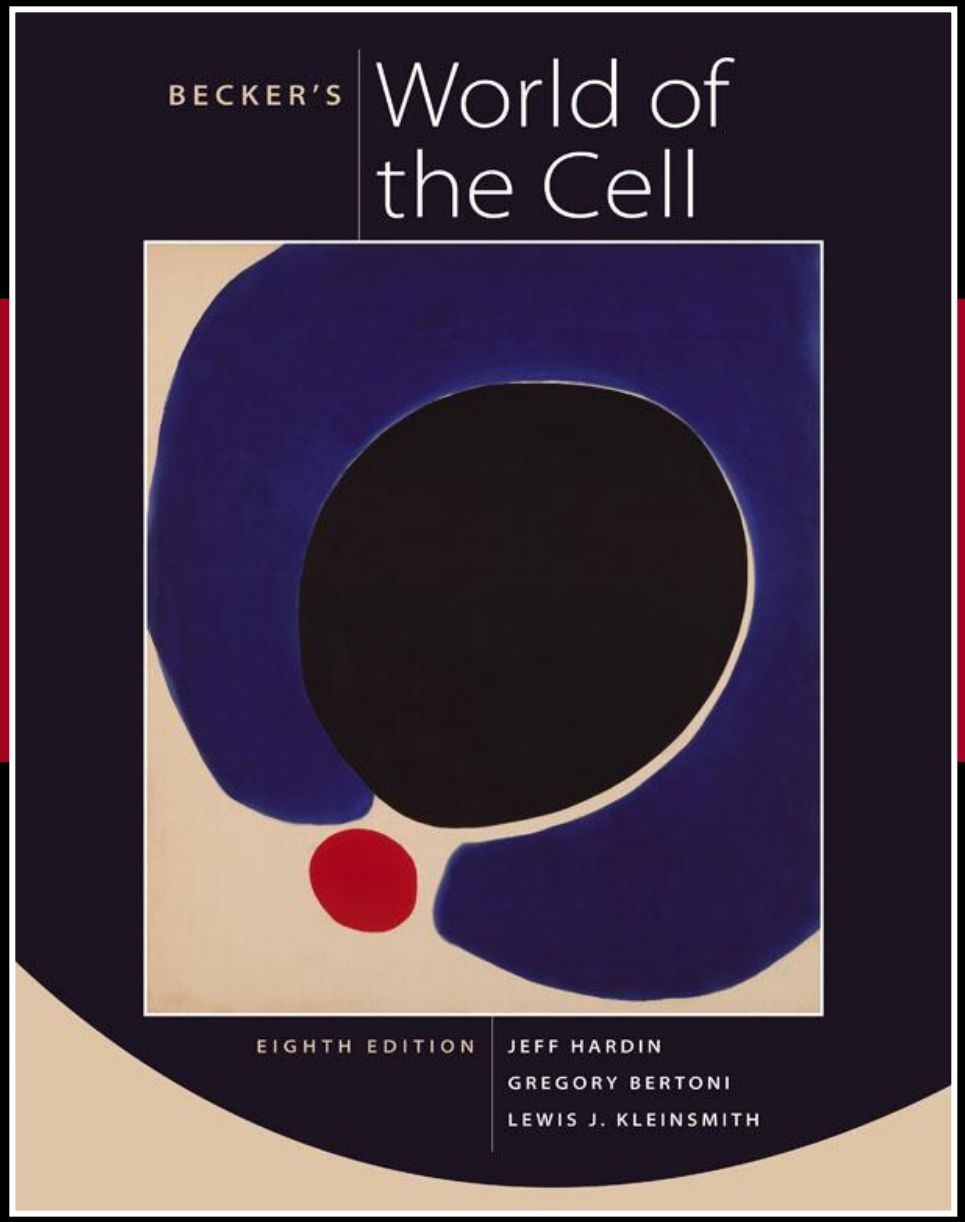


Chapter 1

A Preview of the Cell

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

The Cell Theory: A Brief History

- **Robert Hooke** (1665) observed compartments in cork, under a microscope, and first named **cells** (the basic unit of biology)
- His observations were limited by the low *magnification power* (30X enlargement) of his microscope
- **Antonie van Leeuwenhoek**, a few years later, produced better lenses that magnified up to 300X

Early progress in cell biology was slow

- Two factors restricted progress in early cell biology
  - Microscopes had limited *resolution* (ability to see fine detail)
  - The descriptive nature of cell biology; the focus was on observation, with little emphasis on explanation

Microscopes: essential tools in early cell biology

- By the 1830s, *compound microscopes* were used (two lenses)
  - Increased magnification and better resolution
  - Structures only 1 micrometer in size could be seen
- Using a compound microscope, **Robert Brown** identified the *nucleus,* a structure inside plant cells
- **Matthias Schleiden** concluded that all plant tissues are composed of cells, and **Thomas Schwann** made the same conclusion for animals

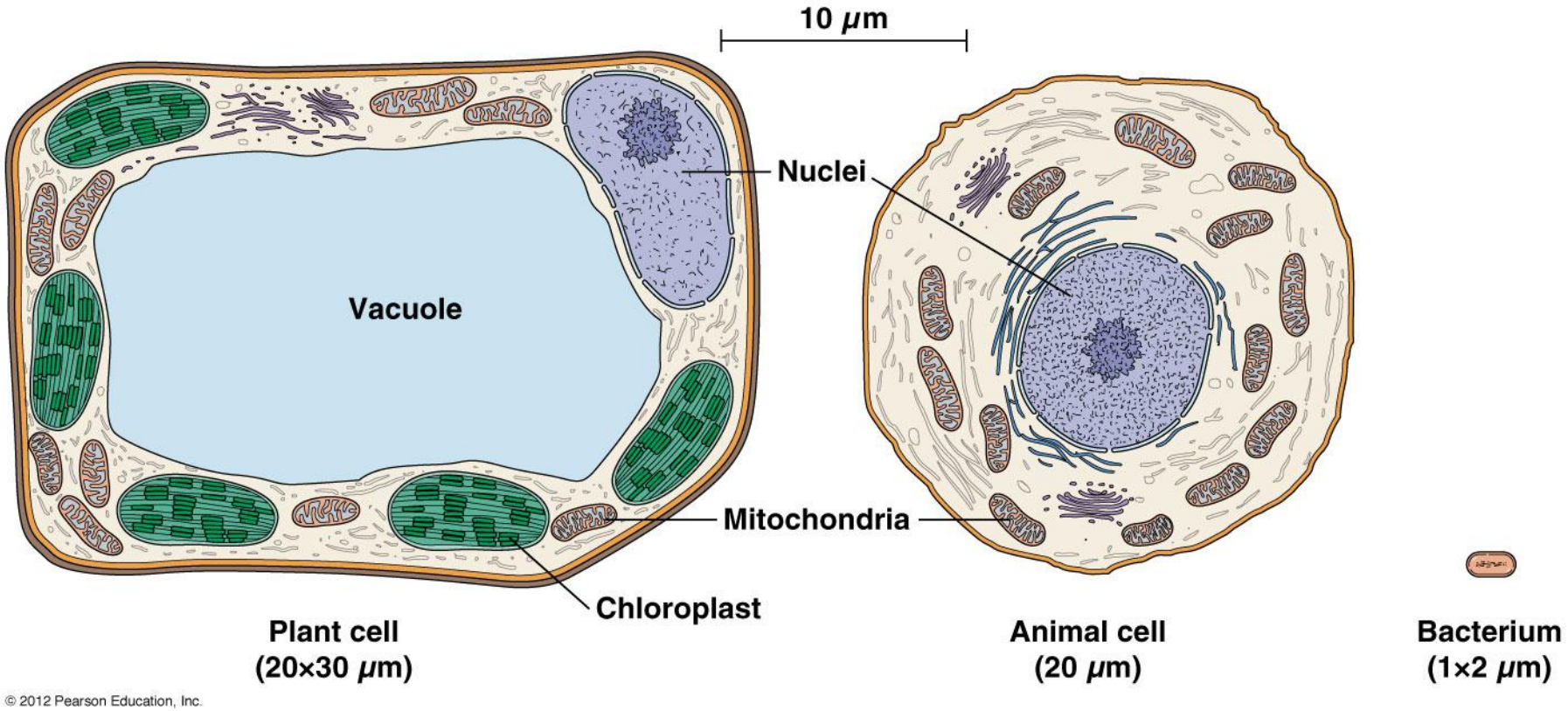
The cell theory

- In 1839, Schwann postulated the **cell theory**
  - 1. All organisms consist of one or more cells
  - 2. The cell is the basic unit of structure for all organisms
- Later, **Virchow** (1855) added
  - 3. All cells arise only from preexisting cells (*Meaning: the cell is the basic unit of reproduction*)

Units of Measurements in Cell Biology

- Most cells are too small → cannot be seen by the naked eye
- Cell size can be expressed by the unit of ***micrometer*** or ***micron*** **(μm)**
- $1\ \mu m = 1 \times 10^{-6}\ m = 1 \times 10^{-3}\ mm$

Figure 1-3A

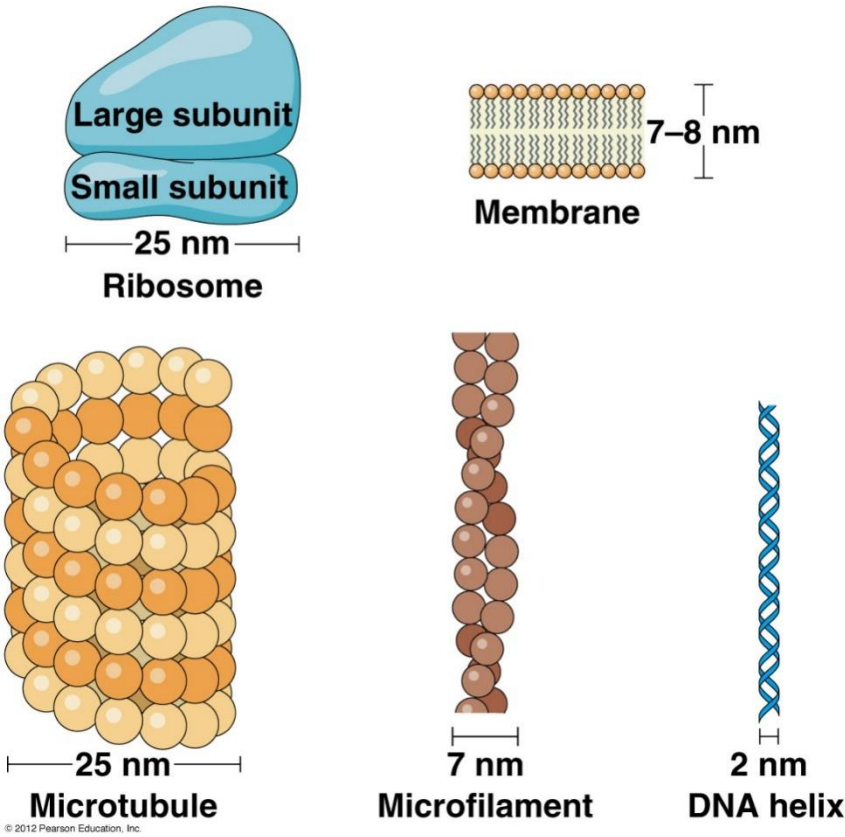


Organelles, e.g. chloroplasts and mitochondria are few μm; comparable size to a bacterium.

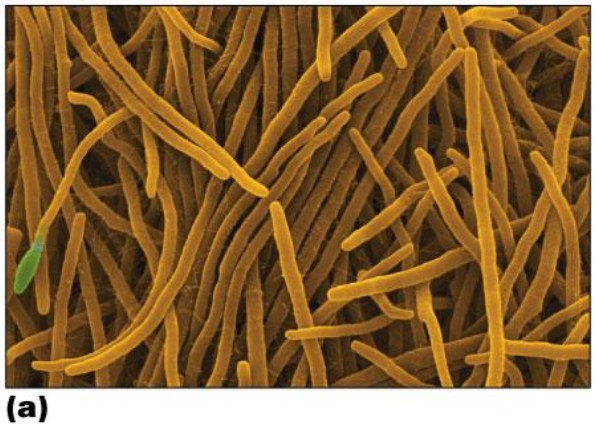
If you see it in a light microscope, its dimensions can be expressed in μm

Subcellular Structure Sizes

- Subcellular structures (structures within the cell) and molecules can be expressed by the unit of ***<u>nanometer</u> (nm)***
- 1 nm = 1 x 10^{-9} m = 1 x 10^{-6} mm = 1 x 10^{-3} μm



Diversity of the Cellular World

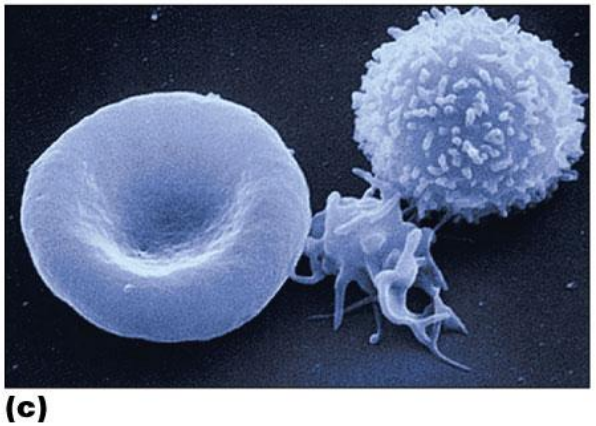


(a)

Filamentous fungal cells

(b)

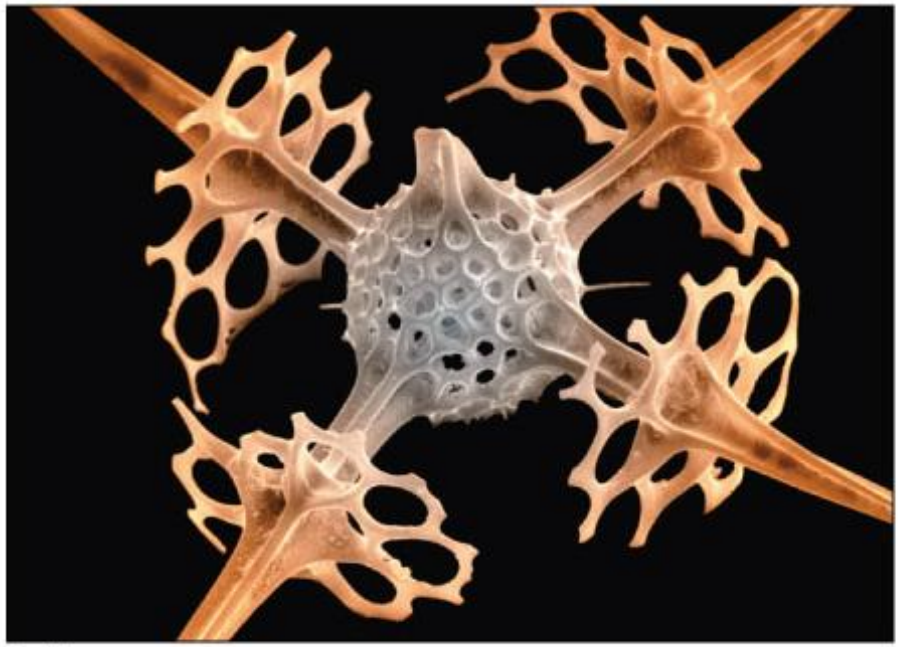
Treponema bacteria



(c)

Human RBC, platelet, WBC

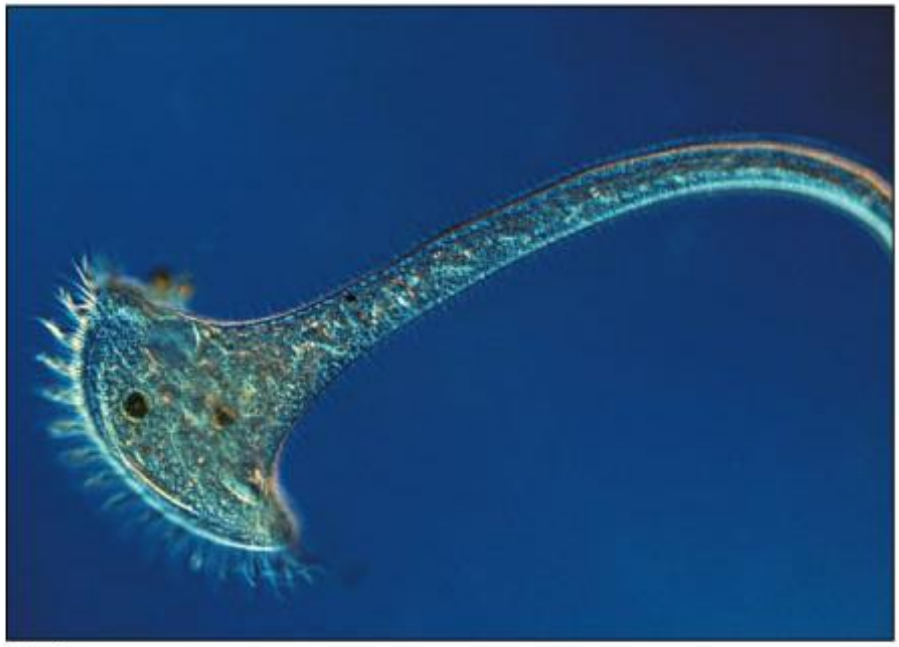
Diversity of the Cellular World



(d)

Radiolarian

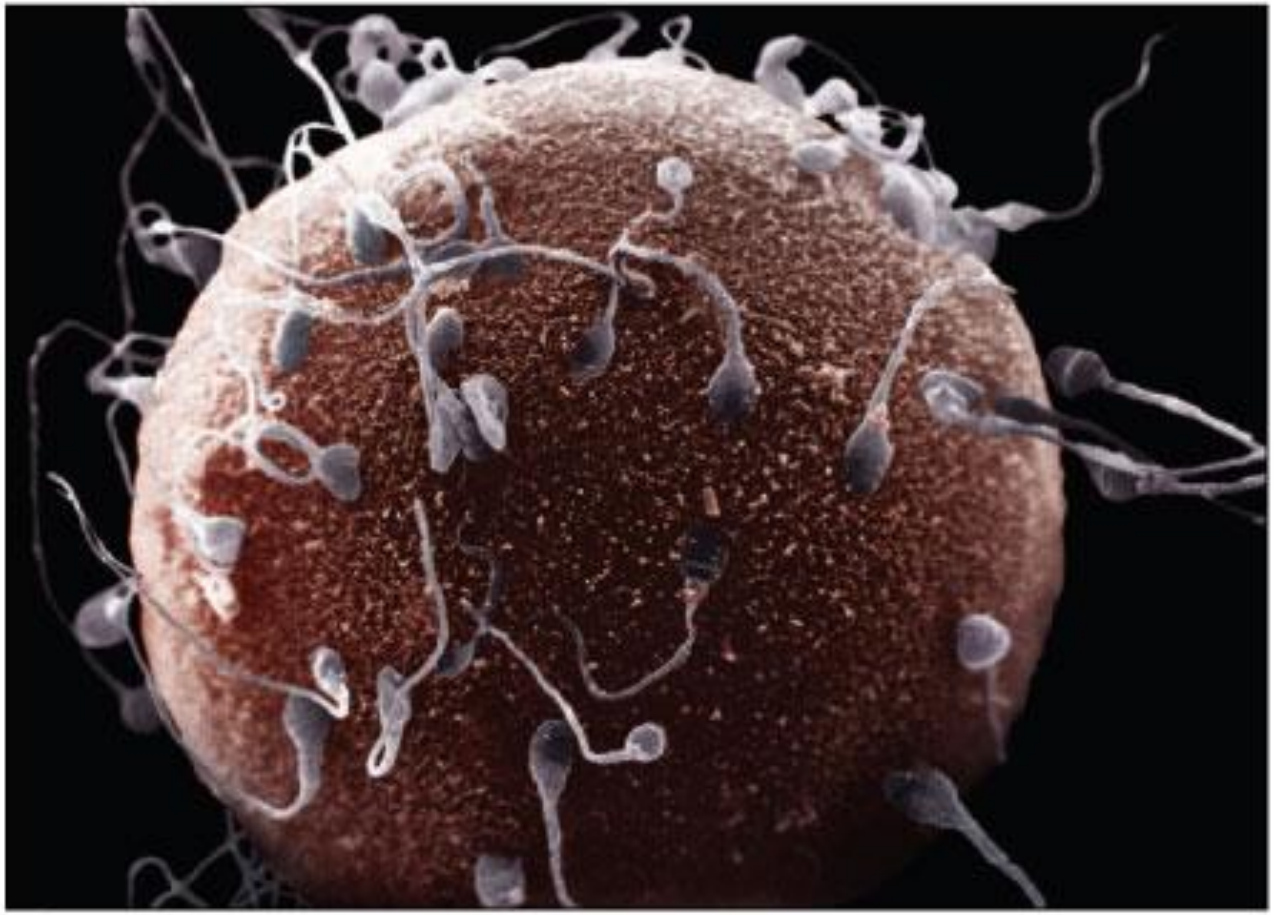
شعاعيات



(e)

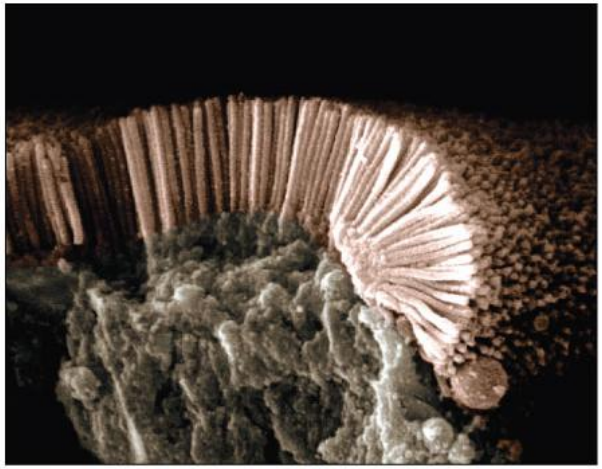
Protozoan

Diversity of the Cellular World



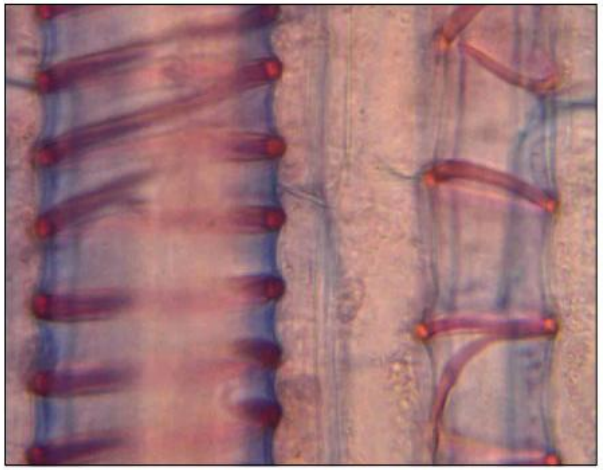
(f) Human egg and sperms

Diversity of the Cellular World



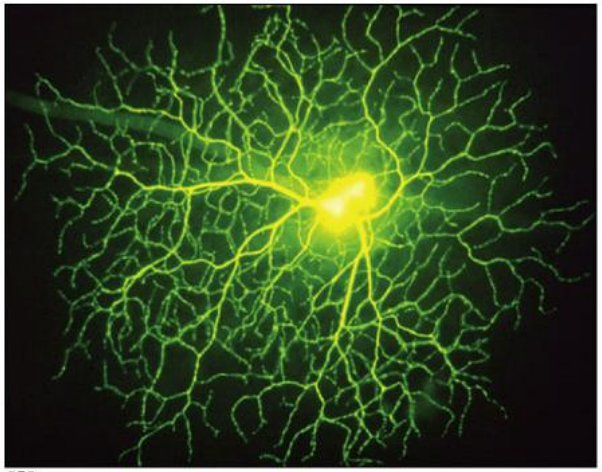
(g)

Intestinal cells



(h)

Xylem cells



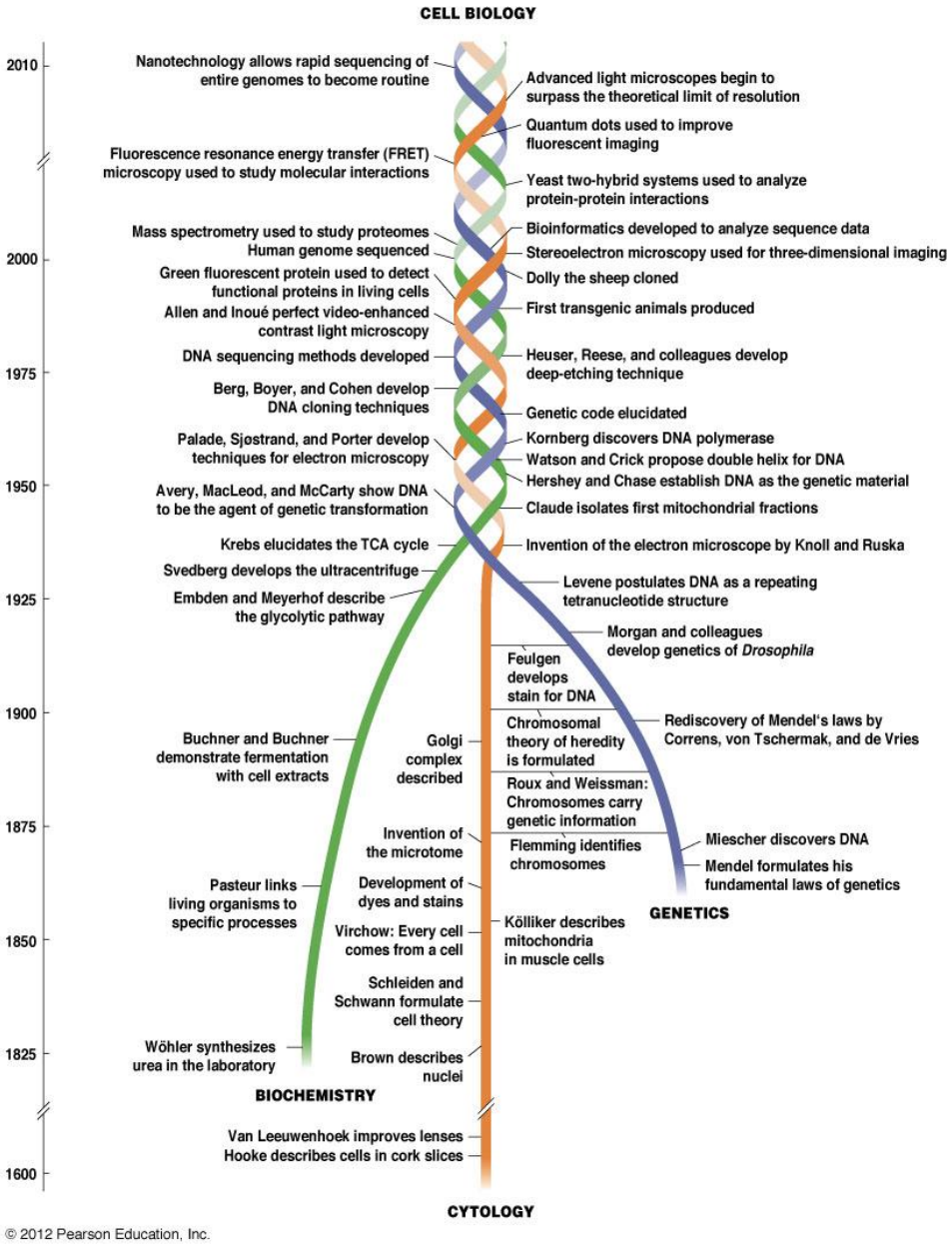
(i)

Retinal neuron

The Emergence of Modern Cell Biology

- Three historical strands weave together into modern cell biology, each with important contributions to understanding cells
- The **cytology** strand focuses mainly on cellular structure, and emphasizes optical techniques
- The **biochemistry** strand focuses on cellular function
- The **genetics** strand focuses on information flow and heredity

Figure 1-2



The Cytological Strand Deals with Cellular Structure

- Historically, cytology deals primarily with cell structure and observation using optical techniques

The Light Microscope

- The **light microscope** was the earliest tool of cytologists
- Allowed identification of *organelles* within cells
- Organelles are membrane-bound structures, such as *nuclei*, *mitochondria,* and *chloroplasts*

Useful tools in early microscopy

- A variety of dyes for staining cells began to be used
- These improved the **limit of resolution** (how far apart objects must be to appear as distinct)
- The smaller the limit of resolution a microscope has, the greater its **resolving power**

Visualization of Cells

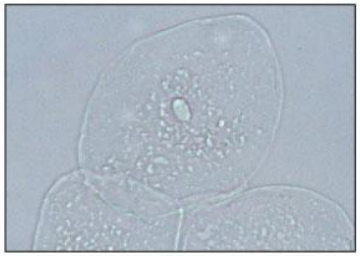
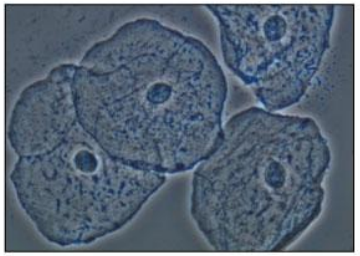
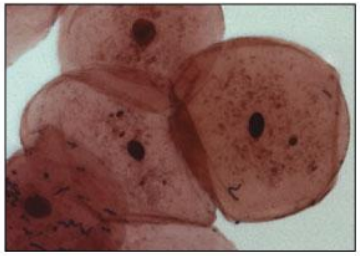
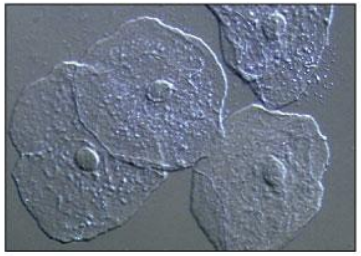
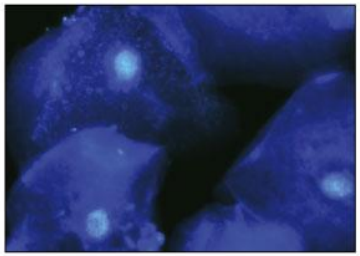
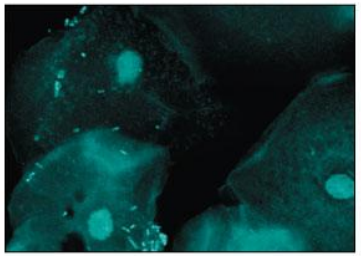
- The light microscopy so far described is called *brightfield* microscopy, as white light is passed through a specimen
- Some preparations (fixing, staining, embedding in plastic) may distort tissues
- Various types of microscopy have been developed to allow observation of living cells

Visualizing living cells

- *Phase contrast/differential interference contrast microscopy* exploit differences in the phase of light passing through a structure with a refractive index different than the surrounding medium
- *Fluorescence microscopy* detects fluorescent dyes, or labels, to show locations of substances in the cell
- *Confocal scanning* uses a laser beam to illuminate a single plane of a fluorescently labeled specimen

Table 1-1

Table 1-1 Different Types of Light Microscopy: A Comparison

Type of Microscopy	Light Micrographs of Human Cheek Epithelial Cells		Type of Microscopy
Brightfield (unstained specimen): Passes light directly through specimen; unless cell is naturally pigmented or artificially stained, image has little contrast.			**Phase contrast:** Enhances contrast in unstained cells by amplifying variations in refractive index within specimen; especially useful for examining living, unpigmented cells.
Brightfield (stained specimen): Staining with various dyes enhances contrast, but most staining procedures require that cells be fixed (preserved).			**Differential interference contrast:** Also uses optical modifications to exaggerate differences in refractive index.
Fluorescence: Shows the locations of specific molecules in the cell. Fluorescent substances absorb ultraviolet radiation and emit visible light. The fluorescing molecules may occur naturally in the specimen but more often are made by tagging the molecules of interest with fluorescent dyes or antibodies.			**Confocal:** Uses lasers and special optics to focus illuminating beam on a single plane within the specimen. Only those regions within a narrow depth of focus are imaged. Regions above and below the selected plane of view appear black rather than blurry.

20 μm

Source: Adapted from Campbell and Reece, *Biology,* 6th ed. (San Francisco: Benjamin Cummings, 2002), p. 110.

The Electron Microscope

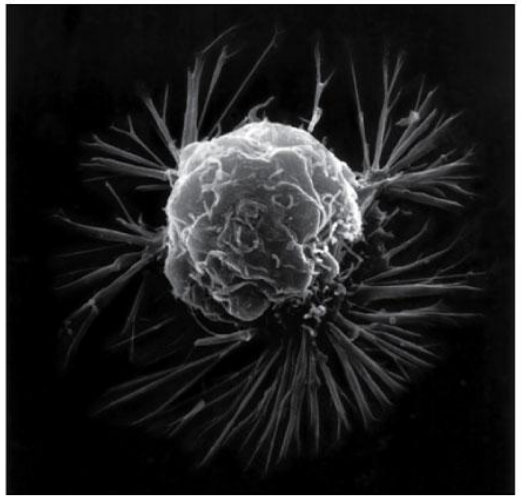
- The **electron microscope**, using a beam of electrons rather than light, was a major breakthrough for cell biology
- The limit of resolution of electron microscopes is around 0.1-0.2 nm
- The magnification is much higher than light microscopes – up to 100,000X

Electron microscopy

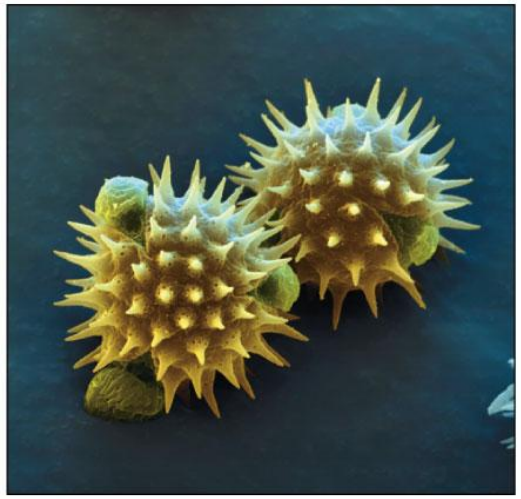
- In **transmission electron microscopy (TEM)**, electrons are transmitted through the specimen
- In **scanning electron microscopy (SEM)**, the surface of a specimen is scanned, by detecting electrons deflected from the outer surface
- Specialized approaches in electron microscopy allow for visualization of specimens in three dimensions, and one allows visualization of individual atoms

Figure 1-5

SEM

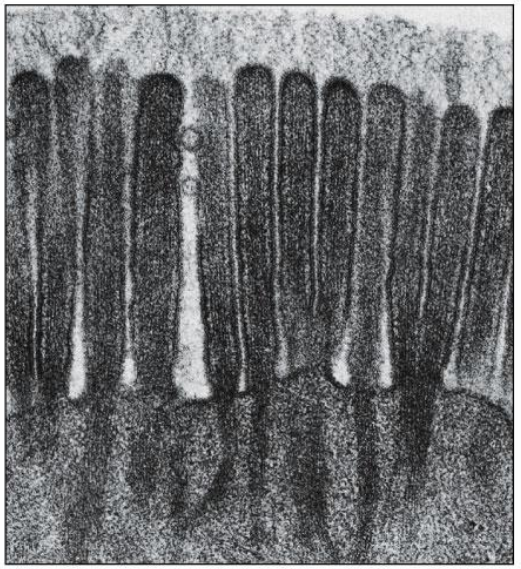


(a) Human cancer cell

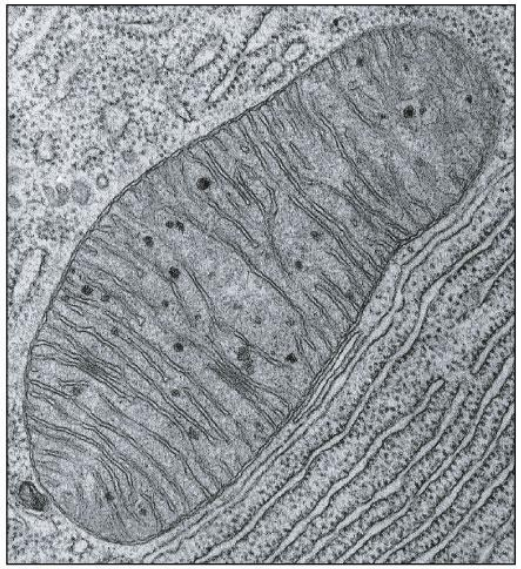


(b) Pollen grains

TEM



(c) Intestinal cell



(d) Mitochondrion

The Biochemical Strand Covers the Chemistry of Biological Structure and Function

- Around the same time cytologists were studying cells microscopically, others began to explore cellular function

- Much of biochemistry dates from the work of **Fredrich Wöhler** (1828), who showed that a compound made in a living organism could be synthesized in the lab

Developments in early biochemistry

- **Louis Pasteur** (1860s) showed that yeasts could ferment sugar into alcohol
- The **Buchners** (1897) showed that yeast extracts could do the same
- Led to the discovery of **enzymes,** biological catalysts

Important advances in biochemistry

- **Subcellular fractionation -** such as *centrifugation* to separate/isolate different structures and macromolecules
- *Ultracentrifuges* - capable of very high speeds (over 100,000 revolutions per minute)

Important advances in biochemistry (continued):

- **Chromatography** - techniques to separate molecules from a solution based on size, charge, or chemical affinity

- **Electrophoresis** - uses an electrical field to move proteins, DNA or RNA molecules through a medium based on size/charge

- **Mass spectrometry** - to determine the size and composition of individual proteins

The Genetic Strand Focuses on Information Flow

- The genetic strand has important roots in the nineteenth century

- **Gregor Mendel**'s experiments with peas (1866) laid the foundation for understanding the passage of "hereditary factors" from parents to offspring

- The hereditary factors are now known to be **genes**

Chromosomes and the genetic material

- **Walther Flemming** (1880) saw threadlike bodies in the nucleus called **chromosomes**
- He called the process of cell division *mitosis*

DNA is the genetic material

- Experiments with bacteria and viruses in the 1940s began to implicate DNA as the genetic material

*****HOMEWORK***** **"AT LEAST 500 WORDS"**

- 1953 - **Watson and Crick**, with assistance from **Rosalind Franklin**, proposed the *double helix model* for DNA structure

- 1960s - many advances toward understanding DNA replication, RNA production, and the genetic code

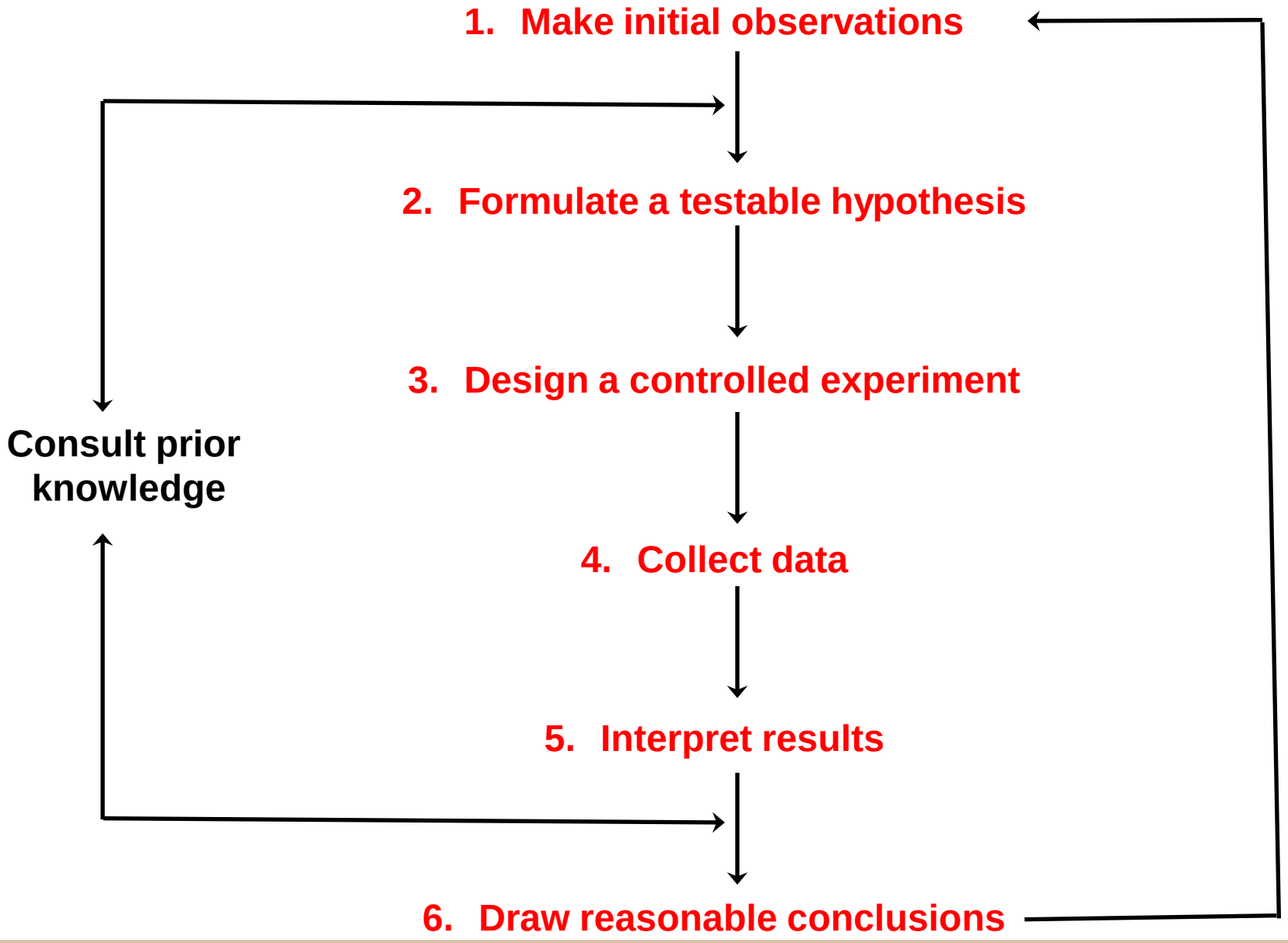
“Facts” and the Scientific Method

- In science, “facts” are tenuous and dynamic
- The *scientific method* is used to assess new information
  - Scientists formulate a *hypothesis* (tentative explanation or model that can be tested)
  - Data are collected and interpreted and the model is accepted or rejected
  - *Occam’s razor* states that the simplest explanation consistent with the observations is most likely to be correct

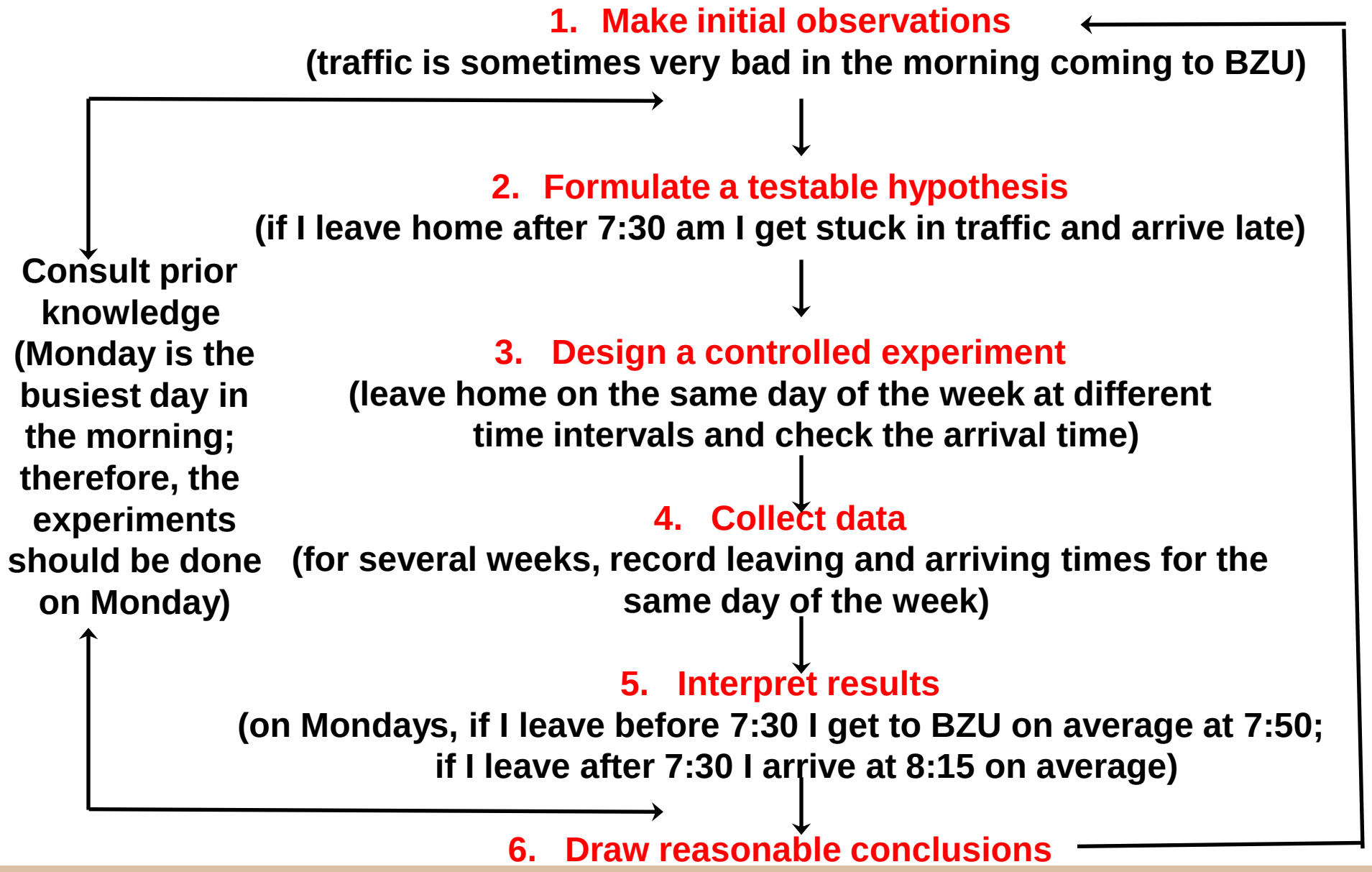
How we explain observations

- **Hypothesis** - statement consistent with most of the data, may take the form of a *model* (an explanation that appears to account for the data); must be testable
- **Theory** - a hypothesis that has been extensively tested by many investigators, using different approaches, widely accepted
- **Law** - a theory that has been tested and confirmed over a long period of time with virtually no doubt of its validity

The Scientific Method

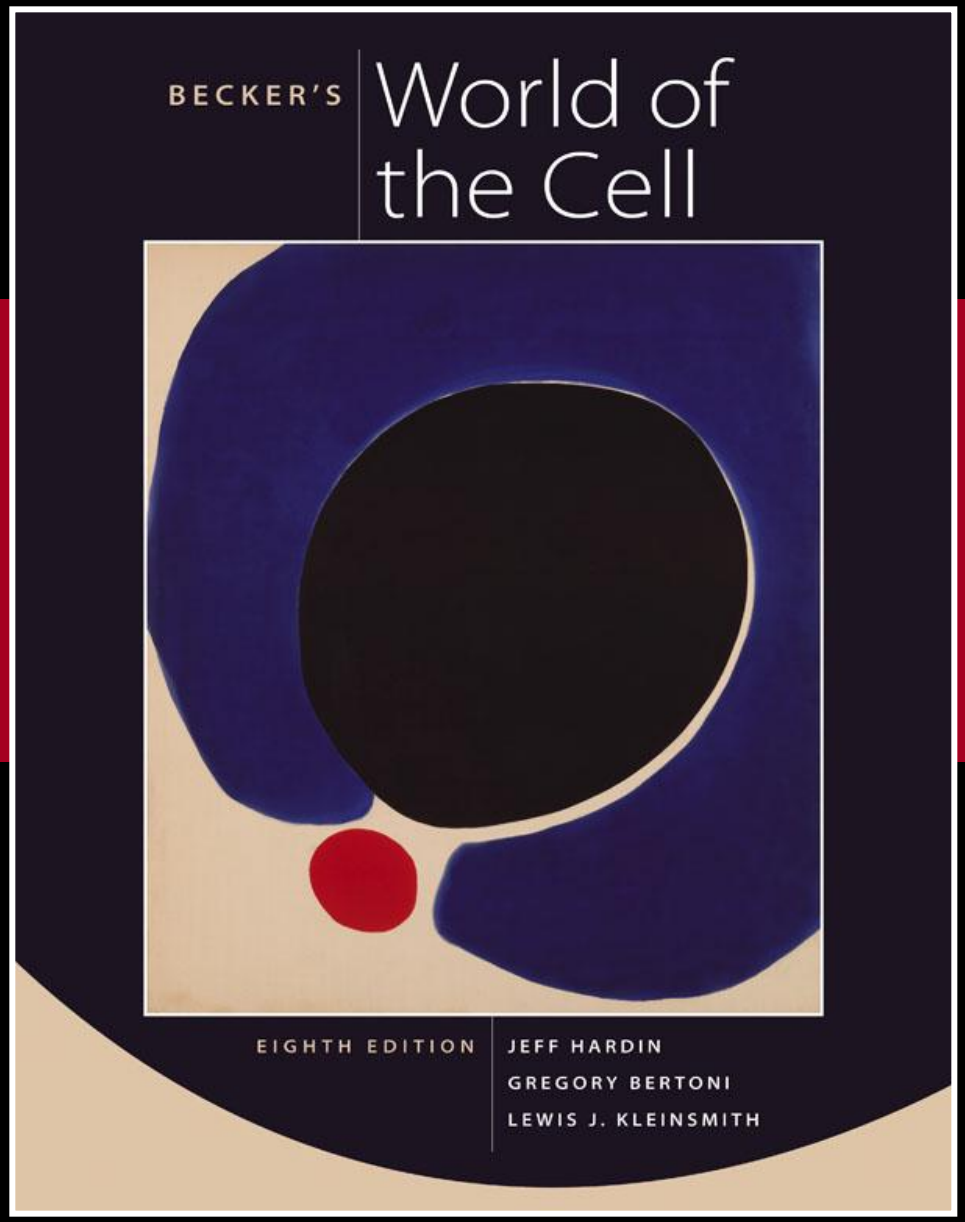


The Scientific Method: Example



Research approaches in cell biology

- Research in laboratories may be
  - *In vitro*, using purified chemicals and cellular components
  - *In vivo*, using live cells or organisms
  - *In silico*, using computer analysis of large amounts of data



Chapter 4

Cells and Organelles

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

All Organisms Are Bacteria, Archaea, or Eukaryotes

- Two types of cells
- The simpler type is characteristic of bacteria (**prokaryotes**) and the more complex type characteristic of plants, animals, fungi, algae and protozoa **(eukaryotes)**
- The main distinction between the two cells types is the membrane-bounded nucleus of eukaryotic cells

A changing view of prokaryotes

- Recently, the term *prokaryote* is unsatisfactory in describing the non-nucleated cells
- Sharing of a gross structural feature is not necessarily evidence of relatedness
- Based on rRNA sequence analysis, prokaryotic cells can be divided into the widely divergent **bacteria** and **archaea**

Three domains

- Bacteria and archaea are as divergent from one another as humans and bacteria are

- Biologists now recognize three domains, the archaea, bacteria and **eukarya** (*eukaryotes)*

Bacteria

- These include most of the commonly encountered single-celled, non-nucleated organisms traditionally called bacteria

- Examples include:
  - *Escherichia coli*
  - *Pseudomonas*
  - *Streptococcus*

Archaea

- Archaea were originally called *archebacteria* before they were discovered to be so different from bacteria

- They include many species that live in extreme habitats and have diverse metabolic strategies

- Types of archaea include:
  - *methanogens* - obtain energy from hydrogen and convert CO_2 into methane
  - *halophiles* - occupy extremely salty environments
  - *thermacidophiles* - thrive in acidic hot springs

Archaea (continued)

- They are considered to have descended from a common ancestor that also gave rise to eukaryotes long after diverging from bacteria

Limitations on Cell Size

- Cells come in various sizes and shapes
- Some of the smallest bacteria are about 0.2 - 0.3 μm in diameter (*mycoplasma*)
- Some highly elongated nerve cells may extend a meter or more
- Despite the extremes, cells in general fall into predictable size ranges

Size ranges

- Bacteria cells normally range from 1 to 5 μm in diameter
- Animal cells have dimensions in the range of 10 - 100 μm
- Cells are usually very small
- There are 3 main limitations on cell size

Limitations on cell size

- Cell size is limited by:
  - The need for **adequate surface area relative to volume**
  - The **rates at which molecules can diffuse**
  - The need to maintain **adequate local concentrations of substances** required for necessary cellular functions

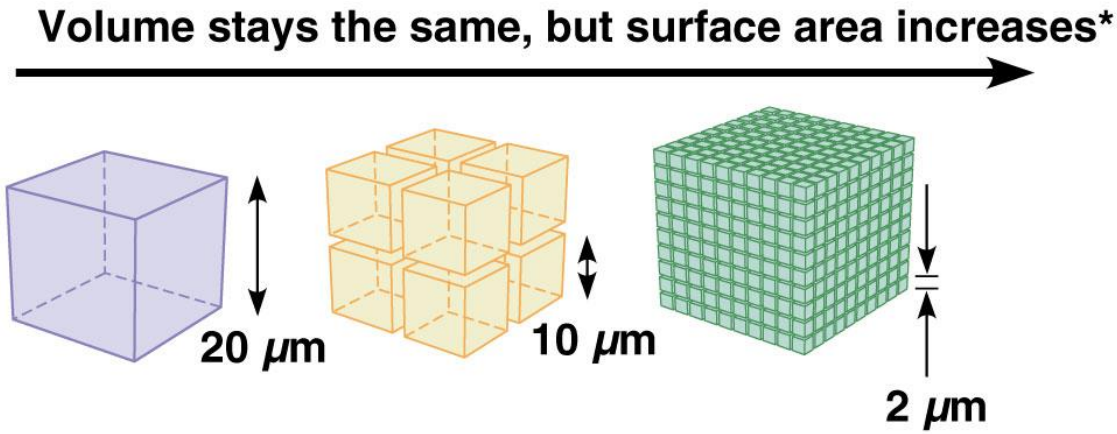
Surface area/volume ratio

- In most cases, the major limit on cell size is set by the need to maintain an adequate surface area/volume ratio
- Surface area is important because *exchanges between the cell and its surrounding* stake place there
- The cell's volume determines the amount of exchange that must take place, across the available surface area

The problem of maintaining adequate surface area/volume ratio

- The volume of a cell increases with the **cube** of its length
- But the surface area of the cell increases with the **square** of its length, *so larger cells have proportionately smaller surface areas*
- Beyond a certain threshold a large cell would not have a large enough surface area to allow for intake of enough nutrients and release of enough wastes

Figure 4-1



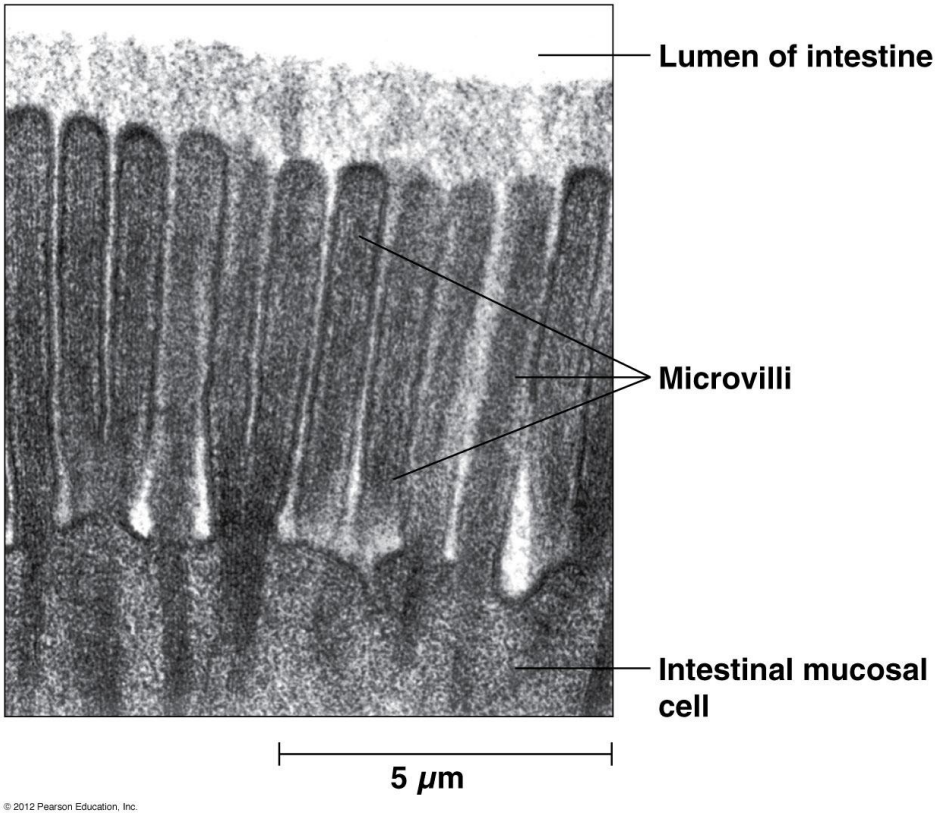
Number of cells	1	8	1000
Length of one side	20 μm	10 μm	2 μm
Total volume	8000 μm^3	8000 μm^3	8000 μm^3
Total surface area	2400 μm^2	4800 μm^2	24,000 μm^2
Surface area to volume ratio	0.3	0.6	3.0

*For a cube having a side with length s, volume = s^3 and surface area = $6s^2$.

Cells specialized for absorption

- Cells that are specialized for absorption have characteristics to maximize surface area/volume ratio

- E.g., cells lining the small intestine have *microvilli,* fingerlike projections that increase the surface area



Diffusion Rates of Molecules

- Many molecules move through the cell by **diffusion**, the unassisted movement of a substance from a region of high concentration to a region of low concentration

- *The rate of diffusion of molecules decreases as the size of the molecule increases*, so the limitation is most important for macromolecules like proteins and nucleic acids

Avoiding limitations of rates of diffusion

- Eukaryotic cells can avoid the problem of slow diffusion rates by using carrier proteins to actively transport materials through the cytoplasm

- Some cells use **cytoplasmic streaming** (**cyclosis** in plants) to actively move cytoplasmic contents

- Other cells move molecules through the cell in vesicles that are transported along microtubules

The Need for Adequate Concentrations of Reactants and Catalysts

- For a reaction to occur, the appropriate reactants must collide with and bind to a particular enzyme
- The frequency of such collisions is greatly increased by higher concentrations of enzymes and reactants
- ↑ cell vol ↓ concentration of molecules → need to produce more molecules to get the same amount of reactions

Eukaryotic Cells Use Organelles to Compartmentalize Cellular Function

- A solution to the concentration problem is the *compartmentalization of activities* within specific regions of the cell

- Most eukaryotic cells have a variety of **organelles**, membrane-bounded compartments that are specialized for specific functions

- e.g. cells in a plant leaf have most of the materials needed for photosynthesis compartmentalized into structures called *chloroplasts*

Bacteria, Archaea, and Eukaryotes Differ from Each Other in Many Ways

- There are shared characteristics among cells of each of the domains, bacteria, archaea and eukarya

- However, each type of cell has a unique set of distinguishing properties

Prokaryotes vs. Eukaryotes

- Membrane-bound nucleus
- Internal membranes to segregate function
- Tubules and filaments
- Exocytosis and endocytosis
- DNA organization
- Segregation of genetic information
- Expression of DNA

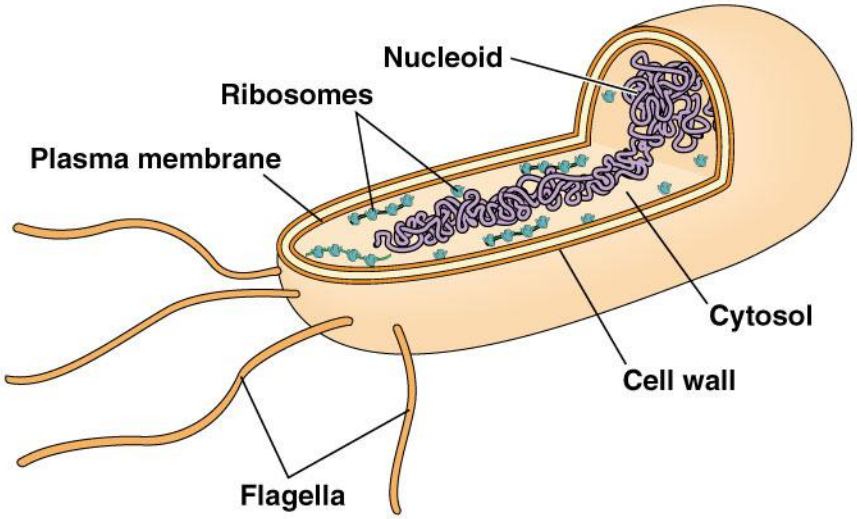
Table 4-1 A Comparison of Some Properties of Bacterial, Archaeal, and Eukaryotic Cells*

Property	Prokaryotes: Bacteria	Prokaryotes: Archaea	Eukaryotes	Refer to:
Typical size	Small (1–5 μm)	Small (1–5 μm)	Large (10–100 μm)	—
Nucleus and organelles	No	No	Yes	Table 4-2
Microtubules and microfilaments	Actin-like and tubulin-like proteins	Actin-like and tubulin-like proteins	Actin and tubulin proteins	Chapter 15
Exocytosis and endocytosis	No	No	Yes	Chapter 12
Cell wall	Peptidoglycan	Varies from proteinaceous to peptidoglycan-like	Cellulose in plants, fungi; none in animals, protozoa	Chapter 17
Mode of cell division	Binary fission	Binary fission	Mitosis or meiosis plus cytokinesis	Chapter 19
Typical form of chromosomal DNA	Circular, few associated proteins	Circular, associated with histone-like proteins	Linear, associated with histone proteins	Chapter 18
RNA processing	Minimal	Moderate	Extensive	Chapter 21
Transcription initiation	Bacterial type	Eukaryotic type	Eukaryotic type	Chapter 21
RNA polymerase	Bacterial type	Some features of both bacterial, eukaryotic types	Eukaryotic type	Chapter 21
Ribosome size and number of proteins	70S with 55 proteins	70S with 65 proteins	80S with 78 proteins	Chapter 22
Ribosomal RNAs	Bacterial type	Archaeal type	Eukaryotic type	Chapter 21
Translation initiation	Bacterial type	Eukaryotic type	Eukaryotic type	Chapter 22
Membrane phospholipids	Glycerol-3-phosphate + linear fatty acids	Glycerol-1-phosphate + branched polyisoprenoids	Glycerol-3-phosphate + linear fatty acids	Chapter 7

*This table lists many features that we have not yet discussed in detail. Its main purpose is to point out that, despite some sharing of characteristics, each of the three main cell types has a unique set of properties.

Presence of a Membrane-Bounded Nucleus

- A eukaryotic cell has a true, membrane bounded nucleus
- The *nuclear envelope* consists of two membranes
- The nucleus also includes the *nucleolus*, the site of ribosomal RNA synthesis and ribosome assembly
- The genetic information of a bacterial or archaeal cell is folded into a compact structure called the *nucleoid* and is attached to the cell membrane



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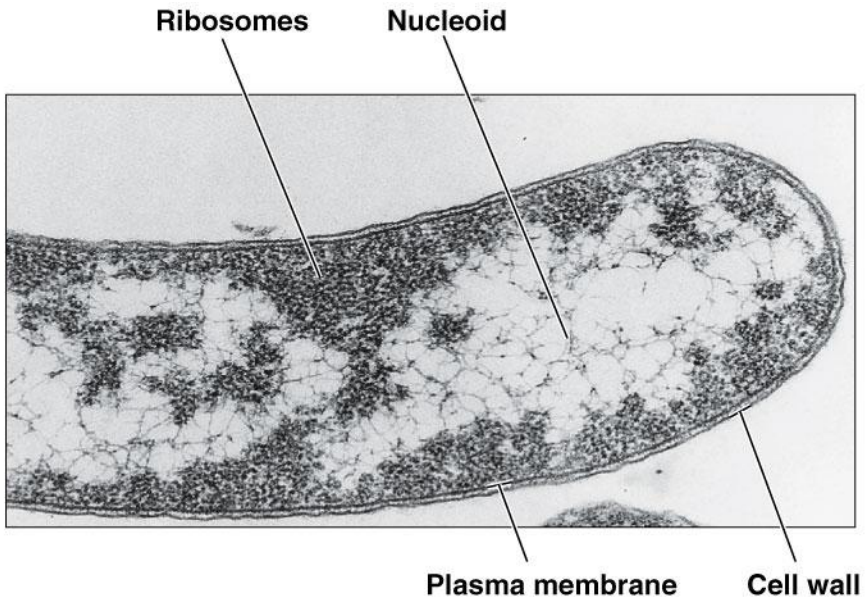

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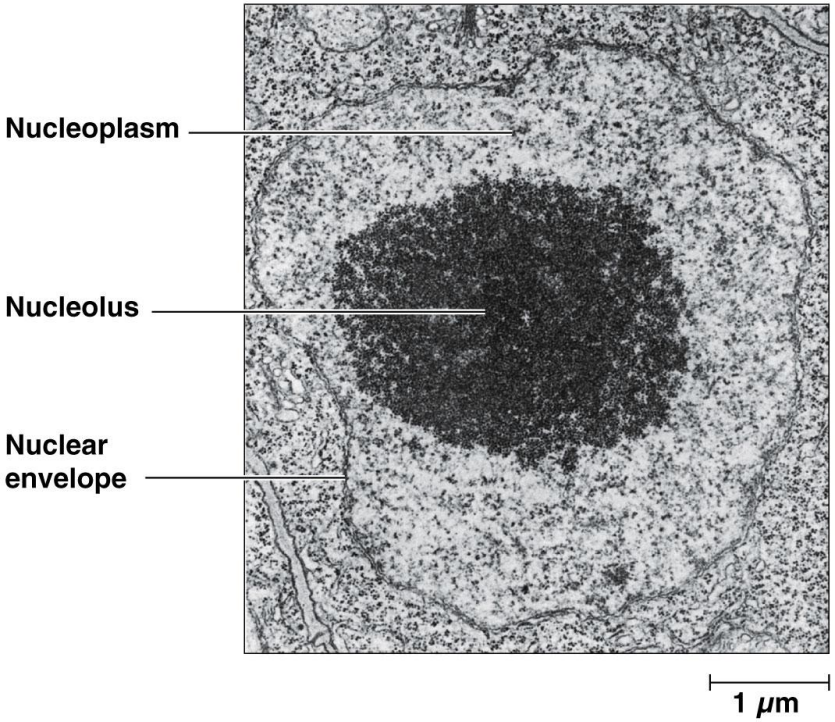


Eukaryote organelles

- Nearly all eukaryotes make extensive use of internal membranes to compartmentalize specific functions and have numerous organelles

- E.g., *endoplasmic reticulum, Golgi complex, mitochondria, chloroplasts, lysosomes, peroxisomes and various types of vacuoles and vesicles*

- Each organelle contains the materials and molecular machinery needed to carry out the functions for which the structure is specialized

Figure 4-5

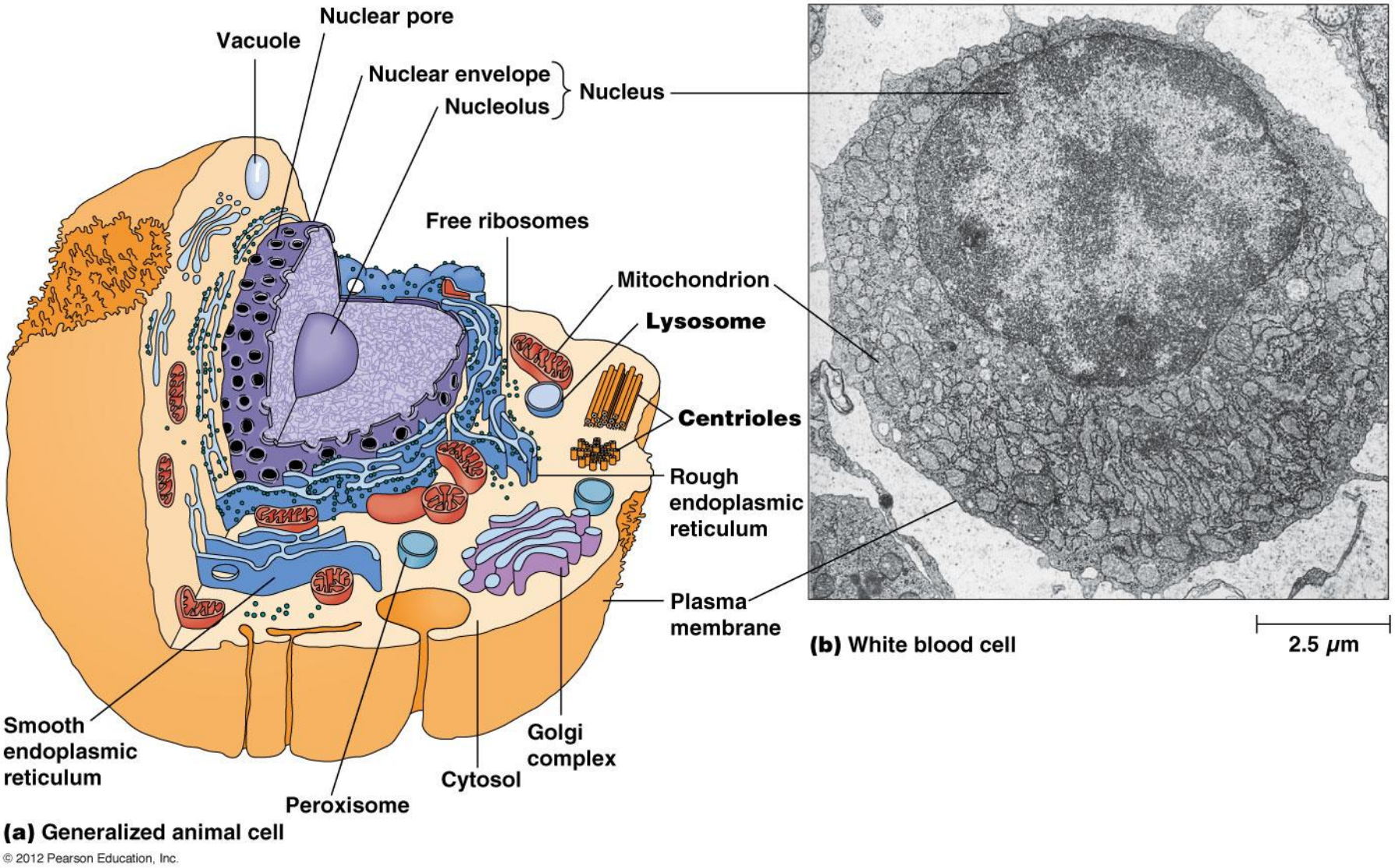
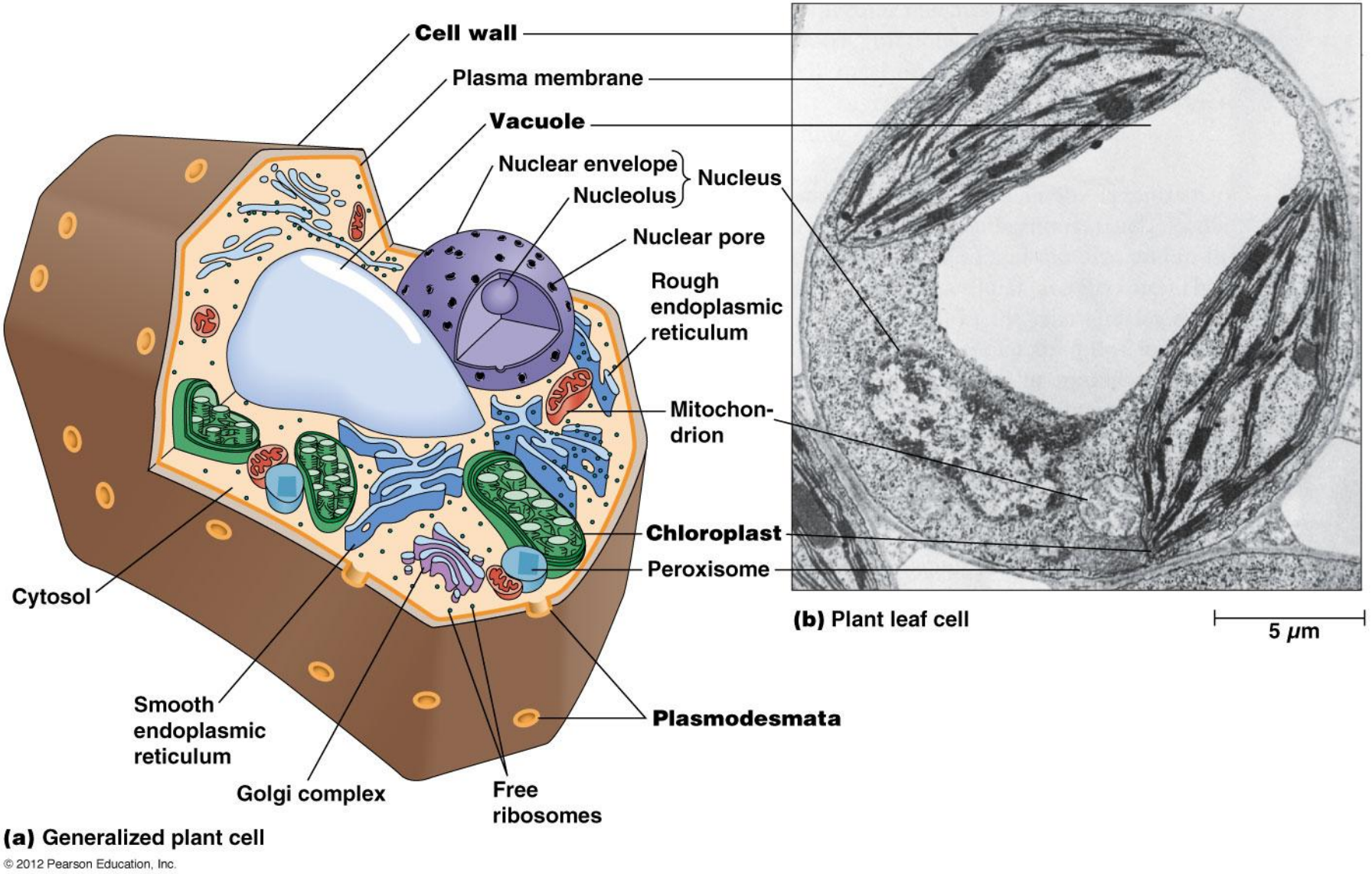


Figure 4-6



The Cytoskeleton

- Several nonmembranous, proteinaceous structures for cellular contraction, motility and support are found in the cytoplasm of eukaryotic cells

- These include: *microtubules*, *microfilaments,* and *intermediate filaments,* all key components of the *cytoskeleton*, which imparts structure and elasticity to most eukaryotic cells

- The cytoskeleton also provides scaffolding for transport of vesicles within the cell

Exocytosis and Endocytosis

- Eukaryotic cells are able to exchange materials between compartments within the cell and the exterior of the cell

- This is possible through *exocytosis* and *endocytosis*, processes involving membrane fusion events unique to eukaryotic cells

Organization of DNA

- Bacterial DNA is present in the cell as a circular molecule associated with few proteins
- Eukaryotic DNA is organized into linear molecules complexed with large amounts of proteins called *histones*
- Archaeal DNA is circular and complexes with proteins similar to eukaryotic histone proteins

DNA packaging

- The circular DNA of bacteria or archaea is much longer than the cell itself and so must be folded and packed tightly, equivalent to packing about 60 feet of thread into a thimble

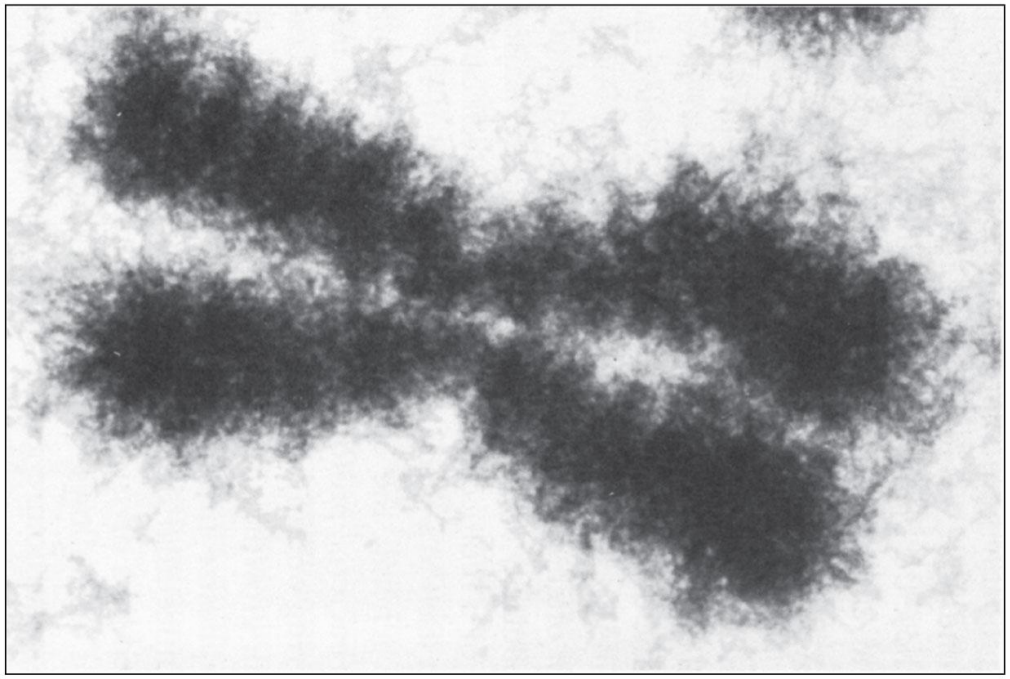
- Most eukaryotic cells have **more than 1000 times more DNA** than prokaryotes and encode only **5-10 times more proteins**

- The excess noncoding DNA has been referred to as junk DNA but may have important functions in gene regulation and evolution

Chromosomes

- The problem of DNA packaging is solved among eukaryotes by organizing the DNA into **chromosomes**

- Chromosomes contain equal amounts of histones and DNA



Segregation of Genetic Information

- Prokaryotes and eukaryotes differ in how genetic information is allocated to daughter cells upon division
- Bacterial and archaeal cells replicate their DNA and divide by *binary fission* with one molecule of the replicated DNA and the cytoplasm going into each daughter cell
- Eukaryotic cells replicate DNA and then distribute their chromosomes into daughter cells by *mitosis* and *meiosis*, followed by *cytokinesis*, division of the cytoplasm

Expression of DNA

- Eukaryotic cells transcribe genetic information in the nucleus into large RNA molecules which are processed and transported into the cytoplasm for protein synthesis
- Each RNA molecule typically encodes one polypeptide
- Ribosome size: large (80S)
- Bacteria transcribe genetic information into RNA, and the RNA molecules produced may contain information for several polypeptides
- In both bacteria and archaea, RNA molecules become involved in protein synthesis before transcription is complete
- Ribosome size: small (70S)

The Eukaryotic Cell in Overview: Pictures at an Exhibition

- The structural complexity of eukaryotic cells is illustrated by the typical animal and plant cells
- A typical eukaryotic cell has: a *plasma membrane*, a *nucleus*, *membrane bounded organelles* and the *cytosol* interlaced by a *cytoskeleton*
- In addition, plant and fungal cells have a rigid cell wall, surrounded by an *extracellular matrix*

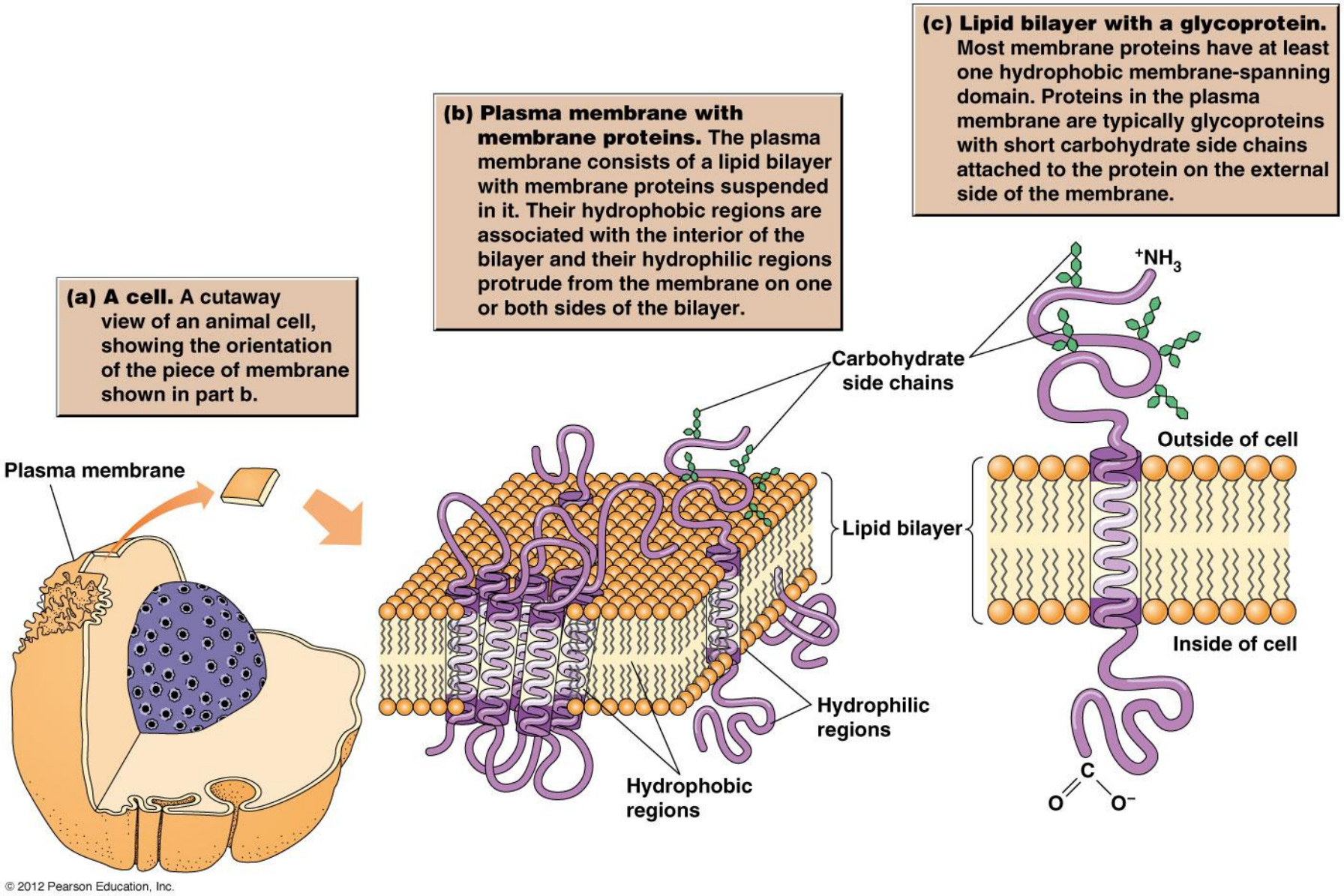
The Plasma Membrane Defines Cell Boundaries and Retains Contents

- The plasma membrane surrounds every cell
- It ensures that the cells contents are retained
- It consists of lipids, including phospholipids and proteins and is organized into two layers

Amphipathic membrane components

- Each phospholipid molecule consists of two hydrophobic “tails” and a hydrophilic “head” and is therefore an ***amphipathic molecule***
- The **lipid bilayer** is formed when the hydophilic heads face outward and the tails face inward
- Membrane proteins are also amphipathic, some, with polysaccharides attached to them, are called *glycoproteins*

Figure 4-10



The Nucleus is the Information Center of the Eukaryotic Cell

- The most prominent structure in the eukaryotic cell is the **nucleus**
- It contains the DNA and is surrounded by the **nuclear envelope**, composed of inner and outer membranes
- The nuclear envelope has numerous openings called *pores,* each of which is a transport channel, lined with a *nuclear pore complex* that regulates movement of macromolecules

The nucleus

- The number of chromosomes in the nucleus is a species-specific characteristic
- Chromosomes are most easily visualized during mitosis, whereas during *interphase*, they are dispersed as **chromatin** and difficult to visualize
- **Nucleoli** are also present in the nucleus

Intracellular Membranes and Organelles Define Compartments

- The internal volume of the cell outside the nucleus is called the *cytoplasm* and is occupied by *organelles* and the semifluid *cytosol*

- A number of heritable human disorders are caused by malfunctions of specific organelles

The Mitochondrion

- **Mitochondria**, found in all eukaryotic cells, are the site of aerobic respiration
- They are comparable in size to bacteria
- Most eukaryotic cells contain hundreds of mitochondria, each of which is surrounded by a *mitochondrial inner* and *outer membrane*

Mitochondrial similarity to bacterial cells

- Mitochondria contain small circular molecules of DNA
- The mitochondrial chromosome encodes some RNAs and proteins needed for mitochondrial function
- They also have their own ribosomes, to carry out protein synthesis

Figure 4-11

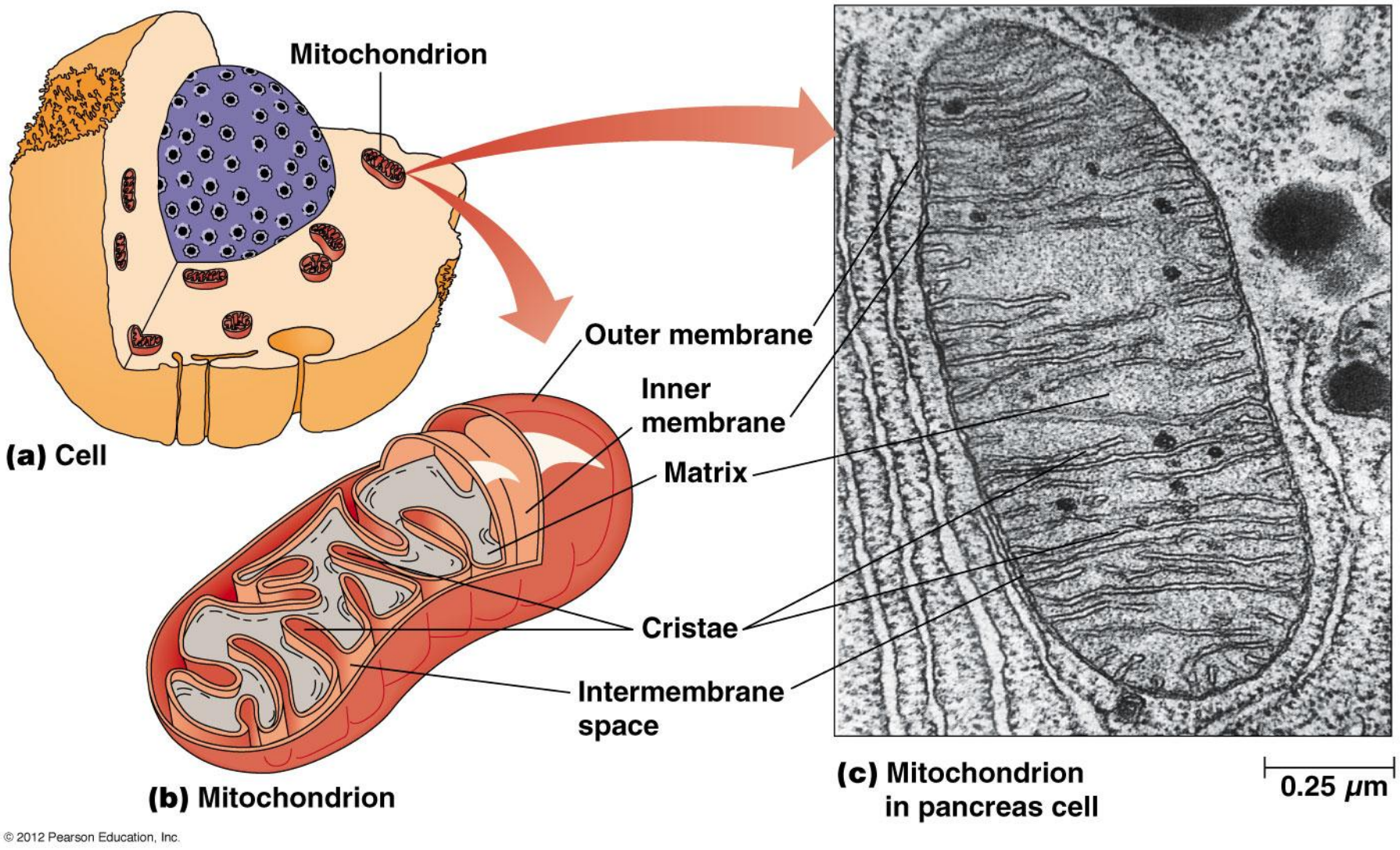
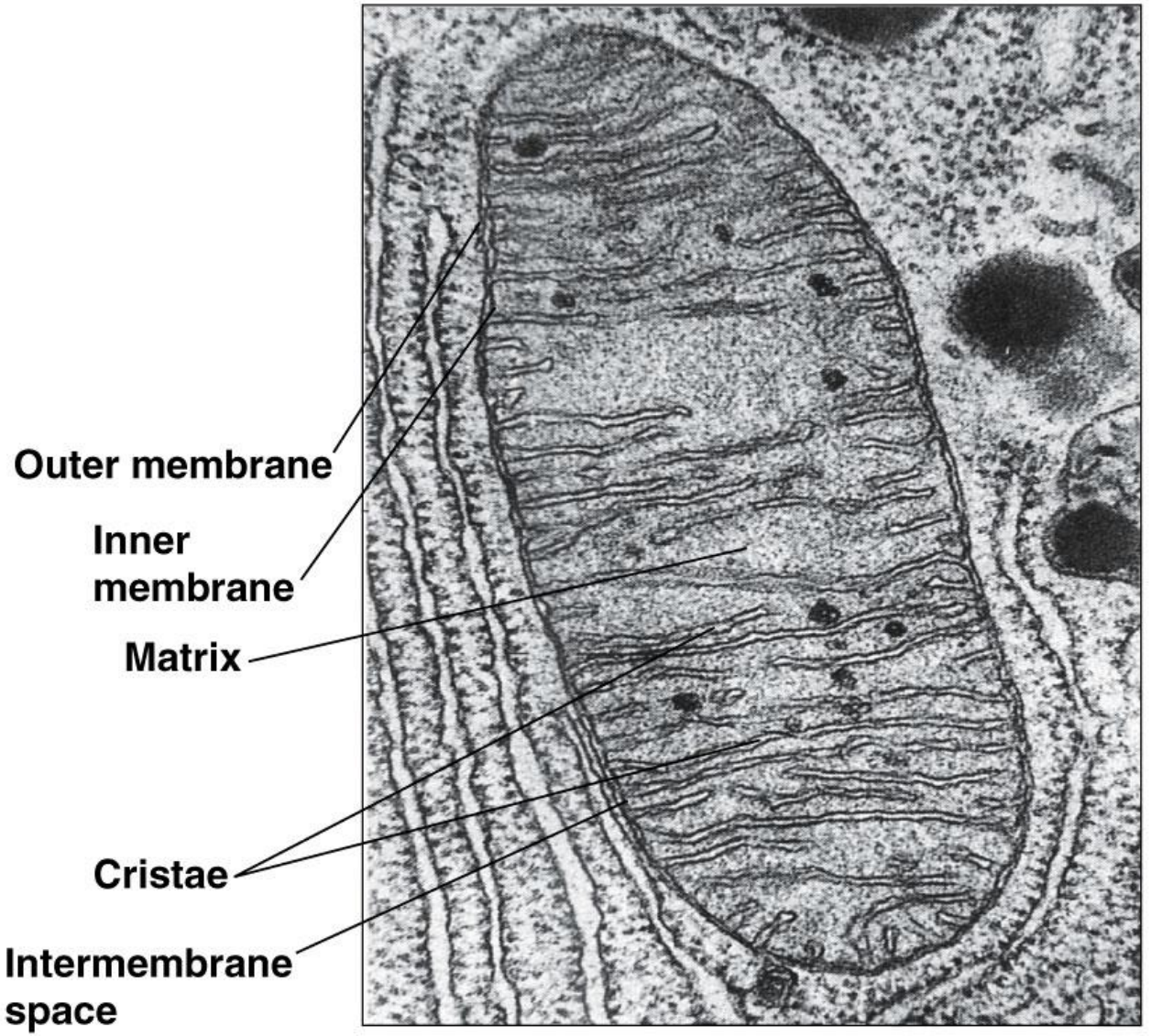


Figure 4-11C



Mitochondrial function

- Oxidation of sugars and other fuel molecules in mitochondria extracts energy from food and stores it in *ATP* (*adenosine triphosphate*)

- Most molecules for mitochondrial function are localized on the **cristae** (infoldings of the inner mitochondrial membrane) or the **matrix** (fluid that fills the inside of the mitochondrion)

Varied number and location of mitochondria

- Number and location of mitochondria varies among cells according to their role in that cell type
- Tissues with high demand for ATP have many mitochondria, located within the cell at the site of greatest energy needs
- E.g., sperm and muscle cells

Figure 4-13

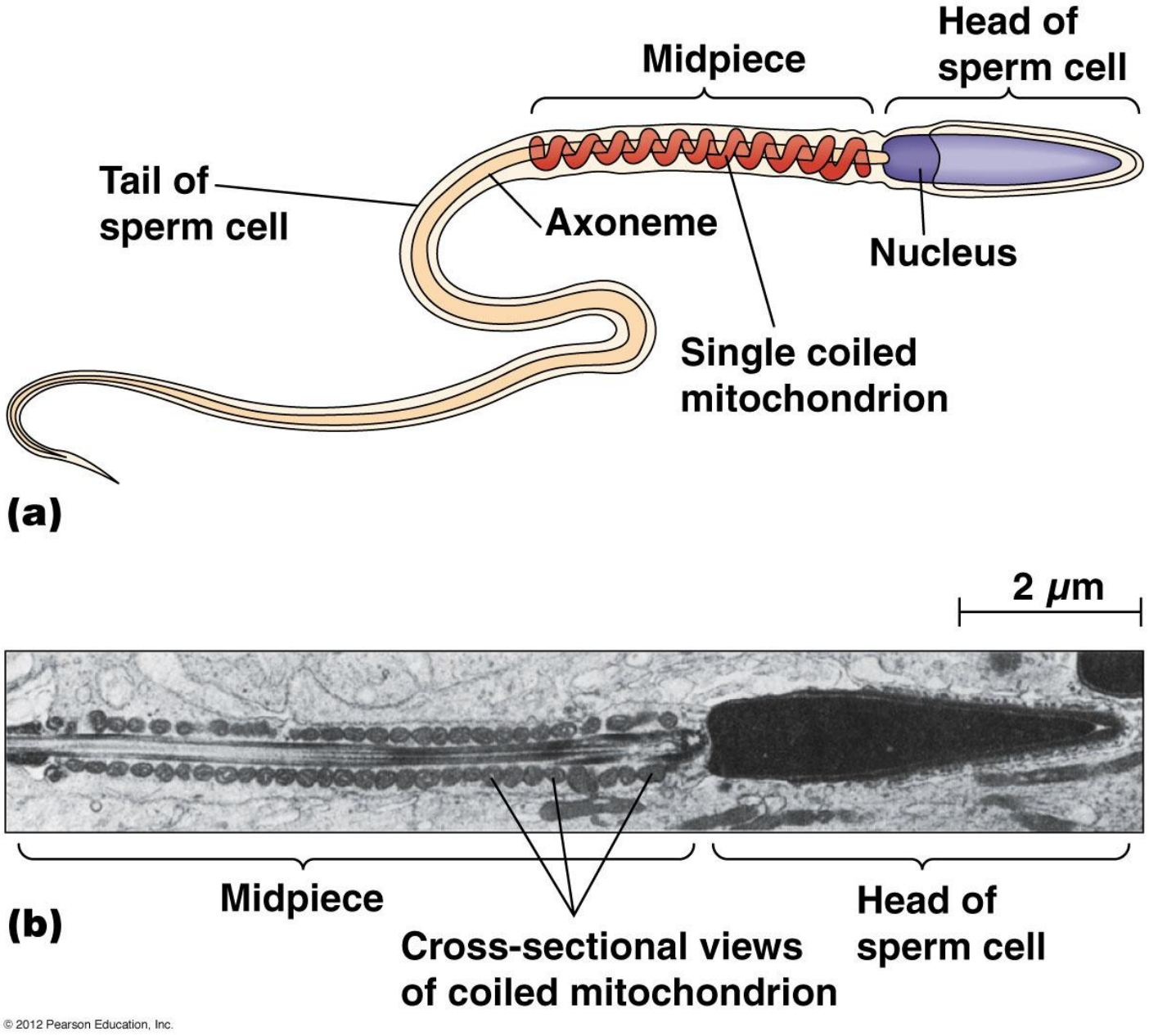
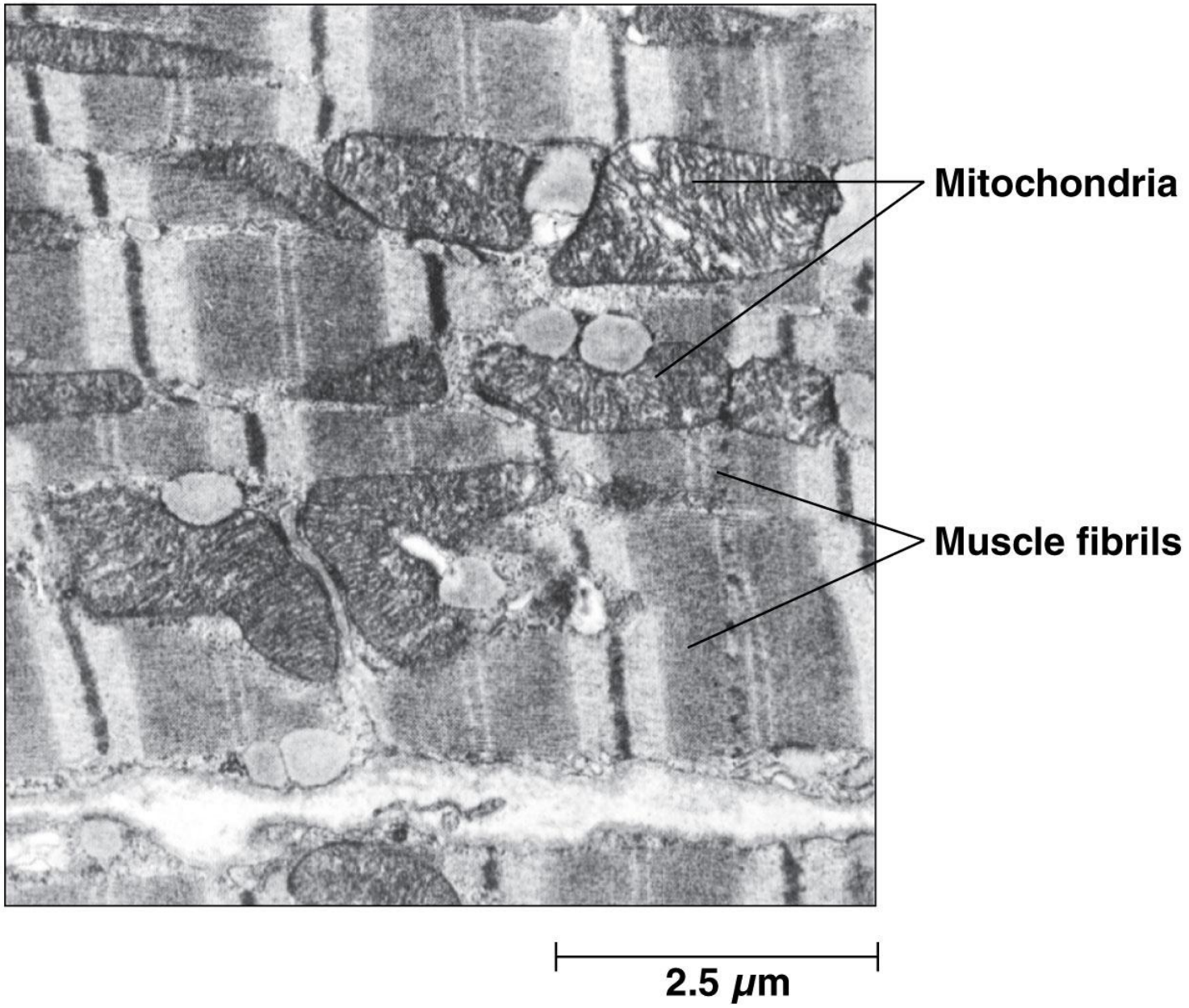


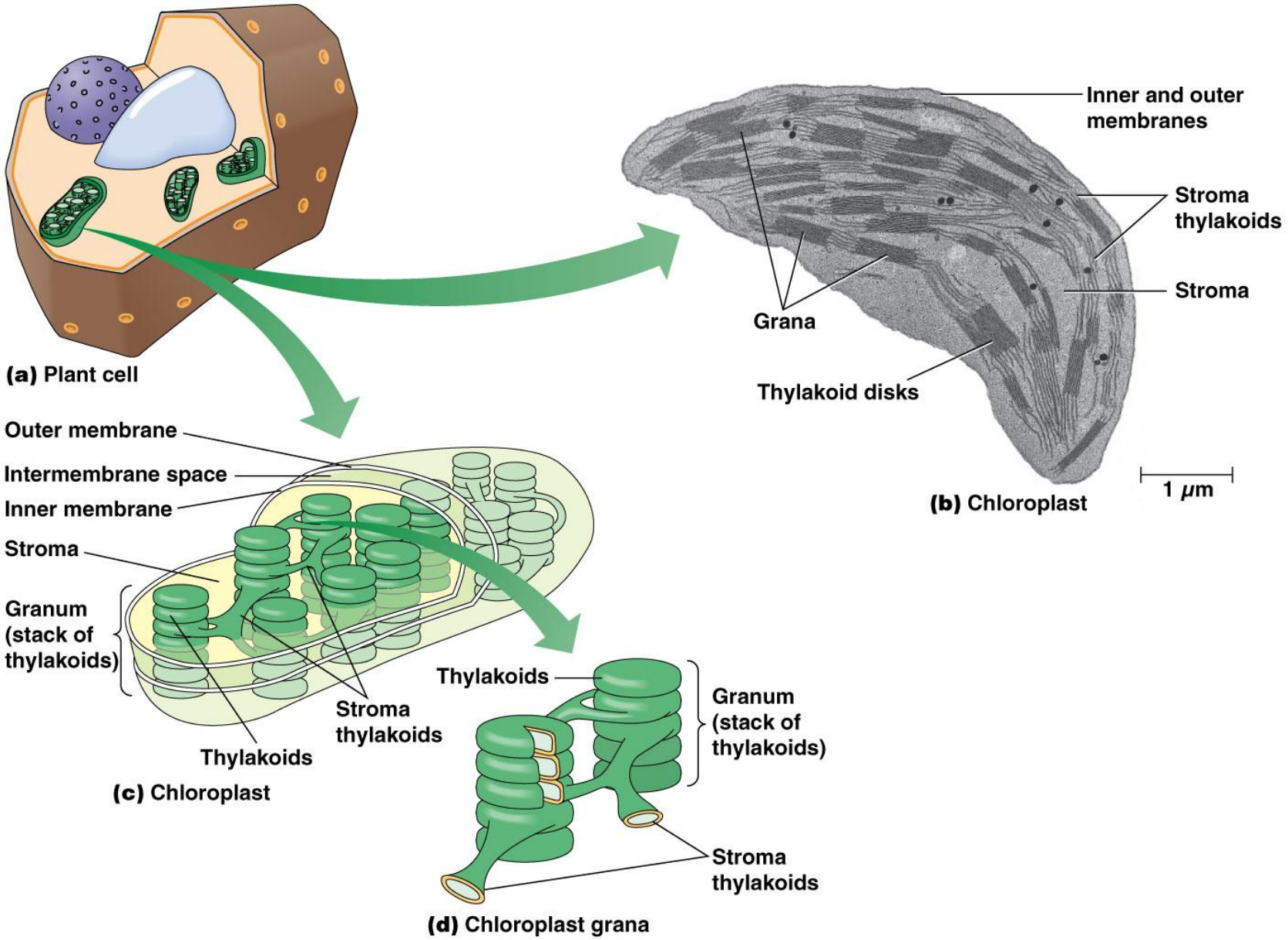
Figure 4-13



The Chloroplast

- The chloroplast is the site of photosynthesis in plants and algae
- They are large, and can be quite numerous in the cells of green plants
- They are surrounded both inner and outer membranes and contain a system of flattened membranous sacs called **thylakoids**, stacked into **grana**

Figure 4-14



Chloroplast function

- Chloroplasts are the site of *photosynthesis*, a process that uses solar energy and CO_2 to produce sugars and other organic compounds
- This process is the reverse of the mitochondrial reactions that oxidize glucose into CO_2
- Chloroplasts are found in photosynthetic cells and contain most of the enzymes needed for photosynthesis

Chloroplast function (continued)

- Reactions that depend on solar energy, take place in or on the thylakoid membranes
- Reactions involved in the reduction of CO_2 to sugar occur within the **stroma**, a semifluid in the interior of the chloroplast
- Like mitochondria, chloroplasts contain their own ribosomes, and a small circular DNA molecule that encodes some RNAs and proteins needed in the chloroplast

The Endosymbiont Theory: Did Mitochondria and Chloroplasts Evolve from Ancient Bacteria?

- Both mitochondria and chloroplasts have their own DNA and ribosomes and can produce some of their own proteins
- However, most of the proteins needed in these organelles are encoded by nuclear genes
- Overall there are many similarities between processes in mitochondria and chloroplasts and those in bacteria

Similarities between mitochondria and chloroplasts and bacteria

- All three have circular DNA molecules without associated histones
- rRNA sequences, ribosome size, sensitivities to inhibitors of RNA and protein synthesis and type of protein factors used in protein synthesis are all similar
- Both resemble bacteria in size and shape and are surrounded by double membranes, the inner of which has bacterial-type lipids

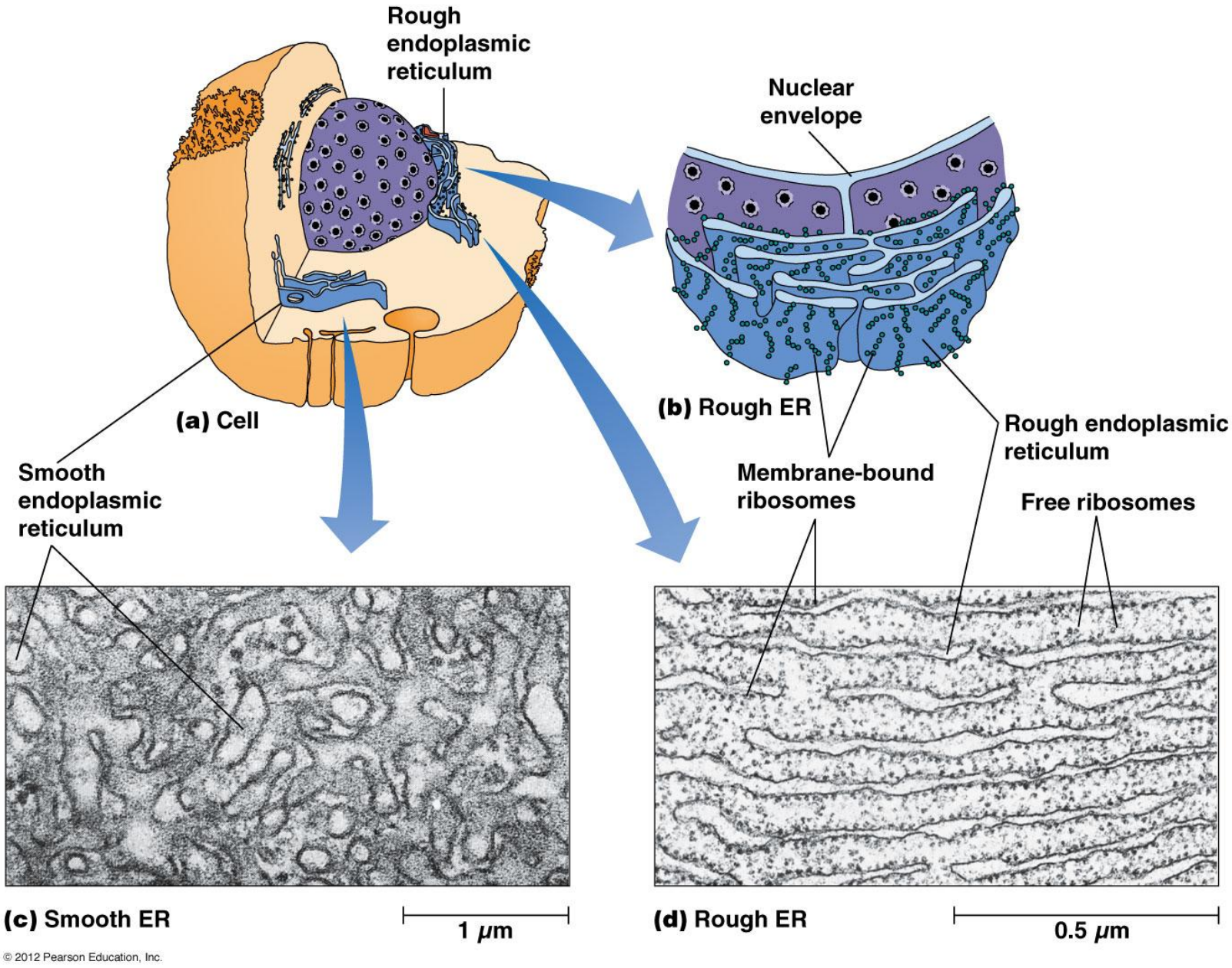
The endosymbiont theory

- The **endosymbiont theory** suggests that mitochondria and chloroplasts originated from prokaryotes
- These gained entry into single-celled organisms called *protoeukaryotes*
- Protoeukaryotes may have ingested bacteria by *phagocytosis* without then digesting them, allowing a symbiotic relationship to develop

The Endoplasmic Reticulum

- Almost every eukaryotic cell has a network of membranes in the cytoplasm, called the **endoplasmic reticulum (ER)**
- It consists of tubular membranes and flattened sacs called **cisternae**
- The internal space of the ER is called the **lumen**
- The ER is continuous with the other membranes in the cell

Figure 4-15



Rough endoplasmic reticulum

- ER can be *rough* or *smooth* in appearance
- **Rough ER** is studded with ribosomes on the cytoplasmic side of the membrane
- These ribosomes synthesize polypeptides that accumulate within the membrane or are transported across it to the lumen
- Free ribosomes are not associated with the ER

Smooth endoplasmic reticulum

- **Smooth ER** has no role in protein synthesis
- It is involved in the synthesis of lipids and steroids such as cholesterol and its derivatives
- Smooth ER is responsible for inactivating and detoxifying potentially harmful substances
- *Sarcoplasmic reticulum* has critical functions in contraction

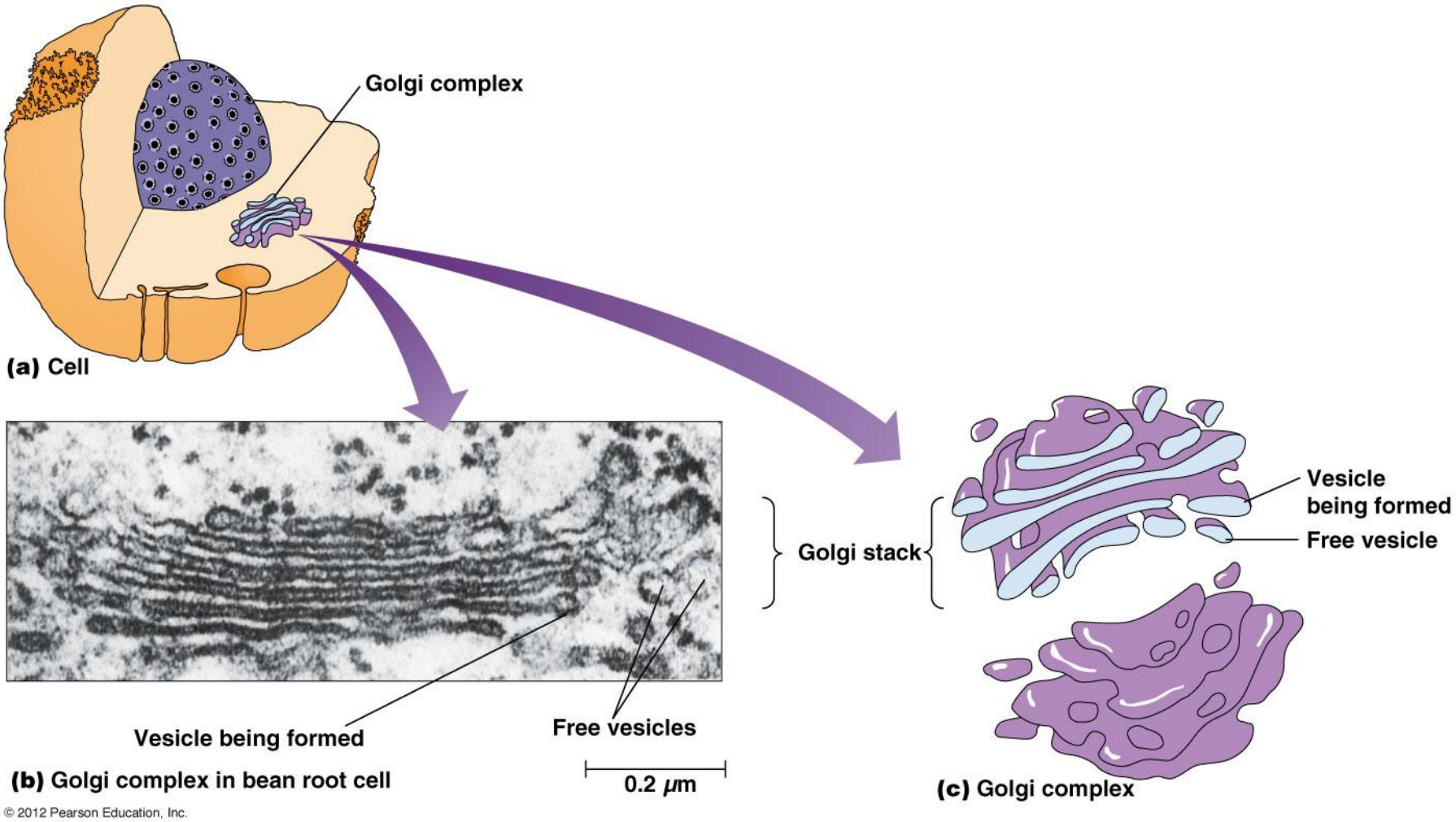
The Golgi Complex

- The **Golgi complex,** closely related to the ER in proximity and function, consists of a stack of flattened vesicles known as *cisternae*
- It plays an important role in processing and packaging secretory proteins, and in complex polysaccharide synthesis
- It accepts vesicles that bud off of the ER

The Golgi complex is like a processing station

- The contents of vesicles from the ER are modified and processed in the Golgi complex
- E.g., secretory and membrane proteins are mainly *glycosylated* (the addition of short-chain carbohydrates), a process that begins in the ER and is completed in the Golgi complex
- The processed substances then move to other locations in the cell through vesicles that bud off of the Golgi complex

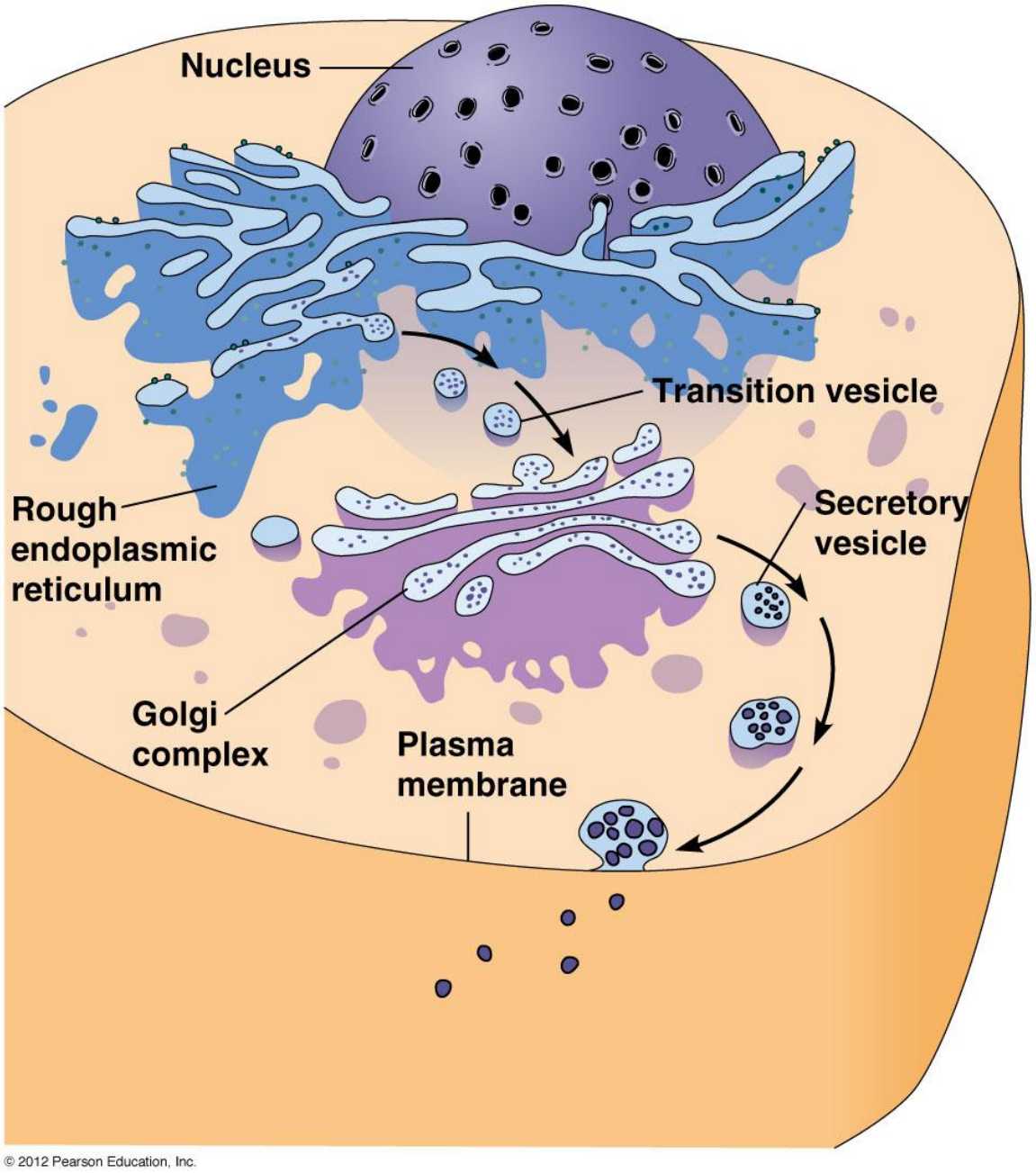
Figure 4-16



Secretory Vesicles

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

Figure 4-17



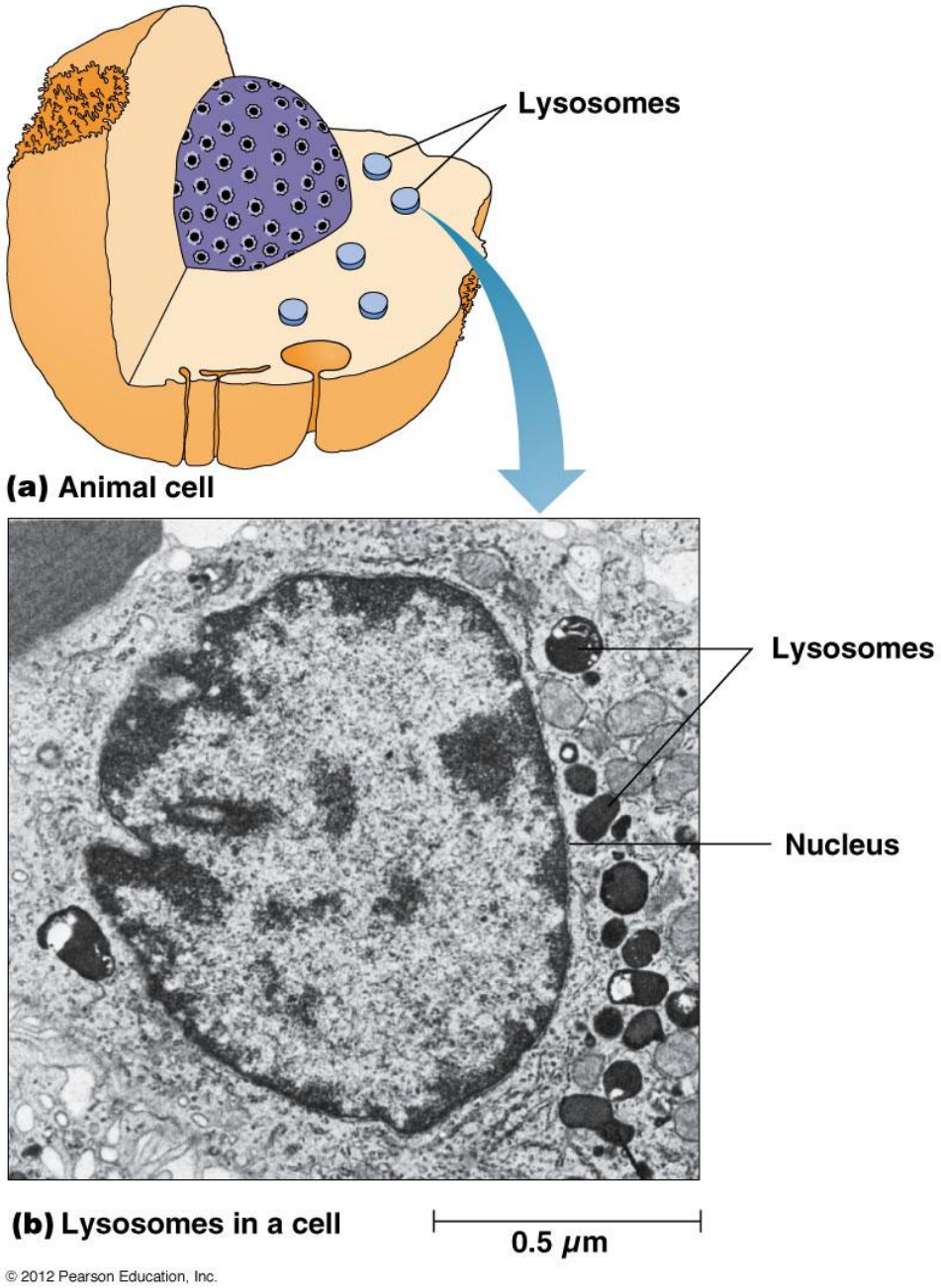
The Lysosome

- **Lysosomes** are single membrane organelles that store *hydrolases*, enzymes that can digest any kind of biological molecule

- These enzymes are sequestered to prevent them from digesting the contents of the cell

- A special carbohydrate coating on the inner lysosome membrane protects it from digestion

Figure 4-18



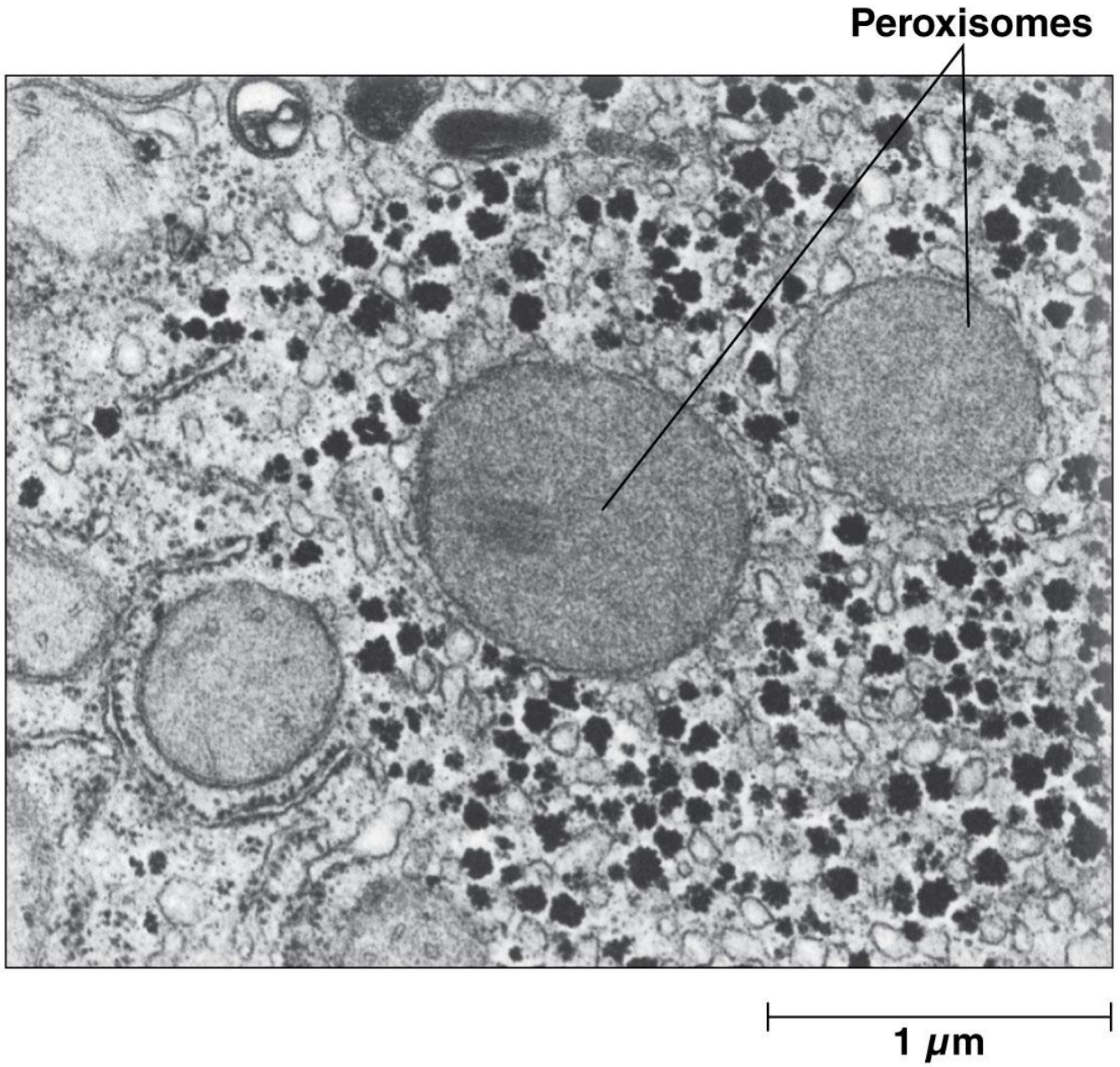
The Peroxisome

- **Peroxisomes** resemble lysosomes in size and appearance
- They are surrounded by a single membrane and perform several functions depending on cell type
- Peroxisomes are especially prominent in the liver and kidney cells of animals

Hydrogen peroxide

- H_2O_2 is highly toxic to cells but can be formed into water and oxygen by the enzyme *catalase*
- Eukaryotic cells have metabolic processes that produce H_2O_2
- These reactions are confined to peroxisomes that contain catalase, so that cells are protected from the harmful effects of peroxide

Figure 4-19



Other functions of peroxisomes

- Peroxisomes detoxify other harmful compounds, and catabolize unusual substances
- In animals, they play roles in oxidative breakdown of fatty acids, especially longer chain fatty acids (up to 22 carbon atoms)
- Some serious human diseases result from defects in one or more peroxisomal enzymes, normally involved in degrading long-chain fatty acids

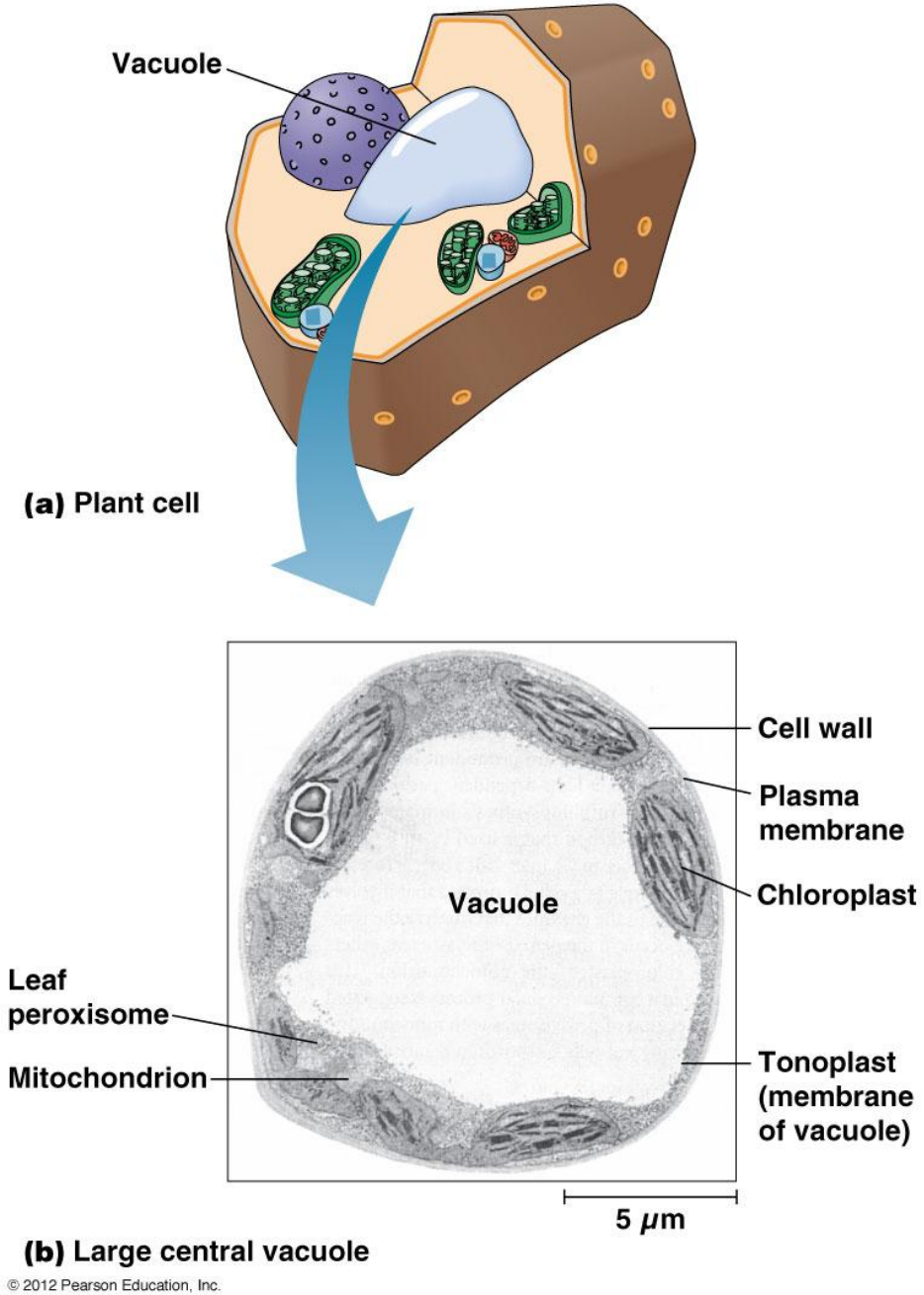
Vacuoles

- Some cells contain a membrane-bounded vacuole
- In animal and yeast cells they are used to temporary storage or transport
- Phagocytosis leads to the formation of a membrane bound particle, called a phagosome
- When this type of vacuole fuses with a lysosome, the contents are hydrolyzed to provide nutrients to a cell

Plant vacuoles

- Most mature plant cells contain a single large vacuole called a **central vacuole**
- The main function of the central vacuole is to maintain the *turgor pressure* that keeps the plant from wilting
- Tissues wilt when the central vacuole no longer presses against the cell contents (fails to provide adequate pressure)

Figure 4-21



Ribosomes

- **Ribosomes** are not really organelles because they are not enclosed by a membrane
- They are found in all cells but differ slightly in bacteria, archaea and eukarya in their size and composition
- Each cell type has a unique type of ribosomal RNA

Ribosomes are very small

- Ribosomes can only be seen under the electron microscope
- They have **sedimentation coefficients** in keeping with their small size
- Sedimentation coefficient: a measure of how rapidly a particle sediments in an ultracentrifuge, expressed in *Svedberg units* (*S)*
- Ribosomes have values of 80S (eukaryotes) or 70S (bacteria and archaea)

Ribosome subunits

- Ribosomes have two subunits, the **large** and **small subunits**, with sedimentation coefficients of 60S and 40S respectively in eukaryotic cells

- Bacteria and archaea have large and small subunits of 50S and 30S, respectively

- The S values of large and small subunits does not add up to the value for the complete ribosome, because S values depend on both size and shape

Ribosome are numerous and ubiquitous

- Ribosomes are much more numerous than most other cellular structures (prokaryote cells contain thousands, eukaryote cells may contain millions)

- Ribosomes in mitochondria and chloroplasts are similar size and composition to those of bacteria

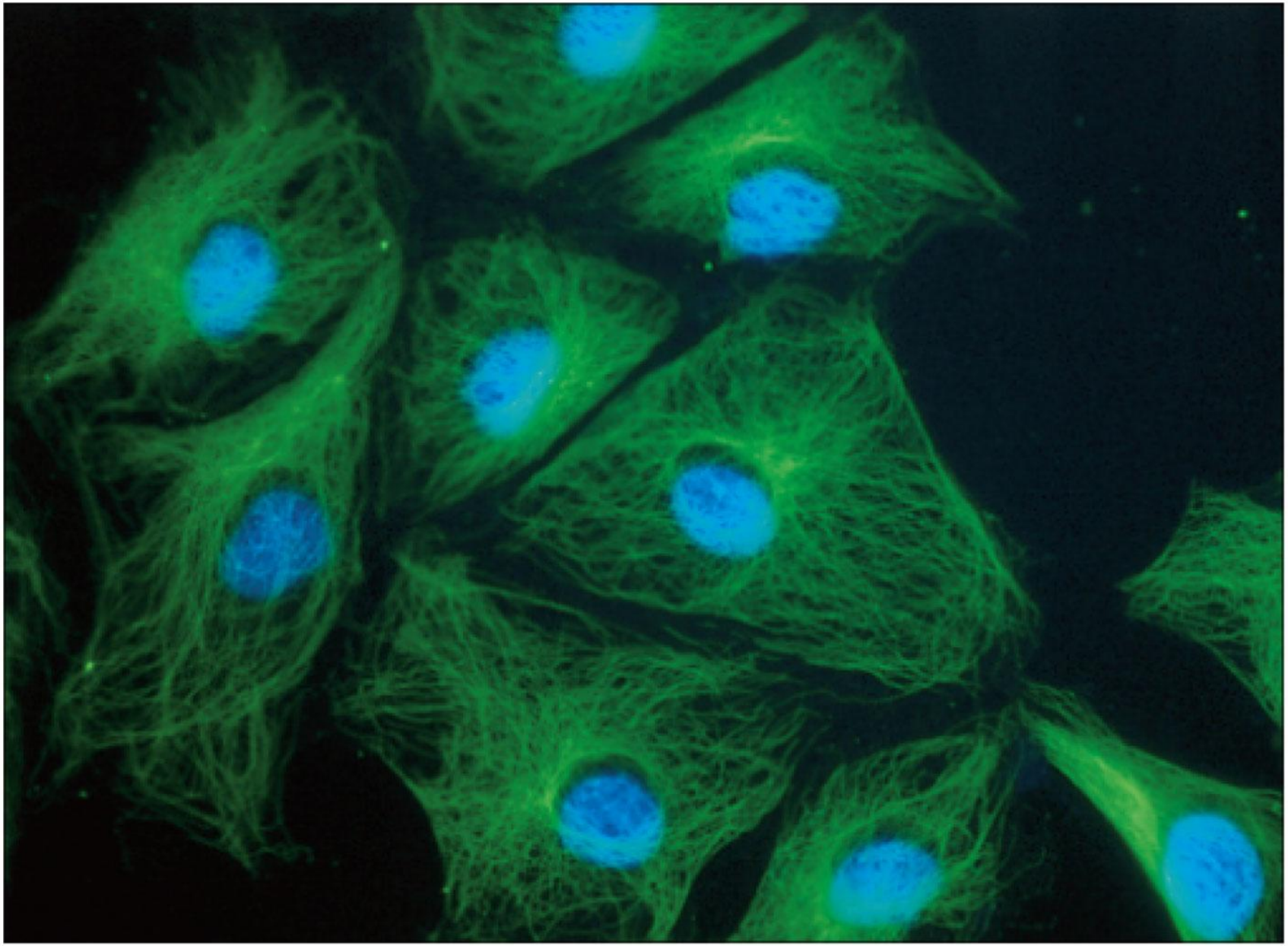
The Cytoplasm of Eukaryotic Cells Contains the Cytosol and Cytoskeleton

- The **cytoplasm** of a eukaryotic cell is the interior of the cell not occupied by the nucleus
- The **cytosol** is the semifluid substance in which the organelles are suspended
- The synthesis of fats and proteins and the initial steps in releasing energy from sugars takes place in the cytosol
- The cytosol is permeated by the **cytoskeleton**

The cytoskeleton

- The cytoskeleton is a three-dimensional array of interconnected microfilaments, microtubules and intermediate filaments
- It gives a cell its distinctive shape and internal organization
- It also plays a role in cell movement and cell division

Figure 4-23



The cytoskeleton (continued)

- The cytoskeleton serves as a framework for positioning and moving organelles and macromolecules within the cell
- It may do the same for ribosomes and enzymes
- Even some of the water within the cell (20-40%) may be bound to microfilaments and microtubules

The Extracellular Matrix and the Cell Wall Are "Outside" the Cell

- Most cells are characterized by extracellular structures
- For many animal cells these structures are called the **extracellular matrix** (**ECM**) and consist mainly of collagen fibers and proteoglycans
- For plant and fungal cells, these are **cell walls**, consisting mainly of *cellulose microfibrils*

Motility and the ECM

- Plant cells are *nonmotile* and thus suited to the rigidity that cell walls confer on an organism
- Animal cells are *motile* and therefore are surrounded by a strong but elastic network of collagen fibers
- Bacteria and archaea may be motile or not; their cell walls provide protection from bursting due to osmotic differences between the cell and the surrounding environment

The ECM

- The primary function of the ECM is support but the types of materials and patterns in which they are deposited regulate a variety of processes
- In animal cells, a network of proteoglyans surrounds the collagen fibers
- In vertebrates, collagen is the most abundant protein in the animal body, as it is also found in tendons, cartilage and bone

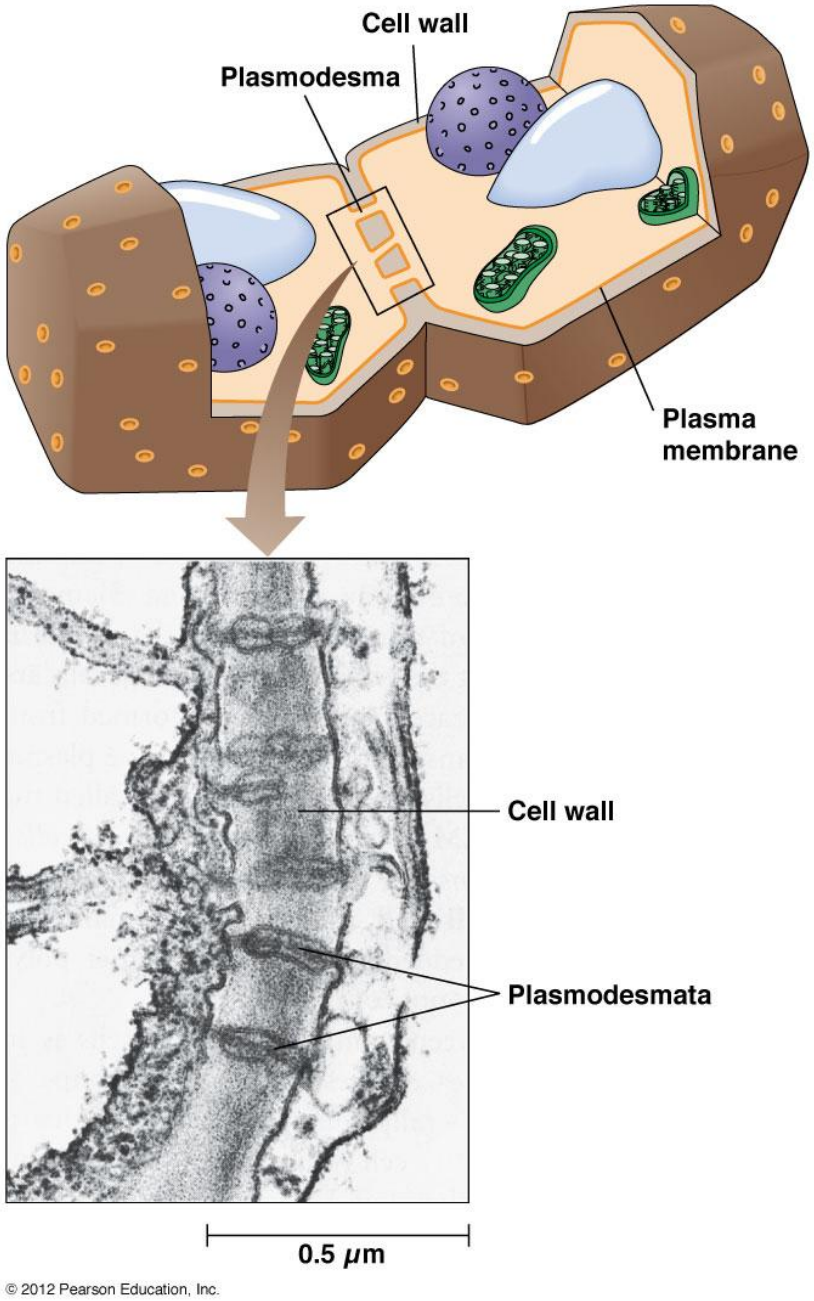
Additional functions of the ECM

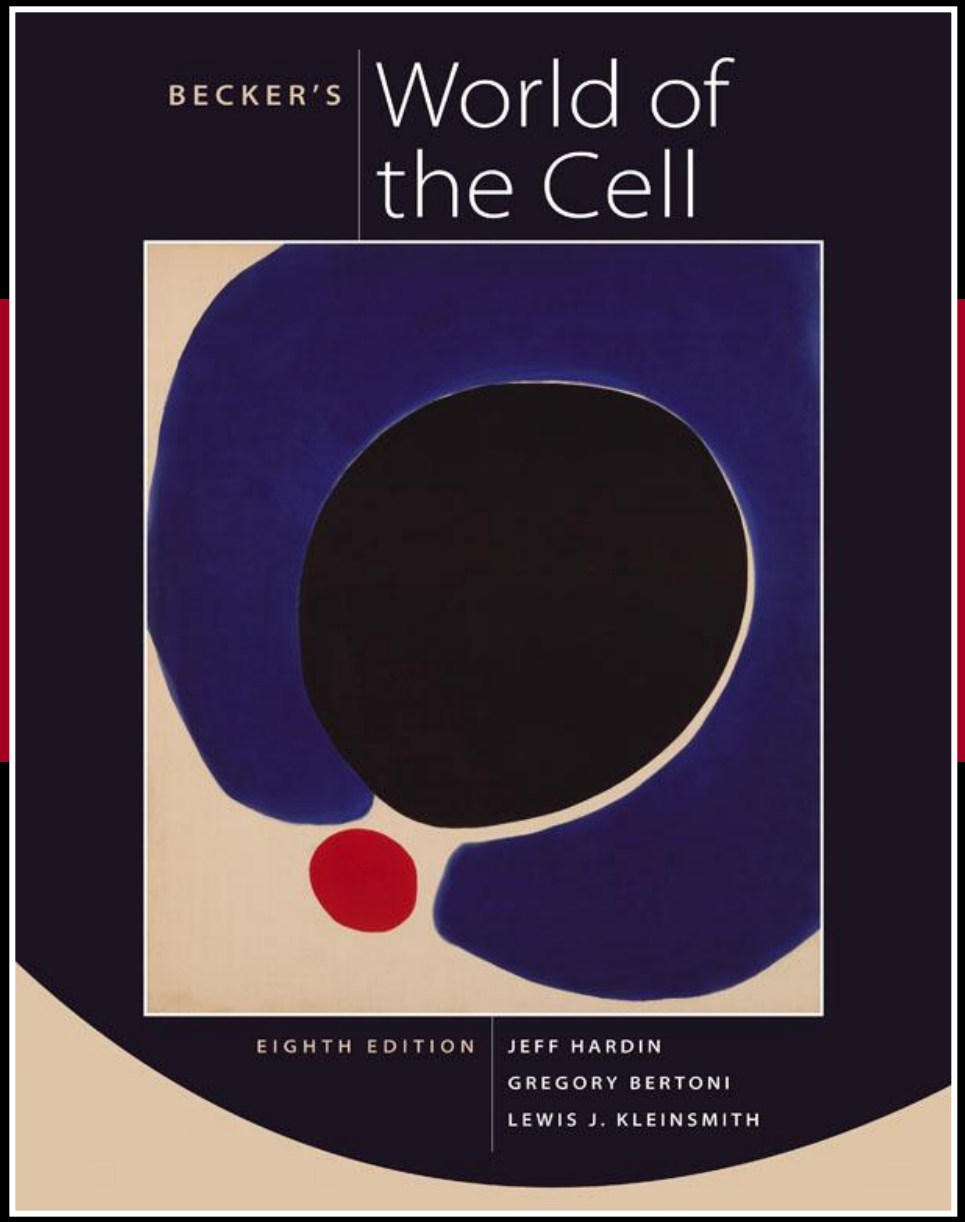
- Processes regulated by the ECM may include:
  - Cell motility and migration
  - Cell division
  - Cell recognition and adhesion
  - Cell differentiation during embryonic development

Cell communication

- Plant cells are connected to neighboring cells by cytoplasmic bridges called **plasmodesmata**, which pass through the cell wall
- Plasmodesmata are large enough to allow the passage of water and small solutes from cell to cell
- Animal cells also communicate with one another through intercellular connections called *gap junctions*
- *Tight junctions* and *adhesion junctions* also connect animal cells

Figure 4-25





Chapter 7

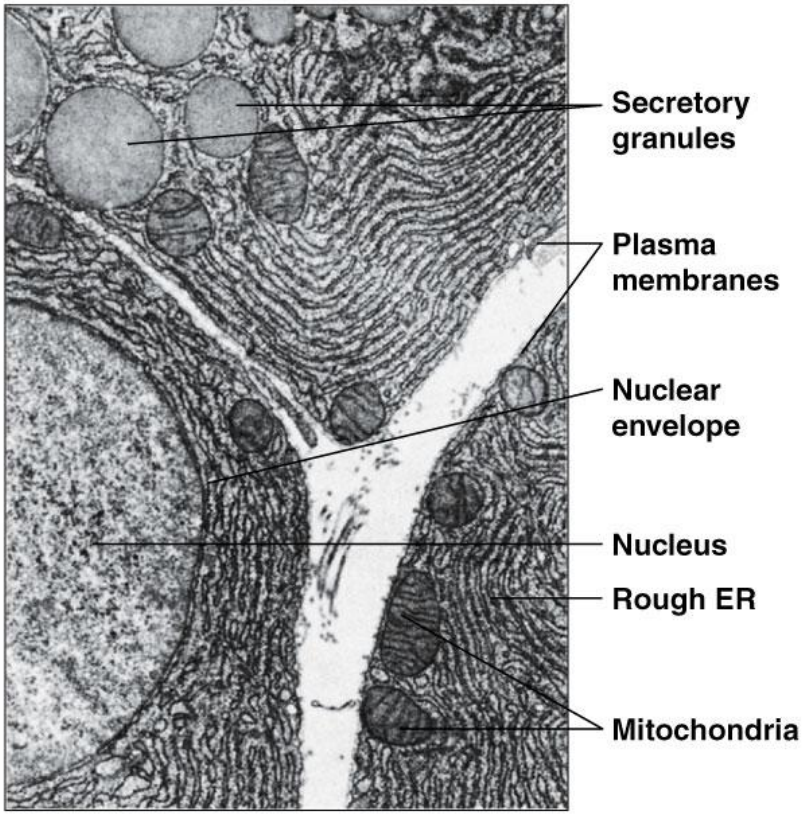
Membranes: Their Structure, Function, and Chemistry

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

Membranes: Their Structure, Function, and Chemistry

- **Membranes** define the boundaries of a cell, and its internal compartments

- Membranes play multiple roles in the life of a cell



The Functions of Membranes

1. Define boundaries of a cell and organelles and act as permeability barriers

2. Serve as sites for biological functions such as electron transport

3. Possess transport proteins that regulate the movement of substances into and out of cells and organelles

4. Contain protein molecules that act as receptors to detect external signals

5. Provide mechanisms for cell-to-cell contact, adhesion, and communication

Membranes Define Boundaries and Serve as Permeability Barriers

- Membranes separate the interior and exterior of cells and organelles
- They are effective permeability barriers due to their hydrophobic interior
- The **plasma membrane** surrounds the whole cell, whereas **intracellular membranes** compartmentalize functions within the cell

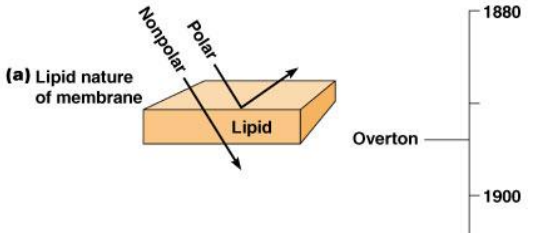
Membranes Are Sites of Specific Proteins and Therefore of Specific Functions

- Membranes are associated with specific functions because the molecules responsible for the functions are embedded in or localized on membranes

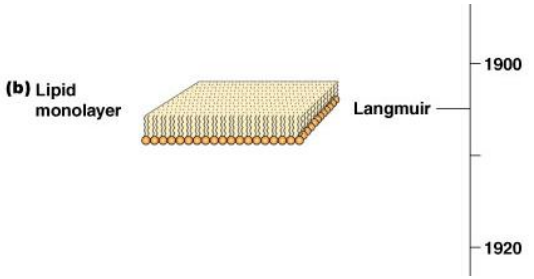
Models of Membrane Structure: An Experimental Approach

Overton and Langmuir: Lipids Are Important Components of Membranes

- In the 1890s Overton observed the easy penetration of lipid-soluble substances into cells and concluded that the cell surface had some kind of lipid “coat” on it

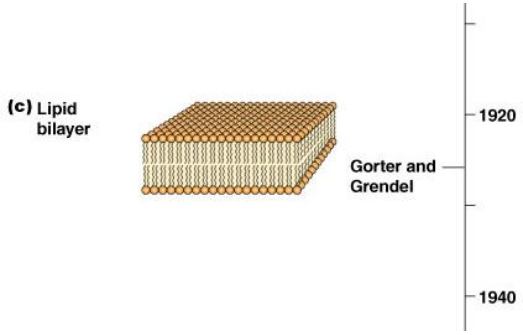


- Langmuir studied phospholipids and found that they were *amphipathic* and reasoned that they must orient on water with the hydrophobic tails away from the water



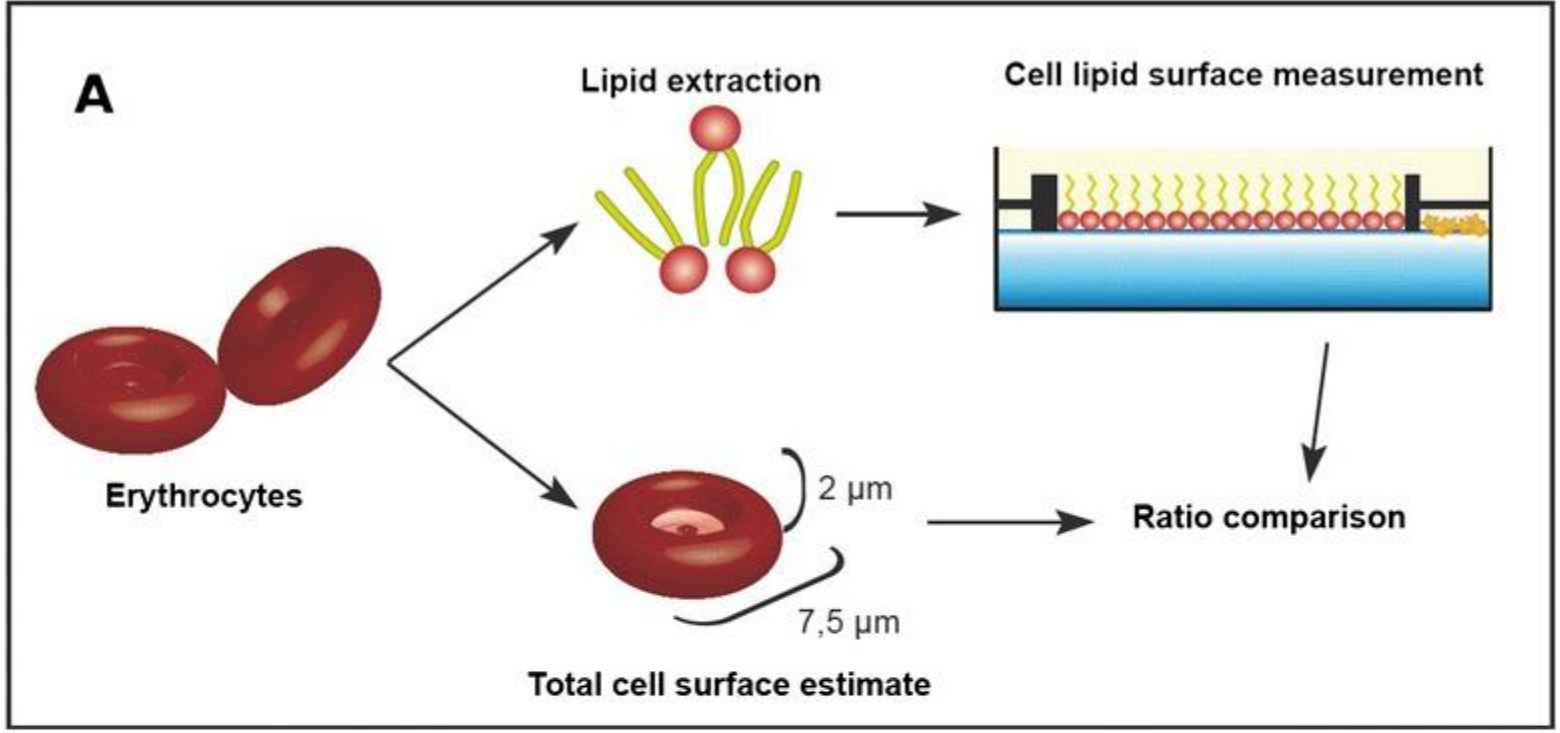
Gorter and Grendel: The Basis of Membrane Structure Is a Bilayer

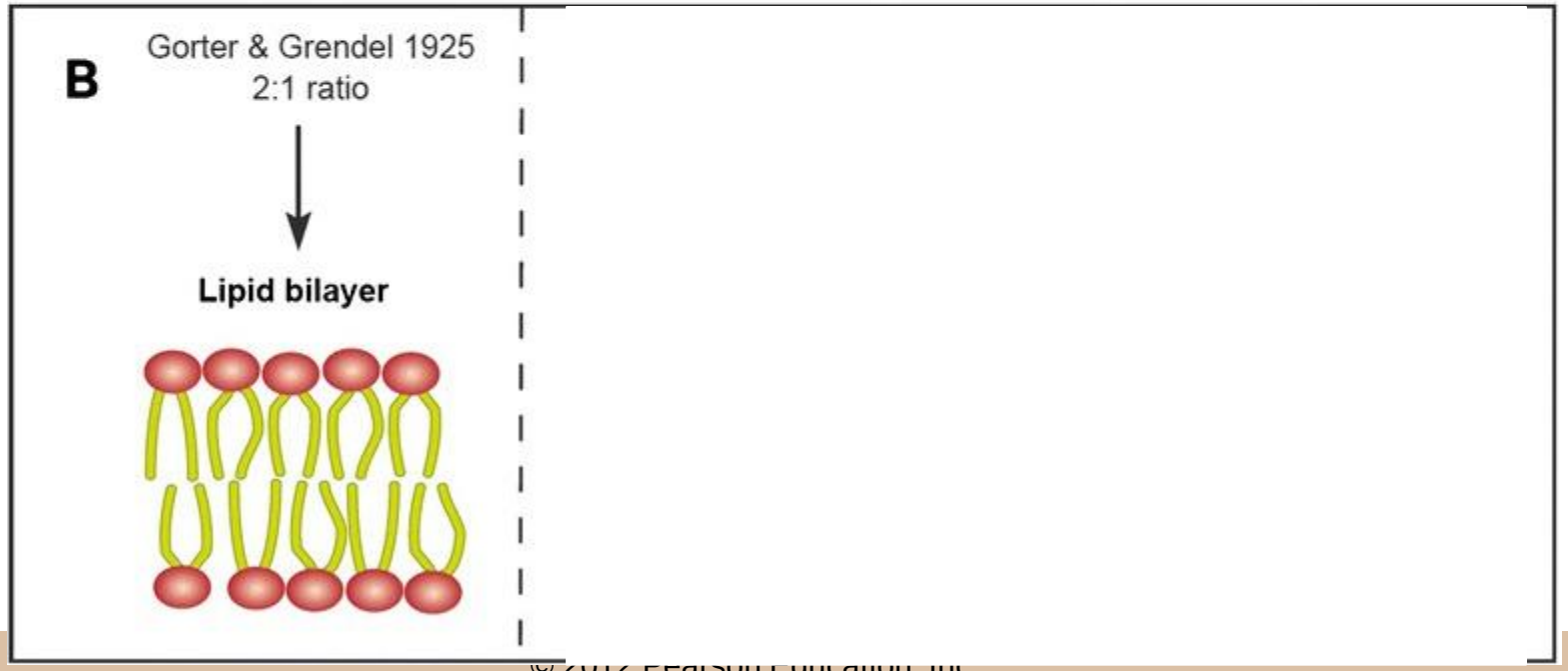
- In 1925, these two physiologists extracted lipids from red blood cells and spread the lipids in a monolayer on a water surface



- The film on the water was twice the surface area of the blood cells, suggesting that lipids on the cell surface consisted of two layers

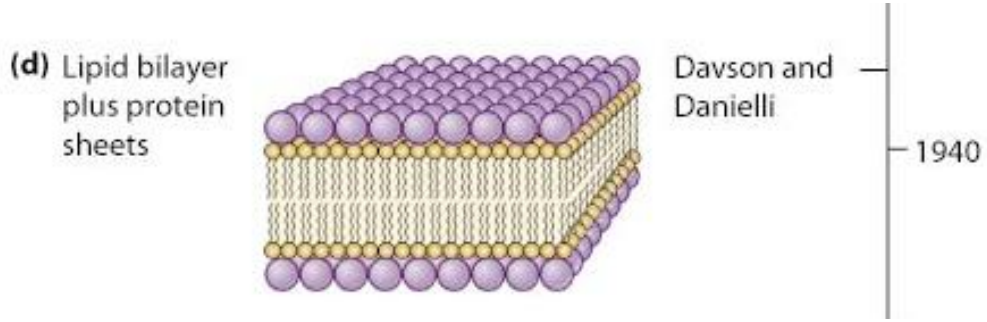
- They suggested that the most favorable structure would be a **lipid bilayer**, with the nonpolar regions of the lipids facing inward. Their data was wrong but the conclusion was correct!!





Davson and Danielli: Membranes Also Contain Proteins

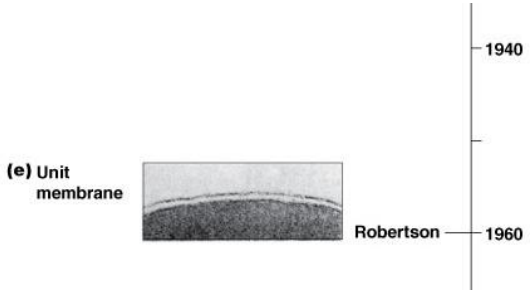
- Davson and Danielli showed that the bilayer alone could not account for all properties of membranes, especially
  - surface tension
  - solute permeability
  - electrical resistance



- They suggested that proteins are present in membranes, as thin sheets, coating the lipids

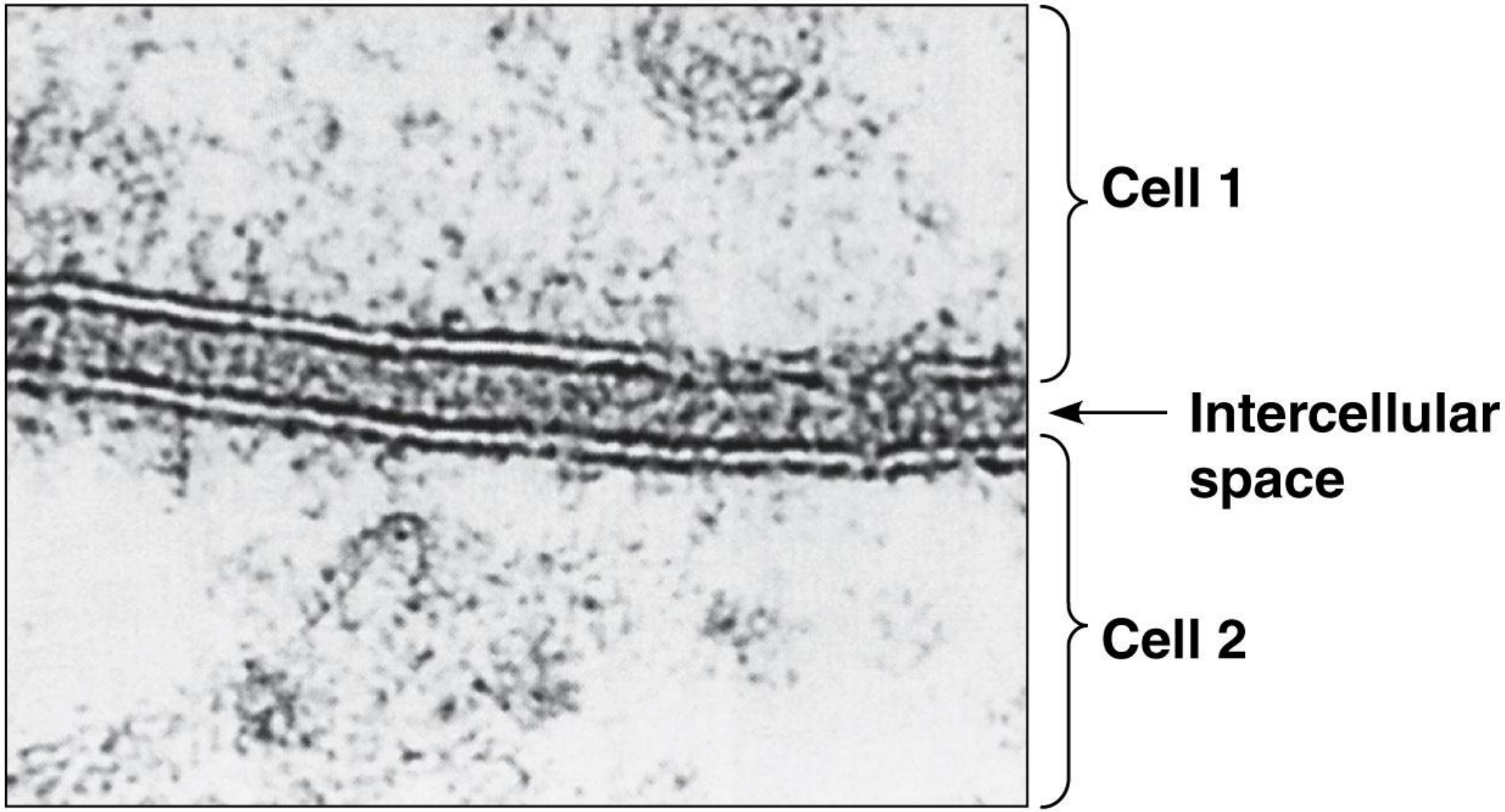
Robertson: All Membranes Share a Common Underlying Structure

- Using electron microscopy, biologists could verify the presence of membranes around cells and organelles



- A trilaminar structure, visible under the TEM, was observed for all membranes, leading to the suggestion of a common membrane structure, called the *unit membrane*

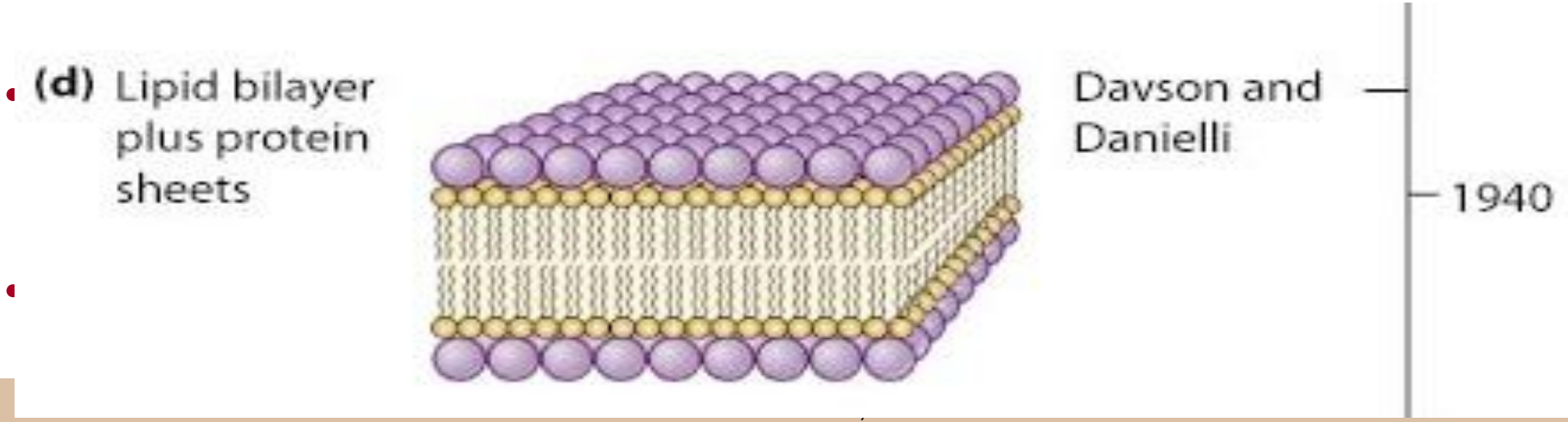
Figure 7-4



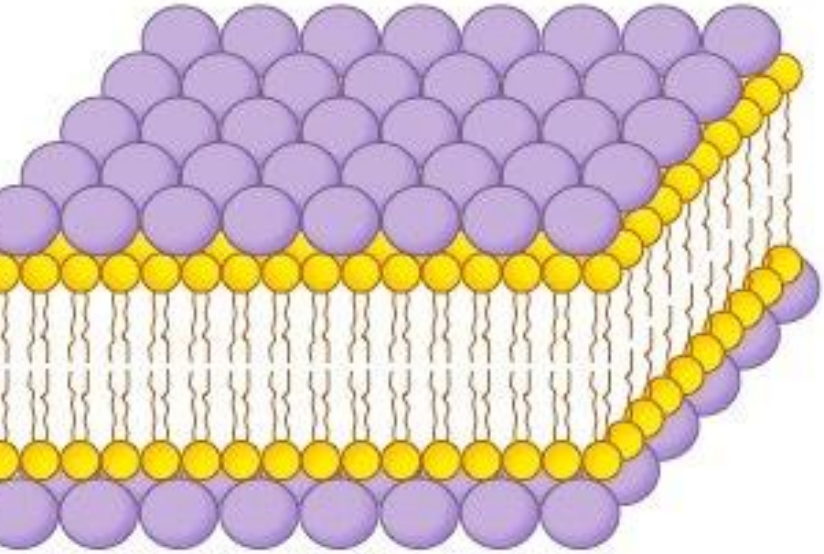
Further Research Revealed Major Shortcomings of the Davson–Danielli Model

- Electron microscopy revealed that there was not enough space to either side of the bilayer for an additional layer of protein

- The Davson–Danielli model also did not account for the chemical distinctiveness of particular types of membranes, especially the protein/lipid ratio

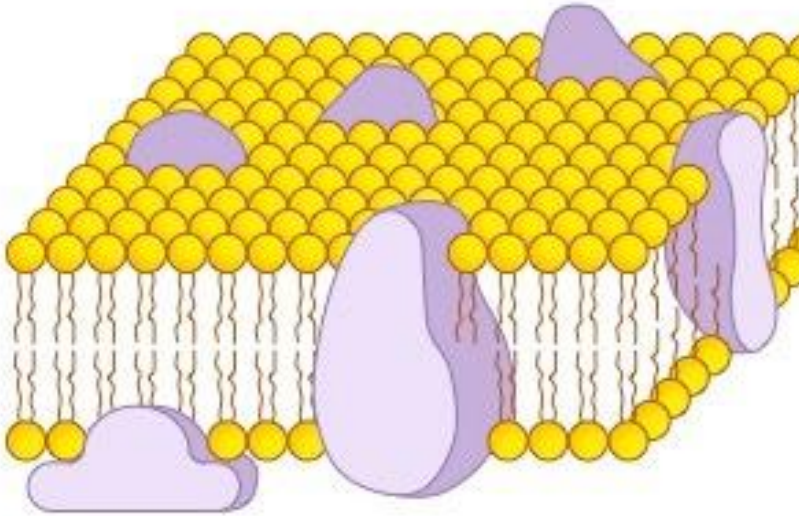


Davson-Danielli Model (1935)



Proteins form distinct layers *(sandwich)*

Singer-Nicolson Model (1972)

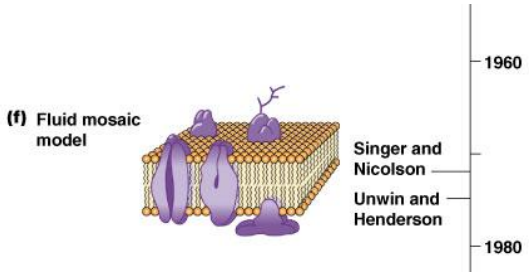


Proteins embedded within bilayer *(fluid-mos*

Singer and Nicholson: A Membrane Consists of a Mosaic of Proteins in a Fluid Lipid Bilayer

- The **fluid mosaic bilayer** model accounts for all the inconsistencies with previous models

- The model has two key features



  – A *fluid* lipid bilayer

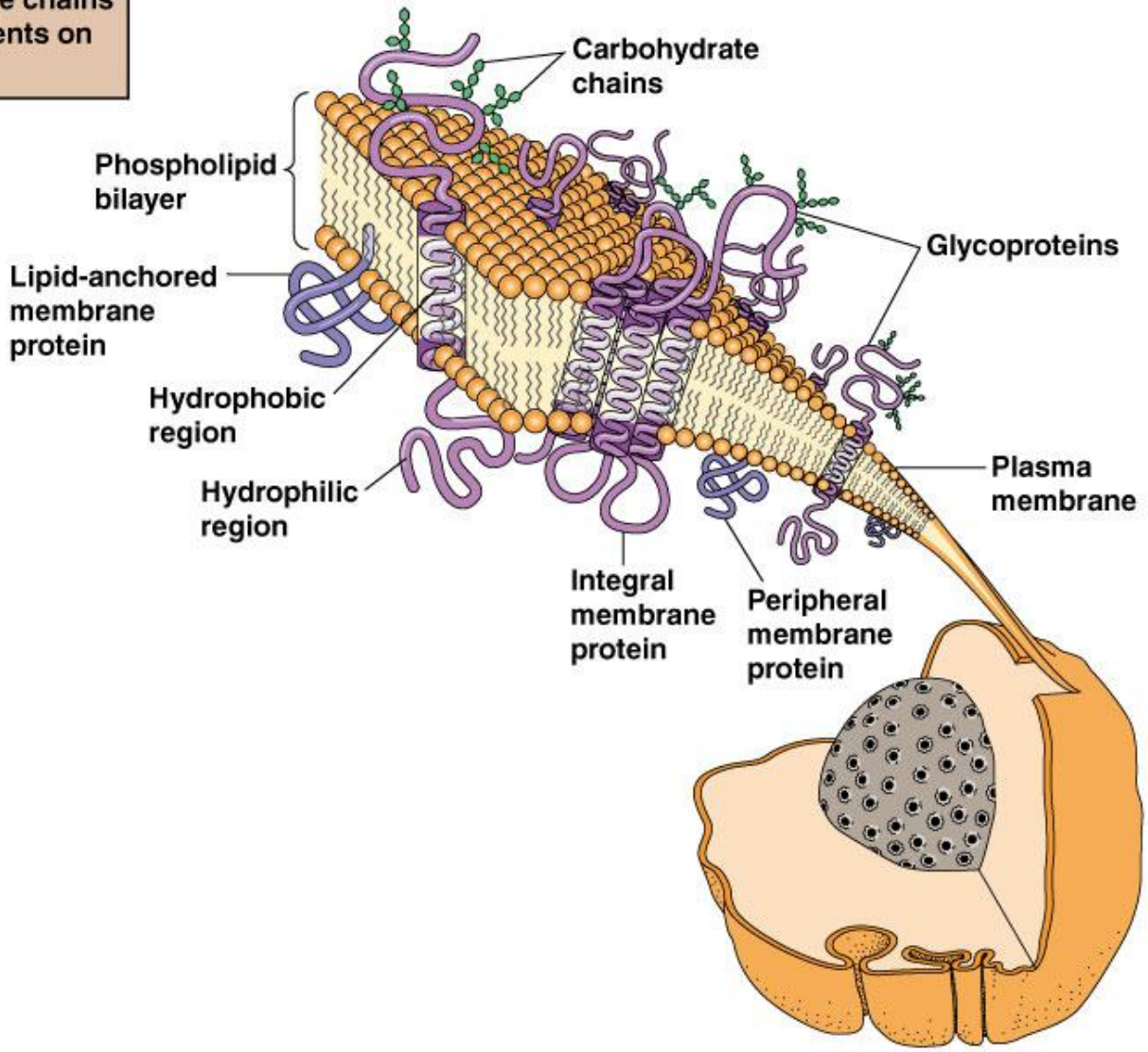
  – A *mosaic* of proteins attached to or embedded in the bilayer

Three classes of membrane proteins

- 1. *Integral membrane proteins* are embedded in the lipid bilayer due to their hydrophobic regions
- 2 *Peripheral proteins* are hydrophilic and located on the surface of the bilayer
- 3. *Lipid-anchored proteins* are hydrophilic and attached to the bilayer by covalent attachments to lipid molecules embedded in the bilayer

Figure 7-5B

(b) An integral membrane protein with multiple α-helical transmembrane segments is shown below. Many integral membrane proteins of the plasma membrane have carbohydrate side chains attached to the hydrophilic segments on the outer membrane surface.

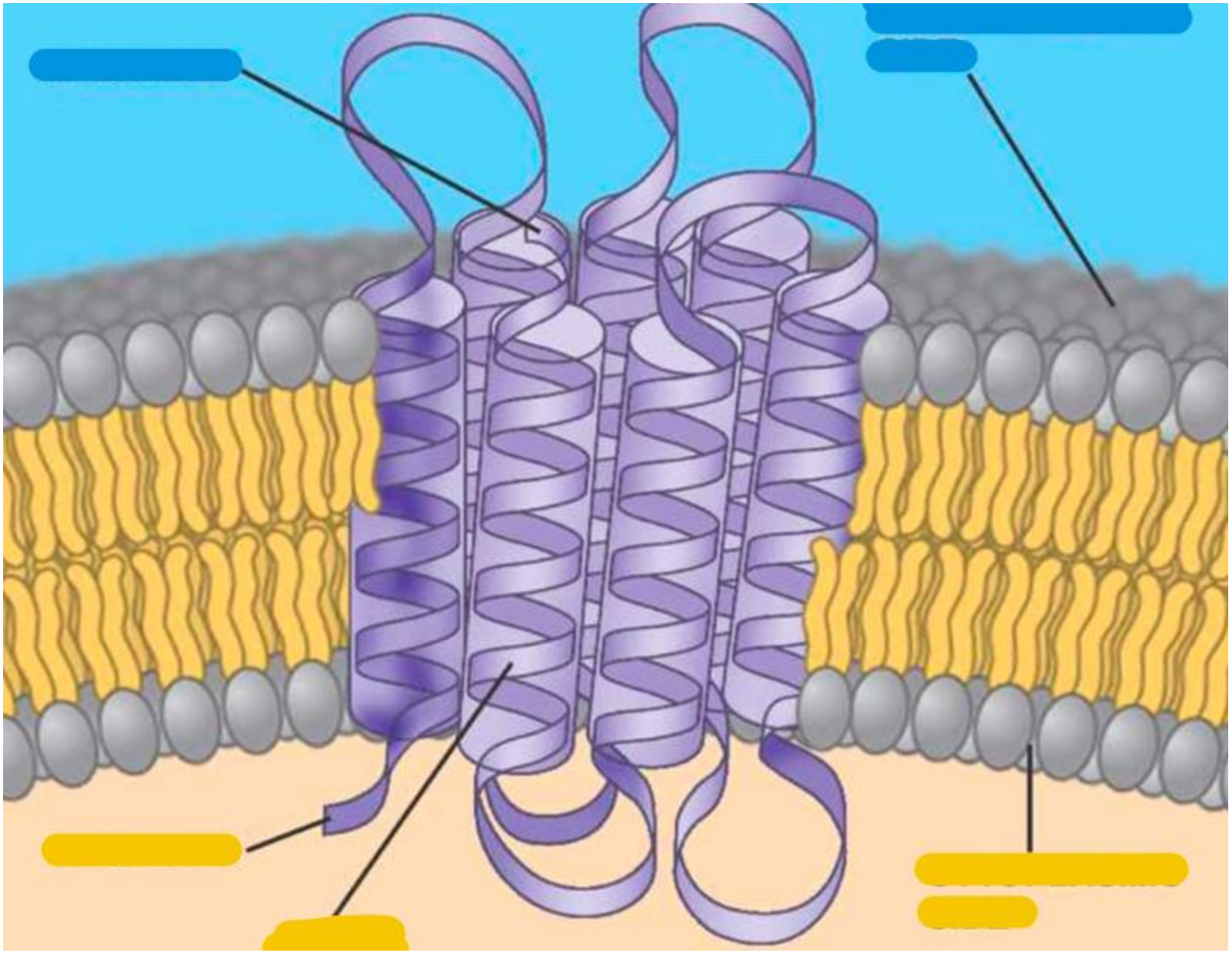


The fluid nature of the bilayer

- Lipids in the bilayer are in constant motion
- Proteins are also able to move laterally within the membrane, though some are anchored to internal structural elements
- Anchored proteins have restricted mobility

Unwin and Henderson: Most Membrane Proteins Contain Transmembrane Segments

- Most integral membrane proteins have one or more hydrophobic segments that span the lipid bilayer
- These *transmembrane segments* anchor the protein to the membrane
- *Bacteriorhodopsin* was the first membrane protein shown to possess this structural feature



Recent Findings Further Refine Our Understanding of Membrane Structure

- Membranes are
  - not homogenous freely mixing
  - ordered through dynamic microdomains called **lipid rafts**
- Most cellular processes that involve membranes depend on structural complexes of specific lipids and proteins

Membrane Lipids: The "Fluid" Part of the Model

- Membrane lipids are important components of the "fluid" part of the fluid mosaic model
- Membranes contain several types of lipids
- The main classes of membrane lipids are *phospholipids*, *glycolipids,* and *sterols*

Phospholipids

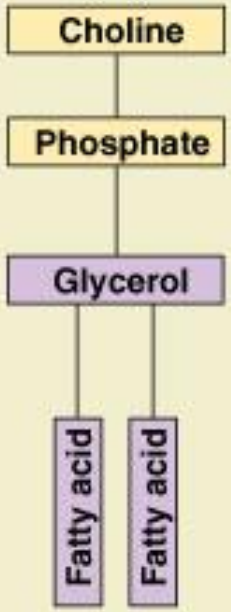
- **Phospholipids** are the most abundant lipids in membranes
- They include the glycerol-based **phosphoglycerides** and the sphingosine-based **sphingolipids**
- The kinds and relative proportions of phospholipids vary greatly among types of membranes

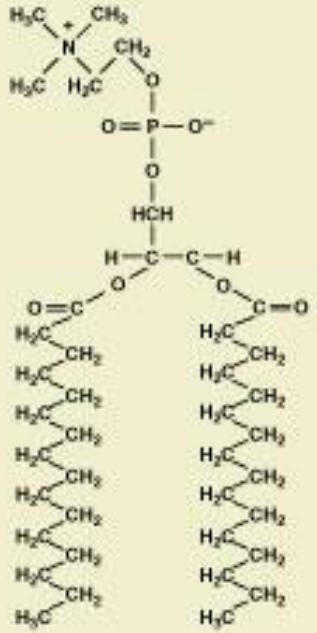
Schematic diagram

Chemical structure

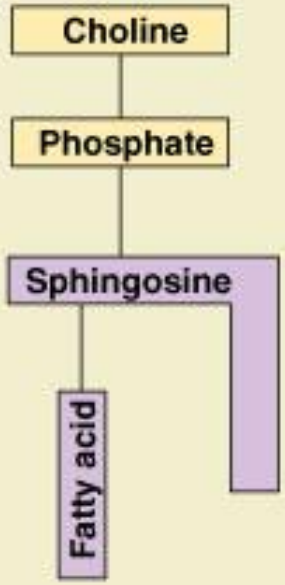
(a) PHOSPHOLIPIDS

Phosphatidylcholine (shown)
Phosphatidylethanolamine
Phosphatidylserine
Phosphatidylthreonine
Phosphatidylinositol
Phosphatidylglycerol
Diphosphatidylglycerol (cardiolipin)





Sphingomyelin (a sphingolipid)



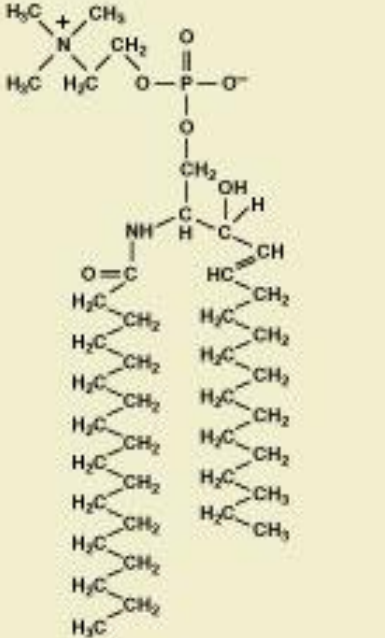


Figure 7-6A

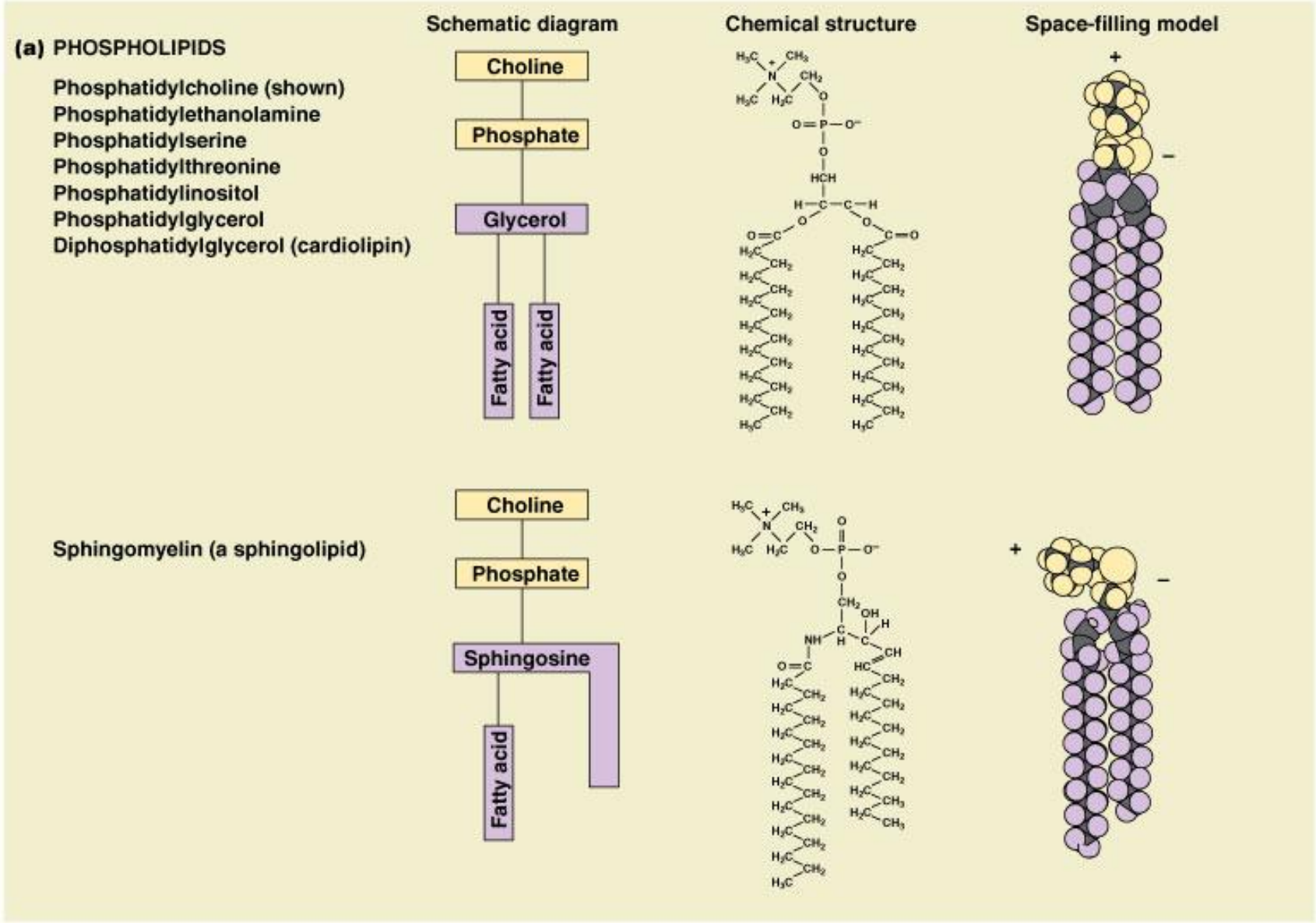
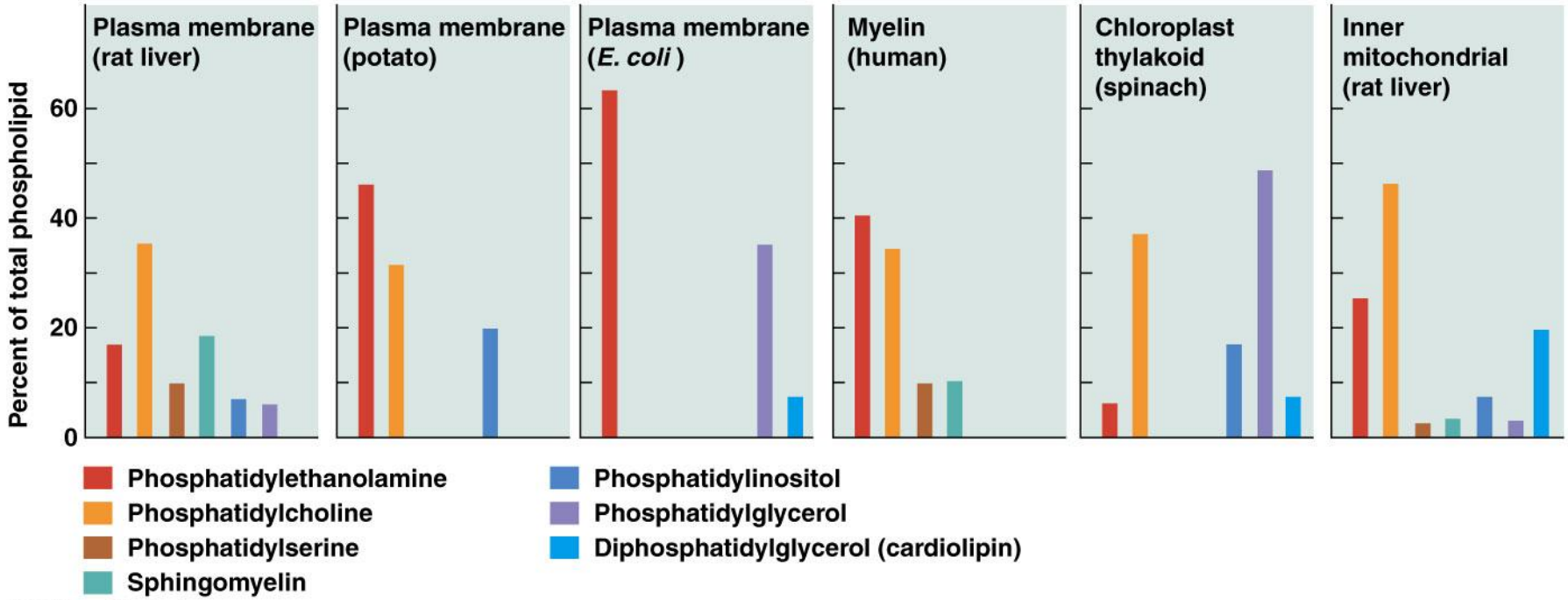


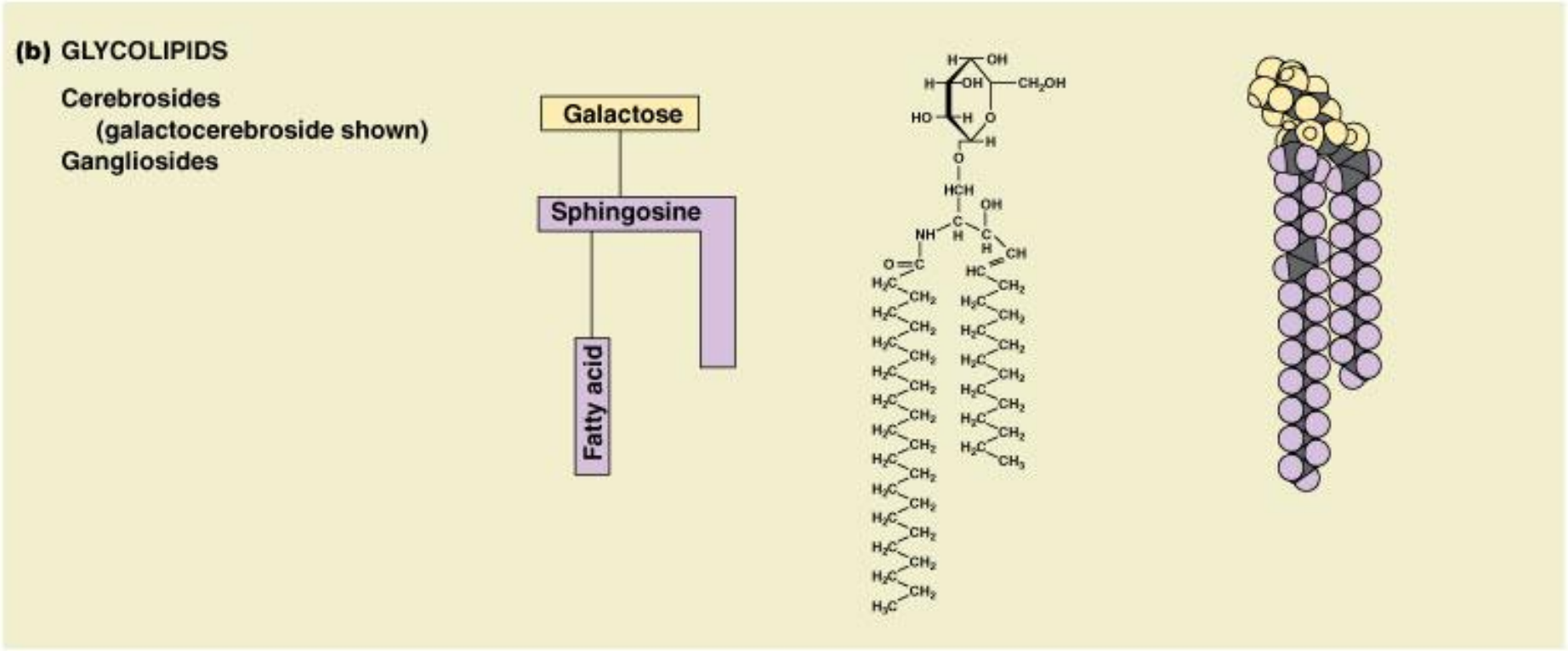
Figure 7-7



Glycolipids

- **Glycolipids** are formed by the addition of carbohydrates to lipids
- Some are glycerol-based and some are sphingosine-based; the *glycosphingolipids*
- The most common glycosphingolipids are **cerebrosides** and **gangliosides**

Figure 7-6B



Cerebrosides and gangliosides

- Cerebrosides are *neutral glycolipids;* each molecule has an uncharged sugar as its head group
- A ganglioside has an oligosaccharide head group with *one or more negatively charged* sialic acid residues
- Cerebrosides and gangliosides are especially prominent in brain and nerve cells

Sterols

- The membranes of most eukaryotes contain significant amounts of **sterols**
- The main sterol in animal cell membranes is **cholesterol,** which is needed to stabilize and maintain membranes
- Plant cell membranes contain small amounts of **phytosterols**, whereas fungal cell membranes contain ergosterol, similar to cholesterol

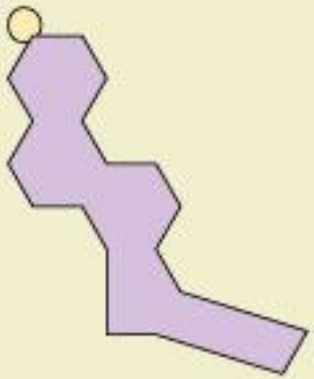
Figure 7-6C

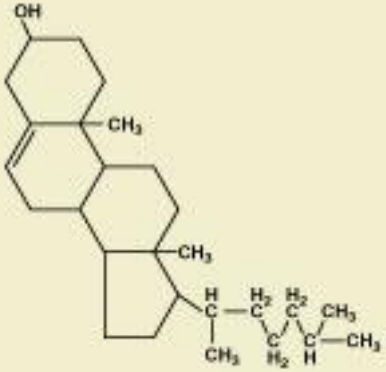
c) STEROLS

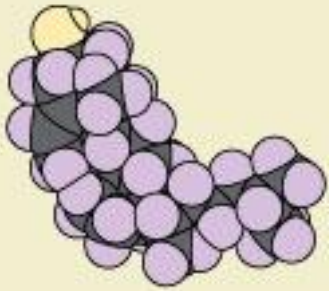
- **Cholesterol (shown)**
- **Campesterol**
- **Sitosterol**
- **Stigmasterol**

Phytosterols

- **Ergosterol**
- **Hopanoids**





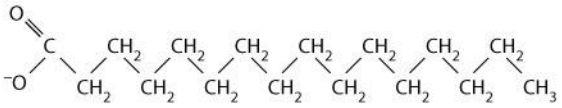
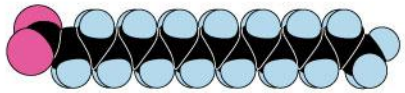
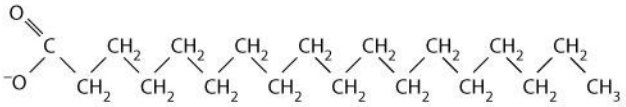
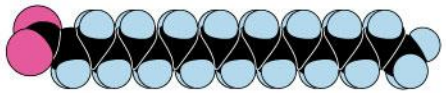
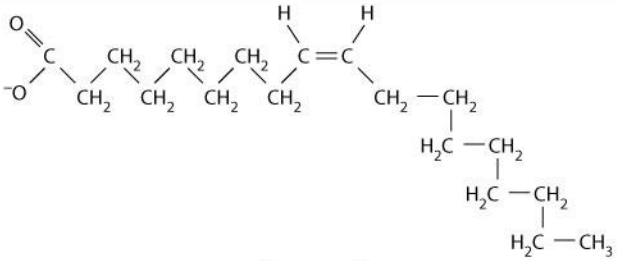
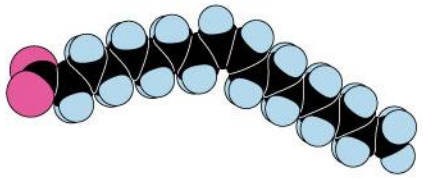
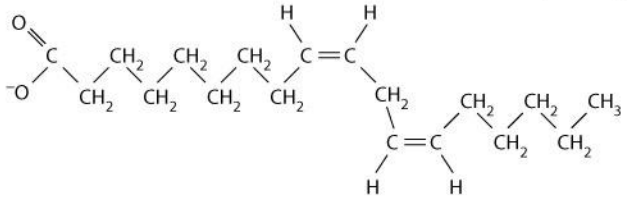
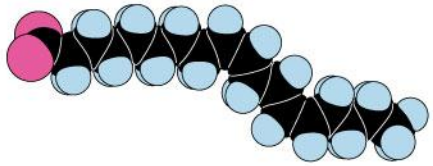


Fatty Acids Are Essential to Membrane Structure and Function

- *Fatty acids* are components of all membrane lipids except the sterols
- Their long hydrocarbon tails provide a barrier to diffusion of polar solutes
- The sizes of membrane fatty acids range between 12–20 carbons long, which is optimal for bilayer formation and dictates the usual thickness of membranes (6–8 nm)

Table 7-2

Table 7-2 Structures of Some Common Fatty Acids Found in Lipid Bilayers

Name of Fatty Acid	Number of Carbon Atoms	Number of Double Bonds	Structural Formula	Space-Filling Model
Saturated				
Palmitate	16	0		
Stearate	18	0		
Unsaturated				
Oleate	18	1		
Linoleate	18	2		

Fatty acids vary in degree of saturation

- Fatty acids vary considerably in the presence and number of double bonds
- *Palmitate* (16C) and *stearate* (18C) are common saturated fatty acids
- *Oleate* (one double bond) and *linoleate* (two double bonds), are both 18C unsaturated fatty acids

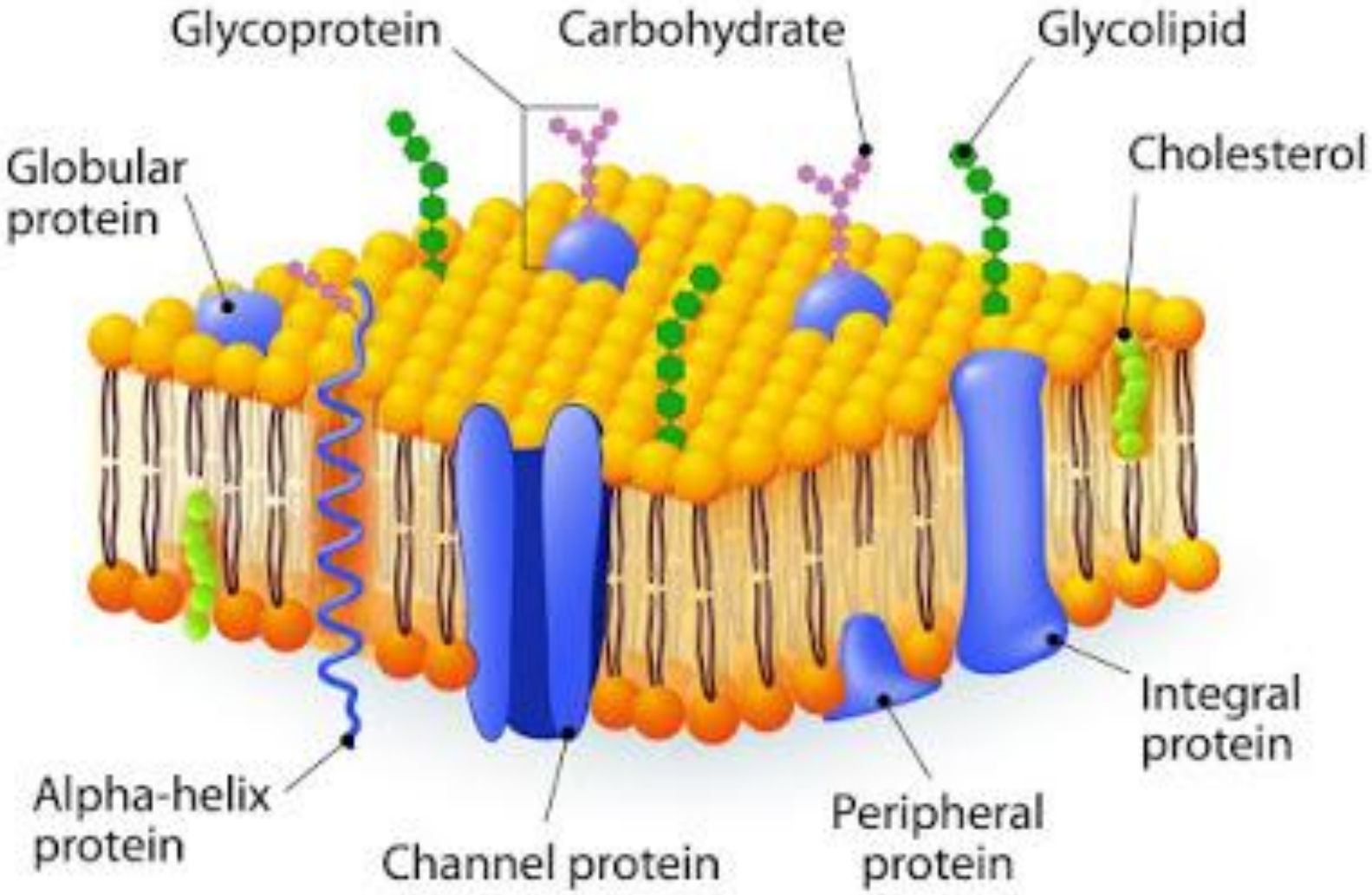
Membrane Asymmetry: Most Lipids Are Distributed Unequally Between the Two Monolayers

- Membrane asymmetry is the difference between the monolayers regarding the

1. kind of lipids present
2. the degree of saturation of fatty acids in the phospholipids

- Most of the glycolipids in the plasma membrane of animal cells are in the outer layer

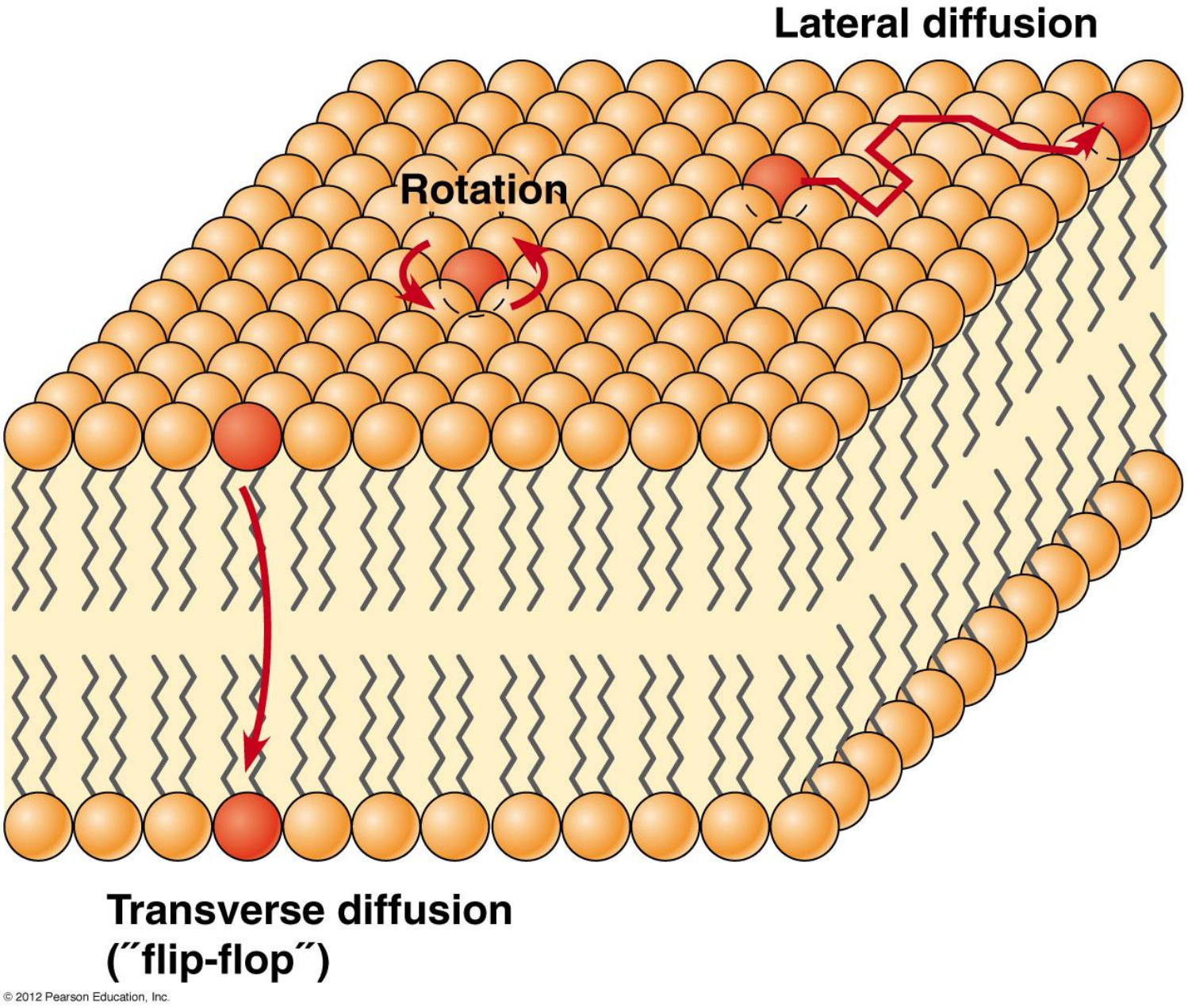
CELL MEMBRANE



Membrane asymmetry tends to be maintained

- Once established, membrane asymmetry does not change much
- The movement of lipids from one monolayer to another requires their hydrophilic heads to move all the way through the hydrophobic interior of the bilayer
- This **transverse diffusion** (or “flip-flop”) is relatively rare

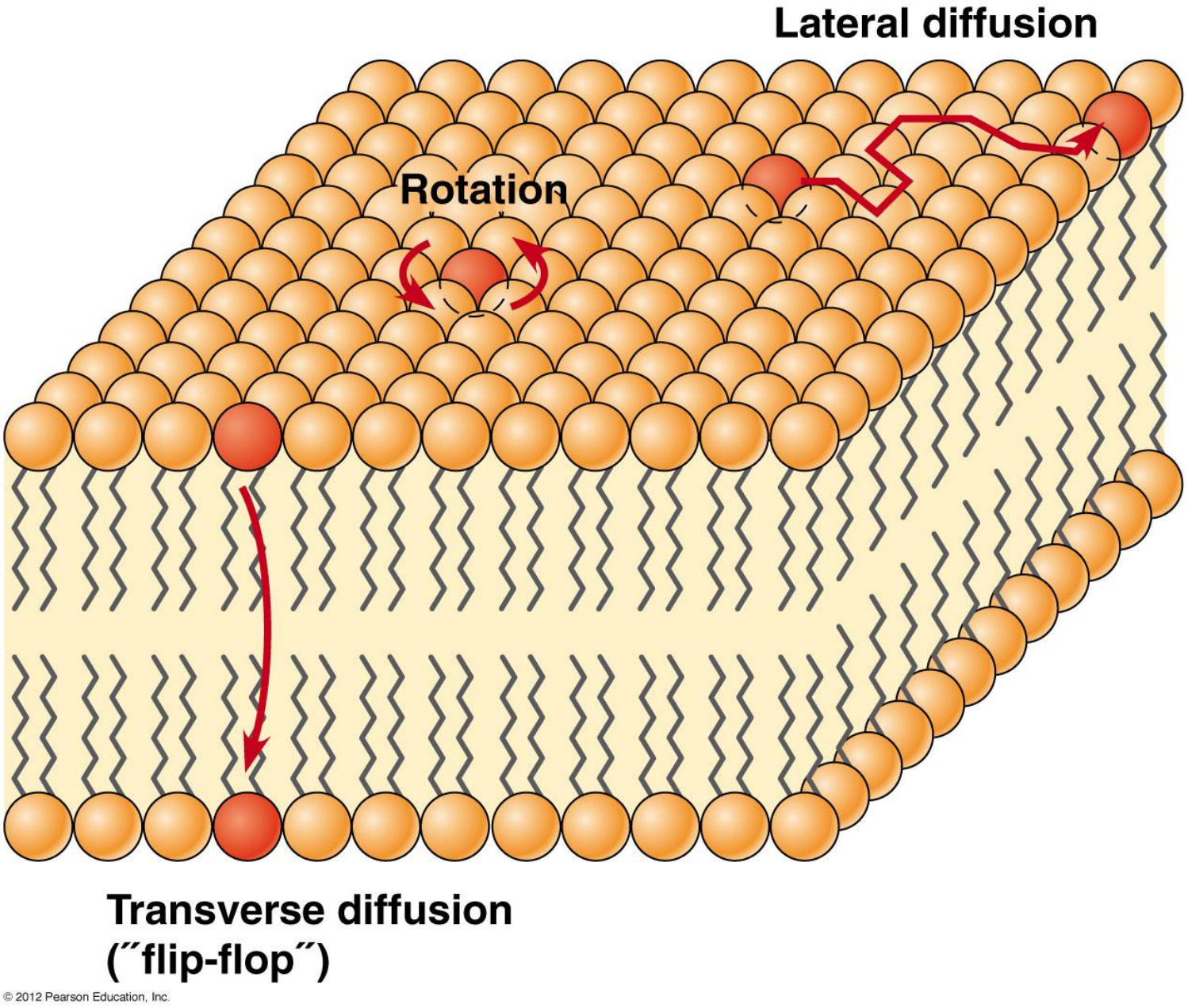
Figure 7-10



Lipids move freely within their monolayer

- Lipids are mobile within their monolayer
- **Rotation** of phospholipids about their axes can occur
- Phospholipids can also move within the monolayer, via **lateral diffusion**
- Both types of movement are rapid and random

Figure 7-10



Transverse diffusion does occur

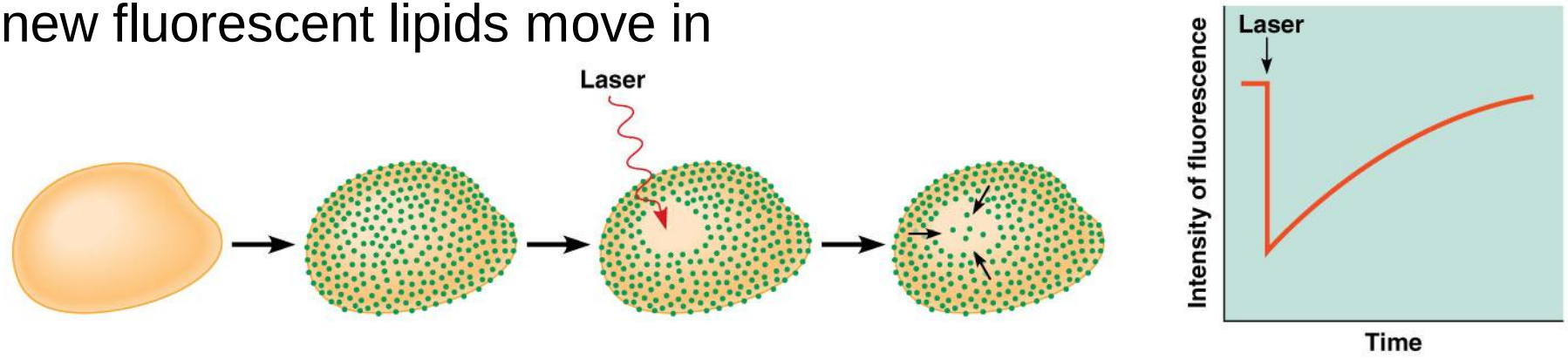
- Though rare, phospholipid flip-flop does occur in natural membranes
- Some membranes, in particular the smooth ER membrane, have proteins that catalyze the flip-flop of membrane lipids
- These proteins are called **phospholipid translocators** or **flippases**

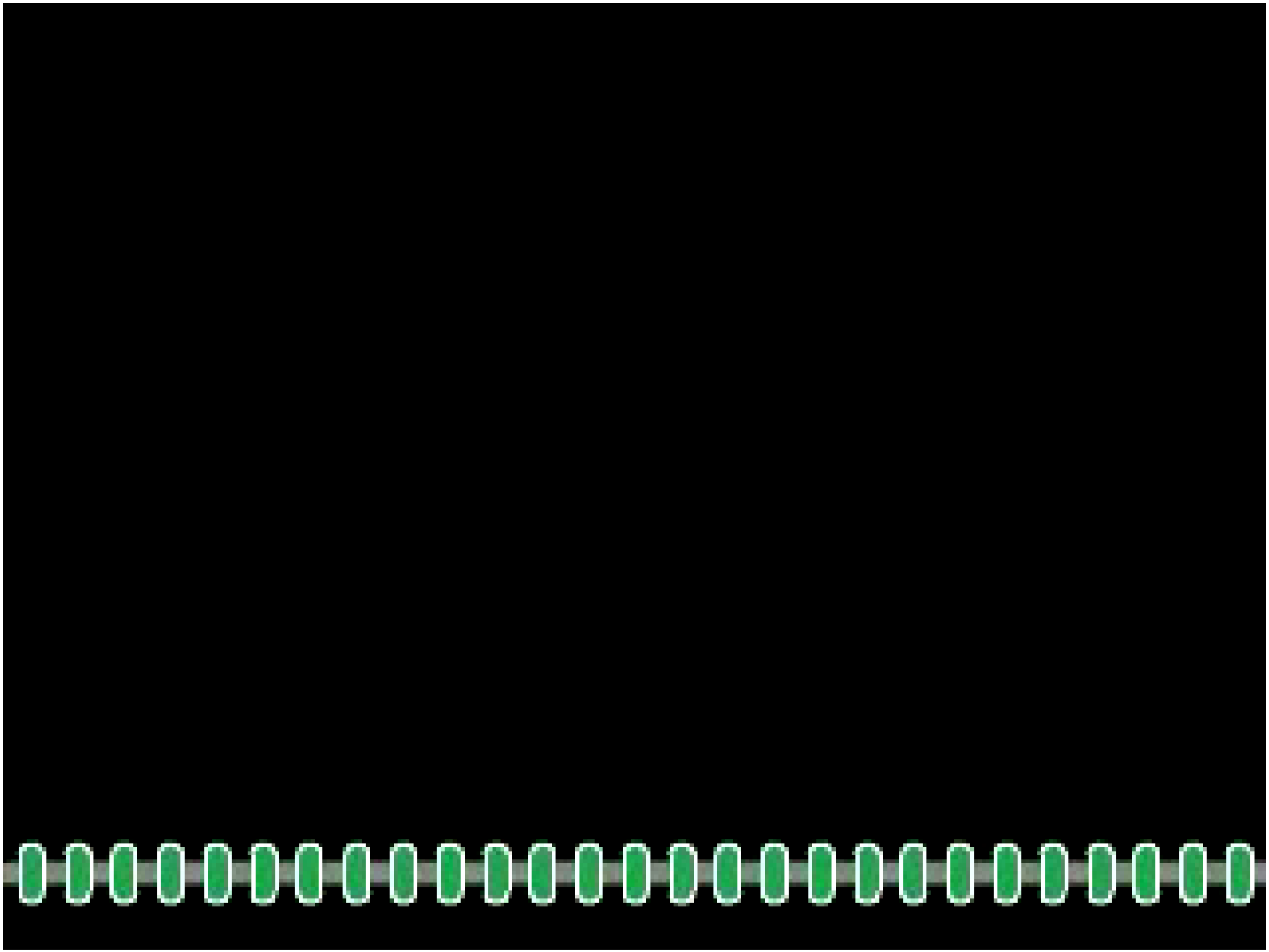
The Lipid Bilayer Is Fluid

- The lipid bilayer behaves as a fluid that permits the movement of both lipids and proteins
- Lipids can move as much as several μm per second within the monolayer
- Lateral diffusion can be demonstrated using **fluorescence recovery after photobleaching** (FRAP)

Measuring lipid mobility with FRAP

- Investigators label lipid molecules in a membrane with a fluorescence dye
- A laser beam is used to bleach the dye in a small area, creating a dark spot on the membrane
- The membrane is observed afterward to determine how long it takes for the dark spot to disappear, a measure of how quickly new fluorescent lipids move in





Membranes Function Properly Only in the Fluid State

- Membrane fluidity changes with temperature, decreasing as temperature falls and vice versa

- Every lipid bilayer has a characteristic **transition temperature T_m**, the **temperature at which it becomes fluid**

This change of state is called a **phase transition**, in this case from solid to liquid

Below the T_m, any functions that rely on membrane fluidity will be disrupted

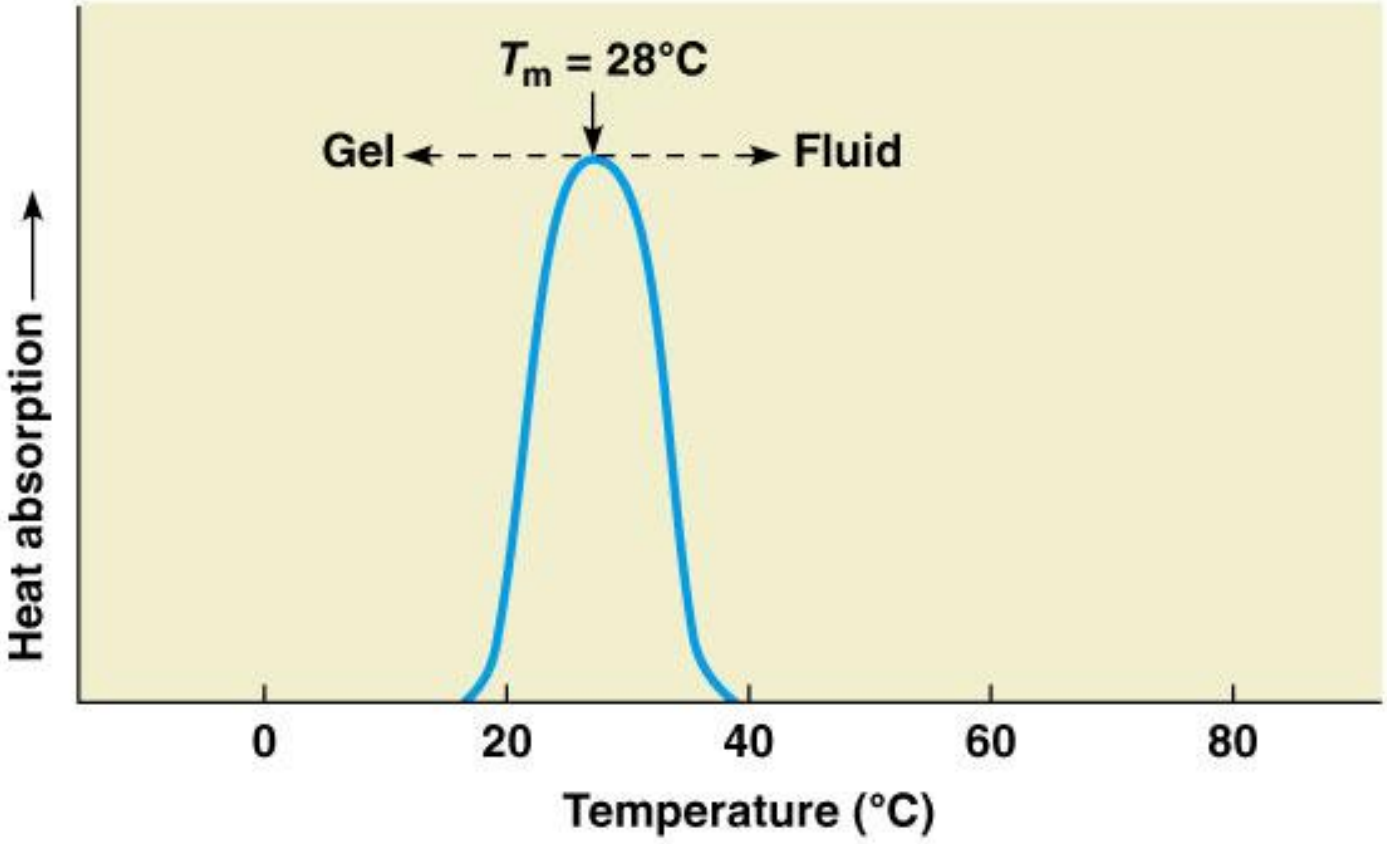
< **Tm** <

Measuring the transition temperature of a membrane

- The transition temperature can be measured by **differential scanning calorimetry**
- The membrane is placed inside a *calorimeter* and the uptake of heat is measured as temperature is increased
- The T_m is the point of maximum heat absorption as the membrane changes from the gel to the fluid state

Figure 7-12A

(a) Normal membrane. When the temperature of a typical membrane preparation is increased slowly in a calorimeter chamber, a peak of heat absorption marks the gel-to-fluid transition temperature, T_m.



Effects of Fatty Acid Composition on Membrane Fluidity

- Fluidity of a membrane depends mainly on the fatty acids that it contains

1. The length of fatty acid chains
2. The degree of saturation

- Long-chain and saturated fatty have higher T_m, whereas short-chain and unsaturated fatty acids have lower T_ms

Figure 7-12B

(b) Membranes enriched in unsaturated or saturated fatty acids. Membranes from cells grown in media enriched in the unsaturated fatty acid oleate (left) are more fluid than normal membranes (lower T_m). Membranes from cells grown in media enriched in the saturated fatty acid stearate (right) are less fluid than normal membranes (higher T_m).

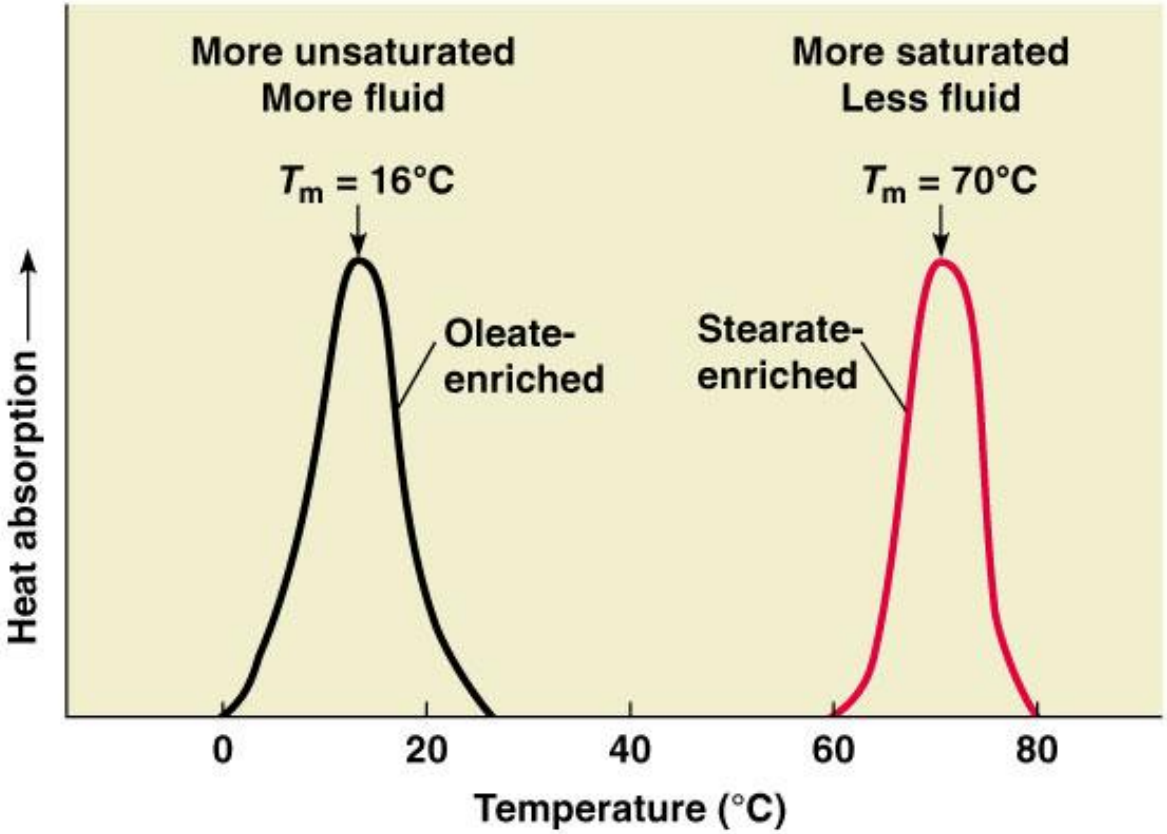
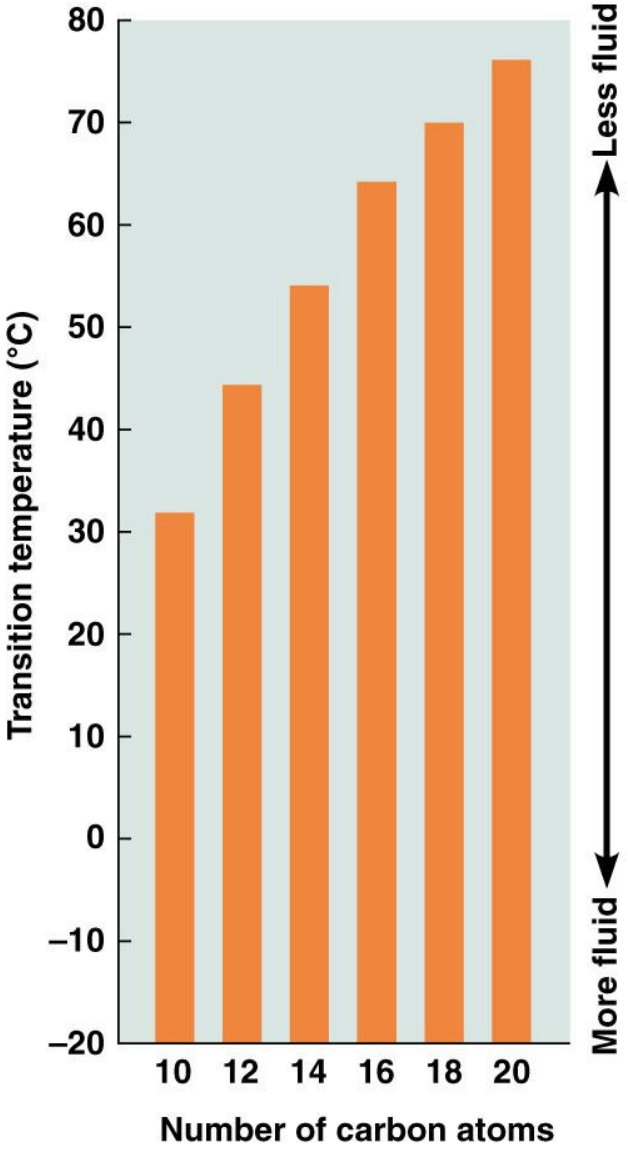
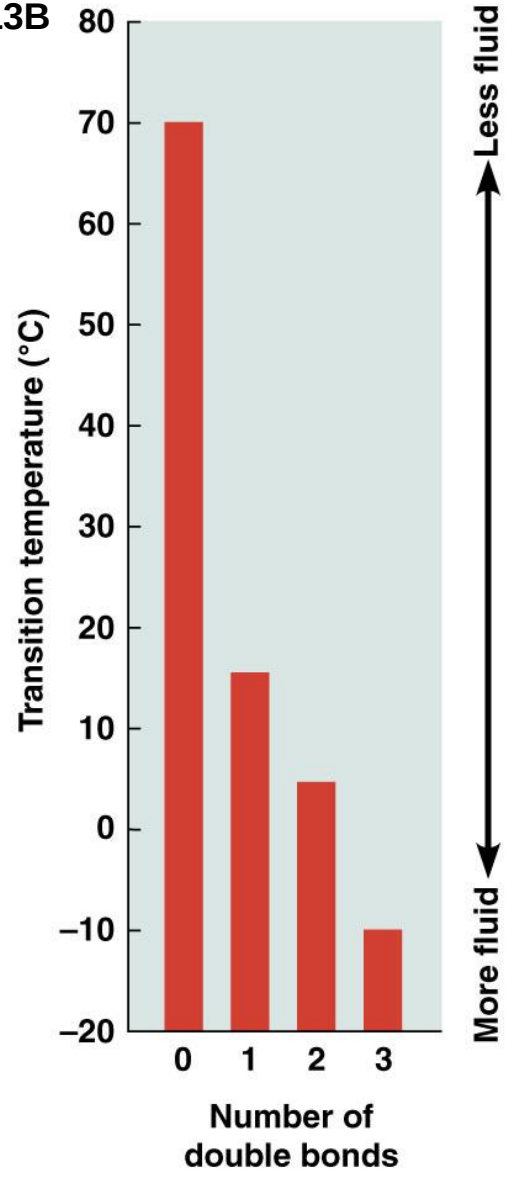


Figure 7-13A



(a) Effect of chain length on the melting point

Figure 7-13B

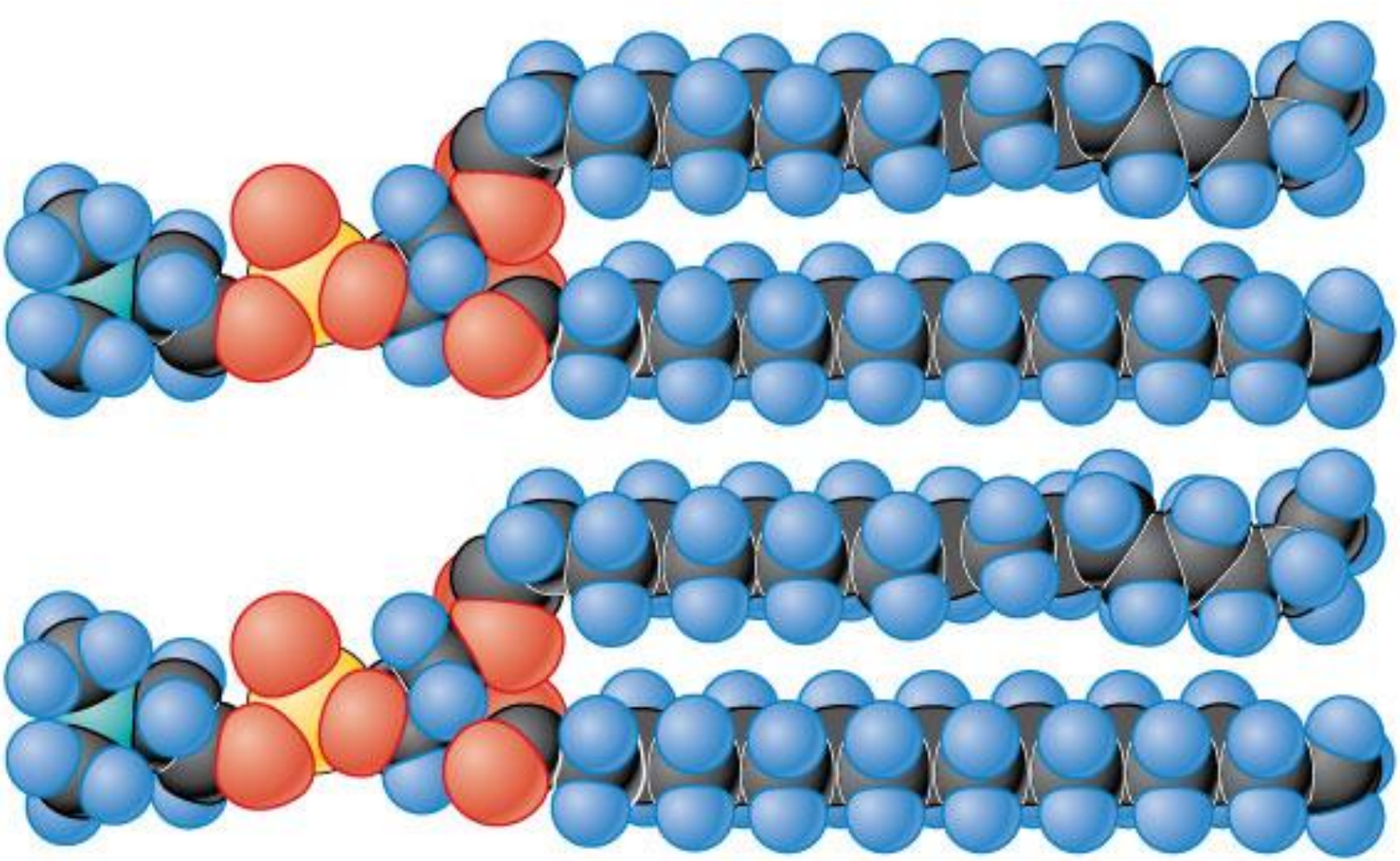


(b) Effect of unsaturation on the melting point

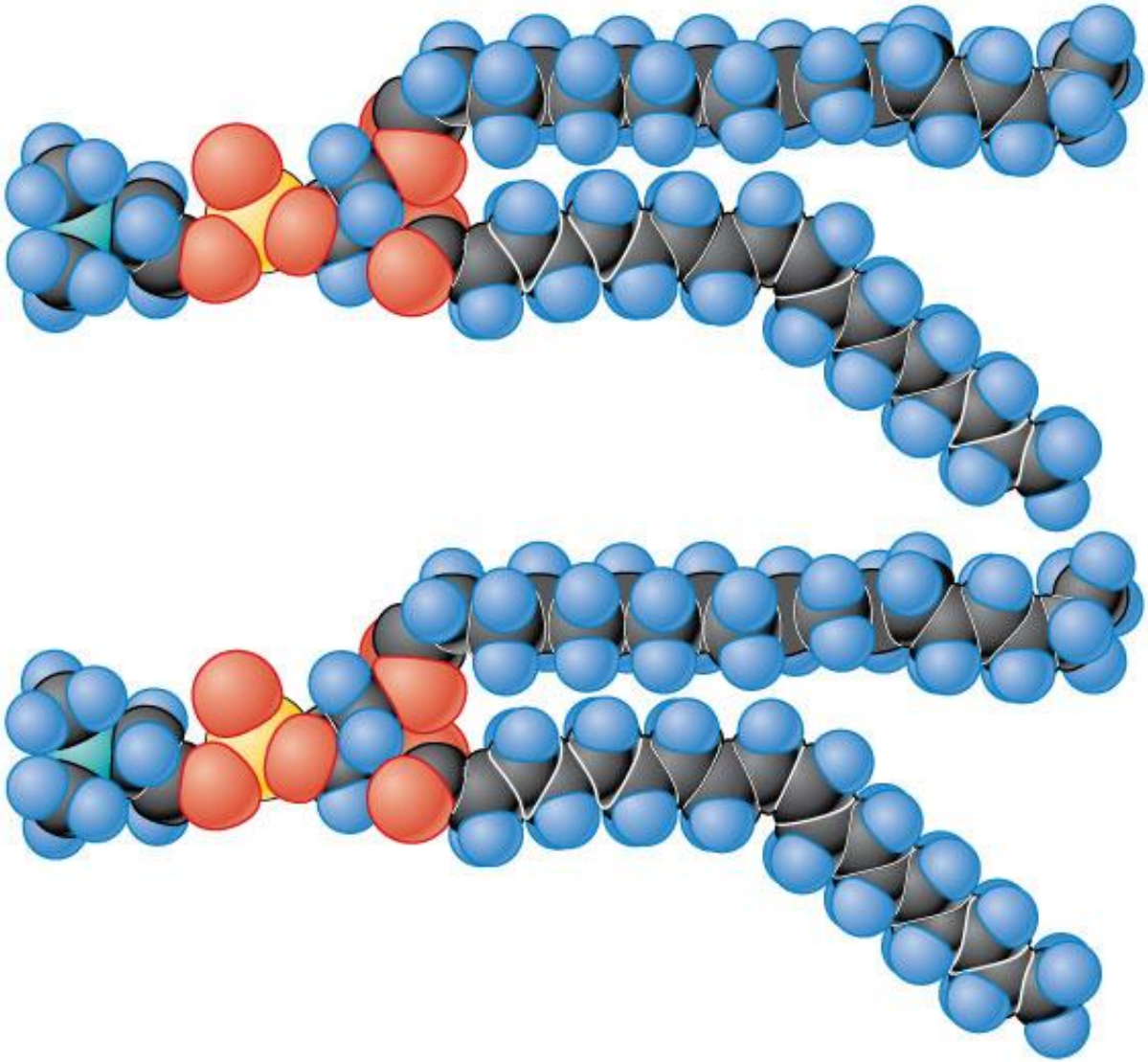
Fatty acid saturation and membrane fluidity

- Saturated fatty acids pack together well in the membrane
- Fatty acids with one or more double bonds have bends in the chains that prevent them from packing together neatly
- Thus unsaturated fatty acids are more fluid than saturated fatty acids, and have a lower T_m

Figure 7-14A

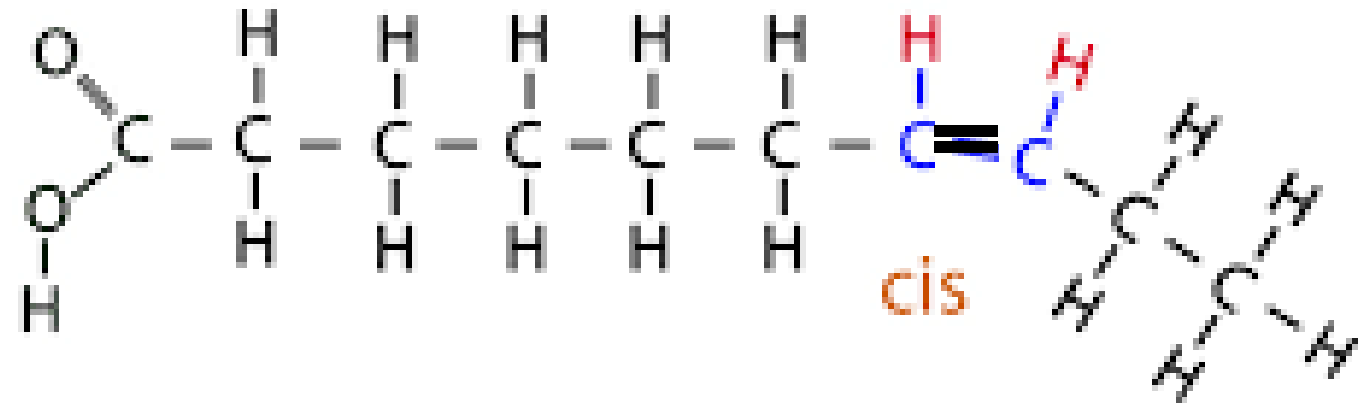


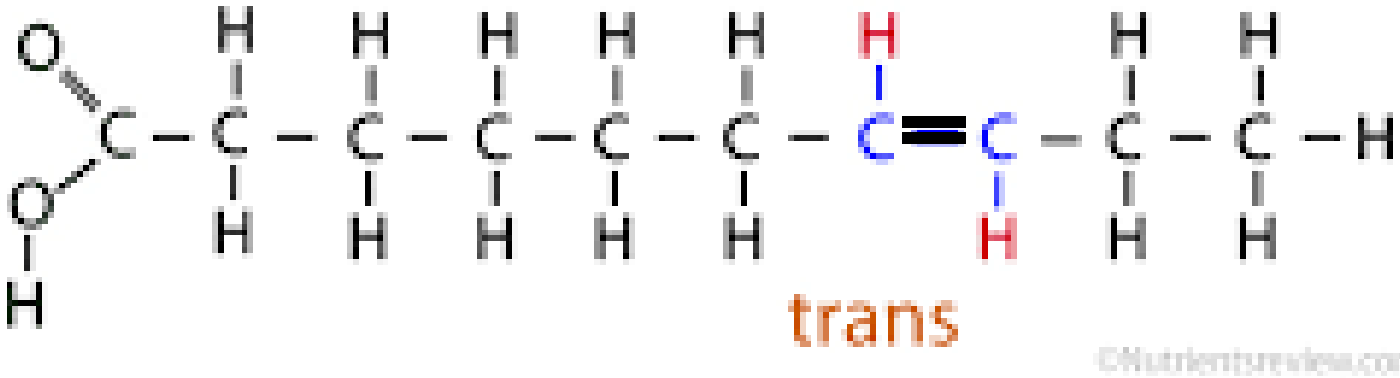
(a) Lipids with saturated fatty acids pack together well in the membrane

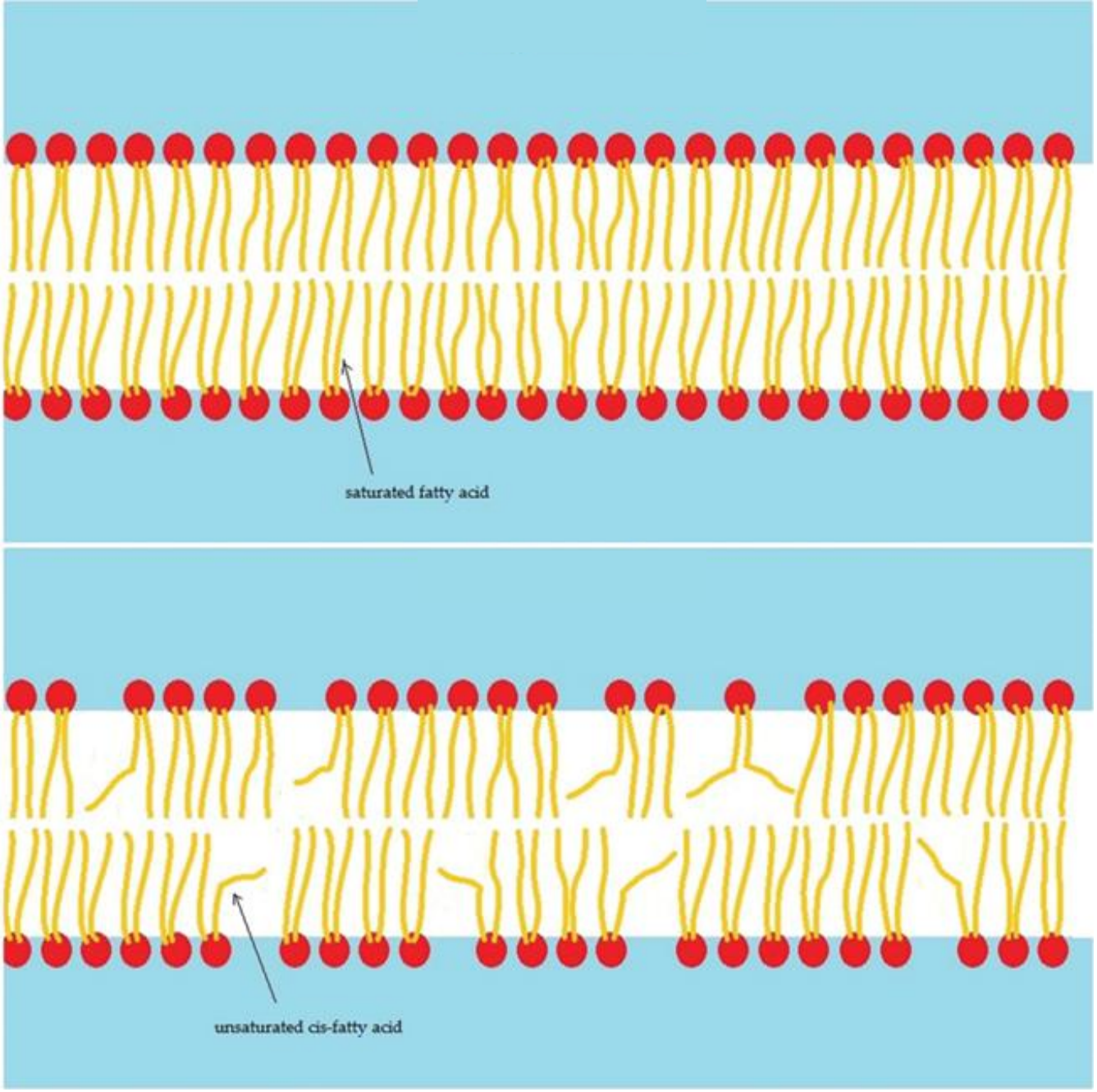


(b) Lipids with a mixture of saturated and unsaturated fatty acids do not pack together well in the membrane

Cis- and Trans-Fatty Acids





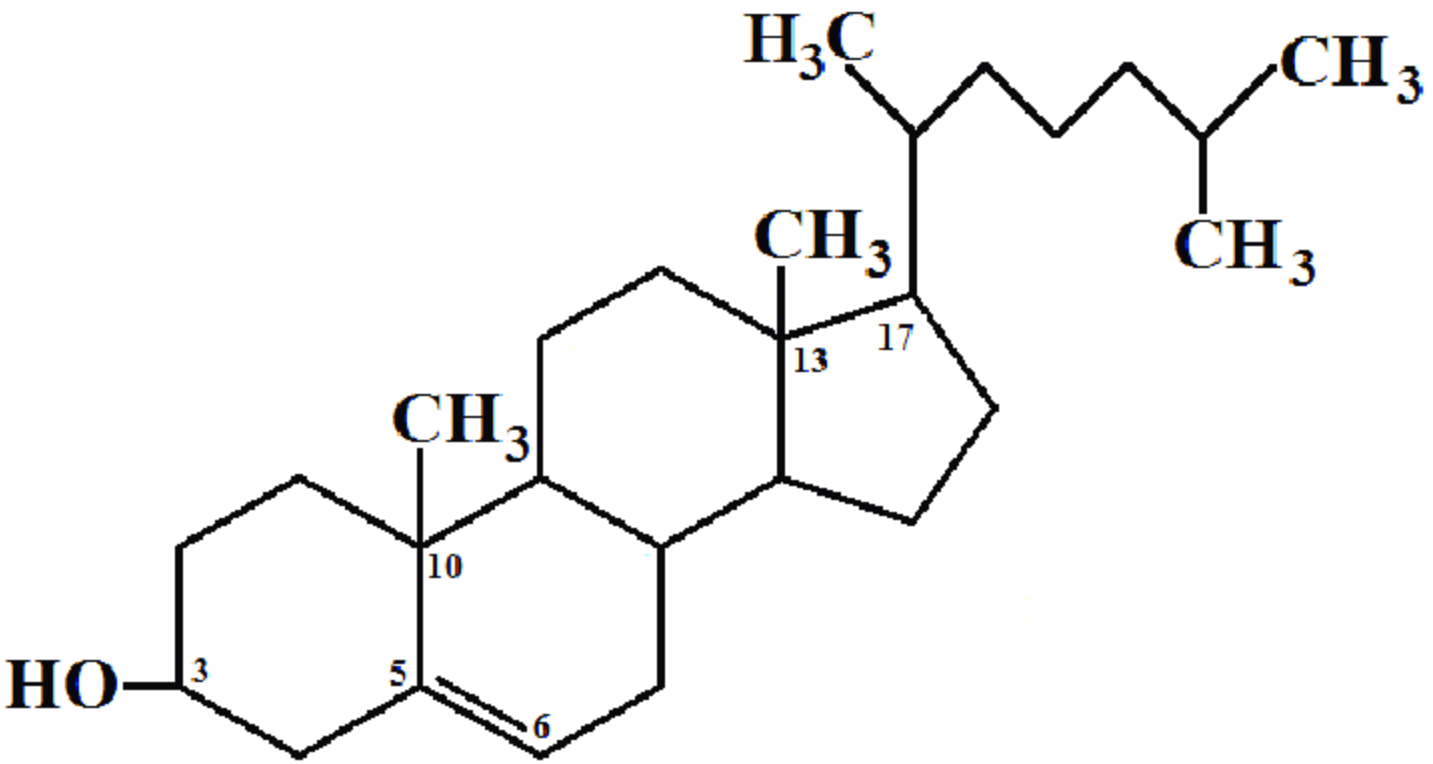


Membrane composition

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

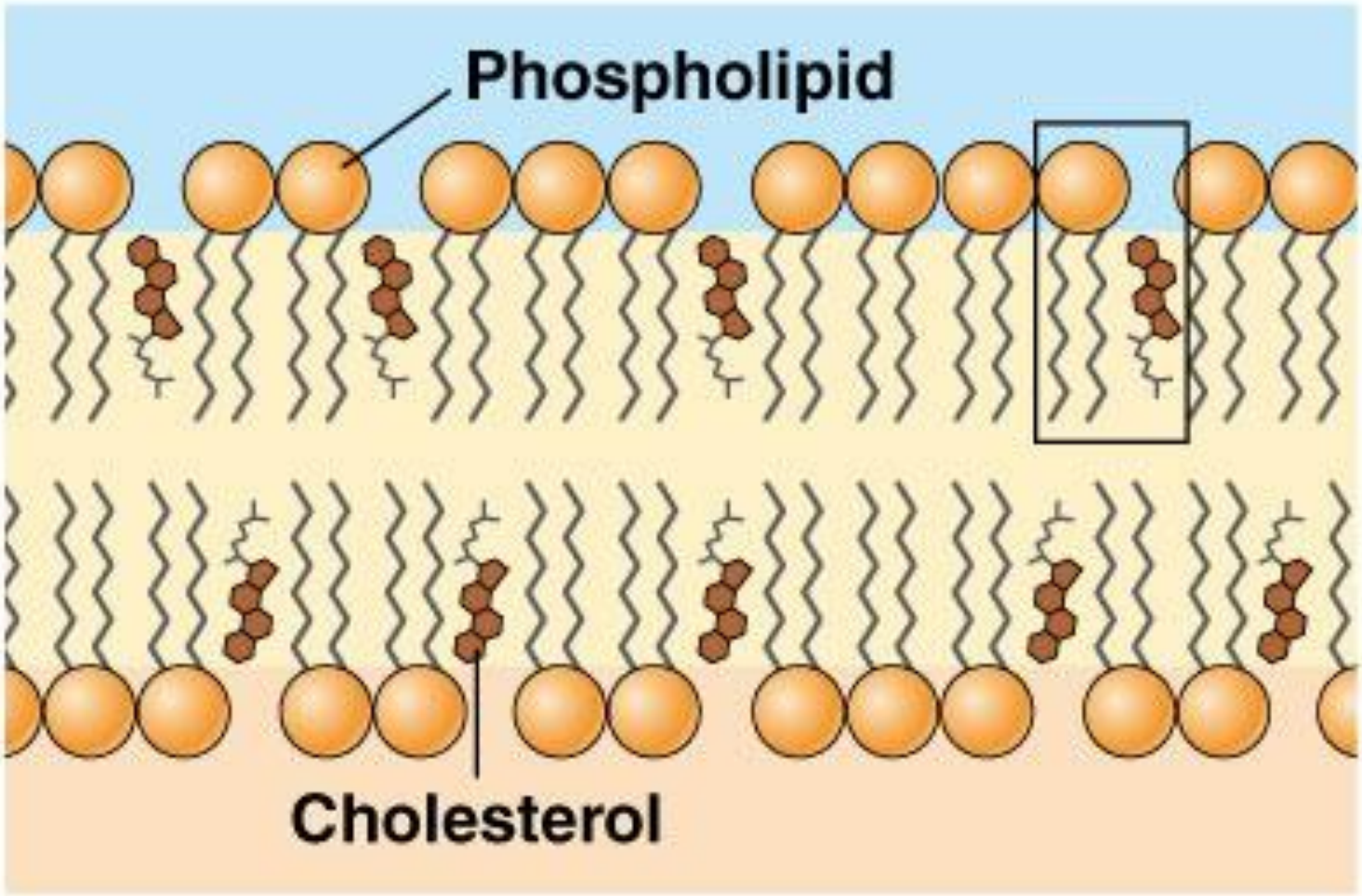
Effects of Sterols on Membrane Fluidity

- Membrane fluidity is influenced by sterols
- The intercalation of rigid cholesterol molecules into a membrane *decreases its fluidity and increases the* T_m
- However, cholesterol also prevents hydrocarbon chains of phospholipids from packing together tightly and so *reduces the tendency of membranes to gel upon cooling*



Cholesterol

Figure 7-15A

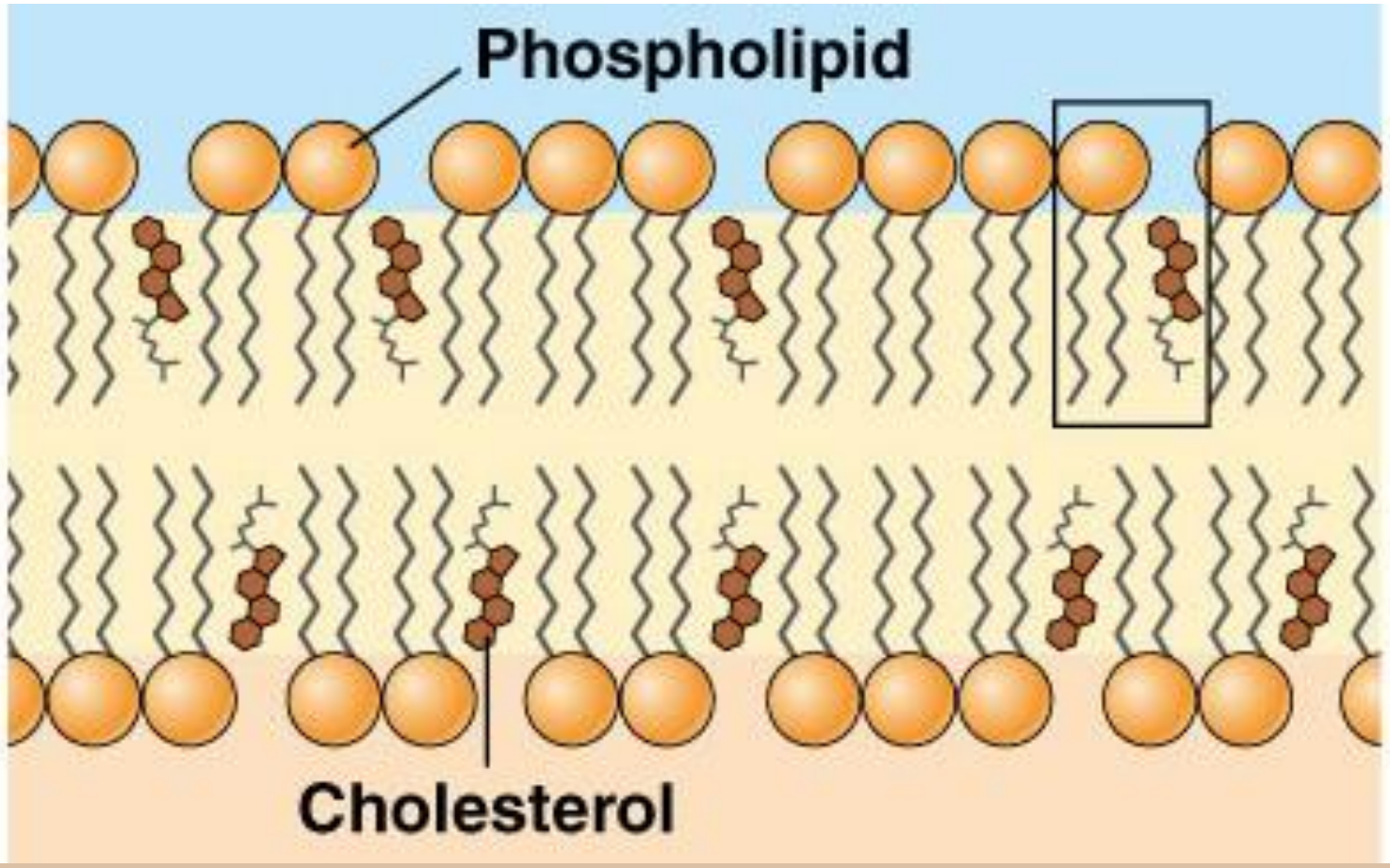


(a) Cholesterol in plasma membrane

Cholesterol is a fluidity buffer

T > T_m Cholesterol **decreases** membrane fluidity

T < T_m Cholesterol **increases** membrane fluidity



Other effects of sterols on membranes

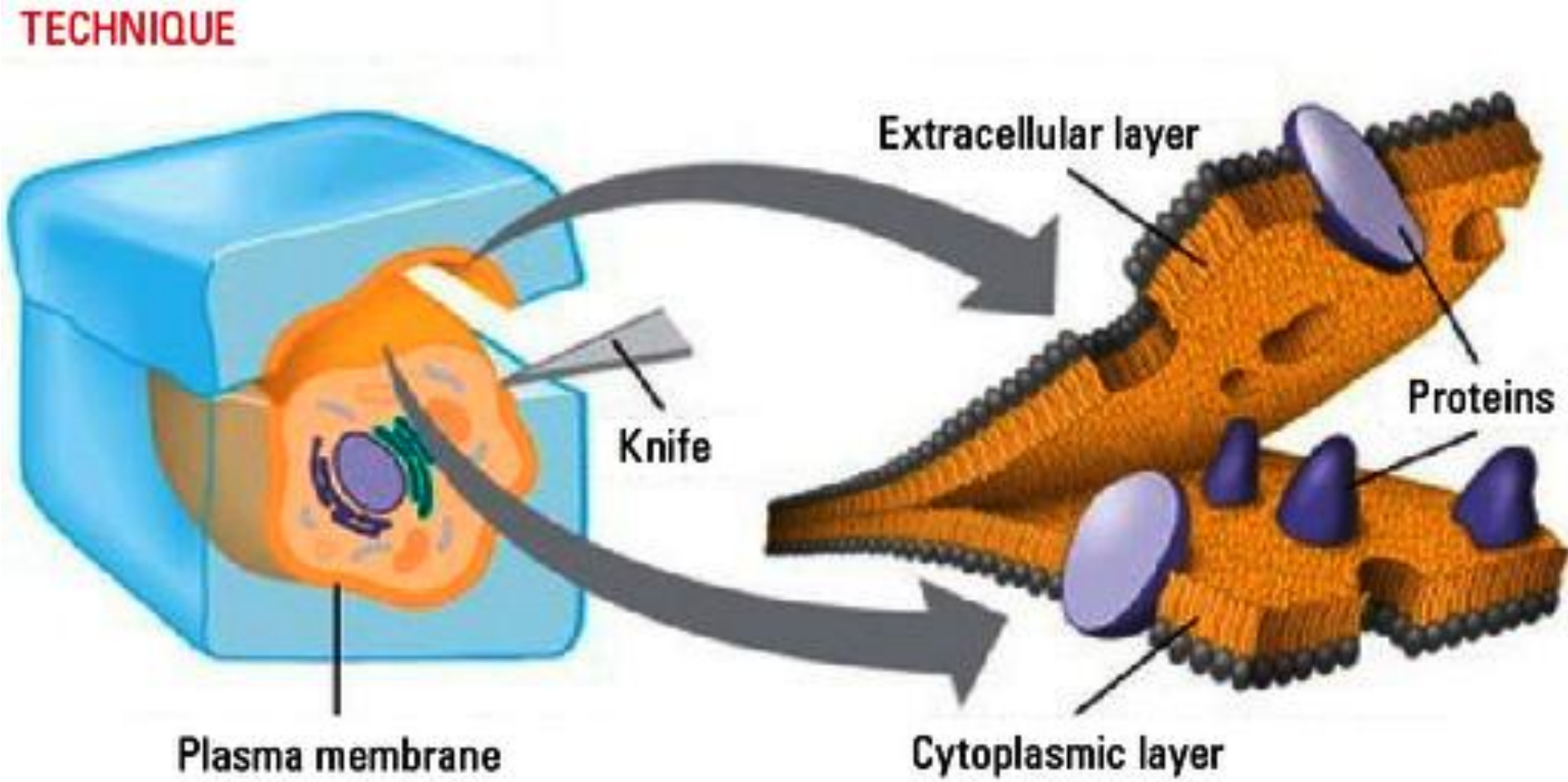
- Sterols decrease the permeability of membranes to ions and small polar molecules
- This is likely because they fill spaces between the hydrocarbon chains of phospholipids
- This effectively blocks the routes that ions and small molecules would take through the membrane

Membrane Proteins: the "Mosaic" Part of the Model

- The mosaic part of the fluid mosaic model includes lipid rafts and other lipid domains

- However, it is membrane proteins that are the main components

The membrane consists of a mosaic of proteins: evidence from freeze facture microscopy



Membranes Contain Integral, Peripheral and Lipid-Anchored Proteins

- Membrane proteins have different hydrophobicites and so occupy different positions in or on membranes
- This, in turn, determines how easily such proteins can be extracted from membranes
- Membrane proteins fall into three categories: *integral, peripheral,* and *lipid-anchored*

Integral Membrane Proteins

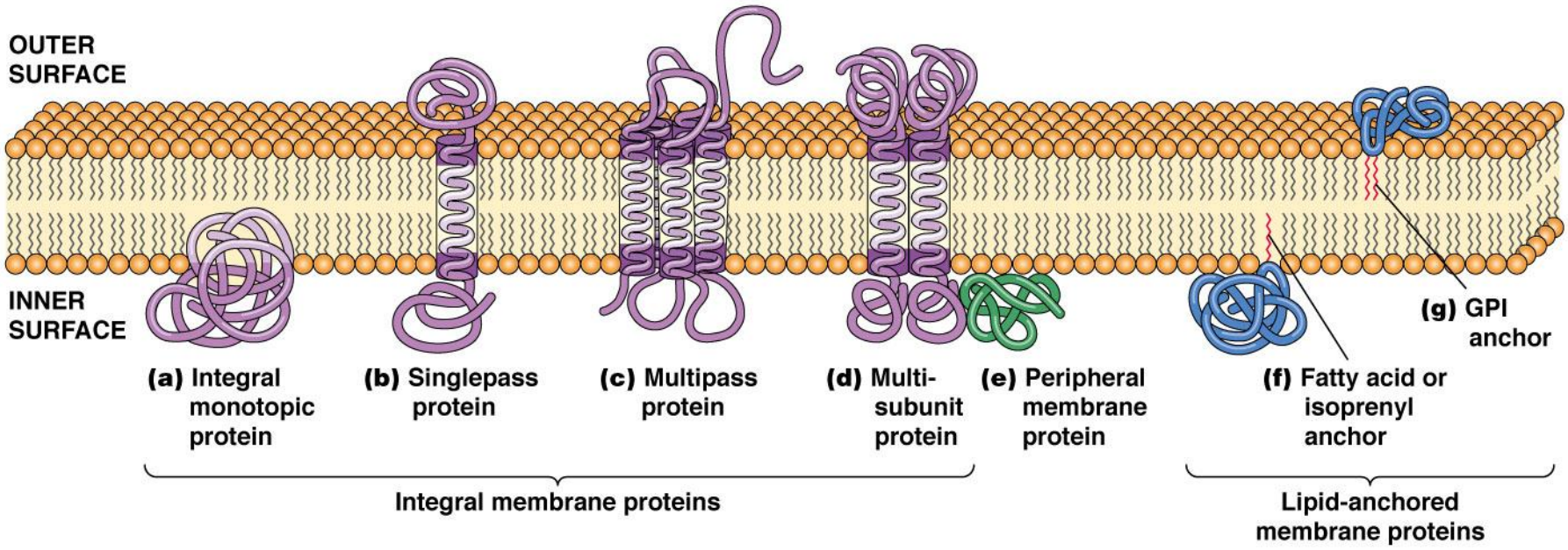
- Most membrane proteins possess one or more hydrophobic regions with an affinity for the interior of the lipid bilayer
- These are **integral membrane proteins**, with hydrophobic regions embedded in the interior membrane bilayer
- They are difficult to remove from membranes by standard isolation procedures

Integral Membrane Proteins

- Some integral membrane proteins, called **integral monotropic proteins**, are embedded in just one side of the bilayer
- However, most are **transmembrane proteins** that span the membrane and protrude on both sides (anchored to the lipid bilayer by one or more hydrophobic **transmembrane segments**)

- Transmembrane proteins cross either once (*singlepass proteins*) or several times (*multipass proteins*)

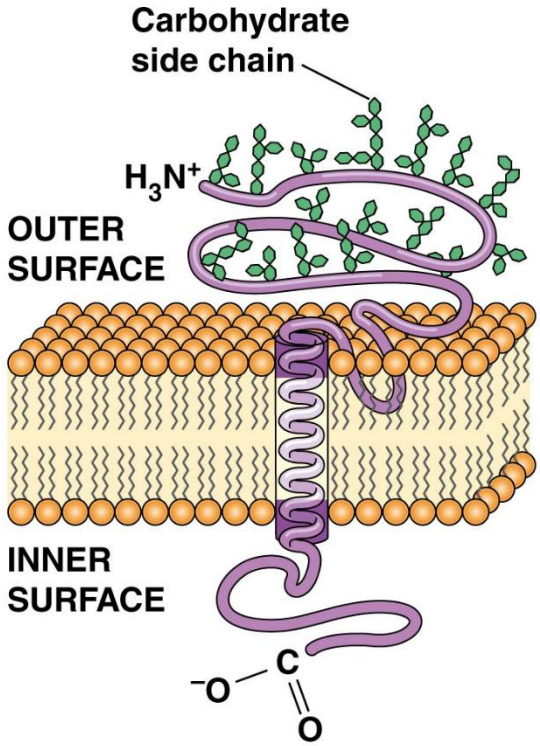
Figure 7-19



Singlepass membrane proteins

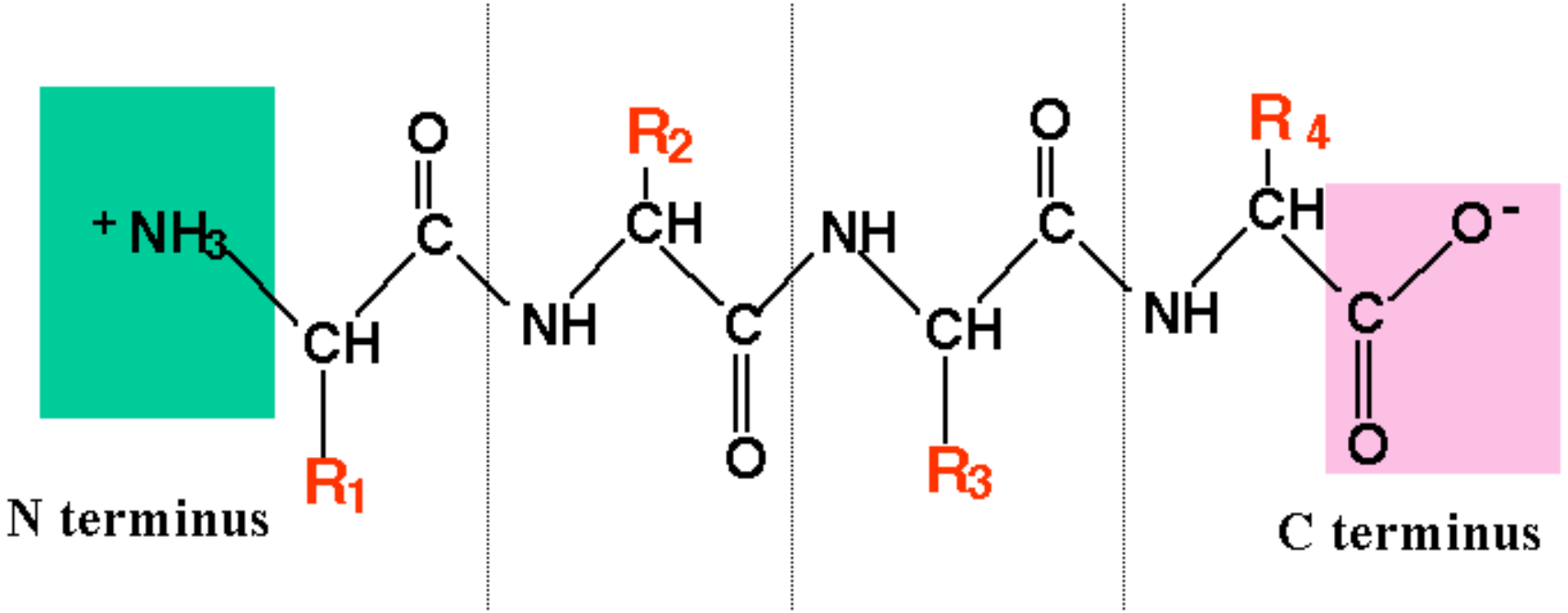
- Singlepass membrane proteins have the
  C-terminus extending from one surface of the membrane and the N-terminus from the other

- For example, *glycophorin* is a singlepass protein on the erythrocyte plasma membrane, that is oriented so the C-terminus is on the inner surface and the N-terminus is on the outer



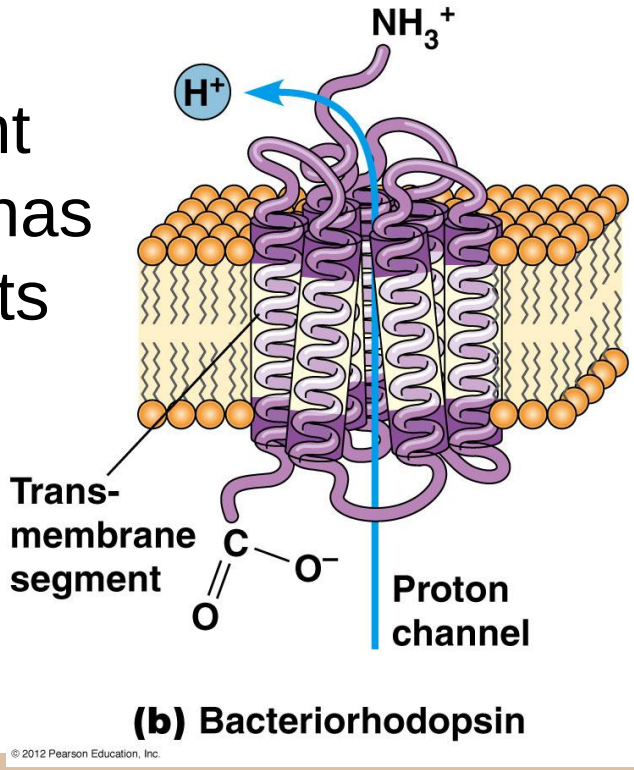
(a) Glycophorin

Peptide = chain of amino acids



Multipass membrane proteins

- Multipass membrane proteins have 2-20 (or more) transmembrane segments
  - For example, *band 3 protein* (*anion exchange protein*) is present in the erythrocyte membrane and has at least 6 transmembrane segments
  - For example, bacteriorhodopsin has 7 transmembrane segments positioned to form a channel



Peripheral Membrane Proteins

- Membrane proteins that lack **discrete** hydrophobic regions do not penetrate the lipid bilayer

- These **peripheral membrane** proteins are bound to membrane surfaces through weak electrostatic forces and hydrogen bonds

- Some hydrophobic residues play a role in anchoring them to the membrane surface

Peripheral membrane proteins (continued)

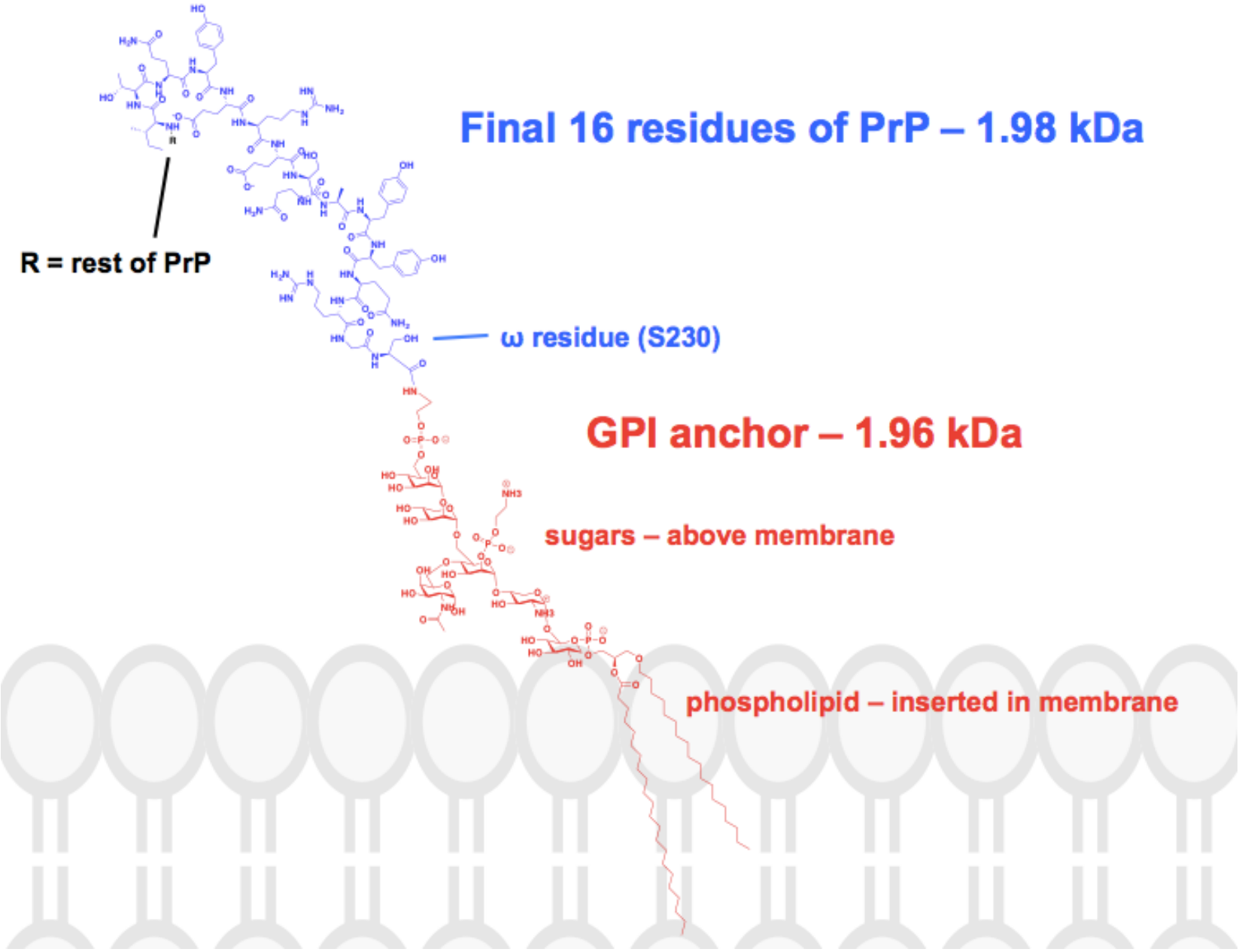
- Peripheral membrane proteins are easily separated from membranes by changing pH or ionic strength
- The main peripheral membrane proteins of erythrocytes are *spectrin*, *ankyrin*, and *band 4.1*
- These are on the inner surface of the plasma membrane

Lipid-Anchored Membrane Proteins

- The polypeptide chains of **lipid-anchored membrane proteins** are located on the surfaces of membranes

- They are *covalently bound* to lipid molecules embedded in the bilayer

- Proteins bound to the inner surface or the external surface of the plasma membrane are linked to fatty acids or GPI
- **GPI-anchored membrane proteins** are covalently linked to *glycosylphosphatidylinositol*



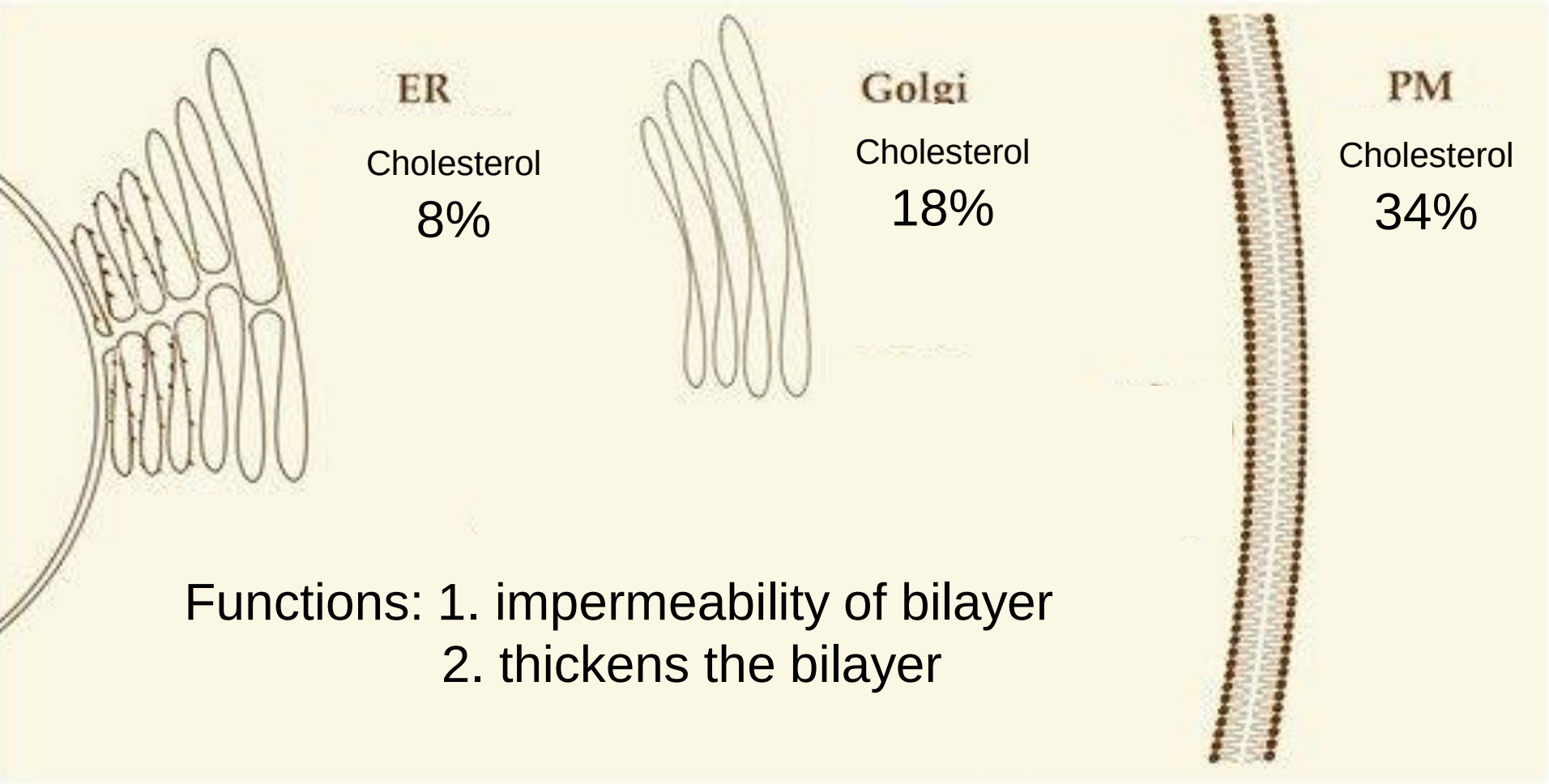
Cellular lipidome vs membrane proteomics

- Proteins received most of the scientific attention
- 1/3 genome encodes for membrane proteins embedded in the bilayer

- Cellular lipidomes may contain up to 7000 distinct lipid species **WHY??**

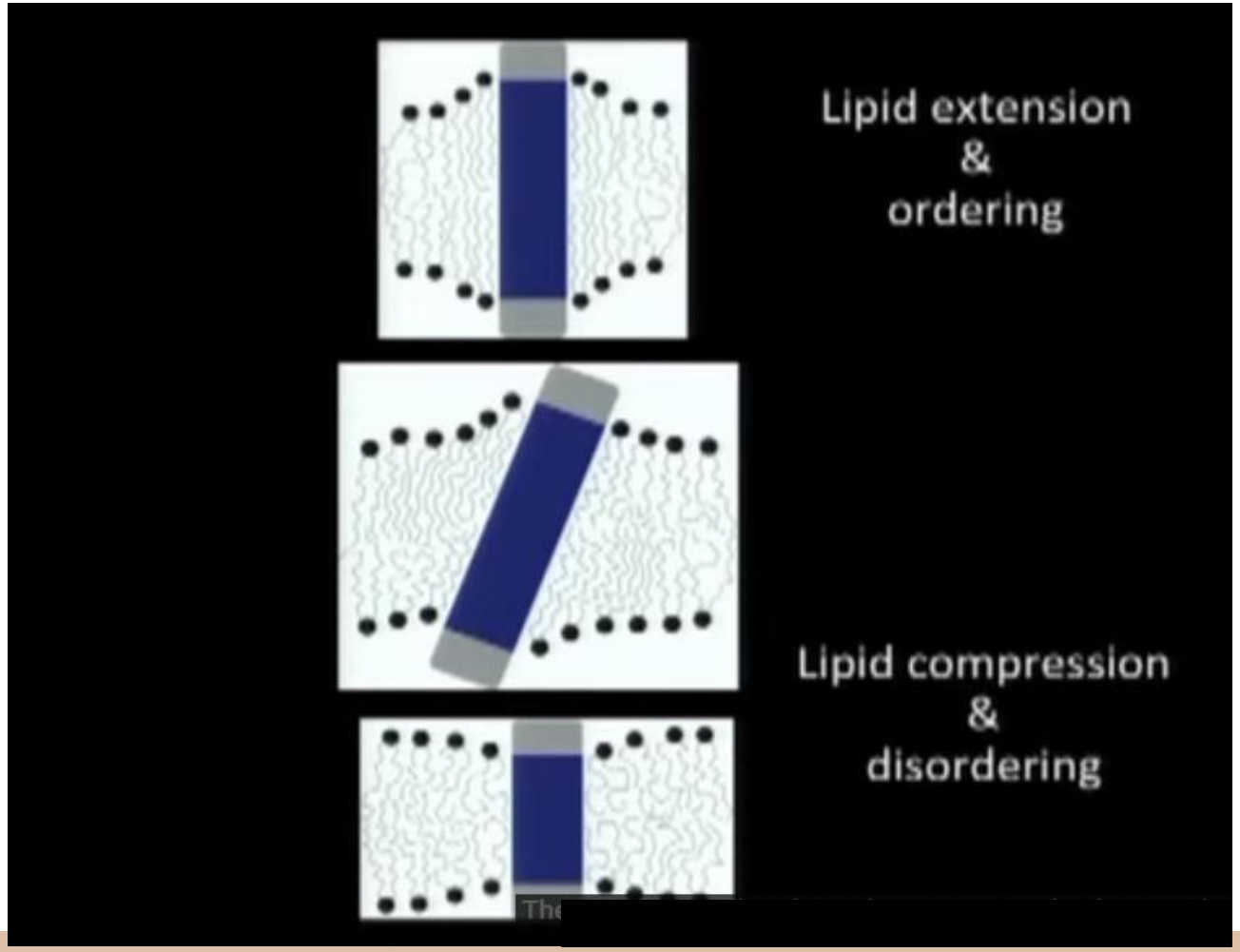
1. Cellular architecture
2. Lipid signaling
3. Regulating membrane proteins
4. Membrane trafficking
5. Part of SUBCOMPARTMENTALIZATION in cells

Lipids are distributed heterogeneously in several ranges: subcellular organelles show lateral differences

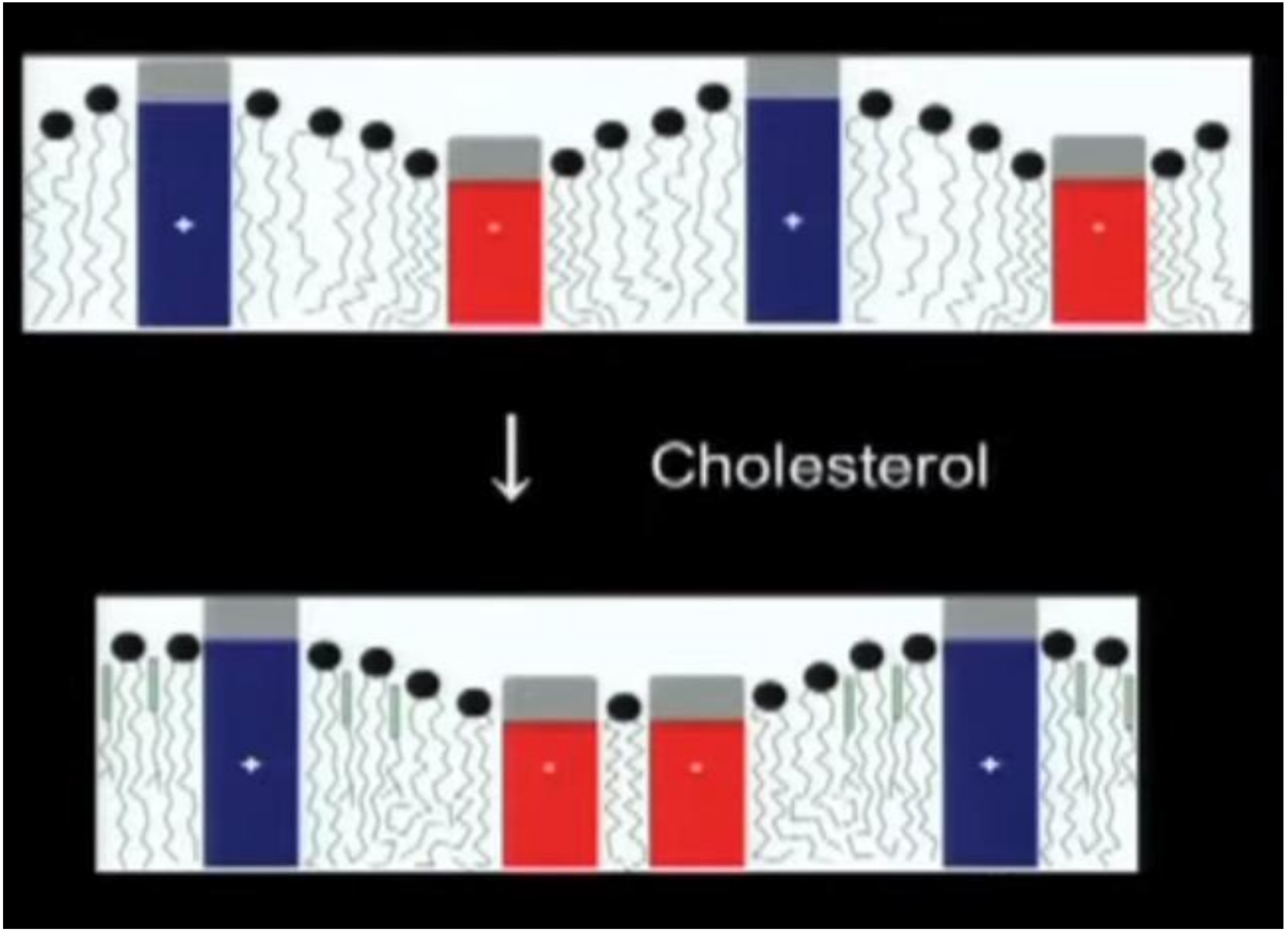


Functions: 1. impermeability of bilayer
2. thickens the bilayer

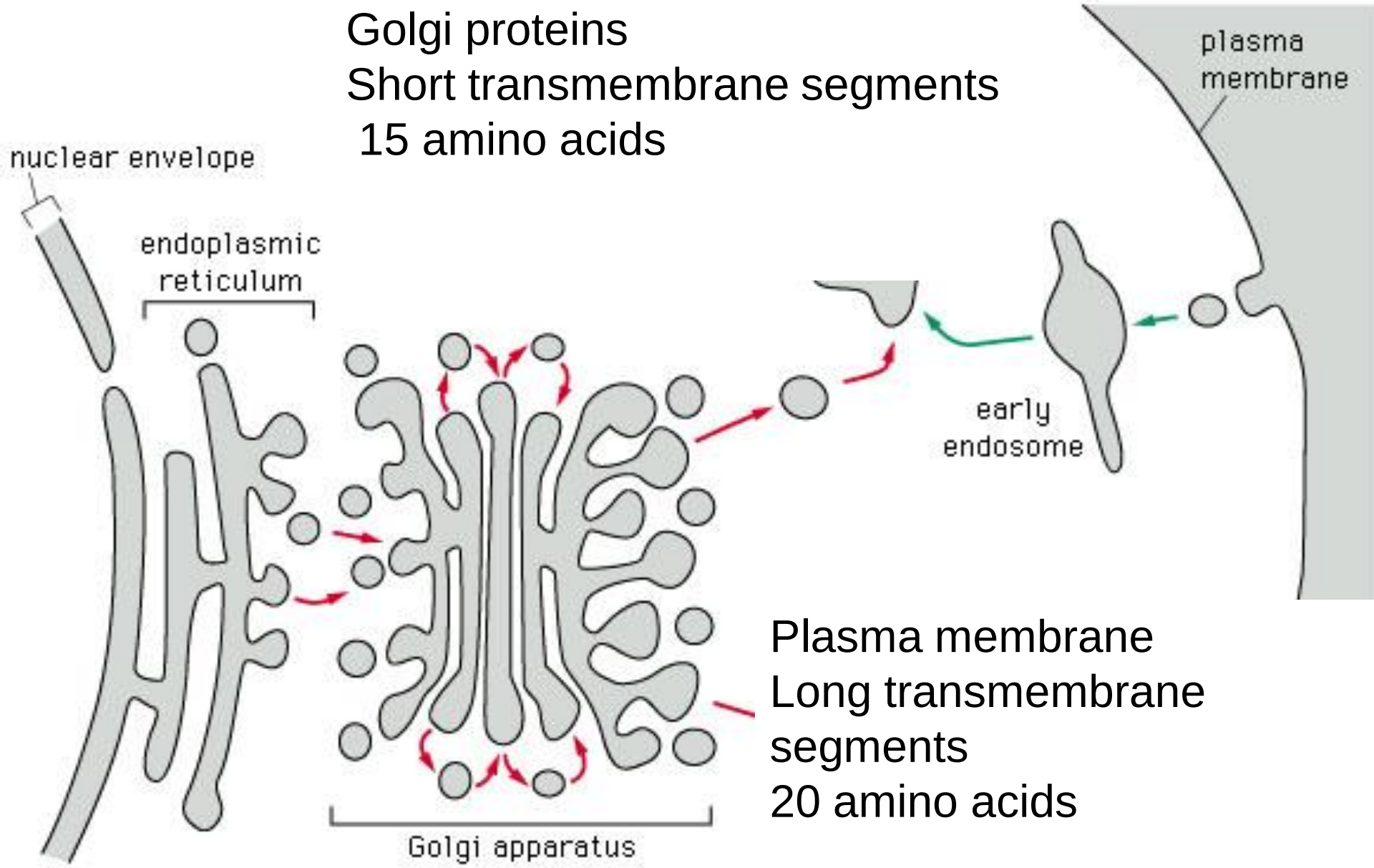
Matching the thickness of the bilayer with hydrophobic transmembrane domains in proteins



Cholesterol induces protein sorting



Golgi proteins
Short transmembrane segments
15 amino acids



Plasma membrane
Long transmembrane segments
20 amino acids

This is NOT enough!!!

ER

Cholesterol 8%
Sphingolipids
(very LOW)

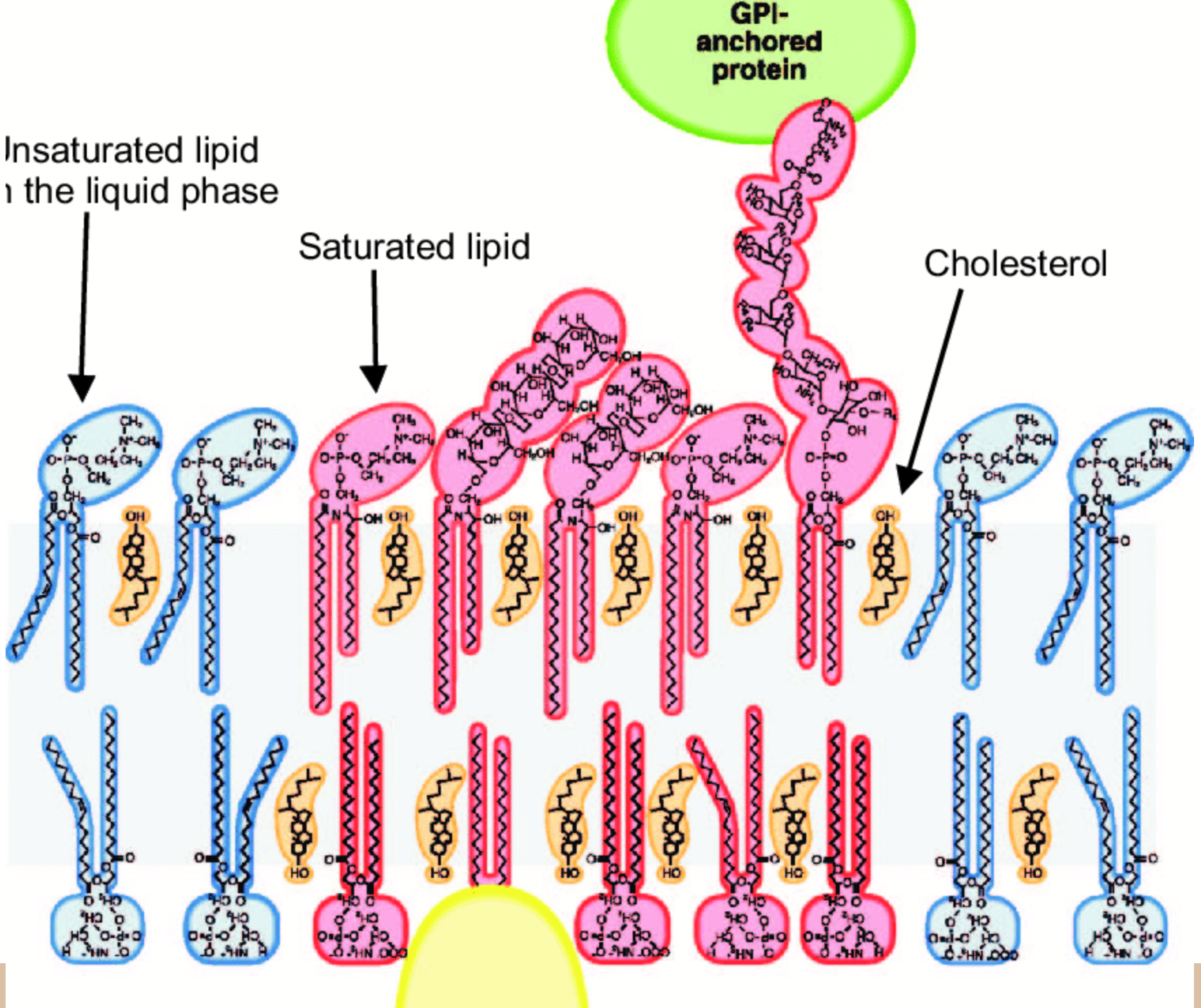
Golgi

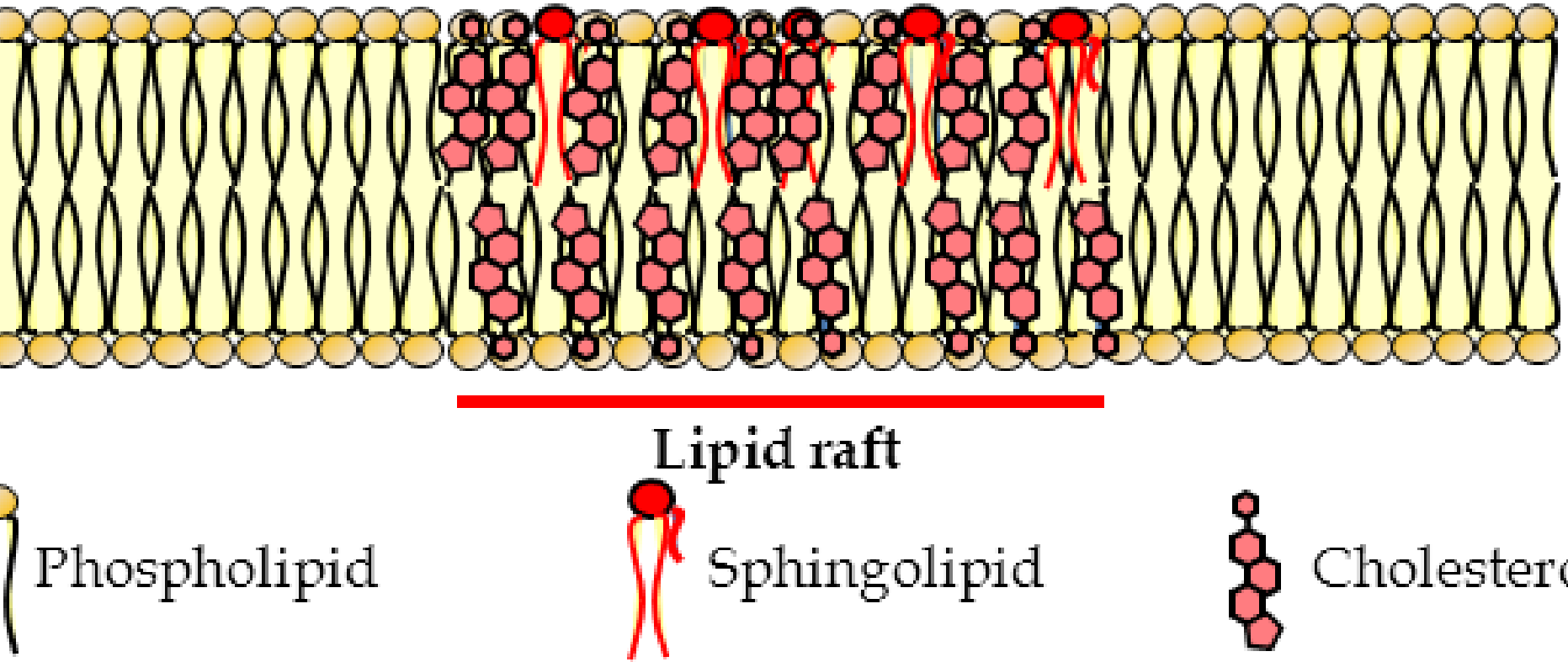
Cholesterol 18%
Sphingolipids
(LOW)

PM

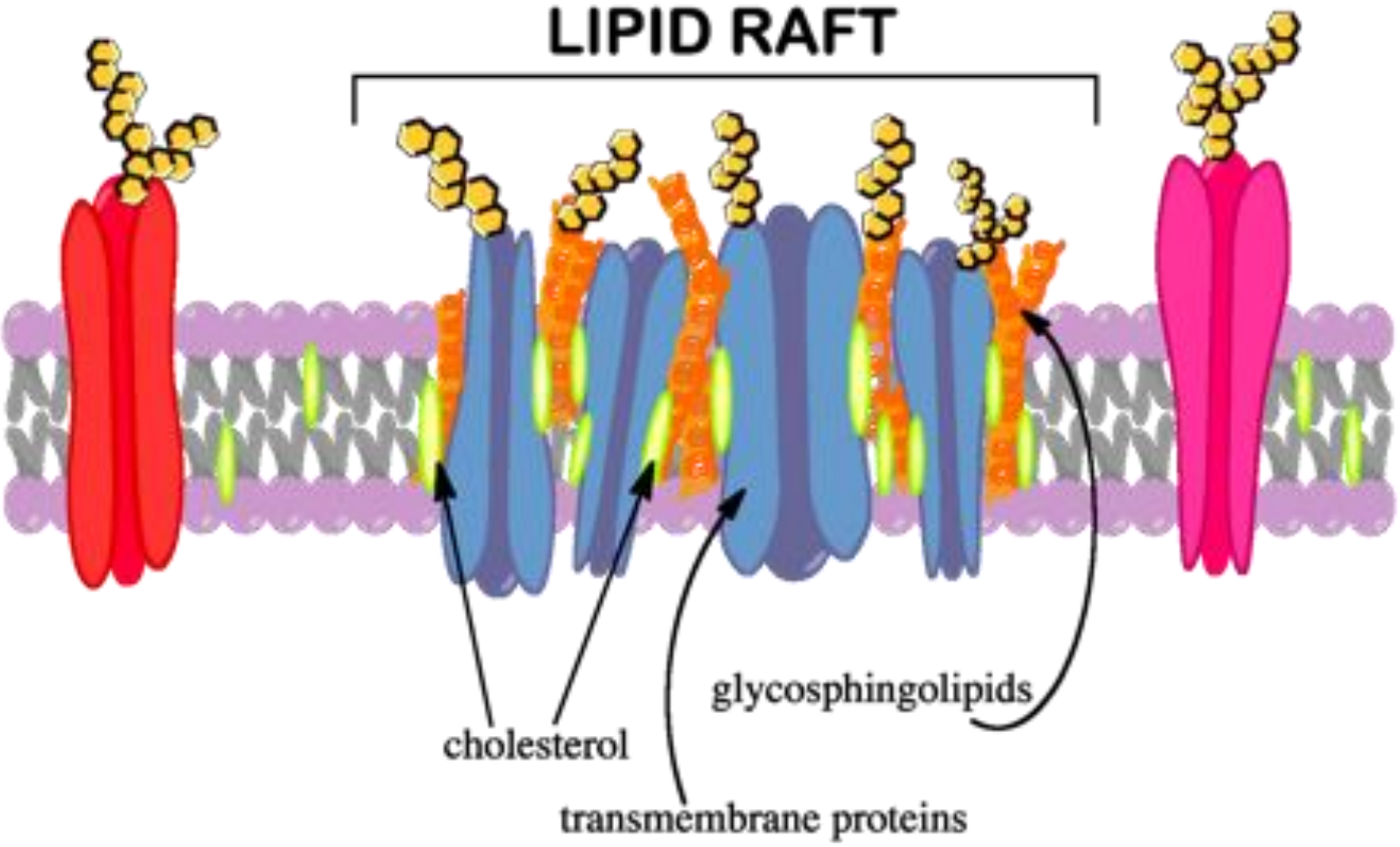
Cholesterol
34%
Sphingolipids
(HIGH)

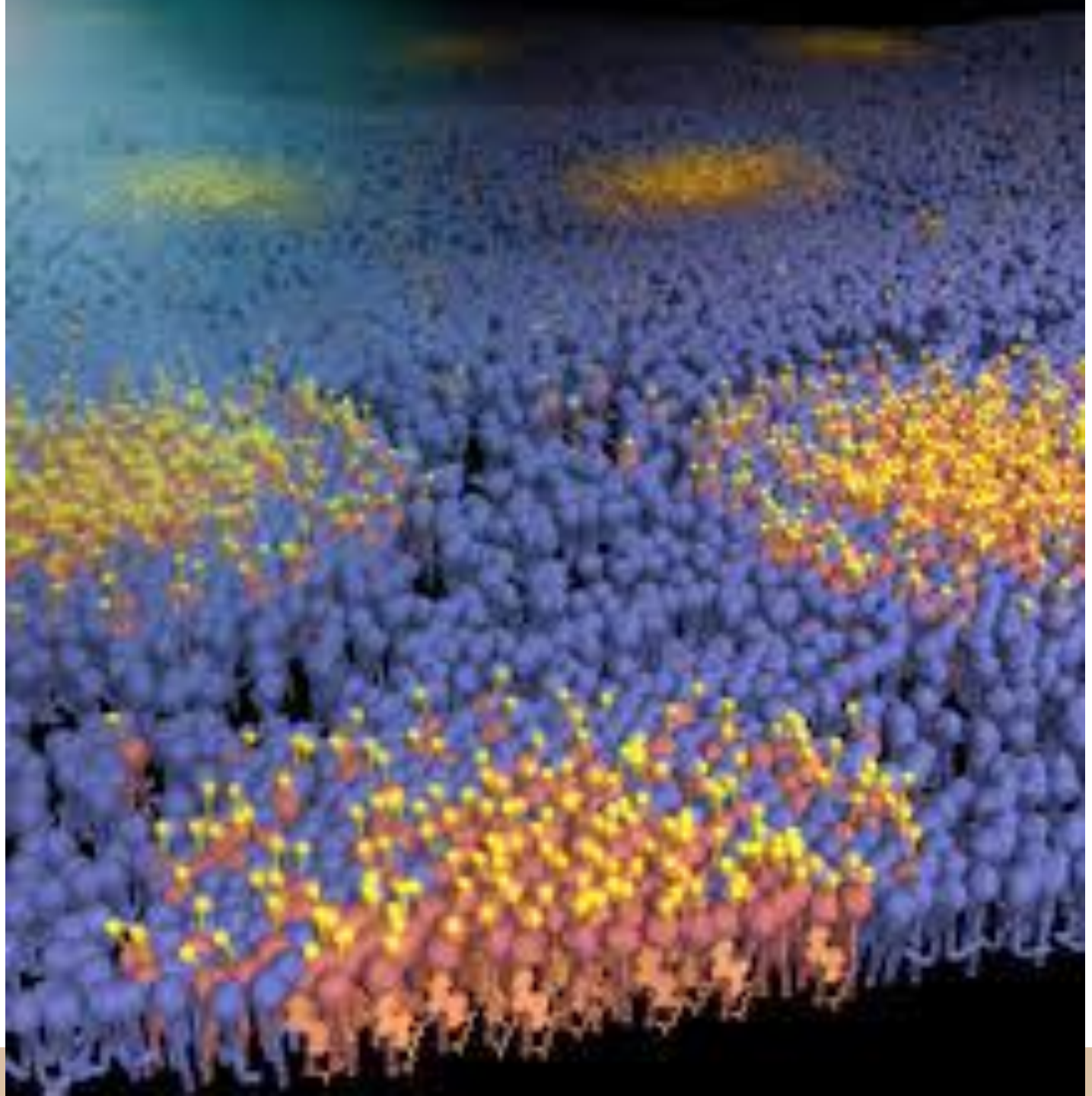
Functions: 1. impermeability of bilayer
2. thickens the bilayer
3. associates with sphingolipids

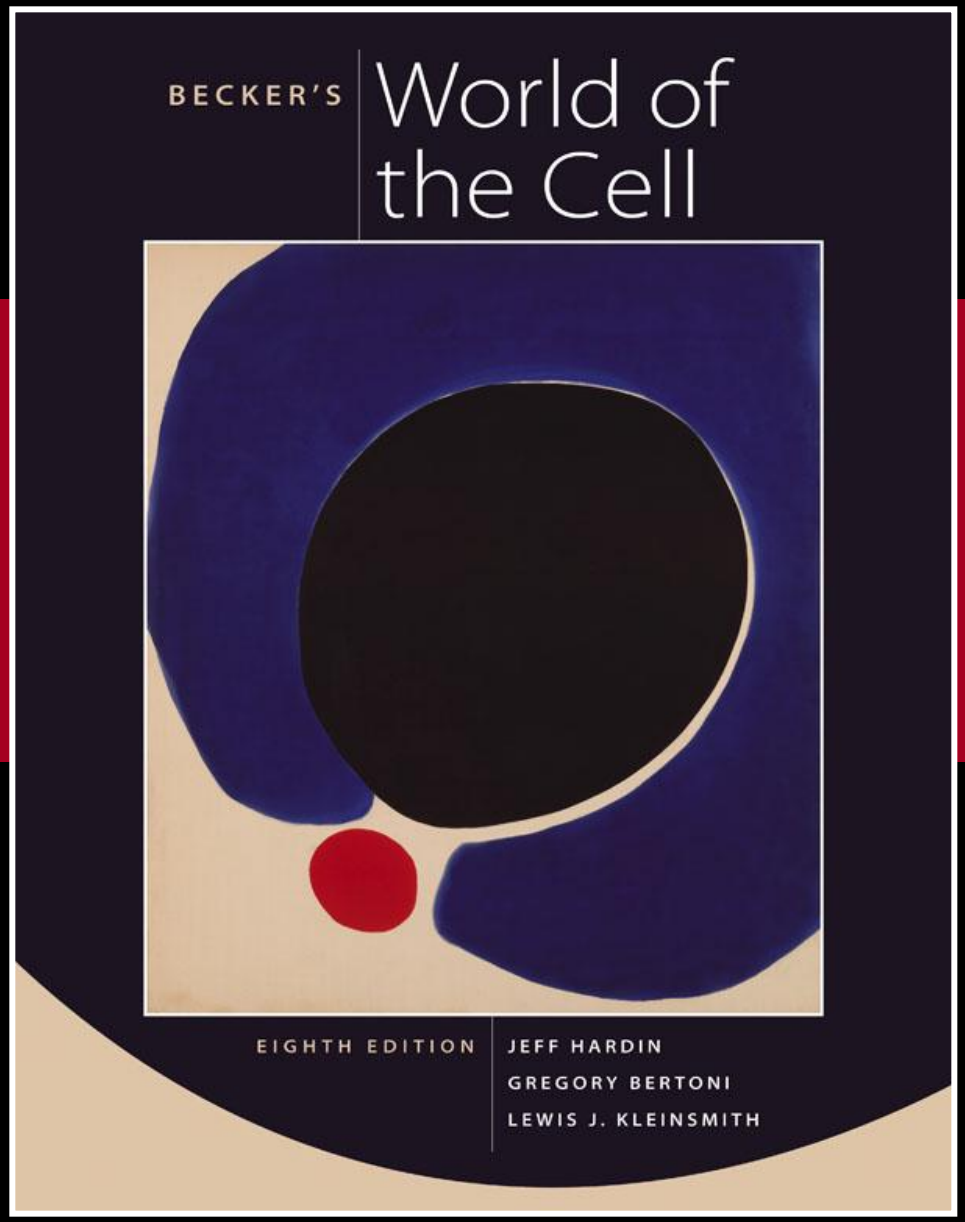

GPI-anchored protein
Insaturated lipid
the liquid phase
Saturated lipid
Cholesterol



Sub-compartmentalization







Chapter 8

Transport Across Membranes

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

Transport Across Membranes: Overcoming the Permeability Barrier

- Overcoming the permeability barrier of cell membranes is crucial to proper functioning of the cell
- Specific molecules and ions need to be selectively moved into and out of the cell or organelle
- Membranes are *selectively permeable*

Cells and Transport Processes

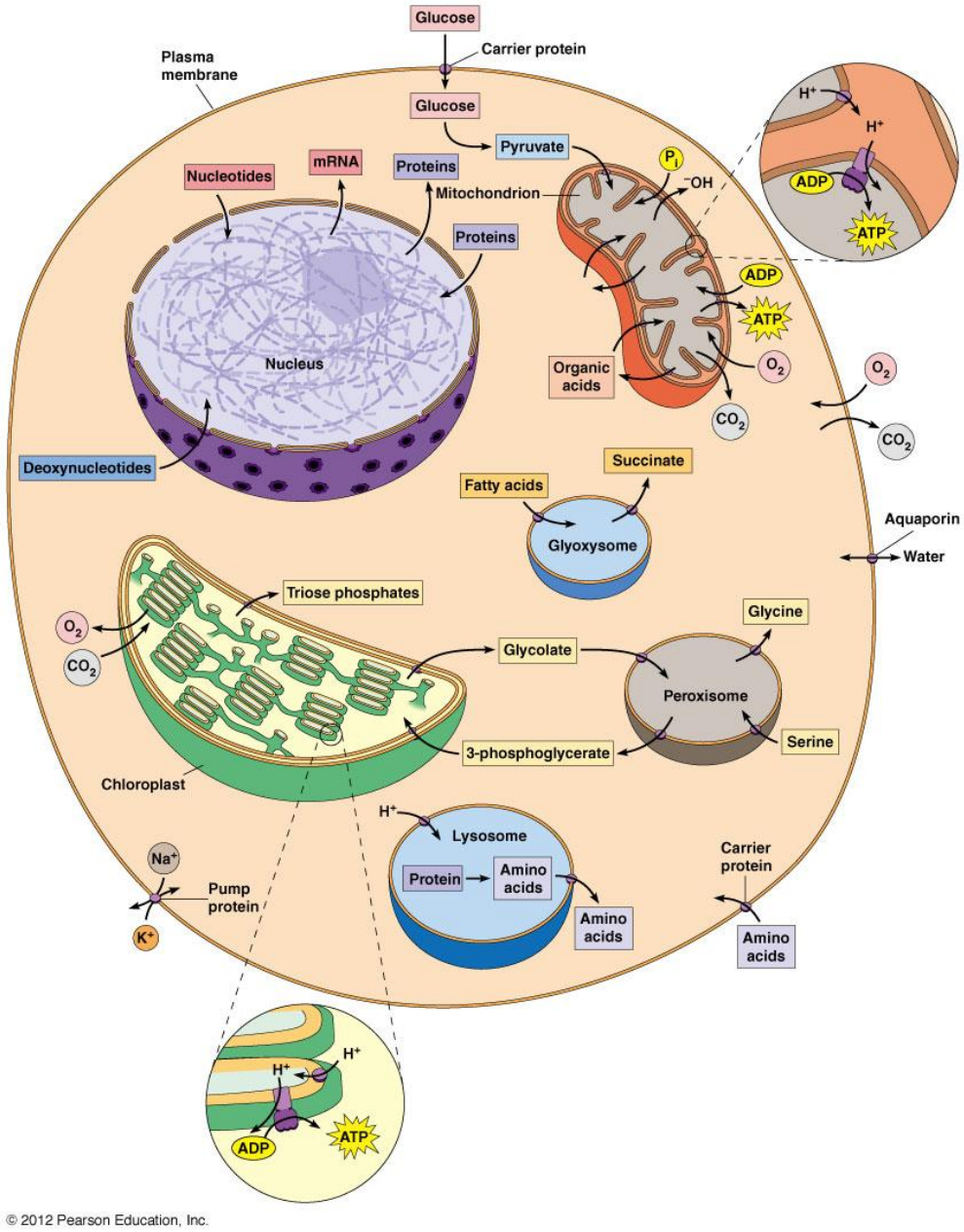
- Cells and cellular compartments are able to **accumulate** a variety of substances in concentrations that are very different from those of the surroundings

- Most of the substances that move across membranes are dissolved gases, ions, and small organic molecules; *solutes*

Transport is central to cell function

- A central aspect of cell function is selective **transport,** the movement of ions or small organic molecules (*metabolites*; components of metabolic pathways)

Figure 8-1



Solutes Cross Membranes by Diffusion (Simple or Facilitated), Active Transport and vesicular transport

- Quite different mechanisms are involved in moving solutes across membranes

- A few molecules cross membranes by *simple diffusion*, the direct unaided movement dictated by differences in concentration of the solute on the two sides of the membrane

- However, most solutes cannot cross the membrane this way

Transport proteins

- *Transport proteins* assist most solute across membranes

- These **integral membrane proteins** recognize the substances to be transported with great **specificity**

- Some move solutes to regions of lower concentration; this *facilitated diffusion* (or *passive transport)* uses no energy

Active transport

- In other cases, transport proteins move solutes against the concentration gradient; this is called *active transport*
- Active transport requires energy such as that released by the hydrolysis of ATP or by the simultaneous transport of another solute down an energy gradient

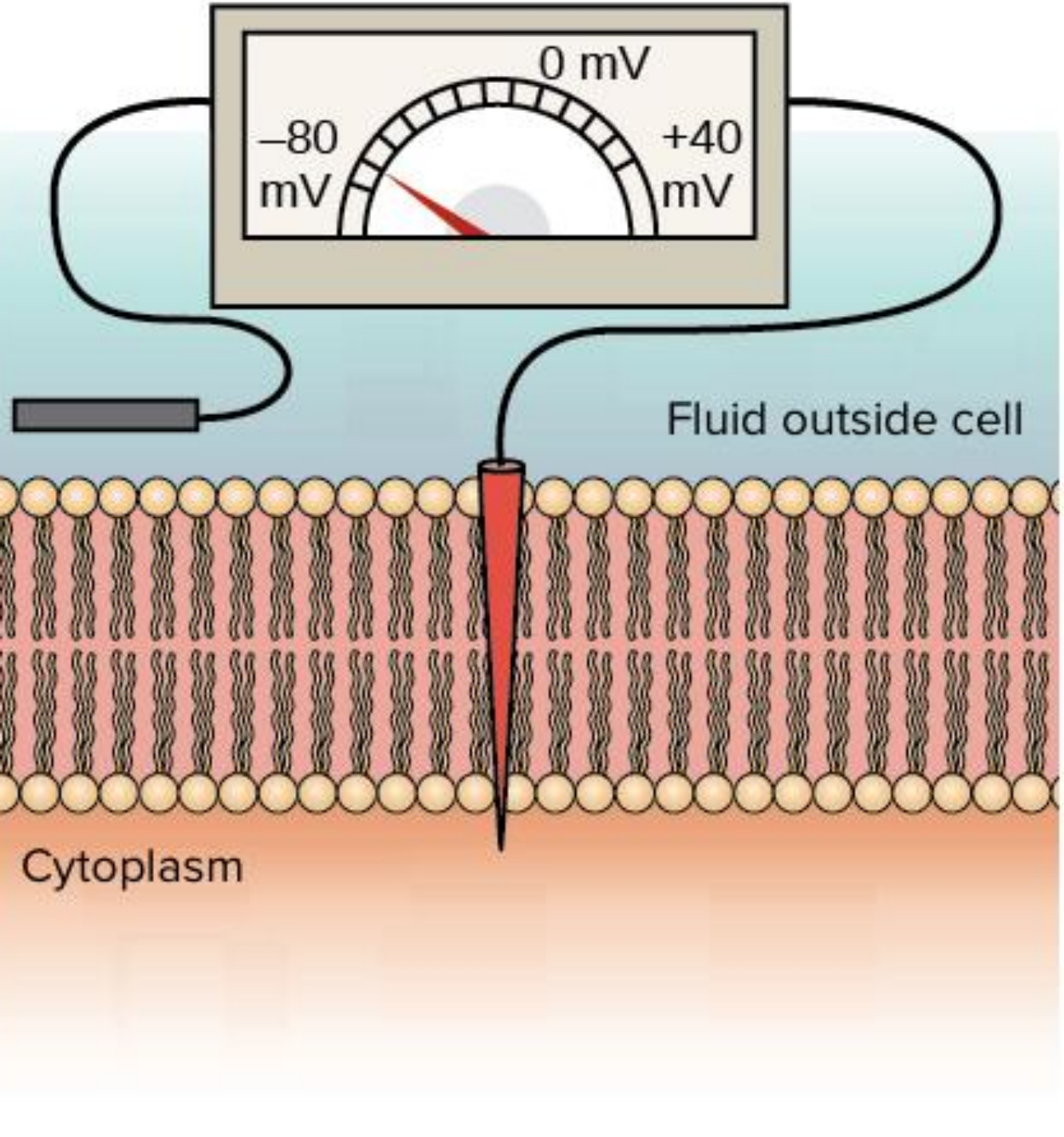
The Movement of a Solute Across a Membrane Is Determined by Its Concentration Gradient or Its Electrochemical Potential

- The movement of a molecule that has no net charge is determined by its **concentration gradient**
- Simple or facilitated diffusion involve **exergonic movement** "down" the concentration gradient (negative ΔG)
- Active transport involves **endergonic movement** "up" the concentration gradient (positive ΔG)

The electrochemical potential

- The movement of an **ion** is determined by its **electrochemical potential**, the combined effect of its concentration gradient and the charge gradient across the membrane

- The active transport of ions across a membrane creates a charge gradient or **membrane potential** ($\boldsymbol{V_m}$) across the membrane


0 mV
–80 mV
+40 mV
Fluid outside cell
Cytoplasm

Active transport of ions

- Most cells have an excess of negatively charged solutes inside the cell

- This charge difference favors the inward movement of cations such as Na^+ and outward movement of anions such as Cl^-

- In all organisms, active transport of ions across the plasma membrane results in **asymmetric distribution of ions inside and outside the cell**

The Erythrocyte Plasma Membrane Provides Examples of Transport Mechanisms

- The transport proteins of the **erythrocyte** plasma membrane are among the best understood of all such proteins
- The membrane potential is maintained by active transport of **potassium ions inward and sodium ions outward**
- Special pores or *channels* allow **water** and **ions** to enter or leave the cell rapidly as needed

Simple Diffusion: Unassisted Movement Down the Gradient

- The most straightforward way for a solute to cross a membrane is through **simple diffusion**, the unassisted net movement of a solute from high to lower concentration

- Typically this is only possible for gases, nonpolar molecules, or small polar molecules such as water, glycerol, or ethanol

Oxygen and the function of erythrocytes

- Oxygen gas traverses the lipid bilayer readily by simple diffusion

- Erythrocytes take up oxygen in the lungs, where oxygen concentration is high, and release it in the body tissues, where oxygen concentration is low

Figure 8-3A

(a) In the capillaries of body tissues (low $[O_2]$ and high $[CO_2]$ relative to the erythrocytes), O_2 is released by hemoglobin within the erythrocytes and diffuses outward to meet tissue needs. CO_2 diffuses inward and is converted to bicarbonate by carbonic anhydrase in the cytosol. Bicarbonate ions are transported outward by the anion exchange protein, accompanied by the inward movement of chloride ions to maintain charge balance. Carbon dioxide therefore returns to the lungs as bicarbonate ions.

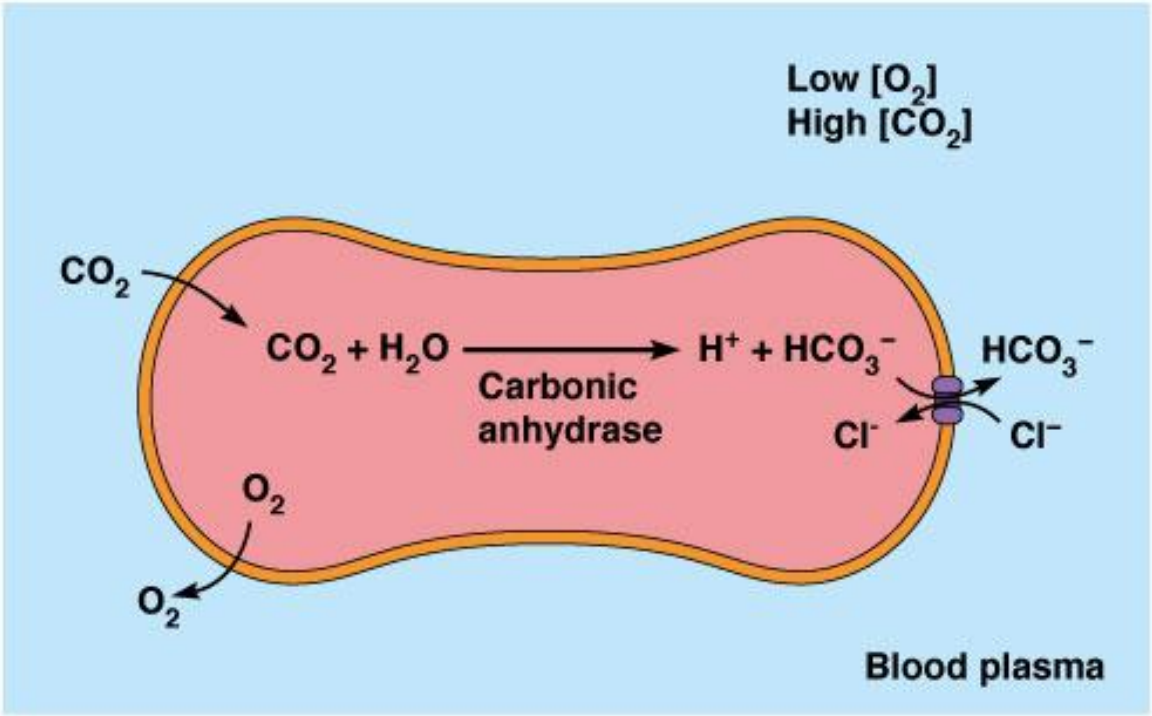
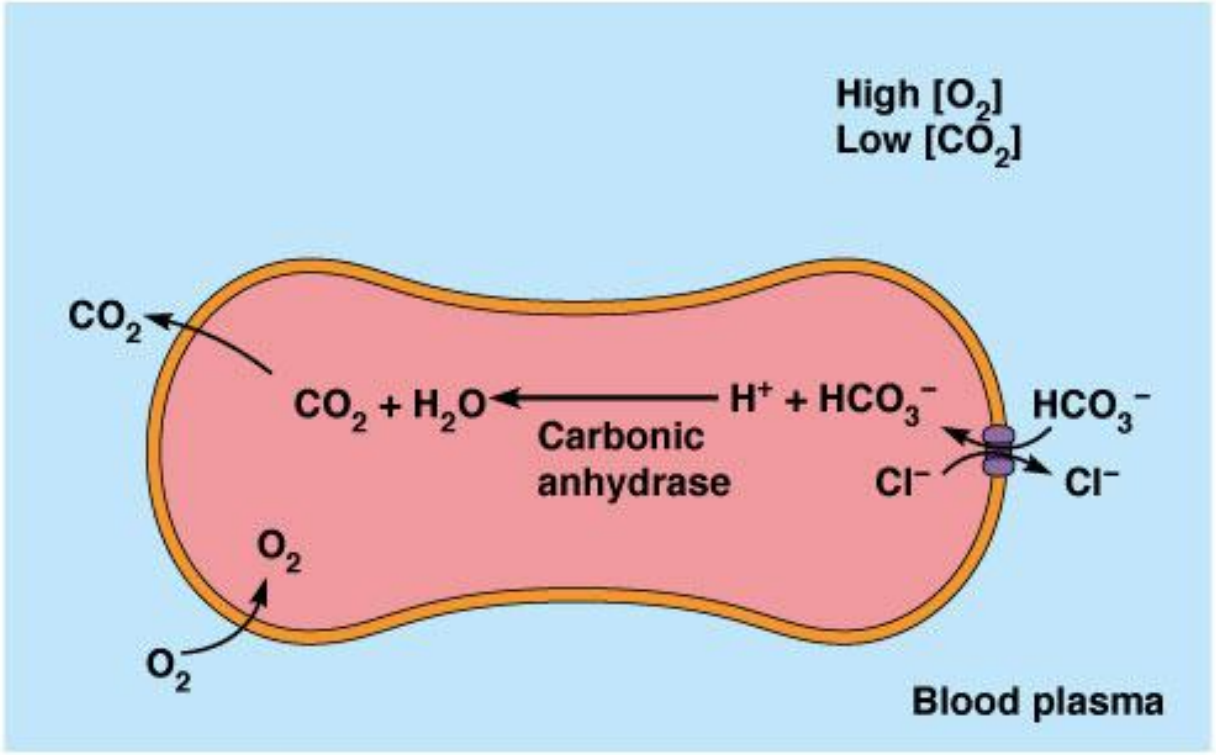


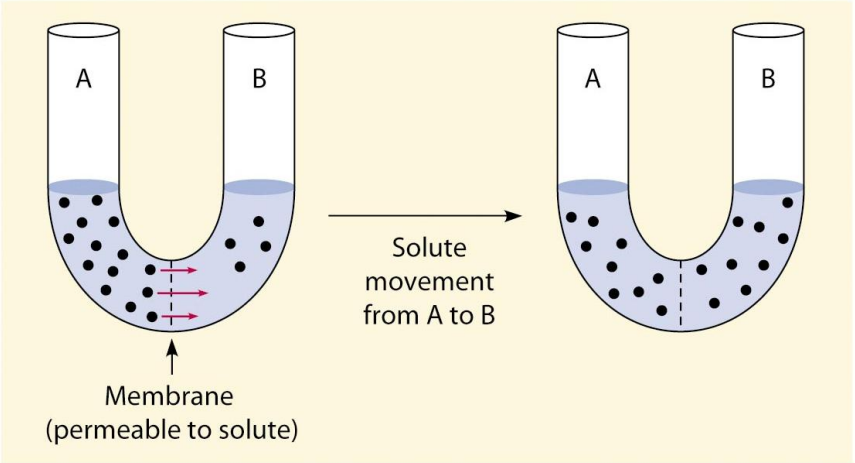
Figure 8-3B



(b) In the capillaries of the lungs (high [O_2] and low [CO_2] relative to the erythrocytes), O_2 diffuses inward and binds to hemoglobin. Bicarbonate moves inward from the blood plasma, accompanied by an outward movement of chloride ions. Incoming bicarbonate is converted into CO_2, which diffuses out of the erythrocytes and into the cells lining the capillaries of the lungs. The CO_2 is now ready to be expelled from the body.

Diffusion Always Moves Solutes Toward Equilibrium

- Diffusion always tends to create a random solution in which the concentration is the same everywhere



- Solutes will move toward regions of lower conc until the concs are equal

- Thus diffusion is always *movement toward equilibrium*

Osmosis Is the Diffusion of Water Across a Selectively Permeable Membrane

- Water diffuses from areas of **lower solute concentration** to areas of **higher solute concentration**
- In cells, water tends to move inward in a **controlled** manner
- Water molecules are **uncharged** and so are not affected by the membrane potential

Osmosis

- If two solutions are separated by a *selectively permeable membrane*, permeable to the water but not the solutes, the water will move toward the region of higher solute concentration
- For most cells, water tends to move inward

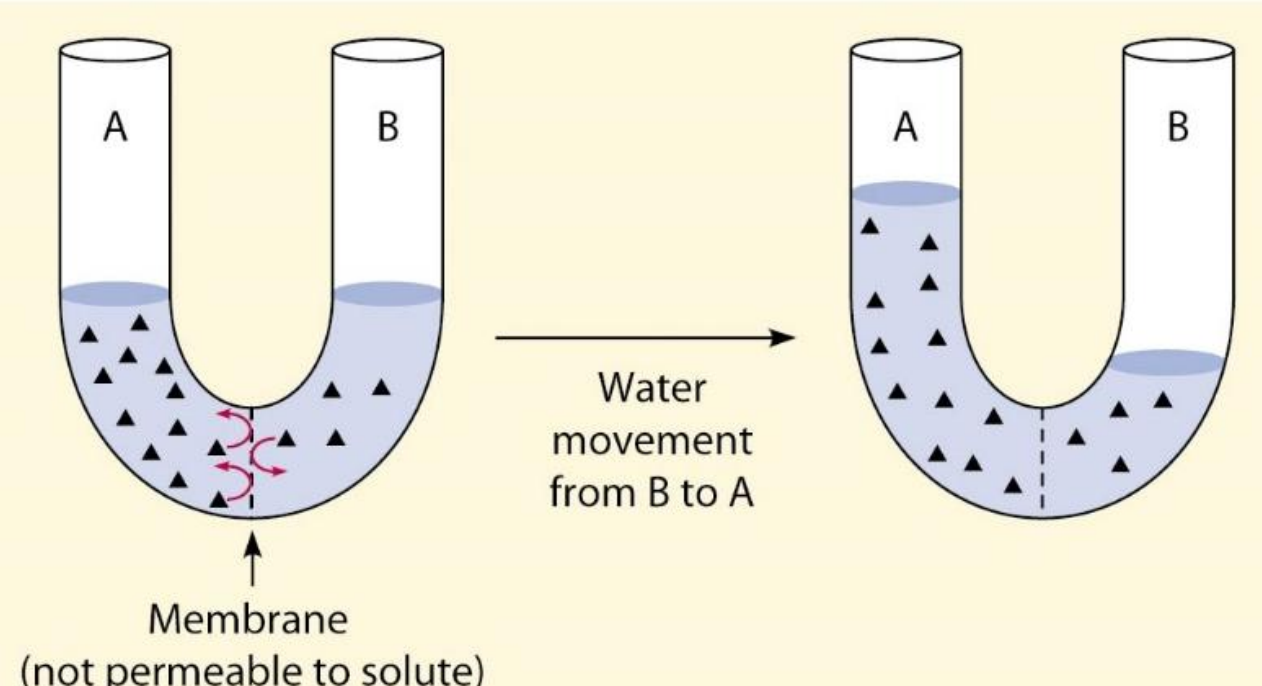
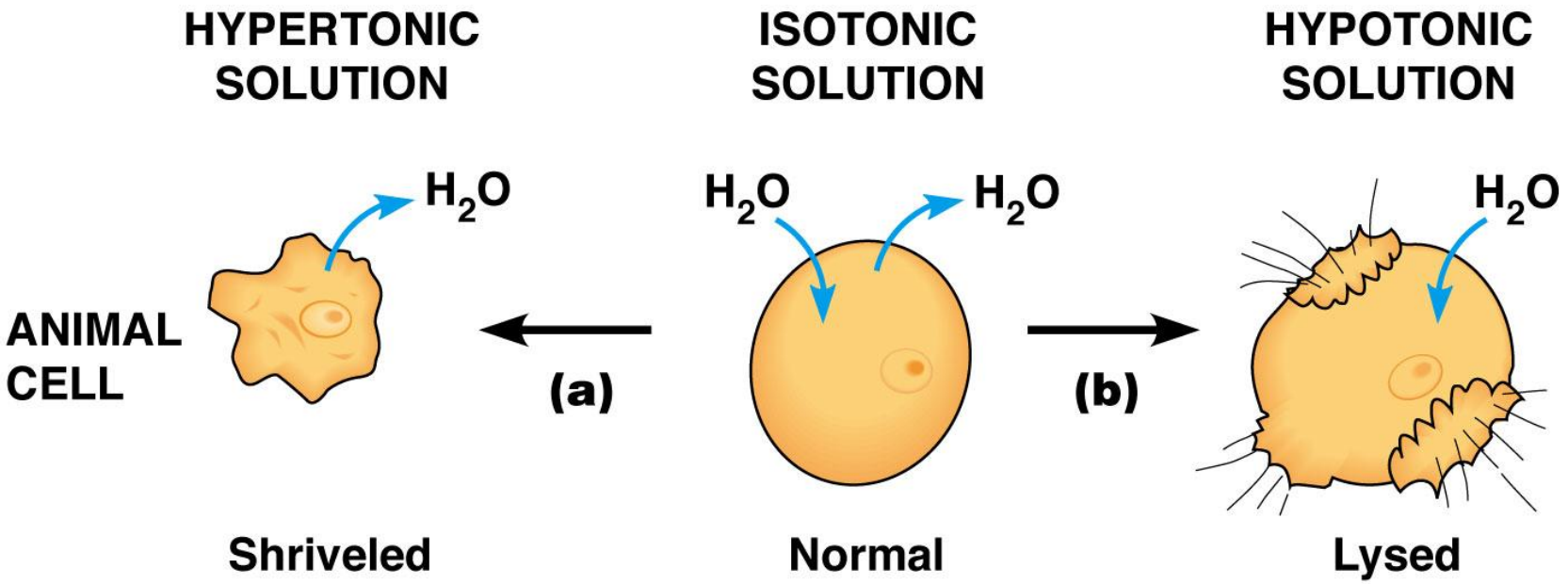
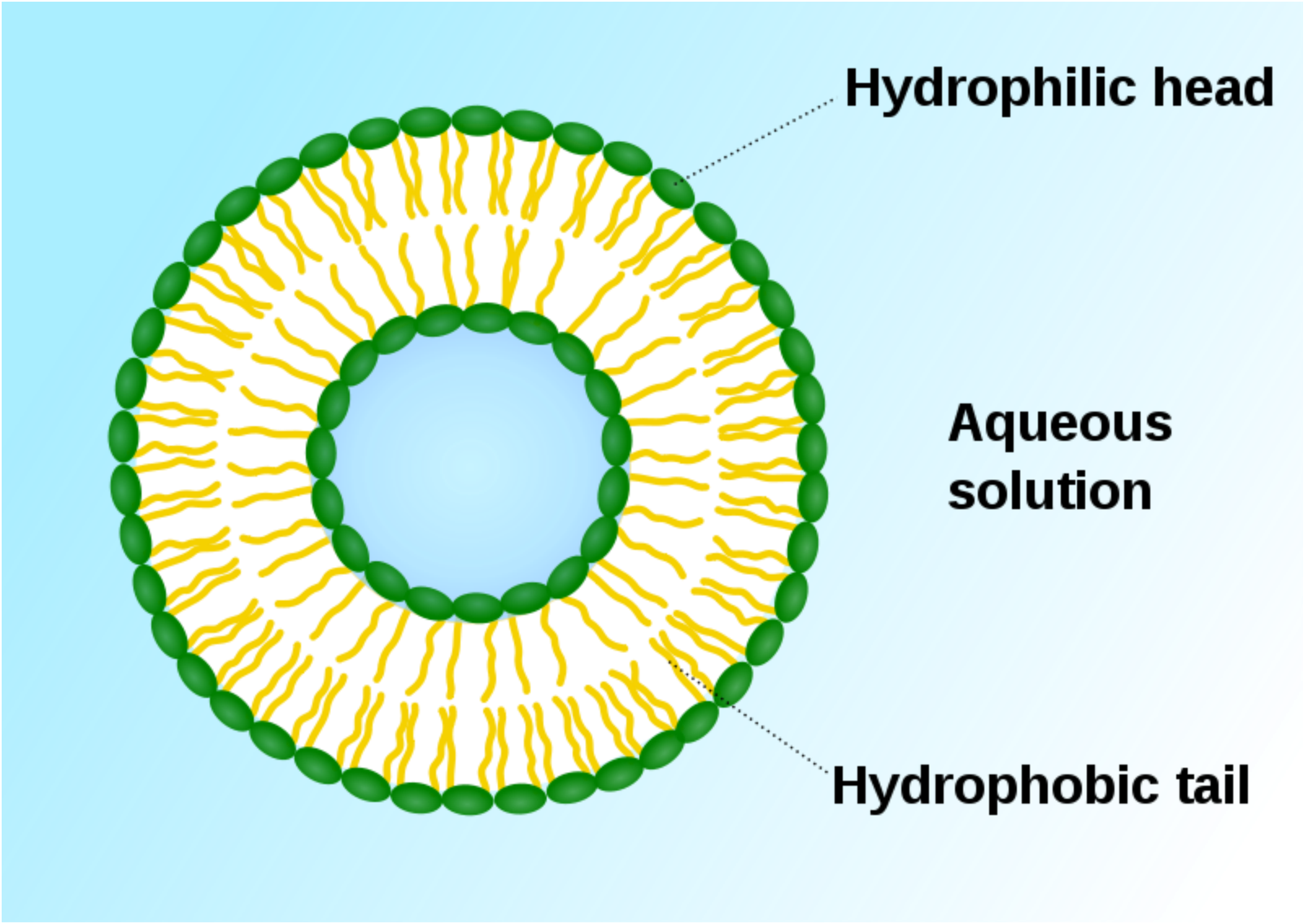


Figure 8-8A-1



Simple Diffusion Is Limited to Small, Nonpolar Molecules

Liposomes – extracted lipids from membranes tend to form small vesicles consisting of spherical lipid bilayer



Simple Diffusion Is Limited to Small, Nonpolar Molecules

Liposomes → trapping solutes and examining escape rates

Entrapped K^+ and Na^+ ions → lasted for days,
O_2 → escaped rapidly (couldn‘t be measured)

Three main factors affecting diffusion of solutes:
1. size
2. polarity
3. charge

Table 8-2 Factors Governing the Rate of Diffusion Across Lipid Bilayers

Factor	Examples		Permeability Ratio*
	More Permeable	Less Permeable	
1. Size: bilayer more permeable to smaller molecules	H_2O (Water)	$H_2N—CO—NH_2$ (Urea)	10^2:1
2. Polarity: bilayer more permeable to nonpolar molecules	$CH_3—CH_2—CH_2—OH$ (Propanol)	$HO—CH_2—CHOH—CH_2—OH$ (Glycerol)	10^3:1
3. Charge: bilayer highly impermeable to ions	O_2 (Oxygen)	OH^- (Hydroxide ion)	10^9:1

*Ratio of diffusion rate for the more permeable solute to the less permeable solute.

Solute Size

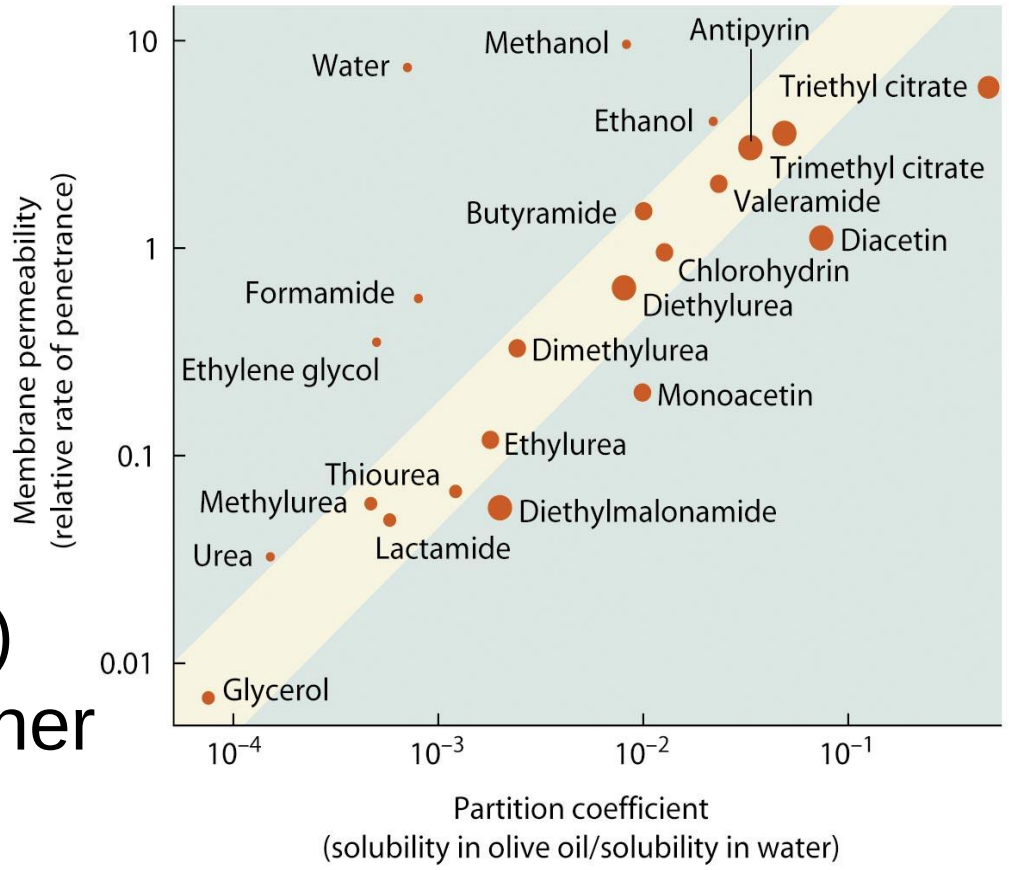
- In general, lipid bilayers are more permeable to small molecules—water, oxygen, carbon dioxide—than larger ones (size up to 100 Da)
- But without a transporter even these small molecules move more slowly than in the absence of a membrane
- Still, water diffuses more rapidly than would be expected for a **polar molecule**

Solute Polarity

- Lipid bilayers are more permeable to nonpolar substances than to polar ones
- Nonpolar substances dissolve readily into the hydrophobic region of the bilayer
- Large nonpolar molecules such as estrogen and testosterone cross membranes easily, despite their large size

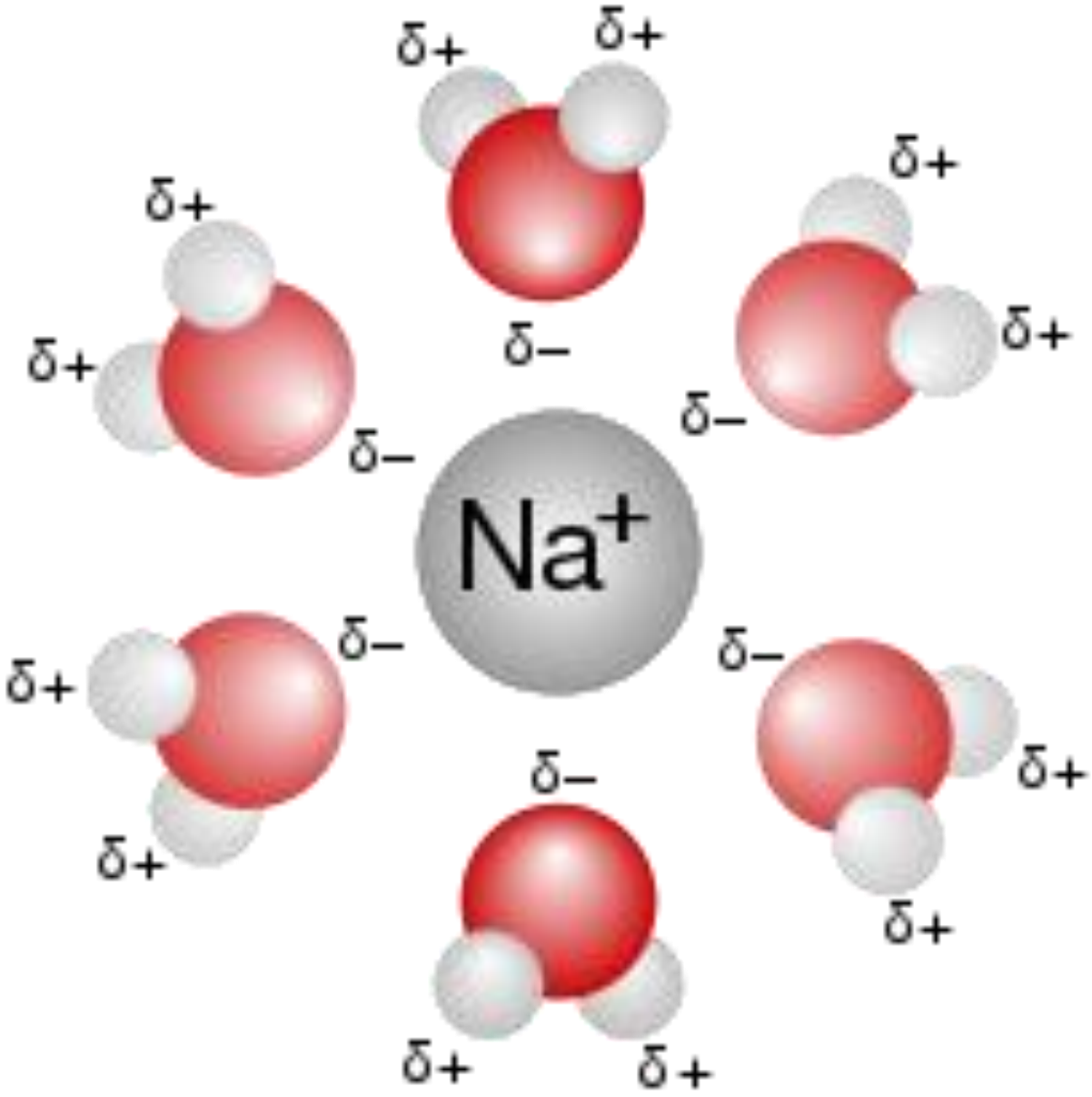
A measure of solute polarity

- Polarity of a solute can be measured by the ratio of its solubility in an organic solvent to its solubility in water
- This is called the *partition coefficient*
- In general, the more nonpolar (hydrophobic) a substance is, the higher the partition coefficient



Solute Charge

- The relative impermeability of polar substances, especially ions, is due to their association with water molecules
- The molecules of water form a *shell of hydration* around polar substances
- In order for these substances to move into a membrane, the water molecules must be removed, which requires energy



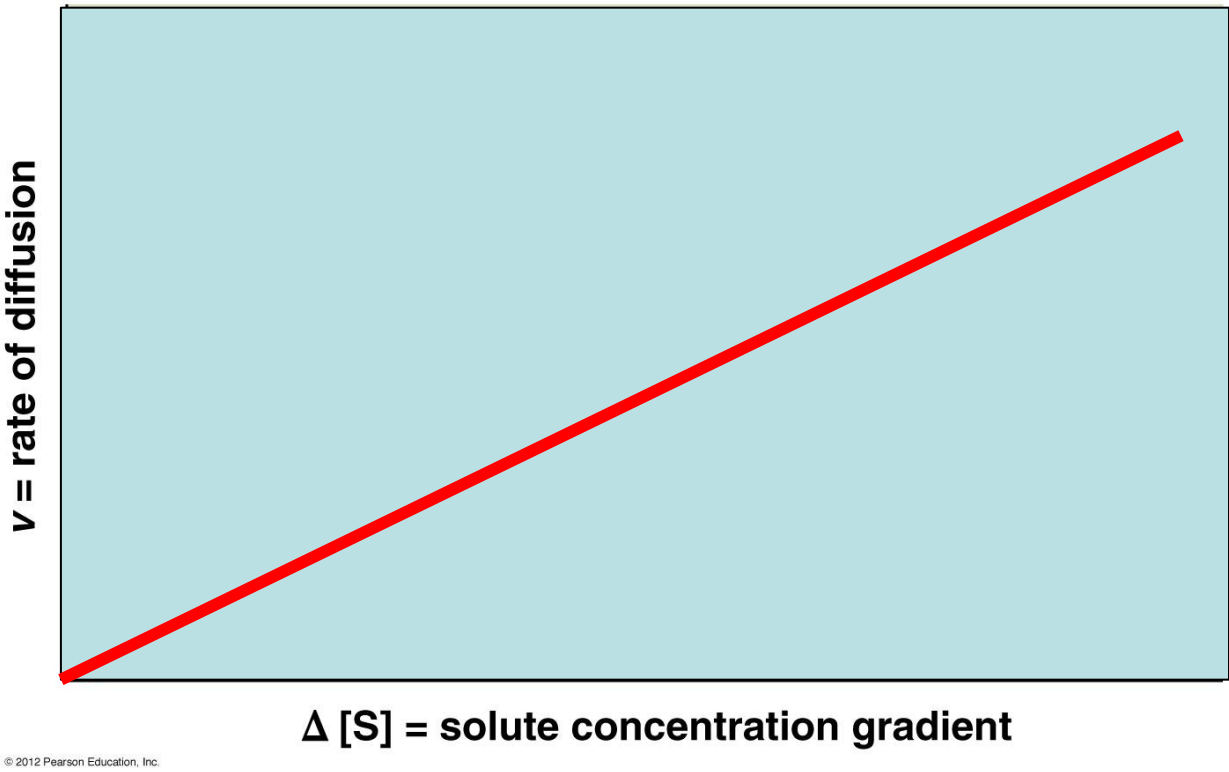
The Rate of Simple Diffusion Is Directly Proportional to the Concentration Gradient

- Thermodynamically, simple diffusion is always an exergonic process, requiring no input of energy

- Kinetically, the net rate of transport for a substance is proportional to its concentration difference across the membrane

- V_{inward} = rate of diffusion in moles/sec.cm^2
- $\Delta[S] = [S]_{outside} - [S]_{inside}$

- Simple diffusion has a linear relationship between inward flux of solute and solute concn



Facilitated Diffusion: Protein-Mediated Movement Down the Gradient

- Most substances in the cell are too large or too polar to cross membranes by simple diffusion
- These can only move in and out of cells with the assistance of **transport proteins**
- If the process is exergonic, it is called **facilitated diffusion;** the solute diffuses as dictated by its concentration gradient

Transport proteins in facilitated diffusion

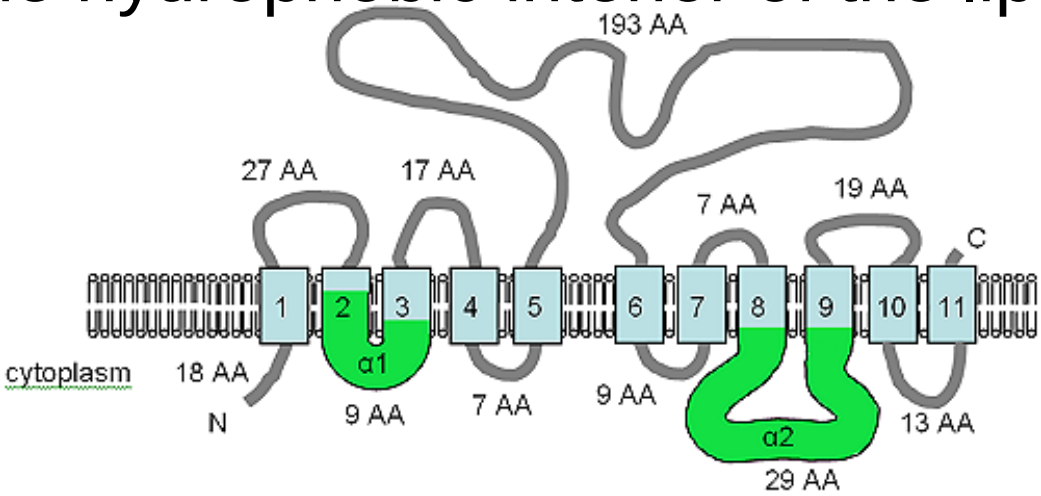
- No input of energy is needed in facilitated diffusion

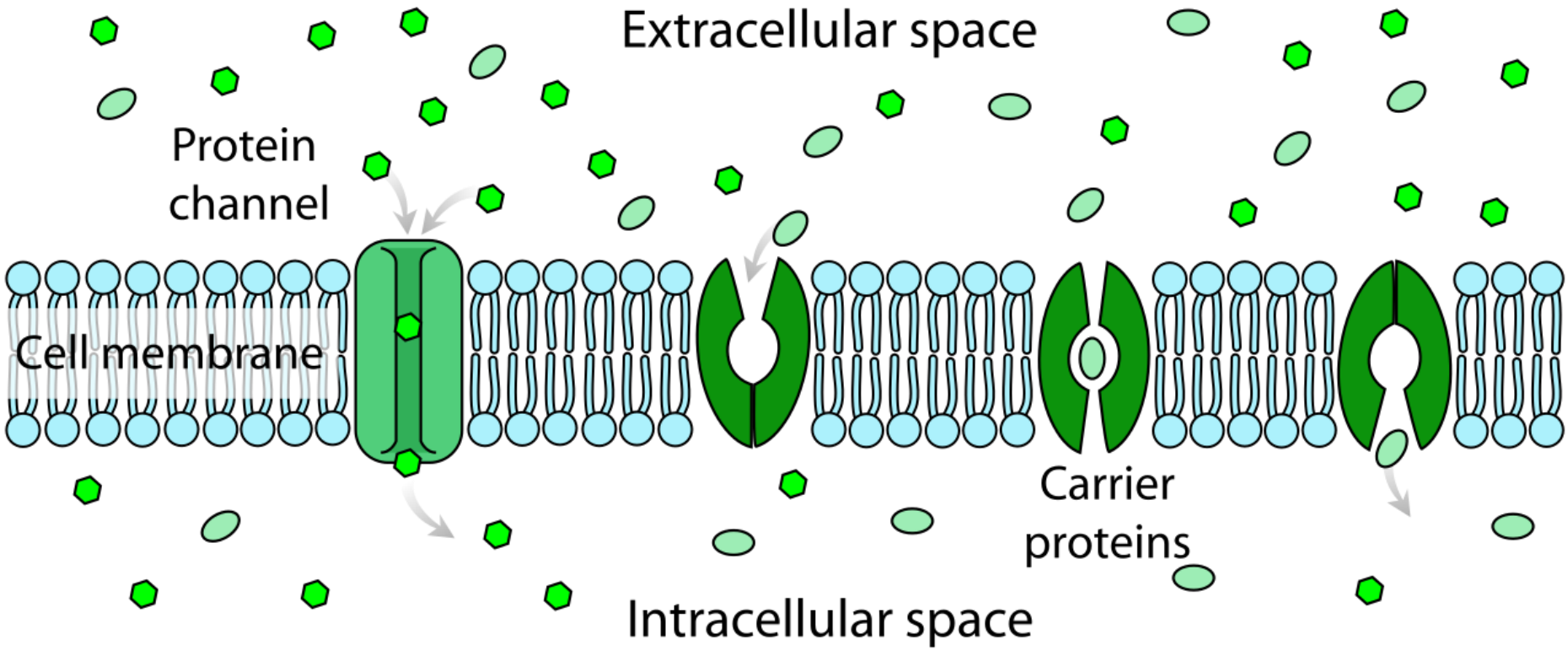
- The role of the transport proteins is just to provide a path through the lipid bilayer, allowing the “downhill” movement of a polar or charged solute

Transport proteins in facilitated diffusion structure

- All membrane transport proteins have been found to be multipass transmembrane proteins.

- These proteins enable specific hydrophilic solutes to cross the membrane without coming into direct contact with the hydrophobic interior of the lipid bilayer.





Carrier Proteins and Channel Proteins Facilitate Diffusion by Different Mechanisms

- **Channel proteins** form hydrophilic *channels* through the membrane to provide a passage route for solutes

- **Carrier proteins** (*transporters* or *permeases*) bind solute molecules on one side of a membrane, **undergo a conformation change**, and release the solute on the other side of the membrane

Channels

- Some channels are large and nonspecific, such as the *pores* on the outer membranes of bacteria, mitchondria, and chloroplasts

- Pores are formed by transmembrane proteins called *porins* that allow passage of solutes up to a certain size to pass (600 Da)

- Most channels are smaller and highly selective

Ion channels

- Most of the smaller channels are involved in ion transport and are called *ion channels*
- *The movement of solutes through ion channels is much faster than transport by carrier proteins*
- This is likely because conformation changes are not required

A channel protein provides a hydrophilic pore or channel into the membrane, allowing ions to pass through without coming into contact with the membrane's hydrophobic interior.

However, channels cannot be coupled to an energy source to perform active transport so the transport that they mediate is always passive (“downhill”).

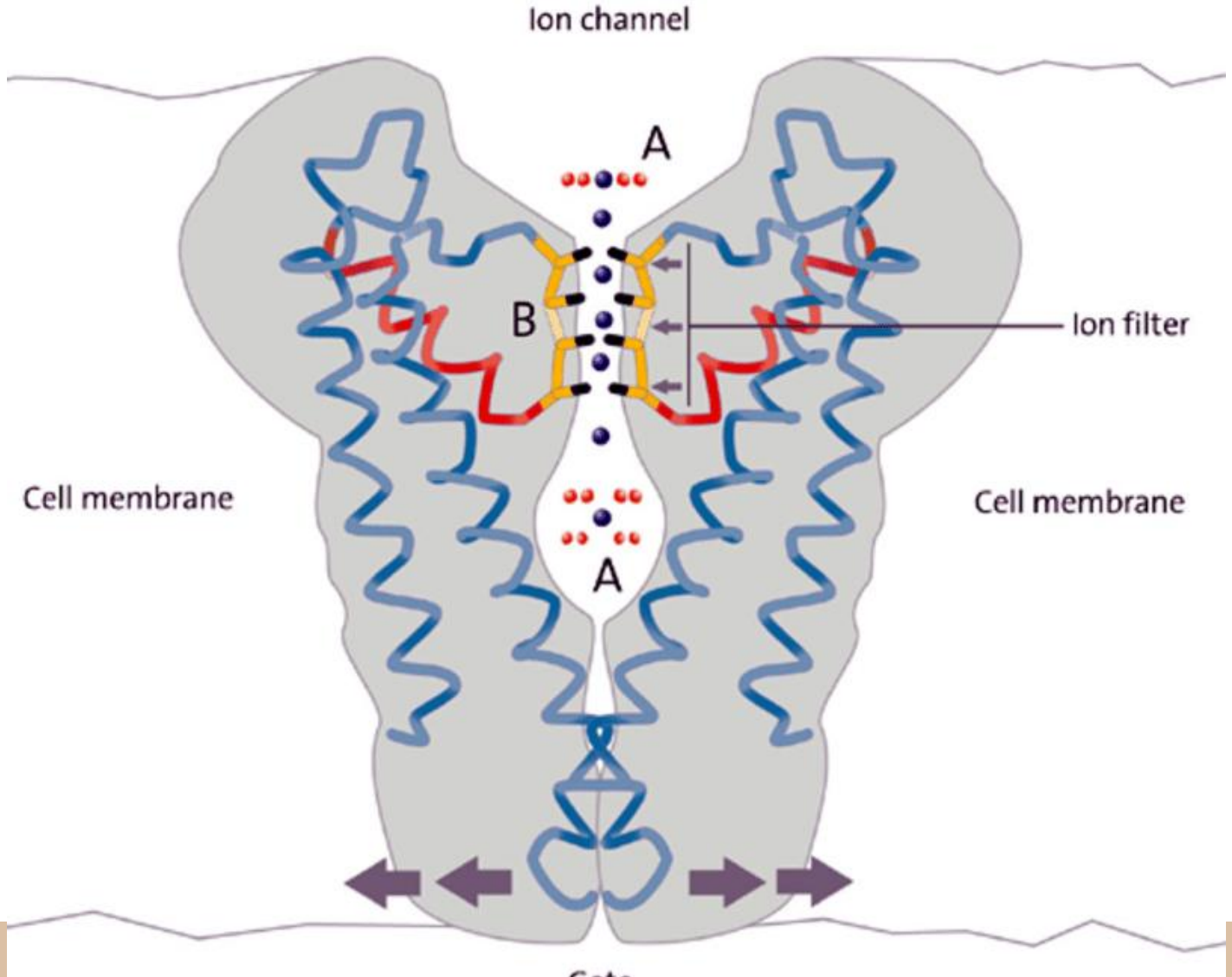
Ion Channels: Transmembrane Proteins That Allow Rapid Passage of Specific Ions

- Because most allow passage of just **one ion**, there are separate proteins needed to transport Na^+, K^+, Ca^{2+}, and Cl^-, etc.

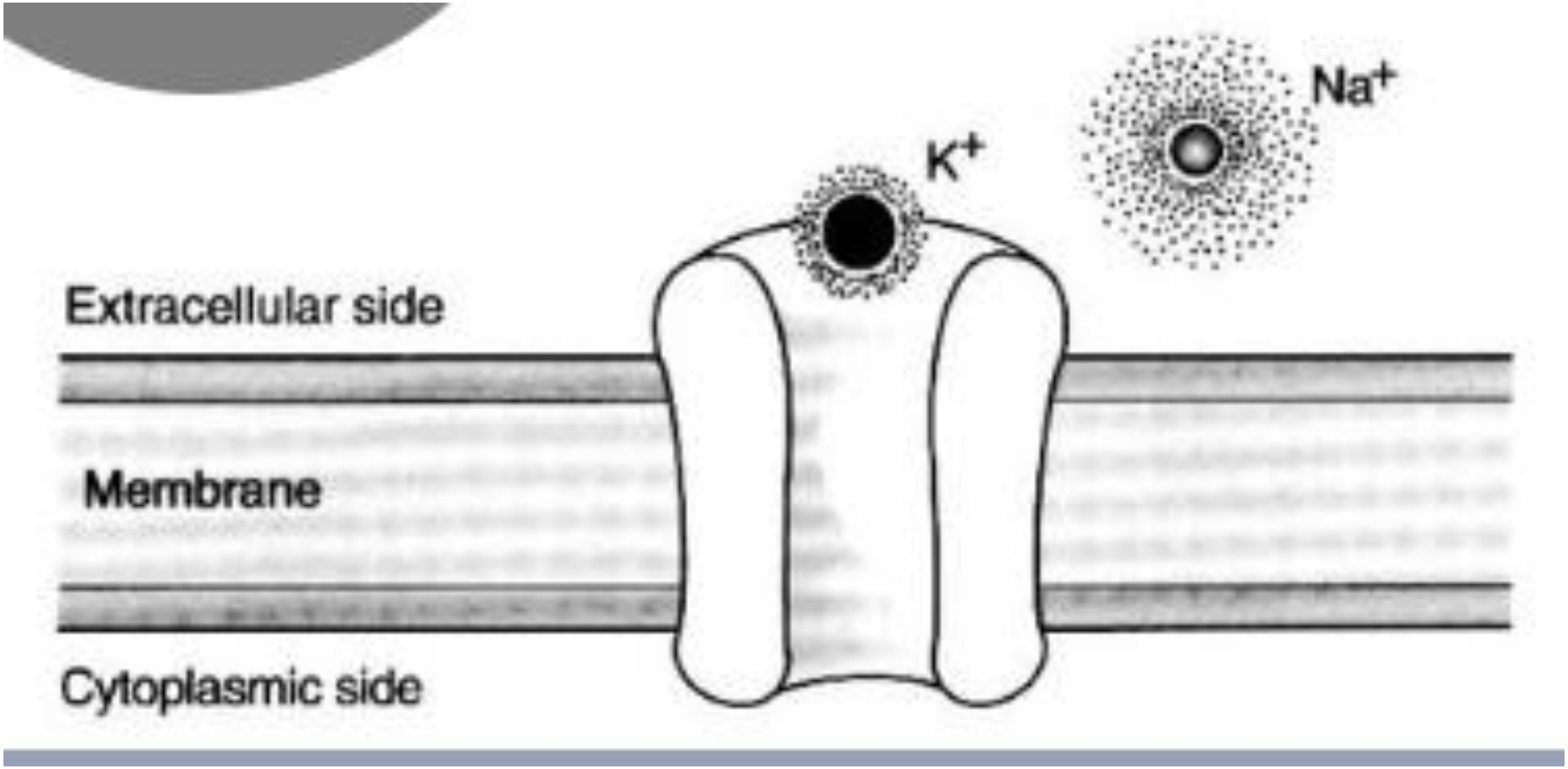
- Selectivity is based on

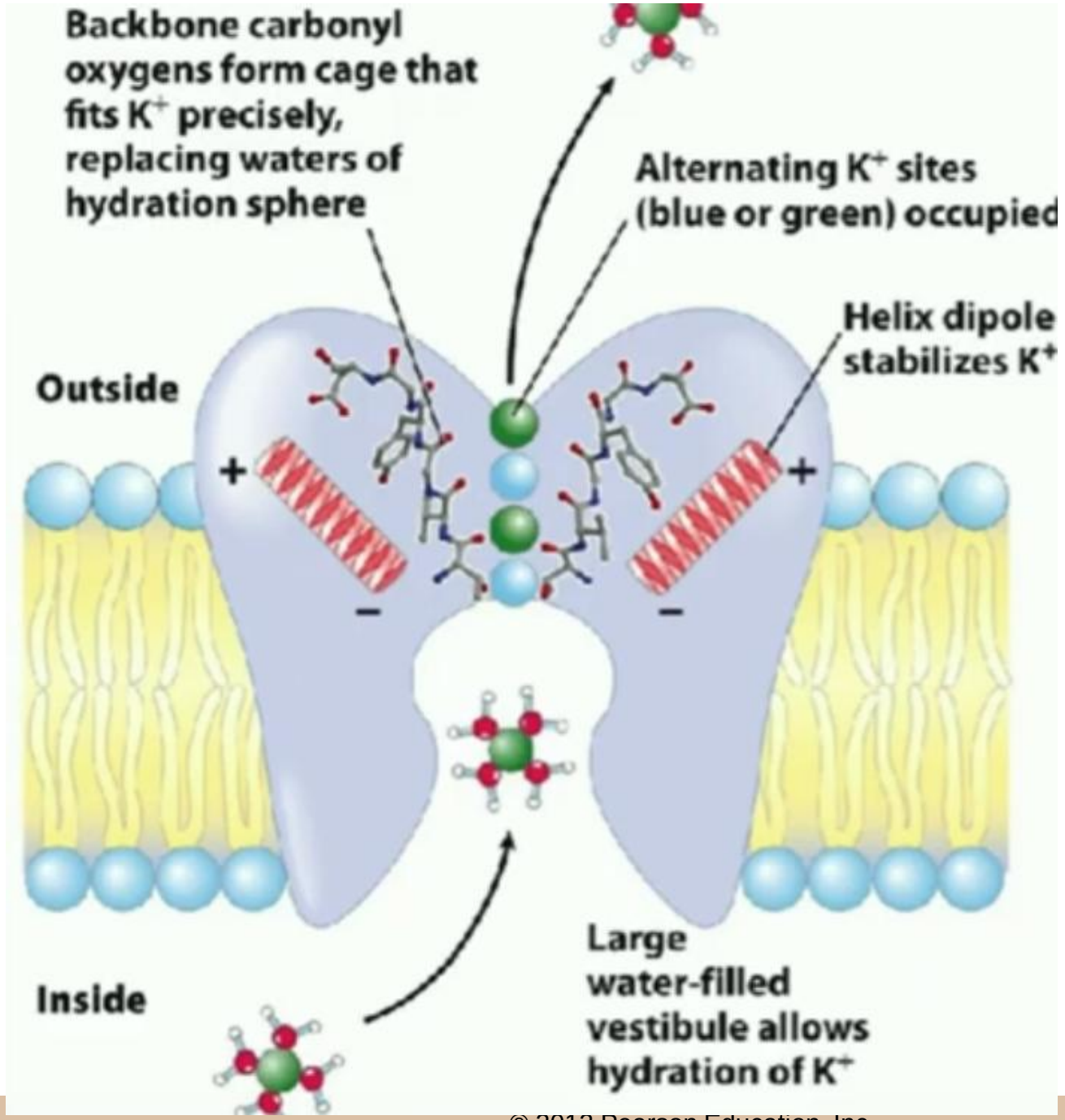
a. binding sites involving amino acid side chains
b. a size filter

Ion channels show *ion selectivity*, permitting some inorganic ions to pass, but not others through a **selectivity filter**



size filter that allows K+ to enter but not Na+





binding sites involving amino acid side chains

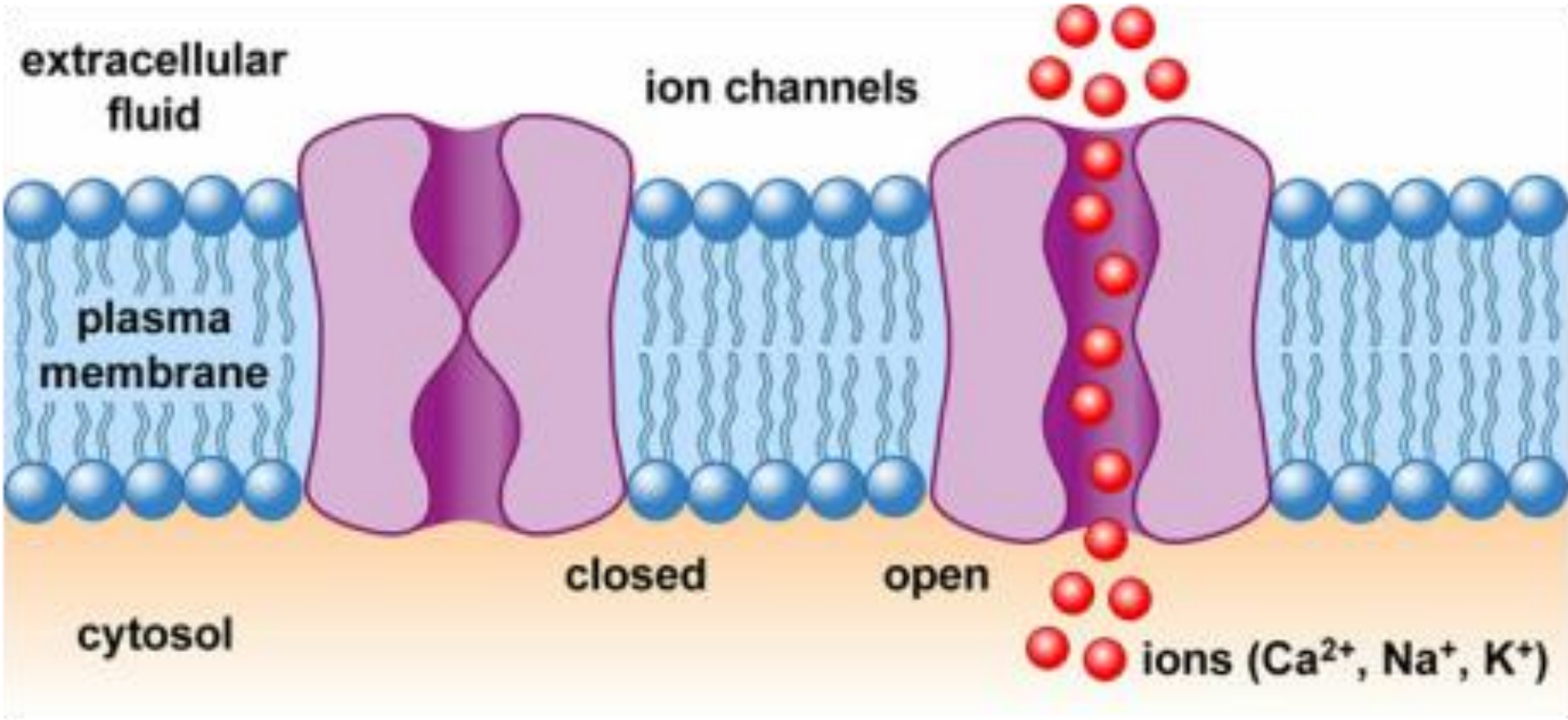
Channels are gated

- Most ion channels are ***gated***, meaning that they **open** and **close** in response to some stimulus

  - *Voltage-gated channels* open and close in response to changes in membrane potential

  - *Ligand-gated channels* are triggered by the binding of certain substances to the channel protein

  - *Mechanosensitive channels* respond to mechanical forces acting on the membrane ex. Piezo channels



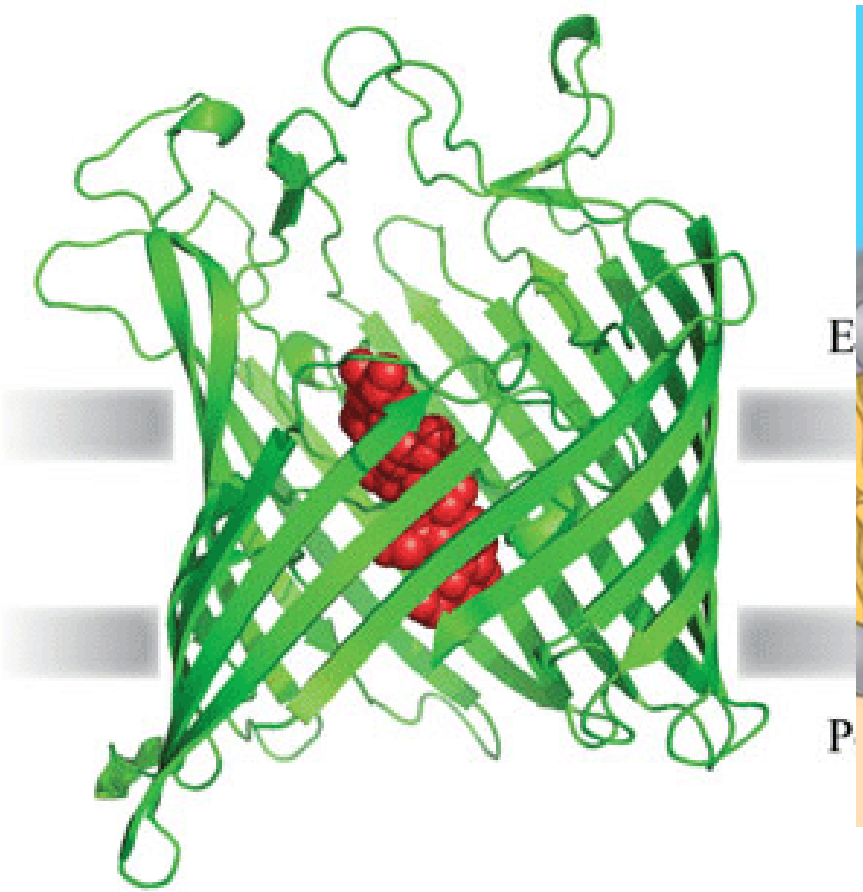
Do channel proteins undergo **any** conformational changes?

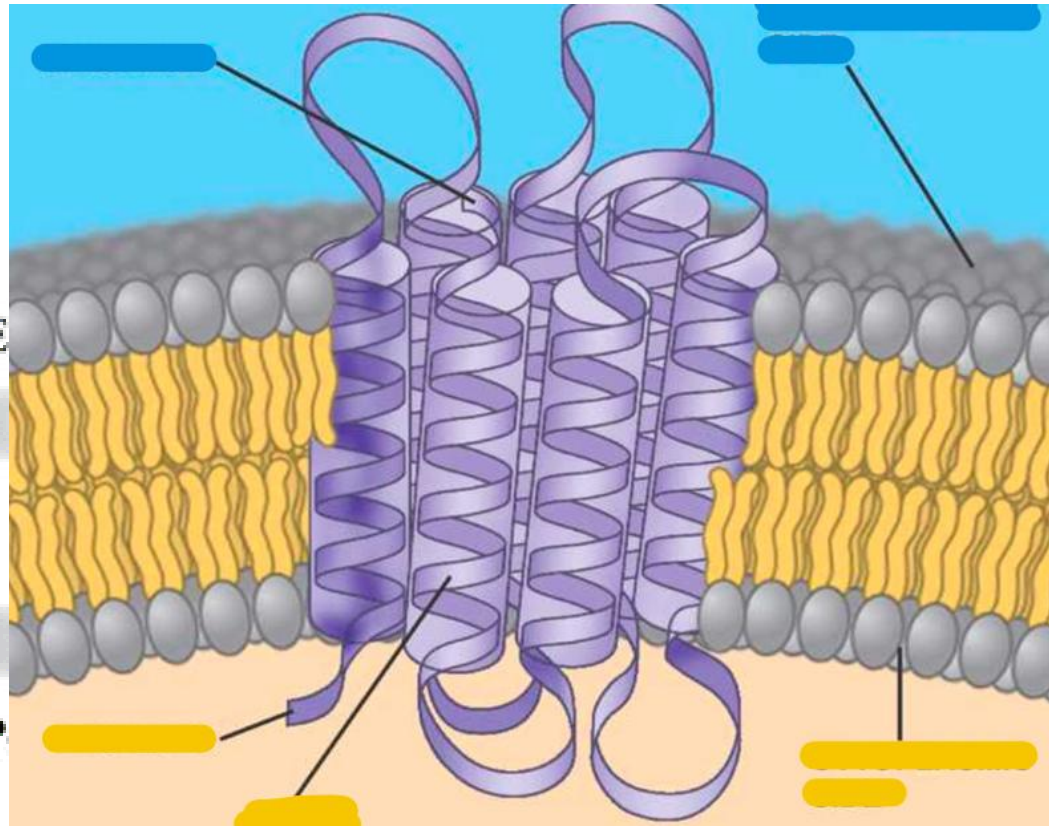
Yes/NO

Porins: Transmembrane Proteins That Allow Rapid Passage of Various Solutes

- Pores on outer membranes of bacteria, mitochondria and chloroplasts are larger and less specific than ion channels
- The pores are formed by multipass transmembrane proteins called **porins**
- The transmembrane segments of porins cross the membrane as β barrels

Porin vs integral protein transmembrane segments

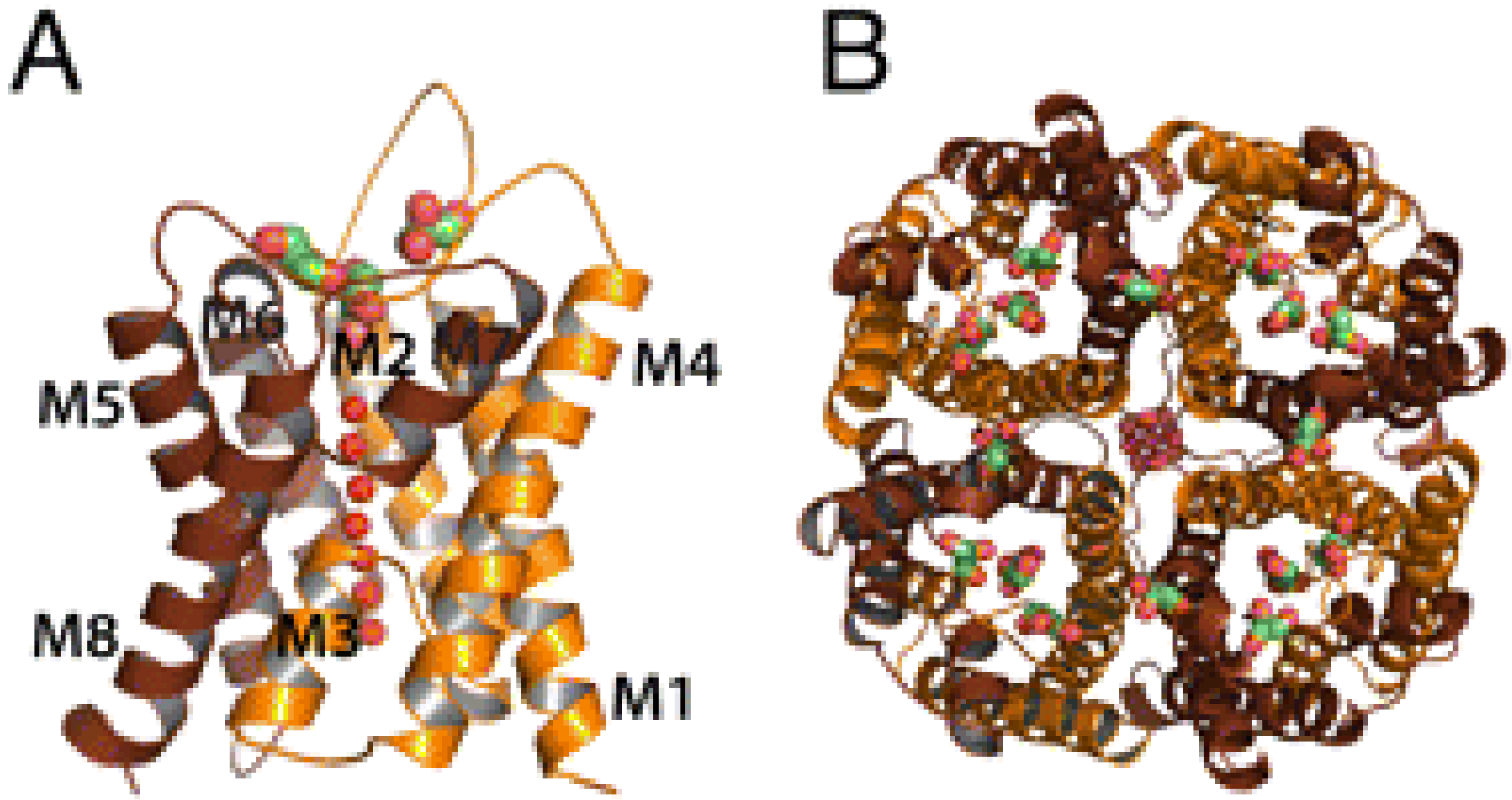




Aquaporins: Transmembrane Channels That Allow Rapid Passage of Water

- Movement of water across cell membranes in some tissues is faster than expected given the polarity of the water molecule
- **Aquaporin (AQP)** was discovered only in 1992
- Aquaporins allow rapid passage of water through membranes of erythrocytes and kidney cells in animals, and root cells and vacuolar membranes in plants

Structure of aquaporin



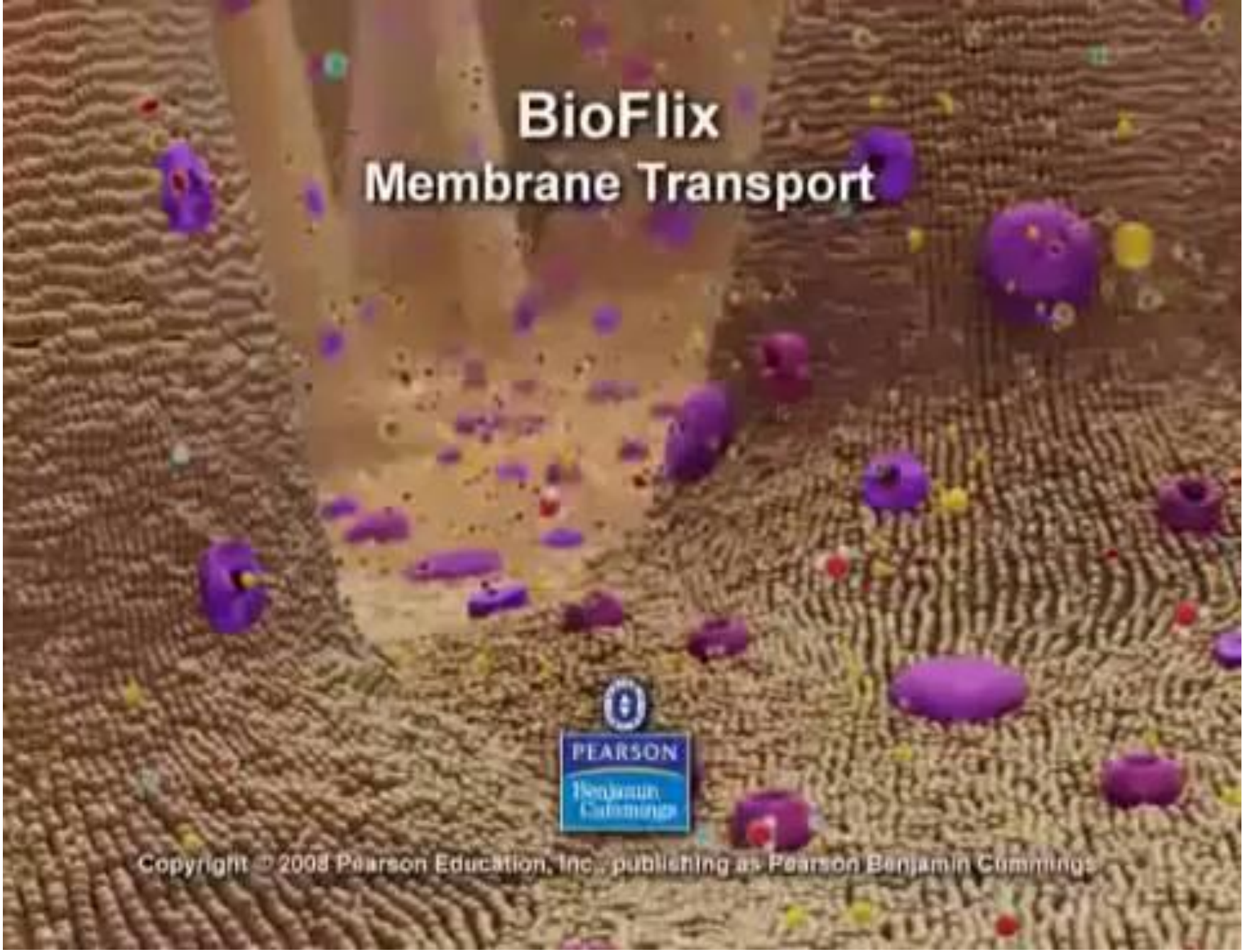
https://www.pnas.org/content/106/18/7437

Carrier Proteins Alternate Between Two Conformational States

- The **alternating conformation model** states that a carrier protein is allosteric protein and alternates between two conformational states

- In one state the solute binding site of the protein is accessible on one side of the membrane

- The protein shifts to the alternate conformation, with the solute binding site on the other side of the membrane, triggering solute release



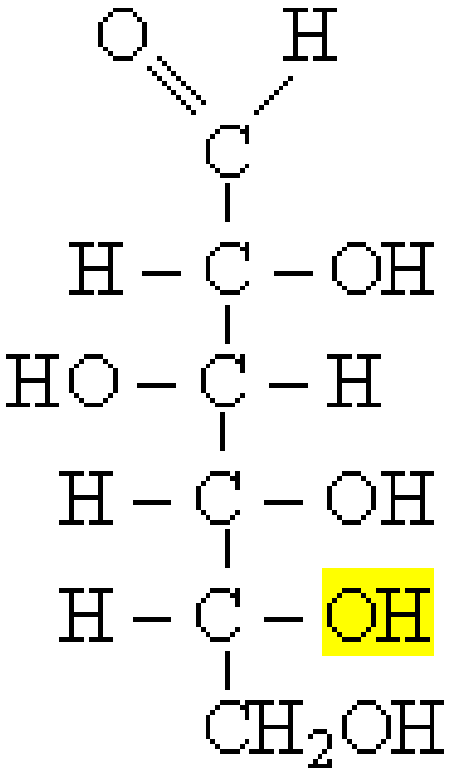
Carrier Proteins Are Analogous to Enzymes in Their Specificity and Kinetics

- Carrier proteins are analogous to enzymes

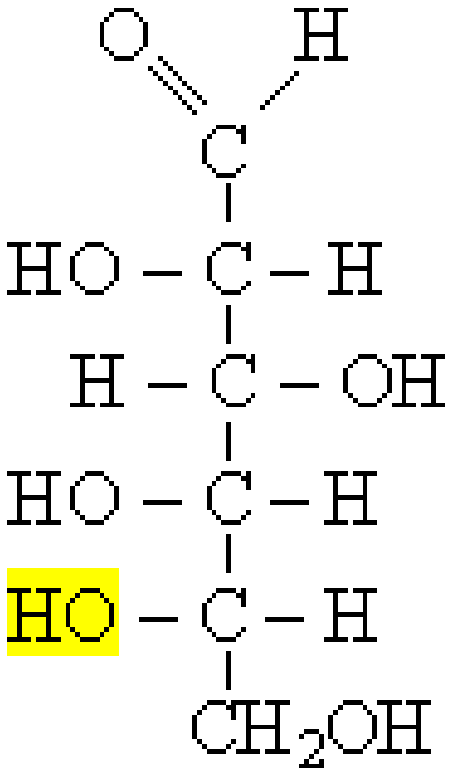
  - Facilitated diffusion involves binding a substrate, on a specific solute binding site
  - The carrier protein and solute form an intermediate
  - After conformational change, the “product” is released (the transported solute)
  - Carrier proteins are regulated by external factors

Specificity of Carrier Proteins

- Carrier proteins share the property of high specificity with enzymes, too
- Transport proteins are often highly specific for a single compound or a small group of closely related compounds
- The carrier protein for glucose in erythrocytes is specific to a few monosaccharides, and is *stereospecific* for only their *D*-isomers



D-glucose

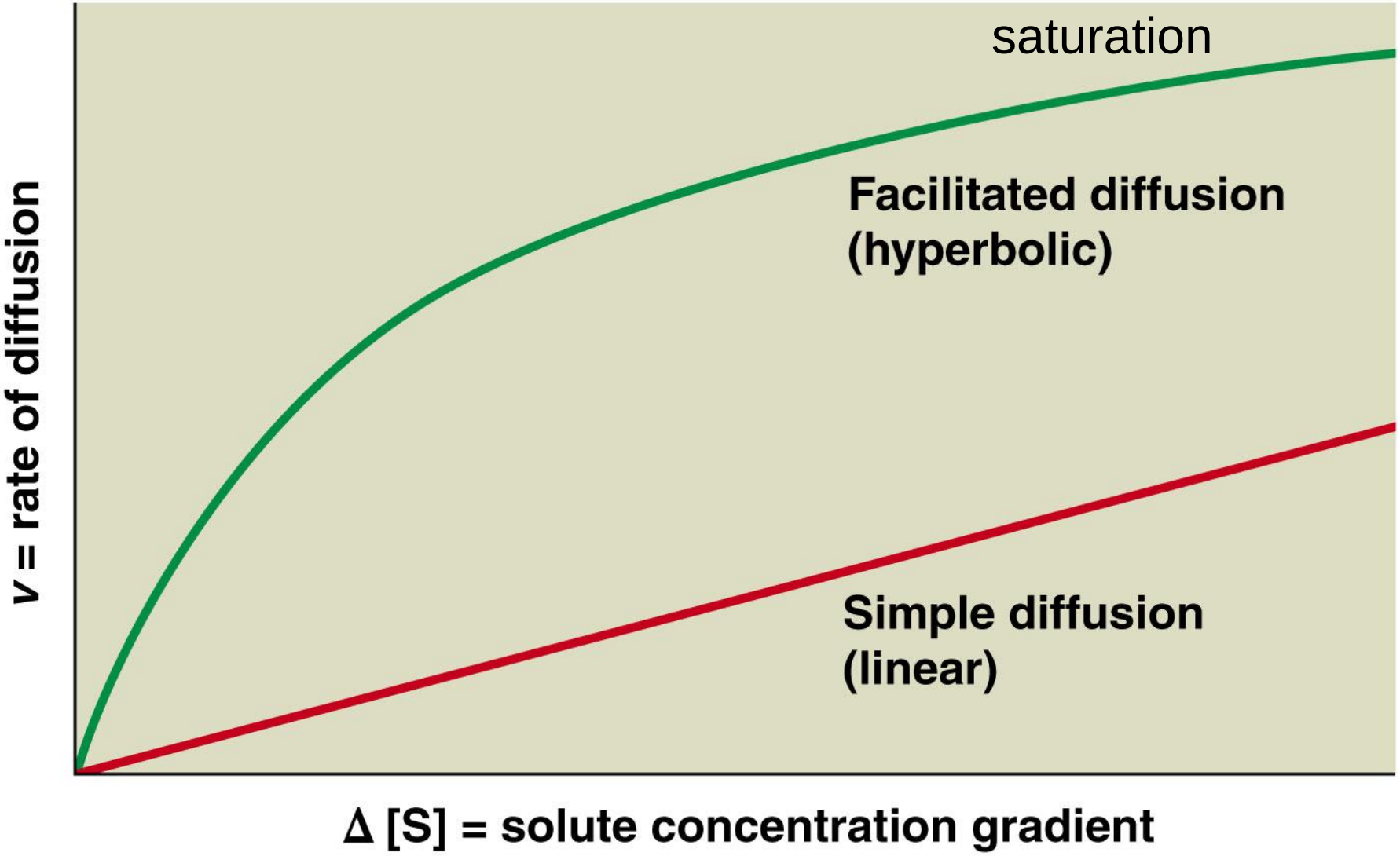


L-glucose

Kinetics of Carrier Protein Function

- Carrier proteins can become **saturated** as the concentration of the solute rises
- This is because the number of carrier proteins is limited and each functions at a finite maximum velocity
- So, carrier-facilitated transport (like enzyme catalysis) exhibits *saturation kinetics*

Figure 8-5



Competitive inhibition of carrier proteins

- *Competitive inhibition* of carrier proteins can occur in the presence of molecules or ions that are structurally related to the correct substrate

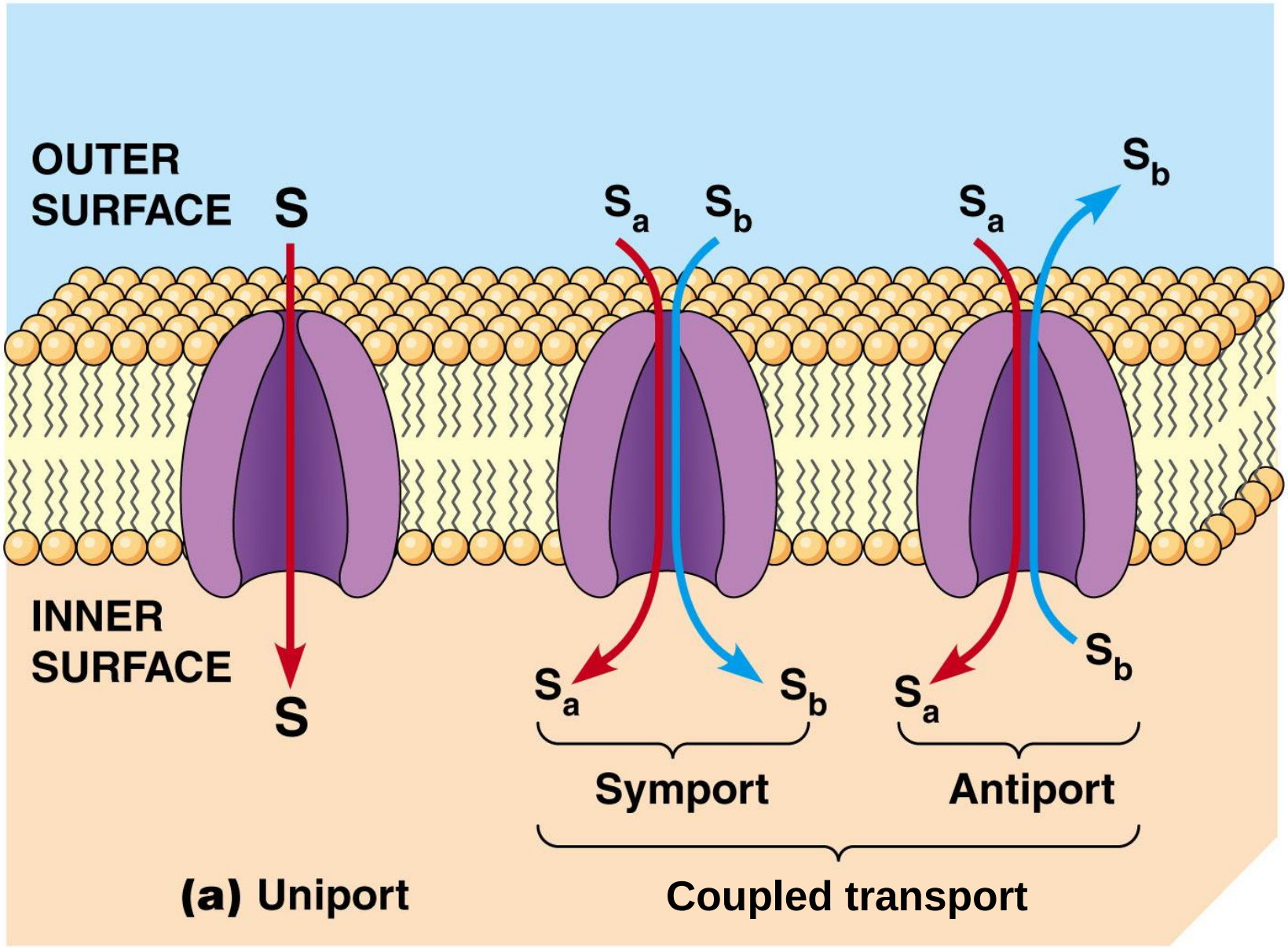
- For example, the transport of glucose by glucose carrier proteins can be inhibited by the other monosaccharides that the carrier accepts (such as mannose and galactose)

Carrier Proteins Transport Either One or Two Solutes

- When a carrier protein transports a single solute across the membrane, the process is called **uniport**

- A carrier protein that transports a single solute is called a *uniporter*

- When two solutes are transported simultaneously, and their transport is coupled, the process is called **coupled transport**



Coupled transport

- If the two solutes are moved across a membrane in the same direction, it is referred to as **symport** (or *cotransport*)
- If the solutes are moved in opposite directions, it is called **antiport** (or *countertransport*)
- Transporters that mediate these processes are *symporters* and *antiporters*

The Glucose Transporter: A Uniport Carrier

- The erythrocyte is capable of glucose uptake by facilitated diffusion because the level of blood glucose is much higher than that inside the cell
- Glucose is transported inward by a **glucose transporter** (**GLUT**; GLUT1 in erythrocytes)
- GLUT1 is an integral membrane protein with 12 transmembrane segments, which form a cavity with hydrophilic side chains

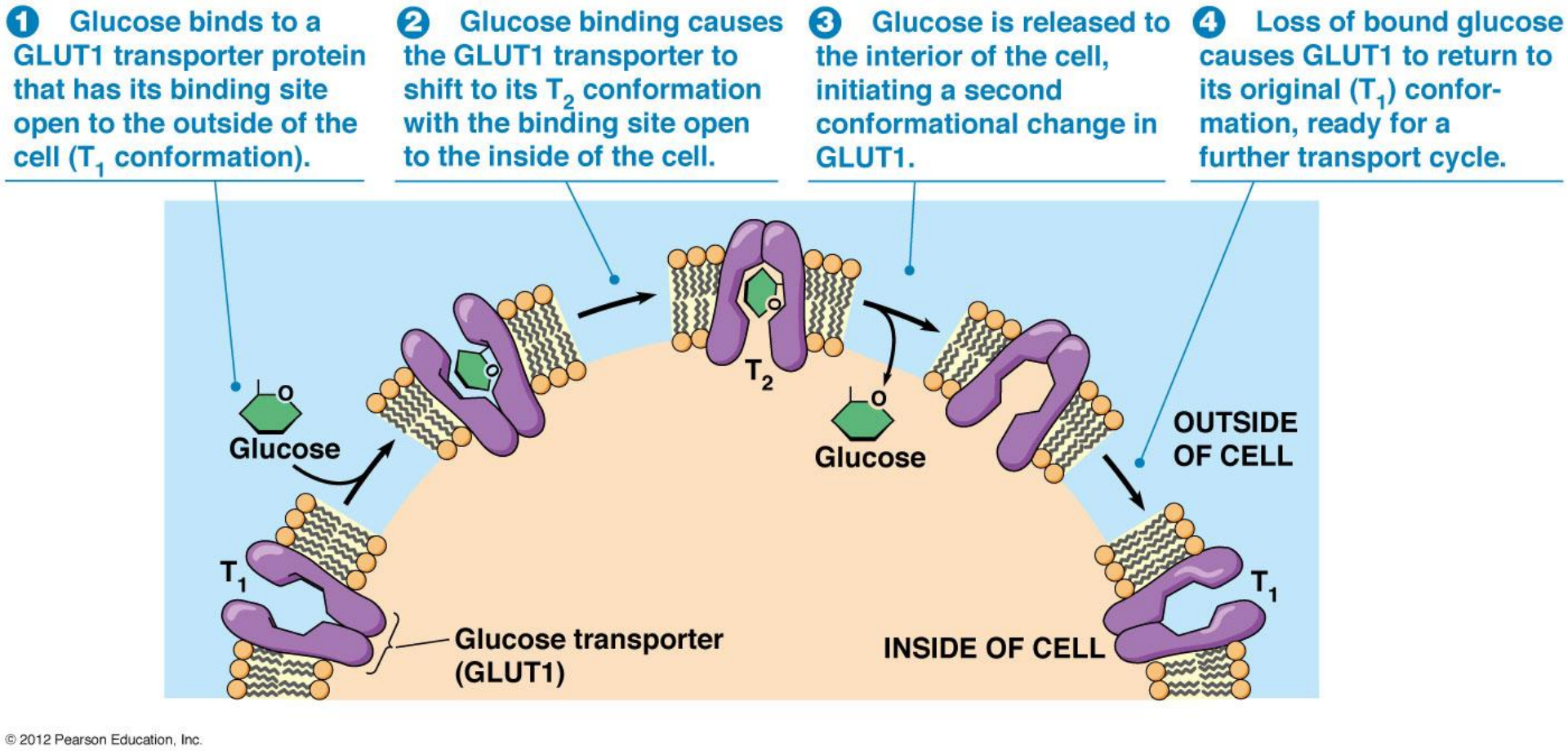
Mechanism of transport by GLUT1

- GLUT1 is thought to transport glucose through the membrane by the alternating conformation mechanism

- One conformational state, T_1, has the binding site for glucose open on the outside of the cell

- The other conformational state, T_2, has the binding site open to the inside of the cell

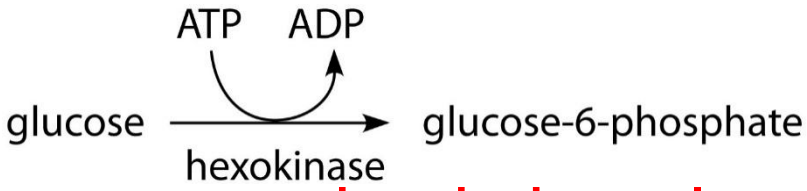
Figure 8-7



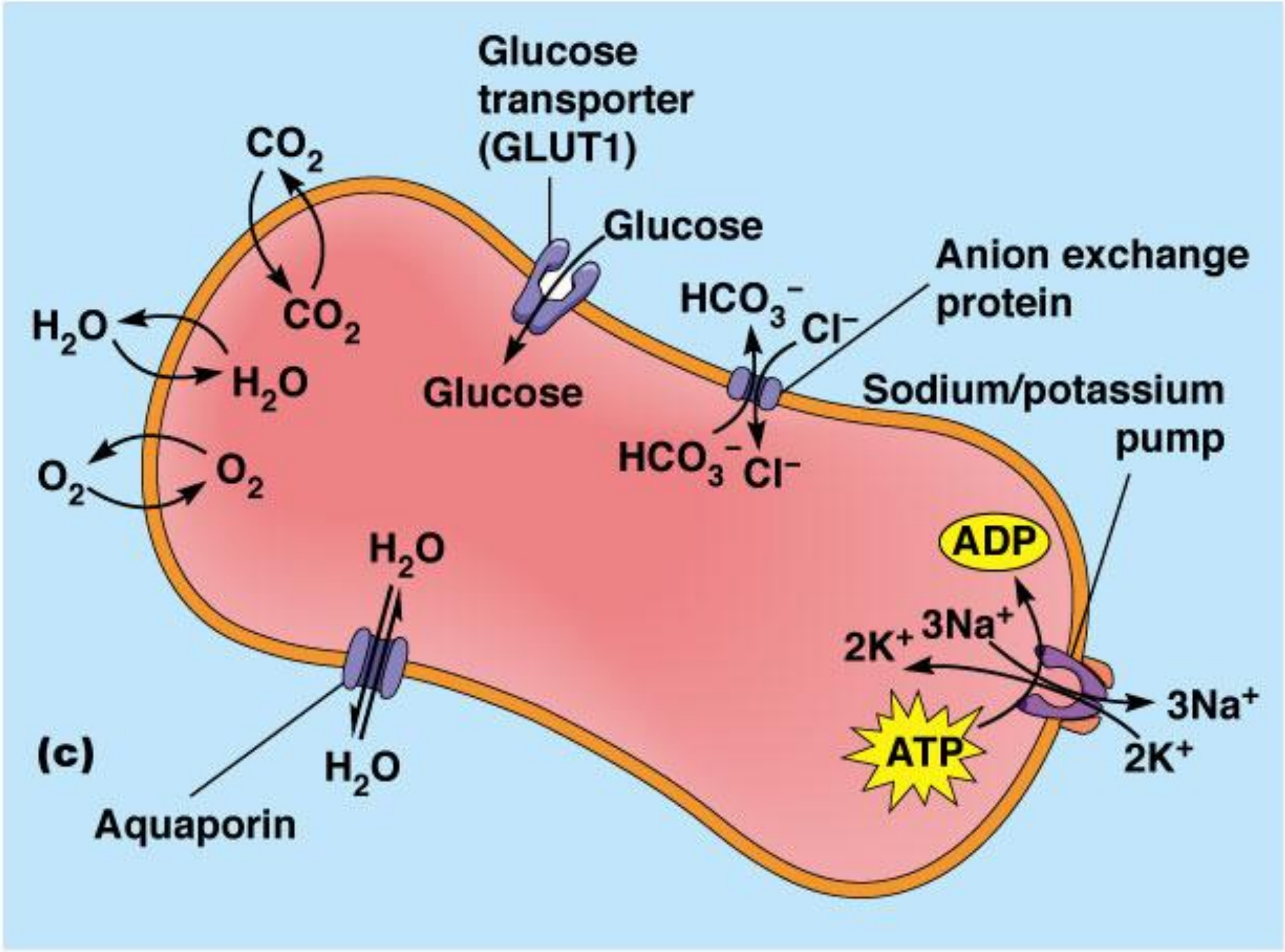
Transport by GLUT1 is reversible

- A carrier protein can facilitate transport in either direction

- The direction of transport is dictated by the relative solute concentrations outside and inside the cell



- Glucose concentration is kept low inside most animal cells (phosphorylation by hexokinase). Once phosporylated, glucose cannot bind the carrier protein any longer, and is effectively locked into the cell



The Erythrocyte Anion Exchange Protein: An Antiport Carrier

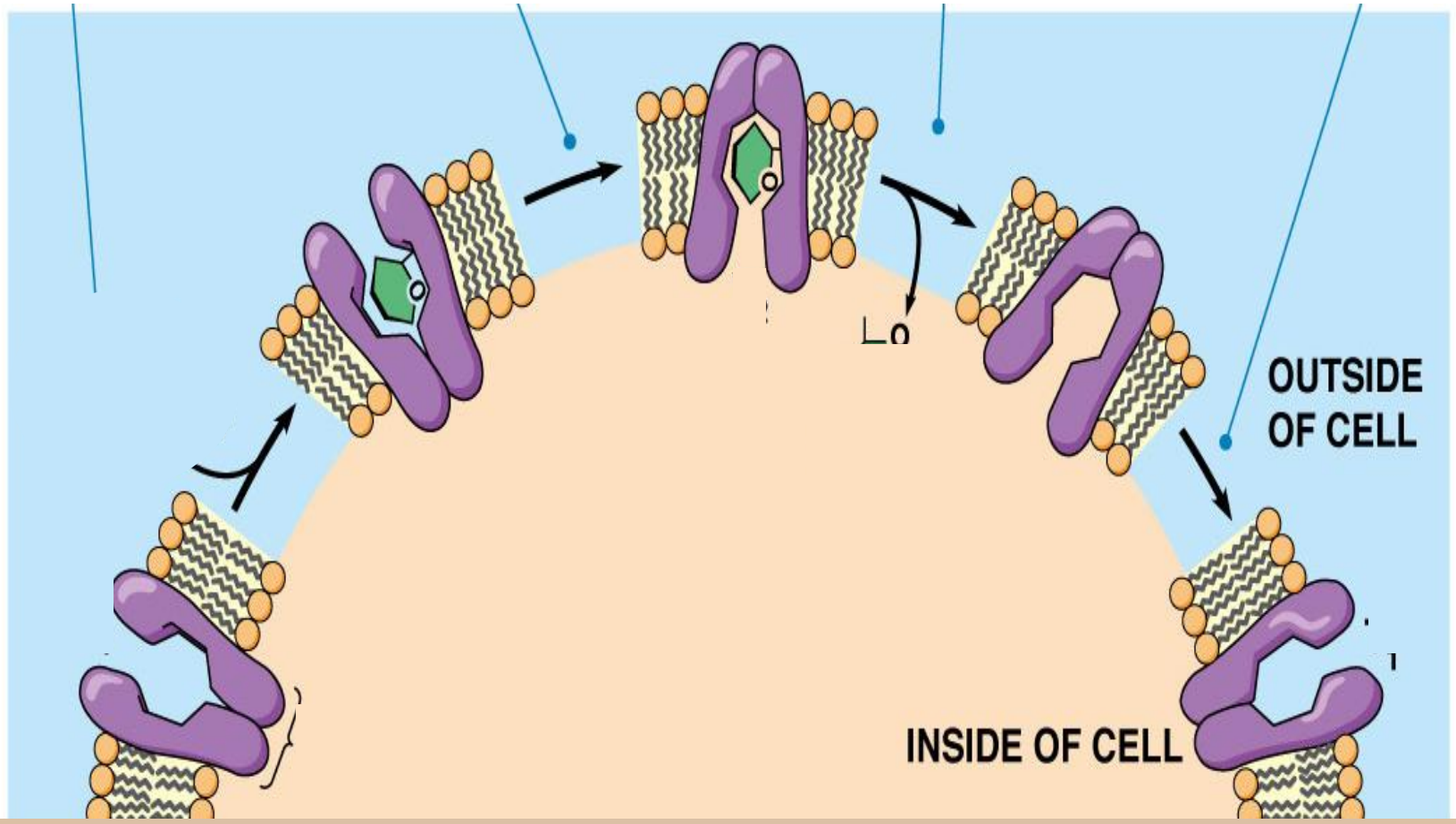
- The anion exchange protein (also called the chloride-bicarbonate exchanger) facilitates reciprocal exchange of Cl^- and HCO_3^- ions only
- Exchange will stop if either anion is absent
- The ions are exchanged in a strict 1:1 ratio

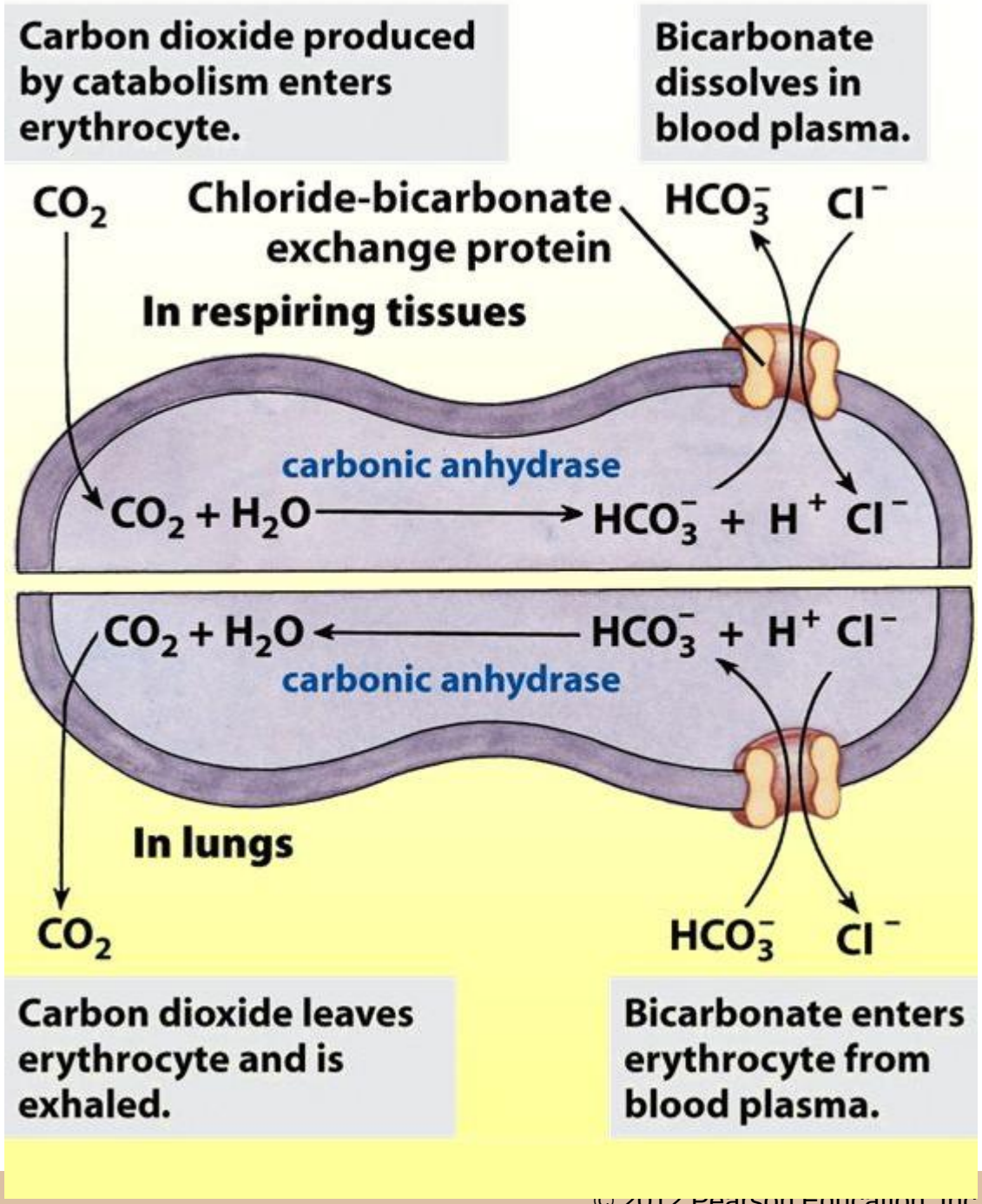
The “ping-pong” mechanism

- The anion exchange protein is thought to alternate between two conformational states
- In the first, it binds a chloride ion on one side of the membrane, which causes a change to the second state
- In the second state, the chloride is moved across the membrane and released

The “ping-pong” mechanism (continued)

- The release of chloride causes the protein to bind bicarbonate
- The binding of bicarbonate causes a shift back to the first conformation
- In this conformation, bicarbonate is moved out of the cell, allowing the carrier to bind chloride again





Biological relevance of anion exchange

- In tissues, waste CO_2 diffuses into the erythrocytes where it is converted to HCO_3^- by the enzyme *carbonic anhydrase*
- As the concentration of bicarbonate rises it moves out of the cell, coupled with uptake of Cl^- to prevent a net charge imbalance
- In the lungs, the entire process is reversed

Active Transport: Protein-Mediated Movement Up the Gradient

- Facilitated diffusion is important, but only accounts for movement of molecules down a concentration gradient, toward equilibrium
- Sometimes a substance must be transported *against* a concentration gradient
- In this case active transport is used to move solutes *up* a concentration gradient, *away* from equilibrium

Functions of active transport

- Active transport couples endergonic transport to an exergonic process, usually ATP hydrolysis

- Active transport performs three important cellular functions

  - Uptake of essential nutrients
  - Removal of wastes
  - Maintenance of non-equilibrium concentrations of certain ions

Nonequilibrium conditions

- Active transport allows the creation and maintenance of an internal cellular environment that differs greatly from the surrounding environment

- Many membrane proteins involved in active transport are called **pumps**, because energy is required to move substances against their concentration gradients

Active transport is *unidirectional*

- Active transport differs from diffusion (both simple and facilitated) in the direction of transport
- Diffusion is ***nondirectional*** with respect to the membrane and proceeds as directed by the concentrations of the transported substances
- Active transport has an intrinsic **directionality**

The Coupling of Active Transport to an Energy Source May Be Direct or Indirect

- Active transport mechanisms can be divided based on the **sources of energy** and whether or not two solutes are transported at **the same time**

- Active transport is categorized as *direct* or *indirect*

Direct active transport

- In **direct active transport** (or *primary active transport*), the accumulation of solute molecules on one side of the membrane is coupled *directly* to an exergonic chemical reaction

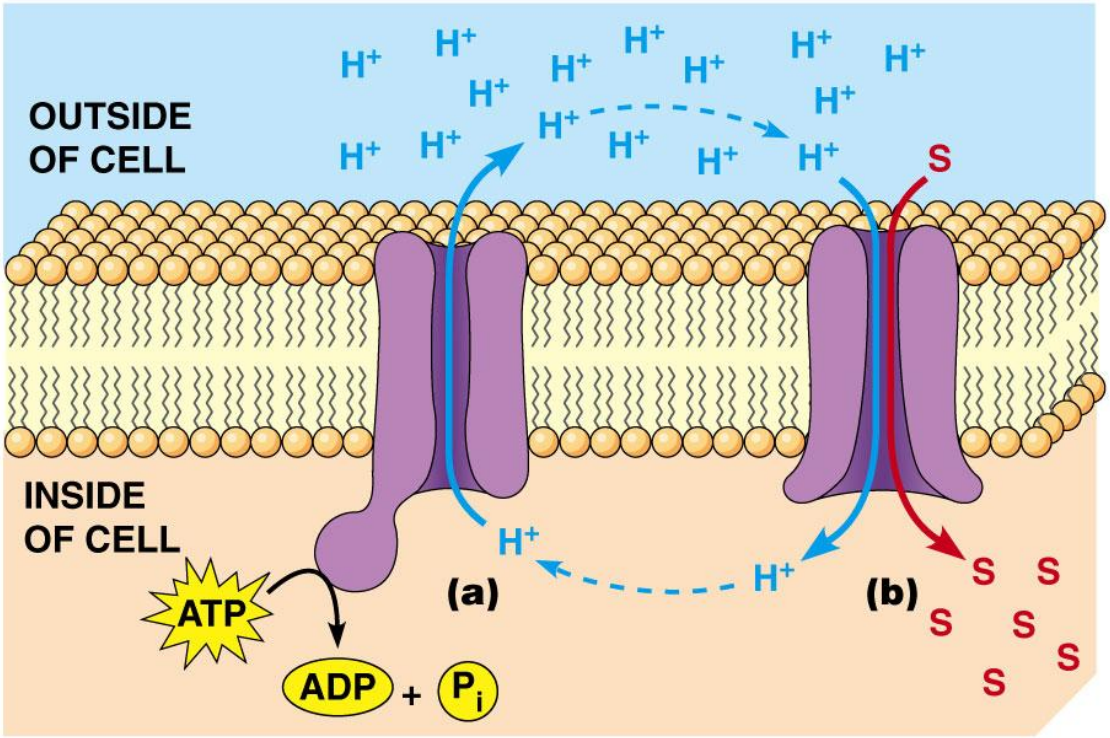
- This is usually hydrolysis of ATP

- Transport proteins driven by ATP hydrolysis are called *transport ATPases* or *ATPase pumps*

Figure 8-9A

(a) Direct active transport involves a transport system coupled to an exergonic chemical reaction, most commonly the hydrolysis of ATP. As shown here, ATP hydrolysis drives the outward transport of protons, thereby establishing an electro-chemical potential for protons across the membrane.

(b) Indirect active transport involves the coupled transport of a solute S and ions—protons, in this case. The exergonic inward movement of protons provides the energy to move the transported solute, S, against its concentration gradient or electrochemical potential.



Indirect active transport

- Indirect active transport depends on the **simultaneous transport of two solutes**

- Favorable movement of one solute *down* its gradient drives the unfavorable movement of the other *up* its gradient

- This can be a ***symport or an antiport***, depending on whether the two molecules are transported in the same or different directions

Figure 8-9A

(a) Direct active transport involves a transport system coupled to an exergonic chemical reaction, most commonly the hydrolysis of ATP. As shown here, ATP hydrolysis drives the outward transport of protons, thereby establishing an electro-chemical potential for protons across the membrane.

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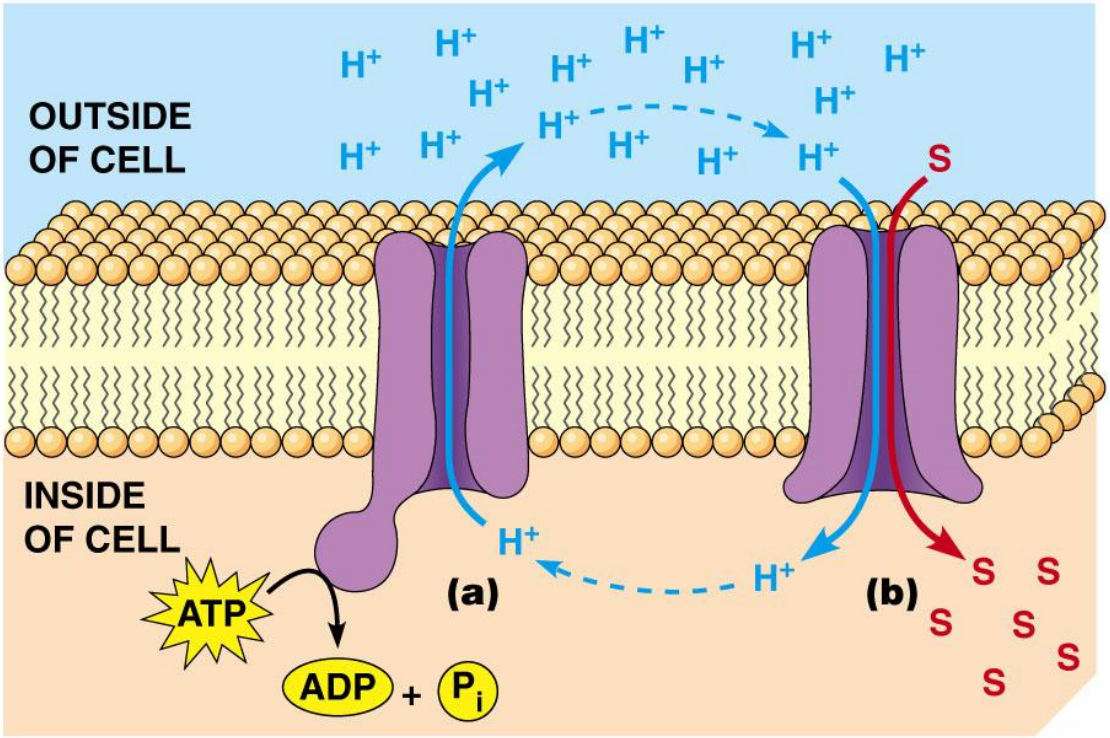


Indirect Active Transport Is Driven by Ion Gradients

- Indirect active transport (or *secondary active transport*) is not powered by ATP hydrolysis

- The inward transport of molecules *up* their electrochemical gradients is often **coupled** to and driven by simultaneous inward movement of Na^+ (animals) or protons (plant, fungi, bacteria) *down* their gradients

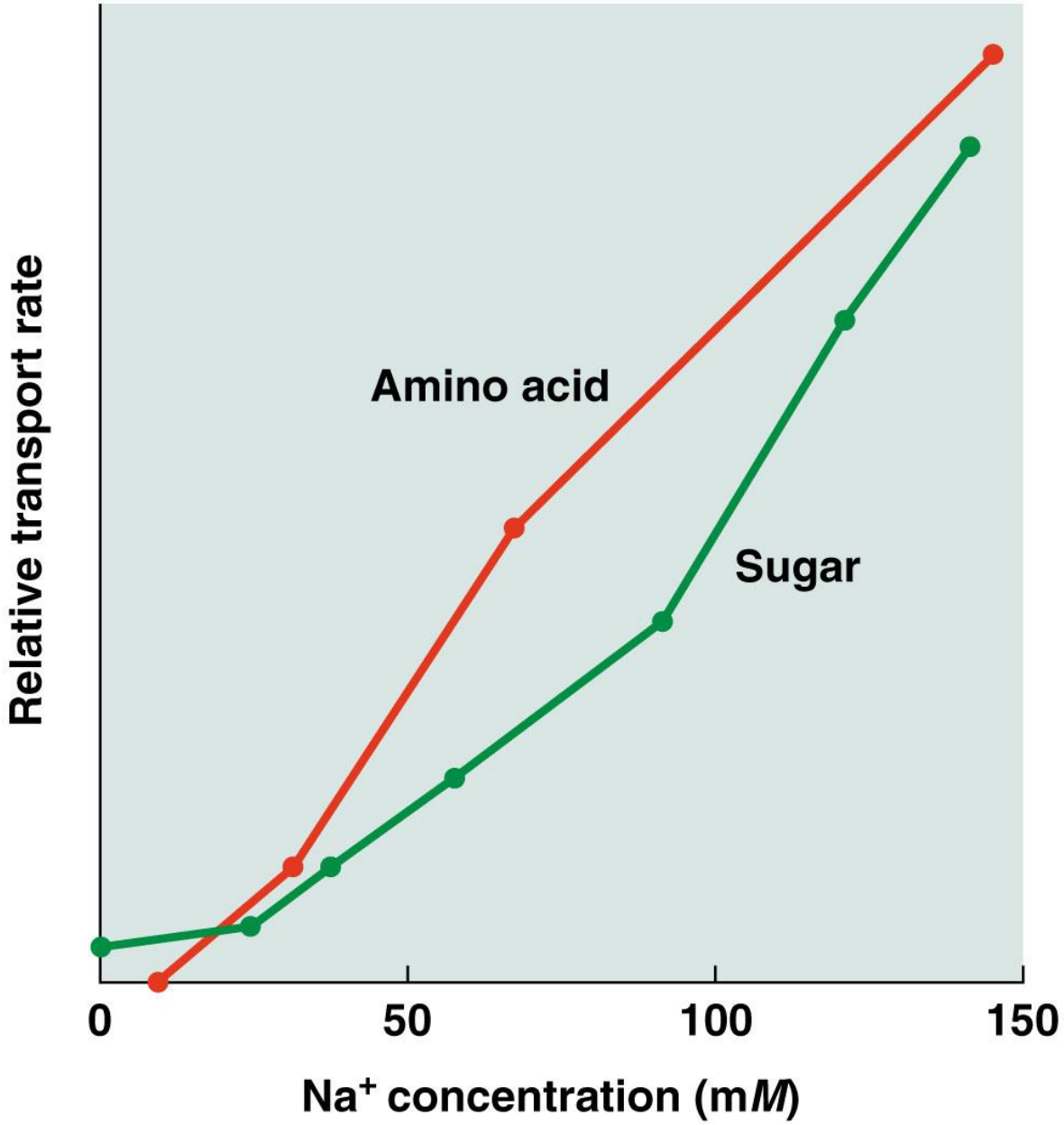
Symport mechanisms of indirect active transport

- Most cells continuously pump either sodium ions or protons out of the cell (e.g., the Na^+/K^+ pump in animals)

- The resulting high extracellular concentration of Na^+ is a driving force for the **uptake of sugars and amino acids**

- This is indirectly related to ATP because the pump that maintains the sodium ion gradient is driven by ATP

Figure 8-10



Proton gradients drive indirect active transport in many organisms

- Most organisms rely on proton gradients rather than the Na^+ gradients used by animals

- For example, fungi and plants use proton symport for the uptake of organic solutes, with ATP driving the proton pump that creates and maintains the proton electrochemical potential

- Proton or ion gradients can be used for *export* as well as *import*

Direct Active Transport: The Na^+/K^+ Pump Maintains Electrochemical Ion Gradients

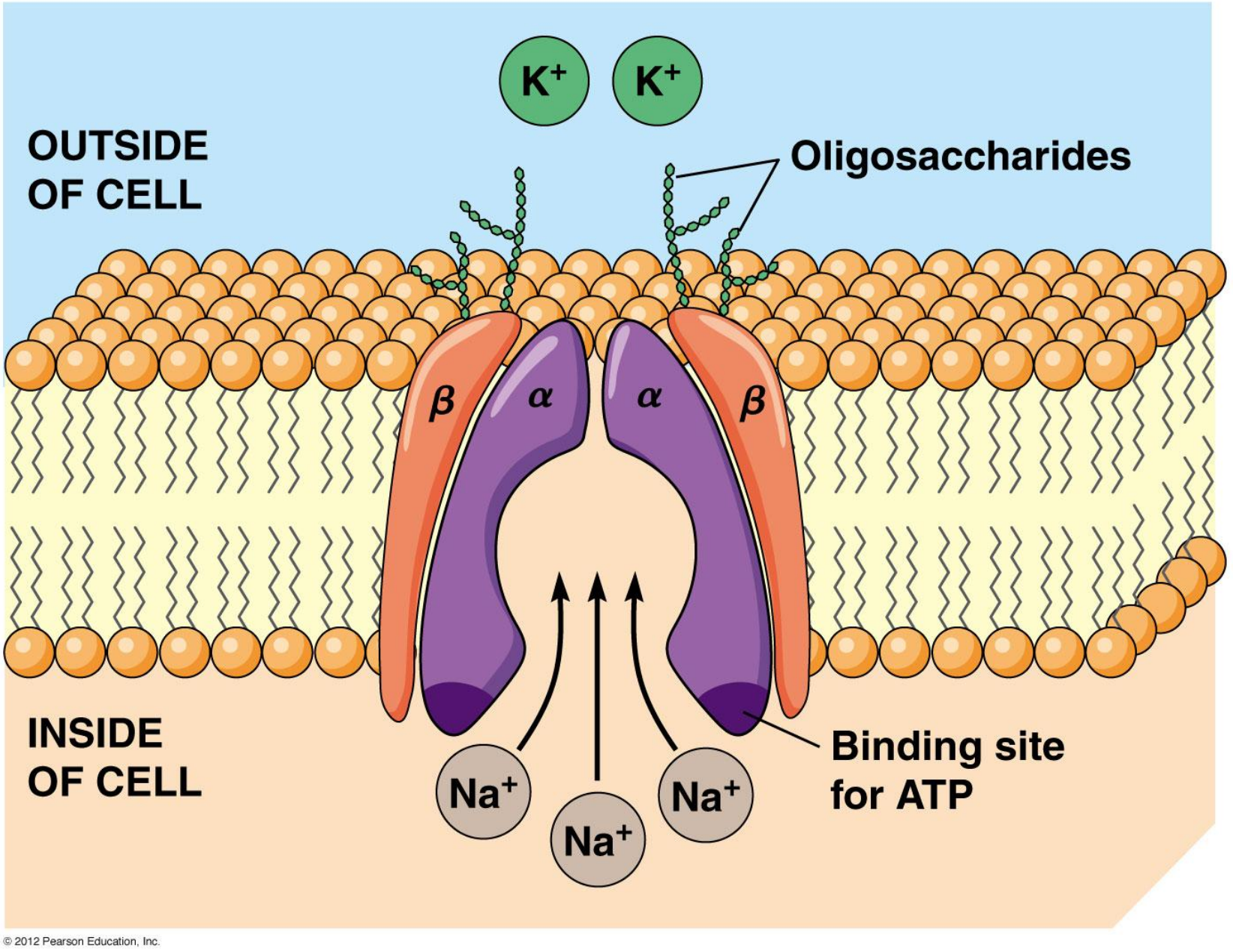
- In a typical mammalian neuron, $[K^+]_{inside}/[K^+]_{outside}$ is about 35:1 and $[Na^+]_{inside}/[Na^+]_{outside}$ is around 0.08:1

- The electrochemical potentials for sodium and potassium are essential as a driving force for coupled transport and for transmission of nerve impulses

Requirement for energy

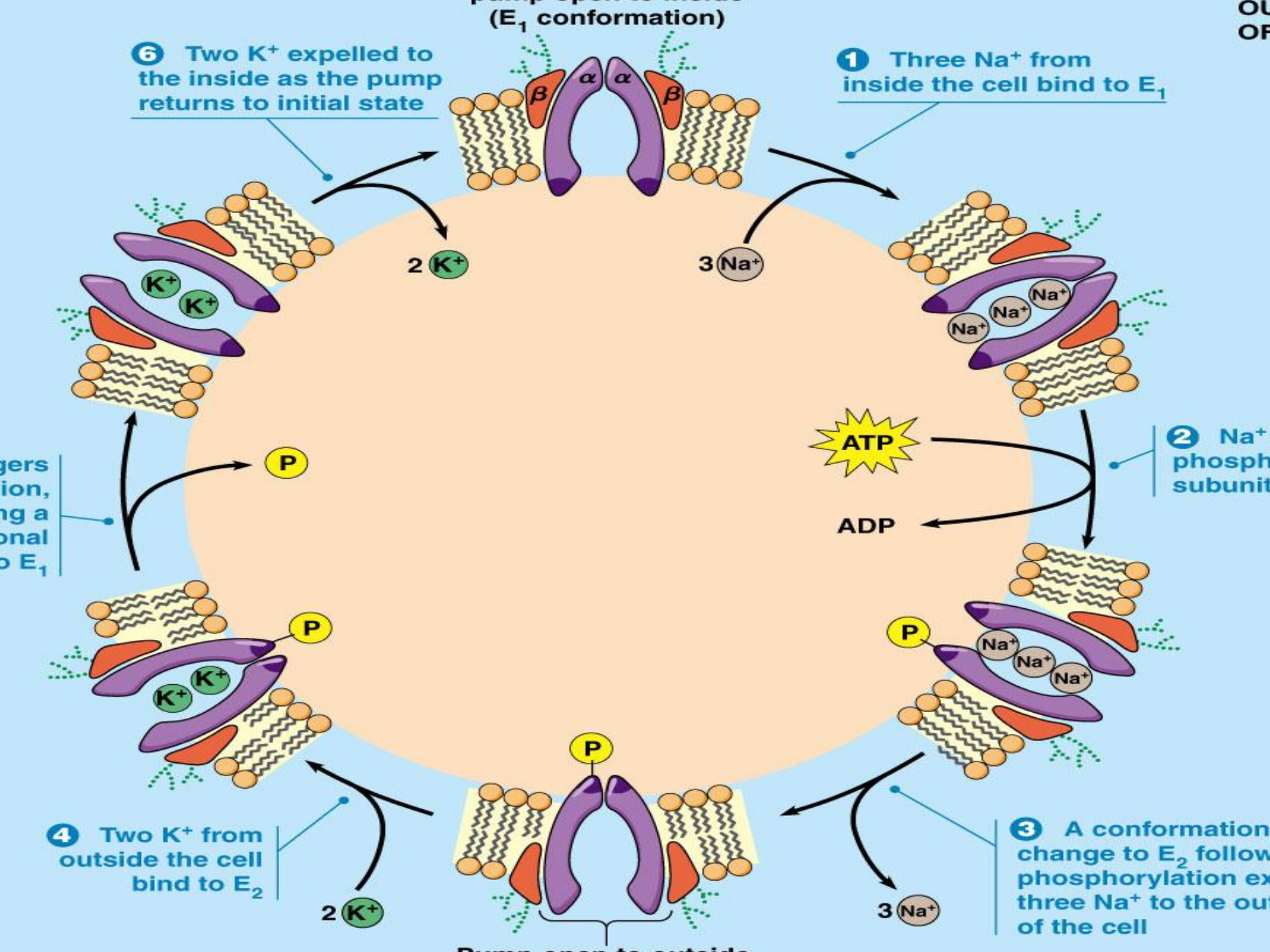
- The pumping of both Na^+ and K^+ ions against their gradients requires energy
- The pump that is responsible, the **Na^+/K^+ ATPase** (or **pump**), uses the **hydrolysis of ATP** to drive the transport of both ions
- It is responsible for the asymmetric distribution of ions across the plasma membrane of animal cells
- Three sodium ions are moved out and two potassium ions moved in per molecule of ATP hydrolysed

Figure 8-11



The Na^+/K^+ pump is an allosteric protein

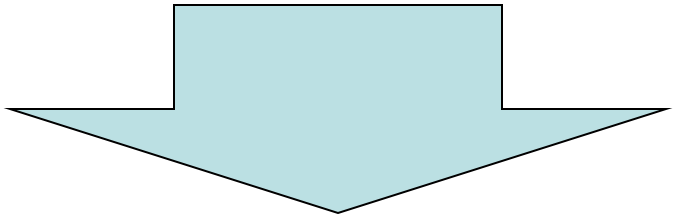
- The Na^+/K^+ pump has two alternative conformational states, E_1 and E_2
- The E_1 conformation is open to the inside of the cell and has high affinity for Na^+ ions
- The E_2 conformation is open to the outside of the cell and has high affinity for K^+ ions


(E1 conformation)
6 Two K+ expelled to the inside as the pump returns to initial state
1 Three Na+ from inside the cell bind to E1
2 K+
3 Na+
K+
K+
Na+
Na+
Na+
ATP
ADP
2 Na+ phosph subunit
gers ion, ng a onal o E1
P
P
K+
K+
P
Na+
Na+
Na+
4 Two K+ from outside the cell bind to E2
2 K+
3 Na+
3 A conformation change to E2 follow phosphorylation ex three Na+ to the ou of the cell
α
α
β
β

The Na^+/K^+ pump

3 Na+ outside
2 K+ inside

1+ charge difference to the outside



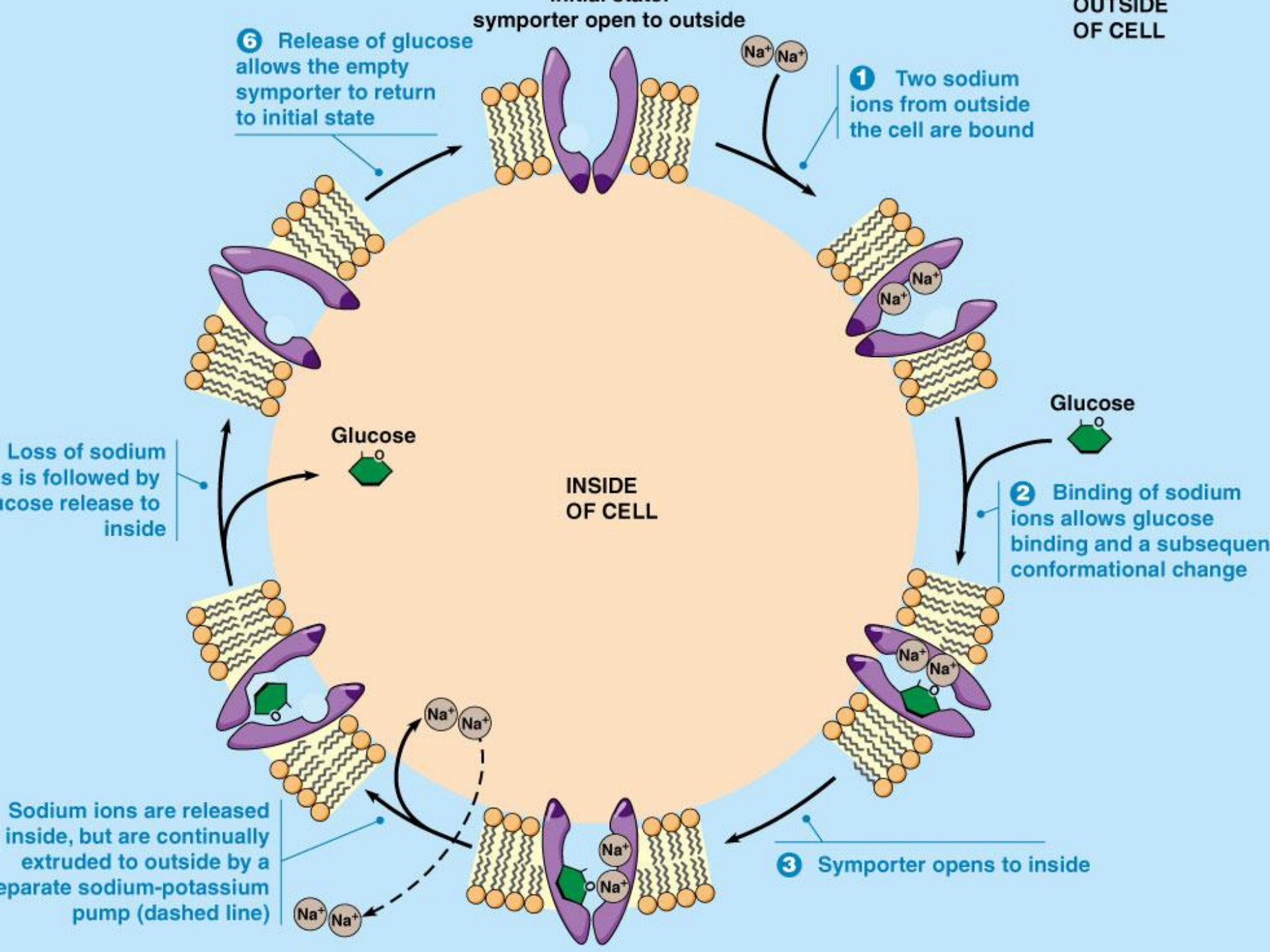
MAINTAIN THE MEMBRANE POTENTIAL

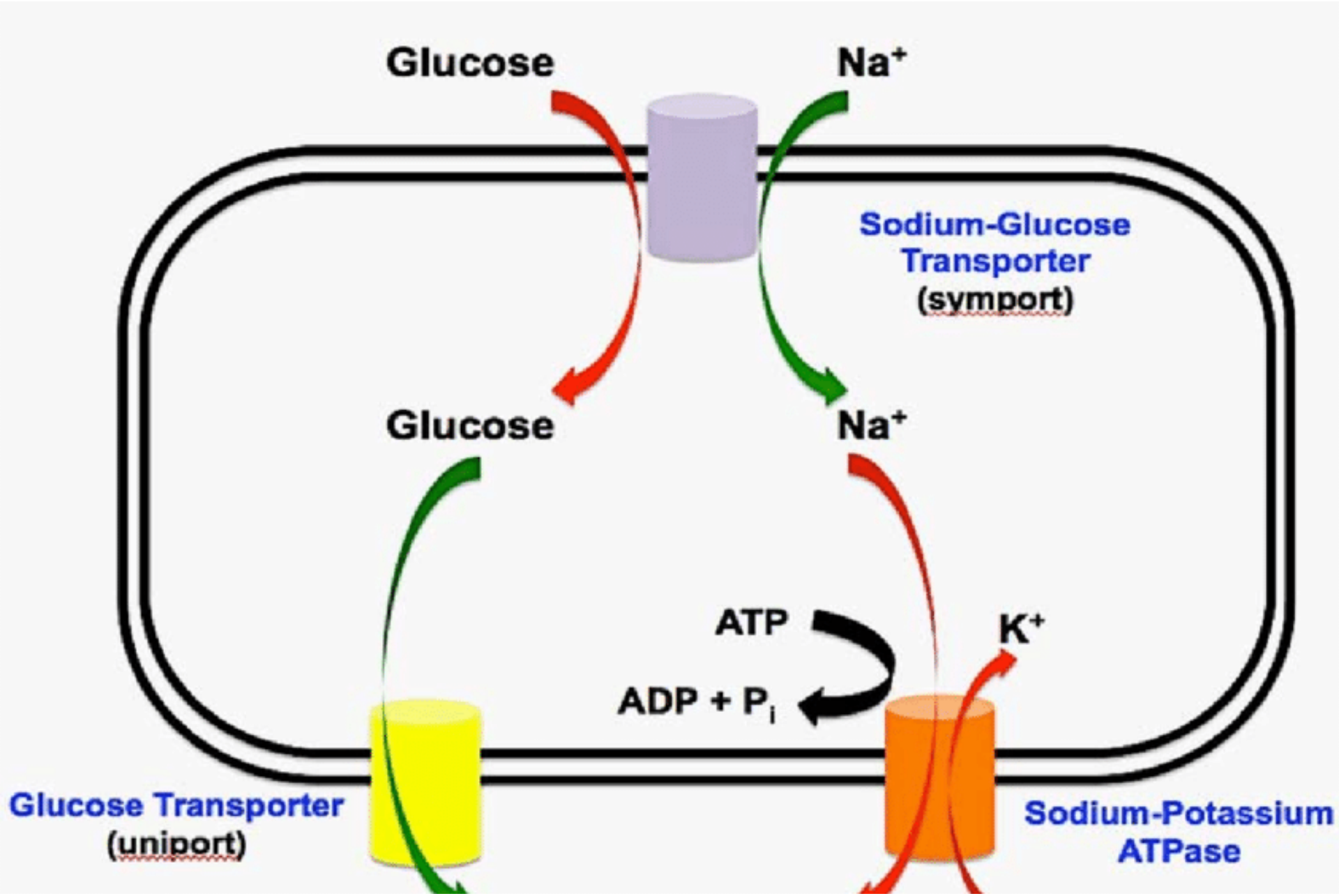
Indirect Active Transport: Sodium Symport Drives the Uptake of Glucose

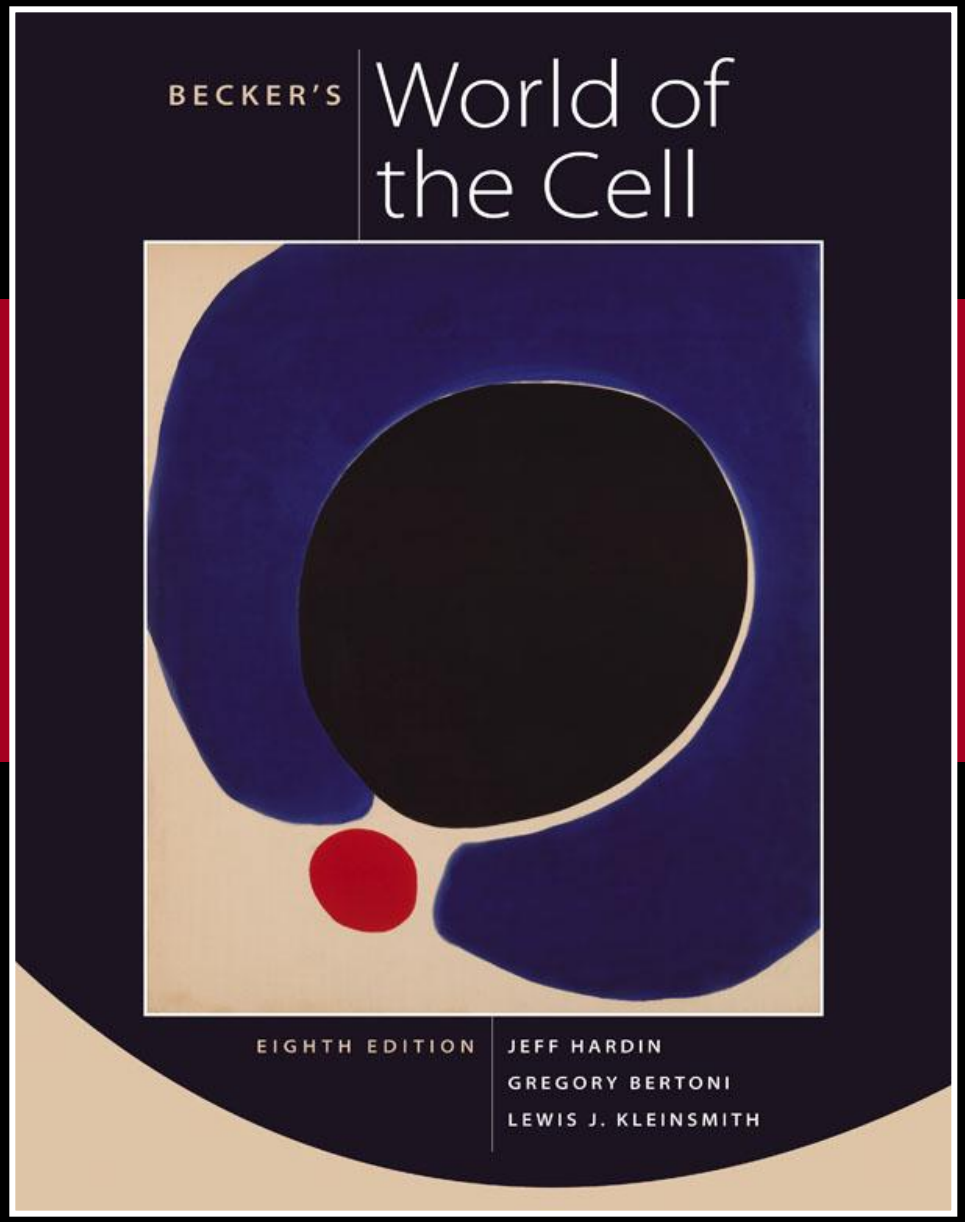
- Although most glucose into and out of our cells occurs by facilitated diffusion, some cells use a **Na^+/glucose symporter**

- For example, the cells lining the intestine take up glucose and some amino acids even when their concentrations are much lower outside than inside the cells

- A steep Na^+ gradient that is maintained across the plasma membrane (via the Na^+/K^+ pump) is used to provide the energy needed


symporter open to outside
OUTSIDE OF CELL
6 Release of glucose allows the empty symporter to return to initial state
1 Two sodium ions from outside the cell are bound
Na+
Na+
Glucose
Loss of sodium
s is followed by
ucose release to
inside
INSIDE OF CELL
2 Binding of sodium ions allows glucose binding and a subsequen conformational change
3 Symporter opens to inside
Sodium ions are released inside, but are continually extruded to outside by a eparate sodium-potassium pump (dashed line)





Chapter 10 + Chapter 11

*Chemotrophic Energy Metabolism
*Phototrophic Energy Metabolism

Lectures by
Kathleen Fitzpatrick
Simon Fraser University

Chemotrophic Energy Metabolism: Aerobic Respiration

- Some cells meet their energy needs through anaerobic fermentation
- However, fermentation yields only modest amounts of energy due to the absence of electron transfer
- ATP yield is much higher in cellular respiration

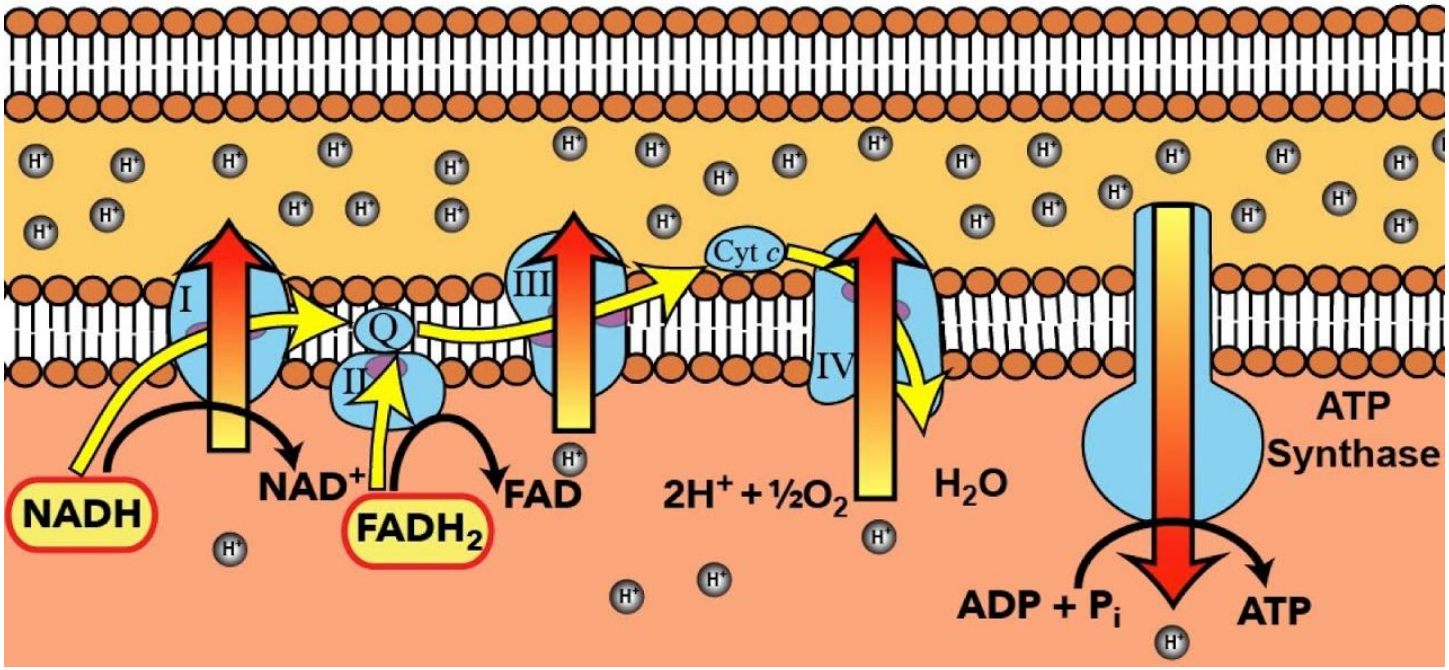
Cellular Respiration: Maximizing ATP Yields

- **Cellular respiration** (or **respiration**) uses an *external* electron acceptor to oxidize substrates completely to CO_2

- External electron acceptor: one that is not a by-product of glucose catabolism

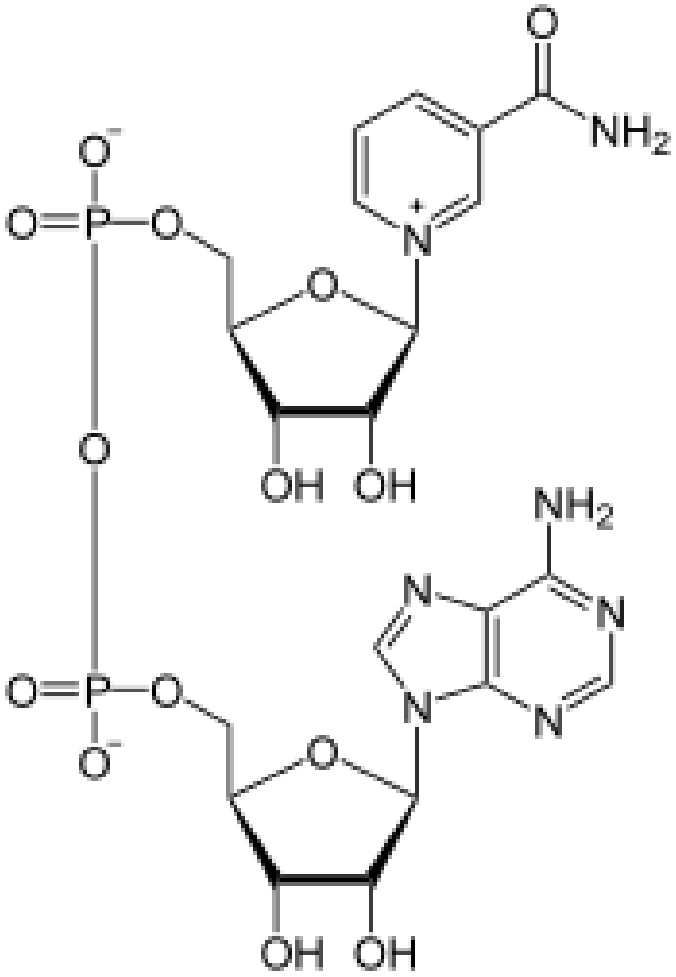
Cellular respiration defined

- Respiration is *the flow of electrons through or within a membrane, from reduced coenzymes to an external electron acceptor usually accompanied by the generation of ATP*



- Coenzymes such as *FAD* (*flavin adenine dinucleotide*), NADH (*nicotinamide adenine dinucleotide*) and *coenzyme Q* (*ubiquinone*) are involved

The structure of NADH



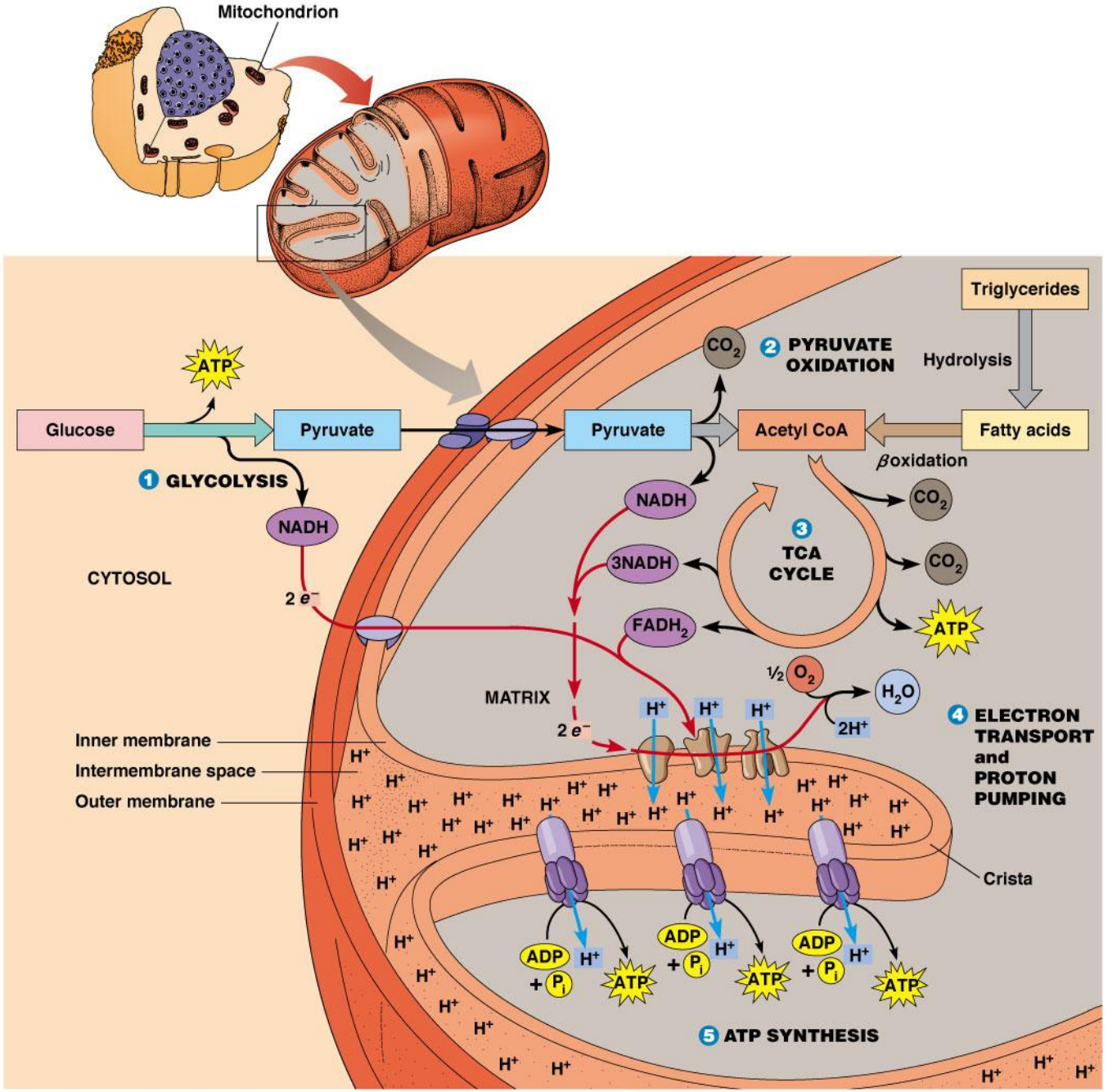
The terminal electron acceptor

- In **aerobic respiration**, the terminal electron acceptor is *oxygen* and the reduced form is *water*
- Other terminal electron acceptors (sulfur, protons, and ferric ions) are used by other organisms, especially bacteria and archaea
- These are examples of **anaerobic respiration**

Mitochondria

- Most aerobic ATP production in eukaryotic cells takes place in the *mitochondrion*
- In bacteria, the plasma membrane and cytoplasm are analogous to the mitochondrial inner membrane and matrix with respect to energy metabolism

Figure 10-1



The Mitochondrion: Where the Action Takes Place

- The **mitochondrion** is called the "energy powerhouse" of the eukaryotic cell
- These organelles are thought to have arisen from bacterial cells
- Mitochondria have been shown to carry out all the reactions of the TCA cycle, electron transport, and oxidative phosphorylation

Mitochondria Are Often Present Where the ATP Needs Are Greatest

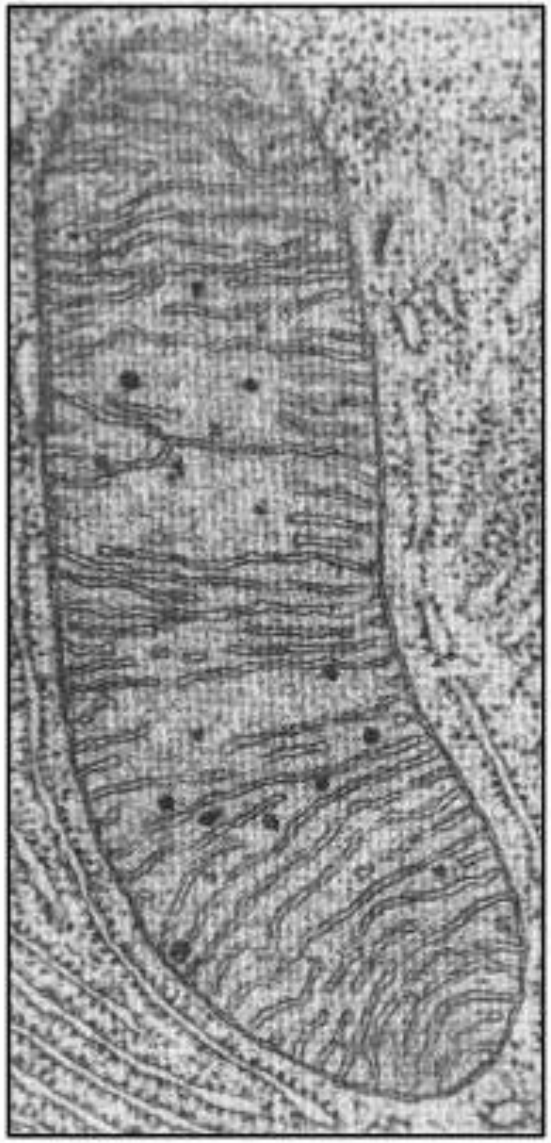
- Mitochondria are found in virtually all aerobic cells of eukaryotes
- They are present in both chemotrophic and phototrophic cells

- Mitochondria are frequently clustered in regions of cells with the greatest need for ATP, e.g., muscle cells

Are Mitochondria Interconnected Networks Rather than Discrete Organelles?

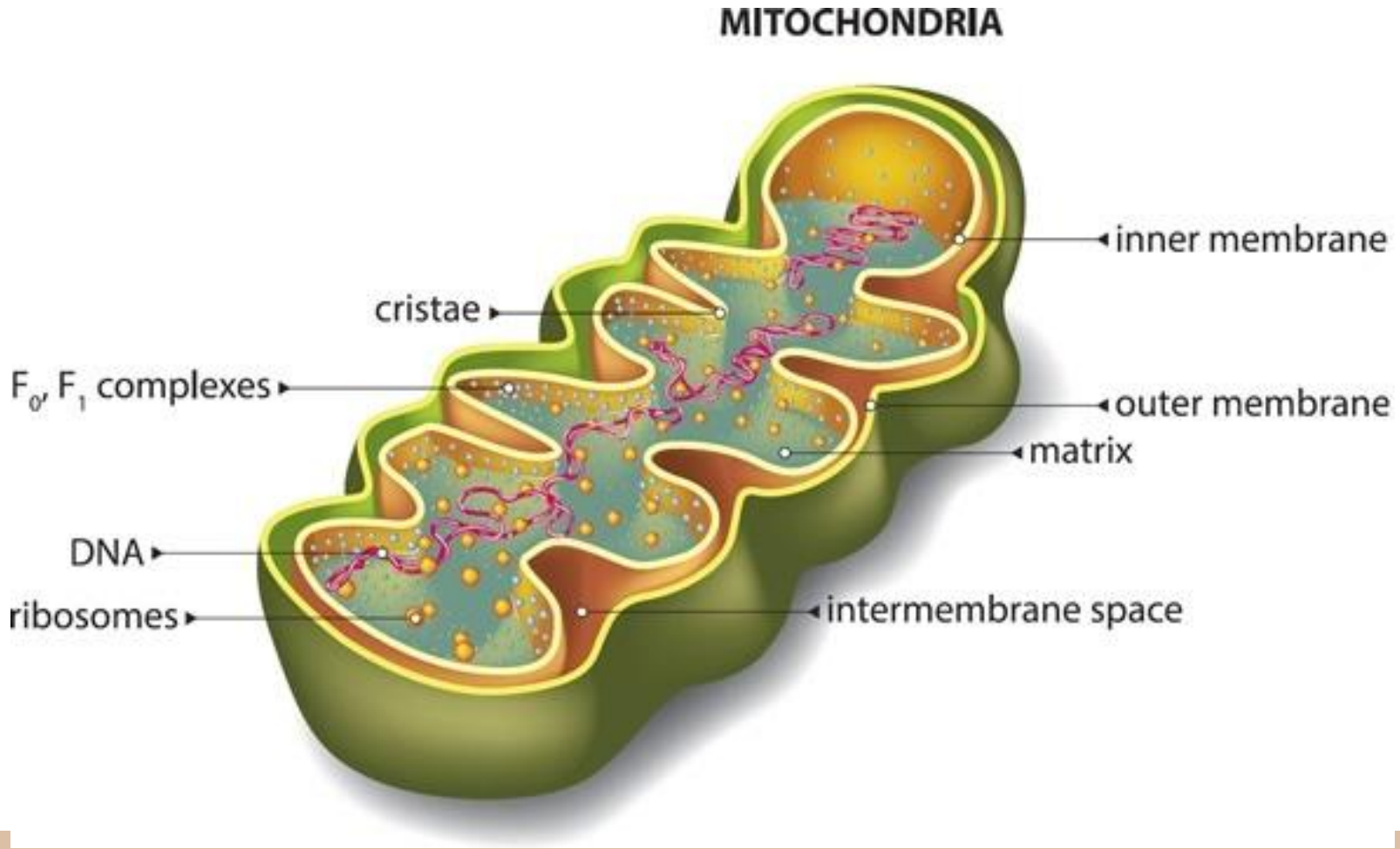
- In electron micrographs, mitochondria usually appear as oval structures
- However they can take various shapes and sizes, depending on the cell type
- Their appearance under EM suggests that they are large, and numerous discrete entities

Mitochondrion seen by TEM



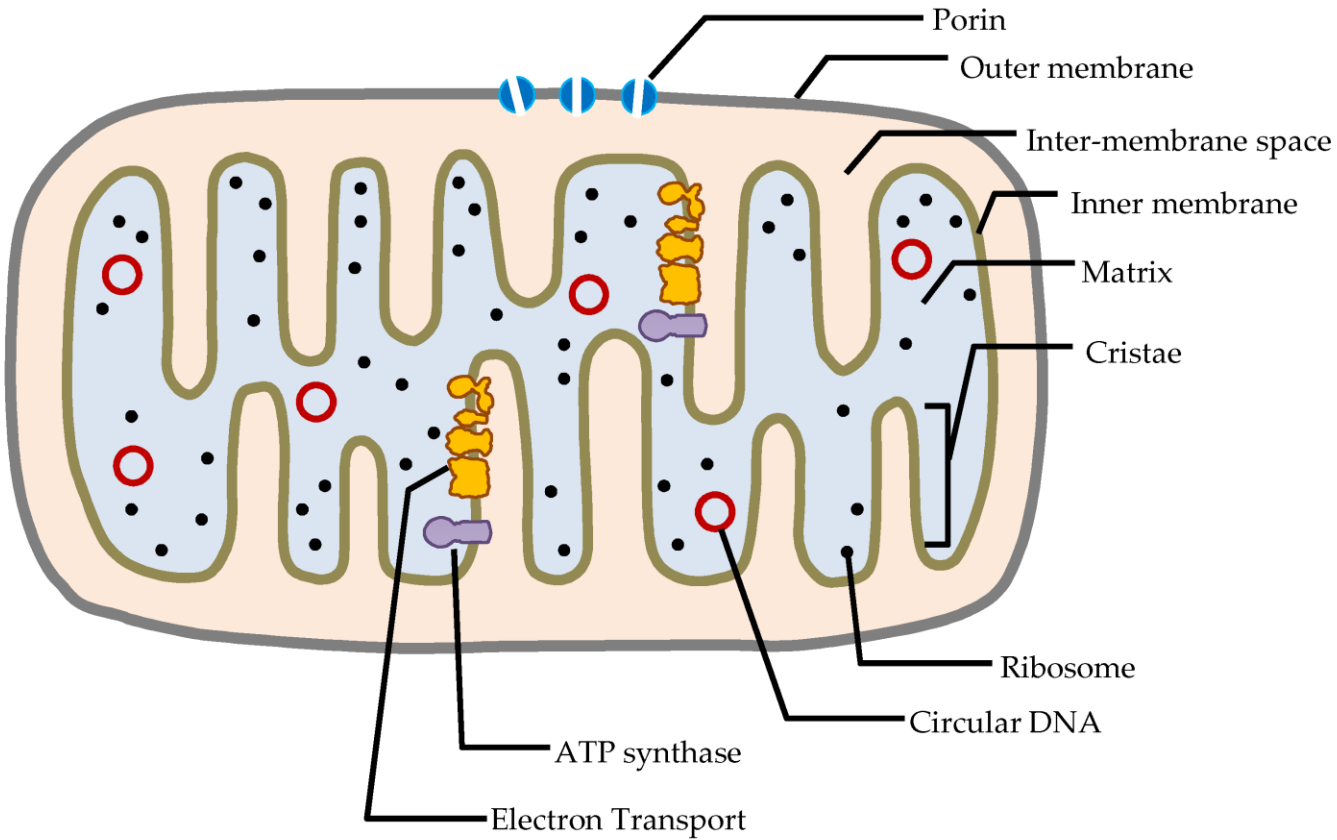
Mitochondrion seen by EM topography

The Outer and Inner Membranes Define Two Separate Compartments and Three Regions



The Outer and Inner Membranes Define Two Separate Compartments and Three Regions

- The **outer membrane** contains **porins** that allow passage of solutes with MW up to 5000 Da



The Outer and Inner Membranes Define Two Separate Compartments and Three Regions

- The **inner membrane** is impermeable to most solutes, partitioning the mitochondrion into two separate compartments

- The **intermembrane space** (IMS) between the MIM and MOM is thus continuous with the cytosol

MOM and MIM lipid composition

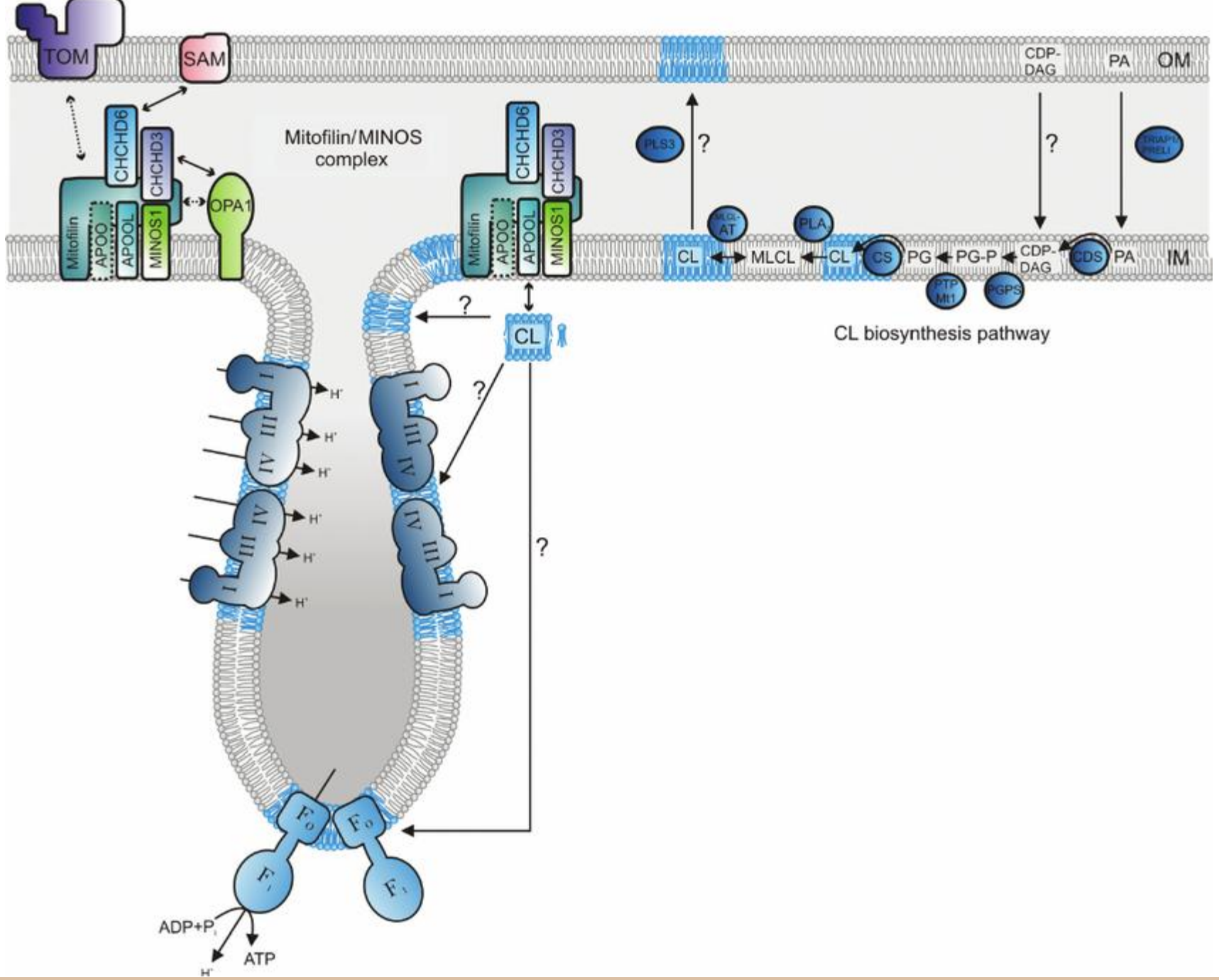
- MOM ➔ 59% lipid and 41% protein

  Rich in phosphotadylcholine, phosphotidylethanolamine, phosphatidylinositol, and very low phosphatidylserine concentration

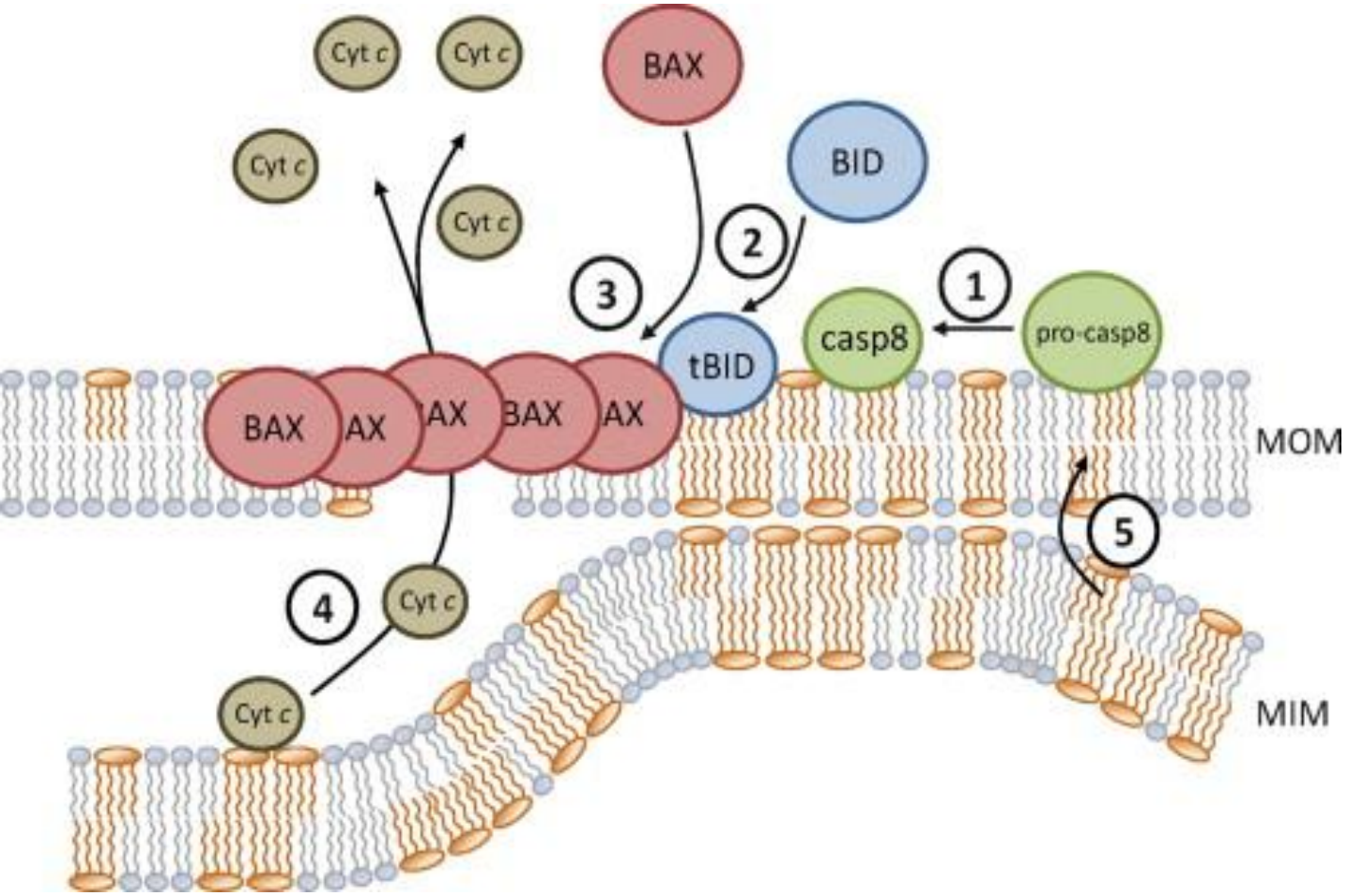
- MIM ➔ 23% lipid and 77% protein

  Rich in diphosphatidylglycerol (cardiolipin) and phosphatidylethanolamine

Cosentino, K., & García-Sáez, A. J. (2014). *Mitochondrial alterations in apoptosis. Chemistry and Physics of Lipids, 181, 62–75.*doi:10.1016/j.chemphyslip.2014.04.001

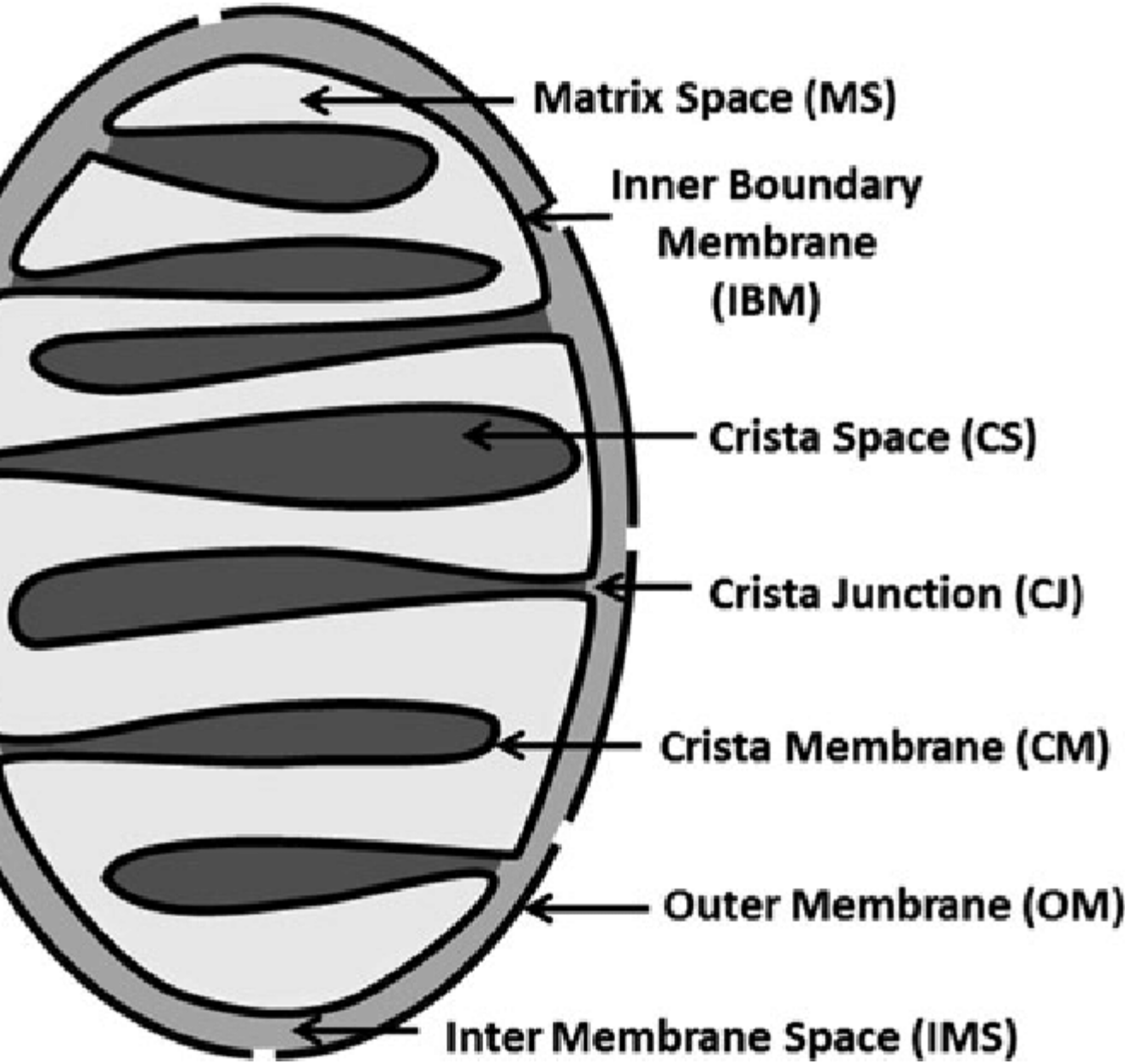


Cardiolipin is redistributed between the MOM and MIM in the early stages of apoptosis

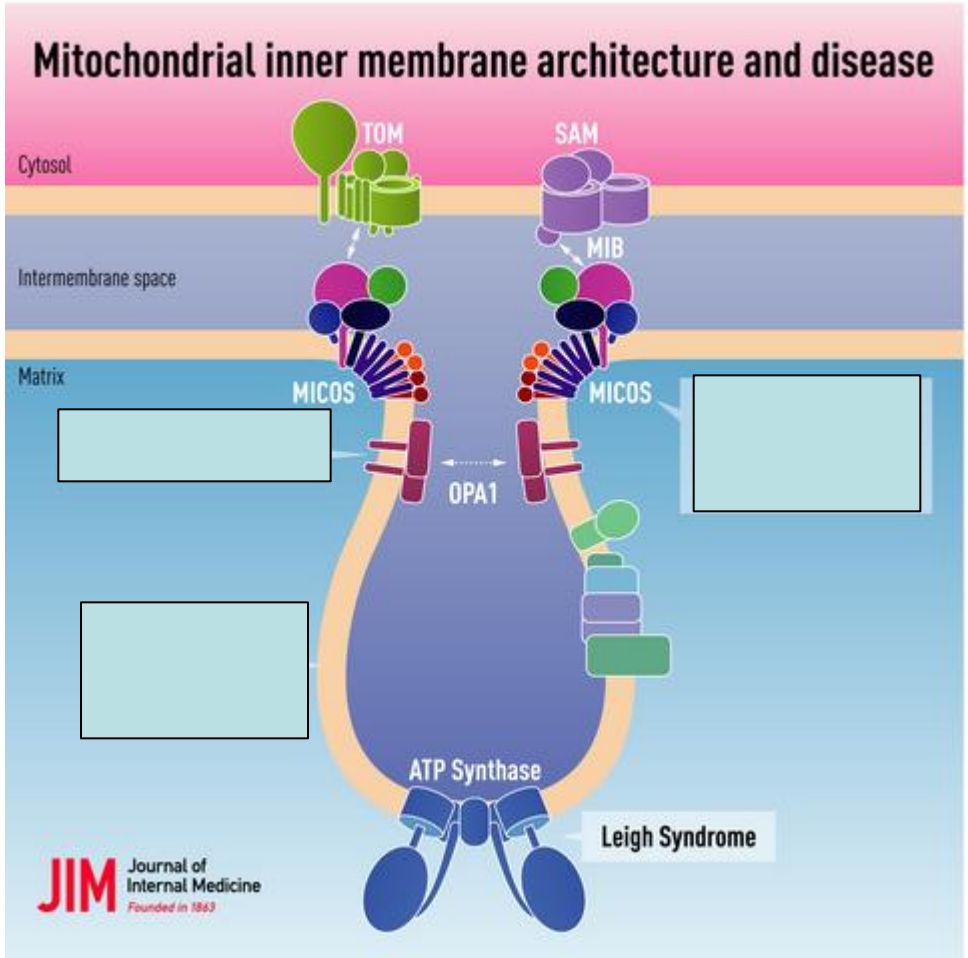


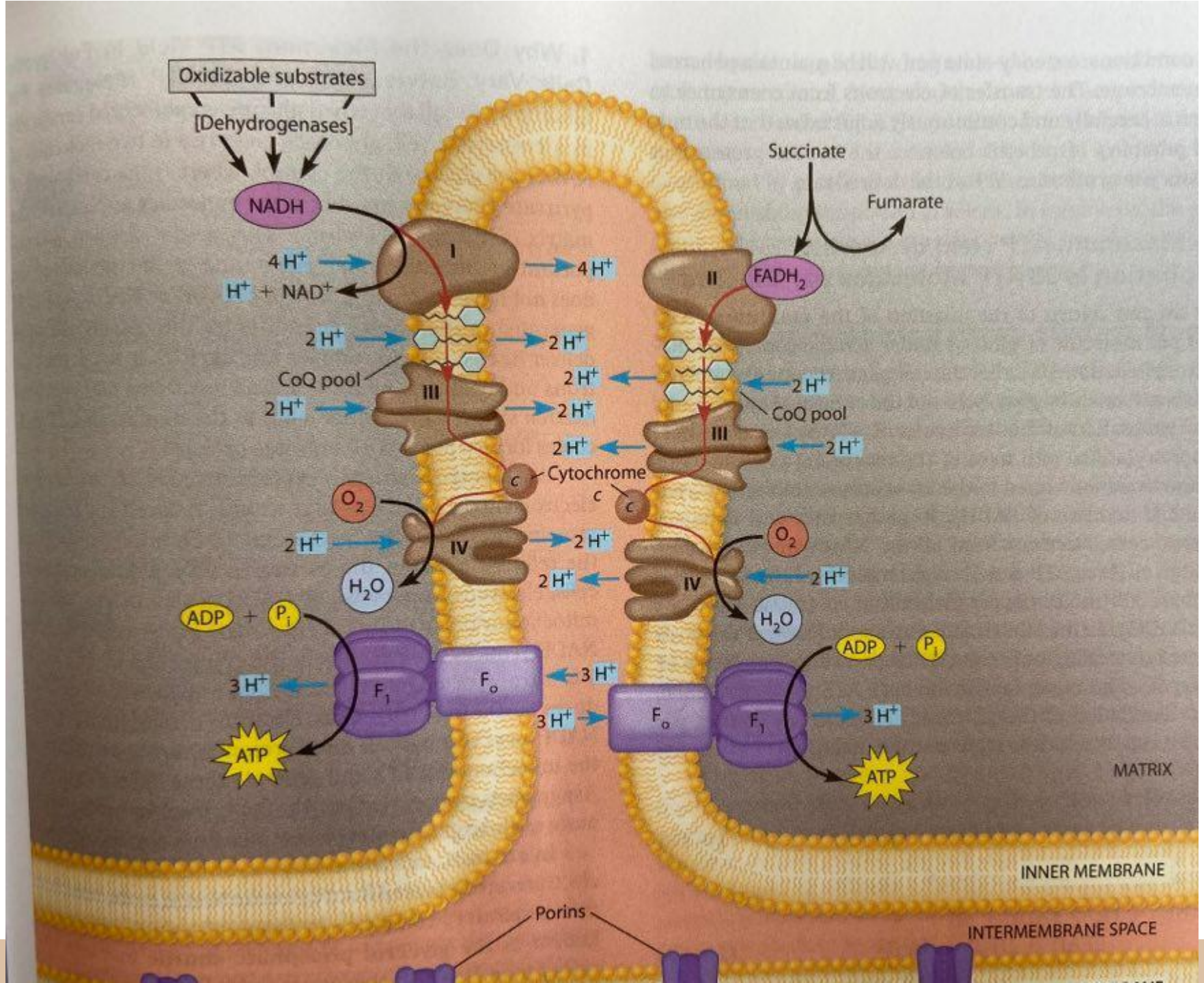
The cristae

- The inner membrane of most mitochondria has many infoldings called **cristae**
- They increase surface area of the inner membrane, and provide more space for electron transport to take place
- They have limited connections to the inner boundary membrane through small openings, *crista junctions*
- Cells with high metabolic activity seem to have more cristae in their mitochondria



Sub-compartmentalization in mitochondria




Oxidizable substrates
[Dehydrogenases]
NADH
4 H+
H+ + NAD+
I
4 H+
2 H+
2 H+
CoQ pool
2 H+
III
2 H+
2 H+
c
Cytochrome
c
O2
2 H+
IV
2 H+
H2O
ADP + Pi
3 H+
F1
Fo
ATP
Succinate
Fumarate
II
FADH2
2 H+
2 H+
CoQ pool
III
2 H+
2 H+
c
O2
IV
2 H+
H2O
ADP + Pi
3 H+
Fo
F1
3 H+
ATP
MATRIX
INNER MEMBRANE
Porins
INTERMEMBRANE SPACE

The mitochondrial matrix

- The interior of the mitochondrion is filled with a semi-fluid **matrix**
- The matrix contains many enzymes involved in mitochondrial function as well as DNA molecules and ribosomes
- Mitochondria contain proteins encoded by their own DNA as well as some that are encoded by nuclear genes

Mitochondrial Functions Occur in or on Specific Membranes and Compartments

- Specific functions and pathways have been localized within mitochondria by fractionation studies
- Most of the enzymes involved in pyruvate oxidation, the TCA cycle, and catabolism of fatty acids and amino acids are found in the matrix
- Most electron transport intermediates are **integral** inner membrane components

Table 10-1 Localization of Metabolic Functions Within the Mitochondrion

Membrane or Compartment	Metabolic Functions
Outer membrane	Phospholipid synthesis Fatty acid desaturation Fatty acid elongation
Inner membrane	Electron transport Oxidative phosphorylation Pyruvate import Fatty acyl CoA import Metabolite transport
Matrix	Pyruvate oxidation TCA cycle β oxidation of fats DNA replication RNA synthesis (transcription) Protein synthesis (translation)

In Bacteria, Respiratory Functions Are Localized to the Plasma Membrane and the Cytoplasm

- Bacteria do not have mitochondria but are capable of aerobic respiration

- Their **plasma membrane** and cytoplasm perform the same functions as the inner membrane and matrix of mitochondria

Phototrophic Energy Metabolism: Photosynthesis

- Most chemotrophs depend on an external source of organic substrates for survival
- Photosynthetic organisms produce the chemical energy and organic carbon required by chemotrophs
- They use solar energy to reduce CO_2 to produce carbohydrates, fats, and proteins

Important terminology

- **Photosynthesis**: the conversion of light energy to chemical energy and its subsequent use in synthesis of organic molecules

- **Phototrophs:** organisms that convert solar energy into chemical energy as ATP and reduced coenzymes

Types of phototrophs

- **Photoheterotrophs**: organisms that acquire energy from sunlight but depend on organic sources of reduced carbon

- **Photoautotrophs**: organisms that use solar energy to synthesize energy-rich organic molecules using starting materials such as CO_2 and H_2O

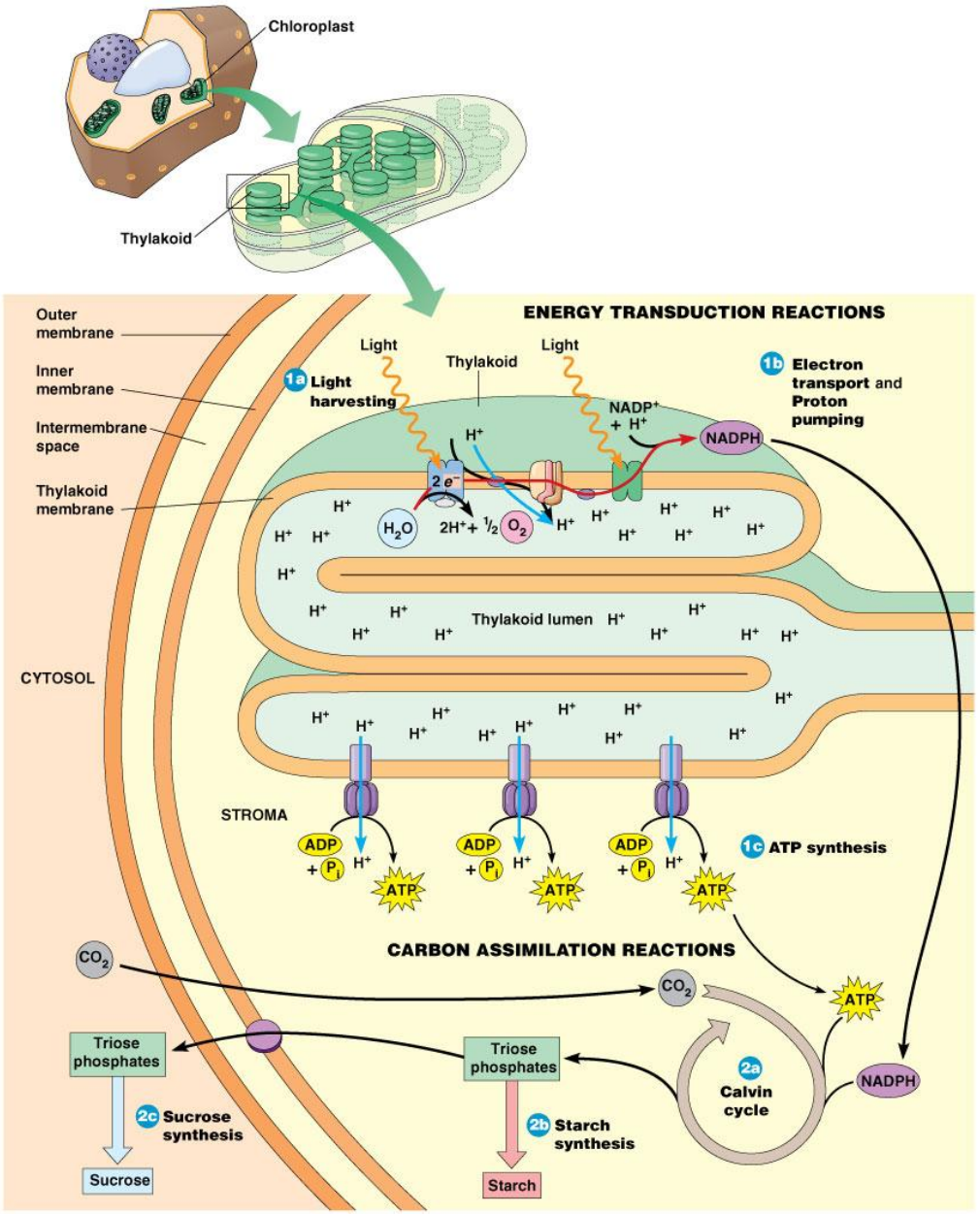
An Overview of Photosynthesis

- Photosynthesis involves two major biochemical processes

  - **Energy transduction reactions**: light energy is captured and converted into chemical energy

  - **Carbon assimilation reactions:** (*carbon fixation reactions*) carbohydrates are formed from CO_2 and H_2O

Figure 11-1



The Energy Transduction Reactions Convert Solar Energy to Chemical Energy

- Light energy is captured by green pigment molecules called chlorophylls, present in the green leaves of plants and the cells of algae and photosynthetic bacteria

- Light absorption by a chlorophyll molecule excites one of its electrons, which is then ejected from the molecule and enters an electron transport system

Unidirectional proton pumping

- The photosynthetic ETC is coupled to unidirectional proton pumping
- The electrochemical gradient produced is used to generate ATP through *photophosphorylation*
- This is similar to *oxidative phosphorylation* in mitochondria

Reduction of carbon

- Photoautotrophs use NADPH to reduce carbon for incorporation into organic molecules
- **Oxygenic phototrophs** (plants, algae, cyanobacteria) use water as the donor of two electrons
- **Anoxygenic phototrophs** (green and purple photosynthetic bacteria) use compounds such as sulfide, thiosulfate, or succinate as donors

The Carbon Assimilation Reactions Fix Carbon by Reducing Carbon Dioxide

- Most of the energy accumulated by the generation of ATP and NADPH is used for carbon dioxide fixation and reduction

$$\text{light} + CO_2 + 2H_2A \rightarrow [CH_2O] + 2A + H_2O$$

- "H_2A" is a suitable *electron donor*, and "A" is the oxidized form of the donor

Oxygenic phototrophs

- For oxygenic phototrophs, in which water is the electron donor, we can summarize the reaction as follows:

$$\text{light} + 6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

- The *intermediate* product of carbon fixation is a triose (3-carbon sugar) rather than the hexose shown in the equation

Production of sugars

- The intermediates of photosynthesis are used for biosynthesis of a variety of products, including glucose, sucrose, and starch

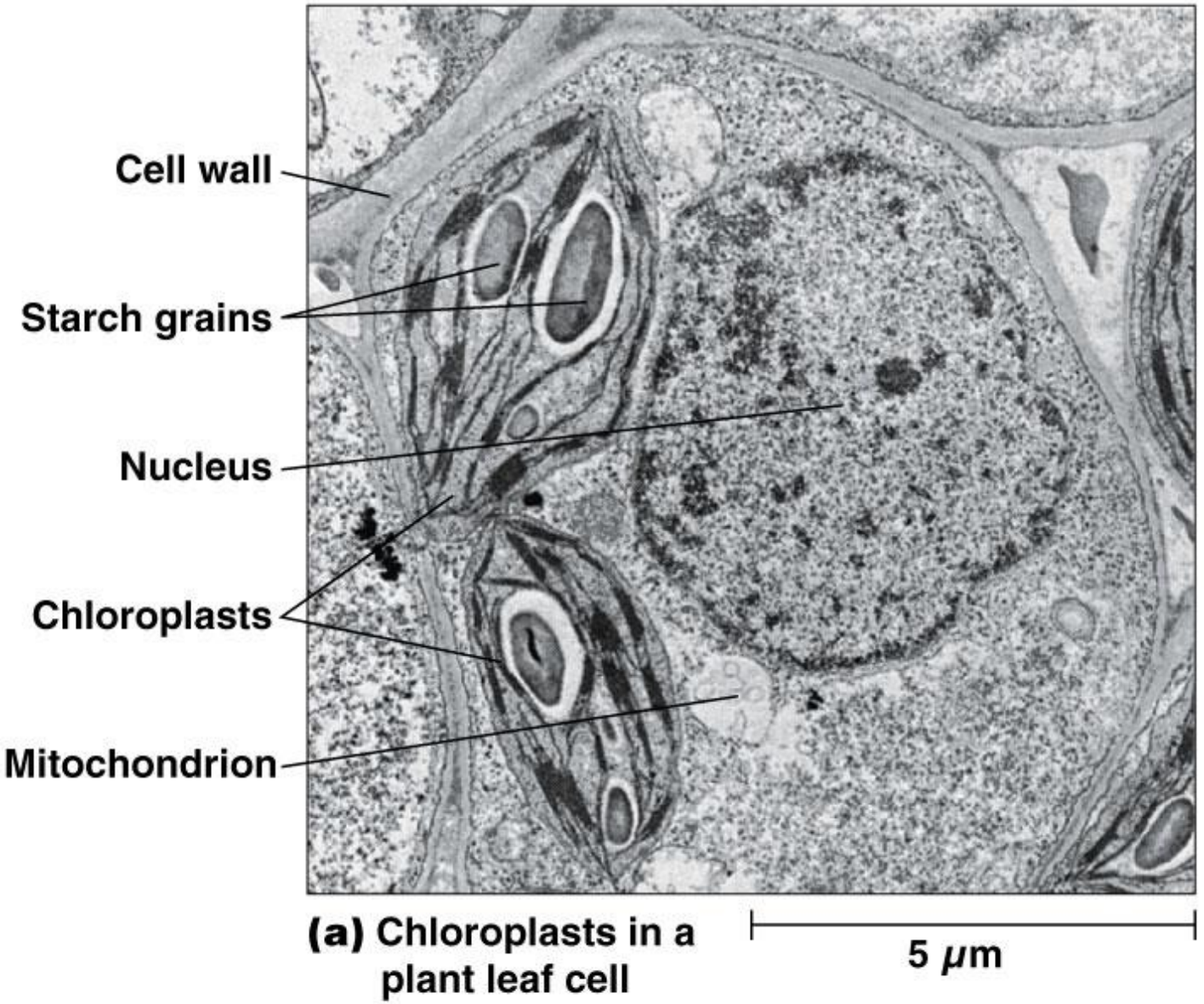
- Sucrose is the major transport carbohydrate in most plants

- Starch is the major storage carbohydrate in most plants

The Chloroplast Is the Photosynthetic Organelle in Eukaryotic Cells

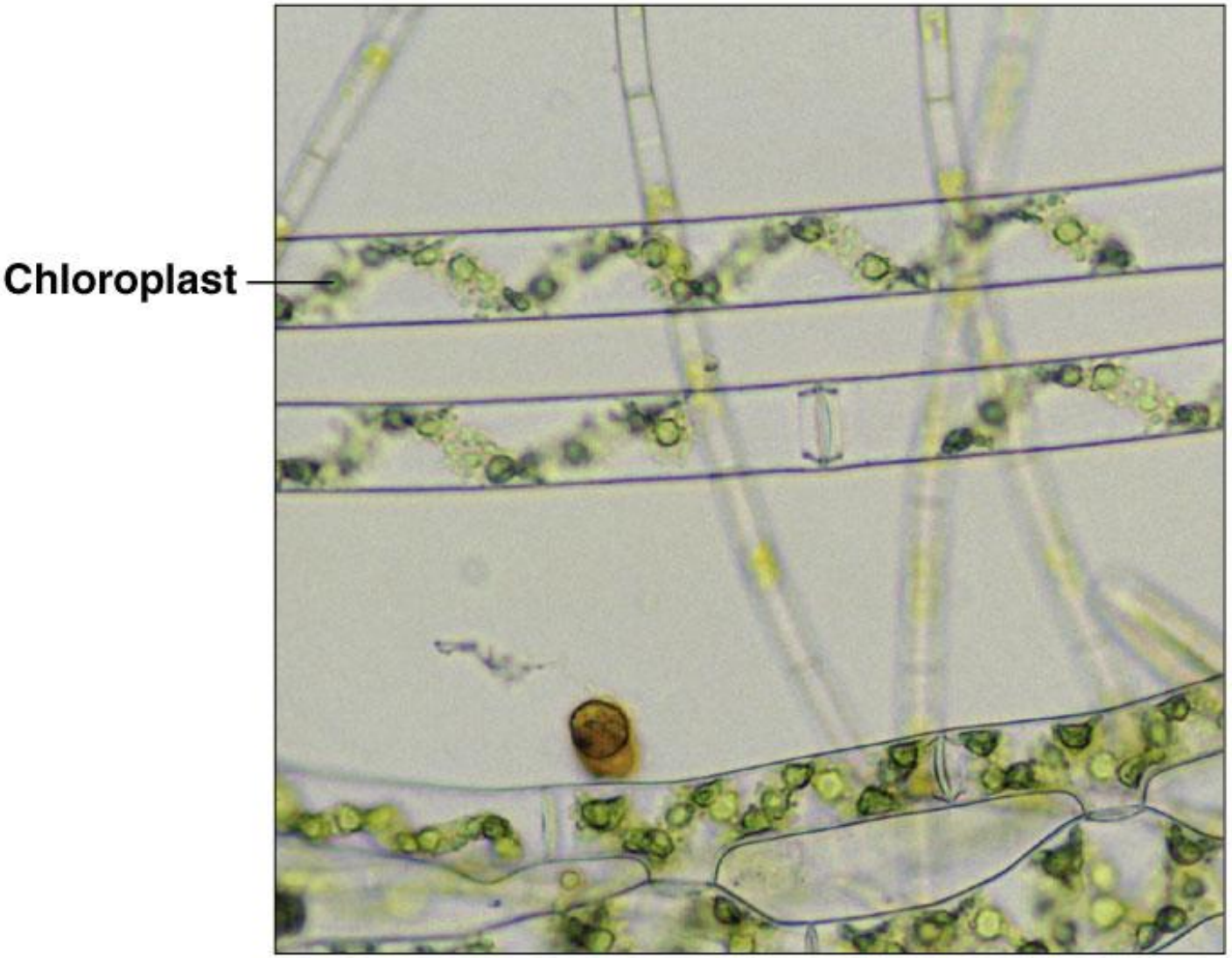
- The most familiar oxygenic phototrophs are the green plants, in which the photosynthetic organelle is the **chloroplast**
- Chloroplasts are large and a mature leaf may contain 20-100
- The shape varies from simple flattened spheres to ribbon-shaped

Figure 11-2A



(a) Chloroplasts in a plant leaf cell

Figure 11-2B



Not all plant cells contain chloroplasts

- Newly differentiated plant cells have smaller organelles called **proplastids**, which may develop into any of several types of **plastids** depending on the function of the cell

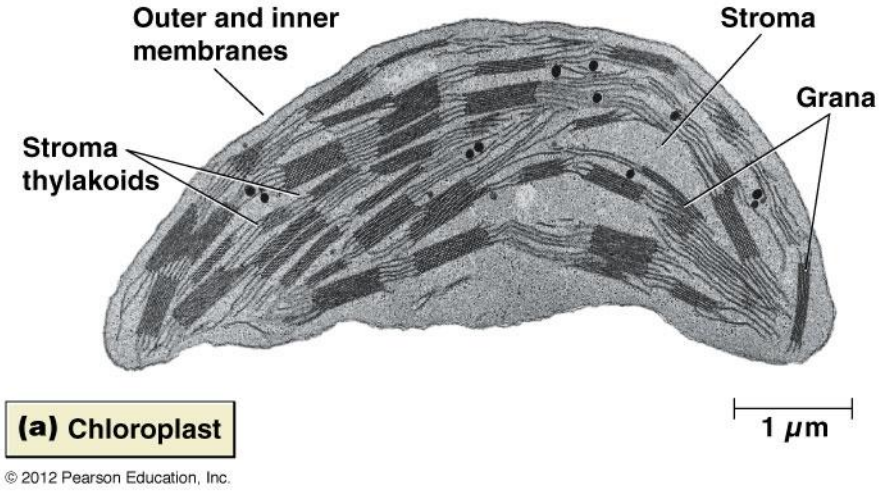
- *Amyloplasts* are specialized for storing starch

- *Chromoplasts* give flowers and fruits their distinctive colors

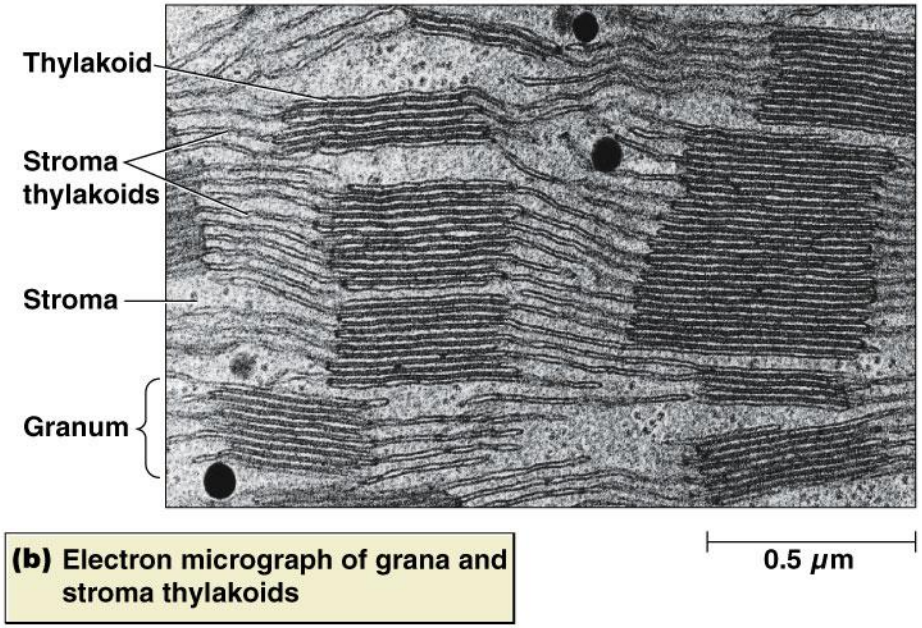
Chloroplasts Are Composed of Three Membrane Systems

- A chloroplast has both an **outer membrane** and an **inner membrane**
- These are usually separated by an **intermembrane space**
- The inner membrane encloses the **stroma**, a gel-like matrix full of enzymes

Figure 11-3A,B

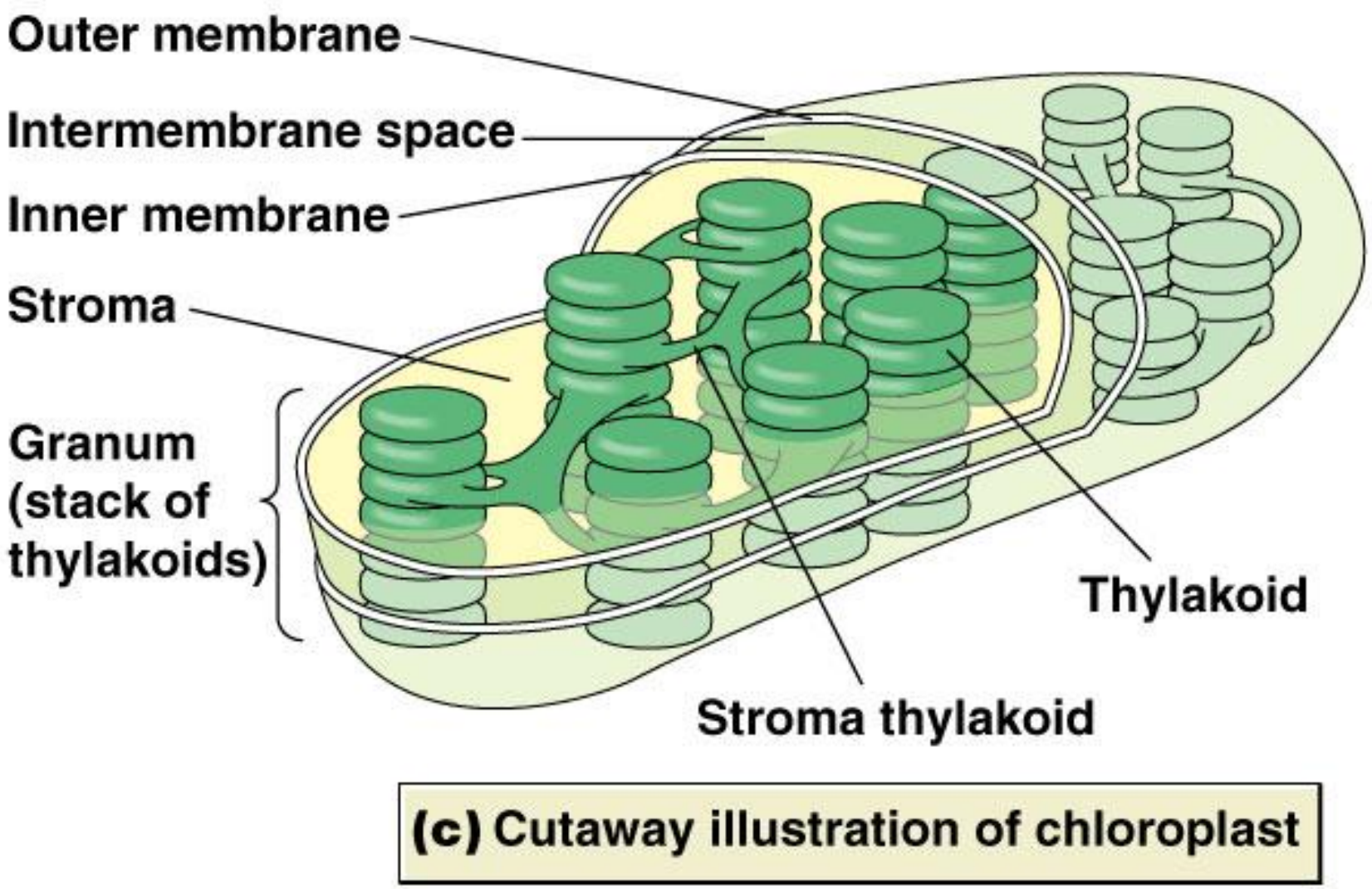


(a) Chloroplast



(b) Electron micrograph of grana and stroma thylakoids

Figure 11-3C



(c) Cutaway illustration of chloroplast

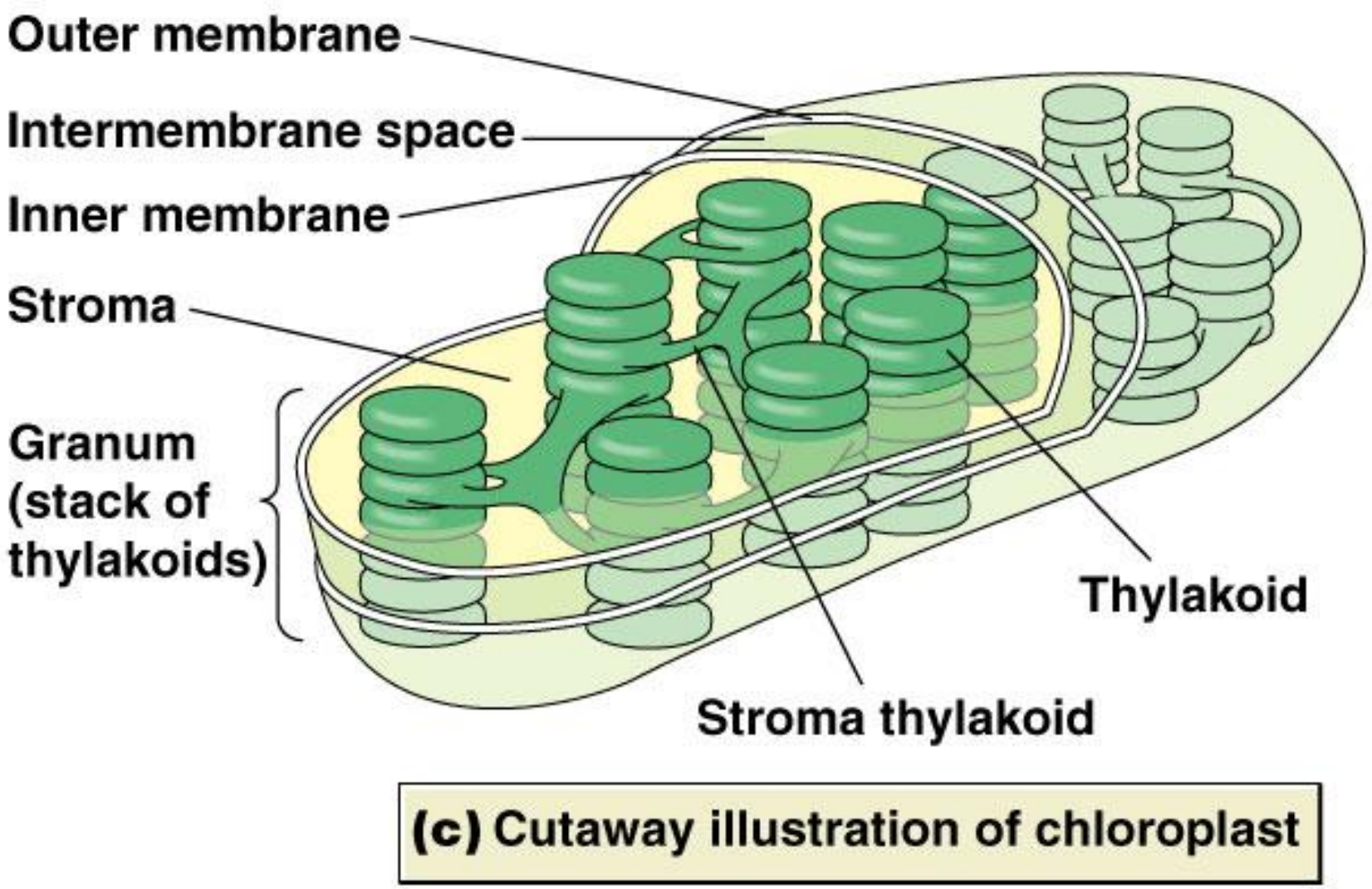
The outer membrane is freely permeable

- The outer membrane contains **porins** similar to those in the mitochondrial outer membrane
- These allow passage of solutes with molecular weights up to 5000 Da
- In the inner membrane transport proteins control the flow of metabolites between the stroma and intermembrane space

Thylakoids

- Chloroplasts have a third membrane system, called **thylakoids**
- These are flat, saclike structures in the stroma, arranged in stacks called **grana**
- Grana are interconnected by **stroma thylakoids**

Figure 11-3C



(c) Cutaway illustration of chloroplast

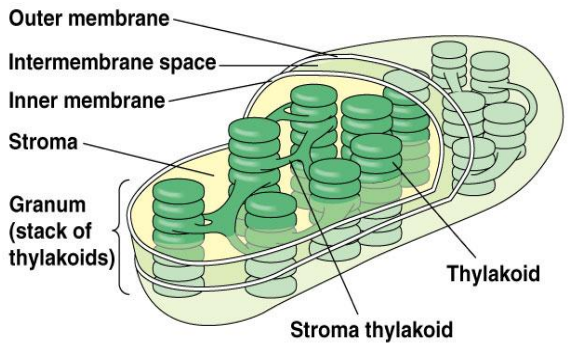
Thylakoids lipid composition

“Thylakoid lipid composition is unusual in that instead of the commonly encountered phosphoglycerides the major components are glycosylglycerides. One uses the word 'unusual' advisedly since, because of the huge amounts of photosynthetic membranes in algae and plants, the glycosylglycerides are, in fact, the most common membrane lipids in the world.”

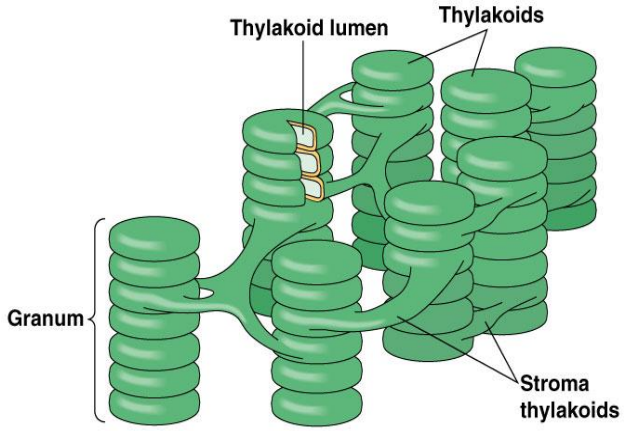
Gounaris, K., Barber, J., & Harwood, J. L. (1986). The thylakoid membranes of higher plant chloroplasts. *The Biochemical journal*, *237*(2), 313-26.

Thylakoid lumen

- Grana and stroma thylakoids enclose a single continuous compartment called the **thylakoid lumen**
- The semipermeable barrier of the thylakoid membrane allows for creation of an electrochemical proton gradient between the lumen and stroma



(c) Cutaway illustration of chloroplast



(d) Illustration of grana and stroma thylakoids

Organisms without chloroplasts

- Photosynthetic bacteria have no chloroplasts
- In some of them, such as the cyanobacteria, the plasma membrane folds inward to form photosynthetic membranes
- To some extent, cyanobacteria appear to be free-living chloroplasts, a resemblance that has contributed to the *endosymbiont theory*

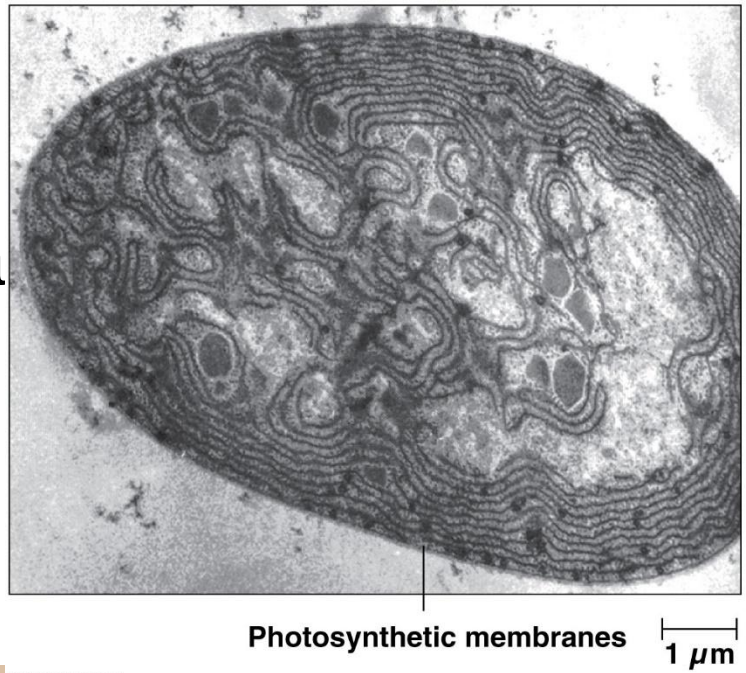
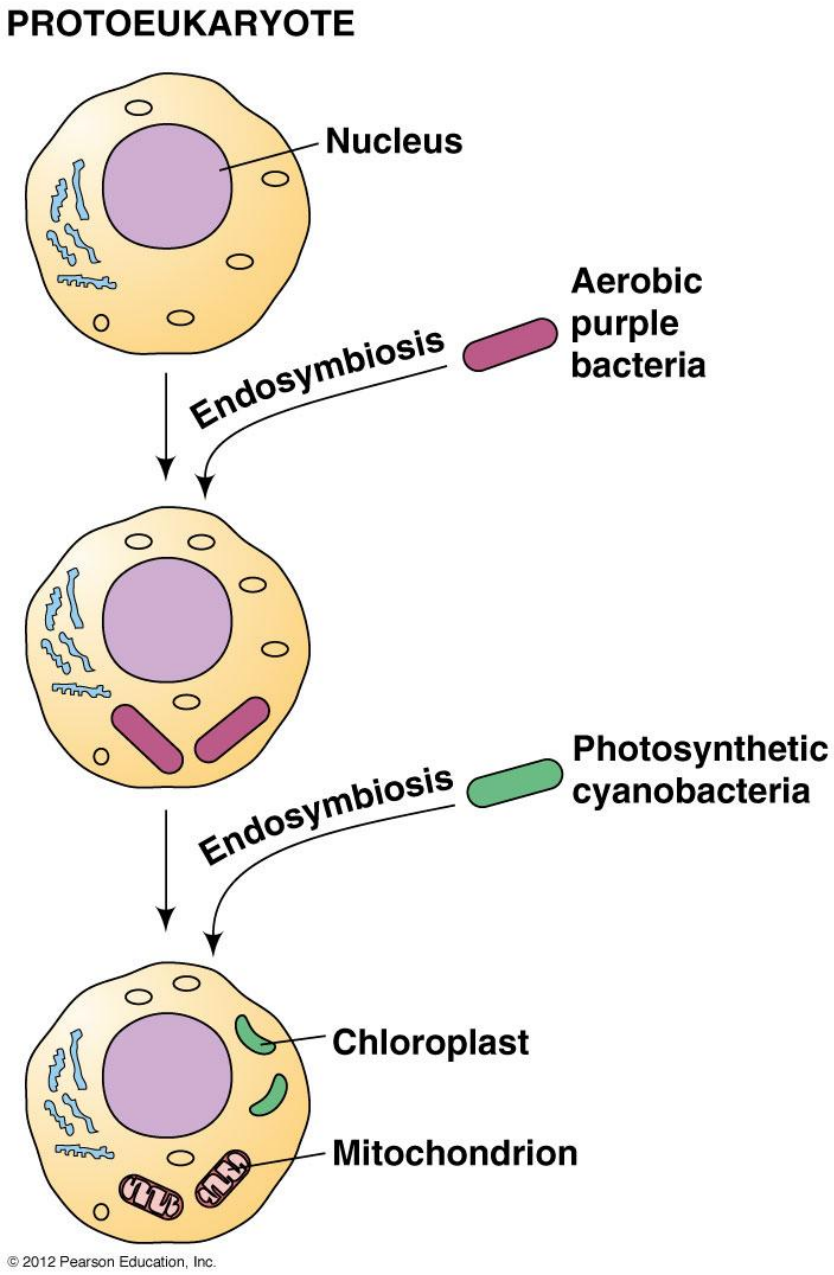
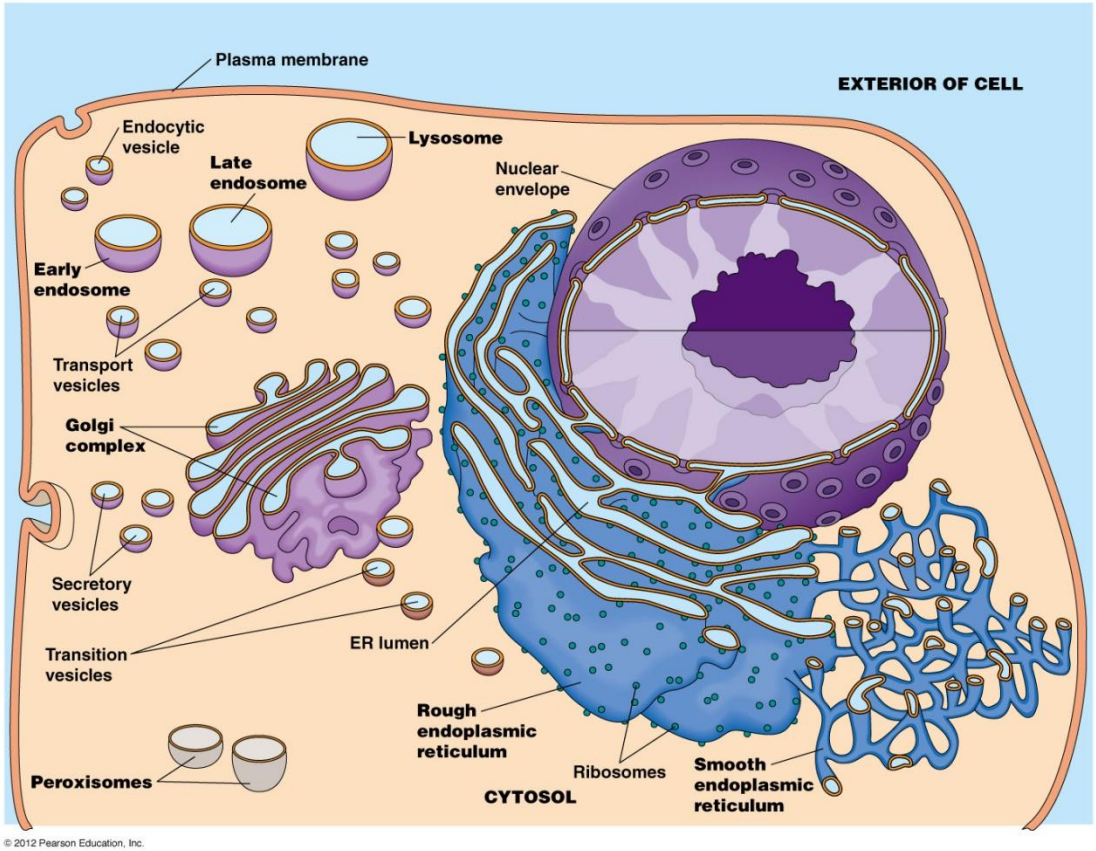


Figure 11A-1



The **endomembrane system** & **peroxisomes**

- *Endosomes* carry and sort material brought into the cell
- *Lysosomes* digest ingested material and unneeded cellular components

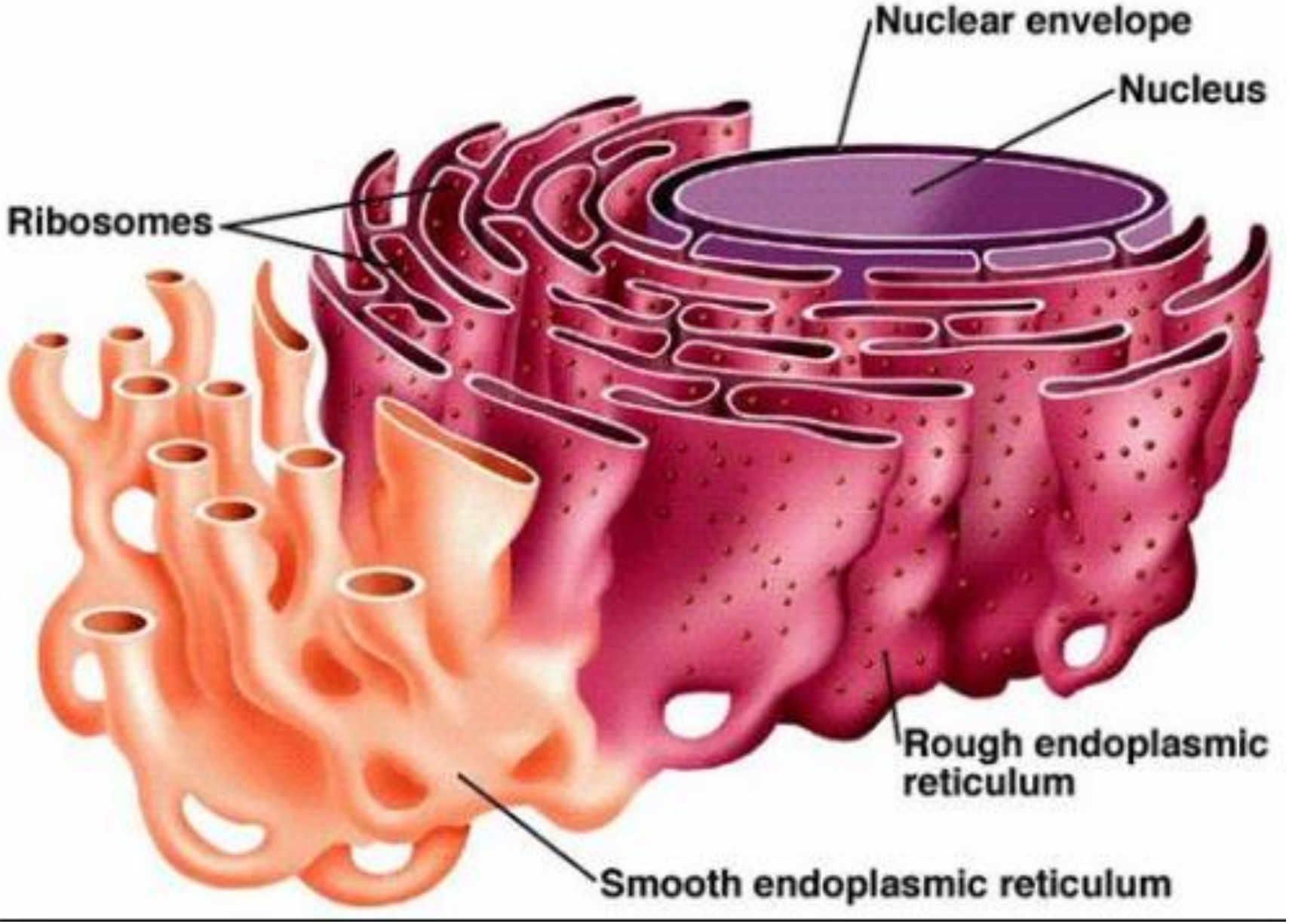


- *Peroxisomes* house hydrogen peroxide generating reactions
- They also perform diverse metabolic functions
- *Rough* and *smooth endoplasmic reticulum* and the *Golgi complex* are sites for protein synthesis, processing, and sorting

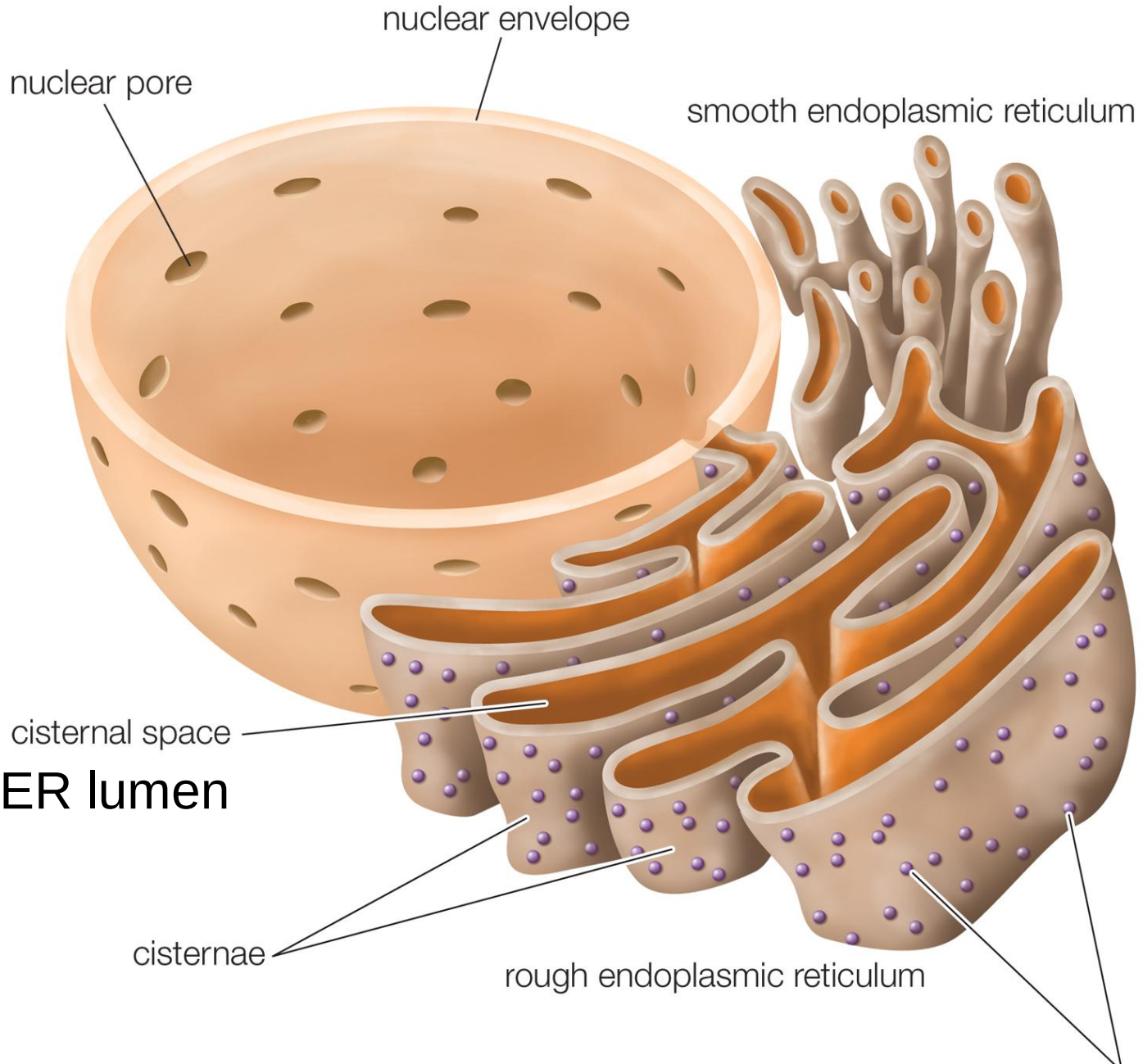
The Endoplasmic Reticulum

- The **endoplasmic reticulum** (**ER**) is a continuous network of flattened sacs, tubules, and vesicles through the cytoplasm of a eukaryotic cell

- The membrane-bound sacs are called **ER cisternae** and the space inside them is the **ER lumen**



Endoplasmic reticulum



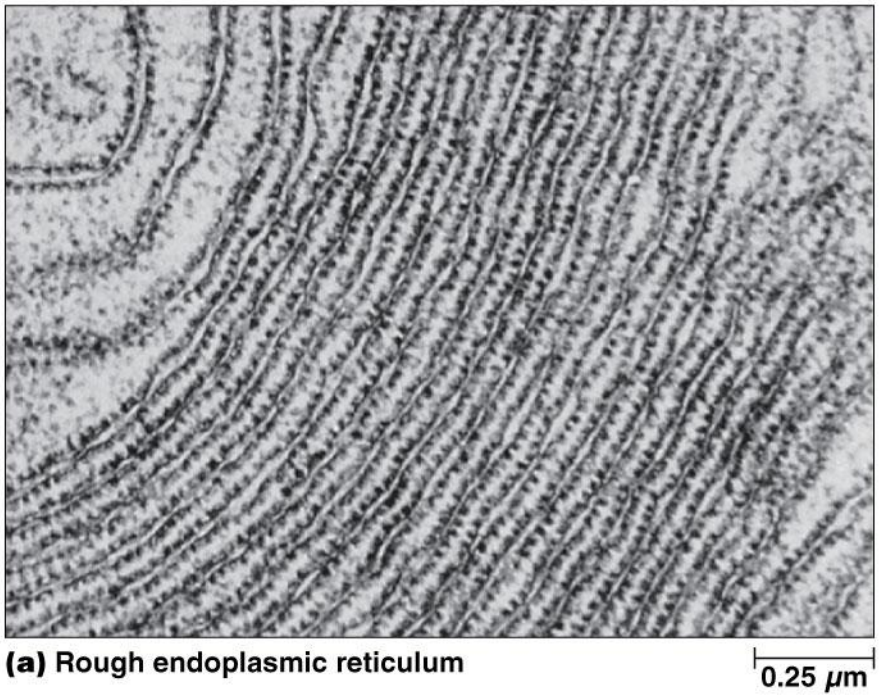
Functions of the ER

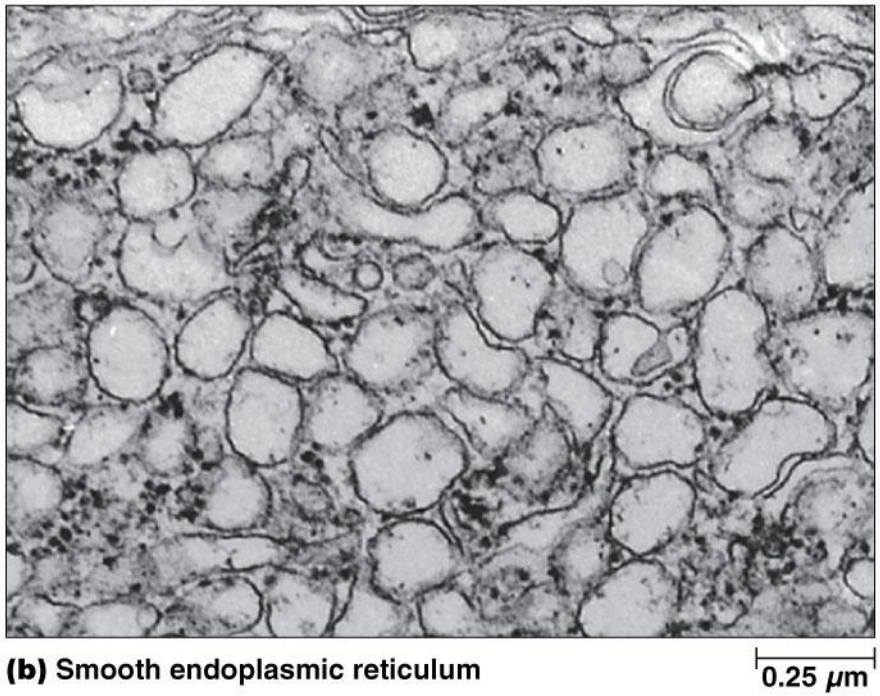
- The enzymes associated with the ER are involved in synthesis of proteins for
  - Incorporation into the plasma membrane
  - Organelles of the endomembrane system
  - Export from the cell
- The ER is also involved in lipid synthesis

The Two Basic Kinds of Endoplasmic Reticulum Differ in Structure and Function

- **Rough endoplasmic reticulum** (**RER**) is characterized by ribosomes on the cytosolic side of the membrane
- A subdomain of rough ER, the **transitional elements** (**TEs**) plays a role in the formation of transition vesicles that shuttle lipids and proteins from the ER to the Golgi complex
- **Smooth ER (SER)** lacks ribosomes and has other roles in the cell

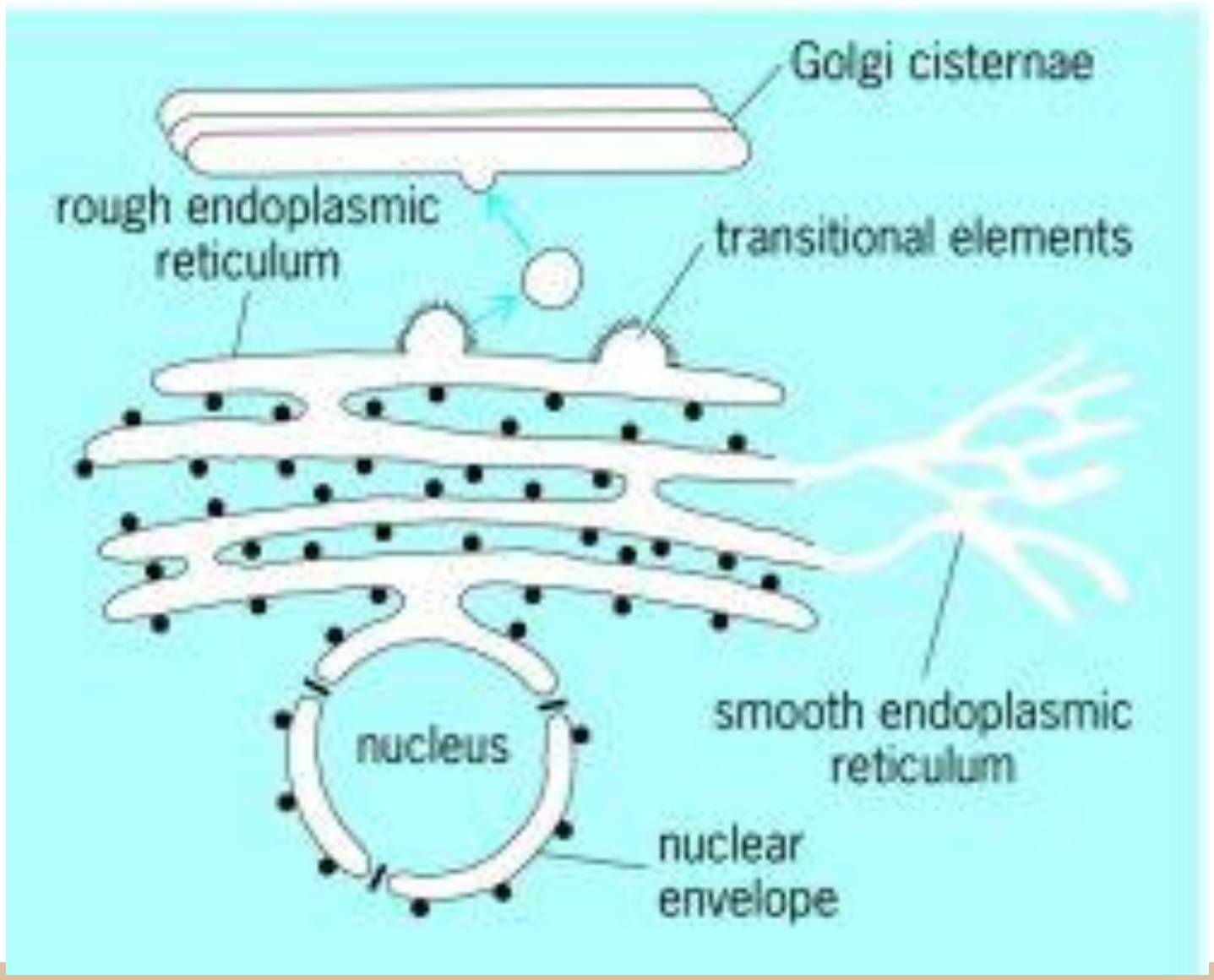
Distinguishing Rough and Smooth ER





- Electron micrograph images of
- RER membranes form large flattened sheets
- SER membranes form tubular structures

Transitional elements of the rough ER are an exception; *they resemble the smooth ER but with ribosomes*



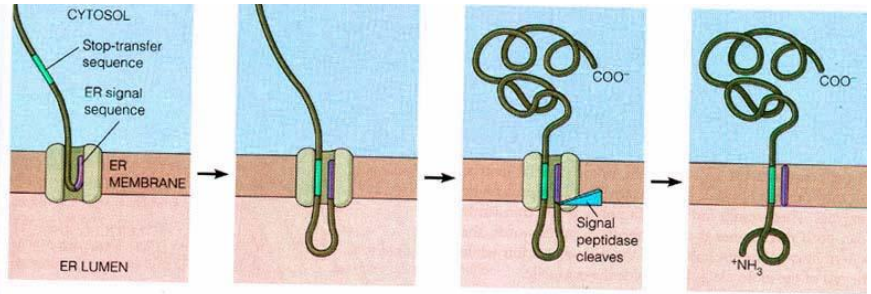
Variation in amounts of rough and smooth ER

- Both types of ER are present in most cells but there is variation in the relative amounts
- Cells involved in synthesis of secretory proteins have **prominent** rough ER networks
- Cells producing steroid hormones tend to have extensive networks of smooth ER

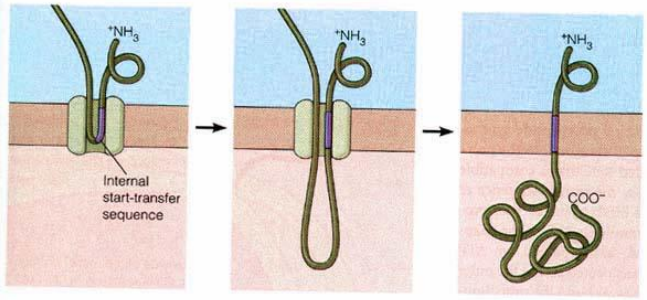
Rough ER Is Involved in the Biosynthesis and Processing of Proteins

- Ribosomes on the cytosolic side of the RER membrane synthesize both **membrane-bound** and soluble proteins for the endomembrane system

- Newly synthesized proteins are inserted into the endomembrane system through a pore complex as they are synthesized (***cotranslationally***)



(a) Polypeptide with an internal stop-transfer sequence and a terminal ER signal sequence



(b) Polypeptide with an internal start-transfer sequence

Rough ER—other functions

- The initial steps of addition of carbohydrates to glycoproteins
- The folding of polypeptides
- Assembly of multimeric protein complexes
- Recognition and removal of misfolded proteins (Quality control)

Smooth ER Is Involved in Drug Detoxification, Carbohydrate Metabolism, Calcium Storage, and Steroid Biosynthesis

- The functions of smooth ER differ from that of rough ER

Drug Detoxification

- Drug detoxification often involves **hydroxylation**
- Adding hydroxyl groups to hydrophobic drugs **increases their solubility**, making them easier to excrete from the body
- Hydroxylation is catalyzed by a member of the **cytochrome P-450** family of proteins, also called *monooxygenases*

Carbohydrate Metabolism

- Smooth ER is involved in breakdown of stored glycogen; it contains ***glucose-6-phosphatase***, an enzyme unique to smooth ER in liver cells

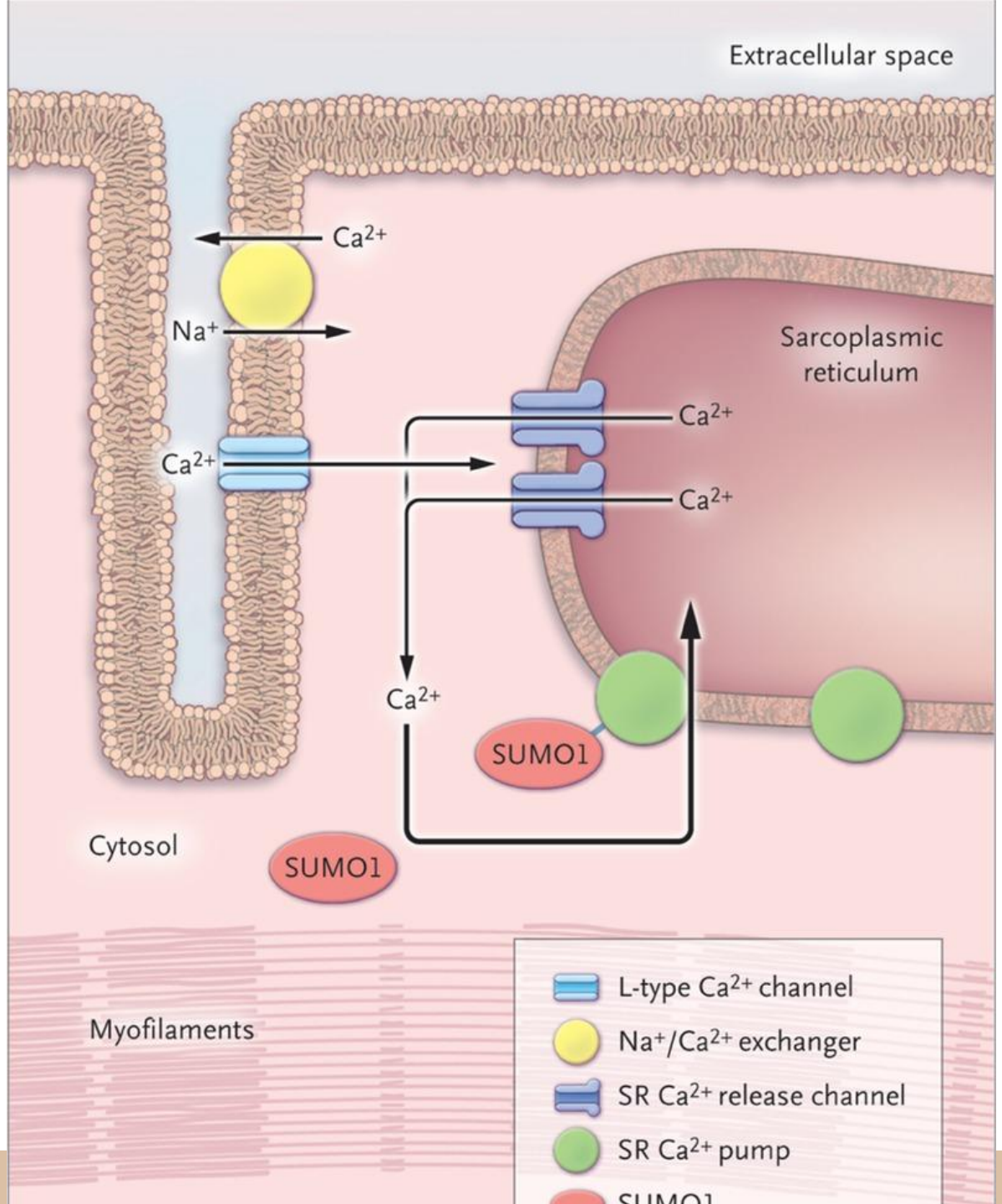
- Glucose-6-phosphatase hydrolyzes the phosphate from glucose-6-phosphate to form free glucose

- 

Calcium Storage

- The *sarcoplasmic reticulum* of muscle cells is an example of smooth ER that specializes in calcium storage

- The ER lumen contains high concentrations of calcium-binding proteins


Extracellular space
Ca^{2+}
Na^{+}
Sarcoplasmic reticulum
Ca^{2+}
Ca^{2+}
Ca^{2+}
Ca^{2+}
SUMO1
Cytosol
SUMO1
Myofilaments
L-type Ca^{2+} channel
Na^{+}/Ca^{2+} exchanger
SR Ca^{2+} release channel
SR Ca^{2+} pump

Steroid Biosynthesis

- Smooth ER in some cells is the site of cholesterol and steroid hormone synthesis
- Large amounts of smooth ER are found in cells that synthesize these

The ER Plays a Central Role in the Biosynthesis of Membranes

- In eukaryotic cells the ER is the primary source of membrane lipids

- Other sources:

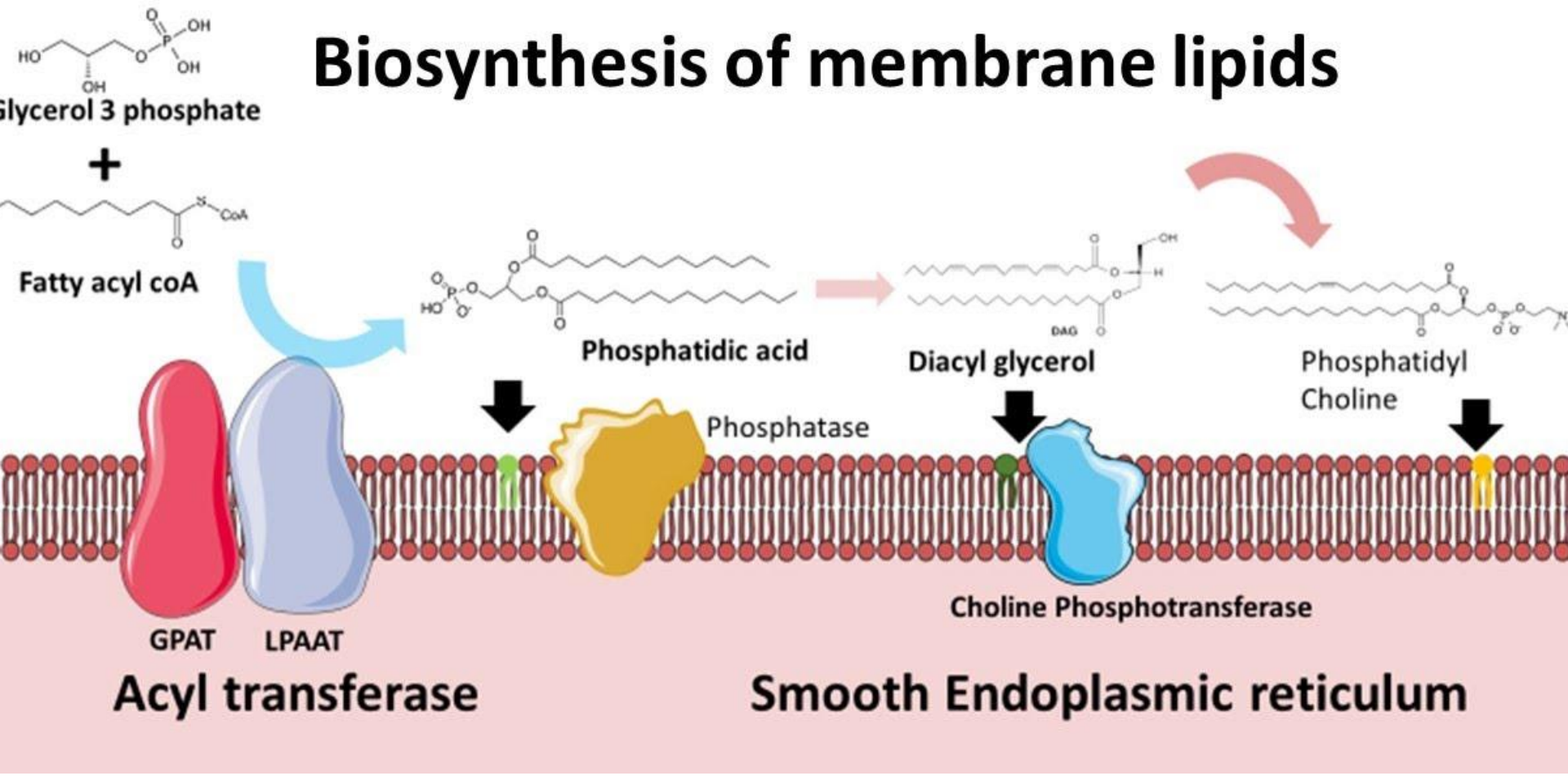
-Mitochondria synthesize phosphatidylethanolamine

-Chloroplasts contain enzymes for chloroplast-specific lipids

Membrane biosynthesis

- Fatty acids for membrane phospholipids are synthesized in the cytoplasm and **incorporated** into the ER membrane on the cytosolic side

Biosynthesis of membrane lipids



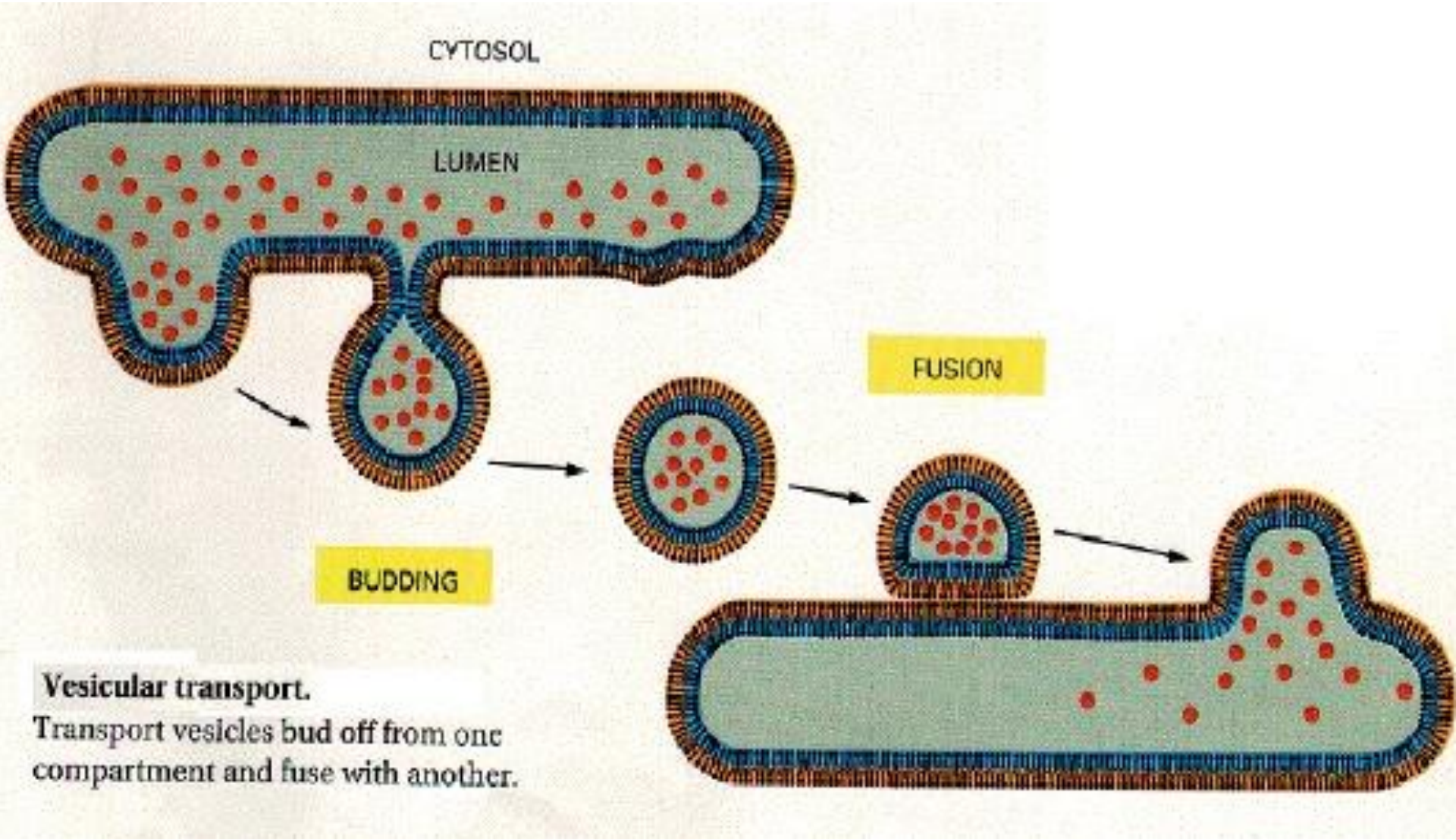
Membrane biosynthesis

- They are transferred to the lumenal side of the *bilayer* by enzymes called **phospholipid translocators** (**flippases**)

- The type of phospholipid molecules transferred across the membrane depends on the particular translocator present, leading to *membrane asymmetry*

Membrane biosynthesis (continued)

- The distinct composition of cytosolic and lumenal monolayers established in the ER is transferred to other cellular membranes
- Movement of phospholipids from ER to mitochondria, chloroplasts, or peroxisomes is problematic
- **Phospholipid exchange proteins** (*phospholipid transfer proteins*) convey specific phospholipids to these organelles



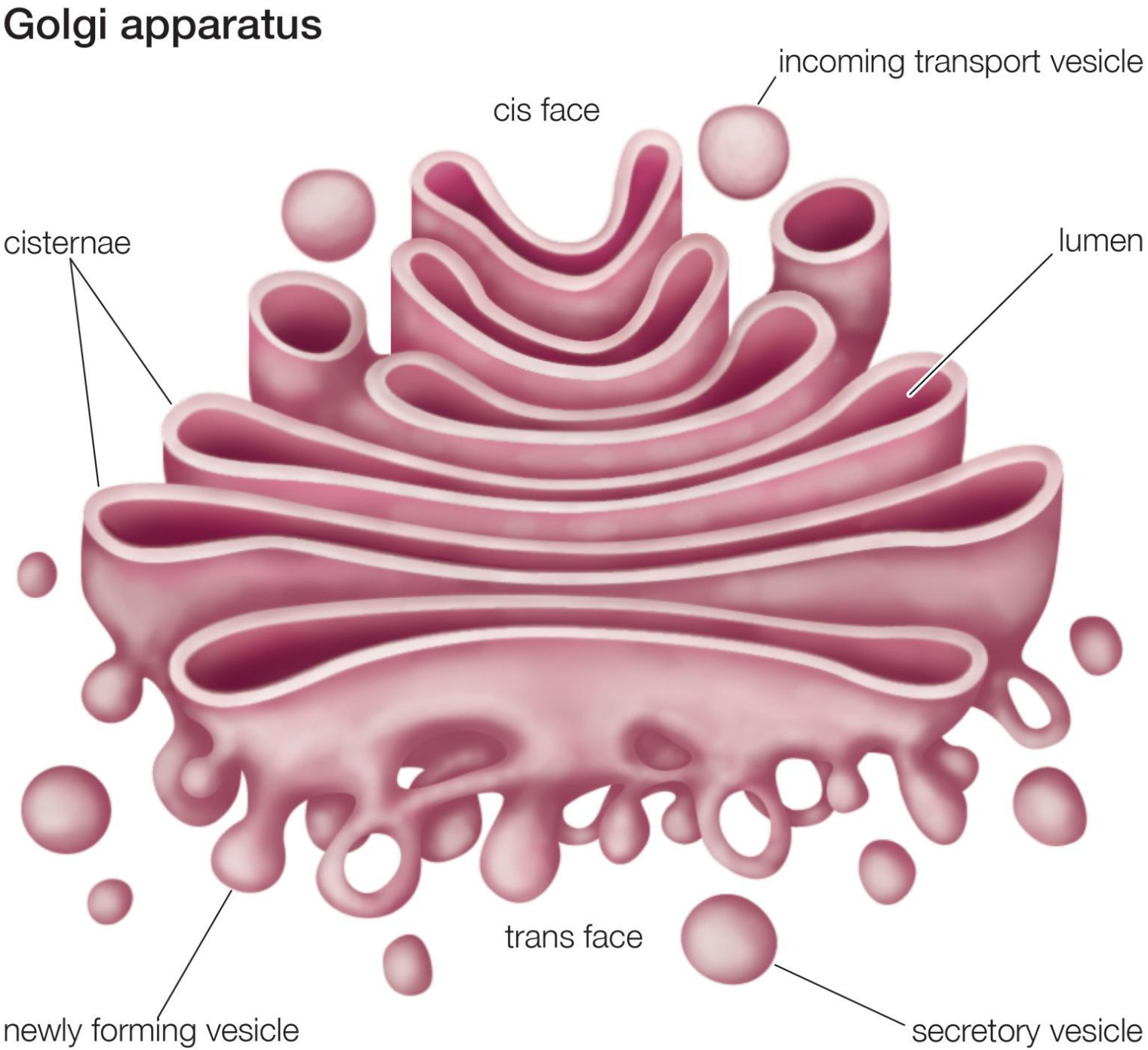
Vesicular transport.
Transport vesicles bud off from one compartment and fuse with another.

The Golgi Complex

- The **Golgi complex** is functionally and physically linked to the ER
- Here, glycoproteins and membrane lipids from the ER undergo further processing and are sorted and packaged for transport
- Thus the Golgi complex plays a central role in *membrane* and *protein trafficking* in eukaryotic cells

The Golgi Complex Consists of a Series of Membrane-Bound Cisternae

- The Golgi complex is a series of flattened membrane-bounded *cisternae*
- A series of cisternae, usually 3-8, is called a *Golgi stack*
- Some cells have one large stack, and others, especially secretory cells, have hundreds or thousands of stacks

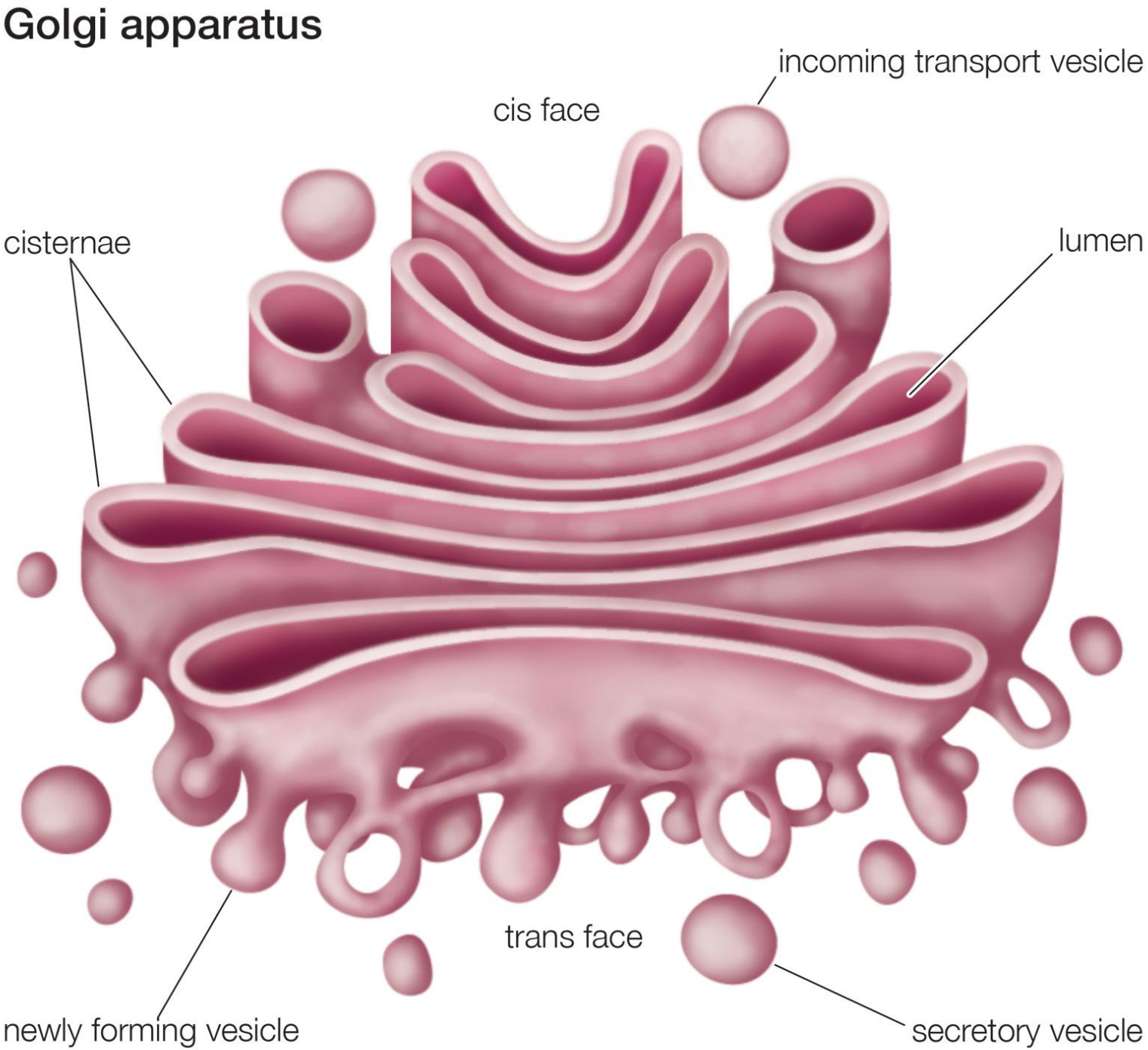


Transport vesicles

- Both ER and the Golgi complex are surrounded by numerous *transport vesicles* that carry lipids and proteins from the ER to the Golgi complex and then to various destinations in the cell

The Two Faces of the Golgi Stack

- Each Golgi stack has two distinct sides, or *faces*
- The *cis* face is oriented toward the ER, the Golgi compartment on this side is called the ***cis*-Golgi network** (**CGN**)
- The opposite side is called the *trans face* and the compartment on this side is called the ***trans*-Golgi network** (**TGN**)



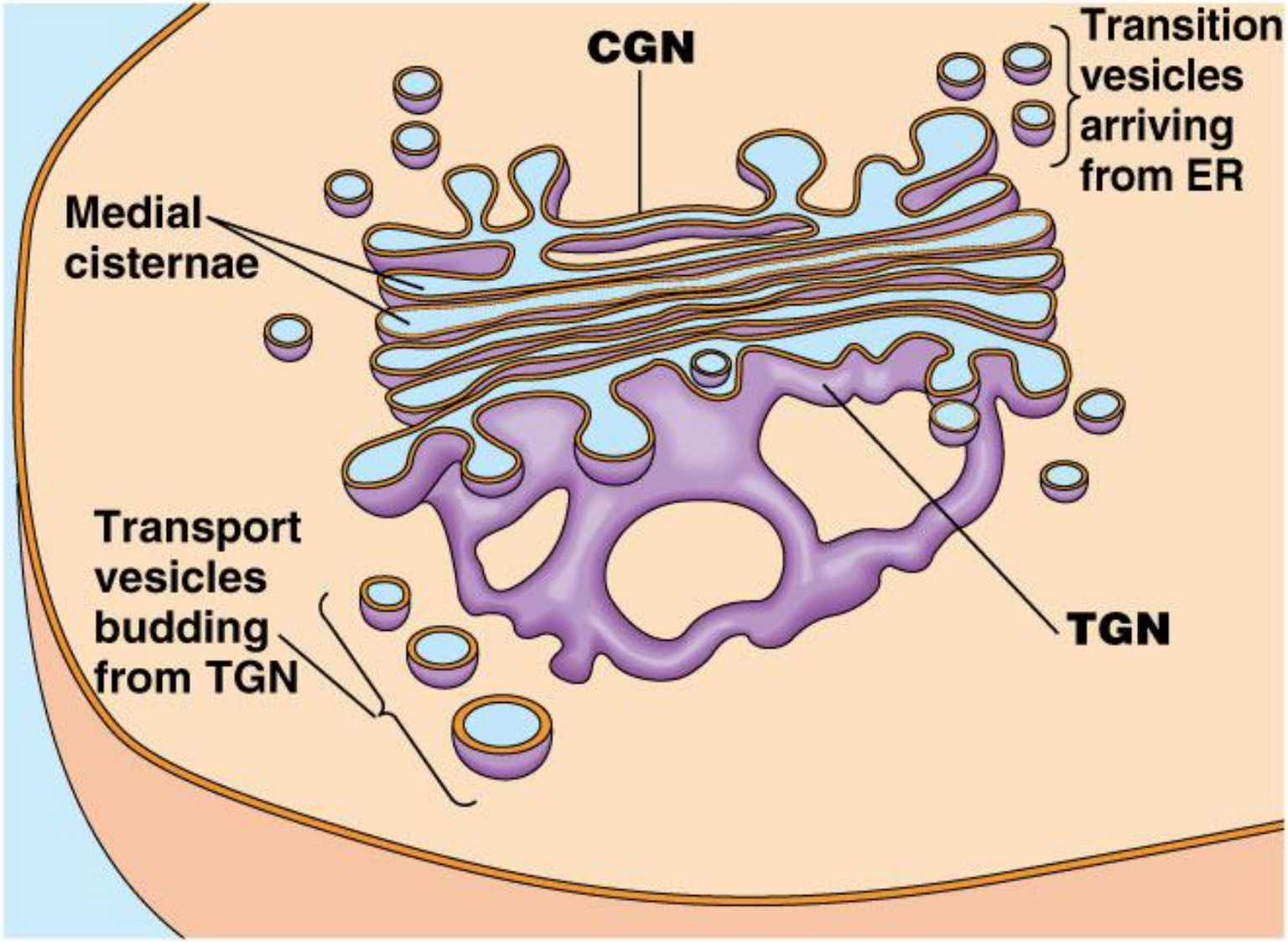
The Two Faces of the Golgi Stack

Proteins and lipids leave the Golgi in **transport vesicles** that continuously bud from the tips of the TGN

Between the TGN and CGN are **medial cisternae**, where much of the processing of proteins occurs

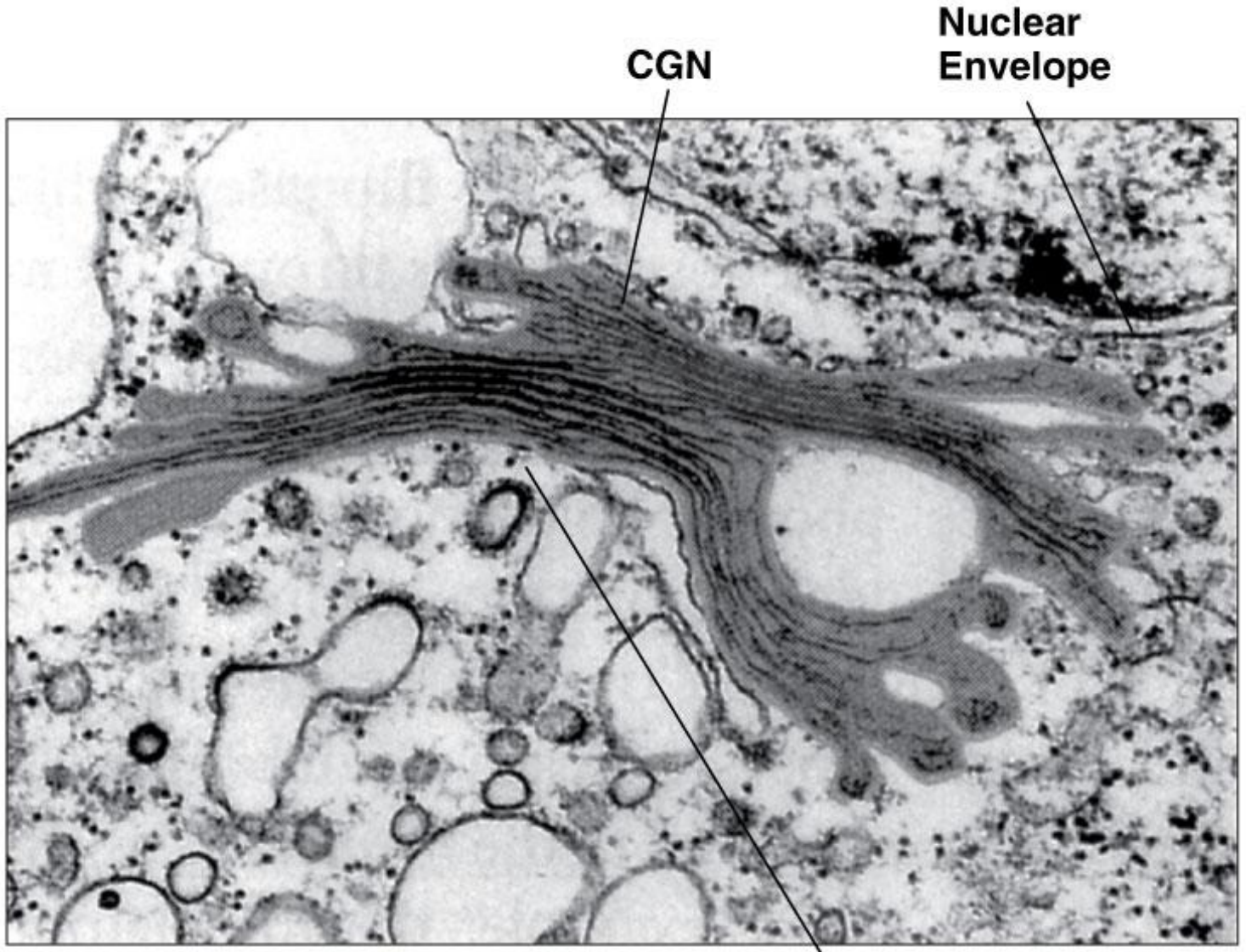
Each compartment shows biochemical *polarity*, containing specific proteins unique to each portion of the network

Figure 12-4A



(a) A Golgi stack in an animal cell

Figure 12-4B



(b) A Golgi stack in an algal cell

Anterograde and Retrograde Transport

- Movement of material toward the plasma membrane is called **anterograde transport**
- As a secretory granule fuses with the plasma membrane and discharges its contents (exocytosis), a bit of membrane from the ER becomes part of the plasma membrane
- This flow of lipids toward the plasma membrane must be balanced

Retrograde Transport

- **Retrograde transport** is the flow of vesicles from Golgi cisternae back to the ER
- This allows the cell to balance the flow of lipids toward the plasma membrane
- It also ensures a supply of materials for forming new vesicles

Roles of the ER and Golgi Complex in Protein Trafficking

- Proteins that are synthesized in the rough ER must have a SIGNAL sequence and then have to be directed to a variety of locations

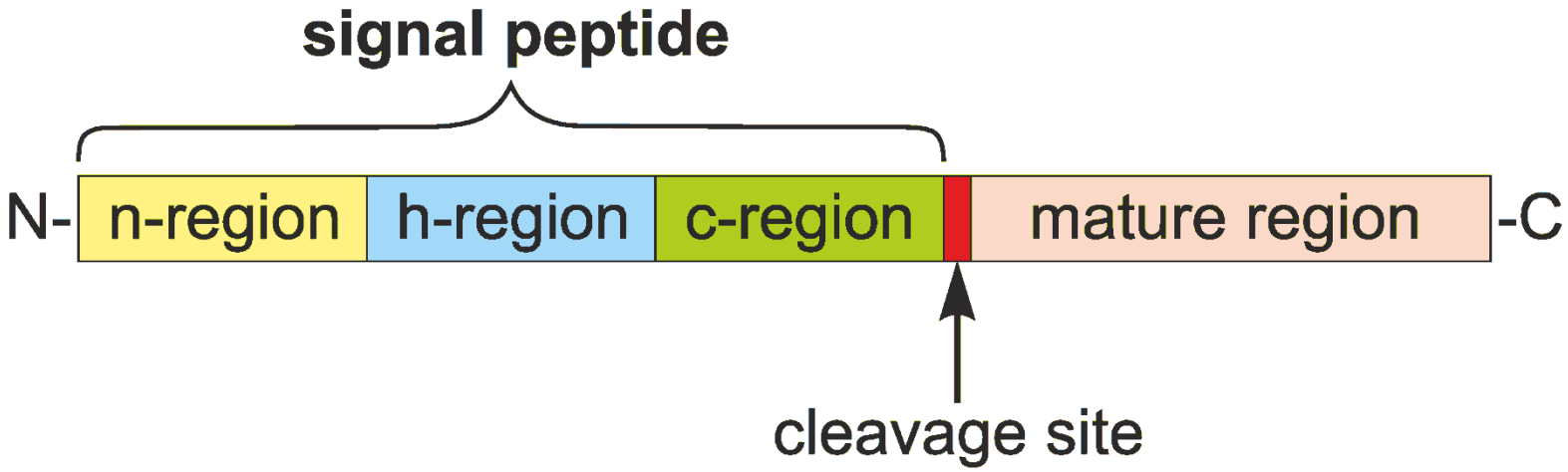
- Once a protein reaches its destination, it must be prevented from leaving

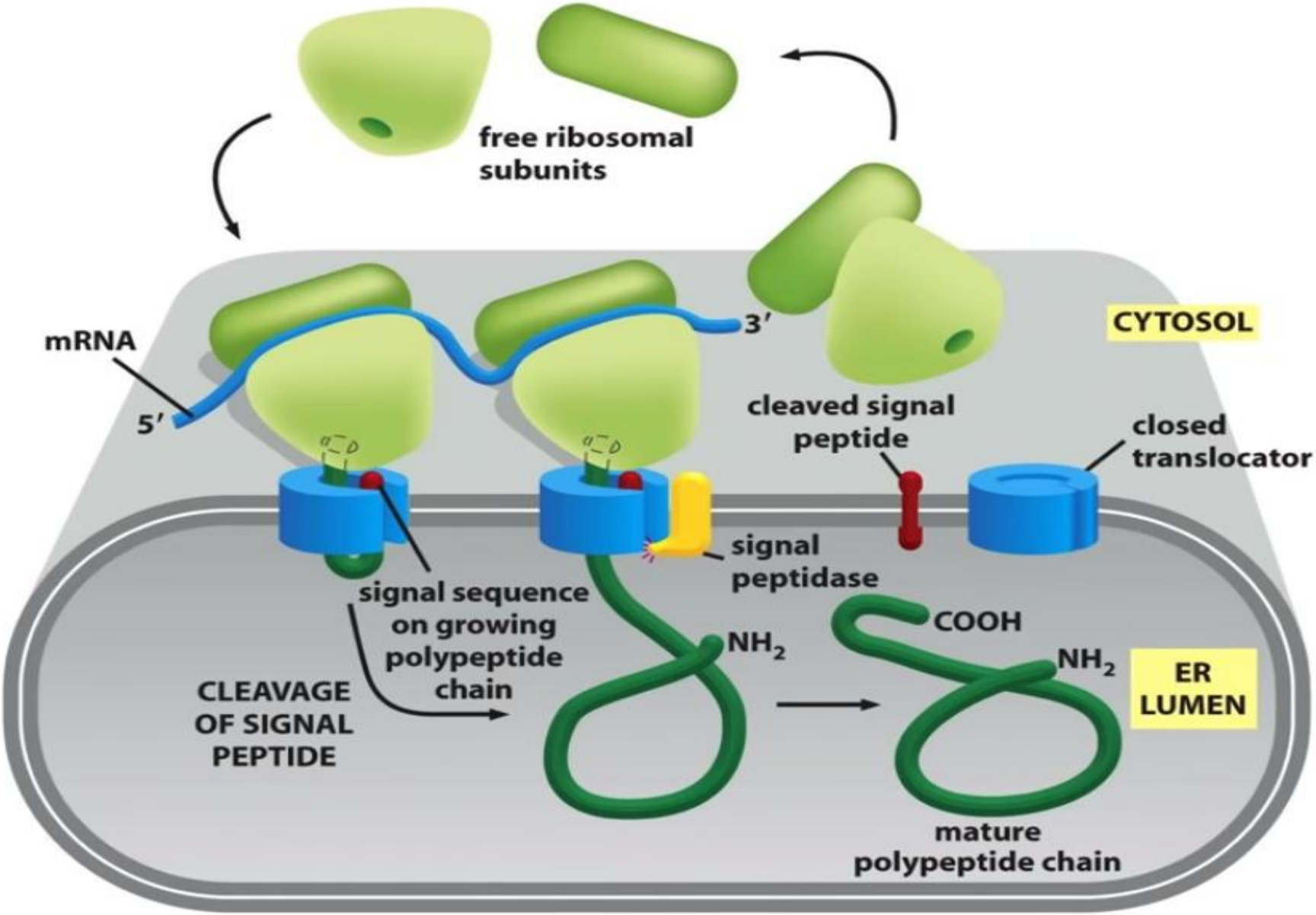
- Each protein contains a specific "tag," targeting it to a transport vesicle that will take it to the correct location

The signal sequence

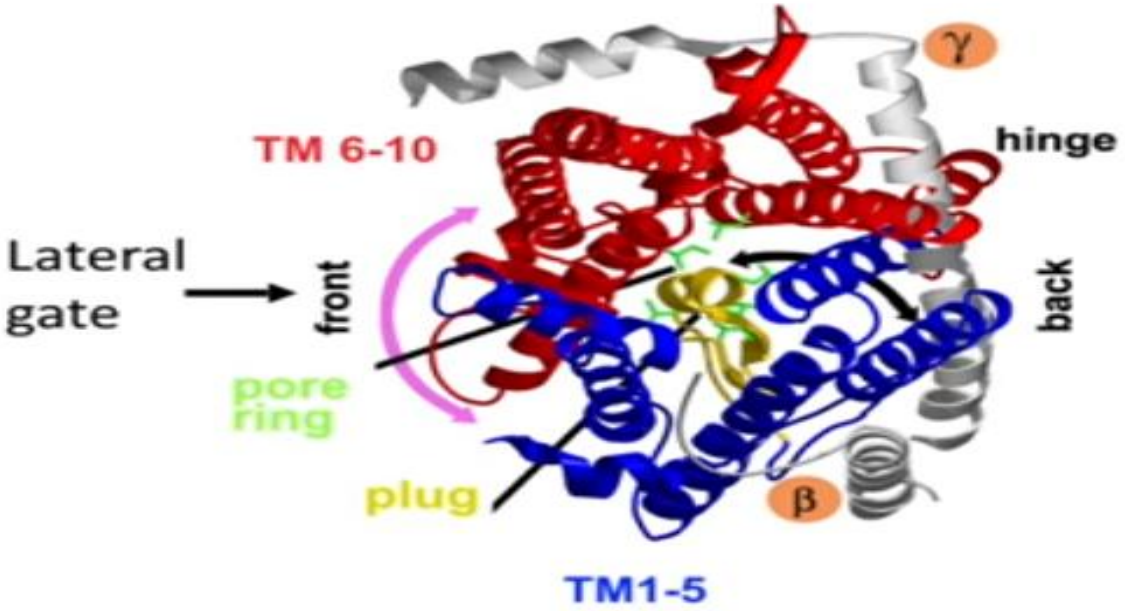
Protein sorting in the cell

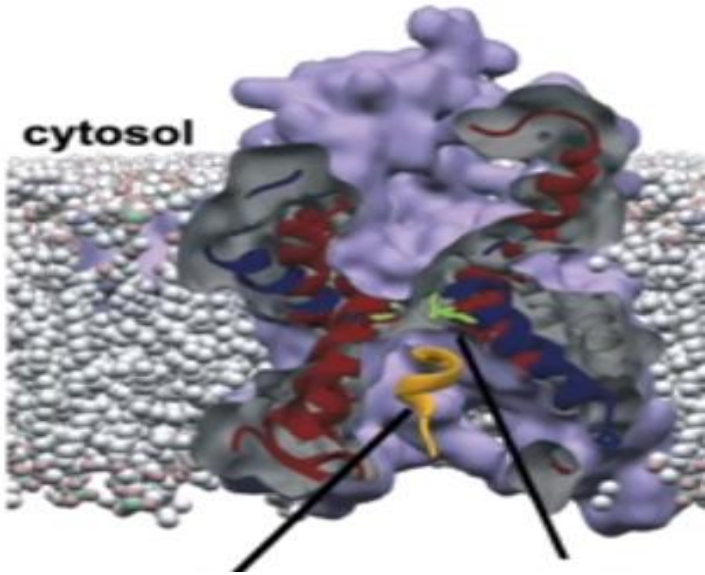
- Cytoplasm ➔ No signal sequence
- Nuclei proteins ➔ Nuclear localization sequence
- Mitochondria ➔ Mitochondrial signal sequence
- Plasma membrane proteins ➔ ER signal sequence

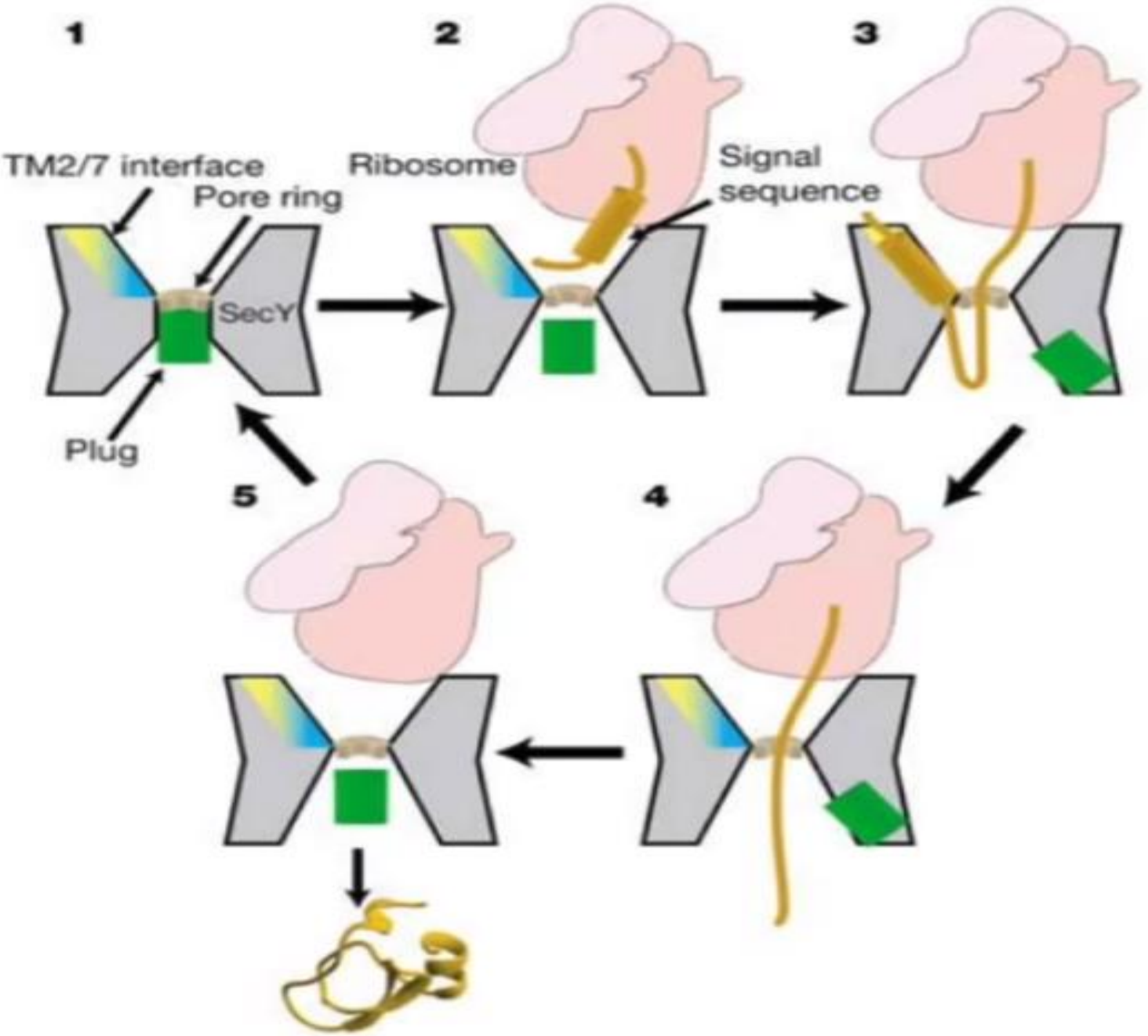


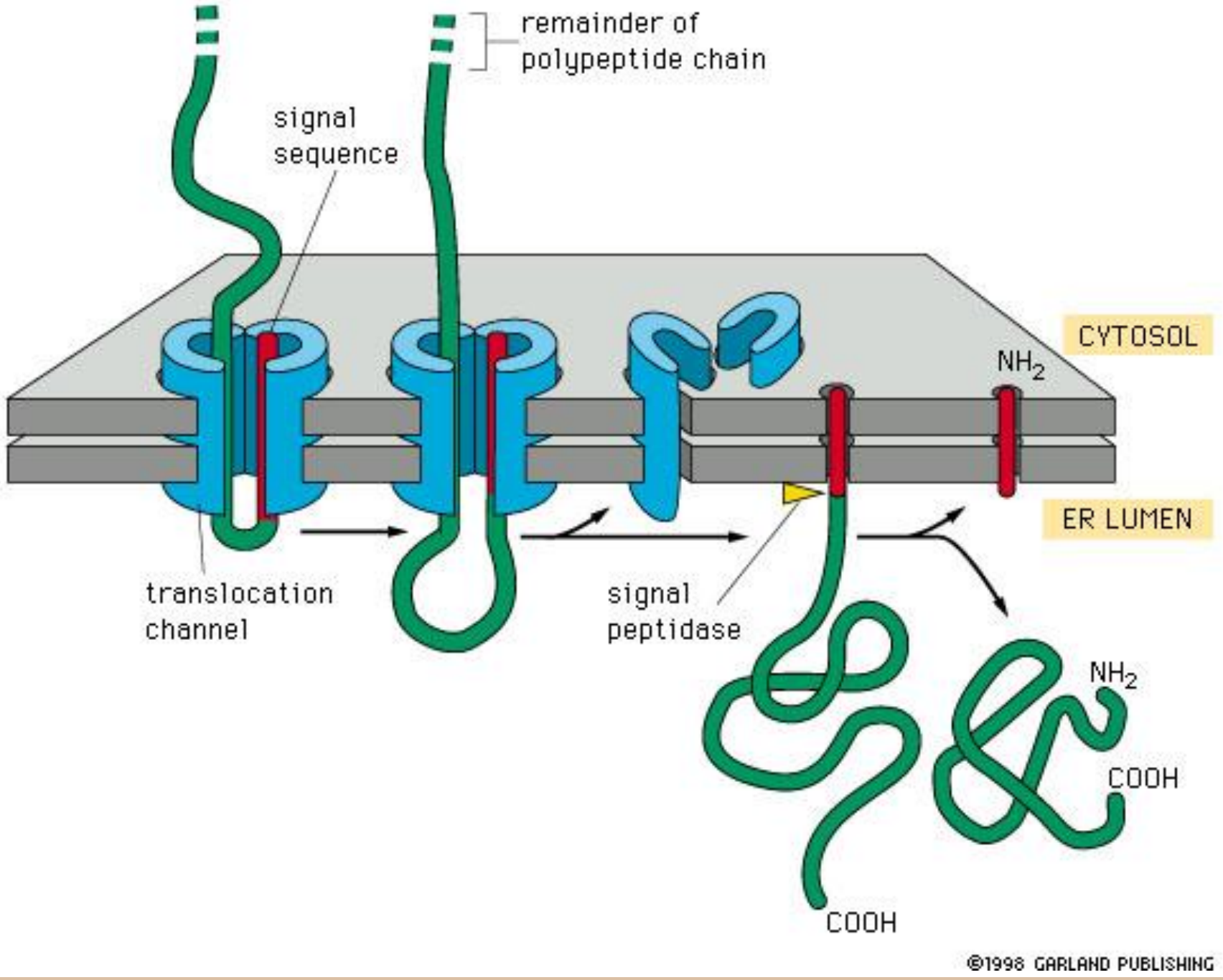


Structure of the channel









Protein tags

- Depending on the protein and destination, a tag may be an
  - amino acid sequence
  - a hydrophobic domain
  - oligosaccharide side chain, or some other feature

- Tags can also be used to exclude material from certain vesicles

Lipid tags

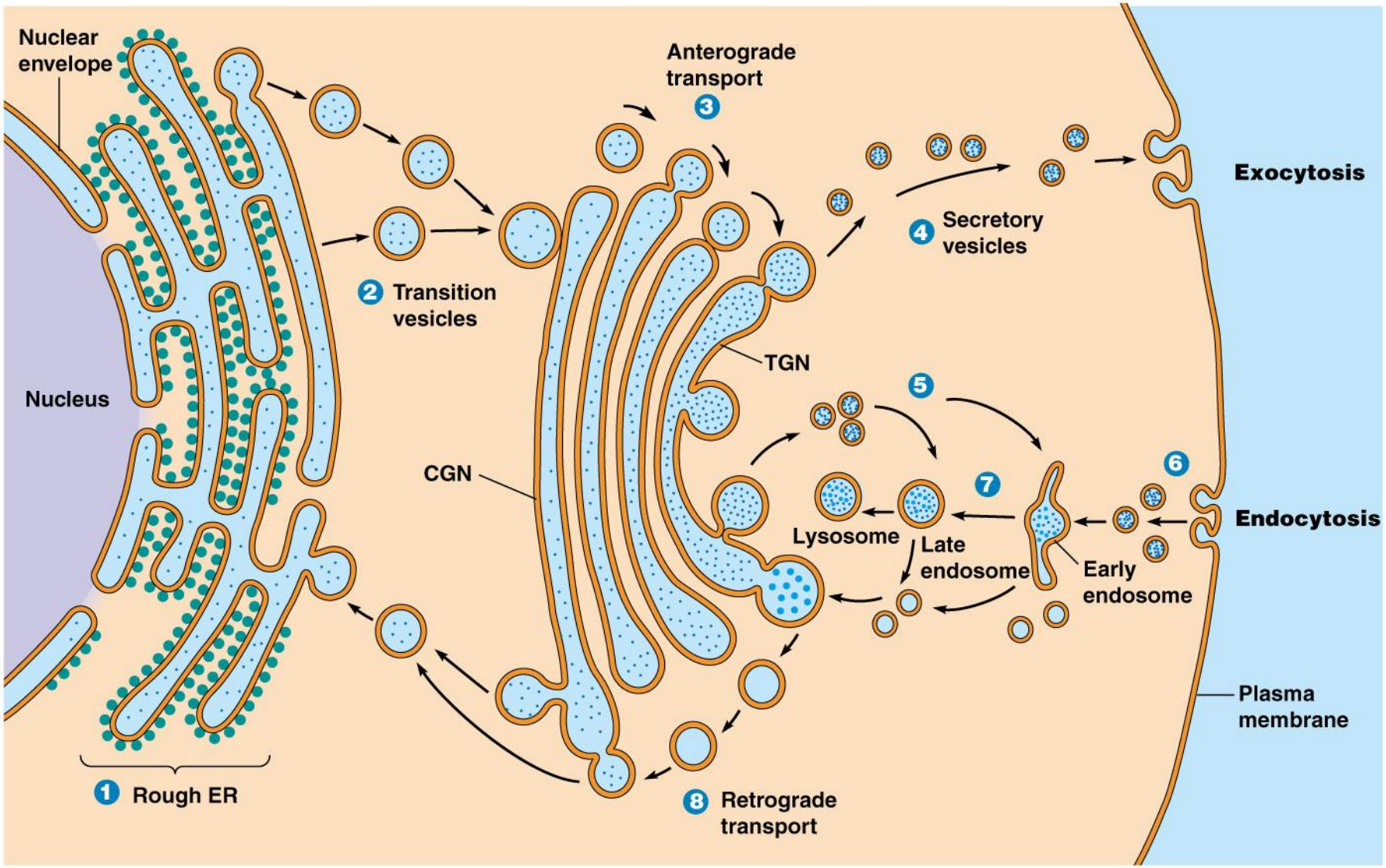
- Membrane lipids may also be tagged to help vesicles reach their destinations

- Lipid tags can be one or more phosphate groups attached to positions 3, 4, and/or 5 of a membrane phosphatidyl inositol

Overview of trafficking

- Sorting of proteins begins in the ER and early compartments of the Golgi
- There are mechanisms to retrieve or retain compartment-specific proteins
- The final sorting of material that will leave the Golgi complex occurs in the TGN

Figure 12-8



ER-Specific Proteins Contain Retention and Retrieval Tags

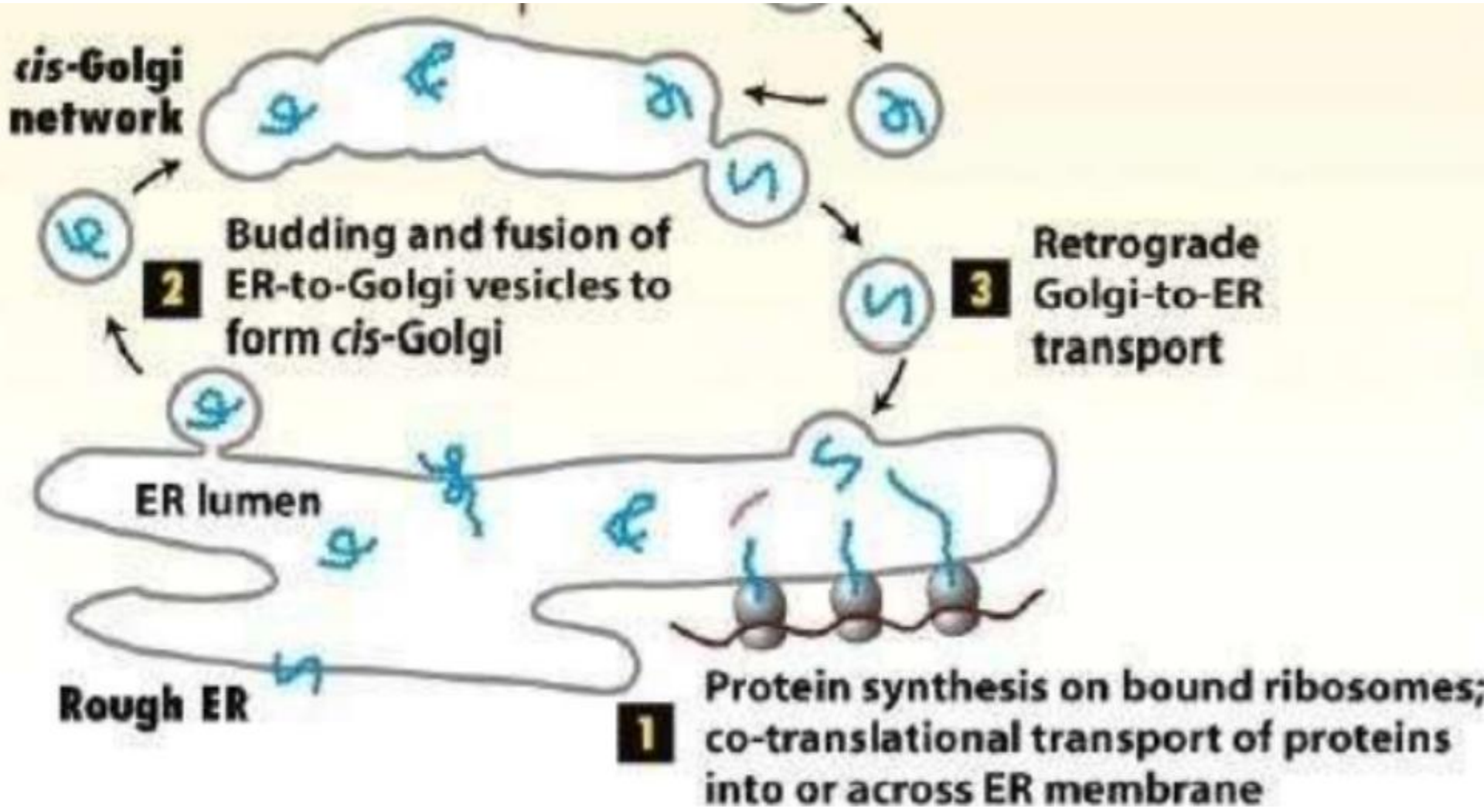
- Protein composition in the ER is maintained by –
  - *preventing some proteins from escaping the ER*
  - *retrieving others from the Golgi*

- Some proteins localized to the ER contain the sequence RXR (Arg-X-Arg; X is any amino acid)

- This is a *retention tag*

Retrieval tags

- Some proteins returned from the Golgi to the ER contain *retrieval tags*
- The tags are short C-terminal sequences such as KDEL (Lys-Asp-Glu-Leu) or KKXX in mammals and HDEL (His-Asp-Glu-Leu) in yeast
- When a **protein with this tag binds a receptor**, the receptor-ligand complex is packaged into a transport vesicle for return to the ER



Protein tags

- A tag may be an
- amino acid sequence
- a hydrophobic domain
- oligosaccharide side chain, or some other feature

Golgi Complex Proteins May Be Sorted According to the Lengths of Their Membrane-Spanning Domains

- Some proteins resident to the **Golgi complex** also contain retention or retrieval tags

- A third mechanism involves ***hydrophobic regions of Golgi proteins***

Golgi-specific proteins are integral membrane proteins

- All Golgi-specific proteins are integral membrane proteins with one or more hydrophobic membrane-spanning domains
- ***The length of the hydrophobic domains may determine into which cisternae each protein is incorporated***
- The thickness of cellular membranes increases progressively from the ER (5nm) to the plasma membrane (8nm)

Length of hydrophobic domains is correlated with location in the Golgi complex

- The thickness of membranes in the Golgi increases from the CGN to the TGN
- Proteins move from compartment to compartment until the membrane thickness exceeds the length of the transmembrane domains
- This blocks further migration

Protein tags

- A tag may be an
- amino acid sequence
- a hydrophobic domain
- oligosaccharide side chain, or some other feature

Targeting of Soluble Lysosomal Proteins to Endosomes and Lysosomes Is a Model for Protein Sorting in the TGN

- Soluble lysosomal enzymes in the ER and early Golgi compartments undergo N-glycosylation followed by removal of glucose and mannose units
- The mannose residues on the side chains are phosphorylated within the Golgi complex, forming an oligosaccharide containing mannose-6-phosphate
- This tag ensures delivery of lysosomal proteins to the lysosomes

Destination of lysosomal proteins

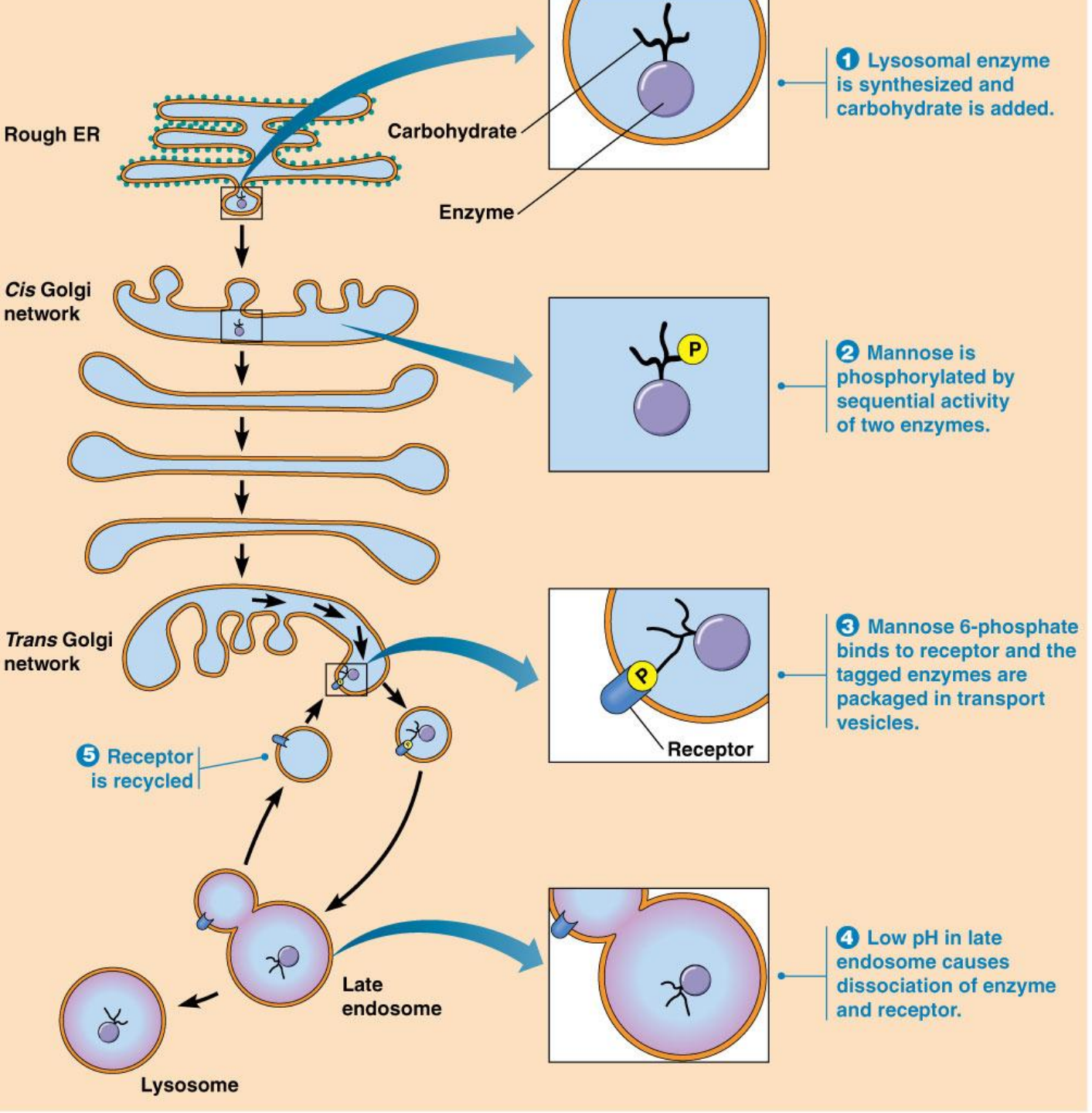
- Tagged lysosomal proteins bind to mannose-6-phosphate receptors (MPRs)
- The receptor-ligand complexes are packaged into transport vesicles and conveyed to an endosome
- In animal cells, lysosomal enzymes are transported from the TGN to organelles known as **late endosomes**

Destination of lysosomal proteins (continued)

- Dissociation of the lysosomal enzymes prevents the retrograde movement of the enzymes back to the Golgi with the receptors

- The late endosome matures to form a new lysosome, or delivers its contents to an active lysosome

Figure 12-


Rough ER
Carbohydrate
Enzyme
1 Lysosomal enzyme is synthesized and carbohydrate is added.
Cis Golgi network
P
2 Mannose is phosphorylated by sequential activity of two enzymes.
Trans Golgi network
P
Receptor
3 Mannose 6-phosphate binds to receptor and the tagged enzymes are packaged in transport vesicles.
5 Receptor is recycled
Late endosome
4 Low pH in late endosome causes dissociation of enzyme and receptor.
Lysosome

Roles of the ER and Golgi in Protein Glycosylation

- Much of the protein processing carried out in the ER and Golgi involves **glycosylation**, the addition of carbohydrate side chains to proteins

- Enzyme-catalyzed reactions involving the resulting **glycoproteins** then modify the oligosaccharide side chain

Two general kinds of glycosylation

- **N-linked glycosylation** (**N-glycosylation**) involves the addition of an oligosaccharide to the *nitrogen* atom of certain asparagine residues

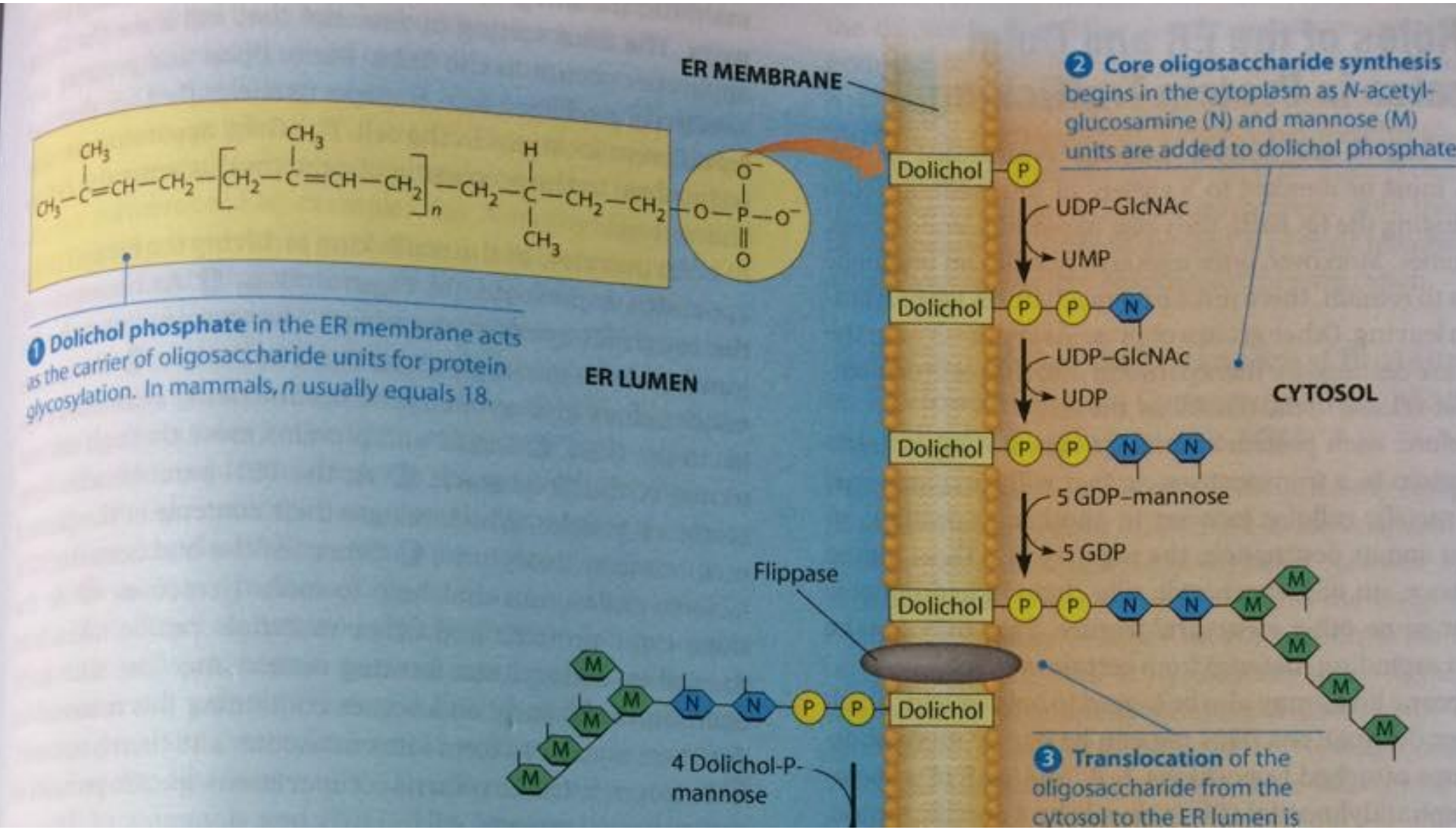
- **O-linked glycosylation** involves addition of the oligosaccharide to the *oxygen* atom on the hydroxyl group of certain serine or threonine residues

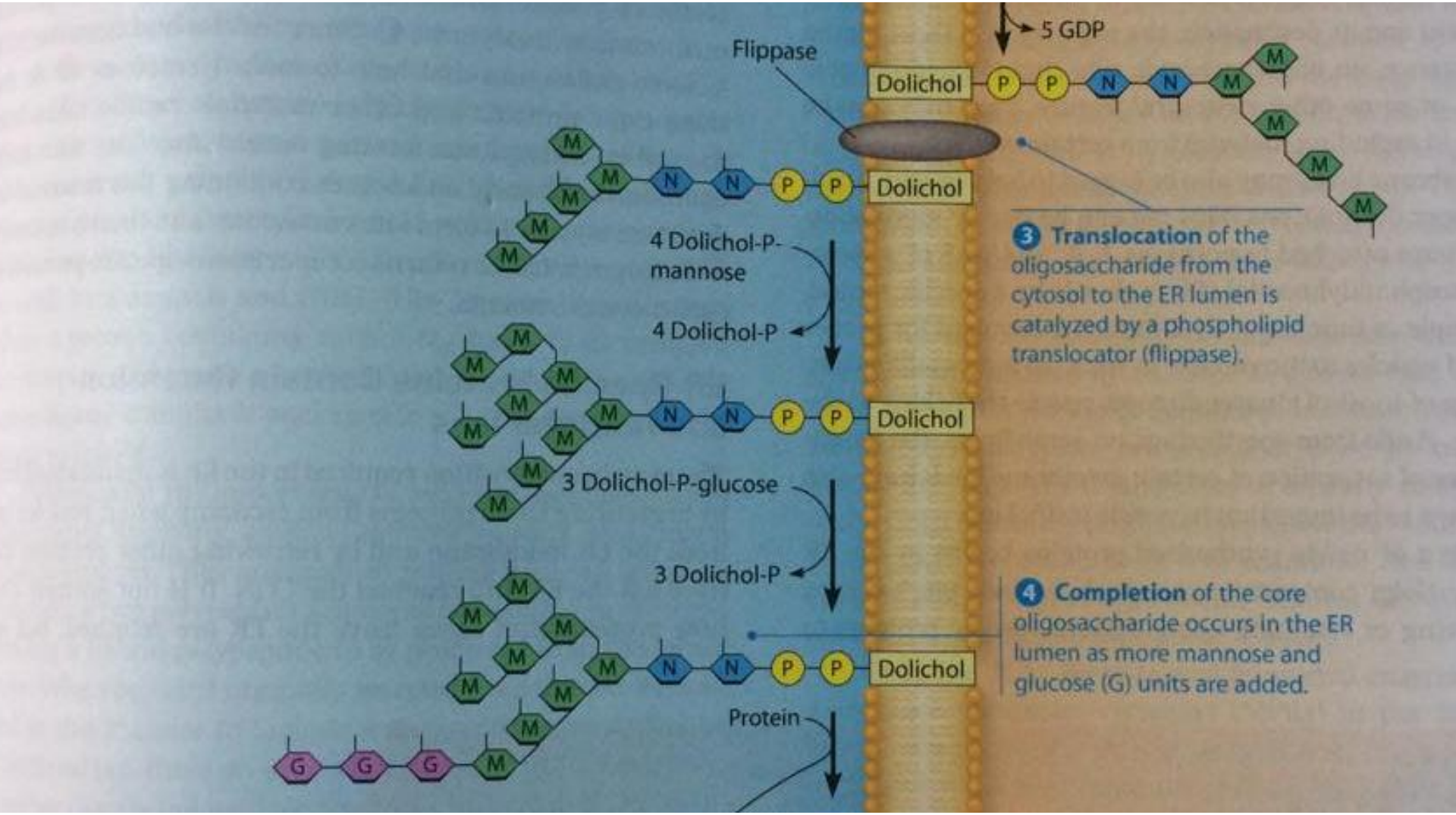
Initial Glycosylation Occurs in the ER

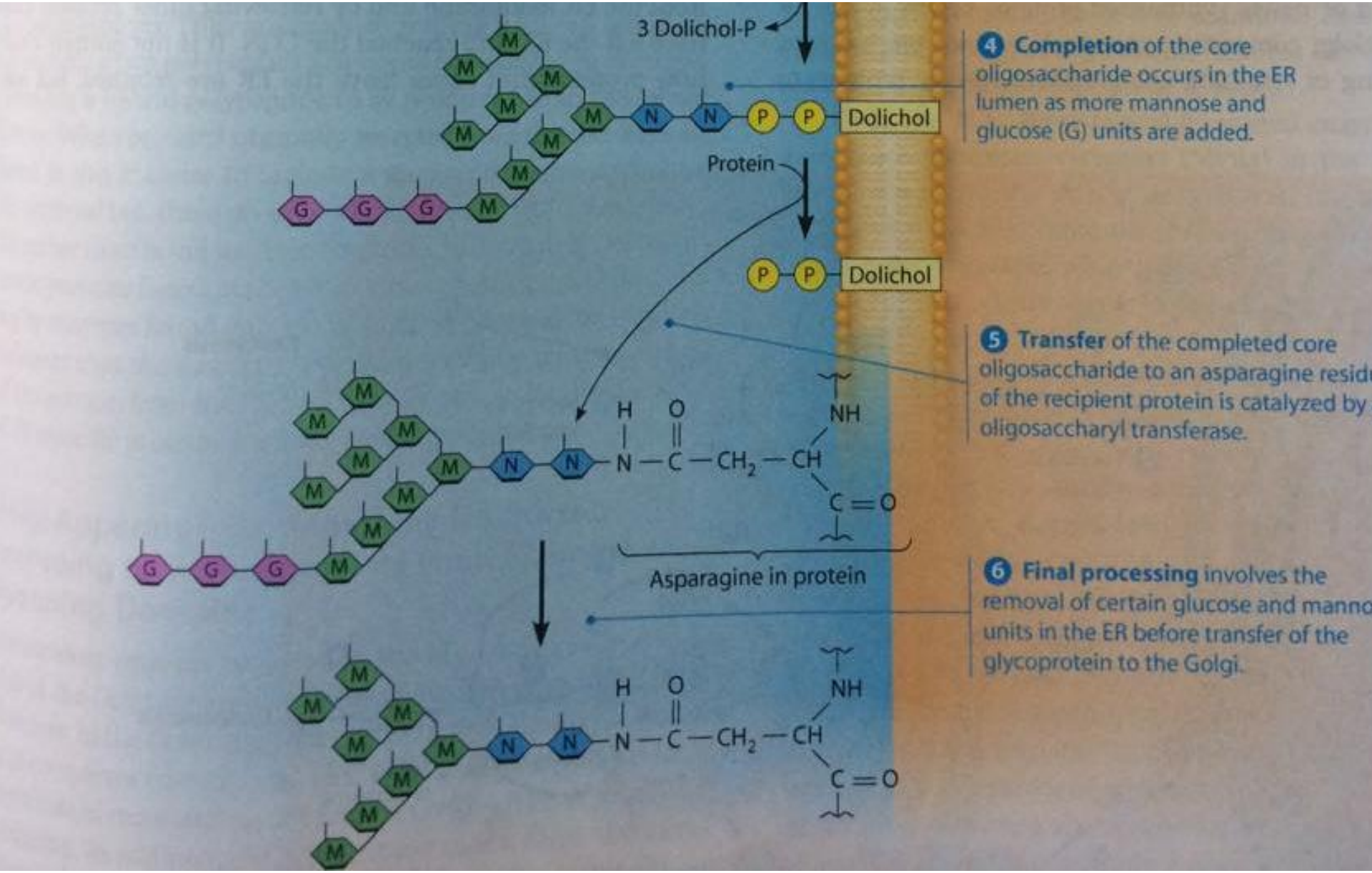
Importantly:

This is **cotranslational glycosylation** and helps in promote protein folding

- All carbohydrate side chains initially have a common **core oligosaccharide** consisting of two units of N-acetylglucosamine, nine mannose units, and three glucose units







Two proteins assist with proper folding

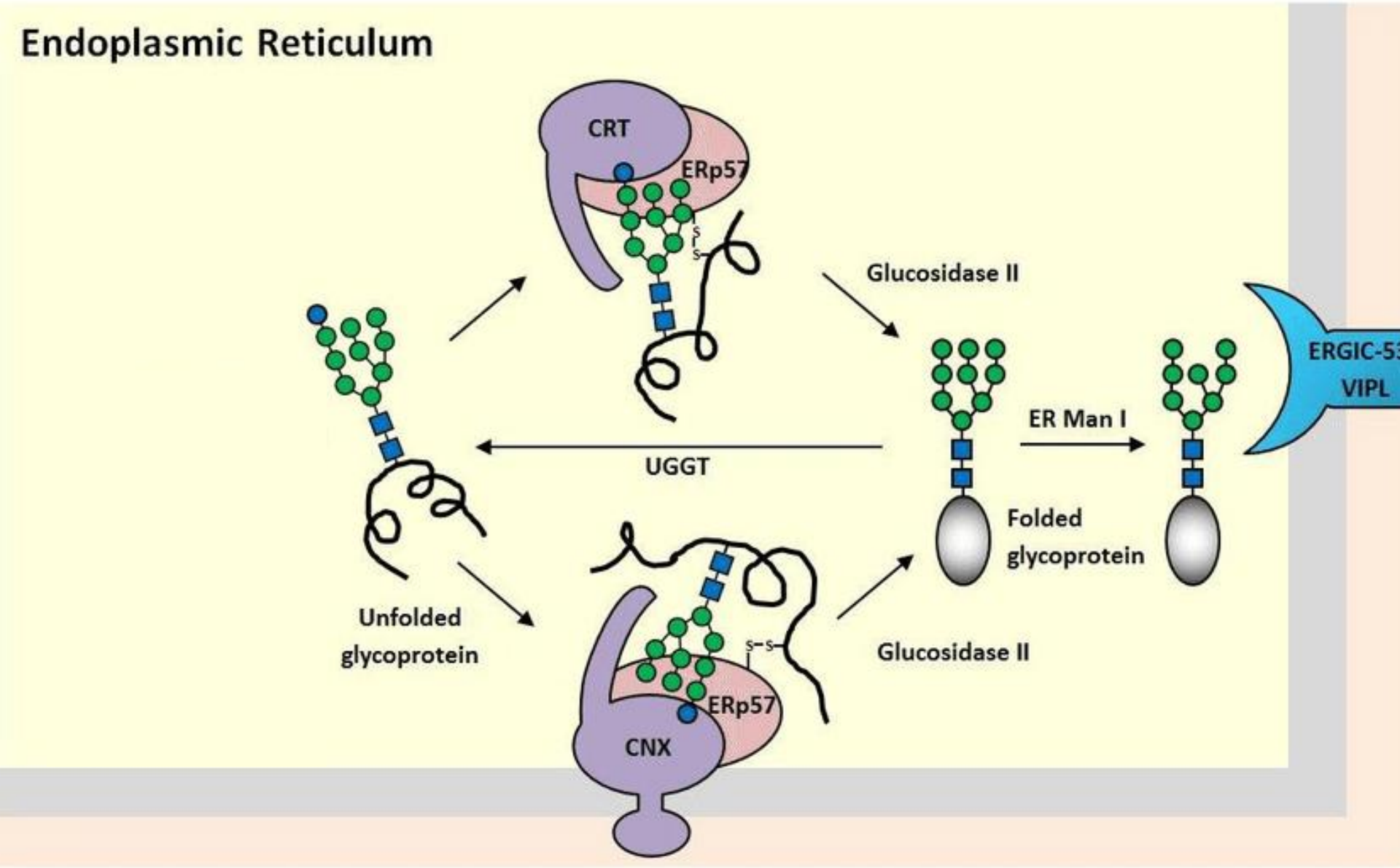
- Two ER proteins, **calnexin** (**CNX**) and **calreticulin** (**CRT**), bind to monoglucosylated proteins and promote disulfide bond formation

- This occurs via a complex that includes a thiol reductase known as *ERp57*

- The complex dissociates and the final glucose is removed by *glucosidase II*

Proper folding in the ER

- A glucosyl transferase, *UGGT*, *UDP-glucose:glycoprotein glucotransferase,* binds to improperly folded proteins
- It adds back a single glucose unit, making the protein a substrate for CNX/CRT binding
- Once proper conformation is achieved, UGGT no longer binds the new glycoprotein, which moves on to the Golgi



2 glc + 1 man removed

CNX/CRT bind to monoglucosylated protein

Promote disulfide bond formation

Protein dissociates

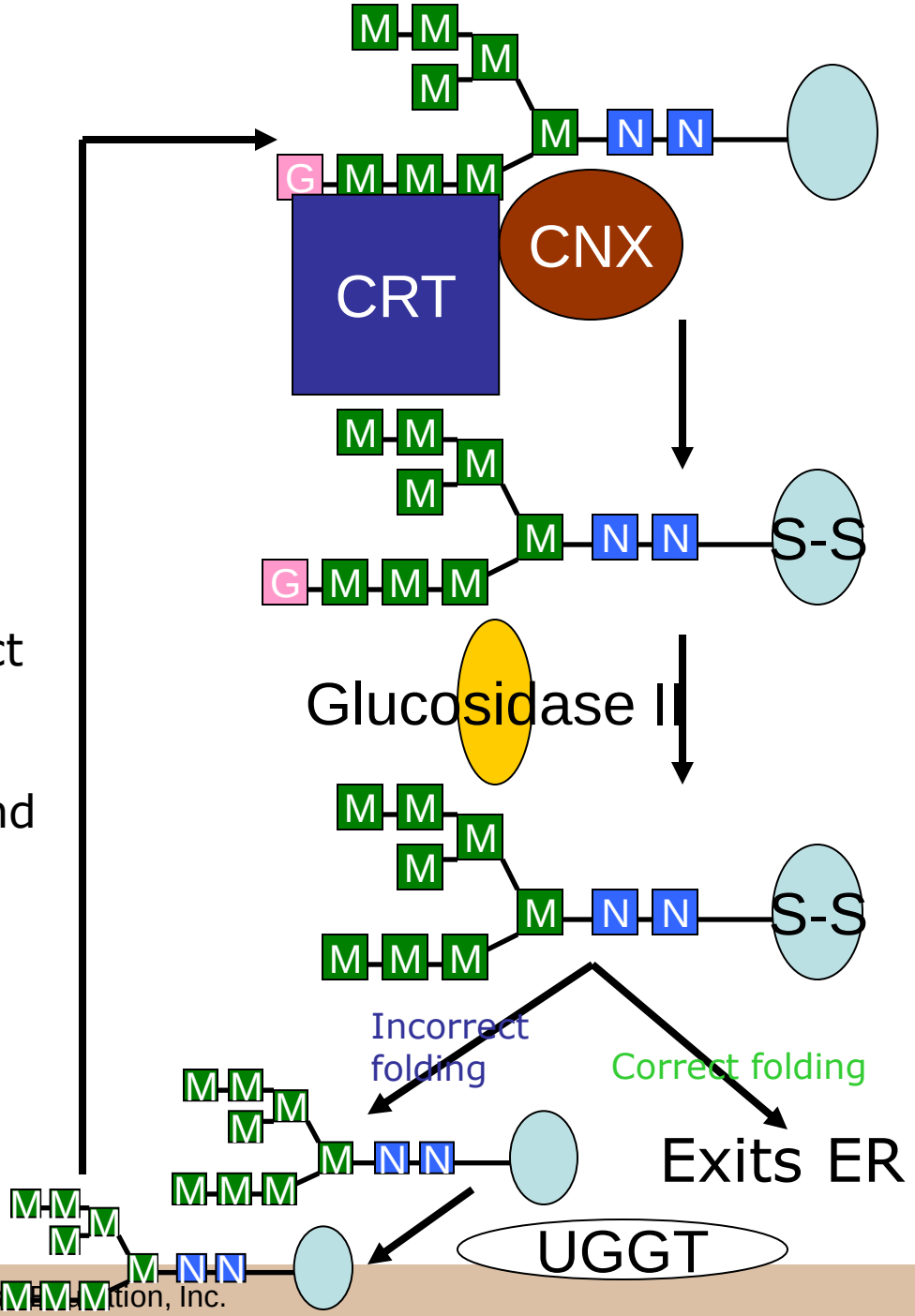
Final glc is removed by glucosidase II

UGGT binds if the folding is not correct (sensor of proper folding)

Binds to improperly folded proteins and adds back glc

Binding of CNX/CRT → another round until the protein is correctly folded

Exits the ER and moves to the GA



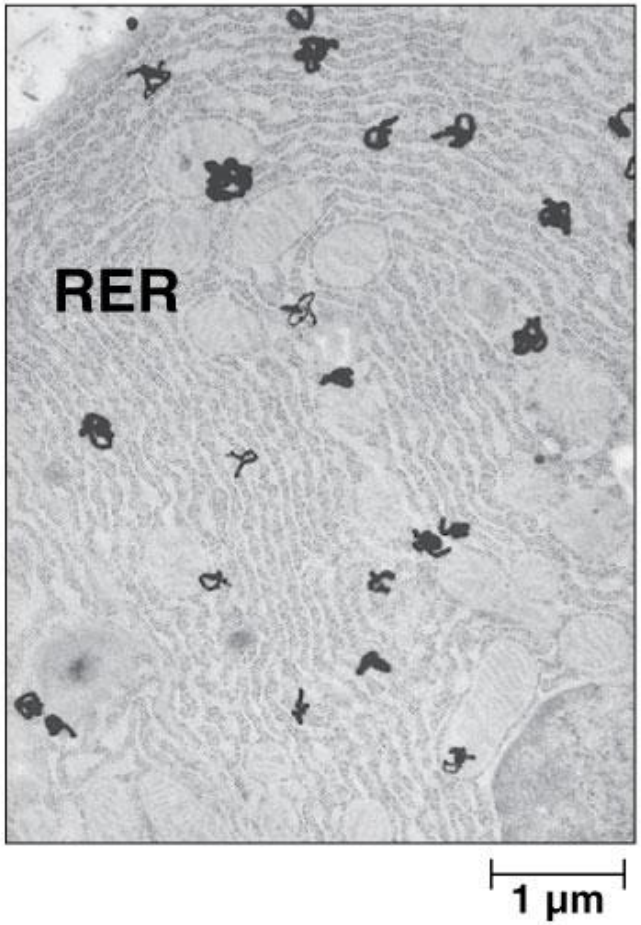
Secretory Pathways Transport Molecules to the Exterior of the Cell

- **Secretory pathways** move proteins from the ER through the Golgi complex to **secretory vesicles** and **secretory granules**
- The secretory granules then discharge their contents to the exterior of the cell
- Experiment using electron microscopy, briefly radiolabeled proteins were followed through secretory pathways

- Proteins were radioactively labeled briefly
- After three minutes the proteins could be seen primarily in the rough ER
- A few minutes later, the proteins began to appear in the Golgi complex

Results of the experiment

(a) After 3 minutes, most of the labeled protein is found in the rough ER where it has just been synthesized.



(b) After 7 minutes, most of the new labeled protein has moved into the adjacent Golgi complexes (arrows).

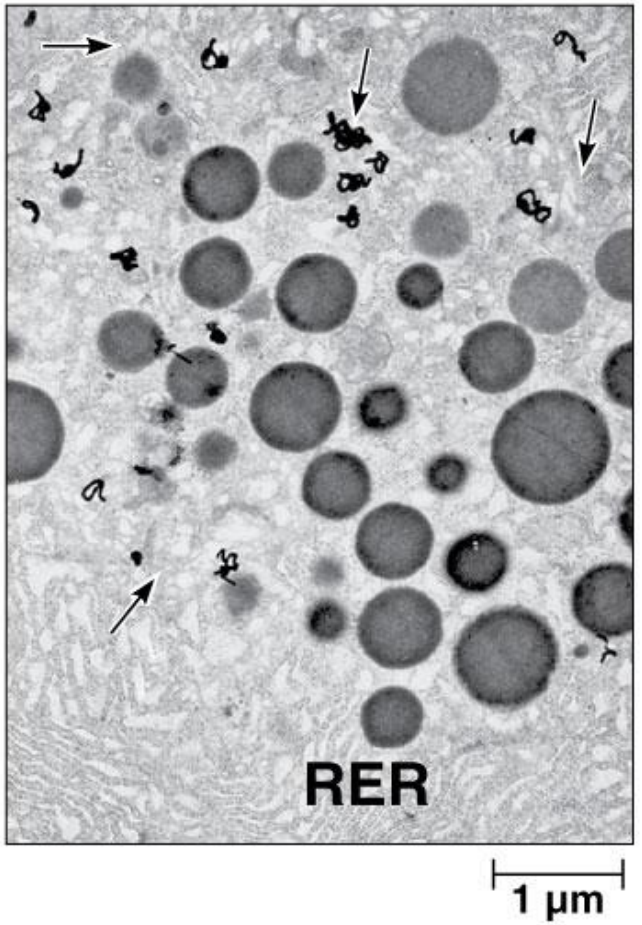
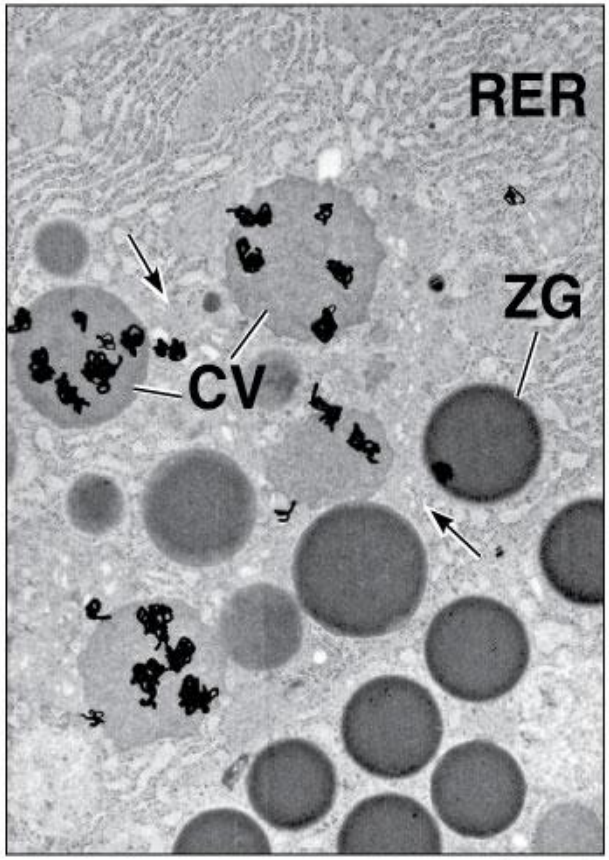
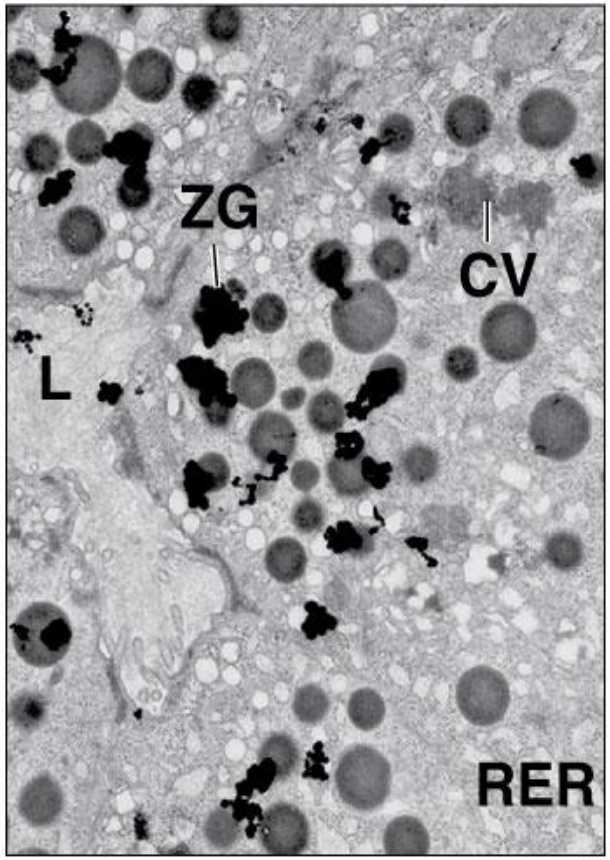


Figure 12-10C, D

(c) After 37 minutes, the labeled protein is being concentrated in condensing vacuoles next to the Golgi.



(d) After 117 minutes, the labeled protein is found in zymogen granules, ready for export to the lumen.



Constitutive Secretion

- After budding from the TGN, some vesicles move directly to the cell surface and immediately fuse with the plasma membrane
- This unregulated process is continuous and independent of external signals
- It is called **constitutive secretion**; one example is mucus secretion by the intestinal lining

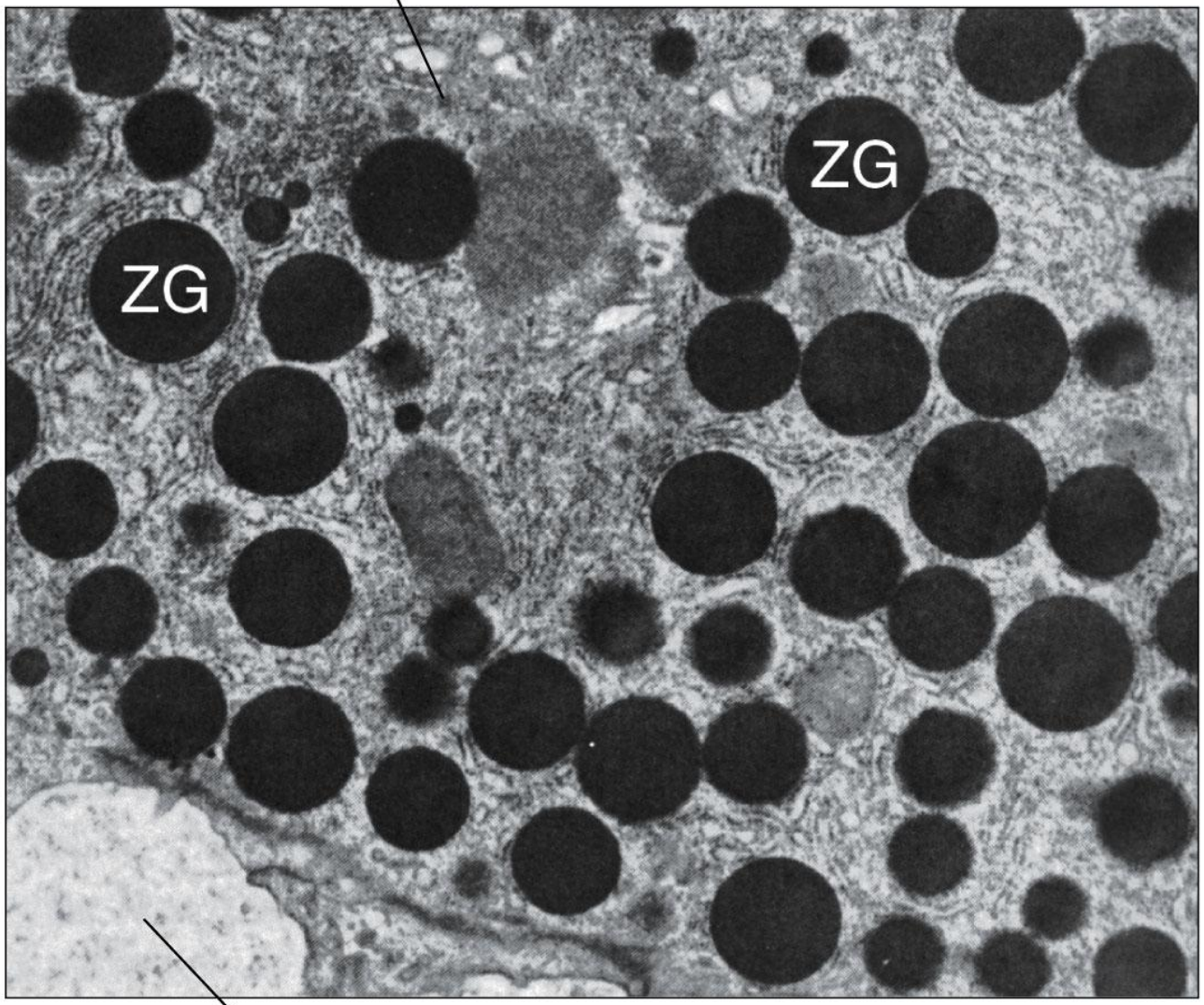
Regulated Secretion

- Secretory vesicles involved in regulated secretion accumulate in the cell and only fuse with the plasma membrane in response to specific signals
- An important example is neurotransmitter release
- Regulated secretory vesicles form by budding from the TGN as immature secretory vesicles

Regulated secretion (continued)

- Maturation of secretory proteins involves their concentration, called *condensation,* and sometimes proteolytic processing
- The mature secretory vesicles move close to the site of secretion and remain there until receiving a signal
- The signal triggers vesicles to release their contents by fusion with the plasma membrane

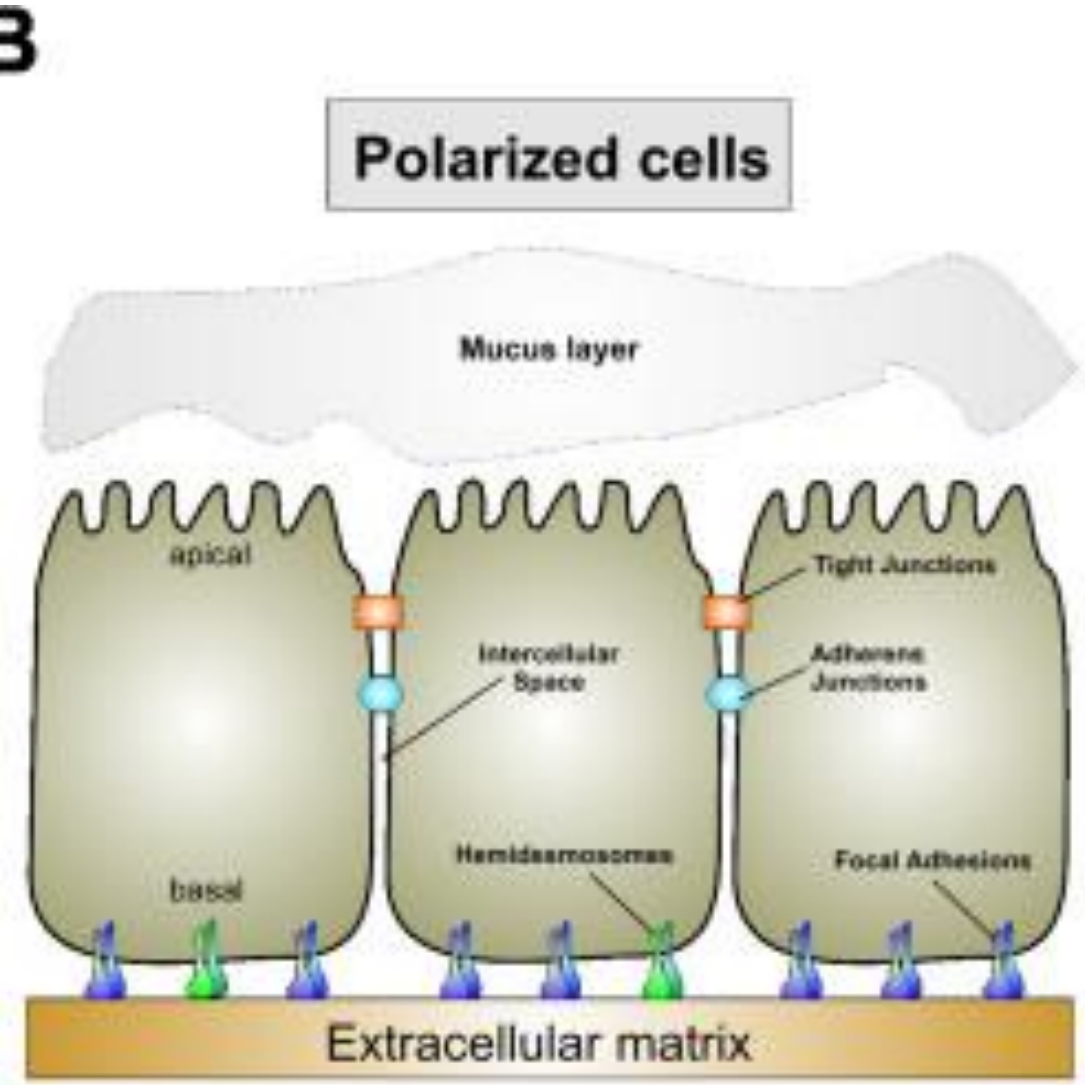
Figure 12-11

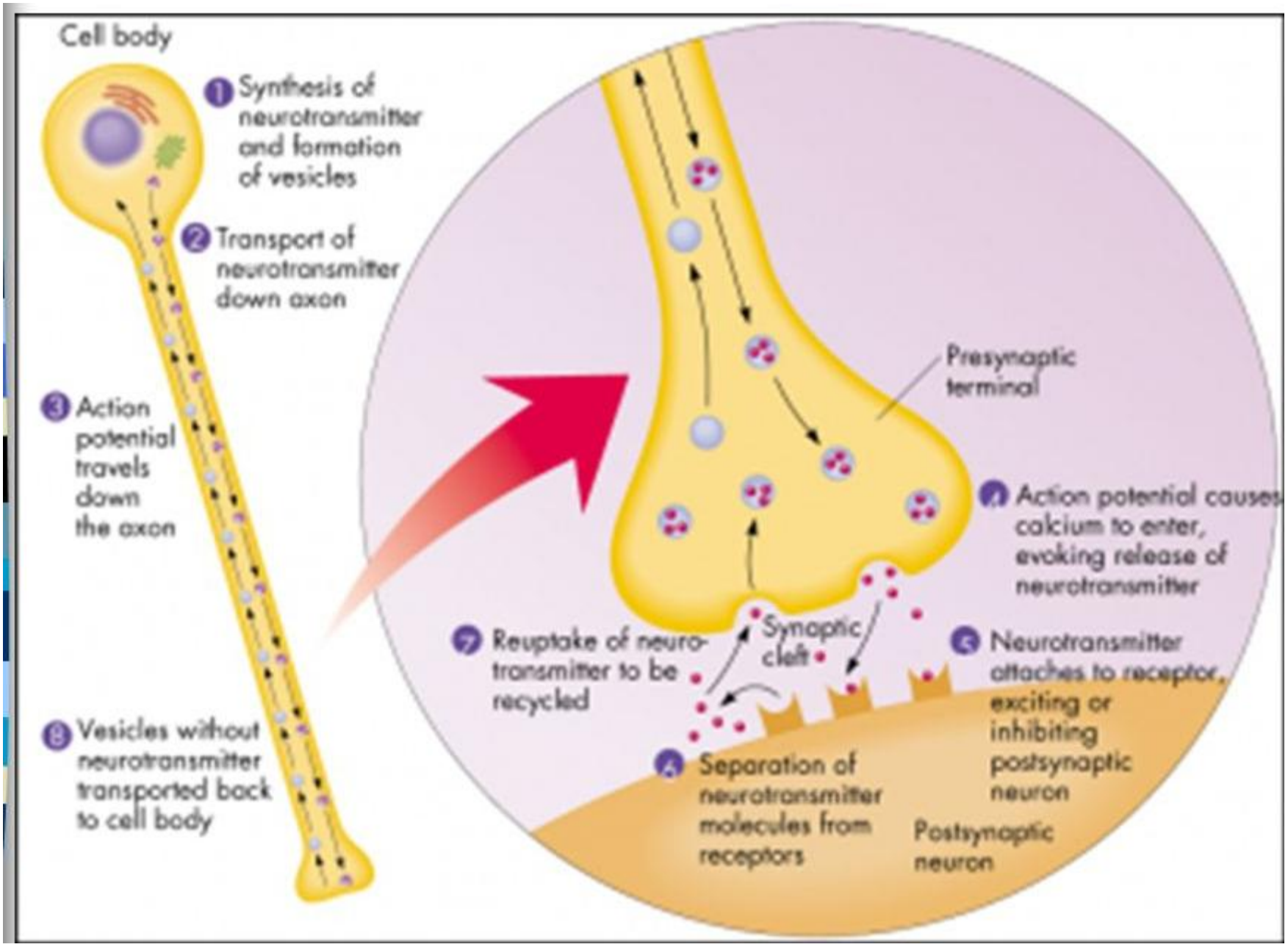


Polarized Secretion

- In many cases, exocytosis of specific proteins is limited to a specific surface of the cell
- For example, intestinal cells secrete digestive enzymes only on the side of the cell that faces into the intestine
- This is called **polarized secretion**

Polarized Secretion





Exocytosis and Endocytosis: Transporting Material Across the Plasma Membrane

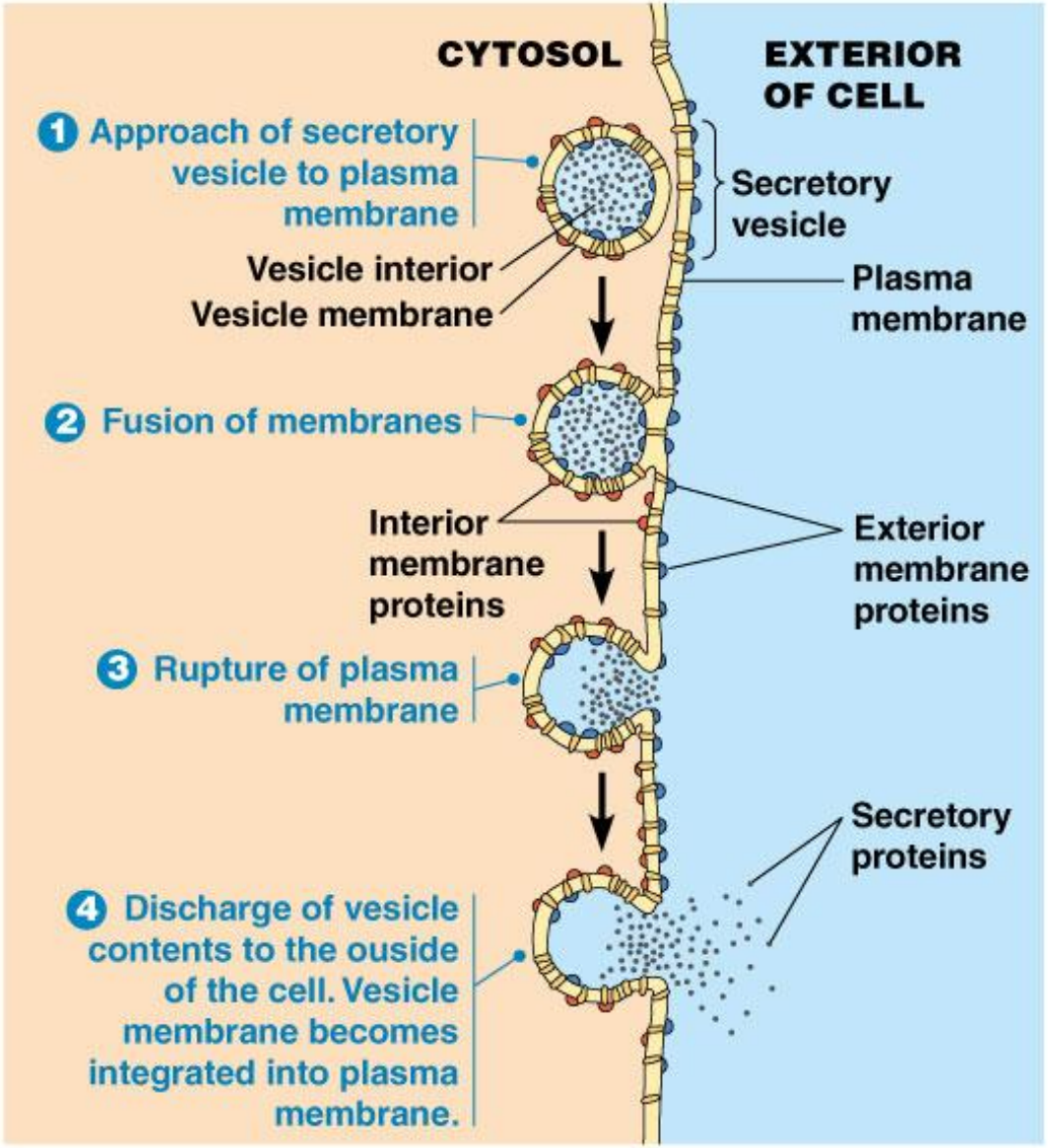
- Two methods (unique to eukaryotes) for transporting materials across the plasma membrane are
  - Exocytosis, the process by which secretory vesicles ***release their contents outside the cell***
  - Endocytosis, the process by which ***cells internalize external materials***

Exocytosis Releases Intracellular Molecules 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

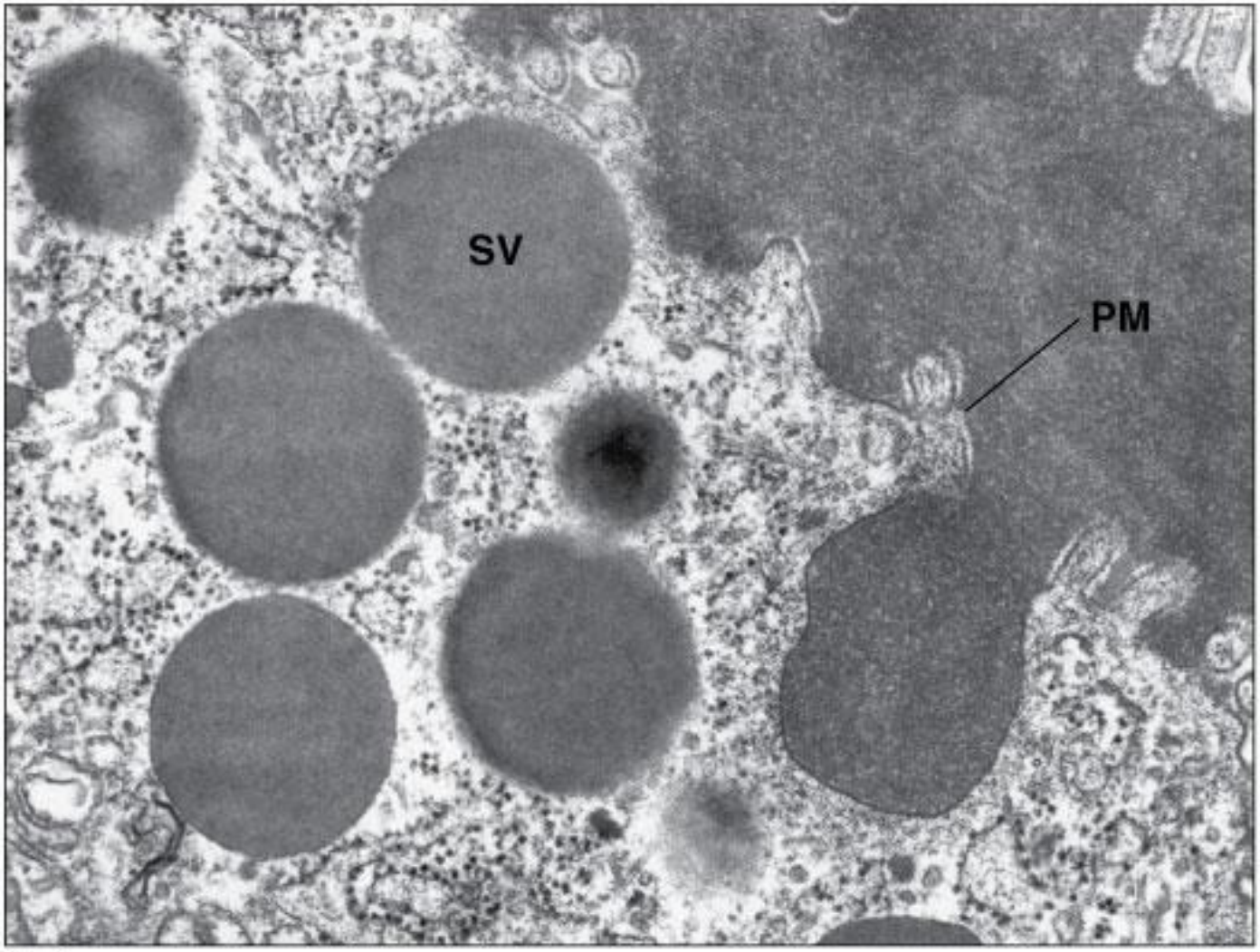
- Animal cells secrete hormones, mucus, milk proteins, and digestive enzymes this way

Figure 12-12A



(a)

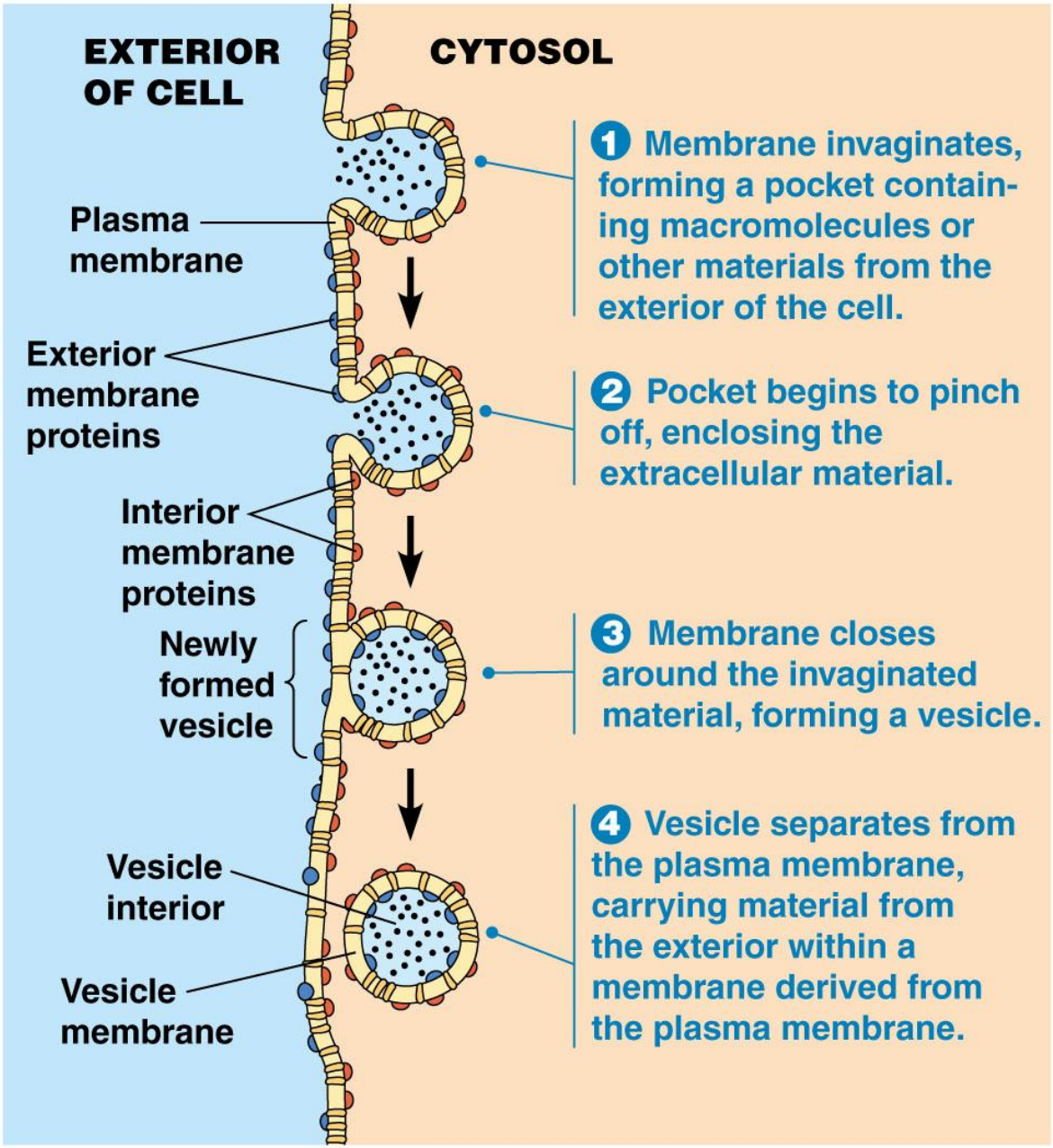
Figure 12-12B



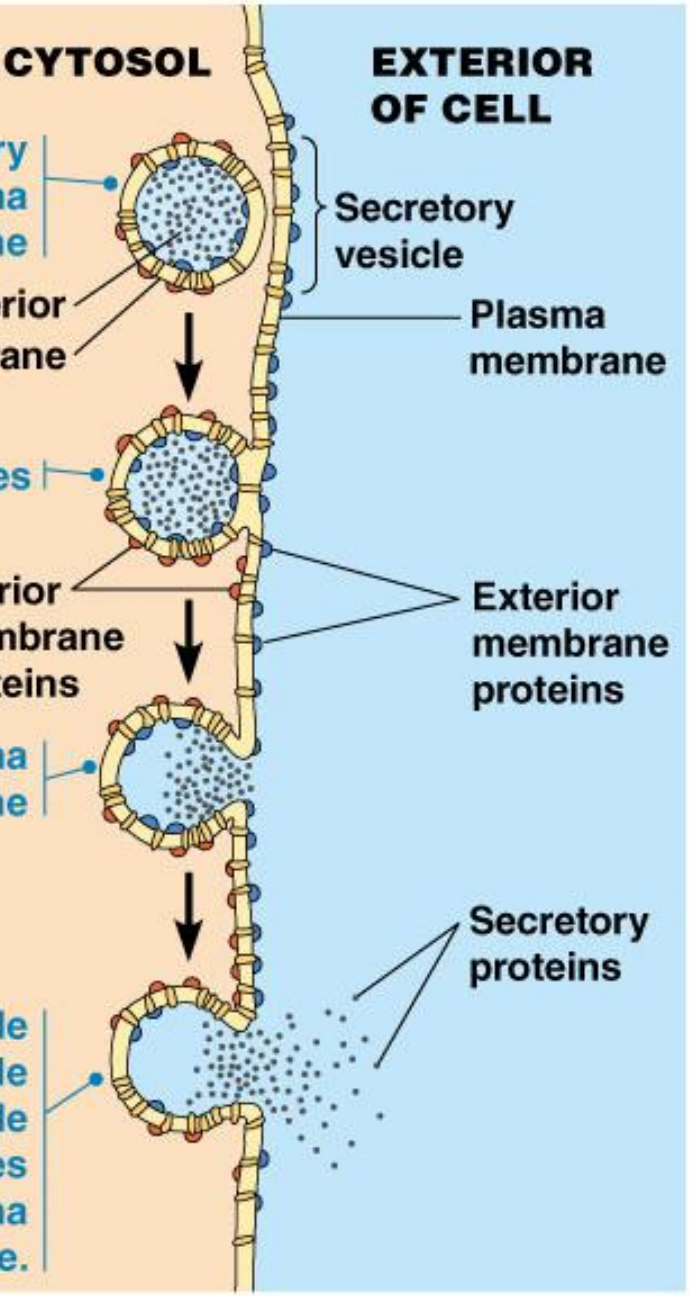
Endocytosis Imports Extracellular Molecules by Forming Vesicles from the Plasma Membrane

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

Figure 12-13

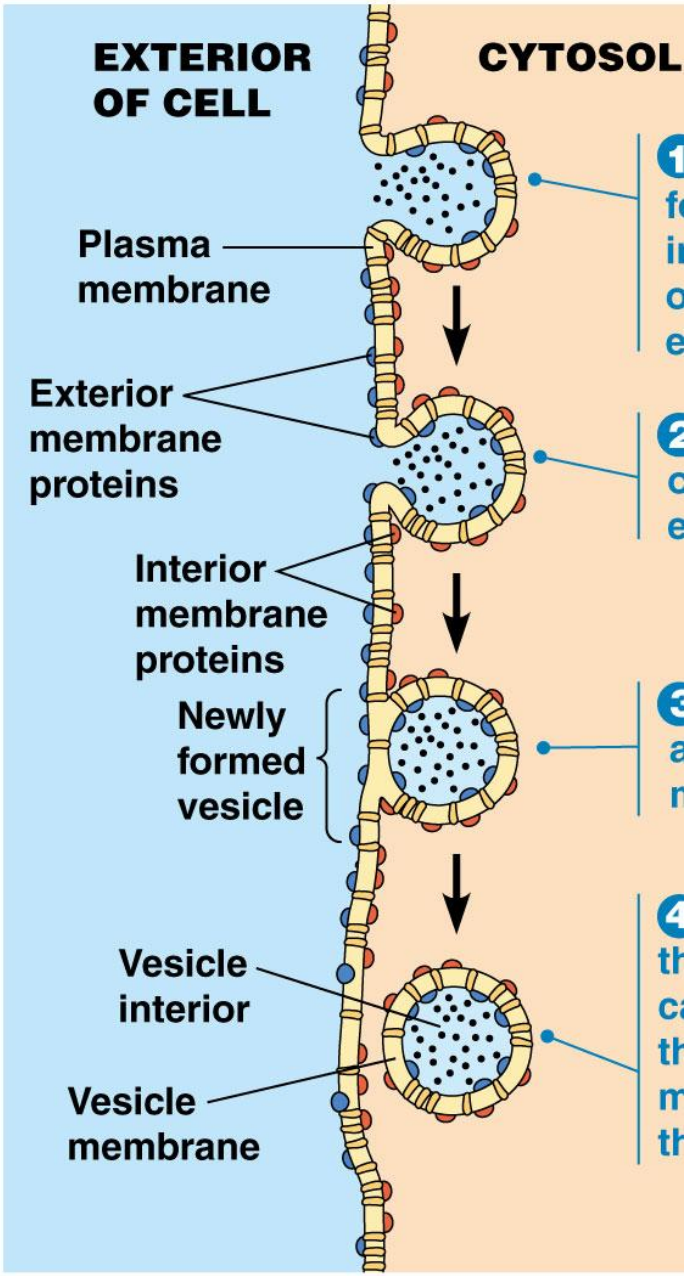


Exocytosis



- Opposite effects
- Steady state composition of plasma membrane
- Balance

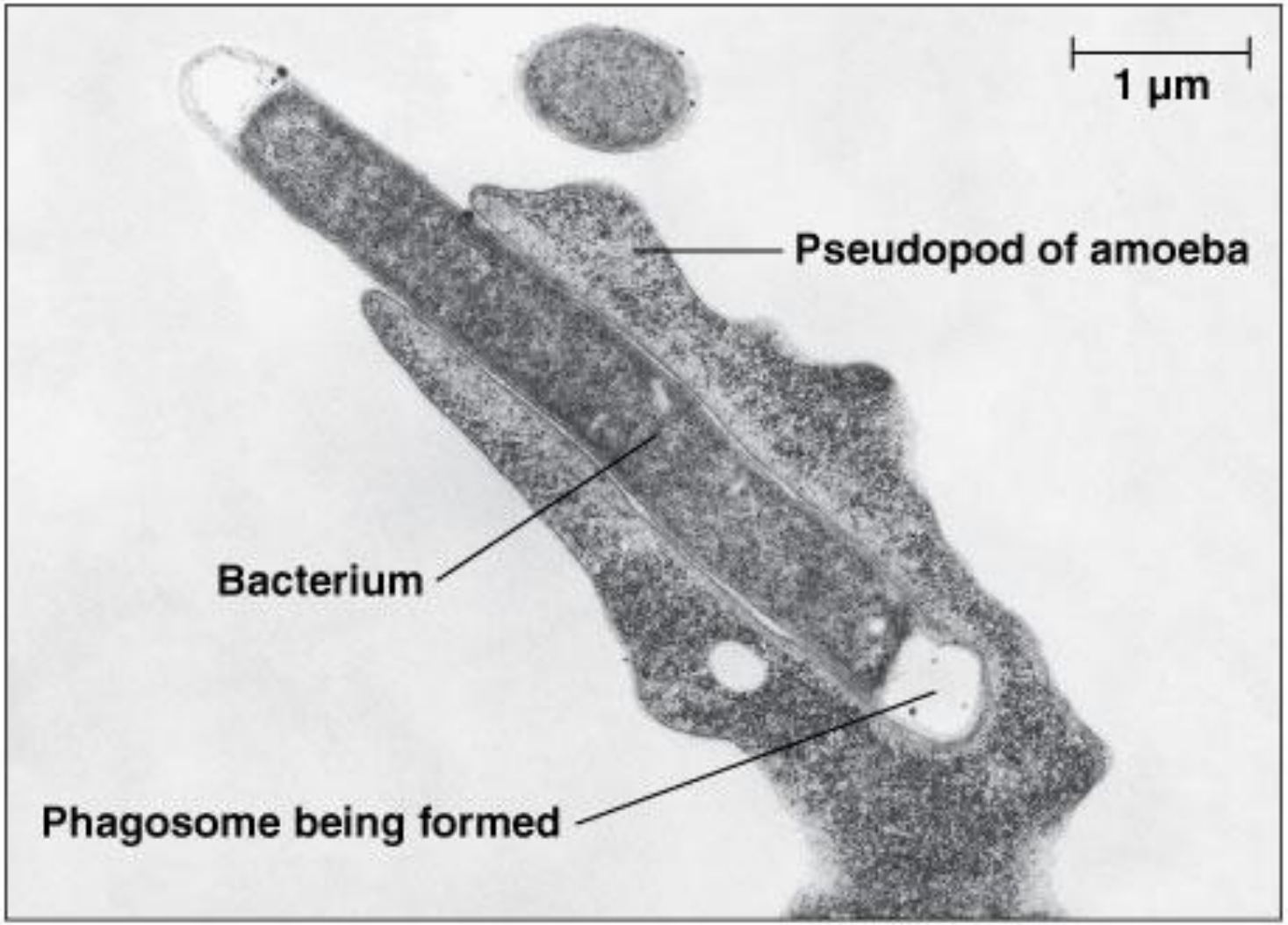
Endocytosis



Phagocytosis

- The ingestion of large particles up to and including whole cells or microorganisms is called **phagocytosis**
- For many unicellular organisms it is a means of acquiring food
- For more complex organisms, it is usually restricted to specialized cells called **phagocytes**

Figure 12-14B



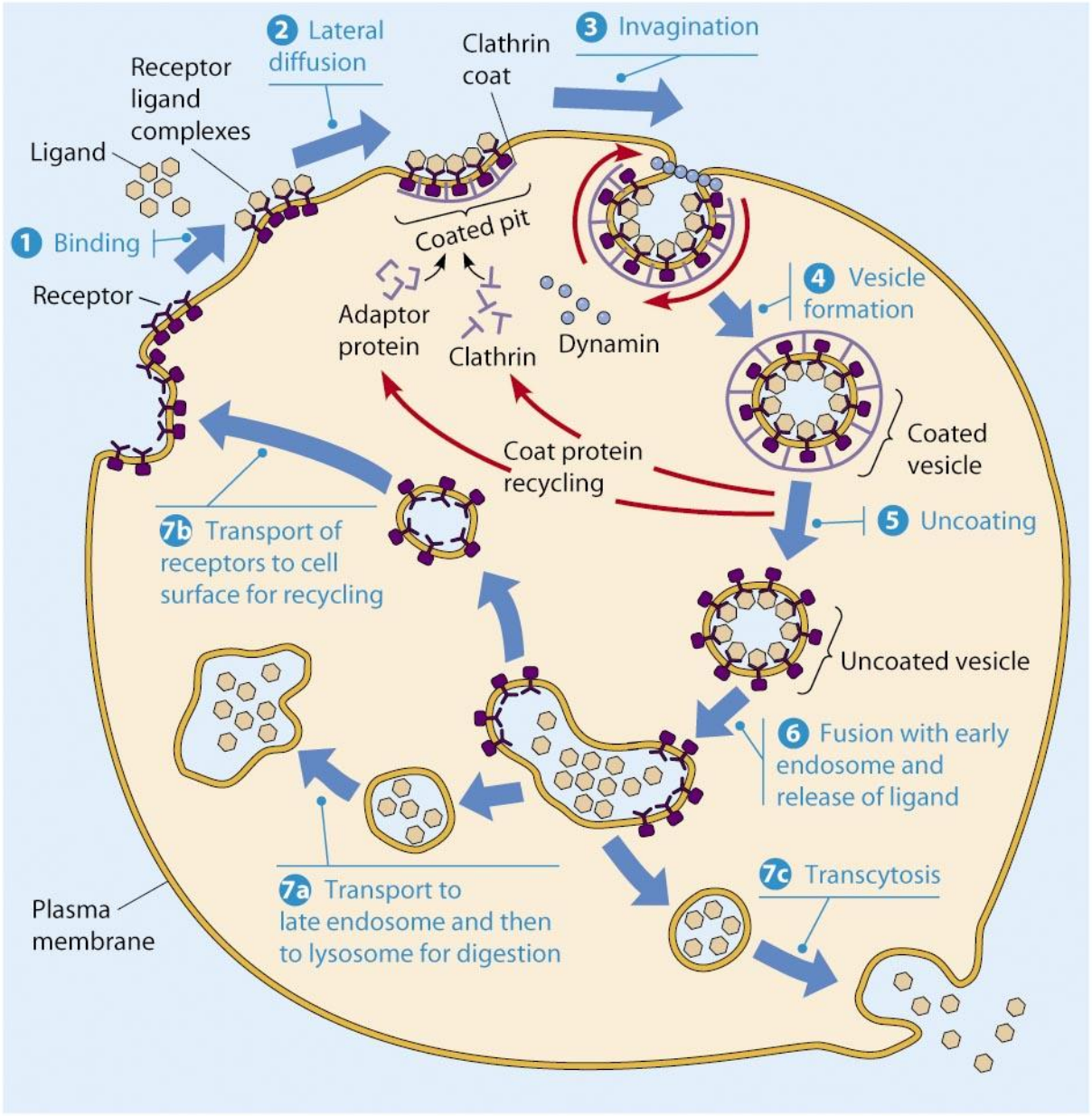
(b)

Receptor-Mediated Endocytosis

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

Process of receptor-mediated endocytosis

- Specific molecules (*ligands*) bind to their *receptors* on the outer surface of the cell (1)
- As the receptor-ligand complexes diffuse laterally they encounter specialized regions called *coated pits,* sites for collection and internalization of these complexes (2)
- In a typical mammalian cell, coated pits occupy about 20% of the total surface area



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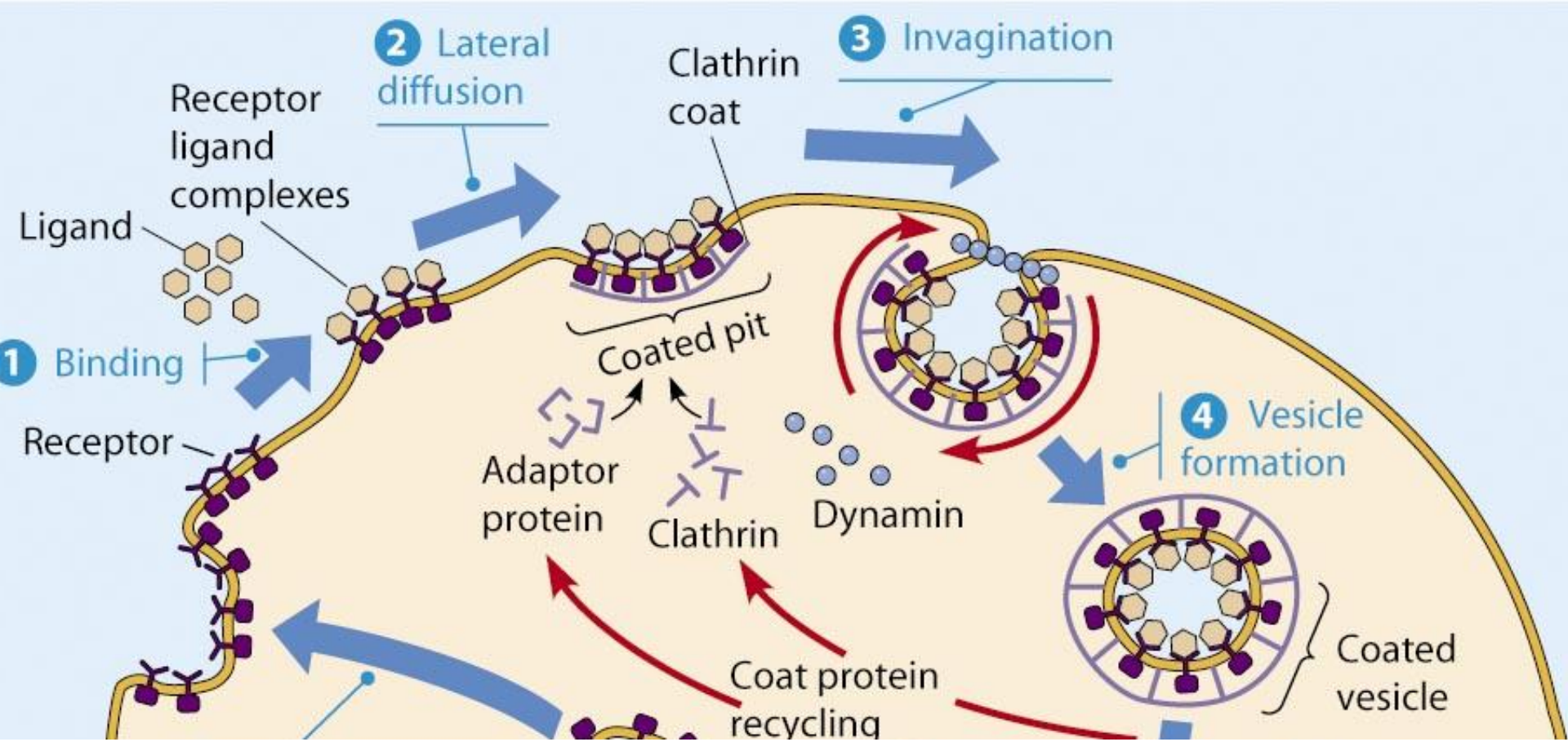
Coated pits ~ 20% of total SA of PM in mammalian cells

~2500 coated pits invaginate per minute

Receptors are concentrated in coated pits when ligands attach or not.

Some are constitutively internalized

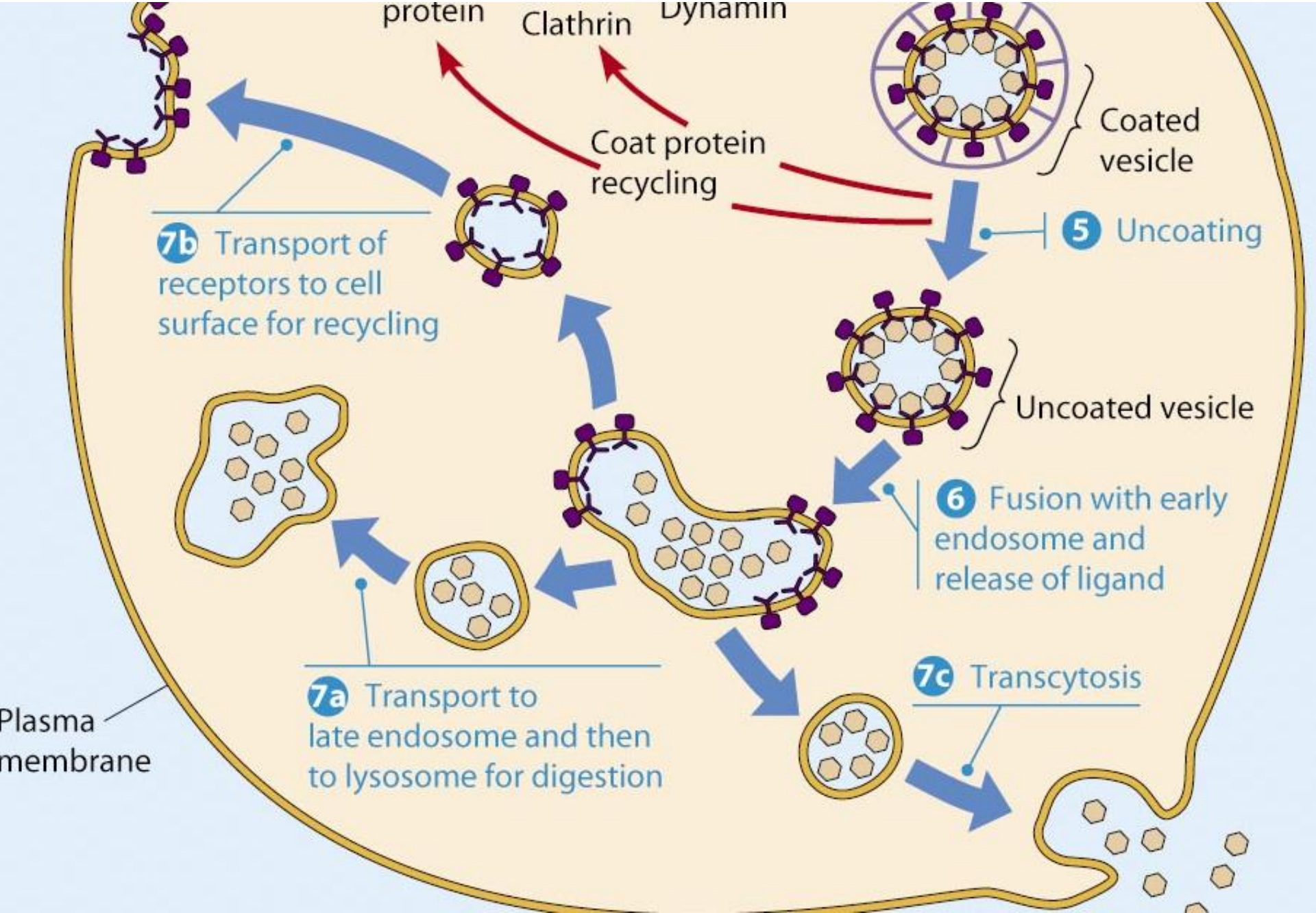


Process of receptor-mediated endocytosis (continued)

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

Process of receptor-mediated endocytosis (continued)

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


protein
Clathrin
Dynamin
Coated vesicle
Coat protein recycling
5 Uncoating
7b Transport of receptors to cell surface for recycling
Uncoated vesicle
6 Fusion with early endosome and release of ligand
7a Transport to late endosome and then to lysosome for digestion
7c Transcytosis
Plasma membrane

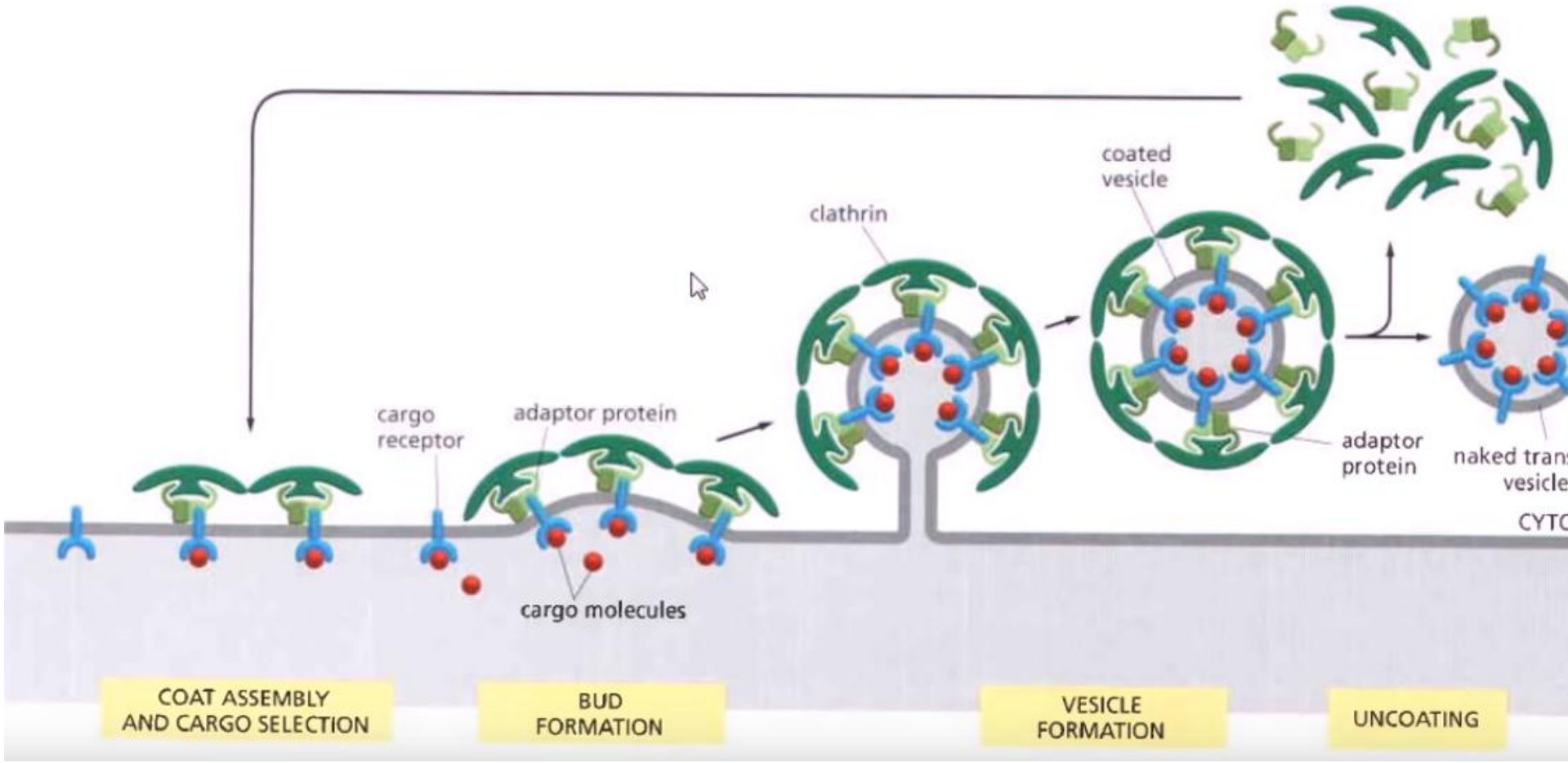
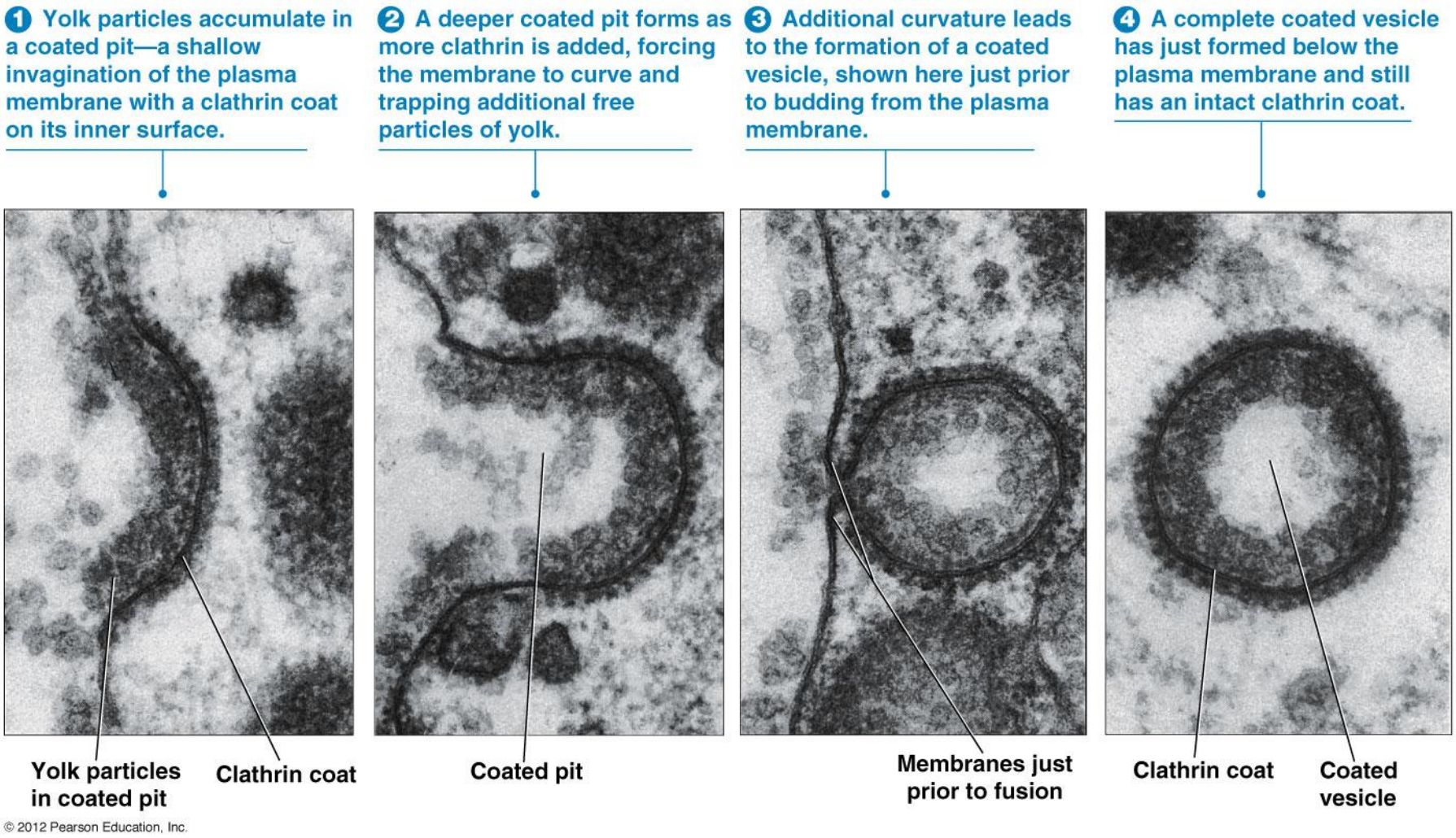


Figure 12-16



After internalization

- Uncoated vesicles fuse with vesicles budding from the TGN to form early endosomes

- Early endosomes continue to acquire lysosomal proteins from the TGN and mature to form late endosomes, which then develop into lysosomes

Recycling plasma membrane receptors

- Receptors from the plasma membrane are recycled due to acidification of the early endosome
- The pH gradually lowers as the endosome matures, facilitated by an *ATP-dependent proton pump*
- The lower pH dissociates ligand and receptors, allowing receptors to be returned to the membrane

Clathrin-Independent Endocytosis

- Fluid-phase endocytosis is a type of pinocytosis for nonspecific internalization of extracellular fluid
- This process does not concentrate the ingested material, and contents are routed to early endosomes
- It proceeds fairly constantly and compensates for membrane segments added by exocytosis

Coated Vesicles in Cellular Transport Processes

- Most vesicles in protein and lipid transport are called *coated vesicles* because of the layers of proteins covering their cytosolic surfaces
- Coated vesicles are involved in **vesicular traffic** throughout the endomembrane system, as well as in exocytosis and endocytosis
- There are several types of coat proteins

Coat proteins

- The most studied coat proteins are *clathrin*, *COPI*, and *COPII*
- The type of coat protein on a vesicle helps to determine the destination of the vesicle
- They also induce curvature needed for the formation of the vesicles and prevent nonspecific fusion of the vesicle with another membrane

Table 12-2 **Coated Vesicles Found Within Eukaryotic Cells**

Coated Vesicle	Coat Proteins*	Origin	Destination
Clathrin	Clathrin, AP1, ARF	TGN	Endosomes
Clathrin	Clathrin, AP2	Plasma membrane	Endosomes
COPI	COPI, ARF	Golgi complex	ER or Golgi complex
COPII	COPII (Sec13/31 and Sec23/24), Sar1	ER	Golgi complex
Caveolin	Caveolin	Plasma membrane	ER?

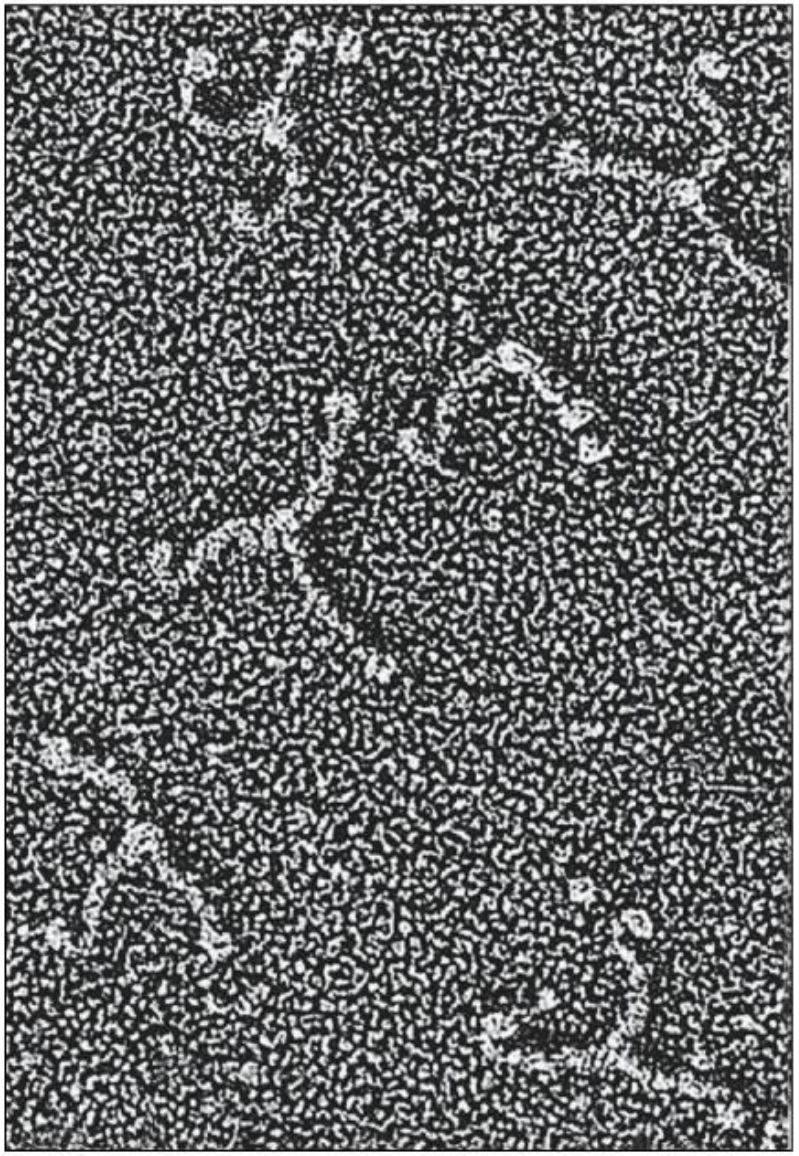
*ARF designates ADP ribosylation factor 1; AP1 and AP2 designate different adaptor protein complexes (also called assembly protein complexes).

Clathrin-Coated Vesicles Are Surrounded by Lattices Composed of Clathrin and Adaptor Protein

- Clathrin-coated vesicles are surrounded by coats made of two multimeric proteins, **clathrin** and *adaptor protein* (*AP*)
- The shape of clathrin proteins and the way they assemble provides the driving force to induce a flat membrane to form a spherical vesicle
- The basic unit of clathrin lattices is a **triskelion**

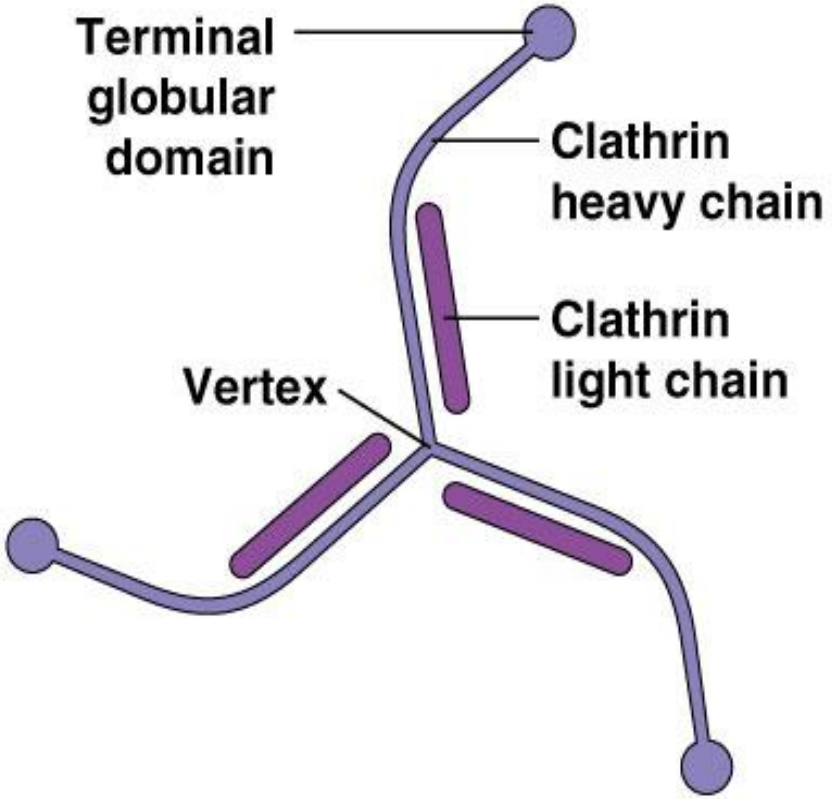
Structure of a triskelion

- Each is a multimeric protein composed of **three heavy chains and three light chain**
- These radiate from a central vertex, with the light chains associated with the inner half of each “leg”
- Triskelions assemble into the hexagons and pentagons of the lattice around clathrin-coated pits and vesicles

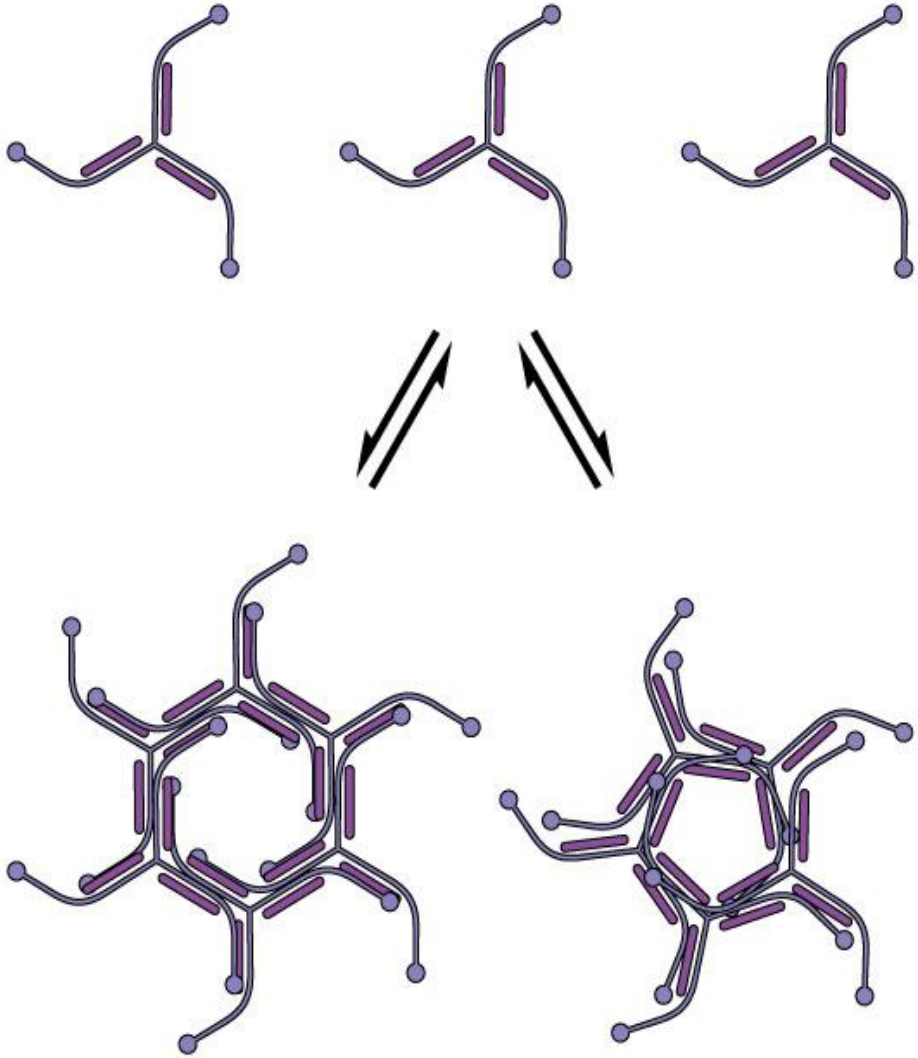


(a) Clathrin triskelions

Figure 12-17B, C



(b) Structure of clathrin triskelion



(c) Model for assembly of clathrin triskelions

The Assembly of Clathrin Coats Drives the Formation of Vesicles from the Plasma Membrane and TGN

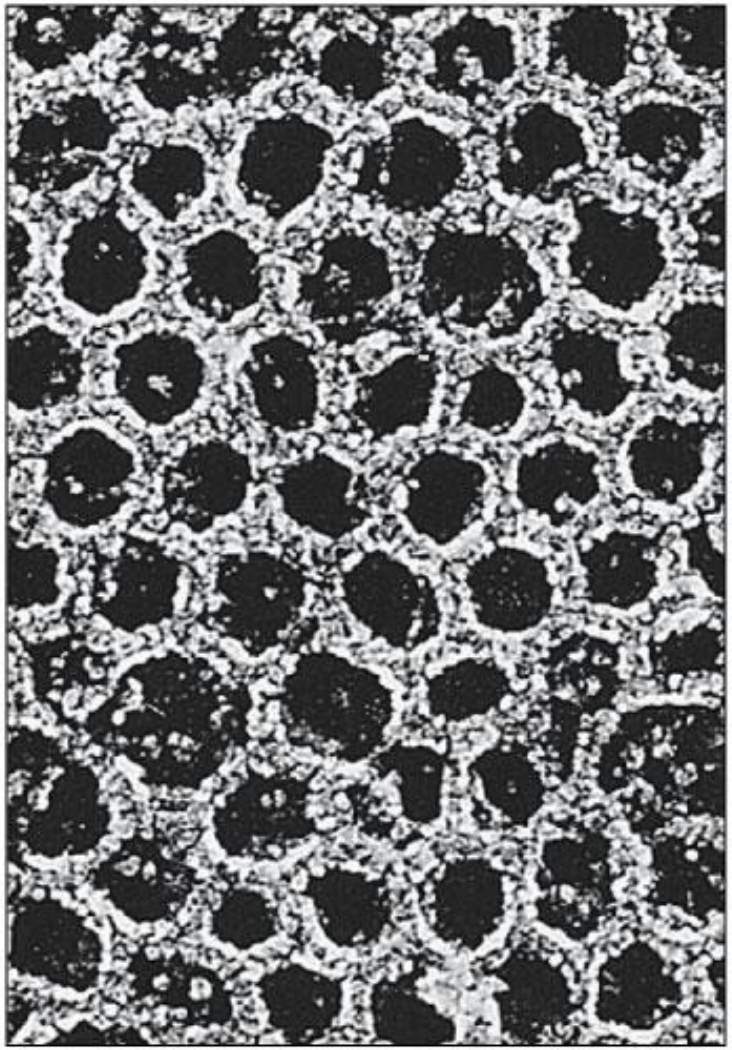
- Binding of AP complexes to the plasma membrane and concentration of receptors or receptor-ligand **complexes require ATP and GTP**

- The assembly of the clathrin coat appears to provide some of the driving force for vesicle formation

Clathrin coat assembly

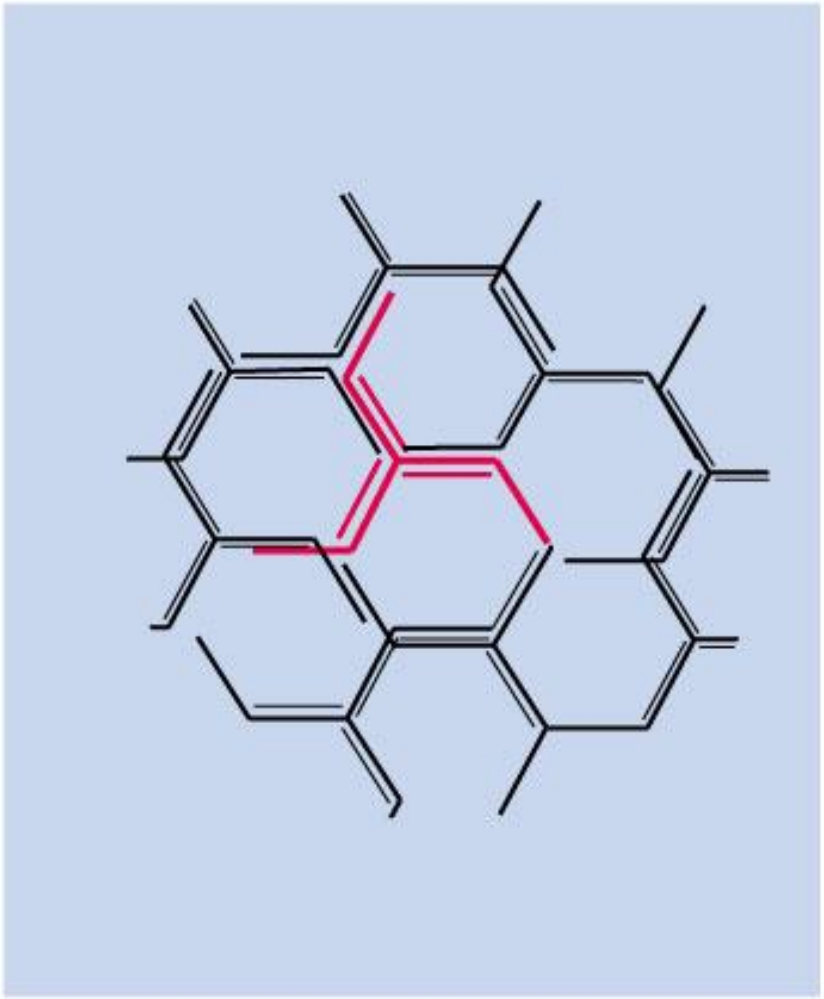
- Initially all clathrin units are hexagonal and form a planar structure
- As more triskelions are incorporated into the growing lattice, some pentagons are formed
- The mixture of pentagons and hexagons allows the new coat to curve around the budding vesicle

Figure 12-18A, C



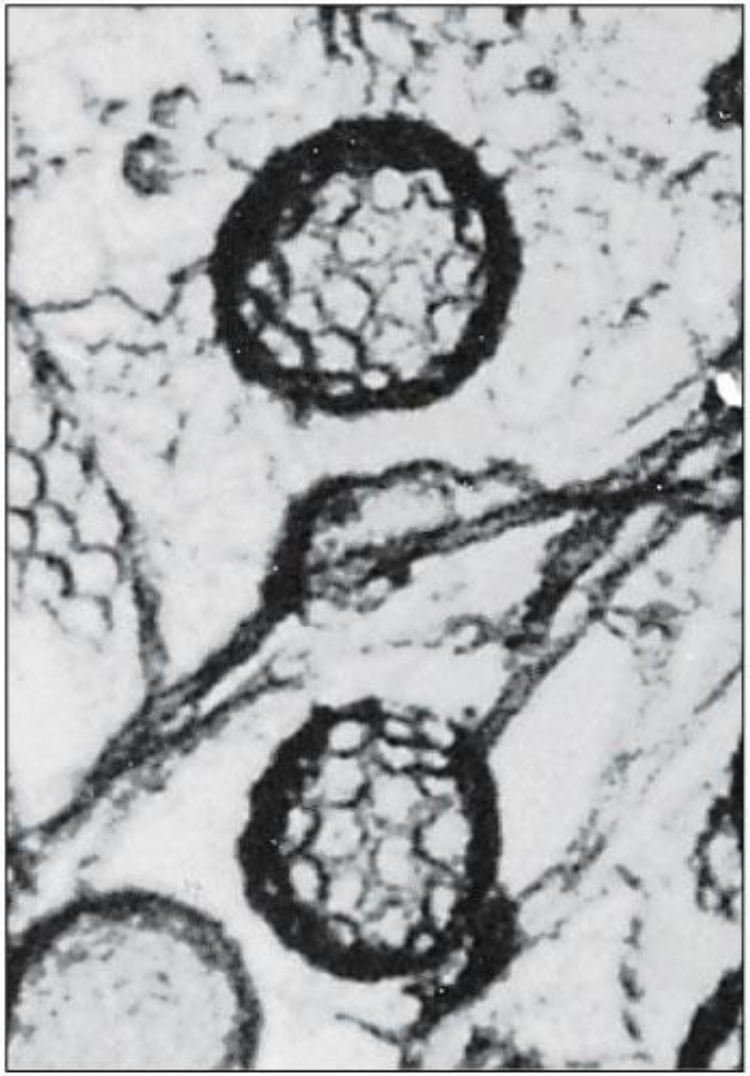
(a)

50 nm



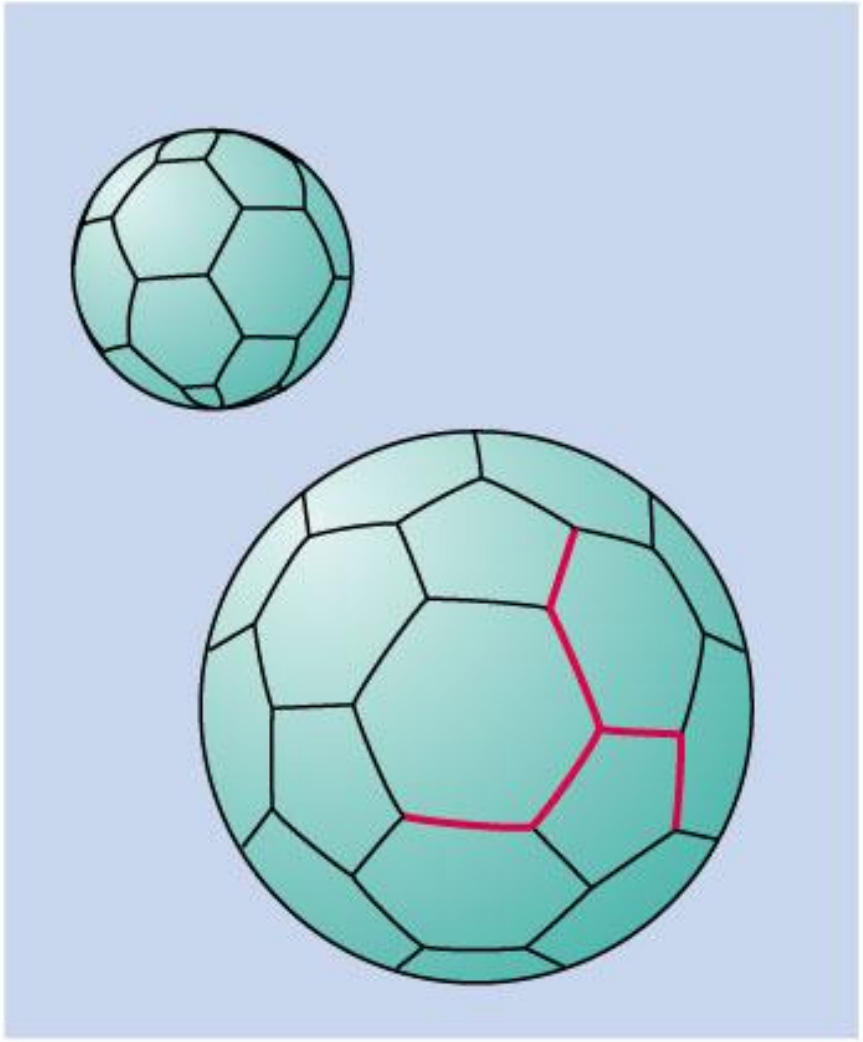
(c)

Figure 12-18B, D



(b)

500 nm

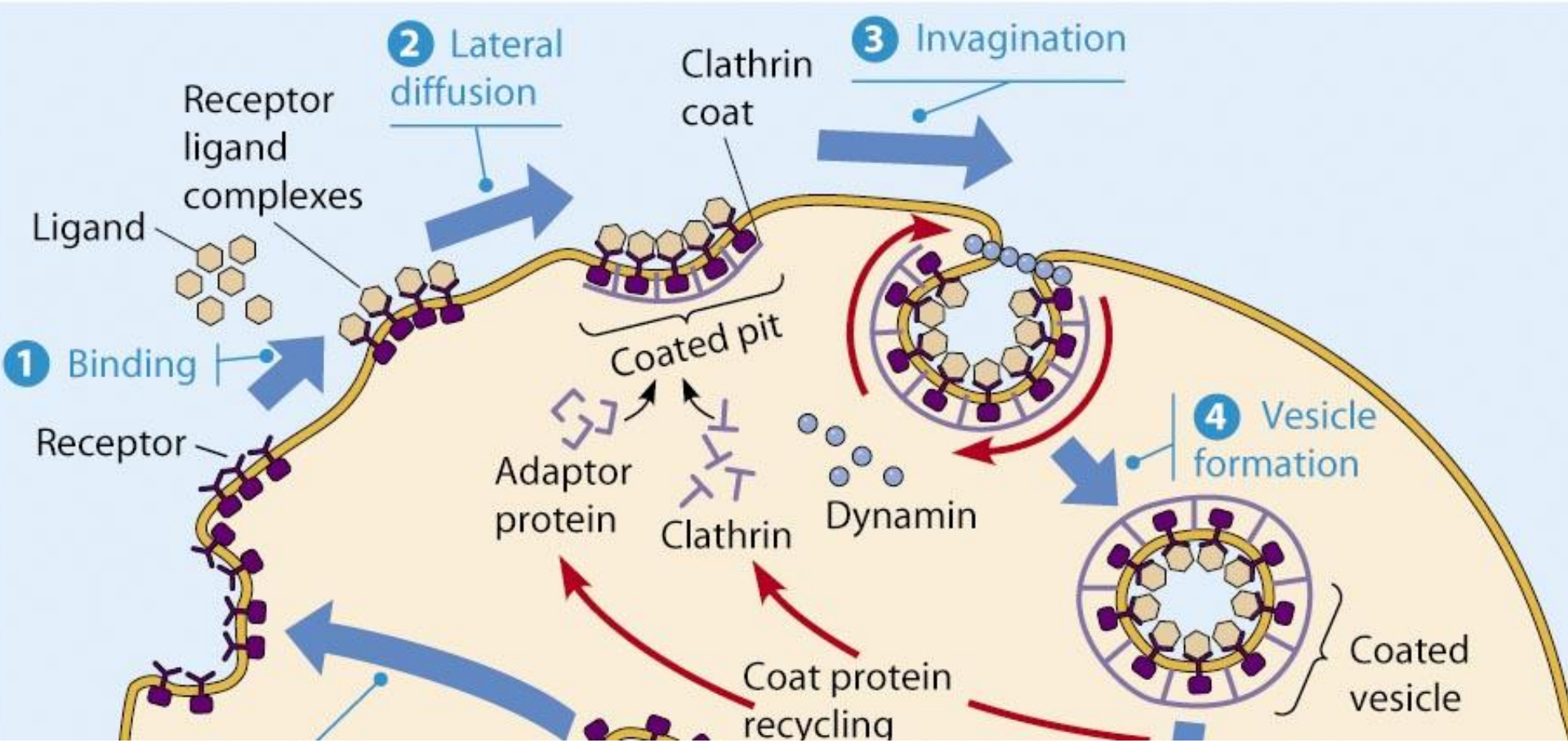


(d)

Dynamin

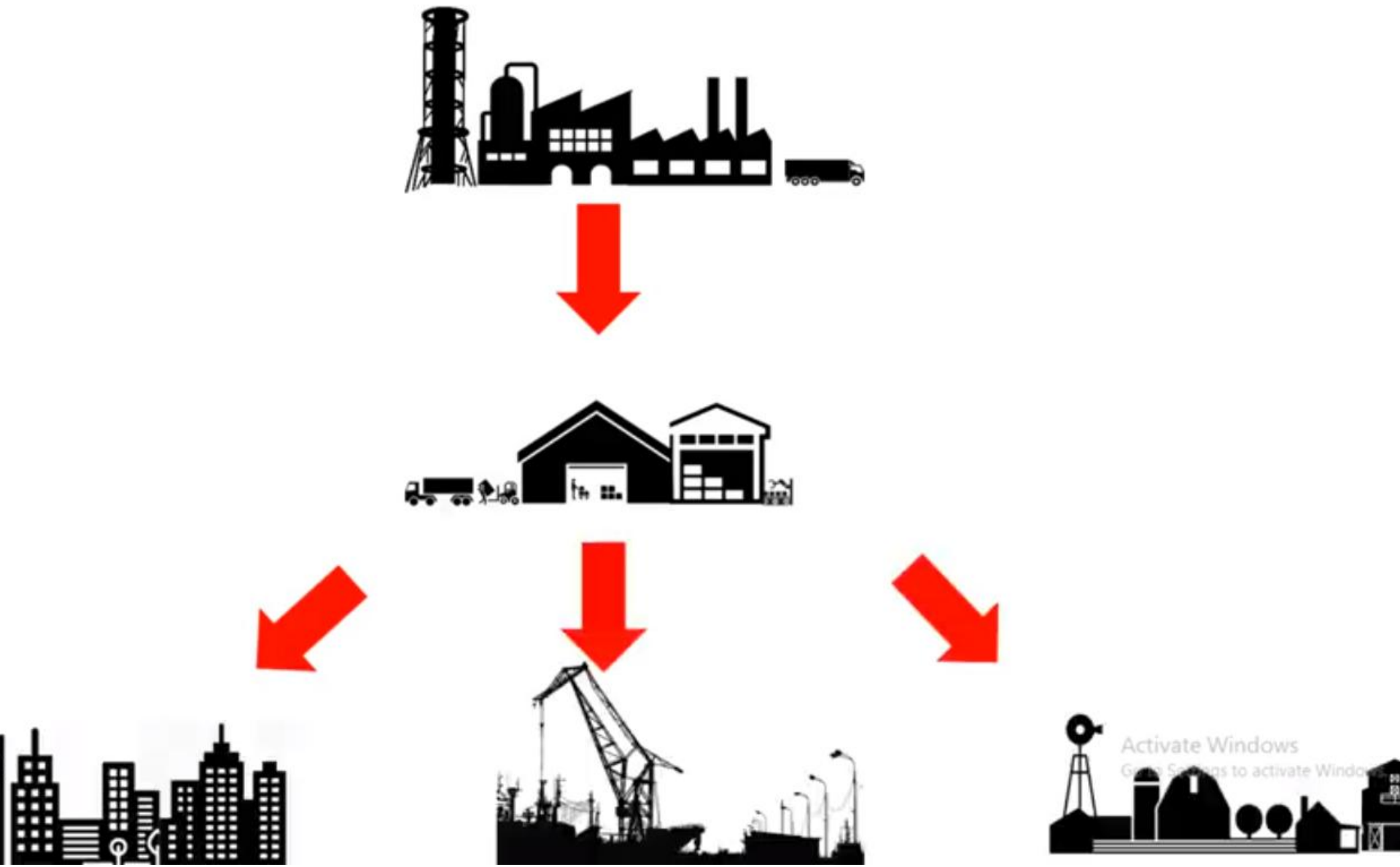
- As clathrin accumulates around the budding vesicle, **dynamin** is required for constricting and closing the vesicle

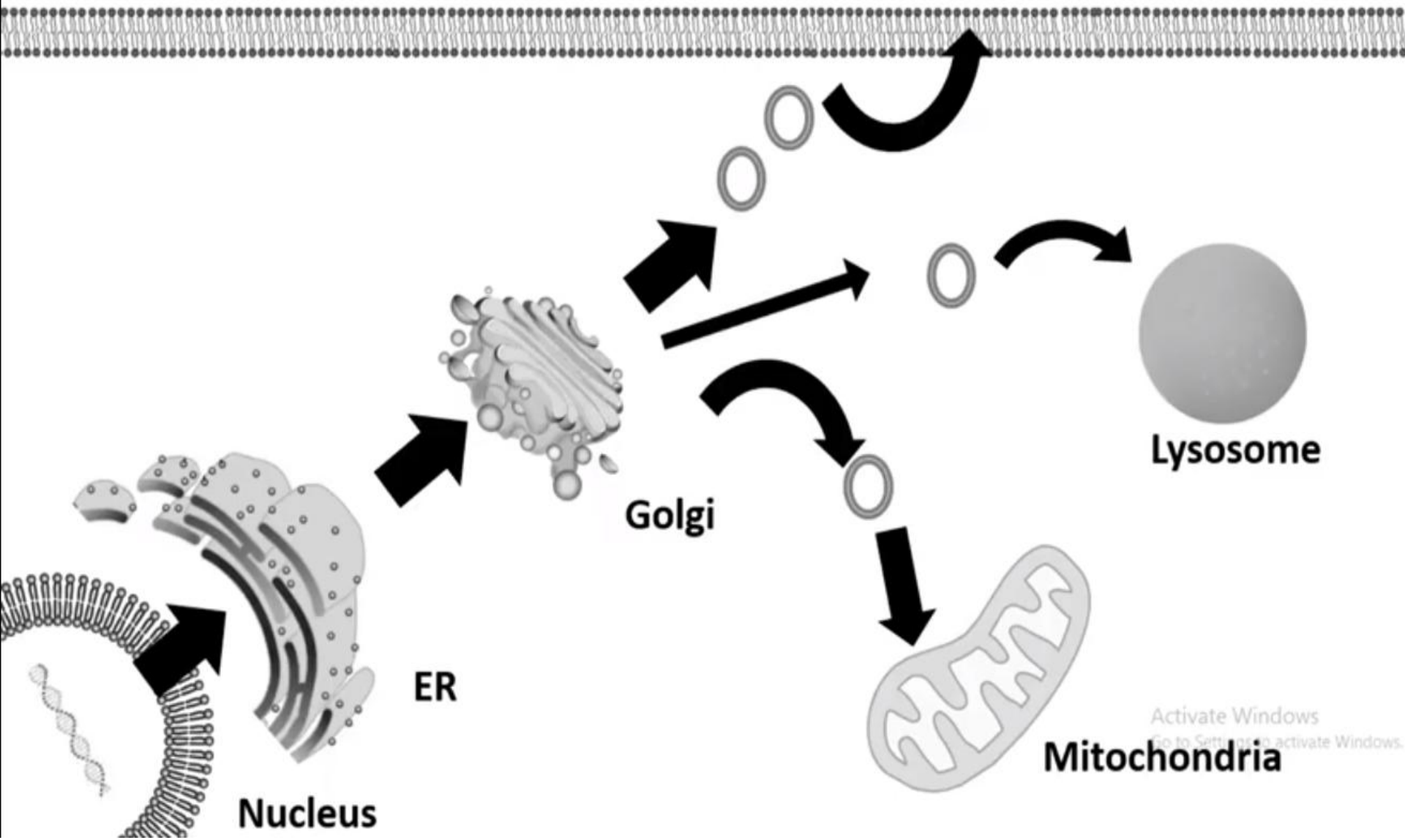
- Dynamin is a cytosolic GTPase; as GTP is hydrolyzed, dynamin rings tighten and separate the vesicle from the plasma membrane

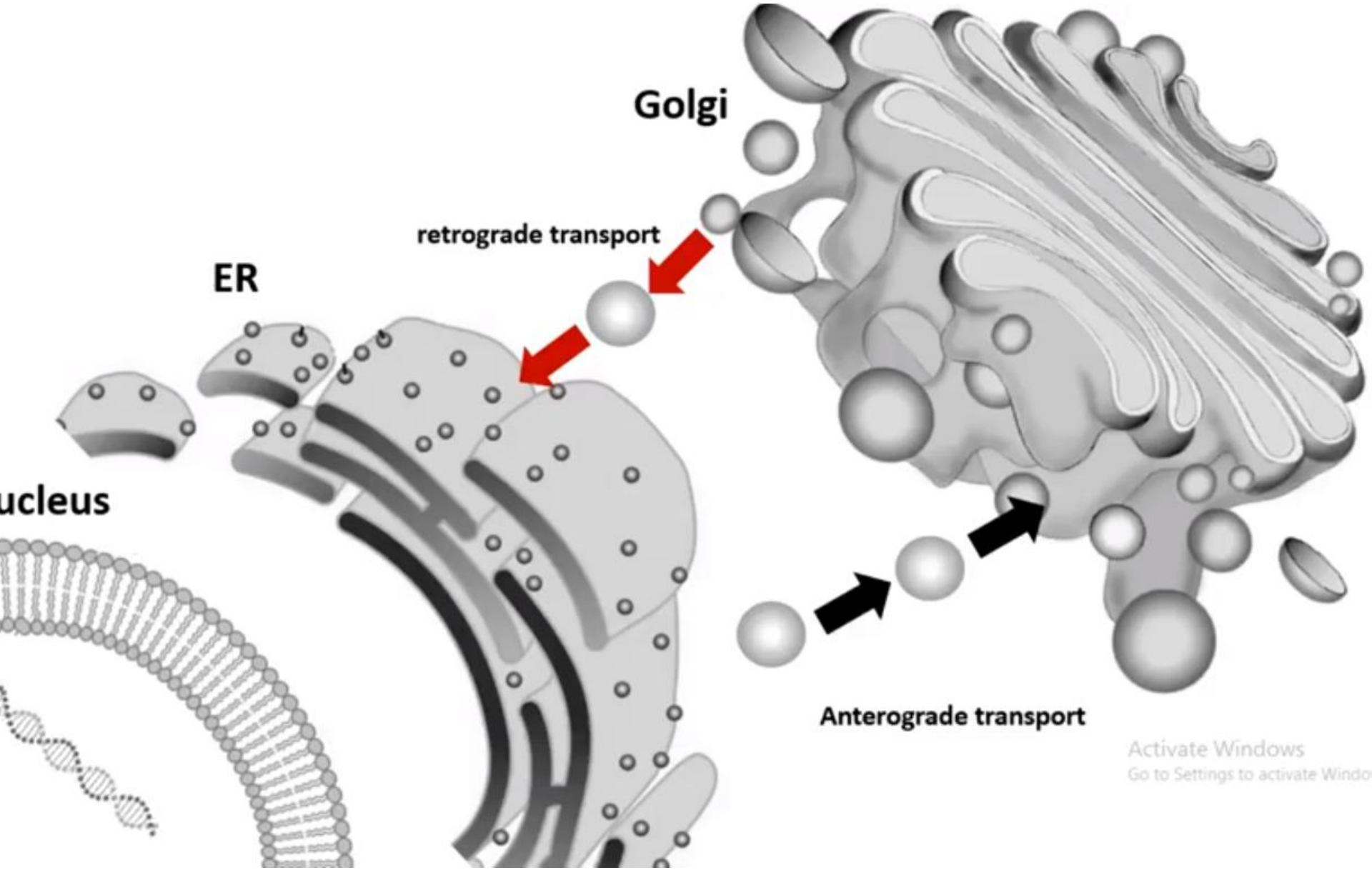


Uncoating the vesicle

- A mechanism is required to remove the clathrin coat (*uncoat*) from the newly formed vesicle
- Clathrin dissociates rapidly once the vesicle is formed
- Uncoating requires energy, provided by an *uncoating ATPase*







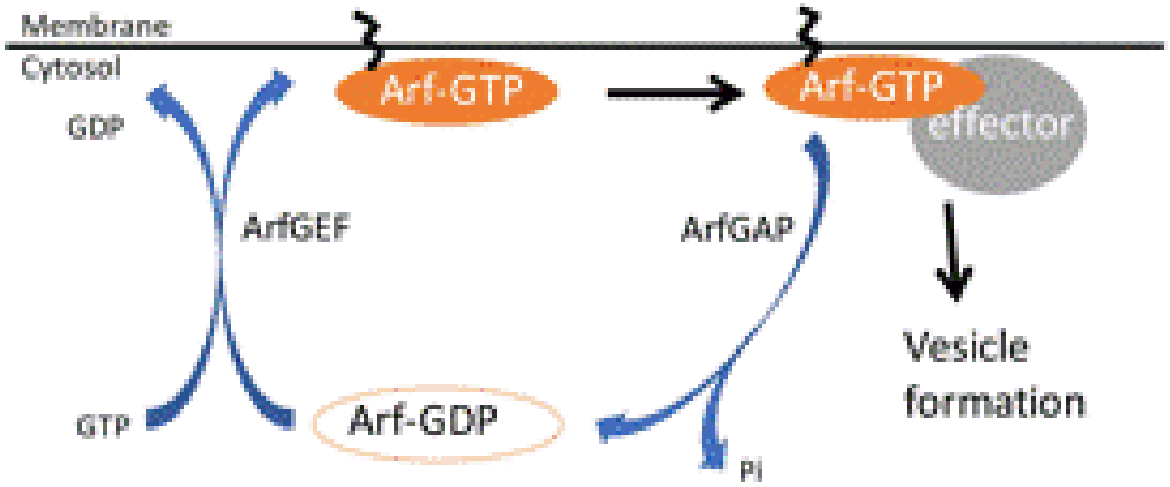
COPI- and COPII-Coated Vesicles Travel Between the ER and Golgi Complex Cisternae

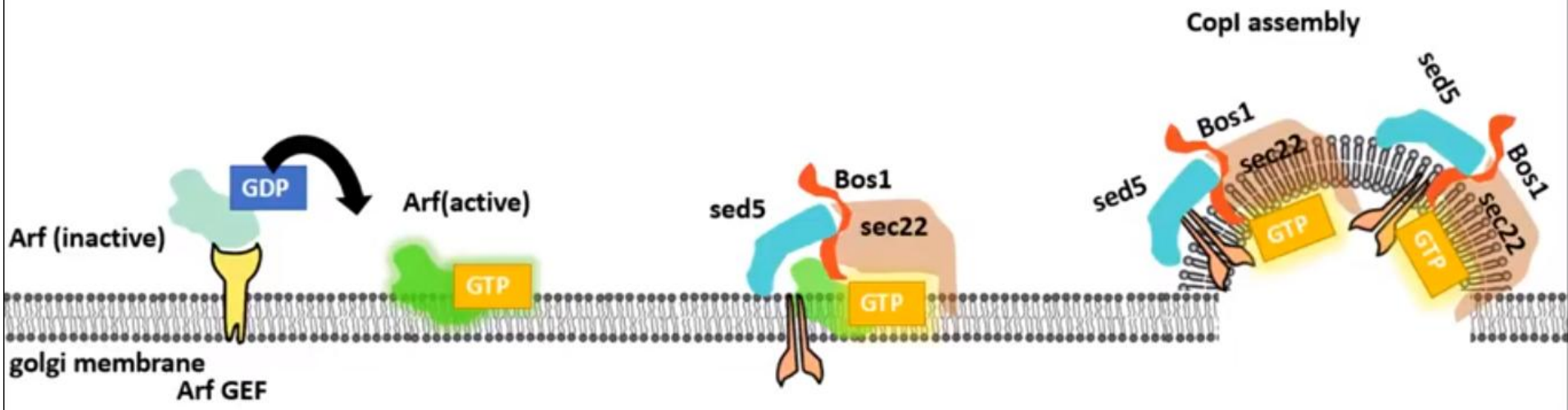
- *COPI-coated vesicles* are found in all eukaryotic cells examined and are involved in retrograde transport from the Golgi back to the ER
- The vesicles are coated with COPI and an **ADP ribosylation factor** (**ARF**), a small GTP-binding protein
- Assembly of the coat is mediated by ARF

Role of ARF

- In the cytosol, it exists as part of an ARF-GDP complex
- Upon meeting a *guanine nucleotide exchange factor* associated with the membrane, the GDP is exchanged for GTP
- The resulting conformational change in ARF attaches it to the lipid bilayer
- Once firmly anchored, ARF binds COPI multimers
- Assembly of the coat drives vesicle formation
- Once the vesicle is formed, a protein in the donor membrane triggers hydrolysis of GTP to GDP, a conformational change in ARF and release of the coat

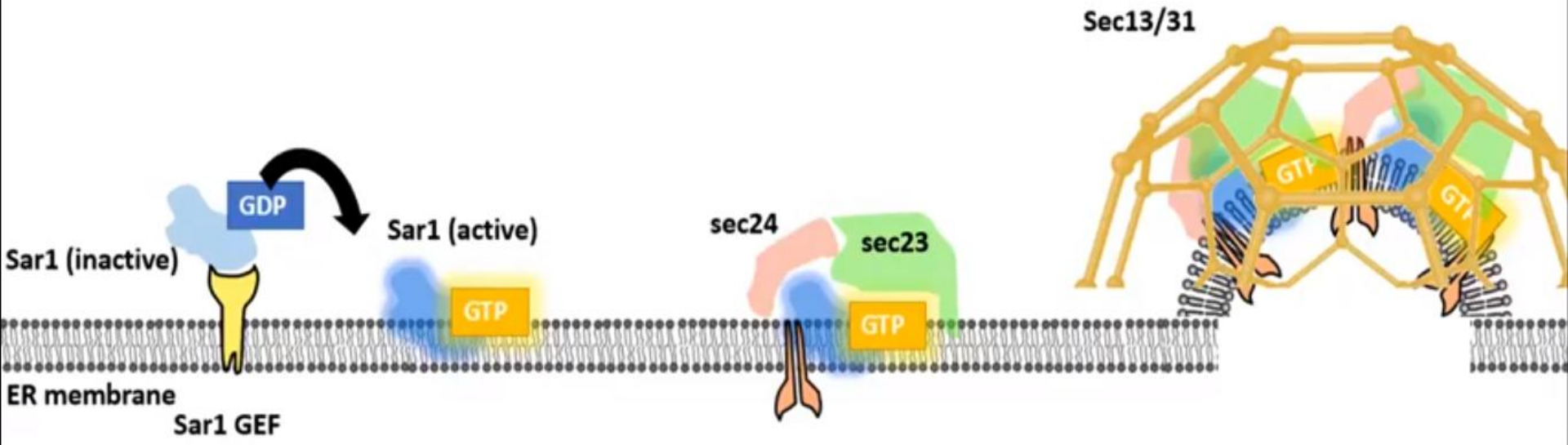
Figure 1

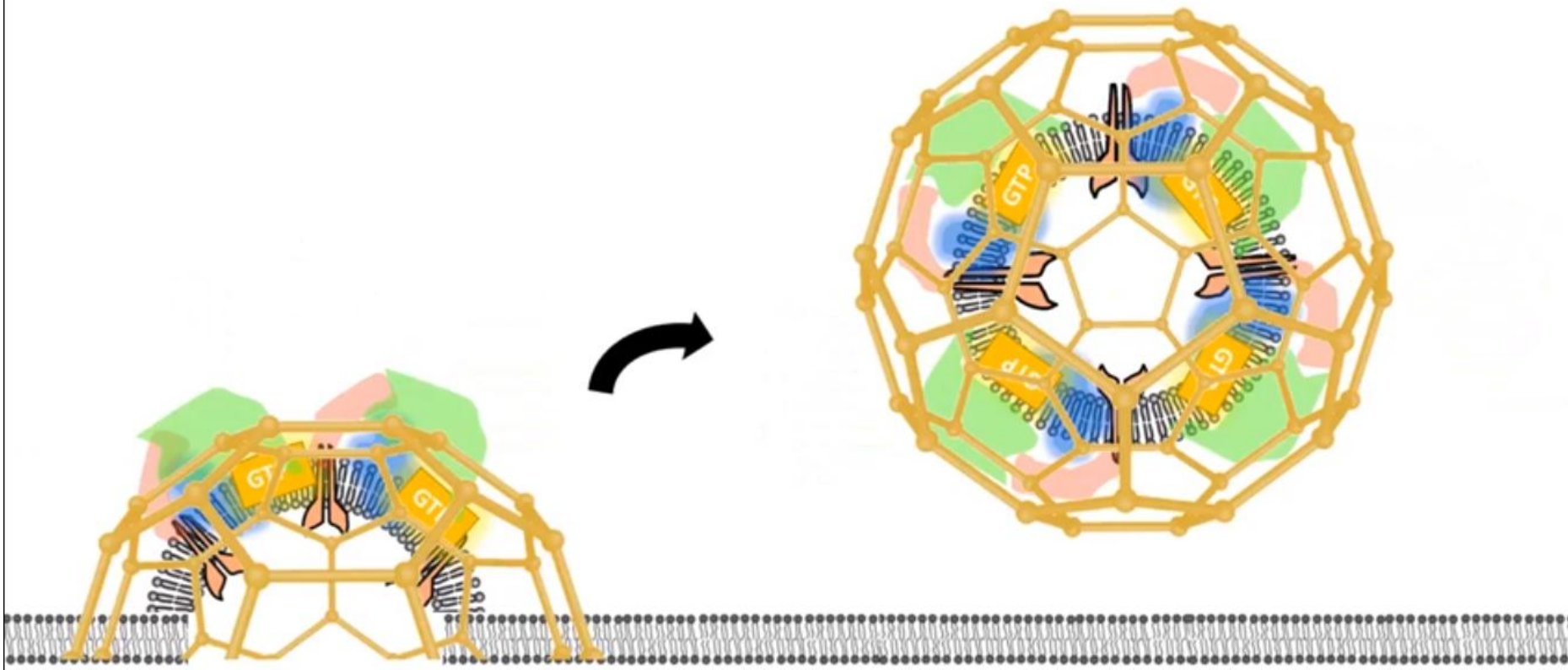




COPII-coated vesicles

- *COPII-coated vesicles* have a role in transport from the ER to the Golgi
- In yeast, the COPII coat is assembled from two protein complexes (*Sec 13/31* and *Sec 23/24*) and a small GTP-binding protein called *Sar1*
- Sar1 is similar to ARF and the process of coat formation is similar to COPI-coated vesicles



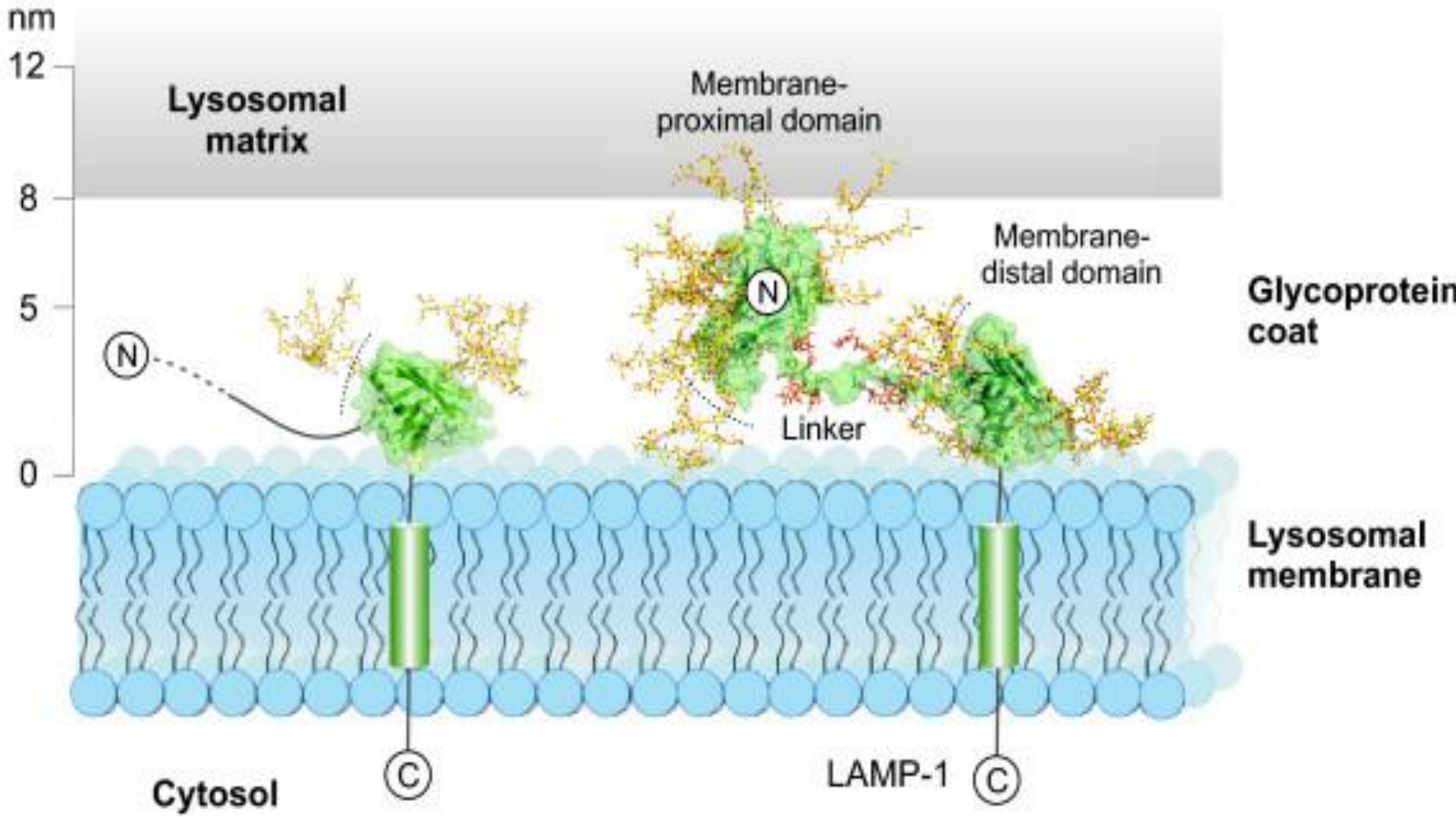


Lysosomes and Cellular Digestion

- The lysosome is an organelle of the endomembrane system that contains digestive enzymes
- It is capable of degrading all the major classes of biological macromolecules

Lysosomes Isolate Digestive Enzymes from the Rest of the Cell

- Lysosomes contain acid phosphatase and several other hydrolytic enzymes
- They vary in size and shape but are usually about 0.5 μm in diameter, bound by a **single membrane**
- **The lumenal side of the membrane is coated with glycoproteins to protect the membrane from degradation**



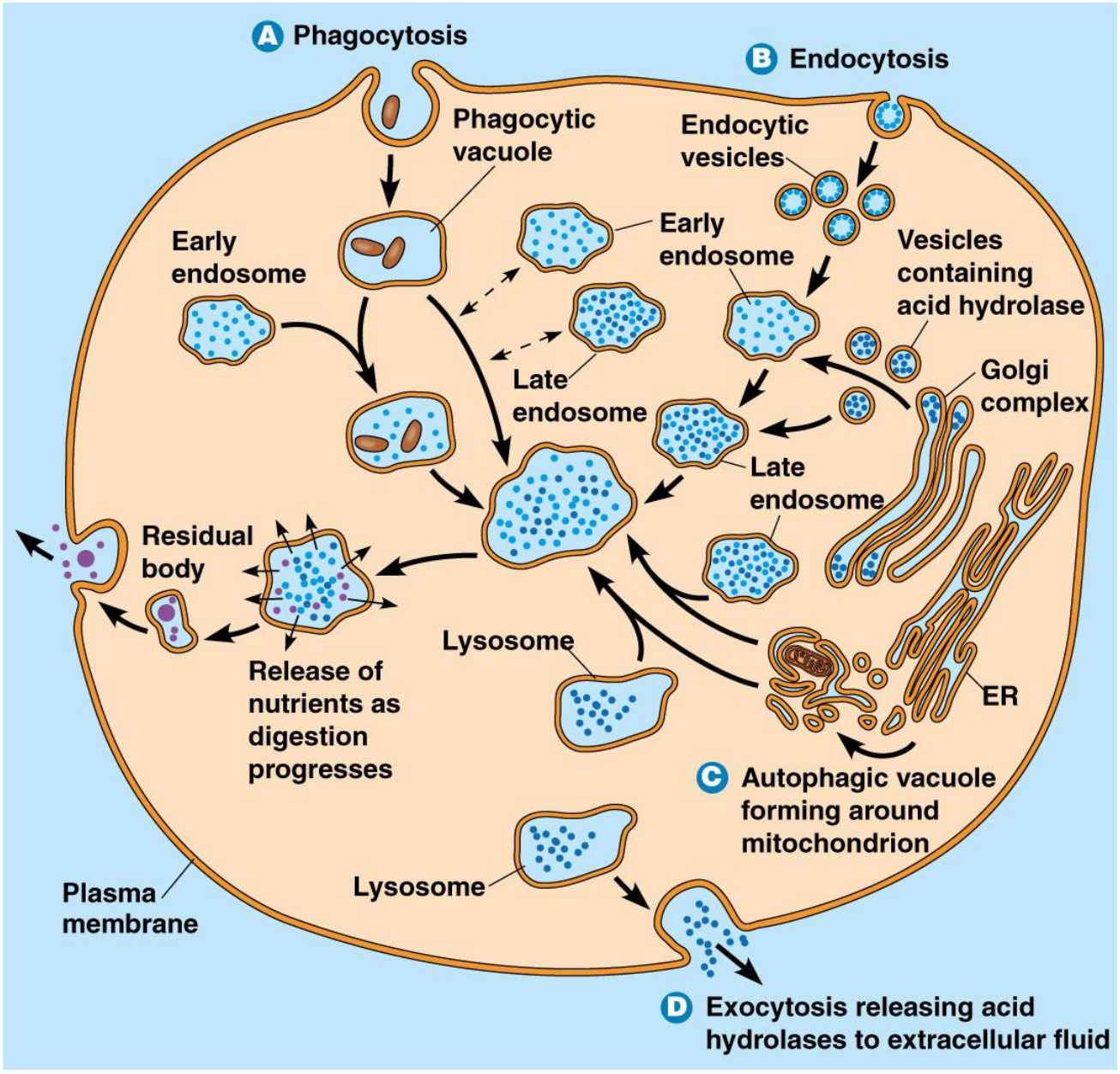
Lysosomes are highly acidic inside

- Lysosomes maintain an acidic environment (pH 4.0–5.0) inside
- ATP-dependent proton pumps in the membrane are responsible for this
- There are numerous enzymes inside lysosomes; all are *acid hydrolases*

Final step of lysosome development

- The last step in development of a lysosome is activation of the acid hydrolases
- This occurs as the internal environment becomes more acidic
- This occurs through the pumping of protons or through fusion with an existing lysosome

Figure 12-21



Lysosomes Develop from Endosomes

- Lysosomal enzymes are synthesized by ribosomes on rough ER, and translocated inside
- Lysosomal enzymes are delivered from the TGN to endosomes in transport vesicles
- Over time endosomes mature into late endosomes, with all the enzymes, but not engaged in digestion

Lysosomal Enzymes Are Important for Several Different Digestive Properties

- Lysosomal material have multiple origins
- Those containing substances that originated outside the cell are called **heterophagic lysosomes**
- Those with materials that originated inside the cell are called **autophagic lysosomes**

Phagocytosis and Receptor-Mediated Endocytosis: Lysosomes in Defense and Nutrition

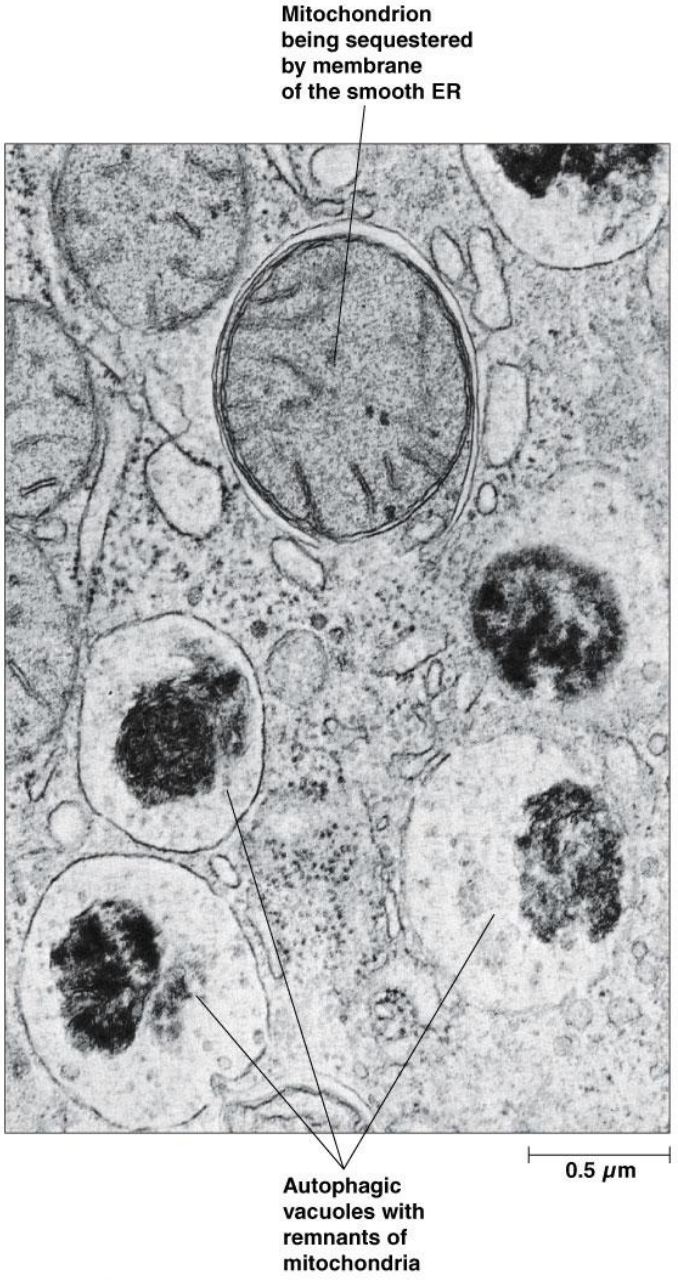
- The degradation of **external materials** brought into the cell occurs by *phagocytosis* and *receptor-mediated endocytosis*
- Phagocytic vacuoles become lysosomes by fusion with endosomes
- Vesicles formed by receptor-mediated endocytosis fuse with vesicles of the TGN containing acid hydrolases

After digestion is complete

- Eventually indigestible material is all that remains in a lysosome
- The lysosome becomes a **residual body** when digestion ceases
- Some cells release the contents by exocytosis; in others, accumulation of debris may contribute to cellular aging

Autophagy: A Biological Recycling System

- Cellular structures that are damaged or unneeded must be broken down, via **autophagy**
- **Macrophagy**: an organelle is wrapped in a double membrane derived from the ER, forming an **autophagic vacuole** (**autophagosome**)
- **Microphagy**: a much smaller vacuole is formed, surrounded by a single-membrane bilayer



Extracellular Digestion

- Most lysosomal digestion occurs inside the cell
- In some cases, lysosomes **discharge their contents outside the cell**, resulting in extracellular digestions
- For example, the head of a sperm releases digestive enzymes to degrade barriers protecting an egg