Molecular biology of the cell
BIO 207

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Intracellular Compartments and Protein Sorting

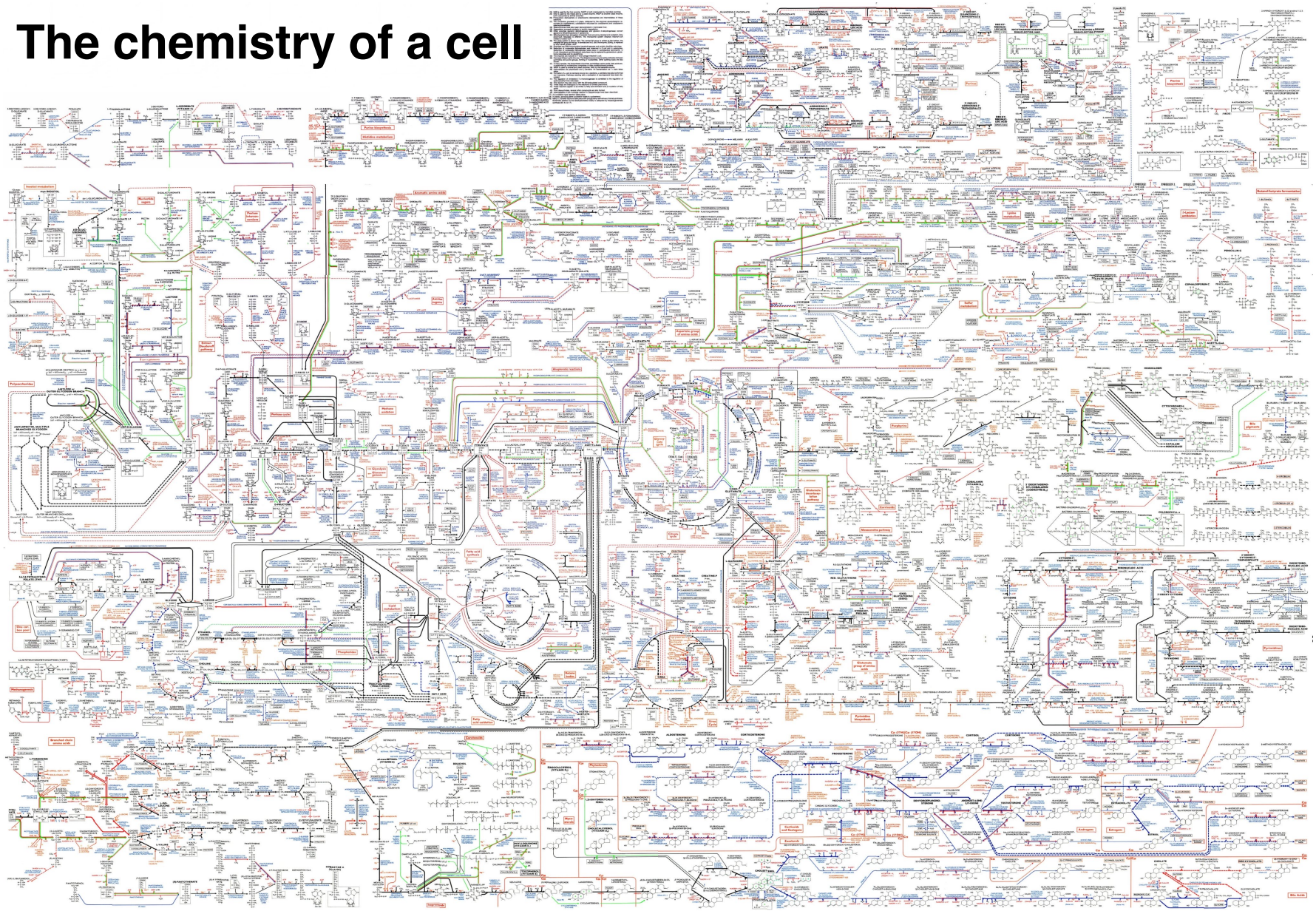
CHAPTER 12

IN THIS CHAPTER

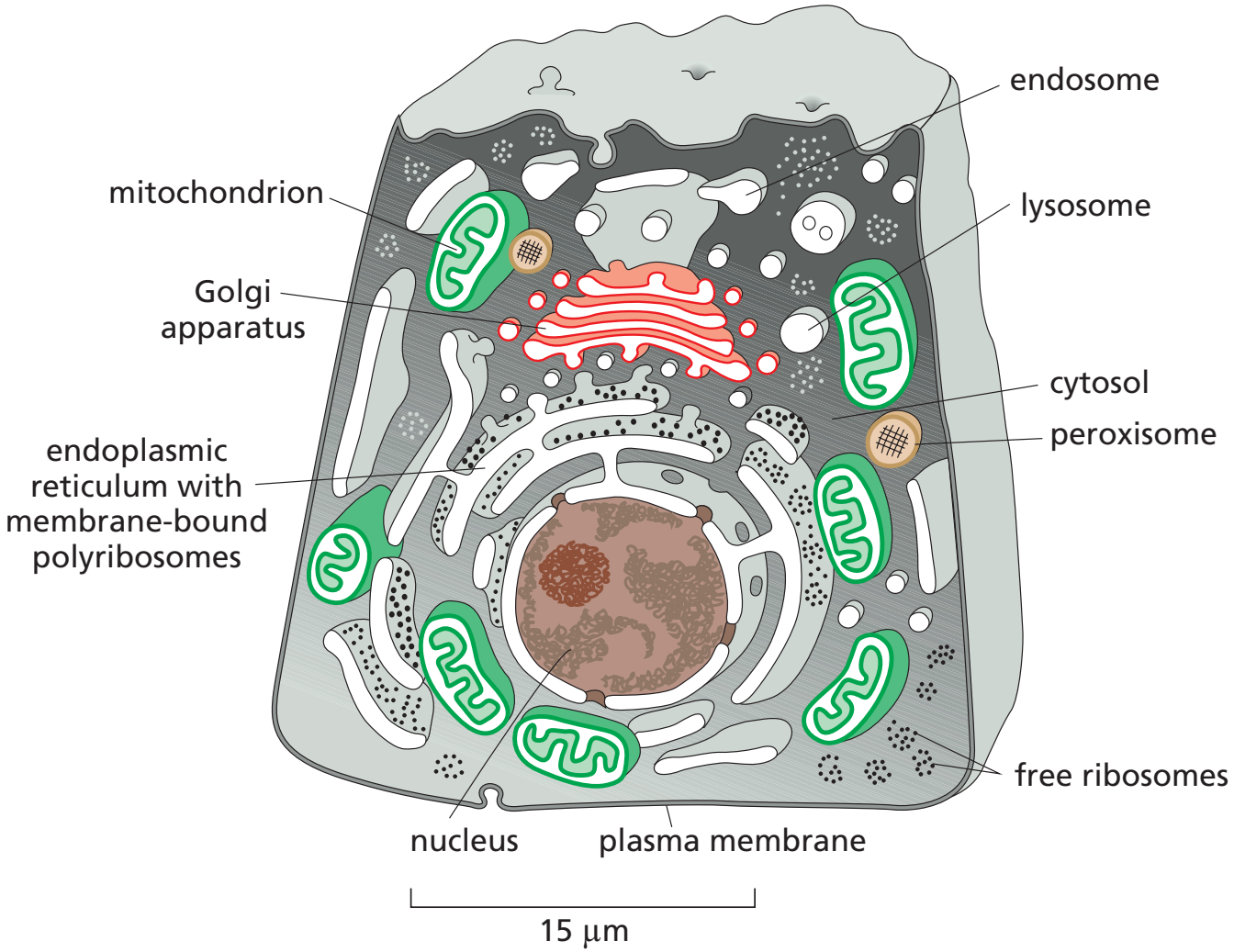
Goals for the coming 3 lectures

i. To know the cellular compartments and their main functions
ii. Have an idea of the distribution of membranes across compartments and their size and quantity
iii. Know there are differences in lipid composition per compartment
iv. Understand the topology of the compartments
v. Know that there are two main forms of exchange between between compartments: by contact and by
vi. vesicular transport
vii. Understand the contribution of vesicular transport to the orientation of proteins and lipids

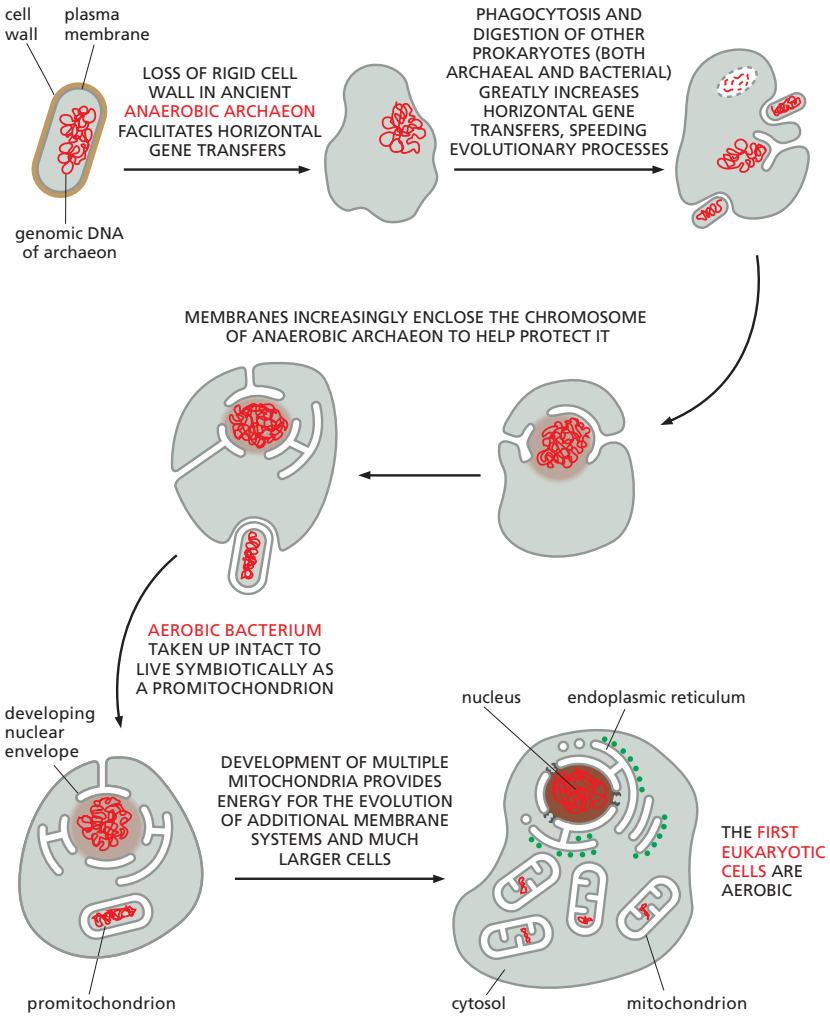
The chemistry of a cell



Intracellular compartments localize the chemistry in an animal cell

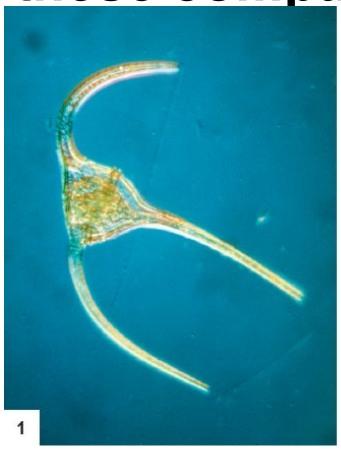


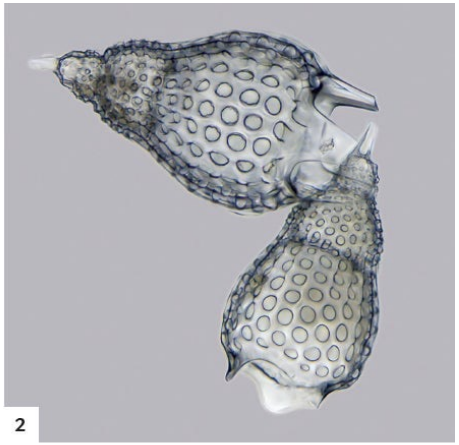
How did these compartments come to be?

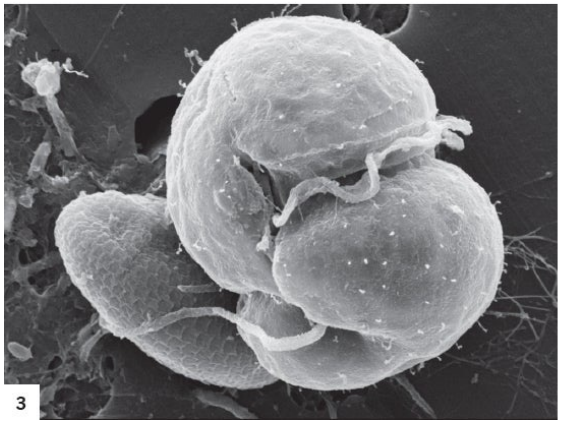


How did these compartments come to be?

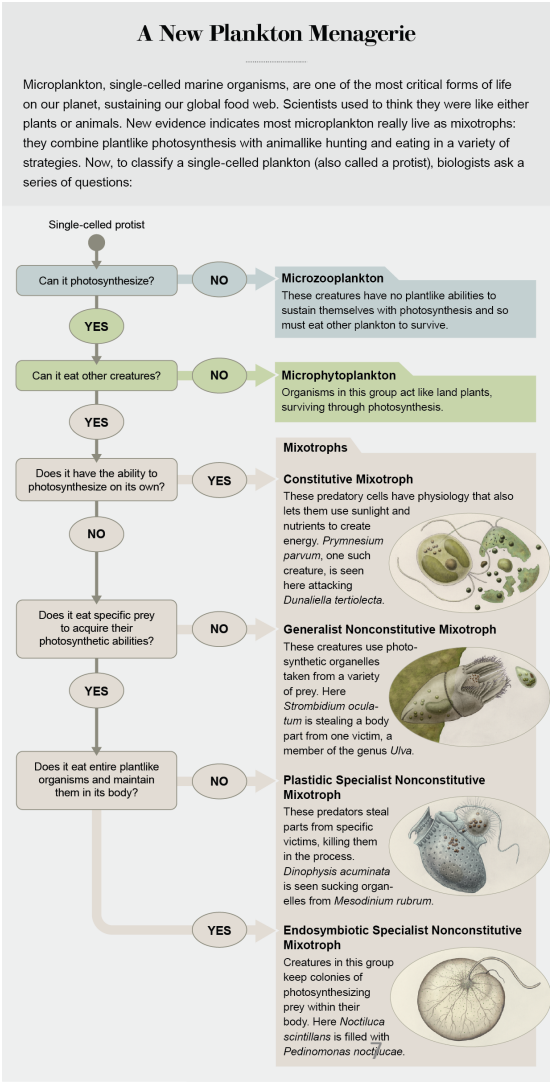
One mixotroph, *Dinophysis* (*right*), sucks photosynthesizing organelles from another, *Mesodinium.* Credit: *Mark Ross Studios*





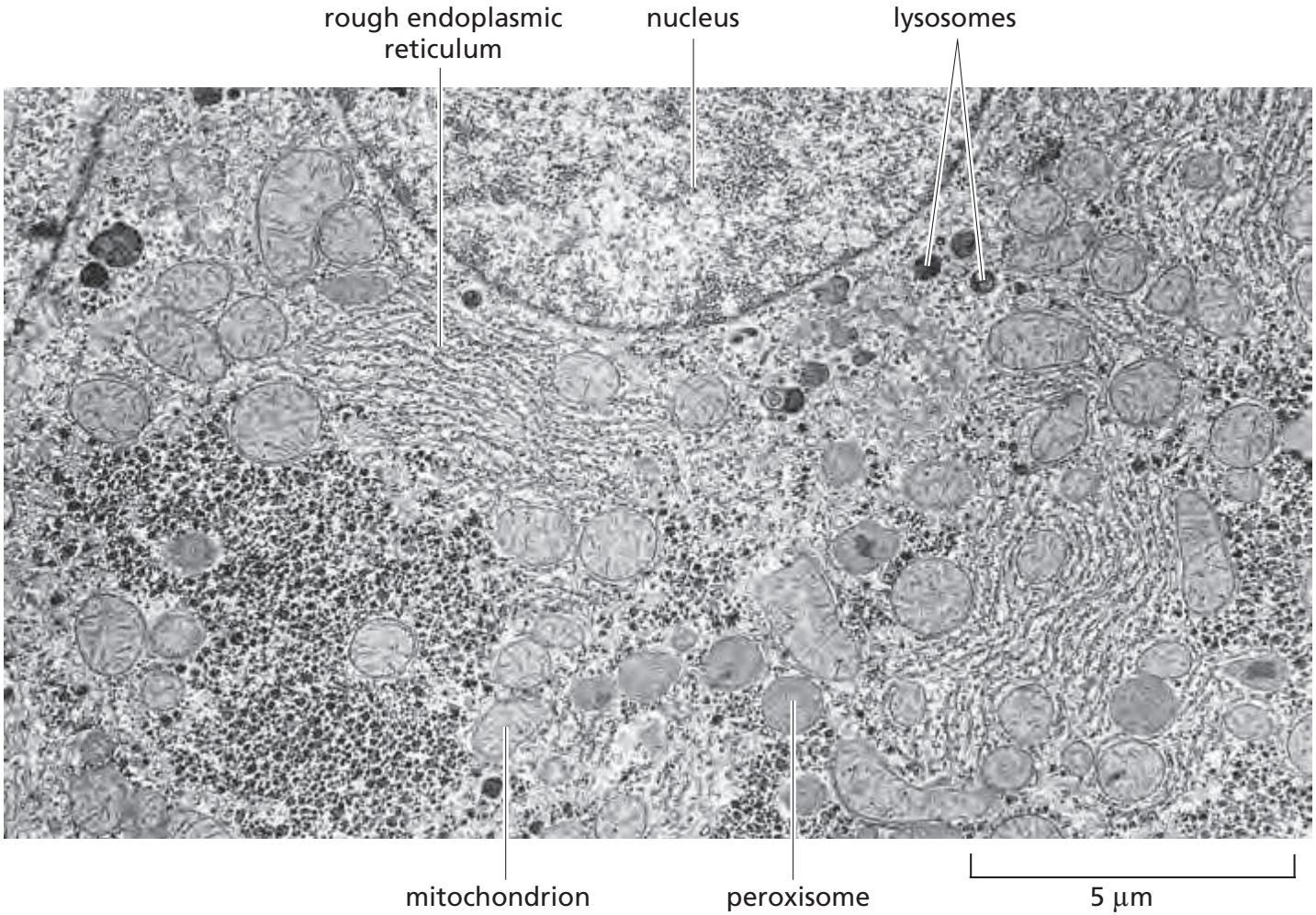


Mixotrophs come in several varieties. Some, such as *Tripos longipes* (*1*), can photosynthesize on their own and eat prey. But members of the order Nassellaria (*2*) steal photosynthetic organs from plankton victims. Species of *Karlodinium* (*3*) act as *T. longipes* does. Credit: Eric Grave *Science Source (1)*; Frank Fox *mikro-foto.de (2)*; Vincent Lovko *Mote Marine Laboratory (3)*



Not part of exam, just curiosity!

Organelles and cellular compartments are densely packed



Cellular compartments

Compartmentalization allows to better regulate metabolic processes, optimize biochemical reactions, sequester toxic products resulting from chemical reactions, etc...

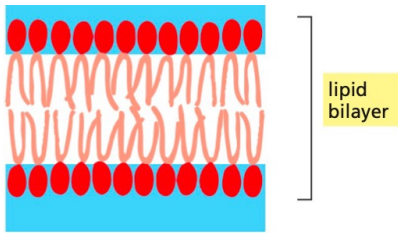
Course focused on the eukaryotic cell

Differences in
function
form
subcellular location
protein composition
lipid composition
ionic composition, e.g:

- pH can vary from 7.2 in the ER to<5 in lysosomes
- Calcium: from nM in the cytoplasm to µM in the ER
- Redox potential: reducing in the cytoplasm or oxidizing in the ER

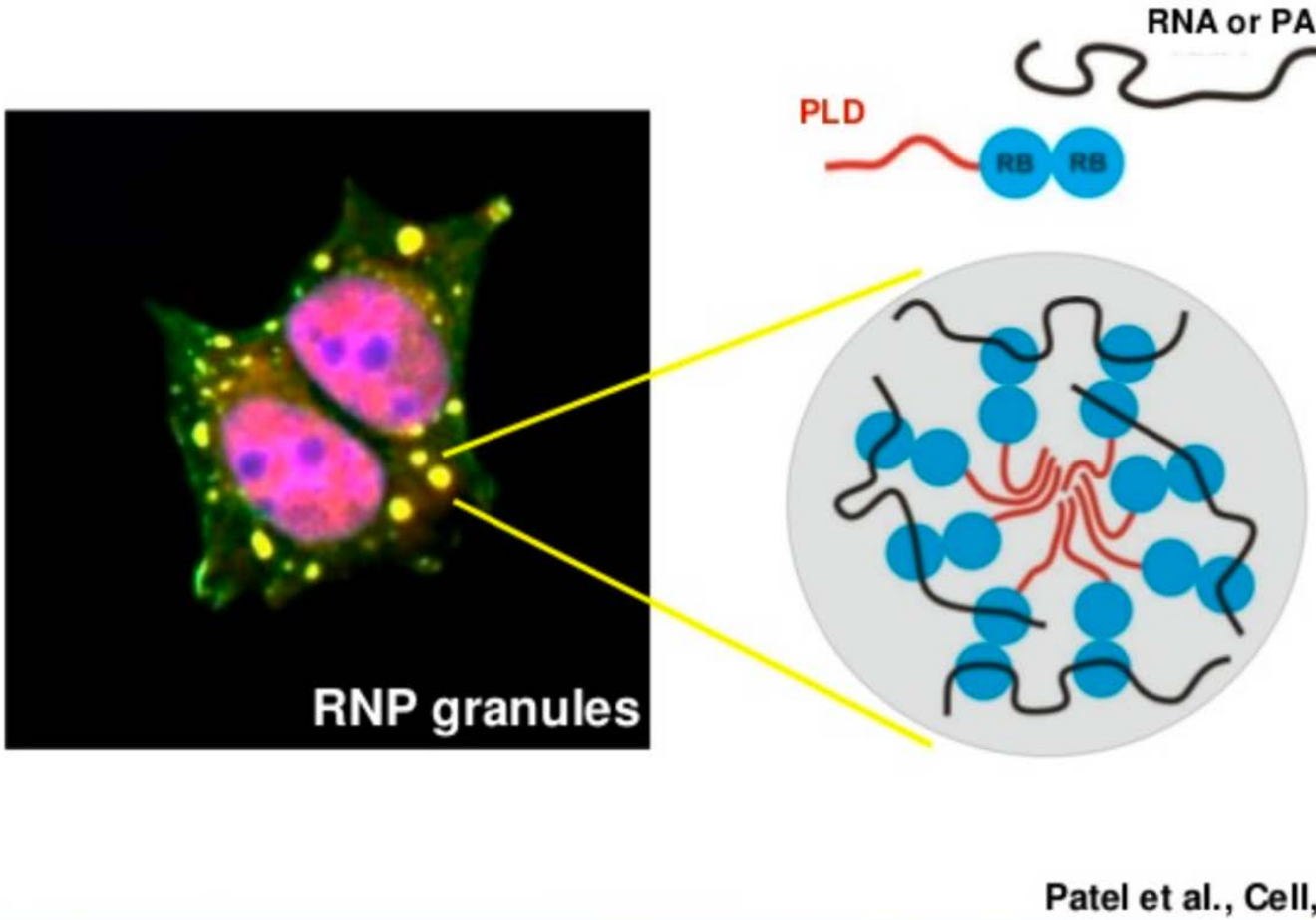
Two kinds of organelles

Membrane
Endoplasmic Reticulum
Nucleus
Golgi System
Peroxisomes
Mitochondria
Lysosome
Endosome



Membrane-less
P-Bodies
Stress Granules
Nucleolus
Many more to be discovered?

Liquid Liquid Phase separation of membraneless compartments

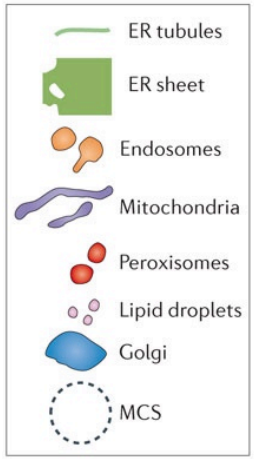


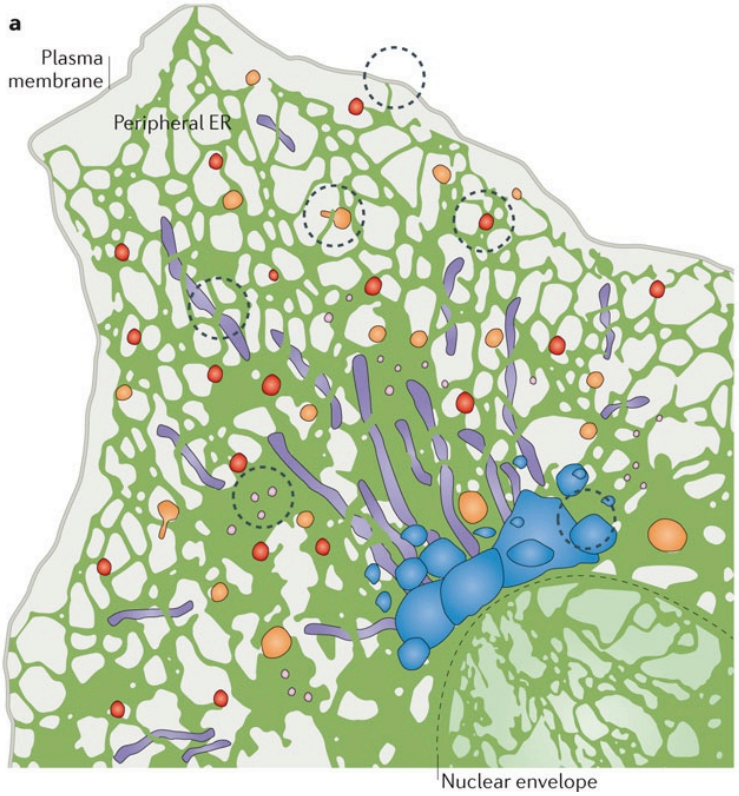
Patel et al., Cell, 2015.

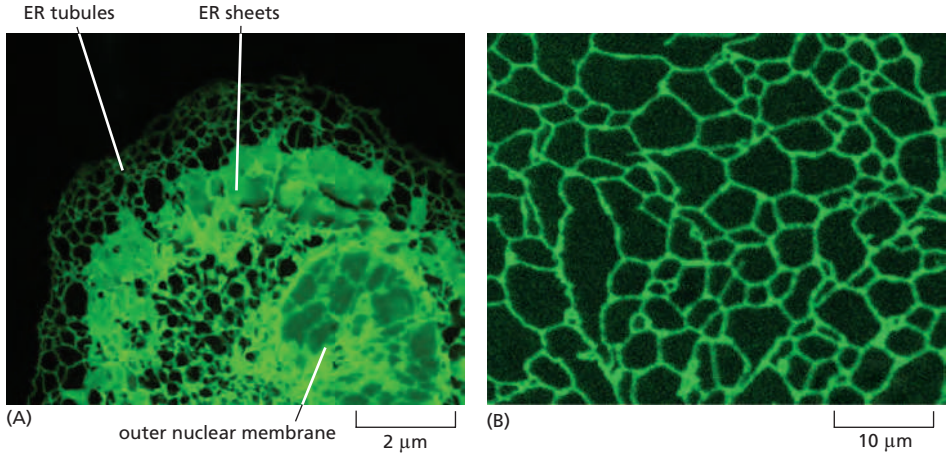
Membraneless organelles

What kind of membrane containing organelles (Endomembrane system) can we find in a cell?

Endoplasmic reticulum







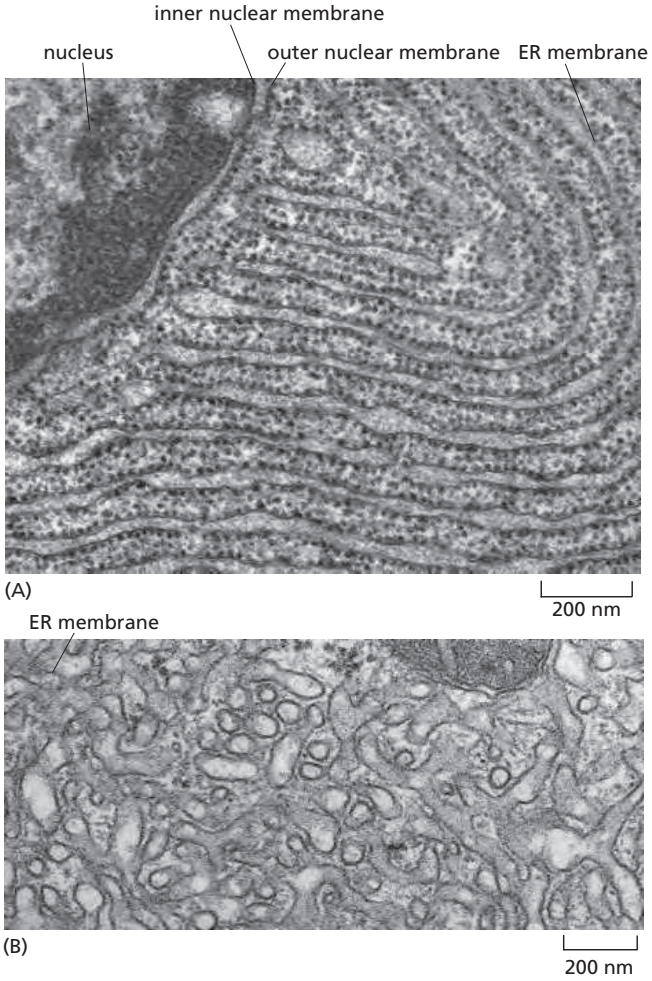
ER lumen = ER cisternal space

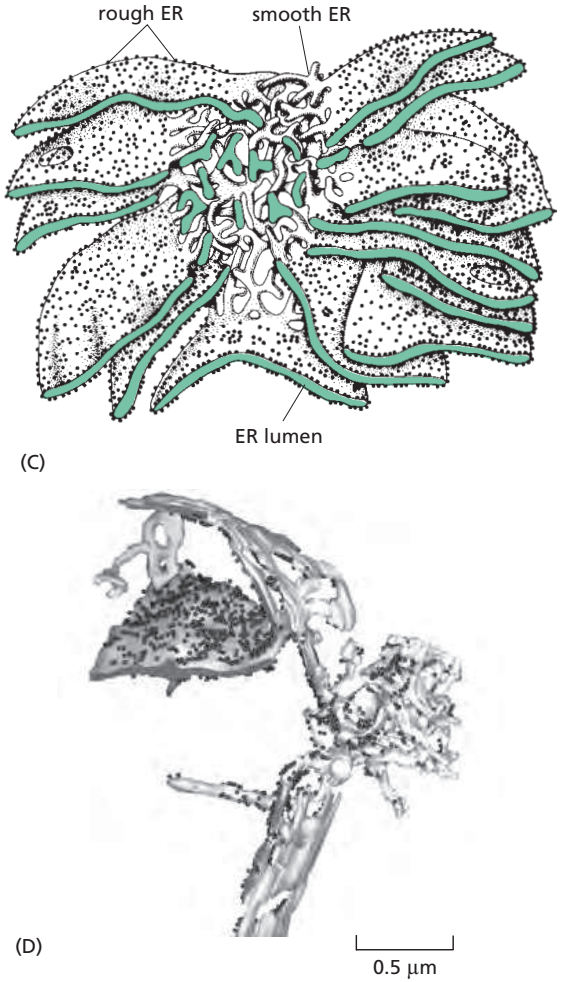
Lipid & Protein Biosynthesis

Site of production of all transmembrane proteins and lipids for all the organelles

Ca2+ storage in the cell (SERCA pump discussed last week!)

The rough and smooth ER

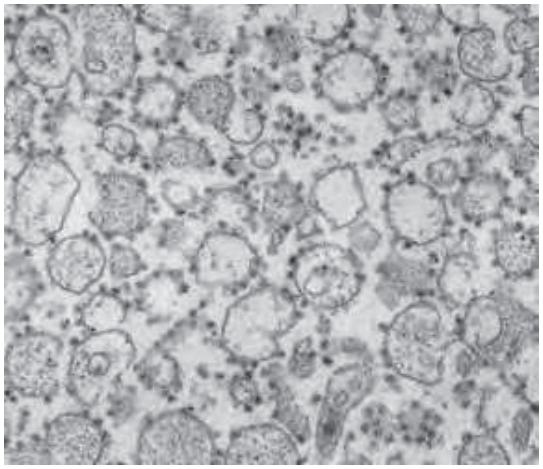




Membrane bound ribosomes = Rough ER

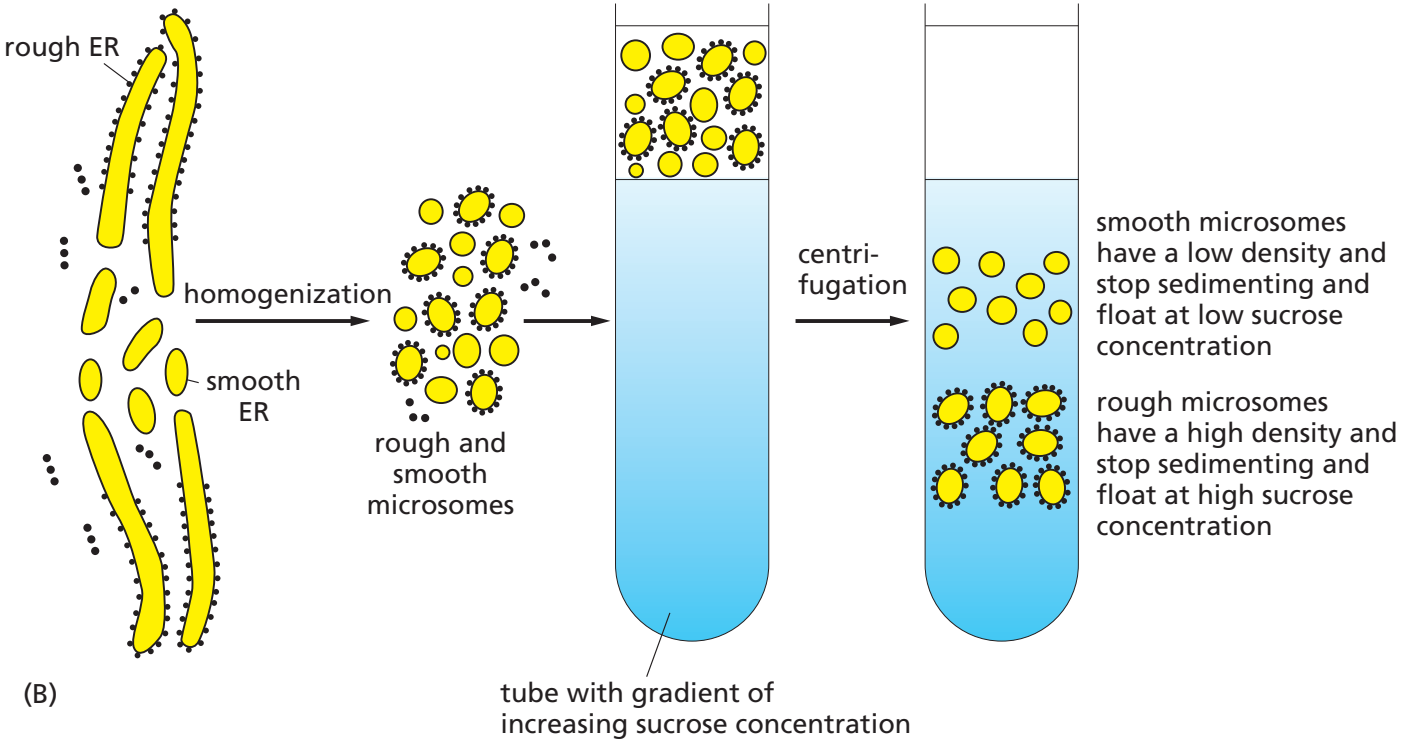
Most cells have both, but ratio differs per cell type

The isolation of purified rough and smooth microsomes from the ER

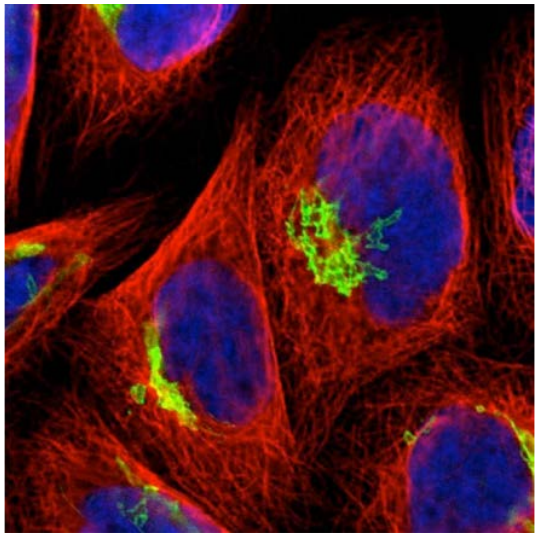


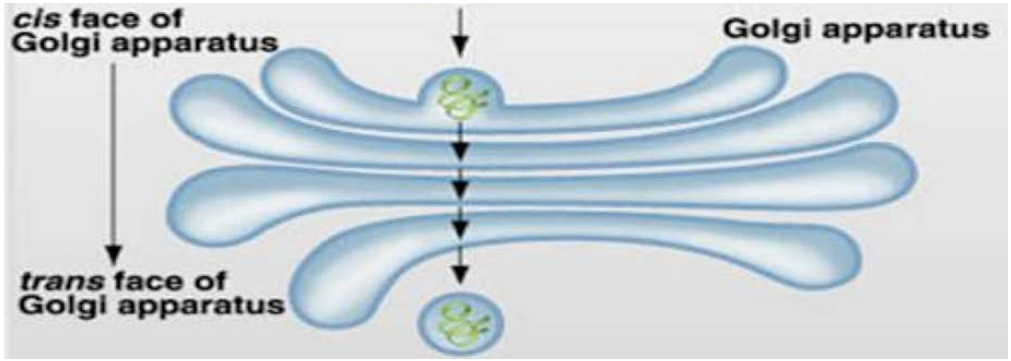
(A)

200 nm



The Golgi system



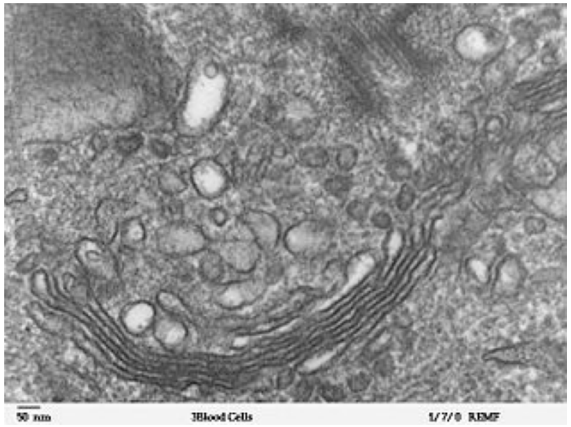


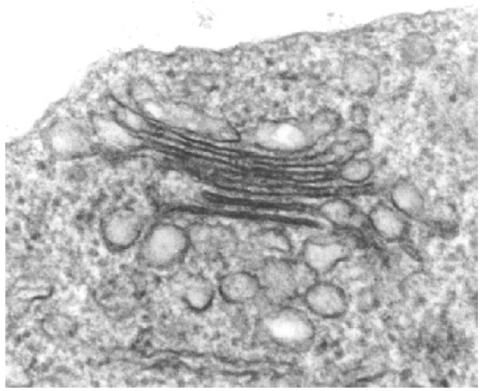
Collection and dispatch station of protein products received from the (ER). Proteins synthesized in the ER are packaged into vesicles, which then fuse with the Golgi apparatus.

Much of the enzymatic processing is post-translational modification of proteins.

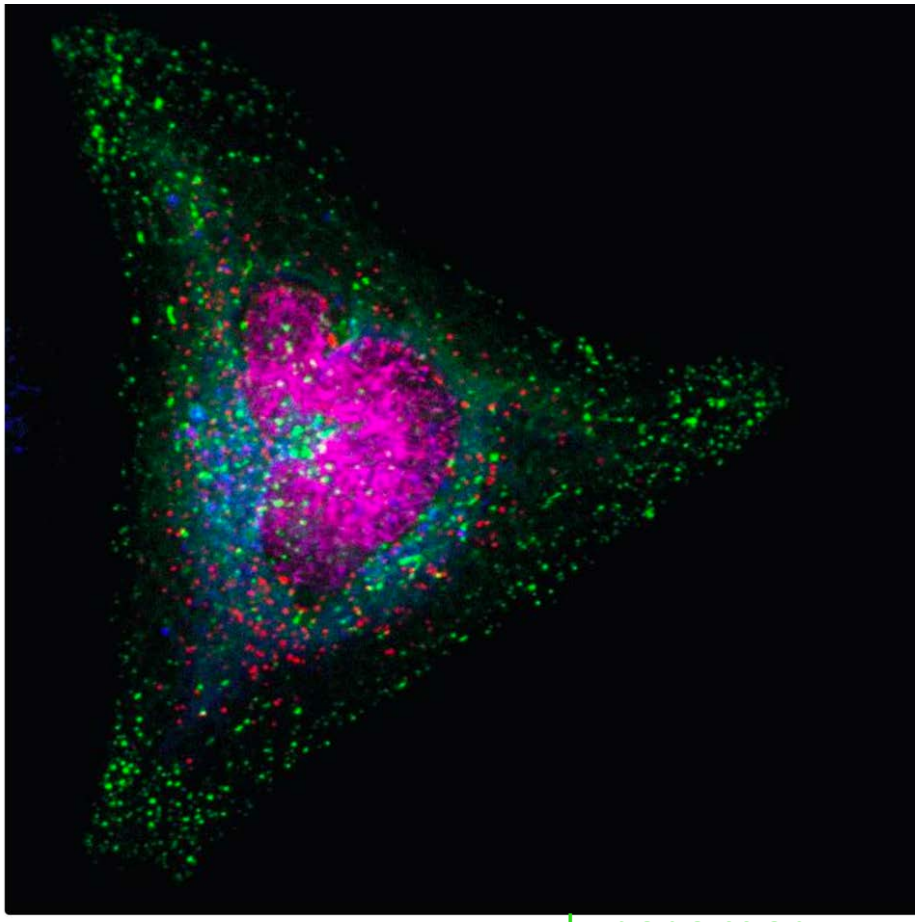
Post-translational modifications of proteins include glycosylation and phosphorylation

These modifications can be a signal for the final destination of the proteins





Lysosomes



Co-localization while using LysoTracker® Deep Red.
HeLa cell expressing CellLight® Peroxisome-RFP and CellLight® Early Endosome-GFP was loade
with 50nM LysoTracker® Deep Red and Hoechst 33342. Images were pseudo colored as Hoechs
33342 (Magenta), LysoTracker® Deep Red (Blue), CellLight® Early Endosome-GFP (green) an
CellLight® Peroxisome-RFP (Red).

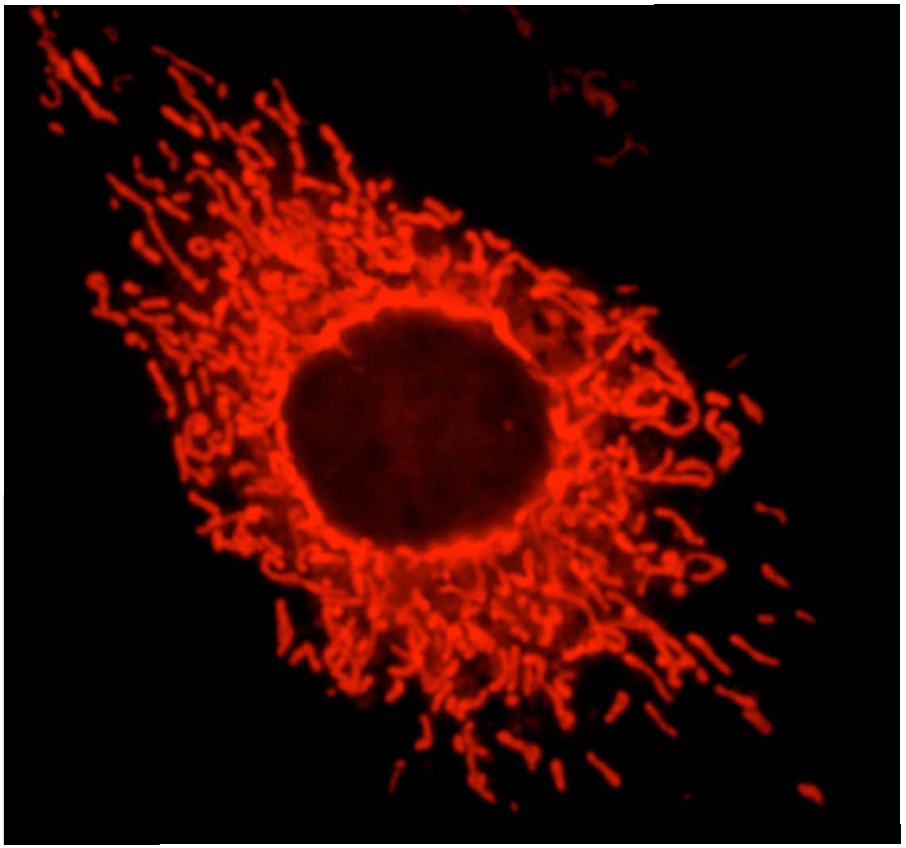
The lumen's pH (~4.5–5.0) is optimal for the enzymes involved in hydrolysis, analogous to the stomach

Lysis of proteins peptides, nucleic acids, carbohydrates and lipids from inside and outside of the cell

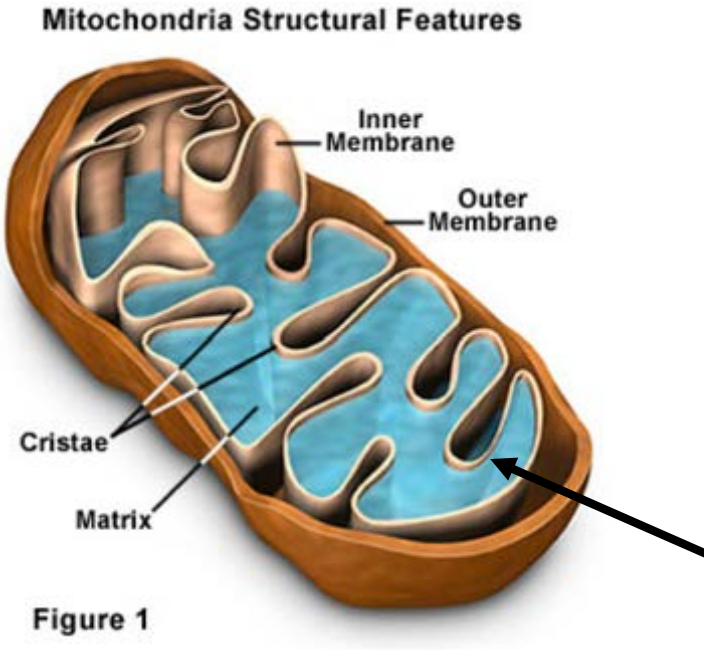
Lysosomes are known to contain more than 60 different enzymes, and have more than 50 membrane proteins

Size is highly variable

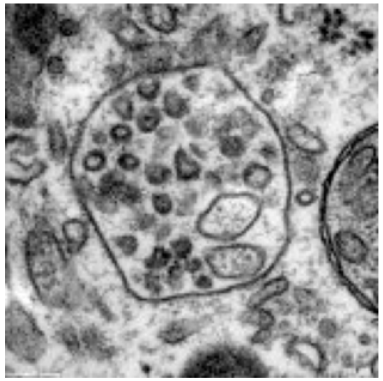
Mitochondrion

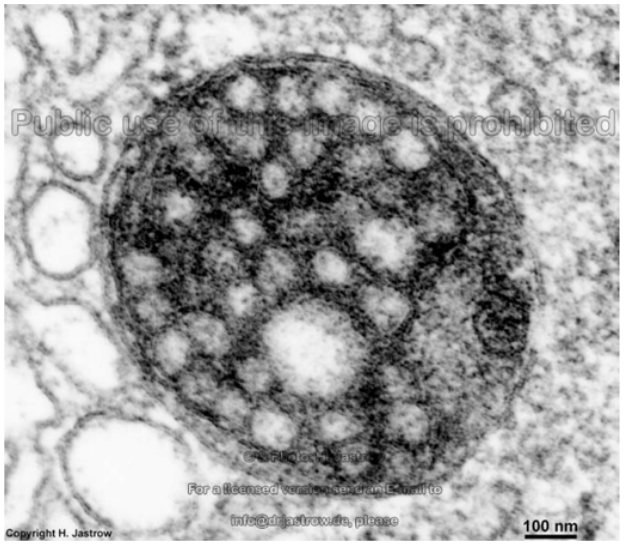


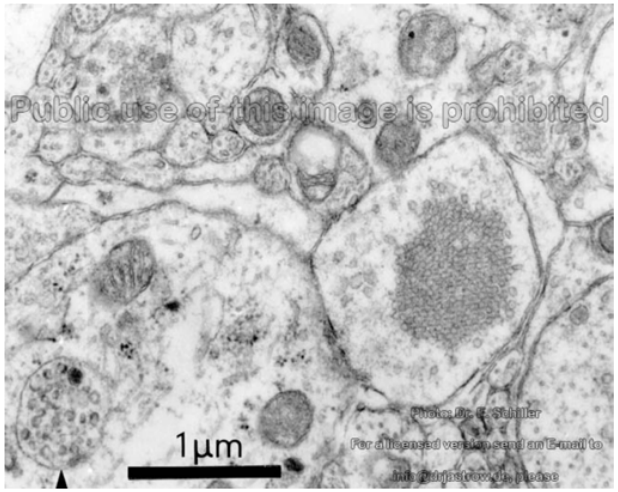
ATP generation
Double membrane structure
Network-like but not continuous



Endosomes







Endosomes are a collection of intracellular sorting organelles in eukaryotes (more on this next week)

peroxisomes

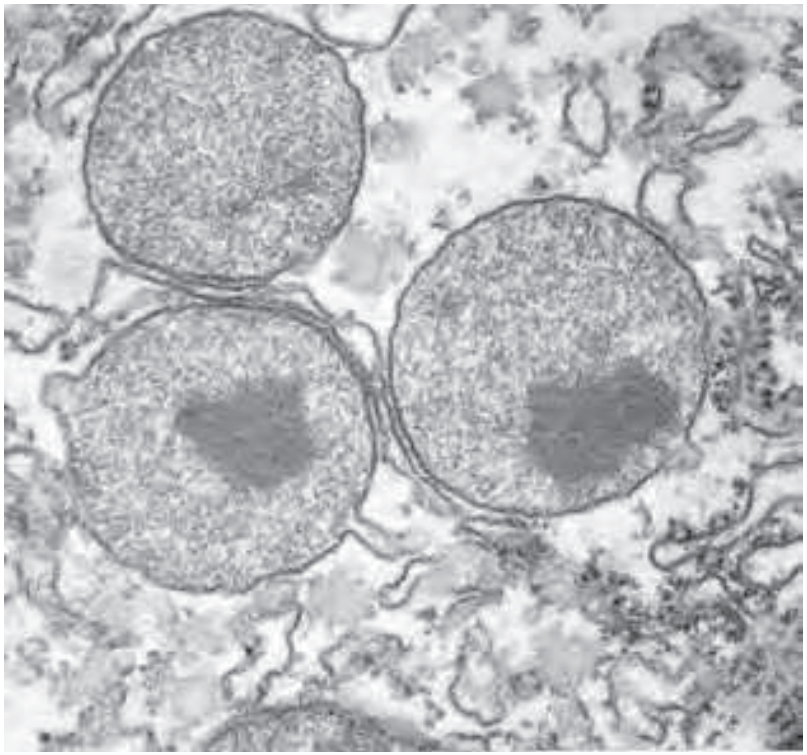


Figure 12–27 An electron micrograph of three peroxisomes in a rat liver cell. The paracrystalline, electron-dense inclusions are composed primarily of the enzyme urate oxidase. (Courtesy of Daniel S. Friend.)

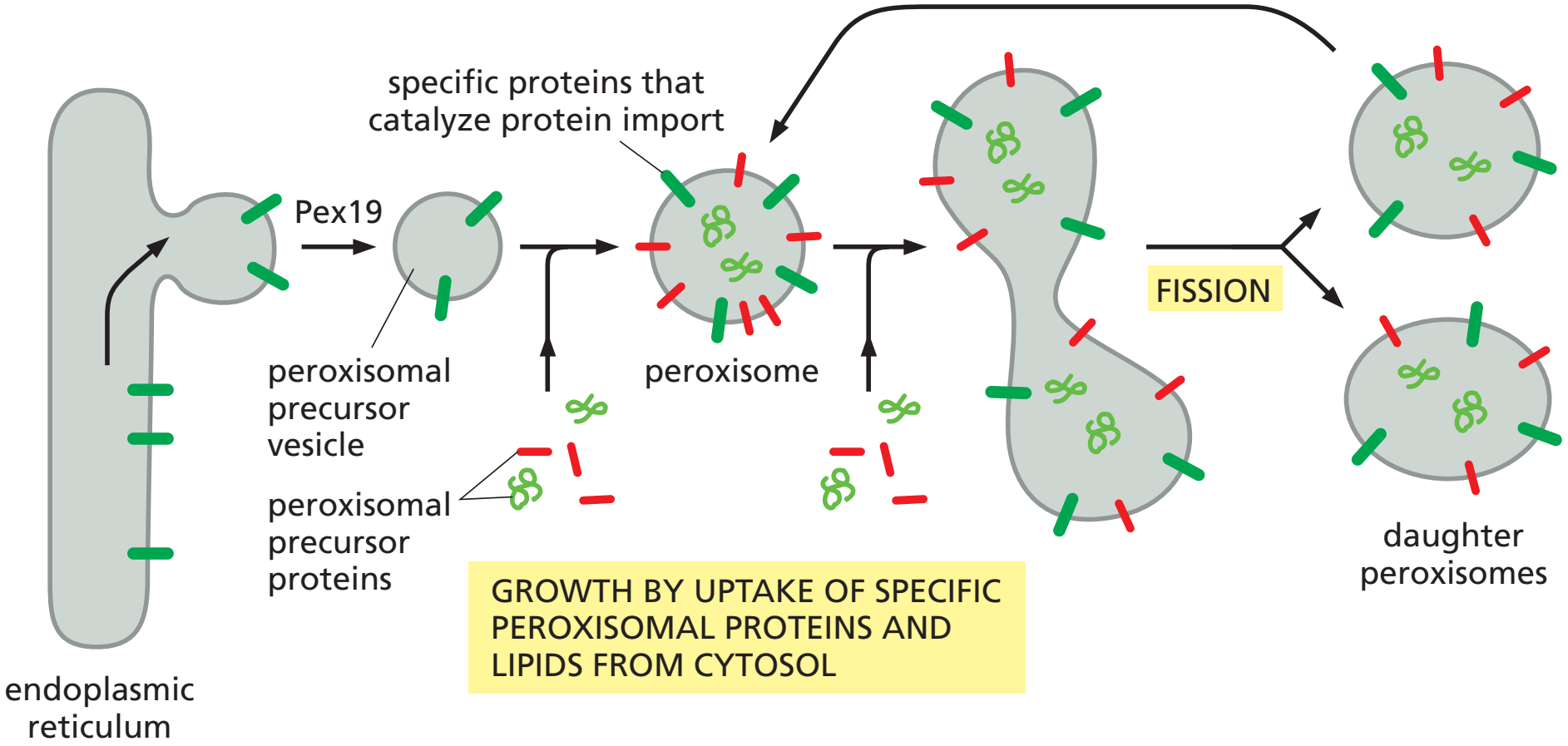
Peroxidation of substrates by catalase (like toxins done by Liver and kidney cells)
$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$

Breakdown of fatty acid molecules by B-Oxidation

Generation of Plasmalogen (a Lipid)

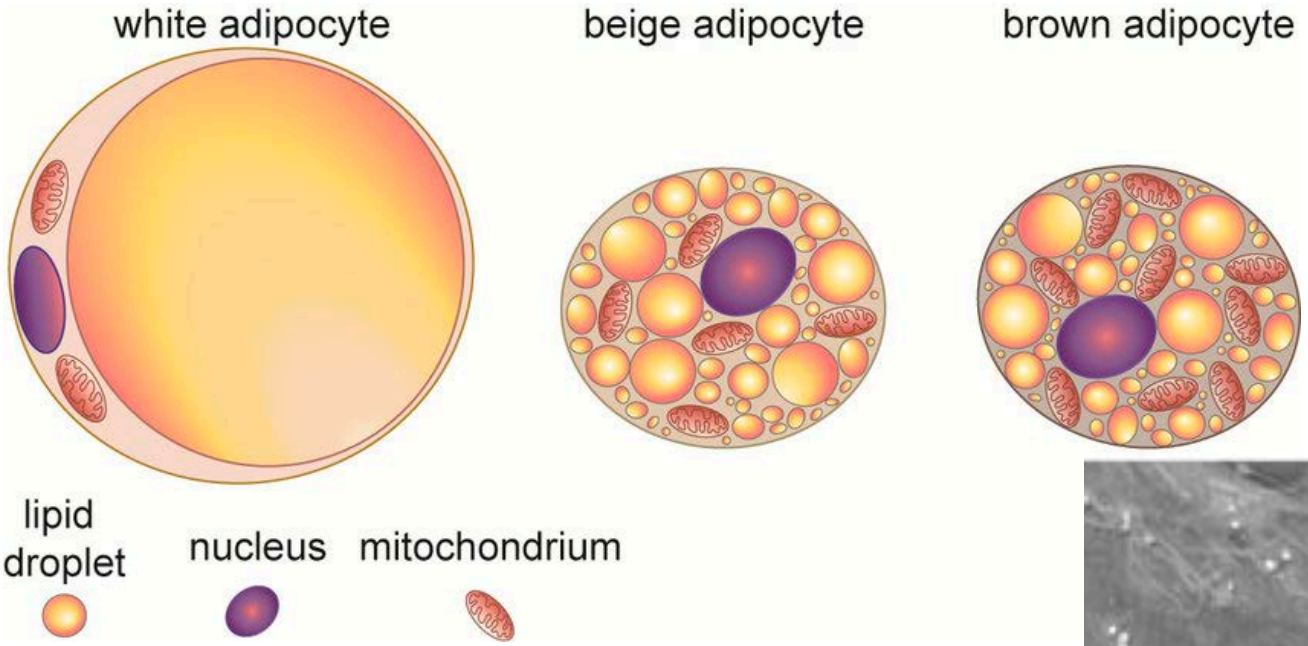
Large variety in composition of proteins

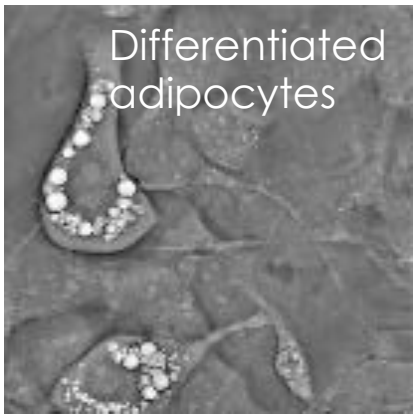
A model that explains how peroxisomes proliferate and how new peroxisomes arise

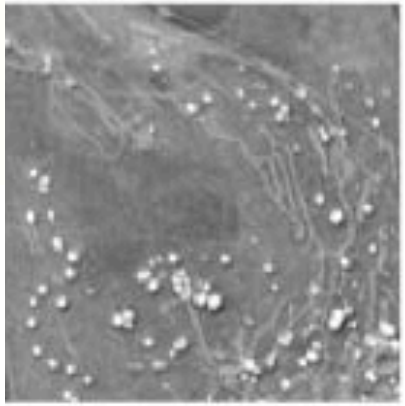


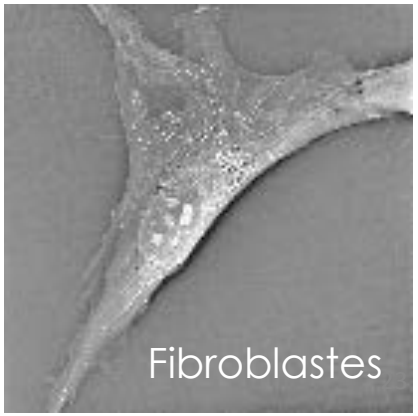
Lipid droplets

Lipid droplet, an organelle present in many eukaryotic cells but particularly abundant/large in adipocytes

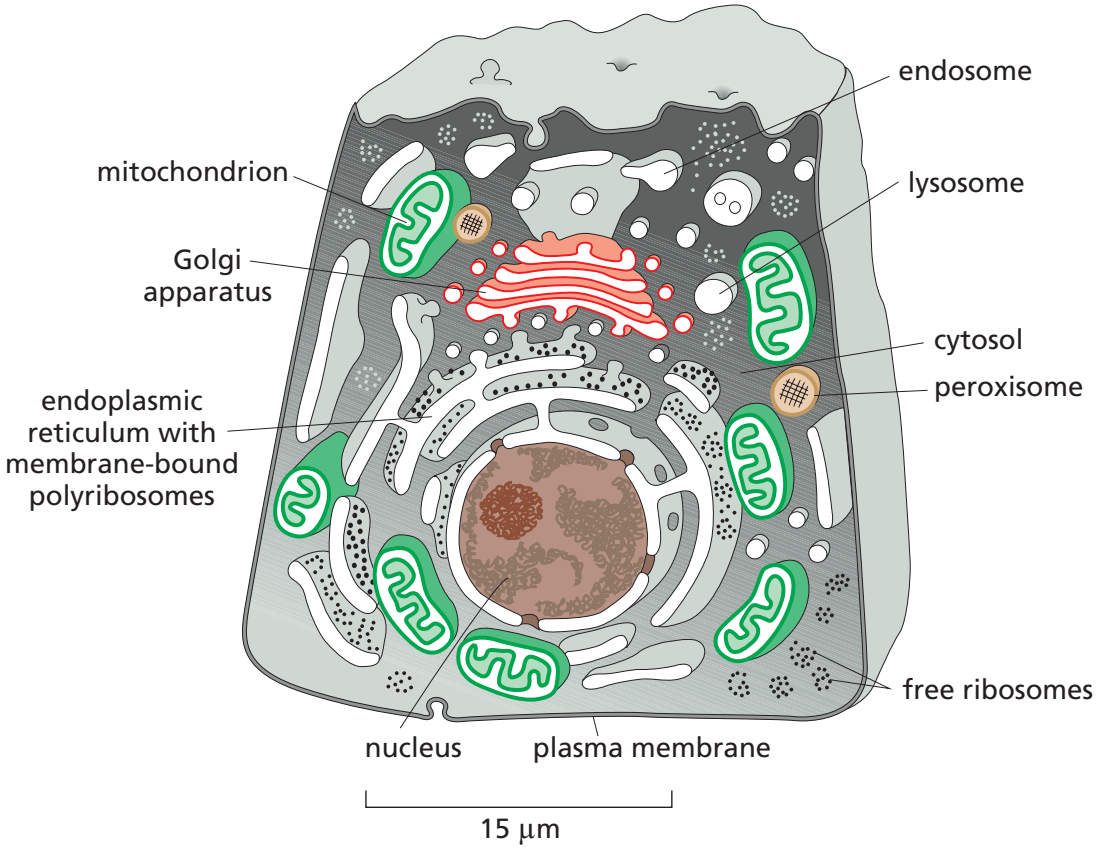








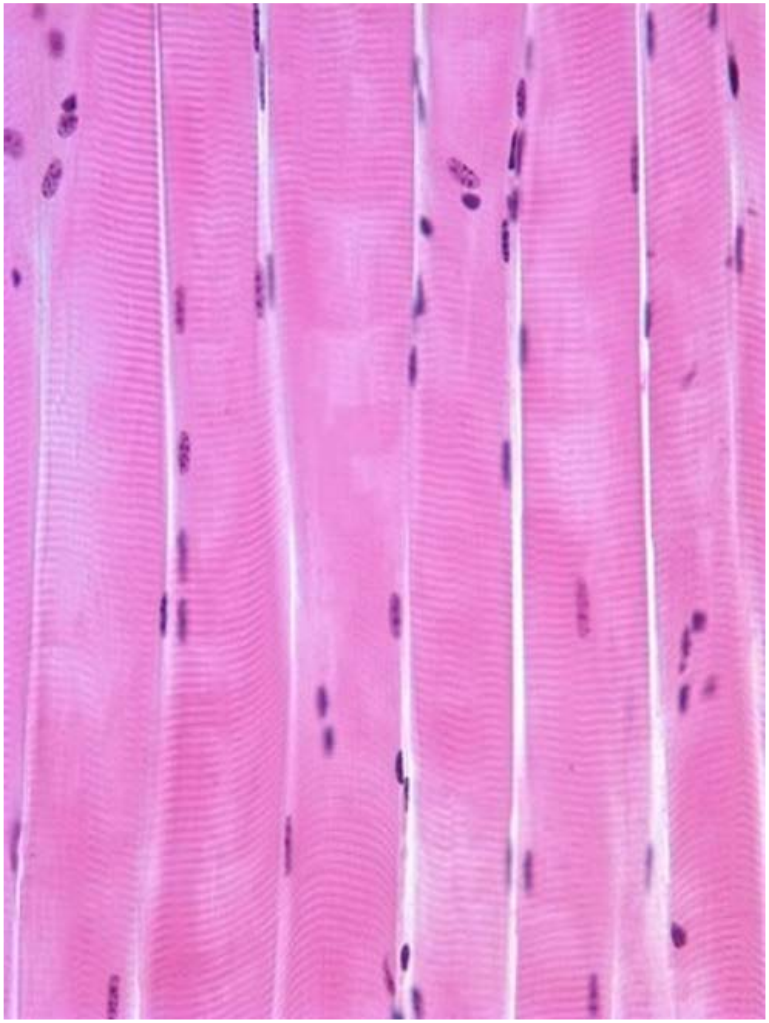
How many organelles compartments per cell?

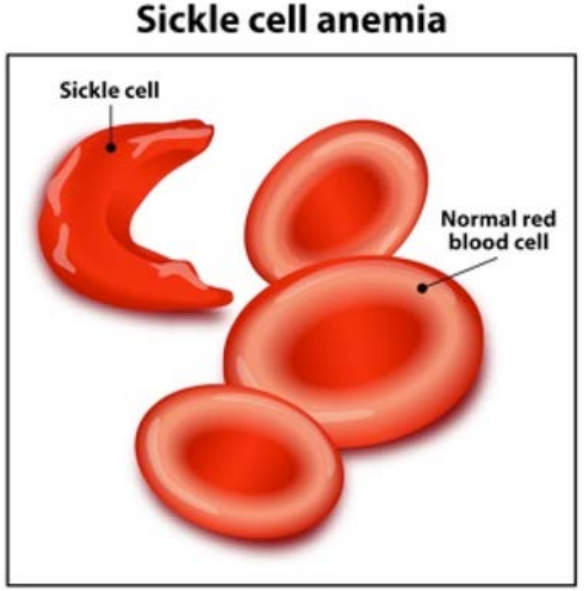


In general:
1 nucleus
1 Golgi
1 endoplasmic reticulum
Hundreds of endosomes/lysosomes
Mitochondria connected in a network that is not necessarily totally continuous
Mebraneless organelles -> many variations

The number of copies of an organelle is not correlated with
to its size, or the amount of membrane/cell

How many organelles compartments per cell?





Two extreme exceptions

Muscle fibers are formed by the fusion of multiple cells resulting in huge multinucleate cells.

During their differentiation, red blood cells lose all their internal membranes, including the nucleus

The volumes and membranes or organelles

TABLE 12–1 Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)

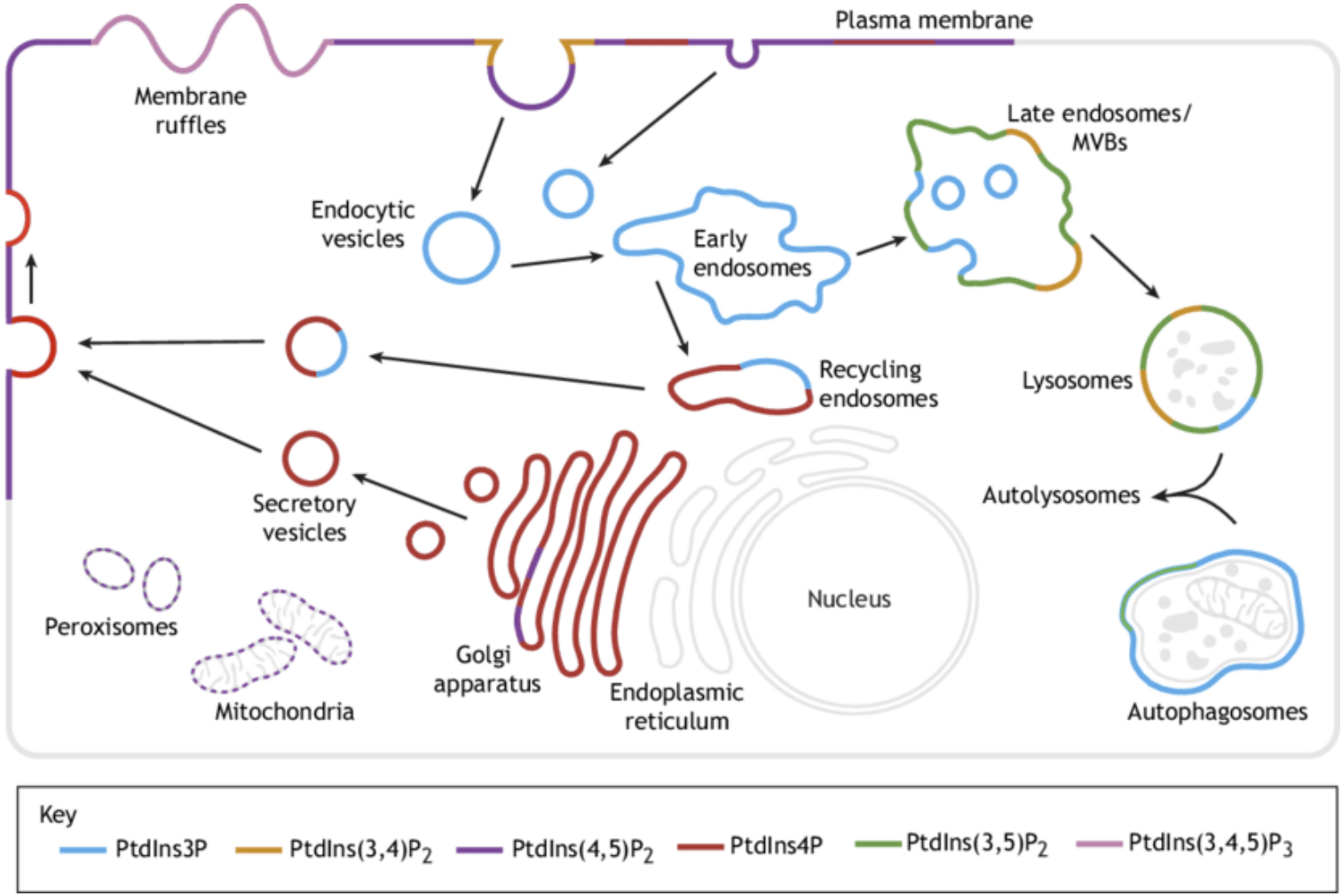
Intracellular compartment	Percentage of total cell volume
Cytosol	54
Mitochondria	22
Rough ER cisternae	9
Smooth ER cisternae plus Golgi cisternae	6
Nucleus	6
Peroxisomes	1
Lysosomes	1
Endosomes	1

TABLE 12–2 Relative Amounts of Membrane Types in Two Kinds of Eukaryotic Cells

Membrane Type	Percentage of total cell membrane	
	Liver hepatocyte*	Pancreatic exocrine cell*
Plasma membrane	2	5
Rough ER membrane	35	60
Smooth ER membrane	16	<1
Golgi apparatus membrane	7	10
Mitochondria Outer membrane Inner membrane	 7 32	 4 17
Nucleus Inner membrane	 0.2	 0.7
Secretory vesicle membrane	Not determined	3
Lysosome membrane	0.4	Not determined
Peroxisome membrane	0.4	Not determined
Endosome membrane	0.4	Not determined

*These two cells are of very different sizes: the average hepatocyte has a volume of about 5000 μm^3 compared with 1000 μm^3 for the pancreatic exocrine cell. Total cell membrane areas are estimated at about 110,000 μm^2 and 13,000 μm^2, respectively.

Specific locations in the cell of the phosphatidylinositols



The lipid composition is different for each organelle

Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT				
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM
Cholesterol	17	23	22	3	6
Phosphatidylethanolamine	7	18	15	28	17
Phosphatidylserine	4	7	9	2	5
Phosphatidylcholine	24	17	10	44	40
Sphingomyelin	19	18	8	0	5
Glycolipids	7	3	28	trace	trace
Others	22	13	8	23	27

Organelles and cellular compartments are dynamic

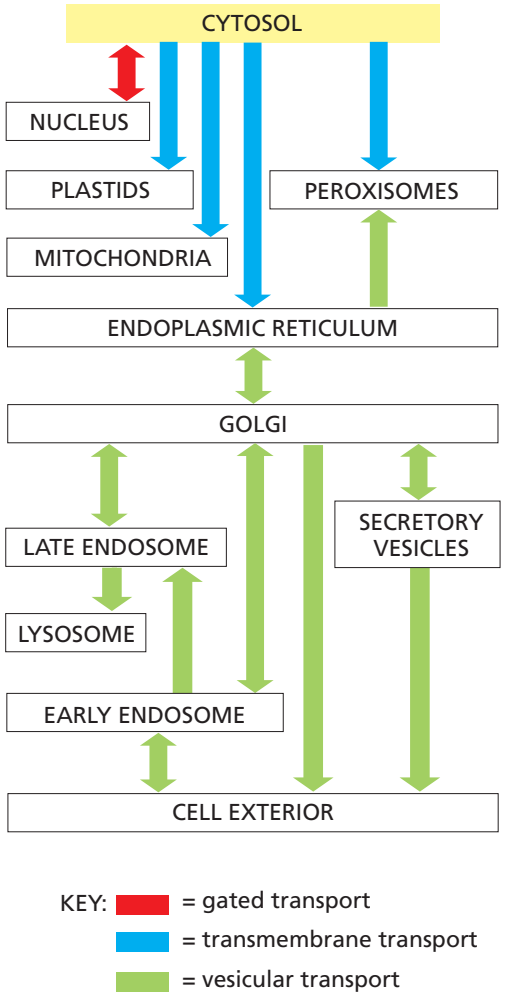
https://www.hhmi.org/insidelook/jennifer-lippincott-schwartz

https://nanolive.ch/mitochondria/

Nucleus during division

How do proteins localize to the different compartments?

A simplified “roadmap” of protein traffic within a eukaryotic cell

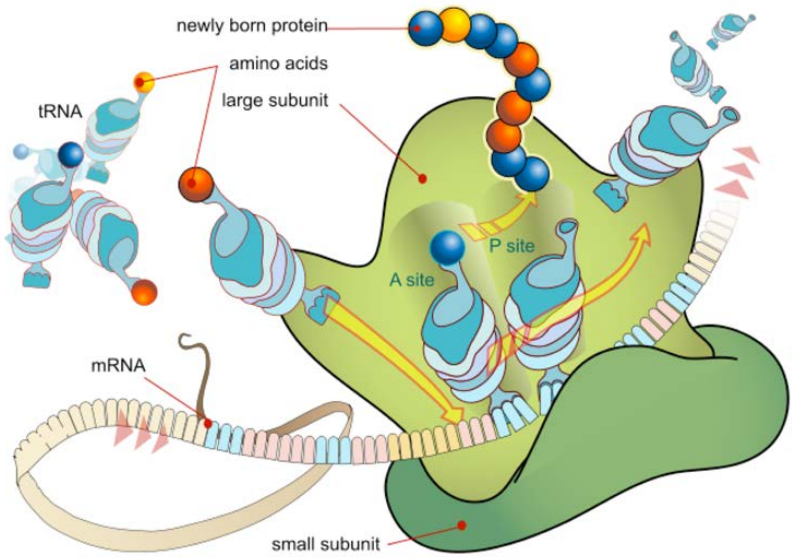


1. Gated transport. In gated transport, proteins and RNA molecules move between the cytosol and the nucleus through nuclear pore complexes in the nuclear envelope. The nuclear pore complexes function as selective gates that support the active transport of specific macromolecules and macromolecular assemblies between the two topologically equivalent spaces, although they also allow free diffusion of smaller molecules.

2. Transmembrane location. In protein translocation, transmembrane protein translocators directly transport specific proteins across a membrane from the cytosol into a space that is topologically distinct. The transported protein molecule usually must unfold to snake through the translocator. The initial transport of selected proteins from the cytosol into the ER lumen or mitochondria, for example, occurs in this way. Integral membrane proteins often use the same translocators but translocate only partially across the membrane, so that the protein becomes embedded in the lipid bilayer.

3. Vesicular transport. In vesicular transport, membrane-enclosed transport intermediates— which may be small, spherical transport vesicles or larger, irregularly shaped organelle fragments—ferry proteins from one topologically equivalent compartment to another. The transport vesicles and fragments become loaded with a cargo of molecules derived from the lumen of one compartment as they bud and pinch off from its membrane; they discharge their cargo into a second compartment by fusing with the membrane enclosing that compartment. The transfer of soluble proteins from the ER to the Golgi apparatus, for example, occurs in this way. Because the transported proteins do not cross a membrane, vesicular transport can move proteins only between compartments that are topologically equivalent

Protein Synthesis (Repeat)



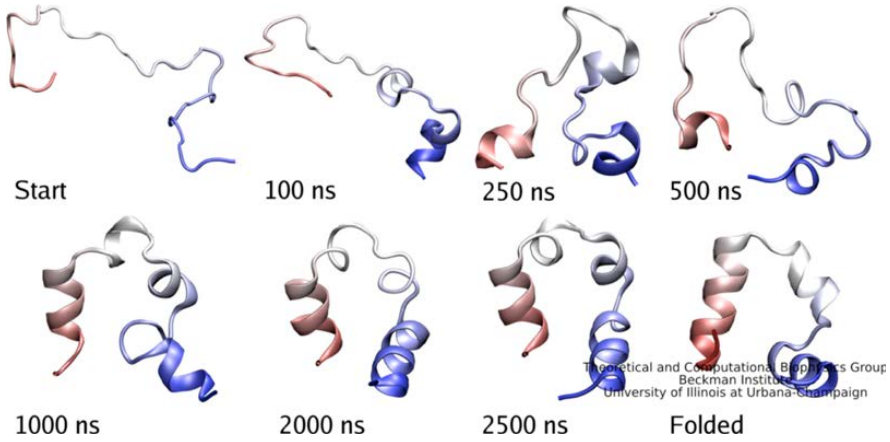
By ribosomes, in the cytoplasm
Synthesis rate in a mammalian cell: 3-5 aa / s
Protein of 500 aa: 2-3 min Protein of 1500 aa: 6-9 min

Protein Synthesis (Repeat)

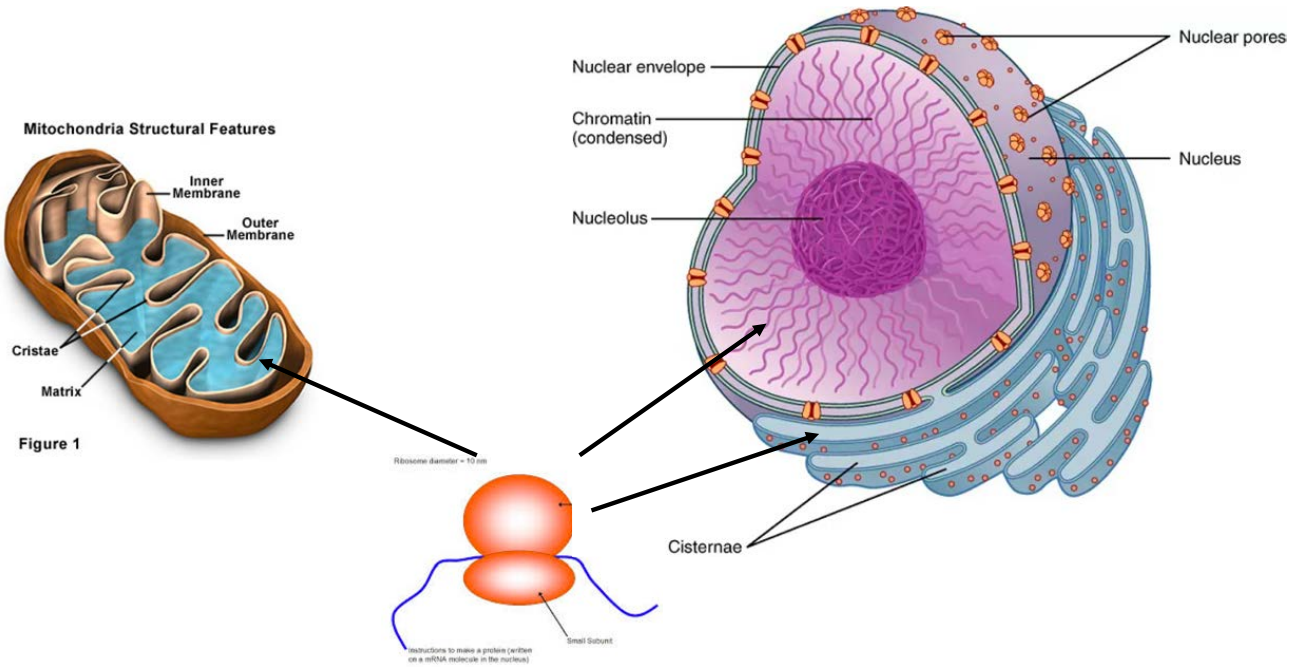
Synthesis and folding follow each other very quickly
Remember that:
Synthesis is vectorial, from the N to the C terminal of the protein

Synthesis is relatively slow (2-3 min for a 550 aa protein)

As soon as a part of the protein is synthesized, it will try to fold, unless a "deliberate" mechanism prevents it



Protein localization

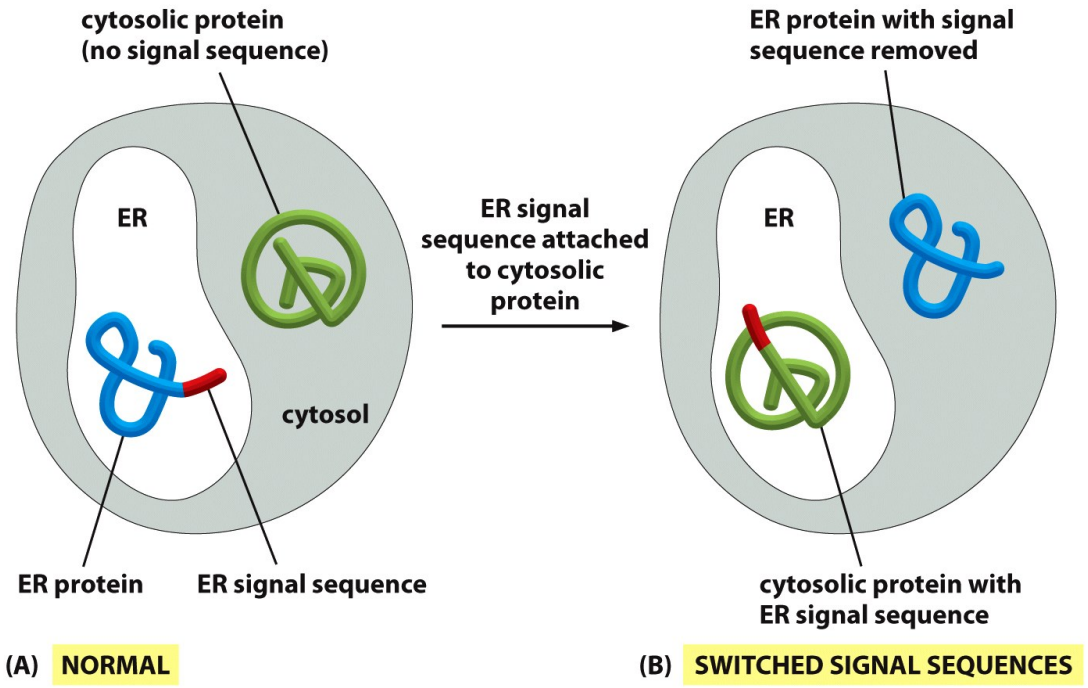


Addressing to a compartment of the endomembrane system

Where is the information that allows a protein to be brought to the right place?

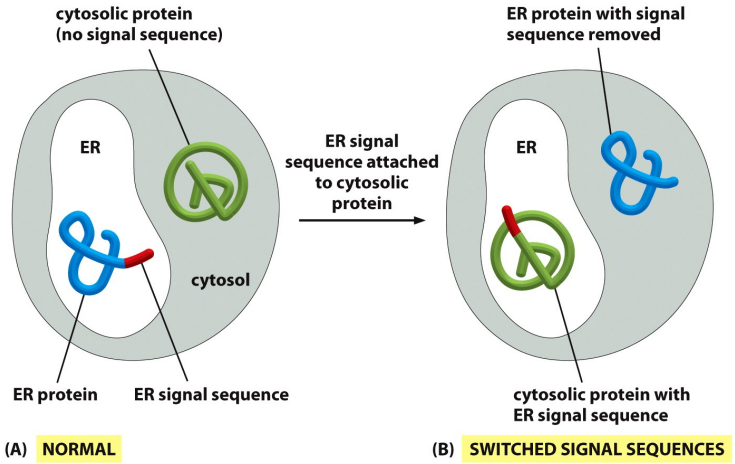
1. Protein destined for the endoplasmic reticulum (or beyond)
2. Protein destined for a nucleus

Protein localization



Addressing to the ER is determined by a signal present in the primary sequence of the protein

Protein localization



- It is present at the N-terminus
- It is composed of hydrophobic amino acids
- It is read during synthesis
- It is removed once "used" (proteins do not go back and forth between
back and forth between ER and cytoplasm)

Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-

Import signals

TABLE 12–3 Some Typical Signal Sequences	
Function of signal sequence	Example of signal sequence
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in *green*. Similarly, important hydrophobic amino acids are shown in *orange* and important hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.

Table 12–3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus Export from nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val- -Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-

Not all signal sequences are played at the same time

At the beginning of the synthesis

1 single reading cut off after -> except for NLS

When the synthesis is finished

Only one ”read” before cleavage : mitochondria, peroxisomes

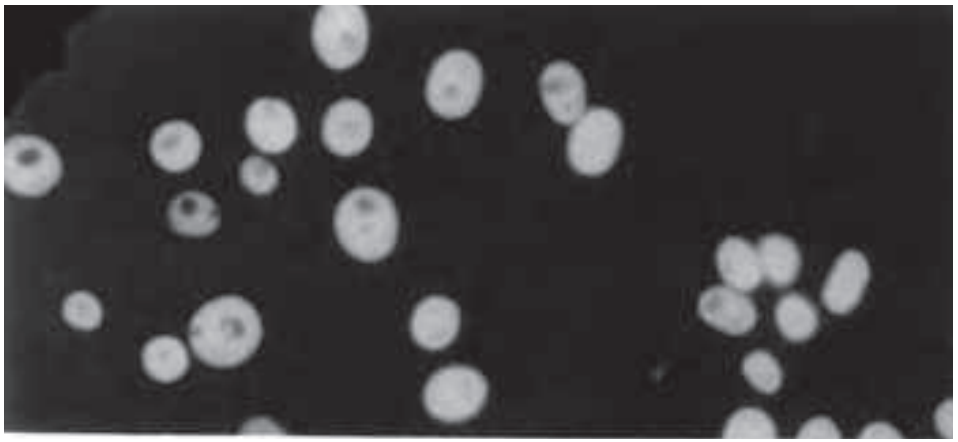
Several “reads” no cleavage: nucleus

How does nuclear import work?

The function of a nuclear localization signal

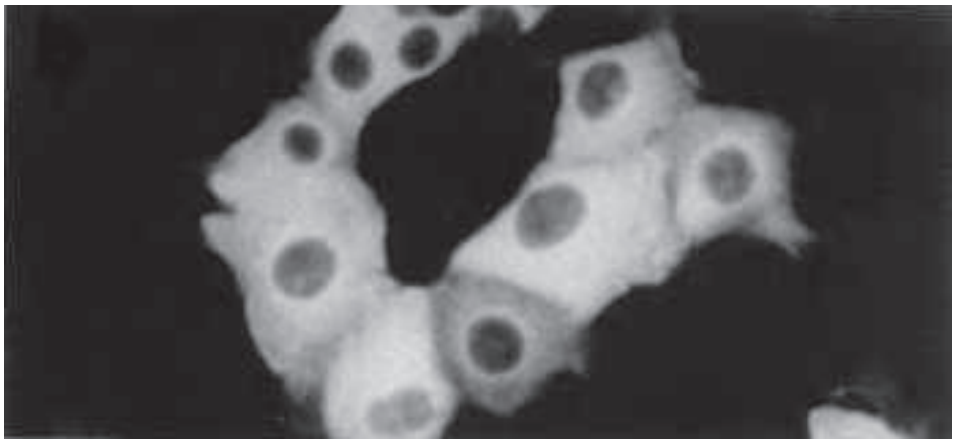
(A) LOCALIZATION OF T-ANTIGEN CONTAINING ITS NORMAL NUCLEAR IMPORT SIGNAL

Pro — Pro — Lys — Lys — Lys — Arg — Lys — Val —

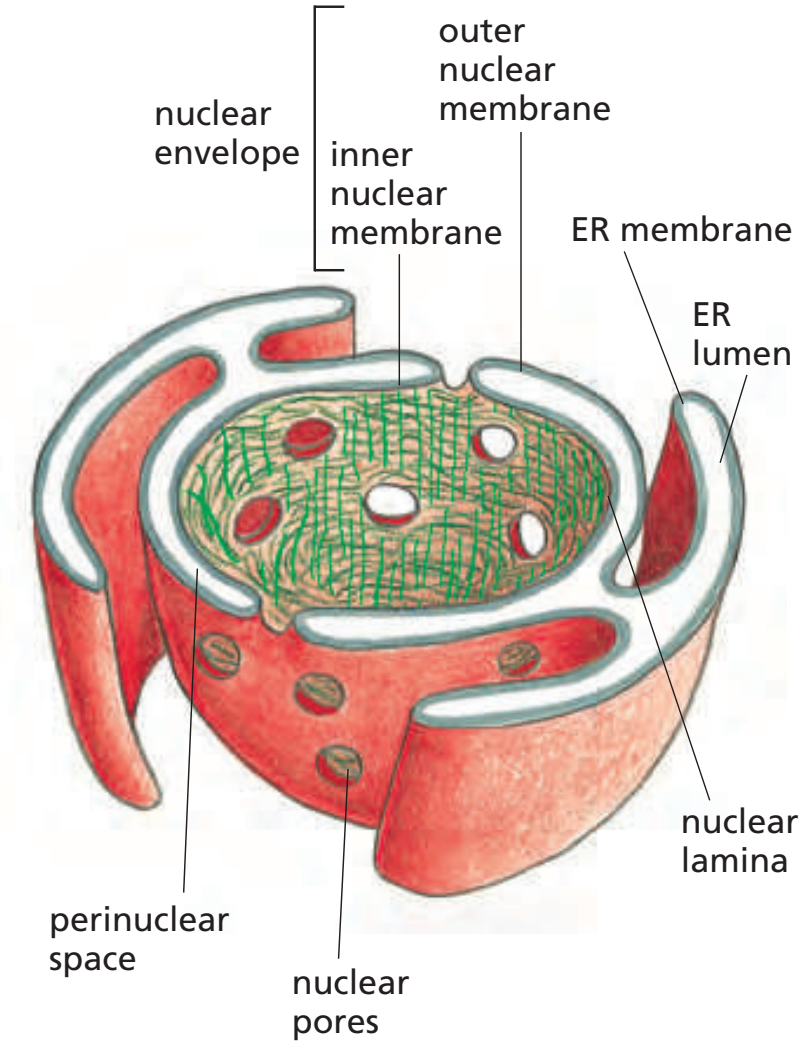


(B) LOCALIZATION OF T-ANTIGEN CONTAINING A MUTATED NUCLEAR IMPORT SIGNAL

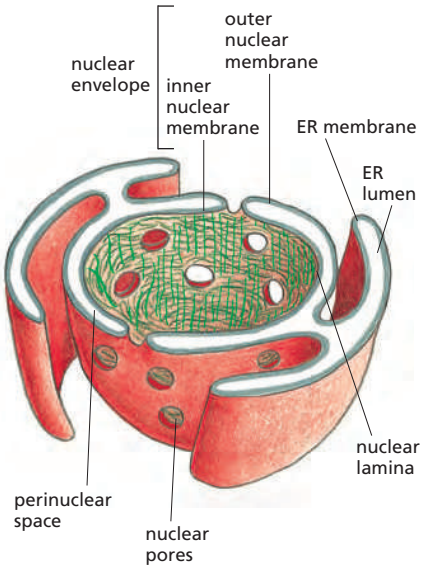
Pro — Pro — Lys — Thr — Lys — Arg — Lys — Val —

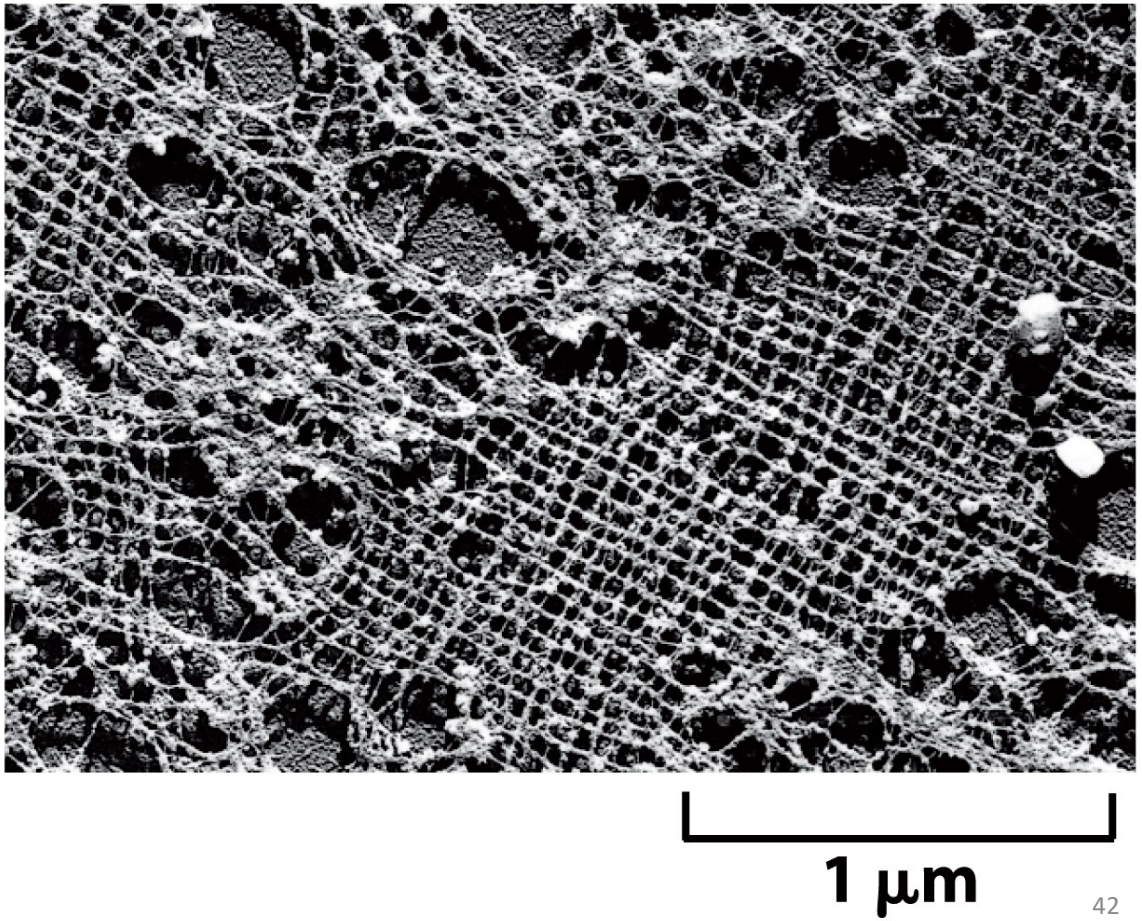


The nuclear envelope

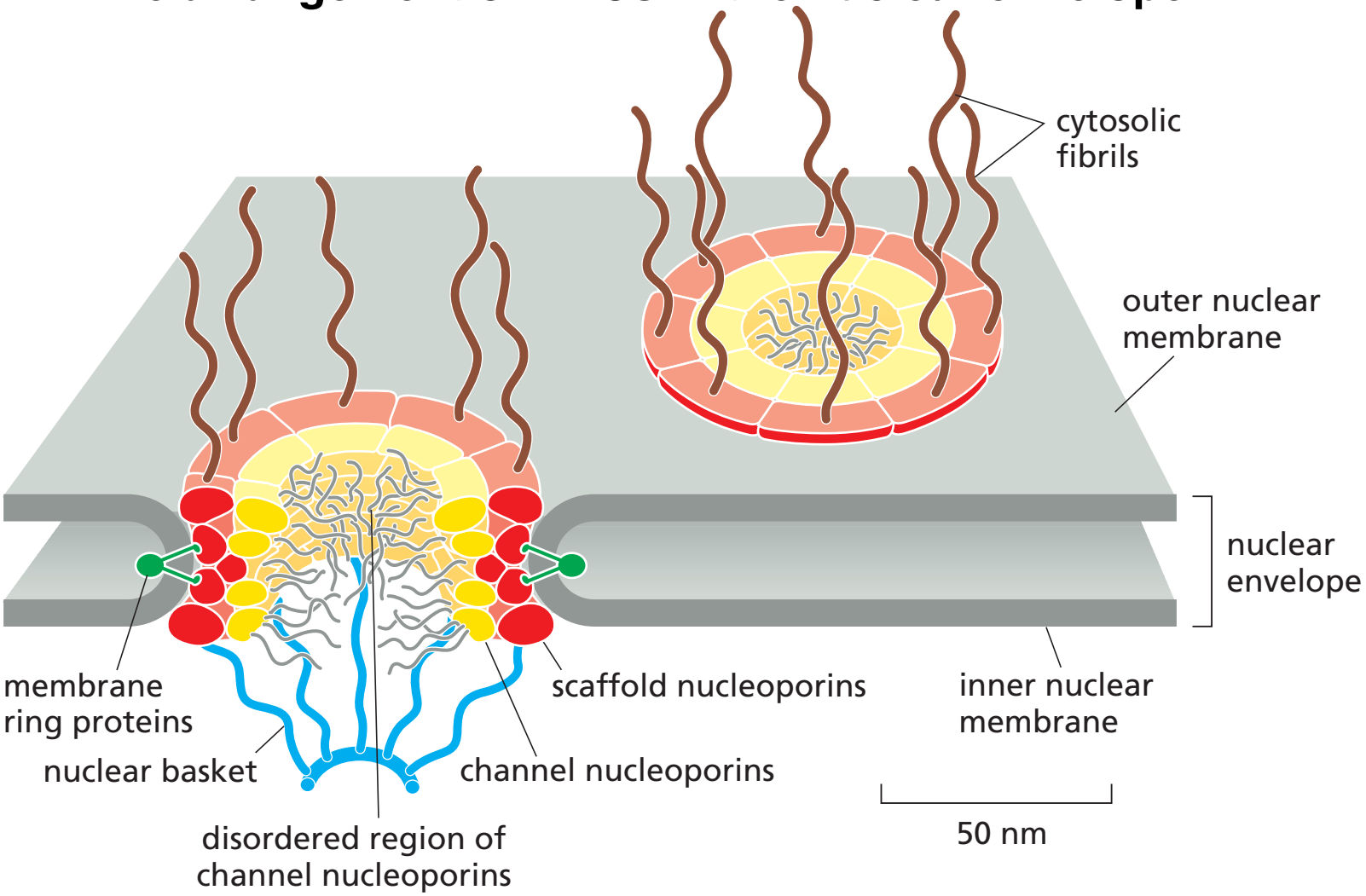


The nuclear Lamina





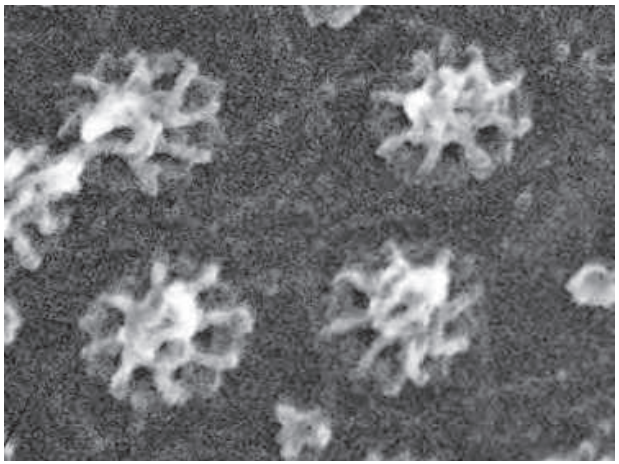
The arrangement of NPCs in the nuclear envelope



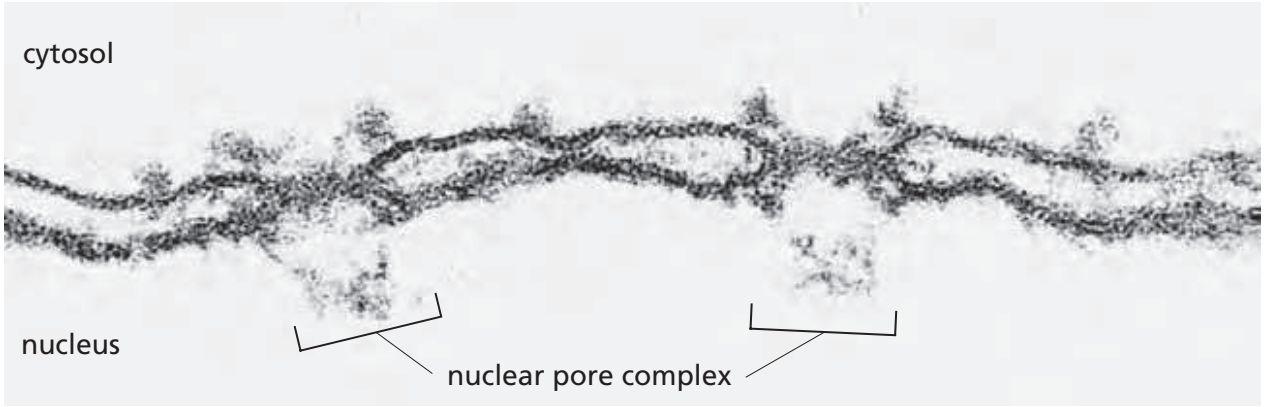
(A)

The arrangement of NPCs in the nuclear envelope

The nuclear membrane is freely permeable to ions and other small molecules under 5000 Dalton

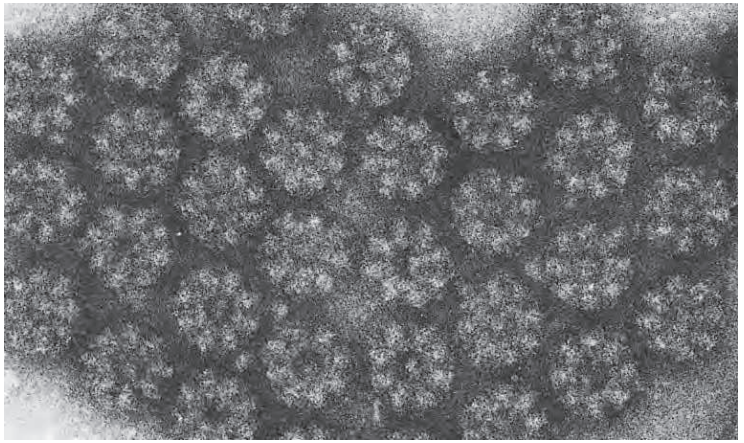


(B)



(C)

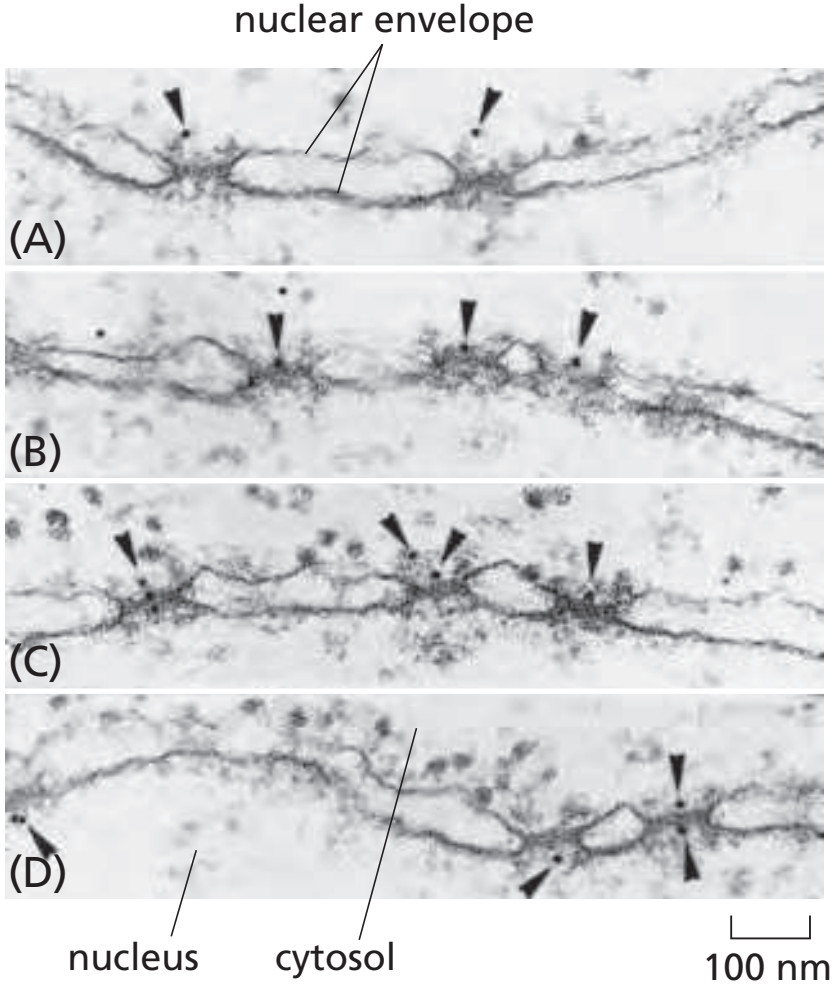
0.1 µm



(D)

0.1 µm

Visualizing active import through NPCs



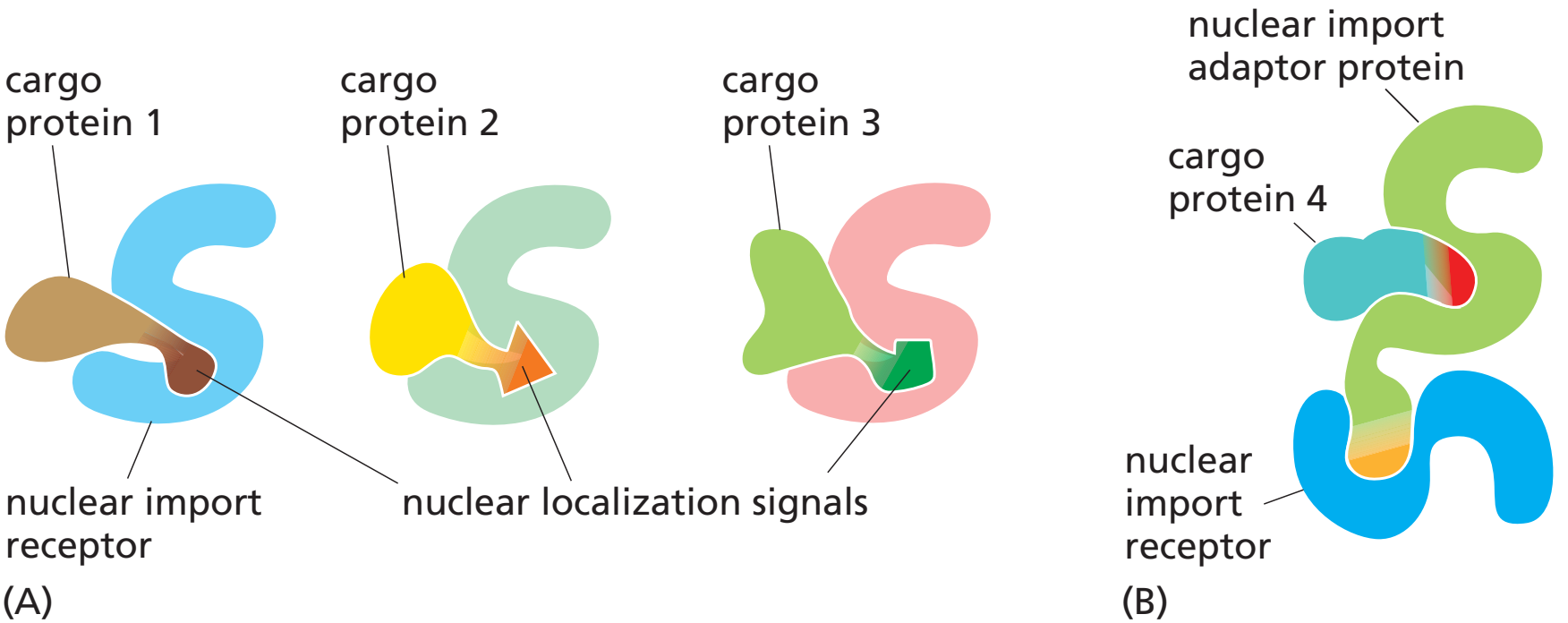
The nuclear membrane is freely permeable to ions and other small molecules under 5000 Dalton

Individual nuclear pores mediate transport in both directions. It is unclear how pores coordinate two-way traffic so as to avoid head-on collisions.

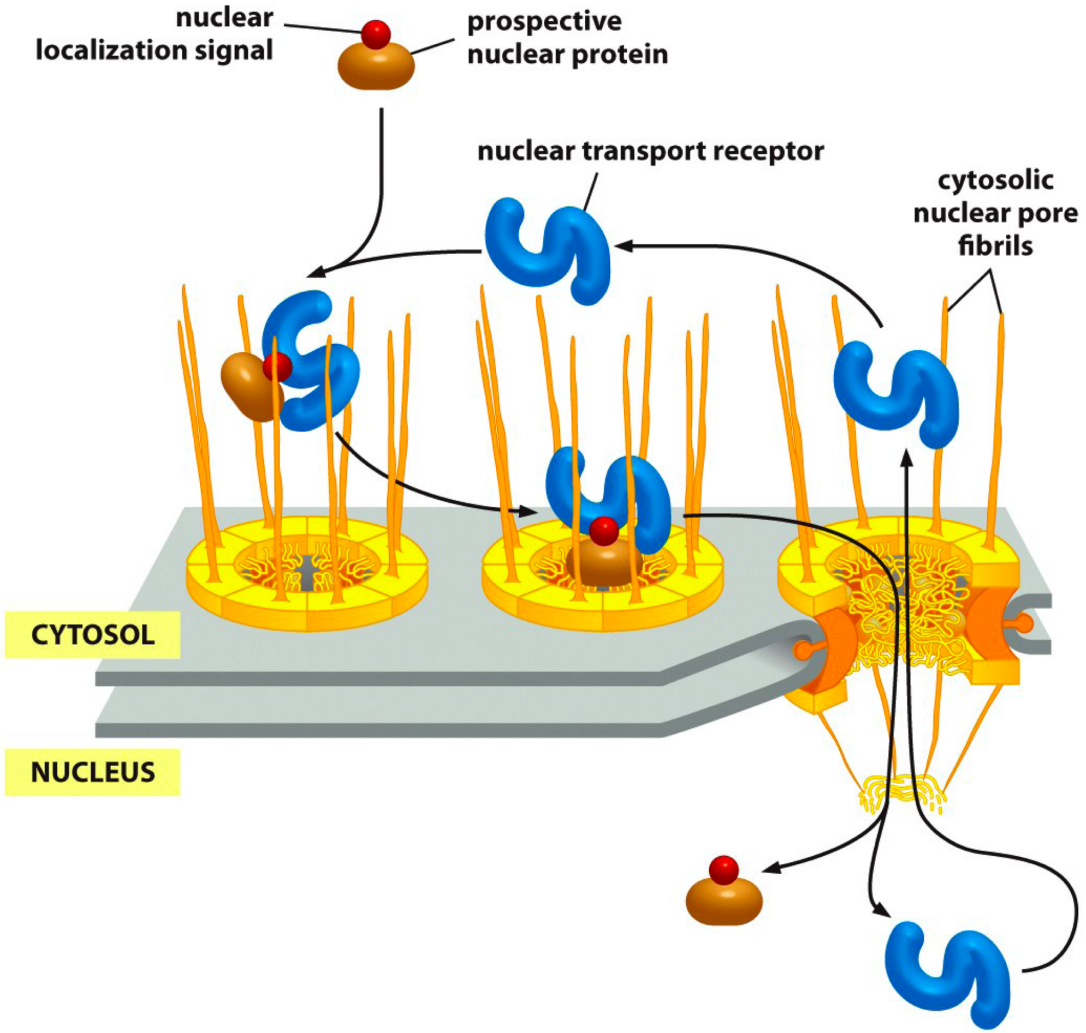
The inside of a pore is a tangled gel-like structure

We do not know if the pores are rigid or elastic?

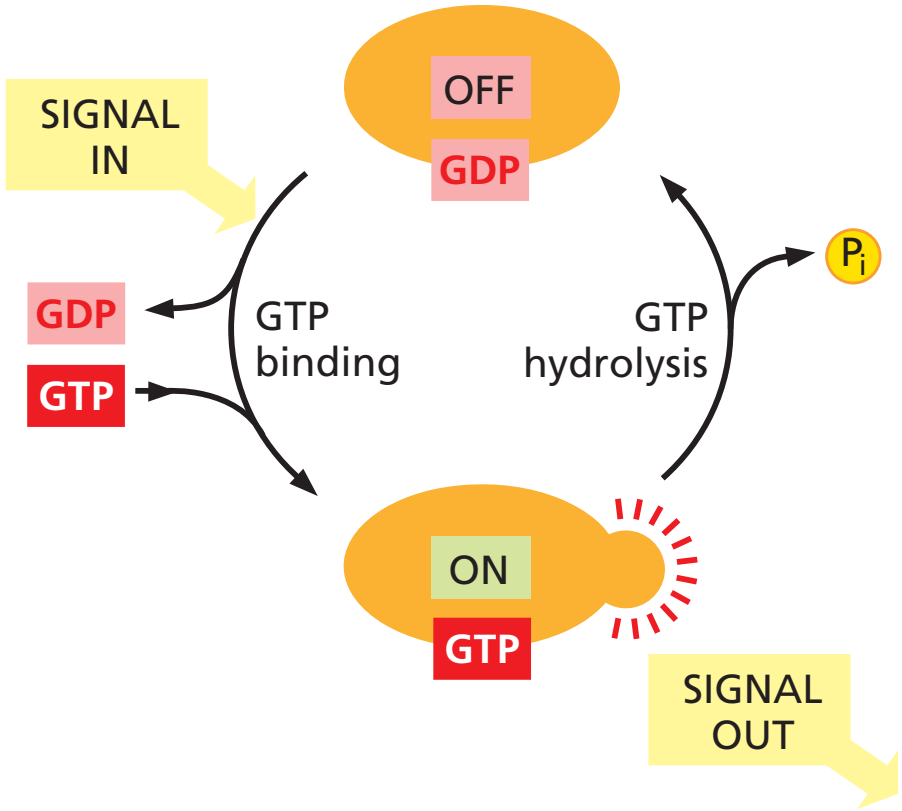
Nuclear import receptors (importins)



Nuclear import receptors (importins)

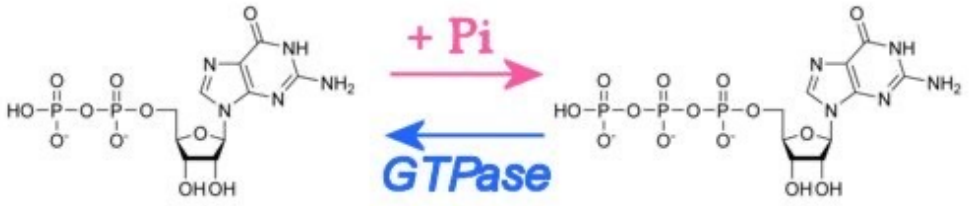


Two types of intracellular signaling proteins that act as molecular switches



(B) SIGNALING BY GTP BINDING

In a cell ratio GTP : GDP = 10:1, so the exchange is efficient



A phosphate is removed from GTP to make GDP

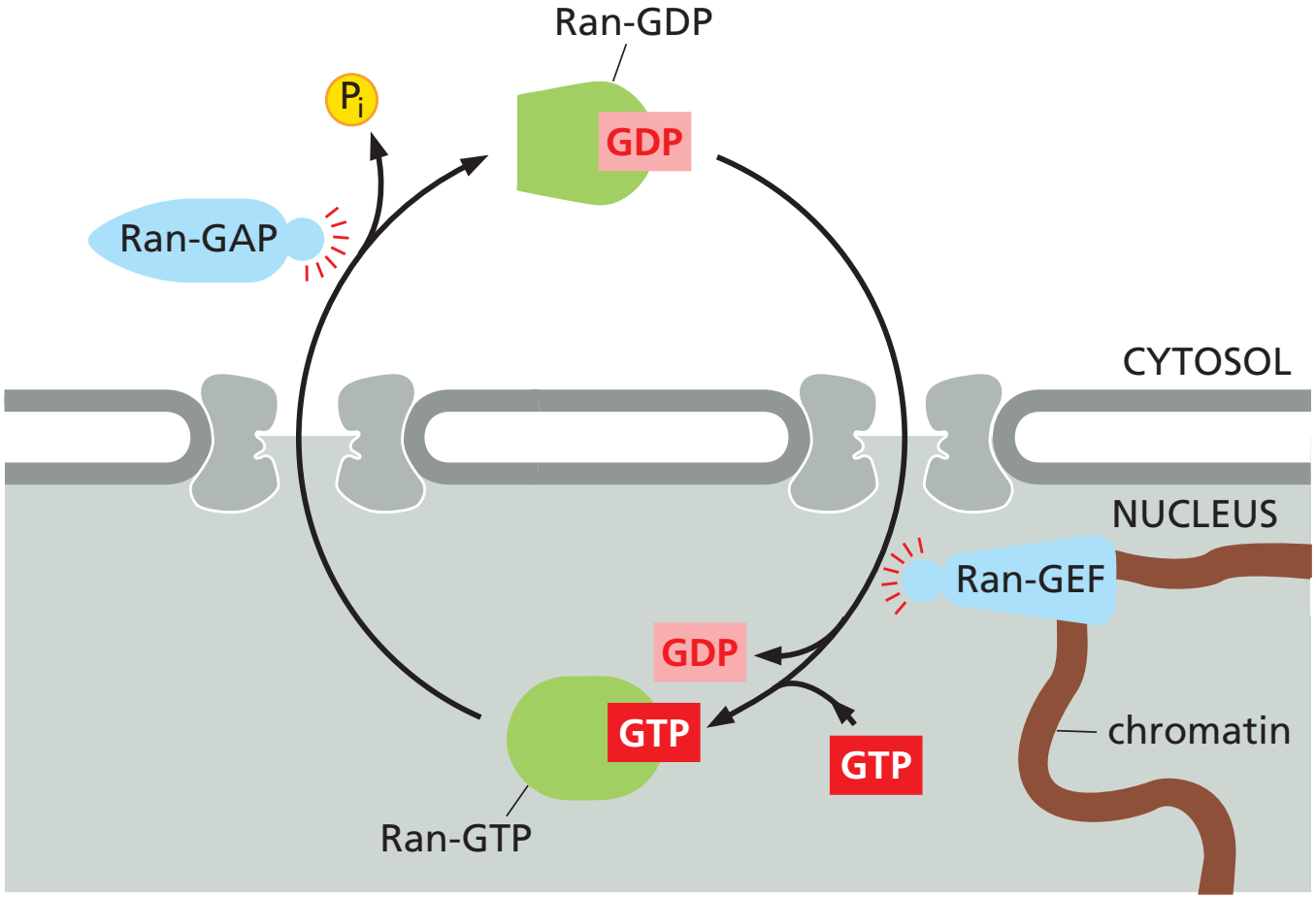
During the conversion from GTP to GDP the molecule stays bound to the protein

A GDP can be exchanged for a GTP

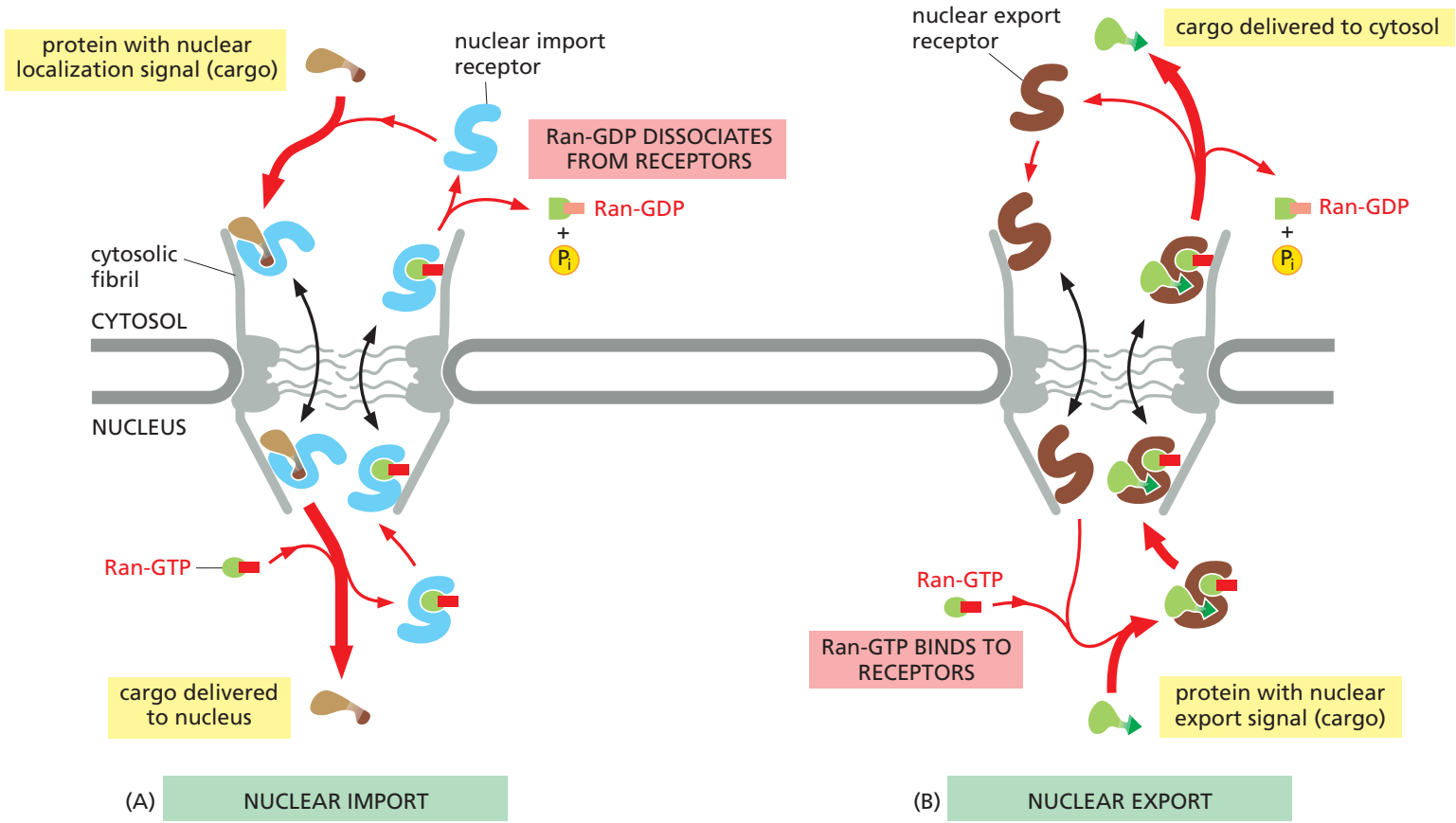
Whether the protein is interacting with GDP or GTP defines whether is signals

These are also G proteins (guanine nucleotide-binding proteins) but a different class monomeric "small" GTPases

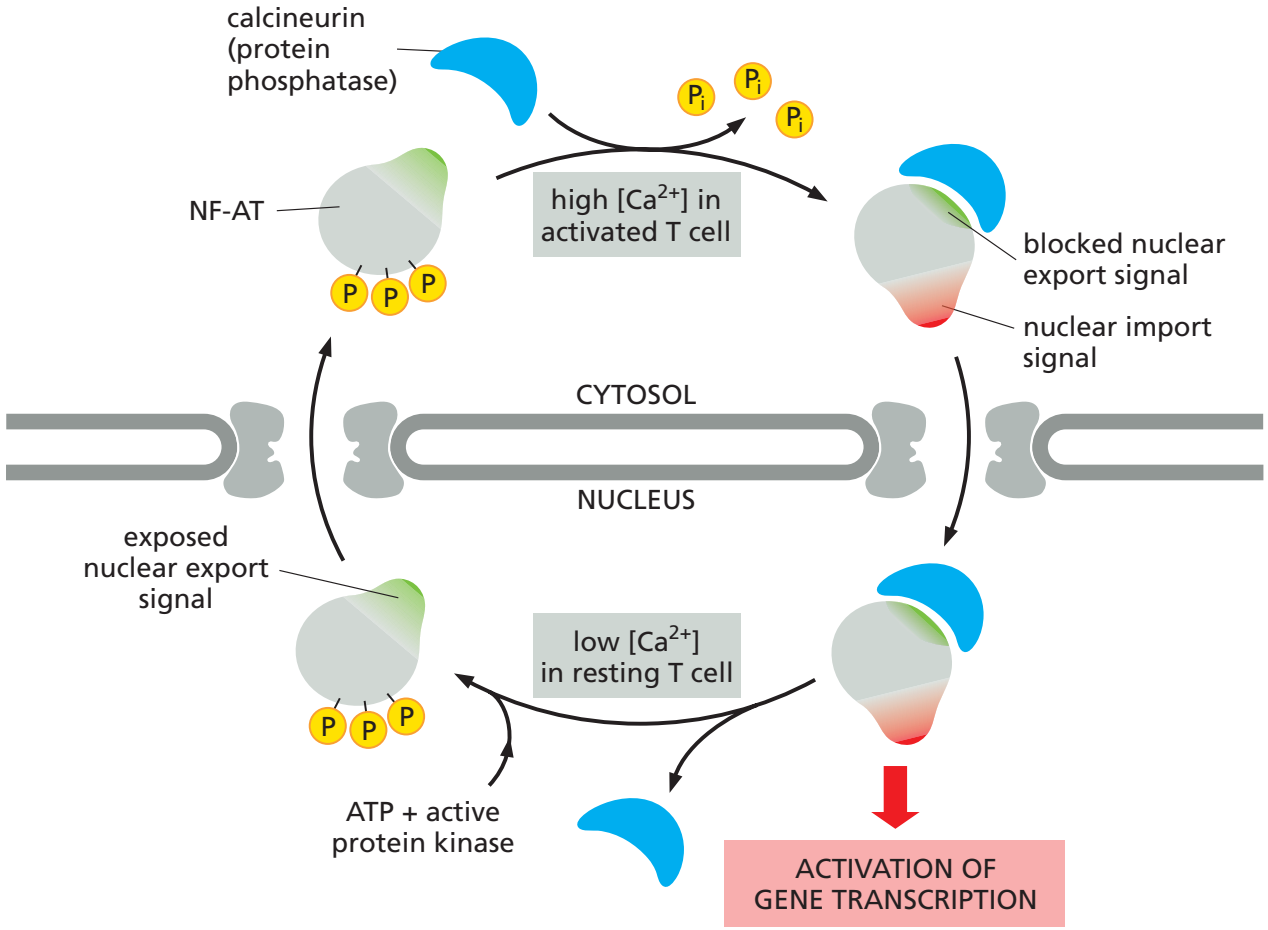
The compartmentalization of Ran-GDP and Ran-GTP



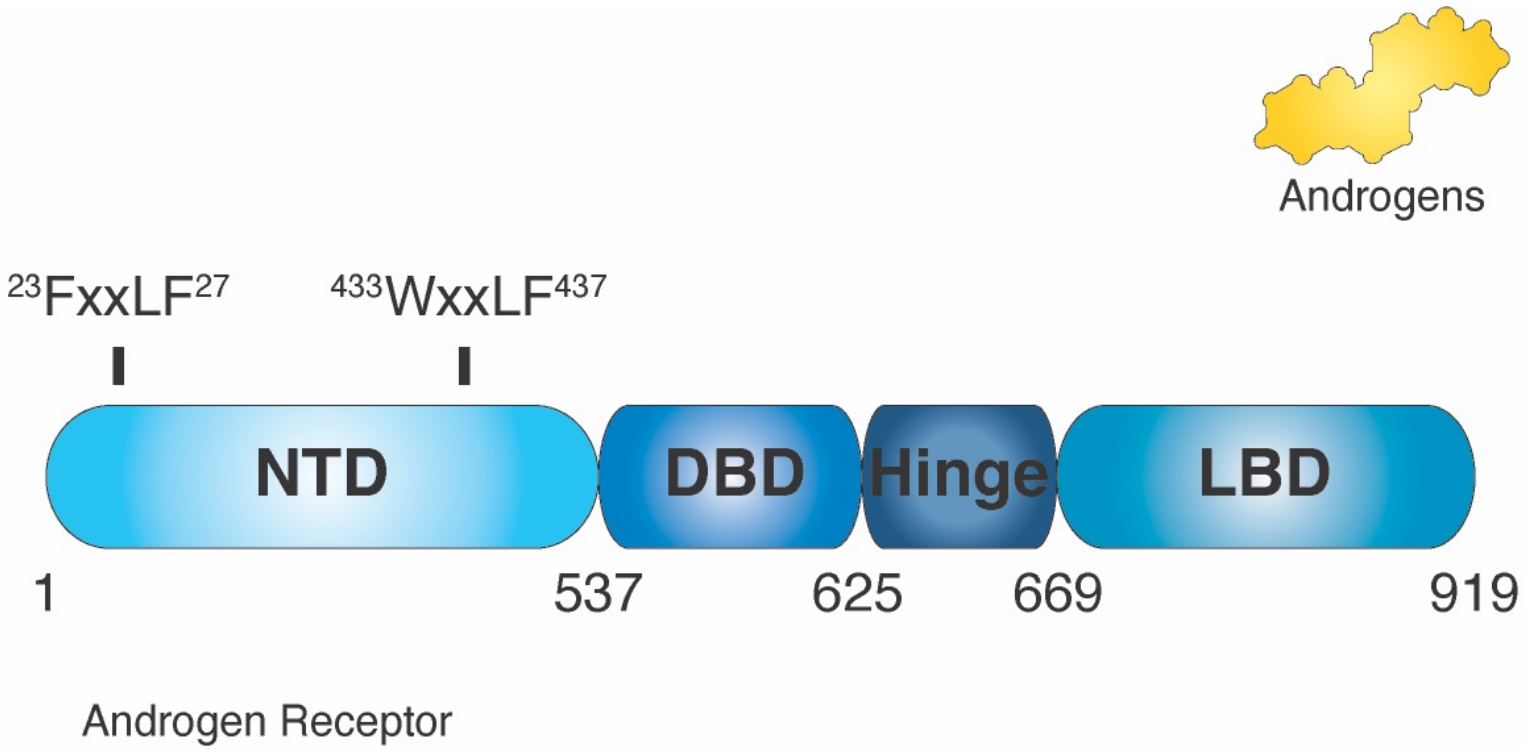
How GTP hydrolysis by Ran in the cytosol provides directionality to nuclear transport

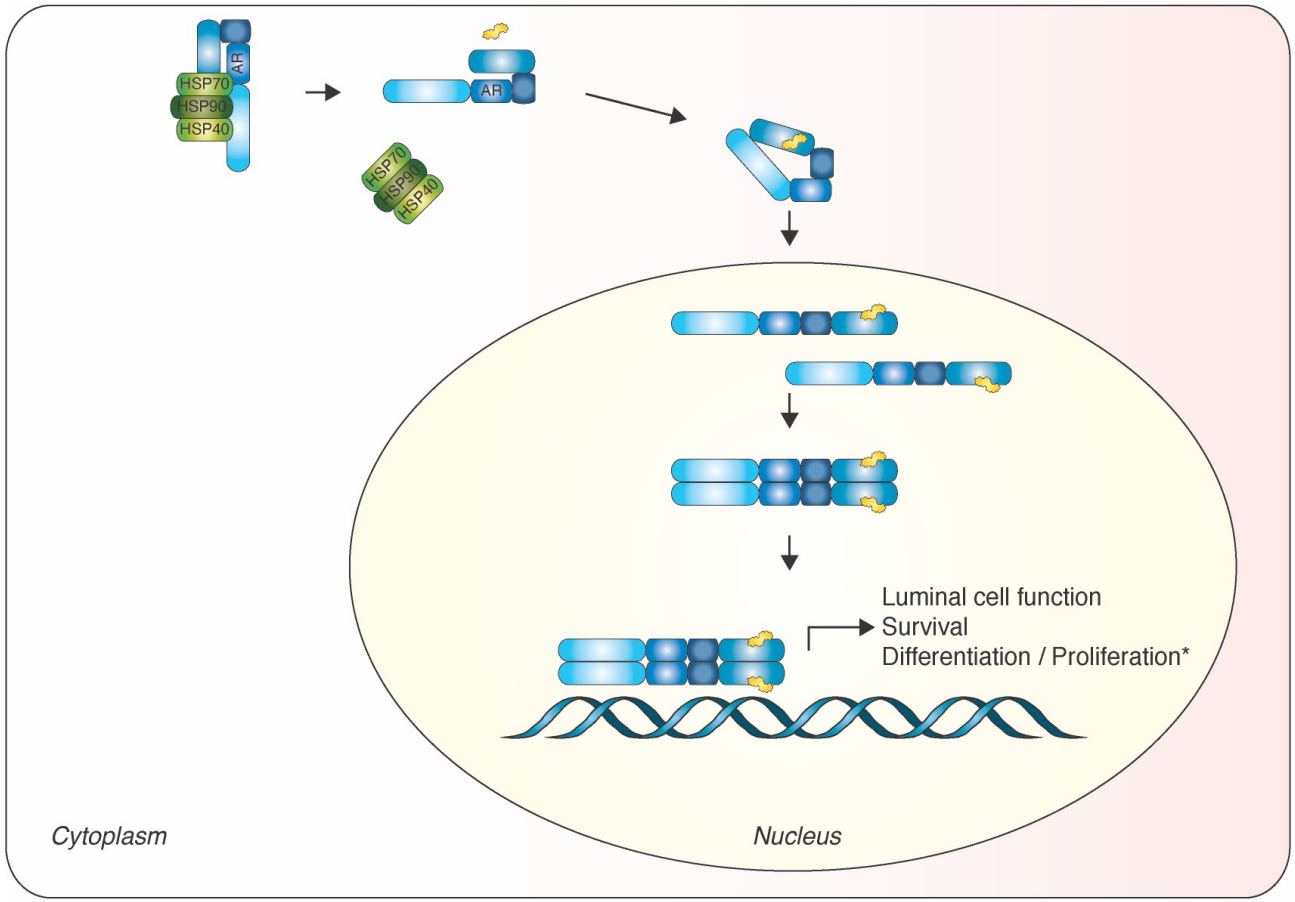


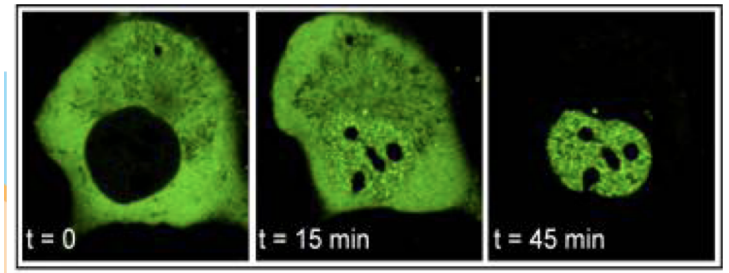
The control of nuclear import during T cell activation



AR signaling is reprogrammed in prostate cancer

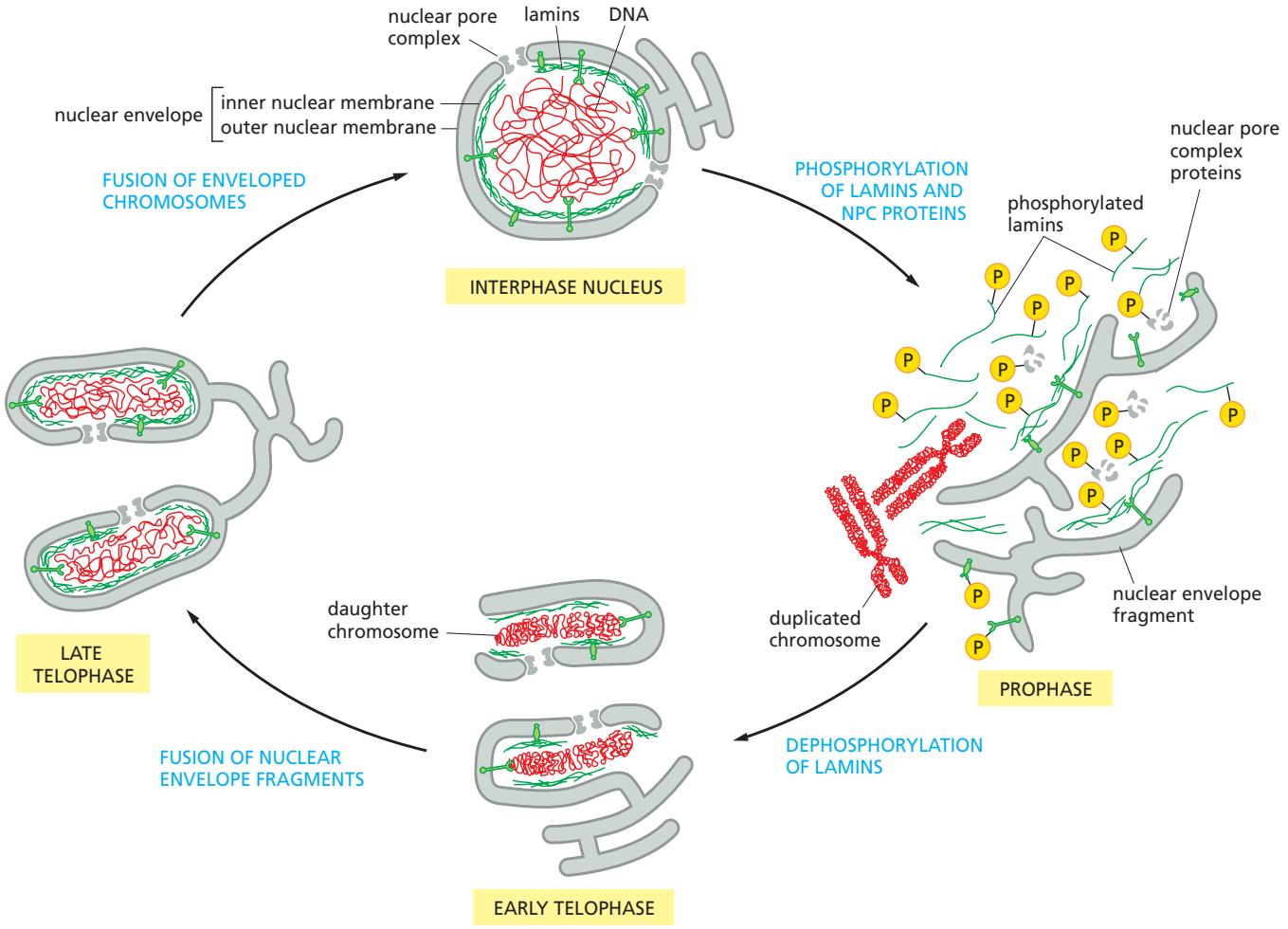






Benign Luminal cell

The breakdown and re- formation of the nuclear envelope and lamina during mitosis

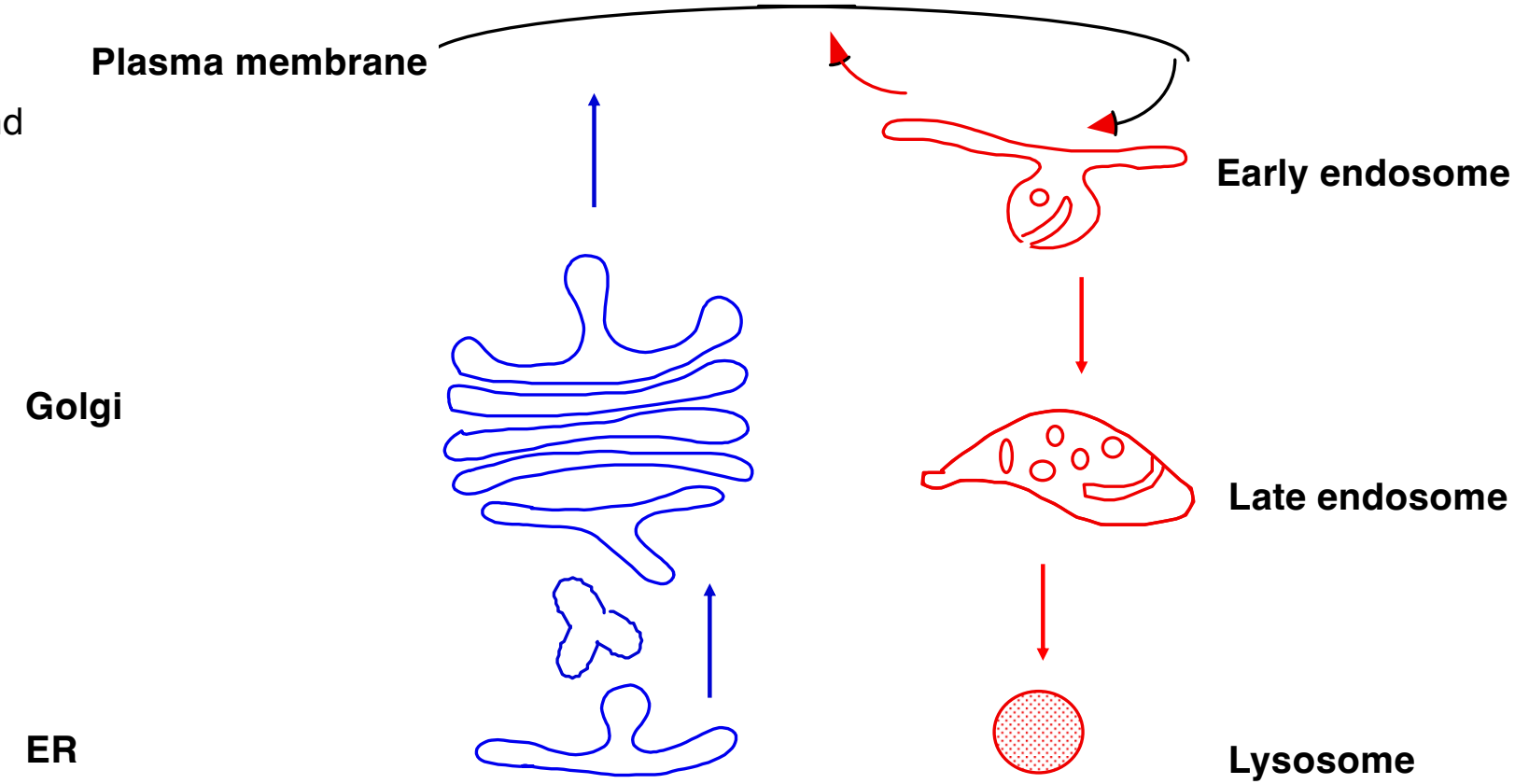


Cytosolic proteins are efficiently excluded from reassembling nuclei by the mechanism of reassembly. The nuclear envelope is initially closely applied to the surface of the chromosomes, excluding all proteins except those bound to the mitotic chromosomes. Once the envelope is complete, other residents of the nucleus are imported via their nuclear localization signals.

The ER, protein folding and directionality of traffic

Transport pathways

two major intracellular transport pathways: biosynthesis and endocytosis



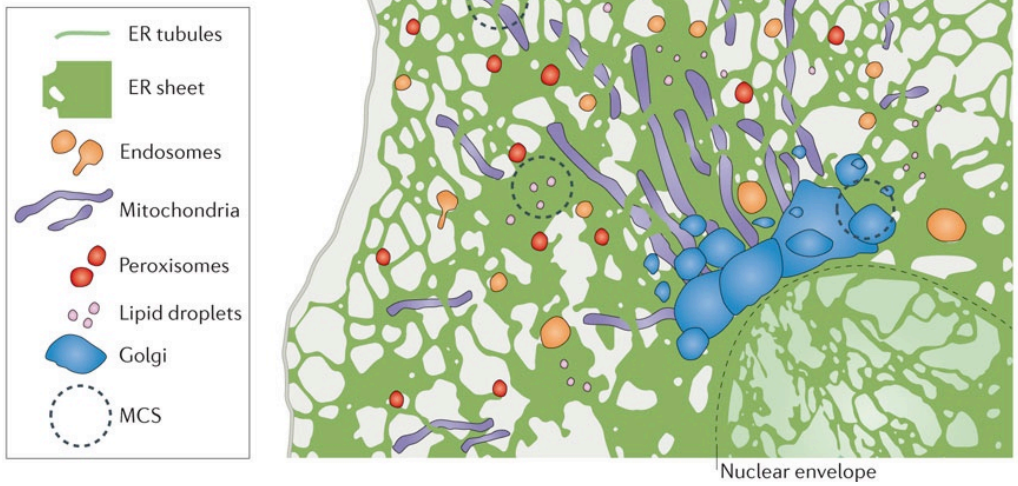
Transport pathways with membranes

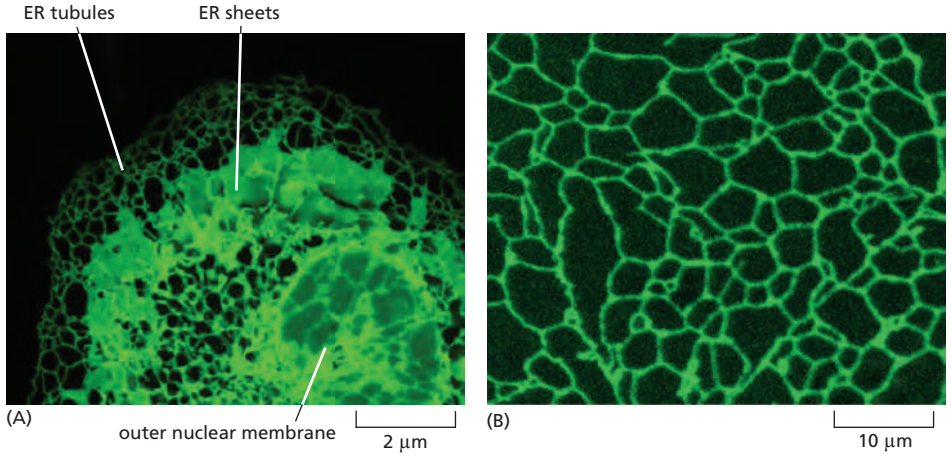
Two major pathways of the endomembrane system

Biosynthesis/secretion
- Endoplasmic reticulum
- Golgi apparatus
- Trans Golgi network
- Plasma membrane

Endocytosis
- Plasma membrane
- Precose endosomes
- Recycling endosomes
- Multivesicular endosomes - Lysosomes
- By contact (very fast transport of lipids, transport of ions like Ca2+)
- By vesicular transport (transport of proteins and lipids)

Endoplasmic reticulum





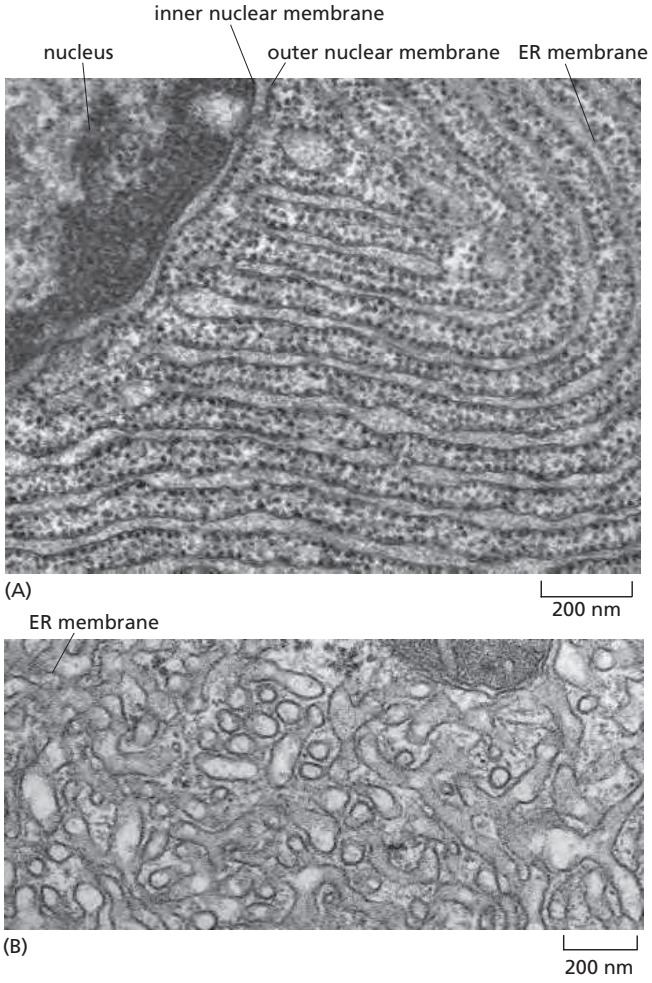
ER lumen = ER cisternal space

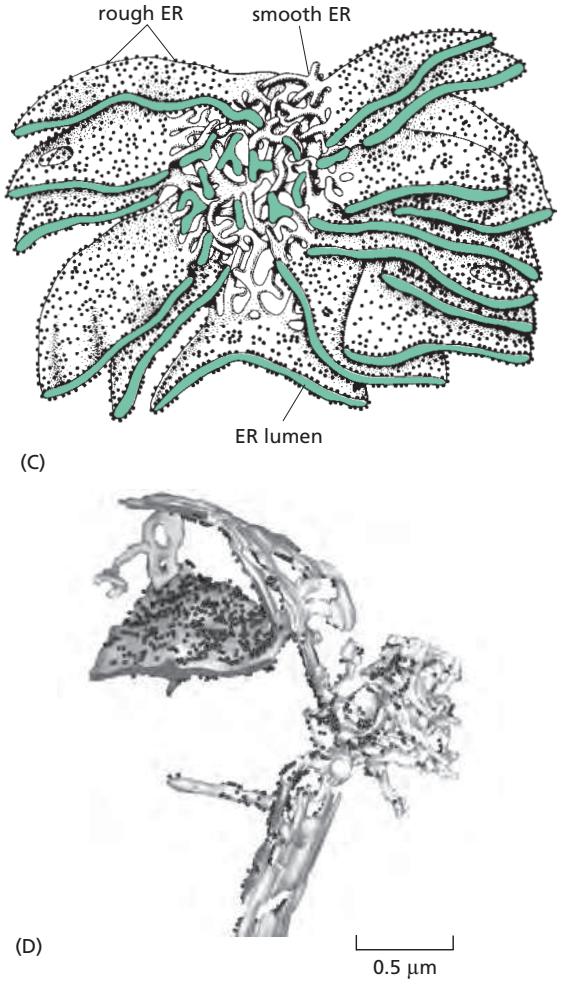
Lipid & Protein Biosynthesis

Site of production of all transmembrane proteins and lipids for all the organelles

Ca2+ storage in the cell (SERCA pump discussed last week!)

The rough and smooth ER

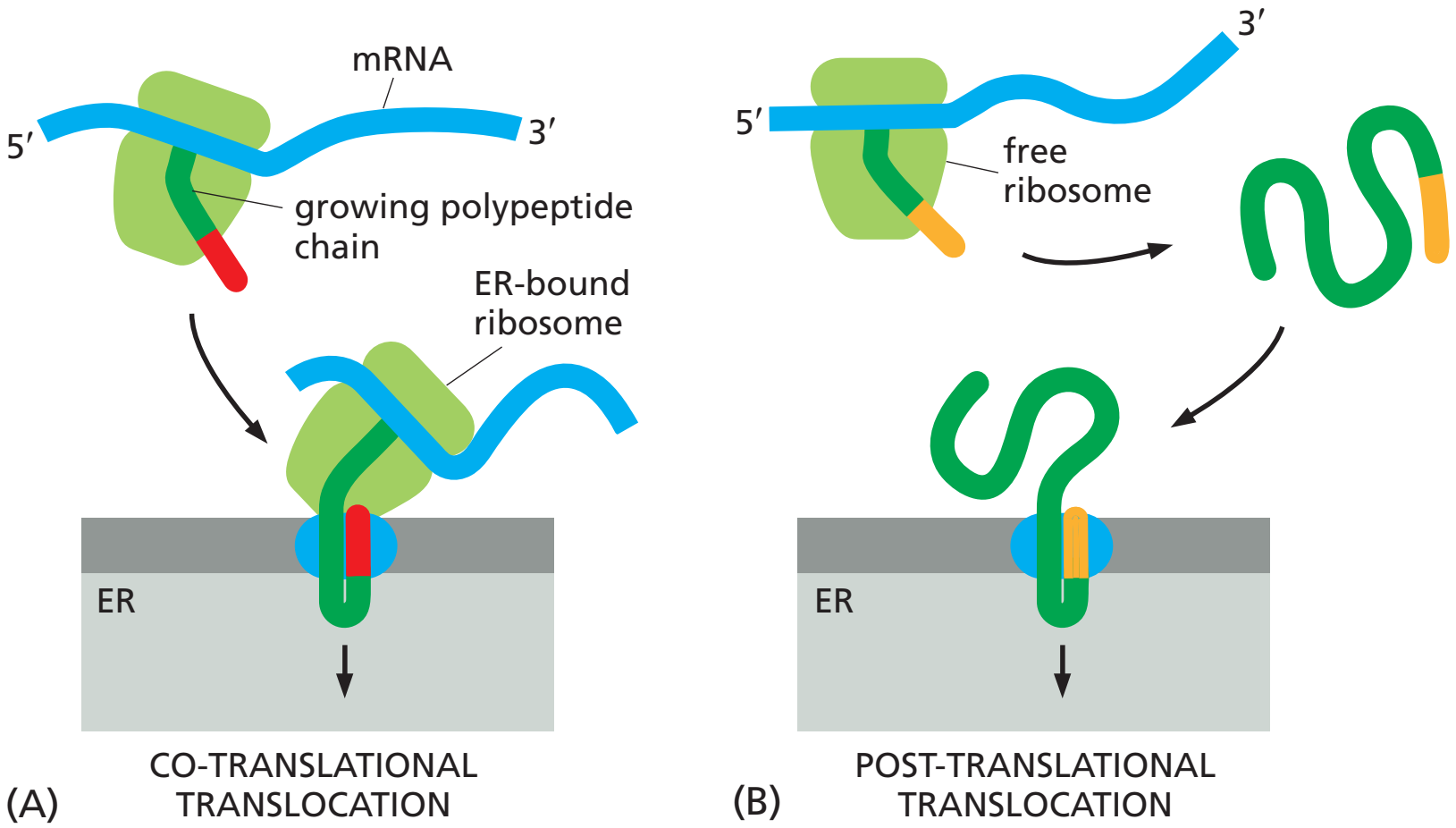




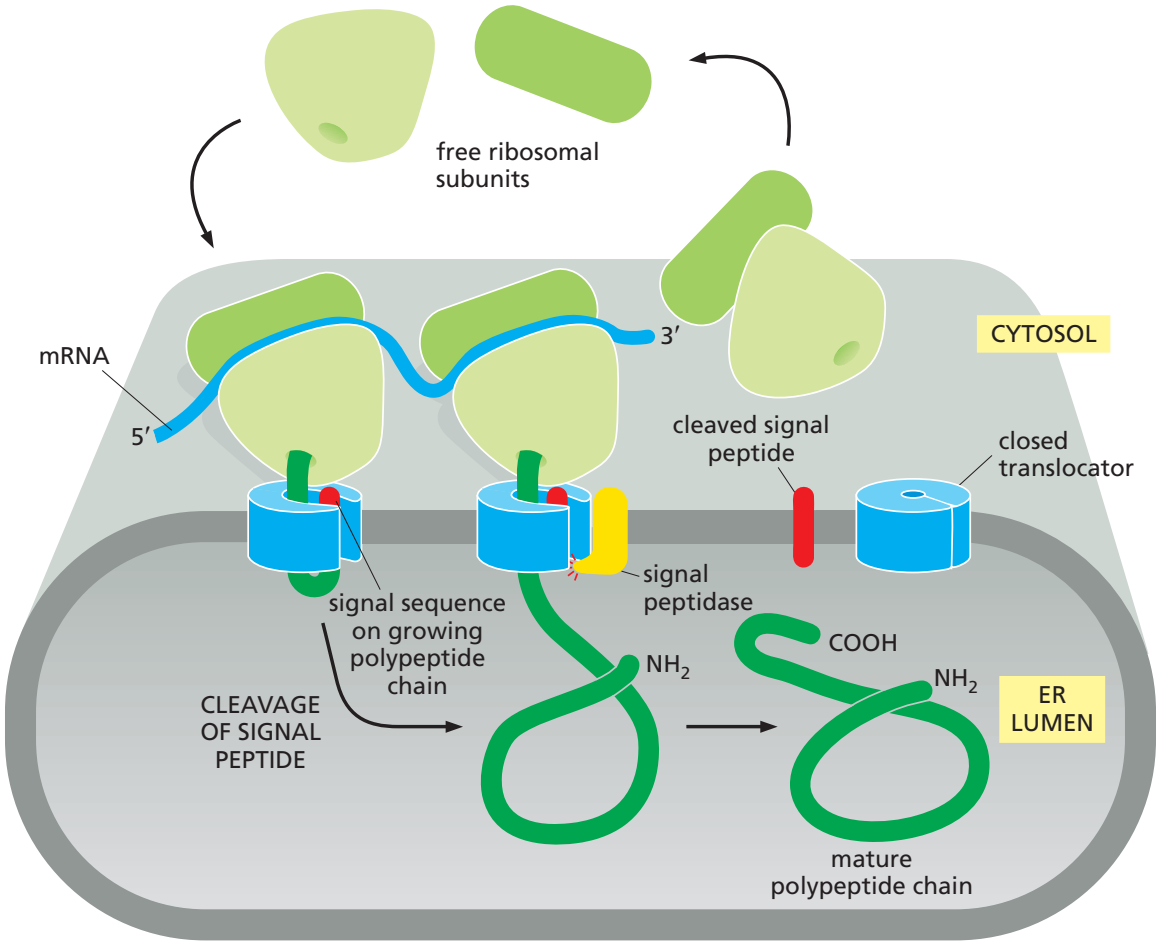
Membrane bound ribosomes = Rough ER

Most cells have both, but ratio differs per cell type

Co-translational and post- translational protein translocation to the ER



Co-translational translocation



The signal-recognition particle (SRP)

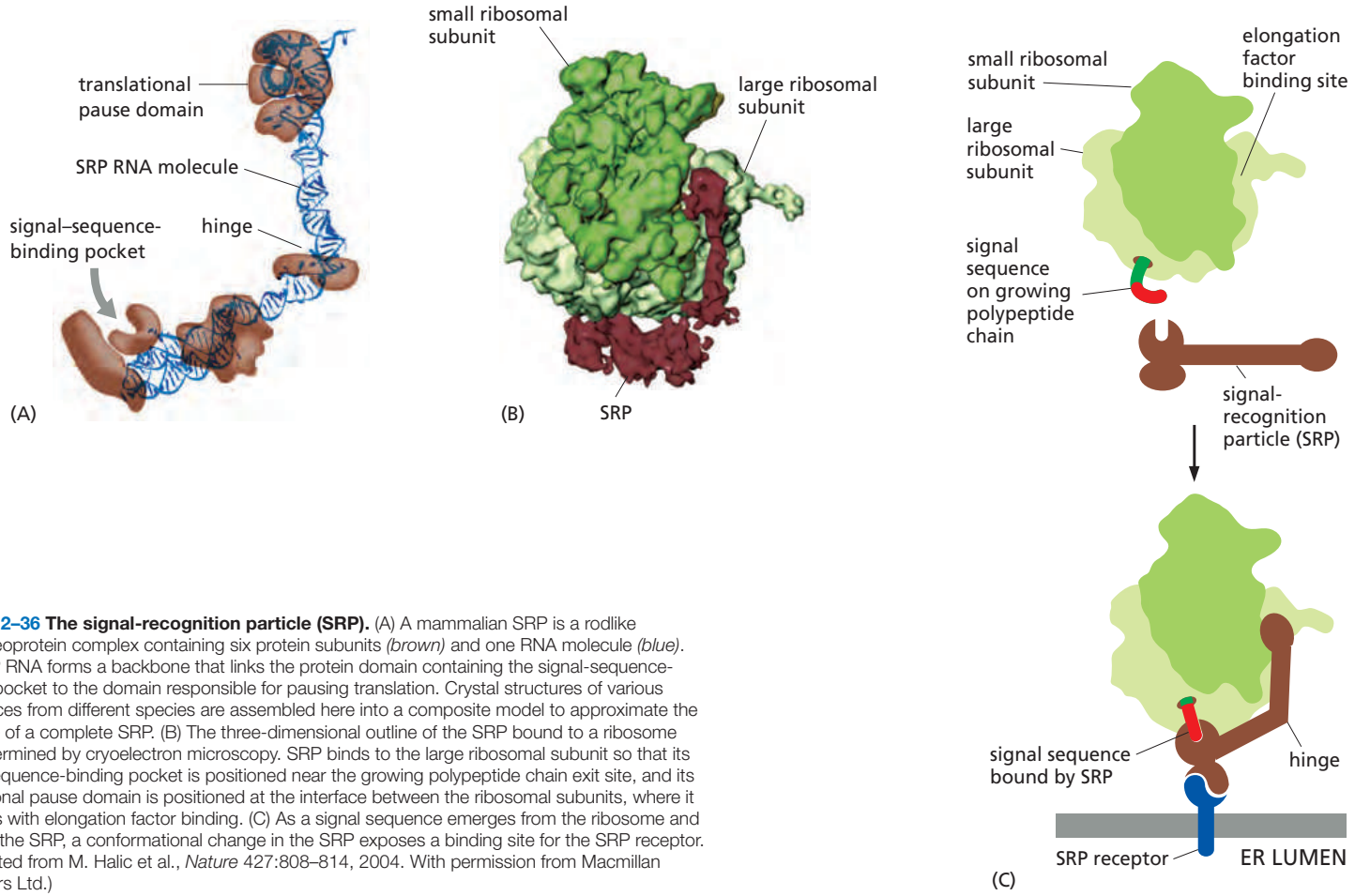
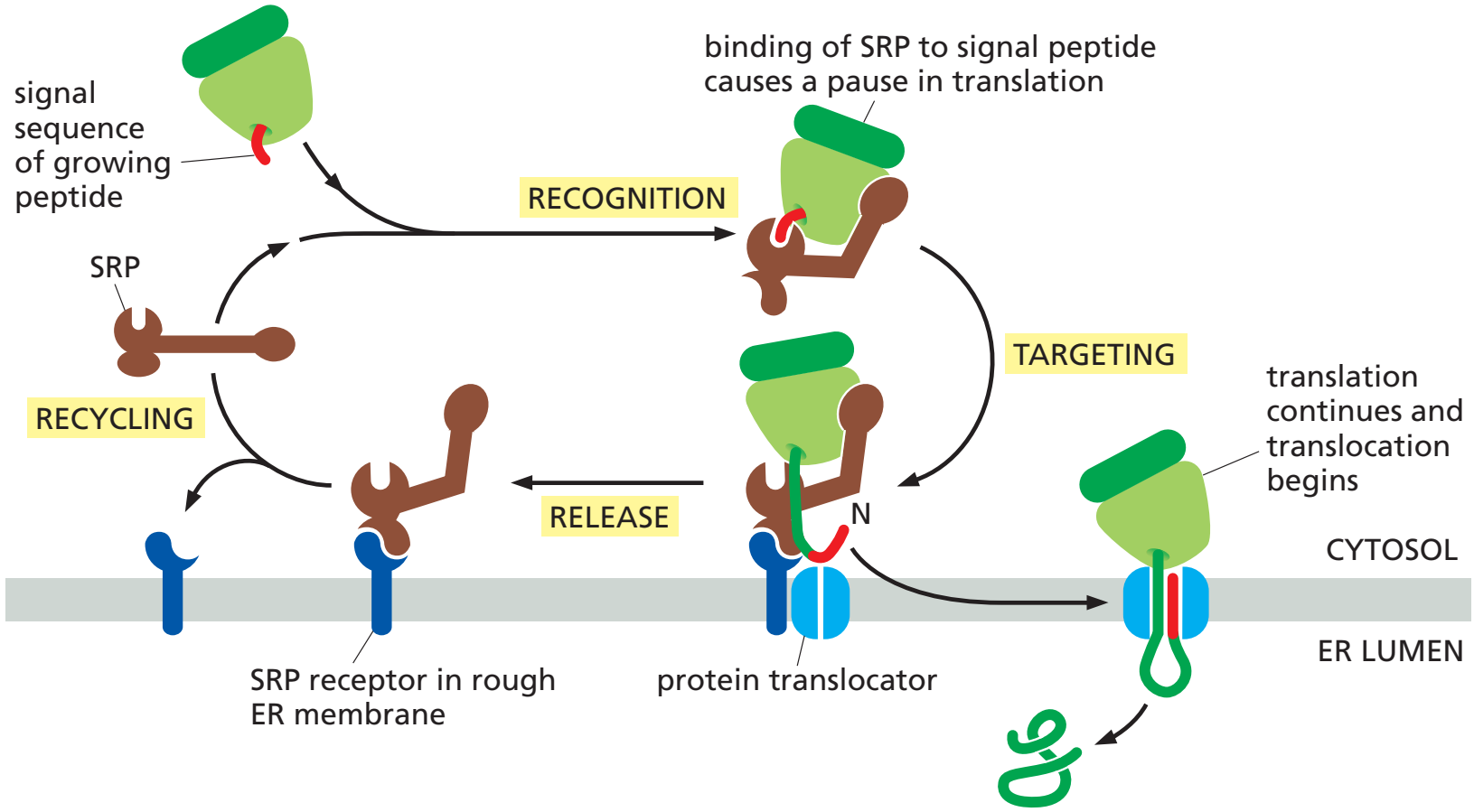
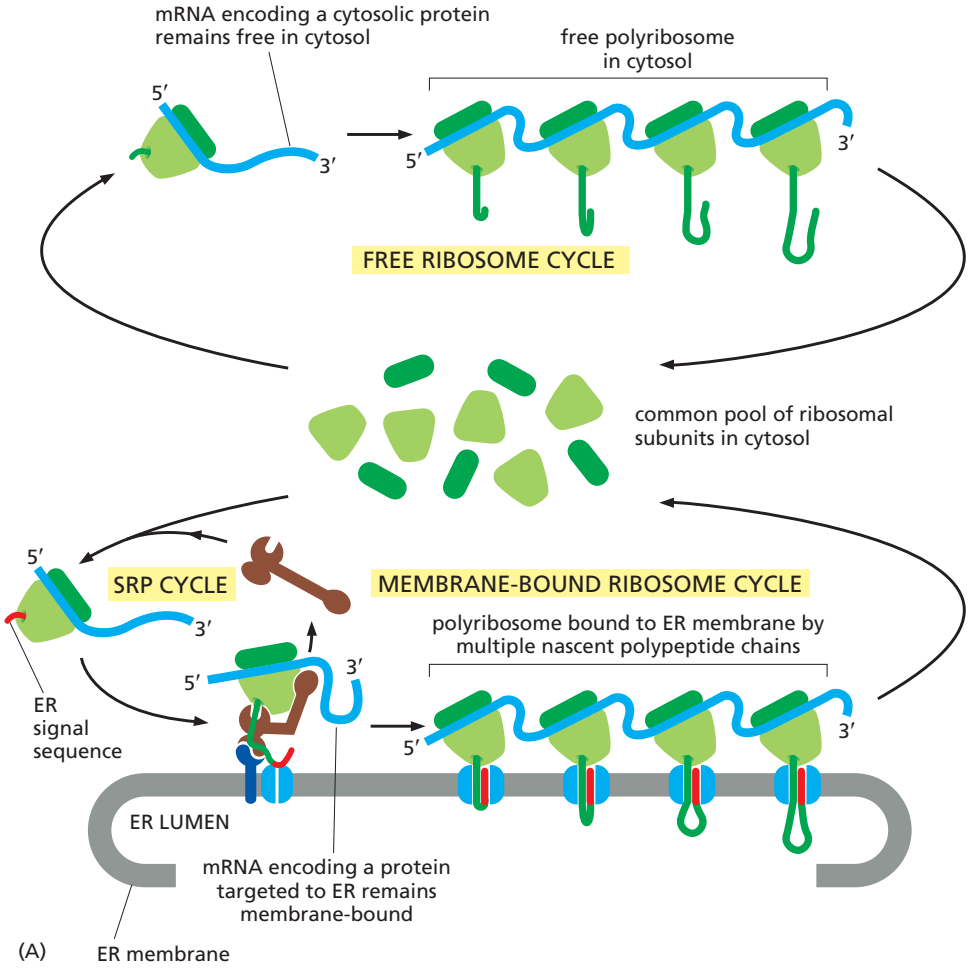


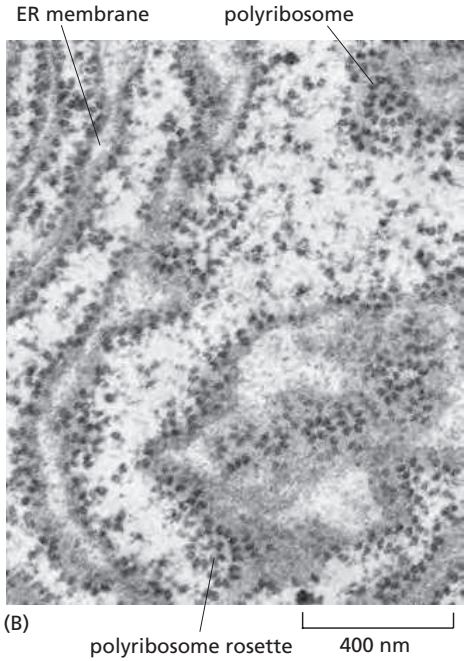
Figure 12–36 The signal-recognition particle (SRP). (A) A mammalian SRP is a rodlike ribonucleoprotein complex containing six protein subunits *(brown)* and one RNA molecule *(blue)*. The SRP RNA forms a backbone that links the protein domain containing the signal-sequence-binding pocket to the domain responsible for pausing translation. Crystal structures of various SRP pieces from different species are assembled here into a composite model to approximate the structure of a complete SRP. (B) The three-dimensional outline of the SRP bound to a ribosome was determined by cryoelectron microscopy. SRP binds to the large ribosomal subunit so that its signal-sequence-binding pocket is positioned near the growing polypeptide chain exit site, and its translational pause domain is positioned at the interface between the ribosomal subunits, where it interferes with elongation factor binding. (C) As a signal sequence emerges from the ribosome and binds to the SRP, a conformational change in the SRP exposes a binding site for the SRP receptor. (B, adapted from M. Halic et al., *Nature* 427:808–814, 2004. With permission from Macmillan Publishers Ltd.)

How ER signal sequences and SRP direct ribosomes to the ER membrane

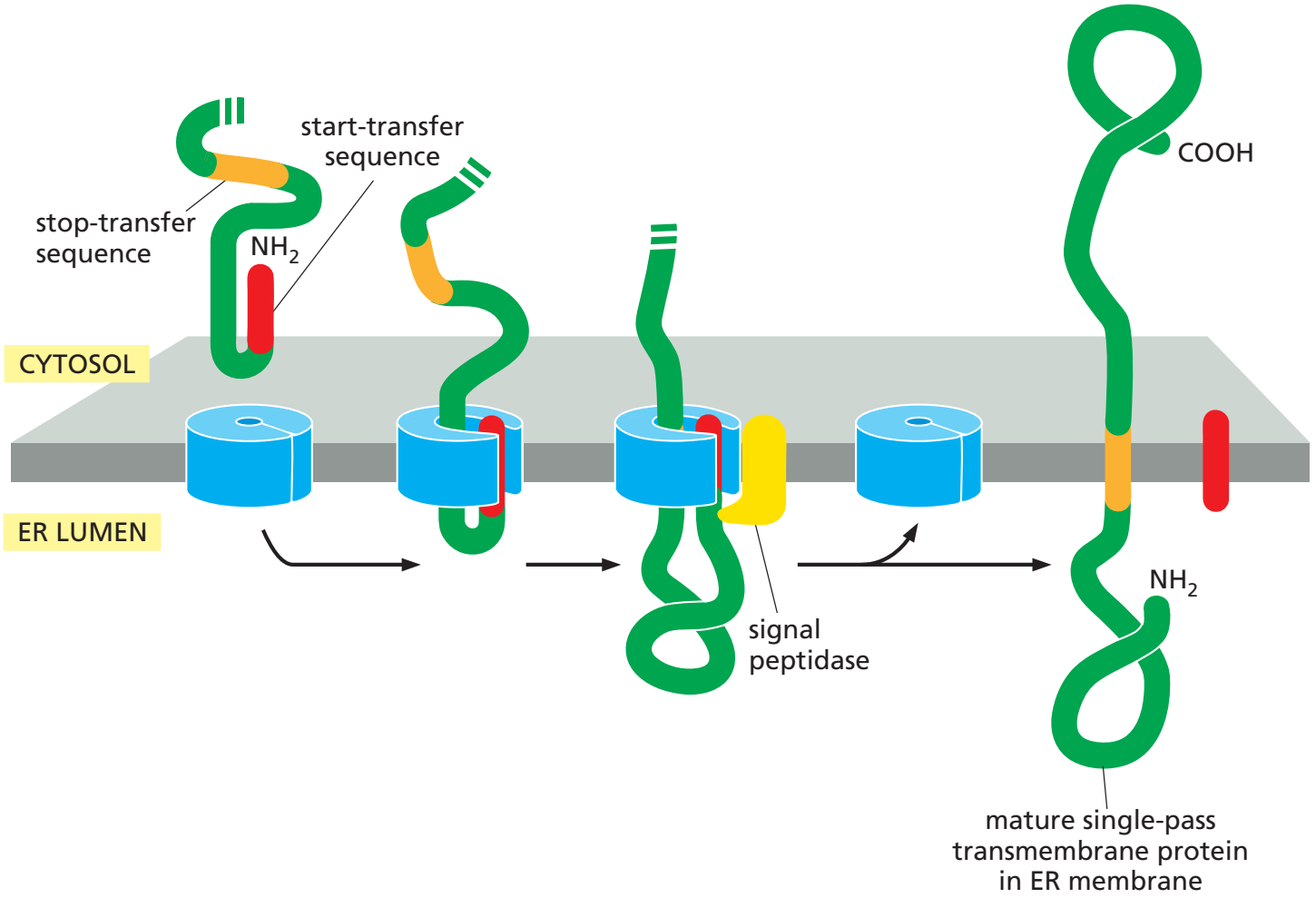


Free and membrane-bound polyribosomes

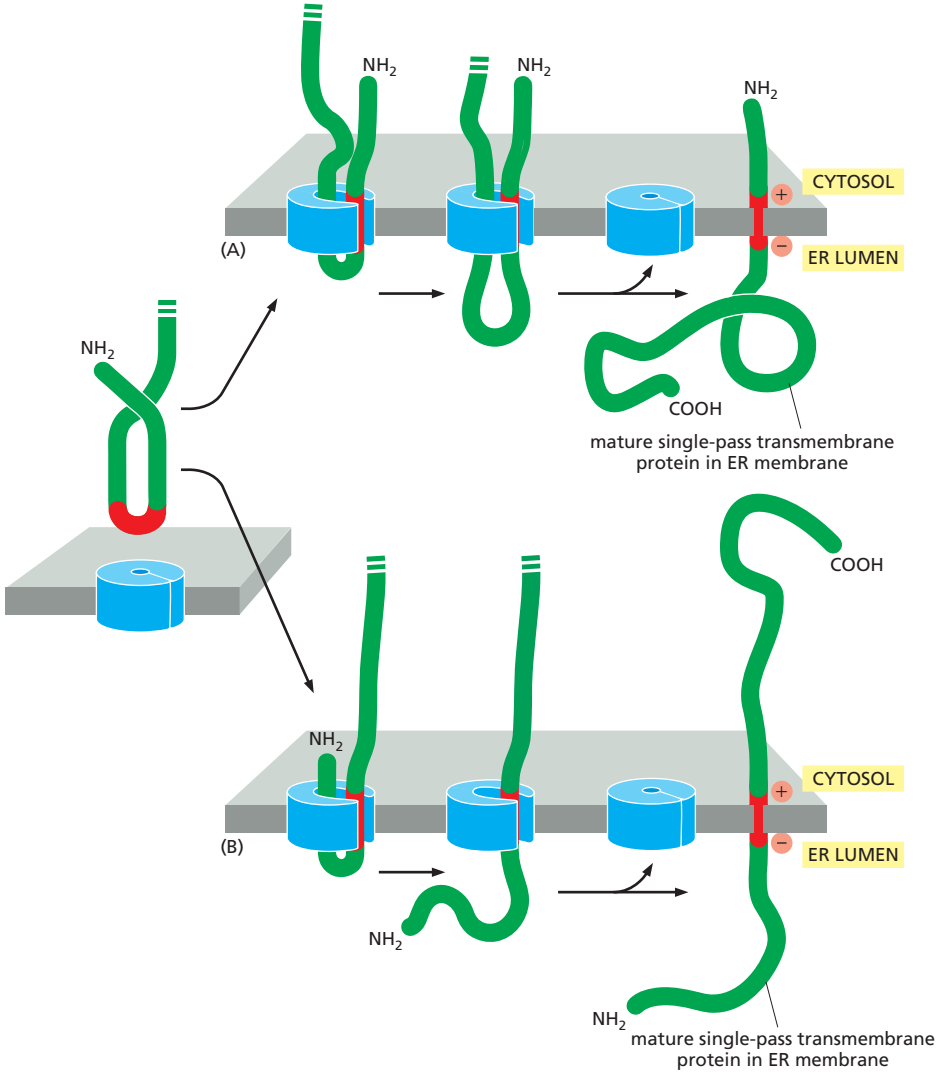




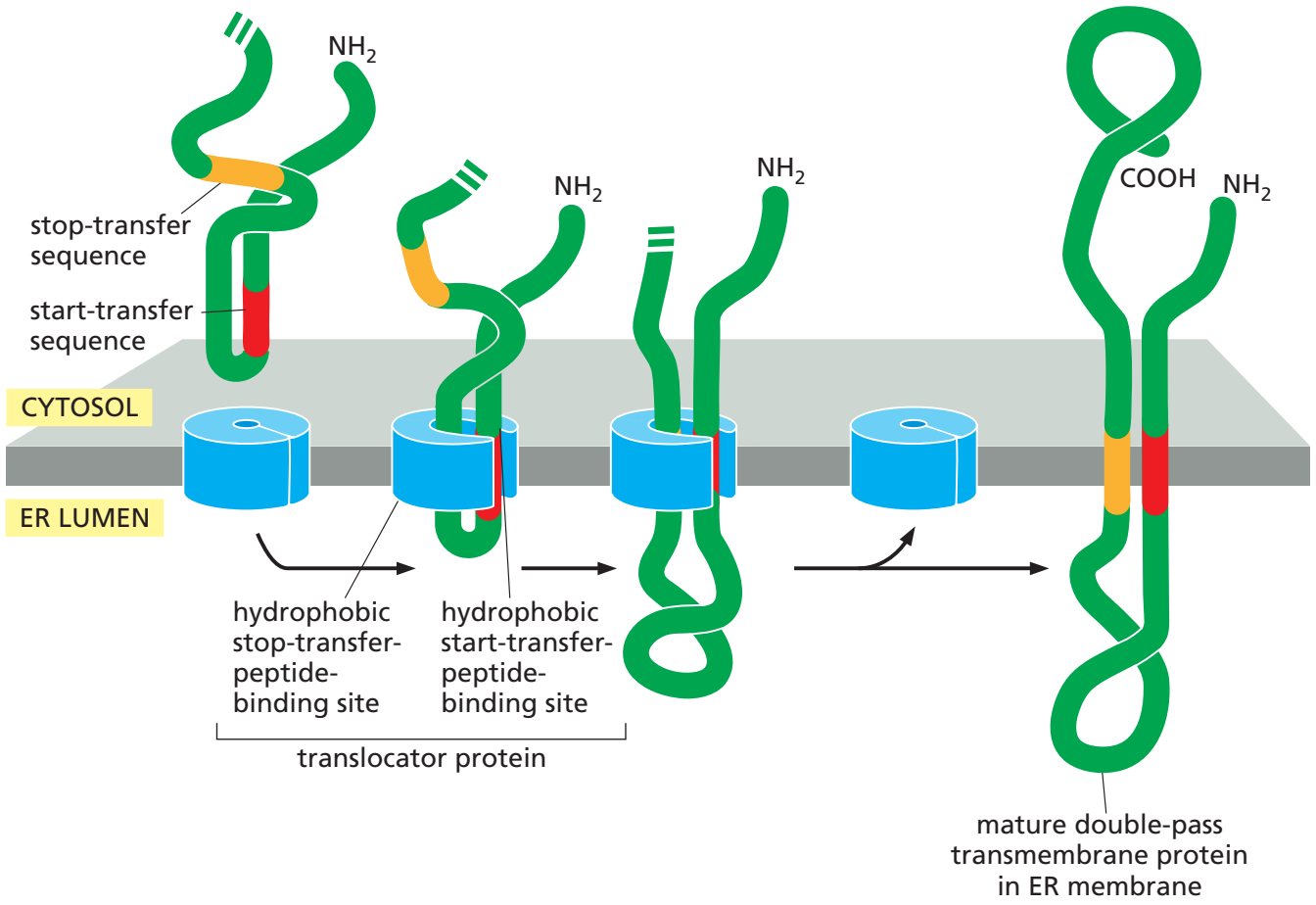
How a single-pass transmembrane protein with a cleaved ER signal sequence is integrated into the ER membrane



Integration of a single- pass transmembrane protein with an internal signal sequence into the ER membrane



Integration of a double- pass transmembrane protein with an internal signal sequence into the ER membrane



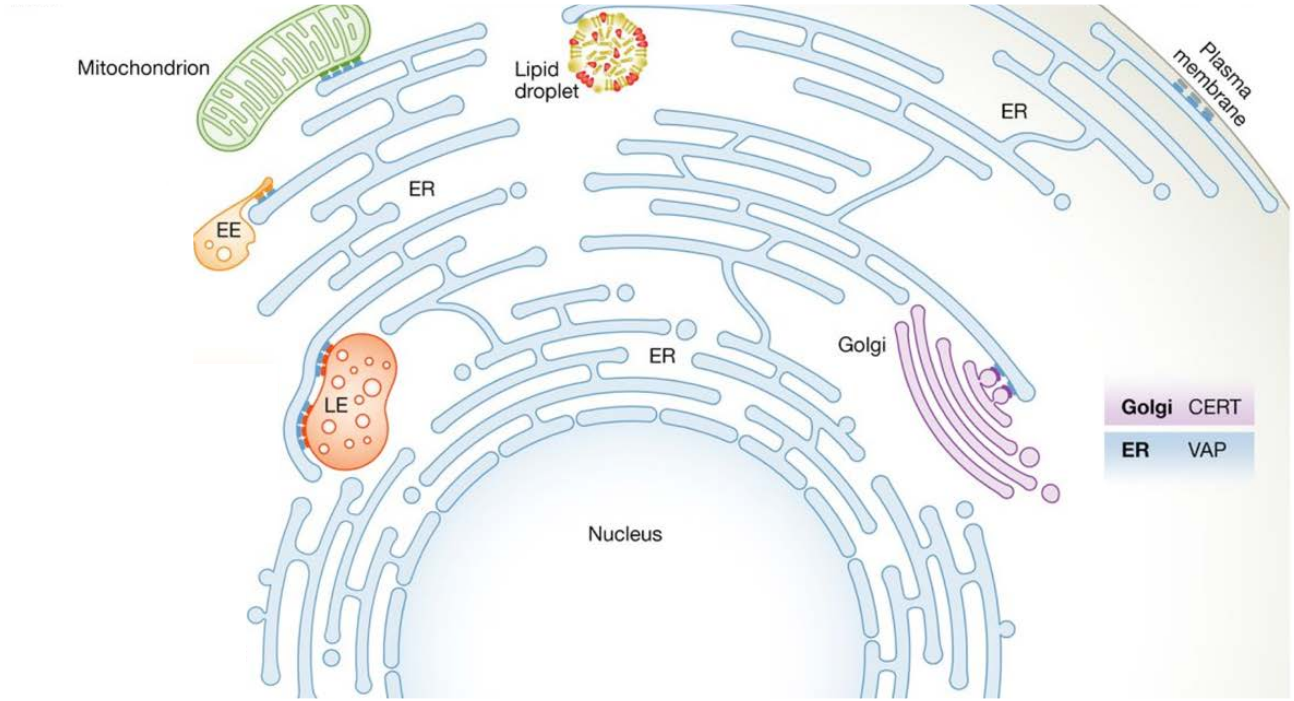
The ER, directionality of traffic

Transport pathways

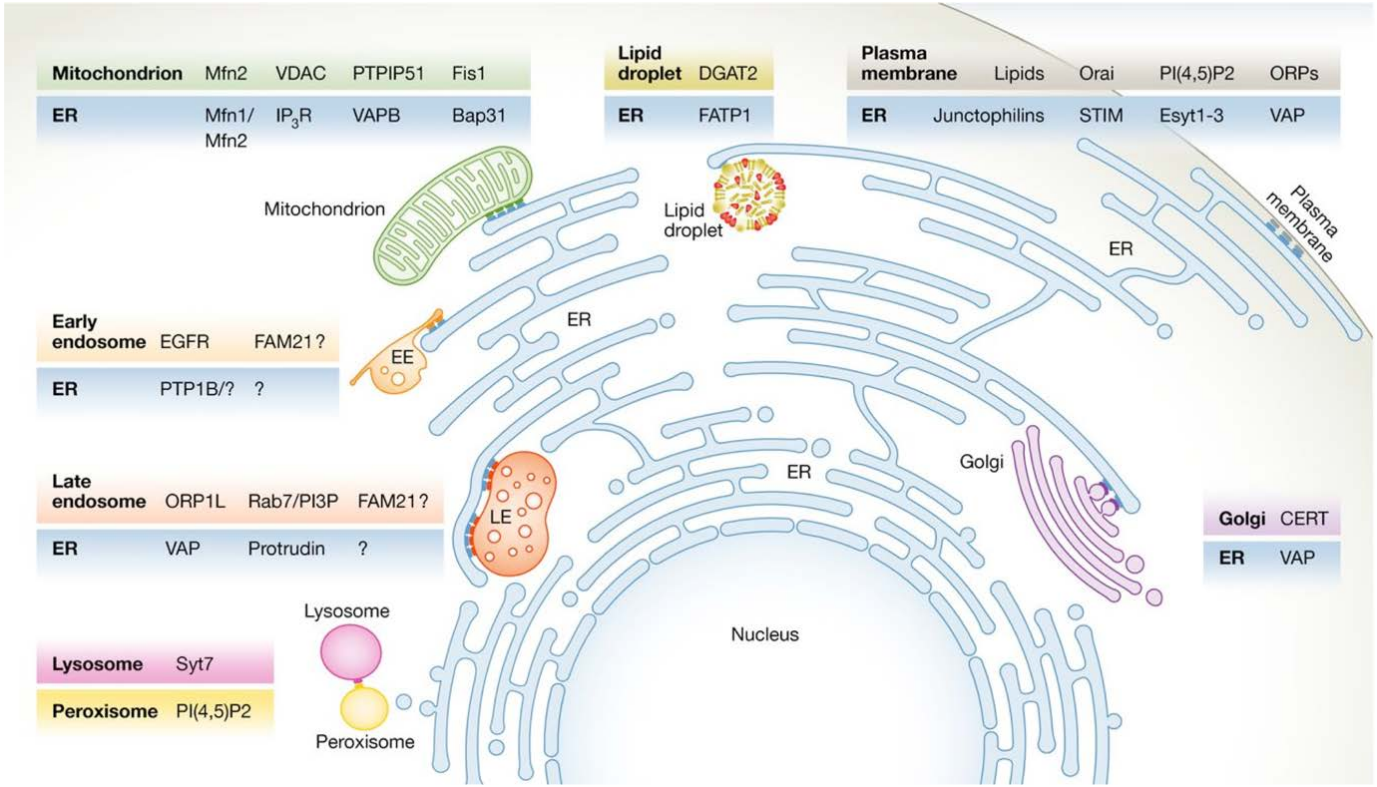
Transport of lipids and proteins
Two modes of transport have been identified:
- via vesicles that shuttle between 2 "distant" compartments
- at the level of contact zones between 2 compartments Vesicular transport, known for a long time, allows to to transport proteins and lipids
Transfer at the level of contact zones: recognized very recently, not yet in the textbooks

Contact between organelles

contact areas
Between compartments of the endomembrane system but
also with the mitochondria

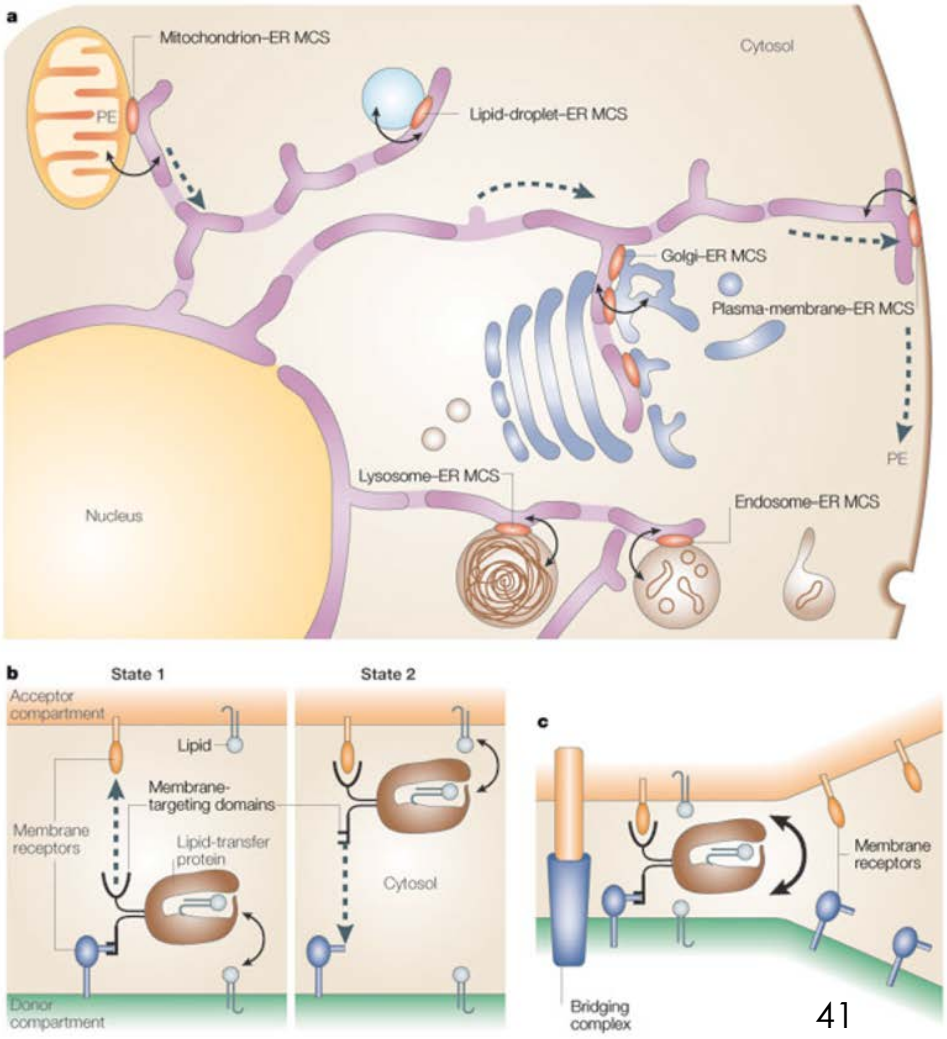


Contact areas



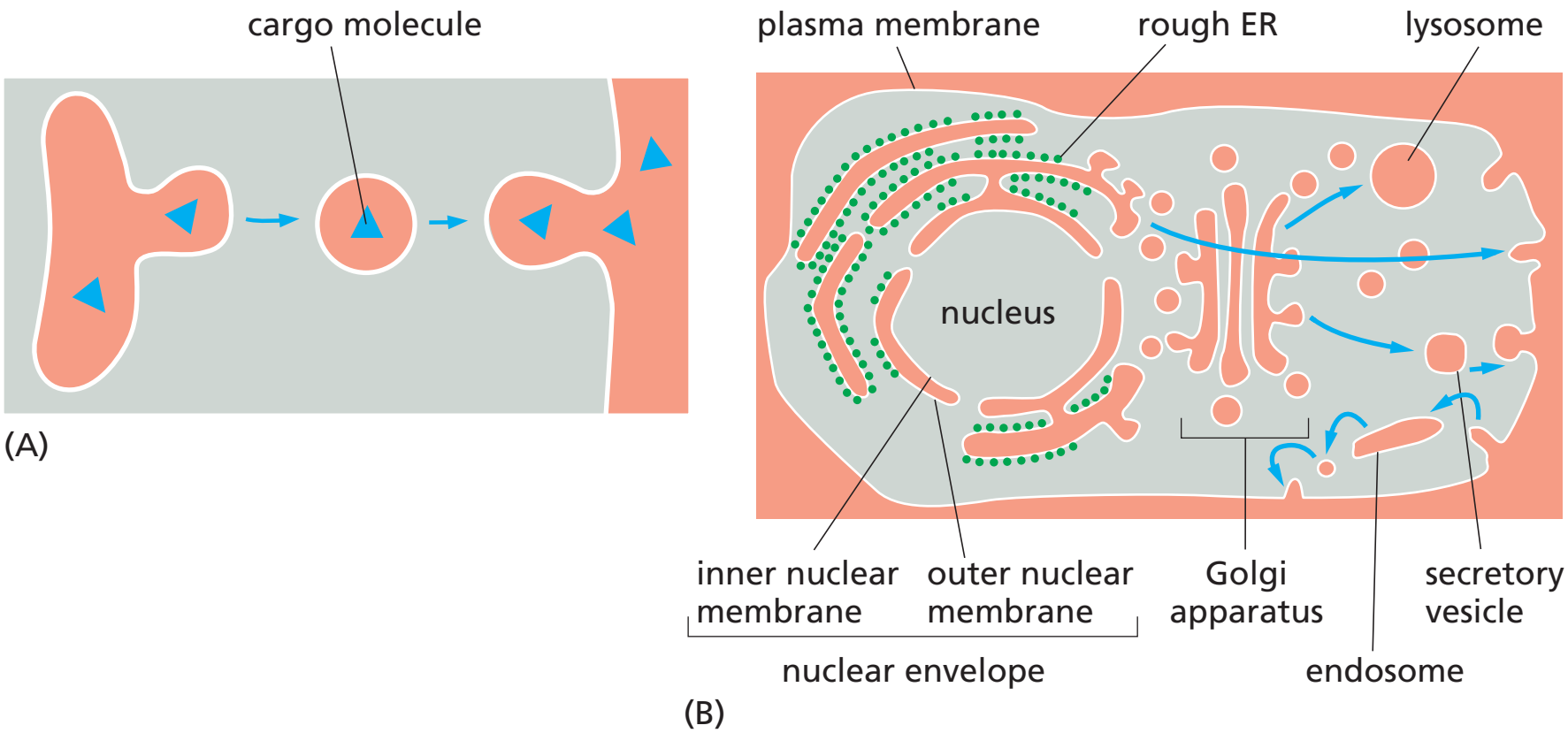
These are not just organelles that touch each other but specific and controlled interactions

Contact areas



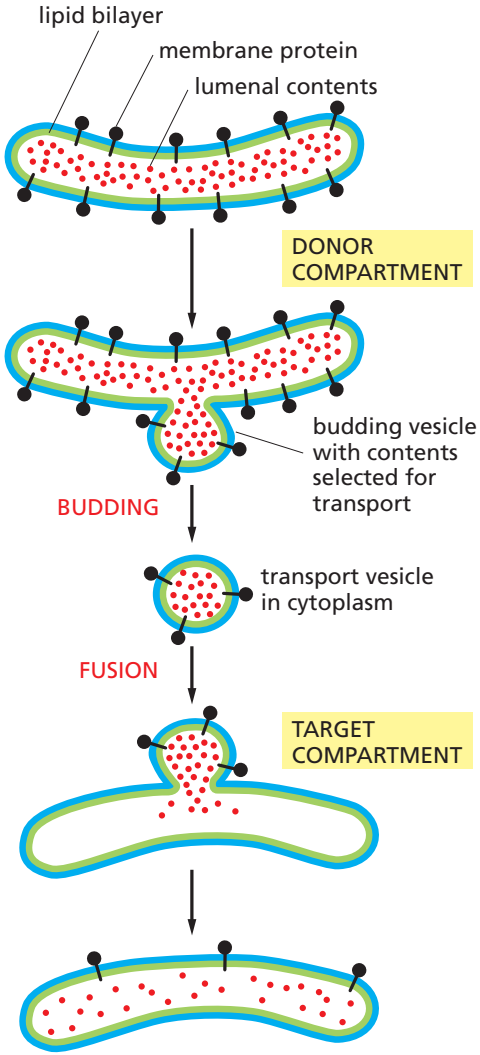
Example of lipid transfer

Topologically equivalent compartments, are able to “communicate”



Topology of cellular spaces: the interior of the compartments of the endomembrane system is topologically equivalent to the extracellular space This is a consequence of vesicular transport

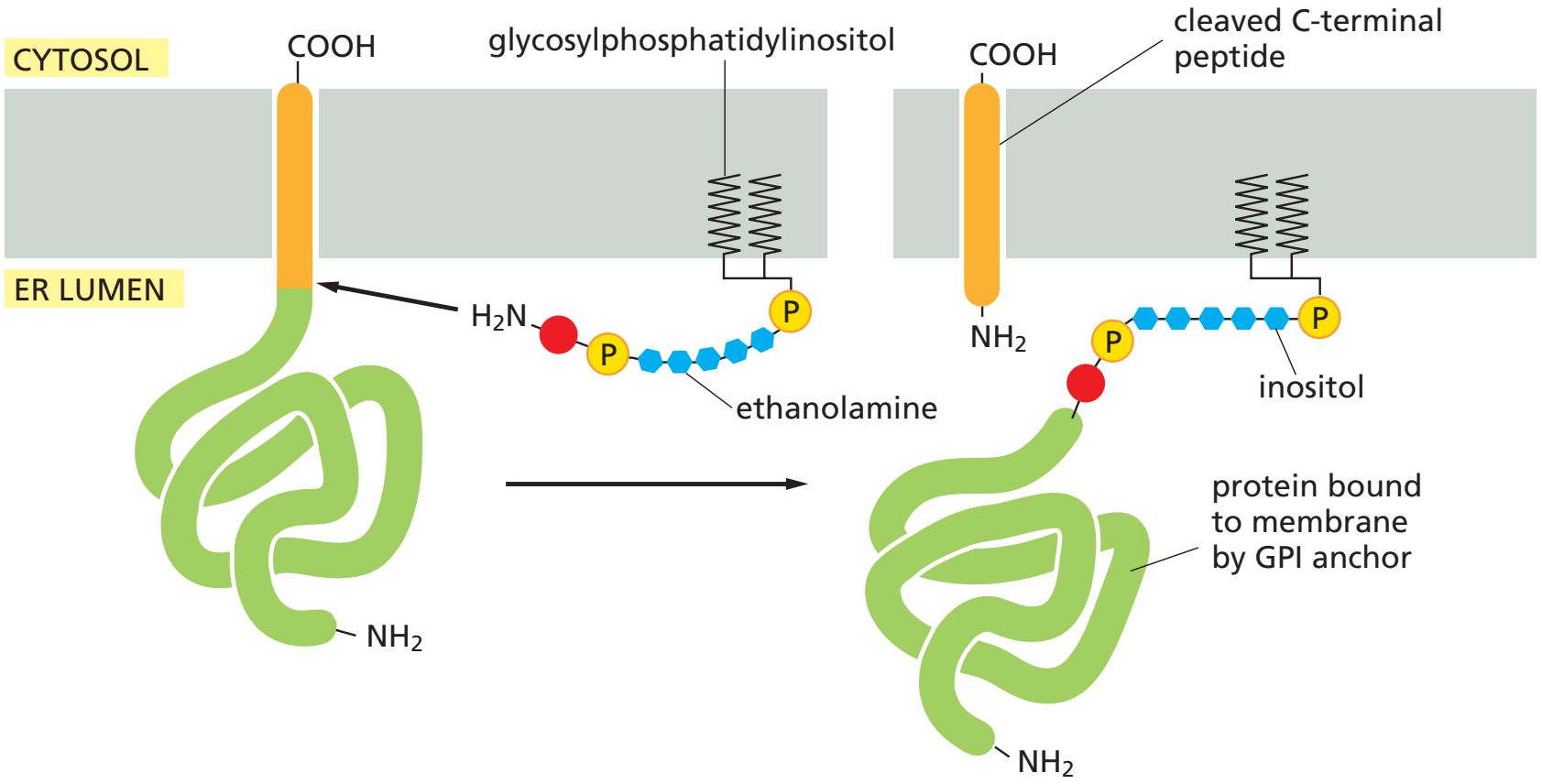
Vesicle budding and fusion during vesicular transport



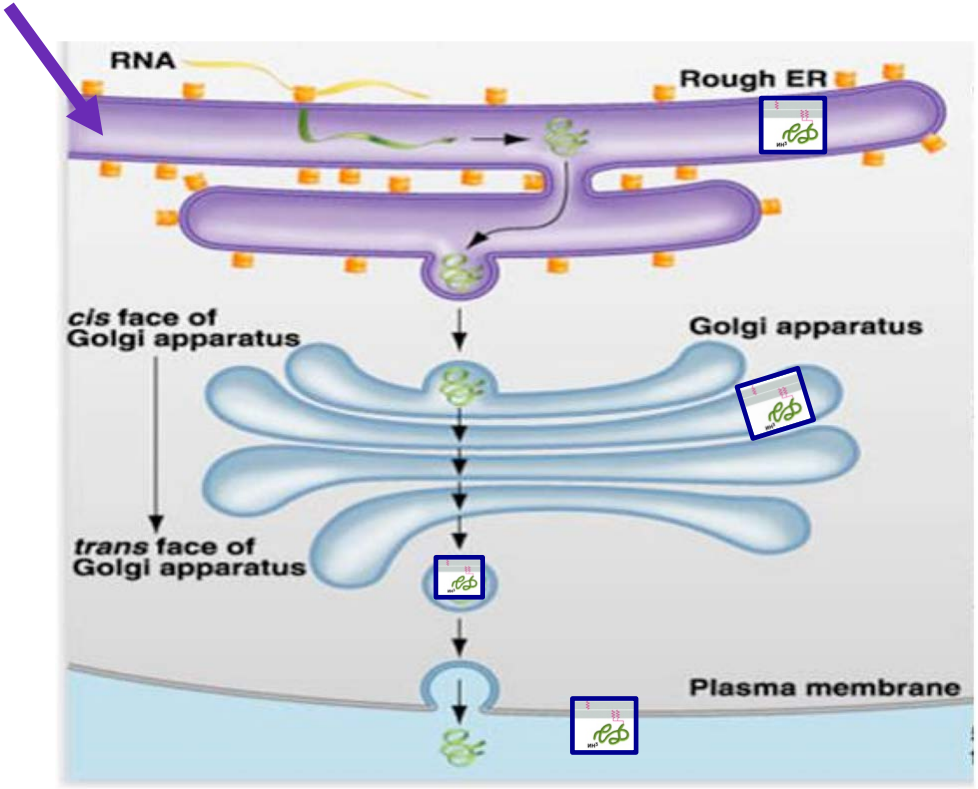
in principle, a protein can access the lumen of any other lumen of the endomembrane system by vesicular transport
But ribosomes are cytoplasmic!
How do proteins get into lumens?
The problem of topology is solved as soon as the protein is synthesized, at the same place, with the same machinery for all.

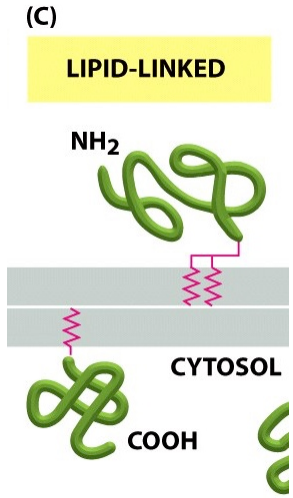
Membrane, luminal, or secreted proteins are synthesized by ribosomes attached to the rough endoplasmic reticulum

The attachment of a GPI anchor to a protein in the ER



Directionality of molecules by synthesis

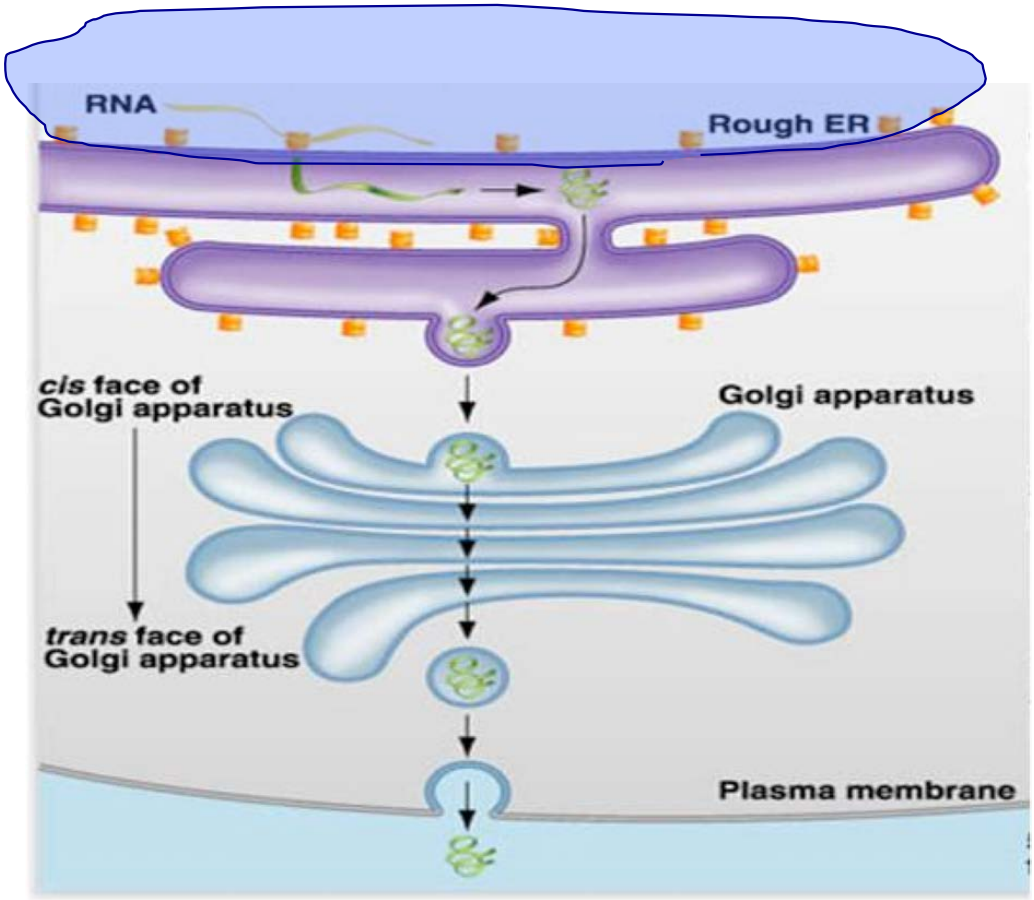




50

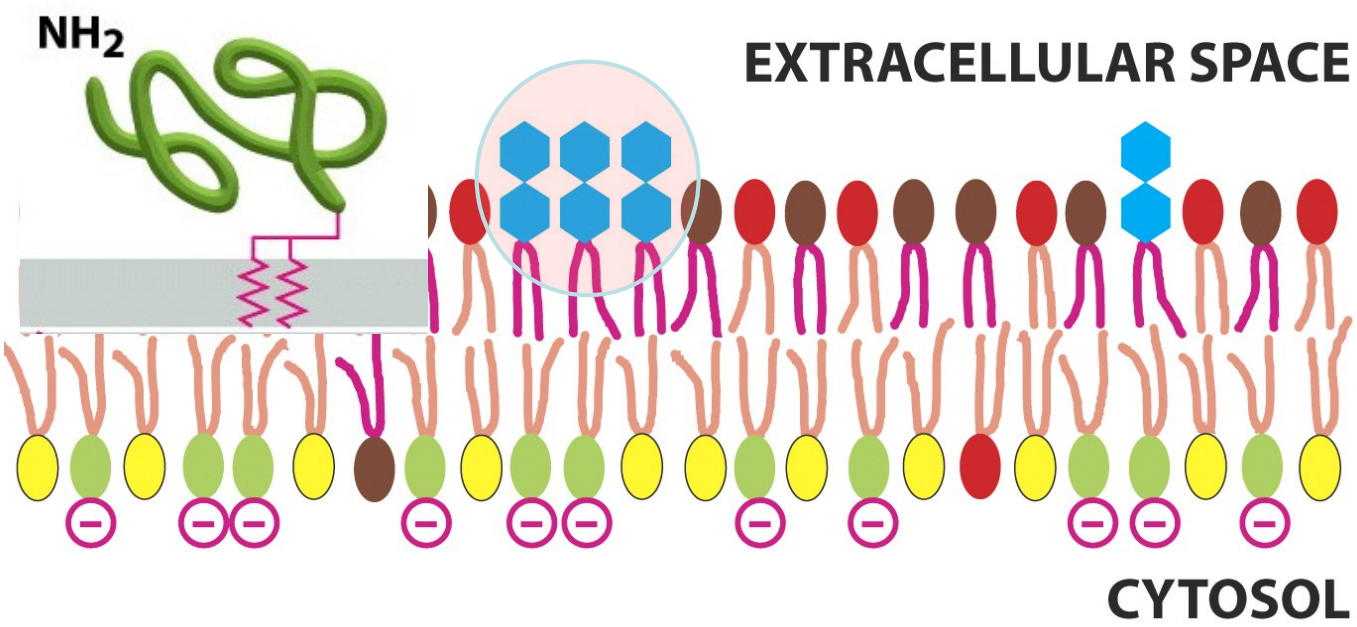
GPI-anchored proteins are therefore always exposed to the outside of the cell

Directionality of molecules by synthesis



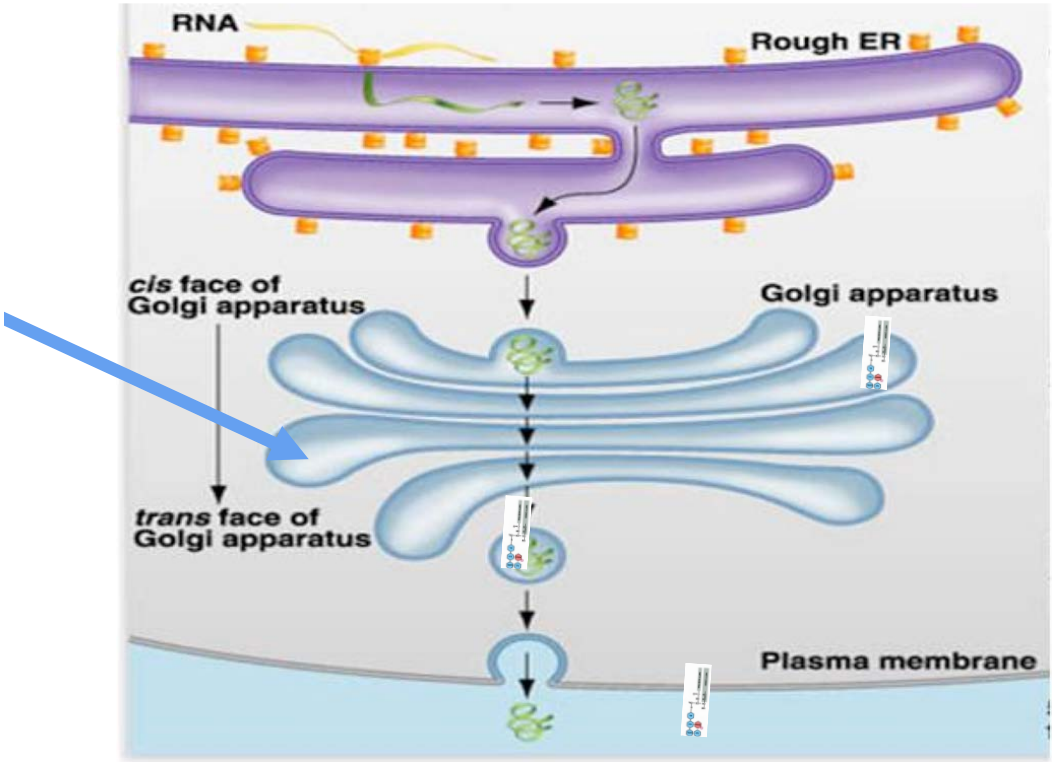
The synthesis of the majority of lipids takes place on the cytosolic side of the ER

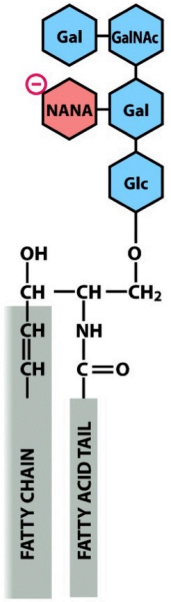
Directionality of molecules by synthesis



Membrane asymmetry is largely due to the topology of lipid synthesis and transport by vesicles or lipid carrier proteins at sites of contact
between compartments

Directionality of molecules by synthesis

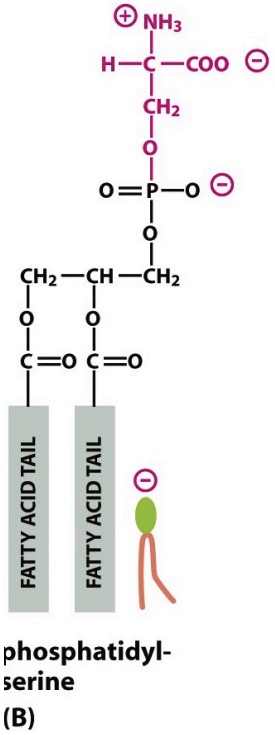




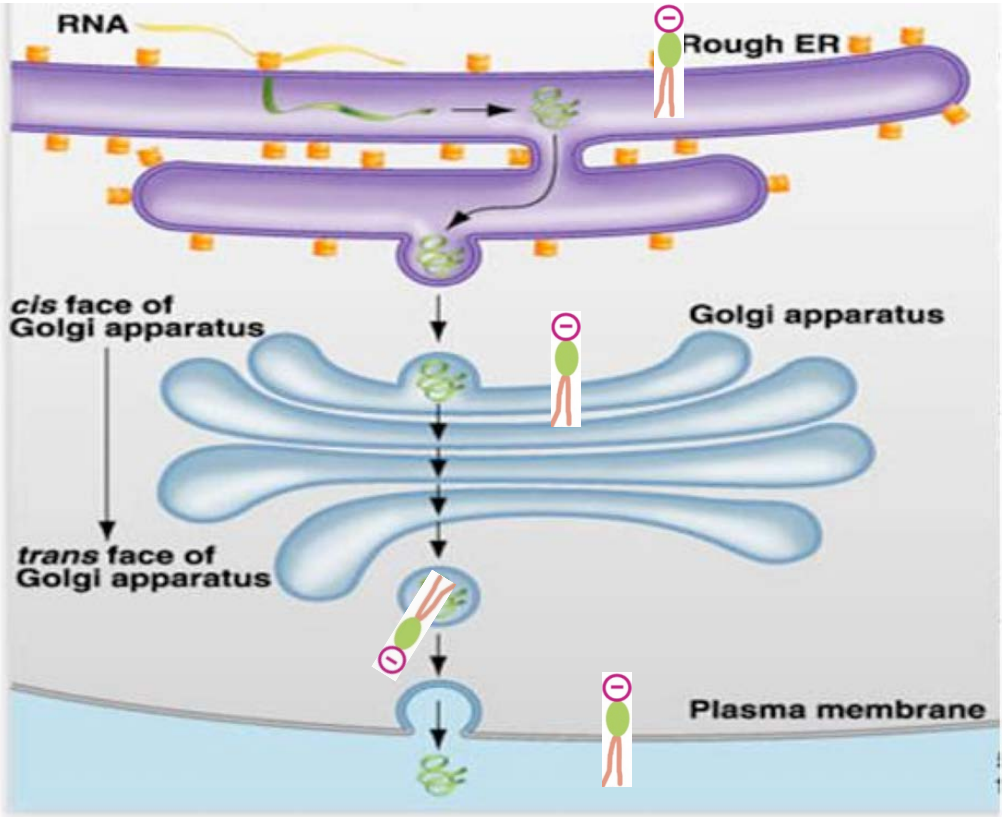
53

Addition of sugars on glycolipids is done in the lumen of the Golgi

Directionality of molecules by synthesis



phosphatidyl-serine (B)



phosphatidyl serine

Summary

1. To know the cellular compartments and their main functions
2. To know how to define the endomembrane system
3. Understand the topology of the compartments
4. Know that there are two main forms of exchange between between compartments: by contact and by vesicular transport
5. Have a notion of the distribution of membranes (in quantity)
6. Understand that the lipid composition differs from one compartment to another
7. Understand the contribution of vesicular transport to the orientation of proteins and lipids