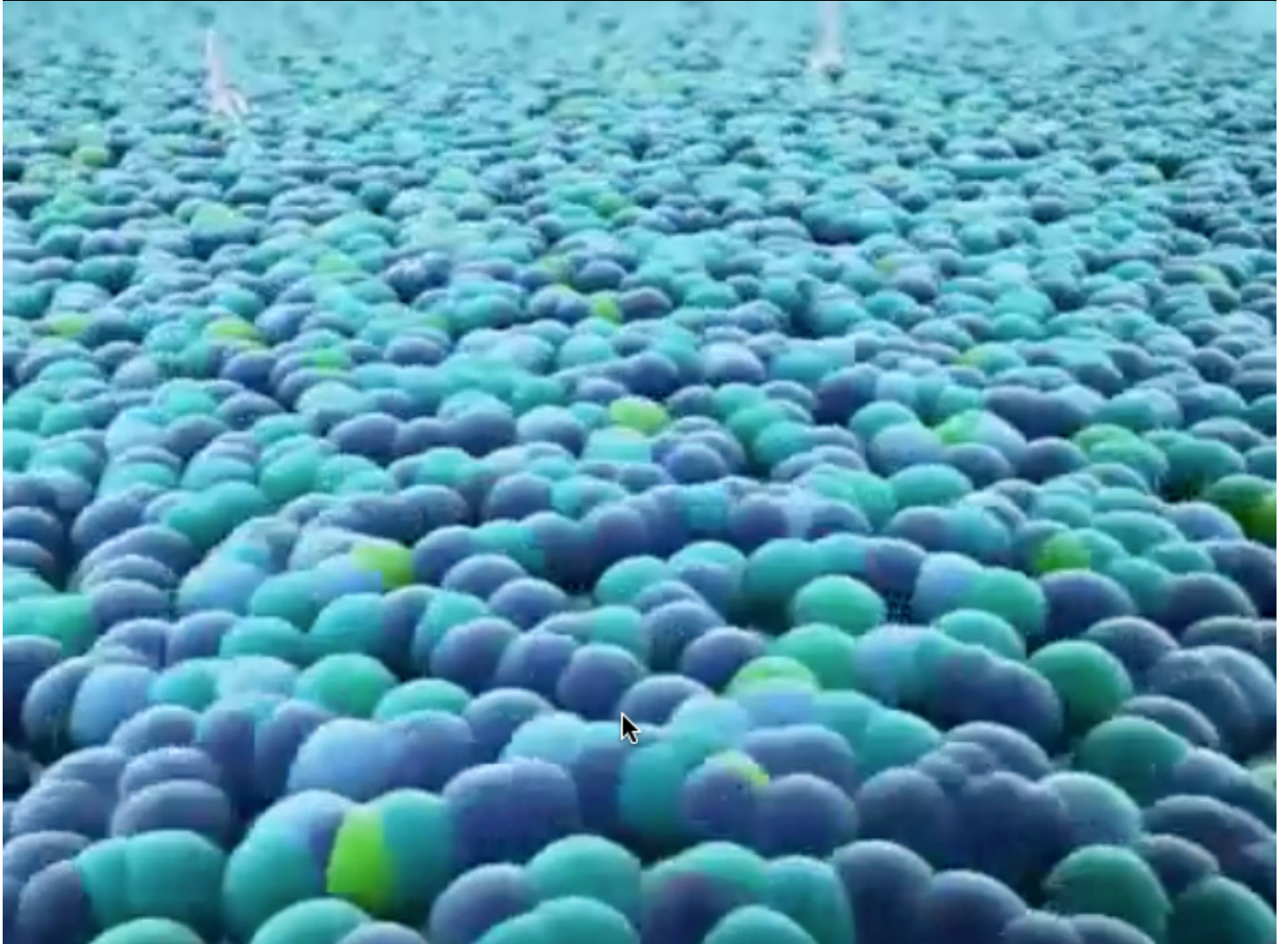
# Composition

*All membranes are lipid-protein assemblies in which the components are held together in a thin sheet by non-covalent bonds*

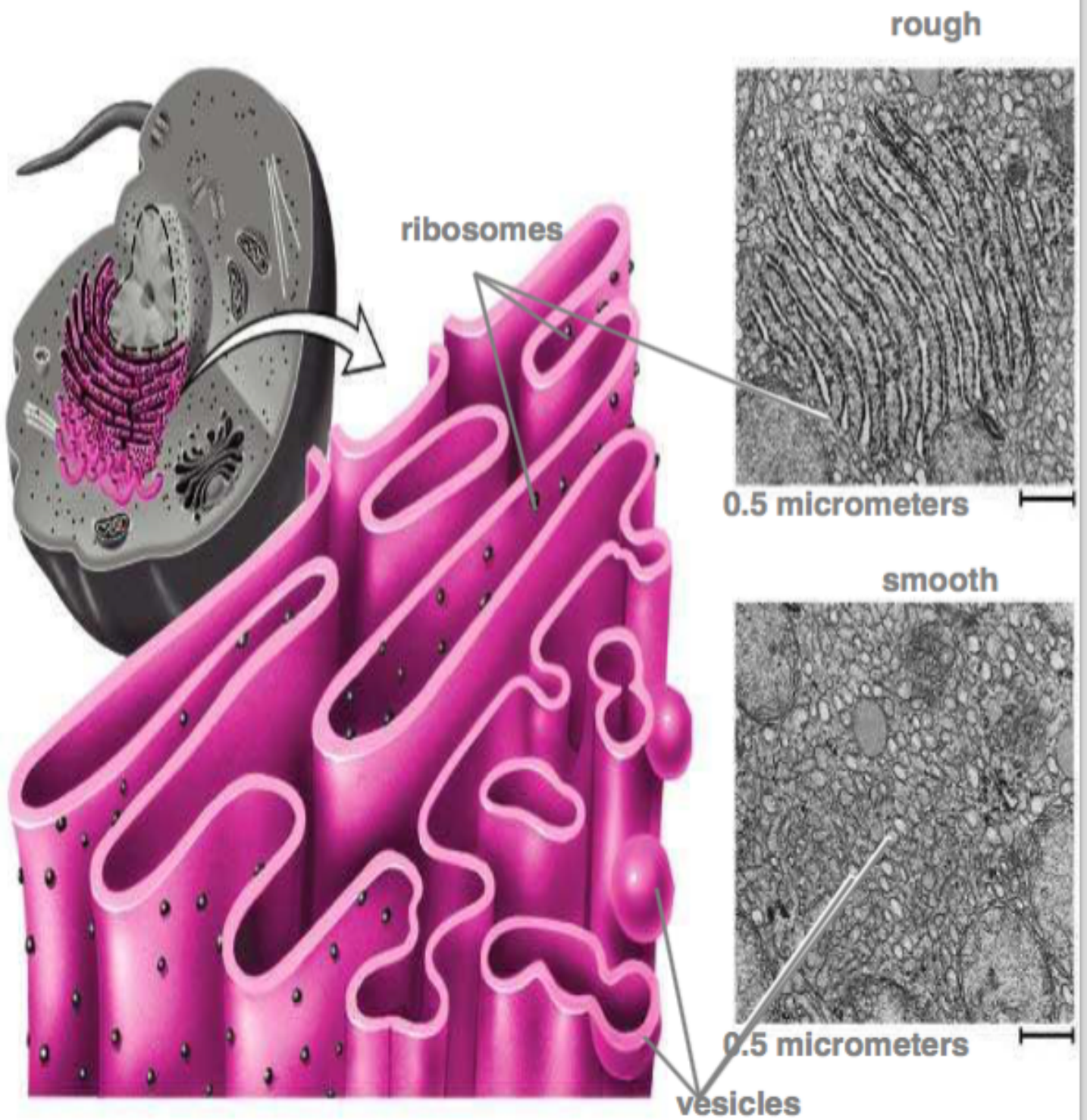
- **Lipids**
  - Phosphoglycerides
  - Sphingolipids
  - Cholesterol
- **Proteins**
- **Carbohydrates**
  - glycolipids
  - glycoproteins

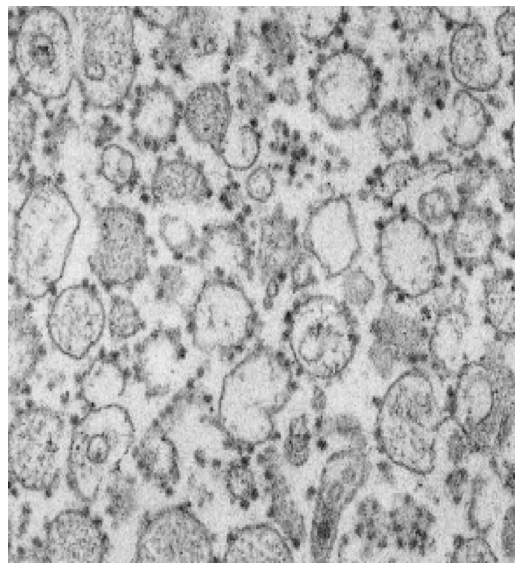
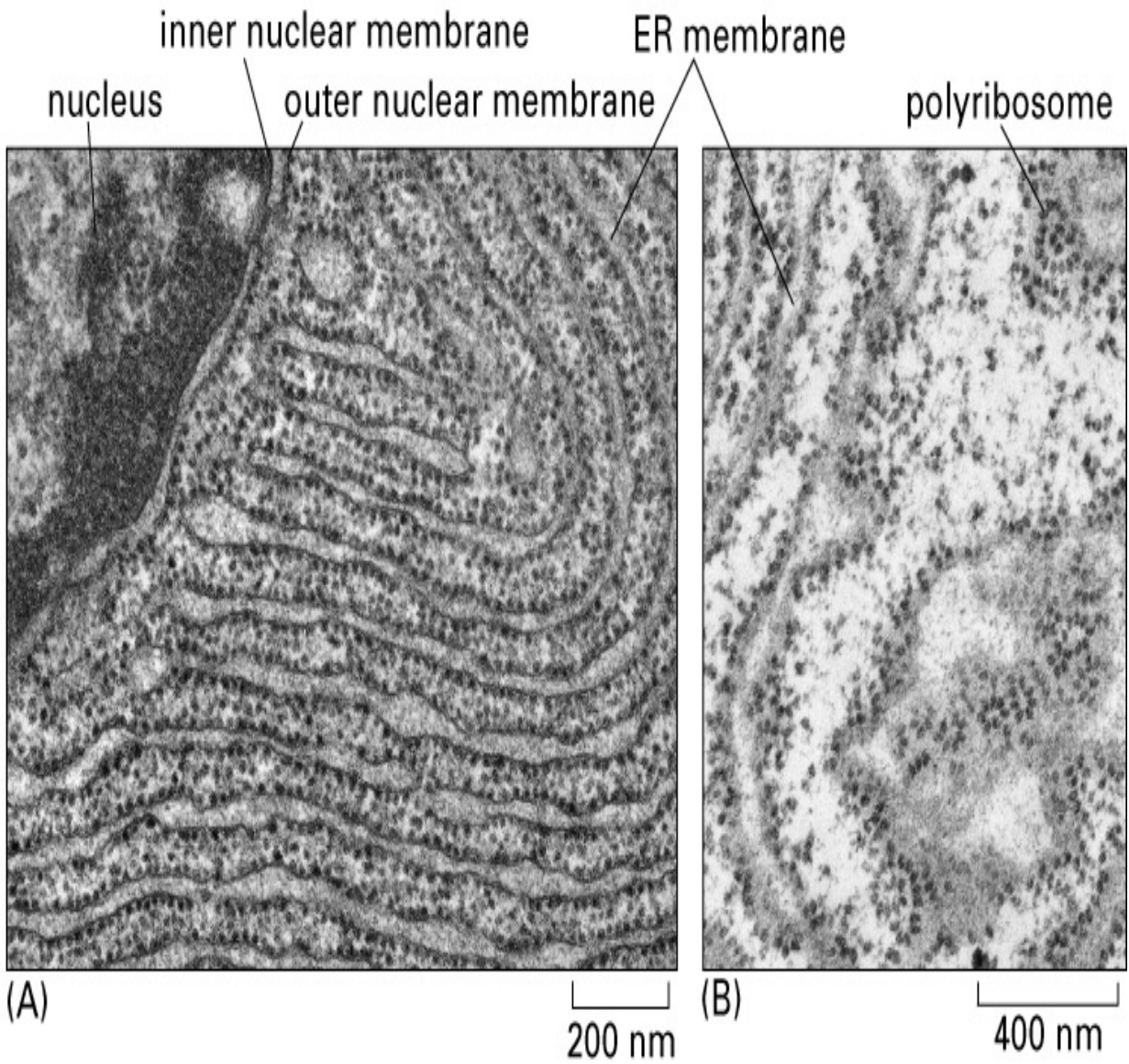


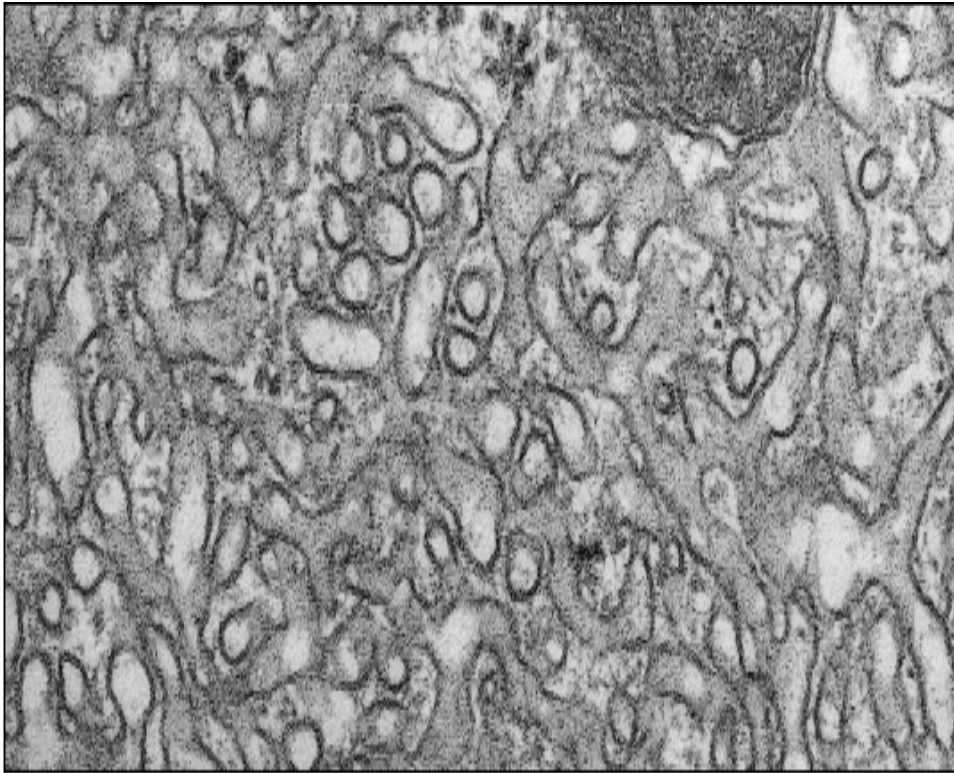
# La composizione della membrana del RE



# Endoplasmic reticulum



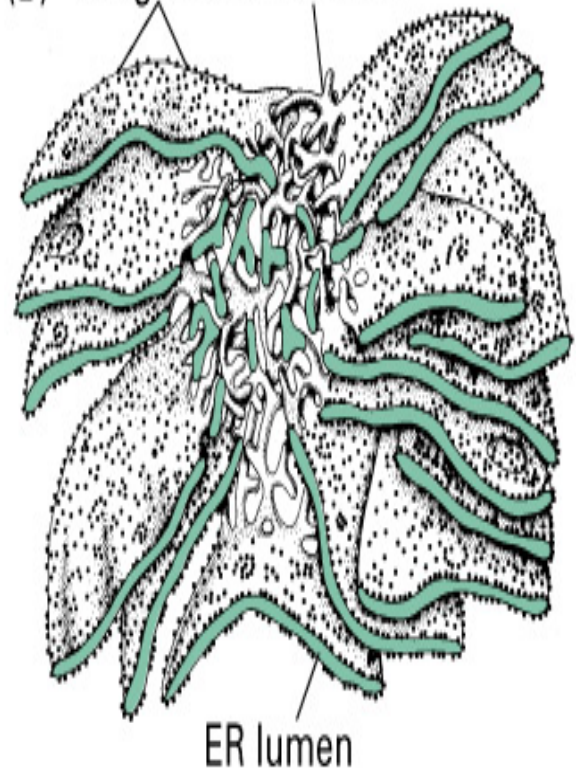




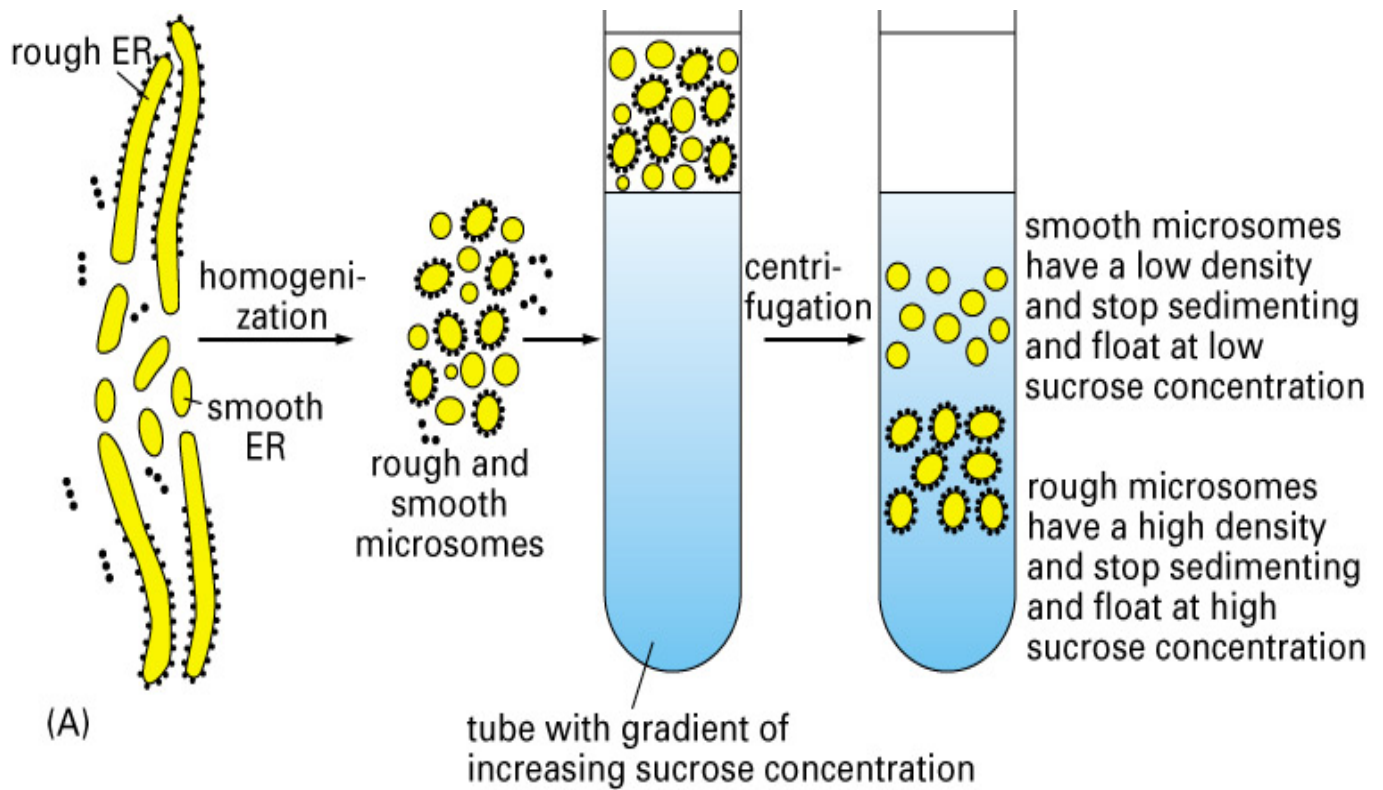
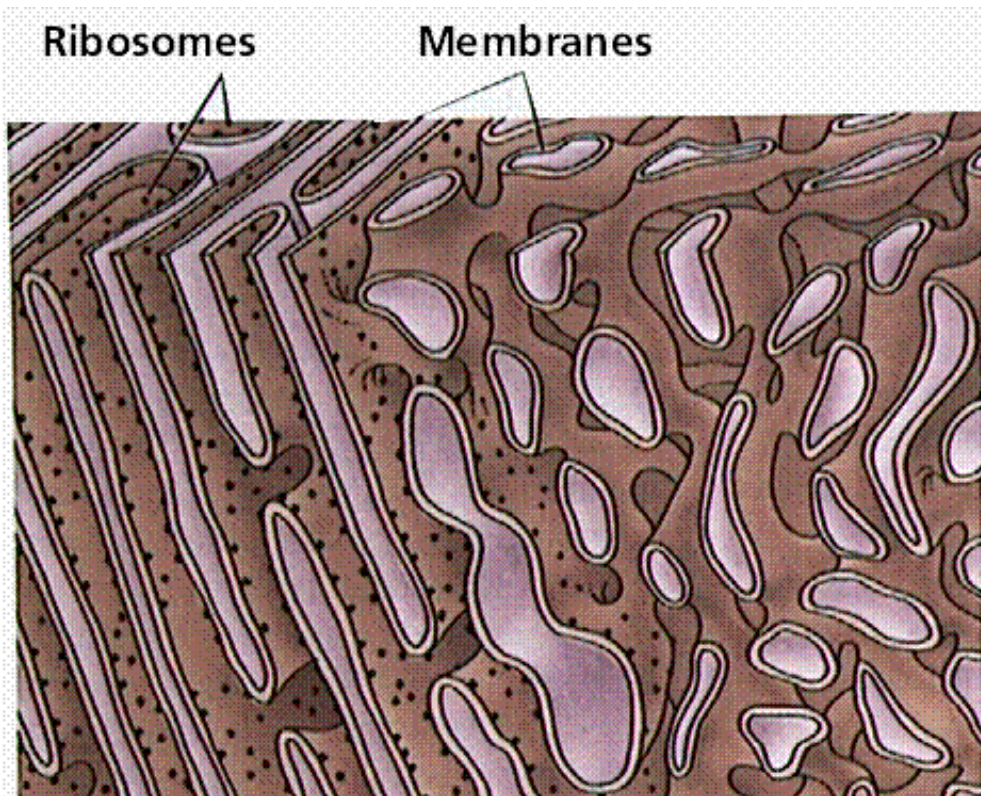
(A)

200 nm

(B) rough ER smooth ER



ER lumen





# ROUGH ENDOPLASMIC RETICULUM

- Flattish sealed sac that is continuous with the nuclear membrane.
- Studded on its outer surface with ribosomes.
- Found throughout the cell but the density is higher near the nucleus and the golgi apparatus.
- It has abundant translocon pores.
- Ribosomes are free to attach at these sites to synthesize proteins and transport them directly into the ER lumen, after which the ribosomes can detach.
- The presence of ribosomes studded on the membranes is what gives the rough ER its name.

# FUNCTIONS OF RER

- Protein folding
- Assembly of multi-subunit proteins
- Disulphide bond formation
- This requires an oxidising environment whereas the cytosol provides a reducing environment. An oxidising environment is found within the ER lumen which also contains disulphide isomerase, an enzyme which facilitates disulphide bond formation.
- Glycosylation – the initial stages occur on specific asparagine residues through the calnexin/calreticulin cycle.
- Degradation of misfolded proteins through the ubiquitin proteasome pathway.

# SMOOTH ENDOPLASTIC RETICULUM

- It is highly curved and tubular.
- It forms an interconnecting system of pipelines which form a network throughout the cytoplasm.
- It is involved in the production of steroid hormones in the adrenal cortex and endocrine glands.
- Lipid composition of the ER membrane is different to that of other cell compartments.
- Has large abundance of phosphatidyl choline and a very low concentration of cholesterol.
- It is also very fluid and disordered due to its large proportion of unsaturated fatty acids.
- It is more prominent in some cells than in others, depending on cell function.

## Contd....

- Synthesizes nearly all major classes of lipids but mainly PC, enzymes are membrane facing the cytosol where their substrates are present.
- Sequesters almost all of the  $\text{Ca}^{2+}$  from the cytosol.
- It is achieved by  $\text{Ca}^{2+}$  pump and high concentrations of calcium binding protein within the lumen.
- Specialized smooth ER is dedicated to the release and reuptake of  $\text{Ca}^{2+}$ .
- Most widely used example of this is the sarcoplasmic reticulum, found in muscle cells.
- Calcium ion release and reuptake triggers myofibril contraction and relaxation respectively in muscle.

# Smooth Endoplasmic Reticulum

- **Functions:**

- **Contains enzymes that detoxify lipid-soluble drugs (liver)**

- Carried out by cytochrome P<sub>450</sub>
- This is a family of enzymes that hydroxylate lipid-soluble drugs
- This makes them water-soluble so they can be secreted
  - (e.g. phenobarbital)

# Smooth Endoplasmic Reticulum

- **Functions:**

- Steroid hormones synthesis in endocrine cells of the gonads and of the adrenal glands

# Smooth Endoplasmic Reticulum

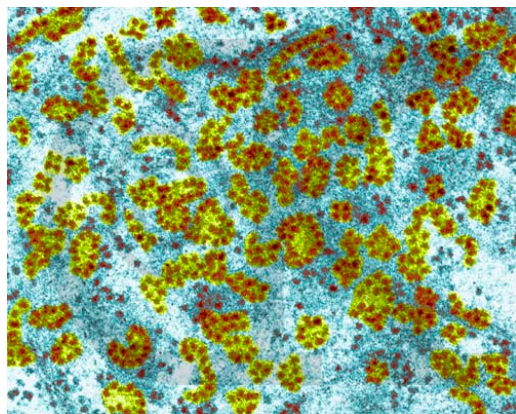
- **Functions:**

- **Sequester  $\text{Ca}^{++}$  from the cytosol**

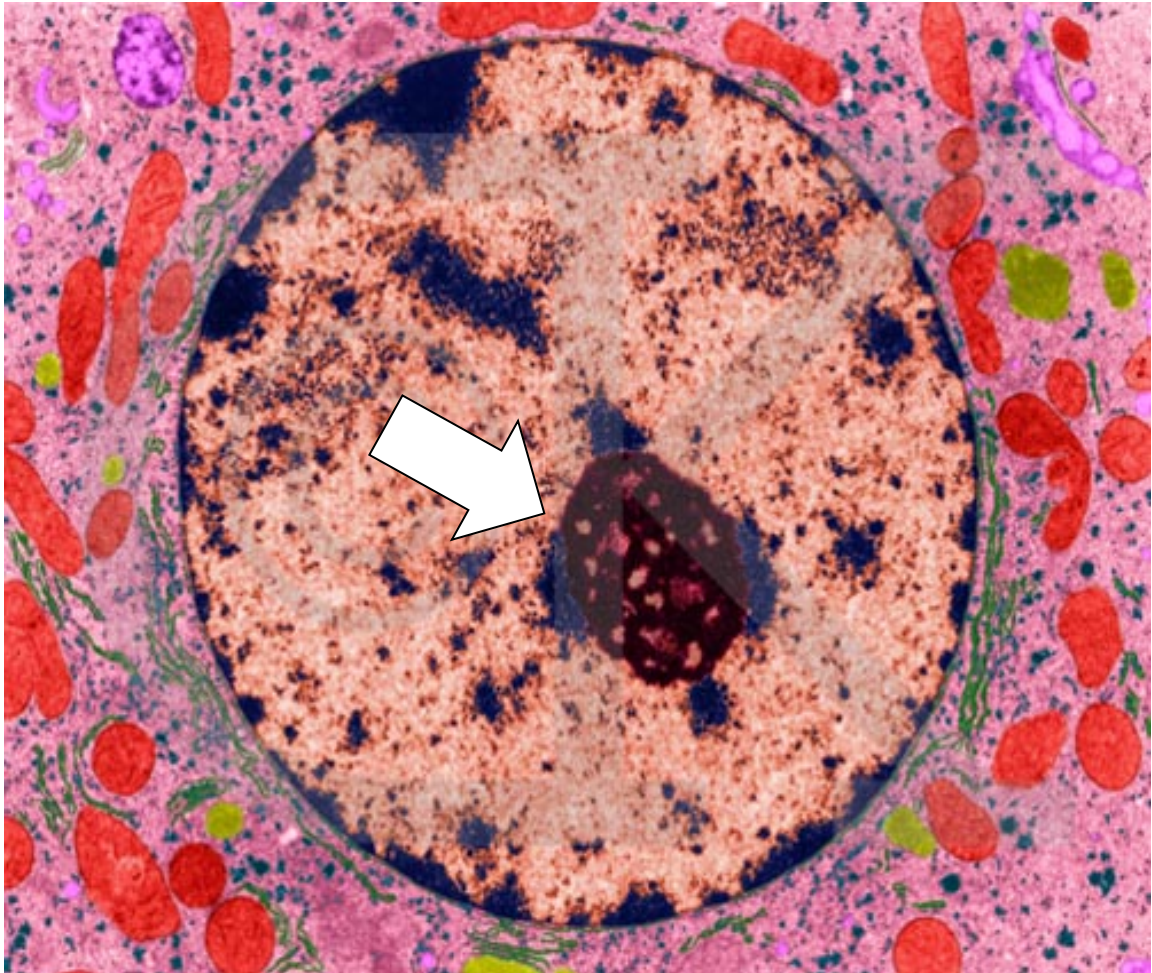
- The release of  $\text{Ca}^{++}$  into the cytosol and its subsequent uptake into the ER are involved in many rapid responses to intracellular (muscle cells) extracellular signals

# I RIBOSOMI

- Sites of **protein synthesis**.
- They are not membrane-bound
- Occur in both **prokaryotes** and **eukaryotes**.
- Eukaryotic ribosomes are slightly larger
- Consists of a small and larger subunit.
- Biochemically the ribosome consists of **ribosomal RNA** (rRNA) and some 50 structural proteins.
- Often ribosomes cluster on the **endoplasmic reticulum**

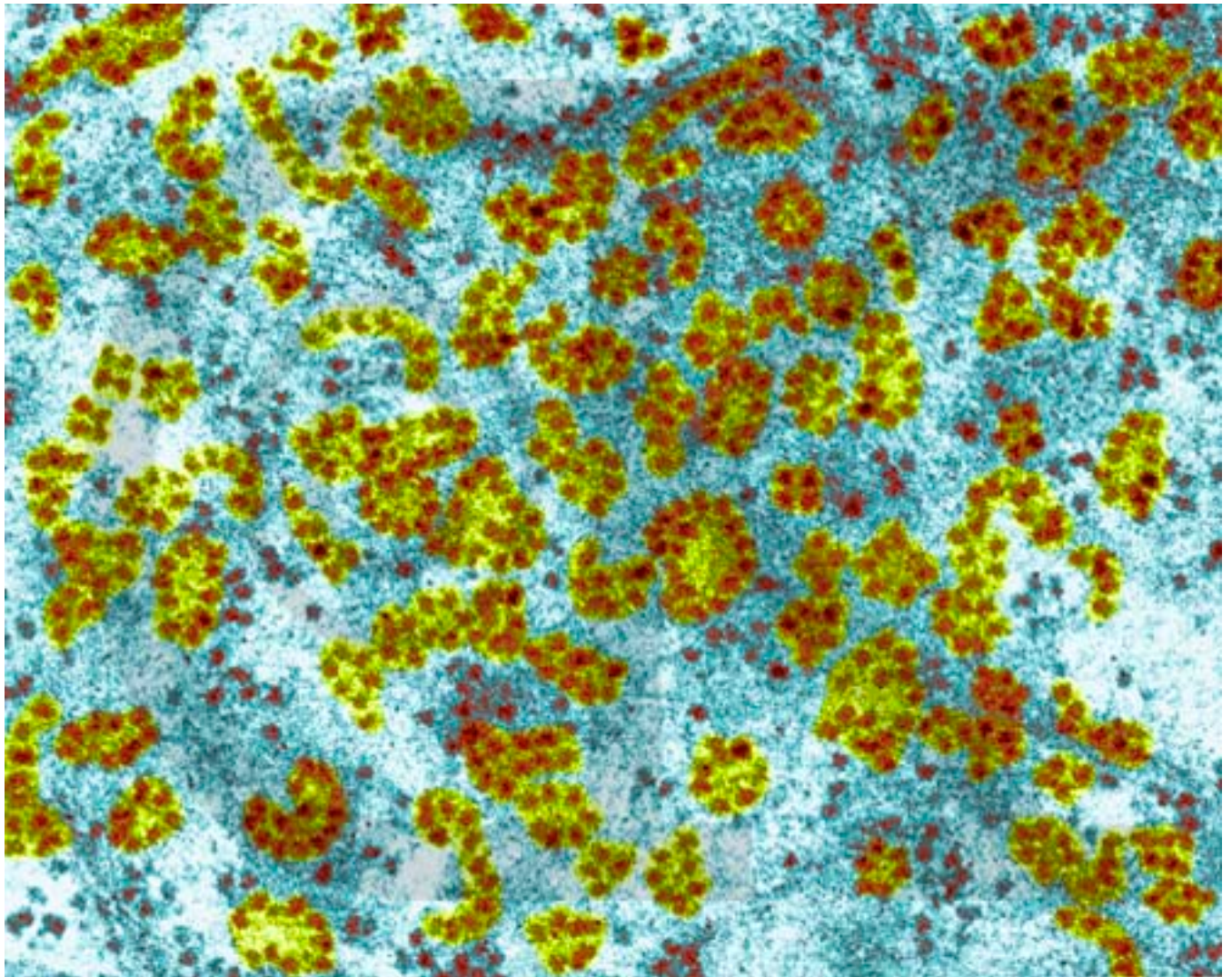
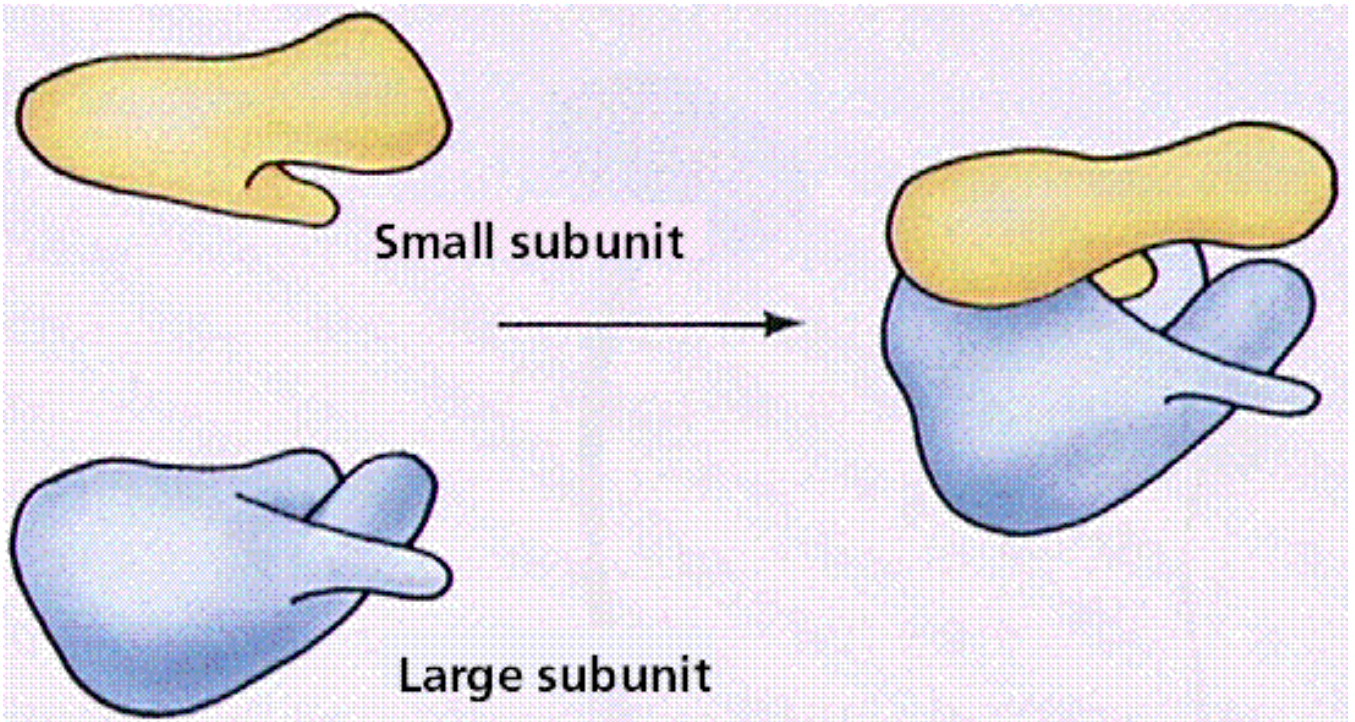


Il nucleolo è la sede dove proteine ribosomiali e rRNA vengono assemblati



**Heterochromatin** stains more strongly and is a more condensed chromatin. **Euchromatin** stains weakly and is more open (less condensed). Euchromatin remains dispersed (uncondensed) during Interphase, when RNA transcription occurs. Some regions of heterochromatin appear to be structural (as in the heterochromatin near the **centromere region**)





Provenienza	Ribosoma	Subunita'	Composizione
Eucarioti	80S	60S	rRNA 28S RNA 5S RNA 5.8S proteine
		40S	rRNA 18S proteine
Procarioti	70S	50S	rRNA 23S RNA 5S proteine
		30S	rRNA 16S proteine
Mitocondri	55S	35S	rRNA 16S proteine
		25S	rRNA 12S proteine

# Types of proteins synthesised

## 1. On ER ribosomes

- Proteins secreted by the cell
- Trans-membrane proteins
- Proteins of Golgi, lysosomes, endosomes, plant vacuoles

## 2. On free ribosomes

- Cytosol proteins (cytoskeleton, glycolysis enzymes)
- Peripheral proteins of the inner plasma membrane (spectrin, ankirin etc)
- Proteins of perossisomes, cloroplasts and mitochondria

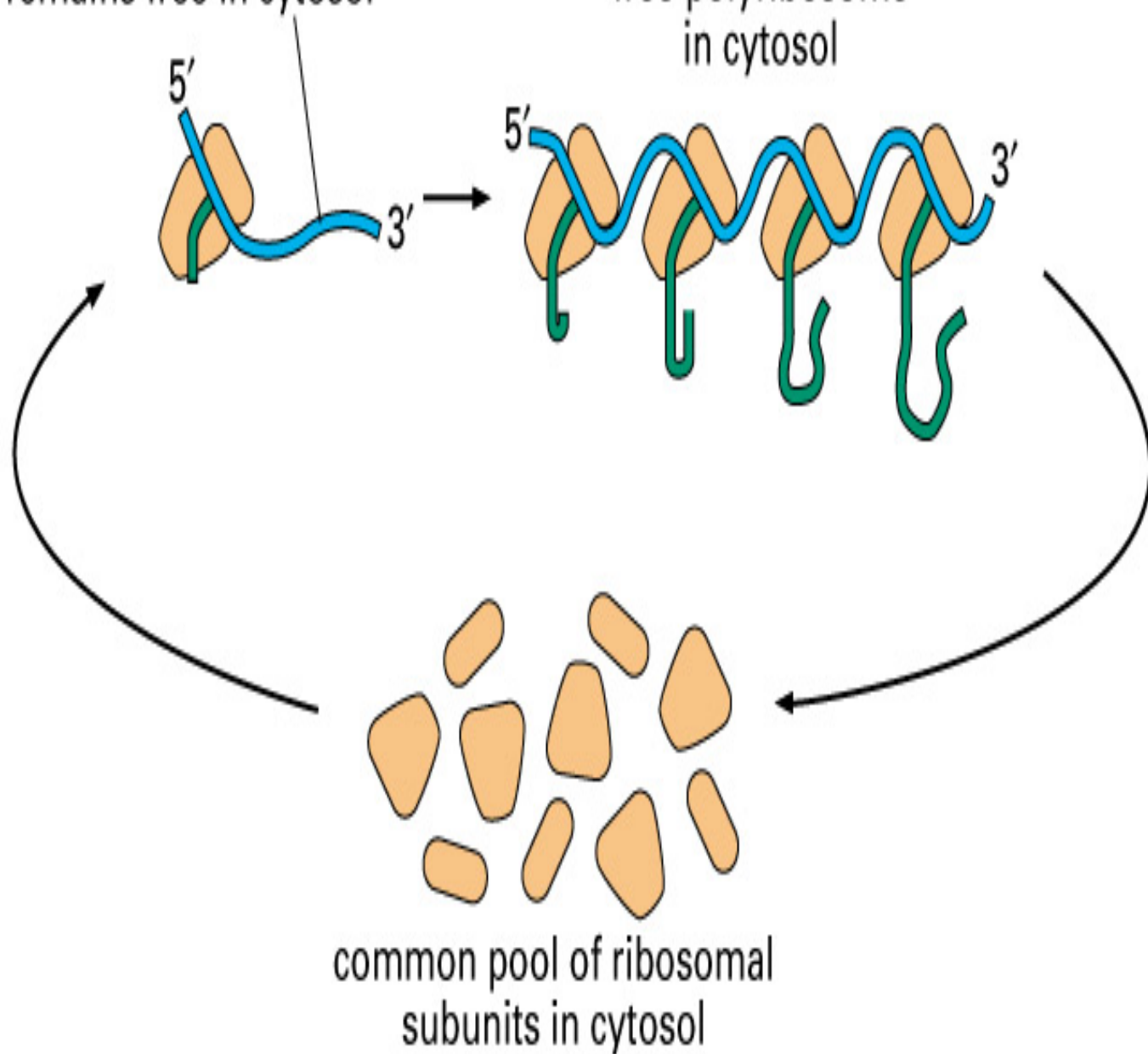
- Import of proteins into the ER begins before they are fully synthesized

Thus import is **co-translational**

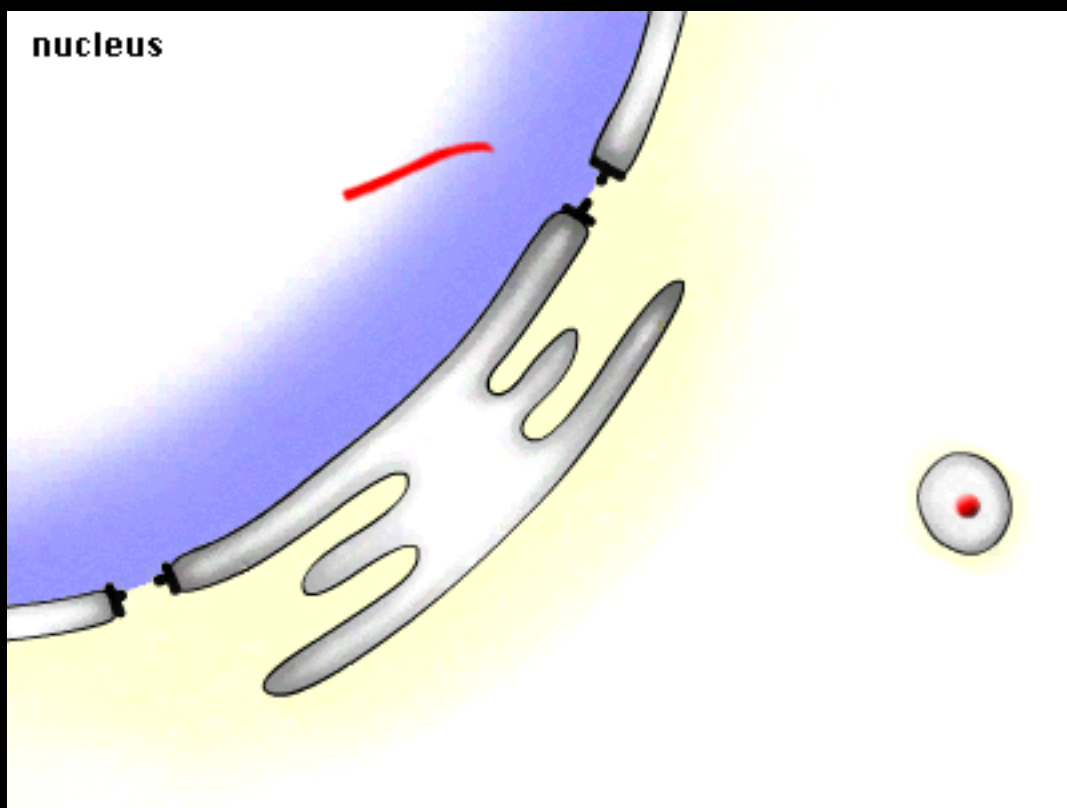
This distinguishes ER import from import into **all** other organelles, which is post-translational

mRNA encoding a cytosolic protein  
remains free in cytosol

free polyribosome  
in cytosol



# Sintesi proteica in ribosomi liberi



# Three types of RNA

- **Ribosomal RNA (rRNA)**

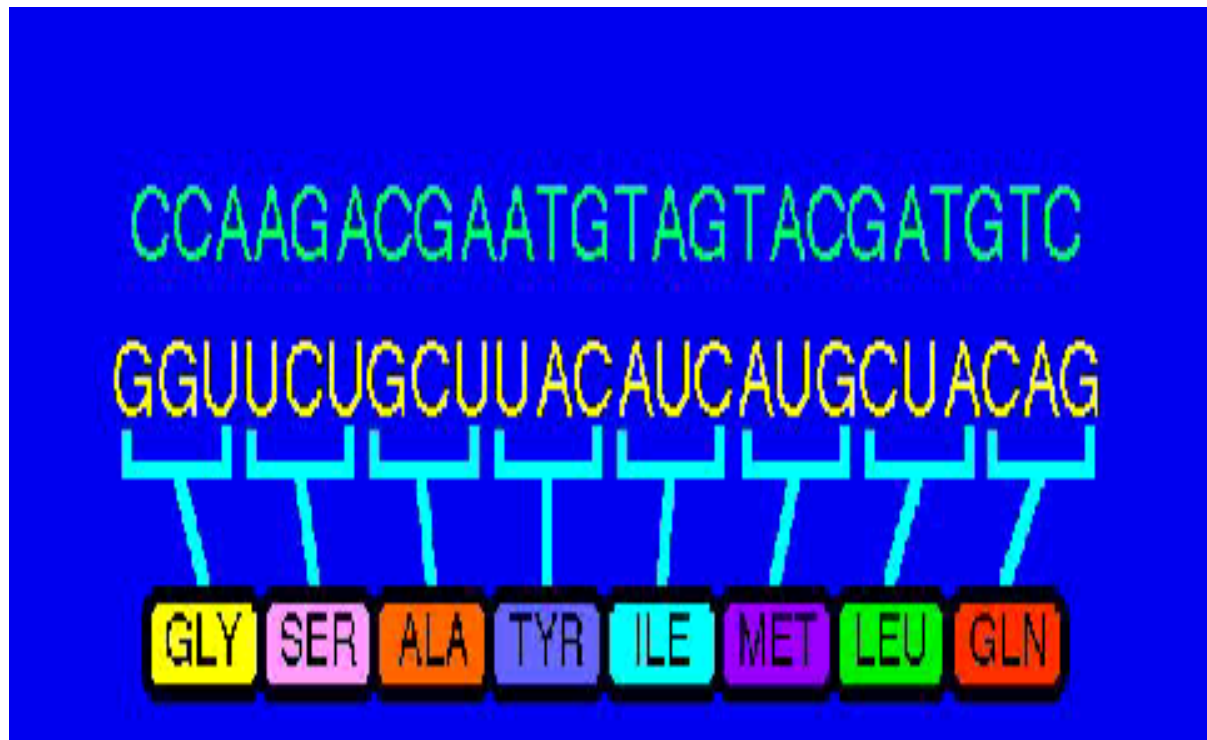
combines with proteins to form the ribosomes

- **Messenger RNA (mRNA)**

contains the instruction for the ordering of amino acids in proteins

- **Transfer RNA (tRNA)**

carries specific amino acids to ribosomes



- The **genetic code** is read in groups of **three nucleotids**, each group representing **one amino acids**.
- Each trinucleotides sequence is called a **codon**.
- A **gene** includes a **series of codons** that is read sequentially from a starting point at one end to a termination point at the other end.



		Second letter				
		U	C	A	G	
First letter	U	UUU UUC	UCU UCC UCA UCG	UAU UAC	UGU UGC	U C
		UUA UUG		UAA UAG	UGA UGG	A G
	C	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU CAC	CGU CGC CGA CGG	U C A G
		AAU AAC		AAA AAG		U C A G
A	AUU AUC AUA	ACU ACC ACA ACG	AAU AAC	AGU AGC	U C A G	
	AUG		AAA AAG	AGA AGG	U C A G	
G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU GAC	GGU GGC GGA GGG	U C A G	
			GAA GAG			

Il codice genetico e'  
 universale,  
 ridondante e degenerato  
 20 aminoacidi - 64 codoni

## The Nobel Prize in Physiology or Medicine 1999



### **Günter Blobel**

Rockefeller University  
New York, NY

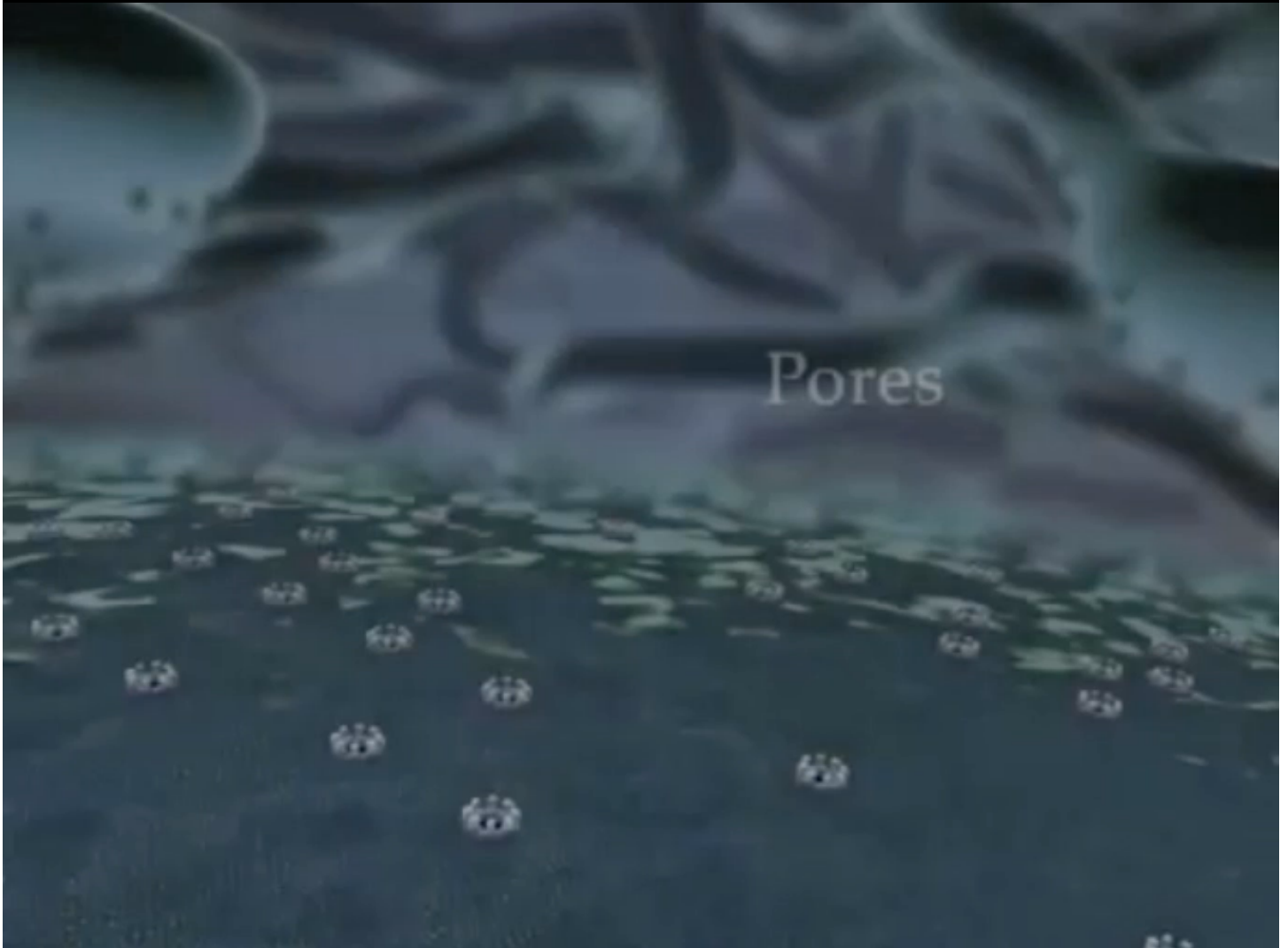
Howard Hughes Medical Institute  
Chevy Chase, MD, USA

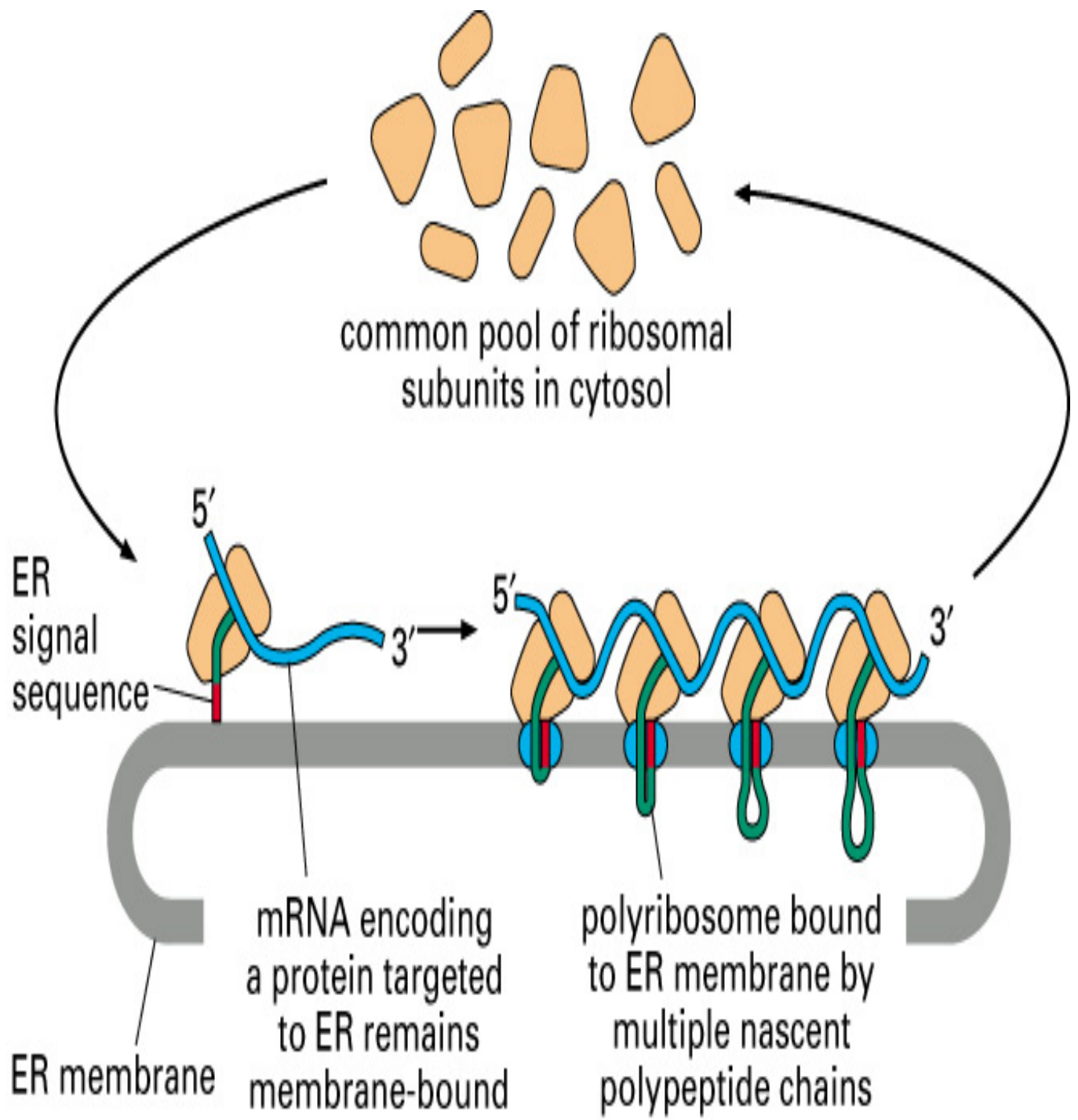
Born 1936  
(in Waltersdorf/Silesia, Germany)



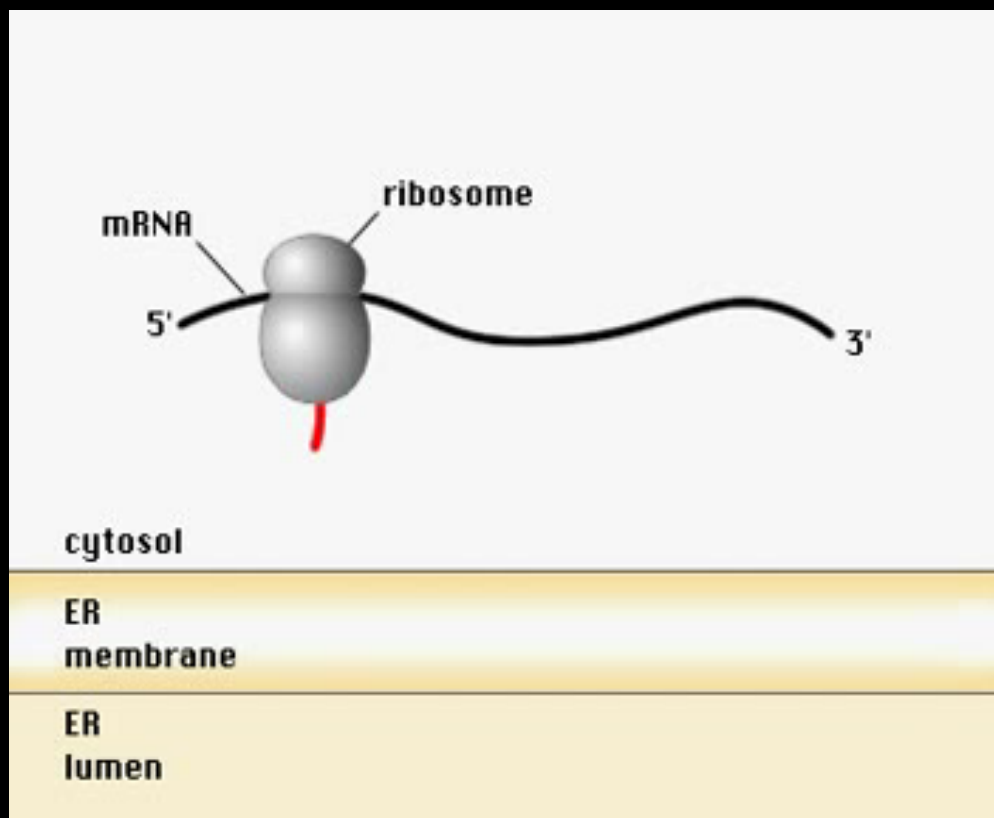
"for the discovery that proteins have intrinsic signals that govern their transport and localization in the cell"

# From mRNA to proteins Transduction on ER-Ribosomes





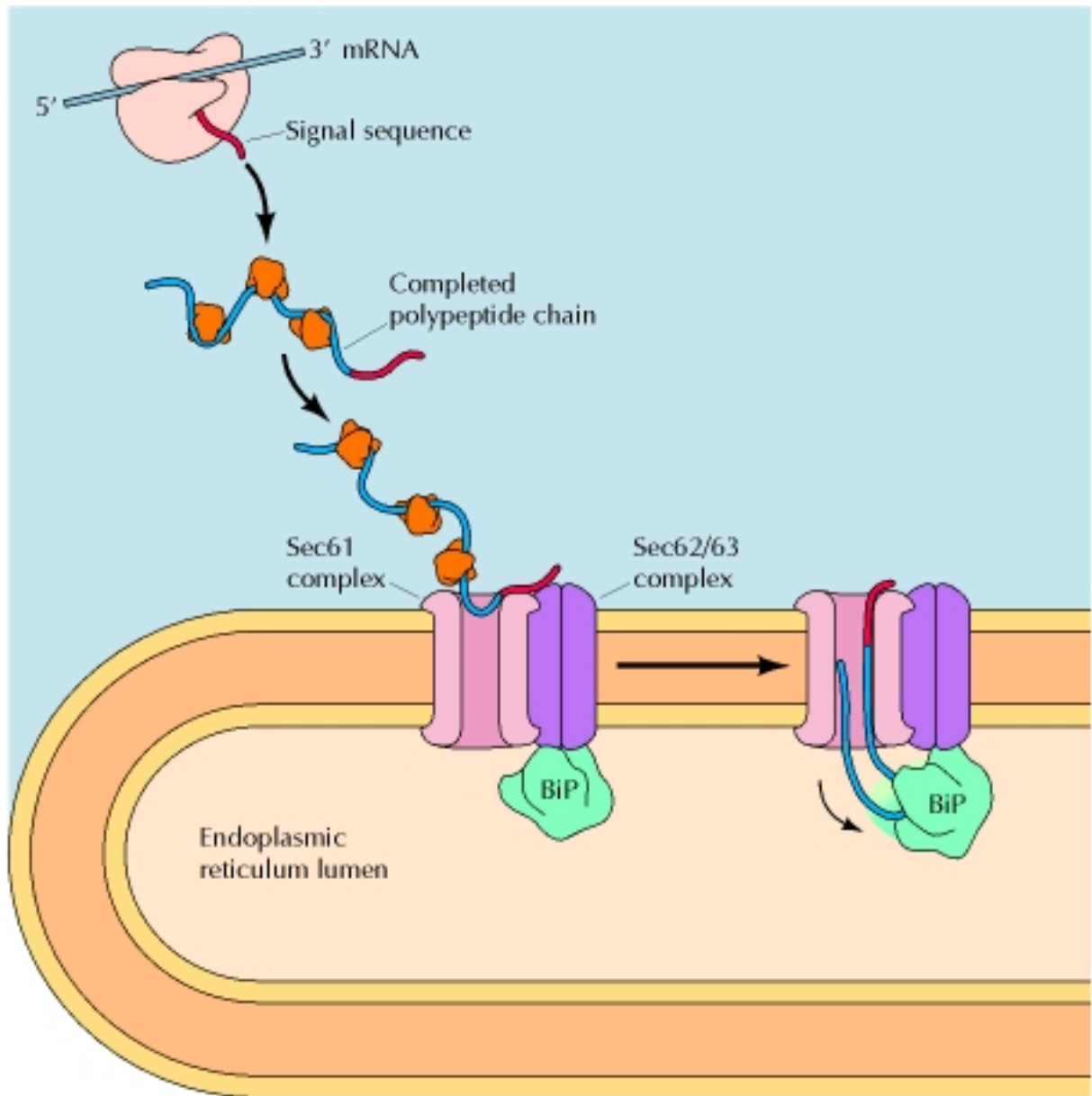
# Sintesi proteica in ribosomi sull'ER



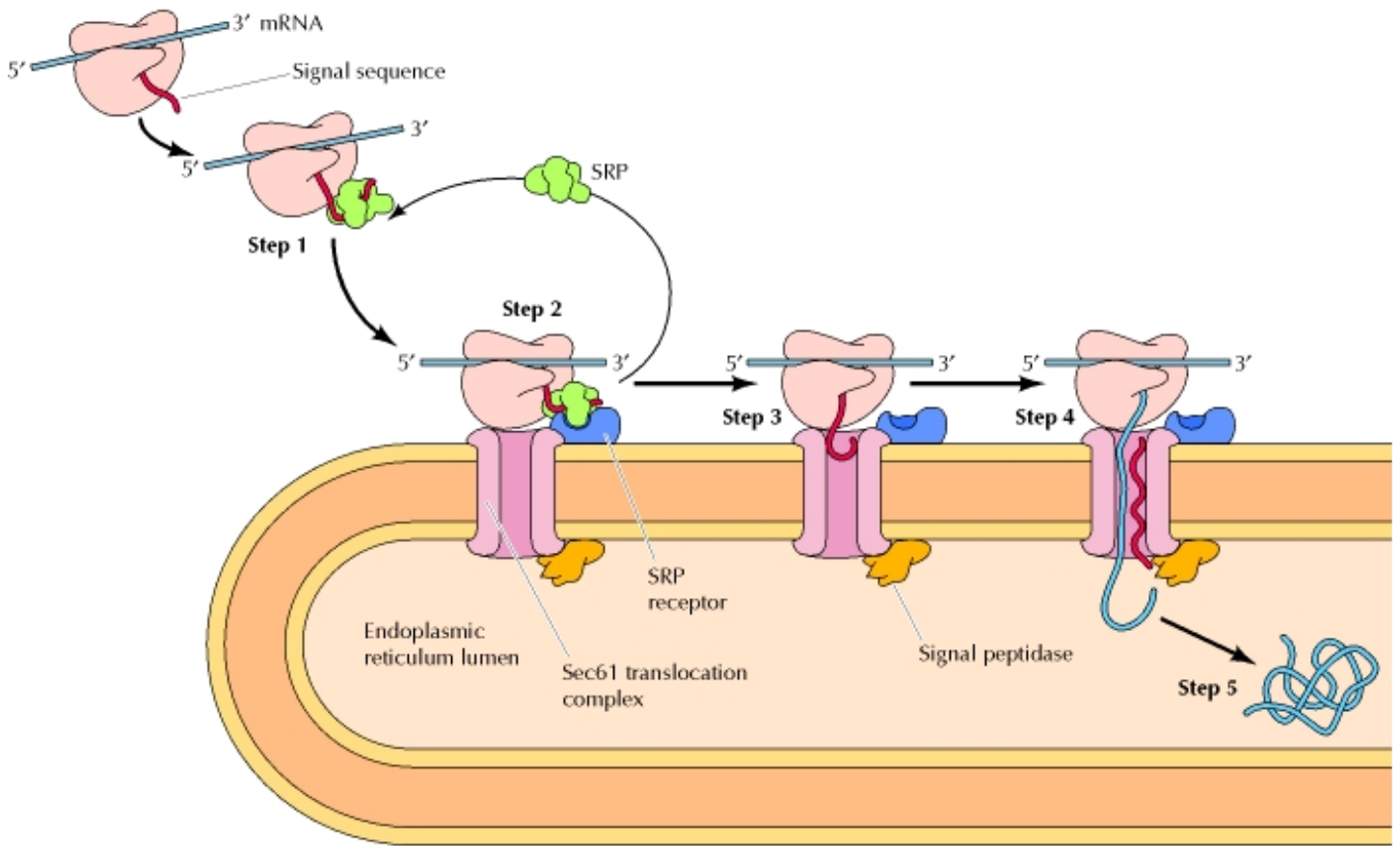
Most of the membrane lipids are synthesised in the ER, with the exception of glycolipids and sphingomyelin which synthesis begins in ER and is completed in the Golgi

Differences in lipids composition in organelles are dependent to:

- intrinsic capacity of organelles to modify lipids
- selection of phospholipids from vesicle membranes during budding
- protein-mediated phospholipids transport from ER to other organelles through the cytosol

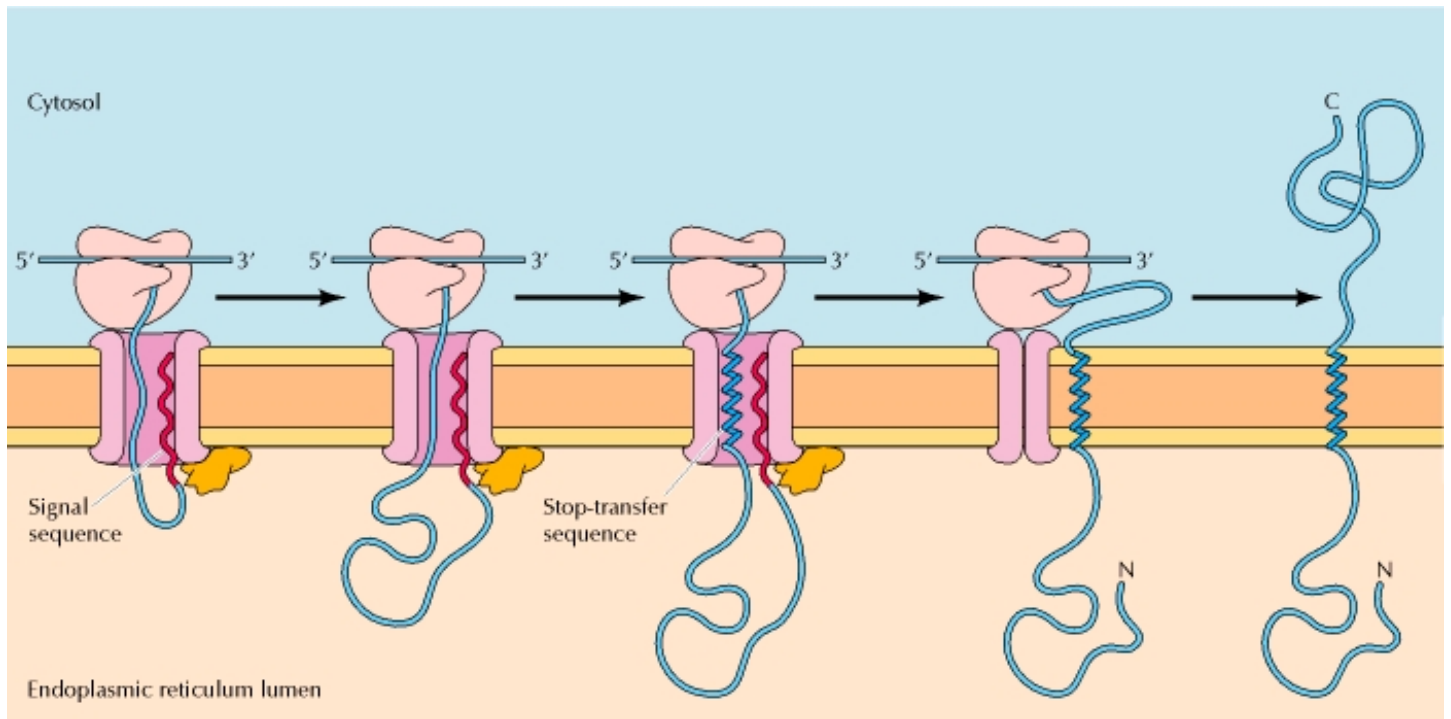


Proteins destined for posttranslational import to the ER are synthesized on free ribosomes and maintained in an unfolded conformation by cytosolic chaperones. Their signal sequences are recognized by the Sec62/63 complex, which is associated with the Sec61 translocation channel in the ER membrane. The Sec63 protein is also associated with a chaperone protein (BiP), which acts as a molecular ratchet to drive protein translocation into the ER.



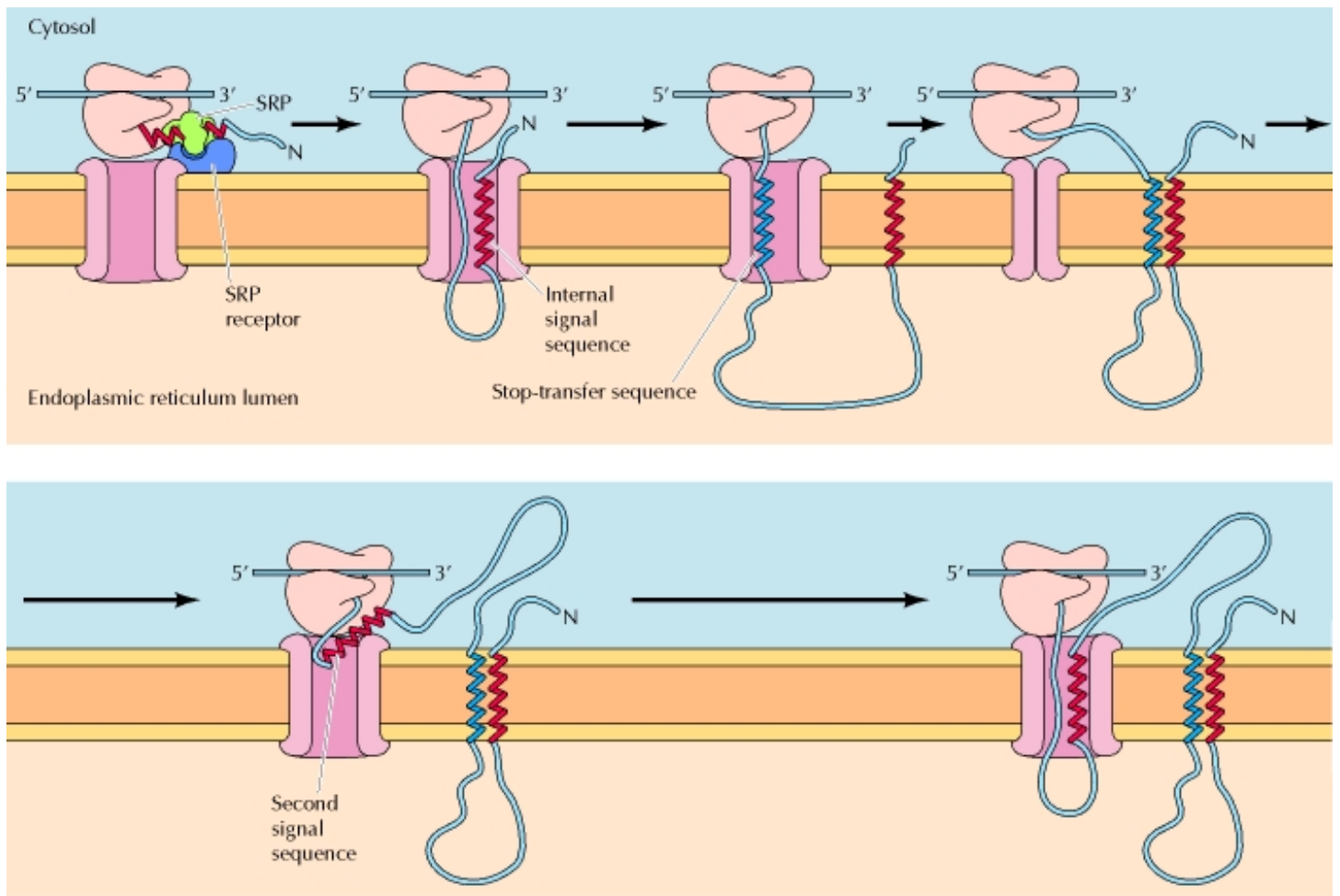
As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP). Step 2: The SRP escorts the complex to the ER membrane, where it binds to the SRP receptor. Step 3: The SRP is released, the ribosome binds to a membrane translocation complex of Sec61 proteins, and the signal sequence is inserted into a membrane channel. Step 4: Translation resumes, and the growing polypeptide chain is translocated across the membrane. Step 5: Cleavage of the signal sequence by signal peptidase releases the polypeptide into the lumen of the ER.





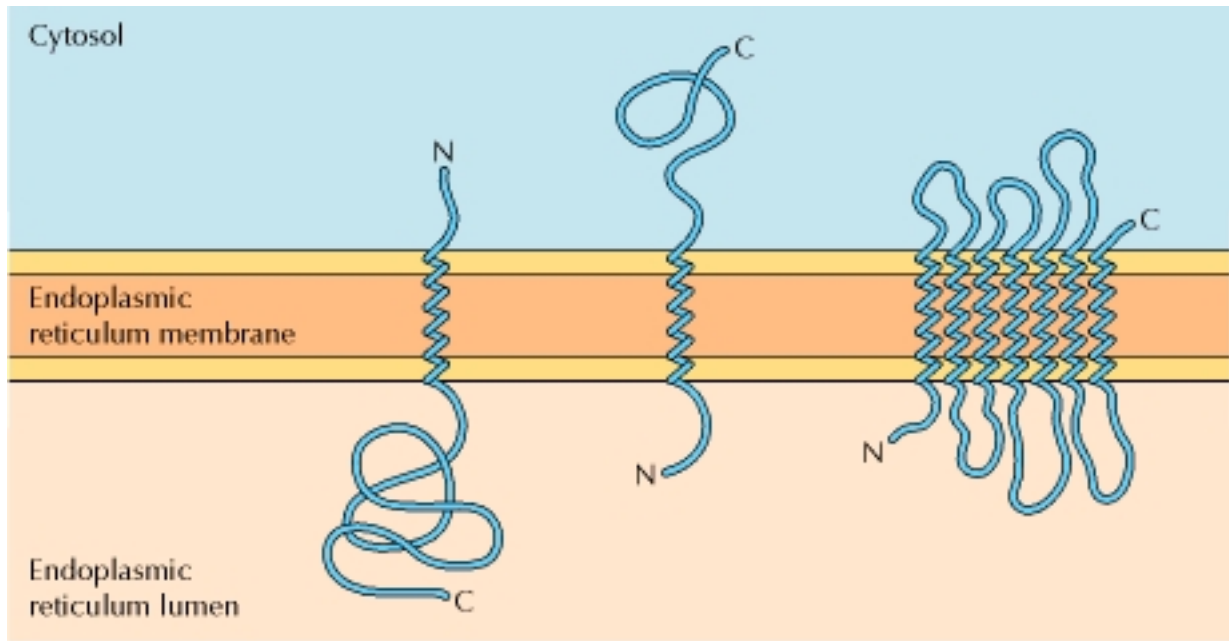
## Insertion of a membrane protein with a cleavable signal sequence and a single stop-transfer sequence

The signal sequence is cleaved as the polypeptide chain crosses the membrane, so the amino terminus of the polypeptide chain is exposed in the ER lumen. However, translocation of the polypeptide chain across the membrane is halted by a transmembrane stop-transfer sequence that closes the Sec61 translocation channel and exits the channel laterally to anchor the protein in the ER membrane. Continued translation results in a membrane-spanning protein with its carboxy terminus on the cytosolic side.

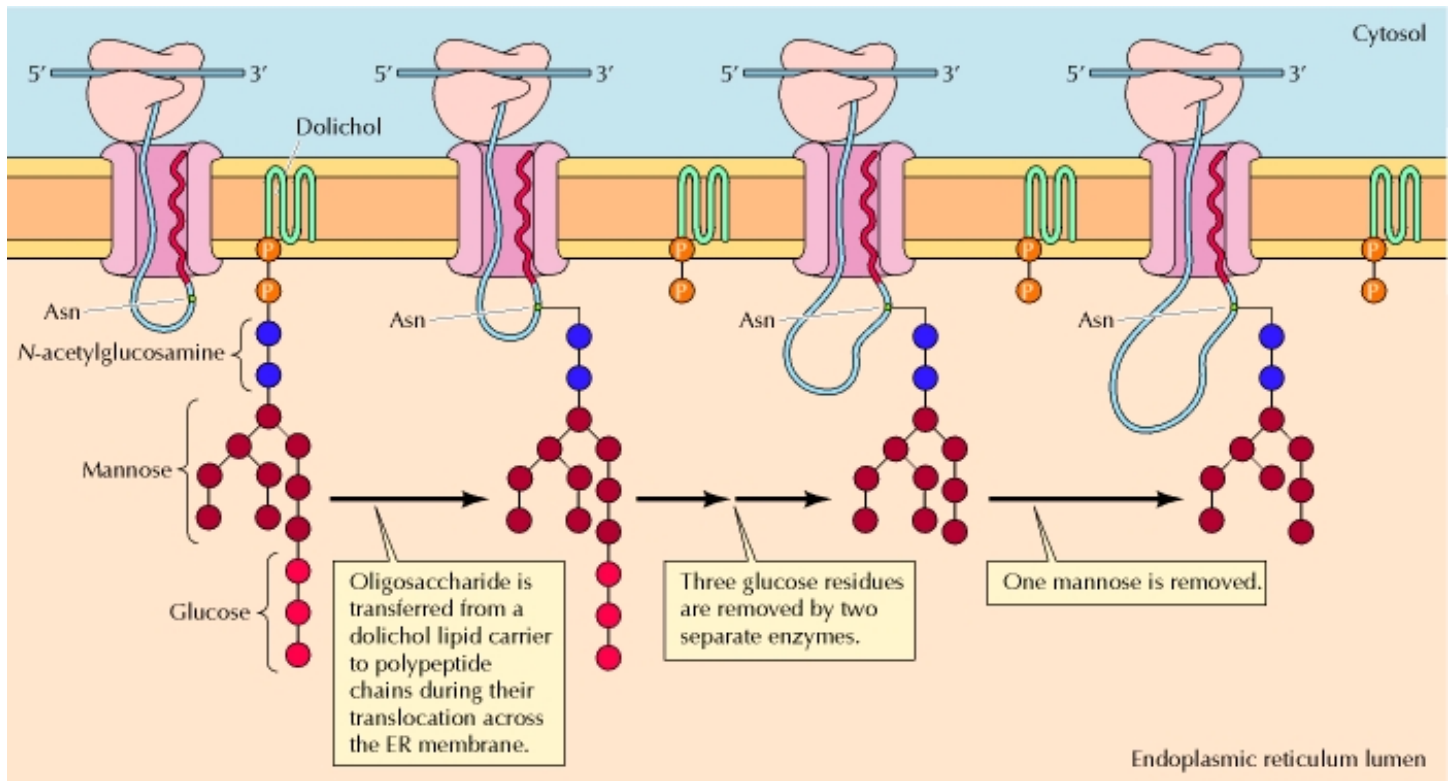


## Insertion of a protein that spans the membrane multiple times

In this example, an internal signal sequence results in insertion of the polypeptide chain with its amino terminus on the cytosolic side of the membrane. A stop-transfer sequence then signals closure of the translocation channel, causing the polypeptide chain to form a loop within the lumen of the ER, and translation continues in the cytosol. A second internal signal sequence reopens the channel, triggering reinsertion of the polypeptide chain into the ER membrane and forming a loop in the cytosol. The process can be repeated many times, resulting in the insertion of proteins with multiple membrane-spanning regions.

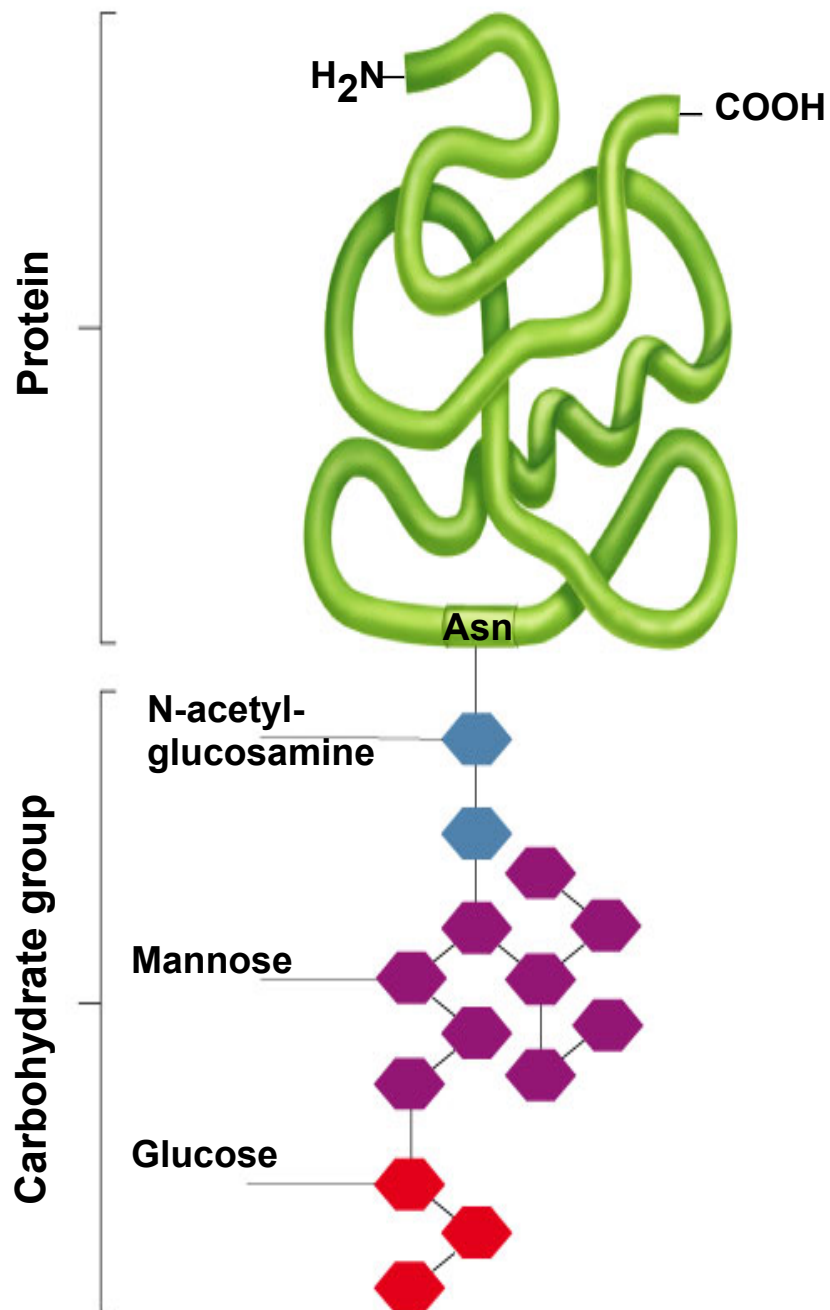


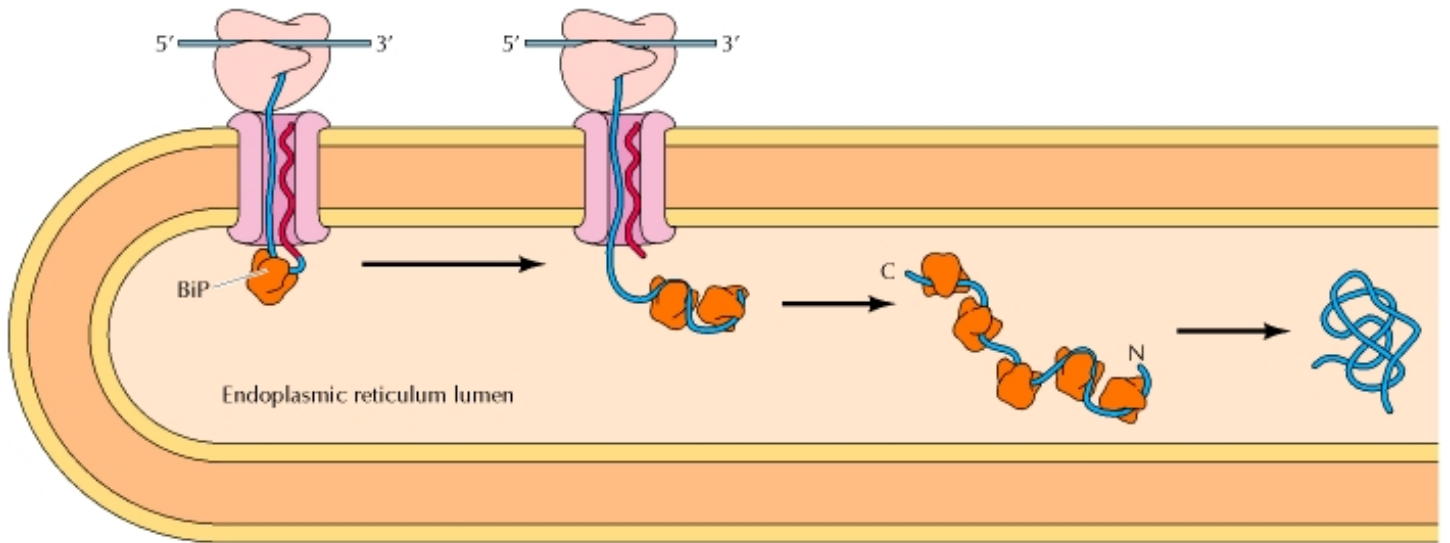
Integral membrane proteins span the membrane via  $\alpha$ -helical regions of 20 to 25 hydrophobic amino acids, which can be inserted in a variety of orientations. The two proteins at left and center each span the membrane once, but they differ in whether the amino (N) or carboxy (C) terminus is on the cytosolic side. On the right is an example of a protein that has multiple membrane-spanning regions.



## Protein glycosylation in the ER

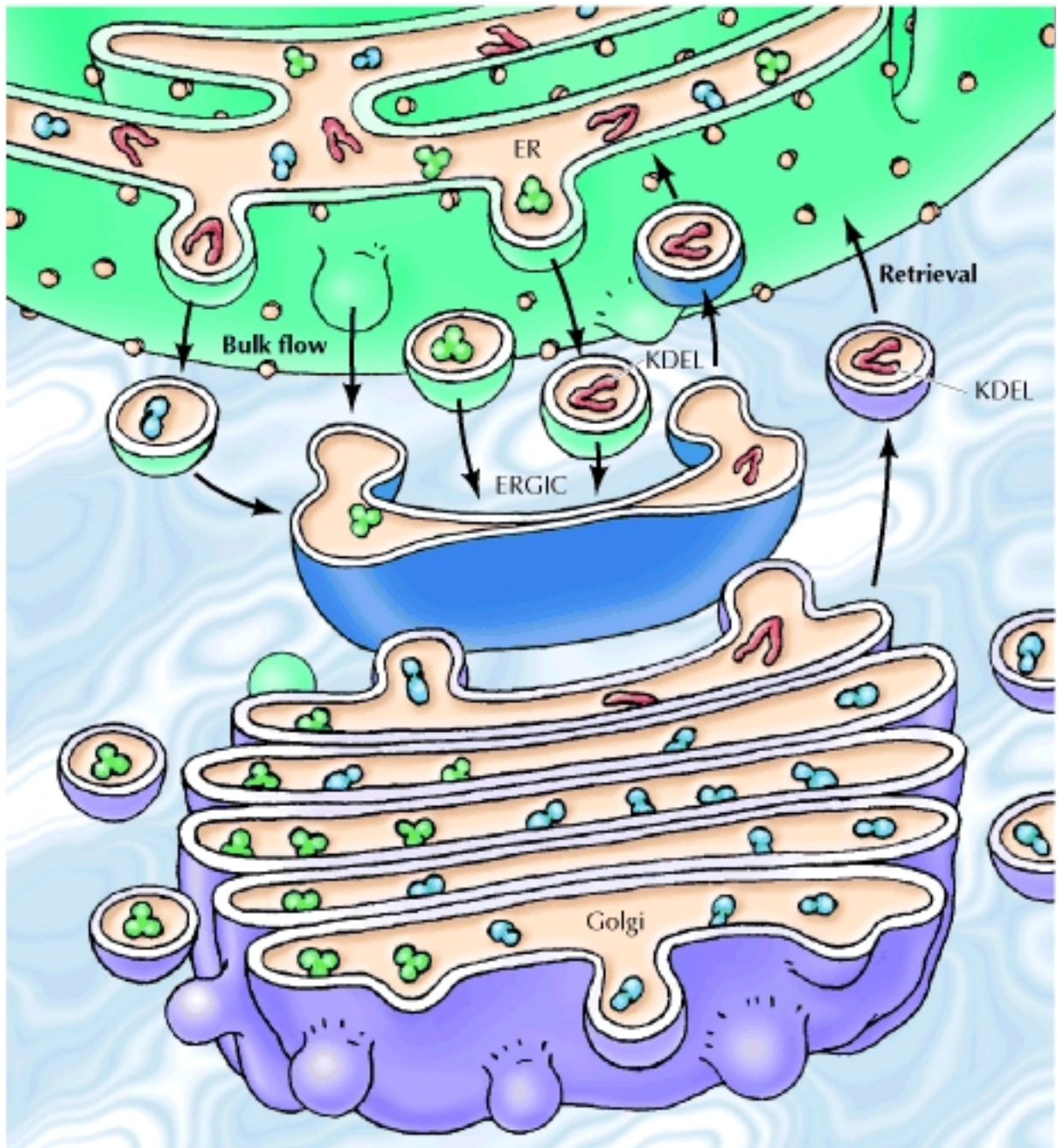
Often associated with transport: "marking" of proteins in the ER **Glycosylation** (sugar-together) involves the addition of carbohydrate monomers >>> glycoproteins





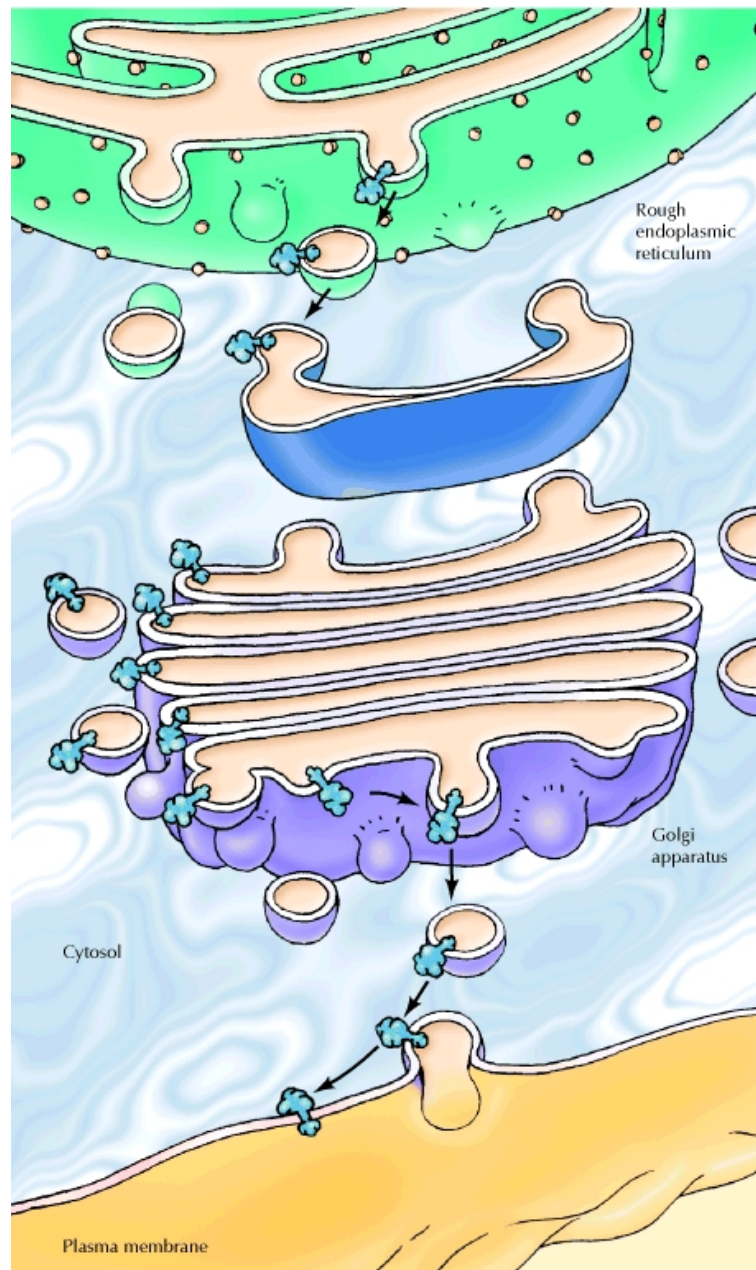
## Protein folding in the ER

The molecular chaperone BiP binds to polypeptide chains as they cross the ER membrane and facilitates protein folding and assembly within the ER.



## Retrieval of resident ER proteins

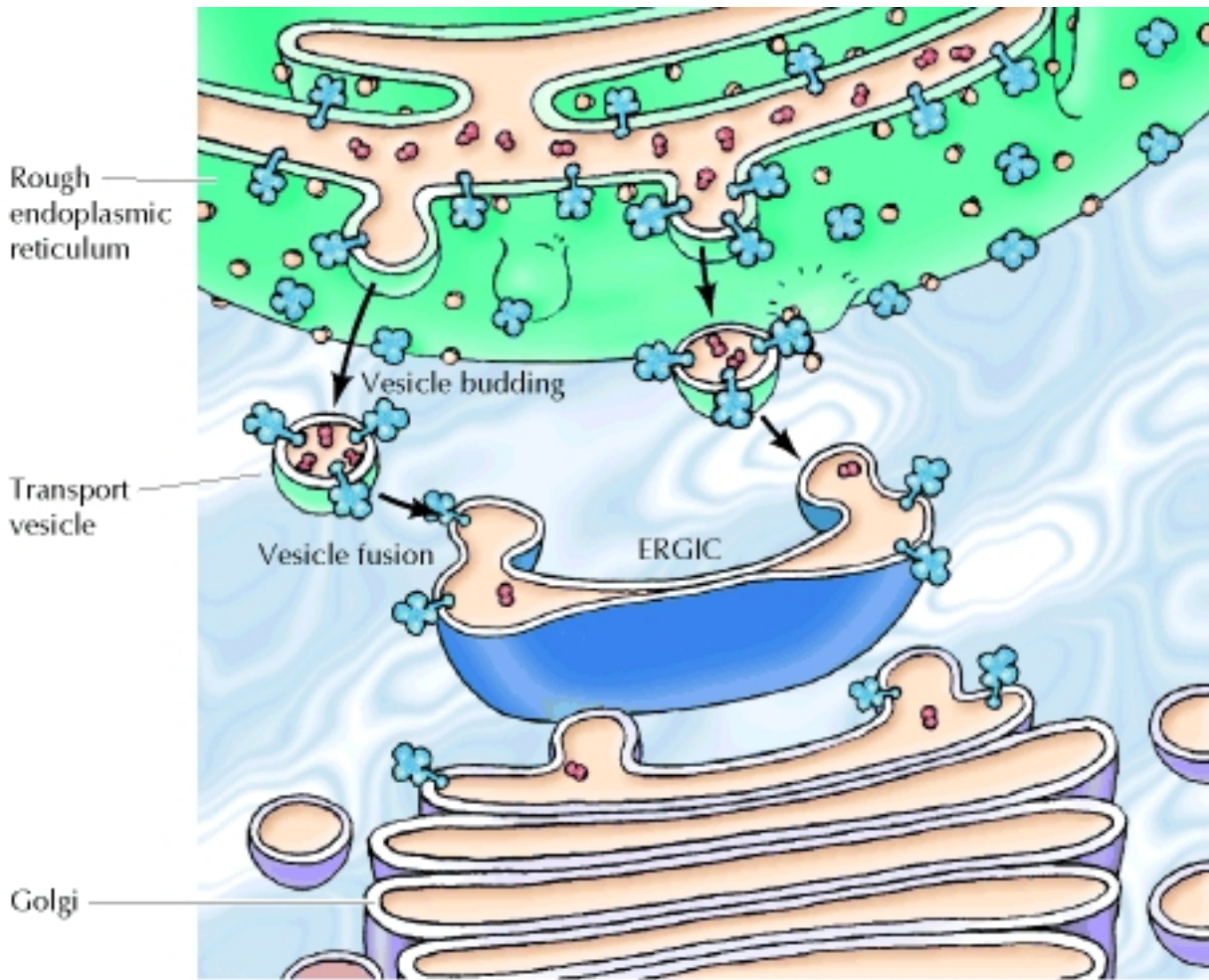
Proteins destined to remain in the lumen of the ER are marked by the sequence **Lys-Asp-Glu-Leu (KDEL)** at their carboxy terminus. These proteins are exported from the ER to the Golgi in the nonselective bulk flow of proteins through the secretory pathway, but they are recognized by a receptor in the ER-Golgi intermediate compartment (ERGIC) or the Golgi apparatus and selectively returned to the ER.



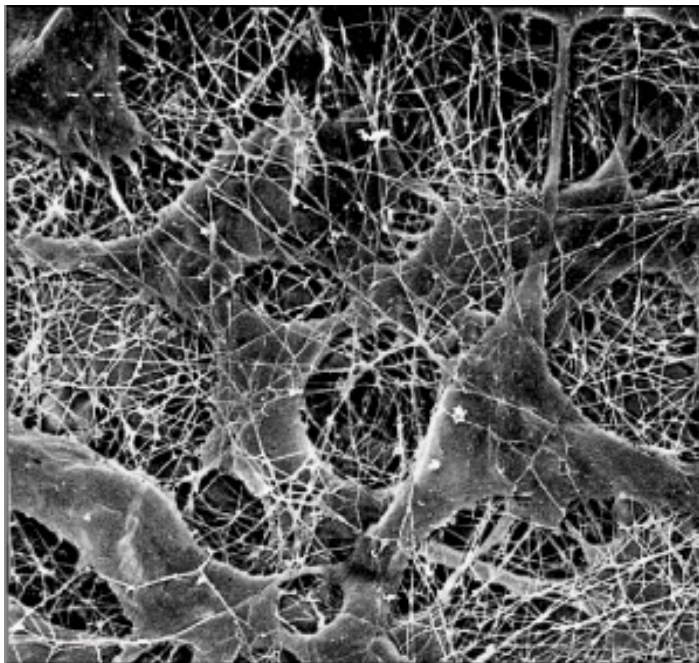
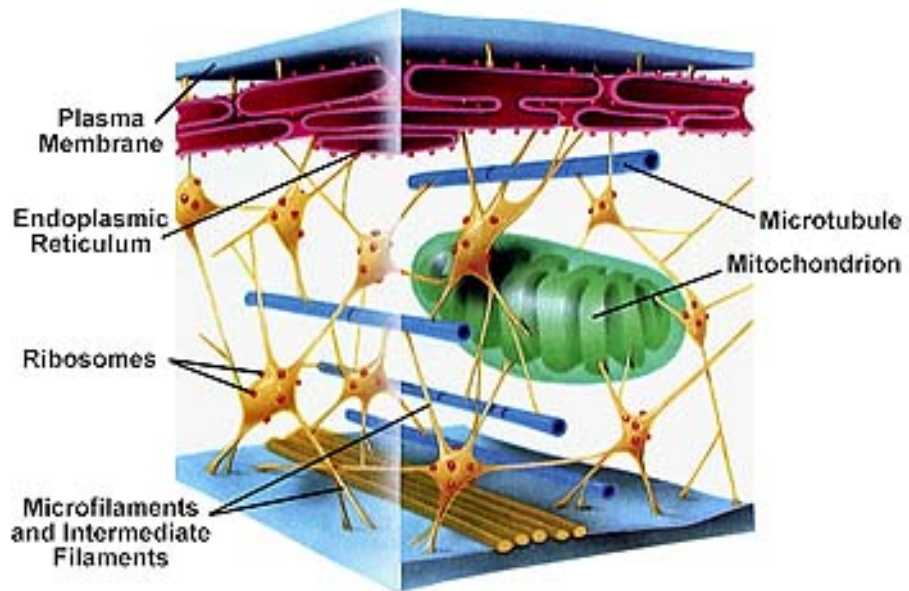
The lumens of the endoplasmic reticulum and Golgi apparatus are topologically equivalent to the exterior of the cell. Consequently, those portions of polypeptide chains that are translocated into the ER are exposed on the cell surface following transport to the plasma membrane.



# Vesicle transport



The cytoskeleton is used to move vesicles



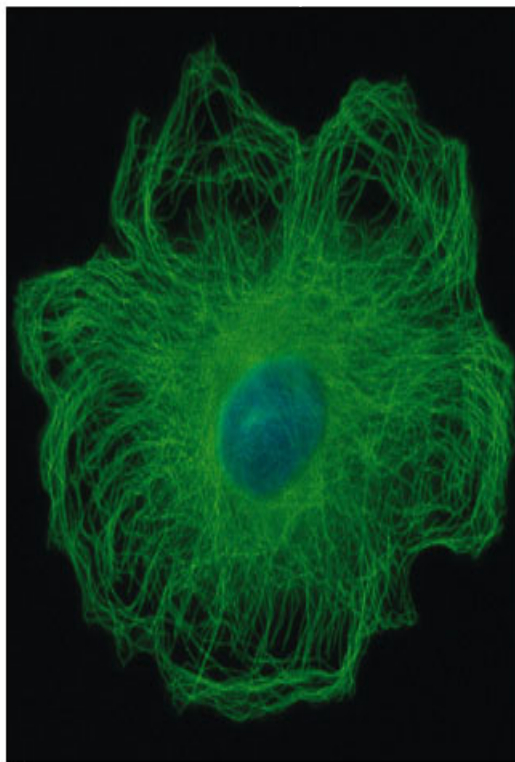
0.1  $\mu\text{m}$

# Cytoskeleton

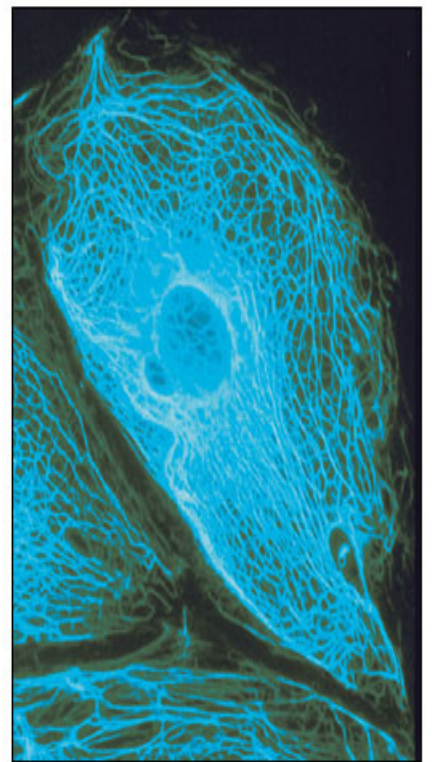


(A)

50 μm



(B)



(C)

## Microfilaments Intermediate filaments

## Microtubules

**Protein subunits**

**Actin**

**Keratin, vimentin, lamin, others**

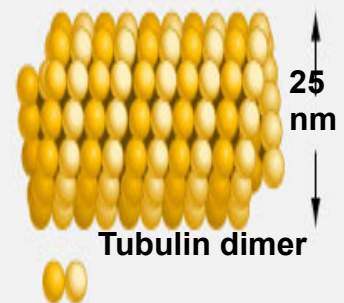
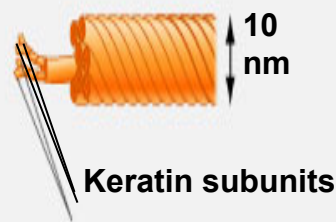
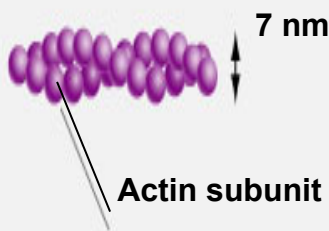
**$\alpha$ -tubulin and  $\beta$ -tubulin dimers**

**Structure**

**Two intertwined strands**

**Fibers wound into thicker cables**

**Hollow tube**



**Functions**

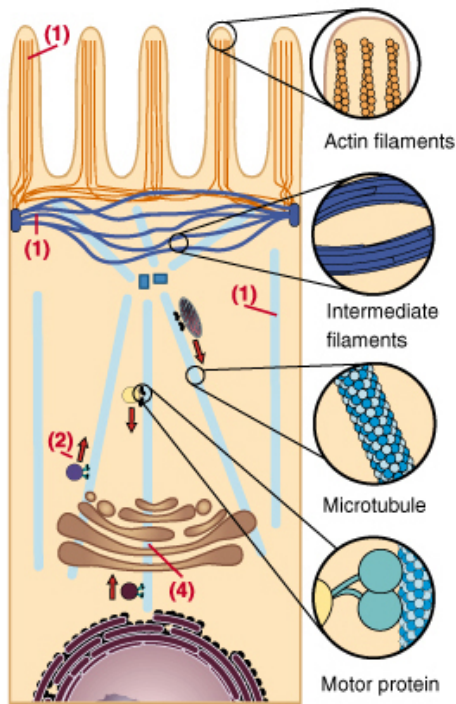
- maintain cell shape by resisting tension (pull)
- motility via pseudopodia
- muscle contraction
- cell division in animals

- maintain cell shape by resisting tension (pull)
- anchor nucleus and some other organelles

- maintain cell shape by resisting compression (push)
- motility via flagella or cilia
- move chromosomes during cell division
- move organelles

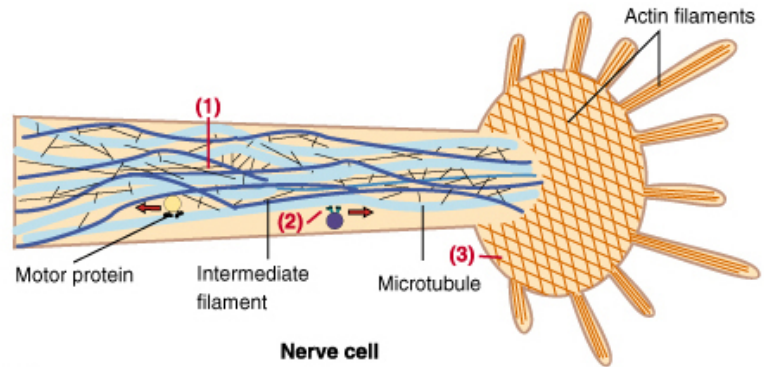
## Key to Cytoskeletal Functions

**(1)** Structure and Support    **(2)** Intracellular Transport    **(3)** Contractility and Motility    **(4)** Spatial Organization



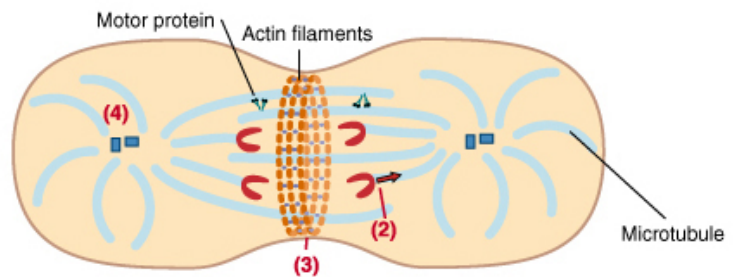
(a) **Epithelial cell**

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**Nerve cell**

(b)



**Dividing cell**

(c)

# Internal cell movement

Grazie ai motori molecolari che lavorano in associazione con il citoscheletro

Tre famiglie:

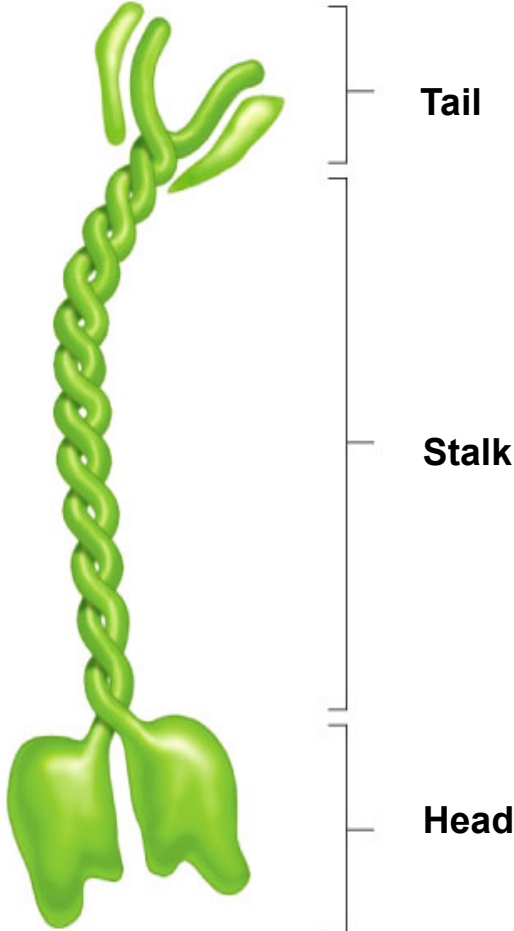
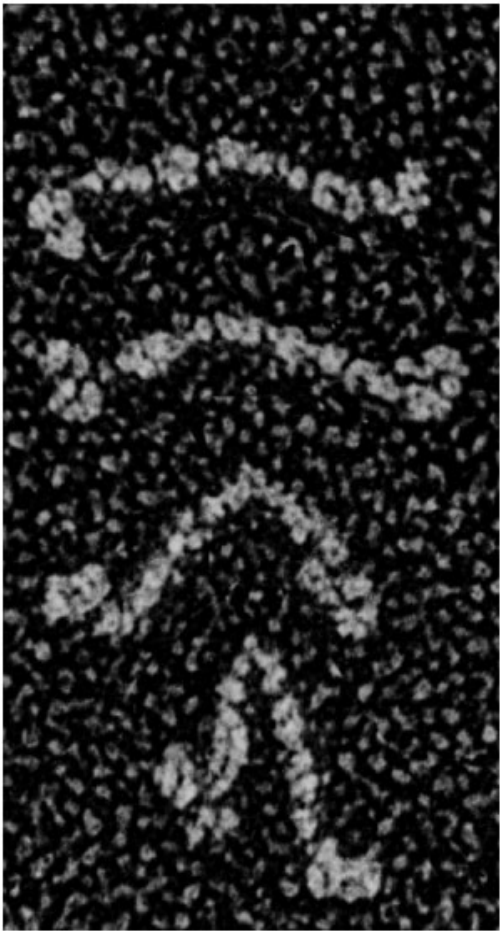
**Miosine** (si muovono lungo i microfilamenti)

**Chinesine** (si muovono lungo i microtubuli)

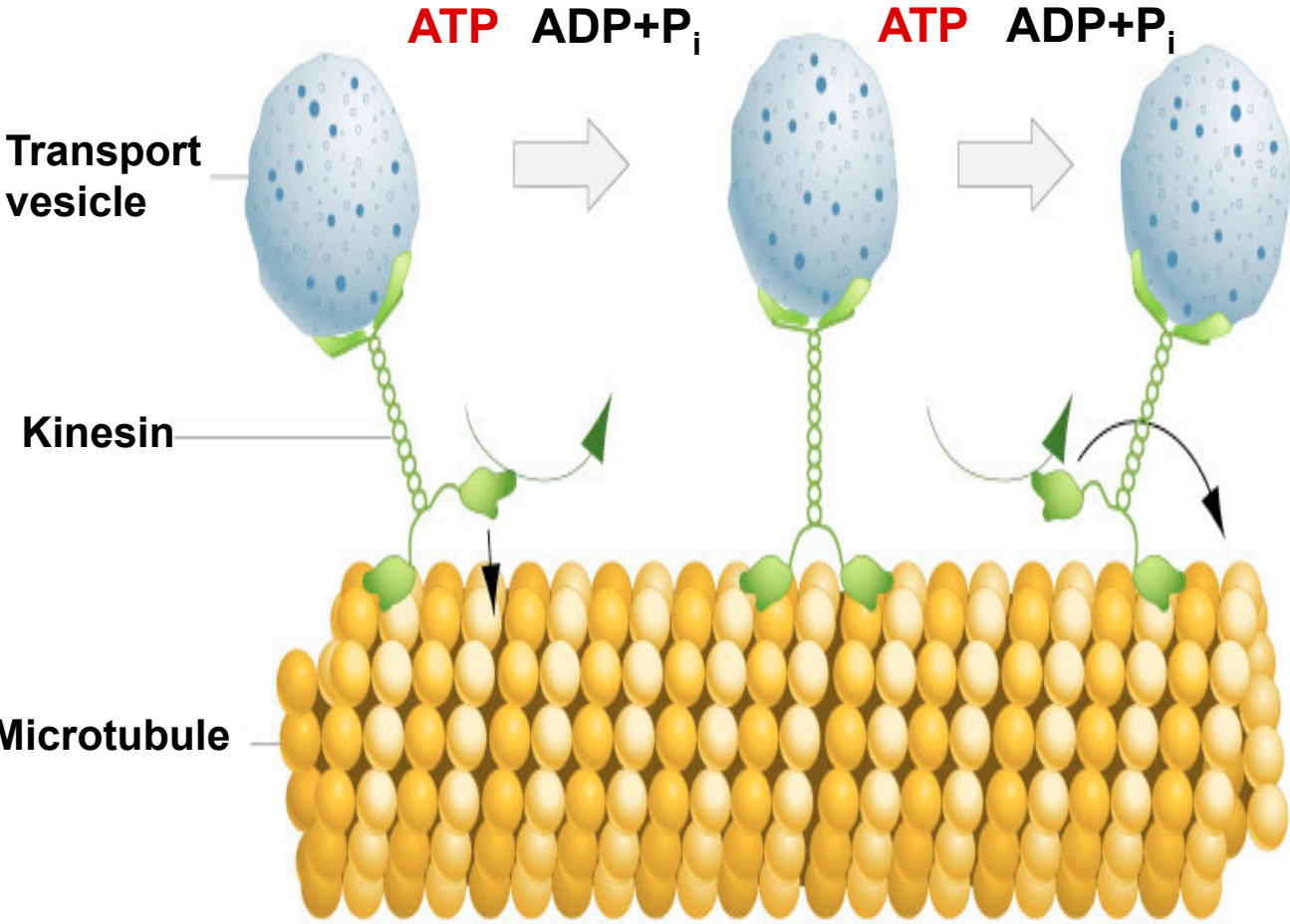
**Dineine** (si muovono lungo i microtubuli)

Le proteine motore trasformano l'energia chimica (**ATP**) in energia meccanica **per muovere** carichi cellulari (**vescicole, mitocondri, lisosomi, cromosomi** etc..)

Structure of **kinesin**: one mechanism of movement

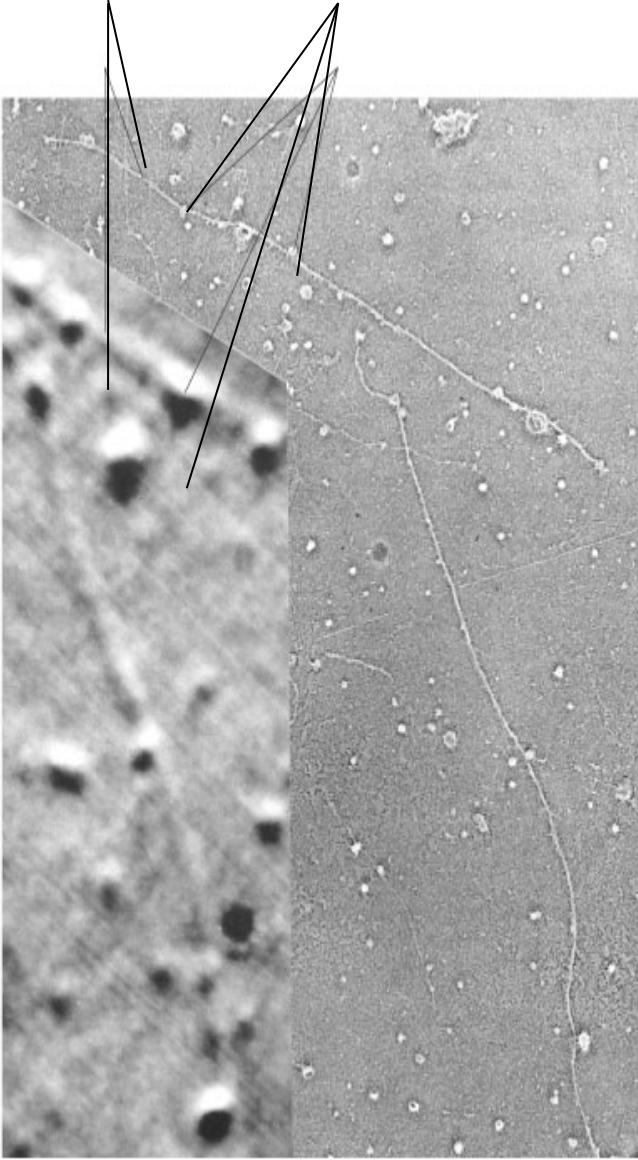


# Kinesin "walks" along a microtubule track

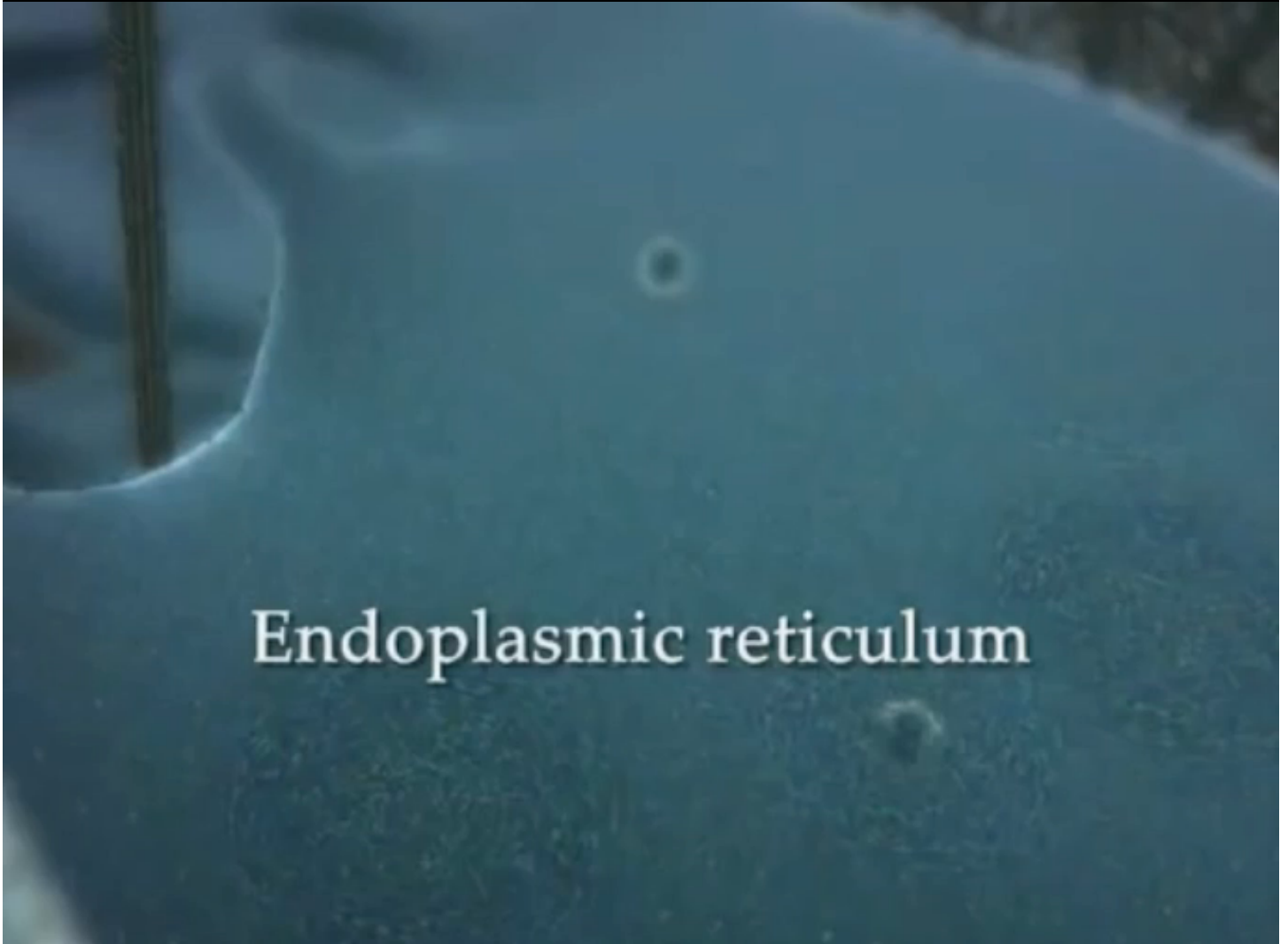




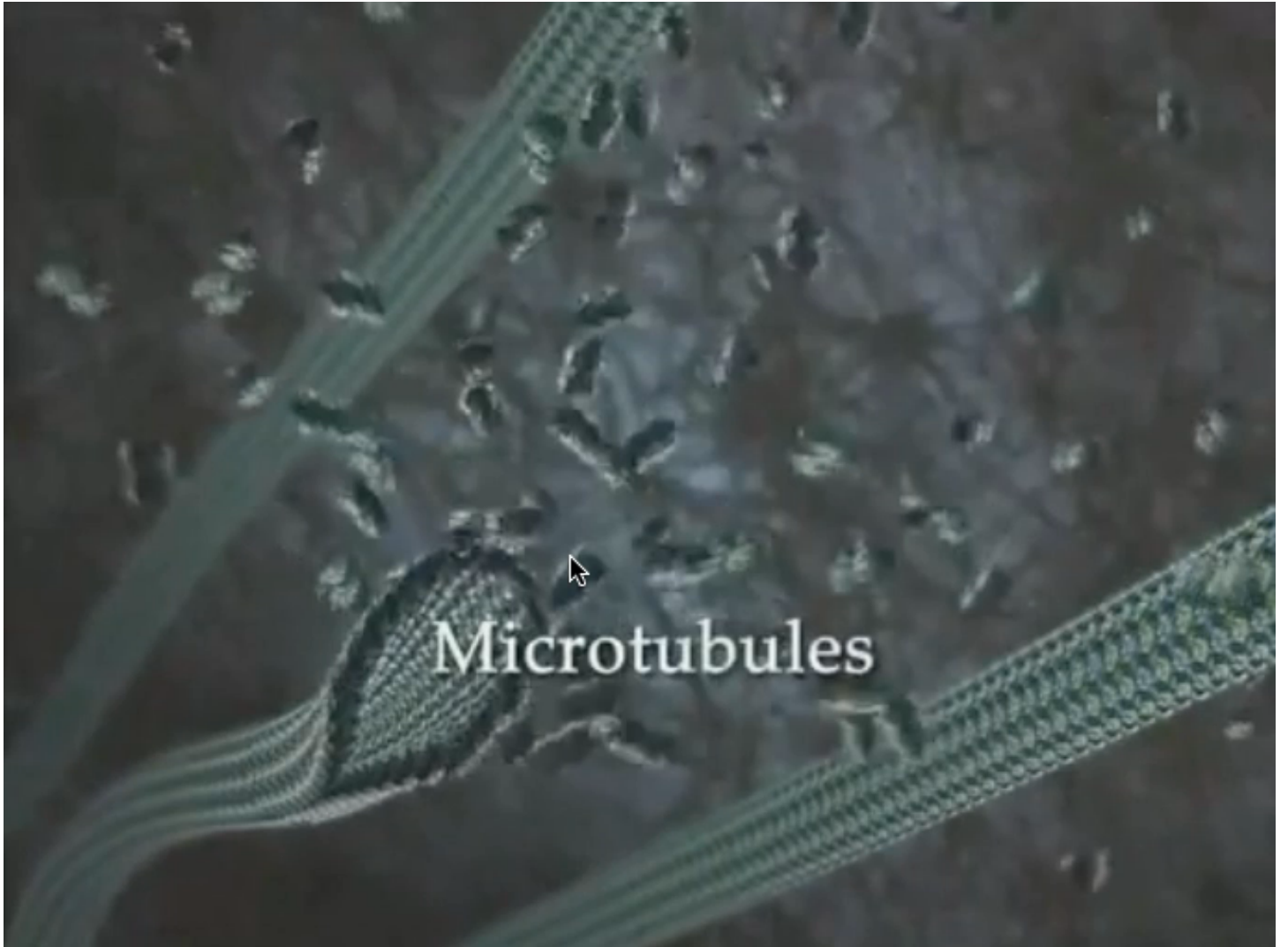
**Microtubule tracks**      **Vesicles**

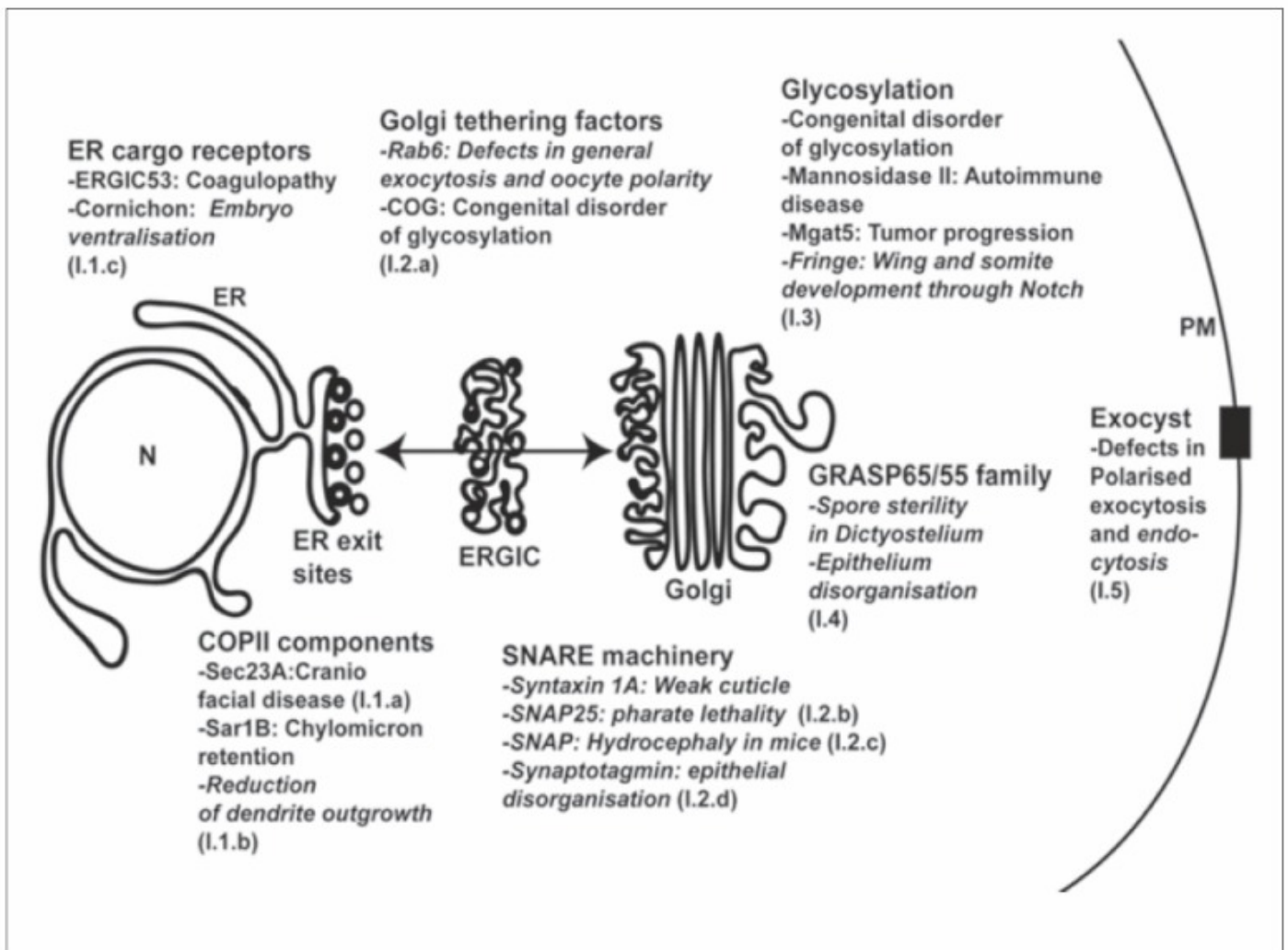


# Vesicle transport



Endoplasmic reticulum





Schematic representation of the exocytic pathway and a summary of mammalian genetic diseases and developmental defects (in italics) occurring when genes encoding for proteins functioning in this pathway are mutated.