

# Johns Hopkins Engineering

## Molecular Biology

Membrane Functions and the Endomembrane System



JOHNS HOPKINS  
WHITING SCHOOL  
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# Outline

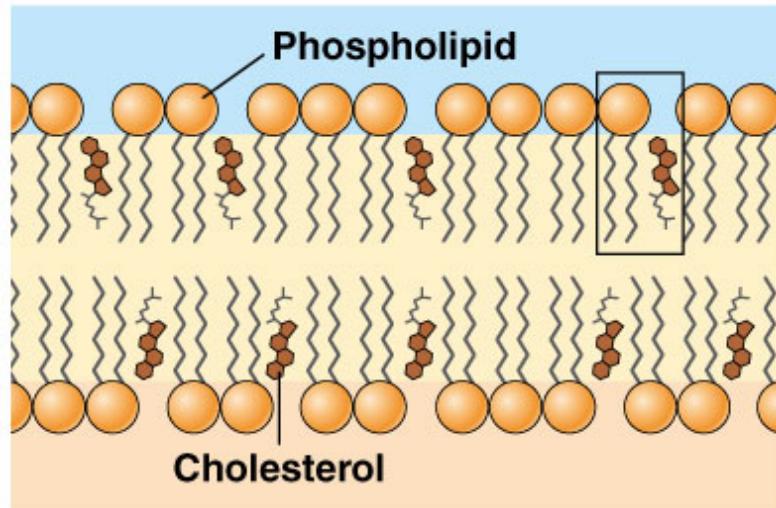
- Membrane composition
- Lipid rafts
- Membrane proteins
- Membrane biosynthesis
- Protein trafficking

# Membrane composition

- Most plasma membrane fatty acids vary in chain length and degree of saturation
- This helps to ensure that membranes are fluid at physiological temperatures
- Most unsaturated fatty acids have *cis* double bonds, unlike the commercially produced *trans* fats, which pack together like saturated fats do (*cis/trans* = relative orientation of functional groups within a molecule)

# Effects of Sterols on Membrane Fluidity

- Membrane fluidity is influenced by sterols
- The intercalation of rigid cholesterol molecules into a membrane decreases its fluidity at high temperatures
- However, cholesterol also prevents hydrocarbon chains of phospholipids from packing together tightly and so reduces the tendency of membranes to gel upon cooling
- Therefore cholesterol is a **fluidity buffer**; sterols in other organisms may function similarly



**(a) Cholesterol in plasma membrane**

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# Other effects of sterols on membranes

- Sterols decrease the permeability of membranes to ions and small polar molecules
- This is likely because they fill spaces between the hydrocarbon chains of phospholipids
- This effectively blocks the routes that ions and small molecules would take through the membrane

# Lipid Rafts Are Localized Regions of Membrane Lipids That Are Involved in Cell Signaling

- Recent theory- localized regions of membrane lipids, in association with specific proteins, are *lipid microdomains*, or **lipid rafts**
- These are dynamic, changing composition as lipids and proteins move into and out of them
- Lipid rafts in the outer monolayer of animal cells have elevated levels of cholesterol and glycosphingolipids and are less fluid than the rest of the membrane

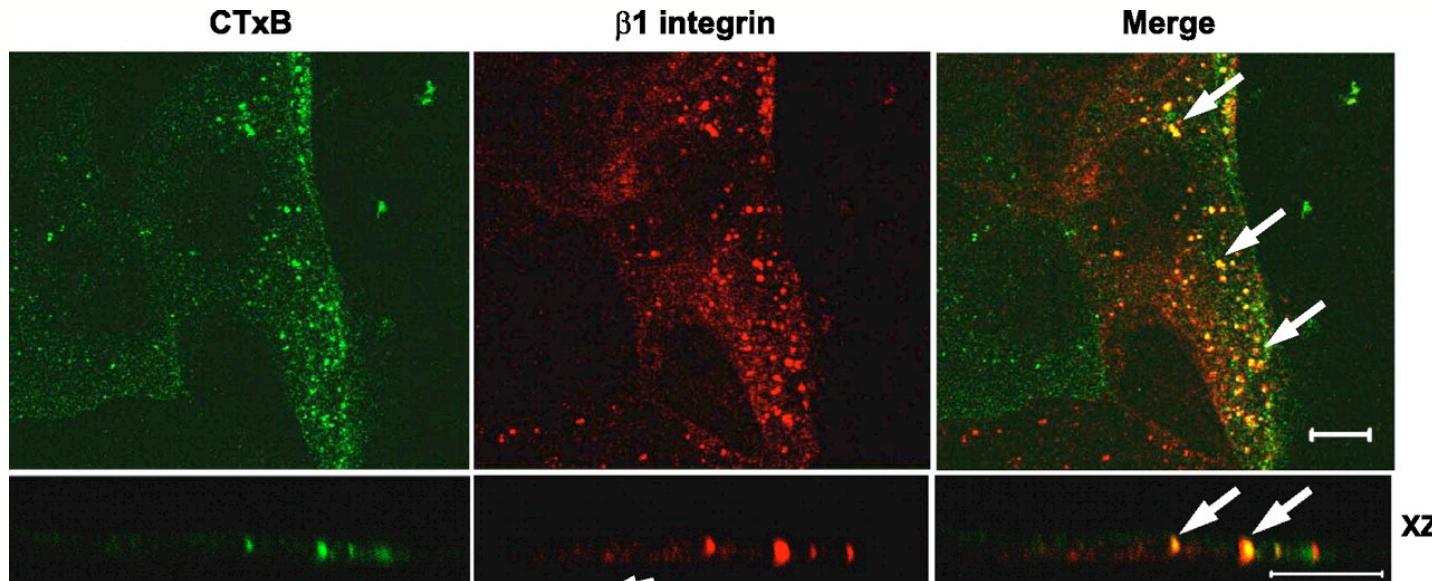
# Functions of lipid rafts

- Lipid rafts are thought to have roles in detecting and responding to extracellular signals
- For example, lipid rafts have roles in
  - transport of nutrients and ions across membranes
  - binding of activated immune system cells to their microbial targets
  - transport of cholera toxin into intestinal cells

# Receptors in lipid rafts

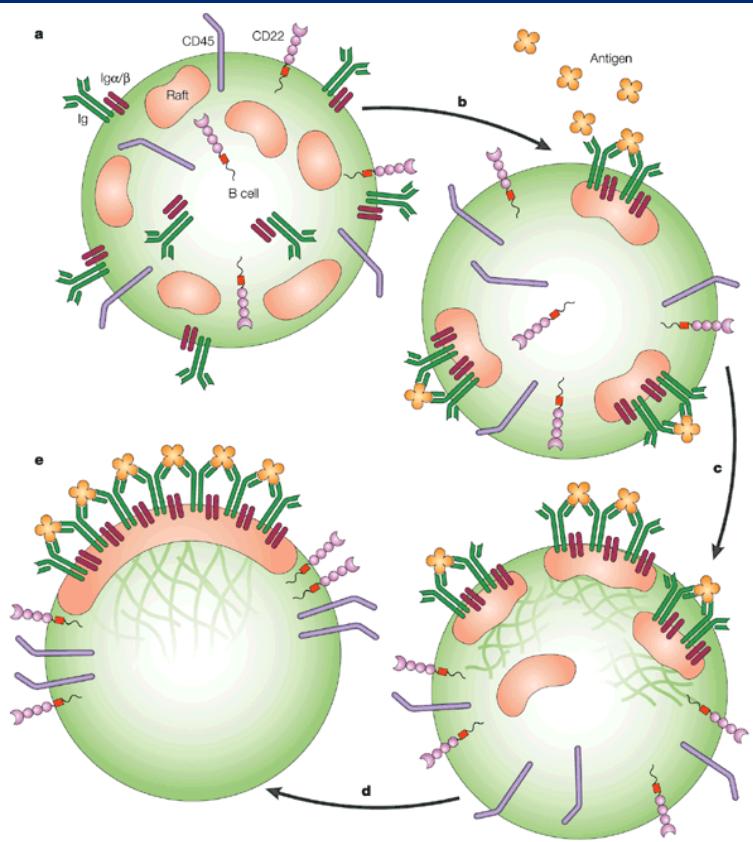
- When a receptor molecule on the outer surface of the plasma binds its ligand, it can move into lipid rafts also located in the outer monolayer
- Lipid rafts containing receptors are coupled to lipid rafts on the inner monolayer
- Some lipid rafts contain kinases, enzymes that generate second messengers in a cell via phosphorylation (addition of a phosphate group) of target molecules

# Lipid Rafts



Cholera toxin subunit B (CTxB) cointernalizes with  $\beta 1$ -integrin at the leading edge of the migrating intestinal epithelial cells (IECs).

[Am J Physiol Gastrointest Liver Physiol](#). 2008 Nov; 295(5): G965–G976.



In resting B cells, the B-cell receptor (BCR) is excluded from lipid rafts along with other membrane proteins, including CD45 and CD22 (a). After antigen binding, the oligomerized BCR associates with rafts by a mechanism that is independent of the actin cytoskeleton and does not require the activity of Lyn (b). Signaling is initiated in rafts, leading to the assembly of signaling complexes and association with the actin cytoskeleton. The initiation of signaling promotes raft clustering (c). Clustering continues as the clustered rafts are moved to one pole of the cell in a process that probably involves the actin cytoskeleton (d). Where antigen is expressed on the surface of another cell, polarization would lead to synapse formation (e).

*Nature Reviews Immunology* volume 2, pages96–105 (2002)

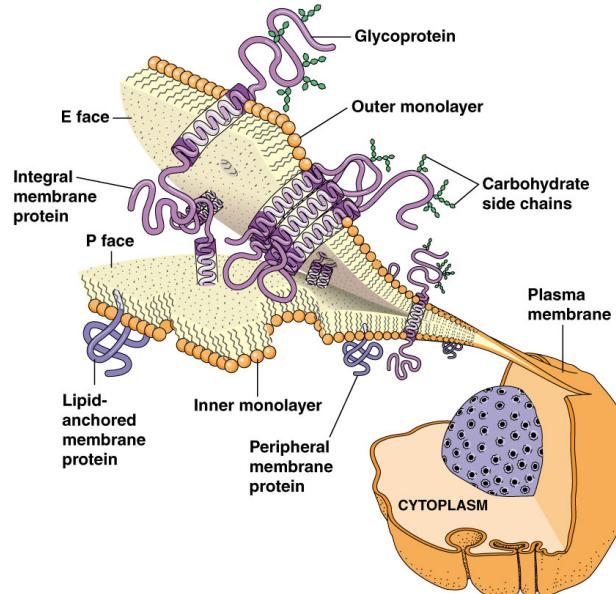
# The Membrane Consists of a Mosaic of Proteins: Evidence from Freeze-Fracture Microscopy

Support for the fluid mosaic model came from studies involving **freeze-fracturing**

A bilayer or membrane is frozen and then hit sharply with a diamond knife

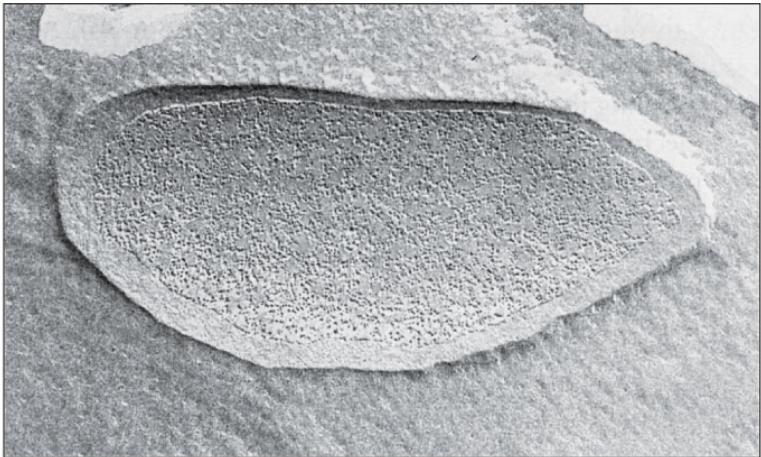
The resulting fracture often follows the plane between the two layers of membrane lipid

(a) Separation of membrane monolayers. Notice how the fracture plane has passed through the hydrophobic interior of the membrane, revealing the inner surfaces of the two monolayers. Integral membrane proteins that remain with the outer monolayer are seen on the E (exoplasmic) face, whereas those that remain with the inner monolayer are seen on the P (protoplasmic) face.



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# Freeze-fracture microscopy



(a) Erythrocyte plasma membrane

0.5  $\mu\text{m}$



(b) Chloroplast membrane

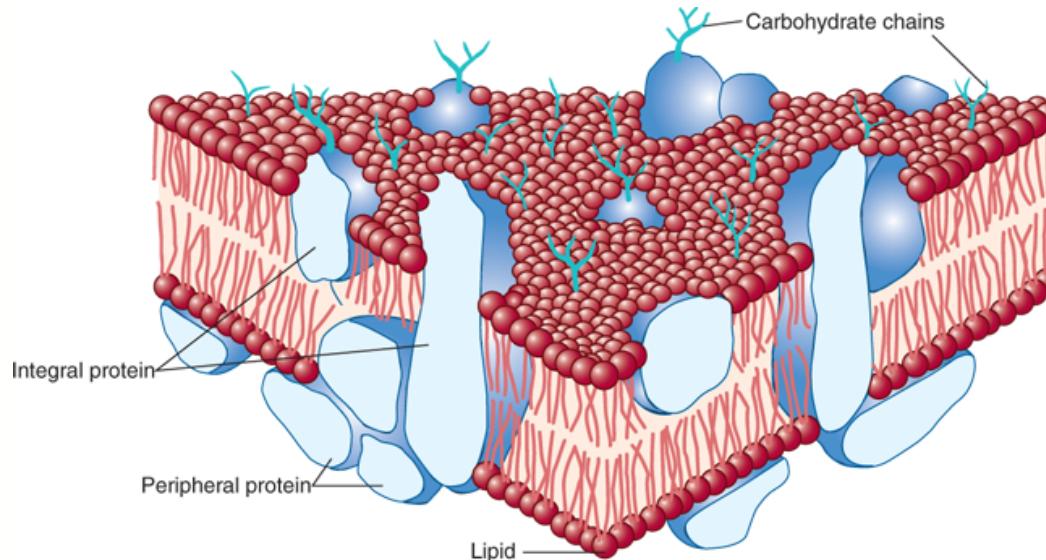
0.2  $\mu\text{m}$

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**Membrane proteins appear as discrete particles within the lipid bilayer**

# Membrane Proteins Are Oriented Asymmetrically Across the Lipid Bilayer

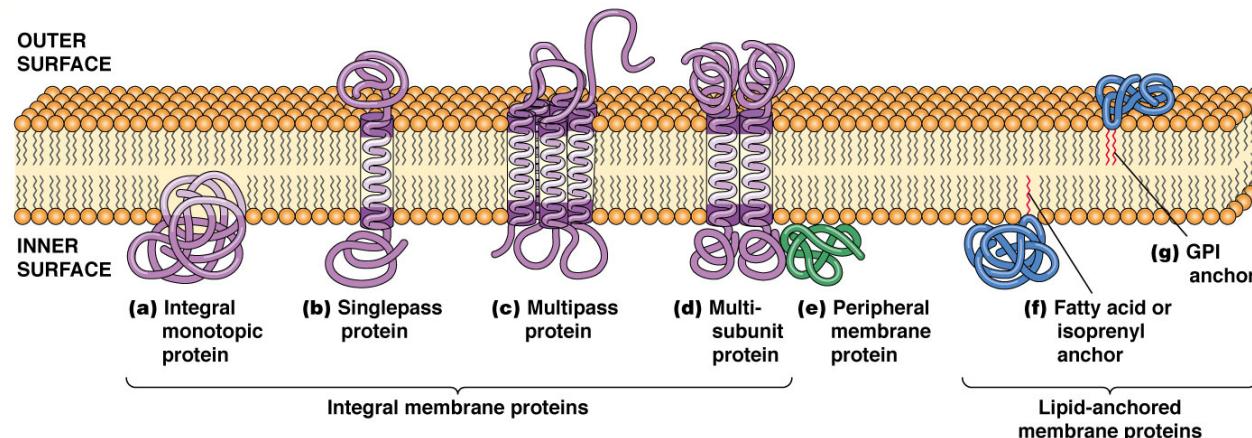
- Once in place, in or on one of the monolayers, proteins cannot move across the membrane from one surface to the other



Source: V. W. Rodwell, D. A. Bender, K. M. Botham, P. J. Kennelly, P. A. Weil: Harper's Illustrated Biochemistry, 13th Edition  
[www.accessmedicine.com](http://www.accessmedicine.com)  
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# Membranes Contain Integral, Peripheral and Lipid-Anchored Proteins

- Membrane proteins have different hydrophobicities and so occupy different positions in or on membranes
- **REVIEW:** Membrane proteins fall into three categories: *integral*, *peripheral*, and *lipid-anchored*



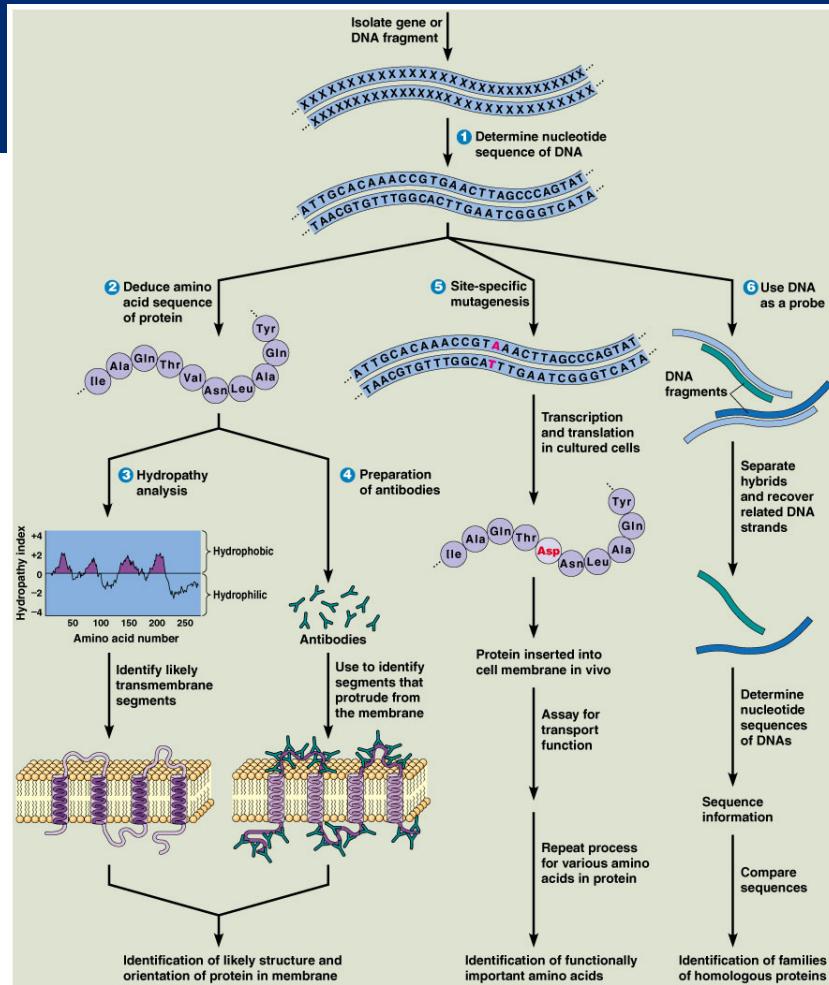
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# Molecular Biology Has Contributed Greatly to Our Understanding of Membrane Proteins

- Within the past three decades the study of membrane proteins has been revolutionized by DNA sequencing and recombinant DNA technology
- Amino acid sequence can be deduced from DNA
- **This increases our understanding of structural and functional relationships among proteins**

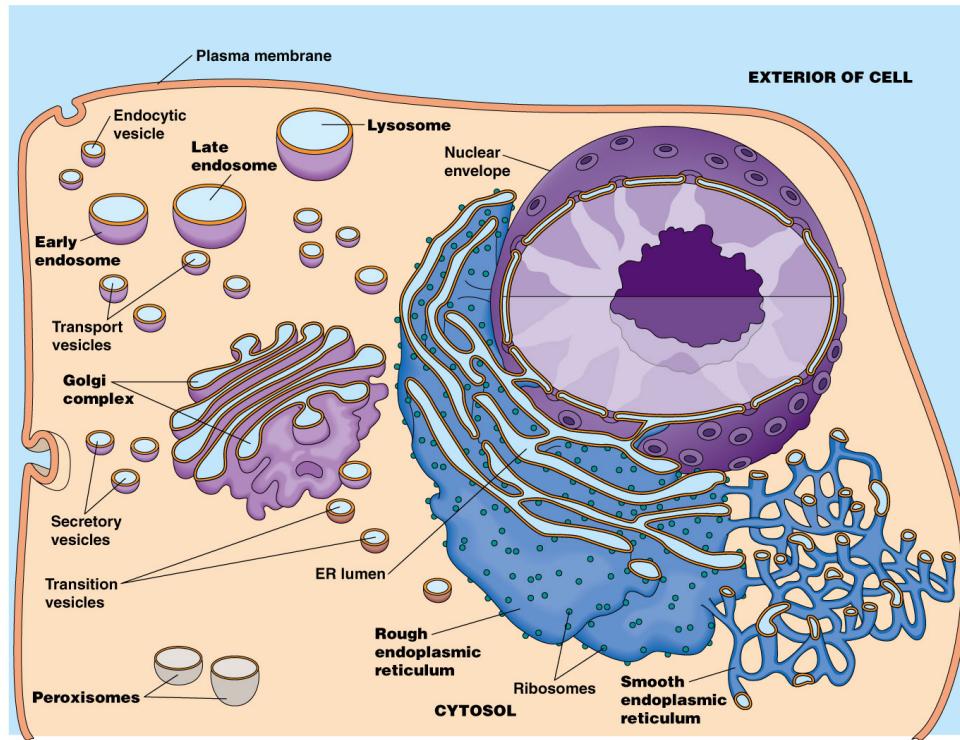
# DNA Sequencing

- Allows determination of the amino acid sequence of a protein without the need to isolate it in purified form
- Reveals evolutionary and functional relationships between proteins
- Allows specific mutation in the protein sequence to allow determination of effects on function (site specific mutagenesis)



# The Endomembrane System

Membranes define cellular borders and organelles but are also involved in transport, signaling, and adhesion



# Membrane biosynthesis

- Fatty acids for membrane phospholipids are synthesized in the cytoplasm and incorporated into the ER membrane on the cytosolic side
- They are transferred to the luminal side of the *bilayer* by enzymes
- The distinct composition of cytosolic and luminal monolayers established in the ER is transferred to other cellular membranes (vesicles from the ER membrane fuse with other organelles of the endomembrane system)

# Variation in amounts of rough and smooth ER

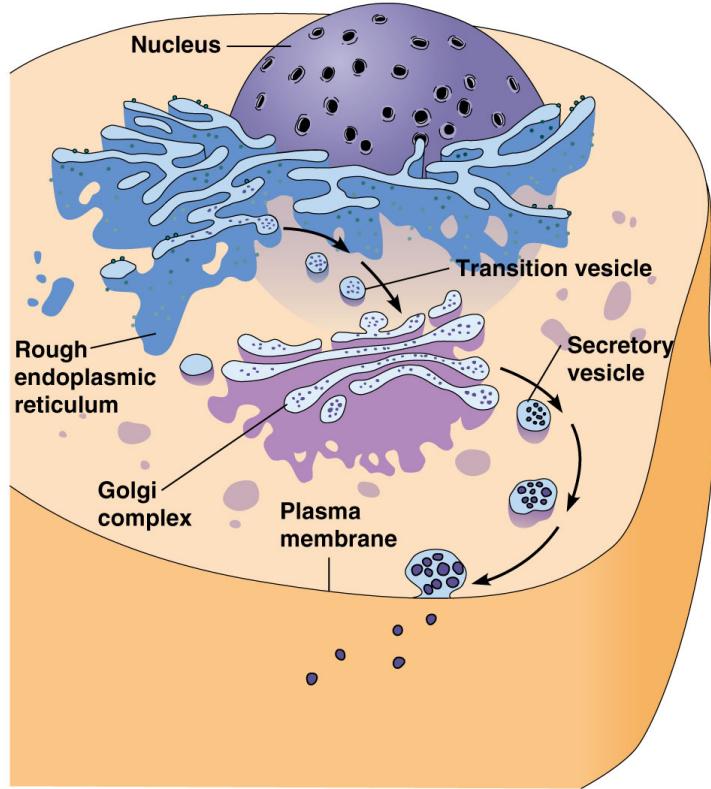
- Both types of ER are present in most cells but there is variation in the relative amounts
- Cells involved in synthesis of secretory proteins have prominent rough ER networks (e.g. fibroblasts in skin secrete collagen)
- Cells producing steroid hormones tend to have extensive networks of smooth ER (e.g. cells of the adrenal gland)

# The Golgi Complex

- The **Golgi complex** plays an important role in processing and packaging secretory proteins, and in complex polysaccharide synthesis
- Here, glycoproteins and membrane lipids from the ER undergo further processing and are sorted and packaged for transport (via the *trans*-Golgi network or TGN)
- Thus the Golgi complex plays a central role in *membrane and protein trafficking* in eukaryotic cells

# Secretory Vesicles

- From the Golgi, materials to be exported from the cell are packaged into **secretory vesicles**
- These move to the plasma membrane and fuse with it, releasing their contents outside the cell
- The ER, Golgi, secretory vesicles and lysosomes make up the *endomembrane system* of the cell, responsible for *trafficking* substances through the cell

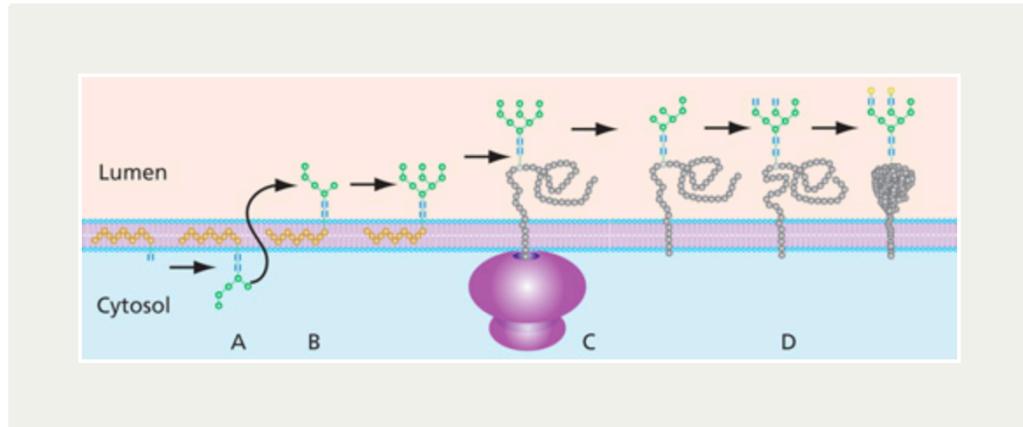


# Roles of the ER and Golgi in Protein Glycosylation

- Much of the protein processing carried out in the ER and Golgi involves **glycosylation**, the addition of carbohydrate side chains to proteins
- This forms **glycoproteins**.
- Enzyme-catalyzed reactions then modify the oligosaccharide side chain
- This increases the diversity of proteins and impacts function

# Initial Glycosylation Occurs in the ER

- The initial steps of N-glycosylation take place on the cytosolic surface of the ER membrane
- Later steps take place in the ER lumen
- All carbohydrate side chains initially have a common **core oligosaccharide**



**Figure 5.** General process of N-linked glycan construction in the ER. The glycan precursor is assembled on a dolichol phosphate base while in the cytoplasm of the ER (**A**). Once the glycan is switched to the lumen side (**B**), the glycan has additional sugars added and removed (trimmed) before being attached to the protein (**C**), where additional processing takes place (**D**).

<https://www.sigmaaldrich.com/technical-documents/articles/biology/glycobiology/n-glycans.html>

# Roles of the ER and Golgi Complex in Protein Trafficking

- Proteins synthesized in the rough ER must be directed to a variety of locations
- Once a protein reaches its destination, it must be prevented from leaving
- Each protein contains a specific “tag,” targeting it to a transport vesicle that will take it to the correct location

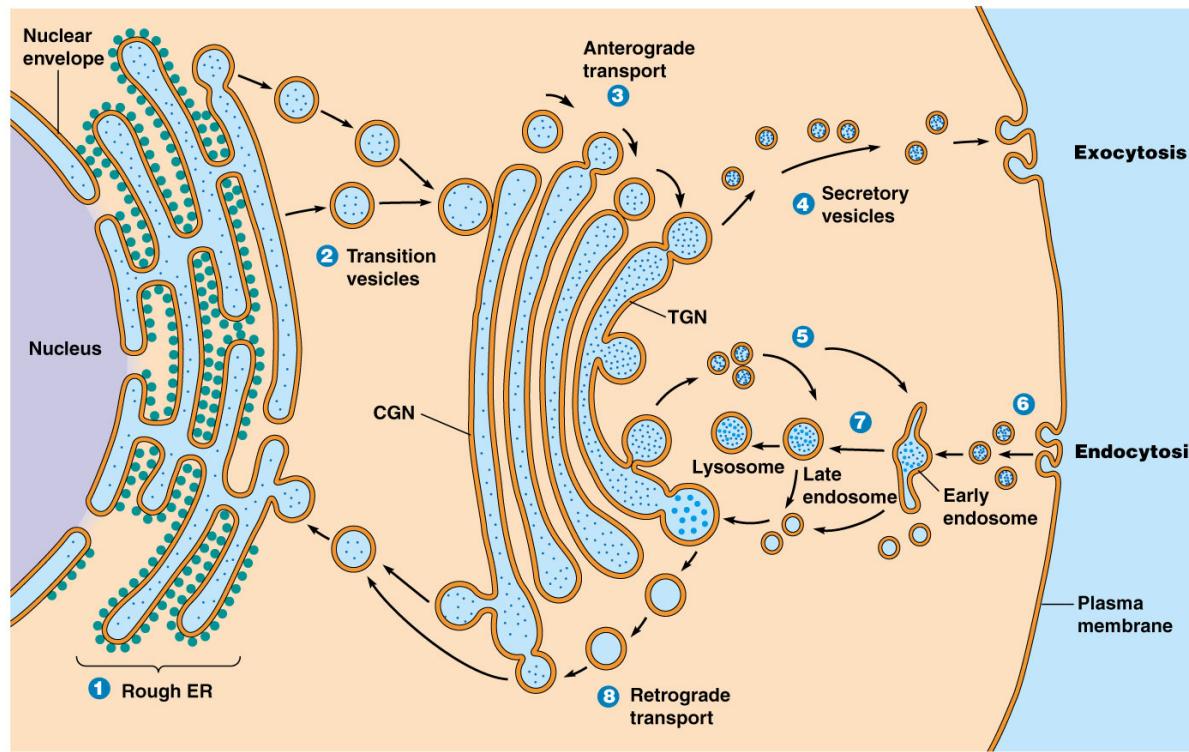
# Protein and Lipid Tags

- Depending on the protein and destination, a tag may be an amino acid sequence, a hydrophobic domain, or oligosaccharide side chain, or some other feature
- Tags can also be used to exclude material from certain vesicles
- Membrane lipids may also be tagged to help vesicles reach their destinations
  - Lipid tags can be one or more phosphate groups

# Overview of trafficking

- Sorting of proteins begins in the ER and early compartments of the Golgi
- There are mechanisms to retrieve or retain compartment-specific proteins
- The final sorting of material that will leave the Golgi complex occurs in the TGN (*trans*-Golgi Network)
- 2010 Nature Review- Golgi transport models
  - <http://www.nature.com/scitable/topicpage/how-do-proteins-move-through-the-golgi-14397318>

# Overview of trafficking through the endomembrane system



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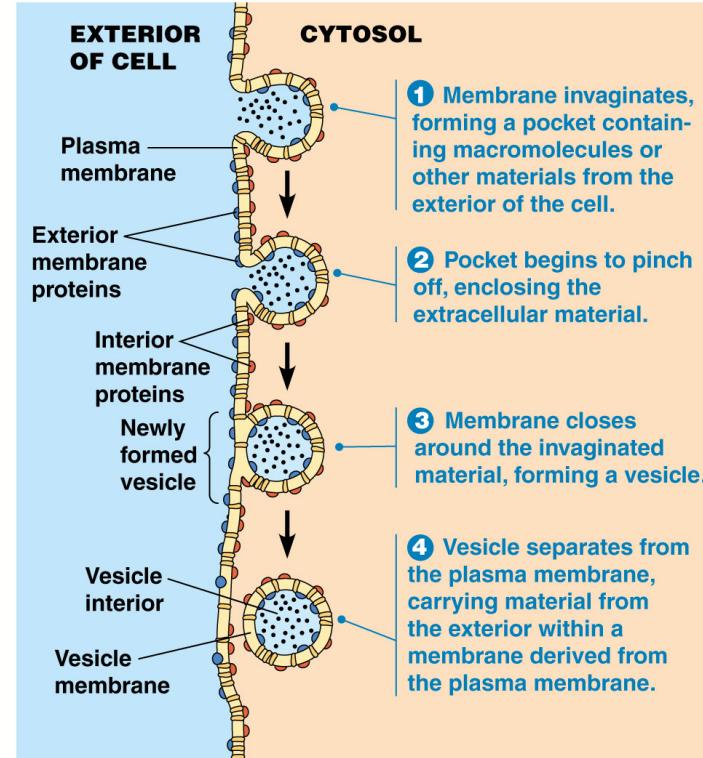
<https://www.youtube.com/watch?v=rvfvrGk0MfA>

# Exocytosis and Endocytosis: Transporting Material Across the Plasma Membrane

- Two methods (unique to eukaryotes) for transporting materials across the plasma membrane are
  - **Exocytosis**, the process by which secretory vesicles release their contents outside the cell
    - Example: Animal cells secrete hormones, mucus, milk proteins, and digestive enzymes this way; Plant and fungal cells secrete enzyme and structural proteins for the cell wall
  - **Endocytosis**, the process by which cells internalize external materials

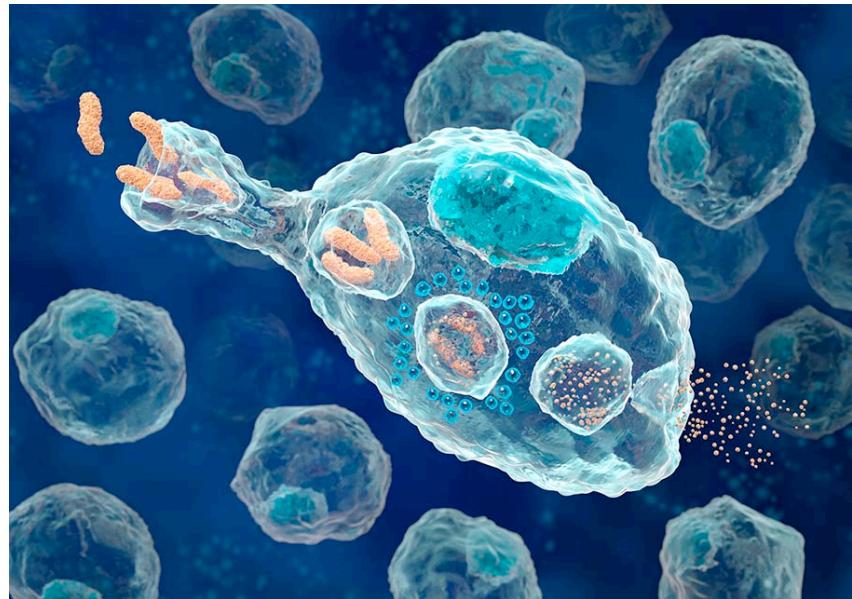
# Endocytosis Imports Extracellular Molecules by Forming Vesicles from the Plasma Membrane

- Most eukaryotic cells carry out one or more forms of endocytosis for uptake of extracellular material
- A small segment of the plasma membrane folds inward (1)
- Then it pinches off to form an **endocytic vesicle** containing ingested substances or particles (2-4)



# Phagocytosis

- The ingestion of large particles up to and including whole cells or microorganisms is called **phagocytosis**, a specific form of endocytosis
- For many unicellular organisms it is a means of acquiring food
- For more complex organisms, it is usually restricted to specialized cells called **phagocytes**
- *Neutrophils, macrophages and dendritic cells* are “professional phagocytes” that engulf and digest foreign materials or invasive microorganisms found in the bloodstream or injured tissues



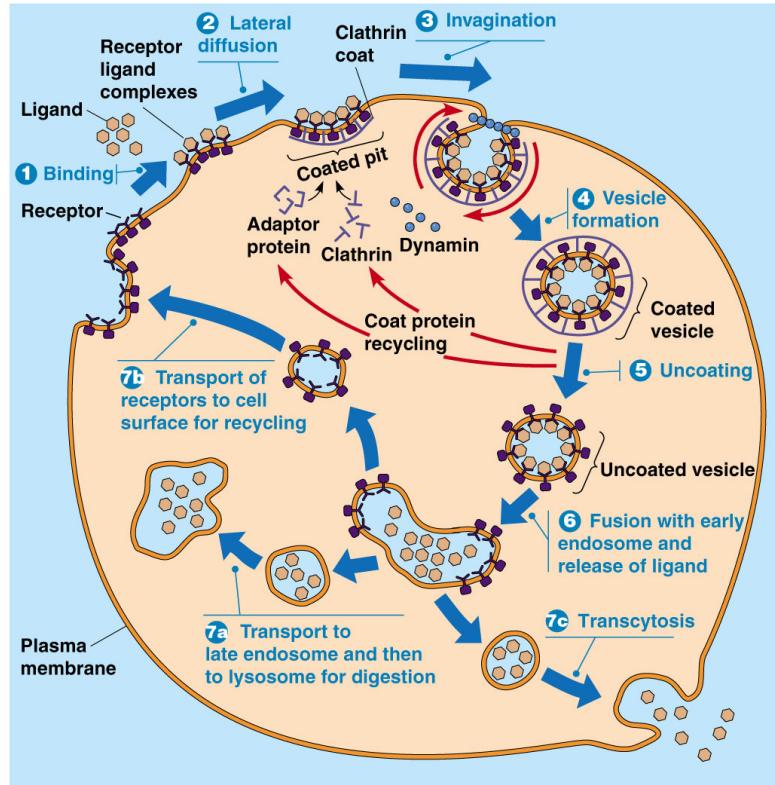
<https://www.creative-biolabs.com>

# Receptor-Mediated Endocytosis

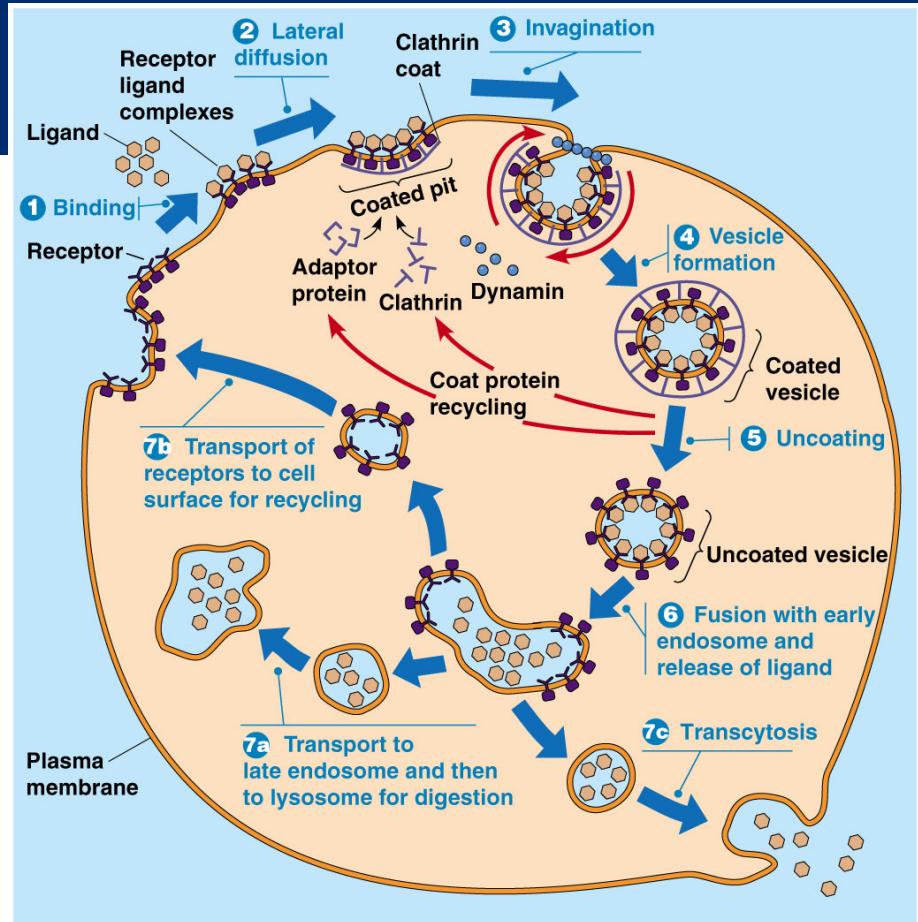
- Cells acquire some substances by **receptor-mediated endocytosis (or clathrin-dependent endocytosis)**
- Cells use receptors on the outer cell surface to internalize many macromolecules
- Mammalian cells can ingest hormones, growth factors, serum proteins, enzymes, cholesterol, antibodies, iron, viruses, bacterial toxins

# Process of receptor-mediated endocytosis

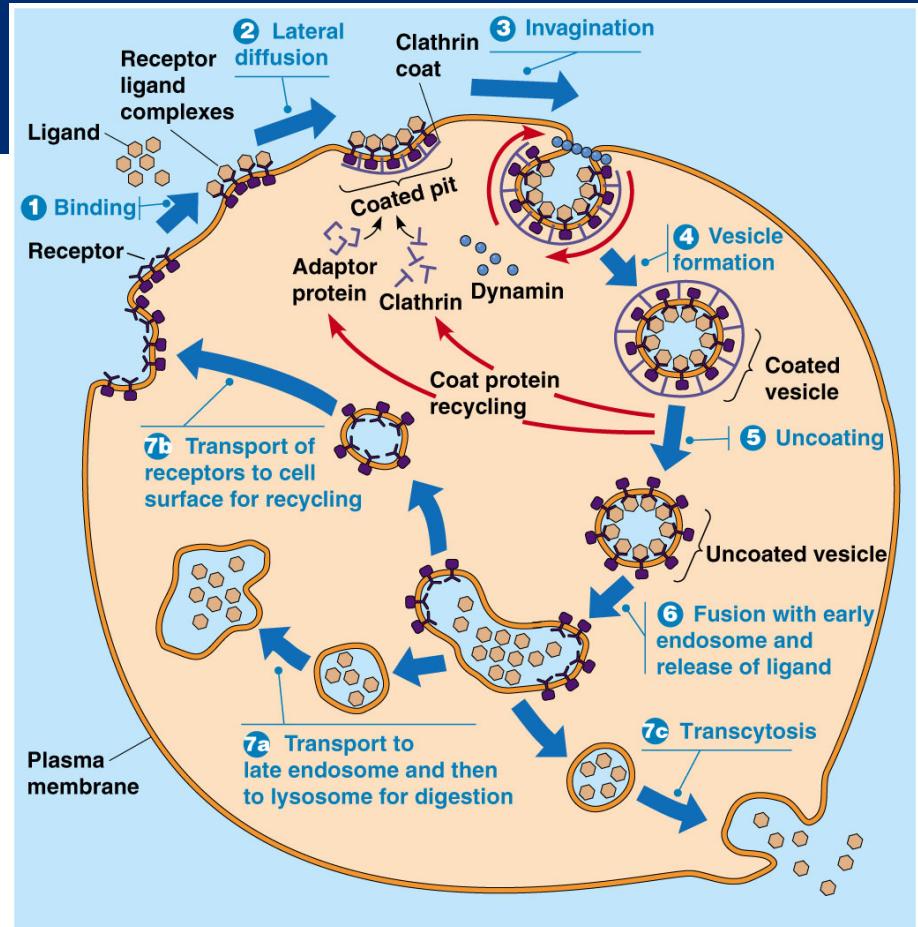
- Specific molecules (*ligands*) bind to their *receptors* on the outer surface of the cell (1)
- The receptor-ligand complexes diffuse laterally into *coated pits* (2)
- In a typical mammalian cell, coated pits occupy about 20% of the total surface area



- Accumulation of complexes in the pits triggers the accumulation of additional proteins on the cytosolic surface of the membrane
- These proteins—*adaptor protein, clathrin, dynamin*—induce curvature and invagination of the pit (3)
- Eventually the pit pinches off (4), forming a **coated vesicle**



- The clathrin coat is released, leaving an uncoated vesicle (5)
- Coat proteins and dynamin are recycled to the plasma membrane and the uncoated vesicle fuses with an early endosome (6)
- The process is very rapid and coated pits can be very numerous in cells



# Summary

- Membrane composition
- Lipid rafts
- Membrane proteins
- Membrane biosynthesis
- Protein trafficking



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