Chapter 9:
Lipids and Biological Membranes

Membrane proteins (pp 251-283)

Membrane proteins

Classification

- Integral (intrinsic)
- Lipid-linked
- Peripheral: soluble

Function

- Enzyme
- Receptor
- Transport
- and more

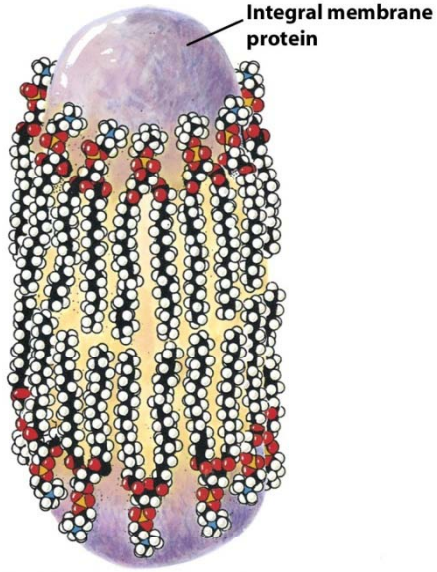
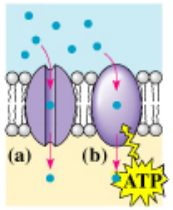
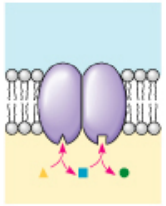


Figure 9-19 Fundamentals of Biochemistry, 2/e

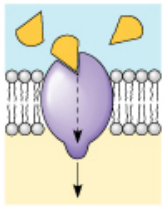
Model of an integral membrane protein



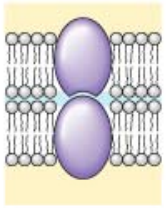
Transport (a) A protein that spans the membrane may provide a hydrophilic channel across the membrane that is selective for a particular solute. (b) Some transport proteins hydrolyze ATP as an energy source to actively pump substances across the membrane.



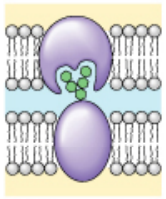
Enzymatic activity A protein built into the membrane may be an enzyme with its active site exposed to substances in the adjacent solution. In some cases, several enzymes in a membrane are ordered as a team that carries out sequential steps of a metabolic pathway.



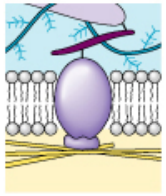
Signal transduction A membrane protein may have a binding site with a specific shape that fits the shape of a chemical messenger, such as a hormone. The external messenger (signal) may cause a conformational change in the protein that relays the message to the inside of the cell.



Intercellular joining Membrane proteins of adjacent cells may be hooked together in various kinds of junctions (see Figure 7.30).



Cell-cell recognition Some glycoproteins (proteins with short chains of sugars) serve as identification tags that are specifically recognized by other cells.



Attachment to the cytoskeleton and extracellular matrix (ECM) Microfilaments or other elements of the cytoskeleton may be bonded to membrane proteins, a function that helps maintain cell shape and fixes the location of certain membrane proteins. Proteins that adhere to the ECM can coordinate extracellular and intracellular changes.

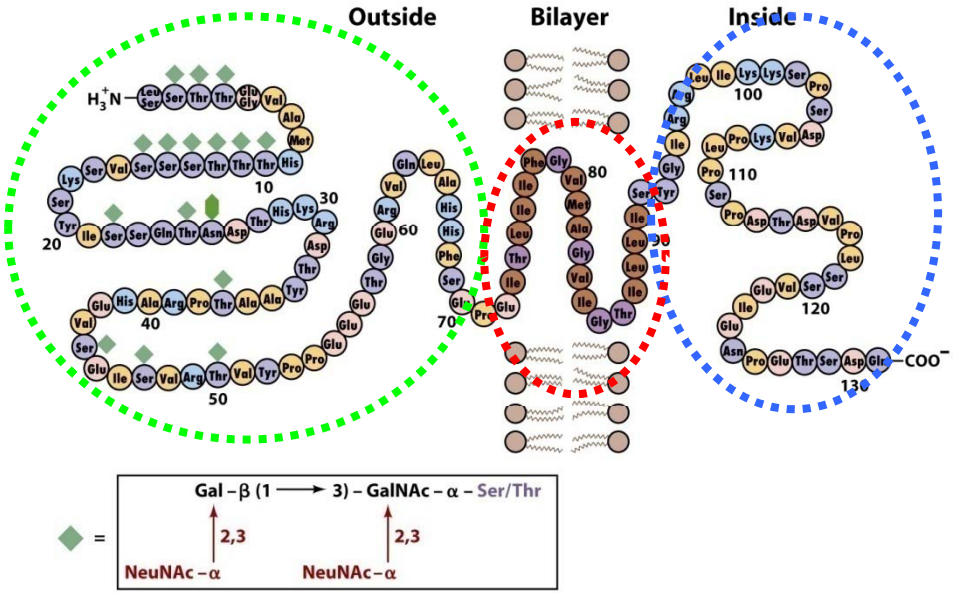
Integral protein

Transmembrane protein: asymmetrically oriented amphiphiles

Transmembrane domain:

alpha helix: one or several

beta sheet: beta barrel



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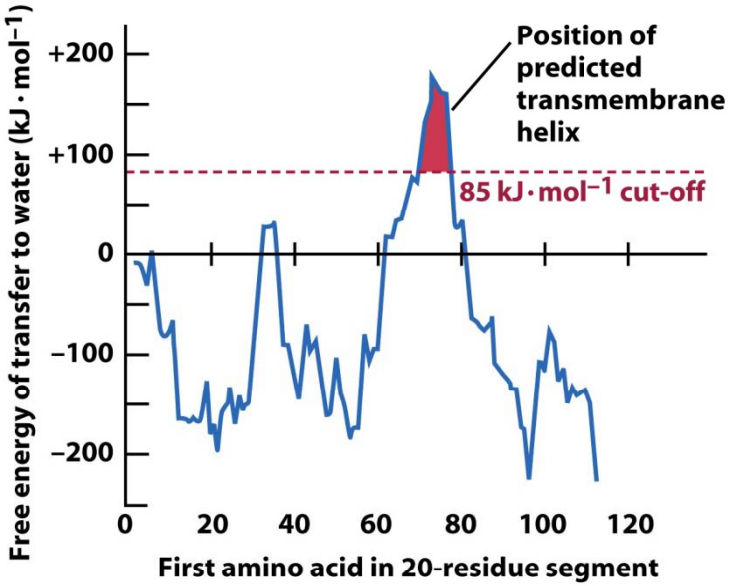

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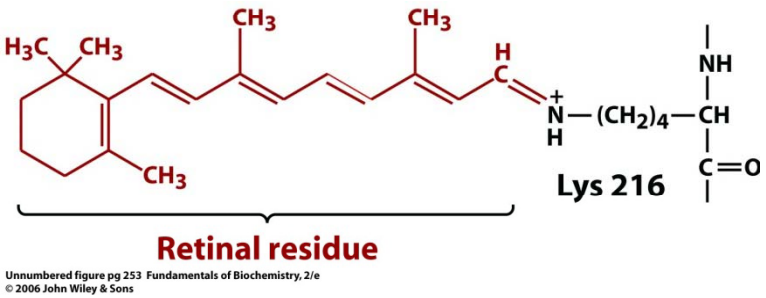
Human erythrocyte glycophorin A

Bacteriorhodopsin: light driven proton pump
Seven transmembrane helices



Unnumbered figure pg 253 Fundamentals of Biochemistry, 2/e

bacteriorhodopsin

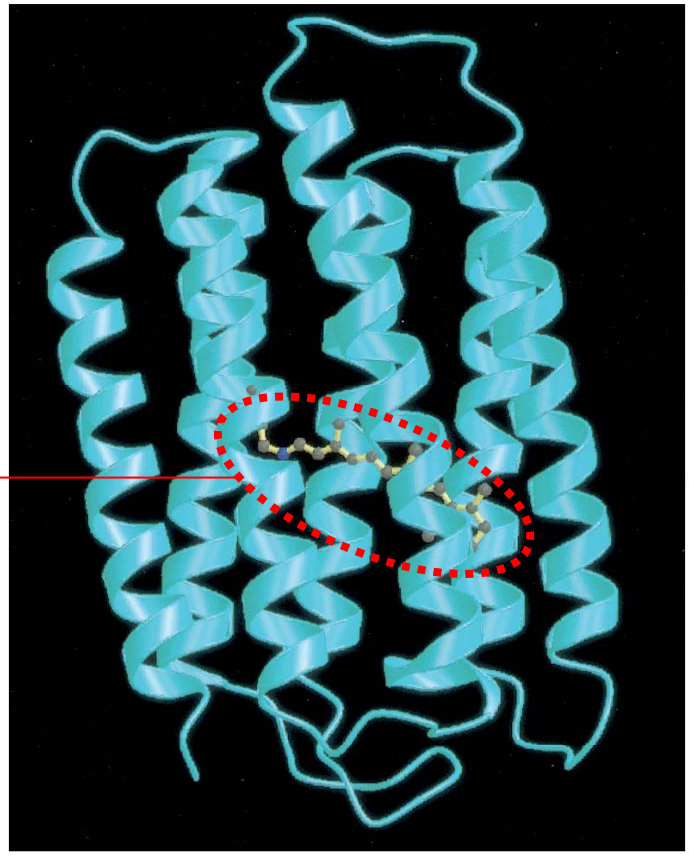


Figure 9-22 Fundamentals of Biochemistry, 2/e

Photosynthetic reaction center

- 11 transmembrane helices
- 4 nonidentical subunits
- 4 chlorophylls
- 4 chromophores
- 1 nonheme Fe atom

X-ray structure of the photosynthetic reaction center of *Rhodopseudomonas viridis*
Nitrogen, blue; oxygen, red; sulfur, yellow

Hydrophobic interior
Hydrophobic exterior

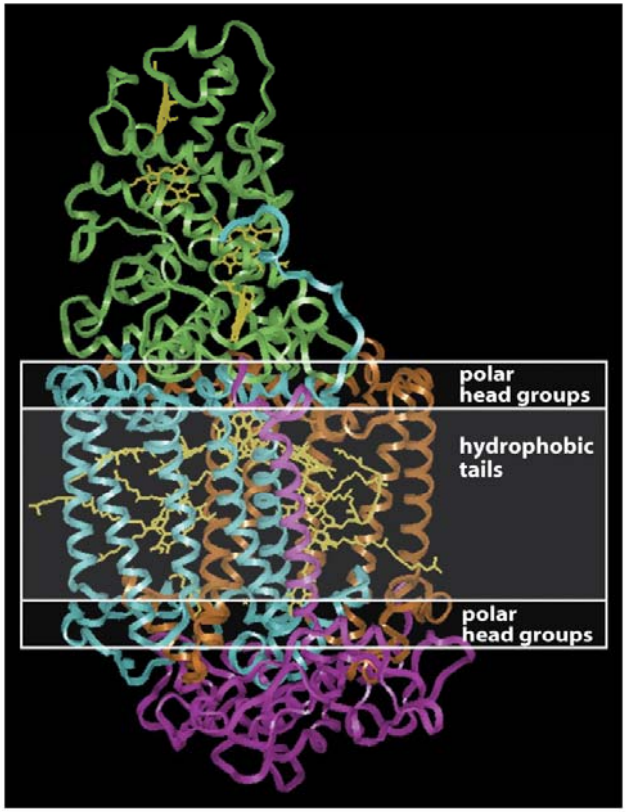


Figure 9-23a Fundamentals of Biochemistry, 2/e

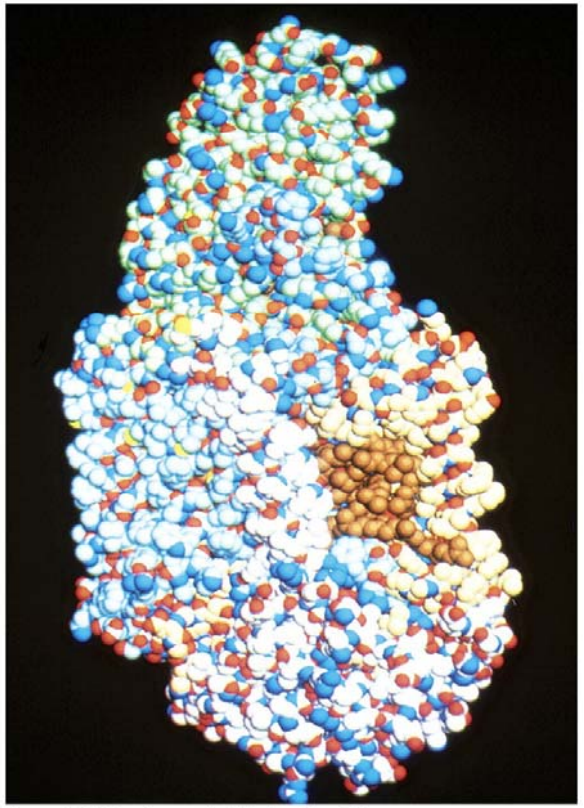


Figure 9-23b Fundamentals of Biochemistry, 2/e

Beta barrel transmembrane proteins

consist of 8~22 beta strands

porins: channel forming proteins

transport of small polar solutes

Figure 9-24a Fundamentals of Biochemistry, 2/e

X-ray structure of the *E. coli* OmpF porin

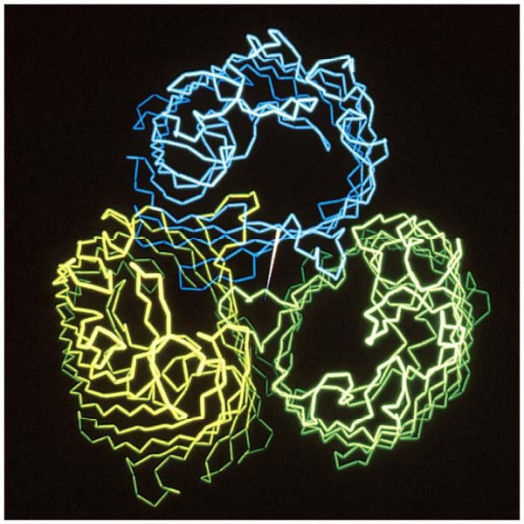


Figure 9-24b Fundamentals of Biochemistry, 2/e

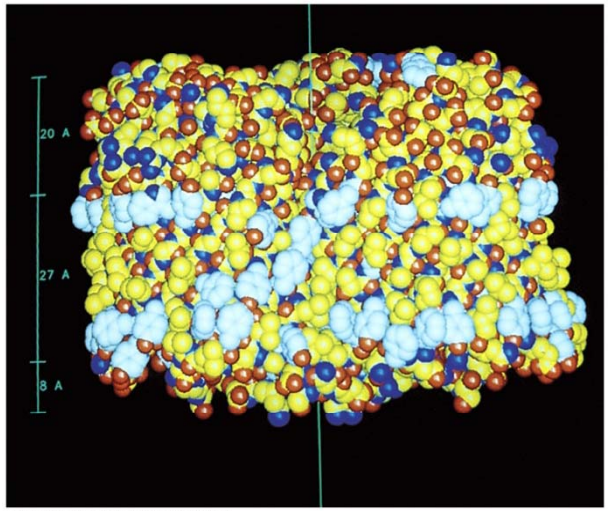
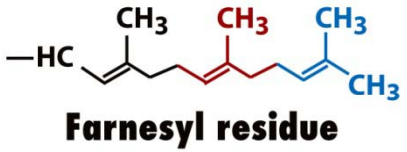


Figure 9-24c Fundamentals of Biochemistry, 2/e

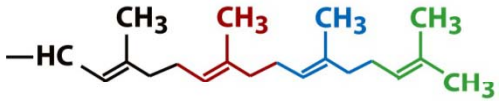
Lipid linked proteins

Prenylated: C-terminal Cys in C-X-X-Y sequence
- Y(A, M, S), farnesyl; Y(L), geranylgeranyl
- X-X-Y is deleted



Farnesyl residue

C15

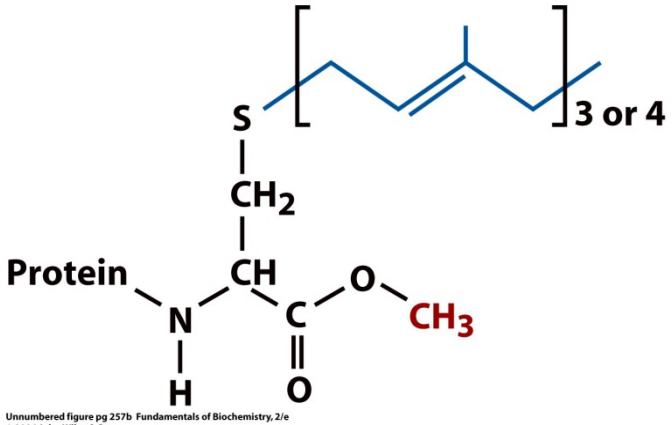


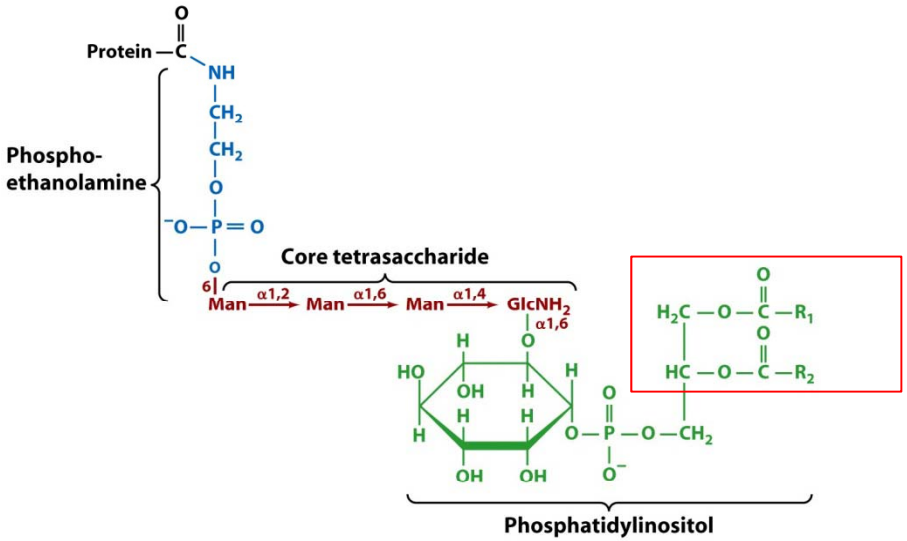
Geranylgeranyl residue

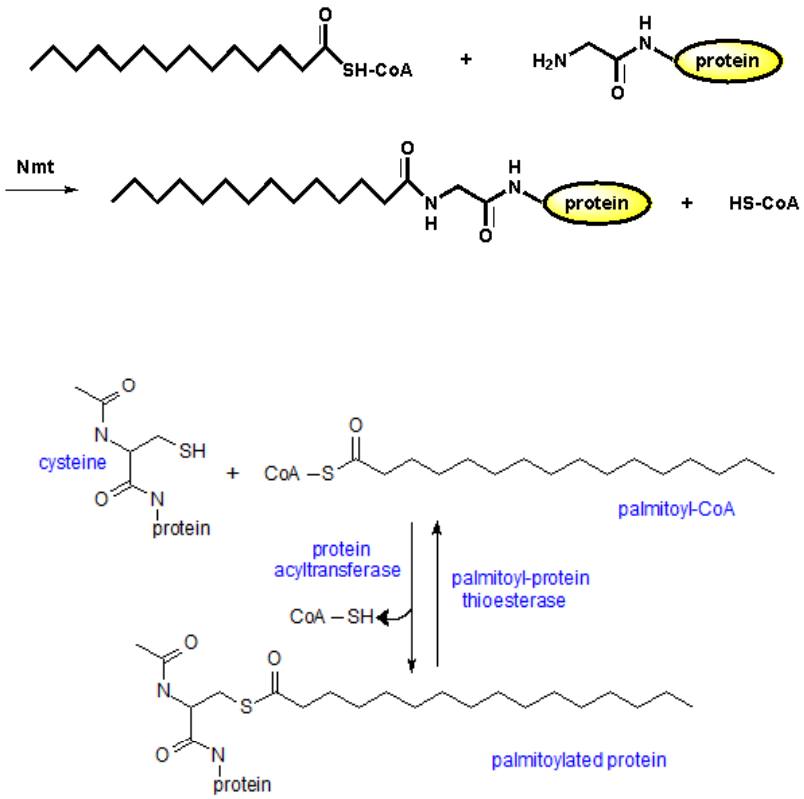
C20

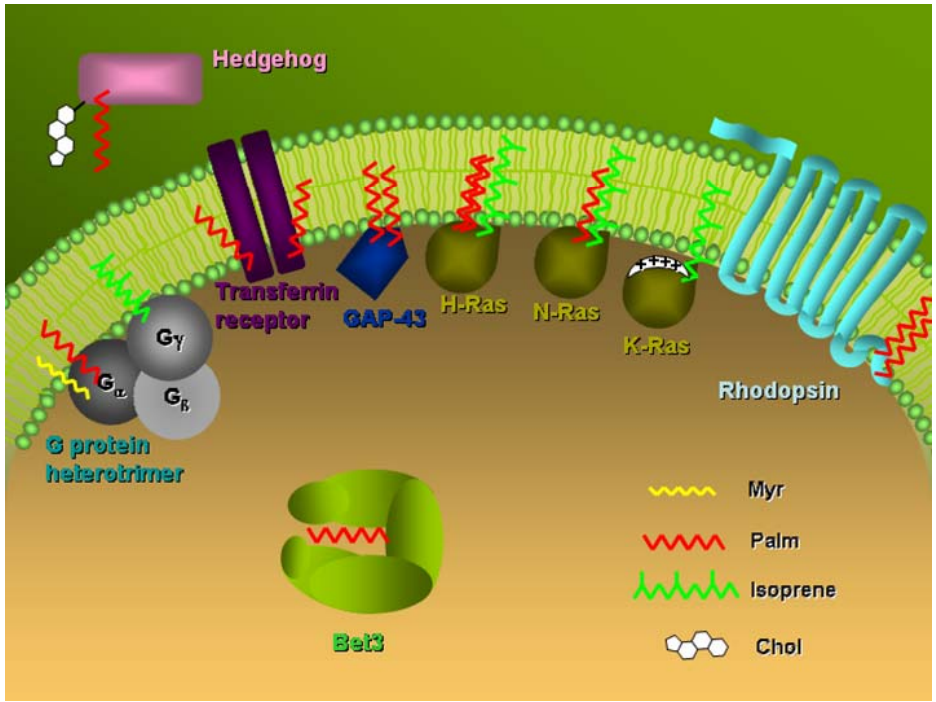
Fatty acylated:
- myristic acid (C14): N-terminal Gly, subcellular, irreversible
- palmitic acid (C16): Cys in cytoplasmic side, reversible

Glycosylphosphatidylinositol (GPI)-linked
- exterior surface of eukaryote plasma membrane









http://lipidlibrary.aocs.org/lipids/protlip/index.htm

Membrane structure and assembly

The fluid mosaic model: 1972, S. Jonathan Singer & Garth Nicholson

- Iceberg floating in a two-dimensional lipid sea
- Lateral diffusion of integral proteins

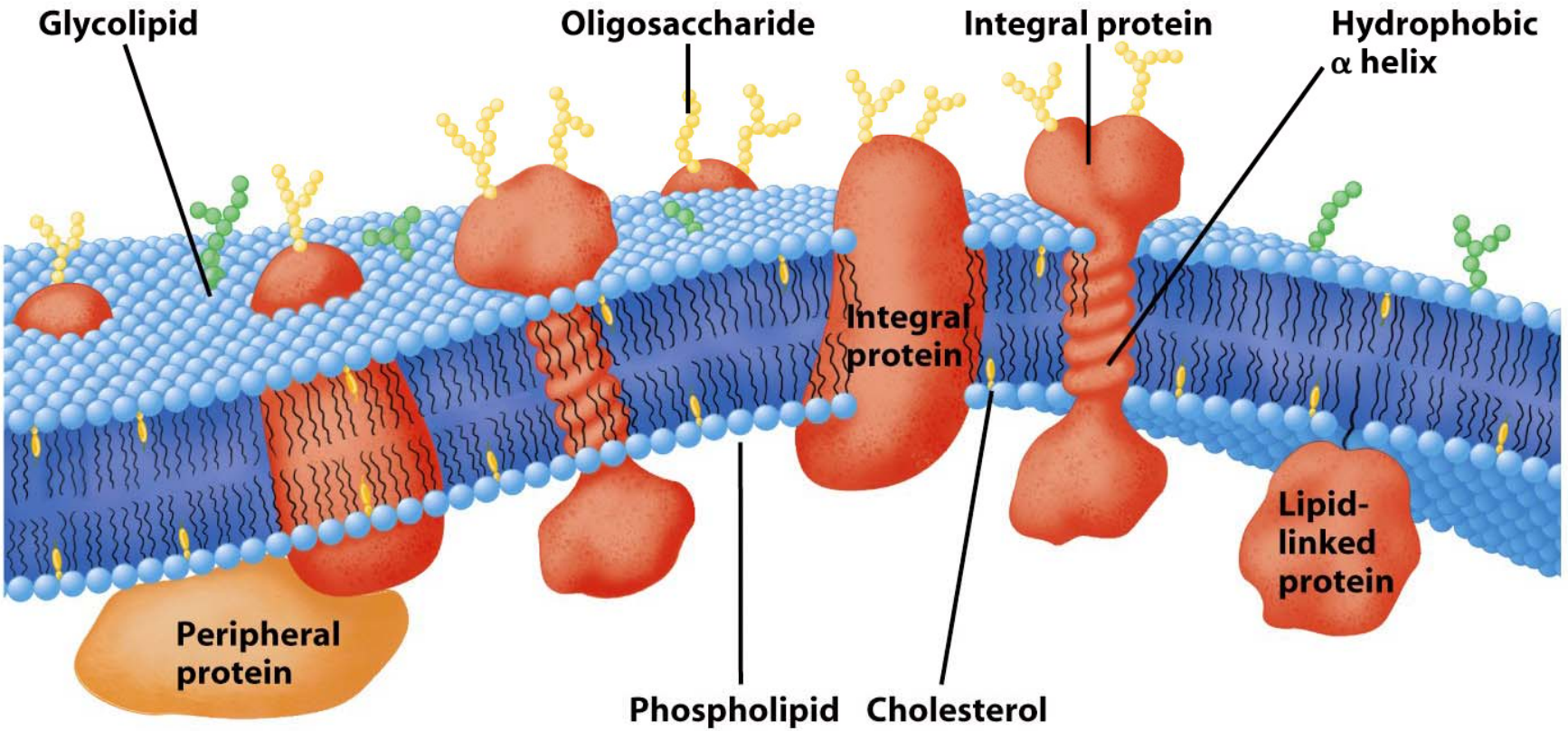
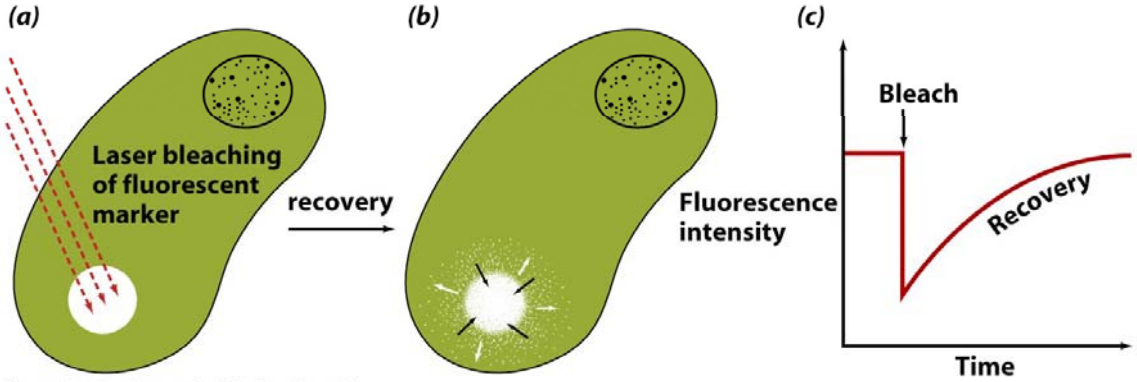


Figure 9-26 Fundamentals of Biochemistry, 2/e

Lateral diffusion of integral proteins

Fusion of mouse & human cells
Fluorescence recovery after photobleaching

Membrane protein vary in their lateral diffusion rates
- 30~90% are freely movable
  - 1 hour to transverse 20 μm length
- Others more slowly
- Some are essentially immobile



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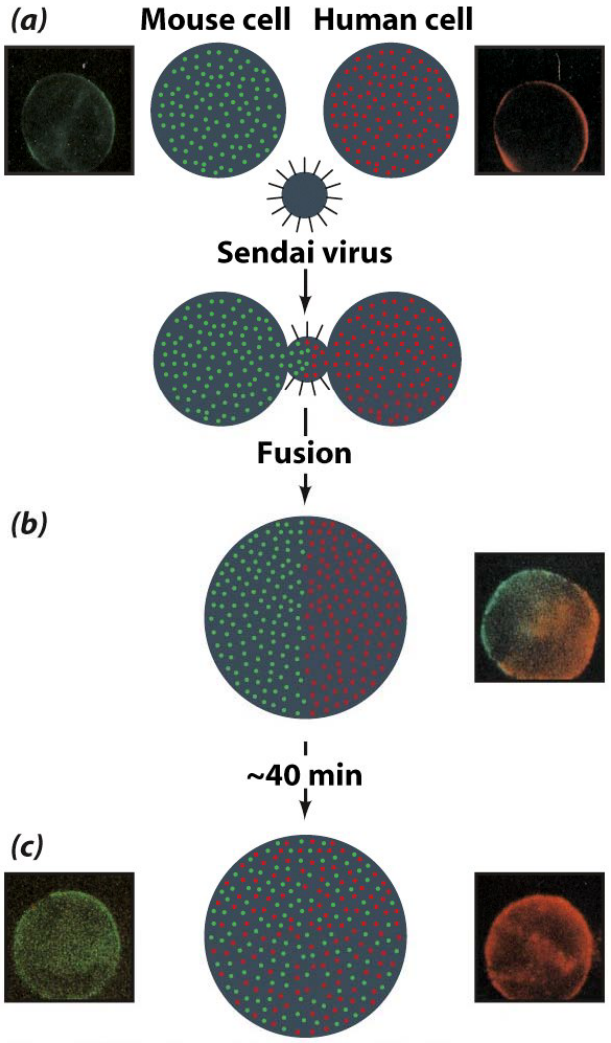

Figure 9-27 Fundamentals of Biochemistry, 2/e

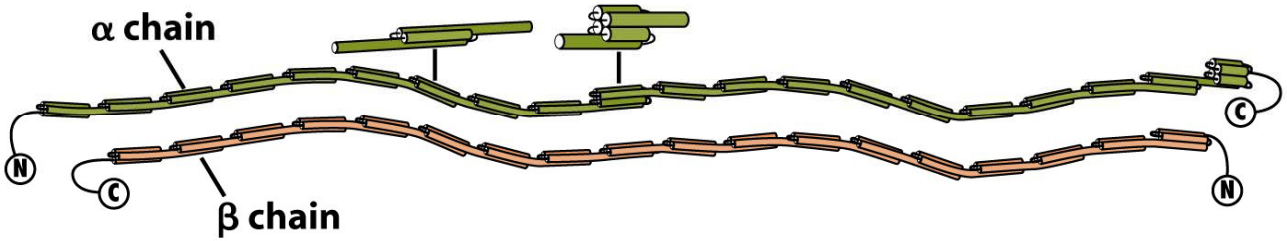

Figure 9-29a Fundamentals of Biochemistry, 2/e
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Structure of spectrin
(αβ)2 tetramer

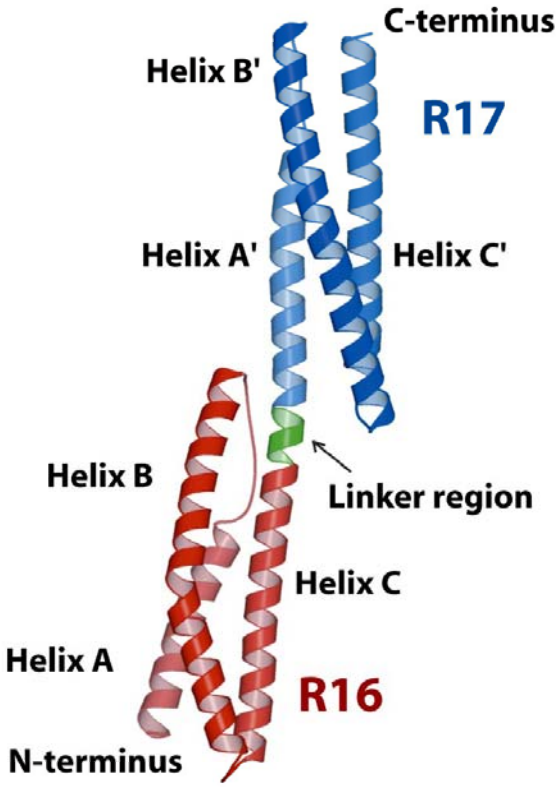
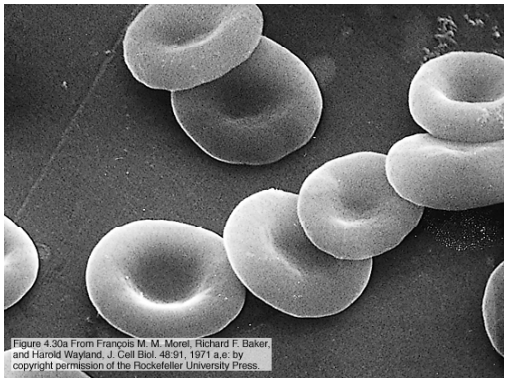
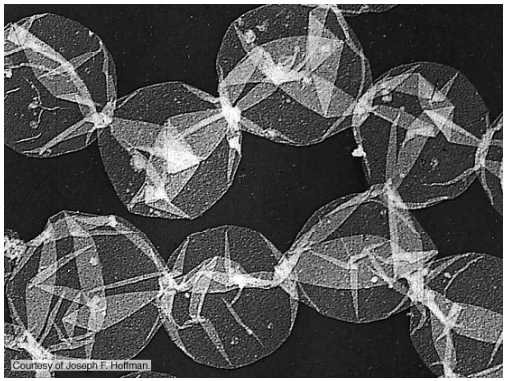


Figure 9-29b Fundamentals of Biochemistry, 2/e

The membrane skeleton

Erythrocyte
a membraneous bag of Hb
Disklike but easily deformable
Membrane rolling like the thread of a tractor





The human erythrocyte membrane skeleton

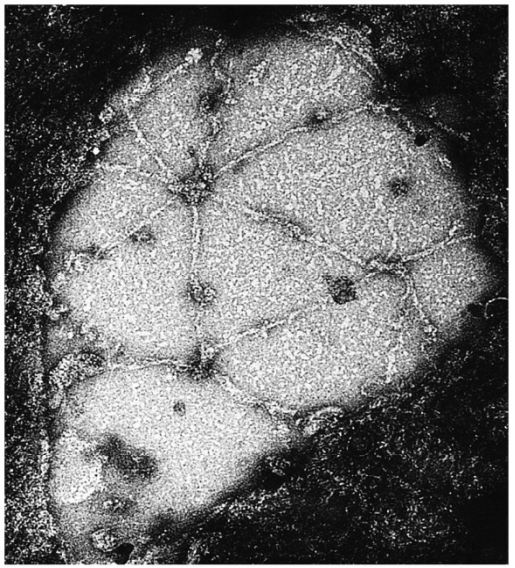


Figure 9-30a Fundamentals of Biochemistry, 2/e

Gates and fence model
various mobilities of membrane proteins

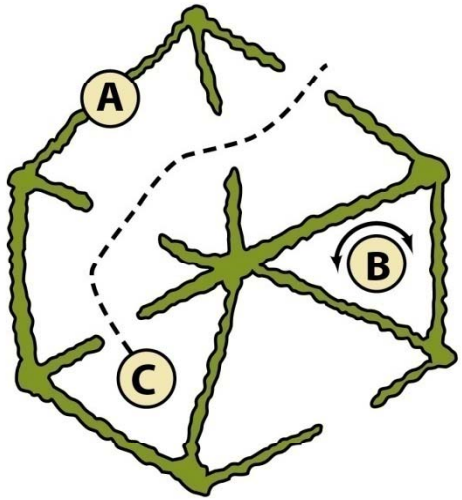


Figure 9-32 Fundamentals of Biochemistry, 2/e

Figure 9-31c Fundamentals of Biochemistry, 2/e

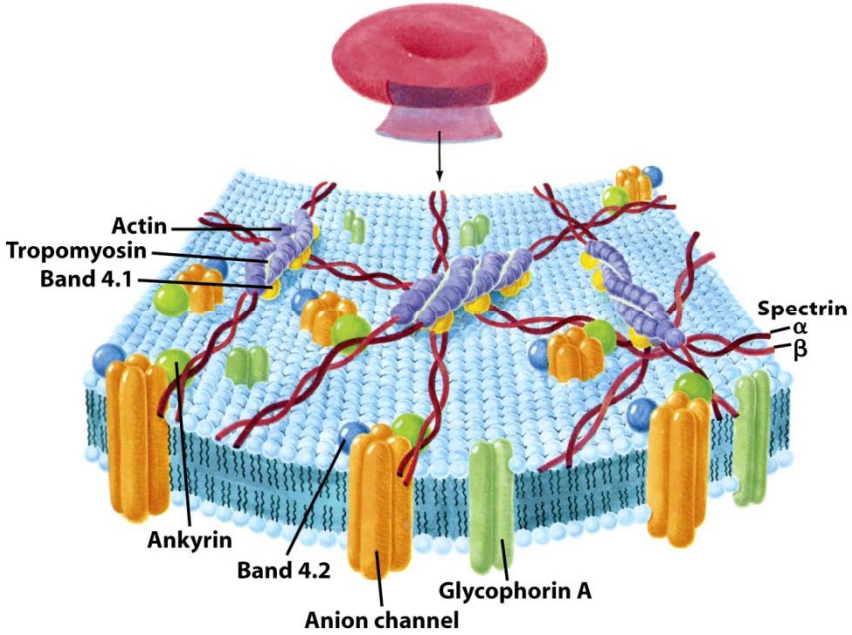
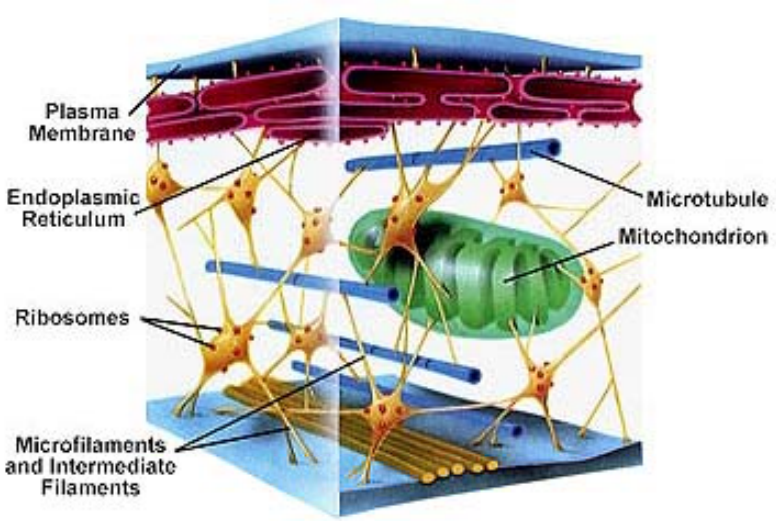
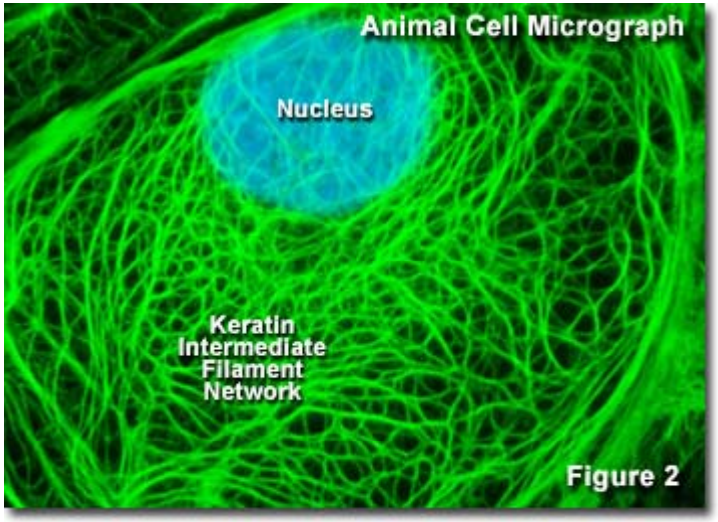


Figure 9-31b Fundamentals of Biochemistry, 2/e

Cytoskeleton: http://www.youtube.com/watch?v=5rqbmLiSkpk&feature=related





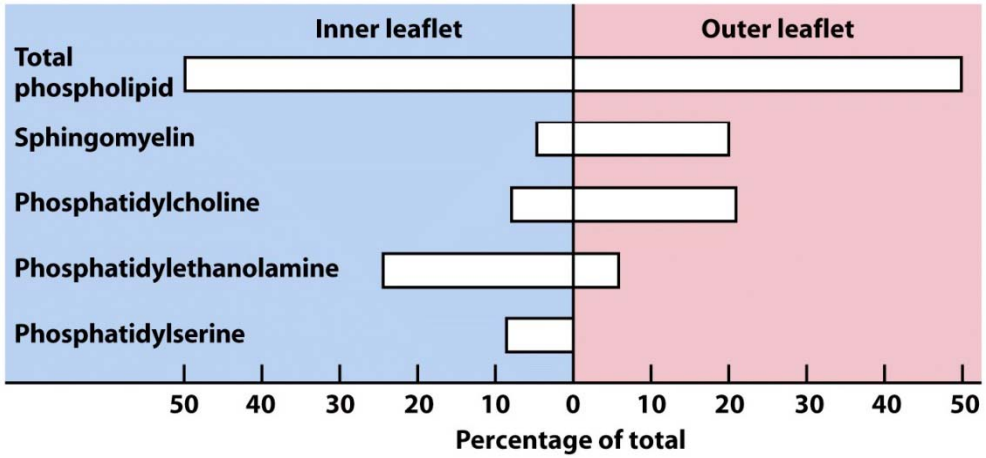
http://micro.magnet.fsu.edu/cells/intermediatefilaments/intermediatefilaments.html

Microfilaments: actins (movement I, movement II)
Intermediate filaments:
Microtubules: alpha- and beta-tubulin
organelle movement on microtubules
kinesin transport protein

Lipid asymmetry

Asymmetry between inner & outer
protein and polar lipid
Larger molecules in outer layer
Carbohydrates in outer layer

The origin of lipid asymmetry
Synthesized by integral membrane proteins
Eukaryotes: ER
Prokaryotes: plasma membrane



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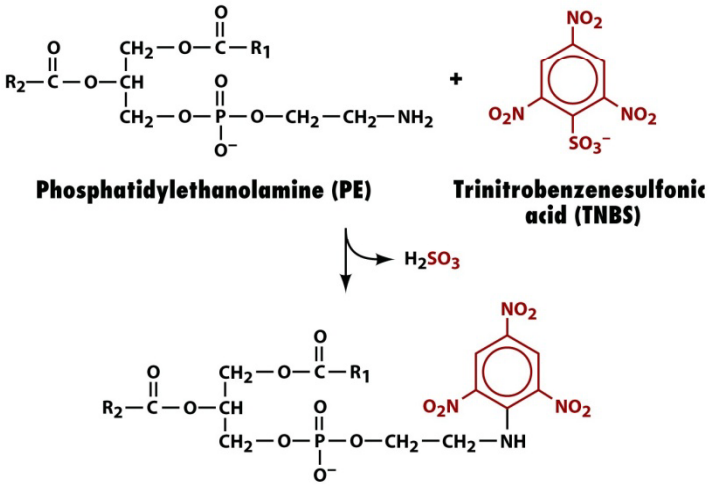
PE labeling with TNBS



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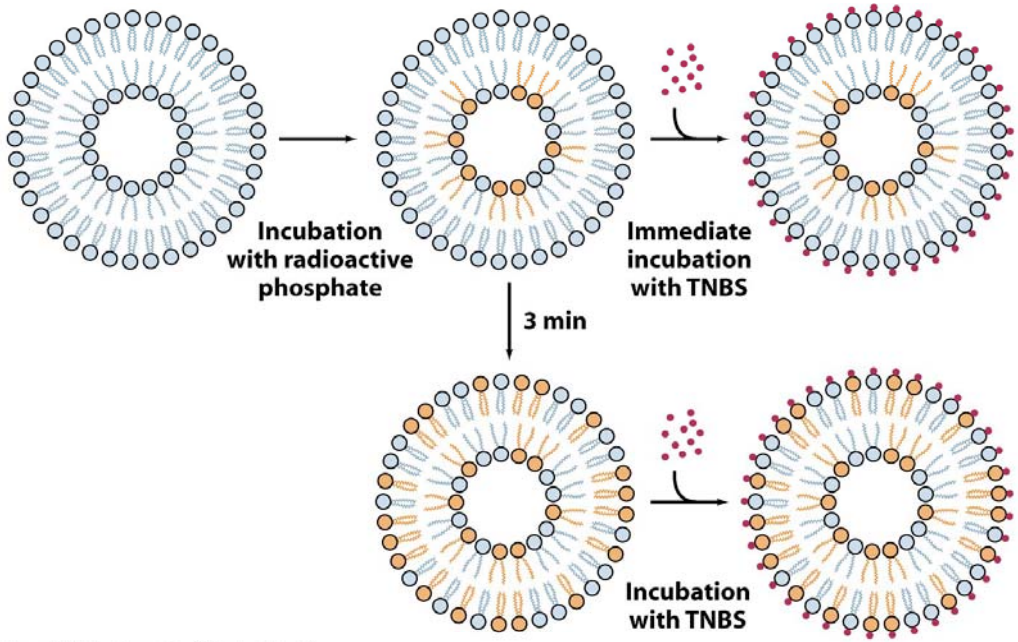

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Flip-flop rate: ~100000 fold greater than in artificial membrane

Flip-flop transport of lipid molecules from cytoplasm

Flipases: facilitated diffusion (from higher conc to lower)

Phospholipid translocases: active transport dependent on ATP

The importance of lipid transport

The presence of phosphatidylserine on the exteriors

of many cells: causes blood clotting (indication of tissue damage)

in erythrocyte: removal signal

<u>Lateral organization of lipids and membranes</u>

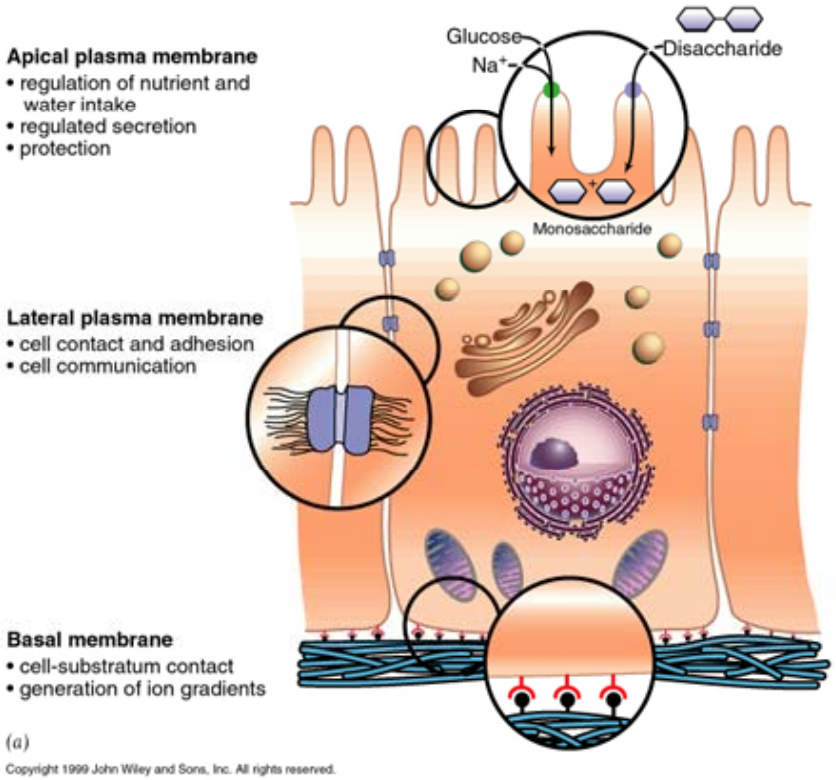
Two or more distinct domains in eukaryotic cells

Ex. epithelial cells:

apical domain

Basolateral domain (basal & lateral)

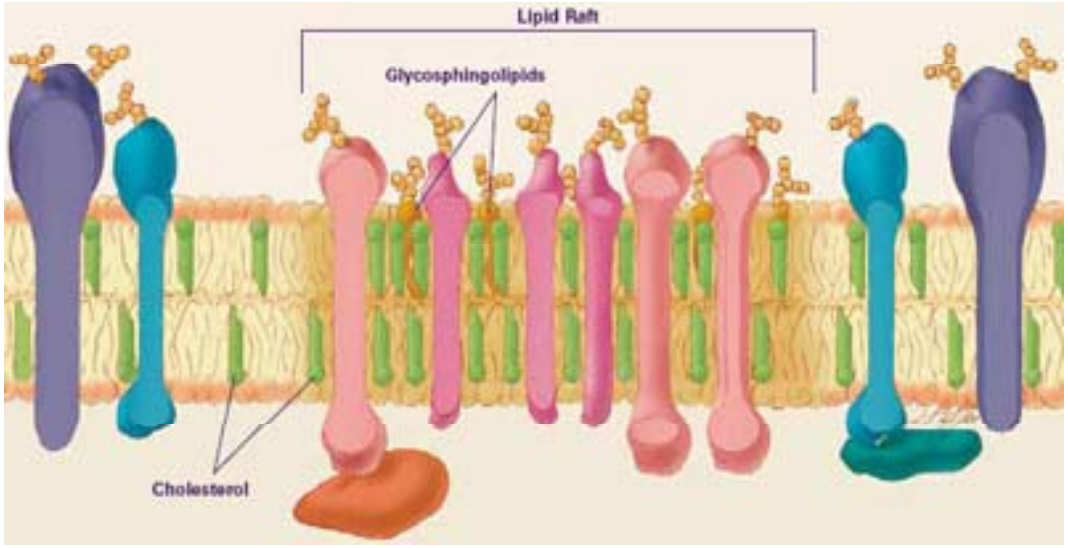
Different compositions of lipids & proteins

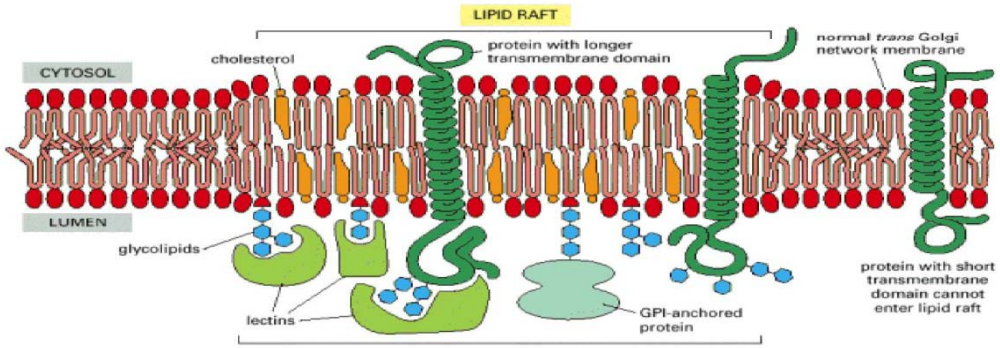


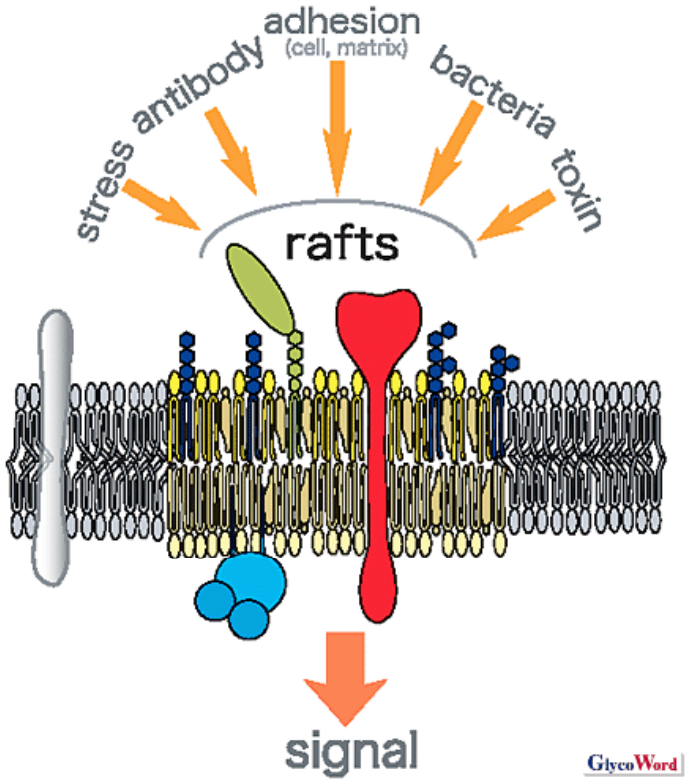
Microdomains: lipid raft

Dynamic structure consisting of
Glycosphingolipids
Cholesterol
GPI-linked proteins

A **lipid raft** is a cholesterol and sphingolipid-enriched microdomain or platform found in cell membranes.



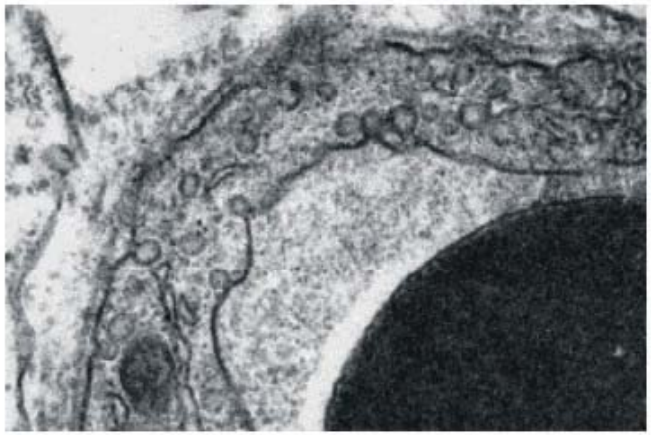




Caveolae

Specialized forms of lipid rafts associated with caveolins
Participate in endocytosis & intercellular signaling

A



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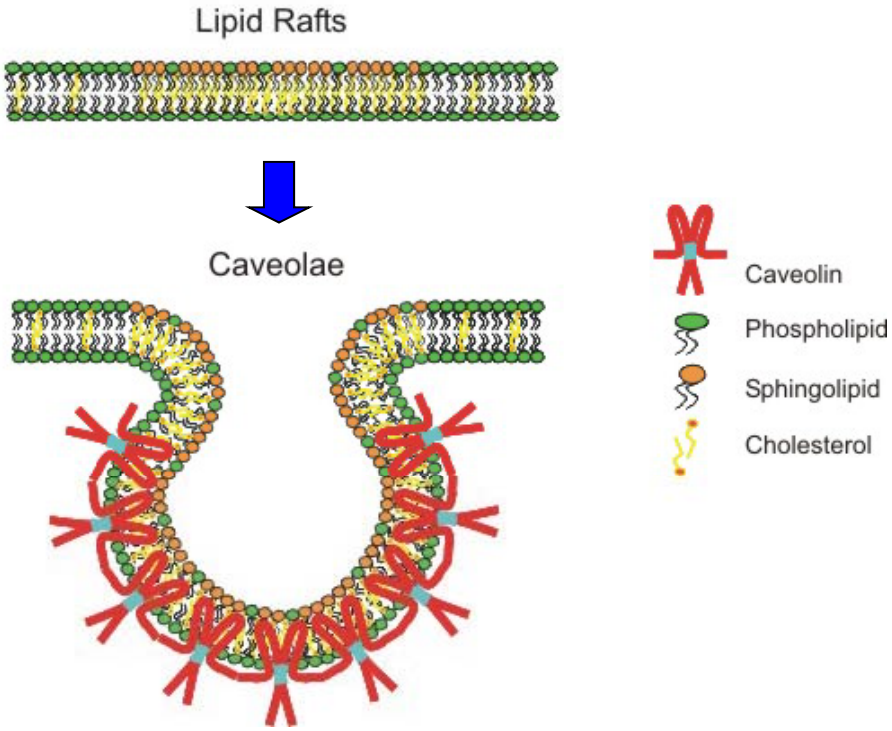


http://www.ruf.rice.edu/~rur/issue1_files/razani.html

Figure 1. Caveolae, a unique "cellular organelle" with a unique "marker protein"
A) Electron micrograph of an endothelial cell showing caveolae, 50-100 nm structures that are either direct invaginations or in close proximity to the plasma membrane. Caveolae are estimated to make-up an estimated 30-70% of the plasma membrane area in certain cells such as endothelial cells, adipocytes, or Type I pneumocytes .
B) Diagram comparing the biochemical composition of lipid rafts and caveolae (adapted from (Galbiati et al., 2001)). Lipid rafts form via a coalescence of cholesterol and sphingolipids; as a result, these microdomains have vastly different biochemical properties than the bulk phospholipids bilayer. Caveolae are generally considered to be "invaginated" lipid rafts primarily due to an enrichment in a family or proteins known as the caveolins. Here, the caveolin oligomer is depicted as a dimer for simplicity.

The secretory pathway

Free ribosomes: soluble & mitcochondrial proteins

ER bound ribosomes: proteins for transmembrane, secretion, ER, lysosomes

~40% of proteins: secretory pathway and some other protein targeting pathway

Signal peptide
Signal recognition particle (SRP)
SRP receptor
GTP hydrolysis
Signal peptidase
Posttranslational modification
Membrane anchor

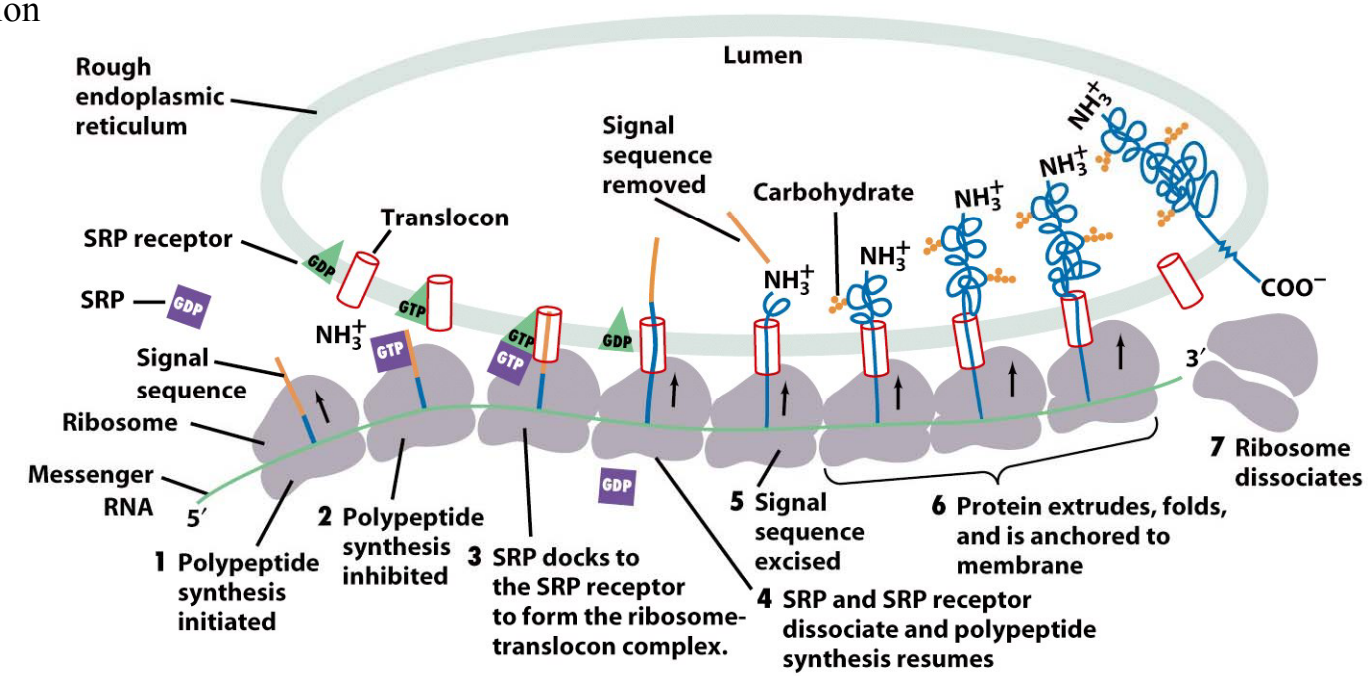


Figure 9-36 Fundamentals of Biochemistry, 2/e

Signal peptides: N-terminal 13~36 residues
Form alpha helices in nonpolar environments

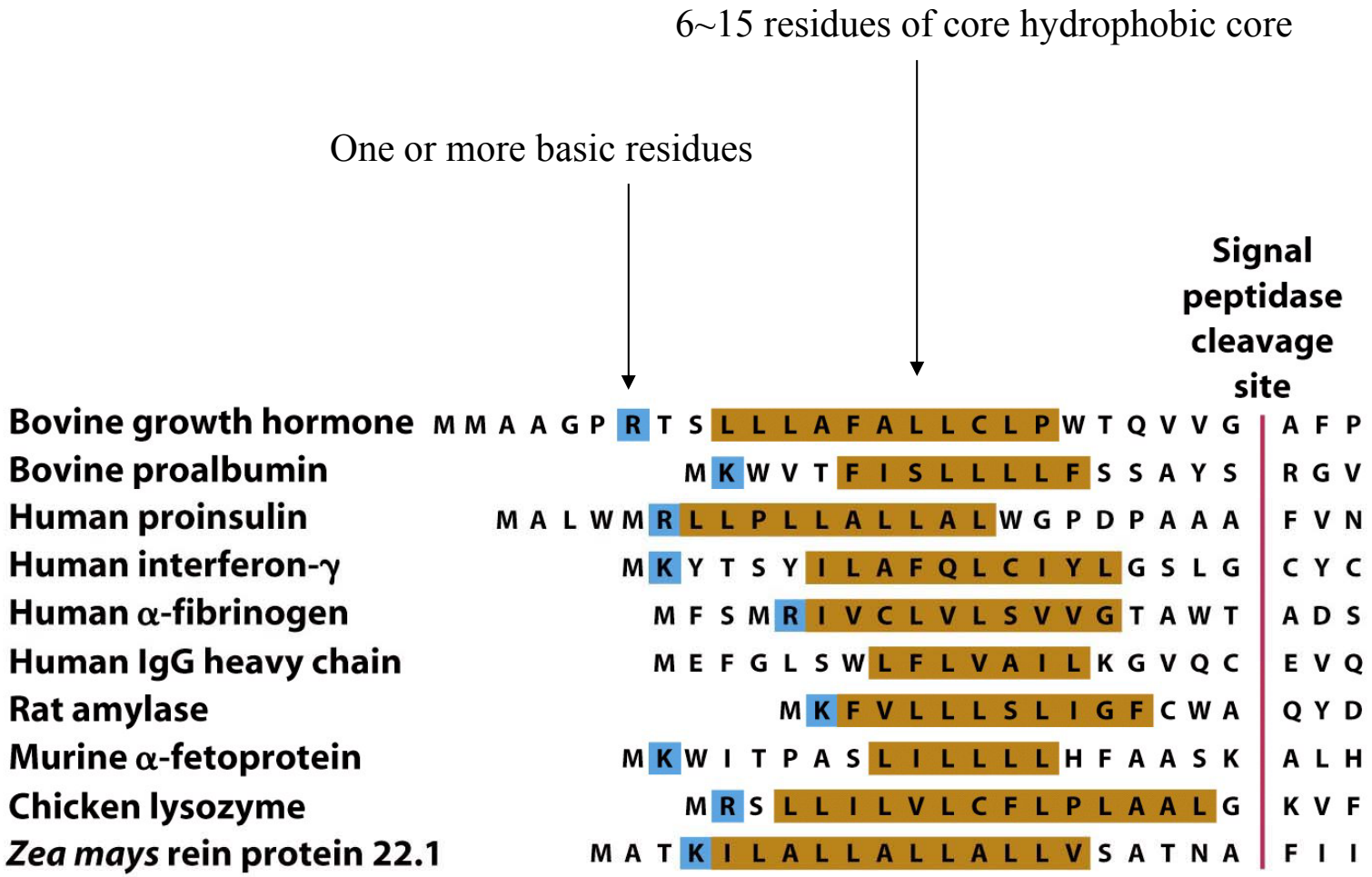


Figure 9-37 Fundamentals of Biochemistry, 2/e

Translocon (translocation channel)

Multifunctional transmembrane channel
Ion-conducting channels in RER: Sec61 (mammals), SecY (pro)
Heterotrimers

Inserts transmembrane helices into the ER membrane

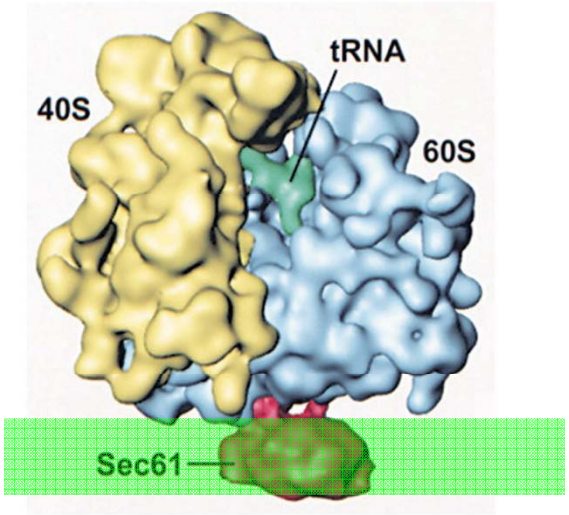


Figure 9-38 Fundamentals of Biochemistry, 2/e

M. jannaschii SecY complex

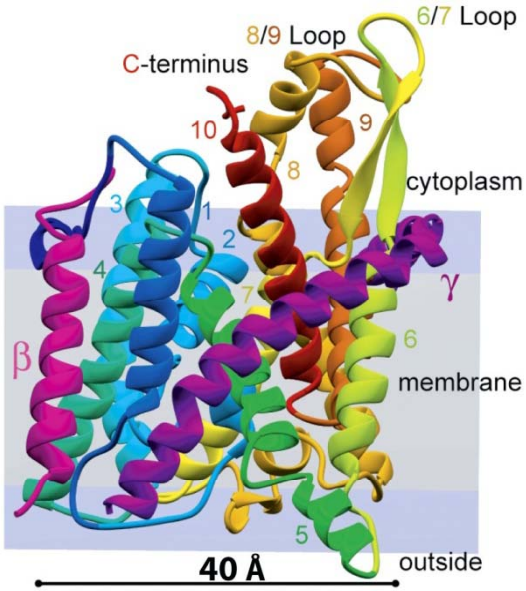


Figure 9-39b Fundamentals of Biochemistry, 2/e

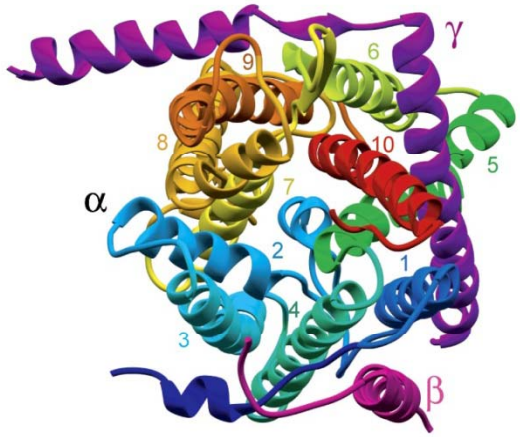


Figure 9-39a Fundamentals of Biochemistry, 2/e

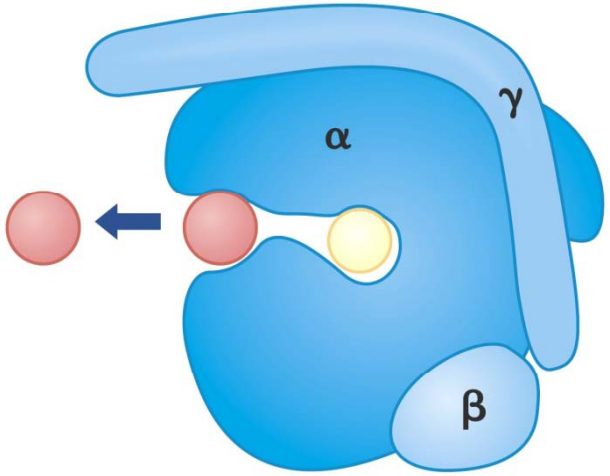
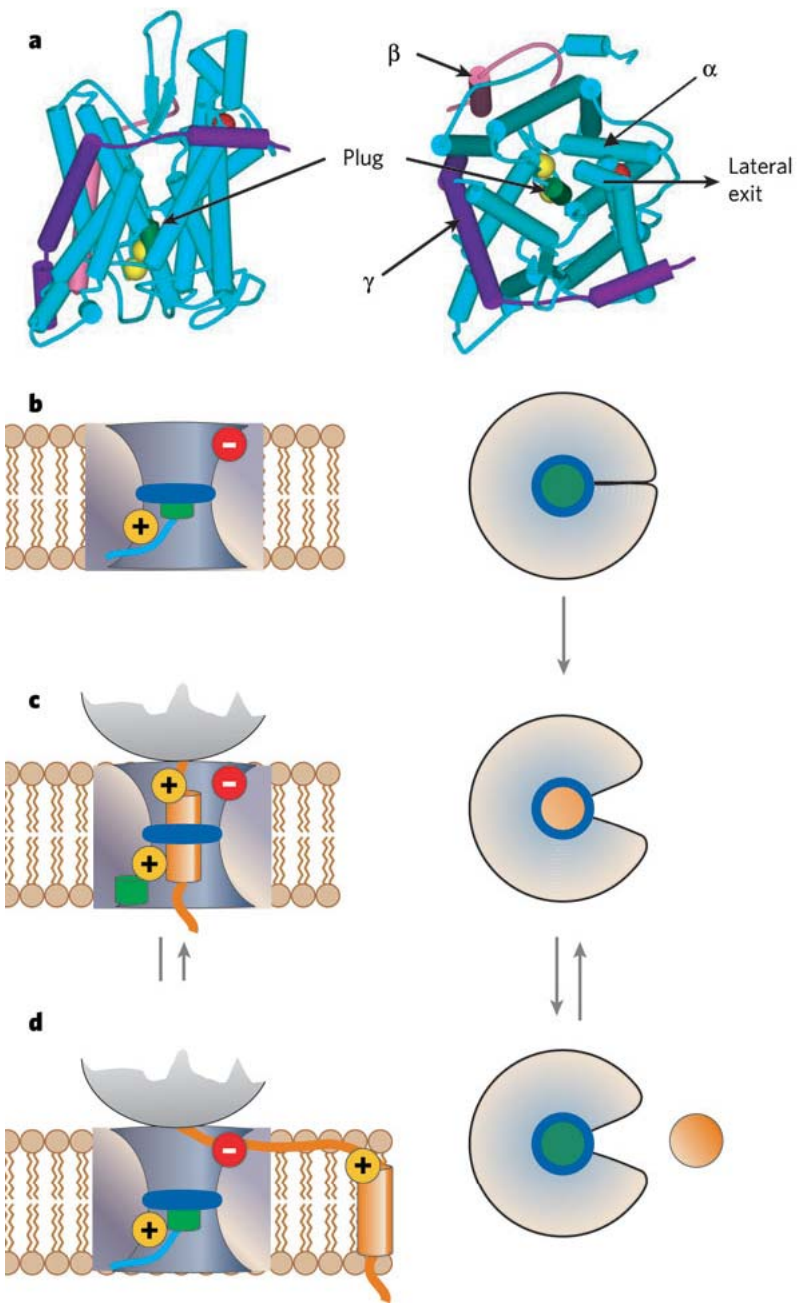


Figure 9-40 Fundamentals of Biochemistry, 2/e

The Sec61 translocon structure and mechanism model



a, The alpha subunit is shown in light blue, the beta subunit in purple and the gamma subunit in pink. The plug helix that blocks the pore is shown in green. Key charged residues that help define the topology of translocating polypetides in *S. cerevisiae* are shown in yellow (positive charge) and red (negative charge). **b**, Schematic side and top views of the Sec61 translocon showing the locations of the charges, the plug helix in green and the hydrophobic collar shown by the dark blue ring. **c**, A schematic view of a the translocon with a nascent polypeptide (orange) emerging from the ribosome (grey). A positively charged residue helps define the topology of this segment as N-terminal first. **d**, A schematic view of the translocon opening the lateral gate so the helix can exit to the membrane if the partitioning is favourable.

Nature 438, 581-589 (1 December 2005)

Vesicle trafficking

Transport to Golgi

Further posttranslational processing (mainly glycosylation)

Cis, medial, trans Golgi networks: different sets of glycoprotein processing enzymes

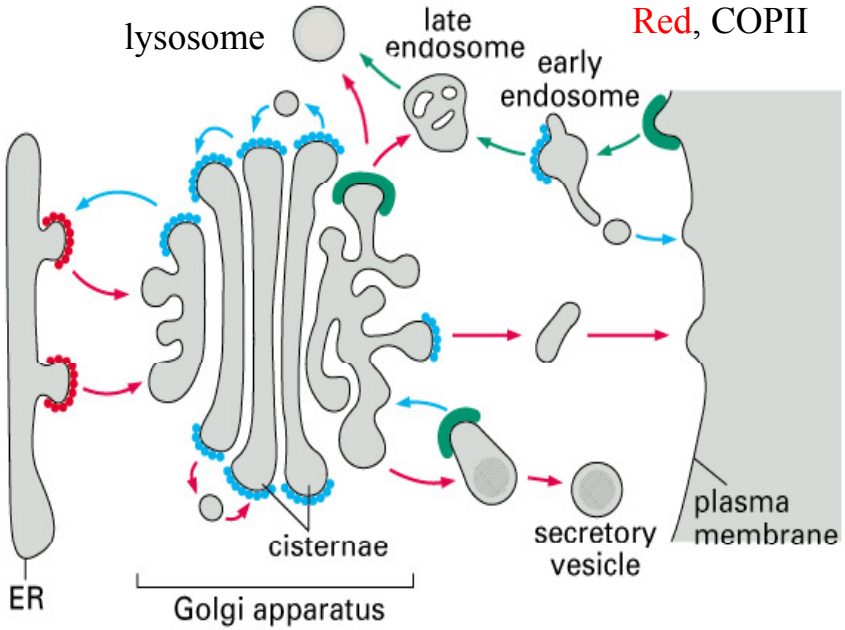
Transport via two mechanisms: as vesicles or golgi stacks

vesicles: anterograde (forward) transport, from cis to trans, bud off and fuse

golgi stacks: cisternal progression or maturation: cis-cisternae eventually becomes trans-cisternae

mediated through retrograde (backward) transport

Green, clathrin
Blue, COPI
Red, COPII



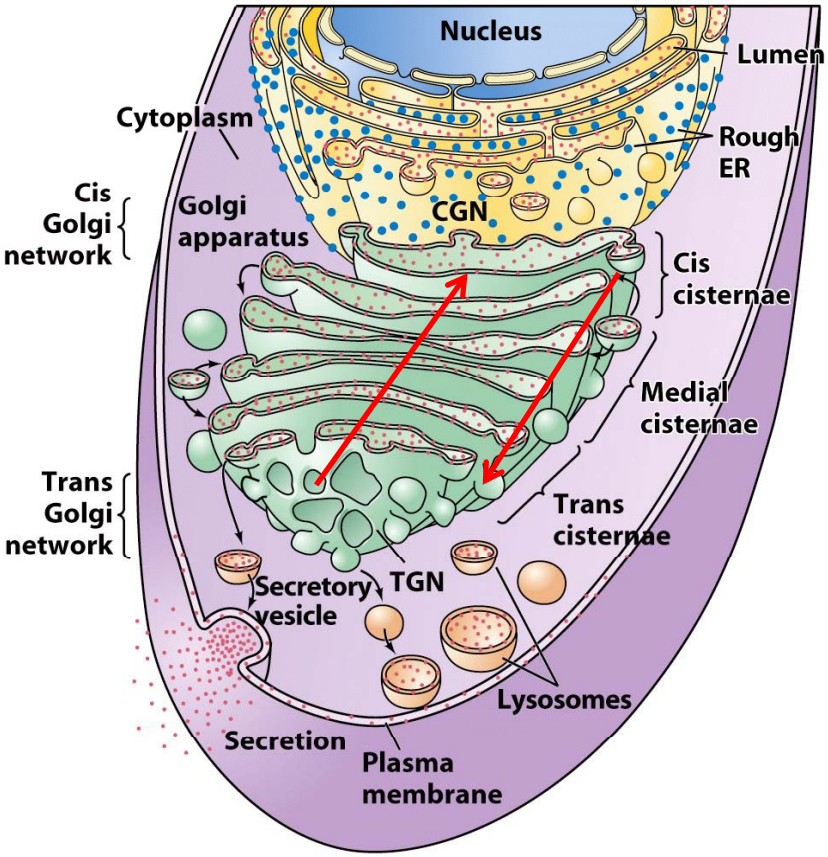
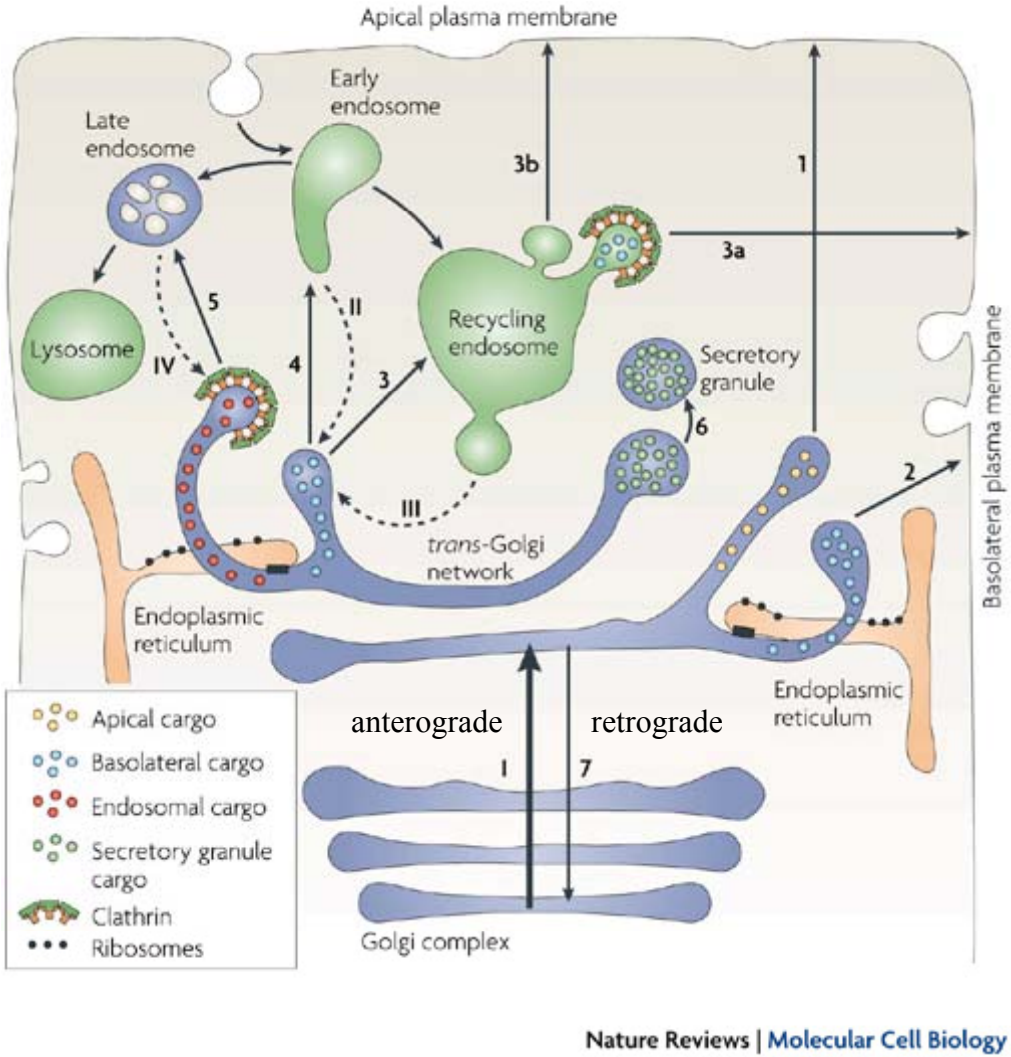


Figure 9-41 Fundamentals of Biochemistry, 2/e



The *trans*-Golgi network (TGN) is a tubular network that originates from the last *trans*-Golgi cisternae. It sorts newly synthesized proteins that arrive from earlier Golgi compartments (I) towards different destinations (1–5). It also receives input from the endocytic pathway (II–IV) and sends back components to the earlier Golgi compartments (7). *Nature Reviews Molecular Cell Biology* **9**, 273-284 (April 2008)

Coated vesicles
60~150 nm diameter membraneous sacs

Three types
- Clathrin
- COPI
- COPII

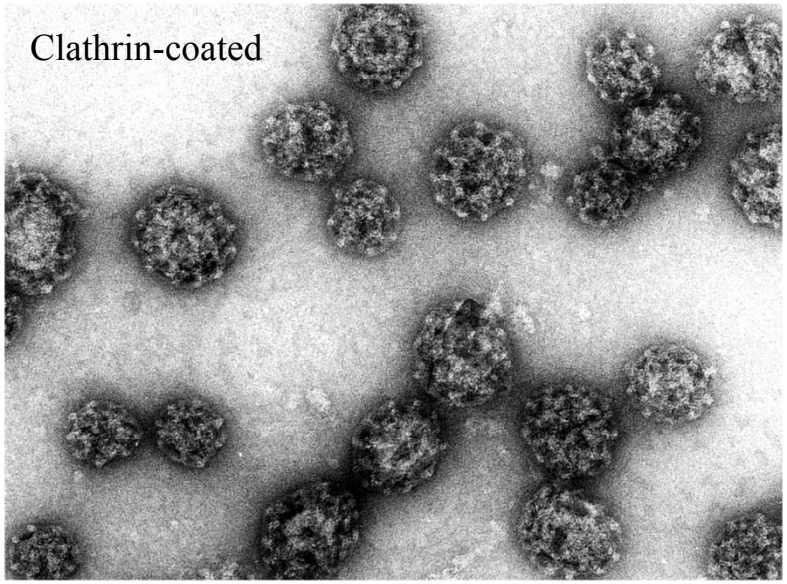


Figure 9-42a Fundamentals of Biochemistry, 2/e

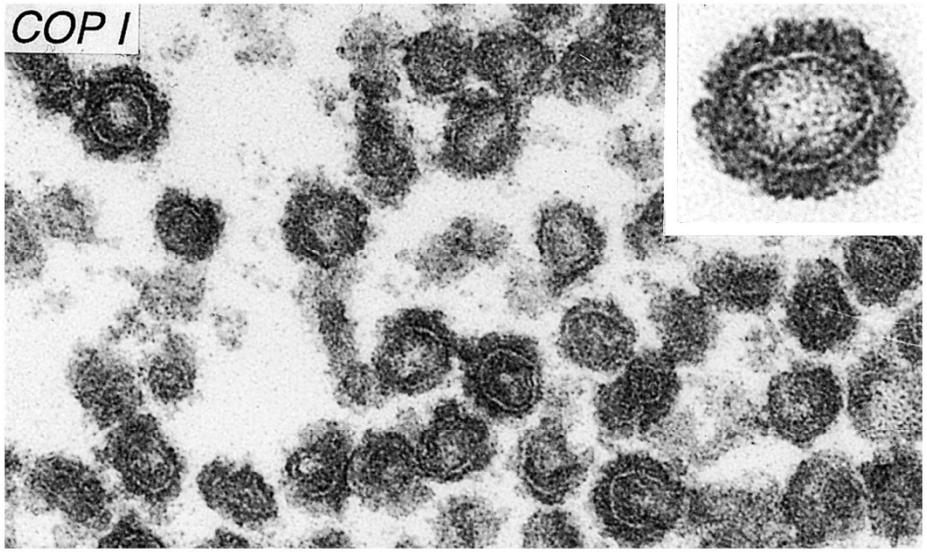


Figure 9-42b Fundamentals of Biochemistry, 2/e

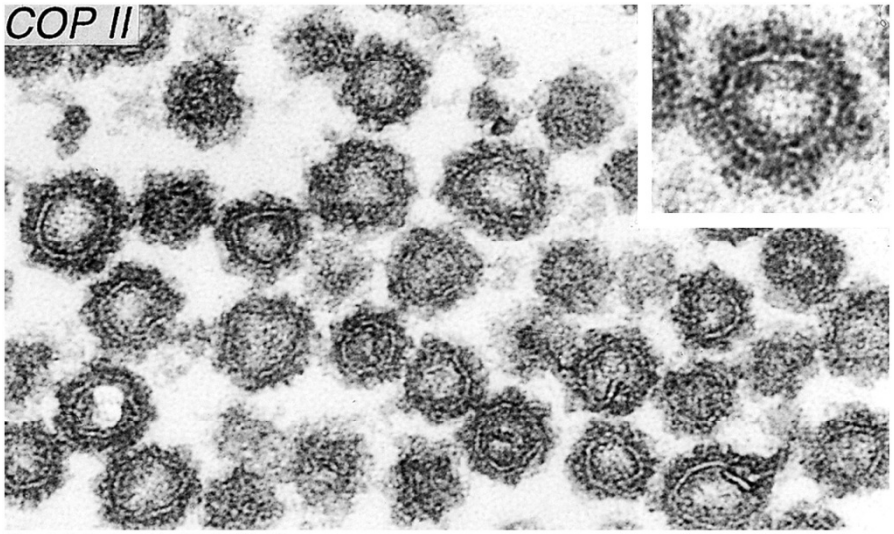


Figure 9-42c Fundamentals of Biochemistry, 2/e

Bud off and fusion

Preservation of
the orientation of the transmembrane protein

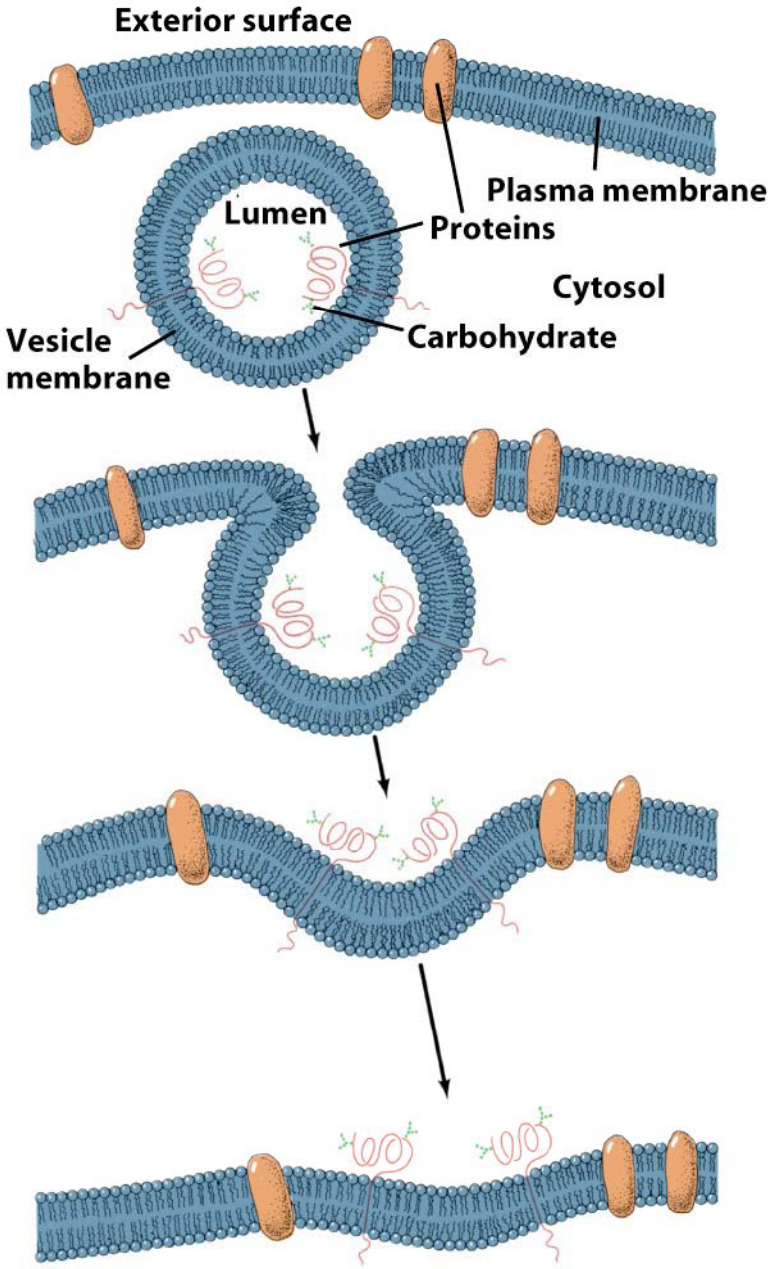


Figure 9-43 Fundamentals of Biochemistry, 2/e

Clathrin cage

Consists of triskelions

Transport TM, GPI-linked, and secretary proteins from the Golgi to the plasma membrane

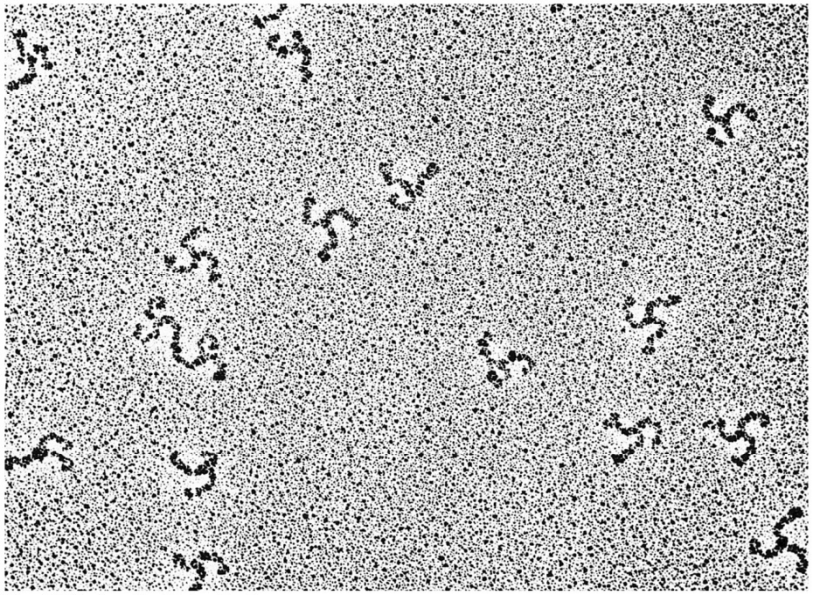


Figure 9-44 Fundamentals of Biochemistry, 2/e

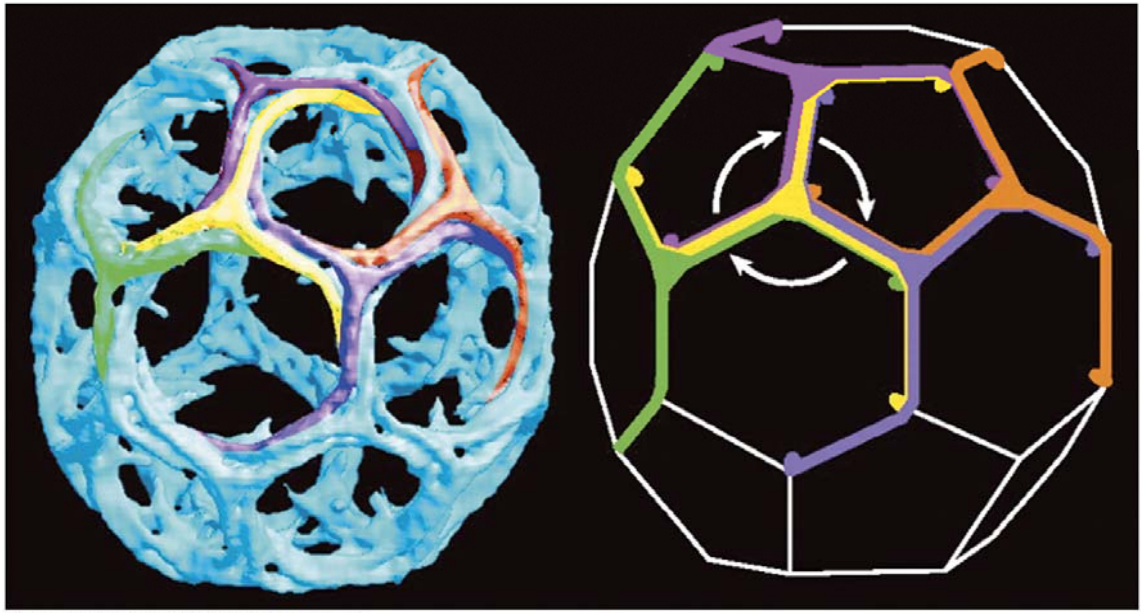


Figure 9-45a Fundamentals of Biochemistry, 2/e

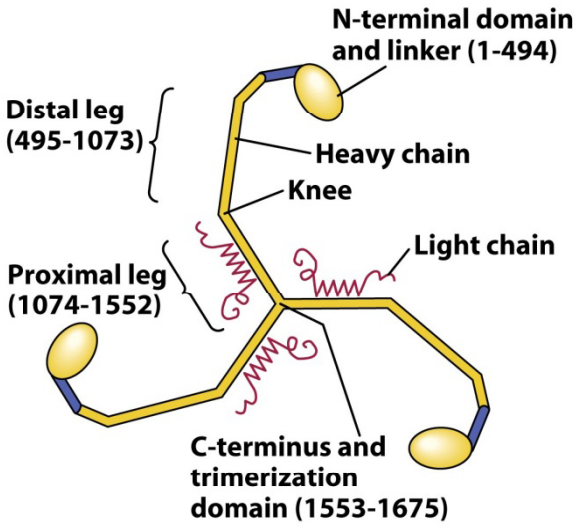


Figure 9-45b Fundamentals of Biochemistry, 2/e

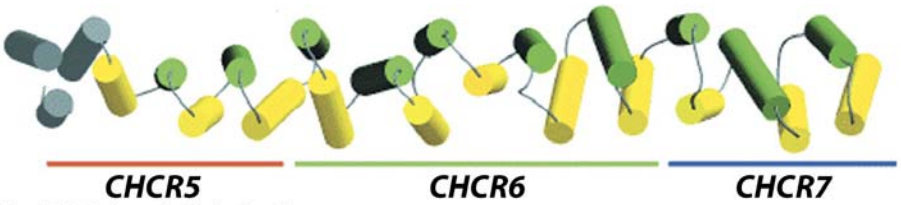


Figure 9-46c Fundamentals of Biochemistry, 2/e

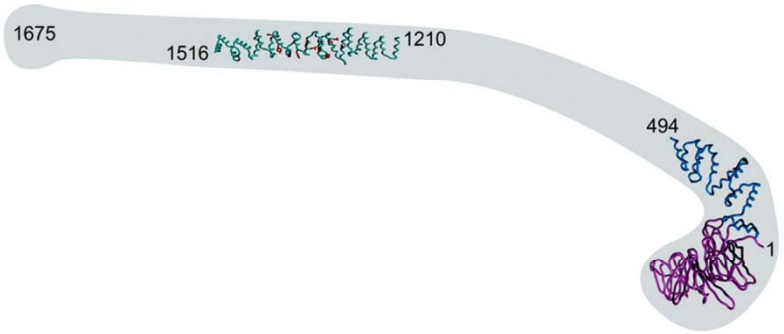
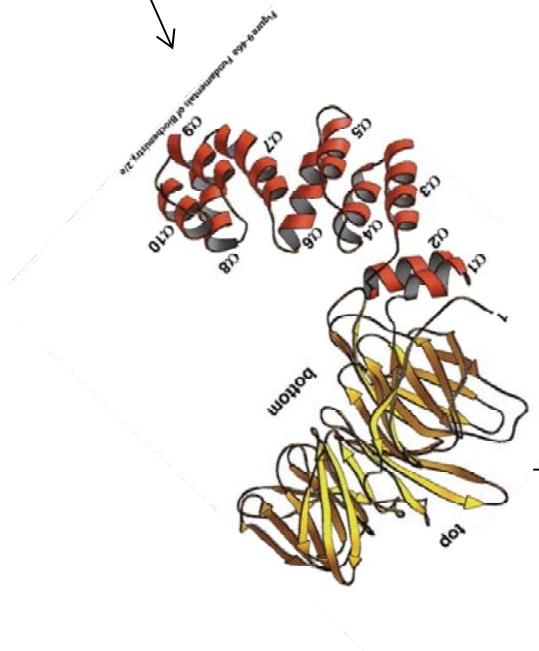


Figure 9-46d Fundamentals of Biochemistry, 2/e



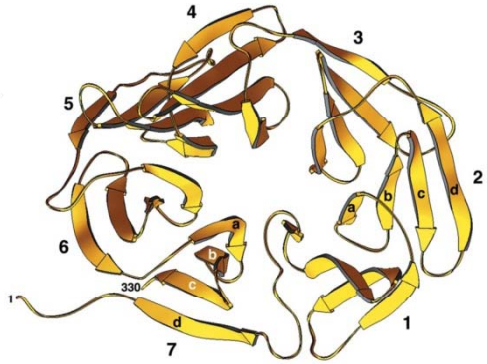
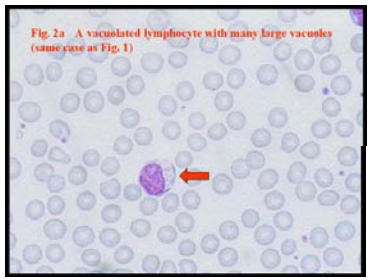


Figure 9-46b Fundamentals of Biochemistry, 2/e



How to know the destination (ER to Golgi to ?)

Proteins are directed to the lysosome by carbohydrate recognition markers

I(inclusion)-cell disease (mucolipidosis II): lysosomal storage disease

- Glycosaminoglycans and glycolipids in the lysosomes of the connective tissue cells
- Absence of a mannose-6-P recognition marker in the proteins due to the deficiency of an enzyme catalyzing the mannose phosphorylation (*N*-acetylglucosamine-1-phosphotransferase)
- Absence of several lysosomal hydrolases: they are secreted into the extracellular

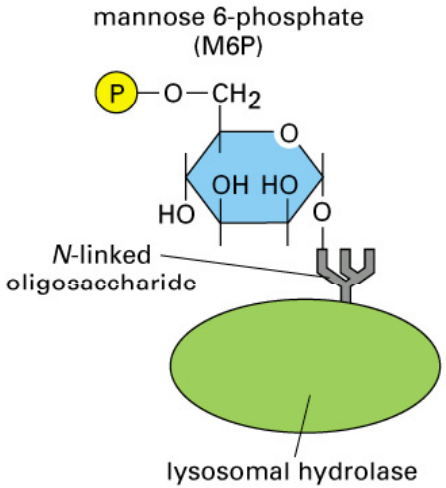


Figure 13–36. Molecular Biology of the Cell, 4th Edition.

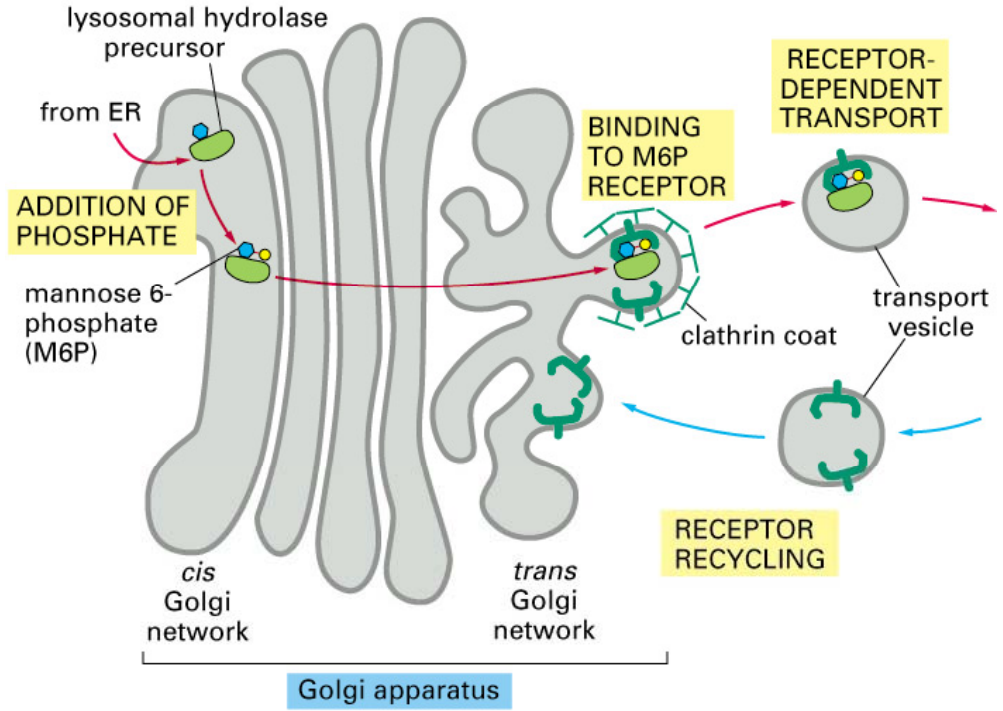


Figure 13–37 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

ER resident proteins

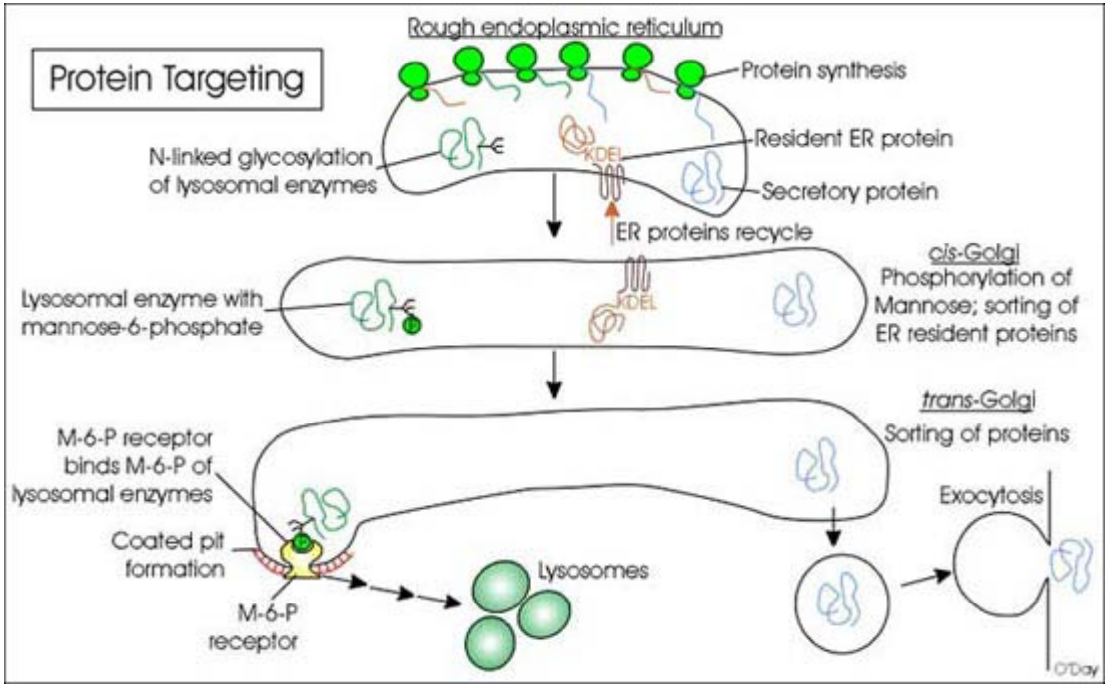
Most of the soluble ER proteins in mammals are retained through a retention motif:

C-terminal KDEL (HDEL in yeast), KKXX, KKKXXX

If not secreted

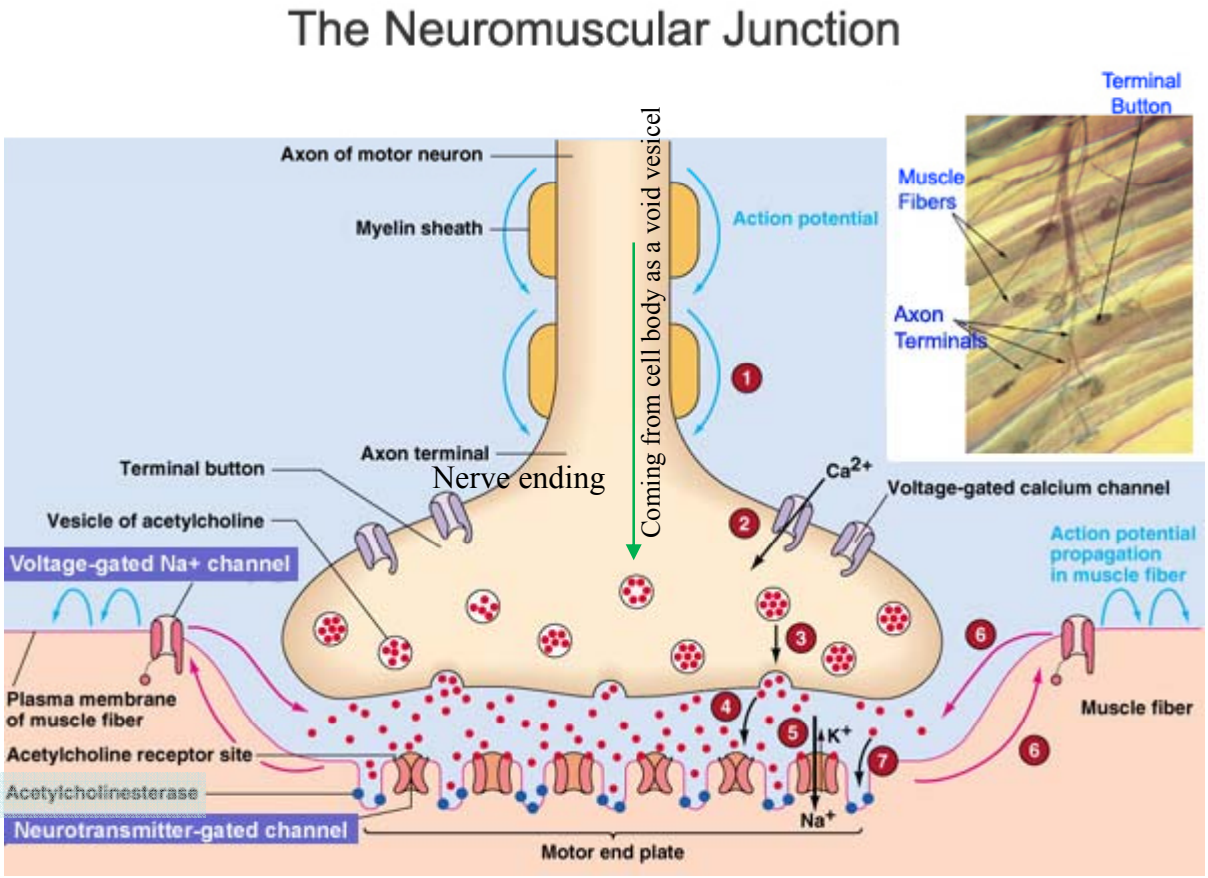
How they are retained in ER?

probably recycling between ER & Golgi via COPII & COPI vesicles, respectively

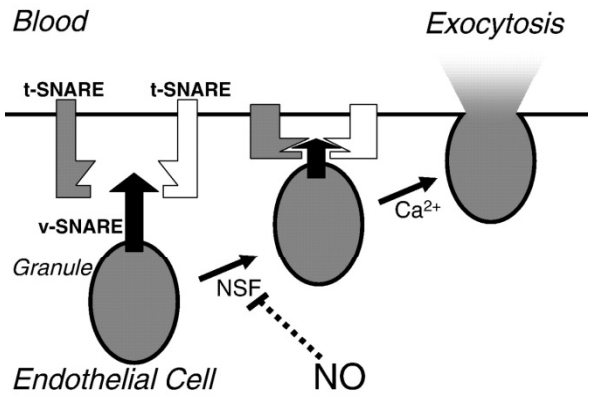


Neuromuscular junction: neuromuscular transmission

Synapse
Presynaptic membrane
Postsynaptic membrane



Choline acetyltransferase
Acetylcholinesterase



Local potential leads to endplate potential (EPP), which is a sum of miniature EPP (very small EPP due to very small influx of Ach)

Vesicle fusion

Membrane turnover via vesicle trafficking

How do they fuse?
why do they fuse only with their target membranes?

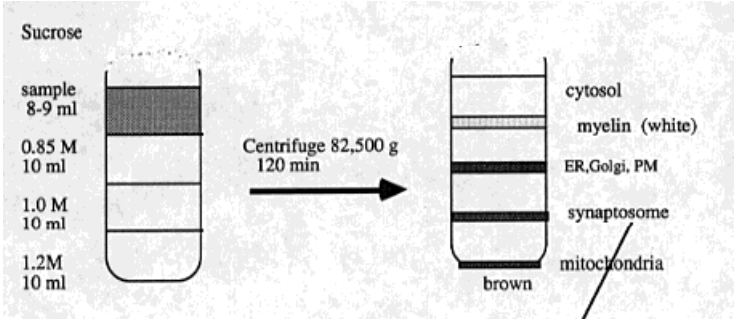
Synaptosome isolation
artificial vesicle prepared from the presynaptic endings

NSF: NEM-sensitive fusion proteins
NEM: *N*-ethylmaleimide alkylating Cys residue

SNAP: soluble NSF attachment protein

SNAREs: SNAP receptors

R-SNAREs: associated with the vesicles
Q-SNAREs: associated with target membranes



Vesicle fusion at the synapse

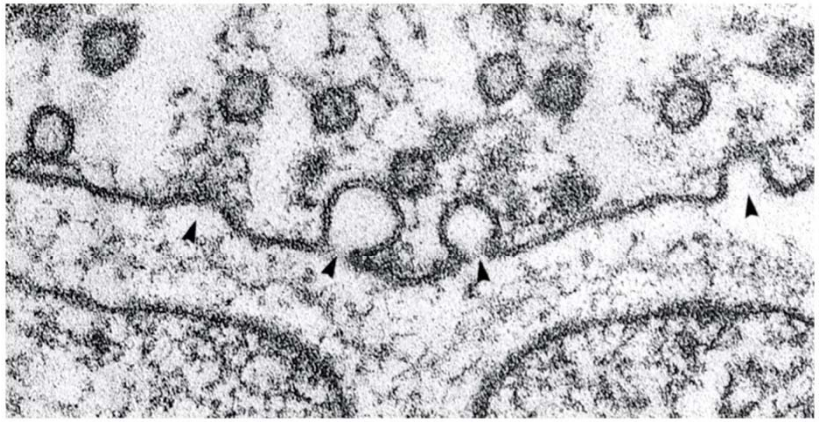


Figure 9-47a Fundamentals of Biochemistry, 2/e

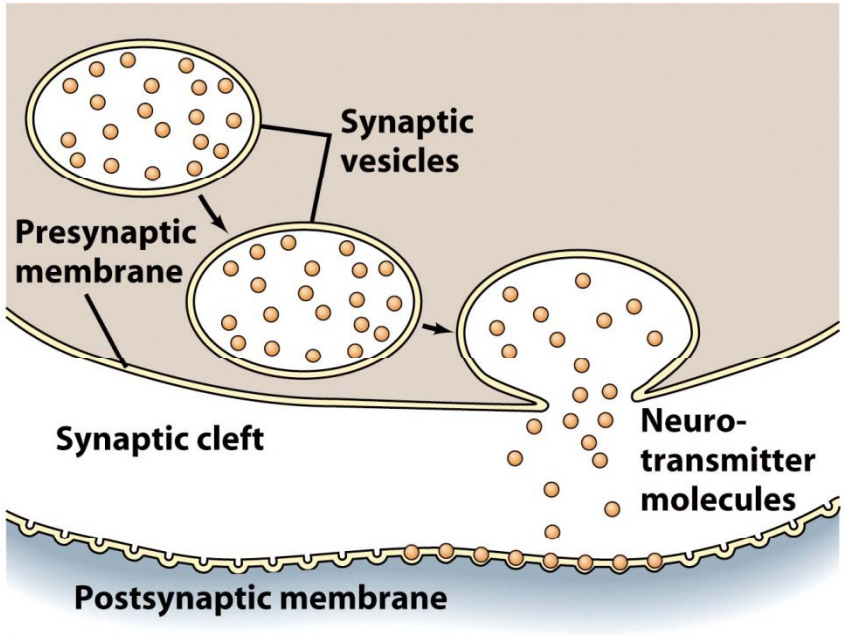
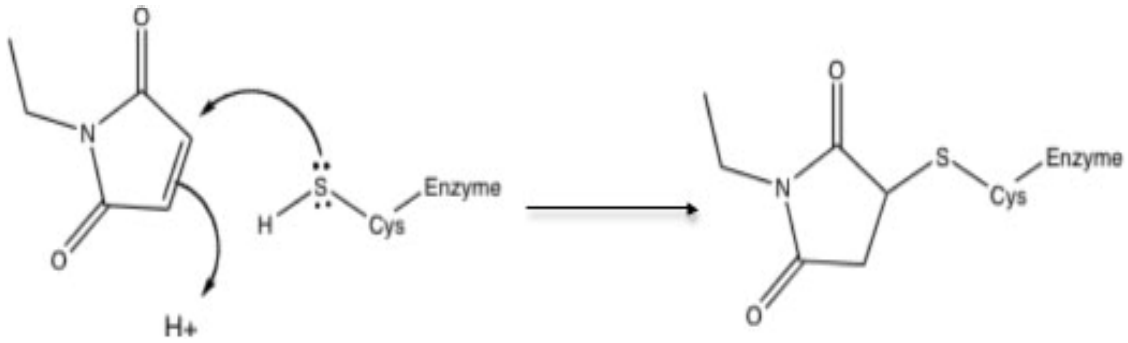


Figure 9-47b Fundamentals of Biochemistry, 2/e

N-Ethylmaleimide (NEM, 1-Ethylpyrrole-2,5-dione) is a irreversible inhibitor of all cysteine proteases, with alkylating occurring at the active site thiol group.(wikipedia)



SNARE complex
R-SNAREs: Synaptobrevin (Sb):
Q-SNAREs: Syntaxin (Sx), SNAP-25 (synaptosome-associated protein of 25 kD, Sn1 & Sn2)

Four helices of the core complex
7-residue repeats, a and d residues are hydrophobic
H-bond between Arg & 3 Gln
Arg from synaptobrevin
Gln from syntaxin, SNAP-25

Responsible for specific transport
numerous different SNAREs

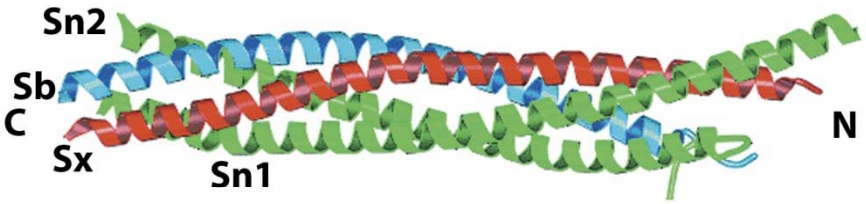


Figure 9-48a Fundamentals of Biochemistry, 2/e

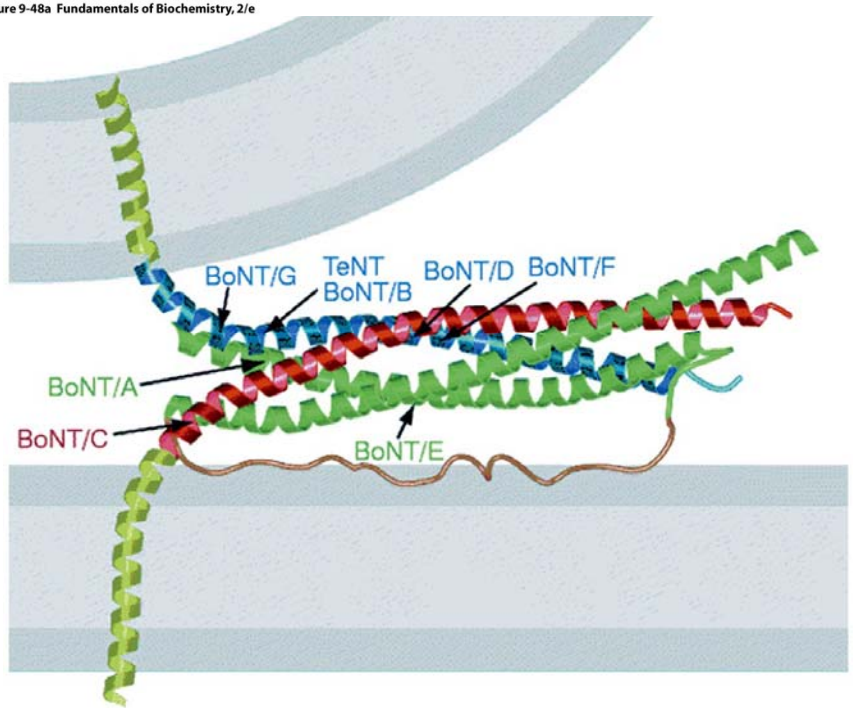


Figure 9-48b Fundamentals of Biochemistry, 2/e

Clostridium botulism, Clostridium tetanus
Botulinum neurotoxin
~150 kD: heavy (~100 kD) + light (~50 kD)
Light chain is a protease targeting SNARE

SNARE complex linking two membranes

A model for SNARE-mediated vesicle fusion

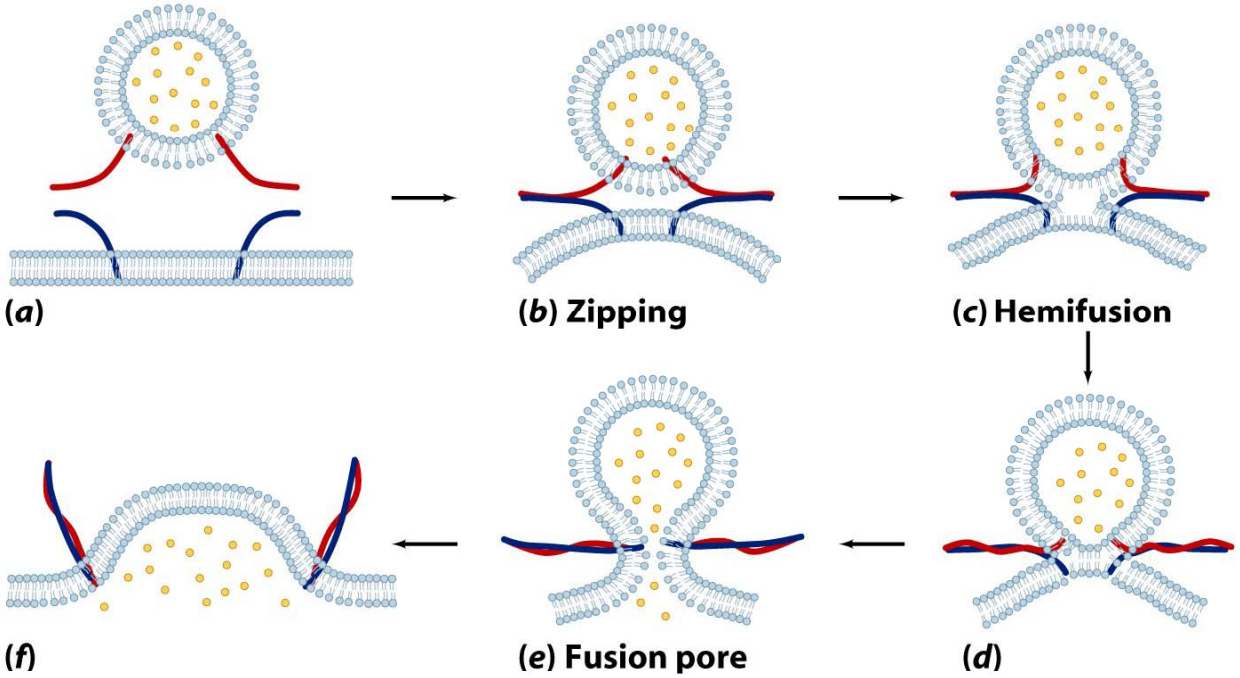
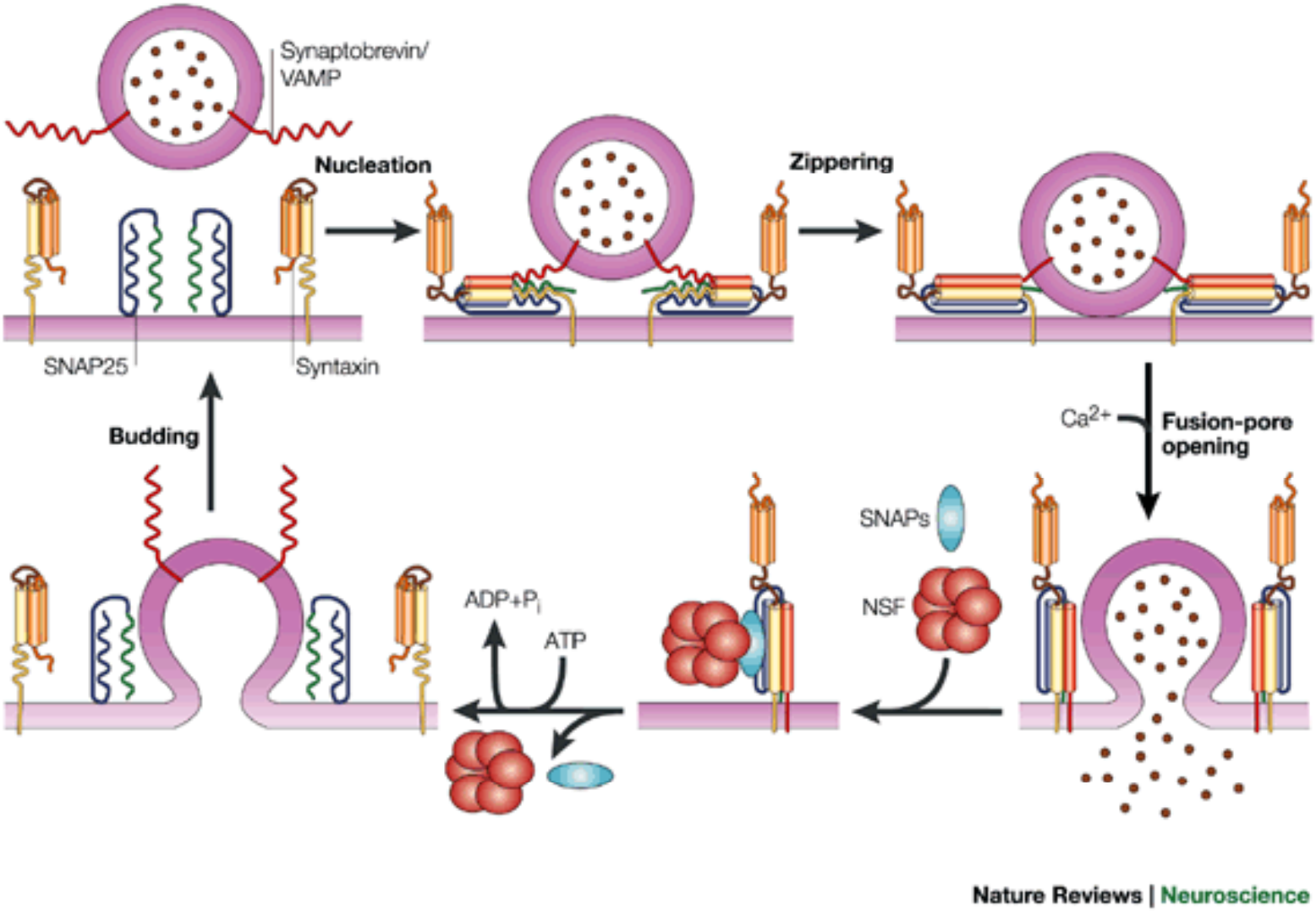
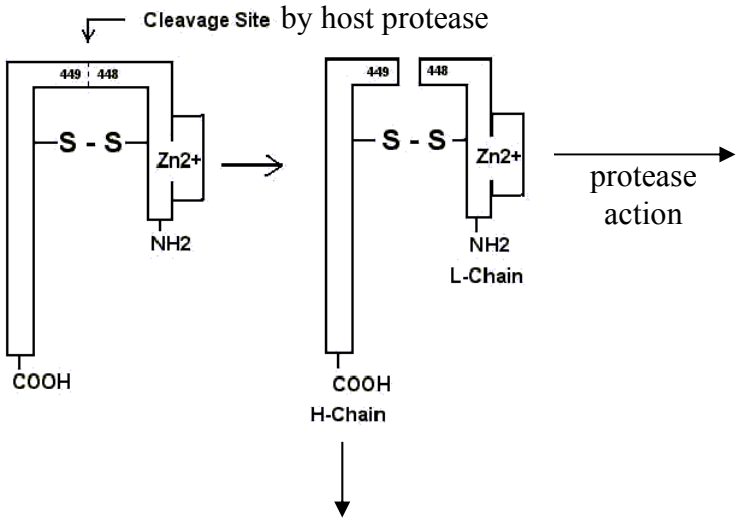


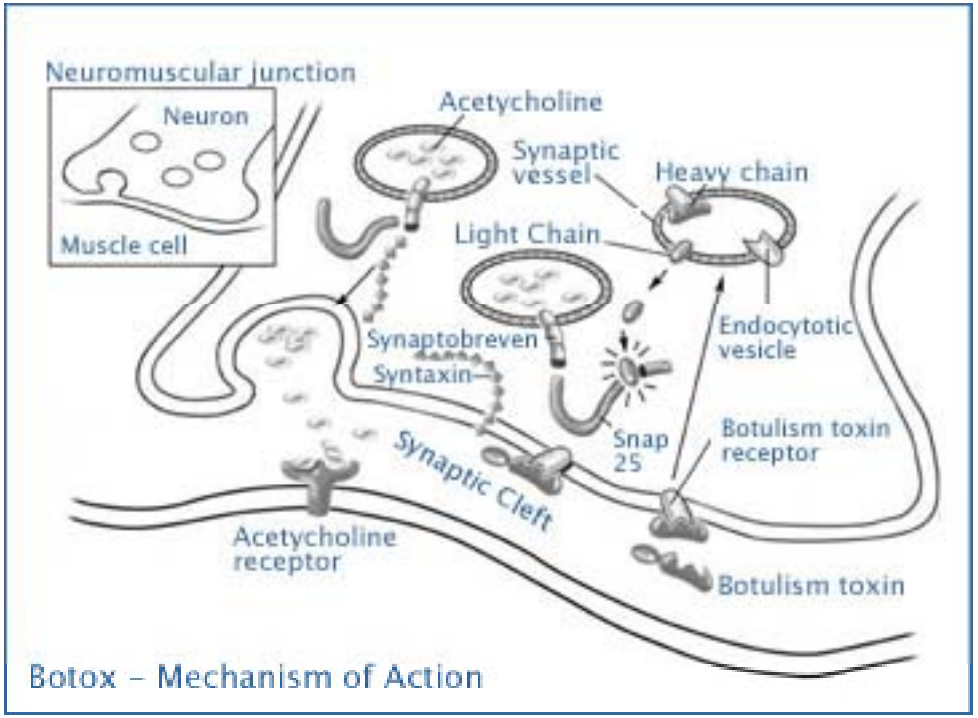
Figure 9-49 Fundamentals of Biochemistry, 2/e



Nature Reviews Neuroscience **3**, **641-653 (August 2002)**

Tetanus and Botulinum toxins: neurotoxins





Tetanus neurotoxin (TeTx) binds to inhibitory neurons, causing spastic paralysis (경련성 마비)
Botulinum neurotoxins (BoNTs) bind to motor neurons, causing flaccid paralysis (이완성 마비)