Cell Biology (BI2213)

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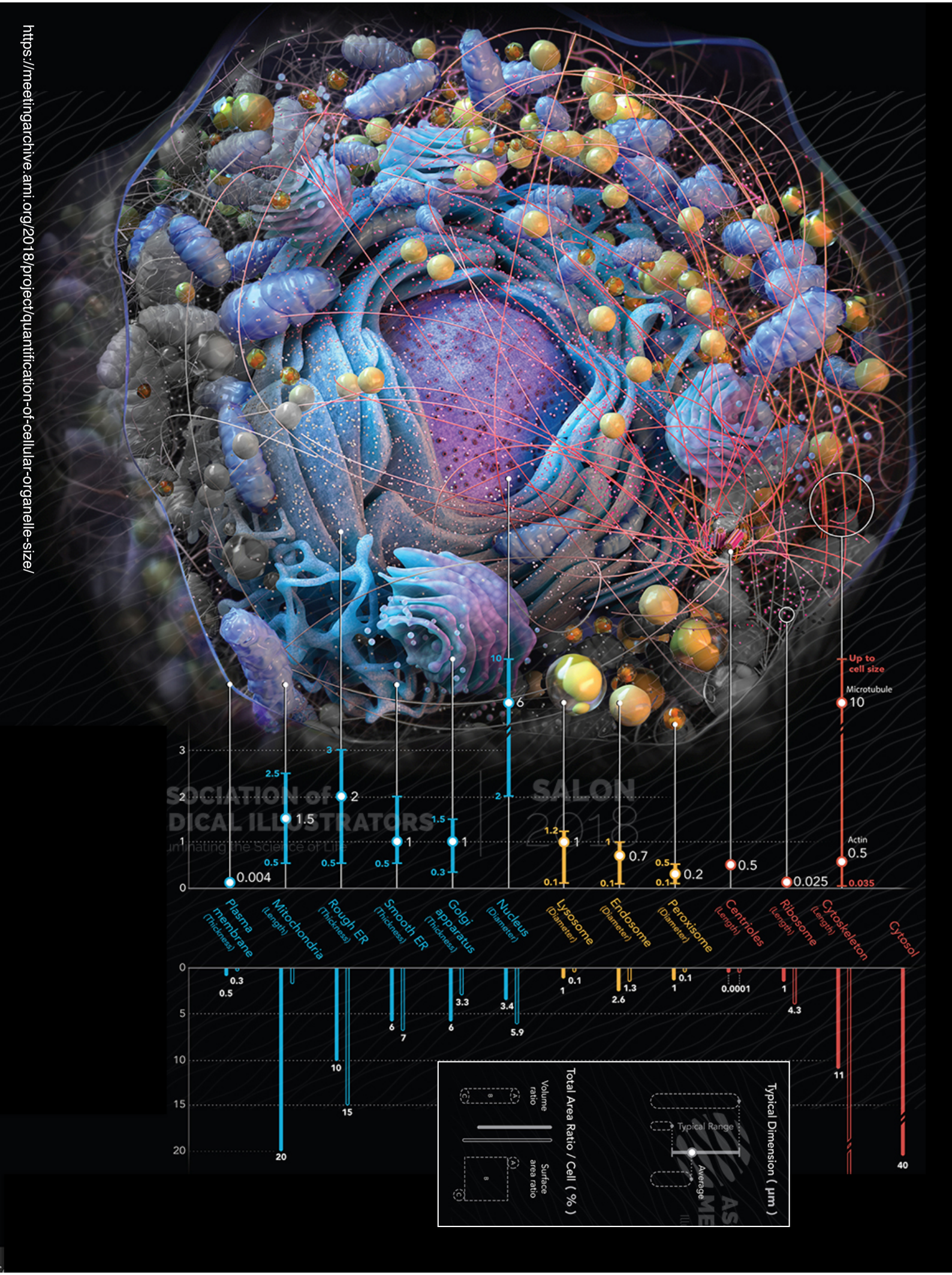
www.pucadyillab.com Lecture notes, reading materials

Intracellular compartmentalisation into organelles

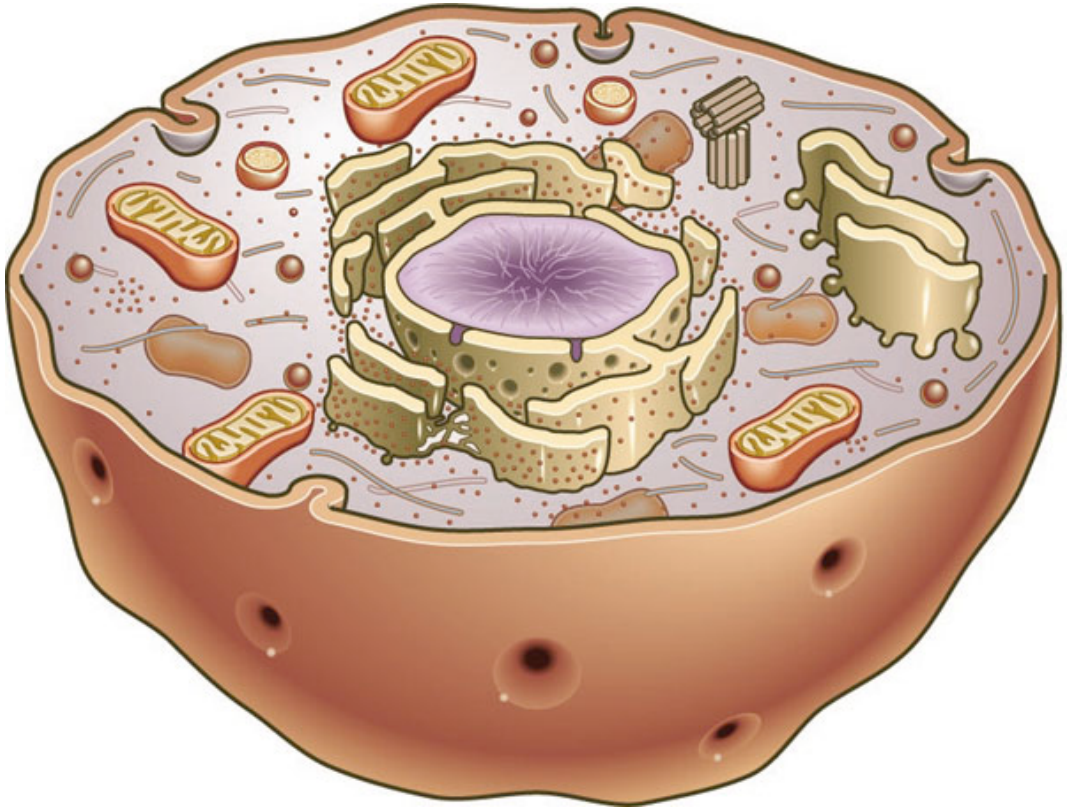
Organelle Structure

How they are formed?

Organelle function in protein sorting

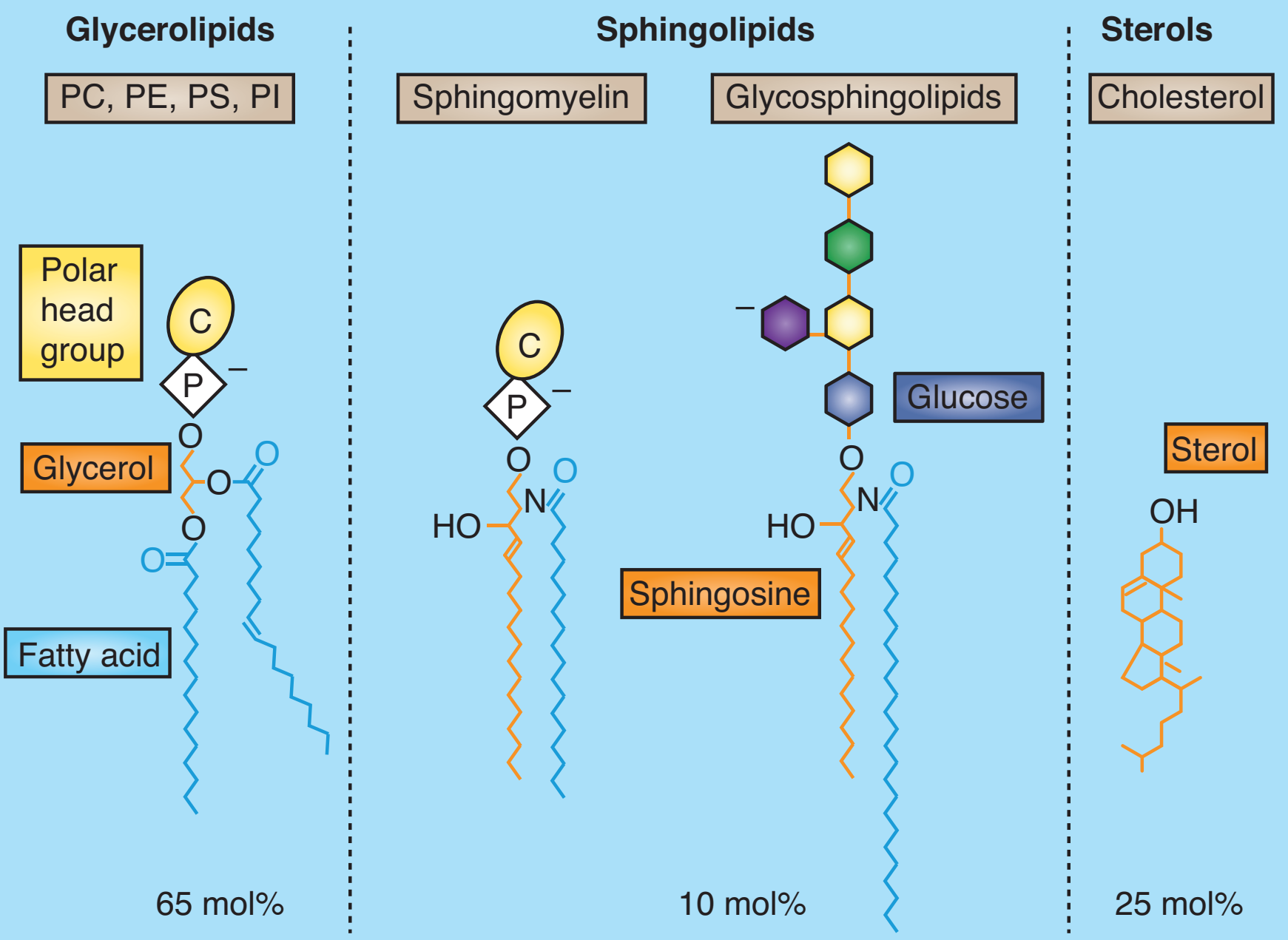

https://meetingarchive.ami.org/2018/project/quantification-of-cellular-organelle-size/
SOCIATION of
DICAL ILLUSTRATORS
SALON
2018
Up to cell size
Microtubule
Actin
Plasma membrane (Thickness)
Mitochondria (Length)
Rough ER (Thickness)
Smooth ER (Thickness)
Golgi apparatus (Thickness)
Nucleus (Diameter)
Lysosome (Diameter)
Endosome (Diameter)
Peroxisome (Diameter)
Centrioles (Length)
Ribosome (Length)
Cytoskeleton (Length)
Cytosol
Total Area Ratio / Cell (%)
Volume ratio
Surface area ratio
Typical Dimension (µm)
Typical Range
Average

Most organelles are membrane-bound structures that compartmentalise the cytoplasm



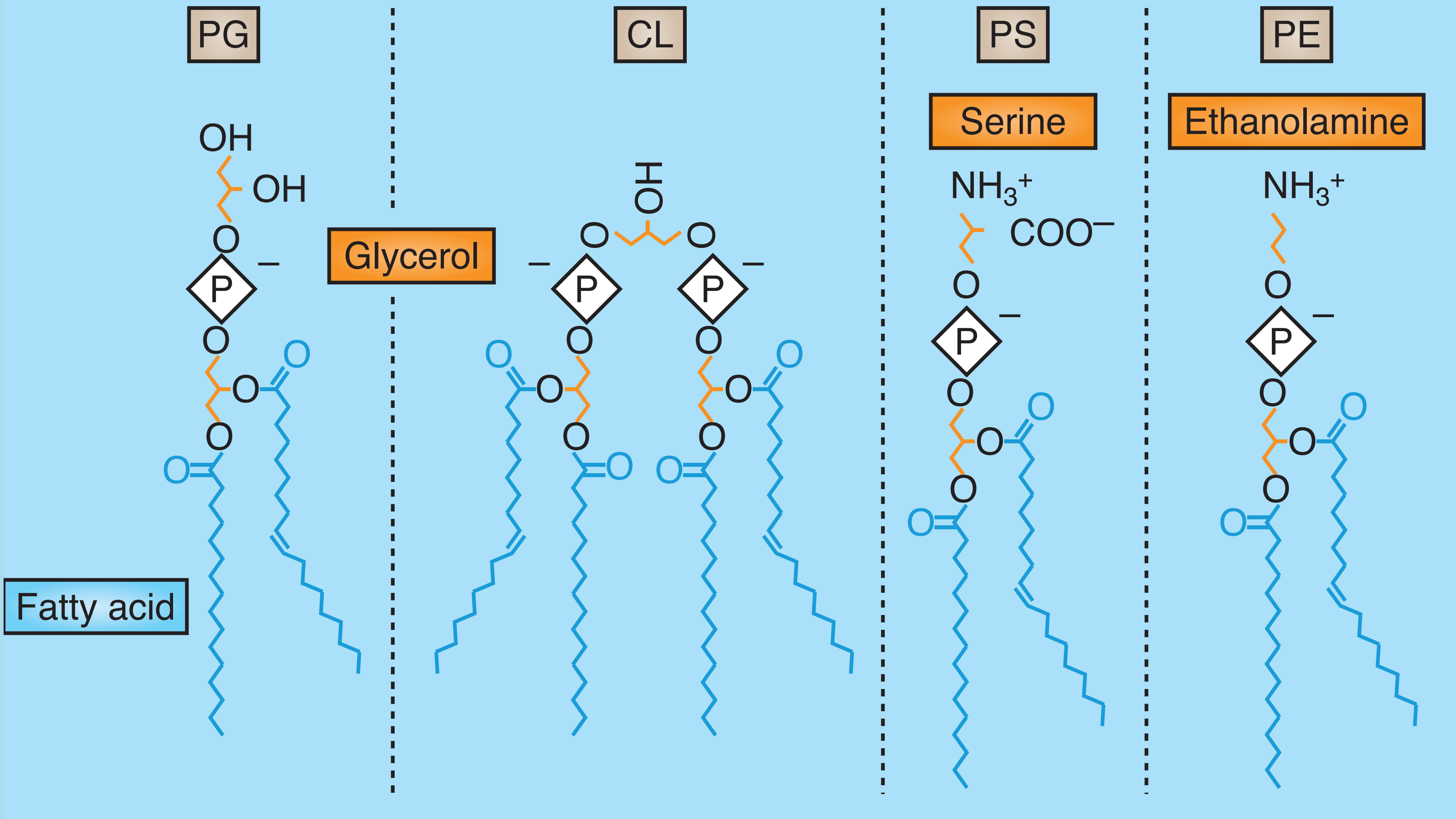
- Very thin, quasi two-dimensional film of lipids and proteins
- Held together by non-covalent interactions
- Membranes are fluid and dynamic
- Enzymes that build and modify lipids are genetically encoded but lipid composition of biomembranes is also dependent on the food we consume

Composition of mammalian lipids



Lipid map of the mammalian cell Gerrit van Meer and Anton I. P. M. de Kroon Bijvoet Center and Institute of Biomembranes, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands (g.vanmeer@uu.nl; a.i.p.m.dekroon@uu.nl) Journal of Cell Science 124, 5-8 © 2011. Published by The Company of Biologists Ltd doi:10.1242/jcs.071233

Mitochondrial glycerolipids of bacterial origin



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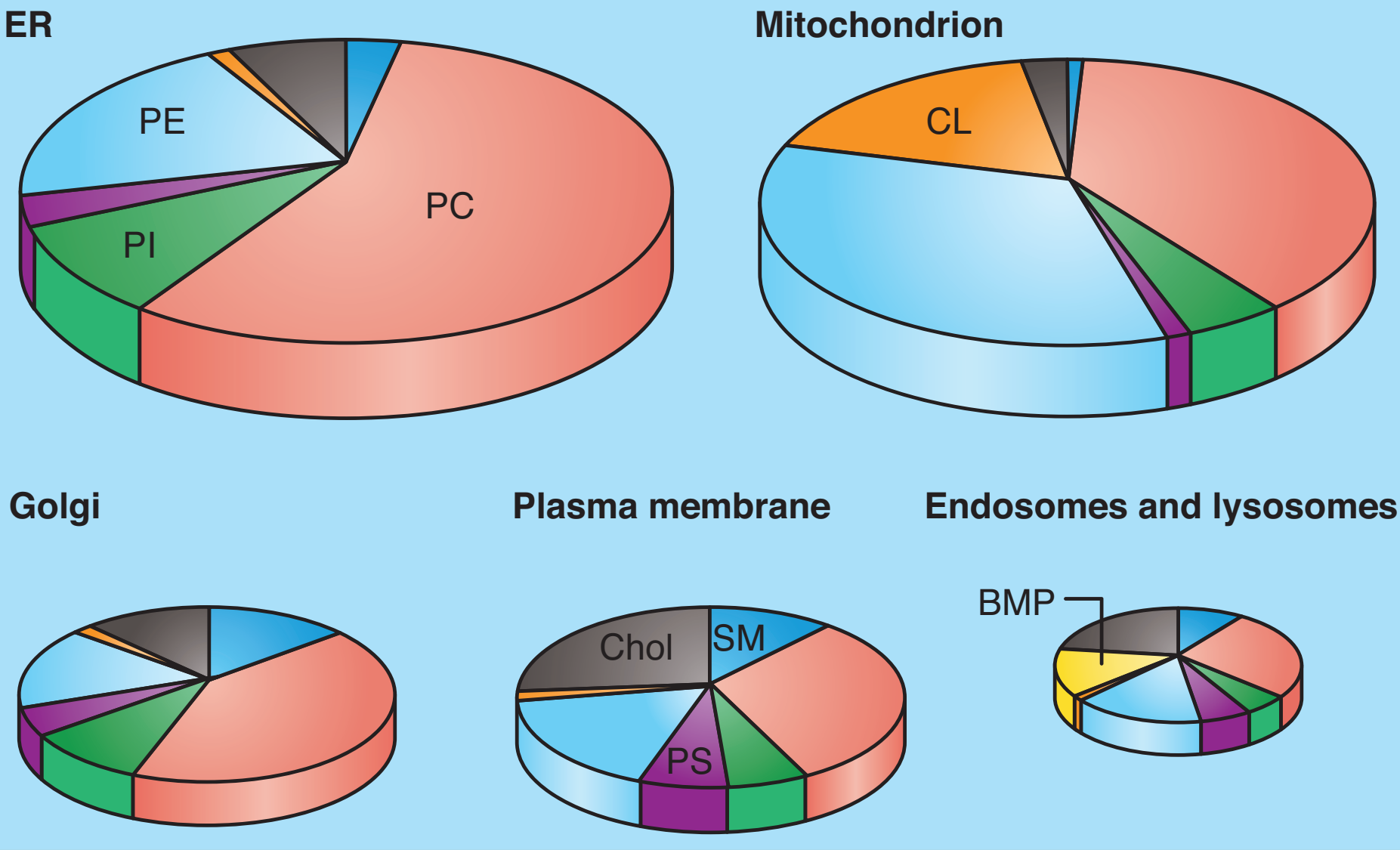
Organelle distribution of phospholipids and cholesterol



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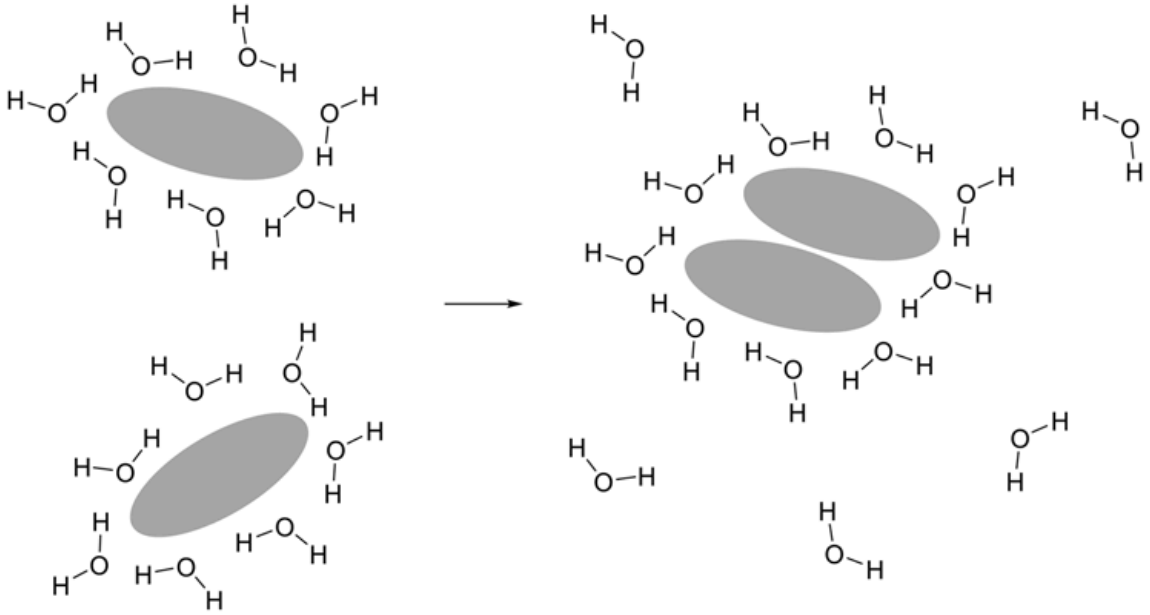
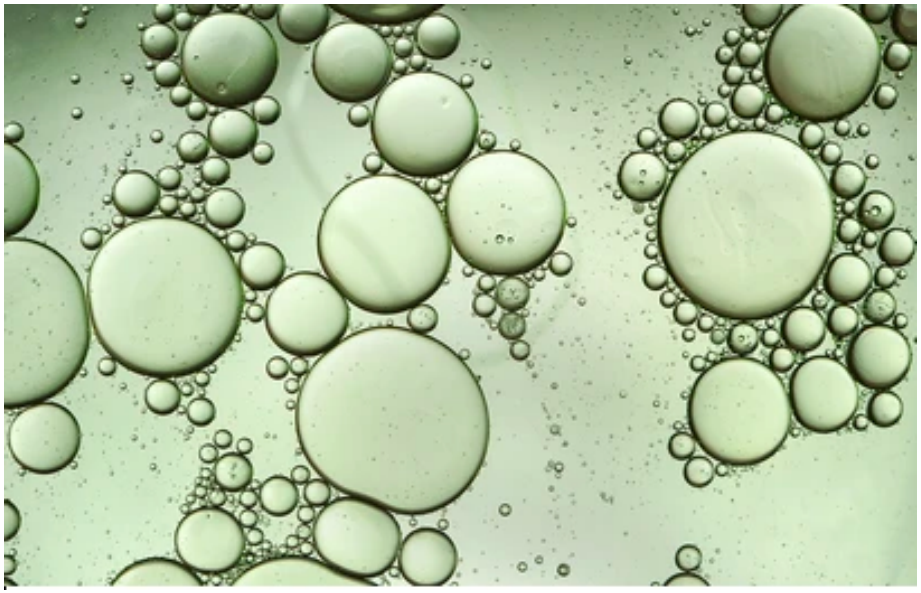
The hydrophobic effect





The hydrophobic effect

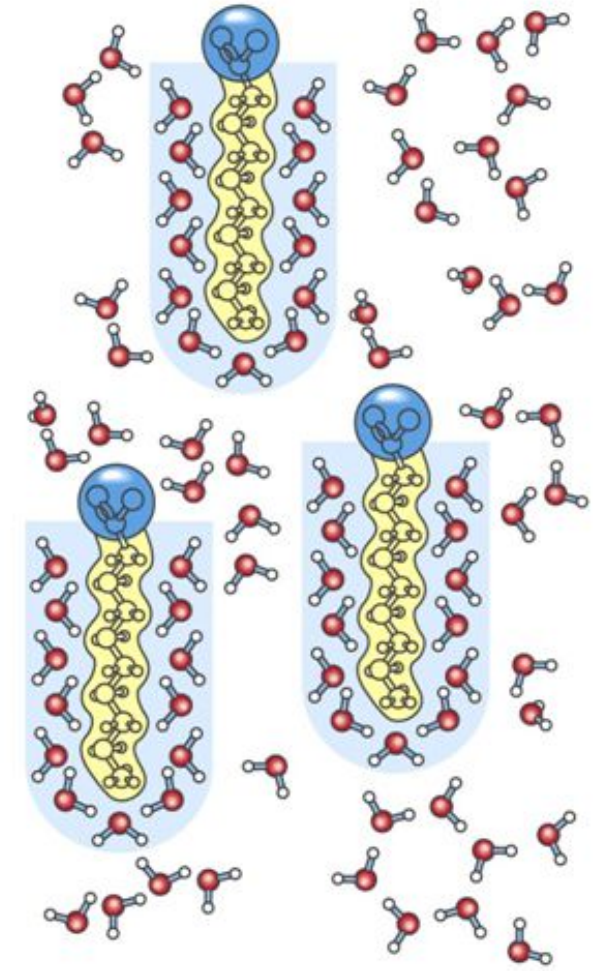


Figure 2-7b part 1
Lehninger Principles of Biochemistry, Fifth Edition

- 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

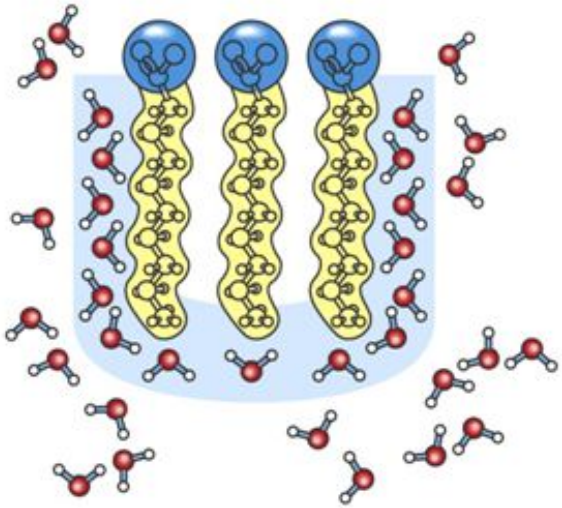
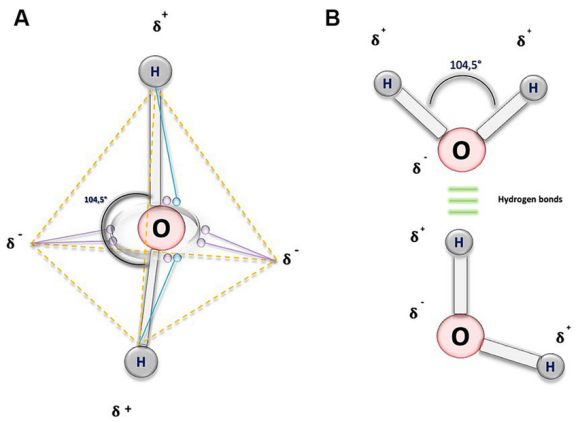
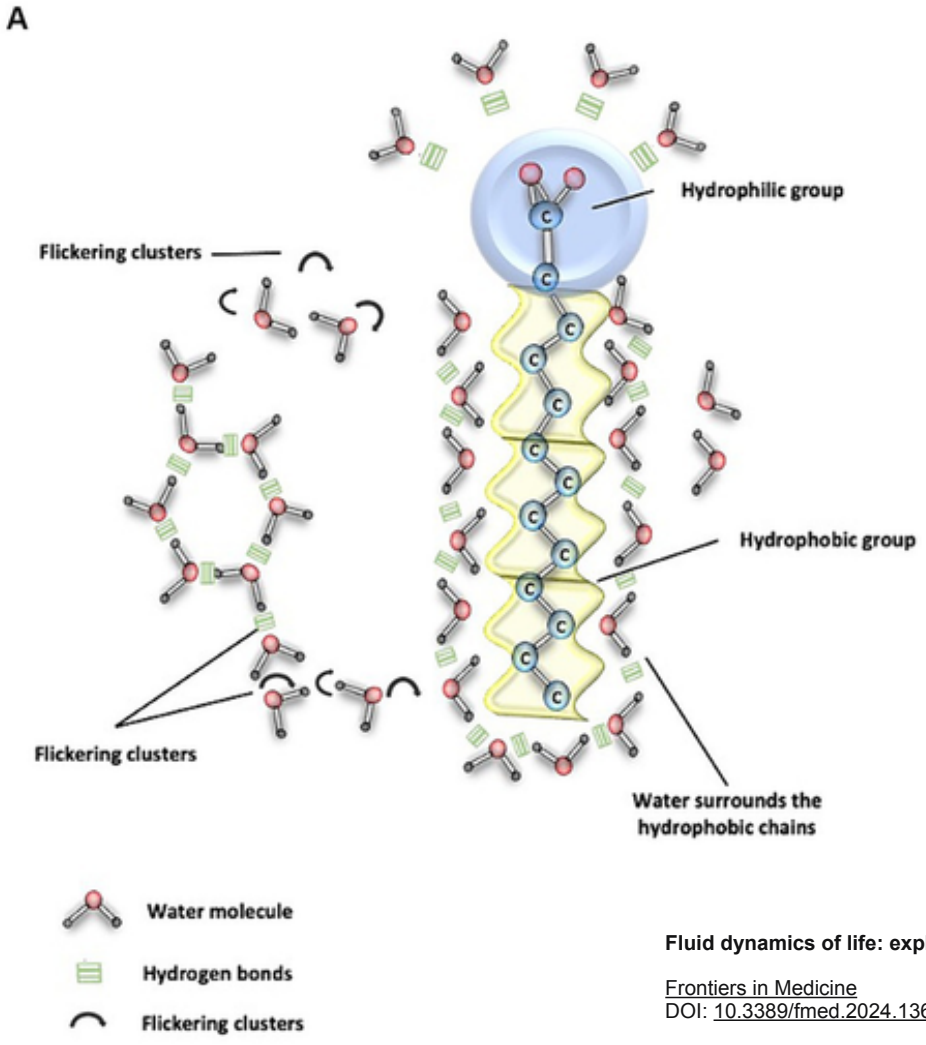


Figure 2-7b part 2
Lehninger Principles of Biochemistry, Fifth Edition

https://www.google.co.in/search?q=hydrophobic+effect&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiRhbOwoM7VAhUCNo8KHYNwDdMQ_AUICigB&biw=1226&bih=654#imgrc=2Toaslh2hDMezM:



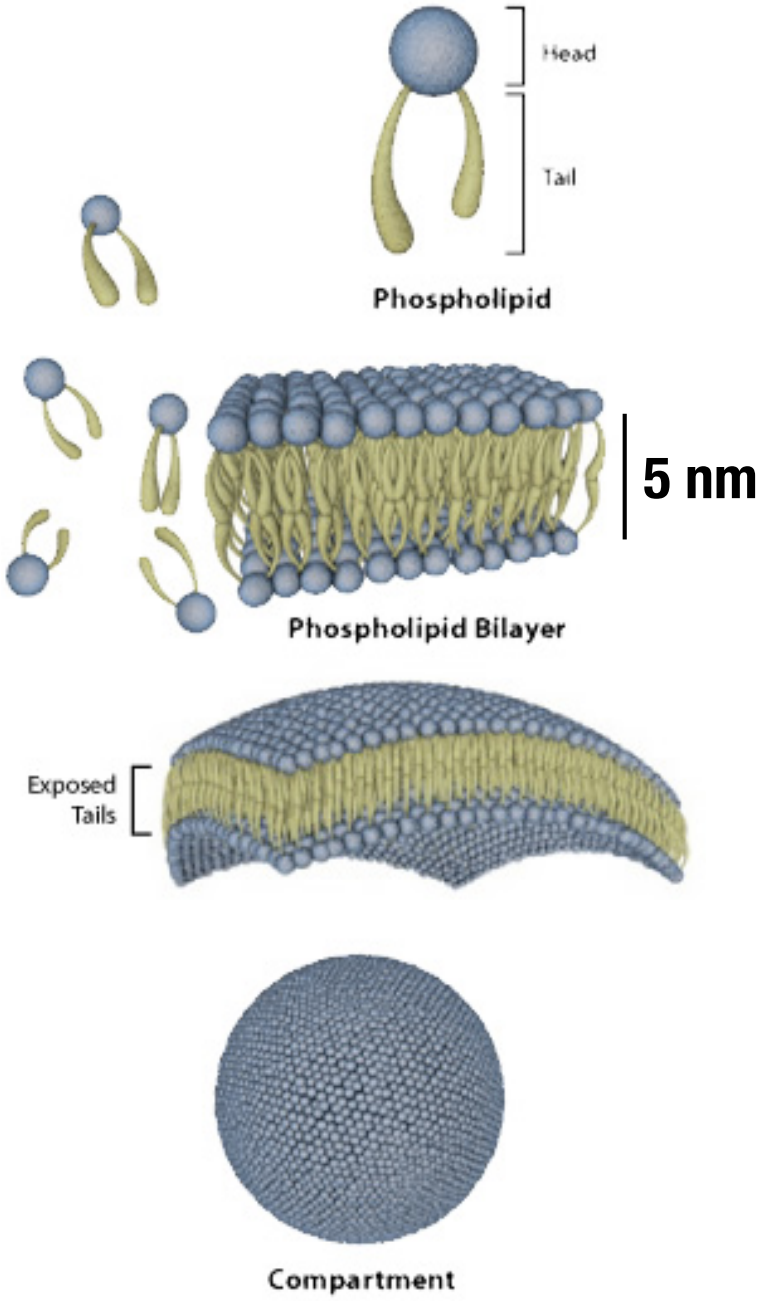
How do lipids come together to form membranes?

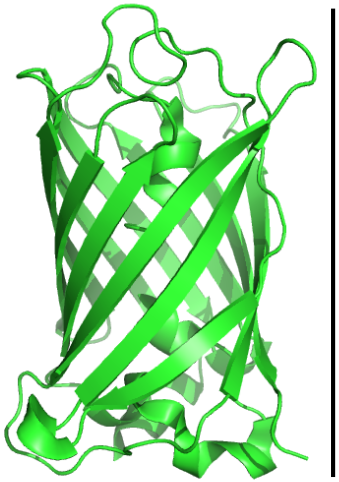


Fluid dynamics of life: exploring the physiology and importance of water in the critical illness

Frontiers in Medicine
DOI: 10.3389/fmed.2024.1368502

Dimensions

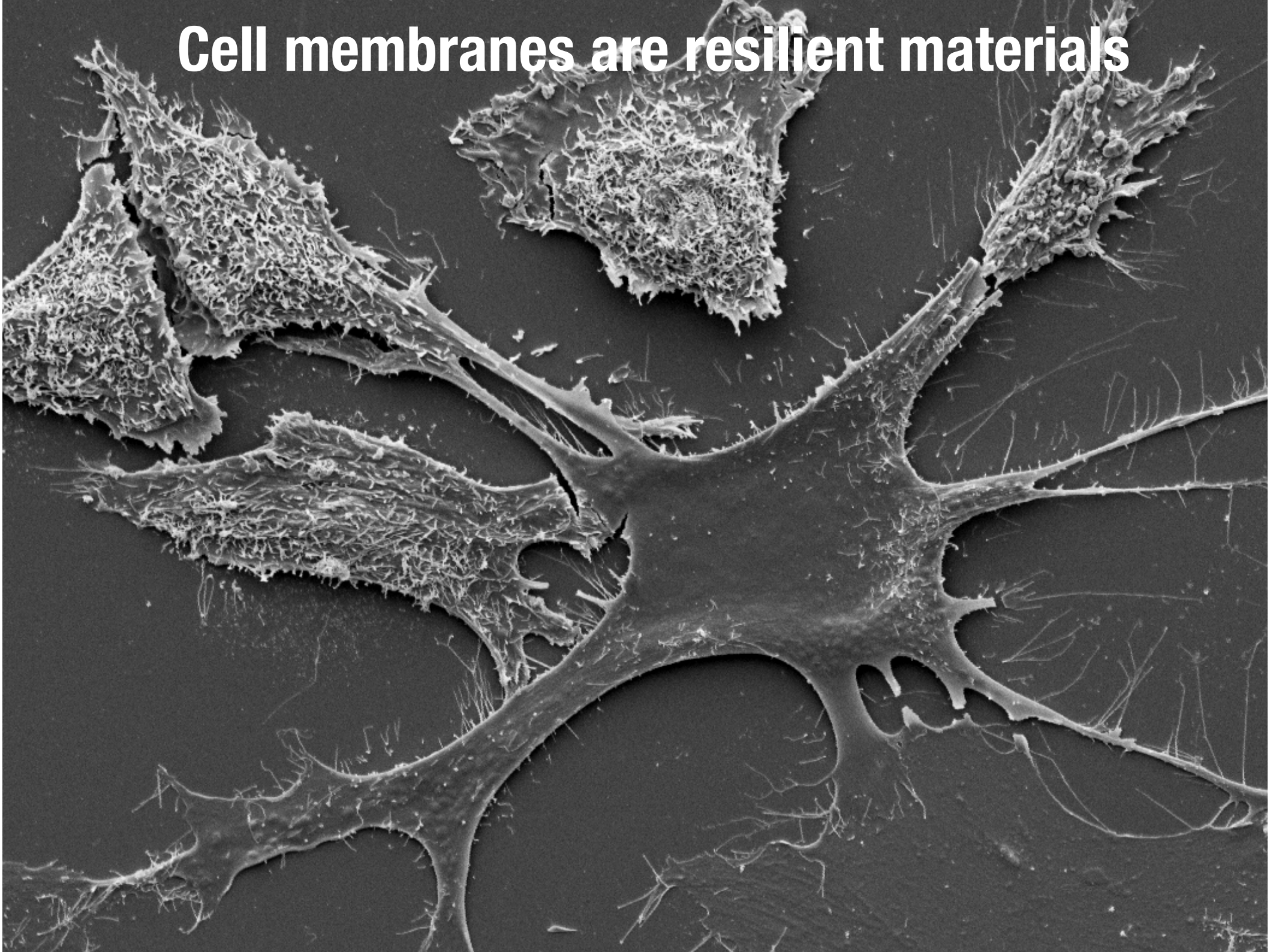


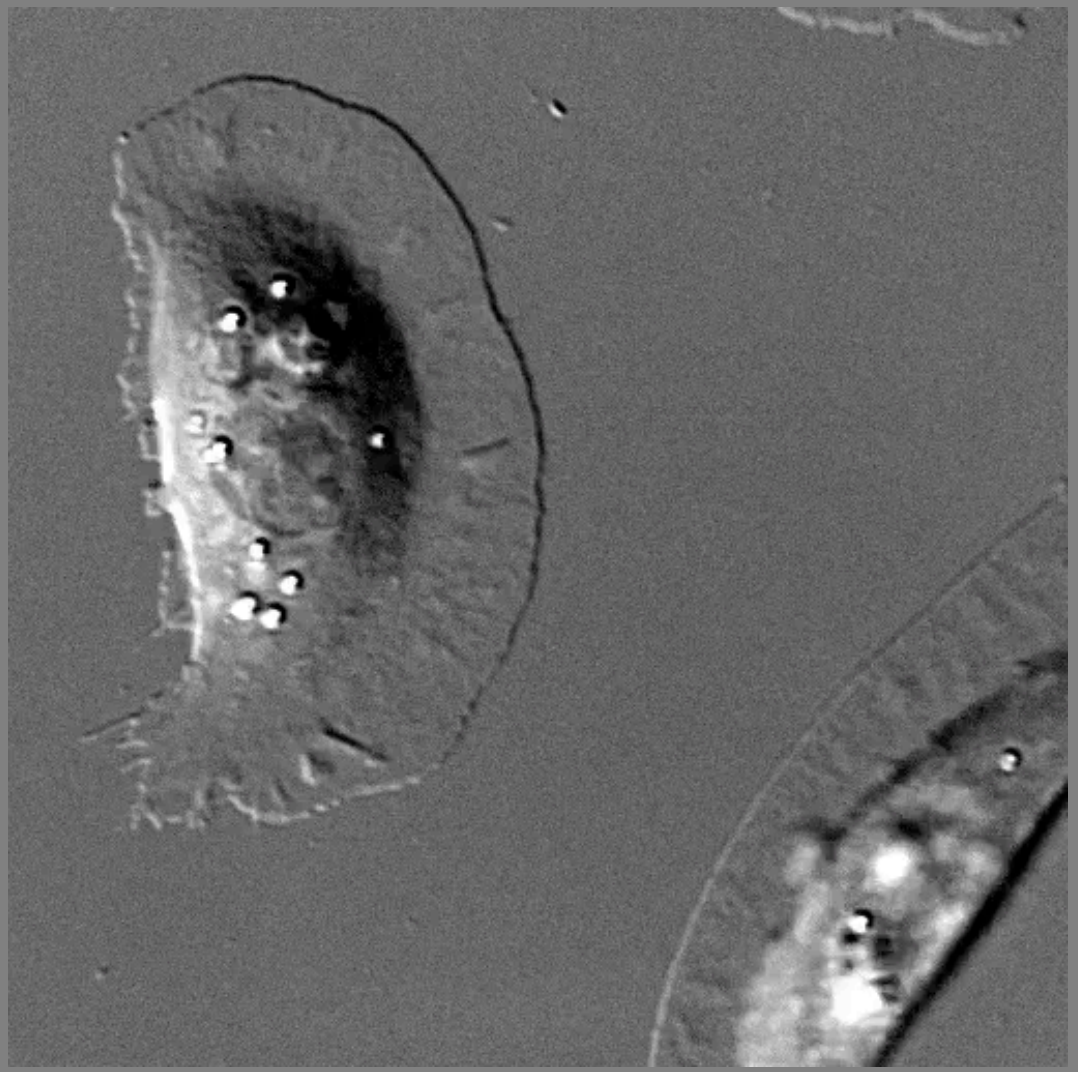


4.6 nm

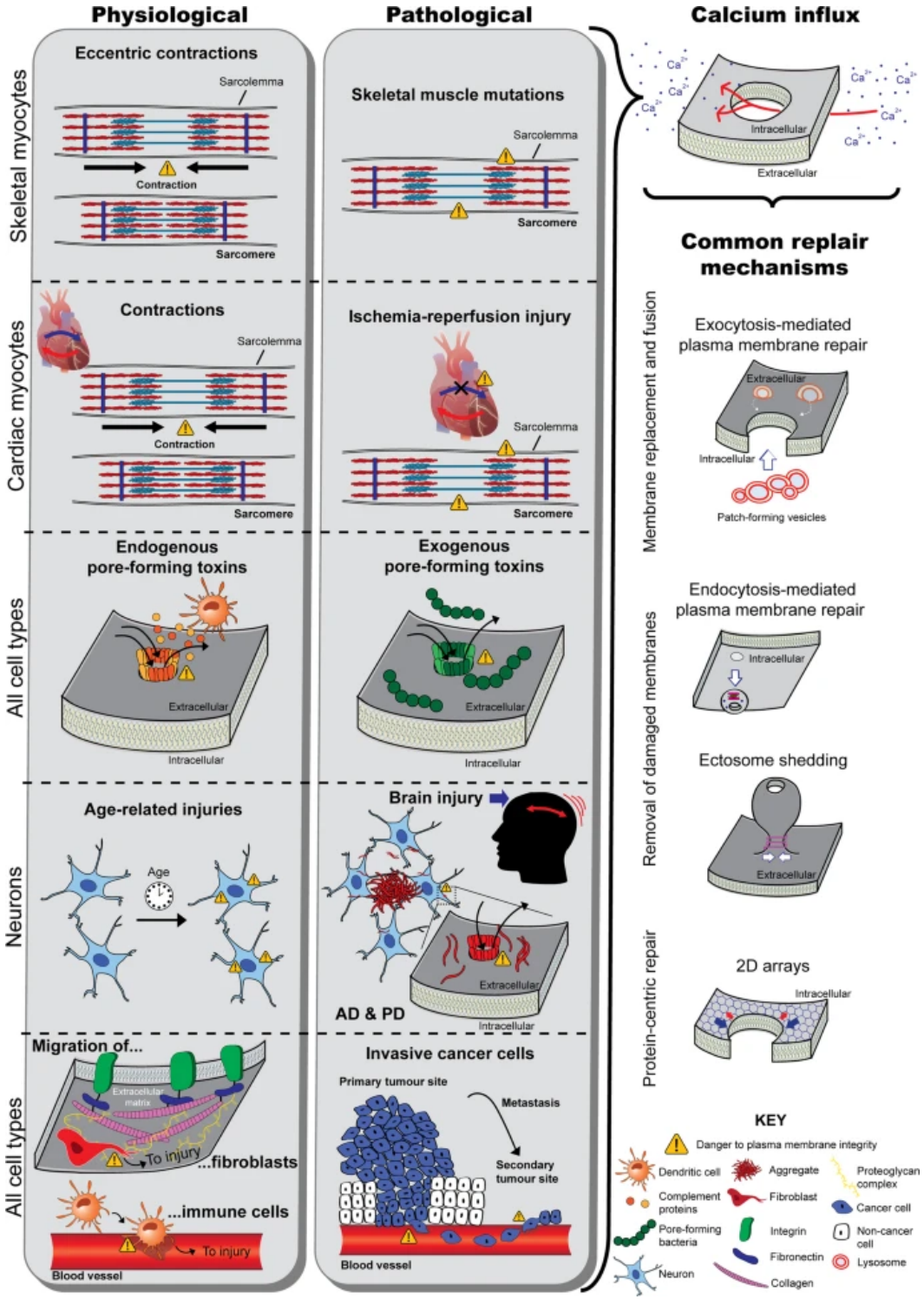
http://learn.genetics.utah.edu/content/cells/vesicles/

Cell membranes are resilient materials





Courtesy: Dylan Burnette (@MAG2ART)

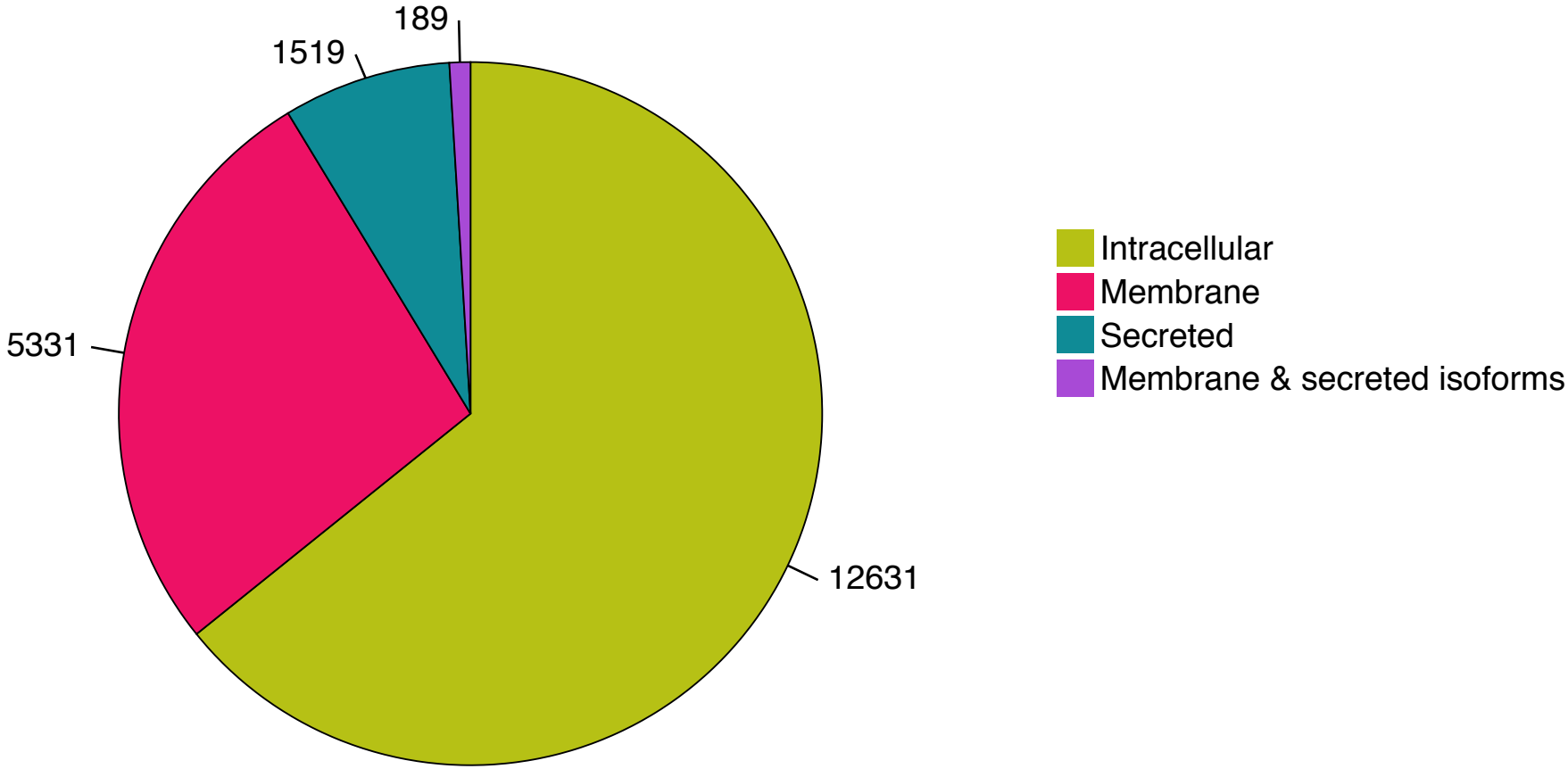


Membranes are resilient materials

and their tearing is energetically expensive

https://www.nature.com/articles/s41421-020-00233-2

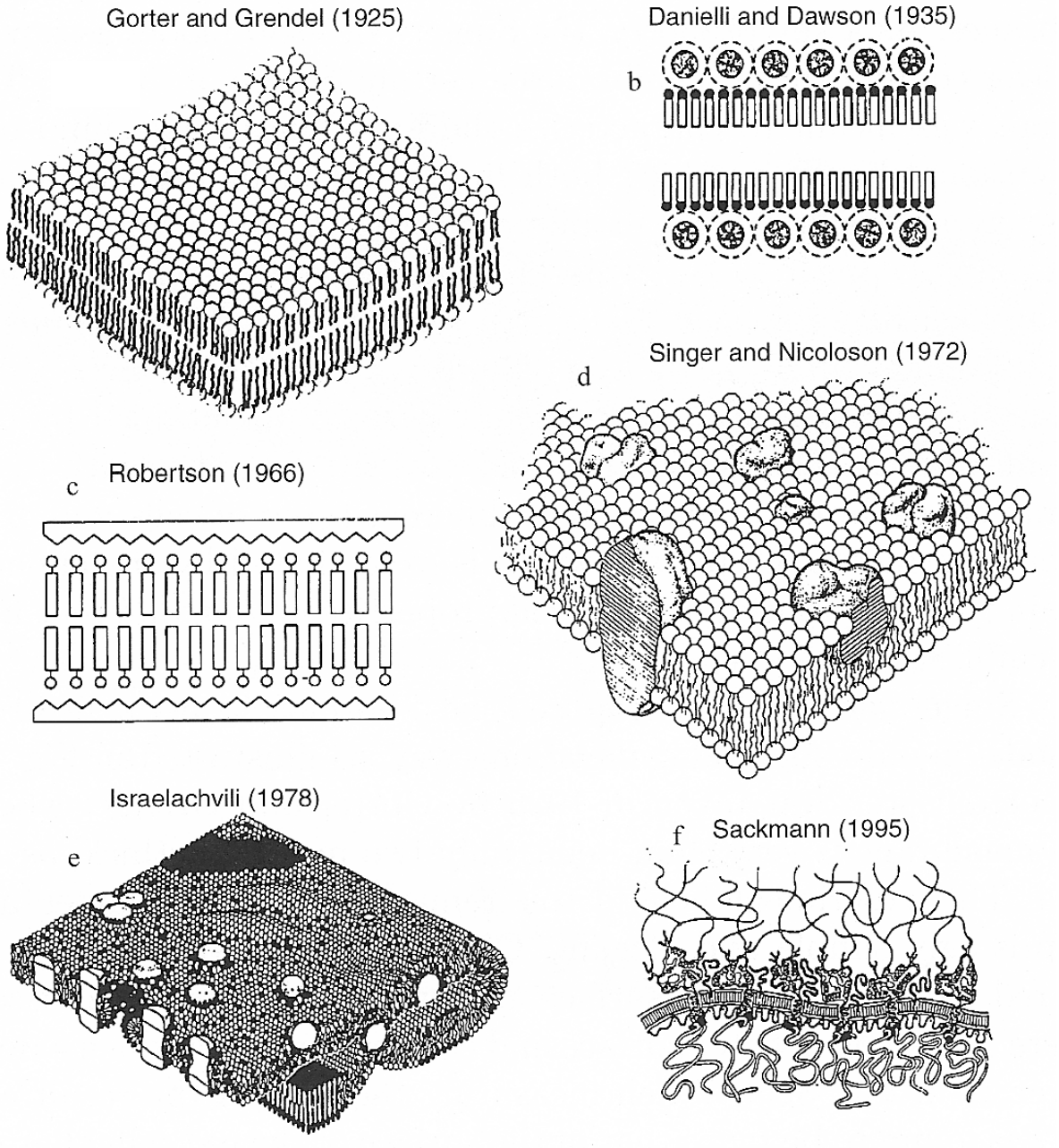
Protein classes



- 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

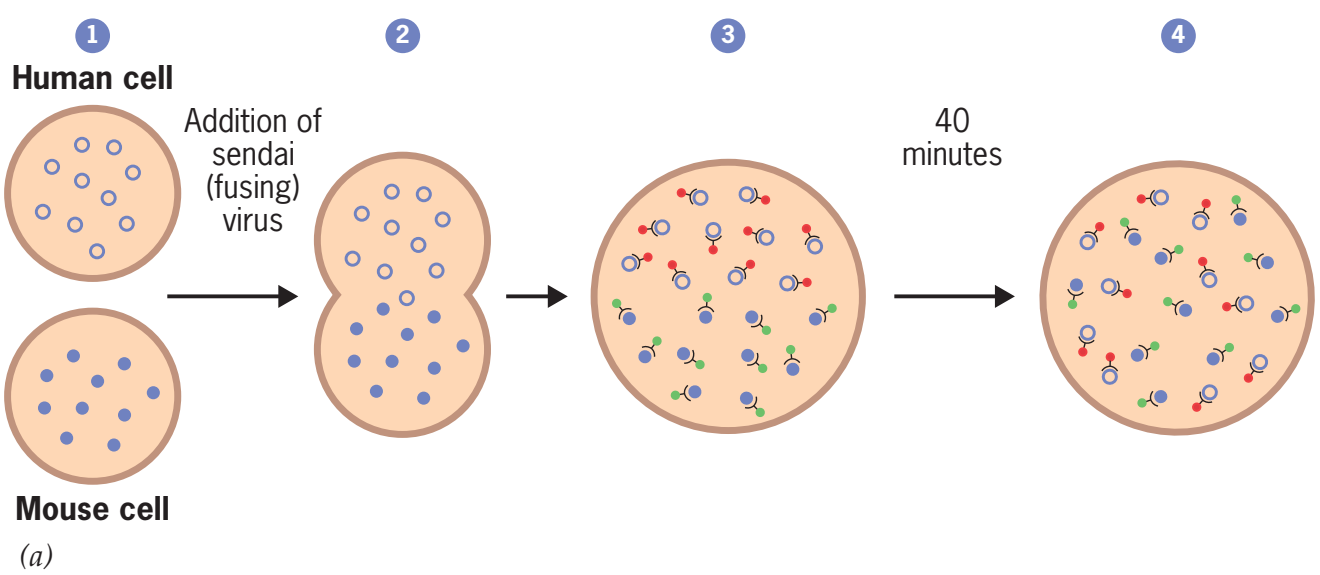
Models of biological membranes

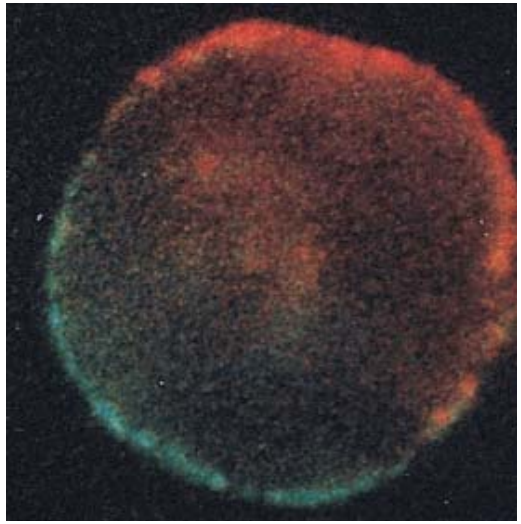


Gorter and Grendel (1925)

- 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

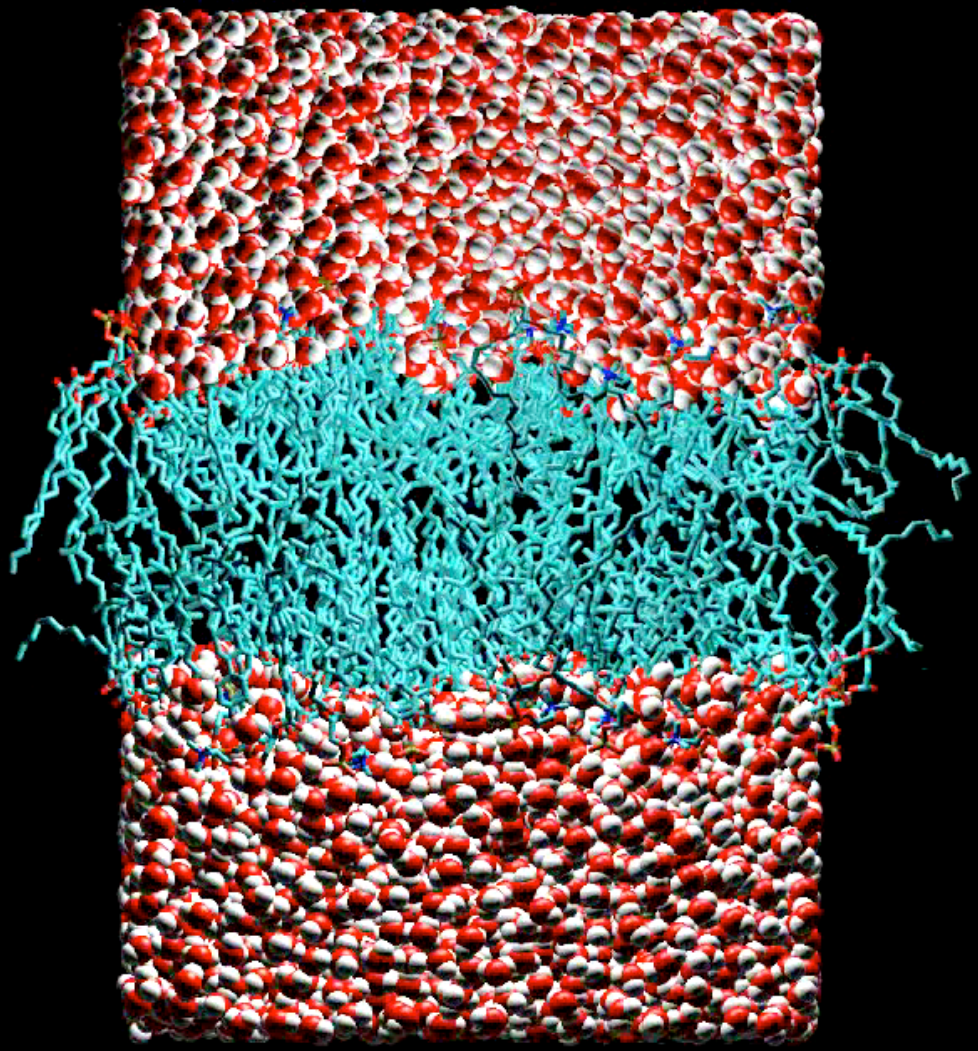
Models of biological membranes





Frye and Edidin (1970)

How is the lipid bilayer organised?



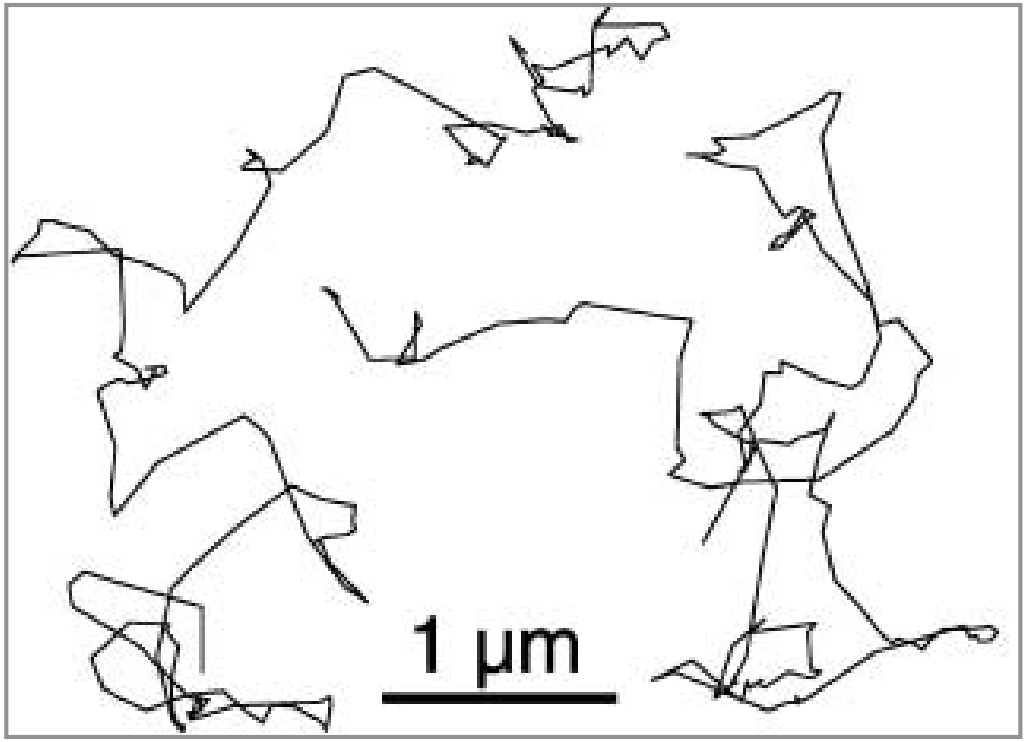
http://www.apmaths.uwo.ca/~mkarttu/membranes.shtml

Membrane Organization analyzed by Single Particle Tracking (SPT)

Cy3-DOPE on NRK cell

Real time

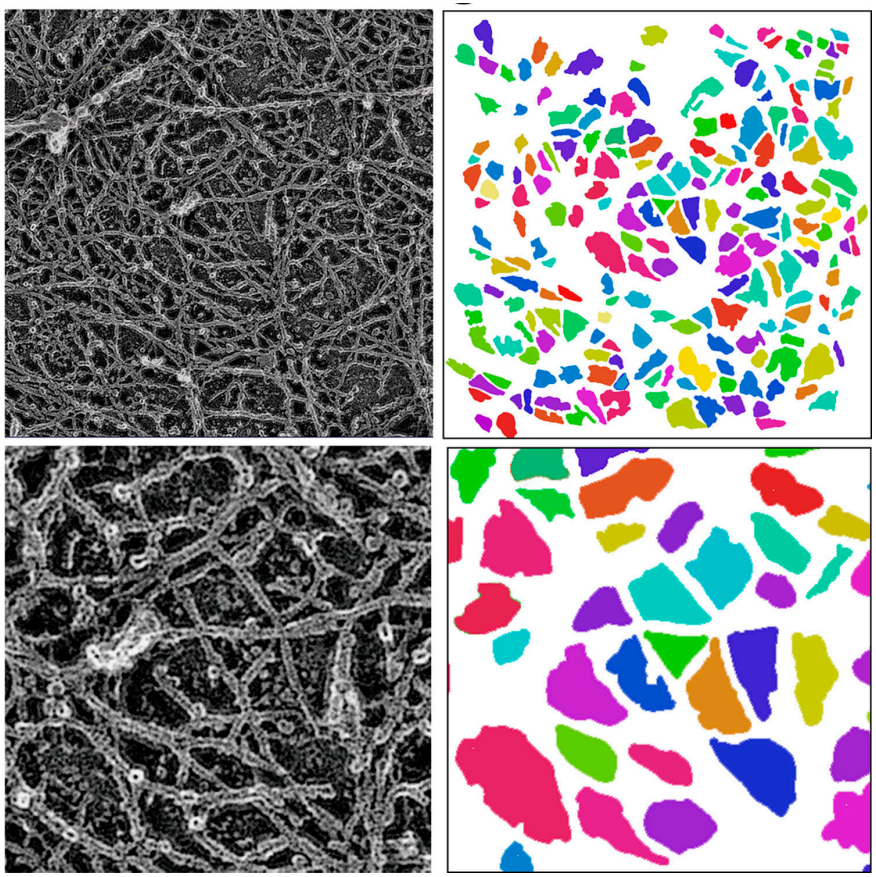
Scale = 1 μm

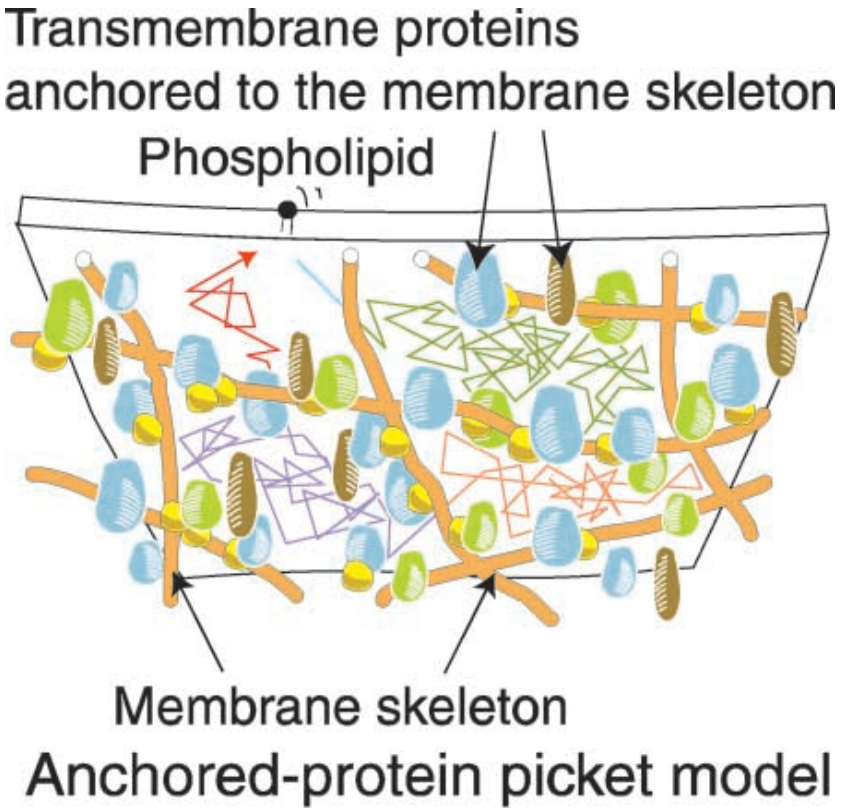


Cy3-DOPE = fluorescent lipid
G40-DOPE = gold particle tagged DOPE

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

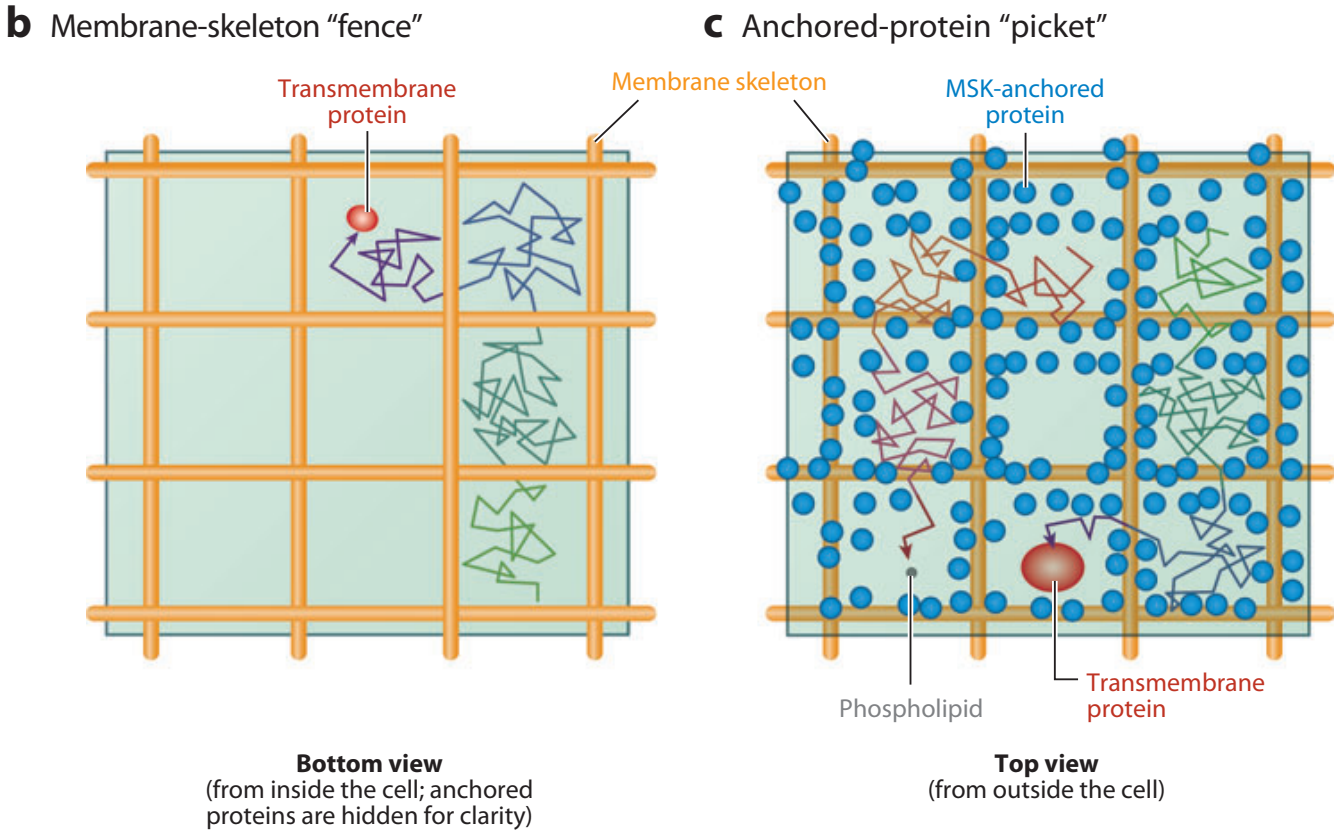
Contemporary model of cell membranes





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

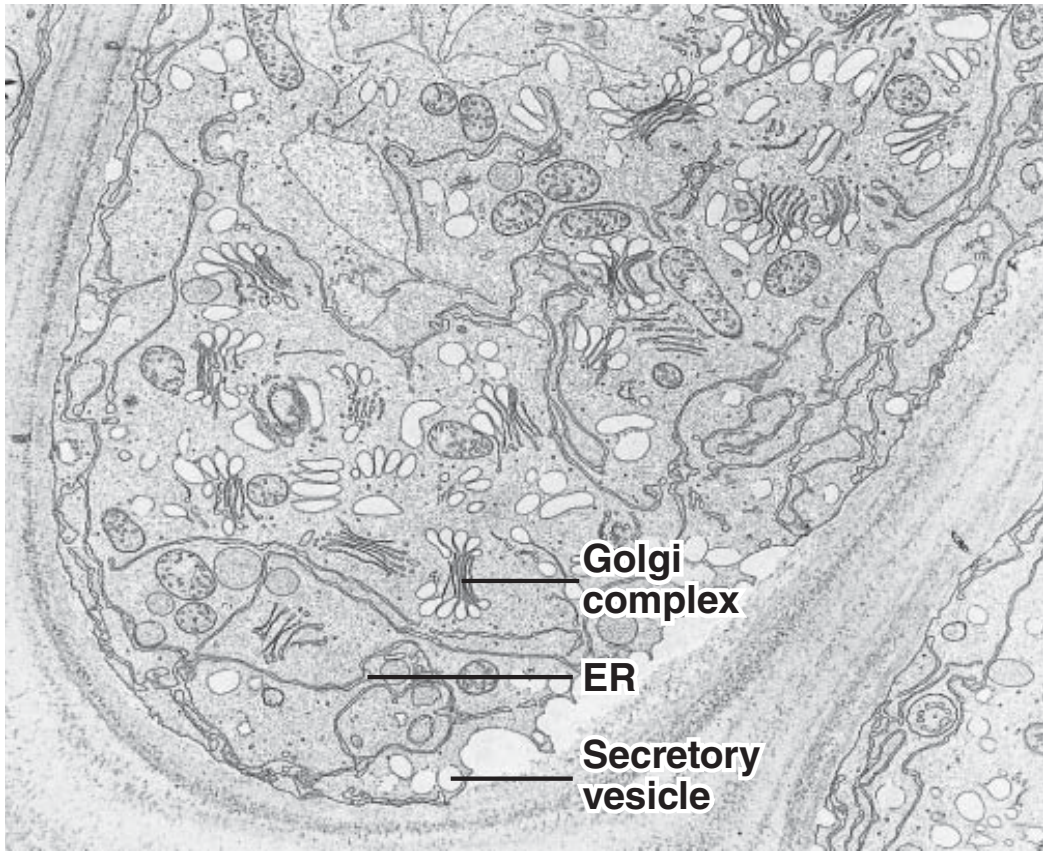
Contemporary model of cell membranes



- 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

Endomembrane system



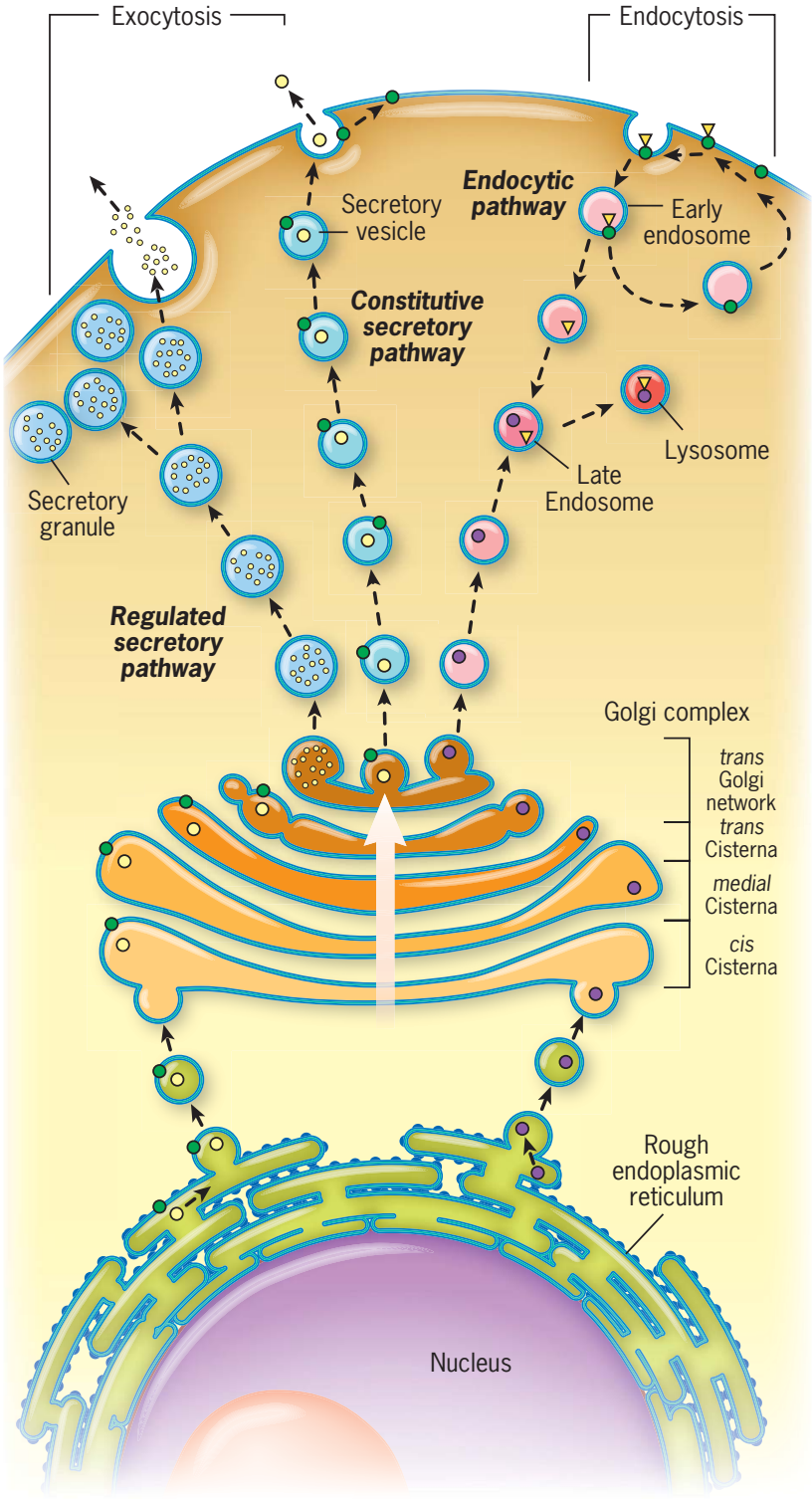
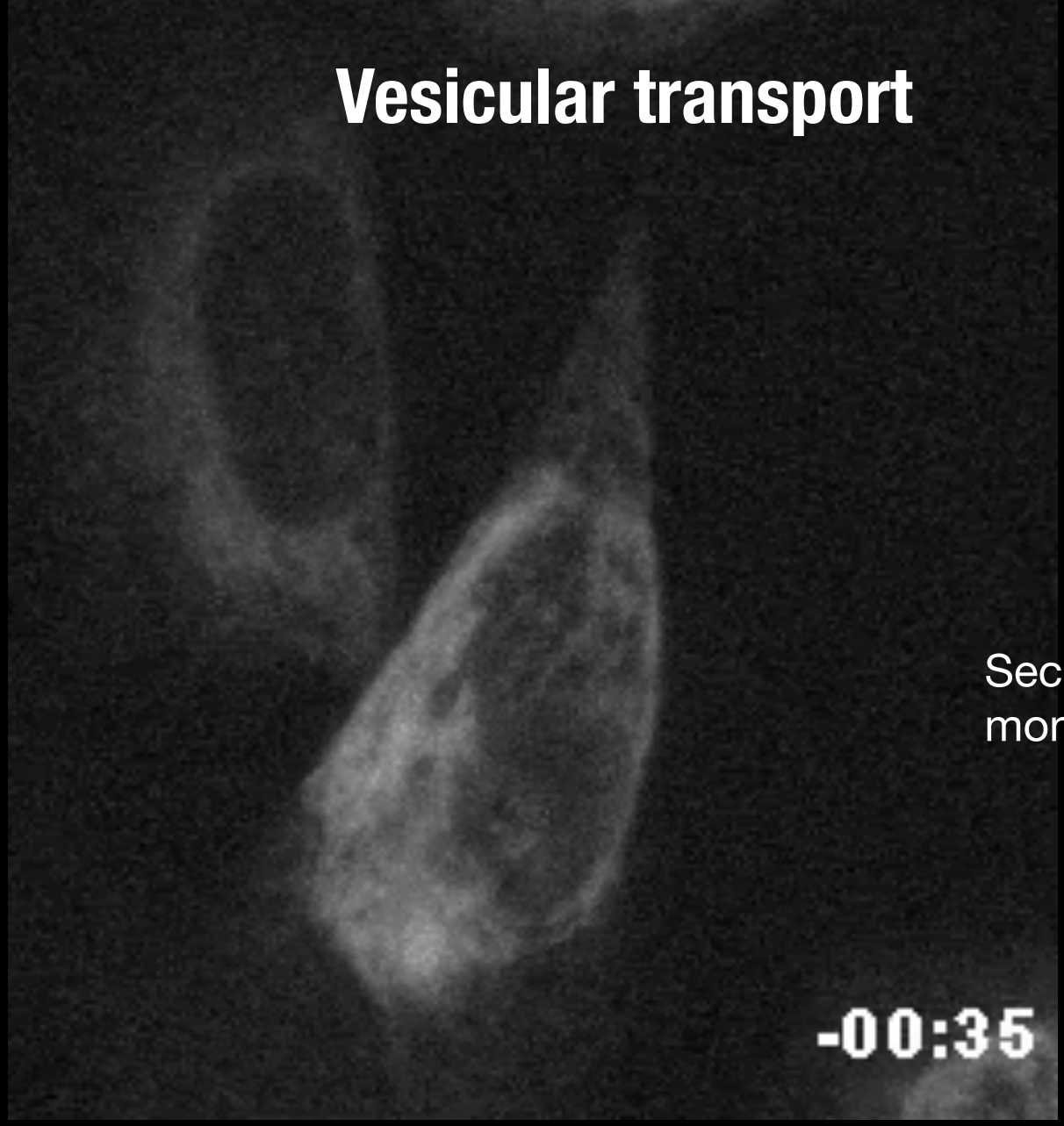


FIGURE 8.1 Membrane-bound compartments of the cytoplasm. The cytoplasm of this root cap cell of a maize plant contains an array of membrane-bound organelles whose structure and function will be examined in this chapter. As is evident in this micrograph, the combined surface area of the cytoplasmic membranes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

SOURCE: Courtesy of Hilton H. Mollenhauer.

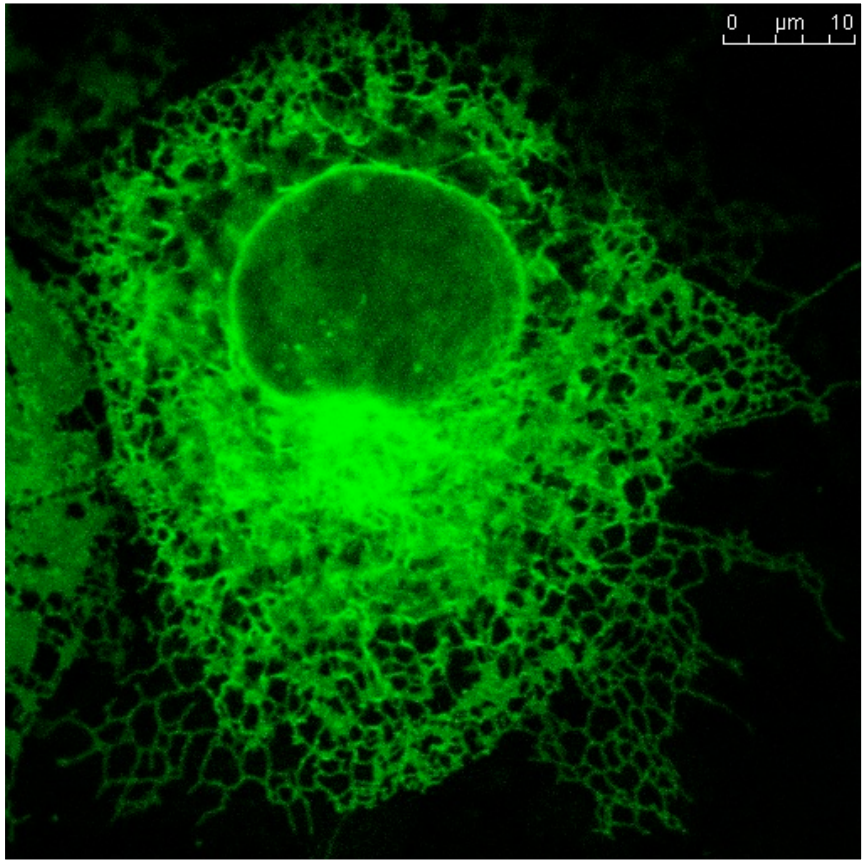
Vesicular transport



Secretory traffic
monitored in HeLa cells

Boncompain et al. (2012) Nature Methods

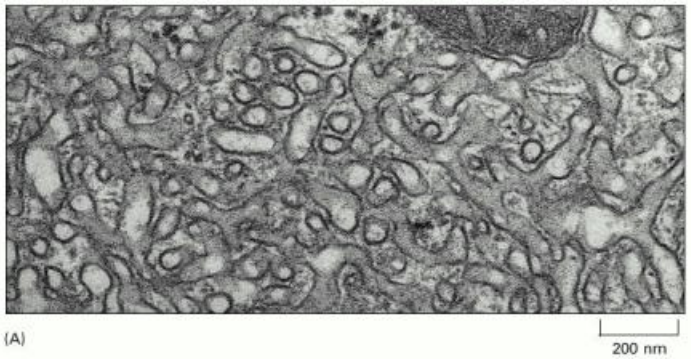
Endoplasmic Reticulum



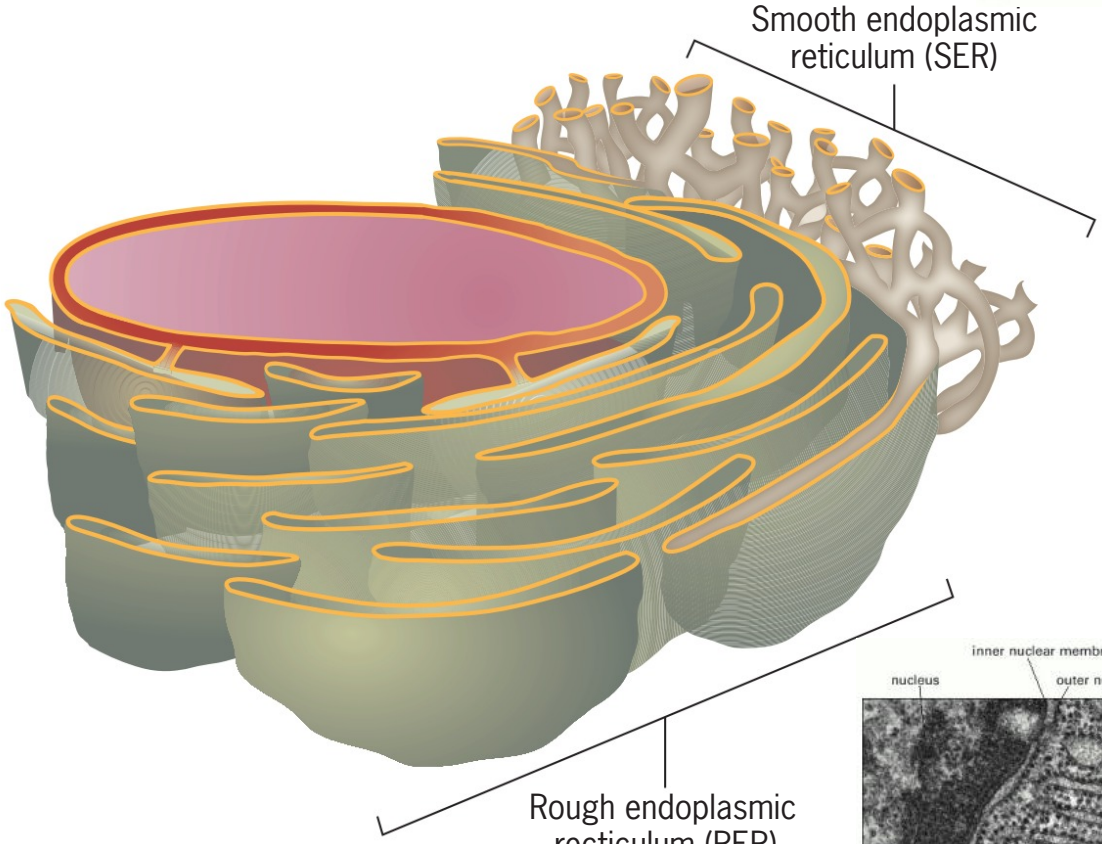
http://www2.warwick.ac.uk/fac/sci/maths/research/events/2009_2010/symposium/netwks/

- 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

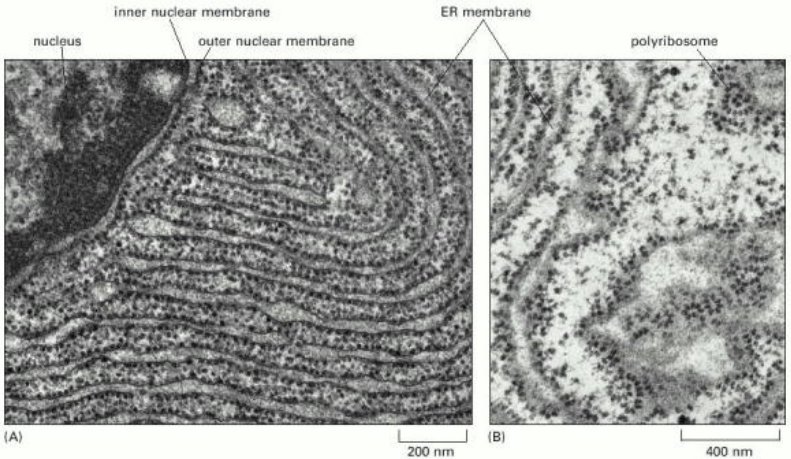


Smooth ER



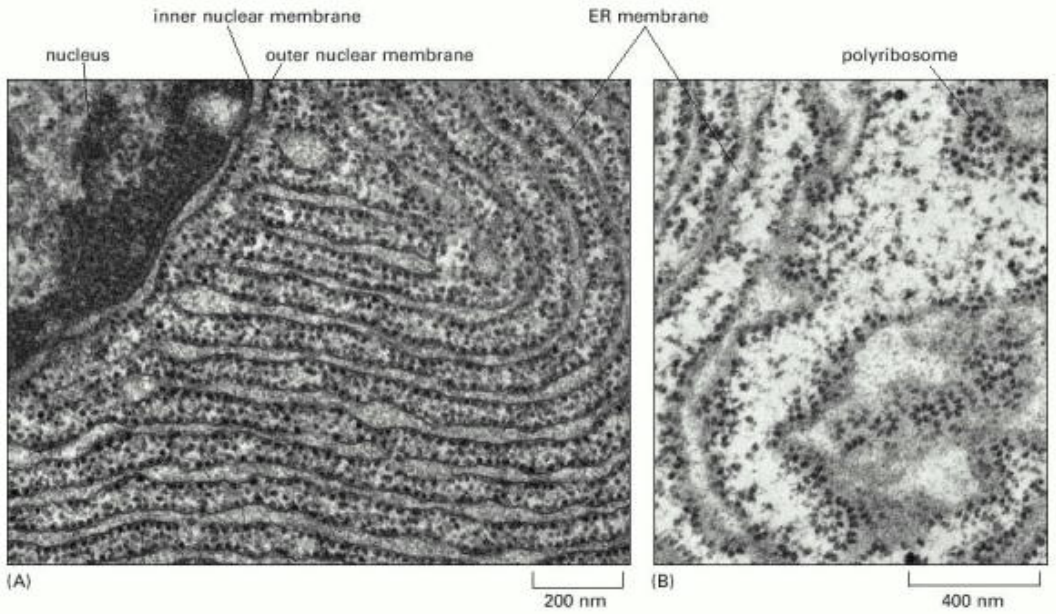
(a)

Rough ER



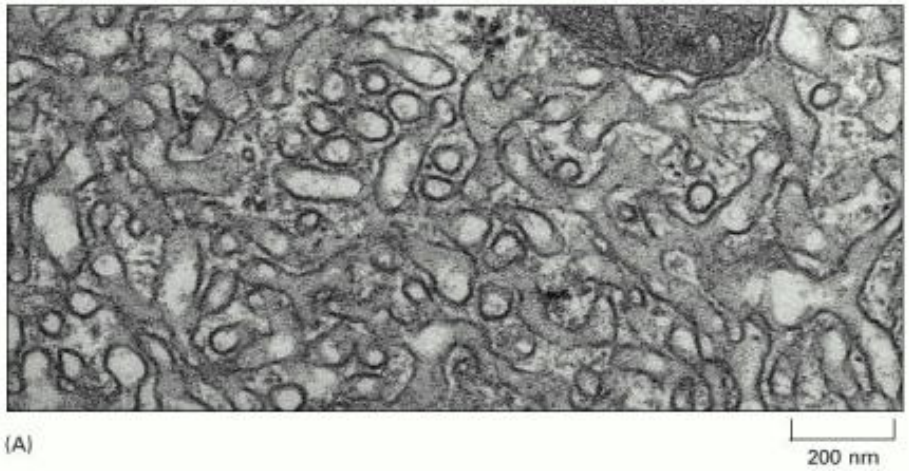
Morphologically divided into two classes

Rough 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

Smooth ER



- Termed smooth ER because they are devoid of bound ribosomes
- Steroid hormone synthesis, detoxification

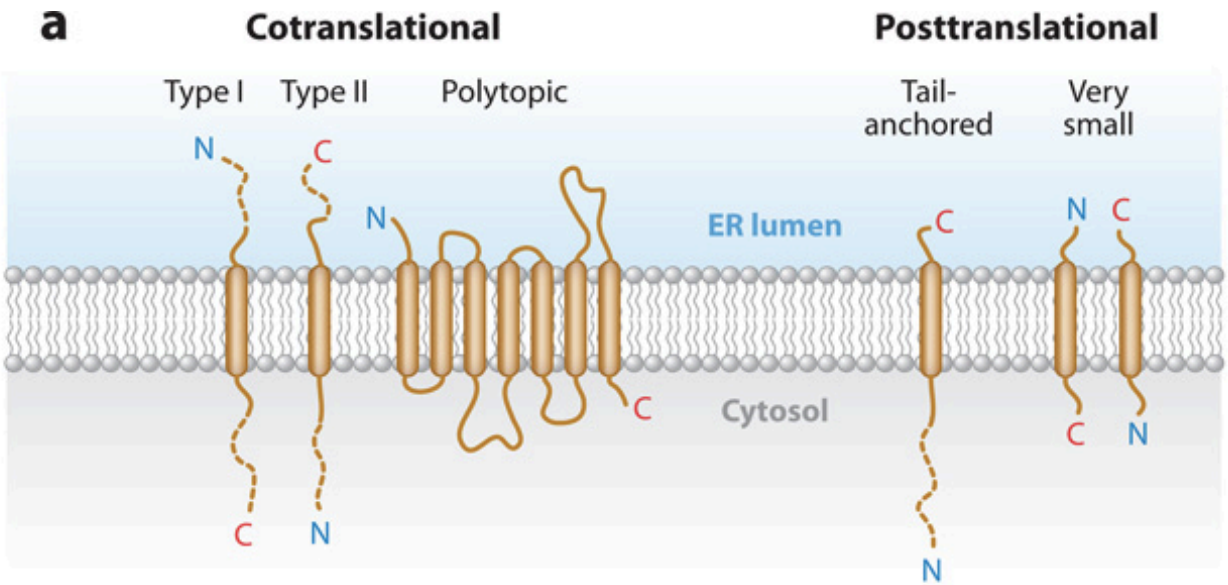
- SER is extensively developed in a number of cell types, skeletal muscle, kidney tubules, and steroid-producing endocrine glands.

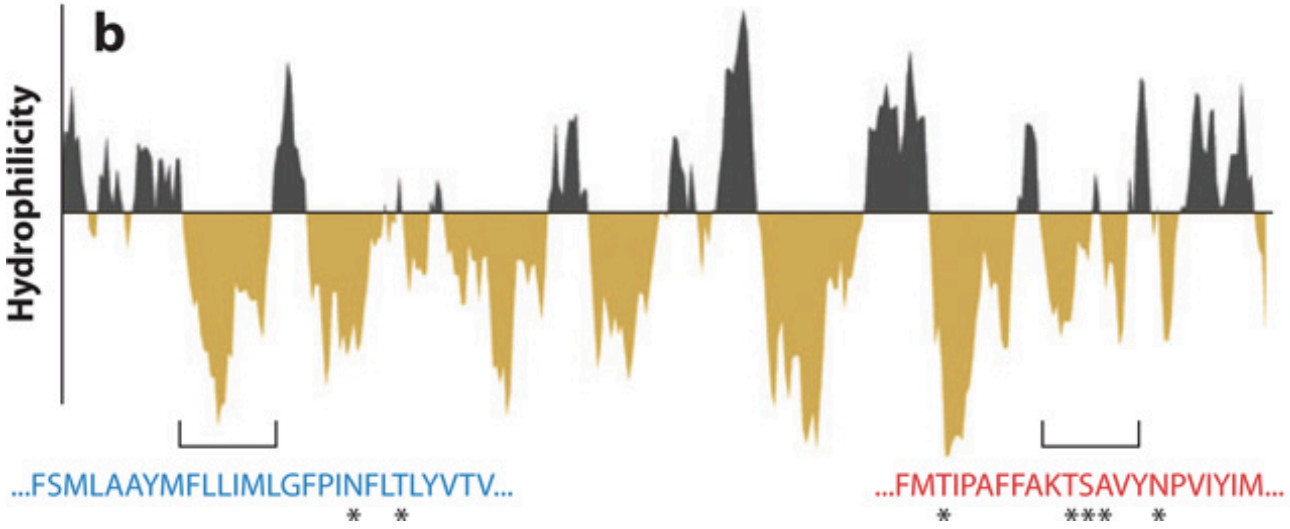
- Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex

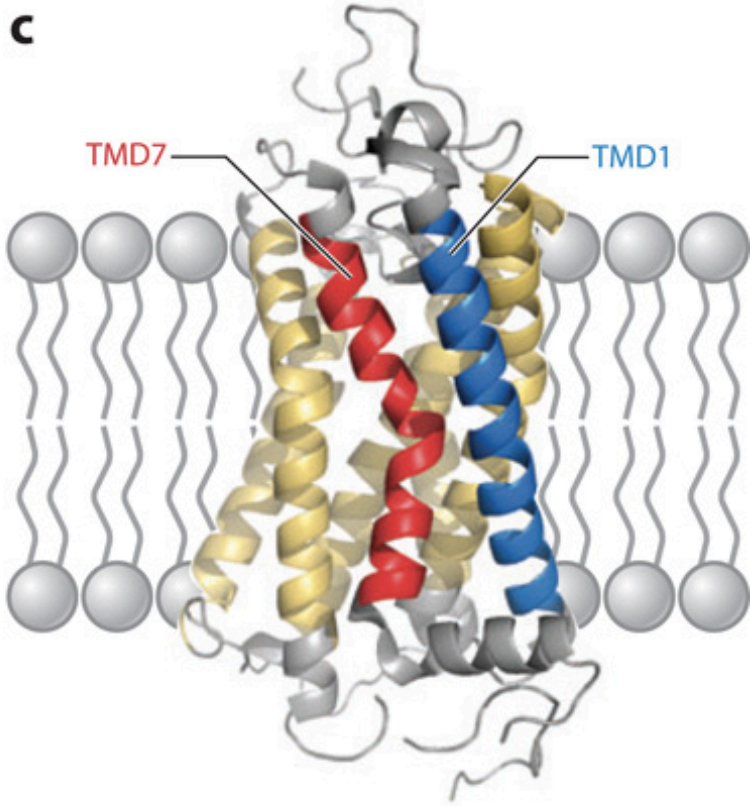
- Detoxification of organic compounds carried out by the ER-localized collection of oxygen-transferring enzymes (oxygenases), including the *cytochrome P450* family. They lack substrate specificity and can oxidize thousands of different hydrophobic compounds and convert them into more hydrophilic entities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



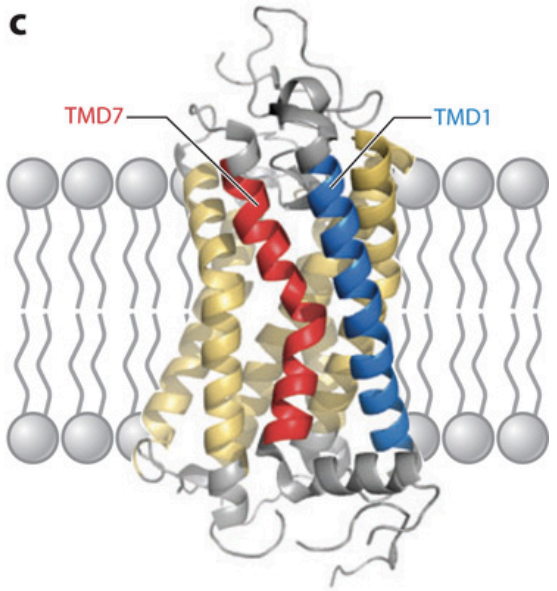




Membrane Protein Insertion at the Endoplasmic Reticulum
Shao and Hegde (2011) Annu. Rev. Cell Dev. Biol.

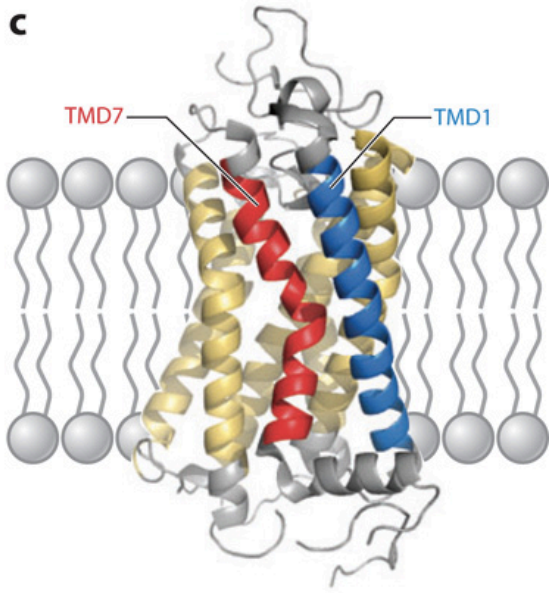
Integral membrane protein (IMP) biogenesis

- 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

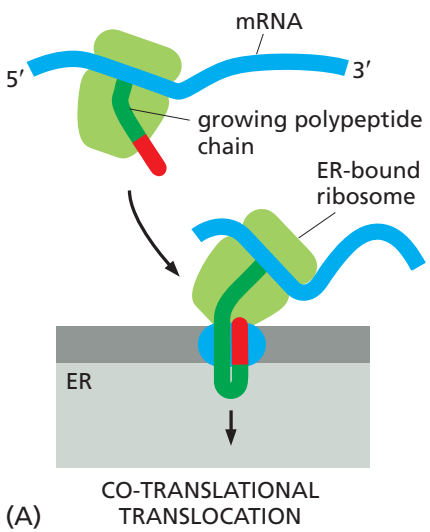
- IMPs destined to be inserted into the ER face a set of challenges
- The hydrophobic TMDs of IMPs must be continuously shielded from the aqueous cytosol (shielding is essential because the tremendously crowded cytosolic environment (~300 mg/ml protein) would promote potentially toxic aggregation)
- 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

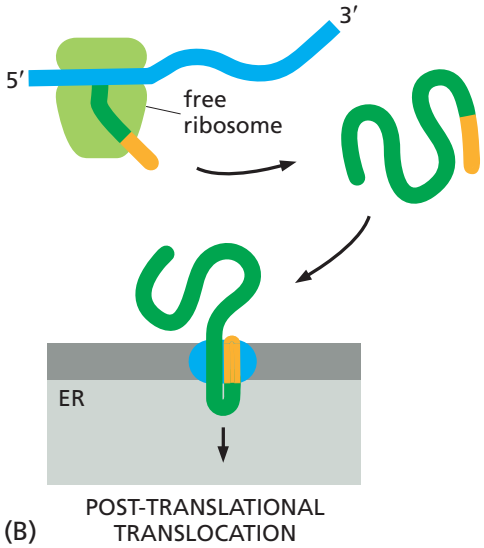
- Co-translational synthesis (translation and insertion are sequential processes)



- Constitutes bulk of the membrane proteins
- Synthesized and assembled at the ER
- Involves the translocon apparatus
- No chaperones necessary for folding

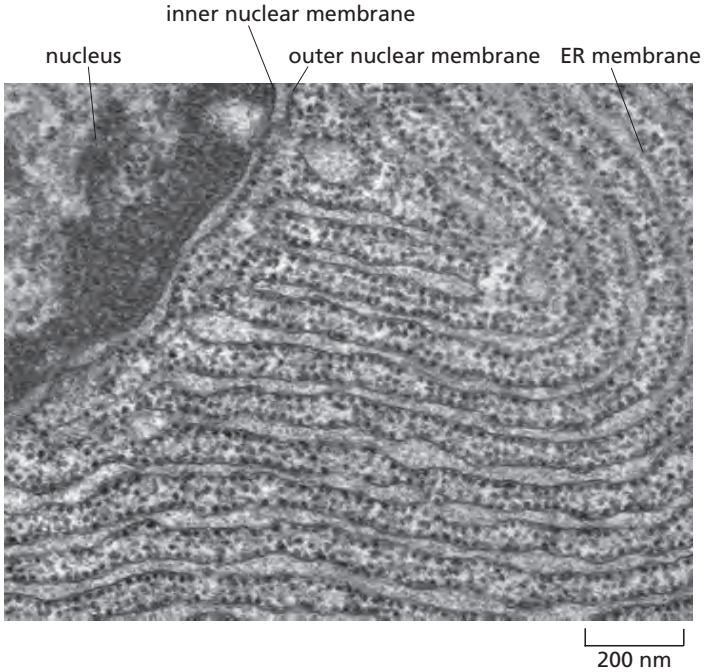
Post-translational

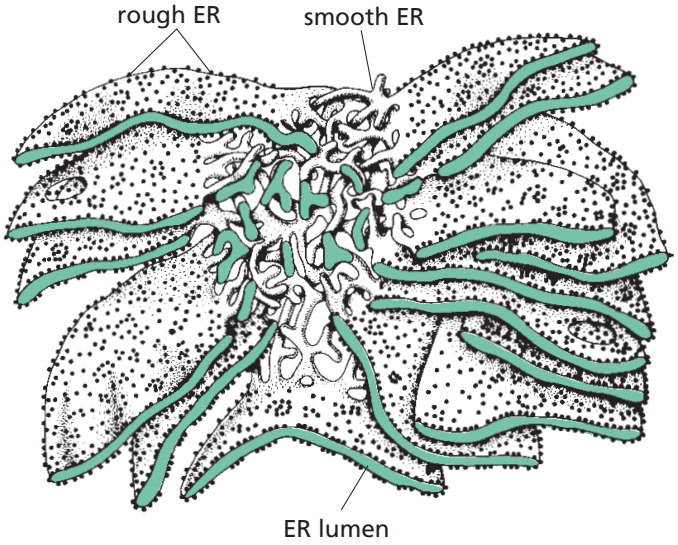
- Post-translational synthesis (translation and insertion are temporally distinct processes)

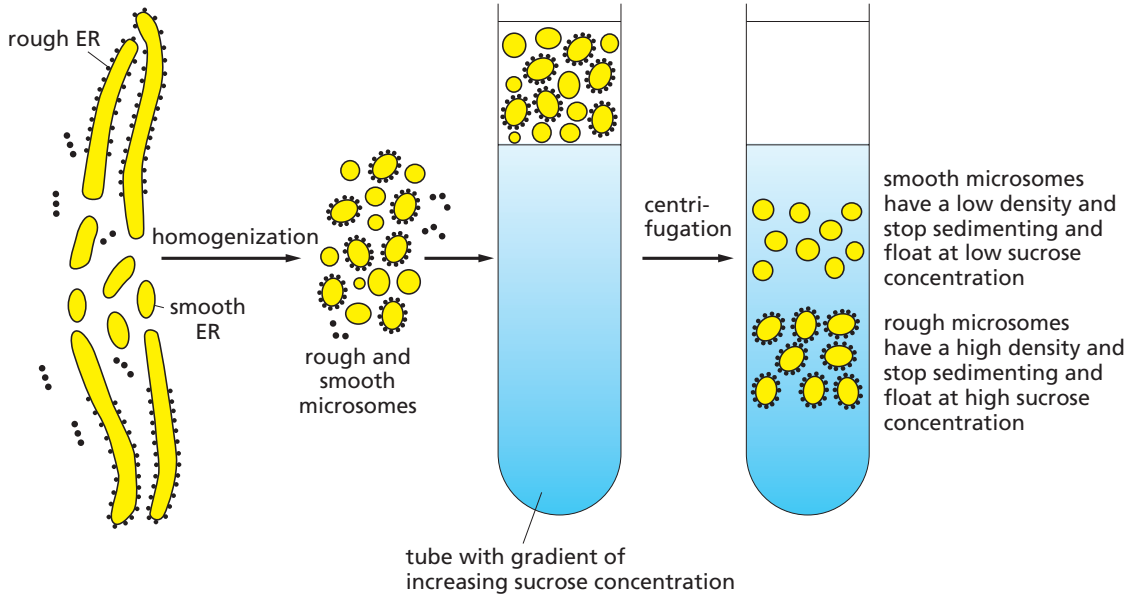


- Constitutes membrane proteins of mitochondria and chloroplast, nuclei and peroxisome and some bacterial toxins for e.g., alpha hemolysin, colicin, melittin and C-terminally anchored proteins found in synaptic vesicles
- Assisted (not involving the translocon apparatus)/ spontaneous insertion into membranes
- Chaperones necessary to maintain solubility in the cytosol

How to study IMP synthesis?



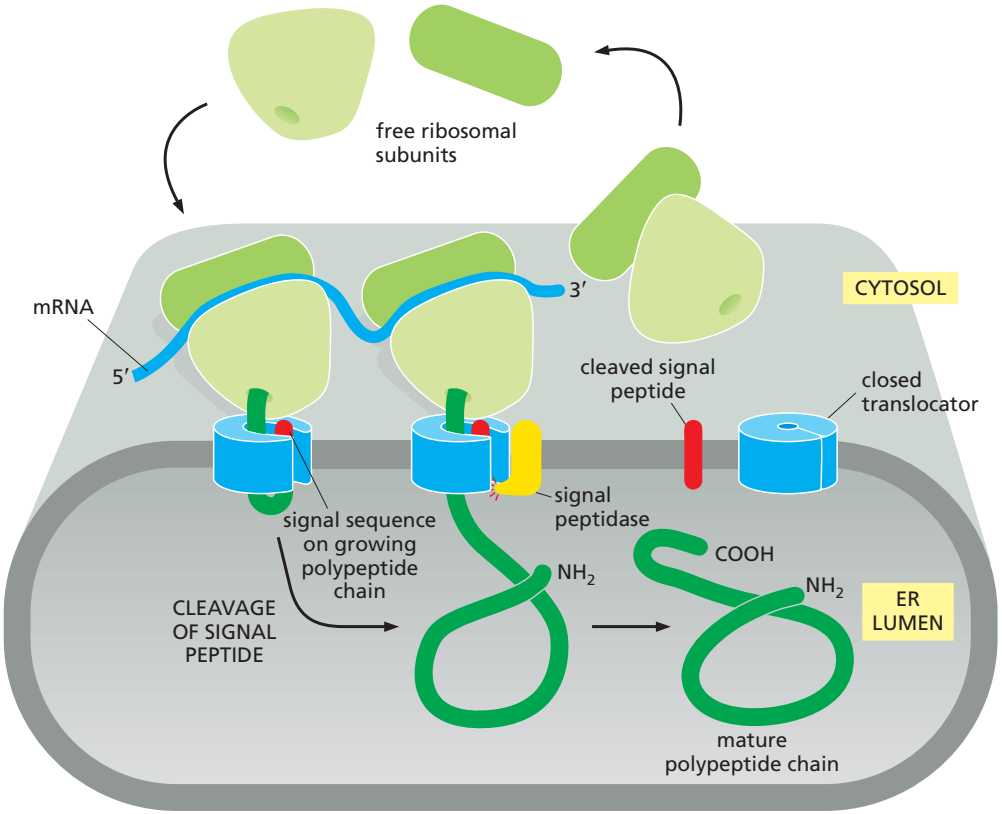




smooth microsomes have a low density and stop sedimenting and float at low sucrose concentration

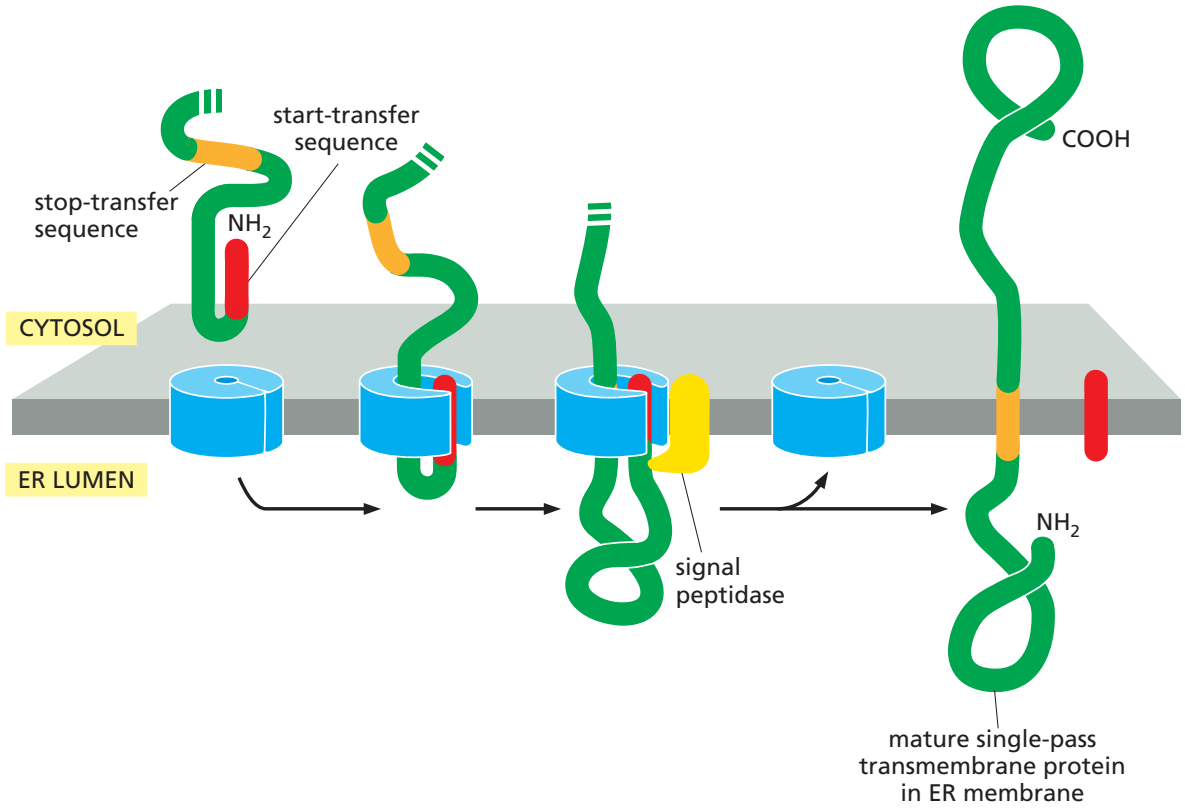
rough microsomes have a high density and stop sedimenting and float at high sucrose concentration

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

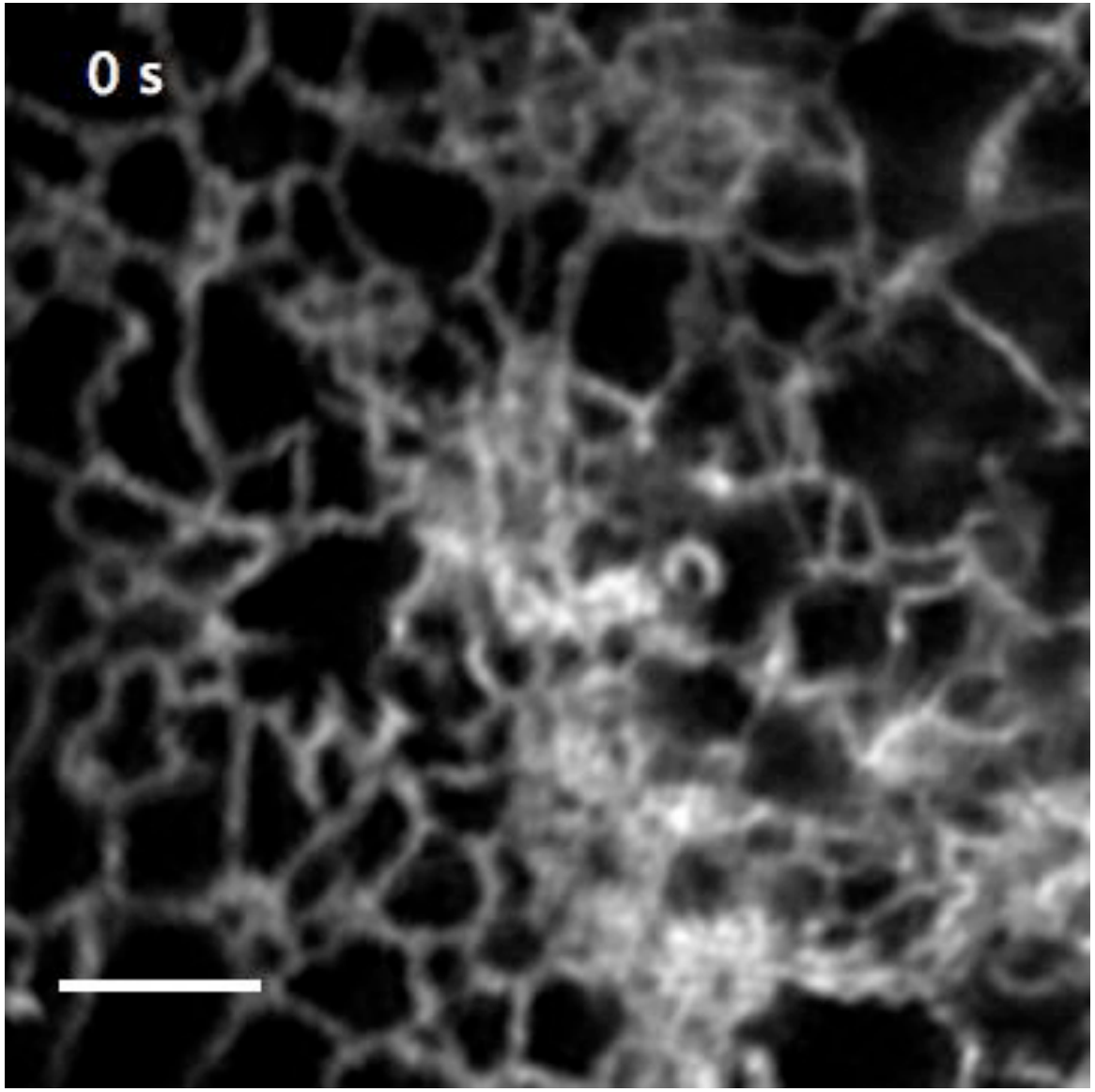
Co-translational protein synthesis and insertion into membranes



- 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

Ref: Jonathon Nixon-Abell et al. Science (2016)



ER organization during cell division

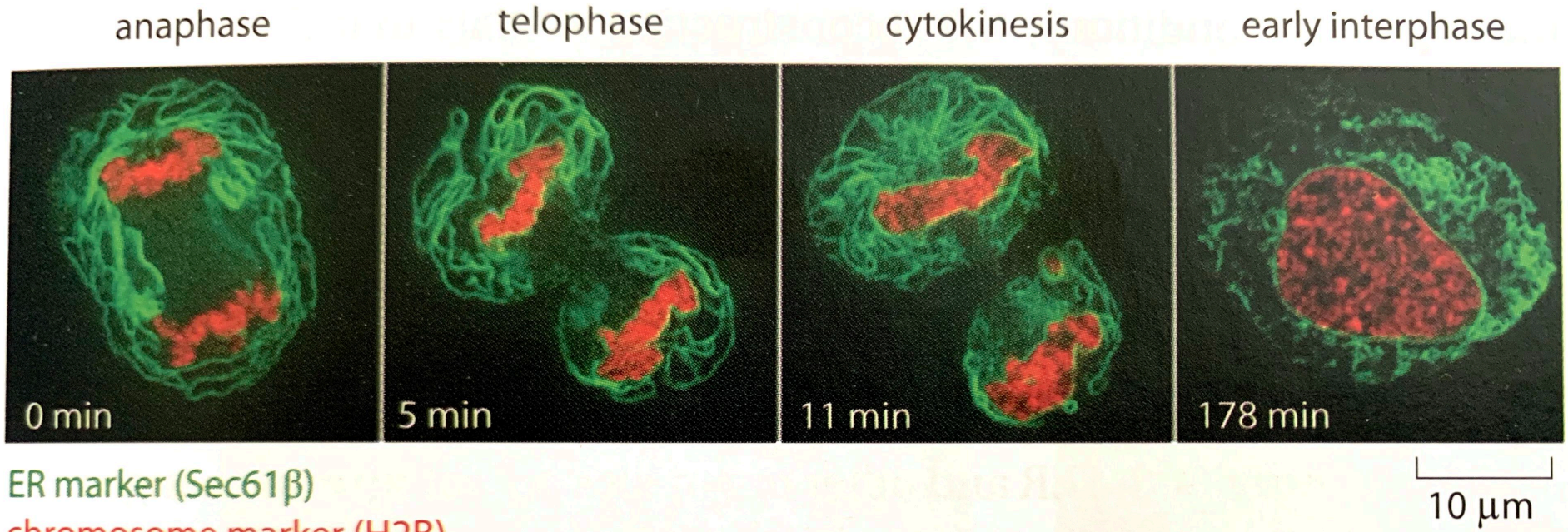
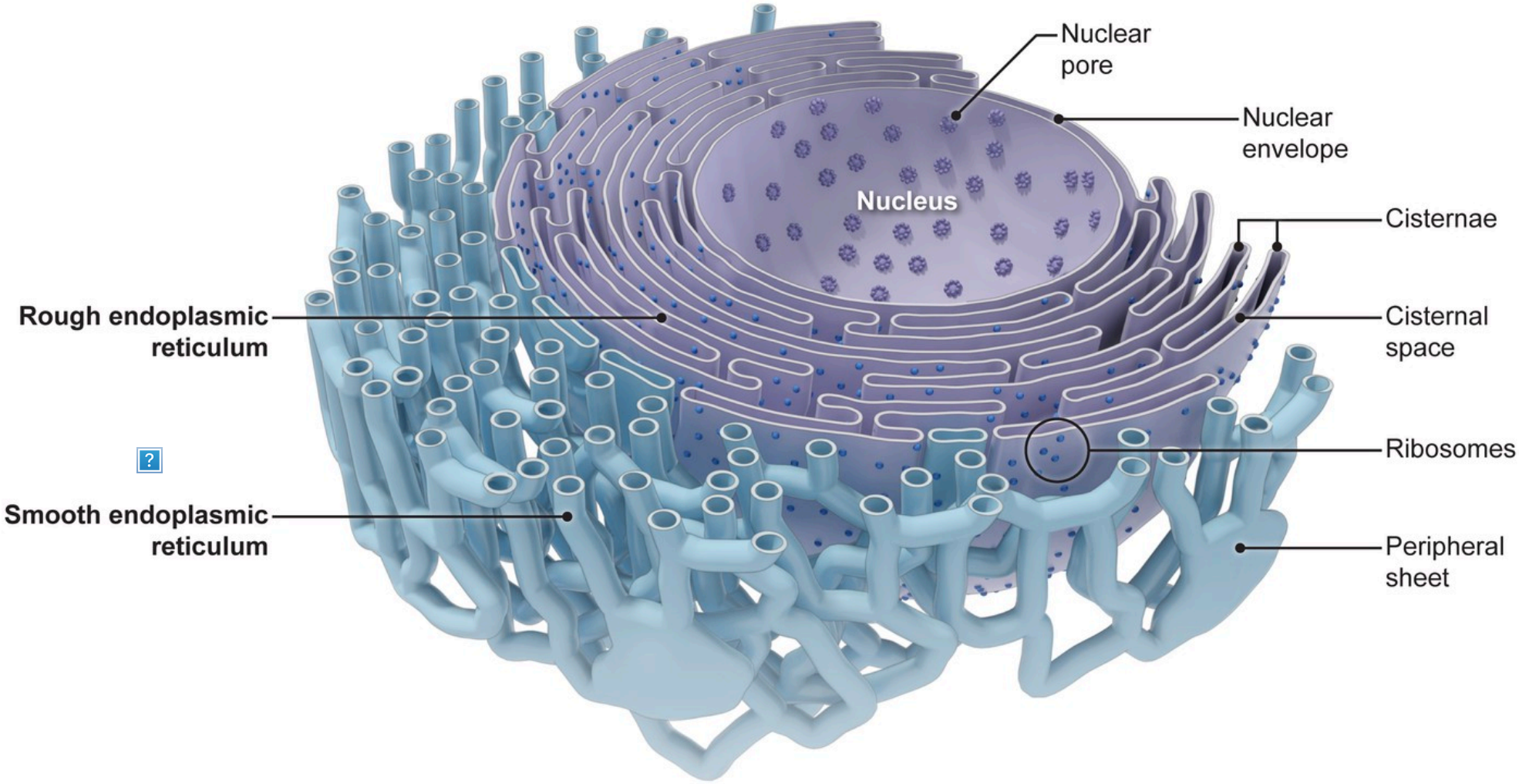


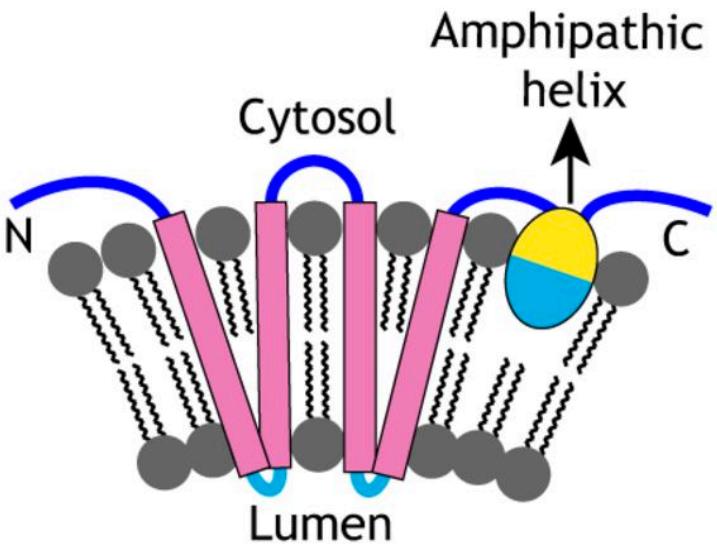
Figure 1-23 Structural dynamics of the endoplasmic reticulum during the cell cycle. Confocal images of HeLa cells. The chromosomes are labeled in red using a fusion of a fluorescent protein with histone H2B. The ER is labeled in green by virtue of a fusion of a fluorescent protein to a molecular member 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?

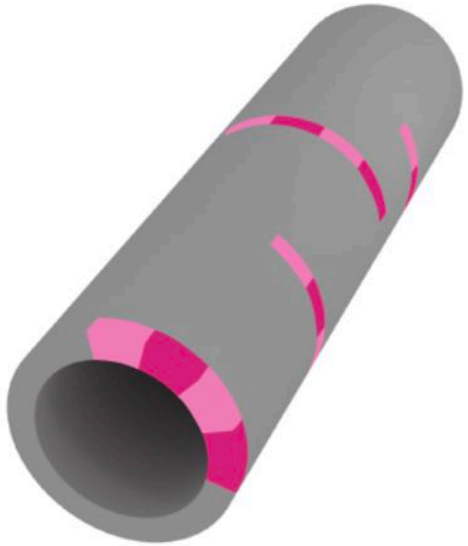


Endoplasmic Reticulum: Keeping in shape. Blackstone and Prinz (2016) eLife

Membrane tubulation



Reticulons

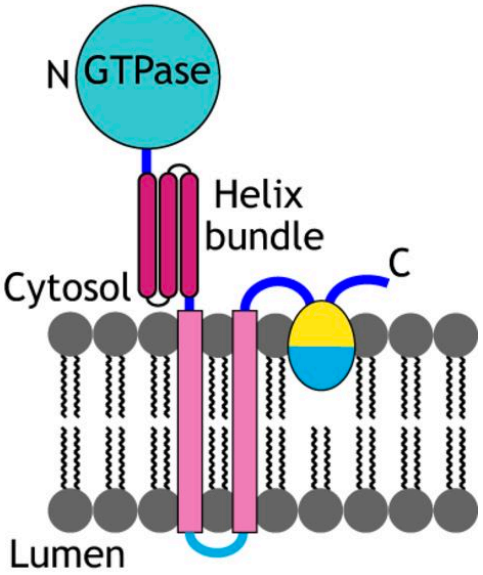


Membrane tubule

Reticulons decide the shape of the ER membrane

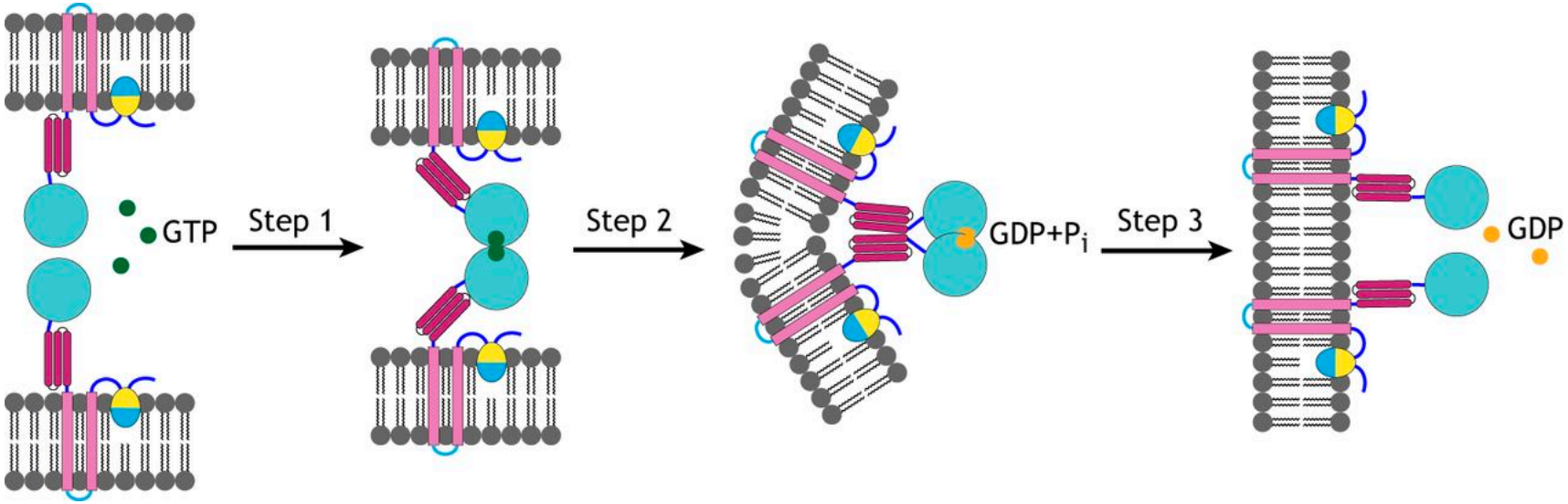
Reconstituting the reticular ER network – mechanistic implications and open questions Wang and Rapoport (2019) J. Cell Sci.

Membrane fusion



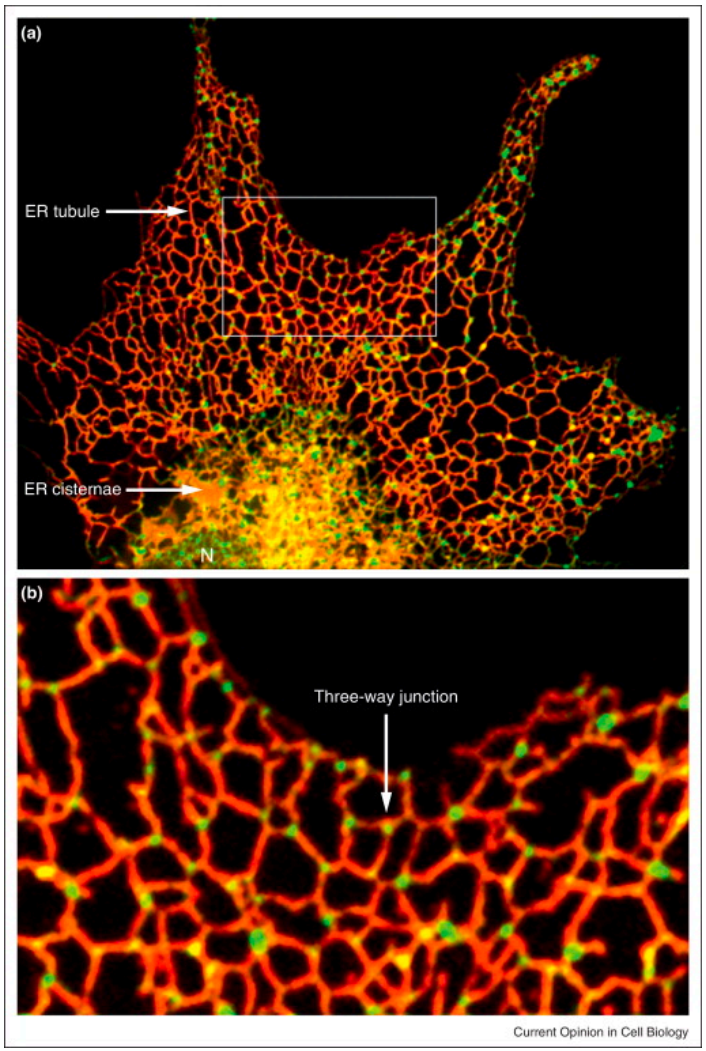
Atlastin

Atlastins fuse membranes

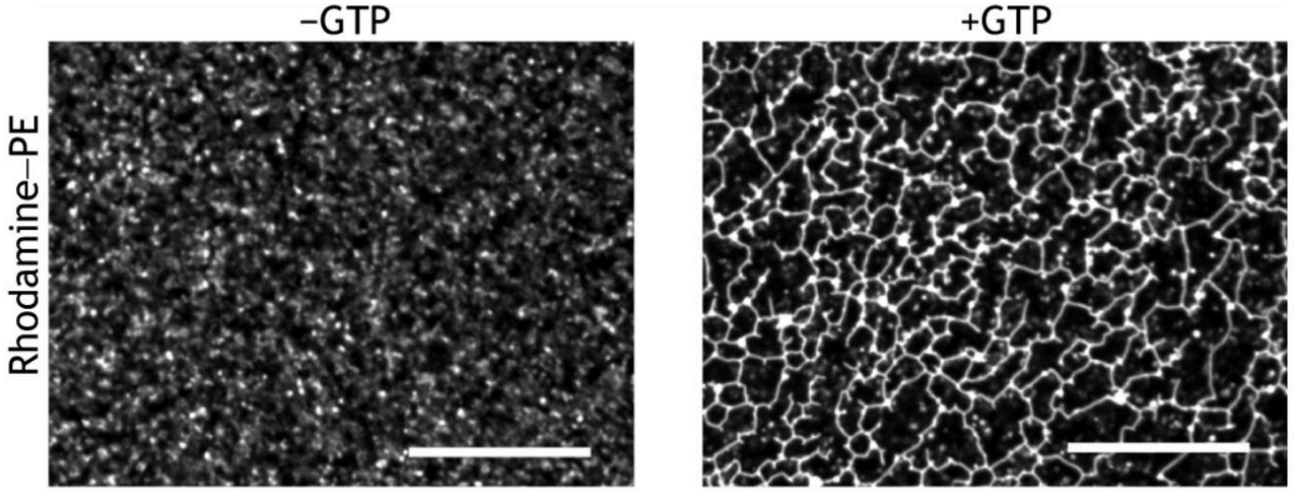


Reconstituting the reticular ER network – mechanistic implications and open questions Wang and Rapoport (2019) J. Cell Sci.

Proof-of-concept



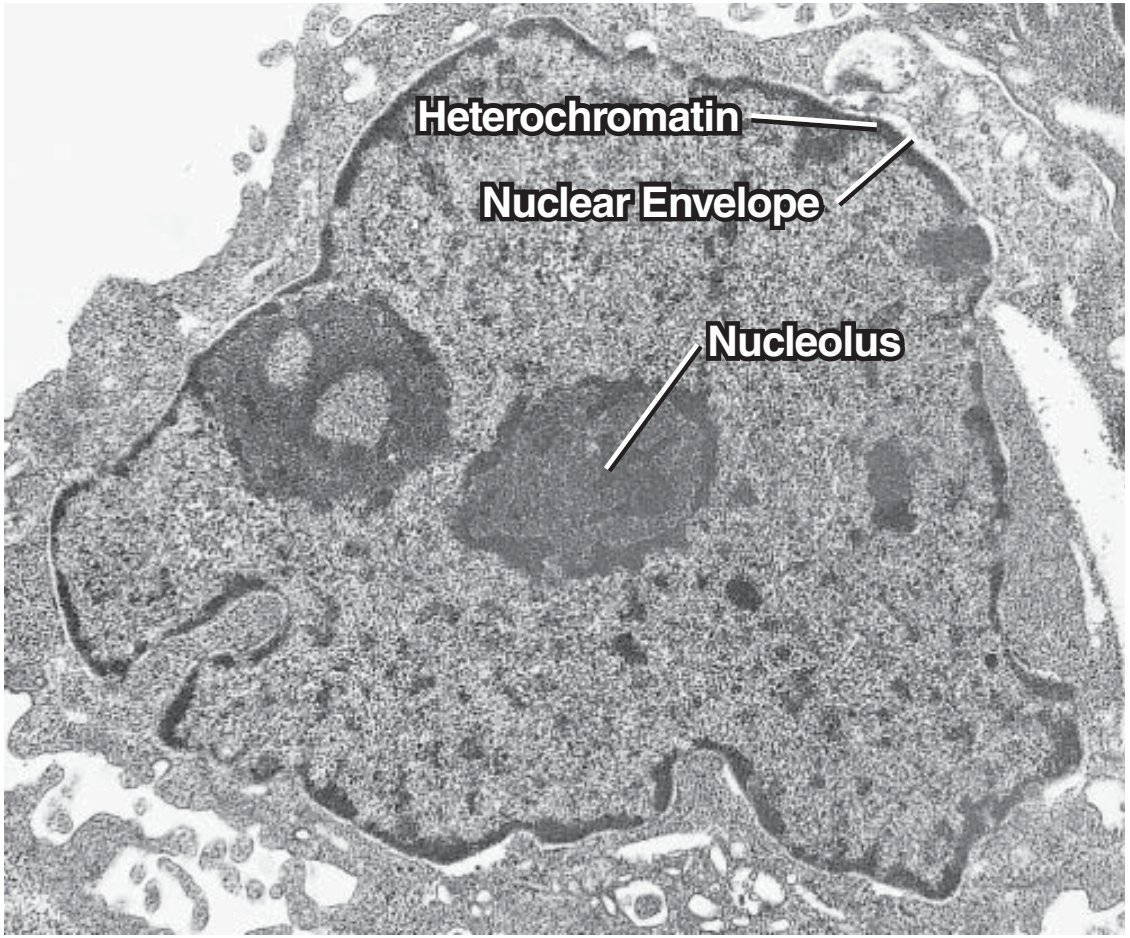
Structure of the ER in cells



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



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*

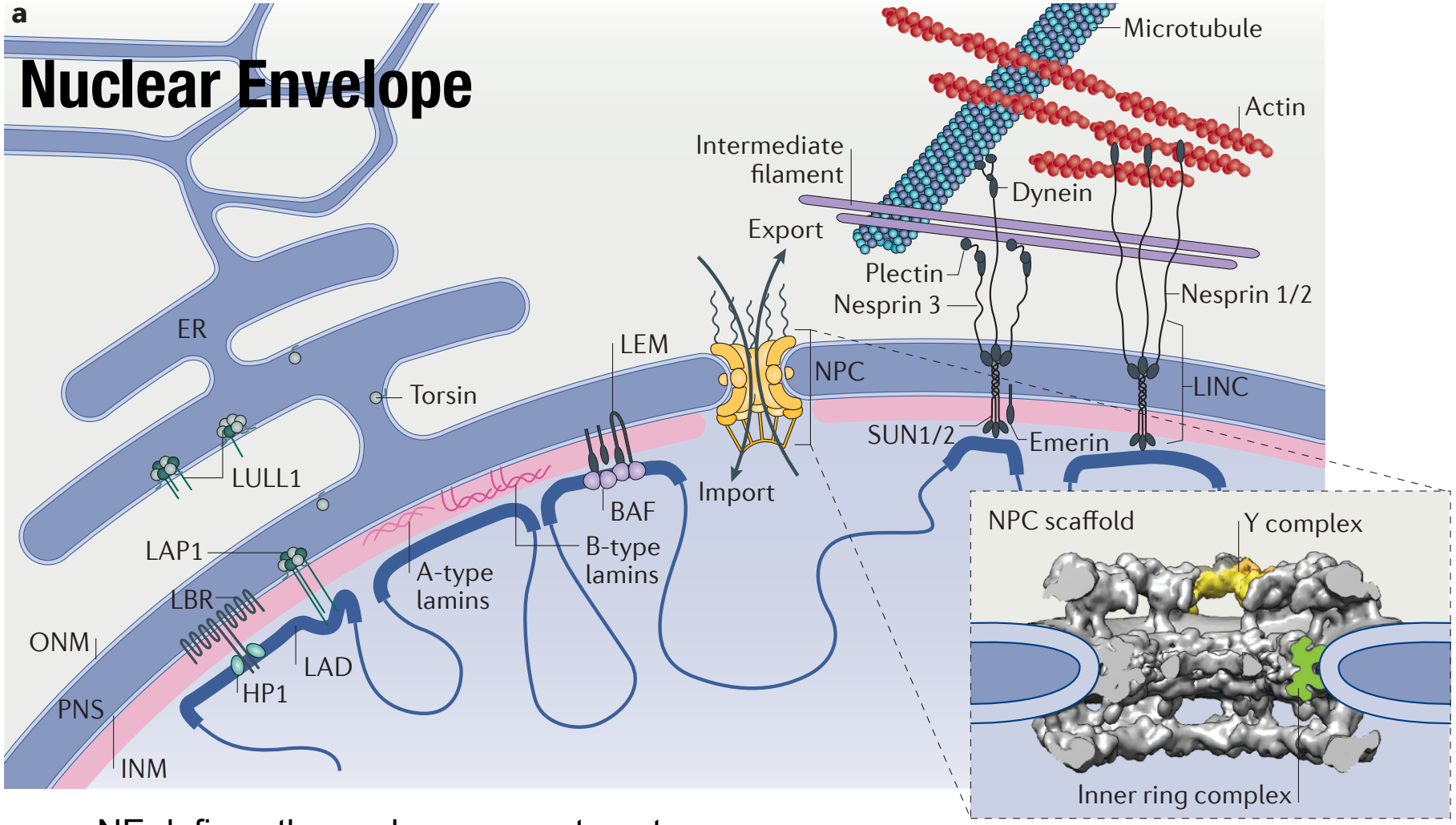
(2) One or more *nucleoli*, which are irregularly shaped electron-dense structures that function in the synthesis of ribosomal RNA and the assembly of ribosomes

(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*

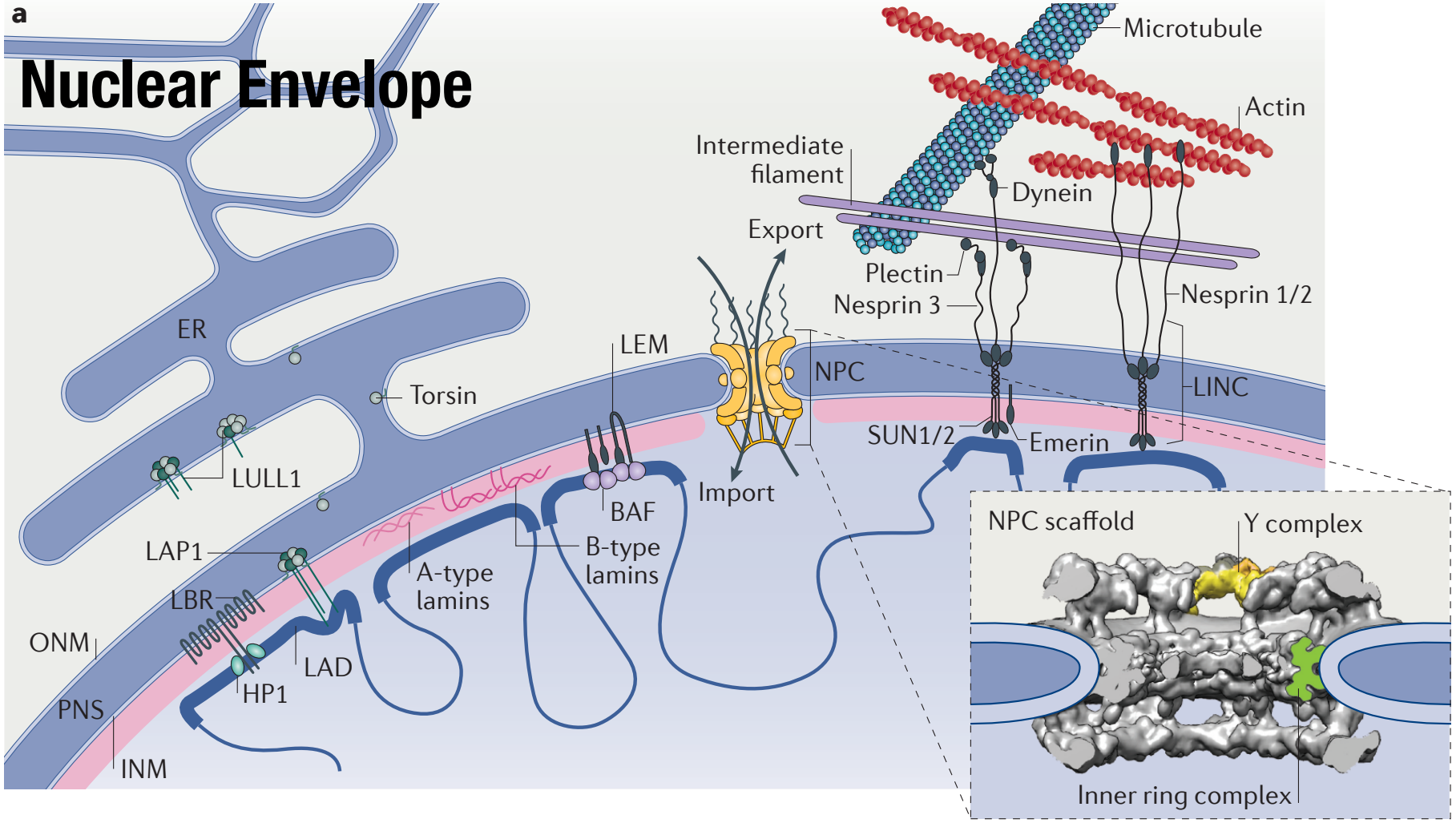
This envelope forms a boundary between the nucleus and cytoplasm.

Karp et al

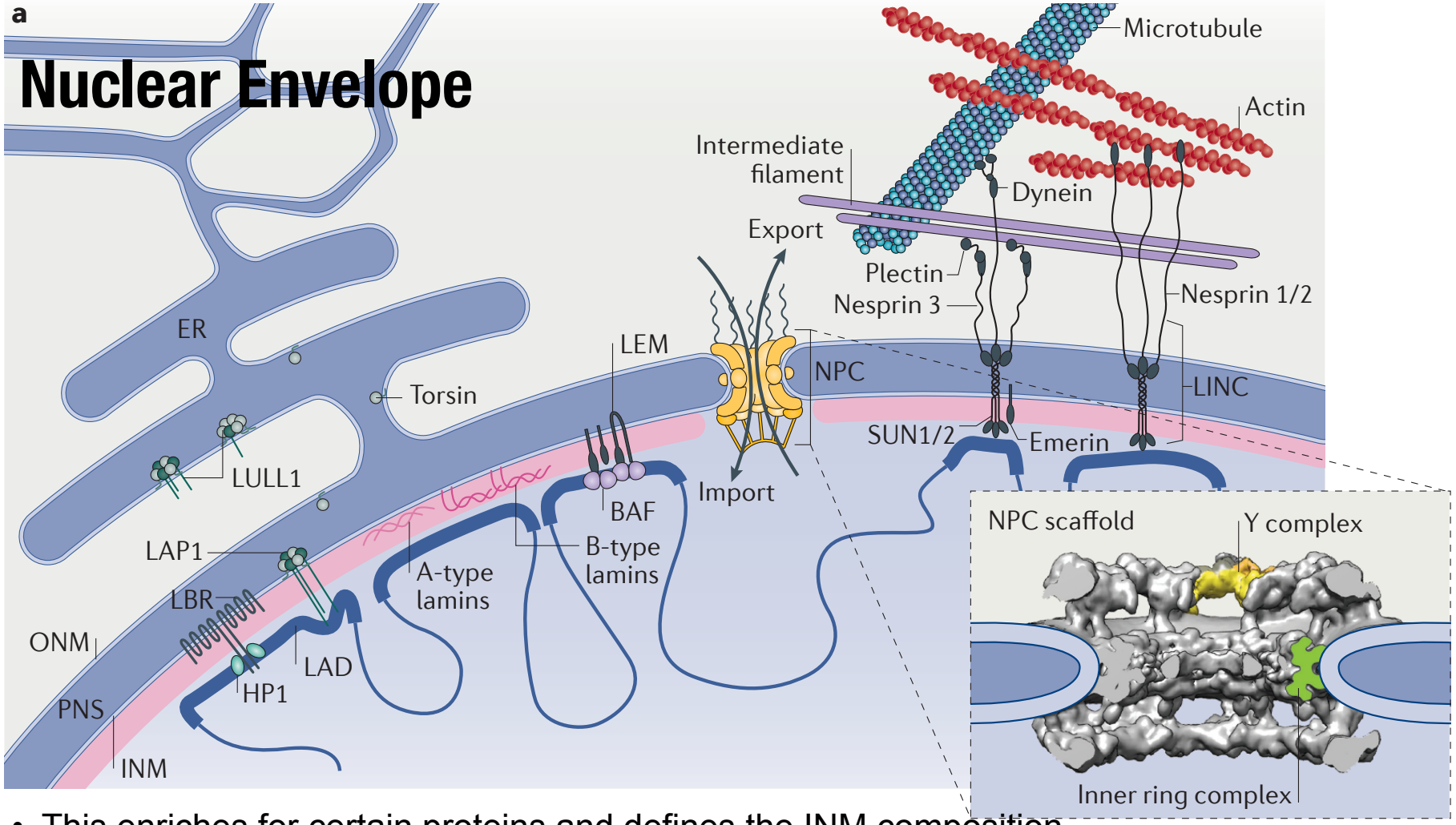


- NE defines the nuclear compartment
- NE is comprised of two concentric membranes - Outer nuclear membrane (ONM) and Inner nuclear membrane (INM)
- ONM and INM are separated by a distance of 10-50 nm
- NE contains about 60 integral membrane proteins
- NE is penetrated by nuclear pore complexes
- ONM 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

Ungricht & Kutay (2017) Nature Reviews Molecular Cell Biology

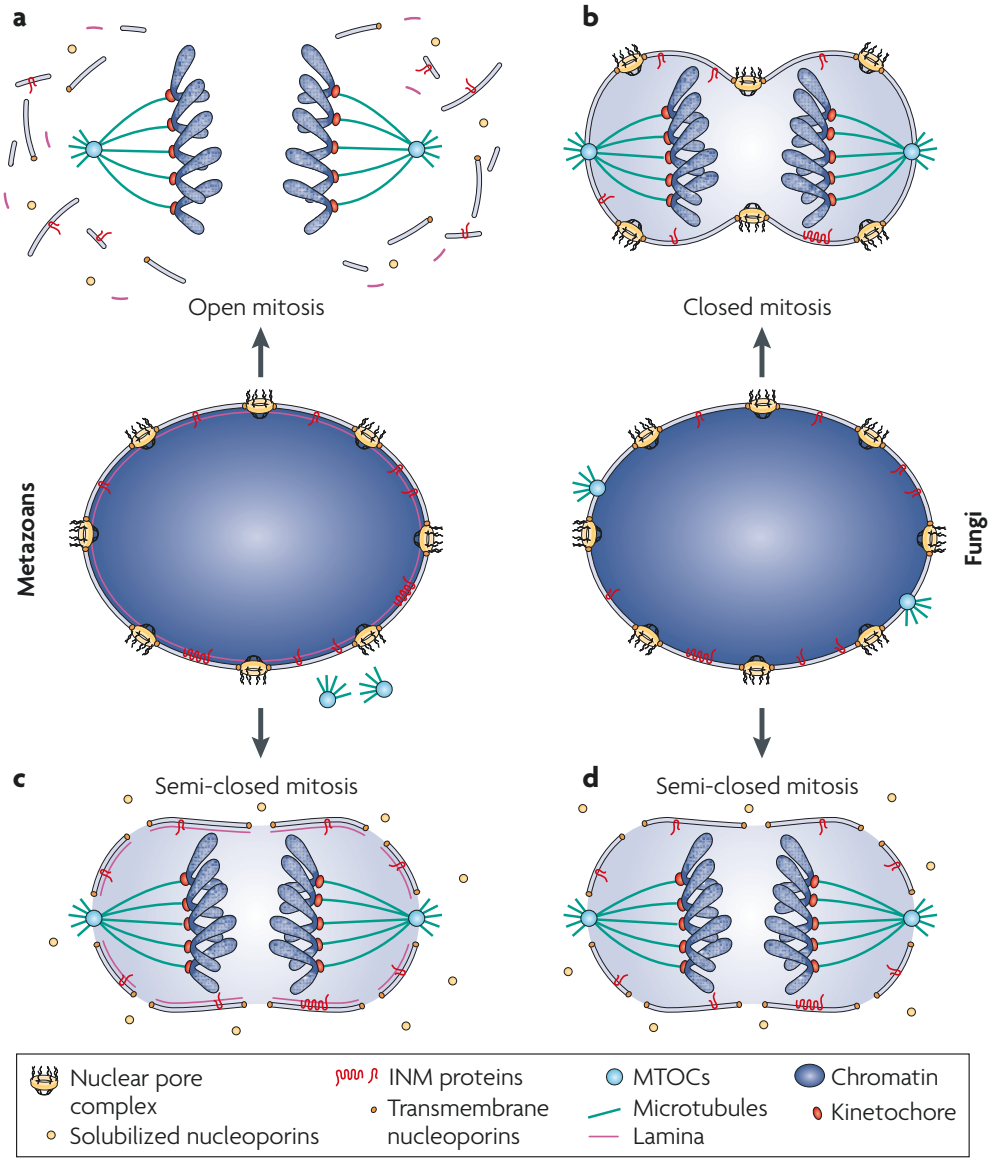


- NE incorporates new components during growth and to replace defective parts
- Adapts to mechanical challenges
- During open mitosis in higher eukaryotes, NE disassembles completely and then reforms
- Integral membrane proteins are co-translationally inserted into the ER network and distribute to the ONM and INM by diffusion
- Some of these proteins are retained by binding to chromatin and/or the nuclear lamina



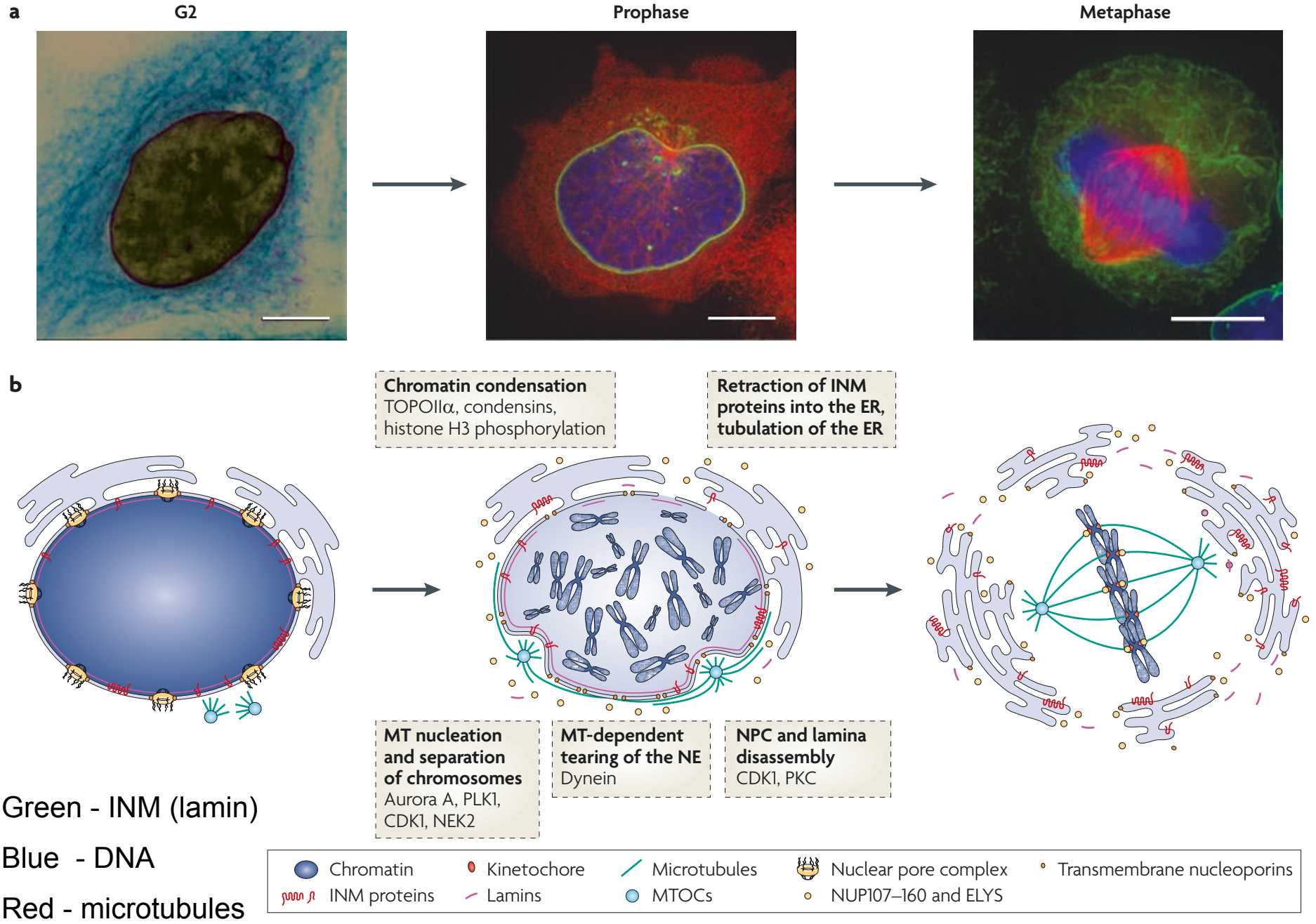
- This enriches for certain proteins and defines the INM composition
- Nuclear pore complexes (NPC) puncture the ONM and the INM
- NPCs restricts free diffusion and prevents the passage of membrane proteins with extralumenal domains larger than approximately 60 kDa to the INM.
- This NPC-based size restriction 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



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

Breakdown of the Nuclear Envelope



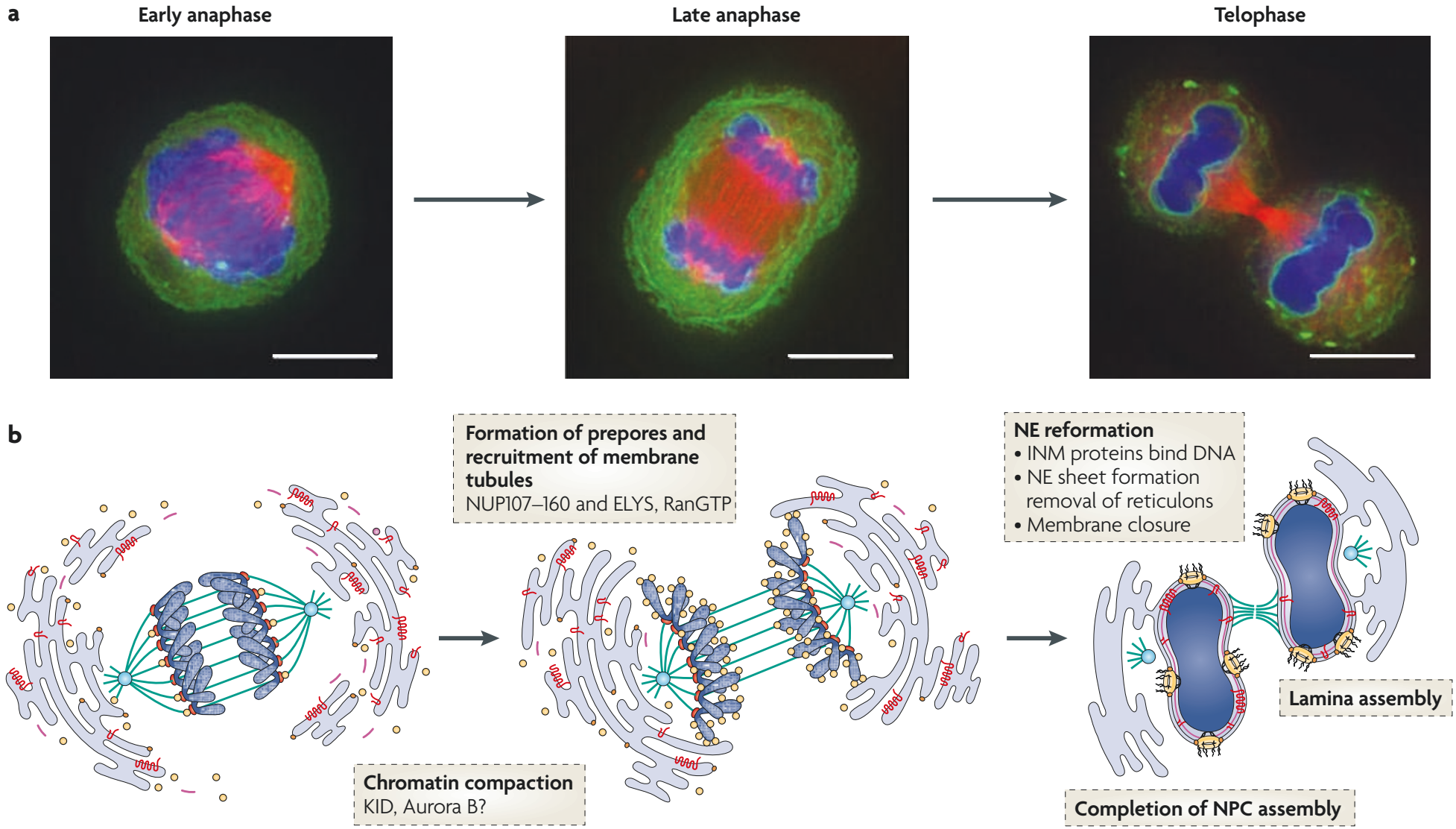
Green - INM (lamin)

Blue - DNA

Red - microtubules

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

Reformation of the Nuclear Envelope



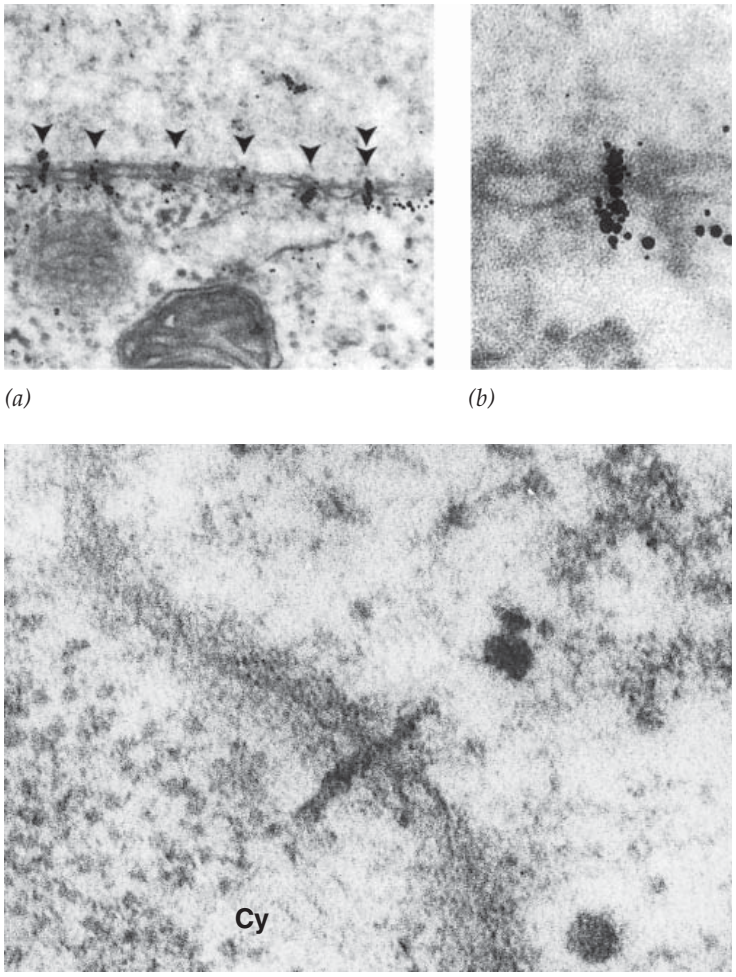
Green - INM (lamin)

Blue - DNA

Red - microtubules

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

Nuclear Transport

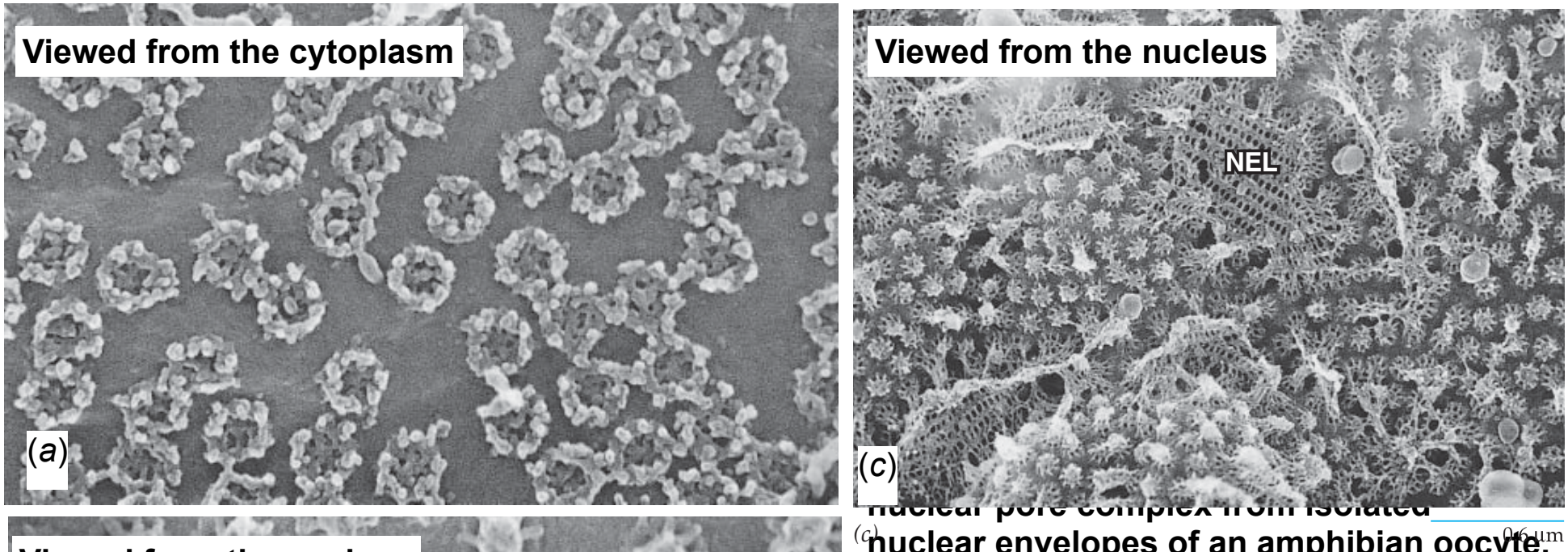


The movement of materials through the nuclear pore. (*a*) Electron micrograph of the nuclear–cytoplasmic border of a frog oocyte taken minutes after injection with gold particles that had been coated with a protein normally found in the nucleus. These particles pass through the center of the nuclear 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."

Ungricht & Kutay (2017) Nature Reviews Molecular Cell Biology

Nuclear pore complex (NPC)



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)

Huge, supramolecular complex - 15 to 30 times the mass of a ribosome (2.7 MDa)

Exhibits octagonal symmetry

Can rearrange to change diameter of the opening from about 20 to 40 nm.

Contain only about 30 different proteins (*nucleoporins)*, which are largely conserved between yeast and vertebrates.

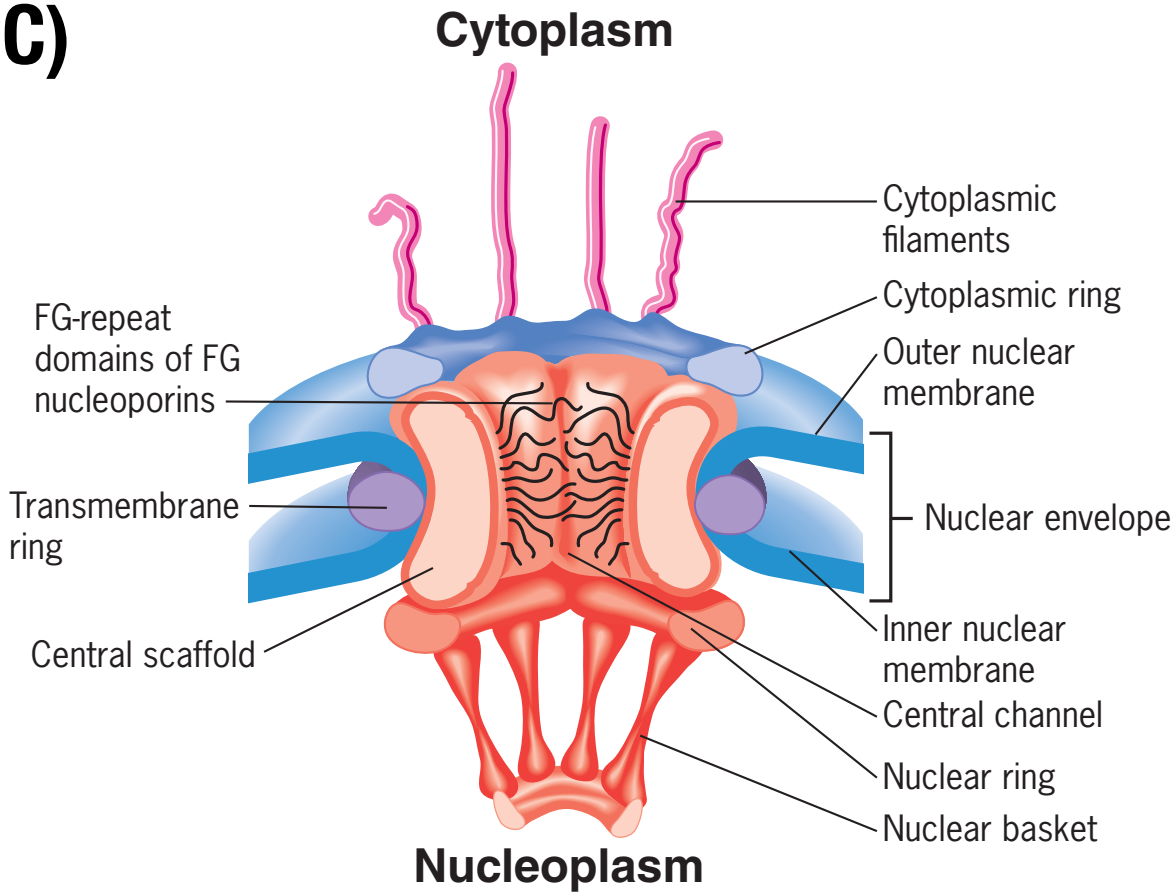
Nucleoporins lining the inner cavity contain stretches of Phe-Gly (FG) repeats

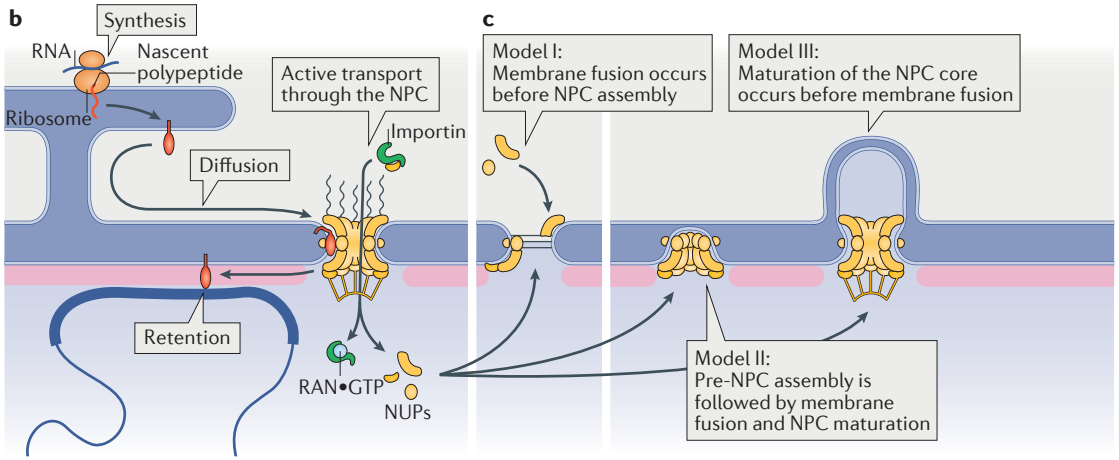
Clustered in a particular region of each nucleoporin to form the FG domain.

Possess a disordered structure that gives them an extended and flexible organization.

The FG domains form a hydrophobic meshwork or sieve that blocks the free diffusion of larger macromolecules (greater than about 40,000 Daltons) between the nucleus and cytoplasm.

Density of nuclear pore complexes correlates with nuclear activity - typical cells contain 3000–4000 complexes





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)

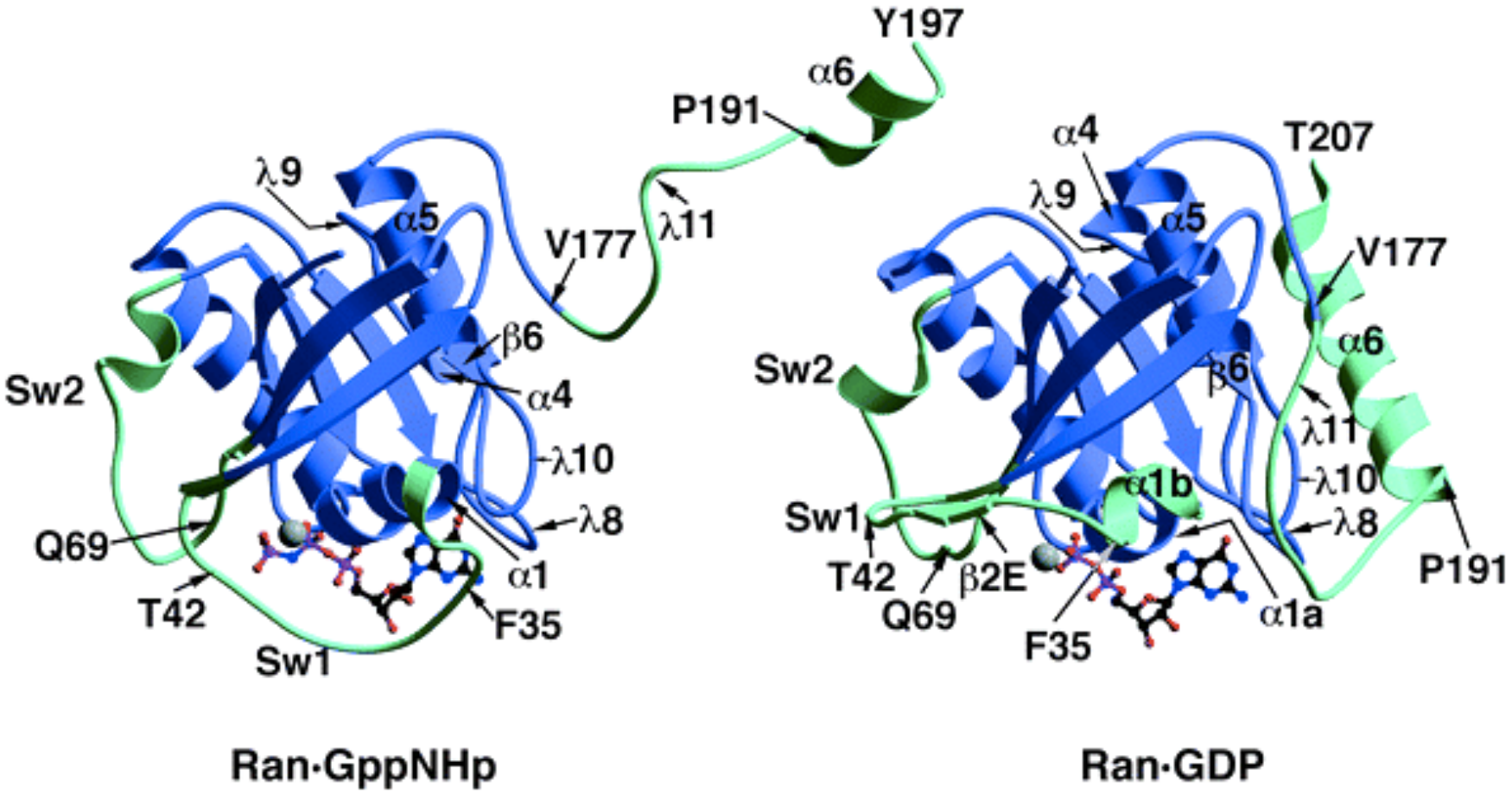
- **Function:** Tag proteins for export from the nucleus to the cytoplasm.
- **Composition:** Short, hydrophobic sequences, often 10-13 amino acids long, with specific hydrophobic residues.

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

Key Differences & Regulation

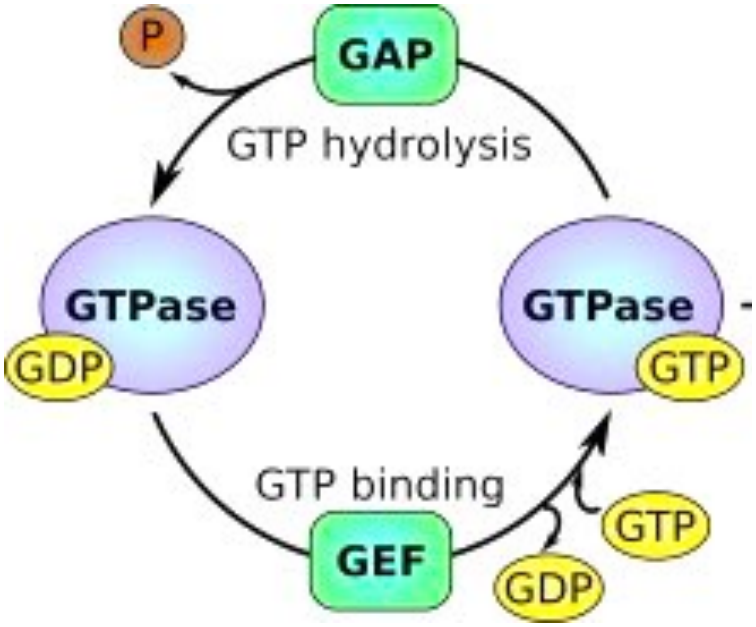
- **Charge:** NLSs are positive; NESs are hydrophobic.
- **Receptors:** Importins for NLSs; Exportins for NESs.

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



Chook and Blobel (1999) Nature

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

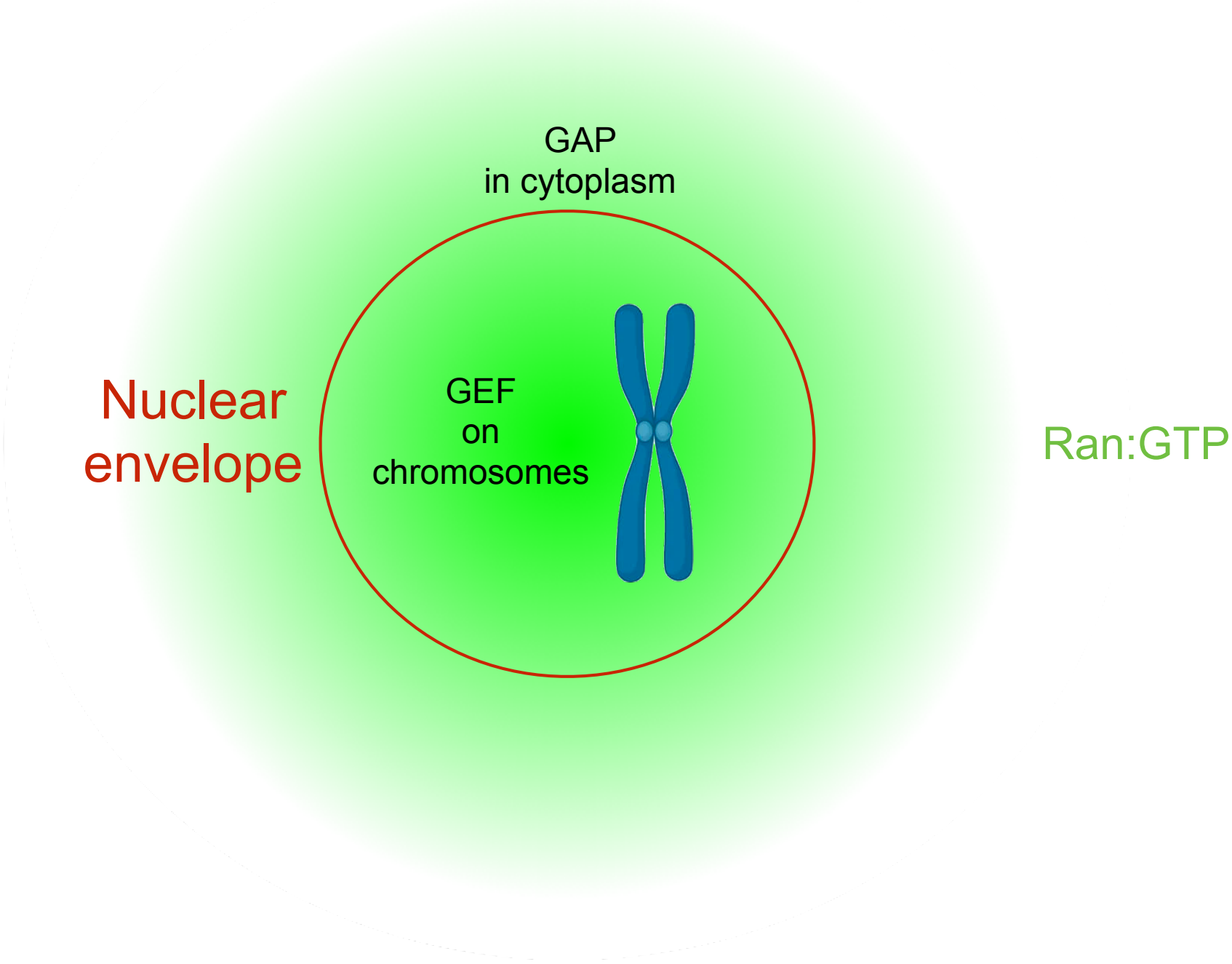
Ran-GEF is a chromatin-associated nuclear protein and facilitates exchange of GDP for GTP

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.

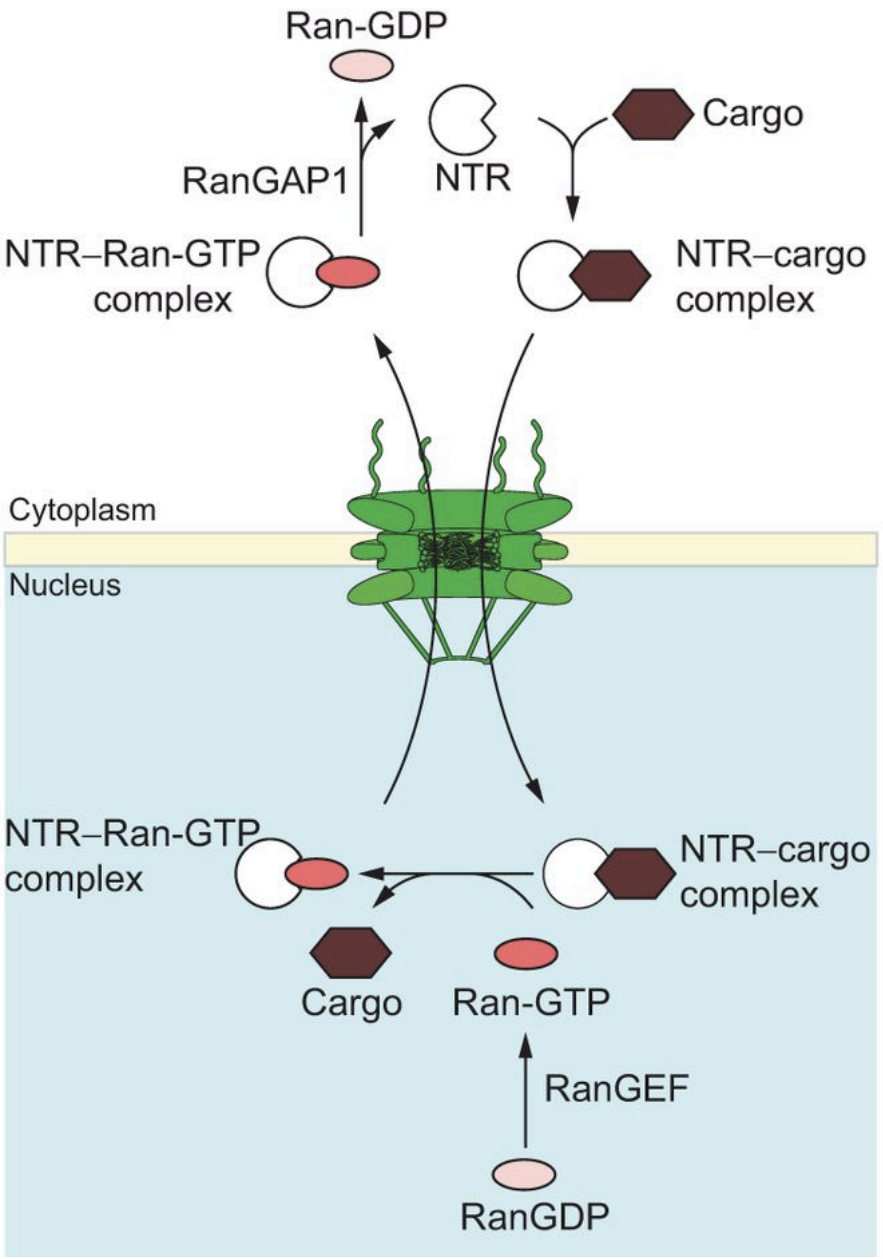
This distribution plays a key role in determining the directionality of nuclear transport.

Dasso (2001) Cell

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



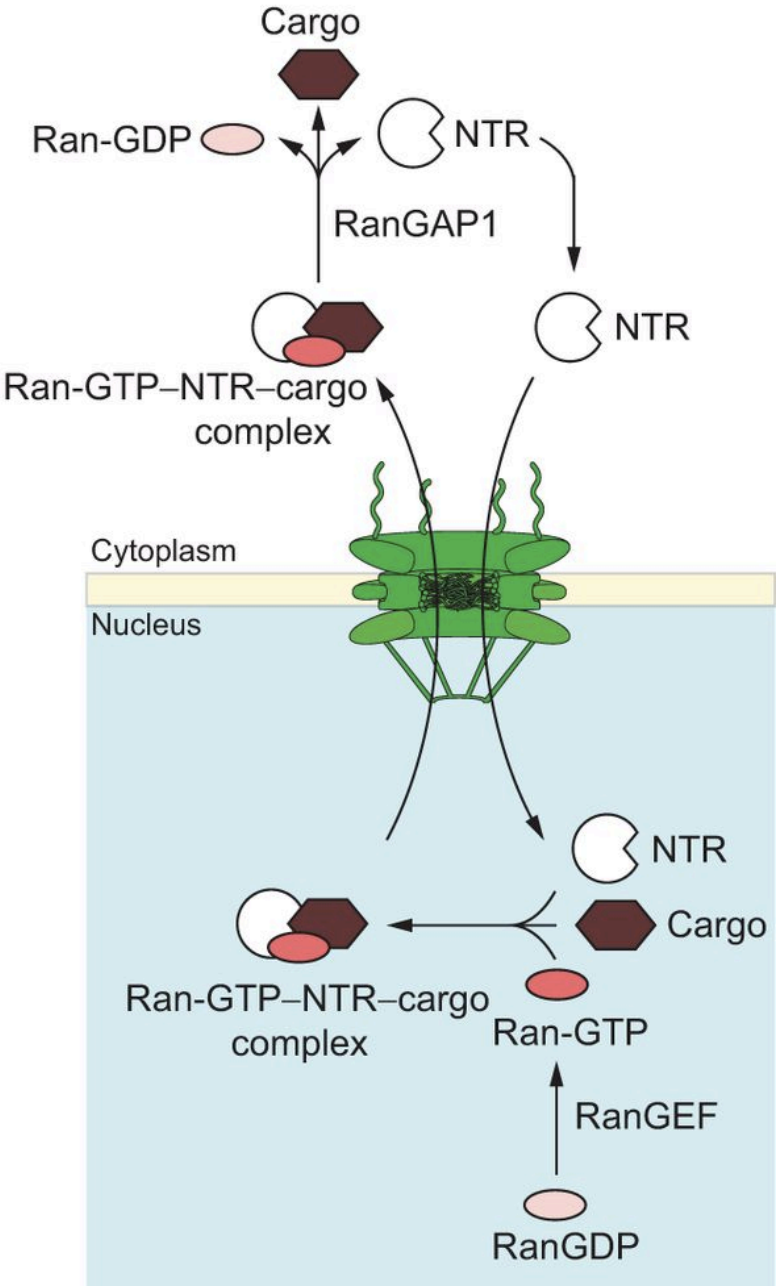
Nuclear Import Cycle



- 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

Journal of Cell Science (2016)

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)