Cell Biology (BI2213)

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IISER Pune
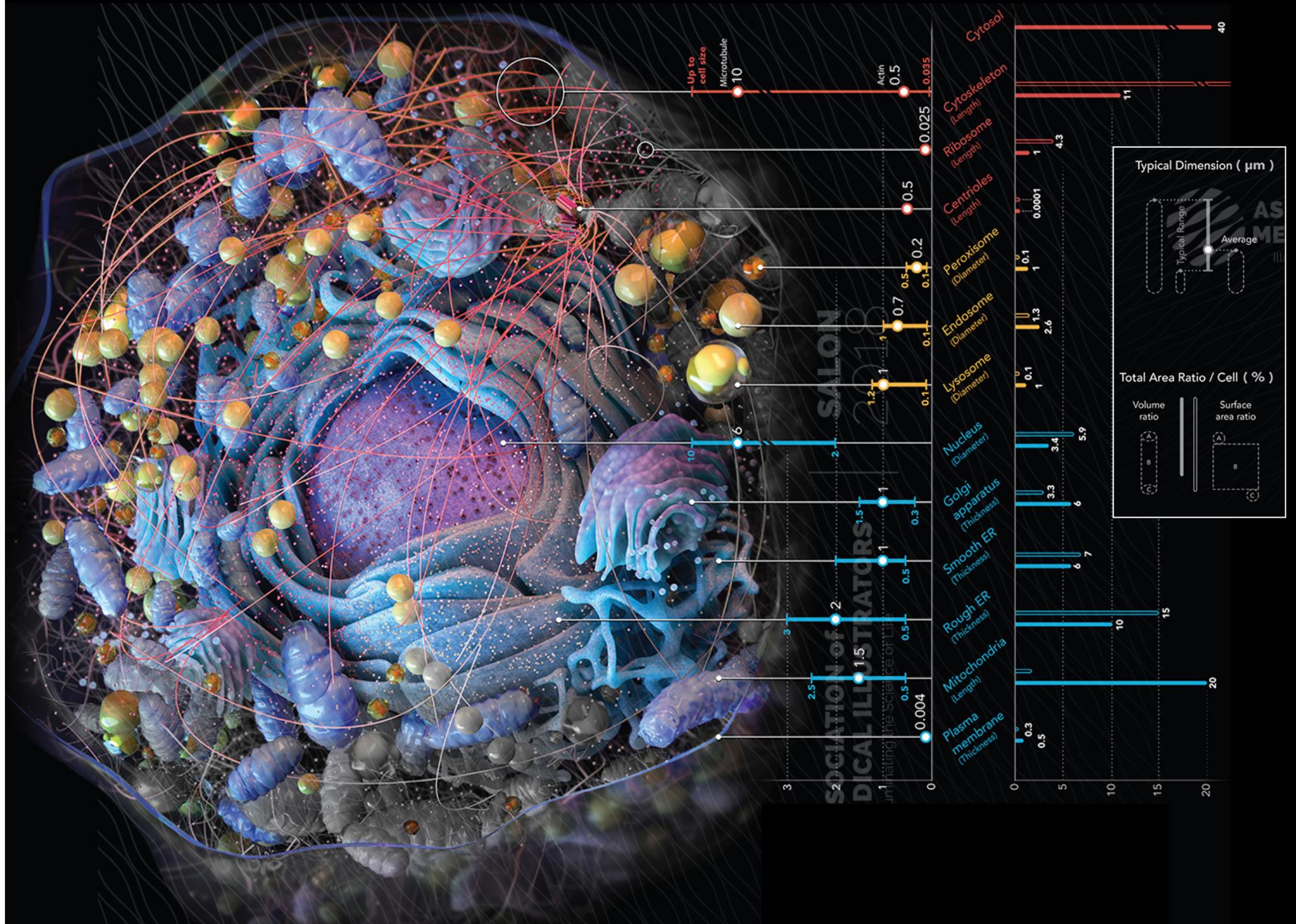**pucadyil@iiserpune.ac.in**

www.pucadyillab.com Lecture notes, reading materials

**Intracellular compartmentalisation into organelles**

Organelle Structure

How they are formed?

Organelle function in protein sorting

Typical Dimension (μm)

Typical Range
Average

Total Area Ratio / Cell (%)

Volume ratio | Surface area ratio

Cytosol — 40
Cytoskeleton (Length) — Microtubule 10 (Up to cell size), Actin 0.5 — 0.035 — 11
Ribosome (Length) — 0.025 — 4.3 / 1
Centrioles (Length) — 0.5 — 0.0001
Peroxisome (Diameter) — 0.2 / 0.5 / 0.1 — 0.1 / 1
Endosome (Diameter) — 0.7 / 0.1 — 1.3 / 2.6
Lysosome (Diameter) — 1 / 1.2 / 0.1 — 0.1 / 1
Nucleus (Diameter) — 6 / 10 / 2 — 3.4 / 5.9
Golgi apparatus (Thickness) — 1 / 0.3 — 3.3 / 6
Smooth ER (Thickness) — 1.5 / 1 / 0.5 — 6 / 7
Rough ER (Thickness) — 2 / 3 / 0.5 — 10 / 15
Mitochondria (Length) — 2.5 / 0.5 — 20
Plasma membrane (Thickness) — 0.004 — 0.3 / 0.5

# Most organelles are membrane-bound structures that compartmentalise the cytoplasm
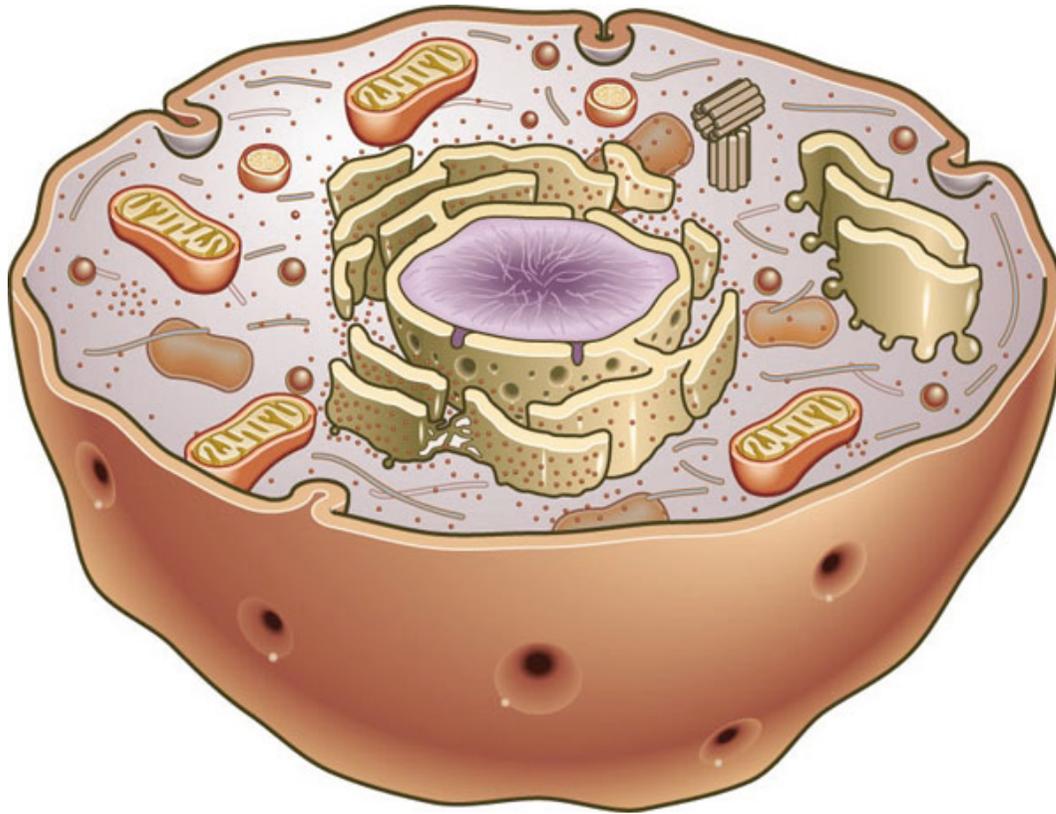
- Very thin, quasi two-dimensional film of lipids and proteins

- Held together by non-covalent interactions

- Membranes are fluid and dynamic

- Enzymes that build and modify lipids are genetically encoded but lipid composition of biomembranes is also dependent on the food we consume

Composition of mammalian lipids
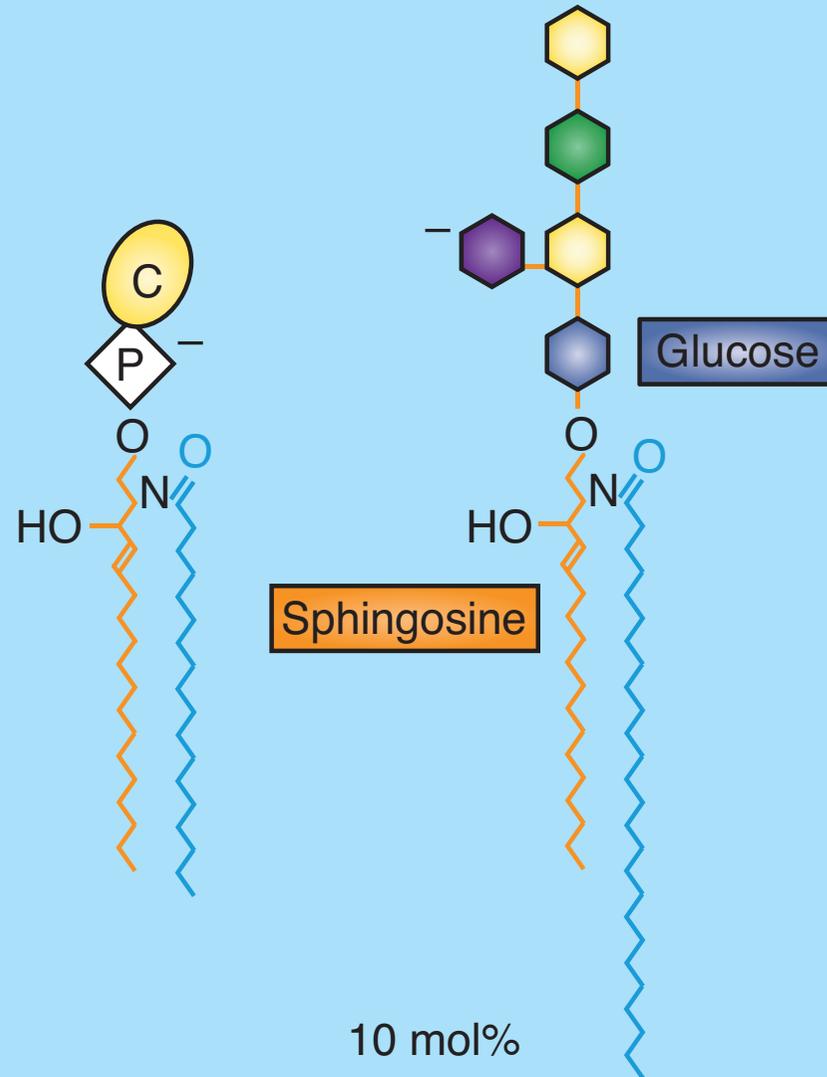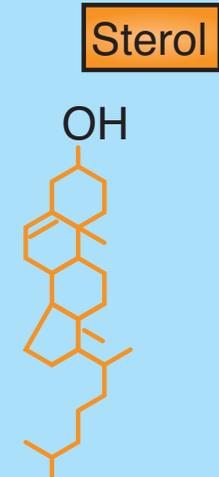
Glycerolipids

PC, PE, PS, PI

Polar head group

Glycerol

Fatty acid
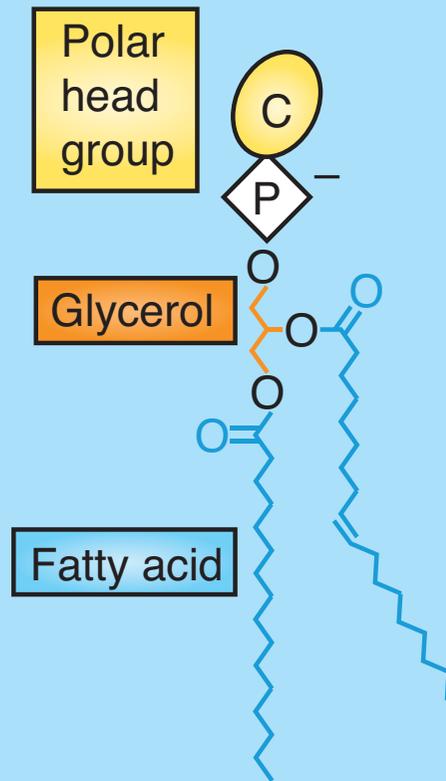
65 mol%

Sphingolipids

Sphingomyelin

Glycosphingolipids
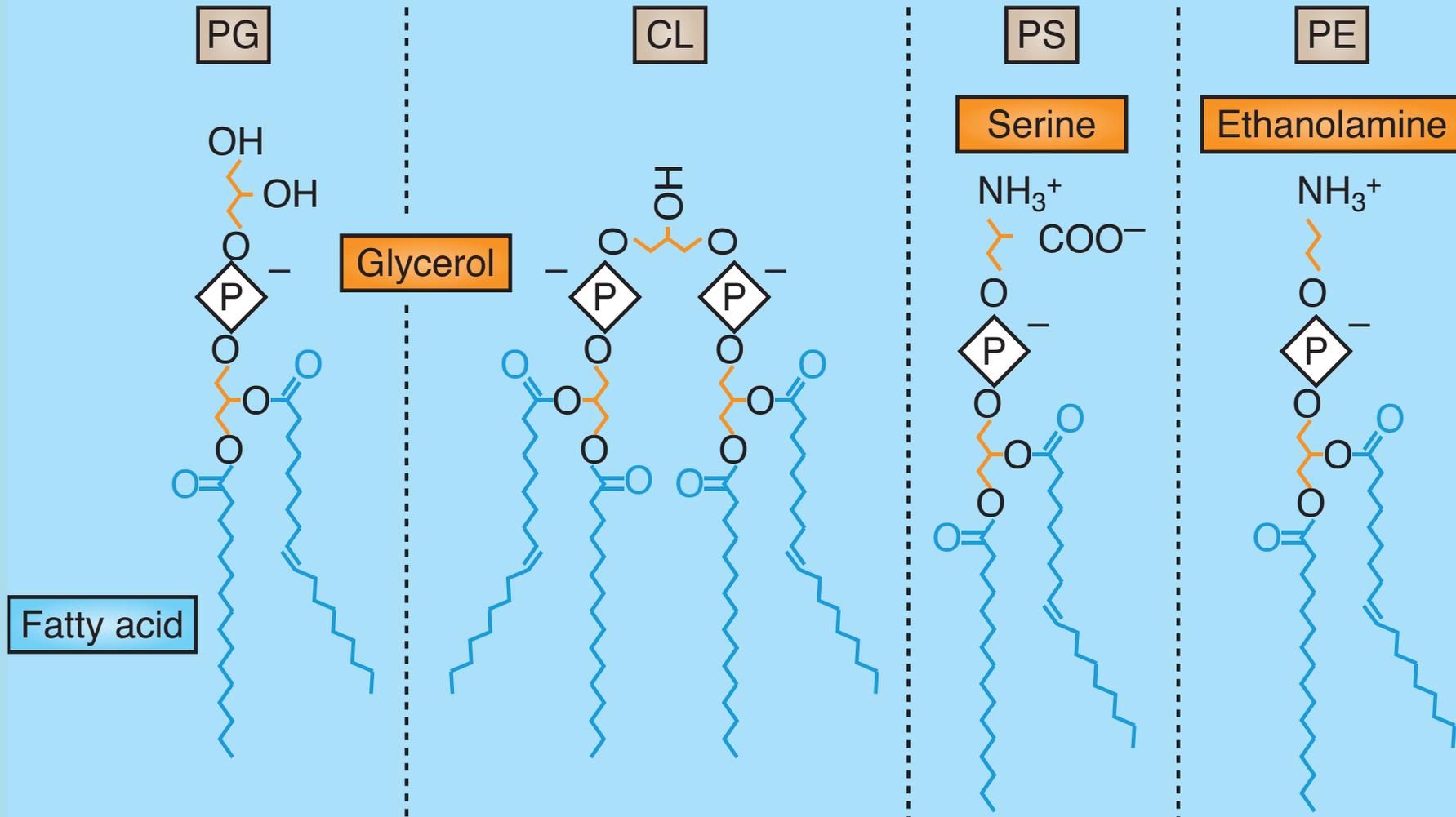
Glucose

Sphingosine
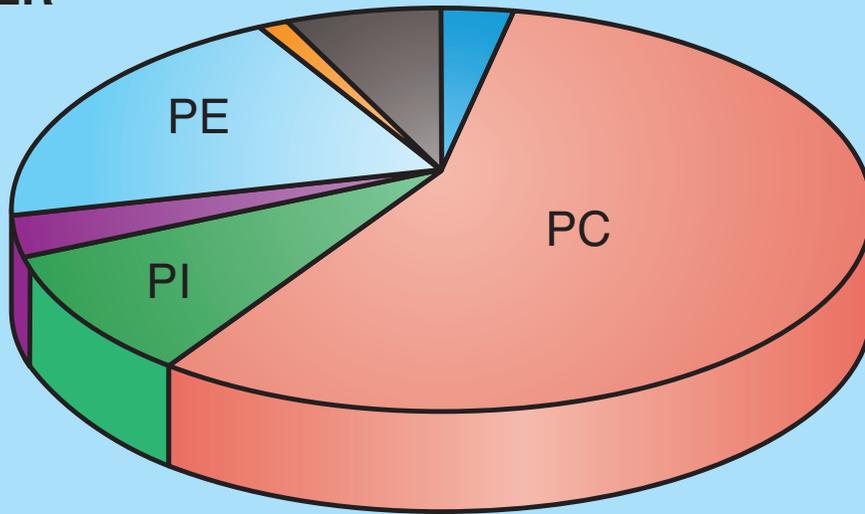
10 mol%

Sterols

Cholesterol

Sterol

25 mol%

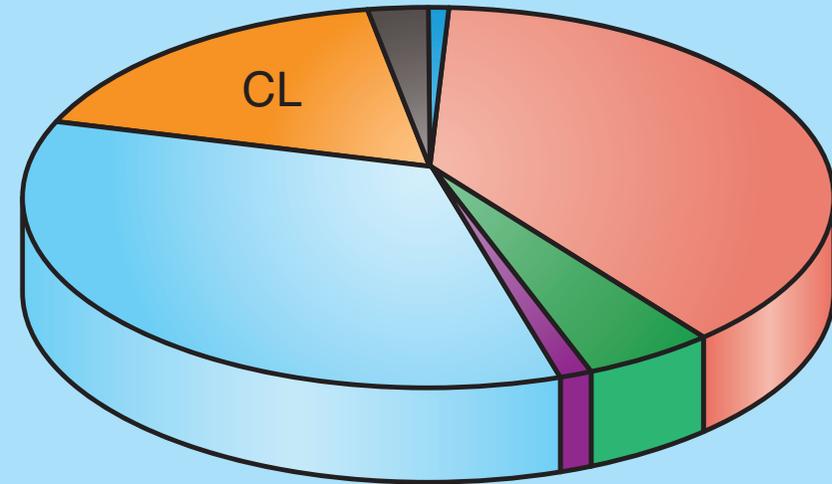# Mitochondrial glycerolipids of bacterial origin

Organelle distribution of phospholipids and cholesterol

ER

Mitochondrion
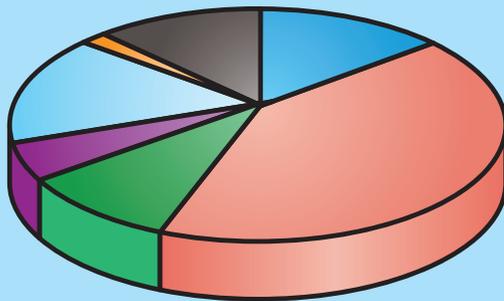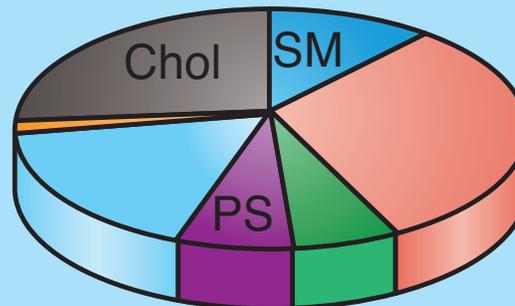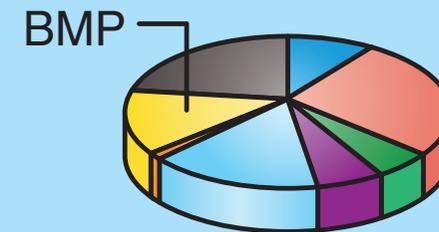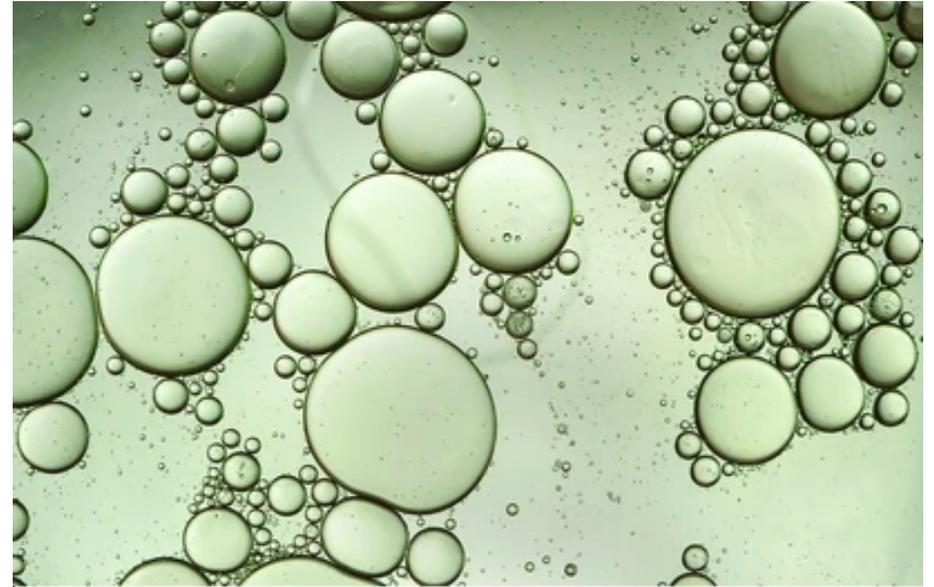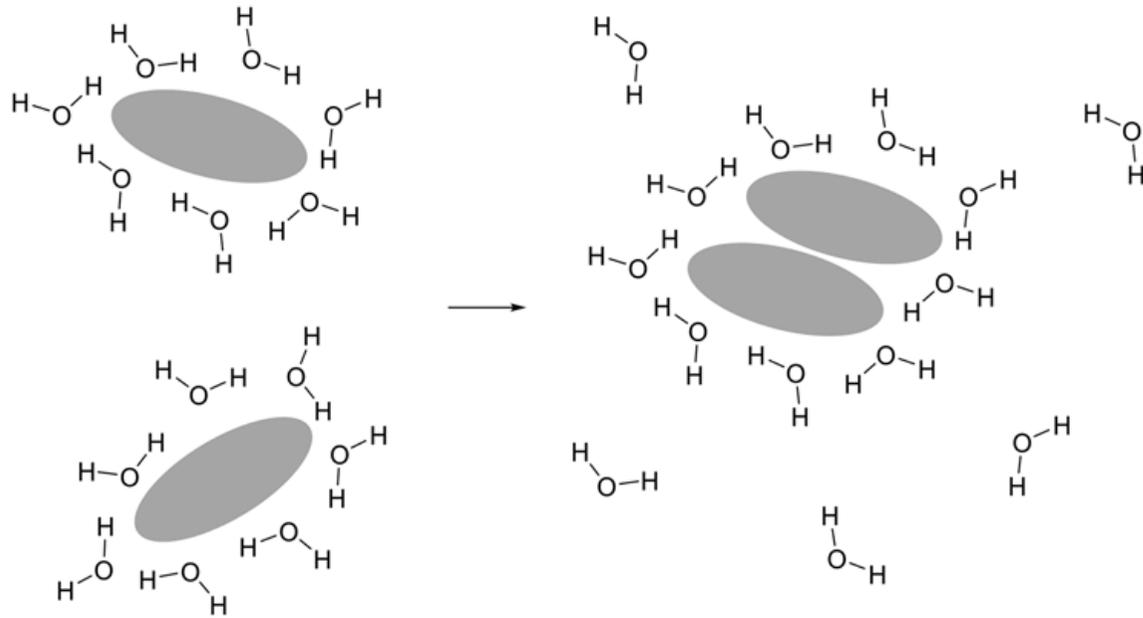
Golgi

Plasma membrane

Endosomes and lysosomes

The hydrophobic effect

The hydrophobic effect

- Lipid molecules disperse in the solution; nonpolar tail of each lipid molecule is surrounded by ordered water molecules

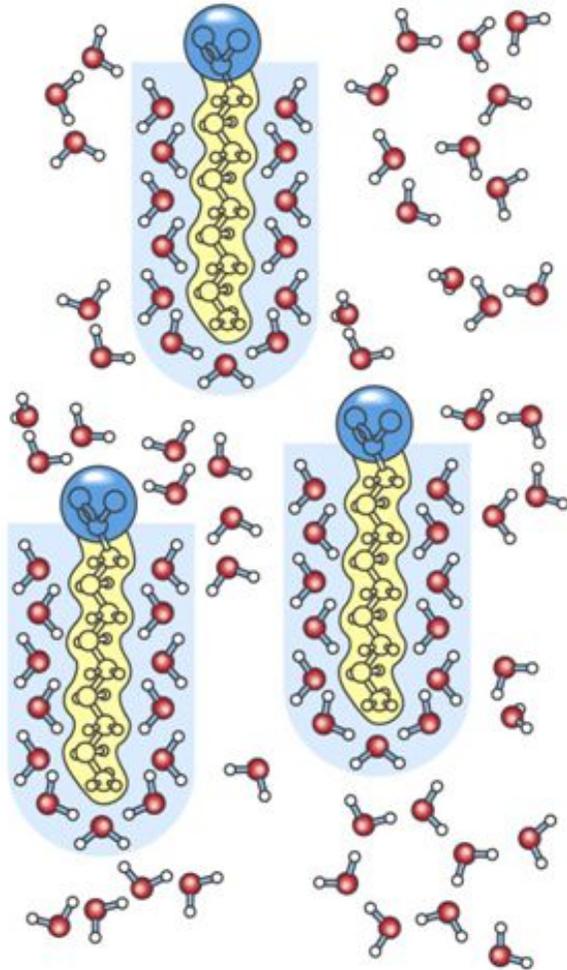- Lipid aggregates – Water released, surface area reduced

Figure 2-7b part 1
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company
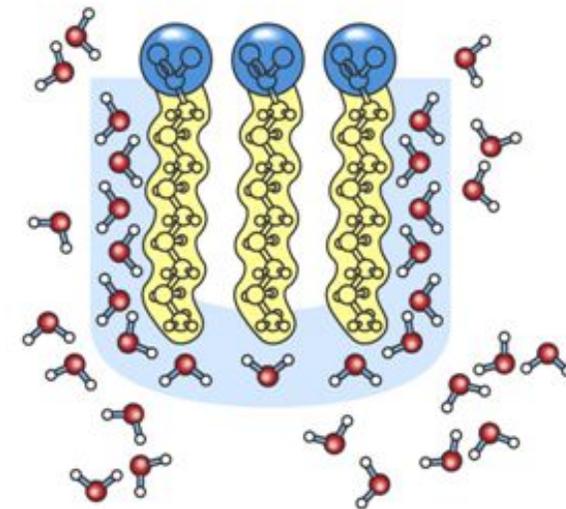
Figure 2-7b part 2
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company
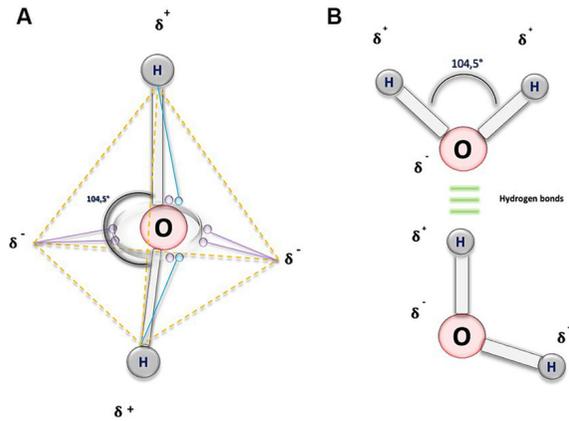
How do lipids come together to form membranes?
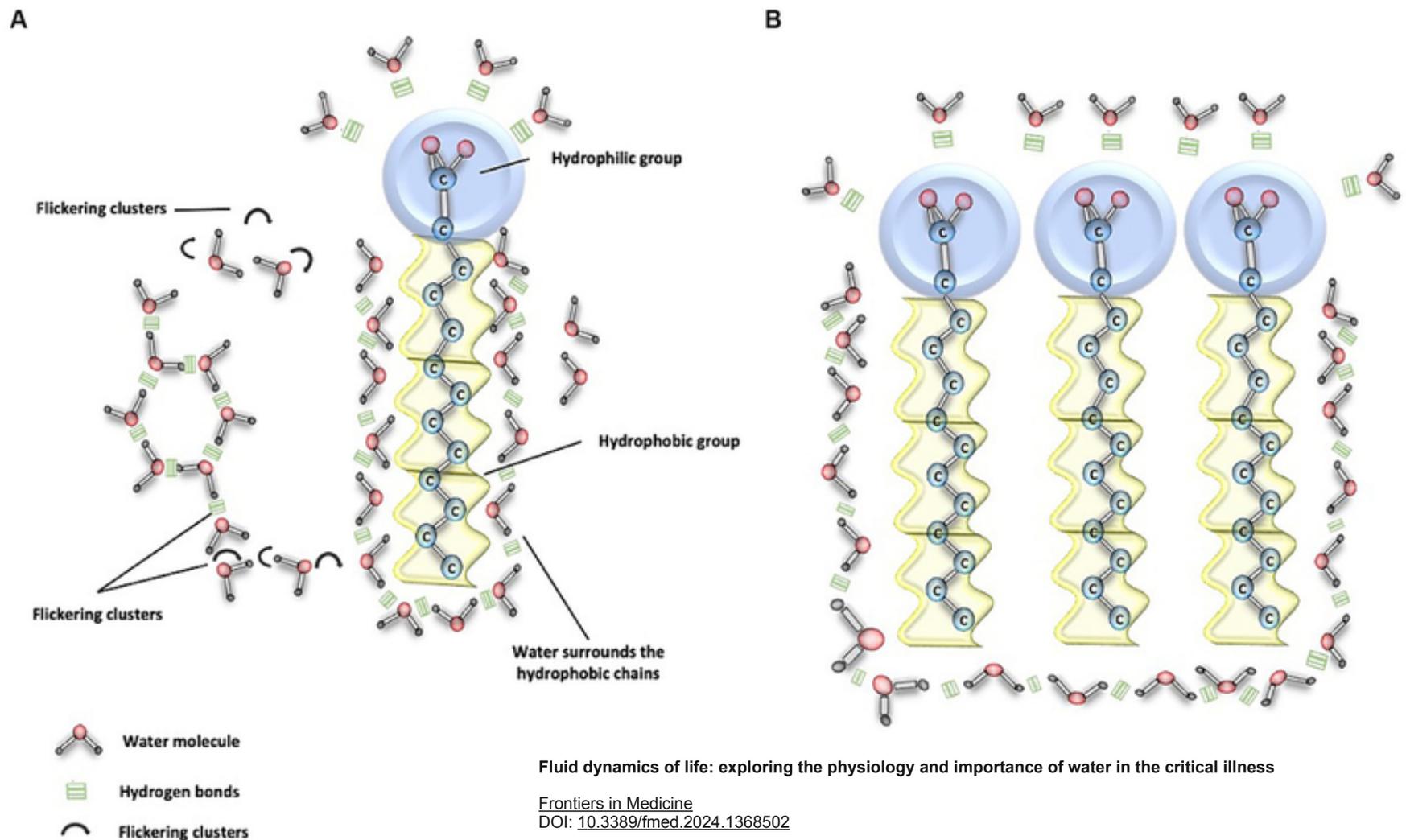
A

Flickering clusters

Flickering clusters

Hydrophilic group

Hydrophobic group

Water surrounds the hydrophobic chains

B

Water molecule

Hydrogen bonds

Flickering clusters

Dimensions

Phospholipid

Head

Tail

Phospholipid Bilayer

5 nm

Exposed Tails

Compartment

4.6 nm
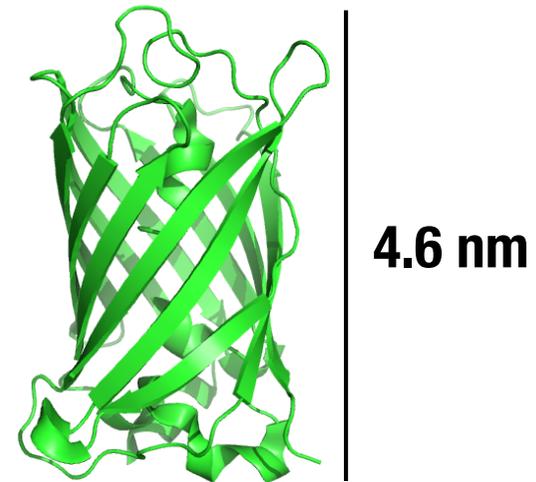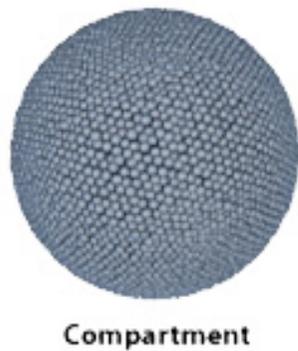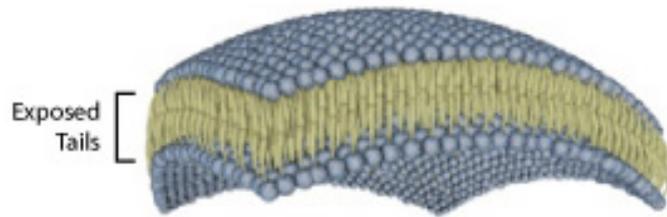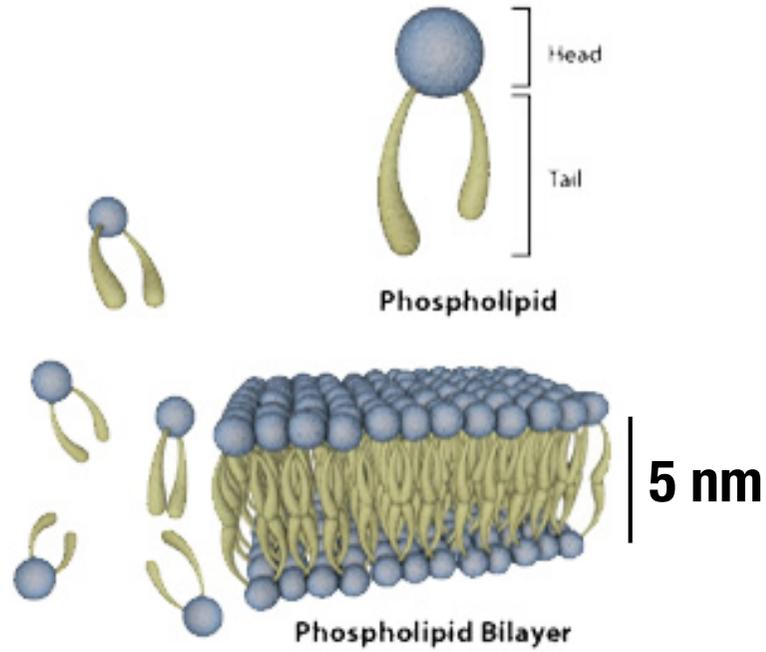
Cell membranes are resilient materials
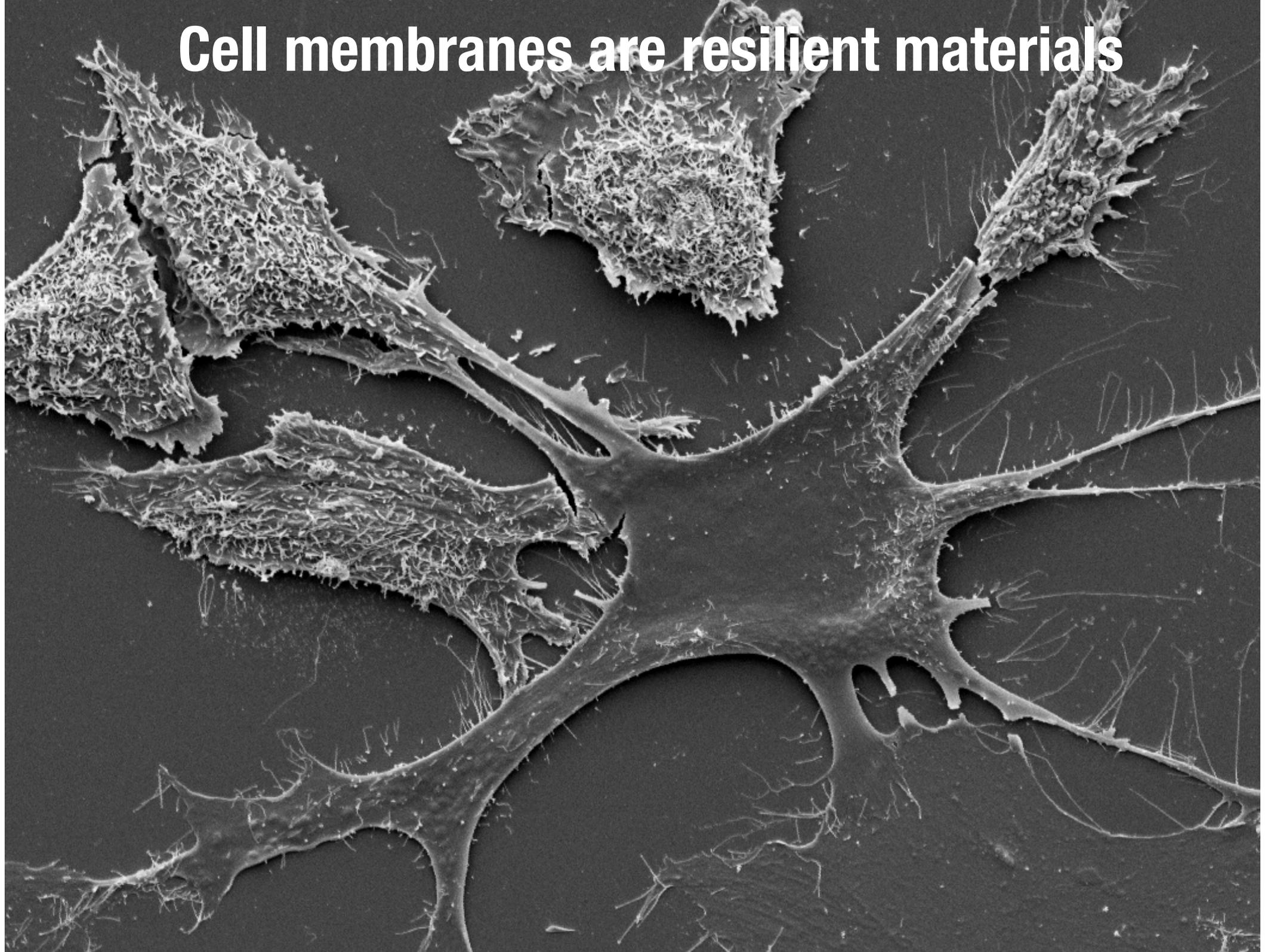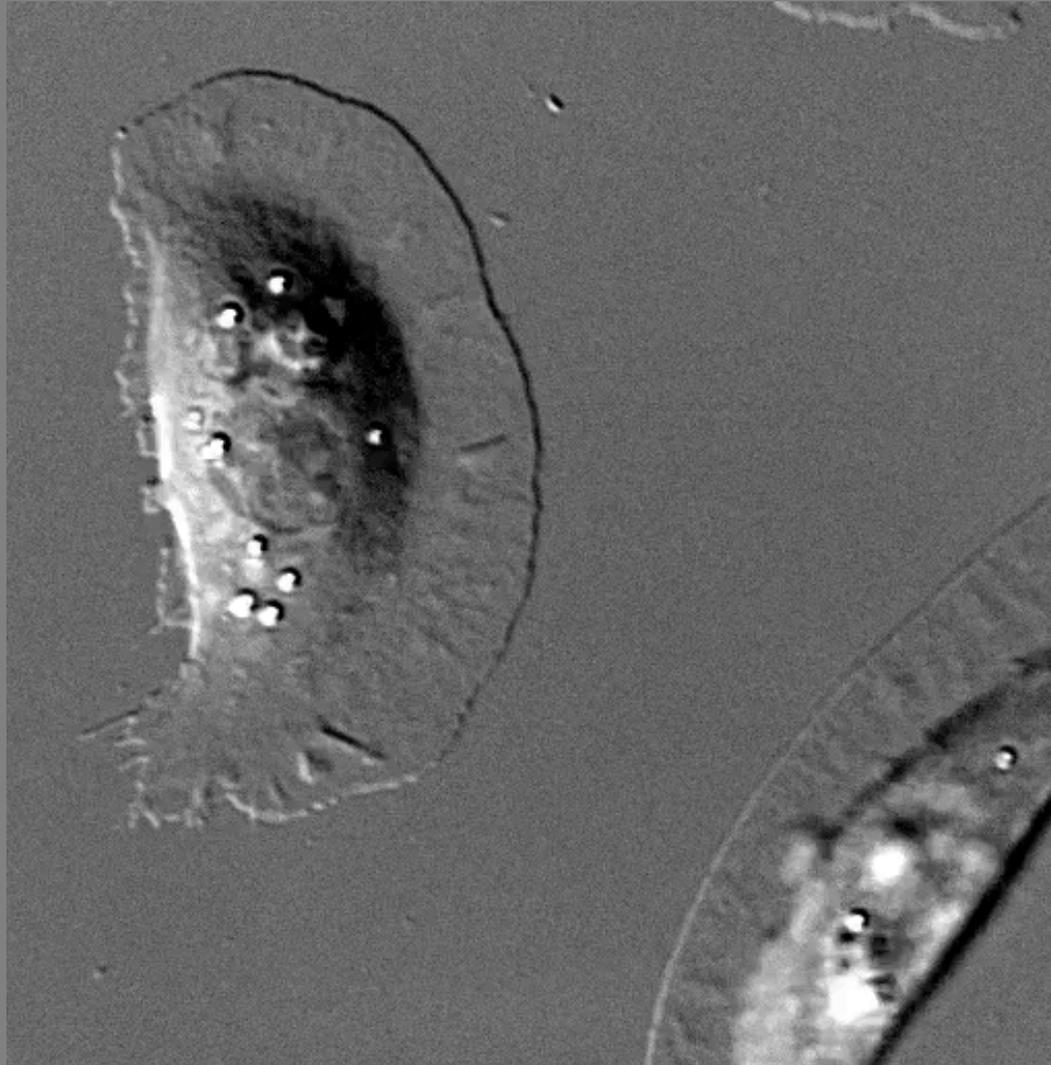
Courtesy: Dylan Burnette (@MAG2ART)
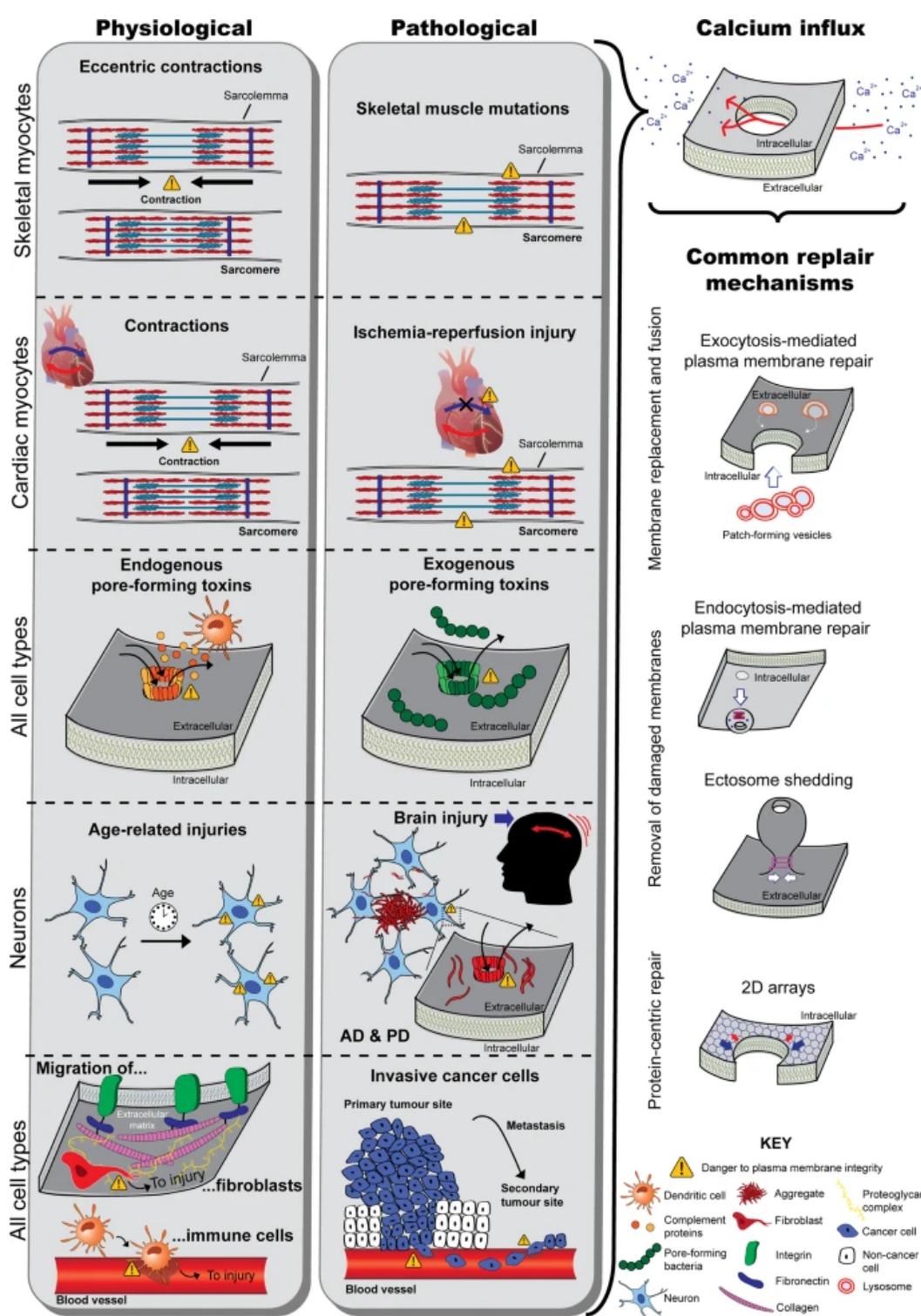
Membranes are resilient materials

and their tearing is energetically expensive

Protein classes
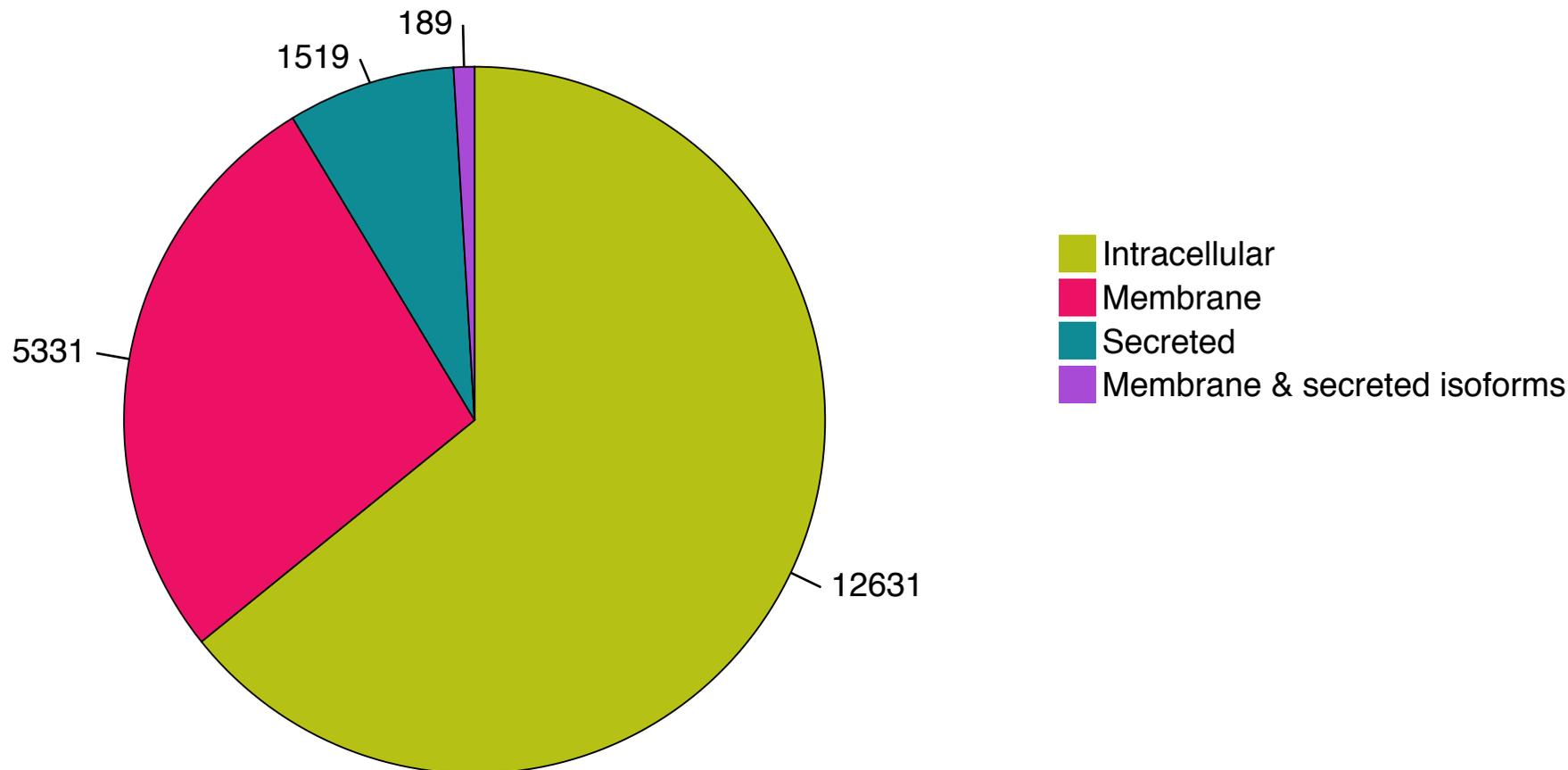
- 19670 human protein-coding genes
- 12631 (64%) genes are predicted to be intracellular
- 5520 (28%) are predicted to have at least one membrane-bound protein product
- 1708 genes (9%) are predicted to have at least one secreted protein product
- 189 genes (1%) have both secreted and membrane-bound isoforms

https://www.proteinatlas.org/humanproteome/tissue/secretome

Models of biological membranes

Gorter and Grendel (1925)
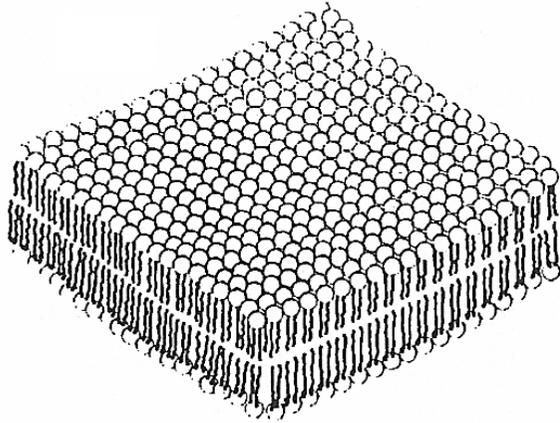
Danielli and Dawson (1935)

b
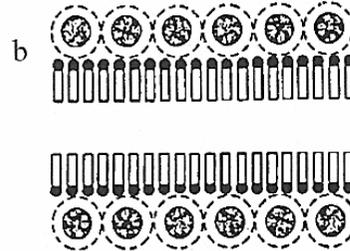
c Robertson (1966)

d Singer and Nicoloson (1972)
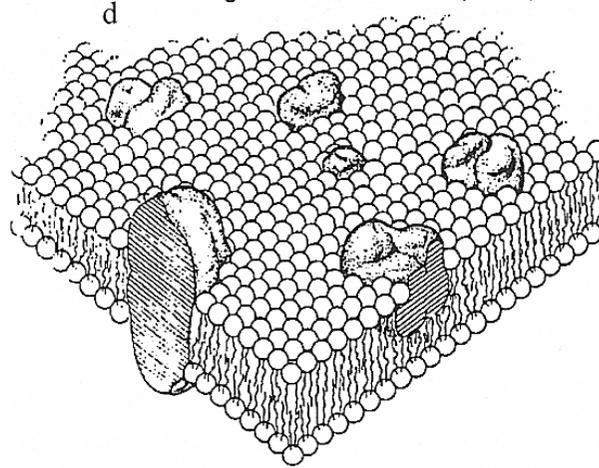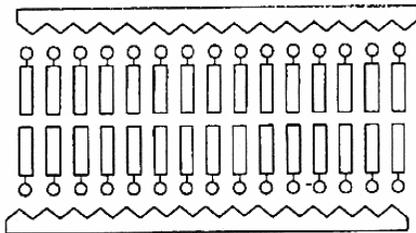
e Israelachvili (1978)
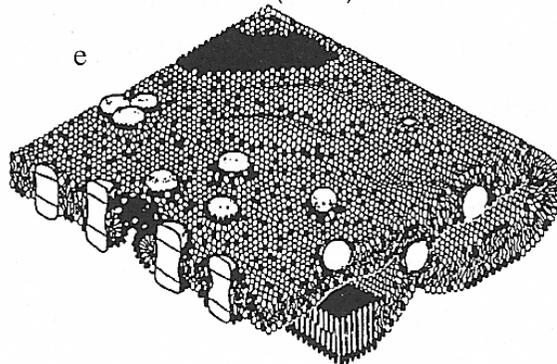
f Sackmann (1995)
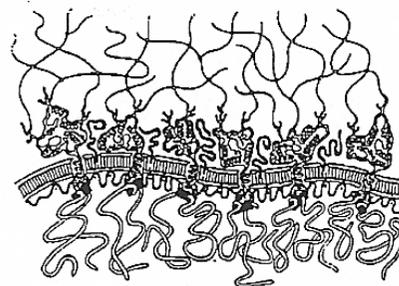
Gorter and Grendel (1925)

- Experimentally investigated the surface area of lipids
- Extracted lipids from red blood cells of man, dog, rabbit, sheep, guinea pig, and goat in acetone
- Spread on a water surface and the area was measured using a Langmuir film balance
- Measured the surface area of the red blood cells from the microscopic images.
- Found that the surface area of the monofilms was within error exactly two times that of the cells
- Concluded that cell membranes are made of two opposing thin molecular layers
- Proposed that two lipid layers form a bilayer with the polar head groups pointing toward the aqueous environment

Models of biological membranes

Human cell

Addition of sendai (fusing) virus

Mouse cell

40 minutes

(a)

(b)

5 μm
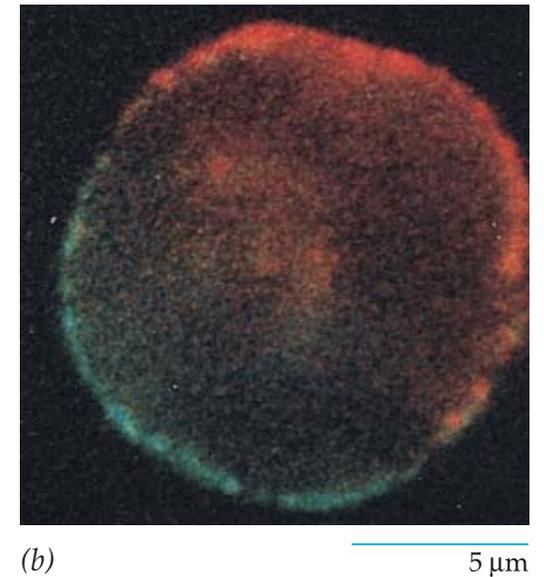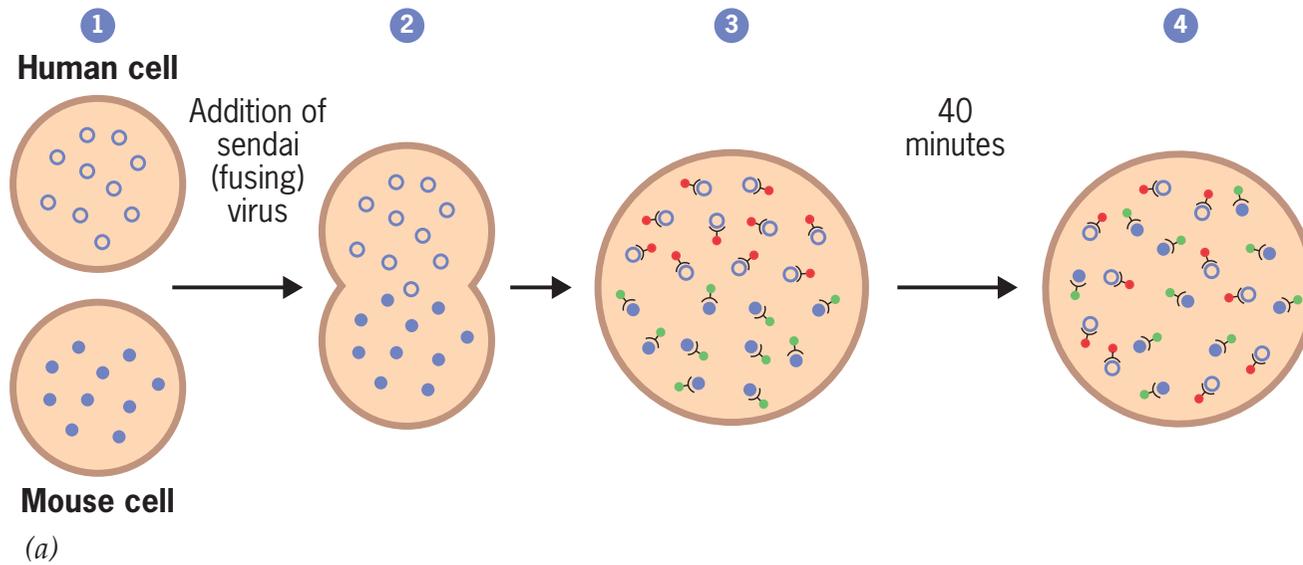
Frye and Edidin (1970)

How is the lipid bilayer organised?
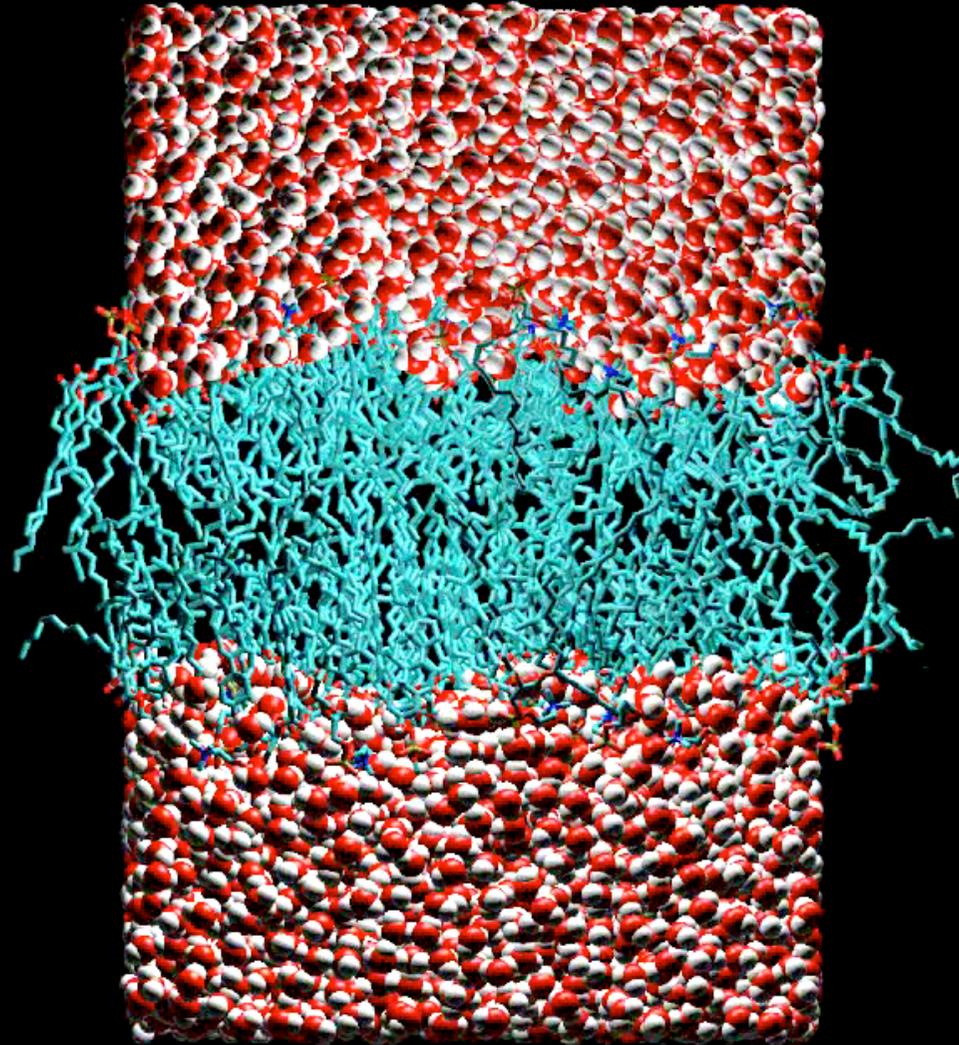
Membrane Organization analyzed by Single Particle Tracking (SPT)
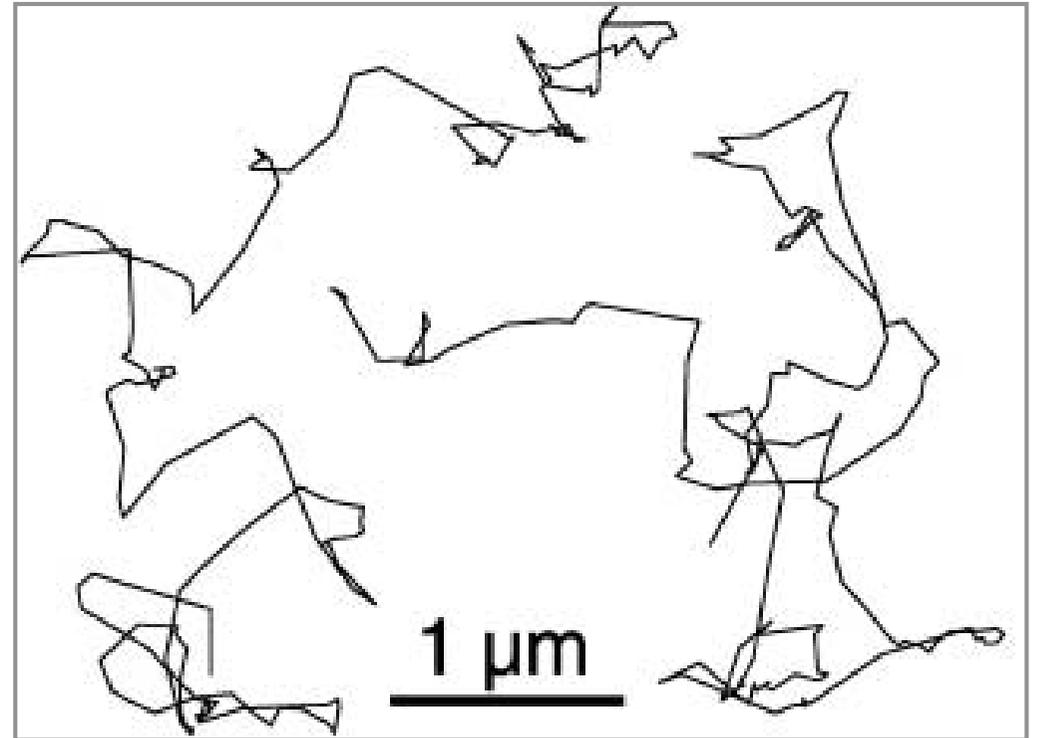
Cy3-DOPE = fluorescent lipid
G40-DOPE = gold particle tagged DOPE

Fujiwara et al. (2002) J. Cell Biol. 157:1071-1081.

Contemporary model of cell membranes
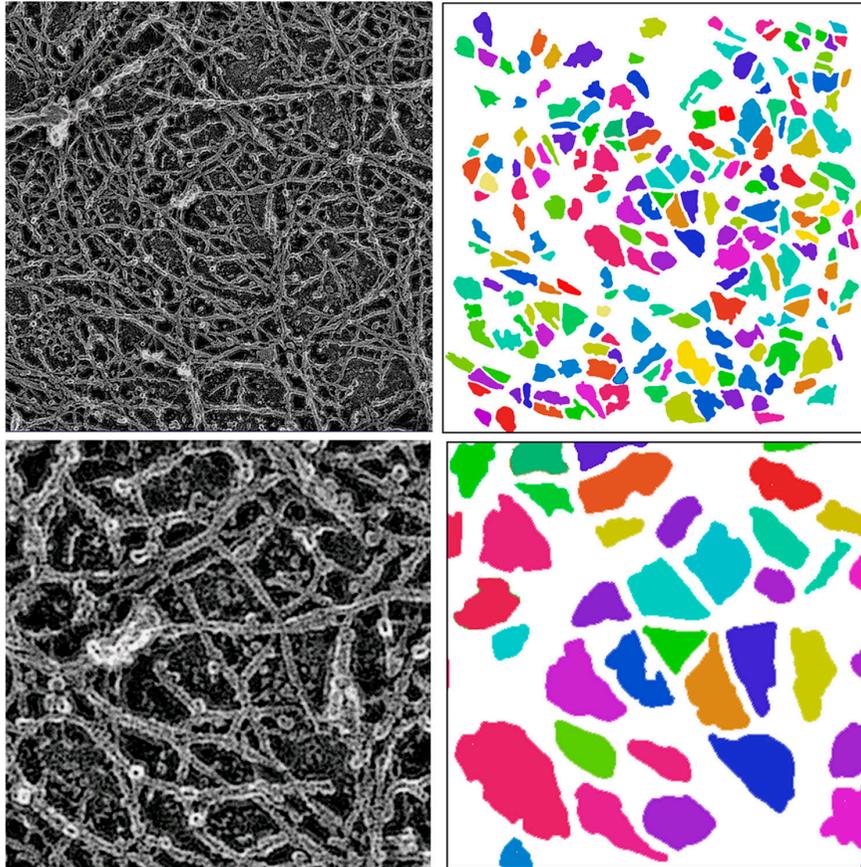
Transmembrane proteins anchored to the membrane skeleton
Phospholipid
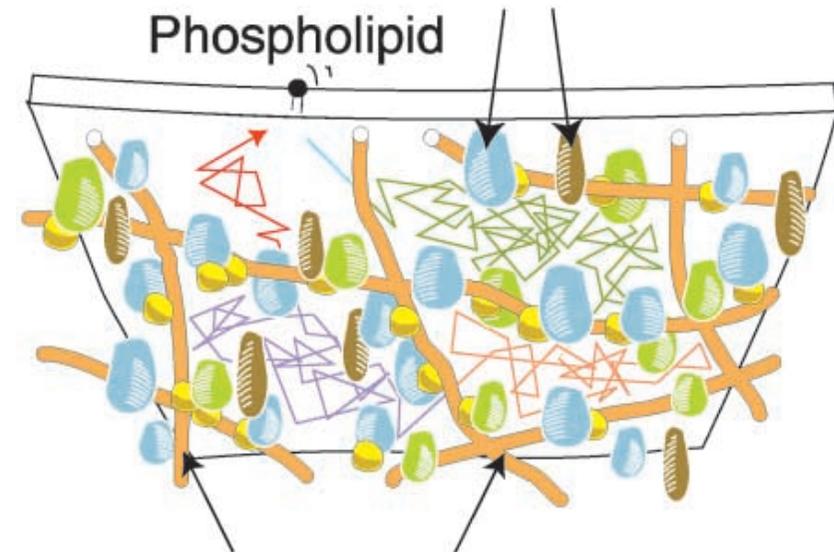
Membrane skeleton
Anchored-protein picket model

Fujiwara et al. (2002) J. Cell Biol. 157:1071-1081
Morone et al. (2006) J. Cell Biol. 174:851-862

Contemporary model of cell membranes

b Membrane-skeleton "fence"

c Anchored-protein "picket"
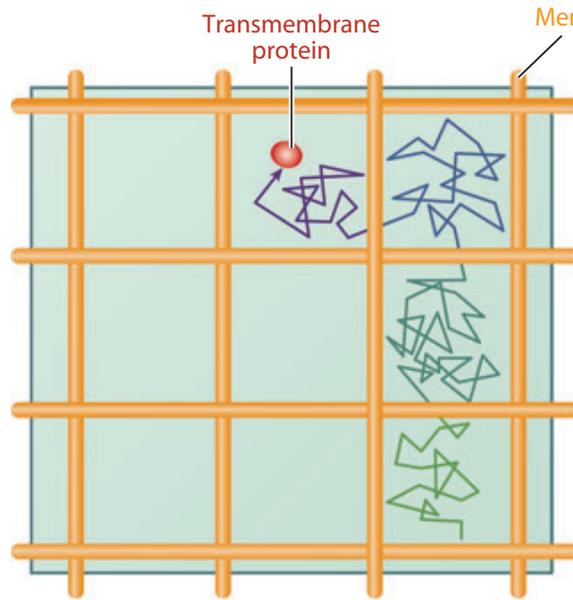
Bottom view
(from inside the cell; anchored
proteins are hidden for clarity)
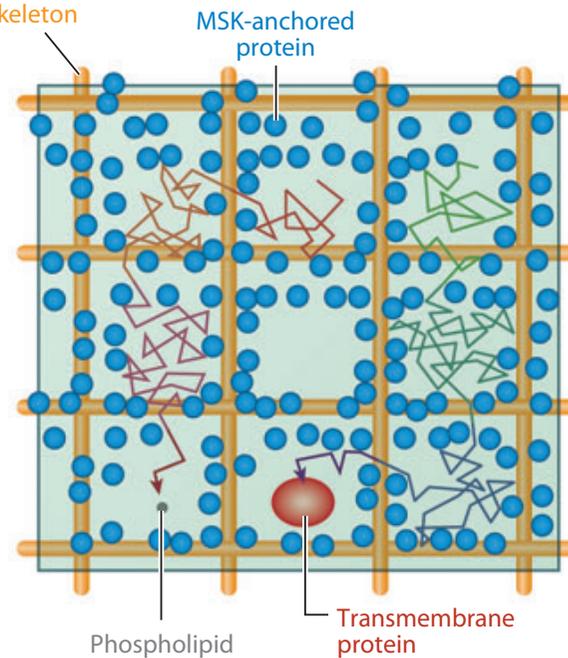
Top view
(from outside the cell)

- Phospholipids undergo hop diffusion within 230 nm confined regions in cell membranes

- As a consequence, long range diffusion is a reflection of diffusion within the confined regions and their tendency to hop across these regions

Endom
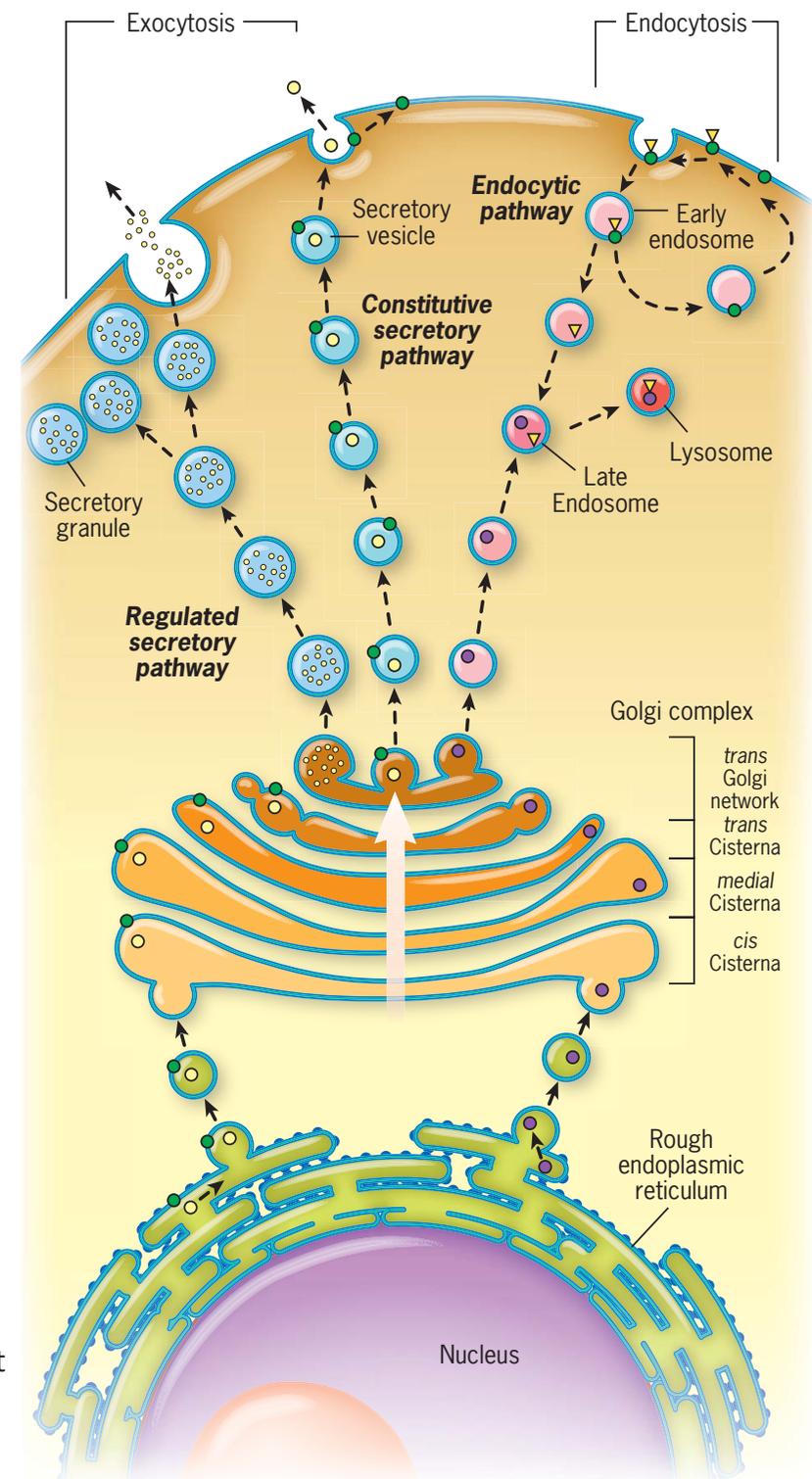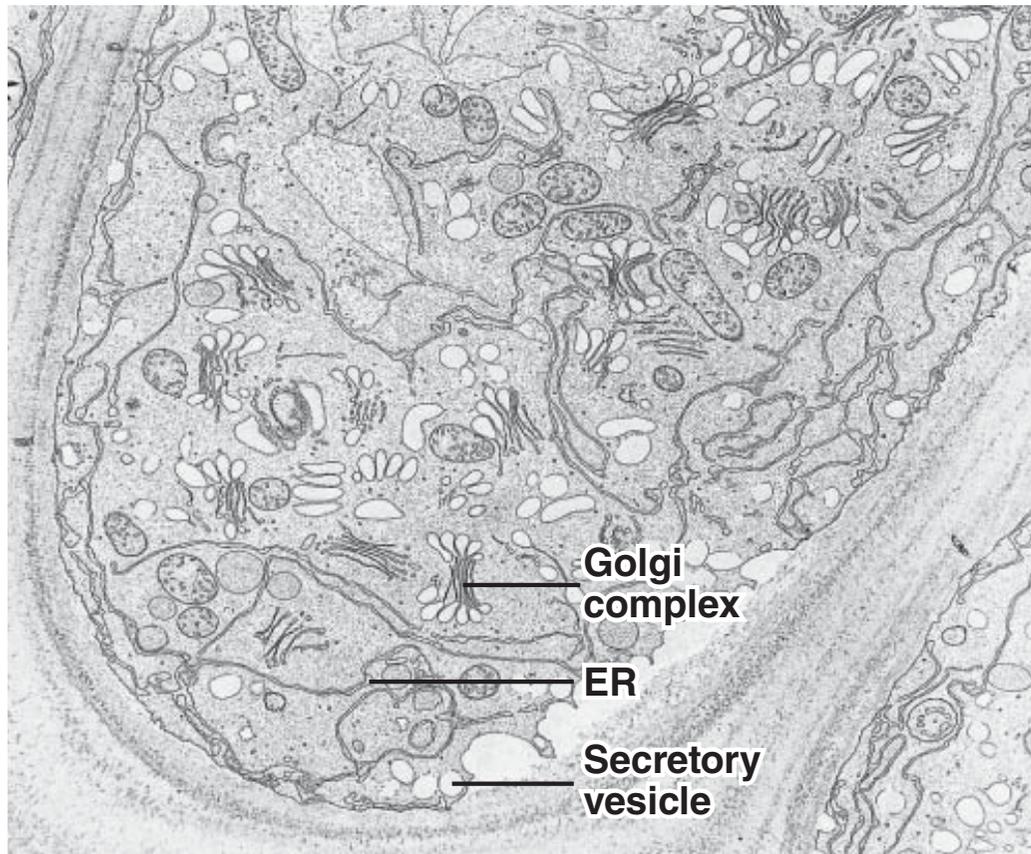
FIGURE 8.1 Membrane-bound compartments of the cytoplasm. The cytoplasm of this root cap cell of a maize plant contains an array of membrane-bound organelles whose structure and function will be examined in this chapter. As is evident in this micrograph, the combined surface area of the cytoplasmic membranes is many times greater than that of the surrounding plasma membrane.

Golgi complex

ER

Secretory vesicle

3 μm

Exocytosis

Endocytosis

Secretory vesicle

Endocytic pathway

Early endosome

Constitutive secretory pathway

Lysosome

Late Endosome

Secretory granule

Regulated secretory pathway

Golgi complex

trans Golgi network

trans Cisterna

medial Cisterna

cis Cisterna

Rough endoplasmic reticulum

Nucleus

Vesicular transport
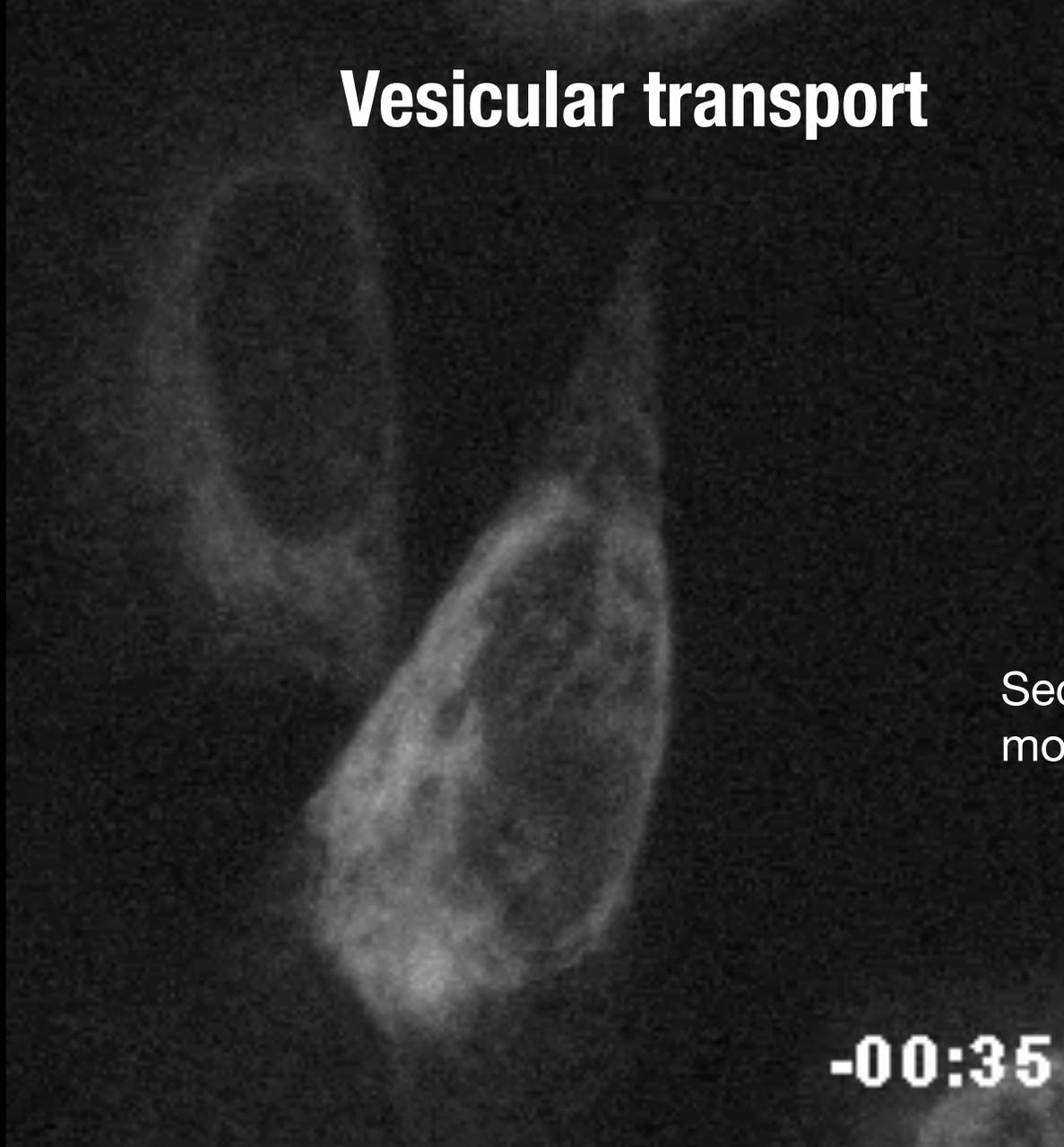
Secretory traffic
monitored in HeLa cells

-00:35

Endoplasmic Reticulum
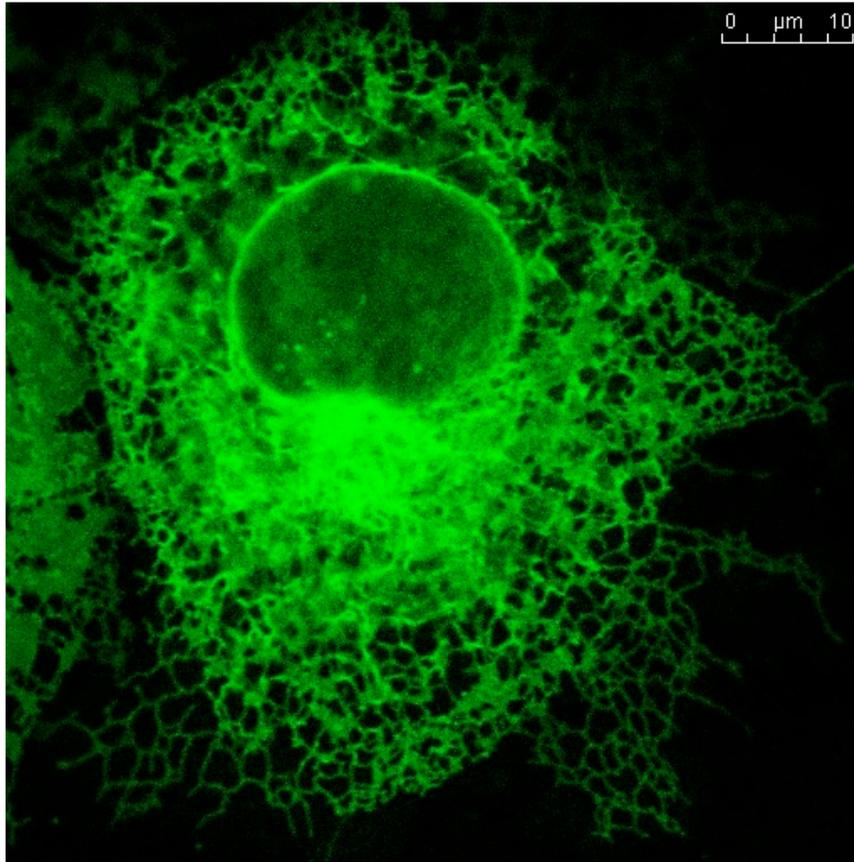
http://www2.warwick.ac.uk/fac/sci/maths/
research/events/2009_2010/symposium/
netwks/

- Constitutes more than half of the total membranes of the cell

- Composed of a network of branching tubules and flattened sacs

- ER membrane connected to the nuclear membrane and lumen connected to extracellular space

- ER membrane is the site of most lipid and membrane protein synthesis in the cell

Morphologically divided into two classes
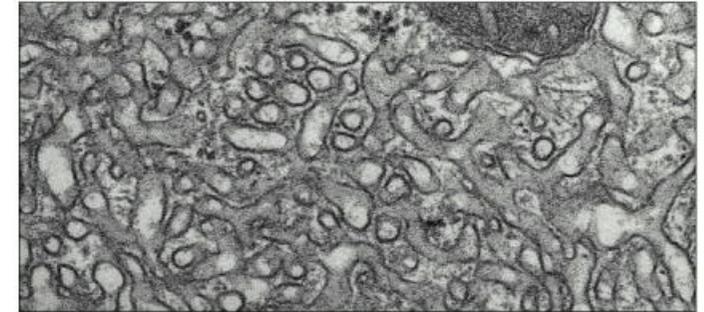
Smooth endoplasmic
reticulum (SER)

Smooth ER

(A)

200 nm

Rough ER

Rough endoplasmic
recticulum (RER)

(a)
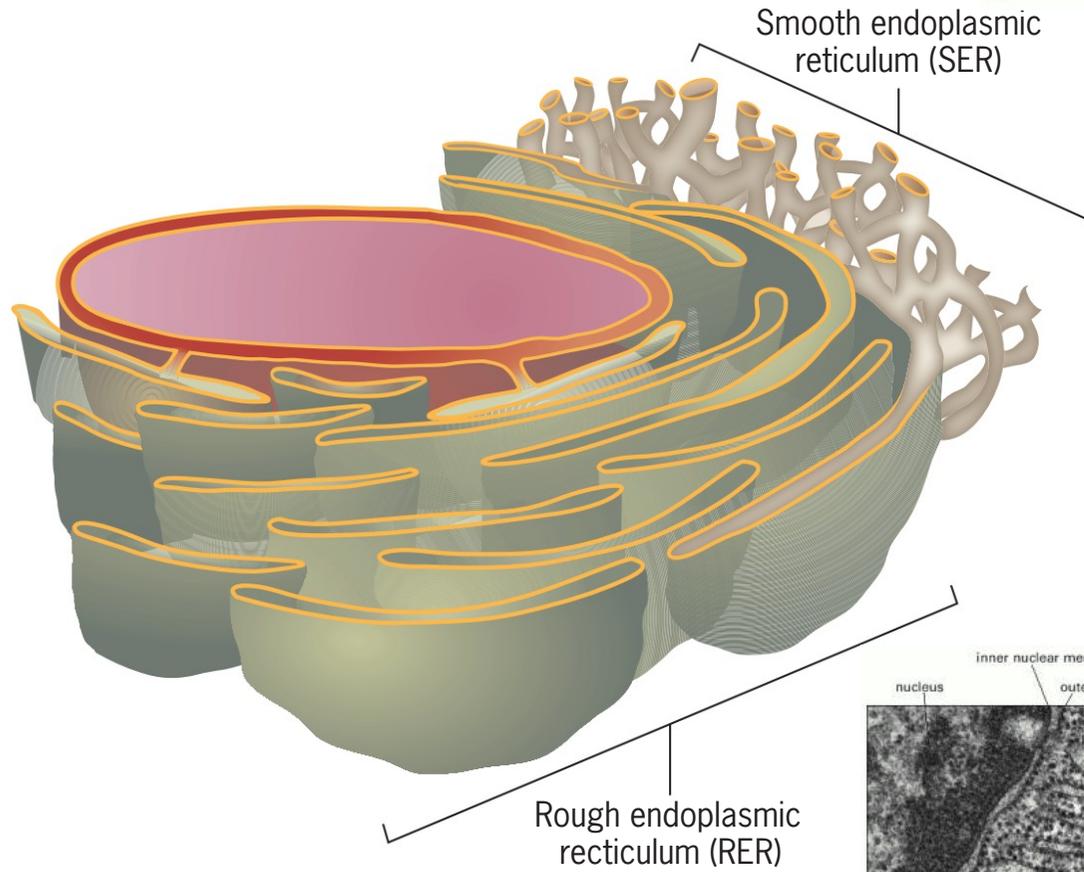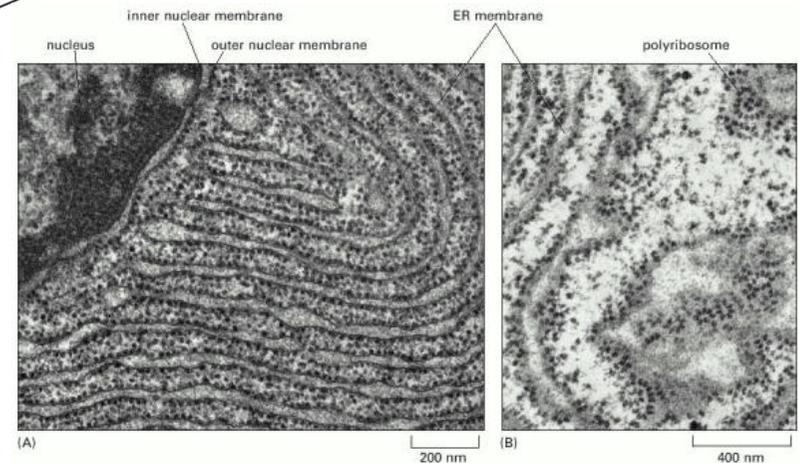
inner nuclear membrane

ER membrane

nucleus

outer nuclear membrane

polyribosome

(A)

200 nm

(B)

400 nm

Morphologically divided into two classes

Rough ER

Smooth ER
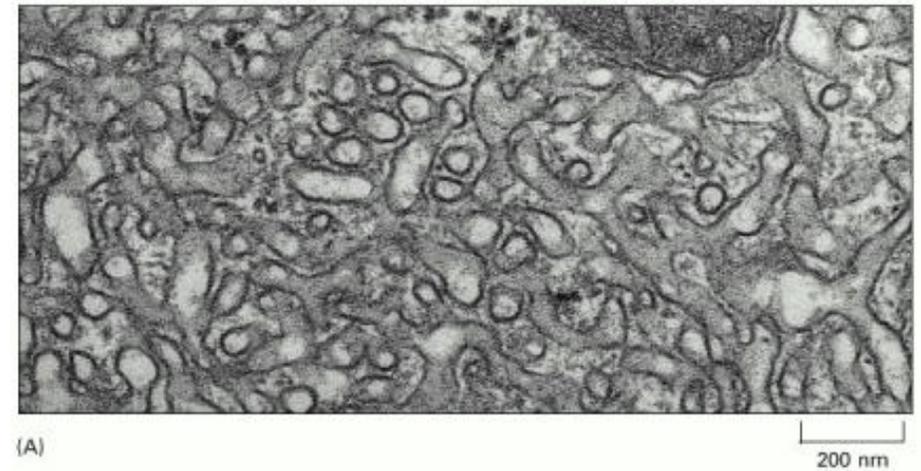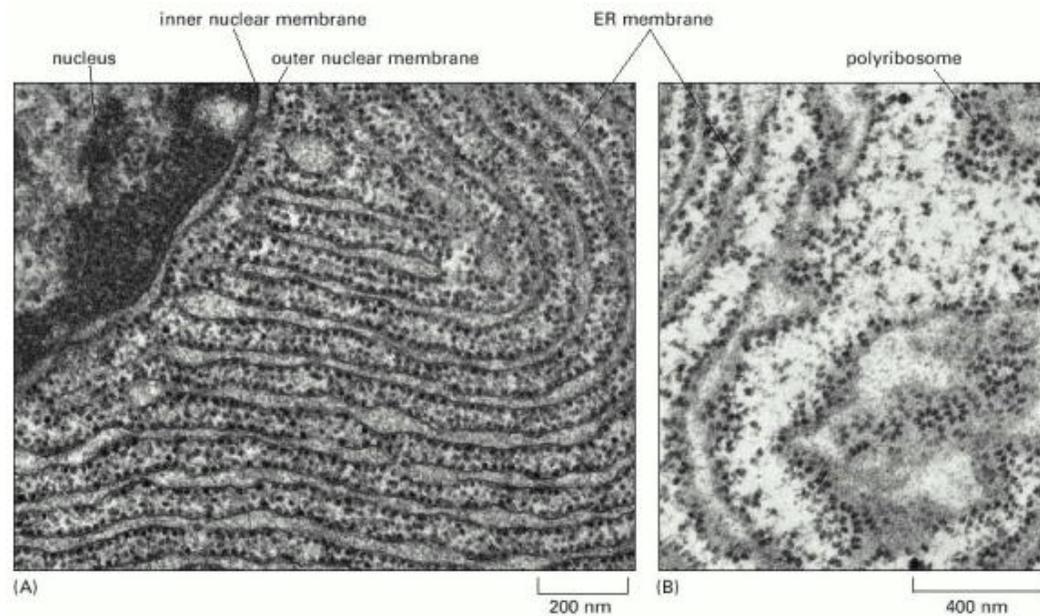
- Termed rough ER because of bound polyribosomes present on the cytosolic side

- Membrane and secreted protein synthesis

- Exocrine cells have a large fraction of rough ER

- Termed smooth ER because they are devoid of bound ribosomes

- Steroid hormone synthesis, detoxification

- SER is extensively developed in a number of cell types, skeletal muscle, kidney tubules, and steroid-producing endocrine glands.

- Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex

- Detoxification of organic compounds carried out by the ER-localized collection of oxygen-transferring enzymes (oxygenases), including the *cytochrome P450* family. They lack substrate specificity and can oxidize thousands of different hydrophobic compounds and convert them into more hydrophilic entities that can be easily excreted.

- Sequestering calcium ions within the cytoplasm of cells. The regulated release of Ca^{2+} from the SER of skeletal and cardiac muscle cells (known as the *sarcoplasmic reticulum* in muscle cells) triggers contraction.

Integral membrane protein (IMP) biogenesis
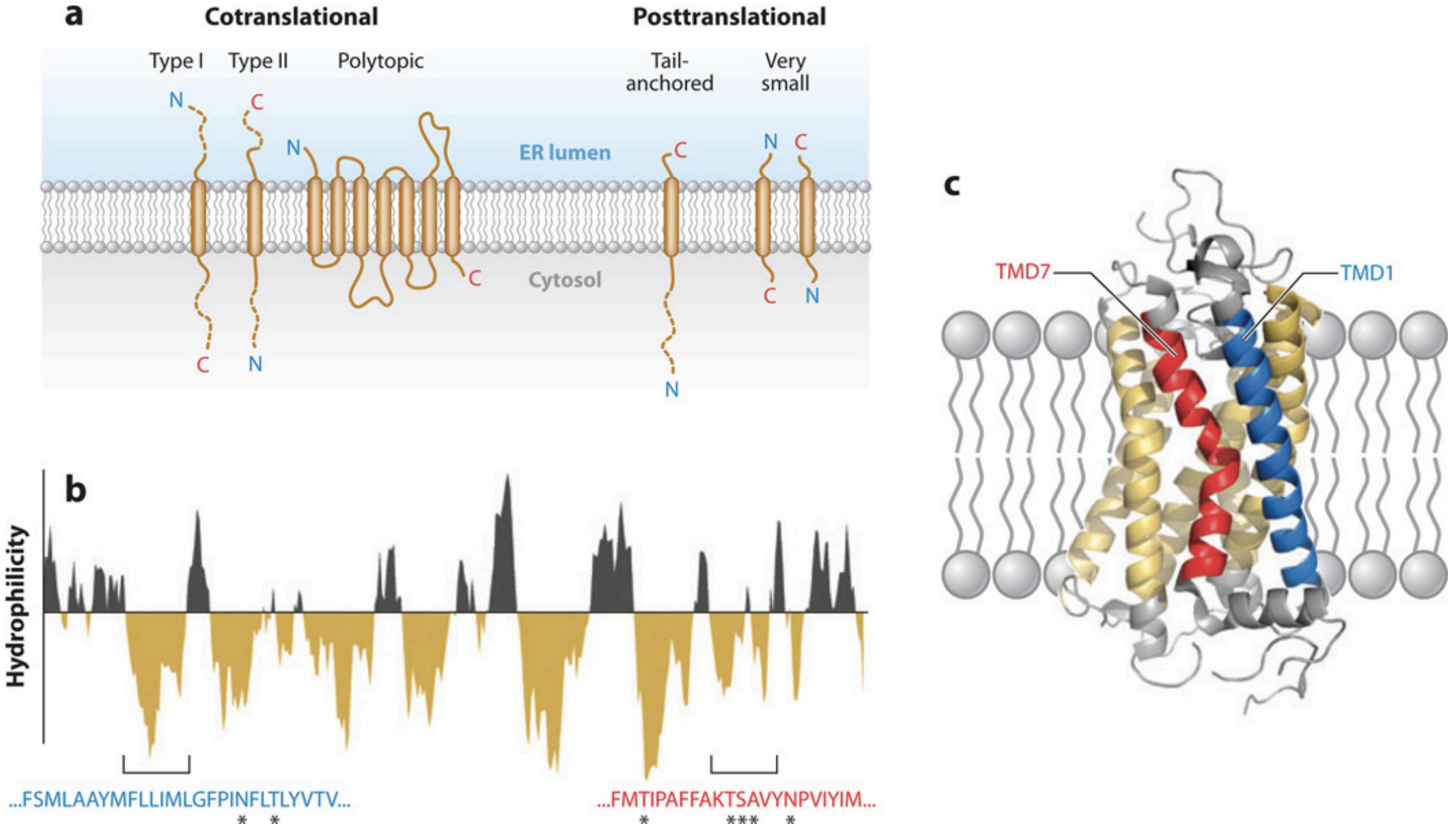
Membrane Protein Insertion at the Endoplasmic Reticulum
Shao and Hegde (2011) Annu. Rev. Cell Dev. Biol.

Integral membrane protein (IMP) biogenesis

- IMPs make up 20–30% of the eukaryotic proteome

- Diverse proteins - signaling receptors, mediate intracellular trafficking, facilitate organelle biogenesis, and transport a variety of molecules across cellular membranes

- IMPs range from having a single transmembrane domain (TMD) that simply anchors a soluble domain to the membrane to having tightly packed bundles containing more than 20 TMDs.

- All IMPs are translated at the ribosome and most IMPs are initially assembled at the endoplasmic reticulum (ER)

- IMP's TMD(s) are integrated into the membrane, final topology is determined, and tertiary and quaternary structures are achieved

- If these steps in IMP biogenesis are successful, the IMP is subsequently sorted to its final location of function

- Otherwise, one of several quality-control pathways routes the IMP for degradation
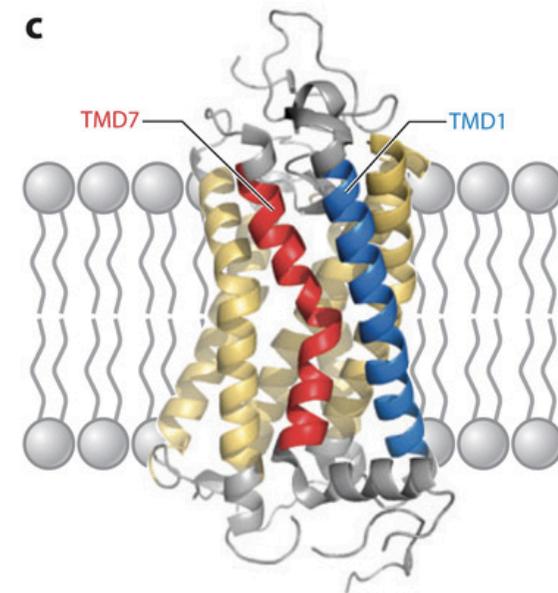
Integral membrane protein (IMP) biogenesis

- IMPs destined to be inserted into the ER face a set of challenges

- The hydrophobic TMDs of IMPs must be continuously shielded from the aqueous cytosol (shielding is essential because the tremendously crowded cytosolic environment (~300 mg/ml protein) would promote potentially toxic aggregation)

- TMDs need to be recognized as they emerge from the ribosome by the targeting machinery.

- IMPs must be targeted to the appropriate organelle, which requires the cytosolic targeting factors to interface with specific membrane receptors.

- TMDs need a route of transport past the highly polar surface of the membrane into the hydrophobic core of the lipid bilayer.

- TMD insertion must be asymmetric, with the final orientation consistent with the IMP's final folded state.

- This means that the insertion machinery must recognize, orient, and provide a potential path into the membrane for a wide range of sequences.
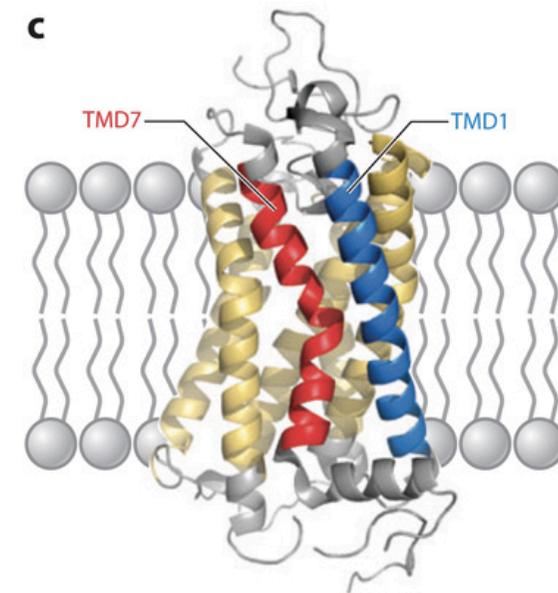
Integral membrane protein (IMP) biogenesis

Co-tanslational

- Co-translational synthesis (translation and insertion are sequential processes)
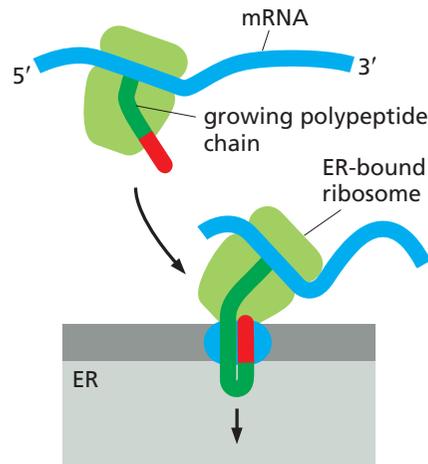
(A) CO-TRANSLATIONAL TRANSLOCATION

- Constitutes bulk of the membrane proteins

- Synthesized and assembled at the ER

- Involves the translocon apparatus

- No chaperones necessary for folding

Post-translational

- Post-translational synthesis (translation and insertion are temporally distinct processes)
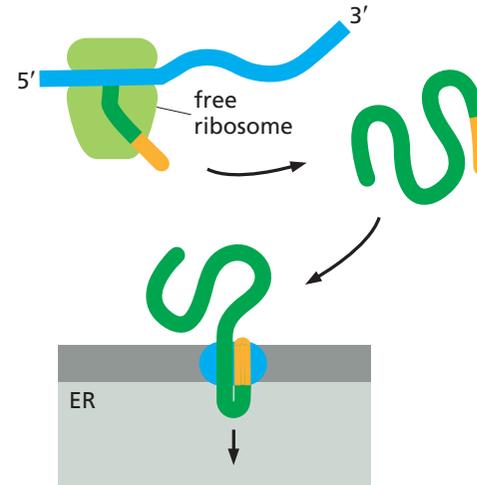
(B) POST-TRANSLATIONAL TRANSLOCATION

- Constitutes membrane proteins of mitochondria and chloroplast, nuclei and peroxisome and some bacterial toxins for e.g., alpha hemolysin, colicin, melittin and C-terminally anchored proteins found in synaptic vesicles

- Assisted (not involving the translocon apparatus)/ spontaneous insertion into membranes

- Chaperones necessary to maintain solubility in the cytosol

How to study IMP synthesis?

nucleus

inner nuclear membrane

outer nuclear membrane   ER membrane

rough ER   smooth ER

200 nm

rough ER

homogenization

smooth ER

rough and smooth microsomes

centri-fugation

smooth microsomes have a low density and stop sedimenting and float at low sucrose concentration

rough microsomes have a high density and stop sedimenting and float at high sucrose concentration

tube with gradient of increasing sucrose concentration

Secretory Protein Biogenesis and the Signal hypothesis
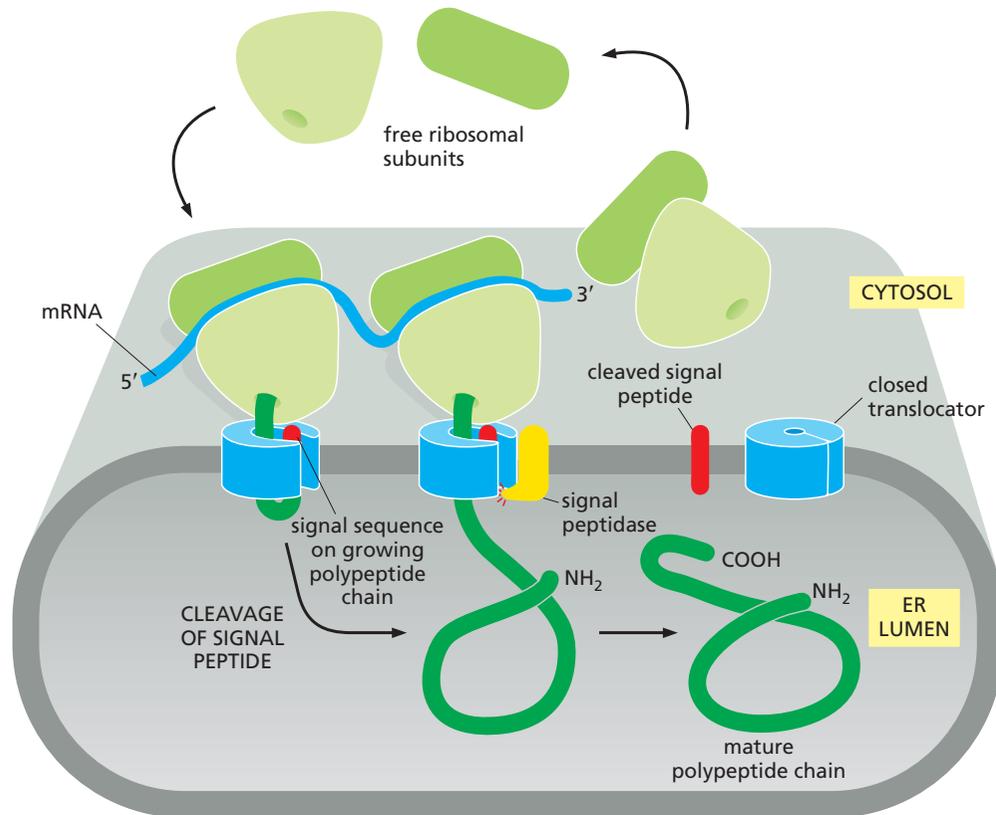
- Secreted proteins contain a ~8 amino acids long hydrophobic segment at the N terminus called the ER signal sequence

- ER signal sequences emerge from the soluble pool of ribosomes

- Signal sequence directs the ribosome to the translocon in the ER membrane

- Protein synthesis and translocation occurs sequentially

- Signal sequence is cleaved off

- Protein is retained in the ER lumen

Co-translational protein synthesis and insertion into membranes

start-transfer sequence

stop-transfer sequence

NH₂

CYTOSOL

ER LUMEN

signal peptidase

COOH

NH₂

mature single-pass transmembrane protein in ER membrane
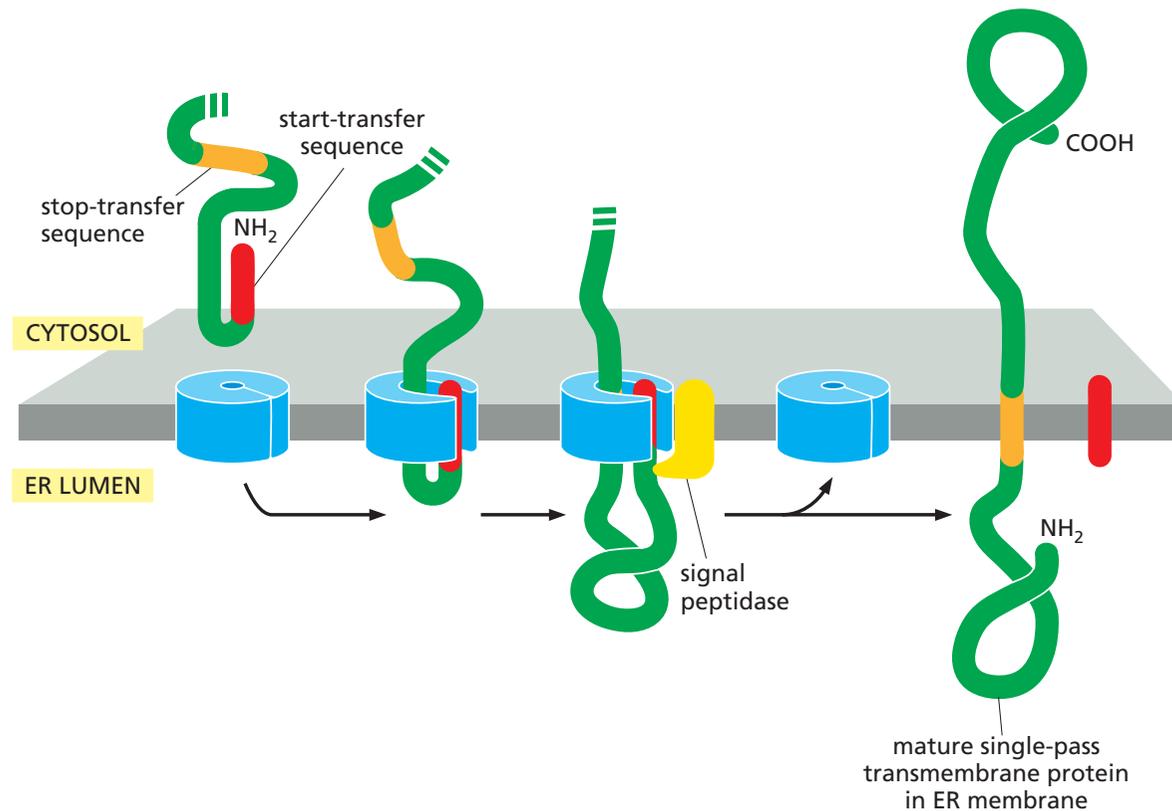
- Events start off like for soluble proteins

- Translocon encounters a stop-transfer hydrophobic segment

- Rest of the protein is translated

- Translocon releases the stop transfer hydrophobic segment into the lipid bilayer (mechanism ?)

Recent Insights into Endoplasmic Reticulum Organization
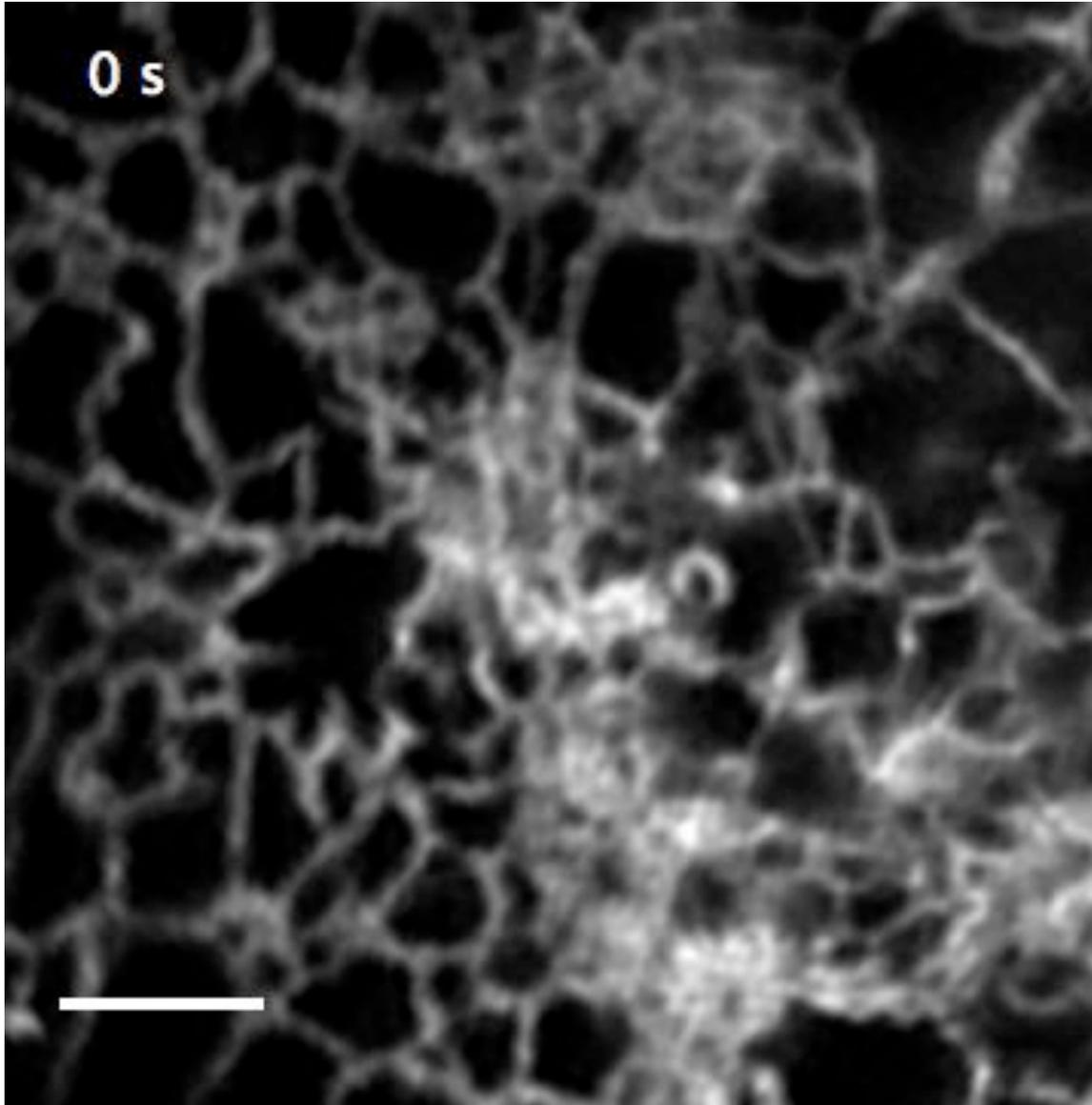
ER organization during cell division
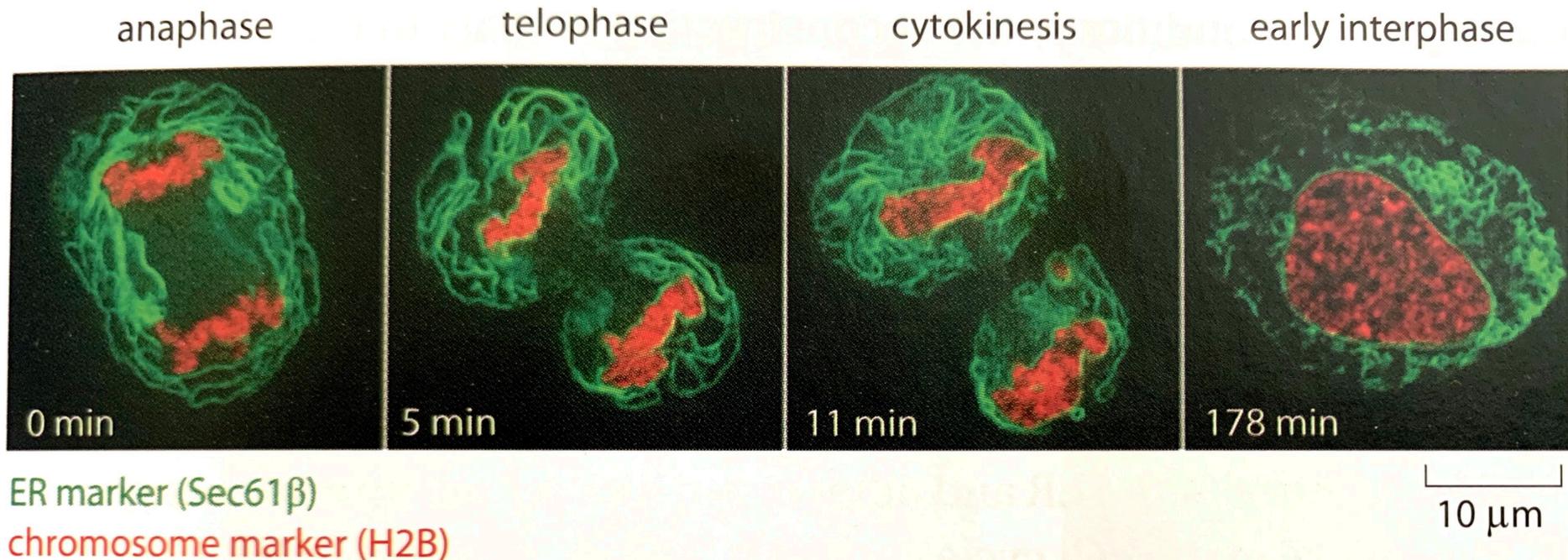
Figure 1-23 Structural dynamics of the endoplasmic reticulum during the cell cycle. Confocal images of HeLa cells. The chromosomes are labeled in red using a fusion of a fluorescent protein with histone H2B. The ER is labeled in green by virtue of a fusion of a fluorescent protein to a molecular member of the ER segregation apparatus (Sec61β-GFP). The sequence of images shows the changes in ER morphology as a function of time during the cell cycle. (Adapted from Lu L, Ladinsky MS & Kirchhausen T [2009] Mol Biol Cell 20:3471–3480.)

How is the ER made?

Endoplasmic Reticulum: Keeping in shape. Blackstone and Prinz (2016) eLife
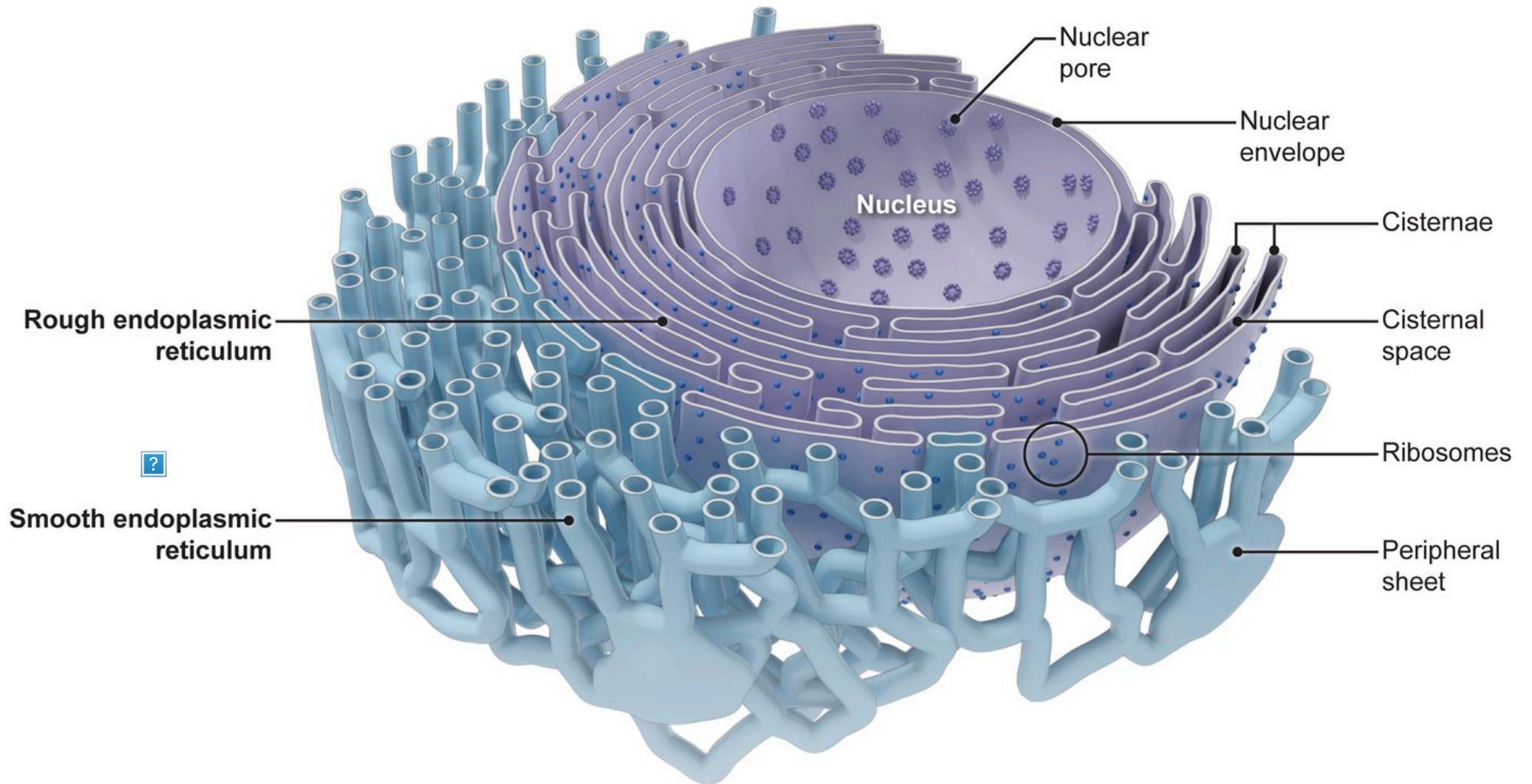
Membrane tubulation

Reticulons

Membrane tubule
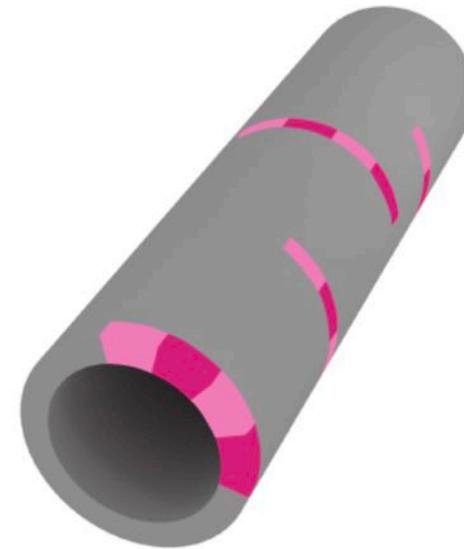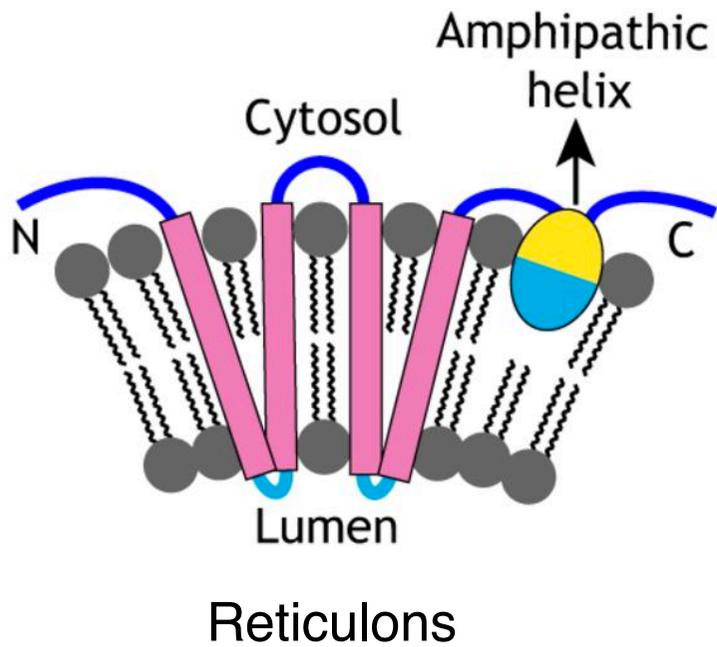
Reticulons decide the shape of the ER membrane

Membrane fusion
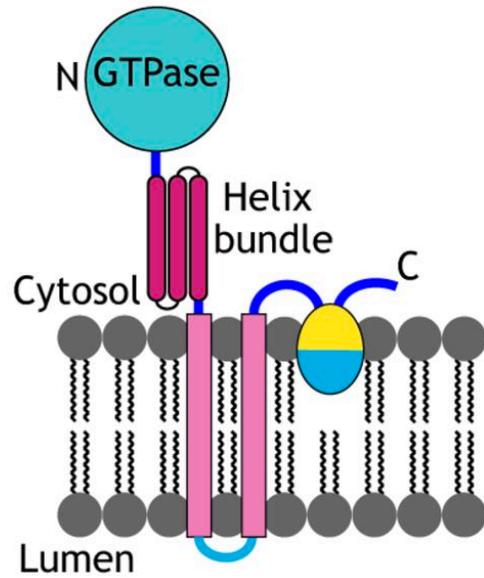
Atlastin

Atlastins fuse membranes
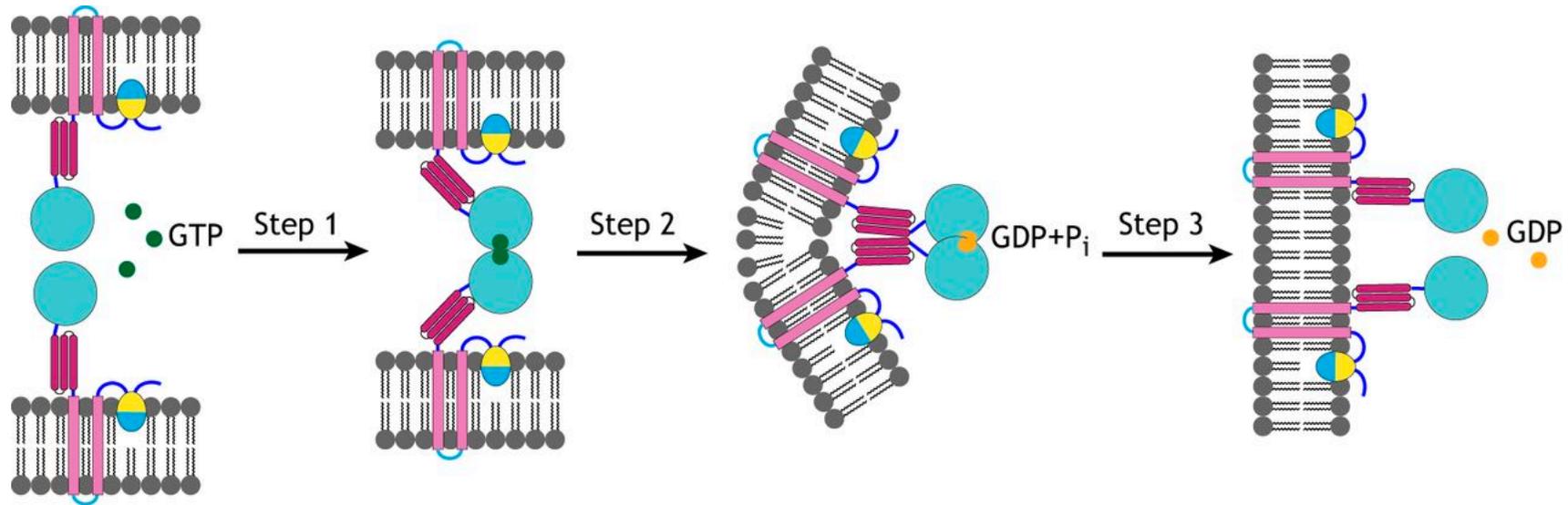
Proof-of-concept
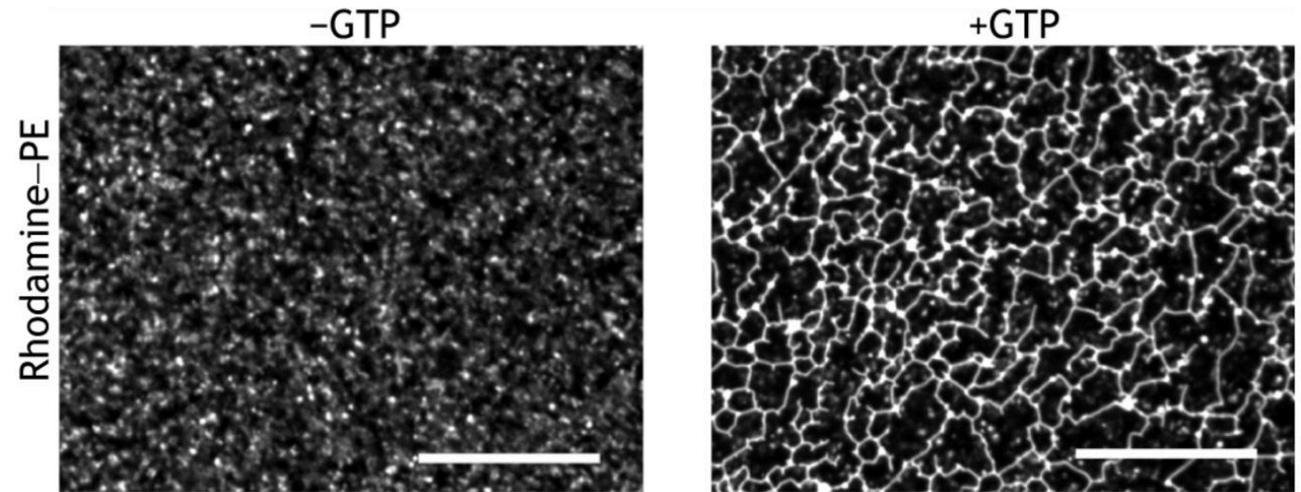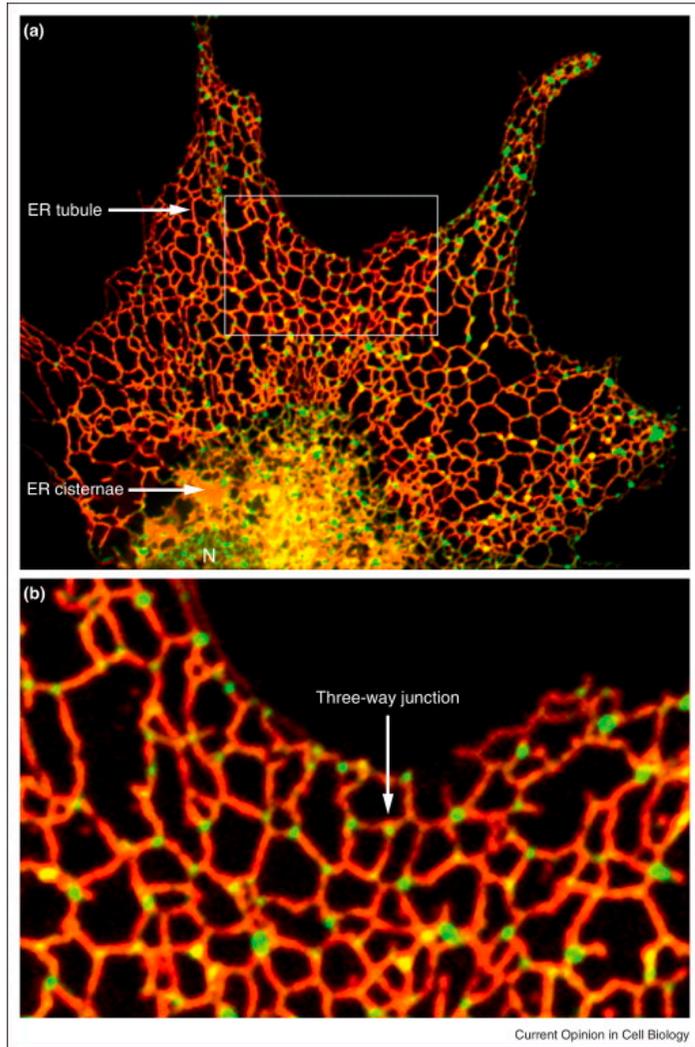
Structure of the ER in cells

Vesicles containing reticulon (Yop1) and atlastin (Sey1)

Reconstituting the reticular ER network – mechanistic implications and open questions Wang and Rapoport (2019) J. Cell Sci.

Nucleus

Heterochromatin

Nuclear Envelope

Nucleolus
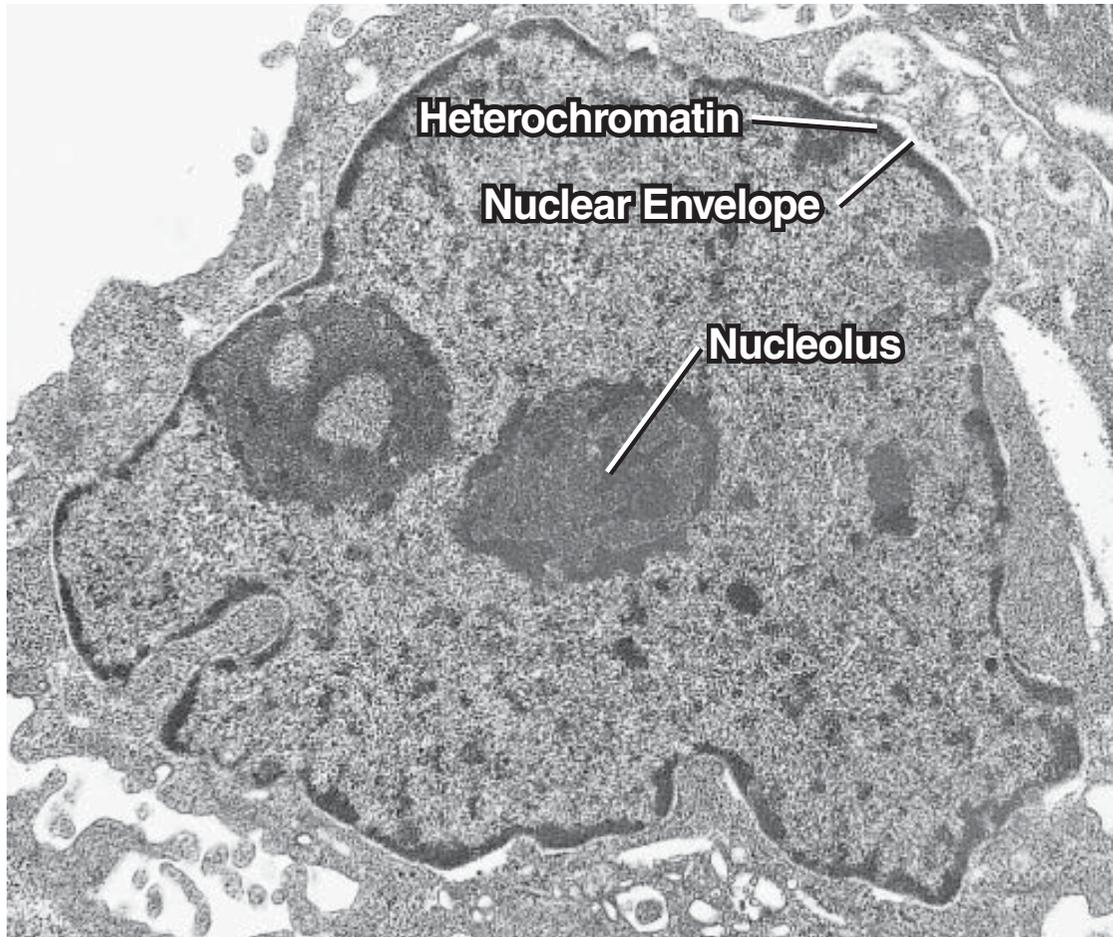
Separation of a cell's genetic material from the surrounding cytoplasm may be the single most important feature that distinguishes eukaryotes from prokaryotes

Makes the appearance of the nuclear envelope a landmark in biological evolution

The nucleus of a typical interphase (i.e., nonmitotic) cell contains

(1) Chromosomes, which are present as highly extended nucleoprotein fibers, termed *chromatin*

(2) One or more *nucleoli*, which are irregularly shaped electron-dense structures that function in the synthesis of ribosomal RNA and the assembly of ribosomes

(3) The *nucleoplasm*, the fluid substance in which the solutes of the nucleus are dissolved.

Contents of the nucleus are present as a viscous, amorphous mass of material enclosed by a complex *nuclear envelope*

This envelope forms a boundary between the nucleus and cytoplasm.

Karp et al

Nuclear Envelope
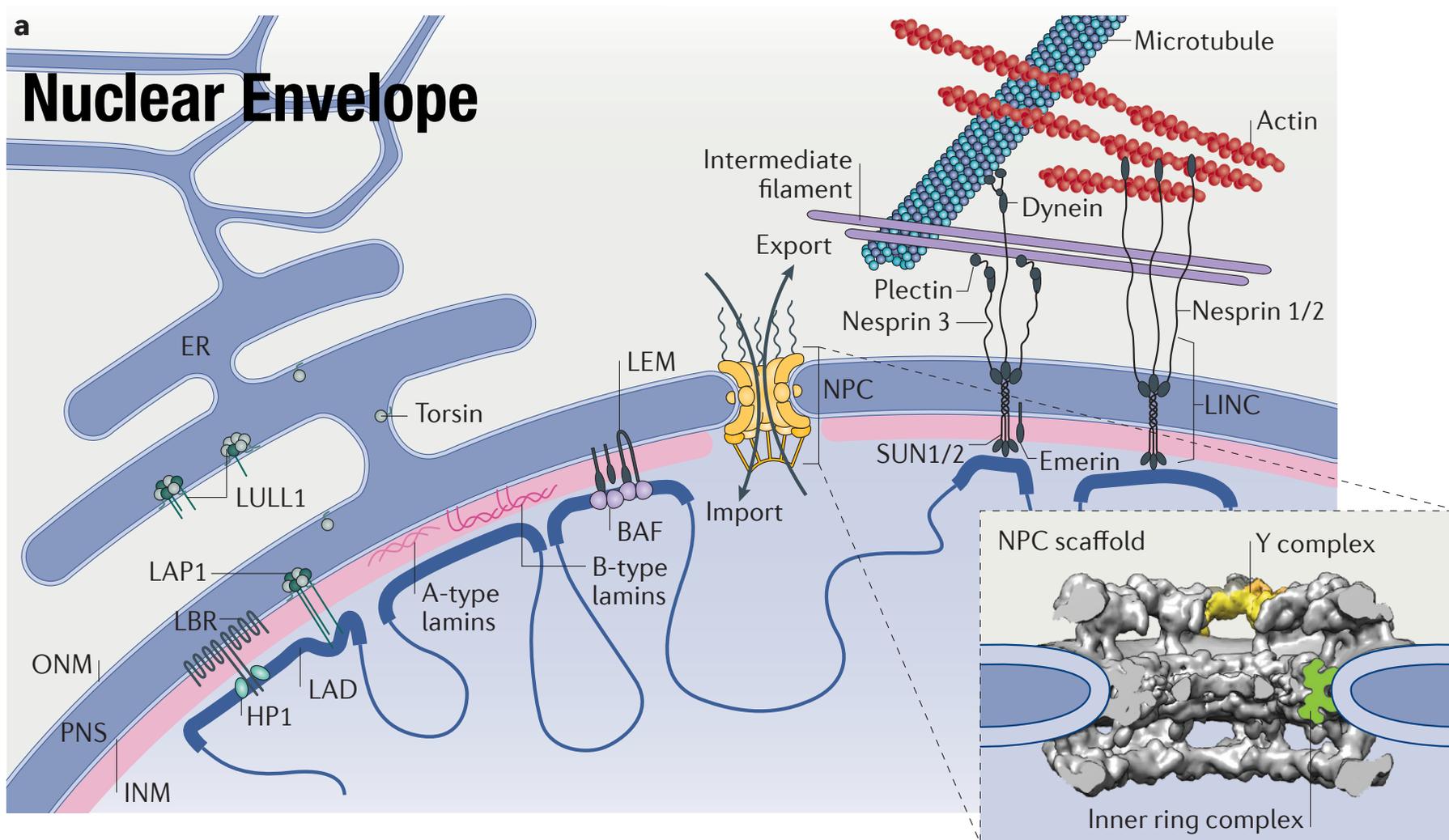
- NE defines the nuclear compartment
- NE is comprised of two concentric membranes - Outer nuclear membrane (ONM) and Inner nuclear membrane (INM)
- ONM and INM are separated by a distance of 10-50 nm
- NE contains about 60 integral membrane proteins
- NE is penetrated by nuclear pore complexes
- ONM and INM are continuous but maintain distinct protein compositions
- INM contains proteins that bind chromatin and nuclear lamina
- ONM is continuous with the ER membrane

Nuclear Envelope
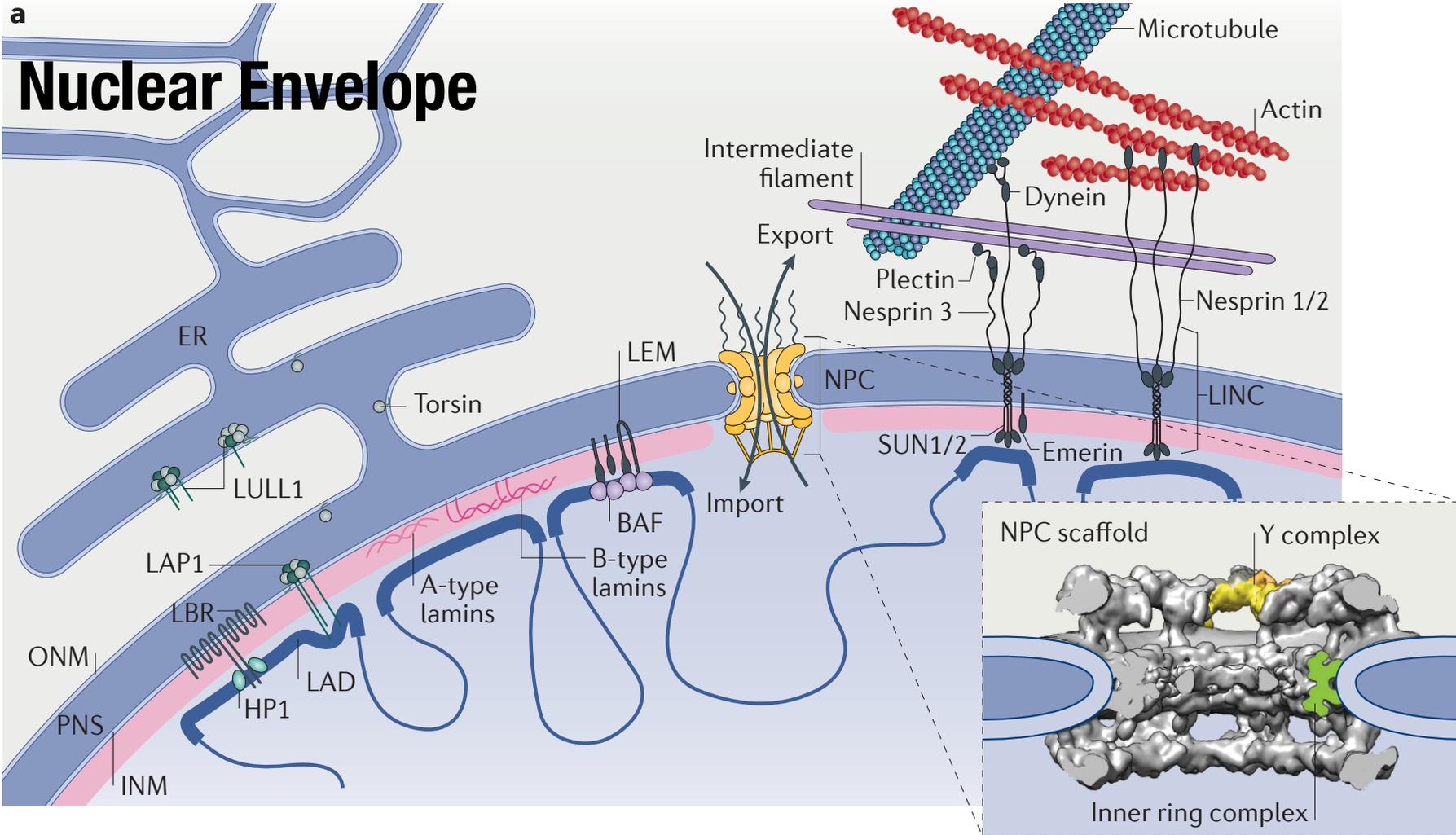
- NE incorporates new components during growth and to replace defective parts

- Adapts to mechanical challenges

- During open mitosis in higher eukaryotes, NE disassembles completely and then reforms

- Integral membrane proteins are co-translationally inserted into the ER network and distribute to the ONM and INM by diffusion

- Some of these proteins are retained by binding to chromatin and/or the nuclear lamina

Nuclear Envelope
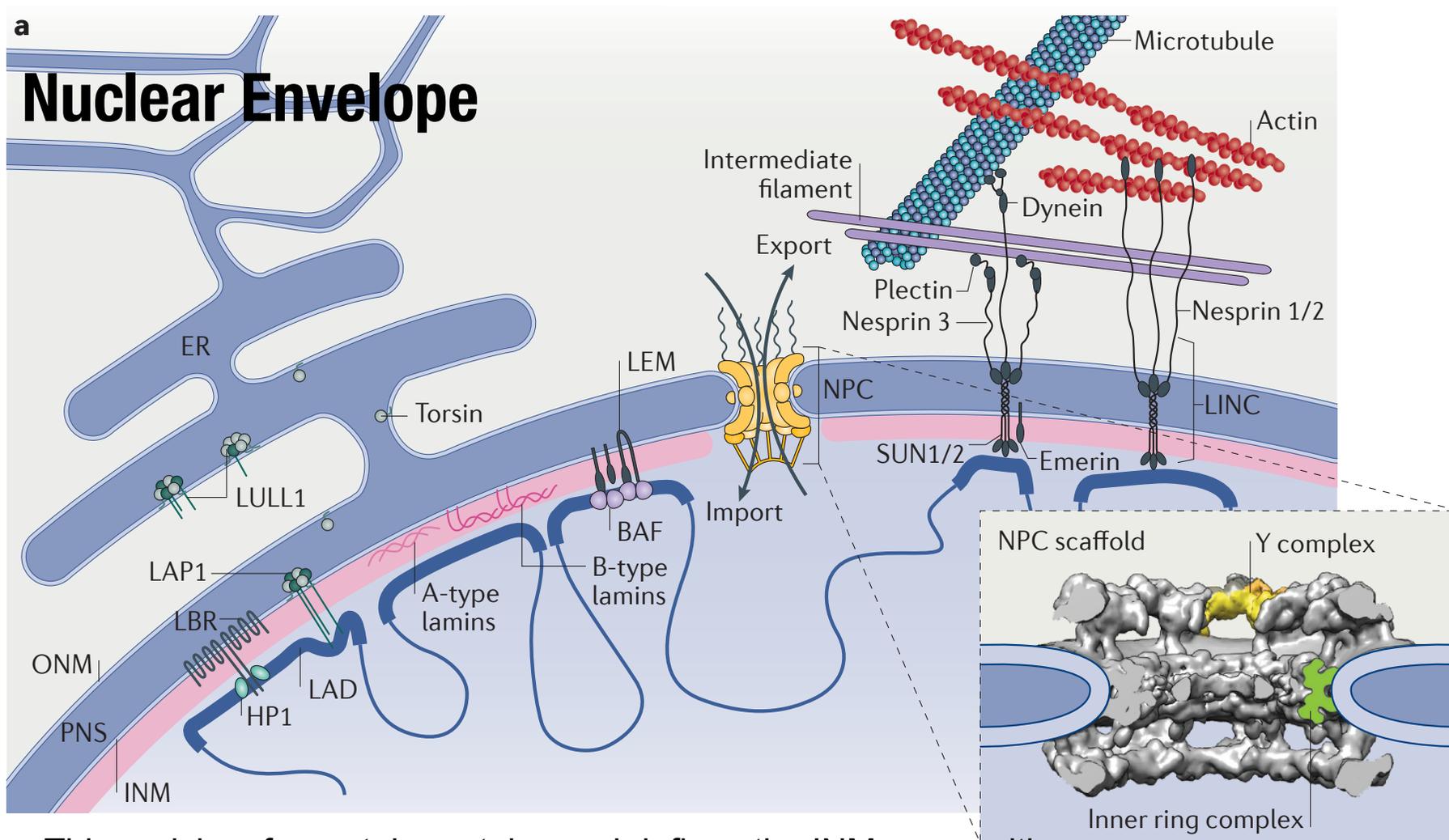
- This enriches for certain proteins and defines the INM composition

- Nuclear pore complexes (NPC) puncture the ONM and the INM

- NPCs restricts free diffusion and prevents the passage of membrane proteins with extralumenal domains larger than approximately 60 kDa to the INM.

- This NPC-based size restriction dictates which proteins can reach the INM. *Thus, the INM can in principle be 'sampled' by ER membrane proteins that fulfill the NPC-based size criterion.*

- However, only proteins that bind efficiently to nuclear components will become enriched in the INM. Thus, the protein composition of the INM is determined by a passive sorting mechanism

Open vs Closed Mitosis and the Nuclear Envelope

a Open mitosis

b Closed mitosis

Metazoans

Fungi

c Semi-closed mitosis

d Semi-closed mitosis

Legend:
- Nuclear pore complex
- Solubilized nucleoporins
- INM proteins
- Transmembrane nucleoporins
- MTOCs
- Microtubules
- Lamina
- Chromatin
- Kinetochore

Güttinger, Laurell & Kutay (2009) Nature Reviews Molecular Cell Biology

Breakdown of the Nuclear Envelope

a

G2 → Prophase → Metaphase

b

Chromatin condensation
TOPOIIα, condensins, histone H3 phosphorylation

Retraction of INM proteins into the ER, tubulation of the ER

MT nucleation and separation of chromosomes
Aurora A, PLK1, CDK1, NEK2

MT-dependent tearing of the NE
Dynein

NPC and lamina disassembly
CDK1, PKC

Green - INM (lamin)

Blue - DNA

Red - microtubules

Legend:
- Chromatin
- INM proteins
- Kinetochore
- Lamins
- Microtubules
- MTOCs
- Nuclear pore complex
- NUP107–160 and ELYS
- Transmembrane nucleoporins

Güttinger, Laurell & Kutay (2009) Nature Reviews Molecular Cell Biology

Reformation of the Nuclear Envelope

a

Early anaphase → Late anaphase → Telophase

b

Formation of prepores and recruitment of membrane tubules
NUP107–160 and ELYS, RanGTP

NE reformation
• INM proteins bind DNA
• NE sheet formation removal of reticulons
• Membrane closure

Chromatin compaction
KID, Aurora B?

Lamina assembly

Completion of NPC assembly

Green - INM (lamin)

Blue - DNA

Red - microtubules

Legend:
— Lamins
• Transmembrane nucleoporins
• NUP107–160 and ELYS
INM proteins
— Microtubules
MTOCs
Chromatin
Kinetochore

Güttinger, Laurell & Kutay (2009) Nature Reviews Molecular Cell Biology

Nuclear Transport

The movement of materials through the nuclear pore. (*a*) Electron micrograph of the nuclear–cytoplasmic border of a frog oocyte taken minutes after injection with gold particles that had been coated with a protein normally found in the nucleus. These particles pass through the center of the nuclear pores (arrows) on their way from the cytoplasm to the nucleus. (*b*) At higher magnification, the gold particles are seen to be clustered in a linear array within each pore. (*c*) Electron micrograph of a section through the nuclear envelope of an insect cell showing the movement of granular material (presumed to be a ribosomal subunit) through a nuclear pore. Karp et al.

"To support growth, a single nucleus must import approximately 560,000 ribosomal proteins and export approximately 14,000 ribosomal subunits every minute."

Nuclear pore complex (NPC)

Viewed from the cytoplasm

(a)

Viewed from the nucleus

(b)

Viewed from the nucleus

NEL

(c)

0.6 μm
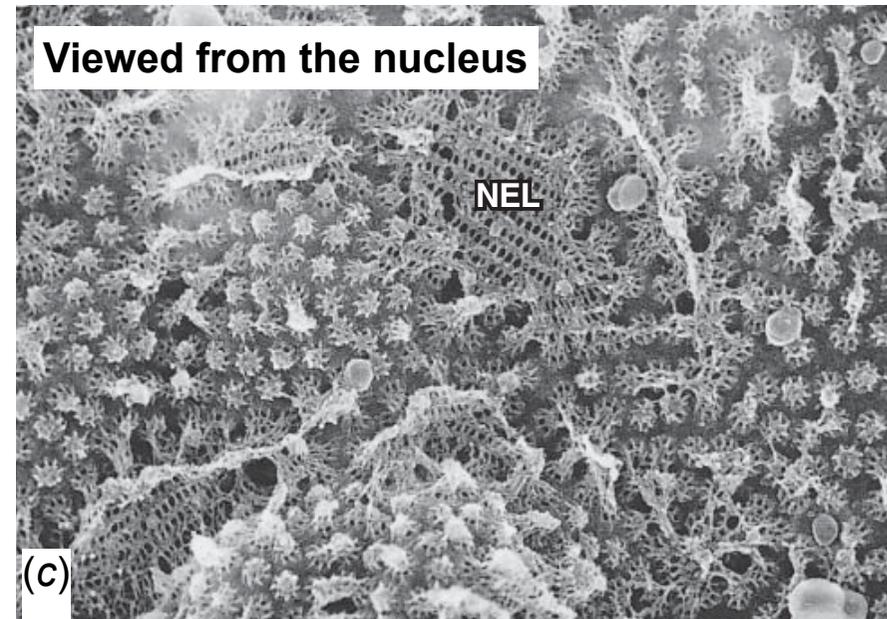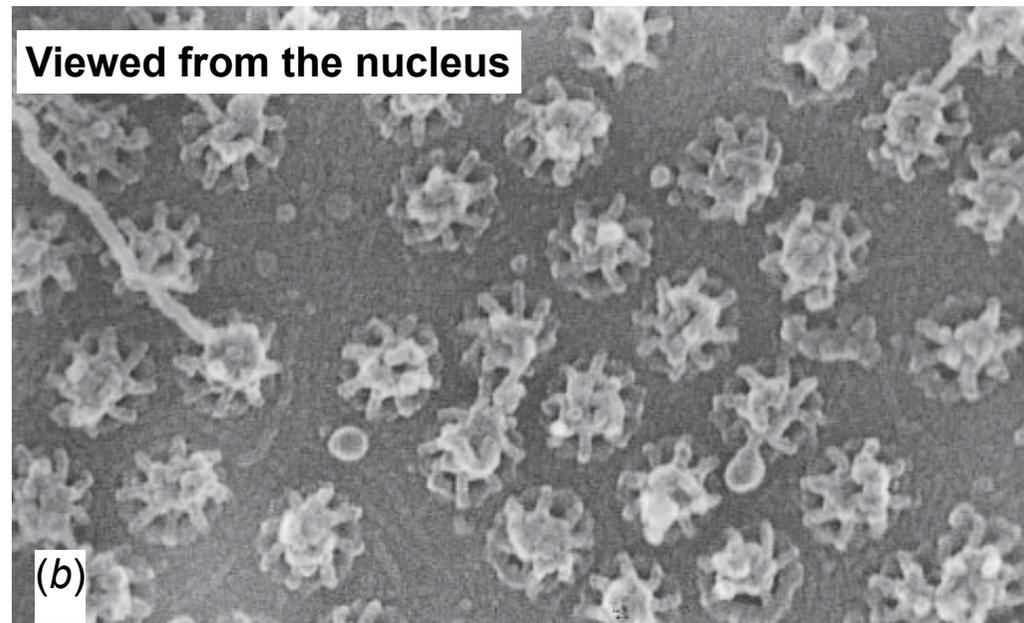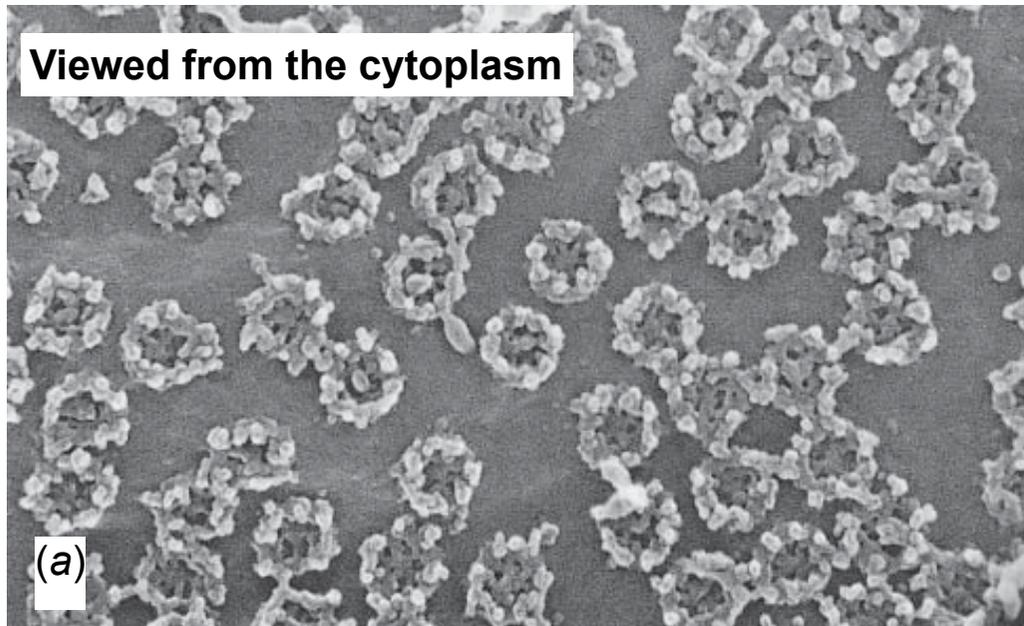
nuclear pore complex from isolated
nuclear envelopes of an amphibian oocyte.
(*a*) The cytoplasmic face of the nuclear
envelope showing the peripheral cytoplasmic
ring of the nuclear pore complex. (*b*) The
nuclear face of the nuclear envelope showing
the basket-like appearance of the inner
portion of the complex. (*c*) The nuclear face of
the envelope showing the distribution of the
NPCs and places where intact patches of the
nuclear lamina (NEL) are retained. In all of
these micrographs, isolated nuclear
envelopes were fixed, dehydrated, dried, and
metal-coated.

Karp et al

Nuclear pore complex (NPC)

30
a)

he

s

vertebrates.

Nucleoporins lining the inner cavity

eats

ves

Density of nuclear pore complexes
correlates with nuclear activity - typical

Cytoplasm

Cytoplasmic filaments

Cytoplasmic ring

FG-repeat domains of G nucleoporins

Outer nuclear membrane

Transmembrane ring

Nuclear envelope

Inner nuclear

Central channel

Nuclear ring

Nuclear basket

Nucleoplasm

b

Synthesis

RNA — Nascent polypeptide

Ribosome

Diffusion

Active transport through the NPC

Importin

c

Model I: Membrane fusion occurs before NPC assembly

Model III: Maturation of the NPC core occurs before membrane fusion

Retention

RAN•GTP

NUPs

Model II: Pre-NPC assembly is followed by membrane fusion and NPC maturation

Nuclear Localization Signals (NLSs)

- **Function:** Tag proteins for import from the cytoplasm into the nucleus.

- **Composition:** Often contain one or more short stretches of basic (positively charged) amino acids (e.g., Lys-Lys-Lys-Arg-Lys).

  Nucleoplasmin KRPAATKKAGQAKKKK
  SV50 T antigen PKKKRKV

- **Mechanism:** Recognized by Nuclear Import Receptors or adaptor proteins (like importin-alpha)

Nuclear Export Signals (NESs)

- **Function:** Tag proteins for export from the nucleus to the cytoplasm.

- **Composition:** Short, hydrophobic sequences, often 10-13 amino acids long, with specific hydrophobic residues.
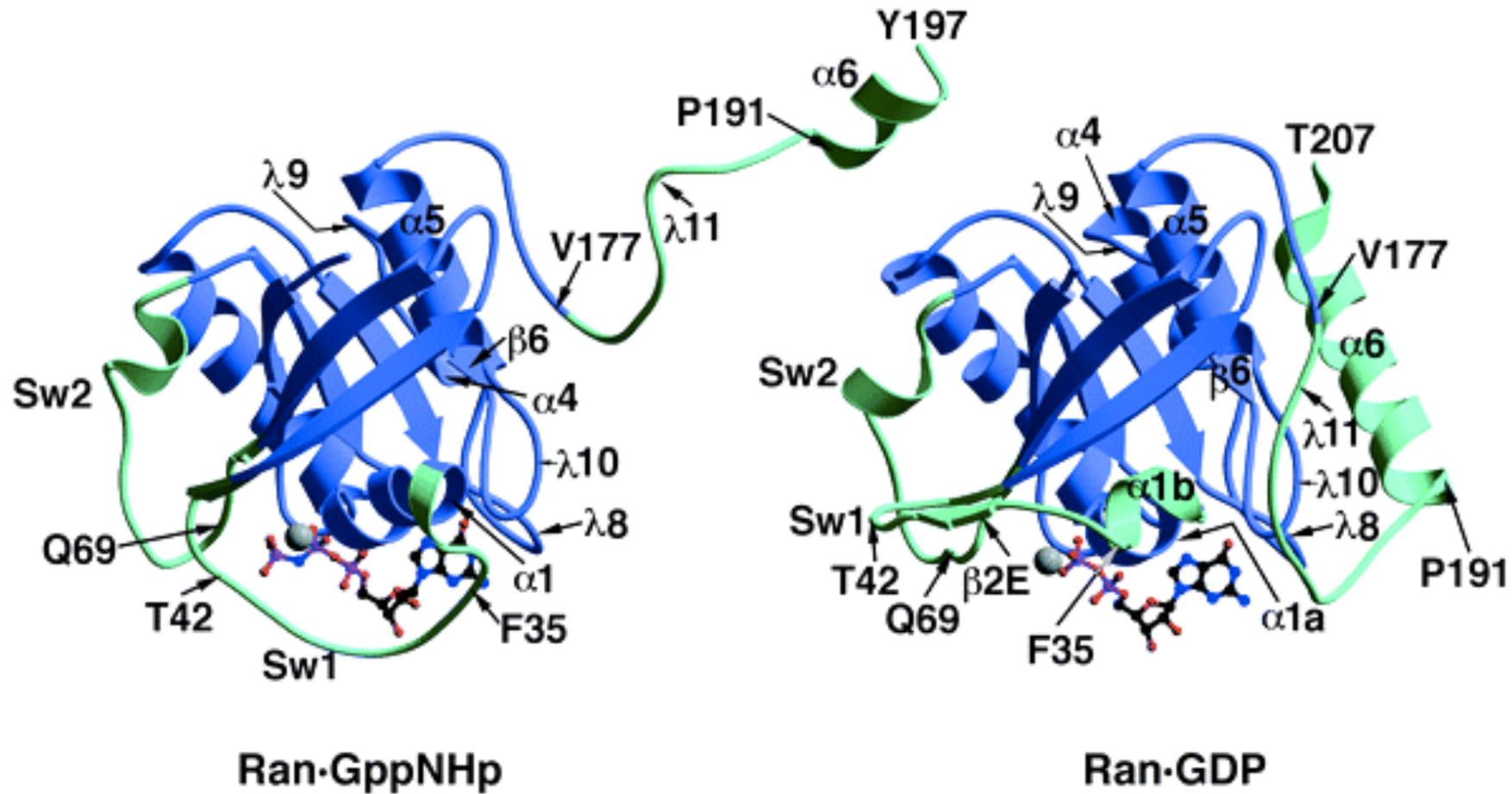
  Rev LPPLERLTL
  PKI LALKLAGLDI

- **Mechanism:** Bind to Nuclear Export Receptors or adaptor proteins (like CRM1)

Key Differences & Regulation

- **Charge:** NLSs are positive; NESs are hydrophobic.

- **Receptors:** Importins for NLSs; Exportins for NESs.

Nuclear localisation relies on the gradient of the small G-protein Ran

Ran·GppNHp

Ran·GDP

Nuclear localisation relies on the gradient of the small G-protein Ran

Ran's intrinsic rates of nucleotide exchange and hydrolysis are slow
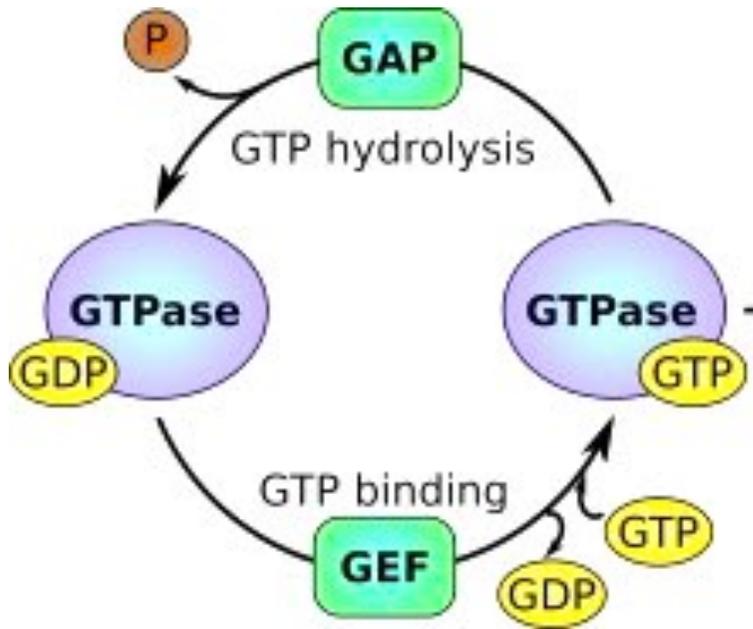
These reactions require a nucleotide exchange factor GEF and a GTPase activating protein (GAP)

Ran-GAP is cytosolic and binds to Ran-GTP with high affinity and increases GTP hydrolysis

Ran-GEF is a chromatin-associated nuclear protein and facilitates exchange of GDP for GTP

This asymmetric distribution of nucleotide exchange and hydrolysis enzymes across the nuclear envelope predicts that Ran-GTP should be largely nuclear and Ran-GDP should be largely cytosolic.
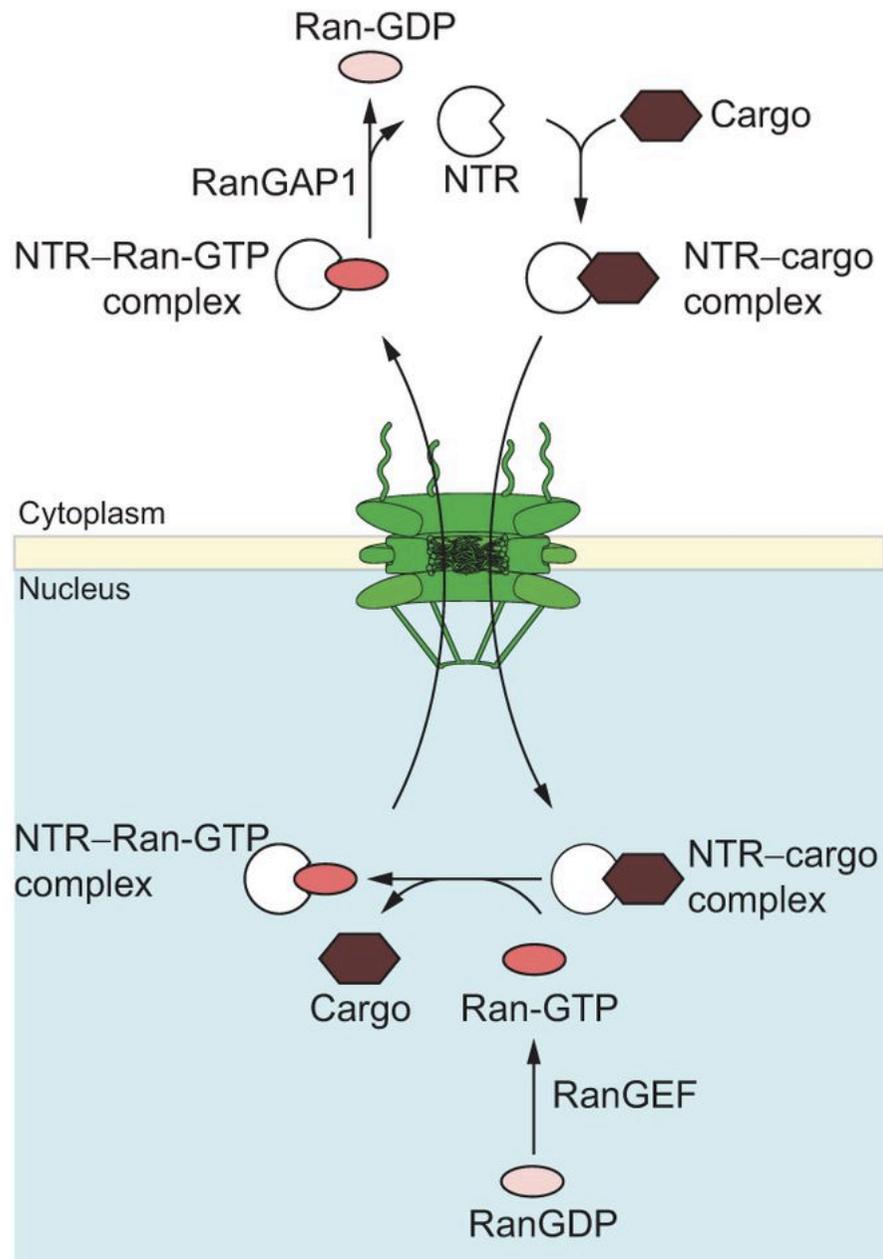
This distribution plays a key role in determining the directionality of nuclear transport.

Dasso (2001) Cell

Nuclear localisation relies on the gradient
of the small G-protein Ran

GAP
in cytoplasm

Nuclear
envelope

GEF
on
chromosomes
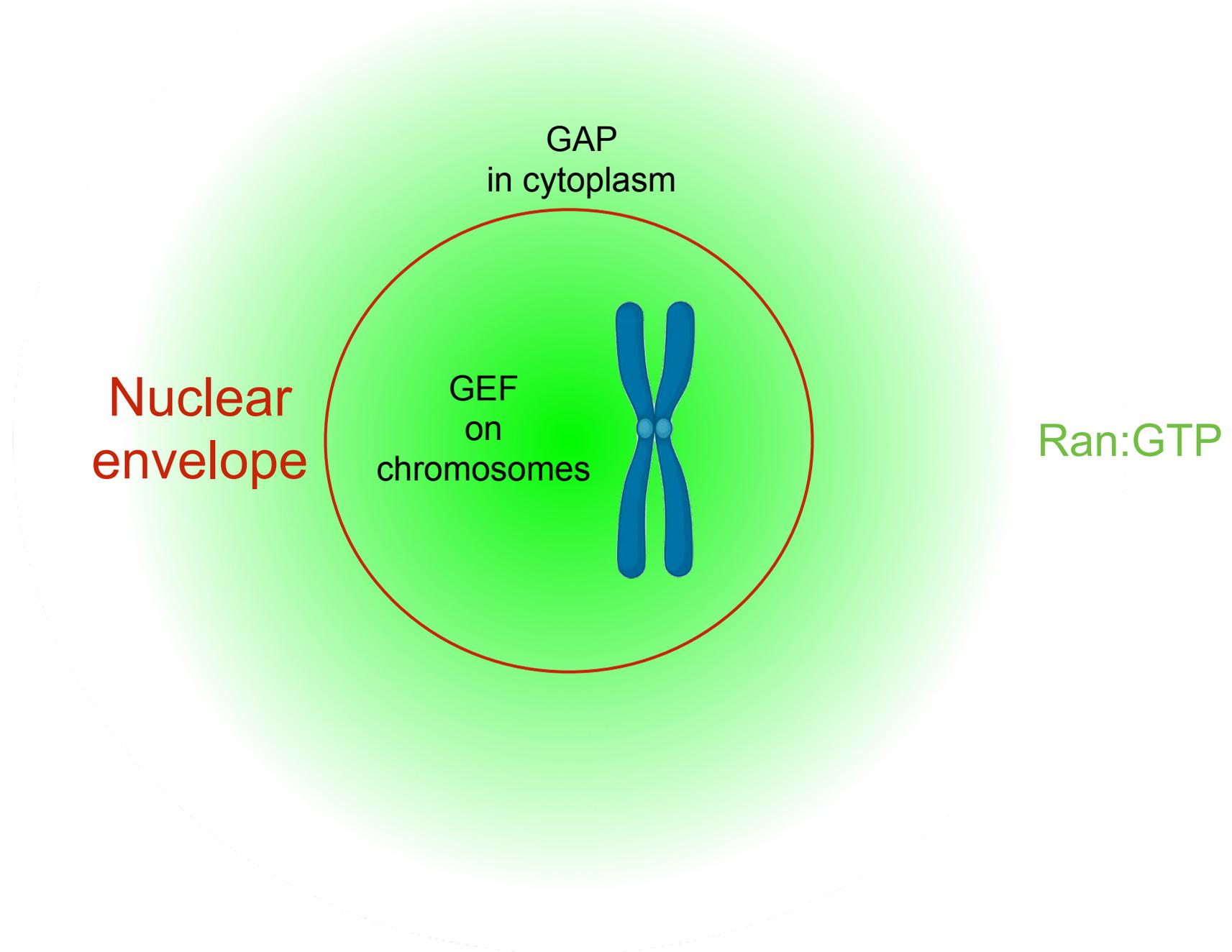
Ran:GTP

Nuclear Import Cycle
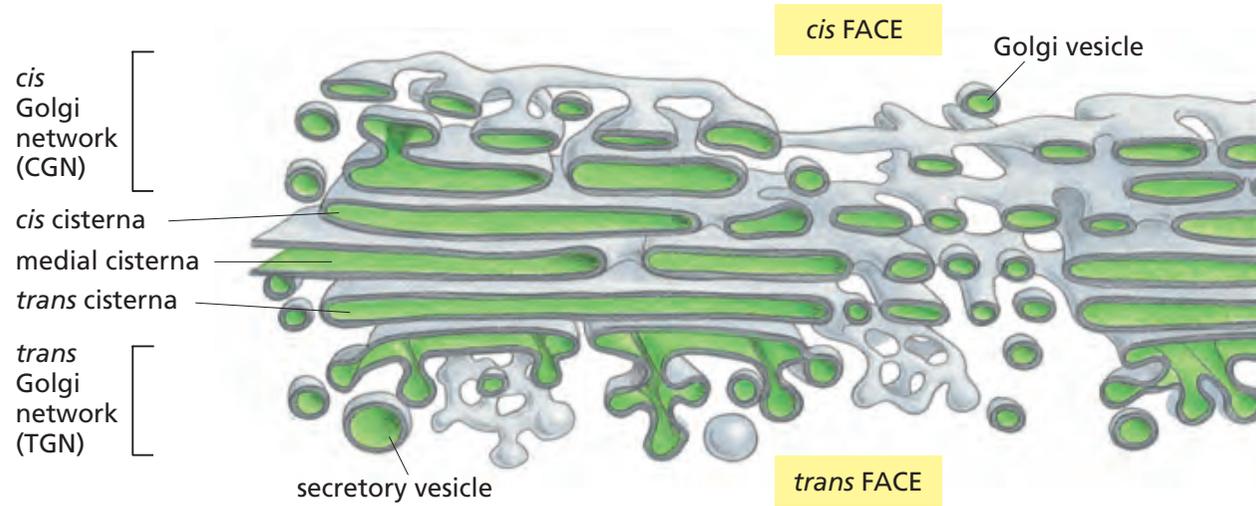

- NLS-bearing cargo binds the nuclear transport receptor (NTR) in the cytoplasm

- This complex binds FG repeats in the NPC and enters into the nucleus

- After reaching the nucleus, Ran-GTP displaces the cargo from the NTR

- NTR–Ran-GTP complex returns to the cytoplasm through the NPC

- RanGAP in the cytosol stimulates GTP hydrolysis on Ran-GTP

- Releases NTR for another import cycle
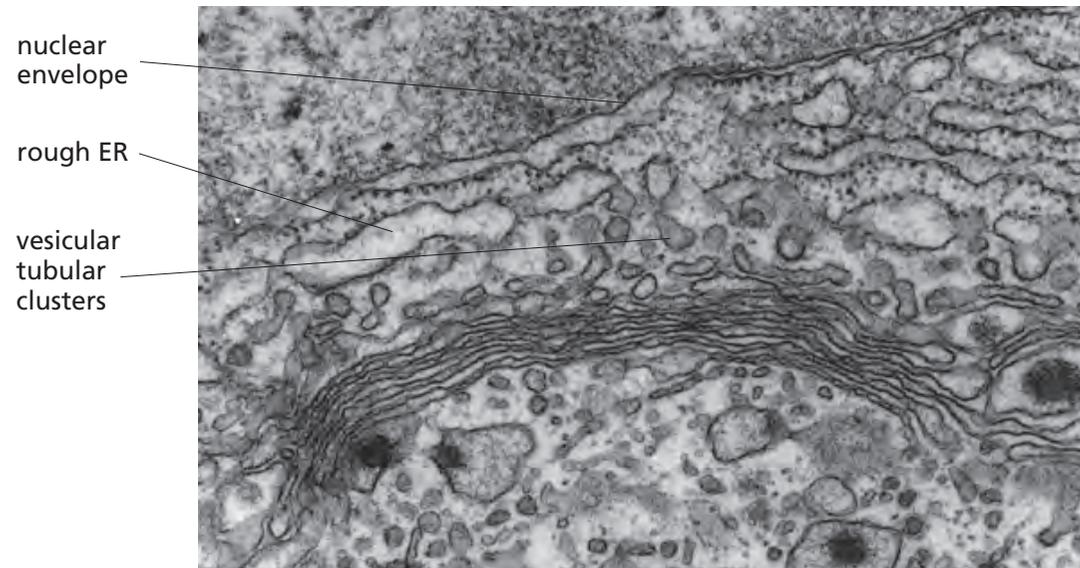
Nuclear Export Cycle
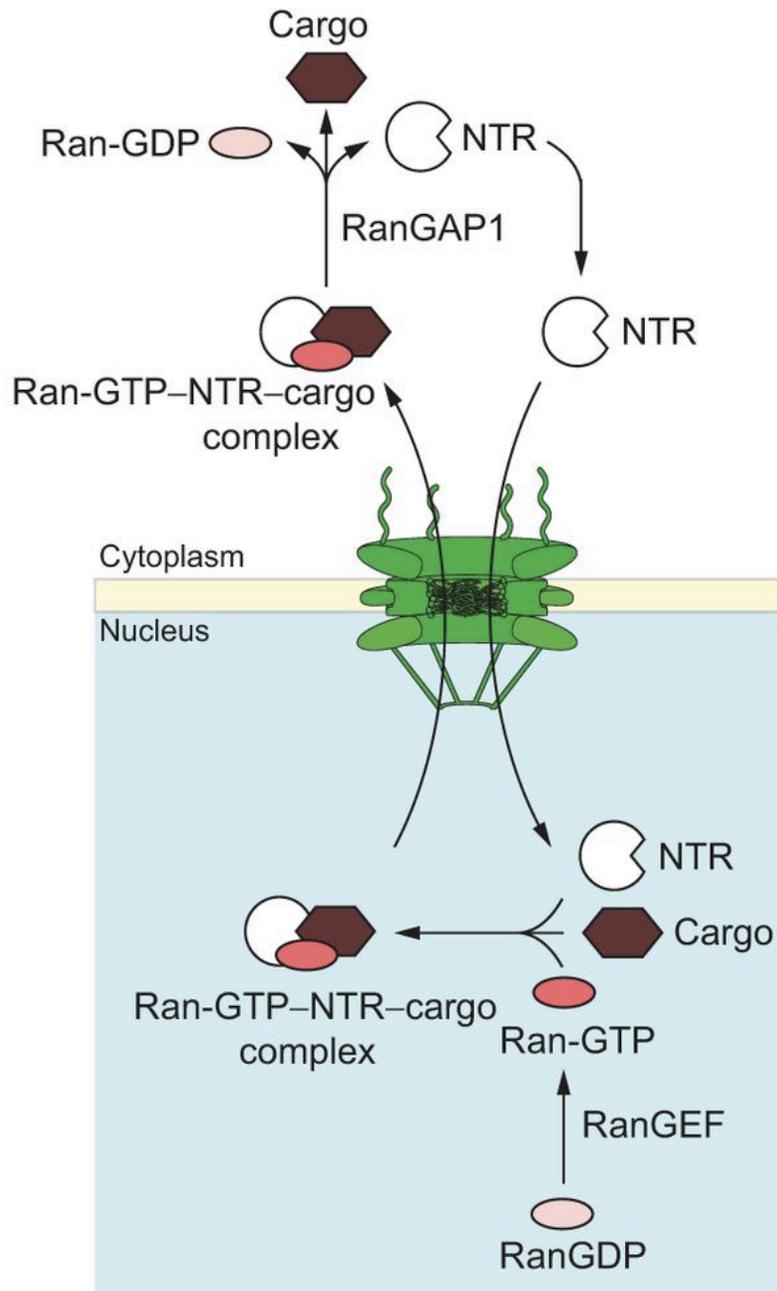
- NES-bearing cargo forms a trimeric complex with NTR and Ran-GTP in the nucleus

- This complex leaves the nucleus and reaches the cytoplasm

- Ran-GTP hydrolysis causes release of the cargo

- Releases NTR for another export cycle

Journal of Cell Science (2016)

The Golgi Apparatus

cis Golgi network (CGN)

cis cisterna

medial cisterna

trans cisterna

trans Golgi network (TGN)

cis FACE

Golgi vesicle

secretory vesicle

trans FACE

(A)

nuclear envelope

rough ER

vesicular tubular clusters

(B)

1 µm

A. Three-dimensional reconstruction from electron micrographs of the golgi apparatus in a secretory animal cell. The cis face of the golgi stack is that closest to the er.
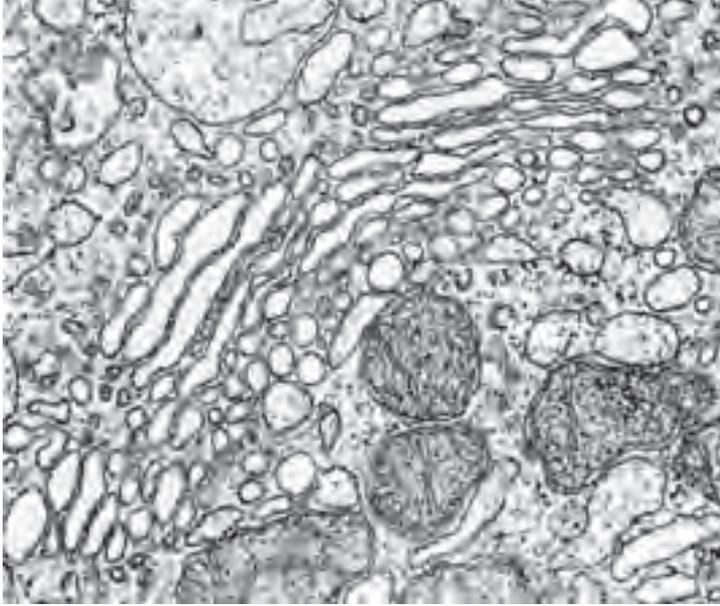
B. A thin-section electron micrograph of an animal cell. In plant cells, the golgi apparatus is generally more distinct and more clearly separated from other intracellular membranes than in animal cells.

Proteins traffic through the Golgi compartment
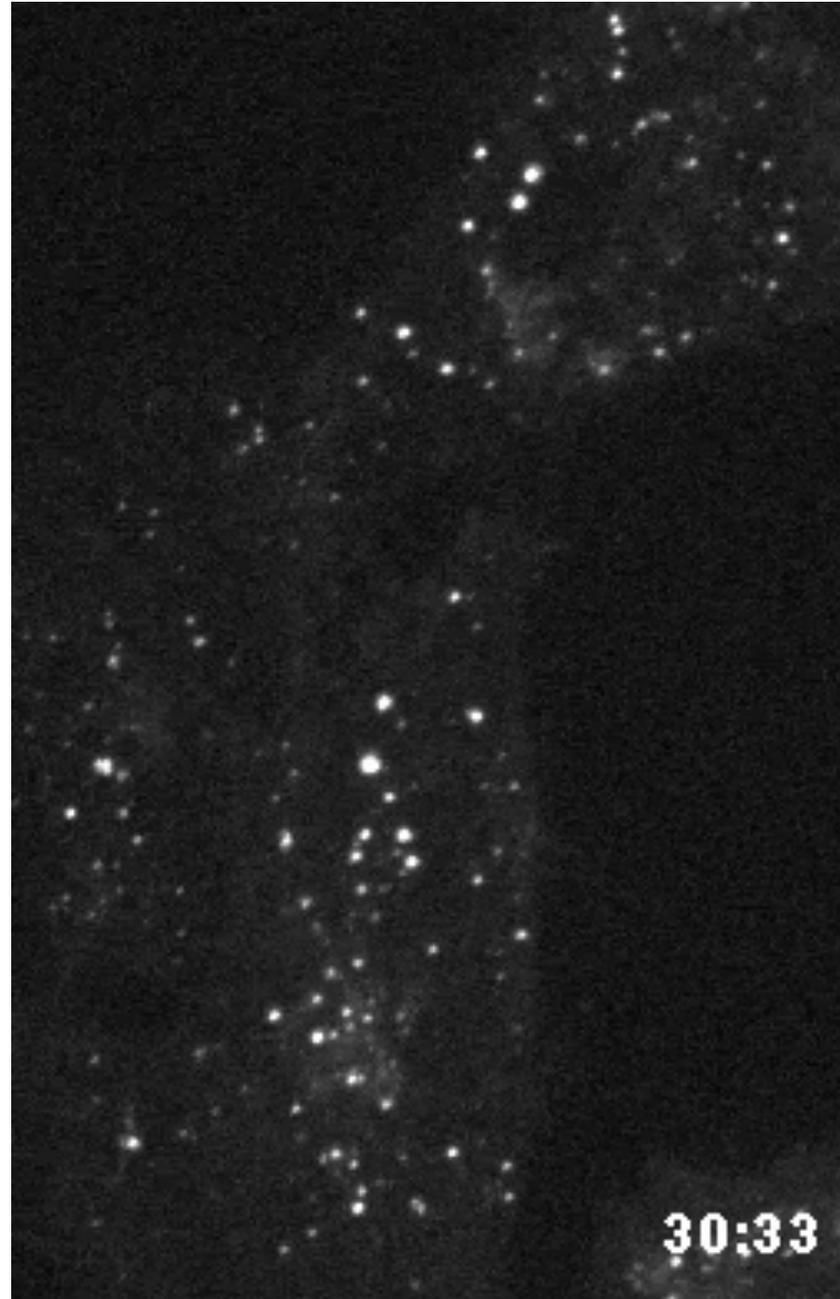
Molecular compartment... ...paratus

Unstained
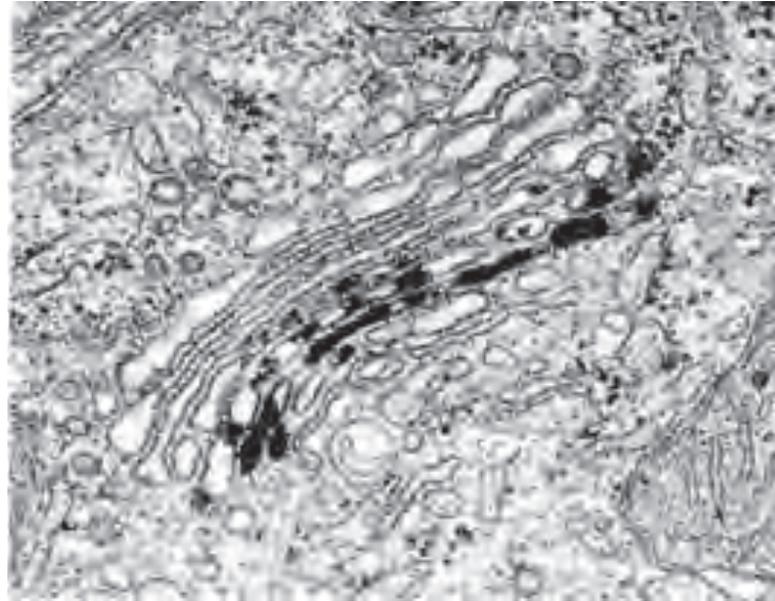
Osmium
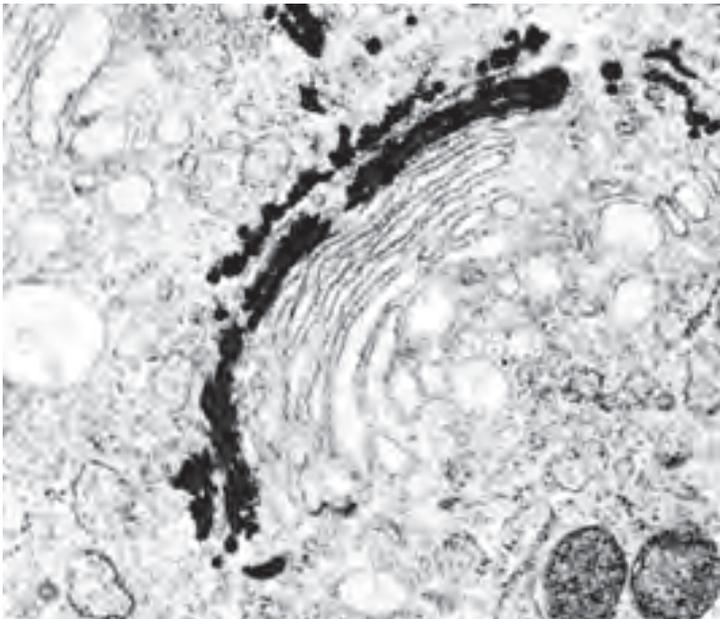
Acid phosphatase
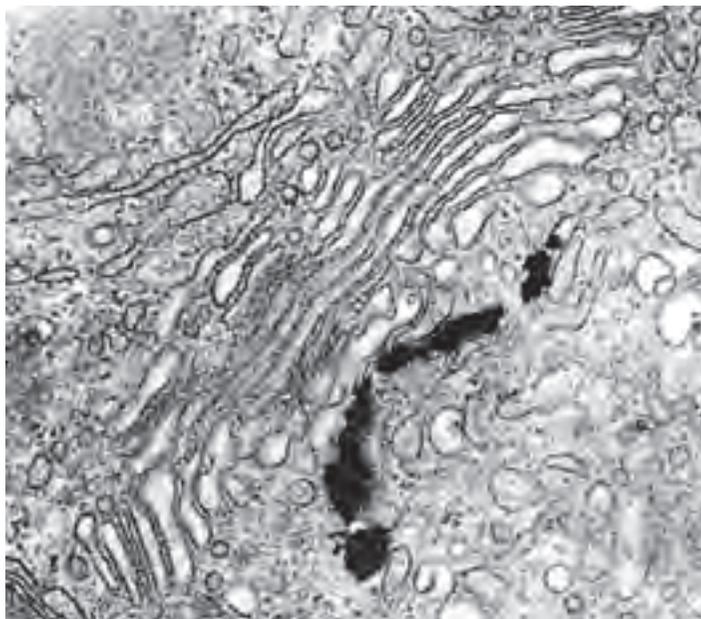
Birth and Maintenance of the Golgi Apparatus

(A) An electron micrograph of vesicular tubular clusters forming around the ER exit sites. (B) vesicular tubular clusters move along microtubules to carry proteins from the ER to the Golgi apparatus. COPI-coated vesicles mediate the budding of vesicles that return to the ER from these clusters and from the Golgi apparatus.

Two Models for Compartmentalisation

(A) CISTERNAL MATURATION MODEL

Labels: cisternae, vesicular tubular cluster, ER, *cis*, *trans*, CGN, medial, TGN, matrix proteins

Cisternal Maturation Model

Cisternae mature in composition as they move along microtubules

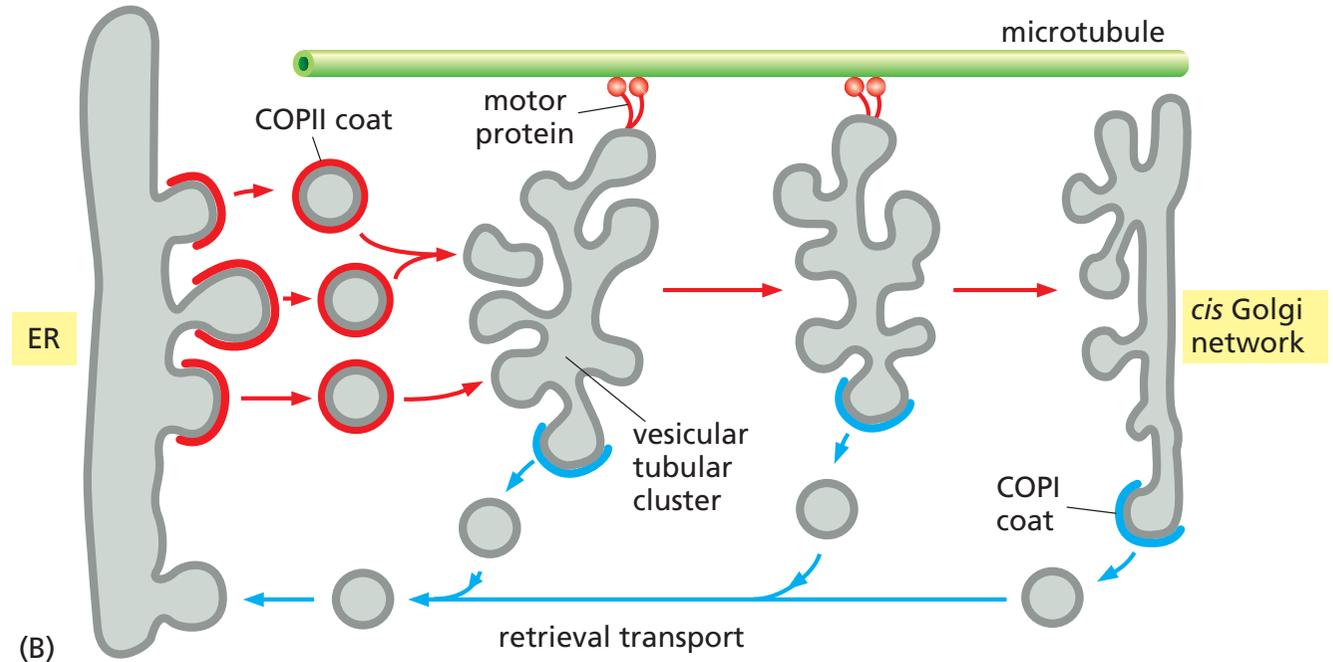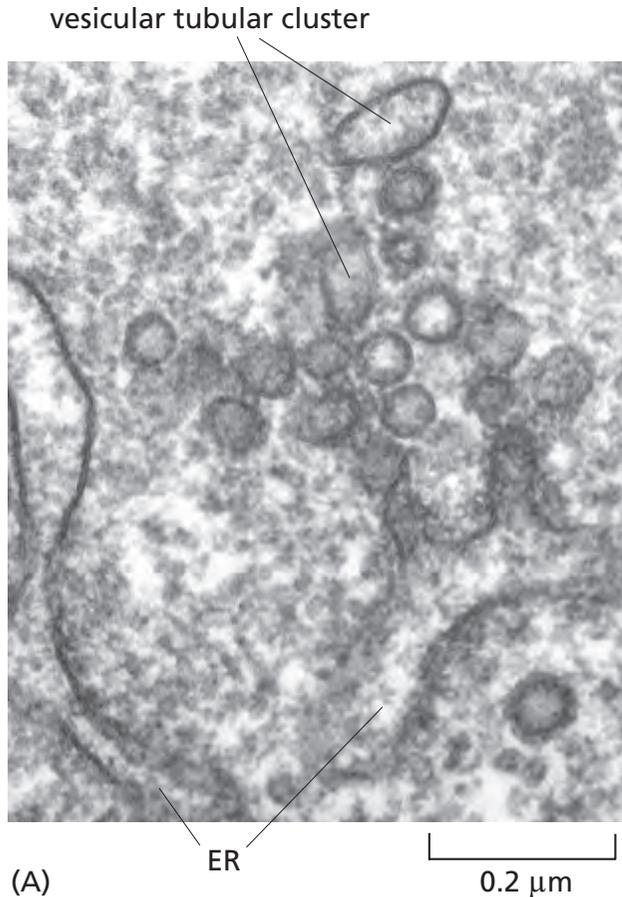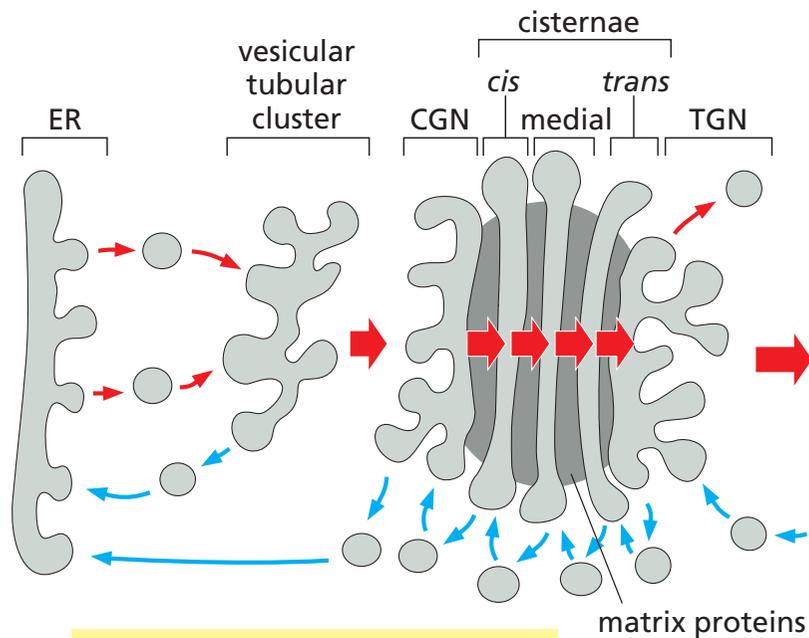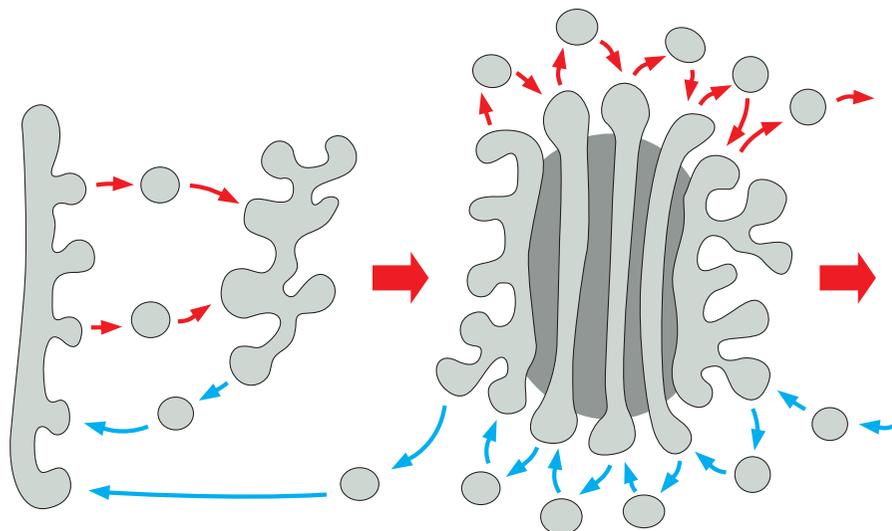Golgi resident proteins that are carried forward in a maturing cisterna are retrieved to an earlier compartment in COPI-coated vesicles.

When a newly formed cisterna moves to a medial position, for example, "leftover" cis golgi enzymes would be extracted and transported retrogradely to a new cis cisterna behind.

Likewise, the medial enzymes would be received by retrograde transport from the cisternae just ahead.

In this way, a cis cisterna would mature to a medial and then trans cisterna as it moves outward.

(B) VESICLE TRANSPORT MODEL

Vesicle Transport Model

Cisternae are static compartments, which contain a characteristic set of resident proteins and enzymes.

The passing of molecules from cis to trans through the golgi is accomplished by forward- moving transport vesicles, which bud from one cisterna and fuse with the next in a cis-to-trans direction.

How do proteins sort between the ER and the Golgi apparatus?
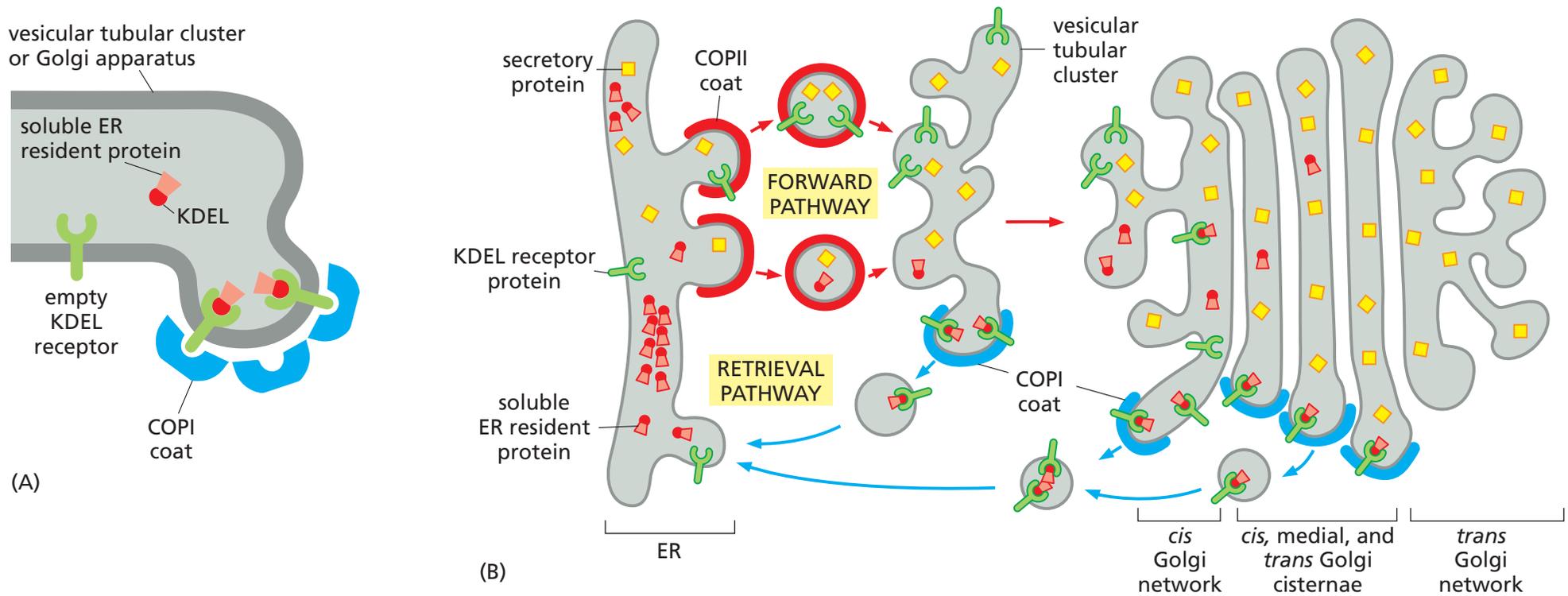
Retrieval of soluble ER resident proteins

ER resident proteins that escape from the ER are returned by vesicle transport

The KDEL receptor present in both vesicular tubular clusters and the golgi apparatus captures the soluble ER resident proteins and carries them in COPI-coated transport vesicles back to the ER

Golgi apparatus functions in glycosylation

ER

SORTING
- phosphorylation of oligosaccharides on lysosomal proteins — *cis* Golgi network
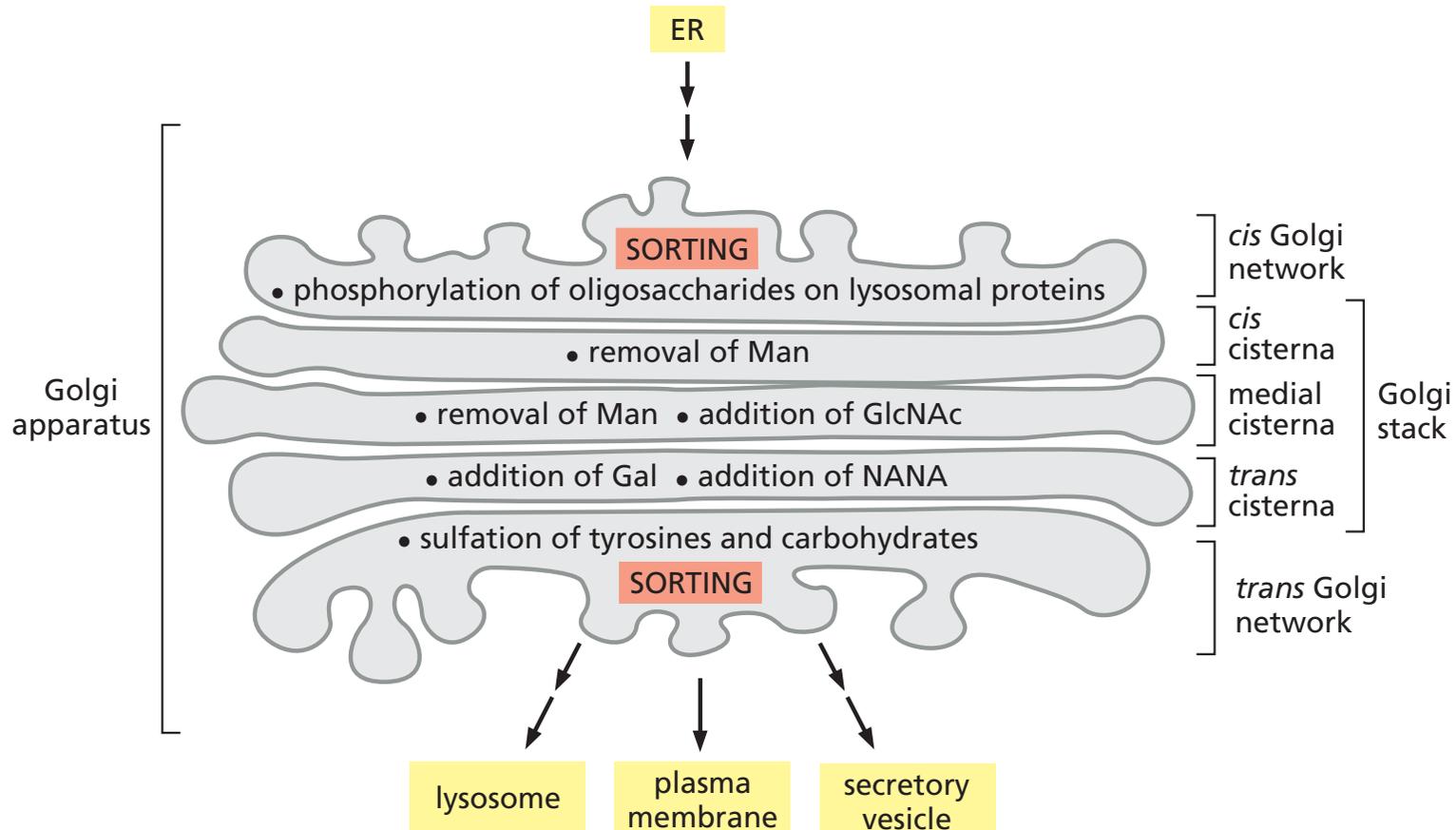
- removal of Man — *cis* cisterna

- removal of Man • addition of GlcNAc — medial cisterna

- addition of Gal • addition of NANA — *trans* cisterna

- sulfation of tyrosines and carbohydrates
SORTING — *trans* Golgi network

Golgi apparatus

Golgi stack

lysosome

plasma membrane

secretory vesicle

The localization of each processing step shown was determined by a combination of techniques, including biochemical subfractionation of the golgi apparatus membranes and electron microscopy after staining with antibodies specific for some of the processing enzymes. processing enzymes are not restricted to a particular cisterna; instead, their distribution is graded across the stack, such that early-acting enzymes are present mostly in the cis golgi cisternae and later-acting enzymes are mostly in the trans golgi cisternae.
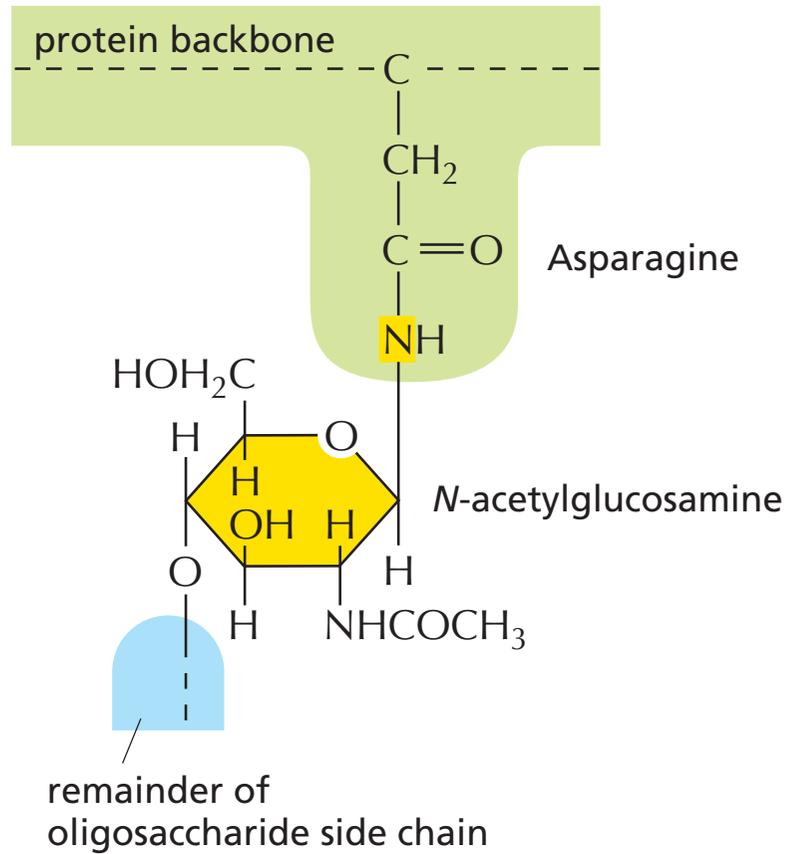
Man - Mannose
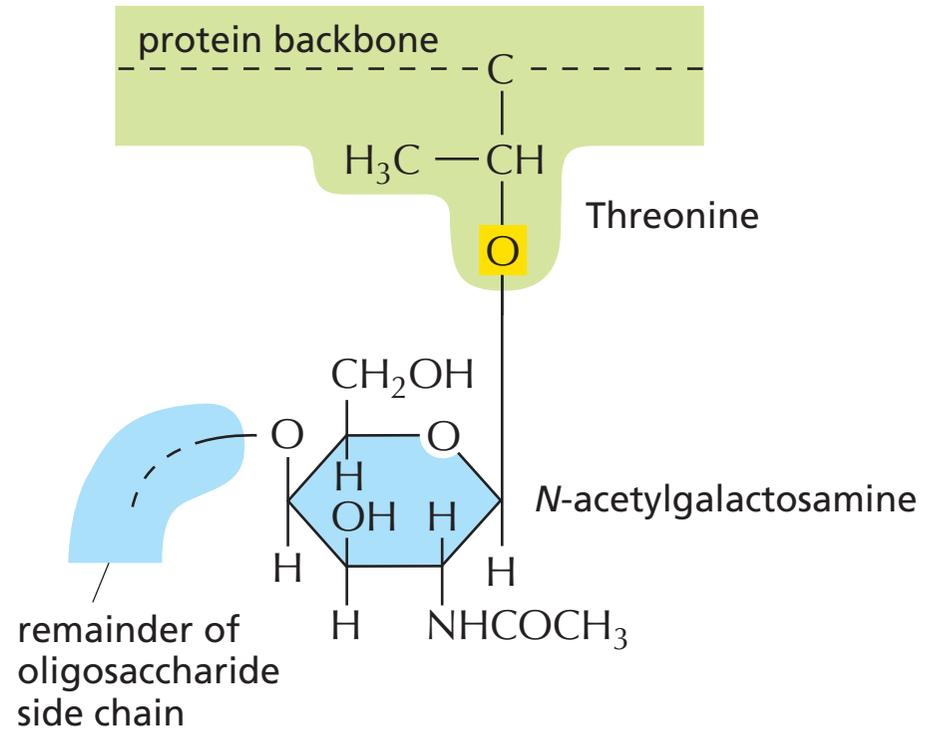
GlcNac - N-Acetylglucosamine

Gal - Galactose

NANA - N-Acetylneuraminic acid (sialic acid)

Two types of glycosylation

N-LINKED GLYCOSYLATION

protein backbone

C

CH₂

C=O   Asparagine

NH

HOH₂C

H   O

H

OH   H   N-acetylglucosamine

O

H   H   NHCOCH₃

remainder of oligosaccharide side chain

O-LINKED GLYCOSYLATION

protein backbone

C

H₃C—CH

O   Threonine

CH₂OH

O   O

H

OH   H   N-acetylgalactosamine

remainder of oligosaccharide side chain

H   H   NHCOCH₃
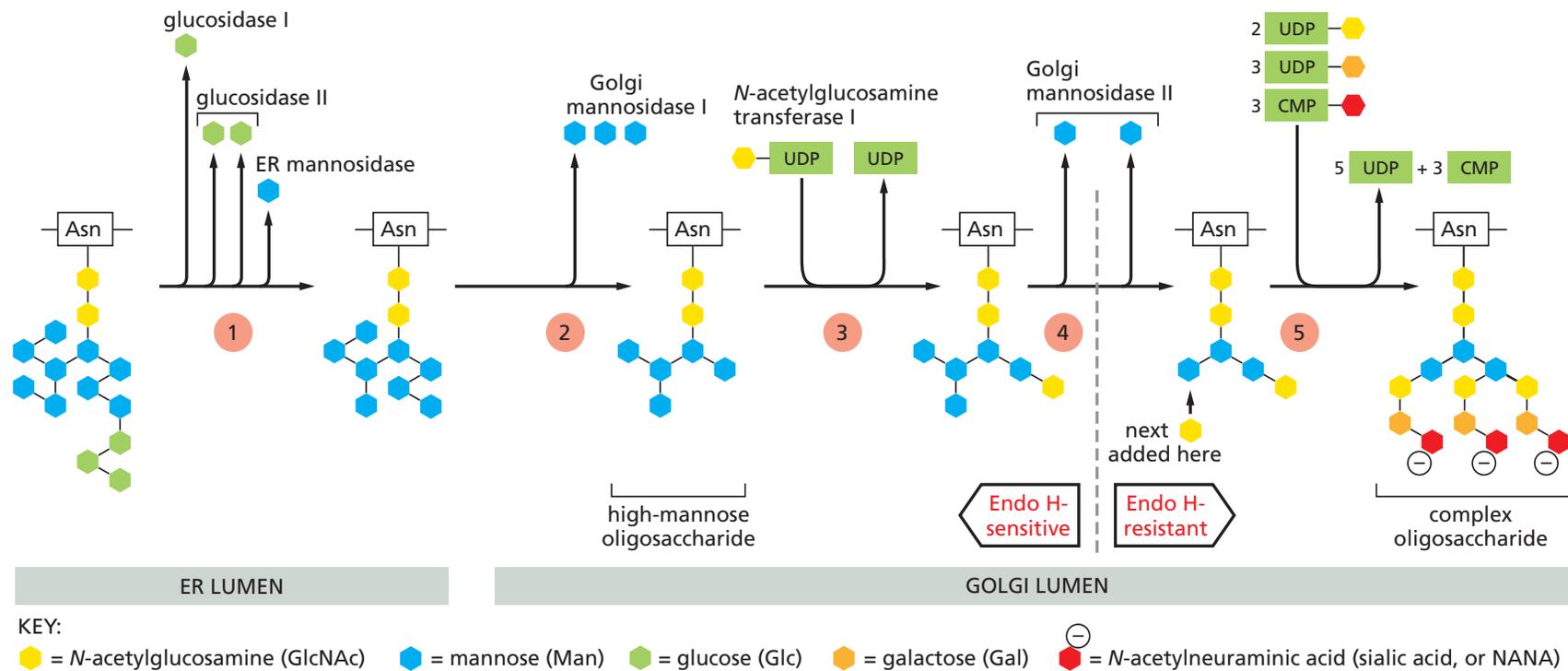
Oligosaccharide processing in the ER and the Golgi apparatus.

The processing pathway is highly ordered, so that each step shown depends on the previous one.

Step 1: processing begins in the ER with the removal of the glucose residues from the oligosaccharide initially transferred to the protein. Then a mannosidase in the ER membrane removes a specific mannose. The remaining steps occur in the Golgi stack.

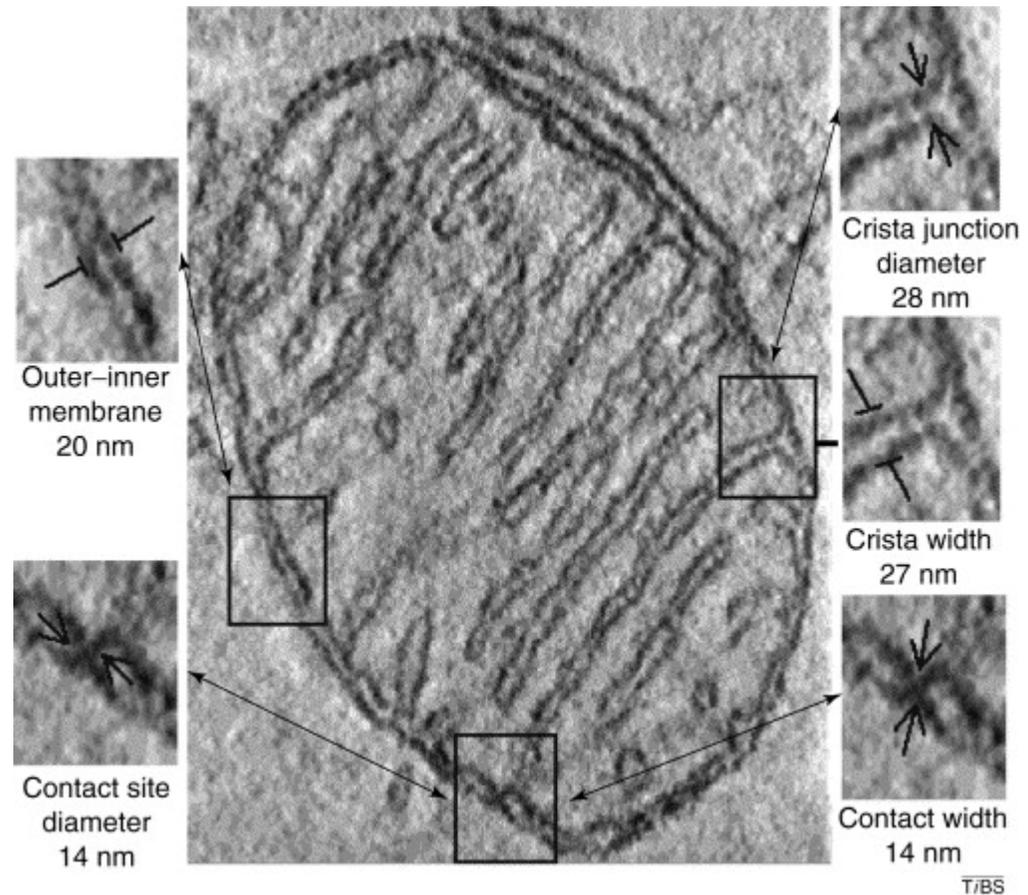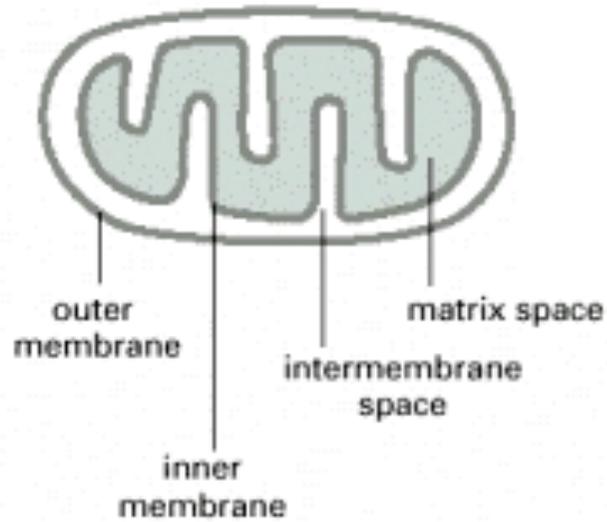Step 2: Golgi mannosidase I removes three more mannose residues.

Step 3: N-Acetylglucosamine transferase I then adds an N-Acetylglucosamine.

Step 4: Mannosidase II then removes two additional mannose residues. This yields the final core of three mannose residues that is present in a complex oligosaccharide.

Step 5: Additional N-Acetylglucosamines, galactoses, and sialic acids are added. These final steps in the synthesis of a complex oligosaccharide occur in the cisternal compartments of the Golgi apparatus. Three types of glycosyl transferase enzymes act sequentially, using sugar substrates that have been activated by linkage to the indicated nucleotide; the membranes of the Golgi cisternae contain specific carrier proteins that allow each sugar nucleotide to enter in exchange for the nucleoside phosphates that are released after the sugar is attached to the protein on the lumenal face.
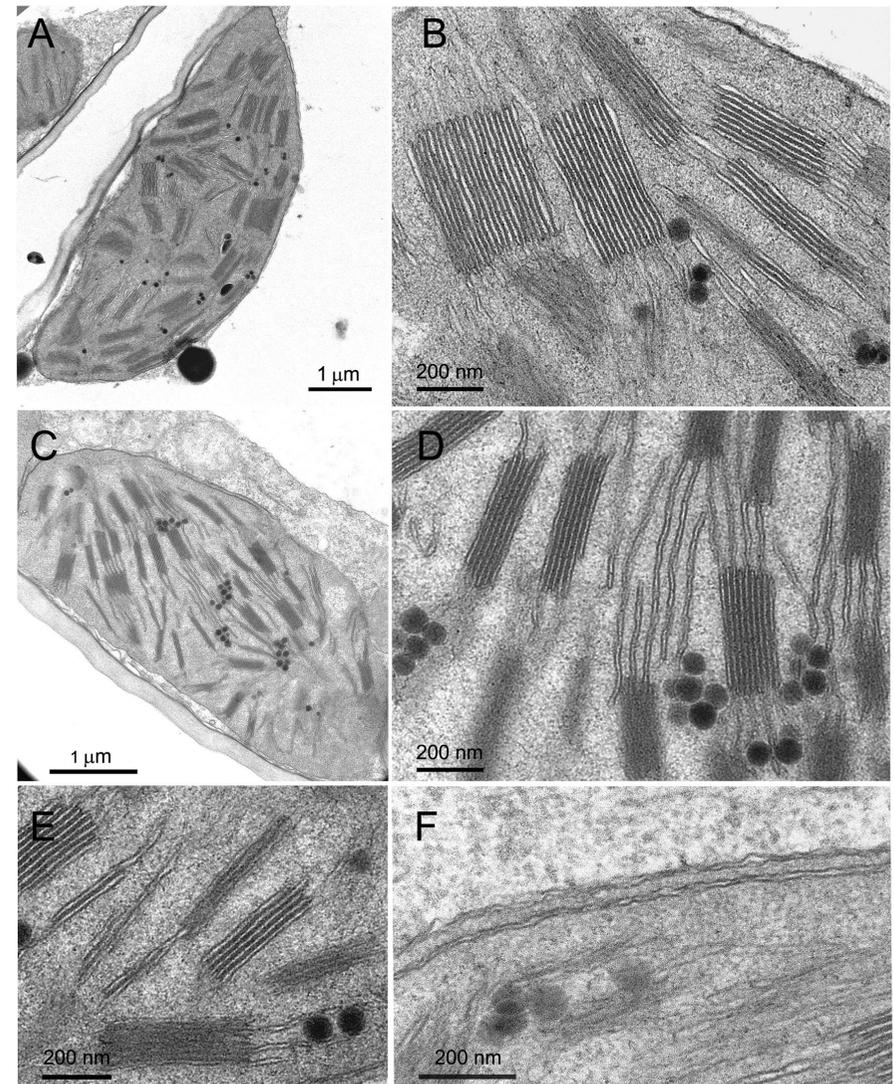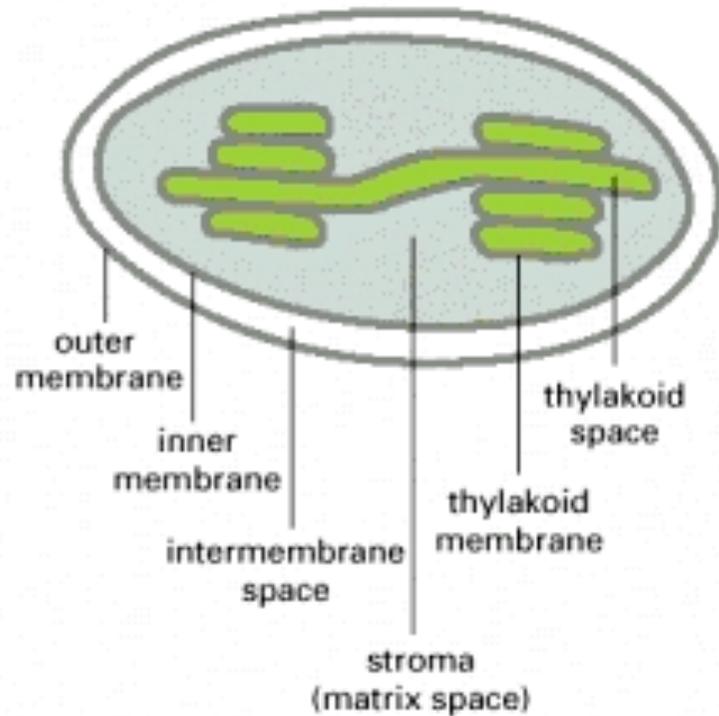
Mitochondria and Chloroplast

(A) MITOCHONDRION

outer membrane

inner membrane

intermembrane space

matrix space

Outer–inner membrane 20 nm

Contact site diameter 14 nm

Crista junction diameter 28 nm

Crista width 27 nm

Contact width 14 nm

• 2 compartments enclosed within 2 membranes

Chloroplast

(B) CHLOROPLAST

- outer membrane
- inner membrane
- intermembrane space
- stroma (matrix space)
- thylakoid membrane
- thylakoid space

- 3 compartments enclosed within 3 membranes

ORIGINAL ARTICLE

Thylakoid ultrastructural variations in chlorophyll-deficient wheat: aberrations or structural acclimation?

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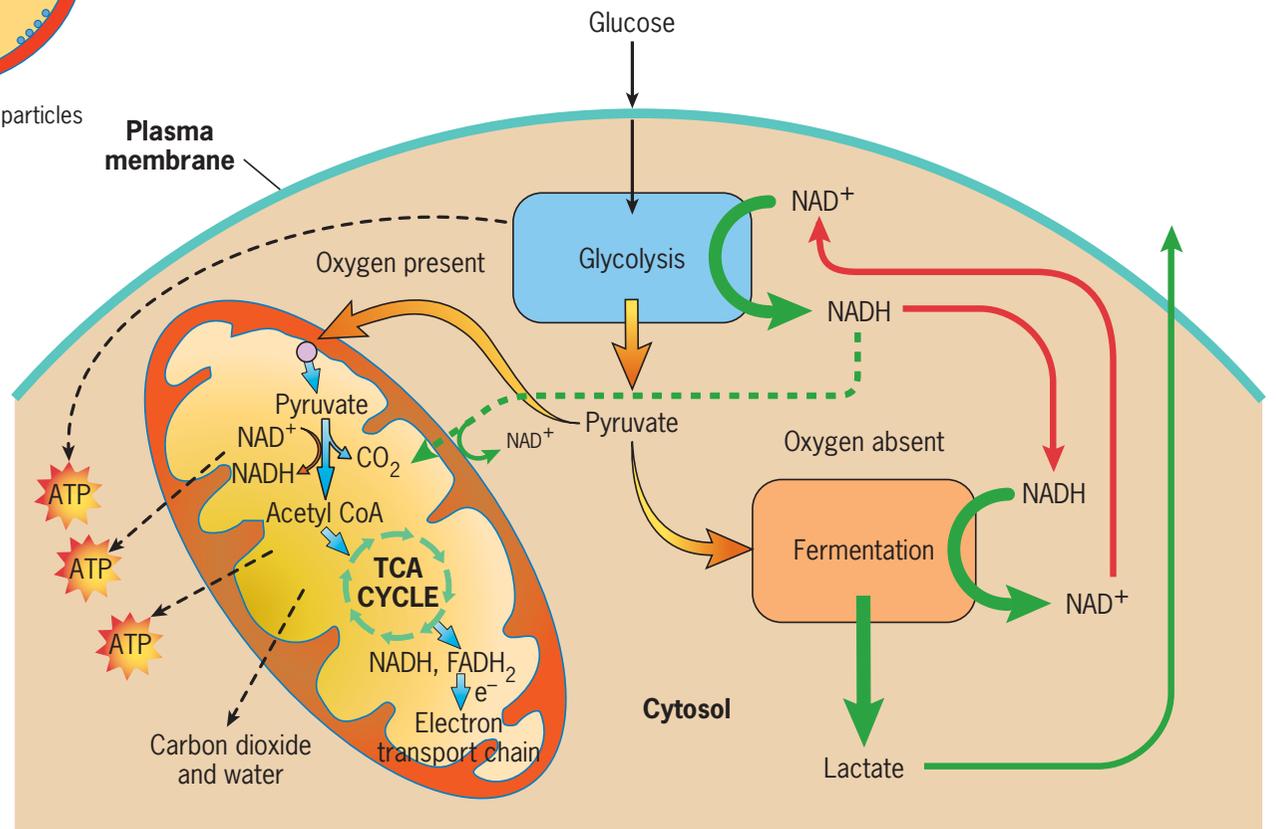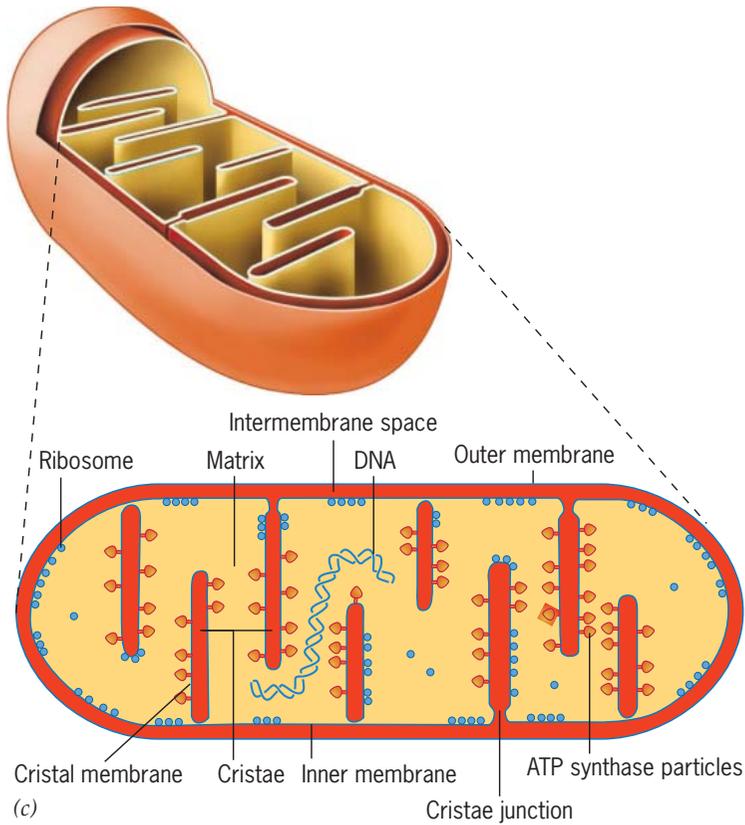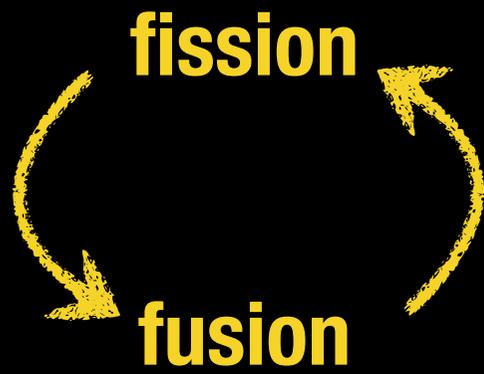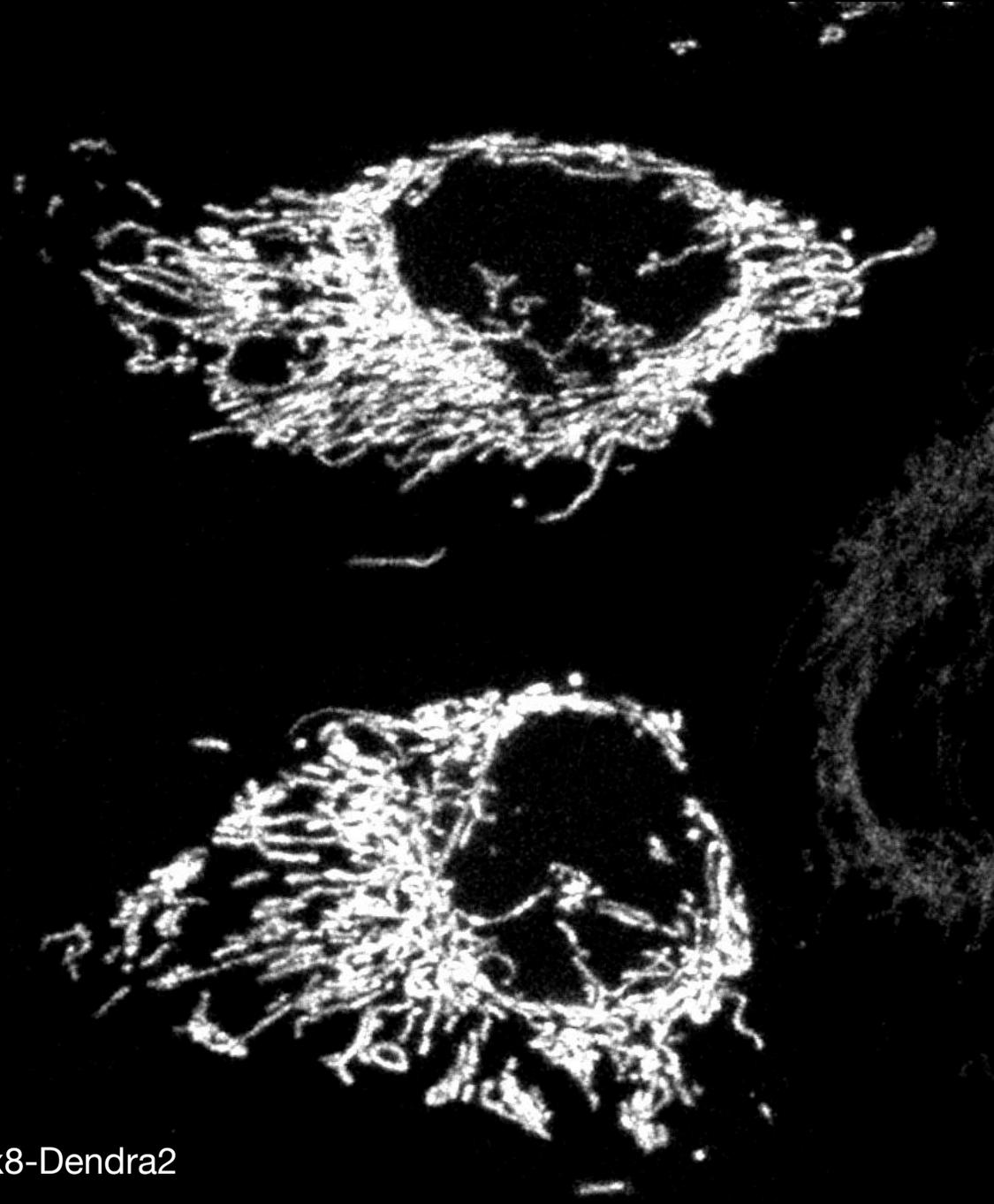
Unique attributes of the mitochondria
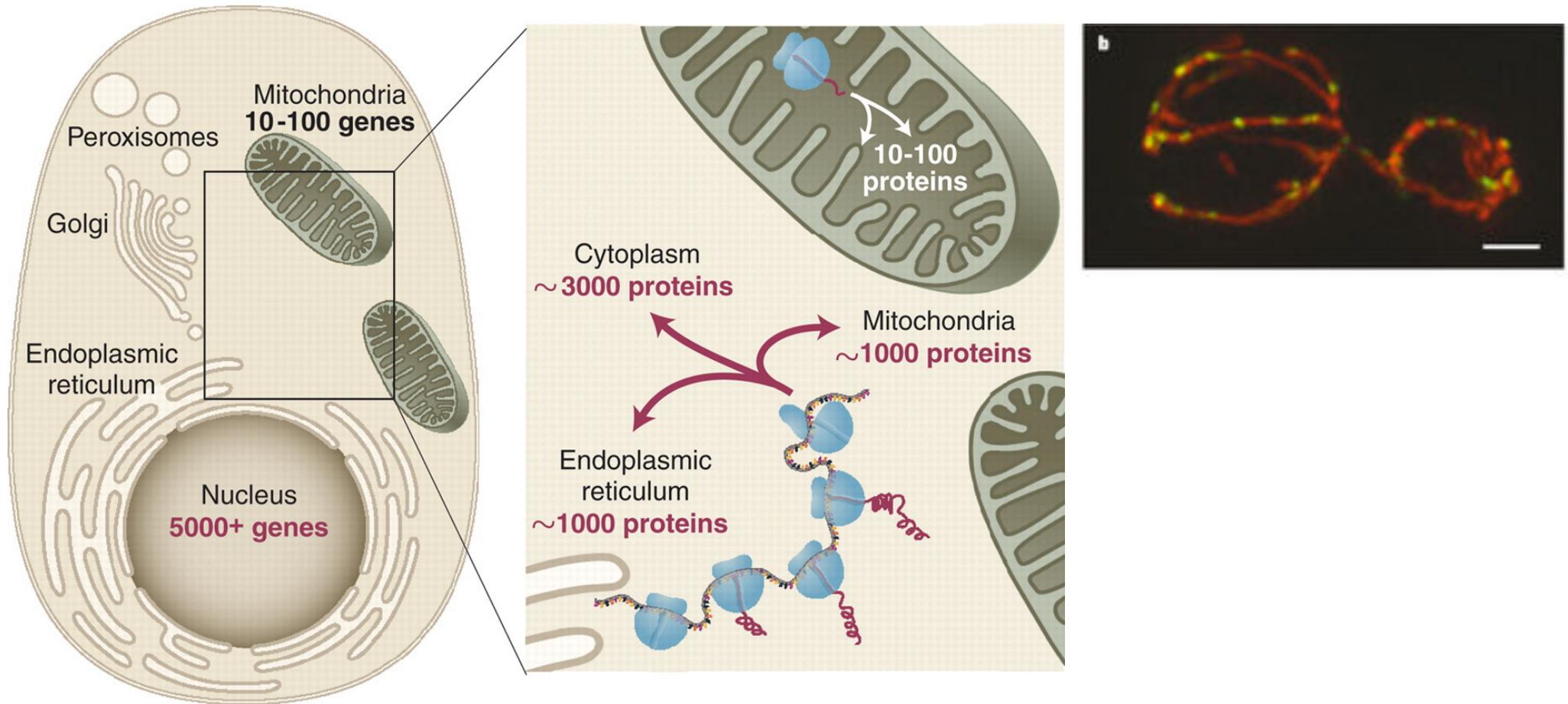
- Arose ~2 billion years ago from the engulfment of an α-proteobacterium by an Asgard archaea
- Maintained core functions of ATP production
- Contains a 16 kilobase, circular genome, which is present in cells in a vast excess of copies relative to nuclear chromosomes
- Nucleus-encoded proteins make up most of the mitochondrial proteome
- Translated on cytosolic ribosomes and actively imported and sorted into mitochondrial sub-compartments by outer and inner membrane translocase machines

Pavel et al. (2006) Science
Friedman and Nunnari (2014) Nature

Ribosome Matrix   Intermembrane space   DNA   Outer membrane

Cristal membrane Cristae Inner membrane ATP synthase particles

Cristae junction

(c)

Glucose

Plasma membrane

Oxygen present

Glycolysis

NAD$^+$

NADH

Pyruvate

NAD$^+$

CO$_2$

NADH

NAD$^+$

Pyruvate

Oxygen absent

Acetyl CoA

NADH

ATP

TCA CYCLE

Fermentation

NAD$^+$

ATP

ATP

NADH, FADH$_2$

e$^-$

Electron transport chain

Cytosol

Carbon dioxide and water

Lactate

Mitochondrial form and dynamics
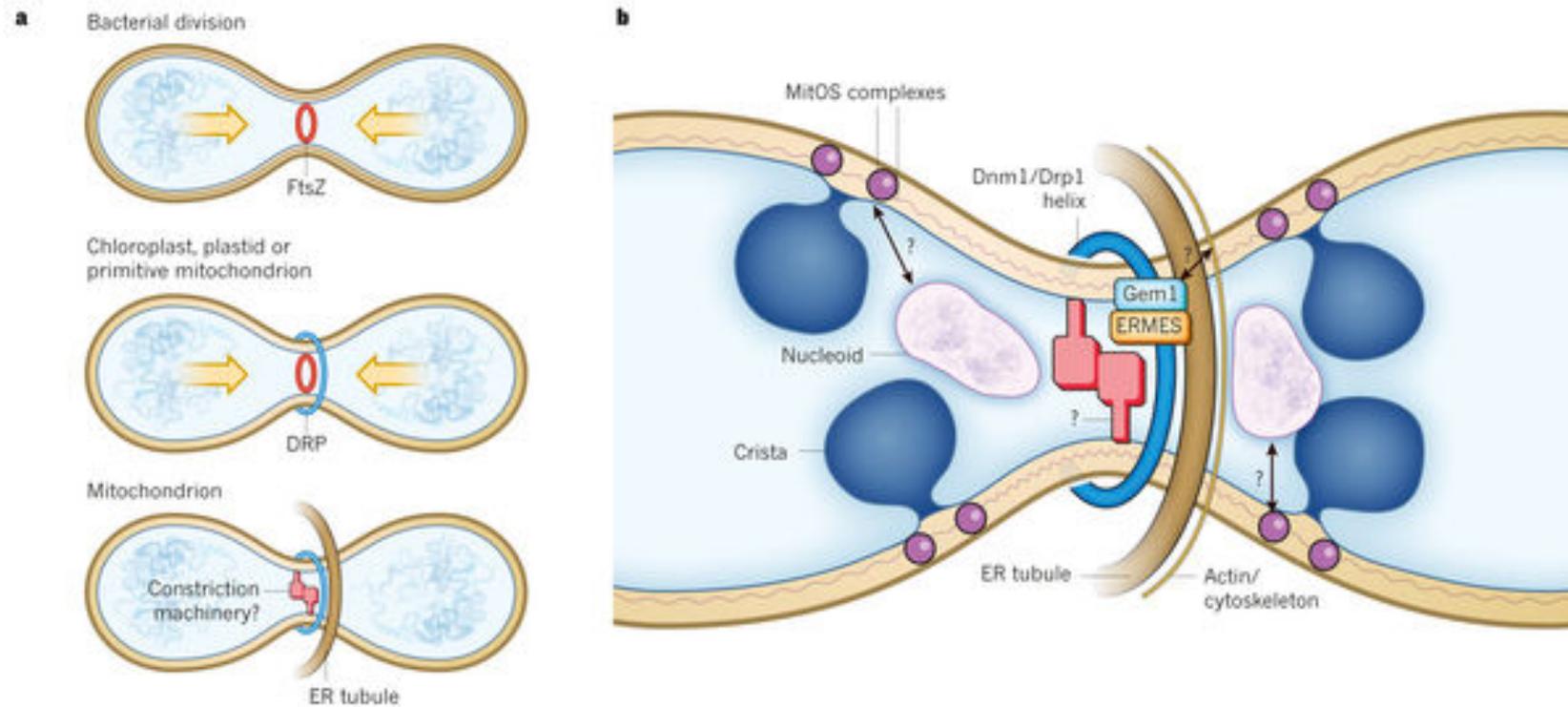

fission

fusion

Mitochondrial labeled with Cox8-Dendra2
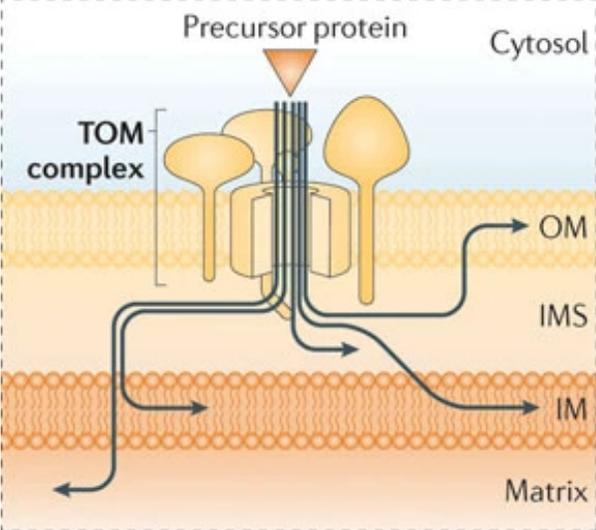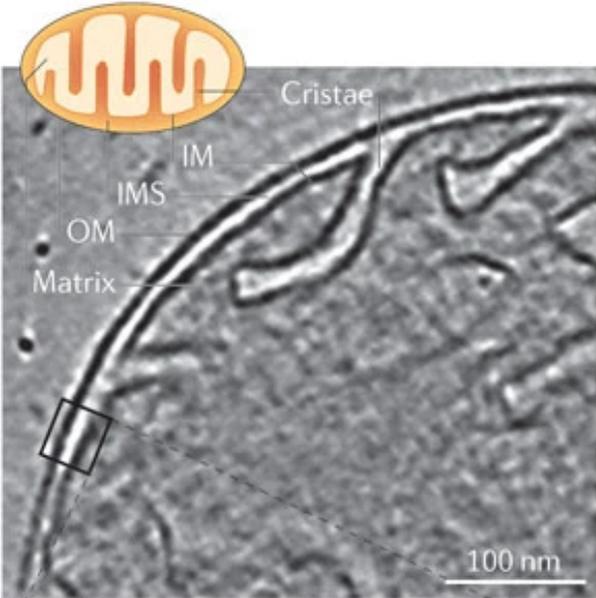
Mitochondrial dynamics
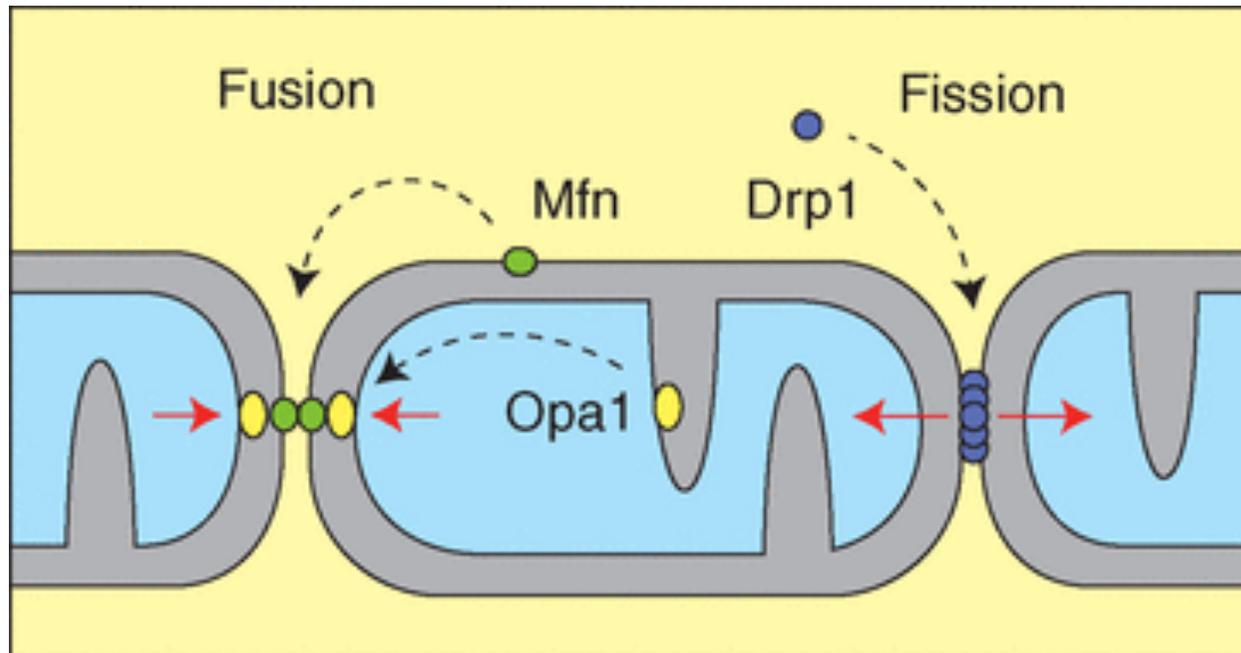
Mitofusins mediate mitochondrial outer membrane fusion in mammals
Opa1 mediates mitochondrial inner membrane fusion
Drp1 mediates mitochondrial fission

van der Bliek et al. 2013
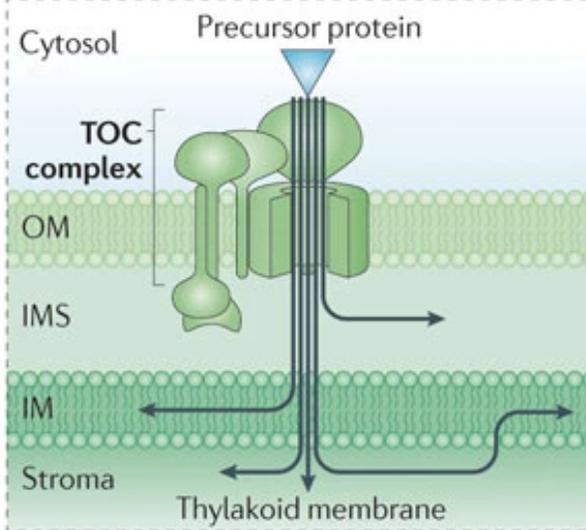
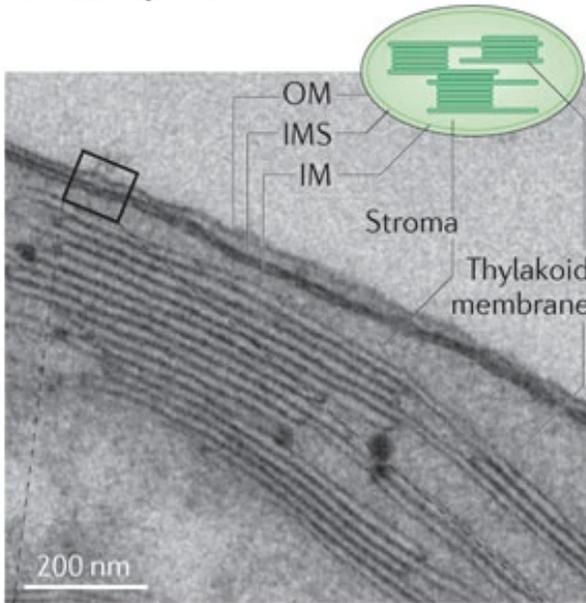# Mitochondrial dynamics

- Algal mitochondria and chloroplasts utilize FtsZ ring for constriction and division of the inner compartment

- FtsZ-dependent placement (indicated by arrows) and division mechanisms on the inside of the organelle have been retained during evolution

- Mammals don't have the FtsZ ring and rely entirely on dynamin-related proteins for division

Friedman & Nunnari (2014) Nature

a Mitochondria

Cristae
IM
IMS
OM
Matrix

100 nm

Precursor protein
Cytosol
TOM complex
OM
IMS
IM
Matrix

b Chloroplasts

OM
IMS
IM
Stroma
Thylakoid membrane

200 nm

Precursor protein
Cytosol
TOC complex
OM
IMS
IM
Stroma
Thylakoid membrane

Nature Reviews | **Molecular Cell Biology**

Common ground for protein translocation: access control for mitochondria and chloroplasts
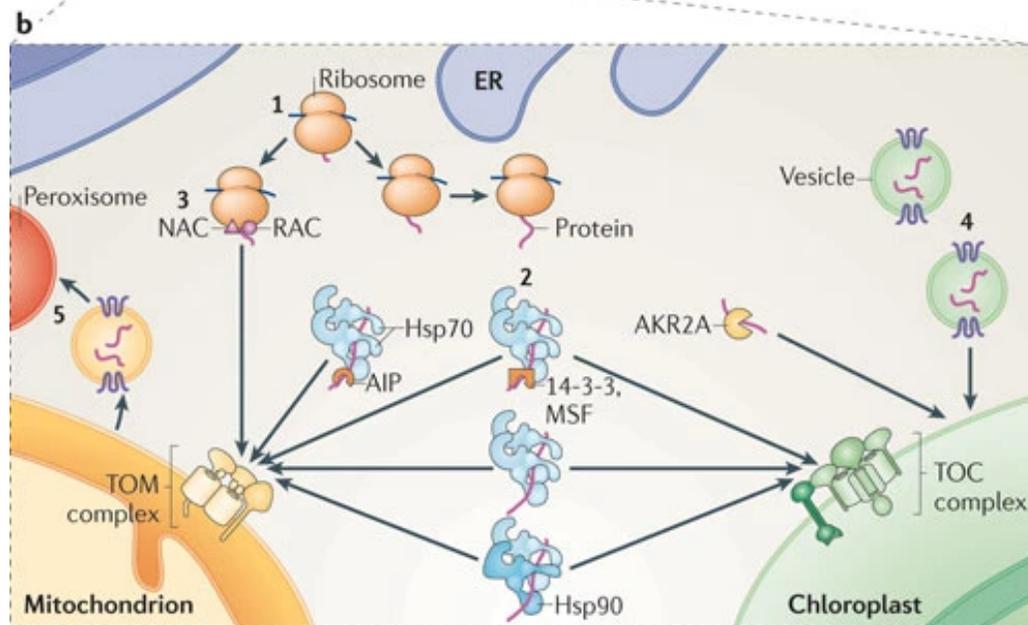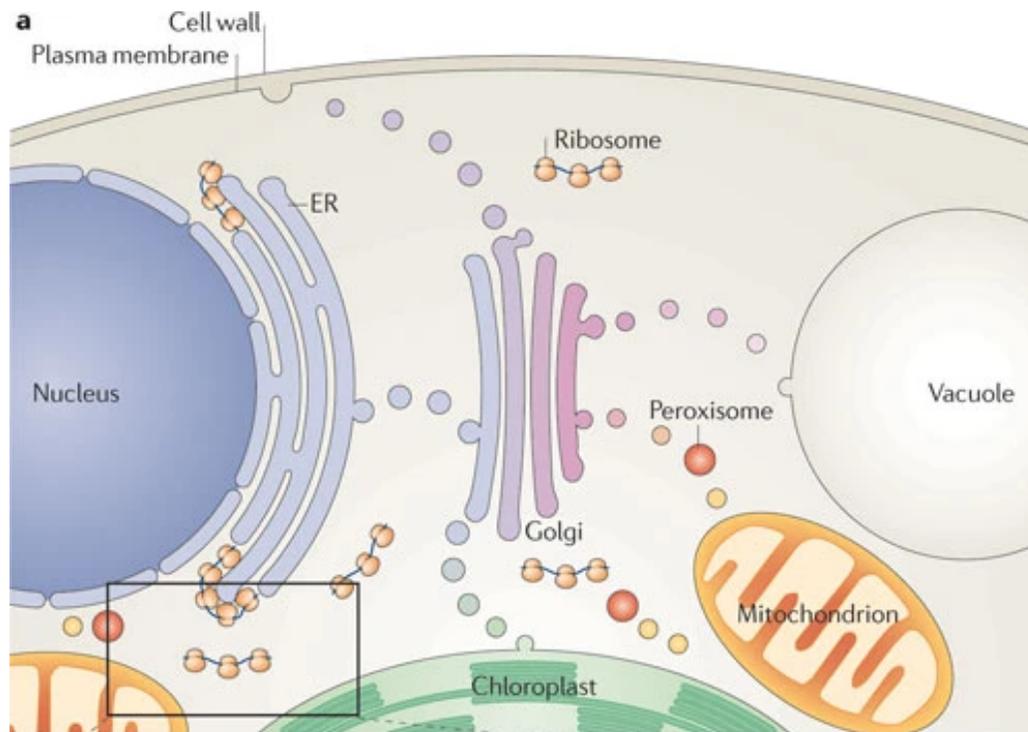
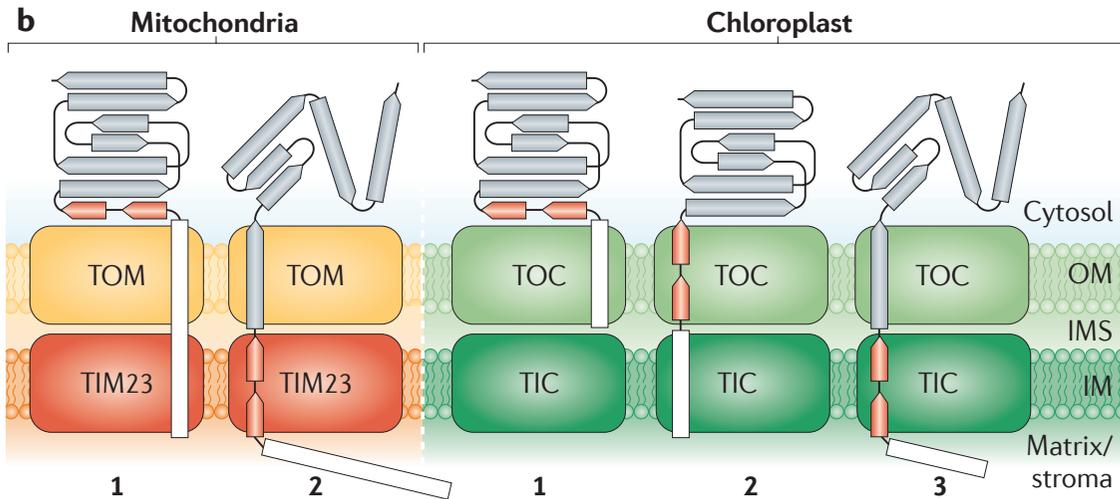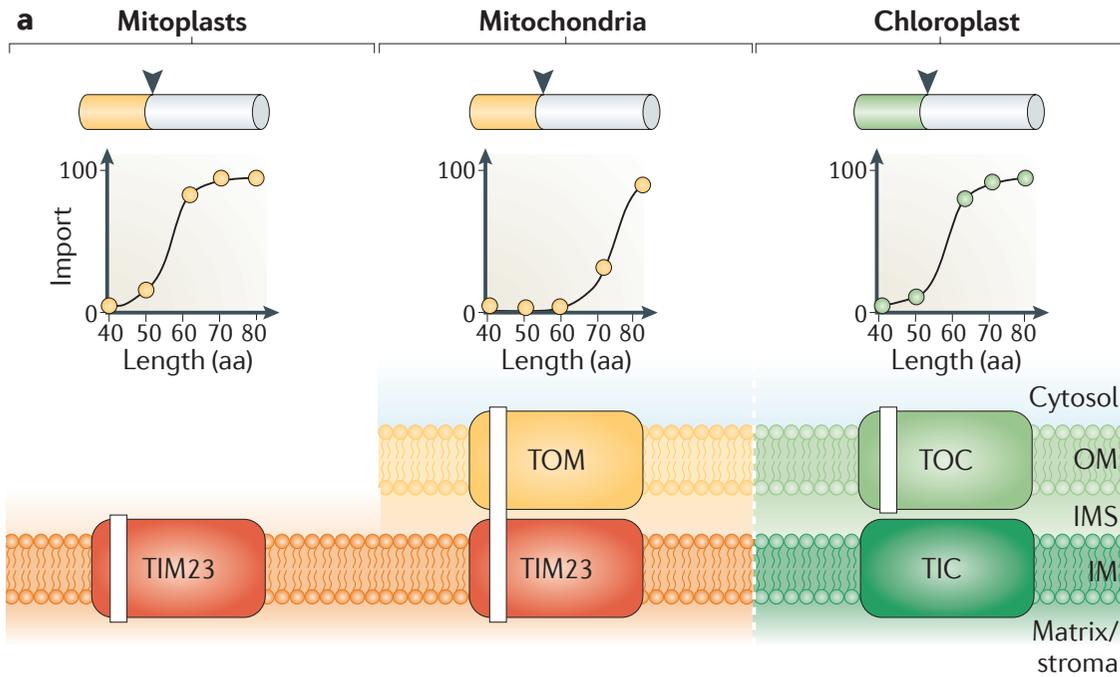*Enrico Schleiff* and Thomas Becker*

Nature Reviews | Molecular Cell Biology

Common ground for protein translocation: access control for mitochondria and chloroplasts

Enrico Schleiff* and Thomas Becker‡

Table 1 | **The import signals of mitochondrial and chloroplast proteins**

Mitochondria	Chloroplasts	Signal	Destination
Cleavable signal sequences			
		Classic presequence	Mitochondrial matrix or chloroplast stroma
		Presequence and non-cleavable hydrophobic sorting signal	Mitochondrial or chloroplast inner membrane
		Presequence and cleavable hydrophobic sorting signal	Mitochondrial intermembrane space or chloroplast thylakoid lumen
Non-cleavable signal sequences			
		Outer-membrane protein (OMP) with signal anchor	Mitochondrial or chloroplast outer membrane
		OMP with internal anchor	Mitochondrial or chloroplast outer membrane
		OMP with carboxy-terminal anchor	Mitochondrial or chloroplast outer membrane
		OMP with multiple anchors	Mitochondrial or chloroplast outer membrane
	?	β-barrel OMP with β-signal	Mitochondrial or chloroplast outer membrane
		Inner membrane carrier with multiple signals	Mitochondrial inner membrane
		Inner membrane protein with internal presequence-like signal	Mitochondrial inner membrane
CCCC		Intermembrane-space protein with Cys-rich motif	Mitochondrial inner membrane
	?	Signal for intermembrane-space targeting	Chloroplast intermembrane space

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Common ground for protein
translocation: access control for
mitochondria and chloroplasts

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