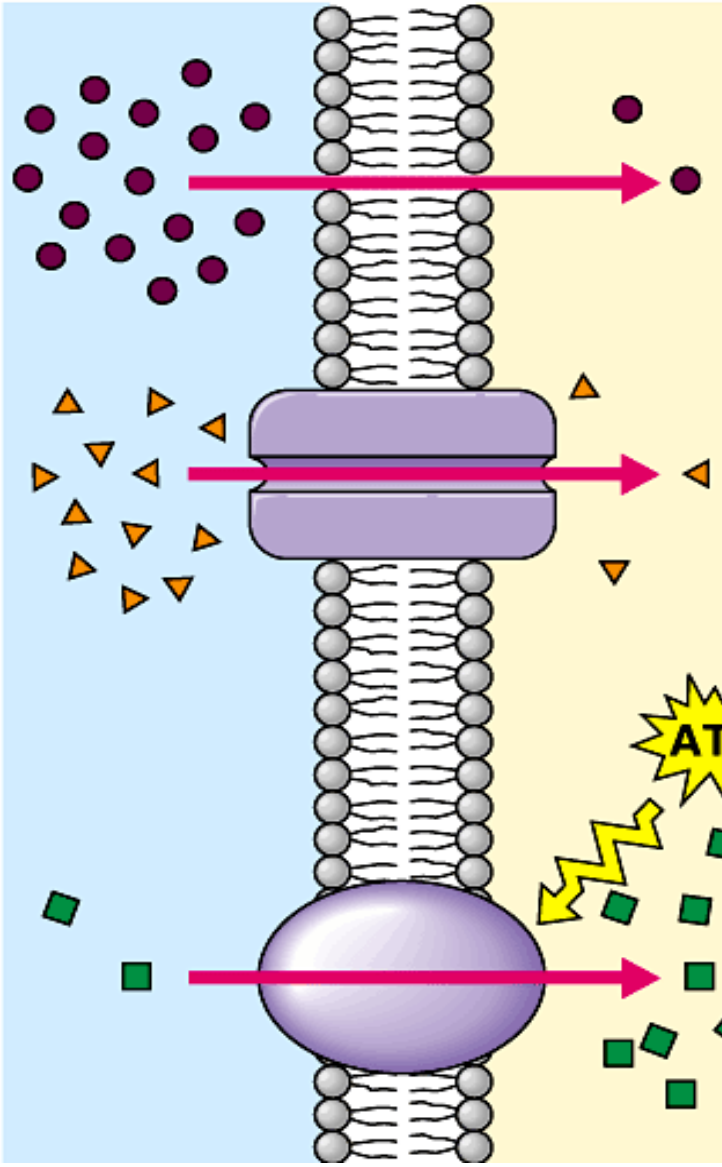
Mechanisms of Transport Across the Cell Membrane



simple diffusion

facilitated diffusion

Passive transport

Professor Nurten ÖZSOY

active transport

Learning objectives

At the completion of this lesson, students will be able to:

- Explain the term concentration gradient
- Describe the process of diffusion
- Understand which substances can pass freely through the lipid bilayer and which cannot.
- Describe the process of osmosis
- Explain how isotonic, hypertonic and hypotonic solutions affect cells
- Distinguish between diffusion and osmosis
- Explain how substances cross the cell membrane through facilitated diffusion
- Distinguish between channel proteins and carrier proteins.
- Be able to distinguish and give examples of ligand-gated, mechanically-gated, and voltage-gated facilitated diffusion channels.

- Distinguish between active and passive transport
- Be able to describe the difference between facilitated diffusion and active transport.
- Understand the role of ATP-binding cassette transporters in, for example, multi-drug resistance and its significance for cancer chemotherapy.
- Understand the difference between direct (ATPase driven) and indirect (co-transport) active transport mechanisms.
- Outline the structure of Na-K ATPase, define what type of transport it mediates
- Explain how large molecules are transported across a cell membrane.
- Distinguish between exocytosis and receptor-mediated endocytosis.

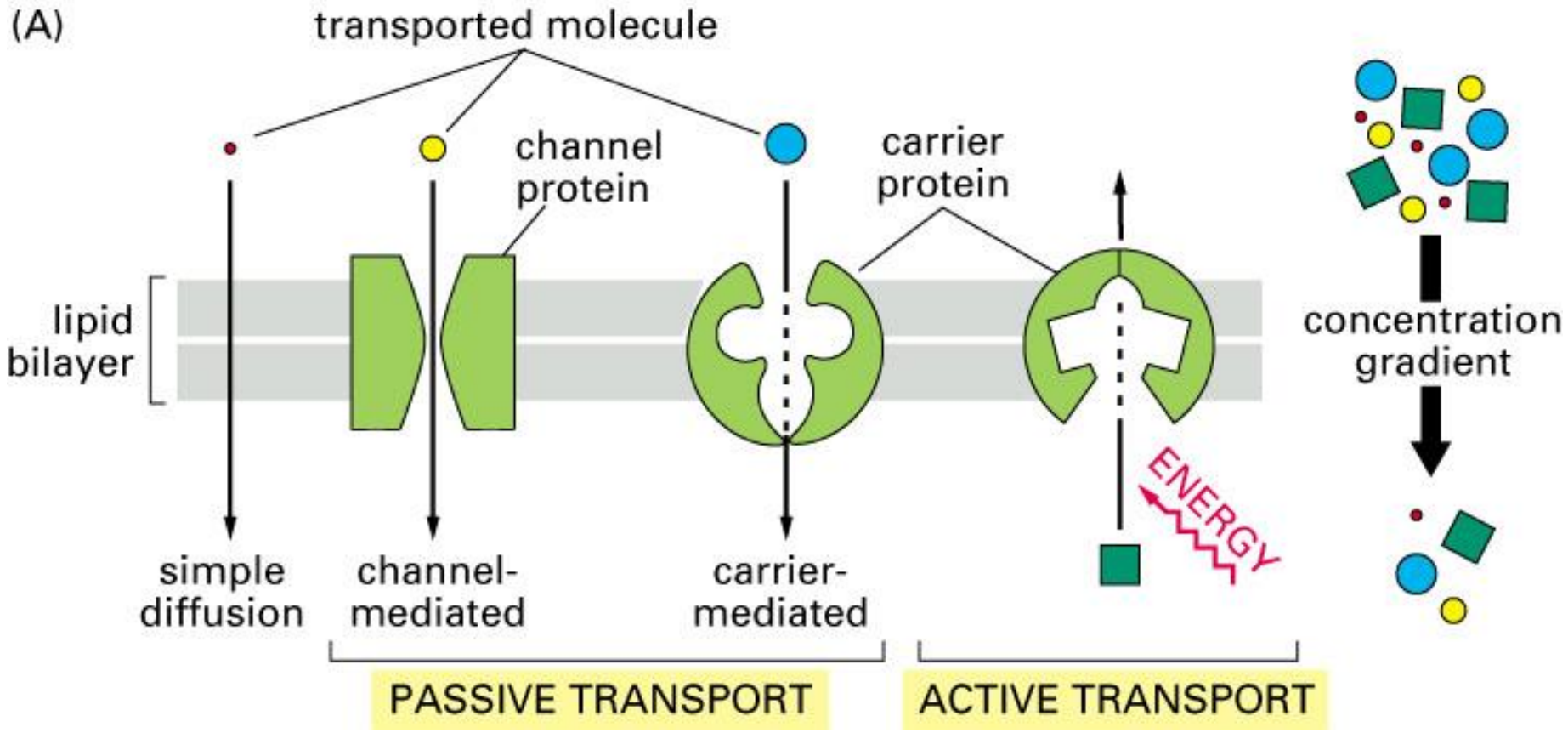
- Describe the different mechanisms by which secretory vesicles fuse with the plasma membrane
- Distinguish between receptor-mediated endocytosis and phagocytosis.
- Be able to discuss the different types of endocytosis and their distinct cellular functions.
- Understand the molecular machinery involved in clathrin- mediated receptor endocytosis.
- Be able to describe the critical role of endosomal pH changes in receptor/ligand interactions and receptor recycling.
- Understand the effects of LDL receptor mutations on endocytic uptake of LDL.

Getting through cell membrane

- Passive Transport
  - Simple diffusion
    - diffusion of nonpolar, hydrophobic molecules
      - lipids
      - high → low concentration gradient
  - Facilitated diffusion
    - diffusion of polar, hydrophilic molecules
    - through a protein channel
      - high → low concentration gradient
- Active transport
  - diffusion *against* concentration gradient
    - low → high
  - uses a protein pump
  - requires ATP

MEMBRANE TRANSPORT PROTEINS: CARRIERS AND CHANNELS

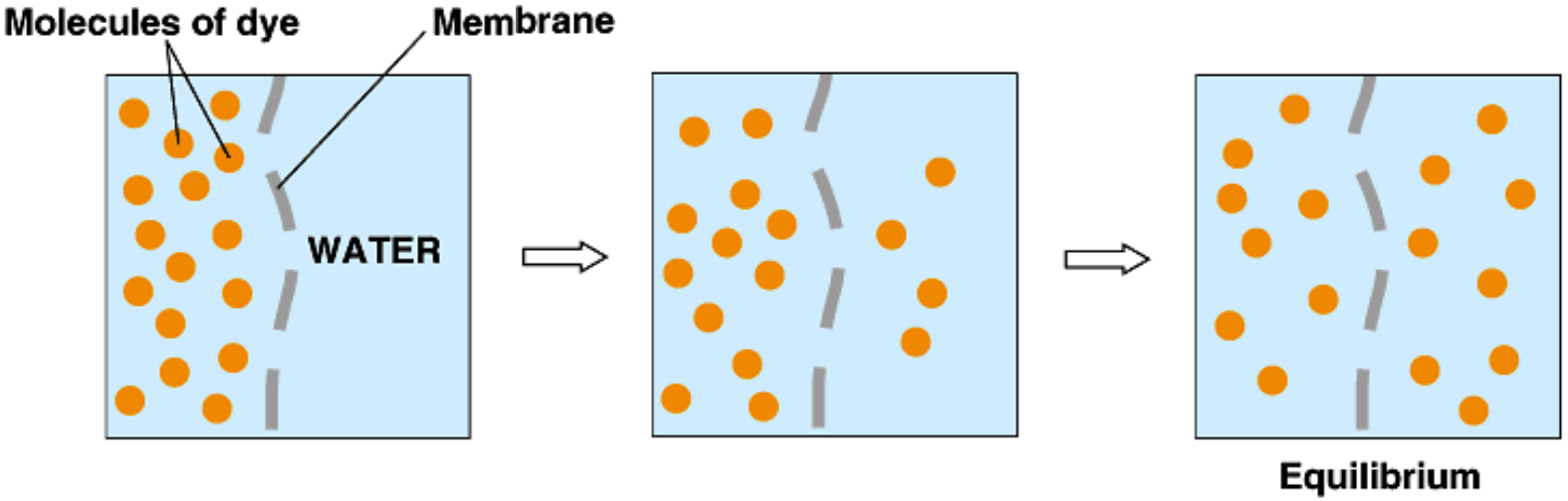
There are two major classes of membrane transport proteins: carrier proteins and channels. Membrane transport proteins can be classified further by whether they mediate active or passive transport.



Diffusion

- **Diffusion**
  - **movement from high → low concentration**

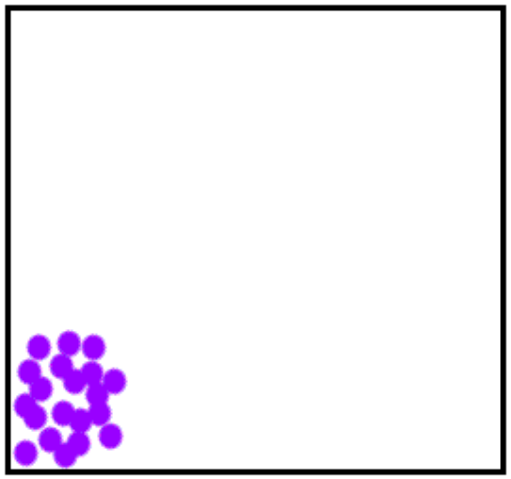
A concentration gradient is the difference in concentration between two areas.



Diffusion is a spontaneous process in which a substance moves from a region of high concentration to a region of low concentration, eventually eliminating the concentration difference between the two regions.

Simple Diffusion

- Movement of molecules from areas of higher concentration to areas of lower concentration
  - "passive transport"
  - no energy needed



diffusion

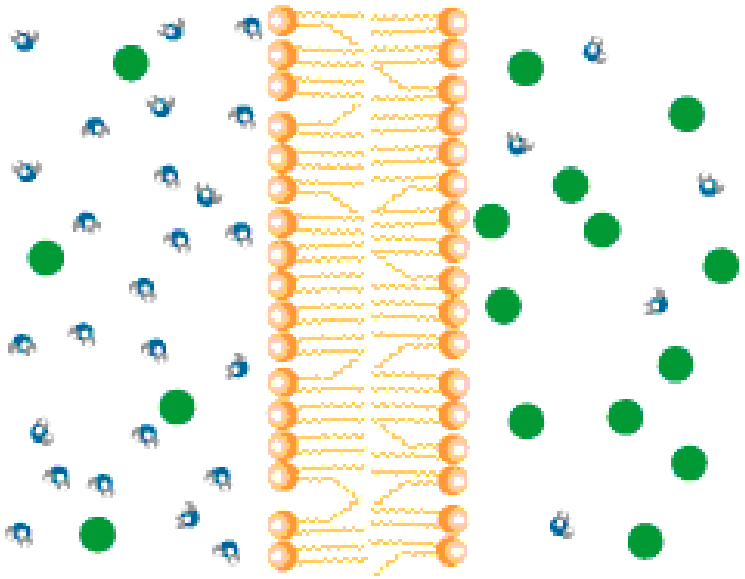
When the substance has fully dispersed through the container, it has reached equilibrium.

When equilibrium has been reached, there is no longer a concentration gradient.

Osmosis

Osmosis is a special example of diffusion. It is the diffusion of water through a partially permeable membrane from a more dilute solution to a more concentrated solution

movement of water



osmosis

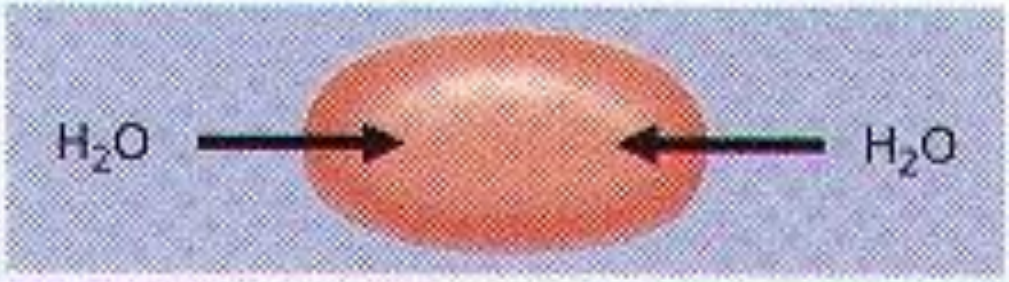
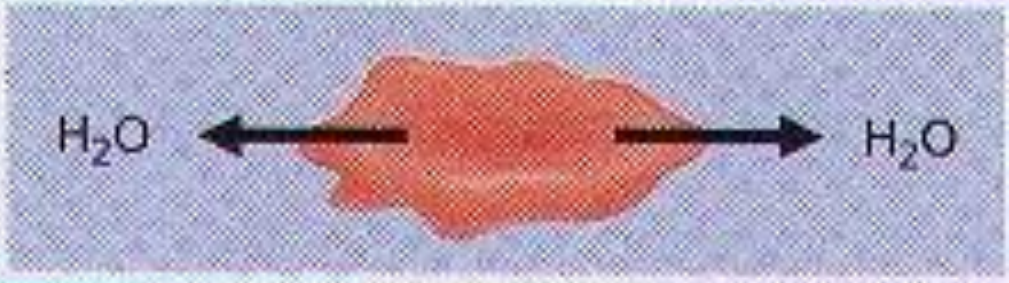
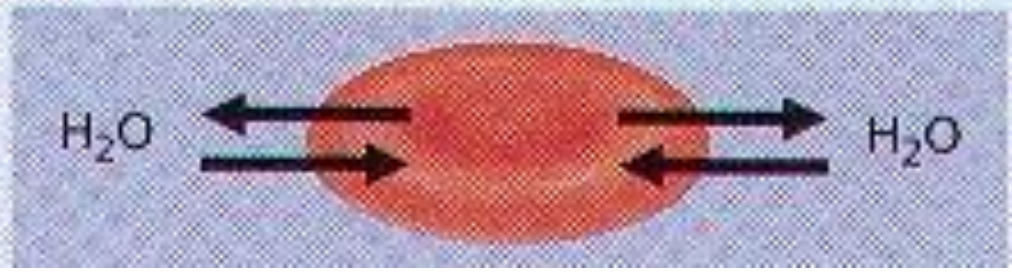
Solution Types

Hypertonic Solutions: contain a **high concentration** of solute relative to another solution (e.g. the cell's cytoplasm). When a cell is placed in a hypertonic solution, the water diffuses **out** of the cell, causing the cell to **shrivel**.

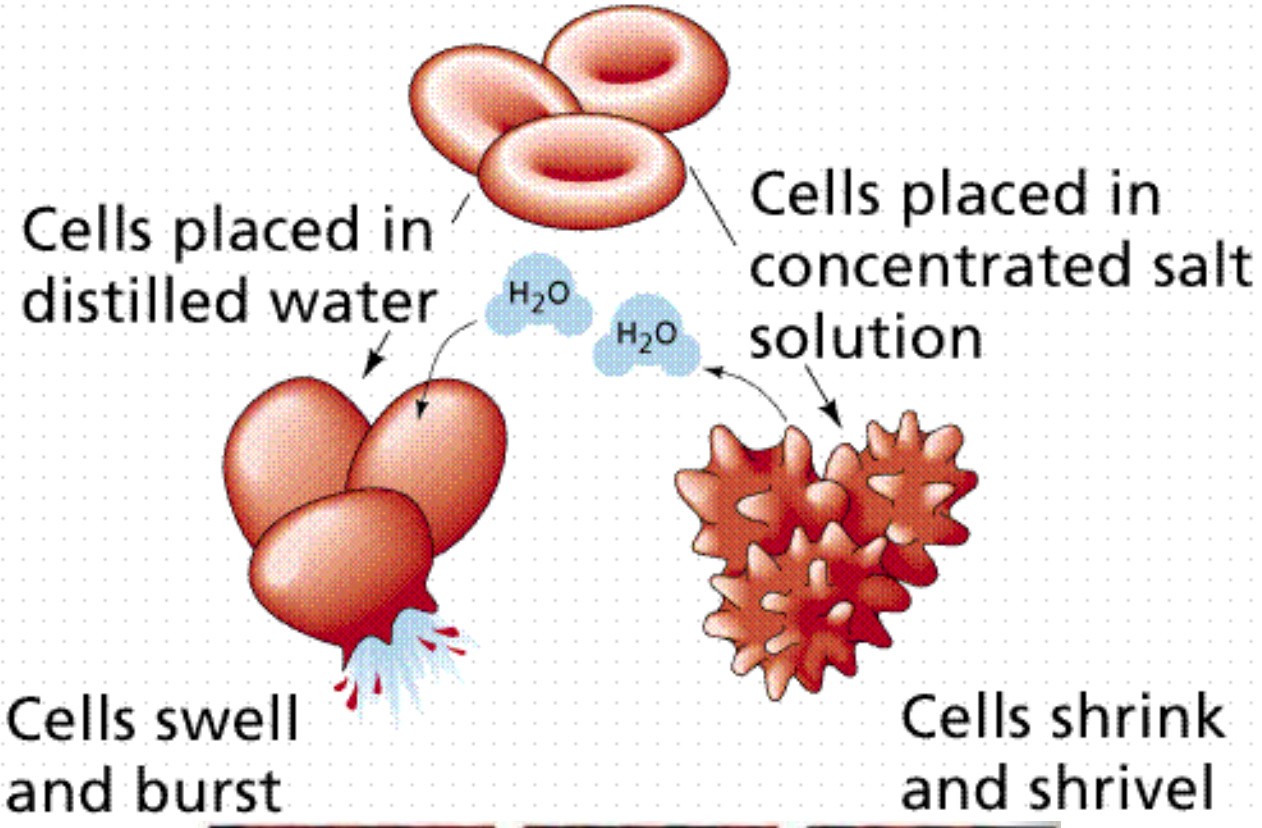
Hypotonic Solutions: contain a **low concentration** of solute relative to another solution (e.g. the cell's cytoplasm). When a cell is placed in a hypotonic solution, the water diffuses **into** the cell, causing the cell to **swell** and possibly **explode**.

Isotonic Solutions: contain the **same concentration** of solute as another solution (e.g. the cell's cytoplasm). When a cell is placed in an isotonic solution, the water diffuses **into and out** of the cell at the same rate. The fluid that surrounds the body cells is isotonic.

Direction of osmosis

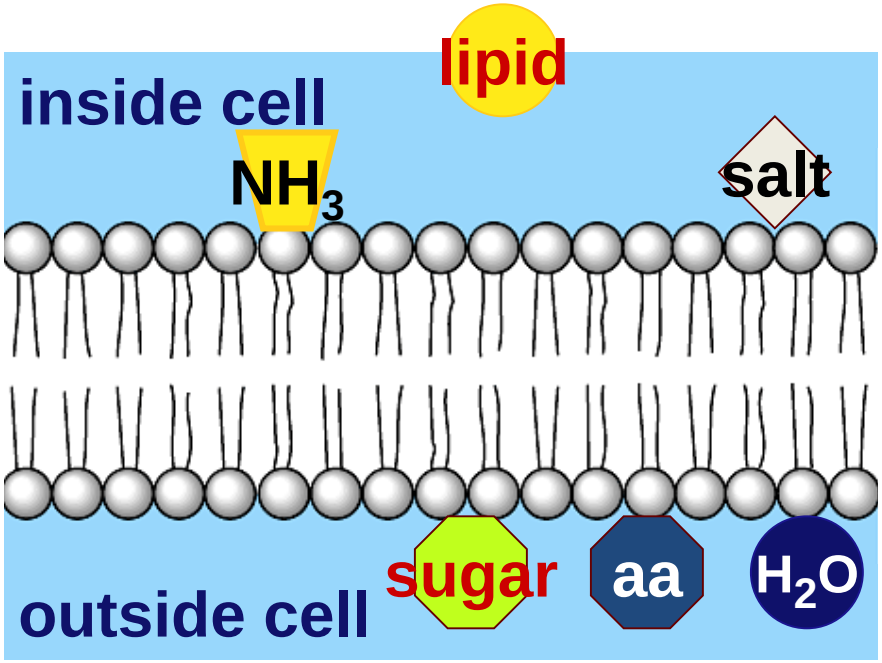
Condition	Net movement of water	
External solution is hypotonic to cytosol	into the cell	
External solution is hypertonic to cytosol	out of the cell	
External solution is isotonic to cytosol	none	

Osmosis in red blood cells



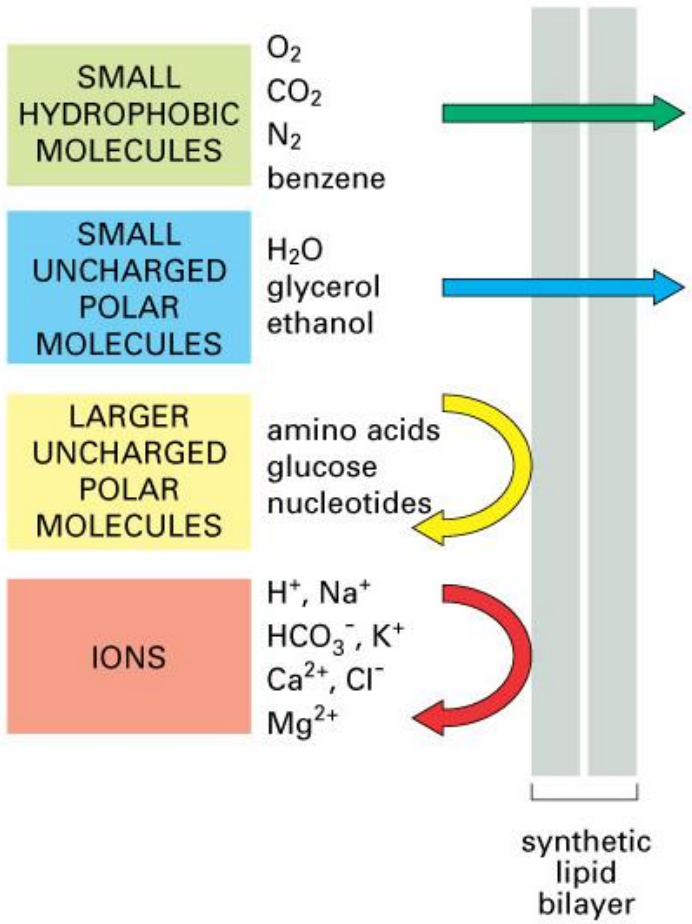
Diffusion through phospholipid bilayer

- What molecules can get through directly?
  - fats & other lipids



- **What molecules can <u>NOT</u> get through directly?**
  - **polar molecules**
    - **H_2O**
  - **ions**
    - **salts, ammonia**
  - **large molecules**
    - **starches, proteins**

Lipid bilayers are *selectively* permeable



ology, 2/e. (© 2004 Garland Science)

Membranes are highly permeable to small inorganic molecules, such as O_2, CO_2, NO, and H_2O

Decreasing permeability

In contrast, larger polar molecules, such as sugars, amino acids, and phosphorylated intermediates, exhibit poor membrane penetrability.

The lipid bilayer is highly impermeable to charged substances, including small ions

Size – polarity - ions

Facilitated Diffusion

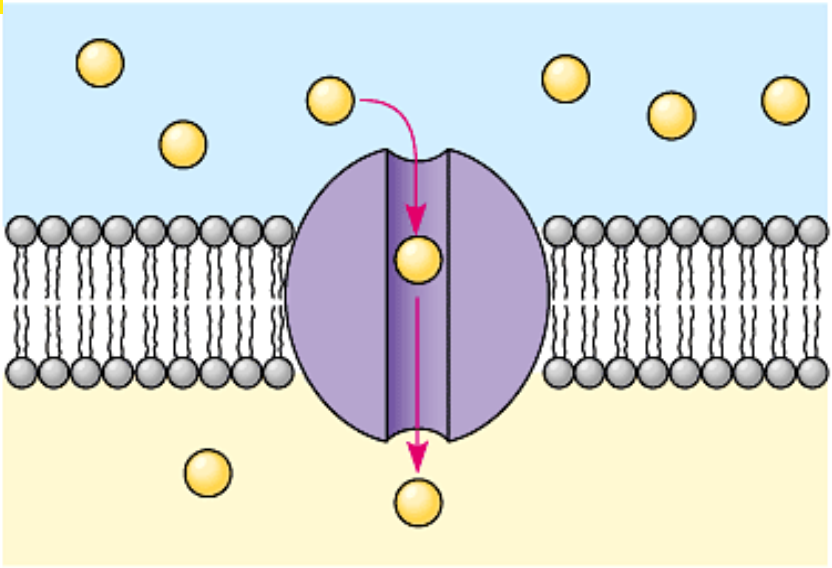
facilitated = with help

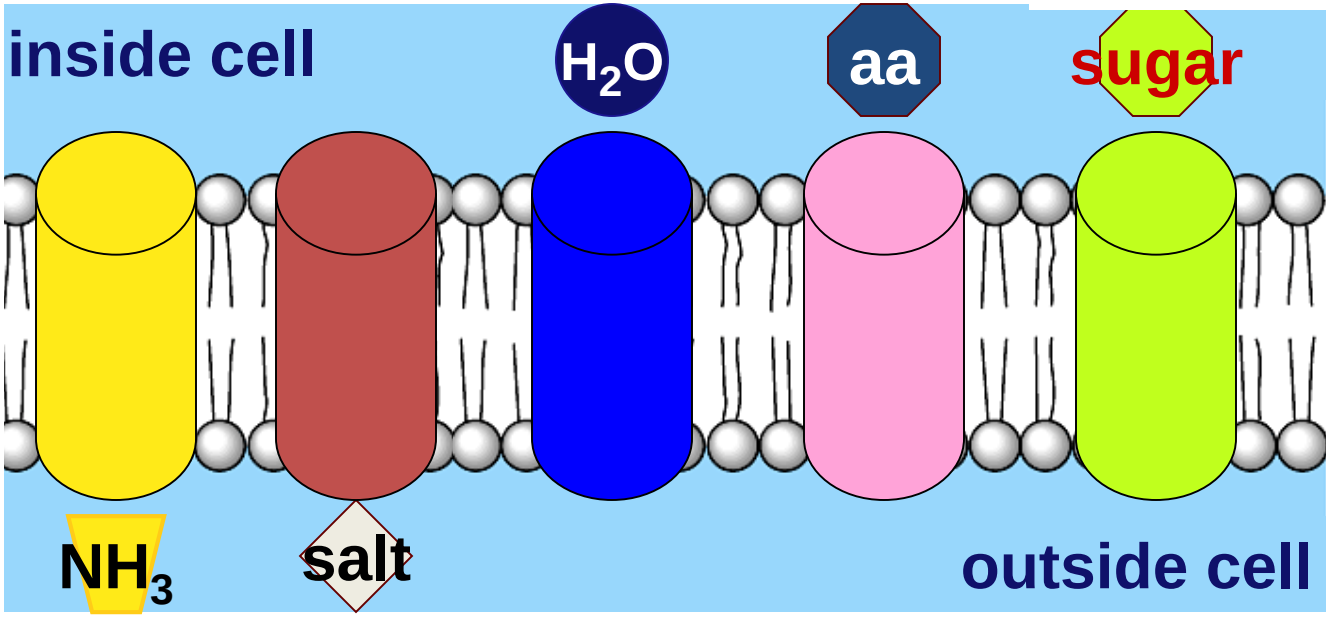
- Some molecules are too large to pass through the cell membrane by diffusion and need help to cross. These molecules use facilitated diffusion.
- This is the movement of specific molecules down a concentration gradient, passing through the membrane via a specific carrier protein
- It is passive and requires no energy from the cell.

high

low

open channel = fast transport





Channels through cell membrane

Channel Proteins

- Channel proteins include:

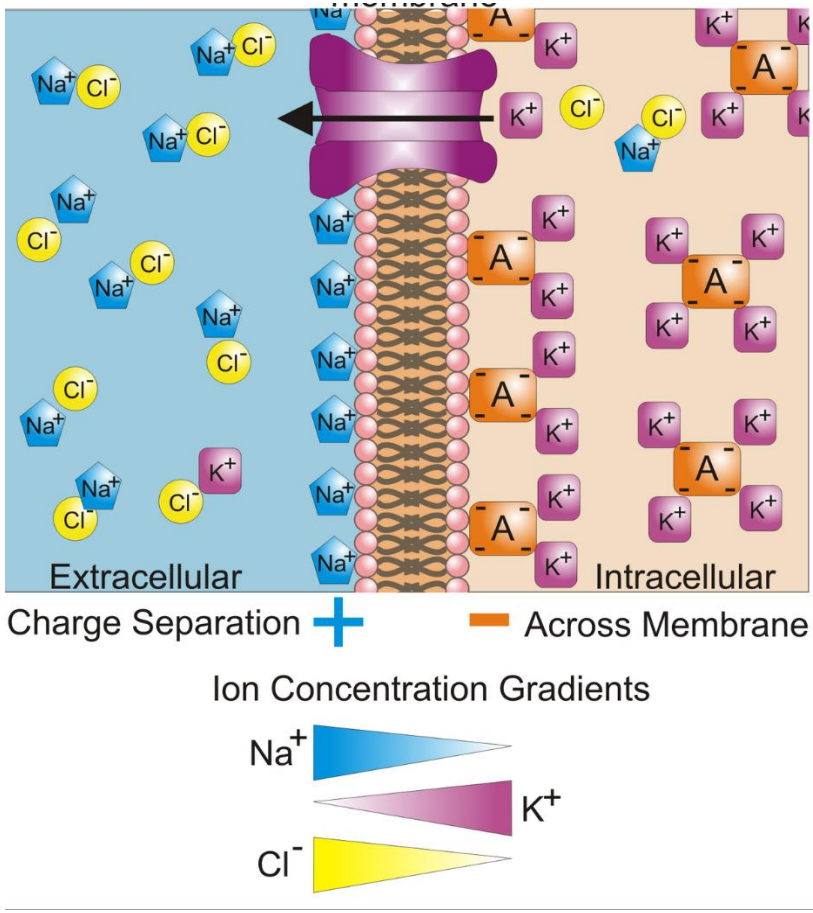
- ion channels - allow the passage of ions (charged atoms or molecules) which are associated with water

- gated channels are opened or closed in response to a stimulus
  - the stimulus may be chemical or electrical

Ion channels

Ions **diffuse** through channels („**Leak channels**“) = constantly open

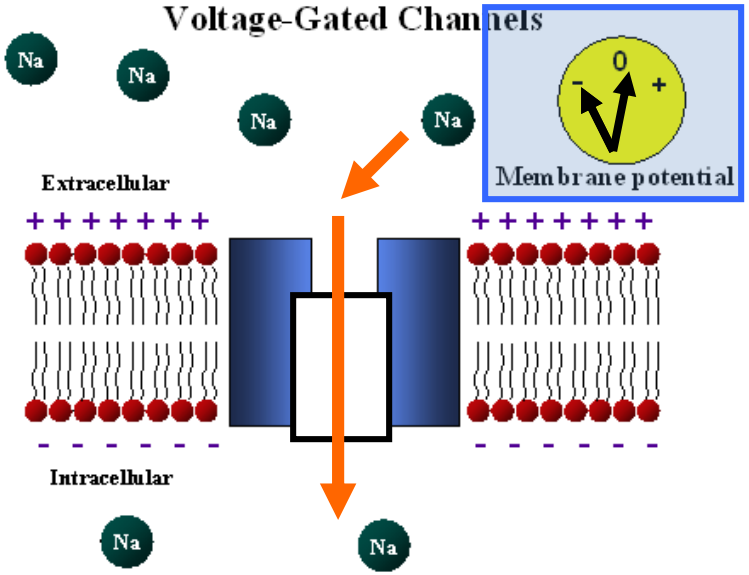
Transport through channels does not require an additional input of energy. Channels have an advantage over carrier proteins in terms of the speed of transport—up to a hundred million ions can pass through an ion channel per second (which is 100,000 times greater than any measured rate of transport via a carrier protein).

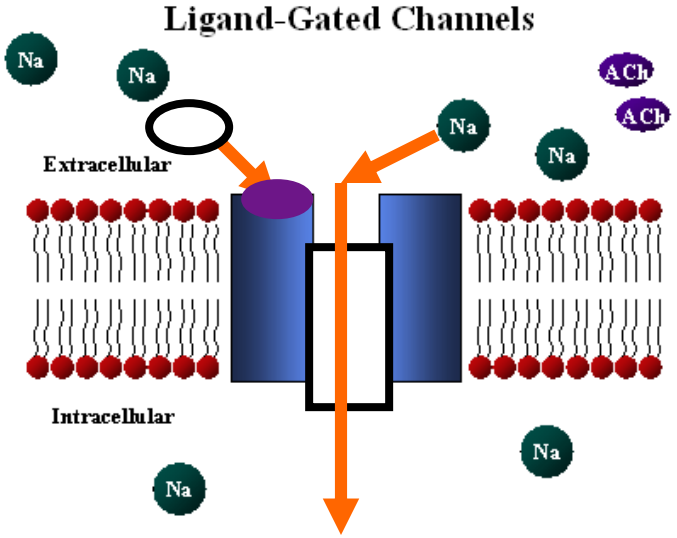


Gated ion channels

- Voltage-gated channels – controlled by membrane potential
- Ligand-gated channels – controlled by binding of a ligand to a membrane protein (either on the outside or the inside)
- Stress activated channel – controlled by mechanical force on the cell

Voltage-gated sodium channels allow the action potential to occur





Voltage-gated channels

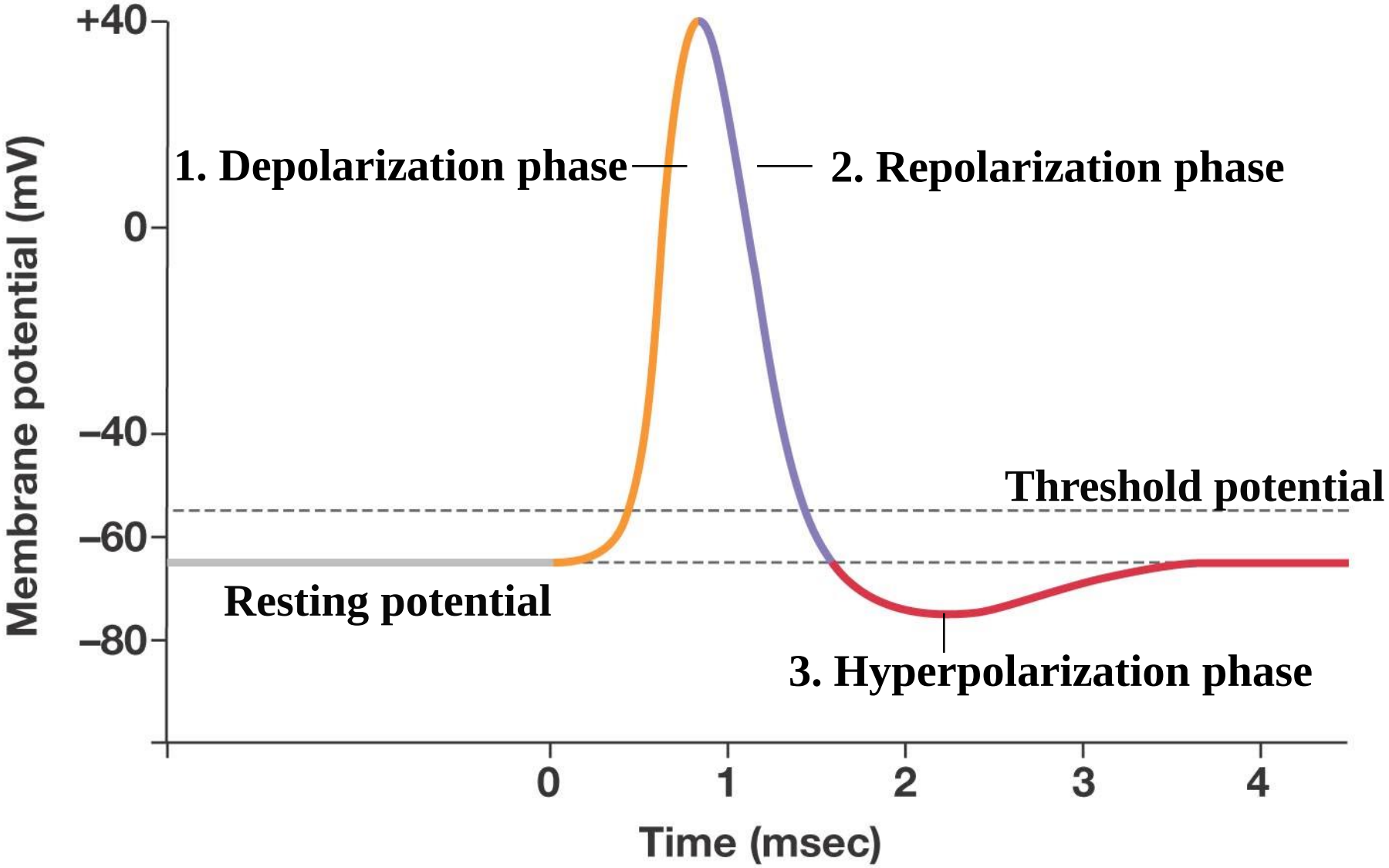
Two important types:

1) Na^+ voltage gated channels
2) K^+ voltage gated channels

How voltage-gated channels work

The Action Potential Is a Rapid Change in Membrane Potential

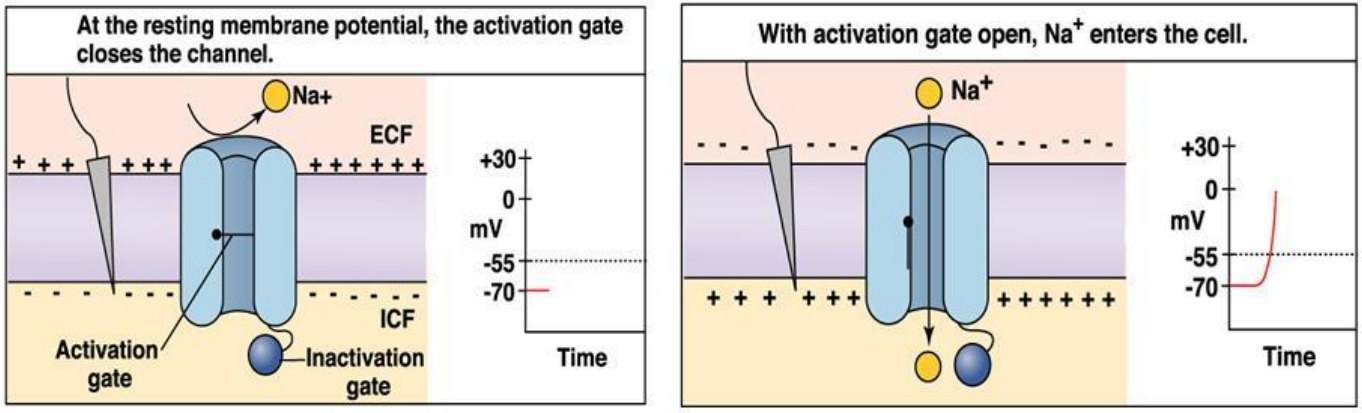
The action potential is initiated when an excitable cell such as a nerve or muscle fiber is depolarized to threshold.



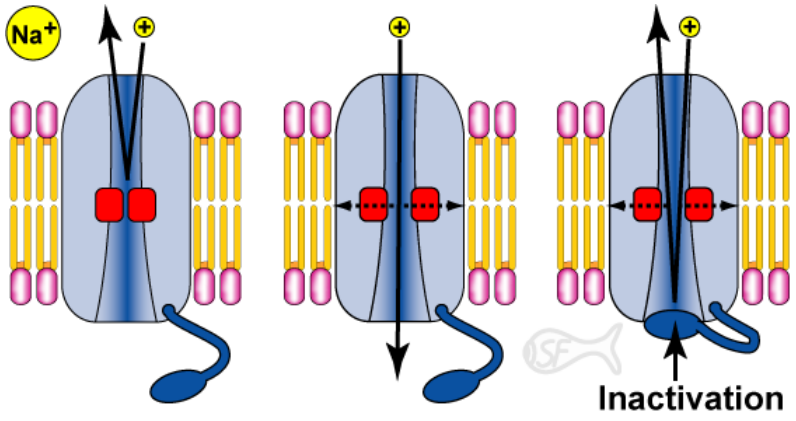
A potential (-70 mV) exists across the membrane of a resting neuron – the membrane is polarized

- Depolarization – inside of the membrane becomes less negative (or even reverses) – a reduction in potential
- Threshold – a critical level of depolarization (-55 to -50 mV) where depolarization becomes self-generating
- Repolarization – the membrane returns to its resting membrane potential
- Hyperpolarization – inside of the membrane becomes more negative than the resting potential – an increase in potential

Depolarization increases the probability of producing nerve impulses. Hyperpolarization reduces the probability of producing nerve impulses.



Depolarization opens the activation Na^+ gate (rapid) and closes the inactivation Na^+ gate (slower). The gate for the K^+ is slowly opened with depolarization.



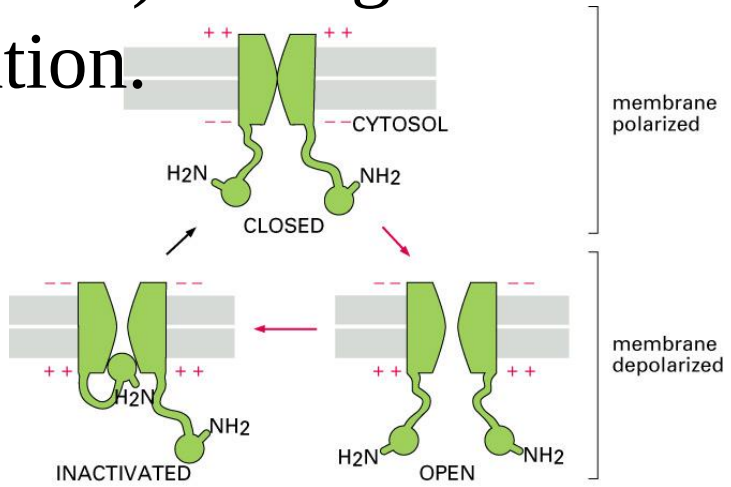
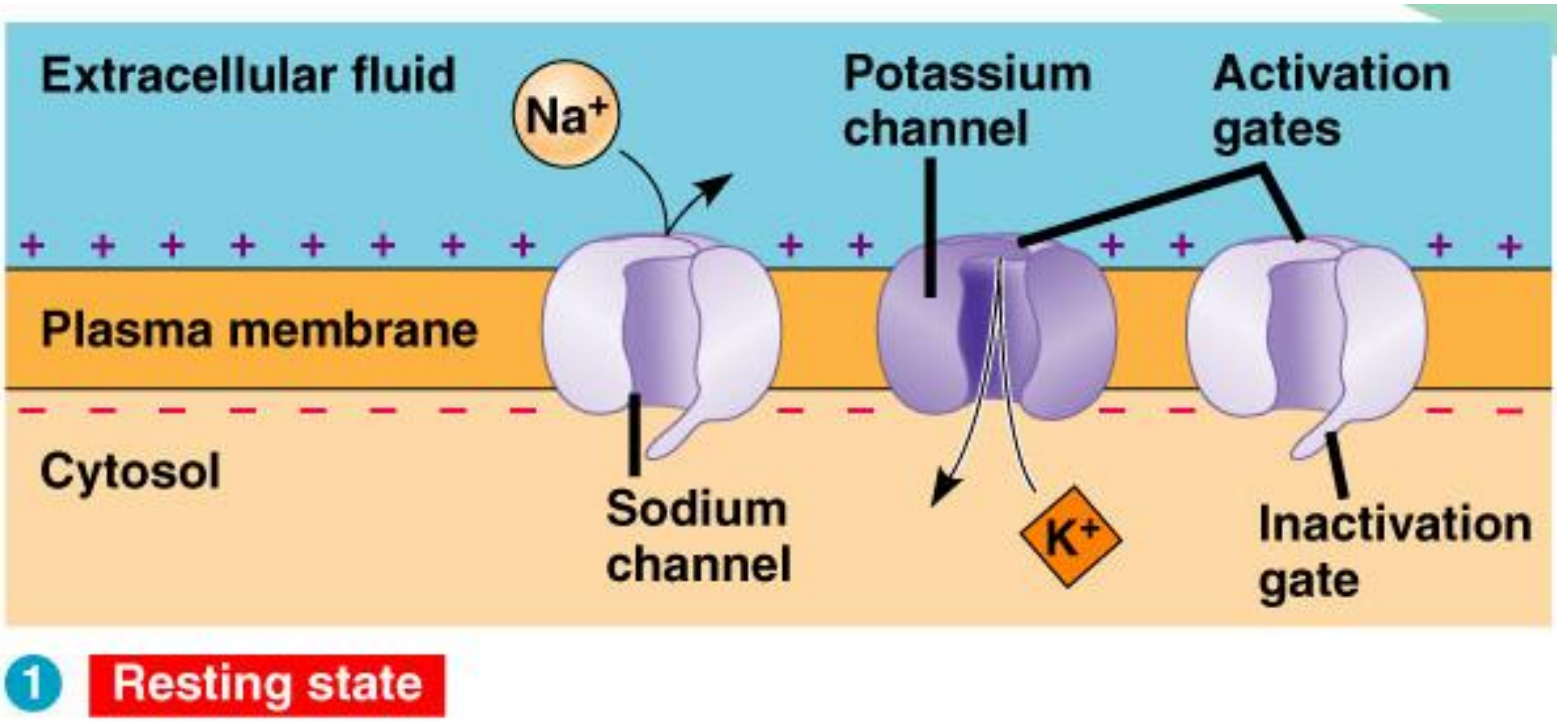
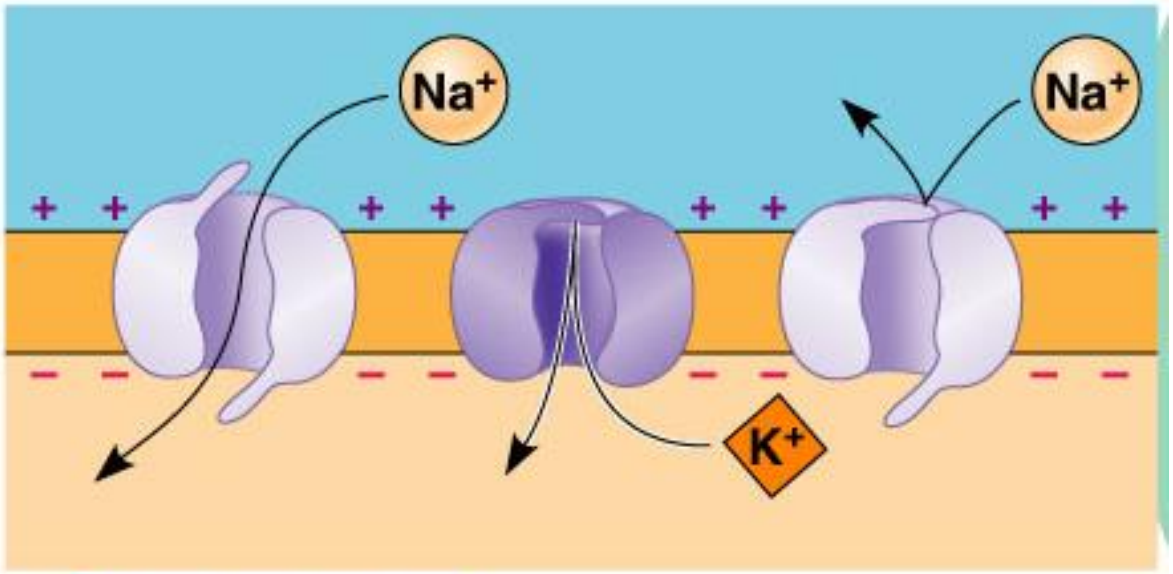
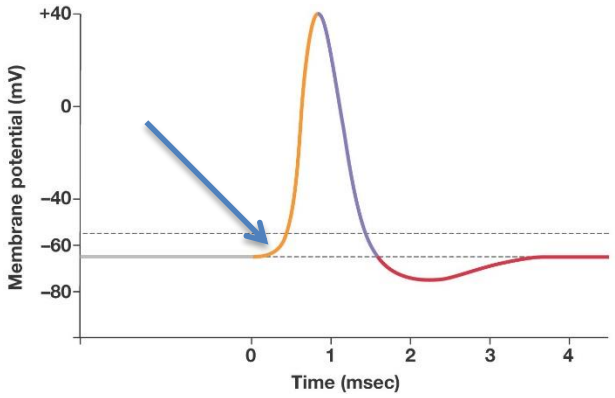


Figure 11–29. Molecular Biology of the Cell, 4th Edition.



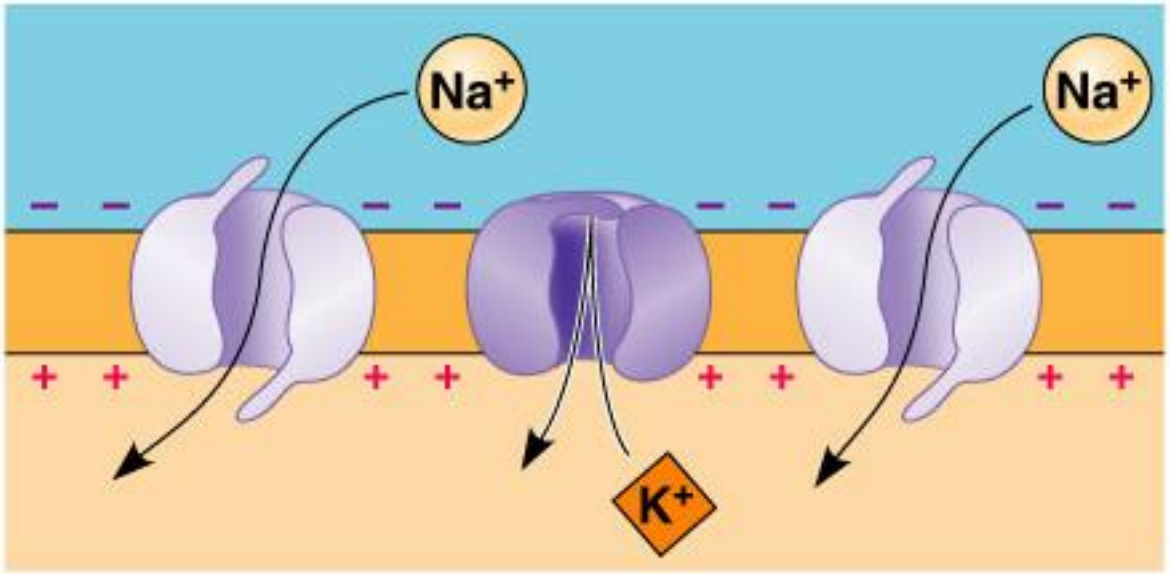
Resting Potential - Both voltage gated Na^+ and K^+ channels are closed.

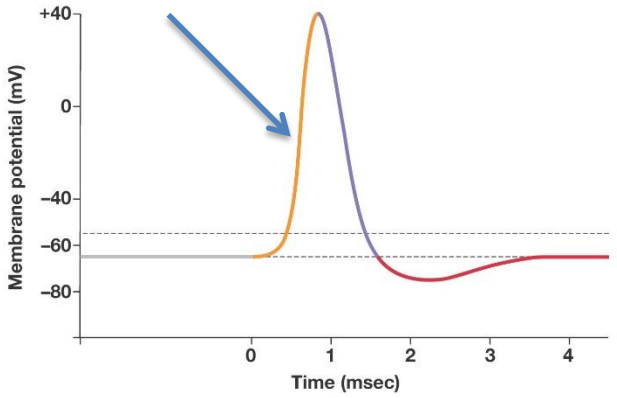




2 Depolarization

Initial Depolarization - Na^+ activation gates open quickly and Na^+ enters causing local depolarization which opens more activation gates and cell interior becomes progressively less negative. If enough Na^+ channels open, then the threshold (-55 mV) is surpassed and an action potential is initiated.





3 Rising phase of the action potential

Na^+ channels open quickly. K^+ channels are still closed.

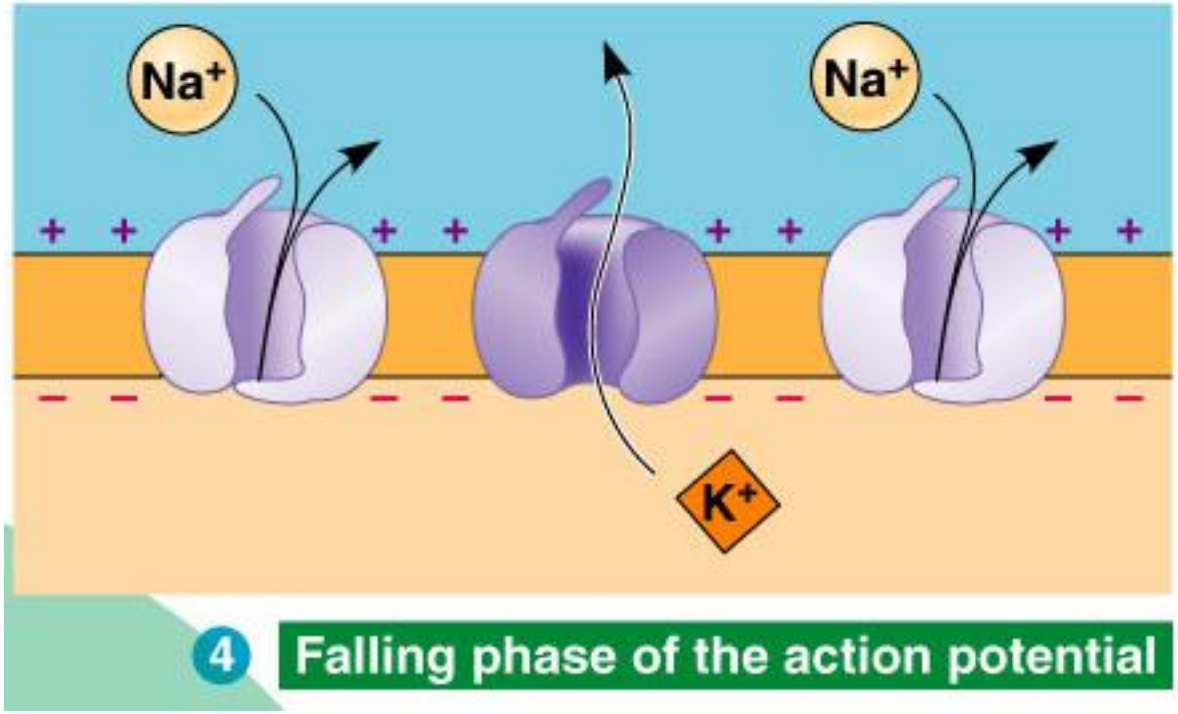
$P_{Na+} > P_{K+}$

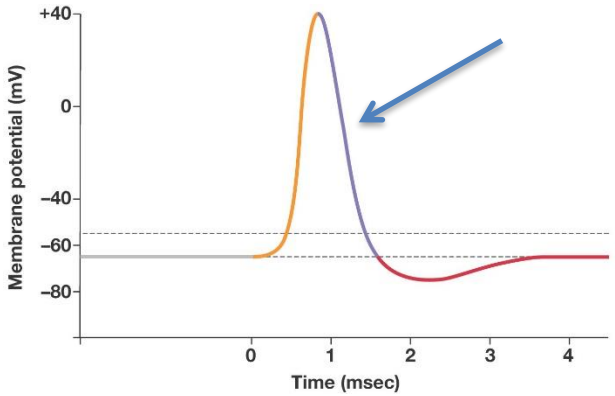
The membrane **potential** continues to depolarize until the peak of the **action potential** is reached at about +40 millivolts (mV) (**overshoot**)

Repolarization Phase

Positive intracellular charge opposes further Na^+ entry. Sodium inactivation gates of Na^+ channels close.

As sodium gates close, the slow voltage-sensitive K^+ gates open and K^+ leaves the cell following its electrochemical gradient and the internal negativity of the neuron is restored.





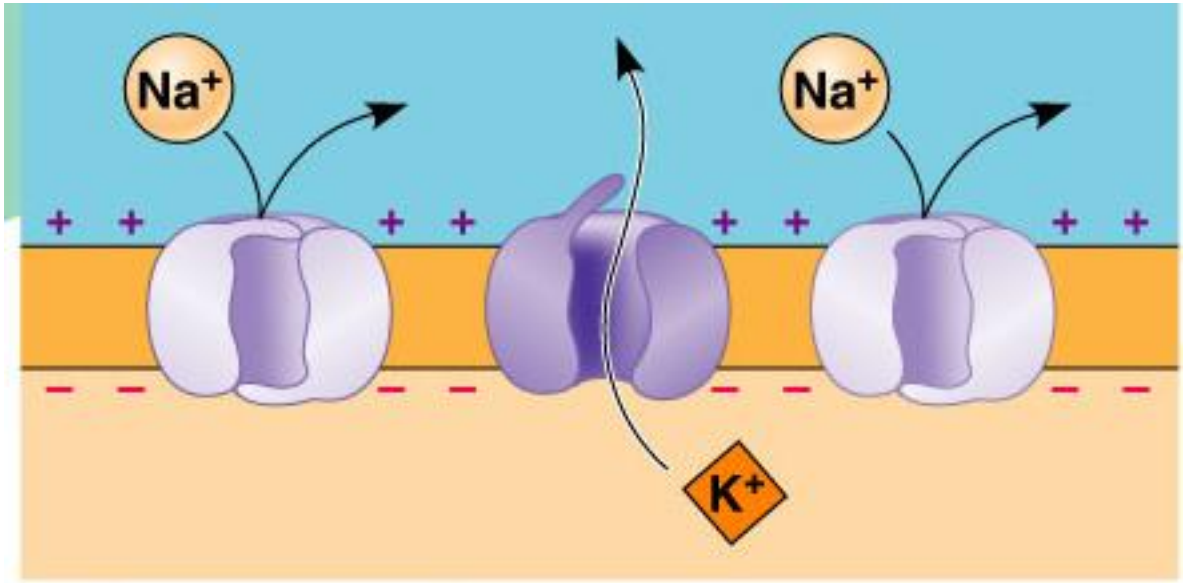
Na^+ channels self-inactivate, K^+ channels are open.

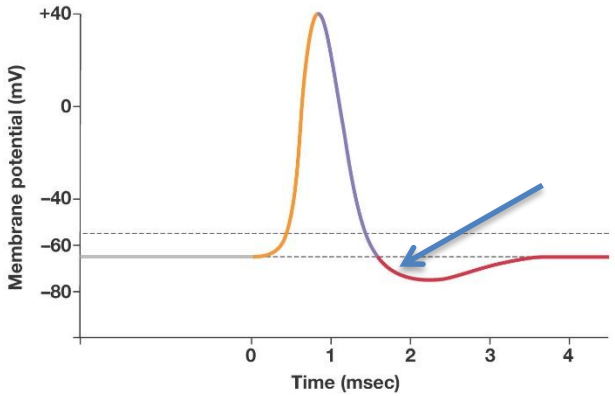
$P_{K+} >> P_{Na+}$

Hyperpolarization

The slow K^+ gates remain open longer than is needed to restore the resting state. This excessive efflux causes hyperpolarization of the membrane.

The neuron is insensitive to stimulus and depolarization during this time.





5 Undershoot

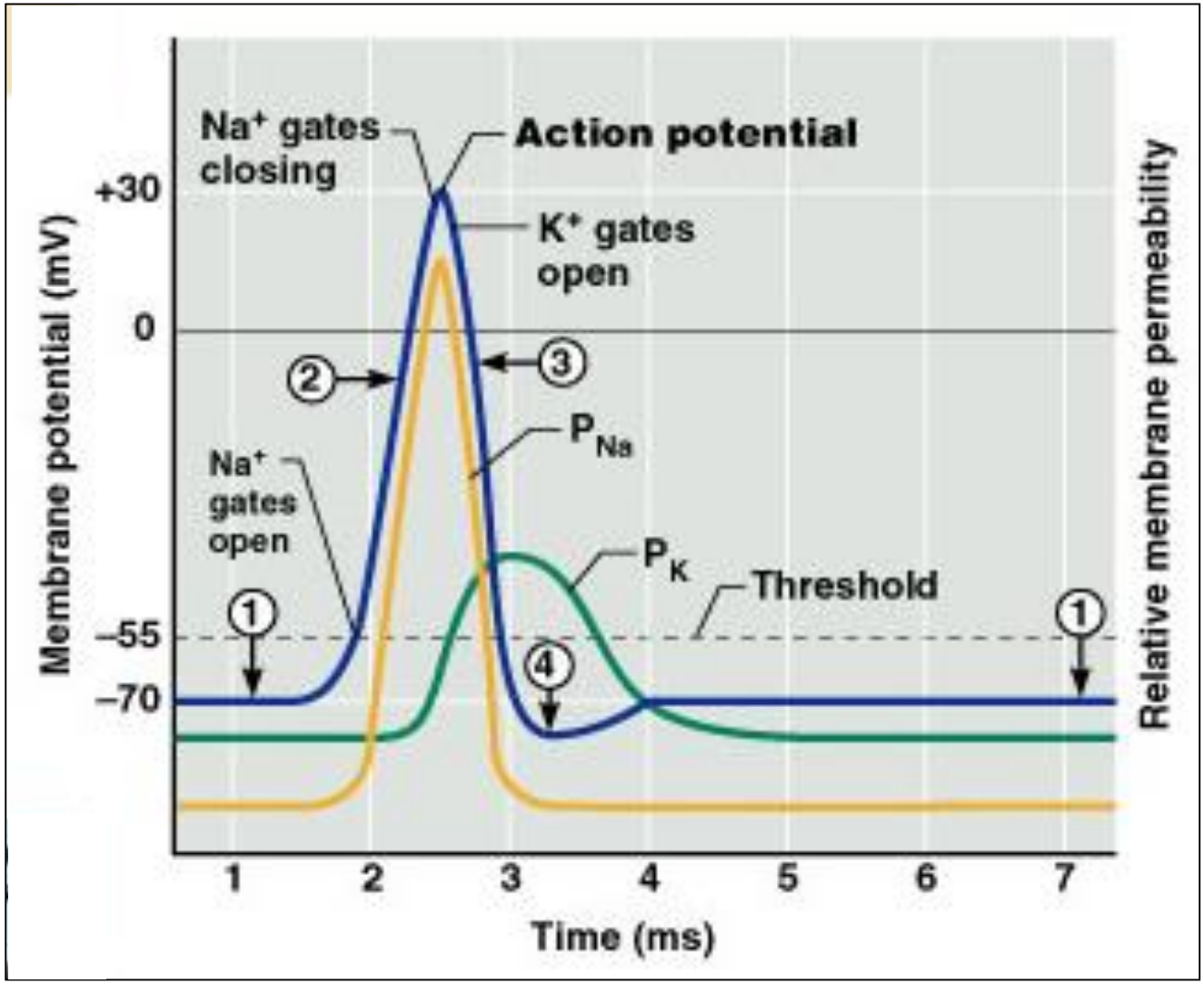
$P_{K+} > P_{K+ \text{ at resting state}}$

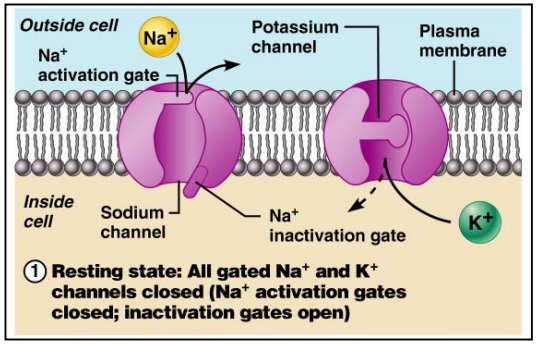
Role of the Sodium-Potassium Pump

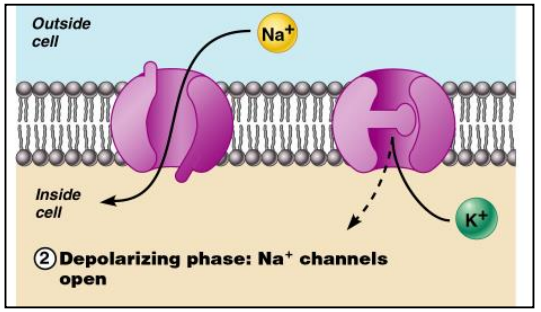
Repolarization restores the resting electrical conditions of the neuron, but does not restore the resting ionic conditions.

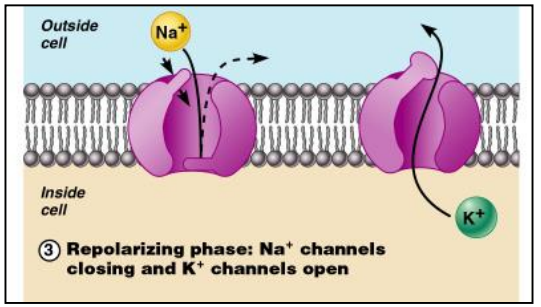
Ionic redistribution is accomplished by the sodium-potassium pump following repolarization.

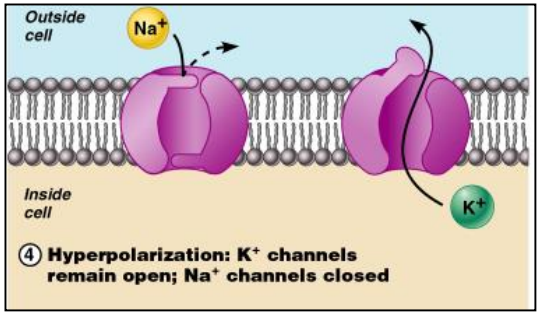
Phases of the Action Potential





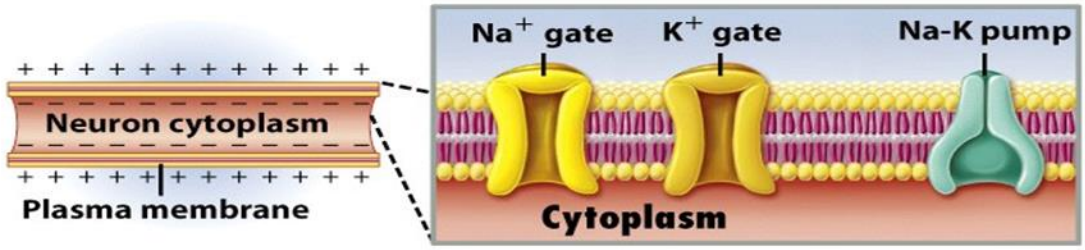






Collectively, these changes in membrane potential are called an **action potential**

Resting Neuron



Action Potential

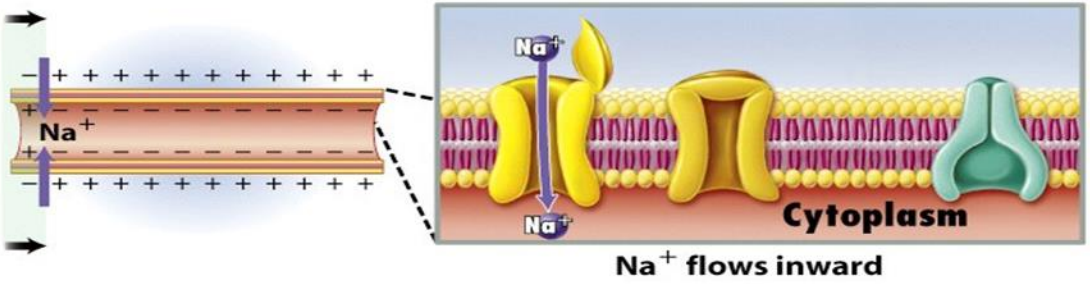
Excitatory stimulus

↓

Sodium channels open

↓

Sodium ions (Na^+) enter the neuron

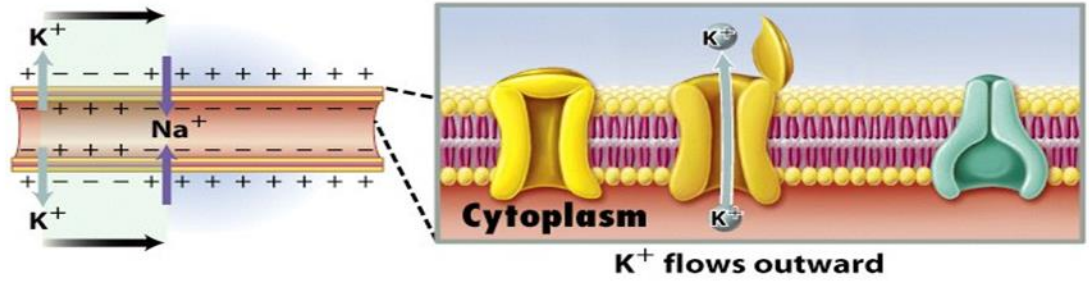


Na^+ flows inward

Potassium channels open

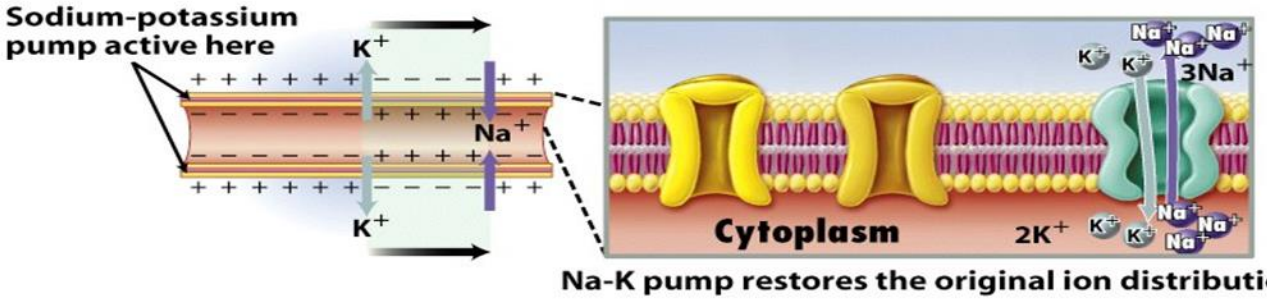
↓

Potassium ions (K^+) leave the neuron



K^+ flows outward

Charge restoration



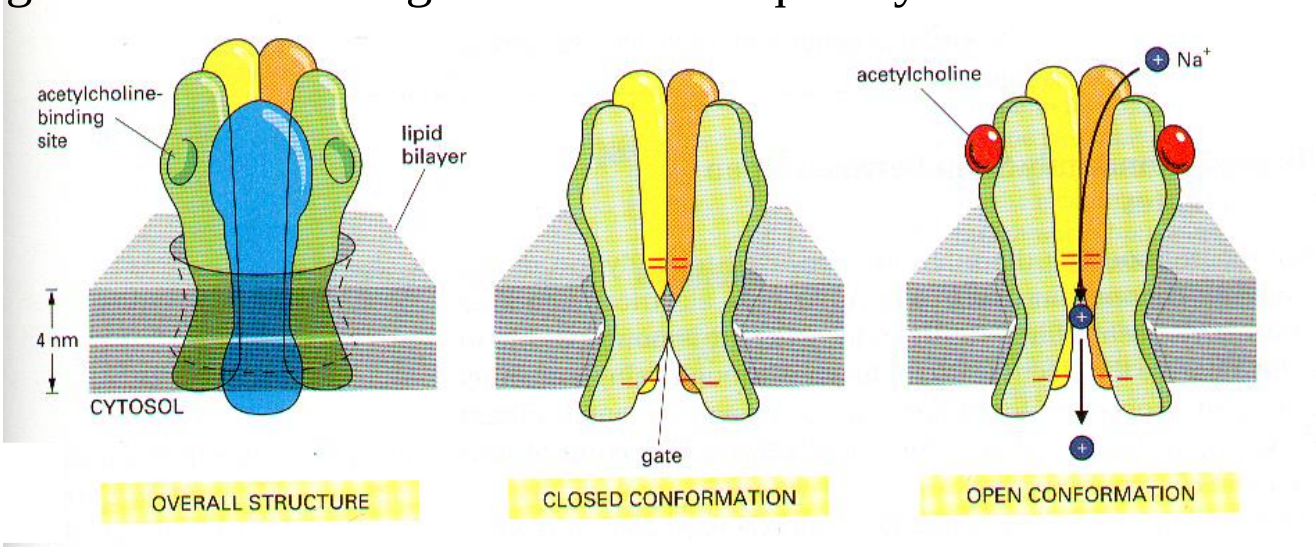
Na-K pump restores the original ion distribution

Figure 7-5a Biology of Humans, 2/e

Ligand-gated ion channel

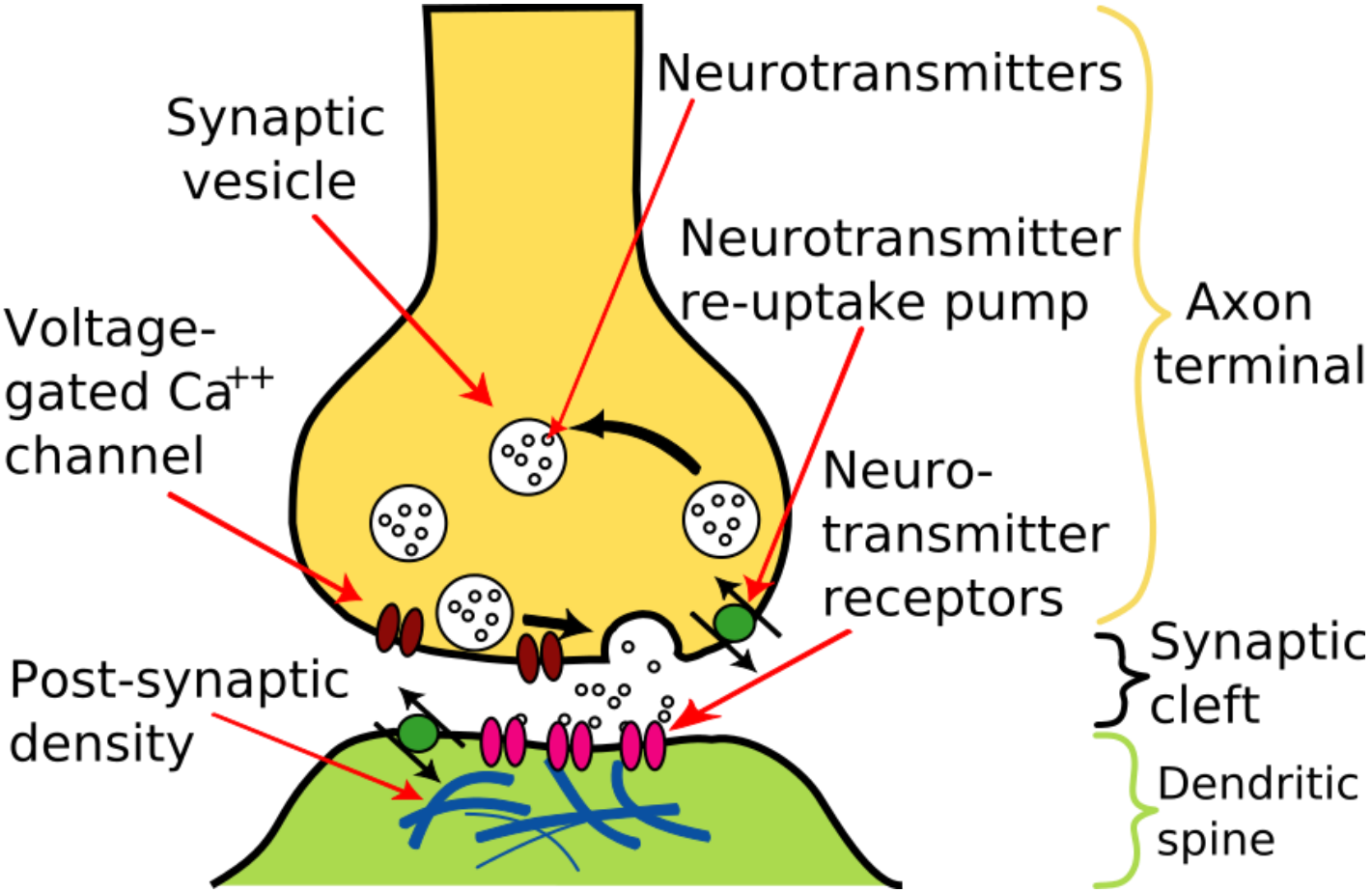
Acetylcholine receptor

- Work very fast; important role in fast neurotransmission
- Each is made of several subunits (together form the complete receptor)
- At center of receptors is a channel or pore to allow flow of neurotransmitter
- At rest - receptor channel is closed
- When neurotransmitter binds -- Ach receptor responds by an extensive change of conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane
- When ligand leaves binding site -- channel quickly closes

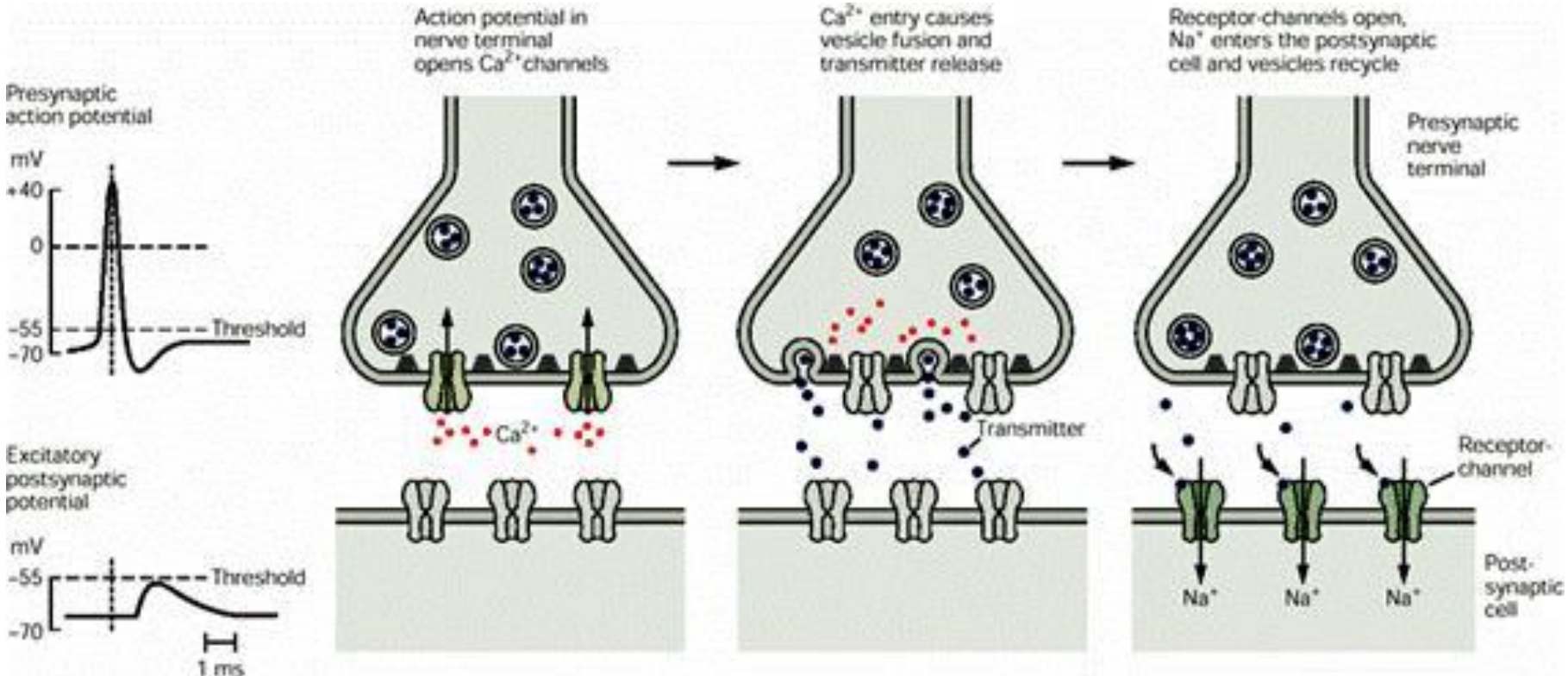


Presynaptic terminal

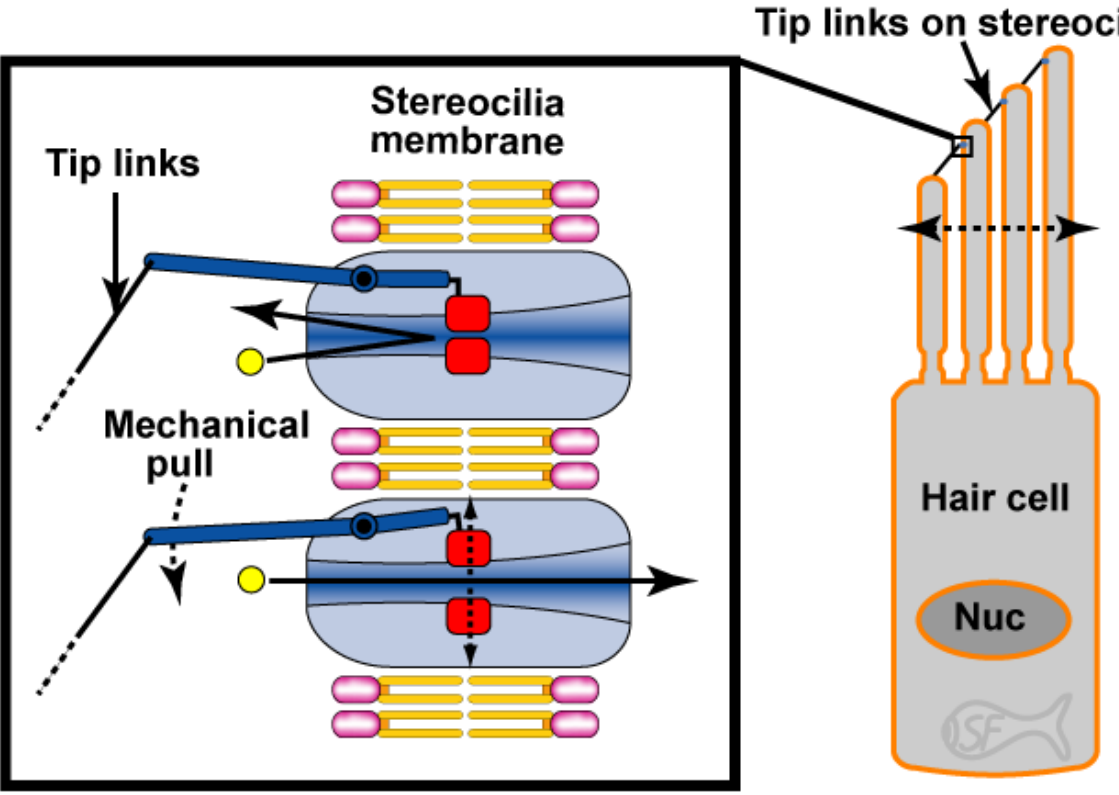
The transfer of information from the end of the axon of one neuron to the next neuron is called synpatic transmition. Synapses are one of the means by which excitable cells communicate with one another.



- The neurotransmitter acethylcholine is stored in vesicles in nerve endings
- When an action potential arrives at presynaptic membrane, voltage gated calcium channels in the presynaptic membrane open and calcium ions enter the presynaptic neurone.
- Calcium ions cause synaptic vesicles to fuse with the presynaptic membrane, releasing acetylcholine into the synaptic cleft.
- Acetylcholine diffuses cross the synaptic cleft and binds to specific neuroreceptor sites in the post synaptic membrane.
- Sodium channels open. Sodium ions diffuse into the postsynaptic membrane causing depolarisation, which may initiate an action potential.
- Acetylcholinesterase breaks down acetylcholine. The products diffuse back into the presynaptic neurone where acetycholine is resynthesised using ATP from the mitochondria.



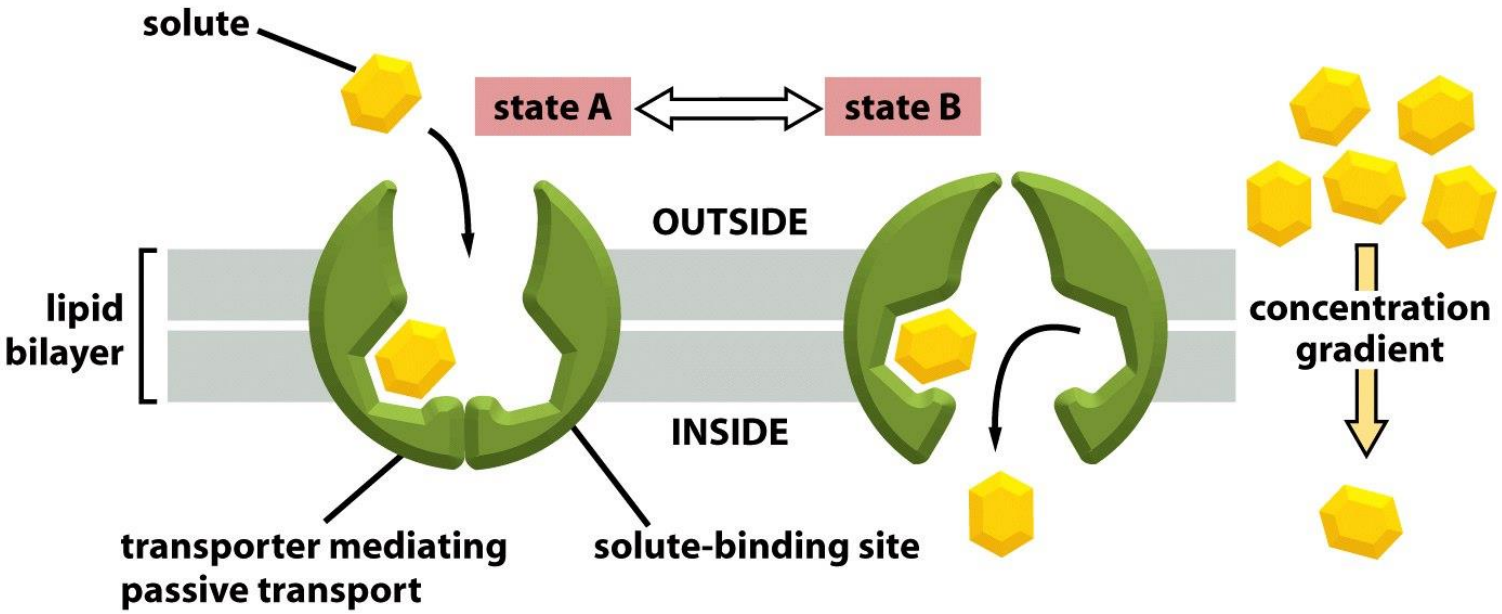
Mechanically gated ion channels



Epithelial hair cells contain a stereocilia bundle that are inter-connected by tip links. The mechanical force imparted by sound waves bends the stereocilia and pulls on the tip links. The strain induced by tip link pulling is thought to open a mechanically activated ion channel complex

- Stress activated
- Sound waves cause the stereocilia to tilt and this causes the channels to open and transport signal to the brain
- Hair cells to auditory nerve to brain

In many cases, the diffusing substance first binds selectively to a **membrane-spanning protein, called a facilitative transporter (carrier proteins)**, that facilitates the diffusion process. Because they operate passively, that is, without being coupled to an energy- releasing system, facilitated transporters can mediate the movement of solutes equally well in both directions. The direction of net flux depends on the relative concentration of the substance on the two sides of the membrane.



A conformational change in a transporter could mediate the <u>passive transport</u> of a solute

Carrier proteins (also called permeases)

Transport solute across membrane by binding it on one side, undergoing a conformational change and then releasing it to the other side

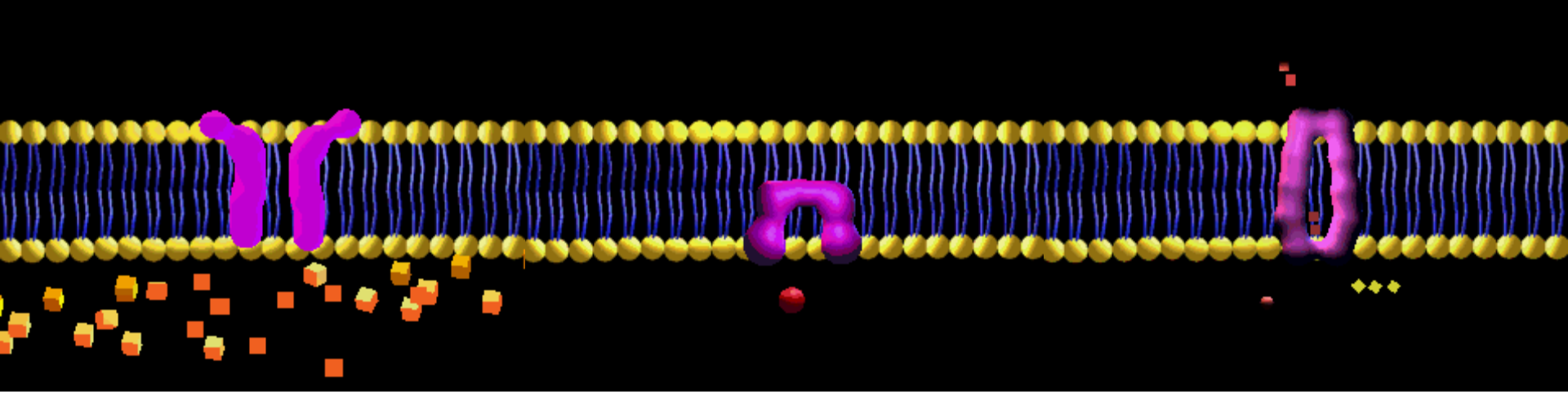
Carrier Proteins can mediate either:

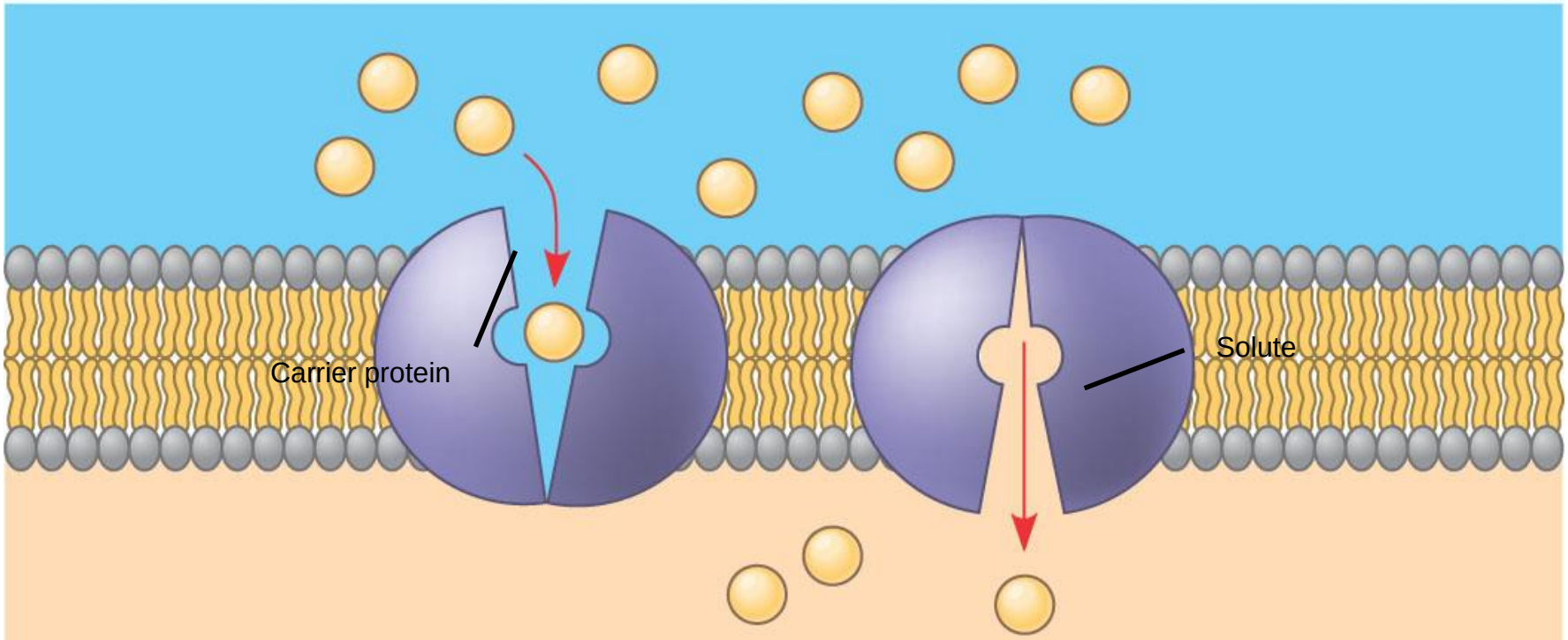
1. Passive transport (Facilitated Diffusion)

OR

2. Active transport

Note: channel proteins mediate only passive transport

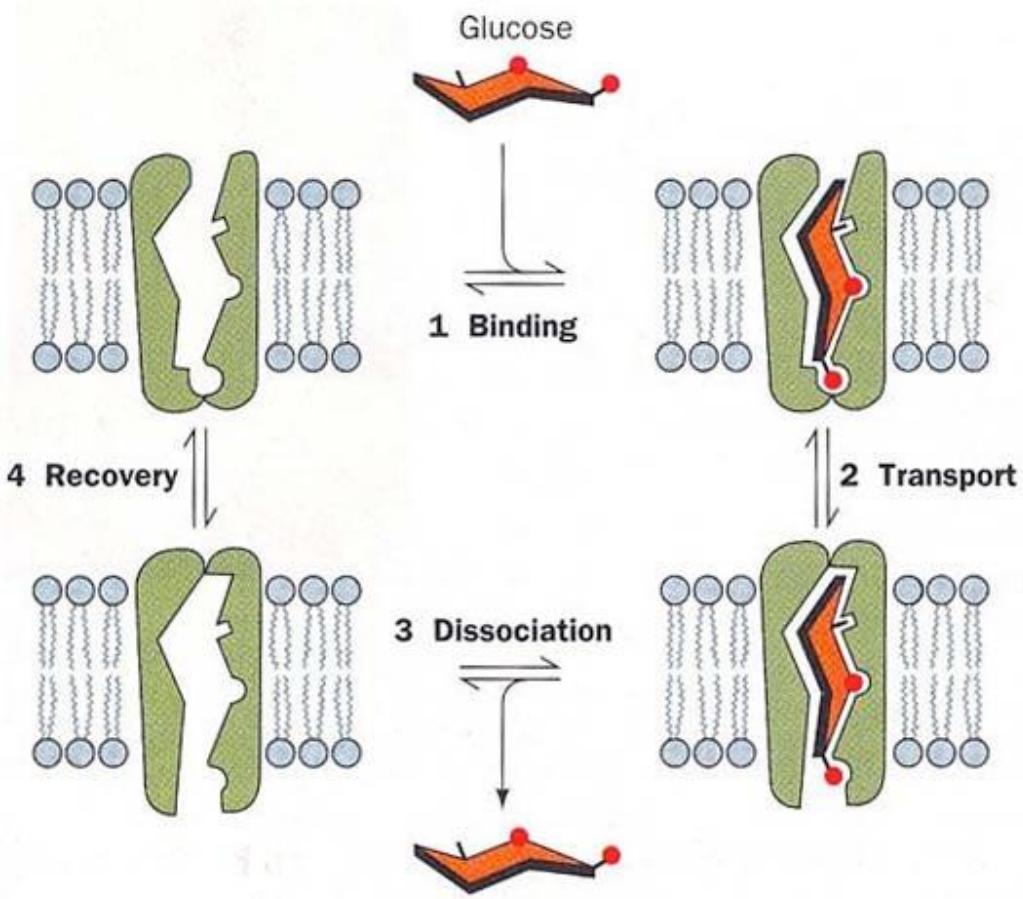




A carrier protein alternates between two conformations, moving a solute across the membrane as the shape of the protein changes. The protein can transport the solute in either direction, with the net movement being down the concentration gradient of the solute.

Passive carriers

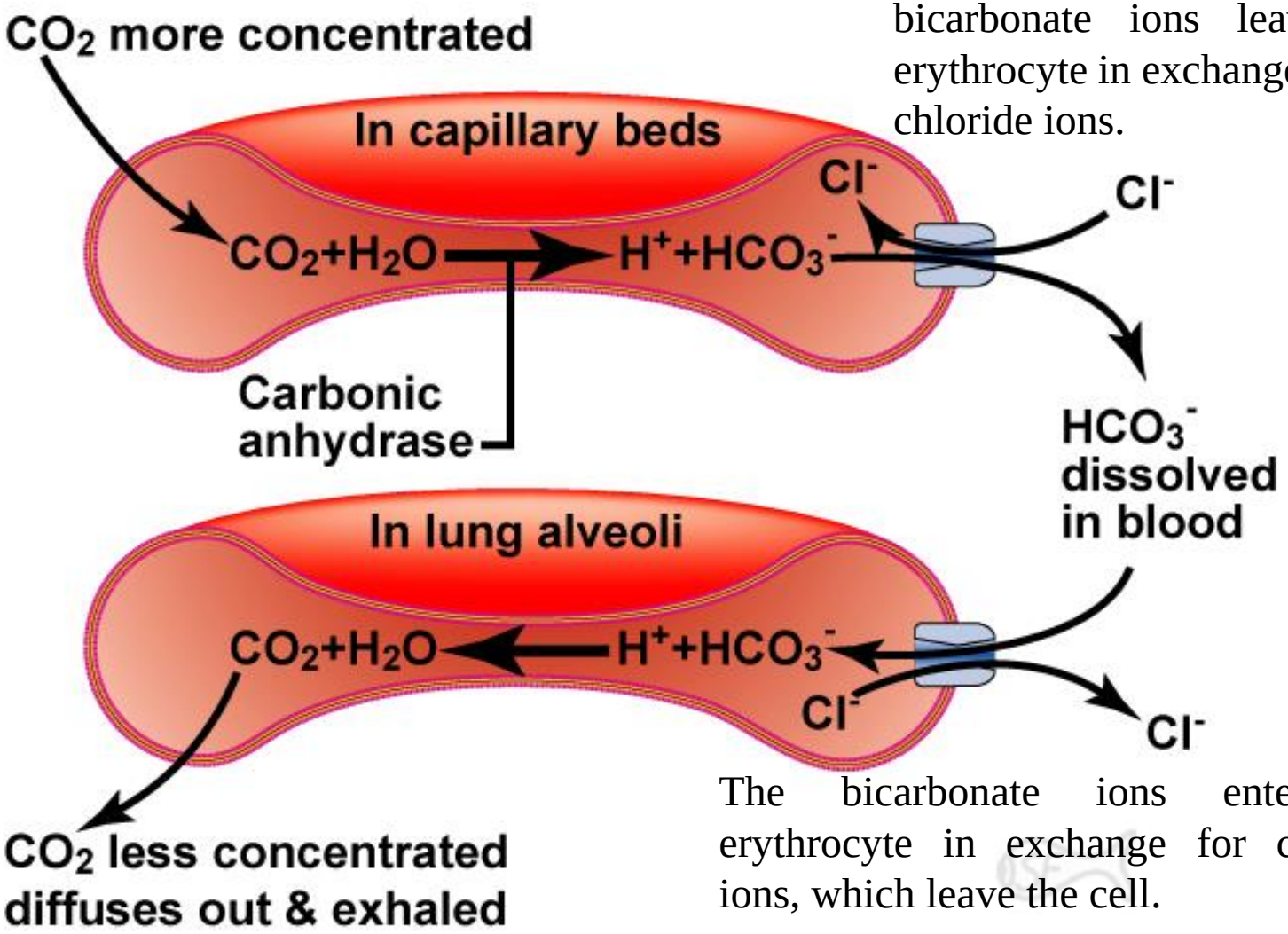
Facilitate the downhill transport of substances across membranes.
The glucose transporter can carry glucose in either direction, depending on the direction of the concentration gradient.



Example: Glucose transporter GluT1 : carrier-mediated facilitated diffusion

Plasma membrane Cl^- /HCO_3-exchangers

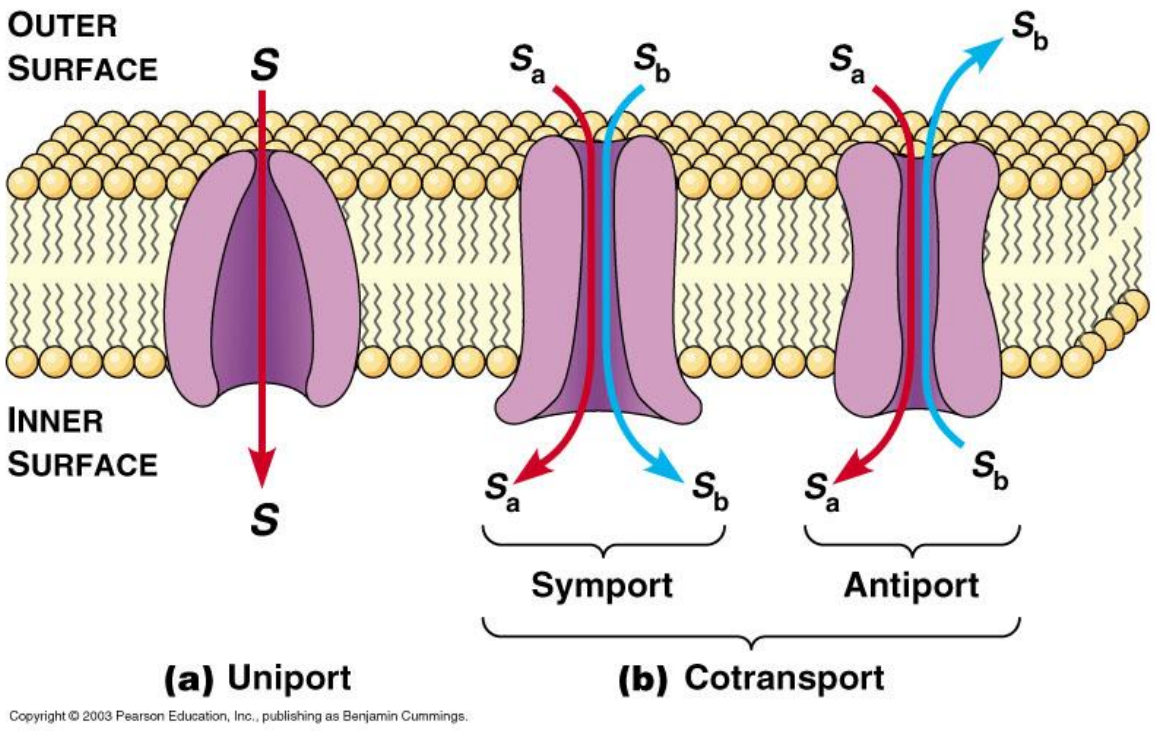
Anion exchanger proteins facilitate the exchange of bicarbonate for chloride across the plasma membrane.



bicarbonate ions leave the erythrocyte in exchange for chloride ions.

The bicarbonate ions enter the erythrocyte in exchange for chloride ions, which leave the cell.

Carrier proteins: three types



Uniport – one solute transported

Symport – two solutes in the same direction

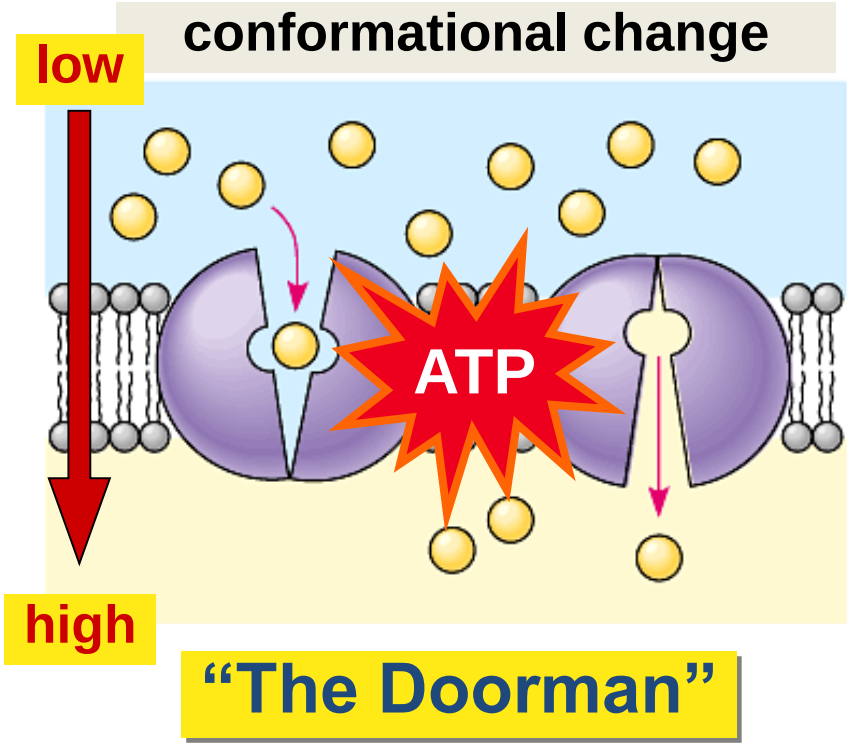
Antiport – two solutes in opposite directions

Active Transport

- Primary active transport utilizes energy in form of ATP to transport molecules across a membrane against their concentration gradient
  - "costs" energy = ATP

Active transport of solutes against their gradients is important for,

- maintaining the balance of ions across membranes,
- concentrating metabolites in certain organs or cellular compartments, and
- exporting foreign substances from cells.



Active Transport

- **Two types of active transport:**
  - **Primary active transport**
    - **Required energy comes *directly* from ATP hydrolysis**
  - **Secondary active transport**
    - **Required energy is obtained *indirectly* from ionic gradients created by primary active transport**

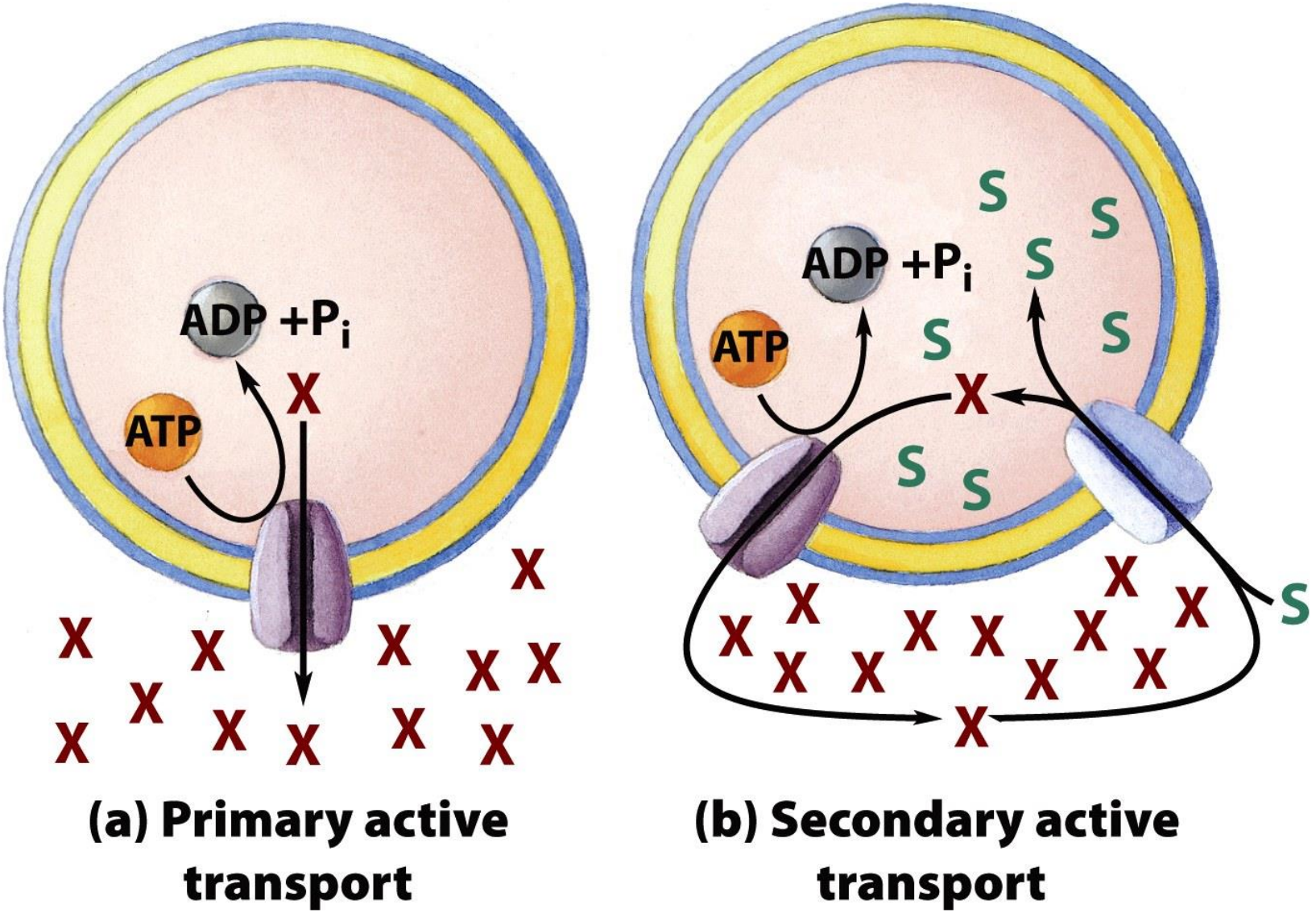
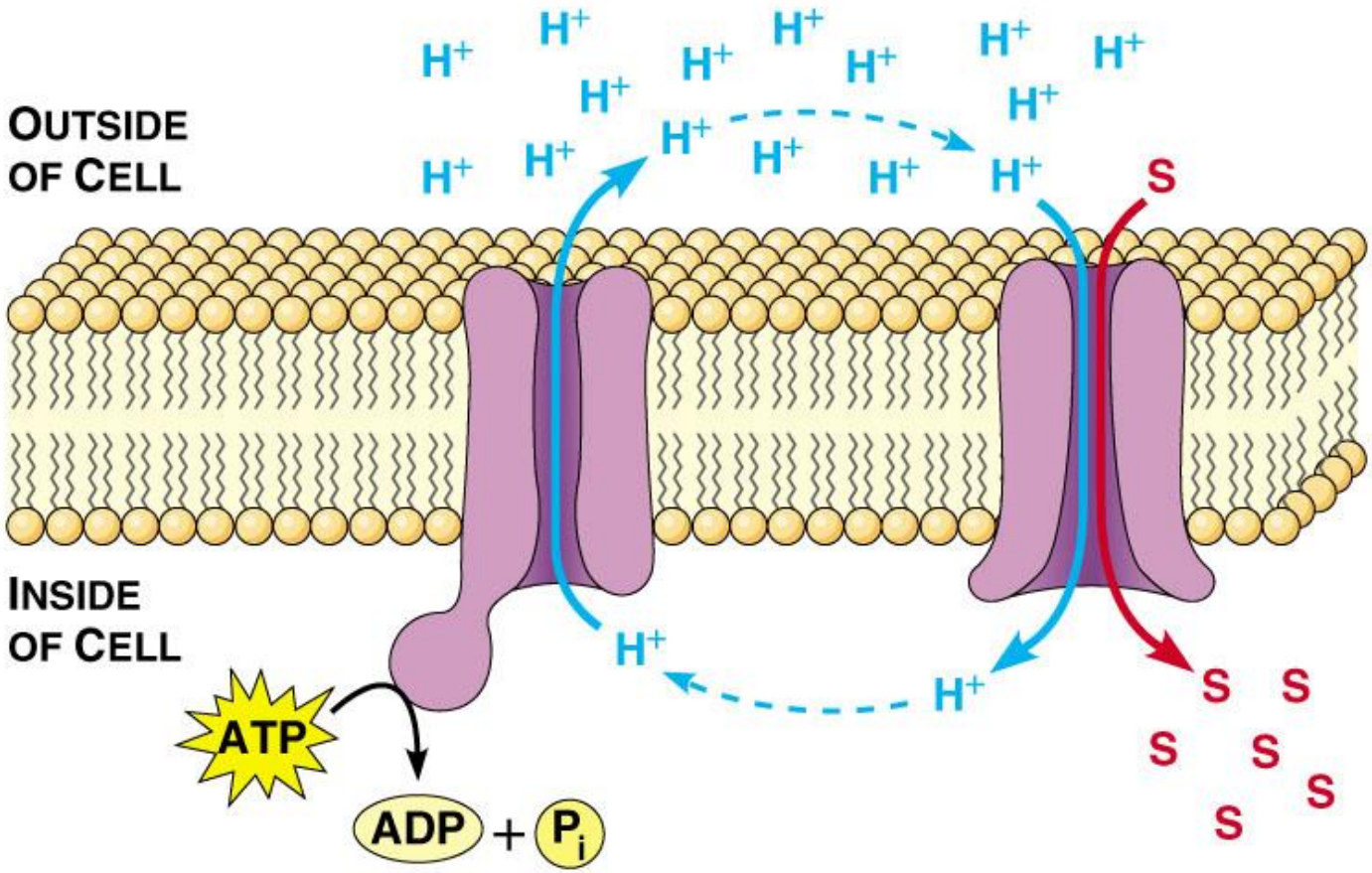


Figure 11-34
Lehninger Principles of Biochemistry, Fifth Edition



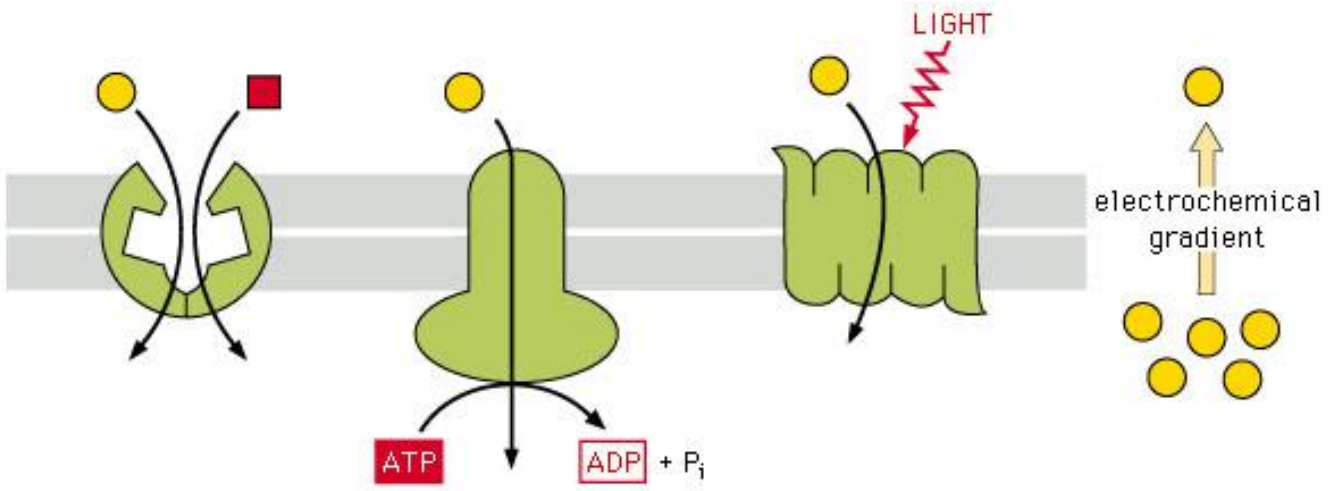
(a) Direct active transport of protons

(b) Indirect active transport of solute molecules

Active Transporters

We will discuss three types of active transporters:

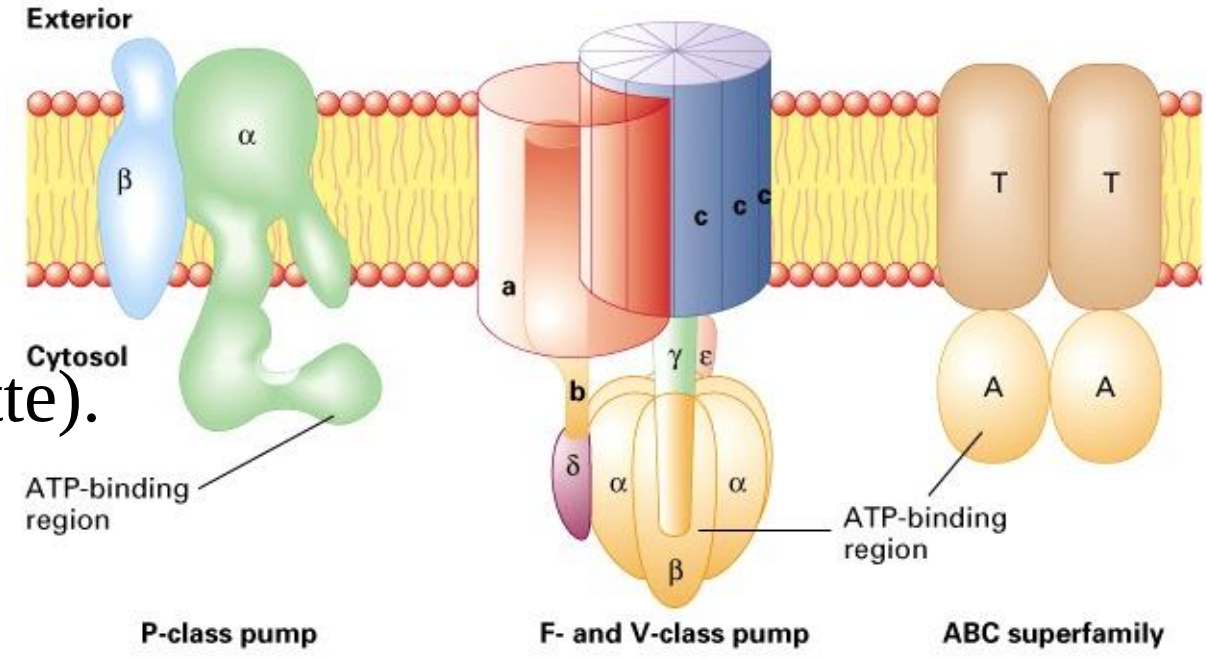
- Coupled transporters – 1 goes down gradient and 1 goes up the gradient
- ATP-driven pumps – coupled to ATP hydrolysis
- Light-driven pumps – uses light as energy, bacteriorhodopsin



ATP-driven ion pumps

Based on the transport mechanism as well as genetic and structural homology, there are considered four classes of ATP-dependent ion pumps:

- P-class pumps
- F-class pumps
- V-class pumps
- ABC transporters (**A**TP-**b**inding **c**assette).



ATP-driven ion pumps utilize the energy liberated by ATP hydrolysis to move ions across membranes, against their gradients. These proteins maintain ion gradients across both the plasma membrane and intracellular membranes

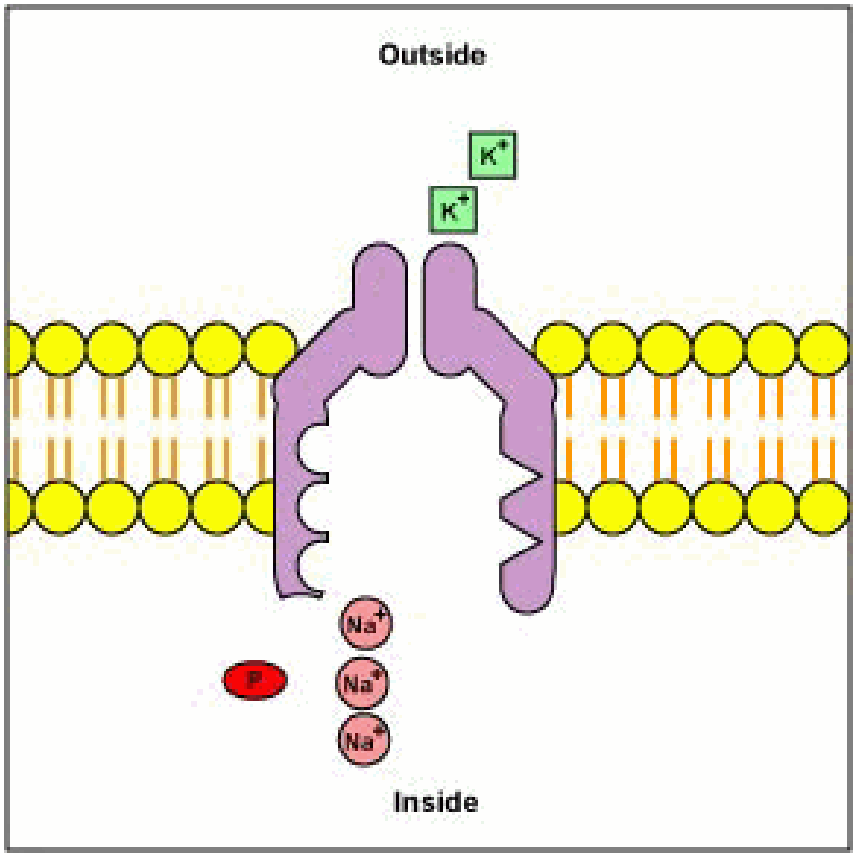
V-type pumps. Membranes of lysosomes and secretory vesicles contain a vacuolar-type H^+-ATPase that pumps H^+ ions from the cytoplasm into the vesicles.

F-type pumps in mitochondria and chloroplasts usually synthesize ATP (proton pump)

P-type ATPases are so named because their reaction mechanism proceeds via a phosphorylated protein intermediates. Among these are **gastric H^+-ATPase** that is responsible for acidification of the stomach contents; **Na^+,K^+-ATPase** that is responsible for maintaining ionic gradients in most cells; **P**lasma **m**embrane **c**alcium **A**TPase (PMCA) responsible for pumping Ca^{2+}out of cells; **S**mooth **e**ndoplasmic **r**eticulum **C**a^{2+}**A**TPase (SERKA), which is responsible for removing Ca^{2+} from the cytosol of a variety of cell types and placing it in storage in internal sacs within cells.

ATP-driven pumps (Na^+-K^+ ATPase)

- *1 ATP moves 3 Na^+ out 2 K^+ in*

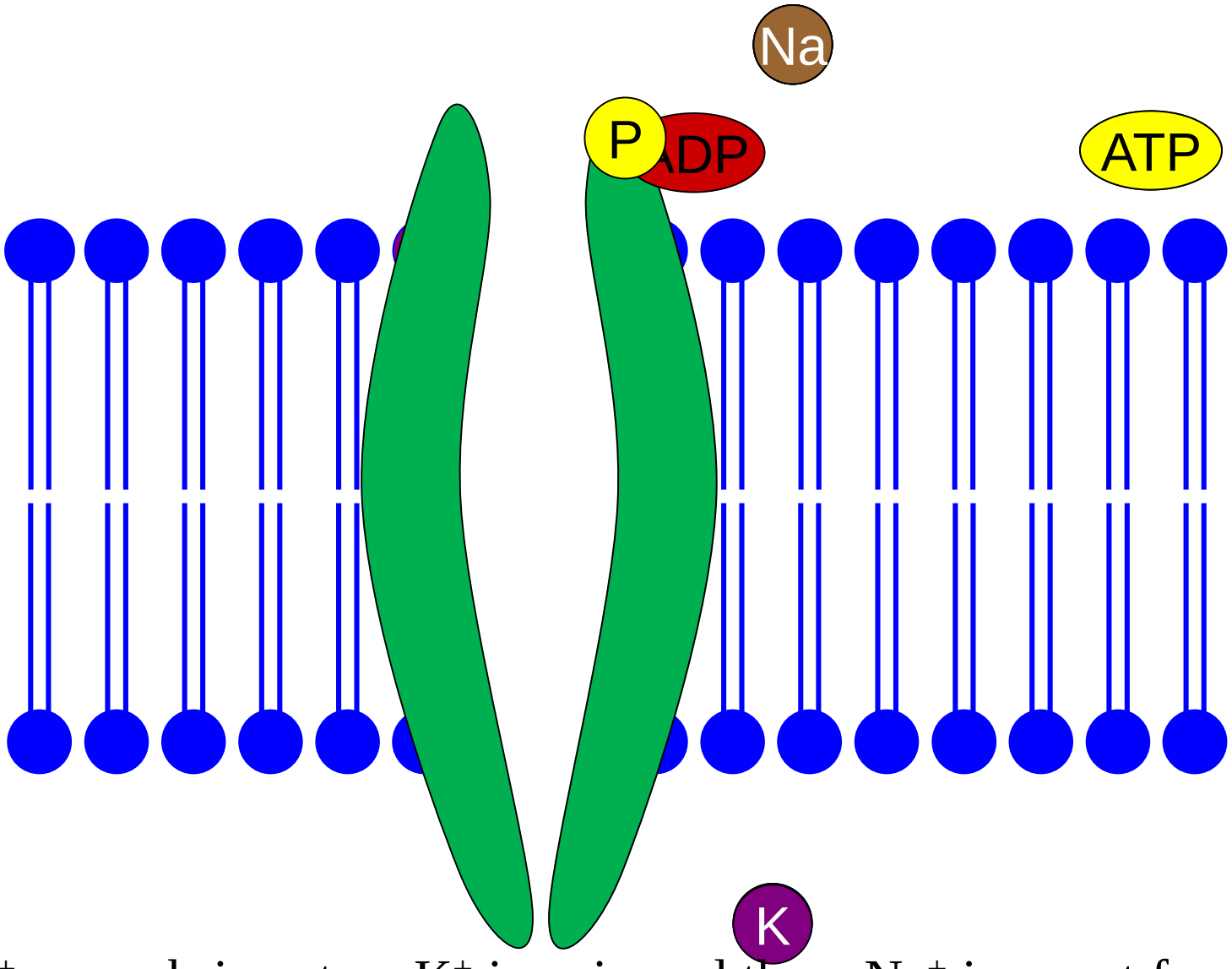


The Na/K-ATPase is an example of a P-type ion pump. The "P" stands for phosphorylation,

- It is pumping $3Na^+$ (sodium ions) out and $2K^+$ (potassium ions) in — against concentration gradients.

This pump is an enzyme embedded in the plasma membrane that hydrolyzes ATP so that Na^+ and K^+ can be transported against their concentration gradients. If Na^+ were not continually pumped out, the gradient would rapidly be lost, and cells would swell and burst.

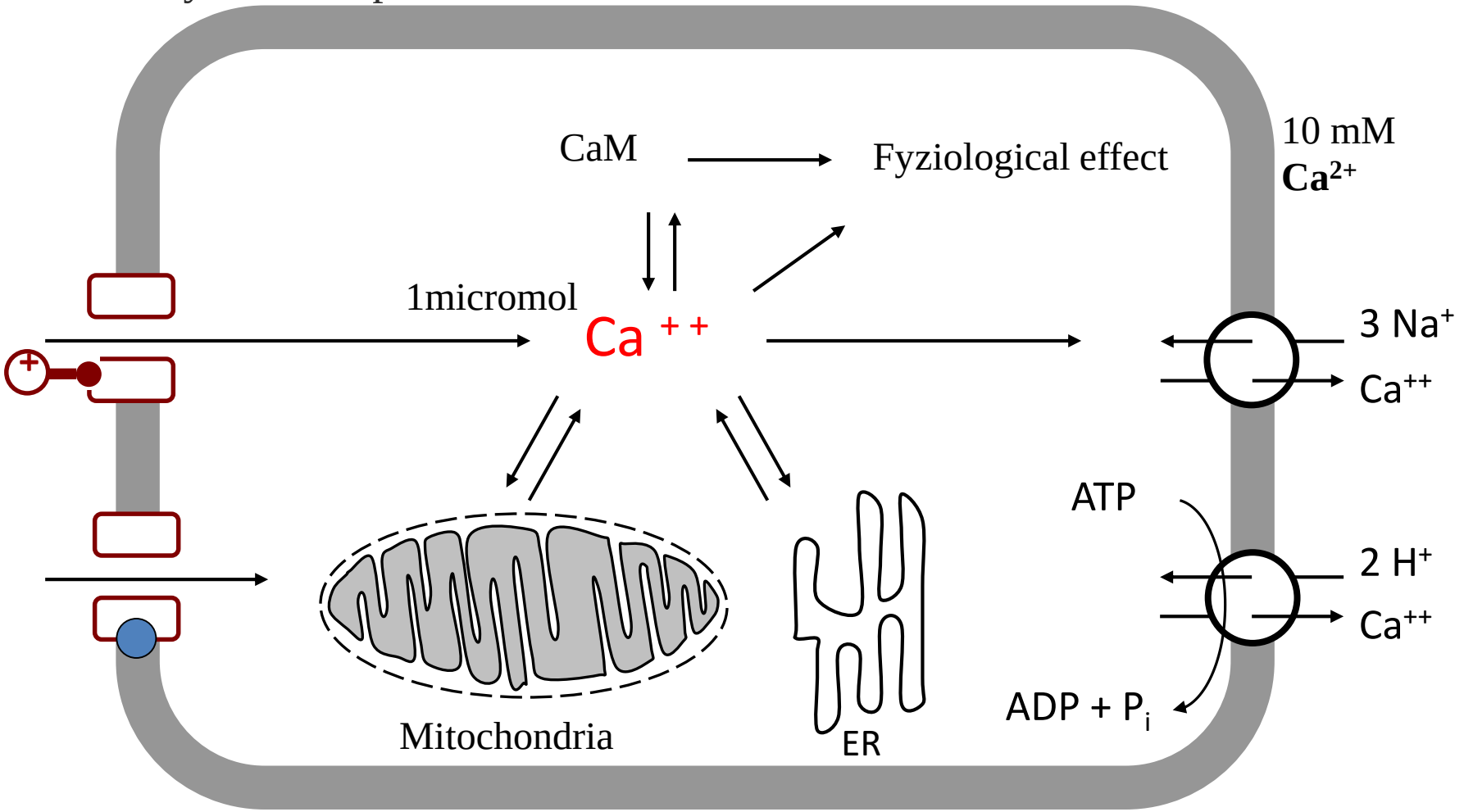
Na^+, K^+ - ATPase



The Na^+/K^+ pump brings two K^+ ions in and three Na^+ ions out for every molecule of ATP hydrolyzed.

Sarcoplasmic Reticulum Ca^{2+} - ATPase

The function of Ca-ATPases is to maintain the intracellular Ca^{2+} concentration at very low levels by active expulsion of Ca^{2+}.



In fact, the intracellular Ca^{2+} concentration is 10 000 times lower than the extracellular concentration. Maintaining a low intracellular Ca^{2+} concentration is critical since Ca^{2+} ions control several intracellular reactions and are toxic at a high concentration.

Gastric mucosal H^+/K^+-ATPase

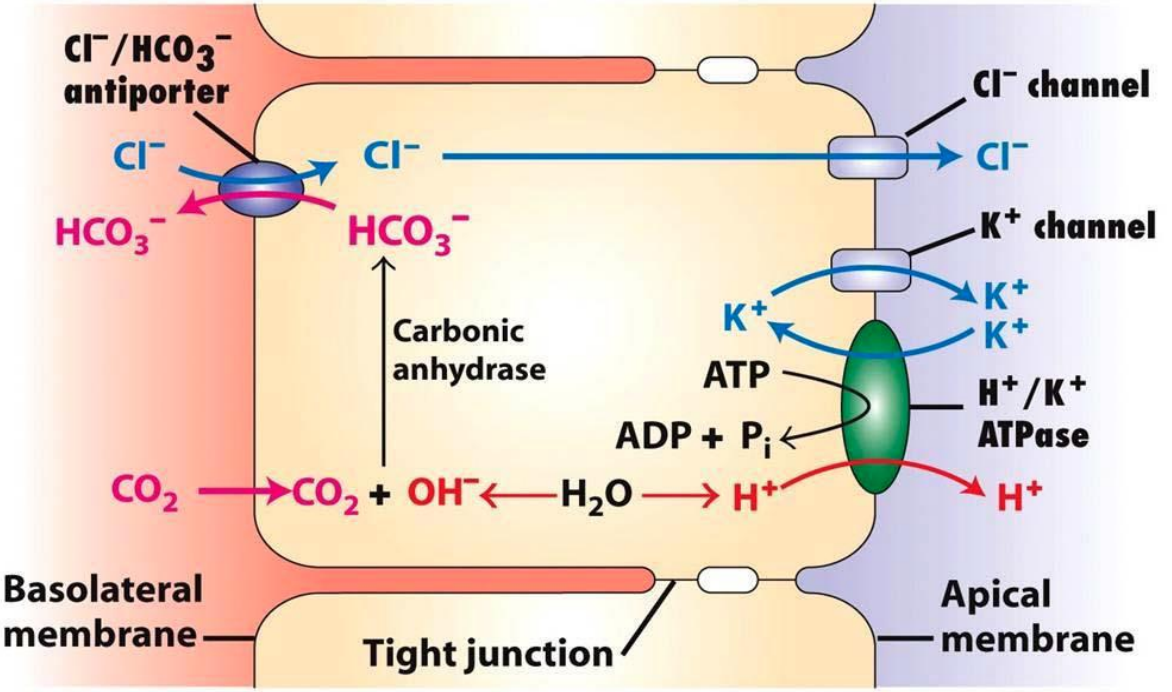


Figure 11-30
Molecular Cell Biology, Sixth Edition

The epithelial lining of the stomach also contains a P-type pump, the H^+/K^+-ATPase, which secretes a solution of concentrated acid (up to 0.16 N HCl) into the stomach chamber. In the resting state, these pump molecules are situated in cytoplasmic membranes of the parietal cells of the stomach lining and are nonfunctional.

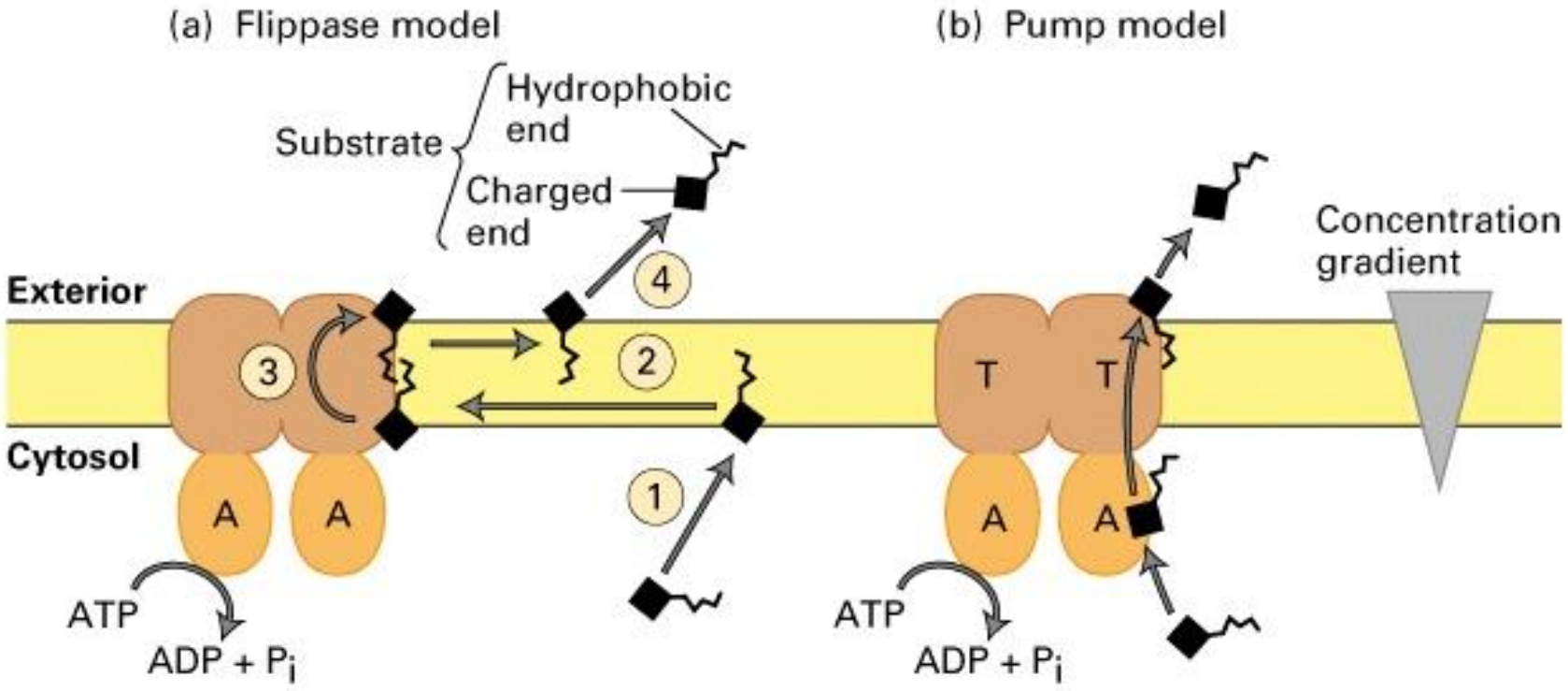
ATP-binding cassette (ABC) transporters

- Facilitate the export of compounds from the cell
- Belong to the ATP-binding cassette (ABC) family
- So called because all of the members of this superfamily share a homologous ATP-binding domain.

Includes

- Multidrug Resistance Pump (p glycoprotein)
- Cystic Fibrosis Transmembrane Conductans Regulator
  - ATP-regulated chloride channel
  - Mutation causes less fluid/salt to be pumped out of cell (Cystic fibrosis)

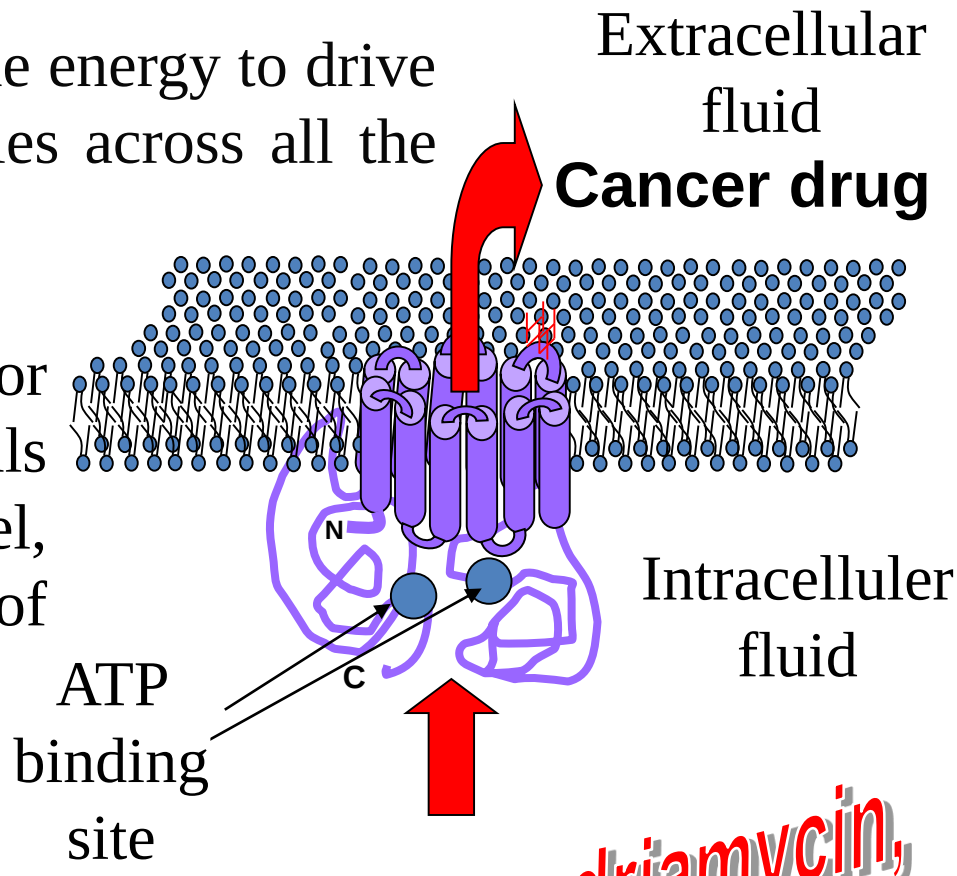
ABC transporters all have a similar structure, consisting of two ATP binding domains facing the cytosol and two transmembrane domains. Similar to the situation seen with ATP-driven ion pumps, the binding and hydrolysis of ATP by ABC transporters is thought to drive conformational changes that transport molecules across the membrane. But while ion pumps transport ions in or out of cells, most ABC transporters in eukaryotes are specialized for pumping small compounds out of cells (these proteins are sometimes referred to as efflux pumps).



P glycoprotein

- This protein bind ATP and use the energy to drive the transport of various molecules across all the cell membranes.

- Initially identified as a major cause of resistance by cancer cells to multiple drugs (e.g., paclitaxel, etoposide) having a variety of structures.

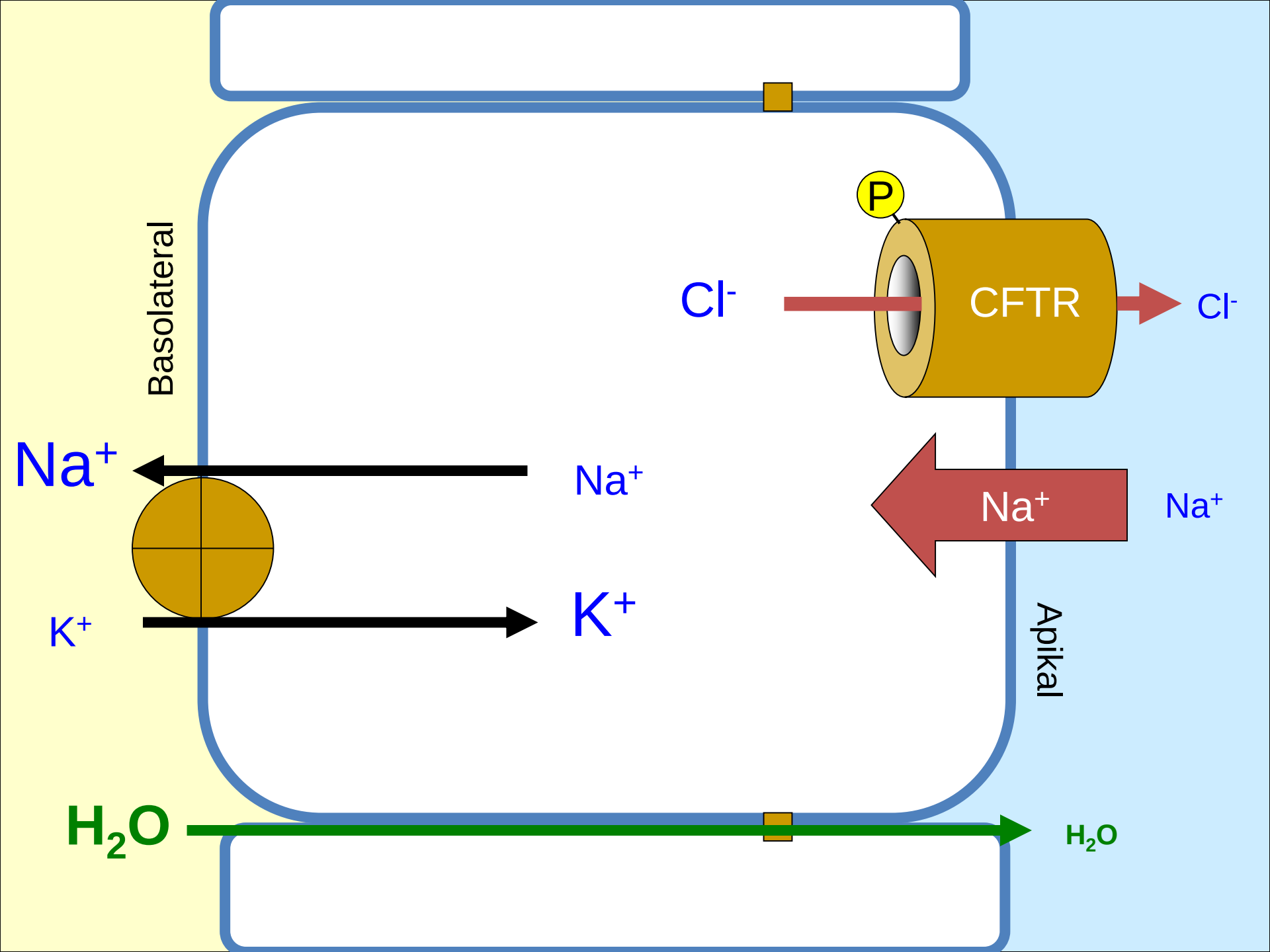


A drug molecule attaches to the binding domain of pgp

2 ATPs bound to the ATP binding regions, become hydrolyzed and induce conformational change to open pathway for the drug molecule to pass through into the extracellular fluid

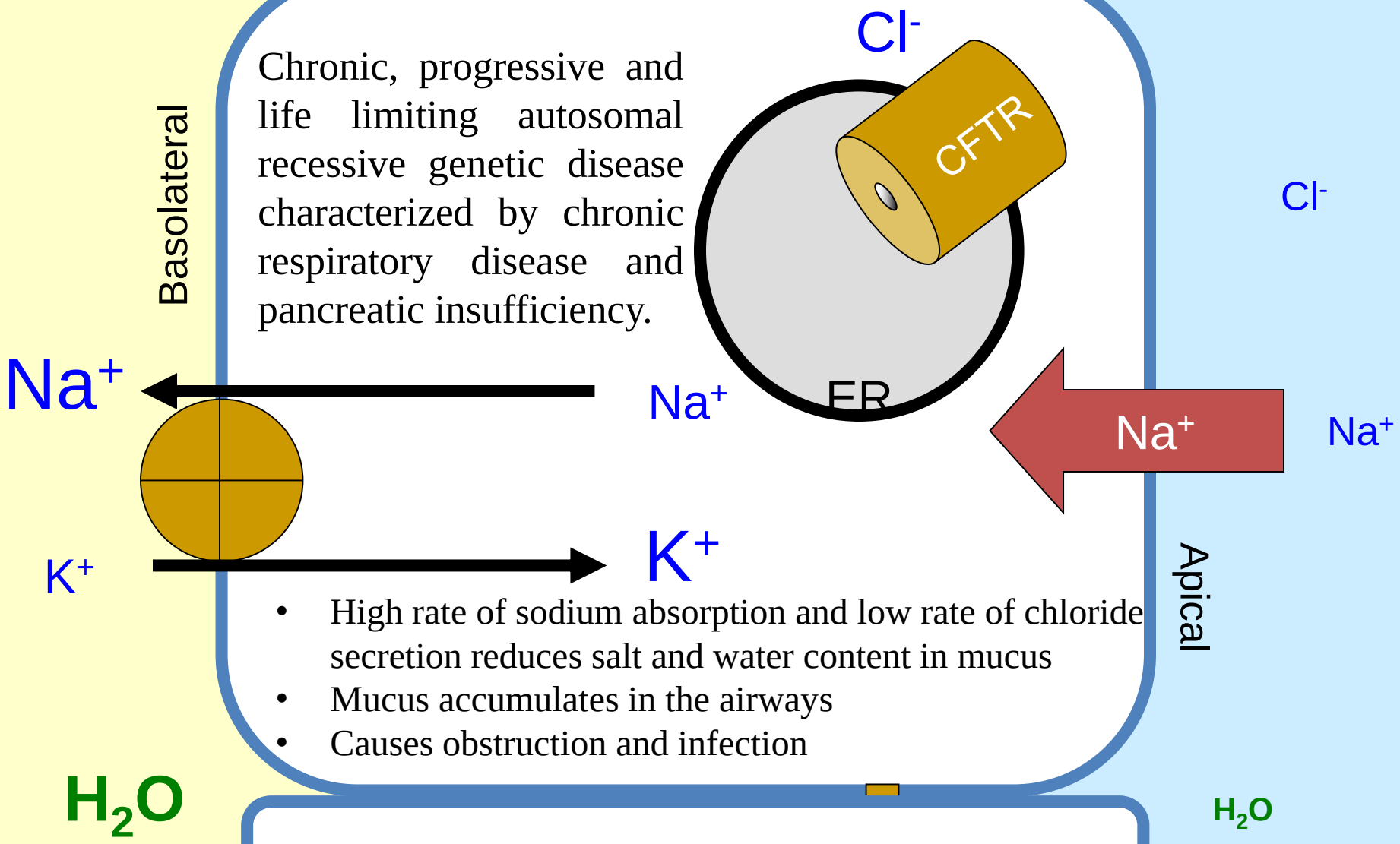
Canser drugs: adriamycin, colchicine, vinblastine

Often, P glycoprotein (P-gp) is found to be overexpressed in human cancer cells. The presence of large amounts of this protein makes the cells resistant to a variety of drugs used in cancer chemotherapy, a phenomenon known as multi-drug resistance (P-gp is also commonly known as the multidrug resistance (MDR) protein.)

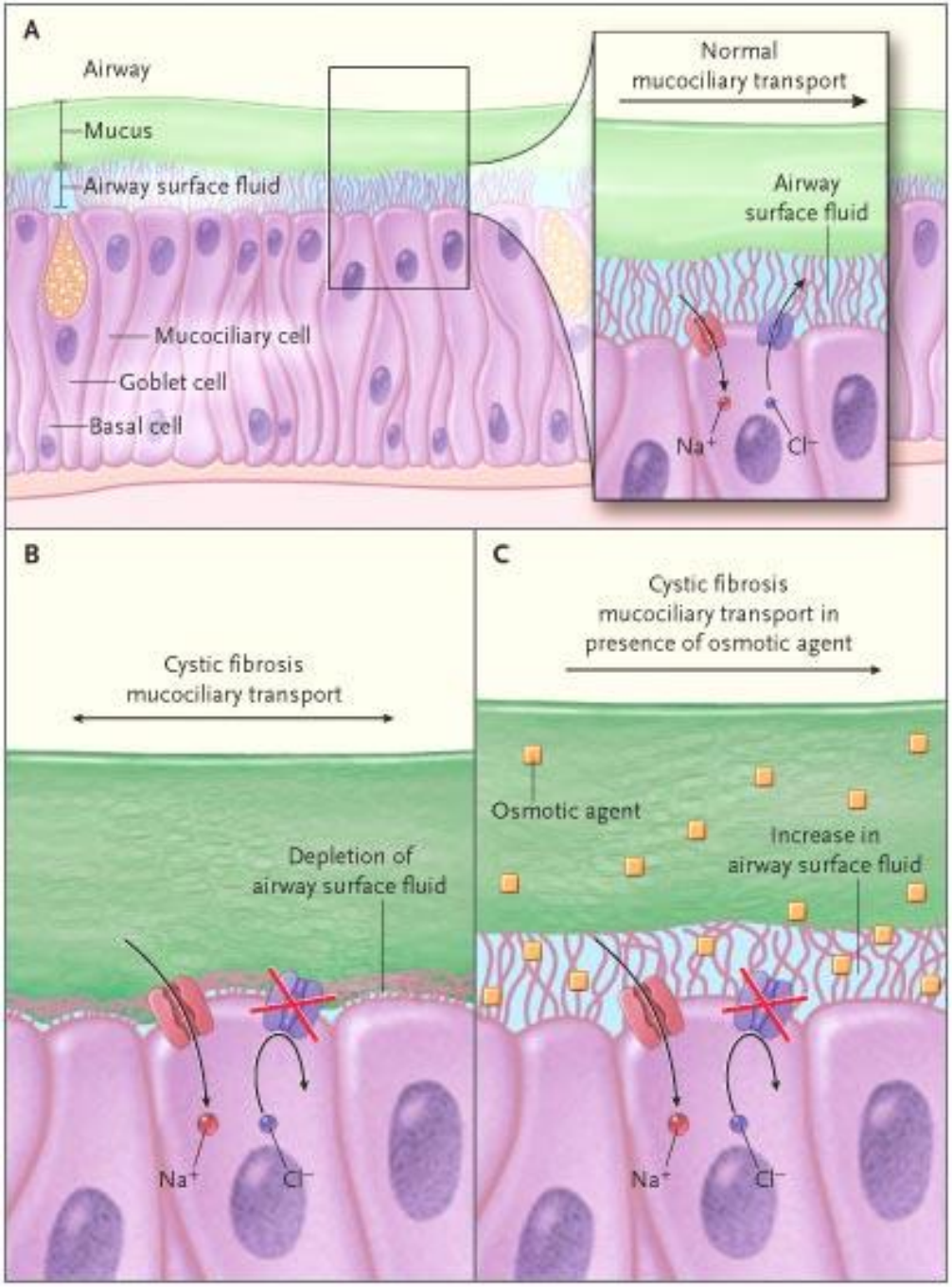

Basolateral
Apikal
P
CFTR
Cl-
Cl-
Na+
Na+
Na+
Na+
K+
K+
H2O
H2O

Cystic fibrosis

Chronic, progressive and life limiting autosomal recessive genetic disease characterized by chronic respiratory disease and pancreatic insufficiency.



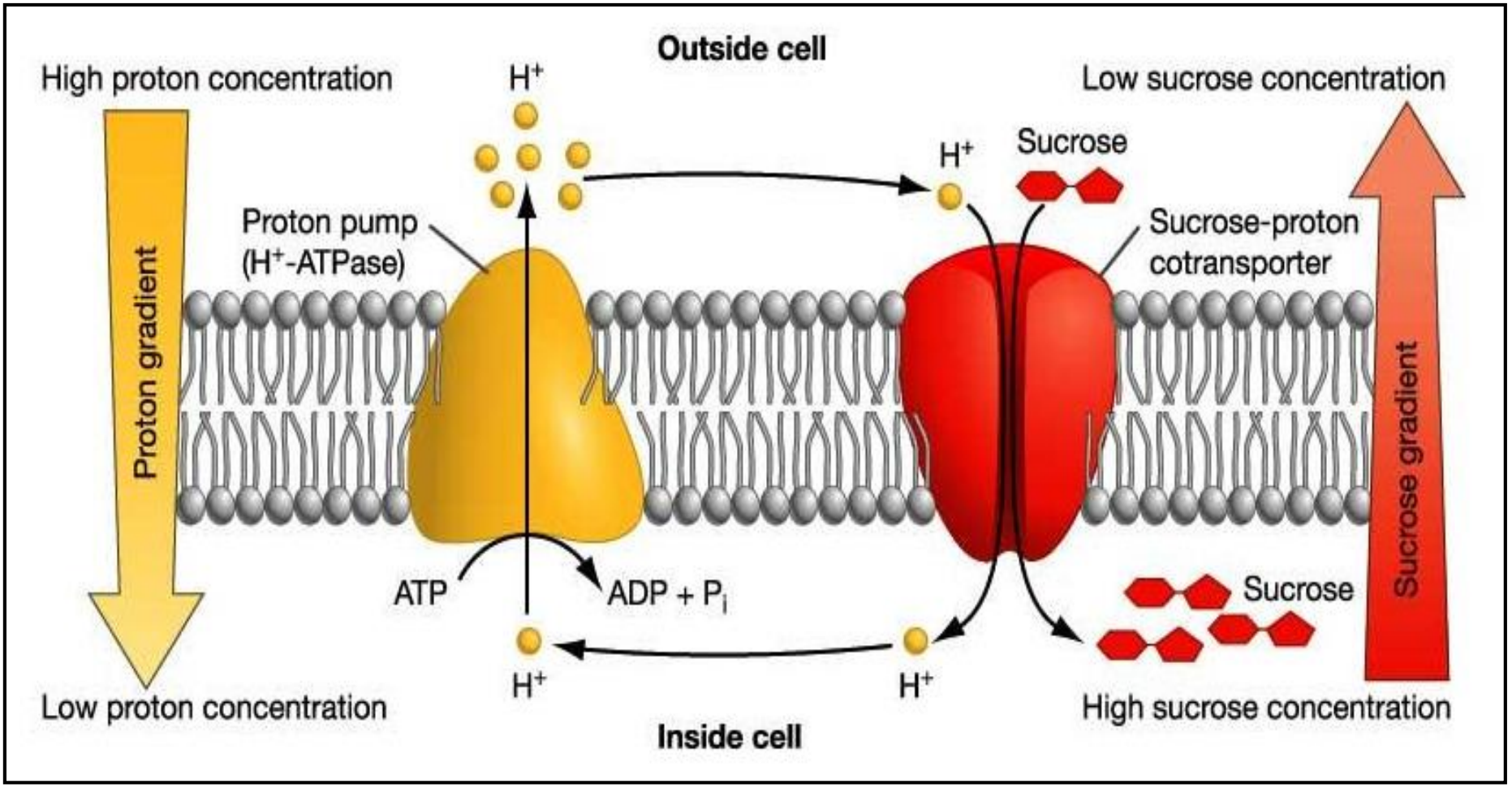
- High rate of sodium absorption and low rate of chloride secretion reduces salt and water content in mucus
- Mucus accumulates in the airways
- Causes obstruction and infection


A
Airway
Mucus
Airway surface fluid
Mucociliary cell
Goblet cell
Basal cell
Normal
mucociliary transport
Airway
surface fluid
Na+
Cl−
B
Cystic fibrosis
mucociliary transport
Depletion of
airway surface fluid
Na+
Cl−
C
Cystic fibrosis
mucociliary transport in
presence of osmotic agent
Osmotic agent
Increase in
airway surface fluid
Na+
Cl−

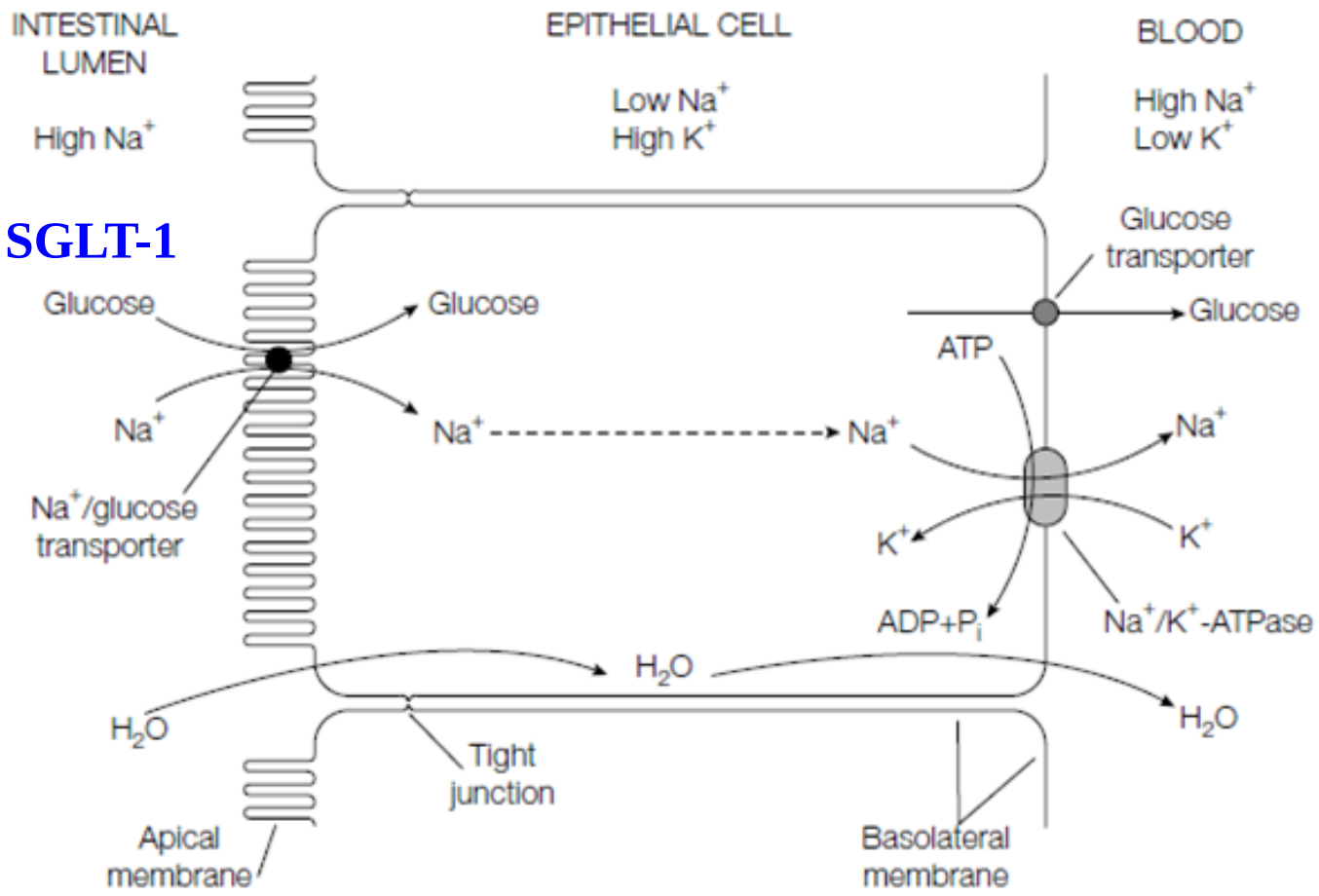
Secondary active transport

(Coupling Active Transport to Existing Ion Gradients)

- Depends on ion gradient that was created by primary active transport system
- Energy stored in gradients is used indirectly to drive transport of other solutes
  - Low Na^+ concentration that is maintained inside cell by Na^+-K^+ pump strengthens sodium's drive to want to enter cell
  - Na^+ can drag other molecules with it as it flows into cell through carrier proteins (usually symporters) in membrane
    - Some sugars, amino acids, and ions are usually transported into cells via secondary active transport


Outside cell
High proton concentration
H+
Low sucrose concentration
H+
Sucrose
Proton pump
(H+-ATPase)
Sucrose-proton
cotransporter
Proton gradient
Sucrose gradient
ATP
ADP + Pi
Sucrose
H+
H+
Low proton concentration
High sucrose concentration
Inside cell

Sodium-glucose symport system of intestinal cells couples the "downhill" transport of two Na^+ ions into the cell to the "uphill" transport of glucose, pumping glucose into the cell against its concentration gradient



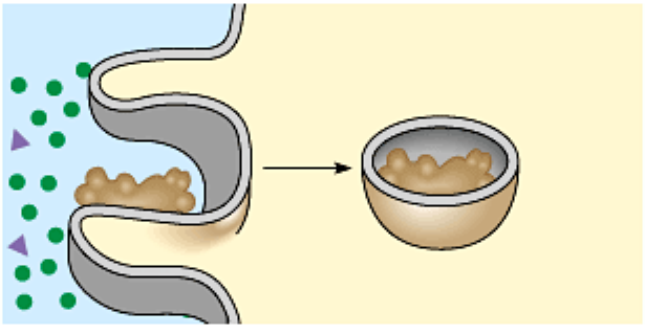
The transport protein, called a *Na/glucose cotransporter*, binds two sodium ions and one glucose molecule on the external surface of the apical plasma membrane. When the sodium ions are released on the inside of the cell into a solution of lesser sodium concentration, the conformation of the protein is changed so that it loses its affinity for the glucose molecule, which is then released into the cell. Once inside, the glucose molecules diffuse through the cell and are moved across the basal membrane by facilitated diffusion.

How about large molecules?

- Moving large molecules into & out of cell
  - through vesicles & vacuoles
  - endocytosis
    - phagocytosis = “cellular eating”
    - pinocytosis = “cellular drinking”
    - receptor-mediated endocytosis
  - exocytosis

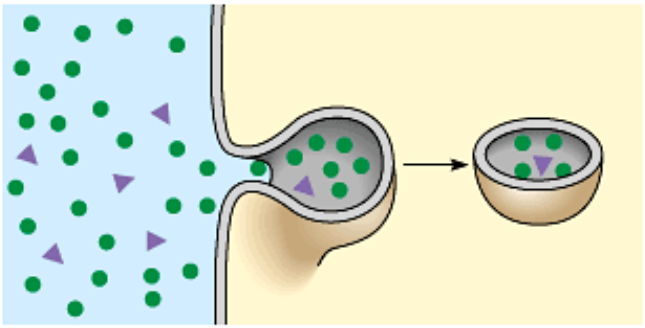
Endocytosis

phagocytosis



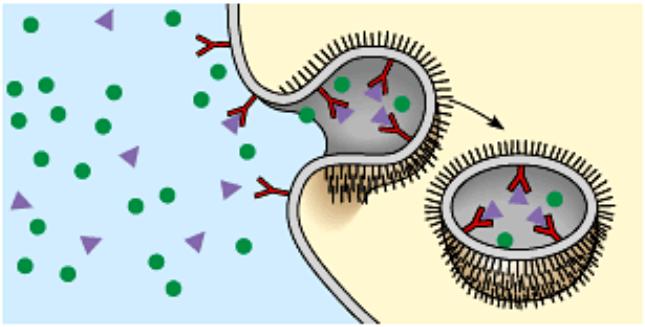
fuse with lysosome for digestion

pinocytosis



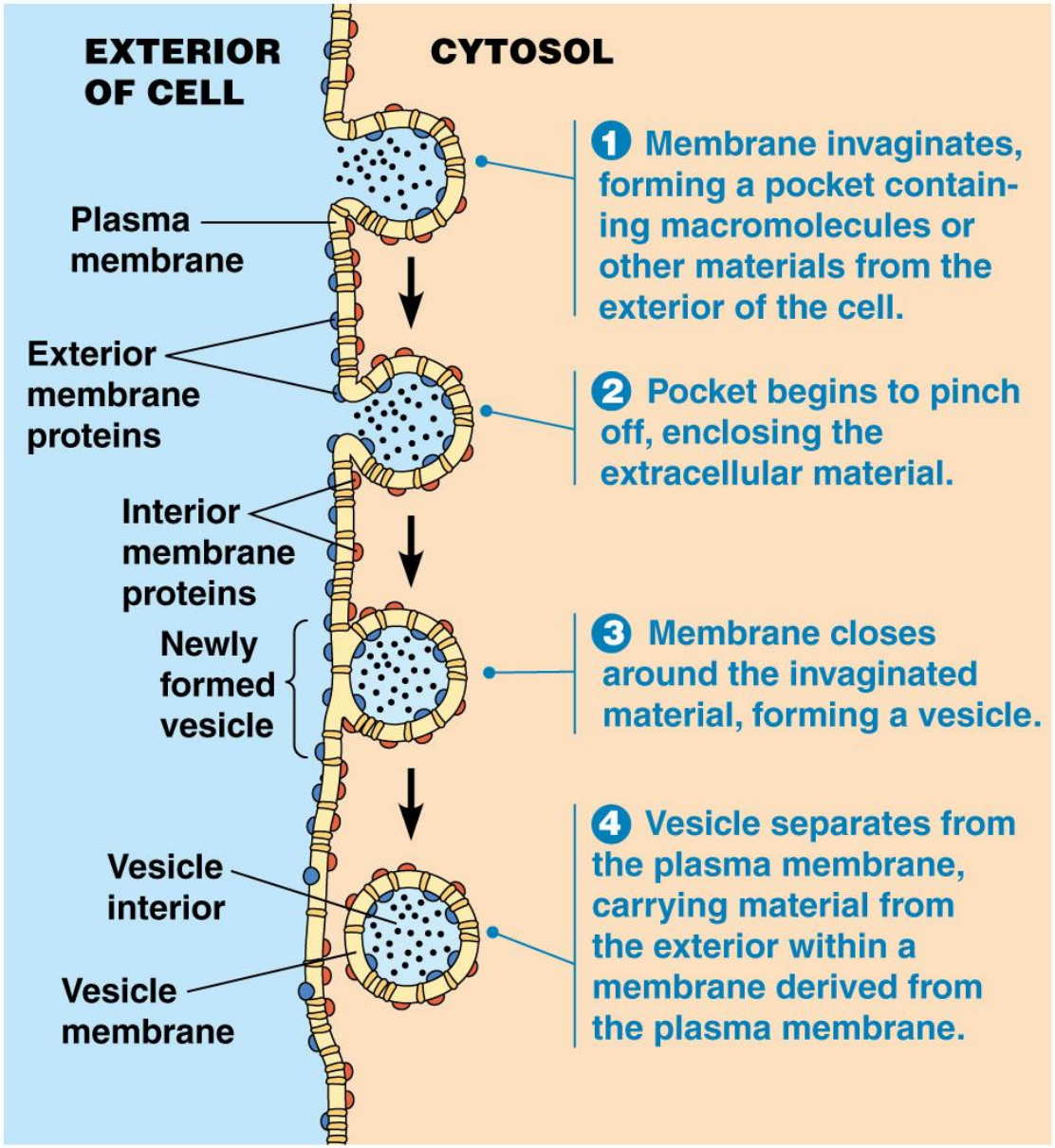
non-specific process

receptor-mediated endocytosis



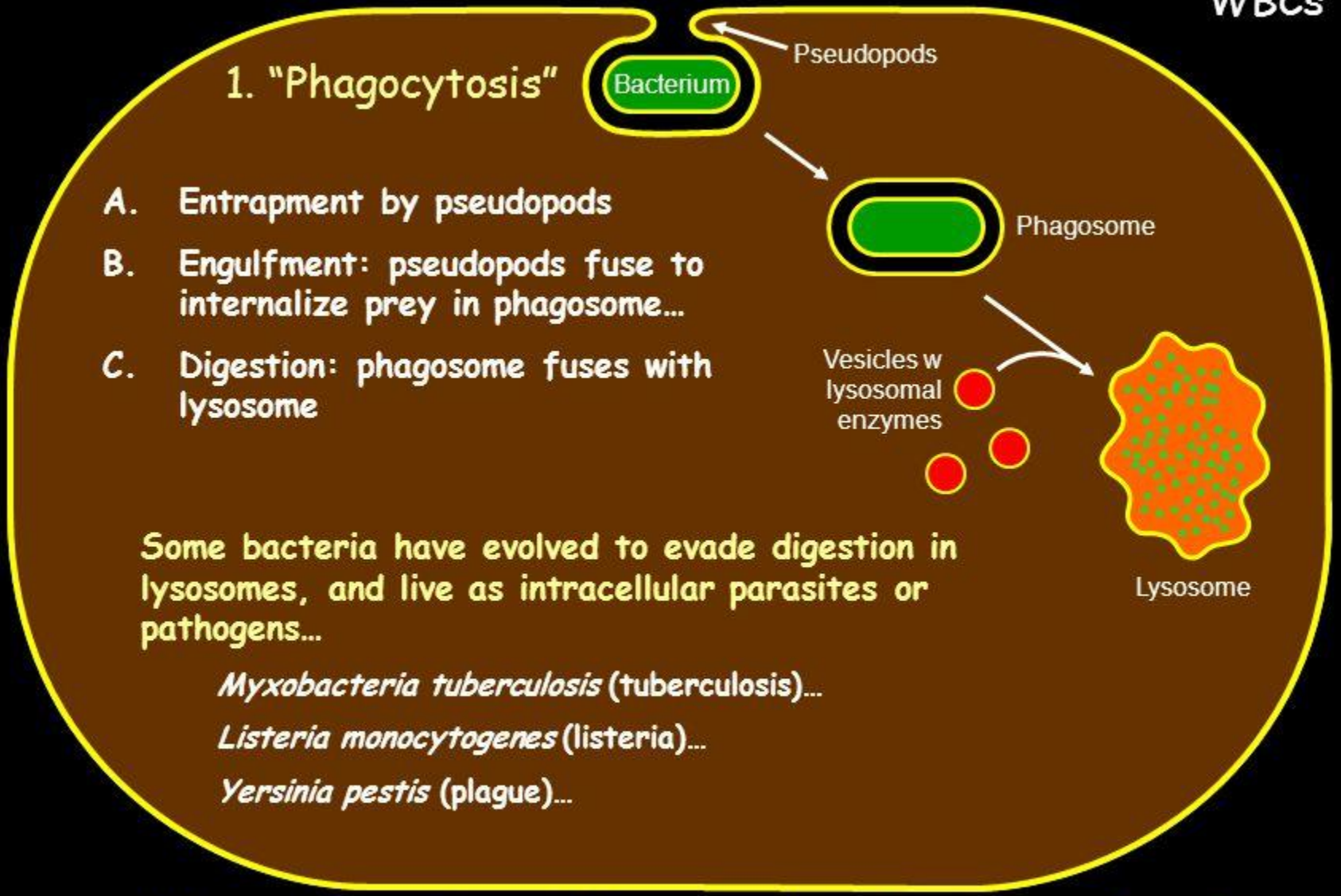
triggered by molecular signal

Uptake of extracellular materials by invagination of plasma membrane to form a small membrane-bounded vesicle (endosome).



- **Phagocytosis: type of endocytosis that is referred to as "cell eating"**
  - **Membrane projections called pseudopods form and flow around solid particles that are being engulfed, forming a vesicle which is pulled into cell**
  - **Formed vesicle is called a phagosome**
  - **Phagocytosis is used by macrophages and certain other white blood cells, which engulf and digest foreign materials or invasive microorganisms found in the bloodstream or injured tissues.**
  - **Macrophages are also scavengers, ingesting cellular debris and damaged cells**

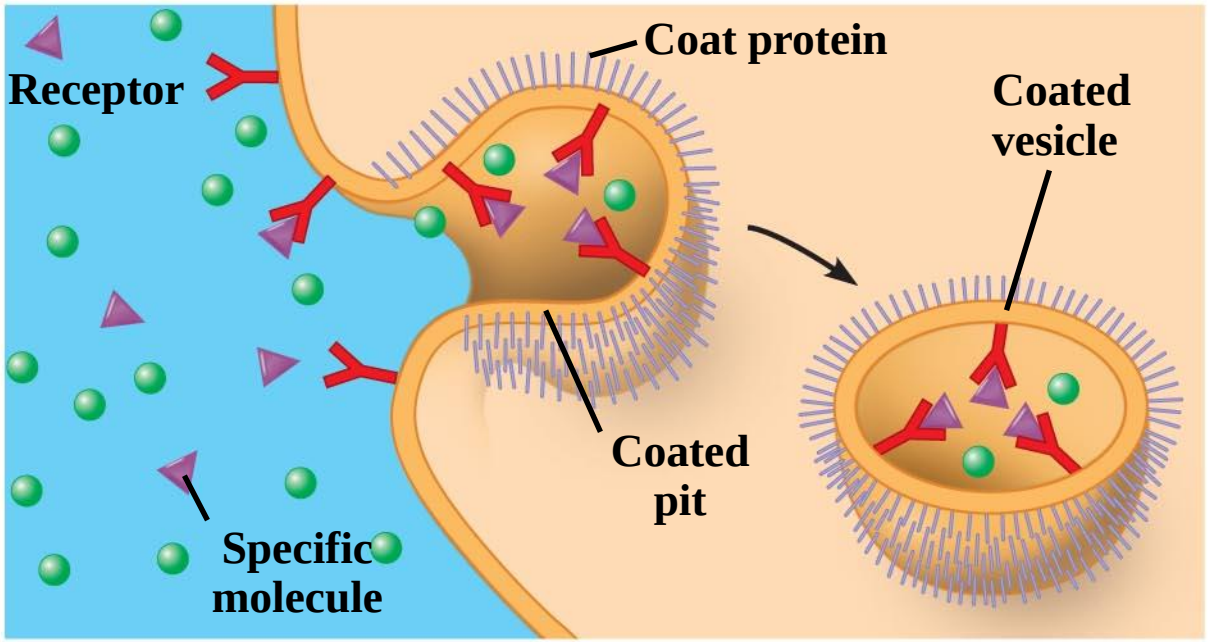
Phagocytosis - "cell eating" Performed by specialized "phagocytes:" WBCs

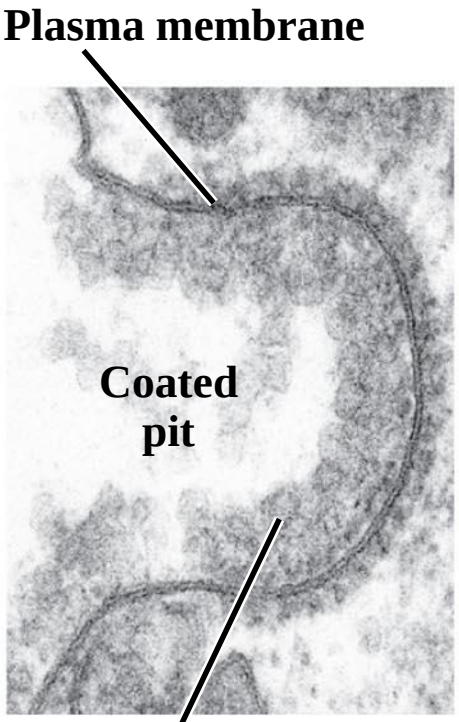


Where do vesicles with lysosomal contents come from?
What is their address label?

- **Pinocytosis: type of endocytosis that is referred to as "cell drinking" or fluid-phase endocytosis**
  - **Plasma membrane infolds, bringing extracellular fluid and dissolved solutes inside cell**
    - **Fuses with endosome**
  - **Main way in which nutrient absorption occurs in the small intestine**
  - **Membrane components are recycled back to membrane**

Receptor-mediated endocytosis





Receptor proteins on the outside of the cell bind specific substances and bring them into the cell by endocytosis

- **Receptor-mediated endocytosis involves endocytosis and transcytosis of specific molecules**
  - **Many cells have receptors embedded in clathrin-coated pits, which will be internalized along with the specific molecule bound**
    - **Examples: enzymes, low-density lipoproteins (LDL), iron, insulin, and, unfortunately, viruses, diphtheria, and cholera toxins may also be taken into a cell this way**

Vesicle budding and membrane curvature

Trafficking through endomembrane system requires vesicle budding

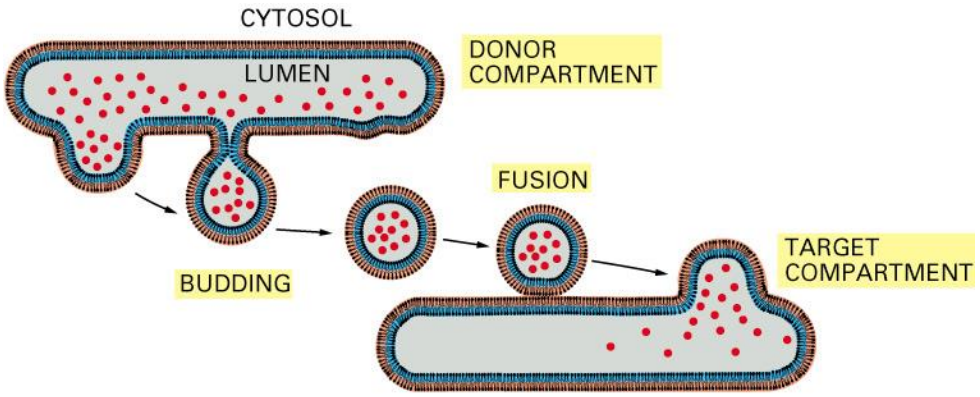


Figure 13–2. Molecular Biology of the Cell, 4th Edition.

1. Vesicle pinches off (budding)
2. Fins way to target compartment
3. Binds (docking)
4. Undergoes membrane fusion

Membrane-Bound Vesicles Transport Materials Through the Endomembrane System

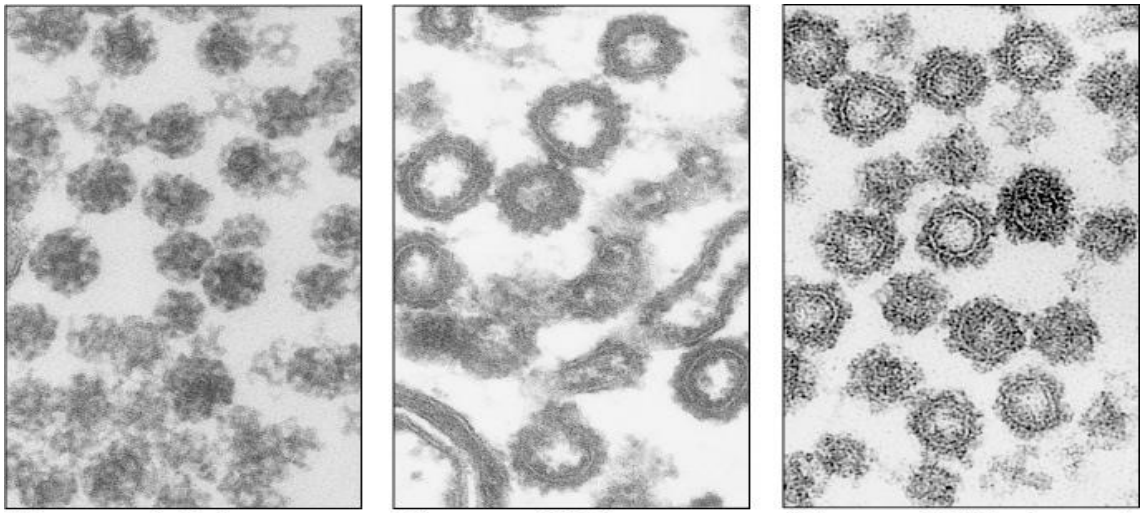


Figure 13–4. Molecular Biology of the Cell, 4th Edition.

Vesicles are enclosed by proteinaceous coats

Identified coats: Clathrin, COPI and COPII

Coat functions:

1) Helps membrane bud to form vesicle
2) Cargo Selection: Captures specific components for onward transport

CLATHRIN COATED VESICLES:

- Have characteristic basket like structure
- Each clatharin subunit – triskelion: comprises 3 heavy and 3 light chains
- Legs of triskelions associate with each other and assemble into a basket like structure of hexagons and pentagons
- Isolated triskelions can self-assemble to form cages in the absence of membrane

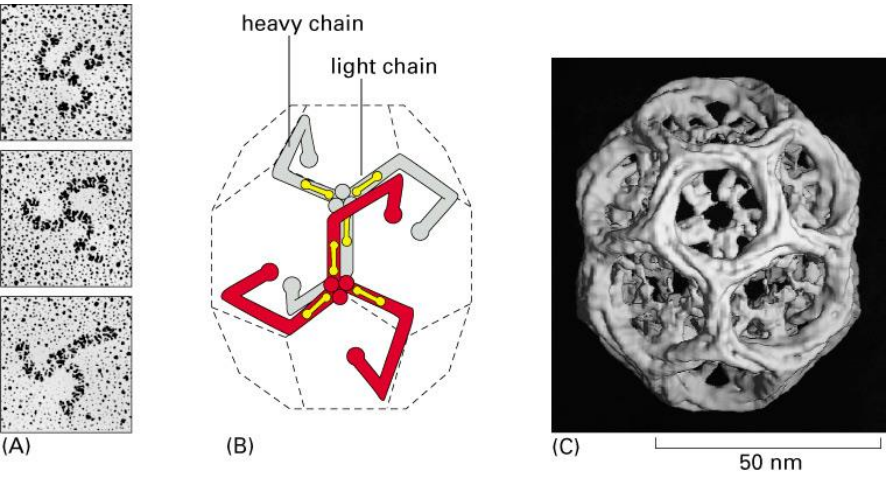
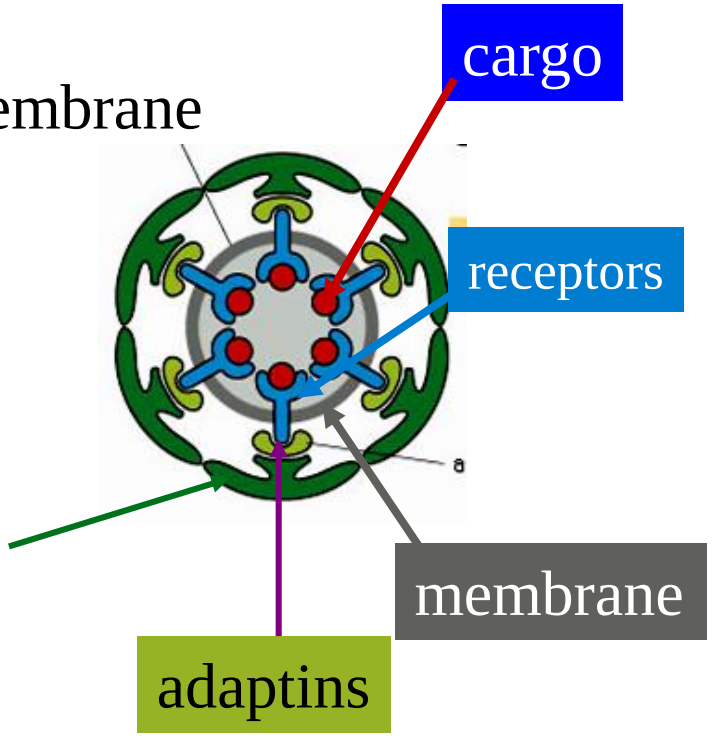
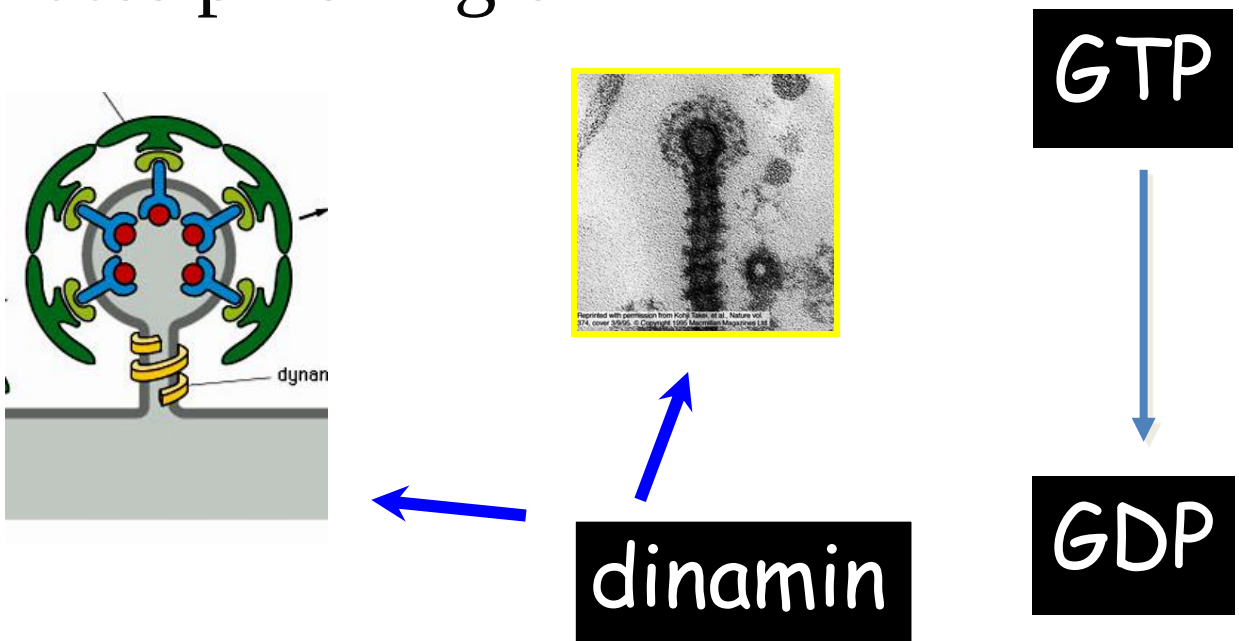


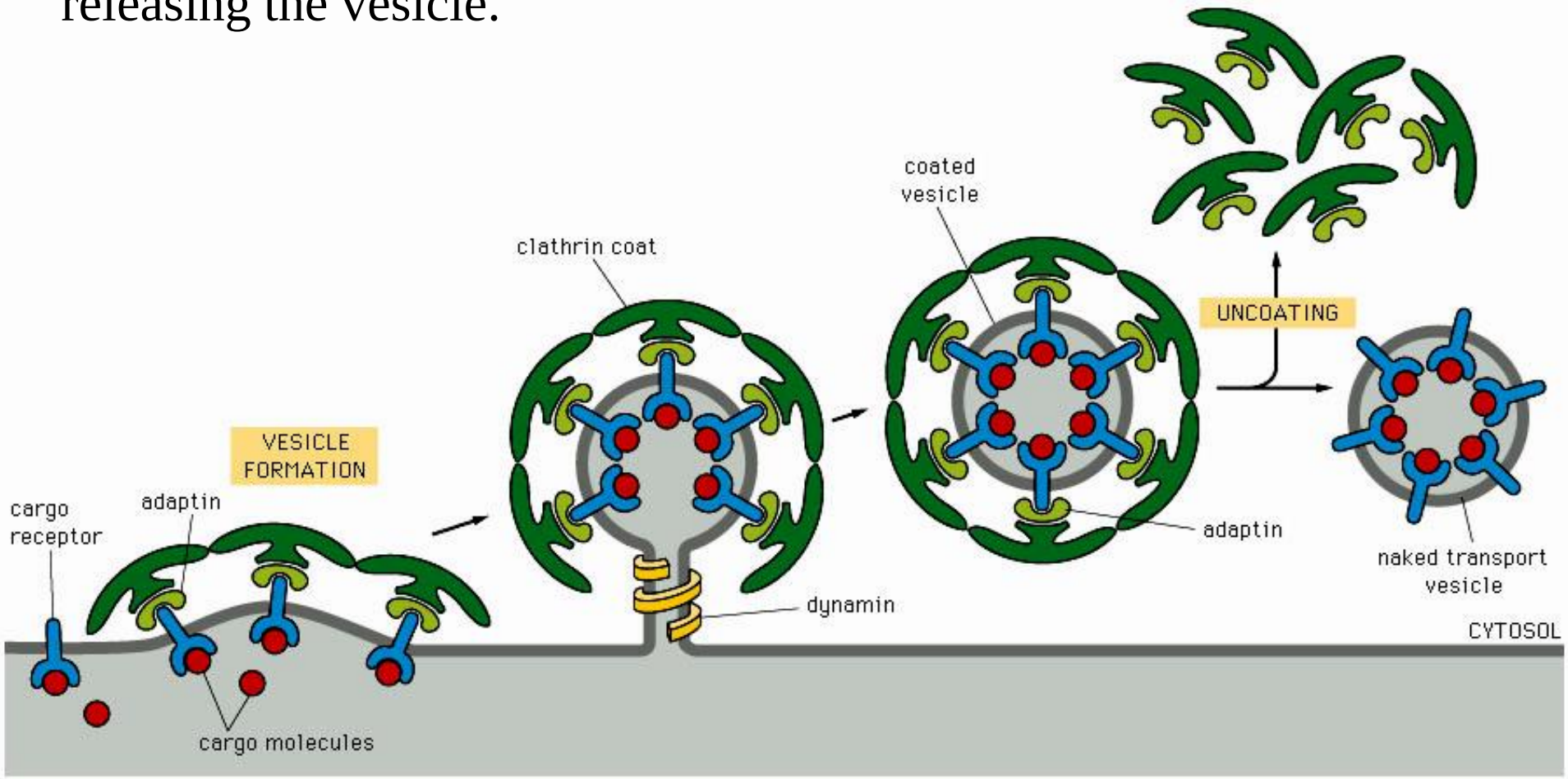
Figure 13–7. Molecular Biology of the Cell, 4th Edition.



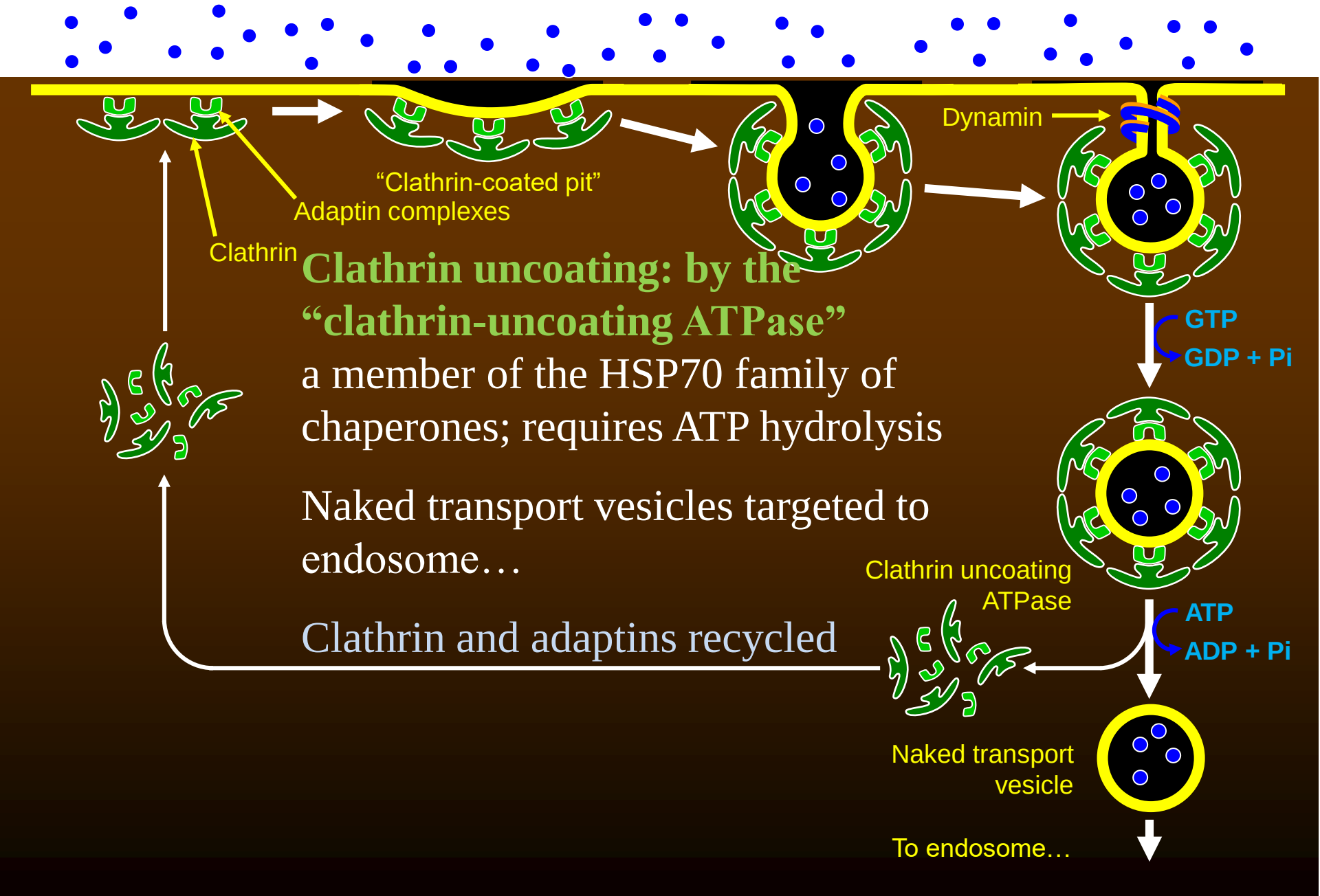
- Clathrin adaptor proteins:
  - Adaptins attach clathrin to membranes and recruit cargo proteins into the vesicle
  - The **adaptor protein** was identified by its ability to promote assembly of clathrin coats and is sometimes called *assembly protein*
  - Dinamin (high molecular weight GTPase) regulates pinching off



- The cargo bind to receptors on the outer face of the plasma membrane.
- Clathrin molecules assemble on the cytosolic surface of the membrane - this starts the shaping of the membrane into a vesicle.
- A small **GTP-binding protein - dynamin** - forms a ring around the neck. GTP is hydrolized, the ring constricts, pinching off the neck and releasing the vesicle.



Clathrin-coated vesicles are rapidly uncoated



The formation of clathrin-coated vesicles from the plasma membrane

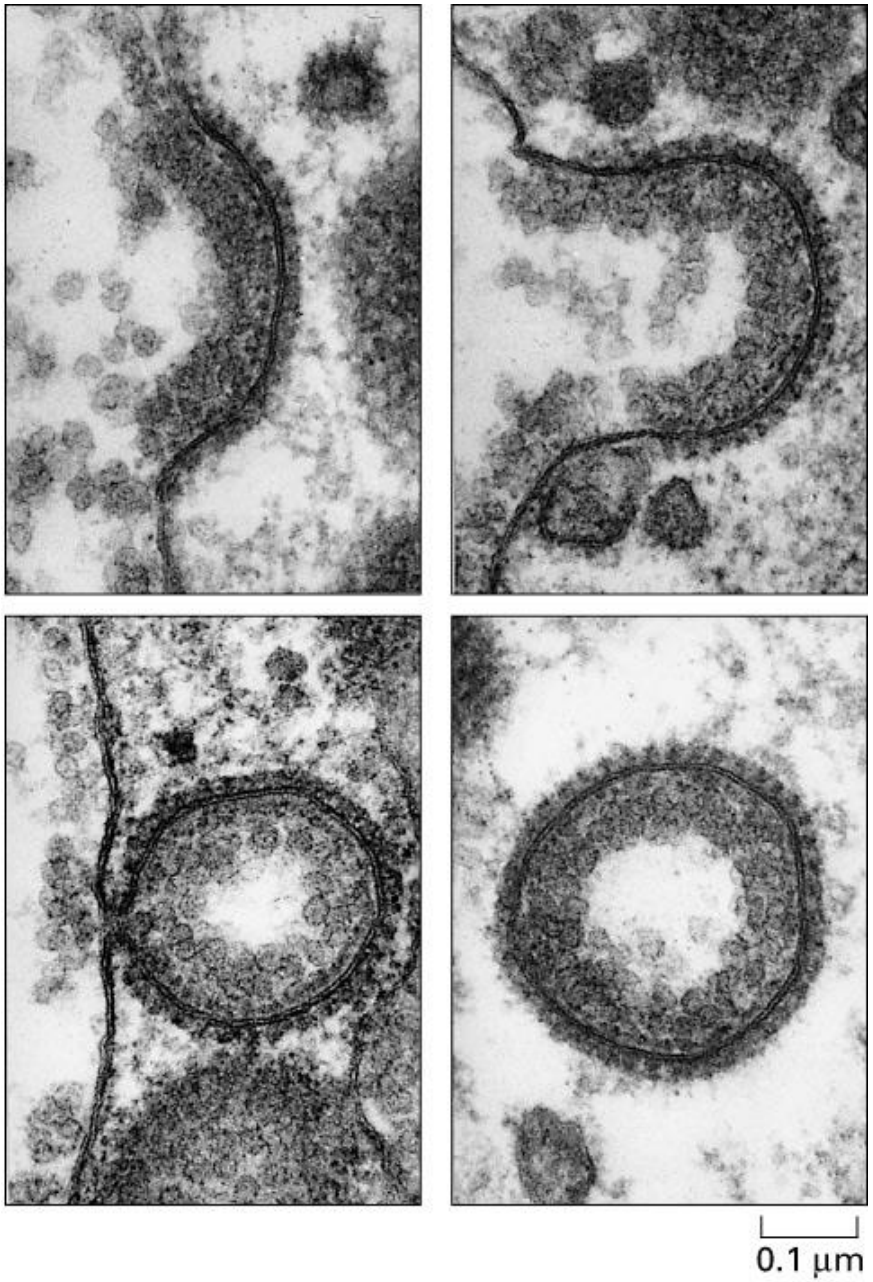


Figure 13–41. Molecular Biology of the Cell, 4th Edition.

Coat assembly is regulated by small GTPases

- Small GTPases act as molecular switches, which are "ON" when they bind GTP, and "OFF" after the GTP has been hydrolyzed to GDP
  - Activation is via a conformational change
- GTPases:

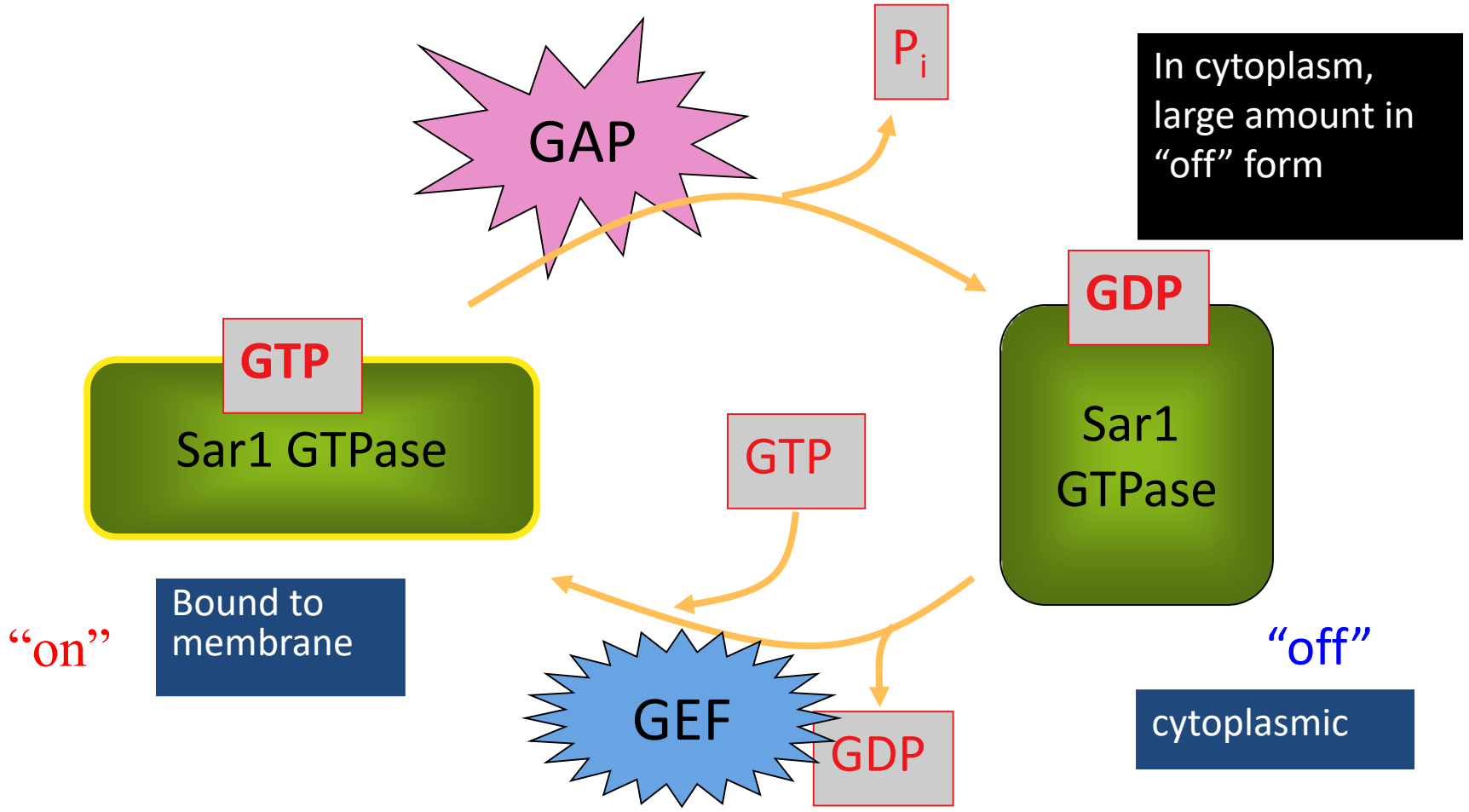
o Sar1(Secretion-Associated RAS related protein) - COPII assembly in ER

o ARF1 (ADP-ribosylation factor 1) - COPI assembly in Golgi, clathrin assembly in trans Golgi

Guanine-nucleotide exchange factors (GEFs), GDP dissociation inhibitors (GDIs), and GTPase-activating proteins are regulators of small GTP-binding proteins

Sar1:GTPase switch on/off

GTPase-activating proteins (GAP inactivate GTPases)



Guanine Exchange Factors (GEFs) activate GTPases

Sar1 activation

COPII vesicle formation is mediated by a monomeric GTPase, Sar1. A GEF in the donor membrane interacts with the GTPase, Sar1, causing GDP/GTP exchange. Sar1-GTP extends a fatty acid tail that inserts into the membrane. COPII assembles on the Sar1 to form a vesicle. COPI vesicle formation involves a protein called ARF that is analogous to Sar1. Uncoating of the COPI and COPII vesicles occurs when the G-protein (ARF or Sar1) hydrolyzes GTP and retracts that fatty acid tail. This may not require a GAP but is instead dictated by the rate of hydrolysis intrinsic to the G-protein.

- GDP-bound SAR1 or ARF are free in cytosol
- Donor membrane contains guanine nucleotide-releasing factor
- Membrane-bound G-protein recruits coat protein subunits

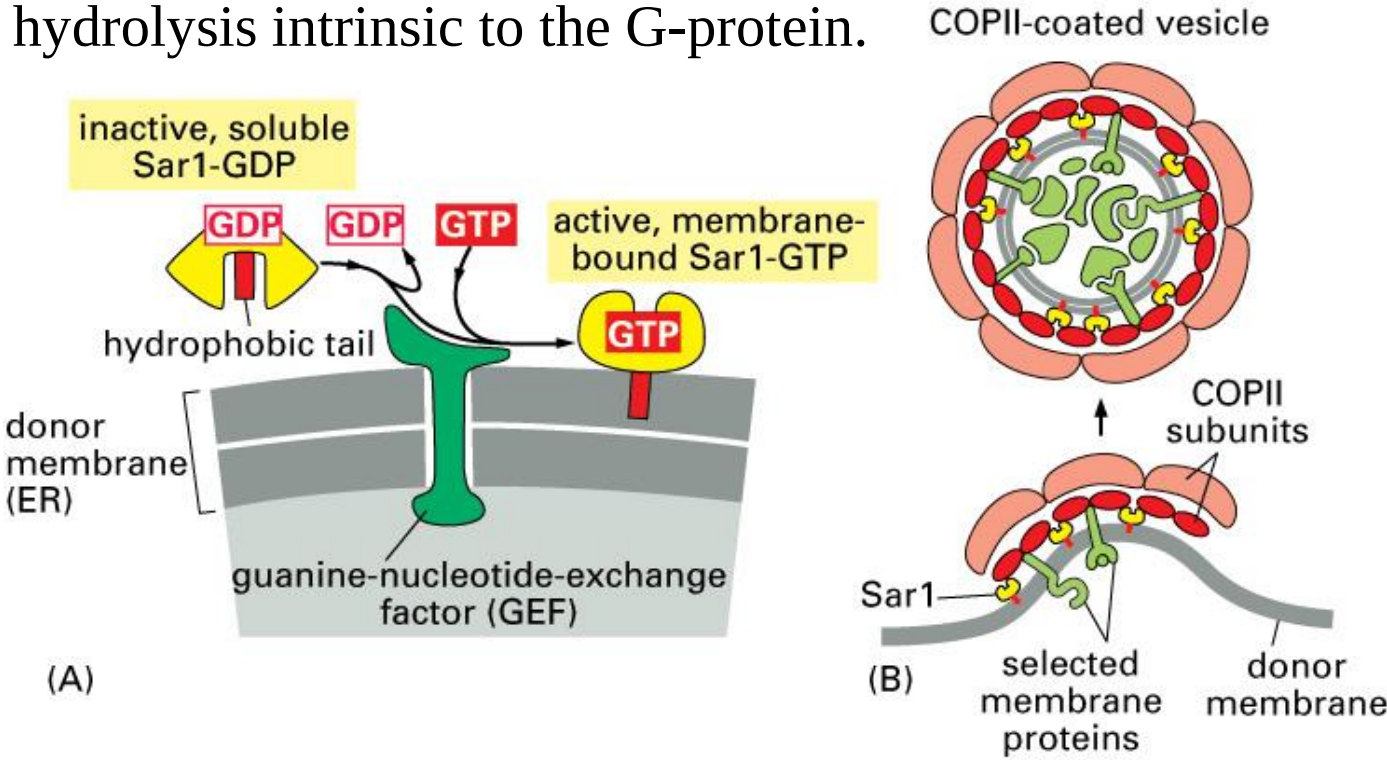


Figure 13–10. Molecular Biology of the Cell, 4th Edition.

Rab-GTPases (small GTP binding proteins on vesicles) Contributes to Accuracy of Vesicle Targeting Before Membrane Fusion is Allowed to Occur

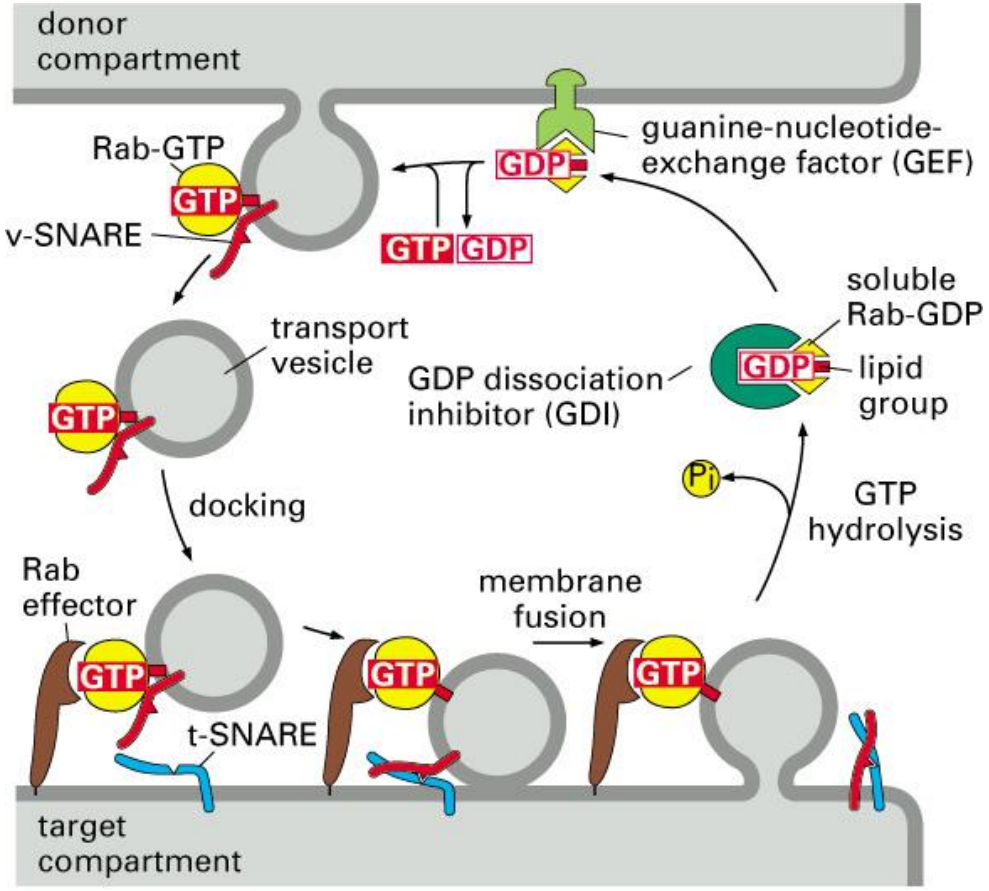


Figure 13–14. Molecular Biology of the Cell, 4th Edition.

- **Rab-GTP on vesicle interacts with Rab effector on target membrane**
- A guanine nucleotide exchange factor (GEF) recognizes a specific Rab proteins and promotes exchange of GDP for GTP.
- Activated Rabs attach to the membrane via covalently attached lipid groups at their C-termini and are incorporated into transport vesicles.
- Rab-GTP recruits effectors that can promote vesicle formation and vesicle fusion with target membranes.
- After fusion Rab-GTP hydrolyzes GTP to GDP and is released from the membrane. GTPase activating proteins (GAPs) accelerate hydrolysis, reducing the avalability of active Rabs.

GTPases cycle between an active GTP-bound state and an inactive GDP-bound state. Guanine Exchange Factors (GEFs) activate GTPases, which in turn interact with specific effectors to mediate downstream pathways. The intrinsic GTPase activity of these small G proteins is stimulated by GAPs, which accelerate the inactivation of the regulatory activity of the GTPases.

SNARE Proteins Mediate Fusion Between Vesicles and Target Membranes

- Once vesicles form, additional proteins ensure delivery to the correct destination
- The **SNARE hypothesis** explains this specificity
- The hypothesis states that sorting and targeting of vesicles involves two families of **SNARE** (**SNAP receptor**) proteins

SNARE proteins

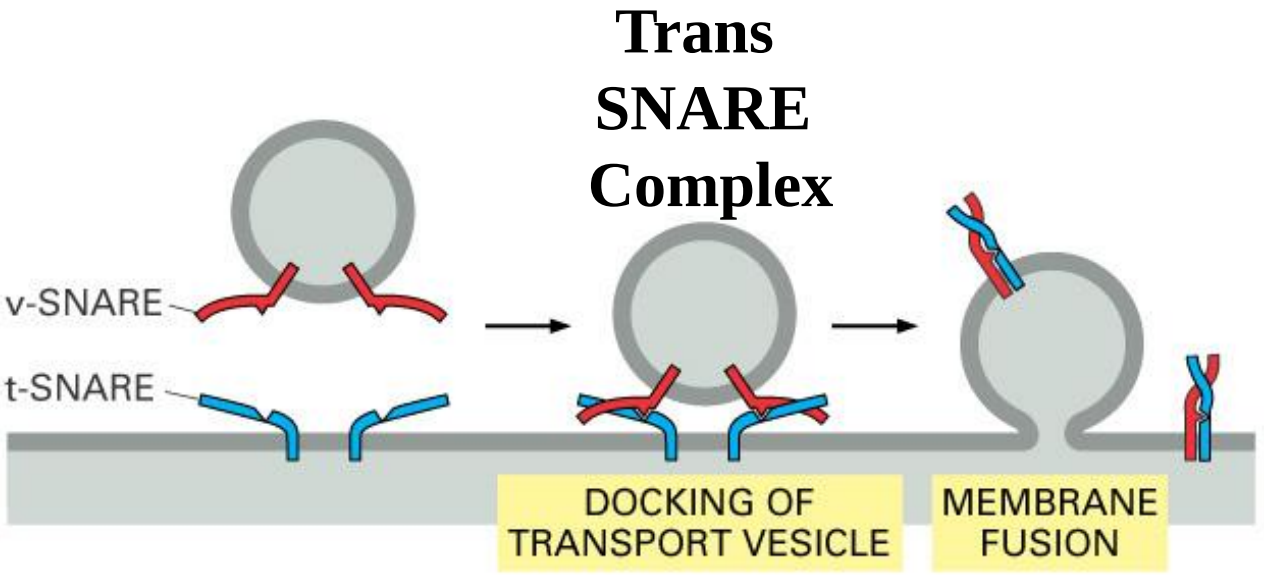
- **v-SNAREs** (**vesicle-SNAP receptors**) are found on vesicles

- **t-SNAREs** (**target-SNAP receptors**) are found on target membranes

- v- and t-SNAREs are complementary molecules that allow recognition between vesicles and their targets

SNAP-NSF Receptors (SNAREs)-integral membrane proteins
SNAPs - (soluble NSF attachment protein). Act as a cofactor mediating NSF attachment to SNAREs.
NSF - (N-ethylmaleimide sensitive factor) binds and hydrolyzes ATP. Required for disassembly of SNARE complex.

SNARE Pairing is Essential for Membrane Fusion



Separation of SNAREs By NSF Requires ATP

SNARE-SNARE pairing provides the energy to bring two bilayers sufficiently close to destabilize them and result in fusion

Figure 15-21 Essential Cell Biology, 2/e. (© 2004 Garland Science)

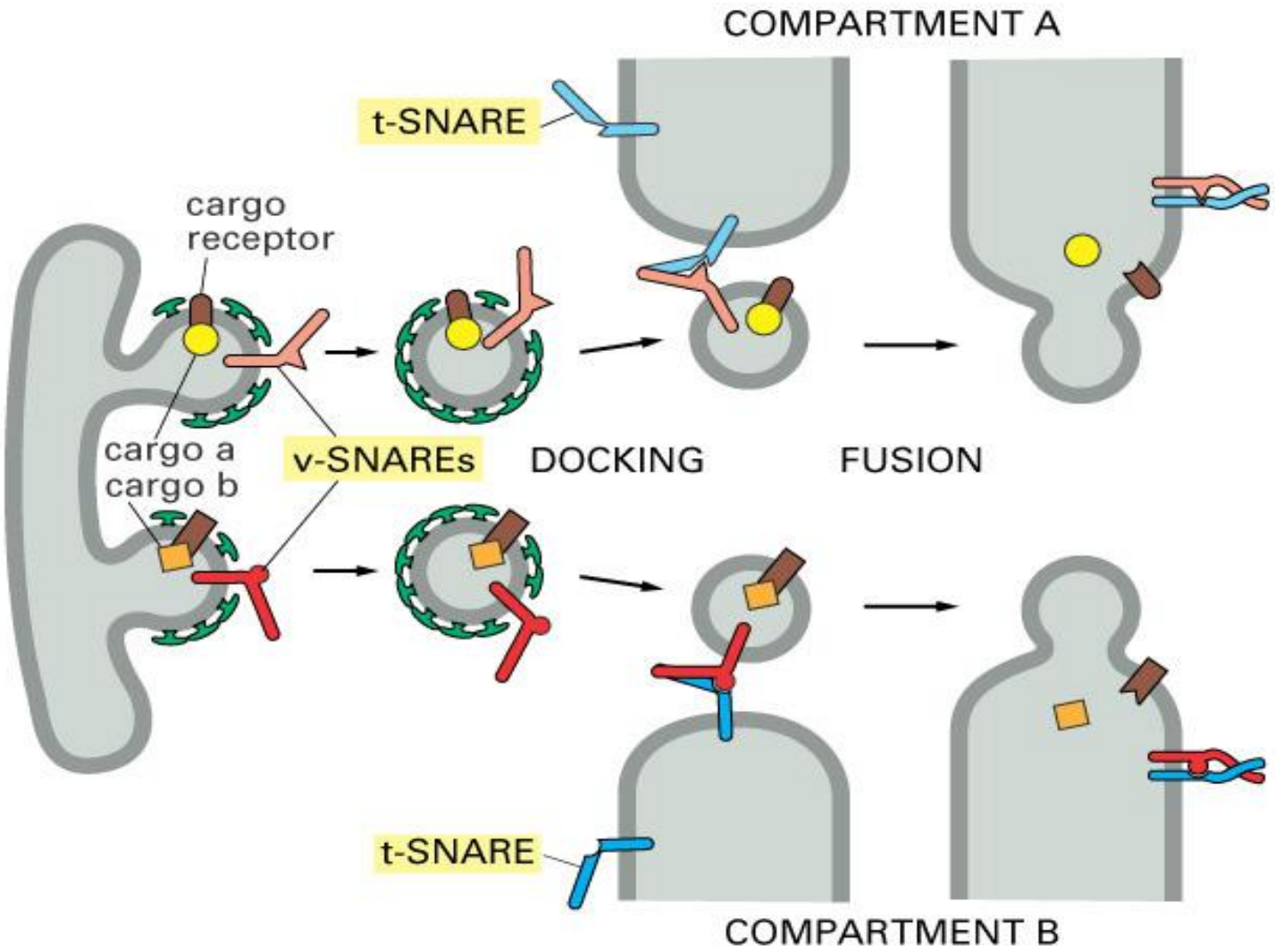


Figure 15-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)

20 Different SNARES (complementary sets) in animal cells each associated with particular membrane bound organelle

v and t SNAREs on opposing membranes interact to initiate formation of 4 helix-bundle trans SNARE complex

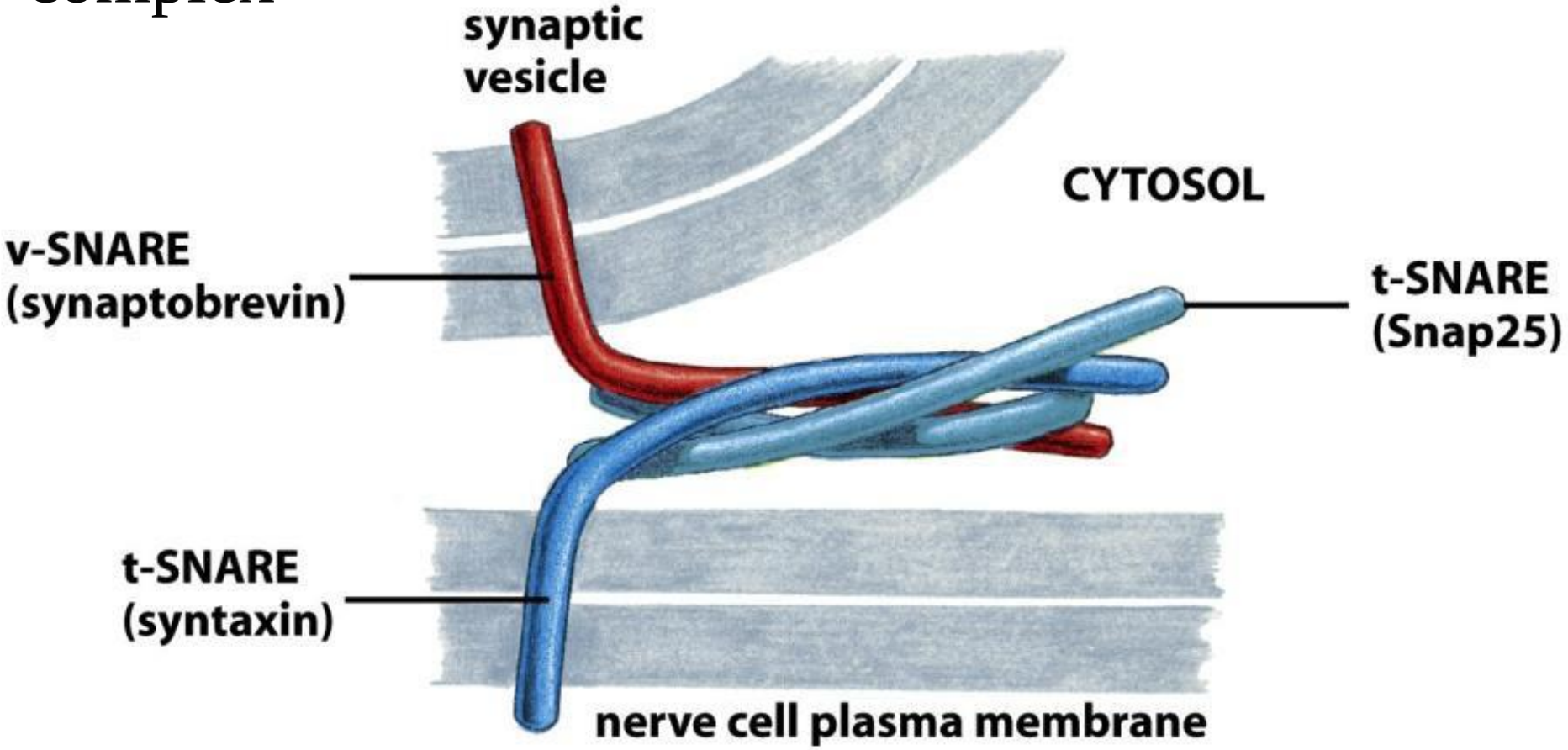
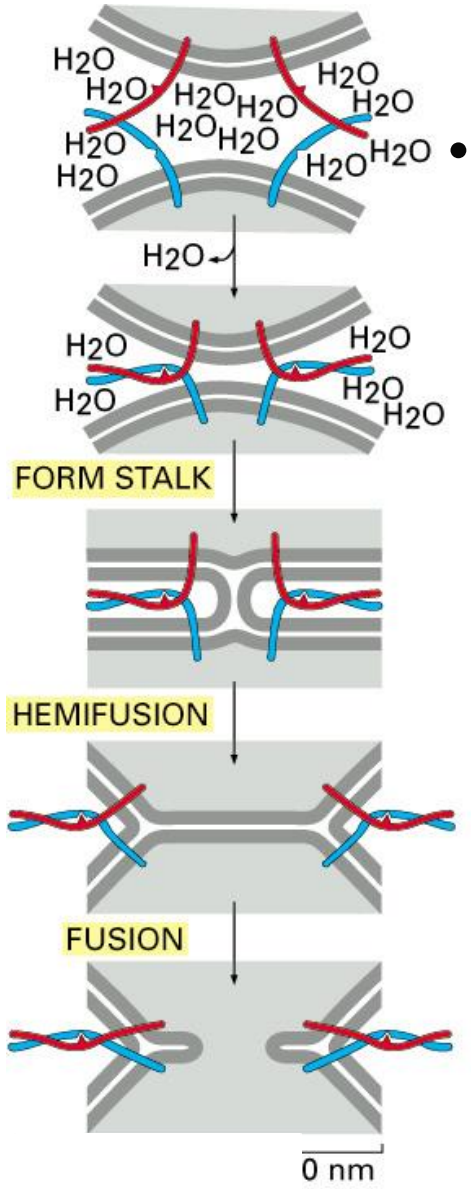


Figure 13-16 Molecular Biology of the Cell 5/e (© Garland Science 2008)

MODEL FOR SNARE MEDIATED MEMBRANE FUSION:



- SNAREs use energy released when interacting helixes wind up to pull membranes together and expel water molecules

r Biology of the Cell, 4th Edition.

DISSOCIATION OF SNARE COMPLEXES AFTER FUSION:

- SNARE complexes are very stable and resistant to boiling
- SNARE complexes must be dissociated for further rounds of transport
- Dissociation is mediated by ATPase NSF

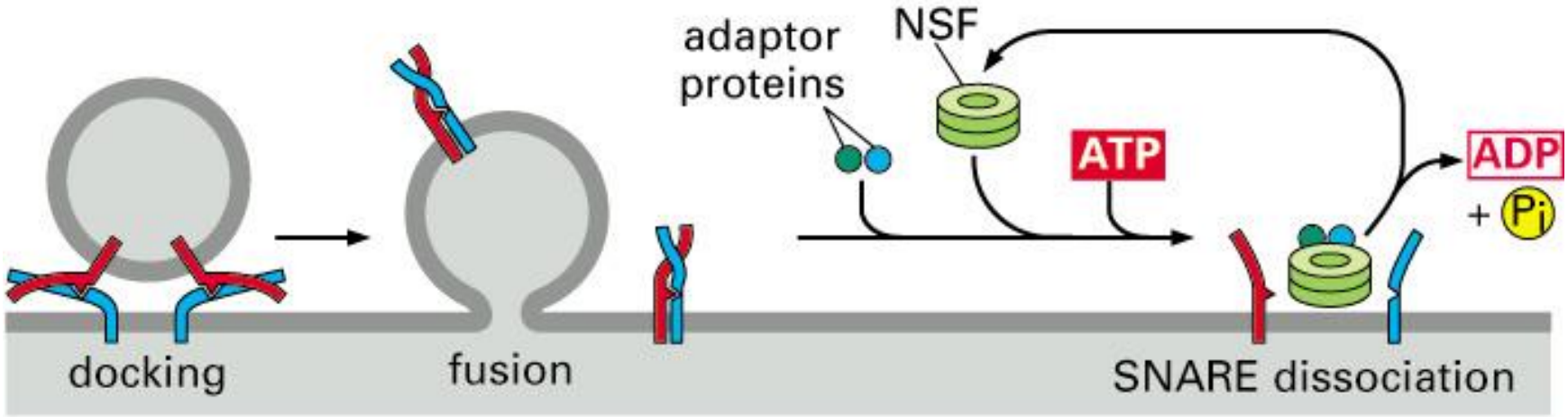
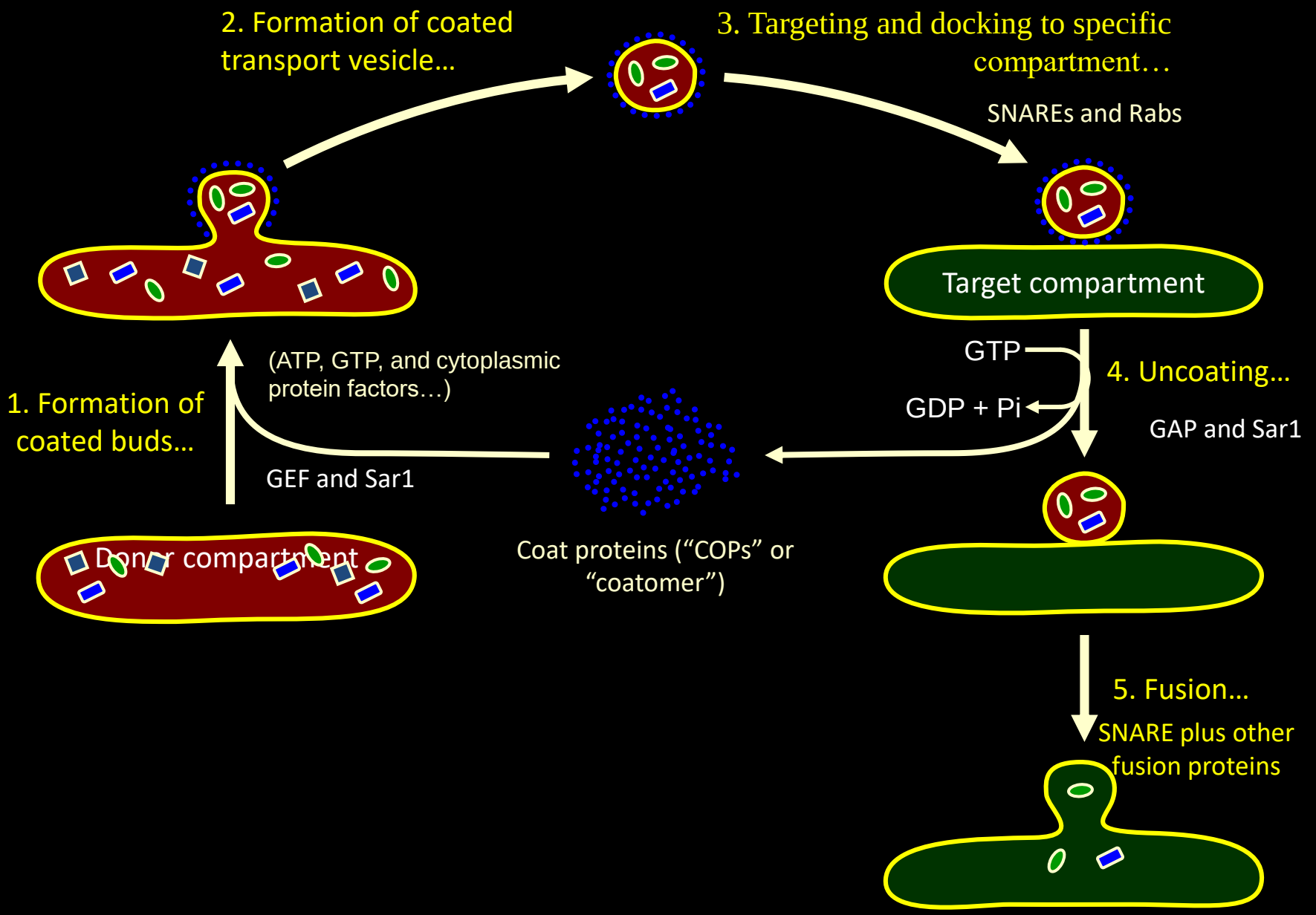


Figure 13–13. Molecular Biology of the Cell, 4th Edition.

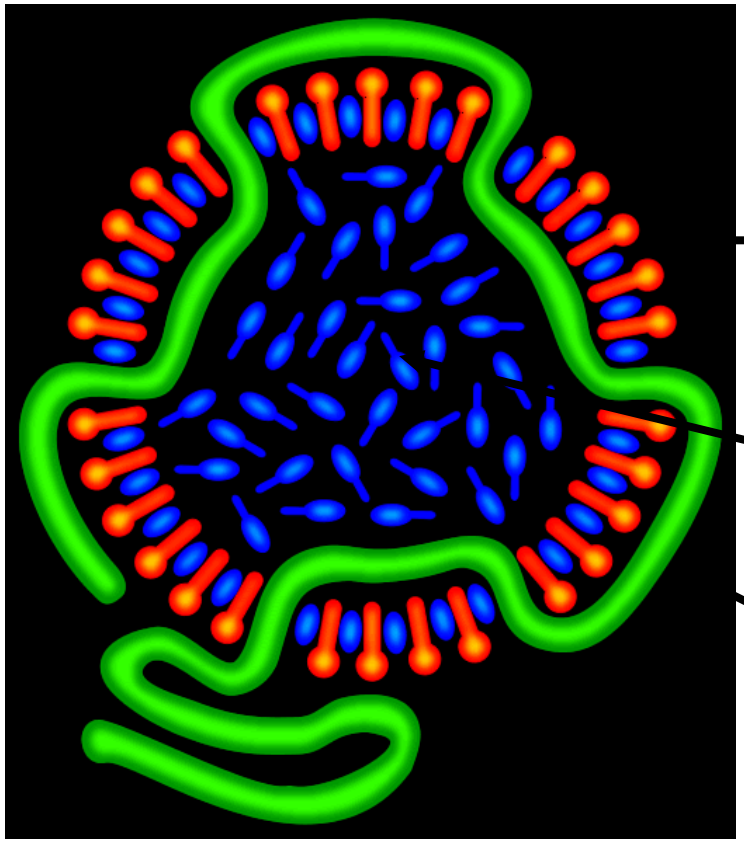
Vesicle formation and targeting is a multi-step process



How do cells take up specific macromolecules?

"Receptor-mediated endocytosis"

Example: Low-density lipoprotein (LDL), structure in which cholesterol is transported through our bodies



Lipid micelle:

~800 phospholipids…

~500 molecules of cholesterol…

~1500 molecules of cholesterol ester

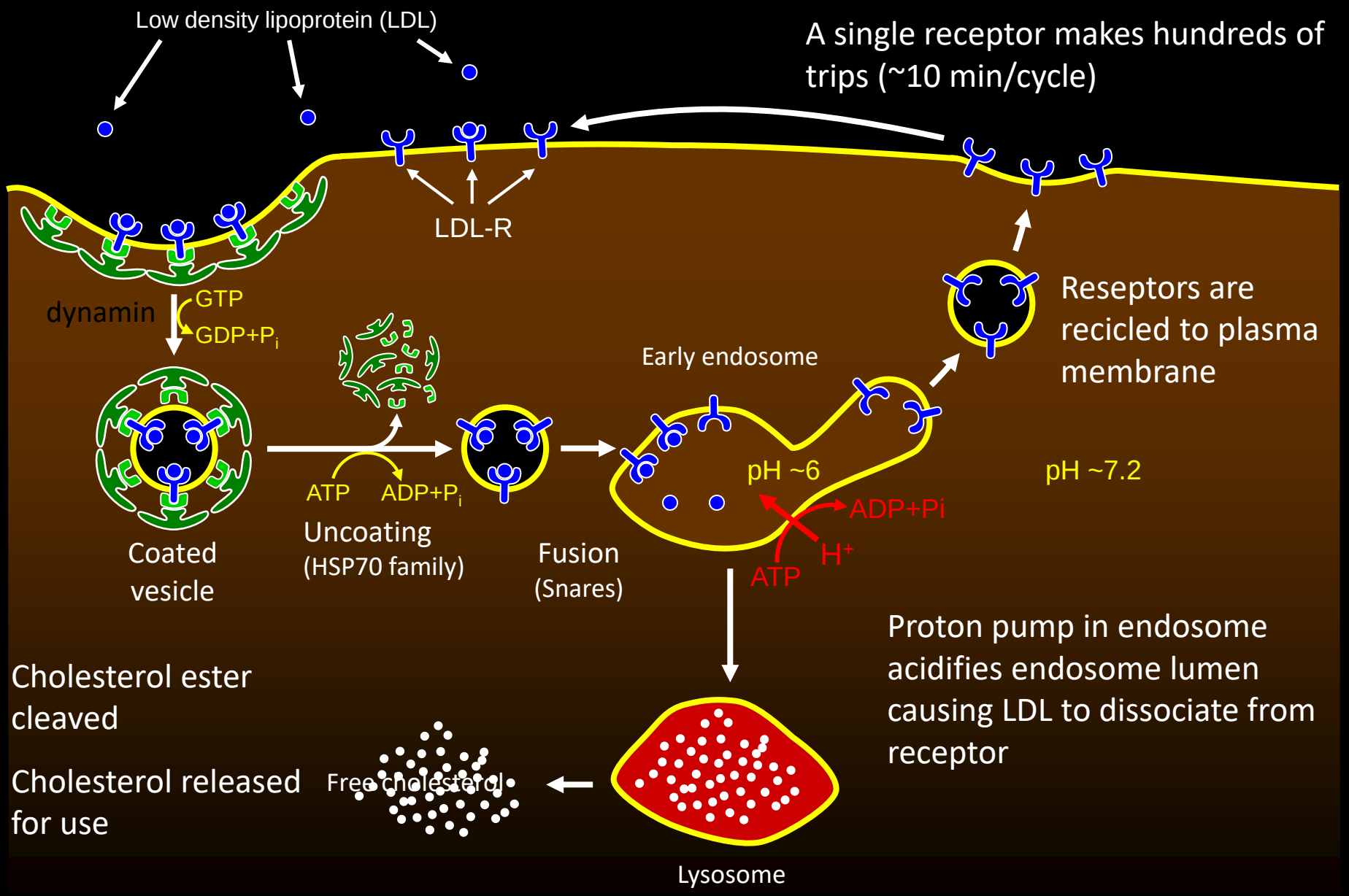
1 copy of apoprotein B…

Total mass: ~ 3 x 10^6 Da

Low-density lipoproteins (*LDL*) are internalized by receptor-mediated endocytosis. The internalization of LDL carries cholesterol into cells

Phases of clathrin-mediated endocytosis:

- Macromolecules (as ligands) bind to the specific cell surface receptors
- Then the receptors are concentrated in specialized regions of plasma membrane and clathrin and adaptor protein bind to these receptors forming clathrin-coated pits
- These pits bud from the membrane and form clathrin-coated vesicles containing receptors, proteins and ligands
- Then these vesicles fuse with early endosomes, in which the contents are sorted for the transport to lysosomes and receptors and proteins are recycled to plasma membrane


Low density lipoprotein (LDL)
A single receptor makes hundreds of trips (~10 min/cycle)
LDL-R
dynamin
GTP
GDP+Pi
Coated vesicle
ATP
ADP+Pi
Uncoating (HSP70 family)
Early endosome
pH ~6
pH ~7.2
ADP+Pi
H+
ATP
Fusion (Snares)
Reseptors are recicled to plasma membrane
Proton pump in endosome acidifies endosome lumen causing LDL to dissociate from receptor
Cholesterol ester cleaved
Cholesterol released for use
Free cholesterol
Lysosome

Defects in LDL endocytosis are associated with "familial hypercholesterolemia"…

- Severe atherosclerosis at early age (strokes and heart attacks in pre-teens)
- Excess LDL in circulating blood
- LDL not properly internalized by cells
- Recessive/single gene… encoding plasma membrane receptor for LDL (LDL-receptor or LDL-R)
- The study of hypercholesterolemia and connection to heart disease led to the discovery of receptor-mediated endocytosis and a Nobel Prize in Physiology and Medicine for Brown and Goldstein (1985).

Normal and mutant LDL receptor

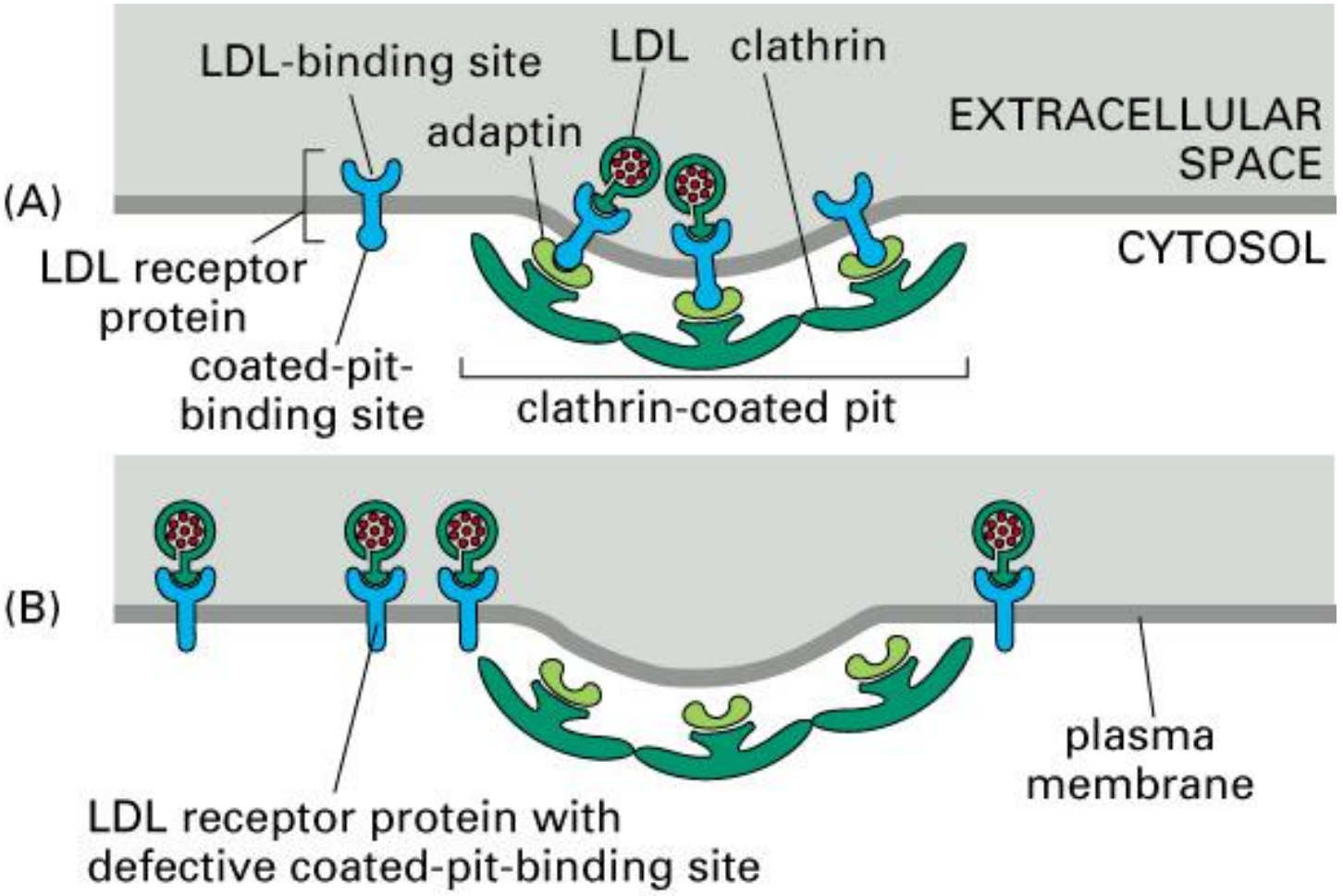
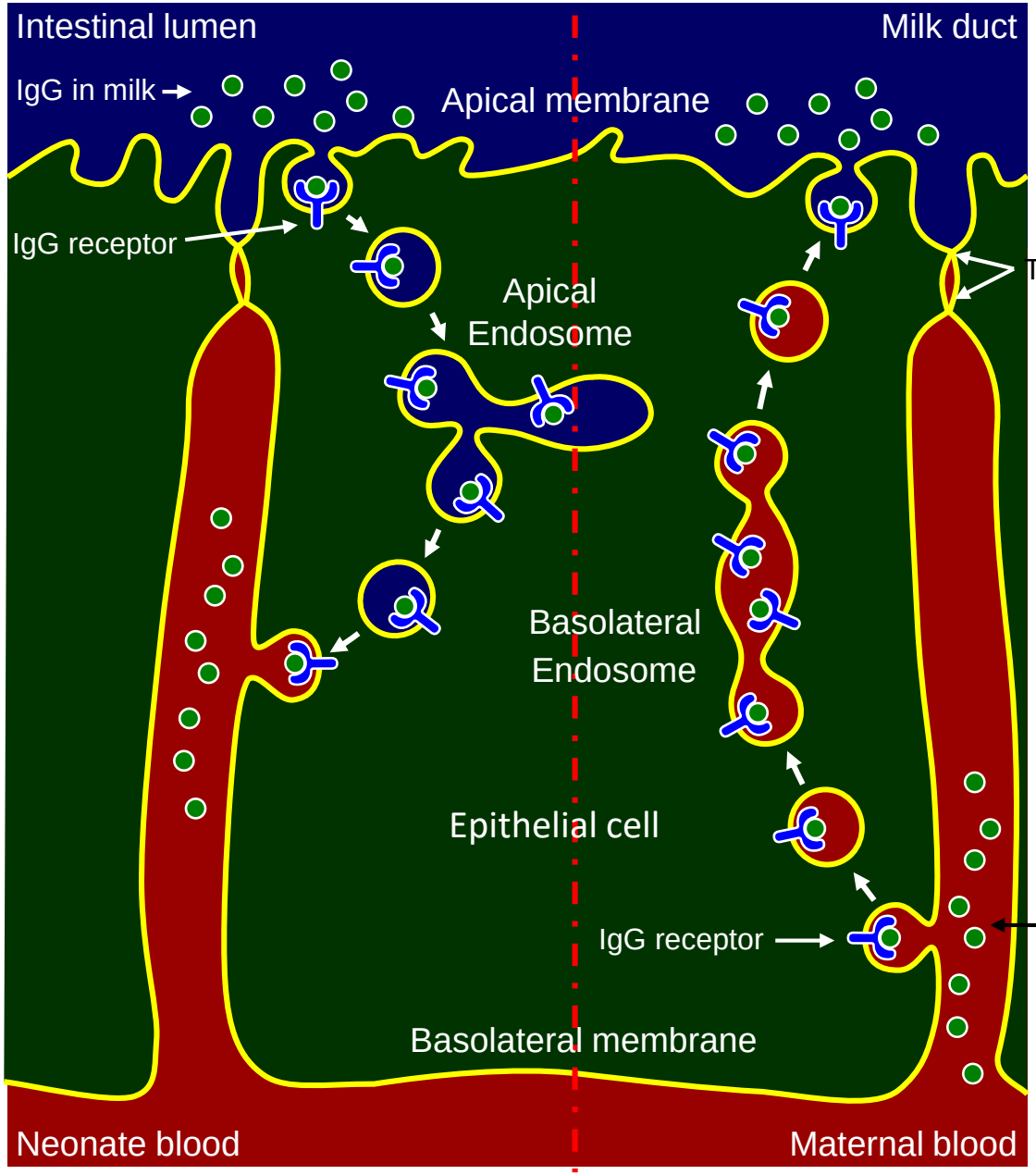


Figure 13–44. Molecular Biology of the Cell, 4th Edition.

“Transcytosis” moves maternal IgG across epithelia



IgG is “secreted” across the mammary epithelium into milk by transcytosis

Tight junctions

Receptor-mediated endocytosis from basolateral domain…

Secretion from apical membrane domain…

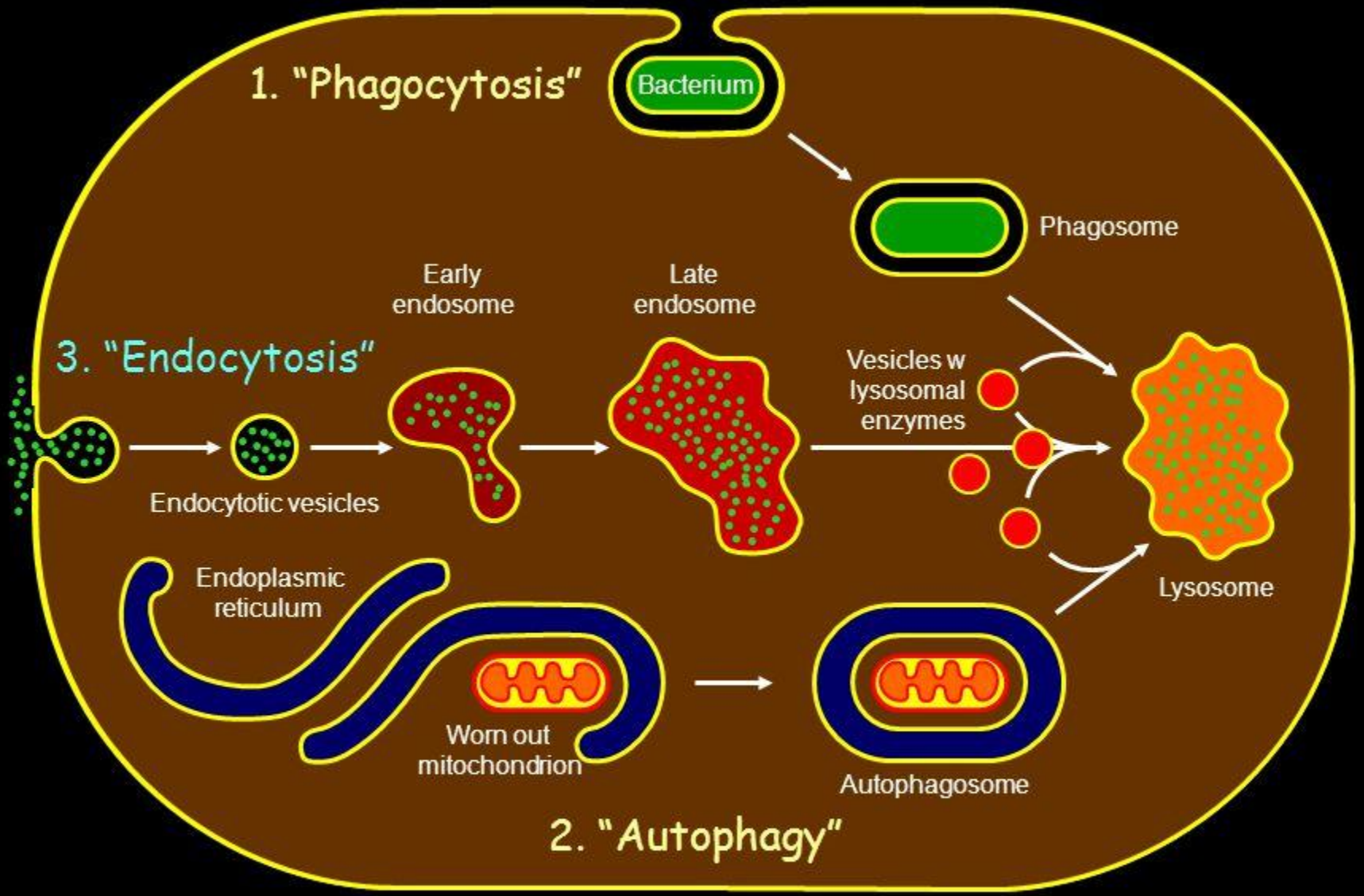
IgG is transcytosed into the neonate blood

Endocytosis from apical domain and secretion to basolateral membrane

IgG in blood

Polarized epithelial cells have distinct apical and basolateral endosome compartments

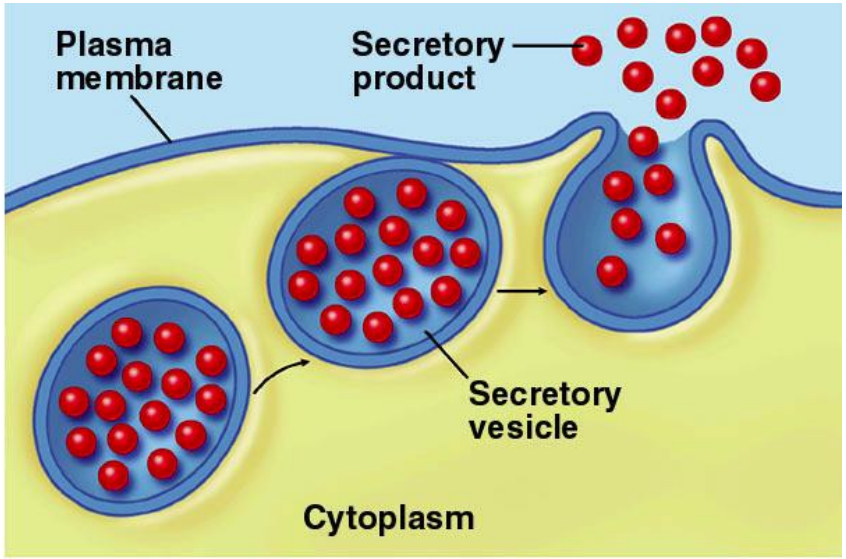
Endocytosis: Pinocytosis ("cell drinking") and "receptor-mediated" endocytosis



Note that vesicles from TGN targeted to lysosome by M6P actually fuse with precursor vesicles/organelles to form lysosome

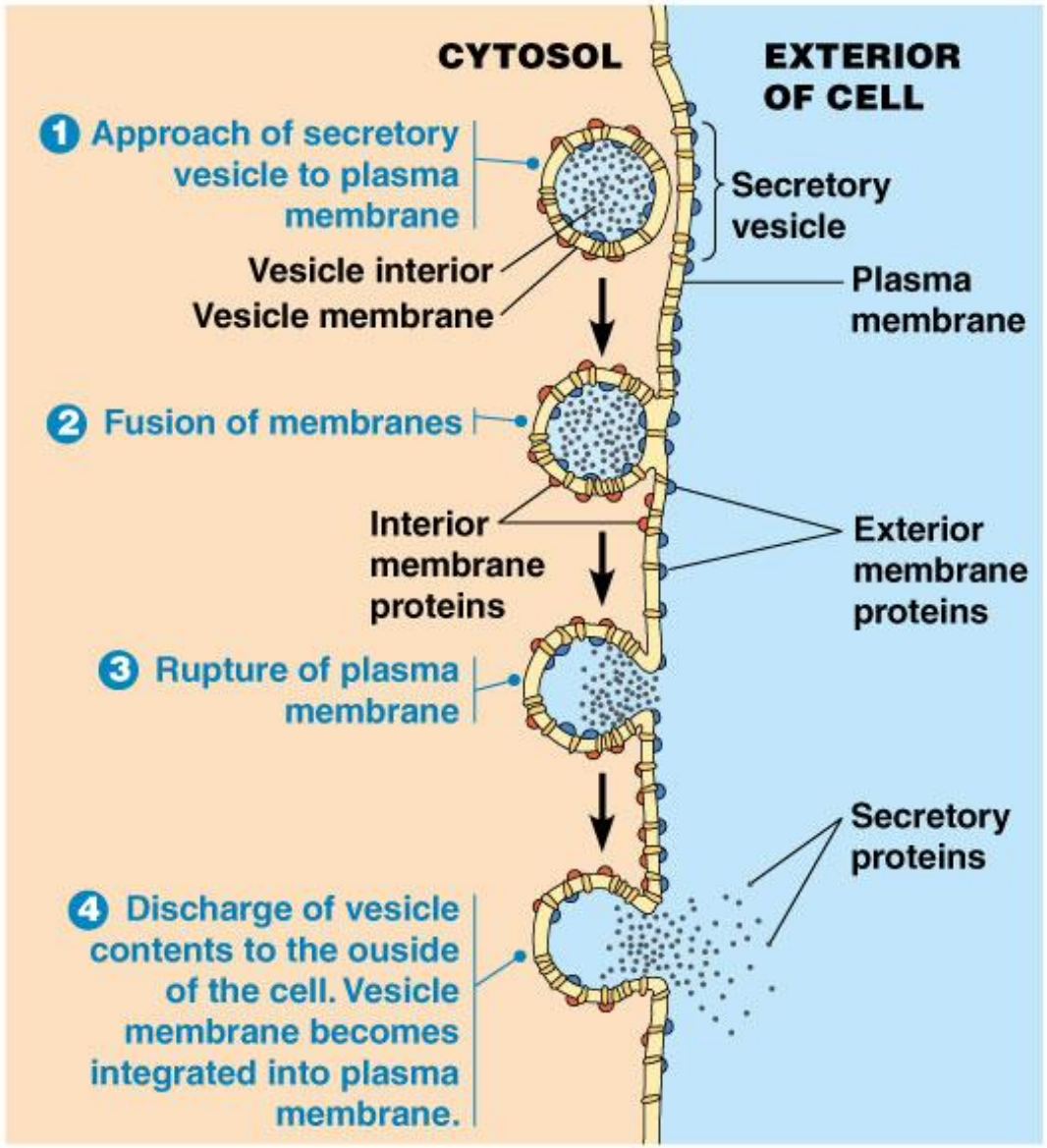
Exocytosis, the process by which secretory vesicles release their contents outside the cell

In exocytosis, proteins in a vesicle are released to the exterior of the cell as the vesicle fuses with the plasma membrane

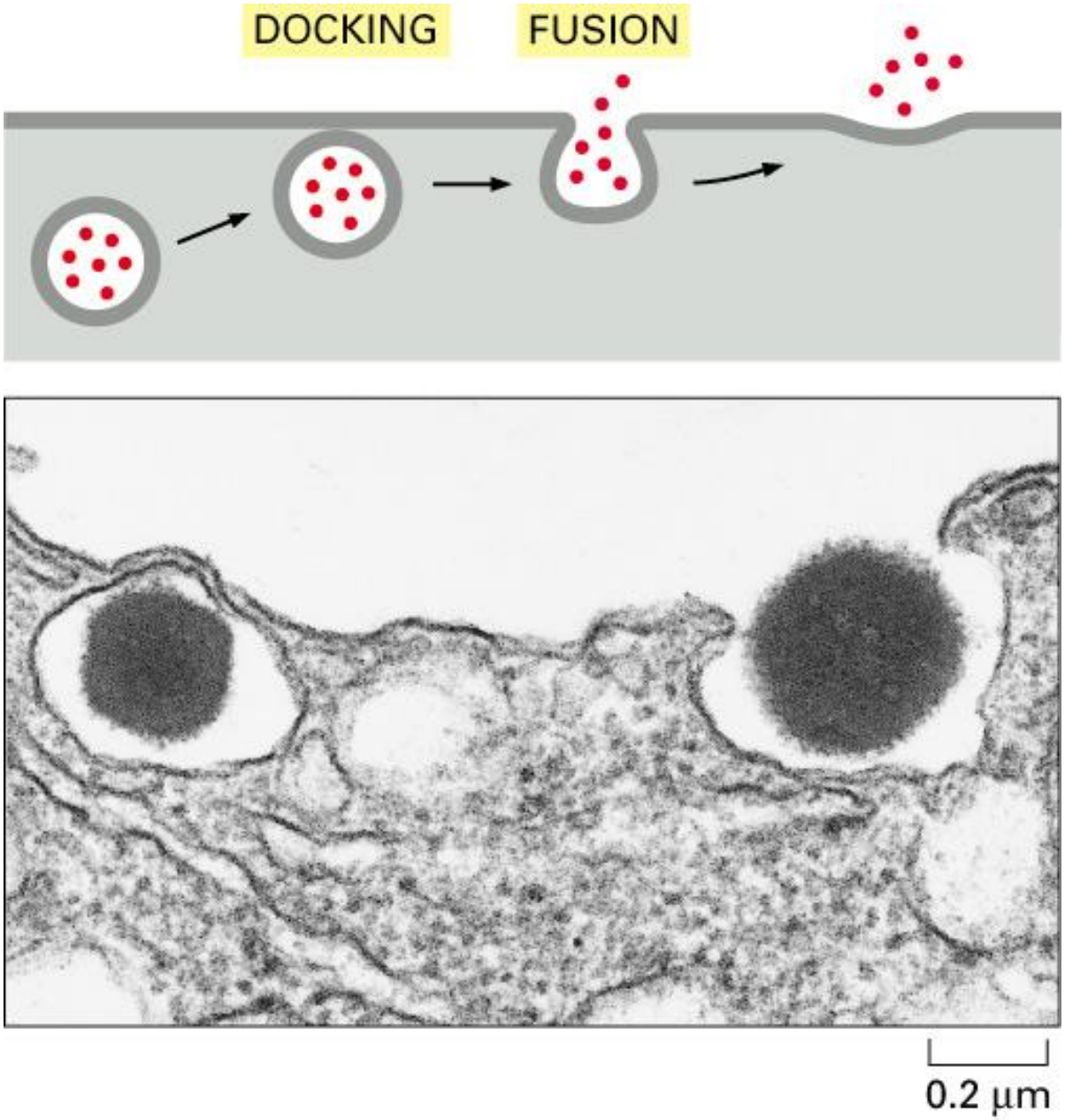


The process of exocytosis

- Vesicles containing products for secretion move to the cell surface (1)
- The membrane of the vesicle fuses with the plasma membrane (2)
- Fusion with the plasma membrane discharges the contents of the vesicle (3)
- The membrane of the vesicle becomes part of the cell membrane (4)



(a)


DOCKING
FUSION
0.2 µm